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COMPARATIVE ANATOMY OF OVULES IN *GALINSOGA*, *SOLIDAGO* AND *RATIBIDA* (ASTERACEAE)

JOLANTA KOLCZYK[1], PIOTR STOLARCZYK[2], AND BARTOSZ J. PŁACHNO[1*]

[1]*Department of Plant Cytology and Embryology, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland*
[2]*Unit of Botany and Plant Physiology, Institute of Plant Biology and Biotechnology, University of Agriculture in Cracow, Al. 29 Listopada 54, 31-425 Cracow, Poland*

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Many Asteraceae species have been introduced into horticulture as ornamental or interesting exotic plants. Some of them, including *Solidago* and *Galinsoga*, are now aggressive weeds; others such as *Ratibida* are not. Special modifications of the ovule tissue and the occurrence of nutritive tissue have been described in several Asteraceae species, including invasive *Taraxacum* species. This study examined whether such modifications might also occur in other genera. We found that the three genera examined – *Galinsoga* (*G. quadriradiata*), *Solidago* (*S. canadensis*, *S. rigida*, *S. gigantea*) and *Ratibida* (*R. pinnata*) – differed in their nutritive tissue structure. According to changes in the integument, we identified three types of ovules in Asteraceae: "Taraxacum" type (recorded in *Taraxacum*, *Bellis*, *Solidago*, *Chondrilla*), with well-developed nutritive tissue having very swollen cell walls of spongy structure; "Galinsoga" type (in *Galinsoga*), in which the nutritive tissue cells have more cytoplasm and thicker cell walls than the other integument parenchyma cells, and in which the most prominent character of the nutritive tissue cells is well-developed rough ER; and "Ratibida" type (in *Ratibida*), in which the nutritive tissue is only slightly developed and consists of large highly vacuolated cells. Our study and future investigations of ovule structure may be useful in phylogenetic analyses.

Key words: Alien plant, Asteraceae, goldenrod, integument, invasive kenophyte, ovule, *Taraxacum*, ultrastructure, weed species.

INTRODUCTION

Galinsoga quadriradiata Ruiz & Pav. (shaggy soldier) grows naturally in Central and South America (from Mexico to Chile) and has been cultivated in Europe since 1849. Now it is a common weed in North America, Europe, Africa and some parts of Asia (Kabuce and Priede, 2010a). The success of *Galinsoga* is most probably associated with its extremely efficient reproduction; even 8 to 9 week-old plant can produce 3,000 flower heads and a huge number of seeds, up to over 7,000 (Kagima, 2000). *Galinsoga* is also a very flexible weed because it produces heteromorphic achenes in a capitulum-type inflorescence, which probably supports survival under variable environmental conditions (Kucewicz et al., 2010). *Galinsoga* species occupy fields, gardens, railways and ruderal sites and may also invade seminatural habitats such as forest paths, clearings and margins in woodlands (Tokarska-Guzik, 2003, 2005; Chmura, 2004; Kabuce and Priede, 2010a; Trzcińska-Tacik et al., 2010). *Galinsoga* species pose a threat to crop production by competing with cultivated plants and also by acting as alternate hosts for many insects, viruses and nematodes that affect crop species (Warwick and Sweet, 1983). Because it is an aggressive weed, *Galinsoga* has attracted the interest of several researchers, including embryologists. *Galinsoga* species most often produce seeds sexually (Dahlgren, 1920; Popham, 1938; Pullaiah, 1977, 1981; Pietrusiewicz et al., 2005; Kang, 2010), and only rarely have other modes of reproduction been recorded, such as the formation of diplosporic embryo sacs (Pietrusiewicz et al., 2005).

Solidago canadensis L. (Canadian goldenrod) is native to North America and occurs across almost all of the USA and Canada (Kabuce and Priede, 2010b). It was introduced to Europe as an easy-to-cultivate ornamental plant as early as the 17th cen-

*e-mail: bartosz.plachno@uj.edu.pl

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tury (Kowarik, 2003). Today, *Solidago canadensis* is present over most of Europe and has also become naturalized in Australia, New Zealand and some parts of Asia. Canadian goldenrod is an aggressive weed that outcompetes native plants (e.g., Guzikowa and Maycock, 1986; Weber, 2000; Kabuce and Priede, 2010b). Only 8 of the ~130 *Solidago* species have been studied embryologically (e.g., Palm 1914; Harling, 1951; Beaudry, 1958; Smith and Johnson, 1980; Małecka, 1989, 1991; Musiał, 1994), including *Solidago canadensis* (Palm, 1914; Carano, 1918; Pullaiah, 1978; Smith and Johnson, 1980; Musiał, 1989). There is a lack of information about the detailed structure of the ovule in this genus.

Members of the coneflower *Ratibida* genus occur on the prairies of North America and Mexico. Two species, *Ratibida columnifera* (Nutt.) Woot. & Standl. and *Ratibida pinnata* (Vent.) Barnhart, are used as ornamental plants in gardens.

Special modifications of the ovule tissue (e.g., the occurrence of nutritive tissue) have been recorded in several Asteraceae genera: *Helianthus* (Newcomb, 1973a), *Bellis* (Engell and Petersen, 1977), *Hieracium* (Koltunow et al., 1998), *Cynara* (Figueiredo et al., 2006), *Taraxacum* (Cooper and Brink, 1949; Musiał et al., 2013a; Płachno et al., 2014), *Chondrilla* (Kościńska-Pająk, 2006; Musiał et al., 2013a). It has even been suggested that in *Hieracium* (Koltunow et al., 1998), *Taraxacum* (van Baarlen et al., 1999; Musiał et al., 2013a) and *Chondrilla* (Musiał et al., 2013a; Musiał and Kościńska-Pająk, 2013) modifications of the ovule tissue may have facilitated the evolution of apomixis in these genera. No such modifications have been recorded in *Rudbeckia* (Musiał, unpublished data, in Musiał et al., 2012). *Ratibida* is closely related to *Rudbeckia* (Urbatsch et al., 2000), raising the question of whether *Ratibida* species indeed lack a special modification of ovule structure.

In this study we examined whether integument modifications also occur in other genera and compared their ovule structure with other Asteraceae species.

MATERIALS AND METHODS

ORIGIN OF PLANT MATERIAL

Galinsoga quadriradiata Ruiz & Pav. [*Galinsoga ciliata* (Raf.) S.F. Blake] – roadsides in Kraków-Podgórze, Poland;

Solidago canadensis L. – Kraków-Podgórze near Vistula River, Katowice on Bankowa Street near the Rawa River, Poland; *S. rigida* L. – Prague Botanical Garden, Czech Republic; *S. gigantea* Aiton – Kraków-Podgórze near the Vistula River, Poland;

Ratibida pinnata (Vent.) Barnhart – Prague Botanical Garden, Czech Republic.

We analyzed 20–30 flowers of each species.

METHODS

LIGHT AND ELECTRON MICROSCOPY

Samples for TEM were prepared as described earlier (Płachno and Świątek, 2009, 2010). Briefly, for electron microscopy the ovaries were fixed with 2.5% formaldehyde and 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.0) or 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4). The material was postfixed in 1% OsO_4 in cacodylate buffer for 2 h at room temperature, rinsed in the same buffer, dehydrated with acetone and embedded with an Epoxy Embedding Medium Kit (Fluka). Semithin sections were stained with methylene blue (Humphrey and Pittman, 1974) and examined with an Olympus BX60 microscope. Ultrathin sections were cut on a Leica ultracut UCT ultramicrotome. After contrasting with uranyl acetate and lead citrate, the sections were examined with a Hitachi H500 electron microscope at 75 kV in the Department of Animal Histology and Embryology, University of Silesia.

Additionally, material embedded in Technovit 7100 (Kulzer, Germany) was also observed. The material was fixed in 2.5% buffered (0.1 M phosphate buffer, pH 7.4) glutaraldehyde, washed four times in the same buffer and dehydrated in a graded ethanol series for 15 min at each concentration and kept overnight in absolute ethanol. Later the samples were infiltrated for 1 h each in 3:1, 1:1 and 1:3 (v/v) mixtures of absolute ethanol and Technovit and stored for 12 h in pure Technovit. The resin was polymerized with the addition of hardener. The material was sectioned 7 μm thick with a rotary microtome (Microm, Adamas Instrumenten), stained with 0.1% toluidine blue O (TBO) and mounted in Entellan synthetic resin (Merck).

RESULTS

GALINSOGA

The flower of *Galinsoga quadriradiata* possesses an inferior and unilocular ovary with a single ovule on the basal placenta (Fig. 1a). The mature ovule is anatropous, unitegmic and tenuinucellate; however, some remnants of nucellus cells persist between the antipodes and integument cells. The ovule is ~507 μm long. The ovule integument shows zonal differentiation (Fig. 1b, c). There are ~5 layers of elongated parenchyma cells subepidermally. These cells have

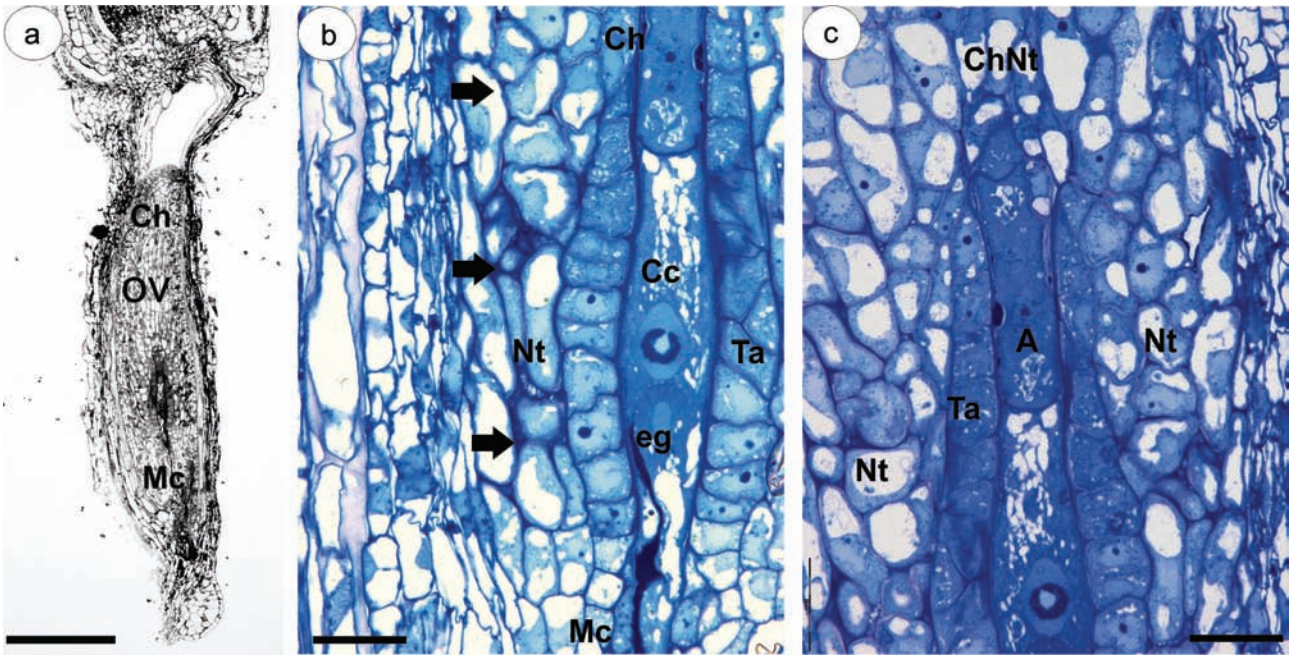

Fig. 1. Ovary and ovule structure of *Galinsoga quadriradiata*. (**a**) Longitudinal section of unilocular ovary with anatropous unitegmic ovule. Mc – micropyle; Ch – chalaza; Ov – ovule. Bar = 200 μm, (**b, c**) Longitudinal section of ovule showing heterogeneous integument structure and embryo sac; arrows indicate zone of the nutritive tissue (Nt). eg – egg cell; Cc – central cell; A – antipodes; ChNt – chalazal nutritive tissue; Ta – integumental tapetum; Mc – micropyle; Ch – chalaza. Bar = 20 μm.

a thin layer of cytoplasm covering the cell wall and nucleus. There are plastids with small starch grains on these cells (Fig. 2a). In addition, the chalazal part of the ovule consists of highly vacuolated, elongated cells (Fig. 2b). The innermost layer of the integument forms the integumental tapetum (endothelium) around the central part of the embryo sac (Fig. 1b, c). The integumental tapetum cells are slightly elongated anticlinally.

The integument parenchyma cells adjacent to the tapetum cells and to the chalazal part of the embryo sac have a unique structure that forms a special tissue (three layers of cells near the central cell and four layers of cells near the antipodes) (Fig. 1b, c). These cells have denser cytoplasm and thicker cell walls than the other integument parenchyma cells (Fig. 2c). The most prominent feature of these cells is their well-developed rough ER. The rough ER cisternae are distended and contain electron-dense material (Fig. 2c). The intercellular spaces contain an accumulation of heterogeneous electron-dense material with rounded or irregular profiles, which seems to be cell debris or secretions (Figs. 2c, 3a). The cell walls between the integumental parenchyma cells have an open, spongy structure. The dictyosomes are well developed and rounded (Fig. 3a). The nucleus is also irregularly shaped. Small oval mitochondria are abundant and have short well-developed cristae. The plastids are inconspicuous and oval, and have electron-dense stroma (Fig. 3a). The differentiation of thick-walled tissue is connected with the ovule and female gametophyte development: at the megaspore tetrad stage, this tissue is still not differentiated (Fig. 3b, c).

SOLIDAGO

The flower of *Solidago canadensis* possesses an inferior and unilocular ovary with a single, anatropous, strongly elongated ovule ~545 μm long. At the mature female gametophyte stage the ovule has a multilayer integument of heterogeneous structure (Fig. 4a). There are 3–4 layers of elongated parenchyma cells subepidermally. These cells have a thin layer of cytoplasm covering the cell wall and nucleus. The cell walls of these cells are thin. The embryo sac is surrounded by a layer of endothelium which differentiates from the inner epidermal cells of the integument (Fig. 4a). There are 3–4 layers of cells with extremely thick cell walls (nutritive tissue) between the external integumentary layers and the endothelium (Fig. 4a). This unique tissue reaches deeply into the chalaza (Fig. 4b) and does not occur near the apical part of the central cell and synergids at the micropylar pole of the ovule (Fig. 4a). The cells of this specialized tissue have a reduced cell lumen and thick swollen cell walls with a unique

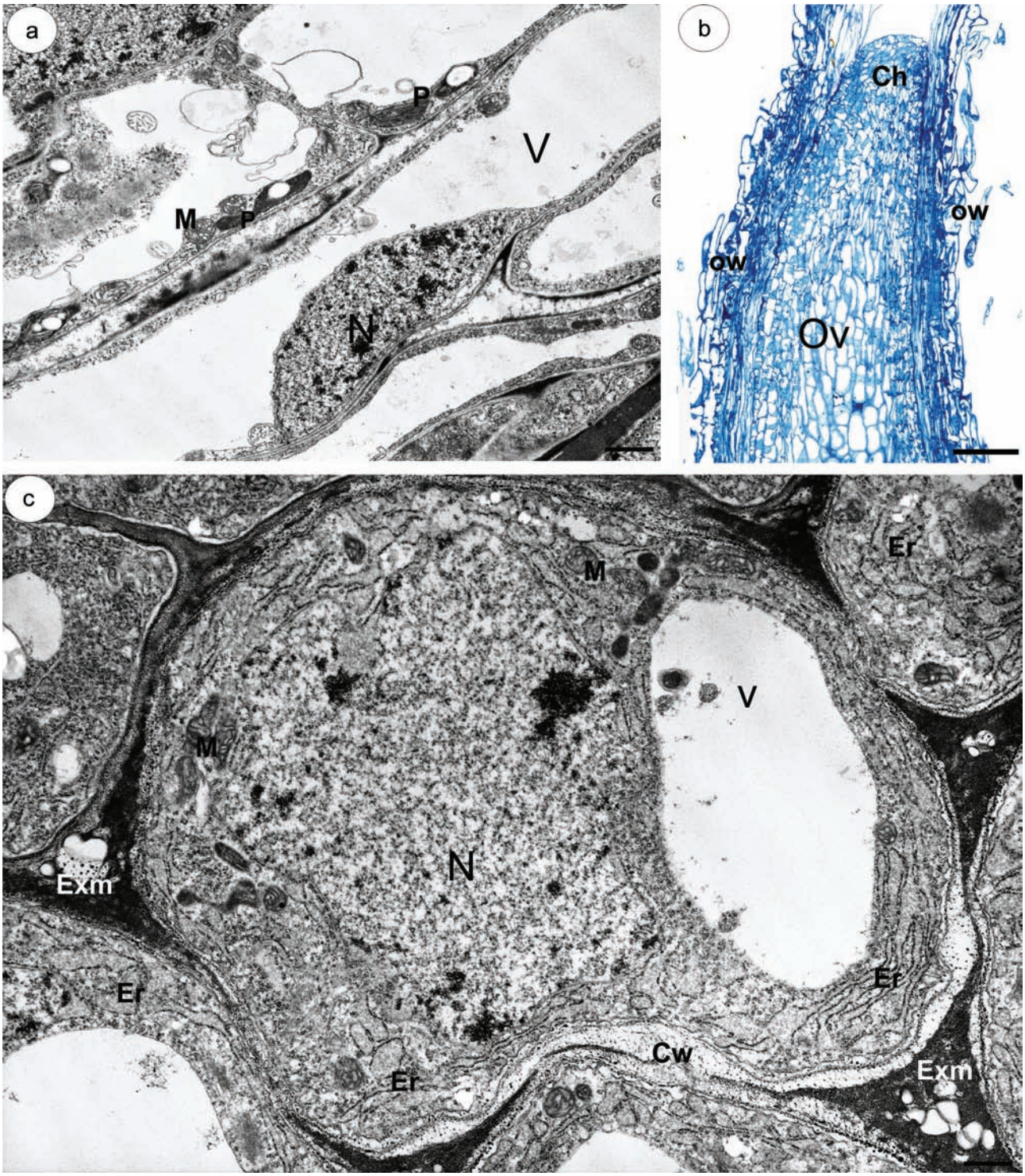

Fig. 2. Ovule structure of *Galinsoga quadriradiata*. (**a**) Ultrastructure of integument parenchyma. P – plastid; M – mitochondrion; N – nucleus; V – vacuole. Bar = 0.8 μm, (**b**) Anatomy of the chalazal part of the ovule. Ov – ovule; Ch – chalaza; ow – ovary wall; Bar = 50 μm, (**c**) Ultrastructure of nutritive tissue. M – mitochondrion; N – nucleus; V – vacuole; Er – endoplasmic reticulum; Exm – extracellular matrix; Cw – cell wall. Bar = 0.6 μm.

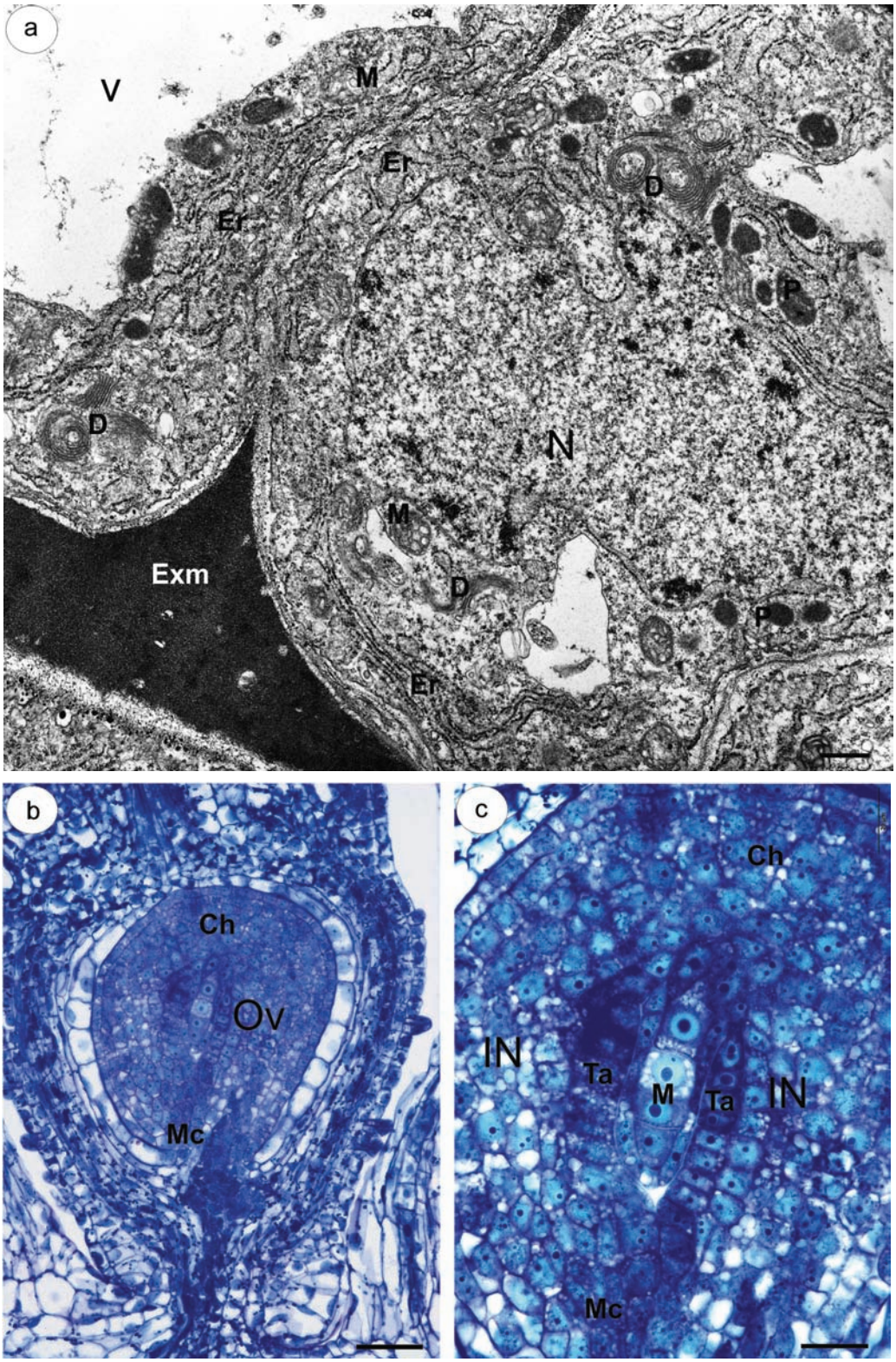

Fig. 3. Ovule structure of *Galinsoga quadriradiata*. (**a**) Ultrastructure of nutritive tissue. M – mitochondrion; N – nucleus; V – vacuole; Er – endoplasmic reticulum; Exm – extra cellular matrix; D – dictyosome; P – plastid. Bar = 2.3 μm, (**b, c**). Section a young ovule showing that the nutritive tissue has not yet differentiated. Ov – ovule; Mc – micropyle; Ch – chalaza; IN – integument; Ta – integumental tapetum; M – tetrad of megaspores. Bars = 50 μm for (b), and Bar = 20 μm for (c).

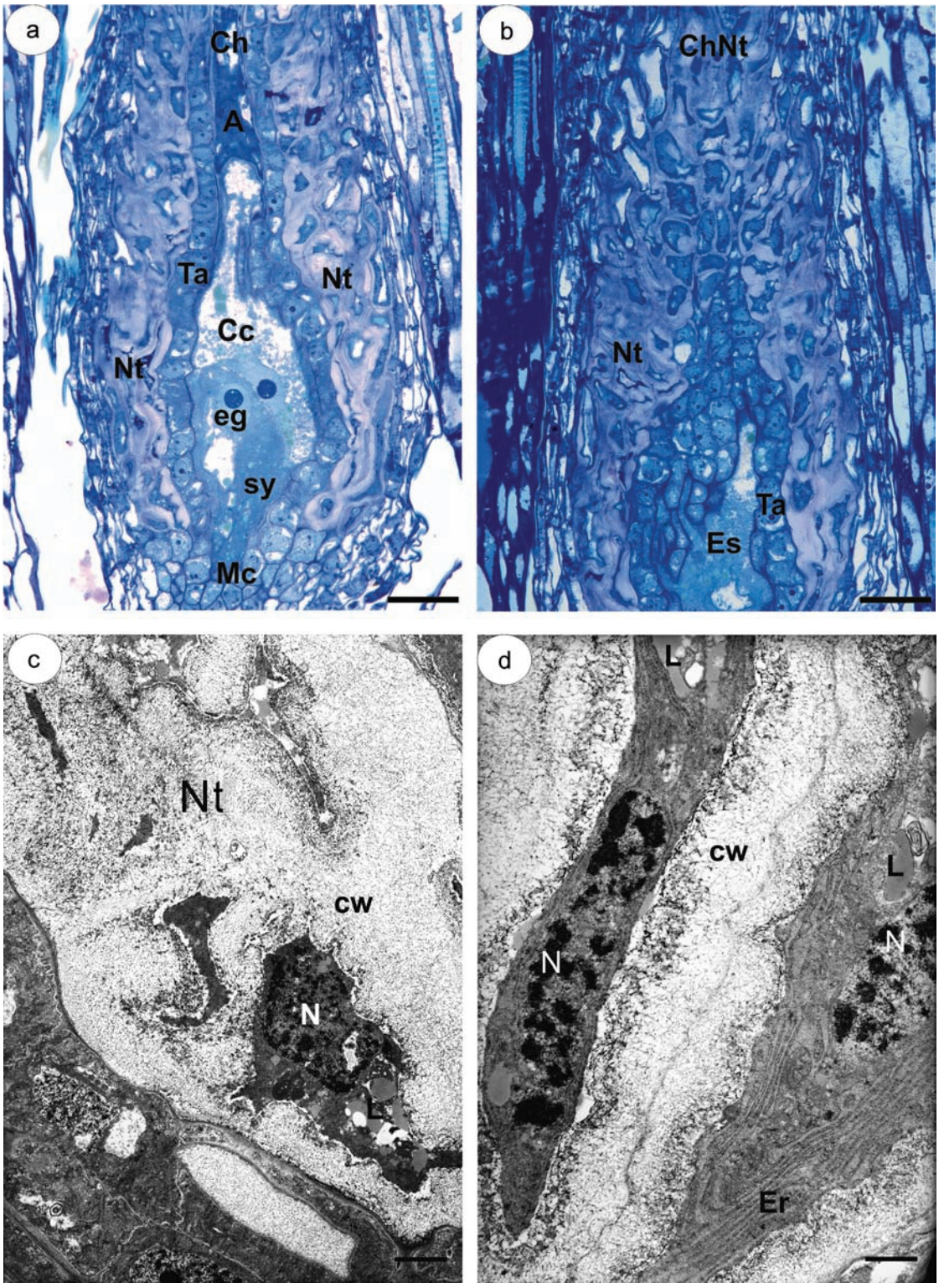

Fig. 4. Ovule structure of *Solidago canadensis*. (**a, b**) Longitudinal sections of anatropous unitegmic ovule showing the heterogeneous integument structure and embryo sac. Nt – nutritive tissue; eg – egg cell; Cc – central cell; A – antipodes; sy – synergids; Ta – integumental tapetum; Es – embryo sac; ChNt – chalazal nutritive tissue; Mc – micropyle; Ch – chalaza. Bar = 20 μm, (**c, d**) Ultrastructure of nutritive tissue. N – nucleus; Er – endoplasmic reticulum; L – lipid droplets; cw – cell wall. Bars = 2 μm for (c) and Bar = 0.9 μm for (d).

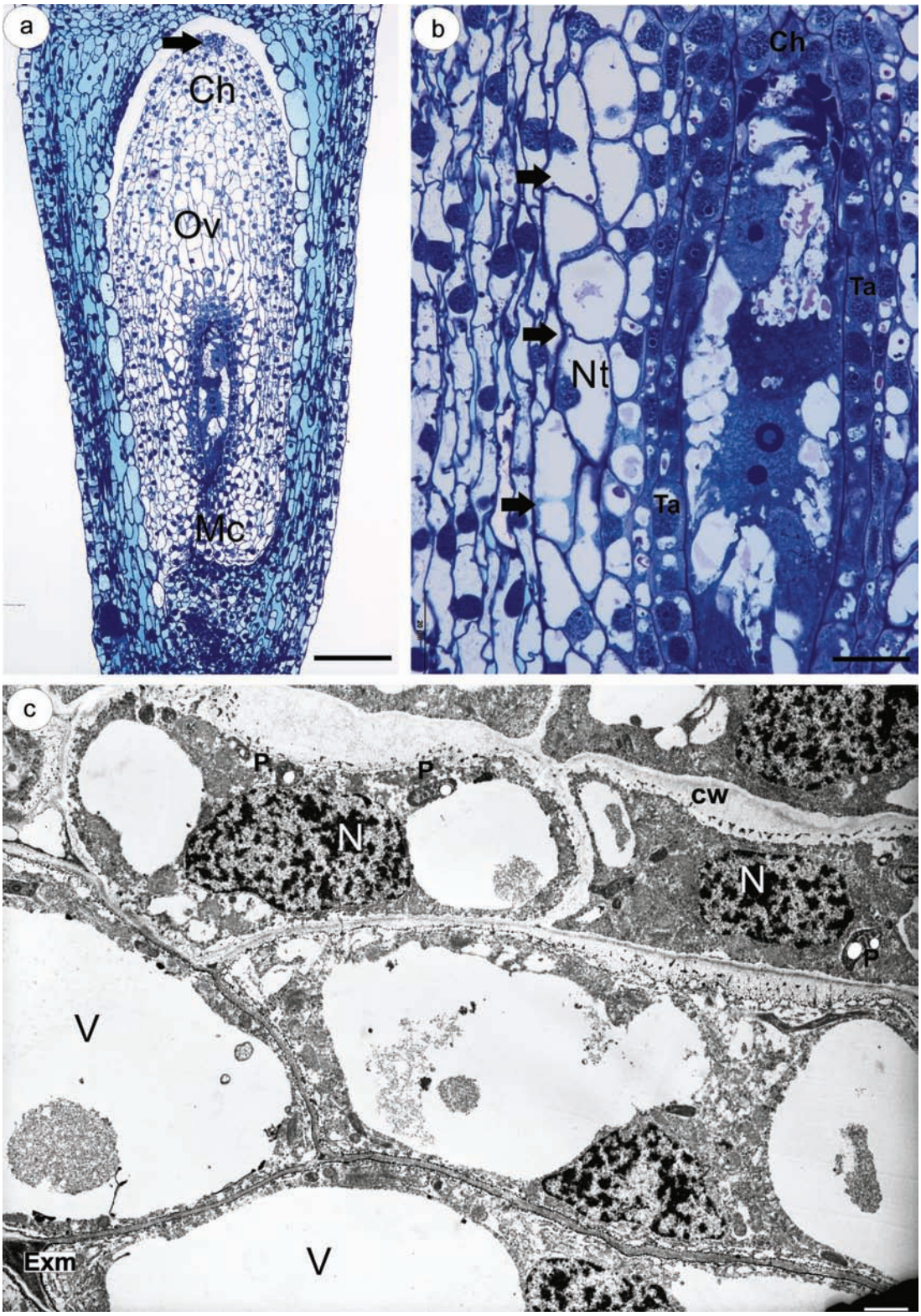

Fig. 5. Ovary and ovule structure of *Ratibida pinnata*. (**a**) Longitudinal section of unilocular ovary with anatropous unitegmic ovule. Mc – micropyle; Ch – chalaza; Ov – ovule; arrow – procambial strand. Bar = 100 μm, (**b**) Part of longitudinal section of ovule, arrows indicate nutritive tissue (Nt); Ta – integumental tapetum; Ch – chalaza. Bar = 20 μm, (**c**) Ultrastructure of nutritive tissue. N – nucleus; V – vacuole; P – plastid; Exm – extra cellular matrix; cw – cell wall. Bar = 1 μm.

ultrastructure (Fig. 4c, d). These walls have an open spongy structure. There are many endoplasmic reticulum cisternae and also accumulations of lipid droplets in the cytoplasm (Fig. 4c, d). *Solidago rigida* and *S. gigantea* have nutritive tissue similar to *S. canadensis* (data not shown).

RATIBIDA

Like the other species studied, the flower of *Ratibida pinnata* possesses an inferior and unilocular ovary with a single, anatropous, unitegmic and tenuinucellate ovule, which is ~690 μm long (Fig. 5a). There is a group of compactly arranged and distinctly smaller cells at the chalazal pole of the ovule (procambial strand), which stands out just below the epidermis (Fig. 5a). The integument shows zonal differentiation: an external epidermis, six layers of elongated parenchyma cells, two layers of large highly vacuolated cells, one layer of elongated cells and inner epidermal cells that forms the endothelium (Fig. 5b). The integument parenchyma cells adjacent to the endothelium have numerous dictyosomes, plastids with small starch grains and thicker cell walls than the other parenchyma cells (Fig. 5c).

DISCUSSION

Embryological characters are useful and important in taxonomical and evolutionary analyses (e.g., Herr, 1984; Prakash, 1987; Tobe, 1989; Igersheim and Endress, 1998; Endress and Igersheim, 2000; Igersheim et al., 2001; Endress 2005; Siuta et al., 2005; Płachno and Świątek, 2010; Płachno, 2011; Kuta et al., 2012). Studies on ovule morphology and histology can also help in understanding evolutionary changes (Soverna et al., 2003; Endress, 2005, 2011; Wang and Ren, 2007; de Toni and Mariath, 2008, 2010; Płachno and Świątek, 2009; Fagundes and Mariath, 2014). According to Anderberg et al. (2007), *Taraxacum* and *Chondrilla* are classified within subfamily Cichorioideae. The genera *Helianthus*, *Galinsoga*, *Solidago*, *Bellis*, *Rudbeckia* and *Ratibida* represent the subfamily Asteroideae. We observed a similar structure of the integument nutritive tissue in *Solidago*, as earlier observed in species of the genera *Helianthus* (Newcomb, 1973a), *Bellis* (Engell and Petersen, 1977), *Taraxacum* (Musiał et al., 2013) and *Chondrilla* (Kościńska-Pająk, 2006; Musiał et al., 2013). Species from these genera have nutritive tissue that consists of extremely thick-walled cells rich in protein (Cooper and Brink, 1949) and carbohydrate (Engell and Petersen, 1977; Musiał et al., 2013a). Thus, some genera from different subfamilies have similar changes in the integument. However, *Galinsoga* has a nutritive tissue structure differing from that in other genera of the same subfamily (Asteroideae) that have been studied. As mentioned earlier, Musiał et al. (2012) did not record any nutritive tissue in *Rudbeckia* (however, no documentation from TEM or resin sections was shown), which is allied to *Ratibida*. We found that the nutritive tissue is only slightly developed in *Ratibida* as compared to other Asteraceae species that have been studied.

Figueiredo et al. (2006) described special ovule tissues in *Cynara cardunculus* (subfamily Carduoideae) but they classified them as a podium and a hypostase, both of nucellar origin. However, the tissue that these authors described as a hypostase is very similar to the nutritive tissue of integument origin that has been described in other Asteraceae such as *Helianthus* (Newcomb, 1973a), *Bellis* (Engell and Petersen, 1977), *Taraxacum* (Musiał et al., 2013a) and *Solidago* (our results). Future studies of ovule development in *Cynara* should help clarify the origin of this tissue, a step needed especially since Goldflus (1899) called the modified integumentary tissue near the antipodes in Asteraceae ovules a "pseudochalaza".

The differentiation of the integumentary nutritive tissue in Asteraceae ovules is related to ovule maturation, as was shown in *Taraxacum* (Cooper and Brink, 1949; Musiał et al., 2013b), *Bellis* (Engell and Petersen, 1977) and *Hieracium* (Koltunow et al., 1998). Our observations in *Galinsoga* agree with this. According to Koltunow et al. (1998), this tissue was utilized during embryo growth and development; it dissipates (undergoes liquefaction) during seed development in *Hieracium*. Degradation of this tissue during embryogenesis has been recorded in *Taraxacum* (Cooper and Brink, 1949), *Bellis* (Engell and Petersen, 1977) and *Helianthus* (Newcomb, 1973a, b). Moreover, Pullaiah (1981) observed that after fertilization some layers of integument cells next to the endothelium disappeared in *Galinsoga parviflora*. Degradation of the integument parenchyma during seed development has been observed in many plants and it is believed that this process is connected with the movement of nutrient resources to the developing embryo (Kapil and Tiwari, 1978).

According to the changes in integument tissue, we propose three types of ovule in Asteraceae (Tab. 1).

In the "Taraxacum" type (recorded in *Taraxacum*, *Bellis*, *Solidago*, *Chondrilla*) the nutritive tissue is well developed and its cells have strongly swollen cell walls with a spongy structure. Koltunow et al. (1998) also observed wall changes in the integument cells near the endothelium in *Hieracium* (subfamily Cichorioideae), and the *Hieracium* ovule probably should also be referred to the Taraxacum type, though more ultrastructural analyses are needed for this.

TABLE 1. Ovule types in Asteraceae family

Ovule type	Type description	Genera and species
"Taraxacum"	The nutritive tissue is well developed and its cells have strongly swollen cell walls with spongy structure	subfamily Cichorioideae genus *Taraxacum* *Taraxacum kok-saghyz* (Cooper and Brink, 1949) *Taraxacum officinale* (Cooper and Brink, 1949) *Taraxacum linearisquameum* (Musiał et al., 2013a) *Taraxacum gentile* (Musiał et al., 2013a) *Taraxacum brandenburgicum* (Płachno et al., 2014) *Taraxacum tenuifolium* (Płachno et al., 2014) *Taraxacum udum* (Musiał et al., 2013b) *Taraxacum alatum* (Musiał and Pająk, 2013) genus *Chondrilla* *Chondrilla juncea* (Kościńska-Pająk, 2006; Musiał et al., 2013a) *Chondrilla brevirostris* (Musiał and Pająk, 2013) genus *Hieracium* ?(Koltunow et al., 1998) - future ultrastructural analyzes are needed subfamily Asteroideae genus *Helianthus* *Helianthus annuus* (Newcomb, 1973a,b) genus *Bellis* *Bellis perennis* (Engell and Petersen, 1977) genus *Solidago* *Solidago canadensis* (our results) *Solidago rigida* (our results) *Solidago gigantea* (our results)
"Galinsoga"	The nutritive tissue cells has more dense cytoplasm and thicker cell walls than other integument parenchyma cells. The most prominent character of the nutritive tissue cells is the well-developed rough ER	subfamily Asteroideae genus *Galinsoga* *Galinsoga quadriradiata* (our results)
"Ratibida"	The nutritive tissue is only slightly developed and consists of large highly vacuolated cells	subfamily Asteroideae genus *Ratibida* *Ratibida pinnata* (Vent.) Barnhart (our results)

In the "Galinsoga" type (in *Galinsoga*) the nutritive tissue cells have more cytoplasm and thicker cell walls than the other integument parenchyma cells. The most prominent character of the nutritive tissue cells was the well-developed rough ER.

In the "Ratibida" type (in *Ratibida*) the nutritive tissue is only slightly developed and consists of large, highly vacuolated cells.

CONCLUSIONS

1) We found that the three studied genera that were examined – *Galinsoga*, *Solidago* and *Ratibida* – differed in their nutritive tissue structure.
2) According to the changes in integument tissue we identified three types of ovules in Asteraceae: "Taraxacum" type, "Galinsoga" type and "Ratibida" type.
3) Some genera from different subfamilies had similar changes in the integument.
4) Our studies and future investigations of ovule structure should be of interest in evolutionary analyses.

AUTHORS' CONTRIBUTION

All authors contributed to the conception and design, acquisition of data, analysis and interpretation of data, and drafting or critical revision of the paper.

The authors declare that they have no conflicts of interest.

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