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EVOLUCIÓ, FILOGÈNIA I SISTEMÀTICA DEL COMPLEX *ARCTIUM-COUSINIA*

Memòria presentada per Sara López Viñallonga per a optar al títol de Doctor per la Universitat de Barcelona

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7. 2. The *Arctium-Cousinia* complex: disentangling *Arctium* and *Cousinia* (Cardueae, Carduinae)

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RESUM. A aquest treball s'ha investigat la filogènia del llinatge Arctioide del complex *Arctium-Cousinia* per tal d'intentar resoldre l'antiga controvèrsia de la delimitació entre els gèneres *Arctium* i *Cousinia*. Per fer-ho s'han analitzat dades moleculars d'una regió nuclear (ITS) i dues cloroplàstiques (*rpS4-trnT-trnL* i *rpl32-trnL*) de 37 espècies que s'han complementat amb evidències morfològiques, quan ha estat possible, gràcies a l'estudi de 323 plecs d'herbari. Com a resultat, s'ha proposat un gènere *Arctium* monofilètic i més ampli de manera que ara comprèn, a més de les espècies del gènere *Arctium* tal i com s'entenia fins ara, tota la resta d'espècies del clade Arctioide pertanyents als gèneres *Anura*, *Cousinia* (subg. *Cynaroides* i *Hypacanthodes*), *Hypacanthium* i *Schmalhausenia*. Aquest, alhora s'ha subdividit en dos nous subgèneres anomenats subg. *Arctium* i subg. *Cynaroides*. Aquests canvis han comportat la supressió de *Cousinia* subg. *Hypacanthodes* i la reordenació del subg. *Cynaroides* donat que tal i com estaven definits no eren monofilètics. En canvi, la classificació seccional tradicional s'ha mantingut en la seva major part. S'inclou una proposta de canvi nomenclatural de totes les espècies transferides a *Arctium* des de la resta de gèneres esmentats. Malgrat tot, degut a la manca de caràcters morfològics prou adients per a recolzar l'aproximació presentada aquí, cal un treball més exhaustiu per tal de poder establir una classificació més robusta en els rangs subgenèric i seccional.

Es preveu enviar aquest article a la revista *Taxon*.

The *Arctium-Cousinia* complex: disentangling *Arctium* and *Cousinia* (Cardueae, Carduinae)

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Abstract

The phylogeny of the Arctioid lineage of the *Arctium-Cousinia* complex is investigated in an attempt to establish the conflictive generic boundaries of *Arctium* and *Cousinia*. This work is based on analyses of one nuclear (ITS) and two chloroplastic DNA regions (*rpS4-trnT-trnL* and *rpl32-trnL*) of 37 species complemented with morphological evidences when possible (323 herbarium sheets are studied). A broadly monophyletic genus *Arctium* is proposed which is divided into two new monophyletic subgenera, subg. *Arctium* and subg. *Cynaroides*. These rearrangements lead to the suppression of subgenus *Hypacanthodes* and the recombination and redefinition of subgenus *Cynaroides* because as presently defined they are not monophyletic. In contrast, the traditional sectional classification is mainly maintained.

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 achenes streaky (with wavy fringes) and very often winged lacking the apical nectary, typical 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 stated by Rechinger (1986) and Knapp (1987), the vast majority of species of this complex is distributed in the Turkestan mountain region (Tien Shan and Pamir-Alay) and the Irano-Turanian region, except *Arctium* s.str. which is Eurosiberian in distribution.

Table 1. Classification of the Arctioid group according to Tscherneva (1962, 1982, 1988a, b, c) and Duistermaat (1996).

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

The genus *Arctium* was first described by Linnaeus (1753) although some authors in the 18th and 19th century kept using the illegitimate prelinnaean synonym *Lappa* Scop. Later, *Cousinia* was described by Cassini (1827) based on *Carduus orientalis* Adam, *Schmalhausenia* was described by Winkler (1892) and finally Juzepczuk (1937) described the genus *Hypacanthium*.

There are a number of works dealing with the *Arctium-Cousinia* complex, most of them based on morphological characters (De Candolle, 1838; Bunge, 1865; Boissier, 1875, 1888; Kuntze, 1891; Winkler, 1892, 1897; Dittrich, 1977; Duistermaat, 1996, 1997; Petit, 1997; Häffner, 2000). Many others also use biogeographical evidences (Takhtajan, 1938; Rechinger, 1953, 1972, 1979, 1986; Tscherneva, 1962, 1974, 1982, 1988a, 1988b, 1988c; Davis, 1975; Knapp, 1987; Tamanian, 1999), palynological data (Schtepa, 1966, 1973, 1976; Kuprianova & Tscherneva, 1982; Qaid, 1990; Petit et 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 et al., 2003b; Ghaffari et al., 2000, 2006; López-Vinyallonga et al., in press) or molecular data (Häffner & Hellwig, 1999; Garcia-Jacas et al., 2002; Susanna et al., 2003a, 2006; López-Vinyallonga et al., 2009).

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

Despite all the aforementioned works, the precise limits between *Arctium* and *Cousinia* are unclear and have not been clearly established yet. All the problems for establishing an accurate delimitation of these genera are circumscribed to the Arctioid group, for two main reasons: firstly because a group of *Cousinia* species share some characters with *Arctium* and secondly both *Cousinia* subgenera *Cynaroides* and *Hypacanthodes* are not monophyletic (López-Vinyallonga et al., 2009). These unclear boundaries are reflected in many reclassifications and even changes of genus adscription such as some illustrative examples we shortly comment.

Duistermaat (1996) included five species of *Cousinia* subg. *Cynaroides* in *Arctium* s.l., which according to her analyses remained monophyletic and easily recognizable by the presence of hooked involucral bracts and would contain 11 species. Duistermaat decided not to include *C. anomala* in *Arctium*, as suggested by Schtepa (1971, 1973) based on pollen type and despite having hooked involucral bracts. Following Duistermaat's cladogram this solution would mean that at least the entire subgenus *Cynaroideae* should be included in the genus *Arctium* leading, according to her, to a poorly recognizable genus. In spite of all these considerations, Duistermaat admitted that accepting *Arctium* s.l. as a separate genus would leave *Cousinia* paraphyletic (Duistermaat, 1996). As an extreme, Kuntze (1891) adopted the solution of merging the whole genus *Cousinia* into *Arctium* in view of the impossibility of establishing clear boundaries between these two genera.

Following the latest molecular phylogeny available (López-Vinyallonga et al., 2009), current classification of the Arctioid group (Table 1) does not reflect natural phylogenetic relationships among its species. Therefore, the main goal of the present work is to establish the generic boundaries of *Arctium* and *Cousinia*. Furthermore, we aim to provide a preliminary infrageneric classification of the Arctioid lineage.

MATERIAL AND METHODS

Molecular phylogenetic study

<u>Plant material</u>

The data described and discussed here are based on a sample of 37 species for which sequences of the nuclear ribosomal internal transcribed spacer region ITS1-ITS2, and the chloroplastic intergenic spacers *rpS4-trnT-trnL* and *rpl32-trnL* were obtained. We have included the four sections of subg. *Hypacanthodes*, the seven sections of subg. *Cynaroides*, four species of the two sections of *Arctium*, the two species of *Hypacanthium* and the unique *Schmalhausenia* species. Two outgroup taxa were selected from *Cousinia* subgenus *Cousinia* on the basis of the previous analyses in López-Vinyallonga et al. (2009): *Cousinia meghrica* Takht. and *Cousinia serawschanica* C. Winkl. All the 37 *rpl32-trnL* sequences included in the analyses are new and so are 10 out of 37 ITS and 10 out of 37 *rpS4-trnT-trnL* sequences. Voucher data, sources of material and GenBank accession numbers of the above 37 species are given in Table 2.

Table 2. Voucher data, sources of material and GenBank accession numbers (ITS1; ITS2; *rpS4-trnT-trnL*; *rpl32-trnL*) of the 37 species studied in the present work. Na means non available data.

Species	Locality	GenBank accessions
A. pallidivirens (Kult.) Tscherneva	Uzbekistan: Sine loc., *Botschantzev s.n.* (LE)	EU923768; EU923882; EU661126; Na
A. lappa L.	Iran: NNE, Khorassan: 25 SW Bodjnourd, 1500 m, *Mehregan 140* (MJG)	EU923773; EU923887; EU661123; Na
Arctium leiospermum Juz. & C. Serg.	Kazakhstan: Zambylskaya oblast, Kurdai pass, 900 m, *Susanna 2154 & al.* (BC)	AY373720; AY373687; EU661121; Na
A. minus (Hill) Bernh.	Belgium: Lovaina Botanical Garden	AF19049; AF19103; EU661122; Na
A. palladini (Marcow.) R.E.Fr. & E.S. Söderb.	Caucasus: sine col. *311* (LE)	Na; Na; Na; Na
C. meghrica Takht.	Armenia: Sjunik, Meghri distr., Agarak village, *Tamanian s.n.* (ERE)	EU923814; EU923928; EU661142; Na
C. serawschanica C. Winkl.	Tajikistan: Voru, 2000–2300 m, *Susanna 2516 & al.* (BC)	EU923874; EU923988; EU661192; Na
C. aurea C. Winkl.	Tajikistan: Schtut, road to Penjikent, *Susanna 2514 & al.* (BC)	Na; Na; Na; Na
C. chlorantha Kult.	Uzbekistan: Malguzar mt., S slope, by Tashkesken ¨say¨, *Kamelin 199* (LE)	EU923864; EU923978; EU661120; Na

C. karatavica Regel & Schmalh.	Kazakhstan: Zambylskaya oblast, Karatau mt., Kuyuk pass, 1000 m, *Susanna 2162 & al.* (BC)	AY373732; AY373699; EU661101; Na
C. korolkowii Regel & Schmalh.	Uzbekistan: N macro-mountainside, Nuratau range, Sintob kishlak surroundings, rocky slopes, *Botschantzev 427* (LE)	EU923865; EU923979; EU661103; Na
C. 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; Na
C. refracta (Bornm.) Juz.	Tajikistan: Kondara river canyon, Varzowski Rayon reservation, *Susanna 2456 & al.* (BC)	EU923867; EU923981; EU661111; Na
C. schmalhausenii C. Winkl.	Uzbekistan: *Botschantzev 275* (LE)	Na; Na; Na; Na
C. anomala Franch.	Tajikistan: slopes above Voru, about 300 m above the kishlak, 2200–2300 m, *Susanna 2521 & al.* (BC)	EU923770; EU923884; EU661115; Na
C. lappacea Schrenk	Kazakhstan: Zambylskaya oblast, Kurdai pass, 900 m, *Susanna 2150 & al.* (BC)	AY373733; AY373700; EU661112; Na
C. 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; Na
C. triflora Schrenk	Iran: Golestan Nat. Park, Yakhbala pass, *Akhani 102* (MJG)	EU923771; EU923885; EU661094; Na
C. albertii Regel & Schmalh.	Kazakhstan: Shimkientskaya oblast, Mashat canyon, 1840 m, *Susanna 2206 & al.* (BC)	AY373721; AY373688; EU661099; Na
C. horrescens Juz.	Kazakhstan: Tien Shan occidentalis, ad declivia saxoso-arcillosa secus canales Bos-su prope pagum Niakbek, *Granitov 478* (LE)	Na; Na; Na; Na
C. pentacantha Regel & Schmalh.	Tajikistan: *Spiridonow* 173 (LE)	Na; Na; Na; Na
C. pentacanthoides Juz. ex Tscherneva	Uzbekistan: sine col. *65* (LE)	Na; Na; Na; Na
C. amplissima (Boiss.) Boiss.	Iran: Dena, 15 km from Meimand to Yassoudj, 2500 m, *Mehregan 174* (MJG)	EU923766; EU923880; EU661098; Na
C. pseudarctium Bornm.	Tajikistan: Vorzov valley, 2 km N kishlak Ziddy, *Susanna 2477 & al.* (BC)	EU923876; EU923990; EU661095; Na
C. tomentella C. Winkl.	Tajikistan: S mountain side of Guissar range, left bank of Varzob river, Deamalik kishlak surroundings, 1800 m, *Tschukavina 10512* (LE)	EU923767; EU923881; EU661097; Na
C. umbrosa Bunge	Kazakhstan: Almatinskaya oblast, Alatau mt. above Almaty, 1200 m, *Susanna 2100 & al.* (BC)	AY373745;AY373712; EU661096; Na
C. abolinii Kult. ex Tscherneva	Kyrgyzstan: SW, Jalal Abad Oblast, Kara Saj Tal, Aksy Rayan, 1030 m, *Lazkov s.n.* (LE)	EU923763; EU923877; EU661113; Na
C. dolichophylla Kult.	Uzbekistan: Ugamsky range above Nanaj, right edge of Pskem valley, “shiblyak”, *Kamelin 265* (LE)	EU923875; EU923989; EU661117; Na
C. egregia Juz.	Uzbekistan: Angren valley, rise to Kamchik pass, rubby slope, *Kamelin 420* (LE)	EU923866; EU923980; EU661196; Na
C. grandifolia Kult.	Kazakhstan: Zambylskaya oblast, Talaski Alatau, 6 km W from Il Tai, 1000 m, *Susanna 2181 & al.* (BC)	AY373730; AY373697; EU661114; Na
C. korshinskyi C. Winkl.	Kyrgyzstan: isolated terrain feature Kanka, upper waters of river Kanka, near snow pot, 2300 m, *E. M. Il’ina s.n.* (LE)	EU923765; EU923879; EU661102; Na

C. macilenta C. Winkl.	Tajikistan: SW, Jugum Hissaricum (Gissar), divorticum aquarum inter flumina Ljuczob et Unou, 3000 m, *Zaprojagaev s.n.* (M)	EU923764; EU923878; EU661119; Na
C. fedtschenkoana Bornm.	Tajikistan: *Romashchenko 632 & Susanna* (BC)	Na; Na; Na; Na
C. vavilovii Kult.	Kazakhstan: prov. Syr-Darja, distr. Aulie-ata. ad declivia saxosa regionis subalpinae in montibus Alexandri prope Utsch-Bulak, *Popov* (B)	Na; Na; Na; Na
H. echinipifolium (Bornm.) Juz.	Kyrgyzstan: sine loc., *Iljin s.n.* (LE)	AY373746; AY373713; EU661125; Na
H. evidens Tscherneva	Uzbekistan: sine loc., sine col. (LE)	Na; Na; Na; Na
S. nidulans (Regel) Petrak	Kazakhstan: Almatinskaya oblast, Alatau mt., above Almaty, *Susanna 2088 & al.* (BC)	AY373752; AY373719; EU661124; Na

DNA extraction, amplification and sequencing strategies

Total genomic DNA was extracted either following the protocol of the CTAB method of Doyle & Doyle (1987) or following the manufacturer's instructions of the NucleoSpin® Plant Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Double-stranded DNA of ITS and *rpS4-trnT-trnL* were amplified by PCR following the protocol given in López-Vinyallonga et al. (2009). The double-stranded *rpl*32-*trn*L DNA region was amplified by PCR using the forward primer *rpl32*F and reverse primer *trnL*(UAG) (Shaw et al., 2007). Reactions were performed in 25.0 µl volumes with 10% 10x AmpliTaq buffer, 10% 50 mM MgCl2, 10% of 20 mM dNTPs mix, 2% of each primer at 25 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 filled up to 25.0 µl with distilled sterilized water. The profile used for amplification consisted of a preheat 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, CA, USA) or DNA Clean & Concentrator-5 kit (Zymo Research, Orange, CA, USA) following the manufacturer's protocols. Direct sequencing of the amplified DNA segments was performed as explained in López-Vinyallonga et al. (2009) at the "Serveis Científico-Tècnics" of the University of Barcelona on an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences were edited with Chromas 2.0 (Technelysium Pty. Ltd., Tewantin, Australia) and Bioedit 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

ITS, *rpS4-trnT-trnL* and *rpl32-trnL* data sets were analyzed by Bayesian Inference one by one independently as well as combined, so we have built seven matrices to work with. All the analyses were performed with MrBayes software package 3.1.2 (Ronquist & Huelsenbeck, 2003) as explained in López-Vinyallonga et al. (2009) using parameters from the model GTR + G as indicated by ModelTest 3.5 (Posada & Crandall, 1998; 2001) as the best fitting model of molecular evolution for all three markers independently and combined. Congruence in the phylogenetic signal of the cpDNA and nuclear datasets was examined by a visual comparison of tree topologies with branch support ≥ 0.95 PP.

Two simultaneous and independent analyses were performed and for each of them the Markov Chain Monte Carlo process was set so that four chains ran simultaneously for 2.000.000 generations sampling one out of every 200 generations, which resulted in a total of 10,000 sample trees in each run. As stationarity was achieved by the 1.000th tree, the first 999 trees were discarded in order 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.

Morphological survey

Those analyses based on molecular data were complemented with morphological evidences based on the study of 323 herbarium sheets, including type specimens, from LE, M and W altogether with some specimens collected by the authors and some colleagues, mentioned in the acknowledgements section, deposited in BC after being revised by Dra. Tscherneva.

RESULTS

As a result of the aforementioned analyses, seven trees have been recovered: ITS (Fig. 1; named nuclear from now), *rpS*4-*trnT*-*trnL* (not shown; named *trnT* from now), *rpl32*-*trnL* (not shown; named *rpl32* from now), *rpS*4-*trnT*-*trnL* + *rpl32*-*trnL* (not shown; named chloroplastic from now), ITS + *rpS*4-*trnT*-*trnL* (Fig. 2; named ITS-*trnT* from now), ITS + *rpl32*-*trnL* (not shown; named ITS-*rpl32* from now) and ITS + *rpS*4-*trnT*-*trnL* + *rpl32*-*trnL* (Fig. 3; named combined from now. The subgeneric classification proposed in the present work is shown in this figure).

By tree topology comparison, it seems like there is a little amount of conflictive signal between the two chloroplastic markers although in general, Bayesian inference analyses do not show significant incongruent topologies. Therefore the data from the three markers can be combined in order to improve the phylogenetic signal of the analyses for the Arctioid lineage.

Each chloroplastic marker by itself provides scarce phylogenetic information and consequently both *trnT* and *rpl32* trees consist of unresolved polytomies (trees not shown). In addition, the trees recovered from the ITS-*rpl32* and combined analyses are essentially identical (that's why the first one is not shown), although with the addition of the third marker clades support increases. Further, in the trees resulting from nuclear (Fig. 1), ITS-*trnT* (Fig. 2), ITS-*rpl32* (tree not shown) and combined (Fig. 3) datasets, relationships among species are reasonably well resolved. To sum up, the discussion of the results is based on nuclear (Fig. 1), ITS-*trnT* (Fig. 2) and combined trees (Fig. 3).

All seven analyses confirm the monophyly of the Arctioid clade with the highest support and in most of the analyses a clear subdivision of the Arctioid lineage into two groups is found (PP = 1.00; Figs. 1 - 3). The first subclade formed by all the species of the genera *Arctium*, *Hypacanthium* and the monotypic *Schmalhausenia* together with *Cousinia arctioides*, *C. vavilovii* and *C. grandifolia* (PP = 1.00 in the four analyses mentioned above; Figs. 1 - 3). This clade is subdivided again into one lineage comprising the genus *Arctium* and *C. arctioides* (PP = 1.00 in the same four analyses; Figs. 1 - 3) and a second one merging *Hypacanthium*, *Schmalhausenia*, *C. vavilovii* and

C. grandifolia (PP = 0.99 and PP = 1.00 for ITS-*rpl32* (tree not shown) and combined (Fig. 3) datasets respectively) which have no statistical support in the nuclear (Fig. 1) and ITS-*trnT* (Fig. 2) analyses.

The second subclade with the remaining *Cousinia* species (PP = 1.00 in the four analyses mentioned above; Figs. 1 - 3) which in turn is grouped in two subgroups. The first one contains most species of the paraphyletic subgenus *Hypacanthodes*, although is not statistically supported in any analysis, and the remaining species group together in a second clade with strong support in the four analyses (PP = 0.97 and PP = 1.00 in the nuclear and the rest of analyses respectively; Figs. 1 - 3). In this second clade, the next subdivision leaves *C. lappacea* apart from the rest of the species (PP = 1.00 in all but the nuclear tree; Figs. 1 - 3). After that, *C. korshinsky*i appears isolated from the rest of the species of the polytomy (PP = 1.00 in the nuclear tree (Fig. 1) and without support in the rest of the analyses; Figs. 2, 3). From the remaining species of this second subclade, all trees agree in the monophyly of sect. *Pseudarctium* which is weakly supported in the two independent chloroplastic analyses while is strongly supported in the nuclear (Fig. 1) and the combined (Figs. 2, 3) analyses with PP = 1.00. The nuclear (Fig. 1) and all the combined analyses but the chloroplastic (Figs. 2, 3), also show sect. *Chrysis* to be monophyletic (PP = 1.00) including *C. anomala*, the only species of section *Ctenarctium*. The species of sect. *Pectinatae* group together with *C. triflora*, from the monotypic section *Oligantha*, with strong support in the nuclear and ITS-*trnT* analyses (PP = 0.99; Figs. 1, 2). In the ITS-*rpl32* (not shown) and combined (Fig. 3) trees, A. *palladivirens* merges with the clade of sect. *Pectinatae* leading to a weaker clade while in the nuclear (Fig. 1) and ITS-*trnT* (Fig. 2) analyses appears isolated and then the support for the sect. *Pectinatae* clade reaches PP = 0.99.

DISCUSSION

As found previously (López-Vinyallonga et al., 2009), the Arctioid lineage is clearly monophyletic with strong support and is sharply divided into two monophyletic subclades which leads to two main conclusions regarding generic and subgeneric adscription. First of all, 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 subgroup (Figs. 1 - 3 clade A) while the species of sect. *Pseudarctium* group in the other highly supported subclade (Figs. 1 - 3 clade B). In addition, subgenera *Cynaroides* and *Hypacanthodes* as established by Tscherneva (1988c) are not monophyletic either (Figs. 1 - 3). *Cousinia arctioides* belongs to the first subgroup whereas the remaining species of Subg. *Cynaroides* belongs to the second one. A parallel case in found in Subg. *Hypacanthodes* where all the species but *C. vavilovii* and *C. grandifolia* fall in the second subclade (Figs. 1 - 3).

Because the two broad sister subclades resulting from the split of the Arctioid lineage have strong statistical support in most of the analyses performed, we have decided to give them subgeneric taxonomic rank and name them Subg. *Arctium* and Subg. *Cynaroides* (Fig. 3). Subgenus *Arctium* comprises all the species of *Arctium* sect. *Arctium* sensu Duistermaat (1996) included in the analyses altogether with *Cousinia arctioides*, *C. vavilovii*, *C. grandifolia*, *Schmalhausenia nidulans* and the two species of *Hypcacanthium*. In turn, all the species of *Arctium* sect. *Arctium* sensu Duistermaat (1996) (*A. minus*, *A. leiospermum*, *A. palladini* and *A. lappa*) and *Cousinia* sect. *Nanarctium* (*C. arctioides*) form a strong supported clade which we give sectional rank as sect. *Arctium*. This section is characterized by involucral bracts ending in hooked apical appendage and achenes oblong with apical ridge. We shall comment that our results indicate that *A. leiospermum* and *A. lappa* are different species against the idea of the former being a synonym of *A. lappa* (Duistermaat, 1996). The rest of the species of Subg. *Arctium* -*C. grandifolia, C. vavilovii*, *S. nidulans*, *H. evidens* and *H. echinopifolium*- are merged in a highly supported group in the analysis with the three markers combined (Fig. 3) but weakly supported in the rest of the analyses (Figs. 1, 2). Taking into account the great morphologic differences between these taxa and the traditional classification, we consider this group as a polytomy. Despite this, *S. nidulans*, *H. evidens* and *H. echinopifolium* are really close to each other as well as quite similar morphologically and therefore we propose grouping them into the newly established sect. *Schmalhausenia* (Fig. 3).

Regarding subgenus *Cynaroides,* it includes most of the species of the Arctioid lineage and is again divided into two subgroups (Fig. 3). The first one, weakly supported, comprises most species of the paraphyletic subgenus *Hypacanthodes*, excluding *C.*

grandifolia and *C. vavilovii* merged in the new Subg. *Arctium*, and *C. korshinskyi*, the type species of this paraphyletic subgenus, which appears separated by two branches with PP = 1.00 from the rest of its species. The solution we suggest, waiting for more studies and given its morphological unity, is maintaining this weakly supported group with lower taxonomic rank as sect. *Hypacanthodes*, comprising *C. abolinii*, *C. dolichophylla, C. egregia, C. fedtschenkoana, C. korshinskyi* and *C. macilenta*.

As far as sectional classification is concerned, many species are merged in our analyses congruently with the sectional classification by Tscherneva (1962, 1988a, b, c) and, as pointed out in the results, most of these sections are monophyletic and therefore should be maintained as currently established. All seven trees obtained agree in the monophyly of sect. *Pseudarctium* (Fig. 3) which is well characterized morphologically by apically hooked involucral bracts with marginal glands. Furthermore, our results suggest that *C. amplissima* and *C. pseudarctium* are different species, as Duistermaat (1996) stated, instead of being *C. amplissima* a synonym of *C. pseudarctium* (Tscherneva, 1962).

Most of our analyses also show the sect. *Chrysis* to be monophyletic although *C. anomala*, the only species of sect. *Ctenarctium*, unexpectedly falls into this clade (Fig. 3). The placement of *C. anomala* leads us to suppress sect. *Ctenarctium*. In addition, a subdivision of this lineage, congruent with morphological evidences, is found between *C. anomala, C. aurea, C. medians, C. refracta* and *C. schmalhausenii* (PP = 0.89, PP = 0.97 and PP = 0.99 in the nuclear, ITS-*trnT* and combined analyses respectively) having entire leaves on the one hand and *C. karatavica*, *C. chlorantha* and *C. korolkovii* with pinnatipartite leaves on the other hand.

Besides, our results suggest that sect. *Pectinatae,* including *C. triflora* from the monotypic sect. *Oligantha*, is monophyletic as well (Fig. 3). Although in some analyses *A. palladivirens* merges with this clade reducing its statistical support, 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 margins, not found in any other species of the entire Arctioid lineage. We propose to suppress sect. *Oligantha* in view of the mentioned placement of *C. triflora* supported by morphological evidence.

According to our results (Fig. 3), we suggest maintaining most of the monotypic sections as currently accepted based on morphological evidences (Tscherneva, 1962, 1988a, b, c): sect. *Amberbopsis (C. vavilovii),* sect. *Serratulopsis (C. grandifolia*) and sect. *Lappaceae (C. lappacea*). Finally, we propose to reduce the taxonomic rank of the monotypic genus *Anura* to sectional level as sect. *Anura*, comprising only *A. pallidivirens*.

At this point the main aim of this work, what means to establish the generic boundaries of *Arctium* and *Cousinia*, must be faced. Taking into account that *Arctium* as currently accepted is not monophyletic, it has to be redefined. If we want to keep together the species belonging to *Arctium* according to Duistermaat (1996), the most parsimonious option is to transfer all the Arctioid species to *Arctium*, turning it into a broader and monophyletic genus, which is supported by our analyses. Moreover, the Arctioid lineage is sharply differentiated from the Cousinioid by some important traits, mainly pollen type as well as basic chromosome number, and the molecular phylogeny supports this great differentiation, showing at the same time the monophyly of both clades and the paraphyly of genus *Cousinia*. Therefore the inclusion of the Arctioid species into a broader genus *Arctium* would turn *Cousinia* into a monophyletic genus and would sort out 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 our molecular analyses. As ascertained in López-Vinyallonga et 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. These characters, among others traditionally used in the definition of taxa of different rank in the *Arctium-Cousinia* complex, appear scattered through all the trees obtained denoting that they evolved several times in parallel. Therefore, we present a redefinition of the Arctioid lineage based on the present molecular phylogeny and supported by morphological evidences when possible. In addition, we provide nomenclatural changes when necessary.

Genus *Arctium* L., Sp. Pl. 2: 816 (1753)
Biennial or perennial with rootstock or taproot, spiny or unarmed suffruticose herbs. Leaves leathery or herbaceous, dentate, lobed, pinnatipartite to pinnatisect or rarely entire. Basal leaves in a rosette, the cauline ones similar to bottom leaves but gradually diminishing to stem apex, the most distal ones usually sessile. Synflorescence paniculate, racemose or corymbose. Capitula homogamous, solitary or in clusters, 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 and 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, limb campanulate. 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 somewhat compressed, smooth, rugose or with longitudinal ridges, brown; often with dark and irregular spots, sometimes with apical rim.

Subg. *Arctium*: Biennial or perennial herbs. Leaves leathery or herbaceous, spiny or unarmed, lobed, two-times pinnatisect or rarely entire. Synflorescence racemose or corymbose. Capitula short to long-pedunculate (0,2 – 8 cm) with more than 20 to more than 100 florets, spherical or subspherical, glabrous, tomentose or lanuginose, sometimes glandular. Involucral bracts apex hooked or ending in a straight spine. Corolla tubulose-campanulate, pink to purple, sometimes whitish or yellow, glabrous or with glandular hairs. Anthers with basal appendages entire to fringed.

Subg. *Cynaroides*: Perennial herbs. Leaves leathery or herbaceous, spiny or unarmed, dentate, lobed, pinnatipartite to pinnatisect or rarely entire. Synflorescence paniculate, racemose or corymbose. Capitula sessile to long-pedunculate (up to 10 cm) with three to more than 50 florets, usually ovoid, sometimes subespherical or oblong, glabrous to densely arachnoid. Involucral bracts apex hooked, curved or ending in a straight spine. Corolla tubulose-campanulate, usually yellow and sometimes white or pink, glabrous. Anthers with basal appendages fringed.

Next we list the nomenclatural changes derived from the present work. We provide synonyms only when necessary for avoiding possible confusions. New proposals are boldfaced.

Genus *Arctium*

Arctium Subgenus *Arctium*

Arctium* Sect. *Amberbopsis (Tscherneva) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia* Sect. *Amberbopsis* Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. SSR 17: 83 (1962)
Arctium grandifolium (Kult.) S. López, Susanna & N. Garcia, ***comb. nov***.
Basionym: *Cousinia grandifolia* Kult. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 6: 9 (1929)

Arctium Sect. *Arctium*
Arctium arctioides (Schrenk) Kuntze, Revis. Gen. Pl. 1: 307 (1891)
Basionym: *Cousinia arctioides* Schrenk in Bull. Phys.-Math. Acad. Petersb. 2: 115 (1844)

Arctium atlanticum (Pomel) H. Lindb. in Acta Soc. Sci. Fenn. n. s. B, 1, 2: 153 (1932)

Arctium lappa L., Sp. Pl. 2: 816 (1753)

Arctium leiospermum Juz. & Ye. V. Serg. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk SSSR 18: 299 (1957)

Arctium minus (Hill) Bernh., Syst. Verz. Erfurt: 154 (1800)

Arctium nemorosum Lej. in Mag. Hort. 1: 289 (1833)

Arctium palladini (Marcow.) R. E. Fr. & E. S. Söderb. in Delect. Spor. Sem. Hort. Bot. Berg. (1923)

Arctium tomentosum Mill., Gard. Dict., ed. 8, 3 (1768)

Arctium* Sect. *Schmalhausenia S. López, Susanna & N. Garcia, ***comb.* et *stat. nov.***
Basionym: Gen. *Schmalhausenia* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 12: 281 (1892)

Arctium echinopifolium (Juz.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia echinopifolia* Bornm. in Beih. Bot. Centralbl. 34: 192 (1916).
Synonym: *Hypacanthium echinopifolium* (Bornm.) Juz. in Trudy Bot. Inst. Akad. Nauk SSSR, Ser. 1, Fl. Sist. Vysš. Rast. 3: 324 (1937)

Arctium eriophorum (Regel & Schmalh.) Kuntze, Revis. Gen. Pl. 1: 307 (1891)
Basionym: *Cousinia eriophora* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 313 (1879)
Synonym: *Schmalhausenia nidulans* Petr. in Allg. Bot. Z. Syst. 20: 117 (1914)

Arctium evidens (Tscherneva) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Hypacanthium evidens* Tscherneva in Bot. Žhurn. (Moscow & Leningrad) 68: 634 (1983)

Arctium* Sect. *Serratulopsis (Tscherneva) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia* Sect. *Serratulopsis* Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. SSR 17: 83 (1962)

Arctium vavilovii (Kult.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia vavilovii* Kult. in Bjull. Sredne-Aziatsk. Gosud. Univ. 12. Suppl. 15 (1926)

Arctium* Subgenus *Cynaroides (Tscherneva) S. López, Susanna & N. Garcia, ***comb. nov.***

Basionym: *Cousinia* Subg. *Cynaroides* Tscherneva in Bot. Žhurn. (Moscow & Leningrad) 73: 594 (1988)

Arctium* Sect. *Anura S. López, Susanna & N. Garcia, ***comb. nov.***

Basionym: *Cousinia* Sect. *Anura* Juz. in Trudy Tadžikistansk. Bazy 8: 512, 546 (1940)

Synonym: Gen. *Anura* (Juz.) Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. SSR 17: 107 (1962)

Arctium pallidivirens (Kult.) S. López, Susanna & N. Garcia, ***comb. nov.***

Basionym: *Cousinia pallidivirens* Kult. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 6: 3 (1929)

Synonym: *Anura pallidivirens* (Kult.) Tscherneva, Fl. URSS 27: 134 (1962)

Arctium* Sect. *Chrysis (Juz.) S. López, Susanna & N. Garcia, ***comb. nov.***

Basionym: *Cousinia* Sect. *Chrysis* Juz. in Trudy Tadžikistansk. Bazy 8: 515, 548 (1940)

Arctium anomalum (Franch.) Kuntze, Revis. Gen. Pl. 1: 307 (1891)

Basionym: *Cousinia anomala* Franch. in Ann. Sci. Nat. Bot. 6: 316 (1883)

Arctium aureum (C. Winkl.) Kuntze, Revis. Gen. Pl. 1: 307 (1891)

Basionym: *Cousinia aurea* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 10: 475 (1887)

Arctium chloranthum (Kult.) S. López, Susanna & N. Garcia, ***comb. nov.***

Basionym: *Cousinia chlorantha* Kult. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 6: 4 (1929)

Arctium haesitabundum (Juz.) S. López, Susanna & N. Garcia, ***comb. nov.***

Basionym: *Cousinia haesitabunda* Juz. in Trudy Tadžikistansk. Bazy 8: 516, 549 (1940)

Arctium karatavicum (Regel & Schmalh.) Kuntze, Revis. Gen. Pl. 1: 308 (1891)
Basionym: *Cousinia karatavica* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 317 (1879)

Arctium korolkowii (Regel & Schmalh.) Kuntze, Revis. Gen. Pl. 1: 308 (1891)
Basionym: *Cousinia korolkovii* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 315 (1879)

Arctium medians (Juz.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia medians* Juz. in Trudy Bot. Inst. Akad. Nauk SSSR, Ser. 1, Fl. Sist. Vysš. Rast. 3: 297 (1936)

Arctium refractum (Bornm.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia aurea β refracta* Bornm. in Beih. z. Bot. Centralbl., Bd. 34: 138 (1917)
Synonym: *Cousinia refracta* (Bornm.) Juz. in Trudy Bot. Inst. Akad. Nauk SSSR, Ser. 1, Fl. Sist. Vysš. Rast. 3: 297 (1937)

Arctium schmalhausenii (C. Winkl.) Kuntze, Revis. Gen. Pl. 1: 308 (1891)
Basionym: *Cousinia schmalhausenii* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 10: 474 (1887)

Arctium* Sect. *Hypacanthodes S. López, Susanna & N. Garcia, ***comb. et stat. nov.***
Basionym: *Cousinia* Subg. *Hypacanthodes* Tscherneva in Bot. Žhurn. (Moscow & Leningrad) 73: 594 (1988)

Arctium abolinii (Kult. ex Tscherneva) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia abolinii* Kult. ex Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. SSR 17: 88 (1962)

Arctium dolichophyllum (Kult.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia dolichophylla* Kult., Trudy Turkestansk. Naučn. Obšč. 1: 112 (1923)

Arctium egregium (Juz.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia egregia* Juz. in Bot. Mater. Gerb. Glavn. Bot. Sada RSFSR 5: 110 (1924)

Arctium fedtschenkoanum (Bornm.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia fedtschenkoana* Bornm. in Beih. Bot. Centralbl. 34: 199 (1916)

Arctium korshinskyi (C. Winkl.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia korshinskyi* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 14: 236 (1897)

Arctium macilentum (C. Winkl.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia macilenta* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 14: 222 (1897)

Arctium pterolepidum (Kult.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia pterolepida* Kult. in Trudy Sredne-Aziatsk. Gosud. Univ., Ser. 8b, Bot. 6: 27 (1929)

Arctium ugamicum (Karmysch.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia ugamica* Karmysch., Fl. Kazakhst. 9: 228, 568 (1966)

Arctium Sect. *Lappaceum* (Bunge) Duist.

Arctium lappaceum (Schrenk) Kuntze, Revis. Gen. Pl. 1: 308 (1891)
Basionym: *Cousinia lappacea* Schrenk, Enum. Pl. Nov. 1: 42 (1841)

Arctium* Sect. *Pectinatae (C. Winkl.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia* Sect. *Pectinatae* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 12: 263 (1892)
Arctium alberti (Regel & Schmalh.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia alberti* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 315 (1879)

Arctium horrescens (Juz.) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia horrescens* Juz. in Trudy Bot. Inst. Akad. Nauk SSSR, Ser. 1, Fl. Sist. Vysš. Rast. 3: 322 (1937)

Arctium pentacanthoides (Juz. ex Tscherneva) S. López, Susanna & N. Garcia, ***comb. nov.***
Basionym: *Cousinia pentacanthoides* Juz. ex Tscherneva in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. SSR 17: 102 (1962)

Arctium pentacanthum (Regel & Schmalh.) Kuntze, Revis. Gen. Pl. 1: 308 (1891)
Basionym: *Cousinia pentacantha* Regel & Schmalh. in Trudy Imp. S.-Peterburgsk. Bot. Sada 6: 315 (1879)

Arctium triflorum (Schrenk) Kuntze, Revis. Gen. Pl. 1: 308 (1891)
Basionym: *Cousinia triflora* Schrenk in Bull. Cl. Phys.-Math. Acad. Imp. Sci. Saint-Pétersbourg 3: 108 (1845)

Arctium Sect. *Pseudarctium* (Juz.) Duist. in Gorteria 3: 112 (1996)
Arctium amplissimum (Boiss.) Kuntze, Revis. Gen. Pl. 1: 307 (1891)
Basionym: *Cousinia amplissima* Boiss., Fl. Orient. [Boissier] 3: 462 (1875)

Arctium pseudarctium (Bornm.) Duist. in Gorteria 3: 114 (1996)
Basionym: *Cousinia pseudarctium* Bornm. in Beih. Bot. Centralbl. 34: 135 (1916)

Arctium tomentellum (C. Winkl.) Kuntze, Revis. Gen. Pl. 1: 308 (1891)

Basionym: *Cousinia tomentella* C. Winkl. in Trudy Imp. S.-Peterburgsk. Bot. Sada 10: 469 (1887)

Arctium umbrosum (Bunge) Kuntze, Revis. Gen. Pl. 1: 308 (1891)
Basionym: *Cousinia umbrosa* Bunge, Gatt. Cous. 10

Table 3. Classification of the Arctioid group suggested according with the present results and based on Tscherneva (1962, 1982, 1988a, b, c) and Duistermaat (1996).

Species	Section	Subgenus
Arctium grandifolium (Kult.) S. López, Susanna & N. Garcia	*Amberbopsis* (Tscherneva) S. López, Susanna & N. Garcia	*Arctium* S. López, Susanna & N. Garcia
Arctium arctioides (Schrenk.) Kuntze	*Arctium* L.	
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. et E.S. Söderb.		
Arctium tomentosum Mill.		
Arctium vavilovii (Kult.) S. López, Susanna & N. Garcia	*Serratulopsis* (Tscherneva) S. López, Susanna & N. Garcia	
Arctium echinopifolium (Juz.) S. López, Susanna & N. Garcia	*Schmalhausenia* S. López, Susanna & N. Garcia	
Arctium eriophorum (Regel & Schmalh.) Kuntze		
Arctium evidens (Tscherneva) S. López, Susanna & N. Garcia		
Arctium pallidivirens (Kult.) S. López, Susanna & N. Garcia	*Anura* S. López, Susanna & N. Garcia	*Cynaroides* (Tscherneva) S. López, Susanna & N. Garcia
Arctium anomalum (Franch.) Kuntze	*Chrysis* (Juz.) S. López, Susanna & N. Garcia	
Arctium aureum (C. Winkl.) Kuntze		
Arctium chloranthum (Kult.) S. López, Susanna & N. Garcia		
Arctium haesitabundum (Juz.) S. López, Susanna & N. Garcia		
Arctium karatavicum (Regel & Schmalh.) Kuntze		
Arctium korolkowii (Regel & Schmalh.) Kuntze		
Arctium medians (Juz.) S. López, Susanna & N. Garcia		
Arctium refractum (Bornm.) S.		

López, Susanna & N. Garcia		
Arctium schmalhausenii (C. Winkl.) Kuntze		
Arctium abolinii (Kult. ex Tscherneva) S. López, Susanna & N. Garcia	*Hypacanthodes* S. López, Susanna & N. Garcia	
Arctium dolichophyllum (Kult.) S. López, Susanna & N. Garcia		
Arctium egregium (Juz.) S. López, Susanna & N. Garcia		
Arctium fedtschenkoanum (Bornm.) S. López, Susanna & N. Garcia		
Arctium korshinskyi (C. Winkl.) S. López, Susanna & N. Garcia		
Arctium macilentum (C. Winkl.) S. López, Susanna & N. Garcia		
Arctium pterolepidum (Kult.) S. López, Susanna & N. Garcia		
Arctium ugamicum (Karmysch.) S. López, Susanna & N. Garcia		
Arctium lappaceum (Schrenk.) Kuntze	*Lappaceum* (Bunge) Duist.	
Arctium albertii (Regel & Schmalh.) S. López, Susanna & N. Garcia	*Pectinatae* (C. Winkl.) S. López, Susanna & N. Garcia	
Arctium horrescens (Juz.) S. López, Susanna & N. Garcia		
Arctium pentacanthoides (Juz. ex Tscherneva) S. López, 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		

CONCLUDING REMARKS

Based on the present molecular analyses a broadly monophyletic genus *Arctium* is proposed which is divided into two new monophyletic subgenera, Subg. *Arctium* and Subg. *Cynaroides*. These rearrangements lead to the suppression of subgenus *Hypacanthodes* and the recombination and redefinition of subgenus *Cynaroides* because as previously defined they were paraphyletic. In contrast, the traditional sectional classification is mainly maintained. Further work is needed in order to establish a definitive and robust subgeneric and sectional classification of the Arctioid lineage.

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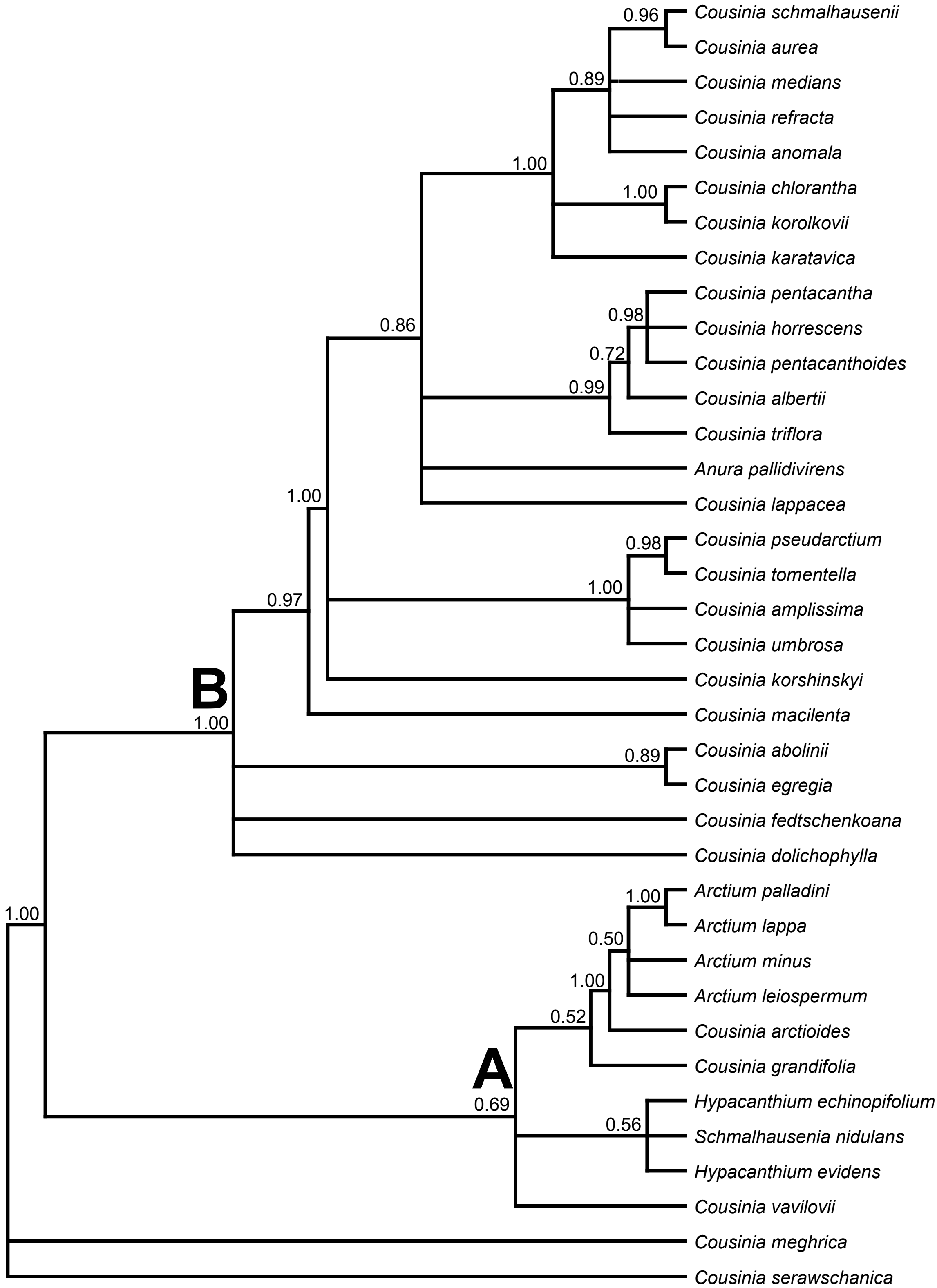

Fig. 1. 50% Majority rule consensus tree obtained from the Bayesian analysis of the ITS data set (namely nuclear tree). Numbers on branches are posterior probabilities. Clade A: subgenus *Arctium*, clade B: subgenus *Cynaroides*.

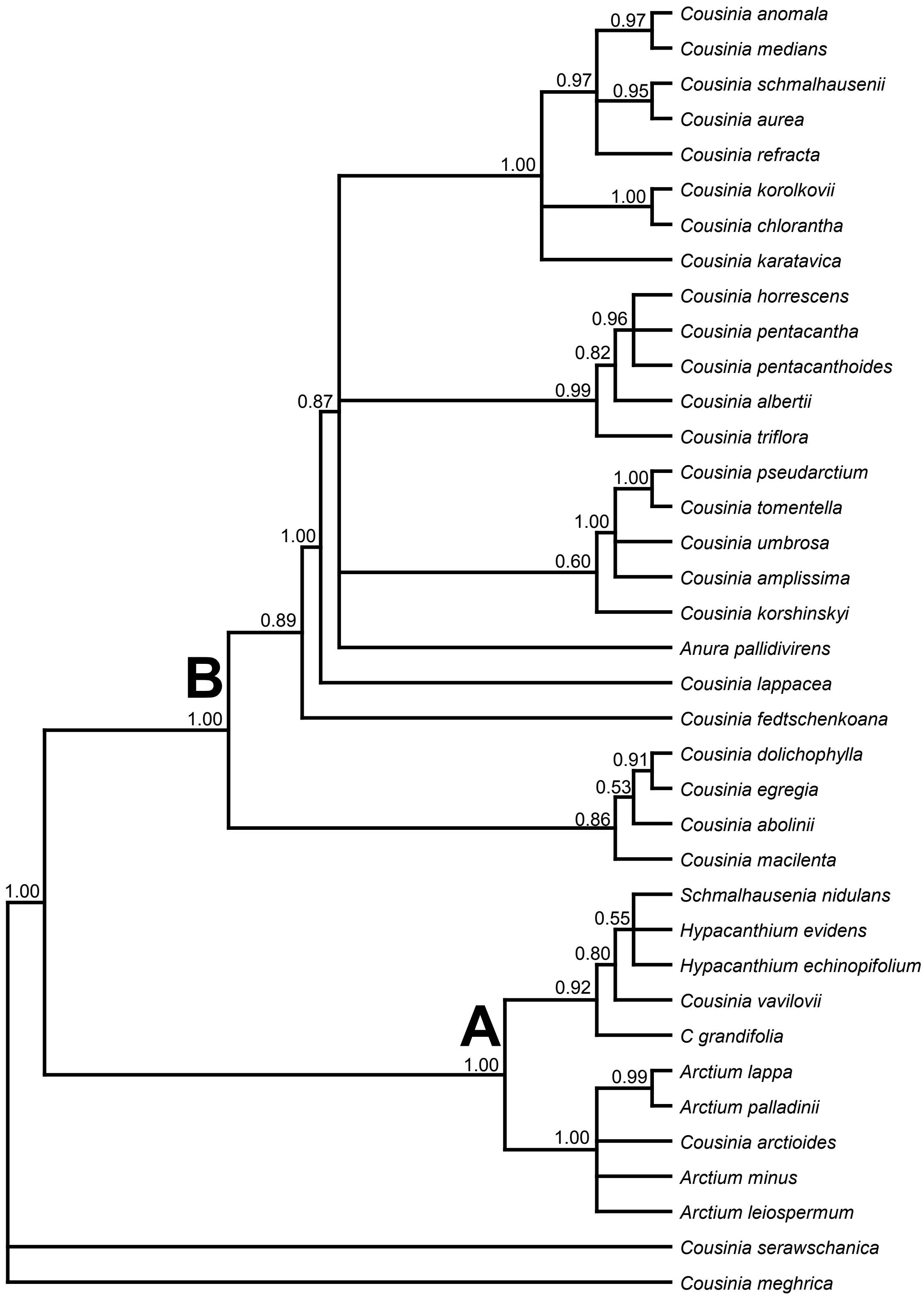

Fig. 2. 50% Majority rule consensus tree obtained from the Bayesian analysis of the ITS + rpS4-*trnT*-*trnL* data set (namely ITS-*trnT* tree). Numbers on branches are posterior probabilities.
Clade A: subgenus *Arctium*, clade B: subgenus *Cynaroides*.

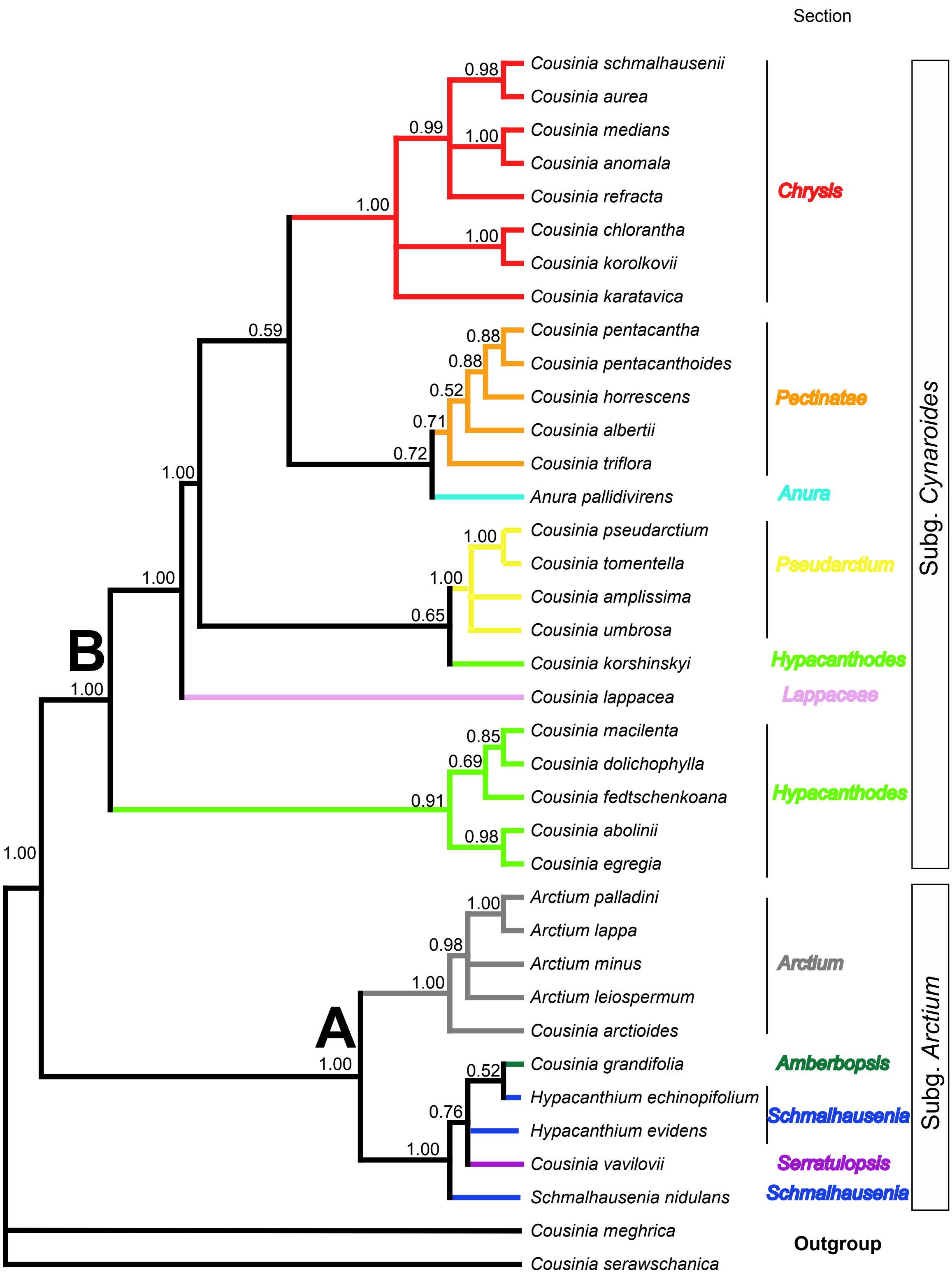

Fig. 3. 50% Majority rule consensus tree obtained from the Bayesian analysis of the ITS + *rpS4-trnT-trnL* + *rpl32-trnL* data set (namely combined tree). Numbers on branches are posterior probabilities. The sectional classification proposed is shown in different colors. Clade A, clade B.