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Taxonomic problems in *Carthamus* (Asteraceae): RAPD markers and sectional classification

ROSER VILATERSANA*, TERESA GARNATJE, ALFONSO SUSANNA and NÚRIA GARCIA-JACAS

Botanic Institute of Barcelona (CSIC), Passeig del Migdia s.n., E-08038 Barcelona, Spain

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Recent molecular studies have clarified the problem of the generic limits of *Carthamus*. However, taxonomic problems remain in the sectional classification: section *Carthamus* was confirmed as a natural group, but molecular phylogenies based on DNA sequences did not resolve the other sections, resulting in a confusing conglomerate. A survey based on RAPD markers has been carried out in this conflicting group of sections, with the aim of evaluating the usefulness of these markers at the section and species levels. On the basis of the results, correlated with morphological and karyological characters, some recommendations are made in relation to the taxonomy of *Carthamus*. The number of sections is reduced from five to two: section *Thamnacanthus* is currently classified as a different genus, *Phonus*; section *Carthamus s.s.* is retained in its present delimitation, and section *Atractylis* is redefined, including current sections *Atractylis*, *Lepidopappus* and *Odontagnathius*. The commonly accepted treatment of some taxa as subspecies is not well founded, and *C. alexandrinus*, *C. creticus*, *C. tenuis* and *C. turkestanicus* should be considered as independent species. Our results confirm that methodological problems posed by RAPD markers can be avoided by careful laboratory procedures and appropriate data analyses; they also suggest that this kind of marker is useful at low taxonomic levels and is, furthermore, complementary to DNA sequence analysis.

ADDITIONAL KEYWORDS: Cardueae – Centaureinae – Compositae – molecular markers.

INTRODUCTION

The genus *Carthamus* L. is composed of 15 species of east Mediterranean origin. Most are diploid, but there are three polyploids (*C. creticus* L., *C. lanatus* L. and *C. turkestanicus* Popov). Some species have spread far beyond the eastern Mediterranean as noxious weeds: *C. lanatus* is widespread in all the Mediterranean region and, together with *C. creticus* and *C. leucocaulos* Sibth. & Sm., in all the Mediterranean climate regions of the world (Argentina, Australia, California, Chile and South Africa; cf. Ashri & Knowles, 1960; Hanelt, 1963; Estilai & Knowles, 1978).

Carthamus tinctorius L. (safflower) is widely grown as an important source of oil in subtropical countries (Hanelt, 1963) and as a substitute for saffron; the wild origin of this cultivated species is unknown.

The natural delineation of *Carthamus* and *Carduncellus* Adans. was recently clarified by Vilatersana *et al*. (2000a), and the next challenge was the sectional classification of *Carthamus*. Knowles (1958) informally established four 'sections' mainly on the basis of chromosome number: section I, species with $n = 12$; section II, species with $n = 10$; section III, species with $n = 22$ (comprising only *C. lanatus*); and section IV, species with $n = 32$ (formed by two species, *C. creticus* and *C. turkestanicus*). Later, Estilai (1977) created section V for *C. divaricatus* Beg. & Vacc., the only species of *Carthamus* with $n = 11$ (Table 1).

Hanelt (1963) formally divided the genus *Carthamus* into five sections on the basis of morphological characters: section *Thamnacanthus* (DC.) Šostak. ex Hanelt ($n = 12$), which included species now classified in a different genus, *Phonus* Hill (López González, 1990; Vilatersana *et al*., 2000a); section *Carthamus* ($n = 12$), made up of the group of species closely related to *C. tinctorius*; section *Odontagnathius* (DC.) Hanelt ($n = 10$), with the single species *C. dentatus* Vahl.; section *Lepidopappus* Hanelt ($n = 10$), with the rest of the species with $n = 10$ and *C. nitidus* Boiss. ($n = 12$), owing

*Corresponding author. E-mail: laboratori@ibb.csic.es

Table 1. Different sectional classification of the genus *Carthamus*

Knowles (1958), Estilai (1977)	Hanelt (1963)
Section I (2*n* = 24)	Sect. *Thamnacanthus* (DC.) Sostak (2*n* = 24)
C. arborescens L.	*C. arborescens* L.
C. riphaeus Font Quer & Pau	*C. riphaeus* Font Quer & Pau
C. oxyacantha M. Bieb.	
C. flavescens Willd. (= *C. persicus* Willd. ex Boiss.)	
C. palestinus Eig	
C. tinctorius L.	
C. nitidus Boiss.	
Section II (2*n* = 20)	Sect. *Carthamus* (2*n* = 24)
**C. alexandrinus* (Boiss. & Heldr.) Bornm.	*C. persicus* Willd. ex Boiss.
**C. boissieri* Halácsy	*C. palestinus* Eig
**C. dentatus* Vahl	*C. gypsicola* Iljin
**C. glaucus* M. Bieb.	*C. curdicus* Hanelt
**C. leucocaulos* Sibth. & Sm.	*C. oxyacantha* M. Bieb.
C. syriacus (Boiss.) Dinsm. (= *C. glaucus* M. Bieb. ssp. *glandulosus* Hanelt)	*C. tinctorius* L.
**C. tenuis* (Boiss. & Blanche) Bornm.	
Section III (2*n* = 44)	Sect. *Odontagnathius* (DC.) Hanelt (2*n* = 20)
**C. lanatus* L.	*C. dentatus* Vahl
	ssp. *dentatus*
	*ssp. *ruber* (Link) Hanelt
Section IV (2*n* = 44)	Sect. *Lepidopappus* Hanelt (2*n* = 20, 24)
**C. creticus* L.	*C. glaucus* M. Bieb.
**C. turkestanicus* Popov	*ssp. *glaucus* (2*n* = 20)
	ssp. *glandulosus* Hanelt (2*n* = 20)
	ssp. *anatolicus* (Boiss.) Hanelt (2*n* = 20)
	*ssp. *alexandrinus* (Boiss & Heldr.) Hanelt (2*n* = 20)
	**C. boissieri* Halácsy (2*n* = 20)
	**C. tenuis* (Boiss. & Blanche) Bornm. (2*n* = 20)
	**C. leucocaulos* Sibth. & Sm. (2*n* = 20)
	C. nitidus Boiss. (2*n* = 24)
Section V (2*n* = 22)	Sect. *Atractylis* Rchb. (2*n* = 22, 44, 64)
C. divaricatus Beg. & Vaccari (= *C. lanatus* L. ssp. *creticus* var. *divaricatus* (Beg. & Vaccari) Pamp.)	*C. lanatus* L.
	*ssp. *lanatus* (2*n* = 44)
	*ssp. *creticus* (L.) Holmb. (2*n* = 64) [including var. *divaricatus* (Beg. & Vaccari) Pamp. (2*n* = 22)]
	ssp. *montanus* (Pom.) Jahand & Maire (2*n* = ?)
	*ssp. *turkestanicus* (Popov) Hanelt (2*n* = 64)

* Species included in the present study.

to the close morphological similarity to *C. leucocaulos*; and finally section *Atractylis* Rchb. (*n* = 11, 22 and 32), which includes *C. divaricatus*, *C. lanatus*, *C. creticus* and *C. turkestanicus* (Table 1).

Besides differences in sectional delineation, there are important variations in the treatment of some species, such as *Carthamus alexandrinus*, *C. creticus*, *C. tenuis* and *C. turkestanicus*, which are diversely treated either as subspecies (Hanelt, 1963) or as independent species (Knowles, 1958; Estilai, 1977).

Our previous analysis using the ITS region of the nuclear-ribosomal DNA in *Carthamus* (Vilatersana *et al*., 2000a) established two different well-supported clades: on the one hand, *Carthamus s.s.* (sect. *Carthamus sensu* Hanelt), which emerged as a well-defined natural group, and on the other, the remaining species of the genus, a conglomerate that has been considered by some authors to be a different genus, *Kentrophyllum* Neck. (De Candolle, 1810; Cassini, 1819). Within this group, ITS phylogeny was not correlated to sectional classification. We decided to try a different approach using molecular markers (RAPD, random amplified polymorphic DNA, Williams *et al*., 1990). Notwithstanding their limitations (reviewed in Riese-

berg, 1996; Gillies & Abbott, 1998), RAPD analysis has proven to be a useful tool in systematics, because it can detect low levels of genetic variability (Hilu, 1995; Bartish, Jeppsson & Nybom, 1999), often matching consistently the results of molecular systematics and morphology (Esselman *et al*., 2000; Nebauer, del Castillo-Agudo & Segura, 2000). Thereafter, we carried out an RAPD analysis on representatives of all the sections of *Carthamus* that constituted the '*Kentrophyllum*' group (sections *Atractylis*, *Lepidopappus* and *Odontagnathius*) with the objectives of evaluating the potential of RAPD markers in systematics at the genus, species and population levels, and also of verifying sectional classification within the group.

MATERIAL AND METHODS

Plant material

The study included two populations each of *C. creticus*, *C. glaucus* M. Bieb. ssp. *glaucus*, *C. lanatus* and *C. turkestanicus*, and one population each of *C. alexandrinus* (Boiss. & Heldr.) Asch., *C. boissieri* Halácsy, *C. dentatus* ssp. *ruber* (Link) Hanelt, *C. leucocaulos* and *C. tenuis* (Boiss. & Blanche) Bornm. Populations included in the study are listed in Table 2. Herbarium vouchers are deposited at the Botanic Institute of Barcelona (BC).

DNA extraction and RAPD amplifications

Seeds collected in the field were germinated and DNA was extracted from a fully developed cotyledon of four or five plants from each population. A duplicate DNA extraction from one plant per population served as a control. Total genomic DNA was isolated following the protocol of Doyle & Doyle (1987), as modified by Cullings (1992). Nineteen primers (A1, A3, A13, A20, B7, B8, D10, E5, G2, G19, J1 and J3–J10; Operon Technologies) were screened to obtain nine primers (A1, A3, A13, A20, B7, B8, G2, J5, and J9; Table 3) that produced reproducible, polymorphic bands. Primers were tested with different DNA concentrations to determine the concentration for optimal RAPD patterns while minimizing artefactual bands. DNA amplification was carried out as described in Garnatje *et al*. (2002). Absence of contamination in each run

Table 2. Species and populations included in the study and vouchers and number of individuals analysed

Species	Voucher	Individuals
C. alexandrinus (Boiss. & Heldr.) Bornm.	Egypt, Alexandria: between El Amiriya & Burg-el-Arab, Susanna 1835 & Vilatersana, 07.vi.1998 (BC).	4
C. boissieri Halácsy	Greece, Kriti, Hania: Drapanon peninsula, Vilatersana 36, 09.vii.1996 (BC).	5
C. creticus L.	Population A: Egypt, Alexandria: near El Amiriya, Susanna 1851 & Vilatersana, 07.vi.1998 (BC).	5
	Population B: Morocco, Al-Hoceima: between Tiztoutine and Al-Hoceima, road from Nador to Al-Hoceima km 417, Garnatje, Susanna 1772 & Vilatersana, 15.vi.1997 (BC).	5
C. dentatus Vahl ssp. *ruber* (Link) Hanelt	Greece, Kriti, Rethimnon: road N-97 between Rotosí and Mesohorio, Vilatersana 44, 14.vii.1996 (BC).	4
C. glaucus M. Bieb. ssp. *glaucus*	Population A: Armenia, Ekhegnadzor: near the village of Agarakadzor, Fajvush, Gabrielyan, Garcia-Jacas, Guara, Hovannisyan, Susanna 1551, Tamanyan & Vallès, 20.viii.1995 (BC).	5
	Population B: Iran, Azarbaijan-e Shargui: near Tatar, Garcia-Jacas, Mozaffarian, Susanna 1678 & Vallès, 07.viii.1996 (BC).	5
C. lanatus L.	Population A: Spain, Málaga: near Antequera, at the foot of the Peña de los Enamorados, Garcia-Jacas, Susanna 1609 & Vilatersana, 23.vi.1996 (BC).	5
	Population B: Greece, Kriti, Rethimnon: between road N-77 and the Necropolis Minois, Vilatersana 27, 07.vii.1996 (BC).	5
C. leucocaulos Sibth. & Sm.	Greece, Kriti, Hania: foot of mount Hrissoskalitissas, Vilatersana 40, 11.vii.1996 (BC).	5
C. tenuis (Boiss. & Blanche) Bornm.	Israel: Jordan valley, R. Levy, ix.1997 (BC).	5
C. turkestanicus Popov	Population A: Armenia, Ararat: near Surenavan, Fajvush, Gabrielyan, Garcia-Jacas, Guara, Hovannisyan, Susanna 1532, Tamanyan & Vallès, 19.viii.1995 (BC).	5
	Population B: Iran, Azarbaijan-e Shargui: 35 km from Tabriz on the road to Ahar, Garcia-Jacas, Mozaffarian, Susanna 1656 & Vallès, 05.viii.1996 (BC).	5

Table 3. Description of the RAPD fragments generated and sequences of the nine primers

Primer	Sequence (5′ → 3′)	Size (bp) min.–max.	No. of bands		
			Polymorphic	Monomorphic	Total
OPA1	CAGGCCCTTC	350–2400	18	0	18
OPA3	AGTCAGCCAC	275–1890	19	0	19
OPA13	CAGCACCCAC	275–2000	15	0	15
OPA20	GTTGCGATCC	370–1800	13	0	13
OPB7	GGTGACGCAG	280–2000	19	1	20
OPB8	GTCCACACGG	340–1880	15	1	16
OPG2	GGCACTGAGG	225–1900	19	1	20
OPJ5	CTCCATGGGG	390–2000	13	1	14
OPJ9	TGAGCCTCAC	330–2300	23	1	24
Total			154	5	159

was verified by including a negative control in which DNA was omitted. Reproducibility was confirmed by duplicating randomly selected reactions for DNA samples of each population for each primer.

Amplification products were resolved by electrophoresis in 2% agarose gels in 0.25× TBE buffer run at 10 V cm^{-1} for about 5 h in 0.25× TBE, and visualized by staining with ethidium bromide. PGEM DNA marker (Promega Corp.) was used as a molecular size marker. The gels were photographed under UV light with a Kodak DC220 digital camera (Eastman-Kodak).

For each primer, all the individuals from a population, plus one repetition, were run on a single electrophoresis gel. A subset of two individuals from the different populations was amplified again and run in a new electrophoresis gel, following the scheme outlined by Crawford *et al*. (1998). This procedure had two advantages: first, to check the reproducibility of the polymorphic bands; second, to increase the precision of detecting population differences, as all the band comparisons were carried out on the same gel.

Data analysis

Analysis of the RAPD products (loci) included only reliably scored and reproducible bands. For comparison among populations, a representative of the dominant pattern was selected for use from each population. Each band was assigned a molecular weight using Lane Manager 2.1a (T.D.I.S.A.), which performs a semi-logarithmic regression with the DNA molecular weight standard on each gel. Bands were then manually scored for each population, 1 for presence and 0 for absence, to generate a binary matrix.

In view of the different opinions on the intensity of bands that should be included in RAPD analysis (Weeden *et al*., 1992; Lamboy, 1994), we followed Hilu & Stalker (1995) and we generated three different matrices: for matrix 1, all DNA fragments were scored as present; for matrix 2, the faint bands were scored as absent, and for matrix 3, only the most intense bands were scored as present, and we carried out the following analyses:

(a) UPGMA. The three binary matrices of polymorphic bands were analysed using the program NTSYS-pc (Rohlf, 1997) with the routine SIMQUAL (similarity for qualitative data), using Dice's similarity coefficient (Dice, 1945) in order to generate an unweighted pair-group (UPGMA) dendrogram, through the SAHN program (sequential agglomerative hierarchical nested cluster analysis) implemented in NTSYS. According to Whitkus, Doebley & Wendel (1994) and Lamboy (1994), Dice's coefficient is the best choice for RAPD.

According to the results of the Mantel test (Mantel, 1967) as implemented in NTSYS-pc, the correlation between cophenetic values of the dendrogram based on matrices 1, 2 and 3 was high ($r = 0.99$, 0.93 and 0.92, respectively) in all the cases, following Rohlf (1997). Thereafter, all our discussion and graphics will centre on the analysis of matrix 2 (strongest and medium bands scored) because artefactual bands can be false positives (presence) as well as false negatives (absence), according to Lamboy (1994).

Matrix 2 was also analysed excluding the individuals of the two taxa of purported allopolyploid origin according to Khidir & Knowles (1970a, b), *C. creticus* and *C. turkestanicus*. We carried out a third analysis excluding *C. lanatus* too, a suspected polyploid. All these verifications were made so as to ascertain whether the hybrid populations were causing 'capture' of the nonhybrid populations (McDade, 1997).

(b) Neighbour-joining analysis (NJ). Neighbour-joining analysis (Saitou & Nei, 1987) was carried out

with the software TREECON for Windows version 1.3b (Van de Peer & De Wachter, 1994) with Nei & Li index (Nei & Li, 1979). The NJ algorithm does not assume that all the evolutionary changes have the same rate, and Gillies & Abbott (1998) suggested that NJ was appropriate for RAPD because of the random nature of RAPD bands and the different rate of evolution between DNA from different parts of the genome (nuclear/organellar, repetitive/not repetitive). As a check of the support for the branches in the NJ tree, we carried out a bootstrap analysis with 100 replicates.

RESULTS

The number and characters of the amplification products are detailed in Table 3. The level of polymorphism was high, with only 3.14% of bands being monomorphic and only four being uninformative. The average number of polymorphic loci per primer was 17.

Dice's similarity coefficient between species ranged from 0.434 (between *C. tenuis* and population B of *C. creticus*) to 0.747 (between population A of *C. lanatus* and population A of *C. creticus*). Interpopulational similarity coefficients were high (from 0.871 in *C. lanatus* to 0.952 in *C. turkestanicus*) as were intrapopulational similarity coefficients (0.987 in population B of *C. lanatus* and 1.000 in *C. tenuis*, *C. creticus* and in population B of *C. turkestanicus*) (Table 4).

Figure 1 shows the UPGMA dendrogram with two well-differentiated clusters. The first one was formed by *C. tenuis*, *C. boissieri*, *C. dentatus* ssp. *ruber* (marked *C. ruber* in all the figures) and *C. alexandrinus*. The second cluster included all the populations of *C. lanatus*, *C. creticus* and *C. leucocaulos*, on the one hand, and the populations of *C. turkestanicus* and *C. glaucus* ssp. *glaucus* (marked *C. glaucus* in all the figures), on the other.

Phenetic dendrograms excluding the purportedly hybrid species were similar to those including all the species; the capture effect suggested by McDade (1997) was not interfering with our results.

The unrooted NJ tree is shown in Figure 2. The results were similar to the UPGMA dendrogram. For a better reading of this tree, the different individuals of each population are not detailed.

DISCUSSION

Genetic variability

Genetic similarity values are always difficult to compare with other studies because genetic variability depends heavily on factors including the history of the species, the reproductive system and ecology (Hamrick, 1989). However, we have found extremely scarce or no intrapopulational genetic variability in our study, partly, no doubt, because many of the included species are colonizing pioneers, in some cases (*C. creticus* or *C. turkestanicus*) with high levels of self-compatibility (Khidir & Knowles, 1970b). In the four cases where more than one population was studied, interpopulational variability was also low (genetic similarity ranged between 0.871 and 0.952, see Table 4), even though the geographical origins of the compared populations were distant from each other: for *C. creticus*, one population was from Egypt and another from Morocco, the extremes of its natural range. In the case of *C. lanatus*, the extreme genetic similarity is not correlated with the high morphological diversity found across the range of this species

Table 4. Average Dice's similarity coefficient

	tenuis	leuco	cretiA	cretiB	lanatA	lanatB	glaucA	glaucB	turkesA	turkesB	boiss	ruber	alex
tenuis	1.000												
leuco	0.505	0.997											
cretiA	0.471	0.720	1.000										
cretiB	0.434	0.694	0.892	1.000									
lanatA	0.469	0.496	0.715	0.747	0.996								
lanatB	0.478	0.526	0.690	0.723	0.871	0.987							
glaucA	0.476	0.564	0.533	0.569	0.577	0.583	0.993						
glaucB	0.515	0.524	0.508	0.579	0.572	0.576	0.896	0.991					
turkesA	0.494	0.567	0.618	0.642	0.644	0.679	0.737	0.763	0.998				
turkesB	0.496	0.591	0.638	0.676	0.663	0.671	0.737	0.743	0.952	1.000			
boiss	0.575	0.528	0.465	0.505	0.493	0.474	0.537	0.565	0.541	0.500	0.987		
ruber	0.578	0.527	0.504	0.508	0.488	0.465	0.545	0.544	0.513	0.543	0.603	0.993	
alex	0.467	0.522	0.481	0.503	0.501	0.494	0.573	0.550	0.585	0.601	0.602	0.534	0.998

Acronyms: alex = *Carthamus alexandrinus*; boiss = *C. boissieri*; creti = *C. creticus*; glauc = *C. glaucus*; lanat = *C. lanatus*; leuco = *C. leucocaulos*; turkes = *C. turkestanicus*. A and B indicate the population.

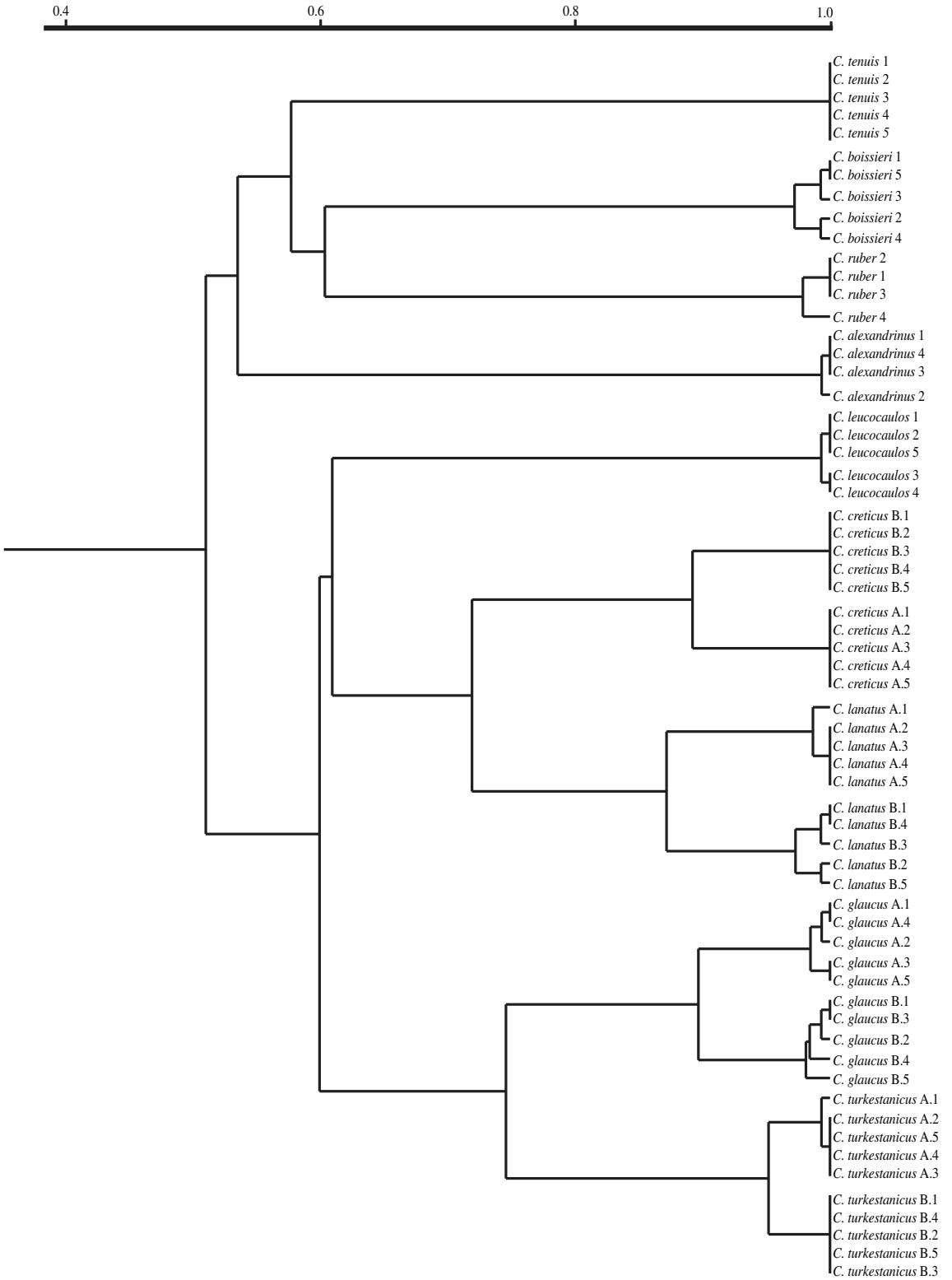

Figure 1. UPGMA dendrogram generated by NTSYS using 159 RAPD markers and Dice's coefficient of genetic similarity. Numbers on the taxa labels indicate the individuals from each population; A and B indicate the population.

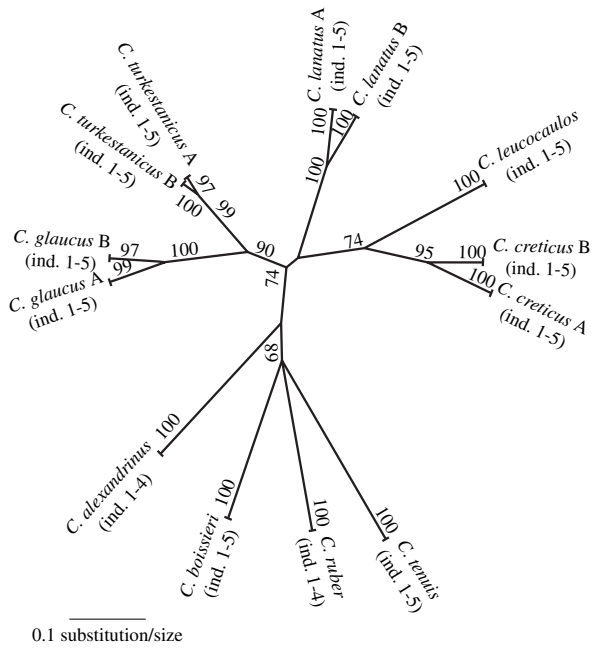

Figure 2. Unrooted neighbour-joining tree generated by NTSYS using 159 RAPD markers and the Nei–Li index. Numbers above branches are bootstrap values; A and B indicate the population.

(Harvey & Knowles, 1965). This pattern of variability often appears in colonizing species of wide distribution (Barrett & Shore, 1989). Contrary to our results, Ash, Raman & Crump (2003) found high levels of genetic variability in colonizing Australian populations of *C. lanatus*. One of the reasons for this variability could be the inclusion of populations of *C. creticus* in their study, which must be considered a different allopolyploid species.

Species delimitation

An important conclusion is that the RAPD analyses do not support the usually adopted subspecific treatment for *C. glaucus* ssp. *alexandrinus* (Hanelt, 1963), *C. glaucus* ssp. *tenuis* (Schank & Knowles, 1964), *C. lanatus* ssp. *creticus* and *C. lanatus* ssp. *turkestanicus* (Hanelt, 1963). RAPD markers strongly favour the specific treatment because the four purported subspecies do not form groups together with the species to which they are subordinated (Figs 1, 2). Only *C. creticus* is always associated to *C. lanatus* (Figs 1, 2), but in this case the subspecific treatment was already inadequate: *C. lanatus* is one of the progenitors of the allopolyploid *C. creticus* (Khidir & Knowles, 1970b) and, on this basis, *C. creticus* cannot be treated as a subspecies of *C. lanatus*.

Sectional classification

Our results do not support the traditional division of the species of *Carthamus* with $n = 10$ into two sections, section *Lepidopappus* and section *Odontagnathius*, as suggested by Hanelt (1963). In the UPGMA dendrogram and NJ tree (Figs 1, 2), all these species are always grouped together. This relationship was anticipated by Schank & Knowles (1964) and Estilai & Knowles (1978) as a result of their hybridization studies in both sections: they found a certain rate of meiotic pairing in crosses between *C. dentatus* and the rest of the species of *Carthamus* with $n = 10$. Later, López González (1990) suggested merging all the species of *Carthamus* with $n = 10$ into a single section, *Odontagnathius*.

All three species of section *Atractylis*, *C. lanatus*, *C. creticus* and *C. turkestanicus*, are polyploid. In the case of *C. lanatus*, it has been suggested that it could be an autopolyploid ($2n = 4x = 44$). On the other hand, *C. creticus* and *C. turkestanicus* would be allopolyploids by hybridization of *C. lanatus* ($n = 22$) and species with $n = 10$. The species of section *Atractylis* appear closely related to the species of *Carthamus* with $n = 10$, as was demonstrated by Ashri & Knowles (1960), Harvey & Knowles (1965) and Khidir & Knowles (1970b), again on the basis of artificial crosses. The analyses of RAPD markers (Figs 1, 2) confirm this close relationship between *C. lanatus*, *C. creticus* and *C. turkestanicus* and species of *Carthamus* with $n = 10$ such as *C. leucocaulos* and *C. glaucus*. The assessment of the origin of polyploidy in section *Atractylis* is the subject of a different study (R. Vilatersana, unpubl. data).

In view of the coincident relationships suggested both by RAPD markers and ITS sequences, and having also in mind the results of previous authors, we suggest the following sectional classification for the genus *Carthamus*:

1. Section *Carthamus*. Type: *Carthamus tinctorius*. Other species: *C. curdicus* Hanelt, *C. gypsicola* Iljin, *C. oxyacantha* M. Bieb., *C. palestinus* Eig, and *C. persicus* Willd. All the species of section *Carthamus* have the base chromosome number $n = 12$. This definition coincides with the delimitation by Hanelt (1963), followed by López González (1990) and confirmed on molecular grounds by Vilatersana *et al*. (2000a).
2. Section *Atractylis*. Type: *Carthamus lanatus*. This section has clear support both from the RAPD analyses and from hybridization and chromosome pairing studies by Schank & Knowles (1964), Harvey & Knowles (1965), Khidir & Knowles (1970a, b) and Estilai & Knowles (1976, 1978). As defined here, this section comprises sections *Atractylis*, *Odontagnathius* and *Lepidopappus* in the classification of

Hanelt (1963), and coincides with the old genus *Kentrophyllum*. Perhaps the only point of dispute in this redefined section *Atractylis* is the inclusion of *C. nitidus*, which was sister to the rest of the species of this group of *Carthamus* in our ITS tree (Vilatersana *et al*., 2000a). The isolated position of this species is also supported by hybridization studies by Knowles & Schank (1964) and Estilai & Knowles (1978), and by its divergent base chromosome number $n = 12$ (a number found in section *Carthamus*), whereas the rest of the species of the section have $n = 11$ and $n = 10$. This relationship between species with $n = 10$, 11 and 12 is a priori disconcerting but it could be explained in terms of descending dysploidy (Vilatersana *et al*., 2000b), with the exclusion of the hybridogenic basic number $n = 32$ from the dysploid series. The pappus of *C. nitidus* is also different, in some aspects intermediate between sections *Carthamus* and *Atractylis* (Hanelt, 1963; Vilatersana, 2002). However, in the final instance, this is the only morphological difference between *C. nitidus* and the rest of the species of section *Atractylis*, and this character alone does not justify a different section. A comparison of RAPD bands between *C. nitidus* and the remaining species could perhaps have helped us to clear up its status, but difficulties in assessing homology of bands made us discard its inclusion in the analysis.

As a concluding remark, RAPD markers have been useful in the study of sectional, species and subspecies classification in *Carthamus*. When comparing RAPD results and the ITS-based phylogeny (Vilatersana *et al*., 2000a), we find a good complementarity because they work at different taxonomic levels. Our results are also coincident with the classic studies of hybridization and chromosome pairing between species by Schank & Knowles (1964), Harvey & Knowles (1965), Khidir & Knowles (1970a, b) and Estilai & Knowles (1976, 1978).

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