

SECRETORY STRUCTURES OF SUBTERRANEAN ORGANS OF SOME SPECIES OF THE CARDUEAE, AND THEIR DIAGNOSTIC VALUE

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Secretory ducts and cavities of roots and rhizomes are typical features of the Cardueae tribe in the Asteraceae family. We used light microscopy to analyze the anatomy of the subterranean organs of 21 species of 13 genera of the Cardueae, with particular attention to the secretory system, interpreted in taxonomic terms. The anatomy of secretory ducts varied greatly. A new measurement quotient, C1 [length of epithelial cells (longitudinal section)] and C2 [length of adjacent cells (longitudinal section)] was established. Different types of ducts are described based on type of development and the size ratios among epithelial cells. Detailed anatomical descriptions of the ducts are given, together with their occurrence in particular taxa. The simultaneous presence of various secretory ducts within a single species and their spatial position relative to other prominent anatomical features provide valuable characters for discriminating the studied Cardueae species. These analyses are of particular interest for identification of herbal drugs as, besides chemical analytical techniques such as chromatographic fingerprinting, light microscopy is a common method for purity controls and thus required in official pharmacopeias.

Key words: Root anatomy, microscopy, Asteraceae, Cardueae, secretory ducts.

INTRODUCTION

The Asteraceae are among the largest families of flowering plants, comprising more than 23,000 species and about 1600 genera (Jeffrey, 2007). Many species such as *Taraxacum officinale* (L.) Weber, *Cichorium intybus* L., *Carlina acaulis* L. and *Silybum marianum* L. are well known for their use in both traditional and western medicine (Wichtl, 2009). The tribe Cardueae comprises over 2360 species organized in 73 genera (Susanna and Garcia-Jacas, 2007), holding some of the largest genera of the Asteraceae.

The Asteraceae, and within that family the Cardueae, are particularly rich in internal secretory tissues. Secretory ducts or canals and secretory cavities are widespread anatomical structures within the plant kingdom and occur in almost all plant organs. They are either initiated by mechanical injury (Fahn, 1988) as so-called induced or traumatic ducts or cavities, or are formed without external stimulus. Ducts are usually oriented parallel to the longitudinal axis of the organ, whereas cavities possess an irregular lumen with no principal extension (Col, 1904; Nair, 1995).

Three developmental types are distinguished in the literature (Nair, 1995; Evert, 2006):

1. The schizogenous type originates from dissolution of the middle lamella of duct initials, resulting in the formation of an intercellular space. The cells may further divide and become epithelial cells.
2. The formation of ducts or cavities through dissolution and autolysis of entire cells, which release their content into the resulting space (holocrine secretion), is known as lysigenous development.
3. Schizolysigenous canals result from a combination of the two processes, with initial schizogenous development followed by lysigenous widening of the duct lumen.

Characteristic of the Cardueae among European species are endodermal resin ducts within their roots and rhizomes (van Tieghem, 1883; Col, 1903; Solereder, 1908). Endodermal resin ducts emerge from the endodermis by doubling of endodermal cells, forming an intercellular space (schizolysigenous development). In the shoot axis or leaves, endodermal resin ducts may be surrounded by special cells smaller than endodermal cells. In roots the

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ducts emerge directly from the endodermis without such specialized surrounding cells (van Tieghem, 1883, 1884; van Vuillemin, 1884). The ducts can widen by further divisions of the surrounding cells. This kind of secretory duct may occur in the shoot axis and in leaves, but always within the root of all species of the Cardueae. Interestingly, doubling of the endodermis, though without the formation of ducts, was also found in the Cichorieae tribe within subterranean parts of *Cichorium intybus* L., *Lapsana communis* L. and *Podospermum laciniatum* (L.) DC., although endodermal resin ducts do not exist within that tribe (van Tieghem, 1883; Solereder, 1908).

In addition to the mentioned endodermal resin ducts, various species of the Cardueae may develop schizogenous secretory ducts within the pith and the cortex. Ducts can also be found within secondary tissues as described from *Carlina acaulis* L. and *Centaurea atropurpurea* Waldst. & Kit. by Col (1903). *Echinops exaltatus* Schrad. possesses resin cells in the secondary phloem (Solereder, 1908).

In general, comparative taxonomic studies on ducts and cavities are rare (reviewed in Pickard, 2008). Most literature deals with the development of diverse secretory reservoirs of single species (e.g., Bennici and Tani, 2004) or concentrates on conifers and legumes, as their gumresins or resins are of commercial importance (Hong Wu and Zheng-hai Hu, 1997; Rodrigues and Machado, 2009; Bollschweiler et al., 2008 and others). Previous literature on the Cardueae deals mainly with the occurrence of secretory ducts or cavities on aerial organs, especially those of leaves and shoots, and the transition between root and shoot (Col, 1903, 1904). Details on the structure of the ducts and cavities of subterranean parts have been neglected; there are only a small number of published studies on the subject and they deal mostly with single species (e.g., Ragonese, 1988; Melo-de-Pinna and Menezes, 2002; Hayashi and Appezzato-da-Gloria, 2007). Comparative studies are few. Melo-de-Pinna (2002) listed about 50 species of the Asteraceae studied for the position of secretory canals in the roots. Appezzato-da-Gloria (2008) and Cury and Appezzato-da-Glória (2009) investigated the secretory structures of 13 additional Asteraceae species.

Insufficient knowledge of the root anatomy of the Asteraceae is particularly troublesome for pharmaceutical research and practice, as identification of root drugs and the required purity tests strongly rely on accurate discriminative anatomical characters as well as chemical analysis techniques. In a study (Fritz, 2011), I endeavored to clarify whether Asteraceae species used pharmaceutically can be anatomically discriminated by means of light microscopy for the purpose of guaranteeing the purity of a declared drug. I examined 59 species of

33 genera of the Cardueae and Cichorieae and created a database of anatomical characters.

Here we focus on the anatomy of the secretory structures in order to reveal additional features for discriminating single species as possible sources of herbal drugs. The work concentrates on species formerly and currently used medicinally: *Carlina acaulis* L., *C. vulgaris* L., *Arctium lappa* L., *A. tomentosum* Mill. (e.g., Berger, 1960; Gerlach et al., 2006; Saric-Kundalic et al., 2010) and their relatives comprising diverse taxa of all subtribes of the Cardueae (Echinopsidinae, Carlininae, Carduineae, Centaureinae; Susanna and Garcia-Jacas, 2009) occurring in Austria according to Fischer et al. (2008).

Though it does not belong to the Cardueae but to the tribe Cichorieae, we examined the roots of *Cichorium intybus* L. as representative of a species exhibiting doubling of the endodermal cells but not possessing secretory canals.

MATERIAL AND METHODS

PLANT MATERIAL

The plant material comprised 21 Cardueae species naturally occurring in Austria (Tab. 1, taxonomy follows Fischer et al., 2008). We studied one to four accessions per species, and analyzed the anatomy of three to six specimens per accession. Taxa were chosen according to their medicinal use and their role as possible adulterants. Fully developed roots and rhizomes were collected during or following anthesis.

Vouchers are deposited in the herbarium of the Department of Pharmacognosy, University of Vienna (WUP). The plant material was taxonomically determined using floristic treatments covering the sampled geographic areas (Pawłowski and Jasiewicz, 1972; Szafer et al., 1976; Lauber and Wagner, 2007; Fischer et al., 2008).

ANATOMICAL ANALYSIS

The samples for light microscopy examination were prepared by the following variant of a traditional method used by the University of Vienna: desiccated roots were boiled in water for 10 min to soften the tissue and then immersed in 96% ethanol for induration. After evaporating the alcohol for a few minutes, transverse and longitudinal sections were cut by hand ~1.5 cm below the hypocotyl, as this site provided specimens with the clearest delimitation of root anatomy. Sections were embedded in a few drops of chloral hydrate (60% in water) and examined with a Nikon Optiphot-2 light microscope equipped with a Samsung Digimax V50 digital camera.

TABLE 1. List of species examined (plants arranged according to Fischer et al., 2008); plant material collected by Elisabeth Fritz (E.F.), Christoph Dobeš (C.D.), Johannes Saukel (J.S.), Valerie Klatte-Asselmeyer (V.K.), Silvia Fialova (S.F.), Werner Lahner (W.L.), Günther Stadler (G.S.); all vouchers are deposited in WUP

Genus	Species	Collection sites
<i>Echinops</i>	<i>E. sphaerocephalus</i> L.	Austria, Vienna, E.F. -, Lower Austria, Tullnerbach, E.F. -, -, Karnabrunn, C.D. -, Tyrol, Fließ, V.K.
<i>Carlina</i>	<i>C. acaulis</i> L.	-, Vienna, E.F. Germany, Baden-Württemberg, Schwäbische Alb, Herbarium of Hohenack, Nr. 652
	<i>C. vulgaris</i> L.	Poland, Gutkowo, Olsztyn, E.F.
<i>Arctium</i>	<i>A. lappa</i> L.	Austria, Vienna, E.F.
	<i>A. tomentosum</i> Mill.	-, Lower Austria, Traiskirchen, E.F.
<i>Saussurea</i>	<i>S. discolor</i> (Willd.) DC.	-, Carinthia, Lesachtal, J.S.
	<i>S. pygmaea</i> (Jacq.) Spr.	-, Styria, Schneealpe, E.F.
<i>Jurinea</i>	<i>J. mollis</i> Rchb.	-, Vienna, E.F. -, Burgenland, Winden, J.S.
<i>Carduus</i>	<i>C. personata</i> (L.) Jacq.	-, Styria, Schneealpe, E.F.
	<i>C. defloratus</i> L. (<i>C. crassifolius</i> Willd.)	-, Lower Austria, Gippel, C.D. -, -, Araburg castle, E.F.
<i>Cirsium</i>	<i>C. arvense</i> (L.) Scop.	-, Vienna, E.F. Slovakia, Modra, S.F. U.S.A., New York, Peekskill, Planta America Septentrionalis, Le Roy
	<i>C. vulgare</i> (Savi) Ten.	Poland, Gutkowo, Olsztyn, E.F.
	<i>C. erisithales</i> (Jacq.) Scop.	Austria, Styria, Schneealpe, E.F.
<i>Onopordum</i>	<i>O. acanthium</i> L.	-, Lower Austria, Buchberg, W.L. Italy, Southern Tyrol, Vinschgau, C.D.
<i>Silybum</i>	<i>S. marianum</i> (L.) Gaertn.	Austria, Lower Austria, Buchberg, W.L. Slovakia, Botanical Garden of Bratislava, S.F.
<i>Serratula</i>	<i>S. tinctoria</i> L.	Austria, Vienna, J.S.
<i>Rhaponticum</i>	<i>R. scariosum</i> Lam.	Liechtenstein, Saminatal, G.S.
<i>Centaurea</i>	<i>C. jacea</i> L.	Austria, Karnabrunn, C.D. -, Vienna, E.F.
	<i>C. scabiosa</i> L.	-, Vienna, J.S. Poland, Gutkowo, Olsztyn, E.F. Switzerland, Graubünden, Lavin, C.D.
	<i>C. cyanus</i> L. (= <i>Cyanus segetum</i> Hill., Fischer et al., 2008)	Austria, Vienna, E.F. Germany, Baden-Württemberg, Kronau, C.D. Poland, Mazury, Zabie, E.F.
	<i>C. montana</i> L. (= <i>Cyanus montanus</i> Hill., Fischer et al., 2008)	Austria, Lower Austria, Unterberg, E.F. Slovakia, Modra, S.F.
<i>Cnicus</i>	<i>C. benedictus</i> L.	Austria, Vienna, Botanical Garden of the Department of Pharmacognosy, University of Vienna
<i>Cichorium</i> (tribe <i>Cichorieae</i>)	<i>C. intybus</i> L.	Austria, Vienna, Botanical Garden of the Department of Pharmacognosy, University of Vienna: seeds of the Botanical Garden Berlin-Dahlem: DE-0- B-2003105: Brandenburg, Kreis Havelland, Falkensee, leg. Dürbye 3090

The following statistical measure was calculated: length of cells (longitudinal section) surrounding the lumen of the secretory ducts (C1) divided by the length of adjacent cells (C2) (Fig. 1). Calculations employed Statistica®.

Additionally, seeds of *Cichorium intybus* L. were cultivated in the garden to observe one species in which doubling of the endodermis is not accompanied by the formation of endodermal resin ducts, as described by van Tieghem (1883). The first sec-

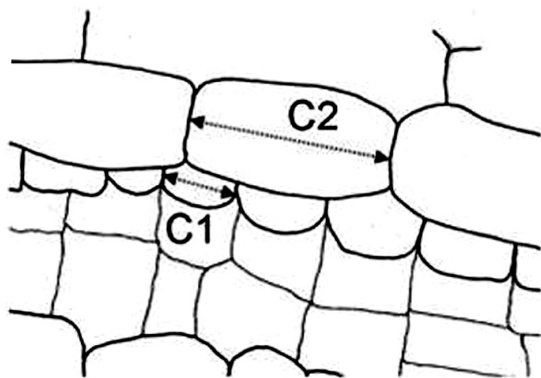


Fig. 1. Secretory duct (transverse section) showing inner and outer layers of epithelial cells, conspicuously differing in length. The measures C1 and C2 were used to calculate a quotient describing that proportion.

tions were made upon the appearance of the first leaf rosette, and then 3 weeks later.

RESULTS

Among the species analyzed, three principal types of secretory tissue were identified: endodermal resin ducts, secretory ducts and secretory cavities.

The secretory structures were not equally distributed among the studied species. While endodermal resin ducts are ubiquitous features, secretory ducts were observed only in a subset of the species, and they varied between taxa, differentiating into five anatomical types which could be found in combination or singly in a given species. Species were characterized by the presence of one or more types of secretory ducts and their position relative to each other and/or to prominent anatomical elements.

ENDODERMAL RESIN DUCTS (endoSD)

Because all species of the tribe Cardueae contain endodermal resin ducts within their roots and rhizomes (van Tieghem, 1883; Col, 1903; Solereder, 1908), this kind of duct usually has been considered of no taxonomic value. However, endoSDs can be used to make some distinction between single species. In particular, the size of the duct versus that of the cells of the surrounding cortex (transverse section) proved important. In *Jurinea mollis* Rchb., for example, the diameter of endodermal resin ducts is several times the size of the surrounding parenchyma cells (Fig. 2a, b). As *Jurinea mollis* possesses a permanent cortex, the endoSDs provide a reliable feature for recognition of this species. Likewise, *Carlina acaulis* develops endoSDs of very large diameter. Since the cortex is lost in the course

of rhytidome formation, the diagnostic value of endoSDs in this species is less. However, the secretory ducts of tissues other than the cortex can be used.

Other species examined in this study which can be characterized to some extent by endoSDs are *Onopordum acanthium* L., *Centaurea jacea* L., *Centaurea cyanus* L., and *Serratula tinctoria* L.. While these species could not be discriminated based on duct diameter, the number of surrounding cells forming the lumen (transverse section) may be decisive. In fully developed roots, the number of cells lining the ducts of *C. cyanus* is usually up to 10 maximum, up to 6–7 in *C. jacea*, and up to 6 in *Onopordum acanthium*. In *Serratula tinctoria*, endoSDs with a small lumen and usually only 4 surrounding cells occur (Fig. 2c,d), as this species possesses a fibrous root system whose roots do not develop secondary growth.

Interestingly, in *Cichorium intybus*, in which doubling of the endodermis was said to occur without the formation of secretory structures, our work documented the formation of endodermal resin ducts within the roots of *Cichorium intybus* at an early state of growth, which vanished with the beginning of secondary growth. Secretory canals were observed when the first leaf rosette appeared (Fig. 3a,b)

OTHER SECRETORY DUCTS (SD)

Many species of the Cardueae do not possess SDs but endoSDs [e.g., *Arctium lappa* L., *Cirsium erisithales* (Jacq.) Scop., *Onopordum acanthium*, etc.]. Other species develop further ducts or cavities within the cortex, pith or secondary tissues. Here we provide a new classification of the SDs of the Cardueae based on anatomical features (Fig. 4):

1. Secretory duct types SD1 and SD2:

Both type SD1 and type SD2 are of schizogenous origin. According to the C1:C2 quotient (see Material and Methods, Fig. 1), the ducts we investigated can be divided clearly into two categories:

- a. Within the first group, the plants develop ducts with characteristic epithelial cells much smaller in length than the other cells of the tissue and an average C1:C2 quotient of <0.3 (secretory ducts of type SD1).
- b. Within the second category (secretory ducts of type SD2), the C1:C2 quotient exceeds 0.4. Our results further suggest a division of this second category into one group of ducts having an average quotient of >0.8 (e.g., *Saussurea* sp., 0.88–1.07; *Cirsium arvense* (L.) Scop. rhizome, 0.81–1.17, located at the boundary between vascular bundle and pith), type SD2b (Fig. 6e-h), and a second group with a quotient of 0.4 to 0.7 (e.g., *Centaurea jacea*, 0.58–0.7;

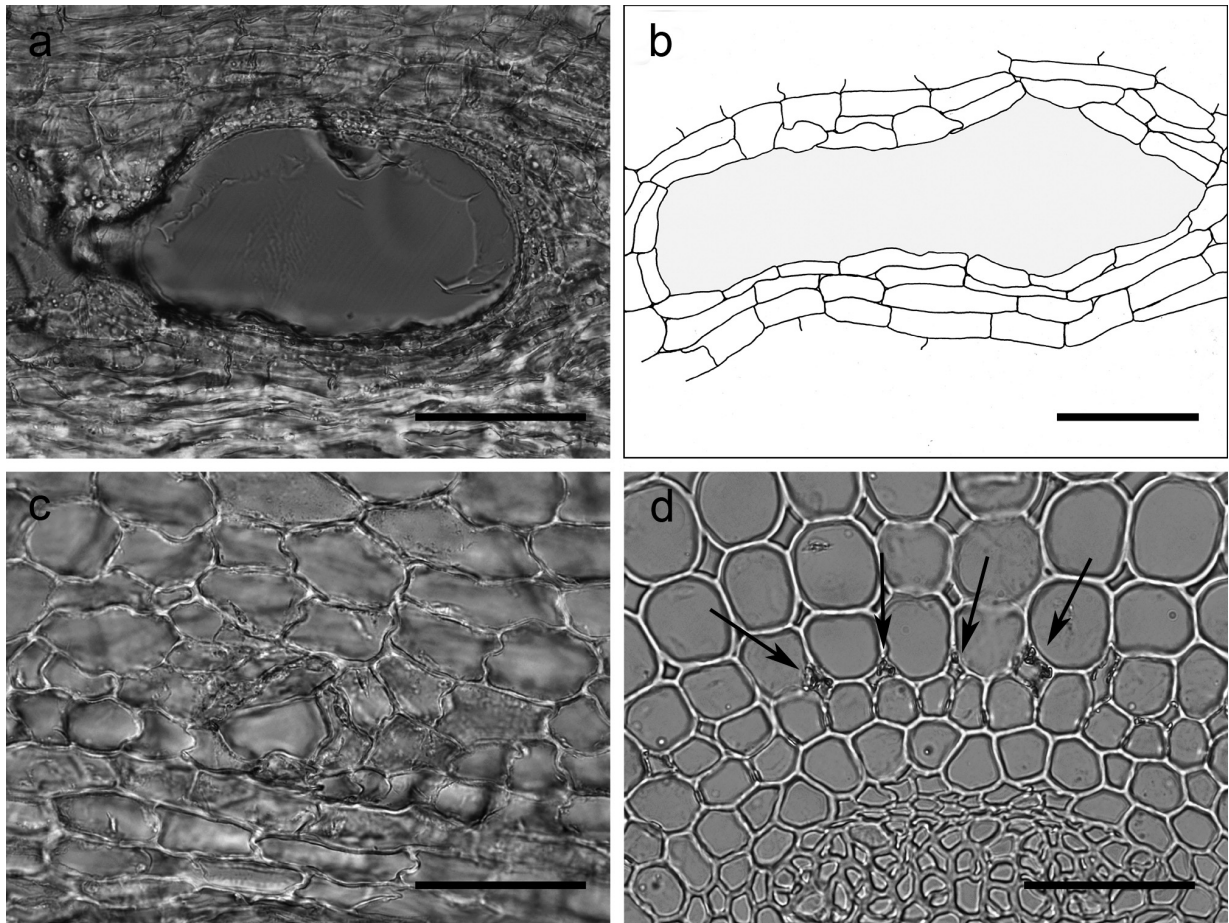


Fig. 2. Endodermal resin ducts of (a,b) *Jurinea mollis*, (c) *Onopordum acanthium*, (d) *Serratula tinctoria* in transverse sections. Bar = 50 μ m.

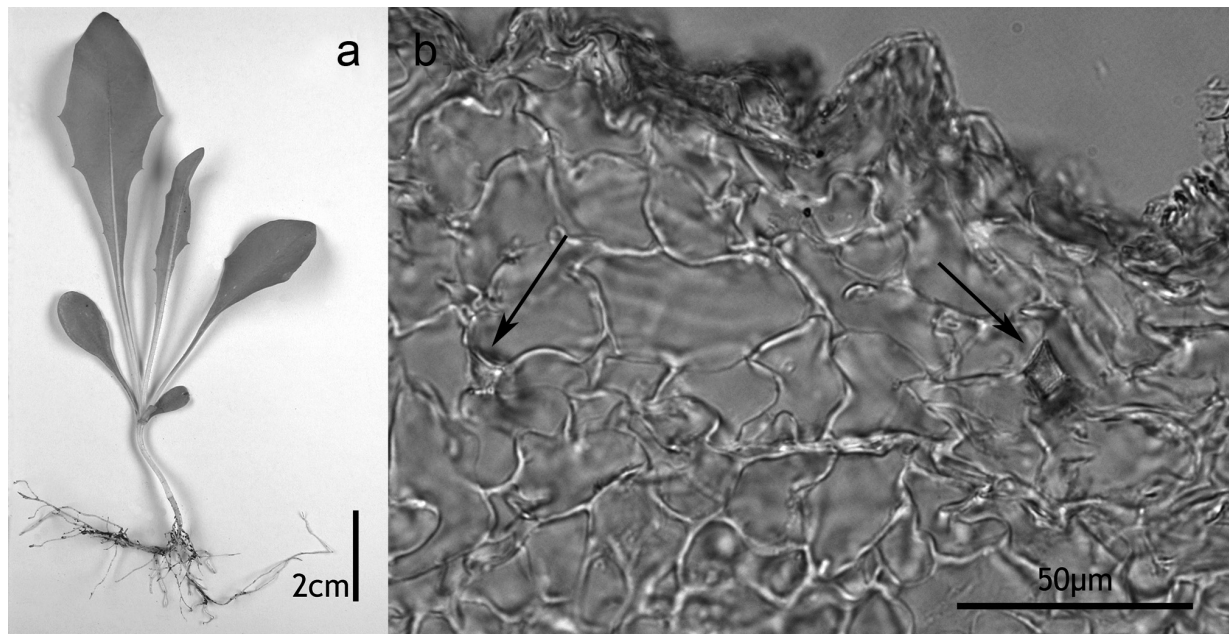


Fig. 3. (a) *Cichorium intybus*, cultivated in the garden: habitus, first rosette of leaves developed, (b) Resin ducts observed in transverse section in the root of the plant shown in (a). Black arrows show secretory ducts.

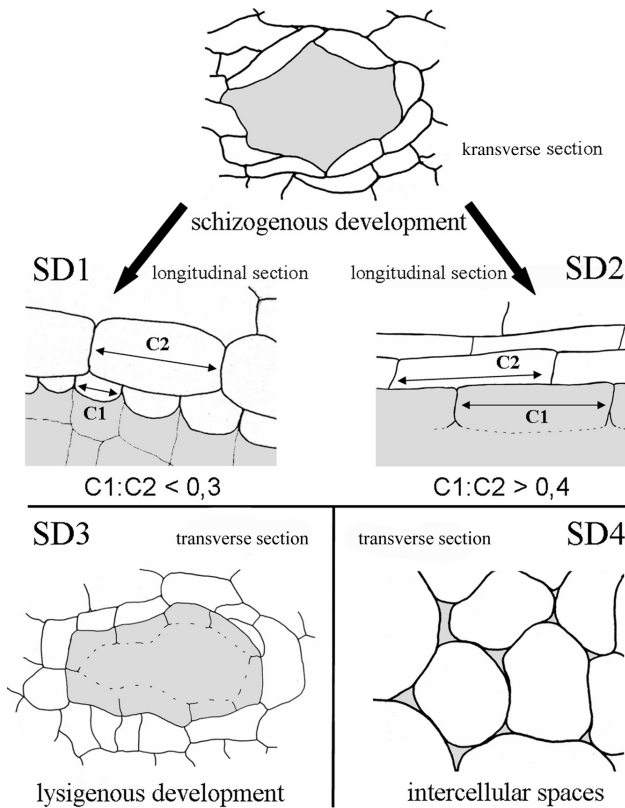


Fig. 4. Overview of the 4 types of secretory ducts occurring in the vascular cylinder.

Rhaponticum scariosum Lam., 0.49–0.7), type SD2a. Figure 5 demonstrates the value of these data with a scatterplot showing that the formation of the diverse types of ducts probably is based on different regularities (Saukel, 1984). Additional work is needed to show whether the division of the second category is justified.

Three species we examined possess ducts of type SD1. *Centaurea montana* L. develops secretory ducts of type SD1 (0.24–0.27) which appear to have colorless content and are always found within the secondary phloem close to the endodermis (Fig. 6a-d). Ducts of both types occur in *Centaurea scabiosa* L. (0.98–0.99, 0.22–0.64), in which case they are located in the fascicular position within the secondary phloem between the phloem rays, forming a triangular pattern around secretory ducts of type SD3 (see below).

In contrast to *C. montana* and *C. scabiosa*, *Centaurea cyanus* and *C. jacea* possess ducts not of type SD1 but of type SD2 (and also SD4 in *C. montana* and *C. jacea*, see below). Hence, the four studied *Centaurea* species can be distinguished based on their secretory ducts, particularly if the C1:C2 quotient is taken into account (C1:C2 = 1–1.01 in *C. cyanus*, 0.58–0.7 in *C. jacea*).

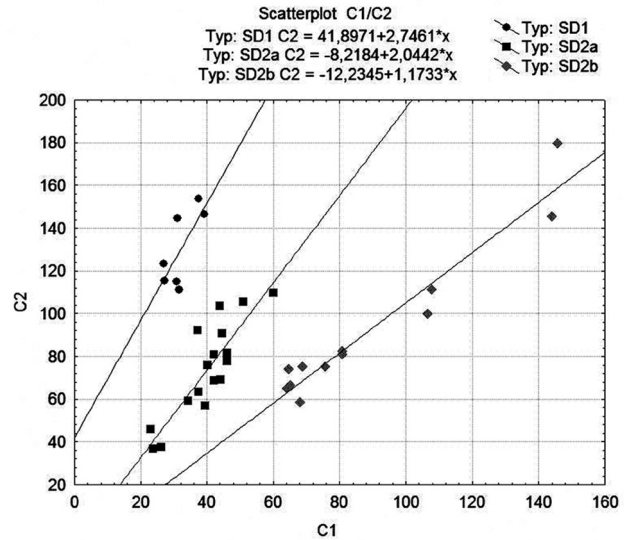


Fig. 5. Scatterplot of C1 (x axis) against C2 (y axis) of different types of secretory ducts (SD1, SD2a, SD2b) of schizogenous origin occurring within the vascular cylinder.

Within *Carlina acaulis*, ducts of a diameter several times that of the surrounding parenchyma cells were observed. The ducts were located in the medullary rays and phloem rays. *C. acaulis* shows ducts of type SD1 and also SD2a, but they may be used to discriminate it from *C. vulgaris*, as type SD1 ducts cannot be found within the roots of the latter (C1:C2 = 0.24–0.4 in *C. acaulis*, 0.48–0.62 in *C. vulgaris*).

Rhaponticum scariosum possesses ducts of type SD2a, circularly arranged and located within the secondary phloem and secondary xylem in a fascicular position (missing in phloem rays and medullary rays). This taxon differs from all other species investigated and provides an example of the importance of the actual position of ducts.

2. Secretory ducts of type SD3 (Fig. 7a,b) arise by lysigenous development and can easily be distinguished from SD1 and SD2. Occurring in *Centaurea scabiosa*, the ducts are located within the phloem in a fascicular position (between the rays). They develop a large interior diameter, and remnants of cells are found in the lumen. They may occupy the whole space between one phloem ray and the next (recognizable in both transverse and longitudinal sections).

3. Secretory structures of type SD4 are not quite ducts but ordinary intercellular spaces filled with various more or less unknown substances secreted by parenchyma cells (Fig. 7c-f, recognizable in longitudinal section). At first sight, however, SD4 may resemble ducts in transverse sections, and for this reason they are included in this paper. Within *Centaurea jacea*, SD4 seems to comprise

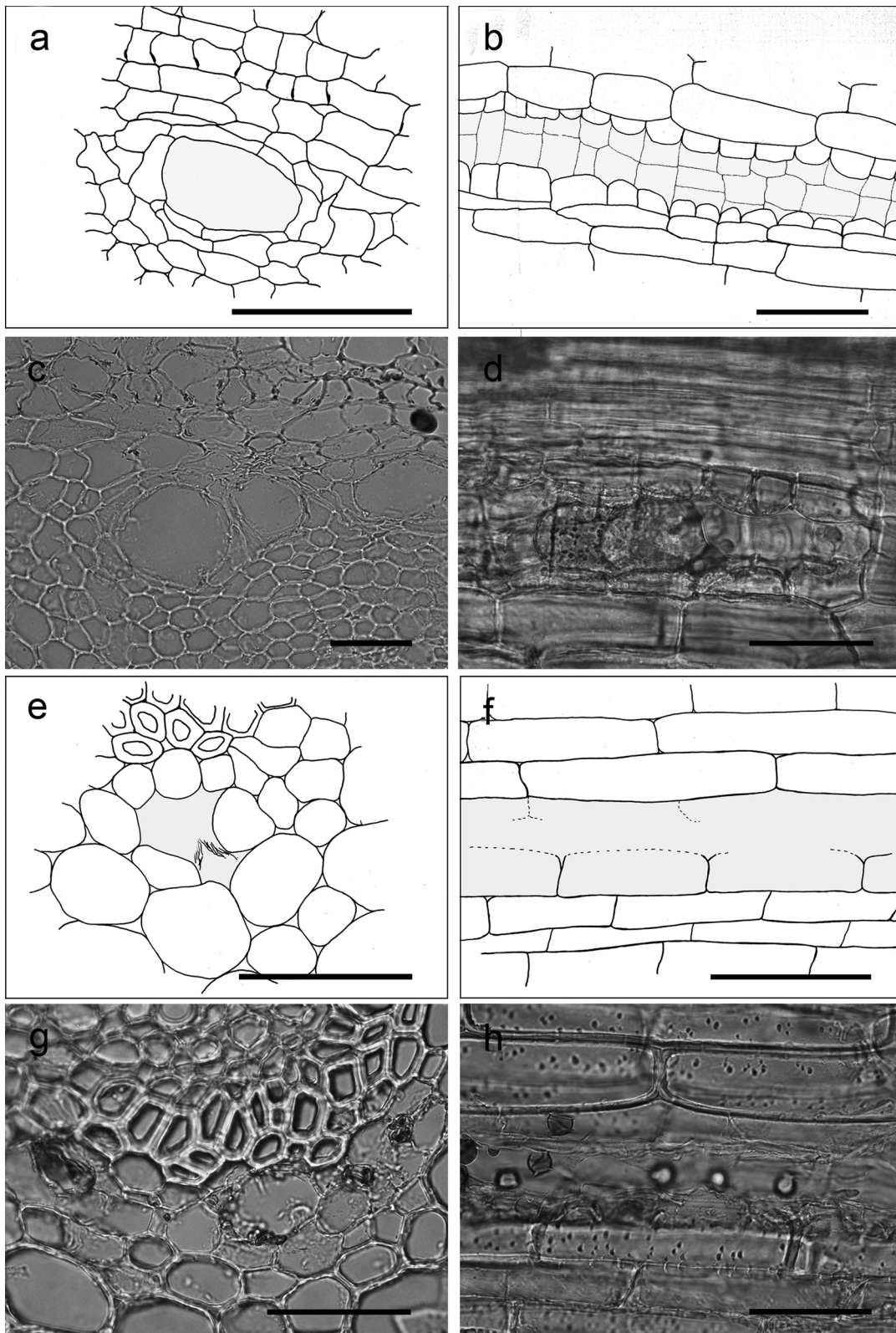


Fig. 6. (a-d) Secretory ducts of type SD1 in *Centaurea montana*, (e-h) Secretory ducts of type SD2b in *Cirsium arvense*. a,c,e,g – transverse sections; b,d,f,h – longitudinal sections. Bar = 50 μm.

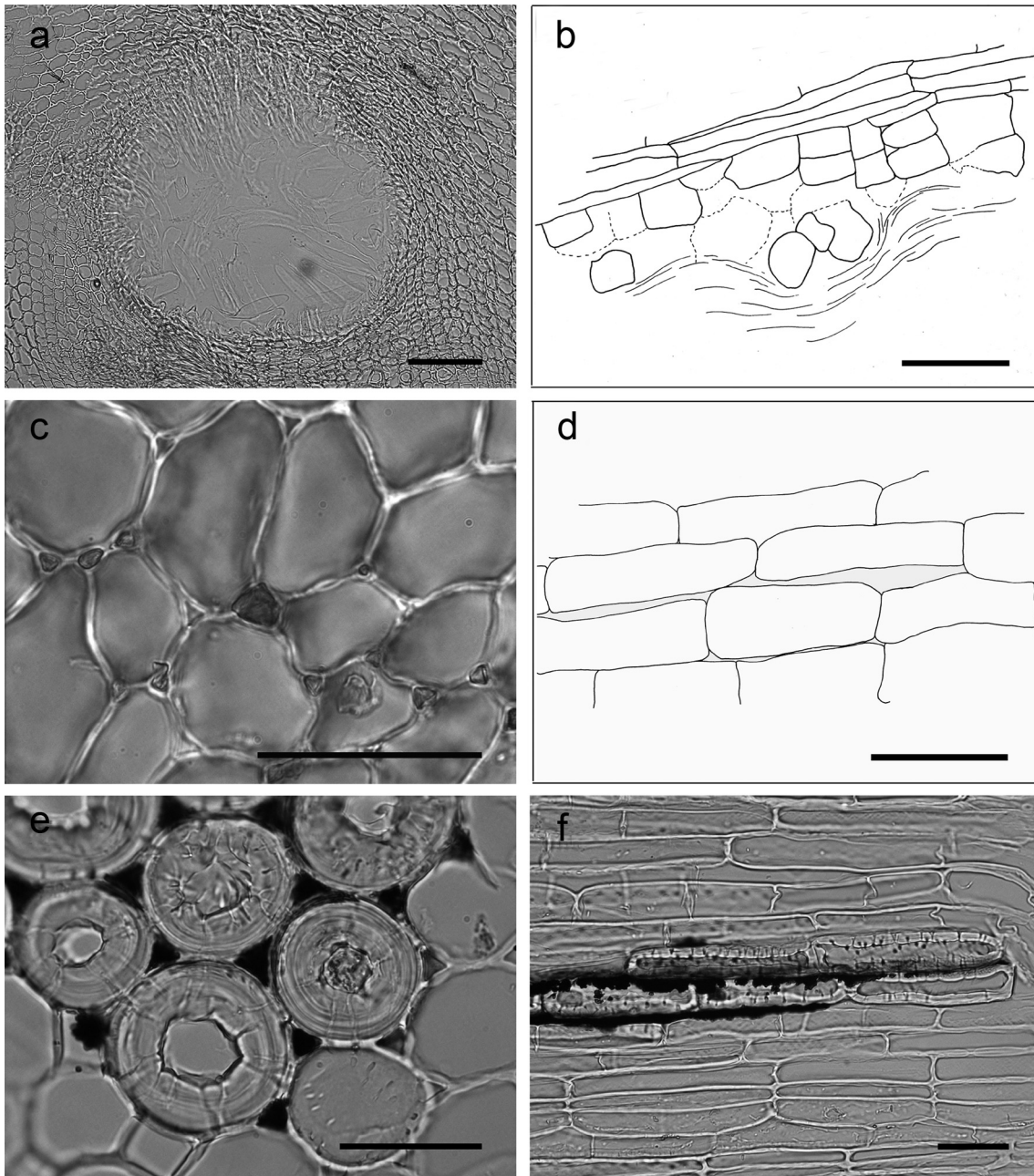


Fig. 7. (a,b) Secretory ducts of type SD3 of *Centaurea scabiosa*, (c-f) Secretory ducts of type SD4 of *Centaurea montana* (c,d) and of *Centaurea jacea* (phytomelanin-coated cells) (e,f). a,c,e – transverse sections; b,d,f – longitudinal sections. Bar = 50 μ m.

phytomelanin-coated cells with numerous pit channels. Similar structures were shown by Upton et al. (2011) in *Parthenium integrifolium* L. and *Echinacea angustifolia* DC. The phytomelanin is always associated with sclereids located in the cortex, secondary phloem and pith (of the root and rhizome).

Type SD4 is also found in any tissue of *Centaurea montana*, though the chemical nature of the substance within the intercellular spaces is

questionable as its light brown color does not correspond to brown-to-black phytomelanin. In this analysis of anatomical structures its appearance is relevant, rather than its chemical composition.

SECRETORY CAVITIES (SC)

Of all the examined taxa, secretory cavities were absent in all species but *Echinops sphaero-*

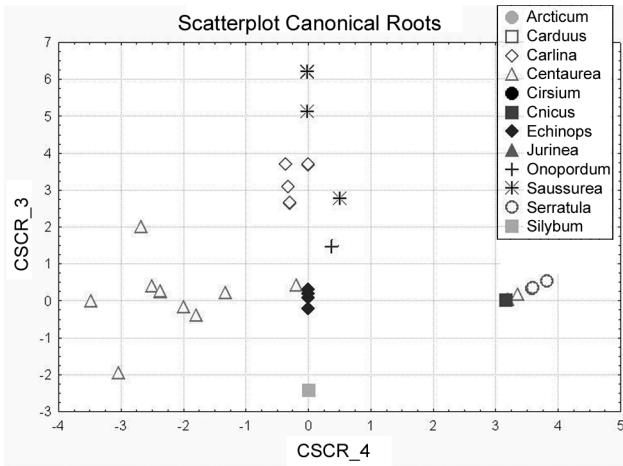


Fig. 8. Scatterplot of discriminant analysis using only features related to secretory structures (23 characters).

cephalus. The cavities were found in the secondary phloem and secondary xylem (missing in the rays).

Table 2 gives an overview of the occurrence of the various types of ducts and cavities observed in the examined species.

STATISTICAL ANALYSIS

Figure 8 is a scatterplot of the results of discriminant analysis of the database (188 investigated samples, 93 features; Fritz, 2011) using only the features related to secretory structures (23 characters): dimension of endoSDs and the number of their endothelial cells (4, >4); the presence of SD1, SD2, SD3, SD4 and secretory cavities, and their location within the root (tissue; fascicular/interfascicular). The separation of the 12 genera is more or less evi-

TABLE 2. Overview of the occurrence of the various types of secretory ducts or cavities within roots and rhizomes of Cardueae species

Species	endoSD	SD1	SD2a	SD2b	SD3	SD4	sec.cav	Position of SD
<i>Echinops sphaerocephalus</i>	+						+	interfasc. in sec. phloem, medullary rays
<i>Carlina acaulis</i>	+ → -	+	+					interfasc. in sec. phloem, medullary rays
<i>Carlina vulgaris</i>	+		+					sec. phloem
<i>Arctium lappa</i>	+ → -							-
<i>Arctium tomentosum</i>	+ → -							-
<i>Saussurea discolor</i>	+ → -			+				sec. phloem
<i>Saussurea pygmaea</i>	+ → -			+				sec. phloem
<i>Jurinea mollis</i>	+							-
<i>Carduus personata</i>	+ → -							-
<i>Carduus defloratus</i>	+ → -							-
<i>Cirsium arvense</i>	+ → -				+			in pith at border with xylem
<i>Cirsium vulgare</i>	+ → -							-
<i>Cirsium erisithales</i>	+ → -							-
<i>Onopordum acanthium</i>	+							-
<i>Silybum marianum</i>	+ → -							-
<i>Serratula tinctoria</i>	+							-
<i>Rhaponticum scariosum</i>	+		+					fasc. in sec. phloem, xylem
<i>Centaurea jacea</i>	+		+			+		SD2: sec. phloem SD4: cortex, sec. phloem, pith
<i>Centaurea scabiosa</i>	+ → -	+	+	+	+			fasc. in sec. phloem
<i>Centaurea montana</i>	+	+				+		SD1: sec. phloem, pith SD4: cortex, phloem, wood
<i>Centaurea cyanus</i>	+			+				fasc. in sec. phloem
<i>Cnicus benedictus</i>	+							-

+ secretory structure present; + → endodermal resin ducts lost in the course of rhytidome formation; sec. cav – secretory cavity; SD – secretory duct

dent. If the calculation is done with all anatomical characters (38) related to the Cardueae, complete discrimination between all examined genera is possible, but among all the features the secretory structures proved very important in this context. A more detailed analysis incorporating all collected data is in preparation.

DISCUSSION

The secretory system, particularly the ducts within the secondary tissues of roots and rhizomes, showed considerable differentiation among the studied Cardueae species. The anatomy and spatial position of secretory canals relative to prominent anatomical elements such as vascular bundles (e.g., adjacent to the vascular bundle as in *Centaurea scabiosa*, or interfascicular as in *Carlina acaulis*) provide valuable characters to discriminate the subterranean parts of species.

Secretory ducts of type SD2 can be classified based on the C1:C2 quotient, as exemplified by the genera *Centaurea* and *Carlina*. This is of special interest in the case of *Carlina*, as the roots of the medicinally important *C. acaulis* may be sufficiently characterized based on SD types. The herbal drugs of *Carlina acaulis* and *Carlina vulgaris* can be differentiated easily from each other and from the other examined species. Future studies covering more species and individuals are needed to verify whether discrimination based on this feature can be justified and extended to other relatives.

The new classification of secretory ducts does not provide characters for discrimination of the medicinally used species *Arctium lappa* and *A. tomentosum*. Because endodermal resin ducts are ubiquitous within the subterranean organs of the Cardueae (van Tieghem, 1883; Col, 1903; Solereder, 1908), and with the endoSDs having no remarkably individual characteristics and the roots possessing no other secretory ducts, the species of this genus cannot be distinguished on that basis.

The type SD4 secretory ducts of *Centaurea jacea* seem to be phytomelanin-coated sclereids similar to those of, for example, *Parthenium integrifolium* L. and *Echinacea angustifolia* DC. (Upton et al., 2011). As phytomelanin is dark brown to black (Pandey and Dhakal, 2001), it is not clear whether it is the same as the light brown substance filling the intercellular spaces of *Centaurea montana*.

Secretory cavities are another type of secretory tissue occurring within the roots of Cardueae, as reported by Col (1904) for *Echinops sphaerocephalus* L. and *Carlina acaulis*. Our studies confirm the presence of SCs in *Echinops*, but SDs are found in the roots of *Carlina*.

The secretory system alone cannot be used to distinguish all the taxa studied, as made evident by our discriminant analysis, but they provide important additional anatomical characters.

In addition to studying the secretory structures of the Cardueae, we examined the subterranean parts of *Cichorium intybus* as a member of the tribe Cichorieae, which is not supposed to carry secretory ducts. Earlier literature (van Tieghem, 1883; Solereder 1904) reported that *C. intybus*, *Lapsana communis* and *Podospermum laciniatum* (Cichorieae) exhibit only doubling of the endodermis without the presence of secretory canals, but we found endodermal resin ducts in *C. intybus* at an early age of the root. Since the doubling of endodermal cells is similar in all three of the species described in that early work, it is reasonable to suggest that resin ducts may occur in young roots of *Lapsana communis* and *Podospermum laciniatum* as well.

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