On the embryology of *Leontodon autumnalis* (*Asteraceae*)

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Abstract. A study of the male and female generative spheres of *Leontodon autumnalis* has been carried out. The anther wall is four-layered and its development follows the Dicotyledonous-type. After simultaneous microsporogenesis, predominantly tetrahedral tetrads are formed in the anthers. Mature pollen is three-celled at the time of shedding. The embryo sac development runs after the *Polygonum* (monosporic)-type. It has been observed that the antipodal complex is the most polymorphous structure in the mature ES. Embryo develops after the Asterad-type. Initially nuclear endosperm transforms during endospermogenesis into cellular one. It has been established that *L. autumnalis* is a sexually reproducing species. Embryological data suggest a more clear affinity of *L. autumnalis* to *L. hispidus* as well as to other earlier studied *Hypochoeridinae* taxa.

Key words: *Asteraceae*, embryology, embryogenesis, endospermogenesis, *Leontodon autumnalis*, male and female gametophyte

Introduction

Genus *Leontodon* L. belongs to the tribe *Lactuceae* Cass., subtribe *Hypochoeridinae* Less. (syn. *Leontodontinae* O. Hoffm.) of *Asteraceae* Dumort. (Tomb 1977; Bremer 1994) and includes about 50 species spread "throughout Europe, North Africa and Southwest Asia to Iran, mainly in the Mediterranean region, one widely distributed and introduced species (*L. autumnalis* L.)" (Bremer 1994:169).

Five species of this genus are reported in the Bulgaria flora (Finch & Sell 1976; Kuzmanov 1985). According to Kuzmanov (1985), three species – *L. hispidus* L., *L. crispus* Vill. and *L. autumnalis* – are widely distributed, whereas *L. fasciculatus* (Biv.) Nyman and *L. riloensis* Hayek have a more district occurrence in our country.

Despite the significant phylogenetic position of the genus *Leontodon* within the limits of the subtribe *Hypochoeridinae* (Stebbins & al. 1953), it has been very poorly understood especially in an embryological aspect. According to Solntseva (1987), out of all species of this genus distributed all over the world only three – *L. crispus* , L. *hispidus* and *L. autumnalis* – are studied embryologically, but the data are still insufficient for their detailed embryological characteristic. On the contrary, genus *Leontodon* is profoundly studied morphologically, cytotaxonomically and phytochemically (Stebbins & al. 1953; Rousi 1973; Seaman 1982; Bremer 1994), including the studies on the Bulgarian representatives of this genus carried out by Kuzmanov (1985).

On the basis of cytotaxonomy and reproductive behavior of eight diploid species of genus *Leontodon,* including *L. autumnalis*, *L. hispidus* and *L. crispus*, Rousi (1973) had found that these species are strongly sexually reproducing and without any indication of apomixis observed. As a result of a comparative embryological investigation of the Bulgarian populations of *L. crispus* and *L. hispidus,* Yurukova-Grancharova

(1978) established that they are strongly proterandrous and sexually reproducing. Although a tendency to somatic apospory was found sporadically in some individuals of one population of *L. hispidus* out of the four examined, no embryo formation proceeded.

The object of this study, *L. autumnalis*, belonging to the section *Oporinia* (D. Don) Koch (Finch & Sell 1976) is distributed in Bulgaria on the moderately moist grassy places in the plains and mountains at altitudes from 50 m to 1800 m (Kuzmanov 1985). Karyological studies on the Bulgarian populations of *L. autumnalis* have revealed this species as a diploid with 2n = 12 (Kuzmanov & Georgieva 1976; Kuzmanov & al.1993).

The aim of the present study is to supply some additional data enriching the embryological characteristic of genus *Leontodon*, to contribute to a more clear delimitation of its taxa within the subtribe *Hypochoeridinae* and tribe *Lactuceae,* as well as to elucidate their relationships and the trends of evolution on the basis of the embryological features.

Material and methods

Flower buds, open capitula and achenes in various stages of development were collected from two populations in their natural habitats (Western Rhodopes, near Sarnitsa village, SOM 97020; Vitosha region, Mt Vitosha, village Dragalevtsi, SOM 97400) and were fixed in FAA (formalin: glacial acetic acide: ethanol-5: 5: 90 parts with 70 % ethanol) or Navashin's mixture (Nikolov & Daskalov 1964). The material was embedded in paraffin, cut into 8–14 μm sections and treated according to classical paraffin methods. The sections were stained with Heidenhain's hematoxylin. The permanent slides were mounted in Canada balsam. Squash preparations of the young anthers with 1 % acetocarmin were also made for more detailed study of the microsporogenesis and microgametogenesis.

Observations were carried out with the Amplival light microscope. The microphotos were made with Nf-matic.

The embryological characteristics reported in the following descriptions are common for the two studied populations of *L. autumnalis*, unless particular comments are given. The embryological data received during this study are compared with the available data on the other *Lactuceae,* predominantly the *Hypochoeridinae* representatives, such as *L. hispidus*, *L. crispus* (Yurukova-Grancharova 1978, 1979), *H. radicata* L., *Picris echioides* L. (Yurukova-Grancharova 1979) and *Urospermum picroides* (L.) Scop. ex F. W. Schmidt (Yurukova-Grancharova (1979, 1983) that were previously studied.

Results and discussion

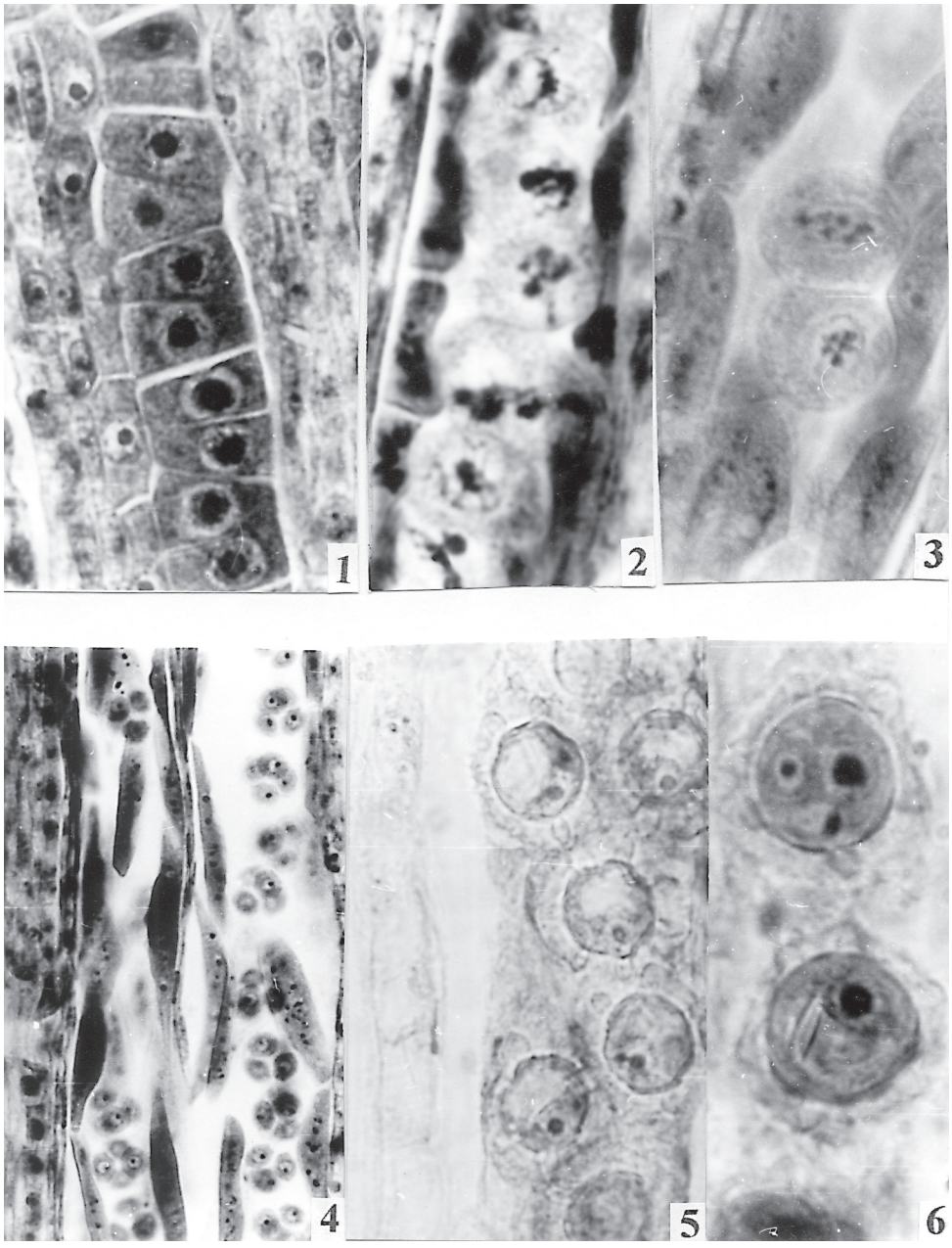
Anther, microsporogenesis and development of the male gametophyte

The anthers of *L. autumnalis* are tetrasporangiate. In the longitudinal sections the anther locules are morphologically almost equal, strongly cylindrical and comparatively narrow. Prior to maturity, the anther wall comprises four layers: epidermis, endothecium, middle layer and tapetum that develop centrifugally (Plate I, Fig. 1). Since the endothecium and the middle layer have histologically a common origin, the anther wall formation is regarded as conforming to the Dicotyledonous- type (Davis 1966; Poddubnaya-Arnoldi 1976). Clear delimitation of the four anther layers becomes possible even during the earlier stages of the prophase I (leptotene-zygotene) of the meiosis in the microspore mother cells (MMCs).

The epidermis consists of large, almost rectangular one-nucleate cells, with thin walls and almost transparent cytoplasm that enlarge tangentially and round off outside. The middle layer is ephemeral and crushes or completely degenerates towards metaphase I of the meiosis in MMCs. During maturation of the anther (after the one-celled pollen stage), the cells of the endothecium enlarge tangentially and develop fibrous thickenings that often are not very typical of all its cells.

The anther tapetum is initially glandular. Its one-nucleate cells (Plate I, Fig. 1) more or less enlarge and become four-, six-, seldom eight-nucleate during the anther development as a result of successive mitotic divisions. The same was found too in the anther tapetum cells of the other studied Bulgarian *Hypochoeridinae* (Yurukova- Grancharova 1979). In some tapetal cells often a polyploidization, as well as pyknosis have been observed during anther ontogenesis, when the meiosis in the MMCs runs (Plate I, Fig. 2). Glandular tapetum transforms into ameboid false periplasmodium after the formation of one-celled pollen (Plate I, Fig. 5), as previously established too in *L. crispus* and *L. hispidus* (Yurukova-Grancharova 1979). At the mature pollen stage, out of the four layers of the anther wall only the epidermis and endothecium are preserved, but of-

Plate I



Figs 1-6. Anther, microsporogenesis and male gametophyte in *L. autumnalis*.
1, four-layered anther wall and one-layered sporogenous tissue. ×160; **2,** polyploid and pyknotic tapetal cells and prophase I in MMC. ×100; **3,** diakinesis in MMC with six bivalents. ×160; 4, tetrahedral and isobylateral microspore tetrads in an anther with glandular tapetum. ×63; **5,** one-celled pollen and ameboid tapetum. ×160; **6,** mature three- and two-celled pollen. ×160.

ten with destroyed entirety, while the middle layer has degenerated and the tapetum was already completely disorganised.

The sporogenous tissue in *L. autumnalis* is usually organised in one (occasionally two) layer (Plate I, Fig. 1). Of the other embryologically studied *Hypochoeridinae* (Yurukova- Grancharova 1979), only in *L. crispus* two-layered sporogenous tissue was occasionally observed. Initially polygonal one-nucleate sporogenous cells very quickly enlarge, round up and directly function as MMCs (Plate I, Fig. 2). The meiosis in MMCs runs normally and during diakinesis six bivalents are clearly counted, which shows a haploid chromosome number n = 6 (Plate I, Fig. 3). Insignificant deviations of the meiosis (more often observed during its heterotypic division) in some MMCs are expressed mainly in the presence of individual lagging chromosomes, chromosomes out of the spindle, as well as chromosome bridges, especially during anaphase I.

The meiosis in MMCs is accompanied by simultaneous cytokinesis and the resultant microspore tetrads are "usually" tetrahedral, "occasionally" isobilateral (Plate I, Fig. 4) and "rarely" decussate on the basis of 50 selected tetrads of the two studied populations (expressions of frequency follow Schmid 1982: 90). The microspores in the tetrads are morphologically uniform, almost equal in size and shape and in seldom some of them micronuclei have been found.

The mature pollen is 3-celled (Plate I, Fig. 6), 3-colporate, with echinate exine that is characteristic of most representatives of the *Asteraceae*. Often in some anther locules two-celled pollen has been observed too at the time of shedding (Plate I, Fig. 6). In the anthers, fertile pollen is formed in a high degree (80–90 %), although in individual anther locules sterile and degenerating pollen grains have been found. An insignificant asynchrony has been observed during the meiosis in MMCs and microsporogenesis of the individual flowers of a capitulum that defines *L. autumnalis* as not so strongly proterandrous in comparison with *L. crispus* and *L. hispidus* (Yurukova-Grancharova 1978).

Ovule, megasporogenesis and development of the female gametophyte

In the unilocular inferior ovary of *L. autumnalis* only one ovule develops. The well-developed ovule is anatropous, tenuinucellate and unitegmic. Its nucellus is poor, completely formed from a single archesporial cell and one-layered nucellar epidermis consisting of a small number of cells around it. Consequently, the nucellar cells elongate and enlarge tangentially, more obviously those located towards the micropylar side of the ovule. The nucellar epidermis cells do not divide at any stage of the ovule development.

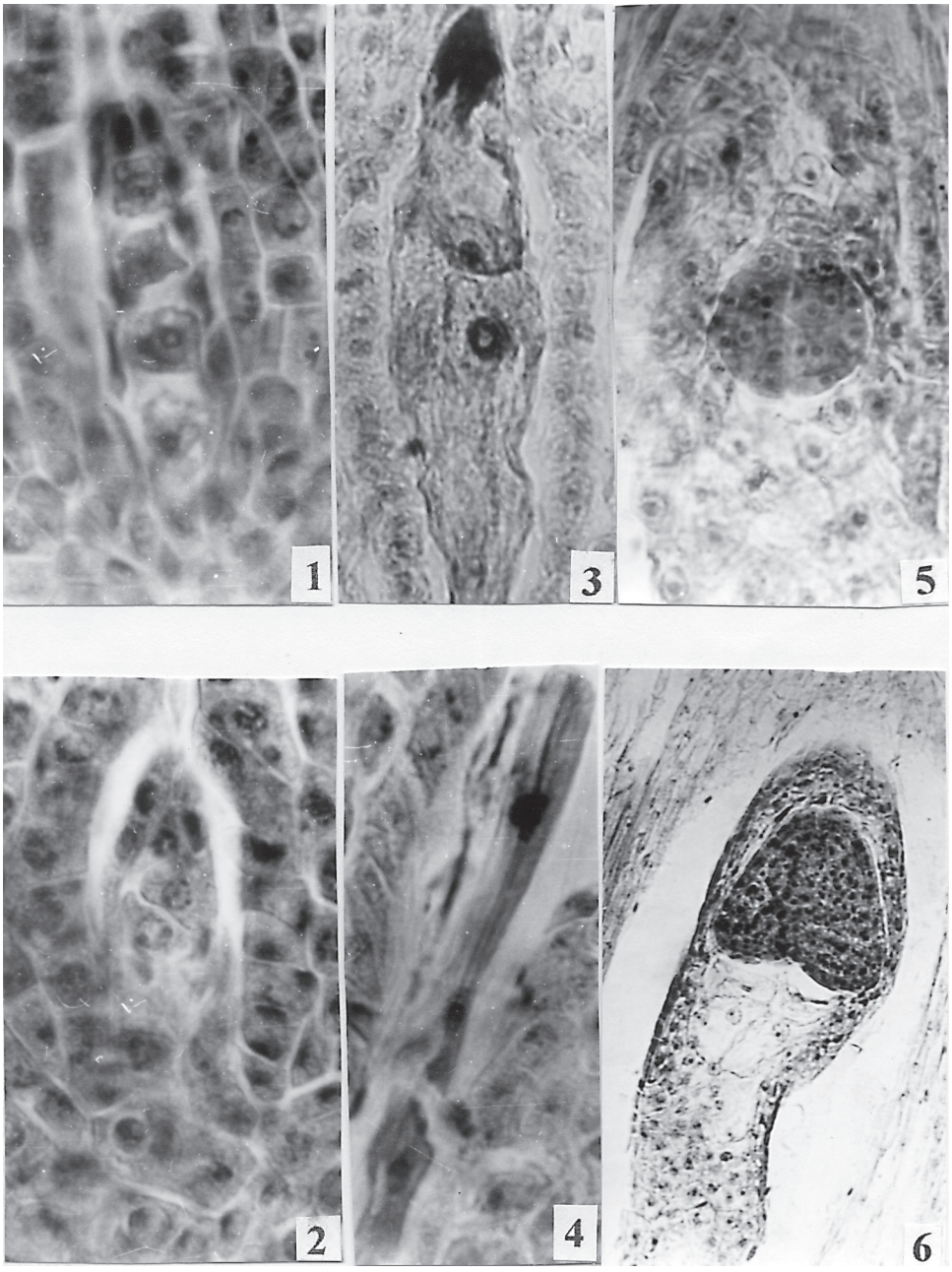
In the still young ovule hypodermally one-celled archesporium forms. No case has been established in which two or more archesporial cells are borne in a single ovule primordium. Occasionally, two archesporial cells were observed in the ovule only in *Sonchus asper* (L.) Hill (Yurukova-Grancharova 1979, 1997). The archesporogenesis runs without formation of parietal cells, which is typical for the representatives of the evolutionary advanced and specialized Angiosperms families, and in particular of *Asteraceae* as Poddubnaya-Arnoldi (1976, 1982) reported. The primary sporogenous cell directly functions as megaspore mother cell (MMC), with one intensively stained nucleus that later undergoes meiosis to produce a tetrad of megaspores with linear arrangement (Plate II, Fig. 1). Mention deserves the fact that in three ovules (out of 18 observed at this stage) a triad of megaspores has been present (Plate II, Fig. 2), instead of a tetrad, as a result of inhibition of the meiosis within MMC.

In some megaspore tetrads, the third and fourth megaspores (in the direction from micropyle to chalaze) are equally vital, both showing a potential for possible development, although only one of them further develops into an ES.

In a linear tetrad, usually the chalazal megaspore becomes functional as an embryo sac mother cell (EMC) that develops successively into a two-, four- and eight-nucleate embryo sac (ES) by three consecutive mitotic divisions. Thus, the mode of ES formation is of the *Polygonum*-(monosporic) type. The remnants of the degenerating three sister megaspores have been visible sometimes even up to the four-nucleate ES stage.

The single integument usually consists of 6–7 rows of somatic cells during prophase I of the meiosis in the MMCs, but after the one-nucleate ES stage it is already sufficiently big, comprising 10–14 layers of somatic cells. During megasporogenesis and megagametogenesis, the nucellar epidermis preserves for comparatively long time even after the differentiation of the last (chalazal) megaspore into an EMC. Remnants of the nucellar epidermis are usually presented only at the

Plate II



Figs 1-6. Ovule, megasporogenesis and female gametophyte in *L. autumnalis.*
1, a linear megaspore tetrad in the ovule. ×100; **2,** a linear triad in the ovule. ×100; **3,** egg cell, central cell and parts of pollen tube in the micropyle of a mature ES with endothelium. ×160; **4,** two- celled antipodal complex with a haustorized two-nucleate upper antipodal. ×320; **5,** a globular embryo with suspensor and nuclear endosperm. ×100; **6**, a heart-shaped embryo and cellular endosperm. ×100.

chalazal pole at the one-nucleate stage of ES, when the endothelium has already formed from the innermost layer of a single integument of the ovule. The endothelium is one-layered and consists of one-nuclear cells that considerably elongate radially during ES development (Plate II, Fig. 3).

Polarization of the nuclei in the two-, four-, and eight- nucleate ES is typical of the *Polygonum*-type female gametophyte and vacuolization is usually clearly expressed for each of these stages of the ES development. Mention deserves the fact that the nuclei at two- and four-nucleate ES often consist of two nucleoli of equal or different size. In 50 ovules (out of 26 observed in that stage) four-nucleate ESs with untypical polarization of the nuclei have been found, which is probably due to some unfavorable environmental circumstances.

At first, the egg apparatus is differentiated in the mature eight-nucleate ES, then the two polar nuclei move to the center of ES cavity, and finally the antipodal complex is formed deeply into the chalazal pole of the ES (Plate II, Fig. 3). After differentiation of the elements in a mature ES, it quickly enlarges considerably and by the beginning of fertilization is usually five or six times larger in size in comparison with the one-nucleate ES stage. The egg cell is large, piriform, with a big nucleus and typical vacuolization (Plate II, Fig. 3). The two synergids are sometimes different in shape, from almost cylindrical to piriform, and their vacualization is not always clearly pronounced. The two polar nuclei moving towards the central part of ES soon fuse and the resulting central cell is located before fertilization close to the wide basal end of the egg cell (Plate II, Fig. 3).

Antipodals are the most polymorphous cells in the ES. Apparently, the major function of antipodals is to provide and support a better trophycal function of ES, especially during its maturity, as many authors have reported (Maheshwari 1950; Poddubnaya-Arnoldi 1976; Kordijum, 1978). The three antipodals are usually with linear, or more seldom with T-shape arrangement. Besides, in some ES the antipodal complex comprises two, instead of three cells as it was found in *Sonchus asper* (Yurukova-Grancharova 1997). Bergman (1935) had noticed a two-celled antipodal complex in *L. hispidus* L. var. *vulgaris* too. In the fully differentiated ES of *L. autumnalis* the upper antipodal is usually two-nucleate as a result of inhibited cytokinesis. The antipodals have often haustorized to a different degree. Haustorization of some antipodals in the mature ES (more often in the population 97 400 from Mt Vitosha) is considerable and in these cases a typical antipodal haustorium forms (Plate II, Fig. 4) that has not been found in other *Lactuceae* taxa (Poddubnaya-Arnoldi 1976). Mention deserves the fact that in some ES, particularly in the same population, the three antipodals exist only as free nuclei and no cytokinesis runs subsequently between them. To sum up, the antipodals in the ES of population 97400 are more polymorphous as compared with those of population 97 020.

The legitimate embryo and endosperm form after double porogamous fertilization and the penetration of the pollen tube has been often observed (Plate II, Fig. 3), especially in the micropylar part of ES in population 97 400 from Mt Vitosha. The zygote and primary endosperm nucleus have been usually observed to divide almost simultaneously. The first mitotic division of the zygote is transversal and has been observed more often, especially in the mature ES of population 97400. Embryogenesis runs according to the Asterad-type as in most *Asteraceae* representatives (Johansen 1950; Maheshwari 1950; Poddubnaya–Arnoldi 1982; Solntseva 1987). The multicellular embryo has a clearly expressed suspensor consisting of 4–5 cells. Occasionally, a more massive two-layered suspensor has been observed in some ES (especially in the part closely connected with the embryo), probably as a result of additional mitotic divisions of the primary suspensor cells.

Initially, the endosperm consists of free nuclei and usually becomes completely cellular after the wall formation between them and the differentiation of the heart-shaped embryo, as in the other two earlier studied *Leontodon* species (Yurukova-Grancharova 1978).

During an earlier embryological study of *L. hispidus* in Bulgaria (Yurukova-Grancharova 1978) a somatic apospory was found occasionally in the ovule, which subsequently never resulted in embryo and seed formation. Because of that, somatic apospory in this species was considered only as a tendency, i.e. non- functional, "untypical", according to Czapik (1996), and a "*Leontodon*-type" somatic apospory, according to Battaglia (1963). During the present study, no somatic aposporous ES or adventitious embryos have been observed in *L. autumnalis*. Rousi (1973: 210-211) had noticed the following for eight diploid species of the examined genus *Leontodon* (including *L. autumnalis, L. hispidus* and *L. crispus*): "No seed formed without pollination, whether the flower heads were emasculat-

ed or not. This means that autonomous apomixis can be ruled out from the possible mode of reproduction".

As a result of this study it has been established that *L. autumnalis* is a strongly expressed sexually reproducing species. This fact, the stable embryological processes and structures, lack of apomixis and polyploidy suggest that *L. autumnalis* is probably an old diploid species in which no active processes of intraspecies formation take place. The embryological results obtained during this study and the available data reveal a clear affinity between *L. autumnalis, L. hispidus* and *L. crispus*. Furthermore, concerning their main embryological features and especially those of taxonomical value, these *Leontodon* species show a clear affinity to the other earlier studied *Hypochoeridinae* taxa (*Hypochoeris radicata*, *Picris echioides*, *Urospermum picroides*) and define this group of *Lactuceae* as monophyletic which was already proved by morphological, karyological and phytochemical evidences.

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