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Phylogeny of the *Cardueae* (*Asteraceae*) based on analysis of morphological and palynological characters

Abstract

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Between 1955 and 1977, the main contributions to the systematics of the *Cardueae* were made by Wagenitz (pollen: 1955), Dittrich (histology of cypselas: 1966, 1968a, 1968b, 1969, 1977, 1984) and Meusel & Kohler (growthform: 1960).

Since 1980, new approaches have provided an impetus to a new look at the group. Different authors used cladistic methods to analyse morphological, palynological and molecular characters [Bremer (1994), Susanna & al. (1995), Petit (1997) and Petit & al. (1996)].

We here present results of a cladistic analysis based on two characters sets, one morphological (33 characters), the other palynological (41 characters), of 34 taxa close to *Centaurea*. The morphological characters come mostly from previous published studies, whereas the palynological ones, defined in a previous work, are applied to the *Centaureinae* for the first time. Special emphasis is placed on the *Carthamus* complex. Several approaches were investigated: two outgroups (*Jurinea* and *Saussurea*) and, with respect to the pollen analysis, different qualitative and quantitative character codings.

The very high sensibility of obtained topologies when the data sets are considered alone or together is remarkable. We interpret the lack of robustness of relationships between clades by the probable "burst" nature of evolution in this group; the origin of the group is doubtless in the Mediterranean region. This "star-like" phylogeny, the probable result of ecological factors, has yet to be explored.

Introduction

The tribe *Cardueae* s. l. is a basal clade within *Asteraceae* and belongs to the *Cichorioidea* subfamily (Karis & al. 1992, Bremer 1994). It comprises about 2500 species distributed across 83-90 genera (Bremer 1994).

Previous cladistic analysis of morphological and palynological data has argued for the recognition of 2 monophyletic groups, the *Echinopeae* and the *Cardueae* s. str. (Petit 1987, 1988, 1997, Petit & al. 1996). On this basis the following classification was proposed:

tribe *Echinopeae* Cass. *Acantholepis*, *Cousiniopsis*, *Cardopatum*, *Echinops*
tribe *Cardueae* Cass.

Subtribe *Carlininae* Dumort.: *Carlina*, *Atractylis*, *Chamaeleon*, *Atractylodes*, *Thevenotia*

Subtribe *Carduinae* Dumort.

Series 1 (*Xeranthemum* group): *Staehelina*, *Amphoricarpos*, *Xeranthemum*, *Siebera*, *Chardinia*

Series 2 (core group of *Cynareae*): about 74 genera.

This classification differs from Dittrich's one (1977) in several important points:

- the reduction of "*Carlineae*" sensu Dittrich (1977) into five very close genera (*Carlininae*), the *Xeranthemum* group being included in *Carduinae*, and *Cardopatium* and *Cousiniopsis* joined to *Echinops-Acantholepis* to form the *Echinopeae*;
- series 2 contains the "*Cardueae*" sensu Dittrich (1977) and is composed of (i) a paraphyletic group, the "*Carduinae* Dumort." sensu Dittrich (species close to *Carduus*) and (ii) the "*Centaureinae* Dumort." sensu Dittrich (1977) (species close to *Centaurea*).

Dittrich (1977) and Petit (1997) considered the genera *Saussurea* and *Jurinea* as phylogenetically at the base of series 2. Moreover, the cladistic analysis (with branch swapping) of Internal Transcribed Spacers (ITS1 and ITS2) sequences suggested that *Jurinea*, close to *Galactites*, is at the base of the "*Centaureinae*" (Susanna & al. 1995); these last authors did not study *Saussurea*. We continue to use here the well-known designation "*Centaureinae*", the content of which is non-ambiguous, despite the fact that in our opinion its rank is far below that of subtribe.

We have conducted a cladistic analysis of genera belonging to "*Centaureinae*" using morphological and palynological characters. Much of the information for this study comes from the works of Dittrich (1966, 1968a, 1968b, 1969, 1977, 1984) on carpology and of Qaid (1990, unpublished thesis) on pollen. The important contribution of Wagenitz (1955) on the pollens of this group, although quite valuable, predates the now common usage of scanning electronic microscopy, and for this reason is hard to exploit for our purpose.

Material and methods

Most of the plant material used in the present study is deposited in the herbarium of the "Biologie cellulaire et Valorisation des espèces ligneuses" laboratory (University of Limoges), and was collected and determined by D. Petit. Remaining materials come from specimens held in the herbarium of the University of Montpellier II (Institut de Botanique, MPU). The precise list of localities with dates of collection of samples is given in table 3.

Morphological and palynological characters here described follow the order, coding and definitions of previous works (Petit 1997, Petit & al. 1996), a very few cases excepted (Table 1). Correspondence between new and ancient character numbers is given in table 2. Some precisions and corrections are added.

Palynological data were collected by Qaid for his thesis (1990), completed and corrected by the authors. Pollen grains, extracted from a mature flower bud of one individual for each species, were mounted in glycerine jelly after acetolysis and observed using light microscope. In order to investigate exine ornamentation details, scanning electron microscope observations were made on acetolyzed or non-acetolyzed grains, and exine structure was studied after ultrasonic fracture. Each dimension taken in account is the average of measures of 25 grains randomly selected on the same slide.

In order to apply cladistic and phenetic (Correspondence Analysis) methods to the whole of Qaid's data set for 156 species of *Cardueae*, each quantitative continuous palynological variable was transformed into a qualitative one by grouping its values in a little number of classes which became the different modalities of the qualitative variable. To satisfy the specific constraints of Correspondence Analysis, limits of classes were established in such a way that frequencies in different classes of the same variable are as equal as possible. In order to give them the same weight, the different quantitative variables were divided in the same number of classes. Three seemed to be a reasonable number, however, visual examination of frequency histograms induced to establish a different number of classes for a few variables, such as character 65 with four classes. Quantitative data preparation was achieved with Bioméco package, developed by investigators of the Unité de Biométrie (CEFE-CNRS, Montpellier).

Cladistic analyses were performed with Hennig86 software (Farris 1988). A non-exhaustive search with branch breaking option (mh* bb) was done, followed by the production of a consensus tree (nelsen option). The successive weighting (xsteps option) allows the weakening of characters that are subject to many state changes, something revealed by previous analysis. We estimated the strength of monophyletic groups with the Jackknife Monophyly Index (JMI) computed by the Lanyon program (Siddal 1994, 1995). It calculates the proportion of nodes in agreement with the results given by n-1 analyses executed with the withdrawal of one taxon. The closer the JMI value approaches 1, the greater the stability of the node. The visualisation of character states on trees were highly facilitated by the use of Tree Gardener vers. 2.2 program (Ramos 1997), run in a Windows (Microsoft TM) environment. Trees were edited with TreeView vers. 1.5.2 (Page 1998).

Table 1. Definitions of characters.

Morphological characters	15. lateral hilum (0), basal hilum (1) 16. lateral hilum (0), caudate hilum (1)
leaves and bracts	17. detachment basal (0), lateral (1)
0. leaves thorny(0), unarmed (1)	18. crown epidermis with thin cells (0), thick cells (1)
1. fleshy juvenile and spiny axillary leaves (1), leaves different (0)	pappus
2. involucre simple (0), complex (1)	19. 1-2 whorls (0), numerous whorls (1)
3. absence (1), presence (0) of lateral spiny lobes on involucre bracts	20. pappus simple (0), double (1)
4. absence (0), presence (1) of scarious margin of involucre bracts	21. pappus non deciduous (0), deciduous as a whole (1), deciduous by elements (2)
cypsela wall	florets
5. pericarp hairs absent (0), short-acute (1), cylindrical (2), pedicellate (3)	22. indentation of the limb more than 3/4 of its length (1), less than 3/4 (0)
6. secondary walls of pericarp lignified (1), not (0)	23. tube glabrous (0), hairy (1)
7. radial wall right-angled (0), oblique (1)	24. peripheral florets fertile (0), sterile (1)
8. cells less than 40µm long (1), more than 80 µm long (0)	25. filaments glabrous (0), papillose (1); hairy (2)
9. hypoderm differentiated (1), not (0)	26. filament hairs sparse (0), dense (1)
10. absence (0), presence (1) of excretion layer (phytomelan)	27. apical appendage with a sword-like form (0), mucronate (1)
elaiosome and other formations	28. anther tails long and ciliate (0), short and scarcely denticulate (1)
11. elaiosome developed at the detachment region of cypsela (1), not developed at this region (0)	29. style branches free (0), fused (1)
12. tissue of elaiosome differentiated (1), not (0)	miscellaneous
13. absence of deformation linked to elaiosome (0), presence (1)	30. peripheral cypselas with pappus (0), without pappus (1)
14. nectary absent (0), Jurinea-type nectary (1)	31. tube less than 10 mm long (0), more than 10 mm long (1)
	32. crystals in the bracts absent (0), present (1)

Table 1 (continued).

Palynological characters	
<u>Qualitative characters</u>	
33. A: form in polar view; 4 ordered states 1: circular; 2: convex circular; 3: convex triangular; 4: triangular	45. M: height of columellae; 3 states 1: larger under spines; 2: uniform; 3: absent
34. B1: edge of ectoaperture; 2 states 1: undulated; 2: straight	46. N1: distribution of columellae; 4 non-ordered states 1: homogenous; 2: heterogenous; 3: restricted to the spines; 4: absent
35. B2: outline of ectoaperture; 3 non-ordered states 1: oblong; 2: elliptic; 3: rectangular	47. N2: diameter of columella; 3 ordered states 1: 0.1-0.5 µm; 2: 0.55-1 µm; 3: >1 µm
36. D1: constriction of endoaperture; 3 non-ordered states 1: obtuse; 2: acute; 3: absent	48. O: caveae; 4 non-ordered states 1: present; 2: more or less visible; 3: with vestigial columellae; 4: absent (thus columellae present)
37. D2: outline of endoaperture; 4 non-ordered states 1: elliptic; 2: rectangular; 3: losangic; 4: circular with acute endings	49. P: form of spines; 5 non-ordered states 1: concave conical acute; 2: conical acute; 3: convex conical acute; 4: convex obtuse; 5: smooth
38. E: intersection of ecto- and endoaperture; 2 states 1: transversally lengthened; 2: radially lengthened	50. Q: structure of spine tip; 2 states 1: empty; 2: wide
39. F: thickness of exine in aperture; 2 states 1: equal to the rest; 2: thicker	51. R: space between spines; 3 ordered states 1: concave and smooth; 2: flat and smooth; 3: flat and tuberculate
40. H: thickness of tectum; 3 ordered states 1: > 5 µm; 2: 2-5 µm; 3: <2 µm	52. S: ridges between spines; 4 ordered states 1: ridges totally absent; 2: ridges often absent; 3: ridges weakly developed; 4: ridges very developed
41. I: tectum structure; 5 ordered states 1: spongy; 2: striate; 3: columellae in 1 layer; 4: columellae in 2 layers 5: columellae in 3 layers	53. T: distribution of spines; 2 states 1: regular; 2: irregular
42. J: ramification of columellae; 3 ordered states 1: absent; 2: variable; 3: present	54. U: thickness of tectum in equatorial optical section; 3 non-ordered states 1: maximum in the intercolpus axis; 2: maximum in mesocolpium; 3: uniform
43. K: tectal columellae in the spines; 4 ordered states 1: absent; 2: <1/3 of the spine height; 3: 1/3-2/3 of the spine height; 4: >2/3 of the spine height	73. PP: pollen polymorphism 0: absent; 1: present
44. L: form of columellae; 4 non-ordered states 1: thick base; 2: thick tip; 3: thick base and tip; 4: cylindrical; 5: columellae absent	
<u>Quantitative characters</u>	
Excepting eh (65)), all characters are considered to show 3 ordered states	
55. p: polar axis 1: < 41 µm; 2: 41-50 µm; 3: > 50 µm	64. tpe: tp/te 1: < 0.85; 2: 0.85-1.19; 3: > 1.19
56. e: equatorial diameter 1: < 38 µm; 2: 38-45 µm; 3: > 45 µm	65. eh: spine height; 4 ordered states 1: < 1.0 µm; 2: 1.0-2.0 µm; 3: 2.0-4.5 µm; 4: > 4.5 µm
57. p/e: pollen form 1: brevixial, < 1.02; 2: equiaxial, 1.02-1.21; 3: longiaxial, > 1.21	66. eb: diameter of spine base 1: < 2.0 µm; 2: 2.0-4.0 µm; 3: > 4.0 µm
58. cp: length of polar columellas 1: < 2.0 µm; 2: 2.0-3.5 µm; 3: > 3.5 µm	67. ev: distance between spine tips 1: < 5.0 µm; 2: 5.0-7.5 µm; 3: > 7.5 µm
59. ce: length of equatorial columellas 1: < 2.0 µm; 2: 2.0-3.5 µm; 3: > 3.5 µm	68. enm: number of spines in a meridian 1: < 9; 2: 9-11; 3: > 11
60. cpe: cp/ce: polar columellas / equatorial col. 1: < 0.9; 2: 0.9-1.5; 3: > 1.5	69. acL: length of ectoaperture 1: < 19 µm; 2: 19-24 µm; 3: > 24 µm
61. cd: distance between columellas 1: < 0.2 µm; 2: 0.2-1.2 µm; 3: >1.2 µm	70. acl: width of ectoaperture 1: <2.0 µm; 2: 2.0-5.0 µm; 3: > 5.0 µm
62. tp: thickness of polar tectum 1: < 1.5 µm; 2: 1.5-2.0 µm; 3: >2.0 µm	71. anl: length of endoaperture 1: < 14 µm; 2: 14-17 µm; 3: > 17 µm
63. te: thickness of equatorial tectum	72. anl: width of endoaperture 1: < 5 µm; 2: 5-7 µm; 3: > 7 µm

Results

The data matrix (table 2) comprises 34 taxa, two of which being out-groups (*Saussurea* and *Jurinea*), and 74 characters, divided into 33 for morphology and 41 for pollen (23 qualitative ones and 18 quantitative ones). The characters 5, 21, 35, 36, 37, 44, 46, 48, 49 and 54 are non-additive, i.e. transition between states is not linear.

Analysis of the whole data set (total evidence) gives three equivalent trees, having 378 steps, of consistency index (CI) 0.30 and retention index (RI) 0.60 (fig. 1). The J.M.I. reported in front of each branch highlights several strongly supported clades. We focus on

Table 2. Data Matrix (34 taxa and 74 characters).

	Morphological characters	palynological characters	
	hennig86 coding	qualitative	quantitative
	00000000001111111112222222222333	3333333444444444455555555556666666667777	
	012345678901234567890123456789012	3456789012345678901234567890123456789012345678901234567890123	
SAAL	10010000000000000000000000000000	22231223533312242222231121213112222232110	
JUHU	100100000000001000010000000000010	1223221333331234322123222223212233321110	
SECO	10010000000000001010000110011000	????????????????4????????????????????	
KLBA	1001?000000000101010000010011000	11231223434412342122233323322313332121111	
KLFL	1001?000000000101010000010011000	12231223434312342122233322222112333133331	
KLPI	1001?000000000101010000010011000	12231123434112242122233322313112223113231	
KLNU	1001?000000000101010000010011000	11211223434312342122232222312112221313320	
CHEP	10010000000000101011000100011000	31212112534112245122232313322322321213220	
CEAF	100100010001100011011000110011000	????????????????????????????????????	
CEMA	100002110001100001111000110011001	12212113333534?2412113113????212111?23110	
CESP	100002110001100001111000110011001	12232123333421244121131132222112111?13110	
CETR	100002110001100001111000120011001	22212223313421244121131132221112221?13110	
CIBE	101002110001100001111000120011001	12231113413434?2212223222????212221313110	
MASA	1001000010010101010101000100011000	3223121342411214222232223321112212223330	
VOTU	10010300100101010101111001100110000	42211223424312242122231312221112421113230	
VOLI	10010300100101010101111001100110000	32211223433112241222231312311212332122230	
CYMU	10010300100101010101111000110110000	3221121353311224122323131121111332112230	
CRCR	10010100000000001?011001110011000	21322212531322142231233321122322211323320	
CRVU	100101000000000011011001110011000	21222212531322142231233231122322322331230	
SETI	10010000000000001010000000010000	12231213424112342122231122312212322132230	
LECO	10011000000000101010100010001010	31231222524112243113233233131322211212230	
LEBE	100110000000000101010100010001010	3123422252411224511323323313132221312230	
STAC	100110000000000101011000000011010	3221211243311214322123221222322321213310	
CDPO	001000000110000001010110021011011	12231212534112242114233232132322332213220	
CDER	001000000110000001010110021011011	1123122353331224221423323313221222312330	
CDPI	001000000110000001010110021011011	31231223434112243114233321123212333212230	
LACO	001000000110000001011010021011111	11211223434312242114233233133112322212210	
LACA	001000000110000001011010021011111	1223112343331224221423213112311222213230	
CALA	001000000110000001111010021011111	12231123434112243114233231121212322213330	
CATI	1011000001100000011?0100210111111	1123122333411224411323313313121222212110	
FEFR	01100000000000001011000021011011	11211223434312242114232221121112111312320	
FEBA	01100000000000001000100021011011	????????????????4????????????????????	
PHRH	0010000000000000101?200021011011	112312234331122422142332311212121212220	
PHAR	0010000000000000101?200021011011	11211213333112242214232132131112322312110	
code following the work of Petit & al. (1996)			
	000001112223333334445555566667777	ABBDDEFHIJKLMNPNPQRSTU	pepcccttteeaaaaaP
	123457890231234781380345912560678	1212 12	/pepdpephvncnnp
			e e e mLlLl
	non additive characters: 5 21 35 37 44 48 49 54		
	non informative characters : 14 35 54		

Code of species	
CALA	<i>Carthamus lanatus</i> L. Malaga, 10 km N., Spain, 33.VII.1986. D. Petit rec., DPE s.n.
CATI	<i>Carthamus tinctorius</i> L. Cultivated, Morocco, 1986. D. Petit rec., DPE s.n.
CDER	<i>Carduncellus eriocephalus</i> Boiss. Erfoud, Morocco, 1.V.1986. D. Petit rec. DPE s.n
CDPI	<i>Carduncellus pinnatus</i> (Desf.) Boiss. Ait Ourir, 20 km, Morocco, 5.IV.1987. D. Petit rec., DPE s.n.
CDPO	<i>Carduncellus pomelianus</i> Batt. Midelt, 50 km W, Morocco, IX 1985, D. Petit rec., DPE s.n.
CEAF	<i>Centaurea africana</i> Lam. Jbel Tazekkan Near Bab-Azhar, 1200 m, Morocco, 29.VI.1926, L.Emberger rec., RAB 48686.
CEAF	<i>Centaurea maroccana</i> Ball. Khouribga, Morocco, 25 V 1985, D. Petit rec., DPE s.n.
CESP	<i>Centaurea sphaerocephala</i> L. Casablanca, Morocco, V 1985, D. Petit rec., DPE s.n.
CETR	<i>Centaurea triumfetti</i> All. Aït-Mohammed, Morocco, 25 V. 1985, D. Petit rec., DPE s.n.
CHET	<i>Cheirolophus tananicus</i> (Maire) Holub . Tizi-n-Test, Morocco, 15 VI 1987, D. Petit rec., DPE s.n.
CIBE	<i>Centaurea benedicta</i> (L.) L. Atlas of Blida near Aken-Ferraoun, Algeria, 15.V 1916, MPU s.n.
CRCR	<i>Crupina crupinastrum</i> (Moris)VisIS. Algérie, 1923, L. Durando rec., MPU s.n.
CRVU	<i>Crupina vulgaris</i> CassASS. Assif Arrous amont, Haut Atlas, Morocco, VI 1985, D. Petit rec., DPE s.n.
CYMU	<i>Cyanopsis muricata</i> (L.) Dostal. Tazeroalt, Morocco, 12 IV 1934, MPU s.n. - Rabat, Morocco, 13.V.1980, D. Petit rec., DPE s.n.
FEBA	<i>Femeniasia balearica</i> (Rodríguez y Femenías) Susanna, MPU
FEFR	<i>Femeniasia fruticosa</i> (Maire) Petit. Boumalne du Dadès, Morocco, 1.V.1986, D. Petit rec., DPE s.n.
JUHU	<i>Jurinea humilis</i> (Desf.) DC. Loukaïmeden, Morocco, 16.IV.1928., Maire rec., MPU s.n.
KLBA	<i>Klasea baetica</i> (Boiss.) Holub. Jbel Er-Rgel, 500 m, Morocco, 1930, Font Quer rec., MPU s.n.
KLFL	<i>Klasea flavescens</i> (L.) Holub. Haute Moulouya, Morocco, 8 VII 1923, H. Humbert rec., MPU s.n.
KLNU	<i>Klasea nudicaulis</i> (L.) Holub. Moyen Atlas, Morocco, 29 VI 1931, Maire rec., MPU s.n.
KLPI	<i>Klasea pinnatifida</i> (Cav.) Holub. Near Constantine, Algeria, 16 VI 1853, Balansa rec., MPU s.n.
LACA	<i>Lamottea calva</i> (Boiss. & Reuter) Pomel. Moyen Atlas, Morocco. 13.VI.1923. MPU s.n.
LACO	<i>Lamottea coerulea</i> (L.) Pomel. Casablanca, 28 km S, Morocco. 4.IV.1987. D. Petit rec. DPE s.n.
LEBE	<i>Leuzea berardioides</i> Cosson. Oued Tarhia, Haut Atlas, Morocco, 22 V 1952, Maire rec., RAB
LECO	<i>Leuzea conifera</i> (L.) DC. Azegour, Morocco, 14 V 1916, Maire rec., MPU s.n.
MASA	<i>Mantiscalca salmantica</i> (L.) Briq. & Cav. Outerbate, 36 km W, Morocco, V 1987, D. Petit rec., DPE s.n.
PHAR	<i>Phonus arborescens</i> (L.) López. Malaga, 10 km N, Spain, 2 VII 1985, D. Petit rec., DPE s.n.
PHRH	<i>Phonus rhiphaeus</i> (Font Quer & Pau) López. Beni Urriaguel, 1928, Sennen and Mauricio rec., MPU
SAAL	<i>Saussurea alpina</i> (L.) DC. Col d'Isère, Savoie, France, VIII 1954, De Retz rec., MPU s.n.
SECO	<i>Serratula coronata</i> L. Morioka, Japan, 27.VIII 1894, Faurie 13524, P s.n.
SETI	<i>Serratula tinctoria</i> L. Aigoual, France, 1984, D. Petit rec., DPE s.n.
STAC	<i>Stemmacantha acaulis</i> (L.) Dittrich. Fès, 19 km S, Morocco, 30 III 1987, D. Petit rec., DPE s.n.
VOLI	<i>Volutaria lippii</i> (L.) Maire. El-Jadida, 13 Km S, Morocco, 8 VI 1986, D. Petit rec., DPE s.n.
VOTU	<i>Volutaria tubuliflora</i> Mürb. Kasba-Tadla, 21 km N, Morocco, 1.V.1986, D. Petit rec., DPE s.n.

the clades that have a J.M.I. exceeding 0.9:

Klasea group, but unexpectedly excluding *K. nudicaulis*;

Mantiscalca group, with *Volutaria* and *Cyanopsis*;

Carthamus complex, with *Carduncellus*, *Lamottea*, *Phonus* and *Femeniasia*;

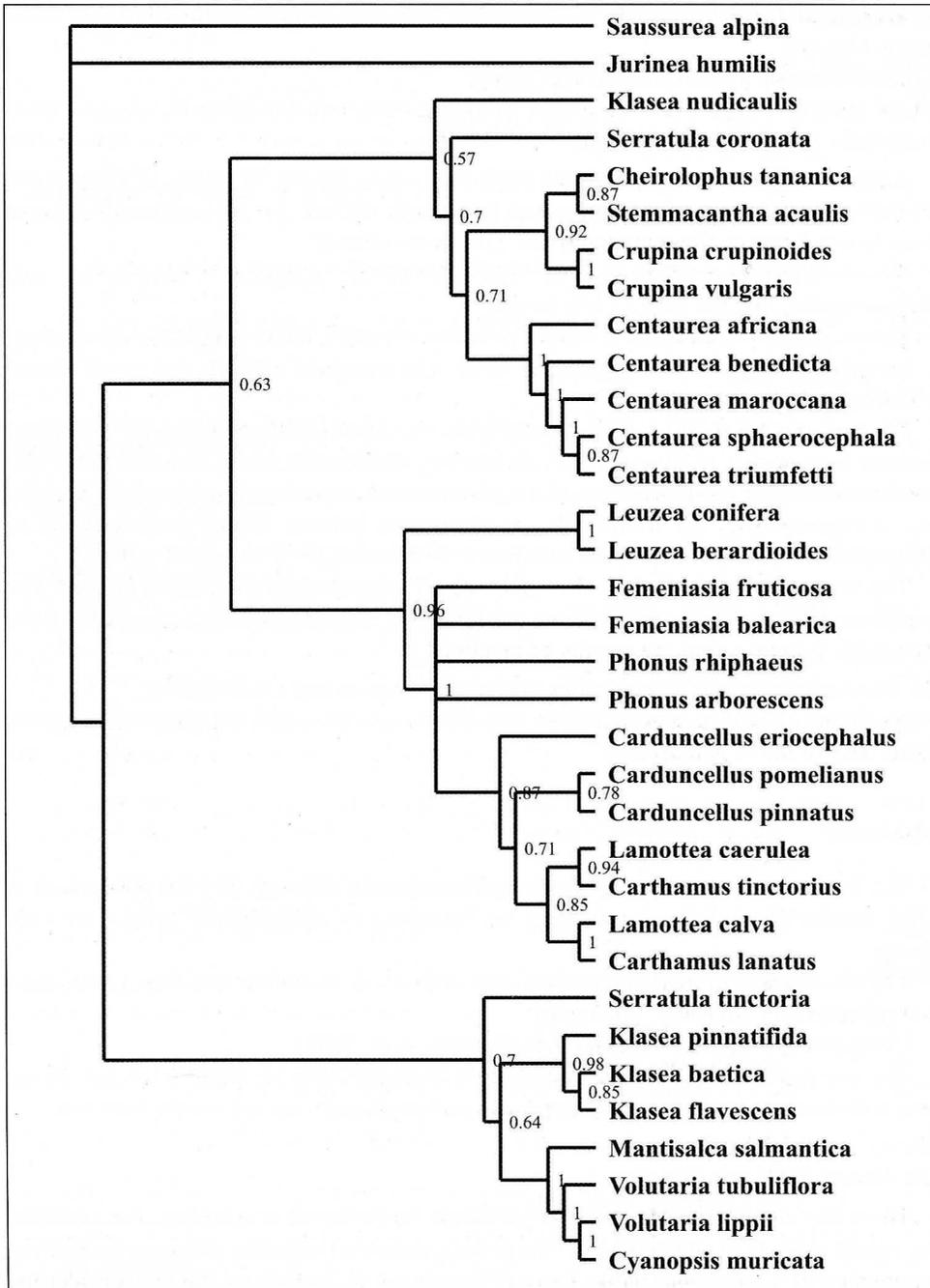


Figure 1. Consensus tree of 3 cladograms of the Centaureinae. Characteristics: search algorithm = mh* bb* option (Hennig86); total evidence; outgroup = Saussurea and Jurinea; length = 388 steps; C.I. = 0.29; R.I. = 0.60. The Jackknife monophyly indexes are given in front of the nodes.

genus *Leuzea*. It is unexpectedly linked to the *Carthamus* complex, with a J.M.I. of 0.96; genus *Crupina*;
Crupina-Stemmacantha-Cheirolophus group;
 all the species in the *Centaurea* group, including *Centaurea benedicta* (L.) L. (= *Cnicus benedictus* L.; see Bremer 1994 and Petit 1997).

Analysis with successive weighting produces 16 trees having 533 steps, of CI 0.68 and RI 0.88. The seven monophyletic groups previously defined are all confirmed, whereas associations between these monophyletic groups are altered:

Mantiscalca group is associated with *Centaurea*, *Crupina*, *Cheirolophus* and *Stemmacantha*, and not with *Klasea* group;

Klasea group is close to *Leuzea* and *Carthamus* complex, rather than *Mantiscalca* group;

Serratula tinctoria is sister species to other "Centaureinae" species, and not to *Klasea* and *Mantiscalca* groups.

We performed a third analysis by removing the quantitative palynological characters because they appear to show the less satisfactory distribution in the obtained trees. The consensus tree (out of 3) looks very like the consensus tree produced by successive weighting, as expected (fig. 2a). It differs by an intervension between *Klasea nudicaulis* and *K. pinnatifida*. The characteristics are as follows: 197 steps, CI = 0.39 and RI = 0.71.

The last analysis was obtained by removing all palynological characters (fig. 2b). The consensus tree (out of the 96) presents the following characteristics: 68 steps, CI = 0.54, RI = 0.85. Several points are worthy of mention:

the *Mantiscalca* group is associated to *Centaurea*, *Crupina* and *Cheirolophus*;

the positions of *Leuzea*, *Stemmacantha* and *Klasea* are not stable, but nonetheless appear at the base of the "Centaureinae".

Discussion

The four analyses produced globally conflicting trees although they are concordant in some details. The conflicts concerning the branching of monophyletic groups are very strong.

The discussion will treat first comparisons with previous authors and then a more general reflection on "star-like" phylogeny.

Comparison with ITS molecular data (Susanna & al. 1995)

The tree produced here by the complete 74 characters data set (total evidence) agrees best with those deduced from ITS sequences and emphasizes an association between:

Klasea and *Volutaria*

Carthamus and *Centaurea*

There do exist differences, some of which can be attributed to sampling. For example, our results indicate the monophyly of the genus *Centaurea* (including subgenus *Centaurea*, represented by *C. africana*) unlike those of Susanna & al. In fact, we did not consider the same species, for example: (i) we did not study *Acroptilon*, *Centaurea dealbata*, *C. clementei*, *C. americana*, nor the same species of *Cheirolophus* (ii) Susanna & al. did not study sequences of *Leuzea*, *Serratula tinctoria*, *S. coronata*, *Mantiscalca*. Further, their

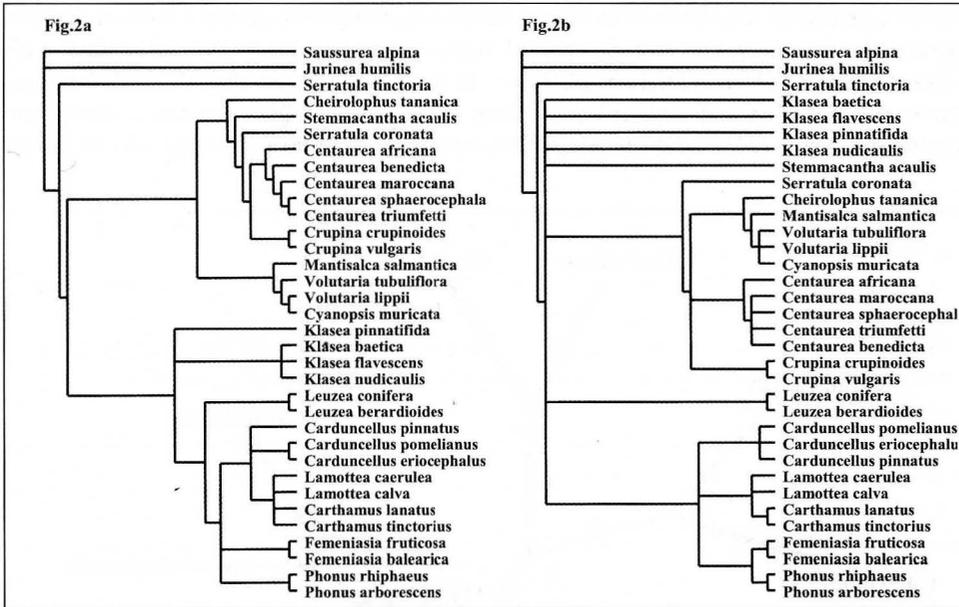


Fig. 2 - Consensus trees of cladograms produced after removing the quantitative palynological characters (Fig. 2a : 3 trees ; 194 steps ; CI = 0.39 ; RI = 0.71) or all the palynological characters (Fig. 2b : 68 trees ; 68 steps ; CI = 0.54 ; RI = 0.85).

results placed the larger part of *Centaurea* (sub-genus *Centaurea* excepted) closer to *Carthamus*, *Femeniasia* and *Carduncellus* than *Crupina* and *Cheirolophus*. Otherwise, they placed *Stemmacantha* near the base of *Centaureinae*. In addition, it should be stressed that their bootstrap indexes (Felsenstein 1985) for nucleotides were rather low. For example, the clade *Femeniasia-Carduncellus* is supported by a bootstrap of 0.42, and associated with *Carthamus* and most *Centaurea* species in a clade supported by a bootstrap of 0.24.

Another source of discrepancy comes from the kind of data. If ITS sequences has proved to be good tools to investigate the phylogeny of close species, it is not certain that they provide a reliable information when addressed to distantly related taxa. Instead, how morphological character states are adaptative is still poorly known except in few cases.

Why is the *Carthamus* complex not monophyletic in the consensus tree produced by Susanna & al. (1995)? Many characters studied here argue in favour of monophyly: thorny leaves (0), complex involucre (2), presence of lateral spiny lobes on involucral bracts (3), hairy filaments (25²) with dense hairs (26), crystals in the bracts (32), exine ridges very developed between spines (52⁴). We could accept the criticism that several characters (0, 2, 3, 31) are more or less functionally linked in the sense of an adaptation against grazing but nevertheless monophyly of the *Carthamus* complex is well supported.

Another example illustrates contradictions between both sources of data. From ITS sequences, *Carthamus* s. l. is closer to the *Centaurea* clade (subgenus *Centaurea* excluded) than to any other genus. Morphological and palynological characters plead for a weaker relationship between *Carthamus* s. l. and *Centaurea*. These taxa do share lateral spiny lobes on involucral bracts (3), crystals in the bracts (32); crown epidermis with thick cells

(18) is a character state limited to highly derived species of the genus *Carthamus* s. str. Instead, in the three analyses performed here, *Centaurea* is always associated with *Cheirolophus*, *Crupina* and *Stemmacantha*. It must be stressed that the link between *Carthamus* complex and *Leuzea* comes from weak morphological evidence: deciduous pappus as a whole (21¹) is clearly adaptative; otherwise, both taxa share the tube of florets

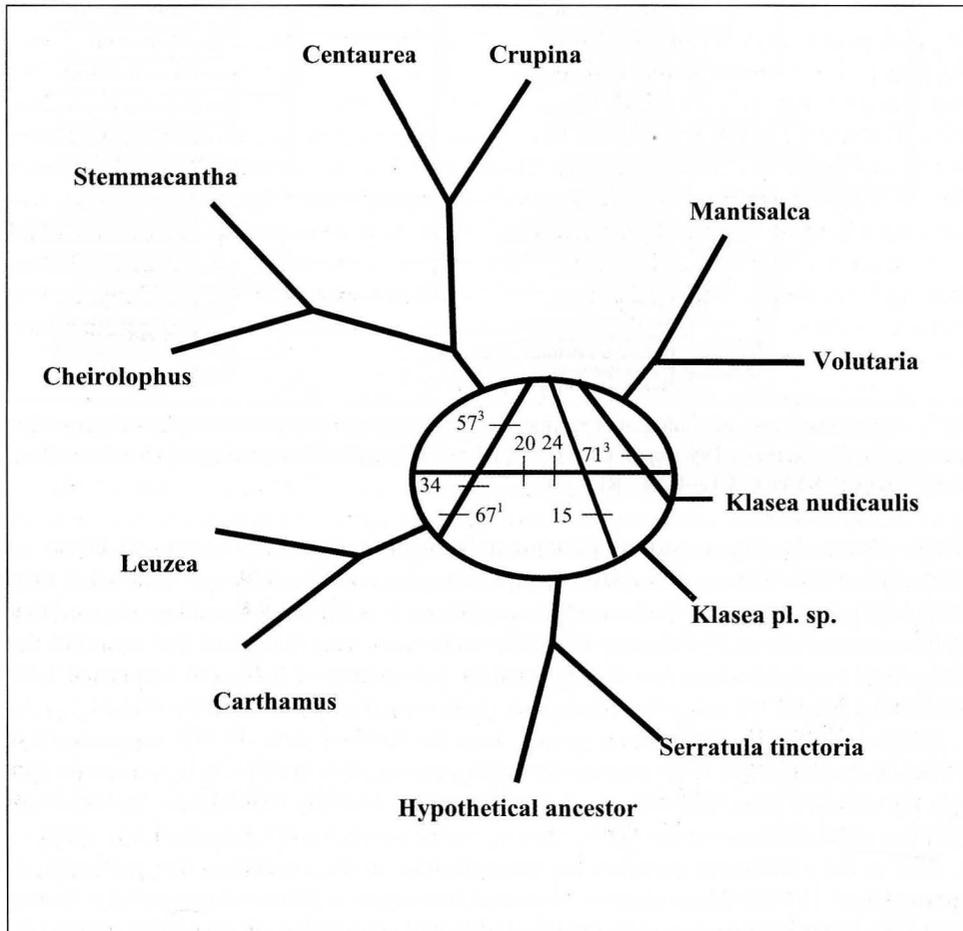


Figure 3. Synthetic distribution of characters in the Centaureinae, after the populational evolution method of Dutrillaux.

The drawing must be read from bottom to top. All taxa above a segment share the derived states of the characters given by the numbers. For example, Mantisalca, Volutaria and *Klasea nudicaulis* share the same apomorphy of character 713 (endoaperture long). Otherwise, Mantisalca is characterized by the derived states of 15, 20, 24, and 71.

15: basal hilum

20: pappus double

24: peripheral florets sterile

34: ectoaperture edge without undulations

573: longiaxial pollen

671: distance between spines >5µm

713: endoaperture long

which is more than 1 mm long. Palynological arguments are stronger: ridges between exine spines more or less developed (52^{3-4}), polar axis $>50 \mu\text{m}$ (55^3) and length of polar columellas $<2 \mu\text{m}$ (59^1).

Organization of the *Carthamus* complex proposed by López (1989)

He recognized the distinction between the genera *Phonus* (*P. riphaeus*, *P. arborescens*), *Carduncellus* (*C. eriocephalus*, *C. pomelianus*...), *Lamottea* (*L. coerulea*, *L. calva*) and *Carthamus* s. str. (*C. tinctorius*, *C. lanatus*...). Moreover, the genus *Femeniasia*, comprising *F. balearica* and *F. fruticosa* (Susanna 1987, Wagenitz & Hellwig 1996, Petit 1997), belongs to this complex. In our analysis based on total evidence, the genus *Femeniasia* has the strongest support with a JMI of 0.79. The genus *Phonus* is recognized with a JMI of 0.62. The species in the genera *Lamottea* and *Carthamus* s. str. are consistently associated but do not form any distinct clades, confirming Hanelt's conception (1963). This suggests that these species form a monophyletic group and a single genus (to be named *Carthamus*, because this name has priority). According to this arrangement, the group *Carduncellus*-*Carthamus* is stable with a JMI of 0.81. In our opinion, the validity of the genus *Phonus* remains to be demonstrated because the two concerned species share few, if any, derived characters states (21 , 43^3 and 50^2). Appropriate taxonomic decisions on this question must await analyses of additional east Mediterranean taxa.

The "*Centaureinae*" treatment of Wagenitz & Hellwig (1996)

If we compare our results with their synthesis, the discrepancy of cladograms is important although the sources of data were the same. They proposed a hypothetical phylogeny close to Susanna & al.'s one without being aware of their works. They retained clearly 8 characters even if many more were described. Maybe they are right in explaining very global views of character changes. For example, they treated pollen data by following the 5 types of organization described by Wagenitz (1955): *Carthamus* and *Serratula* belongs to the same type. There is a great loss of information by doing this way. The advantage of our detailed data set is that we are able to assess the monophyly of *Carthamus* complex (data not shown). Otherwise, they take into account basic chromosome numbers, data that we neglected.

As Wagenitz & Hellwig (1996) pointed out, many convergent evolution occurred in the "*Centaureinae*". Which characters are good ones? Which are badly homoplasic? Unfortunately, the explanation of tendencies in character evolution supposes that phyletic relationships are robust. In this case, it is absolutely not the case. We are still far from elucidating the phylogeny of the group. We must wait for new molecular analyses given by several sequences with fast and slow evolutionary rates to understand the signification of characters.

What can we do with a "star-like" phylogeny ?

In fact, our results portray the following: few clades of reasonable stability but of variable position to each other. This is indicative of a "star-like" phylogeny. Cladograms by their intrinsic nature are poorly adapted for representation of this kind of evolution: the consensus tree looks like a "rake", that is, each tine of the rake is robust but they can't be ordered. For this reason, we have included a depiction of relationships following the system of Dutrillaux (1975), wherein hypothetical relationships are illustrated by a system of lines, more or less crossed, included in a circle. Each line corresponds to a series of derived character states. The line segments limited by the circle isolate sectors corresponding to taxa sharing common characters states. A given taxon can be related by two or more

crossed segments if it possesses two or more groups of derived character states. This system has been applied to numerous examples of chromosomal evolution in Primates (Dutrillaux & al. 1982) or Rodentia (Benazzou & al. 1984, Petit & Dutrillaux 1985).

The links between the clades of the "*Centaureinae*" are illustrated using this method (fig. 3). It gives a very synthetic view of the data distribution although not all character changes have been included.

The origin of the presumed evolutionary "explosion" in the "*Centaureinae*" is largely hypothetical: it possibly relates to the development of chemical compounds such as new types of sesquiterpen lactones (Wagner 1977), which in turn conferred greater protection against herbivory.

In conclusion, we would like to state that the results presented here are provisional and hope a larger sampling to include genera from the former USSR, Arabian Peninsula, Turkey and Iran will provide the basis of an improved conception of the group.

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References

- Benazzou, T., Viegas-Pequignot, E., Prod'homme, M., Lombard, M., Petter, F. & Dutrillaux, B. 1984: Phylogénie chromosomique des *Gerbillidae*. III. Etude d'espèces des genres *Tatera*, *Taterillus*, *Psammomys* et *Pachyuromys*. — Ann. Génét. **27**(1): 17-26.
- Bremer, K. 1994: *Asteraceae*: cladistics and classification. — Portland, Oregon.
- Dittrich, M. 1966: Karpologische Untersuchungen zur Systematik von *Centaurea* und verwandten Gattungen. 1-2. — Bot. Jahrb. Syst. **88**: 70-162.
- 1968 a. - Morphologische Untersuchungen an den Früchten der Subtribus *Cardueae-Centaureinae* (*Compositae*). — Willdenowia **5**: 67-107.
- 1968 b. - Fruchtanatomische und zytologische Untersuchungen an einigen Arten der Gattungen — *Adans.* und *Leuzea* DC. (*Compositae*). — Österr. Bot. Z. **115**: 379-390.
- 1969: Anatomische Untersuchungen an den Früchten von *Carthamus* L. und *Carduncellus* Adans. (*Compositae*). — Candollea **24**(2): 263-277
- 1977: *Cynareae*, systematic review. — Pp. 999-1015 in Heywood, V.H., Harborne, J. B. & Turner, B. L., (ed): The Biology and chemistry of the *Compositae*. — London, New-York, San-Francisco.
- 1984. Neukombinationen in der Gattung *Stemmacantha* Cass. (*Compositae*) mit Bemerkungen zur Typisierung einiger ihrer Arten. — Candollea **39**: 45-49.
- Dutrillaux, B. 1975: Sur la nature et l'origine des chromosomes humains. Monographie des Annales de Génétique. Expansion scientifique française.
- , Couturier, J., Muleris, M., Lombard, M. & Chauvier, G. 1982: Chromosomal phylogeny of forty-two species or subspecies of Cercopithecoidea (Primates *Catarrhini*). — Ann. Génét. **25**(2): 99-109.
- Farris, J. S. 1988: Hennig86. Program and documentation. — Port Jefferson Station, New York.
- Felsenstein, J. 1985: Confidence limits on phylogenies: an approach using the bootstrap. — Evolution **39**: 783-791.

- Hanelt, P. 1963: Monographische Übersicht der Gattung *Carthamus* L. — Feddes Repert. **67(1-3)**: 41-179.
- Karis, P. O., Källersjö, M., Bremer, K. 1992: Phylogenetic analysis of the *Cichorioideae*. — Ann. Missouri Bot. Garden **79**: 416-427.
- López, G.. 1989: Acerca de la clasificación natural del género *Carthamus* L., s. l. - Anales Jardin Bot. Madrid **47(1)**: 11-34.
- Meusel, H. & Kohler, E. 1960: Die Blattbildung von *Carlina vulgaris* L. — Bot. Jahrb. Syst. **79**: 102-207.
- Page, R. D. M. 1998 : TreeView vers. 1.5.2. Program available on the web at the address <http://taxonomy.zoology.gla.ac.uk/rod/rod.html>
- Petit, D. P. 1987: Analyse cladistique du genre *Atractylis* L. (*Compositae*, *Cardueae*). — Bull. Soc. Bot. France, **134**, Lettres botaniques: 165-184.
- 1988: Le genre *Echinops* L. 1. Position phylétique et interprétation de l'incapitulescence. — Candollea **43**: 467-481.
- , Dutrillaux, B. 1985: Phylogénie chromosomique de 7 espèces de *Sciurinae*. — Ann. Génét. **28(1)**: 13-18.
- , Mathez, J., Qaid, A. 1996: Early differentiation of *Cardueae* sensu lato: morphology and pollen. — Pp. 79-93 in Hind, D. J. N., Jeffrey, C., Pope, G. V. (ed.): Proceedings of the International *Compositae* Conference, Kew, 1994. **1**.
- 1997: Generic interrelationships of the *Cardueae* (*Compositae*): a cladistic analysis of morphological data. - Pl. Syst. Evol. **207**: 173-203.
- Qaid, A. 1990: Contribution palynologique à l'étude de la taxinomie du groupe des "Cynarées" (famille des Composées). 1, 2. — Montpellier: Thèse Doct. Sc. U.S.T.L.
- Ramos, T. C. 1997: Tree gardener vers. 2.2.1. Package available on the web at the address <http://www.ib.usp.br/~christy/tree.html>
- Siddal, M. E. 1994, 1995: Random Cladistics vers. 4.0.3. Package and manual available on <ftp://zoo.toronto.edu/pub>
- Susanna, A. 1987: *Femeniasia*, novus genus *Carduearum*. — Collectanea Botanica, Barcelona, **17**: 83-88.
- , Garcia-Jacas, N., Soltis, D.E. et Soltis, P.S. 1995: Phylogenetic relationships in tribe *Cardueae* (*Asteraceae*) based on ITS sequences. — American J. of Botany **82(8)**: 1056-1068.
- Wagenitz, G. 1955: Pollenmorphologie und Systematik in der Gattung *Centaurea* L. s. l. — Flora **142**: 213-279.
- , Hellwig, F. 1996: Evolution of characters and systematics of the *Centaureinae*. - In Hind, D.J.N (Ed.): *Compositae*, Systematics, Biology, Utilisation. - Richmond: Royal Botanic Gardens, Kew.
- Wagner, H. 1977: *Cynareae* — Chemical review. — Pp. 1017-1038 in Heywood, V. H., Harborne, J. B., & Turner, B. L., (ed.): The Biology and Chemistry of the *Compositae*, — London, New-York.

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