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The role of hybridization in the evolution of *Cousinia* s.str. *(Asteraceae, Cardueae)*

Abstract

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In order to assess the possible role of interspecific hybridization for the evolution of the high diversity of *Cousinia* subg. *Cousinia* (*Cousinia* s.str., c. 600 species), we examined the frequency of hybridization in this taxon. For this, hypothetical hybrid combinations published in the literature (28 putative hybrids and 11 intermediate forms) were critically examined. In addition, two hybrids were identified in the present study by their morphological intermediacy, geographical distribution, additivity of ITS sequences and reduced pollen fertility, and were included in our analysis. To examine the relationships among the presumably hybridizing species, a Bayesian analysis of nuclear-ribosomal ITS of 214 species of *Cousinia* and related genera was performed, being the largest ITS phylogeny of *Cousinia* s.str. published to date, with 78 species having been included for the first time. As a result, neither hybridization between *Cousinia* s.str. and other main clades of the '*Arctium-Cousinia* complex', nor between annual and perennial species of *Cousinia* s.str. is revealed by our analysis. Otherwise, our results clearly show that hybridization in *Cousinia* occurs between species of the same clade and of different clades. An extrapolation of the results indicates that somewhat more than 10 % of the species of *Cousinia* s.str. may be involved in interspecific hybridization. This figure together with the complete absence of polyploids in *Cousinia* s.str. leads to the conclusion that the role of interspecific hybridization for the evolution and diversity of the group is likely to have been minor.

Additional key words: *Compositae, Carduinae, Cousinia* subg. *Cousinia,* ITS, phylogeny, systematics

Introduction

Cousinia Cass. is the largest genus of the *Asteraceae* tribe *Cardueae* and contains about 630 species in SW and C Asia (*C.* subg. *Cousinia* c. 600, *C.* subg. *Hypacanthodes* 10, *C.* subg. *Cynaroides* c. 20 species). Within *Cardueae, Cousinia* belongs to the '*Arctium-Cousinia* complex'. The genus is exceptional in containing a large number of species in a comparatively small geographical area. Eight areas of exceptionally high diversity can be observed. These are the western Tien Shan (c. 60 species), Pamir-Alay (c. 170 species), NE Afghanistan (eastern Hindu Kush; c. 80 species), NW Afghanistan (western Hindu Kush; c. 45 species), Kopetdagh (c. 70 species in Iran, 30 species in Turkmenistan), Elburz (N Iran, c. 70 species), N Zagros (W Iran, c. 45 species) and E Anatolian mountains and Azerbaijan (W Turkey and NW Iran, c. 40 species) (Rechinger 1986; Knapp 1987; Tscherneva 1988, 1990; López-Vinyallonga & al. 2009: fig. 1).

Monophyly of the '*Arctium-Cousinia* complex' has been demonstrated in previous studies based on nuclear and chloroplast DNA sequences (Susanna & al. 2003a). The complex consists of two distinct groups supported by characters such as stigma morphology, pollen type and chromosome number: (1) the arctioid group includes *Arctium* L., *Hypacanthium* Juz., *Schmalhausenia* C. Winkl., *Cousinia* subg. *Cynaroides* and *C.* subg. *Hypacanthodes;* all its members have a diploid chromosome number of $2n = 36$; (2) the cousinioid group includes only *C.* subg. *Cousinia* (= *Cousinia* s.str.) with chromosome numbers of $2n = 18$, 20, 22, 24 and 26 (Kupria-

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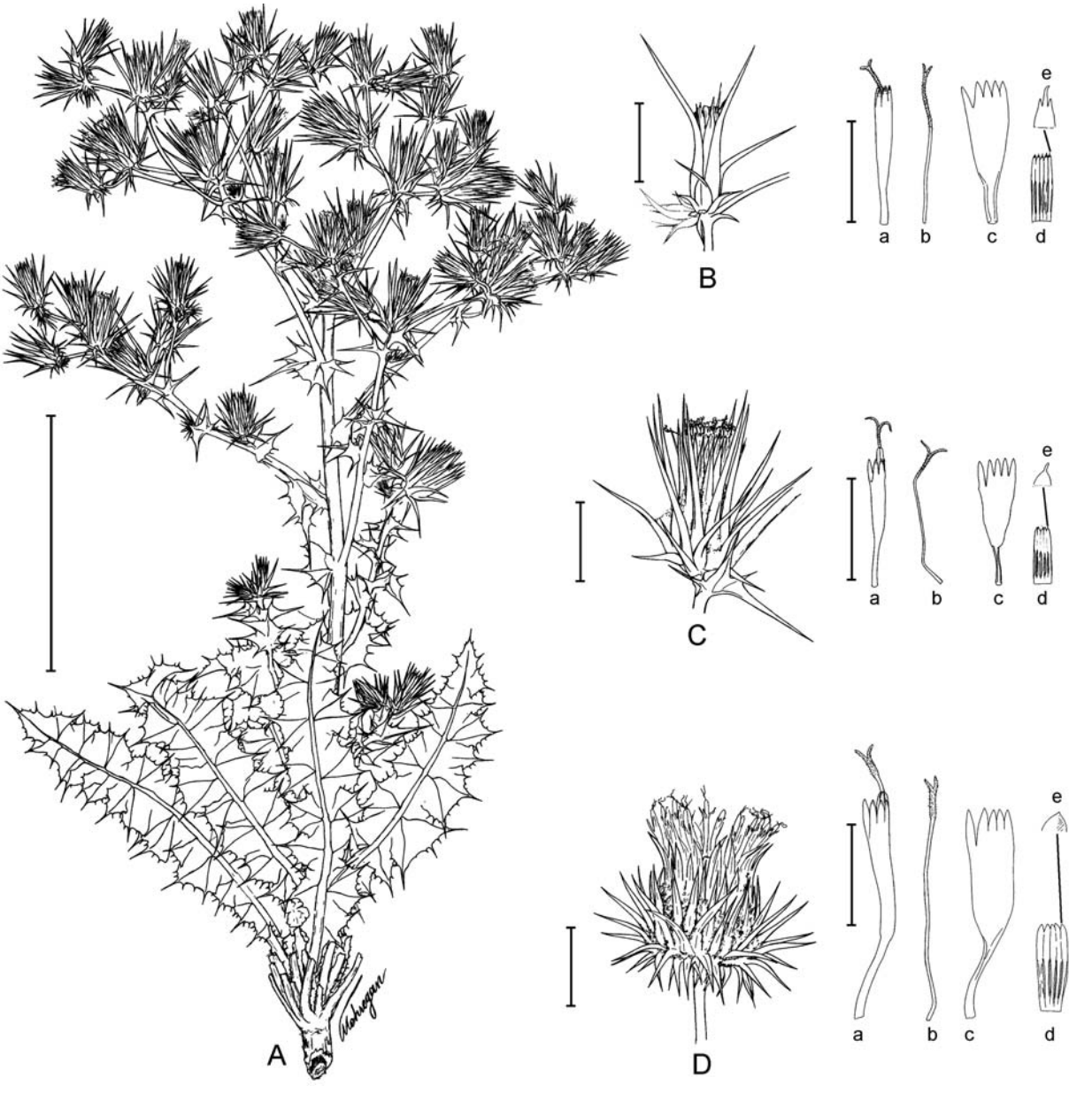

Fig. 1. Morphological intermediacy of the hybrid between *Cousinia eryngioides* and *C. chrysochlora* – A: habit of *C. eryngioides* × *chrysochlora*; B-D: capitulum and flower morphology of *C. eryngioides* (B), *C. eryngioides* × *chrysochlora* (C) and *C. chrysochlora* (D). – a = flower, b = style, c = opened and flattened corolla, d = opened anther tube, e = anther apex. – Scale bars: A = 10 cm, B-D + a-d = 1 cm.

nova & Tscherneva 1982; Duistermaat 1996; Susanna & al. 2003b; Ghaffari & al. 2006; López-Vinyallonga & & al. 2009).

Interspecific hybridization plays an important role in plant and animal evolution (Arnold 1997) and can result in the formation of new taxa via introgressive hybridization and homoploid and polyploid hybrid speciation (Stebbins 1950, 1959; Abbott 1992; Rieseberg 1997; Ramsey & Schemske 1998). Based on a detailed analysis of five floras of Europe (British Isles, Scandinavia), North America (Great Plains, Intermountain West) and the Hawaiian Islands, Ellstrand & al. (1996) estimated the frequency and taxonomic distribution of interspecific hybridization. They suggested that the number of hybrids (expressed as a percentage of the total number of species in these floras) ranges between c. 6 and 21.8 %. They also found hybridization to be non-randomly distributed among taxa. In all five floras analysed *Asteraceae* are among the six families with the highest number of hybrids. According to Rieseberg (1997), the above figures may substantially underestimate true hybrid frequency because a large number of hybrids may be undetected due to the inadequacy of taxonomic knowledge in certain groups and some of the floras analysed. In the flora of the British Isles, one of the best-known floras in the world, 61 (c. 22.8 %) of

Table 1. Published *Cousinia* s.str. hybrids (designated with H) and intermediate forms of possible hybrid origin (designated with I) with references, assumed parents, the latter's current sectional position and the position of the parents in the ITS tree (if data missing: [1)] = one parent only included in tree, [2)] = both parents not included in tree). References: (1) = Rechinger 1950, (2) = Tscherneva 1962, (3) = Rechinger 1972, (4) = Kamelin 1973, (5) = Rechinger 1979.

No.	Confirmed or possible parents	Sectional position of parents	Reference, nothospecies	Position of parents in ITS tree
H 1	*C. decumbens* Rech. f. *C. shahvarica* Rech. f.	*Decumbentes* *Sphaerocephalae*	current paper, –	in different clades
H 2	*C. chrysochlora* Rech. f. & Koie *C. eryngioides* Boiss.	*Lachnosphaerae* *Sciadocousinia*	current paper, –	not resolved
H 3	*C. crispa* Jaub. & Spach *C. gmelini* C. Winkl.	*Serratuloides* *Eriocousinia*	(1), *C.* ×*kamarbandensis* Rech. f.	in same clade
H 4	*C. crispa* Jaub. & Spach *C. sphaerocephala* Jaub. & Spach	*Serratuloides* *Sphaerocephalae*	(1), *C.* ×*hybrida* Rech. f.	–[1)]
H 5	*C. behboudiana* Rech. f. & Esfand. *C. belangeri* DC.	*Cynaroideae* *Pugioniferae*	(5), *C.* ×*mesomorpha* Rech. f.	not resolved
H 6	*C. chlorosphaera* Bornm. *C. orthoclada* Hausskn. & Bornm.	*Cynaroideae* *Pugioniferae*	(3), *C.* ×*subinflata* Rech. f.	not resolved
H 7	*C. turkestanic*a (Tegel) Juz. *C. scabrida* Juz.	*Cousinia* *Cousinia*	(2), –	–[2)]
H 8	*C. resinoa* Juz. *C. maracandica* Juz.	*Cousinia* *Cousinia*	(2), –	–[2)]
H 9	*C. coronata* Franch. *C. microcarpa* Boiss.	*Coronophora* *Microcarpae*	(2), –	in different clades
H 10	*C. radians* Bunge *C. microcarpa* Boiss.	*Coronophora* *Microcarpae*	(4), *C.* ×*paraatripurpurea* Kamelin	in different clades
H 11	*C. alpestris* Bornm. *C. submutica* Franch.	*Coronophora* *Jurineopsis*	(2), *C.* ×*heteromorpha* Bornm.	–[2)]
H 12	*C. alpina* Bunge *C. submutica* Franch.	*Carduncellus* *Jurineopsis*	(2), *C.* ×*iskanderi* Bornm.	–[2)]
H 13	*C. microcarpa* Boiss. *C. submutica* Franch.	*Microcarpae* *Jurineopsis*	(2), –	–[1)]
H 14	*C. outichaschensis* Franch. *C. microcarpa* Boiss.	*Carduncellus* *Microcarpae*	(2), –	–[1)]
H 15	*C. outichaschensis* Franch. *C. pulchella* Bunge	*Carduncellus* *Pulchellae*	(2), *C.* ×*heterogenetos* Bornm.	–[1)]
H 16	*C. microcarpa* Boiss. *C. pulchella* Bunge	*Microcarpae* *Pulchellae*	(2), –	in different clades
H 17	*C. microcarpa* Boiss. *C. sewerzowi* Regel	*Microcarpae* *Pulchellae*	(2), –	in different clades
H 18	*C. ninae* Juz. *S. sewerzowi* Regel	*Microcarpae* *Pulchellae*	(2), *C.* ×*cana* Juz.	in different clades
H 19	*C. jassyensis* C. Winkl. *C. ninae* Juz.	*Jurineopsis* *Microcarpae*	(2), –	in different clades
H 20	*C. xanthina* Bornm. *C. laetevirens* C. Winkl.	*Alpinae* *Alpinae*	(2), –	–[2)]
H 21	*C. trichophora* Kult. *C. newesskyana* C. Winkl.	*Alpinae* *Alpinae*	(2), –	–[2)]
H 22	*C. verticillaris* Bunge *C. laetevirens* C. Winkl	*Alpinae* *Alpinae*	(2), –	–[1)]
H 23	*C. myrioglochis* Rech. f. *C. kataghanica* Rech. f.	*Alpinae* *Alpinae*	(3), –	–[1)]
H 24	*C. ramulosa* Rech. f. *C. schugnanica* Juz.	*Alpinae* *Alpinae*	(3), –	–[2)]

Table 1, continued from preceding page

No.	Confirmed or possible parents	Sectional position of parents	Reference, nothospecies	Position of parents in ITS tree
H 25	*C. auriculata* Boiss. *C. chionophila* Rech. f. & Kopie	*Eriocousinia* *Eriocousinia*	(3), –	in same clade
H 26	*C. urumiensis* Bornm. *C. tenuifolia* C. A. Mey.	*Cousinia* *Cousinia*	(3), –	not resolved
H 27	*C. aggregata* DC. *C. cylindracea* Boiss.	*Stenocephalae* *Stenocephalae*	(3), –	–[1)]
H 28	*C. ecbatanensis* Bornm. *C. kopi-karadaghensis* Rech. f. or *C. inflata* Boiss. & Hausskn.	*Cynaroideae* *Cynaroideae*	(3), –	–[1)]
I 1	*C. hypopolia* Bornm. *C. albiflora* (Bornm. & Sint.) Bornm.	*Stenocephalae* *Stenocephalae*	(2), –	–[2)]
I 2	*C. oxiana* Tschern. *C. turkmenorum* Bornm.	*Chrysoptera* *Chrysoptera*	(2), –	in same clade
I 3	*C. lamakini* C. Winkl. *C. erivanensis* Bornm.	*Cousinia* *Cousinia*	(2), –	–[2)]
I 4	*C. fetissowi* C. Winkl. *C. schischkinii* Juz.	*Carduncellus* *Carduncellus*	(2), –	–[2)]
I 5	*C. verticillaris* Bunge *C. blepharobasis* Rech. f. & Gilli	*Alpinae* *Alpinae*	(3), –	–[1)]
I 6	*C. heterophylla* Boiss. *C. miserabilis* Rech. f.	*Lepidae* *Lepidae*	(3), –	–[1)]
I 7	*C. lachnosphaera* Bunge *C. myriolepis* Rech. f. & Koie	*Lachnosphaerae* *Lachnosphaerae*	(3), –	not resolved
I 8	*C. polyneura* Rech. f. *C. multiloba* DC.	*Eriocousinia* *Alpinae*	(3), –	not resolved
I 9	*C. carduchorum* C. Winkl. & Bornm. *C. algurdina* Rech. f.	*Cynaroideae* *Cynaroideae*	(3), –	–[2)]
I 10	*C. macrolepis* Boiss. & Hausskn. *C. phyllocephala* Bornm. & Gauba	*Cynaroideae* *Cynaroideae*	(3), –	–[1)]
I 11	*C. odontolepis* DC. *C. leatherdalei* Rech. f.	*Cynaroideae* *Cynaroideae*	(3), –	–[2)]

268 species of *Asteraceae* (excl. *Hieracium* and *Taraxacum*) are involved in the formation of 43 hybrids. In British *Cardueae,* 12 (35 %) of 40 species form 14 different hyrids (Stace 1991).

On this background, we here examine the frequency of interspecific hybridization in *Cousinia* s.str. and attemp to assess its possible role in the evolution of the high specific diversity of this taxon. Many species of *Cousinia* s.str. are distributed sympatrically, and there exist no obvious mechanisms of prezygotic reproductive isolation between sympatric species. Hybridization between the arctioid species of *Cousinia* has been analysed by Duistermaat (1996).

Material and methods

Published accounts of *Cousinia* were critically examined for reports of hybrids or intermediate forms.

In addition, two putative hybrids found by us with clearly intermediate characters were examined both morphologically and molecularly along with their suspected parents (Fig. 1, Table 2). For DNA extraction, silica-gel dried leaves of these two hybrids and their putative parents were used. Pollen fertility was determined by aceto-carmine staining and light microscopy (Tyagi & Singh 1998), and the total number of pollen grains per flower was counted.

Phylogenetic relationships between hypothetical parents of hybrids were analysed. For this, we constructed an ITS phylogeny of 214 species representing 63 sections of *Cousinia* out of c. 70 described by Rechinger (1972) and Tscherneva (1988), three accessions of *Arctium,* one species each of *Hypacanthium* and *Schmalhausenia* and two outgroups (*Jurinea albicaulis* Bunge and *Saussurea elegans* Ledeb.). It represents the largest ITS phylogeny of *Cousinia* s.str. published to date; 78

species were included for the first time. The material used for DNA extraction and the published sequences with their Genebank accession numbers are listed in Appendix 1. Original sequences were isolated from material provided by the herbaria B, IRAN, JE, KAS, M, TARI and W (abbreviations according to Holmgren & Holmgren 1998+) and H. Freitag, A. Susanna, D. Podlech and H. Akhani.

Total genomic DNA extraction, ITS (ITS1 and ITS2) amplification and sequencing were performed as described by López-Vinyallonga & al. (2009). Bayesian analyses of the ITS1 + ITS2 data sets were performed with MrBayes software package 3_0b4 (Huelsenbeck & Ronquist 2001). In order to select the best-fit model of substitution, the program ModelTest 3.5 was used (Posada & Crandall 1998, 2001). Following the results for DNA evolution of our data as indicated by ModelTest and using the Akaike Information Criterion (AIC), the model SYM+I+G was selected. Accordingly, ML parameters were set as follows: Nst = 6, rates=gamma, Rmat = (A-C = 0.7992, A-G = 2.8354, A-T = 1.5721, C-G = 0.2990, C-T = 11.8310, G-T = 1.0000), proportion of invariable sites (I) = 0.1827, gamma distribution shape parameter = 0.6222. The number of generations was set to 2 000 000, with trees being sampled every 100 generations for a total of 20 000 trees in the initial sample. The first 3000 trees were discarded and the posterior probability of the phylogeny and its branches was determined from the remaining 17 000 trees.

ITS sequences of the two putative hybrids identified by ourselves and their parents were examined for additivity of diagnostic nucleotide positions.

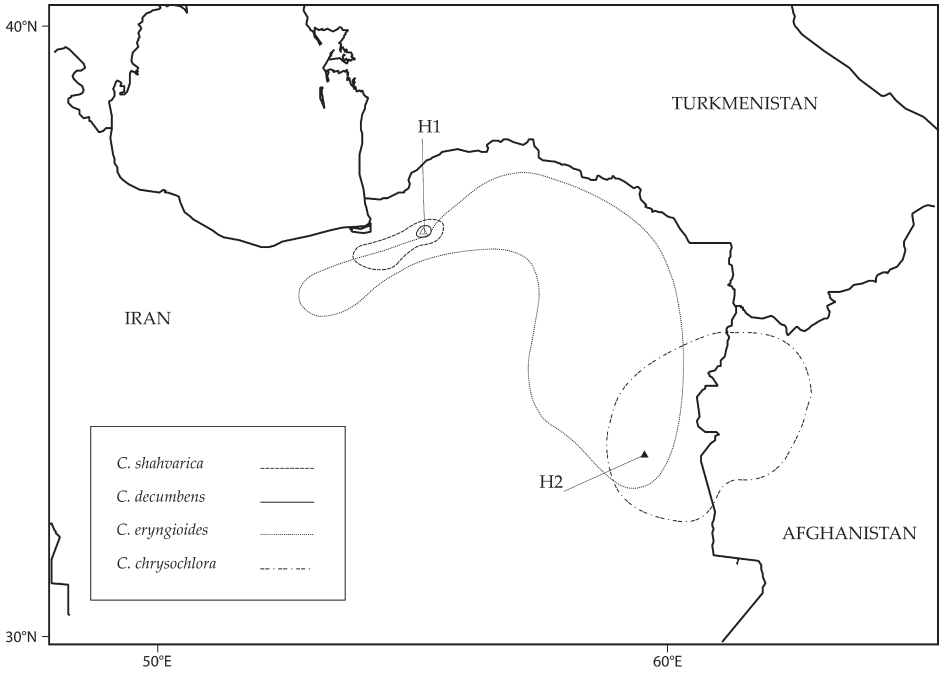

Fig. 2. Geographical distribution of *Cousinia shahvarica, C. decumbens, C. eryngioides* and *C. chrysochlora* (data from Rechinger 1972, 1979). – Triangles indicate collection sites of hybrids H 1 *(C. shahvarica* × *decumbens)* and H 2 *(C. eryngioides* × *chrysochlora)*.

Table 2. ITS positions showing sequence additivity in the hybrids between *Cousinia eryngioides* and *C. chrysochlora* and *C. shahvarica* and *C. decumbens.*

Taxon	Nucleotide position																								
	4	5	6	8	8	1	1	1	1	2	2	2	2	2	3	3	3	4	4	4	4	4			
	5	1	8	0	1	1	2	4	8	0	0	2	8	9	0	0	0	1	6	6	6				
					4	0	1	9	6	7	2	8	0	4	7	8	3	2	3	4					
C. eryngioides	C	G	Y	C	T	C	A	T	C	Y	Y	T	Y	G	T	C	T	Y	T	G	Y				
hybrid H2	Y	S	Y	Y	Y	Y	R	Y	M	Y	Y	Y	Y	K	Y	Y	Y	Y	Y	K	Y				
C. chysochlora	Y	C	C	T	C	Y	G	C	A	T	C	C	Y	K	C	T	C	Y	C	T	T				
	9	1	1	1	1	1	1	2	2	2	3	3	3	3	4	4	4	4	4	4	4				
	1	0	2	2	3	4	9	7	8	9	0	0	4	9	4	4	4	5	6	6	7				
		4	1	7	8	3	8	4	7	1	7	8	1	7	0	1	4	2	2	4	0				
C. shahvarica	C	G	G	T	G	G	A	C	T	T	C	T	C	C	C	A	G	T	Y	C	T				
hybrid H1	Y	R	R	Y	R	R	R	Y	Y	Y	Y	Y	Y	M	Y	R	R	Y	Y	Y	Y				
C. decumbens	T	A	A	C	A	A	G	Y	C	C	T	C	T	A	T	G	A	C	C	T	C				

Table 3. Number of pollen grains per flower and percentage of fertile pollen (pollen fertility) in *Cousinia eryngioides, C. chrysochlora* and their hybrid (H 2) and in *C. shahvarica, C. decumbens* and their hybrid (H 1).

Taxon	Pollen grains per flower (× 10^3)	Pollen fertility (%)
C. shavarica	9.1	95.4
hybrid H 1	4.4	77
C. decumbens	11.6	92.3
C. eryngioides	7	96.3
hybrid H 2	6.2	86
C. chrysochlora	5.4	95

Results

Two new hybrids

The intermediate plants identified by us were found in areas of geographical overlap of the suspected parents and show morphological intermediacy. Hybrid H 1 (Table 1) was collected on Mt Shahvar in NNE Iran where the ranges of the narrowly endemic *Cousinia decumbens* and the more widely distributed *C. shahvarica* overlap (Fig. 2). *C. decumbens* is a small plant with

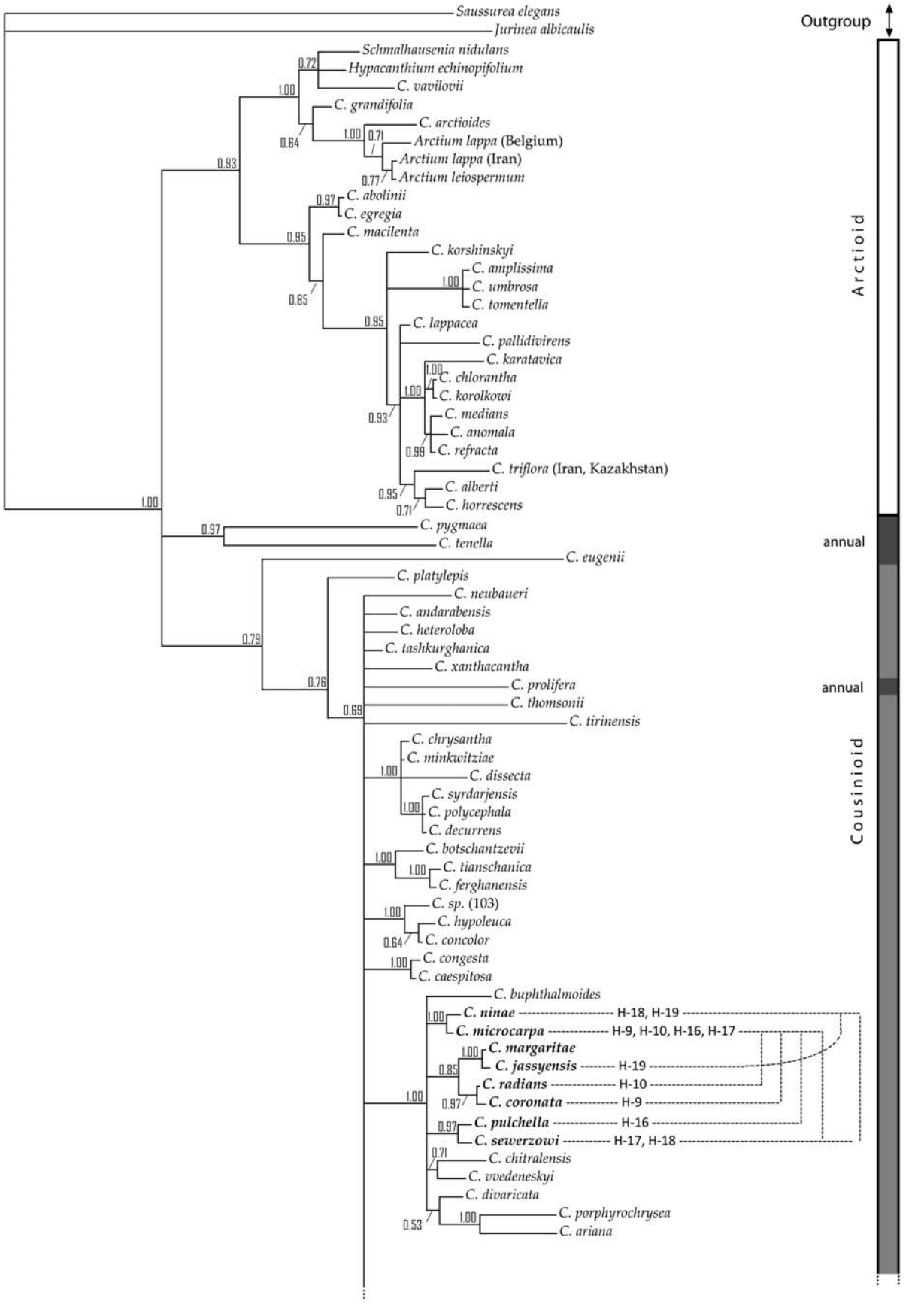

Fig. 3. 50 % majority rule consensus tree from the Bayesian analysis of the ITS data set. – Posterior probabilities (PP) are given above branches. The positions in the phylogenetic tree of suspected or conclusively identified hybrid parents are indicated by lines. The numbers above lines refer to hybrids or intermediate forms compiled in Table 1.

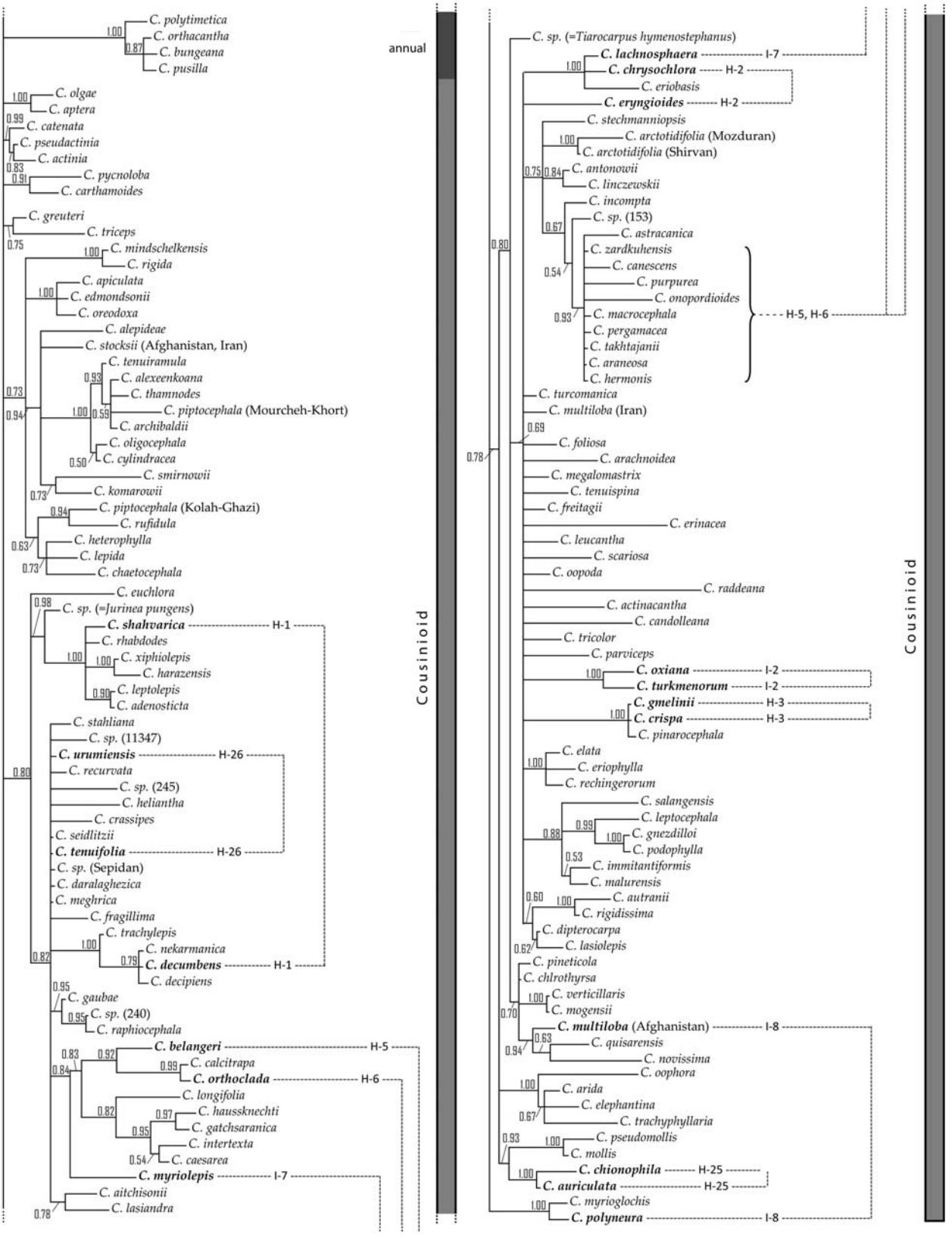

Fig. 3. continued from preceding page.

decumbent and flexuous stems of 10-25 cm length, linear-lanceolate, 5-10 cm long leaves, and deciduous capitula with subulate, recurved phyllaries and yellow flowers. *C. shahvarica* is erect and 30-60 cm high, has lanceolate leaves of up to 35 cm length, and persistent capitula with narrowly lanceolate phyllaries and purple flowers (Rechinger 1972 and own observations). The hybrid has decumbent stems of 2-35 cm length, lanceolate leaves which are 15-20 cm long, and persistent capitula with linear-subulate, slightly recurved phyllaries with purple flowers.

Hybrid H 2 (Table 1) was collected in E Iran in an area of overlap of its parents *Cousinia eryngioides* and *C. chrysochlora* (Fig. 2). Capitula of *C. eryngioides* have 15-18 phyllaries and 8-12 flowers, and those of *C. chrysochlora* have 80-90 phyllaries and 60-80 flowers (Rechinger 1972). The capitula of the hybrid individuals are intermediate and have 20-60 phyllaries and 10-40 flowers (Fig. 1). The apex of the anther in the hybrid indicates morphological intermediacy. Whereas the apex of the anthers of *C. eryngioides* are cuspidate with two small lateral teeth (Fig. 1Bd), and acute in *C. chrysochlora* (Fig. 1Dd), they are acuminate in the hybrid (Fig. 1Cd).

Pollen fertility of the hybrid individuals was clearly reduced (*Cousinia shahvarica:* 95.4 %, *C. decumbens:* 92.3 %, hybrid: 77 %; *C. eryngioides:* 96.3 %, *C. chrysochlora:* 95 %, hybrid: 86 %; Table 3). The hybrid between *C. shahvarica* and *C. decumbens,* but not that between *C. eryngioides* and *C. chrysochlora,* also showed a reduced number of pollen grains (Table 3). The hybrid status of these plants was further confirmed by the analysis of ITS sequences. In most positions the hybrids show additivity in those nucleotide positions in which the parental species differ (Table 2).

Phylogenetic position of the parents of published hybrids and intermediate forms

Twenty-eight hybrids and 11 intermediate forms in *Cousinia* s.str. have been published in the literature (Rechinger 1950, 1972, 1979; Tscherneva 1962; Kamelin 1973) and are compiled in Table 1.

A 50 % majority rule consensus tree from the Bayesian analysis of the ITS data set is shown in Fig. 3. Posterior probabilities (PP) are given above branches. This analysis of the ITS region supports previous studies and confirms the monophyly of the group. It shows a clear subdivision into an arctioid group *(Arctium, Cousinia* subg. *Cynaroides* and subg. *Hypacanthodes, Hypacanthium, Schmalhausenia)* and a cousinioid group *(Cousinia* subg. *Cousinia).* Relationships among species in the cousinioid clade are essentially unresolved. Phylogenetic relationships within the *Arctium-Cousinia* complex have been discussed in detail by López-Vinyallonga & al. (2009).

The position of suspected or conclusively identified hybrid parents in the phylogenetic tree is indicated (Fig. 3). Hybrids or intermediate forms result from parents of the same and different sections following the traditional taxonomy of the genus (Table 1), and from parents of the same and different clades in our phylogeny (Fig. 3). Seventeen of the reported 28 hybrids are suspected (H 3-6, 9-19) or known (H 1-2) to have parents belonging to different sections of *Cousinia* s.str. (Table 1). From the two probably (H 1-2, Table 1) and 11 presumably (H 3, 5-6, 9-10, 16-19, 25-26, Table 1) hybridizing species pairs included in our ITS analysis, one (H 1, Fig. 3) and six (H 9-10, 16-19, Fig. 3), respectively, belong to different clades when clades are defined as lineages with ≥ 0.95 PP. Two of the presumably hybridizing species pairs (H 3, 25, Fig. 3) belong to the same clades. Otherwise relationships are either not resolved (H 2, 5-6, 26) or one or both parents of the suspected hybrids were not included in our analysis. All but one (I 8) of the 11 intermediate forms refer to species pairs of the same section (Table 1). None of the three species pairs for which intermediate forms have been reported and which are represented in our ITS phylogeny (I 2, 7, 8) falls into different clades, when clades are defined as lineages with ≥ 0.95 PP (Fig. 3). Neither hybrids between *Cousinia* s.str. and other main clades of the '*Arctium-Cousinia* complex' nor between annual and perennial species of *Cousinia* s.str. have been reported.

Discussion

Our results, particularly those related to the two hybrids identified by ourselves, clearly show that hybridization in *Cousinia* is possible. These two hybrids have parents that belong to different sections and one of them (H 1) has parents that fall into different clades in our phylogeny (Table 1, Fig. 3). This picture is supported by hybrid combinations reported in the literature, which involve species belonging to the same and different sections and to the same and different clades (clades are defined as lineages with ≥ 0.95 PP). As regards phylogenetic relationships between clades in those instances where hybrid parents belong to different clades, the basally largely unresolved topology of *Cousinia* s.str. does not allow us to draw any firm conclusions. Our results also show that the two hybrids that could be examined in detail show reduced pollen fertility, indicating the presence of postzygotic reproductive barriers.

In case all reported hybrids were correctly identified, and the intermediate forms reported were also hybrids, altogether 64 of c. 600 species of *Cousinia* s.str., i.e., little more than 10 % are involved in interspecific hybridization. If this count is limited to only the 28 hybrids reported, excluding the intermediate forms, the number of species involved is 42 or c. 7 %. Both figures meet those average figures across different floras reported by Ellstrand & al. (1996), but are much lower than those found in *Asteraceae* (c. 23 %) and *Cardueae* (35 %) of the British Isles, or found in *Arctium* s.str.

(five of six species) by Duistermaat (1996). *Cousinia* s.str. is a large group of often rather similar and partly not well-known species, and the figures of total species number and hybrids/intermediates presented here may be either over- or underestimates. The estimated ratio of hybrids may nevertheless be largely correct. If so, it would illustrate that interspecific hybridization is not more frequent in this group than expected from average values, and substantially less frequent than in *Asteraceae* and *Cardueae* in the British Isles.

In *Cousinia* the chromosome numbers of 148 species have been determined (Watanabe 2008), and not a single polyploid chromosome number has been reported to date. As polyploidy probably more often than not arises from allopolyploid hybrid speciation (Levin 2002), the apparent absence of polyploids in conjunction with the above observation of average rates of interspecific hybridization may indicate that the role of interspecific hybridization for the evolution and diversity of the group has been minor.

Our revision of *Cousinia* s.str. sect. *Cynaroideae,* which is likely to represent a monophyletic group (Mehregan & Kadereit 2008), indicates that the very large majority of its 31 species are distributed allo- or parapatrically, and that species ranges often are separated by prominent geographical barriers. This, if true for other clades of *Cousinia* s.str., might explain the apparently minor evolutionary role of interspecific hybridization in this taxon, although, as shown above, hybridization between clades seems possible when species of such clades grow sympatrically.

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Appendix 1. List of taxa and sources of plant material or sequence data (Genebank accession number) used in the molecular phylogenetic analysis. For original sequences both the Genebank accession number and the specimen data are given.

Arctium lappa L., Belgium: AF319048, AF319102. ***A. lappa*** L., Iran: EU923773, EU923887. ***A. leiospermum*** Juz. & Ye. V. Sergievskaja, Kazakhstan: AY373720, AY373687. ***Cousinia abolinii*** Tscherneva (subg. *Hypacanthodes* sect. *Abolinia),* Kyrgyzstan: EU923763, EU923877. ***C. actinacantha*** Rech. f., (subg. *Cousinia),* Afghanistan: Fariab, Maimana, 20 km SSE, 1100 m, *Freitag 6693* (KAS), EU923626, EU923705. ***C. actinia*** Boiss. (subg. *Cousinia* sect. *Actinia*), Afghanistan: Kabul, Korogh Koh, 2600 m, *Freitag 1570* (KAS), EU923640, EU923718. ***C. adenosticta*** Bornm. (subg. *Cousinia* sect. *Sphaerocephalae*), Iran: EU923827, EU923941. ***C. aitchisonii*** C. Winkl. (subg. *Cousinia* sect. *Lasiandrae*), Afghanistan: 70 km WNW Herat, near Tirpul, 780 m, *Freitag 5461* (KAS), EU923676, EU923755. ***C. alberti*** Regel & Schmalh., (subg. *Cynaroides* sect. *Pectinatae*), Kazakhstan: AY373721, AY373688. ***C. alepideae*** Boiss. (subg. *Cousinia* sect. *Lasiandrae*), Afghanistan: EU923794, EU923908. ***C. alexeenkoana*** Bornm. (subg. *Cousinia* sect. *Stenocephalae*), Iran: EU923799, EU923913. ***C. amplissima*** (Boiss.) Boiss. (subg. *Cynaroides* sect. *Pseudarctium*), Iran: EU923766, EU923880. ***C. andarabensis*** Rech. f. (subg. *Cousinia*), Afghanistan: Baghlan, Andarab-Tal, 2600 m, *Podlech 11984* (M), EU923634, EU923713. ***C. anomala*** Franch. (subg. *Cynaroides* sect. *Ctenarctium*), Tadjikistan: EU923770, EU923884. ***C. antonowii*** C. Winkl. (subg. *Cousinia* sect. *Leiocaules*), Iran: EU923849, EU923963. ***C. apiculata*** Tscherneva (subg. *Cousinia* sect. *Pseudactinia*), Iran: EU923792, EU923906. ***C. aptera*** Aitch. & Hemsl. (subg. *Cousinia* sect. *Olgaeanthe*), Afghanistan: 44 km NW of Ghazni, 2780 m, *Freitag 1408* (KAS), EU923636, EU923715. ***C. arachnoidea*** Fisch. & C. A. Mey. (subg. *Cousinia* sect. *Microcarpae*), Kazakhstan: AY373722, AY373689. ***C. araneosa*** DC. (subg. *Cousinia* sect. *Cynaroideae*), Iran: EU923870, EU923984. ***C. archibaldii*** Rech. f. (subg. *Cousinia* sect. *Stenocephalae*), Iran: 28 km S of Nahavand, 1800-2400 m, *Terme & Moussavi 33893-E* (IRAN), EU923656, EU923735. ***C. arctioides*** Schrenk (subg. *Cynaroides* sect. *Nanarctium*), Kazakhstan: EU923772, EU923886. ***C. arctotidifolia*** Bunge (subg. *Cousinia* sect. *Leiocaules*), Iran: Khorassan, Bazangan lake, 750 m, *Mehregan 119* (MJG), EU923621, EU923700 & Iran: Shirvan, EU923848, EU923962. ***C. ariana*** Bornm. (subg. *Cousinia* sect. *Carduncellus*), Afghanistan: EU923788, EU923902. ***C. arida*** C. Winkl. (subg. *Cousinia* sect. *Neurocentrae*), Afghanistan: Herat, 70 km WNW, near Torpul, 780 m, *Freitag 5455* (KAS), EU923680, EU923759. ***C. astracanica*** (Spreng.) Tamamsch. (subg. *Cousinia* sect. *Leiocaules*), Kazakhstan: AY373723, AY373690. ***C. auriculata*** Boiss. (subg. *Cousinia* sect. *Eriocousinia*), Afghanistan: EU923830, EU923944. ***C. autranii*** C. Winkl. (subg. *Cousinia* sect. *Rigidissimae*), Afghanistan: EU923860, EU923974. ***C. belangeri*** DC. (subg. *Cousinia* sect. *Pugioniferae*), Iran: EU923819, EU923933. ***C. botschantzevii*** Tscherneva (subg. *Cousinia* sect. *Regelianae*), Uzbekistan: EU923782, EU923896. ***C. bungeana*** Regel & Schmalh. (subg. *Cousinia* sect. *Dichotomae*), s.loc.: EU923786, EU923900. ***C. buphthalmoides*** Regel (subg. *Cousinia* sect. *Carduncellus*), Afghanistan: EU923789, EU923903. ***C. caesarea*** Boiss. & Balansa (subg. *Cousinia* sect. *Cousinia*), Turkey: Sivas, 58 km E Kangal, 1280 m, *Nydegger 17136* (M), EU923669, EU923748. ***C. caespitosa*** C. Winkl. (subg. *Cousinia* sect. *Eriocousinia*), Kazakhstan: AY373724, AY373691. ***C. calcitrapa*** Boiss. (subg. *Cousinia* sect. *Pugioniferae*), Iran: EU923820, 923934. ***C. candolleana*** Jaub. & Spach (subg. *Cousinia* sect. *Myriotomae*), Iran: EU923858, EU923972. ***C. canescens*** DC. (subg. *Cousinia* sect. *Cynaroideae*), Iran: AF319068, AY319122. ***C. carthamoides*** Aitch. & Hemsl. (subg. *Cousinia* sect. *Eriocousinia*), Afghanistan: Paktia, Jaji, Shotor Gardan pass, 2950 m, *Freitag 5737* (KAS), EU923643, EU923722. ***C. catenata*** Rech. f. (subg. *Cousinia* sect. *Actinia),* Afghanistan: SE Sar-e-Pol, 1330 m, *Freitag 6565* (KAS), EU923638, EU923717. ***C. chaetocephala*** Tscherneva (subg. *Cousinia*), Iran: EU923806, EU923920. ***C. chionophila*** Rech. & Koie (subg. *Cousinia* sect. *Eriocousinia*), Afghanistan: EU923829, EU923943. ***C. chitralensis*** Rech. f.

(subg. *Cousinia*), Afghansitan: Badakhshan, Darya-e Ptukh Tal, 3400 m, *Anders 7248* (M), EU923650, EU923729. ***C. chlorantha*** Kult. (subg. *Cynaroides* sect. *Chrysis*), Uzbekistan: EU923864, EU923978. ***C. chlorothyrsa*** Rech. f. & Koie (subg. *Cousinia* sect. *Racemosae*), Afghanistan: Kabul, 12 km WNW, 1950 m, *Freitag 1983* (KAS), EU923605, EU923684. ***C. chrysantha*** Kult. (subg. *Cousinia* sect. *Alpinae*), Kazakhstan: AY373725, AY373692. ***C. chrysochlora*** Rech. f. & Koie (subg. *Cousinia* sect. *Lachnosphaerae*), Iran: EU923856, EU923970. ***C. chrysochlora* × *C. eryngioides,*** Iran: E, Khorassan, 30 km from Sedeh to Asad Abad, 2000 m, *Mehregan 238* (MJG), EU923612, EU923691. ***C. concolor*** Bunge (subg. *Cousinia* sect. *Serratuloides*), Iran: EU923808, EU923922. ***C. congesta*** Bunge (subg. *Cousinia* sect. *Congestae*), Uzbekistan: AY373726, AY373693. ***C. coronata*** Franch. (subg. *Cousinia* sect. *Coronophora*), Uzbekistan: AY373727, AY373694. ***C. crassipes*** Kult. (subg. *Cousinia* sect. *Homalochaete*), Iran: EU923811, EU923925. ***C. crispa*** Jaub. & Spach (subg. *Cousinia* sect. *Serratuloides*), Iran: EU923843, EU923957. ***C. cylindracea*** Boiss. (subg. *Cousinia* sect. *Stenocephalae*), Iran: EU923797, EU923911. ***C. daralaghezica*** Takht. (subg. *Cousinia* sect. *Cousinia*), Armenia: EU923813, EU923927. ***C. decipiens*** Boiss. & Buhse (subg. *Cousinia* sect. *Stenocephalae*), Iran: Mazandaran, 50 km S of Kord-Koui, 2000 m, *Mehregan 131* (MJG), EU923675, EU923754. ***C. decumbens*** Rech. f. (subg. *Cousinia* sect. *Decumbentes*), Iran: EU923821, EU923935. ***C. decurrens*** Regel (subg. *Cousinia* sect. *Congestae*), Afghanistan: Ghazni, 10 km NW, 2400 m, *Freitag 1371* (KAS), EU923635, EU923714. ***C. dipterocarpa*** Bornm. & Rech. f. (subg. *Cousinia* sect. *Alpinae*), Iran: EU923862, EU923976. ***C. dissecta*** Kar. & Kir. (subg. *Cousinia* sect. *Chrysoptera*), Kazakhstan: AY373728, AY373695. ***C. divaricata*** C. Winkl. (subg. *Cousinia* sect. *Leiacanthos),* Tadzhikistan: Darvaz, between Zikar and former village Yovgar, *Kamelin 656* (LE), EU923647, EU923726. ***C. edmondsonii*** Rech. f. (subg. *Cousinia* sect. *Pseudactinia*), Iran: Khorassan, Bojnourd, between Zard and Hastan, 2500 m, *Assadi 101* (MJG), EU923655, EU923734. ***C. egregia*** Juz. (subg. *Hypacanthodes* sect. *Abolinia),* Uzbekistan: EU923866, EU923980. ***C. elata*** Boiss. & Buhse (subg. *Cousinia* sect. *Platyacanthae*), Iran: Khorassan, between Kadkan and Esfiz, 1900 m, *Mehregan s.n.* (MJG), EU923616, EU923695. ***C. elephantina*** Rech. f. (subg. *Cousinia* sect. *Leiocaules*), Afghanistan: between Herat and Karakh, 1300 m, *Freitag 6809* (KAS), EU923681, EU923760. ***C. erinacea*** Jaub. & Spach (subg. *Cousinia),* Iran: Guilan, Deylaman, *Saeedi 18814* (TUH), EU923615, EU923694. ***C. eriobasis*** Bunge (subg. *Cousinia* sect. *Lachnosphaerae*), Iran: AY373729, AY373696. ***C. eriophylla*** (Kult.) Bornm. (subg. *Cousinia* sect. *Platyacanthae*), Iran: EU923840, EU923954. ***C. eryngioides*** Boiss. (subg. *Cousinia* sect. *Sciadocousinia*), Iran: EU923836, EU923850. ***C. euchlora*** Bornm. & Rech. f. (subg. *Cousinia* sect. *Eriocousinia*), Iran: Khorassan, 30 km W of Torbat-e Djam,1800 m, *Mehregan 136* (MJG), EU923659, EU923738. ***C. eugenii*** Kult. (subg. *Cousinia* sect. *Dichotomae*), Uzbekistan: Arenae Kyzylkum, prope Baskara, *Poretzky 4643* (M), EU923633, EU923712. ***C. ferghanensis*** Bornm. (subg. *Cousinia* sect. *Cousinia*), Tadjikistan: Fergana valley, 22.5.1967, *Ouczinnikov 102* (TAD), EU923641, EU923720. ***C. foliosa*** Boiss. & Balansa (subg. *Cousinia* sect. *Stenocephalae*), Turkey: Malatya, Pötürge, 1680 m, *Nydegger 17205* (M), EU923610, EU923689. ***C. fragillima*** Rech. f. (subg. *Cousinia* sect. *Fragillimae*), Iran: Khorassan, Khaur, 7000 ft., *Koelz 16834* (W), EU923667, EU923746. ***C. freitagii*** Rech. f. (subg. *Cousinia* sect. *Leucocaulon*), Afghanistan: Baghlan, Kataghan, inter Pol-e Khumri et Samangan, 1410 m, *Freitag 3147* (KAS), EU923614, EU923693. ***C. gatchsaranica*** Mehregan & al. (subg. *Cousinia* sect. *Haussknechtianae*), Iran: EU923816, EU923930. ***C. gaubae*** Bornm. (subg. *Cousinia* sect. *Stenocephalae*), Iran: EU923818, EU923932. ***C. gmelini*** C. Winkl. (subg. *Cousinia* sect. *Eriocousinia*), Iran: EU923842, EU923956. ***C. gnezdilloi*** Tscherneva (subg. *Cousinia* sect. *Homalochaete*), Uzbekistan, range Kugitang, near village Hodzha-fil'-ata, *Trans-Cauc.-Middle-Asian Group 798* (LE), EU923618, EU923697. ***C. grandifolia*** Kult. (subg. *Hypacanthodes* sect. *Amberbopsis*), Kazakhstan: AY373730, AY373697. ***C. greuteri*** Rech. f. (subg. *Cousinia* sect. *Congestae*), Afghanistan: EU923783, EU923897. ***C. harazensis*** Rech. f. (subg. *Cousinia* sect. *Spaherocephalae*), Iran: EU923825, EU923939. ***C. haussknechti*** C. Winkl. (subg. *Cousinia* sect. *Haussknechtianae*), Iran: Kuhe Gerri, *Strauss s.n.* (JE), EU923668, EU923747. ***C. heliantha*** Bunge (subg. *Cousinia* sect. *Helianthae*), Iran: EU923810, EU923924. ***C.*** sp. (240) (subg. *Cousinia* sect. *Haussknechtianae*), Iran: Isfahan, Mt Karkas, from Tameh, 2300 m, *Mehregan 240* (MJG), EU923671, EU923750. ***C.*** sp. (11347) (subg. *Cousinia* sect. *Haussknechtianae*), Iran: Isfahan, Mt Karkas, from Tameh, 2300 m, *Mehregan 240* (MJG), Eu 923661, EU923740. ***C. hermonis*** Boiss. (subg. *Cousinia* sect. *Cynaroideae*), Israel: Mt Hermon, 1700 m, *Fragmom-Sapir s.n.* (cult. from achenes, MJG), EU923627, EU923706. ***C. heteroloba*** Rech. f. (subg. *Cousinia* sect. *Molles*), Afghanistan: EU923776, EU923890. ***C. heterophylla*** Boiss. (subg. *Cousinia* sect. *Lepidae*), Afghanistan: EU923804, EU923918. ***C. horrescens*** Juz. (subg. *Cynaroides* sect. *Pectinatae*), Kazakhstan: Tian-schan occ., prope pagum Niazbek, *Granitov s.n.* (TAD), EU923630, EU923709. ***C. hypoleuca*** Boiss. (subg. *Cousinia* sect. *Serratuloides*), Iran: EU923807, EU923921. ***C. immitantiformis*** Rech. f. (subg. *Cousinia* sect. *Immitantes*), Afghanistan: EU923846, EU923960. ***C. incompta*** DC. (subg. *Cousinia* sect. *Pugioniferae*), Iran: EU923851, EU923965. ***C. intertexta*** Freyn & Sint. (subg. *Cousinia* sect. *Cousinia*), Turkey: EU923817, EU923931. ***C. jassyensis*** C. Winkl. (subg. *Cousinia* sect. *Jurineopsis*), Kyrgyzstan: EU923790, EU923904. ***C. karatavica*** Regel & Schmalh. (subg. *Cynaroides* sect. *Chrysis*), Kazakhstan: AY373732, AY373699. ***C. komarowii*** C. Winkl. (subg. *Cousinia* sect. *Stenoloma*), Iran: EU923803, EU923917. ***C. korolkowi*** Regel & Schmalh. (subg. *Cynaroides* sect. *Chrysis*), Uzbekistan: EU923865, EU923979. ***C. korshinskyi*** C. Winkl. (subg. *Hypacanthodes* sect. *Lacerae*), Kirgizstan: EU923765, EU923879. ***C. lachnosphaera*** Bunge (subg. *Cousinia* sect. *Lachnosphaerae*), Iran: EU923857, EU923971. ***C. lappacea*** Schrenk (subg. *Cynaroides* sect. *Lappaceae*), Kazakhstan: AY373733, AY373700. ***C. lasiandra*** Bunge (subg. *Cousinia* sect. *Lasiandrae*), Iran: EU923822, EU923936. ***C. lasiolepis*** Boiss. (subg. *Cousinia* sect. *Alpinae*), Iran: EU923863, EU923977. ***C. lepida*** Boiss. (subg. *Cousinia* sect. *Lepidae*), Iran: EU923805, EU923919. ***C. leptocephala*** Fisch. & C. A. Mey. (subg. *Cousinia* sect. *Stenocephalae*), Turkmenistan: Krasnovodsk, inter Basch mygura & Bala-Ischen, *Korovin s.n.* (B), EU923617, EU923696. ***C. leptolepis*** (Bornm. & Gauba) Rech. f. (subg. *Cousinia* sect. *Sphaerocephalae*), Iran: EU923826, EU923940. ***C. leucantha*** Bornm.

& Sint. (subg. *Cousinia* sect. *Stenoloma*), Iran: EU923837, EU923951. ***C. linczewskii*** Juz. (subg. *Cousinia* sect. *Leucocaulon*), Iran: EU923850, EU923964. ***C. longifolia*** C. Winkl. & Bornm. (subg. *Cousinia* sect. *Spinuliferae*), Iran: EU923815, EU923929. ***C. macilenta*** C. Winkl. (subg. *Hypacanthodes* sect. *Lacerae*), Tadjikistan: EU923764, EU923878. ***C. macrocephala*** C. A. Mey. (subg. *Cousinia* sect. *Cynaroideae*), Iran: EU923852, EU923966. ***C. malurensis*** Rech. f. (subg. *Cousinia*), Afghanistan: Fariab, Maimana, 45 km S, above 1700 m, *Freitag 6721* (KAS),. EU923619, EU923698 ***C. margaritae*** Kult. (subg. *Cousinia* sect. *Jurineopsis*), Kyrgyzstan: 25 km SW Talas, 1600 m, *Martins 909* (JE), EU923649, EU923728. ***C. medians*** Juz. (subg. *Cynaroides* sect. *Chrysis*), Uzbekistan: EU923769, EU923883. ***C. megalomastrix*** Rech. f. (subg. *Cousinia),* Afghanistan: Khesht Pol, between Bale Murghab and Maimana, 800 m, *M. Amin 6223* (KAS), EU923611, EU923690. ***C. meghrica*** Takht. (subg. *Cousinia* sect. *Cousinia*), Armenia: EU923814, EU923928. ***C. microcarpa*** Boiss. (subg. *Cousinia* sect. *Microcarpae*), Iran: Khorassan, 50 km from Mashhad to Neishabour, 1500 m, *Mehregan 169* (MJG), EU923646, EU923725 & Kazakhstan: AY373734, AY373701. ***C. mindshelkensis*** B. Fedtsch. (subg. *Cousinia* sect. *Lopholepis*), Kazakhstan: Mt Karatan, Rustem-Mazar, *Bolchovitina s.n.* (M), EU923654, EU923733. ***C. minkwitziae*** Bornm. (subg. *Cousinia* sect. *Cousinia*), Kazakhstan: AY373735, AY373702. ***C. mogensii*** Rech. f. (subg. *Cousinia* sect. *Alpinae*), Afghanistan: Ghazni, Hajigak-pass, 3300 m, *Freitag 6376* (KAS), EU923607, EU923686. ***C. mollis*** Schrenk (subg. *Cousinia* sect. *Molles*), Afghanistan: Mazar Sharif, Shibaghlan, 600 m, *Rechinger 34270* (M), EU923683, EU923762. ***C. multiloba*** DC. (subg. *Cousinia* sect. *Alpinae*), Afghanistan: Sar-e Pol, Sangcharak, 40 km SSE, above Damardan, 2600-2800 m, *Freitag 6645* (KAS), EU923608, EU923687 & Iran: EU923835, EU923949. ***C. myrioglochis*** Rech. f. (subg. *Cousinia* sect. *Alpinae* Bunge), Afghanistan: Kabul, upper S Salang valley, above 3700 m, *Freitag 3647* (KAS), EU923637, EU923716. ***C. myriolepis*** Rech. & Koie (subg. *Cousinia* sect. *Lachnosphaerae*), Afghanistan: Parvan, Ghowrband valley, 49 km W of Charikar bridge, 1920 m, *Freitag 1088* (KAS), EU923629, EU923708. ***C. nekarmanica*** Rech. f. (subg. *Cousinia* sect. *Stenocephalae*), Iran: Semnan, Shahroud, above Nekarmann, 2100 m, *Mehregan 137* (MJG), EU923674, EU923753. ***C. neubaueri*** Rech. f. (subg. *Cousinia*), Afghanistan: EU923775, EU923889. ***C. ninae*** Juz. (subg. *Cousinia* sect. *Microcarpae*), Kyrgyzstan: EU923868, 923982. ***C. novissima*** Rech. f. (subg. *Cousinia*), Afghanistan: Herat, 15 km SSE Farsi, 2400-2700 m, *Freitag 6822* (KAS), EU923609, EU923688. ***C. olgae*** Regel. & Schmalh. (subg. *Cousinia* sect. *Olgaeanthe*), Iran: EU923780, EU923894. ***C. oligocephala*** Boiss. (subg. *Cousinia* sect. *Stenocephalae*), Iran: EU923796, EU923910. ***C. onopordioides*** Ledeb. (subg. *Cousinia* sect. *Cynaroideae*), Iran: AF319070, AF319122. ***C. oophora*** Rech. f. (subg. *Cousinia* sect. *Leiocaules*), Afghanistan: Fariab, Qaisar, above Shakh, 1850-2500 m, *Freitag 6742* (KAS), EU923679, EU923758. ***C. oopoda*** Juz. (subg. *Cousinia* sect. *Actinia)*, Tadjikistan: EU923839, EU923953. ***C. oreodoxa*** Bornm. & Sint. (subg. *Cousinia* sect. *Pseudactinia*), Iran: EU923793, EU923907. ***C. orthacantha*** Tscherneva (subg. *Cousinia* sect. *Dichotomae*), Afghanistan: 20 km E of Mazar-i-Sharif, 330 m, *Uotila 16964* (W), EU923644, EU923723. ***C. orthoclada*** Hausskn. & Bornm. (subg. *Cousinia* sect. *Pugioniferae*), Iran: Bakhtiari, 10 km from Dehnou to Shahreiarri, 2100 m, *Mehregan 147* (MJG), EU923670, EU923749. ***C. oxiana*** Tscherneva (subg. *Cousinia* sect. *Chrysoptera*), Afghanistan: EU923854, EU923968. ***C. pallidivirens*** Kult. (subg. *Cynaroides*), Uzbekistan: EU923768, EU923882. ***C. parviceps*** Rech. f. & Koie (subg. *Cousinia* sect. *Parvicipites*), Afghanistan: Ghazni, 50 km WNW of Ghazni, 2960 m, *Freitag 1412* (KAS), EU923628, EU923707. ***C. pergamacea*** Boiss. & Hausskn. (subg. *Cousinia* sect. *Cynaroideae*), Iran: Kurdistan, Mt E of Baneh, 2200 m, *Mehregan 208* (MJG), EU923624, EU923703. ***C. pinarocephala*** Boiss. (subg. *Cousinia* sect. *Serratuloides*), Iran: EU923844, EU923958. ***C. pineticola*** Rech. f. & Gilli (subg. *Cousinia* sect. *Racemosae*), Afghanistan: EU923832, EU923946. ***C. piptocephala*** Bunge (subg. *Cousinia* sect. *Badghysia*), Iran: Mourcheh-Khort, EU923801, EU923915 & Iran: Kolah-Ghazi, AY373736, AY373703. ***C. platylepis*** Schrenk (subg. *Cousinia* sect. *Microcarpae*), Kazakhstan: AY373737, AY373704. ***C. podophylla*** Tscherneva (subg. *Cousinia* sect. *Homalochaete*), s.loc., EU923847, EU923961. ***C. polycephala*** Rupr. (subg. *Cousinia* sect. *Cousinia*), Kazakhstan: AY373738, AY373705. ***C. polyneura*** Rech. f. (subg. *Cousinia* sect. *Eriocousinia*), Afghanistan: EU923781, EU923895. ***C. polytimetica*** Tscherneva (subg. *Cousinia* sect. *Dichotomae*), Uzbekistan: EU923785, EU923899. ***C. porphyrochrysea*** Rech. f. (subg. *Cousinia* sect. *Pulchellae*), Afghanistan: Badakhshan, 30 km S of Qeshm, 500 m, *Hedge & al. 9288* (B), EU923645, EU923724. ***C. prolifera*** Jaub. & Spach (subg. *Cousinia* sect. *Microcousinia*), Iran: EU923779, EU923893. ***C. pseudactinia*** Rech. f. (subg. *Cousinia* sect. *Actinia*), Afghanistan: Samangan, 10 km E, 1200 m, *Freitag 3159* (KAS), EU923639, EU923718. ***C. pseudomollis*** C. Winkl. (subg. *Cousinia* sect. *Molles*), Afghanistan: Kataghan, Kalate Robatak, NW Pole Khumrie, 1410 m, *Freitag 3147* (KAS), EU923682, EU923761. ***C. pulchella*** Bunge (subg. *Cousinia* sect. *Pulchellae*), Afghanistan: Badakhshan, Kuh-e Chungar, NW Pol-e Khumrie, 2000-2400 m, *Freitag 6497* (KAS), EU923648, EU923727. ***C. purpurea*** C. A. Mey. (subg. *Cousinia* sect. *Cynaroideae*), Armenia: AY373739, AY373706. ***C. pusilla*** C. Winkl. (subg. *Cousinia* sect. *Dichotomae),* Tadjikistan: EU923787, EU923901. ***C. pycnoloba*** Boiss. (subg. *Cousinia* sect. *Alpinae*), Afghanistan: Paktia, Gardez, SE side of Altimur Kotal, 2600 m, *Freitag 1611* (KAS), EU923642, EU923721. ***C. pygmaea*** C. Winkl. (subg. *Cousinia* sect. *Chrysantha*), Afghanistan: Kataghan, Baghlan, Dashte Gawar, 700-750 m, *Tonchev 2230* (KAS), EU923631, EU923710. ***C. qaisarensis*** Rech. f. (subg. *Cousinia* sect. *Spinuliferae*), Afghanistan: EU923833, EU923947. ***C. raddeana*** C. Winkl. (subg. *Cousinia* sect. *Hoplophylla*), Iran: EU923853, EU923967. ***C. radians*** Bunge (subg. *Cousinia* sect. *Coronophora*), Iran: EU923791, EU923905. ***C. raphiocephala*** Rech. f. (subg. *Cousinia* sect. *Haussknechtianae*), Iran: Isfahan, 12 km NW Muteh, 2150 m, *Mehregan 88247* (MJG), EU923923672, EU923751. ***C. rechingerorum*** Bornm. *(*subg. *Cousinia* sect. *Platyacanthae*), Iran: EU923841, EU923955. ***C. recurvata*** DC. (subg. *Cousinia* sect. *Stenocephalae*), Iran: Khorassan, 17 km from Esfarayen to Bojnourd, 1450 m, *Mehregan 149* (MJG), EU923662, EU923741. ***C. refracta*** (Bornm.) Juz. (subg. *Cynaroides* sect. *Chrysis)*, Tadjikistan: EU923867, EU923981. ***C. rhabdodes*** Bornm. & Rech. f. (subg. *Cousinia* sect. *Spaherocephalae*), Iran: Semnan, Ahovan pass, 35 km E of Semnan, 2100 m,

Mehregan 118 (MJG), EU923678, EU923757. ***C. rigida*** Kult. (subg. *Cousinia* sect. *Lopholepis*), Kyrgyzstan: EU923869, EU923983. ***C. rigidissima*** Rech. f. (subg. *Cousinia* sect. *Rigidissimae*), Afghanistan: EU923861, EU923975. ***C. rufidula*** Bornm. (subg. *Cousinia* sect. *Lepidae*), Afghanistan: Herat, 10 km W, 1150 m, *Freitag 5346* (KAS), EU923657, EU923736. ***C. salangensis*** Rech. f. (subg. *Cousinia* sect. *Immitantes* Rech. f.), Afghanistan: EU923845, EU923959. ***C.*** sp. (103), (subg. *Cousinia* sect. *Eriocousinia*), Iran: Khorassan, SW Bojnourd, Salouk Mt, 2400 m, *Mehregan 103* (MJG), EU923658, EU923737. ***C. scariosa*** Regel (subg. *Cousinia* sect. *Scariosae*), Afghanistan: EU923838, EU923952. ***C. seidlitzii*** Bunge (subg. *Cousinia* sect. *Cousinia*), Iran: Azebaijan, 7 km from Khalkhal to Asalem, 1900 m, *Mehregan 200* (MJG), EU923664, EU923743. ***C. sewerzowi*** Regel (subg. *Cousinia* sect. *Pulchellae*), Kazakhstan: AY373740, AY373707. ***C. shahvarica*** Rech. f. (subg. *Cousinia* sect. *Sphaerocephalae*), Iran: EU923823, EU923937. ***C. shahvarica* × *C. decumbens***, Iran: Semnan, Shahroud, Mt Shahvar, 3600 m, *Mehregan 244* (MJG), EU923665, EU923744. ***C. smirnowii*** Trautv. (subg. *Cousinia* sect. *Kopetdagia*), Iran: EU923802, EU923916. ***C.*** sp. (Sepidan) (subg. *Cousinia*), Iran: Fars, 15 km from Sepidan, 2300 m, *Ghareman & al. 25177* (MJG), EU923666, EU923745. ***C.*** sp. (153), (subg. *Cousinia*), Iran: Khorassan, 80 km from Darreh Gaz to Ghoutchan, 2100 m, *Mehregan 153* (MJG), EU923622, EU923701. ***C.*** sp. (245), (subg. *Cousinia* sect. *Haussknechtianae*), Iran: Arak, Komijan, Kuh-e Ghalanje, 2500 m, *Mehregan 245* (MJG), EU923663, EU923742. ***C.*** sp. (= ***Jurenia pungens*** Boiss.), (subg. *Cousinia*), Iran: Khorassan, pass inter Fariman ad Zharf, 2200 m, *Mehregan s.n.* (MJG), EU923677, EU923756. ***C.*** sp. (= ***Tiarocarpus hymenostephanus*** Rech. f.), (subg. *Cousinia*), Afghanistan: EU923831, EU923945. ***C. stahliana*** Bornm. & Gauba (subg. *Cousinia* sect. *Stenocephalae*), Iran: Khorassan, Shirvan, 10 km from Ziarat to Lojelli, 1215 m, *Mehregan 121* (MJG), EU923660, EU923739. ***C. stechmaniopsis*** Rech. f. (subg. *Cousinia* sect. *Badghysia*), Afghanistan: Badghys, 20 km W of Gulran, 900 m, *Freitag 5626a* (KAS), EU923620, EU923699. ***C. stocksii*** C. Winkl. (subg. *Cousinia* sect. *Stocksianae*), Afghanistan: EU923795, EU923909 & Iran: Baft, Rabor to Saruieh, before Geluchar, 2400 m, *Ghahreman & al. 28618* (TUH), EU923795, EU923909 [as same as that of previous locality]. ***C. syrdarjensis*** Kult. (subg. *Cousinia* sect. *Cousinia*), Kazakhstan: AY373741, AY373708. ***C. takhtajanii*** Tamanyan (subg. *Cousinia* sect. *Cynaroideae*), Armenia: Sjunik province, N Kapan, 46°29'28"E, 39°16'53", 1650 m, *Vitek & al. 04-0991* (MJG), EU923625, EU923704. ***C. tashkurghanica*** Rech. f. (subg. *Cousinia* sect. *Actinia*), Afghanistan: EU923777, EU923891. ***C. tenella*** Fisch. & C. A. Mey. (subg. *Cousinia* sect. *Tenellae*), Iran: EU923774, EU923888. ***C. tenuifolia*** C. A. Mey. (subg. *Cousinia* sect. *Cousinia*), Iran: EU923812, EU923926. ***C. tenuiramula*** Rech. f. (subg. *Cousinia* sect. *Stenocephalae*), Iran: EU923798, EU923912. ***C. tenuispina*** Rech. f. (subg. *Cousinia* sect. *Parvicipites*), Afghanistan: Bamian, near Bande amir, 3000 m, *Japan. Mycol. 7045* (KAS), EU923613, EU923692. ***C. thamnodes*** Boiss. & Hausskn. (subg. *Cousinia* sect. *Stenocephalae*), Iran: EU923800, EU923914. ***C. thomsonii*** Clarke (subg. *Cousinia* sect. *Alpinae*), Pakistan: Chitral, Golen (Krui Uts), 36°1'N, 72°10'E, 13000 ft., *Lyon 133* (W), EU923652, EU923731. ***C. tianschanica*** Kult. (subg. *Cousinia* sect. *Carduncellus*), Kazakhstan: AY373743, AY373710. ***C. tirinensis*** Rech. f. (subg. *Cousinia* sect. *Pugioniferae* Bunge), Afghanistan: Orozgan, 5 km NE Faramoz, 2150 m, *Podlech 31699* (M), EU923653, EU923732. ***C. tomentella*** C. Winkl. (subg. *Cynaroides* sect. *Pseudarctium*), Tadjikistan: EU923767, EU923881. ***C. trachylepis*** Bunge (subg. *Cousinia* sect. *Badghysia*), Iran: Semnan, 10 km from Shahroud to Semnan, 1000 m, *Mehregan 214* (MJG), EU923673, EU923752. ***C. trachyphyllaria*** Bornm. & Rech. f. (subg. *Cousinia* sect. *Platyacanthae*), Iran: EU923828, EU923942. ***C. triceps*** Kult. (subg. *Cousinia* sect. *Leiacanthos),* Uzbekistan: EU923784, EU923898. ***C. tricolor*** Rech. f. (subg. *Cousinia* sect. *Tricolores*), Afghanistan: Ghorat, Dolaini, Darreh Garmak, 2540 m, *Rechinger 18904-II* (W), EU923859, EU923973. ***C. triflora*** Schrenk (subg. *Cynaroides* sect. *Oligantha*), Iran: EU923771, EU923885 & Kazakhstan: AY373744, AY373711. ***C. turcomanica*** C. Winkl. (subg. *Cousinia* sect. *Leucocaulon*), Iran: EU923834, EU923948. ***C. turkmenorum*** Bornm. (subg. *Cousinia* sect. *Chrysoptera*), Iran: EU923855, EU923969. ***C. umbrosa*** Bunge (subg. *Cynaroides* sect. *Pseudarctium*), Kazakhstan: AY373745, AY373712. ***C. urumiensis*** Bornm. (subg. *Cousinia* sect. *Cousinia*), Iran: EU923809, EU923923. ***C. vavilovii*** Kult. (subg. *Hypacanthodes* sect. *Serratulopsisang),* Kyrgyzstan: Syr-Darja, distr. Aulie-ate, prope Utsch-Bulak, *Popov s.n.* (W), EU923632, EU923711. ***C. verticillaris*** Bunge (subg. *Cousinia* sect. *Alpinae*), Afghanistan: Paktia, 10 km E of Dohmandi, 2750 m, *Freitag 3536* (KAS), EU923606, EU923685. ***C. vvedenskyi*** Tscherneva (subg. *Cousinia* sect. *Cousinia*), Uzbekistan: Surhandarya region, Chul'-Bair, near village Cina, *Bochanzev 111 (*LE), EU923651, EU923730. ***C. xanthacantha*** Regel (subg. *Cousinia* sect. *Eriocousinia*), Afghanistan: EU923778, EU923892. ***C. xiphiolepis*** Boiss. (subg. *Cousinia* sect. *Sphaerocephalae*), Iran: EU923824, EU923938. ***C. zardkuhensis*** Attar & Ghahreman (subg. *Cousinia* sect. *Cynaroideae*), Iran: Bakhtiari, Farsan to Filabad, 2400 m, *Mehregan 205* (MJG), EU923623, EU923702. ***Hypacanthium echinopifolium*** (Bornm.) Juz., Kyrgyzstan: AY373746, AY373713. ***Jurinea albicaulis*** Bunge, Greece: AY373747, AY373714. ***Saussurea elegans*** Ledeb., Kazakhstan: AY373750, AY373717. ***Schmalhausenia nidulans*** (Regel) Petr., Kazakhstan: AY37352, AY373719.