



## **Phylogeny of the tribe Cardueae (Compositae) with emphasis on the subtribe Carduinae: an analysis based on ITS sequence data**

Authors: Häffner, Eva, and Hellwig, Frank H.

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EVA HÄFFNER &amp; FRANK H. HELLOWIG

## Phylogeny of the tribe *Cardueae* (*Compositae*) with emphasis on the subtribe *Carduinae*: an analysis based on ITS sequence data

### Abstract

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The bulk of genera within the *Cardueae* is placed in the subtribes *Carduinae* and *Centaureinae*, which together probably form a monophyletic group. Especially within the *Carduinae*, the phylogenetic relationships are unclear. The elucidation of generic affinities within the group is of both evolutionary and systematic interest: understanding the basal relationships could give insight in the diversification processes, the place of origin and the relative age of individual subgroups. It would further contribute to clarify subtribal classification, since it is not clear whether the *Carduinae* are monophyletic or paraphyletic with the segregation of the *Centaureinae*. Eventually, genus delimitation could be tested in the case of larger complexes of closely related genera. In order to reconstruct phylogenetic relationships, DNA sequence data of the Internal Transcribed Spacer (ITS) regions of the nuclear ribosomal DNA were determined. Phylogenetic analysis using PAUP resulted in five most parsimonious trees. Several larger groups of related genera could be reliably identified. Often the phylogenetic patterns coincide with geographical distribution. The results strongly suggest the paraphyly of the *Carduinae*. The *Centaureinae* are most closely related to the large *Carduinae* genera *Jurinea*, *Saussurea* and *Cousinia*, which are all centred in Central Asia. Within the mainly Mediterranean *Carduus-Cirsium* complex, genus delimitation is critically addressed.

### Introduction

The *Cardueae* are a large tribe within the subfamily *Cichorioideae* of the family *Compositae*, comprising about 2500 species in 83 genera (Bremer 1994) with a mainly northern hemispheric Old World distribution. Some 1600 species in about 36 genera are placed in the subtribe *Carduinae* (Bremer 1994). Within the subtribe, some of the most successful thistles are found, e.g. in the diverse genera *Carduus* (approx. 90 species), *Cirsium* (200-300 species) or *Onopordum* (approx. 60 species), which have a large natural distribution area and have been distributed further by human influence. Due to excellent dispersal capacities, long-lived seed banks, their relative drought tolerance and their spiny habit many of them are serious weeds (Baker 1974, Groves & Kaye 1989, Briese & al. 1994). Other large genera within the subtribe predominantly

occur in mountain areas and often show high degrees of endemism, like *Cousinia* (approx. 700 species), *Saussurea* (approx. 400 species) or *Jurinea* (approx. 200 species) (Il'in 1962, Rechinger 1972, Lipšić 1979, Knapp 1987). Most genera within the *Carduinae*, however, are small, 22 containing less than five species, 13 of them being monotypic.

The *Cardueae* are traditionally subdivided into the four subtribes *Echinopinae*, *Carlininae*, *Carduinae* and *Centaureinae* (Bentham 1873, Hoffmann 1890-94). This classification goes back to Cassini (1819), who treated the respective groups as separate tribes, emphasizing the close relationship of the latter two. The smaller subtribes *Echinopinae* and *Carlininae* are regarded as basal within the *Cardueae*, whereas the *Carduinae* and *Centaureinae* together probably constitute a more advanced monophyletic group (Bremer 1994, Susanna & al. 1995, Petit 1997). The subdivision of this large group into *Carduinae* and *Centaureinae* is not entirely satisfactory. While there is hardly any doubt about the monophyly of the *Centaureinae* (Susanna & al. 1995, Hellwig 1996), the *Carduinae* are most likely a paraphyletic assemblage. The phylogenetic relationships between the genera are unclear and the immediate sister group of the *Centaureinae* is unknown. Dittrich (1970, 1977) has proposed a subdivision of the *Carduinae* into four groups based on carpological characters. This classification is, however, not phylogenetic and some of the groups are certainly artificial (Bremer 1994, Häffner unpubl.). The conclusions of previous phylogenetic studies of the *Cardueae* (Bremer 1994, Petit 1997) are in parts contradictory, and the reliability of the postulated relationships especially within the *Carduinae* is not very high. Furthermore, there are several genera especially from Central or E Asia which have not been included in the respective analyses but may play a key role in the evolution of the group. A better understanding of the diversification processes within the *Carduinae*+*Centaureinae* is therefore needed.

An additional problem concerns generic delimitations and the monophyly of some of the larger genera within the *Carduinae*. Many complexes within this group consist of one large genus and several smaller satellite genera, which are closely related to the respective large genus but differ conspicuously in one or another character (Häffner unpubl.). A special case is the *Carduus-Cirsium* group, because this complex consists of two closely related large genera and some smaller ones. It is not clear whether *Carduus* and *Cirsium* are sister groups or whether at least one of them is paraphyletic or even polyphyletic. Their separation is mainly based on a single character (scabrous or plumose pappus bristles respectively, e.g. Bentham 1873) and is often regarded as artificial (e.g. Bremer 1994).

In the present study, the following questions are addressed by the reconstruction of the phylogenetic relationships within the *Carduinae*:

- How can the early diversification processes of the *Carduinae*+*Centaureinae* be characterised and which are the most basal representatives within this group?
- How are the different genera of the *Carduinae* phylogenetically related?
- Are the *Carduinae* paraphyletic, and if so, what is the sister group of the *Centaureinae*?
- Are the large and closely related genera *Carduus* and *Cirsium* monophyletic?

The reconstruction of the phylogenetic relationships is based on DNA sequence data of the Internal Transcribed Spacer (ITS) regions. The ITS regions are located in the nuclear ribosomal DNA (ITS 1 between 18S and 5.8S rDNA, ITS 2 between 5.8S and 26S rDNA). They show sufficient variation to be informative on the lower taxonomic levels, but contain enough more constrained regions to allow unambiguous alignment in most cases (Baldwin & al. 1995). Within the *Centaureinae*, the ITS region has proved to be a useful source of characters for phylogenetic reconstruction (Susanna & al. 1995, Hellwig 1996) and is therefore applied in the present study to resolve phylogenetic relationships around and within the *Carduinae*.

## Material and methods

### Plant material

DNA was extracted from herbarium specimens or from dried leaf material from plants collected in the wild or raised in the botanical gardens of Göttingen and Berlin from seed material col-

lected in the field or obtained from the seed exchange. Plants from the seed exchange were only used when they could be reliably determined. Vouchers of the investigated plants are deposited in the herbaria of Berlin (B), Göttingen (GOET) and Jena (JE). A list of the investigated material is given in Tab. 1.

Tab. 1. Investigated species and voucher information.

- Alfredia cernua* (L.) Cass. – Seed exchange BG Halle, cult. in BG Berlin, Acc. No. 092-98-97-70, 7.1998, Häffner (B).
- Ancathia igniaria* (Spreng.) DC. – Russia, Tuva, okr. g. Kyzyl, zalez', 6.6.1986, Ždanova & Šaulo (B).
- Berardia subacaulis* Vill. – France, Dpt. Hautes Alpes, N Gap, am Weg zum Col de Gleize, Felsspalten, c. 2130 m, 12.8.1995, Ochsmann 95652 & Dreyer (herb. Ochsmann).
- Carduus acanthoides* L. – Germany, Berlin, Schönholz, ehem. Grenzstreifen, Brache, Sandboden, 26.7.1996, Häffner 7 (B).
- Carduus crispus* L. – Finland, Uusimaa, Helsinki, Toukola, Arabianranta, wasteland, edge of ditch, 17.9.1995, Hiltunen 14, seed exchange BG Helsinki, cult. in BG Berlin, Acc. No. 128-07-96-10, 18.6.1997, Häffner (B).
- Carduus defloratus* L. – Italy, Alpi Carniche, Croce di Cormelico, seed exchange BG Uppsala, cult. in BG Berlin, Acc. No. 130-02-96-10, 16.6.1997, Häffner (B).
- Carduus leptacanthus* Fresen. – Ethiopia, Menagesha State Forest, located on an extinct volcano about 30 km SW of Addis Ababa between 38°32' & 38°56'E, 8°56' & 9°00'N, 2720 m, 18.3.1998, Sukopp (UBT).
- Carduus nutans* L. – Germany, Hessen, Kreis Gießen, zw. Holzheim und Pohlheim, 15.7.1994, Kuschel, seed exchange BG Hamburg, cult. in BG Berlin, Acc. No. 120-06-96-10, 18.6.1997, Häffner (B).
- Carlina vulgaris* L. – Germany, Göttingen, Neuer Botanischer Garten, 6.1993, Hellwig (GOET).
- Cirsium ciliatum* (Murr.) M. Bieb. – Russia, Kaukasus, Klin-Jar, c. 4 km NW Kislovodsk, Weide, 1996, Reinhold (B).
- Cirsium eriophorum* (L.) Scop. – Italy, Aymavilles, Ozein 1900 m, seed exchange BG Cogne, cult. in BG Berlin, Acc. No. 169-02-96-10, 15.7.1997, Häffner (B).
- Cirsium texanum* Buckl. – USA, Texas, Austin, 11.1993, Hellwig (GOET).
- Cirsium tuberosum* (L.) All. – France, Elsaß-Lothringen, bei Marckolsheim, 28.6.1994, Kuschel, seed exchange BG Hamburg, cult. in BG Berlin, Acc. No. 120-11-96-10, 4.9.1996, Häffner (B).
- Cousinia hystrix* C. A. Mey. – Cult. in BG Berlin, Acc. No. 92-99-97-70, 26.6.1998, Häffner (B).
- Echinops exaltatus* Schrad. – DNA provided by R. K. Jansen, Austin, Texas, U.S.A.
- Jurinea macrocephala* DC. – Turkey, Vil. Nidge, road to Alihoca, 1350 m, 14.8.1994, Hellwig & Kaya (GOET).
- Jurinea mollis* (L.) Reichenb. – Österreich, Bisamberg bei Wien, Hang oberhalb Langenzersdorf, 24.6.1956, Wagenitz 3018 (GOET).
- Klasea radiata* (Waldst. & Kit.) Á. & D. Löve [= *Serratula radiata* (Waldst. & Kit.) M. Bieb.] – Ex sem. BG Cluj, No. 516 cult. in BG Göttingen, 6.1993, Hellwig (GOET).
- Lamyropsis cynaroides* (Lam.) Dittrich – Greece, Kreta, Nomos Lasithiou, Eparchia Sitia, Kato-Zaleros-Schlucht, 35°06'07"N, 26°14'20"E, 65-70 m, Geröllpediment, 15.7.1997, Böhling 6720, cult. in BG Berlin, Acc. No. 086-05-98-10 (B), 10.6.1999, Häffner (B).
- Notobasis syriaca* (L.) Cass. – Seed exchange BG Dijon, cult. in BG Berlin, Acc. No. 158-07-96-70, 1.8.1996, Häffner (B).
- Olgaea pectinata* Iljin – Kazachstan, Aksu-Džabagliskij zapovednik, kamenistyj južnyj sklon protiv ust'ja r. M. Kaindy, 17.8.1954, Šreter (B).
- Onopordum boissieri* Willk. – Cult. in BG Göttingen ex sem.: Türkei, Side, Strand und Ruderalflächen, 30.5.1991, Ochsmann (GOET).

## Tab. 1 continued

- Ptilostemon afer* (Jacq.) Greuter – Seed exchange BG Krefeld, cult. in BG Berlin, Acc. No. 155-02-96-70, 1.7.1997, Häffner (B).
- Saussurea riederi* Herder – Japan, Hokkaido seed exchange BG Uppsala, cult. in BG Göttingen, 6.1993, Hellwig (GOET).
- Serratula coronata* L. – Cult. in BG Göttingen, 7.1994, Hellwig (GOET).
- Stephanochilus omphalodes* (L.) Maire – Algerien, Wilaya Bechar, Montagnes du Zeramma, E-Abfall der Berge 19 km SW Zeramma ('Kheneg et Tlaia'), c. 55 km WSW Beni Abbes, Felsen und sandiges Oued, 500 m, 1.4.1980, Podlech 33680 (M).
- Stemmacantha rhapontica* (L.) Dittrich – Cult. in BG Göttingen, 6.1993, Hellwig (GOET).
- Synurus deltoides* (Aiton) Nakai – Russia, Nertschinsk, in Berghälern und schattigen, buschigen Schluchten, 1189, Karo, Pl. *Dahuricae* 84a (GOET).
- Tyrimnus leucographus* Cass. – Seed exchange BG Dijon, cult. in BG Berlin, Acc. No. 158-09-96-70, 5.8.1996, Häffner (B).

A selection of species representing all major groups in the *Carduinae* was investigated. Special attention was paid to the representation of formerly poorly investigated taxa of uncertain position such as *Alfredia*, *Ancathia* and *Olgaea*.

*Berardia subacaulis* as a member of unknown subtribal assignment was included. The genus *Synurus*, which has been regarded until recently as a member of the *Centaureinae* (e.g. Bremer 1994), was represented because in the analysis of Hellwig (1996) it proved to be a member of the *Carduinae*.

Smaller genera within the *Carduinae*, which are unambiguously attributable to a certain group of genera on the grounds of morphological characters (Häffner unpubl.), were not regarded except for the representatives of the *Carduus-Cirsium* group. Within this group, species of the small genera *Galactites*, *Notobasis* and *Tyrimnus* were investigated. From the genera *Carduus* and *Cirsium*, potentially distant species were chosen. From *Cirsium*, two members of *C. sect. Epitrachys* DC. (*C. eriophorum* and *C. ciliatum*), one representative of *C. sect. Cirsium* (*C. tuberosum*) and one New World species (*C. texanum*) were included. *C. texanum*, like many New World species of *Cirsium*, is characterised by aneuploid reductions in chromosome numbers as compared to the Old World species (Ownbey & Olson 1969). From *Carduus*, the European species *C. defloratus*, *C. crispus* and *C. nutans* were selected, all belonging to *C. subg. Carduus*, and, as a member of *C. subg. Afrocarduus* Kazmi, *C. leptacanthus* was included.

In order to represent the *Centaureinae*, species of the genera *Serratula*, *Klasea* and *Stemmacantha* were included. All three genera are regarded as basal within the subtribe (Susanna & al. 1995, Hellwig 1996, Wagenitz & Hellwig 1996). *Stephanochilus omphalodes* represents the more advanced groups of the *Centaureinae* with derived pollen types (Wagenitz 1955, Wagenitz & Hellwig 1996).

As outgroups, *Carlina* and *Xeranthemum* from the *Carlininae*, and *Echinops* as a member of the *Echinopinae* were included in the analysis.

All sequences were determined in the laboratory of the second author except for the following: *Arctium lappa* (EMBL Acc. No. L35888), *Cynara humilis* (EMBL Acc. No. L35875), and *Galactites duriaei* (EMBL Acc. No. 35860).

### DNA extraction, amplification and sequencing

Total cell DNA was extracted from leaf tissue following the protocol given in Hellwig & al. (1999). Likewise, the amplification of ITS 1 and 2 and subsequent purification of the amplification products were performed using the protocol given in Hellwig & al. (1999).

Cycle-sequencing was performed following the instructions in the "sequence brochure", Version 3 of MWG-Biotech (Anonymus 1997). The products of sequencing were separated and de-

tected in a LI-COR DNA-sequencer 4000 L. Primers 2 and 4 were labelled with IRD 700 and Primers 1 and 3 with IRD 41. For primer information see Hellwig & al. (1999).

### Sequence alignment

Multiple sequence alignment was done using the program CLUSTAL W (Thompson & al. 1994) to homologue the bases of the sequences. The result was corrected by hand. The alignment is given in the data matrix (characters 1-503), see <http://www.bgbm.fu-berlin.de/bgbm/library/publikat/willd29/haeffner&hellwig.htm>.

### Phylogenetic analysis

The data matrix was phylogenetically analysed using PAUP\*, version 4.0b2 (Swofford 1999) on a Macintosh PowerPC. In order to find the most parsimonious trees, we performed a heuristic search with 100 random addition sequences of taxa input, and applying the TBR-option as a swapping algorithm with the MULPARS option and the steepest descent options in effect.

Gaps were treated as missing data but were coded as binary characters and included in the analysis (characters 504-549 in data matrix). All characters were weighted equally. In order to estimate the reliability of the resulting branching patterns in the trees, a bootstrap analysis with 1000 replicates and a decay analysis were performed. For the latter we used the program Auto-decay, version 3.0 (Eriksson & Wikström 1995).

## Results

### ITS sequence data

ITS 1 and ITS 2 of all taxa except *Alfredia cernua* were sequenced successfully and could be included in the cladistic analysis. For *Alfredia cernua*, only ITS 1 could be obtained. There is a large deletion of 46 bp in ITS 1 of *Carlina vulgaris* (Hellwig 1996), but the ingroup taxa differ by very few major insertion/deletion events. The remarkable exception is a 4+2 bp deletion in ITS 2 that characterises the taxa of the subtribe *Centaureinae* (Hellwig 1996) and the genera *Jurinea*, *Saussurea*, *Arctium* and *Cousinia*. The lengths of ITS 1 and 2 varied slightly between 251 and 259 bp for ITS 1 and 216 and 225 bp for ITS 2. The data matrix contains 549 characters, of which 275 are phylogenetically informative.

### Phylogenetic analysis

Phylogenetic analysis resulted in five equally most parsimonious trees of a length of 1092 steps. The trees have a consistency-index value of 0.4762 and a retention index value of 0.5493. The strict consensus tree is given in Fig. 1.

### Basal relationships

The *Carduinae* and *Centaureinae* together form a monophyletic group with moderate support from bootstrap analysis. Within the outgroups, *Carlinae* and *Echinopinae* are not clearly separated, *Echinops* being united in a monophyletic group together with *Xeranthemum* with considerable support. Within the ingroup consisting of *Carduinae* and *Centaureinae*, *Berardia* holds an isolated position, not being associated in a clade with any other genus. Among the remaining ingroup genera, four major clades can be distinguished (Fig. 1):

- (1) A clade consisting of *Onopordum* and the small Central and E Asian genera *Ancathia*, *Synurus*, *Olgaea* and *Alfredia* receives good support from bootstrap and decay analyses.
- (2) All included genera of *Centaureinae* are united in a monophyletic group with 100 % bootstrap support.
- (3) The clade containing the representatives of the genera *Jurinea*, *Saussurea*, *Cousinia* and *Arctium* is only weakly supported.
- (4) The clade consisting of the representatives of the genera *Carduus* and *Cirsium* and their close relatives and the smaller Mediterranean genera *Cynara*, *Lamyropsis* and *Ptilostemon* receives moderate support from bootstrap and decay analyses.

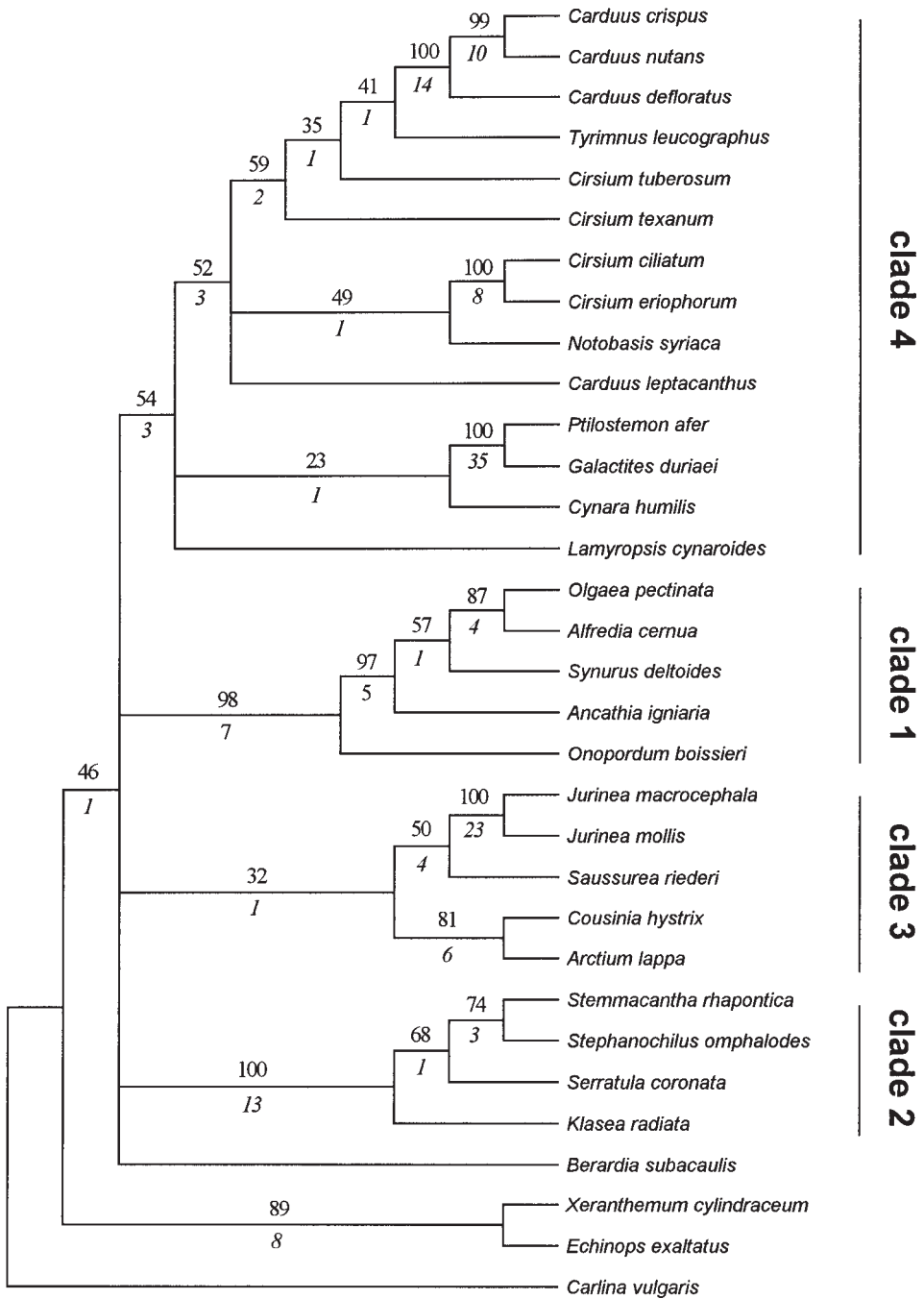


Fig. 1. Strict consensus tree of five most parsimonious trees based on the data matrix (see <http://www.bgbm.fu-berlin.de/bgbm/library/publikat/willd29/haeffner&hellwig.htm>). – Numbers above branches are bootstrap values, numbers in italics below branches are decay values.



The relationships between these basal clades are not resolved in the consensus tree, but nevertheless there are relationships in individual trees which to some degree receive support either from bootstrap and decay analyses, or bring up hypotheses which must be discussed in the light of additional information (see discussion). For example, in four of the five most parsimonious trees, *Berardia* is the sister group of all remaining ingroup generac. A clade uniting the *Centaureinae* (2) and the *Jurinea-Saussurea-Cousinia* clade (3) in a monophyletic group receives a bootstrap support value of 35 % and is present in four of the five most parsimonious solutions. The relationships between this group and the other two major clades (1 and 4) are unclear. Fig. 2 shows one of the four most parsimonious trees in which clades 2 and 3 are sister groups.

### Interrelationships of genera within the groups

(1) In the first of the basal clades, the relationships between genera are well resolved. *Onopordum* is located at the base and the small Central and E Asian genera are united in a further well-supported monophyletic group.

(2) In the *Centaureinae* clade, *Klasea* and *Serratula* (*Serratula* s.l) represent the most basal taxa.

(3) The representatives of the large genera *Cousinia*, *Saussurea* and *Jurinea* together with *Arctium* form a monophyletic group, which is the sister group of the *Centaureinae*. However, this sister-group relationship is only weakly supported by bootstrap analysis. The genus *Arctium* is most closely related to *Cousinia*, and *Saussurea* forms a monophyletic group together with *Jurinea* with moderate reliability.

(4) The fourth complex contains *Cynara*, *Ptilostemon* and *Galactites* united in one clade with low support. The data suggest a remarkably close relationship between *Ptilostemon* and *Galactites* within this first group. *Lamyropsis* occupies an isolated position. The included representatives of *Carduus* and *Cirsium* plus the small genera *Notobasis* and *Tyrinnus* form a monophyletic group.

### Generic delimitations within the *Carduus-Cirsium* complex

According to the relationships within the clade containing *Carduus* and *Cirsium* plus the monotypic genera *Notobasis* and *Tyrinnus* the genera *Carduus* and *Cirsium* are not monophyletic groups. The tropical E African *Carduus leptacanthus* holds an isolated position within the clade. The rest of the included *Carduus* species, however, do form a monophyletic group with a bootstrap support of 100 %. The genus *Cirsium* is paraphyletic in more than one dimension when the *Tyrinnus-Carduus* clade and *Notobasis* are excluded. *Cirsium ciliatum* and *C. eriophorum* both belong *C. sect. Epitrachys*, which is a natural group according to the data of the present analysis. *Notobasis* is most closely related to this section. The American *Cirsium texanum* and the European *Cirsium tuberosum* form a paraphyletic grade at the base of the *Tyrinnus-Carduus* clade.

## Discussion

### Basal relationships

The monophyly of the *Carduinae+Centaureinae* is only moderately supported in the present study, but gets additional support from morphological and chemical points of view. The main morphological characters delimiting this group are achenes without twin-hairs, a pluriseriate pappus, an apical pericarpal rim clearly separated from the pappus tissue and long style branches (Dittrich 1977, Bremer 1994, Petit 1997). The monophyly is further confirmed by a chemical marker, Arctiine, which occurs with high consistency among the *Carduinae* and *Centaureinae*, but is not known from any other *Compositae* (Hänsel & al. 1964).

Within the ingroup, several groups of genera can be distinguished, among which the basal relationships are unclear. The poor resolution of the basal clades might be due to quick divergent evolution following the origin of the clade. Such a radiation may occur shortly after an evolutionary line has disclosed new opportunities to diversify. In cladograms, this condition is reflected in a preponderance of autapomorphies at the respective level compared to synapomorphies between groups (Eldredge & Cracraft 1980). Nevertheless, some hypotheses concerning basal relationships shall be discussed in the light of additional data.



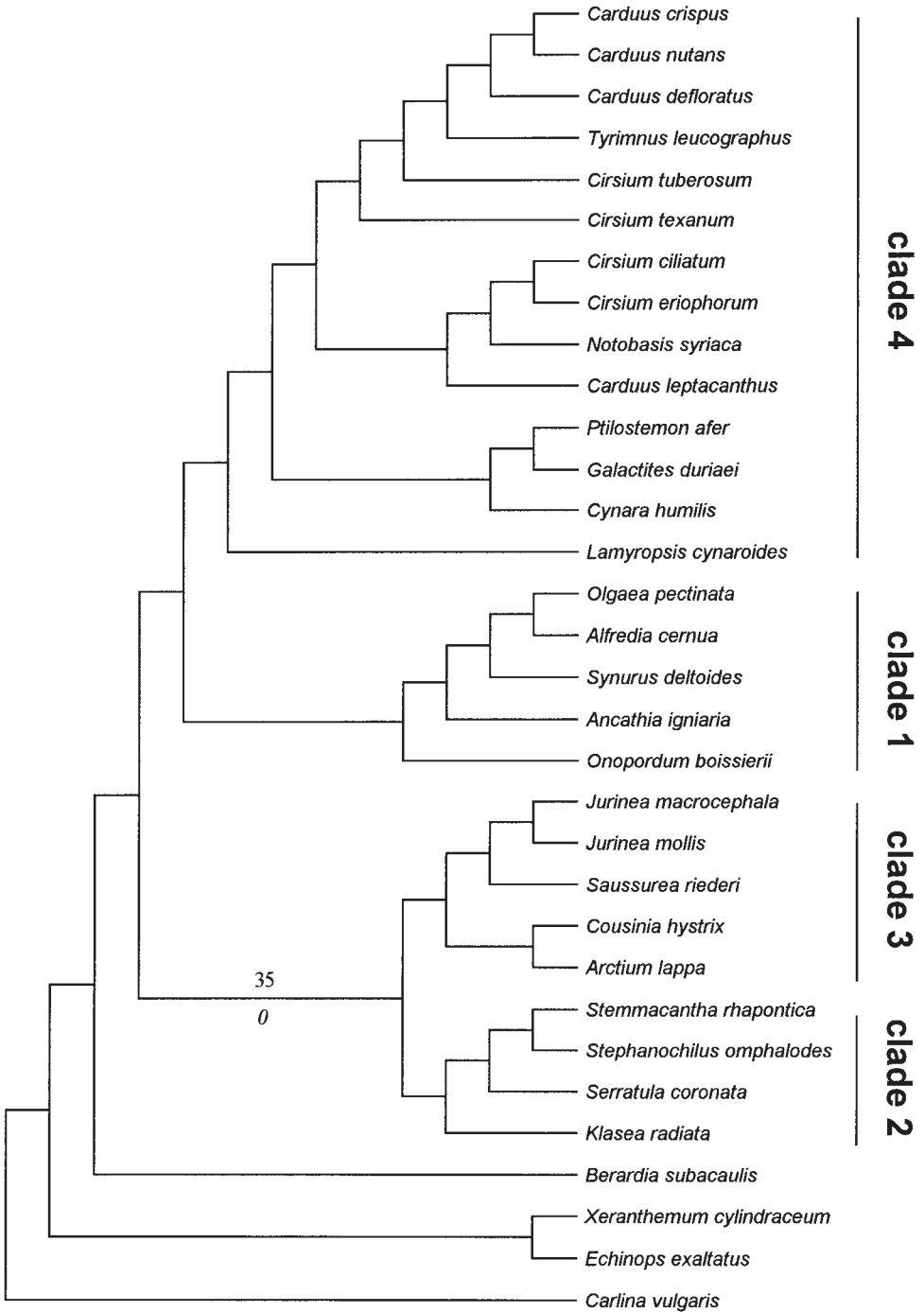


Fig. 2. One of the five most parsimonious trees based on the data matrix (see <http://www.bgbm.fu-berlin.de/bgbm/library/publicat/willd29/haeffner&hellwig.htm>). – Number above the branch is a bootstrap value, number in italics below the branch is a decay value.

There is some evidence that *Berardia*, a monotypic genus endemic to the SW Alps, has a position near the base of the ingroup. The position of *Berardia* has been discussed for a long time. In the past some authors included it in the *Mutisieae* (Bentham 1873, Hoffmann 1890-94). Even after its grouping in the *Cardueae* was recognised (e.g. Markgraf 1968, Dittrich 1977), its position within the tribe remained unclear (Bremer 1994). Morphological evidence is consistent with the results of the present study, in which *Berardia* constitutes the sister group of the remaining *Carduinae* and *Centaureinae* (see Fig. 2). The genus exhibits a morphological character profile, which is intermediate between the *Carlininae* on one hand and the *Carduinae*+*Centaureinae* on the other. With the *Carlininae*, *Berardia* shares short obtuse style branches and a pericarp which is directly continued into the pappus tissue (Bremer 1994, Petit 1997). A bi-layered endosperm present in *Berardia* (Häffner unpubl.) is otherwise only known from some *Carlininae* genera (Dittrich 1996). With the *Carduinae*+*Centaureinae*, *Berardia* is connected by a pluriseriate pappus consisting of free bristles (Bremer 1994, Petit 1997). *Berardia subacaulis* can be described as a relict, which is possibly the closest extant relative to the common ancestor of the *Carduinae*+*Centaureinae*.

### Interrelationships of genera

#### 1) *Onopordum* clade

The large genus *Onopordum* and the smaller E and Central Asian genera *Ancathia*, *Synurus*, *Olgaea* and *Alfredia* are united in a stable monophyletic group. The position of *Onopordum* varied in previous cladistic studies (Bremer 1994, Petit 1997), probably mainly due to its basal position and because its closest relatives have never been included in the respective analyses. Fischer & Jensen (1990) conclude a basal position of *Onopordum* within the *Carduinae* on the basis of serological findings, which is confirmed by the present results.

The smaller genera in the clade are morphologically and geographically so isolated that in the past they have often been misplaced with respect to their relationships. *Ancathia*, a monotypic genus with a main distribution area in Kazakhstan, the Siberian and Mongolian Altai and with an outpost in Azerbaijan and Dagestan, had been included in *Cirsium* (Sprengel 1826) due to its plumose pappus. *Alfredia* and *Olgaea*, two exclusively Central Asian genera, were included in *Carduus* quite recently (Kazmi 1963-64). The E Asian *Synurus deltoides* was originally described as *Onopordum* (Aiton 1789), but afterwards *Synurus* has long been regarded to belong to the *Centaureinae* (e.g. Bremer 1994). Chloroplast restriction site and ITS sequence data have revealed the close affinity with *Onopordum* and its position in the *Carduinae* (Hellwig 1996). Additionally, there are morphological characters which betray the phylogenetic affinity of the genera contained in the well-supported clade. They share a yellow, highly fragile pappus, the achenes of most genera have a rugose testa epidermis and an abaxial carpopodium consisting of sclerified epidermal cells (Häffner unpubl.).

Most genera within the clade have a strongly relictual character, which becomes obvious in relatively small and isolated distribution areas and low species numbers (Simpson 1944). Only *Onopordum* shows greater present diversification and a larger distribution area in Anatolia and the Mediterranean region.

#### 2) The *Centaureinae* and their potential sister group

According to the topology of the tree in Fig. 2, the *Carduinae* are paraphyletic, since only a part of them constitutes the sister group of the *Centaureinae*. Although the sister group relationship between the *Centaureinae* and the complex of the genera *Cousinia*, *Arctium*, *Saussurea* and *Jurinea* does not appear in the consensus tree, it receives moderate support from bootstrap analysis (Fig. 2). Furthermore, a remarkable synapomorphy, a 4+2bp deletion in ITS 2 (Hellwig 1996), leaves little doubt about the monophyly of the group, especially as insertion/deletion events are rare in ITS compared to point mutations (Baldwin & al. 1995). A possible morphological synapomorphy of the clade are extremely short outer pappus bristles, which are shared by all *Centaureinae* and the genera *Jurinea*, *Saussurea*, *Cousinia* and *Arctium* (Häffner unpubl.). The

clade is geographically coherent, as the complexes around the genera *Cousinia*, *Saussurea* and *Jurinea* as well as the basal *Centaureinae* genera *Serratula* and *Stemmacantha* have their centre of diversity in the Central Asian mountain areas of Tien Shan, Pamiroalai and W Himalayas (Meusel & Jäger 1992). Whereas the three large *Carduinae* genera are still centered in this area, the *Centaureinae* have diversified in many different regions, one of them being the Mediterranean basin. Since the relationships between the genera of the potential sister group of the *Centaureinae* are not well supported, it might still be possible that only a part of the *Jurinea*-*Saussurea*-*Cousinia* complex is the real sister group of the *Centaureinae*. For example, the genus *Jurinea* displays some morphological, serological and chemical similarities to the *Centaureinae* such as a lateral detachment area of the fruit, which is a traditional character used to delimit the *Centaureinae* (Bentham 1873), a legumine that corresponds well with that of the *Centaureinae* (Fischer & Jensen 1990), and the presence of certain acetylene glycosides, which are otherwise only found in *Serratula* (Wagner 1977). The phylogenetic proximity of *Arctium* and *Cousinia* within the clade is not surprising as the delimitation of the two genera is diffuse (e.g. Duistermaat 1996, 1997).

### 3) *Carduus*-*Cirsium* clade

The clade formed by the larger genera *Carduus* and *Cirsium* and the smaller genera *Cynara*, *Ptilostemon*, *Lamyropsis*, *Galactites*, *Notobasis* and *Tyrinnus* is a geographically homogeneous entity. All genera have their centres of distribution in the Mediterranean area (Greuter 1973, Meusel & Jäger 1992, Wiklund 1992).

The fact that *Ptilostemon* and *Cynara* are united in one clade corresponds with morphological findings: both genera lack lacticifers, which are present in other *Carduinae* genera (Greuter 1973, Petit 1997). An unexpected result is the remarkably close relationship between *Ptilostemon* and *Galactites* as inferred from the ITS sequence data. *Galactites* shares a number of morphological similarities with *Carduus*, *Cirsium* and related genera, which assign it a firm position within this group in morphological analyses (Petit 1997, Häffner unpubl.). Two explanations for this phenomenon are possible: either one of the data sets shows a large amount of concerted homoplasy, or *Galactites* is a product of reticulate evolution. The latter possibility makes the small genus a rewarding object for further investigation. Maternally inherited chloroplast sequence data could cast some light on this question.

The position of *Lamyropsis* is isolated within the clade. This relatively small genus with an E Mediterranean centre of distribution has been regarded as the closest relative of the genus *Ptilostemon* (Dittrich 1971, Greuter 1973), with which it shares a great habitual similarity. The two genera, however, are never united in a monophyletic group and it is therefore possible that the similarities are symplesiomorphies rather than synapomorphies.

Greuter (1973) emphasizes the relictual nature of *Ptilostemon* and *Lamyropsis*. The present analysis supports the view that *Ptilostemon* and *Lamyropsis* like *Cynara* represent early evolutionary lines in the diverse and widespread clade, which contains some of the presently most successful thistle representatives in the genera *Carduus* and *Cirsium*.

### Generic delimitation within the *Carduus*-*Cirsium* complex

Bremer (1994) emphasizes that the separation of *Carduus* and *Cirsium* is possibly not natural as it is based on a single character, scabrous and plumose pappus bristles respectively (e.g. Bentham 1873, Kazmi 1963-64, Talavera & Valdés 1976). This character must be treated with care in systematic studies (Dittrich 1977). According to the results of the present study, both genera are not natural monophyletic groups in their present circumscription: *Carduus* is polyphyletic, as the tropical African *Carduus leptacanthus*, a representative of *C.* subg. *Afrocarduus* (Kazmi 1963-64), is the sister group of all remaining taxa in 47 % of the bootstrap cycles (data not shown). The other included *Carduus* species, which are all members of *C.* subg. *Carduus* (Kazmi 1963-64), do form a monophyletic group. *C.* subg. *Afrocarduus* has been recognised as a separate entity on the basis of a brown pappus, only shortly pilose filaments and a clear geographical separation (Kazmi 1963-64). The species of this subgenus are distributed exclusively

in mountainous regions of tropical E Africa, where hardly any other representative of the tribe *Cardueae* occurs. Häffner (unpubl.) has found carpological features, which suggest an affinity of the African *Carduus* species to *Cirsium*, like a persistent pericarp and the shape of the cell walls in the outer epidermis of the corolla lobes. There are also cytological differences. The tropical African *Carduus* species have diploid chromosome numbers of 32 or 34 (Hedberg & Hedberg 1977), the latter number being also typical for all Old World species of *Cirsium* (e.g. Talavera 1974), whereas the representatives of *C.* subg. *Carduus* are characterised by aneuploid reduction series of chromosome numbers reaching from  $2n = 22$  to  $2n = 16$  (e.g. Devesa 1981).

The widespread genus *Cirsium* contains about 200-300 species (Bremer 1994), and compared with that diversity, only few representatives have been included in the present study. Nevertheless it becomes obvious that the genus is paraphyletic, having a common ancestor but not comprising some derived groups, which have arisen within *Cirsium*. A particularly well-defined subgroup is *C.* sect. *Epitrachys*, which is characterised by spinules on the upper leaf surface. The two included representatives, *Cirsium eriophorum* and *C. ciliatum*, form a monophyletic group with strong support. The sister group of the clade is the monotypic genus *Notobasis*. The annual, mainly E Mediterranean thistle *Notobasis syriaca* is morphologically and cytologically similar to *Cirsium* (e.g. Moore & Frankton 1962, Petit 1997), but differs in some respects, e.g. the rounded shape and the abaxial detachment area of the achenes (Cassini 1822). This somewhat isolated morphological situation is confirmed by the ITS sequence data: *Notobasis* is characterised by a large number of molecular autapomorphies compared to most other taxa. The same situation can be stated for *Tyrinnus*, which is also a monotypic genus containing one annual species, *T. leucographus*. Both taxa are examples for the fact that annuals often undergo faster evolutionary change than perennials, possibly mainly because of a lack of competition between generations, bottle-neck events and autogamy (Hellwig 1996). Other factors may play a role too. Stebbins (1952) discusses aridity as a stimulus for evolutionary progress in several respects. The fact that both thistles inhabit arid or semiarid areas agrees with that.

*Tyrinnus* is at the base of the monophyletic clade containing the species of *Carduus* subg. *Carduus* in the present study. Morphologically, it resembles *Carduus*, e.g., in its scabrous, not plumose pappus bristles, cytologically it is closer to *Cirsium*, having a diploid chromosome number of 34. These findings support the intermediate position occupied by *Tyrinnus* in the present study.

## Conclusions

The *Carduinae* and *Centaureinae* together form a monophyletic group. *Berardia* is probably the closest extant relative of the common ancestor of the *Carduinae*+*Centaureinae*, a result that is confirmed by morphological findings.

A particularly well-supported clade is constituted by the large and widespread genus *Onopordum* and some smaller genera with a strongly relictual character, which suggests that the group is possibly amongst the oldest in the *Carduinae*.

The remaining clades of the ingroup have clearly separated centres of diversity in Central Asia and the Mediterranean region respectively.

The *Centaureinae* are most closely related to the Central Asian clade. It is concluded that the subtribe has its origin in Central Asia and has then spread to wide areas of the Eurasian continent following an extensive radiation. The sister group of the *Centaureinae* must be sought for among the complexes around the genera *Cousinia*, *Arctium*, *Saussurea* and *Jurinea*. The *Carduinae* are paraphyletic because only a part of them constitutes the sister group of the *Centaureinae*.

In the Mediterranean clade, the smaller genera *Cynara*, *Ptilostemon* and *Lamyropsis* occupy relatively basal positions and the diverse and widespread genera *Carduus*, *Cirsium* and some closely allied smaller genera together form a monophyletic group. The position of *Galactites* deserves further investigation.

The genus *Carduus* is monophyletic only when *C.* subg. *Afrocarduus* Kazmi is excluded. *Cirsium* is paraphyletic, because at least two derived groups, viz. the *Tyrinnus*-*Carduus* clade and *Notobasis*, have originated within this genus.

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Addresses of the authors:

- E. Häffner, Botanischer Garten und Botanisches Museum Berlin-Dahlem, Freie Universität Berlin, Königin-Luise-Str. 6-8, D-14191 Berlin, Germany; e-mail: haeffner@zedat.fu-berlin.de
- F. H. Hellwig, Institut für Spezielle Botanik, Universität Jena, Philosophenweg 16, D-07743 Jena, Germany; e-mail: hellwig@otto.biologie.uni-jena.de