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Evolution in *Carex* L. sect. *Spirostachyae* (Cyperaceae): A molecular and cytogenetic approach

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Abstract

Carex sect. *Spirostachyae* comprises 25 species displaying the centre of diversity in Eurasia. Phylogenetic analysis of the ITS nrDNA region of 20 species of sect. *Spirostachyae*, six species of sect. *Ceratocystis*, five species of sect. *Elatae*, and eight outgroup species reveals that neither sect. *Spirostachyae* nor sect. *Elatae* is monophyletic. With the exclusion of *Carex cretica*, the 19 species of sect. *Spirostachyae* studied form a clade with the five species of tropical-subtropical sect. *Elatae*. Taxonomy of the core Spirostachyae is not only mostly in agreement with our phylogenetic hypothesis, but also with ecological and new cytogenetic results. Two main groups with different chromosome numbers and edaphic preferences are identified in the core Spirostachyae. One includes primarily acidophilous species with high chromosome numbers ($2n = (64)68–84$), whereas the other one includes mainly basophilous species with lower chromosome numbers ($2n = 60–74(75)$). Chromosome-number variation is extremely different in the core Spirostachyae, showing great stability in some widespread species (e.g. *Carex extensa*) but an active chromosome evolution – faster chromosomal rearrangements, fusion and fission events than ITS nucleotide substitutions – in more restricted species (e.g. *Carex troodi*). Biogeography of the two amphiatlantic pairs of species reveals two independent colonizations of South America from the European continent. The geographical barrier of the Strait of Gibraltar has played different roles in the course of evolution of this section, acting as an effective barrier to gene flow in one case (*Carex helodes*) but as a limited barrier or recent separation in two others (*Carex distans*, *Carex punctata*).
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Introduction

Carex L. consists of over 2000 species distributed worldwide and displaying high species diversity in temperate latitudes of the northern hemisphere (Reznicek 1990). Five subgenera (*Carex, Kreczetoviczia, Psillophora, Vignea,* and *Vigneastra*) and nearly 130

sections are recognized based on a comprehensive taxonomic account (Egorova 1999).

Section *Spirostachyae* Drejer ex L.H. Bailey belongs to the subgenus *Carex* and traditionally has been considered closely related to sects. *Elatae* Kükenth. and *Ceratocystis* Dumort (Kükenthal 1909; Luceño and Castroviejo 1993). Most of the species of sect. *Spirostachyae* occurs in mesic places across Eurasia and N Africa, with few representatives distributed in Australia, South Africa or South America (Kükenthal 1909;

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Egorova 1999). Eight morphological characters define sect. *Spirostachyae*: (1) primarily rhizomes with short internodes; (2) presence of leaf anteligule; (3) lowest bract of the inflorescence leaf-like, sheathing; (4) three stigmas; (5) glabrous, and smooth utricles; (6) epidermal cells of utricles with red crystalloid bodies; (7) bifid or bidentate utricle beak; and (8) ellipsoid achenes (Chater 1980; Crins and Ball 1988). Several taxonomic treatments have been proposed for sect. *Spirostachyae*, differing in the number of taxa and taxonomic circumscription regarding to sects. *Elatae* and *Ceratocystis* (Table 1). Kükenthal (1909) recognized 20 species in sect. *Spirostachyae*, including *C. durieui*, *C. hostiana*, and *C. flava* group, which have been recently circumscribed within sect. *Ceratocystis* (Table 1), based on morphology (Chater 1980; Crins and Ball 1988; Egorova 1999; Crins 2002) and molecular data (Hendrichs et al. 2004b). Analyses of two species of sect. *Elatae* (*C. camposii*, *C. laevigata*) in the first phylogenetic approach for sect. *Spirostachyae* (Luceño and Castroviejo 1993) revealed important cytogenetic and morphological affinities between the two sections. As a result, these two species were placed in sect. *Spirostachyae* (Table 1; Luceño and Castroviejo 1993). In addition to these changes, four new species have been described within this section (*C. idaea*, Greuter et al. 1985; *C. lainzii*, Luceño et al. 1988; *C. paulo-vargasii*, Luceño and Marín 2002; *C. troodi*, Turril 1930), and the taxonomic status of three other taxa has been re-evaluated (*C. fuscula* spp. *catharinensis*, Luceño and Alves 1999; *C. helodes*, Luceño 1992b; and *C. vixdentata*, Wheeler 1988).

The genus *Carex*, together with other genera of Cyperaceae and Juncaceae, is characterised by certain cytological peculiarities of particular evolutionary interest: (i) three of the four nuclei resulting from meiotic division degenerate in Cyperaceae, which implies lack of formation of a functional tetrad; (ii) auto-orientation of chromosomes during the first metaphase leading to equational separation at Anaphase I (post-reductional meiosis); and (iii) presence of holocentric chromosomes promoting the formation of aneuploid series via fission (agmatoploidy; Malheirós Gardé and Gardé 1950, Davies 1956b), fusion (symploidy; Luceño and Guerra 1996), and chromosomal rearrangement (Greilhuber 1995) processes. Due to these characteristics, observation of irregularities in chromosome pairing, such as univalents and heteromorphic trivalents, is common (Heilborn 1924; Wahl 1940; Faulkner 1972; Luceño and Castroviejo 1991). Cytogenetic variation caused by fission, fusion, and chromosomal rearrangement events provides a valuable source of data in evolutionary studies of *Carex*. Reznicek (1990) compiled previous caryological data to infer evolutionary patterns and proposed the decrease of chromosome numbers as a major mode of evolution in the genus. Alternatively, some authors reported increase of the number of chromosomes

as a primary pattern in the evolution of sects. *Ceratocystis* (Crins and Ball 1988) and *Spirostachyae* (Luceño and Castroviejo 1993). Chromosome numbers of most species in sect. *Spirostachyae* vary between $2n = 68$ and 74; deviations from this number range are found in *C. extensa* ($2n = 60$), and in *C. laevigata* (one individual is $2n = 80$) (Luceño and Castroviejo 1993).

Several molecular (nuclear and plastid) studies have been conducted to infer phylogenetic relationships within the family (Muasya et al. 1998; Yen and Olmstead 2000; Starr et al. 2003, 2004). Molecular phylogenetic approaches early revealed non-monophyletic groups not only for three of the four subgenera (*Primocarex*, *Indocarex* and *Carex*), but also for most of the sections studied (Roalson et al. 2001; Hendrichs et al. 2004a, b). Some molecular phylogenetic studies have been conducted at sectional level (sect. *Limosae*, Waterway et al. 1997; sect. *Hymenochlaenae*, Waterway and Olmstead 1998; sect. *Phyllostachys*, Starr et al. 1999; sect. *Acrocystis*, Roalson and Friar 2004a, b). However, there has not been any molecular phylogenetic attempt to test monophyly and species relationships of sect. *Spirostachyae*.

In this study we analyse cytogenetics and the nuclear ITS region in sects. *Spirostachyae* and two taxonomically related sections, *Ceratocystis* and *Elatae*, to investigate sectional limits as well as to clarify phylogenetic relationships within sect. *Spirostachyae*. Particular issues of sect. *Spirostachyae* addressed in this paper are to: (i) shed light on the taxonomic circumscription of the section; (ii) infer phylogenetic species relationships using molecular and cytogenetic markers; (iii) describe evolutionary patterns based on levels of congruency between morphology, sequence variation and cytogenetics; and (iv) identify biogeographic patterns of widely distributed and disjunct species.

Material and methods

Ingroup circumscription

We established the delimitation of sects. *Ceratocystis* and *Spirostachyae* based on the last taxonomical treatment published (Egorova 1999), with some additional modifications (Turril 1930; Levyns 1950; Greuter et al. 1985; Wheeler 1988; Luceño 1992b; Luceño and Castroviejo 1993; Luceño and Alves 1999; Luceño and Marín 2002), as shown in Table 1. Egorova (1999) did not include sect. *Elatae* in her revision; for this section we followed Kükenthal's (1909) proposal with the specifications mentioned in Table 1.

Our study ingroup is primarily focused on sect. *Spirostachyae*, but we have also included some representatives of the taxonomically related sections *Ceratocystis* and *Elatae*.

Table 1. Taxonomic background of sects. *Ceratocystis, Elatae*, and *Spirostachyae*: names of taxa studied (far left column), sectional circumscriptions according to three standard treatments, and additional taxonomic references (far right column)

Taxon	Egorova (1999)	Chater (1980)	Kükenthal (1909)	Other notes
***Carex* sect. *Ceratocystis* Dumort.**				
C. demissa Honerm.	*Ceratocystis*	*Ceratocystis*	*Spirostachyae* (*C. flava* f. *demissa*)	
C. flava L.	*Ceratocystis*	*Ceratocystis*	*Spirostachyae*	
C. hostiana DC.	*Ceratocystis*	*Ceratocystis*	*Spirostachyae* (*C. hornschuchiana*)	
C. lepidocarpa L.	*Ceratocystis*	*Ceratocystis*	*Spirostachyae*	
C. nevadensis L.	*Ceratocystis*	*Ceratocystis*	*Spirostachyae* (*C. lepidocarpa* var. *nevadensis*)	
C. viridula Michx.	*Ceratocystis*	*Ceratocystis* (*C. serotina*)	*Spirostachyae* (*C. oederi*)	
***Carex* sect. *Elatae* Kükenth.**	Not treated			
C. aethiopica Sckuhr		Not in Europe	*Elatae*	
C. hochstetteriana J. Gay ex Seub.		*Elatae*	*Elatae*	
C. lowei Bech.		Not in Europe	*Elatae* (*C. elata* Lowe)	Becherer (1939)
C. manni E.A. Bruce		Not in Europe	*Elatae* (*C. boryana* var. *simplissima*)	Cabezas et al. (2004)
C. perraudieriana J. Gay		Not in Europe	*Elatae*	
***Carex* sect. *Spirostachyae* Drejer ex Bailey**				
C. binervis Smith	*Spirostachyae*	*Spirostachyae*	*Spirostachyae*	
C. blakei Nelmes	*Spirostachyae*	Not in Europe	Not treated	
C. burchelliana Boeck.	*Spirostachyae*	Not in Europe	*Spirostachyae*	
C. camposii Boiss. & Reut.	Not treated	*Elatae*	*Elatae*	Luceño and Castroviejo (1993)
C. cretica Gradst. & J. Kern	*Spirostachyae*	*Spirostachyae*	Not treated	
C. diluta M. Bieb.	*Spirostachyae*	*Spirostachyae*	*Spirostachyae*	
C. distans L.	*Spirostachyae*	*Spirostachyae*	*Spirostachyae*	
C. distenta Kunze ex Kunth	*Spirostachyae*	Not in Europe	*Spirostachyae* (*C. fuscula* var. *distenta*)	
C. ecklonii Nees	Not treated	Not in Europe	*Spirostachyae* (*C. extensa* var. *ecklonii* (Nees) Kükenth.)	Levyns (1950)
C. extensa Good.	*Spirostachyae*	*Spirostachyae*	*Spirostachyae*	
C. fissirostris Ball	*Spirostachyae*	Not in Europe	*Spirostachyae* (*C. diluta* var. *fissirostris* (Ball) Kükenth.)	
C. fuscula ssp. *catharinensis* (Boeck.) M. Luceño & M. Alves	Not treated	Not in Europe	Not treated	Luceño and Alves (1999)
C. fuscula d′Urv ssp. *fuscula*	*Spirostachyae*	Not in Europe	*Spirostachyae*	
C. gunniana Boot.	*Spirostachyae*	Not in Europe	*Spirostachyae*	
C. helodes Link	Not treated	Not treated	Not treated	Luceño (1992b)
C. idaea Greuter et al.	Not treated	Not treated	Not treated	Greuter et al. (1985)
C. laevigata Sm.	Not treated	*Elatae*	*Elatae* (*C. helodes* Link)	Luceño and Castroviejo (1993)
C. lainzii Luceño, E. Rico & T. Romero	*Spirostachyae*	Not treated	Not treated	
C. mairii Coss. & Germ.	*Spirostachyae*	*Spirostachyae*	*Spirostachyae*	
C. montis-eeka Hillebrand	*Spirostachyae*	Not in Europe	*Spirostachyae*	
C. munroi Boott ex C.B. Clarke	*Spirostachyae*	Not in Europe	*Spirostachyae*	
C. paulo-vargasii Luceño & Marín	Not treated	Not in Europe	Not treated	Luceño and Marín (2002)
C. punctata Gaud.	*Spirostachyae*	*Spirostachyae*	*Spirostachyae*	
C. tasmanica Kük.	*Spirostachyae*	Not in Europe	Not treated	
C. troodi Turril	Not treated	Not treated	Not treated	Turril (1930)
C. vixdentata (Kük.) G.A. Wheeler	Not treated	Not in Europe	Not treated	Wheeler (1988)

Plant material and sampling strategy

Molecular study

A total number of 94 populations of 38 species were included in the molecular analyses. We made a special effort to sample sect. *Spirostachyae*, which resulted in the inclusion of 20 of the 25 species considered in this section (Table 2). We were unable to obtain material from restricted endemics such as *C. blakei* (SE Australia), *C. distenta* (C Chile), *C. ecklonii* (South Africa), *C. montis-eeka* (Hawaii), and *C. munroi* (E Himalaya). To investigate sister-group relationships, we also included samples of sects. *Ceratocystis* and *Elatae,* consisting of six (of 15) and five (of 17) species to represent the taxonomic diversity of both groups. Outgroup taxa included seven species of different sections and subgenera (Table 2). Six of them represent six different sections of the subgenus *Carex*: *C. falcata* (sect. *Paniceae*), *C. humilis* (sect. *Digitatae*), *C. lemmonii* (sect. *Ferrugineae*), *C. pallescens* (sect. *Porocystis*), *C. serratodens* (sect. *Racemosae*), and *C. umbrosa* (sect. *Mitratae*). One more outgroup species was *C. leporina* (sect. *Ovales*), which belongs to the distant subgenus *Vignea* (Table 2).

Populations sampled within sect. *Spirostachyae* were chosen to represent the geographical, morphological, and cytological variability of each taxon. The number of populations included per taxon varies from one to ten, although for the most part (15 species) at least two populations were sampled (Table 2). Widespread species were sampled in more detail: *C. distans* (eight populations), *C. extensa* (5), *C. fuscula* (10), and *C. punctata* (5). More than two populations were also sequenced for controversial, poorly known and highly variable species (*C. cretica* (3), *C. helodes* (4), *C. idaea* (3), *C. laevigata* (5), *C. lainzii* (3), *C. mairii* (4), and *C. troodi* (3)), as well as for the recently described species *C. paulo-vargasii* (4).

Cytogenetic study

Thirty-seven populations from nine species of sect. *Spirostachyae* plus two populations of one species of sect. *Elatae* were included in the cytogenetic study. Sampling strategy for the caryological study considered the inclusion of six species never counted before, two well-known variable species, and two poorly known species (Table 2). Cytogenetic sampling was designed to include the same individuals and populations used for sequencing, when possible. As a result, the number of populations sampled per species varied from one (*C. cretica*, C. *extensa*, C. *idaea*, and *C. punctata*) to 16 (*C. laevigata*) (Table 2). The number of individuals sampled in each population varied from one (six species) to eight in the special case of *C. laevigata* (Table 2).

Molecular analysis

PCR amplification and sequencing

Sixty-eight ITS accessions representing 20 species and 21 taxa of sect. *Spirostachyae*, together with eleven accessions representing six species of sect. *Ceratocystis* and seven accessions representing five species of sect. *Elatae*, were included as the ingroup in the molecular study. Eight accessions from different sections were included as the outgroup (Table 2). Fifteen ITS sequences (three of sect. *Spirostachyae*, seven of sect. *Ceratocystis*, and five of the outgroup) were taken from previous molecular studies (Roalson et al. 2001; Hendrichs et al. 2004b) via the GenBank database (http://www.ncbi.nlm.nih.gov/).

Total DNA was extracted from silica-dried material collected in the field as well as from herbaria (B, CAMB, CBG, CONC, EOO, HO, KMG, MA, UPOS, VAL). DNA was extracted using DNeasy Plant Mini Kit (Qiagen, California). ITS-A and ITS-4 standard primers were used for the direct and reversal PCR amplifications of the ITS region (Blattner 1999; White et al. 1990) in a Perkin Elmer PCR-system 9700 (California). After 1 min pre-treatment at 94 °C, PCR conditions were: 24 cycles of 1 min at 94 °C, 30 s at 50 °C and 1 min at 72 °C. ITS amplifications from DNA extractions of old herbarium material were obtained by using puReTaq^{TM} Ready-To-Go^{TM} PCR Beads (Amersham Biosciences). In these cases the PCR conditions remained equal, except for the 94 °C pre-treatment time (5 min) and the number of cycles (30). Amplified products were cleaned using spin filter columns (PCR Clean-up kit, MoBio Laboratories, California) following the manufacturer's protocols. Cleaned products were sequenced using dye terminators (Big Dye Terminator v. 2.0, Applied Biosystems, California) and run in polyacrylamide electrophoresis gels (7%) using an Applied Biosystem Prism Model 3700 automated sequencer. Sequences were edited using the program Seqed (Applied Biosystems, California) and applying the IUPAC symbols to represent nucleotide ambiguities. Limits of the ITS region were determined as specified in Starr et al. (1999).

Sequence analyses

Maximum Parsimony (MP) and Bayesian Inference (BI) analyses were performed in the manually aligned 94 ITS-sequence matrix. Parsimony analyses were conducted under Fitch parsimony as implemented in PAUP* (Swofford 1999) with equal weighting of all characters, and transitions: transversions. Heuristic searches were replicated 100 times, with Random Taxon-Addition sequences, Tree-Bisection-Reconnection (TBR) branch swapping, MulTrees and Steepest Descent options. For cases in which the run was interrupted due to a memory fault, a second heuristic search was performed retaining only 500 trees per replicate with a number of steps equal

Table 2. List of studied material, including taxon names (column A), population number within each species (B), locality (C), sample number within each population (D), voucher (E), natural distribution of each taxon (F), meiotic configuration (G), deduced diploid chromosome number (H), and ITS GenBank accession number (I). Asterisks indicate material deposited in the herbarium of the Pablo de Olavide University (UPOS), where some of it has been cultivated in a greenhouse

(A) Taxon	(B)	(C) Locality/source	(D)	(E) Voucher	(F) Natural distribution	(G)	(H)	(I)
Carex subgenus Carex L.								
Carex sect. Ceratocystis Dumort.								
C. demissa Honerm.	1	ES, Àvila, 40°16′N 05°30′W	1	*J. Fernández et al., 50JFA03(1)	Europe, Morocco, Azores Islds., Madeira	–	–	DQ384119
C. demissa	2	DE	1	HeRB 2761		–	–	AY278307
C. flava L.	1	RU	1	Svortsov s.n. (RSA 545715)	N & E Europe, North America	–	–	AT285007
C. flava	2	DE	1	HMH 1869		–	–	AT278310
C. gr. flava	1	ES, Huesca, Pyrenees, Ordesa; 42°38′N 0°60′W	1	*J. Fernández et al., 69JFA03		–	–	DQ384144
C. hostiana DC.	1	FR	1	HMH 2140	C & S Europe, Turkey, NE North America	–	–	AY278309
C. lepidocarpa Tausch.	1	ES, Valladolid, Encinas de Esgueva, 41°45′N 4°6′W	1	J.L. Fernández s.n. (342466MA)	Europe, E North America	–	–	DQ384164
C. lepidocarpa	2	DE	1	HeRB 1511		–	–	AY278293
C. nevadensis Boiss. & Reut.	1	ES, Granada, Sierra Nevada, 37°3′N 3°19′W	1	M. Luceño et al., 6900ML (276UPOS)	SE Spain	–	–	DQ384172
C. viridula Michx.	1	CH	1	HMH 1856	Europe, Asia, NE North America	–	–	AY278290
C. viridula	2	CA, NWT	1	TUB		–	–	AY238708
Carex sect. Digitatae (Fries) Christ.								
C. humilis Leyss.	1	GR	1	Hartvig et Franzen 8844	Mediterranean coast of Europe, W Asia	–	–	AF285008
Carex sect. Elatae Kükenth.								
C. aethiopica Sckuhr	1	ZA, Hewu, Port Elizabeth, 32°13′S 26°35′E	1	P. Vargas, 464PV00 (90UPOS)	South Africa	–	–	DQ384111
C. hochstetteriana J. Gay ex Seub.	1	PT, Azores, Isld. of the Pico, 38°28′N 28°23′W	1	J.M. Pereira s.n. (AZU)	Portugal: Azores Islds.	–	–	DQ384151
C. hochstetteriana	2	PT, Azores, S. Miguel Isld., Ferreirao, 37°47′N 25°29′W	1	E. Sjögrem, F366C (522690MA)		–	–	DQ384152
C. lowei Bech.	1	PT, Madeira Isld., Encumeada, 32°44′N 16°58′W	2	*J.M. Marín, 103JMM(1)	Portugal: Madeira	38[II]	76	DQ384166
C. lowei	2	PT, Madeira Isld., Santana, 32°47′N 16°52′W	1	*M. Luceño et al., 203ML(1)		38[II]	76	DQ384167
C. manni Bech.	1	GQ, Bioco, 03°35′N 08°46′E	1	Carvalho 3677 (512419MA)	Tropical Africa	–	–	DQ384171
C. perraudieriana J. Gay	1	ES, Canary Islds., Tenerife Isld., Anaga reserve, 28°33′N 16°13′W	1	M. Luceño et al., (470007MA)	Spain: Canary Islds.	–	–	DQ384177
Carex sect. Ferrugineaea (Tuckerman) Kükenth.								
C. lemmonii W. Boot	1	US, California	1	Sanders 15031 (UCR)	N America: California	–	–	AF284971

Table 2. (*continued*)

(A) Taxon	(B)	(C) Locality/source	(D)	(E) Voucher	(F) Natural distribution	(G)	(H)	(I)
Carex sect. Mitratae Kükenth.								
C. umbrosa Host	1	RU, Siberia	1	Murray et al., 344	N & W Asia	–	–	AF285042
Carex sect. Paniceae G. Don.								
C. falcata Turcz.	1	RU, Madagan Oblast	1	Dokuchaeva s.n. (RSA 533281)	N Russia	–	–	AF285016
Carex sect. Porocystis Dumort.								
C. pallescens L.	1	FI	1	Hinneri s.n. (RSA 302781)	Europe, E Asia, E North America	–	–	AF284997
Carex sect. Racemosae G. Don								
C. serratodens W. Boott.	1	US, California, Contra Costa Co., Mt Diablo, 37°50′N 121° 57′W	1	*B. Baldwin s.n.	N America: California	–	–	DQ384183
Carex sect. Spirostachyae Drejer ex Bailey								
C. binervis Smith	1	ES, Àvila, Navalguijo, 40°16′N 05°30′W	1	*J. Fernández et al., 51JFA03(1)	W Europe	–	–	DQ384112
C. burchelliana Boeck.	1	ZA, Eastern Cape, Maluti/Maclear, Killarney Farm, 30°20′S 28°17′E	1	A.A. Gubb 13930 (KMG25525)	W Griqualand, steppes of South Africa	–	–	DQ384114
C. camposii Boiss. & Reut.	1	ES, Granada, Sierra Nevada, 36°57′N 3°33′W	1	*J.M. Marín s.n.	SE Spain	–	–	DQ384115
C. cretica Gradst. & J. Kern	1	GR, Crete, Eparchía Kissámou, reforestation area NW of Vlatos, 35°23′N 23°39′E	1	10 0089447B	Crete, (Greece)	–	–	DQ384116
C. cretica	2	GR, Crete, Eparchía Kissámou, partial cut at Vigla N slope W Sembronas, 35°22′N 23°47′E	1	Ralf Jahn s.n. (10 0089448B)		–	–	DQ384117
C. cretica	3	GR, Crete, Kissamos W Vlatos, 35°23′N 28°37′E	1	*S. Martín-Bravo et al., 389SMB05(2)		–	–	DQ384118
C. cretica	4	GR, Crete, Kissamos, Koutsomatados, 35°23′N 23°40′E	1	*S. Martín-Bravo et al., 385SMB05(1) 385SMB05(2)		29^{II}	58	–
			2			29^{II}	58	–
C. diluta M. Bieb.	1	IR, Kerman, Kuh-e Hezar, 29° 59′N 51°34′E	1	J.R. Edmonson et A.G. Miller, 1609 (168695EOO)	C & W Asia	–	–	DQ384120
C. distans L.	1	MA, Nord Ouest, Kenitra, 34°15′N 6°34′W	1	P. Vargas et al., 2742(1)PV (515133 MA)	C & W Asia, Europe, N Africa	$32^{II}+1^{III}$	70	DQ384121
C. distans	2	TR, Anatolia, 39°56′N 32°47′E	1	M. Nydegger 42455 (145370VAL)		–	–	DQ384122
C. distans	3	ES, Sevilla, 37°21′N 5°56′W	1	*J. Fernández et al., 48JFA03(1)		35^{II}	70	DQ384123
C. distans	4	GR, Crete, road Iraklio-Faistos, 35°09′N 25°01′E	1	*S. Martín-Bravo et al., 35SMB05		–	–	DQ384124
C. distans	5	DE	1	HMH 1854		–	–	AY278312
C. distans	6	ES, Madrid, Mar de Ontígola, 40°01′N 03°21′W	1	M. Luceño et al., s.n.(314697MA)		–	–	DQ384125
			2	M. Luceño et al., 4487(1)ML		37^{II}	74	–
C. distans	7	BG, Black sea coast, 7 km NNW of Nessebar, 42°42′N 27°41′E	1	Dimitar Uzunov et al., 97-5341 (624474MA)		–	–	DQ384126
C. distans	8	GR, Dion, 40°10′N 22°29′E	1	*P. Vargas et al., 230PV04(1)		36^{II}	72	DQ384127
C. distans	9	GR, Epiro, Metsovo, 39°46′N 21°10′E	1	*P. Vargas et al., 237PV04(7)		35^{II}	70	–
C. distans	10	GR, Epiro, Cocito River, Kipsali, 39°30′N 22°38′E	1	*P. Vargas et al., 267PV04		35^{II}	70	–
C. distans	11	GR, Crete, Nycritos	1	*S. Martín-Bravo et al., 347SMB05		$36^{II}+1^{III}$, $2^{I}+32^{II}+3^{III}$	75	–

Taxon		Locality		Collector	Distribution			Accession
C. distans	12	MA, Rif, Chefchaouen-Ksar el Kebir, 34°05′N 05°22′W	1	*J. Fernández et al., 34JFA03(9)		35^{II}	70	–
C. extensa Good.	1	FI, Ahvenanmaa, Eckerö, 60°12′N 19°37′E	1	R. Lampinen 13364 (562431MA)	Europe, N Morocco, W Caucasus	–	–	DQ384128
C. extensa	2	ES, Almería, Adra, 36°44′N 3°1′W	1	Fernández-Casas 18402 (386894MA)		–	–	DQ384129
C. extensa	3	IT, Sicily, Ragusa, 36°52′N 14°26′E	1	J. Güemes, JGH-3409 (119531VAL)		–	–	DQ384130
C. extensa	4	GR, Amondia, Aqueronte River	1	*P. Vargas et al., 259PV04(1)		–	–	DQ384131
			2	*P. Vargas et al., 259PV04(3)		30^{II}	60	–
C. extensa	5	DE	1	HeRB 1557		–	–	AY278311
C. fissirrostris Ball	1	MA, Tensift, Marrakech, Okaïmeden, 31°12′N 7°51′W	1	P. Vargas et al., 2715PV(1) (481590MA)	Morocco: Great and Middle Atlas	36^{II}	72	DQ384132
C. fissirrostris	2	MA, Great Atlas, El Haoz, 31°14′N 07°48′W	1	P. Jiménez-Mejías et al., 199PJM05(5)				DQ384133
C. fuscula ssp. catharinensis (Boeck.) Luceño & M. Alves	1	BR, Itatiaia	1	*M.V. Alves et al., 2764MAV	SE Brazil			DQ384134
C. fuscula ssp. catharinensis	2	BR, Santa Catarina, Bom Retiro, 27°48′S 49°31′W	1	*J.M. Marín et al., 3002JMM		–	–	DQ384135
C. fuscula ssp. catharinensis	3	BR, Itatiaia, environs of Itatiaia National Park, 2500–2600 m	1	*M.V. Alves et al., 2766MAV		–	–	DQ384136
C. fuscula ssp. catharinensis	4	Brazil, Itatiaia, environs of the National Park of Itatiaia, 2100–2200 m	1	*M. V. Alves et al., 2758MAV	SE Brazil	–	–	DQ384137
C. fuscula d'Urv ssp. fuscula	1	CL, Tierra del Fuego, section Vicuña, 54°08′S 68°40′W	1	Pisano et al., 7410 (141320CONC)	Chile & Argentina	–	–	DQ384138
C. fuscula ssp. fuscula	2	CL, Nahuelbuta National Park	1	*J.M. Marín et al., 3502JMM(1)		–	–	DQ384139
C. fuscula ssp. fuscula	3	CL, Nahuelbuta National Park	1	*J.M. Marín et al., 3302JMM		–	–	DQ384140
C. fuscula ssp. fuscula	4	CL, Tierra del Fuego, Río Grande, 53°52′S 68°55′W	1	O. Skewes s.n. (149905 CONC)		–	–	DQ384141
C. fuscula ssp. fuscula	5	CL, Concepción, natural reserve of Botany Dept., Concepcion Univ.	1	*J.M. Marín et al., 3202JMM(1)		33^{II}	66	DQ384142
C. fuscula ssp. fuscula	6	CL, Concepción, Cerro Caracol	1	*J.M. Marín et al., 3602JMM		–	–	DQ384143
C. fuscula ssp. fuscula	7	CL, Nabuelbuta National Park	1	*J.M. Marín et al., 3402JMM(1)		32^{II}	64	–
C. gunniana Boot.	1	AU, Tasmania, Point Hibbs, 42°36′S 145°16′E	1	A. Moscal 5606 (97723HO)	SE Australia	–	–	DQ384145
C. gunniana	2	AU, Tasmania, Lambert Park, 42°52′S 147°19′E	1	A.M. Buchanan 11814 (126614HO)		–	–	DQ384146
C. helodes Link	1	PT, Alentejo, Vilanova de Milfontes, 37°47′N 02°43′W	1	*J.M. Marín et al., 8801JMM(2)	S W Iberian Peninsula, N Morocco	–	–	DQ384147
C. helodes	2	MA, Tanger-Tetouan, Chefchaouen, 35°07N 05°21′W	1	*P. Jiménez-Mejías, 29PJM04(2)		–	–	DQ384148
C. helodes	3	PT, Algarve, Córtelha, 37°15′N 7°57′W	1	*J.M. Marín et al., 7901JMM(7)		–	–	DQ384149
C. helodes	4	MA, Tanger-Tetouan, Chefchaouen, 35°05′N 05°22′W	1	*J. Fernández-Mejías et al., 34JFA03(5)		–	–	DQ384150
C. idaea Greuter et al.	1	GR, Crete, Kamares; GR, Crete, Paleochora, Azogres, 35°16′N 23°43′E	1	*S. Martín-Bravo et al., 350SMB05(4)	Greece, Crete	37^{II}	74	–
			2	350SMB05(9)		–	–	DQ384153
C. idaea	2	GR, Crete, Base of Idaea Cave mount, 32°12′N 24°50′N	1	*S. Martín-Bravo et al., 392SMB05(1)		–	–	DQ384154
C. idaea	3	GR, Crete, Base of Idaea Cave mount, 32°12′N 24°50′N	1	*S. Martín-Bravo et al., 375SMB05(8)		–	–	DQ384155

Table 2. (*continued*)

(A) Taxon	(B)	(C) Locality/source	(D)	(E) Voucher	(F) Natural distribution	(G)	(H)	(I)
C. laevigata Sm.	1	ES, Cádiz, La Albina, 36°52′N 05°39′W	1	*V. Valcárcel et al., 01VV03(1)	W Europe, N Morocco	–	–	DQ384156
			2	*J.M. Marín et al., 402JMM(2)		38^{II}	76	–
			3	402JMM(3)		37^{II}	74	–
			4	402JMM(4)		37^{II}	74	–
			5	402JMM(5)		37^{II}	74	–
C. laevigata	2	ES, Asturias, La Caridá-Moutas, 43°21′N 6°7′W	1	*P. Jiménez-Mejías et al., 446PJM05		–	–	DQ384157
C. laevigata	3	ES, Cádiz, Alcalá de los Gazules, 36°08′N 05°38′W	1	*J.M. Marín et al., 6701JMM(4)		40^{II}	80	DQ384158
C. laevigata	4	PT, Foia, Algarve, 37°11′N 7°45′W	1	*V. Valcárcel et al., 12VV03(1)		–	–	DQ384159
C. laevigata	5	ES, Ciudad Real, Sierra Madrona, 38°25′N 04°02′W	1	*M. Luceño et al., 100ML(1)		37^{II}	74	–
			2	100ML(2)		37^{II}	74	–
			3	100ML(3)		37^{II}	74	DQ384160
			4	*M. Escudero et al., 02ME05(1)		37^{II}	74	–
			5	02ME05(5)		37^{II}	74	–
			6	02ME05(8)		37^{II}	74	–
			7	02ME05(A)		37^{II}	74	–
			8	02ME05(B)		37^{II}	74	–
C. laevigata	6	PT, Serra de Ossa, Redondo-Entremoz, 38°44′N 07°35′W	1	*P. Jiménez-Mejías et al., 01PJM05(6)		37^{II}	74	–
C. laevigata	7	PT, Algarve, Caldas de Monchique, 37°17′N 8°33′W	1	*V. Valcárcel et al., 14VV03(2)		37^{II}	74	–
C. laevigata	8	PT, Sierra de Monchique, Alferce, road to Fontesanta, 37°19′N 8°26′W	1	*J.M. Marín et al., 8501JMM(1)		37^{II}	74	–
			2	8501JMM(3)		37^{II}	74	–
			3	8501JMM(4)		37^{II}	74	–
C. laevigata	9	PT, from Caldas de Monchique to Serra de Monchique, 37°18′N 08°31′W	1	*J.M. Marín et al., 8301JMM(1)		38^{II}	76	–
			2	8301JMM(2)		38^{II}	76	–
			3	8301JMM(3)		38^{II}	76	–
			4	8301JMM(4)		38^{II}	76	–
			5	8301JMM(5)		38^{II}	76	–
C. laevigata	10	PT, Baixo Alentejo, between Sao Luiz and Odemira, 37°38′N 08°37′W	1	*J.M. Marín et al., 8901JMM(1)		38^{II}	76	–
			2	8901JMM(2)		38^{II}	76	–
			3	8901JMM(3)		38^{II}	76	–
			4	8901JMM(4)		38^{II}	76	–
			5	8901JMM(5)		38^{II}	76	–
C. laevigata	11	ES, Cádiz, Alcalá de los Gazules, Pico del Aljibe, 36°28′N 5°44′W	1	*P. Jiménez-Mejías et al., 34PJM04(3)		37^{II}	74	–
C. laevigata	12	ES, Cádiz, road Puerto Galis-Ubrique, km 6, 36°41′N 5°27′W	1	*J.M. Marín et al., 6201JMM		38^{II}	76	–

Taxon		Locality		Collection		Chromosome		GenBank
C. laevigata	13	ES, Cádiz, road Puerto Galis-Ubrique, km 10	1	*J.M. Marín et al., 7601JMM(3)		37^{II}	74	–
			2	7601JMM(B1)		37^{II}	74	–
			3	7601JMM(B2)		37^{II}	74	–
			4	7601JMM(B3)		37^{II}	74	–
			5	7601JMM(B4)		36^{II}	72	–
			6	7601JMM(B5)		37^{II}	74	–
C. laevigata	14	ES, Cádiz, road Puerto Galis-Ubrique, km 18	1	*J.M. Marín et al., 7501JMM(1)		37^{II}	74	–
			2	7501JMM(2)		37^{II}	74	–
			3	7501JMM(4)		37^{II}	74	–
			4	7501JMM(5)		37^{II}	74	–
C. laevigata	15	ES, Cádiz, Fascinas-Los Barrios, 36°11′N 05°34′W	1	*E. Caballero et al., 03ECG03(5)		41^{II}	82	–
			2	03ECG03(9)		41^{II}	82	–
			3	03ECG03(11)		41^{II}	82	–
C. laevigata	16	ES, Cádiz, Facinas, 36°52′N 05°41′W	1	*J.M. Marín et al., 6601JMM(2)		42^{II}	84	–
			2	6601JMM(3)		42^{II}	84	–
C. laevigata	17	ES, Málaga, La Sauceda, 36°31′N 05°35′W	1	*P. Jiménez-Mejías et al., 19PJM03(4)		37^{II}	74	–
C. laevigata	18	MA, Tetouan-Ceuta, D'jebel Zem-Zem, 36°04′N 05°31′W	1	*J.M. Fernández et al., 26PJM03(4)		$35^{II} + 2^{III}$	76	–
			2	26PJM03(10)		40^{II}	80	–
			3	27PJM03(3)		$37^{II} + 1^{III}$	77	–
			4	27PJM03(3b)		38^{II}	76	–
			5	27PJM03(6)		38^{II}	76	–
C. lainzii Luceño, E. Rico & T. Romero	1	ES, Valladolid, 41°43′N 04°12′W	1	*R. Àlvarez et al., 2RAD02(8)	C Spain	–	–	DQ384161
C. lainzii	2	ES, Segovia, 41°18′N 04°02′W	1	*R. Àlvarez et al., 1RAD02(3)		–	–	DQ384162
C. lainzii	3	ES, Valladolid, 41°43′N 04°12′N	1	*R. Àlvarez et al., 2RAD02(4)		–	–	DQ384163
C. mairii Coss. & Germ.	1	ES, Huesca, Panticosa, 42°43′N 00°16′W	1	*J. Fernández et al., 60JFA03	France, Spain, Morocco	–	–	DQ384168
C. mairii	2	ES, Albacete, Riopar, 38°30′N 2°27′W	1	A. Aparicio et al., s.n. (00153UPOS)		–	–	DQ384169
C. mairii	3	FR, Pyrenees-Orientales, Sahorre, 42°32′N 2°22′E	1	J. Lambinon 91/152 (42229VAL)		–	–	DQ384170
C. mairii	4	FR	1	FO 9499b		–	–	AY278253
C. paulo-vargasii Luceño & J.M. Marín	1	MA, Rif, Mor Dha El Assif, 35°06′N 05°20′W	1	*J. Fernández et al., 35JFA03(3)	Morocco	37^{II}	74	–
			2	35JFA03(8)		37^{II}	74	–
			3	35JFA03(10)		37^{II}	74	–
			4	35JFA03(12)		–	–	DQ384173
C. paulo-vargasii	2	MA, Oukaïmaden, 31°12′N 7°51′W	1	M. Sequeira et al., 3348MS (41516VAL)		–	–	DQ384174
C. paulo-vargasii	3	MA, Chefchaouen-Fez, Zeida, 34°52′N 04°31′W	1	*V. Valcárcel et al., 24VV03(2)		37^{II}	74	DQ384175
C. paulo-vargasii	4	MA, Rif, road Chefchaouen – Ketama, Km 55, 34°38′N 04°05′W	1	*E. Narbona et al., 02EN03(2)		37^{II}	74	–
			2	02EN03(10)		37^{II}	74	DQ384176
C. paulo-vargasii	5	MA, Rif, Targuist – Ketama, 34°55′N 04°32′W	1	*E. Narbona et al., 04EN03(6)		37^{II}	74	–
			2	04EN03(7)		37^{II}	74	–
			3	04EN03(11)		37^{II}	74	–

Table 2. (*continued*)

(A) Taxon	(B)	(C) Locality/source	(D)	(E) Voucher	(F) Natural distribution	(G)	(H)	(I)
C. paulo-vargasii	6	MA, Rif, road Chefchaouen to Ketama, Km 75, 34°54′N 04°46′W	1	*E. Narbona et al., 03EN03(5)		37^{II}	74	–
			2	03EN03(8)		37^{II}	74	–
C. punctata Gaud.	1	GR, Crete, Lango, closer Skines, 35°25′N 23°53′E	1	*S. Martín-Bravo et al., 381SMB05	Europe, Turkey, N Morocco	–	–	DQ384178
C. punctata	2	FR, Saint-Pée-sur-Nivelle, 43°23′N 01°32′W	1	J.J. Lazare, s.n. (42233VAL)		–	–	DQ384179
C. punctata	3	ES, Guipúzcoa, Irún, 43°19′N 01°50′W	1	P. Catalán, s.n. (342695MA)		–	–	DQ384180
C. punctata	4	FR, Corse, Bitalza, Montagne de Cagna	1	J. Lambinon et al., 118 (693775MA)		–	–	DQ384181
C. punctata	5	MA, Tánger, Cabo Espatel, 35°47′N 05°36′W	1	*J. Fernández et al., 17JFA03(4)		34^{II}	68	–
			2	17JFA03(5)		34^{II}	68	–
			3	17JFA03(7)		–	–	DQ384182
C. tasmanica Kük.	1	AU, Tasmania, Catwright res., 42°56′S 147°21′E	1	E. Hayward, s.n. (441490HO)	SE Australia, Tasmania	–	–	DQ384184
C. tasmanica	2	AU, Tasmania, Hobart, Domain, 42°92′S 147°19′E	1	M.M. Richarson, 372a (9012238CBG)		–	–	DQ384185
C. troodi Turril	1	CY, inter Troodos and Platres, 34°54′N 32°52′E	1	*V. Valcárcel et al., 06VV05(1)	Cyprus	–	–	DQ384186
			2	06VV05(12)		34^{II}, 31^{II} + 2^{III}, 28^{II} + 4^{III}	68	–
C. troodi	2	CY, Pano Amiantos – Troodos, 34°56′N 32°53′E	1	*V. Valcárcel et al., 09VV05(12)		34^{II}	68	–
			2	09VV05(2B)		–	–	DQ384187
C. troodi	3	CY, Tripylos, 34°59′N 32°41′E	1	*V. Valcárcel et al., 15VV05(1)		34^{II}, 31^{II} + 2^{III}, 33^{II} + 2^{I}	68	–
			2	15VV05(A)		–	–	DQ384188
C. troodi	4	CY, inter Prodromos and Kakopetria, 34°57′N 32°50′W	1	*V. Valcárcel et al., 08VV05		33^{II} + 1^{III}	69	–
C. vixdentata (Kük.) G.A. Wheeler	1	AR, Mar de Ajo, 36°43′S 56°40′W	1	*J.M. Marín et al., 3102JMM	C & N Argentina, Uruguay, S Brazil	–	–	DQ384189

Carex subgenus Vignea (Beauv. Ex Lestib.) Peterm.

Carex sect. Ovales Kunth.

C. leporina L.	1	ES, Àvila, Sierra de Béjar, 40°19′N 05°42′W	1	*J. Fernández et al., 54JFA03(1)	Europe, W Asia, N Africa	–	–	DQ384165
C. leporina L.	2	ES, Panticosa, Pyrenees, 42°45′N 00°14′W	1	*J. Fernández et al., 62JFA03(1)		–	–	DQ384113

Chromosome data taken from Luceño and Castroviejo (1993) indicated in italics.

to the one found in the previous heuristic search (Schultheis 2001). Clade supports were assessed by fast bootstrapping with 100,000 re-samplings. All these analyses were conducted both with the complete ITS data matrix (94 sequences) and with a reduced matrix (63 sequences) resulting from the deletion of identical sequences and the inclusion of two sequences maximum per taxon.

The complete 94 ITS matrix was split into two different matrices, one including the ITS-1 and ITS-2 spacers, the other including the 5.8S region. Each matrix was analysed to determine the simplest model sequence evolution that best fit the data, both under the Hierarchical Likelihood Ratio Test (hLRT) and the Akaike Information Criterion (AIC), as implemented in MrModeltest 1.1b (Nylander 2002). When each criterion selected a different evolutionary model, Bayesian analyses were performed under both models using MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003). Four Markov Chain Monte Carlo runs were performed simultaneously in each Bayesian analysis for 5,000,000 generations with an interval of 100 generations. Burn-in was evaluated over generations. After discarding trees yielded before the Likelihood stationary, the remaining trees were compiled in a majority rule consensus tree, using posterior probability (pp) as a measure of clade support (Alfaro et al. 2003). Because tree topologies depicted by different evolutionary models selected by each criterion were similar, only differing in clade supports, the pairwise differences, tree topology and posterior probabilities herein shown are those obtained when applying the simplest model selected by the AIC.

Cytogenetic analysis

For preparation of meiotic plates, glumes were removed from staminate spikes. Once the glumes were eliminated, the remaining material was fixed in a solution of absolute ethanol/glacial acetic acid/acetocarmine 2% (10:4:1, v/v/v/) with a drop of ferric acetate per 15 ml of solution as mordant. After 6–24 h of fixation the material was stained in acetocarmine for 15–30 min and finally squashed (Luceño 1988). Meiotic plates were observed in a microscope (Nikon eclipse E400) and photographed by means of a Nikon DXM1200F digital camera and using the Nikon ACT-1 software. Diploid numbers were deduced from meiotic configurations in metaphase I of pollen mother cells (PMC).

Results

Phylogenetic relationships

Characterization of ITS sequences

Due to the lack of monophyly of two sections (see the discussion), the ITS sequence characteristics are not referred to sections but to two main inclusive groups: (1) the complete data matrix including the 94 ITS sequences, and (2) the core Spirostachyae, which includes 19 species of sect. *Spirostachyae* and five species of sect. *Elatae* (Fig. 1). ITS sequence length varied between 607 (*C. serratodens*) and 613 (*C. burchelliana*, *C. vixdentata*) base pairs (bp) for the complete ITS matrix, and from 611 bp (34 accessions, 11 species) to 613 bp (*C. burchelliana*, *C. vixdentata*) within the core Spirostachyae (Table 3). The ratio of variable to informative characters when comparing the 94 sequences of the complete matrix was 176/145, distributed as follows: 100/85 in the ITS-1, 5/5 in the 5.8S region, and 71/55 in the ITS-2 spacer. Within the core Spirostachyae the ratio for the complete ITS was 111/97, of which 62/56 were found in the ITS-1, 4/4 in the 5.8S region, and 45/37 in the ITS-2 spacer. A total number of 54 additivities in 30 polymorphic sites were detected in the core Spirostachyae, distributed in 28 accessions representing 13 species, most of which (25) were present in the ITS sequences of the *C. laevigata* populations 1, 3, 4, and 5.

Corrected GTR + G pairwise distances of ITS sequences varied between 0% (92 pairs of sequences) and 32% (*C. leporina* (sect. *Ovales*, subgen. *Vignea*)) and the two populations of *C. tasmanica* (sect. *Spirostachyae*, subgen. *Carex*). Within the core Spirostachyae, the minimum pairwise distance (0%) was found between 68 pairs of sequences; the maximum (15%) was found between populations 3 and 4 of *C. fuscula* ssp. *catharinensis* and the two populations of *C. tasmanica*. The highest genetic distance between sequence pairs of the same species in core Spirostachyae (2%) was found when comparing populations 3 and 4 of *C. fuscula* ssp. *catharinensis* and *C. fuscula* ssp. *fuscula* population 5.

Phylogenetic analysis

Phylogenetic reconstructions using Maximum Parsimony analyses of the complete 94 ITS matrix and the reduced 63 ITS matrix were identical with respect to the topology and similar in bootstrap clade supports and fit measures of trees. The MP analysis of the complete 94 ITS matrix retained 41,554 optimal trees with 394 steps (CI excluding uninformative characters (e.u.c.) = 0.53, RI = 0.89). In the BI analysis conducted implementing the GTR + G evolutionary model for the ITS-1 and ITS-2 spacers and the K80 + I + G model for the 5.8S region, the stationary of the Likelihood scores was reached at 40,000 generations. Accordingly, the first 400 trees were discarded. The MP strict consensus tree was mainly in agreement with the one inferred from the BI majority rule consensus. The few incongruencies found did not affect topology but concerned clade supports (Fig. 1).

Two well-supported clades were retrieved (Fig. 1). The first one (94% bs, 100% pp) included the 11 accessions of the six species of sect. *Ceratocystis* (core

Ceratocystis). The second clade (65% bs, 100% pp), hereafter called core Spirostachyae, included 65 accessions of 19 species of sect. *Spirostachyae* and the seven accessions of the five species of sect. *Elatae* (Fig. 1). The species *C. cretica* (3 accessions) of sect. *Spirostachyae* was unrelated to the core Spirostachyae. Species assembled in the core Spirostachyae split into two major clades (A, B; Fig. 1). Clade A was formed by the five species of sect. *Elatae* and 10 species of sect. *Spirostachyae* (58% bs, 100% pp). Clade B (< 50% bs, 100% pp) contained all the accessions of the remaining nine species of sect. *Spirostachyae* included in the core Spirostachyae. Within clade A, nine well-supported clades were formed by all the accessions of the same species: *C. fissirrostris* (100% bs, 100% pp), *C. fuscula* (80% bs, 100% pp), *C. gunniana* (98% bs, 100% pp), *C. helodes* (100% bs, 100% pp), *C. hochstetteriana* (98% bs, 100% pp), *C. lowei* (100% bs, 100% pp), *C. mairii* (88% bs, 100% pp), *C. paulo-vargasii* (85% bs, 100% pp), and *C. punctata* (90% bs, 99% pp). Four well-supported clades contained all the accessions of the same species within clade B: *C. extensa* (95% bs, 99% pp), *C. lainzii* (99% bs, 100% pp), *C. tasmanica* (100% bs, 100% pp), and *C. troodi* (71% bs, 100% pp).

Cytogenetic analysis

Chromosome numbers are herein reported for the first time in six species (Table 2): *C. cretica* ($2n = 58$; Fig. 2A), *C. fuscula* ($2n = 64$, Fig. 2D; $2n = 66$), *C. idaea* ($2n = 74$, Fig. 2E), *C. lowei* ($2n = 76$, Fig. 2(H), *C. paulo-vargasii* ($2n = 74$, Fig. 2I), and *C. troodi* ($2n = 68$, Fig. 2L; $2n = 69$), which displayed meiotic irregularities (pop. 1, 3, and 4).

The remaining 65 counts complemented the previous cytogenetic data of four species (*C. distans* (6 counts; Fig. 2J), *C. extensa* (1; Fig. 2C), *C. laevigata* (57), and *C. punctata* (2; Fig. 2(K); Table 2), and reported new numbers in two of them, *C. distans* ($2n = 75$ with meiotic irregularities; Fig. 2B) and *C. laevigata*. Among the 57 individuals counted of the 16 populations of *C. laevigata* (Fig. 2F and G) studied, three new numbers were found for this species ($2n = 77, 82, 84$; Fig. 2G); three populations (1, 13, 18) of nine showed different numbers ($2n = 74, 76$; $2n = 72, 74$; $2n = 76, 77, 80$; respectively) after screening two or more individuals. Meiotic irregularities in *C. laevigata* were only detected in population 18 (Morocco) (Table 2).

Discussion

Systematic implications

Circumscription of taxonomic sections

Traditionally, sect. *Spirostachyae* has been related to sects. *Ceratocystis* and *Elatae* (Kükenthal 1909; Luceño and Castroviejo 1993). While sectional limits between sects. *Ceratocystis* and *Spirostachyae* have been determined (Crins and Ball 1988), no clear limits have been proposed for *Spirostachyae* and *Elatae*. Considering previous cytogenetic and morphological data (Schmid 1982; Crins and Ball 1988), and molecular results (Hendrichs et al. 2004b), sect. *Ceratocystis* (Table 1) appeared to be a monophyletic group clearly independent from sect. *Spirostachyae*. Our results confirm a well-supported monophyly and independence of sect. *Ceratocystis*, whereas sects. *Elatae* and *Spirostachyae* as herein considered (Table 1) are not monophyletic (Fig. 1). The major clade depicted in the ITS tree (core Spirostachyae), contains most (19) of the species traditionally considered within sect. *Spirostachyae* and the five species of sect. *Elatae* included in this study (Tables 1 and 2; Fig. 1). The core Spirostachyae is divided into two lineages (clades A and B; Fig. 1). The five representatives of sect. *Elatae* are placed in independent sublineages embedded in the basal polytomy of clade A of the core Spirostachyae (Fig. 1). Relatedness of these two sections was previously reported based on morphology, cytogenetics, and phytogeography for *C. laevigata* and *C. camposii*, which were already moved from sect. *Elatae* to *Spirostachyae* (Luceño and Castroviejo 1993).

One species traditionally included in sect. *Spirostachyae* (*C. cretica*; Chater 1980) does not form part of the core Spirostachyae (Fig. 1). This result was somehow expected, as the species presents some but not all of the eight diagnostic characters that define sect.

Fig. 1. Majority rule consensus tree of the 49,600 trees retained in the Bayesian Inference analysis of the 94 ITS sequences of genus *Carex* (eleven of sect. *Ceratocystis*, seven of sect. *Elatae*, 68 of sect. *Spirostachyae*, six from other sections of subgen. *Carex*, and two of *C. leporina* – *C. leporina* pop. 1 as the outgroup). Posterior probabilities and bootstrap values are given above and below branches, respectively. Diploid numbers given right after terminal labels represent: (i) counts obtained from the same individual sequenced (in boldface), (ii) counts obtained from a different individual of the same population (in italics); asterisks indicate counts taken from previous studies (Luceño and Castroviejo 1993). Country sources are mentioned. Chromosome numbers reported for each species either in this or in previous studies (Heilborn 1924, 1928; Tischler 1935; Wulff 1937a, b; Löve 1954; Davies 1955, 1956a, b; Kjellvist and Löve 1963; Dietrich 1972; Favarger et al. 1980; Queirós 1980; Strid and Franzen 1981; Schmid 1982; Stoeva and Stepankova 1990; Luceño 1992a, b, 1993; Luceño and Castroviejo 1991, 1993; Luceño et al. 1987), are specified with the most frequent cytotype underlined. Vertical bars assemble taxa from the same section.

Spirostachyae (see the introduction). This acidophilous endemic to W Crete appears outside the core Spirostachyae and weakly related to sect. *Ceratocystis* (Fig. 1). Morphological analysis of the material sequenced revealed that no red crystalloid bodies are

present in the epidermic cells of the utricles as typically occurs in sect. *Spirostachyae* (Crins and Ball 1988), and truncate or irregularly broken beaks of the utricles were found instead of the bifid or bidentate beaks in sect. *Spirostachyae*. In addition to this, the chromosome
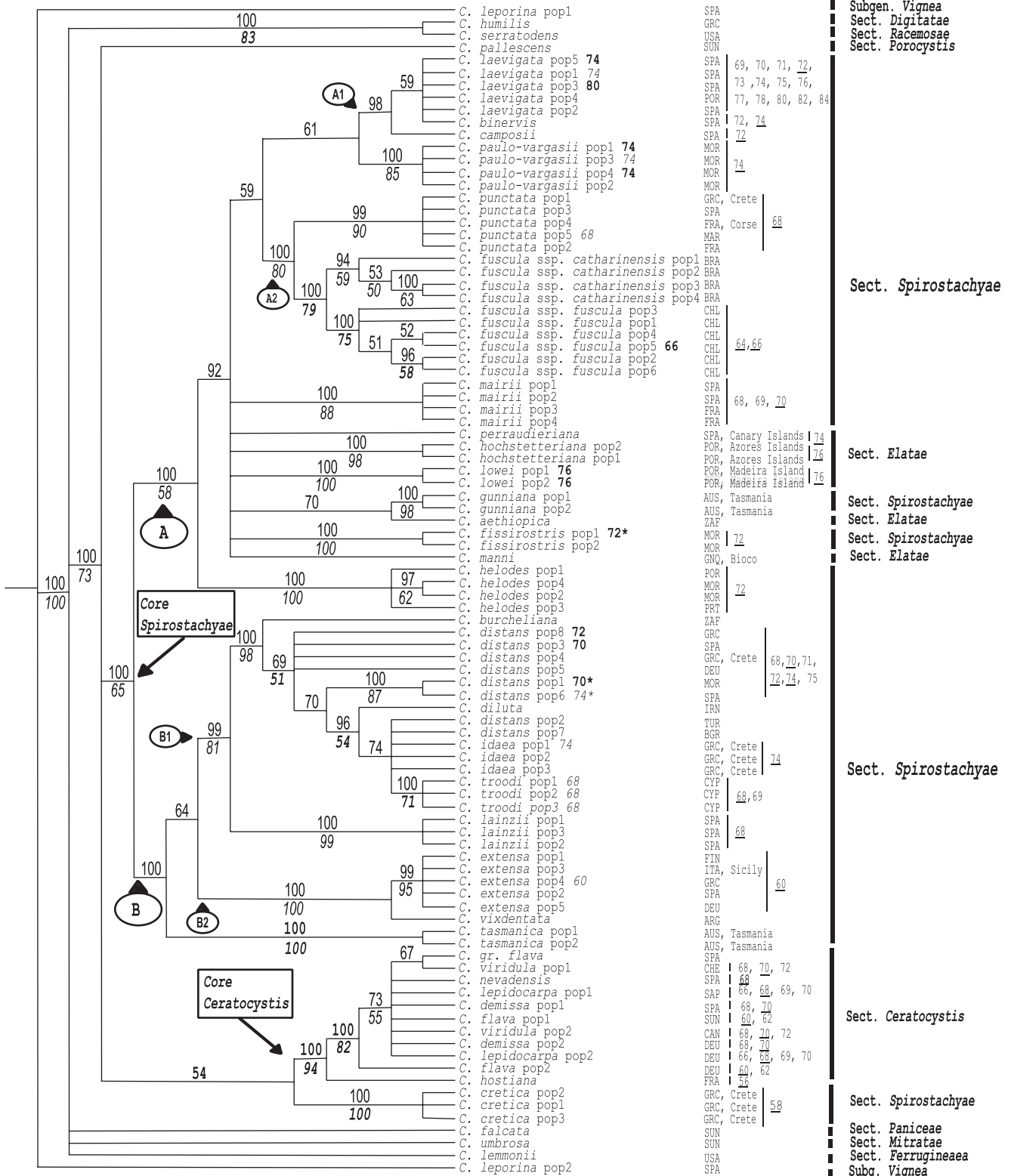
Table 3. General characteristics of the Internal Transcribed Spacer sequences specified for: (i) the complete 94 ITS matrix, including sects. *Ceratocystis*, *Elatae*, and *Spirostachyae* plus the outgroup; (ii) all 72 accessions of the core Spirostachyae; and (iii) the 39 accessions resulting from the inclusion of one single sequence per taxon of the core Spirostachyae

	ITS	ITS-1	5.8 S	ITS-2
Complete 94 ITS matrix				
Aligned length [bp]	630	228	166	236
Number of variable/informative characters	176/145	100/85	5/5	71/55
Core Spirostachyae (all)				
Range of sequence lengths [bp]	611–613	220–222	166	223–227
Number of variable/informative characters	111/97	62/56	4/4	45/37
Number of transversions/transitions in informative positions	62/19	38/11	4/0	20/8
Mean G + C content [%]	63.84	63.47	51.70	70.58
Number of additive polymorphic sites	54	17	4	33
Number of accessions with additive polymorphic sites	28	15	3	19
Core Spirostachyae (one sequence per taxon)				
Number of variable/informative characters	108/63	62/33	3/2	43/28

number found in *C. cretica* (2*n* = 58; Fig. 2A) is outside of the range in sect. *Spirostachyae* ((60)68–74(84)).

In summary, the study of the ITS region and cytogenetic data contributed greatly to establishing confines for sect. *Spirostachyae*. Firstly, one taxon (*C. cretica*) should be excluded from this section in the interest of a more natural (monophyletic) sectional taxonomy in *Carex*. Secondly, the five species of the sect. *Elatae* analysed in this study, and possibly the remaining members of this section, should be circumscribed within sect. *Spirostachyae*. This is not surprising, considering that the species of sect. *Elatae* included in this study display the eight morphological characters defining sect. *Spirostachyae* (see the introduction; Kükenthal 1909).

Species relationships in the core Spirostachyae

Two major lineages can be distinguished within core Spirostachyae: clades A and B (Fig. 1). From an evolutionary point of view, this biphyletic topology is consistent with principal cytogenetic trends and edaphic preferences of taxa grouped in each clade (Luceño and Castroviejo 1993, personal observations and communications). Clade A basically includes acidophilous taxa, except for the basophilous *C. mairii*, and is characterised by a wide range in chromosome number (2*n* = (64)68–84). On the other hand, clade B groups basophilous and halophilous taxa together – with the exception of the ultrabasic *C. troodi* from Cyprus – and shows lower chromosome numbers (2*n* = 60–74(75)). This ecological differentiation is particularly surprising in the case of *C. binervis* and *C. distans*, two species extremely similar (sometimes morphologically indistinguishable) but with different edaphic preferences that appear completely separated in the phylogeny (clades

A and B, respectively). This macro-evolutionary differentiation in the core Spirostachyae parallels similar ecological speciation due to niche separation associated with substrate differentiation as a driving micro-evolutionary force in *C. curvula* (Choler et al. 2004).

Carex helodes is a well-supported monophyletic taxon sister to the remaining taxa of clade A (Fig. 1). This species is endemic to the SW Iberian Peninsula and N Morocco (Luceño and Escudero 2006). Although it is morphologically and cytogenetically well characterised (Luceño 1992b), it has been confused very often with the Atlantic *C. laevigata* (Kükenthal 1909; Vicioso 1959). However, the present phylogeny reveals its monophyly and independence from *C. laevigata* (Fig. 1).

A large polytomy obscures the phylogenetic relationships of Macaronesian species. Only two subclades can be identified within clade A: the *C. laevigata* group (subclade A1) and the *C. fuscula–C. punctata* subclade (A2). Low resolution within subclade A1, formed by *C. laevigata*, *C. camposii* and *C. binervis*, seems to be the result of active hybridization processes between *C. laevigata* and *C. binervis*, as well as between cytological races of *C. laevigata* itself (see the following section of this discussion). The fact that most of the *C. laevigata* populations sequenced (populations 1, 3, 4, 5) present multiple ITS copies detected by nucleotide additive patterns is congruent with the great cytogenetic variability exhibited by this species (Table 2; Luceño and Castroviejo 1991), and may reflect a general pattern of hybridization in this group. The second and well-defined subclade (A2) contains the Old World *C. punctata* and the South American *C. fuscula* s.l. The widely distributed *C. punctata* constitutes a morphologically well-defined species. A remarkable morphological stability is congruent with identity of the ITS sequences and lack of cytogenetic variation (Table 2). On the other hand *C. fuscula* exhibits great
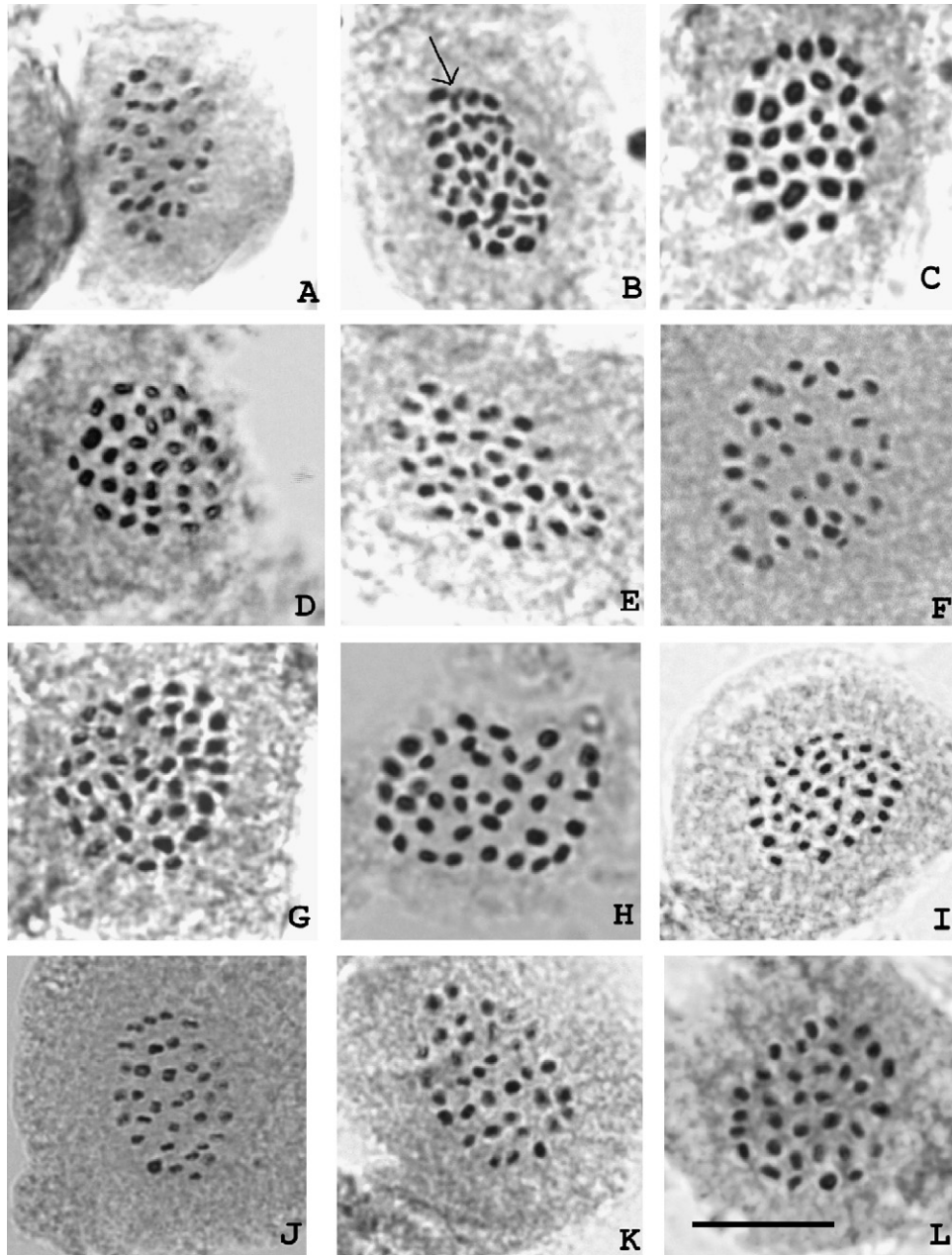
Fig. 2. Meiotic configurations in genus *Carex*: (A) *C. cretica* population 1, sample 2 ($2n = 29^{II} = 58$); (B) *C. distans* pop.11, sam.1 ($2n = 36^{II} + 1^{III} = 75$); (C) *C. extensa* pop.4, sam.2 ($2n = 30^{II} = 60$); (D) *C. fuscula* ssp. *fuscula* pop.7, sam.1 ($2n = 32^{II} = 64$); (E) *C. idaea* pop.1, sam.1 ($2n = 37^{II} = 74$); (F) *C. laevigata* pop.5, sam.2 ($2n = 37^{II} = 74$); (G) *C. laevigata* pop.15, sam.3 ($2n = 41^{II} = 82$); (H) *C. lowei* pop.1, sam.1 ($2n = 38^{II} = 76$); (I) *C. paulo-vargasii* pop.5, sam.3 ($2n = 37^{II} = 74$); (J) *C. distans* pop.12, sam.1 ($2n = 35^{II} = 70$); (K) *C. punctata* pop.5 sam.2 ($2n = 34^{II} = 68$); (L) *C. troodi* pop.2 sam.1 ($2n = 34^{II} = 68$). All cells are in metaphase I. Arrows show irregularities. Scale bar $= 10\,\mu m$.

morphological variability, as was expected because of the numerous taxa described (*C. catharinensis* Boeck., *C. distenta* Kunze, *C. hieronymi* Boeck., and *C. inconspicua* Steud.). The analysis of the ITS sequences reveals two groups within *C. fuscula*: Brazilian and Chilean populations (Fig. 1). Previously, this differentiation was based on morphological differences (Luceño and Alves 1999). However, taking into account the number of morphological similarities between populations from Brazil and Chile, a subspecific treatment is appropriate (ssp. *catharinensis* and ssp. *fuscula*). Three different species have been considered within Chilean populations of *C. fuscula* s.l. (*C. distenta*, *C. fuscula* s.s., and *C. inconspicua*; Wheeler and Zöllner 1996), but no morphological or molecular evidence supports this subdivision (Fig. 1).

Taxonomy and monophyletic groups in clade A are highly congruent. All accessions of five species were

resolved into well-supported monophyletic groups: *C. mairii* from the W Mediterranean basin; *C. hochstetteriana* and *C. lowei* from Macaronesia; *C. gunniana* from SE Australia and Tasmania; and *C. paulo-vargasii*, endemic to Morocco. The latter has been misidentified frequently as *C. binervis* and *C. distans* in herbaria. The ITS phylogeny, however, clearly reveals its monophyly, independence from *C. distans*, and certain relationships with *C. binervis*.

Accessions of four species in clade B form highly supported monophyletic groups (*C. tasmanica*, *C. extensa*, *C. lainzii*, *C. troodi*). Monophyly of the basophilous Iberian *C. lainzii* is supported by morphology (small caules of 4–14(24) cm, utricles with only two veins and gradually narrowed in a short bidentate and aculeolate beak; Luceño 1994). The Cypriot endemic *C. troodi* is well characterised by soft leaves, dark purple female and male glumes, and edaphic preferences restricted to ultrabasic substrates. The South American *C. vixdentata* is clearly the closest relative to the Old World *C. extensa*, as was expected considering that they only differ in a few quantitative morphological characters (length of female spikes and beak teeth of the utricles; Wheeler 1988).

Within clade B, there is one major subclade B1, including the basophilous Spanish endemic *C. lainzii*, sister to the *C. distans* group. The *C. distans* group branched off in the course of speciation: *C. burchelliana* (South Africa), *C. diluta* (C and W Asia), *C. distans* s.l. (Europe, Asia and N Africa), and the Mediterranean island endemics *C. troodi* (Cyprus) and *C. idaea* (Crete). *Carex burchelliana*, sister to the remaining *C. distans* group, is defined morphologically by its coriaceous and glaucous utricles gradually narrowed in a shortly bidentate beak, and by its separate female spikes. Placement of this taxon distant to *C. diluta* in subclade B1 is relatively surprising regarding its morphological affinities to the latter species, from which it differs only in the arrangement of the female spikes, scattered and separated from the male spike. The widely distributed and morphologically and genetically highly variable species *C. distans* s.l. is not monophyletic, although it is characterised morphologically by membranous greenish, brownish or straw-coloured utricles with short bifid beaks and separate female spikes. ITS sequences from the eastern-most populations of *C. distans* (populations 2, 7) are identical to those of *C. idaea*, a midland mountain species from Crete, and appear in the same clade as the Cypriot endemic *C. troodi* and the Iranian *C. diluta*. Association of eastern populations of *C. distans* with *C. idaea* is not surprising if morphology is considered. *Carex idaea* is characterised by rigid leaves, a character also found in a few individuals from Turkish and Bulgarian populations of *C. distans* analysed. Additionally, its dark purplish glumes have also been reported from some deviant individuals of

western Turkish *C. distans* by Nilsson (1985). Interestingly, the lowland population of *C. distans* from Crete (population 4), as well as the Greek population (8) and the western-most populations (1, 3, 5, 6), appear unresolved in a polytomy sister to, but clearly segregated from, the eastern clade. The geographical and altitudinal pattern related to genetic variability of *C. distans*, together with cytogenetic and morphological variability, appear to be the result of an active allopatric speciation process.

Cytogenetics of the core Spirostachyae

Chromosomal evolution

Taxa with holocentric chromosomes, such as those of Cyperaceae (Davies 1956b; Faulkner 1972; Schmid 1982), are characterised by frequent meiotic irregularities. The latter are due to chromosome changes (reciprocal translocations, fissions, and fusions) that can explain the extremely high variability in chromosome number that occurs in some species. The fact that different chromosome numbers have been detected within the same PMC in MI may indicate that such chromosome shifts also occur during Prophase I of the meiosis (Schmid 1982; Luceño 1992a). Lability in producing new chromosome numbers and different meiotic configurations confers cytogenetic variation reliability as a source of data to infer evolutionary patterns in Cyperaceae (Faulkner 1972; Cayouette and Morisset 1985; Vanzela et al. 1998).

Few cytogenetic studies have been conducted within sect. *Spirostachyae* (Luceño and Castroviejo 1991, 1993). In these studies, $2n = 72$ was suggested as the most probable ancestral cytotype to *C. laevigata*, *C. binervis*, *C. camposii*, and *C. helodes*, and chromosome fission was proposed as the principal mechanism involved in cytogenetic evolution of sect. *Spirostachyae*. Based on our ITS results, the hypothesis that $2n = 72$ is the ancestral cytotype can be extended to the whole clade A, in which this cytotype is the most frequent (Fig. 1). Analysis of the ITS sequences has revealed that, in addition to fusion and fission mechanisms, hybridization may also have played an important role in the evolution of some species. This is the case for *C. laevigata*, which displays one of the most remarkable chromosome number variations in *Carex* ($2n = 69$–84) (Figs. 1, 2F, G; Table 2). The variability shown by this species was associated with geographical structure in a north-to-south fission gradient (Luceño and Castroviejo 1991), in which the most common cytotype ($2n = 72$) was found in northern populations (Atlantic Europe, and N Iberian Peninsula) with a gradual increase to S Iberia (up to $2n = 80$) (Luceño and Castroviejo 1991). The addition in the present paper of 10 new southern populations, nine from the Iberian Peninsula and one

from Morocco, precludes accepting or rejecting this geographic-pattern hypothesis. Most of the new numbers found fit the gradient very well, raising the upper range limit to $2n = 84$ (S Iberian population 16; Table 2). However, unexpected numbers for specific geographic locations have been found, such as low numbers in the south of Spain (north of Cádiz), likely related to hybridization events. Incongruent patterns of the 25 additivities detected across the 11 polymorphic sites found in *C. laevigata* (populations 1, 3, 4, and 5) could be interpreted as evidence of active hybridization processes. Individuals of *C. laevigata* sequenced may be the result of multiple hybridization events between different cytological races of *C. laevigata*, as well as with *C. binervis* which appears unresolved in the same clade as *C. laevigata*. In fact, a hybrid between *C. laevigata* and *C. binervis* has been formally described (*C.* x *deserta* Merino). Meiotic behaviour of the F_1 hybrid between both species displayed several configuration irregularities (Luceño and Castroviejo 1991). Interestingly, ITS sequences of population 3 (sample 1) and population 5 (sample 3) showed seven and three additivities, respectively, but they displayed regular meiotic configurations (40^{II} and 37^{II}, respectively). This may indicate that chromosome complement stabilization evolves at a faster rate than ITS concerted evolution.

Caryological data confirm phylogenetic relations between *C. fuscula* and *C. punctata* (Fig. 1). *Carex punctata* displayed $2n = 68$ exclusively, both in North Morocco (Table 2) and in Europe (Heilborn 1924; Davies 1956a, b; Dietrich 1972; Luceño and Castroviejo 1993). On the other hand, the Chilean populations of *C. fuscula* had $2n = 64$ and 66 (Table 2). All these results allow us to describe a new fusion pattern involved in the evolution of *C. fuscula*–*C. punctata* (see the "Biogeographic patterns" section below).

Despite the existence of 18 variable and 14 parsimony-informative ITS characters in the three Macaronesian taxa of the former sect. *Elatae*, no relationship can be inferred among them, since they appear unresolved at a basal polytomy within clade A of the core Spirostachyae (Fig. 1). Macaronesian taxa of sect. *Elatae*, and the whole section itself, have been considered relicts from Pliocene tropical woodlands, as most of the section's extant representatives occur in tropical and subtropical island-like systems (Cronk 1992). Low caryological variability ($2n = 74$ and 76, Figs. 1 and 2H), together with high ITS variation and divergence, supports relictualism for the three Macaronesian species.

Clade B is characterised by taxa with significantly lower chromosome numbers ($2n = 60–74(75)$), $2n = 68$ being the most frequent cytotype. Luceño and Castroviejo (1993) hypothesized that lower numbers may be the oldest in this group and that higher numbers may

have been the result of fission processes. They concluded that *C. lainzii*, an endemic restricted to the central Iberian Peninsula with a stable chromosome number of $2n = 68$ (Luceño et al. 1987), may be a case of paleoendemism. The analysis of the ITS sequences revealed no molecular variation in *C. lainzii*. Moreover, when comparing this taxon to the *C. distans* group, 24 variable characters were detected, 10 of them being synapomorphies of *C. lainzii*, resulting in minimum and maximum pairwise genetic distances of 3–4%, significantly higher than those found among pairs of accessions in the *C. distans* group (0–2%). These results together with the well-defined sister-group relationship to a lineage of five widespread species (Fig. 1) highlight the relatively long-term isolation of the species and are consistent with the hypothesis of paleoendemism.

Identity of ITS sequences in five distant populations of *C. extensa*, together with the constant chromosome number $2n = 60$ (Fig. 2C) and regular meiotic configurations found across marshlands of Europe (Wulff 1937a; Rodrigues 1953; Davies 1956a, b; Dietrich 1972; Labadie 1976; Queirós, 1980; Luceño and Castroviejo 1993), is in agreement with relatively rapid range expansion in contrast to linear biogeographic patterns found in other coastal species (Clausing et al. 2000; Kadereit et al. 2005).

Carex distans s.l. is a morphologically complex taxon distributed from C and W Asia to the W Mediterranean Basin. Although no chromosome counts have been obtained from Asian populations, the great cytogenetic variability revealed by European and African numbers (Table 2) is in agreement with the taxon's considerable morphological variability and complexity. This variability is reflected by the lack of monophyly when analysing the ITS sequences. However, no coherent pattern can be described when analysing morphological, molecular and cytological data together. From a molecular point of view, *C. distans* population 1 from N Africa is the closest relative to population 6 from the central Iberian Peninsula, whereas chromosome numbers from both populations are extremely different ($2n = 70$ vs. 74).

Molecular and cytogenetic evolutionary rates

Interestingly, our results suggest that chromosome shift rate is higher than ITS substitution rate in the core Spirostachyae. There are well-documented examples in which different chromosome numbers and meiotic configurations occur in individuals of the same population of numerous species of *Carex* (Faulkner 1972; Schmid 1982; Cayouette and Morisset 1985). In fact, even different configurations and, rarely, different chromosome numbers have been observed in the same individual (Table 2; Schmid 1982; Luceño 1992a). Surprisingly, this extremely high chromosomal variability is not correlated with ITS variation, at least not in

our dataset. This can be inferred from cytogenetically well-studied species (*C. distans*, *C. mairii*, *C. troodi*, and species of sect. *Ceratocystis*) exhibiting very similar ITS sequences but several different chromosome numbers as well as meiotic configurations (Table 2; Schmid 1982; Luceño and Castroviejo 1993). We are not aware of any flowering plant displaying such an unbalanced degree of ITS nucleotide stability in contrast to high levels of chromosome shift within the same species.

Biogeographic patterns

Despite the lack of long-distance dispersal mechanisms in *Carex*, many species have reached remote areas such as the oceanic archipelagos of Galapagos, Hawaii, Macaronesia, and Tristan da Cunha. Numerous species or groups of closely related species exhibit wide and disjunct distributions in multiple cases, including bipolar (*C. canescens*, *C. magellanica*, *C. microglochin*; Rietz 1940), and amphiatlantic (*C. lepidocarpa*, *C. nigra*, *C. salina*; Hulten and Fries 1986) disjunctions. In recent decades an increasing interest in Cyperaceae dispersal has greatly contributed to descriptions of its mechanisms and syndromes (reviewed by Allessio Leck and Schütz 2005). These studies have revealed that almost every kind of dispersal syndrome is present in the genus (anemochory, autochory, endozoochory, epizoochory, hydrochory, and myrmecochory). Some syndromes are associated with special devices in utricles, achens and flowering stems (inflated or roundish utricles, limp flowering stems, coloured seeds, acute beaks, corky pericarps, and elaiosomes). In some other species of *Carex*, however, no correlation between syndrome and particular dispersal mechanism has been observed (Allessio Leck and Schütz 2005).

Species of the core Spirostachyae provide an ideal case for studying disjunctions and dispersability. Not only are wide disjunctions frequently found and shared by related species within this group, but also some areas were independently colonized by different groups of the species of the core Spirostachyae. Two independent dispersal events are inferred to account for the colonization of Oceania in the core Spirostachyae, because the two Southern Hemisphere species (*C. gunniana* from SE Australia, and *C. tasmanica* from Tasmania) are placed in different clades (A and B, respectively; Fig. 1). The same geographic pattern of distribution is found between two southern African species of the core Spirostachyae: *C. aethiopica* placed in clade A, and *C. burchelliana* in clade B.

One of the most striking cases of geographic separation is the amphiatlantic disjunction of two groups of Old World and South American species. In both groups the American taxa (*C. fuscula*, *C. vixdentata*) are placed in different subclades (A, B; Fig. 1), and clustered together with their respective morphological Old World congeners (*C. punctata*, *C. extensa*). The strong sister-group relationship of *C. fuscula*–*C. punctata* and *C. extensa*–*C. vixdentata*, together with independent placements in the phylogeny, lead us to suggest two long-distance colonization events. The most plausible explanation in both disjunctions is for the Old World to represent the area of origin, as the diversity centre of the section is located in Eurasia. If we assume this origin, two long-distance dispersal events may have taken place in the colonization of South America from the Mediterranean basin to account for the occurrence of *C. fuscula* and *C. vixdentata*. Afterwards, reproductive isolation due to geographic distance may have facilitated morphological and genetic divergence probably accelerating the speciation process, the chromosomes fusion mechanism being an important driving force in the evolution of *C. fuscula* (see "Cytogenetics of the core Spiostachyae" above). Multiple and independent colonization events connecting European and African with American floras have been documented for other amphiatlantic sedges (sect. *Acrocystis*; Roalson and Friar 2004a), in contrast to a single amphiatlantic colonization in other families (*Hypochaeris*; Tremetsberger et al. 2004).

Shorter but not less interesting disjunctions are those involving populations of the same species or closely related taxa on both sides of the Strait of Gibraltar. No more than 14 km long and 5 million years old, the Strait of Gibraltar has stimulated molecular evolutionary studies of its role in present-day differences and similarities between floras of S Iberia and N Morocco. To date, no general pattern can be established correlating genetic structure with reproductive characteristics in cases exhibiting such disjunction (Toumi and Lumaret 1998; Vargas et al. 1999; Fiz et al. 2002; Lumaret et al. 2002; Caujapé-Castells and Jansen 2003; Hampe et al. 2003; Burban and Petit 2003; Tremetsberger et al. 2004). Our results also show multiple patterns, even within a single group (sect. *Spirostachyae*) of species (*C. distans*, *C. helodes*, and *C. punctata*), with similar reproductive syndromes. For example, the ITS sequence of the Moroccan population of *C. punctata* (population 5) is identical to the ones obtained from European populations (1–4), which could be interpreted as evidence for recent origin of the *C. punctata* disjunction on both sides of the Strait of Gibraltar. A similar situation is found in *C. distans* s.l., in which the ITS sequence of the Moroccan population (1) is identical to that of the C Iberian Peninsula (population 6). An interesting situation is that of the restricted SW Iberian and Moroccan endemic *C. helodes*. This taxon, recently discovered in Morocco (Luceño and Escudero 2006), displays a geographic pattern of genetic distribution in which the Strait of Gibraltar seems to have acted as an effective barrier to gene flow. The two Moroccan

populations (2, 4) analysed are clustered in the same clade, which is supported by one nucleotide substitution (Fig. 1). This nucleotide substitution difference between Moroccan and Portuguese populations, though small, is not negligible given that 0(1) nucleotide changes were found at the infraspecific level in populations of 12 of the 14 species of the core Spirostachyae with more than one population analysed.

With respect to the Macaronesia colonization history, our results are not compatible with a single colonization event to Macaronesia. Instead we hypothesize three dispersal events: one involved in the colonization of Azorean islands, which gave rise to the endemic *C. hochstetteriana*; a different one to reach Madeira (*C. lowei*); and a third dispersal to the Canary Islands (*C. perrauderiana*). However, the unresolved placement of the three-species lineages in a basal polytomy impedes description of robust colonization patterns.

Acknowledgements

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