Endive, Chicory, and their wild relatives

A systematic and phylogenetic study of Cichorium (Asteraceae)

I – General part

II – Taxonomic part

Abstract

This thesis describes a systematic and phylogenetic analysis of the genus *Cichorium*. *Cichorium* is a small genus within the Asteraceae family (tribe Lactuceae) and is well known because of its two widely cultivated species: *C. endivia* (endive) and *C. intybus* (chicory). The genus occurs in the Old World and has been introduced in temperate and semi-arid regions elsewhere. An important diagnostic character at the genus and species level is the unique structure of the pappus, which, at the same time, causes difficulties regarding the relationship of *Cichorium* with other Lactuceae genera because of the unknown homology of it with other pappus structures. From a morphological point of view the genus is thus rather isolated. Our molecular phylogenetic analyses based on chloroplast (RFLP and sequence data) and nuclear ribosomal (ITS sequence data) DNA of *Cichorium* and nine other genera (*Agoseris, Catananche, Chondrilla, Lactuca, Microseris, Prenanthes, Scolymus, Scorzonera* and *Taraxacum*) confirm the isolated position of *Cichorium* within the Lactuceae.

Besides the two cultivated species *C. endivia* and *C. intybus*, four wild species are recognised here: *C. pumilum, C. calvum, C. bottae,* and *C. spinosum*. The latter two species are easily distinguished by their cushionlike growth form and spiny terminal branches, respectively. The remaining two wild species are morphologically very similar to the two cultivated species *C. endivia* and *C. intybus*, because they differ primarily in quantitative features. However, in a multivariate analysis of morphological characters the species do not extensively overlap. The most important morphological character for species delimitation is the pappus, but the differences in life span and reproductive system help to distinguish *C. intybus* (self-incompatible perennial) from *C. endivia, C. pumilum,* and *C. calvum* (all self-compatible annuals).

The results of the phylogenetic analysis of *Cichorium* based on both chloroplast and nuclear ribosomal DNA agree with the genetic relationships among the species determined with AFLP markers. The most important results are (1) *C. bottae* is sister to the remaining *Cichorium* species, (2) *C. spinosum* is the closest relative of *C. intybus*, and (3) *C. endivia* is sister to *C. pumilum* and *C. calvum* in the chloroplast based phylogeny, but the relationships among these three species are unresolved in the phylogeny based on nuclear ribosomal DNA. Based on the congruence between phylogenetic and genetic analysis, unique markers were expected for all species. Surprisingly, species-specific markers are nearly absent except for *C. bottae* which proved to have seven unique markers. Apart from that, a single species-specific marker has been found for *C. endivia* and one diagnostic marker has been found for *C. intybus* and *C. spinosum* together, confirming their close relationship. In addition to the species analysis, the genetic relationships among the cultivar groups of *C. intybus* (Root Chicory Group, Witloof Group, Pain de Sucre Group, and Radicchio Group) and *C. endivia* (Scarole Group, Frisé Group, and Endivia Group) are established using AFLP markers. The analysis of *C. intybus* cultivar groups resembles the species analysis in two respects: (1) grouping of cultivars according to cultivar groups, and (2) lack of markers unique to cultivar groups. In

contrast to *C. intybus*, the cultivar groups of *C. endivia* do not form distinct groups, which reflects that crosses have been made among the various cultivar groups. The relationships among *Cichorium* species and cultivars will be useful for setting up a core genebank collection of *Cichorium*. The importance of inclusion of the wild species in such a collection is emphasised.

Despite the high morphological resemblance of *C. endivia* and *C. intybus*, all three DNA-based analytical methods (chloroplast and nuclear DNA based phylogenetic analysis and the multivariate analysis based on AFLP markers) do not support a close relation between the two. The wild progenitor of *C. endivia* remains uncertain. It is usually suggested that *C. pumilum* is *C. endivia*'s wild progenitor, but also the poorly known species *C. calvum* should be taken into consideration. As yet the proper phylogenetic relationships between the three species is not clear.

Finally it is concluded that genetically modified organisms of both *C. intybus* and *C. endivia* have very high D_{pdf}-codes, which means that field experiments with genetically modified endive and chicory plants are to be treated with great caution.

I – GENERAL PART

1 – Introduction

1.1 - General background and aims

Although *Cichorium* is a small genus within the Asteraceae family, it is well known because of its two edible species: *C. endivia* (endive) and *C. intybus* (chicory), which are cultivated world-wide. The leaves of both species are used as salad or cooked as vegetable, while the roots of *C. intybus* are roasted and used as a coffee substitute or additive. The genus occurs in the Old World, and has been introduced in temperate and semi-arid regions elsewhere. Despite the fact that two species of *Cichorium* are widely cultivated, the genus hardly received attention from systematists. Moreover, the distinction between the species is far from clear, which has often led to uncertainties. Besides a revision of the species, the relationships among the species are analysed in this study. Special emphasis is put on the relationship of *C. endivia* with the remaining *Cichorium* species. *Cichorium endivia* is the only species of the genus which is not known from the wild flora. As its closest wild relatives both *C. intybus* and most often *C. pumilum* are suggested, but a phylogenetic analysis including these species has never been performed. Finally, the relationships among the various cultivar groups within *C. intybus* and *C. endivia* are studied here as well.

1.2 - History

The name *Cichorium* goes back to Horatius (65–8 BC), but was also used with a slightly different spelling by Nikandros (kíchóra; 2nd century BC), Dioscorides (kichóre and kichórion; c. 40–90 AD), Pollux (kichóreia and kichória; 2nd century AD), and Theophrastus (373–285 BC). The name has probably been derived from the Greek words 'kío'='go' and 'chorı́on'='from the field', which points to the occurrence of the plant in fields and along roads. This reference is still present in the common German vernacular name of the genus 'Wegwarte' (Hegi, 1987).

Linneaus (1753) included three species in the genus: *C. intybus, C. endivia,* and *C. spinosum*. Later, Lessing (1832) considered *C. spinosum* to be different enough to make it a separate genus named *Acanthophyton,* but this was not followed by others. De Candolle (1838) transferred the genus *Acanthophyton* to *Cichorium* as a monotypic section next to the (illegitimately named) section *Eucichorium* which consisted of four species: *C. intybus, C. endivia, C. pumilum,* and *C. glabratum.* This classification has been followed by Hoffmann (1894). Later the division into sections has fallen into disuse.

The considerable morphological variability led to the description of over 40 species since Linnaeus (1753) of which only half were described legitimately. In 1936, Shicheva recognised nine species in his revision. Unfortunately, this revision is in Russian, which does not make it easily accessible. The most recent revision of the genus is made by Bedarff (1985) and part of it was also published later by Wagenitz and Bedarff (1989). Here, a total of seven species were described, while the two cultivated species *C. intybus* and *C. endivia* were further divided into two subspecies each.

1.3 - Taxonomic position of Cichorium in Lactuceae

The genus *Cichorium* belongs to the family Asteraceae, which is the largest family of flowering plants with about 23,000 species in 1535 genera (Bremer, 1994). The family is easily recognised by the presence of capitula in which a number of flowers (florets) are placed on a common receptacle. These flowers are surrounded by one or several rows of involucral bracts (phyllaries), and consist of an inferior ovary, sympetalous corolla, five usually fused anthers, and a style with two branches (Duistermaat, 1996).

The founder of the Asteraceae classification was Cassini, who described between 1816 and 1830 numerous genera in a total of 19 tribes of which many are still accepted today. Based on his work, Bentham and Hooker (1873) reduced the number of tribes to 13, which, with some modifications by Hoffmann (1894), have been widely used until recently the number of tribes has been extended to 17 (Bremer 1994). The tribal interrelationships of the family have been subject of discussion but the division into two main subfamilies, Asteroideae and Cichorioideae, seemed the best way to represent the relationships (Carlquist 1976). This classification was followed until Bremer and Jansen in 1992 raised the subtribe Barnadesiinae to the rank of subfamily as a result of which the family now consist of three subfamilies: Barnadesioideae, Cichorioideae, and Asteroideae.

The subfamily Cichorioideae, to which *Cichorium* belongs, is a paraphyletic assemblage of what is left of the family when the two monophyletic subfamilies Barnadesioideae and Asteroideae are distinguished. Interrelationships within the Cichorioideae are, however, not well understood, which makes it not yet possible to replace the Cichorioideae by monophyletic subfamilies. The subfamily is recognised as such mainly by absence of the features characterising the Asteroideae (Bremer 1994). Hitherto, the Cichorioideae have been further divided into six tribes of which the tribe Lactuceae is the second largest with 1550 species and 98 genera. In contrast to its subfamily, the Lactuceae are very well supported as a distinct monophyletic group (Bremer, 1994; Whitton et al. 1995).

The Lactuceae (formerly known as Cichorieae or Cichoriaceae, Solbrig 1963) were first recognised as a natural group ("ordo") by de Jussieu (1789) and since then have always been considered as the most distinctive and easily recognisable subdivision of the family (Stebbins 1953). Until the 1970s the distinctness of the tribe was reason to consider it as a different family or subfamily (Liguliflorae; e.g. Hoffmann 1890; Leonhardt 1949), but they are now universally considered a tribe well nested within the Asteraceae (Bremer 1994). The Lactuceae's best characters are ligulate capitula and milky latex, but these features do not distinguish the Lactuceae unequivocally from other tribes (Bremer 1987). The distribution of the Lactuceae is predominantly in the Northern Hemisphere, with concentrations of genera and species in the Mediterranean area, Central Asia, and southwestern North America.

Although *Cichorium* is well nested within the tribe Lactuceae (Kim et al. 1992; Kim and Jansen 1995), its closer relation to other genera within the tribe has never been clear. *Cichorium* was placed close to *Hymenonema* and *Catananche* in the subtribe Cichoriinae by Stebbins (1953), who grouped the genera according to the highest resemblance in external morphology, chromosomes and geographic distribution. The subtribe was considered to be closely related to the Microseridinae based on the shape of the stigma branches and the orange color of the pollen mass. In addition to Stebbins' (1953) characters, style and corolla hair variation were considered to be important by Jeffrey (1966), which resulted in the placement of *Cichorium* in a monogeneric subgroup next to the *Crepis* subgroup in the *Cichorium* group. This means *Cichorium* was considered to be most closely related to, among others, *Lactuca, Prenanthes, Taraxacum* and *Chondrilla.* The first classification based on phylogenetic analysis of the tribe using morphological characters revealed a large polytomy including *Cichorium* (Bremer 1994). Bremer therefore concluded that *Cichorium* cannot be placed in a subtribe as yet, but suggested that it might be a relative or even a member of the Stephanomeriinae or Crepidinae, or it may be a genus derived early in the phylogeny of the Lactuceae. The first molecular analysis of *Cichorium* and related genera was performed by Vermeulen et al. (1994) using restriction fragment length polymorphisms (RFLPs) of the mitochondrial genome. Their results showed that *Cichorium* is most similar to *Lactuca* and *Cicerbita.* A first molecular-based, phylogenetic analysis of the tribe Lactuceae using chloroplast DNA RFLP data revealed a weakly supported topology suggesting that *Cichorium* is sister to Microseridinae and Stephanomeriinae sensu Bremer (1994; Whitton et al. 1995). Most recently, a nuclear ribosomal DNA (rDNA) sequence analysis of the internal transcribed spacer 1 (ITS-1; Koopman et al. 1998) suggested that *Cichorium* is closely related to *Lactuca*, which agrees with Vermeulen et al. (1994). However, this topology was also poorly supported.

Until now there is no consensus about the position of *Cichorium* within the Lactuceae. Therefore, nine genera that were considered to be closely related to *Cichorium* based on morphological and molecular data are included in a phylogenetic analysis and the results are described in this thesis.

2 – Macromorphological variation and reproduction in Cichorium

2.1 - Macromorphological characters

General habit and life span – There is a considerable difference in habit among the *Cichorium* species, varying from a cushion-like growth form (*C. bottae*) to up to 2 m high, bushy herbs (*C. intybus, C. endivia*). All species have a taproot, but three types can be distinguished: (1) a normal, unbranched taproot, (2) a thick, sugarbeet-like root, which occurs in root chicory cultivars of *C. intybus*, and (3) a tree-like, branched taproot ending in several rosettes found in *C. bottae*. The most common habit is a main axis arising from a rosette of leaves which is usually much branched, but several axes may also arise together from the rosette. The stem and branches are either glabrous or hairy, the hairs being short and sometimes glandular. However, in *C. bottae* the main axis is normally absent and the capitula are thus situated between the rosette leaves and are either sessile or stalked. The terminal branches in *C. spinosum* often become spiny after the immature capitula have fallen off. *Cichorium intybus, C. spinosum,* and *C. bottae* are perennials, while *C. endivia, C. calvum,* and *C. pumilum* are annuals, although some *C. intybus* and *C. endivia* cultivars are biennial.

Leaves – The rosette or basal leaves are always sessile and attenuate at the base, while the size and shape vary between and within species. The basal leaves of *C. bottae* and *C. spinosum* are always considerably smaller than those of the other species and oblanceolate to nearly linear. The largest basal leaves are found in the cultivated species *C. endivia* and in some *C. intybus* cultivars grown for their leaves and they are obovate. The leaves vary from entire, to irregularly dentate or runcinate, but the basal leaves of *C. calvum* are usually only irregularly dentate. The indumentum of the leaves also varies within and between species from glabrous to puberulous with the exception of *C. endivia*, which usually has glabrous basal leaves.

The cauline leaves are also sessile and often semi-amplexicaul, spiral and smaller towards the apex. In *C. endivia* and *C. calvum*, the cauline leaves are usually larger in the upper part of the inflorescence than in the other species of the genus.

Capitula – The capitula are all sessile, often clustered in the axils of stemleaves in groups of 2–8. However, in all species there are always some capitula that are situated at the end of peduncles. Those peduncles vary in size and are the longest in *C. endivia* and *C. calvum*. The capitula are very similar in all species and are 2.0–2.7 cm diameter in *C. spinosum*, which also has the lowest (i.e. 5–7) number of florets per capitule. The capitula of the remaining species vary from 3.0–5.0 cm in diameter. The number of florets is 9–14 in *C. bottae, C. pumilum* and *C. calvum*; *C. endivia* has 15–20 florets per capitule, while *C. intybus* has the widest range (12–)15–19(–25).

The phyllaries (or involucral bracts) are imbricate and placed into two rows. The outer row always consists of 5 bracts. The bracts are often reflexed in the upper part, (broadly) ovate or elliptic to (ovate-)lanceolate and acute to acuminate. The lower part of the phyllaries is fleshy in flowering time and rigid in fruiting time. Furthermore, the outer phyllaries are shorter to nearly twice as long as the inner phyllaries, and the largest outer phyllaries occur in *C. calvum*, where there is always at least one single phyllary 8–15 mm wide (in contrast to other species).

The inner row usually consists of 8 phyllaries with the exception of *C. spinosum*, which has only 5. Both inner and outer phyllaries are glabrous or set with long glandular or non-glandular hairs except for *C. spinosum*, which is usually glabrous. The inner phyllaries are villous outside at the acute apex in all species.

Corolla – The corolla is ligulate, consisting of a tube and a 5-lobed plate. This type of corolla is characteristic of the tribe Lactuceae, but also occurs in *Fitchia* (related to Coreopsideae), *Stokesia* (Vernonieae) and some Mutisieae, e.g. *Catamixis, Hyaloseris, Dinoseris* and *Glossarion* (Bremer 1987). The ligulate corolla is considered to be derived from an actinomorphic, 5-lobed corolla (Jeffrey 1977; Bremer 1987). In *Cichorium* the tube is straight and hairy all round in apical part. On the partition between the tube and the plate the filaments of the stamens are inserted. The lobes of the plate are unequal in size within and between florets in all species. They have an acute tip, which is villous, mainly outside. The size varies, with the smallest ligules in *C. spinosum* and the largest in *C. intybus*.

Stamens – The anthers of the 5 stamens are fused and form a tube as in nearly all Asteraceae (except *Xanthium* and *Iva*). The structure of the anthers varies within the family, and they may have apical and/or basal appendages. The basal appendages are much more variable than the apical ones and may even be absent. They are considered to be useful to characterise genera and subtribes by various authors such as Cassini (1816–1830), Bentham (1873), and Bremer (1987), and the form and degree of development of the basal appendages were considered to give some clues as to the evolution of the family (Small 1919). As in most genera, the anthers of all *Cichorium* species show no diagnostic differences; the anthers have rounded apical appendages and sterile, frayed basal appendages.

Style – The style of *Cichorium* is of the so-called ‘vernonioid’ type, which is present in the tribes Vernonieae, Liabeae and Lactuceae (Bremer 1987). The style is slender and ends in two branches, which are called the stigmatic lobes. In *Cichorium* the style always contains spreading hairs in the upper part just below the stigmatic lobes as well as on the outside of the stigmatic lobes, while the stigmatic lobes are pappillose over the entire inner side of the lobes. The spreading hairs on style and lobes have the function to push the pollen out of the anther tube. When the stigmas become susceptible, the stigmatic lobes usually curl outwards and sometimes touch their own pollen which is situated on the upper part of the style just below the stigmatic lobes and by this way can pollinate themselves.

Pappus – The pappus of the Asteraceae might be considered to be positionally as well as process orthologous with sepals (Albert et al. 1998) and it is an important character for classificationhas been considered to be homologous with the calyx in related families. However, no studies are known to confirm or reject this hypothesis. Nevertheless, the pappus is an important character for classification within the Asteraceae, mainly at the generic and species level (Bremer 1987; Bremer 1994). However, the pappus of *Cichorium* has been a point of discussion for a long time since it seems to be unique within the Asteraceae. Because of the scaly pappus it was associated with *Catananche, Hymenonema,* and *Rothmaleria* by Stebbins (1953) and earlier authors and most recently, Bremer (1994) described the pappus of *Cichorium* as a minute scaly pappus of which the homology is unknown.

The *Cichorium* pappus consists of numerous, irregularly shaped scales. Of course, due to the large number of pappus scales per floret, and the scarcity of space within a capitule, the scales vary among the florets of one capitule and within an individual floret. The longest scales will always be found in the florets situated in the middle of the capitule, and in the outer florets the pappus scales are usually wider and blunt to very blunt at the centrifugal side. Within *C. intybus* (Fig. 2.1c) and *C. spinosum* (Fig. 2.1e) the pappus scales are never longer than 0.6 mm and situated in one or two rows. The pappus of *C. bottae* (Fig. 2.1f & g) is very similar to that of *C. intybus* and *C. spinosum*, but rarely the scales of the inner florets are ending in 0.6–1.7 mm long tips. The pappus scales of *C. endivia* (Fig. 2.1a & h) and *C. pumilum* (Fig. 2.1b) are situated in one to three rows. They are always longer than 0.7 mm in the inner florets and are often ending in long tips like in *C. bottae*. The pappus of *C. calvum* (Fig. 2.1d) is very different from the other species, because at first sight it seems to be completely absent in nearly all plants I have seen: it consists usually of a fringe of minute scales (much) less than 0.1 mm long. However, one exception has been found in a specimen from Germany (leg. O. Angerer) in which I observed pappus scales of 1 mm including a 0.3 mm long tip. When comparing SEM photographs it is easily visible that those long scales point in the same direction, i.e. outside, like the minute scales in other collections of *C. calvum*.

Achenes – The fruit of the Asteraceae is a 1-seeded, dry fruit developed from an inferior ovary, with the pericarp more or less adhering to the testa. De Candolle (1813) clearly restricted the term ‘achene’ to this type of fruit and thus excluded all other dry and closed fruits, which were originally also included in this term. Two years later Mirbel (1815; from Wagenitz 1976) introduced the term ‘cypsela’ for the Asteraceae fruit, and this term has been used mainly in Anglo-Saxon literature, especially when Lindley (1832; from Wagenitz 1976) used the term ‘cypsela’ for fruits developed from an inferior ovary and ‘achene’ for fruits developed from a superior ovary. These definitions are in disagreement with De Candolle’s (1813) definition. However, until today both terms are used side by side, but I prefer to use the term ‘achene’ as defined by De Candolle (1813), which agrees with the discussion of Wagenitz (1976), as this seems the most useful and widespread term.

The achenes of *Cichorium* are obovoid to cylindrical and basally indistinctly ribbed, only in *C. calvum* the achenes are shortly obconical. The fruits are light brown with dark brown spots to completely brown when ripe. They are usually glabrous, but in *C. bottae* the fruits can be covered with extremely short, appressed hairs.

2.2 - Pollen ontogeny and morphology

The ontogeny of *C. intybus* pollen is very similar to that of the other Asteraceae investigated so far, i.e. *Catananche caerulea, Cosmos bipinnatus, Helianthus annuus, Leontodon autumnalis* (Varotto et al. 1996). According to Varotto et al. (1996), *C. intybus* shows an amoeboid, periplasmodial tapetum although in an earlier study by Pacini and Keijzer (1989) no periplasmodial tapetum could be found in their *C. intybus* sample, but no explanation for this incongruence has been offered.

Pollen of *C. intybus* has been described by Blackmore (1981, 1984, 1986), who also described pollen of *C. spinosum* (Blackmore 1981). Both *C. intybus* and *C. spinosum* have 3-colporate, echinolophate pollen, which is characterised by having spines on ridges surrounding spineless depressions, termed lacunae. This pollen-type is also found in the remaining species, i.e. *C. endivia, C. calvum, C. pumilum,* and *C. bottae*, als illustrated in Fig. 2.2. This is the most common pollen type in the tribe Lactuceae. It occurs in every subtribe of the Lactuceae, except the Scorzonerinae and the Scolyminae, in which a derivative is found. The echinolophate pollen of *C. intybus* and *C. spinosum* can be more specifically defined as the *Cichorium* type (Blackmore 1986), which is equivalent to the *Taraxacum* type described by Wodehouse (1935; see also Blackmore 1986). The *Cichorium* type is characterised by the possession of a distinct equatorial ridge and 15 lacunae, and is the most common echinolophate pollen type of the tribe Lactuceae. Besides the *Cichorium* type eight other types can be distinguished within the tribe (Blackmore 1986). In each type, there is a spine-covered area of varying extent at each pole of the grain, which is termed the 'polar thickening' and has been used as a diagnostic feature by Blackmore (1981). Within Cichorium this 'polar thickening' is small but more extensive than in *Hymenonema*. The exine stratification of the Hyoseridinae appeared to be complicated and cannot easily be classified into one of the four major exine types of the Asteraceae (Skvarla et al. 1977). Blackmore (1981) therefore, merely described the exine stratification and distinguished four patterns among the Hyoseridinae genera (tribe Lactuceae): the *Catananche* type, the *Scorzonera* type, the *Scolymus* type, and the *Cichorium* type. The *Cichorium* type lacks internal foramina but has several internal tecta in the ridges giving the ectexine a spongy appearance. Besides *Cichorium* also *Hispidella, Arnoseris, Tolpis,* and *Hyoseris* show this type of exine stratification. Unfortunately, the pollen morphology and exine stratification is more diverse in subtribe Hyoseridinae than in any other subtribe of the Lactuceae and the affinities of *Cichorium* to the other genera in the subtribe cannot be deduced from its pollen (Blackmore 1981).

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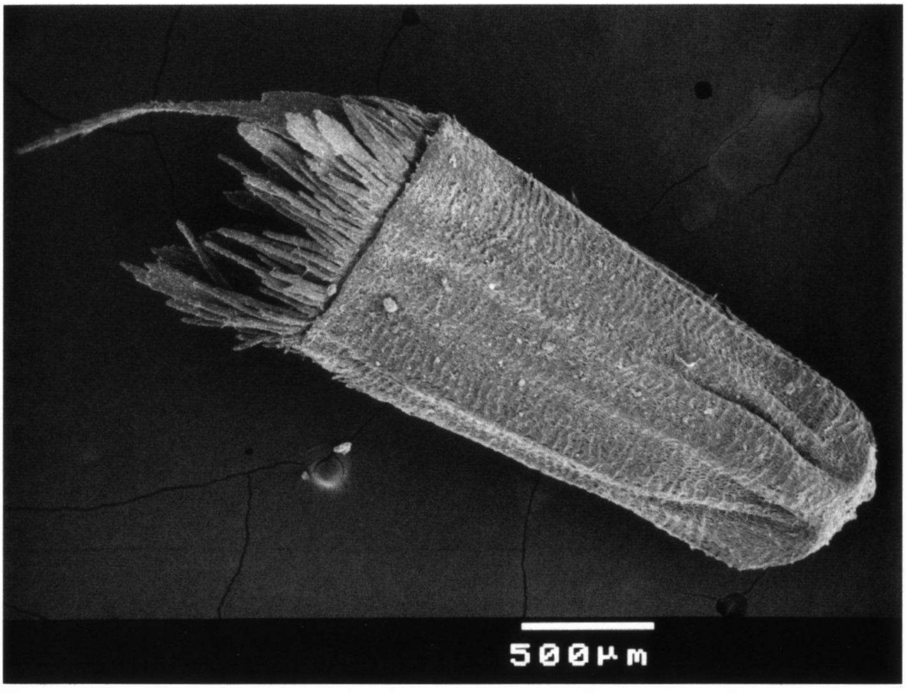
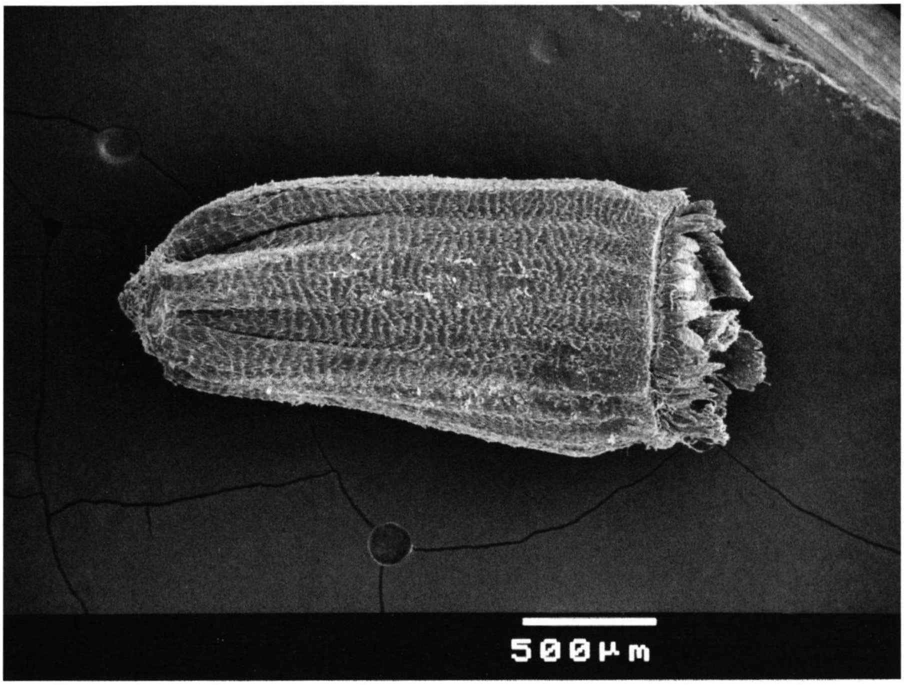
Fig. 2.1.Scanning electron microscope (SEM) photographs of *Cichorium* achenes with pappus. a. *C. endivia*. b. *C. pumilum*, one pappus scale ending in a long tip. c. *C. intybus*. d. *C. calvum*.

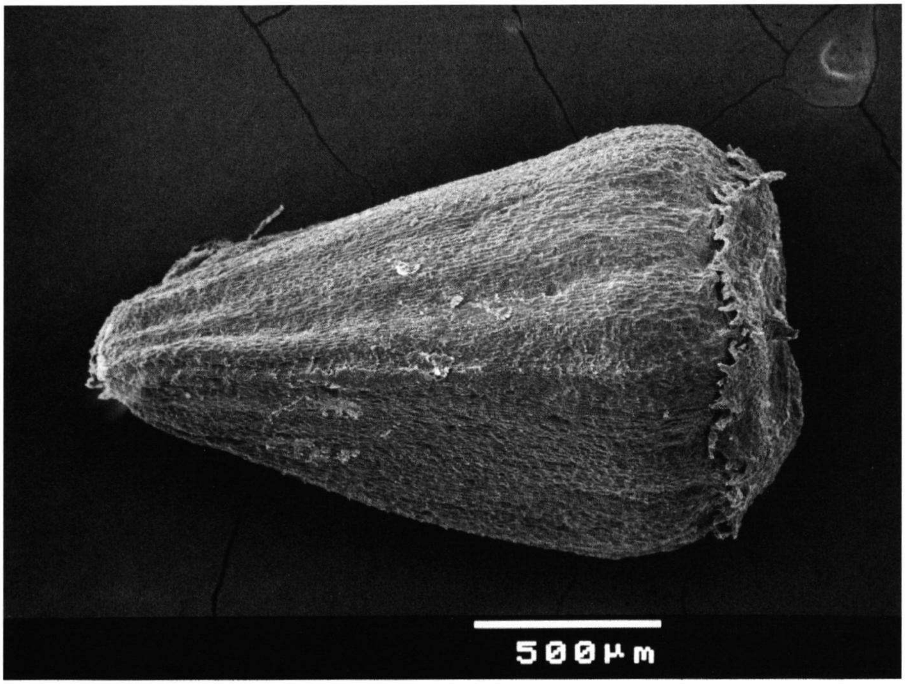
a.



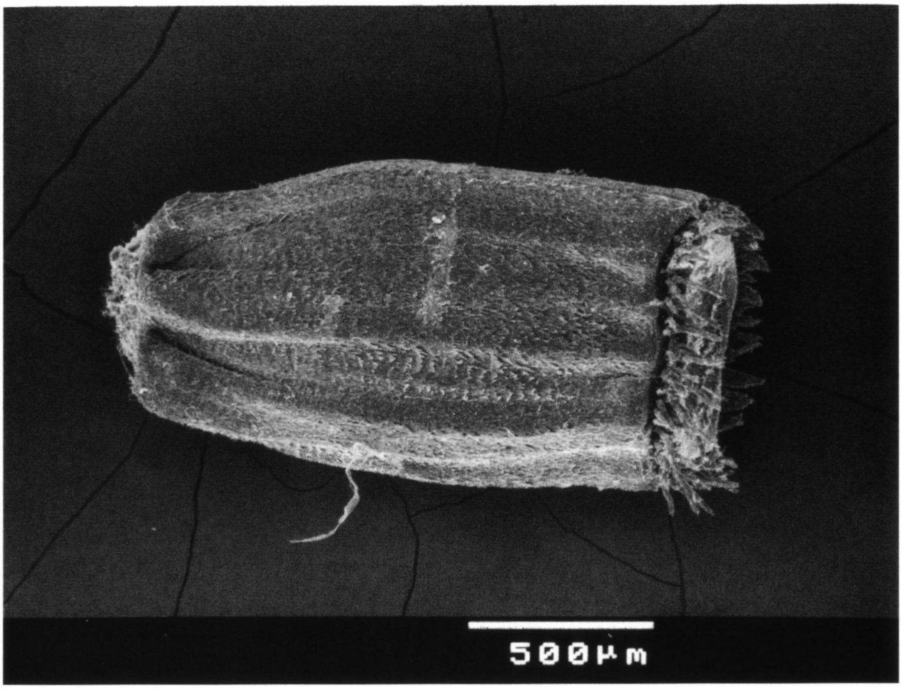
b.



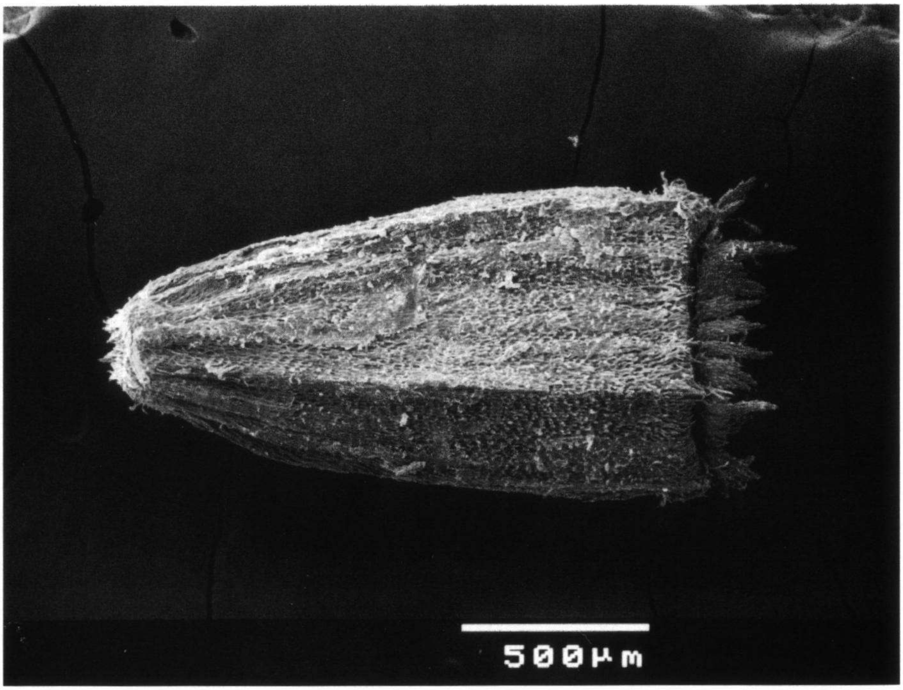
c.



d.

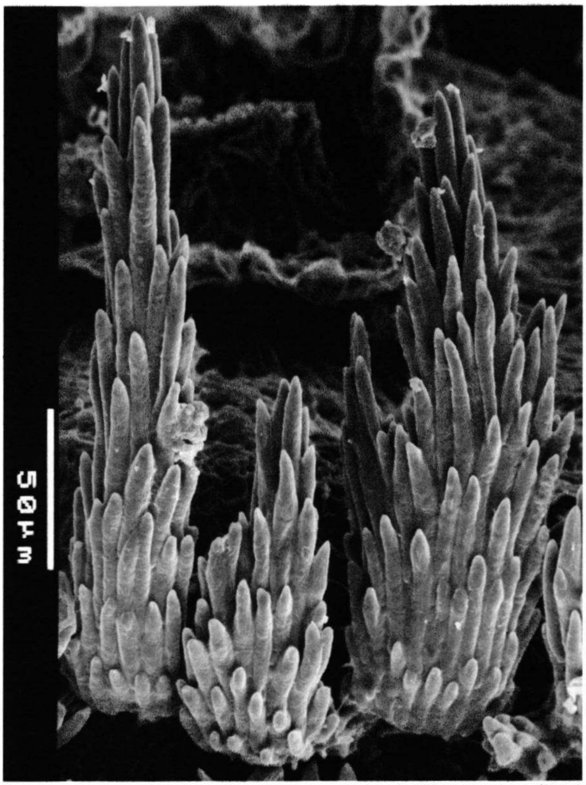


e.

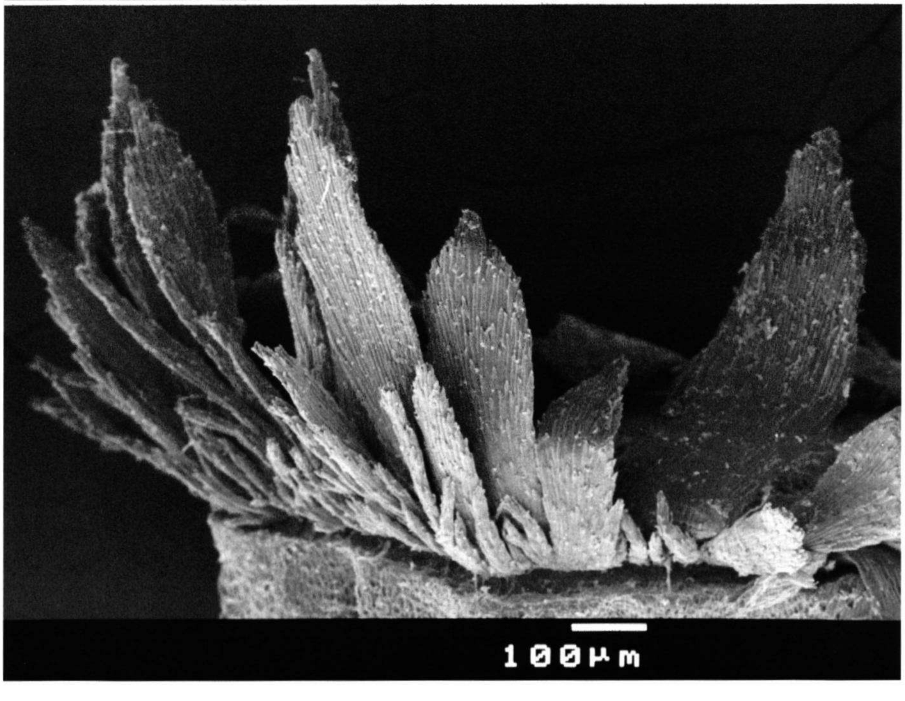


f.

g.



h.



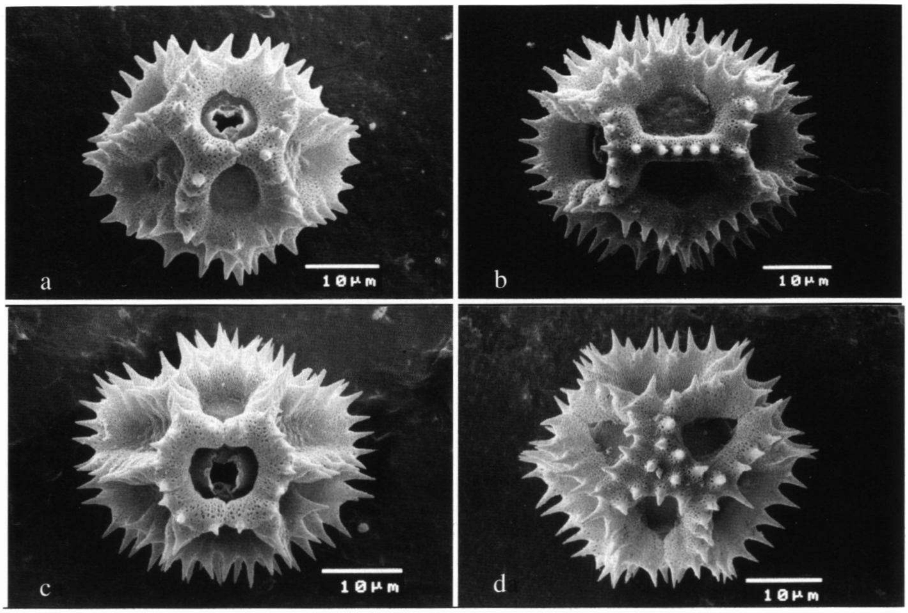


Fig. 2.2. Scanning electron microscope (SEM) photographs of *Cichorium* pollen grains.
a. *C. spinosum*, equatorial view of aperture. b. *C. calvum*, equatorial view of mesocolpium
c. *C. bottae*, equatorial view of aperture. d. *C. bottae*, polair view.

2.3 - Chromosomes

The chromosome numbers of all six species has been assessed using young root tips of three plants per species using the squash technique (Dyer 1979). For all species 18 chromosomes were counted in the diploid phase ($2n=2x=18$), which agreed in case of *C. intybus, C. endivia* and *C. spinosum* with literature (IPCN; Sell 1976), while for the other species the chromosome number had not been counted before.

2.4 - Reproduction

An extensive study of the self-incompatibility system of *C. intybus* has been performed by Eenink (1981a, 1981b, 1982). He concluded that the incompatibility system of *C. intybus* is sporophytically controlled, and furthermore, is based on one locus with different dominance and codominance relationships between the S-alleles in pollen and style. The sporophytic incompatibility system occurs in many Asteraceae species (De Nettancourt, 1977). The presence of this system in *C. intybus* had been suggested before by Pécaut (1958, 1962), but Bannerot and Fouilloux (1970) found some indications for a gametophytic system. However, no evi-

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Fig. 2.1.Scanning electron microscope (SEM) photographs of *Cichorium* achenes with pappus. e. *C. spinosum*. f. *C. bottae*. g. *C. bottae*, detail of pappus scales. h. *C. endivia*, detail of pappus scales.

dence for a gametophytic incompatibility system has been found since. The self-incompatibility system can break down, however, especially as a result of artificial selection in cultivated plants (De Nettancourt 1977). The occurrence of a breakdown of the self-incompatibility in populations of *C. intybus* has been described by Cichan (1983). He found that at least 70% of the *C. intybus* individuals tested produced some seeds in response to selfing in wild populations as well as in cultivars. The occurrence of self compatibility seems not to be affected by temperature, relative humidity, light intensity or end of flowering season, as has been observed in other crops (Eenink, 1981a; Cichan 1983).

In contrast to *C. intybus, C. endivia* is self-compatible and mainly self-pollinating (Rick 1953 and references therein). However, the mode of reproduction of the wild species of *Cichorium* is not known and therefore has been investigated here. Five plants of each species were checked on spontaneous seed set in an insect free greenhouse and 5 capitula per plant were actively selfed by hand. Two wild species, *C. bottae* and *C. spinosum*, showed no seed set under those circumstances, demonstrating that both are self-incompatible species, like *C. intybus*. The remaining three species, *C. endivia, C. pumilum* and *C. calvum* are self compatible as they had almost 100% seed set irrespective of the treatment.

3 – Domestication

3.1 - Archaeological evidence

The origin of cultivation of chicory and endive lies most probably in the Mediterranean Centre, although also the Asian Centre has been suggested for *C. intybus* (De Candolle 1884, Zeven and De Wet 1982, Vavilov 1992). The oldest archaeological evidence of the use of *C. intybus* dates from the Bronze Age and has been found in the Alpenquai site (Zürich, Switzerland). The next proof comes from Italy, where in the Roman period Plinius (23–79 AD) registered chicory together with three kinds of lettuce (Desfontaines 1829 in Nuñez and De Castro 1996). Both Plinius and Dioscorides suggested that the origin of domestication of chicory and endive have taken place in Egypt, but no archaeological evidence has been found (Nuñez and De Castro 1996; Vartavan and Amorós 1997). In the Medieval period and later, chicory has been found often and seems a common plant used mainly for food, medicine, magic, and seasoning, while endive was primarily used for food and magic (Nuñez and De Castro 1996).

3.2 - Early and medicinal use

Already in the medieval period, chicory and endive were used for medicinal purposes (Dodonaeus 1554, 1644; Munting 1696; Uyldert 1948). An extract from the root, stem or flowers were used pure or in combination with wine to clear blood, spleen, liver, and kidneys. It stimulates appetite because it was supposed to dissolve gastric mucus, activate urine production, and heal jaundice and eye diseases. Finally, it was used to suppress fever, headaches, liver condition and piles. In the Middle Ages endive was used to make a kind of eau-de-cologne: eau d'Andive.

Besides *C. intybus* and *C. endivia*, another species has been used too. In an addition to the original text in the Cruydt boeck by Dodonaeus (1644) the use of *C. spinosum* has been described. The Greek at the leaves of this species, which are more bitter and tough than the leaves of *C. endivia* and *C. intybus*, as a salad. The vegetable is called 'stamnangathi' in Greek and is still eaten today as salad or cooked vegetable (Akeroyd and Hogan 1996). However, there is no cultivation of *C. spinosum*, but the rosette leaves are sought-after in the wild flora together with other so-called 'wild greens', which is a popular activity.

3.3 - Classification of the present cultivar groups

According to their use and selection by man, several different cultivars can be distinguished. Formerly, these cultivars were grouped into several varieties. Within *C. intybus*, the root chicory cultivars were classified into var. *sativum*, while the various cultivars cultivated for their leaves are all grouped into var. *foliosum*. In *C. endivia*, three varieties are distinguished: var. *latifolium* (broad-leaved types), var. *crispum* (crispy types), and var. *endivia* (old type, hardly cultivated nowadays). However, the inclusion of cultivars into the nomenclature of plants found in nature is often difficult and confusing as discussed by e.g. Hetterscheid and Brandenburg (1995) and Hetterscheid et al. (1996). These authors also proposed a new, separate classification of cultivated plants with 'cultivar' and 'cultivar group' as the most important ranks.

Within this new system, the cultivar is the basal rank that cannot be subdivided. The cultivar group serves to assemble cultivars on the basis of one or more user criteria and is preferably assessed on an international basis, in order to enhance the nomenclatural stability. The link with the taxonomic hierarchy should be made between cultivar groups on the one hand and species or genera on the other.

In case of *Cichorium* the varieties have been transformed by Siemonsma and Piluek (1993) into cultivar groups with the following names: *C. intybus* var. *foliosum* = cultivar group Foliosum, *C. intybus* var. *sativum* = cultivar group Sativum, *C. endivia* var. *latifolium* = cultivar group Escarole, *C. endivia* var. *crispum* = cultivar group Curled Endive, and *C. endivia* var. *endivia* = cultivar group Small Endive. This classification is rather satisfactory for *C. endivia*, but leads to some problems in *C. intybus*, however, because cultivar group Foliosum is a very heterogeneous group in which several subgroups can be recognised which are used as such in practice (e.g. Baes and Van Cutsum 1993a&b; Bellamy et al. 1995; Bellamy et al. 1996; Van Kruistum 1997, Van Wijk 1999). For that reason, a new classification is proposed here, conform to the rules of the ICPCN (1995), in which the various cultivars are grouped in accordance with the practical use. Within *C. intybus* four cultivar groups are distinguished: (1) Root chicory Group, (2) Witloof Group, (3) Pain de Sucre Group, and (4) Radicchio Group. The three groups within *C. endivia* are (1) Scarole Group, (2) Frisé Group, and (3) Endivia Group. Finally, it has to be noted that although the Radicchio Group is always linked to *C. intybus*, it originates from a cross between *C. intybus* and *C. endivia* and thus should be more properly linked to the genus instead of a single species. However, as it is always associated with C. intybus and is also cultivated similarly it will be linked to *C. intybus* here as well.

3.4 - Development and description of the present cultivar groups of C. intybus

Root Chicory Group – Root chicory or chicory is mainly cultivated for its large roots. The latter are, due to breeding, now very similar to sugarbeets. The roots were roasted and used as a coffee surrogate or additive already as long ago as the Napoleonic times, because they are much cheaper than original coffee beans. In times of wheat scarcity, the roots were also used to bake bread (Kops and Gevers-Deijnoot 1853). Nowadays, the roots are hardly used for coffee surrogate or bread anymore, but root chicory obtained a new use in the production of inulin.

Inulin has been defined as carbohydrate material consisting mainly, if not exclusively, of $\beta(2\rightarrow1)$fructosyl-fructose links (De Leenheer 1996). The collective term for any compound in which one or more fructosyl-fructose links constitute the majority of linkages is fructan and another member of this group is levan. After starch, fructans are the most abundant non-structural polysaccharides found in many Asteraceae (De Leenheer 1996; Koch and Jung 1997). Inulin is a food ingredient with interesting nutritional and health promoting characteristics: it is a soluble dietary fibre, a promotor of the friendly intestinal *Bifidus* bacteria, and it is suited for consumption by diabetics. It has a neutral flavour and it is an ideal fat-replacer (De Leenheer, 1996). Today, inulin can already be found in some yoghurt products as an additional fibre and a certain kind of dogfood, increasing the levels of so-called friendly bacteria in order to improve the overall digestion.

Furthermore, chemicals from the chicory root are developed which can be used to replace petrochemical derivatives used for cosmetics and detergents or bleaching agents for cotton (Volkskrant 6-12-1997, The Netherlands). Finally, chicory has become (again, see e.g. Kops and Gevers-Deijnoot 1853; De Candolle 1884) useful as a forage crop. The cultivar 'Grasslands Puna' was developed in New Zealand. It is usually planted with clover or grass and clover and is currently being tested as a forage crop in the USA as well (Ryder, 1999).

Witloof Group – Witloof is the forced stage of the root chicory. The apical bud (chicon) is produced in the dark, in sand, soil or in hydroculture and is composed of white or yellow-tipped leaves folded over each other, often very tightly.

A first possible indication of a primitive production of witloof has been described in the Cruydt boeck by Dodonaeus (1644). However, the cradle of the witloof (also known as Brussels chicory or French endive) production is Belgium and the origin of the witloof form has been attributed to M. Bréziers who was working in the State Botanical Garden in Brussels. He discovered that some 'forgotten roots' had sprouted in the dark, forming white leaves. The leaves were elongated and loose and the product was known as Barbe de Capucin. In 1846, the first witloof chicory entered the market in Brussels. Around 1890 witloof was a relatively important crop in Belgium, and early 1900 also The Netherlands and France started to produce witloof. The first witloof was a loose head held together by the top of the root. Through mass-selection breeders tried to develop more compact heads and used the Magdeburger type (a root chicory cultivar type) as a starting point. Goal-oriented research started only after the Second World War in 1948 in order to improve the quality of the crop and the production. The original cultivation of witloof took place in a pit covered with sand or soil. After the Second World War dr. Huyskes (IVT, now PRI in Wageningen, The Netherlands; Van Kruistum 1997) selected the first witloof plants suitable for forcing in a pit without etching ground. After 1970 forcing in water became popular and nowadays this is the main type of witloof cultivation. Thanks to a combination of the hydroculture and a good root storage strategy, the cultivation of the crop is now no longer restricted to the winter (Van Kruistum 1997; Ryder 1999).

A cross between Radicchio and witloof resulted in a red chicory forcing type (or "roodlof"), which belongs to the witloof cultivar group. This type has been developed and cultivated in The Netherlands in the 1980's, but it is unclear whether it is cultivated elsewhere. Although the breeding of hybrids suitable for forcing in water around 1990 led to an increase of the red chicory cultivation, the root- and loof-production remains far behind the witloof production, and thus resulted in a loss of interest in the cultivation of red chicory (Van Kruistum 1997).

Pain de Sucre Group – The origin of 'Pain de Sucre' is often described as unknown, although it is also suggested to be a type of Radicchio with completely green leaves (Van Kruistum 1997, Ryder 1999). It is described as an old vegetable known as 'Zuckerhut' in German speaking countries and 'Pain de Sucre' in French speaking countries. Both names suggest that the vegetable tastes sweet, but actually it can have a very bitter taste. The plants are robust, with large leaves and longitudinal, somewhat open heads. The plants are not suitable for forcing in darkness.

Radicchio Group – Originally, radicchio or radicchio rosso, is an Italian vegetable, already cultivated in the 16th century around Venice. Nowadays, Italy is still the most important country for radicchio production, but in Switzerland, South-Germany, and The Netherlands some radicchio is produced as well. The main features of radicchio are the red to dark-red leaves, the white nerves and the rather bitter taste. The vegetable is primarily used as a salad, sometimes pure, but mainly mixed with other salad crops.

Three types can be distinguished within radicchio according to colour and shape: the Treviso type, the Castelfranco type, and the Chioggia type. The Treviso type (e.g. 'Rouge de Verone') has a loose head of erect, long, and usually narrow leaves. Early types are cultivated in the field, but late Treviso types are forced. A cross between Treviso and endive (*C. endivia* Scarole Group) has led to the Castelfranco type. This type has a more compact head compared to the Treviso type. The outer leaves are green, while the inner leaves are yellow with light red spots. This type is also called radicchio biondo. The Chioggia type has been selected from the Castelfranco type and is the most popular type of all radicchio's. The short, dark red leaves which form a more or less rounded head are characteristic for this type (Van Wijk 1999).

3.5 - Development and description of the present cultivar groups of C. endivia

The origin of *C. endivia* is uncertain. De Candolle (1884) described in his 'Origin of Cultivated Plants' that *C. endivia* was first thought to have an Indian origin, because of the high resemblance between *C. endivia* known to De Candolle and *C. casnia (=* C. endivia*)* which was found in India. Later, however, endive was compared with*C. pumilum*, and the few differences between these species convinced him that both species are very closely related (and even treated as one species) and thus the origin of *C. endivia* should be sought in the Mediterranean area where the wild *C. pumilum* occurs. The names '*intybus*' and '*endivia*' are very closely related (Uyldert 1948) and therefore it is impossible to make sure that this plant was used by the Greeks and Romans (De Candolle 1884). However, Ryder (1999) describes that endive was used as a salad vegetable at 'a very early period' in Egypt and was also known by Greeks and Romans, because it was mentioned by Ovidius (43 BC–17 AD), Plinius (23–79 AD) and Columella (1st century AD).

Within *C. endivia* three groups of cultivars can be distinguished: the Scarole Group, the Frisé Group, and the Endivia Group. The Scarole Groups consists of plain endive cultivars, characterised by a loose head of broad leaves with entire to slightly frilled margins. The Frisé Group contains narrow-leaved endives known as curly or crispy endive or "frisé". The leaves are narrower than the leaves of "escarole" and are much more frilled. The leaves form a loose head and are usually more bitter than "escarole". Finally, the Endivia Group includes an old type cultivar type called "endivia", with narrow, incised leaves, but this type is hardly cultivated nowadays (Schultze-Motel 1986, Poll 1994, Ryder 1999).

3.6 - Remarks on the risk assessment of genetically modified C. intybus and C. endivia

The relationship of *C. endivia* and *C. intybus* was of interest to a study on the risk assessment of genetically modified organisms (GMO's) in The Netherlands (Vries et al. 1992; Frietema de Vries 1996). A practical classification of the possibilities of cultivated plants to cause gene dispersal to the wild flora of The Netherlands was developed and resulted in a numerical code system named Dispersal (D) codes. The Dispersal code consists of three separate indices: D_p for pollen dispersal of the cultivated plant, D_d for diaspore dispersal of the cultivated plant, and D_f for frequency of distribution of the wild relatives, summarised as D_{pdf} code. A higher code (range from zero to five) indicates a potentially larger effect of the cultivated plant on the wild flora. For *C. intybus* the code could easily be established, because the species is native to The Netherlands. The code is $D_{pdf} = 5.5.4$ which means that crosses are possible but morphologically undetectable ($D_p = 5$), escapability is untraceable ($D_d = 5$) and the nearest wild relative is rather common ($D_f = 4$), altogether indicating a great potential effect on the flora of The Netherlands (Frietema de Vries 1996). In contrast to *C. intybus*, the code of *C. endivia* could not be established properly. The main reason was the unknown relationship between *C. endivia* and *C. intybus* (which is the only wild species that occurs in the Netherlands) and the suggestion that they might belong to one species (Frietema de Vries 1996). Therefore *C. endivia* was coded as $D_{pdf} = 9.9.4$ which indicates that crosses with wild relatives are unknown, because information is absent or incomplete (D_p = 9), escapability is untraceable for the same reason ($D_d = 9$), and the nearest wild relative is rather common ($D_f = 4$). However, spontaneous crosses between *C. endivia* and *C. intybus* occur in cultivated fields (Rick 1953) and the *C. intybus* cultivar type 'Castelfranco' (Radicchio Group) is suggested to be originated from a spontaneous cross between *C. intybus* type Treviso (Radicchio Group) and endive (*C. endivia* Scarole Group; De Simone et al. 1997). Moreover, detection of hybrids between both species in the wild is very difficult because of the high resemblance between the species. The proper code for pollen (D_p) and diaspore (D_d) dispersal is therefore five, but this code only applies to undetectable hybrids between plants of the same species. A broadening of this code to hybrids between species of the same genus is therefore suggested here and the new code for *C. endivia* is $D_{pdf} = 5.5.4$, which is similar to *C. intybus* and indicates that a substantial effect on the flora can be expected by genetically modified endive.

The described D_{pdf} code is only suitable for the flora of The Netherlands. However, Frietema de Vries (1996) has made suggestions for a similar code for Europe. An important complication is that Europe is too varied to summarise all different possibilities in the different areas. It is therefore suggested by Frietema de Vries (1996) to divide Europe into six plant vegetational regions with a separate European Dispersal Code for each region. For *C. endivia* and *C. intybus* the code will be mainly dependent on the frequency of distribution of *C. intybus* as this is the most common wild species. However, *C. intybus* can also hybridise with *C. spinosum* (Vermeulen et al. 1994) and hybridisations between other species of *Cichorium* might be possible as well (A.M. Kiers, unpubl. data). The detection of such hybrids is obviously hampered by the high resemblance between the species. In regions where other wild relatives of *C. intybus* and *C. endivia* occur the possible interspecific hybrids should be included in the dispersal code as was suggested here for *C. endivia.*

4 – Morphologically defined Cichorium species reflect lineages based on chloroplast and nuclear (ITS) DNA data

Abstract

Cichorium spinosum and *C. bottae* are morphologically well diagnosed species. The remaining four species in the genus, *C. intybus, C. endivia, C. pumilum,* and *C. calvum*, are usually difficult to distinguish because they differ primarily in quantitative characters. Our study indicates however, that these species are not intermixed in a multivariate analysis of morphological characters. A cladistic analysis of restriction fragment length polymorphisms and *trn*L-*trn*F sequences of the chloroplast genome and nuclear ribosomal DNA sequence data obtained from the six Cichorium species and nine possible outgroup genera indicates that 1) *Cichorium* has an isolated position within the tribe Lactuceae, 2) *C. bottae* is sister to all other species of *Cichorium* and 3) the remaining species are divided into two main clades, one consisting of *C. calvum, C. pumilum,* and *C. endivia* and the other consisting of *C. intybus* and *C. spinosum.*

4.1 - Introduction

The genus *Cichorium* (Lactuceae, Asteraceae) is economically important because of two widely cultivated species: *C. endivia* (endive and curly endive) and *C. intybus* (witloof, radicchio, and root chicory). Although since Linnaeus (1753) the two species have been distinguished, they resemble each other to a great extent in morphological characters. *C. endivia* differs from *C. intybus* only clearly in long pappus scales, annual life span, and self-compatibility. In contrast to *C. endivia* which is not known from the wild, the wild form of *C. intybus* (wild chicory) has a wide geographic distribution and is the most variable species of *Cichorium. C. intybus* (Matthews 1975) and *C. pumilum* (Boissier 1875; De Candolle 1884; Meikle 1985; Wagenitz and Bedarff 1989) have been suggested as the closest wild relative of *C. endivia.* Although an explicit phylogenetic analysis of *Cichorium* has not been performed to date. The result of such an analysis could be of importance to breeders interested in broadening the gene pool of *C. endivia* and *C. intybus.*

In a monographic study of the genus *Cichorium* (A.M. Kiers, this thesis) four wild species were distinguished apart from the two cultivated species mentioned above: *C. pumilum, C. calvum, C. spinosum,* and *C. bottae.* The latter two species can be easily identified on the basis of unique spiny terminal branches and a cushion-like growth form, respectively. The remaining two wild species, *C. pumilum and C. calvum,* are morphologically very similar to *C. endivia* and *C. intybus,* but they resemble *C. endivia* in annual life span and self compatibility. The high intraspecific variation in quantitative characters hampers species delimitation and identification (Wagenitz and Bedarff 1989).

The relationship of *Cichorium* to other genera within the tribe Lactuceae was uncertain, with no consensus about its position. Stebbins (1953) believed *Cichorium* to be close to *Hymenonema* and *Catananche* in Cichoriinae and the subtribe as a whole to be closely related to Microseridinae. Jeffrey (1966), however, made *Cichorium* a monogeneric subgroup of the *Cichorium* group next to the *Crepis*

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subgroup which, among other genera, contained *Lactuca, Prenanthes, Taraxacum,* and *Chondrilla.* Bremer's (1994) phylogeny of the tribe Lactuceae based on morphological data revealed a large polytomy including *Cichorium* with a monophyletic sister group consisting of *Catananche, Hymenonema,* and *Rothmaleria* (Catananchinae). Due to this large polytomy, Bremer (1994) did not assign *Cichorium* to a subtribe. However, he suggested *Cichorium* to be closely related to either Crepidinae or Stephanomeriinae, or considered the genus to be an early divergent branch in the Lactuceae phylogeny. A phenetic study based on restriction fragment length polymorphism (RFLP) data of the mitochondrial genome showed that *Cichorium* is most similar to *Lactuca* and *Cicerbita* (Vermeulen et al. 1994). A weakly supported topology based on chloroplast DNA RFLP data suggests that *Cichorium* is sister to Microseridinae and Stephanomeriinae sensu Bremer (1994; Whitton et al. 1995). Most recently, a nuclear ribosomal DNA (rDNA) sequence analysis of the internal transcribed spacer 1 (ITS-1) (Koopman et al. 1998) suggested that *Cichorium* is closely related to *Lactuca,* which agrees with Vermeulen et al. (1994). However, this topology was also poorly supported (Koopman et al. 1998). The different positions of *Cichorium* within the tribe Lactuceae make it impossible to select an unequivocal outgroup for *Cichorium.* Therefore, representatives of the mentioned subtribes (Catananchinae, Microseridinae, Stephanomeriinae, Crepidinae, and Lactucinae) are included.

In this study we examine phylogenetic relationships among the six *Cichorium* species. The relationship of *Cichorium* to other genera of the tribe Lactuceae are studied as well. First, a morphological analysis of the *Cichorium* species is presented to examine variation in morphological characters. Second, our estimation of relationships among *Cichorium* species based on chloroplast DNA RFLP data and sequence data from the *trn*L-*trn*F intergenic spacer is compared to our phylogenetic analysis of nuclear rDNA sequence data. The results are interpreted in the light of the relationships among the cultivated species and their closest wild relatives.

4.2 - Materials and Methods

Plant Material – Specimens from various herbaria (B, BM, G, K, L, M, W, WU) with mature florets and ripe achenes (or 'cypselas'; see Wagenitz (1976) for discussion) were used in the morphological analysis. One morphologically representative individual of each of the six *Cichorium* species was selected for an analysis of chloroplast DNA RFLPs and sequence variation. Another 74 individuals of the various *Cichorium* species and cultivars were analysed for the presence or absence of chloroplast and nuclear RFLPs discovered from exemplars of each species. Based on studies by Bremer (1994), Whitton et al. (1995), and Koopman et al. (1998), one representative each of nine outgroup genera was chosen, i.e., *Taraxacum officinale, Lactuca perennis, Microseris laciniata, Agoseris retrorsa, Chondrilla juncea, Catananche caerulea, Scorzonera hispanica, Prenanthes purpurea,* and *Scolymus hispanicus.* The same *Cichorium* samples were used in the chloroplast and nuclear DNA analyses. ITS sequences of the outgroup taxa *Lactuca sativa, Prenanthes purpurea, Krigia montana, Microseris bigelovii* and *Agoseris*
Amplification was performed in a MJ Research Peltier Thermo Cycler-100 set for 3

Table 4.1. Plant material of *Cichorium* and related genera used. The *Cichorium* accessions used in the chloroplast and nuclear DNA analysis are indicated in **bold**. The accessions of *C. endivia*, *C. pumilum*, and *C. calvum* mentioned in the discussion are underlined.

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[4] N. I. Vavilov Institute of Plant Industry (VIR), Herzen str. 44, St. Petersburg 190000, USSR.

Herbarium material

***Cichorium* L.**

C. calvum Sch.Bip. **Egypt.** E. Burdet 918 (G). **Germany.** Jun 1979, Botanic Garden München s.n. (M). June 1975, Lindenbein s.n. (M). 1 Oct. 1984, O. Angerer s.n. (M). E. Walter MTB 6232/2 (M). J.E. Krach MTB7033/2 (M). Koepff and Krach 13562A (M).

C. pumilum Jacq. ALBANIA. A. H. G. Alston and N. Y. Sandwith 1366 (BM). ARMENIA. G. Woronow 500 (K). **Balearic Islands: Menorca.** A. Charpin and D. Masson AC19286 (G). **Canary Islands: Tenerife.** E. Asplund 1156 (G). **Croatia.** B.E.E. Duyfjes et al. 250 (L). **Cyprus.** E.C. Casey 711 (K). H. Maedeverd 54 (K). **Iran.** Davis and Bokhari D56088 (K). **Iraq.** F.A. Barkley and A.D.Q. Agnew 6016B (K). Alizzi and Omar 34882 (K). W. Thesiger 763 (BM). **Israel.** I. Amdursky 478 (BM). F.S. Meijers and J.E. Dinsmore 8083 (L). J. Bornmueller 971 (G). I. Amdursky 478 (B). **Jordan.** F.S. Meijers and J.E. Dinsmore 4992 (K). **Sicily.** Todaro 527, (BM). E. and A. Huet du Pavillon 125 (G). **Syria.** E. Peyron 425 (G). Post 114 (G). 19 May 1865, C. Haussknecht s.n. (BM). **Turkey.** E. Hennipman et al. 1190 (L). Davis 43185 (K).

C. endivia L.

'Frisé Group': **Croatia.** 5 June 1926, E. Korb. s.n. (W). **Germany.** Schreber 24494 (M).

'Scarole Group': **Republic Congo.** Menyhart 500 (WU). **Unknown.** June 1856, E. Rostan s.n. (BM). 5 Sep. 1929, J. Schneider s.n. (W).

C. intybus L.

'Wild chicory': **Aegean Islands: Lemnos.** Foster 22.17 (K). **Bulgaria.** J. Bornmueller 2642 (B). France. L.W. van Soest 22416 (L). **Germany.** Inst. v. Prehistorie 575 (L). W.D.J. Koch 1844 (L). D. Reichenbach in W.D.J. Koch s.n. (L). 4 Aug. 1914, R. Schulz s.n. (B). **Morocco.** Font Quer 716 (BM). E. Johandiez 580 (G). **Netherlands.** 10 Aug. 1992, R. van der Meijden s.n. (L). **Poland.** M. Cegnowa-Giddon 318 (L). SWEDEN. 14 Sep. 1919, J. Erikson s.n. (L). **Turkey.** P.C. van Welzen 77 (L). J. M. Winter 151 (G). P.C. van Welzen 161 (L). J. Bornmueller 5232 (B).

C. spinosum L. **Crete.** E. Reverchon 93 (G). **Cyclades: Naxos.** Chr. Leonis 1339 (G). **Cyprus.** A. Genneon 1553 (K). P. Laukkonen 442 (K). **Greece.** B. Verdoort 4136 (K). **Unknown.** De Ventenat s.n. (G).

C. bottae Deflers. **Saudi Arabia.** D. Vesey-FitzGerald 16090/2 (BM). J.D. Tothill 125 (BM). G. Popov 185/2 (BM). J.P. Mandaville 2567 (BM). 26 Sep. 1979, B. Vincett s.n. (BM). **Yemen.** F.N. Hepper 6176 (K). H. Scott and E.B. Britton 527 (BM).

Living material

***Cichorium* L.**

C. calvum Sch.Bip. **Iraq.** Rawa, IPK[1] CICH100/88. Israel. Florimond Desprez[2] **C2.**

C. pumilum Jacq. **Crete.** Lasithi, A.M. Kiers **P4**, P6, P12, P13, P18. Pedeada, IPK CICH94/214. **Denmark.** Botanic Garden University of Kopenhagen, IPK CICH87/88. **Italy.** IPK CICH161/94. Bianco, IPK CICH67/88. Botanic Garden, University of Palermo, IPK CICH50/85. Palermo, IPK CICH66/89. **Morocco.** Volubilis, A.M. Kiers P21. **Portugal.** Luz, A. and D. van der Steen I230, I232, I233, I234. **Sweden.** Botanic Garden University of Uppsala, IPK CICH86/76.

C. endivia L.

'Frisé Group': **France.** 'Chicorée frisée', Florimond Desprez E2. **Germany.** 'Imperial', IPK CICH16/75. 'Italienischer Grüne', IPK CICH11/77. **Greece.** IPK CICH1/73. IPK CICH4/73. **Italy.** 'Cicoria Riccia', IPK CICH46/81. IPK CICH110/89. 'Di Pancalieri a costa bianca', IPK CICH29/85. IPK CICH99/86. IPK CICH189/94. **Spain.** IPK CICH27/87. **Syria.** IPK CICH192/94.

'Scarole Group': **France.** 'Chicorée Scarole' Florimond Desprez **E3**. **Germany.** 'Escariol Grüner', IPK CICH42/76. **Italy.** IPK *CICH39/79.* 'Full Heart', IPK CICH171/94.

C. intybus L.

'Wild chicory': **Cyprus.** NCRPIS[3] PL432336. **Ethiopia.** NCRPIS PL196841. **France.** Sampigny, J.C.M. den Nijs I150. **Germany.** Botanic Garden, University of Jena, IPK CICH79/82. Botanic Garden, University of Dresden, IPK CICH8/92. **Hungary.** NCRPIS PL531292. **India.** NCRPIS PL274288. **Italy.** Palermo, P. Mazzola I15. Senise, IPK CICH127/92. **Netherlands.** Apeldoorn, A.M. Kiers I107. Heteren, A.M. Kiers I125. **Pakistan.** NCRPIS PL269459. **Turkey.** NCRPIS PL279705. **Uruguay.** NCRPIS PL162665. **USSR.** VIR[4] CP380, CP381.

'Root Chicory Group': **Belgium.** 'Dageraad', IPK CICH205/94. **France.** 'Orchies', Florimond Desprez I19. **Germany.** 'Echte Zylinderförmige Spitzköpfige Magdeburger Riesen', IPK CICH71/95. **Hungary.** 'Slezka', IPK CICH75/93. 'Horpacsi', IPK CICH80/92. **Poland.** 'Polanowicka', IPK CICH76/93.

'Witloof Group': **Netherlands.** A. and J. van de Woude I18. **Sweden.** 'Brüsseler Witloof', IPK CICH52/94.

'Pain de Sucre Group': **France.** 'Pain de Sucre', Florimond Desprez I1. **Germany.** 'Zuckerhut', IPK CICH62/94. **Italy.** 'Pan di Zucchero', IPK CICH97/86.

'Radicchio Group': **England.** 'Rossa de Verona', IPK CICH128/92. **France.** 'Chioggia', Florimond Desprez I4. 'Rouge de Vérone' Florimond Desprez I20. **Italy.** 'Cicoria rossa di Treviso', IPK CICH119/83. 'Cicoria spadona di taglio', IPK CICH98/86. IPK CICH143/92.

C. spinosum L. **Crete.** along the road around Pahía Amos, A.M. Kiers S1, S2, **S3**, S7. Along the coast of Almirida, A.M. Kiers S16-1, 16-2.

C. bottae Deflers. **Yemen.** Near Mathna along the road to Bajil, A.M. Kiers B27, **B31**, B38. 3 km after Hajer Saeed along the road to Sanía, A.M. Kiers B41. Near Hajer Saeed along the road to Bajil, A.M. Kiers B43, B59.

Other genera

Chloroplast DNA analysis

Agoseris retrorsa Greene. **USA.** K. Vijverberg A10. *Catananche cearulea* L. **Netherlands.** Commercially available CC1. *Chondrilla juncea* L. **Italy.** IPK CHON 6/96. *Lactuca perennis* L. **France.** W.J.M. Koopman CGN 9318. *Microseris laciniata* (Hook.) Sch.Bip. **USA.** K. Vijverberg A82. *Prenanthes purpurea* L. **France.** W.J.M. Koopman W9534. *Scolymus hispanicus* L. **Belgium.** IPK SCOL 3/95. *Scorzonera hispanica* L. **Germany.** 'Schwarzer Peter', IPK SCOR 3/85. *Taraxacum officinale* F.H.Wigg. **Denmark.** R.G.M. van der Hulst TKX1370.

Nuclear rDNA analysis (ITS-1 and ITS-2; GenBank accessions)

Agoseris heterophylla Greene. L13954. *Lactuca sativa* L. L13957. *Microseris bigelovii* (Gray) Sch.Bip. U69703. *Prenanthes purpurea* L. L48151 and L48152.

heterophylla were obtained from GenBank. The sources and accession numbers of samples of the investigated taxa are listed in Table 1. Voucher specimens of the Gatersleben accessions are deposited in GAT, while all others are deposited in L. Chloroplast and nuclear rDNA sequences are available from GenBank under the accession numbers AF118899 to AF118913 and AF118914 to AF118919, respectively.

Morphological Analysis – All macromorphological leaf and indumentum characters were not scored, because they appeared to be too variable within and between individuals. A list of the characters used in the PCA is given in Table 4.2. Two principal component analyses were performed using the Macintosh version of SYSTAT 5.2.1. The first analysis included only diagnostic characters that could be scored directly from the herbarium specimens. The second analysis also included the characters 'life span' and 'reproductive system', which were scored from living plants in the field and in an insect-free greenhouse, respectively.

DNA Isolation – DNA was isolated from one or two fresh young leaves without the main nerve as described by Doyle (1991) with minor modifications. Tissues were ground to a fine powder in liquid nitrogen and incubated at 65°C in 1 ml 2x CTAB extraction buffer with 0.5% β-mercaptoethanol for 1 hr. The lysate was extracted twice with 450 µl chloroform/isoamyl alcohol (24:1). After an isopropanol precipitation DNA was dissolved in 300 µl TE including 3 mg RNAse A and incubated for 1 hr at 37°C. DNA was precipitated with 2.5 M NH_4Ac and cold 76% ethanol and subsequently redissolved in 200 µl TE.

Chloroplast DNA RFLP Analysis – Three chloroplast coding regions, i.e. *16S* rDNA, *psbD*, *psbA* (Tsumura et al. 1995) and nine non-coding regions, i.e. the spacers *trnT-trnL*, *trnL-trnF* (Taberlet et al. 1991), *trnC-trnD*, *trnS-psaA* (Demesure et al. 1995), *atpB-rbcL* (Savolainen et al. 1995), *psbA-trnH* (Sang et al. 1997), and *petA-psbE* (Fofana et al. 1997), and the introns *trnL* (Taberlet et al. 1991), and *trnK* (Demesure et al. 1995), were amplified and digested with the following 19 restriction enzymes: *Bam*HI, *Bgl*II, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Nde*I, *Pst*I, *Pvu*II, *Sac*I, *Sca*I, *Ssp*I, *Xba*I (6bp-recognition sites) and *Alu*I, *Hae*III, *Taq*I, and *Tru*1I (4bp-recognition sites). The PCR was carried out in 25 µl reactions containing 2 ng plant genomic DNA, 5 pmol of each primer, 1x PCR buffer (HT Biotechnology Ltd.), 100 µm of each dNTP, and 1.5 unit STaq (SphaeroQ).

Table 4.2. Morphological characters of the *Cichorium* species used in a Principal Component Analysis. The input order of the herbarium specimens is identical to that of the specimens in Table 1. cal=*Cichorium calvum*, pum=*C. pumilum*, end=*C. endivia*, int=*C. intybus*, spi=*C. spinosum*, bot=*C. bottae*, ann=annual, per= perennial, m=maximum, o phyll=outer phyllaries, i phyll=inner phyllaries, flor=florets, cap=capitulum, pap=pappus, elong pap sc=elongated pappus scales, abs=absent, and pres=present .

specimen	life span 0=ann 1=per	repr system 0=SC 1=SI	spiny shrub 0=no 1=yes	cushion like 0=no 1=yes	plant height (cm)	m length o phyll (mm)	m width o phyll (mm)	m length fleshy part o phyll (mm)	m length i phyll (mm)	m width i phyll (mm)	# flor per cap	m pap length (mm)	elong pap sc 0=abs 1=pres
cal-40	0	0	0	0	40	8.0	3.9	4.1	10.0	2.3	12	0.0	0
cal-2	0	0	0	0	64	6.0	8.0	3.0	10.0	3.0	11	0.0	0
cal-3	0	0	0	0	55	12.0	6.4	2.5	9.0	2.1	10	0.0	0
cal-4	0	0	0	0	75	9.0	6.0	1.5	8.1	2.5	11	1.0	0
cal-5	0	0	0	0	60	6.0	14.0	3.4	8.0	2.0	9	0.2	0
cal-6	0	0	0	0	60	15.0	6.0	3.0	9.2	3.0	9	0.0	0
cal-7	0	0	0	0	70	14.0	8.0	3.0	9.2	2.5	9	0.0	0
pum-34	0	0	0	0	30	8.5	4.4	5.0	9.5	2.0	12	1.0	0
pum-27	0	0	0	0	30	11.8	3.0	5.0	10.5	2.8	11	0.8	0
pum-30	0	0	0	0	27	8.8	3.4	4.7	9.5	2.8	11	1.0	0
pum-33	0	0	0	0	30	7.9	3.0	4.5	9.5	2.0	10	0.8	0
pum-9	0	0	0	0	8	7.4	4.5	4.5	9.5	2.3	12	0.9	1
pum-36	0	0	0	0	12	8.3	3.5	4.2	9.0	2.5	12	0.9	0
pum-37	0	0	0	0	14	9.8	3.5	4.5	9.2	2.3	15	0.9	0
pum-18	0	0	0	0	6	10.5	3.3	4.0	10.0	2.8	12	0.9	1
pum-38	0	0	0	0	23	15.5	4.0	3.5	9.3	2.0	9	0.8	0
pum-24	0	0	0	0	40	11.0	3.4	4.0	9.0	2.7	11	0.8	1
pum-21	0	0	0	0	13	7.3	2.2	4.0	9.0	2.0	11	0.7	1
pum-8	0	0	0	0	22	5.3	3.4	3.0	10.0	2.8	12	1.2	1
pum-19	0	0	0	0	15	9.2	4.5	3.3	10.4	2.5	11	1.2	1
pum-20	0	0	0	0	37	6.9	2.9	3.5	11.5	2.5	12	1.2	1
pum-42	0	0	0	0	30	5.5	3.5	4.0	10.0	1.5	13	0.9	1
pum-10	0	0	0	0	30	9.0	3.0	5.0	10.2	2.5	16	1.1	1
pum-22	0	0	0	0	23	5.0	2.9	2.5	7.2	2.3	13	0.8	1
pum-31	0	0	0	0	27	10.5	4.0	4.8	9.0	2.1	9	0.8	0
pum-16	0	0	0	0	30	8.3	3.0	3.5	10.0	2.5	11	0.9	1
pum-32	0	0	0	0	20	9.0	3.0	4.0	7.5	2.5	10	0.8	0
pum-25	0	0	0	0	50	9.0	2.5	3.5	8.5	2.0	11	0.8	0
pum-17	0	0	0	0	40	7.0	3.3	4.3	9.0	2.0	14	0.9	1
pum-29	0	0	0	0	30	8.0	4.2	3.8	10.0	2.5	10	0.8	0
end-5	0	0	0	0	65	9.0	4.0	4.5	9.0	2.1	15	0.7	0
end-4	0	0	0	0	70	10.0	8.6	3.2	10.8	2.0	17	0.8	0
end-3	0	0	0	0	60	10.2	6.0	3.8	10.0	2.5	20	0.9	0
end-2	0	0	0	0	70	10.1	3.3	4.0	10.0	2.1	17	0.5	0
end-23	0	0	0	0	58	11.0	4.5	4.5	10.5	2.5	20	1.0	1
int-39	1	1	0	0	37	10.8	2.1	3.0	12.0	2.7	12	0.5	0
int-45	1	1	0	0	75	7.0	2.8	3.5	10.0	2.5	18	0.2	0
int-7	1	1	0	0	64	12.0	4.0	3.5	12.0	2.4	18	0.4	0
int-3	1	1	0	0	70	9.0	2.5	4.0	11.0	2.0	16	0.3	0
int-5	1	1	0	0	60	10.5	2.8	4.0	12.8	2.5	18	0.3	0
int-6	1	1	0	0	60	12.5	3.3	3.8	12.5	2.8	15	0.5	0
int-44	1	1	0	0	70	7.0	2.5	2.5	12.0	2.5	19	0.3	0
int-13	1	1	0	0	31	5.5	2.8	3.5	9.2	1.9	18	0.3	0
int-15	1	1	0	0	37	6.0	2.5	3.8	9.5	2.2	11	0.6	0
int-4	1	1	0	0	78	9.5	3.8	4.0	14.5	3.0	17	0.4	0
int-1	1	1	0	0	50	11.5	3.1	3.2	12.0	2.6	19	0.2	0
int-2	1	1	0	0	45	16.0	3.9	5.0	16.5	3.0	15	0.3	0
int-11	1	1	0	0	22	4.7	2.3	3.5	9.8	2.5	14	0.4	0
int-12	1	1	0	0	35	7.5	3.0	4.0	13.5	4.0	16	0.4	0
int-14	1	1	0	0	40	4.7	2.2	3.0	10.0	2.8	14	0.4	0
int-43	1	1	0	0	79	5.5	2.0	3.0	10.0	2.0	13	0.3	0
spi-6	1	1	1	0	9	3.1	2.0	2.0	7.5	2.1	6	0.3	0
spi-5	1	1	1	0	11	2.9	1.8	2.0	7.5	2.0	6	0.4	0
spi-3	1	1	1	0	27	4.2	3.8	4.1	7.8	3.0	5	0.2	0
spi-7	1	1	1	0	9	3.7	2.0	2.5	8.0	2.1	5	0.4	0
spi-1	1	1	1	0	12	2.6	2.0	2.0	7.0	2.3	6	0.1	0
spi-2	1	1	1	0	25	3.0	1.5	1.8	7.2	2.1	6	0.2	0
bot-1	1	1	0	1	0	5.0	1.5	3.0	8.0	1.9	10	0.2	0
bot-3	1	1	0	1	0	6.3	1.9	3.8	9.7	2.8	12	0.3	0
bot-7	1	1	0	1	0	5.0	2.0	3.0	9.0	2.0	12	0.3	0
bot-8	1	1	0	1	0	5.9	2.0	3.8	10.5	2.5	13	0.3	0
bot-9	1	1	0	1	4.5	5.2	1.4	3.0	9.3	2.0	10	0.2	0
bot-5	1	1	0	1	0.5	5.5	1.5	3.2	9.2	2.0	11	0.3	0
bot-6	1	1	0	1	0.5	6.2	2.0	2.3	9.5	2.1	11	0.2	1

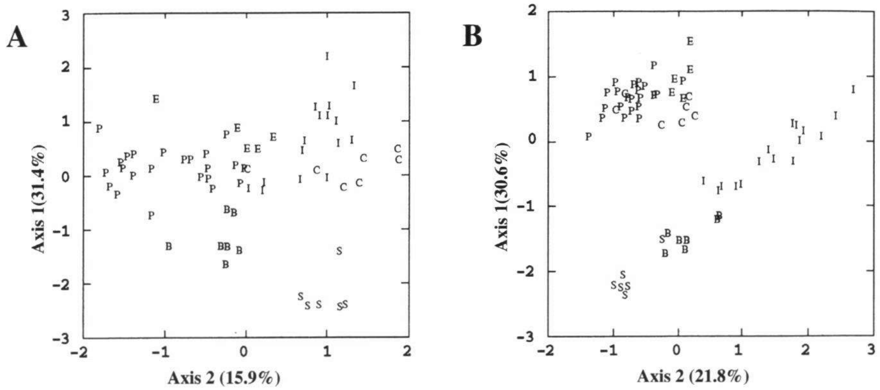


Fig. 4.1. Principal Component Analysis of the morphological data (Table 2) for the *Cichorium* species. A. Analysis without 'life span' and 'reproductive system' characters. B. Analysis including all characters of Table 2. C = *Cichorium calvum*, P = *C. pumilum*, E = *C. endivia*, I = *C. intybus*, S = *C. spinosum* and B = *C. bottae*.

min at 94°C; 37 cycles of 45 sec at 94°C, 1 min at 57°C, and 2 min at 72°C; followed by 10 min at 72°C. The amplified products were separated on 1.5–2% (w/v) agarose and bands were visualized by ethidium bromide staining and illumination under UV light. Restriction site analysis was performed using 1–2 µl amplification product and 0.5–2 units restriction enzyme. Reactions were incubated for 3 hours at 37°or 65°C depending on the optimal temperature of the restriction enzyme used. The restriction fragments were separated as described for the PCR-amplified products. Within *Cichorium* both site and length variation were scored, whereas in the outgroups only restriction site variation was coded. The list of mutations is available from the first author.

Chloroplast DNA And Nuclear rDNA Sequence Analysis – The *trnL-trnF* intergenic spacer region (Taberlet et al. 1991) of each individual was amplified as described above. Sequences of both strands were obtained using the primers E and F (Taberlet et al. 1991). Different PCR amplified regions were used in the sequence analysis. The ITS region, including ITS-1, ITS-2, and the 5.8S gene (White et al. 1990) was amplified on a MJ Research Peltier Thermo Cycler-100 set for 3 min at 94°C; 40 cycles of 1 min 94°C, 1 min at 52°C, and 2 min at 72°C; followed by 10 min at 72°C. Both strands of different PCR amplified products were sequenced using the primers ITS2, ITS3, ITS4, and ITS5 (White et al. 1990). The sequence reactions were performed by BaseClear (Leiden, The Netherlands) on an ABI 310 Genetic Analyzer using dye-terminator chemistry. Sequences were aligned using the EditSeq and MegAlign programs included in the DNAstar package version 3.01 (Lasergene) with subsequent manual adjustments.

Phylogenetic Analysis – A data matrix including only characters informative for parsimony analysis was generated from both the RFLPs and sequences. Phylogenetically informative indels across ingroup and outgroup and present in two or more taxa, were coded as binary characters in the chloroplast DNA sequence analysis. In the nuclear rDNA analysis, only phylogenetically informative indels within *Cichorium* were binary coded (data available from first author). The outgroup sequences were highly divergent from each other and from the ingroup sequences, causing alignment ambiguities at some positions. Gaps in the outgroup sequences were therefore coded as missing data, because leaving out the highly variable portions of the alignment from the analysis would also result in deleting all informative positions within *Cichorium*. All data matrices were analyzed with PAUP 3.1.1 using the branch and bound search algorithm and the MULPARS option in effect (Swofford 1993). Bootstrap analysis (Felsenstein 1985) was conducted with the branch and bound search option and 1,000 replicates. Decay analysis (Donoghue et al. 1992) was performed with the branch and bound search option. Trees up to 5 steps longer

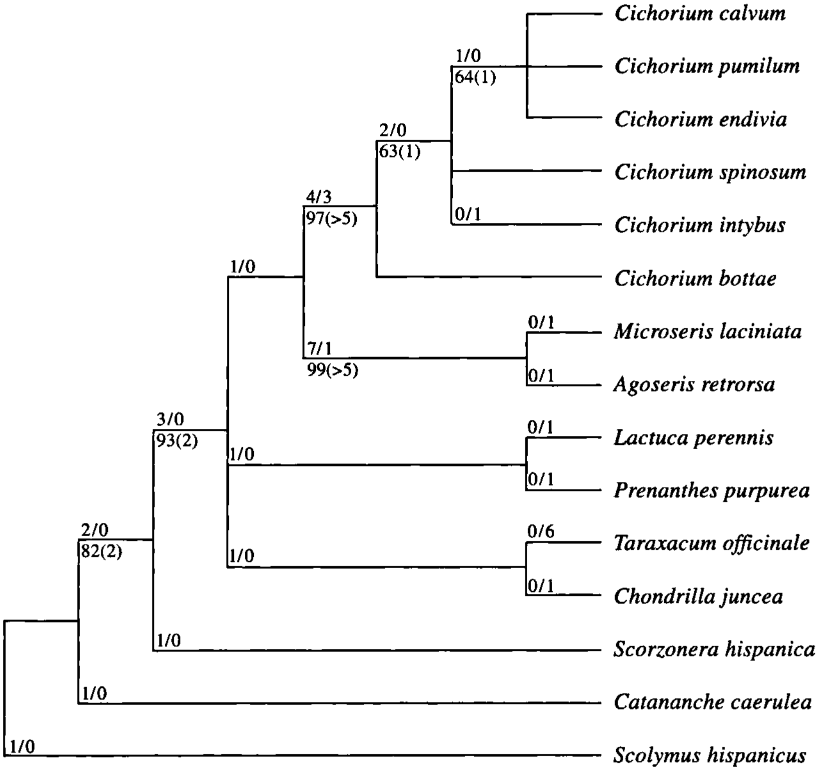


Fig. 4.2. The single most parsimonious tree based on sequences of the chloroplast DNA intergenic spacer *trnL-trnF* with a length of 41 steps and a consistency index of 0.79. Bootstrap values above 50% of 1,000 replicates are given below the branches along with the decay values between brackets. The character state changes are shown above the branches. (First, the number of apomorphic changes, followed by the total number of parallellisms and reversals.)

than the most parsimonious tree were generated. In the chloroplast analysis, trees were rooted using *Scolymus hispanicus* as the outgroup (Bremer 1994; Whitton et al. 1995). In the ITS analysis all five taxa outside *Cichorium* constitute the outgroup.

4.3 - Results

Morphological Analysis – Spontaneous seed set was found in representatives of *C. endivia, C. pumilum,* and *C. calvum,* which we determined to be annuals. The other three species, *C. intybus, C. spinosum,* and *C. bottae,* are all perennials and self-incompatible. The 'life span' and 'reproductive system' characters obtained from individual plants are characteristic for the species they represent.

The two principals component axes of the morphological characters are shown in Fig. 1A. The two axes explain 47.3% of the variation, with the first component explaining 31.4%. Only *C. spinosum* forms a well-separated cluster (Fig 4.1A). All other species, including the morphologically well-defined species *C. bottae*, form a large cluster, although the various species do form groups and are not completely intermixed. Inclusion of the characters referring to 'life span' and 'reproductive system' (Fig. 4.1B), demonstrates their usefulness: *C. pumilum, C. endivia,* and *C. calvum* form one cluster and *C. intybus* constitutes a separate cluster close to *C. bottae* and *C. spinosum.*

The problematic species delimitation based on morphological characters is illustrated in Fig. 4.1A, where only *C. spinosum* forms a clearly separated cluster. This resulted in many misidentifications in the field. Though the 'life span' and 'reproductive system' characters cannot be used in the field, they aid in distinguishing *C. intybus from C. endivia, C. pumilum* and *C. calvum.*

Chloroplast DNA Sequence Analysis – The *trnL-trnF* intergenic spacer region is 324 bp (*Taraxacum officinale*) to 429 bp (*Scorzonera hispanica*) long. A total of 80 variable sites were found, of which 29 were phylogenetically informative. Five phylogenetically informative indels were coded. Phylogenetic analysis of the sequence data revealed one most parsimonious tree (MPT) of 41 steps, with a consistency index of 0.79. The tree shows that *Cichorium* is a highly supported monophyletic genus (Fig. 4.2). *Cichorium bottae* appears to be sister to the other species based on a synapomorphic one bp substitution for the remaining species. The clade consisting of *C. pumilum, C. endivia,* and *C. calvum* shares a unique 11 bp insertion, which is a tandem repeat. Relationships among *C. spinosum, C. intybus* and the clade comprising *C. pumilum, C. endivia,* and *C. calvum* clade, are unresolved. In the *C. spinosum* individual sequenced, a 12 bp deletion was found. However, sequencing of four additional *C. spinosum* individuals, sampled from the same and another population, indicates that this deletion characterizes only a subset of the individuals of *C. spinosum.* All five individuals share a unique one bp deletion and a one bp substitution. The 12 bp deletion of *C. spinosum* was also found in the *Taraxacum officinale* accession used. In several other individuals of *Taraxacum officinale* this deletion is not present (T.H.M. Mes, pers. comm.).

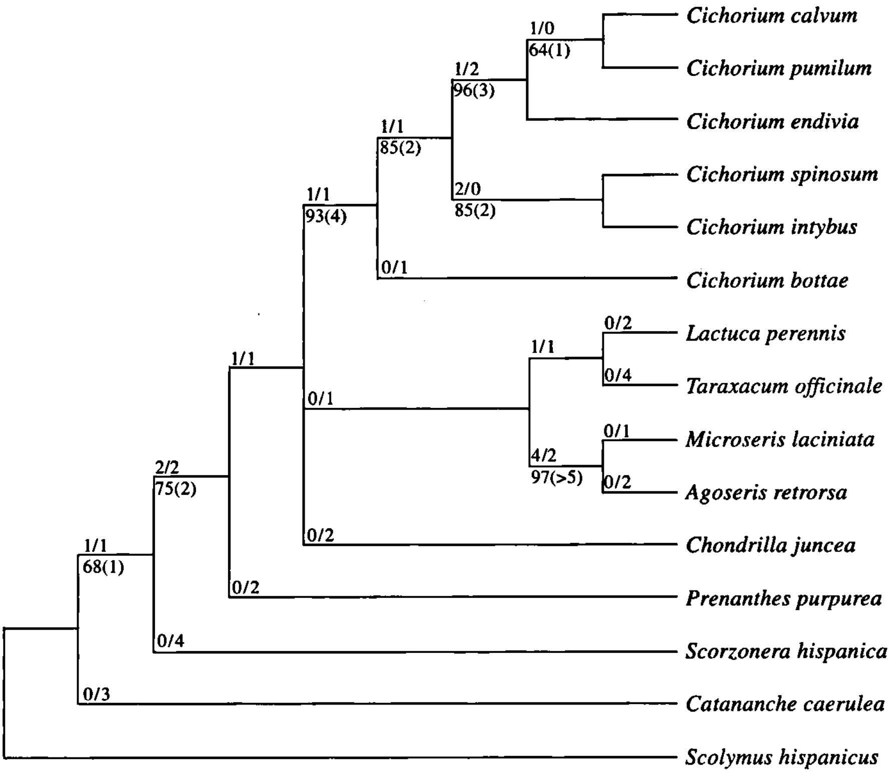


Fig. 4.3. The single most parsimonious tree based on chloroplast DNA RFLP data. The length is 51 steps and the consistency index is 0.65. Bootstrap values above 50% of 1,000 replicates are given below the branches along with the decay values between brackets. The character state changes are shown above the branches, first the number of apomorphic changes followed by the total number of parallellisms and reversals.

The relationship between *Cichorium* and the other genera within the Lactuceae remains poorly supported. Only *Scorzonera hispanica* and *Catananche caerulea* are well-supported branches as is the *Microseris laciniata/Agoseris retrorsa* clade. Sequences of the *trnL-trnF* intergenic spacer region contains a 77 bp tandem repeat of the *trnF* gene in *Scorzonera hispanica* and *Taraxacum officinale*. In both taxa, the *trnF* copy closest to the *trnL* gene shows several substitutions compared to the *trnF* gene of *Nicotiana tabacum* L. (Shinozaki et al. 1986). Probably the degeneration of the 'F'-primers site, which is due to some base substitutions in the 'F'-primer site, caused the absence of a mixture of amplification products after PCR. The occurrence of tandemly repeated *trnF* genes in the Asteraceae family was recently described in *Microseris*, where several copies of this gene were found (Vijverberg et al., 1999). However, the pseudogenes found in *Microseris* are situated between *trnF* and *ndhJ*, while the pseudogenes described here are between *trnL* and *trnF*. Sequencing towards *ndhJ* might reveal the presence of *trnF* pseudogenes in other Lactuceae genera as well, which might be useful for phylogenetic reconstructions at and below the generic level.

Chloroplast DNA RFLP Analysis – Of the twelve regions amplified for RFLP analysis, three appeared to be uninformative for parsimony analysis, i.e. the *trnT-trnL* spacer, *16S*, and *psbA*. The chloroplast spacer region *trnS-psaA* of *Scolymus hispanicus* and the *atpB-rbcL* spacer region of *Scolymus hispanicus* and *Catananche caerulea* could not be amplified and were coded as missing data. A total of 57 mutations were scored, of which 33 were phylogenetically informative. Thirty-one are restriction site mutations and two are length mutations. The length mutations are informative within the genus *Cichorium* and were confirmed by at least two enzymes within each of the two chloroplast DNA regions, i.e. the *trnK* intron and the *petA-psbE* spacer. Because of difficulty of homology assessment, these length variants were scored as missing in the outgroup taxa. All missing data (7.3% of the data points in the matrix) are restricted to the outgroup species.

Phylogenetic analysis of the RFLP data revealed only one MPT of 51 steps, with a consistency index of 0.65 (Fig. 4.3). Again, *Cichorium* is a highly supported mono-phyletic group. *C. bottae* is sister to all other *Cichorium* species. The relationships between the remaining species are more resolved than in the chloroplast DNA sequence-based tree (Fig.4. 2): i.e., *C. spinosum* and *C. intybus* form a strongly supported sister clade to *C. pumilum, C. calvum,* and *C. endivia*. Within the latter clade, *C. endivia* is sister to *C. pumilum* and *C. calvum*. The single character change on the *C. pumilum/C. calvum* branch is a length mutation which was scored unambiguously within *Cichorium*, but could not be scored confidently in the outgroup species.

In addition to the representatives of each species used, 74 individuals from different locations, including representatives of the various cultivar groups within *C. endivia* and *C. intybus*, were checked for the presence or absence of the 11 bp insertion in the *trnL-trnF* intergenic spacer region (Table 4.1). These accessions were also checked for the presence or absence of the five informative restriction mutations previously found. Four times, the informative characters appear to be characteristic for a particular species or clade and agree with the results of the phylogenetic analysis of the complete RFLP data set. However, the length variation characteristic for the clade consisting of *C. calvum* and *C. pumilum* is also present in two *C. endivia* accessions and absent in two *C. pumilum* accessions, all from the Genebank in Gatersleben, Germany (Table 4.1). The origin of these accession are not clear and the voucher specimens were incompletely sampled which prevents proper identification by the authors. Therefore, the accessions were identified according to the herbarium labels.

The relationship between *Cichorium* and the outgroup genera remains poorly supported just as in the sequence analysis of the *trnL-trnF* intergenic spacer region. The relationship among the outgroup genera is also similar to the former analysis with *Catananche caerulea* and *Scorzonera hispanica* being well-separated basal lineages compared to the other taxa, and *Agoseris retrorsa* and *Microseris laciniata* forming a strongly supported group.

Nuclear rDNA Analysis – Within *Cichorium*, ITS-1 is 253 bp long and ITS-2 is 223 bp long, which is in the range of ITS length variation described by Baldwin et al. (1995). A total of six variable sites were found in ITS-1 and two in ITS-2. In ITS-2 a one-bp gap shared by *C. intybus* and *C. spinosum* was coded as an extra binary character. Eight of these nine characters are phylogenetically informative within *Cichorium*. One MPT of 141 steps with a consistency index of 0.81 resulted from parsimony analysis (Fig. 4.4B). The cladogram is in agreement with the tree obtained from a combined chloroplast analysis based on the RFLP and the *trnL-trnF* intergenic spacer sequence analysis (Fig. 4.4A), except that the relationships among *C. endivia, C. pumilum,* and *C. calvum* are unresolved. Within ITS-1, a one-bp mutation resulted in the loss of a restriction site, synapomorphic for *C. endivia, C. pumilum,* and *C. calvum*. Checking for presence or absence of the restriction site in another 74 *Cichorium* individuals confirmed the restriction site loss in *C. endivia, C. pumilum,* and *C. calvum*.

Based on the absence of homoplasy in *Cichorium* in all three data sets and the congruence among the phylogenies based on these data sets, a combined phylogenetic analysis of all three datasets seems justified. Of all outgroup species used, only *Prenanthes purpurea* was included in every analysis. To maximize outgroup representation in the combined analysis, genera that were present in all three datasets, but represented by different species were included as outgroups as well, i.e. *Lactuca, Microseris* and *Agoseris*. The analysis resulted in 1 MPT of 261 steps and a consistency index of 0.78 and is identical to the combined chloroplast DNA cladogram, but with higher bootstrap values as is shown in Fig. 4.4A.

4.4 - Discussion

Position Of Cichorium Within Lactuceae – The uncertain position of the genus *Cichorium* within the tribe Lactuceae is underscored by both the chloroplast DNA and the nuclear rDNA phylogenies described here. In the last 45 years, *Cichorium* has been suggested to be closely related either to Microseridinae (Stebbins 1953; Whitton et al. 1995) or to Crepidinae (Jeffrey 1966; Vermeulen et al. 1994; Koopman et al. 1998). None of these studies however, resulted in strong evidence for the relationship between *Cichorium* and other Lactuceae genera. Possible reasons for this problem may be, among others, reticulate patterns of evolution, insufficient sampling, a high level of homoplasy in characters, and rapid radiation of generic lineages in Lactuceae.

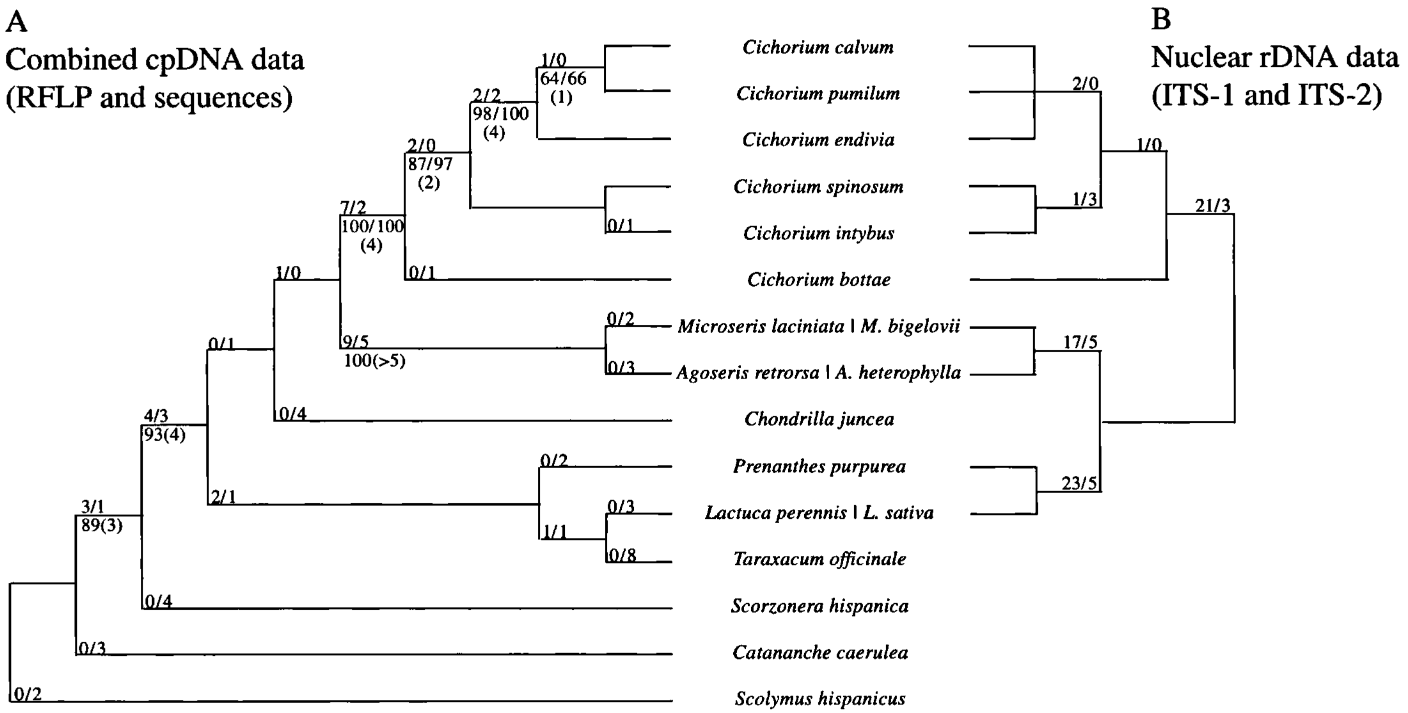
Our results do not confirm nor reject the suggestion of *Cichorium* being close to Microseridinae or Crepidinae, but reaffirm the statement of Bremer (1994) that *Cichorium* cannot be placed in any subtribe at present. Although the relationships between *Cichorium* and the other Lactuceae genera are not clear, justification for placing *Cichorium* within the Lactuceae was provided by Kim et al. (1992) and Kim and Jansen (1995) based on *rbcL* and *ndhF* trees, respectively. In these studies, *Cichorium* forms a highly supported monophyletic clade with *Lactuca* and *Tragopogon*. Both Bremer (1994) and Whitton et al. (1995) found that *Catananche* has a basal position within the Lactuceae, which is confirmed in our chloroplast analyses. We found strong evidence for a basal position of *Scorzonera* compared to the other genera studied which agrees with the chloroplast analysis of Whitton et al. (1995). Our data support the hypothesis that Crepidinae and Lactucinae are artificial (Kim et al. 1996). Only Microseridinae (*Microseris* and *Agoseris*) forms a highly supported clade.

Phylogenetic Relationships Within Cichorium – The genus *Cichorium* is monophyletic according to our chloroplast and nuclear DNA analyses. Within the tribe Lactuceae, the genus is well defined morphologically, which is rather exceptional within Asteraceae (Cronquist 1985). At the specific level, however, the taxa of *Cichorium* are difficult to delimit except for *C. bottae* and *C. spinosum*. On the basis of differences in pappus length, life span, and reproductive system, *C. intybus* can be distinguished from *C. endivia, C. pumilum,* and *C. calvum,* which reflects the phylogenetic relationships uncovered by our molecular data. The latter three species, however, differ primarily in a few quantitative characters (A. M. Kiers, this thesis).

In our analyses *C. bottae* is sister to the other species of *Cichorium.* This species is endemic to Yemen and Saudi-Arabia and its cushionlike growth form appears to be highly adapted to environmental circumstances of the Arabian Peninsula. The remaining five species are divided into two highly supported sister clades, one consisting of *C. intybus* and *C. spinosum* and the other of *C. endivia, C. pumilum,* and *C. calvum.*

While *C. intybus* has been often confused with *C. endivia, C. pumilum,* or *C. calvum,* this species appears to be the closest relative of *C. spinosum.* The grouping of *C. spinosum* and *C. intybus* was found before using RFLP data of the mitochondrial genome (Vermeulen et al. 1994). Based on the estimation of relatedness between cytotypes and the fact that the two species could easily be crossed to produce fertile F_1 offspring, Vermeulen et al. (1994) suggested *C. spinosum* to be an ecotype of *C. intybus* rather than a separate species. A fertile F_1 offspring can also be obtained from crosses between *C. intybus* and *C. endivia,* and possibly also from all other interspecific crosses within *Cichorium* (Rick 1953; A. M. Kiers, unpubl. data). However, because the difference between an ecotype or a taxon lies in the fact that, in contrast to a taxon, an ecotype can have multiple independent origins of the same genetic condition, high genetic similarities and fertile offspring are inconclusive with regard to a taxonomic or an ecotypic designation of *C. spinosum.*

Fig. 4.4. Comparison of chloroplast and nuclear DNA based phylogenies. A. The single most parsimonious tree based on a combined dataset of chloroplast DNA RFLP and trnL-trnF sequence data with a length of 83 steps and a consistency index of 0.70. Bootstrap values above 50% of 1,000 replicates are given below the branches, followed by similarly obtained bootstrap values of a combined analysis including both chloroplast DNA datasets and sequences of ITS-1 and ITS-2. The decay values of the combined chloroplast DNA analysis are given between brackets. Above the branches are the character state changes, first the number of apomorphic changes followed by the total number of parallellisms and reversals. B. The single most parsimonious tree based on sequences of ITS-1 and ITS-2 with a length of 141 steps and a consistency index of 0.81. Indications of bootstrap and decay values as well as the number of character state changes are similar to Fig 4.4A.


A
Combined cpDNA data
(RFLP and sequences)
B
Nuclear rDNA data
(ITS-1 and ITS-2)
1/0
64/66
(1)
2/2
98/100
(4)
2/0
87/97
(2)
7/2
100/100
(4)
0/1
0/1
1/0
9/5
100(>5)
0/2
0/3
0/1
0/4
4/3
93(4)
2/1
0/2
0/3
1/1
0/8
3/1
89(3)
0/4
0/3
0/2
Cichorium calvum
Cichorium pumilum
Cichorium endivia
Cichorium spinosum
Cichorium intybus
Cichorium bottae
Microseris laciniata | M. bigelovii
Agoseris retrorsa | A. heterophylla
Chondrilla juncea
Prenanthes purpurea
Lactuca perennis | L. sativa
Taraxacum officinale
Scorzonera hispanica
Catananche caerulea
Scolymus hispanicus
2/0
1/3
1/0
21/3
17/5
23/5

The remaining clade comprising *C. endivia, C. pumilum,* and *C. calvum* is strongly supported. Within this clade one synapomorphic character (chloroplast DNA length polymorphism in the *trnK* region after digestion with *Alu*I) characterized *C. pumilum* and *C. calvum.* As mentioned in the introduction, *C. endivia* was assumed to be very closely related to either *C. intybus* (Matthews 1975) or *C. pumilum* Boissier 1875; de Candolle 1884; Meikle 1985; Wagenitz and Bedarff 1989) on the basis of morphology. Instead, our chloroplast and nuclear DNA data both show that *C. endivia* is most closely related to *C. pumilum* and *C. calvum.*

Although a close relationship between *C. endivia* and *C. calvum* was hypothesized earlier (Wagenitz and Bedarff 1989), *C. calvum* has never been considered to be a possible wild ancestor of *C. endivia,* while this possibility has been suggested for *C. pumilum* (Boissier 1875; De Candolle 1884; Meikle 1985; Wagenitz and Bedarff 1989). The neglect to consider *C. calvum* may be due to the fact that the species is rarely mentioned in the literature as well as in floras. The obscurity of *C. calvum* may explain why plant material of this species from Israel was not recognized as such by Vermeulen et al. (1994), but was assigned to *C. endivia* despite the absence of pappus.

Because Vermeulen et al. (1994) did not recognize their wild *C. endivia* accession as *C. calvum,* they concluded that pappus is not a suitable character for identifying the various *Cichorium* species. However, the clear difference in pappus length among *C. intybus* and *C. spinosum* at one hand and *C. endivia* and *C. pumilum* on the other demonstrates the opposite. In contrast, pappus is not a very clear character for identifying *C. calvum.* Absence of pappus is the main character by which this species is distinguished (Merxmüller 1957; Wagenitz and Bedarff 1989), because it is easily determined. However, the presence of minute scales in various herbarium specimens (Hoffmann 1897; Wagenitz and Bedarff 1989) and even long (1–1.0 mm) scales in a herbarium specimen used by the authors (Table 4.1) should be taken to indicate that for *C. calvum* pappus might not be the best diagnostic character and additional characters such as achene shape, and maximum size of outer phyllaries should be examined as well.

If the pappus character is plotted on the *Cichorium* phylogeny as presented in Fig. 4.4A, long pappus scales (>0.7 mm) appears to be a derived character state, present in both *C. pumilum* and *C. endivia.* Although *C. calvum* usually has no pappus, one specimen has long pappus scales similar to *C. endivia* and *C. pumilum.* Therefore, it seems likely that long pappus scales is a derived character state within *Cichorium.*

Two more characters can be plotted on the phylogenetic tree: *C. endivia, C. pumilum* and *C. calvum* are annual and self-compatible while the remaining species (*C. intybus, C. spinosum,* and *C. bottae*) are perennial and self-incompatible. Within *Cichorium* both annual and self-compatible are derived character states, that were present in the common ancestor of *C. endivia, C. pumilum,* and *C. calvum.* The occurrence of clusters of closely related, annual and self-compatible taxa in the Mediterranean region has been found before in various genera. Though multiple hypotheses of the influence of autogamy on speciation have been described (Zohary 1997), explanations are lacking.

Despite the resemblance between the cultivated species *C. intybus* and *C. endivia* they are not sister species. However, the possible intercrossability or interfertility of the species within *Cichorium* opens new opportunities for plant breeders. In order to get more insight into the relationships among the species and the various cultivar groups of *C. intybus* and *C. endivia*, supplemental markers are needed.

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5 – A search for diagnostic AFLP markers in Cichorium species with emphasis on endive and chicory cultivar groups

Abstract

The genus *Cichorium* consists of two widely cultivated species *C. intybus* (chicory) and *C. endivia* (endive) and four wild species, *C. bottae, C. spinosum, C. calvum,* and *C. pumilum.* A multivariate and a UPGMA analysis based on AFLP markers were used to establish the genetic relationships among the species and cultivar groups of *C. intybus* and *C. endivia.* At the species level the results correspond with previously obtained phylogenetic relationships in that *C. bottae* is the most divergent species, and *C. intybus* and *C. spinosum* on the one hand, and *C. endivia, C. pumilum,* and *C. calvum* on the other form a cluster. Based on the congruence between phylogenetic and genetic analysis, unique markers were expected for all species. However, hardly any specific marker was found except for *C. bottae.* The analysis of cultivar groups of *C. intybus* resembled the species analysis in two respects: (1) grouping of cultivars according to cultivar groups, and (2) lack of markers unique to cultivar groups. In contrast to *C. intybus,* the cultivar groups of *C. endivia* do not form distinct groups, which would reflect that crosses have been made among the various cultivar groups. The relationships among *Cichorium* species and cultivars will be useful for setting up a core collection of *Cichorium* and stresses the importance of inclusion of the wild species in the collection.

5.1 - Introduction

Cichorium endivia (endive; Asteraceae) and *C. intybus* (chicory) are widely cultivated in the temperate and semi-arid climate zones in the world. Several cultivar groups are distinguished to be referred here according to the rules of the International Code of Nomenclature for Cultivated Plants (Trehane et al. 1995). *C. endivia* is a diploid ($2n=2x=18$), annual, and self-compatible (SC) species (Rick 1953) that is only known from cultivation. The cultivars of this species can be divided into three groups. (1) The Scarole Group consists of broad-leaved or plain endives. (2) The Frisé Group contains crispy or curly endives with narrow, frilled leaves. (3) The Endivia Group consists of ancient cultivars with narrow, incised leaves that are rarely on the market nowadays (Schultze-Motel 1986; Ryder 1999).

Cichorium intybus is a diploid ($2n=2x=18$), perennial, and self-incompatible (SI) species (Rick 1953; Eenink 1981; Cichan 1983) containing three cultivar groups. (1) The cultivars of the Root Chicory Group are cultivated for their large roots that were formerly used as a coffee substitute or additive. Today, they are mainly cultivated for the production of inulin (a β-(2,1)-linked fructan), the major reserve carbohydrate in many Asteraceae and for food and non-food applications (De Leenheer 1996; Koch and Jung 1997). (2) The Witloof Group contains witloof or Brussels chicories, a common vegetable in Belgium, France and The Netherlands, which are used for producing ‘witloof’ or ‘French endive’ under artificial conditions. The ancestral type of both witloof and root chicory is suggested to be the ‘Magdeburger type’ (Baes and Van Cutsem 1993b; Bellamy et al. 1995; Bellamy et al. 1996; Van Kruistum 1997). (3) The Pain de Sucre Group comprises the green-leaved cultivars which are mainly

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cultivated in NW-Europe (Van Kruistum 1997). In contrast to what their name suggests, the cultivars have a bitter taste. (4) The Radicchio Group consists of leaf chicories that are essentially bred for their blond, red or variegated leaves that are used as fresh or cooked food (Baes and Van Cutsem 1993) originating from Northern Italy. In contrast to the original 'Treviso' type, whose origin is unknown, the 'Chioggia' type resulted from a cross between 'Radicchio' and an endive cultivar of the Scarole Group (Van Kruistum 1997; Van Wijk 1999). It has to be noted that a cross between 'Radicchio' and a witloof cultivar has led to a red chicory forcing type which belongs to the Witloof Group instead of the Radicchio Group (Van Kruistum 1997).

The genetic relationships among the various cultivar groups of *C. endivia* and *C. intybus* are of practical interest for the management of chicory genetic resources. In Europe, this is coordinated by the Group for Testing and Control of Varieties and Seeds (GEVES) at Brion (France; Kelechian-Cadot and Boulineau 1996). Because there are only a few morphological characters available for estimation of genetic variability in *Cichorium*, the genetic variation within the genus is best studied with molecular markers. Genetic variation of witloof cultivars was studied using isozymes, but it appeared to be very limited compared to other crops such as tomato, maize, wheat, rice, and sugar beet (Baes and Van Cutsem 1993a/b). Restriction fragment length polymorphisms (RFLPs) of nuclear and cytoplasmic DNA in witloof cultivars were used by Bellamy et al. (1995), but here a low level of variation was obtained as well. Similar results were obtained with RFLP data of the mitochondrial genome of *C. endivia, C. intybus* and *C. spinosum* (Vermeulen et al. 1994). In contrast, random amplified polymorphic DNA markers (RAPDs) revealed a much higher polymorphism rate in witloof cultivars (Bellamy et al. 1996; Demeulemeester et al. 1997) and root chicory cultivars (Koch and Jung 1997). A comparison of RAPDs with amplified fragment length polymorphism markers (AFLPs) indicated that both marker systems are equally useful for the establishment of genetic diversity in root chicory cultivars (Koch and Jung 1997). The sensitivity of RAPDs to experimental conditions, however, limits their usefulness (Jones et al. 1997; Milbourne et al. 1998). A comparison of AFLP markers with RAPDs, single sequence repeats (SSRs), and RFLPs revealed that AFLPs have the highest multiplex ratio (=number of loci simultaneously analysed per experiment) of these marker systems which makes them suitable for germplasm identification (e.g. Powell et al. 1996; Milbourne et al. 1998).

Although various reports concerning the genetic variability of witloof and root chicory were recently published, nothing is known about the variability of *C. endivia* or any of the other wild species of the genus, i.e. *C. bottae, C. spinosum, C. calvum,* and *C. pumilum.* Although only *C. endivia* and *C. intybus* are widely cultivated, one of these wild species, *C. spinosum* ('spiny chicory'; Greek: 'stamnangathi') is a popular wild food on Crete (Greece). It is sought-after for the winter rosettes and eaten as salad or boiled vegetables, separate or in combination with other wild greens (Akeroyd and Hogan 1996). Food applications of the remaining wild species are not known to the authors. The genetic variation within and among the cultivar groups and species as well as a detailed AFLP marker distribution were studied. The results were interpreted in the light of a molecular based phylogeny of the genus (Kiers et al. 1999), and underscore the usefulness of including accessions of wild *Cichorium* species in the chicory genetic resources collection.

5.2 - Material and methods

Plant material – A total of 56 accessions (i.e. seed samples from individual plants or a population) of the *Cichorium* species and cultivar groups comprising 160 individual plants were used for this study. The sources, accession numbers, and the number of individual plants analysed per accession are listed in Table 5.1. All plants were grown in an insect-free greenhouse with a minimum temperature of 4°C and under field conditions.

AFLP analysis – DNA isolation was performed as described by Doyle (1991) with minor modifications (Kiers et al. 1999). The AFLP protocol of Vos et al. (1995) was followed with minor changes (Van der Hulst et al. 2000). The primer combination *Eco*RI+ACA, *Mse*I+CAG was selected from a primer screening with (γ-^{32}P-labeled) *Eco*RI+ACA in combination with *Mse*I+(CAA, CAC, CAG, CAT, CTA, CTC, CTG, or CTT) on a representative of each species. Selection was based on the number of well-separated, polymorphic bands present. Bands were scored as binary characters: 1=present, 0=absent. In case of ambiguous presence/absence of a band missing values were used. If the alignment of group specific markers (i.e. markers present in every individual of an accession and in every accession of the particular group of species) was ambiguous, the particular samples were run again next to each other. The data matrix is available from the first author.

Data analysis – The variation within accessions was determined by studying two to four plants of each accession. The percentage of polymorphic bands of each accession was calculated as the number of polymorphic markers divided by the total number of scored markers of the accession. Variation within species was calculated similarly. The AFLP banding patterns of individuals were pooled per accession so that a band present in one or more individuals was scored as present for the accession. This reduced data set (with respect to the number of individuals) was used in further analysis.

Three Principal Coordinates analyses (PCO) were performed with the NTSYS-PC package (Rohlf 1993), one on the complete data set, one using the cultivars of *C. endivia* and one using the cultivars of *C. intybus*. Dissimilarities between taxa were calculated with the Euclidean distance coefficient. The eigenvectors were calculated using the programs 'Double Center' and 'Eigenvectors'. A dendrogram was constructed using the unweighted pair group method average (UPGMA) method. Similarities between pairs were calculated for the complete data set using the Jaccard coefficient.

5.3 - Results

Distribution of AFLP markers in Cichorium species and cultivars – Analysis of the 56 accessions resulted in a total of 184 scorable AFLP markers of which only one was monomorphic in all individuals. These markers could distinguish 152 of the 160 individual plants, and 55 of the 56 accessions. Two identical accessions were present in *C. pumilum* (E11 + E12). Identical individuals were found in *C. pumilum* (3) and *C. endivia* (2). The number of scored bands per individual varied from 14 (*C. pumilum*) to 47 (*C. intybus*), while the number of scorable bands per accession varied from 16 (*C. pumilum*) to 61 (*C. intybus*).

Table 5.1. Plant material of *Cichorium*. The number of individual plants used in the analysis are indicated between brackets.

[1] Institute for Plant Genetics and Crop Plant Research (IPK), Corrensstraβe 3, D-06466 Gatersleben, Germany.

[2] Florimond Desprez, B.P. 41, 59242 Cappelle-en-pévèle, France.

[3] USDA/ARS North Central Regional Plant Introduction Station, Iowa State University, Ames, IA 50011, USA.

Cichorium calvum Sch.Bip. **Iraq.** Rawa, IPK[1] CICH100/88, I154(2). **Israel.** Florimond Desprez[2] C2(2).

Cichorium pumilum Jacq. **Crete.** Lasithi, A. M. Kiers P4(1), P12(3), P13(3), P18(3). IPK CICH94/214, G30(3). **Denmark.** Botanic Garden University of Kopenhagen, IPK CICH87/88, E12(3). **Italy.** Botanic Garden, University of Palermo, IPK CICH50/85, G26(3). Palermo, IPK CICH66/89, G27(3). **Morocco.** Volubilis, A. M. Kiers P21(3). **Sweden.** Botanic Garden University of Uppsala, IPK CICH86/76, E11(3).

Cichorium endivia L.

Scarole Group: **Germany.** 'Escariol Grüner' IPK CICH42/76, G20(3). **Italy.** IPK CICH39/79, G17(3). 'Full Heart', IPK CICH171/94, G2(3).

Frisé Group: **Germany.** 'Imperial', IPK CICH16/75, G1(3). **Italy.** IPK CICH110/89, G10(3). 'Di Pancalieri a costa bianca', IPK CICH29/85, G24(3). 'Cicoria Riccia', IPK CICH46/81, G25(2). **Spain.** IPK CICH27/87, G19(3). **Syria.** IPK CICH192/94, G3(3).

Endivia Group: **Greece.** IPK CICH1/73, G6(3). IPK CICH4/73, G9(3). **Italy.** IPK CICH99/86, G7(3). IPK CICH189/94, G8(3). 'Italienischer Grüne', IPK CICH11/77, G16(3). IPKCICH67/88, G18(3).

Cichorium intybus L.

Wild chicory: **Cyprus.** NCRPIS[3] PL432336, I40(3). **Germany.** Botanic Garden, University of Jena, IPK CICH79/82, G12(5). Botanic Garden, University of Dresden, IPK CICH8/92, G22(3). **Hungary.** NCRPIS PL531292, I42(3). **Italy.** Palermo, P. Mazzola I15(3). **Netherlands.** Heteren, A. M. Kiers I125(2). **Pakistan.** NCRPIS PL269459, I35(3). **Turkey.** NCRPIS PL279705, I39(3). **Uruguay.** NCRPIS PL162665, I33(3).

Root Chicory Group: **Belgium.** 'Dageraad', IPK CICH205/94, G23(3). **Germany.** 'Echte Zylinderförmige Spitzköpfige Magdeburger Riesen', IPK CICH71/95, I152(3). **Hungary.** 'Slezka', IPK CICH75/93, G4(3). 'Horpacsi', IPK CICH80/92, G14(3). **Poland.** 'Polanowicka', IPK CICH76/93, G15(3).

Witloof Group: **Netherlands.** A. and J. van de Woude I18(3). **Sweden.** 'Brüsseler Witloof', IPK CICH52/94, G29(3).

Pain de Sucre Group: **Italy.** 'Pan di Zucchero', IPK CICH97/86, I153(3).

Radicchio Group: **England.** 'Rossa de Verona', IPK CICH128/92, G5(3). **Italy.** 'Cicoria rossa di Treviso', IPK CICH119/83, G11(2). 'Cicoria spadona di taglio', IPK CICH98/86, G13(3). IPK CICH143/92, G21(3).

Cichorium spinosum L.

Crete. Along the road around Pahía Amos, A.M. Kiers S1(3), S2(3), S3(5). Along the coast of Almirida, A.M. Kiers S16(1).

Cichorium bottae A.Deflers.

Yemen. Near Mathna along the road to Bajil, A.M. Kiers B27(4), B31(3). 3 km after Hajer Saeed along the road to San'a, A.M. Kiers B41(1). Near Hajer Saeed along the road to Bajil, A.M. Kiers B59(1).

Many (7) species-specific AFLP markers, i.e. markers present in every individual of the species or group in question, were obtained for *C. bottae*, but only one specific marker was found for all *C. endivia* cultivars and one specific marker for all *C. intybus* and *C. spinosum* individuals together (Table 5.2). Markers restricted to cultivar groups within *C. endivia* and *C. intybus* were not found if analysed together with the other species or in a separate analysis. Only one marker was unique to all accessions of the Radicchio Group in the analysis restricted to cultivated *C. intybus* accessions. The total number of bands per accession of the SC species *C. pumilum, C. calvum* and *C. endivia* are lower than in the SI species *C. intybus, C. spinosum* and *C. bottae*, as is the percentage of polymorphic bands within accessions. However, the SC species *C. endivia* and *C. pumilum* and SI species *C. intybus* have a similar and relatively high percentage of polymorphic bands within the species, due to the high amount of variation between the accessions of the SC species. The percentage of polymorphic bands within *C. spinosum* (SI), C. bottae (SI), and *C. calvum* (SC) is (much) lower. Of these species only 2–4 accessions were included instead of 10–21 in the highly polymorphic species *C. endivia, C. pumilum, and C. intybus*. An increasing percentage of polymorphism is expected after adding more accessions to each species.

PCO and cluster analysis – The distribution of the AFLP markers of all species of Cichorium over the first two PCO axes is shown in Fig. 5.1. The two axes explain 46.9% of the variation with the first coordinate explaining most (30.7%) of the variation. Two main clusters are formed, one including *C. intybus* and *C. spinosum*, and the other including *C. bottae, C. pumilum, C. calvum,* and *C. endivia*. On the third axis (explaining 6.3% of the variation), *C. bottae* is clearly distinguished from all other species (not shown). Although *C. pumilum, C. calvum* and *C. endivia* form one cluster, the different species are separated within the cluster. Individuals of *C. spinosum* and *C. intybus* however, are slightly mixed which can be found out from the position of the two *C. intybus* individuals (I153 and I15).

UPGMA analysis of the *Cichorium* accessions revealed one dendrogram, which is shown in Fig. 5.2. Similar to the PCO, three main clusters are found with the individual accessions of every species grouping together. This also holds true for the

Table 5.2. Levels of AFLP variation within and among *Cichorium* species. *=only calculated for accessions consisting of more than one individual, i.e. three accessions for *C. spinosum* and two accessions for *C. bottae*; acc=accession(s); spec=species.

species	nr of acc	nr of unique bands	nr of bands per spec	% polymorph per spec	nr of bands per acc	mean nr of bands	% polymorph per acc	mean % polymorph
C. calvum	2	0	32	34.4	25-31	28.0	8.0-19.4	13.7
C. pumilum	10	0	62	93.5	16-31	22.7	0-25.8	14.2
C. endivia	15	1	75	82.7	22-37	29.0	11.5-48.6	25.2
C. intybus	21	0	137	92.0	35-61	48.8	19.4-70.5	43.6
C. spinosum	4	0	63	63.5	45-53	50.0	52.8-59.6*	56.0*
C. bottae	4	7	52	53.8	43-44	43.5	40.9-41.9*	41.4*

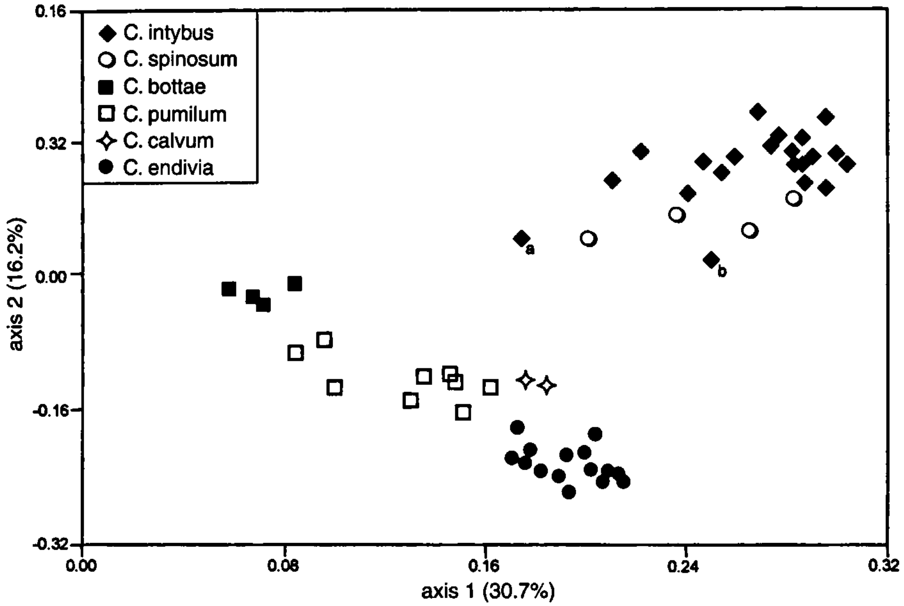

Fig. 5.1. Principal Coordinates Analysis (PCO) based on AFLP markers including all *Cichorium* species. a=I153, b=I15, see text for details.

two *C. intybus* accessions (I153 and I15) that were intermixed with *C. spinosum* in the PCO. The dendrogram including all accessions analysed shows that none of the various cultivar groups of *C. endivia* and *C. intybus* form distinct groups. In a separate PCO analysis of the *C. intybus* cultivars, the accessions of each cultivar group do cluster together which is in contrast to the *C. endivia* accessions (Fig. 5.3).

5.4 - Discussion

The genetic relationships among the *Cichorium* species determined here with AFLP markers are congruent with the phylogenetic relationships among the species based on chloroplast (cp) and nuclear ribosomal (nr) DNA data (Kiers et al. 1999). The high divergence of *C. bottae* according to the AFLP data corresponds with its position as sister to the other *Cichorium* species in the phylogenetic analysis. The remaining species form two distinct groups similar to the two clades found, i.e. *C. intybus* and *C. spinosum* on the one hand, and *C. endivia, C. pumilum,* and *C. calvum* on the other. A congruence of genetic relationships determined with AFLPs with those determined with other marker systems was found before (e.g. Powell et al. 1996; Milbourne et al. 1998). Specific AFLP markers were expected for the species within *Cichorium*, because of the similar grouping of species obtained with AFLPs compared to cpDNA and nrDNA RFLP and sequence data, and the use of a more variable marker system. However, even in our restricted sample this only holds true for *C. bottae* where seven species-specific markers are found. *C. bottae*

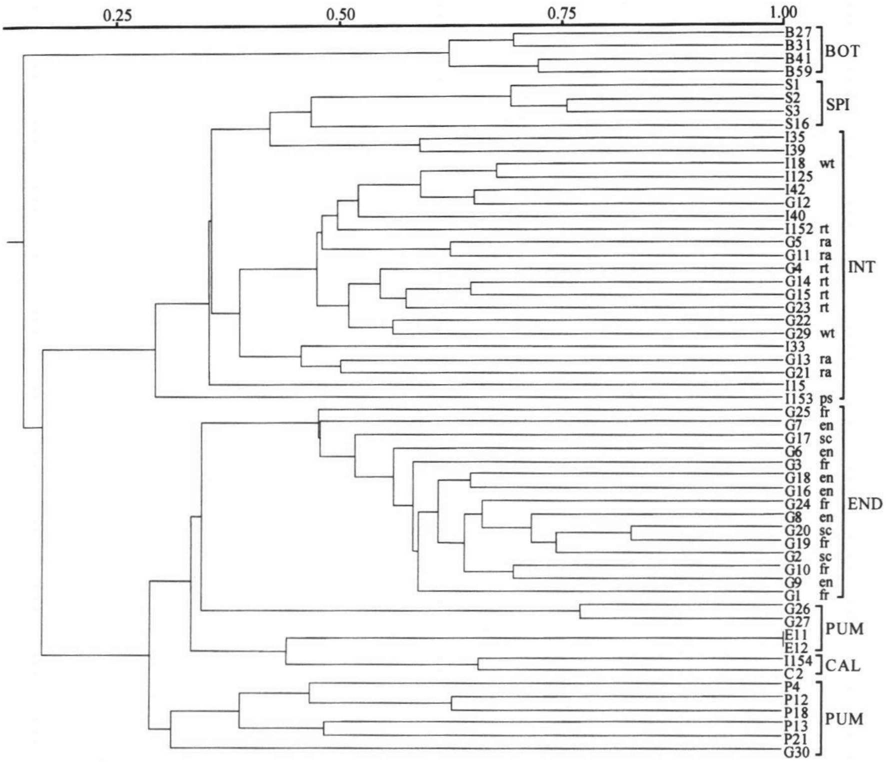

Fig. 5.2. UPGMA dendrogram of Cichorium species and cultivar groups based on AFLP markers. BOT=*C. bottae*; SPI=*C. spinosum*; INT=*C. intybus*; END=*C. endivia*; PUM=*C. pumilum*; CAL=*C. calvum*; wt=Witloof Group; rt=Root Chicory Group; ra=Radicchio Group; ps=Pain de Sucre Group; sc=Scarole Group; fr=Frisé Group; en=Endivia Group; no indication=wild.

is endemic to Yemen and Saudi-Arabia and is the only species that occurs outside the distribution area of *C. intybus*. The isolated position of *C. bottae* might explain the high number of specific markers found for this species relative to the other species of *Cichorium*. Although one specific marker has been found for *C. endivia* and one specific marker for *C. intybus* and *C. spinosum* together, no specific markers were found for *C. intybus, C. spinosum, C. pumilum,* and *C. calvum*. In a comparable study on wild and cultivated species of *Lactuca*, 320 markers were scored of which many markers were restricted to a single species, and in a separate analysis of the cultivated species *L. sativa*, out of 119 scored markers 1–6 unique markers were found for every cultivar group (Hill et al. 1996). In addition, the presence of unique markers has been described in similar studies including wild and cultivated species, e.g. *Manihot* (Roa et al. 1997), *Oryza* (Zhu et al. 1998), and *Olea* (Angiolillo et al. 1999), while many other studies do not mention the presence or absence of specific bands. However, comparison of the various studies is difficult, because it is unclear how specific markers are defined in the various studies (e.g. present in every individual or in most individuals of an accession). Moreover,

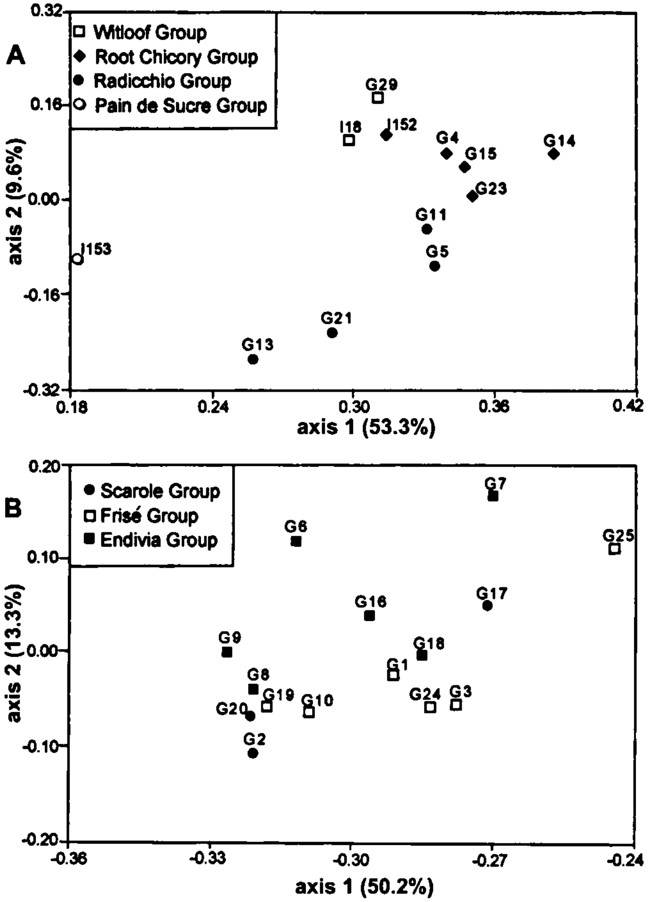

Fig. 5.3. Principal Coordinates Analysis (PCO) based on AFLP markers. A. *C. intybus* cultivars. B. *C. endivia* cultivars.

it is not clear how the results of different taxa can be compared. The absence of species-specific markers in *Cichorium* might be explained by the occurrence of interspecific crosses, which is a question deserving further investigation.

The relationships among cultivar groups, if analysed separately, are only resolved in *C. intybus* where the various cultivar groups correspond to different clusters in a PCO (Fig.5. 3A). Witloof and root chicories are closely related clusters. The root chicory accession 'Echte Zylinderförmige Spitzköpfige Magdeburger Riesen' (I152) is most closely related to the witloof cultivars. This corresponds with the idea that witloof and root chicories are derived from the 'Magdeburger type' (Baes and Van Cutsem 1993b; Bellamy et al. 1995; Bellamy et al. 1996; Van Kruistum 1997). The remaining accessions belong to the Radicchio Group and the Pain de Sucre Group, which are both cultivated for their leaves. The different breeding history of the two groups is confirmed by the clear separation of the Pain de Sucre accession from the accessions belonging to the Radicchio Group (Fig. 5.3A). The suggested hybrid origin of the Radicchio type 'Chioggia' as a result of a spontaneous cross between 'Radicchio' and an endive cultivar of the Scarole Group (Van Kruistum 1997; Van Wijk 1999) cannot be confirmed or rejected from this analysis (McDade 1997). However, all chicory cultivars cluster together with the wild chicory accessions and are clearly separated from the *C. endivia* accessions included (Fig. 5.2). In a separate analysis of the cultivated *C. intybus* accessions, a specific marker is found for all four accessions that belong to the Radicchio Group. However, due to our limited sample it is not yet known whether this marker can be useful as a diagnostic tool. Interestingly, this marker was further only found in four *C. pumilum* and two *C. endivia* accessions. In contrast to *C. intybus*, the cultivar groups of *C. endivia* do not form separate groups in a PCO (Fig 5.3B). The most common explanation for this is that crosses have been made among the various cultivar groups.

The relationships among *Cichorium* species and cultivars (Fig. 5.1–5.3) based on AFLPs will be useful for setting up a core collection of *Cichorium*, which is currently being undertaken in France (Kelechian-Cadot and Boulineau 1996). Because identification of species and cultivars appeared to be difficult due to the absence of specific AFLP markers, a reference collection seems indispensable for the correct identification of (anonymous) accessions. When this research on *Cichorium* was started, it soon became clear that genebank collections consist predominantly of *C. intybus* and *C. endivia* accessions. Besides a few additional *C. pumilum* accessions, none of the other wild species are included. However, the breeding potential of e.g. witloof chicory is limited (Bellamy 1996; Demeulemeester et al. 1997) and the need for diversification of the gene pool and the production of new genotypes is evident and can be achieved by using other species of the genus. Additionally, the close relationships of the cultivated species and their wild relatives as well as the intercrossability of the species (Kiers et al. 1999) stress the importance of inclusion of the remaining wild species in the collection.

Acknowledgements

The authors wish to thank Gerlof Pielage and Peter Kuperus for providing part of the AFLP data, and all persons and institutes listed in Table 1 for providing seeds and/or DNA. Staff members of the Hortus Botanicus Leiden (The Netherlands) and the greenhouse of the University of Amsterdam (The Netherlands) are greatly acknowledged for taking care of plants used in this study.

6 - General discussion

The morphological delimitation of the genus *Cichorium* has never been a point of discussion, and its monophyly has been confirmed by our molecular analysis (Chapter 4). The genus has a pappus with a unique structure of which the homology is unknown. The pappus is not only useful for recognising the genus, but it is also one of the few important diagnostic characters at the species level. However, the unique pappus structure hampers the choice of a suitable outgroup based on morphological characters, which is necessary for a phylogenetic analysis. Several studies, based on morphological or molecular data, are known in which the relation of *Cichorium* to other Lactuceae genera is discussed and the two main hypotheses are that *Cichorium* is either close to Microseridinae (Stebbins 1953; Whitton et al. 1995) or Crepidinae (Jeffrey 1966; Vermeulen et al. 1994; Koopman et al. 1998) sensu Bremer (1994). But none of these studies provide a strong evidence for the position of *Cichorium* within the Lactuceae. In our chloroplast-based phylogeny *Cichorium* is either sister to Microseridinae (*Microseris* and *Agoseris*), but with very low support, or included in a polytomy with Microseridinae and Crepidinae (*Chondrilla, Lactuca, Prenanthes,* and *Taraxacum*). Thus we can not confirm or reject the suggestion of *Cichorium* being close to Microseridinae or Crepidinae, but reaffirm the statement of Bremer (1994) that *Cichorium* can not be placed in any subtribe at present. The topology of the phylogenetic tree is congruent with the results of Whitton et al. (1995), who found that *Catananche* and *Scorzonera* have a basal position within the tribe.

Within *Cichorium* six species are recognised here (Chapter 9), two of which are easily recognisable. The first species is *C. bottae*, which has a cushionlike growth form and is endemic to Yemen and Saudi Arabia. The second species is *C. spinosum*, which is easily identified on the basis of unique spiny terminal branches. The remaining four species, *C. intybus, C. endivia, C. pumilum,* and *C. calvum* resemble each other to a great extent in morphological characters. A multivariate analysis (PCA) of the morphological characters shows, however, that although they form one cluster, the species do form groups and are not completely intermixed (Chapter 4). Moreover, including 'life span' and 'reproductive system' characters in the PCA resulted in a gap between *C. intybus* and a cluster containing *C. endivia, C. pumilum,* and *C. calvum*. The latter three species are all self-compatible annuals while *C. intybus* (along with *C. spinosum* and *C. bottae*) is a self-incompatible perennial. Obviously, the characters concerning 'life span' and 'reproductive system' aid in distinguishing *C. intybus* from *C. endivia, C. pumilum,* and *C. calvum,* but they are difficult to apply in the field, especially the mode of reproduction. Therefore, the most important morphological character for distinguishing the four species is the pappus, which is relatively large (min. 0.7 mm) in *C. endivia* and *C. pumilum*, small (max. 0.6 mm) in *C. intybus* and nearly absent (max. 0.1 mm) or absent in *C. calvum*. The pappus of *C. spinosum* and *C. bottae* are similar to *C. intybus*, but in *C. bottae* sometimes pappus scales with long tips occur, similar to *C. endivia* and *C. pumilum*.

The results of the phylogenetic analysis of *Cichorium* based on chloroplast and nuclear ribosomal DNA (Chapter 4) agree with the genetic relationships among the species determined with AFLP markers (Chapter 5). The most important results are (1) *C. bottae* is sister to the remaining *Cichorium* species, (2) *C. spinosum* is the closest relative of *C. intybus*, and (3) *C. endivia* is sister to *C. pumilum* and *C. calvum* in the chloroplast based phylogeny, but the relationships among these three species are unresolved in the phylogeny based on nuclear ribosomal DNA. Surprisingly, species-specific markers are nearly absent except for *C. bottae* where seven unique markers were found. Additionally, one species-specific marker has been found for *C. intybus* and *C. spinosum* together, confirming their close relationship, and one species-specific marker has been found for *C. endivia*. The high divergence of *C. bottae* according to AFLP data corresponds to its position as sister to the other *Cichorium* species. This species is also highly adapted to the environmental circumstances of the Arabian Peninsula and the only species that occurs outside the distribution area of *C. intybus*. The isolated position might explain the relatively high number of specific markers found in this species. The fact that *C. bottae* has a basal position within *Cichorium* is remarkable regarding its different geographic distribution compared to the remaining species which all have a predominantly Mediterranean distribution. A similar situation in other genera is not known to me. The close relationship between *C. intybus* and *C. spinosum* was found before based on RFLP data of the mitochondrial genome (Vermeulen et al. 1994). Both species share one specific AFLP marker and relatively short pappus scales. The remaining three species, i.e. *C. endivia, C. pumilum,* and *C. calvum*, are all self-compatible annuals, which are derived character states in *Cichorium*. A close relationship between *C. endivia* and *C. pumilum* has been hypothesised before (Boissier 1875; de Candolle 1884; Meikle 1985; Wagenitz and Bedarff 1989) as well as a close relationship between *C. endivia* and *C. calvum* (Wagenitz and Bedarff 1989). But in contrast to *C. pumilum, C. calvum* has never been suggested as a possible wild ancestor of *C. endivia*. This might be due to the fact that the species is rarely mentioned in literature or floras.

Despite the high morphological resemblance of *C. endivia* and *C. intybus*, a close relation between the species was not found by the chloroplast and nuclear DNA based phylogenetic analysis and the multivariate analysis based on AFLP markers. Further elucidation of the relationships between *C. endivia* and its closest wild relatives *C. pumilum* and *C. calvum* might be obtained by additional markers such as sequences of the chloroplast region of matK, which appeared to be useful in similar studies (e.g. Padgett et al. 1999, Denda et al. 1999).

In addition to the species analysis the genetic relationships among the cultivar groups of *C. intybus* (Root Chicory Group, Witloof Group, Pain de Sucre Group, and Radicchio Group) and *C. endivia* (Scarole Group, Frisé Group, and Endivia Group) are established using AFLP markers. In *C. intybus* the cultivar groups are resolved, if analysed separately, and the various cultivar groups correspond to different clusters in a PCO. A root chicory accession related to the Magdeburger type has an intermediate position between root chicory and witloof chicory cultivars. This confirms the idea that both cultivar groups are derived from the Magdeburger type (Baes and Van Cutsem 1993b; Bellamy et al. 1995; Bellamy et al. 1996; Van Kruistum 1997). The remaining accessions belong to the heterogeneous group of leaf chicories in which the 'Pain de Sucre' accession is clearly separated from the 'radicchio' accessions. In contrast to *C. intybus*, the cultivar groups of *C. endivia* do not form distinct groups, which most probably indicate that crosses have been made among the various cultivar groups.

The relationships among *Cichorium* species and cultivars based on AFLPs will be useful for setting up a core collection of *Cichorium*, which is currently being undertaken in France (Kelechian-Cadot and Boulineau 1996). Besides accessions of *C. intybus* and *C. endivia*, and a few of *C. pumilum*, none of the other wild *Cichorium* species are included. However, the close relation between the species and the intercrossability stresses the importance of inclusion of all species in the collection. Moreover, *C. spinosum* is already declining rapidly on Crete (Greece) where it is one of the most popular sought-after greens (Akeroyd and Hogan 1996), and the distribution of *C. calvum* is still unclear, which stresses the significance of preservation of the few collections which are known at present.

For the risk assessment of genetically modified *C. intybus* or *C. endivia* it has been suggested here that the developed code by Frietema de Vries (1996) should be broadened in such a way that (hardly) undetectable hybrids between species of the same genus are included in the code (Chapter 3). In the original code it was assumed that only hybrids between plants of the same species are undetectable. In *Cichorium* however, the occurrence of interspecific hybrids of various origins should be taken into account, but the detection of these hybrids is hampered by the high morphological resemblance between *C. intybus, C. endivia, C. calvum,* and *C. pumilum.* Because of the ability to hybridise, and the difficulty to detect hybrids as such, the effect on the wild flora of genetically modified *C. intybus* and *C. endivia* will be difficult to monitor. Field experiments involving genetically modified *C. intybus* or *C. endivia* should be treated with great caution.

II – TAXONOMIC PART

Introduction

The taxonomic part of this thesis starts with a description of the genus *Cichorium* and is followed by the key to the species. The species are described in order of their phylogenetic relationship and are followed by the hybrid, dubious names, and excluded species, which are listed in separate chapters.

The description of the species and genus starts with the synonymy including the most common infraspecific taxa in chronological order. The morphological descriptions are based on herbarium material and living material which was maintained in the greenhouse of the University of Amsterdam (The Netherlands) and in the Hortus Botanicus (Leiden, The Netherlands). Information from field observations and herbarium labels were included as well. The notes on the distribution, habitat, ecology and altitude are mainly from herbarium labels and flora's. The chromo-some number and the mode of reproduction was, respectively, counted and observed by me. Finally, the cited collections include all available specimens of *C. calvum* and *C. bottae*. For *C. spinosum* and *C. endivia* a representative sample of the available collections is listed including the collections used for the morphological analysis (described in Chapter 2 and 4). The remaining two species *C. intybus* and *C. pumilum* are the most common specimens in the collection and for these species only the material which was studied in detail and used for a morphological analysis (described in Chapter 2 and 4) is included.

7 – Generic description

Cichorium L.

Cichorium L., Sp. Pl. (1753) 813. – sect. *Eucichorium* DC., Prodr. 7 (1838) 84, nom. illeg. – Lectotype: *Cichorium intybus* L., vide M. L. Green, Prop. Brit. Bot. (1929) 178.

Acantophyton Less., Syn. Gen. Compos. (1832) 128. – sect. *Acantophyton* (Less.) DC., Prodr. 7 (1838) 84 – Type: *Acantophyton spinosum* Less.

Annual to perennial, lacticiferous herbs with taproots. *Basal leaves* in a rosette, sessile, attenuate at base. *Cauline leaves* alternate. *Capitula* sessile or on long peduncles, solitary or clustered. *Involucrum* cylindrical, consisting of 2 rows of imbricate bracts. *Outer phyllaries* 5, often reflexed in upper part, ovate to ovate-lanceolate, acute to acuminate, shorter to nearly twice as long as inner phyllaries, the lower part fleshy in flowering time and hard in fruiting time. *Inner phyllaries* 8 or (in *C. spinosum*) 5, reflexed after opening of the florets, like the outer but narrower, lower part often appressed against outer achenes, entire, villous outside at the acute apex. *Receptacle* flat, smooth or with irregularly shaped, acute, 0.2–1.5 mm long scales. *Florets* 5–20, ligulate; tubular part mostly hairy all round in apical part, the hairs multicellular, 0.05–0.80 mm long; plates 5-lobed, basally hairy over 0.5–5 mm, lobes acute and unequal in size in and between plates, villous at apex mainly outside. *Filaments* 0.4–1.0 mm long, glabrous; anthers 3.3–6.0 mm long, caudate, with rounded 0.15–0.40 mm long apical appendages, and 0.3–0.6 mm long frayed basal appendages. *Discus* ring-shaped, ± 0.2 mm high. *Style* slender with patent to erecto-patent sweeping hairs all round over 1.5–3 mm just below the stigmatic lobes and on the outside of the stigmatic lobes, stigmatic lobes inside papillose. *Pappus* present (or in *C. calvum* apparently absent), consisting of 1–3 rows of irregularly shaped, erect to erecto-patent, rounded to acute scales, equal in all florets or in the outer florets wider and blunt to very blunt at centrifugal side. Pappus scales of inner florets usually larger and sometimes ending in long tips. *Achenes* obovoid to cylindrical, weakly ribbed, light brown with dark brown spots to completely brown when ripe.

Distribution – Europe, North-Africa, Arabic Peninsula, West & Central Asia.

Field note – On a bright day, the flowers usually close before noon, but on a cloudy day they may flower all day.

8 – Key to the species

1a. Stems always well-developed. Capitula never situated among basal leaves. Basal leaves often absent during flowering time. Style and stigmatic lobes blue 2

1b. Plants growing in a cushion-like form, rarely with a few short stems arising from the cushion. Capitula sessile among basal leaves. Basal leaves always present during flowering time. Style and stigmatic lobes white .... 1. *C. bottae*

2a. Capitula with at least 9 florets. Inner phyllaries 8. Terminal branches never spiny 3

2b. Capitula with up to 7 florets. Inner phyllaries 5. Most terminal branches spiny .... 2. *C. spinosum*

3a. Most scales of the pappus stiffly erect to erecto-patent. All outer phyllaries of long peduncled capitula up to 5 mm wide 4

3b. Pappus scales apparently absent or smaller than 0.1 mm and then patent. One of the 5 outer phyllaries of most of the long peduncled capitula 8–15 mm wide .... 6. *C. calvum*

4a. Pappus scales of inner florets longer than 0.7 mm and often ending in 0.5–2.0 mm long tips. Florets, when fully expanded, together forming a wide cup. Plants annual 5

4b. Pappus scales always shorter than 0.6 mm. Florets, when fully expanded, forming a flat circle. Plants perennial .... 3. *C. intybus*

5a. Florets 9–14. Capitula clustered in groups of 2(–4). Longest peduncles 4.0–8.0 (–10) cm .... 5. *C. pumilum*

5b. Florets 15–20. Capitula clustered in groups of 4–6(–8). Longest peduncles 9.0–20 cm .... 4. *C. endivia*

Plate 9.1. a. *Cichorium bottae* with part of the root (Yemen). b. *C. endivia*, habitus. c. *C. calvum*, habitus. d. *C. calvum*, detail of capitulum with large outer phyllaries (photo B. Kieft). b, c, d: Hortus Botanicus Leiden, The Netherlands.

Plate 9.2. a. *Cichorium bottae*, habitus (Yemen). b. *C. endivia*, a fully flowering capitulum among several immature capitula (Hortus Botanicus Leiden, The Netherlands).

9 – Species descriptions

1. **Cichorium bottae** A.Deflers – Plate 9.1a & 9.2a

Cichorium bottae A.Deflers, Voy. Yemen (1889) 159. – Lectotype (here designated): A. Deflers 539, 10–06–1887, Yemen, Sana'a valley, SSW of the town at 2300 m alt. (P!)

Cushion-shaped perennial herb with stout taproot. The taproot much branched in the upper part with every branch giving rise to one or few rosettes. *Stem* usually not developed, otherwise up to 5, very rarely up to 25 cm long. *Basal leaves* oblanceolate to nearly linear, 0.5–7.5 x 0.1–0.8 cm, rounded to acute, entire to irregularly dentate or occasionally deeply incised, glabrous to puberulous. *Cauline leaves*, if present, like basal leaves but smaller towards apex. *Capitula* solitary or (along stems) in clusters of 2 or 3, sessile or on 0.5–1 cm long peduncles among basal leaves or rarely also along stems. *Involucrum* c. 4.5 mm wide. *Outer phyllaries* $^1/_2$–$^3/_4$ times as long as inner phyllaries, erect or upper part reflexed, ovate to ovate-lanceolate, 3.7–6.3 x 1.2–2 mm, the lower 1.8–3.8 mm part fleshy, upper part acute to acuminate or occasionally rounded, entire; glabrous or set with 0.05–0.35 mm long glandular or non-glandular hairs. *Inner phyllaries* 8, reflexed in flowering time and erect in fruiting time, and with lower part appressed against outer achenes, 7.5–10.5 x 1.1–2.8 mm; hairs absent or up to 0.6 mm long, glandular or not. *Florets* 10–13, when fully flowering maximally expanded in one (flat) plane; tube 1.7–3 mm long; plate 8.5–13.3 x 2.5–4.3 mm with acute, 0.6–2.0 mm long lobes. *Style* 6–9 mm long, upper part with spreading hairs over 2–3.5 mm, white; stigmatic lobes 1–2.4 mm long, white. *Pappus* consisting of a single row of patent to erecto-patent, irregularly shaped, but often ± 3-lobed, 0.1–0.35 mm long scales, those of inner florets rarely ending in 0.6–1.7 mm long tips. *Achenes* 1.5–2.2 x 1.0–1.3 mm, basally indistinctly ribbed, in the outer florets obovoid, the inner cylindrical.

Distribution – Yemen (high mountains, mainly around Sana'a), Saudi Arabia (Asir mountains) (Fig. 9.1).

Habitat & Ecology – Along roadsides and in disturbed areas, often found around irrigated fields. Alt.: 1500–3000 m. The plants flower from April till October.

Reproduction: self-incompatible.

Chromosome number: $2n=2x=18$.

Notes – 1. The achenes are normally very shortly and appressedly hairy in contrast to the other species.

2. Only blue florets have been found, but very rarely the lower part of the plate has a violet colour.

3. The cushions of rosettes can be quite large in diameter. I saw cushions of up to 80 cm diameter in Yemen. However, it is nearly impossible to assess whether such large cushions are formed by one or more individual plants, because of the complicated rootsystem.

4. Seeds of *C. bottae* which I collected in Yemen were sown in a greenhouse in Amsterdam (The Netherlands). The seedlings were grown in a cold green-house (min. 4°C) and in the Hortus Botanicus in Leiden (The Netherlands). In all, 45 specimens were followed during their growth season. All plants retained the cushion-like form under cultivation, but many formed short stems (2–5, rarely up to

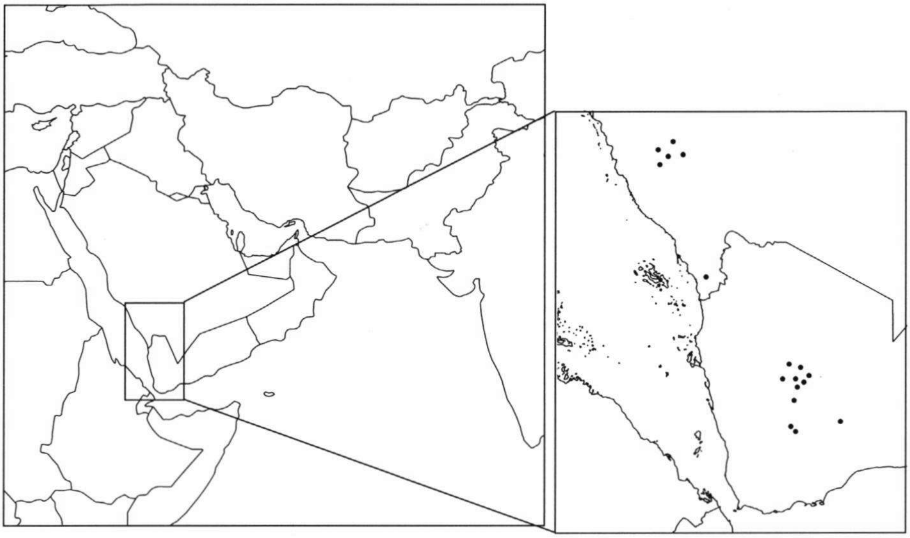

Fig. 9.1. Distribution map of *C. bottae*.

15 cm long). I observed this phenomenon also in the field in Yemen; those plants were growing on small ridges. In the greenhouse and in the Hortus Botanicus, the plants die back during winter. It is not known whether this also occurs in the wild.

5. On some herbarium labels it was indicated that the rosette leaves were eaten by cattle like goats and sheep. However in Yemen I did not observe any traces of damage by herbivores.

Collections – **Saudi Arabia.** D. Vesey-FitzGerald 16090/2 (BM). D. Vesey-FitsGerald 17064/7 (BM). J.D. Tothill 125 (BM). J.D. Tothill 200 (BM). G. Popov 185/2 (BM). G. Popov 96/321 (BM). J.P. Mandaville 2567 (BM). 26 Sep. 1979, B. Vincett s.n. (BM). L. Boulos and A.S. Ads 13845 (K). L. Boulos and A.S. Ads 13983 (K). 1838, M. Botta s.n.(P). W. Rauh 13093 (K). **Yemen.** F.N. Hepper 6176 (K). H. Scott and E.B. Britton 527 (BM). D. Podlech 36237 (G). 2 Apr. 1977, Chaudhary s.n. (G). J.R. Ironside Wood 72/33a (BM). J.R. Ironside Wood 1596 (BM). L. Boulos, A.K. Rowaished, W.A. Saeed, and M.A. Hussein 17320 (BM). A. Radcliffe-Smith and S.J. Henchie 4596 (K). A. Deflers 539 (P). 15 Mar. 1944, A. Khattal s.n. (G). Mar. 1983, U. Beil s.n. (B).

2. Cichorium spinosum L. – Plate 9.3a, b & 9.4a

Cichorium spinosum L., Sp. Pl. (1753) 813. – *Acantophyton spinosum* Less., Syn. Comp. (1832) 128. – Lectotype: [Alavi, Fl. Libya 107 (1983) 322.]: Hb. Linn. 962.4 (LINN, IDC microfiche L!)

Perennial herb with slender taproot. *Stem* 6–15(–27) cm long with many lateral branches or directly branching from the base; terminal branches often lose immature capitula and become spiny afterwards. *Basal leaves* 5–14 x 0.5–1.0 cm, narrowly obovate, obtuse to acute, runcinate to entire, glabrous. *Upper leaves* like basal ones

Plate 9.3. a. *Cichorium spinosum*, habitus (Hortus Botanicus Leiden, The Netherlands). b. *C. spinosum*, detail of capitulum with five inner phyllaries and two terminal branches with immature capitula (Greenhouse, Amsterdam, photo S. Luijten).

Plate 9.4. a. *Cichorium spinosum*, capitulum with five florets. b. *C. intybus*, fully flowering capitulum (Hortus Botanicus Leiden, The Netherlands).

but smaller towards apex. *Capitula* sessile, solitary or clustered in groups of 2–6. *Involucrum* 2–2.7 mm wide. *Outer phyllaries* up to half as long as inner phyllaries, appressed or upper part reflexed, (broadly) ovate to (broadly) elliptic, 1.8–4.2 x 1–3.8 mm, acute to acuminate, the lower 1.2–2.5 mm part fleshy, margins membraneous over 0.1–0.4 mm, glabrous. *Inner phyllaries* 5, 6.3–7.8 x 1.2–3.0 mm, margins membraneous over 0.1–0.5 mm. *Florets* 5 or 6, rarely 7, glabrous or occasionally with 1 row of 0.2–0.5 mm long hairs at centripetal side of lower part of the plate, when fully flowering maximally expanded in one (flat) plane; tube 1.4–2.0 mm long; plate 7.2–9.8 x 2.3–3.6 mm, basally with 1 row of 0.2–0.5 mm long hairs, lobes 0.4–2.0 mm long. *Style* 5.5–6.7 mm long, upper part with spreading hairs over 1.5–2.1 mm, blue; stigmatic lobes 1.4–2.0 mm long, blue. *Pappus* consisting of 1 or 2 rows of ± erect, irregularly shaped, rounded to acute, 0.1–0.4 mm long scales, the scales narrower at centripetal side of the fruit. *Achenes* cylindrical, 1.5–2.2 x 0.7–1.2 mm.

Distribution – Greece (Peloponnesos), Crete, Rhodes, Cyprus, and on many or all islands of the Ionian and Aegean seas, Sicily, Malta, and in NE-Libya (Fig. 9.2).

Habitat & Ecology – Along roadsides and coasts, on flat clayey areas, maritime sands and rocks, on Crete also in the mountains. Alt. 0–1700(–2100) m. Flowering from June till October.

Reproduction: self-incompatible.

Chromosomes: $2n=2x=18$.

Notes – 1. Despite the clear distinction of *C. spinosum* from all other *Cichorium* species, both our molecular based phylogeny and the estimation of mitochondrial diversity of Vermeulen et al. (1994) reveal, surprisingly, a close relation of *C. spinosum* and *C. intybus*. The conclusion of Vermeulen et al. (1994) that *C. spinosum* is merely an ecotype of *C. intybus* is not based on a careful consideration of all possibilities. Using the term 'ecotype' they even imply that the spinosum-type has arisen independently on different localities. They obviously overestimate the value of their DNA-comparisons as a taxonomic tool.

2. The hybrid *C. spinosum* x *C. pumilum* resembles *C. spinosum* most. It varies in the number of inner phyllaries from 5 (as in *C. spinosum*) to 8 (as in *C. pumilum*), as well as in number of florets per capitule from 5–10. The terminal branches are less often spiny. See H1.

Variation – The flower colour is usually blue, but also violet or white flowering individuals have been described, e.g. by Polunin (1997).

Use – The rosette leaves are a popular wild vegetable ('horta' in Greek) on the island of Crete (Greece). However, due to its popularity, the species is fastly disappearing.

Vernacular name – (Greek) stamnangathia (Akeroyd and Hogan 1996; Polunin 1997).

Collections – **Crete.** E. Reverchon 93 (G, K). C. Barclay 265 (K). M. Gandoger 4793 (K). **Cyclades: Naxos.** Chr. Leonis 1339 (G). **Karpathos.** P. H. Davis 18034 (K). **Cyprus.** A. Genneon 1553 (K). P. Laukkonen 442 (K). **Dodecanese (Sporadhes): Samos.** K. H. Rechinger and F. Rechinger 3831 (K). **Greece.** B. Verdoort 4136 (K). 15 June 1896, E. Saint-Lager s.n. (G). **Malta.** 1926, R. Bankart s.n. (BM). **Rhodes.** E. Bourgeau 87 (BM). **Sicily.** D. Davis and S. Sutton D63020. **Unknown.** De Ventenat s.n. (G).

3. **Cichorium intybus** L. – Plate 9.4b & 9.5a-d

Cichorium intybus L., Sp. Pl. (1753) 813. – *Cichorium rigidum* Salisb., Prod. (1796) 183, nom. illeg. – *Cichorium perenne*, Stokes, Bot. Mat. Med. (1812) 133. nom. illeg. – *Cichorium sylvestre* Garsault, Fig. Pl. Anim. Med. t. 222; Descr. Pl. Anim. (1767) 145; Thell., Bull. Herb. Boiss. Sér. II., 8. (1908) 789. nom. illeg. – *Cichorium sylvestre* (Tourn.) Lam., Fl. Fr. (1778) 120, nom. illeg. – Lectotype (Lack, Fl. Iranica 122 (1977) 6): Hb. Linn. 962.1 (LINN, see note 2).

Cichorium commune Pall., Reise, 3. (1772–1773) 655, nom. nud. – see note 3.

Cichorium glabratum C.Presl, Fl. Sicul. I (1826) XXXII. – *Cichorium intybus* subsp. *glabratum* (C.Presl,) Wagenitz & Bedarff, Davis & Hedge Festschrift (ed. Kit Tan) (1989) 12. – Type: unknown.

Cichorium cicorea Dumort., Fl. Belg. Prod. (1827) 64. – Type: unknown (see note 4).

Cichorium byzantinum Clementi, Mem. Accad. Sci. Torino, Cl. Sci. Fis. Mat., Ser. 2. 16: 285 (1855). – Type: unknown.

Perennial herb with taproot. *Stem* 30–200 cm, often branched from the base. *Basal leaves* obovate to oblanceolate, 13–60 x 5–12 cm, rounded to acute, entire to runcinate, glabrous to puberulous. *Cauline leaves* like basal leaves, but smaller towards apex. *Capitula* solitary or clustered in groups of 2–4(–8), sessile or on 4–7 (rarely up to 13) cm long peduncles, which are 2–3 mm in cross-section. *Involucrum* $3^{1}/_{2}$–5 mm wide. *Outer phyllaries* half as long to slightly longer than inner phyllaries, upper part reflexed, ovate or elliptic to (ovate-)lanceolate, 4.7–12.5(16.0) x 2–4 mm, the lower 2–5 mm part fleshy, upper part acute to acuminate, rarely mucronate or cuspidate, more or less entire; glabrous or set with 0.1–1 mm long, glandular or non-glandular hairs. *Inner phyllaries* 8, 7.5–14.5(16.5) x 1.9–3.0(4.0) mm, entire; hairs absent or up to $2^{1}/_{4}$ mm long, glandular or not. *Florets* (12)15–19(25), when fully flowering maximally expanded in one (flat) plane; tube 1.5–3.2 mm long; plate 10–19 x 2–4.5 mm with acute, 0.3–1.3 mm long lobes. *Style* 6.5–10.5 mm long, upper part with spreading hairs over 2–3.5 mm, blue; stigmatic lobes 1.5–3.0 mm long, blue. *Pappus* attached in 1 or 2 rows of erect to erecto-patent, irregularly shaped, rounded to acute, 0.2–0.5(0.6) mm long scales, in the outer florets often wider and blunt to very blunt at centrifugal side. Pappus scales of inner florets usually larger than scales of outer florets. *Achenes* 1.8–2.7 x 1–1.5 mm, obovoid to cylindrical, weakly ribbed.

Distribution – Europe up to 62°N, N-Africa, West & Central Asia (Fig. 9.2, p. 68); introduced elsewhere in temperate and semi-arid regions.

Habitat & Ecology – Along roadsides, and rivers, in disturbed areas, on dry slopes and pastures, and on open stony ground. On calcareous or nitrogenous soil. Alt.: 0–1500 m, in Turkey up to 3050 m. Flowering from May till October.

Reproduction: self-incompatible.

Chromosomes: $2n=2x=18$.

Notes – 1. Apart from *C. bottae* and *C. spinosum*, the remaining *Cichorium* taxa resemble each other more or less closely. Based on a morphological study, Wagenitz and Bedarff (1989) distinguished 5 taxa: *C. calvum, C. intybus* subsp. *intybus, C. intybus* subsp. *glabratum, C. endivia* subsp. *endivia,* and *C. endivia* subsp. *divaricatum.* As a result of my study, also including DNA characters, I distinguish only 4 taxa (as species) in this group, combining the *intybus* subspecies into one taxon. In the Mediterranean area, small specimens of *C. intybus* resemble *C. pumilum* (= *C. endivia* subsp. *divaricatum*), and are often confused with it.

Plate 9.5. a. *Cichorium intybus*, habitus (Hortus Botanicus Leiden, The Netherlands). b. *C. intybus*, Root Chicory Group (Greenhouse, Amsterdam). c. *C. intybus*, Pain de Sucre (Hortus Botanicus Leiden, The Netherlands). d. *C. intybus*, Witloof Group, red chicory cultivar (photo B. Kieft).

Plate 9.6. a. *Cichorium pumilum*, habitus. b. *C. pumilum*, close-up of apically thickened peduncles. a & b: Hortus Botanicus Leiden, The Netherlands.

2. For a proper identification of this type specimen the length of the pappus should be measured. However, I have examined a detailed colour image of the type specimen of Linnaeus, which leaves no doubt that this specimen belongs to *C. intybus*.

3. Pallas (1772–1773) named *C. commune* as one of the species which he found during his travel through Russia, but he did not give a description of the species. However, only *C. intybus* is known from Russia and therefore I treat *C. commune* as a synonym of *C. intybus*.

4. Dumortier (1827) described *C. cicorea* separately from *C. intybus*, but his description applies to *C. intybus*. Moreover, no other wild species is known from Belgium.

5. This species is the most variable one of the genus and therefore difficult to characterise. The height, type of branching, indumentum and form of leaves are all variable between and within populations. However, the length of the pappus scales, the often stout taproot, and the flat shape of the flowering heads are useful characters to distinguish this species from *C. endivia, C. pumilum,* and *C. calvum*.

6. Besides the common blue flower colour, also pink and white colours are found.

7. The genus is widely cultivated in the temperate and semi-arid climate zones in the world and comprises four cultivar groups according to their use: (1) The Root Chicory Group is cultivated for its large roots that were formerly used as a coffee substitute or additive, but at present mainly cultivated for the production of inulin, which is the major reserve carbohydrate in many Asteraceae (formerly named "var. *sativum*"); (2) The Witloof Group consists of witloof or Brussels chicory, which is used for producing 'witloof'or 'French endive' under artificial conditions (formerly "var. *foliosum*"); (3) The Pain de Sucre Group contains green-leaved cultivars with a rather bitter taste, which are hardly cultivated nowadays (formerly "var. *foliosum*"); (4) Radicchio Group consists of leaf chicories which are essentially bred for their large, blond, red or variegated leaves that are used as fresh or cooked food (formerly "var. *foliosum*"). Within this group three types can be distinguished: (a) Treviso, (b) Castelfranco, and (c) Chioggia.

Vernacular names (mainly after Kays and Silva Dias, 1995) – *Cultivars:* (Dutch) cichorei, wortelcichorei, witlof, groenlof, roodlof; (English) Brussels chicory, witloof, witloof-chicory, redleaf chicory, radicchio; (French) chicorée à feuilles vertes, chicorée intybe, chicorée witloof, endive, chicorée sauvage à feuilles rouges; (German) Chicorée, Zichorienwurzel, Fleichkraut, Radicchio, Rosettenzichorie; (Portugese) chicória do café, chicória amarga, chicória selvagem, almeirão, chicória vermelha, chicória de folhas roxas, radichio; (Spanish) achicoria silvestre, achicoria de raíz, achicoria de hojas verdes, achicoria de bruselas, amargón, almirón, achicoria de hoja roja, endibia acogollada roja. *Wild:* (Dutch) wilde cichorei; (English) chicory; (French) chicorée sauvage, chicoré intybe; (German) (Blaue) Wegwarte; (Spanish) achicoria azul.

Collections – **Aegean Islands: Lemnos.** Foster 22.17 (K). **Bulgaria.** J. Bornmueller 2642 (B). France. L.W. van Soest 22416 (L). **Germany.** Inst. v. Prehistorie 575 (L). W.D.J. Koch 1844 (L). D. Reichenbach s.n. (L). 4 Aug. 1914, R. Schulz s.n. (B). **Morocco.** Font

Quer 716 (BM). E. Johandiez 580 (G). **Netherlands.** 10 Aug. 1992, R. van der Meijden s.n. (L). **Poland.** M. Cegnowa-Giddon 318 (L). **Sweden.** 14 Sep. 1919, J. Erikson s.n. (L). **Turkey.** P.C. van Welzen 77 (L). J.M. Winter 151 (G). P.C. van Welzen 161 (L). J. Bornmueller 5232 (B).

4. Cichorium endivia L. – Plate 9.1b & 9.2b

Cichorium endivia L., Sp. Pl. (1753) 813; *C. intybus* b. *endivia* (L.) C.B.Clarke, Comp. Ind. (1876) 250. – *Cichorium esculentum* Salisb., Prod. (1796) 183, nom. illeg. – *Cichorium endivia* var. *sativa* DC., Prodr. 7 (1838) 84. – *Cichorium casnia* C.B.Clarke, Comp. Ind. (1876) 250, nom. illeg. - Lectotype [Alavi, Fl. Libya 107 (1983) 320]: Hb. Linn. 962.3 (LINN, see note 3).

Cichorium crispum Mill., Gard. Dict. ed. VIII (1754) n. 4, nom. illeg.

Annual herb with taproot. *Stem* 100–170 cm, with many lateral branches, often branched from the base. *Basal leaves* obovate, 30–45 x 10–18 cm, rounded to acute, entire to runcinate, glabrous to slightly puberulous. *Cauline leaves* like basal leaves, but smaller towards apex. *Capitula* solitary or clustered in groups of 4–6, sessile or on up to 9–20 cm long, apically thickened peduncles, which are 3–4 mm in cross-section. *Involucrum* 3$^1/_2$–5 mm wide. *Outer phyllaries* about as long as inner phyllaries, upper part reflexed, ovate to ovate-lanceolate, or elliptic to lanceolate, 7–10(15) x 2–5(10) mm, more or less entire, the lower 3.2–4.5 mm part fleshy, the upper part acute to acuminate; glabrous or set with up to 2.0 mm long, glandular or non-glandular hairs. *Inner phyllaries* 8, slightly reflexed after opening of the flowers, 8–12 x 1–3 mm; hairs absent or up to 2 mm long, glandular or not. *Florets* 15–20, when fully flowering half-patent, together forming a wide cup; tube 2–3.2 mm long; plate 10–19 x 2–4.5 mm, with 0.3–1.3 mm long, acute lobes. *Style* 6$^1/_2$–10$^1/_2$ mm long, upper part with spreading hairs over 2–3.5 mm, blue; stigmatic lobes 1$^1/_2$–3 mm long, blue. *Pappus* consisting of 1 to 3 rows of irregularly shaped, erect to erecto-patent, (0.5)0.7–1.0 mm long, rounded to acute scales, in the outer florets narrower at centripetal side, pappus of inner florets larger and often ending in $^1/_2$–1$^1/_2$ mm long tips. *Achenes* 2.0–2.8 x 1–1.5 mm, obovoid to cylindrical, weakly ribbed.

Distribution – Species only known from cultivation.

Notes – 1. The species has been confused with *C. intybus* (see e.g. Wagenitz and Bedarff 1989), but is clearly different in pappus length (longer than 0.7 mm), form of the flowering heads, annual life span and self compatibility as well as on DNA level (see this study, Chapter 4 and 5).

2. The relationship between *C. endivia* and its presumed ancestor *C. pumilum* is discussed under *C. pumilum.*

3. For a proper identification of this specimen the length of the pappus should be measured. However, I have examined a detailed colour image of the type specimen of Linnaeus, which leaves no doubt that this specimen belongs to *C. endivia.* Furthermore, the available capitula are immature which hampers a correct measurement of the pappus.

4. Besides the common blue flower colour also white flowering cultivars are produced.

5. The cultivars of this species are usually divided into three groups. (1) The

Scarole Group consists of the plain endive cultivars with broad leaves, which are also known as 'escarole' (formerly named "var. *latifolium*"). (2) The Frisé Group consists of the crispy or curly endives termed Frisée (formerly "var. *crispum*"). (3) The Endivia Group contains ancient, narrow-leaved cultivars that are rarely on the market nowadays (formerly "var. *endivia*").

Vernacular names (mainly after Kays and Silva Dias, 1995) – (Dutch) andijvie, krulandijvie; (English) endive, escarole; (French) chicorée, chicorée endive, chicorée frisée, cichorée escarole, scarole; (German) Endivie, Winterendivie, Endiviensalat; (Portugese) endívia, escarola, chicória escarola; (Spanish) endivia, endibia, escarola.

Collections – Frisé Group. **Croatia.** 5 June 1926, E. Korb. s.n. (W). **France.** 24 Sep 1878, F. Gravet s.n. (L). **Germany.** Schreber 24494 (M). **Netherlands.** Ann. 185 (L). – Scarole Group. **Austria.** Sep. 1910, Schiffner s.n. (L). 9 Aug. 1904, E. Korb. s.n. (W). **Morocco.** 1879, Kok Ankersmit s.n. (L). **Netherlands.** W.C. van Heurn s.n. (L). **Republic Congo.** Menyhart 500 (WU). **Switzerland.** Aug. 1878, A. E. Ayasse s.n. (G). **Unknown.** June 1856, E. Rostan s.n. (BM). 5 Sep. 1929, J. Schneider s.n. (W).

5. **Cichorium pumilum** Jacq. – Plate 9.6a & b

Cichorium pumilum Jacq., Obs. 4 (1771) 3 t. 80. – *Cichorium endivia* subsp. *pumilum* (Jacq.) C.Jeffrey, Candollea 34 (1979) 309. – Lectotype: t. 80.

Cichorium divaricatum Schousb., Vextr. Marok. (1800) 197. – "*Cichorium dichotomum* Schousb." Link, Handb. 1 (1829) 811, sphalm. – *Cichorium endivia* L. subsp. *divaricatum* (Schousb.) P.D.Sell, Bot. J. Linn. Soc. 71 (1975) 240. – Type: Schousboe s.n. (n.v.).

Cichorium glaucum Hoffmans. & Link, Fl. Port. 2 (1809) 178. – Type: Tab. 95 (see note 3).

Cichorium ambiguum Schult., Obs. (1809) 170. – Type: unknown.

Cichorium minimum Port., Enum. Pl. Dalm. (1824) 16. – Type: Tab XII, fig. II.

Cichorium glandulosum Boiss. & Huet., Diagn., Ser. II. 3 (1856) 87. – Lectotype: Kotschy 138, s.d., vide. Boissier (holo W!, iso W!).

Cichorium noeanum Boiss, Fl. Orient. 3 (1867) 717. – Type: Noe 58, Kutt ad Tigrim (n.v.) (see note 4).

Cichorium polystachyum Pomel, Nouv. Mat. Fl. Atl. (1874) 18. – Type: unknown (see note 5).

Annual herb with slender taproot. *Stem* 6–40 cm , the main axis with usually 2–3 lateral branches or rarely branched from the base. *Basal leaves* ovate to obovate, rounded to acute, $2\frac{1}{2}$–20 x 0.7–4 cm, irregularly dentate to deeply incised, glabrous to puberulous. *Cauline leaves* like basal leaves but much smaller and ovate with entire to dentate margins, in upper half of inflorescence always smaller than 1 x $\frac{1}{2}$ cm. *Capitula* solitary or clustered in groups of 2(–4), sessile or on up to 4–8(10) cm long, apically thickened peduncles, which are 3–4 mm in cross-section. *Involucrum* 3–$4\frac{1}{2}$ mm wide. *Outer phyllaries* $\frac{3}{4}$ times as long to slightly longer than inner phyllaries, upper part reflexed, ovate or elliptic to (ovate-)lanceolate, $3\frac{1}{2}$–12 x $1\frac{1}{2}$–$4\frac{1}{2}$ mm, the lower 2–5 mm part fleshy, upper part acute to acuminate, rarely rounded or mucronate, more or less entire or dentate in upper part; glabrous or set with 0.10–2.5(3.9) mm long, glandular or non-glandular hairs. *Inner phyllaries* 8, 6–11.5 x 0.8–2.8 mm; hairs absent or up to $2\frac{1}{4}$ mm long, glandular or not, situated outside at midvein in upper part. *Florets* 9–14, when fully flowering half-patent, together forming a wide cup; tube 2.2–4.5 mm long; plate 8.8–14.5 x 2.5–4.5 mm, with 0.2–1 mm long lobes. *Style* 6–$10\frac{1}{2}$ mm long, upper part with spreading hairs over 1.1–3.5 mm, blue; stigmatic lobes 0.8–2.5 mm long, blue. *Pappus* consisting of 1 to 3 rows of irregularly shaped, erect to erecto-patent, 0.15–1.2 mm long, rounded to acute scales, in the outer florets narrower at centripetal side, pappus of inner florets narrower and often ending in $^1/_2$–2 mm long tips. *Achenes* 2.0–2.5 x 1–1.5 mm, obovoid to cylindrical, weakly ribbed.

Distribution – Mediterranean region (Morocco, Portugal, Spain, France, Italy, Greece, Crete, Rhodes, Turkey, Lebanon, Syria, Israel), S. Arabia (middle part), Iraq (Fig. 9.2).

Habitat & Ecology – Along roadsides, in disturbed areas, fields, and fallows. Usually on calcareous soil. Alt: up to 1000 m. Flowering from May till September.

Reproduction: self-compatible.

Chromosomes: $2n=2x=18$.

Notes – 1. This species has often been confused with *C. intybus* (see e.g. Wagenitz and Bedarff 1989), but is clearly different in pappus length, form of the flowering head, life span and reproductive system, as well as on DNA level (see this study Chapter 4 and 5).

2. Many authors regard this species as the representative of the ancestor of *C. endivia* (e.g. Boissier 1875, De Candolle 1884, Meikle 1985, Wagenitz and Bedarff 1989). Although the resemblance between *C. pumilum* and *C. endivia* is clear, they do differ in number of florets per capitule, the number of clustered capitula and the length of the peduncles. Apart from that, the molecular based phylogenetic analysis shows that *C. endivia* is equally closely related to both *C. pumilum* and *C. calvum*. Although DNA-based differences should not be used as more decisive than macromorphological characters (but compare note 1 under *C. spinosum*), they are surely of great importance in combination with macromorphological characters. A subspecies classification of *C. endivia* and *C. pumilum* (as adopted by Wagenitz and Bedarff 1989) suggests a closer relation between these two species than between *C. endivia* and *C. calvum*. This, I feel, is not justified by the results from the phylogenetic analysis. Therefore, I treat *C. endivia* and *C. pumilum* as two different species.

3. The drawing given of *C. glaucum* shown in Hoffmansegg and Link (1809) resembles *C. pumilum* in habitus, but the detailed drawings of the phyllaries and pappus at the bottom of the page definitely do not belong to *Cichorium*.

4. I have not seen the material cited by Boissier, but I examined two collections of *C. pumilum* from the same location [Noe 30 (WAG) and Noe 993 (P)].

5. Pomel (1874) mentioned that his *C. polystachyum* resembles *C. pumilum* in capitula and achenes, but differed in the high number of capitula per cluster. I have seen equally high numbers of clustered capitula in both *C. pumilum* and *C. intybus*. Because of the high resemblance in achenes, I think that *C. polystachyum* should be included in *C. pumilum*.

6. The florets are often blue with a violet lower part of the plate.

7. The achenes are enclosed after fruitset and are very difficult to remove.

Collections – **Albania.** A.H.G. Alston and N.Y. Sandwith 1366 (BM). **Armenia.** G. Woronow 500 (K). **Balearic Islands: Menorca.** A. Charpin and D. Masson AC19286 (G). **Canary Islands: Tenerife.** E. Asplund 1156 (G). **Croatia.** B.E.E. Duyfjes et al. 250 (L). **Cyprus.** E.C. Casey 711 (K). H. Maedeverd 54 (K). **Iran.** Davis and Bokhari D56088 (K). **Iraq.** F.A. Barkley and A.D.Q. Agnew 6016B (K). Alizzi and Omar 34882 (K). W. Thesiger 763 (BM). **Israel.** I. Amdursky 478 (B, BM). F.S. Meijers and J.E. Dinsmore 8083 (L).

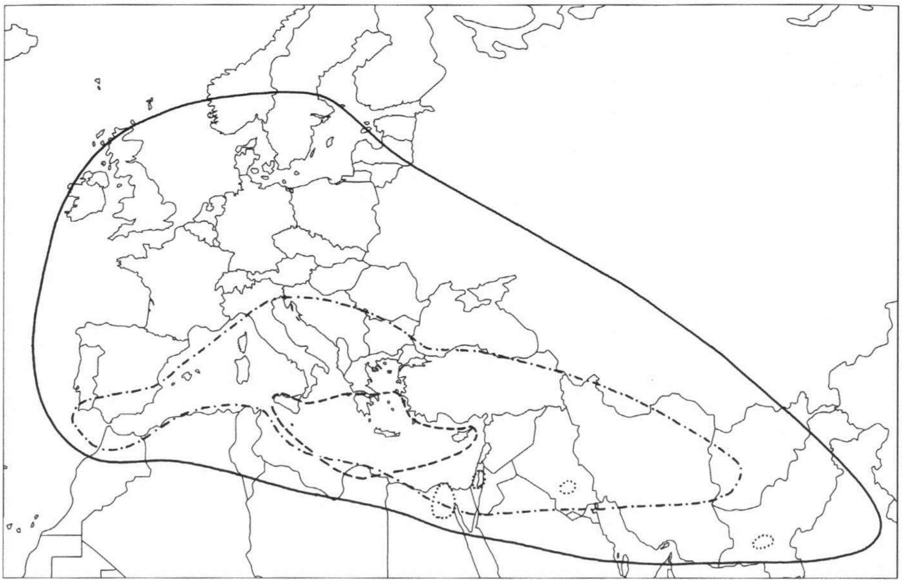

Fig. 9.2. Distribution map of four *Cichorium* species.
—— = *C. intybus*, .—.—. = *C. pumilum*,
------ = *C. spinosum*, ... = *C. calvum.*

J. Bornmueller 971 (G). **Jordan.** F.S. Meijers and J.E. Dinsmore 4992 (K). **Sicily.** Todaro 527, (BM). E. and A. Huet du Pavillon 125 (G). **Syria.** E. Peyron 425 (G). Post 114 (G). 19 May 1865, C. Haussknecht s.n. (BM). **Turkey.** E. Hennipman et al. 1190 (L). Davis 43185 (K).

6. **Cichorium calvum** Sch. Bip. ex Asch. – Plate 9.1c & d

Cichorium calvum Sch. Bip. ex Asch. in Schweinf., Beitr. Fl. Aethiop. (1867) 143; *Cichorium calvum* Sch. Bip., Ind. Sem. Hort. Berol. (1859) 7, nom. nud. – Lectotype: [Lack in Rech., Fl. Iranica 122 (1977) 8]: anon. s.n., s.d. (P, n.v.).

Annual herb with slender taproot. *Stem* 40–80 cm, much branched from the base, slender. *Basal leaves* oblanceolate to lanceolate, 10–25 x 1.8–4 cm, rounded, irregularly dentate, glabrous to puberulous. *Cauline leaves* like basal leaves, but in upper part of inflorescence somewhat triangularly shaped, smaller towards apex, often with long hairs on margins and surface. *Capitula* solitary or clustered in groups of 4–9, sessile or on 10 to 20 cm long, slender peduncles, which are $1^1/_2$–2 (rarely up to $3^1/_2$) mm in cross-section. *Involucrum* 4–5 mm wide. *Outer phyllaries* very unequal in size, $^1/_2$–$1^1/_2$ times as long as inner phyllaries, upper part reflexed, broadly ovate to ovate-lanceolate, 6–20 x 2–15 mm, the lower 1.5–4.1 mm part fleshy, upper part acute to acuminate, sometimes mucronate, entire; glabrous or set with few to many, 0.1–2.5 mm long, glandular or non-glandular hairs; one of the phyllaries usually much larger than the others, especially so in long peduncled capitula and then ca. 10–20 x 8–15 mm. *Inner phyllaries* 8, slightly reflexed after opening of the flowers, 8–10 x 1.0–3.0 mm; hairs absent or up to 2.0 mm long, glandular or not. *Florets* 9–13, when fully flowering half-patent, together forming a wide cup; tube 2–4 mm long; plate 6.2–12.5 x 2.4–4.1 mm with acute, 0.15–0.85 mm long lobes. *Style* 6–10$^1/_2$ mm long, upper part with spreading hairs over 1.1–3.5 mm, blue; stigmatic lobes 0.8–2.5 mm long, blue. *Pappus* apparently absent, consisting of a single row of minute, patent, irregularly shaped, rounded to acute, up to 0.1 mm long scales; scales rarely longer (1.0 mm including a 0.3 mm long apical tip). *Achenes* shortly obconical (i.e. widest near top), 2–2.5 x 1.2–1.7 mm and mainly light brown.

Distribution – Egypt, Israel, Irak, and Pakistan (Fig. 9.2); introduced in Austria and Germany as a contaminant of *Trifolium alexandrinum* and *Trifolium resupinatum* seeds. However, since the *C. calvum* seeds are removed from the *Trifolium* seed packages before selling, *C. calvum* has not been found in Austria and Germany anymore. The species is hardly known from literature and floras and therefore it is not clear whether it also occurs outside the given area.

Habitat & Ecology – Along roadsides, in disturbed areas and in *Trifolium* fields. There are no indications about altitude and flowering time found in literature or on herbarium labels. The plants grown in the Hortus Botanicus in Leiden (The Netherlands) and in the greenhouse, however, flower at the same time as the other *Cichorium* species, i.e. from May till September.

Reproduction: self-compatible.

Chromosomes: $2n=2x=18$.

Notes – 1. *C. calvum* resembles *C. endivia* and to a lesser extent *C. pumilum*, but differs clearly by the apparent absence of pappus scales, the shortly obconical achenes, and the width of the outer phyllaries.

2. The main character by which *C. calvum* has been distinguished from the remaining species in literature and floras is the absence of pappus scales. After analysing SEM photographs of the achenes, however, a rudimentary pappus was recognisable, consisting of <0.1 mm long, scales which were appressed outwards on the apex of the achene; in one collection (Angerer, s.n., M!) some pappus scales reached up to 1 mm length (including a 0.3 mm long tip). I may conclude that *C. calvum* has a very reduced pappus indeed which is a unique character in the genus.

3. The achenes are enclosed after fruit set, but they can easily be removed just like the inner phyllaries.

4. The outer phyllaries vary much in size within a capitule as well as within an individual, but they are largest in long peduncled capitula. Of these long peduncled capitula, at least one capitule has an outer phyllary of 8–15 mm wide, which is diagnostic for this species.

5. The florets are usually blue with a violet lower part of the plate.

6. In 'Weeds of Pakistan' (Khalid 1995) the occurrence of *C. intybus* has been described, but the given picture clearly shows a *C. calvum* individual, with large outer phyllaries and an achene without pappus. Moreover, the species has been described as an annual or a perennial. *C. calvum* is indeed an annual, but *C. intybus* is a perennial species. It is likely that the *C. calvum* individuals have not been recognised as such, because it is a rather obscure species, and are joined with *C. intybus*.

Collections – **Egypt.** E. Burdet 918 (G). May 1876, E. Leeds s.n. (K). N.D. Simpson 4804a (K). 12 June 1971, Soliman Sisi s.n. (G). **Germany.** June 1979, Botanic Garden München s.n. (M). June 1957, Lindenbein s.n. (M). 1 Oct. 1984, O. Angerer s.n. (M). E. Walter MTB 6232/2 (M). J. Ernst Krach MTB 7033/2 (M). Koepff and Krach 13562A (M). E. Dörr MTB 8527/2 (M). W. Lippert and O. Angerer 20475 (M). July 1979, Ann. s.n. (M). Oct. 1978, Ann. s.n. (M). **Pakistan.** S.M.A. Kazmi 5421 (M). G. Popov 226 (BM). **Unknown.** 26 Sep. 1859, Schultz-Bipontus s.n. (G, L).

10 – Hybrid description

H1. Cichorium spinosum x C. pumilum = C. xhybridum Heldr. ex Halácsy

Cichorium xhybridum Heldr. ex Halácsy, Consp. Fl. Gr. (1902) 179. – Lectotype (Halácsy): Heldreich 1662, **Cyclades: Mykonos**, at roadsides and in cultivated places in Anomerà, among the parents, (holo WU!, iso WU!).

Stem 14–30 cm with many lateral branches or directly branching from the base; terminal branches sometimes lose immature capitula and become spiny afterwards. *Basal leaves* 3–7 x 1–1$^1/_2$ cm, narrowly obovate, obtuse to acute, runcinate, puberulous. *Upper leaves* like basal but entire and much smaller towards apex. *Capitula* sessile, solitary or clustered in groups of 2–6. *Involucrum* 2–3 mm wide. *Outer phyllaries* up to half as long as inner phyllaries, appressed or upper part reflexed, (broadly) ovate to (broadly) elliptic, 1.8–6.0 x 1–3.0 mm, acute to acuminate, the lower 1–2$^1/_2$ mm part fleshy, glabrous. *Inner phyllaries* 5–8, 6.5–8 x 1.2–3 mm. *Florets* 5–10. *Pappus* consisting of 1 or 2 rows of ± erect, irregularly shaped, rounded to acute, 0.1–0.5 mm long scales, the scales narrower at centripetal side of the fruit. *Achenes* cylindrical,1.5–2.5 x 0.8–1.3 mm.

Note – The hybrid was found by Heldreich in 1901, but I have not seen any collection of it afterwards. Halácsy mentioned that the seeds are abortive, but I have seen well-developed achenes in the type material.

11 – Dubious names

D1. *Cichorium callosum* Pomel, Nouv. Mat. Fl. Atl. (1874) 17. – Type: unknown.

Note – It is not clear from the description whether this is *C. endivia* or *C. pumilum*. The observation that the plant is biennial refers most probably to *C. endivia*, but the latter is not known from the wild flora. The description of long, apically thickened peduncles may apply to *C. endivia* or *C. pumilum*.

D2. *Cichorium balearicum* Porta, Nuov. Giorn. Bot. Ital. 19 (1887) 311. – Type: unknown.

Note – The description of a thick root refers most likely to *C. intybus*.

13 – Excluded names

E1. *Cichorium aposeris* E.H.L.Krause, in Sturm, Fl. Deutschland, ed. 2, 14 (1906) 179, nom. illeg. = **Aposeris foetida** (L.) Less.

E2. *Cichorium arnoseris* E.H.L.Krause, in Sturm, Fl. Deutschland, ed. 2, 14 (1906) 180, nom. illeg. = **Arnoseris minima** (L.) Schweigger & Koerte

E3. *Cichorium barbatum* E.H.L.Krause, in Sturm, Fl. Deutschland, ed. 2, 14 (1906) 181. = **Tolpis barbata** (L.) Gaertn.

E4. *Cichorium rhagadiolus* E.H.L.Krause, in Sturm, Fl. Deutschland, ed. 2, 14 (1906) 183, nom. illeg. = **Rhagadiolus stellatus** (L.) Gaertn.

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