TAXONOMY

Systematics of the Arctioid group: Disentangling *Arctium* and *Cousinia* (Cardueae, Carduinae)

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Abstract We investigated the phylogeny of the Arctioid lineage of the *Arctium-Cousinia* complex in an attempt to clarify the conflictive generic boundaries of *Arctium* and *Cousinia*. The study was based on analyses of one nuclear (ITS) and two chloroplastic (*trnL-trnT-rps4*, *rpl32-trnL*) DNA regions of 37 species and was complemented with morphological evidence where possible. Based on the results, a broadly redefined monophyletic genus *Arctium* is proposed. The subgenera *Hypacanthodes* and *Cynaroides* are not monophyletic and are suppressed. In contrast, the traditional sectional classification of the genus *Cousinia* is maintained. The genera *Anura*, *Hypacanthium* and *Schmalhausenia* are reduced to sectional level.

Keywords *Anura*; *Arctium*; *Cousinia*; *Hypacanthium*; ITS; molecular phylogeny; nomenclature; *rpl32-trnL*; *trnL-trnT-rpS4*; *Schmalhausenia*

Supplementary Material Appendix 2 is available in the free Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

INTRODUCTION

The *Arctium-Cousinia* complex (Cardueae, Carduinae) is a large group containing between 500 and 600 species. According to Susanna & al. (2003a), it is a monophyletic complex characterized by a receptacle with strongly twisted scales, pappus formed by free deciduous bristles, and streaky achenes (with wavy fringes), which are very often winged, without the apical nectary of the true thistles (Susanna & Garcia-Jacas, 2007). It comprises the genera *Anura* (Juz.) Tscherneva, *Arctium* L., *Cousinia* Cass., *Hypacanthium* Juz. and *Schmalhausenia* C. Winkl. (Table 1). As reported by Rechinger (1986) and Knapp (1987), the vast majority of species of this complex are distributed in the Turkestan mountain region (Tian Shan and Pamir Alai) and the Irano-Turanian region. The only exception is *Arctium* s.str., which is native to the Eurosiberian region and subcosmopolitan in distribution.

The genus *Arctium* was first described by Linnaeus (1753), although some authors in the 18th and 19th centuries persisted in using the illegitimate synonym *Lappa* Scop., based on a pre-Linnaean name. *Cousinia* was later described by Cassini (1827) based on *Carduus orientalis* Adams, and *Schmalhausenia* was described by Winkler (1892). In the 20th century, two more genera were described: *Hypacanthium* (Juzepczuk, 1937) and *Anura* (Tscherneva, 1962).

A number of studies have addressed the *Arctium-Cousinia* complex, and most have been based on morphological characters (Candolle, 1838: 552–557; Bunge, 1865; Boissier, 1875, 1888; Kuntze, 1891: 306–308; Winkler, 1892, 1897; Dittrich, 1977; Duistermaat, 1996, 1997; Petit, 1997; Häffner, 2000). Many others also use biogeographical evidence (Rechinger, 1953, 1972, 1979, 1986; Tscherneva, 1962, 1974, 1982, 1988a,b,c; Davis, 1975; Takhtajan, 1978; Knapp, 1987; Tamanian, 1999), palynological data (Schtepa, 1966, 1973, 1976; Kuprianova & Tscherneva, 1982; Qaid, 1990; Duistermaat, 1996; Petit & al., 1996), karyological information (Poddubnaja-Arnoldi, 1931; Koul, 1964; Fedorov, 1969; Podlech & Dieterle, 1969; Podlech & Bader, 1974; Aryavand, 1975, 1976; Ghaffari, 1984; Tscherneva, 1985; Susanna & al., 2003b; Ghaffari & al., 2000, 2006; López-Vinyallonga & al., 2010) or molecular data (Häffner & Hellwig, 1999; Garcia-Jacas & al., 2002; Susanna & al., 2003a, 2006; López-Vinyallonga & al., 2009).

Recent studies (Susanna & al., 2003a, 2006; López-Vinyallonga & al., 2009) have shown a clear subdivision of the *Arctium-Cousinia* complex into two monophyletic lineages. The first is the Arctioid group with *Arctium* (6 sp.), *Cousinia* subg. *Cynaroides* Tscherneva (20 sp., including the monospecific genus *Anura* ascribed to this subgenus by Susanna & al., 2006), and subg. *Hypacanthodes* Tscherneva (10 sp.), *Hypacanthium* (2 sp.) and the monotypic *Schmalhausenia*. The group is characterized by a chromosome number of $x = 18$, Arctiastrum pollen type (Kuprianova & Tscherneva, 1982) (orbicular and spiny) and a glabrous and cylindrical style with a ring of sweeping hairs at the thickened articulation. The second is the Cousinioid group, which comprises only *Cousinia* subg. *Cousinia* (ca. 500 species) and is characterized by a dysploid series of chromosome numbers of $x = 9, 10, 11, 12$ and 13, Cousinioid pollen type (Kuprianova & Tscherneva, 1982) (oblong and smooth) and long hairs covering the apical part of the style and stylar branches.

Despite the large body of work on these species, the precise limits between *Arctium* and *Cousinia* have not yet been clearly established. The main problems in establishing an accurate delimitation of these genera are centered in the Arctioid group, for two main reasons: first, a group of *Cousinia* species share some characters with *Arctium*, and second, the *Cousinia* subgenera *Cynaroides* and *Hypacanthodes* are both non-monophyletic (López-Vinyallonga & al., 2009). Indeed, as presently defined, *Arctium* and *Cousinia* are not monophyletic either (López-Vinyallonga & al., 2009). These unclear boundaries are reflected in many reclassifications and changes of generic placement of species in the past.

The most extreme reclassification was proposed by Kuntze (1891: 306–308) who merged the entire genus *Cousinia* into *Arctium* in view of the impossibility of establishing clear boundaries between the two genera. A more limited and more sensible proposal was that of Duistermaat (1996) who concluded, based on morphological cladistic analysis, that five species of *Cousinia* subg. *Cynaroides* should be placed in *Arctium*, which would thus include eleven species. This would make *Arctium* monophyletic and easily recognizable by involucral bracts with hooked apical appendages. Duistermaat (1996) did not include *C. anomala* in *Arctium*, as was suggested by Schtepa (1971, 1973) based on pollen morphology, because this would necessitate moving the entire subgenus *Cynaroides* to *Arctium* as indicated by the cladogram. Such a move would result in a poorly recognizable genus *Arctium*, because some species of the subgenus *Cynaroides* do not have glochidiate involucral bracts. Therefore, three species with hooked bracts (*C. anomala*, *C. pentacantha* and *C. tomentella*; the latter included as a synonym of *C. umbrosa*) were kept outside *Arctium*. Duistermaat (1996) also argued that defining *Arctium* in a broader sense would leave *Cousinia* paraphyletic.

In sum, the current classification of the Arctioid group (Table 1) does not reflect natural phylogenetic relationships among its genera and species, as shown by molecular phylogenic analysis (López-Vinyallonga & al., 2009). Therefore, the goal of the present work was to establish the generic boundaries of *Arctium* and *Cousinia* using molecular data and morphological evidence.

■ MATERIALS AND METHODS

Plant material. — The dataset comprises 37 specimens for which we sequenced the nrDNA ITS region (ITS1 and ITS2 are presented separately) as well as the *trnL-trnT-rps4* and *rpl32-trnL* cpDNA regions. The species included in the analyses represent the four sections of *Cousinia* subg. *Hypacanthodes*, the seven sections of *Cousinia* subg. *Cynaroides*, four species of *Arctium*, the two species of *Hypacanthium* and the representatives of the monotypic genera *Anura* and *Schmalhausenia*. For a few species there was no material available, and for others, the amplification and/or sequencing was not successful, and therefore these were not included in the molecular supply. Two outgroup taxa were selected from *Cousinia* subg. *Cousinia* on the basis of previous analyses by López-Vinyallonga & al. (2009): *Cousinia meghrica* Takht. and *Cousinia serawschanica* C. Winkl. All 37 *rpl32-trnL* sequences included in the analyses, 10 out of 37 ITS and 10 out of 37 *trnL-trnT-rps4* sequences are new. Voucher data, sources of material and GenBank accession numbers of the above 37 species are given in Appendix 1.

The analyses based on molecular data were complemented with morphological evidence. A total of 380 herbarium sheets were examined, including type specimens, from the herbaria B, JE, LE, M, MJG, W and WU, together with specimens collected by the authors and collaborators deposited in BC after being revised by Dr. Tscherneva. For most species, the herbarium sheets examined represent the only material available. Information on specimens examined is given in the supplementary Appendix 2.

DNA extraction, amplification and sequencing strategies. — Depending on the quality of the material available, total genomic DNA was extracted either following the protocol of the CTAB method of Doyle & Doyle (1987) as modified by Cullings (1992) for new collections (deposited in BC, ERE and MJG), or following the manufacturer's instructions of the NucleoSpin Plant Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) for the old specimens from herbarium (B, JE, LE and M). Double-stranded DNA of ITS and *trnL-trnT-rps4* was amplified by PCR following the protocol given in López-Vinyallonga & al. (2009). The double-stranded *rpl32-trnL* DNA region was amplified by PCR using the forward primer rpl32F and the reverse primer trnL(UAG) (Shaw & al., 2007). Reactions were performed in 25.0 µl volumes with 10% 10× AmpliTaq buffer, 10% 50 mM $MgCl_2$, 10% of 2 mM dNTP mix, 2% of each primer at 5 pmol/µl conc., 1.0 unit (0.2 µl) AmpliTaq DNA polymerase (Applied Biosystems, Foster City, California, U.S.A.), and 5.0 µl of template DNA (30–60 ng/µl). The volume was brought to 25.0 µl with distilled sterilized water. The profile used for amplification consisted of preheating for 3 min at 95°C, followed by 34 cycles of 94°C for 40 s, 54°C for 40 s and 72°C for 1 min 40 s and a post-treatment of 10 min at 72°C.

All PCR products were purified with either the QIAquick Purification Kit (Qiagen Inc., Valencia, California, U.S.A.) or DNA Clean & Concentrator-5 kit (Zymo Research, Orange, California, U.S.A.) following the manufacturer's protocols. Direct sequencing of the amplified DNA segments was performed as reported in López-Vinyallonga & al. (2009) at the "Serveis Científico-Tècnics" of the University of Barcelona on an ABI PRISM 3700 DNA analyzer (Applied Biosystems). Nucleotide sequences were edited with Chromas v.2.0 (Technelysium Pty. Ltd., Tewantin, Australia) and Bioedit v.7.0.1 (Hall, 1999) and aligned manually by sequential pairwise comparison (Swofford & Olsen, 1990). Data matrices are available on request from the corresponding author.

Phylogenetic analyses. — We used Bayesian inference and maximum likelihood for the analysis of ITS, *trnL-trnT-rps4* and *rpl32-trnL* datasets. All regions were analyzed separately as well as in combination.

Bayesian analyses were conducted with the MrBayes software package v.3.1.2 (Ronquist & Huelsenbeck, 2003) as described by López-Vinyallonga & al. (2009) using parameters from the substitution model GTR+G as indicated by ModelTest v.3.5 (Posada & Crandall, 1998, 2001) as the best-fitting model of molecular evolution for all three markers independently and combined. Two simultaneous and independent analyses were performed, and the Markov chain Monte Carlo process was set

Table 1. Previous classifications of the Arctioid group. *Anura*, *Cousinia*, *Hypacanthium* and *Schmalhausenia* according to Tscherneva (1962, 1982, 1988a,b,c); *Arctium* s.str. following Duistermaat (1996).

Species	Genus and subgenus	Section
Anura pallidivirens (Kult.) Tscherneva	*Anura* (Juz.) Tscherneva	
Arctium atlanticum (Pomel) H. Lindb.	*Arctium* L.	*Arctium*
Arctium lappa L.		
Arctium minus (Hill) Bernh.		
Arctium nemorosum Lej.		
Arctium palladini (Marcow.) R.E. Fr. & E.S. Söderb.		
Arctium tomentosum Mill.		
Cousina aurea C. Winkl.	*Cousinia* subg. *Cynaroides* Tscherneva	*Chrysis* Juz.
Cousinia chlorantha Kult.		
Cousinia haesitabunda Juz.		
Cousinia karatavica Regel & Schmalh.		
Cousinia korolkowii Regel & Schmalh.		
Cousinia medians Juz.		
Cousinia refracta (Bornm.) Juz.		
Cousinia schmalhausenii C. Winkl.		
Cousinia anomala Franch.		*Ctenarctium* Juz.
Cousinia lappacea Schrenk		*Lappaceae* Bunge
Cousinia arctioides Schrenk		*Nanarctium* Juz. ex. Tscherneva
Cousinia triflora Schrenk		*Oligantha* Juz.
Cousinia albertii Regel & Schmalh.		*Pectinatae* C. Winkl.
Cousinia horrescens Juz.		
Cousinia pentacantha Regel & Schmalh.		
Cousinia pentacanthoides Juz. ex Tscherneva		
Cousinia amplissima (Boiss.) Boiss.		*Pseudarctium* Juz.
Cousinia pseudarctium Bornm.		
Cousinia tomentella C. Winkl.		
Cousinia umbrosa Bunge		
Cousinia abolinii Kult. ex Tscherneva	*Cousinia* subg. *Hypacanthodes* Tscherneva	*Abolinia* Tscherneva
Cousinia dolichophylla Kult.		
Cousinia egregia Juz.		
Cousinia grandifolia Kult.		*Amberbopsis* Tscherneva
Cousinia pterolepida Kult.		*Lacerae* C. Winkl.
Cousinia korshinskyi C. Winkl.		
Cousinia macilenta C. Winkl.		
Cousinia fedtschenkoana Bornm.		
Cousinia ugamensis Karmysch.		
Cousinia vavilovii Kult.		*Serratulopsis* Tscherneva
Hypacanthium echinopifolium (Bornm.) Juz.	*Hypacanthium* Juz.	
Hypacanthium evidens Tscherneva		
Schmalhausenia nidulans (Regel) Petrak	*Schmalhausenia* C. Winkl.	

for each so that four chains ran simultaneously for 2,000,000 generations, sampling one out of every 200 generations, resulting in a total of 10,000 sample trees in each run. As stationarity was achieved by the 1000th tree, the first 999 trees were discarded to avoid those trees that might have been sampled prior to convergence of the Markov chains. Therefore, the posterior probability of the phylogeny and its branches was determined from the remaining 19,000 trees. Internodes with posterior probabilities ≥95% were considered to be well-supported.

Maximum likelihood (ML) analyses were performed using the GARLI v.0.951 software (Zwickl, 2006). Both independent and combined analyses were run under the model GTR+I+G as implemented in GARLI. The ML bootstrap (BS) analyses were performed with 1000 replicates and 10 random addition sequences per replicate. The output file containing the best trees (1000 replicates) found for each BS dataset was read into PAUP* v.4.0b10 (Swofford, 2002) where the majority-rule consensus tree was constructed and bootstrap support values were calculated. Tree lengths, consistency index (CI), retention index (RI) and homoplasy index (HI) were calculated excluding uninformative characters.

Congruence in the phylogenetic signal of the cpDNA and nuclear datasets was examined using the incongruence length difference (ILD) test (Farris & al., 1995a,b) as implemented in Winclada v.1.00.08 (Nixon, 2002). It was conducted with 10,000 replicates and 10 random addition sequences per replicate, holding two trees at each step and saving two trees per replicate. In addition, data congruence was examined by a visual comparison of tree topologies with branch support ≥0.95 PP.

■ RESULTS

As a result of the aforementioned analyses, seven trees were recovered: ITS (termed "nuclear"; Fig. 1), *trnL-trnT-rps4* (termed "*trnT*"; not shown), *rpl32-trnL* (termed "*rpl32*"; not shown), *trnL-trnT-rps4+rpl32-trnL* (termed "chloroplastic"; not shown), ITS+*trnL-trnT-rps4* (termed "ITS-*trnT*"; Fig. 2), ITS+*rpl32-trnL* (termed "ITS-*rpl32*"; not shown) and ITS+*trnL-trnT-rps4+rpl32-trnL* (termed "combined"; Fig. 3). The sectional classification proposed in the present work is shown in Fig. 3. Tree topologies are those recovered from the

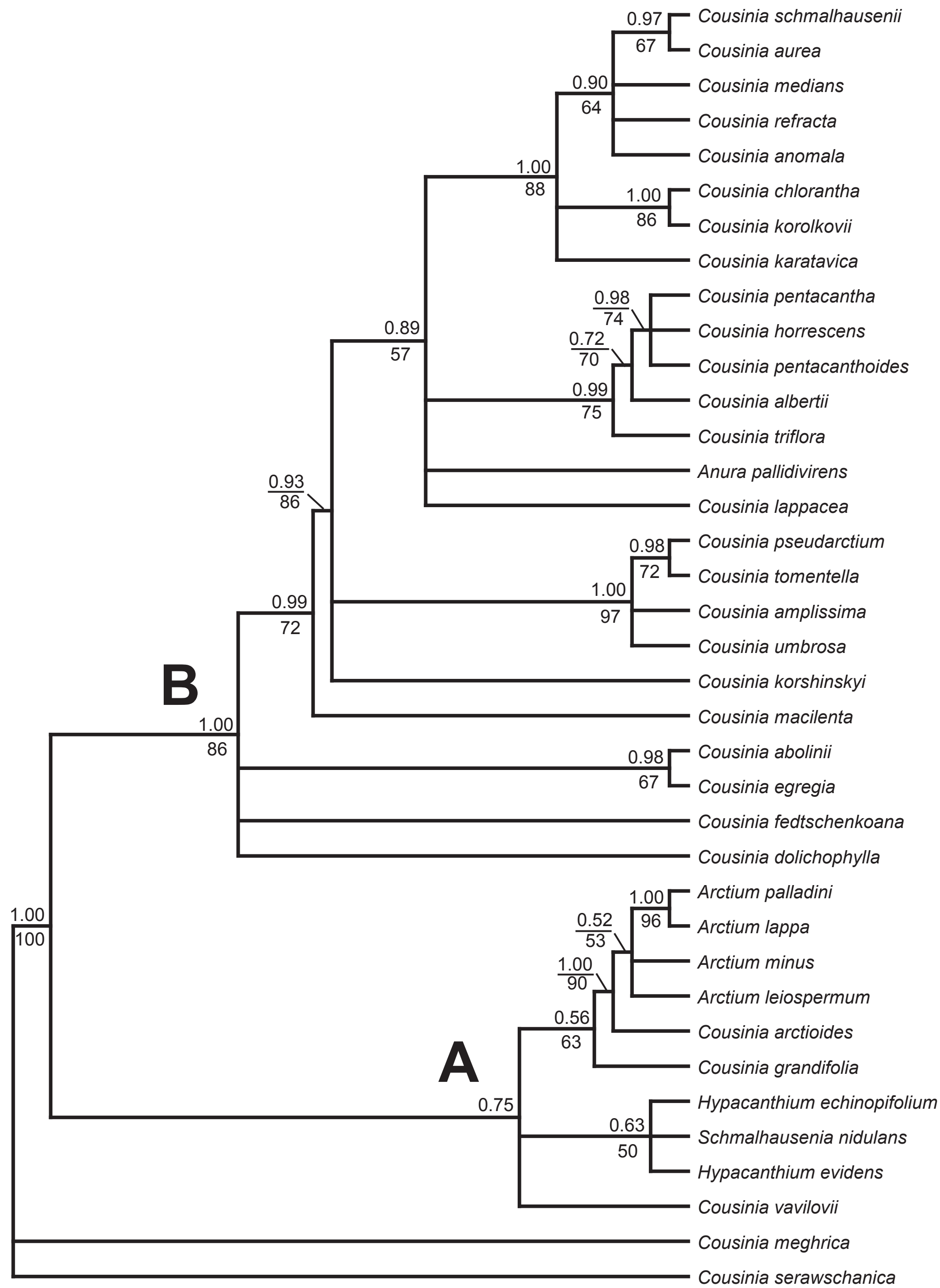

Fig. 1. 50% majority-rule consensus tree obtained from the Bayesian analysis of the ITS dataset (nuclear tree). Numbers above branches are posterior probabilities and numbers below branches are bootstrap percentages. **A,** clade A; **B,** clade B.

Bayesian analysis and both posterior probabilities and bootstrap percentages are provided when the clades from Bayesian inference and maximum likelihood were coincident. The numerical results of the statistics of the PAUP* analyses are given in Table 2.

There was some conflictive signal between the markers used in the ILD analysis ($P = 0.001$). Nevertheless, the tree topologies that resulted from the different phylogenetic analyses were mainly in agreement. Therefore, the data from the three markers were combined to improve the phylogenetic signal of the analyses for the Arctioid lineage.

Each chloroplastic marker by itself provided scarce phylogenetic information, presumably due to the small amount of informative characters (Table 2). Consequently, both *trnT* and *rpl32* trees consisted of unresolved polytomies and the chloroplastic combined tree revealed some meaningless clades (trees not shown). In addition, the trees recovered from the ITS-*rpl32* and combined analyses were essentially identical (hence, the first is not shown), although with the addition of the third marker clade support increases. Furthermore, in the trees resulting from nuclear (Fig. 1), ITS-*trnT* (Fig. 2), ITS-*rpl32* (not shown) and combined (Fig. 3) datasets, relationships among

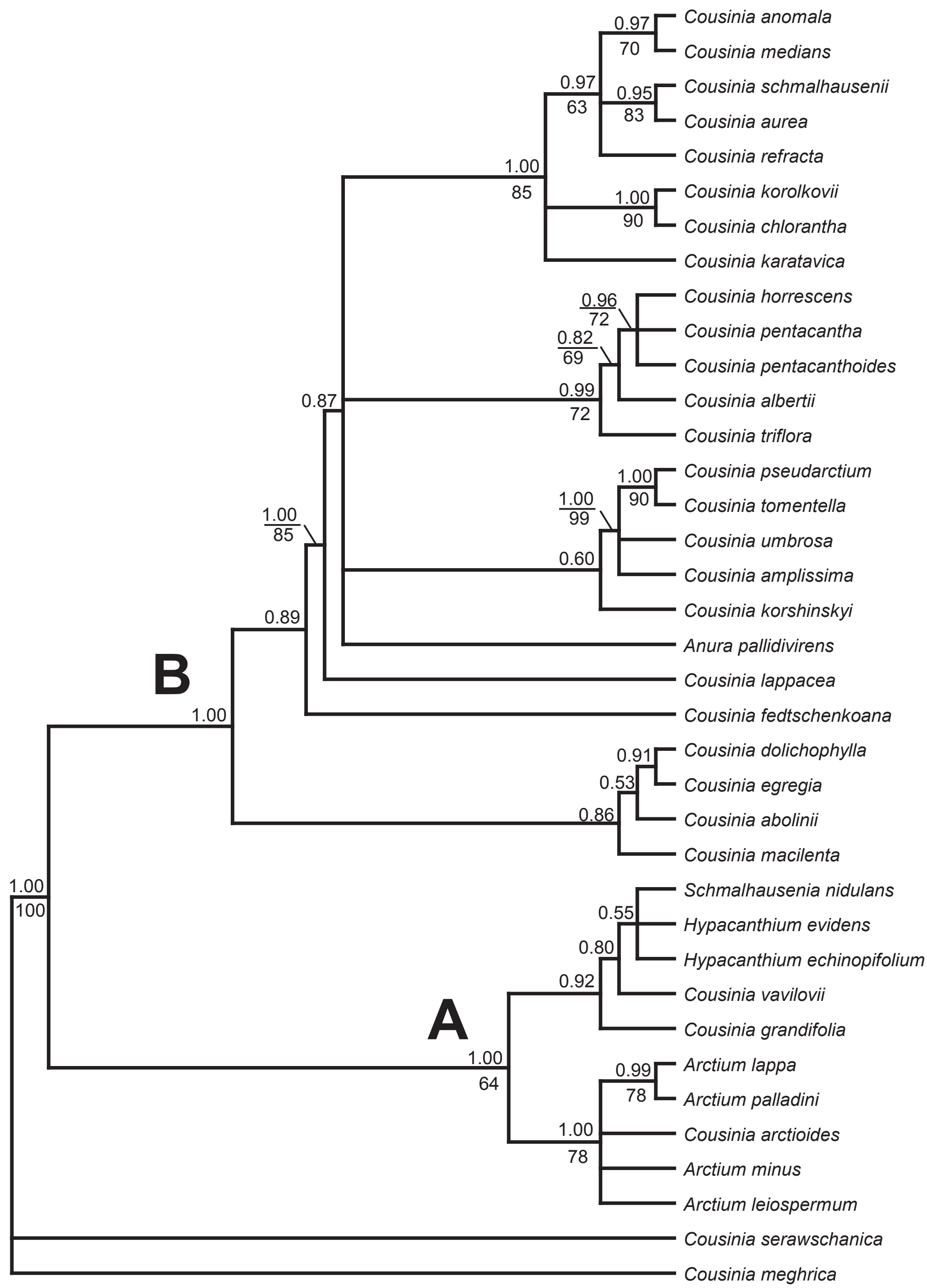

Fig. 2. 50% majority-rule consensus tree obtained from the Bayesian analysis of the ITS+*trnL-trnT-rps4* dataset (ITS-*trnT* tree). Numbers above branches are posterior probabilities and numbers below branches are bootstrap percentages. **A,** clade A; **B,** clade B.

species are reasonably well resolved. Consequently, the discussion of the results is based mainly on the combined tree (Fig. 3).

All the analyses confirmed the monophyly of the Arctioid clade with the highest support, and most of the analyses showed a clear subdivision of the Arctioid lineage into two groups (BS = 100%, PP = 1.00, Fig. 3). The first clade is formed by all the species of the genera *Arctium*, *Hypacanthium* and the monotypic *Schmalhausenia* together with *Cousinia arctioides*, *C. vavilovii* and *C. grandifolia* (BS = 91%, PP = 1.00, Fig. 3, clade A). This clade is subdivided again into one lineage comprising the genus *Arctium* and *C. arctioides* (BS = 86%, PP = 1.00, Fig. 3) and a second one merging *Hypacanthium*, *Schmalhausenia*, *C. vavilovii* and *C. grandifolia* (PP = 1.00, Fig. 3), which have no statistical support in any of the ML tests.

The second clade with the remaining *Cousinia* species (BS = 86%, PP = 1.00, Fig. 1; PP = 0.99, Fig. 3, clade B) has most species of the polyphyletic *C.* subg. *Hypacanthodes* in its base, and they are either ungrouped or gathered without statistical support in any analysis. The rest of the species fall in a clade with strong support only in the Bayesian analyses (BS = 72%, PP = 0.99, Fig. 1; PP = 0.99, Fig. 3). The most remarkable results in this clade concern the sectional classification.

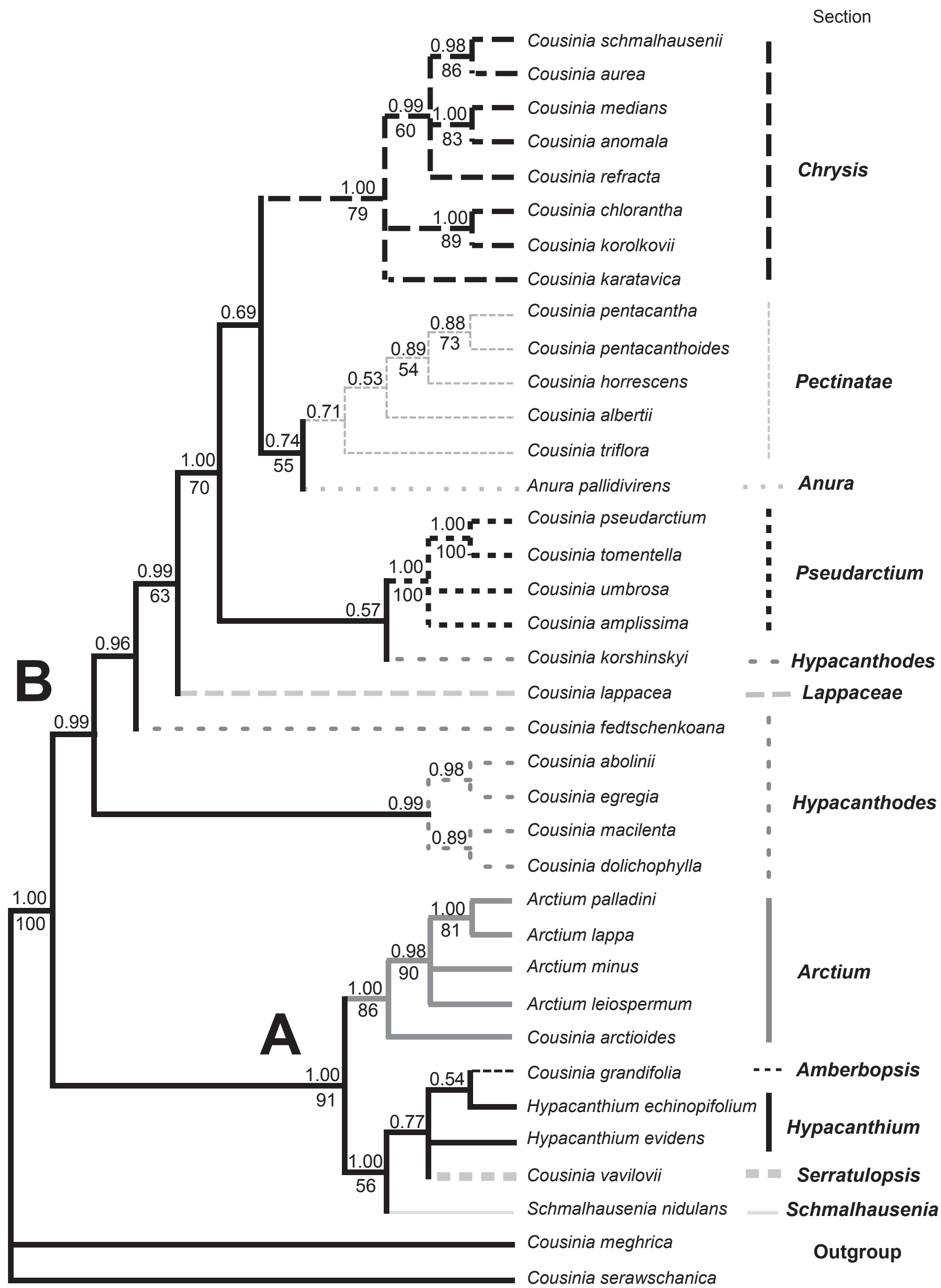

Fig. 3. 50% majority-rule consensus tree obtained from the Bayesian analysis of the ITS+*trnL-trnT-rps4+rpl32-trnL* dataset (combined tree). Numbers above branches are posterior probabilities and numbers below branches are bootstrap percentages. The sectional classification proposed is shown. **A,** clade A; **B,** clade B.

Table 2. Numerical results of the analyses of the ITS, *trnL-trnT-rps4*, *rpl32-trnL* and combined ITS+*trnL-trnT-rps4*+*rpl32-trnL* datasets.

	ITS	*trnL-trnT-rps4*	*rpl32-trnL*	ITS + *trnL-trnT-rps4*	ITS + *rpl32-trnL*	*trnL-trnT-rps4* + *rpl32-trnL*	ITS + *trnL-trnT-rps4* + *rpl32-trnL*
Total characters	484	1063	933	1547	1417	1996	2480
Phylog. inf. chars.	69	15	32	84	101	47	116
Tree length	135	26	45	174	194	99	235
Consistency index	0.5852	0.6538	0.7778	0.5517	0.5876	0.6118	0.5574
Retention index	0.8654	0.8043	0.8851	0.8312	0.8410	0.3882	0.8106
Homoplasy index	0.4148	0.3462	0.2222	0.4483	0.4124	0.3333	0.4426

Phylog. inf. chars., phylogenetically informative characters. CI and RI are reported with uninformative sites excluded.

The monophyly of *C.* sect. *Pseudarctium* is strongly supported (BS = 100%, PP = 1.00, Fig. 3). *Cousinia* sect. *Chrysis* is also monophyletic (BS = 79%, PP = 1.00, Fig. 3), including *C. anomala*, the only species of the *C.* sect. *Ctenarctium*. The species of *C.* sect. *Pectinatae* were grouped together with *C. triflora*, from the monotypic *C.* sect. *Oligantha*, with strong support in the nuclear and ITS-*trnT* analyses (BS = 75% and 72%, respectively, PP = 0.99; Figs. 1 and 2). In the ITS-*rpl32* (not shown) and combined (Fig. 3) trees, *Anura palladivirens* merges with the clade of *C.* sect. *Pectinatae*, leading to a weaker clade.

■ DISCUSSION

As previously reported (López-Vinyallonga & al., 2009), the Arctioid lineage is clearly monophyletic with high support and is sharply divided into two strongly supported monophyletic clades. These results lead to two main conclusions regarding generic and subgeneric assignment. First, the genus *Arctium* as established by Duistermaat (1996) is not monophyletic because the species of *Arctium* sect. *Arctium* and sect. *Nanarctium* fall into the first strongly supported group (Figs. 1–3, clade A), while the species of sect. *Pseudarctium* are placed in the other highly supported clade (Figs. 1–3, clade B). Second, neither *C.* subg. *Cynaroides* nor subg. *Hypacanthodes* as established by Tscherneva (1988c) are monophyletic (Figs. 1–3). *Cousinia arctioides* belongs to clade A, whereas the remaining species of subg. *Cynaroides* belong to clade B. A similar situation was found in subg. *Hypacanthodes*, where all species except *C. vavilovii* and *C. grandifolia* fall into clade B (Figs. 1–3).

The sister clades resulting from the split of the Arctioid lineage appear in all the analyses performed. The *Arctium* lineage (Figs. 1–3, clade A) comprises the species of *Arctium* sect. *Arctium* sensu Duistermaat (1996) included in the analyses together with *Cousinia arctioides*, *C. vavilovii*, *C. grandifolia*, *Schmalhausenia nidulans* and the two species of *Hypacanthium*. In turn, the species of *Arctium* sect. *Arctium* sensu Duistermaat (1996) (*A. minus*, *A. leiospermum*, *A. palladini*, *A. lappa*) and *C.* sect. *Nanarctium* (*C. arctioides*) form a strong supported clade, which is treated here as sect. *Arctium*. The thus redefined sect. *Arctium* is characterized by involucral bracts ending in a hooked appendage and oblong achenes with apical ridge. Our results also indicate that *A. lappa* and *A. leiospermum* are different species, as opposed to the latter being a synonym of *A. lappa*, as stated by Duistermaat (1996). The rest of the species of the subclade *Arctium* (*C. grandifolia*, *C. vavilovii*, *S. nidulans*, *H. evidens*, *H. echinopifolium*) are merged into a highly supported group in the combined 3-marker analysis (Fig. 3), but weakly supported in the other analyses (Figs. 1 and 2). Taking into account the tree topologies and the great morphological differences between these taxa, we consider this group as a polytomy (see below).

The second main lineage, the clade *Cynaroides* (Figs. 1–3, clade B), includes most of the species of the Arctioid group and is likewise divided into two subgroups (Fig. 3). The first, which is weakly supported, comprises most species of the polyphyletic *C.* subg. *Hypacanthodes*, excluding *C. grandifolia* and *C. vavilovii*, merged in the *Arctium* subclade, *C. fedtschenkoana* and *C. korshinskyi*. The latter is the type of the subgeneric name and appears separated by two branches with PP > 0.95 from the rest of the species. Despite the lack of monophyly of subg. *Hypacanthodes*, our provisional solution (pending further study) is to maintain the group but at a lower taxonomic rank, as sect. *Hypacanthodes*, suppressing sect. *Abolinia* and sect. *Lacerae*. The redefined section includes *C. abolinii*, *C. dolichophylla*, *C. egregia*, *C. fedtschenkoana*, *C. korshinskyi* and *C. macilenta*. Although this solution is not optimal, the alternative would be to create new monotypic sections without morphological support, which seems less desirable.

The second well-supported subgroup contains all the species of *C.* subg. *Cynaroides* except *C. arctioides*, one species of *C.* subg. *Hypacanthodes* (*C. korshinskyi*) and the monotypic genus *Anura*. We therefore suggest to suppress subg. *Cynaroides* because, as presently resolved, it is not monophyletic.

In terms of sectional classification, many species that are merged in our analyses are in keeping with the sectional classification described by Tscherneva (1988a,b,c, 1993). Indeed, most of these sections are monophyletic and therefore should be maintained as currently established. All seven trees obtained agree in the monophyly of *C.* sect. *Pseudarctium* (Fig. 3), which is well characterized morphologically by apically hooked involucral bracts with marginal glands. Furthermore, our results confirm that *C. amplissima* and *C. pseudarctium* are different species, as previously suggested by Duistermaat (1996), instead of *C. amplissima* being a synonym of *C. pseudarctium*

(Tscherneva, 1962). Also in agreement with Duistermaat (1996), we find that these species differ in the diameter of the capitules, the number of flowers per capitule and the length of achenes, corolla lobes and stylar apex.

Most of our analyses show *C.* sect. *Chrysis* to be monophyletic, although *C. anomala*, the only species of *C.* sect. *Ctenarctium*, unexpectedly falls into this clade (Fig. 3). This section is characterized by short head peduncles and yellow corollas. The placement of *C. anomala* leads us to suppress sect. *Ctenarctium*. The molecular and morphological data also indicate separating *C. anomala*, *C. aurea*, *C. medians*, *C. refracta* and *C. schmalhausenii* with entire leaves from *C. karatavica*, *C. chlorantha* and *C. korolkovii* with pinnatipartite leaves (Figs. 1–3).

Furthermore, our results suggest that *C.* sect. *Pectinatae*, including *C. triflora* from the monotypic *C.* sect. *Oligantha*, is monophyletic (Figs. 1 and 2). Although in some analyses *Anura pallidivirens* merges with this clade reducing its statistical support (Fig. 3), we prefer to maintain sect. *Pectinatae* because all its species (including *C. triflora*) have very characteristic involucral bracts with 2 to 6 pairs of spines along the margins, a character not found in any other species in the entire Arctioid lineage. These results indicate that sect. *Oligantha* should be suppressed.

According to our results (Fig. 3), we suggest maintaining most of the monotypic sections as currently accepted based on morphological evidence (Tscherneva, 1962, 1988a,b,c): *C.* sect. *Amberbopsis* (*C. grandifolia*), sect. *Serratulopsis* (*C. vavilovii*) and sect. *Lappaceae* (*C. lappacea*). Finally, we propose to reduce the taxonomic rank of the genera *Anura*, *Hypacanthium* and *Schmalhausenia* to sectional level, leading to sect. *Anura* (*A. pallidivirens*), sect. *Hypacanthium* (*H. evidens*, *H. echinopifolium*) and sect. *Schmalhausenia* (*S. nidulans*). On molecular grounds, the only problem is posed by sect. *Hypacanthium* which is not monophyletic (Fig. 3). However, we prefer to keep *H. echinopifolium* and *H. evidens* in a single section rather than accepting two monotypic sections which would be untenable on morphological grounds.

Since *Arctium* and *Cousinia* as currently defined are not monophyletic, the two genera should be redefined. When the species belonging to *Arctium* would be kept together according to Duistermaat (1996), the most parsimonious option would be to transfer all Arctioid species to *Arctium*, turning it into a broader and monophyletic genus. This option is supported by our analyses. Moreover, the Arctioid lineage is sharply differentiated from the Cousinioid lineage by several important traits, mainly pollen type and basic chromosome number. Indeed, the molecular phylogeny supports this distinction, while also showing the monophyly of both lineages and the paraphyly of the genus *Cousinia*. Inclusion of the Arctioid species into a broader genus *Arctium* would thus turn *Cousinia* into a monophyletic genus and would resolve the ancient entanglement between these two genera.

Despite the undeniable monophyly of the Arctioid group and its uniformity regarding chromosome number, pollen type and style morphology, there are no morphological characters suitable enough to establish a strong classification based on the molecular data. For example, the two highly supported sister clades obtained in the present molecular phylogeny could be interpreted as subgenera. However, given the fact that we could not find morphological characters suitable to define these clades, we prefer not to establish any subgeneric classification. As demonstrated by López-Vinyallonga & al. (2009), the distribution of characters such as spiny vs. unarmed leaves or apically hooked vs. straight involucral bracts, runs across generic and subgeneric boundaries and suggest that these characters evolved several times in parallel.

■ TAXONOMIC CONCLUSIONS

The preferred taxonomic solution with respect to the lack of monophyly of the main genera belonging to the *Arctium-Cousinia* complex, is transferring all Arctioid species to *Arctium*. This resolves the ancient entanglement between *Arctium* and *Cousinia*, turning them into monophyletic lineages. The redefinition of the Arctioid lineage, as based on the molecular data and supported by morphological evidence where possible, is presented below (see also Tables 3 and 4).

Synonyms are mentioned only when necessary to avoid possible confusion. Citation of type materials is orientative and mostly limited to citation of protologue data and herbaria where types might be preserved. For formal designations of type specimens we may refer to Duistermaat (1996).

Arctium L., Sp. Pl. 2: 816. 1753.

Biennial or perennial, spiny or unarmed suffruticose herbs with rootstock or taproot. Leaves leathery or herbaceous, dentate, lobed, pinnatipartite, pinnatisect or entire. Basal leaves in a rosette, the cauline ones similar to bottom leaves but gradually diminishing towards the apex, the most distal ones usually sessile. Synflorescence paniculate, racemose or corymbose. Capitula homogamous, solitary or in clusters, from sessile to long pedunculate with three to more than 100 florets, spherical to ovoid, glabrous to densely arachnoid. Involucral bracts pluriseriate, imbricate, basally appressed, apex hooked, curved or ending in a straight spine. Receptacle densely covered with rough or smooth bristles. Bristles of pappus scabrous, free, deciduous. Florets uniform, hermaphrodite. Corolla tubulose-campanulate, white, yellow or pink to purple, glabrous or with glandular hairs, with campanulate limb. Anthers with basal appendages fringed (occasionally entire); apical appendages deltoid, glabrous or dorsally villose. Style glabrous with a ring of sweeping hairs at the thickened articulation. Achenes glabrous, oblong or ovate, sometimes slightly compressed, smooth, rugose or with longitudinal ridges; brown, often with dark and irregular spots, sometimes with apical rim. Diploid plants with basic chromosome number $x = 18$. Pollen orbicular and spiny of the Arctiastrum type.

Arctium sect. ***Amberbopsis*** (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia* sect. *Amberbopsis* Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 17: 83. 1962 – Type:

Table 3. New classification of the former Arctioid group based on the present results.

Species	Section
Arctium grandifolium (Kult.) S. López, Romaschenko, Susanna & N. Garcia	*Amberbopsis* (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia
Arctium pallidivirens (Kult.) S. López, Romaschenko, Susanna & N. Garcia	*Anura* S. López, Romaschenko, Susanna & N. Garcia
Arctium arctioides (Schrenk.) Kuntze	*Arctium*
Arctium atlanticum (Pomel) H. Lindb.	
Arctium lappa L.	
Arctium leiospermum Juz. & Ye. V. Serg.	
Arctium minus (Hill) Bernh.	
Arctium nemorosum Lej.	
Arctium palladini (Marcow.) R.E. Fr. & E.S. Söderb.	
Arctium tomentosum Mill.	
Arctium anomalum (Franch.) Kuntze	*Chrysis* (Juz.) S. López, Romaschenko, Susanna & N. Garcia
Arctium aureum (C. Winkl.) Kuntze	
Arctium chloranthum (Kult.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium haesitabundum (Juz.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium karatavicum (Regel & Schmalh.) Kuntze	
Arctium korolkowii (Regel & Schmalh.) Kuntze	
Arctium medians (Juz.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium refractum (Bornm.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium schmalhausenii (C. Winkl.) Kuntze	
Arctium echinopifolium (Juz.) S. López, Romaschenko, Susanna & N. Garcia	*Hypacanthium* S. López, Romaschenko, Susanna & N. Garcia
Arctium evidens (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia	
Arctium abolinii (Kult. ex Tscherneva) S. López, Romaschenko, Susanna & N. Garcia	*Hypacanthodes* S. López, Romaschenko, Susanna & N. Garcia
Arctium dolichophyllum (Kult.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium egregium (Juz.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium fedtschenkoanum (Bornm.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium korshinskyi (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium macilentum (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium pterolepidum (Kult.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium ugamense (Karmysch.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium lappaceum (Schrenk) Kuntze	*Lappaceum* (Bunge) Duist.
Arctium albertii (Regel & Schmalh.) S. López, Romaschenko, Susanna & N. Garcia	*Pectinatae* (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia
Arctium horrescens (Juz.) S. López, Romaschenko, Susanna & N. Garcia	
Arctium pentacanthoides (Juz. ex Tscherneva) S. López, Romaschenko, Susanna & N. Garcia	
Arctium pentacanthum (Regel & Schmalh.) Kuntze	
Arctium triflorum (Schrenk.) Kuntze	
Arctium amplissimum (Boiss.) Kuntze	*Pseudarctium* (Juz.) Duist.
Arctium pseudarctium (Bornm.) Duist.	
Arctium tomentellum (C. Winkl.) Kuntze	
Arctium umbrosum (Bunge) Kuntze	
Arctium eriophorum (Regel & Schmalh.) Kuntze	*Schmalhausenia* S. López, Romaschenko, Susanna & N. Garcia
Arctium vavilovii (Kult.) S. López, Romaschenko, Susanna & N. Garcia	*Serratulopsis* (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia

Cousinia grandifolia Kult. ≡ *Arctium grandifolium* (Kult.) S. López, Romaschenko, Susanna & N. Garcia.

Perennial hemicryptophytes. Leaves entire, lobed, unarmed. Capitule solitary, ovoid. Involucral bracts ending in a straight spine. Florets yellow. Endemic to the Tian Shan.

Arctium grandifolium (Kult.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia grandifolia* Kult. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 6: 9. 1929 – Type (Tscherneva, 1962): Kazakhstan. In distr. Tschimkent, in montibus Duany-tau, in angust. Fl. Daubaba, 30.V.1924, fl. fr., *M. Sovetkina 98* (TASH).

Arctium sect. ***Anura*** (Juz.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia* sect. *Anura* Juz. in Trudy Tadzhikistansk. Bazy 8: 512. 1940 ≡ *Anura* (Juz.) Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 17: 107. 1962 – Type: *Anura pallidivirens* (Kult.) Tscherneva ≡ *Arctium pallidivirens* (Kult.) S. López, Romaschenko, Susanna & N. Garcia.

Perennial hemicryptophytes. Leaves entire, ovate, with the margin finely toothed, unarmed. Synflorescence dense, corymbose-paniculate. Capitula numerous, deciduous, with 3–5 whitish or pallid-yellow florets. Involucral bracts ending in a straight spine. Endemic to the Pamir Alai.

Arctium pallidivirens (Kult.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia pallidivirens* Kult. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 6: 3. 1929 ≡ *Anura pallidivirens* (Kult.) Tscherneva, Fl. URSS 27: 134. 1962 – Type: Uzbekistan. Serawschan, Dist. Katta-kurgan, Montes Ak-tau, prope Tamany, 24.V.1925, *M.G. Popov 343, 355, 384, 356* (TASH).

Arctium sect. *Arctium*

Biennial hemicryptophytes. Leaves very large (40–80 cm long), hastate, entire, unarmed. Capitules solitary or in clusters, sessile to long pedunculate. Synflorescence racemose or corymbose. Involucral bracts apically hooked. Florets light pink to deep purple (except *A. arctioides* with yellow florets). Achenes

Table 4. Main diagnostic characters and geographical distribution of the sections recognized in this study.

	Morphological characters		
Section	Life form	Leaves	Capitule
Amberbopsis (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia	Perennial hemicryptophyte	Entire, lobed, unarmed	Solitary, ovoid
Anura (Juz.) S. López, Romaschenko, Susanna & N. Garcia	Perennial hemicryptophyte	Entire, ovate, margin finely toothed, unarmed	Numeorus, deciduous, few-flored (3–5)
Arctium	Biennial hemicryptophyte	Entire, very large, hastate, unarmed	Solitary or in clusters, sessile to long pedunculate
Chrysis (Juz.) S. López, Romaschenko, Susanna & N. Garcia	Perennial hemicryptophyte	Entire or not, unarmed	Numerous, medium to long pedunculated
Hypacanthium S. López, Romaschenko, Susanna & N. Garcia	Perennial hemicryptophyte	Bipinnatisect, narrowly lanceolate in outline, spiny	Solitary or in pairs
Hypacanthodes (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia	Perennial hemicryptophyte	Pinnatifid to pinnatepartite, very spiny	Solitary, ovoid
Lappaceum (Bunge) Duist.	Perennial hemicryptophyte	Entire, broadly oblong-lanceolate, unarmed or spiny dentate	Numerous, sessile, deciduous, few-flored (up to 10)
Pectinatae (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia	Perennial hemicryptophyte	Entire, margin spiny-toothed	Numerous, up to 50 florets
Pseudarctium (Juz.) Duist.	Perennial hemicryptophyte	Irregularly undulate, unarmed	Sessile to short pedunculated, in clusters, up to 50 florets
Schmalhausenia (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia	Perennial hemicryptophyte	Bipinnatisect, very spiny	Clusters of 5 to 10, sessile, woolly
Serratulopsis (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia	Perennial hemicryptophyte	Entire, ovate, unarmed	Solitary, long pedunculate, oblong-ovoid, glabrous

oblong with apical ridge. Subcosmopolitan distribution. – Type (Duistermaat, 1996): *Arctium lappa* L.

Arctium arctioides (Schrenk) Kuntze, Revis. Gen. Pl. 1: 307. 1891 ≡ *Cousinia arctioides* Schrenk in Bull. Phys.-Math. Acad. Petersb. 2: 115. 1844 – Type: Kazakhstan. Flussufer der Saryssu, *A. Schrenk s.n.* [s.d.] ex herb. Krzisch. (LE, W!).

Arctium atlanticum (Pomel) H. Lindb. in Acta Soc. Sci. Fenn., Ser. B, Opera Biol. 1, no. 2: 153. 1932 – Neotype (Duistermaat, 1996: 73): Algeria. Tlemcen, *Faure s.n.* (P).

Arctium lappa L., Sp. Pl. 2: 816. 1753 – Lectotype (Duistermaat, 2003: 851): [illustration] "Bardana, siue Lappa maior", Dodoens, Stirpium Hist. Pempt.: 38. 1583 – Epitype (Duistermaat, 2003: 851): France, S. of Macon, roadside, 30 Jul 1990, *H. Duistermaat & J.J. Vermeulen 137* (L), illustrated in Duistermaat (1996: 77, fig. 18.2).

Arctium leiospermum Juz. & Ye.V. Serg. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 18: 299. 1957 – Type: Kirghizia. Jugum montium Alaicum, inter pag. Gulcza et Langar, 15 VIII 1901, *O. et B. Fedtschenko* (LE).

Arctium minus (Hill) Bernh., Syst. Verz.: 154. 1800 – Duistermaat (1996) cites an icon in Hill, Veg. Syst. 4: 28, pl. 25, fig. 3a. 1762.

Arctium nemorosum Lej. in Mag. Hort. (Liège) 1: 289. 1833 – Type (Duistermaat, 1996): *Lejeune s.n.*, s.d. (holotype: BR; isotype: W).

Arctium palladini (Marcow.) R.E. Fr. & E.S. Söderb. in Delect. Spor. Sem. Hort. Bot. Berg. 1923 – Lectotype (Duistermaat, 1996: 98): Russia. Ossetien, Czecznia und Imeretien, [1899], *B. Marcowicz s.n.* (LE!).

Arctium tomentosum Mill., Gard. Dict., ed. 8, 3. 1768 – Neotype (Duistermaat, 1996): Herb. Linnaeus 964.1 (LINN).

Involucral bracts	Florets	Other characters	Geographical distribution
Apical spine straight	Yellow		Tian Shan (endemic)
Apical spine straight	Whitish or pallid-yellow	Synflorescence dense, corymbose-paniculate	Pamir Alai (endemic)
Apically hooked	Light pink to deep purple (yellow in *A. arctioides*)	Synflorescence racemose or corymbose	Subcosmopolitan
Apical spine straight	Yellow	Synflorescence paniculate or corymbose	Pamir Alai (endemic), except *A. karatavicum*, also found in the Tian Shan
Apical spine straight	Purple		Tian Shan (endemic)
Apical spine straight	Whitish or pink		Tian Shan (endemic), except *A. fedtschenkoanum* and *A. macilentum*, endemic to the Pamir-Alai
Coriaceous, apically hooked	Purple	Synflorescence racemose	Tian Shan and Pamir Alai
2 to 6 pairs of spines along margins, apical spine straight (apically hooked in *A. pentacanthum*)	Yellow	Synflorescence paniculate	Tian Shan (endemic), except *A. triflorum* also found in the Pamir Alai and the Kopet-Dagh
Marginal glands, apically hooked	Whitish or purple	Synflorescence paniculate	Tien Shan, Pamir Alai, Turkmenistan Mountain Range, north Iran and Afghanistan
Apical spine straight	Purple	Densely tomentose, stems and nerves purple, scape hollow 2 to 3 cm diam.	Tian Shan (endemic)
Apical spine straight, inner bracts with a scarious, purple apendage	Whitish		Tian Shan (endemic)

Arctium sect. ***Chrysis*** (Juz.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia* sect. *Chrysis* Juz. in Trudy Tadzhikistansk. Bazy 8: 515. 1940 – Type (Tscherneva, 1962): *Cousinia aurea* C. Winkl. ≡ *Arctium aureum* (C. Winkl.) Kuntze.

Perennial hemicryptophytes. Leaves entire or not, unarmed. Capitula medium to long pedunculated in paniculate or corymbose synflorescences. Involucral bracts ending in a straight spine. Florets yellow. Endemic to the Pamir Alai (except *A. karatavicum*, also found in the Tian Shan).

Arctium anomalum (Franch.) Kuntze, Revis. Gen. Pl. 1: 307. 1891 ≡ *Cousinia anomala* Franch. in Ann. Sci. Nat., Bot., ser. 6, 16: 316. 1883 – Type: Tajikistan. Environs of Voru (Kohistan), 11 juillet, *G. Capus 664* (P).

Arctium aureum (C. Winkl.) Kuntze, Revis. Gen. Pl. 1: 307. 1891 ≡ *Cousinia aurea* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 10: 475. 1887 – Type: Tajikistan. Regioni fluminis Sarawschan ad rivulum fluvium Woru influentem, inter pagum Sänturutsch et trajectum Kschtut et in montibus prope Kschtut sitis altitudine 5–7000′ Junio et Julio anni 1882, *A. Regel s.n.* (LE).

Arctium chloranthum (Kult.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia chlorantha* Kult. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 6: 4. 1929 – Type (Tscherneva, 1962): Uzbekistan. Sarawschan, inter Gop et Suforidum, 27.V.1919, *G. Balabaëv 1655* (TASH).

Arctium haesitabundum (Juz.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia haesitabunda* Juz. in Trudy Tadzhikistansk. Bazy 8: 516, 549. 1940 – Type: Uzbekistan. Kaytash Mts., near the kishlak Daratash, without collector (LE).

Arctium karatavicum (Regel & Schmalh.) Kuntze, Revis. Gen. Pl. 1: 308. 1891 ≡ *Cousinia karatavica* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 317. 1879 – Type: Kazakhstan. In Turkestaniae montibus karatavicis prope Ischtübe, *A. Regel s.n.* (LE).

Arctium korolkowii (Regel & Schmalh.) Kuntze, Revis. Gen. Pl. 1: 308. 1891 ≡ *Cousinia korolkovii* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 315. 1879 – Type: Uzbekistan. Ad Chiwam inter Chala-Ati et Adam kiri ullan, *Korolkow s.n.* (LE).

Arctium medians (Juz.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia medians* Juz. in Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 3: 297. 1936 – Type: Turkmenistan. Kuhitang Mts., near Kuhitang Village, 22 VI 1931, Nº 376, *S. Nevski* (LE).

Arctium refractum (Bornm.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia aurea β refracta* Bornm. in Beih. Bot. Centralbl. 34: 138. 1917 ≡ *Cousinia refracta* (Bornm.) Juz. in Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 3: 297. 1937 – Type: Tajikistan. Hissar-Gebiet, Hakimi im Thale des Karatag, 5000′, 8.–20.VI.1889, fl. fr. imm., *A. Regel s.n.* (LE).

Arctium schmalhausenii (C. Winkl.) Kuntze, Revis. Gen. Pl. 1: 308. 1891 ≡ *Cousinia schmalhausenii* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 10: 474. 1889 – Type: Uzbekistan. Valle Sarawschan, altitudine 2–3000′ ubi plantam Majo mense anui 1869 clarissima *O. Fedtschenko s.n.* (LE).

Arctium sect. ***Hypacanthium*** (Juz.) S. López, Romaschenko, Susanna & N. Garcia, **comb. et stat. nov.** ≡ *Hypacanthium* Juz. in Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 3: 324. 1937 – Type: *Hypacanthium echinopifolium* (Bornm.) Juz. ≡ *Arctium echinopifolium* (Bornm.) S. López, Romaschenko, Susanna & N. Garcia

Perennial hemicryptophytes. Leaves spiny, bipinnatisect, narrowly lanceolate in outline. Capitula solitary or in pairs, 2.5 to 4 cm in diameter (excluding spines). Involucral bracts ending in a straight spine. Florets purple. Endemic to the Tian Shan.

Arctium echinopifolium (Bornm.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia echinopifolia* Bornm. in Beih. Bot. Centralbl. 34: 192. 1916 ≡ *Hypacanthium echinopifolium* (Bornm.) Juz. in Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 3: 324. 1937 – Type: Kirghizia. Fergana Region, in distr. Namangan, [Chanach Pass,] 06.07.1912, *O. von Knorring 67*, Herb. J. Bornmüller (holotype: LE; isotype: B!).

Arctium evidens (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Hypacanthium evidens* Tscherneva in Bot. Zhurn. (Moscow & Leningrad) 68: 634. 1983 – Type: Uzbekistan. Declive australe jugi Czatkalensis, in fluxu superiore rivuli Katta-Akar (systema Irtasch-saj), 2700 m, ad schistoso-lapidosa, 20 IX 1979, *R.G. Jussupov s.n.* (LE).

Arctium sect. ***Hypacanthodes*** (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia, **comb. et stat. nov.** ≡ *Cousinia* subg. *Hypacanthodes* Tscherneva in Bot. Zhurn. (Moscow & Leningrad) 73: 594. 1988 – Type: *Cousinia korshinskyi* C. Winkl. ≡ *Arctium korshinskyi* (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia.

Perennial hemicryptophytes. Leaves very spiny and pinnatifid to pinnatepartite. Capitula solitary, ovoid. Involucral bracts ending in a straight spine. Endemic to the Tian Shan (except *A. fedtschenkoanum* and *A. macilentum*, endemic to the Pamir-Alai).

Arctium abolinii (Kult. ex Tscherneva) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia abolinii* Kult. ex Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 17: 88. 1962 – Type: Kirghizia. Kyzyl-Jar region, Naryn basin, Kurpsai river, rocky slope, 1927.IX.11 fl. et fr., *R.I. Abolin 717* (TASH).

Arctium dolichophyllum (Kult.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia dolichophylla* Kult. in Trudy Turkestansk. Nauchn. Obshch. 1: 112. 1923 – Type: Uzbekistan. Karzhantau, environs of Khumsan (TASH).

Arctium egregium (Juz.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia egregia* Juz. in Bot. Mater. Gerb. Glavn. Bot. Sada R.S.F.S.R. 5: 110. 1924 – Type: Uzbekistan. Turkestania rossica, syst. fl. Tshirtshik, in declivibus faucium fl. Nurek-ata, 3.VIII.1914, *Z. v. Minkwitz, Iter ad distr. Taschkent 1914, Nº1155* (LE).

Arctium fedtschenkoanum (Bornm.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia fedtschenkoana* Bornm. in Beih. Bot. Centralbl. 34: 199. 1916 – Type: Tajikistan. Samarkand, alpes Sarawschan, in jugis excelsis inter Simarl et Ansob, 3200 m. 26.7.1913, *J.F.N. Bornmüller 498* (isotypes: B!, LE).

Arctium korshinskyi (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia korshinskyi* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 14: 236. 1897 – Type: Kirghizia. Trajectu Kugart montium Ferghanensium altitudine 8–9000 s. m., VIII.1895, *Korshinsky s.n.* (LE)

Arctium macilentum (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia macilenta* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 14: 222. 1897 – Type: Tajikistan. Prope pagum Sarczob, 06–1896, *Lipsky s.n.* (LE).

Arctium pterolepidum (Kult.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia pterolepida* Kult. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 6: 27. 1929 – Lectotype (Tscherneva, 1962): Uzbekistan. Ugam Range, Kurum-Dzhul Pass, without collector (TASH).

Arctium ugamense (Karmysch.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia ugamensis* Karmysch., Fl. Kazakhst. 9: 228, 568. 1966 – Type: Kazakhstan. Declivia septentrionalia jugi Ugamica fl. Kaskasu, declivum orientale argilloso-schistosum ad orientem vallis Shalpaksai dispositum, 15.VI.1956, fl., *G. Sinicin s.n.* (AA).

Arctium sect. *Lappaceum* (Bunge) Duist.

Perennial hemicryptophyte. Leaves broadly oblong-lanceolate, unarmed or spiny dentate, unarmed. Capitula with up to 10 florets, numerous, sessile, deciduous. Synflorescence racemose. Involucral bracts coriaceous, apically hooked. Florets purple. Distributed in Tian Shan and Pamir Alai. – Type: *Arctium lappaceum* (Schrenk) Kuntze.

Arctium lappaceum (Schrenk) Kuntze, Revis. Gen. Pl. 1: 308. 1891 ≡ *Cousinia lappacea* Schrenk in Fischer & Meyer, Enum. Pl. Nov. 1: 42. 1841 – Type: Kazakhstan. Radicem Montium Alatau, d. 11 Junii m., *Schrenk s.n.* (LE).

Arctium sect. ***Pectinatae*** (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia* sect. *Pectinatae* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 12: 263. 1892 – Type (Tscherneva, 1962): *Cousinia albertii* Regel & Schmalh. ≡ *Arctium albertii* (Regel & Schmalh.) S. López, Romaschenko, Susanna & N. Garcia.

Perennial hemicryptophytes. Leaves entire, prickly-toothed along margin. Capitula with up to 50 florets, numerous. Synflorescence paniculate. Involucral bracts with 2 to 6 pairs of spines along margins ending in a straight spine (apically hooked in *A. pentacanthum*). Florets yellow. Endemic to the Tian Shan (except *A. triflorum* also found in the Pamir Alai and the Kopet-Dagh).

Arctium albertii (Regel & Schmalh.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia albertii* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 315. 1879 – Type: Kazakhstan. In Turkestaniae montibus karatavicis prope Boroldai, *A. Regel s.n.* (LE).

Arctium horrescens (Juz.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia horrescens* Juz. in Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 3: 322. 1937 – Type (Tscherneva, 1962): Uzbekistan. Ad declivia saxoso-argillosa sec. canalem Bos-su prope pag. Niazbek, 19.V.1926, fl., *Granitov 478* (holotype: TASH; isotypes: B!, LE!).

Arctium pentacanthoides (Juz. ex Tscherneva) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia pentacanthoides* Juz. ex Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 17: 102. 1962 – Syntypes: Uzbekistan. Kuraminshiy Range, mountains between Kurashi-Kuduk and Kaynar river, porphyric scree, 1924, *E.P. Korovin 114862, 114863, 114864* (TASH).

Arctium pentacanthum (Regel & Schmalh.) Kuntze, Revis. Gen. Pl. 1: 308. 1891 ≡ *Cousinia pentacantha* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 315. 1879 – Type: Uzbekistan. Prope Samarkand ad fluvium Maili, *Krause s.n.* (LE).

Arctium triflorum (Schrenk) Kuntze, Revis. Gen. Pl. 1: 308. 1891 ≡ *Cousinia triflora* Schrenk in Bull. Cl. Phys.-Math. Acad. Imp. Sci. Saint-Pétersbourg 3: 108. 1844 – Type: Kazakhstan. Chantau, *Schrenk 250* (WU!, probably also in LE, non vid.).

Arctium sect. *Pseudarctium* (Juz.) Duist. in Gorteria 3: 112. 1996

Perennial hemicryptophytes. Leaves irregularly undulate, unarmed. Synflorescence paniculate. Capitula sessile to short pedunculated, with up to 50 florets. Involucral bracts with marginal glands, apically hooked. Florets whitish or purple.

Present in Tien Shan, Pamir Alai, Turkmenistan Mountain Range, north Iran and Afghanistan. – Type (Duistermaat, 1996): *Arctium umbrosum* (Bunge) Kuntze

Arctium amplissimum (Boiss.) Kuntze, Revis. Gen. Pl. 1: 307. 1891 ≡ *Cousinia amplissima* Boiss., Fl. Orient. 3: 462. 1875 – Type (Duistermaat, 1996): prope pagum Dusekurd ad radice montis Kuh-Daëna in Persia Australis, 16/6/1842, *Kotschy 735* (holotype: G; isotypes: BM, K, LE, P, W).

Arctium pseudarctium (Bornm.) Duist. in Gorteria Suppl. 3: 114. 1996 ≡ *Cousinia pseudarctium* Bornm. in Beih. Bot. Centralbl. 34: 135. 1916 – Lectotype (Duistermaat, 1996: 115): Tajikistan. Prov. Samarkand, alpes Sarawschan, ditionis fluvii Jagnob in herbidis ad pag. Warsout, 2400 m. 27.07.1913, *Bornmüller, Plantae Turkestanicae 562*, (LE; isolectotypes, B!, W). Syntypes: Ibid., *Bornmüller 564* (B!, LE, W); inter Piskan et Nowobad, 2800 m, 28.07.1913, *Bornmüller 614* (B!); 15.07.1913, *B.A. Fedtschenko 340a* (B!, LE, W).

Arctium tomentellum (C. Winkl.) Kuntze, Revis. Gen. Pl. 1: 308. 1891 ≡ *Cousinia tomentella* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 10: 469. 1889 – Type: Uzbekistan. M. Hissar, 1882, *Regel s.n.* (B!, probably also in LE, non vid.).

Arctium umbrosum (Bunge) Kuntze, Revis. Gen. Pl. 1: 308. 1891 ≡ *Cousinia umbrosa* Bunge, Mém. Acad. Sci. Saint Pétersbourg, Sér. 7, 9: 10. 1865 – Type: Iran. Prope Dschegar in prov. Khorassan Persiae bor-orientalis, 06.VII.1858, *Bunge s.n.* (LE).

Arctium sect. ***Schmalhausenia*** (C. Winkl.) S. López, Romaschenko, Susanna & N. Garcia, **comb. et stat. nov.** ≡ *Schmalhausenia* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 12: 281. 1892 – Type: *Schmalhausenia nidulans* (Regel) Petr. ≡ *Arctium eriophorum* (Regel & Schmalh.) Kuntze.

Perennial hemicryptophyte, densely tomentose. Stems and nerves purple, scape hollow 2 to 3 cm in diameter. Leaves very spiny, bipinnatisect. Compact synflorescences with sessile heads, in clusters of 5–10, woolly. Involucral bracts ending in a straight spine. Florets purple. Endemic to the Tian Shan.

Arctium eriophorum (Regel & Schmalh.) Kuntze, Revis. Gen. Pl. 1: 307. 1891 ≡ *Cousinia eriophora* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 313. 1879 ≡ *Schmalhausenia nidulans* (Regel) Petr. in Allg. Bot. Z. Syst. 20: 117. 1914 – Type (Tscherneva, 1962): Kok-Dzhar Pass, without collector (LE).

Arctium sect. ***Serratulopsis*** (Tscherneva) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia* sect. *Serratulopsis* Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 17: 83. 1962 – Type: *Cousinia vavilovii* Kult. ≡ *Arctium vavilovii* (Kult.) S. López, Romaschenko, Susanna & N. Garcia

Perennial hemicryptophyte. Leaves ovate, entire, unarmed. Capitula solitary, long pedunculate, oblong-ovoid, glabrous. Involucral bracts ending in a straight spine, the inner ones with a scarious, purple appendage. Florets whitish. Endemic to the Tian Shan.

Arctium vavilovii (Kult.) S. López, Romaschenko, Susanna & N. Garcia, **comb. nov.** ≡ *Cousinia vavilovii* Kult. in Byull. Sredne-Aziatsk. Gosud. Univ. 12(Suppl.): 15. 1926 – Type: Kazakhstan. Prov. Syr-Darja, distr. Aulie-ata., ad declivia saxosa regionis subalpinae in montibus Alexandri prope Utsch-Bulak, 7.VII.1924, *Popov s.n.* (B!, TASH, W!).

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Appendix 1. Voucher data, sources of material, and GenBank accession numbers (ITS1; ITS2; *trnL-trnT-rpS4*; *rpl32-trnL*) of the 37 species included in this work. Asterisks (*) indicate new sequences.

Anura pallidivirens Kult., Uzbekistan: sine loc., *Botschantzev & Kamelin s.n.* (LE), EU923768; EU923882; EU661126; HQ184233*. ***Arctium lappa*** L., Iran: NNE, Khorassan, 25 SW Bodjnourd, 1500 m, *Mehregan 140* (MJG), EU923773; EU923887; EU661123; HQ184229*. ***Arctium leiospermum*** Juz. & C. Serg., Kazakhstan: Zambylskaya oblast, Kurdai pass, 900 m, *Susanna 2154 & al.* (BC), AY373720; AY373687; EU661121 ; HQ184230*. ***Arctium minus*** (Hill.) Bernh., Belgium: Lovaina Botanical Garden, AF19049; AF19103; EU661122 ; HQ184231*. ***Arctium palladini*** (Marcow.) R.E. Fr. & E.S. Söderb., Caucasus: *sine coll. 311* (LE), HQ184214*; HQ184221*; HQ184205*; HQ184232*. ***Cousinia abolinii*** Kult. ex Tscherneva, Kyrgyzstan: SW, Jalal Abad Oblast, Kara Saj Tal, Aksy Rayan, 1030 m, *Lazkov s.n.* (JE), EU923763; EU923877; EU661113; HQ184234*. ***Cousinia albertii*** Regel & Schmalh., Kazakhstan: Shimkientskaya oblast, Mashat canyon, 1840 m, *Susanna 2206 & al.* (BC), AY373721; AY373688; EU661099; HQ184235*. ***Cousinia amplissima*** (Boiss.) Boiss., Iran: Dena, 15 km from Meimand to Yassoudj, 2500 m, *Mehregan 174* (MJG), EU923766; EU923880; EU661098; HQ184236*. ***Cousinia anomala*** Franch., Tajikistan: slopes above Voru, about 300 m above Kishlak, 2200–2300 m, *Susanna 2521 & al.* (BC), EU923770; EU923884; EU661115; HQ184237*. ***Cousinia arctioides*** Schrenk, Kazakhstan: Dzhezkazganskaya reg., Turgajskaya lowland, 49 km to SW from Dzhezkazgana, right bank of Kumula river, *Tamarix* bushland, *Kamelin 6434* (LE), EU923772; EU923886; EU661118; HQ184238*. ***Cousinia aurea*** C. Winkl., Tajikistan: Schtut, road to Penjikent, *Susanna 2514 & al.* (BC), HQ199843*; HQ184222*; HQ184206*; HQ184239*. ***Cousinia chlorantha*** Kult., Uzbekistan: Malguzar mt., S slope, by Tashkesken ¨say¨, *Kamelin 199* (LE), EU923864; EU923978; EU661120; HQ184240*. ***Cousinia dolichophylla*** Kult., Uzbekistan: Ugamsky range above Nanaj, right edge of Pskem valley, "shiblyak", *Kamelin 265* (LE), EU923875; EU923989; EU661117; HQ184241*. ***Cousinia egregia*** Juz., Uzbekistan: Angren valley, rise to Kamchik pass, rubby slope, *Kamelin 420* (LE), EU923866; EU923980; EU661196; HQ184242*. ***Cousinia fedtschenkoana*** Bornm., Tajikistan: *Romaschenko 632 & Susanna* (BC), HQ184215*; HQ199842*; HQ184207*; HQ184243*. ***Cousinia grandifolia*** Kult., Kazakhstan: Zambylskaya oblast, Talaski Alatau, 6 km W from Il Tai, 1000 m, *Susanna 2181 & al.* (BC), AY373730; AY373697; EU661114; HQ184244*. ***Cousinia horrescens*** Juz., Kazakhstan: Tian Shan occidentalis, ad declivia saxoso-arcillosa secus canales Bos-su prope pagum Niakbek, *Granitov 478* (LE), HQ184216*; HQ184223*; HQ184208*; HQ184245*. ***Cousinia karatavica*** Regel & Schmalh., Kazakhstan: Zambylskaya oblast, Karatau mt., Kuyuk pass, 1000 m, *Susanna 2162 & al.* (BC), AY373732; AY373699; EU661101; HQ184246*. ***Cousinia korolkowii*** Regel & Schmalh., Uzbekistan: N macro-mountainside, Nuratau range, Sintob kishlak surroundings, rocky slopes, *Botschantzev 427* (LE), EU923865; EU923979; EU661103; HQ184247*. ***Cousinia korshinskyi*** C. Winkl., Kyrgyzstan: isolated terrain feature Kanka, upper waters of river Kanka, near snow pot, h. 2300 m, *E.M. Il'ina s.n.* (LE), EU923765; EU923879; EU661102; HQ184248*. ***Cousinia lappacea*** Schrenk, Kazakhstan: Zambylskaya oblast, Kurdai pass, 900 m, *Susanna 2150 & al.* (BC), AY373733; AY373700; EU661112; HQ184249*. ***Cousinia macilenta*** C. Winkl, Tajikistan: SW, Jugum Hissaricum (Gissar), divorticum aquarum inter flumina Ljuczob et Unou, 3000 m, *Zaprojagaev s.n.* (M), EU923764; EU923878; EU661119; HQ184250*. ***Cousinia medians*** Juz., Uzbekistan: S, Supkhandarbinskaya reg., between kishlaks Sajrob and Shurob, 121–122 km of the road from Termez, Speckled rock outcrops, *Botschantzev 240* (LE), EU923769; EU923883; EU661100; HQ184251*. ***Cousinia meghrica*** Takht., Armenia: Sjunik, Meghri distr., Agarak village, *Tamanian s.n.* (ERE), EU923814; EU923928; EU661142; HQ184252*. ***Cousinia pentacantha*** Regel & Schmalh., Tajikistan: *Spiridonow* 173 (LE), HQ184217*; HQ184224*; HQ184209*; HQ184253*. ***Cousinia pentacanthoides*** Juz. ex Tscherneva, Uzbekistan: *sine coll. 65* (LE), HQ184218*; HQ184225*; HQ184210* HQ184254*. ***Cousinia pseudarctium*** Bornm., Tajikistan: Vorzov valley, 2 km N kishlak Ziddy, *Susanna 2477 & al.* (BC), EU923876; EU923990; EU661095; HQ184255*. ***Cousinia refracta*** (Bornm.) Juz., Tajikistan: Kondara river canyon, Varzowski Rayon reservation, *Susanna 2456 & al.* (BC), EU923867; EU923981; EU661111; HQ184256*. ***Cousinia schmalhausenii*** C. Winkl., Uzbekistan: *Botschantzev 275* (LE), HQ184219*; HQ184226*; HQ184211*; HQ184257*. ***Cousinia serawschanica*** C. Winkl., Tajikistan: Voru, 2000–2300 m, *Susanna 2516 & al.* (BC), EU923874; EU923988; EU661192; HQ184258*. ***Cousinia tomentella*** C. Winkl., Tajikistan: S mountainside of Guissar range, left bank of Varzob river, Deamalik kishlak surroundings, 1800 m, *Tschukavina 10512* (LE), EU923767; EU923881; EU661097; HQ184259*. ***Cousinia triflora*** Schrenk, Iran: Golestan Nat. Park, Yakhbala pass, *Akhani 102* (MJG), EU923771; EU923885; EU661094; HQ184260*. ***Cousinia umbrosa*** Bunge, Kazakhstan: Almatinskaya oblast, Alatau mt. above Almaty, 1200 m, *Susanna 2100 & al.* (BC), AY373745; AY373712; EU661096; HQ184261*. ***Cousinia vavilovii*** Kult., Kazakhstan: prov. Syr-Darja, distr. Aulie-ata. ad declivia saxosa regionis subalpinae in montibus Alexandri prope Utsch-Bulak, *Popov s.n.* (B), EU923632; EU923711; HQ184227*; HQ184212*. ***Hypacanthium echinopifolium*** (Bornm.) Juz., Kyrgyzstan: sine loc., *Iljin s.n.* (LE), AY373746; AY373713; EU661125; HQ184262*. ***Hypacanthium evidens*** Tscherneva, Uzbekistan: sine loc., *sine coll., s.n.* (LE), HQ184220*; HQ184228*; HQ184213*; HQ184263*. ***Schmalhausenia nidulans*** (Regel) Petrak, Kazakhstan: Almatinskaya oblast, Alatau mt., above Almaty, *Susanna 2088 & al.* (BC), AY373752; AY373719; EU661124; HQ184264*.