DISSERTATION

Titel der Dissertation

Comparative anatomical studies of medicinally used drugs of the Asteraceae and their possible adulterations

Verfasserin

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2000 B.C. – Here – eat this root.
1000 A.D. - That root is heathen. Here – say this prayer.
1850 A.D. - That prayer is superstition. Here – drink this potion.
1940 A.D. - That potion is snake oil. Here – swallow this pill.
1985 A.D. - That pill is ineffective. Here – take this antibiotic.
2000 A.D. - That antibiotic is artificial. Here – eat this root.

- *A Short History of Medicine* (author unknown)

in: L. Snyder (ed). 2007. *Biomedical Ethics Reviews: Complementary and Alternative Medicine: Ethics, the Patient, and the Physician.* Humana Press, Towota, NJ.

CONTENTS

ABSTRACT

Apart from chemical methods, light microscopic analyses are of great significance and rank first in the official pharmacopoeia for the identification and purity control of medicinally used drugs. Detailed knowledge of the anatomy of used plant organs, hence, is crucial for a reliable quality control. Although descriptions of the most important medicinally used drugs are available, respective data from related plant taxa are rarely investigated. As a consequence, the anatomical differentiation of pharmaceutically used and inconsiderable plants remained in many cases dubious and it's questionable to which extend differences between the single species / genera exist.

This work concentrates on the subterranean organs of the Asteraceae, a highly diverse plant family of wide geographic distribution (it occurs on all continents except Antarctica) which comprises a large number of medicinal plants. The study aims to widen the knowledge on the root anatomy of the Asteraceae as a basis for the identification and the detection of adulterations by means of light microscopy, one of the basic methods of the pharmacognosy. The roots and rhizomes of 59 species out of 33 genera / 12 subtribes of the tribes Cardueae and Cichorieae, comprising medicinally used taxa such as *Taraxacum*, *Carlina acaulis*, *Cichorium intybus,* and their pharmaceutically insignificant close relatives, were analysed by microscopic means and the anatomical data compared to each other (transverse and longitudinal sections of each sample, databank comprising about 5500 pictures). Intraspecific variation of anatomical features was addressed by examination of several vouchers coming in most cases from different locations. The plant material was mainly collected in Austria, Italy, Germany, Slovakia and Poland. Additionally, various species were cultivated in the experimental garden of the department to study the developmental aspect.

In order to verify if root anatomy supports the current prevalently morphology, karyology and DNA-molecular based taxonomy of the studied tribes, the anatomical features were compared to phylogenies reconstructed from sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (including ITS1, 5.8S and ITS2 obtained from the GenBank). 12 different computations dealing with the DNA and microscopical data (either referring to cluster analyses [Ward, Complete Linkage] or to maximum parsimony) have been performed, revealing similarities and connections between the different calculations and samples. A classification matrix of a discriminant function analysis showed an a priori classification probability of 100% of all subtribes except the Hypochaeridinae (with 96.77 %; with one sample from Prenanthes purpurea being misclassified as member of the Lactucinae).

The discrimination of the tribes Cardueae and Cichorieae based on the studied European species is particularly possible due to the occurrence of secretory ducts (Cardueae) and lactifers (Cichorieae). In contrast to the published literature though, further, the development of endodermal resin ducts within young roots of *Cichorium intybus* L. could be proofed.
Detailed investigations into the structure and spatial position of the secretory ducts provide additional characters for discrimination of single species and genera. The secretory structures can be classified into diverse types based on developmental features, their extension, and form parameters of the cells lining the ducts.
Additional important characters of discriminative power – besides the arrangement and extension of principal tissues within the underground organs – are the presence and spatial position of sclereids, fibers and vessels including the fine structure of wall thickenings and pits. On the contrary, the diameter of the largest vessels has to be considered with care – though often stressed in literature – since this character can vary largely within a single root (e.g. as in *Silybum marianum* and *Hypochaeris radicata*). *Saussurea discolor* and *S. pygmaea* were special among the examined taxa due to the occurrence of interxylary cork.

In summary, the anatomy of the subterranean organs allows a delimitation of the studied medicinally used taxa from their close relatives, even though discriminative characters did not become available for all possible species comparisons. Discrimination of species within a single genus based on anatomical features was thus not possible in some cases (e.g. *Taraxacum, Arctium, Tragopogon*), yet in others it is (e.g. *Scorzonera*).

The enlarged anatomical dataset holds strong implications for the identification of possible adulterations of plant drugs, which, consequently, may be avoided for the sake of safety of herbal drugs.

ZUSAMMENFASSUNG

Für die Identifikation und Reinheitsprüfungen von Arzneidrogen ist neben chemischen Methoden die mikroskopische Analyse von großer Bedeutung und steht auch in den Pharmacopoen an erster Stelle. Voraussetzung für eine zuverlässige Qualitätskontrolle ist somit die detaillierte Kenntnis der anatomischen Strukturen der verwendeten Pflanzenorgane der einzelnen Heilpflanzen. Obwohl Beschreibungen der wichtigsten Arzneidrogen vorliegen, sind deren nahe Verwandte kaum untersucht und es stellt sich die Frage, ob eine Differenzierung zur medizinisch genutzten Stammpflanze möglich ist und inwieweit Unterschiede der einzelnen Arten / Gattungen in ihrer anatomischen Struktur existieren.

Diese Arbeit konzentriert sich auf die unterirdischen Organe der Asteraceae – eine vielfältige Pflanzenfamilie, die nicht zuletzt aufgrund ihrer weiten Verbreitung auf allen Kontinenten (ausgenommen der Antarktis) eine große Anzahl an Heilpflanzen liefert, - und erweitert das Wissen über die Wurzelstockanatomie der Asteraceae, um die Identifizierung bzw. Feststellung von Verfälschungen mithilfe einer der grundlegenden Methoden der Pharmakognosie – der Mikroskopie – zu ermöglichen. Die Wurzeln / Rhizome von 59 Arten aus 33 Gattungen / 12 Subtriben der Tribe Cardueae and Cichorieae, beinhaltend arzneilich genutzte Taxa (beispielsweise *Taraxacum* sp., *Carlina acaulis*, *Cichorium intybus*) und deren nahe Arten bzw. Gattungen, wurden mit mikroskopischen Methoden analysiert und im Sinne der vergleichenden systematischen Pflanzenanatomie einander gegenübergestellt. Um eine Variation der Merkmale innerhalb einer Art zu berücksichtigen, wurden jeweils mehrere Belege, sofern möglich von unterschiedlichen Standorten, untersucht (Quer- und Längsschnitte jedes Beleges, Datenbank beinhaltend etwa 5500 Fotos). Das Pflanzenmaterial stammt großteils aus Eigenaufsammlungen in Österreich, Italien, Deutschland, Slowakei und Polen, jedoch wurden auch Belege des Herbariums des Department of Pharmacognosy, Universität Wien (WUP) miteinbezogen. Zusätzlich wurden diverse Arten im Arzneigarten des Departments angebaut, um die Entwicklung der Pflanzen mitverfolgen zu können.

Um zu sehen, ob die anatomische Wurzelstrukturen die aktuelle Taxonomie, die vorwiegend auf morphologischen, karyologischen und molekularen Daten beruht, unterstützt, wurden die einzelnen neu erhobenen Merkmale mit DNA-Daten (DNA-Sequenzen von der Internal Transcribed Spacer (ITS)-Region [ITS1, 5.8S und ITS2]) aus der GenDatenbank abgeglichen. 12 verschiedenen Berechnungsmethoden (sich beziehend auf Cluster-Analysen [Ward, Complete Linkage] und Maximum Parsimony) wurden mit den DNA- und mikroskopischen Daten durchgeführt, um sie miteinander zu vergleichen und Gemeinsamkeiten und Verbindungen zwischen den einzelnen Berechnungsmethoden bzw. den Arten aufzuzeigen. Die Klassifikationsmatrix einer Diskriminanzanalyse wies eine a priori Klassifikationswahrscheinlichkeit von 100% aller Subtribe bis auf die Hypochaeridinae (96.77 %; ein Sample von *Prenanthes purpurea* wurde fälschlich zu den Lactucinaen gezählt) auf.

Die Unterscheidung der zwei Triben Cardueae and Cichorieae ist bei europäischen Arten durch das Auftreten von Harzgängen (Cardueae) einerseits und Milchröhren (Cichorieae) andererseits leicht möglich. Im Unterschied zur bisherigen Literatur konnte jedoch das Auftreten von endodermalen Harzgängen bei jungen Wurzeln von *Cichorium intybus* L. nachgewiesen werden.
Genauere Untersuchungen zur Struktur und Position der Sekretgänge machen eine weiterführende Unterscheidung zwischen einzelnen Arten / Gattungen möglich. Aufgrund der Entstehung, der Ausdehnung und der Gestalt, der an die Sekretgänge angrenzenden Zellen, können die Sekretgänge / -behälter in verschiedene Typen unterteilt werden.
Als weitere wichtige Differenzierungsmerkmale erwiesen sich neben der allgemeinen Aufteilung der Gewebe u.a. die Präsenz und Lokalisation von Sklereiden, Fasern und Tracheen und deren Wandverstärkungen / Tüpfel. Der größte gemessene Durchmesser von Tracheen hingegen – obwohl in der Literatur vielfach als Identifikationsmerkmal angegeben – muss mit Vorsicht betrachtet werden, da große Unterschiede selbst innerhalb einer einzelnen Wurzel (beispielsweise *Silybum marianum*, *Hypochaeris radicata*) auftreten können.
Als Sonderfall unter den untersuchten Arten zeigten sich *Saussurea discolor* und *S. pygmaea* durch das Auftreten von interxylärem Kork.
Die Anatomie der unterirdischen Organe ermöglicht eine eindeutige Abgrenzung der arzneilich genutzten Pflanzen von ihren nahen Verwandten, wenn auch nicht aller untersuchten Arten voneinander. Innerhalb einer Gattung ist eine weiterführende

Differenzierung anhand anatomischer Kriterien manchmal nicht durchführbar (z. B. *Taraxacum*, *Arctium*, *Tragopogon*), manchmal hingegen durchaus (z. B. *Scorzonera*).

Durch das nun erweiterte Wissen über die Anatomie bislang noch nicht untersuchter Arten, können etwaige Verfälschungen vermieden und dadurch die Sicherheit der Verwendung von Arzneidrogen erhöht werden.

INTRODUCTION

Comprising more than 23 000 species and about 1 600 genera (Jeffrey, 2007), the Asteraceae represent one of the largest families of plants constituting approximately 8% of all eudicot plants with its species distributed all over the world except for Antarctica (Stevens, 2001 onwards), many of which are popular among people since ancient times with a widespread use in society as important sources of for various medicinal and food purposes. *Taraxacum officinale* F.H. Wigg., *Silybum marianum* L. and *Cichorium intybus* L. are just a few examples out of the existing diversity with a long tradition in both conventional and traditional medicine all over the world (Wichtl, 2009, Kelly, 2008).

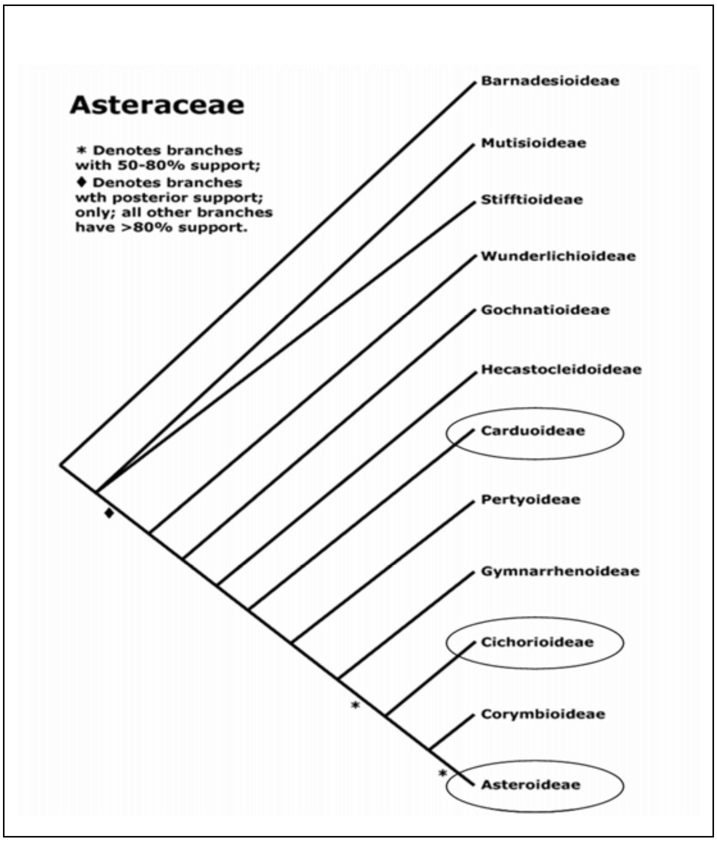

FIGURE 1: The 12 major lineages of the Asteraceae (Stevens, 2001 onwards)

The plant family contain 12 major lineages with three of them (Asteroideae, Cichorioideae, Carduoideae) occurring in Western Europe (Panero and Funk, 2008; Stevens, 2001 onwards) (fig. 1). This work concentrates on species growing naturally in Central Europe of the two tribes Cardueae (subtribe Carduoideae) and Cichorieae (subtribe Cichorioideae).

The tribe Cardueae (= Cynareae [Fischer *et al.*]) encompasses over 2,360 species organized in 73 genera (Susanna and Garcia, 2007), accounting for more than 90% of the species diversity of the Carduoideae (Panero and Funk, 2008). The taxonomy of the tribe containing some of the largest genera of the Asteraceae and due to its high morphological diversity is complicated.

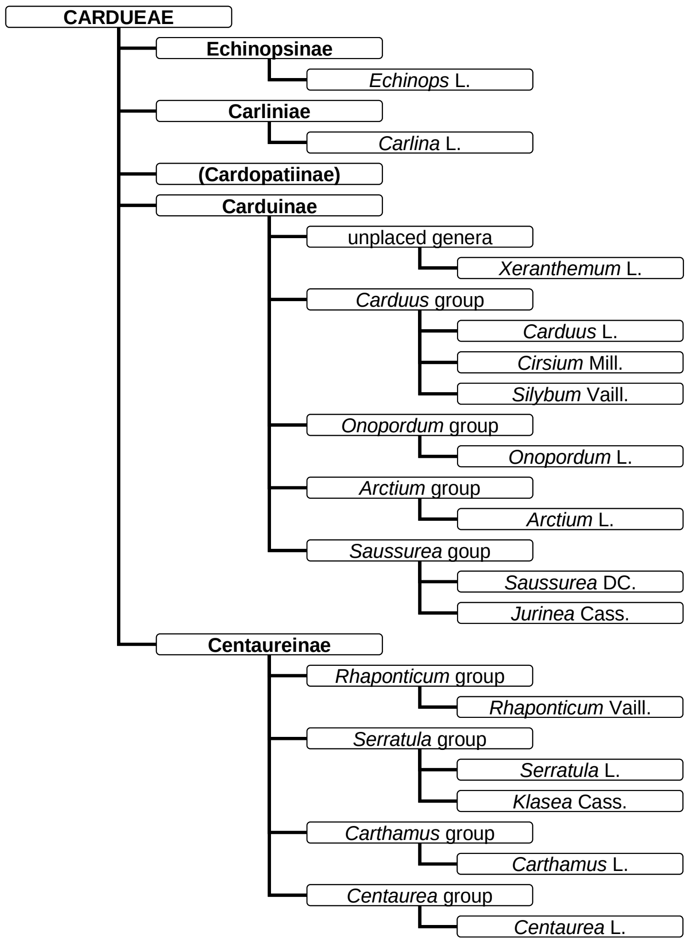

FIGURE 2: Division of the tribe Cardueae as suggested by Susanna and Garcia, 2007; only taxa occurring in Austria are presented

Traditionally, the Cardueae were subdivided into the four subtribes Echinopsinae, Carlininae, Carduinae and Centaureinae (Hoffmann, 1890-94). This tribal classification was subject of various discussions in the past, e.g. with the subtribe Echinopsinae being raised to the level of a separate tribe by Wagenitz, (1976). Recently, Susanna and Garcia (2007) modified the old classification of the Cardueae – the four subtribes mentioned above were joined by the fifth subtribe Cardopatiinae.

The tribe Cichorieae Lam. & DC. (1806) (= Lactuceae Cass. [1819]) comprises about 93 genera with the number of species differing widely due to different views on their circumscription (particularly genera *Hieracium*, *Pilosella* and *Taraxacum* with large numbers of hybridogenous and / or apomictic species) (Funk et al, 2009). Excluding these three genera the tribe encompasses approximately 1500 species (Lack, 2007).

Much work has been done recently regarding the Cichorieae, according to which the tribe is now divided into eleven subtribes: Warioniinae, Scorzonerinae, Scolyminae, Lactucinae, Hyoseridinae, Crepidinae, Hypochaeridinae, Chondrillinae, Hieraciinae, Microseridinae, Cichoriinae – in comparison to the classification of Bremer (1994) and Lack (2007) two new subtribes were recognized (Warioniinae and Chondrillinae) with diverse subtribes not maintaining (Funk et al, 2009).

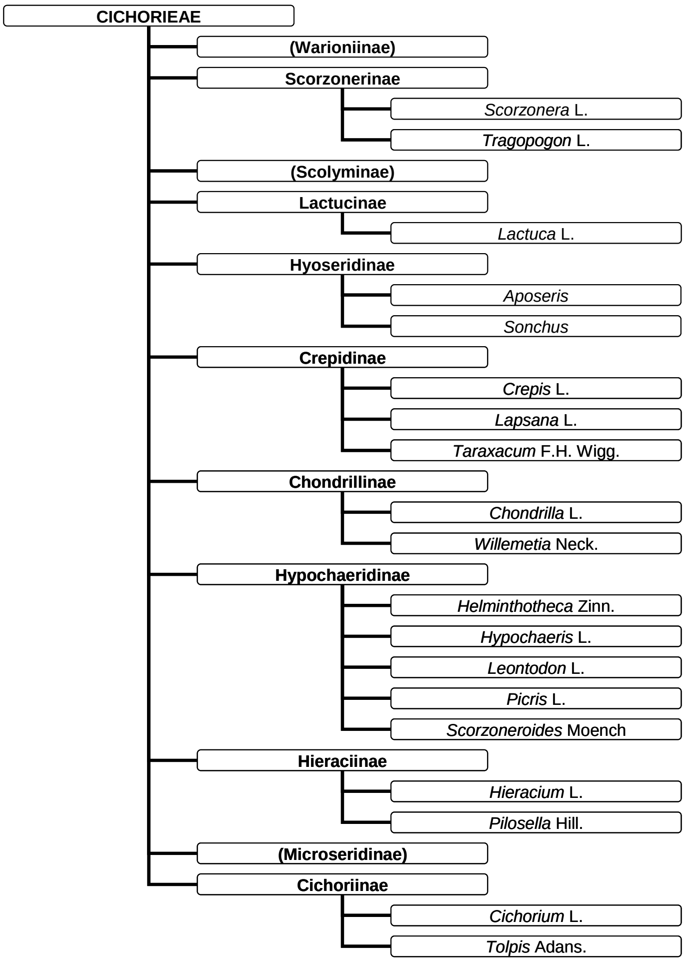

FIGURE 3: Division of the tribe Cichorieae as suggested by (Funk, Susanna, Stuessy & Bayer, 2009; only taxa examined in this study are presented

The use of plants in medicine is as old as mankind. Both in conventional and traditional medicine, the diverse plant organs are used in varying formulations, usually originating from the cutted or powdered drug. Besides chemical analytical techniques, eg chromatographical fingerprints, the identification of drugs is mainly done by microscopic means as anatomical structures of plants are widely unchangeable and provide reliable features for required purity and quality control and thus, are required in the official pharmacopoeas (e.g. OEAB, 2009). Furthermore, light microscopy is a cheap method and can be easily performed given that reliable literature is available (Hohmann *et al.*, 2001). Detailed knowledge on the anatomy of the diverse organs of both medicinally used plants and related but inconsiderable species as their possible adulterations is required.

Yet, detailed descriptions regarding the anatomy of subterranean organs of pharmaceutically exploited Asteraceae species and their close relatives are mainly missing. The main work on this subject dates back to the second half of the 19th century and deals with the secretory structures of the family, concentrating in particular on the taxa of the two tribes Cardueae (resin ducts) and Cichorieae (laticiferous vessels) (Col, 1903; Col, 1904; Van Tieghem, 1883; Van Tieghem, 1884; Van Vuillemin, 1884).

Secretory structures are common anatomical features throughout the plant kingdom and may occur in various plant organs. Whereas articulated anastomosing laticifers (e.g. well known of *Taraxacum* sp.) are typical for the Cichorioideae, for the tribe Cardueae – among European species – endodermal resin ducts within their subterranean organs are characteristic (Van Tieghem, 1883; Col, 1903; Solereder, 1908). They result from a doubling of the endodermal cells, thus forming intercellular spaces which may widen further by the divisions of the surrounding cells (schizolysigenous development) (Van Tieghem, 1883; Van Vuillemin, 1884; Van Tieghem, 1884). Yet not belonging to the tribe Cardueae but instead Cichorieae, in literature, the doubling of the endodermis is also described for the roots of *Cichorium intybus* L., *Lapsana communis* L. and *Podospermum laciniatum* (L.) DC. – though without formation of ducts (Van Tieghem, 1883; Solereder, 1908).
Besides endodermal resin ducts, secretory ducts, cavities or resin cells may develop within cortex, pith and secondary tissues as reported for instance for *Carlina acaulis* L. and *Centaurea atropurpurea* Waldst. & Kit. (ducts in secondary xylem) (Col, 1903) or for *Echinops exaltatus* Schrad. (resin cells in secondary phloem) (Solereder, 1908).

However, though the secretory structures on aerial vegetative organs of Asteraceae species – especially of leaves and shoots and the transition between root and shoot – have been intensively studied (Col, 1903; Col, 1904), respective comparative studies on subterranean organs are rare. Some literature deal with the resin ducts and cavities of single species (amongst others: Ragonese, 1988; Melo-de-Pinna and Menezes, 2002; Hayashi and Appezzato-da-Gloria, 2007). Melo-de-Pinna (2002) listed about 50 species of the Asteraceae studied for the position of secretory ducts in the roots, Appezzato-da-Gloria *et al.* (2008) and Cury and Appezzato-da-Glória (2009) investigated 13 additional taxa. Nevertheless, species occurring in Western Europe and interesting with respect to their use in medicine are not covered and their anatomy is still insufficiently described as the existing studies were not targeted at a pharmaceutically useful discrimination between the taxa.
Information concerning the anatomy of the Cichorieae is even rarer. A single comparative study (Länger, 1990) deals with the roots of the medicinally used taxa *Taraxacum officinale* and their differentiation to some related species (*Leontodon* sp., *Aposeris foetida* and *Hypochaeris* sp.). Important thereby showed to be the presence or lack of libriform fibers and

bordered vessels, the diameter of the vessels and the arrangement of laticifers. However, it seems questionable if these anatomical features can persist in a wider range of species.

Thus, as a broader knowledge of the root anatomy of the Asteraceae would be useful to pharmaceutical practise for the identification of drugs and required purity tests, within this research the subterranean organs of 59 species out of 33 genera of the Cardueae and Cichorieae have been examined (appendix I: list of all examined species with collection history; appendix II: anatomical descriptions of all investigated taxa) and a database of anatomical characters has been created. Intraspecific variation was carefully considered in order to identify reliable taxonomically informative characters. Based on these results, characters useful for the discrimination of the analysed species were extracted aiming to clarify if a distinct differentiation between medicinally used plants is possible and if the purity of a declared drug can be guaranteed by means of light microscopy. The taxa involve pharmaceutically useful plants as well as inconsiderable relatives, all naturally occurring in Austria.

In addition, the anatomical features were connected with molecular data available of GenBank (DNA sequence data from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, including ITS1, 5.8S and ITS2; GenBank accession numbers listed in appendix I, alignment in appendix III) to show (1) if there is a connex between microscopy of the subterranean organs and the phylogeny, and (2) if some characters are phylogenetically informative and valuable for inferring the phylogenetic relationships among the taxa studied. So far, there is not much information concerning the correlation between phylogeny and microscopy. For taxonomy just a few micro-morphological characters such as length of trichomes, trichome shapes, pollen anatomy or stomata indices / stomata length are used. A recent study deals with the root anatomy of Cranichideae (Orchidaceae) and phylogeny and could show some anatomical features to be phylogenetically informative (e.g. tilosome distribution, lamellate type, velamen cell-wall thickenings) (Figueroa *et al.*, 2008). This is the first study in this context regarding the Asteraceae.

CONCLUSION

In the scope of this study, 59 species out of 33 genera / 12 subtribes of the two tribes Cardueae and Cichorieae have been examined by means of light and fluorescence microscopy. A databank of the resulting anatomical features was created and statistically analysed.
All taxa can be allocated to the subtribes accordingly to the currently valid phylogeny (Funk et al., 2009) except for *Prenanthes purpurea*. Interestingly, Funk et al. (2009) indicate *Prenanthes* L. as questionable inclusion to the subtribe Hypochaeridinae Less. instead of the Lactucinae Dumort. This study emphasizes the affiliation of Prenanthes to the Lactucinae Dumort. on behalf of the anatomical features (paper V).

In the course of the work, the occurrence of interxylary cork could be observed within the two taxa *Saussurea discolor* DC. and *S. pygmaea* Spreng. Both species develop a periderm within the secondary xylem which connects locally with cortical periderm. Separating the originally solid xylem cylinder, it results into a splitting of the root into various strands. So far, this anomalous phenomenon has not been described for a species of the genus Saussurea (paper I).

The discrimination between the tribes Cardueae and Cichorieae (regarding taxa growing naturally in Austria), is particularly possible due to the occurrence of secretory ducts (Cardueae) and lactifers (Cichorieae), although it could be shown that, in contrast to published literature, in *Cichorium intybus*, which is said to just develop a doubling of the endodermis without the development of ducts (Van Tieghem, 1883; Solereder, 1908), endodermal resin ducts may occur within young roots. Detailed examinations of the structure and spatial location of the secretory ducts of the Cardueae provided new features for a differentiation between the species (paper II). Especially the ducts within the secondary tissues of the subterranean organs are of great relevance. According to their anatomical structure, the secretory structures can be classified into diverse types:

- Secretory duct types **SD1** and **SD2:**

According to the quotient of **C1:C2** [longitudinal section: length of the cells surrounding the lumen of the secretory ducts (C1) divided by the length of adjacent cells (C2)], the ducts are divided into two categories:

SD1: average quotient **C1:C2** of < 0.3

SD2: average quotient **C1:C2** > 0.4. A further division with one group of ducts of an average quotient of > 0.8 (type **SD2b)** and a second group with a quotient of 0.4 to 0.7 (type **SD2a**) has yet to be confirmed by additional studies.

- Secretory ducts of type **SD3**: lysigenous development, large inner diameters;
- Secretory ducts of type **SD4**: Actually, SD4 are not ducts in the common sense but intercellular spaces filled with more or less unknown substances secreted by the parenchyma cells. As not the chemical composition but the visual nature is crucial for this anatomical work, SD4 has been included in this context. In the case of *Centaurea jacea*, it's likely to be a matter of phytomelanin-coated sclereids.
- Secretory cavities

With the help of this classification, the separation of the 12 investigated genera of the Cardueae is more or less evident but not possible in all cases. However, the secretory structures showed to be very important in this context, although additional anatomical features are necessary for a complete discrimination between all examined genera.

Important characters of discriminative value - besides the arrangement and extension of principal tissues within the underground organs and besides the diverse secretory structures discussed above - are the tissue-dependent occurrence of sclereids and fibers and the tracheids and their fine structure of wall thickenings and pits. Moreover, the ratio of the length and width of the vessel elements can be considered as useful. In contrast, the diameter of the largest vessels which is an often used parameter for quality control of medicinal drugs, may vary largely even within one single root (e.g. *Silybum marianum*: 245 µm near the tip of the root to 113 µm 1.5 cm below the hypocotyle), and can only be referred to if the exact position along the root axis and the developmental stage of the analysed root sample is defined. Furthermore, a possible influence of varying preparation methods on the dimensions has to be kept in mind.

Comprising all collected data, the identification of most investigated taxa is possible, though some species are indistinguishable from others. For instance, the genera *Taraxacum*, *Arctium* and *Tragopogon* can be clearly discriminated from the other genera examined. A further differentiation within the single genera, however, is thus not possible. On the contrary though, members of other genera can be further discriminated by using diverse anatomical features like e.g. the formation of the phellem (*Scorzonera*) or the tissue-dependant occurrence and type of secretory ducts (*Carlina*) (paper III, IV).

The collected data widen the knowledge of the anatomy of so far not investigated taxa that are important for the identification of medicinally used herbs and purity controls in the pharmaceutical industry. Adulterations may be avoided and the safety of herbal drugs increased.

REFERENCES

APPEZZATO-DA-GLORIA B, HAYASHI AH, CURY G, SOARES MKM, ROCHA R. 2008. Occurrence of secretory structures in underground systems of seven Asteraceae species. *Botanical Journal of the Linnean Society* 157: 789-796.

BREMER K. 1994. *Asteraceae. Cladistics and classification*. Timber Press, Portland, Oregon.

COL MA. 1903. Recherches sur l'appareil sécréteur interne des composées. *Journal de Botanique* 17: 252-318.

COL MA. 1904. Recherches sur l'appareil sécréteur interne des composées (Suite). *Journal de Botanique* 18: 110-174.

CURY G, APPEZZATO-DA-GLÓRIA B. 2009. Internal secretory spaces in thickened underground systems of Asteraceae species. *Australian Journal of Botany*, 57, 229–239.

FIGUEROA C, SALAZAR A, ZAVALETA HA, ENGLEMAN EM. 2008. Root Character Evolution and Systematics in Cranichidinae, Prescottinae and SPiranthinae (Orchidaceae, Cranichideae. *Annals of Botany* 101: 509-520.

FISCHER MA, OSWALD K, ADLER W. 2008. *Exkursionsflora für Österreich, Liechtenstein und Südtirol.* 3rd ed. Austria: OÖ Landesmuseen, Land Oberösterreich.

FUNK, SUSANNA, STUESSY, BAYER. 2009. *Systematics, Evolution, and Biogeography of the Compositae*. International Association for Plant Taxonomy, Washington.

HAYASHI HA, APPEZZATO-DA-GLORIA B. 2007. Anatomy of the underground system in *Vernonia grandiflora* Less. and *V. brevifolia* Less. (Asteraceae). *Brazilian Archives of Biology and Technology* 50: 979-988.

HOHMANN B, REHER G, STAHL-BISKUP E. 2001. *Mikroskopische Drogenmonographien der deutschsprachigen Arzneibücher*. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

HOFFMANN O. 1890-94: Compositae. – 87-387 in Engler A & Prantl K, eds. *Die natürlichen Pflanzenfamilien 4* (5). Leipzig.

JEFFREY C. 2007. Compositae. In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg , 61-77.

KELLY G. 2008. Inulin-Type Prebiotics – A Review: Part 1. *Alternative Medicine Review*, 13, 4: 315-329.

LACK HW. 2007. Tribe Cichorieae Lam. & DC. In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg, 180-199.

LÄNGER R. 1990. Orientierende Untersuchungen zur Wurzelanatomie einiger Cichorieaceen. *Scientia Pharmaceutica*, 58, 417-422.

MELO-DE-PINNA G, MENEZES N. 2002. Vegetative organ anatomy of *Ianthopappus corymbosus* Roque and Hind (Asteraceae-Mutisieae). *Revista Brasileira de Botânica* 25: 505-514.

PANERO JL, FUNK VA. 2008. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. *Molecular Phylogenetics and Evolution*. 47: 757–782.

RAGONESE AM. 1988. Canales secretores en los organos vegetativos de *Eupatorium inulaefolium* H.B.K. (Compositae). *Acta Farmaceutica Bonaerense* 7: 161-168.

SOLEREDER H. 1908. *Systematische Anatomie der Dicotyledonen*. Verlag von Ferdinand Enke, Stuttgart.

STEVENS. 2001 onwards. *Angiosperm Phylogeny Website*. Version 9, June 2008 (and more or less continuously updated since).

SUSANNA A, GARCIA-JACAS N. 2007. Tribe Cardueae Cass. (1819). In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg, 123-146.

VAN TIEGHEM M. 1883. Sur la situation de l'appareil sécréteur dans les composées. *Bulletin de la Société Botanique de France* 30: 310-313.

VAN TIEGHEM M. 1884. Sur la situation de l'appareil sécréteur dans la racine des composées. *Bulletin de la Société Botanique de France* 31: 112-116.

VAN VUILLEMIN M. 1884. Remarques sur la situation de l'appareil sécréteur des composées. *Bulletin de la Société Botanique de France* 31: 108-110.

WAGENITZ G. 1976. Systematics and phylogeny of the Compositae (Asteraceae). *Plant Systematics and Evolution* 125: 29-46.

WICHTL MAX. 2009. *Teedrogen und Phytopharmaka: ein Handbuch für die Praxis auf wissenschaftlicher Grundlage*. 5th ed., Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

LIST OF ORIGINAL PAPERS

ELISABETH FRITZ, JOHANNES SAUKEL (published). Interxylary cork of *Saussurea discolor* DC. and *S. pygmaea* DC. (Asteraceae). Biologia, Vol. 66, 3, 2011.
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Own contribution: 90%

ELISABETH FRITZ, JOHANNES SAUKEL (to be submitted). Phylogeny and root anatomy of various plants of the Cardueae and Cichorieae.
Own contribution: 50%

LIST OF MEETING CONTRIBUTIONS

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Interxylary cork of *Saussurea discolor* and *S. pygmaea* (Asteraceae)

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Abstract: The root anatomy of the subalpine to alpine plant species *Saussurea discolor* (Willd.) DC., and *Saussurea pygmaea* (Jacq.) Spreng., (Asteraceae) has been investigated by means of light and fluorescence microscopy on specimens of Austrian provenance. Both species develop a so called interxylary cork which mediates the splitting of the root into various strands. This phenomenon takes place in the secondary xylem and involves the development of a periderm separating the originally solid xylem cylinder. Interxylary cork is currently known from approximately 40 species of the Dicotyledones. This is the first report of this specific anatomical structure from the two studied species.

Key words: Asteraceae; *Saussurea*; interxylary cork; root anatomy

Introduction

The Asteraceae is a wide-spread, taxonomically highly diverse plant family (Jeffrey 2007) which comprises also numerous taxa used in medicine (Blaschek et al. 2007). Knowledge about the anatomy of roots and rhizomes of medicinally used species and their adulterations in the Asteraceae is still limited although it is important for the identification and purity of herbal drugs.

The genus *Saussurea* belongs to the *Carduinae*, a subtribe of the taxonomically extremely diverse *Cardueae* (Susanna & Garcia 2007). *Saussurea* DC. comprises about 300 species mainly distributed in the temperate regions of Europe and particularly Asia with more than two-thirds of the species occurring on the Qinghai-Tibet Plateau (Wang & Liu 2004). Species of the genus grow up to the altitudinal limits of plant life (e.g. *S. triactyla* 5,800 m a.s.l.). In the Alps, three representatives of the genus can be found: *S. pygmaea* (sect. *Caulescentes* Hooker) and *S. discolor* and *S. alpina* (both sect. *Corymbiferae* Hooker; Gander-Thimm 1963).

In scope of a systematic project (Fritz & Saukel unpubl.) on the anatomy of underground parts, various species from the tribes *Cardueae* and *Cichorieae*, which comprise several taxa of pharmaceutical value, have been analysed microscopically to create a database of their anatomical features. These activities included the investigation of two subalpine to alpine European *Saussurea* species, *Saussurea discolor* and *S. pygmaea*, the root anatomy of which has not been reported so far.

An unusual anatomical differentiation taking place in the secondary root could be observed. Here we present the primary and secondary root anatomy of these species analysed from Austrian accessions and discuss the obtained results based on the literature record.

Material and methods

The plant material was collected in Austria in Tuffbad (Lesachtal, Carinthia): *S. discolor* (Willd.) DC., July 2009; and on the Schneealpe (limestone Alps, Styria, at the border to Lower Austria): *S. pygmaea* (Jacq.) Spreng., September 2009. Vouchers are deposited in the herbarium of the Department of Pharmacognosy, University of Vienna (WUP).

The anatomical preparations (seven specimens per accession) were examined by means of light and fluorescence microscopy. The desiccated roots were boiled in water for 10 minutes to soften the tissue and subsequently embedded in 96% ethanol for dehydration. After evaporating the alcohol for a few minutes, transverse and longitudinal sections were obtained by free hand sectioning. Roots were cut by standard about 1cm below the hypocotyle as this region provided the most information about the root anatomy. The obtained sections were embedded in few drops chloral hydrate (60% in water) prior to light microscopic examination.

Additionally, in order to get insights into the ontogenesis of the roots, fruits of *S. pygmaea* collected in September 2009 from the mount Schneealpe at about 1,760 a.s.l. were grown in the greenhouse. Seeds germinated following vernalisation (3 weeks at $-20\,^{\circ}C$, 2 weeks at $4\,^{\circ}C$) and treatment with aqueous solution of gibberellic acid (Fluka – BioChemika) (24 hours, 1 ppm). Like the desiccated root samples, transverse and longitudinal sections were obtained by free hand sectioning from closely below the hypocotyle, but using fresh roots embedded in 96% alcohol for a few minutes.

A Nikon Optiphot–2 microscope was used for the light microscopy and a Nikon Eclipse E 600 fluorescence microscope with UV Ex filter 330–380 for fluorescence microscopy. Both microscopes were equipped with a Samsung Digimax V50 Digital Camera.

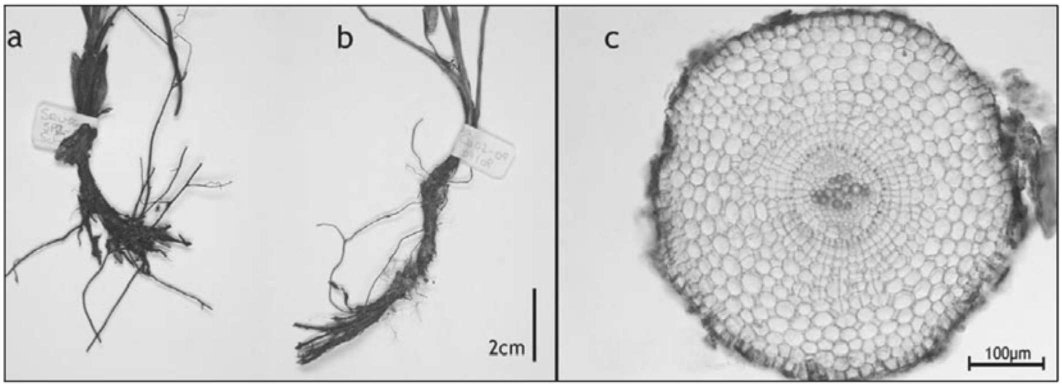

Fig. 1. Root of *Saussurea pygmaea* (a) and *Saussurea discolor* (b); c – overview showing typical structure of a primary root. In the cortex, two layers can be distinguished: the inner cortex with cortical cells arranged in orderly radial rows with a concentric layering and the less orderly appearing outer cortex (seedlings of *S. pygmaea*).

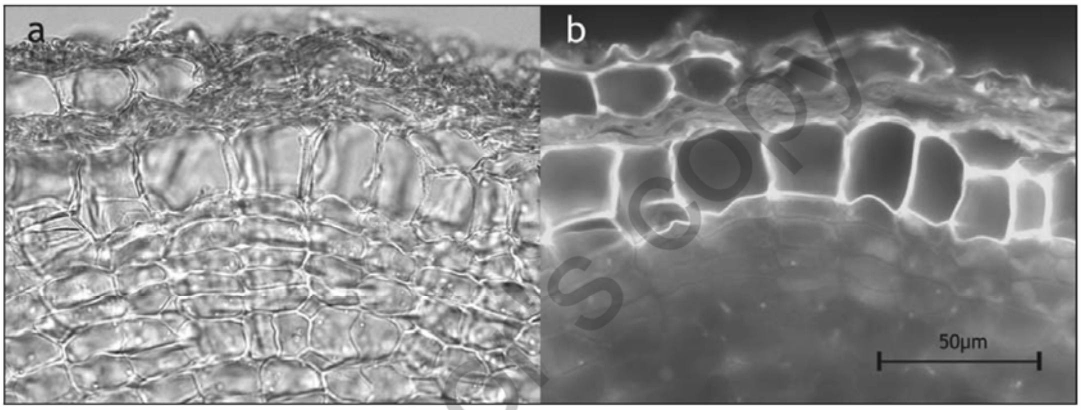

Fig. 2. *Saussurea pygmaea*. Formation of rhytidome: bright field (a), fluorescence microscopy under UV 330–380; blue fluorescence of periderm (b), (plant at about the four-leaf stage).

Results

At the first glance, the rootstock of *Saussurea pygmaea* and *S. discolor* looked quite ordinary (Figs 1a,b). A transverse section though revealed a surprising structure which is not commonly known. To clarify this structure during the process of its ontogenetic development, *S. pygmaea* was cultivated. As the ontogenetic study was only carried out with this species, the observed development can only be reported from this taxon but the similarity of the root anatomy of both *S. discolor* and *S. pygmaea* let presume a similar ontogenetic and the results in the following apply to both species.

In the transverse section of the seedling, the anatomy of a typical primary root can be observed: a brown rhizodermis is followed by the exodermis – the term being used to designate the outermost layer of cortical cells (Evert 2006) –, which encloses a broad cortex. The cortex can be separated into an inner cortex with cortical cells arranged in orderly radial rows and concentric layers, and an outer cortex of less orderly tissue structure. The endodermis, as the innermost layer of the cortex, showing the Casparian stripe in its anticlinal walls followed by a multiseriate pericycle composed of thin-walled parenchyma, surrounds the vascular cylinder (Fig. 1c). Endodermal resin ducts can be observed.

Starting at about the four-leaf stage of plants, a repeating production of periderms starts first within the cortex, later on within the secondary phloem. The outer tissues become isolated and consequently cease functioning resulting in a root dominated by secondary xylem in extension. Secretory ducts may occur in the secondary phloem.

In an older stage, the anatomy of the main root becomes anomalous. An additional periderm develops in the secondary xylem. The periderm shows light blue fluorescence, which eases the study of its development (Fig. 2). Across the medullary rays, the interxylary periderm connects with the cortical periderm (Fig. 3). Thereby, the thus surrounded tissues get isolated from the rest of the root but keep functioning. The repeated production of interxylary periderm results in the splitting of the root into several separated strands, each comprising of secondary phloem and secondary xylem surrounded by numerous periderms forming a rhytidome (Fig. 3f). The central part of the xylem gets isolated too, forming a separate strand composing of

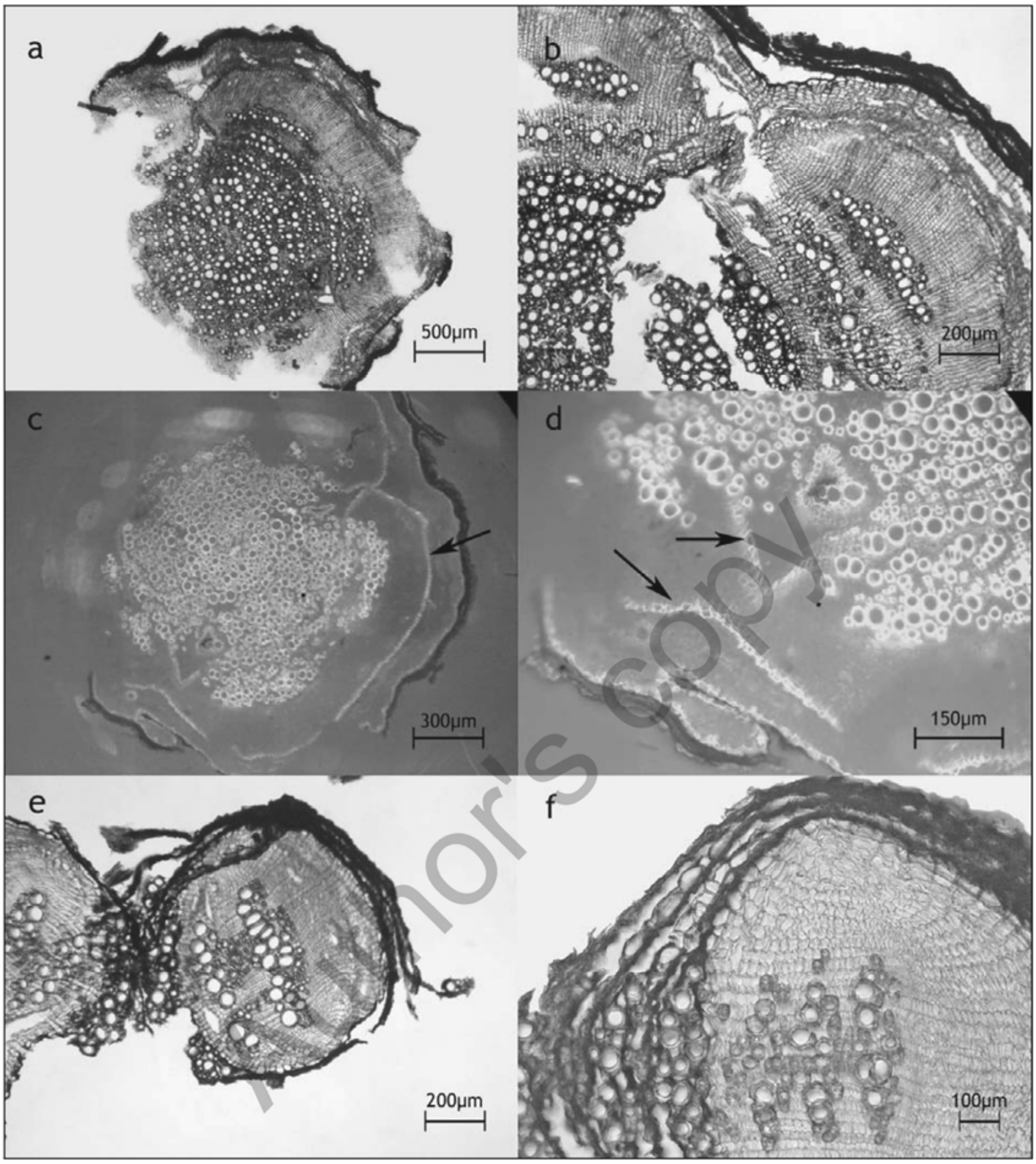

Fig. 3. *Saussurea discolor.* a–c – overview showing arrangement of tissues of an older root: Due to formation of rhytidome, the cortex is already missing. The production of interxylary periderm and the ablation of single strands of the root has started; the interxylary periderm connects with the cortical periderm; bright field (a,b), fluorescence microscopy under UV 330–380 nm: blue fluorescence of periderm (c); d – beginning of connection between interxylary and cortical periderm across medullary ray under fluorescence UV330–380 nm; e, f – ablation of single strands almost completed, each comprising secondary phloem and secondary xylem surrounded by numerous periderms forming a rhytidome (bright field); c, d: Black arrows show the periderm.

vessels and (in the case of *S. discolor*) fibers. This part of the root, finally, ceases functioning and gets eliminated.

The lateral roots show the formation of a rhytidome, but without interxylary phloem and the splitting of the root (Fig. 4).

Discussion

In contrast to other *Saussurea* species from which information of their root anatomy was available such as *S. costus* DC., a species used in traditional Chinese medicine, (Blaschek et al. 2007) the two species examined in this study exhibited a splitting of the root into several separated units mediated by a interxylary periderm.

Searching further corresponding books (e.g. Evert 2006) and their references, literature dating back to the middle of the last century concerning similar phenomena within other genera could be found. Like-

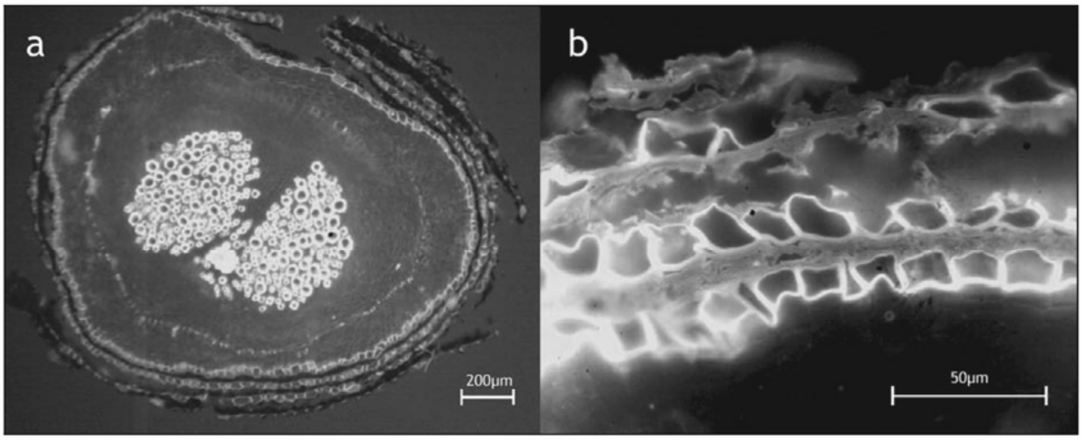

Fig. 4. *Saussurea discolour.* a – overview of lateral root showing the formation of outer bark, no production of interxylary cork; b – rhytidome of lateral root (blue fluorescence of periderm under UV 330–380).

wise root development has been described for about 40 species of the Dicotyledones within the genera *Gentiana*, *Aconitum*, *Corydalis*, *Salvia*, *Sedum* (Jost 1890) and *Artemisia* (Moss & Gorham 1953).

So far, little is known about the reasons for the observed fission of roots. Jost (1890) postulates a presumably correlation between the formation of interxylary periderm and the death of annual parts of e.g. *Gentiana cruciata*. The parts of roots / rhizomes which affiliate directly to dead parts of the plants, get eliminated by the process of the generation of interxylary peridem. Moss & Gorham (1953) mention the importance of the phenomenon as protection against desiccation and for the adaptation of plants to their habitats, mainly dry and wind-blown soil. The splitting of a plant helps it surviving under difficult environmental circumstances, as each single strand may face another microhabitat with its advantages and disadvantages (Ginzburg 1963). In the case of *Saussurea* sp., both supposed causes seem reasonable. Mechanical improvement may be another reason, as the separation of the main root into smaller strands will make it more flexible compared to a massif solid body. This principle is well known from many lianas. The holding on of the plants to rocky grounds and crevices may be one reason for the splitting of the roots as well as forces in wind blown soil as already mentioned by Moss & Gorham (1953).

Further studies are needed to clarify the taxonomical significance of the described anatomical structures and developments for the genus *Saussurea*. Although interxylary periderm is regarded as anomalous phenomenon, it has to be assumed that, due to the limited number of studies, it may occur more often than expected.

References

Evert R. 2006. Esau's Plant Anatomy, 3^{rd} edition, John Wiley & Sons, New Jersey, 601 pp.

Gander-Thimm I. 1963. Zur Verbreitung der Gattung Saussurea DC. in den Ostalpen. Berichte des naturwissenschaftlichen-medizinischen Vereins in Innsbruck **53:** 77–88.

Ginzburg C. 1963. Some anatomic features of splitting of desert shrubs. Phytomorphology **13:** 92–97.

Blaschek W., Ebel S., Hackenthal E., Holzgrabe U., Keller K., Reichling J. & Schulz V. (eds) 2007. Hagers Enzyklopädie der Arzneistoffe und Drogen, 14, Rut-Suf, 6. neu bearbeitete und ergänzte Auflage, Wissenschaftliche Verlagsgesellschaft mbH Stuttgart.

Jeffrey C. 2007. Compositae, pp. 61–87. In: Kubitzki K. (ed.), The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots, Springer Verlag, Berlin Heidelberg.

Jost L. 1890. Die Zerklüftungen einiger Rhizome und Wurzeln. Botanische Zeitung **48:** 434–445, 453–462, 469–480, 485–494, 501–512.

Moss E.H. & Gorham A.L. 1953. Interxylary cork and fissions of stems and roots. Phytomorphology **3:** 285–294.

Susanna A. & Garcia-Jacas N. 2007. Tribe Cardueae Cass. (1819), pp. 123–146. In: Kubitzki K. (ed.), The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots, Springer Verlag, Berlin Heidelberg.

Wang Y.J. & Liu J.Q. 2004. Phylogenetic analyses of *Saussurea* sect. Pseudoeriocoryne Asteraceae Cardueae based on chloroplast DNA trnL_F sequences. Biochem. System. Biol. **32:** 1009–1023.

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Secretory structures of subterranean organs of some species of the Cardueae and their value for discrimination

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Running title: Secretory structures of the Cardueae

Key words: root anatomy, microscopy, asteraceae, secretory ducts

ABSTRACT
Secretory ducts and cavities of the roots and rhizomes are typical features of the tribe Cardueae from the Asteraceae family. Within this study, the anatomy of the subterranean organs of 21 species out of 13 genera of the Cardueae was analysed by means of light microscopy. Particular attention was spent to the secretory system, which was interpreted in a taxonomic context. Secretory ducts were of particular variable anatomy. A new measurement, the quotient of C1 [length of the epithelial cells (longitudinal section)] and C2 [length of adjacent cells (longitudinal section)], was established. Different types of ducts are described based on the type of their development and the size ratios among epithelial cells. Detailed anatomical descriptions of the defined ducts together with their taxonomic occurrence are provided. The combined presence of the various secretory ducts within single species and their spatial position relative to other prominent anatomical features provided valuable characters to discriminate among the studied Cardueae species. These analyses are of particular interest to the identification of herbal drugs, as light microscopy, besides chemical analytical techniques, e.g. chromatographical fingerprints, is a common method for purity controls and thus required in the official pharmacopoeas (e.g. OEAB, 2009).

INTRODUCTION

The Asteraceae are among the largest families of flowering plants comprising more than 23 000 species and about 1 600 genera (Jeffrey, 2007). Numerous species such as *Taraxacum officinale* (L.) Weber, *Cichorium intybus* L.*, Carlina acaulis* L. and *Silybum marianum* L., to mention only a few, are well known for their use in both traditional and western medicine (Wichtl, 2009). The tribe Cardueae thereby comprises over 2 360 species organized in 73 genera (Susanna and Garcia-Jacas, 2007), thus holding some of the largest genera of the Asteraceae.

The Asteraceae – and therefore 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) – so called induced or traumatic ducts or cavities – or are formed without external stimulus. Ducts are usually orientated parallel to the longitudinal axis

of the organ whereas cavities possess an irregular lumen of no prevalent extension (Nair, 1995; Col, 1904).
In literature, three developmental types are distinguished (Nair, 1995, Evert, 2006):

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

Characteristic for the Cardueae – among European species – are their 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 the endodermal cells thus forming an intercellular space (schizolysigenous development). In shoot axis or leaves the endodermal resin ducts may be surrounded by special cells smaller in size compared to the endodermal cells. In roots, in contrast, the ducts emerge directly from the endodermis without such specialised surrounding cells (Van Tieghem, 1883; Van Vuillemin, 1884; Van Tieghem, 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, the doubling of the endodermis, though without formation of ducts, was also found in the tribe Cichorieae within the subterranean parts of *Cichorium intybus* L., *Lapsana communis* L. and *Podospermum laciniatum* (L.) DC. although within that tribe, endodermal resin ducts don't exist (Van Tieghem, 1883; Solereder, 1908).

Additionally to the mentioned endodermal resin ducts, various species of the Cardueae may develop schizogenous secretory ducts within the pith and the cortex. Moreover, ducts can 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 (review article of Pickard, 2008). Most literature deals with the development of the 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 etc.). Concerning the Cardueae, the former literature mainly deals with the occurrence of secretory ducts or cavities on aerial organs, especially of leaves and shoots and the transition between root and shoot (Col, 1903; Col, 1904). Details on the structure of the ducts and cavities of the subterranean parts have been neglected and there exist only a few published studies regarding this subject (amongst others: Ragonese, 1988; Melo-de-Pinna and Menezes, 2002; Hayashi and Appezzato-da-Gloria, 2007) which deal with single species. Comparative studies are largely missing. 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.

The limited knowledge on the root anatomy of the Asteraceae is particularly troublesome to pharmaceutical research and practise, as the identification of root drugs and required purity tests, apart from chemical analytical techniques, strongly rely on accurate discriminative anatomical characters. In scope of a study (Fritz and Saukel, unpubl) we aim to clarify, if

pharmaceutically used Asteraceae species can be anatomically discriminated by means of light microscopy and thereby the purity of a declared drug guaranteed. 56 species out of thirty-three genera of the Cardueae and Cichorieae have been examined and a database of anatomical characters created. In the present work the focus is laid on the anatomy of the secretory structures only, with the intention to get additional features for discrimination between single species and thus, possible herbal drugs. The work concentrates on the formerly and currently medicinally used species *Carlina acaulis* L., *C. vulgaris* L., *Arctium lappa* L. and *A. tomentosum* Mill. (among others: Saric-Kundalic et al., 2010, Berger, 1960, Gerlach et al., 2006) and their relatives comprising diverse taxa of all subtribes of the Cardueae (Echinopsidinae, Carlininae, Carduineae, Centaureineae [Susanna & Garcia-Jacas, 2009]) occurring in Austria (country of investigation) according to Fischer et al. (2008).
In addition, though not belonging to the Cardueae but instead to the tribe Cichorieae, the roots of *Cichorium intybus* L. as a representative for a species exhibiting the doubling of the endodermal cells but with secretory canals missing (see above), have been examined.

MATERIAL AND METHODS

Plant Material

The plant material comprised 21 Cardueae species naturally occurring in Austria (table 1, taxonomy follows Fischer et al., 2008). One to four accessions were studied per single species and the anatomy of three to six specimens per accession analysed. Taxa were chosen respectively to their medicinal use and their role as possible adulterations. Fully development 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 (Fischer, Oswald, Adler, 2008; Pawłowskiego and Jasiewicza, 1972; Szafer et al., 1976; Lauber and Wagner, 2007).

TABLE 1. List of species examined (plants arranged accordingly 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 kept in the herbarium WUP;

Genus	Species	Collection sites
Echinops	*E. sphaerocephalus* L.	Austria, Vienna, E.F. -, Lower Austria, Tullnerbach, E.F. -, -, Karnabrunn, C.D. -, Tyrol, Fließ, V.K.
Carlina	*C. acaulis* L.	-, Vienna, E.F. Germany, Baden-Württemberg, Schwäbische Alb, Herbarium of Hohenack, Nr. 652
	C. vulgaris L.	Poland, Gutkowo, Olsztyn, E.F.
Arctium	*A. lappa* L.	Austria, Vienna, E.F.
	A. tomentosum Mill.	-, Lower Austria, Traiskirchen, E.F.

Saussurea	*S. discolor* (Willd.) DC.	-, Carinthia, Lesachtal, J.S.
	S. pygmaea (Jacq.) Spr.	-, Styria, Schneealpe, E.F.
Jurinea	*J. mollis* Rchb.	-, Vienna, E.F. -, Burgenland, Winden, J.S.
Carduus	*C. personata* (L.) Jacq.	-, Styria, Schneealpe, E.F.
	C. defloratus L. (*C. crassifolius* Willd.)	-, Lower Austria, Gippel, C.D. -, -, Araburg castle, E.F.
Cirsium	*C. arvense* (L.) Scop.	-, Vienna, E.F. Slovakia, Modra, S.F. USA, New York, Peekskill, Planta America Septentrionalis, Le Roy
	C. vulgare (Savi) Ten.	Poland, Gutkowo, Olsztyn, E.F.
	C. erisithales (Jacq.) Scop.	Austria, Styria, Schneealpe, E.F.
Onopordum	*O. acanthium* L.	-, Lower Austria, Buchberg, W.L. Italy, Southern Tyrol, Vinschgau, C.D.
Silybum	*S. marianum* (L.) Gaertn.	Austria, Lower Austria, Buchberg, W.L. Slovakia, Botanical Garden of Bratislava, S.F.
Serratula	*S. tinctoria* L.	Austria, Vienna, J.S.
Rhaponticum	*R. scariosum* Lam.	Liechtenstein, Saminatal, G.S.
Centaurea	*C. jacea* L.	Austria, Karnabrunn, C.D. -, Vienna, E.F.
	C. scabiosa L.	-, Vienna, J.S. Poland, Gutkowo, Olsztyn, E.F. Switzerland, Graubünden, Lavin, C.D.
	C. cyanus L. (= *Cyanus segetum* Hill., Fischer et al., 2008)	Austria, Vienna, E.F. Germany, Baden-Württemberg, Kronau, C.D. Poland, Mazury, Zabie, E.F.
	C. montana L. (= *Cyanus montanus* Hill., Fischer et al., 2008)	Austria, Lower Austria, Unterberg, E.F. Slovakia, Modra, S.F.
Cnicus	*C. benedictus* L.	Austria, Vienna, Botanical Garden of the Department of Pharmacognosy, University of Vienna
Cichorium (tribe Cichorieae)	*C. intybus* 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

Anatomical analysis

The samples were examined by means of light microscopy. For preparation, the following variation of a traditional method of the University of Vienna was used: The desiccated roots were boiled in water for 10 minutes to soften the tissue and subsequently submersed in 96% ethanol for dehydration. After evaporating the alcohol for a few minutes, transverse and longitudinal sections were obtained by free hand sectioning about 1.5 cm below the hypocotyle as this position proofed to provide the best information about the root anatomy. Sections were embedded in few drops of chloral hydrate (60% in water) and examined using a Nikon Optiphot–2 light microscope equipped with a Samsung Digimax V50 Digital Camera.

The following statistic measure was calculated: length of the cells (longitudinal section) surrounding the lumen of the secretory ducts (C1) divided by the length of adjacent cells (C2) (fig. 1). Calculations were done using the software package Statistica®.

Additionally, seeds of *Cichorium intybus* L. were cultivated in the garden to observe one species with a doubling of the endodermis without formation of endodermal resin ducts as cited in literature (Van Tieghem, 1883). First sections were made at the appearance of the first rosette of leafs and repeated after 3 weeks.

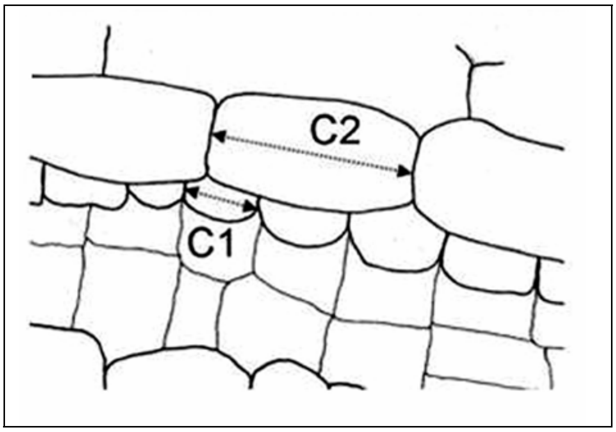

Fig. 1: Secretory duct showing an inner and outer layer of epithel cells which conspicuously differ in length. The measures C1 and C2 were used to calculate a quotient, describing the proportion.

RESULTS

Within the number of species successfully analysed, 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 the endodermal resin ducts are ubiquitous features, secretory ducts were observed in a subset of the species only and showed additional variation among taxa by differentiation into five anatomical types. These types could further be found in combination or alone thus that single species were characterised 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)

Due to the fact that all species of the tribe Cardueae contain **endoSD**s within their roots and rhizomes (Van Tieghem, 1883; Col, 1903; Solereder, 1908), usually, this kind of duct was considered to be of no taxonomic value. Still, some distinction between single species can be made based on the **endoSD**s. Particularly, the size of the duct in comparison to the cells of the surrounding cortex (transverse section) turned out to be important. *Jurinea mollis* Rchb. may serve as an example for endodermal resin ducts of a diameter multiple of the size of the surrounding parenchyma cells (fig. 2A,B). As *Jurinea mollis* possesses an enduring cortex, the **endoSD**s provide a permanent feature for the recognition of this species. Likewise, *Carlina acaulis* develops **endoSD**s with an outstanding large diameter. Since the cortex gets lost in course of rhytidome formation, the value as differentiation character of the **endoSD**s for this species is of less importance. However, the secretory ducts of other tissues than the cortex alternatively can be taken into account.

Other species examined in this study, which can be characterised to some extent by the **endoSD**s, are *Onopordum acanthium* L., *Centaurea jacea* L., *Centaurea cyanus* L., and *Serratula tinctoria* L.. While these species could not be discriminated based on the diameter of the duct, the number of the surrounding cells forming the lumen (transverse section) may be decisive. Thus, in fully developed roots, the number of cells lining the ducts of *C. cyanus* is usually up to 10 at maximum, up to 6-7 in *C. jacea*, and up to 6 in *Onopordum acanthium*. Within *Serratula tinctoria*, **endoSD**s with a small lumen with usually only four surrounding cells (fig. 2C, D) do occur, as this species possesses a fibrous root system which roots don't develop secondary growth.

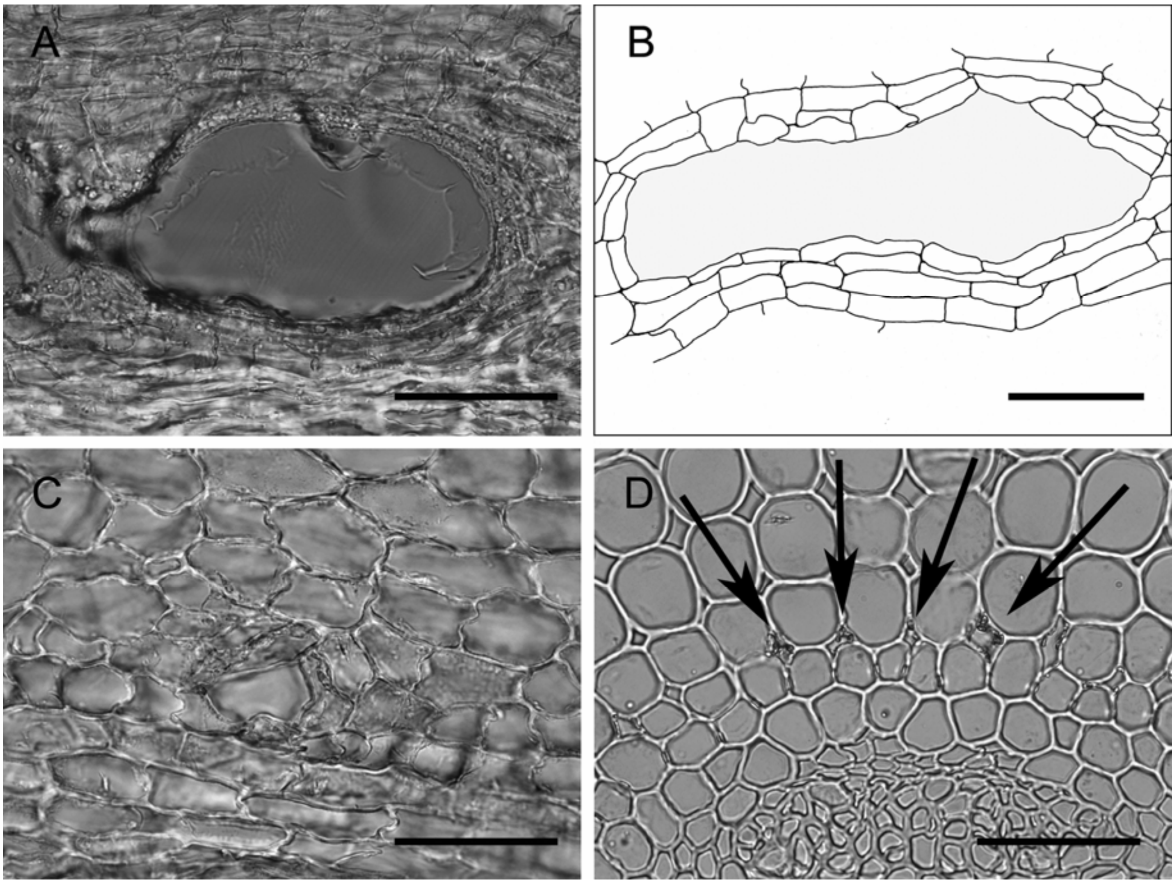

Fig. 2: Endodermal resin ducts of **(A,B)** *Jurinea mollis*, **(C)** *Onopordum acanthium*, **(D)** *Serratula tinctoria*
A-D: transverse sections; Bar = 50µm

Regarding the doubling of the endodermis without formation of secretory structures, interestingly, our studies document the formation of endodermal resin ducts within the roots of *Cichorium intybus* at an early state of growth which vanish with the beginning of secondary growth. Secretory canals could be observed when the plant gets their first rosette of leaves (fig. 3A, B)

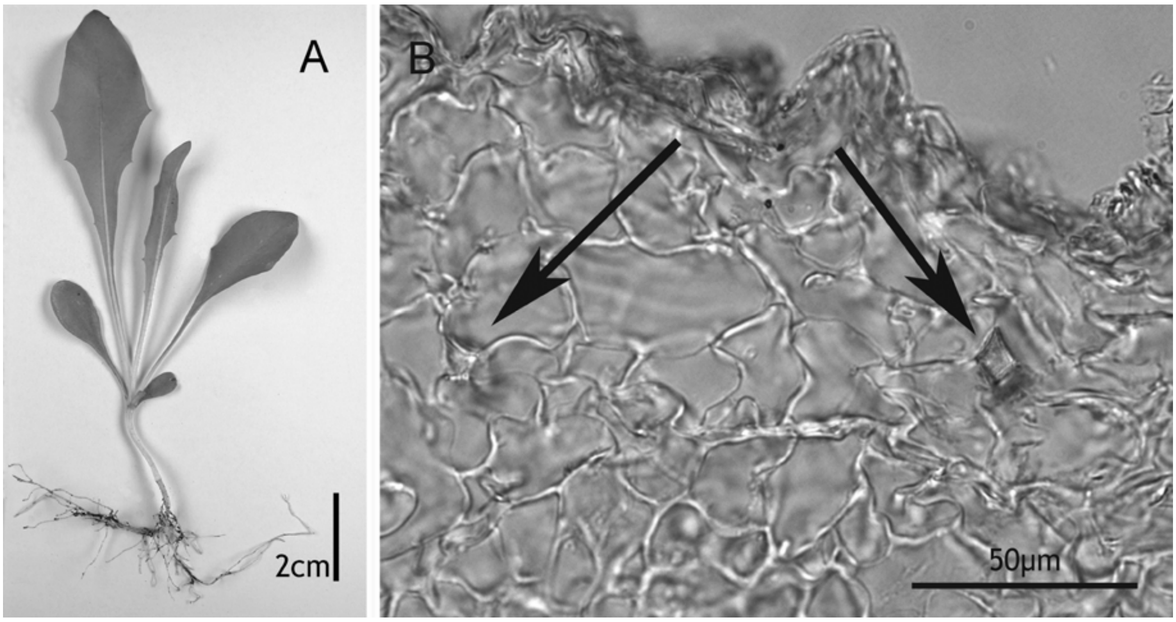

Fig. 3: (A) *Cichorium intybus,* cultivated in the garden: habitus, first rosette of leafs developed, **(B)** Resin ducts observed in the root of the plant shown in (A)
The black arrows show the secretory ducts. B: transverse section;

Other Secretory Ducts (SD)

Many species of the Cardueae don't possess **SD**s but **endoSD**s (e.g. *Arctium lappa* L., *Cirsium erisithales* (Jacq.) Scop., *Onopordum acanthium*, etc.). Others, though, can develop further ducts or cavities within the cortex, the pith or secondary tissues. Here we provide a new classification of the **SD**s of the Cardueae based on anatomical features (fig. 4):

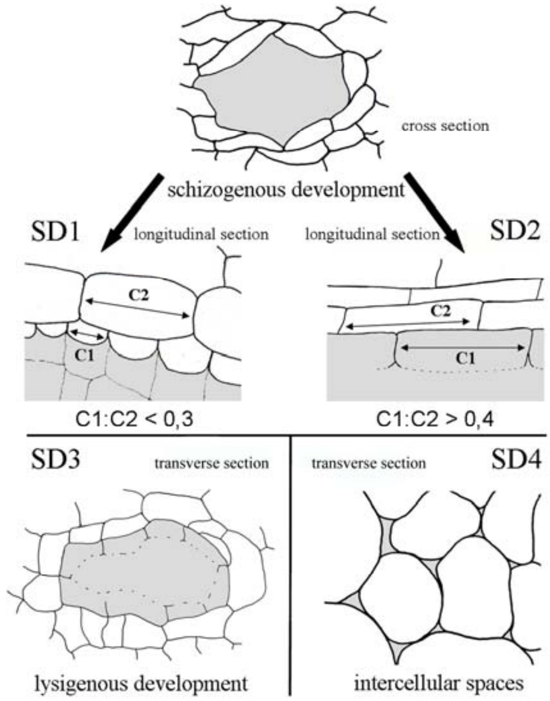

Fig. 4: Overview of the 4 types of secretory ducts occurring in the vascular cylinder

1. Secretory duct types **SD1** and **SD2**:

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

- 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 quotient **C1:C2** of <0.3 (secretory ducts of type **SD1**).
- Within the second category (secretory ducts of type **SD2**), the quotient **C1:C2** exceeds 0.4. Our results further suggest a division of this second category by the **C1:C2**-quotient with one group of ducts of an average quotient of > 0.8 (e.g., *Saussurea* sp. (0.88-1.07), ducts of the rhizome of *Cirsium arvense* (L.) Scop. (0.81-1.17) located at the border

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), *Rhaponticum scariosum* Lam. (0.49-0.7)), type **SD2a.** For demonstrating that these data are of actual value, a scatterplot is added (fig. 5) which shows that the formation of the diverse types of ducts is likely to be based on different legalities (Saukel, 1984). Additional studies have to show if the differentiation within the second category can be justified.

Three species examined in this study possess ducts of type **SD1**.
Centaurea montana L. develops secretory ducts of type **SD1** (0.24-0.27) which appear to possess a colourless content and always can be 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 fascicularly positioned between the phloem rays forming a triangular pattern around secretory ducts of type SD3 (see below).

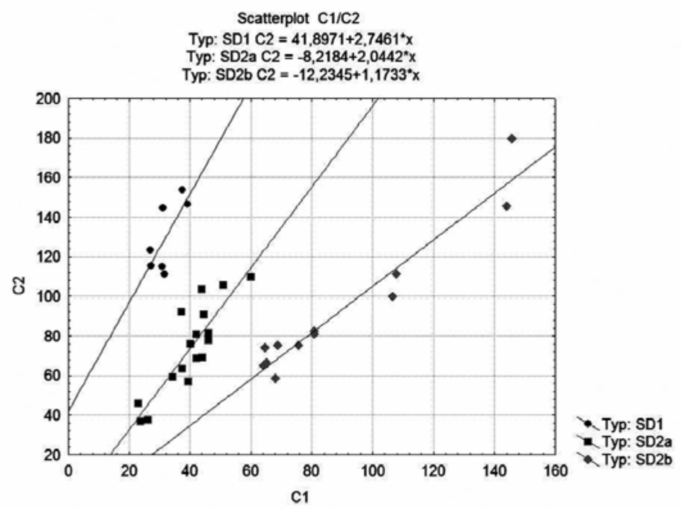

Fig. 5: Scatterplot C1 (x-axis) against C2 (y-axis) of different types of secretory ducts (SD1, SD2a, SD2b) with schizogenous origin occurring within the vascular cylinder

In contrast to *C. montana* and *C. scabiosa*, *Centaurea cyanus* and *C. jacea* don't possess ducts of type **SD1** but **SD2** (*C. jacea* additionally SD4 – 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*).

Within *Carlina acaulis,* ducts of a multiple of the diameter of the surrounding parenchyma cells were observed. The ducts were located in the medullary rays and in the phloem rays. Although *C. acaulis* shows ducts not only of type **SD1** but of **SD2a** as well, they may serve to discriminate against *C. vulgaris* as within the roots of this species ducts of type **SD1** cannot be found (**C1:C2**-ratio of *C. acaulis*: 0.24-0.4:, *C. vulgaris*: 0.48-0.62).

Rhaponticum scariosum possesses ducts of type **SD2a** circularly arranged located in fascicular position in the secondary phloem and the secondary xylem. This taxon differs from all other species investigated and provides an example which underpins the importance of the actual position of ducts.

2. Secretory ducts of type **SD3** (fig. 7A, B) have a lysigenous development and can easily be distinguished from **SD1** and **SD2**. Occurring in *Centaurea scabiosa*, the ducts are located in fascicular position within the phloem between the rays, they develop large inner diameters with remnants of the cells being found in the lumen. They may occupy the whole space from one phloem ray to the next (recognizable in both transverse and longitudinal section).

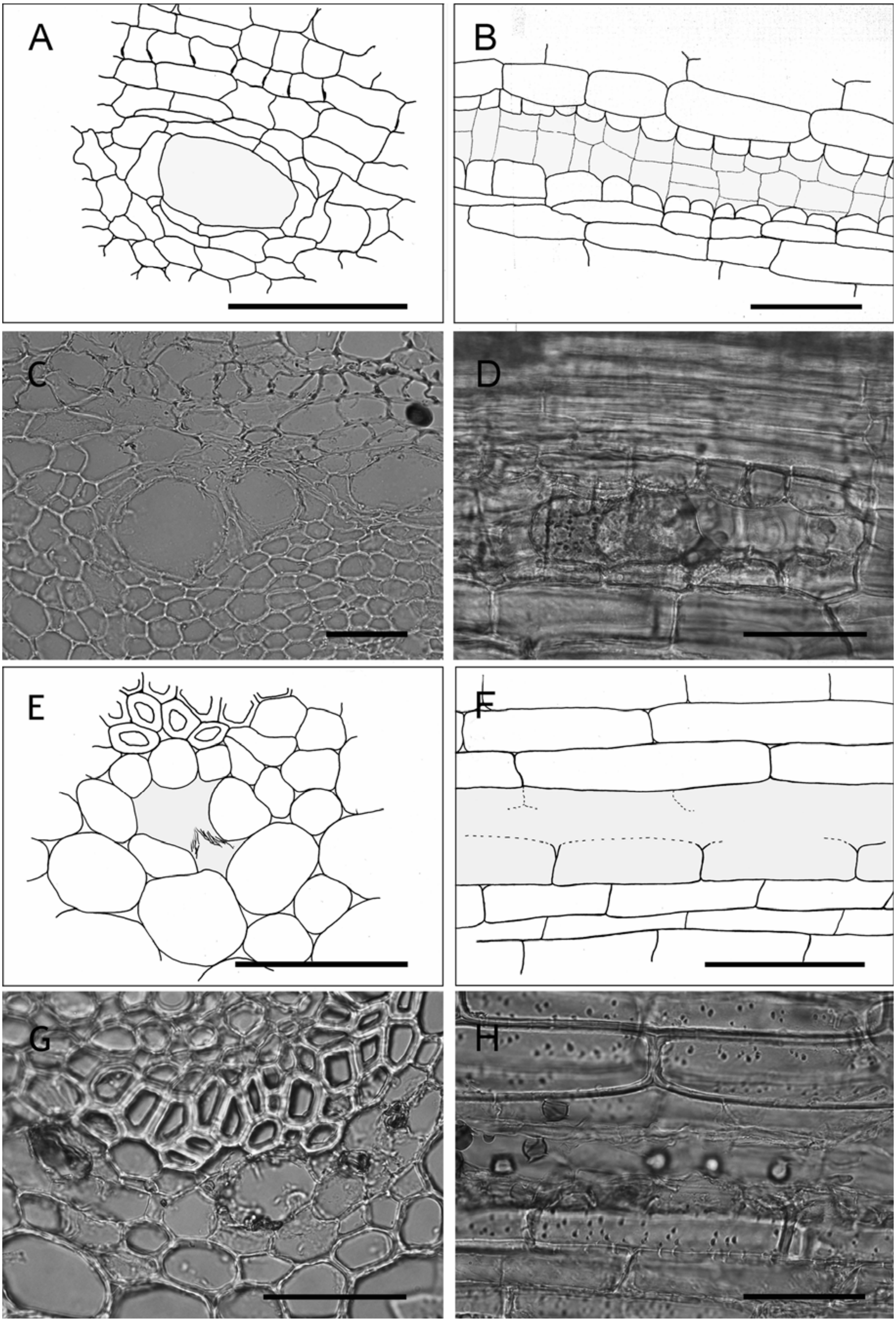

Fig. 6: (A-D) Secretory ducts of type SD1 in *Centaurea montana,* **(E-H)** Secretory ducts of type SD2b in *Cirsium arvense*;
A,C,E,G: transverse sections, B,D,F,H: longitudinal sections; Bar = 50µm

3. Secretory structures of type **SD4** are not quite ducts but ordinary intercellular spaces, filled by various, more or less unkown substances secreted by the parenchyma cells (fig. 7C-F, recognizable in longitudinal section). At the first sight though, in transverse section, **SD4** may appear like ducts and for this reason, they are included in this paper. Within *Centaurea jacea*, SD4 seems to be a matter of phytomelanin-coated cells with numerous pit channels. Similar structures are shown by Upton et al. (2011) in *Parthenium integrifolium* L., *Echinacea angustifolia* DC.. The phytomelanin is always associated with sclereids located in the cortex, secondary phloem (in fascicular and interfascicular position) and pith (of the root and rhizome).

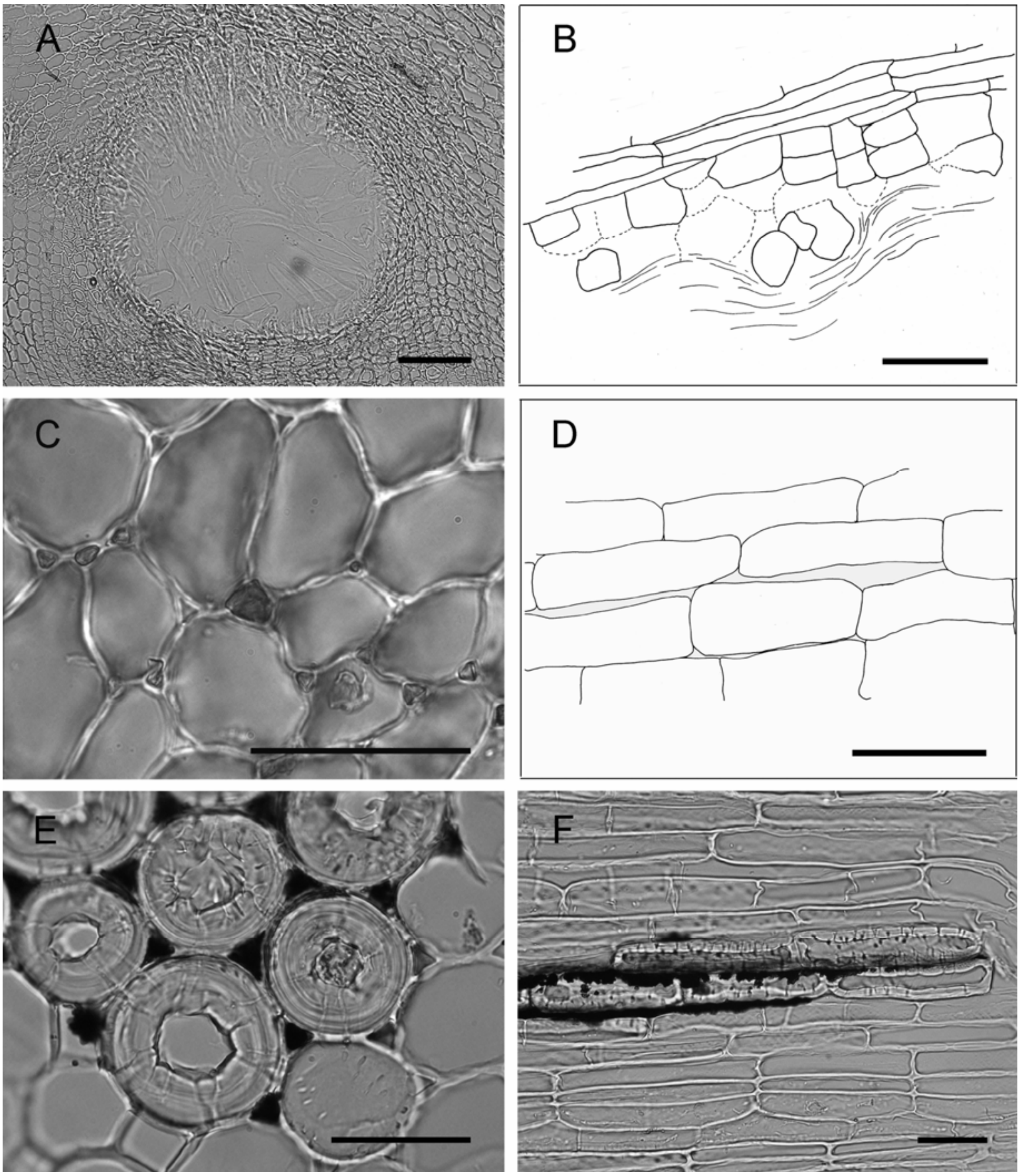

Fig. 7: (A,B) Secretory ducts of type SD3 of *Centaurea scabiosa,* **(C-F)** Secretory ducts of type (phytomelanin-coated cells) (SD4) of *Centaurea montana* **(C,D)**, of *Centaurea jacea* **(E,F)**;
A,C,E: transverse sections, B,D,F: longitudinal sections; Bar = 50µm

SD4 may be also found in any tissue of *Centaurea montana,* though the chemical nature of the substance found within the intercellular spaces is questionable as the light brown colour doesn't correlate with the brown-to-black phytomelanin.
For the present question of the analysis of anatomical structures though, not the chemical composition but the visual nature is relevant.

Secretory cavities (SC)
Of all the examined taxa, secretory cavities were absent in all species but *Echinops sphaerocephalus*. Cavities could be found in fascicular position within the secondary phloem and secondary xylem.

An overview of the occurrence of the various types of ducts and cavities observed in the examined species is provided in table 2.

Statistical Analysis
Fig. 8 presents a scatterplot demonstrating the results of a discriminant analysis of the database (188 investigated samples, 93 features; Fritz & Saukel, unpubl.) using only features concerning secretory structures (23 characters): the dimension of the endoSDs and their number of 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 evident. If the calculation is done with all anatomical characters (38) regarding the Cardueae, a complete discrimination between all examined genera is possible but among the other features, the secretory structures showed to be very important in this context. A more detailed analysis comprising all collected data is still under preparation.

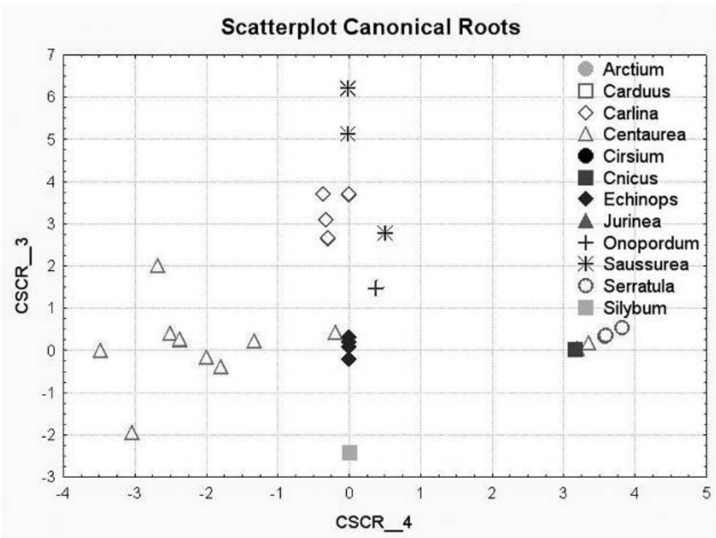

Fig. 8: scatterplot of a discriminant analysis (using Statistica®) using only features concerning secretory structures (23 characters)

TABLE 2. Overview of the occurrence of the various types of secretory ducts or cavities within roots and rhizomes of Cardueae species
+ = secretory structure exists
+ → - = endodermal resin ducts lost in consequence of formation of rhytidome;
sec. cav = secretory cavities; SD = secretory duct;

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

DISCUSSION

The secretory system, particularly the ducts within the secondary tissues of the 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., facing the vascular bundle: *Centaurea scabiosa*, or having an interfascicular position: *Carlina acaulis*) provide valuable characters to discriminate the subterranean parts of species. Furthermore, the 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 case of *Carlina*, as the roots of the medicinally important *C. acaulis* may be sufficiently characterised based on **SD**-types. The herbal drugs of *Carlina acaulis* and *Carlina vulgaris* can be differentiated easily from each other and clearly stand out from the other examined species. Future studies covering more species and individuals have to verify if a discrimination based on this feature can be justified and extended to other relatives.

In contrast, the new classification of secretory ducts doesn't provide characters for the discrimination of the medicinally used species *Arctium lappa* and *A. tomentosum*,. Due to the general presence of endodermal resin ducts within the subterranean organs of the Cardueae (Van Tieghem, 1883; Col, 1903; Solereder, 1908), the endoSDs being without remarkable specifics and the roots without further secretory ducts, the species of this genus cannot be distinguished.

The secretory ducts of type SD4 of *Centaurea jacea* seem likely to be phytomelanin-coated sclereids similar to e.g. *Parthenium integrifolium* L. and *Echinacea angustifolia* DC. (Upton et al., 2011). As Phytomelanin has a dark brown to black colour (Pandey & Dhakal, 2001), it is questionable if it is identical to the light brown substance that fills the intercellular spaces of *Centaurea montana*.

Secretory cavities are a further 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 existence of **SC**s in *Echinops* but **SD**s are found in the roots of *Carlina*.

The secretory system alone cannot be used to distinguish among all taxa studied as visible in our disciminant analysis, but constitute important additional anatomical characters.

Additionally to the secretory structures of the Cardueae, the subterranean parts of *Cichorium intybus* as a member of the tribe Cichorieae, which is not supposed to carry secretory ducts, has been investigated. In contrast to earlier results (Van Tieghem, 1883, Solereder 1904) that report *C. intybus*, *Lapsana communis* and *Podospermum laciniatum* (Cichorieae) to exhibit just the doubling of the endodermis with secretory canals missing, endodermal resin ducts can be found in *C. intybus* at an early age of the root. Since the doubling of the endodermal cells is similar in all three of the mentioned species, resin ducts may occur in young roots of *Lapsana communis* and *Podospermum laciniatum* as well.

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REFERENCES

APPEZZATO-DA-GLORIA B, HAYASHI AH, CURY G, SOARES MKM, ROCHA R. 2008. Occurrence of secretory structures in underground systems of seven Asteraceae species. *Botanical Journal of the Linnean Society* 157: 789-796.

BENNICI A, TANI C. 2004. Anatomical and ultrastructural study of the secretory cavity development of *Citrus sinensis* and *Citrus limon*: evaluation of schizolysigenous ontogeny. *Flora - Morphology, Distribution, Functional Ecology of Plants* 199: 464-475.

BERGER F. 1960. *Handbuch der Drogenkunde. Erkennung, Wertbestimmung und Anwendung. V. Radices*. Wilhelm Maudrich Verlag, Wien.

BOLLSCHWEILER M, STOFFEL M, SCHNEUWLY DM, BOURQUI K. 2008. Traumatic resin ducts in *Larix decidua* stems impacted by debris flows. *Tree physiology*. 28: 255-263.

COL MA. 1903. Recherches sur l'appareil sécréteur interne des composées. *Journal de Botanique* 17: 252-318.

COL MA. 1904. Recherches sur l'appareil sécréteur interne des composées (Suite). *Journal de Botanique* 18: 110-174.

CURY G, APPEZZATO-DA-GLÓRIA B. 2009. Internal secretory spaces in thickened underground systems of Asteraceae species. *Australian Journal of Botany*, 57, 229–239.

EVERT R. 2006. *Esau´s Plant Anatomy*, 3rd edition, John Wiley & Sons, New Jersey.

FAHN A. 1988. Secretory tissues in vascular plants. *New Phytologist* 108: 229-257.

FISCHER MA, OSWALD K, ADLER W. 2008. *Exkursionsflora für Österreich, Liechtenstein und Südtirol.* 3rd ed. Austria: OÖ Landesmuseen, Land Oberösterreich.

SUSANNA A, GARCIA-JACAS N. 2009. Tribe Cardueae. In: Funk VA, Susanna A, Stuessy TF & Bayer RJ (eds.). *Systematics and Evolution of the Compositae*. International Association for Plant Taxonomy IAPT, Vienna: 293-313.

GERLACH S, SAUKEL J, KUBELKA W. 2006. Pflanzen in der österreichischen Volksmedizin - die „Volksmed-Datenbank". SciPharm, 74**,** Suppl. 1: 36.

HAYASHI HA, APPEZZATO-DA-GLORIA B. 2007. Anatomy of the underground system in *Vernonia grandiflora* Less. and *V. brevifolia* Less. (Asteraceae). *Brazilian Archives of Biology and Technology* 50: 979-988.

HONG WU, ZHENG-HAI HU. 1997. Comparative anatomy of resin ducts of the Pinaceae. *Trees* 11: 135-143.

JEFFREY C. 2007. Compositae. In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg , 61-77.

LAUBER K, WAGNER G. 2007. *Flora Helvetica*. 4th ed. Haupt Verlag, Bern.

MELO-DE-PINNA G, MENEZES N. 2002. Vegetative organ anatomy of *Ianthopappus corymbosus* Roque and Hind (Asteraceae-Mutisieae). *Revista Brasileira de Botânica* 25: 505-514.

MELO-DE-PINNA G, MENEZES N. 2003. Meristematic endodermis and secretory structures in adventitious roots of *Richterago* Kuntze (Mutisieae-Asteraceae). *Revista Brasileira de Botânica* 26: 1-10.

NAIR MNB. 1995. Some notes on gum and resin ducts and cavities in angiosperms. In: M. Iqbal, eds, *The cambial derivatives*. Gebr. Borntraeger, Berlin Stuttgart, 317-340.

OESTERREICHISCHES ARZNEIBUCH (OEAB) (Pharmacopoea Austriaca). 2009. Wien: Verlag Österreich GmbH.

PAWŁOWSKIEGO B, JASIEWICZA A. 1972 *Flora Polska TOM XIII*. Polska Akademia Nauk, Warszawa.

PICKARD W. 2008. Laticifers and secretory ducts: two other tube systems in plants. *New Phytologist* 177: 877-888.

RAGONESE AM. 1988. Canales secretores en los organos vegetativos de *Eupatorium inulaefolium* H.B.K. (Compositae). *Acta Farmaceutica Bonaerense* 7: 161-168.

RODRIGUES TM, MACHADO SR. 2009. Developmental and structural features of secretory canals in root and shoot wood of *Copaifera langsdorfii* Desf. (Leguminosae – Caesalpiniodeae). *Trees* 23: 1013-1018.

SAUKEL J. 1984. Pharmakobotanische Untersuchungen von Arzneidrogen II. Herba Epilobii. 6. Mitteilung. *Scientia Pharmaceutica* 52: 196-220.

ŠARIĆ-KUNDALIĆ B, DOBEŠ C, KLATTE-ASSELMEYER V, SAUKEL J. 2010. Ethnobotanical survey of traditionally used plants in human therapy of east, north and north-east Bosnia and Herzegovina. *Journal of Ethnopharmacology*. doi:10.1016/j.jep.2010.11.033.

SOLEREDER H. 1908. Systematische Anatomie der Dicotyledonen. Verlag von Ferdinand Enke, Stuttgart.

SUSANNA A**,** GARCIA-JACAS N. 2007. Tribe Cardueae Cass. (1819). In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg, 123-146.

SZAFER W, KULCZYŃSKI S, PAWŁOWSKI B. 1976. *Rośliny Polskie*, 4th ed. Panstwowe Wydawnictwo Naukowe, Warszawa.

UPTON R, GRAFF A, JOLLIFFE G, LAENGER R, WILLIAMSON E. 2011. *American Herbal Pharmacopoeia. Botanical Pharmacognosy – Microscopic Characterization of Botanical Medicines*. CRC Press.

VAN TIEGHEM M. 1883. Sur la situation de l'appareil sécréteur dans les composées. *Bulletin de la Société Botanique de France* 30: 310-313.

VAN TIEGHEM M. 1884. Sur la situation de l'appareil sécréteur dans la racine des composées. *Bulletin de la Société Botanique de France* 31: 112-116.

VAN VUILLEMIN M. 1884. Remarques sur la situation de l'appareil sécréteur des composées. *Bulletin de la Société Botanique de France* 31: 108-110.

WICHTL MAX. 2009. *Teedrogen und Phytopharmaka: ein Handbuch für die Praxis auf wissenschaftlicher Grundlage*. 5th ed., Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.

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Anatomy of Subterranean Organs of Medicinally Used Cardueae and Related Species and its Value for Discrimination

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Abstract

Numerous species of the Asteraceae, the composites, are famous for their use in both traditional and conventional medicine. Reliable anatomical descriptions of these plants and of possible adulterations provide a basis for fast identification and cheap purity controls of respective medicinal drugs by means of light microscopy. Nevertheless, detailed comparative studies on root and rhizome anatomy of valuable as well as related inconsiderable composite plants are largely missing yet. The presented study aims to narrow this gap by performing anatomical analyses of roots and rhizomes of 16 species belonging to the tribe Cardueae, of formerly and currently used drugs as well as their near relatives as potential adulterations (*Carlina acaulis* L., *Carlina vulgaris* L., *Arctium lappa* L., *Arctium tomentosum* Mill., *Carduus defloratus* L., *Carduus personata* (L.) Jacq, *Cirsium arvense* (L.) Scop., *Cirsium vulgare* (Savi) Ten., *Cirsium erisithales* (Jacq.) Scop., *Onopordum acanthium* L., *Silybum marianum* (L.) Gaertn., *Rhaponticum scariosum* Lam., *Centaurea jacea* L., *Centaurea scabiosa* L., *Centaurea cyanus* L., *Cnicus benedictus* L.). A detailed verbal and graphical survey of the analysed anatomical features is provided. Several characters were finally extracted which allow for discrimination of the examined species and may be effectively used for drug quality controls.

Keywords

Root anatomy • Asteraceae • Microscopy • Plant anatomy

Introduction

The Asteraceae represent one of the largest plant families comprising at least 23,000 species and about 1,600 genera [1]. Numerous composites such as *Carlina acaulis, Arctium lappa* and *Taraxacum officinale,* to mention only a few, have a long history in both traditional and conventional medicine. Light microscopy is a common and effective method for the identification of pharmaceutically useful plants and their adulterations. Hence, detailed knowledge of the anatomy of the diverse plant parts used as drugs is required for quality control. Yet, reliable comparative studies of the anatomy of subterranean organs of medicinally exploited Asteraceae species and their close relatives are rare. Most studies date back to about 1900 and concentrate on the secretory system of the family tribes Cardueae (characterized by resin ducts) and Cichorieae (laticifers) in particular [2–5]. Most work, however, concerned the aerial vegetative organs and did not aim to discriminate species. Some recent studies deal with the subterranean organ anatomy of diverse Asteraceae species [e.g. 6–11]. However, the investigated species were of no medicinal value and not of a Western European origin. An additional recent study concentrates on the secretory structures of the subterranean organs of various species of the tribe Cardueae and demonstrated - based on five identified types of secretory ducts - the value of anatomical characters for the discrimination of composite plants [12]:

- Secretory ducts of type SD1 and SD2a / SD2b are of schizogenous origin and distinguished based on the C1:C2-quotient (i.e. the length of the cells lining the ducts [C1] divided by the length of adjacent cells [C2], longitudinal section, see fig. 1 [fig cited: 12]).

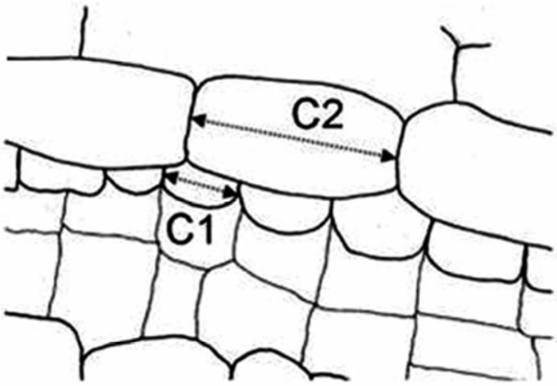


Fig. 1. Secretory duct in longitudinal section build from two adjacent layers of cells differing in length. The quotient C1:C2 is used to discriminate between three types of secretory ducts (fig. cited: [12]).

- Secretory ducts of type SD3 show cell lysis: the large lumen is filled with the remnants of the cells.

- Secretory ducts of type SD4 seem to be actually intercellular space, filled with diverse substances secreted by the adjacent parenchyma cells.

Although the secretory system proofed valuable for species discrimination, the anatomy of entire roots and rhizomes is still insufficiently described. So far a single study only focuses on medicinal drugs [13] by comparing the root anatomy of *Taraxacum* spp., *Leontodon* sp., *Aposeris foetida,* and *Hypochaeris* sp..

In order to narrow this gap of knowledge, a comprehensive study on the anatomy of the subterranean organs of 32 genera and 57 species from the tribes *Cardueae* (22 species) and *Cichorieae* (35 species) used in medicine or occurring as potential adulterations was initiated. The anatomy of these plants was analysed in detail and a database of typical anatomical features created [14].

The present work deals with the tribe Cardueae and analyses the underground part anatomy of species possessing a taproot or dominating rhizome. Taxa with a fibrous root system were excluded. The focus thereby lies on formerly or currently medicinally used species of the Cardueae, which have been used for medical purposes since long:

Thus already Hieronymus Bock [15] mentions *Carlina acaulis* L. as a plant highly valued by people as a diaphoretic and diuretic medicine and against worms. The roots soaked in vinegar were also known as appropriate remedy against scabies and tetter [16]. Today, this plant is still used in traditional medicine and serves as an ingredient of various liquids such as Swedish bitters elixir, as a diuretic as well as a stomachic remedy and against skin diseases [17, 18].

Even though rarely used nowadays, another species of the genus *Carlina, Carlina vulgaris* L., still has a place in traditional medicine. The species is applied against nocturnal enuresis and to cure frightened babies [19].

The medicinally use of *Arctium lappa* L. reaches back to the ancient times. Dioskurides [20] praises the effects of its roots as an expectorans (the antitussive activity was confirmed by Kardošová [21]) and its application on luxations and sprains. Lonicerus [22] mentions its effects against asthma. The usage of *A. lappa* oil as hair restorer was first mentioned in 1673 [Pankovius, 1673 in: 23] and is still popular among people. In traditional medicine, *A. lappa* – along with *Arctium tomentosum* Mill. – is still valued as a plant with antirheumatic, diuretic and diaphoretic effects [17, 19]. Recent studies document a gastroprotective activity [24] and hepatoprotective effects [25].

Although officinal in former times (Radix et Herba Spinae albae seu Cardui tomentosi; [23]), these days, *Onopordum acanthium* is no longer used except in anthroposophical medicine (Onopordi acanthi herba) [17]. The roots (together with the sap of the fresh leaves) were reported to be useful against stiff neck and opisthotonus [26, 20] and as a diaphoretic and laxative remedy [23].

Rhaponticum has been used in Chinese, Tibetan and Mongol medicine for more than 5,000 years [27]. *Rhaponticum carthamoides* (Willd.) Iljin. was studied currently as it is supposed to possess various positive effects on memory, blood, cardiovascular and nervous system and on physiological functions such as work capacity and sexual function (reviewed by Kokoska [28]). Łotocka & Geszprych [27] dealt with the anatomy and secretory structures of *R. carthamoides* but the identification of the plant and its roots remained problematic as comparative material from other species of the genus is missing.

On that account the anatomy of *Rhaponticum scariosum* Lam. – the only species of the genus native to Austria (the area mainly addressed here) – has been included in our studies. Though *R. scariosum* has been used in former times as substitute to *Rheum palmatum* [29] it is not applied any longer.

Carduus defloratus L., Carduus personata (L.) Jacq, Cirsium arvense (L.) Scop., Cirsium vulgare (Savi) Ten., Cirsium erisithales (Jacq.) Scop. Silybum marianum L., Centaurea jacea L., Centaurea scabiosa L., Centaurea cyanus and Cnicus benedictus L. have been examined as near relatives of the taxa mentioned above.

Results

Reliable anatomical root and rhizome characters discriminating between the various species investigated in this study were identified. These features are proofed valuable for the characterisation of the drugs and may be used for quality control:

The following main anatomical features of roots and rhizomes can be used for purity and quality control in pharmacy: the overall distribution and proportions of the principal tissues in transverse section, the fine structure of the cork, vessel types, the occurrence of fibers including their maximum diameter and wall structure, and, finally, the occurrence of sclereids and secretory ducts in diverse tissues.

Our studies demonstrated that even within one single plant the diameter of the largest vessels may differ largely depending on their position along the root axis. In transverse sections the dimension of the largest vessels of *Silybum marianum*, for instance, varied largely with their position along the taproot: the maximum diameter decreased from 245 μm near the tip of the root to 113 μm 1.5 cm below the hypocotyle. However, in other samples of the same species this parameter varied insignificantly only.

The example of *S. marianum* points out that the diameter of vessels, though a popular feature used for quality control of drugs, has to be carefully considered as preparations of drugs usually are available as a cut formulation. Therefore, standardisation of the analysed root parts appears impossible.

For the purpose of standardization measured values given in the following microscopical descriptions always refer to a section of the root axis just below the hypocotyle (see Experimental). As the occurrence of secretory ducts is of particular importance for the taxonomic identification of the species described in the following, respective data have been included from [12].

Microscopical descriptions and discriminative anatomical characters

Carlina acaulis L. (fig. 2)

Secondary root: cork thin-walled; cortex durable or lost in course of rhytidome formation; endodermal resin ducts are in size a multiple of the diameter of the surrounding parenchyma cells, lost together with the cortex; secondary phloem dominant, comparable in extension to the vascular cylinder, with fibers arranged in bundles, rays with crystalline needles; secondary xylem consists of fibers and vessels; vessels reticulate and simple, strongly bordered, up to 114 μm in diameter; medullary rays multiseriate, parenchymatous, with crystalline needles of the same type occurring in the phloem; secretory ducts of type **SD1** and **SD2a** (average quotient **C1:C2** <0,4 [12]), size a multiple of the diameter of the surrounding parenchyma cells, located in the medullary rays and in the phloem rays; pith missing; sclereids missing;

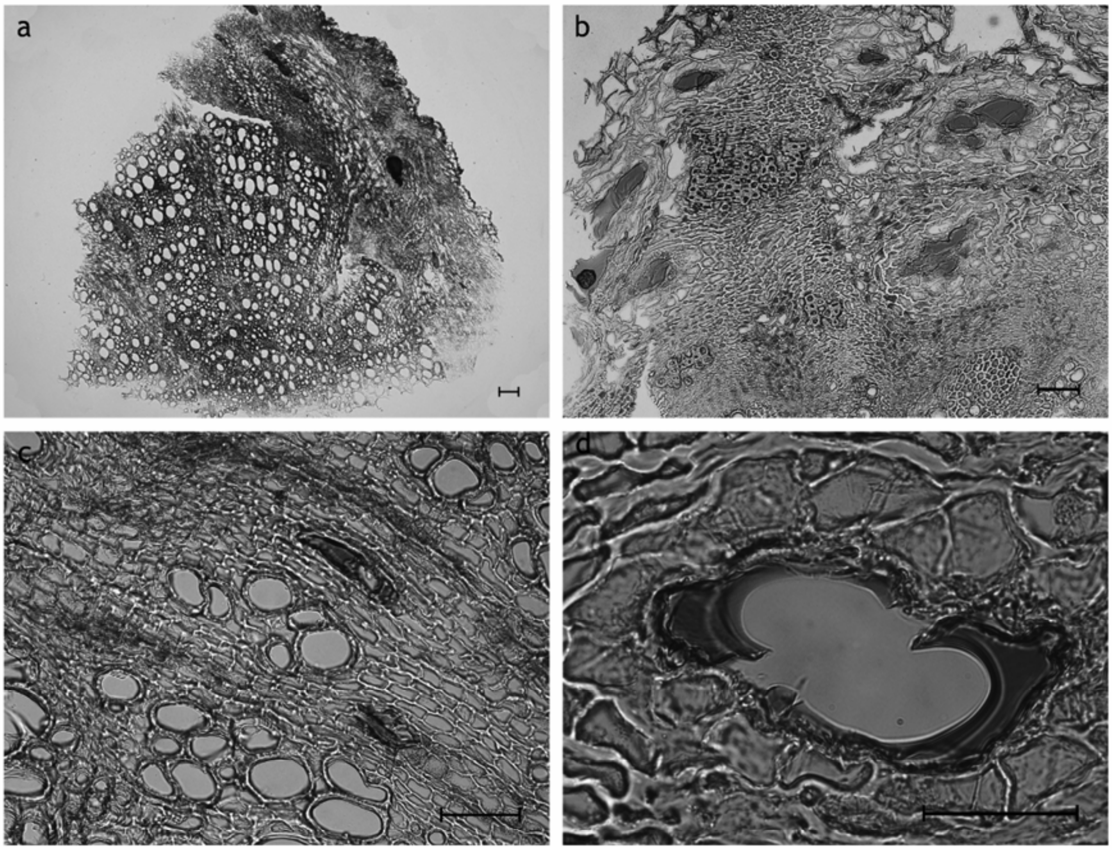


Fig. 2. *Carlina acaulis* root: a: Overview showing the extension and arrangement of the participating tissues: the secondary xylem is the most prominent component followed in extension by the secondary phloem; cortex conspicuously small or lost in course of rhytidome formation; small cortex with endodermal resin ducts, secondary phloem and xylem with secretory ducts type SD1 within the medullary rays; b: secondary phloem having an expansion a multiple of the diameter of the surrounding parenchyma cells and with fibers arranged in bundles; c: SD1 and crystalline needles in medullary rays of the vascular cylinder; d: secretory ducts type SD1 of secondary phloem; a–d: transverse sections; scale bars are 50 µm

Carlina vulgaris L. (fig. 3)

Secondary root: cork thin-walled sometimes with crystalloids; cortex enduring; distinct endodermis with endodermal resin ducts – usually up to 10 surrounding cells at maximum; secondary phloem broad, but of lesser radial extension than the vascular cylinder, with secretory ducts of type **SD2** (C1:C2 >0,48); secondary xylem dominated by fibers, few vessels dispersed over the transverse section, strongly bordered, up to 59 µm in diameter; medullary rays biseriate; pith missing; sclereids, laticifers missing; fibers in secondary phloem missing;

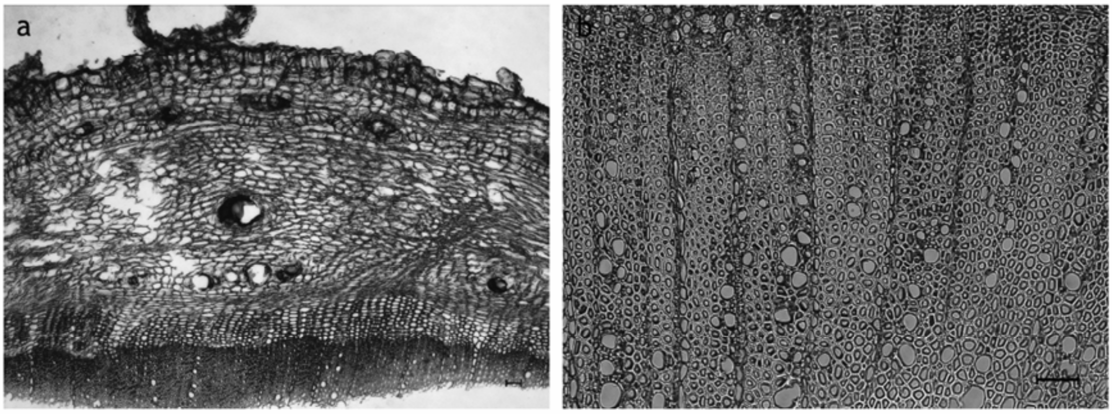


Fig. 3. *Carlina vulgaris* root: a: overview showing distinct endodermal resin ducts and secretory ducts of the type SD2b in the secondary phloem; b: xylem dominated by fibers and with dispersed vessels, biseriate medullary rays in regular arrangement; a, b: transverse sections; scale bars are 50 µm

Arctium lappa L. and A. tomentosum Mill. (fig. 4)

The following description applies to both examined species:

Secondary root: broad phellem thin-walled – cortex lost in course of rhytidome formation; endodermal resin ducts lost together with the cortex; secondary phloem broad, but of lesser radial extension than the vascular cylinder, possibly with fibers arranged in bundles; secondary xylem mostly with vessels in rows – in the center single-rowed, in the outer part multiple-rowed, often combined with fibers, mainly reticulate, also weakly bordered up to 89 µm (*A. lappa*) / 125 µm (*A. tomentosum*) in diameter; medullary rays multiseriate, unlignified; cells of medullary rays often obliterating and easily ripping apart; pith missing; sclereids, crystalloids missing; secretory ducts besides endodermal resin ducts missing;

The early loss of the cortex with its endodermal resin ducts deprives the genus *Arctium* of a character otherwise valuable for taxonomic comparisons with taxa outside of the Cardueae.

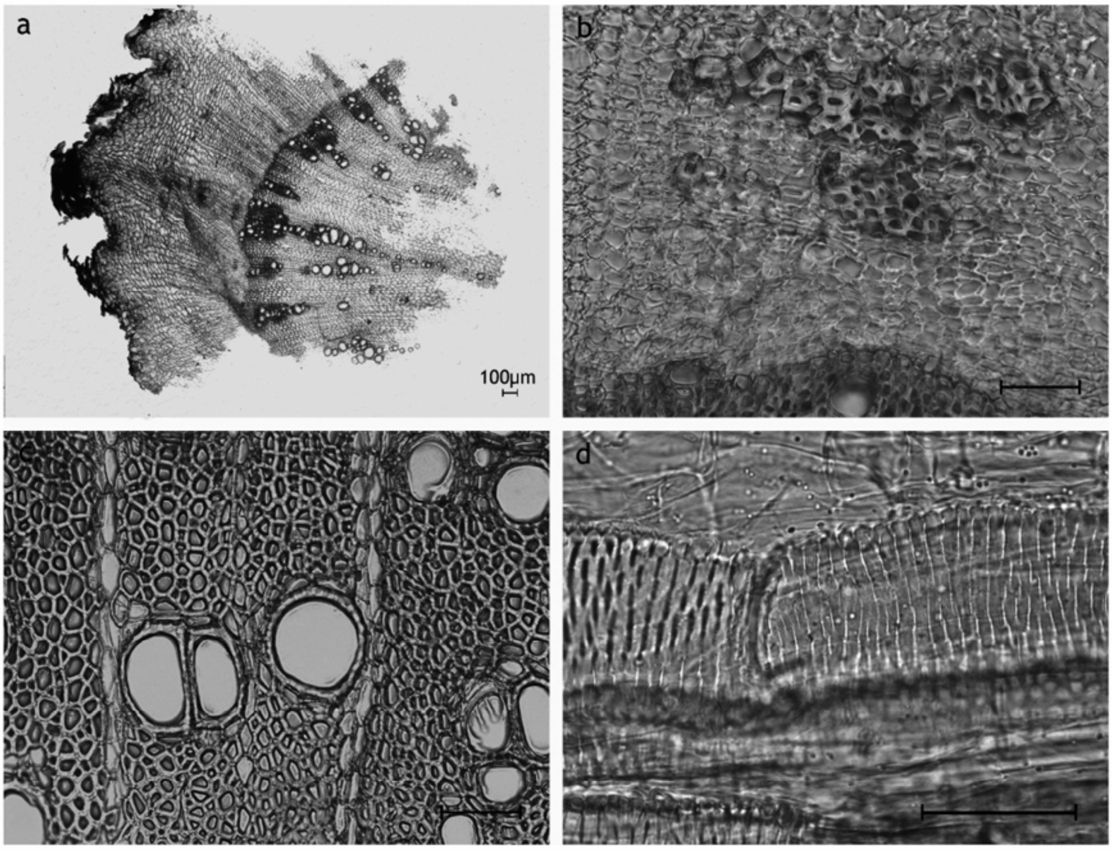


Fig. 4. *Arctium lappa* root: a: Overview demonstrating the extension and arrangement of tissues: the secondary xylem is the most expanded component followed in extension by the secondary phloem; cortex lost in course of rhytidome formation; xylem dominated by unlignified parenchymatous cells, vessels arranged in rows; b: secondary phloem with sclereids and fibers arranged in bundles ; c: xylem with fibers and with dispersed vessels, medullary rays in regular arrangement; d: reticulate vessels; a–c: transverse sections; d: longitudinal section; scale bars are 50 µm

Onopordum acanthium L. (fig. 5)

Secondary root: cork thin-walled, sometimes with crystalloids; small cortex enduring; distinct endodermis with endodermal resin ducts usually up to 6 surrounding cells at maximum; secondary phloem usually broader than cortex, but of lesser radial extension than the vascular cylinder, with fibers single and arranged in bundles; secondary xylem with fibers alternating with parenchymatous cells, vessels dispersed, strongly bordered, up to 150 µm in diameter; multiseriate medullary rays multiseriate; pith missing; sclereids missing; secretory ducts beside endodermal resin ducts missing;

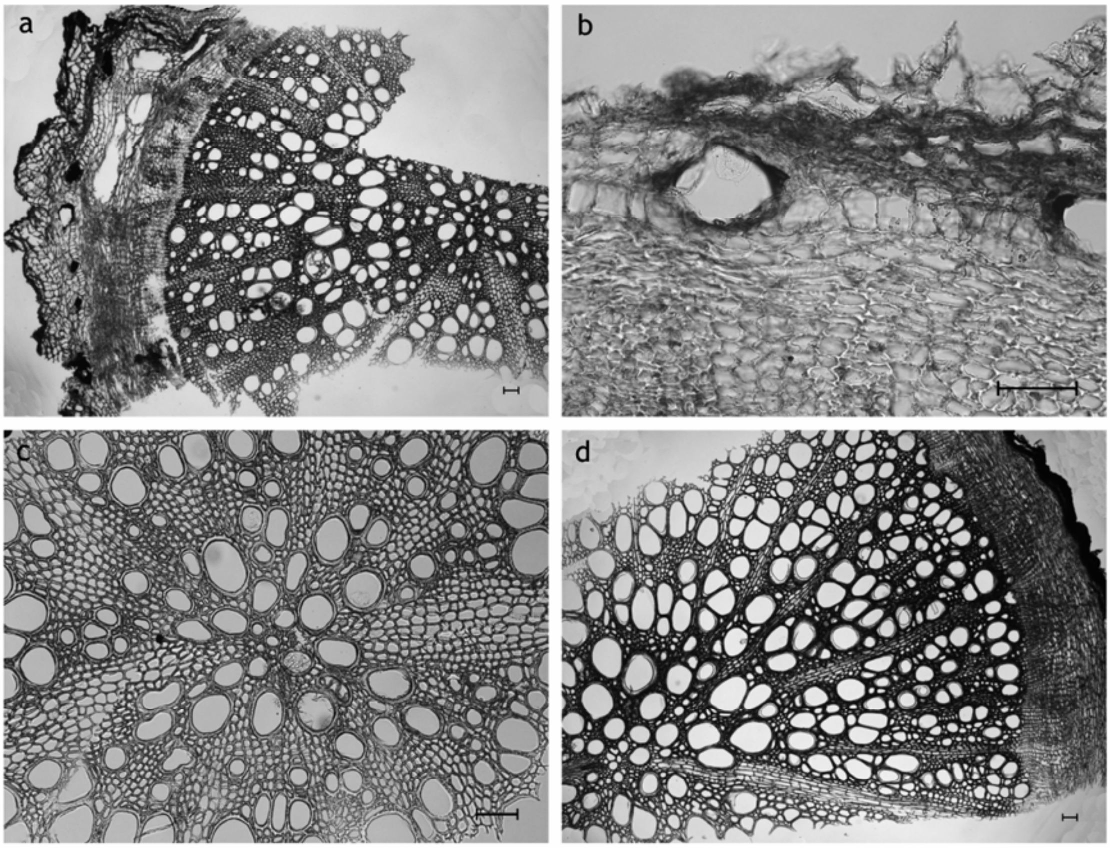


Fig. 5. *Onopordum acanthium* root: a: Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component followed in extension by the secondary phloem; cortex enduring with endodermal resin ducts; vascular cylinder with multiseriate medullary rays (from left to right); b: Distinct endodermis with endodermal resin ducts usually up to 6 surrounding cells at maximum; c Vascular cylinder with multiseriate medullary rays, numerous dispersed vessels, fibers; d: *Silybum marianum* root: cortex lost, secondary phloem broader than cortex but of far lesser radial extension than the vascular cylinder; a–d: transverse sections; scale bars are 50 µm

The root anatomy of *Silybum marianum* resembles that of *O. acanthium*. A distinction among these species may be possible with proceeding secondary growth: rhytidome formation within *S. marianum* versus maintenance of a durable cortex with well visible endodermal resin ducts in *O. acanthium*. The root anatomy of *Cnicus benedictus* and *O. acanthium* is similar. The diameter of the vessels may serve as the only discriminative character available (*C. benedictus*: <100 µm, *O. acanthium* up to 150 µm) with the value of this feature questionable

Rhaponticum scariosum Lam. (fig. 6)

Secondary root: cork thin-walled; cortex enduring; endodermal resin ducts; secondary phloem dominant, often almost comparable in extension to the vascular cylinder, with fibers arranged in bundles possible, secretory ducts type **SD2** located in fascicular position between the phloem rays, circularly arranged, forming a triangle; secondary xylem with bundles of fibers in conjunction with vessels, more or less circularly arranged relative to the centre of the xylem, vessels reticulate, up to 120 µm in diameter, with secretory ducts type **SD2** located in fascicular position in the secondary xylem between the medullary rays; medullary rays broad, multiseriate, unlignified; pith missing; sclereids, crystalloids missing;

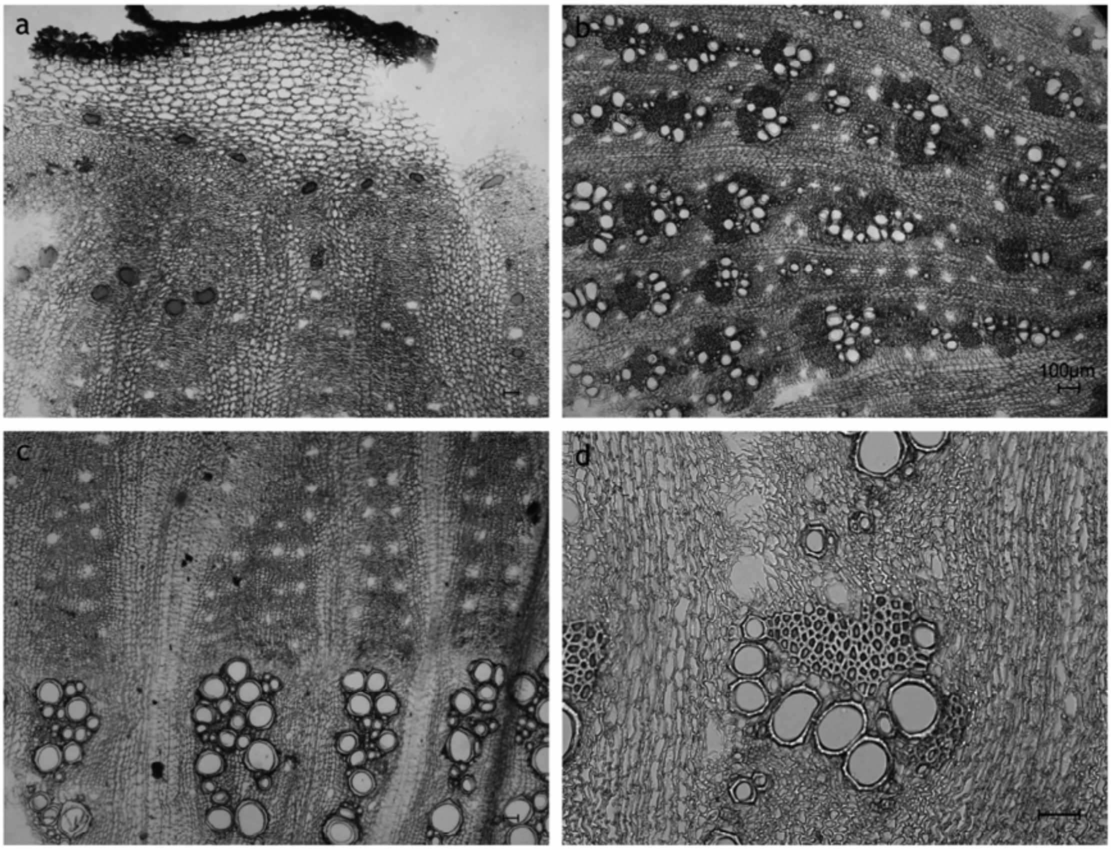


Fig. 6. *Rhaponticum scariosum* root: a: enduring cortex with endodermal resin ducts, SD2 in secondary phloem between phloem rays; b: overview showing the extension of the vascular cylinder with vessels in groups more or less circularly arranged relative to the centre of the xylem, multiseriate medullary rays; c: secretory ducts type SD2 arranged in fascicular position between phloem rays of the secondary phloem and the medullary rays of the secondary xylem; d: group of vessels and fibers of the secondary xylem, secretory ducts SD2; a-d: transverse sections; scale bars are 50 µm

The anatomy of *R. scariosum* clearly differs from all other species examined in this study based on the arrangement of the secretory ducts. Although the roots develop ducts both within the secondary phloem and the secondary xylem like *Carlina acaulis*, in *R. scariosum* ducts are found in fascicular position, i.e. between the medullary rays. In contrast, ducts occur within the medullary in all other taxa examined.

Carduus sp. and Cirsium sp.

Carduus defloratus, *C. personata*, *Cirsium arvense*, *C. vulgare* and *C. erisithales* are close relatives of the medicinally used species described above. These species are representatives of the subtribe Carduinae (just like *Onopordum acanthium* and *Arctium* spp.) and studied given their potential occurrence as adulterations.

The characters of highest value for the discrimination of *Carduus* and *Cirsium* (except *Cirsium erisithales*) from their medicinally used relatives is the exhibition of sclereids (fig. 7). *Cirsium erisithales*, can be distinguished instead based on its rhizome, which replaces the root developed in the other studied species (fig. 8).

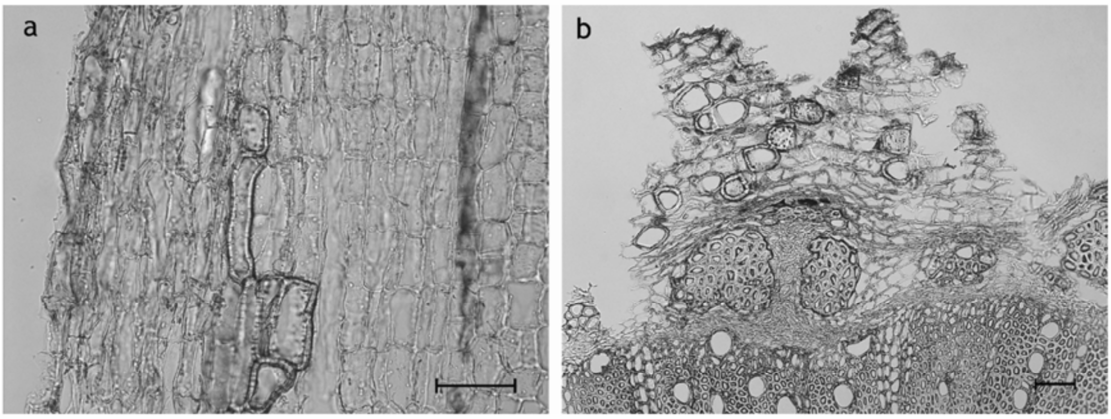


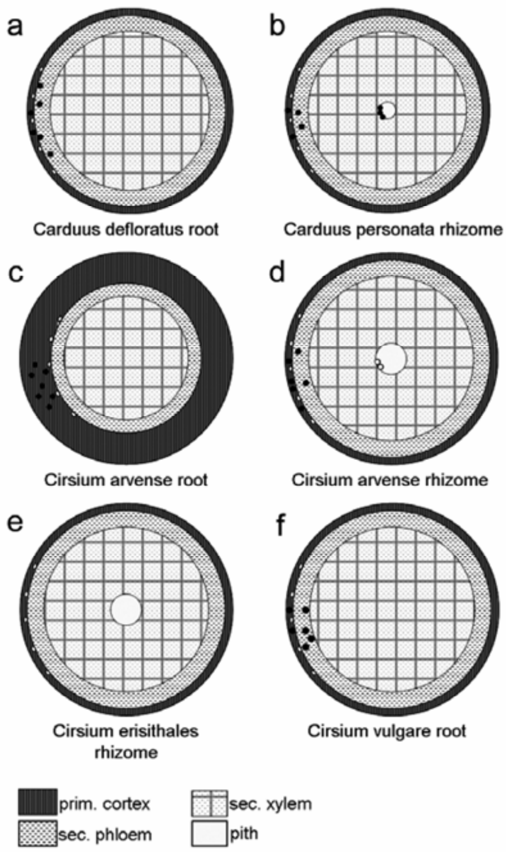
Fig. 7. *Cirsium arvense* root: sclereids (as an important discriminative character) developed within the cortex; a: longitudinal section; b: transverse sections; scale bars are 50 µm

Centaurea sp.

The three species of the genus *Centaurea* investigated in this study – *C. jacea*, *C. cyanus* and *C. scabiosa* – can be well distinguished from each other based on secretory structures [12]:

Centaurea jacea is characterised by sclereids regularly appearing in conjunction with intercellular spaces filled by various substances secreted by the surrounding cells.

In *C. scabiosa,* large secretory ducts with lysigenous development, beside others, can be observed in the secondary phloem in fascicular position. Both mentioned secretory ducts are missing in *C. cyanus*.



a: Carduus defloratus:
Secondary root: sclereids in cortex and sec. phloem, endoSDs lost together with the cortex in course of rhytidome formation; SDs besides endoSDs missing;

b: Carduus personata:
Rhizome with secondary growth: sclereids in cortex, secondary phloem and as transition between vascular bundles and pith; endoSDs or remnants of them visible; SDs besides endoSDs missing;

c,d: Cirsium arvense:
allorhizous: taproot with long part of rhizome; stoloniferous plant – spreading building rhizomes;
Secondary root (c): sclereids in cortex (large intercellular spaces – aerenchyma) and secondary phloem; endoSDs regularly arranged; SDs besides endoSDs missing;
Rhizome with secondary growth (d): sclereids in cortex and secondary phloem; endoSDs lost lost together with the suberizing of the cortex; SDs at the border between vascular bundle and pith;

e: Cirsium erisithales:
Rhizome with secondary growth: endoSDs lost together with the cortex due to rhytidome formation; sclereids missing; SDs besides the endoSDs missing;

f: Cirsium vulgare:
Secondary root: sclereids in cortex and secondary phloem; endoSDs lost together with the cortex due to rhytidome formation; SDs beside endoSDs missing;

Fig. 8. Schematic view of *Carduus* sp. and *Cirsium* sp. (transverse section): small ellipses mark the position of the enodermal resin ducts (endoSDs) of the cortex and the ducts of type SD2, black points represent the sclereids

Discussion

The diameter of vessels is a parameter frequently used for the identification of medicinal drugs [e.g. 13]. Our studies, however, demonstrated that this character may largely vary, aside from the age and development of a root, even within one plant. Therefore, the root part taken for respective analyses should be standardized. This appears highly important, even so most studies did not define the exact spatial localisation and developmental stage of the analysed root sample [13]. In case of cut roots as common with commercially traded drugs, the use of not-standardized values may be misleading. Furthermore, varying preparation methods may influence the dimension of the vessels.

Discrimination between most of the examined species proofed possible based on the observed diversity and differentiation of the various anatomical features analysed. A summary of the root anatomy as seen in transverse sections is presented for these species in figures 9 and 10. This overview is intended to provide a taxonomic key.

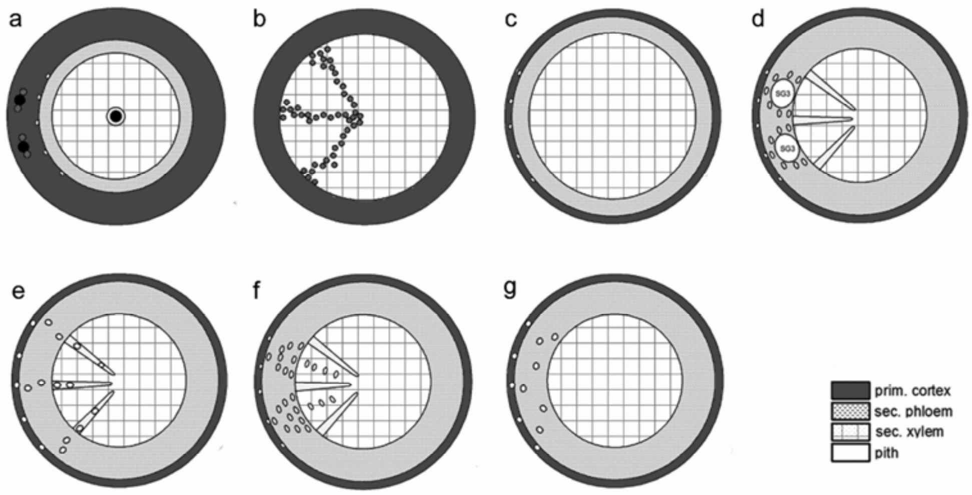


Fig. 9. Schematic view of the various roots studied: small ellipses mark the position of the endodermal resin ducts of the cortex and the ducts of type SD2; a: black points represent the sclereids in conjunction with secretory ducts (grey dots), b: points represent the vessels (b); a: *Centaurea jacea*; b: *Arctium lappa, A. tomentosum*; c: *Onopordum acanthium, Silybum marianum, Cnicus benedictus*; d: *Centaurea scabiosa*; e: *Carlina acaulis*; f: *Rhaponticum scariosum*; g: *Carlina vulgaris, Centaurea cyanus*;

Strikingly, all species of the genera *Cirsium and Carduus* as well as *Centaurea jacea* can be easily identified in medicinal drugs by the unique possession of rhizomes or sclereids. The other taxa can be distinguished based on the presence or absence, type (according to [12]) and spatial location of secretory structures.

Interestingly, the anatomy of several species may vary widely within a single genus (*Centaurea* sp., *Carlina* sp.) or, in contrast, be indifferent and of no discriminative value as in the case of the genus *Arctium*. In addition, species of different genera may be quite similar in root anatomy (*Onopordum acanthium, Cnicus benedictus, Silybum marianum*).

The discrimination between the two examined representatives of the genus *Carlina*, *C. acaulis* and *C. vulgaris*, is easily possible based on anatomical features: The endodermal resin ducts of the enduring cortex of *C. vulgaris*, and *C. acaulis*. are clearly distinct in size. In conjunction with the endodermis – usually visible even in roots showing secondary growth – this feature well characterizes these species. The **C1:C2** quotient of the epithelial cells of the secretory ducts occurring in the secondary phloem as well as their spatial position provide other good discriminative features. The domination of fibers over few vessels in the secondary xylem, vessel diameter (up to 60 µm; about half the value observed in *C. acaulis*), and biseriate medullary rays with ducts missing are additional characters distinguishing the medically inconsiderable species from its valuable relative. Finally, the absence of fibers in the secondary phloem and of crystalline needles may be used for differentiation.

1 Rhizome ....*Cirsium arvense*, *Cirsium erisithales* (fig. 12)
- Taproot..2

2 Sclereids present... *Cirsium* sp., *Carduus* sp. (fig. 12), *Centaurea jacea* (fig. 8a)
- Sclereids absent...3

3 SDs besides endoSDs absent...4
- SDs besides endoSDs present 6

4 Cortex lost with secondary growth, secondary xylem with vessels arranged in rows………. ....*Arctium lappa*, *A. tomentosum* (fig. 8b)
- Cortex remains but becomes part of rhytidome............ *Silybum marianum* (fig. 8c)
- Cortex enduring with distinct endodermis..5

5 Diameter of the largest vessels (1.5cm below hypocotyle) < 100µm....................... *Cnicus benedictus* (fig. 8c)
- Diameter of the largest vessels (1.5cm below hypocotyle) > 100µm....................... *Onopordum acanthium* (fig. 8c)

6 Secretory structures of type SD3 present...................*Centaurea scabiosa* (fig. 8d)
- Secretory structures of type SD2 present, SD3 absent..7

7 SD2 within secondary phloem and secondary xylem 8
- SD2 within secondary phloem..10

8 SD2 interfascicular positioned.. *Carlina acaulis* (fig. 8e)
- SD2 fascicular positioned..9

9 SD2 in secondary phloem with no typical pattern.......*Rhaponticum carthamoides**
- SD2 in secondary phloem forming a characteristic pattern ....*R. scariosum* (fig. 8f)

10 C1:C2 = 1–1.01 ....*Centaurea cyanus* (fig. 8g)
- C1:C2 = 0.48–0.62 ....*Carlina vulgaris* (fig. 8g)

Fig. 10. Key of the subterranean organs of the species examined in this study;
* Data concerning *R. carthamoides* is based on Łotocka & Geszprych [27]

Centaurea cyanus, *C. jacea* and *C. scabiosa* differed from each other in secretory duct type (fig. 8).

According to Łotocka & Geszprych [27] an anatomical differentiation between *R. carthamoides* and *R. scariosum* seems possible. The conspicuous arrangement of the secretory ducts within the secondary phloem, arranged in regular circles around the centre thereby forming together with the phloem rays a kind of triangular pattern, however,, is not described in that study. *Rhaponticum scariosum* appears to develop a much more extensive secondary phloem than the medicinally applied species.

This study on the anatomy of several roots and rhizomes of the Cardueae reveal considerable among-species variation. Important features of discriminative value are the arrangement of principal tissues as observed in transverse section of roots and rhizomes, the tissue-dependent occurrence of fibers and sclereids as well as of various types of secretory ducts. The different duct types [12] provide a particularly valuable character for taxonomic discrimination if the type of surrounding tissue and their position relative to prominent anatomical elements such as vascular bundles is taken into account. Certainly, the arrangement and diameter of vessels and the appearance of medullary rays in the secondary xylem must not be forgotten. However, the largest diameter of vessels though can be only considered a reliable taxonomically informative character if applied to root parts standardized in terms of developmental age and spatial position.

In summary, an identification of most investigated taxa used in medicine proofed possible based on various extracted anatomical characters. Further studies into the root anatomy using a broader and more diverse taxonomic sample have to verify the feasibility of microscopic techniques for the identification of medicinally used genera and species of the Asteraceae.

Experimental

The plant material comprised of 16 Cardueae species naturally occurring in Austria. Species were chosen respectively to their use in medicine (*Carlina acaulis*, *Carlina vulgaris*, *Arctium lappa, Arctium tomentosum, Onopordum acanthium,*) and their role as possible adulterations (*Rhaponticum scariosum, Carduus defloratus*, *Carduus personata*, *Cirsium arvense*, *Cirsium vulgare*, *Cirsium erisithales, Silybum marianum*, *Centaurea jacea*, *Centaurea scabiosa*, *Centaurea cyanus* and *Cnicus benedictus*.). To guarantee full development of the roots, the plants were collected during or following antheses. A list of the studied species including the collection history is provided in Table1 (taxonomy follows Fischer et al., 2008 [31]). Vouchers available for all studied species 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 [31–33].

In order to include modificative effects of the environment (e.g. influence of the soil) on root anatomy, each species was collected from different locations if possible.

Anatomical analysis: The roots were examined by means of light microscopy. For preparation, a traditional method of our department was used: After boiling in water for about 10 minutes to soften the tissues, the roots were embedded in 96% ethanol for dehydration. Transverse and longitudinal sections were obtained by free hand sectioning about 1.5 cm below the hypocotyle as this position proofed to be the furthest developed region, thus providing the most information about the anatomy. The resulting sections were embedded in few drops of a solution of chloral hydrate (60% in water) and examined using a Nikon Optiphot–2 light microscope equipped with a Samsung Digimax V50 Digital Camera.

Tab. 1. List of species examined in this study (accessions are taxonomically arranged following the current systematic concept provided in [31]); AUT: Austria, GER: Germany, ITA: Italy, POL: Poland, SLK: Slovakia, LIE: Liechtenstein; Plant material collected by Elisabeth Fritz (EF), Christoph Dobeš (CD), Silvia Fialova (SF), Werner Lahner (WL), Günther Stadler (GS)

Genus	Species	Location of collection
Carlina	*C. acaulis* L.	AUT, Vienna, EF
		GER, Baden-Württemberg, Schwäbische Alb, Hohenack, Nr. 652 (WUP)
		Samples of a commercially traded product (Kottas Pharma, sample number 1881, 1882)
	C. vulgaris L.	POL, Gutkowo / Olsztyn, EF
Arctium	*A. lappa* L.	AUT, Vienna, Donauinsel, EF
	A. tomentosum Mill.	-, Lower Austria, Traiskirchen, EF
Carduus	*C. defloratus* L.	-, Lower Austria, Gippel, CD
		-, Lower Austria, Araburg, EF
	C. personata (L.) Jacq	-, Styria, Schneealpe, EF
Cirsium	*C. arvense* (L.) Scop.	SLK, Modra, Tochova Chata, SF
		AUT, Vienna, EF
	C. vulgare (Savi) Ten.	POL, Gutkowo / Olsztyn, EF
	C. erisithales (Jacq.) Scop.	AUT, Styria, Schneealpe, EF
Onopordum	*O. acanthium* L.	AUT, Lower Austria, Buchberg, WL
		ITA, Southern Tyrol, Vinschgau, CD
Silybum	*S. marianum* (L.) Gaertn.	AUT, Lower Austria, Buchberg, EL
		SLK, Bratislava, Botanical Garden, SF
Rhaponticum	*R. scariosum* Lam.	LIE, GS
Centaurea	*C. jacea* L.	Austria, Karnabrunn, CD
		-, Vienna, EF
	C. scabiosa L.	-, Vienna, JS
		Poland, Gutkowo, Olsztyn, EF
		Switzerland, Graubünden, Lavin, CD
	C. cyanus L.	Austria, Vienna, EF
	(= *Cyanus segetum*	Germany, Baden-Württemberg, Kronau, CD
	Hill., Fischer *et al.)*	Poland, Mazury, Zabie, EF
Cnicus	*C. benedictus* L.	Botanical Garden of the Department of Pharmacognosy, University of Vienna

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Supporting Information

Detailed descriptions and pictures of all species mentioned in the text are available in the online version (Format: PDF, Size: ca. 5.3 MB): http://dx.doi.org/10.3797/scipharm.1010-05.

Authors' Statement

Competing Interests

The authors declare no conflict of interest.

References

[1] Jeffrey C.
Compositae.
In: Kubitzki K, ed. The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots.
Berlin Heidelberg: Springer Verlag, 2007: 61–77.

[2] Col MA.
Recherches sur l'appareil sécréteur interne des composées.
Journal de Botanique. 1903; 17: 252–318.

[3] Col MA.
Recherches sur l'appareil sécréteur interne des composées (Suite).
Journal de Botanique. 1904; 18: 110-174.

[4] Van Tieghem M.
Sur la situation de l'appareil sécréteur dans les composées.
B Soc Bot Fr. 1883; 30: 310–313.

[5] Van Tieghem M.
Sur la situation de l'appareil sécréteur dans la racine des composées.
B Soc Bot Fr. 1884; 31: 112-116.

[6] Ragonese AM.
Canales secretores en los organos vegetativos de *Eupatorium inulaefolium* H.B.K. (Compositae).
Acta Farm Bonaerense. 1988; 7: 161–168.

[7] Hayashi H.A., Appezzato-da-Gloria B.
Anatomy of the underground system in *Vernonia grandiflora* Less. and *V. brevifolia* Less. (Asteraceae).
Braz Arch Biol Technol. 2007; 50: 979–988.
doi:10.1590/S1516-89132007000700009

[8] Melo-de-Pinna G, Menezes N.
Meristematic endodermis and secretory structures in adventitious roots of *Richterago* Kuntze (Mutisieae-Asteraceae).
Rev Bras Bot. 2003; 26: 1–10.
doi:10.1590/S0100-84042003000100002

[9] Appezzato-da-Gloria B, Hayashi AH, Cury G, Soares MKM, Rocha R.
Occurrence of secretory structures in underground systems of seven Asteraceae species.
Bot J Linn Soc. 2008; 157: 789–796.
doi:10.1111/j.1095-8339.2008.00823.x

[10] Cury G., Appezzato-da-Glória B.
Internal secretory spaces in thickened underground systems of Asteraceae species.
Aust J Bot. 2009; 57: 229–239.
doi:10.1071/BT08139

[11] Fritz E, Saukel J.
Interxylary cork of *Saussurea discolor DC*. and *S. pygmaea* Spreng. (Asteraceae).
Biologia; In print.

[12] Fritz E, Saukel J.
Secretory structures of the Cardueae and their value for discrimination.
In prep.

[13] Länger R.
Orientierende Untersuchungen zur Wurzelanatomie einiger Cichorieaceen.
Sci Pharm. 1990; 58: 417–422.

[14] Fritz E.
PhD-Thesis.
Unpublished, 2010.

[15] Bock H.
Kräutterbuch.
Strassburg: Wilhelm Christian Glaser, 1539, reprint 1630.

[16] Fuchs L.
New Kreuterbuch.
Basel. 1543.

[17] Frohne D.
Heilpflanzenlexikon.
Stuttgart: Wissenschaftliche Verlagsgesellschaft mbH. 2006.

[18] Länger R, Kubelka W.
Phytokodex. Pflanzliche Arzneispezialitäten in Österreich 2001/2002.
Gablitz: Krause&Pachernegg GmbH. 2001.

[19] Gerlach S, Saukel J, Kubelka W.
Pflanzen in der österreichischen Volksmedizin - die „Volksmed-Datenbank".
Sci Pharm. 2006; 74 (Suppl 1): S36.
doi:10.3797/scipharm.oephg.19.OP

[20] Berendes J.
Des Pedanius Dioskurides aus Anazarbos Arzneimittellehre in fünf Büchern.
Stuttgart, 1902.

[21] Kardošová A, Ebringerováa A, Alföldia J, Nosál'ováb G, Fraňovób S, Hříbalovác V.
A biologically active fructan from the roots of *Arctium lappa* L., var. Herkules.
Int J Biol Macromol. 2003; 33: 135–140.
doi:10.1016/S0141-8130(03)00079-5

[22] Lonicerus A.
Kreuterbuch.
Grünwald / München: Kölbl. 1679, reprint 1962.

[23] Madaus G. (1938).
Lehrbuch der Biologischen Heilmittel.
Leipzig: Olms Verlag. 1938.

[24] Dos Santos AC, Baggio CH, Freitas CS, Lepieszynski J, Mayer B, Twardowschy A, Missau FC, dos Santos EP, Pizzolatti MG, Marques MC. Gastroprotective activity of the chloroform extract of the roots from *Arctium lappa* L.
J Pharm Pharmacol. 2008; 60: 795–801.
doi:10.1211/jpp.60.6.0016

[25] Lin SC, Chung TC, Lin CC, Ueng TH, Lin YH, Lin SY, Wang LY.
Hepatoprotective Effects of *Arctium Lappa* on Carbon Tetrachloride- and Acetaminophen-Induced Liver Damage.
Am J Chin Med. 2000; 163–173.
doi:10.1142/S0192415X00000210

[26] Mattioli PA.
New Kreuterbuch.
1563.

[27] Łotocka B, Geszprych A.
Anatomy of the vegetative organs and secretory structures of *Rhaponticum carthamoides* (Asteraceae).
Bot J Linn Soc. 2004; 144: 207–233.
doi:10.1111/j.1095-8339.2003.00251.x

[28] Kokoska L, Janovska D.
Chemistry and pharmacology of *Rhaponticum carthamoides*: A review.
Phytochemistry. 2009; 70: 842–855.
doi:10.1016/j.phytochem.2009.04.008

[29] Machatschek M.
Nahrhafte Landschaft: Ampfer, Kümmel, Wildspargel, Rapunzelgemüse.
3rd ed., Wien: Böhlau Verlag. 2007.

[30] Susanna A, Garcia-Jacas N.
Tribe Cardueae Cass. (1819).
In: Kubitzki K, eds. The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots.
Berlin Heidelberg: Springer Verlag, 2007. 123-146.

[31] Fischer MA, Oswald K, Adler W. (2008).
Exkursionsflora für Österreich, Liechtenstein und Südtirol. 3rd ed.
Austria: OÖ Landesmuseen, Land Oberösterreich. 2008.

[32] Pawłowskiego B, Jasiewicza A.
Flora Polska TOM XIII
Warszawa: Polska Akademia Nauk. 1972.

[33] Szafer W, Kulczyński S, Pawłowski B.
Rośliny Polskie.
4th ed. Warszawa: Panstwowe Wydawnictwo Naukowe. 1976.

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RESEARCH ARTICLE

Microscopical discrimination of the subterranean organs of medicinally used plants of the Cichorieae and their relatives

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Abstract

Context: Light microscopy is in most cases a quick method for the identification and discrimination of medicinally used plant drugs; moreover, this technique is very inexpensive. Reliable descriptions of the anatomy of plants and their adulterations are prerequisites for necessary purity controls.

Objective: The anatomy of the subterranean organs of 18 pharmaceutically useful as well as related but inconsiderable Asteraceae species from nine genera (*Taraxacum* F. H. Wigg., *Leontodon* L., *Scorzoneroides* Moench, *Hypochaeris* L., *Crepis* L., *Aposeris* Neck., *Cichorium* L., *Scorzonera* L., and *Tragopogon* L.; tribe Cichorieae, Asteraceae) is described in detail and graphically illustrated. Features characterizing and discriminating the studied taxa are presented and discussed.

Materials and methods: The roots/rhizomes of various species were examined by means of light microscopy.

Results: Useful anatomical characters were found for the discrimination between the species, and some of them were examined for the first time.

Discussion and conclusion: Discrimination of most genera and species investigated was possibly based on the anatomy of their underground parts. The identified characters may be effectively used for quality control of commercial drugs and the identification of adulterations.

Keywords: Root anatomy, Asteraceae, microscopy

Introduction

The Asteraceae, the sunflower family, comprise about 23,000 species and 1600 genera (Jeffrey, 2007), many of which have long been used for medicinal purposes. *Taraxacum* F. H. Wigg. sp., *Scorzonera* L. sp., and *Cichorium intybus* L. (Asteraceae) are few examples out of the existing diversity with a long tradition in conventional and traditional medicine all over the world.

The genus *Taraxacum* is a widespread taxon, first mentioned against ailments of the eyes in the 13th century (Madaus, 1938), and its species have been used for diverse cures since then without any special differentiation. Until now, *Taraxacum* is popular for its effects as an amarum and cholereticum against absence of appetite and disorders of the gastrointestinal tract (Frohne, 2006). *Taraxacum* is also famous for its use in slimming diets and spring makeovers due to its diuretic effects and against diabetes (Gerlach et al., 2006; Šarić-Kundalić et al., 2010). The herb and the roots are established not only in traditional but also in conventional medicine, for example, compound of species cholagogae (OEAB, 2009) and various preparations against diseases of liver and gall (Länger & Kubelka, 2001).

C. intybus is an ancient medical plant. Dioskurides (according to Berendes, 1902) mentioned it to be an astringent and good for the stomach. Mattioli (1563) applied a kind of syrup of the roots against liver problems and packs of leafs and roots in combination with rose water against skin diseases. The roots also served as a substitute for coffee. Nowadays, the roots are mainly used for dyspeptically disorders and are ingredients of various teas (Frohne, 2006).

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Scorzonera hispanica L. is mainly known as a vegetable used for salads or as a side dish. Like *C. intybus*, its roots are also served as a kind of coffee. Until the 16th century, *S. hispanica* was considered efficacious against snake bites (Diemair, 1945; Genaust, 1996). A study about 10 years ago (Jantzen, 1999) confirmed the potency of this plant against hemorrhages caused by snake venoms. Investigations among people these days showed that *S. hispanica* is still being used in traditional medicine against disorders of stomach, gall bladder, and liver, and against adenophyma and is compiled in the so-called VOLKSMED database (Gerlach et al., 2006).

Both modern and traditional medicine use plant organs for the preparation of drugs, usually as a cut formulation or as a powder. In praxis, the identification of drugs and required purity tests are mainly done by microscopic means based on discriminative anatomical features. Hence, proper knowledge on the anatomy of the diverse parts of the plants used as drugs is required for drug analysis.

However, respective comparative studies on subterranean organs of pharmaceutically exploited Asteraceae species and their close relatives (occurring as adulterations) are rare. Some works have been done on resin ducts and cavities as well as on laticiferous vessels of diverse Asteraceae (Van Tieghem, 1883, 1884; Van Vuillemin, 1884; Col, 1903, 1904) in the second half of the 19th century. Most available studies though dealt with the aerial vegetative organs. A few recent results concern the anatomy, particularly the secretory structures, of roots and rhizomes (Ragonese, 1988; Melo-de-Pinna & Menezes, 2003; Appezzato-da-Gloria et al., 2008), although they do not cover species interesting in the course of medicine and growing in Western Europe.

Comparative studies on the root anatomy of the Cichorieae are available so far for *Taraxacum* species and the genera *Hypochaeris* L., *Leontodon* L., and *Aposeris* Neck. (Länger, 1990). *Taraxacum*, for instance, could be discriminated from the other genera by the lack of libriform fibers and of bordered vessels, the presence of laticifers arranged in concentric circles, and the diameter of the vessels. However, these characters may discriminate among the chosen restricted set of taxa only, but might fail if further species are added. In addition, intraspecific and even intraindividual variation has to be carefully considered in order to identify reliable taxonomically informative characters. Depending on the position along the root axis, the dimensions of vessels, for instance, may differ largely within one plant (Fritz & Saukel, 2011). Hence, standardization of the part of an analyzed root, as well as selection of a representative taxonomic sample, is an urgent requirement in comparative anatomical studies.

In this study, we will analyze the anatomical structure of the subterranean plant parts of nine Asteraceae genera (*Taraxacum*, *Leontodon*, *Scorzoneroides* Moench, *Hypochaeris*, *Crepis* L., *Aposeris*, *Cichorium*, *Scorzonera*, and *Tragopogon* L.) from the tribe Cichorieae (Asteraceae). The anatomy of roots and rhizomes will be analyzed in detail by means of light microscopy and a database of identified anatomical features created. Based on these results, we will try to extract characters useful for the discrimination of the analyzed species. This will involve pharmaceutically useful plants as well as inconsiderable relatives.

Materials and methods

Plant material

The plant material used for examination included 19 species from the Cichorieae occurring naturally in Austria as listed in Table 1 (plants arranged according to classification in Fischer et al., 2008). Taxa were chosen, respectively, to their use in medicine (e.g., *Taraxacum* sp., *C. intybus*) and their role as possible adulteration. Fully development roots were collected during or following antheses.

Vouchers are deposited in the herbarium of the Department of Pharmacognosy, University of Vienna (WUP). The plant material was identified by the authors using floristic manuals covering the sampled geographic areas (Pawłowskiego & Jasiewicza 1972; Szafer et al., 1976; Fischer et al., 2008). As the anatomy may vary with environmental conditions (e.g., soil parameters) and geography, species were collected from three to six localities if possible.

Anatomical analysis

The samples were examined by means of light microscopy. For preparation, based on a traditional method of the University of Vienna, the desiccated roots were boiled in water for 10 min to soften the tissue and subsequently inundated in 96% ethanol for dehydration. After evaporating the alcohol for a few minutes, transverse and longitudinal sections were obtained by free hand sectioning about 1.5 cm below the hypocotyle as this position proofed to provide the most information about the root anatomy. Sections obtained by hand cutting were embedded in few drops of chloral hydrate (60% in water) and examined using a Nikon Optiphot-2 light microscope equipped with a Samsung Digimax V50 Digital Camera.

Results

Taraxacum sp.

In line with the results of Länger (1990), the studied *Taraxacum* species, *Taraxacum cucullatum* Dahlst. and *Taraxacum laevigatum* DC., were indistinguishable from each other based on root anatomy. The following description applies to both taxa: the plants possess an allorhizous root system with a taproot (Figure 1).

Microscopical description of the secondary root (Figures 1B and 2): The root develops a thin-walled

Table 1. List of species examined in this study (plants arranged according to the classification applied by Fischer et al., 2008).

Genus	Species	Location of collection
Aposeris	*A. foetida* Cass.	Austria, Carinthia, Feistritz im Rosental (JS)Germany, Garmisch-Partenkirchen, Wank (CD)
Scorzonera	*S. hispanica* L.	Austria, Lower Austria, Anninger (JS)—, —, Weinling (JS)
	S. humilis L.	—, —, Drosendorf (JS)
	S. purpurea L.	—, Vienna (EF)
	S. aristata Ramond ex DC.	—, Carinthia, Lesachtal, Lumkofel (JS)
	S. austriaca Willd.	—, Vienna (EF)
	S. rosea Waldst. & Kit.	Austria, Carinthia, Lesachtal, Lumkofel (JS)
Tragopogon	*T. orientalis* L.	—, Lower Austria, Araburg (EF)—, —, Laaben (EF)Poland, Gutkowo, Olsztyn (EF)
	T. dubius Scop.	Austria, Vienna (EF)Italy, Vinschgau, Schluderns (CD)
Crepis	*C. pontana* Druce	Austria, Carinthia, Lesachtal, Lumkofel (JS)
Taraxacum	*T. cucullatum* Dahlst.	—, Salzburg, Riedingtal (JS)
	T. laevigatum DC.	—, Vienna (JS)
Leontodon	*Leontodon incanus* Schrank	—, Burgenland, St. Margarethen (JS)—, Steiermark, Oberwölz (JS)—, Lower Austria, Baden (JS)—, —, Anninger (JS)
	Leontodon hispidus Scop.	—, —, Rekawinkel (EF)—, —, Rax (EF)—, Carinthia, Pöllatal (JS)
Scorzoneroides	*Scorzoneroides helvetica* (Merat) Holub	—, Steiermark, Etrachtal (JS)—, Steiermark, Prebertal (JS)—, Anger, Lessachwinkel (JS)
	Scorzoneroides autumnalis (L.) Moench	Poland, Gutkowo, Olsztyn (EF)
Hypochaeris	*Hypochaeris uniflora* Hoffm.	Austria, Salzburg, Lungau (JS)—, Salzburg, Kareck (JS)
	H. radicata L.	Poland, Gutkowo, Olsztyn (EF)Botanischer Garten Berlin-Dahlem; 916; DE-0-B-2421281: Bayern, Kreis Wunsiedel, Fichtelgebirge, leg. HempelItaly, Eisacktal, Blumau-Rielinger-Atzwang (JS)
Cichorium	*C. intybus* L.	—, Lower Austria, Pressbaum (EF)—, Vienna (EF)Poland, Gutkowo, Olsztyn (EF)Botanical Garden Berlin-Dahlem: DE-0-B-2003105: Brandenburg, Kreis Havelland, Falkensee, leg. Dürbye 3090

Plant materials were collected by Elisabeth Fritz (EF), Christoph Dobeš (CD), and Johannes Saukel (JS).

phellem, the cortex gets lost due to the secondary growth and the formation of rhytidome. Within the secondary phloem, which is dominating in extension, laticiferous vessels are arranged in concentric circles. Phloem fibers are absent. The secondary xylem comprises tracheary elements and xylem parenchyma only, with reticulate vessels (in diameter up to 50 µm in *T. cucullatum* and 61 µm in *T. laevigatum*) irregularly arranged. Medullary rays are not found. No secretory structures besides the laticifers are differentiated, crystalloids and sclereids are absent.

The analysis of various roots and rhizomes of *Taraxacum* sp. and related taxa (*Leontodon* sp., *Scorzoneroides* sp., *Hypochaeris* sp., *Crepis pontana* Druce, *Aposeris foetida* Cass.) showed the following anatomical features to be the most important to be used in their identification and differentiation: the overall structure of the vascular cylinder, with the distribution of vessels, and the presence or absence of medullary rays, the occurrence of fibers in the secondary xylem, and the types of perforation plates in the vessels (Figure 3).

The diameter of the largest vessels cannot serve as a useable feature, as the ranges of the diverse species mainly overlap. Besides, our studies confirm the importance to define a point of the root where the cross section for the measurements is to be taken as the diameter of the vessels may vary greatly even within a

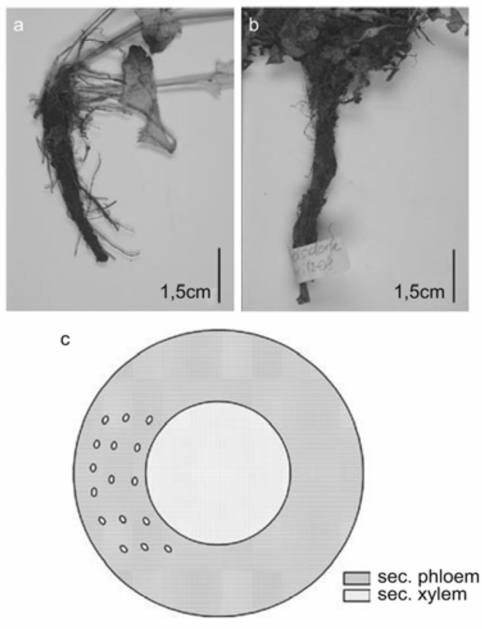


Figure 1. Taproot of (A) *T. cucullatum* and (B) *T. laevigatum*. (C) Schematic view of *Taraxacum* sp. secondary root in transverse section: small ellipses mark the position of the laticifers within the dominating secondary phloem (gray), secondary xylem with vessels dispersed; medullary rays are not found.

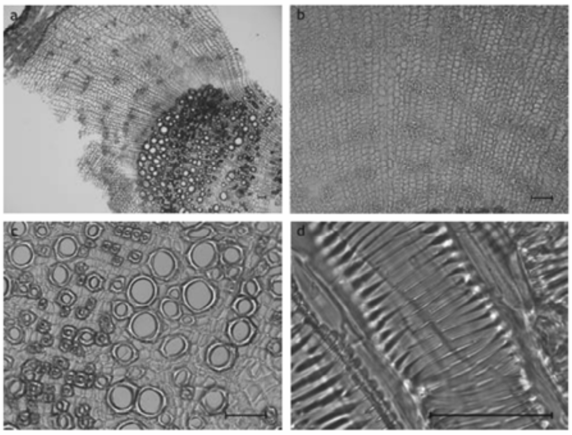


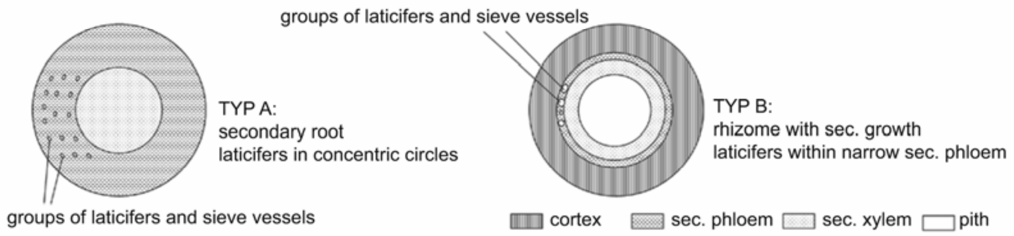
Figure 2. *Taraxacum* roots. (A) Overview showing the extension and arrangement of the diverse tissues: cortex lost due to formation of rhytidome, secondary phloem dominating, laticiferous vessels within secondary phloem circularly arranged, medullary rays in secondary xylem missing; (B) secondary phloem with laticiferous vessels arranged in concentric circles; (C) secondary xylem with vessels irregularly dispersed over transverse section, fibers missing; (D) reticulate vessels of secondary xylem [(A, C) *T. laevigatum* (TL01-08), (B, D) *T. cucullatum* (TC03-08)]; (A–C) transverse sections; (D) longitudinal section; scale bars = 50 μm.

single plant (Fritz & Saukel, 2011), for example, samples of *Hypochaeris radicata* showed a great variability with 34 μm 1.5 cm below the hypocotyle, 72 μm at the tip of the root. The numbers given in Figure 3 were all derived from sections beneath the hypocotyle as mentioned in "Material and Methods."

Scorzonera hispanica

S. hispanica develops a long rhizome, presumably with taproot (Figure 4A).

Microscopical description of the secondary root (Figures 4B and 5): The cortex gets lost in course of the formation of rhytidome; the emerging phellem is thin-walled and thoroughly parallel-laminated, sometimes with crystalloids. The layer of secondary phloem is dominant, of similar extension as the vascular cylinder, with laticiferous vessels arranged in rows in fascicular position in continuation of the vessels of the secondary xylem as well as in interfascicular position, also irregularly dispersed. Outer parts of the secondary phloem often appear obliterated and collapsed. No phloem fibers were found. The secondary xylem is dominated by parenchymatous cells, and the vessels (reticulate, up to 60 μm in diameter) are aggregated into single or double radial rows, separated by wide parenchymatous rays.



	TYP A	TYP B	medullary rays existing	fibers in sec. xylem	vessels bordered	diameter of largest vessels
Taraxacum cucullatum	+	–	–	–	–	27–50 μm
Taraxacum laevigatum	+	–	–	–	–	48–61 μm
Leontodon incanus	+	–	–	+	+	39–54 μm
Leontodon hispidus	–	+	+	+	+	18–30 μm
Scorzoneroides helvetica	–	+	+	+	+	20–23 μm
Scorzoneroides autumnalis	–	+	+	+	+	30–34 μm
Hypochaeris radicata	+	–	+	+	+	34–45 μm
Hypochaeris uniflora	+	–	–	+	+	34–52 μm
Crepis pontana	+	–	+	–	–	52–68 μm
Aposeris foetida	–	+	+	–	+	34–45 μm

Figure 3. Important characters for the discrimination between *Taraxacum* sp. and related taxa.

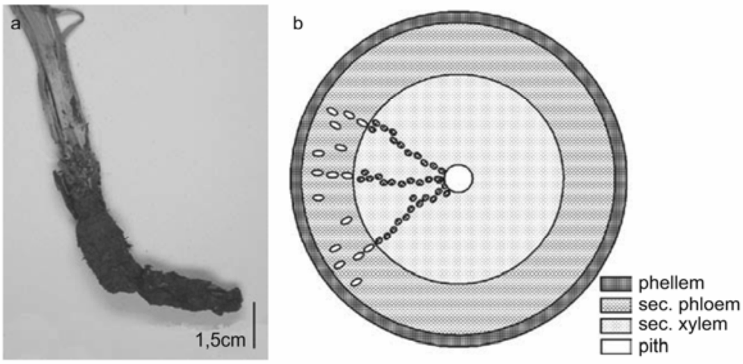


Figure 4. (A) Rhizome of *S. hispanica*. (B) Schematic section of rhizome: small ellipses mark the position of the laticifers; vascular cylinder with vessels (dots) arranged in single or multiple radial rows.

The pith is often collapsed. Xylem fibers and sclereids are absent.

The root anatomy of the other examined species of the genus *Scorzonera* (*Scorzonera humilis* L., *Scorzonera purpurea* L., *Scorzonera aristata* Ramond ex DC., *Scorzonera rosea* Waldst. & Kit.) with the exception of *Scorzonera austriaca* Willd. showed to be similar to *S. hispanica* apart from the structure of the phellem (either thin-walled as within *S. purpurea*, *S. humilis* (additional sclereids) or thick-walled as within *S. aristata*, *S. rosea*).

S. austriaca represented considerably different anatomical structure from all other taxa investigated due to its irregular secondary growth. Bundles of phloem and xylem separated by a cambium are irregularly dispersed over the transverse section, each undergoing secondary growth (illustrated in Figure 8).

Tragopogon orientalis L. and *Tragopogon dubius* Scop.

The following description applies to both examined species of the genus: The root system is allorhizous, and the species possess taproots.

Microscopical description of the secondary root: A periderm with a thin-walled phellem, sometimes containing crystalloids, replaces the rhizodermis. A distinct endodermis separates the small layer of the enduring cortex from the broad secondary cortex, whose extension is comparable with the vascular cylinder. Within the secondary phloem, laticiferous vessels are aggregated into rows in fascicular position "continuing" the formation of the vessels of the secondary xylem, and they may also be irregularly dispersed within the outer part of the phloem. The vessels of the secondary xylem are arranged in single and double rows, cumulative at the outer part, separated by broad, and often obliterated medullary rays. Mainly strongly bordered pits are present. The diameter of the tracheary elements may reach up to 68 µm. Fibers occur particularly within the outer part of the xylem, and sclereids are not found.

Cichorium intybus

Typical for *C. intybus* is its long taproot (Figure 6A).

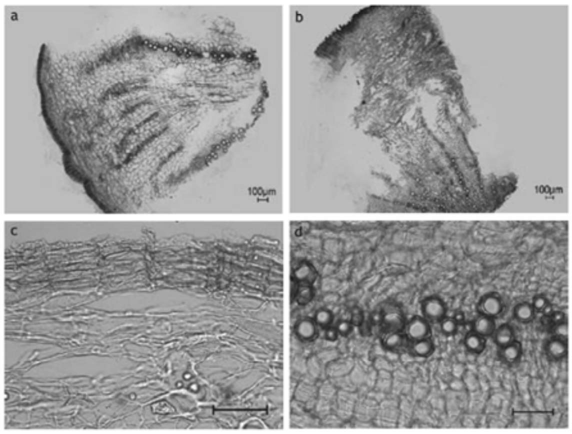


Figure 5. *S. hispanica* rhizome. (A, B) Overview showing the extension and arrangement of tissues: secondary phloem with laticiferous vessels arranged in rows, fibers are missing (A), secondary xylem dominated by parenchymatous cells with fibers missing, vessels arranged in single to multiple rows (B) (SHI03); (C) phellem thin-walled, thoroughly parallel-laminated (SHI02); (D) vessels of secondary xylem more or less in single or double rows (SHI02); (A–D) transverse sections; scale bars = 50 µm.

Microscopical description of the secondary root (Figures 6B and 7): On the surface of the root, a thin-walled phellem, rarely comprising crystalloids, develops. A small layer of the cortex remains despite secondary growth. The secondary phloem is dominant, comparable in extension to the vascular cylinder, with laticiferous vessels arranged in rows. Phloem fibers are missing. The secondary xylem contains fibers, and the vessels are arranged in rows or dispersed groups, short in longitudinal view, reticulate, mainly strongly bordered, up to 109 µm in diameter. The root lacks sclereids.

Discussion

The studied *Taraxacum* species can be characterized as well as distinguished from related genera based

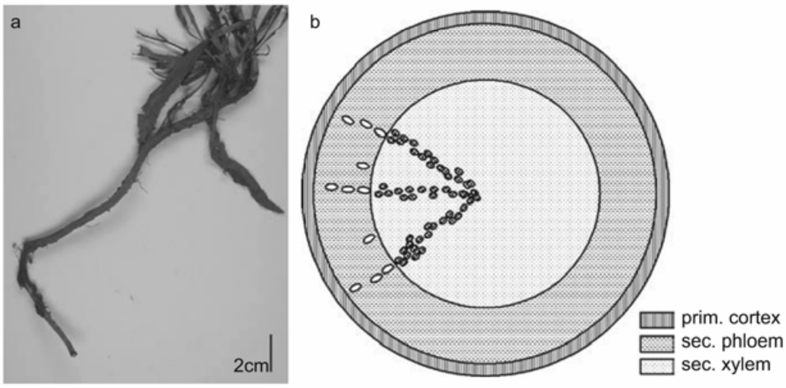


Figure 6. (A) Taproot of *C. intybus*. (B) Schematic view of *C. intybus* root in transverse section: secondary root: small ellipses mark the laticifers, dots within xylem are vessels.

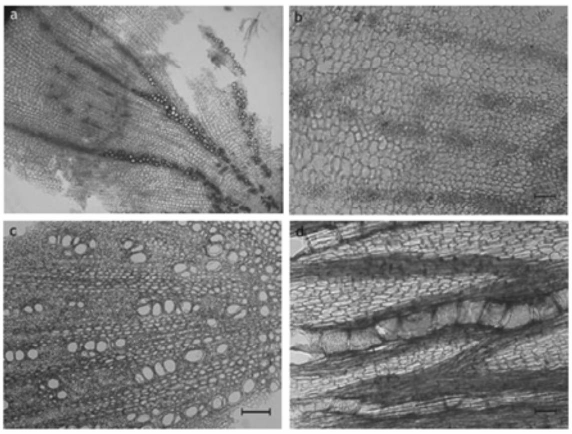


Figure 7. *C. intybus* root. (A) Overview showing the extension and arrangement of tissues: secondary phloem and secondary xylem are of similar extension (S5); (B) secondary phloem with lactiferous vessels arranged in rows (CI1-08); (C) secondary xylem with vessels in groups and short rows (CI1-08); (D) secondary xylem: vessels and rays (CI2-07); (A–C) transverse sections; (D) longitudinal section; scale bars = 50 µm.

on various anatomical root features—amongst others, the absence of medullary rays, the lack of fibers in the secondary xylem, and the types of perforation plates of the vessels (Figure 3). *Taraxacum* is one of the few (along with *A. foetida* and *C. pontana*) plants with underground organs completely missing fibers. Furthermore, root vessels are of the reticulate type in *Taraxacum*, but bordered in all other studied taxa except for *C. pontana*.

The diameter of vessels cannot be used as reliable taxonomic characters as the size ranges of the taxa overlap. Furthermore, our studies confirmed a possibly great variability of the dimensions of the vessels even within one plant depending on the position along the root axis from which the sample is taken (Fritz & Saukel, 2011). Hence, measurements of vessels have to be carefully considered with regard to their pharmaceutical use for the identification of cut roots/rhizomes in which case a localization of a certain position within a root cannot be provided.

The root anatomy of *S. hispanica* was similar to that of the other species of the genus as well as to *Tragopogon*. However, the two genera can be distinguished based on the vessels. In *Scorzonera*, the vessels were reticulate, whereas *Tragopogon* mainly possessed strongly bordered vessels. Nevertheless, no distinction could be made between *T. orientalis* and *T. dubius* by means of light microscopy. In contrast, diverse species of the genus *Scorzonera* could be distinguished based on phellem characteristics (Figure 8).

An exceptional case represents *S. austriaca*, whose anatomical structure differs clearly from all other species investigated due to its irregular secondary growth.

The root of *C. intybus* is anatomically indistinguishable from *Tragopogon*. As these species are morphologically largely distinct, adulterations can easily be identified based on above ground organs.

Conclusions

The identification and discrimination of used drugs by microscopical means is a well-known and often used method in pharmacognosy. According to the diverse anatomical characters identified in this study, the nine investigated genera could be distinguished based on root and rhizome anatomy. *Taraxacum*, as a pharmaceutically important plant, can thus be clearly discriminated from all other genera examined. A mix-up with other medicinally used species may easily be identified, as the root anatomy of *C. intybus* (differing in the arrangement of laticifers, the possession of libriform fibers) and of *Scorzonera* (differing in the formation of vascular cylinder and the phellem) is largely different.

A further discrimination of species within a single genus, however, may be restricted using root anatomy alone. Respective results differed, with *Taraxacum* species being indistinguishable, but members of the genera *Scorzonera* and *Tragopogon* could be differentiated using phellem and vessel characteristics, respectively.

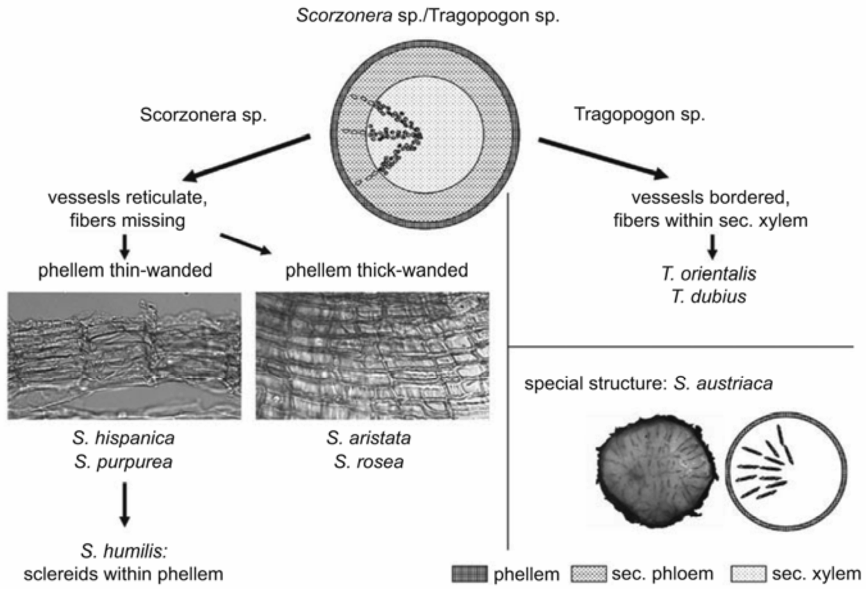


Figure 8. Differentiation of *Scorzonera* sp. and *Tragopogon* sp.; *S. austriaca*: transverse section treated with phloroglucine-HCL, lignified tracheids of the xylem parts red-colored.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Appezzato-da-Gloria B, Hayashi AH, Cury G, Soares MKM, Rocha R. (2008). Occurrence of secretory structures in underground systems of seven Asteraceae species. *Bot J Linn Soc* 157:789–796.

Berendes J. (1902). Des Pedanius Dioskurides aus Anazarbos Arzneimittellehre in fünf Büchern. Stuttgart: *Ferdinand Enke Verlag.*

Col MA. (1903). Recherches sur l'appareil sécréteur interne des composé*es. J Bot* (Desvaux) 17:252–318.

Col MA. (1904). Recherches sur l'appareil sécréteur interne des composé*es (Suite). J Bot* (Desvaux) 18:110–174.

Diemair W. (1945). Die Haltbarmachung von Lebensmitteln. Stuttgart: *Ferdinand Enke Verlag,* p. 52.

Fischer MA, Oswald K, Adler W. (2008). Exkursionsflora für Österreich, Liechtenstein und Südtirol, 3rd ed. Austria: OÖ Landesmuseen, Land Oberösterreich.

Fritz E, Saukel J. (2011). Anatomy of subterranean organs of medicinally used Cardueae and related species and its value for discrimination. *SciPharm*.79:157–174.

Frohne D. (2006). Heilpflanzenlexikon. Stuttgart: *Wissenschaftliche Verlagsgesellschaft mbH.*

Genaust H. (1996). Etymologisches Wörterbuch der botanischen Pflanzennamen, 3rd ed. Basel Boston Berlin: Birkhäuser.

Gerlach S, Saukel J, Kubelka W. (2006). Pflanzen in der österreichischen Volksmedizin—die "Volksmed-Datenbank." *SciPharm* 74 (Suppl. 1):36.

Jantzen K. (1999). Chemische und biochemische Untersuchungen von Pflanzeninhaltsstoffen aus *Calendula officinalis* und *Scorzonera hispanica* und ihre Wirksamkeit gegen Schlangengifte. Ph.D. Thesis, *University of Hannover.*

Jeffrey C. (2007). Compositae. In: Kubitzki K, ed. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Berlin, Heidelberg: Springer Verlag, pp. 61–77.

Länger R. (1990). Orientierende Untersuchungen zur Wurzelanatomie einiger Cichorieaceen. *SciPharm* 58:417–422.

LängerR, KubelkaW. (2001). Phytokodex. PflanzlicheArzneispezialitäten in Österreich 2001/2002. Gablitz: Krause&Pachernegg GmbH.

Madaus G. (1938). Lehrbuch der Biologischen Heilmittel. Leipzig: *Olms Verlag.*

Mattioli PA. (1563). New Kreüterbuch. Prag: Georgen Melantrich von Auentin.

Melo-de-Pinna G, Menezes N. (2003). Meristematic endodermis and secretory structures in adventitious roots of *Richterago* Kuntze (Mutisieae-*Asteraceae). Rev Bras Bot* 26:1–10.

Oesterreichisches Arzneibuch (OEAB). (2009). Pharmacopoeia Austriaca. Wien: Verlag Österreich GmbH.

Pawłowskiego B, Jasiewicza A. (1972). Flora Polska TOM XIII, Warszawa: *Polska Akademia Nauk.*

Ragonese AM. (1988). Canales secretores en los organos vegetativos de *Eupatorium inulaefolium* H.B.K. (Compositae). *Acta Farm Bonaerense* 7:161–168.

Šarić-Kundalić B, Dobeš Ch, Klatte-Asselmeyer V, Saukel J. (2010). Ethnobotanical study on medicinal use of wild and cultivated plants in middle, south and west Bosnia and Herzegovina. *J Ethnopharmacol* 131:33–55.

Szafer W, Kulczyński S, Pawłowski B. (1976). Rośliny Polskie, 4th ed. Warszawa: Panstwowe Wydawnictwo Naukowe.

Van Tieghem M. (1883). Sur la situation de l'appareil sécréteur dans les composées. *B Soc Bot Fr* 30:310–313.

Van Tieghem M. (1884). Sur la situation de l'appareil sécréteur dans la racine des composées. *B Soc Bot Fr* 31:112–116.

Van Vuillemin M. (1884). Remarques sur la situation de l'appareil sécréteur des composées. *B Soc Bot Fr* 31:108–110.

Anatomical Features suitable for Phylogenetic Research?

Elisabeth Fritz, Johannes Saukel

ABSTRACT

Taxonomy mainly relies on micro- and macro-morphology, karyology, DNA-molecular variation and a limited number of microscopic characters such as pollen anatomy or stomata length. This study, in contrast, estimates the taxonomic value of the anatomy of the roots and rhizomes of 59 species and 12 subtribes of the *Cardueae* and *Cichorieae* and compares the anatomy to a nuclear ribosomal DNA sequence-based phylogenetic reconstruction of the tribes. The subterranean organs were analysed by means of light microscopy, supplying a databank of typical anatomical characters. Based hereupon, a binary decision tree to show the separabilities of the species based on the anatomical characters coded as binary variables is presented. Furthermore, a discriminant function analysis (DFA) was performed. Using successive canonical roots, accessions clustered – except one sample – according to their subtribal affiliation. A novel method for the comparison of different categorical information about group membership of OTU´s (CLUST_META) is presented.

INTRODUCTION

The Asteraceae, the sunflower family, represent one of the largest families of plants comprising more than 23,000 species and about 1,600 genera (Jeffrey, 2007). The plant family is currently divided into 12 subfamilies, three of which (Asteroideae, Cichorioideae, Carduoideae) occur in Europe (Panero & Funk, 2008; Stevens, 2001). This study concentrates on native Central European representatives of the tribes Cardueae (Carduoideae) and Cichorieae (Cichorioideae).

In the last few years much phylogenetic work on Asteraceae was carried out, resulting in many taxonomical changes.

The Cardueae comprises over 2,360 species organized in 73 genera (Susanna & Garcia-Jacas, 2007). The tribe exhibits a conspicuous morphological diversity and holds some of the most species-rich genera of the Asteraceae. Traditionally, the Cardueae were divided into four subtribes: Echinopsinae, Carlininae, Carduinae, Centaureinae. However, this tribal classification was diversely discussed in the past (Susanna & Garcia-Jacas, 2009), e.g. the subtribe Echinopsinae was classified as a separate tribe by Wagenitz (1976). After various taxonomic rearrangements, recently, a new classification of the Cardueae comprising five subtribes – the four subtribes mentioned above plus the fifth subtribe Cardopatiinae – was suggested (Susanna and Garcia-Jacas, 2007).

The tribe Cichorieae includes about 93 genera. The reported species numbers, however, differ widely due to the numerous hybrid species and agamospecies described for the genera *Hieracium, Pilosella*, and *Taraxacum* (Kilian et al., 2009). These taxonomically diverse genera excluded, the tribe comprises approximately 1,500 species. Based on a recent molecular study, the Cichorieae are divided into eleven subtribes, eight of which have been included in this study: Scorzonerinae, Lactucinae, Hyoseridinae, Crepidinae, Hypochaeridinae, Chondrillinae, Hieraciinae, and Cichoriinae (Kilian et al., 2009).

The taxonomy is mainly based on morphology, karyology and DNA-molecular data (e.g. Stebbins, 1953, Saukel & Länger, 1990, Guo et al, 2008, Garcia-Jacas et al., 2002, Sánchez-Jiménez et al., 2010). In addition, pollen shape proved taxonomically important in some cases (e.g. genus *Centaurea;* Wagenitz, 1955; Özler et al, 2009). Furthermore, micro-morphological characters such as length of trichomes and trichome shapes play an important role in plant taxonomy (Jeffrey, 1966, in: Susanna & Garcia-Jacas, 2009; Hayat et al., 2009). Rarely only, taxonomic studies dealed with microscopic characters like stomata lengths (e.g. Saukel & Länger, 1992).

In particular, studies combining microscopic characters with molecular data generally were rarely performed. For instance, Figueroa et al. (2008) found anatomical root characters (e.g. tilosome distribution, lamellate type, velamen cell-wall thickenings) to be phylogenetically informative in the Cranichideae (Orchidaceae). Respective studies regarding anatomy of subterranean organs, however, so far were not performed for the Asteraceae.

The present work assesses the value of diverse anatomical features of the subterranean organs to infer the phylogeny of the Cardueae and Cichorieae.

Thus, the anatomy of the subterranean organs of of 59 species (out of 33 genera / 12 subtribes) from the *Cardueae* and *Cichorieae* was analysed in detail and an image database (comprising over 5500 pictures) forming the basis of a databank of typical anatomical features created. Selected parts dealing with main principles of anatomical structures have already been published (Fritz & Saukel, 2011a, b, c, d). The present study is based on the complete data set and deals with its statistically evaluation in combination with DNA data of the taxa.

The molecular data used DNA sequence data from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA, including ITS1, 5.8S and ITS2 taken from NCBI (http://www.ncbi.nlm.nih.gov/genbank/).

In particular, the following aims will be addressed: (1) Is a discrimination of species on the basis of anatomical characters of the subterranean organs by microscopical means possible, (2) which characters are responsible for discrimination and (3) is there any correlation between the anatomy and the ITS-data in respect to the arrangement of species/genera?

MATERIAL AND METHODS

Plant material

The plant material used for examination comprises 59 species from 33 genera and 12 subtribes of the two tribes Cardueae and Cichorieae. Intraspecific variation of anatomical features due to environmental conditions (e.g. soil parameters and geography) was addressed by examination of several vouchers coming in most cases from different locations. In total, 188 specimens were studied. The plant material was collected during or following antheses (Austria, Germany, Italy, Liechtenstein, Slovakia, Switzerland and Poland) and from plants cultivated in the Botanical Garden of the Department of Pharmacognosy (University of Vienna). 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 (Fischer et al., 2008; Tutin et al, 1976; Pawłowskiego & Jasiewicza, 1972; Szafer et al*.,* 1976). A material list including the collection history and voucher numbers is provided in the Appendix.

Anatomical analysis

The preparation for the anatomical analysis was based on a traditional method of the University of Vienna (Fritz & Saukel, 2011a). To enable comparability between the investigated specimen and in order to exclude variation according to the longitudinal position of the root axis in anatomical root characters (e.g. diameter of vessels), the longitudinal position of sections along the root axis was standardized following Fritz & Saukel (2011a). Thus, sections were taken 1.5 – 2.5 cm below the insertion of the rosette leaves or beginning of the superterranean shoot axis, respectively. In addition, plants were collected in the same growth phase, i.e. during or shortly after anthesis. All information collected in the database refers to this part of the plant. Comparisons with sections of the tip of the root and about half in between showed this position providing the best information about the anatomy of subterranean organs. An example of a character with its different states is given in Fig. 1. Sections were embedded in few drops of chloral hydrate (60% in water) and observed using a Nikon Optiphot–2 light microscope and a Nikon Eclipse E 600 fluorescence microscope with UV Ex filter 330–380. Images were taken using a Samsung Digimax V50 Digital Camera.

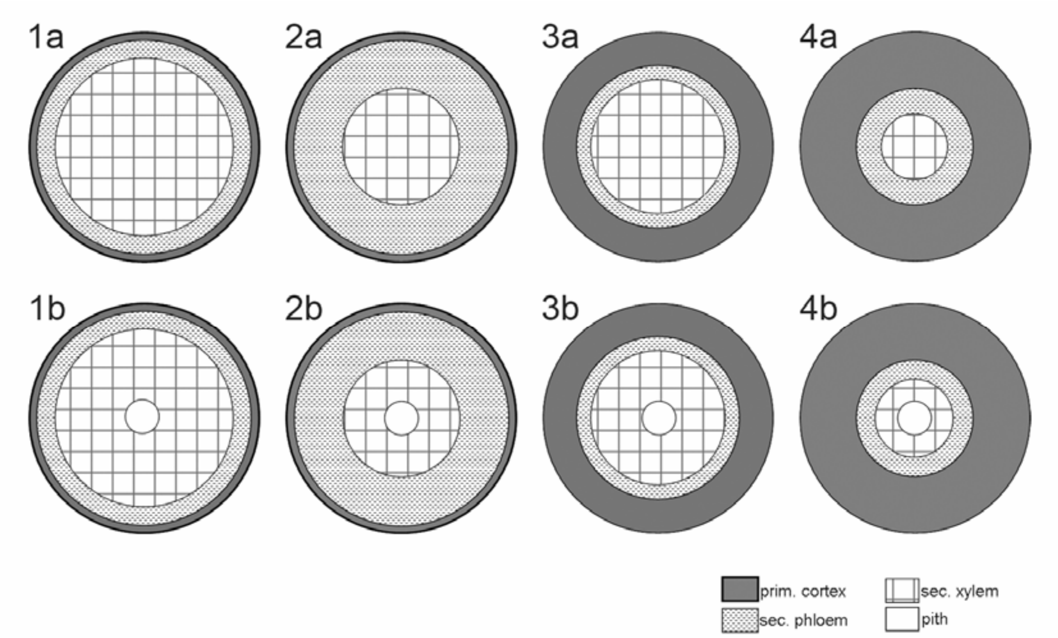

Fig. 1: Schematic view of the different types of specifications resulting of binary characters of the transverse sections (a – root, b- rhizome): 1: secondary xylem dominating in extension; 2: secondary phloem and secondary xylem similar in extension; 3: cortex and secondary xylem similar in extension; 4: cortex dominating;

Compilation of anatomical database

A database of anatomical characters was established based on 5,500 photographs and drawings taken from the prepared sections. First, the optical information obtained was transformed into clearly defined characters. Sixty mostly nominal characters were defined. For computational purposes, all multistated characters (30) were converted into binary-coded variables (0, absent; 1, present), altogether finally resulting into eighty-two traits (1 quantitative and 81 qualitative, Table 1).

Calculations

Ribosomal nuclear DNA sequences including part of the internal transcribed spacer 1 (ITS1), the complete 5.8S gene, and part of the ITS2 were obtained from NCBI (http://www.ncbi.nlm.nih.gov; accession numbers given in the Appendix). In several cases only sequences from taxa of the next higher order or from close relatives were available.

Table 1. Quantitative and qualitative traits recorded for the database (final version) and statistical analysis.

Quantitative character
Diameter of vessels max. (µm)
Qualitative characters
Life form – annual / biennial / plurennial / perennial
Subterranean organ – rhizome / rhizome part of taproot/ taproot / fibrous root system / special forms: interxylary cork results in splitting of the root into various strands (Fritz & Saukel, 2011b); irregularly secondary growth: bundles of phloem and xylem separated by a cambium are irregularly dispersed over the transverse section (Fritz & Saukel, 2011d)
Dominating in extension – xylem / secondary phloem / cortex (see fig. 1)
Cortex enduring
Endodermis clearly visible
Fibers within xylem / secondary phloem
Sclereids within cortex / secondary phloem / xylem / phellem / pith
Size of endodermal resin ducts (multiple of the surrounding cells / not extraordinary / primary state / 4 epithel cells / more than 4 epithel cells / missing)
Secretory ducts of type SD1 (C1:C2 of <0.3: Fritz & Saukel, 2011c) within sec phloem / xylem / position fascicular / interfascicular / independent
Secretory ducts of type SD2 (C1:C2 of >0.4: Fritz & Saukel, 2011c) within sec phloem/ xylem / pith / position fascicular/ interfascicular / independent
Secretory ducts of type SD3 (lysigenous development: Fritz & Saukel, 2011c) within sec phloem / fascicular
Secretory ducts of type SD4 (Fritz & Saukel, 2011c) within cortex / secondary phloem / xylem / pith
Secretory cavities
Lacticifers in concentric circles / radiant rows / within small phloem_ formation not distinguishable / disordered / located above medullary rays / located above medullary rays just within the outer layers / missing
Phellem present / 1-5 layered / multilayered/ layers clearly laminated / cells: thin-walled / thick-walled
Vessels reticulate / simple pits / pits with faint border / pits with conspicuous border
Medullary rays uni- or biseriate / up to 5 rows / more than 5 rows / not visible
Cells walls of pith thin-walled / slightly thickened / irregularly thickened / nodularly thickened / thickened / rarely pitted
Vessels arranged in circles / in groups below or next to each other / in rows (1-2) / in rows (>2) / irregularly
Crystalloids within phellem
Crystal needles
Cortex divided into inner and outer cortex

However, as the emphasis of the analyses was laid on the different subtribes and genera and most times, only 2 species of the same genus were examined, the infrageneric phylogeny could be neglected. The following substitutes for the species lacking available DNA but being investigated by microscopical means were chosen: *Picris hieracioides* subsp. *grandiflora* and *Picris hieracioides* subsp. *hieracioides* were replaced by *Picris hieracioides* subsp. *morrisonensis*, *Crepis pontana* by 22_*Crepis aurea*, *T. cucullatum* by 55_*Taraxacum laevigatum*, *L. saxatilis* by 35_*Leontodon hispidus*, *S. rosea* by 49_*Scorzonera pupurea*, *A. tomentosum* by 3_*Arctium lappa*, *C. personata* by 5_*Carduus crassifolius*, *C. erisithales* by 18_*Cirsium vulgare*, *S. pygmaea* by 43_*Saussurea discolor*, *C. acaulis* by 7_*Carlina vulgaris*. Sequences were aligned using the program GeneDoc® (Nicholas, 1997) and, finally, trimmed to the length of the shortest sequence. The total length of the alignment was 772 bases.

Trees were generated with PAUP® version 4.0b1 (Swofford, 2005) using maximum parsimony analyses. The heuristic search algorithm was chosen, using the RANDOM ADDITION of taxa and the TBR option (tree bisection-reconnection) for branch swapping, which was restricted to 1000 retained trees. Gaps were treated as MISSING. Besides Paup®, cluster analyses using Complete Linkage and Ward-Method as implemented in the software package Statistica® were performed. The numerous analyses were conducted on four different data sets:

(1) The whole alignment consisting of 772 characters. 424 characters were variable and 371 parsimonious informative.

(2) The binary data matrix comprising 81 anatomical variables (only qualitative characters, see above).,In the cluster analyses, the quantitative character (maximal diameter of vessels) was included as well.

(3) The data used in (1) included a lot of ambiguous entries. Therefore intending a combination of DNA-data and anatomical data, in order to achieve a reduction of the influence of DNA-data, the DNA-data was cleaned in such way that only A, C, G, T letters are allowed. All columns with any different letters have been eliminated. In the end, the resulting data matrix consists of 182 characters.

(4) The combined matrices 2 and 3.

DFA - a discriminant function analysis (DFA) using the software package Statistica® was performed on the basis of the whole dataset (188 accessions and 82 characters [table 1]) – (1) to estimate if the studied taxa can be differentiated based on the multivariate anatomical data and (2) to identify potential characters of highest discriminative power. For an optimal performance of the DFA, sufficient variance in each selected taxon is requested. Therefore, subtribes (Hyoseridinae, Carduinae, Carlininae, Centaureinae, Cichoriinae, Chondrillinae, Crepidinae, Echinopsidinae, Hypochaeridinae, Hieraciinae, Lactucinae and Scorzonerinae) instead of species were used as categorical grouping variable.

CLUST_META was developed as a novel method for tree comparisons (cf. Carrizo S.F., 2004, Isenberg P. et al., 2007, Gouret Ph. et al., 2009, Kao M-Y., 2001, Zhong Y. et al., 1997, Sokal et al., 1962, Restrepo G., 2007, Penny D. & Hendy M.D., 1985, Phipps J.B., 1971) for comparing different trees. CLUST_META is a new and very simple method for the comparison of categorical information about group membership of operational taxonomic units (OTU´s) delivered by different algorithms or by manual input after a determination of material or an assignment of OTU´s to higher taxonomical units. The principle of the method will be explained in the following example:
Suggest that we want to compare four different classifications. The alternative classifications can be presented as a data matrix consisting of four rows of categorical vectors, which provide the respectively proposed groups as numbers (up to the maximum number of groups classified) (Table 2). Logically, the matrix expresses information about the relationship of OTU´s only, but is silent about the underlying data or algorithm.

Table 2. Originating data matrix (columns represent the OTU's, rows represent the categorical information)

	1	2	3	4	5
Classification 1	1	1	2	3	2
Classification 2	2	2	1	3	1
Classification 3	3	3	2	1	2
Classification 4	1	1	2	1	2

The main idea is now to disentangle the specific group number from each information vector. For this purpose each line of the initial data matrix will be decomposed in as many single binary vectors as the maximal group number indicates. The decomposition is shown for two classifications only (see Table 3).

Table 3. Decomposition of two classifications of the original data matrix into single binary vectors (columns represent the OTU's, rows represent the categorical information)

	1	2	3	4	5
Classification 1 group 1	1	1	0	0	0
Classification 1 group 2	0	0	1	0	1
Classification 1 group 3	0	0	0	1	0
Classification 2 group 1	0	0	1	0	1
Classification 2 group 2	1	1	0	0	0
Classification 2 group 3	0	0	0	1	0

Now it is easy to see that simple sorting procedures could reveal new pattern in the matrix. Finally, the alternative classifications are sorted by rows and compared to each other (Table 4).

Table 4. Sorted data matrix (columns represent the OTU's, rows represent the categorical information)

	1	2	3	4	5
Classification 1 group 3	0	0	0	1	0
Classification 2 group 3	0	0	0	1	0
Classification 1 group 2	0	0	1	0	1
Classification 2 group 1	0	0	1	0	1
Classification 1 group 1	1	1	0	0	0
Classification 2 group 2	1	1	0	0	0

The algorithm provides some useful features as an option to sort by rows or columns, to move whole blocks of rows and columns as well as some advanced sorting algorithms for an automatic arrangement of the binary matrix (see results).

Two great advantages of this new method can be highlighted:

a) no loss of information at any time of the procedure
b) information about the links between the different information vectors (classifications) and the new group pattern in the sorted data matrix

RESULTS AND DISCUSSION

Three questions were the center of our attention: (1) Is a discrimination of species with the anatomical characters of their subterranean organs by microscopical means possible, (2) which characters are responsible for a discrimination and (3) is there any correlation between the anatomy and the ITS-data in respect to the arrangement of species/genera/subtribes?

To answer question one, a binary decision tree (fig. 2) has been constructed on the basis of the generated database to achieve a better overview over the separabilities of the diverse taxa by microscopical means. It is important to notice that the arrangement of the taxa doesn't represent any phylogenetic relations but instead the arrangement is caused by the order of discrimination characters in the tree. The features used for differentiation were chosen in terms of their unambiguousness and reliability and with the purpose of a quick discrimination. As illustrated, a differentiation between most of the taxa is possible, leaving just a few species not distinguishable. For instance, there is no infrageneric variation of the anatomy of *Arctium* sp. or *Taraxacum* sp. and there are no anatomical characters to discriminate between *Prenanthes purpurea* and *Cicerbita alpina*.

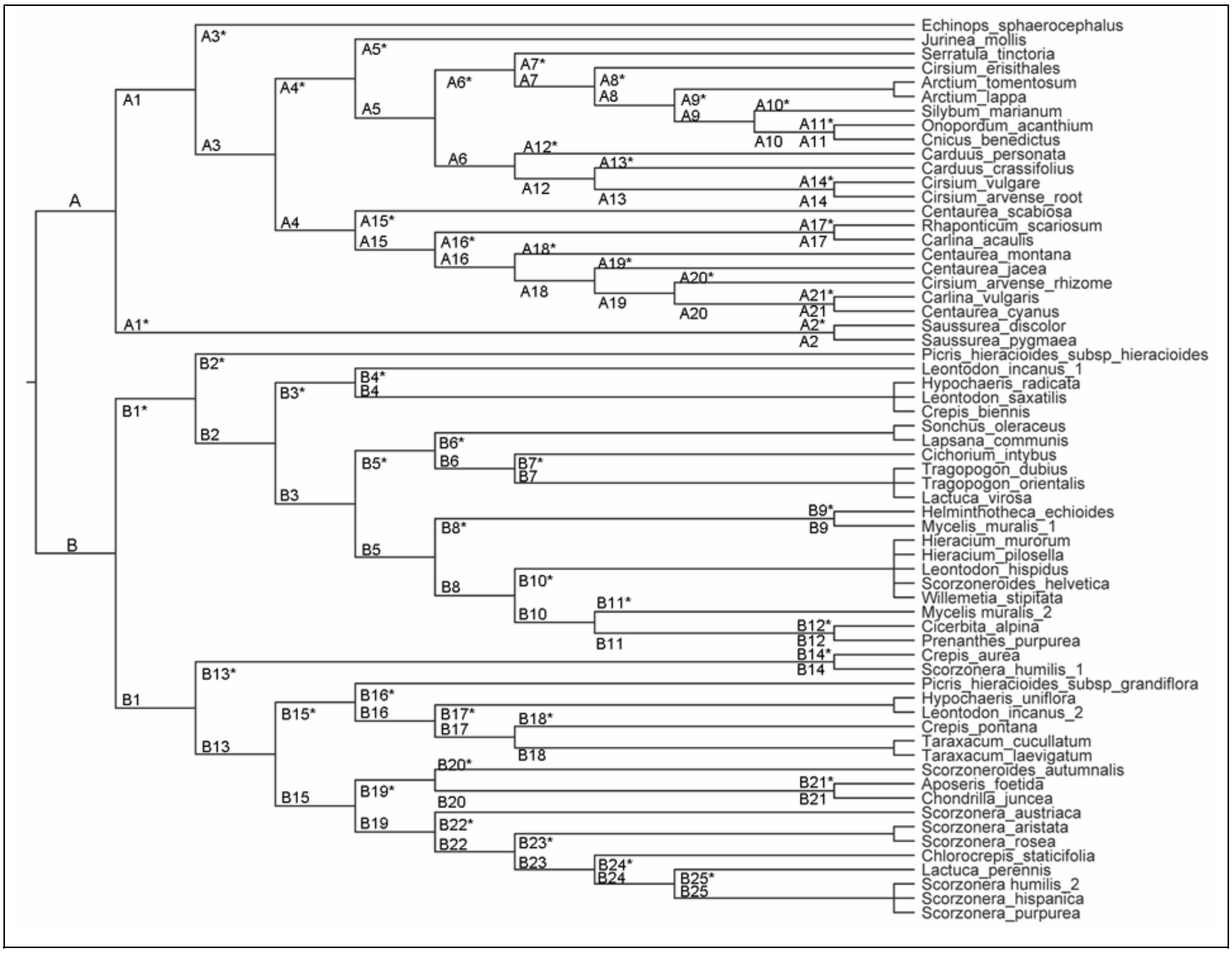

Fig. 2: Binary decision tree concerning the underground organs: More than one way may be possible for the identification of certain taxa. If so, the species appear twice within the tree, marked with _1/_2. The shortest branches mark the taxa which are not distinguishable from each other.
Discrimination characters: A_endodermal resin ducts present; A1*_interxylary cork present; A1_interxylary cork absent; A2*_fibers present; A2_fibers absent; A3*_secretory cavities present; A3_secretory cavities absent; A4*_secretory ducts besides endodermal resin ducts absent; A4_secretory ducts besides endodermal resin ducts present; A5*_endodermal resin ducts of a size multiple of surrounding parenchyma cells; A5_endodermal resin ducts not of a size multiple of surrounding parenchyma cells; A6*_sclereids absent; A6_sclereids present; A7*_fibrous root system, primary structure of roots remains; A7_taproot / rhizome; A8*_rhizome; A8_taproot; A9*_vessels in rows; A9_vessels irregularly dispersed; A10*_no distinct endodermis, cortex becomes rhytidome; A10_enduring cortex with distinct endodermis; A11*_diameter of largest vessels > 100µm; A11_diameter of largest vessels < 100µm; A12*_cells of pith thick-walled, pitted; A12_either no pith or cells of pith thin-walled; A13*_tracheids mainly reticulate and scalariform; A13_tracheids mainly with bordered pits; A14*_cortex without aerenchym; A14_aerenchym in cortex; A15*_secretory ducts of type SD3 present; A15_secretory ducts of type SD3 absent; A16*_secretory ducts of type SD1 / SD2 in wood cylinder present; A16_secretory ducts of type SD1 / SD2 in wood cylinder absent; A17*_SDs in fascicular position; A17_SDs in interfascicular position; A18*_secretory ducts of type SD1 with C1:C2 < 0,3 within secondary phloem present; A18_secretory ducts of type SD1 with C1:C2 < 0,3 within secondary phloem absent; A19*_phytomelanin-coated sclereids present; A19_phytomelanin-coated sclereids absent; A20*_secretory ducts of type SD2 at the border between xylem and pith present; A20_secretory ducts of type SD2 within secondary phloem; A21*_secretory ducts with C1:C2 = 0,40 - 0,70; A21_secretory ducts with C1:C2 > 0,9; B_laticifers present; B1_fibers present; B1_fibers absent; B2*_sclereids present; B2_sclereids absent; B3*_laticifers circular arranged; B3_laticifers in rows or irregularly dispersed; B4*_taproot; B4_fibrous root system; B5*_taproot; B5_fibrous root system / rhizome; B6*_medullary rays < 5 cells in a row; B6_medullary rays > 5 cells in a row; B7*_lenght:width of vessel elements < 2; B7_lenght:width of vessel elements > 3; B8*_fibrous root system; B8_rhizome; B9*_laticifers in rows; B9_laticifers irregularly dispersed; B10*_tracheids mainly reticulate; B10_tracheids mainly with bordered pits; B11*_diameter of largest vessels < 45µm; B11_diameter of largest vessels > 45µm; B12*_lenght:width of vessel elements < 2; B12_lenght:width of vessel elements > 3,5; B13*_sclereids present; B13_sclereids absent; B14*_sclereids in cortex, sec. phloem and xylem; B14_sclereids in phellem; B15*_laticifers circular arranged; B15_laticifers in rows or irregularly dispersed; B16*_fibrous root system; B16_taproot; B17*_vessels with bordered pits; B17_vessels only reticulate; B18*_medullary rays multiseriate; B18_medullary rays absent; B19*_laticifers irregularly dispersed; B19_laticifers in rows; B20*_cells of pith thin-walled; B20_cells of pith thick-walled; B21*_cells of pith with nodular thickening; B21_cells of pith without nodular thickening; B22*_irregularly secondary growth; B22_regularly secondary growth; B23*_phellem thickwalled; B23_phellem thinwalled; B24*_vessels irregularly dispersed; B24_vessels in rows; B25*_special structure of rhytidome / phellem; B25_regularly laminated phellem;

Discriminant function analysis

Concerning question two, a discriminant function analysis (DFA) on the basis of the anatomical data set was performed. As a priori grouping the affiliation to one of the twelve subtribes was used. As one result, the different types of secretory ducts (classified according to the quotient length of the duct epithel cells/length of adjacent cells as seen in longitudinal section; Fritz & Saukel, 2011c) and their exact location with respect to other prominent anatomical structures as well as the structure of the vessels including secondary wall thickenings and pits were identified as the most discriminative characters.

Secondly, the corresponding classification matrix shows an a priori classification probability of 100% of all subtribes except the Hypochaeridinae (with 96.77 %; one sample from Prenanthes purpurea was misclassified as member of the Lactucinae). Interestingly, *Prenanthes* L. formerly used to belong to the subtribe Lactucinae, whereas recently it was transferred to the subtribe Hypochaeridinae Less. (Kilian et al., 2009). The results of the anatomy-based DFA, however, support the former affiliation.

Additionally, the DFA provides a canonical correlation analysis. In scope of these computations, the successive canonical roots were determined. Figure 3 shows scatterplots of the canonical roots 3, 4 and 5. The scatterplots demonstrate that samples cluster according to subtribes.

Phylogenetic analyses versus Cluster analyses

Nuclear DNA- and anatomy-based trees were compared to each other using the new algorithm *CLUST_META*.

Figures 4a and 4b summarise the groupings inferred from 12 different computations: 8 grouping vectors refer to cluster analyses (using the software package Statistica®), carried out on the four data sets described earlier (Complete Linkage [C_772, C_182, C_MI182,C_MI], and Ward [W_772, W_182, W_MI182, W_MI]) using a distance matrix (*p*-distance [Swofford, 2005]) generated by Paup®. The achieved dendrogramms are cut at the level of 4, 5 and 6 groups to create different grouping vectors. The other 4 vectors show groupings suggested by Paup® using maximum parsimony (nrDNA- [P_772 and P_182], anatomy-based analyses [P_Mi], and the combined data [P_MI182]). Here again, the trees are cut at the level of 4, 5 and 6 groups too to achieve comparability with the clusters mentioned above. In fig. 4 (and fig 5) the data matrix was sorted according to the similarity of lines and according to the membership to subtribes: similarities and dissimilarities within single subtribes and connections between can be observed. For instance, a group comprising *Carduus* sp., *Cirsium* sp. *and Silybum marianum* (horizontal lines 6-10) clearly stands out of the other Carduinae (vertical lines 31-48, 123-125, 165-178, referring to both various calculation of pure microscopical data, pure DNA-data and the mix of both). *Prenanthes purpurea* (horizontal line 35) shows its special status within the Hypochaeridinae with closeness to the subtribe Lactucinae, in terms of both microscopy (vertical lines 108-109, 130-133) and DNA-data (vertical lines 61-65, 101-107). Concerning the microscopical data, similarities not only to the Lactucinae but also to *Chondrilla juncea* and *Carduus personata* and *Cirsium* sp. exist (vertical lines 130-133).

Furthermore, some subtribes are clearly defined against others (e.g. Scorzonerinae, Crepidinae).

The reduction of the ITS-data resulting in 182 characters, intentionally performed to be able to combine DNA-data with microscopical data within one tree, reveals not to make much sense regarding some species (and therefore, the mix between 182 and microscopy as well). The reduction seems to be associated with the loss of important information. P_182 (vertical lines 142-144, 154-156) show the differences between *Aposeris foetida* and Sonchus oleraceus (subtribe Hyoseridinae) in contrast to the computations of the DNA-data containing 772 characters.

In Fig. 5, the diverse calculations of the microscopical data (P_MI, W_MI, C_MI) are compared with the complete DNA-data (P_772). Taxa are ordered by similarity only, i.e. neglecting taxonomy. Obviously, in some respects, there is agreement between the trees of phylogeny and subterranean anatomy (e.g. a group with Serratula / Centaurea sp. [vertical lines 4-5, 17-18, 40-45]). Here again, similarities and connections between the different calculations and samples become visible. For another view, fig. 6 presents the same data but with taxa unsorted, sortation just on behalf of the objects (calculation methods). Paup® seems not to be suitable for calculations with microscopical data, cluster analyses using Complete Linkage or Ward are far more congruent with the DNA information.

In conclusion, our results demonstrate that a congruence between the DNA and the microscopical data exist to a certain degree depending on the calculation method. Rather striking is the outcome of the DFA with an a priori classification probability of 100% of the subtribes in almost all cases. Microscopical characters supporting the systematic relationships of the Cardueae and Cichorieae have been successfully identified. However, the number of species that have been examined in this study is small compared with the diversity of the group and an expanded sampling is needed to verify the generality of our observations.

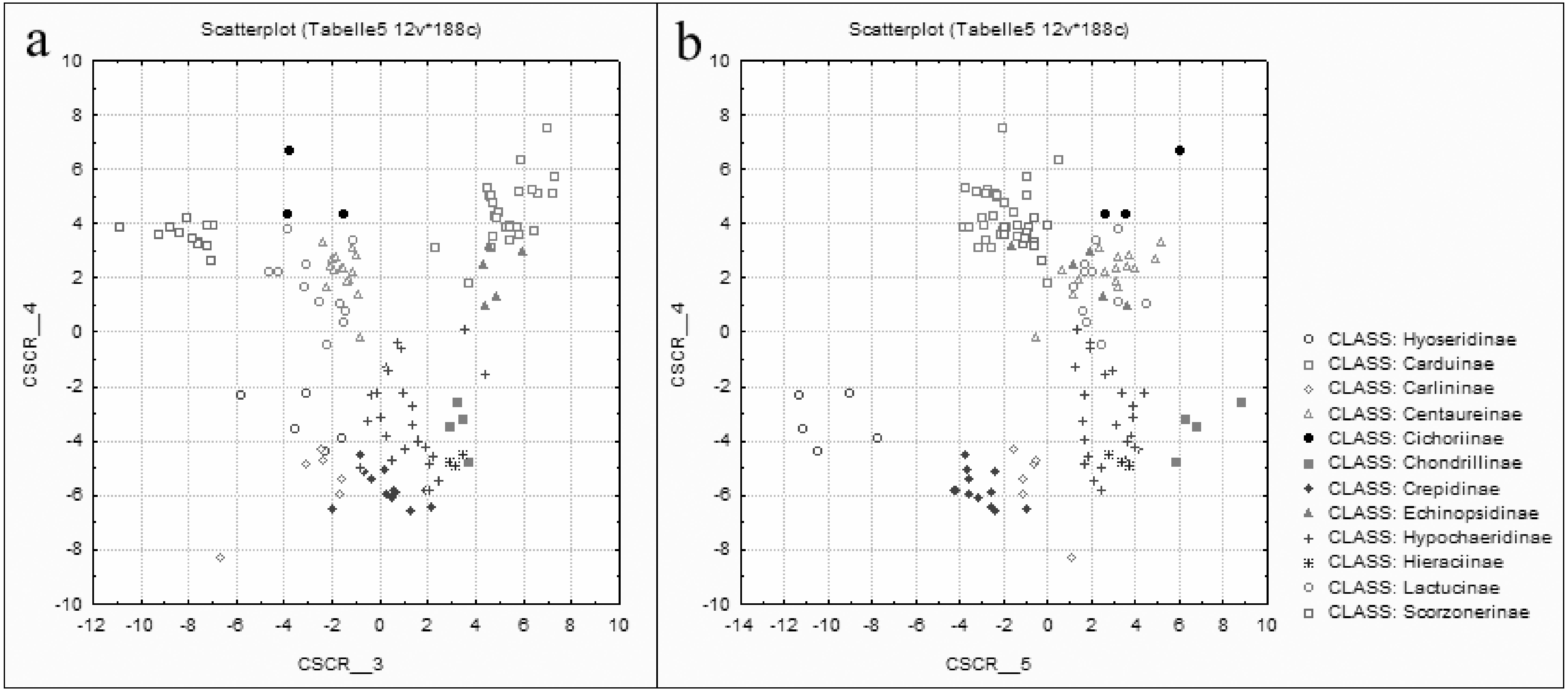

Fig. 3: Scatterplot of the Canoncial roots 3, 4, 5 (CSCR) of the Discriminant Analysis (82 characters used). Individuals from different subtribes are labelled with different symbols.

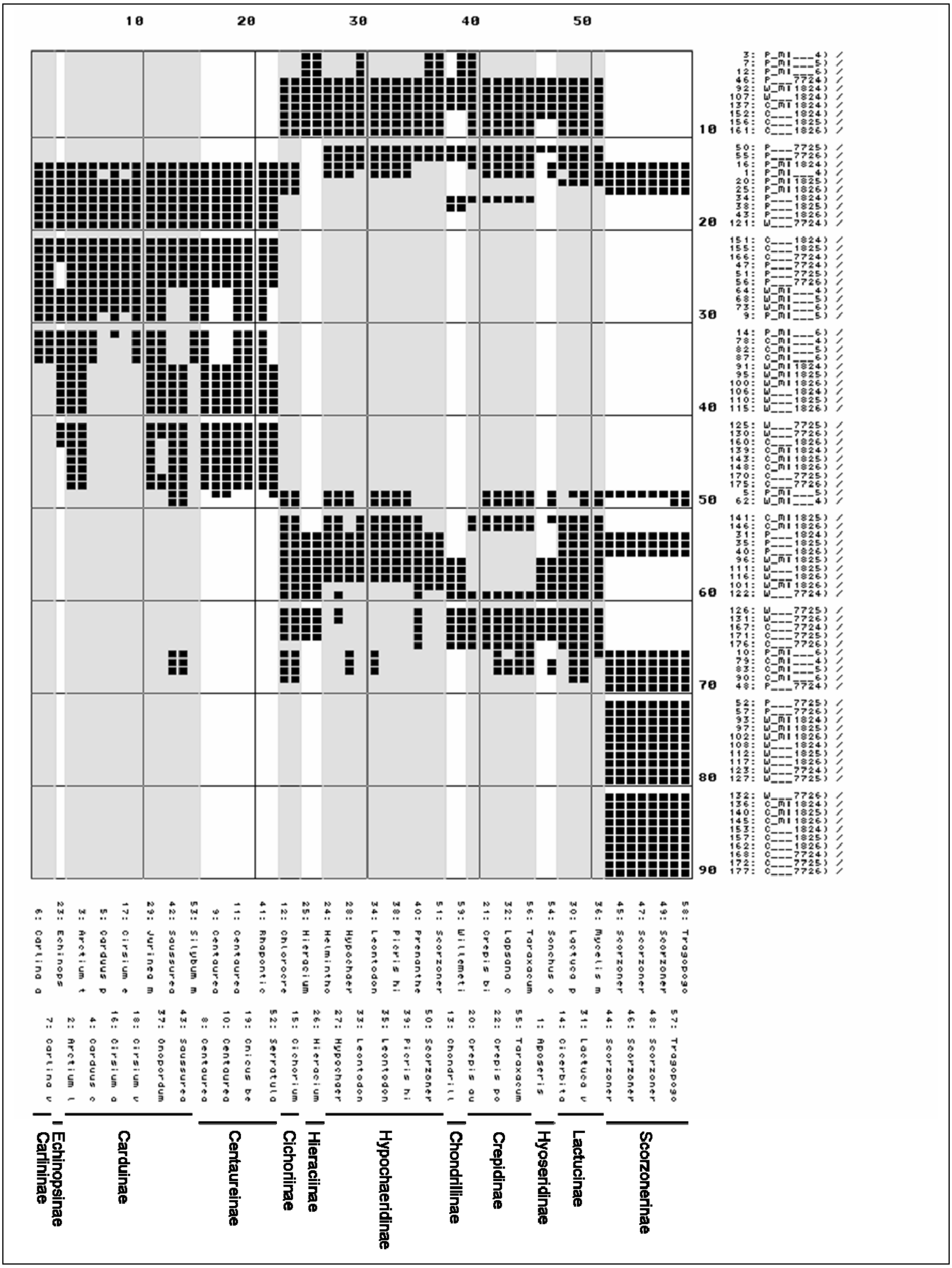

Fig. 4a: Graph achieved by the CLUST_META algorithm (part 1: line 0-90) comparing the generated trees P_772, P_182, P_MI, P_MI182, W_772, W_182, W_MI, W_MI182 and C_772, C_182, C_MI, C_MI182 cut at the levels of 4, 5 or 6 groups. The cipher at the end of the name of a line indicates the number of differentiated groups within this tree. Samples are sorted with regard to their subtribe (complete names are given under their numbers in the appendix), the succession of the subtribes follow the phylogeny given in Funk et al., 2009.

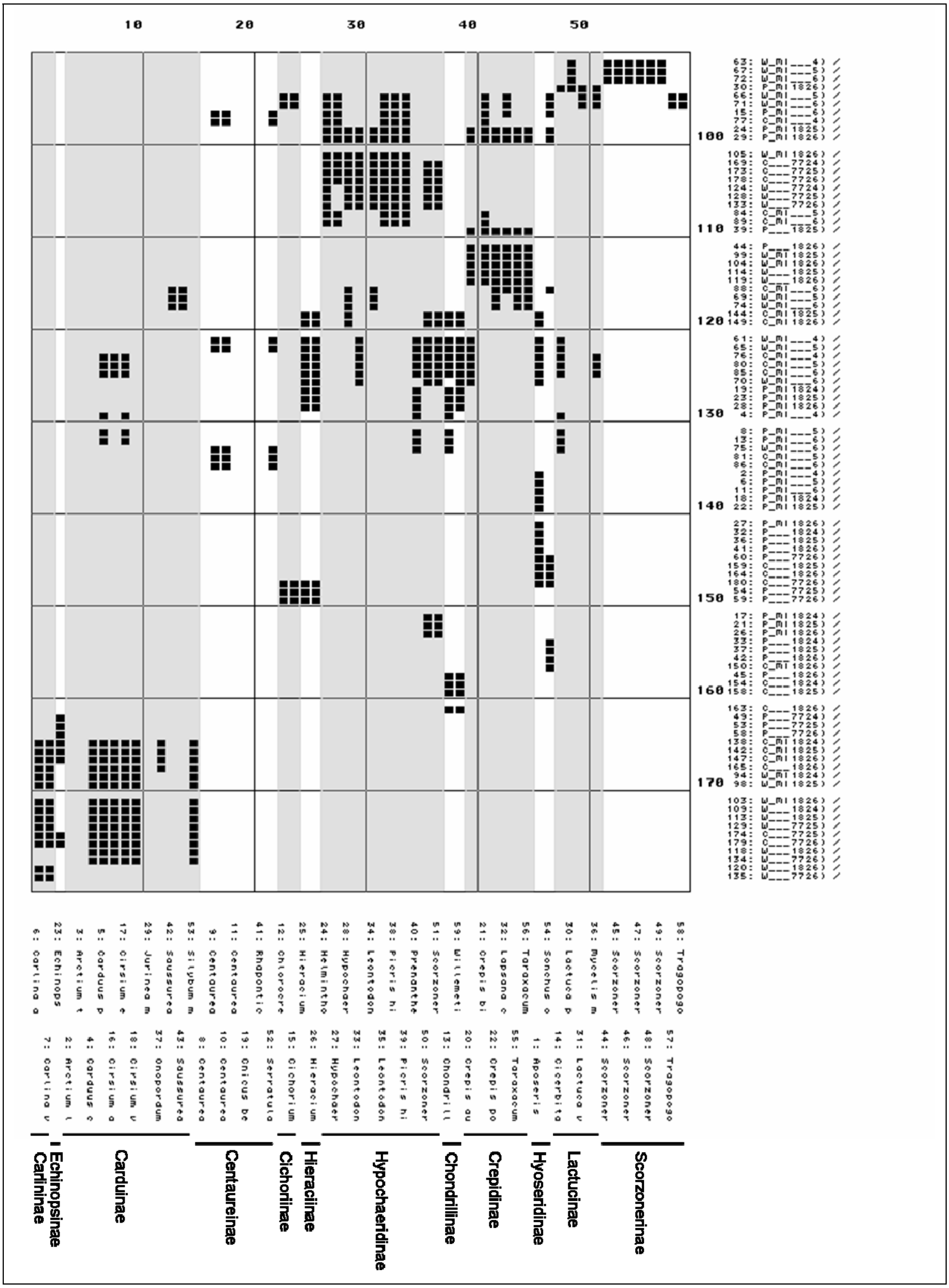

Fig. 4b: Graph achieved by the CLUST_META algorithm (part 2: line 90-180) comparing the generated trees P_772, P_182, P_MI, P_MI182, W_772, W_182, W_MI, W_MI182 and C_772, C_182, C_MI, C_MI182 cut at the levels of 4, 5 or 6 groups. The cipher at the end of the name of a line indicates the number of differentiated groups within this tree. Samples are sorted with regard to their subtribe (complete names are given under their numbers in the appendix), the succession of the subtribes follow the phylogeny given in Funk et al., 2009.

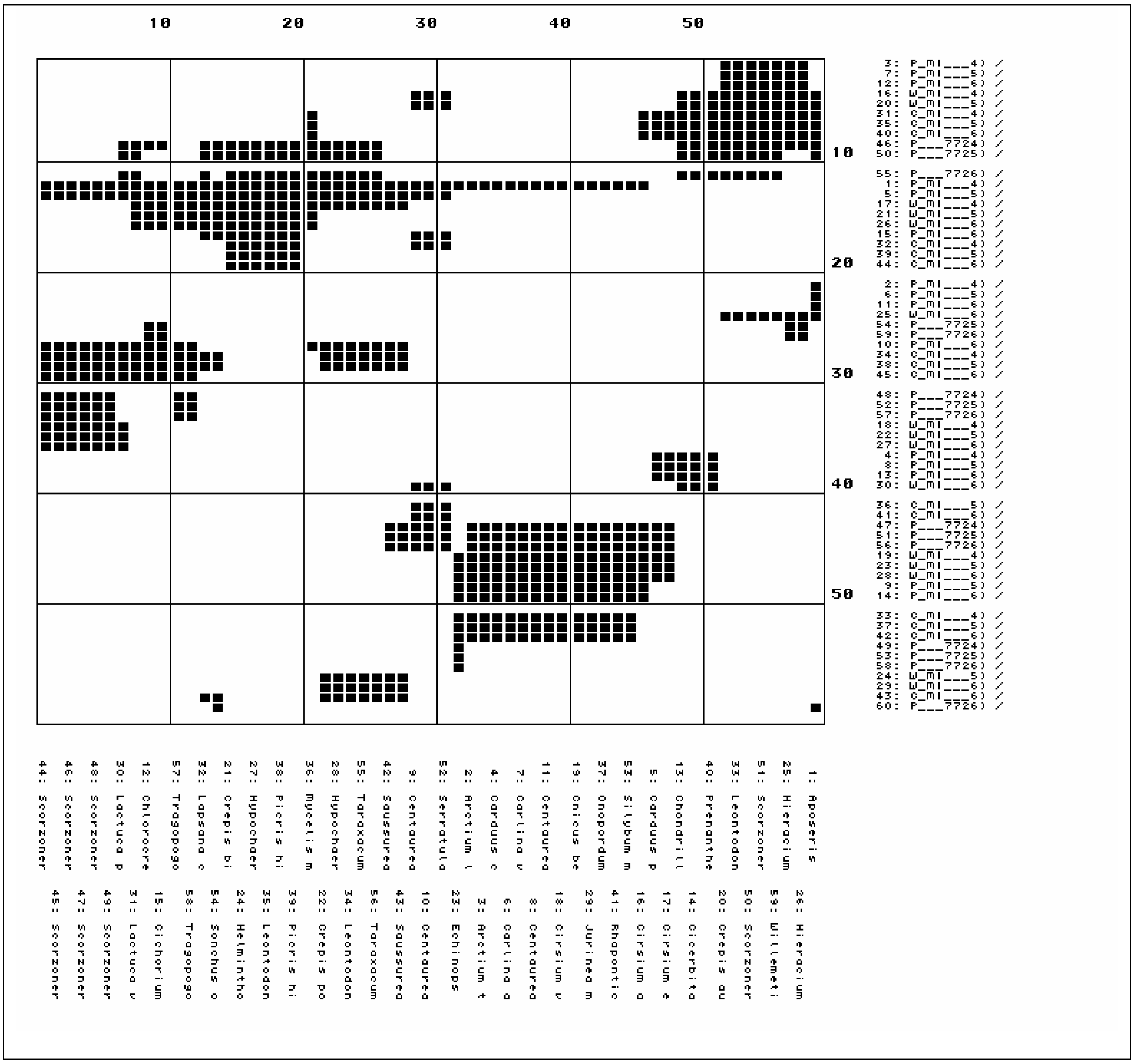

Fig. 5: Graph achieved by the CLUST_META algorithm comparing the generated trees P_772, P_MI, W_MI and C_MI cut at the levels of 4, 5 or 6 groups. The cipher at the end of a name of a line indicates the number of discriminated groups within this tree. Samples are sorted on behalf of similarities of their lines (complete names are given under their numbers in the appendix).

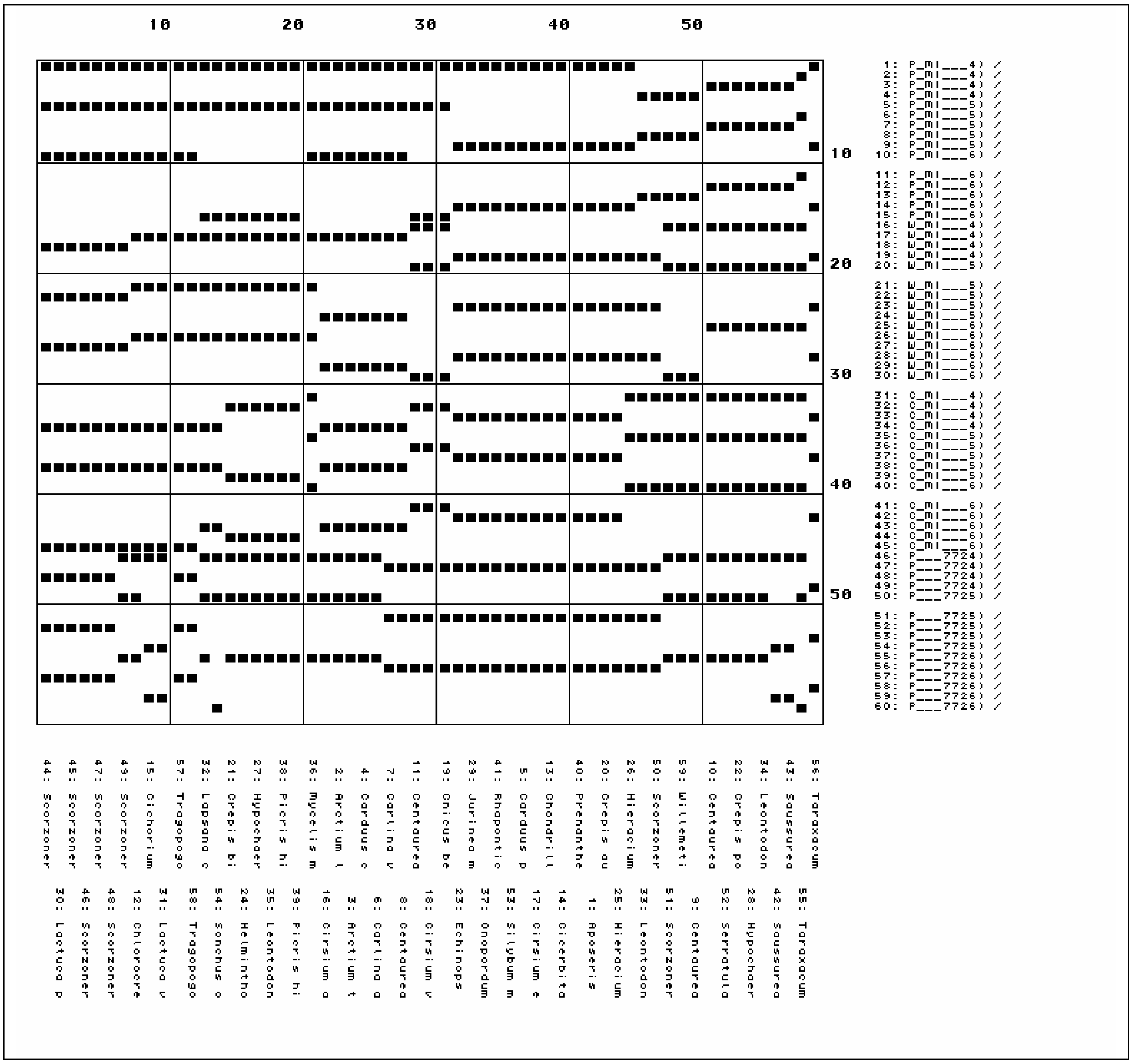

Fig. 6: Graph achieved by the CLUST_META algorithm comparing the generated trees P_772, P_MI, W_MI and C_MI cut at the levels of 4, 5 or 6 groups. The cipher at the end of a name of a line indicates the number of discriminated groups within this tree. Sortation on behalf of the objects (complete names are given under their numbers in the appendix).

APPENDIX

Taxa studied, collection history, voucher numbers and GenBank accession numbers of DNA sequences (taxonomy following Funk et al., 2009); "Nr." indicates the number which the taxa received for the analyses. Plant material collected by Elisabeth Fritz (EF), Christoph Dobeš (CD), Johannes Saukel (JS), Valerie Klatte-Asselmeyer (VK), Silvia Fialova (SF), Werner Lahner (WL), Günther Stadler *(GS)*

Subtribe	Species	Nr.	Origin, Voucher numbers	GenBank accession no.
Echinopsinae	*Echinops sphaerocephalus* L.	23	Austria, Vienna, EF (ES02-09, ES03-09) -, Lower Austria, Tullnerbach, EF (ES06-09) -, -, Karnabrunn, CD (ES07-09) -, Tyrol, Fließ, VK (ES08-09)	AY538637
Carlininae	*Carlina acaulis* L.	6	-, Vienna, EF (CA02, CA05) Germany, Baden-Württemberg, Schwäbische Alb, Herbarium of Hohenack, Nr. 652 (WUP) (CA01, CA04) Samples of a commercially traded product (Kottas Pharma, sample number 1881, 1882)	-
	C. vulgaris L.	7	Poland, Gutkowo, Olsztyn, EF (CV02-09 - CV05-09)	AY826246
Carduinae	*Arctium lappa* L.	2	Austria, Vienna, EF (AL01-09, AL05-09, AL3A-09)	FJ528300
	A. tomentosum Mill.	3	-, Traiskirchen, EF (AT01-10 - AT03-10)	-
	Saussurea discolor (Willd.) DC.	42	-, Carinthia, Lesachtal, JS (SD01-09 - SD07-09)	AF319146, AF319092
	S. pygmaea (Jacq.) Spr.	43	-, Styria, Schneealpe, EF (SaP01-09 - SaP06-09, SaP S1)	
	Jurinea mollis Rchb.	29	-, Vienna, EF (JM01-09) -, Burgenland, Winden, JS (JM03-86, JM04)	AY780404
	Carduus personata (L.) Jacq.	5	-, Styria, Schneealpe, EF (CaP01-09 - CaP03-09)	-
	C. defloratus L. (*C. crassifolius* Willd.)	4	-, Lower Austria, Gippel, CD (CC01-08) -, -, Araburg castle, EF(CC02-08, CC03-08)	AY826241
	Cirsium arvense (L.) Scop.	16	-, Vienna, EF (CiA02-08) Slovakia, Modra, SF (CiA01-08) USA, New York, Peekskill, Planta America Septentrionalis, Le Roy (**WUP**) (CiA03-08)	AF443680, AF443681
	C. vulgare (Savi) Ten.	18	Poland, Gutkowo, Olsztyn, EF (CiV01-09 - CiV03-09)	AF443715
	C. erisithales (Jacq.) Scop.	17	Austria, Styria, Schneealpe, EF (CE01-09 - CE03-09)	-
	Onopordum acanthium L.	37	-, Lower Austria, Buchberg, WL (O01-08, O02-08) Italy, Southern Tyrol, Vinschgau, CD (AST3)	AY914827
	Silybum marianum (L.) Gaertn.	53	Austria, Lower AUT, Buchberg, WL (MD1-08, MD2-08, SM1) Slovakia, Botanical Garden of Bratislava, SF (MD3-08)	AJ831537, AY914831
Centaureinae	*Serratula tinctoria* L.	52	Austria, Vienna, JS (ST01-09 - ST04-09)	AJ868085, AJ868084
	Rhaponticum scariosum Lam.	41	Liechtenstein, Saminatal, GS (RS01-09, RS02-09)	DQ310951, DQ310952
	Centaurea jacea L.	9	Austria, Karnabrunn, CD (CJ01-09, CJ02-09) -, Vienna, EF (CJ8-1)	AM114332, DQ319125
	C. scabiosa L.	11	-, Vienna, JS (CS03-08) Poland, Gutkowo, Olsztyn, EF (CS02-08) Switzerland, Graubünden, Lavin, CD (CS01-08A,	FJ459692

			CS01-08B)	
	C. cyanus L. (= *Cyanus segetum* Hill., Fischer *et al.)*	8	Austria, Vienna, EF (CS3-08) Germany, Baden-Württemberg, Kronau, CD (CS2-08) Poland, Mazury, Zabie, EF (CS1-08)	AY826254, L35879
	C. montana L. (= *Cyanus montanus* Hill., Fischer *et al.*)	10	Austria, Lower Austria, Unterberg, EF (C01-08) -, -, Pressbaum, EF (C03-08) Slovakia, Modra, S.F. (C02-08)	L35887
	Cnicus benedictus L.	19	Botanical Garden of the Department of Pharmacognosy, University of Vienna (CB1 – S3,S4)	DQ319091
Scorzonerinae	*Scorzonera hispanica* L.	46	Austria, Lower Austria, Anninger, JS (SHI01) -, -, Weinling, JS (SHI02, SHI03)	AM117052, AY508186, AJ633472
	S. humilis L.	47	-, -, Drosendorf, JS (SH02, SH03) -, -, Bisamberg (SH01)	AJ633476, AM117053
	S. purpurea L.	48	-, Vienna, EF (SP01-09 - SP03-09)	-
	S. aristata Ramond ex DC.	44	-, Carinthia, Lumkofel, JS (SAr01-09 - SAr03-09)	AY508192
	S. austriaca Willd.	45	-, Vienna, EF (SA01-09, SA04-09, SA05-09)	AY508216, AM117047
	S. rosea Waldst. & Kit.	49	Austria, Carinthia, Lumkofel, JS (SR01-09, SR02-09)	-
	Tragopogon orientalis L.	58	-, Lower Austria, Araburg, EF (TO01-08) -, -, Laaben, EF (TO02-08) Poland, Gutkowo, Olsztyn, EF (TO03-08)	AY508170
	T. dubius Scop.	47	Austria, Vienna, EF (TD1-08) Italy, Vinschgau, Schluderns, CD (AST1)	AY525376, AY645813
Lactucinae	*Lactuca virosa* L.	31	Austria, Vienna, EF (LV01-07, LV02-07)	AJ633335
	L. perennis L.	30	Italy, Eisacktal, JF (LP01) -, Southern Tyrol, Vinschgau, CD (LPAST14A, LPAST14B)	AJ633334
	Mycelis muralis (= Lactuca muralis Dumort., Fischer *et al.*)	36	Austria, Vienna, EF (M01-08, MM01-08) -Lower Austria, Irenental, EF (M02-07) -, Rekawinkel, EF (M01-07)	AJ633338, AJ633339
	Cicerbita alpina Wallr. (= *Lactuca alpina* Benth. & Hook.f., Fischer *et al.*)	14	-, Styria, Schneealpe, EF (CiAl01-09 - CiAl03-09)	AJ633324, AJ633340
Hyoseridinae	*Aposeris foetida* Cass.	1	Austria, Carinthia, Feistritz im Rosental, JS (AF1, AST19) Germany, Garmisch-Partenkirchen, Wank, CD (AST23)	DQ451822
	Sonchus oleraceus L.	54	Austria, Vienna, EF (SO01-07) -Lower Austria, Irenental, EF (SO02-07A, SO02-07B) Italy, Southern Tyrol (CD): seeds planted in Botanical Garden of the Department of Pharmacognosy (AST18)	GQ478113, AY862581
Crepidinae	*Crepis biennis* L.	21	Italy, Southern Tyrol, Vinschgau, CD (AST5) Slovakia, Modra, SF (CB01-08) Botanical Garden Karl Franzens Universität Graz; Mürzsteger Alpen; Seewirtgraben / Mariazell (70) Botanical Garten Berlin-Dahlem; DE-0-B-0164479: Hessen, Werra-Meißner-Kreis, Eschwege, leg. Royl&al. (814)	DQ451818

	C. aurea Rchb.	20	Austria, Salzburg, Mehrlhütte, JS (CrA01-08 - CrA04-08)	AF528483, AJ633359
	C. pontana Druce	22	Austria, Carinthia, Lesachtal, Lumkofel, JS (CP01-09 - CP04-09)	-
	Lapsana communis L.	32	Italy, Southern Tyrol, Vinschgau, CD (AST17) Austria, Vienna, EF (LC01-08, LC02-08B) Germany, Baden-Württemberg, Blankenloch, CD (LC02-08A)	AJ633285, AJ633286
	Taraxacum cucullatum Dahlst.	55	-, Salzburg, Riedingtal, JS (TC01-08 - TC04-08)	-
	T. laevigatum DC.	56	-, Vienna, JS (TL01-08 - TL03-08)	AJ633288
Chondrillinae	*Chondrilla juncea* L.	13	-, Vienna, Kovats (CJ02) Hungary, Holuby (CJ01)	AJ633348
	Willemetia. stipitata Cass.	59	Austria, Salzburg, Maria Pfarr, JS (WS01-08 - WS04-08)	AY508170
Hypochaeridinae	*Helminthotheca echioides* (L.) Holub	24	Botanical Garden of the Univ. Bonn; 1744;DE-0-Bonn-23773: F. Klingenstein&J. Manner,1998, Deutschland, Nordrhein-Westfalen, Bonn-Ippendorf (HE1)	AF422123
	Hypochaeris uniflora Hoffm.	28	Austria, Salzburg, Lungau, JS (V310, V312) -, Salzburg, Kareck, JS (101, HU01-08)	AF528481
	H. radicata L.	27	Poland, Gutkowo, Olsztyn, EF (5-100, 5-16) Botanical Garden Berlin-Dahlem; 916; DE-0-B-2421281: Bayern, Kreis Wunsiedel, Fichtelgebirge, leg. Hempel Italy, Eisacktal, Blumau-Rielinger-Atzwang, JS (H113)	EF107656, GU011987, AF528457
	Leontodon incanus Schrank	34	-, Burgenland, St. Margarethen, JS (L100) -, Steiermark, Oberwölz, JS (L108) -, Lower Austria, Baden, JS (L305) -, -, Anninger, JS (L358, L359)	DQ451772
	L. hispidus Scop.	33	-, -, Rekawinkel, EF (L1-106) -, -, Rax, EF (3-22) -, Carinthia, Pöllatal, JS (L105)	AF528485, DQ451769, DQ451770
	Picris hieracioides subsp. *hieracioides* L.	39	Austria, Vienna, EF (PH02-07A, PH02-07B) Poland, Gutkowo, Olsztyn, EF (PH01-07)	AJ633320, AJ633322
	Picris hieracioides subsp. *grandiflora* (Ten.) Arcang.	38	Botanical Garden of the Friedrich-Schiller-Universität Jena; Weimar:Oberweimar	
	Scorzoneroides helvetica (Merat) Holub	51	-, Styria, Etrachtal, JS (L109) -, -, Prebertal, JS (L112) -, -, Anger, Lessachwinkel, JS (L109)	DQ451766, DQ451767
	S. autumnalis (L.) Moench	50	Poland, Gutkowo, Olsztyn, EF (L102 - L104)	AF528486
	Prenanthes purpurea L.	40	Austria, Styria, Schneealpe, EF (PP01-09 - PP03-09)	AJ633342, AJ633343
Hieraciinae	*Hieracium murorum* L.	25	-, Lower Austria, Hohe Wand, EF (HM01-08) Switzerland, Graubünden, Bos-cha, CD (AST11) Botanischer Garten Karl Franzens Universität Graz; 81; Schillingsdorf, VI-X/2007 (AD) (HM1)	AF528492
	H. pilosella L.	26	Italy, Southern Tyrol, Vinschgau, CD (AST6) Poland, Gutkowo, Olsztyn, EF (01-07); (HP01-86)	AY879161
Cichoriinae	*Cichorium intybus* L.	15	Austria, Lower Austria, Pressbaum, EF (CI1-07)	AY504694,

		-, Vienna, EF (CI1-08) Poland, Gutkowo, Olsztyn, EF (CI2-07) Seeds of the Botanical Garden Berlin-Dahlem:DE-0-B-2003105:Brandenburg, Falkensee, leg. Dürbye 3090 (CI1)	AJ633451
Chlorocrepis staticifolia (All.) Griseb.	12	Italy, Southern Tyrol, Vinschgau, CD (AST7) -, Valbruna, CD (AST22)	AJ633437
Wunderlichia mirabilis Riedel ex Baker (Outgroup)			DQ414742

Carrizo, SF. 2004. Phylogenetic Trees: An Information Visualisation Perspective. In *Proc. Second Asia-Pacific Bioinformatics Conference* (APBC2004), Dunedin, New Zealand. CRPIT, 29. Chen, Y.-P. P., Ed. ACS. 315-320.

Crithclow DE, Li S, Nourijelyani K, Pearl DK. 2000. Some Statistikal Methods for Phylogenetic Trees with Application to HIV Disease, *Mathematical and Computer Modelling* 32: 69-81.

Figueroa C, Salazar A, Zavaleta HA, Engleman EM. 2008. Root Character Evolution and Systematics in Cranichidinae, Prescottinae and Spiranthinae (Orchidaceae, Cranichideae. *Annals of Botany* 101: 509-520.

Fischer MA, Oswald K, Adler W. 2008. *Exkursionsflora für Österreich, Liechtenstein und Südtirol.* 3rd ed. Austria: OÖ Landesmuseen, Land Oberösterreich.

Fritz E, Saukel J. 2011a. Anatomy of subterranean organs of medicinally used Cardueae and related species and its value for discrimination. *Scientia pharmaceutica* Vol. 79, 1.

Fritz E, Saukel J. 2011b. Interxylary cork of *Saussurea discolor* and *S. pygmaea* (Asteraceae). *Biologia* Vol. 66, 3.

Fritz E, Saukel J. 2011c. Secretory structures of subterranean organs of some species of the Cardueae and their value for discrimination. *Acta Biologica Cracoviensia* Vol. 53, 1.

Fritz E, Saukel J. 2011d. Microscopical discrimination of the subterranean organs of medicinally used plants of the Cichorieae and their relatives. *Pharmaceutical Biology* 1–7, Early Online, DOI: 10.3109/13880209.2010.548390

Funk, Susanna, Stuessy, Bayer. 2009. *Systematics, Evolution, and Biogeography of the Compositae*. International Association for Plant Taxonomy, Washington.

Garcia-Jacas N, Garnatje T, Susanna A, Vilatersana R. 2002. Tribal and Subtribal Delimitation and Phylogeny of the Cardueae (Asteraceae): A Combined Nuclear and Chloroplast DNA Analysis. *Molecular Phylogenetics and Evolution* Vol. 22, 1: 51–64.

Gouret Ph, Thompson JD, Pontarotti P. 2009. PhyloPattern: regular expressions to identify complex patterns in phylogenetic trees. *BMC Bioinformatics* 10: 298-310.

Guo Y, Saukel J Ehrendorfer F. 2008. AFLP trees versus scatterplots: evolution and phylogeography of the polyploid complex *Achillea millefolium* agg. (Asteraceae). *Taxon* 57 (1): 1–17.

Hayat MQ, Ashraf M, Akhan M, Yasmin G, Shaheen N, Jabeen S. 2009. Diversity of Foliar Trichomes and their Systematic Implications in the Genus *Artemisia* (Asteraceae) *International Journal of Agriculture & Biology* 09–139/AWB/11–5–542–546.

Isenberg P, Carpendale S. 2007. Interactive Tree Comparison for Co-located Collaborative Information Visualization, *IEEE Transactions on Visualization and Computer Graphics*, 13: 1232-1239.

Jeffrey C. 2007. Compositae. In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg , 61-77.

Kao M-Y, Lam T-W, Sung W-K and Ting H-F. 2001. An Even Faster and More Unifying Algorithm for Comparing Trees vie Unbalanced Bipartite Matchings, *Journal of Algorithms* 40: 212-233.

Kilian N, Gemeinholzer B, Lack HW. 2009. Tribe *Cichorieae*. – In: Funk V. A., Susanna A., Stuessy T. & Bayer R. (ed.), *Systematics, evolution, and biogeography of the Compositae*. – Vienna: IAPT.

Lack HW, 2007. Tribe Cichorieae Lam. & DC. In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg, 180-199.

Nicholas, KB, Nicholas HB Jr, and Deerfield, DW II. 1997. *GeneDoc: Analysis and Visualization of Genetic Variation*, EMBNEW.NEWS 4:14; www.psc.edu/biomed/genedoc

Özler H, Kaya Z, Pehlivan S. 2009. Pollen Morphology of some Centaurea L., Psephellus Cass. and Cyanus Miller Taxa *Acta Biologica Cracoviensia, Series Botanica* 51/2: 53–66.

Pawłowskiego B, Jasiewicza A. 1972. *Flora Polska TOM XIII*. Polska Akademia Nauk, Warszawa.

Panero JL, Funk VA. 2008. The value of sampling anomalous taxa in phylogenetic studies: Major clades of the Asteraceae revealed. *Molecular Phylogenetics and Evolution* 47: 757–782.

Phipps JB. 1971. Dendrogramm Topology, *Systematic Zoology* 20 (3): 306-308.

Penny D, Hendy MD. 1985. The use of Tree Comparison Metrics, *Systematic Zoology* 34 (1): 75-82.

Restrepo G, Mesa H, Llanos EJ. 2007. Three Dissimilarity Measures to Contrast Dendrograms, *J.Chem.Inf. Model*. 47: 761-770.

Sánchez-Jiménez I, Lazkov GA, Hidalgo O, Garnatje T. 2010. Molecular systematics of *Echinops* L. (Asteraceae, Cynareae): A phylogeny based on ITS and *trnL-trnF* sequences with emphasis on sectional delimitation. *Taxon* 59 (3): 698–708.

Saukel J, Länger R. 1992. *Achillea pratensis* SAUKEL & LÄNGER, spec, nova, eine tetraploide Sippe der *Achillea millefolium*-Gruppe. *Phyton* 32 (1): 159-172.

Saukel J, Länger R. 1990. Die *Achillea millefolium* - Gruppe *(Asteraceae)* in Mitteleuropa, 2. *Phyton* 32 (1): 47-78.

Sokal RR, Rohlf FJ. 1962. The comparison of dendrograms by objective methods, *Taxon*, XI: 33-40.

Stebbins, G.L. 1953. A new classification of the tribe Cichorieae, family Compositae. *Madrono* 12: 65-81.

Stevens, PF. 2001 onwards. *Angiosperm Phylogeny Website*. Version 9, June 2008 [and more or less continuously updated since] http://www.mobot.org/MOBOT/research/APweb/

Susanna A**,** Garcia-Jacas N. 2007. Tribe Cardueae Cass. (1819). In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg, 123-146.

Susanna A, Garcia-Jacas N. 2009. Cardueae – In: Funk V. A., Susanna A., Stuessy T. & Bayer R. (ed.), *Systematics, evolution, and biogeography of the Compositae*. – Vienna: IAPT.

Swofford DL. 2005. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.

Szafer W, Kulczyński S, Pawłowski B. 1976. *Rośliny Polskie*, 4th ed. Panstwowe Wydawnictwo Naukowe, Warszawa.

Tutin TG, Heywood VH, Burges NA, Valentine DH. 1976. *Flora Europea 4 - Plantaginaceae To Compositae, Rubiaceae*. Cambridge University Press, Cambridge.

Wagenitz. 1955. Pollenmorphologie und Systematik in der Gattung *Centaurea* L. s.l. *Flora* 142: 213-279.

Wagenitz G. 1976. Systematics and phylogeny of the Compositae (Asteraceae). *Plant Systematics and Evolution* 125: 29-46.

Zhong Y, Meacham ChA, Pramanik S., A general method for tree-comparison based on subtree similiarity and its use in a taxonomic database, BioSystems 42, 1-8, 1997

Investigations of the Underground Parts of Medicinally Used Plants and Possible Adulterations of Various *Cardueae* and *Cichorieae*

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Asteraceae is the taxonomically most diverse family of plants comprising of more than 23,000 species and about 1,600 genera. Numerous representatives such as *Taraxacum officinale*, *Cichorium intybus, Silybum marianum,* and *Hieracium pilosella* to mention only few thereby have a long history in both traditional and western medicine. The anatomy of rhizomes and roots of a representative number of species from the tribes *Cardueae* and *Cichorieae* was analysed in detail as respective comparative studies on underground parts of medicinally used drugs and possible adulterations are missing yet for these diverse taxa. Until now, 28 genera and 37 species have been collected and examined by means of light microscopy and a database of typical anatomical characters created. In addition, some of the studied species were cultivated to follow the ontogenetic development of the underground organs at different states of growth. Particular attention was thereby spent to the secretory system: Endodermal resin ducts are characteristic to the *Cardueae*, whereas, according to literature data, these anatomical elements are restricted within the tribe *Cichorieae* to *Scorzonera hispanica*, *Tragopogon porrifolius*, and the genus *Scolymus*. *Cichorium intybus* and *Lapsana communis* were reported to exhibit the “doubling” of the endodermis, but with ducts missing [1]. In contrast to these published results, our studies unravelled, that the roots of *Cichorium intybus* contain secretory ducts at an early state of growth at least. It seems likely that they occur in young roots of *Lapsana communis* as well.
Based on anatomical structure, we could distinguish different types of resin ducts. These various forms of resin ducts and the structural context of their occurrence, particularly with respect to tissue of origin and their position relative to prominent anatomical elements such as vascular bundles (e.g., a centrifugal position in *Centaurea scabiosa* versus an interfascicular position in *Carlina acaulis*) provided valuable characters to discriminate among the species studied.

1. Van Tieghem, M. Bull. Soc. Botanique de France 1884: 112-116

APPENDIX I

List of all species examined in this work including collection history and GenBank accession number used for phylogeny (cited paper V)

Taxonomy following Funk, Susanna, Stuessy & Bayer, 2009;
Plant material collected by Elisabeth Fritz (EF), Christoph Dobeš (CD), Johannes Saukel (JS), Valerie Klatte-Asselmeyer (VK), Silvia Fialova (SF), Werner Lahner (WL), Günther Stadler (GS);
Numbers in brackets indicate the voucher numbers.

Species	Collection sites, Voucher numbers	GenBank accession no.
Echinops sphaerocephalus L.	Austria, Vienna, EF (ES02-09, ES03-09) -, Lower Austria, Tullnerbach, EF (ES06-09) -, -, Karnabrunn, CD (ES07-09) -, Tyrol, Fließ, VK (ES08-09)	AY538637
Carlina acaulis L.	-, Vienna, EF (CA02, CA05) Germany, Baden-Württemberg, Schwäbische Alb, Herbarium of Hohenack, Nr. 652 (WUP) (CA01, CA04) Samples of a commercially traded product (Kottas Pharma, sample number 1881, 1882)	-
C. vulgaris L.	Poland, Gutkowo, Olsztyn, EF (CV02-09 - CV05-09)	AY826246
Arctium lappa L.	Austria, Vienna, EF (AL01-09, AL05-09, AL3A-09)	FJ528300
A. tomentosum Mill.	-, Traiskirchen, EF (AT01-10 - AT03-10)	-
Saussurea discolor (Willd.) DC.	-, Carinthia, Lesachtal, JS (SD01-09 - SD07-09)	AF319146, AF319092
S. pygmaea (Jacq.) Spr.	-, Styria, Schneealpe, EF (SaP01-09 - SaP06-09, SaP S1)	
Jurinea mollis Rchb.	-, Vienna, EF (JM01-09) -, Burgenland, Winden, JS (JM03-86, JM04)	AY780404
Carduus personata (L.) Jacq.	-, Styria, Schneealpe, EF (CaP01-09 - CaP03-09)	-
C. defloratus L. (*C. crassifolius* Willd.)	-, Lower Austria, Gippel, CD (CC01-08) -, -, Araburg castle, EF(CC02-08, CC03-08)	AY826241

Cirsium arvense (L.) Scop.	-, Vienna, EF (CiA02-08) Slovakia, Modra, SF (CiA01-08) USA, New York, Peekskill, Planta America Septentrionalis, Le Roy (WUP) (CiA03-08)	AF443680, AF443681
C. vulgare (Savi) Ten.	Poland, Gutkowo, Olsztyn, EF (CiV01-09 - CiV03-09)	AF443715
C. erisithales (Jacq.) Scop.	Austria, Styria, Schneealpe, EF (CE01-09 - CE03-09)	-
O. acanthium L.	-, Lower Austria, Buchberg, WL (O01-08, O02-08) Italy, Southern Tyrol, Vinschgau, CD (AST3)	AY914827
Silybum marianum (L.) Gaertn.	Austria, Lower AUT, Buchberg, WL (MD1-08, MD2-08, SM1) Slovakia, Botanical Garden of Bratislava, SF (MD3-08)	AJ831537, AY914831
Serratula tinctoria L.	Austria, Vienna, JS (ST01-09 - ST04-09)	AJ868085, AJ868084
Rhaponticum scariosum Lam.	Liechtenstein, GS (RS01-09, RS02-09)	DQ310951, DQ310952
Centaurea jacea L.	Austria, Karnabrunn, CD (CJ01-09, CJ02-09) -, Vienna, EF (CJ8-1)	AM114332, DQ319125
C. scabiosa L.	-, Vienna, JS (CS03-08) Poland, Gutkowo, Olsztyn, EF (CS02-08) Switzerland, Graubünden, Lavin, CD (CS01-08A, CS01-08B)	FJ459692
C. cyanus L. (= *Cyanus segetum* Hill., Fischer *et al.)*	Austria, Vienna, EF (CS3-08) Germany, Baden-Württemberg, Kronau, CD (CS2-08) Poland, Mazury, Zabie, EF (CS1-08)	AY826254, L35879
C. montana L. (= *Cyanus montanus* Hill., Fischer *et al*.)	Austria, Lower Austria, Unterberg, EF (C01-08) -, -, Pressbaum, EF (C03-08) Slovakia, Modra, S.F. (C02-08)	L35887
Cnicus benedictus L.	Botanical Garden of the Department of Pharmacognosy, University of Vienna (CB1 – S3,S4)	DQ319091
Scorzonera hispanica L.	Austria, Lower Austria, Anninger, JS (SHI01) -, -, Weinling, JS (SHI02, SHI03)	AM117052, AY508186, AJ633472
S. humilis L.	-, -, Drosendorf, JS (SH02, SH03) -, -, Bisamberg (SH01)	AJ633476, AM117053
S. purpurea L.	-, Vienna, EF (SP01-09 - SP03-09)	-
S. aristata Ramond ex DC.	-, Carinthia, Lumkofel, JS (SAr01-09 - SAr03-09)	AY508192

S. austriaca Willd.	-, Vienna, EF (SA01-09, SA04-09, SA05-09)	AY508216, AM117047
S. rosea Waldst. & Kit.	Austria, Carinthia, Lumkofel, JS (SR01-09, SR02-09)	-
Tragopogon orientalis L.	-, Lower Austria, Araburg, EF (TO01-08) -, -, Laaben, EF (TO02-08) Poland, Gutkowo, Olsztyn, EF (TO03-08)	AY508170
T. dubius Scop.	Austria, Vienna, EF (TD1-08) Italy, Vinschgau, Schluderns, CD (AST1)	AY525376, AY645813
Lactuca virosa L.	Austria, Vienna, EF (LV01-07, LV02-07)	AJ633335
L. perennis L.	Italy, Eisacktal, JF (LP01) -, Southern Tyrol, Vinschgau, CD (LPAST14A, LPAST14B)	AJ633334
L. muralis (Mycelis muralis Dumort.*)*	Austria, Vienna, EF (M01-08, MM01-08) -Lower Austria, Irenental, EF (M02-07) -, Rekawinkel, EF (M01-07)	AJ633338, AJ633339
L. alpina Benth. & Hook.f. *(Cicerbita alpina* Wallr.*)*	-, Styria, Schneealpe, EF (CiAl01-09 - CiAl03-09)	AJ633324, AJ633340
Aposeris foetida Cass.	Austria, Carinthia, Feistritz im Rosental, JS (AF1, AST19) Germany, Garmisch-Partenkirchen, Wank, CD (AST23)	DQ451822
Sonchus oleraceus L.	Austria, Vienna, EF (SO01-07) -Lower Austria, Irenental, EF (SO02-07A, SO02-07B) Italy, Southern Tyrol (CD): seeds planted in Botanical Garden of the Department of Pharmacognosy (AST18)	GQ478113, AY862581
Crepis biennis L.	Italy, Southern Tyrol, Vinschgau, CD (AST5) Slovakia, Modra, SF (CB01-08) Botanical Garden Karl Franzens Universität Graz; Mürzsteger Alpen; Seewirtgraben / Mariazell (70) Bot. Garten Berlin-Dahlem; DE-0-B-0164479: Hessen, Werra-Meißner-Kreis, Eschwege, leg. Royl&al. (814)	DQ451818
C. aurea Rchb.	Austria, Salzburg, Mehrlhütte, JS (CrA01-08 - CrA04-08)	AF528483, AJ633359
C. pontana Druce	Austria, Carinthia, Lesachtal, Lumkofel, JS (CP01-09 - CP04-09)	-
Lapsana communis L.	Italy, Southern Tyrol, Vinschgau, CD (AST17) Austria, Vienna, EF (LC01-08, LC02-08B) Germany, Baden-Württemberg, Blankenloch, CD (LC02-08A)	AJ633285, AJ633286
		-

Taraxacum cucullatum Dahlst.	-, Salzburg, Riedingtal, JS (TC01-08 - TC04-08)	
T. laevigatum DC.	-, Vienna, JS (TL01-08 - TL03-08)	AJ633288
Chondrilla juncea L.	-, Vienna, Kovats (CJ02) Hungary, Holuby (CJ01)	AJ633348
Willemetia. stipitata Cass.	Austria, Salzburg, Maria Pfarr, JS (WS01-08 - WS04-08)	AY508170
Helminthotheca echioides (L.) Holub	Botanical Garden of the Univ. Bonn; 1744;DE-0-Bonn-23773: F. Klingenstein&J. Manner,1998, Deutschland, Nordrhein-Westfalen, Bonn-Ippendorf (HE1)	AF422123
Hypochaeris uniflora Hoffm.	Austria, Salzburg, Lungau, JS (V310, V312) -, Salzburg, Kareck, JS (101, HU01-08)	AF528481
H. radicata L.	Poland, Gutkowo, Olsztyn, EF (5-100, 5-16) Botanical Garden Berlin-Dahlem; 916; DE-0-B-2421281: Bayern, Kreis Wunsiedel, Fichtelgebirge, leg. Hempel Italy, Eisacktal, Blumau-Rielinger-Atzwang, JS (H113)	EF107656, GU011987, AF528457
Leontodon incanus Schrank	-, Burgenland, St. Margarethen, JS (L100) -, Steiermark, Oberwölz, JS (L108) -, Lower Austria, Baden, JS (L305) -, -, Anninger, JS (L358, L359)	DQ451772
L. hispidus Scop.	-, -, Rekawinkel, EF (L1-106) -, -, Rax, EF (3-22) -, Carinthia, Pöllatal, JS (L105)	AF528485, DQ451769, DQ451770
Picris hieracioides L.	Austria, Vienna, EF (PH02-07A, PH02-07B) Poland, Gutkowo, Olsztyn, EF (PH01-07)	AJ633320, AJ633322
Scorzoneroides helvetica (Merat) Holub	-, Styria, Etrachtal, JS (L109) -, -, Prebertal, JS (L112) -, -, Anger, Lessachwinkel, JS (L109)	DQ451766, DQ451767
S. autumnalis (L.) Moench	Poland, Gutkowo, Olsztyn, EF (L102 - L104)	AF528486
Prenanthes purpurea L.	Austria, Styria, Schneealpe, EF (PP01-09 - PP03-09)	AJ633342, AJ633343
Hieracium murorum L.	-, Lower Austria, Hohe Wand, EF (HM01-08) Switzerland, Graubünden, Bos-cha, CD (AST11) Botanischer Garten Karl Franzens Universität Graz; 81; Schillingsdorf, VI-X/2007 (AD) (HM1)	AF528492
H. pilosella L.	Italy, Southern Tyrol, Vinschgau, CD (AST6) Poland, Gutkowo, Olsztyn, EF (01-07); (HP01-86)	AY879161
Cichorium intybus L.	Austria, Lower Austria, Pressbaum, EF (CI1-07) -, Vienna, EF (CI1-08)	AY504694, AJ633451

	Poland, Gutkowo, Olsztyn, EF (CI2-07) Seeds of the Botanical Garden Berlin-Dahlem:DE-0-B-2003105:Brandenburg, Falkensee, leg. Dürbye 3090 (CI1)	
Chlorocrepis staticifolia (All.) Griseb.	Italy, Southern Tyrol, Vinschgau, CD (AST7) -, Valbruna, CD (AST22)	AJ633437
Wunderlichia mirabilis Riedel ex Baker (Outgroup)		DQ414742

APPENDIX 2

Supplementary material: Microscopical descriptions of all taxa examined (including material of papers I-IV)

Number of voucher of which the photograph was taken is given in brackets after the legends; bar scales = 50µm

TRIBE CARDUEAE

SUBTRIBE ECHINOPSINAE

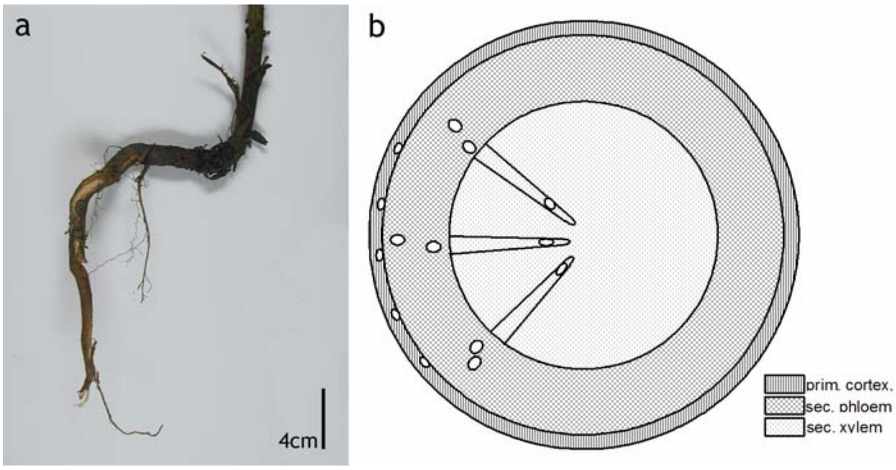
GENUS *ECHINOPS*

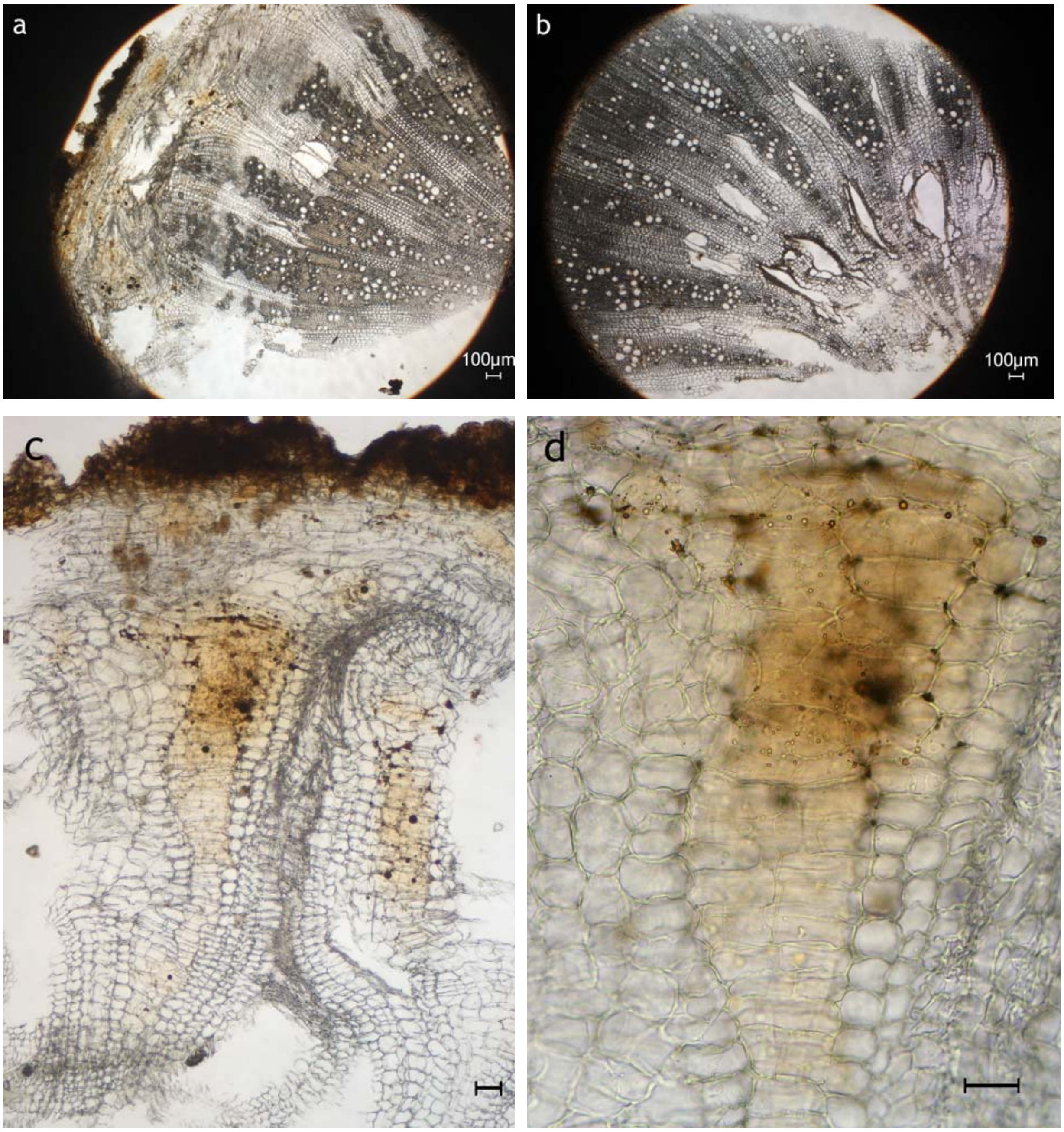
ECHINOPS SPHAEROCEPHALUS L.

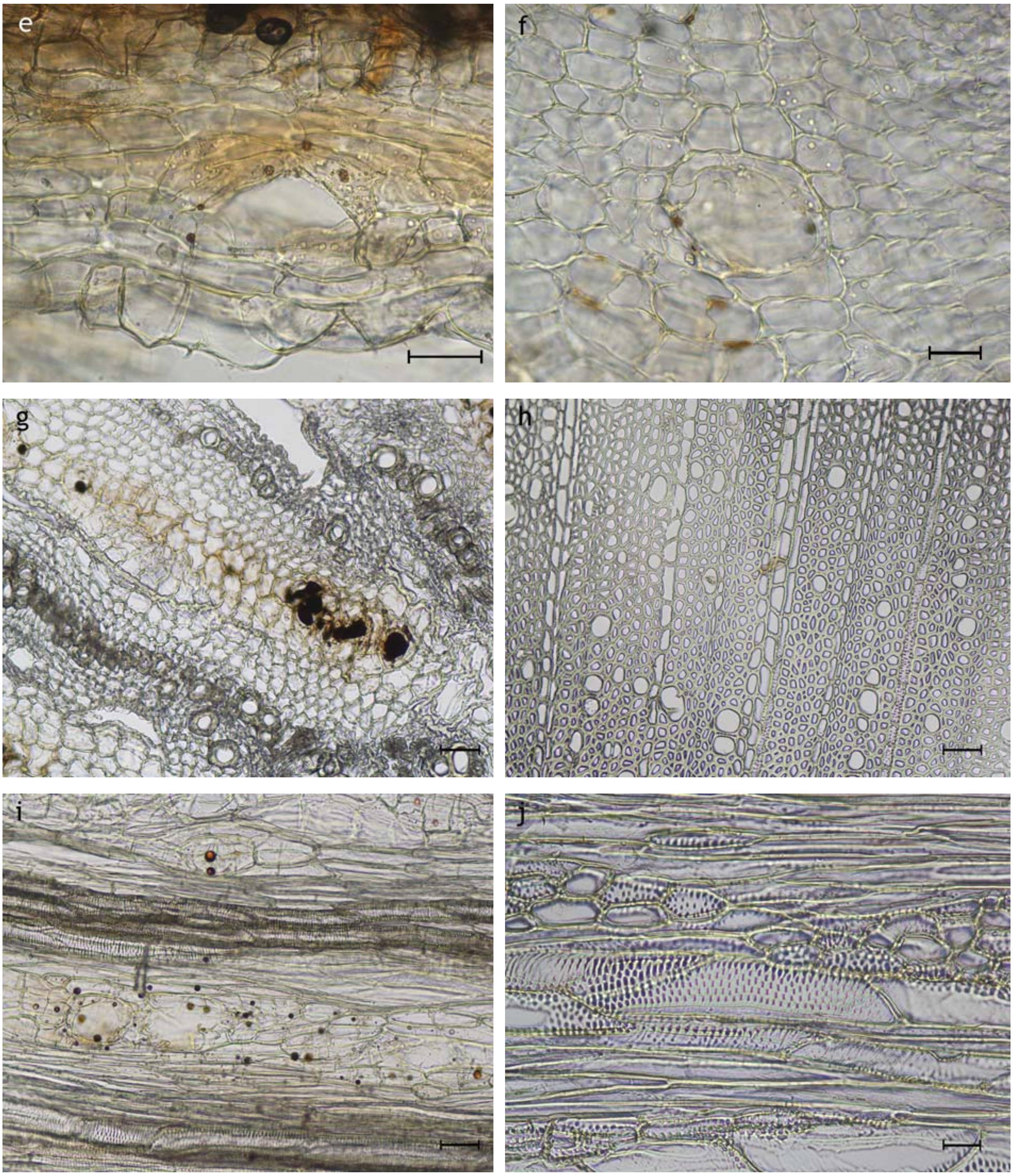
biennial to perennial hemicryptophyte;
allorhizous with taproot or fibrous root system;

secondary root: cork thin-walled, sometimes with crystalloids; **cortex** enduring; **endodermal resin ducts (endoSDs)**; **secondary phloem** broad, but of lesser radial extension than the vascular cylinder, with rare sclereids and with fibers arranged in bundles; **secondary xylem** dominated by fibers, vessels strongly bordered, up to 75µm in diameter, dispersed, sometimes circularly arranged relative to the centre of the xylem; **medullary rays** mainly uni- and biseriate, parenchymatous, getting broader towards the root center; **secretory cavities (SCs)** located in the medullary rays towards the root centre as well as in the rays and the axial parenchyma of the secondary phloem; **pith** missing
laticifers missing;

a: Root of *Echinops sphaerocephalus*, habitus (ES08-09)
b: Schematic view of *E. sphaerocephalus* (transverse section): small ellipses mark the position of the endoSDs of the cortex and SCs in the rays of the sec. phloem and in the medullary rays of the xylem







Echinops sphaerocephalus, root: **a:** Overview showing the extension and arrangement of tissues: the sec. xylem is the most expanded component followed in extension by the sec. phloem; cortex conspicuously small; the torn-apart phloem is an artefact (ES06-09); **b:** vascular cylinder: as a natural process during the development of the root medullary rays are ripped apart near the centre of the root; the xylem consists mostly of fibers interspersed with vessels (ES07-09); **c:** cork, cortex, sec. phloem with SCs (ES02-09); **d:** SC within sec. phloem (ES02-09); **e:** endoSD (ES06-09); **f:** SC of the sec. phloem; (ES06-09); **g:** SC within medullary ray (ES02-09); **h:** xylem dominated by fibers and with dispersed vessels, uni-, bi- and multiseriate medullary rays in regular arrangement (ES08-09); **i:** SC in medullary rays (ES03-09); **j:** strongly bordered vessels (ES08-09);
a-h: transverse sections; i,: longitudinal sections; j: tangential sections

SUBTRIBE CARLININAE

GENUS *CARLINA*

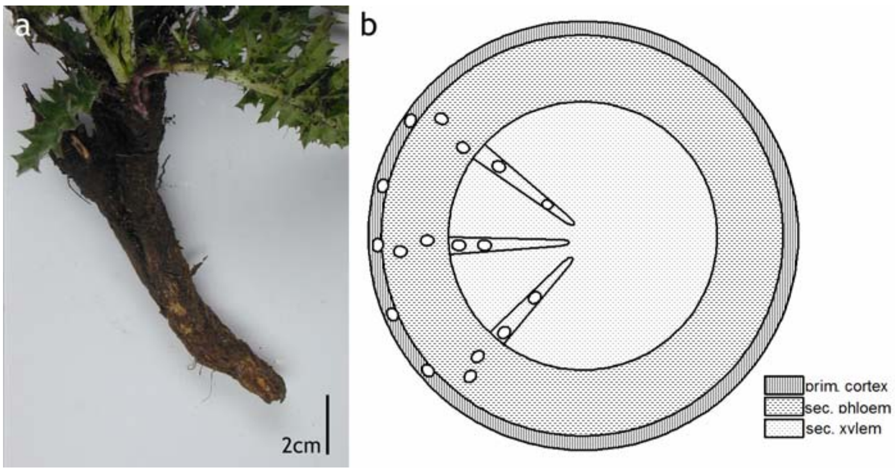
CARLINA ACAULIS L. (cf. Paper III)

perennial hemicryptophyte;

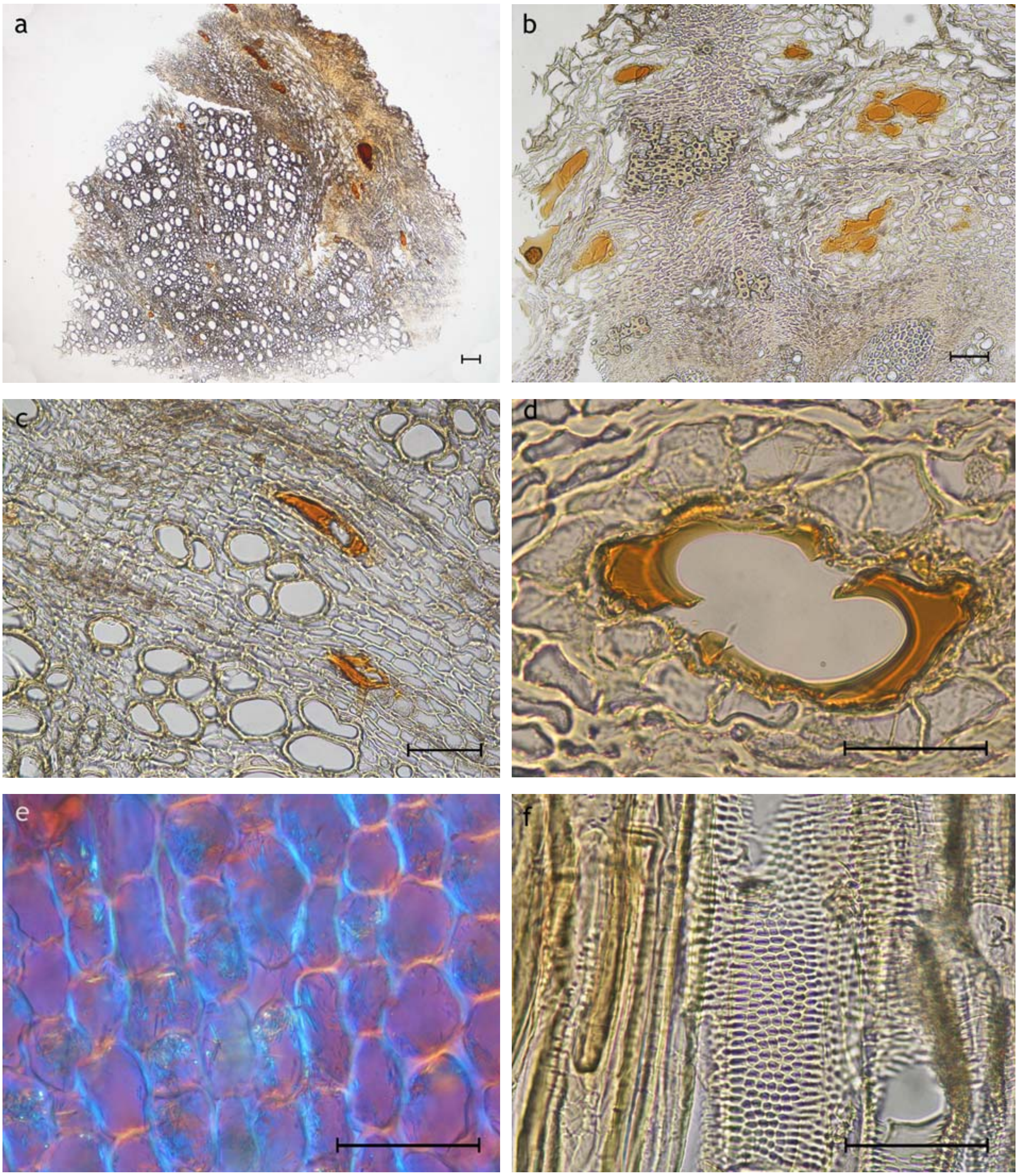
allorhizous, with taproot;

secondary root: cork thin-walled; **cortex** durable or lost in course of rhytidome formation; **endoSDs** are in size a multiple of the diameter of the surrounding parenchyma cells, lost together with the cortex; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with fibers arranged in bundles, rays with crystalline needles; **secondary xylem** consists of fibers and vessels; vessels reticulate and simple, strongly bordered, up to 114µm in diameter; **medullary rays** multiseriate, parenchymatous, with crystalline needles of the same type occurring in the phloem; **secretory ducts** of type SD1 / SD2a, size a multiple of the diameter of the surrounding parenchyma cells, located in the medullary rays and in the phloem rays; **pith** missing

sclereids, laticifers missing;



a: Taproot of *Carlina acaulis*, habitus (CA01-09)
b: Schematic view of the root of *C. acaulis* (transverse section): small ellipses mark the position of the endoSDs of the cortex and SD1/SD2a in phloem rays and in the medullary rays of the xylem



Carlina acaulis root: **a:** Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component followed in extension by the secondary phloem; cortex conspicuously small or lost in course of rhytidome formation; phellem, small cortex with endoSDs, secondary phloem and xylem with secretory ducts type SD1 within the medullary rays (CA02); **b:** secondary phloem with size a multiple of the diameter of the surrounding parenchyma cells and fibers arranged in bundles (CA04); **c:** SD1 and crystalline needles in medullary rays of the vascular cylinder (CA02); **d:** secretory ducts type SD1 of secondary phloem (CA04); **e:** crystalline needles in medullary rays (CA1883); **f:** strongly bordered vessels (CA01)
a-f: transverse sections; g: longitudinal section;

CARLINA VULGARIS L. (cf. Paper III)

perennial hemicryptophyte;

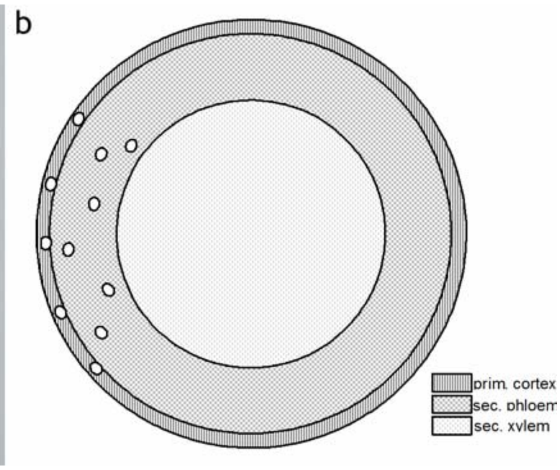
allorhizous, with taproot;

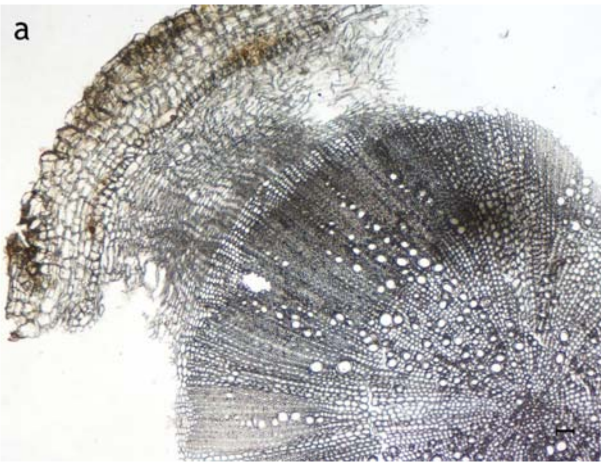
secondary root: cork thin-walled sometimes with crystalloids; **cortex** enduring; distinct endodermis with **endoSDs** – usually up to 10 surrounding cells at maximum; **secondary phloem** broad, but of lesser radial extension than the vascular cylinder; **secondary xylem** dominated by fibers, few vessels dispersed over the transverse section, strongly bordered, up to 59µm in diameter; **medullary rays** biseriate; **secretory ducts** of type SD2a located in the secondary phloem; **pith** missing;

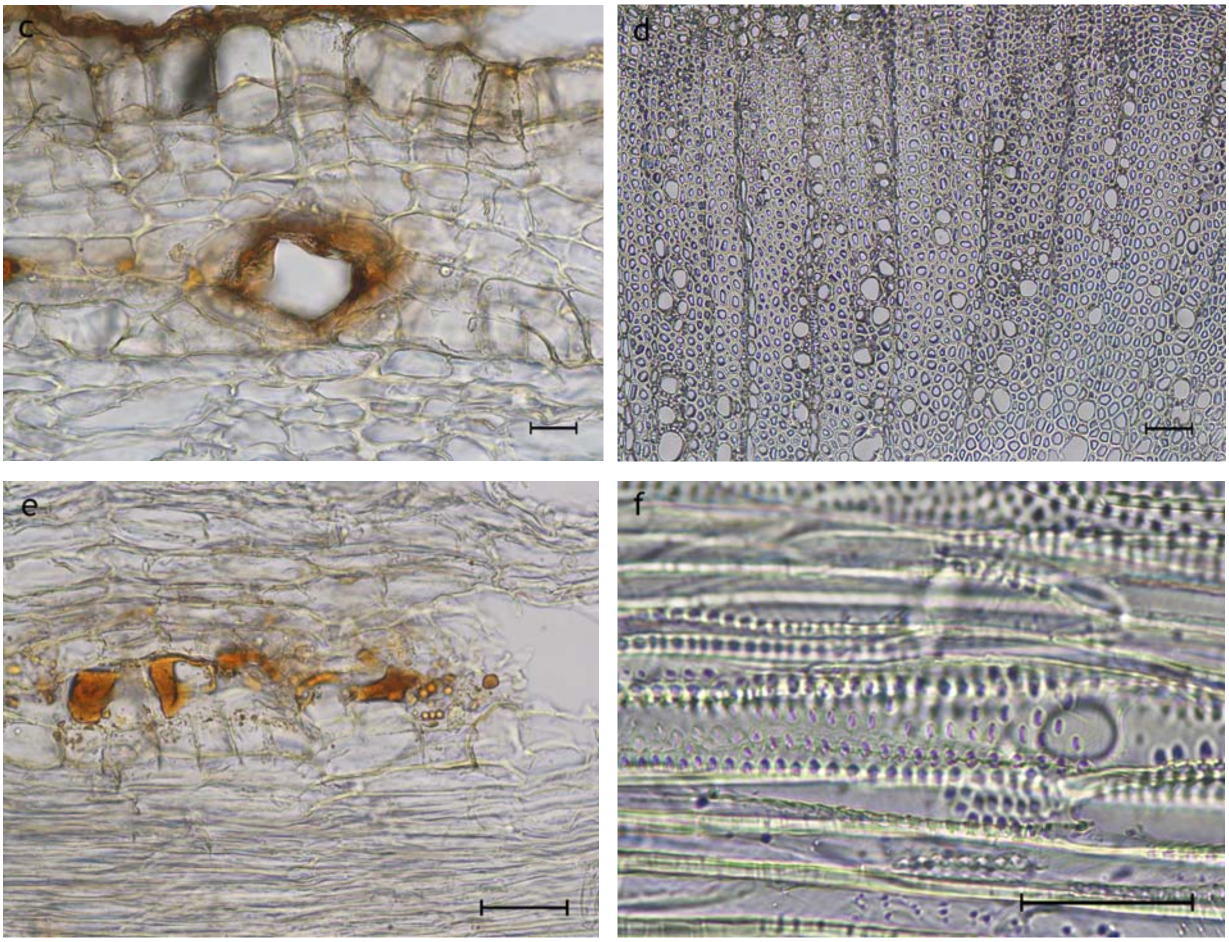
sclereids, laticifers missing;

fibers in sec. phloem missing;

a: Taproot of *Carlina vulgaris,* habitus (CV01-09)
b: Schematic view of *Carlina vulgaris* taproot in transverse section: small ellipses mark the position of the endoSDs of the cortex and type SD2a ducts of the secondary phloem







Carlina vulgaris root: **a:** Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component followed in extension by the secondary phloem; cortex conspicuously small with distinct endodermis (CV01-09); **b:** overview showing distinct endoSDs and secretory ducts of the type SD2a in the secondary phloem (CV03-09) **c:** distinct endodermis with endodermal resin duct (CV03-09); **d:** xylem dominated by fibers and with dispersed vessels, biseriate medullary rays in regular arrangement (CV04-09); **e:** secretory duct type SD2a (CV03-09); **f:** strongly bordered vessels (CV03-09)
a-d: transverse sections; e-f: longitudinal section;

SUBTRIBE CARDUINAE

GENUS *ARCTIUM*

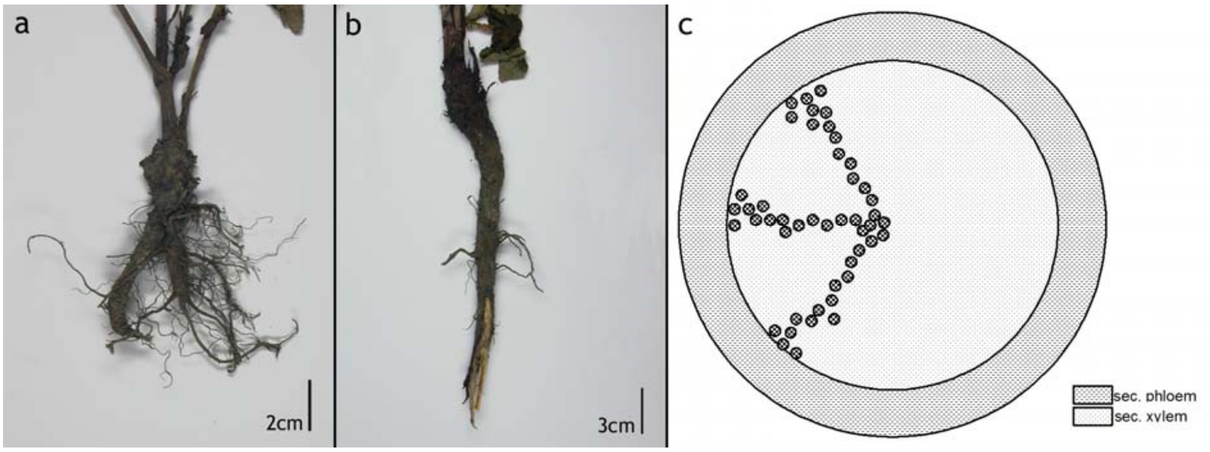
ARCTIUM LAPPA L. / *A. TOMENTOSUM* Mill. (cf. Paper III)

There is no difference of the root anatomy between the two species.

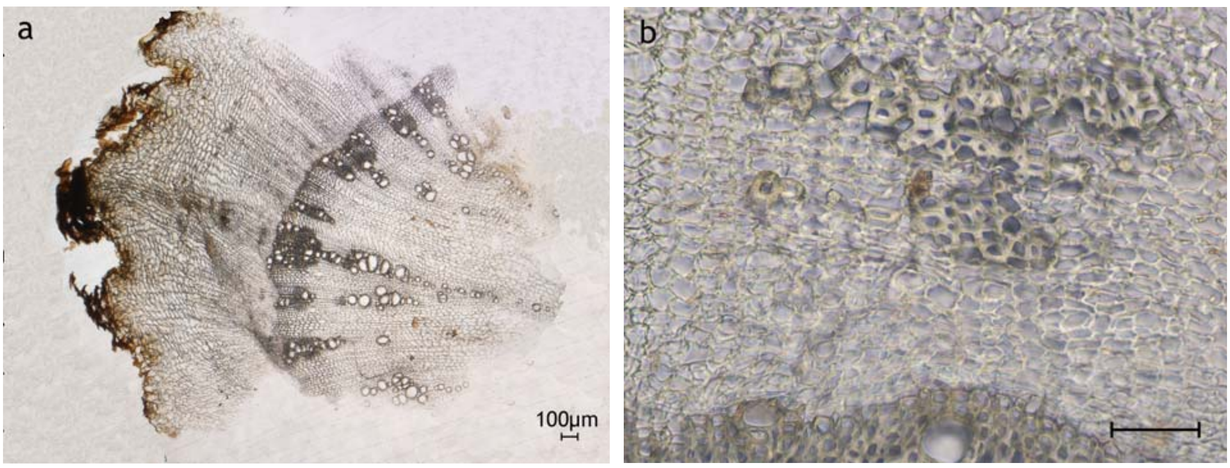
perennial hemicryptophyte;

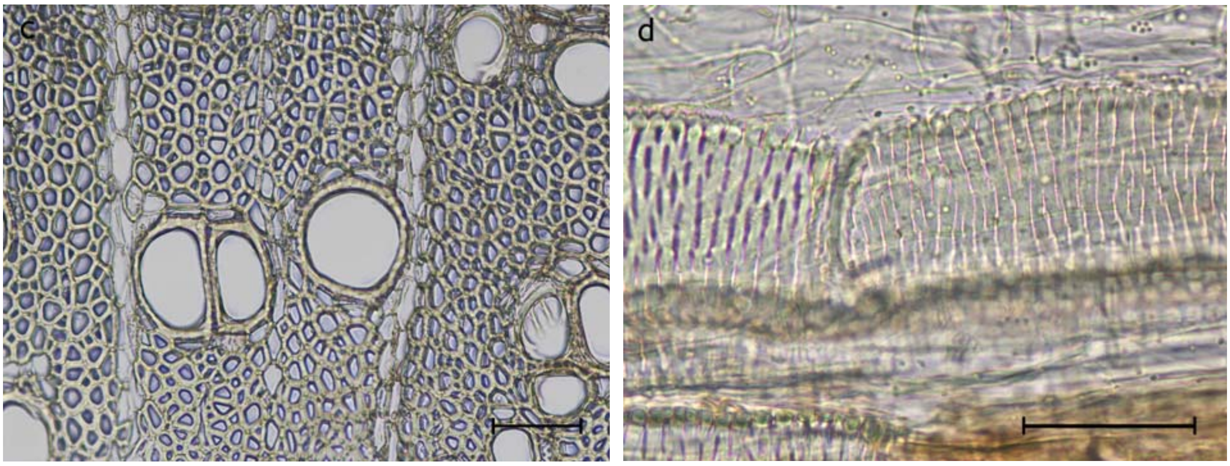
allorhizous, with taproot;

secondary root: broad **cork / bark** thin-walled – **cortex** lost in course of rhytidome formation; **endoSDs** lost together with the cortex; **secondary phloem** broad, but of lesser radial extension than the vascular cylinder, possibly with fibers arranged in bundles; **secondary xylem** mostly with vessels in rows – in the center single-rowed, in the outer part multiple-rowed, often combined with fibers, mainly reticulate, also weakly bordered up to 89µm (*A. lappa*) / 125µm (*A. tomentosum*) in diameter; **medullary rays** multiseriate, unlignified; cells of medullary rays often obliterating and easily ripping apart; **pith** missing;
sclereids, laticifers, crystalloids missing;
secretory ducts besides endoSDs missing;

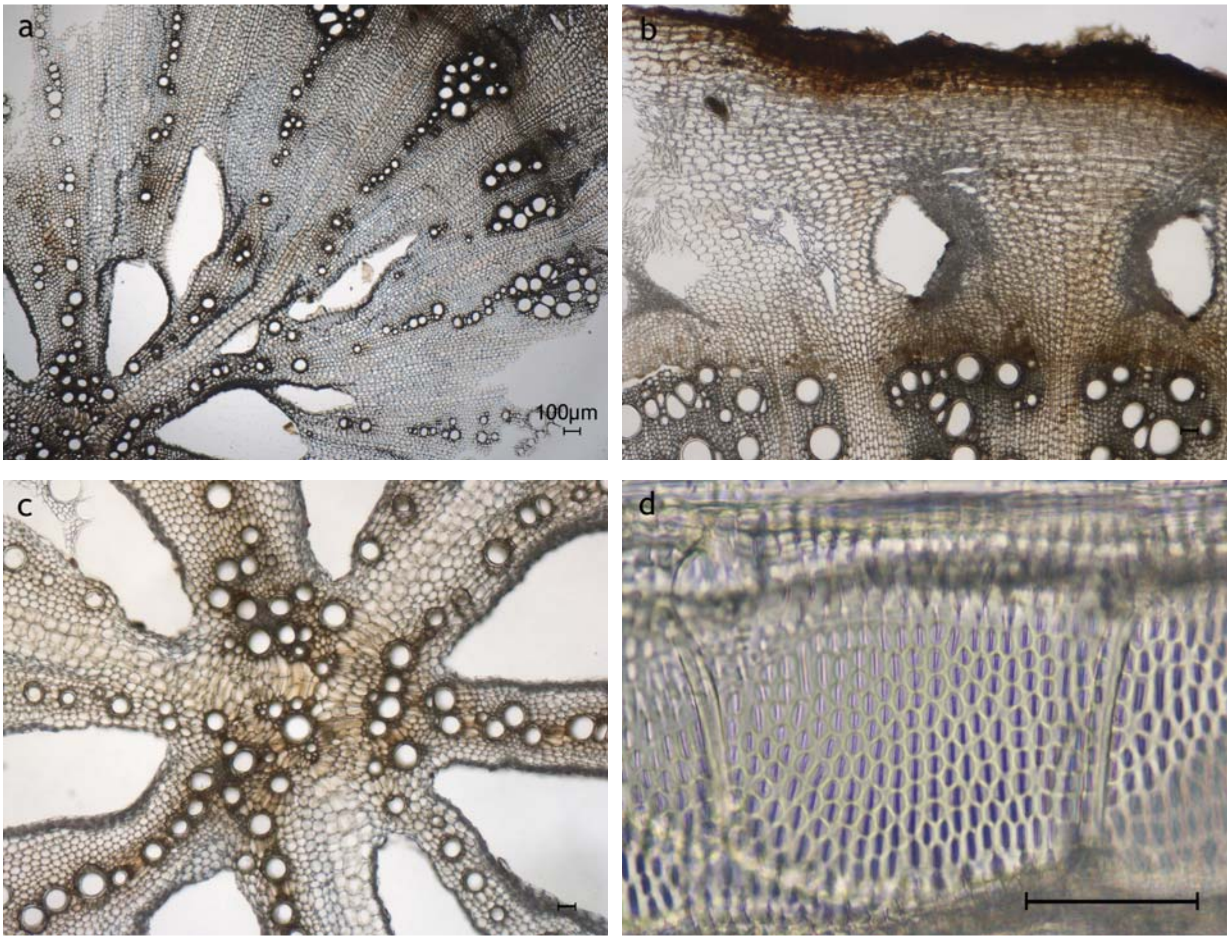


a: Taproot of *Arctium lappa*, habitus (AL3A-09)
b: Taproot of *Arctium tomentosum*, habitus (AT02-10)
c: Schematic view of *Arctium* sp. root (transverse section): black points mark the distribution of vessels; sec. phloem broad, but of lesser radial extension than the vascular cylinder with cortex and therefore endoSDs missing;





Arctium lappa root: **a:** Overview showing the extension and arrangement of tissues: the sec. xylem is the most expanded component followed in extension by the sec. phloem; cortex lost in course of rhytidome formation; xylem dominated by unlignified parenchymatous cells with vessels in rows (AL3A-09); **b:** sec. phloem with sclereids and fibers arranged in bundles (AL01-09); **c:** xylem with fibers and with dispersed vessels, medullary rays in regular arrangement (AL05-09); **d:** reticulate vessels (AL05-09)
a-c: transverse sections; d,e: longitudinal section;



Arctium tomentosum root: **a:** Overview showing the extension and arrangement of the sec. xylem: it is the most expanded component; dominated by unlignified parenchymatous cells with vessels in rows (AT01-10); **b:** sec. phloem ripping apart (AT01-10); **c:** xylem with vessels in rows, medullary rays in regular arrangement (AT01-10); **d:** strongly bordered vessels (AT01-10)
a-c: transverse sections; d: longitudinal section;

GENUS SAUSSUREA

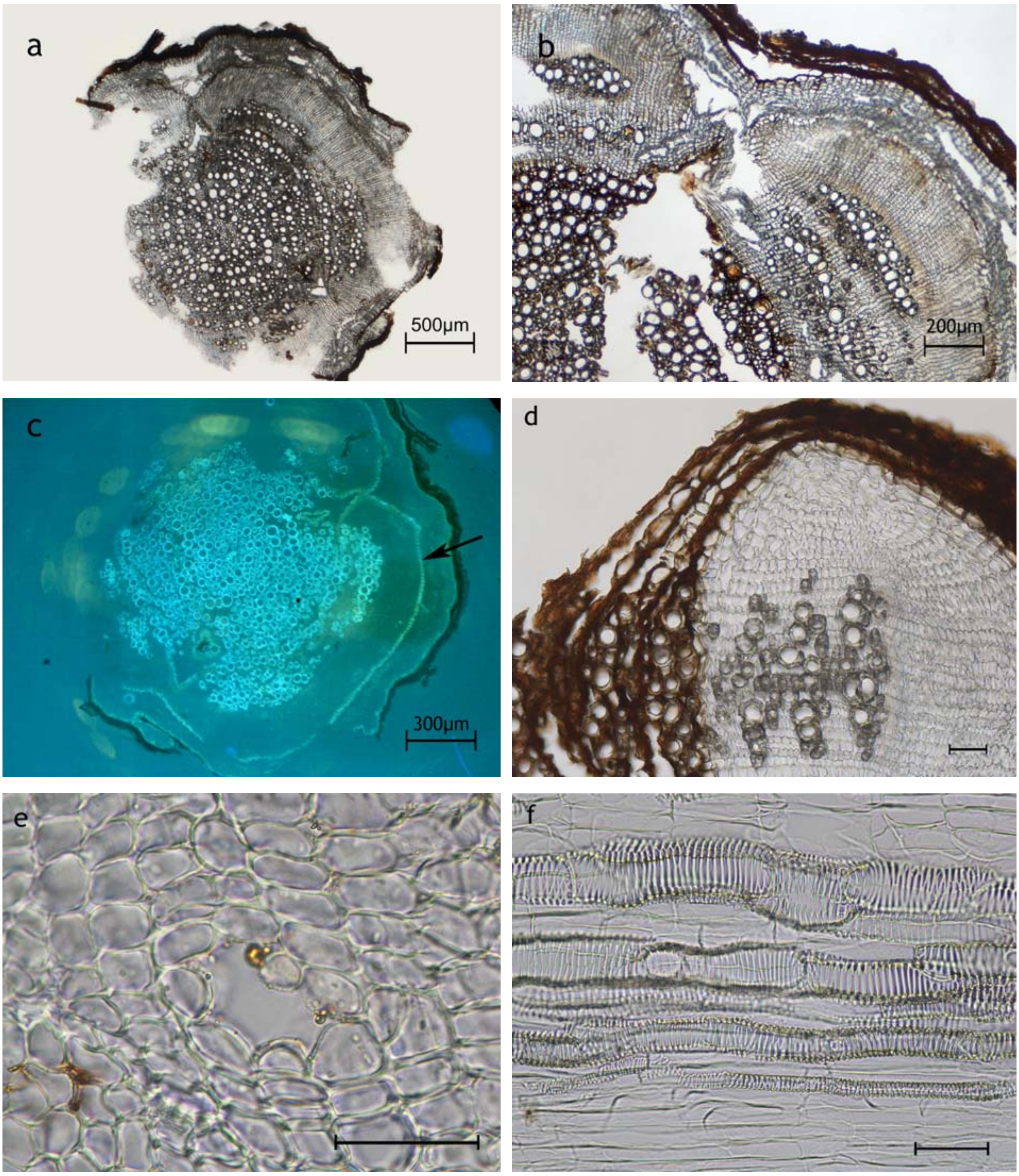
SAUSSUREA DISCOLOR (Willd.) DC. / *S. PYGMAEA* (Jacq.) Spr. (cf. Paper I)

The following description applies to both species. Single difference: In contrast to *S. discolor*, *S. pygmaea* doesn't contain fibers.

perennial hemicryptophyte;
allorhizous, root splitted into strands;

secondary root: interxylary **cork** resulting in the separation of the main root into smaller strands each comprising of secondary phloem and secondary xylem surrounded by numerous periderms forming a rhytidome (Fritz and Saukel, in print); **cortex** lost in course of rhytidome formation; **endoSDs** lost together with the cortex; **secondary phloem** broad, but secondary xylem dominating in extension; **secondary xylem** consists of vessels, irregularly dispersed and (in case of *S. discolor*) bundles of fibers; vessels reticulate (rarely simple), up to 70µm (S. discolor) / 45µm (*S. pygmaea*) in diameter; **secretory ducts** of type SD2 within secondary phloem though ducts not always visible due to elimination of tissues in course of interxylary periderm; **medullary rays** multiseriate; **pith** missing;
sclereids, laticifers missing;
fibers in secondary phloem (both species) and secondary xylem (*S. pygmaea*) missing;

a: Root of *Saussurea pymaea*, habitus (SP02-09)
b: Root of *S. discolor* (SD02-09)



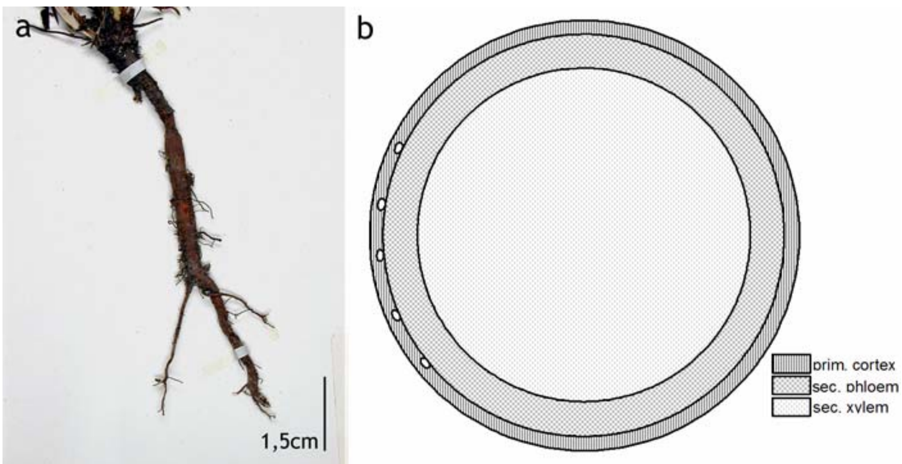
Saussurea discolor root (a-d): **a,b:** Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component followed in extension by the secondary phloem; cortex conspicuously lost in course of rhytidome formation (SD01-09); **c:** fluorescence microscopy under UV 330-380 nm: blue fluorescence of vessels and periderm (arrow), interxylary and cortical periderm begin to connect across medullary rays (SD01-09); **d:** repeated layers of periderm within secondary xylem (SD03-09); *Saussurea pygmaea* root (e,f): **e:** secretory duct type SD2 within secondary phloem (SD01-09); **e:** crystalline needles in medullary rays (SP01-09); **f:** reticulate vessels (SP01-09)
a-f: transverse sections; g: longitudinal section;

GENUS *JURINEA*

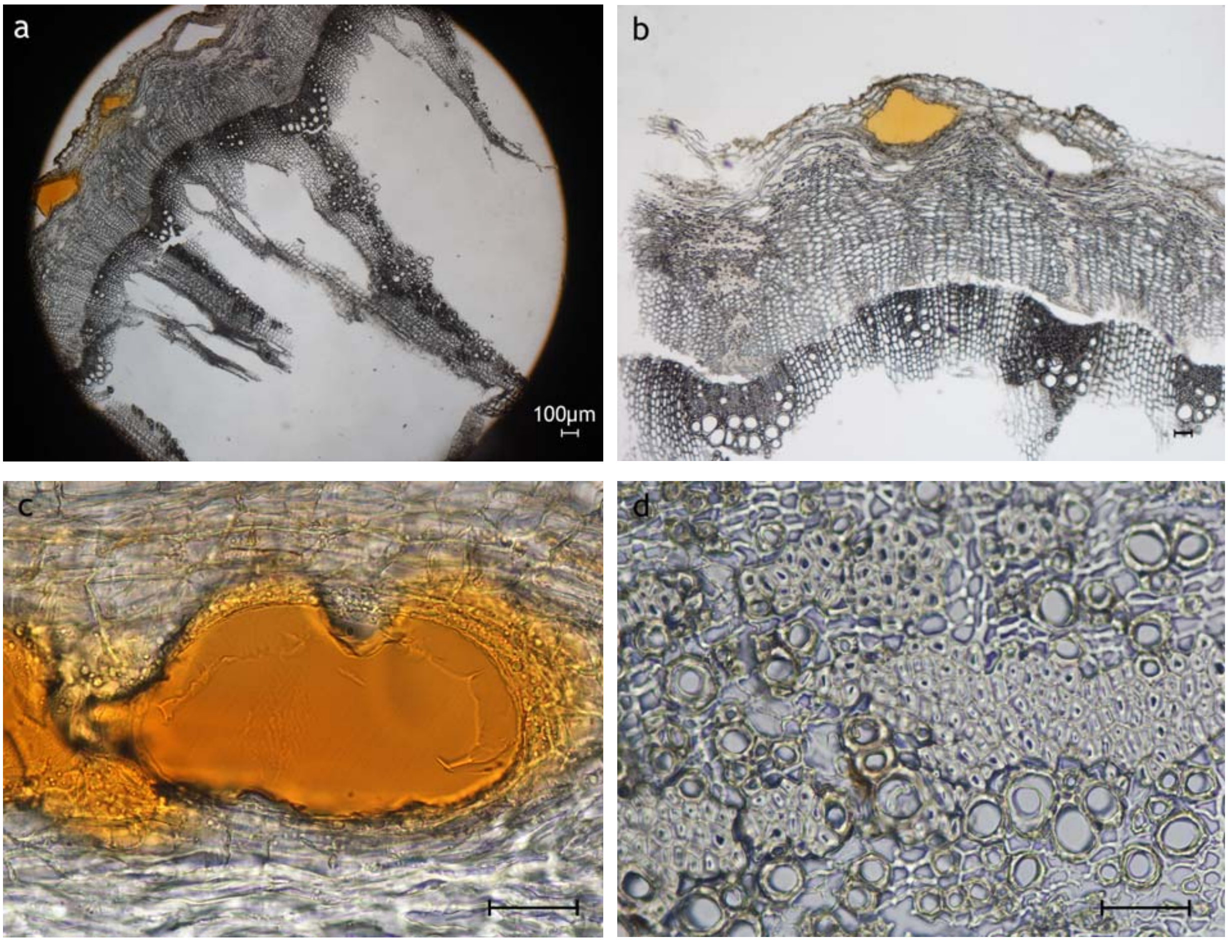
JURINEA MOLLIS Rchb.

perennial hemicryptophyte;
allorhizous, with taproot;

secondary root: cork thin-walled sometimes with crystalloids; **cortex** small, enduring; distinct endodermis with **endoSDs** – in size a multiple of the diameter of the surrounding parenchyma cells; **sec. phloem** broad, but of lesser radial extension than the vascular cylinder, with fibers arranged in groups in fascicular position above the vascular cylinder between phloem rays; **sec. xylem** with **vessels** in broad rows or wedges and fibers in tangential bands alternating with parenchymatous cells; vessels mainly reticulate, also bordered; within the wedges / rows vessels subject to their size circularly arranged, up to 68µm in diameter with the diameter in the outer parts larger than in center; **medullary rays** multiseriate; **pith** missing;
sclereids, laticifers missing;
secretory ducts beside endoSDs missing;



a: Root of *Jurinea mollis*, habitus (JM03-86)
b: Schematic view of *J. mollis* root (transverse section): small ellipses mark the position of the endoSDs of the cortex, sec. phloem of lesser radial extension than the vascular cylinder



Jurinea mollis root: **a:** Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component followed in extension by the secondary phloem; cortex conspicuously small; phellem, small cortex with endoSDs in a size multiple of the diameter of the surrounding parenchyma cells, secondary phloem with further secretory ducts missing, xylem (JM03-86); **b:** cortex with endoSDs, secondary phloem with fibers arranged in groups in fascicular position above the vascular cylinder between phloem rays (JM03-86) ; **c:** endodermal resin duct of a size multiple of the diameter of the surrounding parenchyma cells with content (JM03-86; cited Paper II); **d:** secondary xylem with vessels more or less circularly arranged, fibers in tangential bands alternating with parenchymatous cells (JM03-86);
a-d: transverse sections;

GENUS *CARDUUS*

CARDUUS DEFLORATUS L. (= *C. CRASSIFOLIUS* Willd. [Fischer et al.]) (cf. Paper III)

perennial hemicryptophyte;

allorhizous, with taproot;

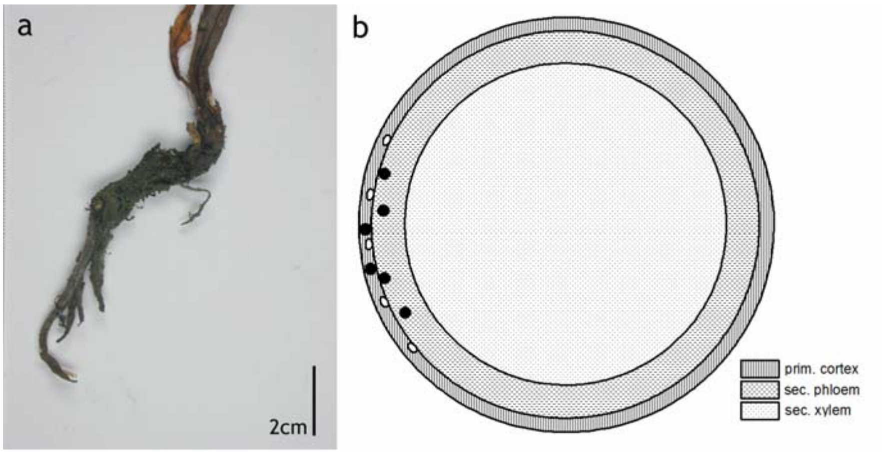
secondary root: bark thin-walled, broad – **cortex** lost in course of rhytidome formation, parts remain with sclereids: in transverse section small lumen, in longitudinal section resembling the adjacent parenchyma cells in size and shape; **endoSDs** lost together with the cortex; **sec.**

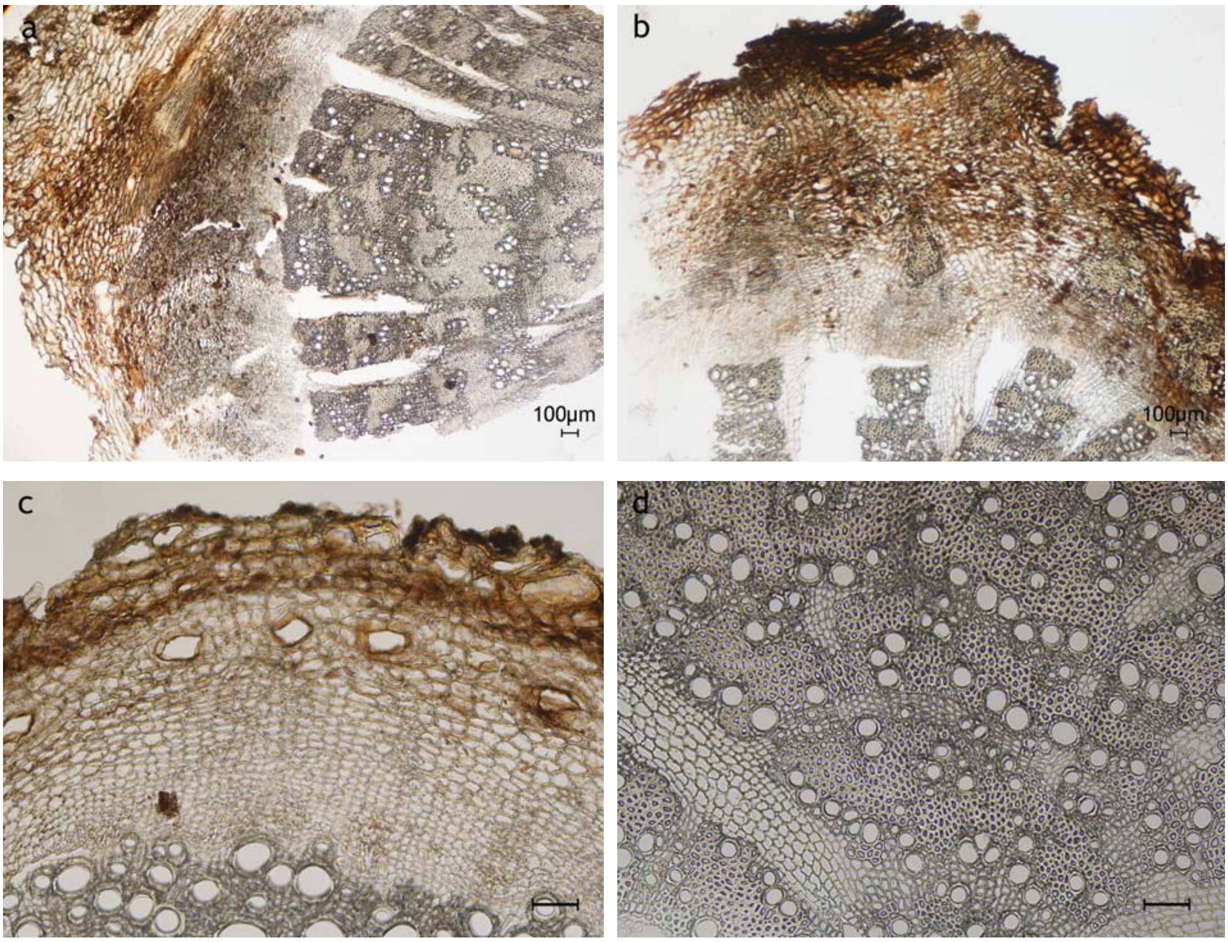
phloem broad, but of lesser radial extension than the vascular cylinder, suberizing, with sclereids of the same type occurring in the cortex and fibers single or arranged in bundles; **sec. xylem** dominated by fibers often arranged in tangential bands alternating with parenchymatous cells; vessels circularly arranged, mainly reticulate, also weak bordered, up to 91µm in diameter; **medullary rays** multiseriate, unlignified; **pith** missing;

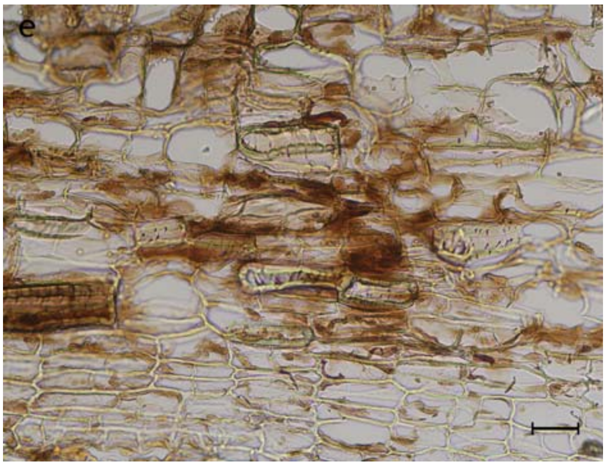
crystalloids, laticifers missing;

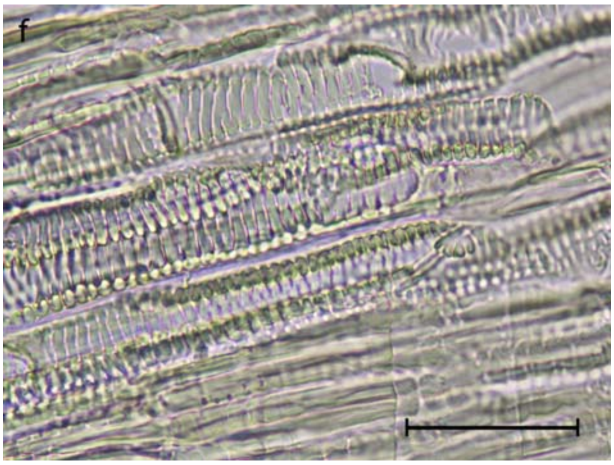
secretory ducts besides endoSDs missing;

a: Root of *Carduus crassifolius*, habitus (CR03-08)
b: Schematic view of *C. crassifolius* root (transverse section): small ellipses mark the position of the endoSDs of the cortex, black points represent the sclereids within the suberized cortex and the sec. phloem









Carduus crassifolius root: **a:** Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component followed in extension by the secondary phloem; cortex and parts of the secondary phloem suberized (CC03-08); **b:** overview showing secondary phloem with fibers arranged in groups, bark (CC02-08); **c:** cortex with regularly arranged endoSDs of a young *Carduus crassifolius* root (CC01-08); **d:** vascular cylinder with vessels circularly arranged, fibers in tangential bands alternating with parenchymatous cells, medullary rays multiseriate (CC01-08); **e:** sclereids in bark resembling the adjacent parenchyma cells in size and shape (CC03-08); **f:** reticulate vessels (CC02-08)
a-d: transverse sections; e,f: longitudinal sections;

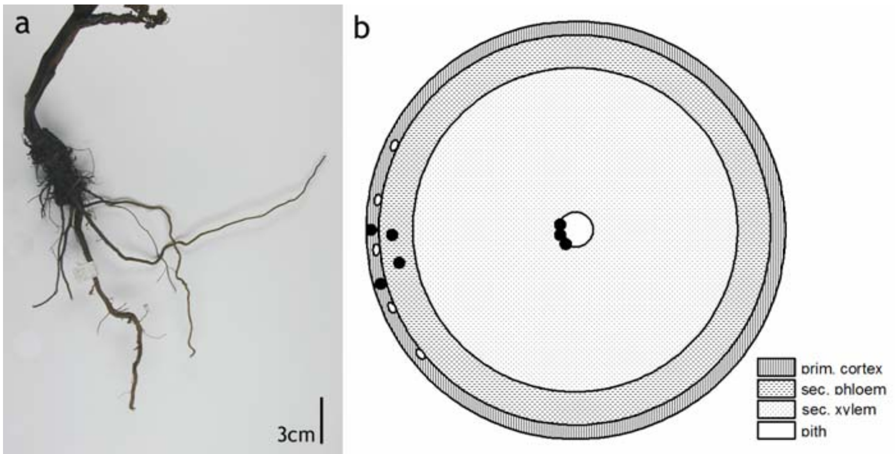
CARDUUS PERSONATA (L.) Jacq. (cf. Paper III)

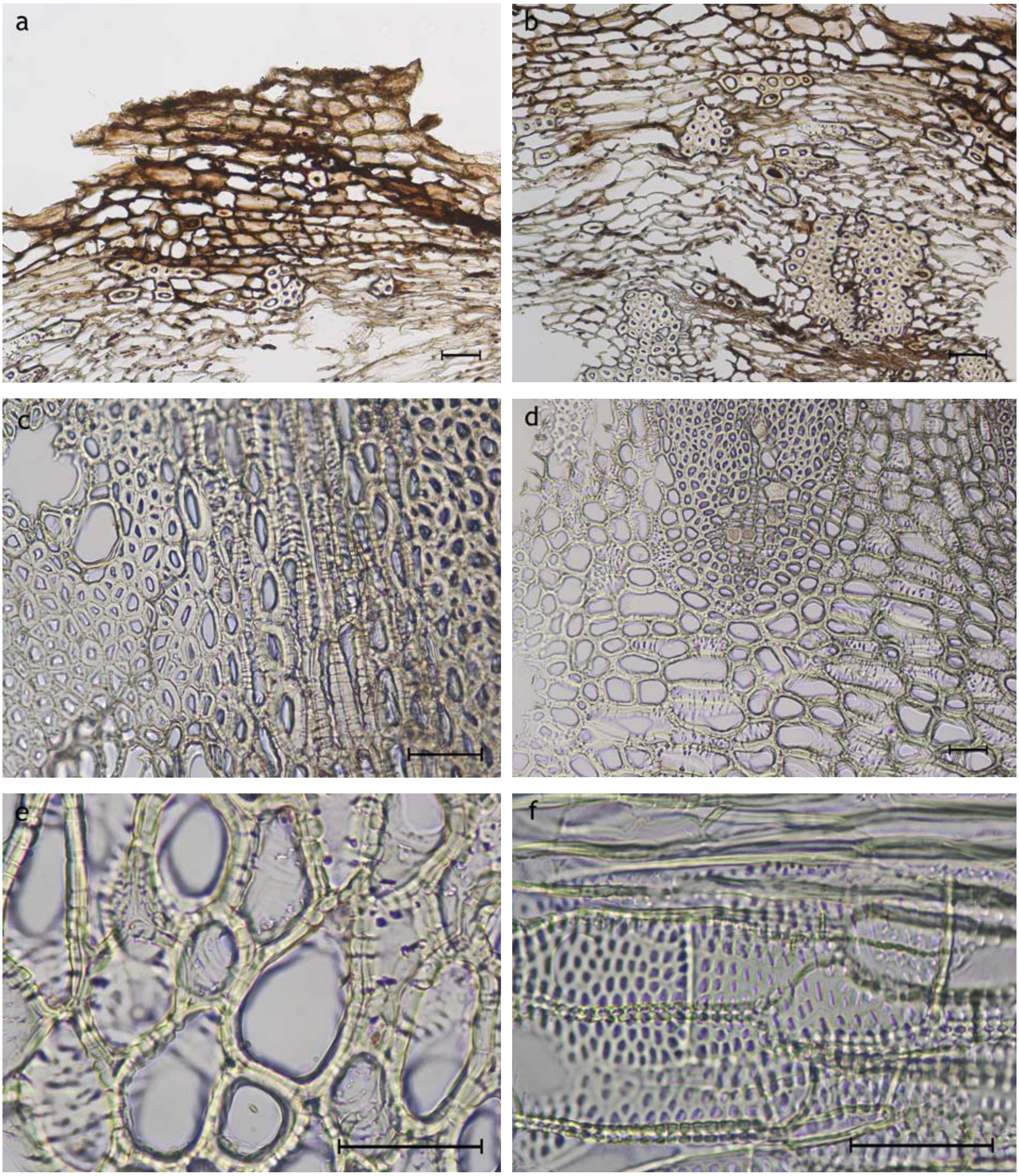
perennial hemicryptophyte;

rhizome;

rhizome with secondary growth: Thin-walled **cork / bark**; **cortex** suberized with sclereids either round and with small lumen or resembling the adjacent parenchyma cells in size and shape; **endoSDs** or remnants of them visible; **secondary phloem** usually broader than cortex, but of lesser radial extension than the vascular cylinder, within older rhizomes partly suberized, with sclereids of the same type occurring in the cortex and fibers single or arranged in bundles; **secondary xylem** with fibers dominating, vessels dispersed, reticulate and strongly bordered, up to 91µm in diameter; sclereids as transition between vascular bundles and pith; **medullary rays** multiseriate with sclerenchymatous thickening; **pith** of thick-wanded, pitted parenchymatous cells;
crystalloids, laticifers missing;
secretory ducts besides endoSDs missing;

a: Rhizome of *Carduus personata*, habitus (CP03-09)
b: Schematic view of *C. personata* rhizome (transverse section): small ellipses mark endoSDs of the cortex, black points represent the sclereids within the suberized cortex, sec. phloem and pith





Carduus personata rhizome: **a:** Sclereids within the suberized cortex and secondary phloem (CP03-09); **b:** sclereids and fibers single and arranged in bundles of the secondary phloem (CP03-09); **c:** cells of medullary ray with sclerenchymatous thickening (CP02-09); **d:** vascular bundle with sclereidal cells as transition to the pith (CP01-09); **e:** pith with cells with pitted and thickended walls (CP03-09); **f:** strongly bordered vessels (CP02-09);
a-e: transverse sections; f: longitudinal section;

GENUS *CIRSIUM*

CIRSIUM ARVENSE (L.) Scop. (cf. Paper III)

perennial geoptophyte;

allorhizous: taproot with long part of rhizome; stoloniferous plant

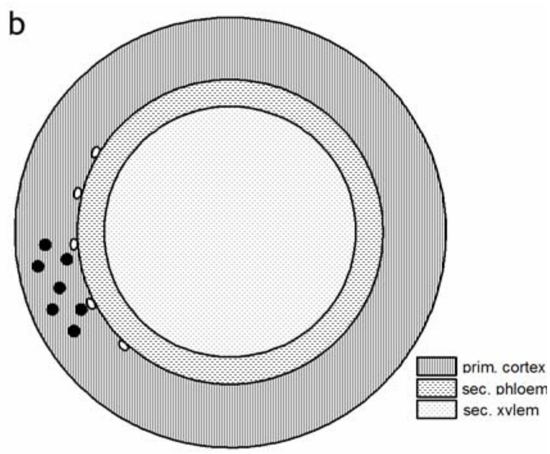
Root:

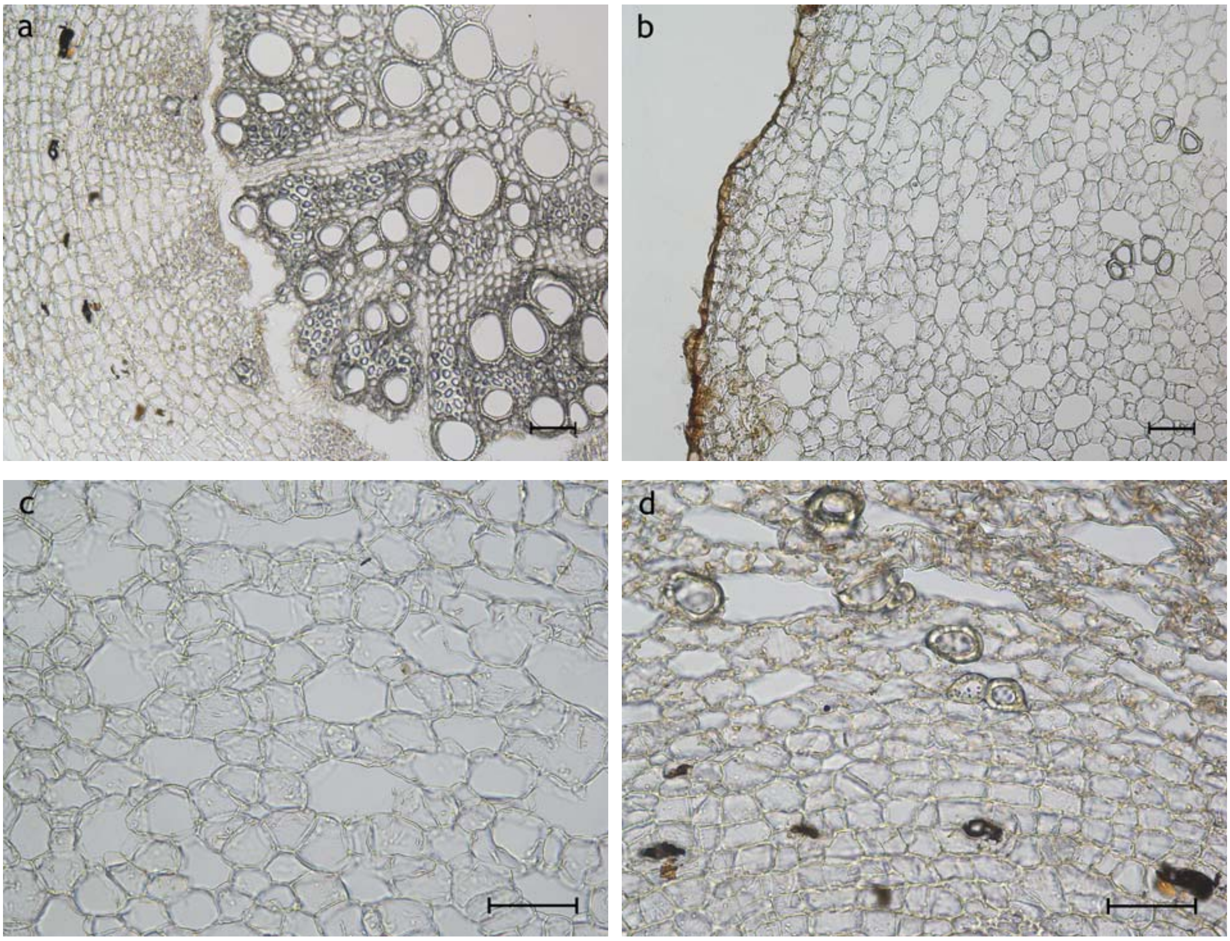
secondary root: Rhizodermis or **cork** thin-walled; **cortex** with large intercellular spaces – aerenchyma, with sclereids resembling the adjacent parenchyma cells in size and shape, large lumen; **endoSDs** regularly arranged; **secondary phloem** usually smaller than cortex, with sclereids of the same type occurring in the cortex and possibly single fibers; vascular cylinder dominating; **secondary xylem** with fibers, vessels strongly bordered, up to 120µm in diameter; **medullary rays** multiseriate; **pith** missing;
crystalloids missing;
secretory ducts besides endoSDs missing;

Rhizome:

rhizome with secondary growth: cork / bark thin-walled; **cortex** suberizing with sclereids resembling the adjacent parenchyma cells in size and shape, large lumen, tissue frequently ruptured; **endoSDs** lost lost together with the suberizing of the cortex; **secondary phloem** conspicuously small with sclereids of the same type occurring in the cortex, fibers building a cap over the vascular bundle; wide radial extension of the dominating vascular cylinder; **secondary xylem** dominated by fibers, vessels dispersed, strongly bordered, up to 79µm in diameter; **secretory ducts** at the border between vascular bundle and pith; **medullary rays** multiseriate; **pith** of cells with slightly thickened, pitted cell wall;
crystalloids, laticifers missing;

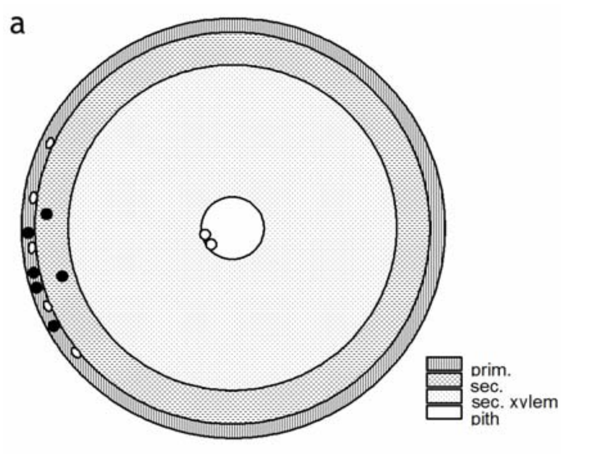
a: Taproot of *Cirsium arvense*, habitus (CiA02-08)
b: Schematic view of *C. arvense* root (transverse section): small ellipses mark the position of the endoSDs, black points represent the sclereids within the broad cortex

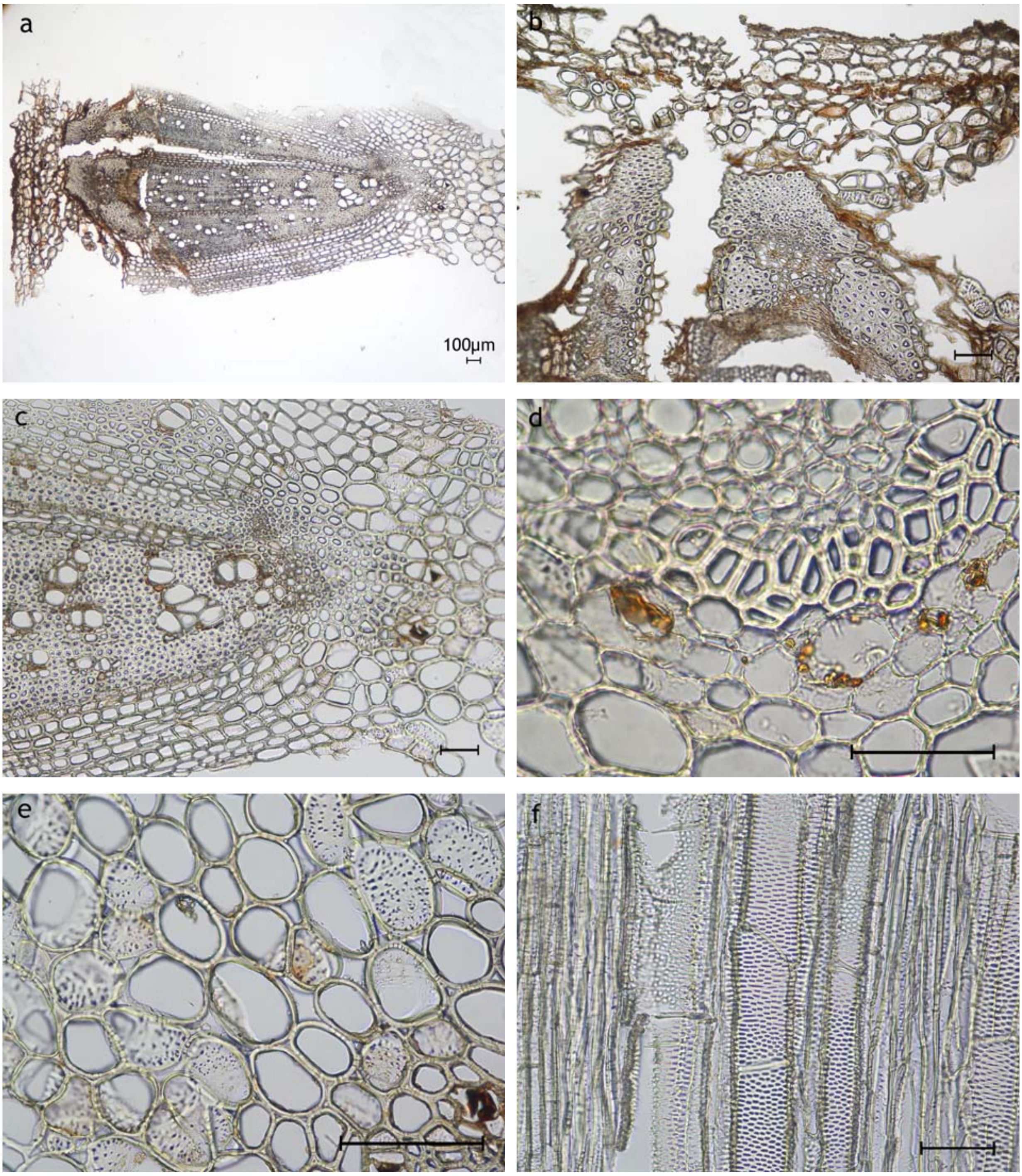




Cirsium arvense root: **a:** Overview showing the extension and arrangement of the vascular cylinder: the secondary xylem is the most expanded component followed in extension by the cortex; regularly arranged endoSDs (CiA01-08); **b:** cortex with sclereids resembling the adjacent parenchyma cells in size and shape, large lumen (CiA01-08); **c:** aerenchyma of the cortex (CiA01-08); **d:** sclereids and endoSDs of the cortex (CiA01-08)
a-d: transverse sections;

a: Schematic view of *Cirsium arvense* rhizome in transverse section: small ellipses mark the position of the endoSDs and secretory ducts of type SD2 at the border between the vascular bundles and the pith, black points represent the sclereids within the cortex and the secondary phloem





Cirsium arvense rhizome: **a:** Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component, cortex and secondary phloem conspicuously small, suberizing (CiA02-08); **b:** sclereids resembling the adjacent parenchyma cells in size and shape within the suberized cortex, secondary phloem with fibers building a cap over the vascular bundle (CiA02-08); **c:** overview of the transition between vascular bundle and pith (CiA02-08); **d:** secretory ducts at the border between vascular bundle and pith (CiA03-08); **e:** pith with slightly thickened cell walls (CiA02-08); **f:** strongly bordered vessels (CiA01-08)
a-e: transverse sections; f: longitudinal section;

CIRSIUM ERISITHALES (Jacq.) Scop. (cf. Paper III)

perennial hemicryptophyte;

rhizome;

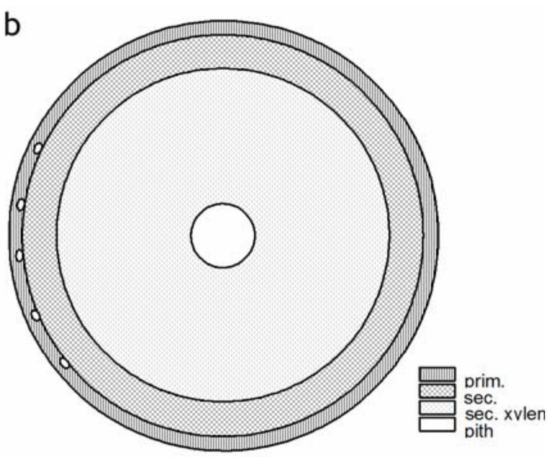
rhizome with secondary growth: cork / bark thin-walled; **cortex** lost in course of rhytidome formation; **endoSDs** lost together with the cortex; **secondary phloem** broad, but of lesser radial extension than the vascular cylinder, with fibers in groups arranged in circular bands in fascicular position above the vascular bundles alternating with bands of parenchymatous cells; **secondary xylem** dominated by fibers, vessels circularly arranged, reticulate, mainly strongly bordered, up to 102µm in diameter; **medullary rays** multiseriate; **pith** of thin-walled cells;

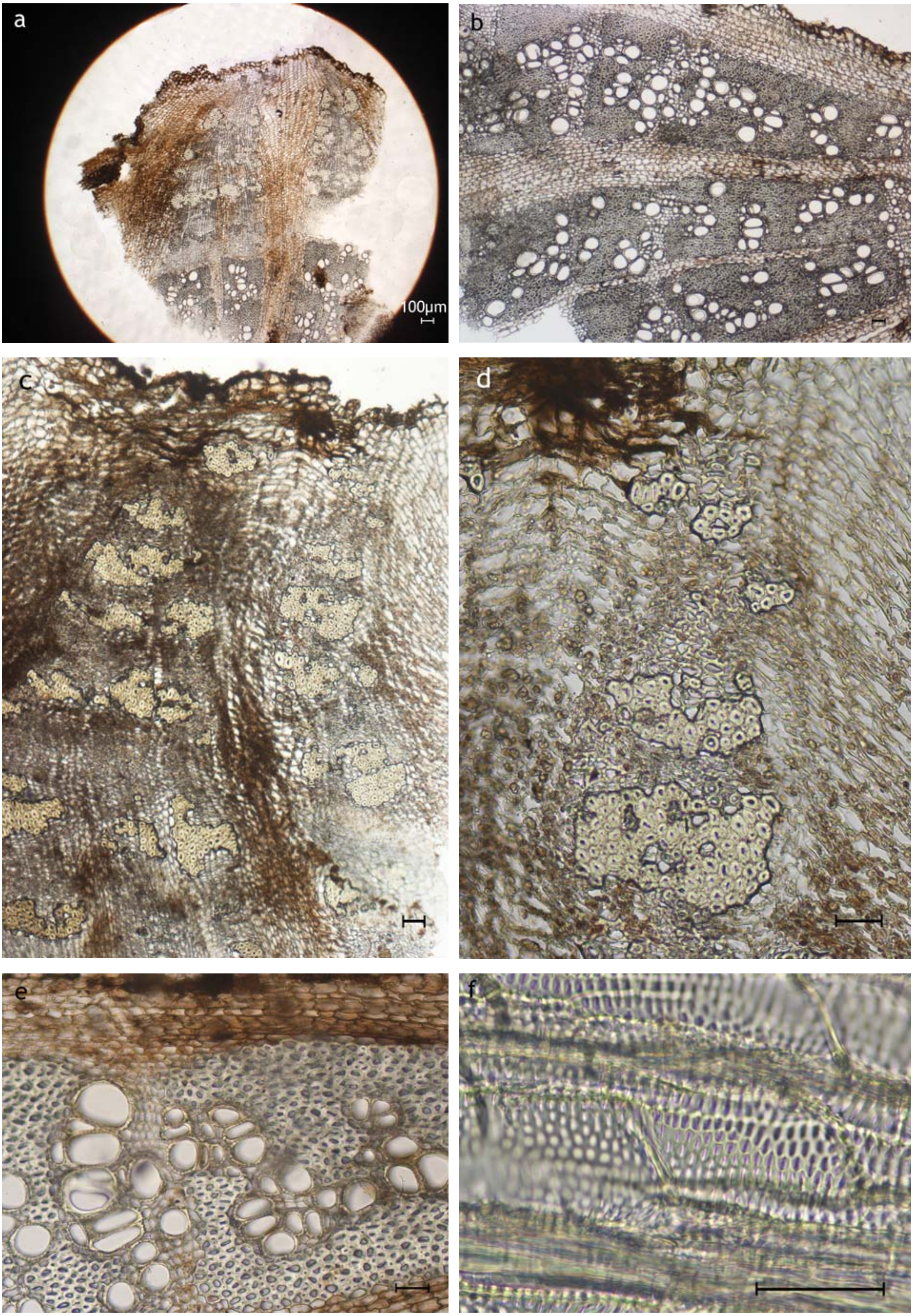
crystalloids missing;

sclereids, laticifers, crystalloids missing;

further secretory ducts besides the endoSDs missing;

a: Rhizome of *Cirsium erisithales*, habitus (CE03-09)
b: Schematic view of *C. erisithales* rhizome (transverse section): small ellipses mark the position of the endoSDs of the cortex, vascular cylinder dominating





Cirsium erisithales rhizome: **a:** Overview showing the extension and arrangement of tissues: cortex lost in course of rhytidome formation, secondary phloem broad, but of lesser radial extension than the vascular cylinder (CE01-09); **b:** vascular cylinder dominated by fibers, vessels circularly arranged,

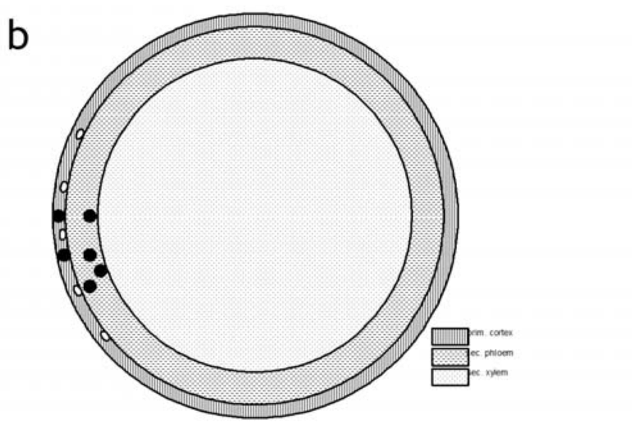
medullary rays multiseriate (CE02-09); **c:** bundles of fibers in arranged in circular bands within the secondary phloem (CE03-09); **d:** fibers of the secondary phloem (CE03-09); **e:** vessels between alternating bands of fibers and parenchymatous cells (CE02-09x); **f:** strongly bordered vessels (CE03-09)
a-e: transverse sections; f: longitudinal sections

CIRSIUM VULGARE (Savi) Ten. (cf. Paper III)

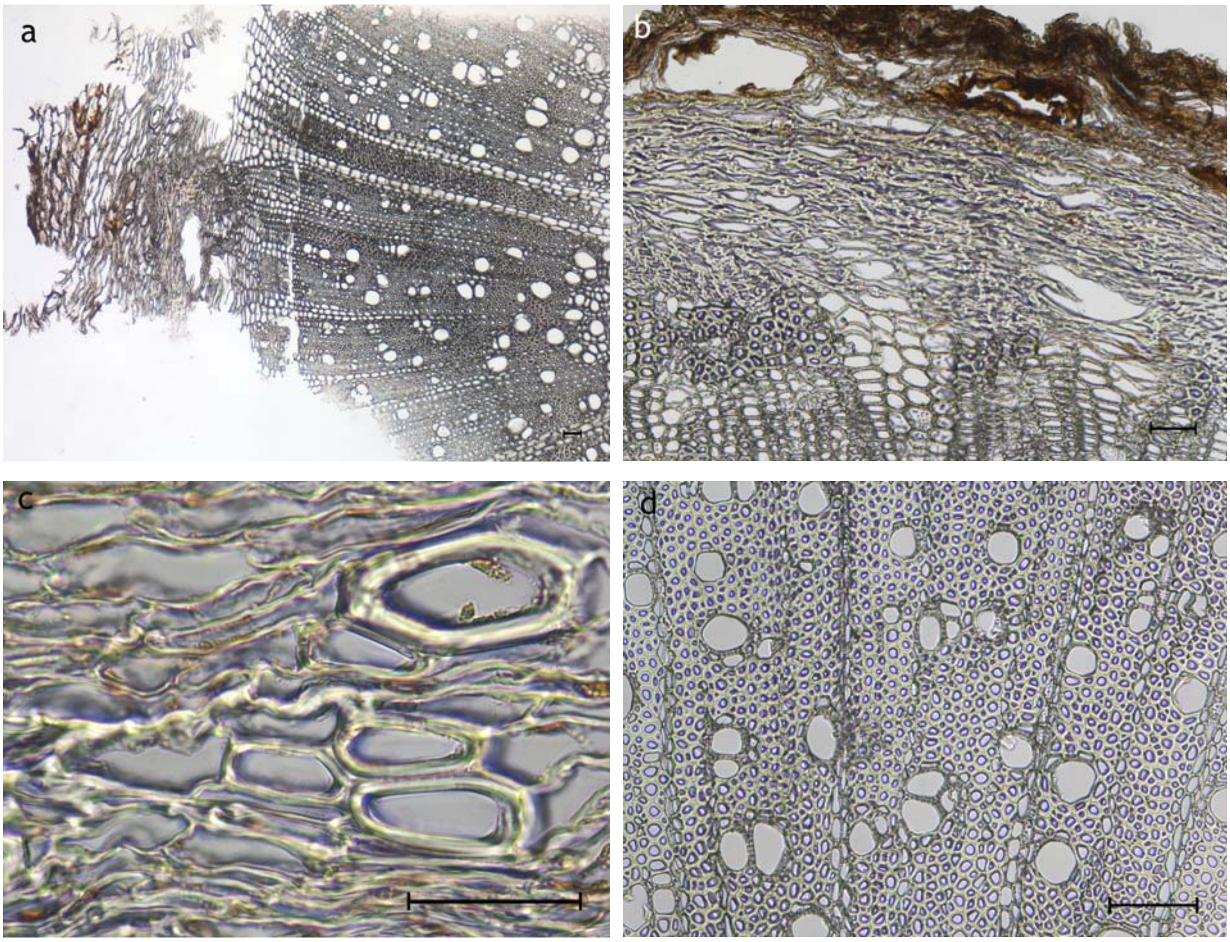
biennial hemicryptophyte;

allorhizous or homorhizous root system;

secondary root: cork / bark thin-walled; **cortex** lost, with sclereids resembling the adjacent parenchyma cells in size and shape, large lumen; **endoSDs** lost together with the cortex; **secondary phloem** broader than cortex, but of lesser radial extension than the vascular cylinder, with sclereids of the same type occurring in the cortex and bundles of fibers arranged in fascicular position above the vascular bundles; **secondary xylem** dominated by fibers, vessels dispersed, reticulate, mainly strongly bordered, up to 79µm in diameter; **medullary rays** bi- and multiseriate; **pith** missing;
crystalloids, laticifers missing;
secretory ducts beside endoSDs missing;



a: Root of *Cirsium vulgare*, habitus (CiV03-09)
b: Schematic view of *Cirsium vulgare* root (transverse section): small ellipses mark the position of the endoSDs, black points represent the sclereids within the suberous cortex and the secondary phloem



Cirsium vulgare root: **a:** Overview showing the extension and arrangement of tissues: narrow cortex enduring, secondary phloem broad, but of lesser radial extension than the vascular cylinder; bark / suberized cortex and secondary phloem with sclereids, vascular cylinder with bi- and multiseriate medullary rays (CiV01-09); **b:** cortex with endoSDs, secondary phloem with fibers arranged in bundles (CiV02-09); **c:** sclereids of secondary phloem (CiV01-09); **d:** xylem dominated by fibers and with dispersed vessels, biseriate medullary rays in regular arrangement (CiV02-09)
a-d: transverse sections;

ONOPORDUM ACANTHIUM L. (cf. Paper III)

biennial – plurennial hemicryptophyte;

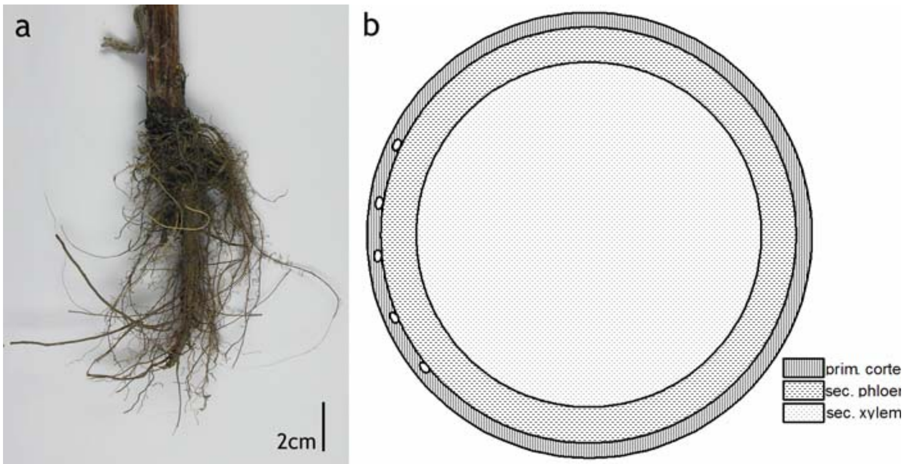
allorhizous, with taproot;

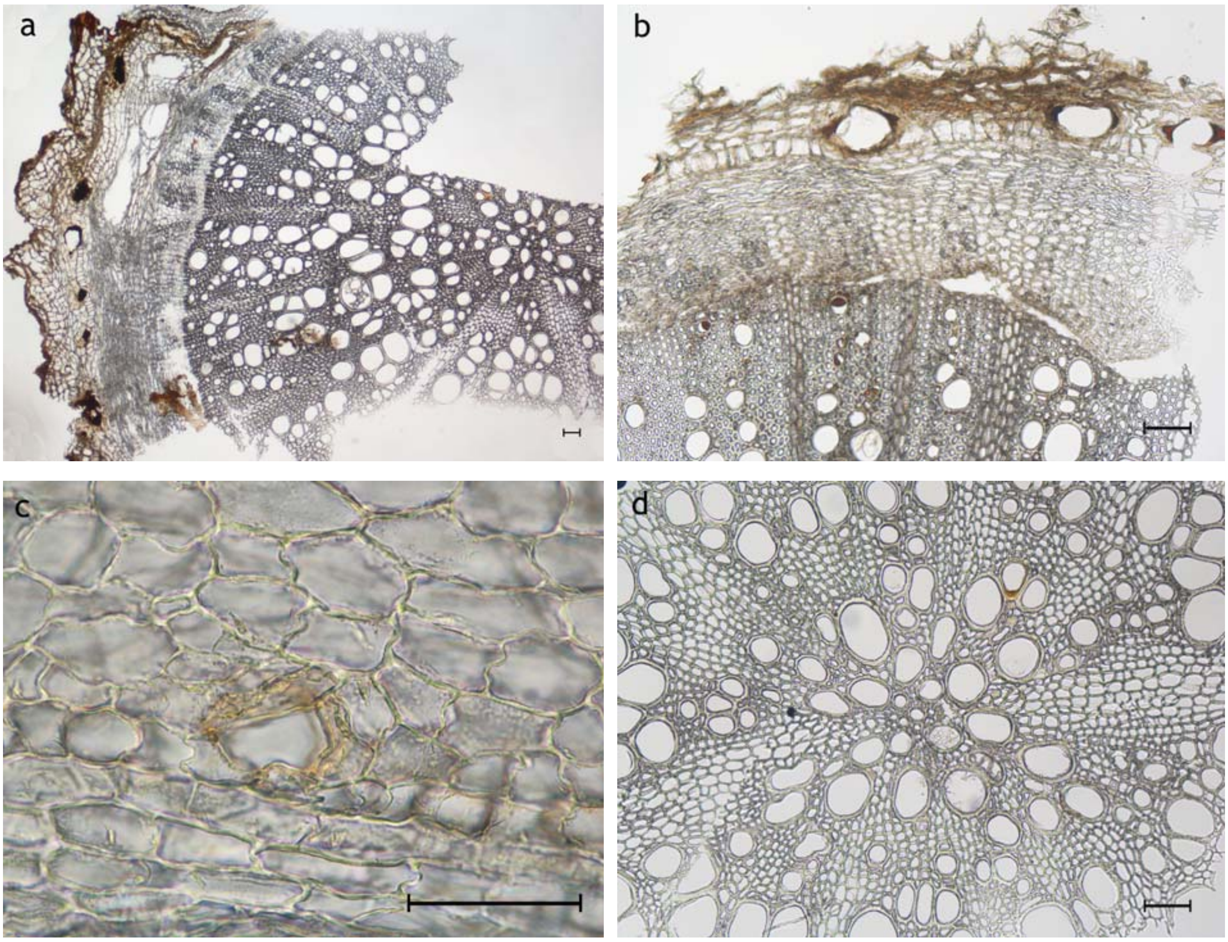
secondary root: cork thin-walled, sometimes with crystalloids; small **cortex** enduring; distinct endodermis with **endoSDs** usually up to 6 surrounding cells at maximum; **secondary phloem** usually broader than cortex, but of lesser radial extension than the vascular cylinder, with **fibers** single and arranged in bundles; **secondary xylem** with fibers alternating with parenchymous cells, vessels dispersed, strongly bordered, up to 148µm in diameter; multiseriate **medullary rays** multiseriate; **pith** missing;

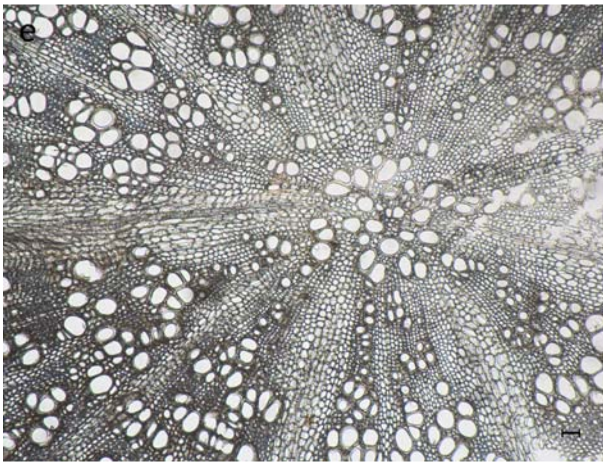
sclereids, laticifers missing;

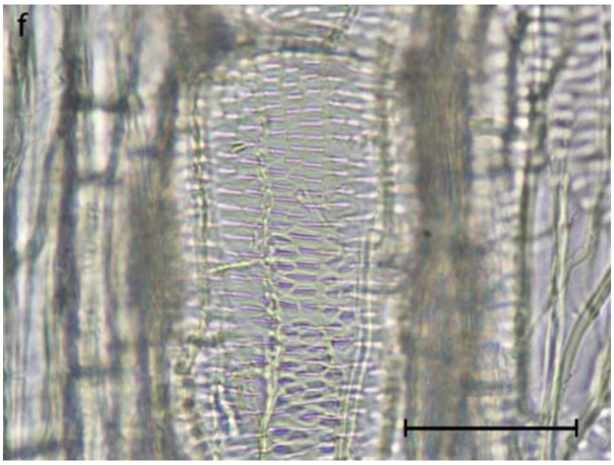
secretory ducts beside endoSDs missing;

a: Taproot of *Onopordum acanthium*, habitus (O02-08a)
b: Schematic view of *O. acanthium* root (transverse section): small ellipses mark the position of the endoSDs within the cortex; vascular cylinder dominating









Onopordum acanthium root: **a:** Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component followed in extension by the secondary phloem; cortex enduring with endoSDs; vascular cylinder with multiseriate medullary rays (from left to right) (O01-08); **b:** overview showing the distinct endodermis with the endoSDs within the cortex, secondary phloem with fibers arranged in bundles (O02-08); **c:** distinct endodermis with endoSDs usually up to 6 surrounding cells at maximum (AST3; cited Paper II); **d,e:** vascular cylinder with multiseriate medullary rays, many vessels dispersed, fibers (O01-08, O02-08); **f:** strongly bordered vessels (O02-08) a-e: transverse sections; f: longitudinal section;

SILYBUM MARIANUM (L.) Gaertn. (cf. Paper III)

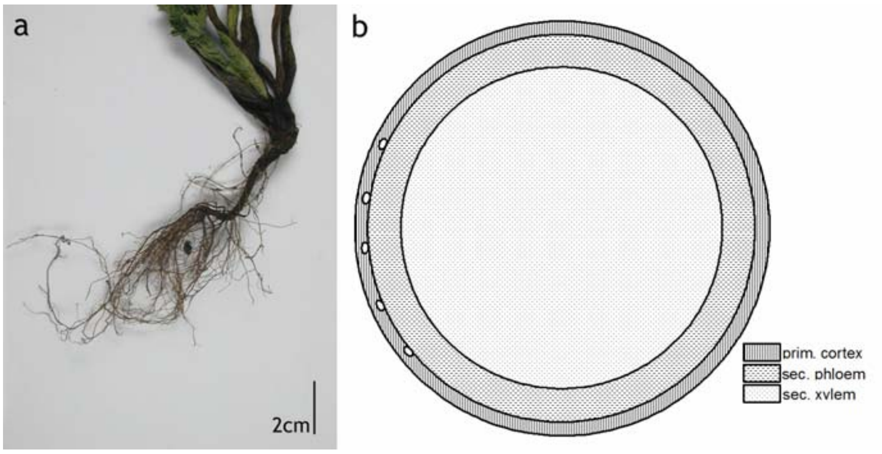
annual – biennial therophyte - hemicryptophyte;

allorhizous, with taproot;

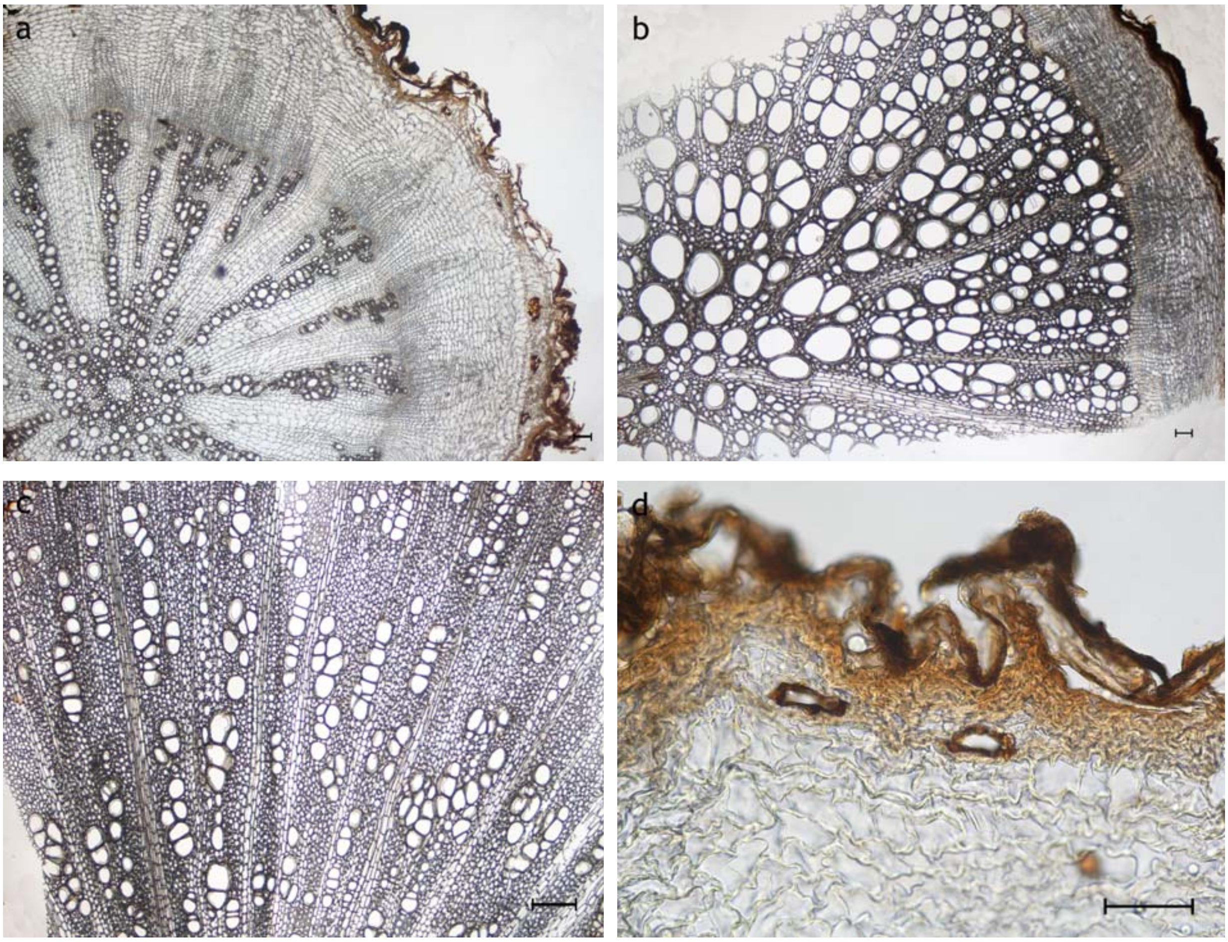
secondary root: cork / bark thin-walled; **cortex** lost in course of rhytidome formation; **endoSDs** lost together with the cortex; small **secondary phloem** usually broader than cortex, but of lesser radial extension than the vascular cylinder, with fibers single and arranged in bundles in fascicular position above the vascular cylinder between the phloem rays; **secondary xylem** with mainly parenchymatous cells (often slightly thickened walls), few fibers; vessels in groups, strongly bordered, up to 136µm in diameter, short in longitudinal section; **medullary rays** usually up to 5 cells in a row, very regularly arranged; **pith** missing;
crystalloids, sclereids missing;
secretory ducts beside endoSDs missing;

Culture: vessels much smaller in diameter (up to 61µm), arranged like rays; less fibers in secondary phloem as well as in vascular cylinder

Diameter of vessels highly variable even within one root: 245µm near the tip of the root, 113µm 1,5cm below the hypocotyle!



a: Root of *Silybum marianum*, habitus (S3-11-08)
b: Schematic view of *S. marianum* taproot (transverse section): small ellipses mark the position of the endoSDs within the cortex; sec. phloem usually broader than the cortex, but of lesser radial extension than the vascular cylinder



Silybum marianum root: **a:** Young root (cultivated plant): overview showing the extension and arrangement of tissues: small cortex with endoSDs, broad sec. phloem, but of lesser radial extension than the vascular cylindervascular cylinder with multiseriate medullary rays, vessels in rows (S4); **b:** older root: overview showing the extension and arrangement of tissues: cortex lost, sec. phloem broader than cortex but of far lesser radial extension than the vascular cylinder (MD2-08); **c:** xylem dominated by fibers, vessels more or less in groups, medullary rays up to 5 cells in a row in regular arrangement (MD1-08); **d:** cortex with endoSDs (S4); **e:** suberized cortex and sec. phloem with fibers arranged in bundles in fascicular position above the vascular cylinder between the phloem rays (MD1-08); **f:** short, strongly bordered vessels (MD2-08)
a-e: transverse sections; f: longitudinal section;

SUBTRIBE *CENTAUREINAE*

GENUS *SERRATULA*

SERRATULA TINCTORIA L.

perennial geophyte - hemicryptophyte;

fibrous root system;

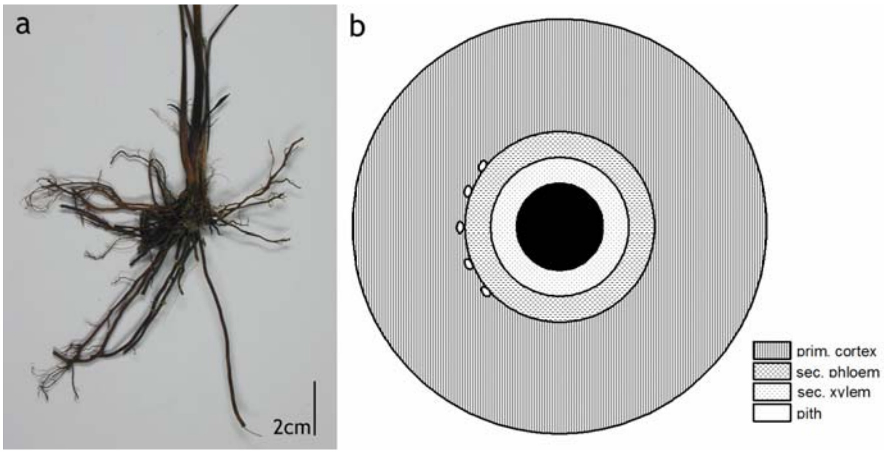
primary root: rhizodermis brown, with thickened cell walls to the outside; distinct brown exodermis with thickened cell walls; **cortex** broad, enduring due to primary root system; distinct endodermis with **endoSDs** with small lumen – usually 4 surrounding cells; **secondary phloem** small; vascular cylinder 4- or 5-radiate; **secondary xylem** dominated by fibers strongly lignified in central position, few vessels, mainly simple, also weakly bordered, up to 34µm in diameter; fibers and vessels mainly of the same size or vessels smaller; **medullary rays** not existing because of primary structure of the roots; **pith** missing;

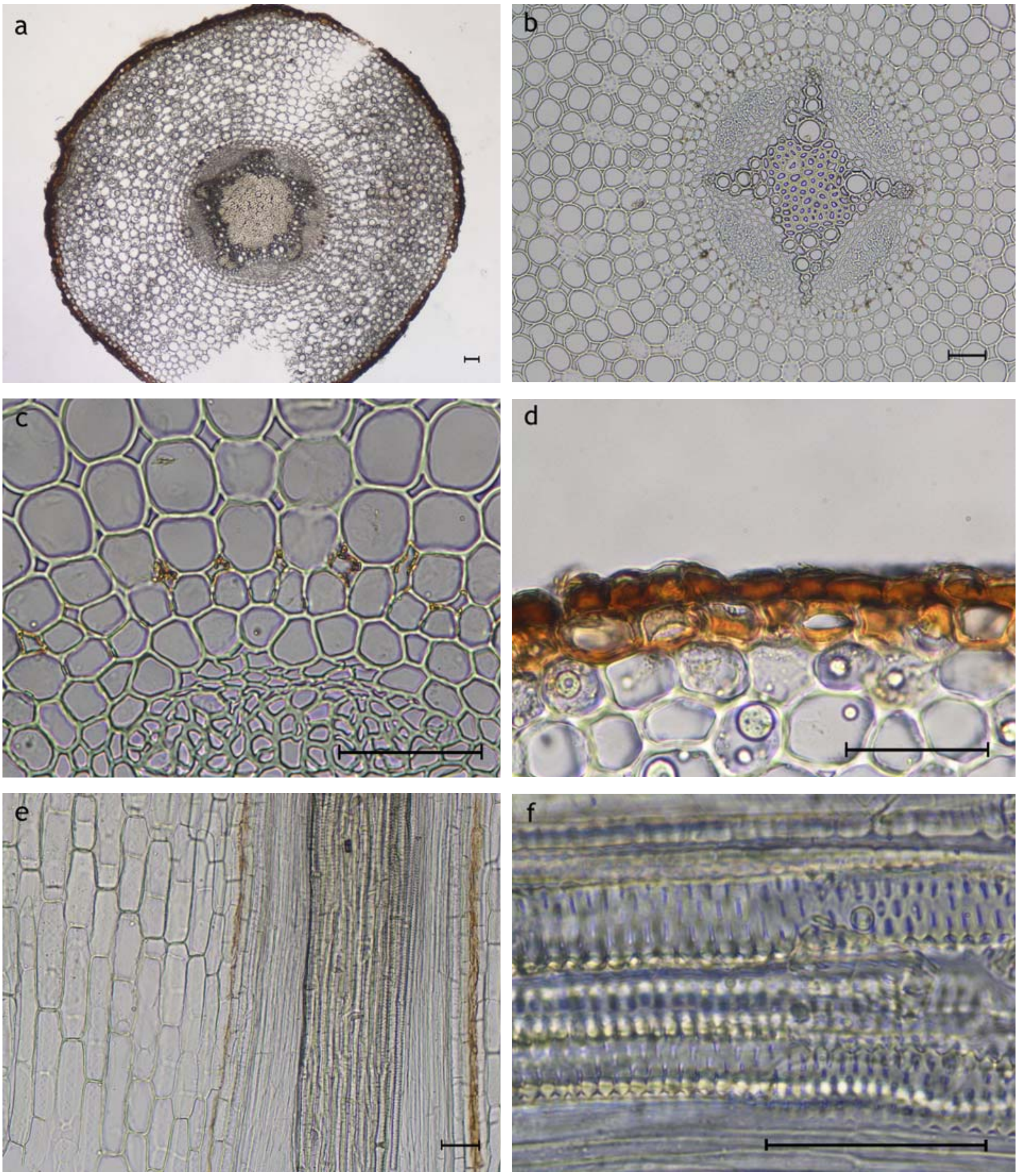
sclereids, laticifers, crystalloids missing;

fibers in secondary phloem missing;

secretory ducts besides endoSDs missing;

a: Fibrous root system of *Serratula tinctoria*, habitus (ST02-09)
b: Schematic view of *S. tinctoria* root (transverse section): primary root: small ellipses mark the position of the endoSDs of the cortex, vascular cylinder with fibers in central position





Serratula tinctoria root: **a:** Overview showing the extension and arrangement of tissues: the cortex is the most expanded component, vascular cylinder (5-radiate) dominated by fibers in central position (ST01-09); **b:** vascular cylinder (4-radiate) surrounded by the distinct endodermis with endoSDs (ST03-09); **c:** distinct endodermis with caspary strips visible and endoSDs – 4 surrounding cells (ST03-09); **d:** brown rhizodermis with thickened cell wall to the outside, distinct brown exodermis with thickened cell walls (ST01-09); **e:** overview showing the arrangement of tissues in longutidinal section: vascular cylinder dominated by fibers, few vessels, small secondary phloem, cortex broad (ST03-09); **f:** weakly bordered vessels (ST01-09)
a-d: transverse sections; e,f: longitudinal sections;

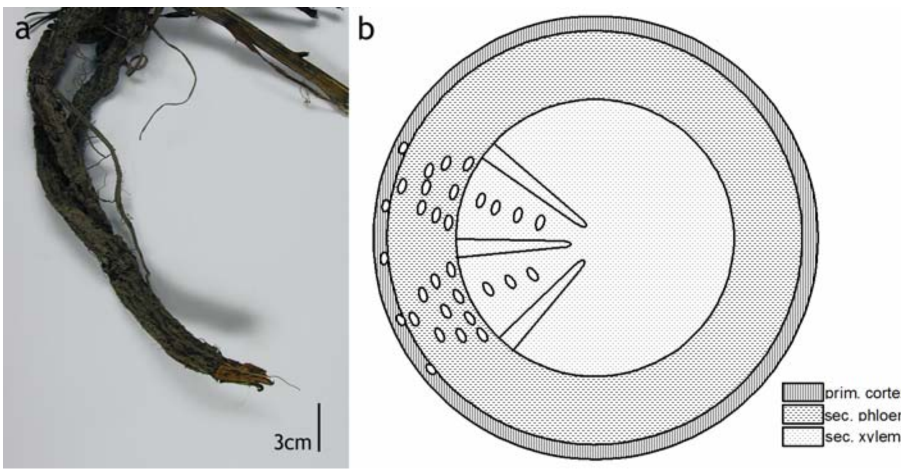
GENUS *RHAPONTICUM*

RHAPONTICUM SCARIOSUM Lam. (cf. Paper III)

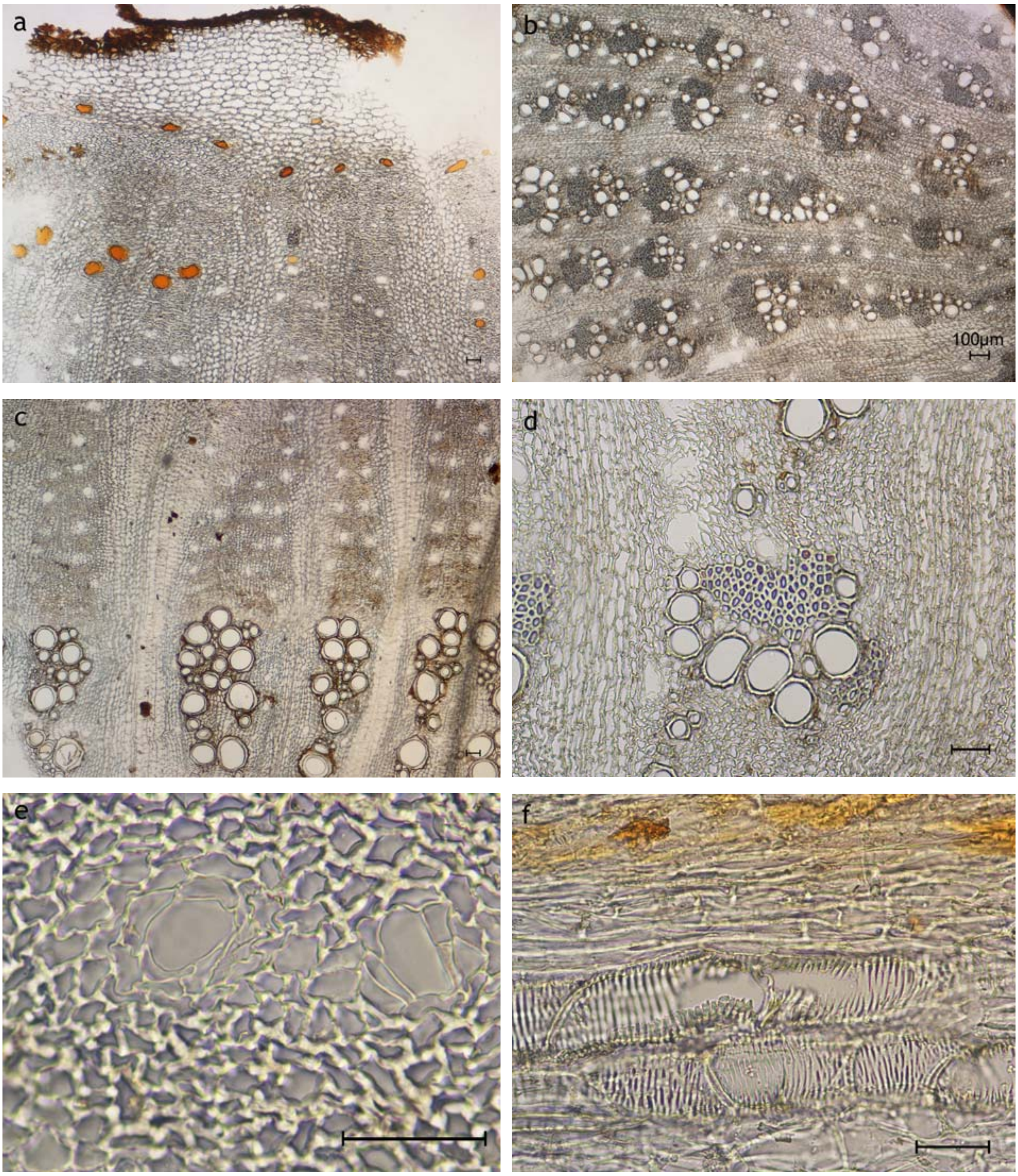
perennial hemicryptophyte;

allorhizous, with taproot;

secondary root: cork thin-walled; **cortex** enduring; **endoSDs**; **secondary phloem** dominant, often almost comparable in extension to the vascular cylinder, with fibers arranged in bundles possible; **secondary xylem** with bundles of fiber in conjunction with vessels, more or less circularly arranged relative to the centre of the xylem, vessels reticulate, up to 118µm in diameter; **medullary rays** broad, multiseriate, unlignified; secretory ducts type **SD2a** located in fascicular position between the phloem rays and within the vascular cylinder in the secondary xylem between the medullary rays, arranged circular; **pith** missing;
sclereids, laticifers, crystalloids missing;



a: Taproot of *Rhaponticum scariosum*, habitus (RS01-09)
b: Schematic view of *R. scariosum* root (transverse section): small ellipses mark the position of the endoSDs of the cortex and type SD2a ducts in fasc. position between the phloem rays and the medullary rays



Rhaponticum scariosum root: **a:** Overview showing the conspicuously small cortex with endoSDs and the wide extension of the secondary phloem; secondary phloem with secretory ducts type SD2a positioned fascicular between phloem rays (2x); **b:** overview showing the extension of the vascular cylinder with vessels in groups more or less circularly arranged relative to the centre of the xylem, multiseriate medullary rays (2x); **c:** cortex with endoSDs, SD2 in secondary phloem between phloem rays(4x); **d:** secretory ducts type SD2a positioned fascicular between phloem rays in the secondary phloem and within the secondary xylem between the medullary rays(10x); **e:** secretory ducts type SD2 in secondary phloem(40x); **f:** group of vessels and fibers of the secondary xylem, secretory ducts SD2a (10x); **g:** secretory ducts in secondary xylem between medullary rays(10x); **h,i:** reticulate vessels(20x,40x)
a-f: transverse sections; g-i: longitudinal sections;

GENUS *CENTAUREA*

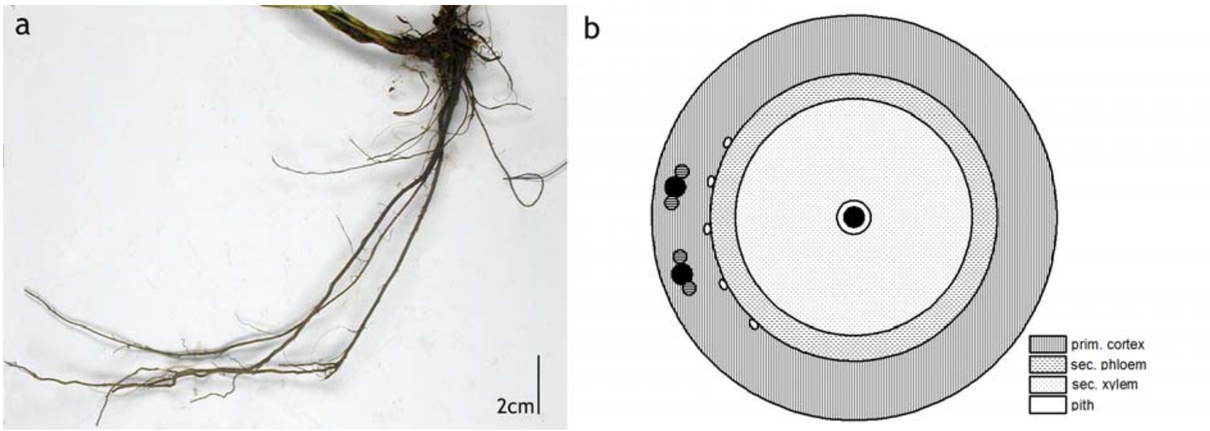
As presently prescribed (Susanna and Garcia-Jacas, 2007) the genus Centaurea contains three subgenera: *Centaurea* (formerly the large *Jacea* group – Garcia-Jacas *et al*., 2000; Garcia-Jacas *et al*., 2006), *Acrocentron* (formed by *Centaurea* sect. *Acrocentron* (Cass.) DC., *Centaurea* sect. *Chamaecyanus* Willk. and *C*. sect. *Stephanochilus* Coss.&Dur.) (Font *et al*., 2009) and *Cyanus*.
In the Austrian Flora (Fischer *et al*., 2008) the group *Acrocentron* is included in *Centaurea* (*Jacea*-group) leaving *Cyanus* as a distinct genus.

CENTAUREA JACEA L. (cf. Paper III)
perennial hemicryptophyte;
most times just very small rhizome - fibrous root system; long, thin rhizome also possible;

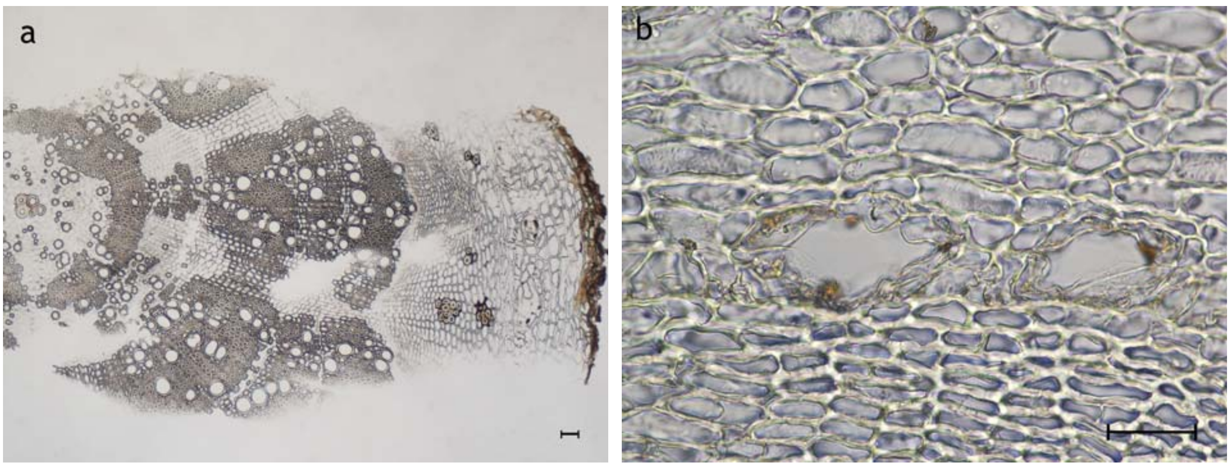
<u>Root: </u>**secondary root: rhizodermis** brown; **cortex** enduring, with distinct sclereids arranged in groups surrounded by parenchyma cells: shape roundish to elliptic in transverse view, in longitudinal view resembling the adjacent parenchyma cells in size and shape, thick-walled, strongly lignified, with simple pits; distinct endodermis with **endoSDs** – usually up to 6-7 surrounding cells at maximum; **secondary phloem** with sclereids of the same type occurring in the cortex, with rarels scattered fibers; broad vascular cylinder dominating in extension with prim. xylem visible; **secondary xylem** dominated by fibers, few vessels dispersed over the transverse section, reticulate, simple or weakly bordered, up to 57µm in diameter; **medullary rays** varying from narrow to broad rays, can even appear almost raylessness in transverse section because of thickened cell walls of ray cells; **secretory ducts** of type SD4 (secretion by parenchyma cells / sclereidal cells into ordinary intercellular spaces) always associated with sclereids occurring in central position of the pith as well as in cortex and secondary phloem; **pith** of unlignified, slightly thickened parenchymous cells with just few pits visible in transverse section, with sclereids;
cristalloids, laticifers missing;

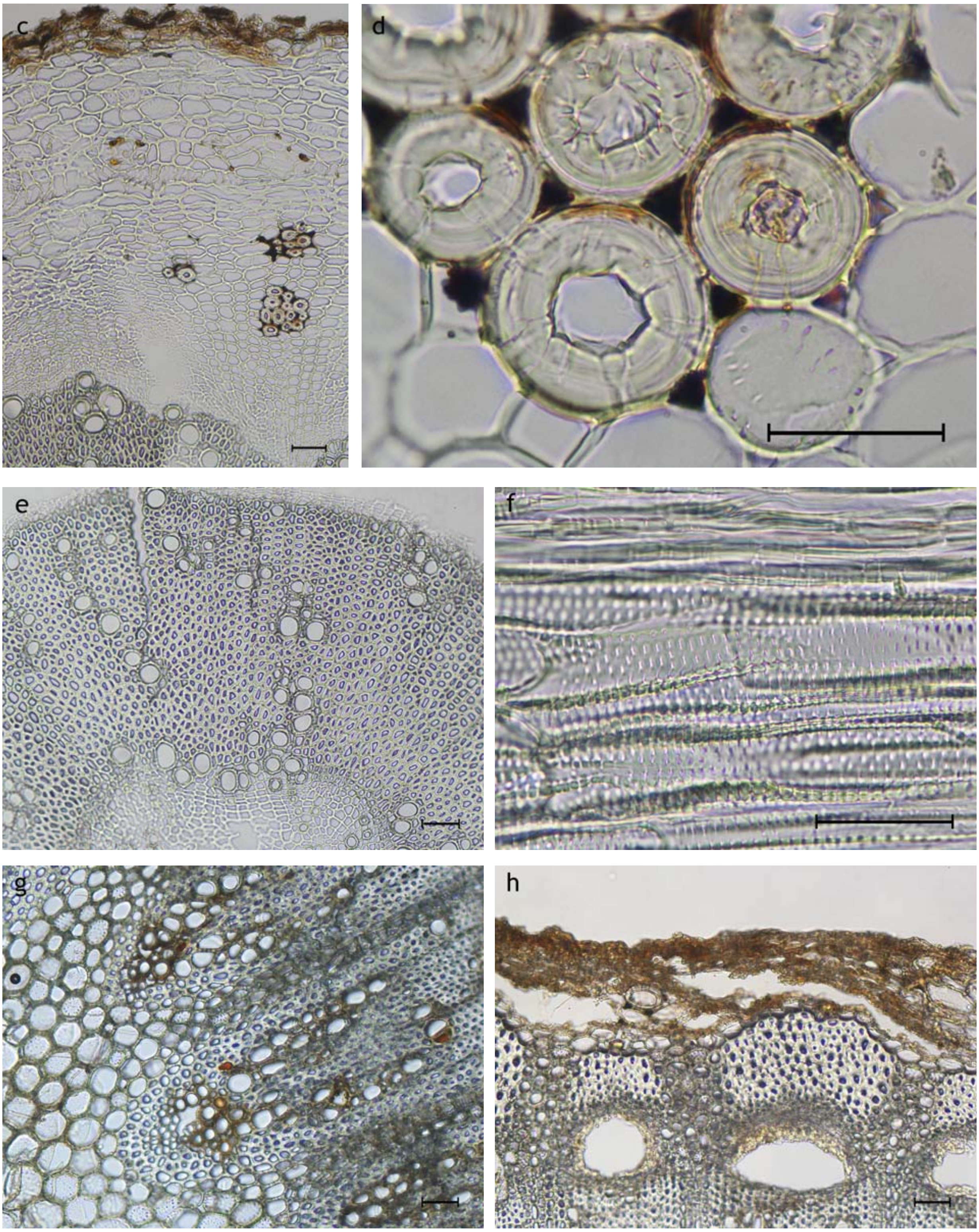
<u>Rhizome:</u> **rhizome with secondary growth: rhizodermis** brown; small **cortex** enduring but tissue frequently ruptured / obliterated, with sclereids single or in groups mainly in

conjunction with SD4 (see above); **endoSDs** lost or crushed together with rupturing of the cortex; **secondary phloem** usually broader than cortex, but of lesser radial extension than the vascular cylinder broad, with sclereids of the same type occurring in the cortex and fibers arranged in bundles which may build caps over vascular bundles; **secondary xylem** with few vessels usually in groups cumulative to the center, reticulate, simple or weakly bordered, up to 45µm in diameter; **medullary rays** with partly thickened cell walls; **secretory** ducts type SD2a possible within secondary phloem, type SD4 in conjunction with sclereids in cortex, secondary phloem fascicular and interfascicular and pith possible; **pith** of cells with slightly thickened, pitted cell wall, with sclereids (as mentioned above);
crystalloids, laticifers missing;



a: Fibrous root system of *Centaurea jacea*, habitus (CJ01-09)
b: Schematic view of *Centaurea jacea* root in transverse section: small ellipses mark the position of the endoSDs of the cortex, black points represent the sclereids in conjunction with SD4 in cortex and pith





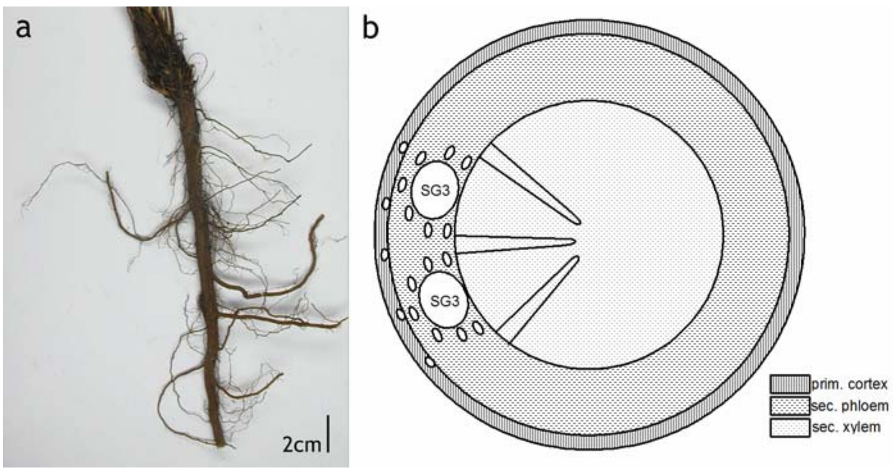
Centaurea jacea root: **a:** Overview showing the extension and arrangement of tissues: the secondary xylem is the most expanded component (CJ02-09); **b:** endoSDs of sec. phloem (CJ02-09); **c:** overview showing sclereids in conjunction with SD4 within the secondary phloem, endodermis with endoSDs, cortex and rhizodermis (CJ02-09); **d:** sclereids positioned in the pith associated with secretory ducts type SD4 (CJ8-1); **e:** secondary xylem dominated by fibers, with few vessels dispersed (CJ01-09); **f:** pitted vessels (CJ8-1); rhizome: **g:** transition between pith and vascular bundles (CJ8-1); **h:** fibers within secondary phloem building caps over vascular bundles, ruptured cortex (CJ8-1)
a-e,g,h: transverse sections; f: longitudinal sections

CENTAUREA SCABIOSA L. (cf. Paper III)

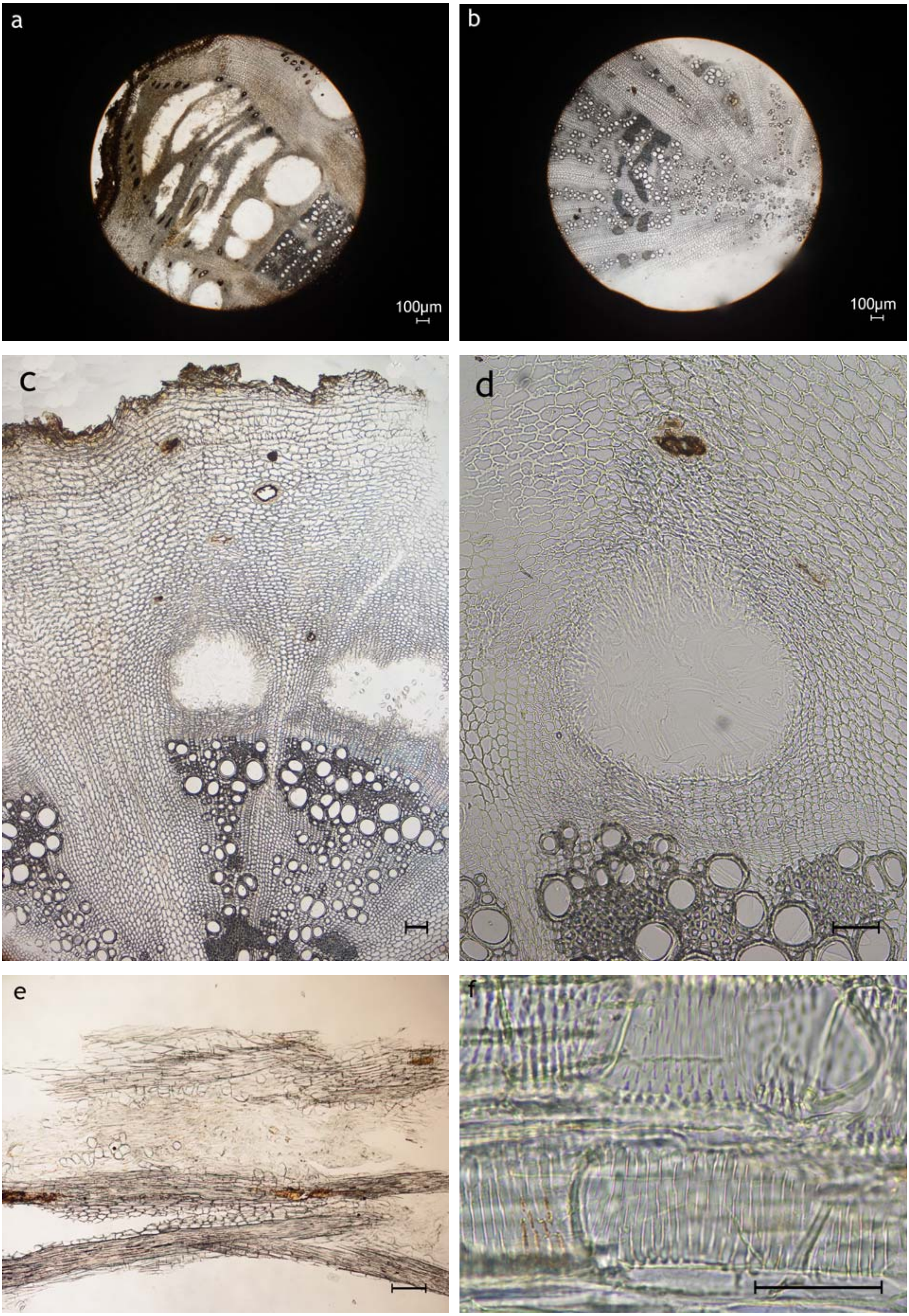
perennial hemicryptophyte;

allorhizous, with taproot;

secondary root: cork thin-walled, sometimes with crystalloids; **cortex** durable or lost in course of rhytidome formation, with occasionally sclereids single or in groups resembling the adjacent parenchyma cells in size and shape; distinct endodermis with **endoSDs** lost together with cortex; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with sclereids of the same type occurring in the cortex and pitted fibers single or in bundles; **secondary xylem** with fibers in tangential bands more or less alternating with bands of parenchymatous cells, vessels circularly arranged relative to the centre of the xylem, reticulate and strongly bordered, up to 114µm in diameter; **medullary rays** broad, multiseriate, unlignified; **secretory ducts** type SD3 (lysigenous development, large inner diameter – can fill the whole space from one phloem ray to the next) located in fascicular position between the phloem rays within the secondary phloem; secretory ducts type SD1 and SD2 forming a triangular pattern around SD3 – fascicular positioned; **pith** missing; laticifers missing;



a: Taproot of *Centaurea scabiosa*, habitus (CS02-08)
b: Schematic view of *C. scabiosa* root (transverse section): small ellipses mark the endoSDs of the cortex and SD1-2 ducts surrounding SD3 between the phloem ray in the sec. phloem



Centaurea scabiosa root: **a:** Overview showing the extension of the secondary phloem with secretory ducts SD1 -2 / SD3; SD1-2 form a triangular pattern around SD3 (CS01-08A); **b:** secondary xylem with fibers in tangential bands more or less alternating with bands of parenchymatous cells (CS01-08A); **c:**

cortex with distinct endodermis and endodermal resin duct, secretory ducts SD3 located in fascicular position between the phloem rays (CS02-08); **d:** secretory duct type SD3 (lysigenous development) with SD2 at the top (CS02-08); **e:** secretory duct type SD3 (CS03-09); **f:** reticulate vessels (CS01-08B)
a-d: transverse sections; e,f: longitudinal sections;

CENTAUREA MONTANA L. (= *CYANUS MONTANUS* Hill. [Fischer *et al*.])

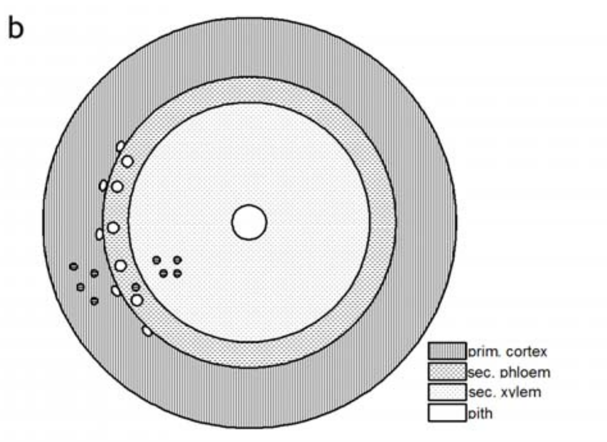
perennial hemicryptophyte;

fibrous root system;

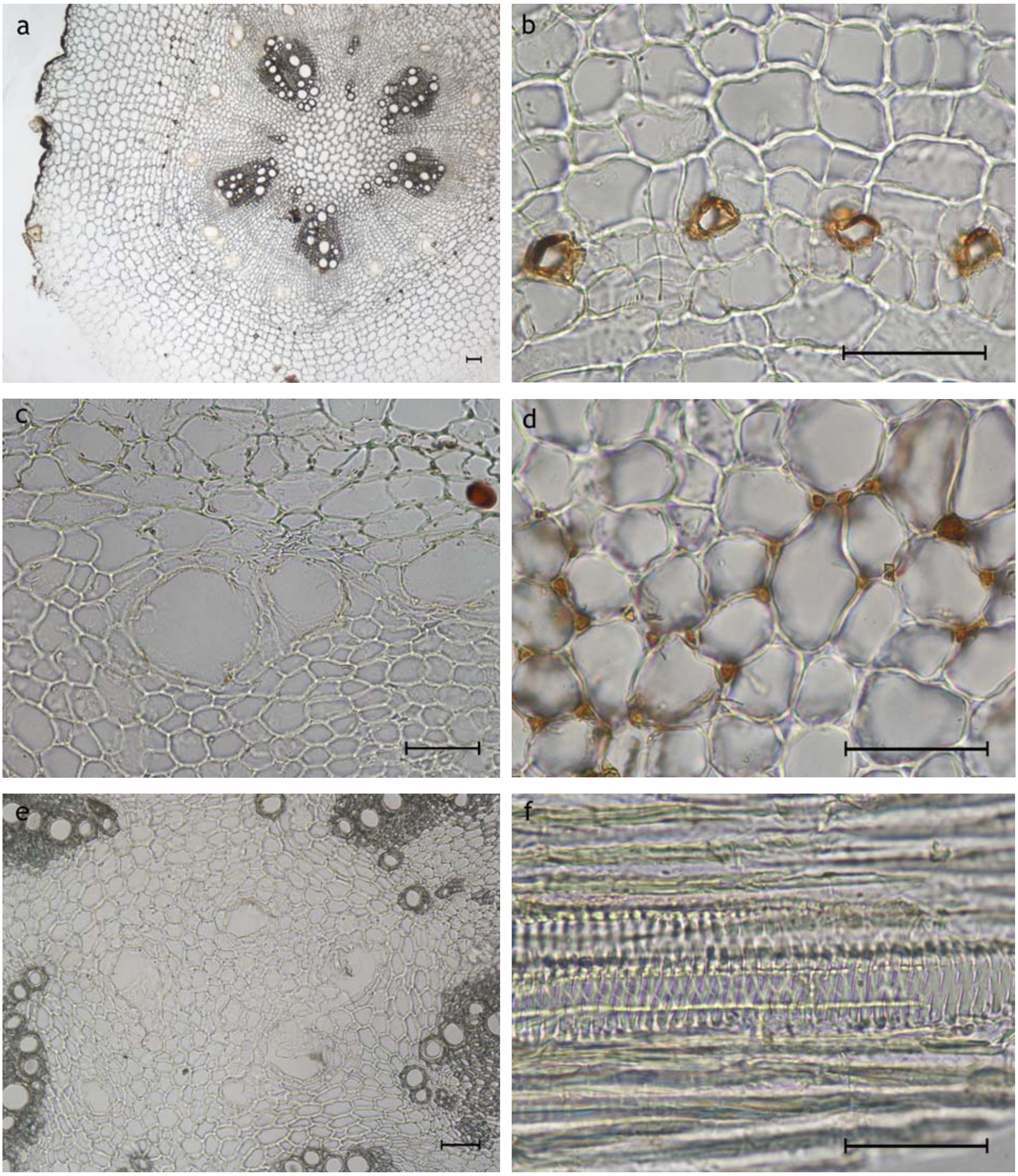
secondary root: rhizodermis brown; **cortex** enduring; distinct **endodermis** with caspary stripe, radial divisions of endodermal cells – extreme growth of endodermis while root is growing; **endoSDs**; **secondary phloem** usually smaller than cortex; broad vascular cylinder with prim. xylem visible; **secondary xylem** dominated by fibers, vessels dispersed, reticulate and weakly bordered, up to 73µm in diameter; **medullary rays** broad, multiseriate; **secretory ducts** type SD1 colourless within the secondary phloem near the endodermis; type SD4 may occur in cortex, secondary phloem, medullary rays; **pith** of thin-wanded parenchymatous cells;

crystalloids, sclereids, laticifers missing;

fibers in secondary phloem missing;



a: Fibrous root system of *Cyanus montanus*, habitus (C01-08)
b: Schematic view of *Cyanus montanus* root in transverse section: small ellipses mark the position of the endoSDs within the cortex and secretory ducts type SD1 of the secondary phloem, small points represent type SD4 in the cortex, secondary phloem and medullary rays



Cyanus montanus root: : **a:** Overview showing the extension and arrangement of tissues: the cortex is the most expanded component, secondary phloem rather small; vascular cylinder with pith, primary xylem visible (C03-08); **b:** distinct endodermis with caspary stripe, endoSDs in an early state (C03-08); **c:** secretory ducts type SD1 located near the endodermis (CM01-08; cited Paper II); **d:** secretory duct type SD4 in medullary ray (CM02-08); **e:** secretory ducts type SD1 located in the pith (C01-08); **f:** weakly bordered vessels (C03-08)
a-e: transverse sections; f: longitudinal section;

CENTAUREA CYANUS L. (= *CYANUS SEGETUM* Hill. [Fischer *et al.*]) (cf. Paper III)

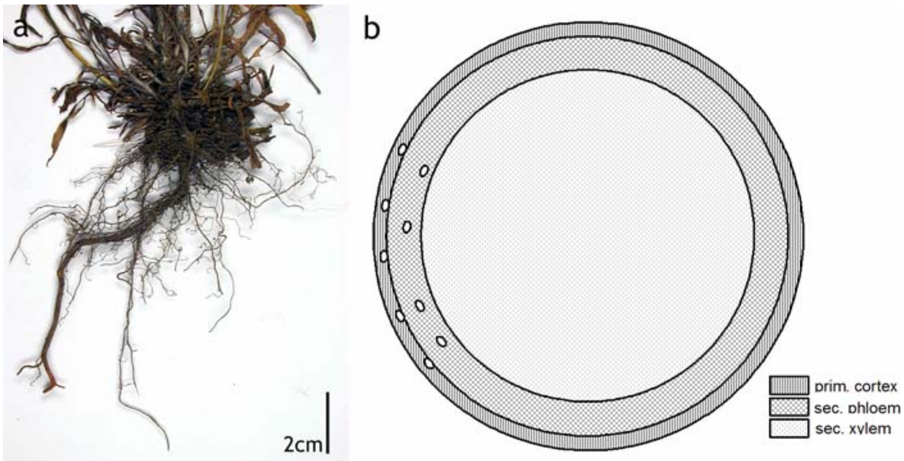
therophyte, annual;

allorhizous, with taproot;

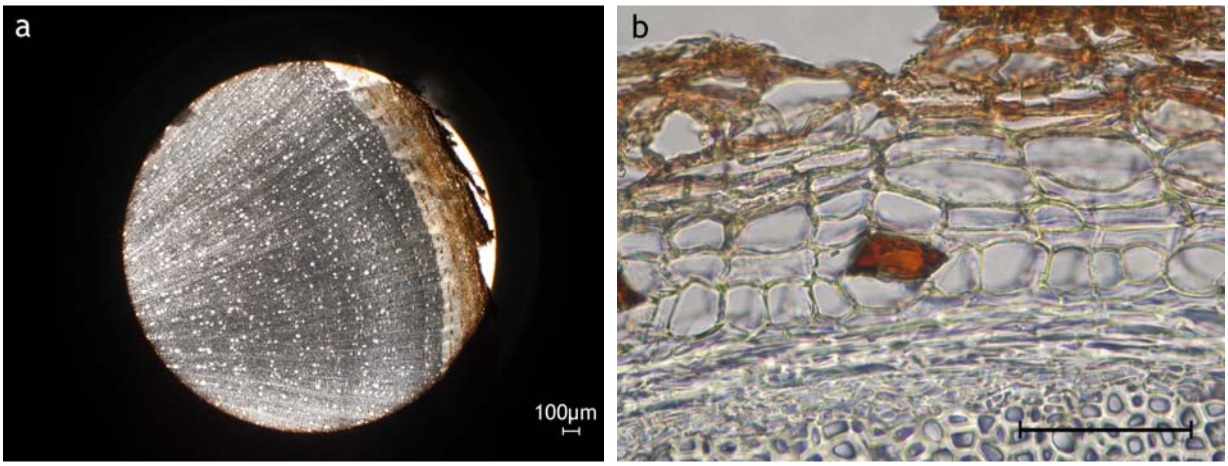
secondary root: cork / bark narrow, thin-walled, sometimes with crystalloids; **cortex** enduring, possibly with sclerenchymatous thickening of cells though distinct sclereids are missing; distinct **endodermis** with **endoSDs** – usually up to 10 surrounding cells at maximum; **secondary phloem** narrow, in younger roots almost invisible; vascular cylinder dominating;; **secondary xylem** dominated by fibers, few vessels either dispersed over the transverse section or arranged in tangential bands, reticulate, mainly strongly bordered, up to 82µm in diameter; **medullary rays** up to 5 cells in one row, can even appear almost raylessness in transverse section because of thickening of cell walls of ray cells; **secretory ducts** type SD2b in fascicular position within secondary phloem; **pith** missing;

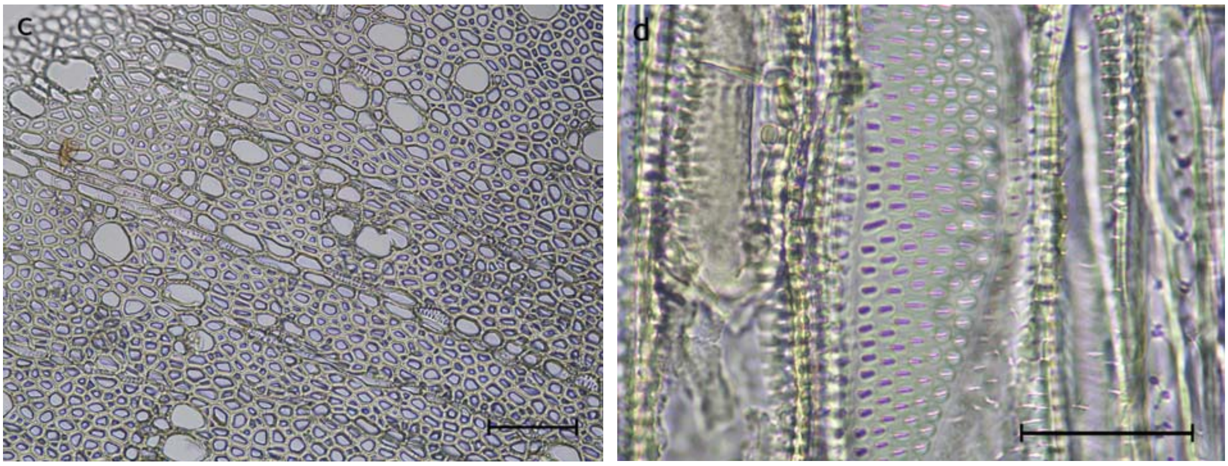
sclereids, laticifers missing;

fibers in sec. phloem missing;



a: *Cyanus segetum,* taproot, habitus (CS03-08)
b: Schematic view of *C. cyanus* root (transverse section): small ellipses mark the endoSDs within the cortex and ducts SD2b of the sec. phloem located fascicular between phloem rays





Cyanus cyanus root: **a:** Overview showing the extension and arrangement of tissues: vascular cylinder dominating, small secondary phloem, cortex partly suberized, endoSDs enduring (CS01-08); **b:** endodermal resin duct (CS02-08); **c:** xylem dominated by fibers, medullary rays mono- and biseriate (CS02-08); **d:** strongly bordered vessels (CS02-08);
a-c: transverse sections; d: longitudinal section;

CNICUS BENEDICTUS L. (cf. Paper III)

annual therophyte;

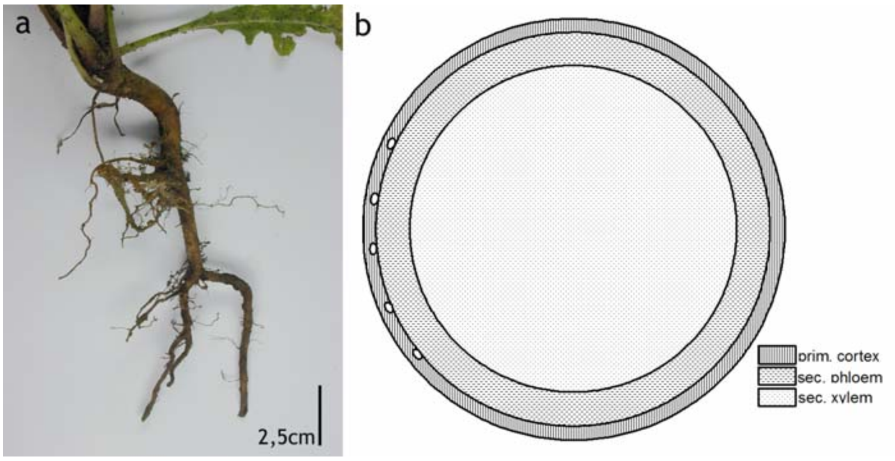
allorhizous, with taproot;

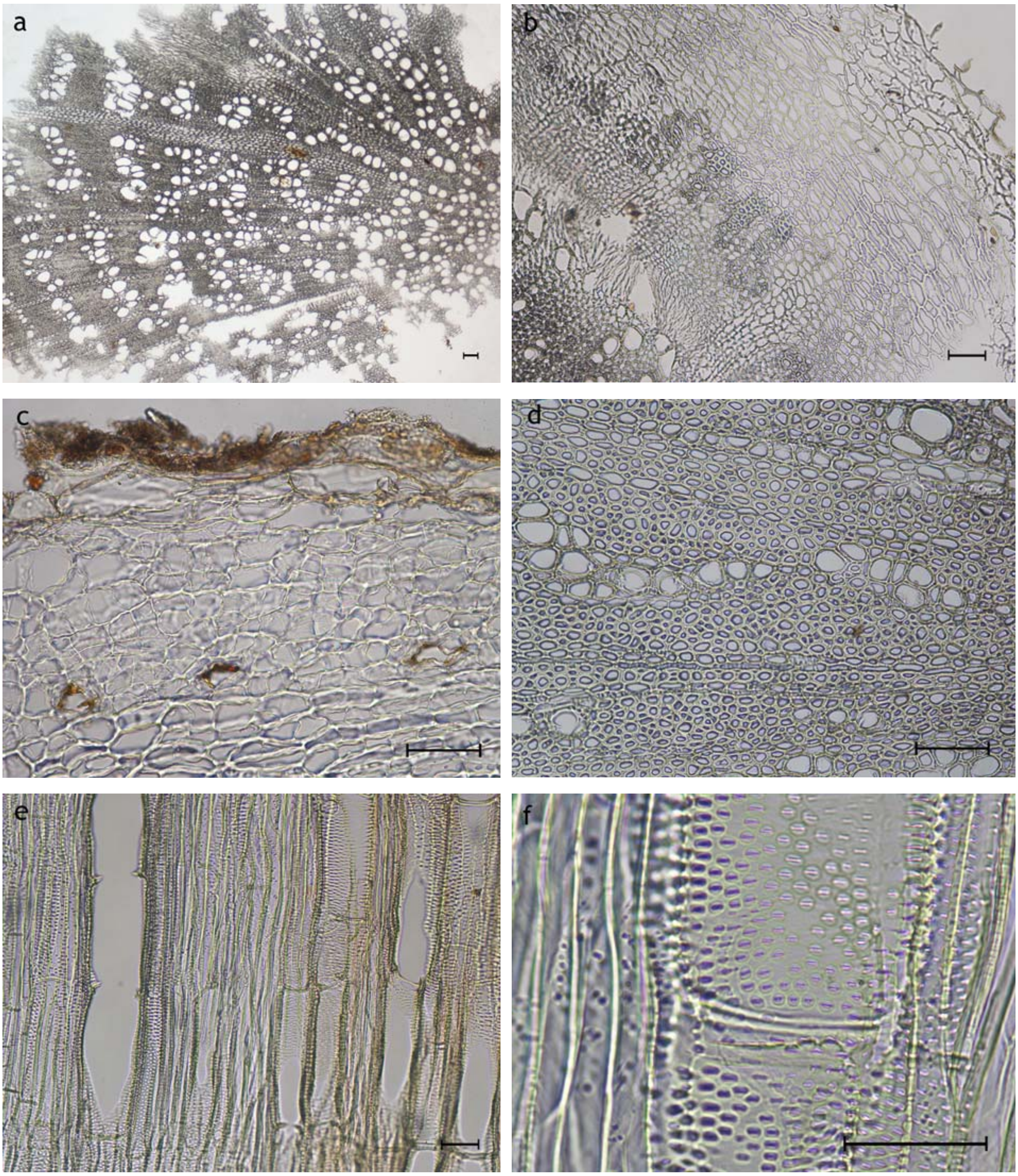
secondary root: cork narrow, thin-walled, sometimes with crystalloids; **cortex** enduring; **endoSDs** with small lumen; small **secondary phloem** usually broader than cortex, but of lesser radial extension than the vascular cylinder, with fibers arranged in bundles in fascicular position above the vascular cylinder between phloem rays; **secondary xylem** with fibers, vessels either more or less circularly arranged relative to the centre of the xylem or dispersed, strongly bordered, up to 93µm in diameter; **medullary rays** usually narrow, bi- or multiseriate, regularly arranged; **pith** missing;

sclereids, laticifers missing;

secretory ducts beside endoSDs missing;

a: Taproot of *Cnicus benedictus,* habitus (S3)
b: Schematic view of *C. benedictus* root (transverse section): small ellipses mark the position of the endoSDs within the cortex; sec. phloem broader than the cortex, but sec. xylem dominating





Cnicus benedictus root: **a:** Overview showing the extension and arrangement of tissues: small cortex with endoSDs, broad secondary phloem, but of lesser radial extension than the vascular cylinder cortex, vascular cylinder (S4); **b:** overview showing the anatomy of the secondary phloem (S2); **c:** endodermis with regularly arranged endoSDs with small lumina (S2); **d:** xylem dominated by fibers, medullary rays mono- and biseriate (S3); **e:** overview of secondary xylem (K30-10-08); **f:** strongly bordered vessels (K30-10-08);
a-d: transverse sections; e,f: longitudinal section;

TRIBE CICHORIEAE

SUBTRIBE SCORZONERINAE

GENUS SCORZONERA

SCORZONERA PURPUREA L.

perennial geophyte - hemicryptophyte;

allorhizous with taproot;

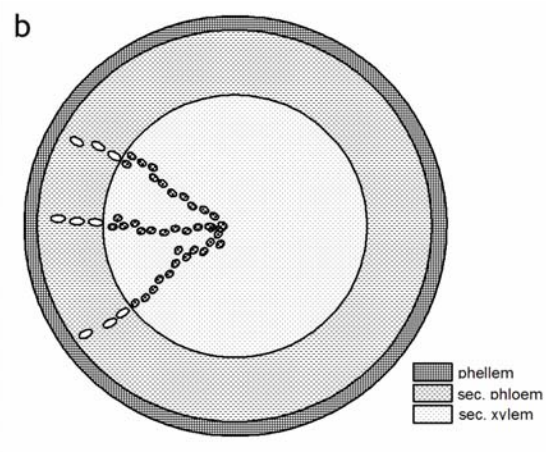
secondary root: phellem thin-walled, thoroughly parallel laminated, sometimes with crystalloids; **cortex** lost in course of rhytidome formation; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with lacticiferous vessels arranged in rows in fascicular position "continuing" the vessels of the secondary xylem; **secondary xylem** dominated by parenchymatous cells with vessels arranged in single rows; vessels reticulate, up to 45µm in diameter; **medullary rays** broad, multiseriate; **pith** missing;

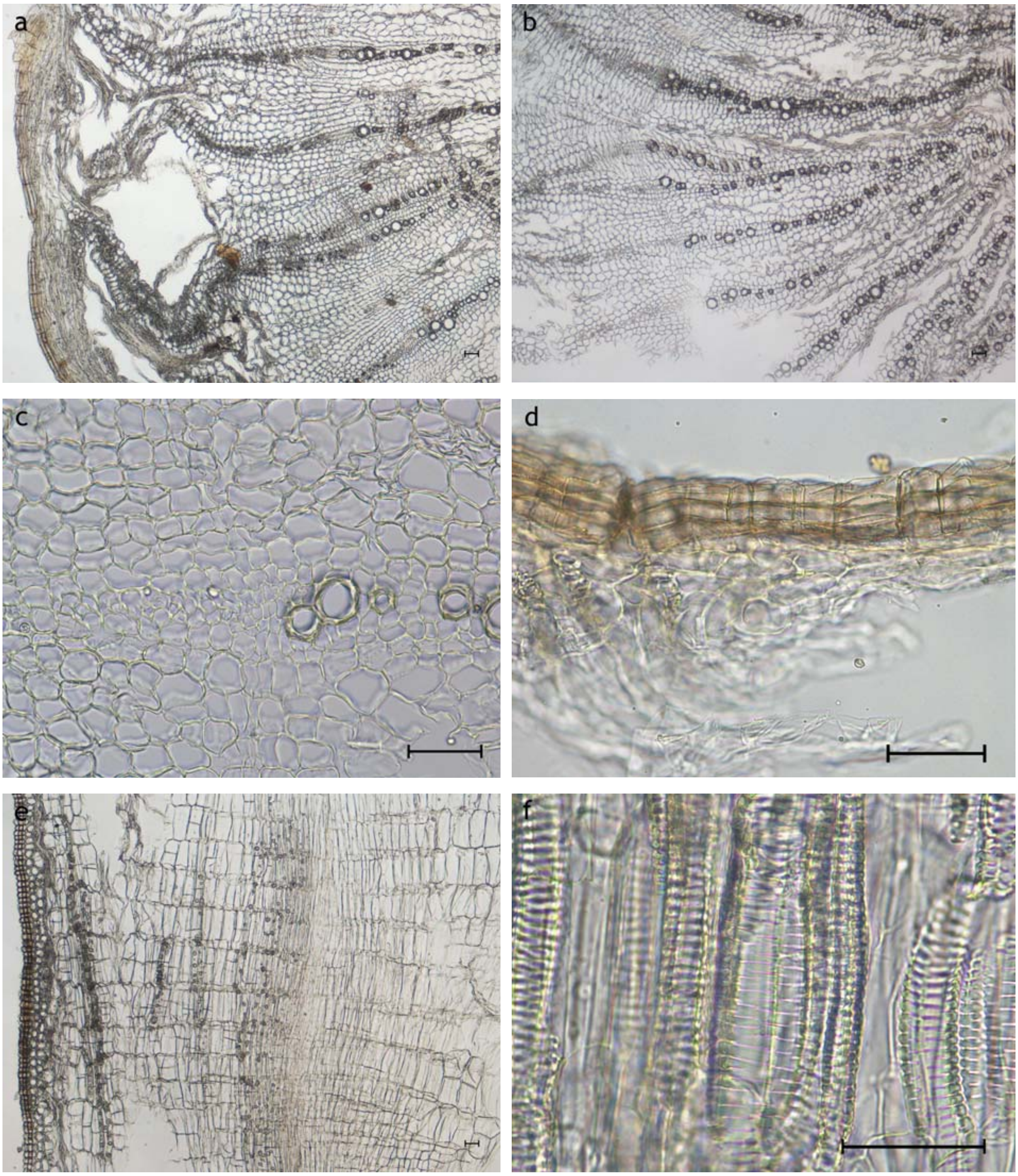
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem and secondary xylem missing;

a: Taproot of *Scorzonera purpurea*, habitus (SP01-09)
b: Schematic view of *S. purpurea* root (transverse section): small ellipses mark the position of the laticiferous vessels of the broad sec. phloem, vessels of the dominating sec. xylem arranged in rows





Scorzonera purpurea root: **a,b:** Overview showing the extension and arrangement of tissues: the secondary phloem is comparable in extension to the vascular cylinder, fibers both in phloem and xylem missing, laticiferous vessels within secondary phloem arranged in rows in fascicular positions above vessels of secondary xylem (SP02-09); **c:** laticiferous vessels within secondary phloem arranged in rows in fascicular positions above vessels of secondary xylem – "continuing the rows of vessels" (SP01-09); **d:** phellem thin-walled, thoroughly parallel laminated (SP02-09); **e:** overview showing the extension and arrangement of tissues: phellem, sec. phloem with laticiferous vessels (40x) 03-09; **f:** reticulate vessels (03-09)
a-d: transverse sections; e, f: longitudinal sections;

SCORZONERA ROSEA Waldst. & Kit. (Fischer *et al*.)

(= *S. purpurea subsp. rosea* (Waldst. & Kit.) Nyman*)*

perennial geophyte - hemicryptophyte;

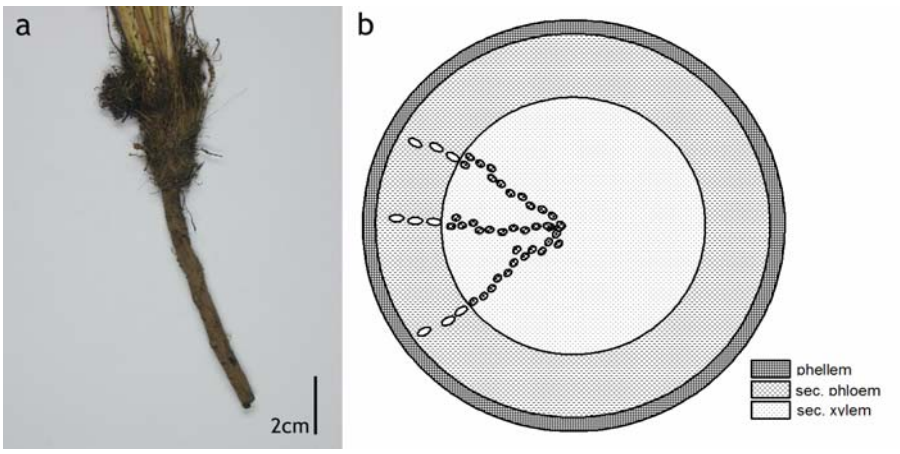
allorhizous with taproot;

secondary root: phellem with slightly thickened cell walls, thoroughly parallel laminated, sometimes with crystalloids; **cortex** lost in course of rhytidome formation; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with lacticiferous vessels arranged in rows in fascicular position "continuing" the vessels of the secondary xylem as well as in interfascicular position above parenchymatous tissues of the secondary xylem; **secondary xylem** dominated by parenchymatous cells with vessels arranged in single or multiple rows; vessels reticulate, up to 57µm in diameter; **medullary rays** broad, multiseriate; **pith** missing;

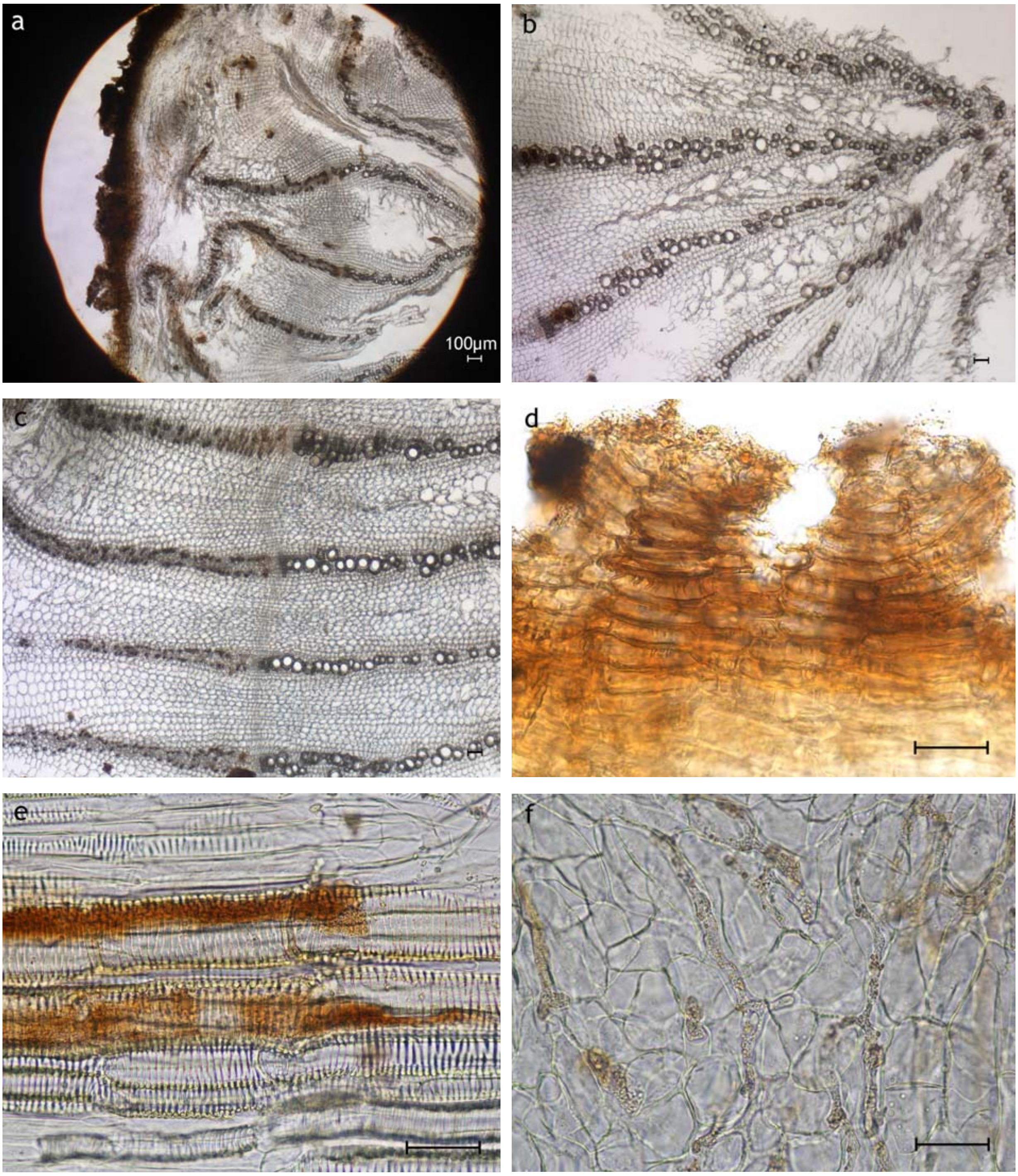
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem and secondary xylem missing;



a: Taproot of *Scorzonera rosea*, habitus (SR01-09)
b: Schematic view of *Scorzonera rosea* root (transverse section): small ellipses mark the position of the lacticifers of the broad sec. phloem, vessels of the broad sec. xylem arranged in rows



Scorzonera rosea root: **a,b:** Overview showing the extension and arrangement of tissues: the secondary phloem is comparable in extension to the vascular cylinder, fibers missing, laticiferous vessels within secondary phloem arranged in rows (SR02-09) (a), secondary xylem dominated by parenchymatous cells with fibers missing, vessels arranged in single to multiple rows (b) (SR01-09); **c:** laticiferous vessels within secondary phloem arranged in rows in fascicular positions above vessels of secondary xylem – "continuing the rows of vessels" (SR02-09); **d:** phellem with slightly thickened cell walls, thoroughly parallel laminated (SR02-09); **e:** reticulate vessels (SR01-09); **f:** laticiferous vessels (SR02-09) a-d: transverse sections; e,f: longitudinal sections;

SCORZONERA ARISTATA Ramond ex DC.

perennial hemicryptophyte;

allorhizous with taproot;

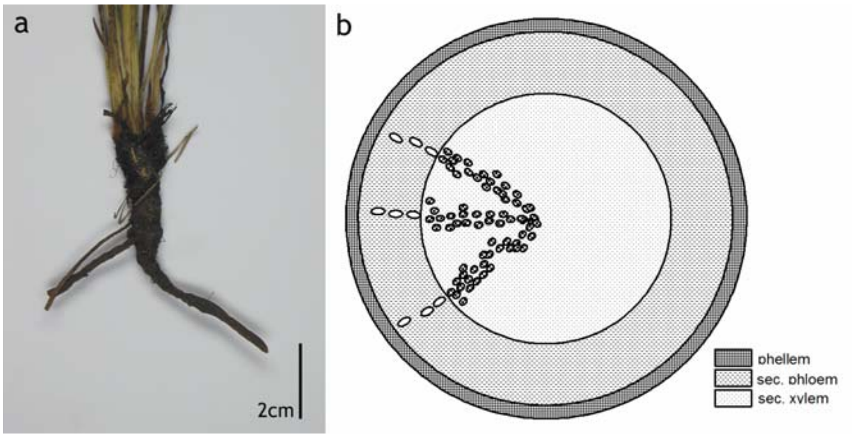
secondary root: phellem with slightly thickened cell walls, thoroughly parallel laminated, sometimes with crystalloids; **cortex** lost in course of rhytidome formation; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with laticifers arranged in rows in fascicular position “continuing” the vessels of the secondary xylem as well as in interfascicular position above parenchymatous tissues of the secondary xylem; **secondary xylem** dominated by parenchymatous cells with vessels arranged in multiple rows; vessels reticulate, up to 59µm in diameter; **medullary rays** broad, multiseriate; **pith** missing;

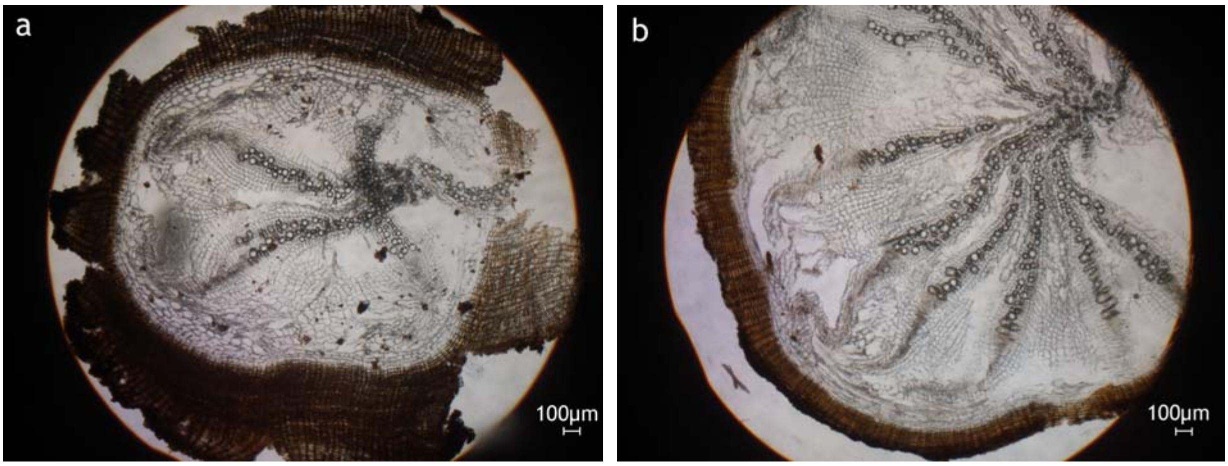
endoSDs, further secretory ducts missing;

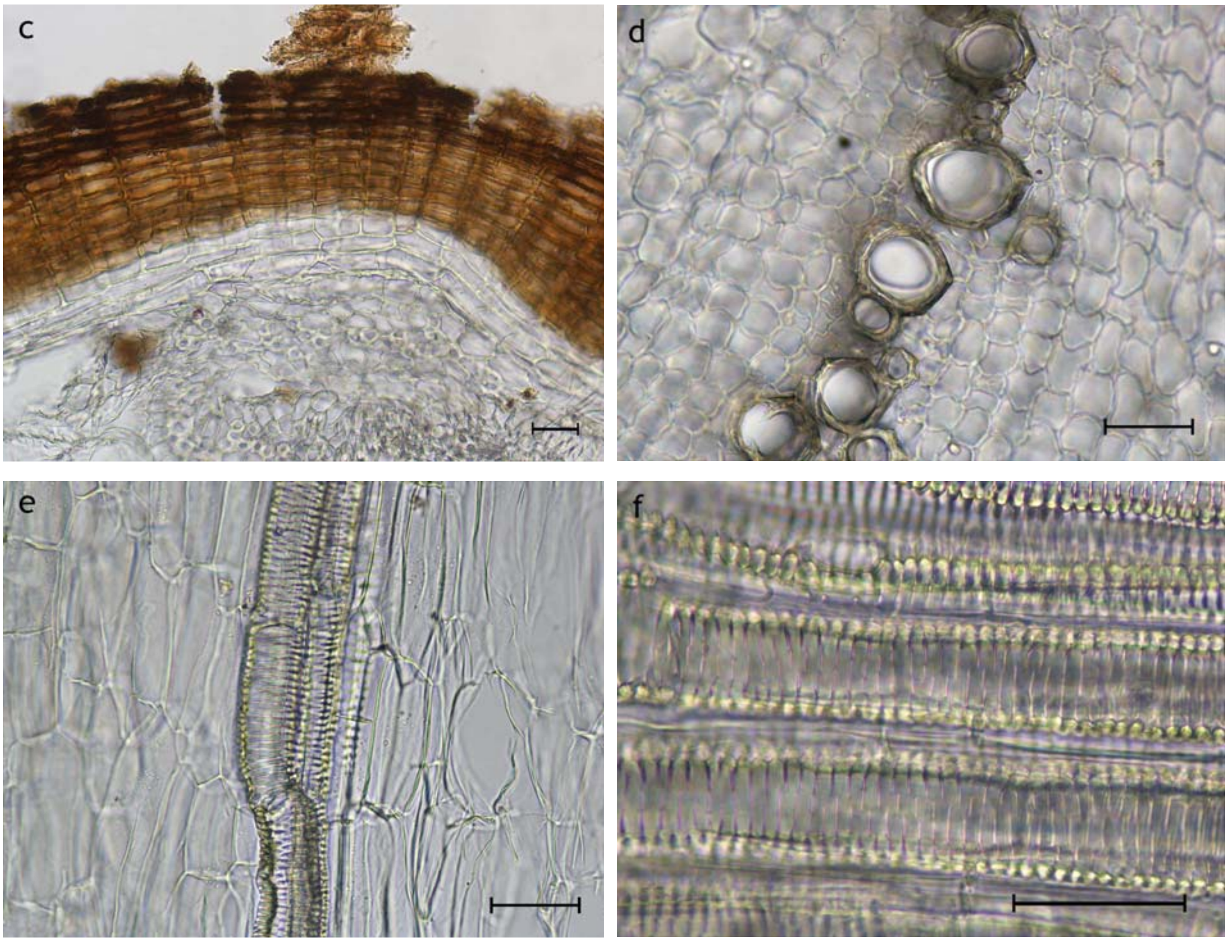
sclereids missing;

fibers in secondary phloem and secondary xylem missing;

a: Taproot of *Scorzonera aristata*, habitus (SAr01-09)
b: Schematic view of *S. aristata* root (transverse section): small ellipses mark the laticifers of the sec. phloem, vessels of the sec. xylem arranged in rows







Scorzonera aristata root: **a,b:** Overview showing the extension and arrangement of tissues: broad phellem, the secondary phloem is comparable in extension to the vascular cylinder, fibers missing, laticiferous vessels within secondary phloem and vessels of secondary xylem arranged in rows (SAr01-09, SAr03-09); **c:** phellem with slightly thickened cell walls, thoroughly parallel laminated (SAr03-09); **d:** vessels of secondary xylem (SAr02-09); **e,f:** reticulate vessels (SAr03-09, SAr01-09)
a-d: transverse sections; e,f: longitudinal sections;

SCORZONERA AUSTRIACA Willd.

perennial hemicryptophyte;

allorhizous with taproot;

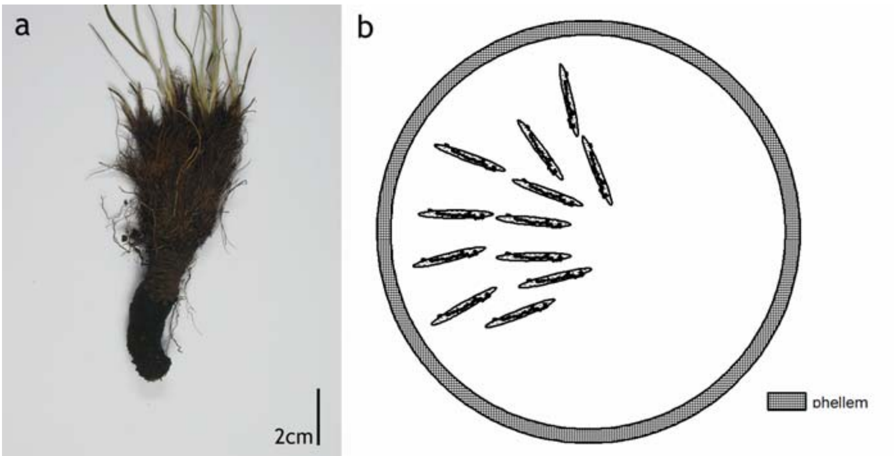
secondary root: phellem broad, with slightly thickened cell walls, thoroughly parallel laminated, sometimes with crystalloids; **cortex** lost in course of rhytidome formation; **secondary phloem** comparable in extension to the vascular cylinder, with lacticiferous vessels arranged in rows in fascicular position “continuing” vessels arranged in single rows of the **secondary xylem**; secondary phloem and secondary xylem building bundles comparable to monocotyles but with secondary growth, irregulary dispersed over the transverse section; vessels reticulate and simple, up to 45µm in diameter; **medullary rays** not existing because of irregular structure; **pith** missing;

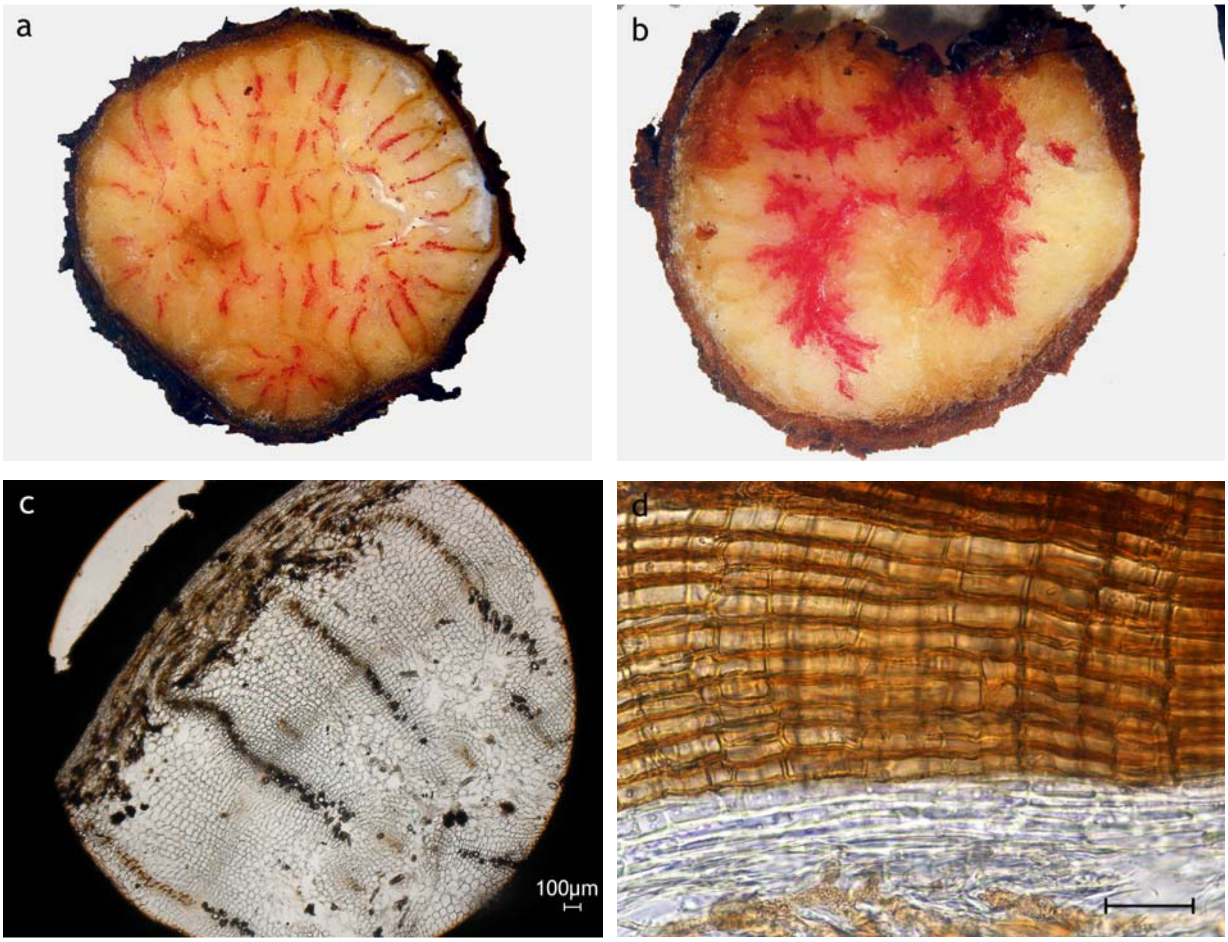
endoSDs, further secretory ducts missing;

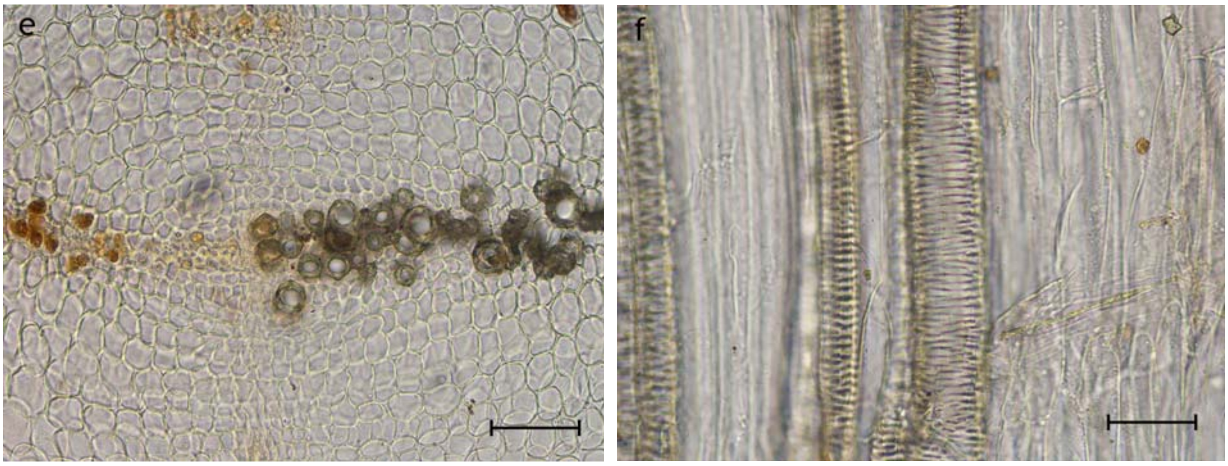
sclereids missing;

fibers in secondary phloem and secondary xylem missing;

a: Taproot of *Scorzonera austriaca*, habitus (SA05-09)
b: Schematic view of *S. austriaca* root (transverse section): vascular bundles bundles with sec. growth, irregulary dispersed over the transverse section







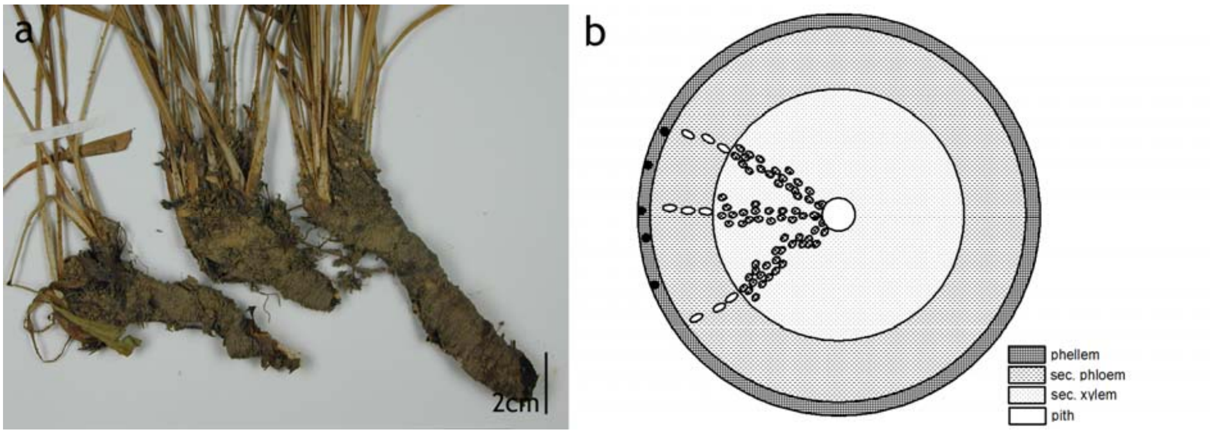
Scorzonera austriaca root: **a,b:** Macroscopic picture of transverse section, treated with Phloroglucin-HCl: secondary xylem red-coloured (SA01-09,SA04-09); **c:** overview showing the extension and arrangement of tissues: broad phellem, the secondary phloem is comparable in extension to the vascular cylinder, irregularly arranged within transverse section, no circular structure of root – "bundles" of secondary xylem and secondary phloem dispersed comparable to monocotyles but with secondary growth (SA01-09); **d:** phellem with slightly thickened cell walls, thoroughly parallel laminated (SA04-09); **e:** laticiferous vessels within secondary phloem arranged in rows in fascicular positions above vessels of secondary xylem – "continuing the rows of vessels" (SA01-09); **f:** reticulate vessels (SA01-09)
a-e: transverse sections; f: longitudinal sections;

SCORZONERA HUMILIS L.

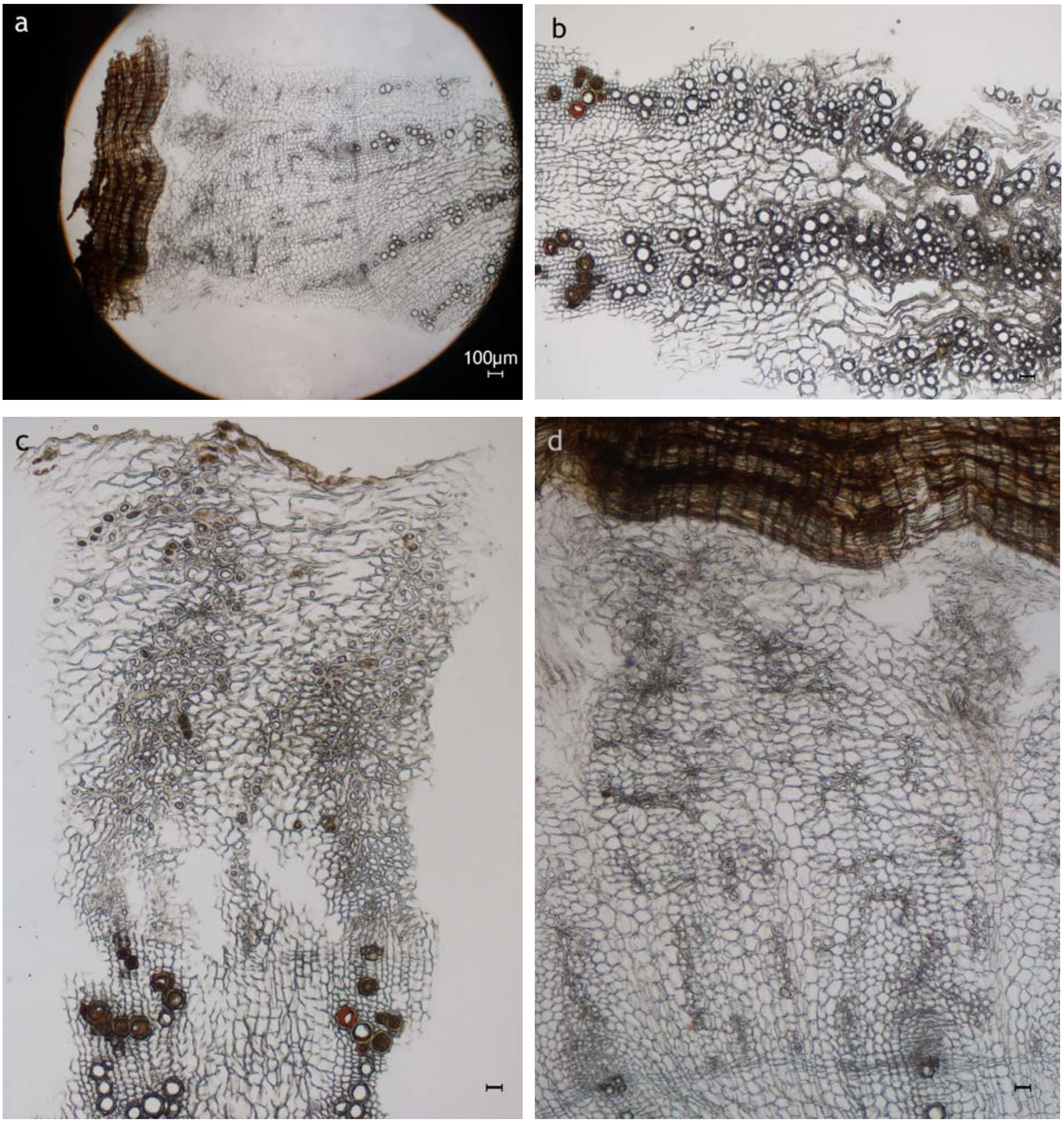
perennial hemicryptophyte;

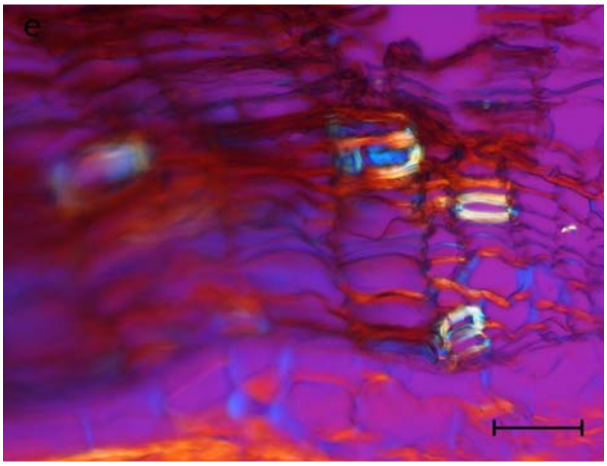
allorhizous, long rhizome presumably with taproot;

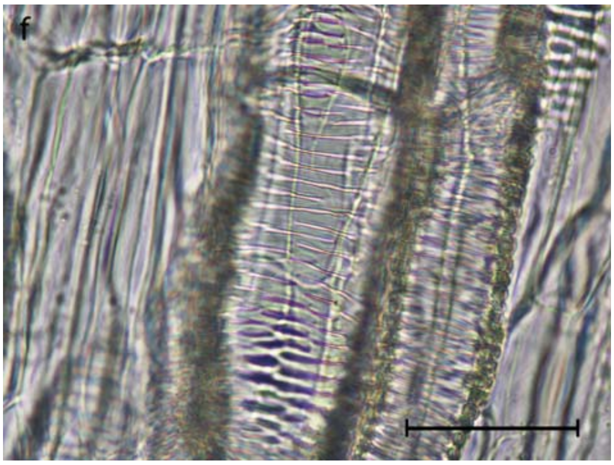
rhizome: phellem thin-wanded, may contain sclereids, thoroughly parallel laminated, sometimes with crystalloids; **cortex** lost in course of rhytidome formation; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with lacticiferous vessels arranged in rows in fascicular position "continuing" the vessels of the secondary xylem as well as in interfascicular position above parenchymatous tissues of the secondary xylem, sometimes also irregularly dispersed; **secondary xylem** dominated by parenchymatous cells with vessels arranged double to multiple rows; vessels reticulate, up to 59µm in diameter; **medullary rays** broad, multiseriate, often collapsed; **pith** collapsed;
endoSDs, further secretory ducts missing;
fibers in secondary phloem and secondary xylem missing;



a: Rhizome of *Scorzonera humilis,* habitus (SH02,SH03)
b: Schematic view of *S. humilis* rhizome in transverse section: small ellipses mark the position of the laticiferous vessels of the broad sec. phloem, comparable in extension to the vascular cylinder with vessels arranged in double to multiple rows, black points represent sclereids of the phellem







Scorzonera humilis: **a:** Overview showing the extension and arrangement of tissues: the secondary phloem is comparable in extension to the vascular cylinder, fibers missing, laticiferous vessels within secondary phloem irregularly dispersed (a) (SH01); **b:** vessels of secondary xylem arranged in multiple rows (SH03); **c:** laticiferous vessels more or less in fascicular position arranged in rows (SH01); **d:** laticiferous vessels irregularly dispersed within the secondary phloem (SH01); **e:** phellem with thin-walled cell walls containing sclereids (SH01); **f:** reticulate vessels (SH02)
a-e: transverse sections; f: longitudinal sections;

SCORZONERA HISPANICA L. (cf. Paper IV)

perennial hemicryptophyte;

allorhizous, long rhizome presumably with taproot;

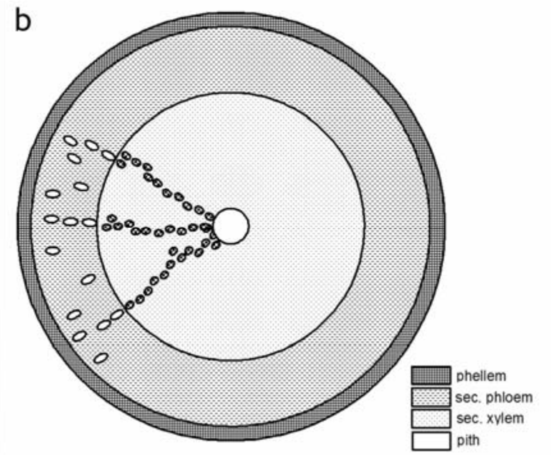
secondary root: phellem thin-walled, thoroughly parallel laminated, sometimes with crystalloids; **cortex** lost in course of rhytidome formation; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with lacticiferous vessels arranged in rows in fascicular position "continuing" the vessels of the secondary xylem as well as in interfascicular position irregularly dispersed; outer parts of secondary phloem often obliterated / collapsed; **secondary xylem** dominated by parenchymatous cells with vessels arranged in single or double rows; vessels reticulate and simple up to 59µm in diameter; **medullary rays** broad, multiseriate; **pith** collapsed;

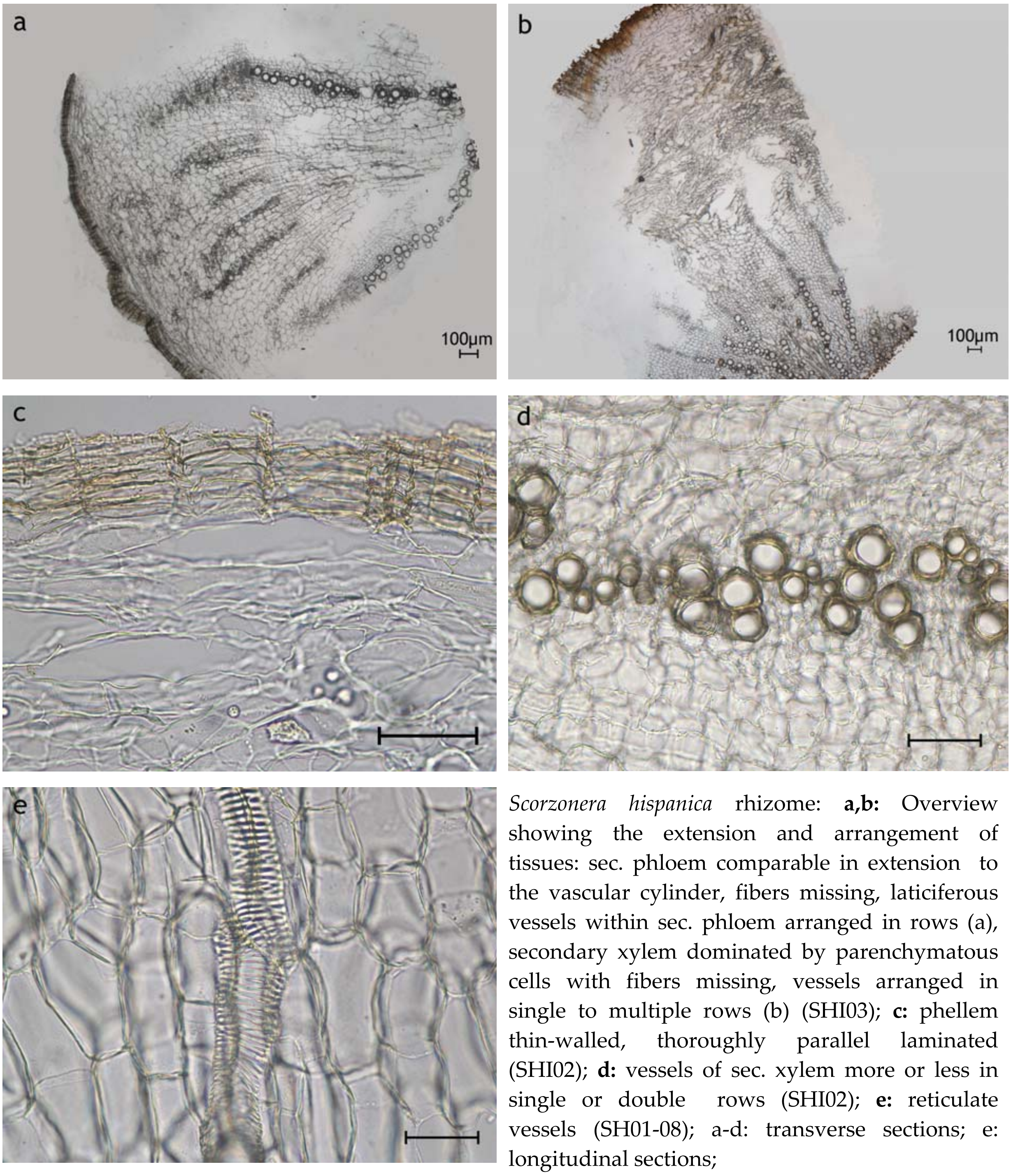
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem and secondary xylem missing;

a: Rhizome of *Scorzonera hispanica*, habitus (SHI01)
b: Schematic view of *S. hispanica* rhizome (transverse section): small ellipses mark the laticifers of the sec. phloem, the vascular cylinder with vessels arranged in single or multiple rows





Scorzonera hispanica rhizome: **a,b:** Overview showing the extension and arrangement of tissues: sec. phloem comparable in extension to the vascular cylinder, fibers missing, laticiferous vessels within sec. phloem arranged in rows (a), secondary xylem dominated by parenchymatous cells with fibers missing, vessels arranged in single to multiple rows (b) (SHI03); **c:** phellem thin-walled, thoroughly parallel laminated (SHI02); **d:** vessels of sec. xylem more or less in single or double rows (SHI02); **e:** reticulate vessels (SH01-08); a-d: transverse sections; e: longitudinal sections;

TRAGOPOGON ORIENTALIS L. / *T. DUBIUS* Scop. (cf. Paper IV)

no differentiation possible between the two species;

perennial hemicryptophyte;

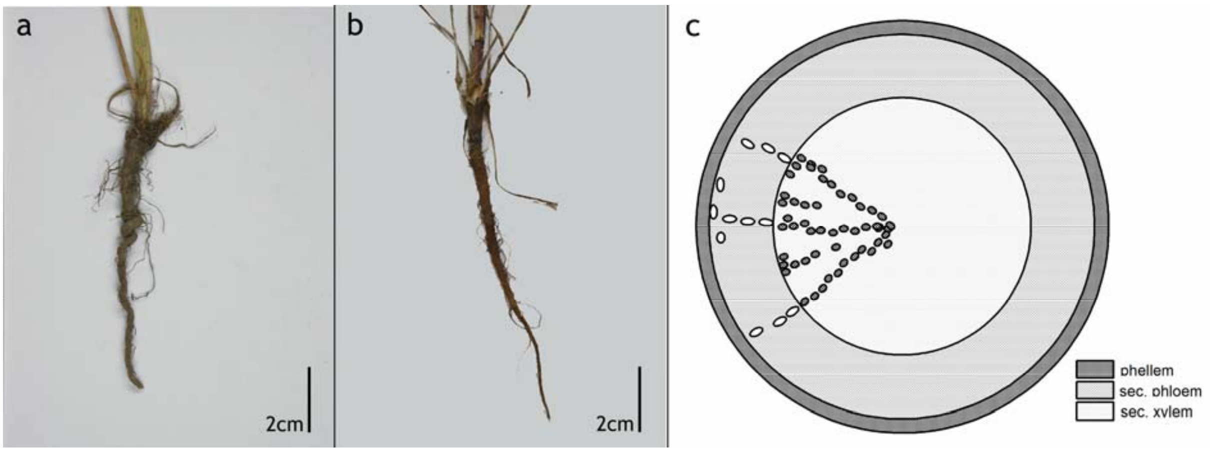
allorhizous, with taproot;

secondary root: phellem thin-walled, sometimes with crystalloids; **cortex** enduring with distinct **endodermis** visible; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with lacticiferous vessels arranged in rows in fascicular position "continuing" the vessels of the secondary xylem, within the outer part of the secondary phloem also irregulary dispersed; **secondary xylem** with vessels arranged in single and double rows, cumulative at the outer part, fibers mainly within the outer part of the xylem circularly lignified relative to the centre of the xylem, inner parts unlignified; vessels mainly strongly bordered, also reticulate, up to 68µm in diameter; **medullary rays** broad, multiseriate, often obliterated and ripped apart; **pith** missing;

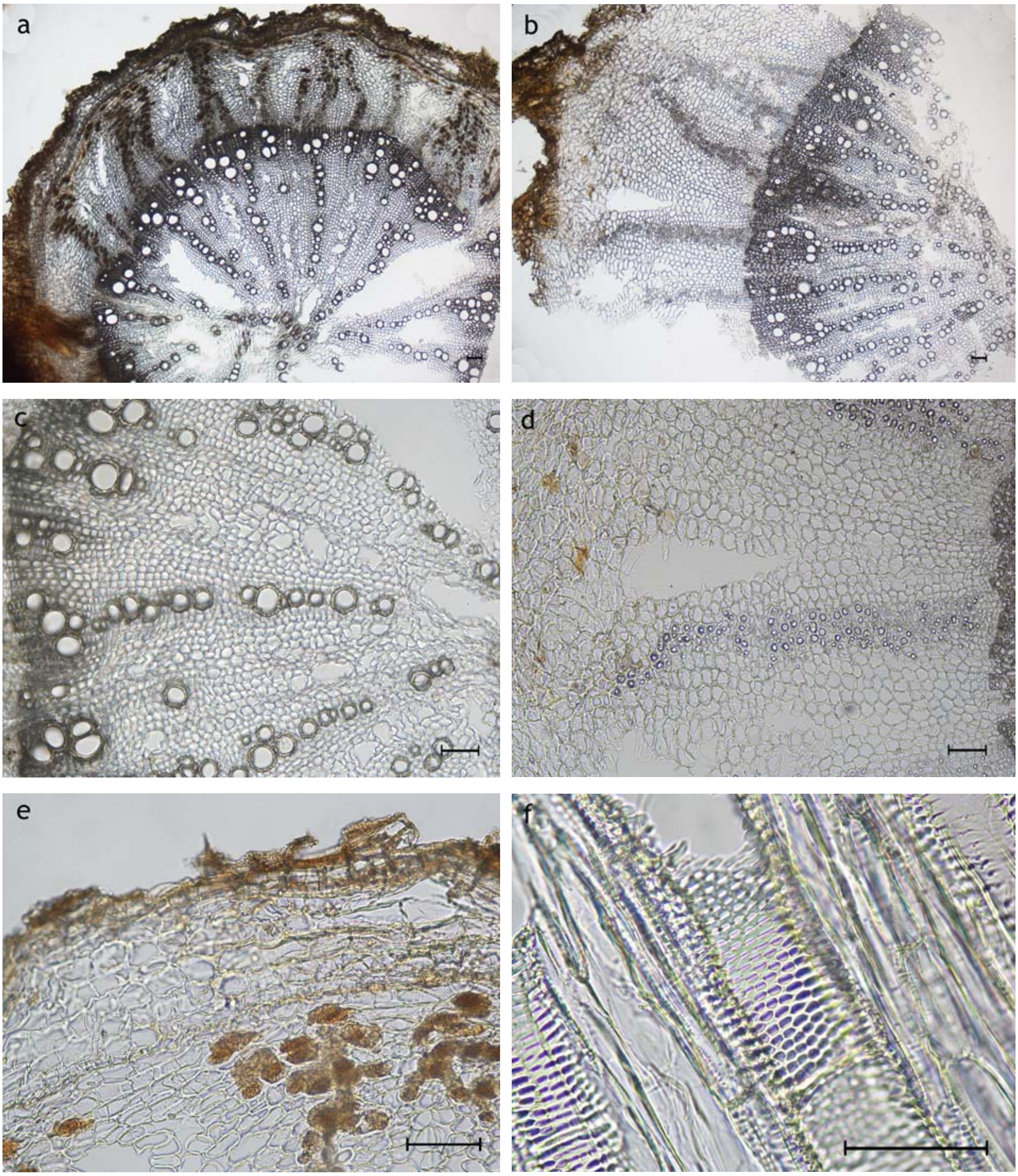
endoSDs, further secretory ducts missing;

sclereids missing;

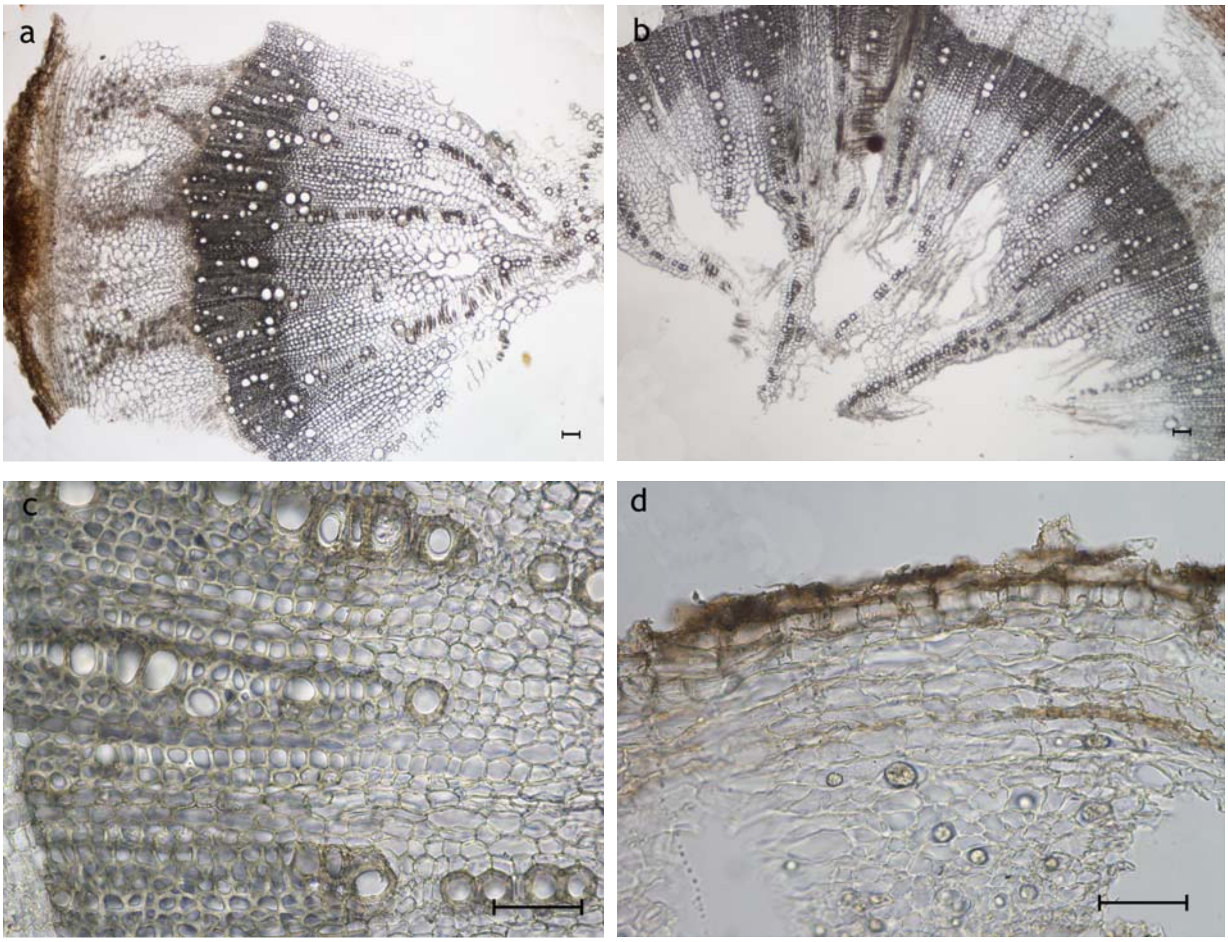
fibers in secondary phloem missing;



a: Taproot of *Tragopogon orientalis*, habitus (TO02-08)
b: Taproot of *Tragopogon dubius*, habitus (TD1-08)
c: Schematic view of *Tragopogon* sp. root (transverse section): sec. root: small ellipses mark the position of the laticifers of the broad sec. phloem, comparable in extension to the vascular cylinder with vessels arranged in single or double rows



Tragopogon orientalis root: **a,b:** Overview showing the extension and arrangement of tissues: the secondary phloem is comparable in extension to the vascular cylinder (the secondary xylem just slightly dominating), fibers in secondary phloem missing, laticiferous vessels within secondary phloem arranged in rows; small phellem (TO03-08, TO01-08); **c:** sec. xylem with vessels more or less arranged in single or double rows, more vessels to the outer part (TO03-08); **d:** secondary phloem with laticiferous vessels in rows (TO01-08); **e:** cortex enduring with endodermis visible (TO03-08); **f:** strongly bordered vessels (TO01-08)
a-e: transverse sections; f: longitudinal sections;



Tragopogon dubius root: **a:** Overview showing the extension and arrangement of tissues: the secondary phloem is comparable in extension to the vascular cylinder (the secondary xylem just slightly dominating), fibers in secondary phloem missing, laticiferous vessels within secondary phloem arranged in rows; small phellem (AST1); **b:** overview showing the extension and arrangement of secondary xylem: broad secondary xylem with fibers within the outer part lignified circularly relative to the centre of the xylem, inner parts unlignified (TD01-08); **c:** secondary xylem with vessels more or less arranged in single or double rows, more vessels to the outer part; fibers within the outer part of the xylem circularly lignified relative to the centre of the xylem, inner parts unlignified (TD01-08); **d:** cortex enduring with endodermis visible (TD01-08);
a-d: transverse sections;

SUBTRIBE LACTUCINAE

GENUS LACTUCA

LACTUCA ALPINA Benth. & Hook.f. (= *CICERBITA ALPINA* Wallr.)

perennial hemicryptophyte;

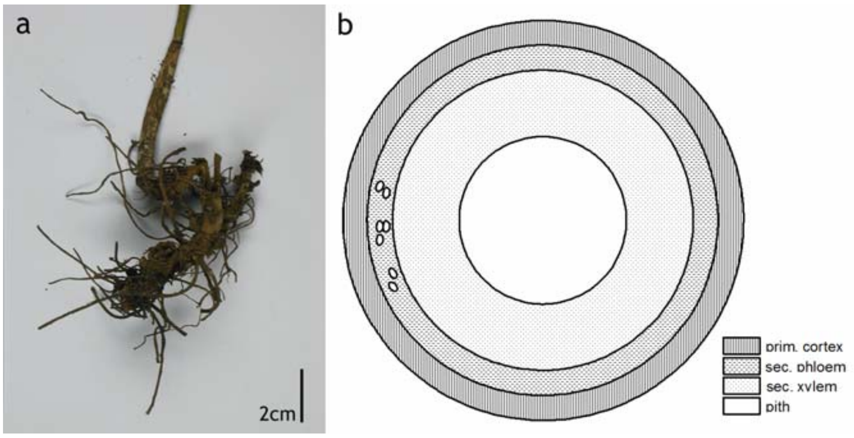
rhizome;

rhizome with secondary growth: Thin-walled **phellem**, subexodermal; **cortex** enduring; cortex and **secondary phloem** of lesser radial extension than the vascular cylinder, with lacticiferous vessels dispersed; **secondary xylem** with fibers, few vessels cumulating at the outer parts of vascular bundles with diameter enlarging, strongly bordered, up to 95µm in diameter; inner part of vascular bundles may show irregular pattern (see figure); medullary rays broad, multiseriate; **pith** of thin-wanded cells;

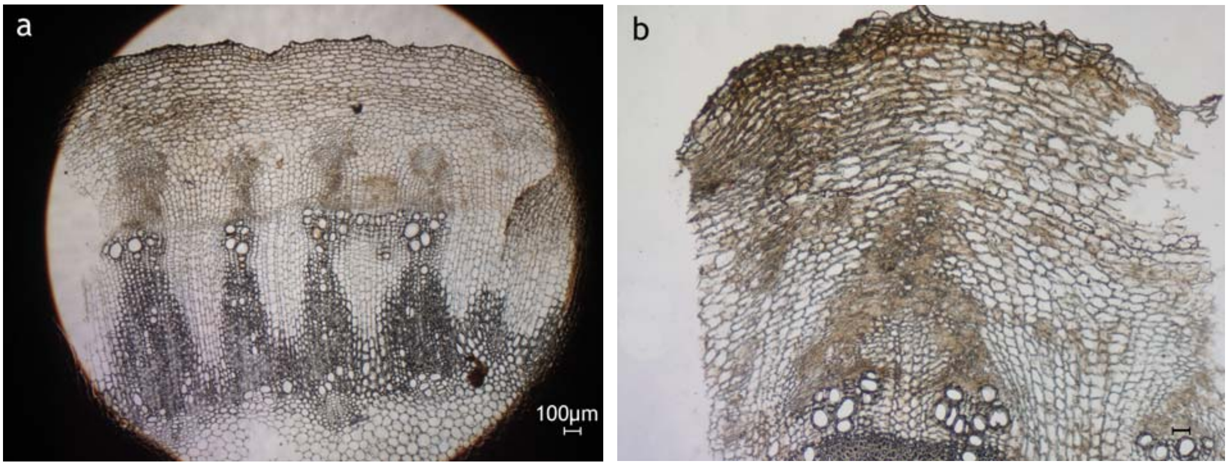
endoSDs, further secretory ducts missing;

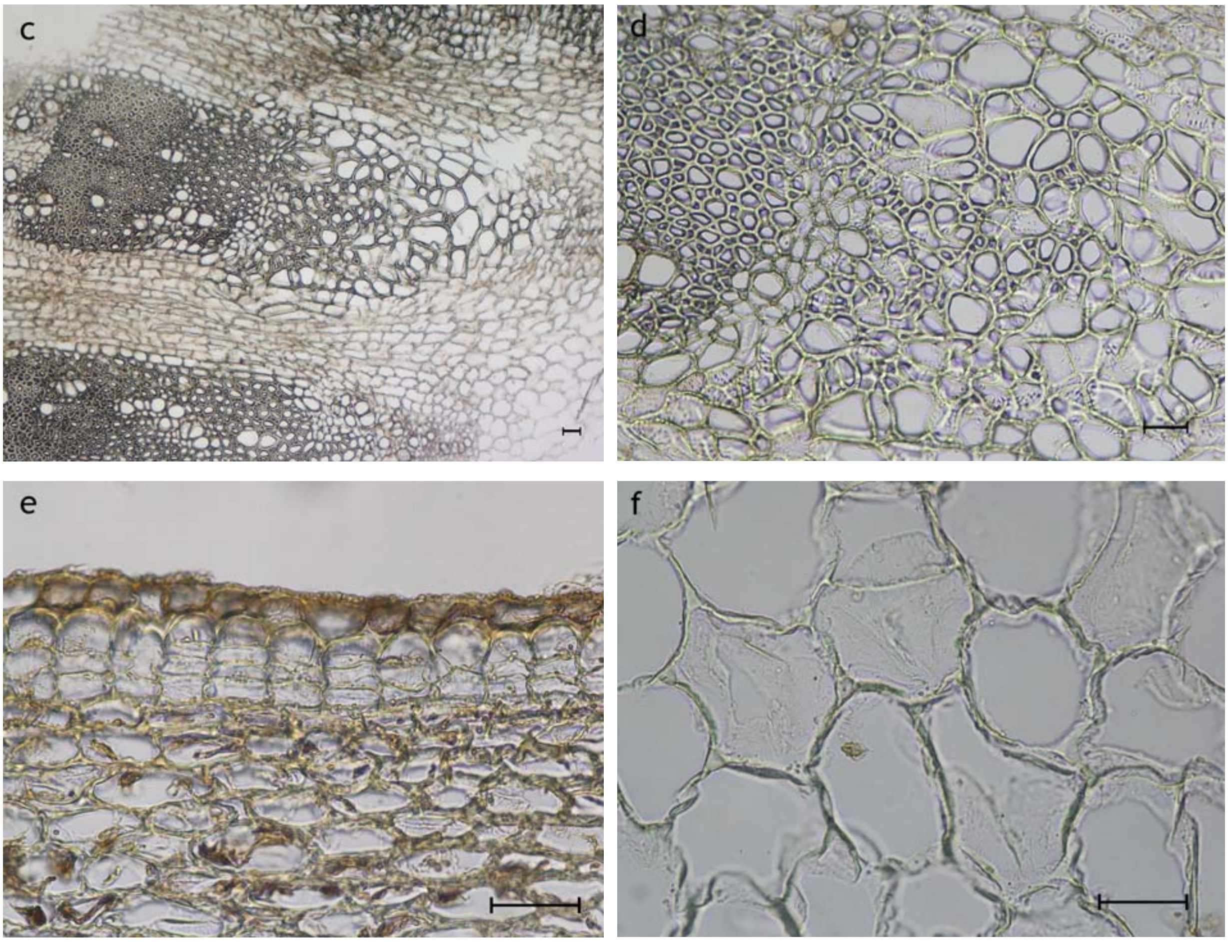
crystalloids, sclereids missing;

fibers in secondary phloem missing;



a: Rhizome of *Cicerbita alpina*, habitus (CiAl01-09)
B: Schematic view of *C. alpina* rhizome (transverse section): small ellipses mark laticifers of the phloem; cortex and sec. phloem of lesser radial extension than the vascular cylinder, large pith





Cicerbita alpina rhizome: **a:** Overview showing the extension and arrangement of tissues: cortex and sec. phloem of lesser radial extension than the vascular cylinder, vessels cumulating at the outer parts of vascular bundles with diameter enlarging, large pith (CiAl01-09); **b:** cortex, sec. phloem with laticifers dispersed (CiAl03-09); **c:** vascular bundle between broad medullary rays and transition to pith (CiAl03-09); **d:** inner part of vascular bundle (CiAl03-09); **e:** subexodermal phellem of thin-wanded cells (CiAl01-09); **f:** pith with thin-walled cells (CiAl03-09)
a-f: transverse sections;

LACTUCA MYCELIS (L.) E.Mey. (= *MYCELIS MURALIS* (L.) Dumort.)

perennial hemicryptophyte;

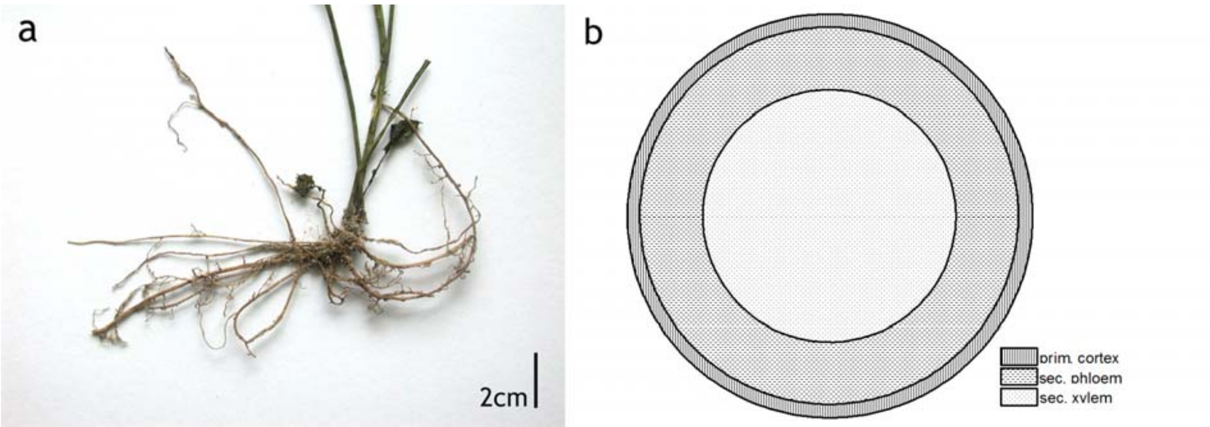
fibrous root system (with rhizome very short, also longer)

secondary root: phellem possible, narrow, thin-walled, sometimes with crystalloids; **cortex** enduring; **secondary phloem** narrow with laticiferous vessels dispersed; vascular cylinder dominating; **secondary xylem** dominated by fibers, few vessels usually arranged in short single rows, reticulate, mainly strongly bordered, up to 45µm in diameter; **medullary rays** may occur broad, multiseriate or biseriate, can even appear almost raylessness in transverse section because of thickening of cell walls of ray cells; **pith** missing;

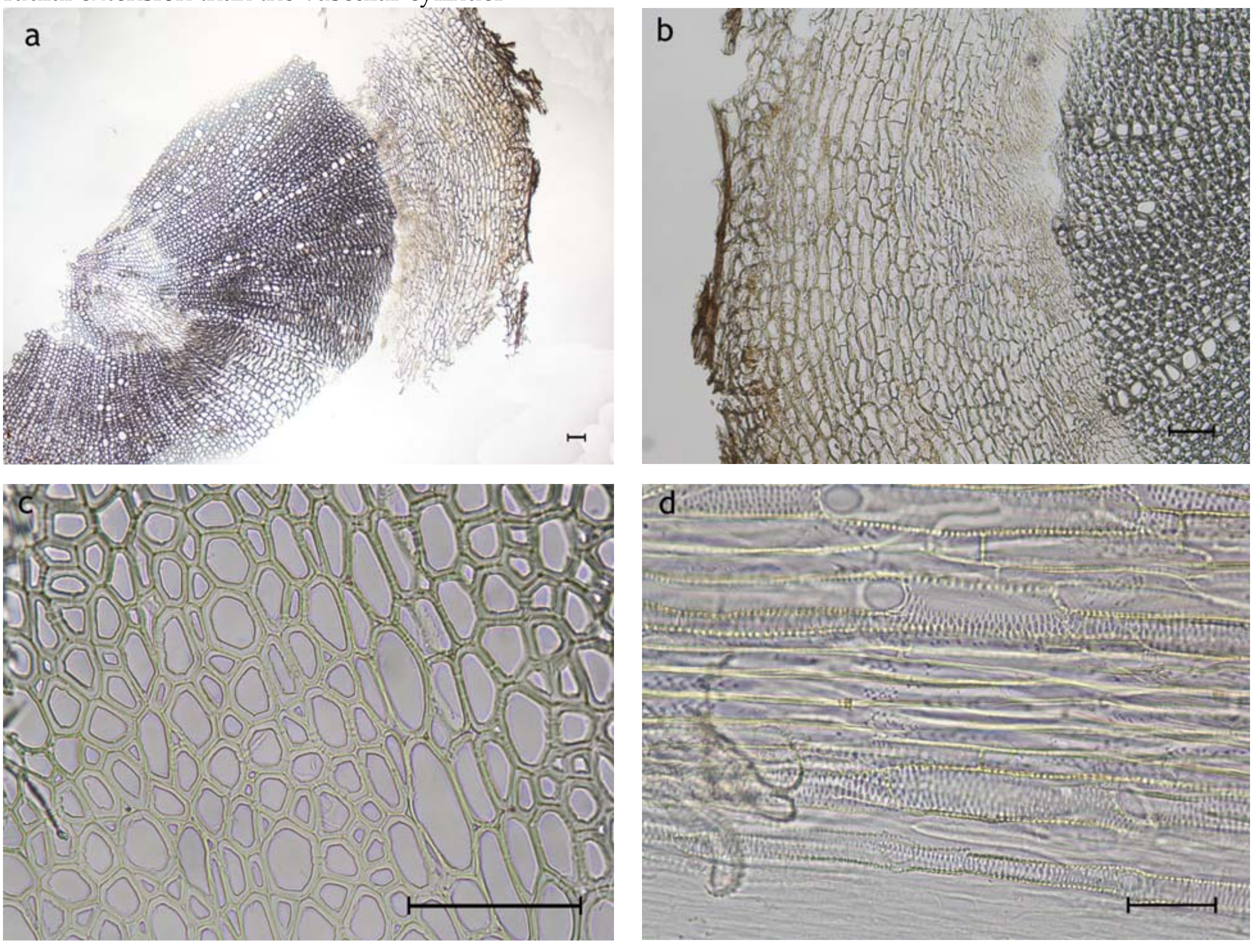
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem missing;



a: Fibrous root system of *Mycelis muralis*, habitus (M01-07)
b: Schematic view of *Mycelis muralis* root in transverse section: durable cortex, secondary phloem of lesser radial extension than the vascular cylinder



Lactuca muralis root: **a:** Overview showing the extension and arrangement of tissues: cortex and secondary phloem of lesser radial extension than the vascular cylinder, secondary xylem dominated by fibers, few vessels arranged in short single rows (M01-08); **b:** cortex and narrow secondary phloem with laticiferous vessels dispersed (M01-08); **c:** secondary xylem with medullary ray of cells with thickened cell walls (M02-07); **d:** secondary xylem with mainly strongly bordered vessels (M02-07) a-c: transverse sections; d: longitudinal section

LACTUCA PERENNIS L.

perennial hemicryptophyte;

allorhizous with taproot

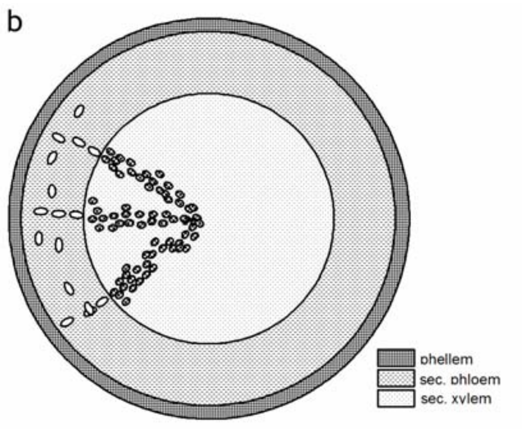
secondary root: phellem, thin-walled, thoroughly parallel laminated, sometimes with crystalloids; **cortex** lost in course of rhytidome formation; **secondary phloem** broad, but of lesser radial extension than the vascular cylinder, with laticiferous vessels in rows, also irregularly dispersed; **secondary xylem** with vessels arranged in multiple rows – often winded, not straight –, reticulate thickening, up to 93µm in diameter; **medullary rays** multiseriate, ray cells often obliterated, ripped apart; **pith** missing;

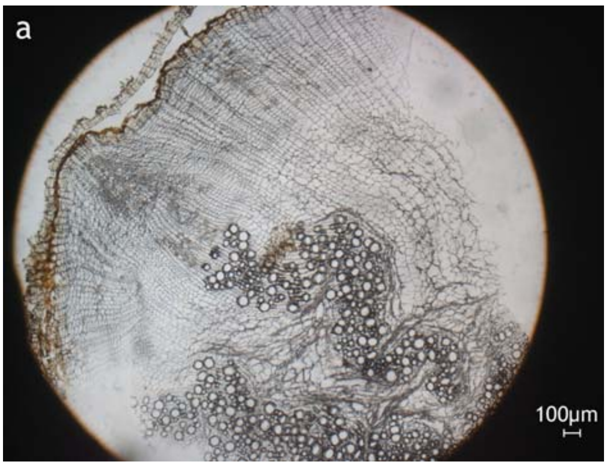
endoSDs, further secretory ducts missing;

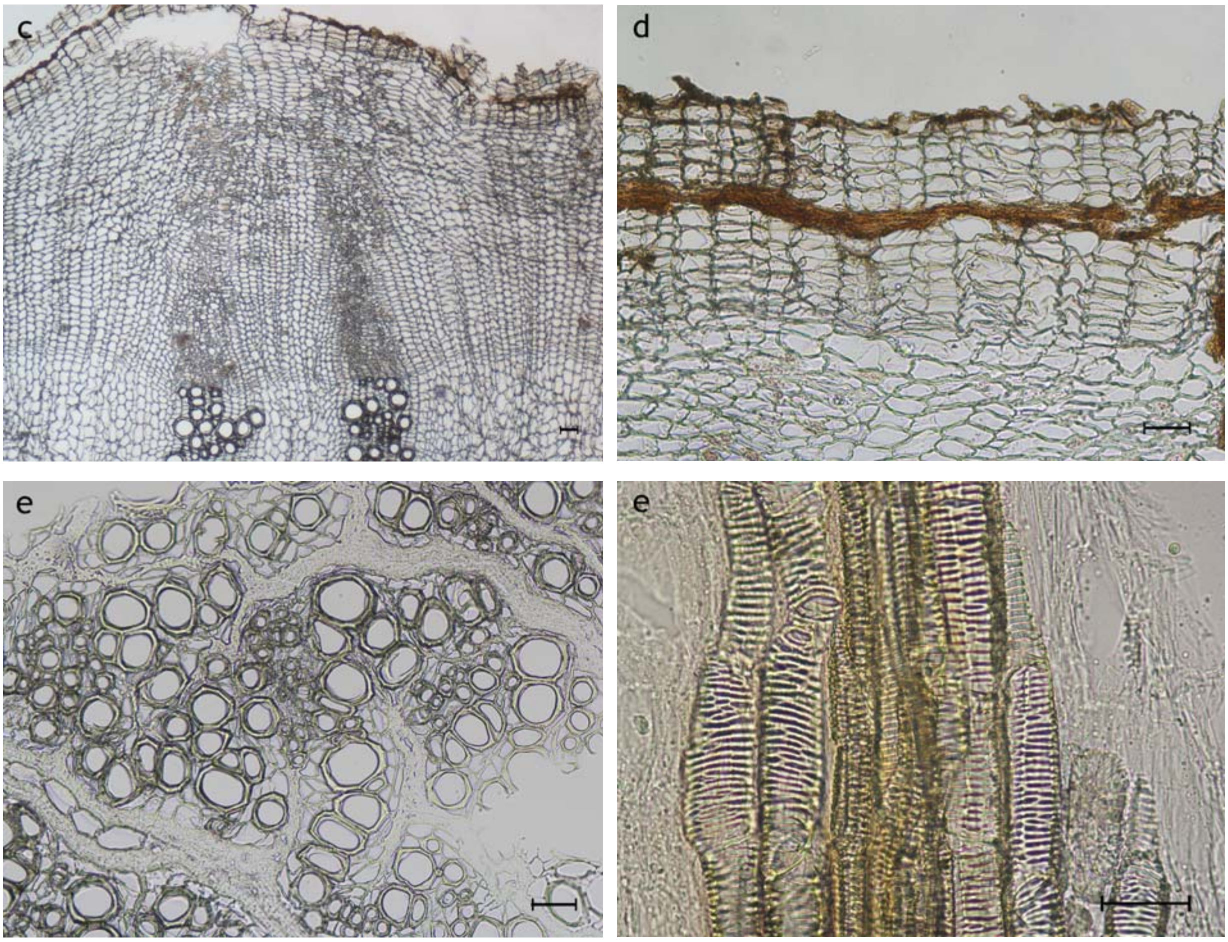
sclereids missing;

fibers in secondary phloem and secondary xylem missing;

a: Taproot of *Lactuca perennis*, habitus (M01-07)
b: Schematic view of *L.* perennis root (transverse section): small ellipses mark the laticifers of the sec. phloem, cortex missing (formation of rhytidome); black points represent the vessels







Lactuca perennis root: **a:** Overview showing the extension and arrangement of tissues: cortex missing due to formation of rhytidome, secondary phloem of lesser radial extension than the vascular cylinder, secondary xylem with vessels arranged in multiple, winded rows (LPAST14B); **b:** secondary xylem with multiple rows, medullary rays multiseriate, tissues ripped apart (LPAST14A); **c:** secondary phloem with laticiferous vessels arranged in rows and irregularly dispersed (LPAST14B); **d:** rhytidome: phellem of thinwalled cells, thoroughly laminated, in between obliterated dead cells (LPAST14A); **e:** secondary xylem with vessels in winded multiple rows and medullary rays with obliterated ray cells (LPAST14B); **f:** reticulate vessels (LPAST14A)
a-e: transverse sections; f: longitudinal section

LACTUCA VIROSA L.

perennial hemicryptophyte;

allorhizous;

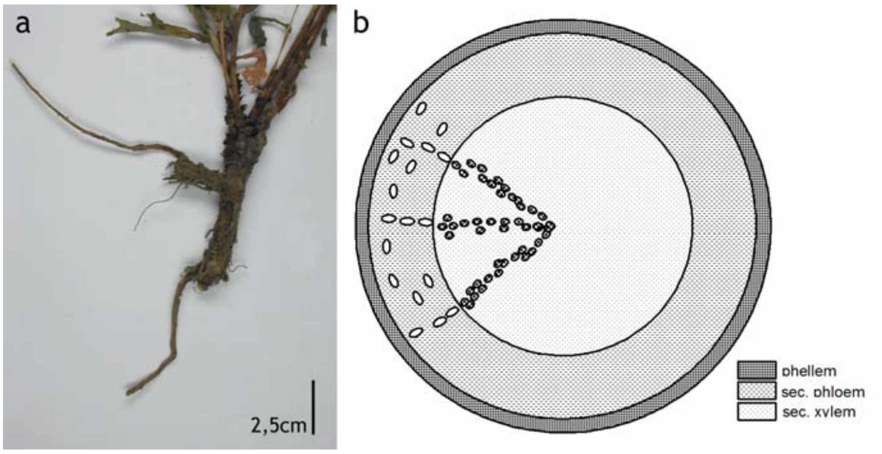
secondary root: phellem, thin-walled, sometimes with crystalloids; **cortex** lost in course of rhytidome formation; **secondary phloem** broad, but of lesser radial extension than the vascular cylinder, with laticiferous vessels in rows, also irregularly dispersed; **secondary xylem** with fibers, vessels arranged in single to double rows, reticulate thickening, simply and strongly bordered pits, mainly simply bordered, up to 93µm in diameter; **medullary rays** multiseriate, often ripped apart; **pith** missing;

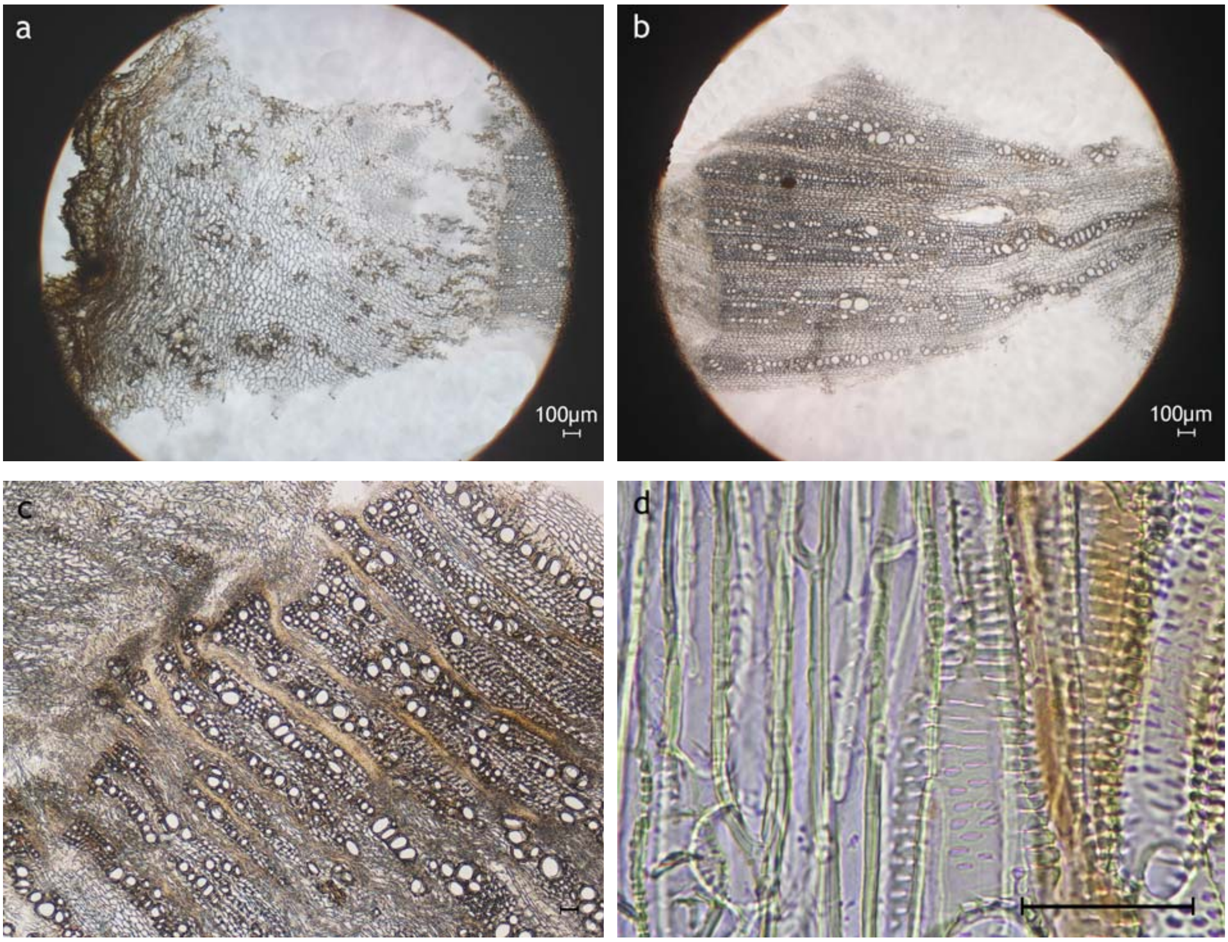
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem missing;

a: Root of *Lactuca virosa*, habitus (LV02-07)
b: Schematic view of *L. virosa* root (transverse section): small ellipses mark the laticifers of the phloem; cortex missing (formation of rhytidome); black points represent the vessels





Lactuca virosa root: **a:** Overview showing the extension and arrangement of tissues: cortex missing due to formation of rhytidome, secondary phloem of lesser radial extension than the vascular cylinder, secondary xylem with vessels arranged in multiple, winded rows (LV02-07); **b:** secondary xylem with multiple rows, medullary rays multiseriate, tissues ripped apart (LV02-07); **c:** secondary xylem with vessels in winded multiple rows and medullary rays with obliterated ray cells (LV01-07); **d:** reticulate vessels (LV02-07)
a-c: transverse sections; d: longitudinal section

SUBTRIBE HYOSERIDINAE

GENUS *APOSERIS*

APOSERIS FOETIDA Cass.

perennial hemicryptophyte;

rhizome;

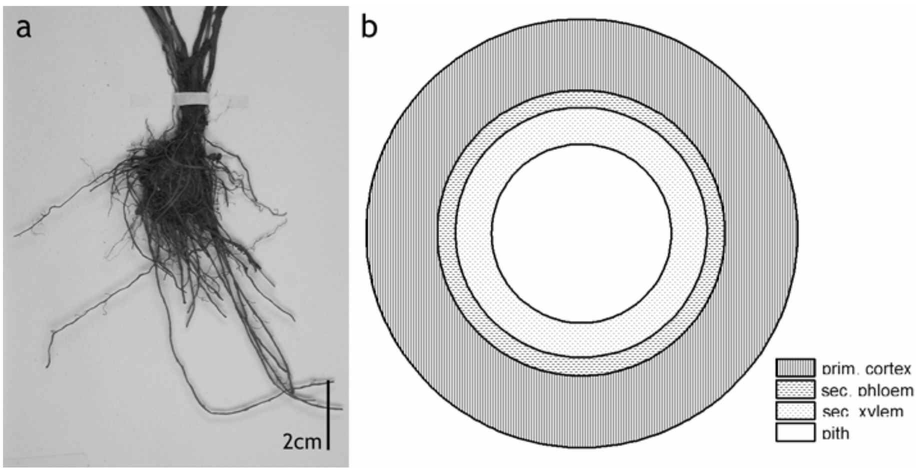
rhizome: phellem thin-walled, sometimes with crystalloids; **cortex** broad, dominating in extension (besides pith), enduring; **secondary phloem** small, comparable in extension to the vascular cylinder, with laticiferous vessels irregularly dispersed; **secondary xylem** with vessels irregularly dispersed; vessels mainly reticulate, also simply bordered, up to 45µm in diameter; **medullary rays** broad, multiseriate; **pith** wide in extension, cells with tuberous thickened walls;

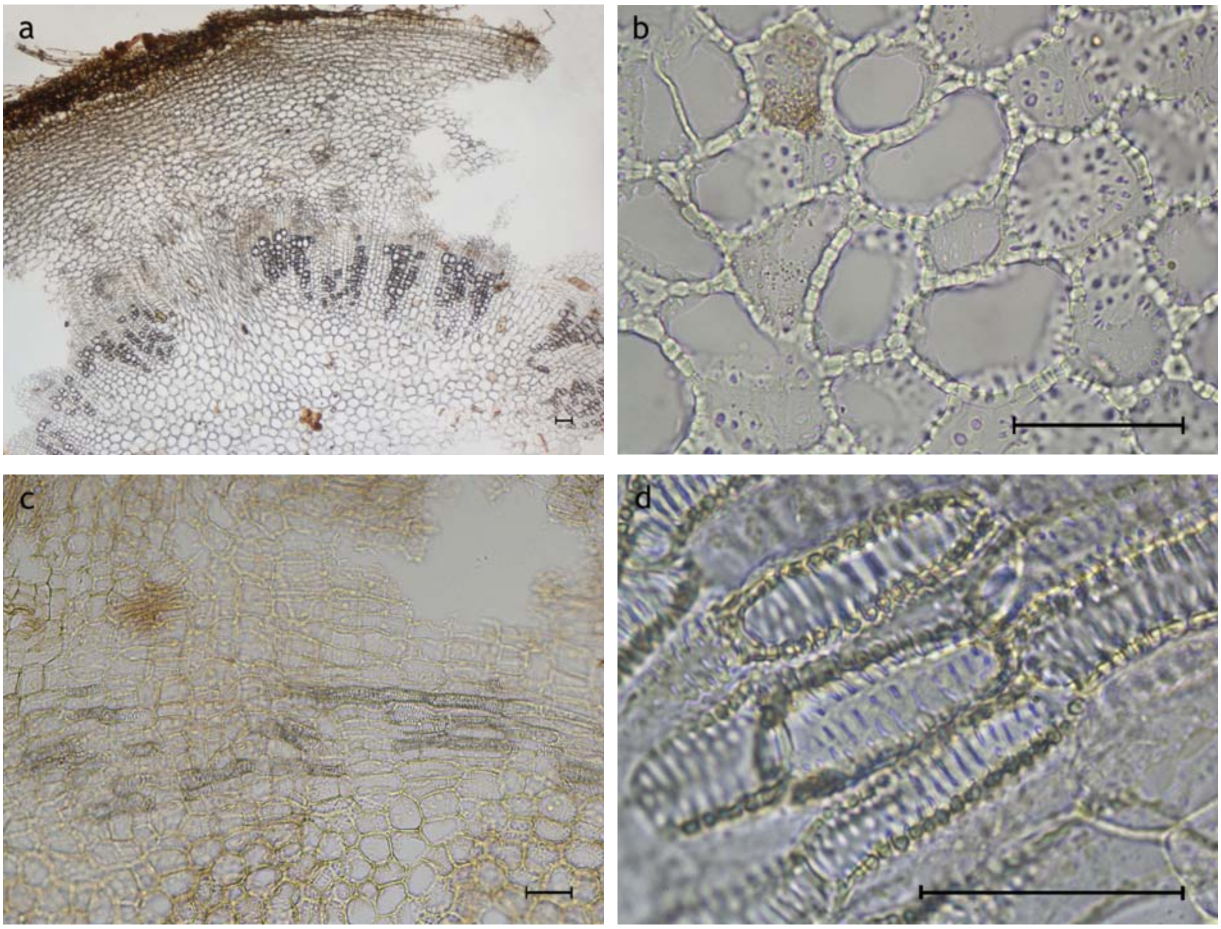
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem and secondary xylem missing;

a: Rhizome of *Aposeris foetida*, habitus (AF1)
b: Schematic view of *A. foetida* rhizome in transverse section: broad cortex enduring, vascular bundles with pith dominating, laticifers within the small secondary phloem irregularly dispersed





Aposeris foetida rhizome: a: Overview showing the extension and arrangement of tissues: broad cortex, vascular cylinder with pith dominating, laticiferous vessels within the small secondary phloem irregularly dispersed (AST19); b: pith with tuberous thickened cell walls (AF1); c: overview showing the extension and arrangement of tissues: short vessels, not straight (AST23); d: reticulate and simply bordered vessels (AST23)
a,b: transverse sections; c,d: longitudinal sections;

SONCHUS OLERACEUS L.

annual therophyte;

allorhizous with taproot;

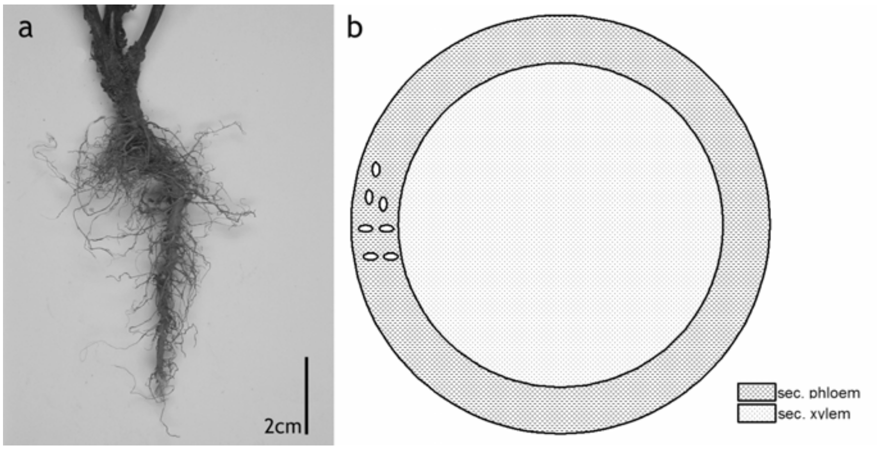
secondary root: phellem thinwalled; **cortex** lost in course of rhytidome formation; **secondary phloem** usually of lesser radial extension than the vascular cylinder, with laticiferous vessels either arranged in rows or irregularly dispersed; **secondary xylem** with fibers, vessels either arranged in rows or irregularly dispersed; vessels mainly weakly bordered, also strongly bordered, up to 70µm in diameter; **medullary rays** multiseriate, up to 5 cells in a row, ray cells may have thickened cell walls, medullary rays sometimes bad visible; **pith** missing;

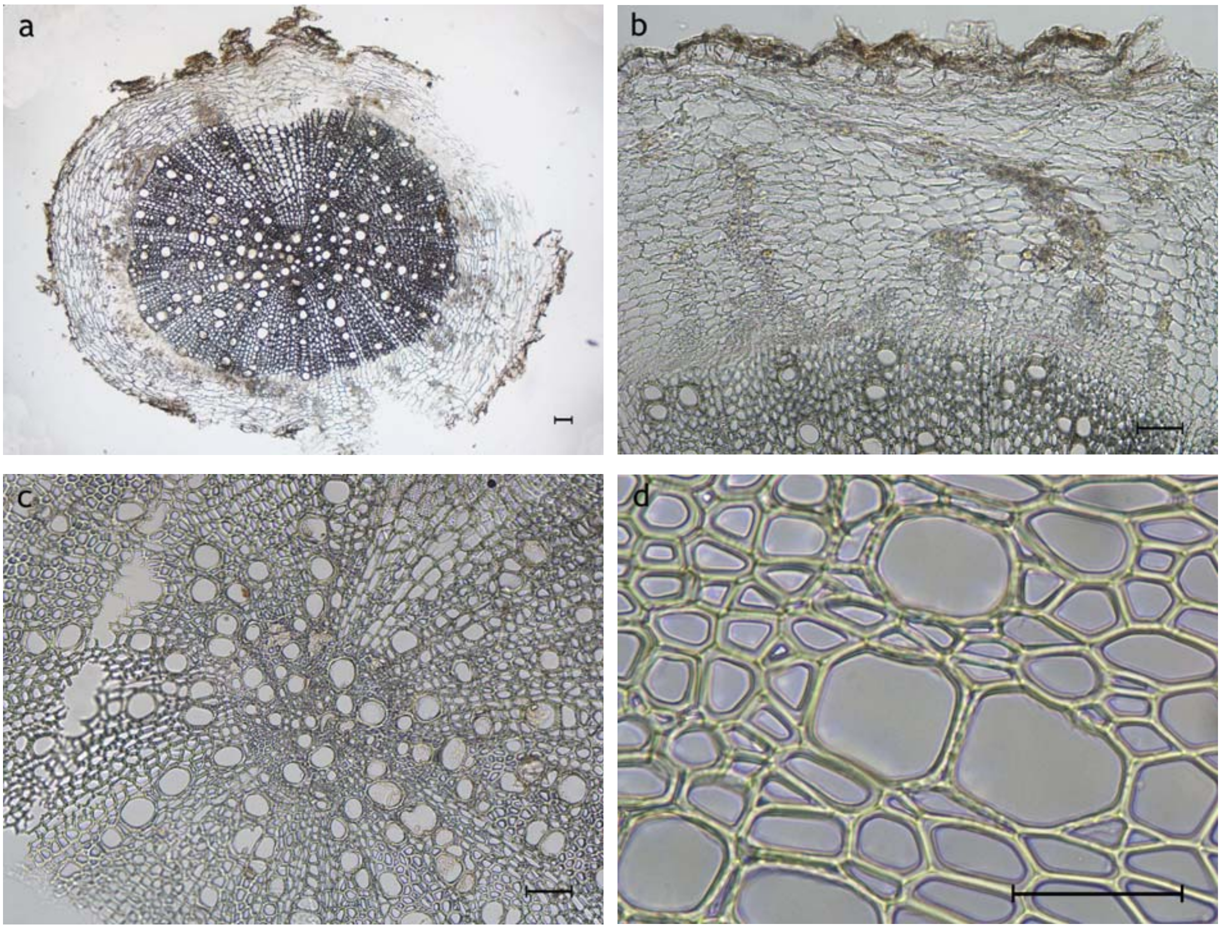
endoSDs, further secretory ducts missing;

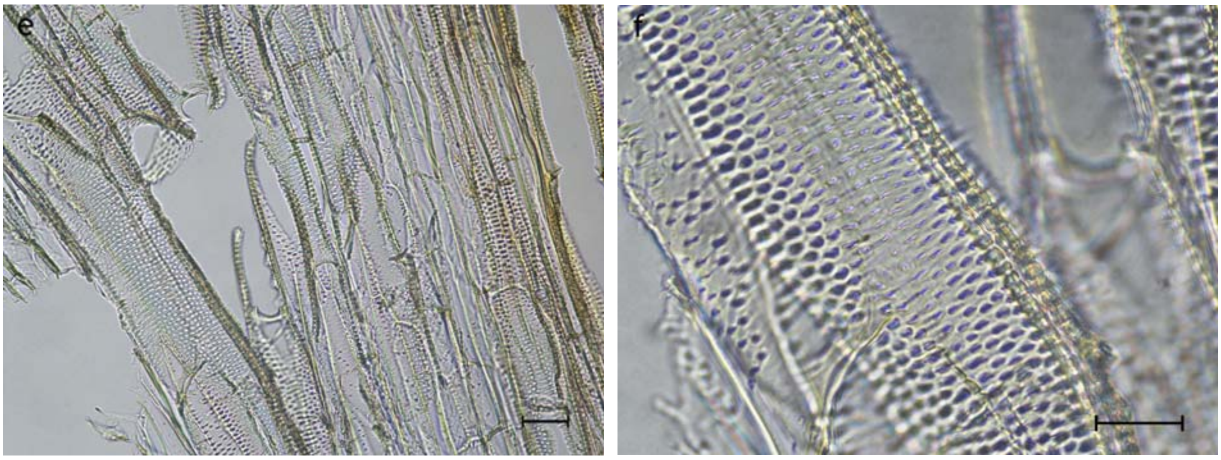
crystalloids, sclereids missing;

fibers in secondary phloem missing;

a: Taproot of *Sonchus oleraceus*, habitus (SO02-07B)
b: Schematic view of *S. oleraceus* root in transverse section: secondary root: small ellipses mark the position of the laticifers of the broad sec. phloem, broad sec. xylem







Sonchus oleraceus root: Overview showing the extension and arrangement of tissues: the secondary phloem is of lesser radial extension than the vascular cylinder, secondary xylem with vessels irregularly dispersed (SO01-07); b: laticiferous vessels within secondary phloem arranged in rows (AST18); c: center of secondary xylem with vessels dispersed and fibers (SO01-07); d: secondary xylem with vessels (SO02-07B); e: overview over secondary xylem (SO02-08B); f: strongly bordered vessels (SO02-08B)
a-c: transverse sections; d-f: longitudinal sections;

SUBTRIBE CREPIDINAE

GENUS *CREPIS*

CREPIS BIENNIS L.

perennial hemicryptophyte;

fibrous root system;

secondary root: phellem thinwanded; **cortex** lost in course of rhytidome formation; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with laticiferous vessels circularly arranged relative to the centre of the xylem in fascicularly positioned; **secondary xylem** with vessels either irregularly dispersed or arranged in groups or rows and fibers; vessels reticulate and weakly bordered, up to 82µm in diameter; **medullary rays** bi- and multiseriate; **pith** missing;

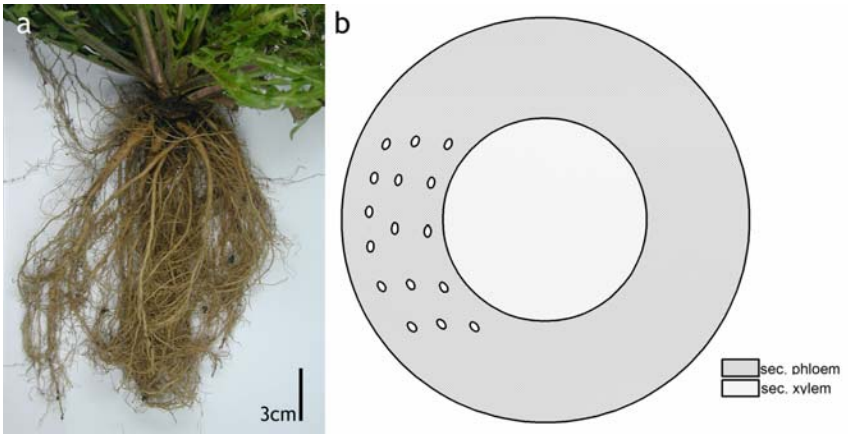
endoSDs, further secretory ducts missing;

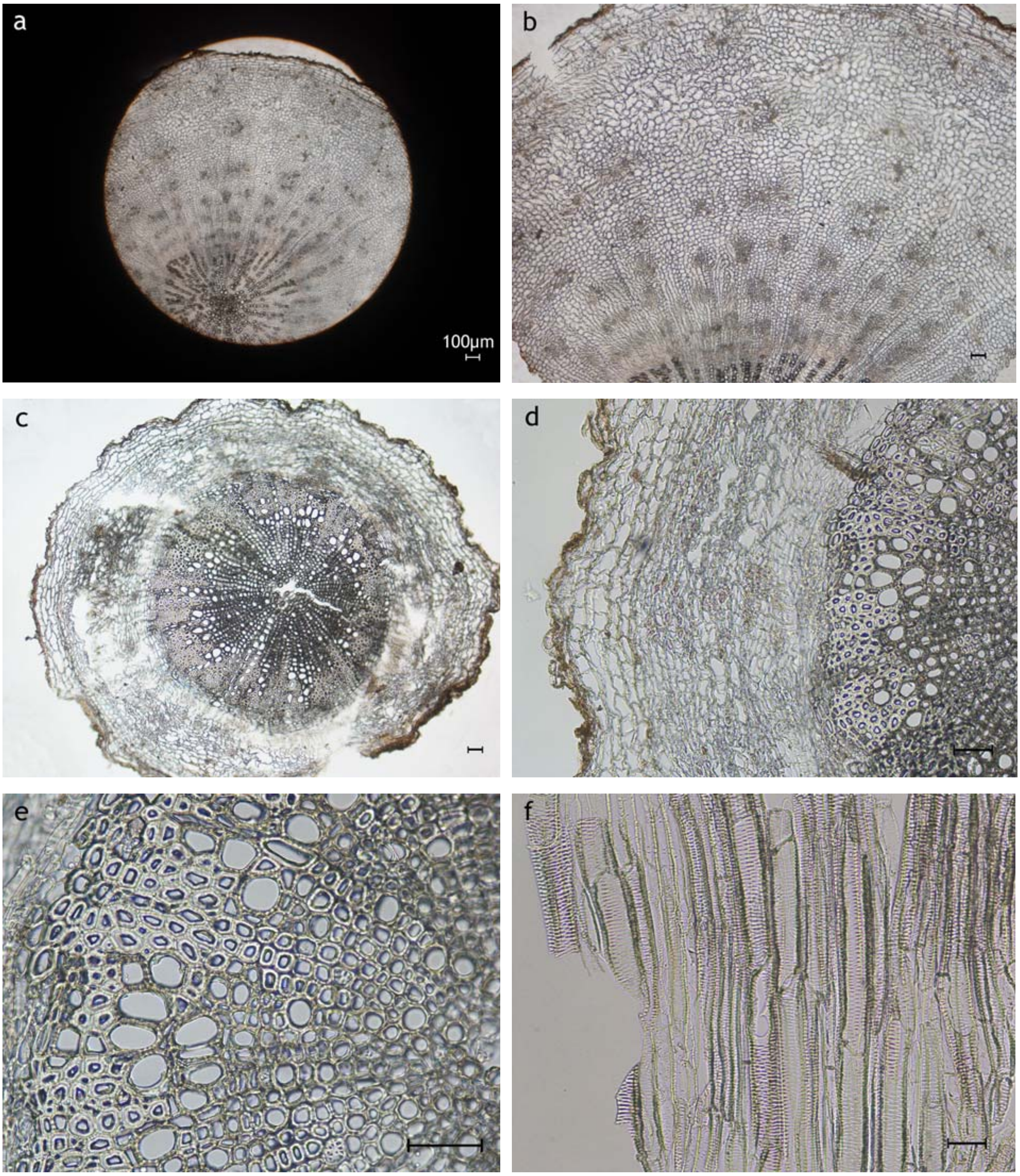
crystalloids and sclereids missing;

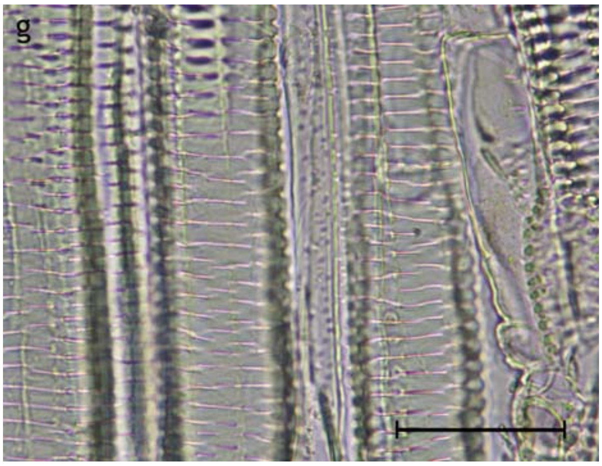
fibers in secondary phloem missing;

Cultivated plants show a broader secondary phloem with far less thickened fibers within the xylem.

a: Fibrous root system root of *Crepis biennis*, habitus (70S4)
b: Schematic view of *C. biennis* root (transverse section): sec. root: small ellipses mark the position of the laticifers of the dominating sec. phloem, sec. xylem with vessels dispersed;







Crepis biennis root: **a,b:** cultivated plant: Overview showing the extension and arrangement of tissues: dominant sec. phloem with fibers missing, laticiferous vessels within sec. phloem circularly arranged, vessels of sec. xylem in rows (a) (70S4); broad sec. phloem with laticifers circularly arranged (b) (70S4); **c-g:** wild plant: overview showing the extension and arrangement of tissues: small cortex, sec. phloem in extension almost comparable to sec. xylem, vessels of sec. xylem irregularly dispersed (c) (AST5), cortex and sec. phloem with laticifers (AST5) (d), sec. xylem with vessels irregularly dispersed, mainly embedded in fibers; medullary rays almost invisible (AST5) (e), Overview of the sec. xylem showing the arrangement of vessels and fibers (CB01-08) (f), reticulate vessels (CB01-08) (g)
a-e: transverse sections; f,g: longitudinal sections;

CREPIS AUREA Rchb.

perennial hemicryptophyte;

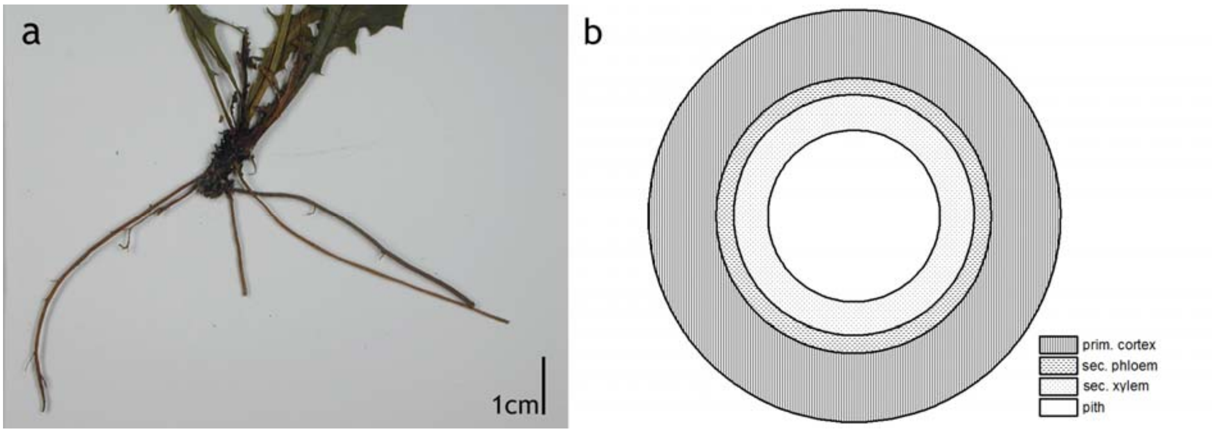
rhizome;

rhizome: phellem thinwanded; **cortex** dominating, enduring; **secondary phloem** small with laticiferous vessels dispersed; **secondary xylem** with parenchymatous cells with sclereidal thickening and sclereids – thickness of cell walls gaining towards the pith; vessels irregularly dispersed over the transverse section, reticulate and weakly bordered, up to 30µm in diameter; **medullary rays** multiseriate; **pith** with lightly and irregularly thickened cell walls;

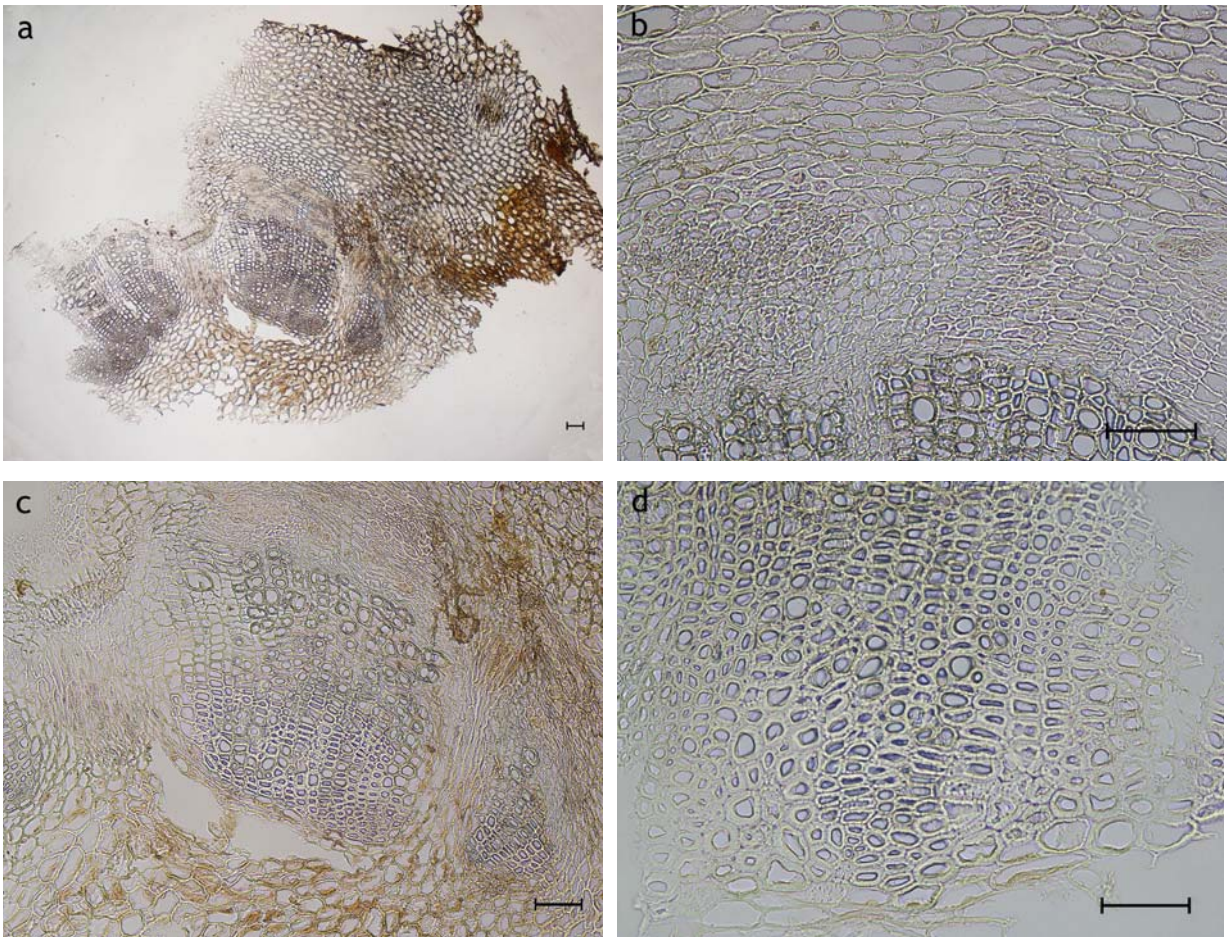
endoSDs, further secretory ducts missing;

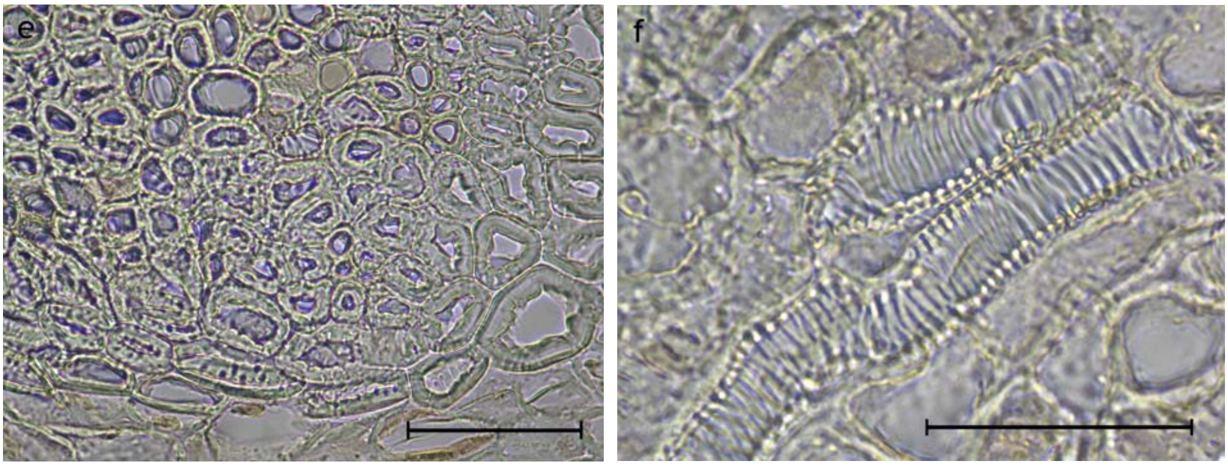
crystalloids missing;

fibers in secondary phloem and secondary xylem missing;



a: Rhizome of *Crepis aurea*, habitus (CrA04-08)
b: Schematic view of *C. aurea* rhizome in transverse section: cortex dominating, laticifers dispersed within small secondary phloem, large pith





Crepis aurea rhizome: **a:** Overview showing the extension and arrangement of tissues: cortex dominating, vascular bundles with small secondary phloem, broad pith (CrA04-08); **b:** secondary phloem with laticiferous vessels irregularly dispersed (CrA03-08); **c:** vascular bundle with sclereidal thickening of cell walls (CrA04-08); **d:** sclereidal thickening of cells of the vascular bundle, thickness of cell walls gaining towards the pith (CrA01-08); **e:** sclereids of the secondary xylem (CrA02-08); **f:** reticulate vessels (CrA01-08)
a-e: transverse sections; f: longitudinal section;

CREPIS PONTANA Druce

perennial hemicryptophyte;

allorhizous, with taproot;

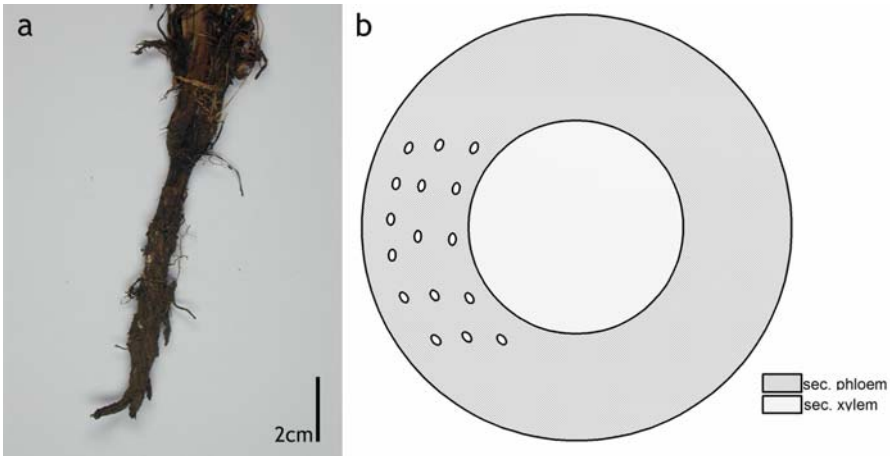
secondary root: phellem thin-walled; **cortex** lost in course of rhytidome formation; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with laticiferous vessels arranged in concentric circles fascicularly positioned; **secondary xylem** with vessels irregularly dispersed over the transverse section, getting larger in diameter towards the outside; vessels reticulate, up to 68µm in diameter; **medullary rays** broad, multiseriate; **pith** missing;

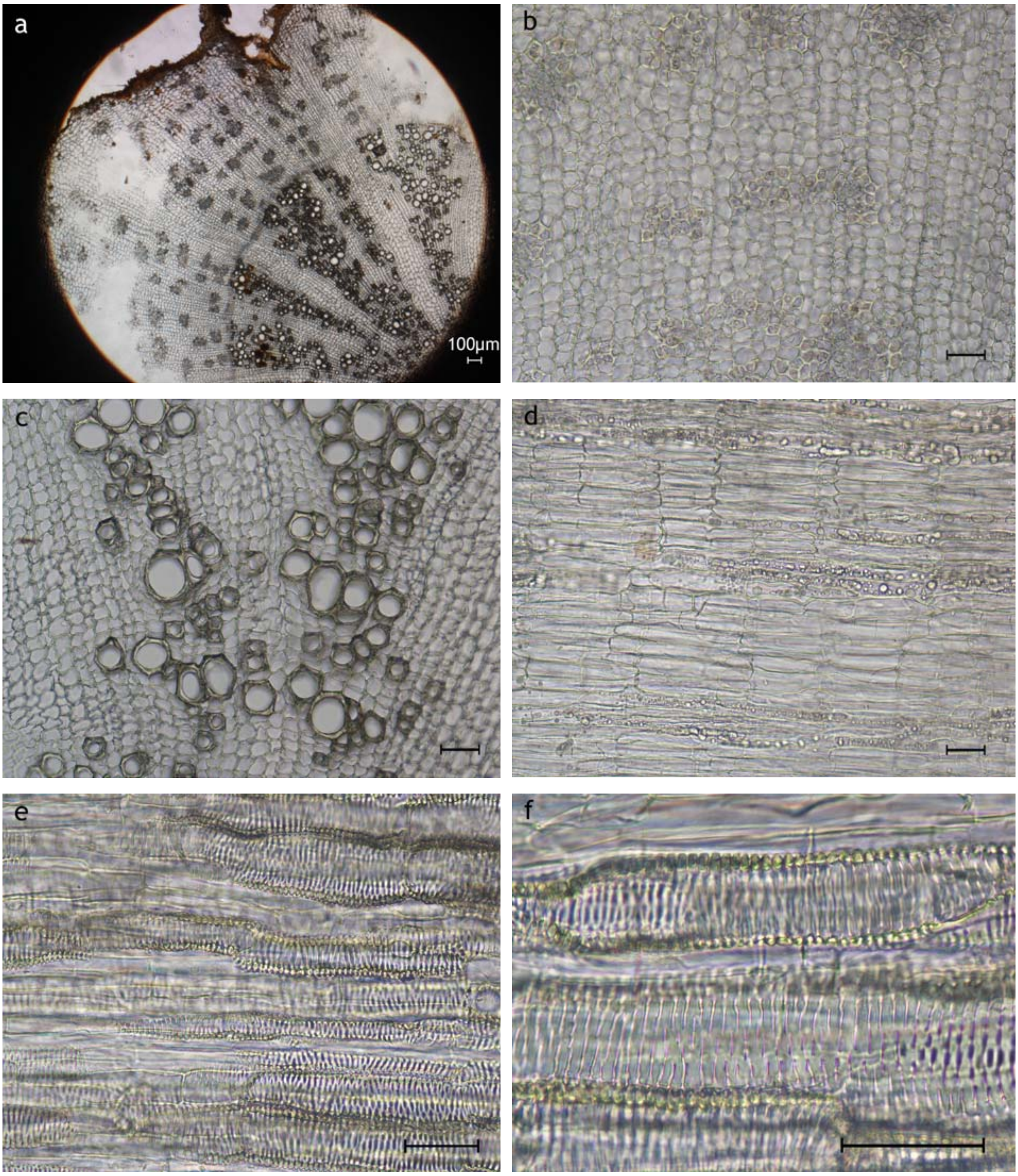
endoSDs, further secretory ducts missing;

crystalloids and sclereids missing;

fibers in secondary phloem and secondary xylem missing;

a: Taproot of *Crepis pontana,* habitus (CP04-09)
b: Schematic view of *C. pontana* root (transverse section): small ellipses mark the laticifers of the broad secondary phloem, comparable in extension to the vascular cylinder





Crepis pontana root: **a:** Overview showing the extension and arrangement of tissues: the secondary phloem is comparable in extension to the vascular cylinder, fibers missing, laticiferous vessels within secondary phloem arranged in concentric circles, broad medullary rays (CP03-09); **b:** laticiferous vessels of the secondary phloem (CP02-09); **c:** vessels of secondary xylem irregularly dispersed (CP01-09); **d:** laticiferous vessels within the secondary phloem (CP04-09); **e:** overview of the secondary xylem showing the arrangement of vessels and fibers (CP02-09); **f:** reticulate vessels (CP02-09)
a-c: transverse sections; d-f: longitudinal sections;

GENUS *LAPSANA*

LAPSANA COMMUNIS L.

perennial hemicryptophyte;

allorhizous, with taproot;

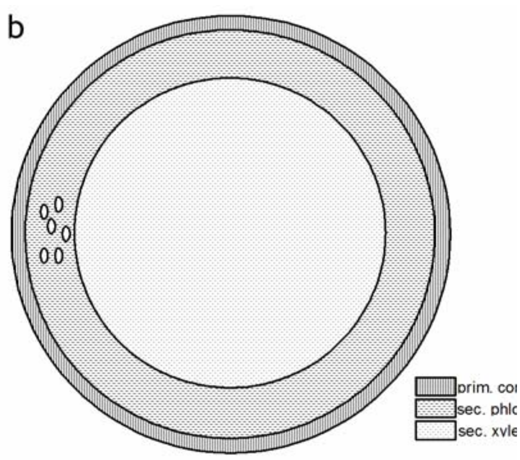
secondary root: small **phellem** thin-walled; **cortex** enduring; **secondary phloem** small, with laticiferous vessels irregularly dispersed; **secondary xylem** dominated by fibers, few vessels arranged in short single rows or irregularly dispersed over the transverse section; vessels reticulate and weakly bordered up to 52µm in diameter; **medullary rays** mainly uniseriate; **pith** missing;

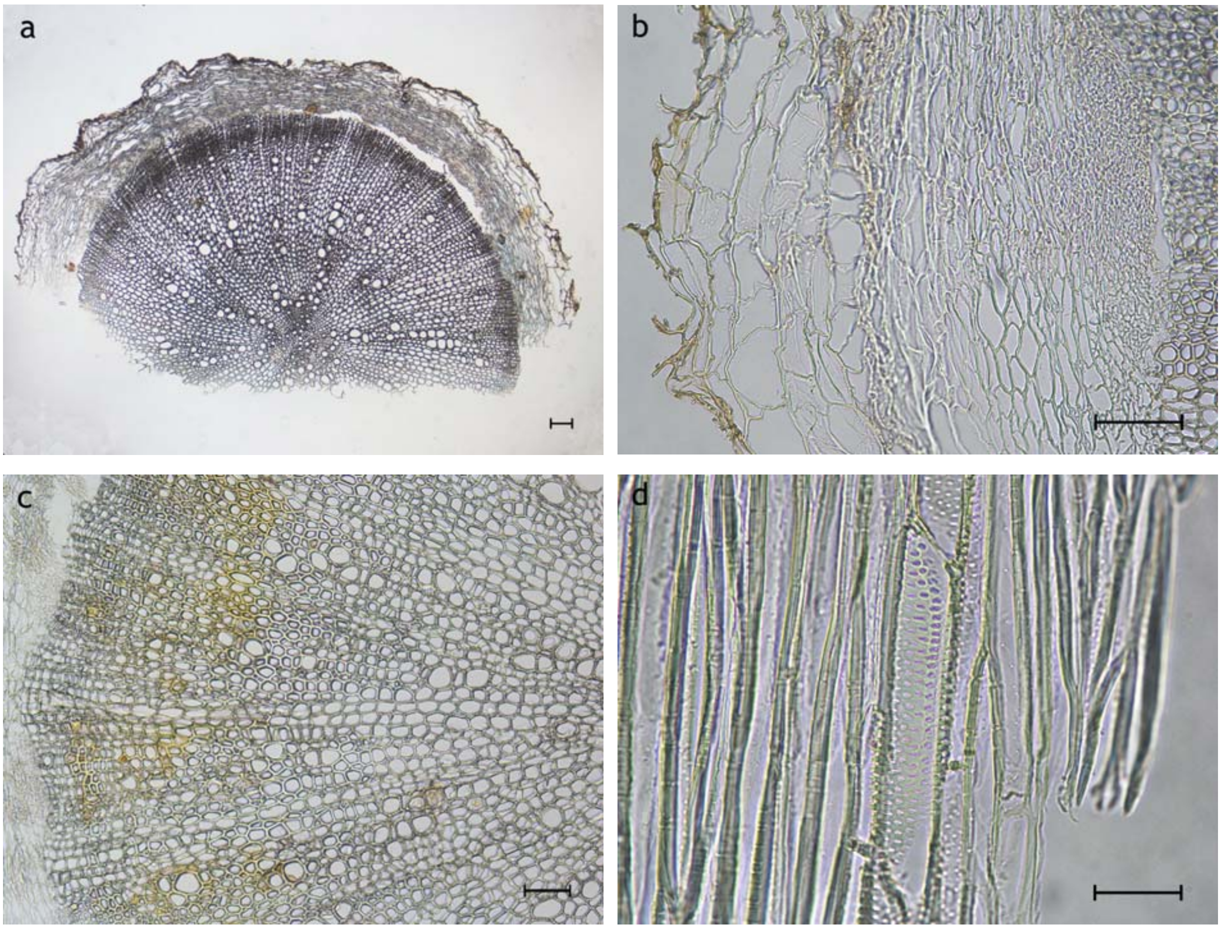
endoSDs, further secretory ducts missing;

crystalloids and sclereids missing;

fibers in secondary phloem missing (in lateral roots possible);

a: Taproot of *Lapsana communis*, habitus (LC02-08)
b: Schematic view of *L. communis* root (transverse section): sec. root: small ellipses mark the position of the laticifers of the small sec. phloem, cortex enduring, sec. xylem dominating





Lapsana communis root: **a:** Overview showing the extension and arrangement of tissues: cortex and secondary phloem small, secondary xylem dominating, relatively few vessels irregularly dispersed (AST17); **b:** durable cortex, small secondary phloem with laticiferous vessels irregularly dispersed (LC02-08B); **c:** secondary xylem with few vessels dispersed over transverse section, many fibers, medullary rays mainly uniseriate (LC02-08A); **d:** fibers and vessels of secondary xylem (LC01-08)
a-c: transverse sections; d: longitudinal section;

GENUS *TARAXACUM*

TARAXACUM CUCULLATUM Dahlst. / *T. LAEVIGATUM* DC. (cf. Paper IV)

The following description applies to both species.

perennial hemicryptophyte;

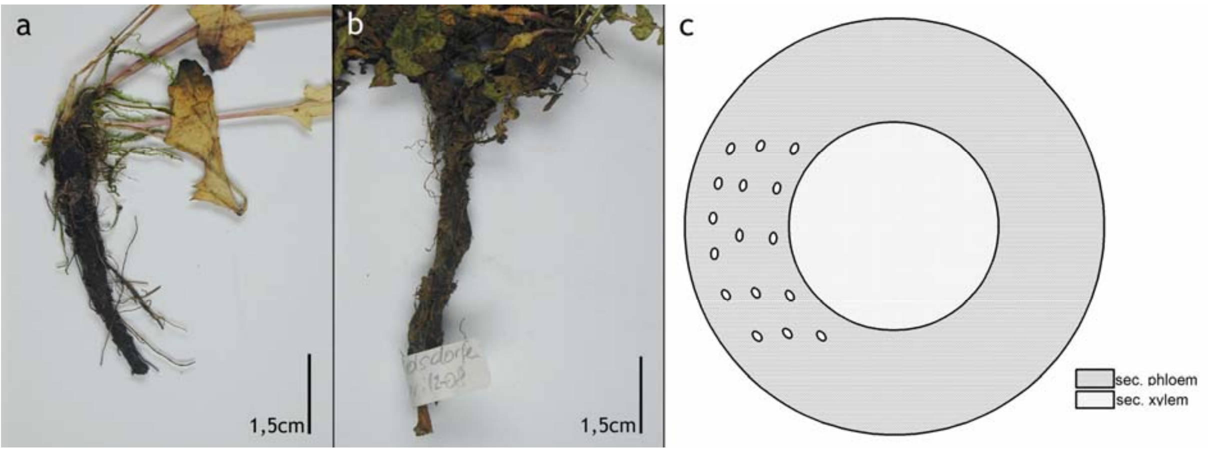
allorhizous, with taproot;

secondary root: phellem thin-walled; **cortex** lost due to formation of rhytidome; **secondary phloem** dominant, with laticiferous vessels arranged in concentric circles; **secondary xylem** with vessels irregularly arranged; vessels reticulate, up to 50µm (*T. cucullatum*) / 61µm (*T. laevigatum*) in diameter; **medullary rays** not existing; **pith** missing;

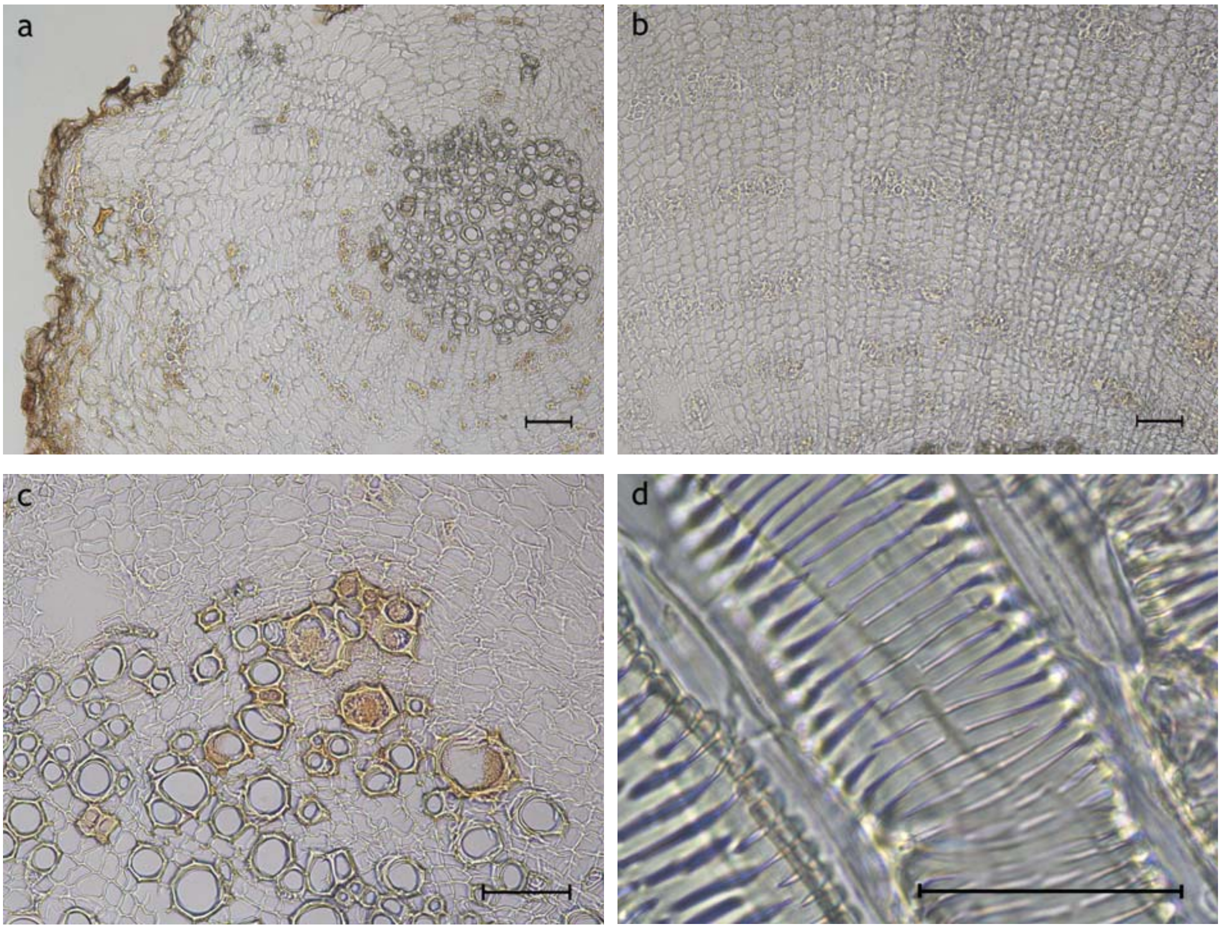
endoSDs, further secretory ducts missing;

crystalloids and sclereids missing;

fibers in secondary phloem and secondary xylem missing;

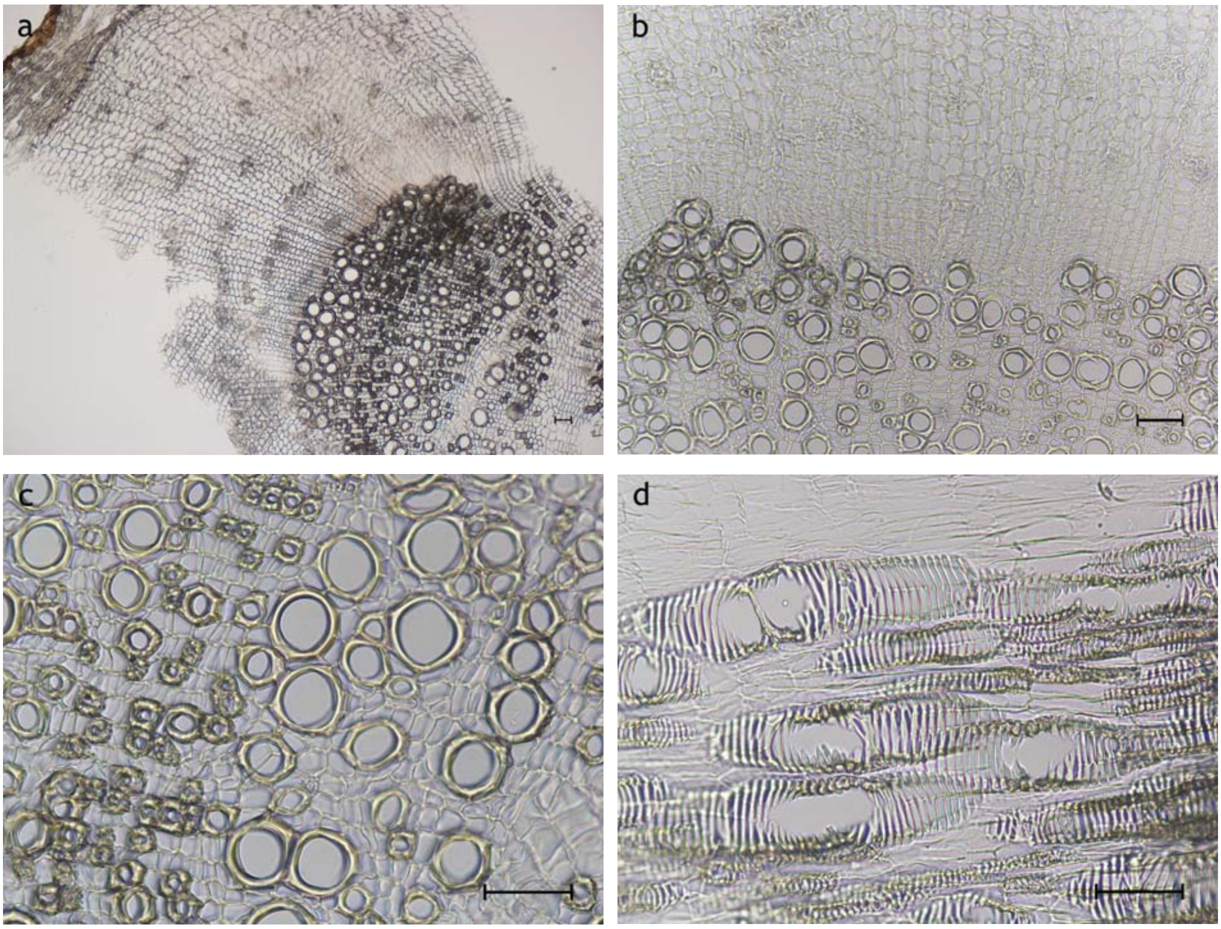


a: Taproot of *Taraxacum cucullatum*, habitus; b: Taproot of *Taraxacum laevigatum*, habitus; c: schematic view of *Taraxacum* sp. root in transverse section: secondary root: small ellipses mark the position of the laticifers of the dominating sec. phloem, sec. xylem with vessels dispersed; medullary rays missing;



Taraxacum cucullatum root: **a:** Overview showing the extension and arrangement of tissues: cortex lost due to formation of rhytidome, secondary phloem dominating, laticiferous vessels within secondary phloem circularly arranged (TO01-08); **b:** laticiferous vessels arranged in concentric circles within

secondary phloem (TC03-08); **c:** secondary xylem with vessels irregularly dispersed, fibers missing (TO03-08); **d:** reticulate vessels (TO03-08)
a-c: transverse sections; d: longitudinal section;



Taraxacum laevigatum root: **a:** Overview showing the extension and arrangement of tissues: cortex lost due to formation of rhytidome, secondary phloem dominating, laticiferous vessels within secondary phloem circularly arranged (TL01-08); **b:** overview showing secondary phloem, cambium and secondary xylem – vessels irregularly dispersed over transverse section, fibers missing (TL03-08); **c:** secondary xylem with vessels irregularly dispersed over transverse section, fibers missing (TL01-08); **d:** reticulate vessels of secondary xylem (TL03-08)
a-c: transverse sections; d: longitudinal section;

SUBTRIBE CHONDRILLINAE

GENUS *CHONDRILLA*

CHONDRILLA JUNCEA L.

perennial hemicryptophyte;

rhizome;

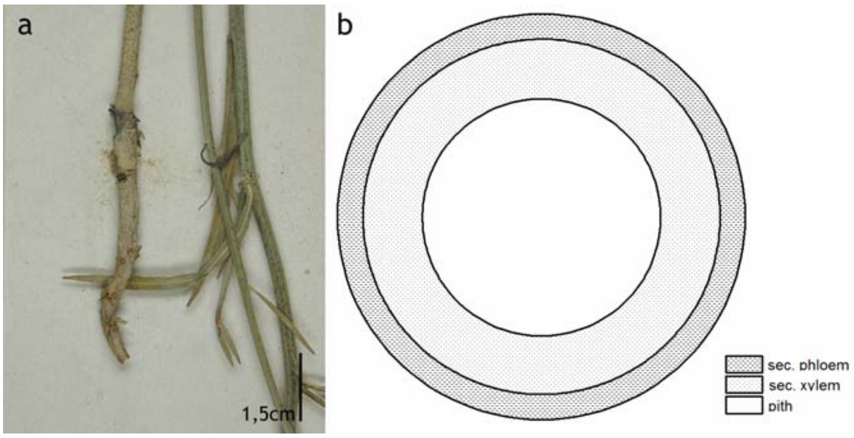
rhizome with secondary growth: small **cortex** either enduring or rather lost/collaborated in course of the formation of a thin-walles phellem **; secondary phloem** small, with laticiferous vessels dispersed; **secondary xylem** relatively few vessels irregularly dispersed, mainly embedded in fibers, weakly bordered, up to 79µm in diameter; **medullary rays** multiseriate; large **pith** of thick-wanded and pitted cells, triangular intercellulars;

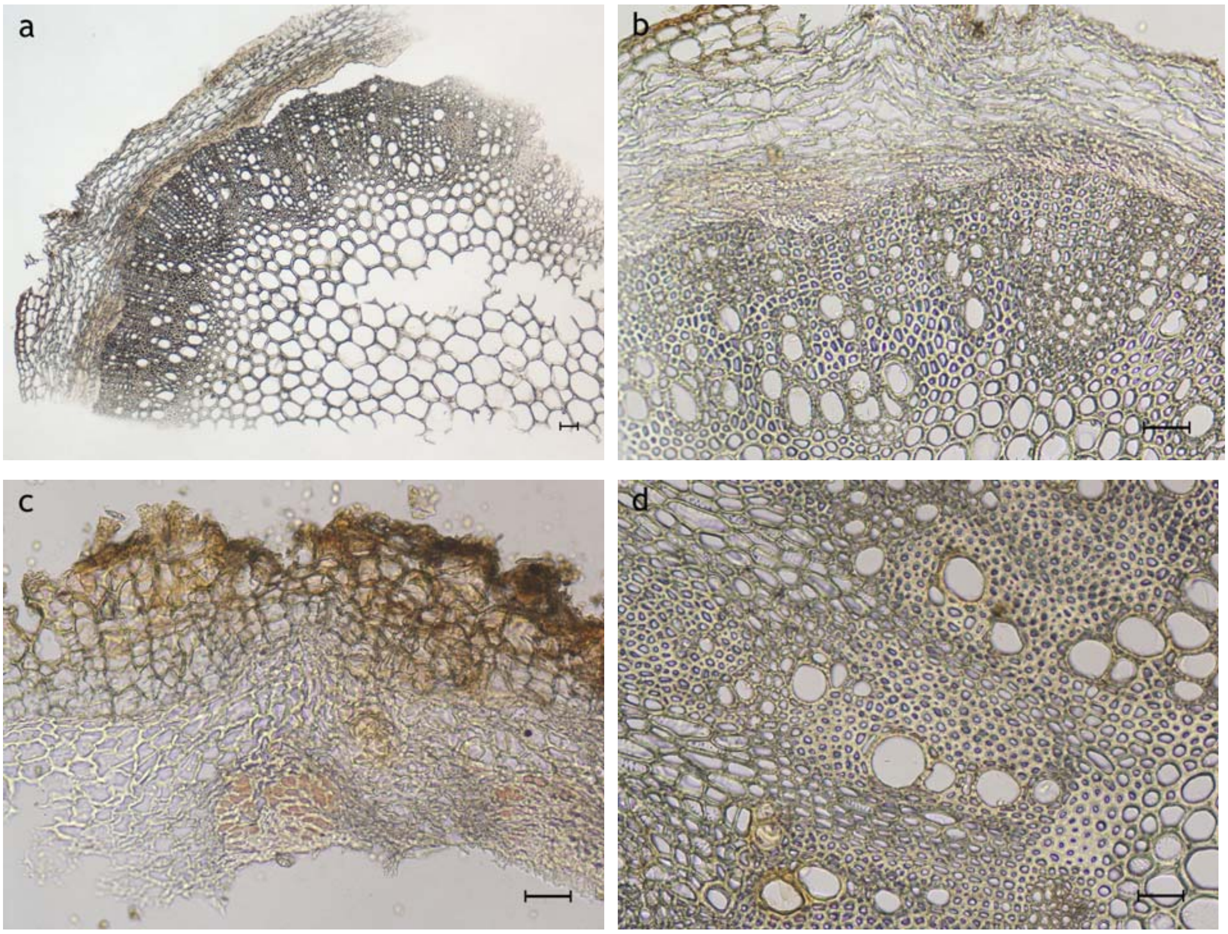
endoSDs, further secretory ducts missing;

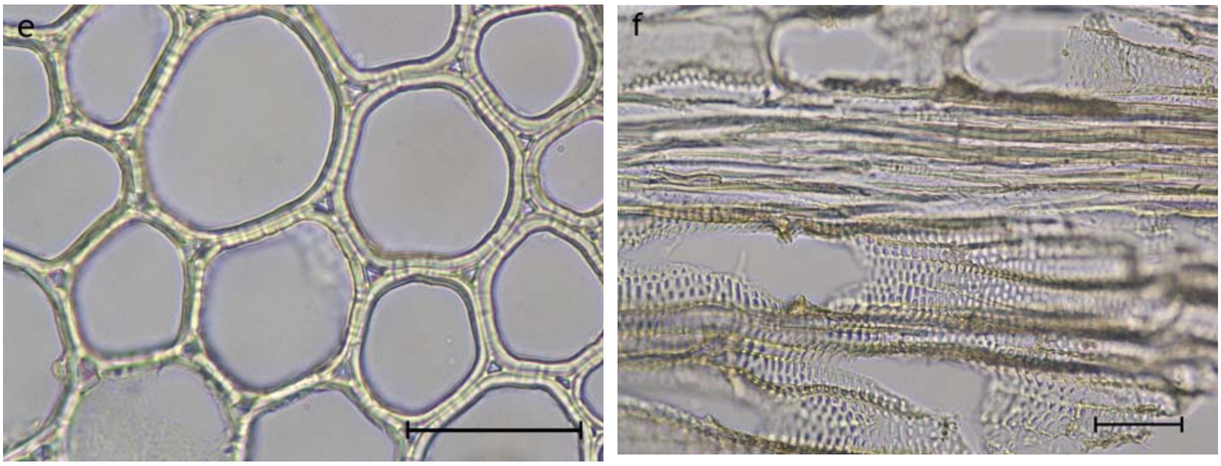
crystalloids, sclereids missing;

fibers in secondary phloem missing;

a: Rhizome of *Chondrilla juncea* habitus (CJ02)
b: Schematic view of *C. juncea* rhizome (transverse section): cortex lost (rhytidome formation), small sec. phloem with laticifers dispersed, sec. xylem and pith dominating







Chondrilla juncea rhizome: **a:** Overview showing the extension and arrangement of tissues: cortex and secondary phloem of lesser radial extension than the vascular cylinder, large pith (CJ01); **b:** small secondary phloem with laticiferous vessels dispersed, vascular bundles with lots of fibers, relatively few vessels (CJ01); **c:** phellem of thin-wanded cells (CJ02); **d:** vascular bundle of rhizome with secondary growth – few vessels embedded in lots of fibers (CJ02); **e:** pith of thick-wanded and pitted cells, triangular intercellulars (CJ01); **f:** weakly bordered vessels (CJ02)
a-e: transverse sections; f: longitudinal section;

GENUS *WILLEMETIA*

WILLEMETIA STIPITATA Cass.

perennial hemicryptophyte;

rhizome;

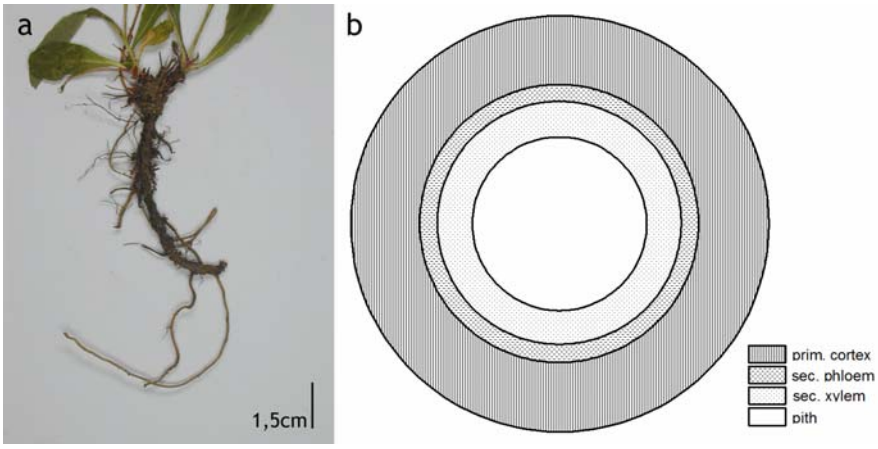
rhizome with secondary growth: cortex enduring, dominating in extension; endodermis visible even wthin older roots; **secondary phloem** small, with laticiferous vessels irregularly dispersed; **secondary xylem** with vessels irregularly dispersed, lignified fibers circularly arranged; vessels reticulate and weakly bordered, rarely strongly bordered, up to 34µm in diameter; **medullary rays** multiseriate; large **pith** of thin-wanded cells;

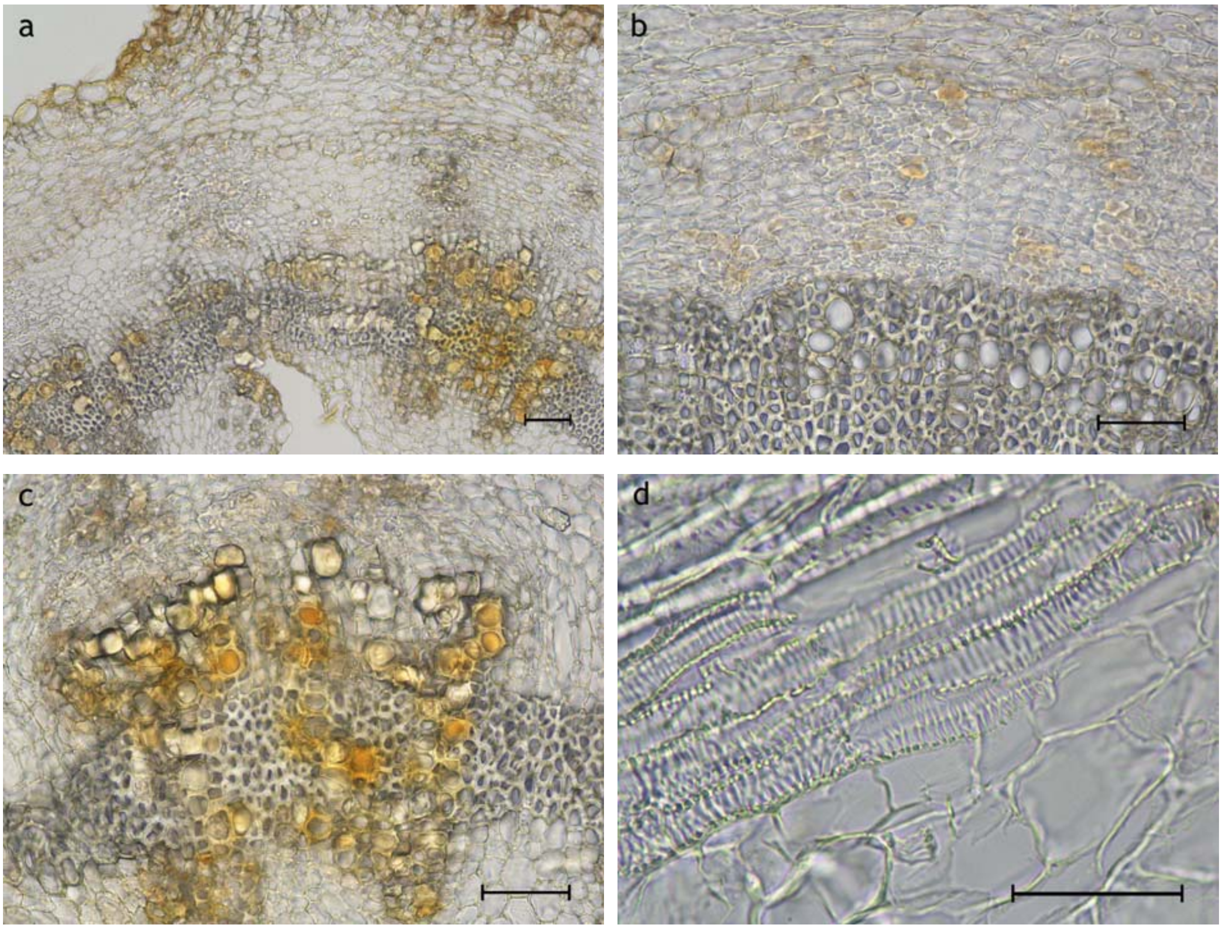
endoSDs, further secretory ducts missing;

crystalloids, sclereids missing;

fibers in secondary phloem missing;

a: Rhizome of *Willemetia stipitata*, habitus (WS03-08)
b: Schematic view of *W. stipitata* rhizome in transverse section: durable cortex dominating, small secondary phloem of lesser radial extension than the vascular cylinder, large pith





Willemetia stipitata rhizome: **a:** Overview showing the extension and arrangement of tissues: broad cortex and secondary phloem, secondary xylem with fibers WS01-08; **b:** secondary phloem with irregularly dispersed laticiferous vessels (WS02-08); **c:** vascular bundle with vessels irregularly dispersed and lignified fibers building a circular band (WS01-08); **d:** reticulate vessels of vascular bundle (WS02-07)
a-c: transverse sections; d: longitudinal section;

SUBTRIBE HYPOCHAERIDINAE

GENUS *HELMINTHOTHECA*

HELMINTHOTHECA ECHIOIDES (L.) Holub

annual to perennial therophyte - hemicryptophyte;

fibrous root system;

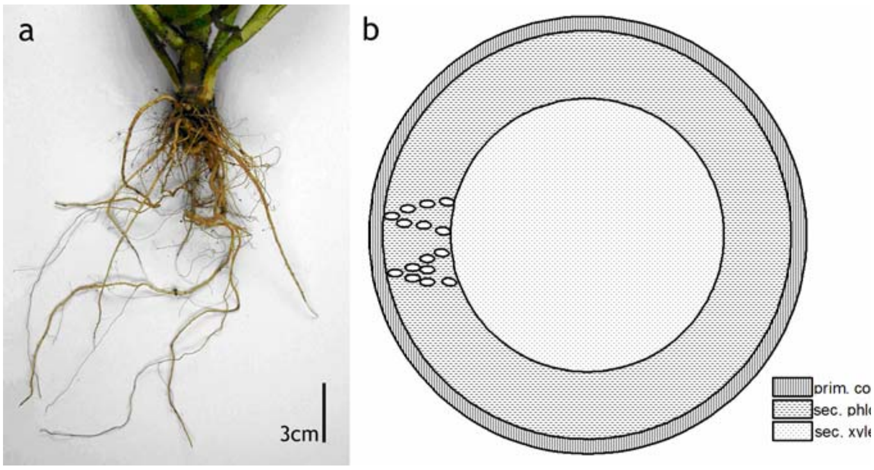
secondary root: rhizodermis; small **cortex** enduring; **secondary phloem** dominating in extension, comparable to the vascular cylinder, with laticiferous vessels mainly in rows – often two rows band together in the outer part of the phloem; **secondary xylem** with vessels irregularly dispersed, fibers; vessels reticulate, also simply and strongly bordered, up to 63µm in diameter; **medullary rays** multiseriate; **pith** missing;

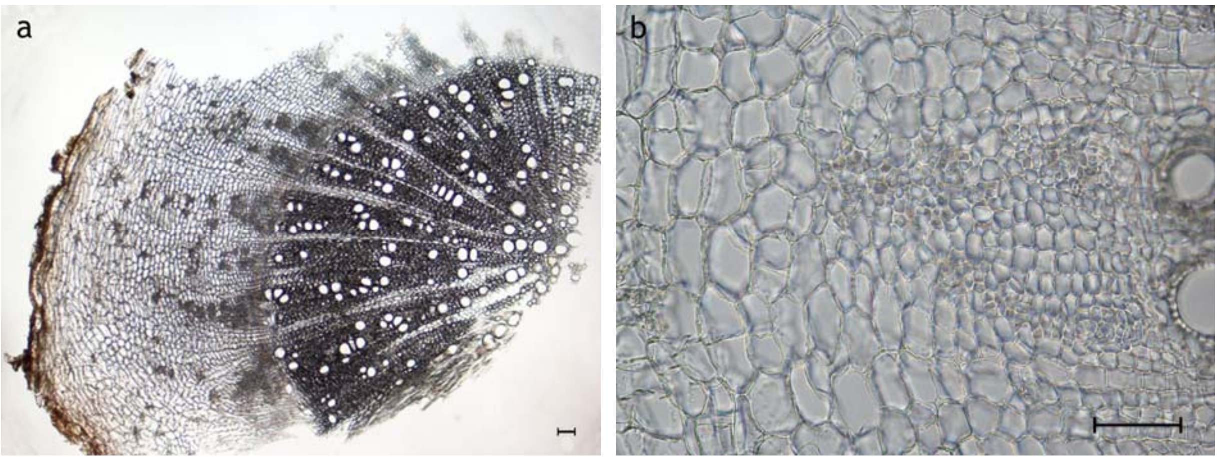
endoSDs, further secretory ducts missing;

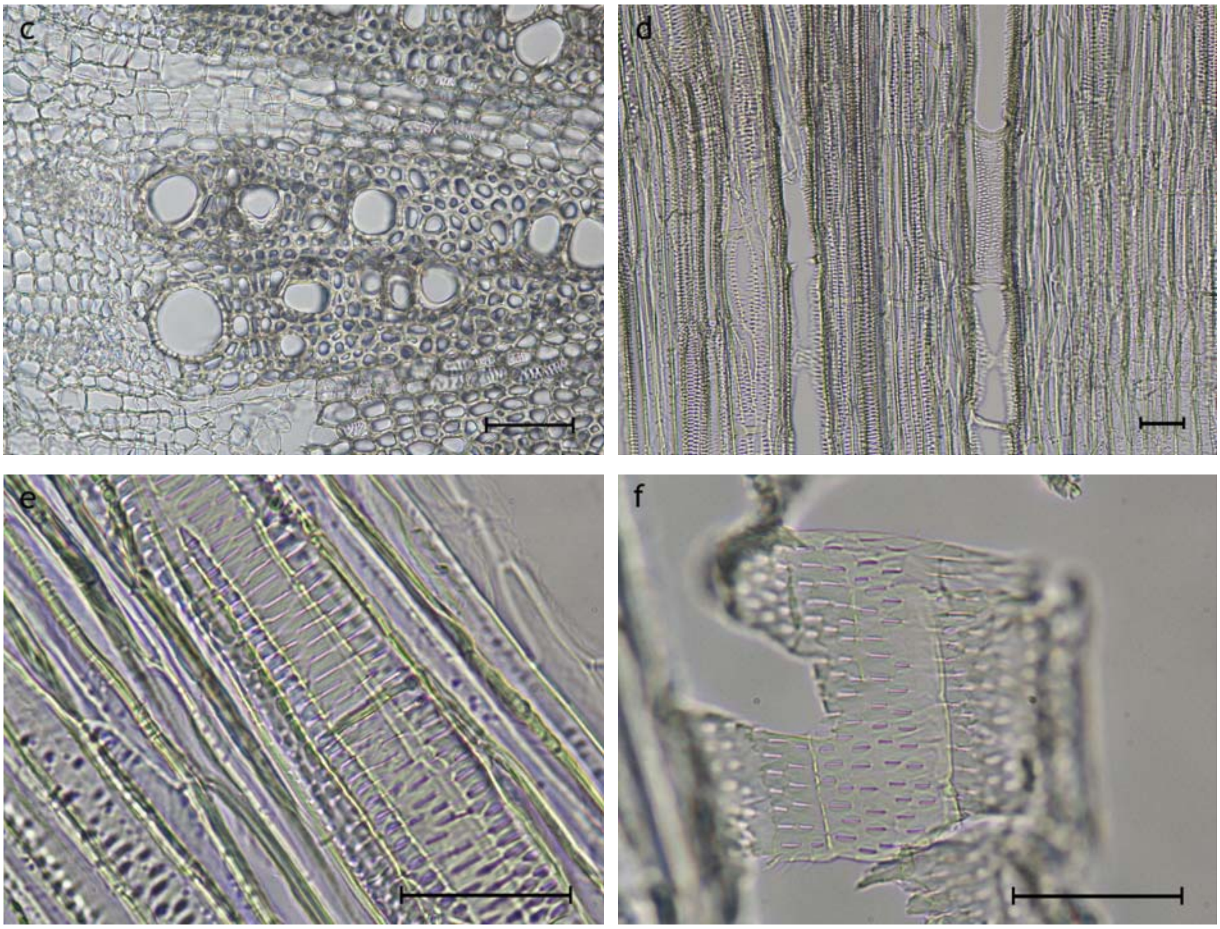
crystalloids, sclereids missing;

fibers in secondary phloem missing;

a: Fibrous root system of *Helminthotheca echioides*,, habitus (KS5)
b: Schematic view of *H. echioides* root (transverse section): cortex enduring, small ellipses mark the laticifers within the sec. phloem, broad sec. xylem







Helminthotheca echioides root: **a:** Overview showing the extension and arrangement of tissues: sec. phloem in extension comparable to sec. xylem, laticifers mainly in rows (S4); **b:** two rows of laticiferous vessels band together to one (S5); **c:** vessels and fibers of secondary xylem (S5); **d:** overview of vascular cylinder (KS5); **e:** reticulate and simply bordered vessels (KS5); **f:** strongly bordered vessels (KS5)
a-c: transverse sections; d-f: longitudinal sections;

GENUS *HYPOCHAERIS*

HYPOCHAERIS UNIFLORA Hoffm.

perennial hemicryptophyte;

allorhizous with taproot;

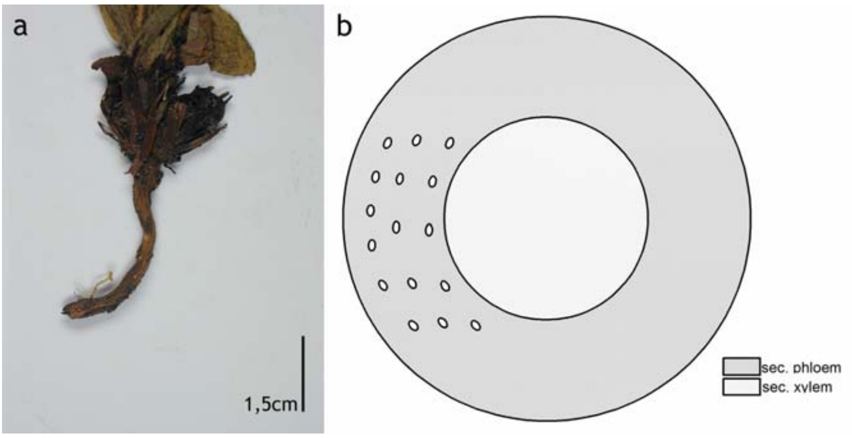
secondary root: phellem thinwalled; **cortex** lost in course of rhytidome formation; **secondary phloem** dominating in extension, laticiferous vessels in concentric circles; **secondary xylem** vessels irregularly dispersed; vessels reticulate and weakly bordered, up to 52µm in diameter; **medullary rays** missing; **pith** missing;

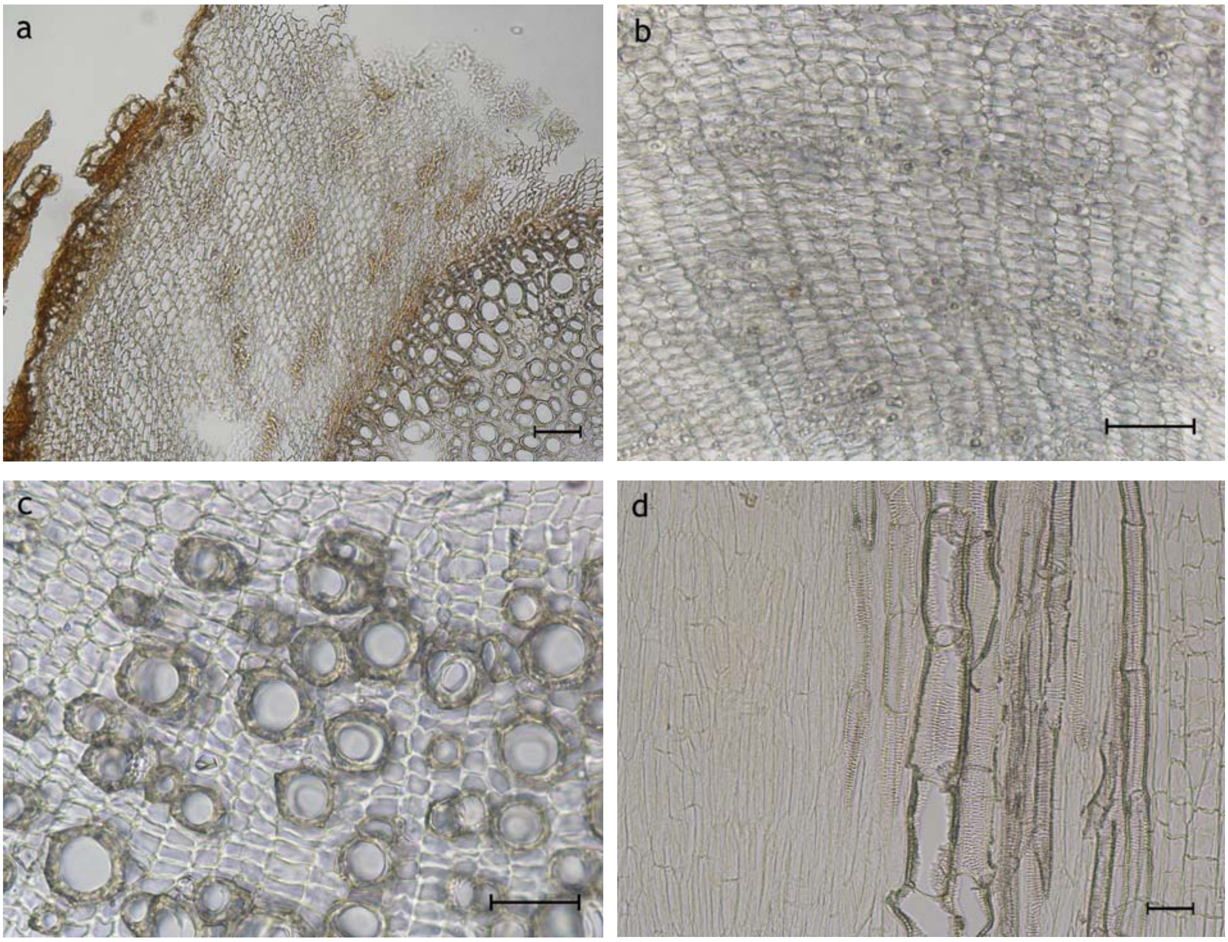
endoSDs, further secretory ducts missing;

crystalloids, sclereids missing;

fibers in secondary phloem missing;

a: Taproot of *Hypochaeris uniflora*, habitus (HU01-08)
b: Schematic view of *H. uniflora* root in transverse section: secondary root: small ellipses mark the laticifers of the broad secondary phloem, comparable in extension to the vascular cylinder





Hypochaeris uniflora root: **a:** Overview showing the extension and arrangement of tissues: the secondary phloem is comparable in extension with the sec. xylem with laticifers in concentric circles (501); **b:** laticiferous vessels within secondary phloem arranged in circles (HU01-08); **c:** vessels within sec. xylem (HU01-08); **d:** Overview over longitudinal section (310)
a-c: transverse sections; d: longitudinal sections;

HYPOCHAERIS RADICATA L.

perennial hemicryptophyte;

fibrous root system;

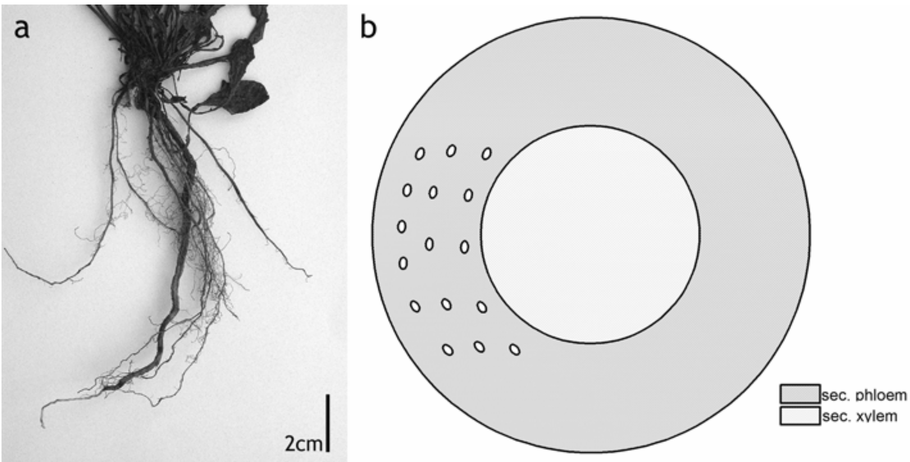
secondary root: phellem thinwanded; **cortex** lost in course of rhytidome formation; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with laticifers circularly arranged relative to the centre of the xylem in fascicularly positioned; **secondary xylem** with vessels either irregularly dispersed or arranged in groups or rows and fibers; vessels reticulate and weakly bordered, up to 45µm in diameter; **medullary rays** bi- and multiseriate; **pith** missing;

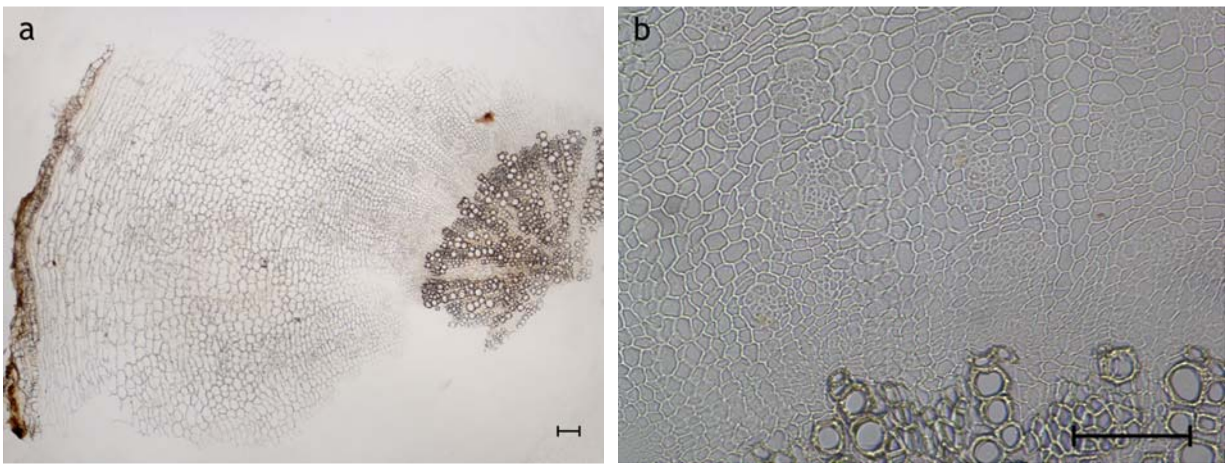
endoSDs, further secretory ducts missing;

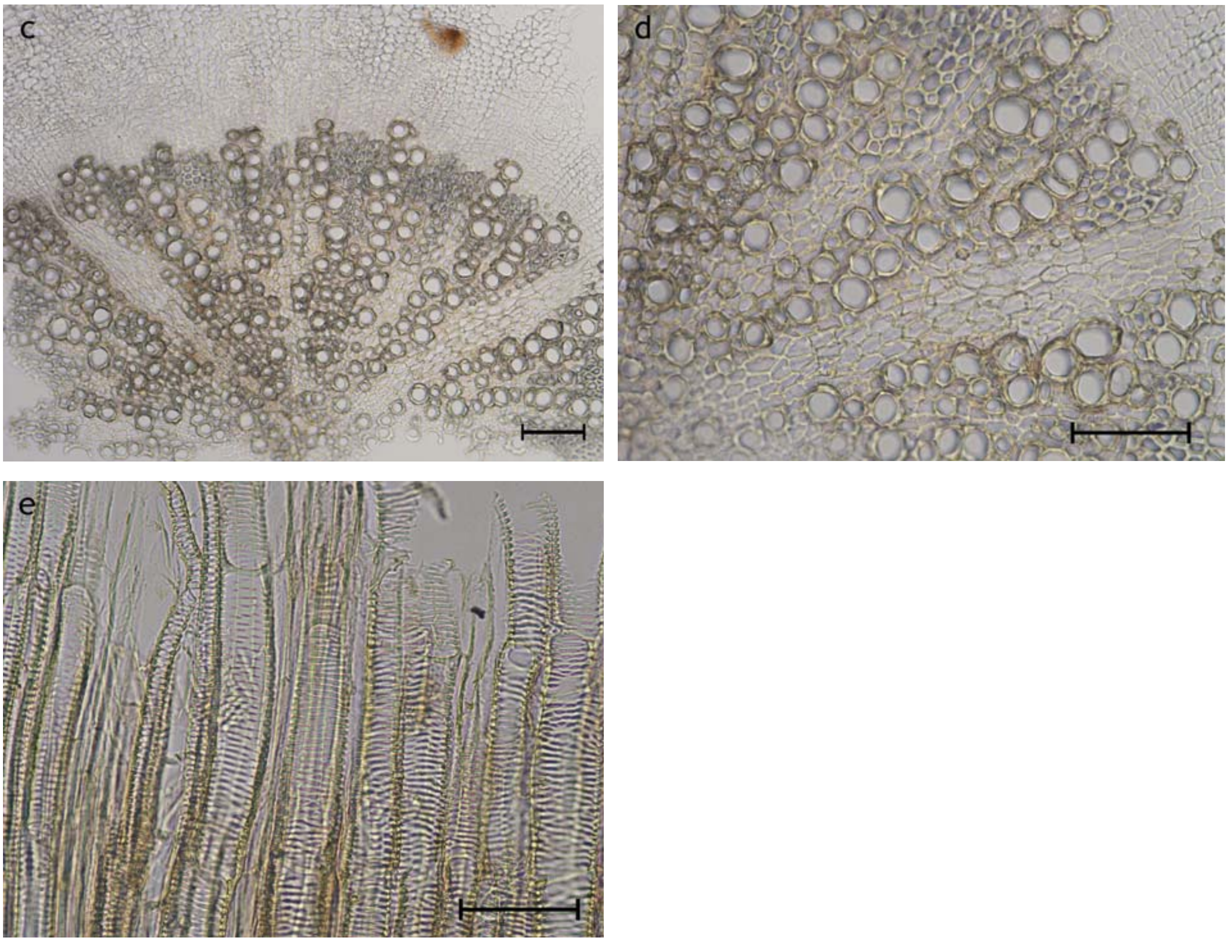
crystalloids and sclereids missing;

fibers in secondary phloem missing;

a: Fibrous root system of *Hypochaeris radicata*, habitus (5-100)
b: Schematic view of *H. radicata* root in transverse section: secondary root: small ellipses mark the position of the laticifers of the broad sec. phloem







Hypochaeris radicata root: **a,b:** cultivated plant: Overview showing the extension and arrangement of tissues: dominant secondary phloem with fibers missing, laticiferous vessels within secondary phloem circularly arranged (a); broad secondary phloem with laticiferous vessels circularly arranged (b) (5-16); **c:** overview over vascular cylinder (5-16); **d:** sec. xylem with vessels and multiseriate medullary rays (5-16); **e:** reticulate vessels (H113)
a-d: transverse sections; e: longitudinal section;

GENUS *LEONTODON*

LEONTODON INCANUS Schrank

perennial hemicryptophyte;

allorhizous, with taproot;

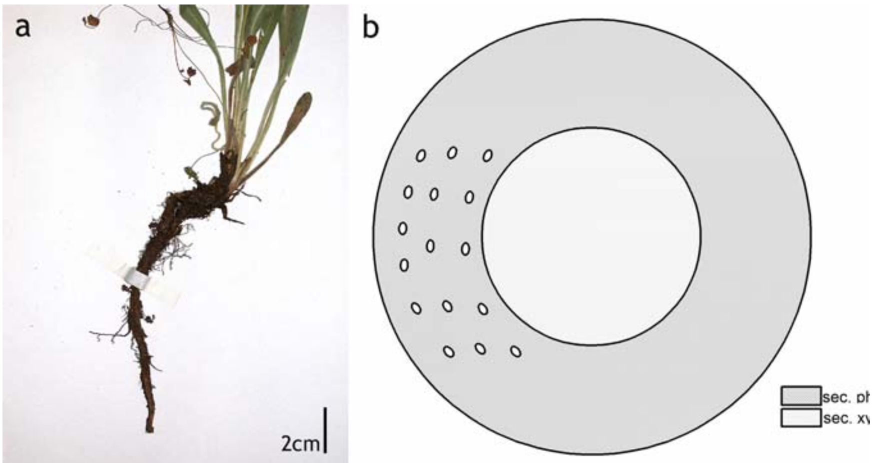
secondary root: phellem thinwanded; **cortex** lost in course of rhytidome formation; **secondary phloem** dominating in extension, with laticiferous vessels arranged in concentric circles; **secondary xylem** with rare fibers, vessels irregularly dispersed over the transverse section, reticulate and bordered, up to 54μm in diameter; **medullary rays** multiseriate; **pith** missing;

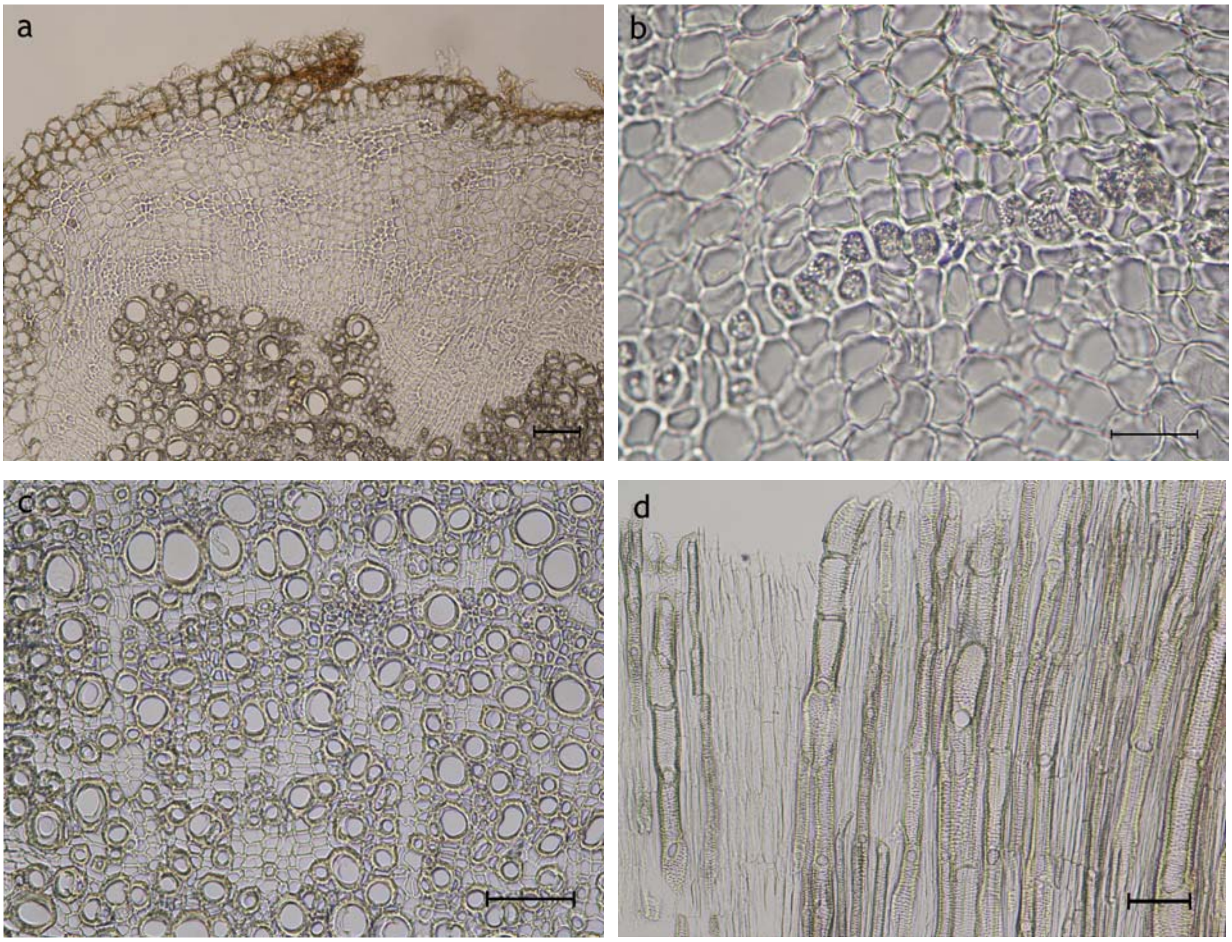
endoSDs, further secretory ducts missing;

crystalloids and sclereids missing;

fibers in secondary xylem missing;

a: Taproot of *Leontodon incanus*, habitus (L100)
b: Schematic view of *L. incanus* root in transverse section: sec. root: secondary phloem dominating, with laticifers in concentric circles, sec. xylem with vessels irregulary dispersed





Leontodon incanus root: **a:** Overview showing the extension and arrangement of tissues: cortex lost in course of rhytidome formation, sec. phloem dominating, secondary xylem with vessels irregularly dispersed, fibers (L107); **b:** secondary phloem with laticiferous vessels in circular lines (L358-1); **c:** secondary xylem with vessels and rarely fibers (L108); **d:** overview showing the secondary xylem (L100);
a-c: transverse sections; d: longitudinal section;

LEONTODON HISPIDUS Scop.

perennial hemicryptophyte;

rhizome;

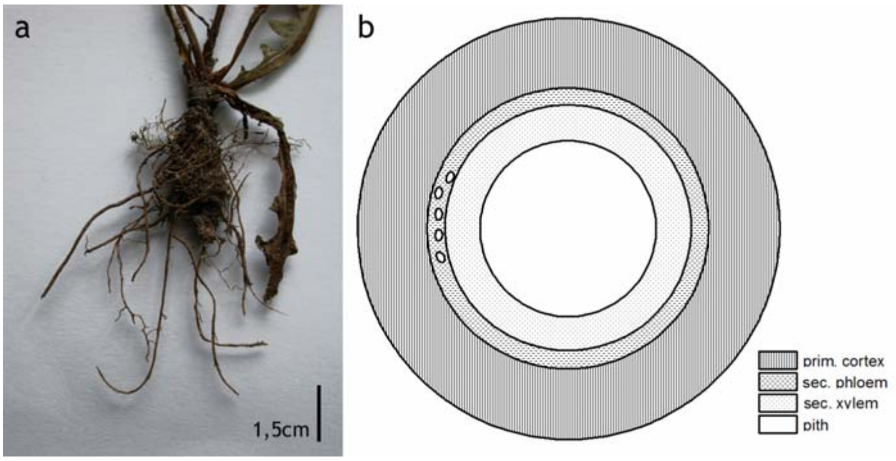
rhizome with secondary growth: phellem thin-walled, sometimes with crystalloids; broad **cortex** enduring, cells towards the outside with thickened cell walls; **secondary phloem** small, comparable in extension to the vascular cylinder, with laticiferous vessels irregularly dispersed; **secondary xylem** with fibers and vessels irregularly dispersed; vessels mainly reticulate, also simply bordered, up to 30μm in diameter; **medullary rays** multiseriate; **pith** with thinwanded cells;

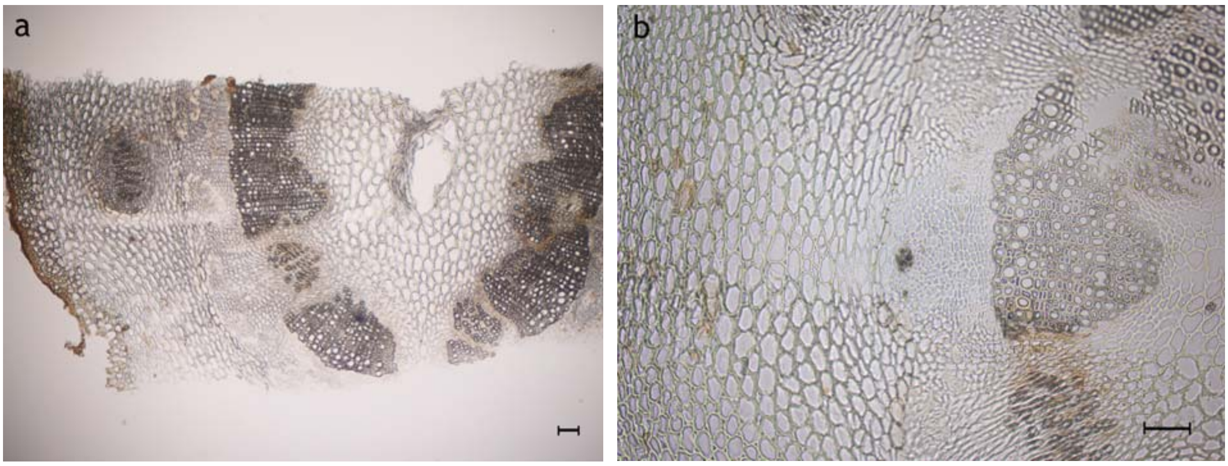
endoSDs, further secretory ducts missing;

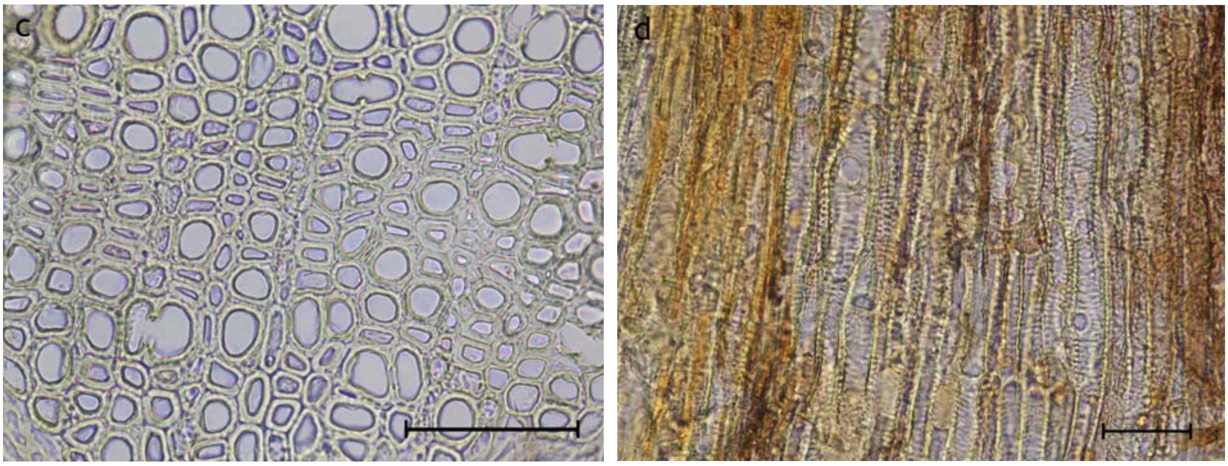
sclereids missing;

fibers in secondary phloem missing;

a: Rhizome of *Leontodon hispidus,* habitus (L105)
b: Schematic view of *L. hispidus* rhizome in transverse section: ellipses mark the position of the laticifers of the small sec. phloem, xylem with vessels dispersed, broad pith







Leontodon hispidus rhizome: **a:** Overview showing the extension and arrangement of tissues: cortex and pith dominating, small vascular bundles (L105); **b:** laticiferous vessels within secondary phloem irregularly dispersed, broad cortex (L105); **c:** vessels of secondary xylem with fibers (L105); **d:** overview of secondary xylem (L4-107);
a-c: transverse sections; d: longitudinal section;

GENUS *PICRIS*

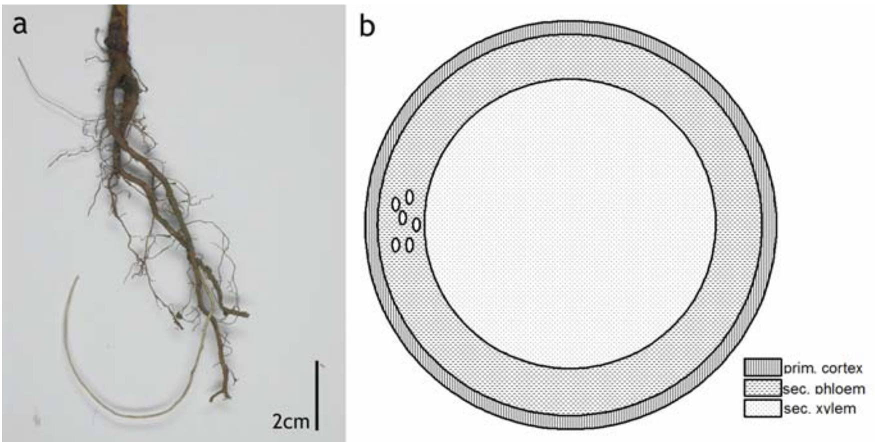
PICRIS HIERACIOIDES subsp. *hieracioides* L.

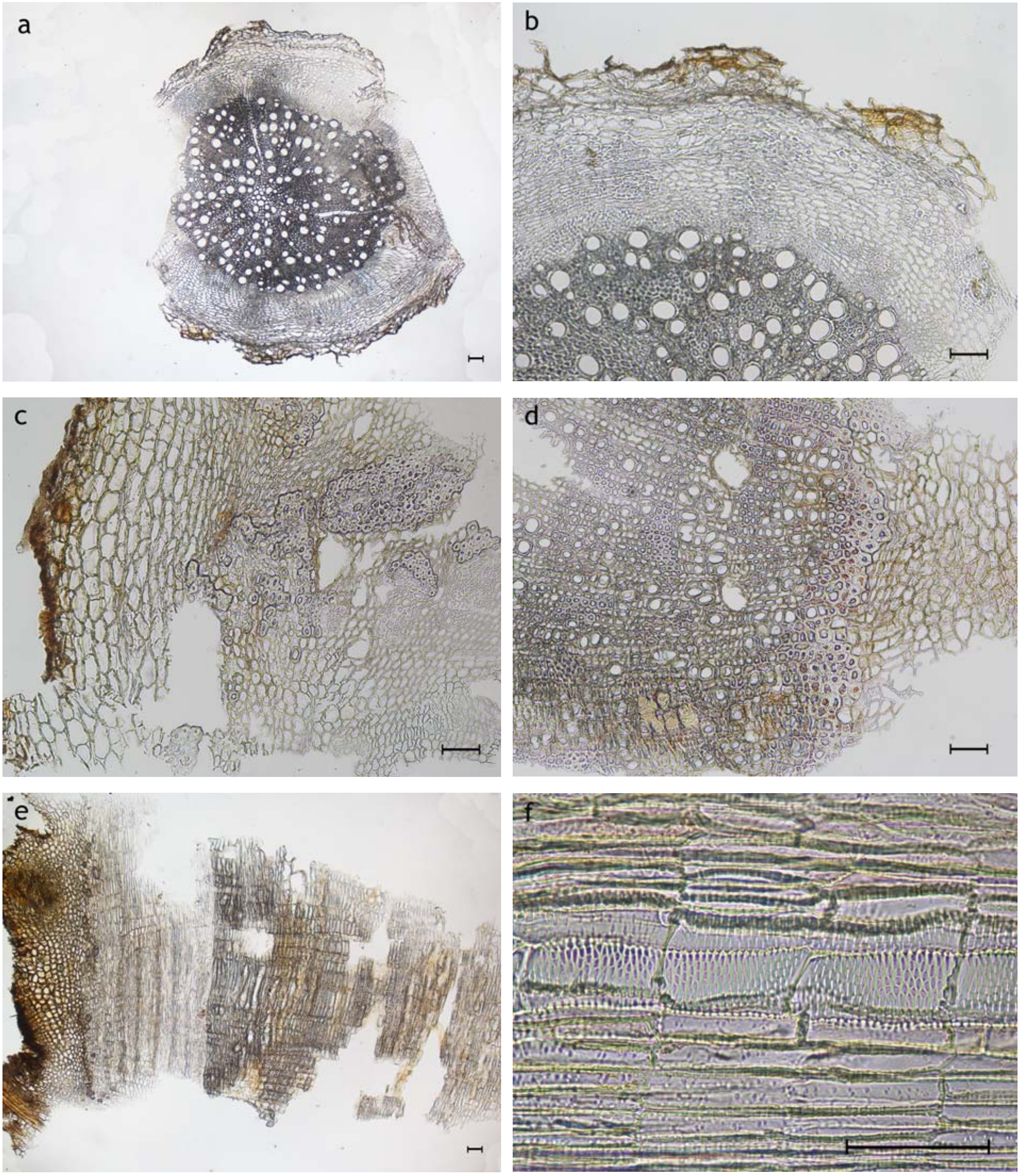
biennal to pluriennal / perennial hemicryptophyte;

fibrous root system, long rhizomes possible;

secondary root: phellem thin-walled, sometimes with crystalloids; **cortex** enduring, with sclereids; **secondary phloem** small, with laticiferous vessels irregularly dispersed, containing sclereids, often in bundles, also bundles of fibers; **secondary xylem** dominating in extension, comprising lots of fibers, vessels irregularly dispersed over the transverse section, reticulate and weakly bordered up to 57µm in diameter; [within rhizome sclereids within the xylem, specially at the transition to pith; pith thin-wanded;] **medullary rays** multiseriate; **pith** missing;
endoSDs, further secretory ducts missing;

a: Root of *Picris hieracioides* subsp. *hieracioides*, habitus (PH02-07)
b: Schematic view of *P. hieracioides root* in transverse section: secondary root: cortex enduring, laticifers irregularly dispersed within small phloem, sec. xylem dominating





Picris hieracioides root / rhizome: **a:** Overview showing the extension and arrangement of tissues: durable cortex and secondary phloem small, secondary xylem dominating, vessels irregularly dispersed (root) (PH02-07); **b:** durable cortex, small secondary phloem with laticiferous vessels irregularly dispersed (root) (PH02-07); **c:** cortex and sec. phloem with sclereids and fibers in bundles (rhizome) (PH01-07); **d:** secondary xylem with few vessels dispersed over transverse section, fibers, sclereids at the transition to pith (rhizome) (PH01-07); **e:** overview showing the extension and arrangement of tissues (root) (PH01-07); **f:** bordered vessels (root) (PH01-07)
a-d: transverse sections; e,f: longitudinal sections;

GENUS *SCORZONEROIDES*

SCORZONEROIDES HELVETICA (Merat) Holub

perennial hemicryptophyte;

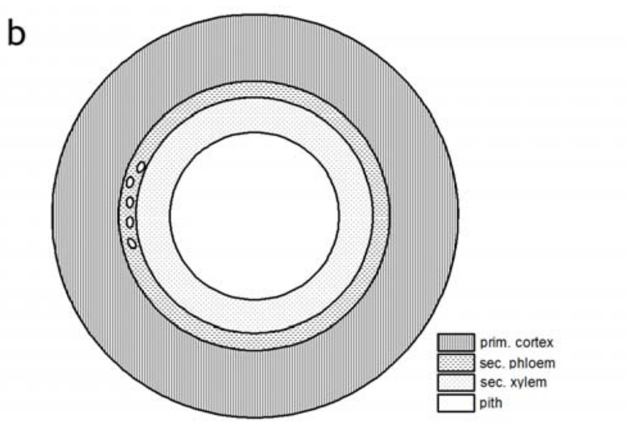
rhizome;

rhizome with secondary growth: phellem thin-walled, sometimes with crystalloids; broad **cortex** enduring; **secondary phloem** small, comparable in extension to the vascular cylinder, with laticiferous vessels irregularly dispersed; **secondary xylem** with fibers and relatively few vessels irregularly dispersed; vessels mainly reticulate, also simply bordered, up to 23µm in diameter; **medullary rays** multiseriate; **pith** with thinwanded cells;

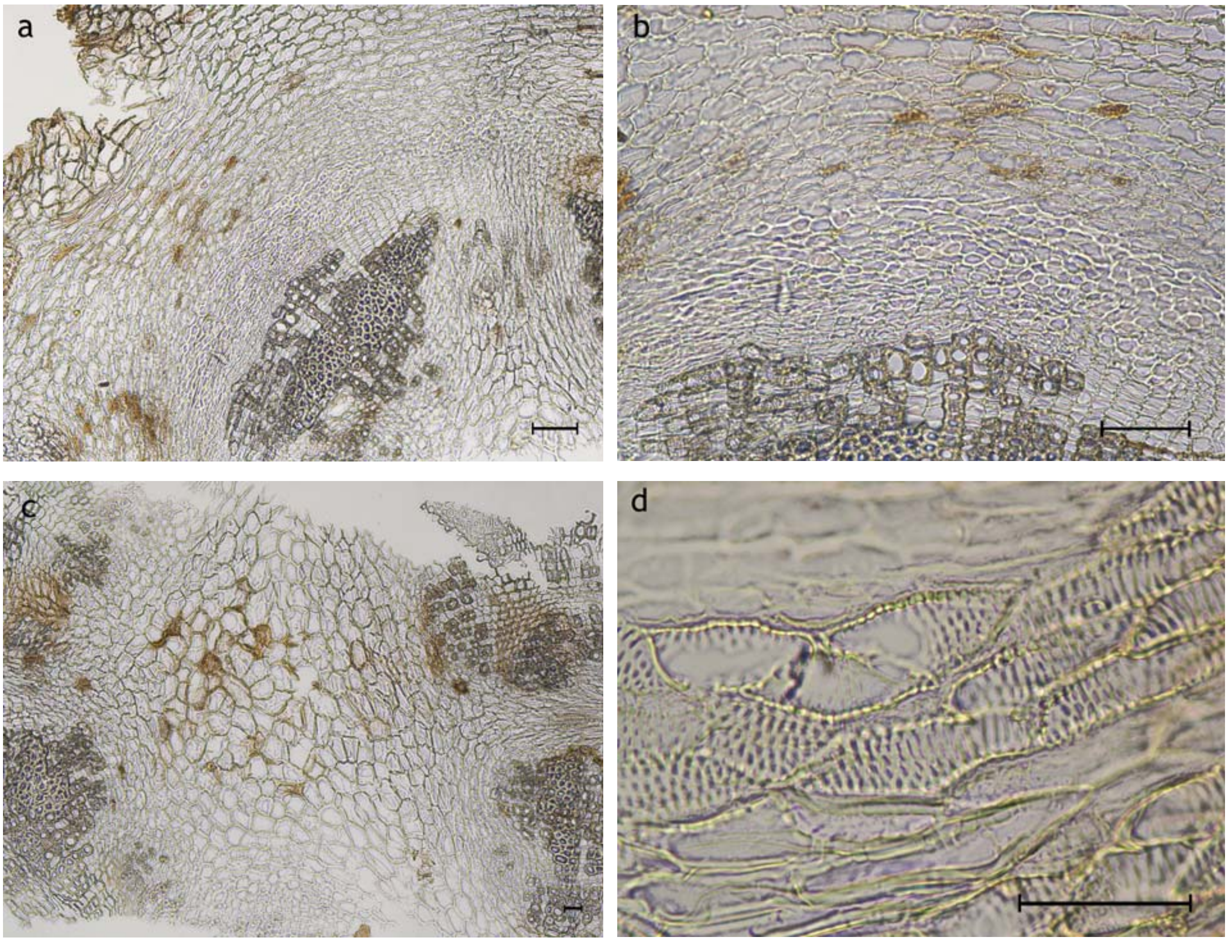
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem missing;



a: Rhizome of *Scorzoneroides helveticus*, habitus (L109)
b: Schematic view of *S. helveticus* rhizome in transverse section: small ellipses mark the position of the laticiferous vessels of the narrow secondary phloem, broad cortex, broad pith



Scorzoneroides helveticus rhizome: **a:** Overview showing the extension and arrangement of tissues: thin-wanded phellem, cortex enduring, small vascular bundles with relatively few vessels, multiseriate medullary rays (L109); **b:** laticiferous vessels within secondary phloem irregularly dispersed (L109); **c:** pith (L109); **d:** simple vessels (L110)
a-c: transverse sections; d: longitudinal section;

SCORZONEROIDES AUTUMNALIS (L.) Moench

perennial hemicryptophyte;

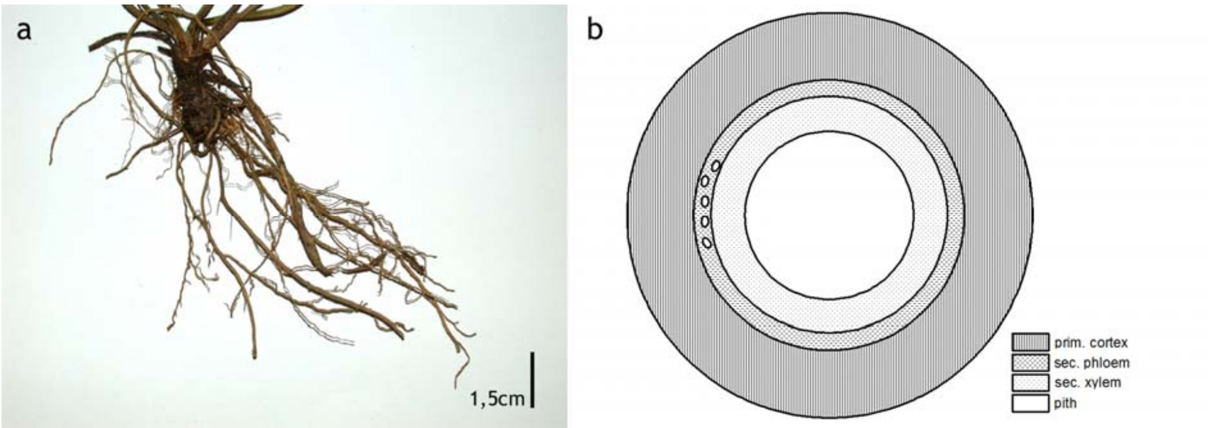
rhizome;

rhizome with secondary growth: phellem thin-walled, sometimes with crystalloids; broad **cortex** enduring with big intercellulars; **secondary phloem** small, comparable in extension to the vascular cylinder, with laticiferous vessels irregularly dispersed; **secondary xylem** with few vessels irregularly dispersed; vessels mainly reticulate, also simply bordered, up to 34µm in diameter; **medullary rays** multiseriate; **pith** with thinwanded cells;

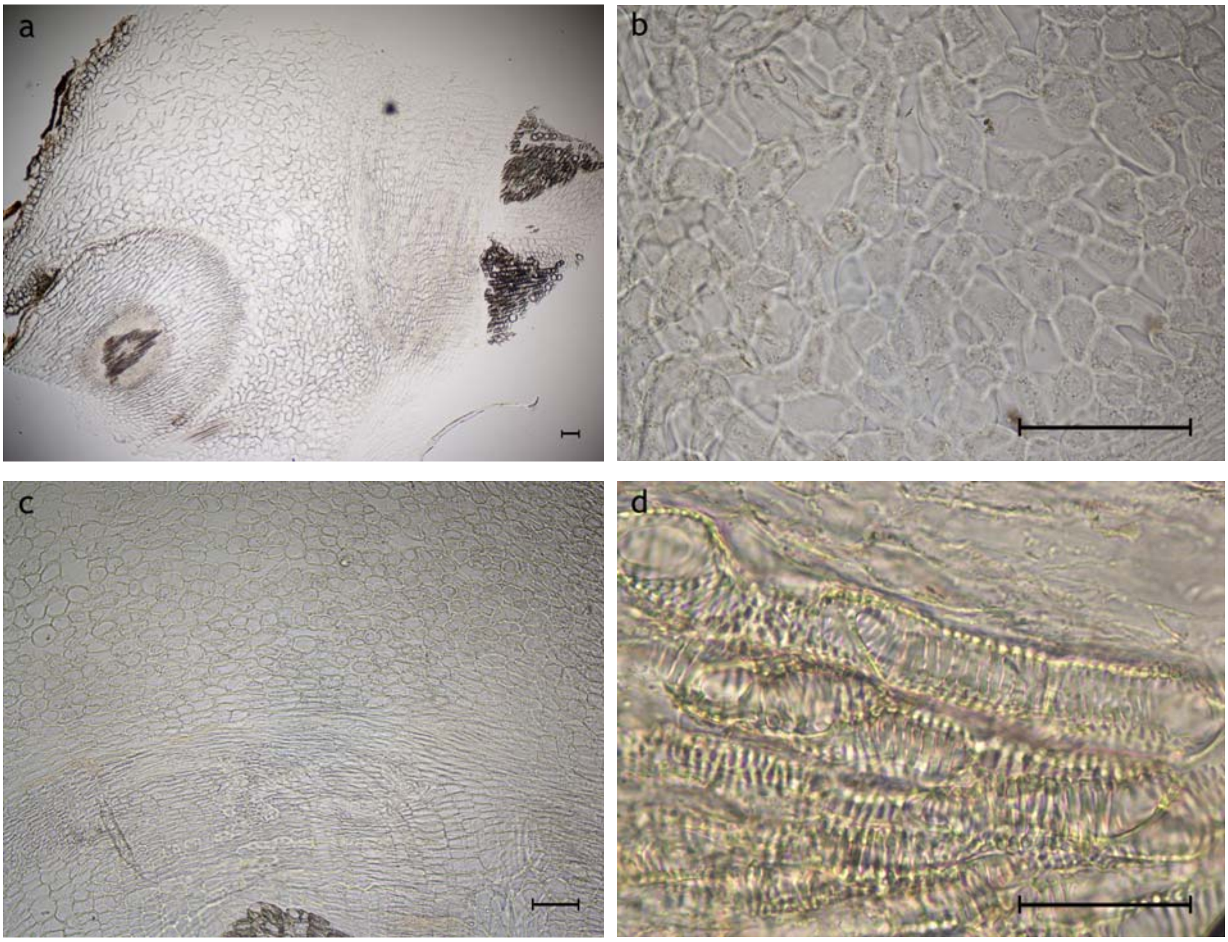
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem and secondary xylem missing;



a: Rhizome of *Scorzoneroides autumnalis*, habitus (L103)
b: Schematic view of *S. autumnalis* rhizome in transverse section: small ellipses mark the position of the laticiferous vessels of the narrow secondary phloem, broad cortex, broad pith;



Scorzoneroides autumnalis rhizome: **a:** Overview showing the extension and arrangement of tissues: thin-wanded phellem, cortex enduring, small vascular bundles, multiseriate medullary rays (L102); **b:** cortex with big intercellulars (L102); **c:** overview of cortex and secondary phloem with laticifers dispersed (L104); **d:** reticulate vessels of secondary xylem (L103)
a-c: transverse sections; d: longitudinal section;

GENUS *PRENANTHES*

PRENANTHES PURPUREA L.

perennial hemicryptophyte;

rhizome;

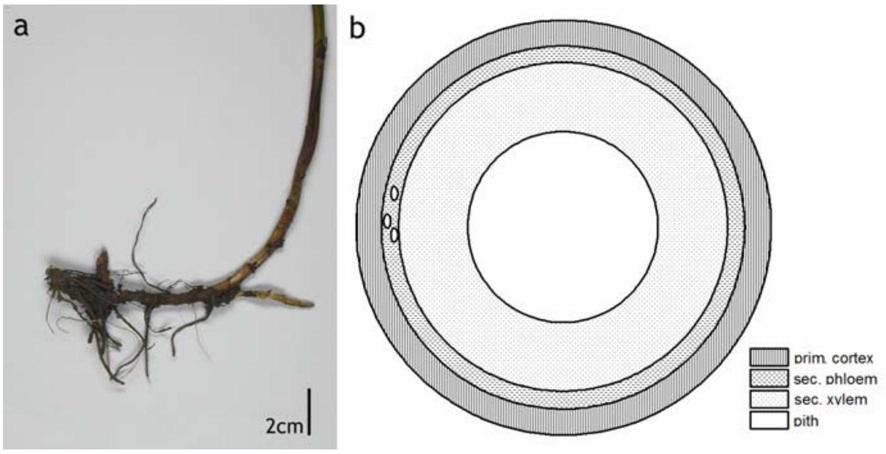
rhizome with secondary growth: phellem thin-walled, exodermal; rhizodermis with thick cuticle, exodermis consisting of large cells; small **cortex; secondary phloem** small, with laticiferous vessels irregularly dispersed; **secondary xylem** together with pith dominating in extension, vessels irregularly dispersed, mainly embedded in fibers, strongly bordered, up to 86µm in diameter; **medullary rays** multiseriate; large **pith** of thin-wanded cells;

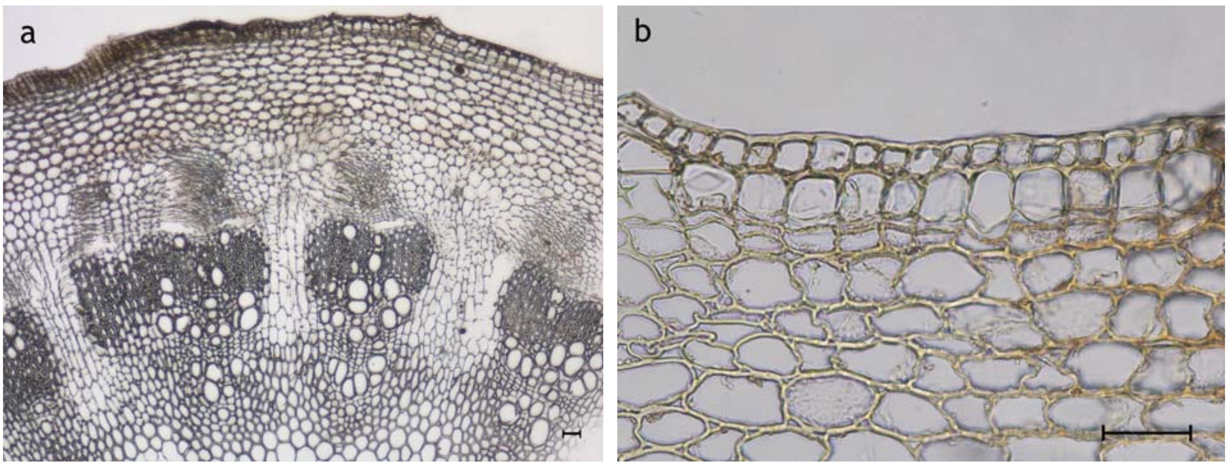
endoSDs, further secretory ducts missing;

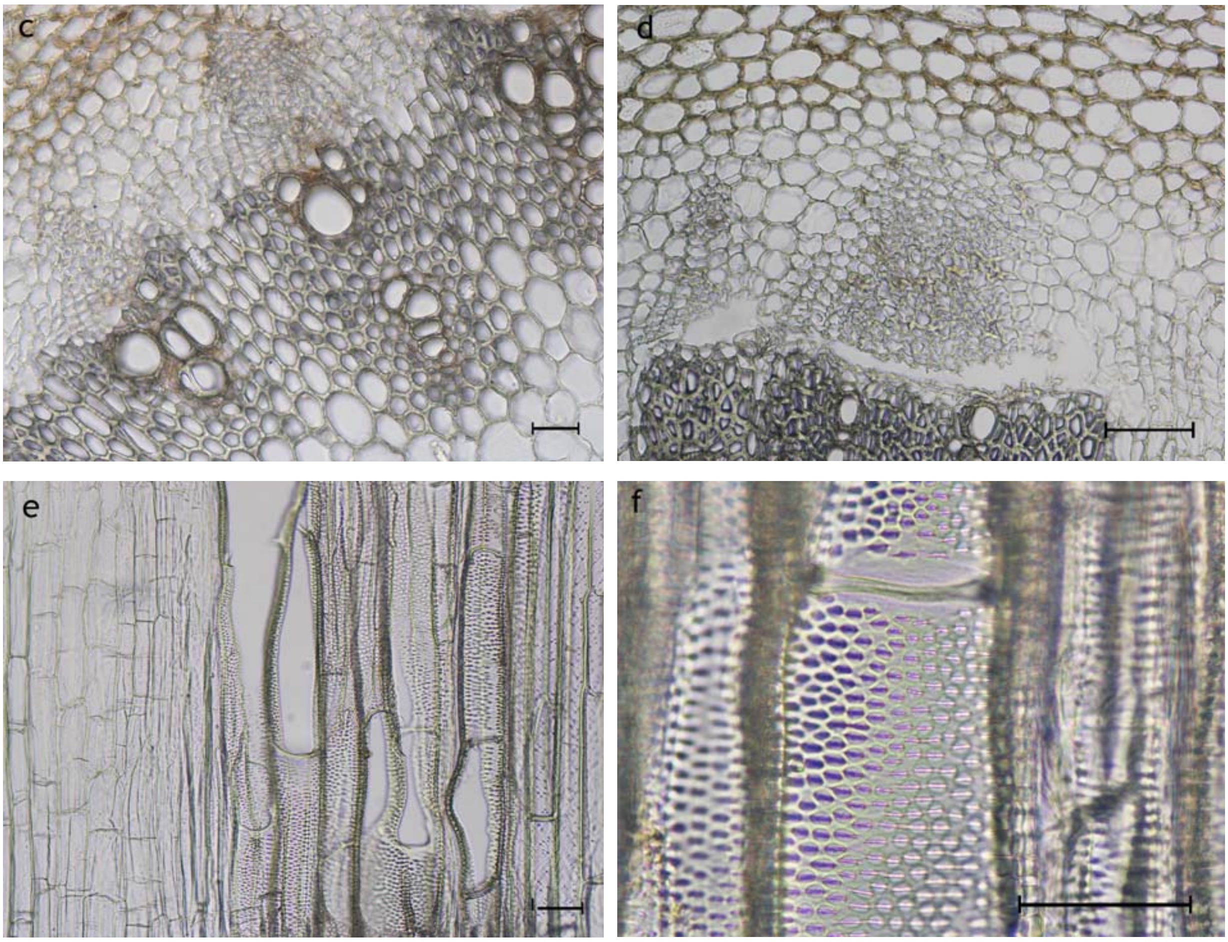
crystalloids, sclereids missing;

fibers in secondary phloem missing;

a: Rhizome of *Prenanthes purpurea*, habitus (PP02-09)
b: Schematic view of *P. purpurea* rhizome (transverse section): small ellipses mark the laticifers of the phloem, broad vascular cylinder and pith







Prenanthes purpurea rhizome: **a:** Overview showing the extension and arrangement of tissues: cortex and secondary phloem of lesser radial extension than the vascular cylinder, large pith (PP03-09); **b:** rhizodermis with thick cuticle, subexodermal phellem starting to develop (PP01-09); **c:** overview over vascular bundles: few vessels embedded in fibers (PP01-09); **d:** laticiferous vessels dispersed in sec. phloem (PP03-09); **e,f:** strongly bordered vessels of sec. xylem (PP01-09)
a-d: transverse sections; e,f: longitudinal sections;

SUBTRIBE HIERACIINAE

GENUS *HIERACIUM*

HIERACIUM MURORUM L.

perennial hemicryptophyte;

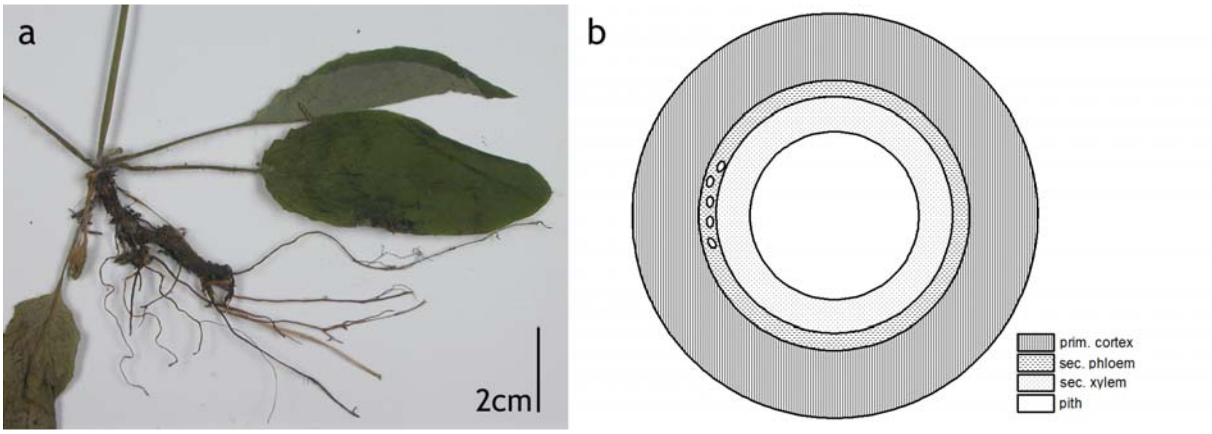
rhizome;

rhizome with secondary growth: cortex enduring, dominating in extension; endodermis visible even within older roots; **secondary phloem** small, with laticiferous vessels irregularly dispersed; **secondary xylem** with vessels irregularly dispersed, fibers; vessels reticulate and weakly bordered, up to 57µm in diameter; **medullary rays** multiseriate; large **pith** of thin-wanded cells;

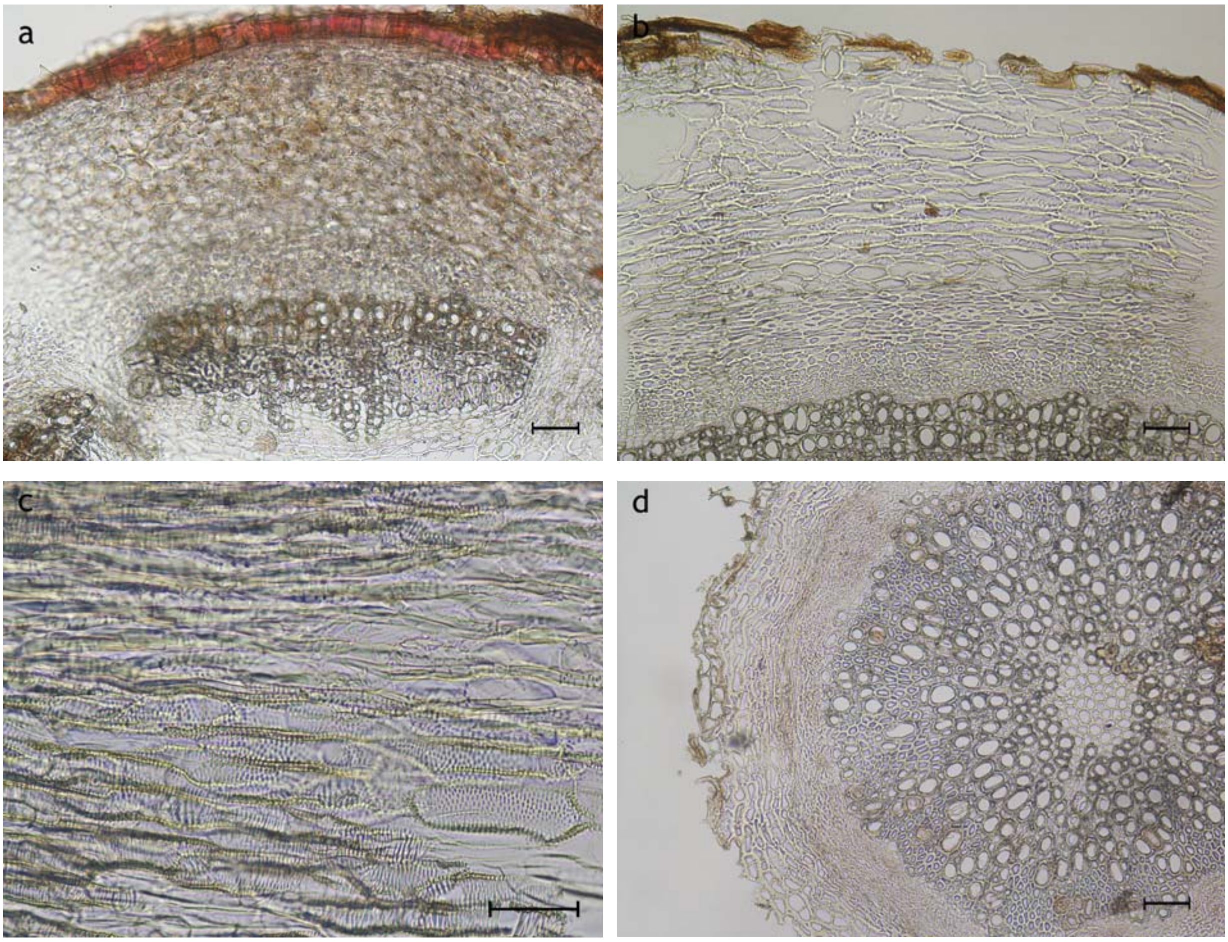
endoSDs, further secretory ducts missing;

crystalloids, sclereids missing;

fibers in secondary phloem missing;



a: Rhizome of *Hieracium murorum*, habitus (HM01-03)
b: Schematic view of *H. murorum* rhizome in transverse section: small ellipses mark the position of the laticiferous vessels of the narrow phloem, durable cortex dominating, secondary phloem of lesser radial extension than the vascular cylinder



Hieracium murorum rhizome: **a:** Overview showing the extension and arrangement of tissues: broad cortex, secondary phloem small, vascular bundle with fibers (HM01-08); **b:** cortex, secondary phloem with irregularly dispersed laticiferous vessels (AST11); **c:** bordered vessels and fibers of the vascular bundle (HM01-08); **d:** overview over adventitious root showing the sec. xylem dominating in extension with a parenchymatous center, small phloem and cortex (S4)
a,b,d: transverse sections; c: longitudinal section;

HIERACIUM PILOSELLA L.

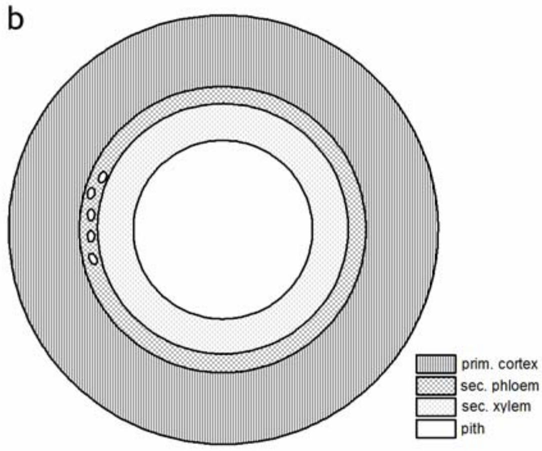
perennial hemicryptophyte;

rhizome;

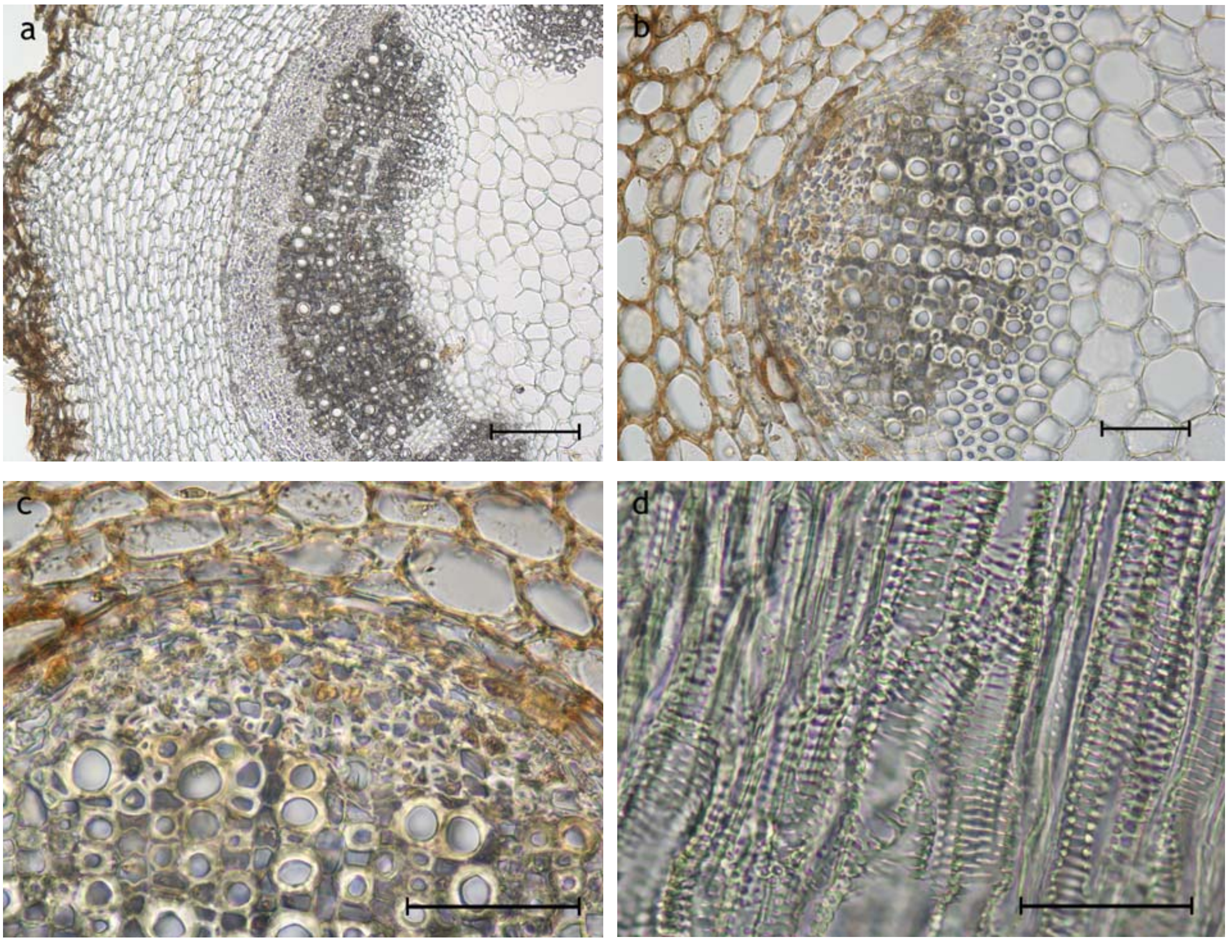
rhizome with secondary growth: phellem thin-wanded; dominate **cortex** enduring; **secondary phloem** small, with laticiferous vessels irregularly dispersed, older roots may contain fibers; **secondary xylem** with fibers and vessels irregularly dispersed; vessels reticulate and weakly bordered, up to 34µm in diameter; **medullary rays** multiseriate; large **pith** of thin-wanded cells;

endoSDs, further secretory ducts missing;

crystalloids, sclereids missing;



a: Rhizome of *Hieracium pilosella*, habitus (HP01-07)
b: Schematic view of *H. pilosella rhizome* (transverse section): small ellipses mark the position of the laticiferous vessels of the narrow phloem, broad durable cortex, broad pith;



Hieracium pilosella rhizome: **a:** Overview showing the extension and arrangement of tissues: broad cortex, narrow secondary phloem, secondary xylem with fibers, broad pith (HP01-07); **b:** vascular bundle (AST6); **c:** secondary phloem with laticifers dispersed in groups with sieve vessels (AST6); **d:** reticulate vessels of vascular bundle (HP01-86);
a-c: transverse sections; d: longitudinal section;

SUBTRIBE CICHORIINAE

GENUS *CICHORIUM*

CICHORIUM INTYBUS L. (cf. Paper IV)

perennial hemicryptophyte;

allorhizous, with taproot;

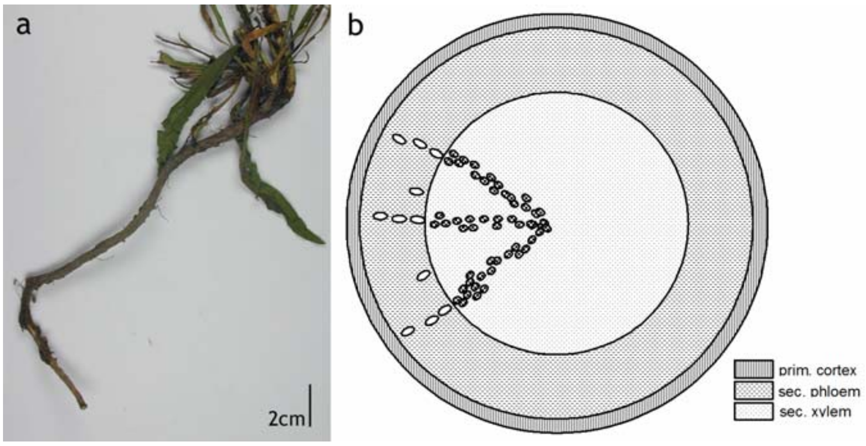
secondary root: phellem thin-wanded, sometimes with crystalloids; small **cortex** enduring; **secondary phloem** dominant, comparable in extension to the vascular cylinder, with laticiferous vessels arranged in rows; **secondary xylem** with fibers, vessels arranged in rows or groups over the transverse section, short in longitudinal view, reticulate, mainly strongly bordered, up to 109µm in diameter; **medullary rays** multiseriate; **pith** missing;

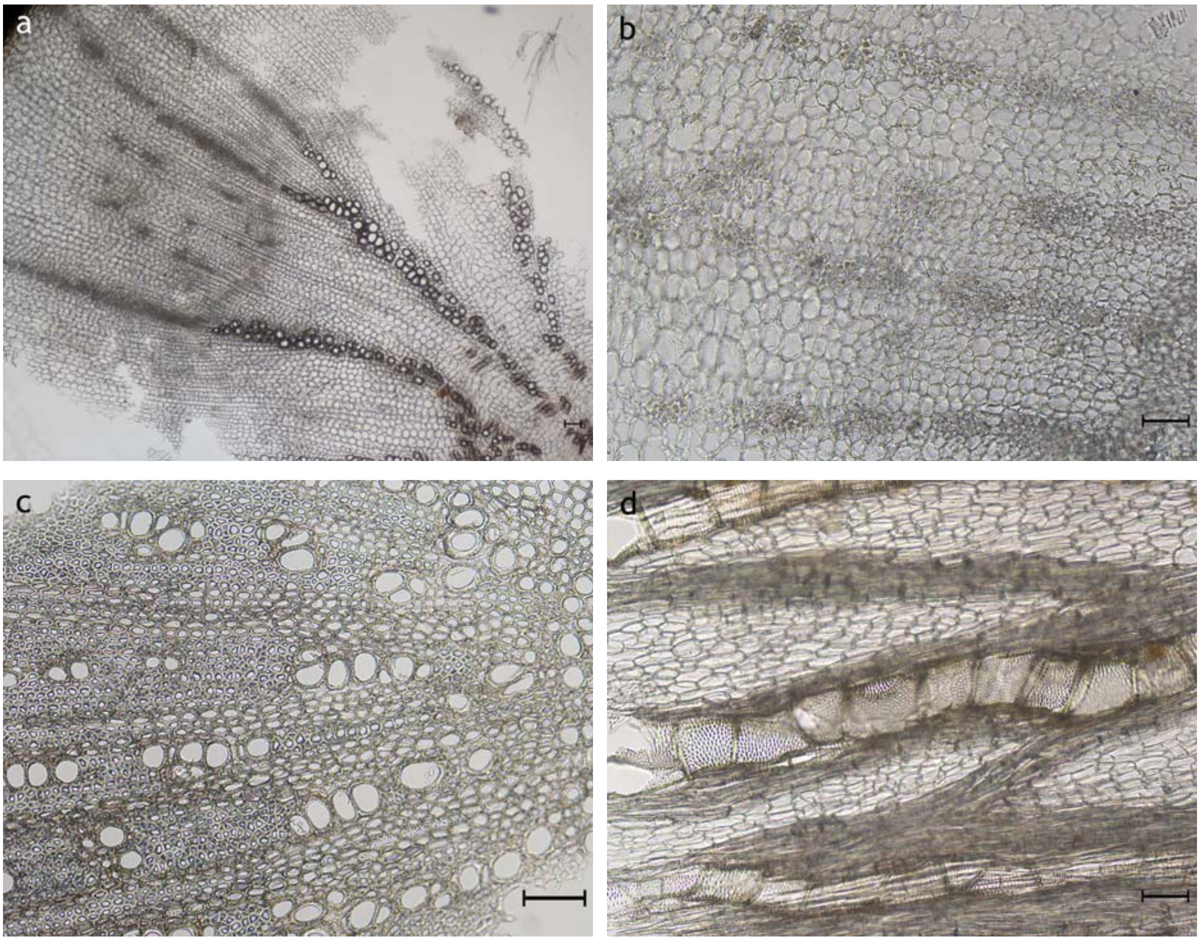
endoSDs, further secretory ducts missing;

sclereids missing;

fibers in secondary phloem missing;

a: Taproot of *Cichorium intybus*, habitus (CI1-08)
b: Schematic view of *C. intybus* root (transverse section): sec. root: secondary phloem dominating comparable in extension to sec. xylem, with laticifers in rows;





Cichorium intybus root: **a:** Overview showing the extension and arrangement of tissues: sec. phloem dominating in extension comparable to secondary xylem (S5); **b:** secondary phloem with laticiferous vessels in rows (CI1-08); **c:** secondary xylem with vessels in groups / short rows, fibers (CI1-08); **d:** overview showing the secondary xylem (CI2-07);
a-c: transverse sections; d: longitudinal section;

GENUS *TOLPIS*

CHLOROCREPIS STATICIFOLIA (All.) Griseb.

perennial hemicryptophyte;

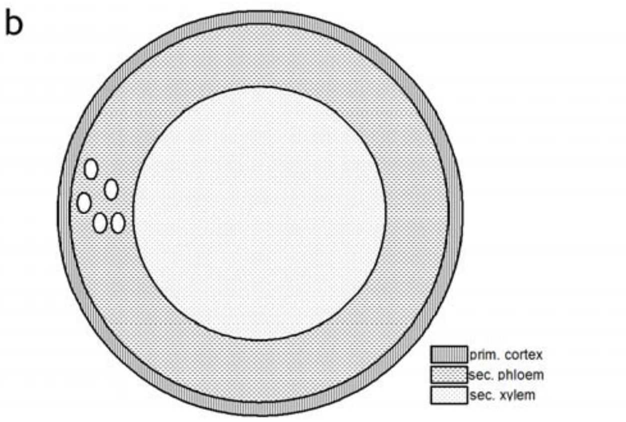
allorhizous;

secondary root: phellem thin-wanded, sometimes with crystalloids; small **cortex** enduring, endodermis visible; **secondary phloem** broad, but sec. xylem dominant in extension, phloem with laticiferous vessels irregularly dispersed; **secondary xylem** with vessels dispersed over the transverse section, mainly reticulate, also strongly bordered, up to 77µm in diameter; **medullary rays** multiseriate; **pith** missing;

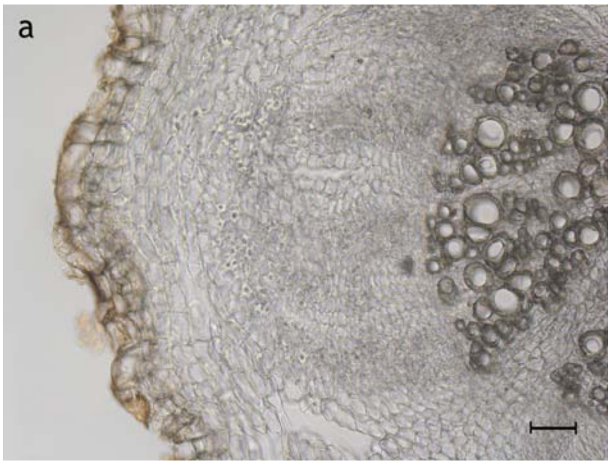
endoSDs, further secretory ducts missing;

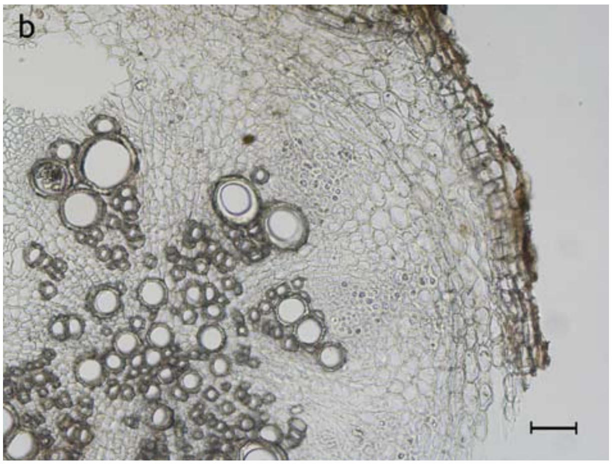
sclereids missing;

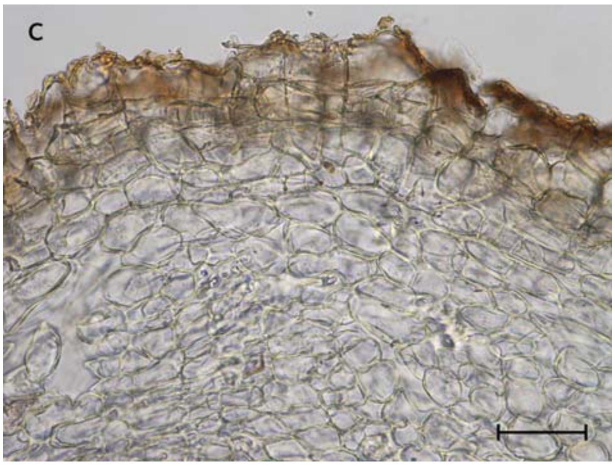
fibers in secondary phloem and secondary xylem missing;

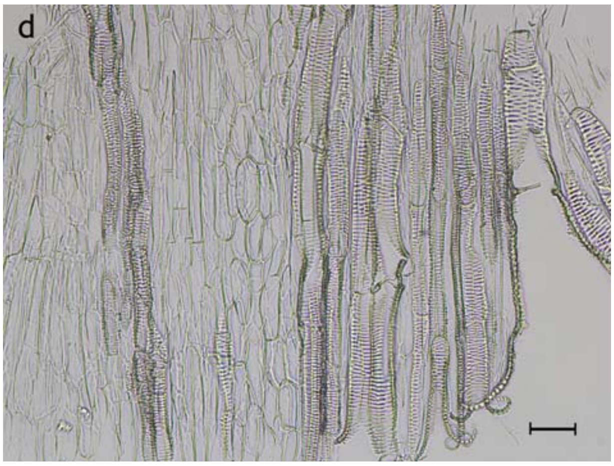


a: Root of *Chlorocrepis staticifolia*, habitus (AST7)
b: Schematic view of C. *intybus* root in transverse section: sec. root: secondary phloem dominating comparable in extension to sec. xylem, with laticifers in rows









Chlorocrepis staticifolia root: **a,b:** Overview showing the extension and arrangement of tissues: sec. phloem broader than cortex but sec. xylem dominating in extension (AST22, AST7); **c:** phellem thin-walled, cortex with endodermis still visible, sec. phloem with laticiferous vessels dispersed (AST22); **d:** overview showing the secondary xylem, fibers missing (AST7)
a-c: transverse sections; d: longitudinal section;

REFERENCES

FISCHER MA, OSWALD K, ADLER W. 2008. *Exkursionsflora für Österreich, Liechtenstein und Südtirol.* 3rd ed. Austria: OÖ Landesmuseen, Land Oberösterreich.

FONT, M. et al., 2009: Evolution and biogeography of *Centaurea* section *Acrocentron* inferred from nuclear and plastid DANN sequence analyses. *Annals of Botany.* 103: 985-997.

GARCIA-JACAS et al., 2006: *Centaurea* Revisited: A molecular Survey of the *Jacea* Group. *Annals of Botany.* 98: 741-753.

GARCIA-JACAS et al., 2000: The natural delimitation of *Centaurea* (Asteraceae: Cardueae): IST sequence analysis of the *Centaurea jacea* group. Plant Syst. Evol. 223: 185-199.

SUSANNA A, GARCIA-JACAS N. 2007. Tribe Cardueae Cass. (1819). In: Kubitzki K, eds. *The Families and Genera of Vascular Plants. VIII Flowering Plants-Eudicots*. Springer Verlag, Berlin Heidelberg, 123-146.

APPENDIX III

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ITS-Alignment, blocksize = 70

1-70
Wunderlichia_mirabilis                      TAAAAC-TTGCATGGN-GAACGACMMCGKT--ACA-TSTCCCG-AGCATYGGGGKTTTGRTSG--GSYGT
1_Aposeris_foetida                          TCGAACCCTGCAAGGCAGAACGACC-C-GTGAACT-TGTAAAA-ACAACTAGGTGATG-GTGTGATGGGC
2_Arctium_lappa                             TCGAAGCCTGCACAGCAGAACGACC-C-GTGAACA-TGTAATC-ACAACCGGGCATCGGGGGGATTGGGT
3_Arctium_lappa                             TCGAAGCCTGCACAGCAGAACGACC-C-GTGAACA-TGTAATC-ACAACCGGGCATCGGGGGGATTGGGT
4_Carduus_crassifolius                      TCGAAGCCTGCACAGCAGAACGACC-C-GTGGACA-TGTAATC-ACAGCCGGGCGTCAAAGGTGTCGGGC
5_Carduus_crassifolius                      TCGAAGCCTGCACAGCAGAACGACC-C-GTGGACA-TGTAATC-ACAGCCGGGCGTCAAAGGTGTCGGGC
16_Cirsium_arvense                          TCGAAGCCTGGACAGCAGAACGACC-T-GTGGACA-CGTAATC-ACAGCCGGGCGTCAACGGGGTCGGGT
18_Cirsium_vulgare                          TCGAAGCCTGCACAGCAGAACGACC-C-GTGGACA-CGTAATC-ACAGCCGGGTGTCAAGGGGGTCGGGC
17_Cirsium_vulgare                          TCGAAGCCTGCACAGCAGAACGACC-C-GTGGACA-CGTAATC-ACAGCCGGGTGTCAAGGGGGTCGGGC
53_Silybum_marianum                         TCGAAGCCTGCACAGCAGAACGACC-C-GCGAACA-CGTAATC-ACAGCCGGGCGTCGAGGGGGTCGGGC
8_Centaurea_cyanus                          TCGAAGCCTGCATAGCAGAACGACC-C-GTGAACA-TGTAACC-AAAACCGGGTGTCGTGGG-GTCGGGT
10_Centaurea_montana                        NCGAAGCCTGCACAGCAGAACGACC-C-GTGAACA-TGTAACC-ACAATCGGGTGTCGTGGG-GTCGGGT
9_Centaurea_jacea                           TCGAAGCCTGCACAGCAGAACGACC-C-GTGAACA-TGTAATC-ACAACCGGGTGTCGTGGG-ATTGGGT
19_Cnicus_benedictus                        TCGAAGCCTGCACAGCAGAACGACC-C-GTGAACA-TGTAATC-ACAACCGGGTGTCGTGGG-ATCGGGT
11_Centaurea_scabiosa                       TCGAAGCCTGCACAGCAGAACGACC---GTGAACA-TGTAATC-ACAACCGGGTGTCGTGGG-ATCGGGT
41_Rhaponticum_scariosum                    TCGAAGCCTGCACAGCAGAACGACC-C-GTGAACA-TGTAATC-ACAATCGGGTGTCGTAGG-ATTGGGT
52_Serratula_tinctoria_subsp_tinctoria      TCGARACCTGCACAGCAGAACGACC-C-GTGAACA-CGTAATT-ACAACCGGGTGTCGTGGG-ATCGGGT
7_Carlina_vulgaris                          TCGANGNCTGCATAGCAGAACGACC---GCGAACG-TGTAAAACACAACCGG------------------
6_Carlina_vulgaris                          TCGANGNCTGCATAGCAGAACGACC---GCGAACG-TGTAAAACACAACCGG------------------
12_Chlorocrepis_staticifolia                TCGAACCCTGCAAAGCAGAACGACC-C-GTGAACA-TGTACCA-ACAACCGGGAGCCGGAGA-GATGGGC
15_Cichorium_intybus                        TCGAACCCTGCAAAGCAGAACGACC-C-GTGAACA-TGTACCT-ATAACCGGGAGTCAGGGG-TATGGGC
25_Hieracium_murorum                        TCGAACCCTGCAAAGCAGAACGACC-CNGTGAACTNTGTACCA-ACAACCGGGAGACGGGGNAGACTGAC
26_Hieracium_pilosella                      TCGAACCCTGCAAGGCAGAACGACC-C-GTGAACT-TGTACCA-ACAACTGGGAGACGAGG-AGACGGAC
13_Chondrilla_juncea                        TCGAACCCTGCGAAGCAGAACGACC-C-GTGAACA-TGTAAAC-ACAACTGGGTTTTG-GGGAAATGGGC
59_Willemetia_stipitata                     TCGAACCCTGCATAGCAGAACGACC-T-GTGAATA-TGTAAAC-ACAATTAGGTTATA-GGGAAATGGGC
54_Sonchus_oleraceus                        TCGAACCCTGCAAAGCAGAACGACC-C-GTGAACA-TGTAAAT-ACAAGTTGGTGCTG-TTGTGATGGGC
20_Crepis_aurea                             TCGAACCCTGCTAAGCAGAACGACC-TNGTGAATCNTGTAAAAAACAACCGGGTGATGGGGNAGATAGAC
22_Crepis_aurea                             TCGAACCCTGCTAAGCAGAACGACC-TNGTGAATCNTGTAAAAAACAACCGGGTGATGGGGNAGATAGAC
21_Crepis_biennis                           TCGAACCCTGCTAAGCAGAACGACC-T-GTGAATT-TGTAAAT-ACAATTGGGTGATGGGG-AGATGGAC
32_Lapsana_communis                         TCGAACCCTGCAAAGCAGAACGACC-C-GTGAACA-TGTAAAT-ACAACTCGGTGATGGGG-AGATGGGC
56_Taraxacum_laevigatum                     TCGAACCCTGCATGGCAGAACGACC-T-GTGAACA-TGTAAAT-ACAATCGGGTGATGGGG-AAATGGAT
55_Taraxacum_laevigatum                     TCGAACCCTGCATGGCAGAACGACC-T-GTGAACA-TGTAAAT-ACAATCGGGTGATGGGG-AAATGGAT
14_Cicerbita_alpina                         TCGAACCCTGCAAGGCAGAACGACC-A-GTGAACA-TGTAACC-ACAACGGGGTGACC-GGGAAATGGGC
36_Mycelis_muralis                          TCGAACCCTGCAAAGCAGAACGACC-T-GTGAACA-TGTAACC-ACACCAGGGTAAAA-GGGAACCGGGC
30_Lactuca_perennis                         TCGAACCCTGCAAGGCAGAACGACC-T-GTGAACA-TGTAAAC-ACAACTGGGTGACA-G-GAAATGGGC
31_Lactuca_virosa                           TCGAACCCTGCAAGGCAGAACGACC-C-GCGAACA-TGTAACC-ACAACGGGGTGAAT-GTGATAAGGGC
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40_Prenanthes_purpurea                      TCGAACCCTGCAAGGCAGAACGACC-C-GTGAACA-TGTAAAA-ACAACTGGGTGATGGGG-TGATGGGC
28_Hypochaeris_uniflora                     TCGAACCCTGCAAGGCAGAACGACC-C-GTGAACANTGTAAAT-ACAACCGGGTGATGGG-TATAATGGC
50_Scorzoneroides_autumnalis                TCGAA-CCTGCAAGGCAGAACGACC-C-GTGAACA-TGTAAAA-ACA-TCCGGTGATGGG--ATATGGTC
51_Scorzoneroides_helvetica                 TCGAA-CCTGCAAGGCAGAACGACC-T-GTGAACA-TGTAAAT-ACAACCCGGTGATGGGG-ATATGGTC
24_Helminthotheca_echioides                 TCGAA-CCTGCAAGGCAGAACGACC-T-GTGAACT-CGTAAAT-ACAACTAGGTGATGGGG-AGATGGGC
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39_Picris_hieracioides_subsp_morrisonensi   TCGAACCCTGCAAAGCAGAACGACC-T-GTGAACA-TGTAAAT-ACAACCGGGTGATGTGG-AGTCGGGC
33_Leontodon_hispidus                       TCGAACCCTGCAAGGCAGAACGACC-TNGTGAACANCGTAAAT-ACAACTGGGTGATGGGGNAGATGGGC
35_Leontodon_hispidus                       TCGAACCCTGCAAGGCAGAACGACC-TNGTGAACANCGTAAAT-ACAACTGGGTGATGGGGNAGATGGGC
34_Leontodon_incanus                        TCGAACCCTGCAAGGCAGAACGACC-T-GTGAACA-CGTAAAT-ACAACTGGGTGGTGGGG-AGATGGGC
27_Hypochaeris_radicata                     TCGAACCCTGCATGGCAGAACGACC-A-GTTAACA-CGTAAAT-ACAACTGGGTGATGGGG-AGATGGGC
44_Scorzonera_aristata                      NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
47_Scorzonera_humilis                       TCGAAACCTGCCAAGCAGAACGACC-C-GTGAACT-TGTACTCAAAATCAGG-TGATGGGGT-AACATTC
45_Scorzonera_austriaca                     TCGAAACCTGCAAAGCAGAACGACC-C-GCGAACA-TGTACTTCTAATTAGG-AGATGGGGG-AATTGGC
46_Scorzonera_hispanica                     TCGAAACCTGCAAAGCAGAACGACC-C-GCGAACC-TGTACTCAAAATC-GGTTAATGGGGG-AAGAGGG
48_Scorzonera_purpurea                      TCGAAACCTGCAAAGCAGAACGACC--TGTGAACT-TGTACTCATAATCAGG-TGATAGGGT-AACAGCC
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57_Tragopogon_dubius                        TCGAAACCTGCAAAGCAGAACGACC-C-GTGAACA-TGTAATTACAATCAC-CTGATGGGG-AAACGGGC
58_Tragopogon_orientalis                    TCGAAACCTGCGAAGCAGAACGACC-C-GTGAACA-TGTAATTACAATCAC-CTGATGGGG--AACGGGC
23_Echinops_sphaerocephalus                 TCGAAGCCTGCATAGCAGAACGACC-C-GTGAACA-TGTAATGACAATCCGCATCAGGGTG--ACCGGG-
29_Jurinea_mollis                           TCGAAGCCTGCATAGCATAACGACC-C-GTGAACA-CGTAATCACAA-CCGGGTGTTGCGGGGGTCGGG-
42_Saussurea_discolor                       NNNNNNNNTGNNCAGCAGNATGACC-C-GTGANCA-CGTMMTCACAN-CAGGGNGTCTAGGGGMTCGGS-
43_Saussurea_discolor                       NNNNNNNNTGNNCAGCAGNATGACC-C-GTGANCA-CGTMMTCACAN-CAGGGNGTCTAGGGGMTCGGS-
37_Onopordum_acanthium                      TCGAAGCCTGCACAGCAGAACGACC-C-GTGAACA-CGTAATCACAA-CCGGGCATCGNGGGGATCGGG-

71-140
Wunderlichia_mirabilis                      CGTCG-GCCCG-TMAACCTYGACGCCCCGSCGGCGTG---CGTGCGTGGCGCCCC-TTCCGGGGCCTCGC
1_Aposeris_foetida                          CTTGG-TCCTGATCAACAAAA---CCTCC-CGGCATT--GCTTTCATGTTGCCTC-ATTTGCGGTACCAT
2_Arctium_lappa                             GCGAG--CCTGAGCCTTGCGATGCTCCGT-CGGCATA---CGTGCATGGTGCCCC-TTACGAGGCATTGT
3_Arctium_lappa                             GCGAG--CCTGAGCCTTGCGATGCTCCGT-CGGCATA---CGTGCATGGTGCCCC-TTACGAGGCATTGT
4_Carduus_crassifolius                      GTGAG--CCCGGTGCTTGTGATGCCTCGT-CGACGTG---TCTCTGCGTTGCCCCGTTTTGGGGCGTCGT
5_Carduus_crassifolius                      GTGAG--CCCGGTGCTTGTGATGCCTCGT-CGACGTG---TCTCTGCGTTGCCCCGTTTTGGGGCGTCGT
16_Cirsium_arvense                          GTGAG--CCCGGTGCCTGTGATGCCTCGT-CGATGAG---CGTCAGCGATGCCCCGTTTTGAGGCGTCGT
18_Cirsium_vulgare                          GTGAG--CCCGGTGCCCGCGATGCCTCGT-CGGCGTG---CGTCTGCGATGCCCCGTTTTGAGGCGTCGT
17_Cirsium_vulgare                          GTGAG--CCCGGTGCCCGCGATGCCTCGT-CGGCGTG---CGTCTGCGATGCCCCGTTTTGAGGCGTCGT
53_Silybum_marianum                         GTGAG--CCCGGTGCCCGCGATGCCTTGT-CGACGTG---CGTCTGCGATGCCCCGTTTCGAGGCGTCGT
8_Centaurea_cyanus                          GCGAG-CCTTTACCCCTGCGATGC-TCGC-CGTCATG---TGTGCAAGGTGCCTA-TCTCTAGGTATCGT
10_Centaurea_montana                        GTGAG-CCTT-AGCCCTACGATGC-TCGC-CGGCATG---CGTGCAAGGTGCCTA-TTTATAGGTTTCGT
9_Centaurea_jacea                           GCGAG-CCTT-AGCCCTGCGATGC-TCGC-CGGCATG---CGTTCAAGGTGCCTA-TCTCTAGGCATCGT
19_Cnicus_benedictus                        GCGAG-CCTT-ATCCTTGCGATGC-TCGT-TGGCATT---CGTGCAAGGTGCCTA-TCTCTAGGCATCTT
11_Centaurea_scabiosa                       GAGAG-CCTT-AGTCCTGCGATGC-TTGC-CGACATG---CGTGCAAGGTGCCTA-TCTCTAGGCGTCGT
41_Rhaponticum_scariosum                    GTGAG-CCCT-AGCCSTGYGATGC-TYGT-CGGCATG---CGTCCAAGGTGCCTA-TCTTTAGGCATCGT
52_Serratula_tinctoria_subsp_tinctoria      GTGAG-CCTG-AGCCTTGCGATGC-TTGT-CGGCATT---CGTGCAAGGCGCCTA-TCTTTAGGCATCGT
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7_Carlina_vulgaris	------------------------------CGGCGTG---CGCGTGTGGC-CTCCCTT-TGGGGTGCCTC
6_Carlina_vulgaris	------------------------------CGGCGTG---CGCGTGTGGC-CTCCCTT-TGGGGTGCCTC
12_Chlorocrepis_staticifolia	CTCGC-TCCCTTTCTCCGGCA--CCCTGC-CGGCGTG---TGTTTTTGGT-CTCCCAATTGGGGCGCCAT
15_Cichorium_intybus	CTTGG--CCCTATCGCTGGCA--CCCCGT-CGGCTTA---TGTTTGTGGTTGCCCCATTTGGGGTGCCAT
25_Hieracium_murorum	CTTGG-TCACGATCCCCGCCA--CCCTCC-CNGNGCTTNTCGTTAATGATGCCCC-ATTCGGGTCGTCAT
26_Hieracium_pilosella	CTTGG-TCCAGATCCCCGCCA--CCCTCC-CGGCTTG---CGTTCATGGTGCCCC-GTTCGGGTCGTCAT
13_Chondrilla_juncea	CTTGG-TCTAAATCTTAAAAA--CCCATC-CGATGTT---CATTTTTGGTGCCCT-GTTTAGGGTATCAT
59_Willemetia_stipitata	CTTGG-TTCAGATCCCGAAAA--CCC-CC-TGACGTG---CATACGTGGTGTCTT-CTTTGGGGCAACAT
54_Sonchus_oleraceus	TTTATGTTTAGATCAGCAA---CGCCATC-CGGTGTG---TTTTCAAGGTATCTC-TTTTGTGGTACCAT
20_Crepis_aurea	CTTGT-TCTTGATCCTCAAA-TACCTCCC-TNGNGCGTNGCAAATATGGTGTCTC-TTTTGGGGGCCCAT
22_Crepis_aurea	CTTGT-TCTTGATCCTCAAA-TACCTCCC-TNGNGCGTNGCAAATATGGTGTCTC-TTTTGGGGGCCCAT
21_Crepis_biennis	CTTGG-TCCTGACCCTCA---TAACCTCC-TGGCGTG---CAAATATGGTGTCGC-TTTTGTGG-CCCAT
32_Lapsana_communis	CTTGG-TCTTGATCCTCAA---AACCTCT-CGATGTG---CAATCGTGGTGTCTC-TTTTGGGGCACCAA
56_Taraxacum_laevigatum	CTTGG-TTCTGATCCTCAA---CACCTCC-TAGCGTG---CCTGCATGCTTTCTC-TTTTGGGCTATCAT
55_Taraxacum_laevigatum	CTTGG-TTCTGATCCTCAA---CACCTCC-TAGCGTG---CCTGCATGCTTTCTC-TTTTGGGCTATCAT
14_Cicerbita_alpina	CTTGG-TCCTGATCCCCCA--ACCCCTCC-CGACGTG---CATTTGTGGTGCCTTGTTTTGAGGC-CCAT
36_Mycelis_muralis	CTTGG-TCCAGATCCCTCG--ACCCCTCC-CGATGTG---CATTTGTGGTGCCTTGTTTTGAGGCGCCAT
30_Lactuca_perennis	CTTGG-TCTTGATCCCCCA--ACCCCTTC-TGATGTG---TATTTGTGGTGCCTT-CTTTTGGGCATCAT
31_Lactuca_virosa	CTTGG-TCCTTATCCCATATAACCCTTCC-TGACGTG---AATTCGTGGTGTCCT-TTTTAGGGCATCAT
40_Prenanthes_purpurea	CTCTG-TCCTTATCCCCAT----CCCTCC-CGGCGTG---CATGCGTGGTGCCTC-GTTCGGGGCACCAT
28_Hypochaeris_uniflora	ATTGG-TTGTTATCCTCAT---CCCCTCC-TGACATG---TGTTTGTGGCGCCTT-GTATAGGGTGCCAT
50_Scorzoneroides_autumnalis	CTTGG-TCCTTATCCCTTT---CCCCTCC-CTGCATG---CGTGTGTGGAATTCC-GTTTGGGGTGCCAT
51_Scorzoneroides_helvetica	CTTGG-TCCTTATCCCCTT---CCCCTCC-CTGCATG---CGTGTGTGGAGCTCC-GTTTGGGGTGCCAT
24_Helminthotheca_echioides	CTTGG-TCCTTGTCCCCAT---CCCCTCC-CGGCGTG---CGTGTGTGGCGCCTC-TTTCGGGGTGCCAT
38_Picris_hieracioides_subsp_morrisonensi	CTTGG-TCCTTATCCCCAT---CCCCTCC-CGGTGTG---TGTTTGTGTTGCCTC-TTTTGGGGTGCCAT
39_Picris_hieracioides_subsp_morrisonensi	CTTGG-TCCTTATCCCCAT---CCCCTCC-CGGTGTG---TGTTTGTGTTGCCTC-TTTTGGGGTGCCAT
33_Leontodon_hispidus	CTTGG-TCCTTTTCCCCAT---CCCCTCC-CGGCGTG---CATGTGTGGTGCCTC-TTCTGAGGTGCCAT
35_Leontodon_hispidus	CTTGG-TCCTTTTCCCCAT---CCCCTCC-CGGCGTG---CATGTGTGGTGCCTC-TTCTGAGGTGCCAT
34_Leontodon_incanus	CTTGG-TCCTTTTCCCCAT---CCCCTCC-CGGCGTG---CATGTGTGGTGCCTC-CTCTGGGGTGCCAT
27_Hypochaeris_radicata	CTTGG-CTCTTTTCCTTAT---CCCCTCT-CGGTGTGTG---TTTGTGATGCCTC-TTTTGGGGCGCCAC
44_Scorzonera_aristata	NNNNNNNNNNNNNNNNNNNNNNNNNNNNGGT-AGGTG-G---CGTTTGTGATGCTT--A-TTGGAGCACCAA
47_Scorzonera_humilis	CTAGG-ATTGGAACCCTGTT--GCC-TGT-CGGCGTG---CGTTTGTGATGCTTC-ATTTGGAGAACCAT
45_Scorzonera_austriaca	GAAGG-CCTCGATCCCTATT--TCC-TGT-TGGTGTG---CGTTTGTGCTGCCCC-ATTTGGAGTGCCAC
46_Scorzonera_hispanica	GAAGC-CCTCGAACCCTGTT--ACC-TGT-TGGCGTG---CATGCGTGTTGCTCC-TTTTGGAGCTCCAC
48_Scorzonera_purpurea	CAAGG-CTTGGTACCTTGTT--GTC-TGT-CGGTGTG---CATTTGTGTTGTTCT-ACTTGGAATGGCAT
49_Scorzonera_purpurea	CAAGG-CTTGGTACCTTGTT--GTC-TGT-CGGTGTG---CATTTGTGTTGTTCT-ACTTGGAATGGCAT
57_Tragopogon_dubius	TACGG-CCTTGATCCTTGTC--CCT-TGT-CGGCGTG---CGTTTGTGCCGCTCC-GAATGGAACGCCAT
58_Tragopogon_orientalis	TATGG-CCTTGATCCTTGTC--CCT-TGT-CGGCATG---CGTTTGTGCCGCTCC-GAATGGAACGCCAT
23_Echinops_sphaerocephalus	TGTGAGCCTGGGAGCT-GTGATGCTTTGT-TGGTGTG---CGCGCACCGGGCCAC-TTT-GTGGCCTTGT
29_Jurinea_mollis	TGTGAGCCT-GGTCCTCGTGATGCTTTGC-TGGCATG---CATGCATGGTGTTTC-TTGCGAGGCACCGT
42_Saussurea_discolor	TGCGAGCC-CGNGCCTCGTGNTGCTCCGC-TGGCATG---CNTCCATGGTGTCCC-TTTTGAGGCATCAT
43_Saussurea_discolor	TGCGAGCC-CGNGCCTCGTGNTGCTCCGC-TGGCATG---CNTCCATGGTGTCCC-TTTTGAGGCATCAT
37_Onopordum_acanthium	TGTGAGCCC-GGTGCCCGCGATGCTCCGT-CGGCGTG---CGTGCAAGATGCCCC-TTCCGGGGCATCGT

141-210

Wunderlichia_mirabilis	GGACG-TCCCATCGGCACCAT-AACAAACCCCCGGMASKGTCTGTGCCAAGGAAGCCCCAA-CTTWAKAG
1_Aposeris_foetida	GAATG-TCATGTTGG-ACCAT-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAATGAAA--ATGA-GA
2_Arctium_lappa	GGATG-TTGTGTCGGCACGAA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACCAAA--CTTAAGA
3_Arctium_lappa	GGATG-TTGTGTCGGCACGAA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACCAAA--CTTAAGA
4_Carduus_crassifolius	GGATG-TCGCGTCGGCACCTA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CATAGGA
5_Carduus_crassifolius	GGATG-TCGCGTCGGCACCTA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CATAGGA
16_Cirsium_arvense	GGATG-TTGCGTCGGCACCTA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CATAGGA
18_Cirsium_vulgare	GGATG-TTGCGTCGGCACCTA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CATAGGA
17_Cirsium_vulgare	GGATG-TTGCGTCGGCACCTA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CATAGGA
53_Silybum_marianum	GGATG-TTGCGTCGGCACCTA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CATATGA
8_Centaurea_cyanus	GGACG-TTGTGTCGGCACAAA-AACAAA-CCCCGGCACGGAATGTGCCAAGGAAAAAGAAA--CTCAAGA
10_Centaurea_montana	GGACG-TTGTGTCGGCACAAA-AACAAA-CCCCGGCACG-AATGTGC-AAGGAAAACAAAA--CTCAAGA
9_Centaurea_jacea	GGATG-TTTTGTCGGCACAAA-AACAAAACCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CTCAAGA
19_Cnicus_benedictus	GGATG-TTATGTCGGCACAAA-AACAAAACCCCGGCACGGCATGTGCCAAGGAAAAATAAA--CTGAAAA
11_Centaurea_scabiosa	GGACG-TTGTGTCGGCACATA-AACAAACCCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CTTAAGA
41_Rhaponticum_scariosum	GGATG-TTTTGTCGGCACAAA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA--CTTAAGA
52_Serratula_tinctoria_subsp_tinctoria	GGACG-TTGTGTCGGCACAAA-ACCAAA-CCCCGGCACGGAATGTGCCAAGGAAAACAAAA--CTTAAGA
7_Carlina_vulgaris	GGGTG-TTTCGTCGCCACGTA-AACAAA-CCCCGGCACAACACGTGCCAAGGAAAACAAAA--CTTAAGA
6_Carlina_vulgaris	GGGTG-TTTCGTCGCCACGTA-AACAAA-CCCCGGCACAACACGTGCCAAGGAAAACAAAA--CTTAAGA
12_Chlorocrepis_staticifolia	GGATG-TCATGCCGGCACCTT-AACAAA-CCCCGGCACGGAATGTGCCAAGGAATAC-AATAAATGA-GA
15_Cichorium_intybus	GGATT-TTAATCCGGCACCAT-AACAAA-CCCCGGCACGGTATGTGCCAAGGAAAAC-AACAAATGA-GA
25_Hieracium_murorum	GGACG-TCATGCCGGAAACAT-AACAAC-CCCCGGCACGGAATGTGCCAAGGAAAAC-AACATATGA-GA
26_Hieracium_pilosella	GGATG-TCATGTCGGAAACAT-AACAAC-CCCCGGCACGGAATGTGCCAAGGAAAAC-AACAAATGA-GA
13_Chondrilla_juncea	GGATGATTTTGTTGG-ACCAT-AACAAA-TCCCGGCACGGCATGTGCCAAGGAAAACATAATATGGAAGA
59_Willemetia_stipitata	GGATGTTTTAGTCGGGACCAT-AACAAA-CCCCGGCACAGCATGTGTCAAGGAATACAAAATATTGA-GA
54_Sonchus_oleraceus	GGATG-TCCCATCGG-ACTAT-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAATTAAA-TAAAA-GA
20_Crepis_aurea	GTATG-TATCGTCAG-ACTA---ACAAA-CCCCGGCACGGAATGTGCCAAGGAAAACATAA-TATGA-GA
22_Crepis_aurea	GTATG-TATCGTCAG-ACTA---ACAAA-CCCCGGCACGGAATGTGCCAAGGAAAACATAA-TATGA-GA
21_Crepis_biennis	GGATG-TCTCGTCAG-ACTA---ACAAC-CCCCGGCACGGAATGTGCCAAGGAAAACATAA-TATGA-GA
32_Lapsana_communis	GGATG-TCTCGTCGG-ACTATTAACAAA-CCCCGGCACGGAATGTGCCAAGGAAAACAAAA-AATGA-GA
56_Taraxacum_laevigatum	GCATG-TATTGTTGG-AATTT-AACAAAACCCCGGCACGGCATGTGCCAAGGAAAACAAAA-AACGA-GA
55_Taraxacum_laevigatum	GCATG-TATTGTTGG-AATTT-AACAAAACCCCGGCACGGCATGTGCCAAGGAAAACAAAA-AACGA-GA
14_Cicerbita_alpina	GGATG-TCCCGCCGG-ACCAT-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACATAA-AATGA-GA
36_Mycelis_muralis	GGATG-TTCTGTCGG-ACCAT-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA-ATTGA-GA
30_Lactuca_perennis	GGAT---CCTGTCAG-ACCAT-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAAA-AATGA-GA
31_Lactuca_virosa	GGAT---TCCGTTGG-ACCAT-AACAAAACCCCGGCACGGTATGTGCCAAGGAAAACAAAA-A-TGA-GA
40_Prenanthes_purpurea	AGATG-TTATGTCGG-ACTAT-AACAAC-CCCCGGCACGGCATGTGCCAAGGAAAACAAATAAAGA-GA
28_Hypochaeris_uniflora	GGATG-TCATGTCGG-ATCTT-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAGAAAAT----A
50_Scorzoneroides_autumnalis	GGATG-TCATGTTGG-GATTT-AACAAA-CCCCGGCACGGAATGTGCCAAGGAAAACAAA--AGTGA-GA
51_Scorzoneroides_helvetica	GCATG-TCATGTTGG-GATTT-AACAAA-CCCCGGCACGGAATGTGCCAAGGAAAACAAA--AGTGA-GA
24_Helminthotheca_echioides	AGATG-TGATGCTGG-ACTAT-AACAAAACCCCGGCACGGCATGTGCCAAGGAAAACGAA--AATGA-GA

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38_Picris_hieracioides_subsp_morrisonensi  AGATG--CATGCTGG-ACCAT-AACAAAACCCCGGCACGGCATGTGCCAAGGAAAACAAA--AATGA-GA
39_Picris_hieracioides_subsp_morrisonensi  AGATG--CATGCTGG-ACCAT-AACAAAACCCCGGCACGGCATGTGCCAAGGAAAACAAA--AATGA-GA
33_Leontodon_hispidus                      GGATG-TCAGGCTGG-ATTAT-AACAAAACCCCGGCACGGCATGTGCCAAGGATAACAATA-AATGA-GA
35_Leontodon_hispidus                      GGATG-TCAGGCTGG-ATTAT-AACAAAACCCCGGCACGGCATGTGCCAAGGATAACAATA-AATGA-GA
34_Leontodon_incanus                       GGATG-TCAGACTGG-ATTAT-AACAAAACCCCGGCACGGCATGTGCCAAGGAAAACAATA-AATGA-GA
27_Hypochaeris_radicata                    TGACG-TCATGCTGA-ACCTT-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAACAAA--ATCGA-GA
44_Scorzonera_aristata                     ATACG-TWCCGCCGACAA-CT-AACCAA-CCCCGGCACGGAATGTGCCAAGGAAAAC-AAAACAAAA-GA
47_Scorzonera_humilis                      ATACG-TTCCGCCGACAAGCT-AACCAA-CCCCGGCACGGAATGTGCCAAGGAAAAC-ATAACAAAA-GA
45_Scorzonera_austriaca                    GGACG-TTTTGCTAACAACCT-AACCAA-CCCCGGCACGGAATGTGCCAAGGAAAAC-AAAACGGAA-GA
46_Scorzonera_hispanica                    GGACG-TTTCGTTGACAGCCT-AACAAA-CCCCGGCACGGAATGTGCCAAGGAAAAC-AAAACAAAA-GA
48_Scorzonera_purpurea                     GGATG-TTTTGCTGACAATCT-AACCAA-CCCCGGCACGGAATGTGCCAAGGAAAAC-AAAACAAAA-GA
49_Scorzonera_purpurea                     GGATG-TTTTGCTGACAATCT-AACCAA-CCCCGGCACGGAATGTGCCAAGGAAAAC-AAAACAAAA-GA
57_Tragopogon_dubius                       AGATG-TTTTGTTGACAAGTT-AACCAA-CCCCGGCACGGAATGTGCCAAGGAAAAAGAAAACAAAA-GA
58_Tragopogon_orientalis                   AGATG-TTTTGTTGACAAGTT-AACCAA-CCCCGGCACGGAATGTGCCAAGGAAAAAGAAAACAAAA-GA
23_Echinops_sphaerocephalus                GGATG-TTATGCCGACACAAA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAAC-AAAACAT----A
29_Jurinea_mollis                          GGGTG-TTGTGTTGGCATGAT-AACAAACCCCCGGCACGGCATGTGCCAAGGAAAAC-AAAACATAA-GA
42_Saussurea_discolor                      GGACG-TTGTSTCGGCATGAA-AACAAACCCCCGGCACGGCATGTGCCAAGGAAAAC-CAAACTTAA-GA
43_Saussurea_discolor                      GGACG-TTGTSTCGGCATGAA-AACAAACCCCCGGCACGGCATGTGCCAAGGAAAAC-CAAACTTAA-GA
37_Onopordum_acanthium                     GTGCG-TCCCGTCGTCACCGA-AACAAA-CCCCGGCACGGCATGTGCCAAGGAAAAC-AAAACTTAA-GA
211-280
Wunderlichia_mirabilis                     GAGCGCGTGCCGCGTY--GCCCCGTAC-GTGGTGTGCACGCGGACCGTCGCTTC-TT-GGAG--TCACAA
1_Aposeris_foetida                         AGGTTTTCACCTGTTTT-GCCCCGTTT-GCGGTGTGCATACAGGTGCTAGCCTCCTT-GGAA--TTACAA
2_Arctium_lappa                            AGGGCGCGTCCTGTGTT-GCCCCGTTT-GCGGTGTGTGCACGGGTCGTGGCCTCTCATCAAA--CCATAA
3_Arctium_lappa                            AGGGCGCGTCCTGTGTT-GCCCCGTTT-GCGGTGTGTGCACGGGTCGTGGCCTCTCATCAAA--CCATAA
4_Carduus_crassifolius                     AGGTCGCGTCTCGTGAT-GCCTCGTTC-GCGGGGTGTGCATGGGTCGTGGCCTCTCAAT-AA--CCATAA
5_Carduus_crassifolius                     AGGTCGCGTCTCGTGAT-GCCTCGTTC-GCGGGGTGTGCATGGGTCGTGGCCTCTCAAT-AA--CCATAA
16_Cirsium_arvense                         AGGGCGCGTCCCGTGTT-GCCCCGTTT-GCGGTGTGCGCACGGGTCGTGGCCTCTCAAT-AA--CCATAA
18_Cirsium_vulgare                         AGGGCGTGTCCCGTGTT-GCCCCGTTC-GCGGTGTGCGCACGGGTCGTGGCCTCTCAAT-AA--CCATAA
17_Cirsium_vulgare                         AGGGCGTGTCCCGTGTT-GCCCCGTTC-GCGGTGTGCGCACGGGTCGTGGCCTCTCAAT-AA--CCATAA
53_Silybum_marianum                        AGGGTGCGTCTCGTGTT-GCCCCGTTC-GCGGTGCGCGCACGGGCCGTGGCCTCTCAAT-AA--CCATAA
8_Centaurea_cyanus                         AGGGTGCGTCTCGTGTT-GCCCCGTAT-TCGGTGTGCACATGGGTCGTGGCCTCTCATT-AA--CCATAA
10_Centaurea_montana                       AGGGTGCGTCTCGTGTT-GCCCCGTTT-TCGGTGTGCACACGGGTCGTGGCCTCTCATT-AA--CCATAA
9_Centaurea_jacea                          AGGGTGCGTCTCGTGTT-GCCCCGTTT-TCGGTGTGCATGCGGGTCGYGGCCTCTCATT-AA--CCATAA
19_Cnicus_benedictus                       AGGGTGTGTCTCGTGTT-GCCCCGTTA-TCGGTGTGCATGCGGGTCGTGGCCYCTTATT-AA--CCATAA
11_Centaurea_scabiosa                      AGGGTGCGTCACGTGTT-GTCCCGTTT-TCGGTGTGCACACGGGTCGTGGCCTCTCATT-AA--CCATAA
41_Rhaponticum_scariosum                   AGGGTGCGTCTCGTGTT-GTCCCGTTT-TCGGTGCGCACACAGGTCGTGGCCTCTCATT-AA--CCATAA
52_Serratula_tinctoria_subsp_tinctoria     AGGGTGCGTCTCGTGTT-GTCCCGTTT-TCGGTGTGCACACGGGTCGTGGCCTCTCA-ATAA--CCA---
7_Carlina_vulgaris                         AGGGCGCGTCCCGTGTT-GCCCCGTTC-GCGGAGCGCGCACGGGGC-TGGCCTCTCT-AGAA--CCACAA
6_Carlina_vulgaris                         AGGGCGCGTCCCGTGTT-GCCCCGTTC-GCGGAGCGCGCACGGGGC-TGGCCTCTCT-AGAA--CCACAA
12_Chlorocrepis_staticifolia               AGGATGCGTCCAGCATC-GCCCCGTTC-GCGGTGTGCGTGCTGGTCGTGGCCTCCTT-GGAA--TCACGA
15_Cichorium_intybus                       AGGATGCGTCCAACTTT-GTCCCGTTC-GCGGTGTGCATGTTGGTCGTGGCCTCCTT-AGAA--TCACAA
25_Hieracium_murorum                       AGGATGCATCCTGTTTT-GTCCCGTTC-GCGGTGTGCTTACAGGATGCGGCCTCCNTTGAAA--TCACAA
26_Hieracium_pilosella                     AGGACTCATCCTGTGTT-GTCCCGTTC-GCGGTGTGCATACAGGATGCGGCCTCCTT-GAAA--TCACAA
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13_Chondrilla_juncea                      TGGACTTGTCTTGTATT-GCCCCGTTT-GCGGTGTGCATTCAAGCTTTGGCCTACTT-AGAA--CAATAA
59_Willemetia_stipitata                   AGGACTTGTCCTGTACT-GCCCCGTTC-GCGGTTAGCATTCGGGTCTTGGCCTACTT-GGAA--CCATAA
54_Sonchus_oleraceus                      TGCTATTTACTTGATTT-GCCCCGTTTTACGGTGTGCATACAGGTGGTAGCCTTCTTT-AAA--ACACTA
20_Crepis_aurea                           AGGACTCGTCCTGTTAT-GTCCCGTTT-TCGGTGTGCATGCTGGCCGTGGCCTCCNTTTGAA--TCACAA
22_Crepis_aurea                           AGGACTCGTCCTGTTAT-GTCCCGTTT-TCGGTGTGCATGCTGGCCGTGGCCTCCNTTTGAA--TCACAA
21_Crepis_biennis                         AGGACATGTCCTGTTAT-GCCCCGTTT-TCGGTGTGCAAGCTGGTCGTGGCCTCCTTT-GAA--TCACAA
32_Lapsana_communis                       AGGACTCGTCCTGTTAT-GTCCCGTAC-GCGGTGTGCATTCTGGTCGTGGCCTCCTT-AGAA--TCATAA
56_Taraxacum_laevigatum                   AGGACTCGACCTGTAAT-GCCCCGTTT-GTGGTGTGCATTCTGAGCGTGTCCTCCTTT-GAA--TCACAA
55_Taraxacum_laevigatum                   AGGACTCGACCTGTAAT-GCCCCGTTT-GTGGTGTGCATTCTGAGCGTGTCCTCCTTT-GAA--TCACAA
14_Cicerbita_alpina                       AGGACACGTCCAGTATC-GCCCCGTTC-GCGGTGTGCGTGCGGGTTGTGGCCTCCTT-GGAA--TCACAA
36_Mycelis_muralis                        AGGGCACGTCCAGTGTC-GCCCCGTTT-GCGGTGTGCGTTCTGGTTGTGGCCTCCTT-GGAA--TCACAA
30_Lactuca_perennis                       AGGACACTTACTGTATT-GCCCCGTTT-GCGGTGTGCGTGC-GGTCGTGGCCTCCTT-GGAA--TCACAA
31_Lactuca_virosa                         AGGACACTTGCTGTTTC-GCCCCGTTC-GCGGTGTGCGTGCAGGTCGTGGCCTCTTT-GGAA--TCACAA
40_Prenanthes_purpurea                    AGGACTCGTCTTGTGTT-GCCCCGTTC-GCGGTGTGCATGCTGGACGTGGCCTCCTT-GGAA--TCACAA
28_Hypochaeris_uniflora                   AGGTCTCATCCTGTGTT-GCCCCGTTT-GCGGTGTGCATGCAGGTTGTGGCCTC-TTAGTAAAATAACAA
50_Scorzoneroides_autumnalis              AGGACTTGTCTTGTCTT-GCCCCGTTC-GCGGTGTGCAAGTGGGTCGTGGCCTCTTT-GTAA--TTAAAA
51_Scorzoneroides_helvetica               AGGACTCGTCTTGTCTT-GCCCCGTTC-GCGGTGTGCAAGCGGGTTGTGGCCTCTTT-GTAA--TTAAAA
24_Helminthotheca_echioides               AGGACTCGTACCGTGCT-GTCCCGTTC-GCGGTGTACATGCGGCTCGTGGACTCTTT-TTAA--TTACAA
38_Picris_hieracioides_subsp_morrisonensi AGGACTCGAACCGTGTT-GCCCTGTTT-GCAGTGTGCATGCGGTTCGTGGACTCTTT-GTAA--TTACAA
39_Picris_hieracioides_subsp_morrisonensi AGGACTCGAACCGTGTT-GCCCTGTTT-GCAGTGTGCATGCGGTTCGTGGACTCTTT-GTAA--TTACAA
33_Leontodon_hispidus                     AGGACTCGTGCCGTGTA-GCCCCGTTC-GCGGTGTGCATGCGGTTCGTGGACTCTTT-GTAA--TTACAA
35_Leontodon_hispidus                     AGGACTCGTGCCGTGTA-GCCCCGTTC-GCGGTGTGCATGCGGTTCGTGGACTCTTT-GTAA--TTACAA
34_Leontodon_incanus                      AGGACTCGTACCGTGTT-GCCCCGTTC-GCGGTGTGCATGCGGCTCGTGGACTCTTT-GTAA--TTACAA
27_Hypochaeris_radicata                   AGGACGCGTCTTGTGTT-GCCCCGTTC-GCGGTGTGCATGCGGGCCGTGGCCTTTTT-ATAA--TTACAA
44_Scorzonera_aristata                    AGACTACGCCTTGTGTT-GCCCCGTTC-GCGGTGTGCATGCGGGTAGTGGCCTCTT--GGAA--ACATAA
47_Scorzonera_humilis                     AGGGTGCGCCTCGTGTT-GCCCCGTTT-GCGGTGTGCATGCGGGTCGTGGCCTCTT--GGAA--ACAAAA
45_Scorzonera_austriaca                   AGGGTGCGCCCCGTGTT-GCCCCGTTT-GCGGTGTGCATGCGGGTCGTGGCCTCCT--GTAA--ACACAA
46_Scorzonera_hispanica                   AGGGCGTGCCCCGTGTT-GCCCCGTTC-GCGGTGTGCACGCGGGTCGTGGCCTCCT--GGAA--ACACAA
48_Scorzonera_purpurea                    AGG-TGCGGTTTGT-TTTGCCCCGTAC-GCGGTGTGCATGCGAATCGTGGCCTCTT--GGAA--ACACAA
49_Scorzonera_purpurea                    AGG-TGCGGTTTGT-TTTGCCCCGTAC-GCGGTGTGCATGCGAATCGTGGCCTCTT--GGAA--ACACAA
57_Tragopogon_dubius                      AGGTTGTACCTGGTTTTTGCCCCGTTC-GCGGTGTGCATGCTG-TTGCGACCTCTTT--CAA--ACAAAA
58_Tragopogon_orientalis                  AGGTTGTACCCGGTTTTTGCCCCGTTC-GCGGTGTGCATGCTGGTTGTGACCTCTTT--AAC--ACAAAA
23_Echinops_sphaerocephalus               AGGGTGCAC-CTGTGTT-GCTCCGTTC-GCGGTATGCGCACGGGTCGTGGCCTCTTT-GAAA--CCATAA
29_Jurinea_mollis                         ATGGCGCGTCCCGTGTT-GCCCCGTTC-GCGGTGTGCGCGTGGGTCGTGTCTTCTCATAAAA--CCATAA
42_Saussurea_discolor                     AGGGNGCTGCTSGTGTT-GCCCCGTTT-NCGGTGTGCACATGGGTCGTGGTCTCTCATTAA---CCATA-
43_Saussurea_discolor                     AGGGNGCTGCTSGTGTT-GCCCCGTTT-NCGGTGTGCACATGGGTCGTGGTCTCTCATTAA---CCATA-
37_Onopordum_acanthium                    AGGGCGCGTCTCGTGTT-GCCCCGTCC-GCGGTGTGCGCACGGGCCGTGGCCTCTCATTAA---CCATAA

281-350
Wunderlichia_mirabilis                    A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
1_Aposeris_foetida                        A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
2_Arctium_lappa                           A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
3_Arctium_lappa                           A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
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4_Carduus_crassifolius                     A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
5_Carduus_crassifolius                     A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
16_Cirsium_arvense                         A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATCAAGAACGTAGCAAAATGCGATACTTGGTG
18_Cirsium_vulgare                         A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
17_Cirsium_vulgare                         A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
53_Silybum_marianum                        A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
8_Centaurea_cyanus                         A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
10_Centaurea_montana                       A-CGACTATCG-----------------------------------------------------------
9_Centaurea_jacea                          A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
19_Cnicus_benedictus                       A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
11_Centaurea_scabiosa                      A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
41_Rhaponticum_scariosum                   A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
52_Serratula_tinctoria_subsp_tinctoria     ----------------------------------------------------------------------
7_Carlina_vulgaris                         A-CGACTCTCGGCAACGGATATCTTGGCTCACGCATCGATGAAGAACGTAGGAAAATGTGATACTTGGTG
6_Carlina_vulgaris                         A-CGACTCTCGGCAACGGATATCTTGGCTCACGCATCGATGAAGAACGTAGGAAAATGTGATACTTGGTG
12_Chlorocrepis_staticifolia               A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
15_Cichorium_intybus                       A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
25_Hieracium_murorum                       A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
26_Hieracium_pilosella                     A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
13_Chondrilla_juncea                       A-TGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
59_Willemetia_stipitata                    A-TGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
54_Sonchus_oleraceus                       A-TGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
20_Crepis_aurea                            A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
22_Crepis_aurea                            A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
21_Crepis_biennis                          A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
32_Lapsana_communis                        A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
56_Taraxacum_laevigatum                    A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
55_Taraxacum_laevigatum                    A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
14_Cicerbita_alpina                        A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
36_Mycelis_muralis                         A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
30_Lactuca_perennis                        A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
31_Lactuca_virosa                          A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
40_Prenanthes_purpurea                     A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
28_Hypochaeris_uniflora                    A-CGACTCTCN-----------------------------------------------------------
50_Scorzoneroides_autumnalis               A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
51_Scorzoneroides_helvetica                A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
24_Helminthotheca_echioides                A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
38_Picris_hieracioides_subsp_morrisonensi  A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
39_Picris_hieracioides_subsp_morrisonensi  A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
33_Leontodon_hispidus                      A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
35_Leontodon_hispidus                      A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
34_Leontodon_incanus                       A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
27_Hypochaeris_radicata                    A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
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44_Scorzonera_aristata                       A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
47_Scorzonera_humilis                        A-CGACTCTCGGCAACGGATATCTTGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
45_Scorzonera_austriaca                      AACGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
46_Scorzonera_hispanica                      A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
48_Scorzonera_purpurea                       A-CGACTCTCGGCAACGGATATCTTGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
49_Scorzonera_purpurea                       A-CGACTCTCGGCAACGGATATCTTGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
57_Tragopogon_dubius                         A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
58_Tragopogon_orientalis                     A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
23_Echinops_sphaerocephalus                  A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
29_Jurinea_mollis                            A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG
42_Saussurea_discolor                        ----------------------------------------------------------------------
43_Saussurea_discolor                        ----------------------------------------------------------------------
37_Onopordum_acanthium                       A-CGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGCAAAATGCGATACTTGGTG

351-420
Wunderlichia_mirabilis                       TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTYGGCCG-AGG
1_Aposeris_foetida                           TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCAGGCTA-AGG
2_Arctium_lappa                              TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
3_Arctium_lappa                              TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
4_Carduus_crassifolius                       TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
5_Carduus_crassifolius                       TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
16_Cirsium_arvense                           TGAATTGCAGAATCCCGTKAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
18_Cirsium_vulgare                           TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
17_Cirsium_vulgare                           TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
53_Silybum_marianum                          TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
8_Centaurea_cyanus                           TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
10_Centaurea_montana                         ----------------------------------------------------------------------
9_Centaurea_jacea                            TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTTGGCCG-AGG
19_Cnicus_benedictus                         TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
11_Centaurea_scabiosa                        TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
41_Rhaponticum_scariosum                     TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATTCGGCCG-AGG
52_Serratula_tinctoria_subsp_tinctoria       ----------------------------------------------------------------------
7_Carlina_vulgaris                           TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTTAGCGCAAGTTGCGCCCGAAGCCATCCGGTTG-AGA
6_Carlina_vulgaris                           TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTTAGCGCAAGTTGCGCCCGAAGCCATCCGGTTG-AGA
12_Chlorocrepis_staticifolia                 TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGCCG-AGG
15_Cichorium_intybus                         TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCCGGCCG-AGG
25_Hieracium_murorum                         TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGCCG-AGG
26_Hieracium_pilosella                       TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGCCG-AGG
13_Chondrilla_juncea                         TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCAGGTTG-AGG
59_Willemetia_stipitata                      TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCTCGAAACCTTCAGGTTG-AGG
54_Sonchus_oleraceus                         TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGCCG-AGG
20_Crepis_aurea                              TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTNGAGG
22_Crepis_aurea                              TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTNGAGG
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21_Crepis_biennis                         TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG-AGG
32_Lapsana_communis                       TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCCGGTTG-AGG
56_Taraxacum_laevigatum                   TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCTGAAGCCATCCGGTTG-AGG
55_Taraxacum_laevigatum                   TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCTGAAGCCATCCGGTTG-AGG
14_Cicerbita_alpina                       TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG-AGG
36_Mycelis_muralis                        TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG-AGG
30_Lactuca_perennis                       TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG-AGG
31_Lactuca_virosa                         TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGCTG-AGG
40_Prenanthes_purpurea                    TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGCTG-AGG
28_Hypochaeris_uniflora                   ----------------------------------------------------------------------
50_Scorzoneroides_autumnalis              TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGTTG-AGG
51_Scorzoneroides_helvetica               -GAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCCGGCCG-AGG
24_Helminthotheca_echioides               TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
38_Picris_hieracioides_subsp_morrisonensi TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGTCG-AGG
39_Picris_hieracioides_subsp_morrisonensi TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGTCG-AGG
33_Leontodon_hispidus                     TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCNGAGG
35_Leontodon_hispidus                     TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCNGAGG
34_Leontodon_incanus                      TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
27_Hypochaeris_radicata                   TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATCCGGCCG-AGG
44_Scorzonera_aristata                    TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATACGGCTS-AGG
47_Scorzonera_humilis                     TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCCGGCCG-AGG
45_Scorzonera_austriaca                   TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCCGGCTA-AGG
46_Scorzonera_hispanica                   TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCCGGCTG-AGG
48_Scorzonera_purpurea                    TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCGTTTGGCCG-AGG
49_Scorzonera_purpurea                    TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCGTTTGGCCG-AGG
57_Tragopogon_dubius                      TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCCGGTTG-AGG
58_Tragopogon_orientalis                  TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCAAAGCCATCCGGTTG-AGG
23_Echinops_sphaerocephalus               TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
29_Jurinea_mollis                         TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
42_Saussurea_discolor                     ----------------------------------------------------------------------
43_Saussurea_discolor                     ----------------------------------------------------------------------
37_Onopordum_acanthium                    TGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAAGTTGCGCCCGAAGCCATTCGGCCG-AGG
421-490
Wunderlichia_mirabilis                    GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCTC-CGACAACACGCGTTCGAGC--GGAACGCGT
1_Aposeris_foetida                        GCACGCCTGCCTGGGCGTCA---CACATCGCGTCGCCCC-CCTCCATACAT-TCCAAAC-TGGAACTATA
2_Arctium_lappa                           GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-CGACCACGCCT-CCCCAGTGGGGATGCGTG
3_Arctium_lappa                           GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-CGACCACGCCT-CCCCAGTGGGGATGCGTG
4_Carduus_crassifolius                    GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCACGCCT-CCATACGGGGGATGTGTA
5_Carduus_crassifolius                    GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCACGCCT-CCATACGGGGGATGTGTA
16_Cirsium_arvense                        GCACGTCTGCCTGGGCGTCA---CGCATTGCGTCGCCCC-AGACTACGCCT-CCCCAACGGGGATGCGTT
18_Cirsium_vulgare                        GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCACGCCTCCCCAACTGGGGATGCGTT
17_Cirsium_vulgare                        GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCACGCCTCCCCAACTGGGGATGCGTT
53_Silybum_marianum                       GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCACGCCT-TCCCTACGGGGATGCGTT
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8_Centaurea_cyanus                     GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCATGC-T-CCCACTTGGG-ATGTGTT
10_Centaurea_montana                   ------------------------------CGTCGCCCC-AGACCAAGC-T-CCCCATCGGG-AGGTGTT
9_Centaurea_jacea                      GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCATGC-T-CCCCCAGAGGGA--CGTT
19_Cnicus_benedictus                   GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCATGC-T-CCTGCATAGAGA--TGTT
11_Centaurea_scabiosa                  GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCATGC-T-CCCCCATGGGGACGTGTT
41_Rhaponticum_scariosum               GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCC-AGACCACGC-T-CCCCCATGGGGATGTGTT
52_Serratula_tinctoria_subsp_tinctoria --------------------------ATCGCGTCGCCCC-AGATCACCC-T-CCCCAATGGGCAGGTGTT
7_Carlina_vulgaris                     GCACATTTTCTTGGGCATCA---CACATCGCGTCTCTCCCA-ACTATGCCT-CCTTCATGCGATGGAGTG
6_Carlina_vulgaris                     GCACATTTTCTTGGGCATCA---CACATCGCGTCTCTCCCA-ACTATGCCT-CCTTCATGCGATGGAGTG
12_Chlorocrepis_staticifolia           GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCAACCCATTCAT-CCCTTATGGTGGTGTTTG
15_Cichorium_intybus                   GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCA--ACCATGCAT-CCCTTATCGGGACACATG
25_Hieracium_murorum                   GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCC--ACCAAACAT-CCCCT-TGGGGATACATG
26_Hieracium_pilosella                 GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCC--ACCAAGCAT-TCCTA-TGGGCATGCTTG
13_Chondrilla_juncea                   GCACGCCTGCCTGGGCGTCA---CACATCGTGTCGCTCCAC-TCCATAGTT-CCCAATTGGGT--TTGTG
59_Willemetia_stipitata                GCACGCCTGCCTGGGCGTCA---CGCATCGTGTCCCTCTAC-ACCACAGTT-CCCTATCGGGTGTTTGTG
54_Sonchus_oleraceus                   GCACGCCTGCCTGGGCGTCA---CGCATCTCGTCGCCCCCT-GCCACACAT-CCTAAAGGTGTATTAATG
20_Crepis_aurea                        GCACGCCTGCCTGGGCGTCA---CGCATCG-GTCGCCTCCC-ACCATACTCCTCCTAACGGGTTGTTTTG
22_Crepis_aurea                        GCACGCCTGCCTGGGCGTCA---CGCATCG-GTCGCCTCCC-ACCATACTCCTCCTAACGGGTTGTTTTG
21_Crepis_biennis                      GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCTC-ACCATACTTAGCCTAACGGGTTGTTTTG
32_Lapsana_communis                    GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCC-ATCATTCCTCTCCTAACAGGTAGGCTTG
56_Taraxacum_laevigatum                GCACGCCTGCCTGGGCGTCA---CGCATCGCGTTGCCCCCC-ATCATACTTCCCTTAA-GGGTAGTCGTG
55_Taraxacum_laevigatum                GCACGCCTGCCTGGGCGTCA---CGCATCGCGTTGCCCCCC-ATCATACTTCCCTTAA-GGGTAGTCGTG
14_Cicerbita_alpina                    GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCCC-ACCATACTTC-CCCAACGG-TAGTAATG
36_Mycelis_muralis                     GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCCC-ACCATACTTC-CTTGACGGGTATTAATG
30_Lactuca_perennis                    GCACGCCTGCCTGGGCGTCA---CACATCGCGTCGCTCCAA-ACCATGCTTC-CCTAACGGGTTGTGATG
31_Lactuca_virosa                      GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCCC-ACCATGCCTC-CCCAACGGGTTGGCATG
40_Prenanthes_purpurea                 GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCC-ACCATATTTC-CCCAAAGGGTAACGATA
28_Hypochaeris_uniflora                ------------GGGCGTCA---CGCATCGCGTCGCCCCCC-ACCACATGGTG---AAGGGG--------
50_Scorzoneroides_autumnalis           GCACGCCTGCCTGGGCGTCA---CGCTTCGCATCGCCCCCC-ACCATACA-TACCCAACGGGTACTAATG
51_Scorzoneroides_helvetica            GCACGCCTGCCTGGGCGTCA---CGCTTCGCGTCGCCCCCC-ACCATATAATACCCAACGGGTACTCATG
24_Helminthotheca_echioides            GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCATCC-ACCATA-ATTACCCAACAGGTACTCATG
38_Picris_hieracioides_subsp_morrisonensi GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCC-CCC-ATCATACA-AACCCAACCGGTTGTCATG
39_Picris_hieracioides_subsp_morrisonensi GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCC-CCC-ATCATACA-AACCCAACCGGTTGTCATG
33_Leontodon_hispidus                  GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCC-ACCATATTA------------ACTCATG
35_Leontodon_hispidus                  GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCC-ACCATATTA------------ACTCATG
34_Leontodon_incanus                   GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCC-ACCATATTA------------ACTCATG
27_Hypochaeris_radicata                GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCC--ACCA-AATTTCC--AA---------ATC
44_Scorzonera_aristata                 GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCC---CCATGTATTCC-TATTTGGGATGCATG
47_Scorzonera_humilis                  GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCC---CCATGTAT-CC-TATTTGGGATGCATG
45_Scorzonera_austriaca                GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCC---CCATGTAT-CC--TTTTGGGATGTATA
46_Scorzonera_hispanica                GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCC---CCATCCAT-CC--TTCAAGGATGTATG
48_Scorzonera_purpurea                 GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCC---CCATGTAT-CC-TATATAGAATGCATG
49_Scorzonera_purpurea                 GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCC---CCATGTAT-CC-TATATAGAATGCATG
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57_Tragopogon_dubius                       GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCCC-CCATGTATATCAGATTTGGGATGCATG
58_Tragopogon_orientalis                   GCACGCCTGCCTGGGCGTCA---CGCATCGCGTCGCTCCC---CCATGTATATCAGATTTGTAATGCATG
23_Echinops_sphaerocephalus                GCACGTCTGCCTGGGCGTCA---CGCATCACATCGCCCCTC-ACCAT---CATCCTAT---GGACAAGTG
29_Jurinea_mollis                          GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCG-ACCACGCCTCCCTCAT-GGGAATGGCTT
42_Saussurea_discolor                      --------------------------ATCGCGTCGCCCCCA-ACAACGCCTCCCTCAT-GGGGATGTGTT
43_Saussurea_discolor                      --------------------------ATCGCGTCGCCCCCA-ACAACGCCTCCCTCAT-GGGGATGTGTT
37_Onopordum_acanthium                     GCACGTCTGCCTGGGCGTCA---CGCATCGCGTCGCCCCCG-ACCACGCCTCTCCTAT-GGGGACGCGTT

491-560
Wunderlichia_mirabilis                     GGCGTCGGGGGCGGAGACTTGG-CCTCCCKTGCCCTCGGCGCGGTTGGCCCAAACAGGAGCCC-CTTTYG
1_Aposeris_foetida                         GGTGTT-GGGGCGGAAA-TTGG-CCTCCCGTTCTTGAAGTGCGGTTAGCCTAAATAGGAGTCC-CCTTCG
2_Arctium_lappa                            TCGTTT-GGGGCGGAGA-CTGG-TCTCCCGTGCCCATGGTGCGGTTGGCCTAAAAAGGAGTCC-CCTTTG
3_Arctium_lappa                            TCGTTT-GGGGCGGAGA-CTGG-TCTCCCGTGCCCATGGTGCGGTTGGCCTAAAAAGGAGTCC-CCTTTG
4_Carduus_crassifolius                     -TGTCT-GGGGCGGAGA-ATGG-TCTCCCGTGCCGTCGGCGCGGTTGGCCTAAAAAGGAGTCC-CCTTCG
5_Carduus_crassifolius                     -TGTCT-GGGGCGGAGA-ATGG-TCTCCCGTGCCGTCGGCGCGGTTGGCCTAAAAAGGAGTCC-CCTTCG
16_Cirsium_arvense                         -CGACT-GGGGCGGAGA-ATGG-TCTCCCGTGTCGTCGGCGTGGTTGGCCTAAAAAGGAGTCC-CCTTCG
18_Cirsium_vulgare                         -CGTCT-GGGGCGGAGA-ATGG-TCTCCCGTGTCGTCGGCGCGGTTGGCCTAAAAAGGAGTCC-CCTTCG
17_Cirsium_vulgare                         -CGTCT-GGGGCGGAGA-ATGG-TCTCCCGTGTCGTCGGCGCGGTTGGCCTAAAAAGGAGTCC-CCTTCG
53_Silybum_marianum                        -CGTCT-GGGGCGGAGA-ATGG-TCTCCCGTGCCGTCGGCGCGGTTGGCCTAAAAAGGAGTCC-CCTTCG
8_Centaurea_cyanus                         TGGTTT-GGGACGGAGA-TTGG-TCTCCCGTGCCTATGGTGCGGTTTGCCTAAAAAAGAGTCC-CCTTTG
10_Centaurea_montana                       TGGTCT-GGGTCGGAGA-CTGG-TCTCCCGTGCCGATGGTGTGGTTGGCCTAAAAAGGAGTCC-CCTTTG
9_Centaurea_jacea                          TGGTTT-GGGACGGATA-CTGG-CCTCCCGTGCCCATGGTGCGGTTGGCCTAAAAAGGAGTCC-CCTTTG
19_Cnicus_benedictus                       TGGTCT-GGGACGGAGA-CTGG-CCTCCCGTGTCGATGGTGCGGTTGGCCTAAAAAGGAGTCC-CCTTTG
11_Centaurea_scabiosa                      TGGTCT-GGGACGGATA-CTGG-GCTCCCGTGCCGATGGTGCGGTTGGCCTAAAAAGGAGTCC-CCTTTG
41_Rhaponticum_scariosum                   TTATCT-GGGACGGAGA-CTGG-TCTCCCGTGCCRACGGCGCGGTTGGCCTAAAAAGGAGTCC-CCTTTG
52_Serratula_tinctoria_subsp_tinctoria     TCGTCT-GGGACGGATA-CTGG-TCTCCCGTGTTGATGGCGCGGTTGGCCTAAAAAGGAGTCC-CCTTTG
7_Carlina_vulgaris                         CTCTT-GGGGGCGGAGA-CTGG-CCTCCCGTGCCTACGGTGTGACTGGCCCAAATAGGAGTCC-CCTTCG
6_Carlina_vulgaris                         CTCTT-GGGGGCGGAGA-CTGG-CCTCCCGTGCCTACGGTGTGACTGGCCCAAATAGGAGTCC-CCTTCG
12_Chlorocrepis_staticifolia               GCATC--GGGGCGGATA-TTGG-TCTCCCGTGCTGTTGGTGCGGTTGGCCTAAACTGGAGTCC-CCTTCG
15_Cichorium_intybus                       GCAAT--GGGGCGGAGA-TTGG-TCTCCCGTGCTTATGGTGCGGTTGGCCTAAACTGGAGTCC-CCTTCG
25_Hieracium_murorum                       GCATC--GGGGCGGAGA-TTGG-CCTCCCATTCTTTT-GGGTGGTTGGCCTAAANTGGAGTCC-CCTTCG
26_Hieracium_pilosella                     GGATC--GGGGCGGAGA-TTGG-CCTCCCATGCCTTCGGTGTGGTTGGCCTAAACCGGAGTCC-CCTTCG
13_Chondrilla_juncea                       GCGTTTTGGGGCGGAGA-TTGG-TCTCCTGTGCTGATGGTGCGGTTGGCCTAAATTAGAGTCC-CCATTT
59_Willemetia_stipitata                    GCGTTT-GGGGCGGAGA-TTGG-CCTCCCGTGCATTTGGTGCGGTTGGCCTAAATTAGAGTCC-CCATTT
54_Sonchus_oleraceus                       GTCAT-GGGGGCGGAAA-TTGG-CCTCCCGTTCTTGT-GTTTGGTTGGCCTAAAGATGAGTCC-CCTTAG
20_Crepis_aurea                            GTGTTT-GGGGC-GAGA-TTGG-CCTCCCATGCTTGTAGTGTGGTTGGCCTAAATATGAGTCC-CCTTCG
22_Crepis_aurea                            GTGTTT-GGGGC-GAGA-TTGG-CCTCCCATGCTTGTAGTGTGGTTGGCCTAAATATGAGTCC-CCTTCG
21_Crepis_biennis                          GTGTTT-GGGGCGGAGA-TTGG-CCTCCCGTGCTTCTAGTGTGGTTGGCCTAAATGTGAGTCC-CCTTCG
32_Lapsana_communis                        GTGATA-GGGGCGGAGA-TTGG-TCTCCCGTGCTTTTTGTGTGGTTGGCCTAAAAAGGAGTCC-CTTTCG
56_Taraxacum_laevigatum                    GTGATTGGGAGCGGAGA-TTGG-CCTCCCGTACTTGTGGTGCGGTTGGTCAAAATAGGAGTCC-CCTTCG
55_Taraxacum_laevigatum                    GTGATTGGGAGCGGAGA-TTGG-CCTCCCGTACTTGTGGTGCGGTTGGTCAAAATAGGAGTCC-CCTTCG
14_Cicerbita_alpina                        GTGTT--GGGGCGGATA-ATGG-CCTCCCGTGCTTGT--CGCGGTTGGCCTAAATAGGAGTCC-CCTTCG
36_Mycelis_muralis                         GTGTT-GGGGGCGGATA-ATGG-CCTCCCGTGCTTGT--TGCGGTTGGCCTAAACAGGAGTCC-CCTTCG
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30_Lactuca_perennis                        GTGTTA-GGGGCGGATA-GTGG-CCTCCCGTTCTTATGTTTCGGTTGGCCTAAATAGGAGTTC-CCTTCA
31_Lactuca_virosa                          GTGTT--GGGGCGGATA-ATGG-CCTCCCGTGCTTGTGTTTCGGTTGGCCTAAATAAGAGTTC-CCTTCG
40_Prenanthes_purpurea                     GTGTT-GGGGGCGGAGA-TTGG-CCTCCTGTGCTTTTGTTGCGGTTGGCCTAAATAGGAGTCC-CCTTCG
28_Hypochaeris_uniflora                    -----------CGGAGA-TTGGNTCTCCCGTAC-TCTGTTGCGGTTGGCCTAAAAATGAGTCC-CCTTTA
50_Scorzoneroides_autumnalis               GTGTTT-GGGGCGGAGA-TTGG-CCTCCCGCACCTCTGATGCGGTTGGCCTAAAAATGAGTCC-CCTTCA
51_Scorzoneroides_helvetica                GTGTTT-GGGGCGGAGA-TTGG-CCTCCCGTACTTGTATTGCGGTTGGCCTAAAAATGAGTCC-CCTTCA
24_Helminthotheca_echioides                GTGAT--GTGGCGGAGA-ATGG-TCTCCCGTACATGT--GGCGGTTGGCCTAAAAAGGAGTCC-CCTTCA
38_Picris_hieracioides_subsp_morrisonensi  GTGAT--GGGGCGGAGA-TTGG-TCTCCCGTACTTGT--TTCGGTTGGCCCAAAAACGAGTCC-CCTTCA
39_Picris_hieracioides_subsp_morrisonensi  GTGAT--GGGGCGGAGA-TTGG-TCTCCCGTACTTGT--TTCGGTTGGCCCAAAAACGAGTCC-CCTTCA
33_Leontodon_hispidus                      GTGAT-GGGGGCGGAGA-TTGG-TCTCCCGTGCCTGT--CGCGGTTGGCCTAAAAAGGAGTCC-CCTTCA
35_Leontodon_hispidus                      GTGAT-GGGGGCGGAGA-TTGG-TCTCCCGTGCCTGT--CGCGGTTGGCCTAAAAAGGAGTCC-CCTTCA
34_Leontodon_incanus                       GTGAT-GGGAGCGGAGA-TTGG-TCTCCCGTGCTTGT--CGCGGTTGGCCTAAAAAGGAGTCC-CCTTCA
27_Hypochaeris_radicata                    TGGTT-GGGGGCGGAGA-TTGG-CCTCCCGTACCTGT--TGTGGTTGGCCTAAAAAGGAGTCC-CCTTCG
44_Scorzonera_aristata                     TTTAT--GGGGCGGAAA-CTGG-CCTCCCGTGCTCTTGGTGTGGTTGGCCCAAACA---GTCC-CCTGTG
47_Scorzonera_humilis                      TTYTT-GGGGGCGGAGA-TTGG-CCTCCCGTTCTCTTGGTGTGGTTGGCCCAAACACGAGTCC-CCTGTG
45_Scorzonera_austriaca                    GTATC--GGGGCGGAGA-TTGG-CCTCCCGTGATCTTATTGCGGTTGGCCCAAACACGAGTCC-CTTTCG
46_Scorzonera_hispanica                    CTACC--GGGGCGGAGA-TTGG-CCTCGTGTGCTCTAGGCGTGCTTGGCCCAAACACGAGTCC-CCTTCG
48_Scorzonera_purpurea                     -TGAC-GGGGGCGGAGA-TTGG-CCTCCTGTGCTCTTTGTGTGGTTGGCCCAAACATGAGTCT--TTGTA
49_Scorzonera_purpurea                     -TGAC-GGGGGCGGAGA-TTGG-CCTCCTGTGCTCTTTGTGTGGTTGGCCCAAACATGAGTCT--TTGTA
57_Tragopogon_dubius                       GTGTC--GGGGCGGAGA-TTGG-TCTCCTGTGCAACTGTCGCGGTTGGCCCAAACATGAGTCA-CCTTCG
58_Tragopogon_orientalis                   GTTTC--GGGGCGGAGA-TTGG-TCTCCCGTGCAACTGCTGCGGTTGGCCCAAACATGAGTCA-CCTTCG
23_Echinops_sphaerocephalus                GTGTA-GGGAGCGGATA-TTGG-TCTCCCGTGCCCATGGTGTGGTTGATCTAAATAGGAGTCCTCCTTCG
29_Jurinea_mollis                          GTGTC-GGGGGCGGAGA-CTGG-CCTCCCATGCCCATGGTGTGGTTGGCCTAAAAAGGAGTCC-CCYTTG
42_Saussurea_discolor                      TTGTTT-GGGGCGGATA-ATGG-TCTCCCGTGCTCATGGTGCGGTTGGCCTAAAAAGGAGTCC-CCTTCG
43_Saussurea_discolor                      TTGTTT-GGGGCGGATA-ATGG-TCTCCCGTGCTCATGGTGCGGTTGGCCTAAAAAGGAGTCC-CCTTCG
37_Onopordum_acanthium                      TGGTCC-GGGGCGGAGA-CTGG-TCTCCCGTGCCCATGGCGCGGTTGGCCTAAAAAGGAGTCC-CCTCCG

561-630
Wunderlichia_mirabilis                     ACG-GGCGCACGGCCAGTGGTGGTTGAC-A-AGG---CCCTCGGTTTGTGCCGTGCGNTCKTG-TGTCRC
1_Aposeris_foetida                         GCG-GACACACAACTAGTGGTGGTTGTA-T-AGA---CCCTCTTCTTGTGTTGTGTGTT-GTA-AGCTGT
2_Arctium_lappa                            ACG-GACGCACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCGTATCGAGCCGTGCG--------GACGC
3_Arctium_lappa                            ACG-GACGCACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCGTATCGAGCCGTGCG--------GACGC
4_Carduus_crassifolius                     ACG-GACGCACGGCTAGTGGTGGTTGAT-A-AGG---CCTTCGTATCGAGCCGTGTGTC-GTT-AGCCGC
5_Carduus_crassifolius                     ACG-GACGCACGGCTAGTGGTGGTTGAT-A-AGG---CCTTCGTATCGAGCCGTGTGTC-GTT-AGCCGC
16_Cirsium_arvense                         GCG-GACGCACGGTTAGTGGTGGTTGTT-A-AGG---CCTTCGTATCGAGCCGTGTGTC-GTT-AGCCGC
18_Cirsium_vulgare                         ACG-GACGCACGGCTAGTGGTGGTTGTT-A-AGG---CCTTCGTATCGAGCCGTGTGTC-GTT-AGCCGC
17_Cirsium_vulgare                         ACG-GACGCACGGCTAGTGGTGGTTGTT-A-AGG---CCTTCGTATCGAGCCGTGTGTC-GTT-AGCCGC
53_Silybum_marianum                        ACG-GACGCACGGCTAGTGGTGGTTGTT-A-AGG---CCTTCGTATCGAGCCGTGTGTC-GTT-AGCCGC
8_Centaurea_cyanus                         GCG-GACGCACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCGTATCGAGCCGTGCT--------AATGC
10_Centaurea_montana                       GCG-GATCGACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCATATCGAGTCGTGCT--------GATGC
9_Centaurea_jacea                          GCG-GACGCACGGCTAGTGGTGGTTGTC-A-AGA---CCTTCGTATCGAGCCGTGGT--------GATGC
19_Cnicus_benedictus                       GCG-GACGCACGGCTAGTGGTGGTTGTC-A-AGA---CCTTCGTATCGAGCCGTGGT--------GATGC
11_Centaurea_scabiosa                      GCG-GACGCACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCGTATCGAGCCGTGCT--------GATGC
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41_Rhaponticum_scariosum                  GCG-GGCGCACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCGTATCGAGCCGTGTT--------GATGC
52_Serratula_tinctoria_subsp_tinctoria    GCG-GAGGCACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCGTATCGAGCCGTGCT--------GAAGC
7_Carlina_vulgaris                        ACG-GACGCACGACTAGTGGTG-TTGTA-A-TGG---CCCTCGTATCGAGTCGTGTGTC-GCG-AGCCGC
6_Carlina_vulgaris                        ACG-GACGCACGACTAGTGGTG-TTGTA-A-TGG---CCCTCGTATCGAGTCGTGTGTC-GCG-AGCCGC
12_Chlorocrepis_staticifolia              GTG-GACGCACAACTAGTGGTGGTTGAA-T-AGA---CCCTCGTCTTGTGTTGTGCGCC-GTG-AGCTGT
15_Cichorium_intybus                      GTG-GACGCACGACTAGTGGTGGTTGAA-A-AGA---CCCTCGTATTGTGTCGTGCGTC-ATG-AGCTGT
25_Hieracium_murorum                      GTGNGACGCACGACTAGTGGTGGTTGAANANAGA---CCCTCTTCCTGTGCCGTGCGTC-TTA-AGCTGT
26_Hieracium_pilosella                    GTG-GACGCACGACTAGTGGTGGTTGAA-T-AGA---CCCTCGTCATGTGTCGTGCGTC-TTG-ATCTGT
13_Chondrilla_juncea                      GCG-GACACACAGTTAGTGGTGGTTGAA-T-AGA---CCTTCGTCTTTGGCTGTGTGTT-TTG-AGCTGC
59_Willemetia_stipitata                   GCG-GACACACGGATAGTGGTGGTTGAA-T-AGA---CCCTTGTCTTTTACCGTGTGTT-GTG-AGCTCC
54_Sonchus_oleraceus                      GGCGGATGCACAACTAGTGGTGGTTGAA-T-AGA---CCCTCGTCTTGTGTTGTGTGTC-GTG-AGCTGT
20_Crepis_aurea                           GTGNGATACACGGCTAGTGGTGGTTGTANTNTGA---CCCTCGTATTGTGCTGTGTGTC-GTG-AGCTGC
22_Crepis_aurea                           GTGNGATACACGGCTAGTGGTGGTTGTANTNTGA---CCCTCGTATTGTGCTGTGTGTC-GTG-AGCTGC
21_Crepis_biennis                         GTG-GATACACGGCTAGTGGTGGTTGTA-T-TGA---CCCTCGTATTGTGCCGTGTGTT-GTG-AGCTGC
32_Lapsana_communis                       GTG-GATACACGGCTAGTGGTGGTTGTA-A-AGA---CCCTCTTTATGTGTTGTGTGTT-GTG-AGCTGC
56_Taraxacum_laevigatum                   GTG-GACACACGGCTAGTGGTGGTTGTA-A-AGA---CCCTTTTCTTTTGCTGTGTGTT-GTG-AGCTGT
55_Taraxacum_laevigatum                   GTG-GACACACGGCTAGTGGTGGTTGTA-A-AGA---CCCTTTTCTTTTGCTGTGTGTT-GTG-AGCTGT
14_Cicerbita_alpina                       GTG-GACGCACGACTAGTGGTGGTTGAA-C-AGA---CCCTCGTCTTGTGTTGTGTGTC-GTG-AGCTGT
36_Mycelis_muralis                        GCG-GACACACGACTAGTGGTGGTTGAA-C-AGA---CCCTCGTCTTGTGTTGTGTGTC-GTG-GGCTGT
30_Lactuca_perennis                       GCG-GACACACAACTAGTGGTGGTTGAA-C-AGA---CCTTCGTCTTGGGTTGTGTGTC-GTG-AGCTGT
31_Lactuca_virosa                         GCG-GACAC--GACTAGTGGTGGTTGAA-T-AGA---CCCTCGTCTTTTGTTGTGTGTC-GTG-AGCTGT
40_Prenanthes_purpurea                    GTG-GACACACGACTAGTGGTGGTTGAG-C-AGA---CCCTCGTCTCGTGTTGTGTGTC-GTG-AGCTGC
28_Hypochaeris_uniflora                   GCG-GACACATGACTAGTGGTGGTTGAANCNTGA---CCCTCGTCTT-TATCGTGTGTC-GTG-AGCTGC
50_Scorzoneroides_autumnalis              GCG-GACACACGACTAGTGGTGGTTGAA-C-AGA---CCCTCGTCCT-TATCGTGTGTC-GTG-AGCTGC
51_Scorzoneroides_helvetica               GCG-GACGCACGACTAGTGGTGGTTGAA-C-AGA---CCCTCGTCCT-TATCGTGTGTC-GTG-AGCTGC
24_Helminthotheca_echioides               GCG-GACACACGACTAGTGGTGGTTGAA-C-AGA---CCCTCGTCTT-TATCGTGTGTC-GTG-AGCTAC
38_Picris_hieracioides_subsp_morrisonensi GTG-GACACACGACTAGTGGTGGTTTAA-C-AGA---CCCTTGTCTT-TATCGTGTGTT-ATG-AGCTGC
39_Picris_hieracioides_subsp_morrisonensi GTG-GACACACGACTAGTGGTGGTTTAA-C-AGA---CCCTTGTCTT-TATCGTGTGTT-ATG-AGCTGC
33_Leontodon_hispidus                     GTGNGACACACGATTAGTGGTGGTTGAANCNAGG---CCTTCG-CTT-TATCGCGTG-C-GTG-AGCT-C
35_Leontodon_hispidus                     GTGNGACACACGATTAGTGGTGGTTGAANCNAGG---CCTTCG-CTT-TATCGCGTG-C-GTG-AGCT-C
34_Leontodon_incanus                      GCG-GACACACGACTAGTGGTGGTTGAA-C-AGA---CCCTCGTCTT-TATCGTGTGTC-GTG-AGCT-C
27_Hypochaeris_radicata                   GTG-GACACACGACTAGTGGTGGTTGAA-C-AGA---CCCTCGTCTT-TATTGTGTGTC-ATG-AGCTGC
44_Scorzonera_aristata                    CTG-GACACACAACANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
47_Scorzonera_humilis                     GTG-GACGCACGACAAGTGGTGGTTGAA-T-AGG---CCCTCGTCTTTGGTCGTGTGTC-GTTTAGCTGC
45_Scorzonera_austriaca                   GTG-GATGCACAACTAGTGGTGGTTGAA-T-AGG---CCCTCTTCTTCTGTTGTGTGTC-GTT-TGCTGC
46_Scorzonera_hispanica                   GTG-GACGCACGACTAGTGGTGGTTGAA-T-AGG---CCCTCGTCTTTTGTCGTGTGCC-GTG-TGCTGC
48_Scorzonera_purpurea                    GTG-AATGCACGACAAGTGGTGGTTGAA-T-AGG---CCCTCGTCTTTAGTCGT-AATA-GTT-TGCTAC
49_Scorzonera_purpurea                    GTG-AATGCACGACAAGTGGTGGTTGAA-T-AGG---CCCTCGTCTTTAGTCGT-AATA-GTT-TGCTAC
57_Tragopogon_dubius                      GTT-GACGCACGGCTAGTGGTGGTTGAA-T-AGG---CCCTCGTCTTTTGCCGTGCGTC-GTT-TGCTGC
58_Tragopogon_orientalis                  GTT-GACGCACGGCTAGTGGTGGTTGAA-T-AGG---CCCTCGTCTTTTGTTGTGCGTC-GTT-TGCTGC
23_Echinops_sphaerocephalus               GTG-GATGCACGGCTAGTGGTGGTTGTA-T-A-----ATCTCGTATCGAGCCGTGTGTT-GTG-AGCCGC
29_Jurinea_mollis                         TCG-GACGCACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCGTAACGAGCCGTGTG--------GATGC
42_Saussurea_discolor                     ACG-GACGCACGGCTAGTGGTGGTTNTC-A-AGG---CCTTCGTATCGAGCYGTGCA--------TACGC
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43_Saussurea_discolor                    ACG-GACGCACGGCTAGTGGTGGTTNTC-A-AGG---CCTTCGTATCGAGCYGTGCA--------TACGC
37_Onopordum_acanthium                   ACG-GACGCACGGCTAGTGGTGGTTGTC-A-AGG---CCTTCGTATCGAGCCGTGCGTC-GTG-AGCCGC
631-694
Wunderlichia_mirabilis                   G-AR-GCGATGCCCTTSG-AAG-NACTTA-GGTTTG-TCTCC------GNGGGAAACTTANNNN
1_Aposeris_foetida                       G-AGGGAAGTTCTCATTAAAAGACCCCACTGTATCA-TCCTT-----GGATG-TTACATC--GA
2_Arctium_lappa                          A-AGGGAAGCGCTCTCCA-ATGACCCCAACGTGTCG-TCTTG-----CAACG-ACGCTTC--GA
3_Arctium_lappa                          A-AGGGAAGCGCTCTCCA-ATGACCCCAACGTGTCG-TCTTG-----CAACG-ACGCTTC--GA
4_Carduus_crassifolius                   A-AGGGAAGCGCNCTCCG-TAGACCCTAATGTGTCG-TCACG-----CGACG-ATGCTTC--GA
5_Carduus_crassifolius                   A-AGGGAAGCGCNCTCCG-TAGACCCTAATGTGTCG-TCACG-----CGACG-ATGCTTC--GA
16_Cirsium_arvense                       A-AGGGAAGCACTTCTTA-AAGACCCCAATGTGTCG-TCTCG-----TGACG-ACGCTTC--GA
18_Cirsium_vulgare                       A-AGGGAAGCGCTCTCTA-AAGACCCTAACGTGTCG-TCTTG-----TGACG-ANGCTTC--GA
17_Cirsium_vulgare                       A-AGGGAAGCGCTCTCTA-AAGACCCTAACGTGTCG-TCTTG-----TGACG-ANGCTTC--GA
53_Silybum_marianum                      A-AGGGAAGCGCTCTCCA-AAGACCCCAACGTGTCG-TCTCG-----CGACG-ATGCTTC--GA
8_Centaurea_cyanus                       T-AGGGAGGTGCTCTCTA-AAGACCCTAATGTGTCG-TGTTA-----TGACG-ATGCTTC--GA
10_Centaurea_montana                     T-AGGGAGTTGCTCTCTA-AAGACCCTAATGTGTNN-N-TTA-----CGACG-ATGCTTC--GA
9_Centaurea_jacea                        T-AGGGAGTNGCTCTCTA-GAGACCCTAACGTGTCG-TCTTA-----CGACG-ATGCTTC--GA
19_Cnicus_benedictus                     T-AGGGGGTCGCTCTCTA-AAGACCCTAACGTGTCG-TCTTG-----CGACG-ATGCTTC--GA
11_Centaurea_scabiosa                    T-AGGGAATCGCACTCTA-TAGACCCTAATGTGTCG-TTTTA-----CGACG-ACGCTTC--GA
41_Rhaponticum_scariosum                 A-AGGGATTTGCWCTCTA-AAGACCCTAACGTGTCG-TCTTA-----CGACG-ATGCTTC--GA
52_Serratula_tinctoria_subsp_tinctoria   A-AGGGAATCRCTCTCTA-TAGACCCTAACGTGTCG-TCTTA-----CGACG-ATGCTTC--GA
7_Carlina_vulgaris                       A-AGGGAATTGCTCGACA-AAGACACCAACGCGTCG--CGAT-----CGACG-ACGCTTC--AA
6_Carlina_vulgaris                       A-AGGGAATTGCTCGACA-AAGACACCAACGCGTCG--CGAT-----CGACG-ACGCTTC--AA
12_Chlorocrepis_staticifolia             G-AGGGAGGCCTTTTACG-AAGACCCCAATGTGTTG-TC-TT----GCGATG-ACGCTTC--GA
15_Cichorium_intybus                     G-AGGGAGGCCCTTGATG-AAGACCCCAATGTATCG-TC-TT----GAGACG-ATGCTTC--GA
25_Hieracium_murorum                     G-AAGGATGTGCTCGATA-AAGA-CCCAATGCGTCG-TCCTT----GC-ACG-ATCTTNC--GA
26_Hieracium_pilosella                   G-AGGGATGTGCTCGATG-AAGACCCCAATGTGTCG-TC-TT----GCGACG-ATGCTTC--GA
13_Chondrilla_juncea                     T-TGGGAAGTACT-AACGAAAGACCCTAA--YATCG-TCATT-----AGACG-ATGCTTC--GA
59_Willemetia_stipitata                  A-TGGGAAGTACTAAAAGAAAGACCCTATTGTATCG-TCTTG-----CGATG-ATGCTTC--GA
54_Sonchus_oleraceus                     G-AGGGAAATTCTCAATT-TAGACCCCACTGTATCG-TTAAA-----AAACG-ATATATC--GA
20_Crepis_aurea                          T-AGGGTAACCCTCATCA-AAGACCCAATCGTATCG-TCTTC-CTTAGGACG-ATGCTTC--GA
22_Crepis_aurea                          T-AGGGTAACCCTCATCA-AAGACCCAATCGTATCG-TCTTC-CTTAGGACG-ATGCTTC--GA
21_Crepis_biennis                        T-AGGGTAACCCTCATACCAAGACCCCATTGTATTG-TCTTCTCTTAAGACG-ATGCTTC--GA
32_Lapsana_communis                      T-AGGGTAACCCTCAACA-AAGACCCCATTGTATCG-TCTTG-----CGACG-ATGCTTC--GA
56_Taraxacum_laevigatum                  T-TGGGAAACCCTCAAAA-AAGACCCCAATGTGTCGTTCTA------GGATG-ATACTTC--GA
55_Taraxacum_laevigatum                  T-TGGGAAACCCTCAAAA-AAGACCCCAATGTGTCGTTCTA------GGATG-ATACTTC--GA
14_Cicerbita_alpina                      T-AGGGAAGCCCTCATTA-AAGACCCCATTGTATCG-TCTAT-----CGACG-ATGCTTC--GA
36_Mycelis_muralis                       A-AGGGATGACCTCATTA-AAGACCCCATTGTATCG-TCTAT-----GGATG-ATGCTTC--GA
30_Lactuca_perennis                      G-AGGGAAGCCATCATCA-ATGACCCCTTTGTATCG-TCTTC-----GGACG-GTGCTTC--GA
31_Lactuca_virosa                        A-GGGGAAGCCCTCATCA-AAGACCCCATTGTGTCG-TCTTC-----GGATG-ATGCTTC--GA
40_Prenanthes_purpurea                   T-AGGGAAACCCTCATCA-AAGACCCCAATGTATCG-TCTTT-----TGACG-ATGCTTY--GA
28_Hypochaeris_uniflora                  T-AGGGAAGCCCTCATAA-TAGNCCCGATCGTATCG-TTTTA-----GTACG-GTGCTTC--GA
50_Scorzoneroides_autumnalis             A-AGGGAAACCCTCACCA-AAGACCCTATTGCATTGTTTTTT-----GGACA-ATGCTTC--GA
51_Scorzoneroides_helvetica              T-AGGGAAGCCCTCACCA-AAGACCCCATTGCATTGTTTTTT-----GGACG-GTGCTTC--GA
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24_Helminthotheca_echioides           GAAGGGAAGCCCTCATAA-AAGACCCTATCGTATCG-TTTGA-----GGACG-GTGCTTC--GA
38_Picris_hieracioides_subsp_morrisonensi T-AGGGAAGCCCTCATCA--AGACCCCATCGTATCG-TTTTA-----GGACG-GTGCTTC--GA
39_Picris_hieracioides_subsp_morrisonensi T-AGGGAAGCCCTCATCA--AGACCCCATCGTATCG-TTTTA-----GGACG-GTGCTTC--GA
33_Leontodon_hispidus                 T-AGGGAA-CCATTATTA-AAGACCCCATGG--TCG-TTTTA-----GGACG-GGGCTTC--GA
35_Leontodon_hispidus                 T-AGGGAA-CCATTATTA-AAGACCCCATGG--TCG-TTTTA-----GGACG-GGGCTTC--GA
34_Leontodon_incanus                  T-AGGGAAGCCATCAACA-AAGACCCCATTGTATCG-TTTCA-----GGACG-GTGCTTC--GA
27_Hypochaeris_radicata               T-AGGGA-GCCCTCATCA-AAGACCCTTT-GTATCGTTTTCG------GACG-GTGCTTC--GA
44_Scorzonera_aristata                NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
47_Scorzonera_humilis                 A-AGGGCTCTTGTATTA---AGACCCTAACGCATTA-TC-CT----ATGATG-ATGCTTC--GA
45_Scorzonera_austriaca               A-TGGGATCCTCTCGTAT-AAGACCCTAATGCATTG-TC-CA----ACGATG-ATGCTTC--GA
46_Scorzonera_hispanica               A-AGGGAACCTCTTTTAT-AAGACCCCAATGCATCT-TC-CT----ATGACG-ATGTTTC--GA
48_Scorzonera_purpurea                A-GGATAACCTCTCGTAT-AAGACCCCAATGCATTG-TC-C--------ATG-ATGCTTT--GA
49_Scorzonera_purpurea                A-GGATAACCTCTCGTAT-AAGACCCCAATGCATTG-TC-C--------ATG-ATGCTTT--GA
57_Tragopogon_dubius                  A-AGGAAAGCTCTTGTAT-AAGACCCTAATGCGTTG-TC-CT----GTGATG-ACGCTTC--GA
58_Tragopogon_orientalis              A-AGGAAAGCTCTTGTAT-AAGACCCTAATGCGTTG-TC-CT----GTGATG-ACGCTTC--GA
23_Echinops_sphaerocephalus           A-AGCGAGTTTCTCTTCA-AAGACCCCATAGTGTCG-TC-TT----GCGACG-ATGCTTC--GG
29_Jurinea_mollis                     A-AAGGGACCGCTCTAAA-AAGANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
42_Saussurea_discolor                 G-AGGGAATCGCTCTCCA-AAGACCCCAACGTGTCG-TC-TT----GCGACG-ACGCTTC--GA
43_Saussurea_discolor                 G-AGGGAATCGCTCTCCA-AAGACCCCAACGTGTCG-TC-TT----GCGACG-ACGCTTC--GA
37_Onopordum_acanthium                G-AGGGAAGCGCTCTCCA-AAGACCCCAACGCGTCG-TC-TC----GCGACG-ATGCTTC--GA
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Professional experience

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