

Female germ unit in *Genlisea* and *Utricularia*, with remarks about the evolution of the extra-ovular female gametophyte in members of Lentibulariaceae

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Abstract Lentibulariaceae is the largest family among carnivorous plants which displays not only an unusual morphology and anatomy but also the special evolution of its embryological characteristics. It has previously been reported by authors that *Utricularia* species lack a filiform apparatus in the synergids. The main purposes of this study were to determine whether a filiform apparatus occurs in the synergids of *Utricularia* and its sister genus *Genlisea*, and to compare the female germ unit in these genera. The present studies clearly show that synergids in both genera possess a filiform apparatus; however, it seems that *Utricularia quelchii* synergids have a simpler structure compared to *Genlisea aurea* and other typical angiosperms. The synergids are located at the terminal position in the embryo sacs of *Pinguicula*, *Genlisea* and were probably also located in that position in common *Utricularia* ancestor. This ancestral characteristic still occurs in some species from the *Bivalvaria* subgenus. An embryo sac, which grows out beyond the limit of the integument and has contact with nutritive tissue, appeared independently in different *Utricularia* lineages and as a consequence of this, the egg apparatus changes position from apical to lateral.

Keywords Egg apparatus · Synergids · Central cell · Ovule · Ultrastructure · Carnivorous plants · Lentibulariaceae

Introduction

During the growth and guidance of the pollen tube, two phases are distinguished: saprophytic and gametophytic. In the latter, embryo sac (ES; female gametophyte) plays a key role. After experiments on the *Torenia fournieri* (Higashiyama et al. 2001; 2003), it is widely accepted that synergids are responsible for the attraction of pollen tubes in angiosperms. An embryo sac with two dead synergids did not attract any pollen tubes (Higashiyama et al. 2001).

However, other studies have also suggested that the egg cell and central cell also play a role in the attraction of pollen tubes; e.g., in maize in both the synergids and egg cell, the expression of Zm EA1 gene, which encodes a small (94 amino acids) transmembrane protein needed for proper micropylar pollen tube attraction, was recorded (Marton et al. 2005). In *Arabidopsis thaliana* female gametophytic central cell guidance mutant, the micropylar pollen tube guidance is absent. Thus, all parts of female germ unit are important in proper pollen tube guidance and bursting to release the sperm cells (Chen et al. 2007). Nevertheless, synergids are essential for the fertilization process of angiosperms (Higashiyama et al. 2001; Punwani and Drews 2008 and reference therein; see also Li et al. 2009). In some plants, e.g., in *Plumbago* and *Plumbagella* where synergids are absent, the egg cell takes over the functions of the synergid (Huang et al. 1990) and also possesses a synergid characteristic—a filiform apparatus (Cass 1974; Cass and Karas 1974; Russell and Cass 1988). The filiform apparatus appears to be a universal structure in angiosperms, and even occurs in the synergids of such ancestral like as the *Amborella*-type female gametophyte (Friedman and Ryerson 2009). However, some rare exceptions also occur, e.g., according to Janson and Willemse (1995) no filiform apparatus was detected in cultivars of *Lilium longiflorum*, and moreover, no synergid degeneration occurred

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prior to pollen tube penetration. Recently, very interesting hypothesis was proposed that some apomictic species from the *Compositae* family (genera *Chondrilla*, *Taraxacum*) have synergids only in the juvenile phase, which lacks a filiform apparatus, and that this is connected with apomixis (Kościńska-Pająk 2006; Kościńska-Pająk and Bednara 2006). There are, however, arguments against this idea because other studies of apomictic species from other families showed the occurrence of the filiform apparatus (e.g., Jankun 1994; Guan et al. 2007).

Khan (1954), and later Shivaramiah (1967), reported that the *Utricularia* species (Lentibulariaceae) also lack a filiform apparatus in synergids. Recently, the family Lentibulariaceae has attracted increased scientific interest because of its carnivory (e.g., Adamec 2006; 2007; 2008; Płachno et al. 2007; Płachno and Wołowski 2008; Sirová et al. 2009; 2010), ultra-small haploid genome (Greilhuber et al. 2006), molecular evolution (Jobson et al. 2002; Laakkonen et al. 2006; Müller et al. 2004; Albert et al. 2010), and unusual embryo evolution (Płachno and Świątek 2010). In relation to the morphological and anatomical transformation of vegetative organs, Lentibulariaceae can only compete with river weeds (Rutishauser et al. 1999, 2005; Kirchoff et al. 2008; Ghogue et al. 2009; Koi and Kato 2010) and some parasitic plants. Also in the case of reproduction, Lentibulariaceae and especially the genus *Utricularia* has many advanced evolutionary characteristics (Khan 1954; Płachno and Świątek 2008; 2009; Płachno et al. 2009). Thus, perhaps the egg apparatus in members of Lentibulariaceae has special characteristics?

The main purposes of this study were to determine whether a filiform apparatus occurs in the synergids of *Utricularia* and its sister genus *Genlisea* and to compare the female germ unit in these genera. Another purpose was to examine the evolution of the extra-ovular female gametophyte in members of Lentibulariaceae.

Materials and methods

Flowers of *Genlisea aurea* A. St.-Hil. (subgen. *Genlisea*, Fischer et al. 2000), Chapada dos Guimarães (Mato Grosso state, Brazil) and Itacambira (Minas Gerais state, Brazil), and *Utricularia quelchii* N.E.Br. (subgenus *Utricularia*, Müller and Borsch, 2005), Ilu Tepui (Venezuela), at the anthesis stage were obtained from the greenhouse collection of Kamil Pásek at Dobroslavice (Ostrava, Czech Rep.).

Light and electron microscopy

For electron microscopy, placentas with ovules were isolated from ovaries and fixed in 2.5% formaldehyde and 2.5% glutaraldehyde in a 0.05 M cacodylate buffer (pH 7.0) for

2 days. The material was postfixed in 1% OsO₄ in a cacodylate buffer for 24 h at ~4°C, rinsed in the same buffer, treated with 1% uranyl acetate in distilled water for 1 h, dehydrated with acetone and embedded in Epon 812 (Fullam, Latham, NY). Semithin sections were stained with methylene blue and examined using an Olympus BX60 microscope. The periodic acid-Schiff (PAS) reaction (Wędzony 1996) was used to detect water insoluble polysaccharides with 1,2-glycol groups. Ultrathin sections were cut on a Leica ultracut UCT ultramicrotome. After contrasting with uranyl acetate and lead citrate, the sections were examined using a Hitachi H500 electron microscope.

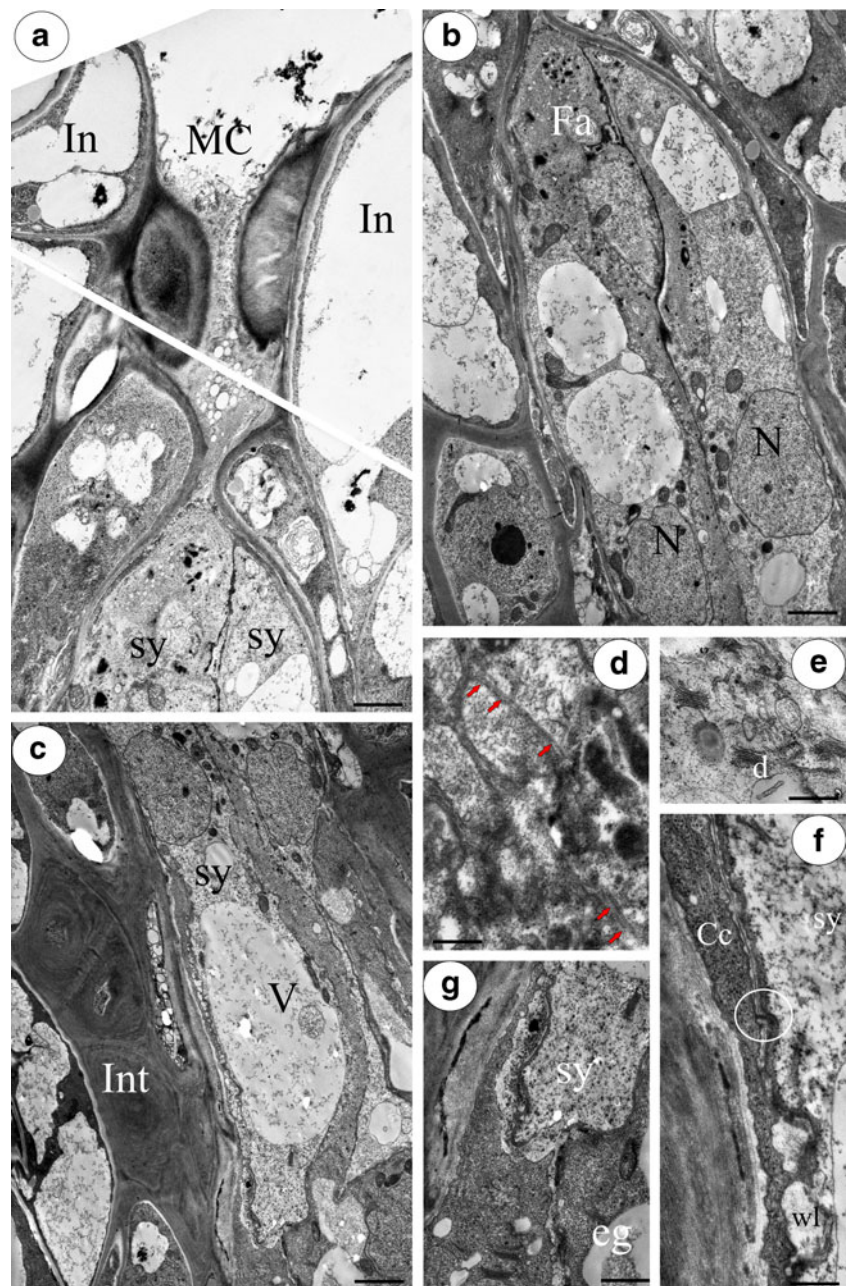
Results

Genlisea aurea (Figs 1, 2, 3, and 4)

The micropylar canal is very short and partially filled with secretion (Fig. 1a). The micropylar part of synergids extends to the micropylar canal. Synergids are elongated and slightly pear-shaped (Fig. 1b–c). There are small vacuoles in the micropylar pole; whereas, a large vacuole occurs in the chalazal pole (Fig. 1c). The synergid nucleus occurs in a mid-longitudinal position within the cell. The filiform apparatus occurs in both synergids in the micropylar and middle region. It is connected with a common synergid cell wall. It is a sponge-like mass wall material (stained by the PAS reaction), contains some osmiophilic inclusion, and is inter-penetrated by a plasma membrane and cytoplasm with microtubules (Fig. 1d). Both synergids have cytoplasm that is poor in ribosomes in comparison to the egg cell and the central cell. Generally, synergids are also poorer with regard to the presence of organelles in comparison to the central cell. Mitochondria have mainly a perinuclear distribution (Fig. 1b) but also occur near the filiform apparatus. Dictyosomes also occur but they are not active; each dictyosome is composed of up to eight cisternae (Fig. 1e). In the middle part of a synergid, between the central cell and the synergid, there is a typical cell wall with middle lamella and plasmodesmata (Fig. 1f); however, in the direction of the chalazal pole this wall disappears, and pockets of fibrillar wall material appear between plasma membranes (Fig. 1f). In the chalazal pole of the synergid between the central cell and synergid, there is an irregular layer of wall material—fine-fibrillar material (Fig. 1g)—which gives a positive result after the PAS reaction (Fig. 2a). This layer also separates the synergid and the central cell on the side of the filiform apparatus (Fig. 2a).

The vase-shaped egg cell is situated below the synergids (Fig. 2b). In the micropylar part of the egg cell, near the attachment of the egg to lateral megagametophyte wall, the wall between the egg cell and the central cell has a middle

Fig. 1 Ultrastructure of the ovule and embryo sac of *G. aurea*. **a** Sections through the micropylar canal and the micropylar part of synergids, *In* integument, *MC* micropylar canal, *sy* synergid, *bar*=1.2 μ m. **b** Sections through the micropylar and the central parts of the synergids, *Fa* filiform apparatus, *N* nucleus, *bar*=1.6 μ m. **c** Chalazal parts of the synergids, *V* vacuole, *Int* thick-walled integument cells, *bar*=2 μ m. **d** A part of a filiform apparatus, *arrow* microtubules, *bar*=0.45 μ m. **e** Synergid cytoplasm with dictyosomes (**d**), *bar*=0.6 μ m. **f** middle part of a synergid, the wall between the central cell and the synergid, *circle* plasmodesmata, *sy* synergid, *Cc* central cell, *wl* wall packet, *bar*=0.4 μ m. **g** Chalazal parts of the synergid, *sy* synergid, *eg* egg cell, *bar*=1.2 μ m

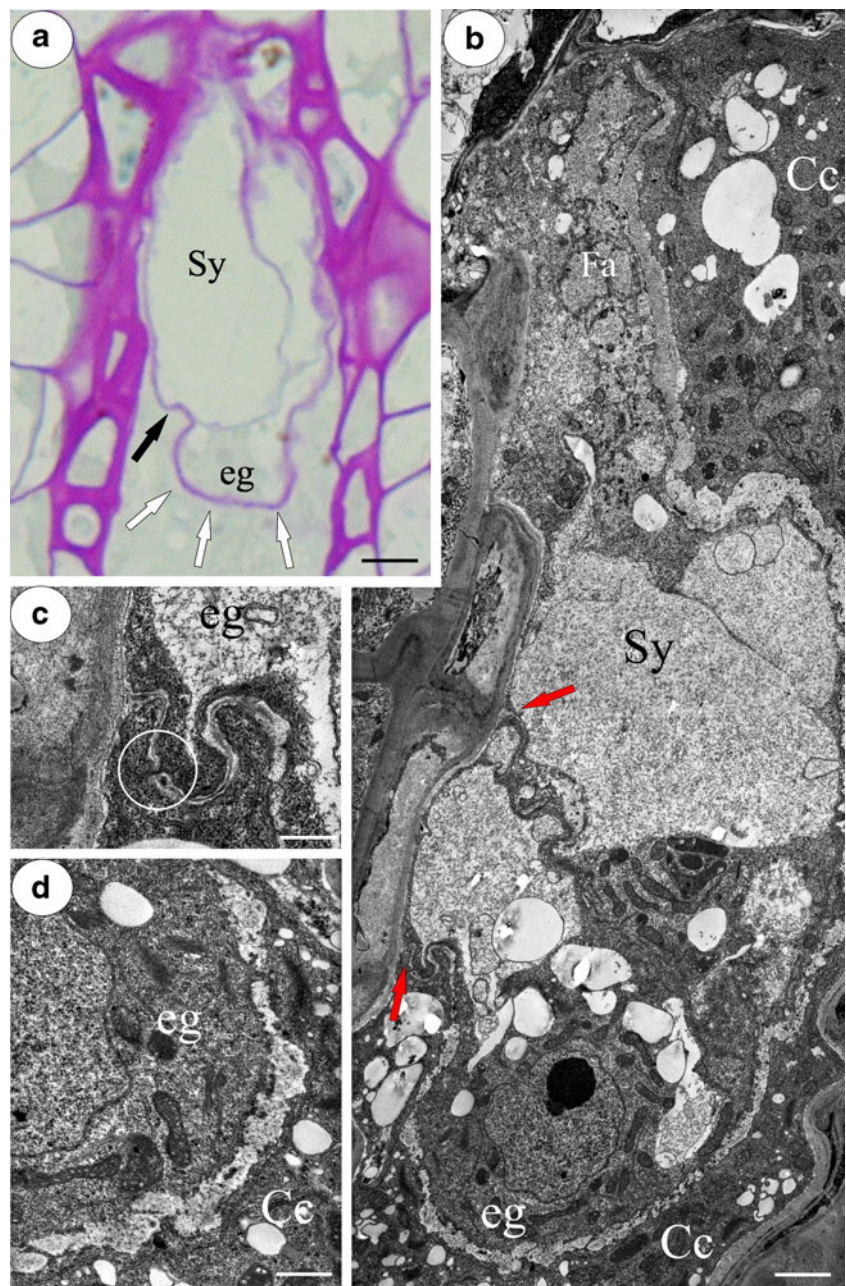


lamella and plasmodesmata (Fig. 2c). In the chalazal pole of the egg cell between the central cell and the egg, there is layer of wall material—fine-fibrillar material that is translucent in the electron matrix (Fig. 2d)—which has a different thickness and gives a positive result after the PAS reaction (Fig. 2a). In some egg cells, osmiophilic deposits are seen in this layer. The micropylar pole of the egg cell is vacuolated (Fig. 2b), but in some egg cells small vacuoles may also occur in a perinuclear position at the chalazal pole. The cytoplasm is rich in ribosomes. Near the nucleus of the egg cell, there are mitochondria, long cisternae of ER, inactive dictyosomes (Fig. 3a), and plastids with dark

stroma. In addition, microtubules are clearly visible in the egg cell cytoplasm (Fig. 3b).

The central cell seems to be the most active part of the female gametophyte. Its cytoplasm is full of mitochondria, dictyosomes, and plastids (Fig. 3c–d), and also in the micropylar part (Fig. 2b). The dictyosomes are very active in the production of large vesicles with an electron-translucent context; these vesicles fuse forming vacuoles. endoplasmic reticulum (ER) and microbodies also occur. Large polar nuclei occur near the vicinity of the egg cell (Fig. 3c); they later fuse creating a secondary nucleus (Fig. 4a–b). There are numerous microtubules in the

Fig. 2 Anatomy and ultrastructure embryo sac of a *G. aurea*. **a** Egg apparatus after PAS reaction, note PAS-positive wall on the chalazal part of the egg cell (white arrow) and synergid (black arrow), bar=4 μm ; **b** egg apparatus *Fa* filiform apparatus, *sy* synergid, *eg* egg cell, red arrows attachment of egg wall to the wall of the embryo sac, *Cc* central cell, bar=1.7 μm ; **c** wall between the central cell and the egg cell, circle plasmodesmata, *eg* egg cell, bar=0.4 μm ; **d** the micropylar part of egg cell, *eg* egg cell, *Cc* central cell, bar=1 μm



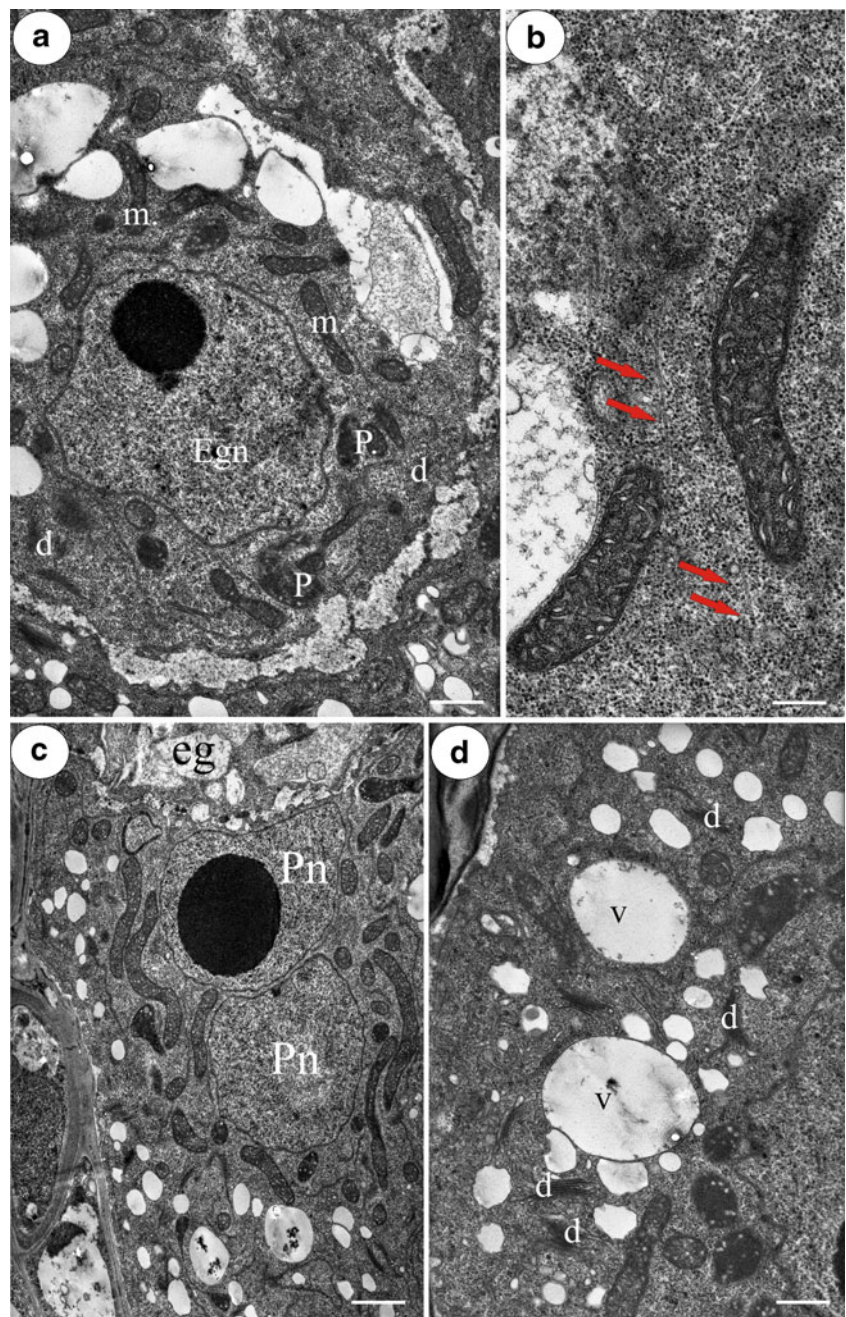
cortical cytoplasm there are numerous microtubules (Fig. 4c), some of them lying parallel to the plasma membrane. The central cell is highly vacuolated in the chalazal part (not shown).

Utricularia quelchii (Figs 5, 6, and 7)

The ovule is very small, tenuinucellate, and unitegmic. The integument is merged with the funicle forming a raphe. The embryo sac is extended and slightly curved. In the middle part, it borders the integumental tapetum. The most impressive part of the mature embryo sac is the micropylar part of the central cell which projects out beyond the limit

of the integument and forms a bulbous apex which grows in the placenta nutritive tissue (Fig. 5a) which is a spherical group of colenchymatous cells that differentiates near the base of the ovule. Thus, a pocket filled with embryo sac is formed in the placenta. This micropylar part of the central cell has a large vacuole traversed by cytoplasmic strands (Fig. 5b) with many mitochondria. The outer cytoplasm is rich in ribosomes; there are also mitochondria, cisternae, and tubules of ER, dictyosomes, microbodies, and lipid bodies. Microtubules are seen (Fig. 5c) near branched tubules of ER. Placental cells in contact with embryo sac are crushed and their remnants are clearly visible. The micropylar part of the central cell has contact with the

Fig. 3 Ultrastructure of a *G. aurea* egg cell and central cell. **a** Micropylar part of egg cell, *Egn* egg nucleus, *m* mitochondrion, *d* dictyosome, *P* plastid, *bar*=0.9 μm ; **b** the part of egg cytoplasm with mitochondria and microtubules (*red arrows*), *bar*=0.3 μm ; **c** the part of the central cell with polar nuclei (*Pn*), *eg* egg cell, *bar*=1.4 μm ; **d** the part of central cell cytoplasm with active dictyosomes (*d*), *v* vacuole, *bar*=0.7 μm

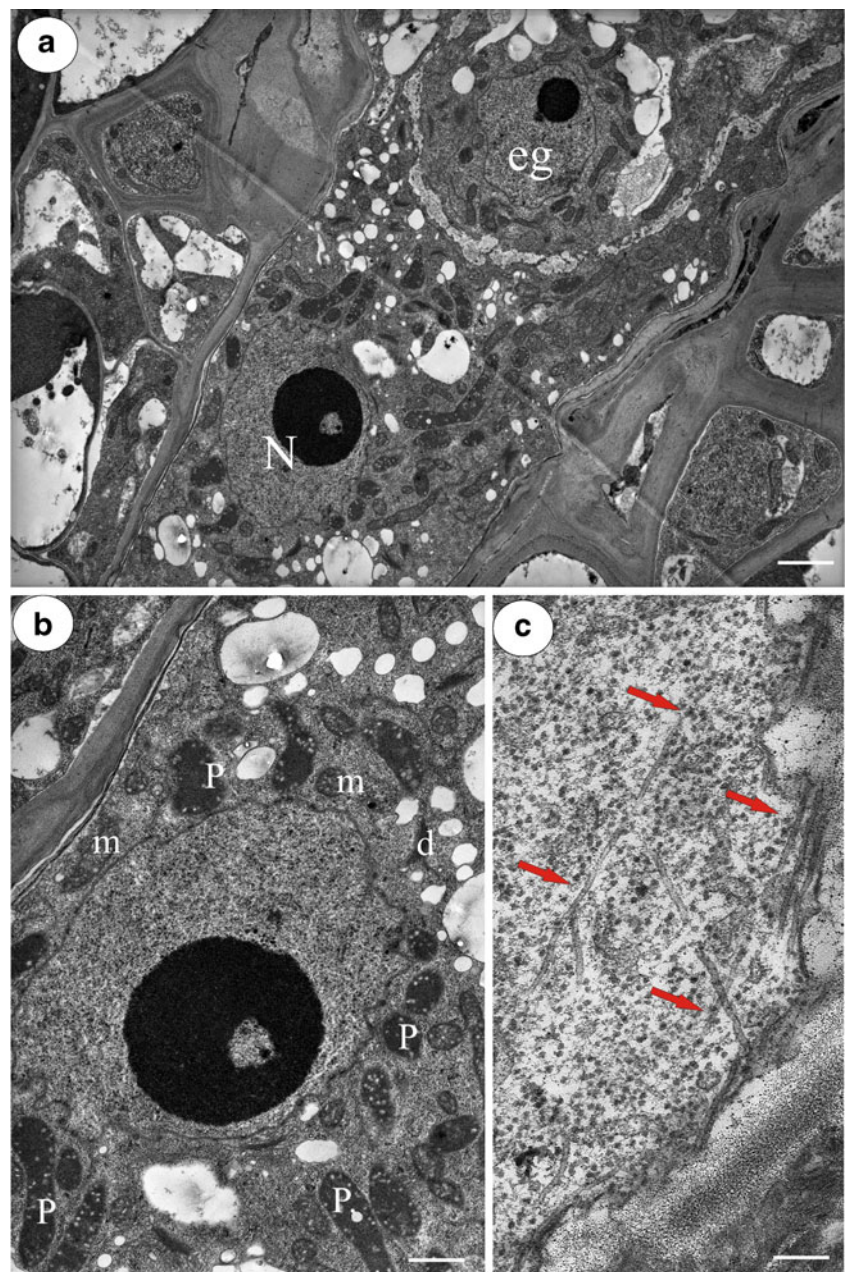


environment of the ovary chamber (Fig. 5d) because between the apex of the integument and the placenta there is a semi-circular slit. This “naked” part of the central cell has a thin layer of peripheral cytoplasm with multivesicular bodies (Fig. 5f). The cell wall here is thin and its external surface is covered by secretion (Fig. 5f).

The egg apparatus is not organized in the micropylar apex but occurs laterally on the raphe side of the embryo sac (Fig. 5a). Synergids occur side by side in the horizontal plane, while the egg cell is situated below them (Fig. 6a). The filiform apparatus occurs in both synergids in the

micropylar region. It forms an irregular layer, which consists of a fine-fibrillar material submerged in an electro-translucent matrix (Figs. 5d and 6b). The material of the filiform apparatus is stained intensely by the PAS reaction (Fig. 6a). Plasmalemma and cytoplasm enters the cavities of the filiform apparatus (Fig. 6b). In the synergids, most of the cytoplasm (with dictyosomes, mitochondria, lipids bodies) and nuclei are shifted towards the micropyle, while the chalazal pole is occupied by large vacuole, while the cytoplasm forms only a thin layer between the tonoplast and plasma membrane. The cell wall of synergids is

Fig. 4 Ultrastructure of a *G. aurea* embryo sac. **a** Egg cell (*eg*) central cell with secondary nucleus (*N*), *bar*=1.8 μm . **b** The part of central cell cytoplasm with secondary nucleus, plastids (*P*), mitochondria (*m*), dictyosomes (*d*), *bar*=0.9 μm . **c** The part of the central cell cytoplasm with numerous cortical microtubules (*red arrows*), *bar*=0.2 μm

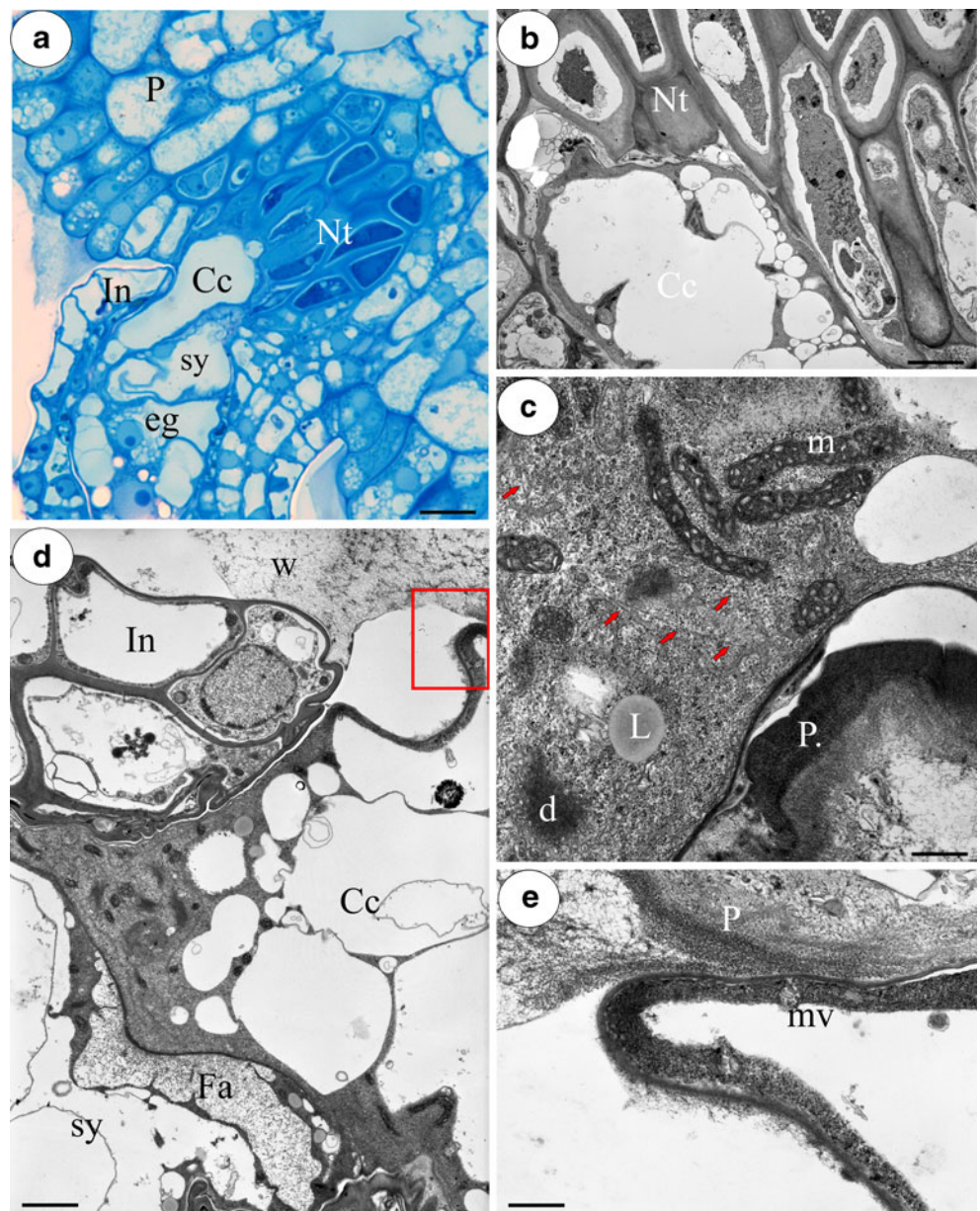


thickest in the micropylar region where it is contiguous with the filiform apparatus, and then this wall becomes thinner towards the chalaza. Between the central cell and the chalazal part of the synergid there is only a very narrow zone between the two plasma membranes of these cells (Fig. 6c) with microtubules occurring near the plasma membrane in the cytoplasm of the central cell.

The egg is vase-shaped and characteristically hooked (Fig. 6d). In the micropylar part of the egg cell, near the attachment of the egg to lateral megagametophyte wall, plasmodesmata between the egg and the central cell occur (Fig. 6e). Also the micropylar region of the egg cell adjacent

to the synergid has a well-developed cell wall with plasmodesmata (Fig. 6f). The egg nucleus and the majority of the egg cytoplasm (which is rich in ribosomes) are located in the chalazal region of the cell with the micropylar and the middle parts of the cell which are occupied mostly by vacuole (Fig. 6d). Mitochondria, large lipid bodies, convoluted plastids (proplastid type), dictyosomes (Fig. 7a) are situated in the perinuclear chalazal region of the egg cell. ER cisternae lie parallel to the plasma membrane of the egg cell. Microtubules also occur near the surface membrane of the egg cell (Fig. 7a, framed part). There is an electron-translucent space filled with membranous material (Fig. 7a)

Fig. 5 Anatomy of the ovule and embryo sac of *U. quelchii*. **a** Sections through placenta with ovule; *P* placenta, *Nt* nutritive tissue, *In* integument, *sy* synergid, *eg* egg cell, *Cc* central cell, *bar*=12 μ m. **b** Sections through thick-walled nutritive tissue (*Nt*) and the extra-ovular part of the central cell (*Cc*), *bar*=3 μ m. **c** The part of the extra-ovular central cell cytoplasm; *m* mitochondrion, *L* lipid body, *d* dictyosome, *P* placental cell, *red arrows* microtubules, *bar*=0.7 μ m. **d** The micropylar part of the embryo sac; *In* integument, *sy* synergid, *Cc* central cell, *Fa* filiform apparatus, *W* secretion, *framed part* shown in Fig. e, *bar*=2 μ m. **e** Extra-ovular “naked” part of the central cell; *mv* multivesicular body, *P* placenta, *bar*=0.6 μ m



between the plasma membranes of the egg cell and central cell, and in some places this space gives a PAS-positive reaction. This space has a different thickness, thus sometimes the plasma membranes are tightly appressed (Fig. 7a).

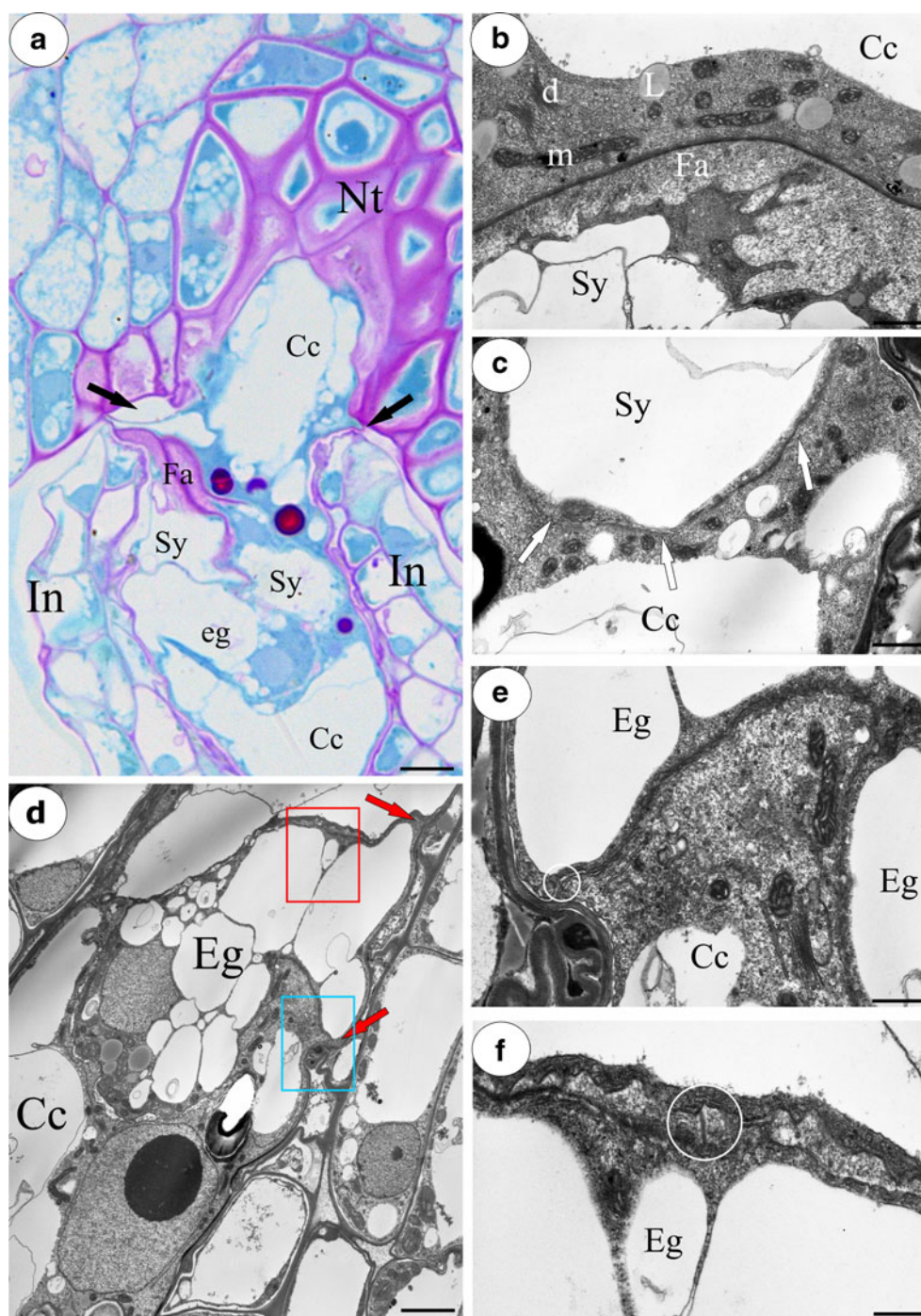
Near the vicinity of the egg cell, the first polar nuclei which after fusing (Fig. 7b) give a large secondary nucleus occur (Fig. 7c), and perinuclearly there are amyloplasts with starch, lipids bodies, mitochondria, and dictyosomes (Fig. 7c). The chalazal part of central cell is strongly vacuolated. The micropylar part of central cell which has contact with synergids deserves special attention; its cytoplasm is rich in ER, mitochondria, dictyosomes producing small-coated vesicles (Fig. 5d). An amyloplast with starch can also occur (Fig. 6a).

Discussion

The position of the egg apparatus and behavior of embryo sac

In most angiosperm plants, synergids occur in the extreme end of female gametophyte below micropylar canal and this position guarantees that one of the synergids will be the first female gametophyte cell that the contact with a pollen tube (Huang and Russell 1992). Of course, it seems logical that the terminal position of synergids is linked with the secretion of pollen tube attractants. The terminal position of synergids is also typical for Lamiales to which Lentibulariaceae belongs (Jobson and Albert 2002; Jobson et al. 2003;

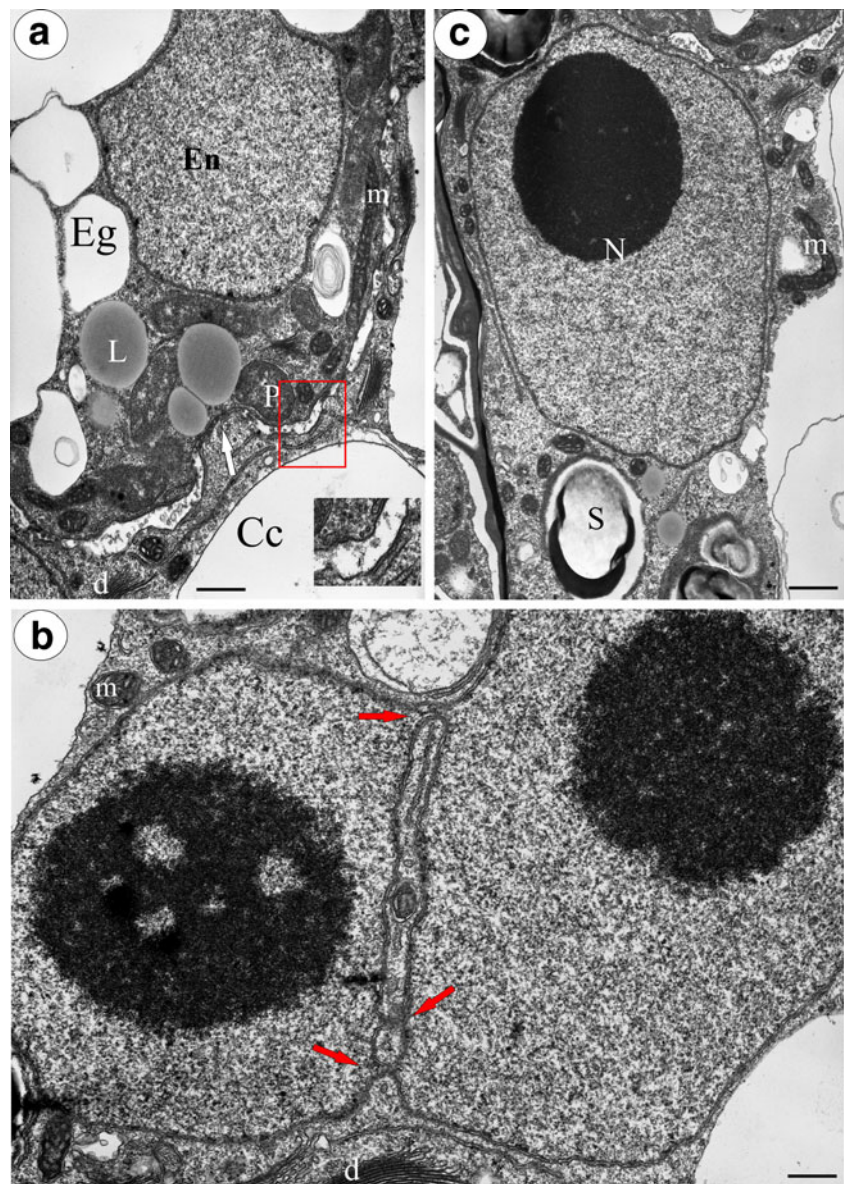
Fig. 6 Anatomy of the ovule and embryo sac of *U. quelchii*. **a** Section through the part of placenta with the ovule in the horizontal plane, *arrows* indicate parts of the central cell which grows into the ovary chamber; *Nt* nutritive tissue, *In* integument, *Sy* synergid, *Fa* filiform apparatus, *eg* egg cell, *Cc* central cell, *bar*=6 μ m. **b** The micropylar part of the synergid; *Sy* synergid, *Cc* central cell, *m* mitochondrion, *L* lipid body, *d* dictyosome, *Fa* filiform apparatus, *bar*=0.9 μ m. **c** The chalazal part of the synergid; *arrows* depict the narrow zone between the two plasma membranes of the synergid and the central cell, *Sy* synergid, *Cc* central cell, *bar*=0.7 μ m. **d** Ultrastructure of the egg cell and the central cell; *Eg* egg cell, *red arrows* attachment of the egg wall to the wall of the embryo sac; *Cc* central cell, *framed parts* shown in Fig. E (*blue*) and F (*red*) *bar*=2 μ m. **e** The cell wall between the egg cell and the central cell; *Eg* egg cell, *Cc* central cell, *circle* plasmodesmata, *bar*=0.9 μ m. **f** The cell wall between the egg cell and the central synergid; *Eg* egg cell, *circle* plasmodesmata, *bar*=0.35 μ m



Müller et al. 2000, 2004), e.g., see position of egg apparatus in *Proboscidea louisianica* (Mogensen 1978) and Orobanchaceae (Fig. 12 in Teryokhin and Nikiticheva 1981). Extreme examples in Lamiales are the genus *Torenia* and some species of *Lindernia* in which the egg apparatus is extra-ovular (Krishna Iyengar 1941; Higashiyama et al. 2006). If we look at the Lentibulariaceae family from a phylogenetic point of view, we find that in *Pinguicula*, which is a sister to *Genlisea-Utricularia* clade (Jobson and Albert 2002; Jobson et al. 2003; Müller et al. 2004; Müller

and Borsch 2005), a micropylar canal occurs and the egg apparatus has an apical position in the embryo sac (Merz 1897; Kopczyńska 1964). In *Genlisea*, the micropylar canal also occurs in the ovule (Merl 1915), and as it is shown, the micropylar part of synergids extends to micropylar canal. In the genus *Utricularia* the situation is more complicated (Table 1). Based on embryological characteristics, Farooq (1965) had already noted that among *Utricularia* he could distinguish two main groups, as well as *Utricularia coerulea*, which bears characteristics of both groups.

Fig. 7 Ultrastructure of a *U. quelchii* egg cell and central cell. **a** The micropylar part of the egg cell; *Eg* egg cell, *En* egg nucleus, *m* mitochondrion, *d* dictyosome, *P* plastid, *Cc* central cell, *framed part* shows microtubules near the egg plasma membrane, *bar*=0.6 μm . **b** Polar nuclei during fusion; *red arrows* nuclear bridges, *m* mitochondrion, *d* dictyosome, *bar*=0.4 μm . **c** The central cell with a secondary nucleus; *m* mitochondrion, *S* starch grain in an amyloplast, *N* secondary nucleus, *bar*=0.9 μm



However, he probably did not know the papers of Merz (1897) and Merl (1915). The first group consisted of *Utricularia uliginosa*, *Utricularia arcuata*, *Utricularia reticulata* (terrestrial species); the second *Utricularia inflata*, *Utricularia aurea*, *Utricularia macrorhiza* (aquatic species). Later, Siddiqui (1978b) proposed that *Utricularia dichotoma* has some embryological characteristics which resemble the aquatic species.

Considering what is currently known about the *Utricularia* phylogeny (Jobson and Albert 2002; Jobson et al. 2003; Müller and Borsch 2005) and embryo sac characteristics in this genus (Table 1), we can see that in all of the *Utricularia* species examined, irrespective of either section or subgenus, the ES surpasses the entire micropylar canal; however, the behavior of the ESs is different among species and this characteristic depends on the sectional position.

According to Jobson and Albert (2002), the clades that comprise section *Polypompholyx* and section *Pleiochasia* form a monophyletic lineage which is a sister to the clade that includes all of the other sections of *Utricularia*. Nevertheless, in this lineage the embryo sac extends beyond the limit of integument and the egg apparatus generally occurs in the lateral position on the funicular side (section *Pleiochasia*, see Siddiqui 1978a). Therefore, the position of the egg apparatus differs from what was reported in *Pinguicula* and *Genlisea*. The subgenus *Polypompholyx* is unique in Lentibulariaceae because of its massive funiculus with nutritive tissue (Siddiqui 1978a; Płachno and Świątek 2008). It seems that this subgenus is characterized by a mixture of primitive and specialized features. In the subgenus *Bivalvaria*, in some species from the section *Oligocista*, the embryo sac is confined within

Table 1 Behavior of *Utricularia* embryo sacs and position of egg apparatus, sections according to Taylor (1989), subgenera according to Müller and Borsch (2005)

Subgenus	Section	Behavior of embryo sac (ES) and position of egg apparatus	Author/s
<i>Utricularia</i>	<i>Vesiculina</i>	ES grows out beyond the limit of the integument and forms bulbous apex which grows in placenta nutritive tissue. Egg apparatus in the lateral position (<i>U. cucullata</i> , <i>Utricularia purpurea</i>).	Merl 1915, Merz, 1897
	<i>Utricularia</i>	ES grows out beyond the limit of the integument and forms bulbous apex which affected placental nutritive tissue—apex is buried in it. Egg apparatus in the lateral position (e.g., <i>U. stellaris</i> , <i>U. inflata</i> , <i>U. aurea</i> , <i>U. macrorrhiza</i>).	eg. Farooq and Siddiqui 1964, Khan 1954, Merz 1897, Wylie and Yocom 1923
	<i>Iperua</i>	ES grows out beyond the limit of the integument and forms bulbous apex which grows in placenta nutritive tissue (reniformis reniformis).	Merl 1915
	<i>Orchidioides</i>	ES grows out beyond the limit of the integument and forms bulbous apex which grows in placenta nutritive tissue (<i>U. quelchii</i>), or even grows on the surface of neighboring ovule (<i>Utricularia alpina</i>). Egg apparatus in the lateral position.	This paper, Płachno and Świątek 2008
	<i>Foliosa</i>	ES grows out beyond the limit of the integument and has a contact with placenta nutritive tissue (<i>Utricularia tricolor</i>).	Merl 1915
<i>Bivalvaria</i>	<i>Oligocista</i>	The ES confines within the ovule or slightly extends the micropyle, the tip of ES contains egg apparatus (<i>Utricularia scandens</i> , <i>U. uliginosa</i> , <i>U. smithiana</i> , <i>U. reticulata</i>). The tip of ES contains egg apparatus, however, protrudes out of micropyle and has contact with the nutritive tissue (<i>Utricularia graminifolia</i>). The micropylar apex of ES becoming extra-ovular, the egg apparatus is situated below it (<i>Utricularia polygaloides</i>).	Farooq 1965, Rajan and Kumar 1974, Shivaramiah 1967, Farooq and Bilquis 1966, Begum 1965, Kausik and Raju 1955
	<i>Calpidisca</i>	The micropylar end of ES slightly extends beyond the limit of integument and touches the funicular nutritive tissue. Synergids in apical part of the ES (<i>Utricularia livida</i> , <i>Utricularia sandersonii</i>).	Płachno 2002
	<i>Nigrescentes</i>	ES grows out beyond the limit of the integument and forms bulbous apex which grows in placenta nutritive tissue, the egg apparatus is beyond the micropyle—exposed (<i>U. coerulea</i>).	Kausik 1938
<i>Polypompholyx</i>	<i>Pleiochasia</i>	The micropylar end of ES slightly extends beyond the limit of integument and touches epidermis of the funicular nutritive tissue (<i>Utricularia menziesii</i> , <i>U. dichotoma</i>). Generally egg apparatus in the lateral position (<i>U. dichotoma</i>).	Merl 1915, Siddiqui 1978a, Płachno and Świątek 2008
	<i>Polypompholyx</i>	The micropylar end of the ES extends beyond the limit of integument and touches epidermis of the funicular nutritive tissue.	Lang 1901

the ovule or slightly extends the micropyle, and its tip contains the egg apparatus (Table 1), and in this characteristic is similar to *Pinguicula* and *Genlisea*. However, in the same subgenus in section *Nigrescentes*, *U. coerulea* has an extra-ovular egg apparatus (Kausik 1938). But, species from sections: *Oligocista*, *Stomoisia*, *Nigrescentes*, and *Calpidisca* are linked by the development of the endosperm—in all these sections the endosperm bends in the shape of a horseshoe (Farooq 1965; Siddiqui 1979; Płachno 2002).

The fact that the embryo sac in aquatic species (section *Utricularia*) is aggressive and invades the placental nutritive tissue at the 4-nucleate stage was well known (Khan 1954; Farooq and Siddiqui 1964; Farooq 1965). Here, it is clear that also in section *Orchidioides*, the embryo sac is aggressive in relation to the placental nutritive tissue. So, in the subgenus *Utricularia*, whichever ecological groups are investigated (aquatic, epiphytic, lithophytic), the egg apparatus has the lateral position (Table 1). However, there is still lack of information about

some other sections, e.g., it would be very interesting to know the behavior of the embryo sac in terrestrial species from section *Setiscapella* from the subgenus *Utricularia*.

Why and how did the extra-ovular embryo sacs evolve in *Utricularia*? It seems that this process occurred in a step-by-step process. In a common ancestor of the *Genlisea-Utricularia* clade, a micropylar canal existed and the embryo sac remained inside the ovule, while the egg apparatus had apical position. The first step, the embryo sac of the common ancestor of *Utricularia* filled the entire micropylar canal, but did not grow out beyond the limit of the integument. The egg apparatus had an apical position. This condition still occurs in some members of the subgenus *Bivalvaria* (Table 1). However, independently in different *Utricularia* lineages an embryo sac appeared which grows out beyond the limit of the integument and has contact with nutritive tissue. The advantage of this stage was the development of another pathway of metabolite transport directly from the placental (funicular in *Polypompholyx*) nutritive tissue to the embryo sac (Khan 1954, 1992; Płachno and Świątek 2008). As a consequence of the extra development of the micropylar part of the embryo sac (central cell), the egg apparatus changed position from apical to lateral.

In angiosperms, the haustoria of embryo sacs have been recorded in about 20 families; however, the most common are the chalazal, but the micropylar such as those in *Utricularia* are rare (Poddubnaya-Arnoldi 1976). It is believed that the enlargement of the central cell increases the abortive surface of the embryo sac (Masand and Kapil 1966). But Czapik

(1987) proposed that formation of the haustorium there is also a process leading to a need for an increase in the volume of the embryo sac before the development of the endosperm and embryo in the *Dryas*. It seems that this also occurs in *Utricularia* because species with a well-developed extra-ovular embryo sac later also have the most well-developed aggressive micropylar endospermal haustorium (and later syncytium; Khan 1954).

Ultrastructure of female germ unit

It should be highlighted that this paper is the first report about the ultrastructure of the egg apparatus in the Lentibulariaceae family. A comparison of the egg apparatus of *G. aurea* and *U. quelchii* is given in Table 2. Surprisingly, the egg apparatus in *G. aurea* is enclosed in the chalazal part by PAS-positive wall. This situation has been recorded in only a few species of angiosperms: *Capsella* (Schulz and Jensen 1968), *Papaver* (Olson and Cass 1981), *Epidendrum* (Cocucci and Jensen 1969), *Ornithogalum* (Tilton 1981) and *Scilla* (Bhandari and Sachdeva 1983). Typically, on the chalazal parts of the synergids and egg cell, only plasma membranes separate these cells from central cell (Huang and Russell 1992). Generally, the ultrastructure of the egg cells of both *Genlisea* and *Utricularia* is similar to other plants. However, the eggs cell of Lentibulariaceae contain only a low number of plastids and a small amount of mitochondria in comparison to *Impatiens* (Richter-Landmann 1959) and *Plumbago* (Russell 1987). But

Table 2 Comparison between egg apparatus of *G. aurea* and *U. quelchii*

	<i>G. aurea</i>	<i>U. quelchii</i>
Synergids		
Position	Micropylar part of synergid extends to micropylar canal.	Lack of micropylar canal, egg apparatus is occurs laterally on the raphe side of the embryo sac.
Filiform apparatus	Occurs	Occurs
Plasmodesmata	Between synergid and central cell	Between synergid and egg cell
Cell wall	In chalazal pole, there is an irregular layer of PAS-positive wall material.	In chalazal pole there is only very narrow zone between two plasma membranes.
Vacuole	Small vacuoles on the middle part, large vacuole in chalazal pole	Synergid strongly vacuolated—most of the cell is filled by vacuole.
Distribution of organelles	Perinuclear distribution and near filiform apparatus	Distribution near filiform apparatus
Egg cell		
Plasmodesmata	Between egg cell and central cell	Between egg cell and central cell Between egg cell and synergids
Cell wall	On micropylar pole typical cell wall is organized. In chalazal pole, there is an irregular layer of PAS positive wall material	On micropylar pole typical cell wall is organized. In chalazal pole, there is a space with some exocytic vesicles
Vacuole	Occurs in the micropylar part. Small vacuoles may occur also in perinuclear position at chalazal pole	Occurs in the micropylar and middle parts
Storage material	—	Large lipid bodies

a low number of plastids were also observed in *Oenothera* (Meyer and Stubbe 1974), and a small amount of mitochondria in *Petunia* and *Agave* (Tilton 1981).

It is worth mentioning that even in the material that was chemically fixed the microtubules are well preserved in female gametophytes, which is in contrast to the results of Thijssen et al. (1997) who observed microtubules only in high-pressure frozen embryo sacs.

It is presented that the filiform apparatus occurs in both genera *Genlisea* and *Utricularia*. So these results are in contrast to the observations of Khan (1954) and Shivaramiah (1967). In *Genlisea aurea*, the apical position of synergids and the filiform apparatus inside them is similar to other angiosperms (see Fig. 6 in Huang and Russell 1992). This might be connected with the synergid's role in the secretion of attractants for pollen tubes by the filiform apparatus to the micropyle, and later with the penetration of the synergid by the pollen tube (e.g., Diboll and Larson 1966; Went 1970; Higashiyama et al. 2001, 2003; Huang and Russell 1992). The results of this research are in agreement with Merl (1915), who observed porogamy in *Genlisea*. However, in *U. quelchii* and some other *Utricularia* species, (Table 1) the egg apparatus is not organized in an apical position and this has the following consequences. Firstly, if synergids produce attractants for pollen tubes, these attractants should be transported via the central cell or placenta cells. Secondly, the central cell, not the synergid, is the first female gametophyte cell which has contact with pollen tube. In relation to the first, Merz (1897) described contact of pollen tubes with extra-ovular embryo sacs in *Utricularia* (*U. inflata*, *Utricularia purpurea*, *Utricularia stellaris*). According to him, a pollen tube enters the central cell and grows inside up to the egg apparatus which is organized on the funicular side of the ovule. In contrast, Khan (1954), in *U. aurea*, did not observe that a pollen tube enters the central cell directly and grows inside this cell. According to him and Farooq (1964), a *U. stellaris* pollen tube has its first contact with the embryo sac and later grows between the wall of the embryo sac and the placenta surface, and finally reaches the funicular side, enters the embryo sac, and penetrates the synergid. Khan (1954) wondered why in *U. aurea*, if synergids emit chemo-attractants, pollen tubes do not grow directly via the central cell using the shortest route to the synergids.

However, it seems that the synergids in *U. quelchii* have a simpler structure when their ultrastructure is compared with *Genlisea* (this paper) and other angiosperms (Huang and Russell 1992). In this case, the micropylar part of the central cell which has contact with the synergids of *U. quelchii* deserves special attention. It is very active and I propose that it may be responsible for liaising between a female gametophyte and a pollen tube. It seems that it could take over the functions of the synergid in this aspect. Still,

synergids with a filiform apparatus are needed for the pollen tube to properly enter the embryo sac. However, this should be proven by using other techniques. Recently, Marton et al. (2005) showed that the central cell also plays a role in attracting pollen tubes. Another question is why the extra-ovular part of the *Utricularia* embryo sac is strongly vacuolated. This vacuolization is probably needed to generate the turgor which helps to crush the placental nutritive cells and later absorbs nutrients.

Conclusions

Synergids in both *Genlisea* and *Utricularia* possess a filiform apparatus; however, it seems that the *U. quelchii* synergids have a simpler structure compared to *G. aurea* and other typical angiosperms.

The terminal position of synergids occurs in the embryo sacs of *Pinguicula*, *Genlisea* and probably occurred in a common *Utricularia* ancestor. This ancestral characteristic still occurs in some species from the *Bivalvaria* subgenus. Independently, in different *Utricularia* lineages, an embryo sac which grows out beyond the limit of the integument and has contact with nutritive tissue has appeared and as a consequence of this, the egg apparatus changes position from apical to lateral.

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Conflict of interest The author declares that he has no conflict of interest.

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