THE CARDUEAE (COMPOSITAE) REVISITED: INSIGHTS FROM ITS, *trnL-trnF*, AND *matK* NUCLEAR AND CHLOROPLAST DNA ANALYSIS^{1, 2}

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ABSTRACT

The new outline of relationships in basal branches of the family Compositae Giseke confirms that the sister group to the tribe Cardueae Cass. are not Mutisieae Cass., but rather a group of African genera now classified as the tribe Tarchonantheae Kostel. This change implies that the monophyly of the Cardueae must be reassessed on a molecular basis. Moreover, new collections in recent years allow us to extend our sampling to 70 of the 74 genera of the tribe. We performed a new molecular study of the tribe using one nuclear region (ITS) and two chloroplastic markers (*trnL-trnF* and *matK*) in addition to a more appropriate outgroup. Our results confirm that the Cardueae is a natural group but indicate some changes in subtribal delineation: the subtribe Cardopatiinae Less. is recognized and some genera are moved to other subtribes (*Myopordon* Boiss., *Nikitinia* Iljin, *Syreitschikovia* Pavlov, and the *Xeranthemum* L. group). A recapitulation of a number of interesting questions that remain unresolved in the classification of some large genera is presented.

Key words: Cardueae, Compositae, genus delineation, ITS, *matK*, phylogeny, tribal delimitation, *trnL-trnF*.

RESUMEN

El nuevo esquema de las relaciones en las ramas basales de la familia Compositae Giseke ha confirmado que el grupo hermano de la tribu Cardueae Cass. no es la tribu Mutisieae Cass., sino más bien un grupo de géneros africanos que ahora se clasifican como tribu Tarchonantheae Kostel. Este cambio implica que la monofilia de las Cardueae establecida sobre bases moleculares debe confirmarse. Además, nuestras nuevas recolecciones en los últimos años hacen que nuestro muestreo se extienda a 70 de los 74 géneros de la tribu. Hemos llevado a cabo un nuevo estudio molecular de la tribu usando una región nuclear (ITS) y dos cloroplásticas, además de seleccionar un grupo externo más adecuado. Nuestros resultados confirman que las Cardueae son un grupo natural, pero aconsejan algunos cambios en la delimitación subtribal: el reconocimiento de la subtribu Cardopatiinae Less. y el cambio de algunos géneros a otras subtribus (*Myopordon* Boiss., *Nikitinia* Iljin, *Syreitschikovia* Pavlov y el grupo *Xeranthemum* L.). Presentamos también una recapitulación sobre ciertas cuestiones interesantes que quedan sin resolver en la clasificación de algunos grandes géneros.

INTRODUCTION

OVERVIEW OF COMPOSITAE SYSTEMATICS

The systematics of Compositae is marked by three milestones, each one involving deep changes in the classification of the family. Since the history of this classification has been revised in depth in Funk et al. (2005), here we give only a short summary. The first attempt to classify Compositae was made by Cassini (1819), who defined 20 tribes. A more synthetic system was proposed by Bentham (1873) and, soon after, Hoffmann (1894). Both authors proposed

a classification of two subfamilies and 16 tribes that gained general acceptance until very recently. The two latest revisions of the family, toward the end of the 20th century (Dittrich, 1977; Bremer, 1994), followed Hoffmann's classification. The third set of large-scale changes was produced by the introduction of methods based on DNA analysis. First came the pioneering study by Jansen & Palmer (1987) using cpDNA restriction site polymorphisms, which led to the description of a third subfamily, Barnadesioideae (Benth. & Hook. f.) K. Bremer & R. K. Jansen; this proposal was reflected in Bremer (1994). Second, the

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Table 1. Outline of two different proposals of classification of Compositae (only basal branches).

BREMER (1994)	PANERO & FUNK (2002)
BARNADESIOIDEAE (Benth. & Hook. f.) K. Bremer & R. K. Jansen • BARNADESIEAE D. Don	BARNADESIOIDEAE (Benth. & Hook. f.) K. Bremer & R. K. Jansen • BARNADESIEAE D. Don
CICHORIOIDEAE Chevall. • MUTISIEAE Cass.	Stiftioideae clade (provisional) • Stifftieae clade (provisional) (S. AMERICA, AFRICA)
• CICHORIEAE Lam. & DC.	MUTISIOIDEAE (Cass.) Lindl.
• CARDUEAE Cass.	• MUTISIEAE Cass. (S. AMERICA)
• VERNONIEAE Cass.	GOCHNATIOIDEAE (Benth. & Hook. f.) Panero & V. A. Funk
• LIABEAE Rydb.	HECASTOCLEIDOIDEAE Panero & V. A. Funk
• ARCTOTEAE Cass.	• HECASTOCLEIDEAE Panero & V. A. Funk
ASTEROIDEAE (Cass.) Lindl.	CARDUOIDEAE Cass. ex Sweet
..... (3 subfamilies, 17 tribes)	• DICOMEAE Panero & V. A. Funk
	• TARCHONANTHEAE Kostel.
	• CARDUEAE Cass.
	 (11 subfamilies, 36 tribes)

latest and more revolutionary study by Panero & Funk (2002) analyzed sequences of nine chloroplast regions across the entire family and proposed a new classification with 11 subfamilies and 35 tribes; this was in some ways closer to Cassini’s analytical views than to synthetic approaches. The dramatic differences between Bremer’s (1994) and Panero and Funk’s (2002) classification are illustrated in Table 1 (only the basal groups are shown). The high statistical support for the latter and its sound correlation with morphology leads us to believe that the new classification of Compositae is near to being definitive.

THE TRIBE CARDUEAE

Cardueae Cass. is one of the largest tribes of Compositae, with ca. 2500 species. Previous studies based on DNA sequence analyses, both nuclear (Susanna et al., 1995) and combined chloroplast and nuclear (Garcia-Jacas et al., 2002), confirm Cardueae as monophyletic. However, the new classification shows that our previous outgroup choice was not the best choice. In the classic system of Compositae (e.g., Heywood et al., 1977; Bremer, 1994), Cardueae were classified in subfamily Cichorioideae Chevall., close to tribes Cichorieae Lam. & DC. and Mutisieae Cass. (Table 1). Therefore, in our first nuclear-DNA-based phylogeny (Susanna et al., 1995), the outgroup was composed of one Cichorieae (*Tragopogon* L.) and three Mutisieae (*Ainsliaea* DC., *Gerbera* L., and *Warionia* Benth. & Coss.). In Garcia-Jacas et al. (2002), we replaced *Ainsliaea*, *Tragopogon*, and *Warionia* because of the increasing difficulties in aligning the ITS region, and we used two Mutisieae as outgroups, *Gerbera* and *Mutisia* L. f. However, according to the new classification by Panero & Funk

(2002), tribe Cichorieae (*Tragopogon*) is derived in relation to Cardueae; *Ainsliaea* and *Warionia* do not belong to Mutisieae but to Perteyeae Panero & V. A. Funk and Gundelieae H. Rob. & Brettell, respectively, both tribes also derived with regard to Cardueae; and *Gerbera* and *Mutisia* are placed in Mutisieae sensu stricto, phylogenetically far from Cardueae (Table 1). With these outgroup species, Cardueae will always be monophyletic, and monophyly of the tribe has always been a controversial issue. The outgroup should be chosen from the clade formed by tribe Tarchonanthaeae plus the genus *Oldenburgia* L., a clade that appears as the true sister group to Cardueae with bootstrap support values of 100% in Panero and Funk (2002). In fact, Cardueae, Dicomeae Panero & V. A. Funk, and Tarchonanthaeae (plus *Oldenburgia*) compose a monophyletic subfamily, Carduoideae Cass. ex Sweet, also with the highest statistical support (Panero & Funk, 2002). Morphological connections between Cardueae and the rest of subfamily Carduoideae are, however, unknown to date.

TRIBAL LIMITS OF CARDUEAE

In the earliest classification (Cassini, 1819), present Cardueae were divided in three tribes: Echinopeae, Carlineae, and Cardueae, the latter with two subtribes: Carduinae and Centaureinae. Bentham (1873) and Hoffmann (1894) proposed grouping the three tribes in a single tribe Cardueae that held four subtribes: Echinopinae (Cass.) Dumort., Carlininae (Cass.) Dumort., Carduinae Cass., and Centaureinae (Cass.) Dumort. This was a conservative approach that was generally accepted for a very long time. However, discussion on the status of Echinopinae restarted when Wagenitz (1976) proposed the segregation of the

subtribe as a separate tribe, Echinopeae. Dittrich (1977) returned to Cassini's early views and proposed the restoration of Echinopeae and Carlineae. Finally, Bremer (1994) reintroduced the conservative approach with only one tribe, Cardueae, which, according to our molecular studies, is a better solution (Susanna et al., 1995; Garcia-Jacas et al., 2002).

SUBTRIBAL CLASSIFICATION

Within Cardueae, there is general agreement in accepting four groups, regardless of the rank (tribe or subtribe) adopted. Three subtribes are natural (Carlininae, Echinopinae, and Centaureinae) and the fourth (Carduinae) is a paraphyletic assemblage (Garcia-Jacas et al., 2002).

Subtribe Carlininae is sister to the rest of the tribe. A striking and probably plesiomorphic character is the presence of true ray florets in at least one genus of Carlininae, *Atractylis* L., while remaining subtribes have only disk florets. Capitula are usually subtended by pectinate-pinnatisect leaf-like bracts; corolla lobes are very short, only 1–3 mm long; and the pappus has long, plumose bristles, often connate at the base forming broader, robust scales (Susanna & Garcia-Jacas, in press).

Subtribe Echinopinae is easily characterized by its second-order inflorescences (uniflowered capitula clustered in a large synflorescence). Our latest molecular phylogeny indicates that Echinopinae should also include the genera of the *Xeranthemum* group and we previously proposed that the small heads of the genus *Xeranthemum* and allies could be interpreted as reduced synflorescences (Garcia-Jacas et al., 2002).

Subtribe Carduinae is a paraphyletic complex of genera with some well defined groups (*Arctium* L. group, *Onopordum* L. group, *Saussurea* DC. group, or the thistles) together with genera of problematic ascription like *Berardia* Vill. or *Stachelina* L. All the genera of Carduinae have basal or basal-abaxial insertion areole of achenes and, usually, a simple pappus, and are often spiny.

Finally, subtribe Centaureinae is the most derived group and is characterized by achenes with lateral-adaxial insertion areole, a double pappus, and, with few exceptions, unarmed leaves. However, examining the limits between Carduinae and Centaureinae represents a challenge, because differences lie in microcharacters of the achene and pappus that are difficult to observe in incomplete or immature herbarium materials. The examples of *Nikitinia* and *Syreitschikovia* illustrate these difficulties (Susanna et al., 2002) and the ascription of these and other genera should be checked against a molecular phylogeny.

In Garcia-Jacas et al. (2002) we suggested that maybe a fifth subtribe could be recognized, Cardopatiinae Less., with two genera: *Cardopatium* Juss. and *Cousiniopsis* Nevski. Cardopatiinae were placed in an intermediate position between Carlininae and the rest of the tribe (Garcia-Jacas et al., 2002). However, we have postponed the restoration of this subtribe until more unambiguous evidence has been collected.

GENERIC LIMITS IN TRIBE CARDUEAE

Other points of interest are genus affinities and limits in Cardueae, a tribe with some of the largest genera of the family. Regarding genus affinities, on the basis of morphology and partial molecular studies, the two largest subtribes (Carduinae and Centaureinae) were subdivided into informal groups (Susanna & Garcia-Jacas, in press), which should be checked against a more comprehensive molecular phylogeny. As to genus limits, in our latest revision of Cardueae (Susanna & Garcia-Jacas, in press) we adopted a broad generic concept for *Cousinia* Cass. (600 species), *Jurinea* Cass. (200), and *Saussurea* (400) because of the lack of recent systematic revisions for all three. Recently, on the basis of a partial study of DNA sequences and achene morphology, Raab-Staube (2003) proposed the restoration of two small genera, *Frolovia* (DC.) Lipsch. and *Lipschitzia* Kamelin, and described a new genus, *Himalaiella* Raab-Staube, all of these within the *Saussurea* group.

SCOPE AND AIMS

With the addition of new materials, our DNA sampling covers 70 of the 74 accepted genera of Cardueae: only *Ancathia* DC. (Carduinae, Central Asia), *Centaurodendron* Johow (Centaureinae, Juan Fernández archipelago), *Goniocaulon* Cass. (Centaureinae, India and East Tropical Africa), and *Takeikadzuchia* Kitag. & Kitam. (Carduinae, Mongolia) are absent. However, the position of these within the tribe and their subtribal ascription has never been challenged on a morphological basis (Susanna & Garcia-Jacas, in press). To test our broad generic concept, we included the genera *Frolovia*, *Lipschitzia*, and *Modestia* Kharadze & Tamamsch., which we submerge in *Jurinea*; and *Anura* (Kult.) Tscherneva and *Tiarocarpus* Rech. f., which we had previously considered in *Cousinia* (Susanna & Garcia-Jacas, in press). We also included the published sequence of the recently described genus *Himalaiella*. For this wide representation of Cardueae, we completed the ITS and *matK* regions and, in view of the low resolution of basal groups in previous analyses, we added a new marker. Low resolution in

many molecular phylogenies may be solved by adding more data to DNA sequence matrices as discussed in Panero & Funk (2002). We used a chloroplast marker, the *trnL-trnF* intergenic region, which is widely utilized in Compositae (Bayer & Starr, 1998; Liu et al., 2002; Oberprieler, 2002; Panero & Funk, 2002). Our goals were to:

- verify monophyly of Cardueae using species from the sister clade Tarchonantheae and *Oldenburgia* as an outgroup;
- clarify subtribal classification and define the position of Cardopatiinae, which might constitute a fifth subtribe;
- examine whether the informal species groups defined in subtribes Carduinae and Centaureinae are natural, and check the systematic position within these groups of genera not included in our previous studies; and
- verify the suitability of a broad generic concept in certain large genera of Cardueae by analyzing species from genera that we had previously rejected on the basis of morphological characters.

MATERIAL AND METHODS

PLANT MATERIAL

Sampling was defined on the basis of Garcia-Jacas et al. (2001), Garcia-Jacas et al. (2002), Susanna et al. (2003), and Susanna & Garcia-Jacas (in press), in order to represent most of the genera of tribe Cardueae. Thirteen accepted genera (*Amphoricarpus* Vis., *Karvandarina* Rech. f., *Lamyropappus* Knorring & Tamamsch., *Lamyropsis* (Kharadze) Dittrich, *Myopordon* Boiss., *Nikitinia*, *Olgaia* Iljin, *Plagiobasis* Schrenk, *Polytaxis* Bunge, *Russowia* C. Winkl., *Syreitschikovia*, *Siebera* J. Gay, *Tricholepis* DC., and *Tugarinovia* Iljin) are sequenced here for the first time. Six other genera that were not accepted in our latest revision of the tribe (Susanna & Garcia-Jacas, in press) are *Aegopordon* Boiss., *Anura*, *Frolovia*, *Lipschitzia*, *Modestia*, and *Tiarocarpus*. Two more genera, *Dolomiaea* DC. and *Himalaiella*, were obtained from sequences published elsewhere. Three outgroup species were chosen, two among Tarchonantheae and another from the genus *Oldenburgia*, because the tribe and the genus form the sister clade to Cardueae (Panero & Funk, 2002). Many of our ITS1 and ITS2 sequences from previous studies (Garcia-Jacas et al., 2001, 2002) have been completed with the sequence of the 5.8 S gene, and some of our old manual ITS sequences (Susanna et al., 1995) were re-sequenced or confirmed by automatic sequencing. Both previously published (Garcia-Jacas et al., 2002) and new sequences of the *matK* gene were used in this analysis. All the *trnL-trnF* sequences analyzed are new, with the exception of *Dolomiaea* (from Liu, unpublished) and some species of *Saussurea* (from Raab-Staube, 2003). The number of new sequences is

283 for a total of 466. The origin of the samples and their GenBank accession numbers are given in Table 2.

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Total genomic DNA was extracted following the miniprep procedure of Doyle & Doyle (1987) as modified by Soltis et al. (1991) and Cullings (1992), from silica gel-dried leaves collected in the field or from fresh leaves of plants cultivated in the Botanic Institute of Barcelona. In some cases, herbarium material was used.

cpDNA *trnL-trnF* REGION STRATEGIES

The plastid *trnL-trnF* region includes the *trnL* intron, the 3' *trnL* (UAA) exon, and the intergenic spacer between *trnL* (UAA) and *trnF* (GAA), that were amplified and sequenced together. Universal primers *trnL-c*, forward, and *trnL-f*, reverse (Taberlet et al., 1991), were used for amplifying the *trnL-trnF* region. In some cases, *trnL-d*, reverse, and *trnL-e*, forward, were used. Polymerase chain reaction (PCR) was conducted in a thermocycler (MJ Research PTC 100). The PCR procedure included a warm start at 95°C for 1 minute 35 seconds, followed by 80°C during which the polymerase (Ecotaq, Ecogen S.R.L., Barcelona, Spain) was added, with 34 cycles of 1 min. denaturation at 93°C, 1 min. annealing at 58°C, 1 min. extension at 72°C, and a final 10 min. extension at 72°C.

PCR products were cleaned with a QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and sequenced with the *trnL-c* and *trnL-f* primers. Direct sequencing of the amplified DNA segments was performed using the BigDye Terminator Cycle Sequencing v3.1 (PE Biosystems, Foster City, CA), following the protocol recommended by the manufacturer. Nucleotide sequencing was carried out at the Serveis Científico-Tècnics of the University of Barcelona on an ABI PRISM 3700 DNA analyzer (PE Biosystems, Foster City, CA).

cpDNA *matK* GENE STRATEGIES

We have sequenced the first 1000 base pairs at the 5' end, because this part includes most of the variability in the *matK* plastid gene (Hilu & Liang, 1997). Partial *matK* was amplified by PCR with the primers *trnK*-710 F (Johnson & Soltis, 1995) and AST-1R (Garcia-Jacas et al., 2002). The PCR procedure included a warm start at 94°C for 1 min. 20 sec., followed by 80°C during which the polymerase (Ecotaq, Ecogen S.R.L., Barcelona, Spain) was

Table 2. Origin of the materials, herbaria where the vouchers are deposited, and GenBank accession numbers (new sequences are boldfaced).

Species	Voucher	ITS accession	<i>trnL-trnF</i> accession	<i>matK</i> accession
<i>Acantholepis orientalis</i> Less.	Garcia-Jacas et al., 2002	AY826222	AY772267	AY785086
<i>Acroptilon repens</i> (L.) DC.	Garcia-Jacas et al., 2001	AY826223	AY772268	AY013489
<i>Alfredia acantholepis</i> Kar. & Kir.	Kazakhstan, <i>Susanna</i> 2092 et al. (BC)	AY826224	—	—
<i>Alfredia cernua</i> (L.) Cass.	Garcia-Jacas et al., 2002	AY826225	AY772269	AY013519
<i>Alfredia nivea</i> Kar. & Kir.	Kazakhstan, <i>Susanna</i> 2090 et al. (BC)	AY826226	AY772270	AY785087
<i>Amberboa turanica</i> Iljin	Garcia-Jacas et al., 2001	AY012275, AY012311	—	—
<i>Amphoricarpus autariatus</i> Blečić & Mayer	Serbia & Montenegro, <i>Stevanovic et al.</i> 4.10.91 (BC)	AY826227	AY772271	AY785088
<i>Amphoricarpus exsul</i> O. Schwarz	Turkey, <i>Susanna</i> 2256 et al. (BC)	AY826228	AY772272	AY785089
<i>Arctium lappa</i> L.	Garcia-Jacas et al., 2002	AY826229	AY772273	AY013520
<i>Arctium minus</i> Bernh.	Garcia-Jacas et al., 2002	AY826230	AY772274	AY013521
<i>Atractylis cancellata</i> L.	Garcia-Jacas et al., 2002	AY826231	AY772275	AY013522
<i>Atractylis carduus</i> (Forssk.) Christ.	Garcia-Jacas et al., 2002	AY826232	AY772276	AY013523
<i>Atractylis humilis</i> L.	Garcia-Jacas et al., 2002	AF319052, AF319106	—	—
<i>Atractylodes japonica</i> Koidz. ex Kitam.	Garcia-Jacas et al., 2002	AY826233	AY772277	AY013524
<i>Berardia subacaulis</i> Vill.	Garcia-Jacas et al., 2002	AY826234	AY772278	AY013525
<i>Brachylaena discolor</i> DC.	South Africa, <i>Ortiz</i> 20.3.02 (NBG)	AY826236	AY772280	AY785090
<i>Callicephalus nitens</i> (M. Bieb. ex Willd.) C. A. Mey.	Garcia-Jacas et al., 2001	AY826237	AY772281	AY013492
<i>Cardopatum corymbosum</i> (L.) Pers.	Garcia-Jacas et al., 2002	AY826238	AY772282	AY013526
<i>Carduncellus duvauxii</i> Batt. & Trab.	Vilatersana et al., 2000; Garcia-Jacas et al., 2001	AY826239	AY772283	AY013493
<i>Carduncellus mareoticus</i> (Delile) Hanelt	Vilatersana et al., 2000	AF140480, AF140481	—	—
<i>Carduus carlinoides</i> Gouan	Garcia-Jacas et al., 2002	AY826240	AY772284	AY013527
<i>Carduus defloratus</i> L.	Spain, <i>Garnatje 18</i> (BC)	AY826241	AY772285	AY785091
<i>Carduus pycnocephalus</i> L.	Garcia-Jacas et al., 2002	AF319111, AY013528	—	AY013528
<i>Carlina acanthifolia</i> All.	Garcia-Jacas et al., 2002	AY826242	AY772286	AY013529
<i>Carlina falcata</i> Svent.	Garcia-Jacas et al., 2002	AY826243	AY772287	AY013530
<i>Carlina gummifera</i> (L.) Less.	Garcia-Jacas et al., 2002	AY826244	AY772288	AY013531
<i>Carlina lanata</i> L.	Garcia-Jacas et al., 2002	AY826245	AY772289	AY013532
<i>Carlina macrophylla</i> (Desf.) DC.	Garcia-Jacas et al., 2002	AF319062, AF319116	—	—
<i>Carlina vulgaris</i> L.	Garcia-Jacas et al., 2002	AY826246	AY772290	AY013533
<i>Carthamus creticus</i> L.	Vilatersana et al., 2000	AY826247	AY772291	AY785092
<i>Carthamus leucocaulos</i> Sibth. & Sm.	Vilatersana et al., 2000	AF140460, AF140461	—	—
<i>Carthamus oxyacantha</i> M. Bieb.	Vilatersana et al., 2000; Garcia-Jacas et al., 2001	AY826248	AY772292	AY013494
<i>Carthamus tinctorius</i> L.	Vilatersana et al., 2000	AF140458, AF140459	—	—
<i>Carthamus turkestanicus</i> Popov	Vilatersana et al., 2000	AY826249	AY772293	AY785093
<i>Centaurea alba</i> L.	Garcia-Jacas et al., 2000, 2001	AY829446	—	AY013495
<i>Centaurea amadanensis</i> Sch. Bip.	Garcia-Jacas et al., 2000	AF058839, AF058864	—	—

Table 2. Continued.

Species	Voucher	ITS accession	<i>trnL-trnF</i> accession	<i>matK</i> accession
<i>Centaurea behen</i> L.	Garcia-Jacas et al., 2000, 2001	AY826250	AY772294	AY013496
<i>Centaurea benedicta</i> L.	France, Nancy Bot. Gard. (BC)	AF058850, AF058875	—	AY013508
<i>Centaurea bruguierana</i> (DC.) Hand.-Mazz.	Garcia-Jacas et al., 2000, 2001	AY826251	AY772295	AY013497
<i>Centaurea calcitrapa</i> L.	Egypt, <i>Susanna 1866</i> & <i>Vilatersana</i> (BC)	AY826252	—	—
<i>Centaurea carolipauana</i> Fern. Casas & Susanna	Garcia-Jacas et al., 2001	AY826253	AY772296	AY013498
<i>Centaurea cyanus</i> L.	Spain, <i>Garcia-Jacas</i> & <i>Susanna 2076</i> (BC)	AY826254	—	—
<i>Centaurea depressa</i> M. Bieb.	Garcia-Jacas et al., 2001	AY826255	AY772297	AY013499
<i>Centaurea involucrata</i> Desf.	Susanna et al., 1995; Garcia-Jacas et al., 2001	AY826256	AY772298	AY013503
<i>Centaurea kotschy</i> (Boiss. & Heldr.) Hayek	Garcia-Jacas et al., 2000	AY829441	—	—
<i>Centaurea lagascana</i> Graells	Garcia-Jacas et al., 2001	AY826257	AY772299	AY013504
<i>Centaurea lingulata</i> Lag.	Garcia-Jacas et al., 2000, 2001	AY826258	AY772300	AY013505
<i>Centaurea linifolia</i> L.	Garcia-Jacas et al., 2000	AF058832, AF058857	—	—
<i>Centaurea macrocephala</i> Muss.- Puschk.	Susanna et al., 1995	L35873	—	—
<i>Centaurea montana</i> L.	Susanna et al., 1995	L35887	—	—
<i>Centaurea polyacantha</i> Willd.	Susanna et al., 1995	L35878	—	—
<i>Centaurea rhizantha</i> C. A. Mey.	Garcia-Jacas et al., 2000	AF058842, AF058867	—	—
<i>Centaurothamnus maximus</i> Wagenitz & Dittrich	Garcia-Jacas et al., 2001	AY826259	AY772301	AY013506
<i>Chardinia orientalis</i> (L.) O. Kuntze	Garcia-Jacas et al., 2002	AY826260	AY772302	AY013534
<i>Cheirolophus benoitii</i> (Humbert) Holub	Susanna et al., 1999	AF045415, AF079942	—	—
<i>Cheirolophus mauritanicus</i> (Font Quer) Susanna	Susanna et al., 1999	AY826261	AY772303	AY013507
<i>Cheirolophus sempervirens</i> (L.) Pomel	Susanna et al., 1999	AF021156, AF021173	—	—
<i>Cheirolophus teydis</i> (C. Smith) G. López	Susanna et al., 1995	AY826262	AY772304	AY785094
<i>Cirsium arvense</i> (L.) Scop.	Susanna et al., 1995	L35867	—	—
<i>Cirsium echinus</i> (M. Bieb.) Hand.-Mazz.	Garcia-Jacas et al., 2002	AY826263	AY772305	AY013535
<i>Cirsium ochrolepidium</i> Juz.	Uzbekistan, <i>Susanna</i> <i>2048 et al.</i> (BC)	AY826264	AY772306	AY785095
<i>Cirsium palustre</i> (L.) Scop.	Garcia-Jacas et al., 2002	AY826265	AY772307	AY013536
<i>Cousinia alberti</i> Regel & Schmalh.	Susanna et al., 2003	AY373721, AY373688	—	—
<i>Cousinia arachnoidea</i> Fisch. & C. A. Mey.	Susanna et al., 2003	AY373722, AY373689	—	—
<i>Cousinia astracanica</i> (Spreng.) Tamamsch.	Susanna et al., 2003	AY826266	AY772308	AY373670
<i>Cousinia caespitosa</i> C. Winkl.	Susanna et al., 2003	AY373724, AY373691	—	—
<i>Cousinia canescens</i> DC.	Garcia-Jacas et al., 2002	AF319068, AF319122	—	—

Table 2. Continued.

Species	Voucher	ITS accession	<i>trnL-trnF</i> accession	<i>matK</i> accession
<i>Cousinia chrysanth</i> Kult.	Susanna et al., 2003	AY373725, AY373692	—	—
<i>Cousinia congesta</i> Bunge	Susanna et al., 2003	AY373726, AY373693	—	—
<i>Cousinia coronata</i> Franch.	Susanna et al., 2003	AY826267	AY772309	AY373662
<i>Cousinia dissecta</i> Kar. & Kir.	Susanna et al., 2003	AY373728, AY373695	—	—
<i>Cousinia esfandiarrii</i> Rech. f. & Aellen	Garcia-Jacas et al., 2002	AF319069, AF319123	—	AY013537
<i>Cousinia grandifolia</i> Kult.	Susanna et al., 2003	AY826268	AY772310	AY373679
<i>Cousinia karatavica</i> Regel & Schmalh.	Susanna et al., 2003	AY373732, AY373699	—	—
<i>Cousinia lappacea</i> Bunge	Susanna et al., 2003	AY826269	AY772311	AY373677
<i>Cousinia microcarpa</i> Boiss.	Susanna et al., 2003	AY826270	AY772312	AY373667
<i>Cousinia neubaueri</i> Rech. f. [= <i>Tiarocarpus neubaueri</i> (Rech. f.) Rech. f.]	Afghanistan, Dieterle 1410 (W)	AY826271	AY772376	AY785096
<i>Cousinia onopordioides</i> Ledeb.	Garcia-Jacas et al., 2002	AF319070, AF319124	—	—
<i>Cousinia pallidivirens</i> Kult. [= <i>Anura pallidivirens</i> (Kult.) Tschern.]	Uzbekistan, Botschantzev 20.6.71 (LE)	AY826272	—	—
<i>Cousinia platylepis</i> Schrenk	Susanna et al., 2003	AY373737, AY373704	—	—
<i>Cousinia polycephala</i> Rupr.	Susanna et al., 2003	AY826273	AY772313	AY373668
<i>Cousinia purpurea</i> C. A. Mey.	Susanna et al., 2003	AY826274	AY772314	—
<i>Cousinia syrdariensis</i> Kult.	Susanna et al., 2003	AY373741, AY373708	—	—
<i>Cousinia tianshanica</i> Kult.	Susanna et al., 2003	AY373743, AY373710	—	—
<i>Cousinia triflora</i> Schrenk	Susanna et al., 2003	AY826275	AY772315	AY373675
<i>Cousinia umbrosa</i> Bunge	Susanna et al., 2003	AY826276	AY772316	AY373676
<i>Cousiniopsis atractylodes</i> (C. Winkl.) Nevski	Garcia-Jacas et al., 2002	AF319071, AF319125	AY772317	AY785097
<i>Crocodylium creticum</i> (Boiss. & Heldr.) N. Garcia & Susanna	Garcia-Jacas et al., 2001 [as <i>Aegialophila cretica</i> (L.) Boiss. & Heldr.]	AY826278	AY772318	AY013490
<i>Crocodylium pumilum</i> (L.) N. Garcia & Susanna	Garcia-Jacas et al., 2001 [as <i>Aegialophila pumilio</i> (L.) Boiss. & Heldr.]	AY012272, AY012308	—	—
<i>Crocodylium syriacum</i> Cass.	Garcia-Jacas et al., 2001 [as <i>Centaurea crocodylium</i> L.]	AY826279	AY772319	AY785098
<i>Crupina crupinastrum</i> (Moris.) Vis.	Susanna et al., 1995	L35884	—	—
<i>Crupina vulgaris</i> Cass.	Garcia-Jacas et al., 2001	AY826280	AY772320	—
<i>Cynara cornigera</i> Lind.	Garcia-Jacas et al., 2002	AY826281	AY772321	AY013538
<i>Cynara humilis</i> L.	Portugal, Blanqué 8 (BC)	AY826282	AY772322	AY785099
<i>Dolomiaea</i> unsp. sp. (<i>D. tibetica</i> in GenBank)	Liu (unpubl.)	AY366334	AY330342	—
<i>Echinops niveus</i> Wall.	Garnatje et al., 2005	AY538634	AY772323	AY785100
<i>Echinops persicus</i> Stev. & Fisch.	Garnatje et al., 2005	AY538639	AY772324	AY785101
<i>Echinops ritro</i> L.	Garcia-Jacas et al., 2002	AF319074, AF319128	—	—
<i>Echinops spinosissimus</i> Turra	Garcia-Jacas et al., 2002	AF319075, AF319129	—	AY013539
<i>Echinops tschimganicus</i> B. Fedtsch.	Garnatje et al., 2005	AY538633	AY772325	AY785102
<i>Echinops viscosus</i> DC.	Garcia-Jacas et al., 2002	AY826283	AY772326	AY013540

Table 2. Continued.

Species	Voucher	ITS accession	<i>trnL-trnF</i> accession	<i>matK</i> accession
<i>Femeniasia balearica</i> (J. J. Rodr.) Susanna	Garcia-Jacas et al., 2001	AY826284	AY772327	AY013509
<i>Galactites tomentosa</i> Moench	Garcia-Jacas et al., 2002	AY826285	AY772328	AY013541
<i>Hypacanthium echinopifolium</i> (Bornm.) Juz.	Susanna et al., 2003	AY826286	AY772329	—
<i>Jurinea albicaulis</i> Bunge	Susanna et al., 2003	AY826287	AY772330	AY373684
<i>Jurinea berardioides</i> (Boiss.) O. Hoffm.	Pakistan, <i>Rechinger 28456</i> (W) [as <i>Aegopordon</i> <i>berardioides</i> Boiss.]	AY826288	AY772331	—
<i>Jurinea carduiiformis</i> Boiss.	Garcia-Jacas et al., 2002 [as <i>Outreya carduiiformis</i> Jaub. & Spach]	AY826289	AY772332	AY785103
<i>Jurinea humilis</i> (Desf.) DC.	Susanna et al., 1995	L35868	—	—
<i>Jurinea lanipes</i> Rupr.	Susanna et al., 2003	AY373748, AY373715	—	—
<i>Jurinea macrocephala</i> DC.	Garcia-Jacas et al., 2002	AF319081, AF319135	—	—
<i>Jurinea modesta</i> Boiss. 2002 [= <i>Hyalochaete modesta</i> (Boiss.) Dittrich & Rech. f.]	Garcia-Jacas et al., 2002	AF319080, AF319134	—	—
<i>Jurinea moschus</i> (Habl.) Bobrov [= <i>Jurinella moschus</i> (Habl.) Bobrov]	Garcia-Jacas et al., 2002	AF319083, AF319137	—	—
<i>Jurinea robusta</i> Schrenk	Susanna et al., 2003	AY826291	AY772333	—
<i>Jurinea</i> sp. ined. 2 [= <i>Modestia</i> <i>darwasica</i> (C. Winkl.) Kharadze & Tamamsch.]	Tadjikistan, <i>Bubanov 27.9.61</i> (LE)	AY826290	—	—
<i>Jurinea</i> sp. ined. 1	Garcia-Jacas et al., 2002	AF319082, AF319136	—	AY013543
<i>Jurinea stoechadifolia</i> (Bieb.) DC.	Ukraine, <i>Romo 10321 et al.</i> (BC)	AY826292	—	—
<i>Jurinea suffruticosa</i> Regel	Kazakhstan, <i>Susanna 2186</i> <i>et al.</i> (BC)	AY826293	—	—
<i>Karvandarina aphylla</i> Rech. f., Aellen & Esfand.	Iran, <i>Soják 8379</i> (W)	AY826294	—	—
<i>Klasea serratuloides</i> (DC.) Greuter & Wagenitz	Garcia-Jacas et al., 2001	AY826295	AY772334	AY013514
<i>Lamyropappus schakaptaricus</i> (B. Fedtsch.) Knorr. & Tamamsch.	Kyrgyzistan, <i>Poljakov 29.8.53</i> (LE)	AY826296	AY772335	AY785104
<i>Lamyropsis cynaroides</i> (Lam.) Dittrich	Turkey, <i>Susanna 2267 et al.</i> (BC)	AY826297	AY772336	AY785105
<i>Leuzea conifera</i> (L.) DC.	Garcia-Jacas et al., 2001	AY826298	AY772337	—
<i>Mantisalca salmantica</i> (L.) Briq. & Cavill.	Garcia-Jacas et al., 2001	AY012292, AY012328	—	—
<i>Myopordon aucheri</i> Boiss.	Iran, <i>Carls s.n.</i> (W)	AY826299	AY772338	—
<i>Myopordon hyrcanum</i> (Bornm.) Wagenitz	Iran, <i>Koelz 16395</i> (W)	AY826300	AY772339	—
<i>Myopordon persicum</i> Boiss.	Iran, <i>Remandieri s.n.</i> (W)	AY826301	—	—
<i>Nikitinia leptoclada</i> (Bornm. & Sint.) Iljin	Turkmenistan, <i>Markova</i> 3.8.54 (LE)	AY829442	—	—
<i>Notobasis syriaca</i> (L.) Cass.	Garcia-Jacas et al., 2002	AY826302	AY772340	AY013545
<i>Oldenburgia intermedia</i> Bond.	South Africa, <i>Ortiz 3.4.02</i> (NBG)	AY826303	AY772341	AY785106
<i>Olgaea baldschuanica</i> (C. Winkl.) Iljin	Tadjikistan, <i>Kamelin et al.</i> 31.5.86 (LE)	AY826304	AY772342	AY785107

Table 2. Continued.

Species	Voucher	ITS accession	<i>trnL-trnF</i> accession	<i>matK</i> accession
<i>Olgaea pectinata</i> Iljin	Kazakhstan, <i>Susanna 2187 et al.</i> (BC)	AY826305	AY772343	AY785108
<i>Oligochaeta divaricata</i> (Fisch. & C. A. Mey.) K. Koch	Garcia-Jacas et al., 2001	AY826306	AY772344	—
<i>Oligochaeta minima</i> (Boiss.) Briq.	Uzbekistan, Tashkent Bot. Gard. (BC)	AY826307	AY772345	—
<i>Onopordum leptolepis</i> DC.	Garcia-Jacas et al., 2002	AF319086, AF319140	—	AY013547
<i>Onopordum nervosum</i> Boiss.	France, Dijon Bot. Gard. (BC)	AY826308	AY772346	AY785109
<i>Onopordum tauricum</i> Willd.	Germany, Berlin Bot. Gard. (BC)	AY826309	AY772347	AY785110
<i>Phonus arborescens</i> (L.) G. López	Vilatersana et al., 2000 [as <i>Carthamus arborescens</i>]	AF140444, AF140445	—	—
<i>Phonus riphaeus</i> (Font Quer & Pau) G. López	Vilatersana et al., 2000 [as <i>Carthamus riphaeus</i>]	AY826310	AY772348	AY013512
<i>Picnomon acarna</i> (L.) Cass.	Garcia-Jacas et al., 2002	AY826311	AY772349	AY013549
<i>Plagiobasis centauroides</i> Schrenk	Kazakhstan, <i>Susanna 2130 et al.</i> (BC)	AY826312	—	—
<i>Polytaxis lehmanii</i> Bunge	Tadjikistan, <i>Kamelin et al.</i> 23.4.86 (LE)	AY826313	AY772350	—
<i>Polytaxis winkleri</i> Iljin	Tadjikistan, <i>Botschantzev et al.</i> 27.4.82 (LE)	AY826314	—	—
<i>Psephellus dealbatus</i> (Willd.) K. Koch	Susanna et al., 1995 [as <i>Centaurea dealbata</i>]	L35886	—	—
<i>Psephellus gilanicus</i> (Bornm.) Wagenitz	Garcia-Jacas et al., 2001 [as <i>Centaurea gilanica</i>]	AY826315	AY772351	AY013501
<i>Psephellus incanescens</i> (DC.) Boiss.	Garcia-Jacas et al., 2001 [as <i>Centaurea incanescens</i>]	AY012283, AY012319	—	—
<i>Psephellus persicus</i> (DC.) Wagenitz	Garcia-Jacas et al., 2001 [as <i>Centaurea gaubae</i>]	AY826316	AY772352	AY013500
<i>Psephellus pulcherrimus</i> (Willd.) Wagenitz	Garcia-Jacas et al., 2001 [as <i>Aetheopappus pulcherrimus</i>]	AY826317	AY772353	AY013491
<i>Psephellus xantocephalus</i> (DC.) Fisch. & C. A. Mey.	Garcia-Jacas et al., 2001 [as <i>Centaurea xantocephala</i>]	AY829445	—	—
<i>Ptilostemon afer</i> (Jacq.) Greuter	Garcia-Jacas et al., 2002	AY826318	AY772354	AY785111
<i>Ptilostemon diacantha</i> (Labill.) Greuter	Turkey, <i>Susanna 2313 et al.</i> (BC)	AY826319	AY772355	AY785112
<i>Ptilostemon echinocephalus</i> (Willd.) Greuter	Ukraine, <i>Romo 10365 et al.</i> (BC)	AY829442	—	—
<i>Ptilostemon hispanicus</i> (Lam.) Greuter	Spain, <i>Mateos & García-García</i> , 18.9.98 (BC).	AY829444	—	—
<i>Rhaponticoides africana</i> (Lam.) M. V. Agab. & Greuter	Susanna et al., 1995	L35863	—	—
<i>Rhaponticoides hajastana</i> (Tzvel.) M. V. Agab. & Greuter	Garcia-Jacas et al., 2001	AY826235	AY772279	AY013502
<i>Rhaponticum acaule</i> DC.	Susanna et al., 1995	AY826334	AY772369	AY013515
<i>Rhaponticum australe</i> (Gaud.) Soskov	Australia, <i>Funk s.n.</i> (BC)	AY826335	AY772370	AY785120
<i>Russowia sogdiana</i> (Bunge) B. Fedtsch.	Tadjikistan, <i>Botschantzev</i> 5.5.75 (LE)	AY826320	—	—
<i>Saussurea alpina</i> (L.) DC.	Garcia-Jacas et al., 2002	AF319091, AF319145	—	—
<i>Saussurea asbukinii</i> Iljin [= <i>Frolovia asbukinii</i> (Iljin) Lipsch.]	Tadjikistan, <i>Kamelin</i> 24.6.70 (LE)	AY826321	AY772356	—

Table 2. Continued.

Species	Voucher	ITS accession	<i>trnL-trnF</i> accession	<i>matK</i> accession
<i>Saussurea carducephala</i> (Iljin) Iljin [= <i>Lipschitzia carducephala</i> (Iljin) Kamelin]	Tadjikistan, <i>Smakov</i> & <i>Dengubianko</i> 6.8.86 (LE)	AY826322	AY772357	—
<i>Saussurea ceratocarpa</i> Decne. [= <i>Lipschitzia ceratocarpa</i> (Decne.) Kamelin]	Raab-Straube, 2003	AJ606170, AJ606210	AJ606138	—
<i>Saussurea deltoidea</i> (DC.) Sch. Bip. [= <i>Himalaiella deltoidea</i> (DC.) Raab-Straube]	Raab-Straube, 2003	AJ606169, AJ606209	AJ606137	—
<i>Saussurea discolor</i> (Willd.) DC.	Garcia-Jacas et al., 2002	AF319092, AF319146	—	—
<i>Saussurea elegans</i> Ledeb.	Susanna et al., 2003	AY826323	AY772358	—
<i>Saussurea frolovii</i> Ledeb. [= <i>Frolovia</i> <i>frolovii</i> (Ledeb.) Raab-Straube]	Raab-Straube, 2003	AJ606171, AJ606211	—	—
<i>Saussurea maximowiczii</i> Herder	Susanna et al., 2003	AY826324	AY772359	—
<i>Schischkinia albispina</i> (Bunge) Iljin	Garcia-Jacas et al., 2001	AY826325	AY772360	AY785113
<i>Schmalhausenia nidulans</i> (Regel) Petr.	Susanna et al., 2003	AY826326	AY772361	AY373681
<i>Serratula coronata</i> L.	Garcia-Jacas et al., 2002	AY826327	AY772362	AY785114
<i>Siebera pungens</i> (Lam.) DC.	Turkey, <i>Susanna 2316 et al.</i> (BC)	AY826328	AY772363	AY785115
<i>Silybum marianum</i> (L.) Gaertner	Garcia-Jacas et al., 2002	AY826329	AY772364	AY013551
<i>Staelia baetica</i> DC.	Garcia-Jacas et al., 2002	AF319095, AF319149	—	—
<i>Staelia dubia</i> L.	France, <i>Garnatje 25 & Luque</i> (BC)	AY826330	AY772365	AY785116
<i>Staelia fruticosa</i> L.	Greece, Kriti, <i>Garnatje 147 &</i> <i>Luque</i> (BC)	AY826331	AY772366	AY785117
<i>Staelia lobelia</i> DC.	Turkey, <i>Susanna et al. 2272</i> (BC)	AY826332	AY772367	AY785118
<i>Staelia uniflorescens</i> Sibth. & Sm.	Greece, <i>Raus & Royle 5479</i> , Berlin Bot. Gard. (BC)	AY826333	AY772368	AY785119
<i>Stizolophus balsamita</i> (Lam.) Cass. ex Takht.	Garcia-Jacas et al., 2001	AY826336	AY772371	AY785121
<i>Stizolophus coronopifolius</i> Cass.	Garcia-Jacas et al., 2001	AY826337	AY772372	AY013516
<i>Synurus palmatopinnatifidus</i> (Makino) Kitam.	Garcia-Jacas et al., 2002	AY826338	AY772373	AY013552
<i>Syreitschikovia spinulosa</i> (Franch.) Paylov	Kazakhstan, <i>Susanna</i> <i>2200 et al.</i> (BC)	AY826339	AY772374	AY785122
<i>Tarchonanthus camphoratus</i> L.	South Africa, <i>Ortiz 17.3.02</i> (NBG)	AY826340	AY772375	AY785123
<i>Tricholepis tibetica</i> Hook. f. & Thomson	Pakistan, <i>Nüsser 1055</i> (B)	AY826341	—	—
<i>Tugarinovia mongolica</i> Iljin	Mongolia, <i>Trubov et al. 1.7.89</i> (LE)	AY826342	AY772377	AY785124
<i>Tyrinnus leucographus</i> (L.) Cass.	Garcia-Jacas et al., 2002	AY826343	AY772378	AY013554
<i>Volutaria crupinoides</i> (Desf.) Maire	Garcia-Jacas et al., 2001	AY826344	AY772379	AY785125
<i>Xeranthemum annuum</i> L.	Turkey, <i>Susanna 2362 et al.</i> (BC)	AY826345	AY772380	AY785126
<i>Xeranthemum cylindraceum</i> Sm.	Denmark, Copenhagen Bot. Gard. (BC)	AY826346	—	—
<i>Xeranthemum inapertum</i> (L.) Miller	Garcia-Jacas et al., 2002	AY826347	AY772381	AY013555
<i>Xeranthemum longepapposum</i> Fisch. & C. A. Mey.	Kazakhstan, <i>Susanna 2182</i> <i>et al.</i> (BC)	AY826348	AY772382	AY785127

Table 2. Continued.

Species	Voucher	ITS accession	<i>trnL-trnF</i> accession	<i>matK</i> accession
<i>Zoegea baldschuanica</i> C. Winkl.	Uzbekistan, <i>Khassanov s.n.</i> (BC)	AY012305, AY012341	—	—
<i>Zoegea lepturea</i> L.	Garcia-Jacas et al., 2000 [as <i>Z. mianensis</i>]	AY826349	AY772383	AY013517

added, and 40 cycles of 45 sec. denaturation at 94°C, 1 min. annealing at 58°C, 2 min. extension at 72°C, and a final 10 min. extension at 72°C. PCR products were cleaned with QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA) and sequenced with *trnK*-710 F and AST-1R primers. Direct sequencing of the amplified DNA segments was performed as for the *trnL-trnF* region.

nrDNA ITS REGION STRATEGIES

The three nuclear ITS1 spacer, 5.8 S gene, and ITS2 spacer (the ITS region) were amplified and sequenced together. The ITS region was amplified by PCR with 1406 F (Nickrent et al., 1994) and ITS1 (White et al., 1990) as forward primers, and ITS4 (White et al., 1990) as reverse primer, referring to the protocol described in Soltis and Kuzoff (1993). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc., Valencia, CA). Sequencing primers 1406 F and ITS4 were used. Direct sequencing of the amplified DNA segments was performed as for the *trnL-trnF* region.

PHYLOGENETIC ANALYSIS

Nucleotide sequences were edited with Chromas 1.56 (Technelysium, Tewantin, Australia). The *trnL-trnF* and *matK* sequences were aligned visually by sequential pairwise comparison (Swofford & Olsen, 1990). The *matK* sequences were translated for their putative proteins with GeneJockey (Biosoft, Cambridge, U.K.) to verify the absence of internal stop codons among those for amino acid codons. Due to the high level of variability of the ITS sequences, our alignment was checked with the ITS alignment for the whole Compositae by Goertzen et al. (2003) and adjusted manually. In order to conserve the phylogenetic information of insertions and deletions that constituted most of the variation of the *trnL-trnF* region, and at the same time avoid an overestimation of lengthy indels, they were coded as presence-absence characters and added to the end of matrices in the combined analyses. The aligned data matrices are available on request from the corresponding author.

The ITS matrix was analyzed by Bayesian inference, because heuristic parsimony search was impossible due to the size of the data matrix (190 species; the search for most-parsimonious trees was too time-consuming and soon became unpractical). Bayesian inference (BI) estimation was calculated using MrBayes 3.01 (Ronquist & Huelsenbeck, 2003). The best-available model of molecular evolution, required for Bayesian estimations of phylogeny, was selected using hierarchical likelihood ratio tests (hLRT) and Akaike information criteria (AIC) as implemented in the software MrModeltest 1.1b (Nylander, 2002), which considers only nucleotide substitution models that are currently implemented in PAUP and MrBayes 3.01 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The best-fit model of nucleotide substitution for the ITS dataset was the same in both methods: the symmetrical model, with some sites assumed to be invariable and variable sites assumed to follow a discrete gamma distribution (GTR+I+G; Yang, 1996). Bayesian inference analyses were initiated with random starting trees and were run for 1×10^6 generations. Four Metropolis-coupled Markov chain Monte Carlo (MCMC) chains were sampled every 100 generations, which resulted in 10,000 sample trees. A critical aspect of the Bayesian analysis is to ensure that the Markov chain has reached stationarity. All sample points prior to stationarity are essentially random and are discarded as “burn-in” 1,000 samples trees, because they do not contain useful parameter estimates. Internodes with posterior probabilities $\geq 95\%$ were considered statistically significant. A majority-rule consensus tree was calculated with PAUP version 4.0b4a (Swofford, 1999). Posterior probability support (PP) was estimated to be significant for nodes with $PP > 0.95$.

For the combined data sets, parsimony analysis involved heuristic searches conducted with PAUP version 4.0b10 (Swofford, 1999) using Tree Bisection Recognition (TBR) branch swapping with character states specified as unordered and unweighted. All most parsimonious trees (MPT) were saved. To locate islands of most parsimonious trees (Maddison, 1991), we performed 100 replicates with random taxon addition, and with TBR branch swapping. Tree lengths, consistency index (CI), and retention index

Table 3. Comparison of results from the ITS, ITS + *trnL-trnF*, and ITS + *trnL-trnF* + *matK* data sets. The consistency and homoplasy indices are calculated by excluding uninformative characters.

Data set	ITS	ITS + <i>trnL-trnF</i>	ITS + <i>trnL-trnF</i> + <i>matK</i>
Number of taxa	190	121	111
Total characters	519	1699	2708
Informative characters	390	493	677
Number of MPTs	—	298	3122
Number of steps	—	2970	3288
Islands	—	8	5
Consistency index (CI)	—	0.2914	0.3259
Retention index (RI)	—	0.6800	0.6783
Homoplasy index (HI)	—	0.7086	0.6741
Range of divergence, ingroup (%)	0–5	0.13–2.43	0.12–1.8

(RI) are always given, excluding uninformative characters. Two combined analyses were performed, with different data sets: the ITS + *trnL-trnF* sequence data and the ITS + *trnL-trnF* + *matK* data.

Bootstrap (BS) and Bremer support (Bremer, 1988; Donoghue et al., 1992) or decay index (DI) were carried out to obtain support estimates for the nodes in the consensus trees. Bootstrap analysis was performed (Felsenstein, 1985) using 1000 replicates and heuristic search with the default options. In the nrDNA ITS data matrix, we used the approach by Lidén et al. (1997) using 1000 replicates, random taxon addition with 20 replicates, and no branch swapping. For the two combined matrices, DI was calculated for each node by successive analyses using the clade constraint approach, as discussed in Morgan (1997), with 10 replicates. ACCTRAN (accelerated transformation) character-state optimization was used for all illustrated trees.

RESULTS AND DISCUSSION

Since we were unable to obtain DNA sequences for all three genic regions for every taxon sampled, we performed three distinct analyses: (1) ITS alone, to examine the position of some genera not included in previous analyses and for which we were not able to amplify any chloroplast region; (2) ITS and *trnL-trnF* regions combined, to study the generic limits in the *Saussurea* group; and (3) the three regions (ITS, *trnL-trnF*, and *matK*) combined, to elucidate subtribal limits and to confirm the naturalness of the informal groups in Carduinae. The numeric results of the three analyses are summarized in Table 3. The resulting trees are shown in Figure 1A and 1B (Bayesian majority rule consensus for the ITS dataset alone), Figure 2 (parsimony strict consensus of the combined ITS and *trnL-trnF* sequences), and Figure 3 (parsimony strict consensus of the combined ITS, *trnL-trnF* and *matK* sequences). The Bayesian majority rule

consensus trees of the two combined data sets are largely coincident with the parsimony consensus trees, and therefore we have added the Bayesian support (PP) to those branches that have PP > 0.95 but are not, or are only weakly, supported by parsimony (Figs. 2, 3). Only the Carlininae branch in the combined Bayesian analysis of the three regions is illustrated (Fig. 4), because it confirms the position of *Tugarinovia* within Carlininae.

DELINEATION OF CARDUEAE

The monophyly of Cardueae was confirmed with the new outgroup in all the analyses with high statistical support: PP = 1.00 (Fig. 1A), BS = 100%, 100% (Figs. 2, 3), and DI = 11 (Figs. 2, 3). Thus, the most appropriate status for Echinopinae and Carlininae is subtribal. Indeed, Cardueae could be divided into five tribes, but we consider it impractical to fragment a natural group that can be so easily recognized on the basis of macromorphology.

SUBTRIBAL CLASSIFICATION

The four subtribes recognized by the latest report on the tribe (Garcia-Jacas et al., 2002), Carlininae, Echinopinae, Carduinae, and Centaureinae, were confirmed. Subtribe Cardopatiinae must be restored and some changes made to correlate molecular phylogeny and subtribal delineation. However, in view of the moderate support for these basal branches (they collapse in a polytomy in all the Bayesian analyses, cf. Fig. 1A), subtribes Carlininae, Echinopinae, and Cardopatiinae should be considered a currently unresolved polytomy basal to Carduinae–Centaureinae.

CARLININAE AND TUGARINOVIA

Our results do not modify the circumscription of Carlininae in our latest surveys of Cardueae (Garcia-

Figure 1. (pp. 162–163).—A (p. 162). Bayesian majority rule consensus tree of the ITS sequence data matrix (basal part of the tree only). Numbers above branches are Bayesian Posterior Probabilities (PP). CARDOPAT = Cardopatiinae. —B (p. 163). Bayesian majority rule consensus tree of the ITS sequence data matrix (upper part of the tree only). Numbers above branches are Bayesian Posterior Probabilities (PP).

genera that were classified by other authors (Dittrich, 1977, 1996b; Bremer, 1994) in Carlininae belong either to Carduinae (*Staelhina* and the *Xeranthemum* group) or Cardopatiinae (*Cardopatium* and *Cousiniopsis*), based on molecular phylogenies as suggested by the sequence data. The classic definition of Carlininae was based mainly on achene characters (Dittrich, 1977, 1996b): parenchymatic pericarp usually hir-

Figure 2. Strict consensus tree of the most parsimonious trees resulting from the ITS and *trnL-trnF* combined data matrix. Bolded numbers are Bayesian Posterior Probabilities (PP) which are detailed only for branches unsupported by parsimony bootstrap. Numbers above branches or before a slash are bootstrap percentages (BS); below branches or after a slash, decay indices (DI). A dash indicates bootstrap support less than 50%. Abbreviations of subtribes: CL = Carlininae; CP = Cardopatiinae; ECH = Echinopinae.

Figure 3. Strict consensus tree of the most parsimonious trees resulting from the ITS, trnL-trnF, and matK combined data matrix. Bolded numbers are Bayesian Posterior Probabilities (PP) detailed only for branches unsupported by parsimony bootstrap. Numbers above branches or below branches are bootstrap percentages (BS); below branches or after a slash, decay indices (DI). A dash indicates bootstrap support less than 50%. Abbreviations of subtribes: CL = Carlininae; CP = Cardopatiinae; ECH = Echinopinae.

Figure 4. Detail of the branch of the subtribe Carlininae from the Bayesian majority rule consensus of the ITS, *trnL-trnF*, and *matK* combined data matrix. Numbers above branches are Bayesian Posterior Probabilities (PP).

sute, and pappus setae very long with plumose directly attached to the pericarp. However, these characters must be interpreted as plesiomorphic, because they appear across all basal subtribes (Carlininae, Cardopatiinae, and Echinopinae) and even in Carduinae. If we rely only on achene characters for classification, the resulting definition of Carlininae (Dittrich, 1977, 1996b) differs greatly from the delineation on the basis of DNA sequence analyses and macromorphology (Susanna & Garcia-Jacas, in press).

Our molecular analyses confirm that *Tugarinovia*, a puzzling monotypic genus of dioecious plants from Mongolia that was placed in Carlininae by Dittrich et al. (1987), belongs to Cardueae. Curiously, this subtribal placement is supported only by the Bayesian combined analysis of the three regions (PP = 1.0, Fig. 4). Indeed, the only subtribe where it can be placed on the basis of morphological affinities (leaves, involucre bracts, and pappus) is Carlininae (Dittrich et al., 1987; Susanna & Garcia-Jacas, in press). Our analyses show no connection of *Tugarinovia* with the only other East Asian representative of Carlininae, *Atractylodes*, or with any other genus of the subtribe, reinforcing its isolated position.

CARDOPATIINAE

This subtribe had moderate support in our parsimony analyses (BS = 65%, 81%; DI = 4, 6; Figs. 2, 3), perhaps due to the different evolutionary rates of annual *Cousiniopsis* and perennial *Cardopatum*. The Bayesian support for this branch, however, is very high (PP = 1.00, Figs. 1A, 3, 4).

Subtribe Cardopatiinae, as first defined, included only the east Mediterranean genus *Cardopatum*. Later, Nevski (1937) described a monotypic genus from central Asia, *Cousiniopsis*, closely related to *Cardopatum* (it was first described as *Cardopatum atractylodes* C. Winkler). Classic monographers of Compositae (Bentham, 1873; Hoffmann, 1894; Dittrich, 1977; Bremer, 1994) consistently placed both genera among Carlininae, but the only characters that connect these two groups are those of achenes, which could equally relate *Cardopatum* and *Cousiniopsis* to

Echinopinae. It is tempting to interpret the corymbose inflorescence of *Cardopatum*, formed by very small, few-flowered capitula, as a first step towards syncephaly. On this basis, Petit (1997) considered *Cardopatum* sister to *Echinops* L. and placed *Cardopatum* and *Cousiniopsis* in Echinopinae. On the basis of our results, we prefer to interpret these similarities as convergence, because syncephalies at various states of development involving small, few-flowered heads occur in all the subtribes across Cardueae (Garcia-Jacas et al., 2002).

ECHINOPINAE

Our results demonstrate, contrary to our previous studies (Garcia-Jacas et al., 2002), that Echinopinae include only *Echinops* s.l. (*Echinops* and *Acantholepis* Less.) with strong support (PP = 1.00, Fig. 1B; BS = 100%, DI = 26, 30, Figs. 2, 3). In fact, a recent molecular study indicates that *Acantholepis* is a reduced, unarmed species of *Echinops* (Garnatje et al., 2005), as originally described (*Echinops acantholepis* Jaub. & Spach). Our combined analyses reveal that the *Xeranthemum* group does not belong to Echinopinae, but rather to Carduinae as sister to the rest of this subtribe (Figs. 1A, 2, 3).

The origin of the compound inflorescence of *Echinops* cannot be tracked on molecular grounds, because the subtribe does not show supported affinity to any other group in Cardueae. Cardopatiinae and Carlininae are the best candidates for sister groups to the subtribe Echinopinae (because the structure of the achenes is very similar, cf. Dittrich, 1977).

CARDUINAE

If monophyletic Centaureinae are recognized as a distinct subtribe, the Carduinae constitute a paraphyletic assemblage (Figs. 1A, 2, 3). However, alternate solutions are not practical. Either a subtribe level is ascribed to all the monophyletic groups recognized in present Carduinae and a fragmented classification results, or a single large subtribe Carduinae, including Centaureinae, is maintained,

which thereby encompasses almost ninety per cent of the species of the tribe (Garcia-Jacas et al., 2002).

Even in this disparate assemblage, some well-defined groups emerge, together with genera without known affinities like *Berardia* or *Staelina*.

BERARDIA AND STAEHELINA

Our molecular analyses show that these two genera present no obvious affinities. They cluster in an isolated position within Carduinae, in both combined analyses, without statistical support (Figs. 2, 3). *Berardia* was ranked among Mutisieae on the basis of achene characters (Dittrich, 1977) and we agree in that the pericarp cells, with thickened u-shaped walls, are very similar to the type found in Gochnatiinae Benth. & Hook. f., a subtribe of Mutiseae (Dittrich, 1996a). Further support for the, albeit weak, relationship between *Berardia* and *Staelina* is that the pericarp of *Staelina* is also "gochnatioid" (Dittrich, 1996a). However, we cannot state whether this similarity represents convergence or is a very old character conserved in these two anomalous genera.

Staelina was previously placed among Carlininae (Bentham, 1873; Hoffmann, 1894; Dittrich, 1977; Bremer, 1994), but Petit (1997) proposed moving it to Carduinae. For Dittrich (1996b), the two species of *Staelina* with hirsute pericarp (*S. fruticosa* L. and *S. lobelii* DC.) should be classified in a distinct genus, *Hirtellina* Cass. All our analyses grouped the included species of *Staelina* (five out of eight) in a robust clade with very high support (PP = 1.00, Fig. 1A; BS = 100%, DI = 34, 47, Figs. 2, 3). However, both combined analyses divided the genus into two well-supported clades that coincide with *Staelina* s.s. and *Hirtellina* (Figs. 2, 3), which is compatible with the division of the genus. Nevertheless, morphological differences other than presence or absence of achene pilosity are virtually non-existent, and we prefer to keep a single genus with *Staelina* and *Hirtellina* recognized at sectional rank within *Staelina* (Susanna & Garcia-Jacas, in press).

THE XERANTHEMUM GROUP

In a previous study, the *Xeranthemum* group (genera *Amphoricarpus*, *Chardinia* Desf., *Siebera*, and *Xeranthemum*) was placed among subtribe Echinopinae. This unexpected result led us to propose that the very small and peculiar heads of the genera of the group, with very large receptacular bracts, could constitute a syncephaly (Garcia-Jacas et al., 2002). Our new analyses show that this view was erroneous and, in fact, Harris (1995) had already demonstrated

that the inflorescence of *Xeranthemum* was not a syncephaly. Indeed, the *Xeranthemum* group appears, in the combined analyses, as part of the Carduinae, sister to the rest of the subtribe, with low parsimony bootstrap support but very high Bayesian support (PP = 1.00, Figs. 1A, 2, 3), in an isolated position. The monophyly of the group also has very high support (PP = 1.00, Fig. 1A; BS = 100%, DI = 23, 28, Figs. 2, 3). Traditional classification (Dittrich, 1977; Bremer, 1994) placed it in Carlininae, and Petit (1997) was the first to suggest Carduinae. Species of three of the genera of the group (*Chardinia*, *Siebera*, and *Xeranthemum*) are annual colonizers of arid and waste-land throughout the Mediterranean region. In contrast, species of the dwarf shrubby genus *Amphoricarpus* are narrow mountain endemics, sister to the rest of the genera of the *Xeranthemum* group in all the analyses (Figs. 1A, 2, 3).

THE ONOPORDUM GROUP

The usual definition of this group is based on the absence of receptacular bracts. A pitted, naked receptacle is otherwise rare in the tribe. However, not all the species of at least one genus (*Alfredia* Cass.) show an epaleate receptacle. In addition to this character, achenes are also peculiar with pericarp diversely pitted, wrinkled, or rugulose (Susanna & Garcia-Jacas, in press), but seldom smooth (*Olgaia* and *Syreitschikovia*). The group has considerable negative importance, because species of *Onopordum* include some highly noxious weeds widespread in the Mediterranean region and the American west like *O. acanthium* L. and *O. nervosum* Boiss., giant thistles that can reach up to 3 m high.

Three genera not included in previous studies, *Lamyropappus*, *Olgaia*, and *Syreitschikovia*, are classified in the *Onopordum* clade in all three analyses, which confirm the group as a natural one with significant support (PP = 1.00, Fig. 1A; BS = 91%, 97%, DI = 6, 4, Figs. 2, 3). *Syreitschikovia* had been previously placed by Dittrich (1977) and Bremer (1994) in Centaureinae. Its classification in Carduinae and its relationship to the *Onopordum* group was reported by Susanna et al. (2002) on the basis of morphology.

Generic definitions in the group are unclear, with the only exception of *Onopordum*. *Alfredia* forms a polytomy with *Lamyropappus*, *Olgaia*, *Synurus* Iljin, and *Syreitschikovia* (Figs. 1A, 2, 3). The inclusion of more species of *Olgaia*, which comprises some 15 taxa from the Tien Shan mountains of Kyrgyzstan and Tajikistan, and its strange relative *Takeikadzuchia* Kitag. & Kitam. from Mongolia, may contribute to a better definition of the genera in the group.

THE *CARDUUS* GROUP

This represents a large complex of very spiny plants which are usually called thistles. All share medium or large-sized heads, spiny leaves, and a long pappus detachable as a single piece. Our results indicate that a large number of the genera (*Carduus* L., *Cirsium* Mill., *Notobasis* Cass., *Picnomon* Adans., *Silybum* Adans., and *Tyrinnus* Cass.) form a natural group with significant parsimony support (BS = 91%, 100%, DI = 10, 14, Figs. 2, 3). Remaining genera, *Cynara* L., *Galactites* Moench, *Lamyropsis* (Kharadze) Dittrich, and *Ptilostemon* Cass., are also placed in this group in all analyses, but this is only supported by the Bayesian analyses (PP = 1.00 in all the cases, Figs. 1A, 2, 3).

As pointed out by Häffner and Hellwig (1999) and Garcia-Jacas et al. (2002), phylogenetic relationships and generic boundaries within the clade are obscure (Fig. 1A, 2, 3). One of the reasons for this is that the co-existence of annual or biennial species (most of *Carduus*, *Galactites*, *Picnomon*, *Silybum*, or *Tyrinnus*) together with perennials (many *Cirsium*, *Cynara*, *Lamyropsis*, and *Ptilostemon*) hinders the assessment of the two aspects from a molecular standpoint. Differences in mutation rates between annuals and perennials (Gaut et al., 1997; Laroche et al., 1997; Andreasen & Baldwin, 2001) make comparison of DNA sequences a less reliable tool. In fact, unexpected results, like the strange position of the annual genus *Galactites*, could be a result of these differences; *Galactites* is placed close to the base of the thistles in the combined analyses, grouped with *Ptilostemon* (Figs. 2, 3), thereby contradicting morphological evidence (*Galactites* is morphologically similar to *Carduus* or *Cirsium*). *Lamyropsis*, the only genus of the thistles missing in our previous studies and sequenced here for the first time, appears related to *Ptilostemon* in the combined analyses, without support (Figs. 2, 3). Species of *Lamyropsis* have dentate-spiny leaves with very prominent veins beneath, similar to many species of *Ptilostemon*. The affinities between the two genera were pointed out by Dittrich (1971).

Taking into account our low sampling for such an enormous group (ca. 500 species in total), any concluding remark on the thistles would be premature. The *Carduus* group, together with the two following ones, requires a more comprehensive molecular analysis.

THE *ARCTIUM* GROUP

This group has been the subject of a recent preliminary molecular survey, using ITS and *matK*

sequences (Susanna et al., 2003). The results herein, including the *trnL-trnF* region (Figs. 2, 3), do not change our previous main conclusions that the limits of *Arctium* L. and *Cousinia* are unclear. Our study (Susanna et al., 2003) demonstrated two principal clades in the *Arctium* group: the Arctioid clade (supported only by the two combined analyses with BS = 85%, 100%, DI = 5, 7, Figs. 2, 3) and the Cousinioid clade (supported by all three analyses with PP = 0.99, Fig. 1A.; BS = 92%, 94%, DI = 4, 3, Figs. 2, 3). The two groups can be segregated by molecular, chromosome, and pollen characters, but this grouping is not consistent with morphology: two genera of the group, *Schmalhausenia* C. Winkl. and *Hypacanthium* Juz., are affined with *Arctium* on the basis of pollen, chromosomes, and DNA sequences (Figs. 1A, 2), but in other respects are morphologically much closer to *Cousinia*. In addition to an Arctioid species group within *Cousinia*, there is also a Cousinioid group seen in *Arctium*. More sampling of the obscure *Cousinia* subgenus *Hypacanthodes* Tscherneva from Central Asia is required, but it is highly probable that all four genera will have to be grouped in *Arctium*.

Finally, our ITS analysis (Fig. 1A) confirms that the purported genera *Anura* and *Tiarocarpus*, as previously proposed by Susanna and Garcia-Jacas (in press), cannot be segregated from *Cousinia*, to which they are united with good support (PP = 1.00 in both cases, Fig. 1A).

THE *SAUSSUREA* GROUP

The only genera placed in the *Saussurea* group by Susanna and Garcia-Jacas (in press) that were not included in our previous studies are *Dolomiaea* and *Polytaxis*. The ITS and the combined ITS + *trnL-trnF* analyses place *Polytaxis* basal to *Saussurea* with high Bayesian (PP = 0.99, Fig. 1A; PP = 1.00, Fig. 2) and good parsimony support (BS = 87%, DI = 6, Fig. 2). Because species of *Polytaxis* are the only annual taxa in this clade, its basal position could originate in the faster evolution of annuals relative to perennials (Gaut et al., 1997; Laroche et al., 1997; Andreasen & Baldwin, 2001), in the same way that annual *Acantholepis* always appears basal to perennial *Echinops* (Fig. 1B, 2, 3). By its side, *Dolomiaea* is grouped with high support (PP = 1.00, Fig. 1A; BS = 94%, DI = 6, Fig. 2) in the ITS and the combined ITS and *trnL-trnF* analyses with *Frolovia* (*Saussurea asbukinii* Iljin and *S. frolovii* Ledeb.). It seems adequate to consider *Frolovia*, a genus restored by Raab-Staube (2003), a synonym of *Dolomiaea*.

Another taxon that was not included in our previous study is the purported genus *Aegopordon* (*Jurinea*

berardioides (Boiss.) O. Hoffm. in Figs. 1A, 2), which, according to Susanna and Garcia-Jacas (in press), should be considered a synonym of *Jurinea*. The combined ITS + *trnL-trnF* analysis (Fig. 2) place it in a robust clade (BS = 91%, DI = 4) with *Jurinea carduiformis* (Jaub. & Spach) Boiss., formerly also considered a distinct genus (*Outreya* Jaub. & Spach) that we merged in *Jurinea* (Garcia-Jacas et al., 2002).

Our results confirm that the limits between *Jurinea* and *Saussurea* are not well established (as pointed out recently by Kita et al., 2004), because some species formerly included in *Saussurea* are grouped in the genus *Jurinea* (Figs. 1A, 2). *Saussurea carduicephala* (Iljin) Iljin and *S. deltoidea* (DC.) Sch. Bip. were considered by Raab-Staube (2003) as the distinct genus *Himalaiella*. *Saussurea ceratocarpa* Decne. was for Raab-Staube (2003) a restored genus *Lipschitzia*. Both purported genera form a robust clade in the ITS and the ITS + *trnL-trnF* analysis (PP = 1.00, Fig. 1A; BS = 98%, DI = 6, Fig. 2). According to this result, if we grant the genus level to this clade, *Himalaiella* should be considered a synonym of *Lipschitzia*. However, we prefer to consider it a synonym of *Jurinea*, because both purported *Himalaiella* and *Lipschitzia* form a monophyletic clade with *Jurinea* s. str., with very high support (PP = 1.00, Fig. 1A; BS = 100%, DI = 15, Fig. 2).

No final conclusions can be drawn from this entanglement of genera, because our sampling of *Jurinea* was very limited. However, a redefinition of the boundaries between *Jurinea* and *Saussurea* is clearly required. The clarification of these limits, and indeed the description of new genera in a complex in which no less than 15 have been already described (Susanna & Garcia-Jacas, in press), calls for a much more comprehensive sampling than any performed to date.

CENTAUREINAE

Our results confirm the general outline of Centaureinae proposed by Garcia-Jacas et al., 2001, this time on the basis of three regions of the genome (Figs. 1B, 2, 3). Here we describe only the most important results, namely the inclusion of two genera formerly classified in Carduinae, *Myopordon* and *Nikitinia*, in the subtribe.

Myopordon was considered related to *Onopordum* (hence the name) and placed in subtribe Carduinae because of the absence of receptacular setae (Wagenitz, 1958; Dittrich, 1977). In contrast, the ITS analysis relates *Myopordon* to the genus *Oligochaeta* K. Koch of subtribe Centaureinae with very high support (PP = 1.00, Fig. 1B), and the combined ITS + *trnL-trnF* analysis places *Myopordon* deeply nested in

the *Rhaponticum* Vaill. group with high support (BS = 85%, DI = 5, Fig. 2). Difficulties in interpretation of, even apparently unambiguous, characters consistently occur in tribe Cardueae: as we have seen above, the naked receptacle is a supported character of the *Onopordum* group (Susanna & Garcia-Jacas, in press), but there are many exceptions. Epaleate genera are present in almost every subtribe: *Tugarinovia* in Carlininae, *Dolomiaea* and part of the *Onopordum* group in Carduinae, and *Myopordon* and *Russowia* C. Winkl. in Centaureinae. To verify the position of *Myopordon* within Centaureinae on morphological grounds, the characters of the achenes are critical; however, we were unable to find herbarium material with mature fruits. Mouterde (1983) described the insertion areole of the achenes as oblique, a character of Centaureinae. This observation contrasts with that of Wagenitz (1958), who reported the insertion as straight, which therefore points towards Carduinae.

Nikitinia was described in Carduinae, and in recent reviews of the tribe was maintained in that subtribe (Dittrich, 1977; Bremer, 1994). However, achene characters are undoubtedly centauroid (especially the double pappus, illustrated in Susanna et al., 2002) and relate it to the genus *Klasea* Cass. as confirmed by molecular analyses (PP = 1.00, Fig. 1B).

CONCLUDING REMARKS

With a more suitable outgroup and with the addition of the *trnL-trnF* region, the systematics of Cardueae now appears to be more fully resolved. However, there are taxonomic issues that remain unclear and their clarification requires better sampling and more morphological and molecular data. In addition to only moderate support for basal branches in the combined analysis of the three regions, doubts remain regarding problems typical of delimitation of very large genera such as are frequently found in Compositae (classic examples are *Aster* L., *Erigeron* L., or *Senecio* L.). In Cardueae, generic boundaries are difficult to establish for *Carduus*, *Cirsium*, *Cousinia*, *Jurinea*, and *Saussurea*. In the case of *Carduus* and *Cirsium*, extensive sampling in Africa and North America is needed. For *Cousinia*, *Jurinea*, and *Saussurea*, which are the easternmost representatives of the tribe in Eurasia, intensive collections are called for in Central and East Asia.

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