Taxonomía y sistemática de las secciones *Ceratocystis* y *Phacocystis* del género *Carex* en Europa y la cuenca mediterránea

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Taxonomía y sistemática de las secciones *Ceratocystis* y *Phacocystis* del género *Carex* en Europa y la cuenca Mediterránea

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A mi Familia,
que son mis Amigos.
A mis Amigos,
que son mi Familia.

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CAPÍTULO 1

Introducción

Motivación y objetivos

El género *Carex* L. (*Cyperaceae*), con unas 2000 especies, se cuenta entre los más diversos de entre los géneros de plantas vasculares no agamospérmicas (Reznicek, 1990). Debido a esta enorme diversificación se pueden identificar situaciones de divergencia muy diferentes entre los distintos linajes evolutivos que lo componen. Así, mientras existen algunos grupos evolutivamente aislados, con pocos táxones y bien caracterizados, en otros conjuntos de especies de diversificación más reciente, los límites taxonómicos están escasamente diferenciados. Es también por ello uno de los géneros más complejos desde el punto de vista taxonómico. A lo largo de la historia taxonómica del género, los distintos autores han considerado diferente número de táxones y, frecuentemente, bajo rangos taxonómicos dispares. La dificultad para la observación de los caracteres que permiten la discriminación de especies y subespecies, unido al polimorfismo que muestran no pocas especies, contribuye a acentuar aún más esta problemática.

El uso de técnicas moleculares y de computación ha permitido el acceso a inmensos y/o complejos conjuntos de datos, cuyo análisis permite obtener parámetros simplificadores de sorprendente exactitud estadística. De este modo podemos aproximarnos de un modo bastante más objetivo a diversas cuestiones biológicas que décadas antes eran aún objeto de discusiones especulativas. Ante semejante horizonte de contundentes perspectivas de proyección científica, el estudio de grupos o táxones modelo se revela de interés general, ya que conclusiones suficientemente sólidas pueden resultar extrapolables a casos similares.

Tenemos la impresión de que la taxonomía, catalogación y ordenación de los sistemas biológicos, es una ciencia en crisis. En un mundo cada vez más competitivo, una disciplina que resulta ser básica entre las ciencias no aplicadas, cobra cada vez menos interés. La sensación de estar cercanos al cierre del inventario de biodiversidad, si no a nivel planetario sí al menos en determinadas regiones, unida al creciente desarrollo de los modernos recursos científicos, hacen parecer a la taxonomía una consecuencia accesoria de estudios de mayor calado. Sin embargo, curiosamente, la inmensa mayoría de los estudios en biología requieren como unidad de trabajo al taxon, entidad que previamente habrá tenido que ser definida con exactitud por los taxónomos. El presente proyecto de tesis trabaja dentro del género *Carex* dos grupos problema, de

diversificación reciente, y con límites taxonómicos escasamente diferenciados: las secciones *Ceratocystis* Dumort. y *Phacocystis* Dumort (ver Figura 1). Pese a que son dos de los grupos mejor estudiados dentro del género, ambos están aún lejanos de una resolución sistemática y taxonómica definitiva. Ambos grupos presentan una serie de problemas comunes en la delimitación de sus miembros: 1- Los límites entre táxones están escasamente diferenciados, bien por la dificultad en la observación de los caracteres diagnósticos, o bien por una aparente variabilidad clinal de los mismos (Luceño 1994). 2- Son frecuentes los fenómenos de hibridación entre sus especies, lo cual dificulta aún más la delimitación precisa de las unidades taxonómicas, lo que a su vez retroalimenta el problema de la continuidad morfológica. 3- Las frecuentes confusiones entre los distintos miembros de estos grupos dificultan tremendamente el trabajo de revisión bibliográfica y oscurecen la definición de las especies, especialmente en lo que a su distribución y ecología se refiere. Autores norteamericanos y del norte y centro de Europa (Sylvén, 1963; Faulkner, 1972,1973; Schmid,1982; Cayouette & Morisset, 1985; Crins & Ball, 1988,1989a,b; Blackstock & Ashton, 2001,2010; Dean & Ashton, 2008; Dragon & Barrington, 2008,2009; Nakamatte & Lye, 2008,2010; entre otros) han trabajado estos grupos dentro de sus respectivos ámbitos geográficos. Sin embargo, su estudio sistemático en el sur de Europa, y la cuenca mediterránea, no ha sido abordado de modo extensivo, y estos táxones se han tratado desconectados, en general, de puntos de vista más integradores: unas veces con criterios sintéticos que consideran táxones en un sentido muy amplio, otras disgregando las especies en entes locales. Sin embargo, estudios biogeográficos basados en datos genéticos de numerosos grupos han establecido que en no pocas ocasiones el sur de Europa muestra valores de diferenciación y heterogeneidad diferentes a los de la Europa fría (Taberlet *et al.* 1998; Hewitt, 1999; Hampe & Petit, 2005; entre otros). Por ello la carencia de tratamientos exahustivos para las áreas meridionales de dos grupos taxonómicamente tan complejos puede estar ocultando parte de la diversidad subyacente. Mediante el uso de técnicas moleculares y de análisis informático hemos pretendido extraer conclusiones que, sin ser definitivas, resulten en el avance de un paso más en la resolución del árbol de la vida. El presente proyecto de tesis hace un énfasis especial en el margen meridional de la distribución de ambas secciones, y lo aborda de un modo integrador junto con los datos generales que ya se conocen. De este modo, los objetivos generales que se proponen son: (1) Aproximar las relaciones filogenéticas de ambas secciones mediante

el uso de marcadores moleculares. (2) Estudiar la estructura filogeográfica de aquellos grupos más complejos para inferir los procesos que han modelado su estructura taxonómica. (3) Reconsiderar del modo más objetivo posible los límites taxonómicos en los conjuntos de táxones que su estudio revele heterogéneos. (4) Proponer las reorganizaciones taxonómicas que se estimen necesarias para aproximar la clasificación a la historia evolutiva y hacer la interpretación de ésta más accesible.

Los objetivos concretos pueden establecerse como sigue:

1- Evaluar la estructura sistemática de las secciones *Ceratocystis* y *Phacocystis*, a raíz de las relaciones filogenéticas que se infieran en las mismas.
2- Estabilizar la taxonomía de la sección *Ceratocystis* en el sur de su área de distribución en el Viejo Mundo mediante una evaluación objetiva de las fronteras morfológicas basada en análisis estadísticos de caracteres cuantitativos: por un lado, abordar globalmente el extremo suroeste de su área de distribución; por el otro, estudiar un conjunto de formas enanas de alta montaña de morfología aparentemente homogénea.
3- Comparar las historias filogeográficas de los táxones considerados similares (*C. elata* All. y *C. reuteriana* Boiss., sección *Phacocystis*), pero de los que se estableció su heterogeneidad, y evaluar los elementos que han modelado dicha historia.
4- Realizar un estudio basado en técnicas de huella genética (*fingerprinting*) para elucidar la estructura taxonómica de un taxon mal delimitado y ampliamente distribuido (*C. nigra* (L.) Reichard s.l., sección *Phacocystis*), así como inferir su historia evolutiva reciente.
5- Revisar aquellos casos taxonómicos dentro de las secciones *Phacocystis* y *Ceratocystis* que se revelen heterogéneos y replantearlos en un contexto crítico, así como llevar a cabo los reajustes taxonómicos que se estimen necesarios.

Estructura de la memoria de tesis doctoral

Para la consecución de los objetivos propuestos se ha estructurado la memoria de tesis en 9 capítulos divididos a su vez en cuatro bloques bien diferenciados: una introducción general, un segundo bloque dedicado a la sección *Ceratocystis*, el siguiente

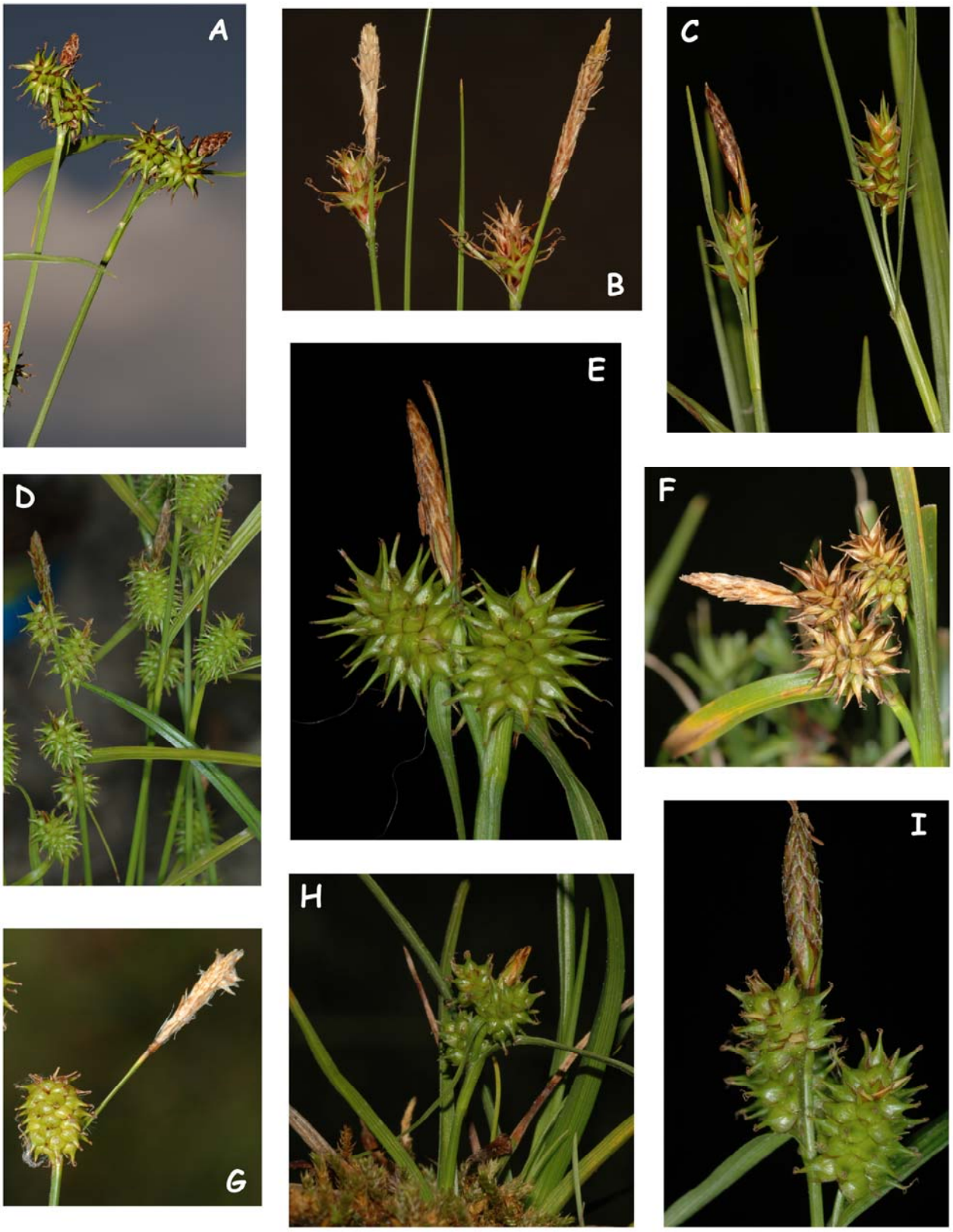
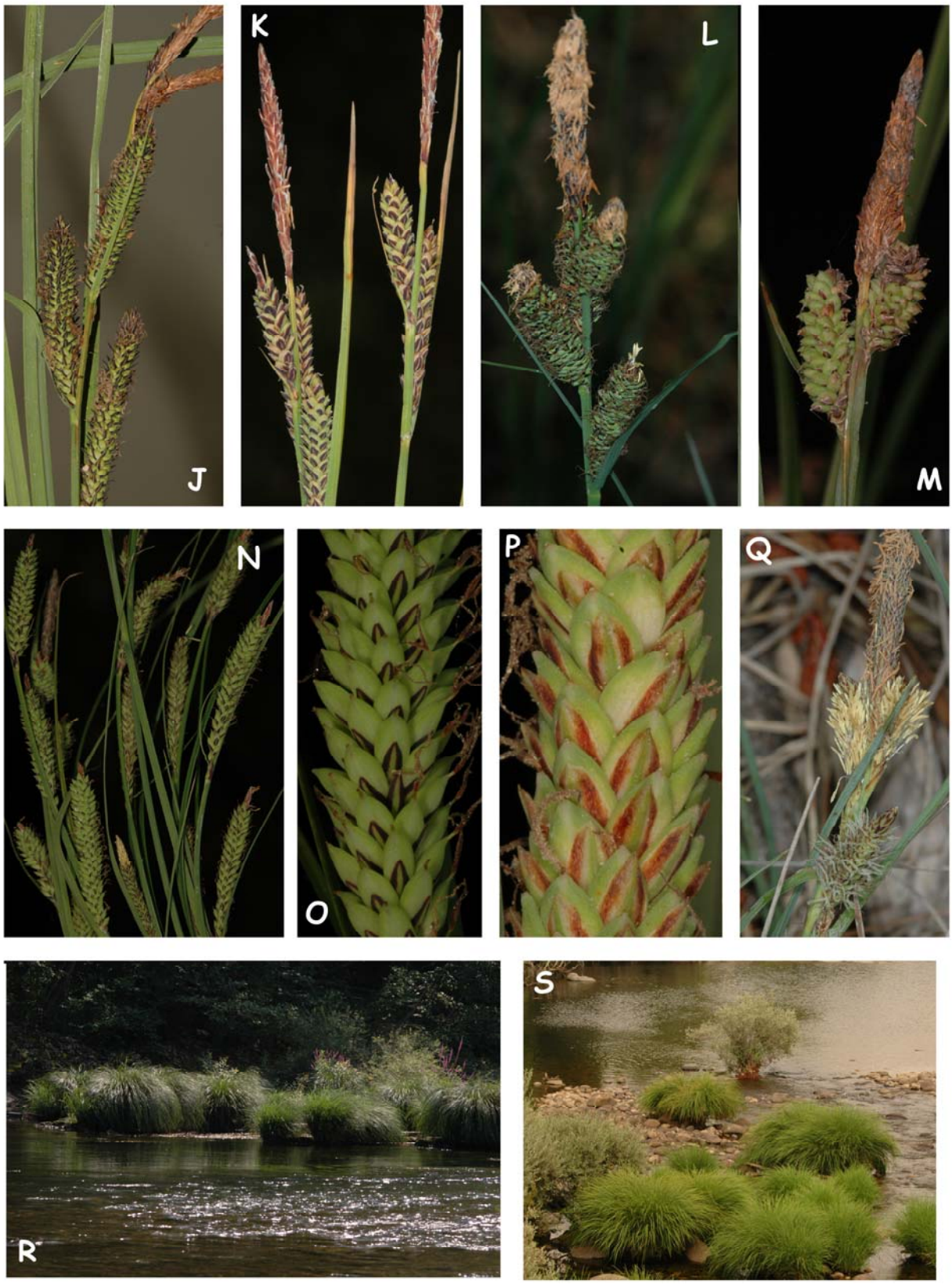


Figura 1. Representantes de las secciones *Ceratocystis* (**A-I**) y *Phacocystis* (**J-S**). **A.** *C. castroviejoi*, Épiro, Grecia; **B.** *C. durieui*, Pontevedra, España; **C.** *C. hostiana*, Highlands, Escocia; **D.** *C. demissa* × *C. viridula*, Alcocer do Sal, Portugal; **E.** *C. flava*, Troms, Noruega; **F.** *C. demissa* × *C. lepidocarpa*, Pirineos orientales, Gerona, España; **G.** *C. lepidocarpa* subsp. *lepidocarpa*, Pirineos centrales, Huesca, España; **H.** *C. viridula*, Épiro, Grecia; **I.** *C. demissa*, Sistema Central, Ávila, España; **J.** *C. acuta*, Durmitor, Montenegro; **K.** *C. nigra*, Troms, Noruega; **L.** *C. elata*, Doñana, Huelva, España; **M.** *C. cespitosa*,



Pirineos occidentales, Navarra, España; **N**. *C. reuteriana* subsp. *reuteriana*, Coimbra, Portugal; **O.** *C. reuteriana* subsp. *reuteriana*, Coimbra, Portugal, detalle de la espiga femenina; **P.** *C. trinervis*, Doñana, Huelva, España, detalle de la espiga femenina; **Q.** *C. trinervis*, Figueira-da-Foz, Portugal; **R.** Población de *C. cespitosa* en el río Bidasoa, Pirineos occidentales, Navarra, España; **S.** Población de *C. reuteriana* subsp. *reuteriana* en el Sistema Central, Ávila, España.

centrado en la sección *Phacocystis*, y una discusión final donde se tratan los resultados obtenidos en conjunto desde un punto de vista evolutivo, taxonómico y sistemático.

En el presente capítulo (Capítulo 1) se incluye una aproximación al conocimiento de las secciones *Ceratocystis* y *Phacocystis*. Para ello se han recopilado los antecedentes citogenéticos y moleculares, así como las aproximaciones taxonómicas previas. En el capítulo 2 se presenta el primer estudio de amplio espectro acerca de en las relaciones filogenéticas dentro de la sección *Ceratocystis* (objetivo 1) mediante el uso de secuencias de ADN (ITS, nuclear; *5'-trnK* y *rps16*, plastidiales). Para complementar los datos moleculares obtenidos, también se incluye un estudio citogenético adicional centrado en la Península Ibérica, una de las áreas taxonómicamente más problemáticas. Los capítulos 3 y 4 consisten en sendos estudios de carácter morfométrico. El primero de ellos reevalúa las fronteras morfológicas dentro de la sección *Ceratocystis* en la Península Ibérica, norte de África y Macaronesia, cuadrante geográfico donde los tratamientos de dicha sección han sido marcadamente discordantes (objetivos 2 y 5); en otros términos: sobre los resultados biosistemáticos del capítulo 2, se establece una base estadística objetiva a partir de la cual se propone una clasificación más estable y duradera. Por otro lado, el capítulo 4 aborda un grupo geográficamente heterogéneo, pero de tal similitud morfológica que no pocos autores han tratado poblaciones dispares como conespecíficas (objetivos 2 y 5). El uso conjunto de filiación taxonómica mediante el uso de las secuencias de ADN plastidial y análisis morfométricos basados en caracteres macro y micromorfológicos, permite caracterizar la heterogeneidad entre las poblaciones de dicho grupo. El capítulo 5, el primero que trata la sección *Phacocystis*, desarrolla un estudio basado en AFLPs y los marcadores plastidiales *ycf6-psbM* y *rpl32-trnL*^{UAG} sobre un grupo de plantas previamente consideradas afines: *C. elata* y *C. reuteriana* (objetivos 3 y 5). El estudio comparado de ambas entidades permite la comparación de historias evolutivas recientes con condicionantes similares. El capítulo 6 estudia las relaciones filogeográficas, cohesión taxonómica y diferenciación dentro de *C. nigra*, un taxon de amplia distribución y notable variación morfológica y ecológica (objetivo 4). Para ello se usaron también AFLPs y secuencias plastidiales de *ycf6-psbM* y *rpl32-trnL*^{UAG}. Se realizó una comparación entre las distintas zonas de su área de distribución, con especial hincapié en las diferencias entre los límites norte y sur. El capítulo 7 aproxima la estructura sistemática de los representantes euroasiáticos que previamente han sido dados como un grupo monofilético (objetivo 1). Para ello se usaron dos marcadores nucleares (ITS y

ETS) y dos plastidiales (*ycf6-psbM* y *rpl32-trnL*^{UAG}). Los capítulos 8 y 9 aúnan algunas de las principales modificaciones taxonómicas de la sección *Phacocystis* fruto de los estudios genéticos y de la revisión de materiales de herbario (objetivo 5). Se presenta en concreto la reevaluación de poblaciones de *C. acuta s.l.* del Mediterráneo y su consideración como tres táxones diferentes: *C. acuta s.s.*, *C. kurdica* Hand.-Mazz. y *C. panormitana* Guss (capítulo 8), y la revisión de la distribución de *C. cespitosa* en el oeste y sur de su área, mal conocida por las confusiones taxonómicas (capítulo 9). Finalmente, en el capítulo 10 se aborda una discusión general con base en los resultados obtenidos, sobre delimitación taxonómica en un contexto sistemático. Se incluyen además 3 apéndices relacionados con la presente tesis doctoral: 1- el tratamiento taxonómico de las secciones *Phacocystis* y *Ceratocystis* del género *Carex* para la obra *Flora Iberica*; 2- la descripción formal de *C. castroviejoi* Luceño & Jim.-Mejías, una especie nueva del este del Mediterráneo; y 3- contribuciones de carácter local a la corología de algunos táxones estudiados.

Antecedentes

EL GÉNERO CAREX *L.*

- Taxonomía

Como ya se ha citado, el género *Carex* es con ca. 2000 sp. (Reznicek, 1990) el de mayor diversificación entre las angiospermas. Su distribución es cosmopolita, aunque apenas habita en las regiones desérticas, y no se conoce de la Amazonía ni de la Antártida; no obstante, la mayor parte de sus especies se distribuyen por las regiones frías y templadas del hemisferio norte. Se engloba en la tribu *Cariceae*, junto con los géneros *Kobresia* Willd., *Schoenoxiphium* Nees y *Uncinia* Pers.

El primer tratamiento global del género se debe a Kükenthal (1909) quien circunscribió 793 especies en cuatro subgéneros: *Psyllophora* (Degl.) Peterm., *Vignea* (Lestib.f.) Peterm., *Vigneastra* (Tuck.) Kükenth. y *Carex* (nomenclatura corregida siguiendo a Egorova, 1999). Las secciones *Ceratocystis* y *Phacocystis* fueron incluídas en el subgénero tipo, que está caracterizado por inflorescencias provistas de brácteas foliáceas, más raramente setáceas, con las espigas pedunculadas, provistas de un profilo

basal, las superiores por lo general enteramente masculinas y las inferiores femeninas o andróginas (Egorova 1999; Starr & Ford 2009).

- Citogenética

El género *Carex*, junto con otros géneros de ciperáceas, se caracteriza por ciertas peculiaridades citogenéticas de interés evolutivo. Entre ellas destaca la presencia de cromosomas holocéntricos, que favorecen la formación de series aneuploides (*s.l.*) mediante fisión (agmatoploidía; Malheiros & Gardé 1950, Davies 1956) o fusión de cromosomas (simploidía; Luceño & Guerra 1996), así como reorganizaciones de los mismos (Greilhuber 1995). La amplia variación citogenética dentro del género no siempre se ajusta al mismo modelo, sino que está en función del grupo taxonómico. De hecho se han demostrado distintas tendencias evolutivas en las secciones *Ovales* (Hipp 2007) y *Spirostachyae* (Escudero *et al.*, 2010).

- Sistemática molecular

El conocimiento de la sistemática del género *Carex* ha cambiado enormemente debido al uso generalizado de las técnicas basadas en marcadores moleculares. Los últimos trabajos que abordan globalmente el género (Starr & Ford, 2009; Waterway *et al.*, 2009; ver figura 2) rechazan la ordenación tradicional del género en cuatro subgéneros desde que la mayoría de estos grupos se han demostrado no monofiléticos y se entremezclan con los otros géneros de cariceas. En el caso del subgénero *Carex* su configuración como grupo natural requiere la inclusión de la mayoría de las especies de *Vigneastra*, así como la exclusión de la sección *Gynobasidae* (Gerkhe *et al.*, 2010). Por otro lado, las relaciones entre las secciones ya incluidas en el subgénero están relativamente bien establecidas, y se han determinado la mayoría de los casos de polifilia o parafilia (cf. Waterway *et al.*, 2009), por lo que el proceso de reestructuración taxonómica y nomenclatural se encuentra en un estado bastante avanzado.

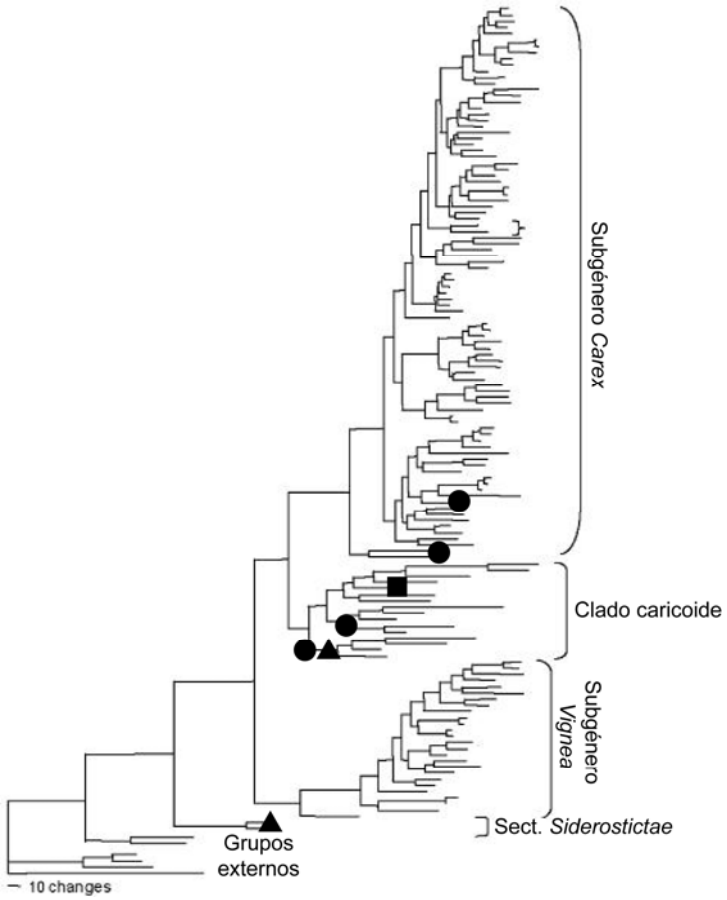


Figura 2. Árbol filogenético extraído de Waterway *et al.* (2009): análisis de máxima parsimonia de las regiones combinadas ITS, ETS, *trnL* y *trnL-trnF* de 140 especies. Se indican los principales linajes de la tribu Cariceae. Bajo la denominación de "clado caricoide" se designa el clado que amalgama los géneros *Kobresia*, *Schoenoxiphium*, *Uncinia* y representantes del subgénero *Psyllophora* del género *Carex*. La ubicación de los representantes del subgénero *Vigneastra* se indica con un círculo. La ubicación de linajes aislados del subgénero *Carex* se indican con un triángulo, y de representantes de *Vignea* con un cuadrado; según la figura se ubique en un terminal del árbol o en un nodo/rama se indica respectivamente si esas muestras se incluyeron en el análisis de Waterway *et al.*(2009) o si su posición sistemática fue revelada en otros trabajos.

CAREX SECT. *CERATOCYSTIS* DUMORT.

- Taxonomía

La sección *Ceratocystis* se incluye en el subgénero *Carex*. Se trata de un grupo pequeño dentro del género, pero de taxonomía muy compleja debido a los frecuentes fenómenos de hibridación y a que los caracteres taxonómicos son poco evidentes. Así, el número de especies varía entre 5 y 18 según los autores (Tabla 1). Se distribuye

Tabla 1. Esquema taxonómico de las tendencias sintéticas y analíticas más aceptadas que abordan el tratamiento global de la sección *Ceratocystis*

Tratamiento sintético [modificado de Schmid (1983) y Crins & Ball (1989b)]	**Tratamiento analítico** [modificado de Nelmes (1955), Chater (1980), Kukkonen (1998), Egorova (1999, 2000), Crins (2002) y Derieg *et al.* (2008)]	**Distribución geográfica**	
C. durieui Steud.	*C. durieui* Steud.	NO de la Península Ibérica	Grupo de *C. flava*
C. hostiana DC.	*C. hostiana* DC.	Europa y E de Norteamérica	
C. flava L.	*C. flava* L.	Europa, Asia Menor y C y E de Norteamérica	
	var. *alpina* Kneuck.	C Europa (Alpes)	
C. lutea LeBlond	*C. lutea* LeBlond	E de Norteamérica (N Carolina)	
C. cryptolepis Mack.	*C. cryptolepis* Mack.	C y E de Norteamérica	
	C. sp. [Derieg *et al.* 2008]	E de Norteamérica (Ohio)	
C. viridula Michx. subsp. *viridula*	*C. viridula* Michx. (=*C. serotina* Mérat; *C. oederi* auct.)	Áreas templadas y subárticas del hemisferio norte	Agregado de *C. viridula*
var. *pulchella* (Lönnr.) B. Schmid	subsp. *pulchella* (Löonr.) Malyschev		
var. *bergrothii* (Palmgr.) B. Schmid	*C. bergrothii* Palmgr.		
	C. philocrena V.I. Krecz.		
subsp. *oedocarpa* (Andersson) B. Schmid	*C. demissa* Hornem.	Europa, N de África y E de Norteamérica	
	subsp. *iranica* Kukonnen	Asia Menor	
	[*C. tumidicarpa*] subsp. *cedercreutzii* Fagerstr.	Azores	
subsp. *brachyrhyncha* (Čelak.) B. Schmid	*C. lepidocarpa* Tausch.	Europa, N de África y NE de Norteamérica	
	subsp. *jemtlandica* Palmgr.	N de Europa	
	C. castroviejoi Luceño & Jim.-Mejías	N de Grecia y Albania	
var. *nevadensis* (Boiss. & Reuter) Crins	*C. nevadensis* Boiss. & Reuter	SE de España	
var. *saxilittoralis* (Robertson) Crins	*C. saxilittoralis* A. Robertson	E de Canadá	
	C. barrosii Nelmes	S de Sudamérica	Táxones del hemisferio sur
	C. cataractae R.Br.	Tasmania y Nueva Zelanda	
	C. flaviformis Nelmes	Nueva Zelanda	
	C. monotropa Nelmes	Sudáfrica	

ampliamente por la Eurasia templada, Norte de África y Norteamérica (Schmid 1983; Crins 2002), con cuatro táxones disyuntos en Sudamérica, Sudáfrica, Tasmania y Nueva Zelanda (Nelmes, 1955) (Figura 3). Todas las especies habitan en suelos húmedos. Ha sido tradicionalmente considerada próxima a la sección *Spirostachyae* Drejer ex Bailey (Crins & Ball, 1988; Luceño & Castroviejo, 1993; Egorova, 1999), donde a veces ha sido incluida (Kükenhal, 1909; Vicioso, 1959).

Los principales caracteres que definen la sección *Ceratocystis* son: 1) rizomas con entrenudos más o menos cortos, 2) bráctea inferior foliácea a setácea, envainante, 3) espigas femeninas de cortamente cilíndricas a subglobosas, 4) tres estigmas, 5) utrículos lisos, atenuados en un conspicuo pico bífido, 6) aquenios obovados y 7) células epidérmicas del aquenio con un cuerpo silíceo central cónico, generalmente rodeado por varios cuerpos satélite de menor tamaño (Crins & Ball 1988; Crins 2002; Luceño & Jiménez-Mejías 2008) (ver Figura 4).

La sección fue dividida formalmente en tres subsecciones por Egorova (1999): subsect. *Hostianae* Egor., monotípica (*C. hostiana*); subsect. *Flavae* Carey, que contendría las especies con el pico de utrículo curvado; y subsect. *Serotinae* Egor., que englobaría las especies con el pico del utrículo recto. Entre los táxones que Egorova considera dentro de la sección *Ceratocystis*, no se pronuncia sobre el emplazamiento subseccional del endemismo ibérico *C. durieui*, y del taxon norteamericano *C. saxilittoralis*.

Debido a los problemas de delimitación taxonómica que presenta, y ante una falta de consenso unánime, los autores suelen referirse a los táxones de la sección *Ceratocystis* –con la excepción de *C. durieui* y *C. hostiana*– como el "grupo de *C. flava*" (Schmid, 1983; Crins & Ball, 1988; Stoeva & Štěpánková, 1990; Pykälä & Toivonen, 1994; Hedrén, 2002; Luceño & Jiménez Mejías, 2008). A grandes rasgos se podrían definir dos grandes tendencias taxonómicas seguidas por la mayoría de autores a la hora de abordar el "grupo de *C. flava*": 1- Un concepto sintético (Schmid, 1983; Crins & Ball, 1989b), que distinguiría dos especies biológicas, *C. flava* s.s. y *C. viridula* en su más amplia circunscripción, englobando el resto de los componentes del grupo. Ello vendría apoyado por la discontinuidad citogenética encontrada entre los dos grupos de táxones, que dificultaría los fenómenos de hibridación entre ellos (ver el apartado de citogenética); 2- Una visión analítica, que considera aquellos táxones más ampliamente aceptados como "buenas" especies morfológicas. Este último criterio ha provocado que diferentes autores hayan considerado tratamientos más o menos diferentes (eg., Chater,

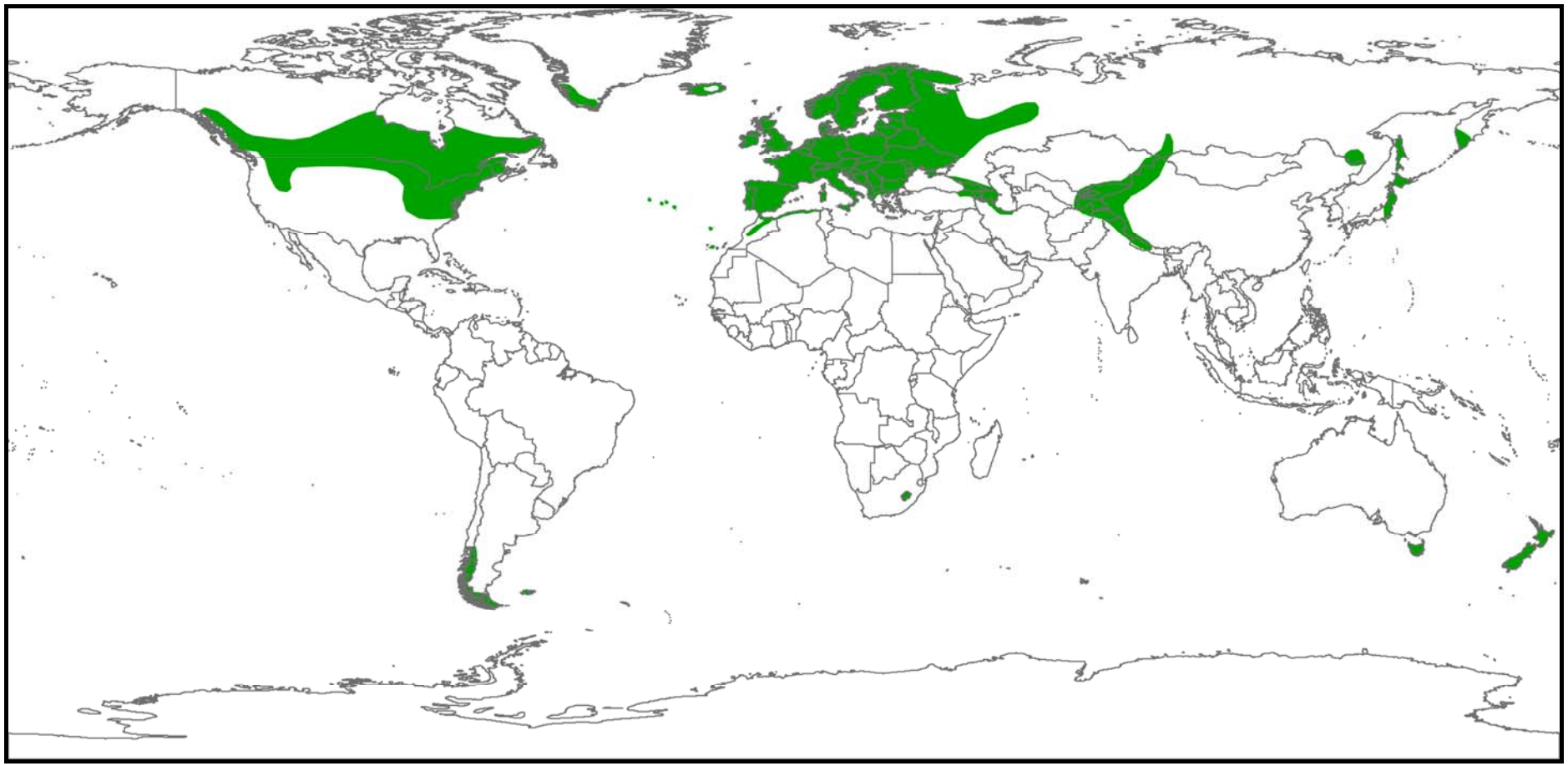


Figura 3. Distribución global aproximada de la sección *Ceratocystis* del género *Carex*.

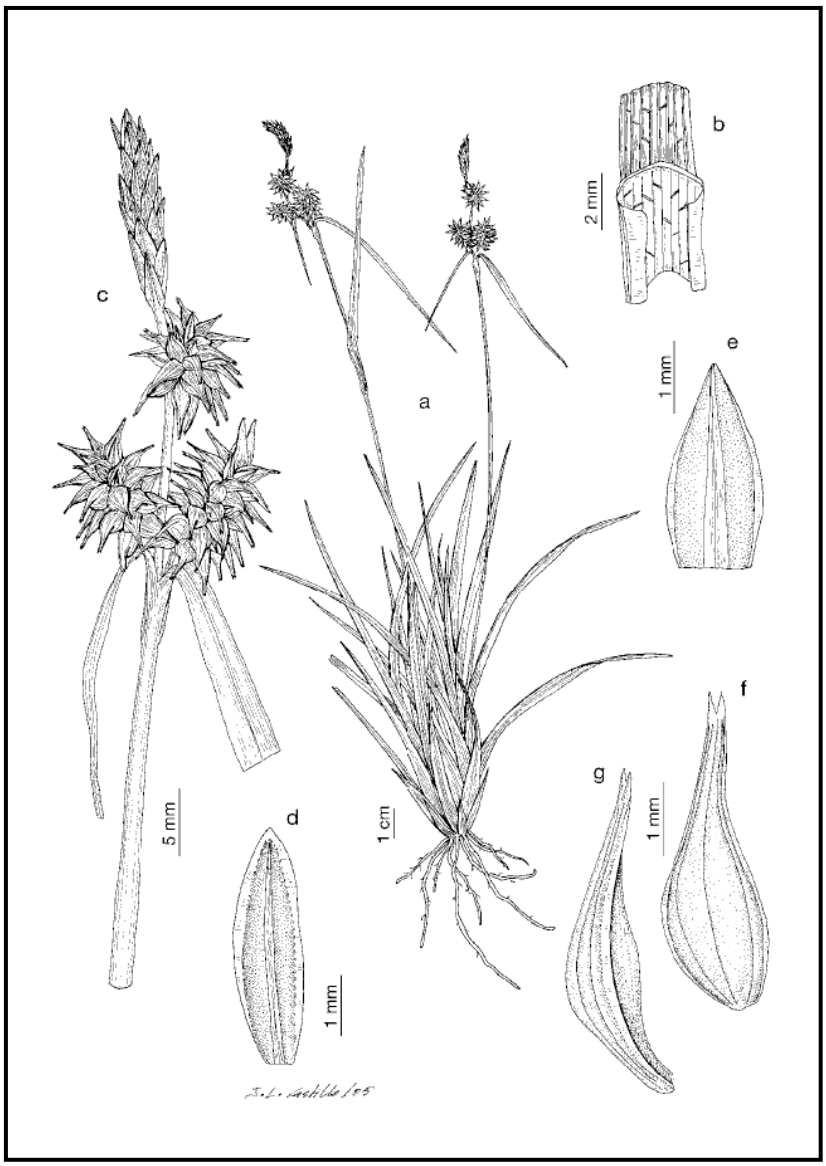


Figura 4. *Carex demissa* × *C. lepidocarpa* (Queralbs, Núria, Gerona, Pirineos): **a.** Porte. **b.** Lígula. **c.** Inflorescencia. **d.** Gluma masculina. **e.** Gluma femenina. **f.** Utrículo, cara abaxial. **g.** Utrículo, vista lateral.

1980; Egorova, 1999). Los apoyos en que se basan los criterios analíticos hay que buscarlos en la diferenciación morfológica de los extremos, diferencias en la distribución geográfica, requerimientos ecológicos y, más recientemente, en estudios de alozimas (Pykälä & Toivonen, 1994; Hedrén, 2004; ver el apartado de sistemática molecular).

La inestabilidad taxonómica de la sección se refleja, además, en la existencia de una serie de morfotipos problemáticos de estatus controvertido. Siguiendo la tendencia general en los estudios taxonómicos de estos grupos (ver motivación y objetivos), se trata de poblaciones meridionales en el continente, a las que los estudios monográficos de investigadores norteamericanos y norte y centroeuropeos no han accedido en profundidad. Por un lado las poblaciones de plantas de montaña, formas enanas muy semejantes entre cordilleras diferentes, han dado origen a numerosas confusiones. Así,

Chater (1980) en *Flora Europaea* las aunó todas bajo un concepto amplio de *C. nevadensis*, mientras otros (Maire, 1957; Vicioso, 1959) reservaron ese nombre para plantas ibéricas y norteafricanas, refiriéndose a las formas de los Alpes como *C. flava* var. *alpina* (Schmid, 1982). Finalmente, *Carex tumidicarpa* subsp. *cedercreutzii* (Fagerström, 1967) es un taxon descrito de Azores cuya reducida distribución no ha impedido su controversia. Mientras Schmid (1983) lo mantiene como subespecie de *C. viridula*, Crins & Ball (1989a,b) no le consideran estatus alguno y lo relegan como parte de la variación de *C. demissa*. Otros autores macaronésicos (León-Arencibia *et al.*, 1990; Press & Short, 1994) admiten su autonomía taxonómica y consideran que las poblaciones de Madeira y Canarias deben incluirse en dicha raza.

- Citogenética

En la sección *Ceratocystis* se ha propuesto que la fisión de cromosomas es el mecanismo más importante de evolución citogenética (Schmid 1982; Crins & Ball 1988), de modo similar a lo propuesto para la cercana sección *Spirostachyae* (Luceño & Castroviejo 1993), por lo que el grupo constituiría una serie agmatoploide. De este modo, los números cromosómicos más bajos corresponderían a estadíos más primitivos en la evolución de la sección (*C. durieui*, 2n = 52, 53; *C. hostiana*, 2n = 56; *C. flava* s.s., 2n = 58,60), mientras que los números más altos caracterizarían a los táxones más derivados [el llamado "agregado de *C. viridula*" (*C. demissa, C. lepidocarpa* y *C. viridula* s.s.), 2n = 66, 68, 70, 72] (Schmid, 1982; Halkka *et al.*, 1992; Luceño & Castroviejo, 1993; Roalson, 2008). Este patrón citogenético fue el apoyo principal para la organización sintética del grupo de *C. flava* en dos especies biológicas (ver el apartado de taxonomía). Así, Schmid (1982) y Halkka *et al.* (1992) mostraron cómo los híbridos entre *C. flava ss* y los miembros del agregado de *C. viridula* desarrollaban meoisis irregulares y una baja fertilidad, mientras que los híbridos entre miembros del agregado de *C. viridula* carecían de tales irregularidades y eran considerablemente más fértiles.

- Sistemática molecular

La monofilia de la sección *Ceratocystis* ha sido demostrada mediante la inclusión de un muestreo representativo en varios estudios.

En la primera filogenia general del género basada en la región nuclear ribosómica ITS (Hendrichs *et al.* 2004), la sección constituyó un clado bien apoyado. Los resultados que obtuvieron apoyan la teoría de la sección como una serie aneuploide de tendencia creciente (ver apartado de citogenética): *C. hostiana* se situaba en posición basal al resto de la sección, y *C. flava* como taxon hermano al agregado de *C. viridula*. Como consecuencia, consideraron adecuado el tratamiento del grupo desde un punto de vista sintético y que *C. demissa* y *C. lepidocarpa* se trataran como subespecies de una *C. viridula* circunscrita en su sentido más amplio.

Las relaciones con las secciones cercanas también están bien establecidas. En filogenias con diversos marcadores nucleares y plastidiales (Escudero & Luceño., 2009; Waterway *et al.*, 2009) la sección *Ceratocystis* se ubica en un clado bien apoyado, aunque no bien resuelto, junto con las secciones *Rhynchocystis* Dumort., *Rostrales* Meinsh., *Spirostachyae* (incl. *Elatae* Kük.) y *Sylvaticae* Rouy, (Figura 5).

Derieg *et al.* (2008) abordaron el estudio de los endemismos norteamericanos de la sección (*C. cryptolepis* y *C. lutea*) con los marcadores nucleares ribosómicos ITS y ETS. Con la inclusión de otros miembros del grupo de *C. flava* como referencia, mostraron que *C. lutea* junto con una nueva especie del este de Norteamérica (aún no descrita) constituyen el grupo hermano al resto de los representantes incluidos de la sección. Sin embargo, no incluyeron *C. durieui* y *C. hostiana*, las especies que se consideran basales según la hipótesis de la sección como serie aneuploide (Crins & Ball, 1988).

Estudios basados en alozimas han abordado la cohesión de los diferentes táxones propuestos (Bruederle & Jensen 1991; Hedrén & Prentice, 1996; Hedrén, 2002), corroborando la independencia de *C. demissa*, *C. flava*, *C. lepidocarpa* y *C. viridula*, así como la naturaleza híbrida de intermedios morfológicos.

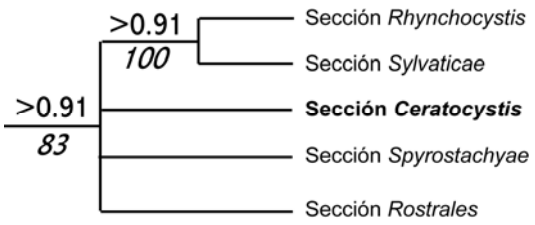


Figura 5. Cladograma simplificado mostrando la posición filogenética relativa de *Carex* sect. *Ceratocystis*, modificado de la filogenia de Waterway *et al.* (2009): análisis de parsimonia y de inferencia bayesiana de las regiones combinadas ITS, ETS, *trnL* y *trnL-trnF*. Sobre y bajo las ramas se muestran respectivamente los apoyos de probabilidad a posteriori bayesiana y *bootstrap*.

CAREX SECT. *PHACOCYSTIS* DUMORT.

- Taxonomía

En su circunscripción moderna, la sección *Phacocystis* (= *Acutae* Fries) es una de las mayores del subgénero *Carex*, con unas 90-130 especies según los distintos tratamientos. Si se considera aparte la sección *Praelongae* (Kük.) Nelmes (ver abajo), la sección *Phacocystis* se distribuye principalmente por las zonas templadas y frías del hemisferio norte, con especies disyuntas en Centro y Sudamérica, Nueva Guinea, sudeste de Australia y Nueva Zelanda (Figura 6). La mayoría de sus especies se encuentran ligadas a medios acuáticos o suelos muy húmedos. Algunas de ellas tienen una enorme importancia ecológica como formadoras de medios, como *C. nigra* en las turberas del *Caricion fuscae* de las montañas ibéricas, *C. elata* en las comunidades turbosas del *Magnocaricion elatae* o *C. reuteriana* en las comunidades de lechos de gargantas del *Caricion broterianae* (Rivas-Martínez *et al.*, 2001). Se trata de otra sección de taxonomía compleja, debido a los fenómenos ya citados para la sección *Ceratocystis*: hibridación frecuente y caracteres taxonómicos poco desarrollados.

Ha sido tradicionalmente asociada a la sección *Bicolores* Fries (cf. Kükenthal, 1909), debido a que ambas son las únicas del subgénero *Carex* con utrículos lenticular-biconvexos, a veces papilosos, con pico poco desarrollado o ausente, y dos estigmas. Sin embargo, los tratamientos más recientes, excluyen de modo inequívoco a *Bicolores* de su circunscripción.

Los principales caracteres que definen la sección *Phacocystis* son: 1) rizomas robustos que permiten la perduración vegetativa de la planta, de entrenudos largos (reptantes) o bien apretados formando densas macollas, 2) bráctea inferior no envainadora, frecuentemente con dos proyecciones membranosas (aurículas) en su base, 3) espigas femeninas cilíndricas, 4) dos estigmas, 5) utrículos lisos o papilosos, con pico muy corto y truncado, o sin pico, 6) aquenios lenticulares (Chater, 1980; Standley *et al.*, 2002; Luceño & Jiménez-Mejías 2008) (Figura 7).

La delimitación actualmente más aceptada para la sección *Phacocystis* incluye a la sección *Temnemis* (Rafin.) V.Krecz. [=*Cryptocarpae* (L.H. Bailey) Mackenzie] (Chater, 1980; Standley *et al.* 2002), que contiene entre otras a las especies halófilas (*C. paleacea* Wahlenb., *C. recta* Boott., *C. subspathacea* Hornem. y afines), un complejo de táxones de origen híbrido de distribución circumboreal. La cercana sección

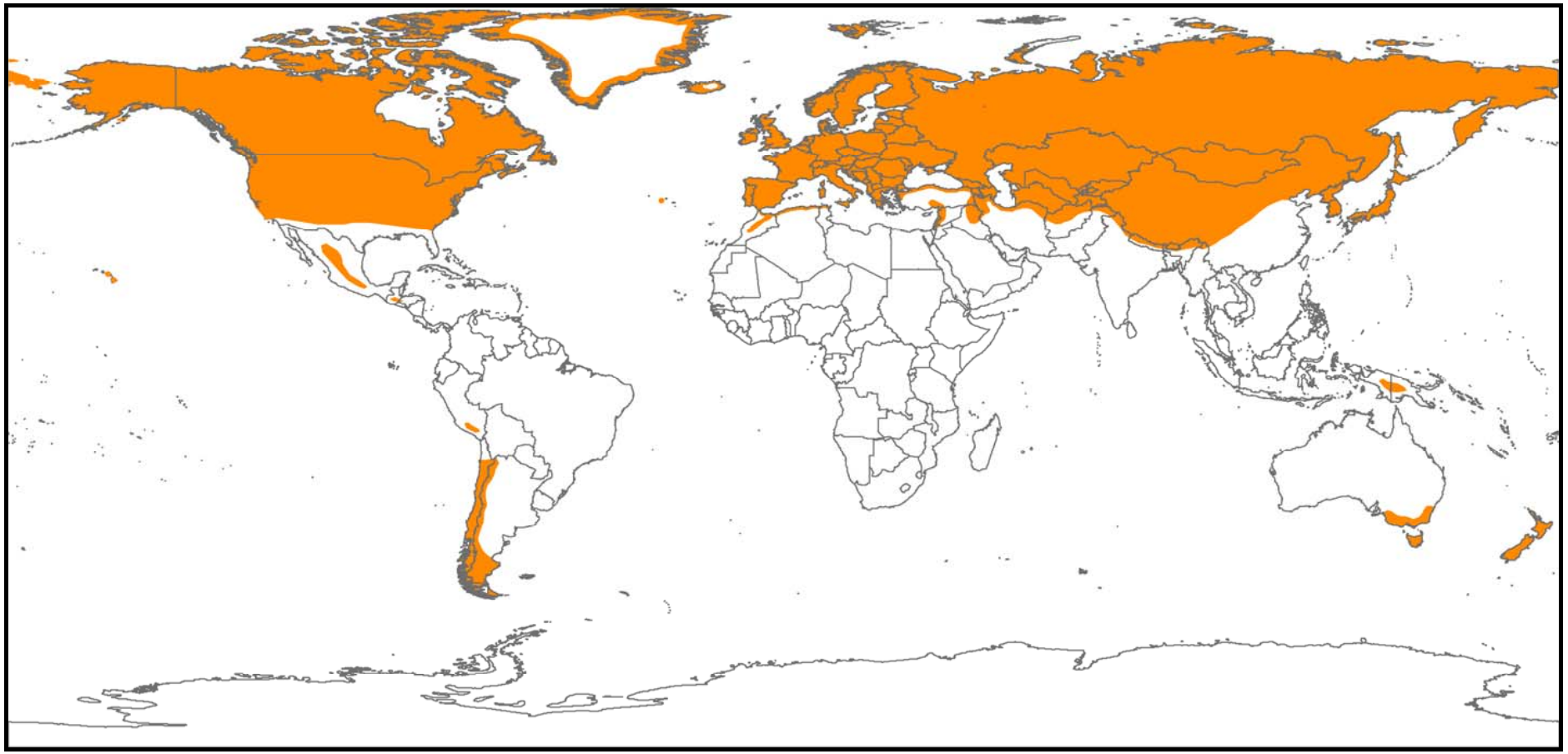


Figura 6. Distribución global aproximada de la sección *Phacocystis* del género *Carex* incluyendo la sección *Temnemis* y excluyendo *Praelongae*..

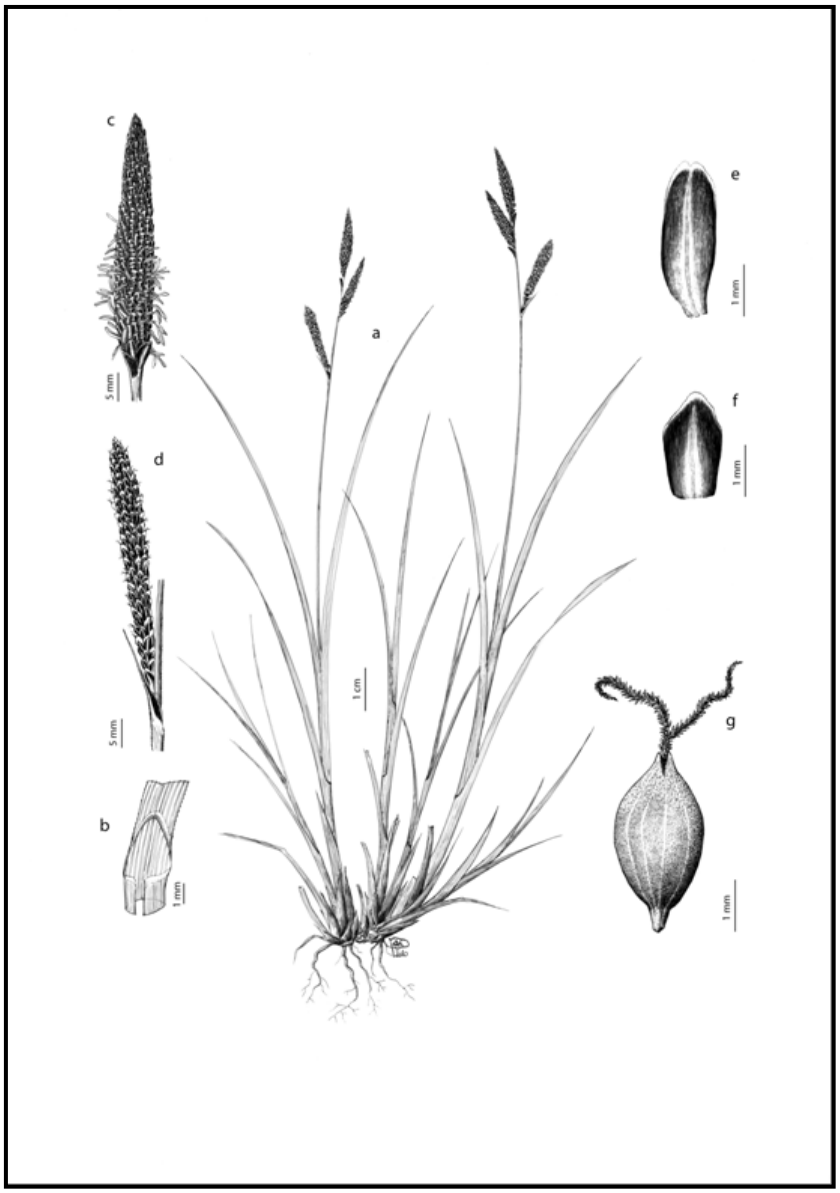


Figura 7. *Carex elata* subsp.*elata* (Cachopo, Tavira, Algarve, Portugal):
a. Porte. **b.** Lígula.
c. Espiga masculina.
d. Espiga femenina.
e. Gluma masculina.
f. Gluma femenina.
g. Utrículo.

Praelongae (Kük.) Nelmes suele ser incluida también en *Phacocystis* (Kükenthal, 1909; Standley *et al.* 2002), grupo fundamentalmente asiático y australasiático, con algunas especies tropicales y subtropicales en el África subsahariana. El tratamiento de Egorova (1999) considera las tres secciones dentro de un subgénero propio, *Kreczetoviczia* Egor., junto con las secciones *Abditispicae* Wheeler, *Forficulae* Raymond, *Graciles* (Kük.) Ohwi y *Tuminenses* Y.L.Chang & Y.L.Yang.

La partición subseccional de *Phacocystis* es igualmente problemática. En su acepción más restrictiva, la sección fue dividida por Egorova (1999) en 8 subsecciones para englobar las 22 especies incluidas en su monografía de la antigua URSS. Por otro lado, Kükenthal (1909), quien considera *Bicolores* Kük., *Forficulae* y *Praelongae* Kük. subsecciones dentro de *Phacocystis*, reparte los miembros integrados hoy en la sección

en 6 de las 7 subsecciones: *Bicolores* (p.p.), *Caespitosae* Fries., *Cryptocarpae* Kük., *Forficulae* Kük. (p.p.), *Rigidae* Kük. y *Vulgares* Aschers.

En Europa, el tratamiento más generalizado es el propuesto por Chater (1980), que considera 16 especies en el continente (ver Tabla 2). Sin embargo los distintos autores llegan a considerar hasta 5 táxones adicionales (especies y subespecies), y 2-3 especies más si tenemos en cuenta Asia Menor, N de África y el Cáucaso. La mayor parte de las diferencias se concentran en los tratamientos del complejo de *C. nigra*, y en las grandes cárices tipo *C. acuta*-*C. elata* en la zona más meridional del área de distribución. De nuevo, como ocurría en los tratamientos taxonómicos de la sección *Ceratocystis* (ver apartado correspondiente dentro de este mismo capítulo) se trata de aquellas poblaciones más meridionales dentro de su área, tratadas con menor profundidad en los estudios abordados por investigadores de zonas más septentrionales de Europa.

Dentro de *C. nigra*, el principal punto problemático se centra en la consideración de *C. juncella* Fr., taxon del Norte de Europa y Siberia, como especie autónoma (Sylvén, 1963; Egorova, 1999) o subordinada a *C. nigra* (Chater, 1980). La única diferencia clara entre ambas plantas es el hábito de crecimiento, con rizomas de entrenudos largos en *C. nigra*, y formando llamativas macollas en *C. juncella*. Sin embargo, estudios biosistemáticos ya demostraron la enorme similitud entre ambas formas, y propusieron su consideración como meros ecótipos (Faulkner, 1973). *Carex nigra* subsp. *intricata* (Tin.) Rivas Mart. (*Carex intricata* Tin.) es el nombre aplicado en tratamientos florísticos más o menos locales a formas enanas de alta montaña que crecen en Córcega, Sicilia, Sierra Nevada y el Norte de África (Maire, 1957; Vicioso, 1959; Pignatti, 1982; Chater, 1980). Otras formas de montaña, menos diferenciadas que las anteriores, se han agrupado bajo *C. nigra* subsp. *alpina* (Gaudin) Lemke (Norte y Centro de Europa; Chater, 1980), "*C. nigra*" subsp. *dacica* (Heuff.) Soó (Balcanes y Turquía (Chater, 1980; Nilsson, 1985); nombre hoy usado para formas de *C. bigelowii*; cf. Egorova, 1999), *C. transcaucasica* T.V.Egorova (Cáucaso; Egorova, 1999) y *C. nigra* subsp. *drukyuliensis* Noltie (Himalaya; Noltie, 1994).

Por otro lado, las grandes cárices de la sección *Phacocystis* han sido objeto de diversas confusiones en la zona más meridional de su área de distribución. A veces tratadas bajo *C. elata*, otras bajo *C. acuta*, y otras como táxones independientes, las poblaciones de la Península Ibérica, Norte de África, Cerdeña, Sicilia, los Balcanes, Turquía y Oriente Próximo se cuentan entre las de posicionamiento taxonómico más inestable. La heterogeneidad que subyace a este grupo de especies similares queda

Tabla 2. Principales tratamientos taxonómicos de la sección *Phacocystis* (excluidas las especies halófilas) en Europa, N de África y Asia Menor

Kükenthal (1909)	**Chater** (1980)	**Luceño & Aedo** (1994) y **Luceño & Jiménez Mejías** (2008)	**Egorova** (1999)	**Nilsson** (1985) y **Kukkonen** (1996)
C. panormitana Guss.	-	-	-	-
C. gracilis Curtis	*C. acuta* L.	*C. acuta*	*C. acuta*	*C. acuta*
		C. mauritanica Boiss.[3]	-	-
		C. elata subsp. *tartessiana* Luceño & Aedo	-	-
C. reuteriana Boiss.	*C. nigra* (L.) Reichard	*C. elata* subsp. *reuteriana* (Boiss.) Luceño	-	-
C. goodenoughii Gay		*C. nigra*	*C. nigra*	*C. nigra*
	subsp. *intricata* (Tineo) Maire[2]	-	subsp. *intricata*	-
	subsp. *alpina* (Gaudin) Lemke[2]	-	subsp. *alpina*	subsp. *alpina*
	subsp. *dacica* (Heuff.) Soó[2]	-	-	subsp. *dacica*
	var. *juncea* (Fries) Hyl.	-	*C. juncella* (Fries) Th.Fries	-
	-	-	*C. transcaucasica* Egor.	-
C. aquatilis Wahlenb.	*C. aquatilis*	-	*C. aquatilis*	-
	subsp. *stans* (Drej.) Hult.	-	subsp. *stans*	-
C. hudsonii A.Bennett	*C. elata* All.	*C. elata* subsp. *elata*	*C. elata*	*C. elata*
C. omskiana Meinsh.	subsp. *omskiana* (Meinsh.) Jalas	-	subsp. *omskiana*	subsp. *omskiana*
C. buekii Wimm.	*C. buekii*	-	*C. buekii*	-
C. cespitosa L.	*C. cespitosa*	*C. cespitosa*	*C. cespitosa*	*C. cespitosa*
C. rigida Good.	*C. bigelowii* Torr. ex Schwein	-	*C. bigelowii*	-
	subsp. *rigida* (Good.) Schultze.Motel	-	subsp. *dacica* (Heuff.) Egor.	-
	subsp. *ensifolia* (Gorodk.) Holub	-	subsp. *ensifolia*	-
	subsp. *arctisibirica* (Jurtz.)A&D.Löve	-	subsp. *arctisibirica*	-
C. orbicularis Boott	-	-	*C. orbicularis* subsp. *kotschyana* (Boiss & Hohen) Kukkonen	*C. orbicularis* subsp. *kotschyana*
C. rufina Drejer	*C. rufina*	-	*C. rufina*[3]	-
C. trinervis Degl.	*C. trinervis*	*C. trinervis*	*C. trinervis*[3]	-
C. kurdica Hand.Mazz.[1]	-	-	*C. kurdica*[2]	*C. kurdica*

[1]Descrita en 1914 como "*Kükenthal ex Handel-Mazzetti*"; [2]enumeradas como variedades sin pronunciarse acerca de su validez taxonómica; [3]No incluida en el tratamiento, pero explícitamente aceptada como especie

puesta de manifiesto con las reestructuraciones recientes que proponen los diversos autores. Así, para los Alpes orientales, en Austria, Alemania y Eslovenia, Wallnöfer (1992, 1993) describió recientemente una especie nueva, *C. randalpina* B. Wall., taxon que luego ha sido encontrado en el Noreste de Italia, Croacia y Hungría (Prosser, 1998; Stančić, 2009; Mesterházy, 2010). Para la Península Ibérica, los táxones hoy considerados como *C. elata* subsp. *reuteriana* y *C. elata* subsp. *tartessiana* han sido la mayor fuente de inestabilidad hasta el estudio biosistemático de Luceño & Aedo (1994). Distribuidas la primera en el noroeste y centro de la Península Ibérica, y la segunda en Andalucía, los autores anteriores las han considerado de forma muy variopinta. Así, Kükenthal (1909) consideró el rango específico para *C. reuteriana*, mientras la segunda la incluyó bajo *C. acuta*; por otro lado, Vicioso (1959) y Chater (1980) subordina ambas a *C. nigra* y *C. acuta* respectivamente. En el norte de África, *C. mauritanica* fue el nombre propuesto para poblaciones luego subordinadas a *C. acuta*. Sin embargo, estas últimas fueron consideradas por Luceño & Aedo (1994) como taxonómicamente independientes. Para Cerdeña y Sicilia los autores italianos admiten la existencia de un taxon endémico, *C. panormitana*, (Pignatti, 1980; Pignatti *et al.* 2001), que ha sido rechazado por autores más sintéticos (Chater, 1980). Finalmente, en Asia Menor, el taxon conocido como *C. kurdica* fue largamente olvidado hasta su recuperación en las obras *Flora of Turkey* (Nilsson, 1985) y *Flora Iranica* (Kukkonen, 1996).

- Citogenética

La primera aproximación citogenética que trató de un modo extensivo la sección *Phacocystis* en Europa fue llevada a cabo por Faulkner (1972). Los números euploides inferidos para cada especie permitieron la agrupación de los táxones de la sección en hasta cinco categorías (Faulkner, 1972; Luceño & Aedo, 1994): *C. bigelowii* (2n = 68-70), *C. paleacea* (2n = 72), *C. aquatilis* y *C. elata s.l.* (2n = 74-76; incl. *C. reuteriana*), *C. cespitosa* (2n = 78-80) y *C. acuta*, *C. nigra s.l.* y *C. trinervis* (2n = 84-85). Dentro de cada taxon, se asume como número euploide aquel encontrado con mayor frecuencia y cuyos cromosomas aparean en su totalidad durante la meiosis. Los números hipo o hiperploides se explican mediante procesos de aneuploidía s.l. Sin embargo, con base en diferencias morfológicas de los cromosomas, Faulkner (1972) avisó que especies con números similares no tendrían por qué tener un origen común, y admitió que la

condición ancestral o derivada de los diferentes números, y por tanto especies o grupos, era en aquel momento irresoluble.

Faulkner (1973) también demostró que cruces entre táxones con números cromosómicos similares resultan en valores de fertilidad mayor. Asimismo, consideró la irregularidad en el apareamiento de los cromosomas homólogos durante meiosis en tales híbridos como indicador de divergencia. De este modo, pese a su relativa fertilidad, las irregularidades detectadas en el cruce entre *C. nigra* y *C. acuta* le llevó a considerarlas especies diferentes. Por el contrario, fue el apareamiento regular encontrado en el cruce entre *C. nigra* y *C. juncella* lo que fundamentó su propuesta de tratar ambas plantas como conespecíficas (*C. nigra s.l.*). Dicha base experimental ha sido usada en otros trabajos como evidencia de hibridación en la sección *Phacocystis* (Cayouette & Morisset, 1985).

La revisión de los táxones ibéricos de la sección *Phacocystis* de Luceño & Aedo (1994) utilizó la información cariológica conocida hasta la fecha como un marcador que permitiera una asignación taxonómica menos equívoca. El resultado más novedoso fue la ruptura de los conceptos tradicionales que consideraban las poblaciones de *C. reuteriana* y las formas andaluzas llamadas *C. mauritanica* como afines a *C. nigra* y/o *C. acuta*. El número cromosomático de tales poblaciones (2n = 74-75(76)) resultó ser mucho más próximo a *C. elata* (2n = 76) que al mostrado en la Península Ibérica por *C. acuta* (2n = 84-86) o *C. nigra* (2n = 84-85). Por ello, ambos grupos de poblaciones fueron finalmente subordinadas bajo *C. elata* en el rango subespecífico: subsp. *reuteriana* y subsp. *tartessiana*. Este número más bajo obtenido para ambos táxones locales, en comparación con la ampliamente distribuida *C. elata s.s.* propició la hipótesis de un origen derivado de las subespecies *reuteriana* y *tartessiana* a partir de fenómenos de fusión cromosomática (Luceño & Aedo, 1994).

- Sistemática molecular

Las primeras aproximaciones moleculares mostraron problemas en la delimitación de la sección *Phacocystis*. El muestreo representativo incluido por Roalson *et al.* (2001) mostró que la sección *Phacocystis* es un grupo parafilético al situarse entre sus miembros *C. podocarpa* R.Br., especie de la sección *Scitae* Kük. Con un muestreo más amplio, el estudio basado en ITS de Hendrichs *et al.* (2004) rechazó distinción entre las secciones *Phacocystis* y *Temnemis*, y excluyó la sección *Bicolores,* que se configuró

como un grupo independiente. Los últimos estudios de Waterway *et al.* (2009) con las regiones nucleares ITS, ETS y la plastidial *trnL-F* constituyen la más completa aproximación a los grupos cercanos a la sección *Phacocystis*, que estaría relacionada con otras secciones de especies predominantemente acuáticas: *Glaucescentes*, *Limosae* y *Squarrosae*.

Los trabajos de Dragon & Barrington (2008 2009), basados en los marcadores nucleares ITS y ETS y el plastidial *psbA-trnH*, enfatizan en la monofilia y estructura interna de la sección *Phacocystis* (Figura 8). El problema de parafilia con la sección *Scitae* se muestra más complejo, ya que este grupo resulta ser polifilético, y anida al menos en dos posiciones dentro del núcleo de la sección *Phacocystis*. Por otro lado, los táxones de la sección *Praelongae* se ubican en las ramificaciones más basales del cladograma, lo que sitúa a los clados de *Phacocystis* como linajes derivados dentro de *Praelongae*. Estos resultados corroboran la heterogeneidad del grupo y dejan a día de hoy la sección aún no circunscrita, a la espera de muestreos masivos que proporcionen una visión más general que permita abordar la partición o reestructuración de este grupo. Si dejamos de lado la circunscripción de la sección y sus relaciones externas, lo que sí deja claro el trabajo de Dragon & Barrington es la organización de *Phacocystis* en cuatro grandes clados: 1- Un clado que engloba la mayoría de las especies americanas, junto con *C. aquatilis*, la anfiatlántica *C. rufina* y las especies de la sección *Temnemis*; 2- un segundo clado que agrupa a *C. bigelowii* y otros táxones relacionados; 3- un clado de distribución predominante en Australasia y Asia oriental; 4- y finalmente un clado que aparece formado por aquellos táxones del grupo predominantemente europeos.

Nakamatte & Lye (2007) abordaron en profundidad los táxones del norte de Europa mediante AFLPs, y los compararon con especies norteamericanas. Sus análisis revelaron un claro agrupamiento de la mayoría de las muestras conespecíficas mientras que demostraron la heterogeneidad de *C. aquatilis* y *C. bigelowii*. Las distintas especies exhibieron entre ellas un agrupamiento laxo, a excepción de los táxones halófilos (*C. subspathacea* y *C. paleacea*), las especies de la sección *Praelongae*, y *C. acuta* y *C. trinervis*, que se recuperaron todas en clados bien apoyados.

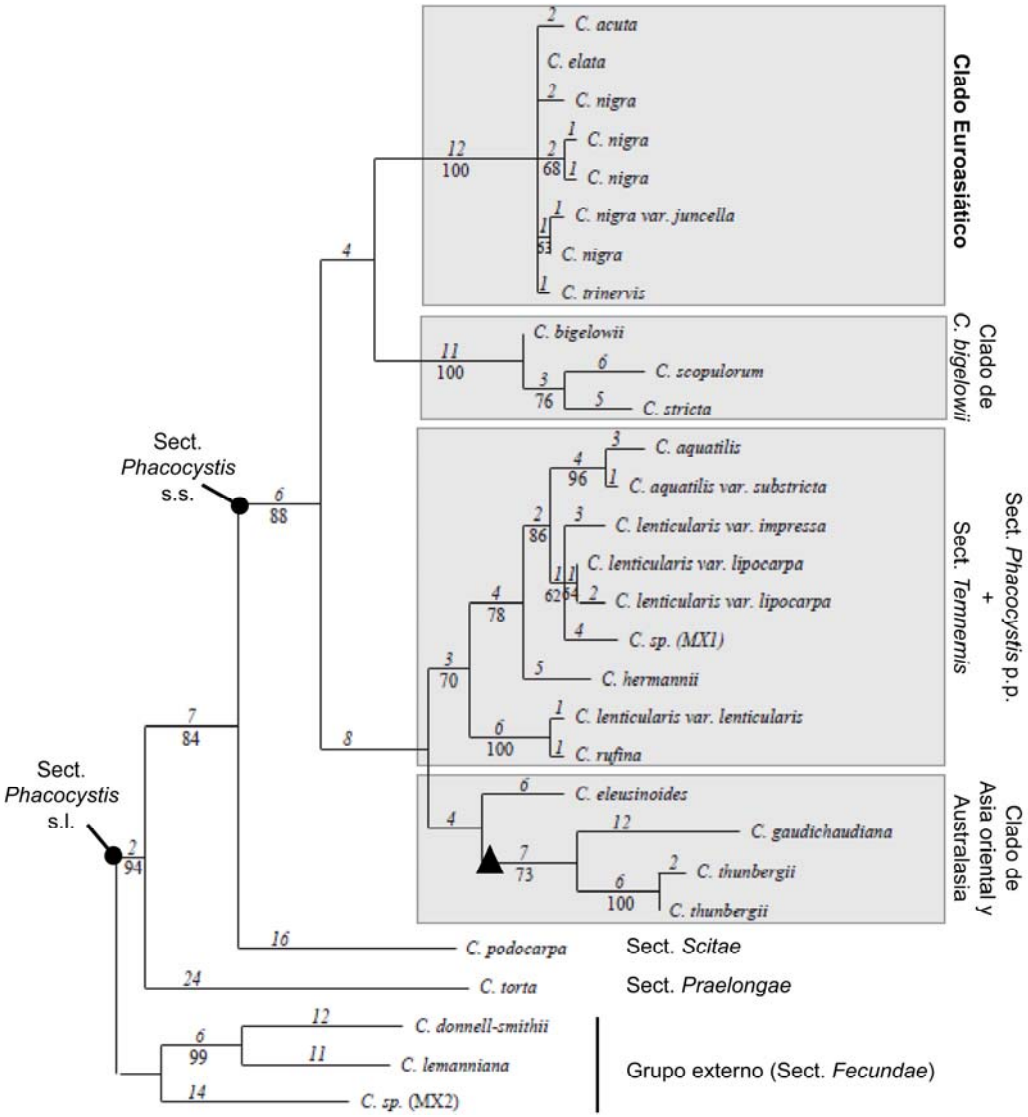


Figura 8. Árbol filogenético extraído de Dragon & Barrington (2008): análisis de máxima parsimonia de las regiones ITS y ETS de 41 muestras. Los números sobre las ramas indican longitud de rama, las cifra debajo apoyos >50% bs. El triángulo indica el clado al que se asocian los otros miembros de la sección *Scitae* en el trabajo de Dragon & Barrington (2009).

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CAPÍTULO 2

Systematics and taxonomy of *Carex* sect. *Ceratocystis* (Cyperaceae) in Europe: a molecular and cytogenetic approach*

P. Jiménez-Mejías, S. Martín-Bravo & M. Luceño

*Under review

Abstract

Carex sect. *Ceratocystis* is distributed in Eurasia and North America, with a few disjunct taxa in the Southern Hemisphere. Despite being one of the most intensively studied groups within *Carex*, its taxonomy remains a complex issue due to hybridization and faint morphological boundaries. Two main contrasting approaches to its taxonomy may be distinguished, synthetic and analytical, widely differing in the number of considered taxa. The status of several morphotypes from Europe and the Mediterranean Basin are particularly problematic. We used phylogenetic analyses of nuclear ITS and plastid *rps16* and *5'trnK* sequences along with cytogenetic data to evaluate the main taxonomic approaches and to infer evolutionary patterns in Europe and North Africa, with a especial focus in the problematic morphotypes. Three major clades were found, which mostly match morphological features of the utricle. *Carex durieui* should be excluded from section *Ceratocystis*. Although a linear agmatoploid series has been generally proposed to account for the cytogenetic evolution of section *Ceratocystis*, our results suggest chromosome number increase but not in a linear fashion. Different extensive hybridization areas in South Europe are suggested for some of the problematic morphotypes (Pyrenean-Cantabrian Mountains, Atlantic-Iberian Strip and Corsica).

Keywords. Aneuploid series, *Carex flava*, ITS polymorphisms, phylogeny, *rps16*, *5'trnK*

Introduction

Plant hybridization and reticulate evolution have been extensively studied and documented since the beginning of evolutionary studies in plants (e.g., Grant 1981; Rieseberg 1997; Hewitt 2001). From taxonomic intuition to molecular revolution, a wide range of biological techniques has been used for this purpose (e.g., Kükenthal 1909; Heiser et al. 1969; Faulkner 1973; Schmid 1982; Fuertes Aguilar et al. 1999a; Runyeon-Lager and Prentice 2000; Comes and Abbott 2001). Molecular techniques have provided highly valuable tools to evaluate the impact of hybridization and the role of genetic introgression in plant evolution. Tandemly repeated nuclear rDNA loci, such as the internal transcribed spacer (ITS), are especially useful markers to detect hybridization because different copies can coexist within a single individual (Baldwin et al. 1995). As a result, it is sometimes possible to identify the putative parental species involved in the hybridization event (Widmer and Baltisberger 1999). Phenomena such as additive polymorphism of single sites (Sang et al. 1995; Fuertes Aguilar and Nieto Feliner 2003; Albaladejo et al. 2005) and recombinant sequences that represent a mixture of parental sequences (Buckler et al. 1997; Álvarez and Wendel 2003) allow for the current or ancient coexistence of more than one ITS copy to be inferred. However, as a result of concerted evolution (Sang et al. 1995; Nieto Feliner et al. 2001, Calonje et al. 2009) or recurrent introgression with one of the parental taxa (Fuertes Aguilar and Nieto Feliner 2003), a copy very often becomes predominant and coexisting copies disappear, hindering the detection of hybridization processes (Álvarez and Wendel 2003). Plastid sequences can also help in the understanding of hybridization patterns when shared haplotypes among taxonomical units exist (e.g., Schaal et al. 1998; Comes and Abbott 2001; Gutiérrez Larena et al. 2002) or when incongruences between the nuclear-plastid phylogenies are found (Rieseberg 1997; Escudero and Luceño 2009; Martín-Bravo and Jiménez-Mejías 2009). In addition, when compared to molecular results, morphological and biogeographical evidence also allows for the detection of extensive interspecific gene flow (Fuertes Aguilar et al. 1999a; Nieto Feliner et al. 2001).

The genus *Carex* L. (*Cariceae*, Cyperaceae) is among the largest non-apomictic angiosperm genus, comprising approximately 2000 species (Reznicek 1990). Recent systematic studies have found frequent incongruences between molecular phylogenies

Table 1. Main approaches to the taxonomy of *Carex* sect. *Ceratocystis*.

Synthetic treatment [modified from Schmid (1983) and Crins & Ball (1989b)]		**Analytical treatment and ingroup definition**[1] [modified from Nelmes (1955), Chater (1980), Kukkonen (1998), Egorova (1999, 2000), Crins (2002), Derieg et al. (2008) and Jiménez-Mejías & Luceño (2009)]	**Geographical range**	
C. durieui Steud.		*C. durieui* Steud.*	NW Iberian Peninsula	*C. flava* group
C. hostiana DC.		*C. hostiana* DC.*	Europe, E North America	
C. flava L.		*C. flava* L.*	Europe, SW Asia, C and E North America	
		var. *alpina* Kneuck.[#]	C and S Europe (Alps)	
C. lutea LeBlond		*C. lutea* LeBlond*	E North America (N Carolina)	
C. cryptolepis Mack.		*C. cryptolepis* Mack.*	C and E North America	
		C. sp. (Derieg et al. 2008)*	E North America (Ohio)	
C. viridula Michx.	subsp. *viridula*	*C. viridula* Michx.* (=*C. serotina* Mérat; *C. oederi* auct.)	Temperate and Subarctic Northern Hemisphere	*C. viridula* aggregate
	var. *pulchella* (Lönnr.) B. Schmid	subsp. *pulchella* (Löonr.) Malyschev		
	var. *bergrothii* (Palmgr.) B. Schmid	*C. bergrothii* Palmgr.		
	-	*C. philocrena* V.I. Krecz.		
	subsp. *oedocarpa* (Andersson) B. Schmid	*C. demissa* Hornem.*	Europe, N Africa, E North America	
		[*C. tumidicarpa*] subsp. *cedercreutzii* Fagrestr.[#]	Azores	
		subsp. *iranica* Kukonnen	W Asia	
	subsp. *brachyrhyncha* (Čelak.) B. Schmid	*C. lepidocarpa* Tausch.*	Europe, NE North America, N Africa	
		subsp. *jemtlandica* Palmgr.*	N Europe	
		C. castroviejoi Luceño & Jiménez Mejías[#]	NW Greece (Pindhos range)	
	var. *nevadensis* (Boiss. & Reuter) Crins	*C. nevadensis* Boiss. & Reuter[#]	SE Spain (Sierra Nevada and Filabres)	
	var. *saxilittoralis* (Robertson) Crins	*C. saxilittoralis* A. Robertson	E Canada	
		C. barrosii Nelmes*	S South America	Southern Hemisphere Taxa
		C. cataractae R.Br.	Tasmania, New Zealand	
		C. flaviformis Nelmes*	New Zealand	
		C. monotropa Nelmes*	S Africa	
		Atlantic-Iberian plants[#] (*C. demissa? C. flava*?)	W Iberian Peninsula	Additional problematic morphotypes
		Atlasic plants[#] (*C. nevadensis*?)	N Africa (High Atlas)	
		Corsican plants[#] (*C. nevadensis*? *C. viridula*?)	Corsica	
		Pyrenean-Cantabrian plants[#] (*C. flava*? *C. nevadensis*?)	Pyrenees and Cantabrian Mountains	

[1]Note: Those well-defined taxa considered in our tentative classification for ingroup definition are marked with an asterisk (*), whereas those considered as problematic morphotypes are marked with hash (#)

and traditional infrageneric classifications based on morphology (Roalson et al. 2001; Hendrichs et al. 2004a, b; Starr et al. 2004; Hipp et al. 2006; Waterway and Starr 2007; Escudero and Luceño 2009; Starr and Ford 2009; Escudero et al. 2010). These cases of homoplasy are related to the difficult taxonomy of *Carex*, which is due mainly to the frequently poor morphological differentiation between species and the reduced inflorescence (Escudero and Luceño 2009; Starr and Ford 2009).

Carex sect. *Ceratocystis* Dumort. is a small group of sedges (6-19 species depending on the treatment; Table 1) mainly distributed in temperate Eurasia, North Africa and North America (Schmid 1983; Crins 2002). Some of them are widely distributed taxa, with a typical amphi-Atlantic disjunction between North America and Europe, whereas others are more or less restricted endemisms. Four additional disjunct taxa are found in South America, South Africa, Tasmania and New Zealand (Nelmes 1955). Although section *Ceratocystis* has been previously included in section *Spirostachyae* Drejer ex L.H. Bailey (Kükenthal 1909; Maire 1957; Vicioso 1959), more recent authors consider section *Ceratocystis* an independent group (Chater 1980; Egorova 1999; Crins 2002; Luceño and Jiménez-Mejías 2008), a view also supported by molecular studies (Hendrichs et al. 2004b; Escudero et al. 2008). The main morphological characteristics of section *Ceratocystis* are obovate achenes, utricles that are attenuated in a long bifid beak and shortly subcylindrical to globose female spikes (Crins and Ball 1988; Crins 2002; Luceño and Jiménez Mejías 2008). Its members display short life cycles (Schmid 1984) and, as reported for other caespitose *Carex*, are predominantly self-compatible (Schmid 1984; Derieg et al. 2008), although Hedrén (2004) proposed a mixed breeding system combining selfing and outcrossing. Hybridization processes have been frequently reported across its range (e.g. Schmid 1982, 1983; Crins and Ball 1989b; Pykälä and Toivonen 1994; Egorova 1999; Jermy et al. 2007), and have been demonstrated to result in morphologically intermediate individuals (Hedrén 2002; Blackstock and Ashton 2010). Most taxa of section *Ceratocystis* have usually been included in a species complex termed the "*C. flava* group" or the "*C. flava* aggregate", due to species circumscription problems, particularly in Europe (Schmid 1983; Stoeva and Štěpánková 1990; Pykälä and Toivonen 1994; Hedrén 2002; Luceño and Jiménez Mejías 2008; Table 1). Two main taxonomic approaches may be inferred from previous treatments of the widely variable, amphi-Atlantic taxa of *C. flava* group (*C. demissa*, *C. flava*, *C. lepidocarpa* and *C. viridula*; Table 1): (1) A synthetic view that distinguishes two "biological species," *C.*

flava s.s. and a broad concept of *C. viridula* (*C. viridula* aggregate) that amalgamates the remaining species (Schmid 1983; Crins and Ball 1989b). This first group of treatments is based on a supposedly difficult cross between these two groups of taxa, due to the cytogenetic discontinuity between them (see below) (Schmid 1982); (2) An analytical approach that considers the amphi-Atlantic taxa as distinct species, and split up to eight additional taxa from their variability at the specific and infraspecific rank (e.g., Chater 1980; Egorova 1999). These analytical treatments are based on morphological differentiation, geographical distribution, ecological requirements and allozyme data (Pykälä and Toivonen 1994; Hedrén 2004). Furthermore, in Europe, the taxonomic complexity of section *Ceratocystis* is depicted by several problematic morphotypes whose status has remained controversial (see Table 1). *Carex flava* var. *alpina* and *C. nevadensis* correspond to mountain dwarf plants described from the Alps and Sierra Nevada, respectively. The former has been also cited from the Pyrenees (Luceño 1994; Bolòs and Vigo 2001), and the latter from other cicum-Mediterranean mountains (Atlas, Cantabrian Mountains, Corsica). *Carex nevadensis* has been both treated as a distinct species (Valdés et al. 2002; Molina et al. 2006; Luceño and Jiménez-Mejías 2008), and at the infraspecific rank (Kükenthal 1909; Maire 1957; Vicioso 1959; Schmid 1983; Crins and Ball 1989b; Luceño 1994, 1999). Chater (1980) united these dwarf mountain taxa under an expanded concept of *C. nevadensis*, whereas Corsican populations were later considered as *C. viridula* (Lambinon et al. 1992; Piquemal and Gamisons 2007). *Carex castroviejoi*, from the Greek Pindhos range, is another recently described mountain taxon (Luceño and Jiménez-Mejías 2008) formerly considered under *C. lepidocarpa* (Chater 1980; Hartvig 1991). Morphologically problematic populations from Portugal were ambiguously adscribed to *C. flava* (Coutinho and Pereira 1939; Sampaio 1947; Chater 1980; Luceño 1994) or *C. demissa* (Franco and Afonso 1994). Finally, populations from the Azores archipelago were recognized at subspecific rank by some authors (*C. tumidicarpa* subsp. *cedercreutzii*; Schmid 1983), and merged under *C. demissa* variability by others (Crins and Ball 1989b).

As the rest of Cyperaceae, *Carex* is characterized by certain cytogenetic peculiarities of evolutionary interest, particularly the presence of holocentric chromosomes, which eases the formation of aneuploid series via fission (agmatoploidy; Malheiros and Gardé 1950; Davies 1956) and fusion (symploidy; Luceño and Guerra 1996). An agmatoploid series resulting in an increase of the chromosome number has

been proposed as a major evolutionary pattern in *Carex* sect. *Ceratocystis* (Schmid 1982; Crins and Ball 1988). Accordingly, taxa with the lowest chromosome numbers have been proposed to be the most primitive (*C. durieui*, 2n = 52, 53; *C. hostiana*, 2n = 56; *C. flava* s.s., 2n = 58,60; *C. cryptolepis*, 2n = 64), while the highest numbers would be found in the most derived taxa [*C. viridula* aggregate (*C. demissa*, *C. lepidocarpa* and *C. viridula* s.s.), 2n = 66, 68, 70, 72] (Schmid 1982; Crins and Ball 1988; chromosome numbers reported by Schmid 1982; Crins and Ball 1989b; Halkka et al. 1992; Luceño and Castroviejo 1993; Roalson 2008). This cytogenetic pattern lends support to the synthetic hypothesis of two biological species for the amphi-Atlantic taxa (Schmid 1983). Thus, hybrids between *C. flava* s.s. and members of the *C. viridula* aggregate show severely disturbed meiosis and low fertility, whereas hybrids among taxa within *C. viridula* aggregate are characterized by more regular meiosis and a higher degree of fertility (Schmid 1982; Halkka et al. 1992).

The main goals of this work are to (1) assess the two main approaches to the taxonomy of section *Ceratocystis* on the basis of phylogenetic data; (2) clarify the taxonomy of the section in Europe and North Africa, with special emphasis on the problematic morphotypes; and (3) elucidate possible hybridization scenarios using molecular and cytogenetic data.

Materials and methods

INGROUP CIRCUMSCRIPTION

For the purpose of this paper, we have distinguished between well-defined taxa and problematic morphotypes within the studied members of section *Ceratocystis*. For the delimitation of well-defined taxa, we have followed an analytical approach mainly based in the taxonomic treatments by Nelmes (1955), Chater (1980) and Crins (2002) (Table 1). Thus, the amphi-Atlantic members of *Carex flava* group (*C. demissa*, *C. flava*, *C. lepidocarpa*, *C. viridula* s.s.) were considered as distinct species. In addition, *C. barrosii, C. cryptolepis, C. durieui, C. flaviformis, C. hostiana, C. lutea, C. monotropa* and a yet to be described North American species (*C. sp.*; Derieg et al., 2008), were also regarded at specific rank. Additionally, we took into account two subspecies within *C. lepidocarpa* (subsp. *lepidocarpa* and subsp. *jemtlandica*), but no

infraspecific division was applied to *C. viridula* s.s, since northern European races have been recently viewed as ecotypes (Hedrén 2002, 2004).

Problematic morphotypes were defined for eight different geographic areas mainly from circum-Mediterranean Mountains and Macaronesia (Table 1). Distinct taxa have been formally described for four of those areas: Greek Pindhos mountains (*C. castroviejoi*), Alps (*C. flava* var. *alpina*), Spanish Sierra Nevada (*C. nevadensis*), and Azores (*C. tumidicarpa* subsp. *cedercreutzii*). Populations for the remaining four areas have not been treated as distinct taxa: western Iberian Peninsula (Atlantic-Iberian plants), Pyrenean-Cantabrian ranges, Atlas and Corsica.

MOLECULAR STUDY

- Sampling

A total of 89 samples (13 from GenBank; see Appendix 1) from sect. *Ceratocystis* were included in the molecular study, of which 59 corresponded to the well-defined taxa and 30 to the problematic morphotypes. A special effort was made to cover the geographical and morphological variation of section *Ceratocystis* in Europe, with emphasis in the problematic morphotypes (Fig. 1). The sampling for plastid sequencing is limited to Europe, North Africa and Macaronesia, whereas for nuclear ITS it also includes sequences from America, S Africa and New Zealand; see Appendix 1). We included one species from each of three sections, *Sylvaticae* (*C. cretica*), *Spirostachyae* (*C. distans*) and *Tumidae* (*C. melanostachya*) as outgroups for the phylogenetic analyses (Appendix 1). Sections *Spirostachyae* and *Sylvaticae* have been found to be closely related to section *Ceratocystis* (Waterway and Starr 2007; Escudero et al. 2008).

- PCR amplification and sequencing

Total DNA was extracted from silica-dried material collected in the field as well as from herbarium specimens (BCC, CONC, E, JACA, M, MA, O, RNG, UPOS) using the DNeasy Plant Mini Kit (Qiagen, California). Field collections were deposited at the UPOS herbarium at Pablo de Olavide University (Seville, Spain).

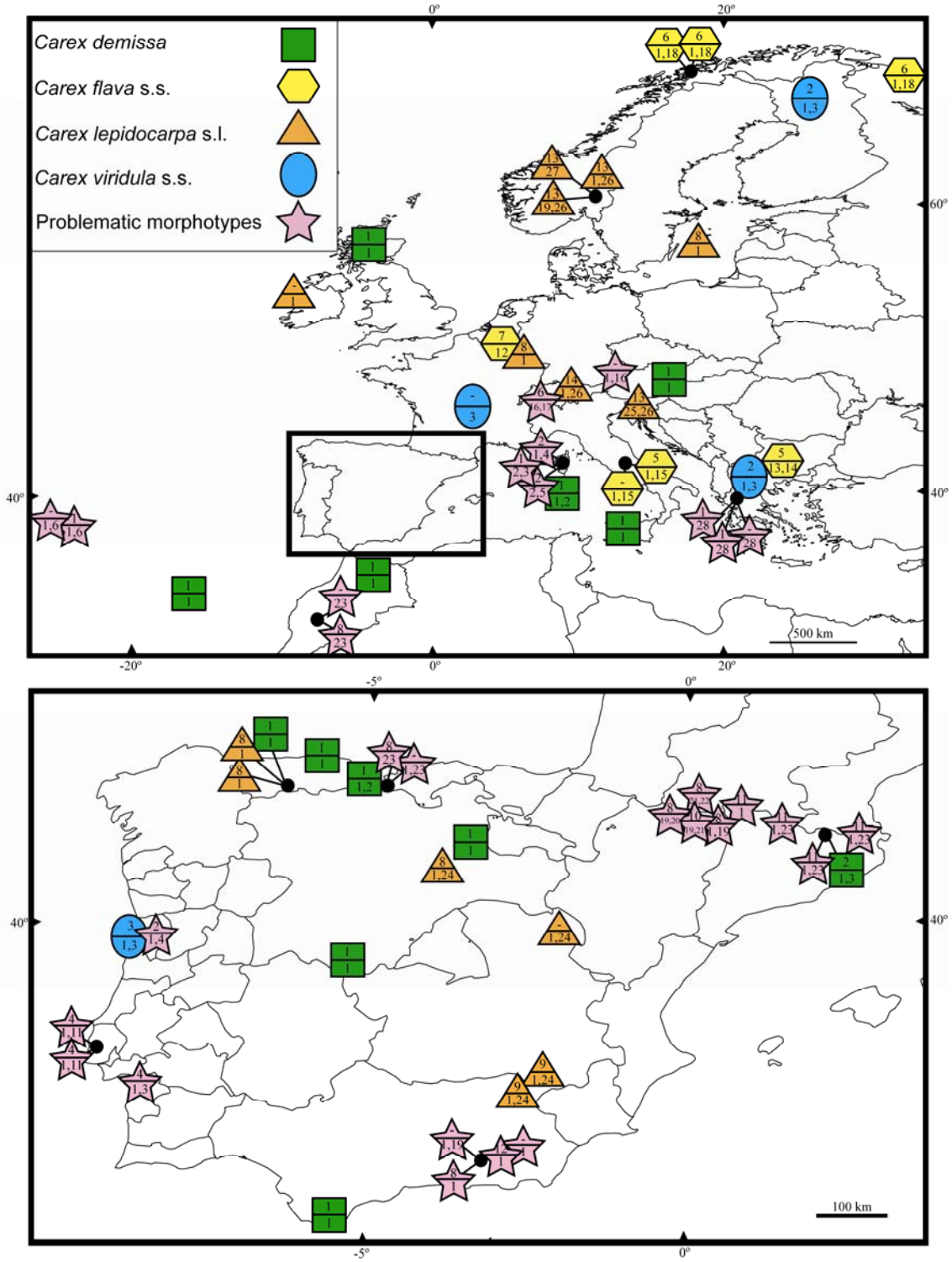

Fig. 1. Geographic distribution of the *5'trnK/rps16* haplotypes (upper numbers) and the ITS ribotypes (lower numbers), including 68 samples from four well-defined species and eight problematic morphotypes from *C. flava* group Only haplotypes and ribotypes from Europe, North Africa and Macaronesia are depicted. Colour key represents the different taxa.

We amplified and sequenced the nuclear ribosomal ITS region and the plastid *rps16* and *5'trnK* introns. These regions have been successfully used to address phylogenetic relationships within *Carex*, including closely related groups to section*Ceratocystis* (Escudero et al. 2008, Escudero and Luceño 2009; Escudero et al. 2010; Gehrke et al. 2010). The PCR conditions and primers followed those described in Escudero et al. (2008) for ITS, Schönswetter et al. (2006) for *rps16* and Escudero and Luceño (2009) for *5'trnK*. The amplified products were cleaned using spin filter columns (PCR Clean-up kit, MoBio Laboratories, California) following the manufacturer's protocol and were sequenced using the CIB Sequencing Service, CSIC, Madrid. The sequences were edited using the program Seqed (Applied Biosystems, California). The limits of the ITS, *rps16* and *5'trnK* region were determined as specified in Starr et al. (1999), Schönswetter et al. (2006) and Escudero and Luceño (2009), respectively. Polymorphic sites (PS) in the ITS sequences were assigned following the criteria in Fuertes Aguilar et al. (1999a, b) and using the IUPAC ambiguity symbols. These PS were considered additive (APS) when the two bases implied were found independently in the same position in other accessions (Fuertes Aguilar and Nieto Feliner 2003). Identification of co-occurring ITS copies (ribotypes) was inferred by subtraction (Clark 1990) using the sequences lacking polymorphisms as reference. Inference of ribotypes from additive sequences has already been used in several studies documenting plant hybridization (Sang et al. 1995; Wichman et al. 2002; Wu and Huang 2004; Albaladejo et al. 2005, Segarra-Moragues et al. 2007; Jones et al. 2008). This method has been recently evaluated as a fairly accurate approximation relative to cloning by Martín-Bravo et al. (2010), since most ribotypes inferred by subtraction were also found among the cloned sequences.

- Sequence analysis

Manual alignment was performed in PAUP* (Swofford 2002). Potentially informative indels were codified as binary characters, except for those resulting from poly-A and poly-T due to their high degree of homoplasy (Kelchner 2000). Maximum parsimony (MP), Bayesian inference (BI) and statistical parsimony (SP) analysis methods were applied to the matrices. The MP and BI analysis were conducted with three different matrices: a) the plastid matrix with all the combined *rps16-5'trnK* plastid sequences (matrix A; 65 sequences; 1515 characters, 0.63% coded as missing data), and

two nuclear matrices, b) a complete ITS ribotype matrix (matrix B; 57 sequences; 614 characters, 0.60% coded as missing data) resulting from the reduction of multiple identical ribotypes to two, and c) a reduced ITS ribotype matrix (matrix C; 26 sequences; 614 characters, 0.61% coded as missing data) including only those sequences that did not show APS, in order to minimize the disruptive effects of reticulation in the phylogenetic reconstructions (Fuertes Aguilar and Nieto Feliner 2003). The maximum parsimony analyses were conducted under Fitch parsimony as implemented in TNT (Goloboff et al. 2003). In the ITS matrix B, non-additive PS were treated as missing data (Albaladejo et al. 2005). Heuristic searches were replicated 10,000 times, retaining a maximum of two trees per replication, with Tree-Bisection-Reconnection branch swapping. A second heuristic search was run based on the trees obtained in the first search. A strict consensus tree was obtained from the heuristic searches. Clade support was assessed using bootstrapping, with 10,000 re-samplings with the same conditions used in the heuristic searches. The Bayesian inference analyses were performed with MrBayes (Ronquist and Huelsenbeck 2003). The analyses were conducted under the simplest model of sequence evolution that best fit the data, which was selected using the Akaike information criterion (AIC) in MrModeltest (Nylander 2004). Models were calculated independently for each ITS region (ITS1, 5.8s and ITS2). When analyzing matrices with coded indels, we selected F81 as the model for the corresponding additional characters, following MrBayes manual (Ronquist and Huelsenbeck 2003). Four simultaneous Markov Chain Monte Carlo were run in each BI analysis for 5,000,000 generations with an interval of 100 generations. Burn-in was evaluated over the generations. After discarding trees identified before the likelihood stationary phase, the remaining trees were compiled in a majority rule consensus tree. Corrected pairwise distances were calculated for each pair of sequences using the evolutionary models implemented by AIC for each region. The model was re-calculated with MrModeltest for the entire ITS region. Statistical parsimony analyses of the ribotypes and the haplotypes for the core *Ceratocystis* (section *Ceratocystis* excluding *C. durieui*; see results) were performed using TCS (Clement et al. 2000), with informative indels coded as described above.

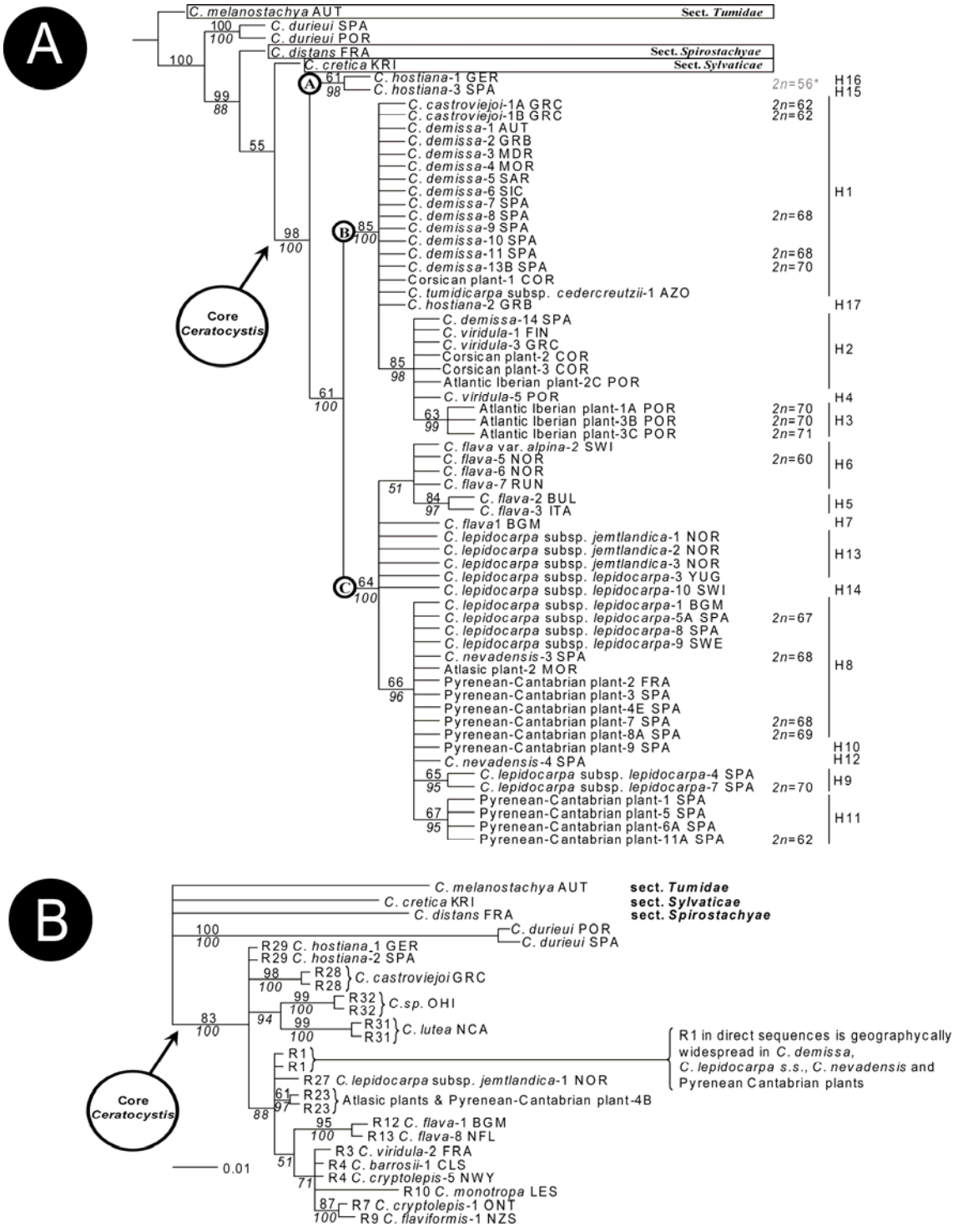

Fig. 2. Majority rule consensus trees of the 49,700 trees retained in the Bayesian inference analysis of the 65 *5'trnK/rps16* sequences (A) and of the 49,850 trees of the 14 sequences included in the reduced ITS matrix B (B). Bootstrap and posterior probabilities values are given above and below branches, respectively. Country sources are indicated following TDWG geographic codes (Brummit 2001). Diploid inferred chromosome numbers (2n) are displayed in the cpDNA tree. The number of *C. hostiana* (depicted with an asterisk) was taken from previous studies (Roalson 2008). Vertical bars assemble samples with the same haplotype.

CYTOGENETIC STUDY

- Sampling

Forty-five samples from 25 populations representing four well-defined taxa and four problematic morphotypes were included in the cytogenetic study (Table 2). Most studied populations (23) were from the Iberian Peninsula, where we conducted a detailed sampling of the problematic Pyrenean-Cantabrian morphotypes. The number of populations sampled per taxon varied from one (*C. castroviejoi*, *C. flava* s.s.) to seven (Pyrenean-Cantabrian plants). The number of individuals sampled in each population varied from one to five.

- Chromosome counts

To prepare meiotic plates, young anthers were fixed in a solution of absolute ethanol, glacial acetic acid and acetocarmine (10:4:1, v/v/v) with a drop of ferric acetate per 15 mL of solution as mordant. After 6-24 hours of fixation at room temperature, the material was stained in acetocarmine for 15-30 min and finally pressed (Luceño 1988). The meiotic plates were observed in a microscope (Nikon eclipse E400) and photographed with a Nikon DxM1200-F digital camera using Nikon ACT-1 software. The diploid numbers were deduced from meiotic configurations in metaphase I of the pollen mother cells (Escudero et al. 2008).

Results

PLASTID SEQUENCES

- Sequence and haplotype characterization

The matrix A was 1512 base pairs long (bp) (*5'trnK*, 682 bp; *rps16*, 830 bp). The core *Ceratocystis* (see below) *5'trnK* length ranged from 662-663 bp, whereas the *rps16* intron was 794-795 bp. Seventeen different haplotypes (labeled H1-H17; Fig. 1, Appendix 2) were identified within the core *Ceratocystis*. There was an important

congruence between the plastid haplotypes and the taxonomy (76.5% of the haplotypes were specific to a single well-defined taxon or a problematic morphotype). The ratio of variable *vs* informative characters (excluding coded indels) was 43/21 for the *5'trnK* intron and 49/19 for *rps16* region, whereas the ratios were 14/9 and 8/5, respectively, if only the core *Ceratocystis* sequences were considered. The corrected haplotype pairwise distances (GTR+I) in the core *Ceratocystis* varied between 0.07% (H2-H3) and 0.65% (H10-H16).

- Phylogenetic analyses

The MP analysis of the matrix A retained 37 most parsimonious trees of 109 steps [CI excluding uninformative characters (e.u.c.) = 0.8; RI = 0.94]. In the BI analysis implementing the GTR+I evolutionary model for both plastid regions, the stationary of the likelihood scores was reached at 50,000 generations. Accordingly, the first 500 trees were discarded. The phylogenetic trees from the MP and BI analyses had similar topologies (Fig. 2A). Sampled *Carex* sect. *Ceratocystis* members constitute a well-supported monophyletic clade (core *Ceratocystis*; 98% bs, 100% pp) once the monophyletic *C. durieui* (100% bs, 100% pp) is excluded. The core *Ceratocystis* is arranged in two main lineages and three major clades. Clade A (61% bs, 98% pp), sister to the remaining core *Ceratocystis*, includes two of the three *C. hostiana* samples. The second lineage (62% bs, 95% pp) comprises two clades: clade B (85% bs, 100% pp), which includes taxa mainly with patent utricles (*C. castroviejoi*, *C. demissa*, *C. viridula* s.s., *C. tumidicarpa* subsp. *cedercreutzii*, Corsican and Atlantic-Iberian problematic morphotypes and a British *C. hostiana* sample), and clade C (64% bs, 100% pp), which contains species with deflected utricles (*C. flava* s.s., *C. flava* var. *alpina*, *C. lepidocarpa* s.l., *C. nevadensis*, Atlasic and Pyrenean-Cantabrian problematic morphotypes). Within clade B, a subclade grouped *C. viridula* s.s. with Corsican and Atlantic-Iberian plants (85% bs, 98% pp). A single well-supported subclade was recovered in clade C containing *C. lepidocarpa* s.s. from Western Europe (including Sweden) plus *C. nevadensis* and the Atlasic and the Pyrenean-Cantabrian problematic morphotypes (66% bs; 96% pp). The SP analysis of core *Ceratocystis* haplotypes matrix yielded a single network (Fig. 3) in which three main groups of haplotypes, separated by between four and eight mutational steps, can be distinguished. They match the three main clades (A, B, C) retrieved in the MP and the BI analyses (Fig. 2A).

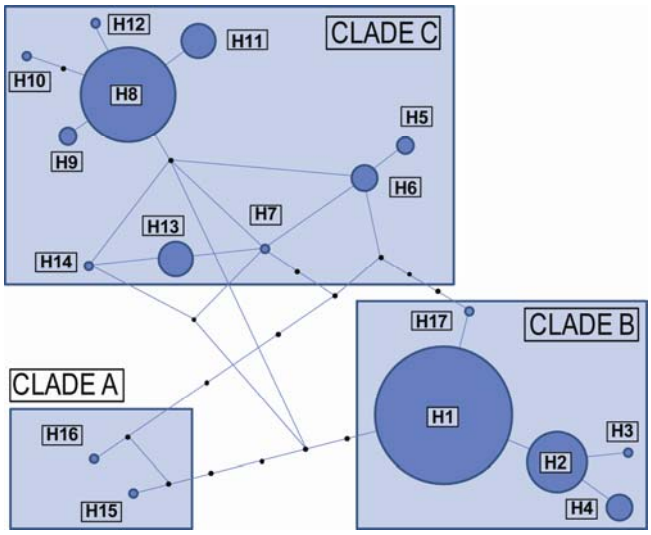

Fig. 3. Statistical parsimony network of the 17 haplotypes retrieved from the analysis of the 65 *5'trnK/rps16* sequences. Small black circles represent extinct or not sampled haplotypes, and each line between the haplotypes represents a sequence mutation step. Circle size is proportional to the number of samples displaying the corresponding haplotype.

ITS SEQUENCES

- Sequence and ribotype characterization

The matrix B containing all ITS ribotypes was 613 bp long (223, 166 and 224 bp for ITS1, 5.8S and ITS2, respectively). The core *Ceratocystis* ITS region ranged between 609 and 611 bp (220-222, 166 and 223 bp, respectively). The ratio of variable *vs.* informative characters in the matrix B was 108/75 (60/41, 4/4, and 44/30, respectively), whereas within core *Ceratocystis* it was 42/34 (25/22, 3/2, 14/10, respectively); 47 PS were detected among the 89 variable sites in the 87 ITS accessions, of which 17 corresponded to APS (11, 2, and 4, respectively). In the matrix C, the ratio of variable *vs.* informative chacters was 107/72 (59/38, 4/4, and 44/30, respectively) and 40/29 within core *Ceratocystis* (23/18, 3/1, and 14/10, respectively). Thirty-two different ribotypes (R1-R32; Fig. 1, Appendix 3) were identified within core *Ceratocystis*. The ribotypes were mainly congruent with the taxonomy, with each ribotype generally confined to a single well-defined taxon or to a problematic morphotype (78.1%). The most frequent ribotype (R1) was widely distributed in 51 samples (57.9% of all samples) belonging to four different well-defined taxa (*C. demissa*, *C. flava* s.s., *C. lepidocarpa* and *C. viridula* s.s.) and six problematic morphotypes (*C. flava* var. *alpina*, *C. nevadensis*, *C. tumidicarpa* subsp. *cedercreutzii*, Corsican, Atlantic-Iberian and Pyrenean-Cantabrian plants).

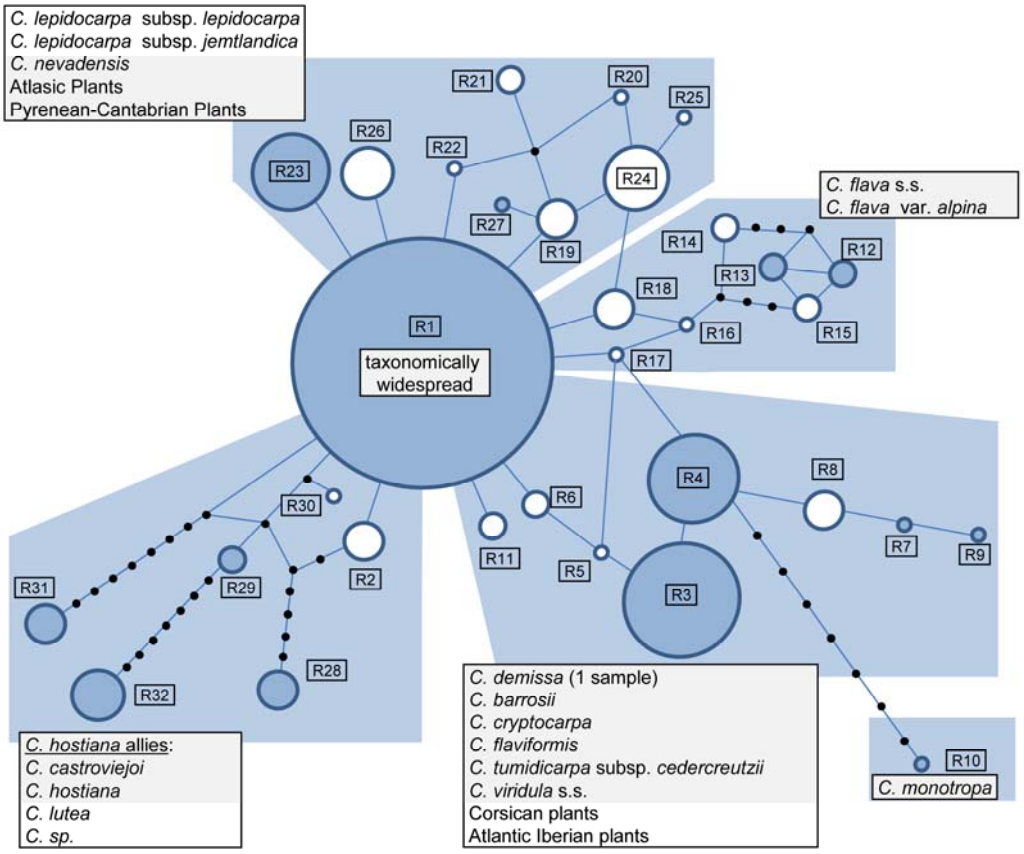

Fig. 4. Statistical parsimony network of the 32 direct (grey circles) and inferred (white circles) ribotypes retrieved from the analysis of the 85 ITS sequences. Small black circles represent extinct or not sampled ribotypes, and each line between the haplotypes represents a mutation step. Circle size is proportional to the number of samples displaying the corresponding ribotype.

The corrected ribotype pairwise distances (GTR+I+G) within the core *Ceratocystis* varied between 0.16% (R12 and R15, two *C. flava* s.s. populations) and 3.33% (*C. monotropa* and *C. sp.*).

- Phylogenetic analyses

The MP analysis of the matrix B retained 320,588 most parsimonious trees of 170 steps [CI e.u.c. = 0.67; RI = 0.81], and eight most parsimonious trees of 155 steps [CI e.u.c. = 0.73; RI = 0.81] for the reduced matrix C. In the BI analyses, the evolutionary models implemented for the matrix B were GTR+G for the ITS1 and ITS2 regions and K80+I for the 5.8s gene; for the matrix C, the models were GTR+I for ITS1, K80+I for 5.8s and GTR+G for ITS2. The stationary point of the likelihood scores was reached at 35,000 generations in the matrix B and at 15,000 in the matrix C.

Accordingly, the first 350 and 150 trees were discarded for the majority rule consensus tree, respectively. Phylogenetic reconstructions from both datasets revealed the monophyly of the sampled members of section *Ceratocystis* (86% bs, 100% pp in matrix B, not shown; 83% bs, 100% pp in matrix C, Fig. 2B) once the monophyletic *C. durieui* was excluded (100% bs, 100% pp in both matrices).

The MP analysis of the matrix B yielded a large polytomy containing the entire core *Ceratocystis*, whereas the BI analysis provided modestly better resolution, but with low posterior probability support (<90%) (results not shown). Only five of the clades in core *Ceratocystis* showed significant support in the MP and / or the BI analyses of matrix B: *C. cryptolepis p.p.* (samples 1-4) and *C. flaviformis* (R7-R9; 71% bs, 99% pp), *C. flava p.p.* (R12, R13 and R15, from samples 1, 8 and 3-4, respectively; 82% bs, 98% pp), *C. castroviejoi* (R28; 97% bs, 100% pp), *C. lutea* (R31; 99% bs, 100% pp) and *C. sp.* (R32; 99% bs, 100% pp). The analysis of matrix C yielded a similar topology, with six well-supported clades (Fig. 2B): *C. cryptolepis* (sample 1) and *C. flaviformis* (87% bs, 100% pp), *C. castroviejoi* (98% bs, 100% pp), *C. flava p.p.* (samples 1, 8), *C. lutea* (99% bs, 100% pp), *C. sp.* (99% bs, 100% pp) and R23 from Pyrenean-Cantabrian and Atlasic plants (61% bs, 97% pp).

The SP analysis of the core *Ceratocystis* ITS ribotype matrix yielded a single network (Fig. 4) that showed two main groups of interconnected ribotypes. Both groups were connected through the widespread R1, which occupied an internal position. The first group (*C. hostiana* allies) contained R2 and R28-R32 (*C. castroviejoi*, *C. hostiana*, *C. lutea*, *C. sp.*). The second group (core *C. flava*) displayed 22 ribotypes connected through loops. Three ribotypes (R11, from Atlantic-Iberian plants samples 3B-3C; R23, for the Pyrenean-Cantabrian and the Atlasic plants, and R26, from *C. lepidocarpa* s.l.) appeared isolated and only linked with R1.

CHROMOSOME COUNTS

The counts reported herein increase the range of the known chromosome numbers for *C. flava* group in the Iberian Peninsula (Table 2, Fig. 5). All taxa investigated displayed more than one diploid number, except for *C. nevadensis* (2n = 68). *Carex demissa* and *C. viridula* s.s. displayed 2n = 68, 70. The Atlantic-Iberian plants showed 2n = 68, 70 with regular configurations, except for one sample from the

Table 2. Diploid deduced numbers and metaphase configuratios from the studied plant material. Counts from non-Iberian samples are marked with an asterisk.

Taxon	Sample number	Diploid number and metaphase configurations
Well-defined taxa		
C. demissa	8	$2n = 68\ (34^{II})$
	11	$2n = 68\ (34^{II})$
	12A,B,C,D	$2n = 70\ (35^{II})$
	13A,B,C	$2n = 70\ (35^{II})$
C. flava s.s.	5	$2n = 60\ (30^{II})$*
C. lepidocarpa subsp. *lepidocarpa*	5A	$2n = 67\ (32^{II} + 1^{III})$
	5B	$2n = 69$ | $34^{II}+1^{I}$; $31^{II}+1^{I}+2^{III}$
	7	$2n = 70\ (35^{II})$
C. viridula s.s.	6	$2n = 70\ (35^{II})$
	7	$2n = 70\ (35^{II})$
	8	$2n = 70\ (35^{II})$
Problematic morphotypes		
C. castroviejoi	1A,B	$2n = 62\ (31^{II})$*
C. nevadensis	3	$2n = 68\ (34^{II})$
	5	$2n = 68\ (34^{II})$
Atlantic-Iberian Plants	1A,B,C	$2n = 70\ (35^{II})$
	2A	$2n = 68\ (34^{II})$
	2B	$2n = 70\ (35^{II})$
	3A,B,D	$2n = 70\ (35^{II})$
	3C	$2n = 71$ | $28^{II} + 6^{I} + 3^{III}$; $28^{II} + 4^{I} + 1^{III} + 2^{IV}$; $29^{II} + 7^{I} + 2^{III}$; $31^{II} + 6^{I} + 1^{III}$
	4	$2n = 70\ (35^{II})$
Pyrenean-Cantabrian Plants	1	$2n = 68\ (34^{II})$
	4A	$2n = 64\ (32^{II})$
	4B	$2n = 68\ (34^{II})$
	4C	$2n = 61$ | $26^{II} + 1^{III} + 1^{V}$; $29^{II} + 1^{III}$
	4D	$2n = 68\ (34^{II})$
	4E	$2n = 62\ (31^{II})$
	6B	$2n = 68\ (34^{II})$
	7	$2n = 68\ (34^{II})$
	8A	$2n = 69$ | $34^{II} + 1^{I}$; $33^{II} + 1^{III}$
	8B,C	$2n = 68\ (34^{II})$
	8D	$2n = 67$ | $32^{II} + 1^{III}$; $31^{II} + 2^{I} + 1^{III}$
	10	$2n = 68\ (34^{II})$
	11A	$2n = 62\ (31^{II})$
	11B	$2n = 70\ (35^{II})$

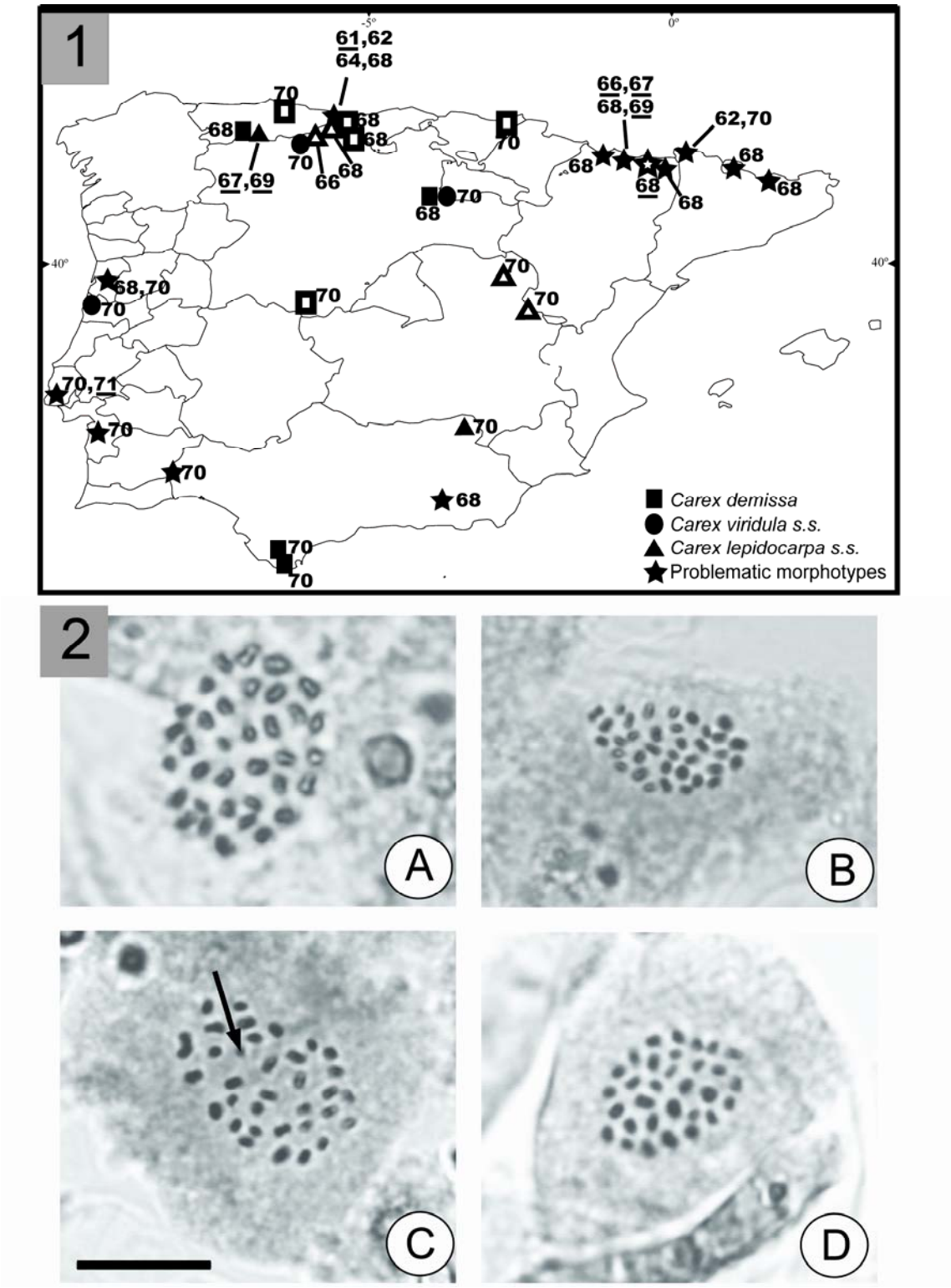

Fig. 5. (1) Cytotype map of the Iberian Peninsula. Full shapes are original counts; empty shapes are numbers reported by Luceño & Castroviejo (1993); Underlined numbers indicate irregular meiotic configurations. (2) Meiotic plates correspond to the following taxa and configurations: A. *C. demissa*-12A, 2n = 70 (35^{II}); B. *C. viridula* s.s.-6, 2n = 70 (35^{II}); C. *C. lepidocarpa* s.s.-5B, 2n = 69 ($34^{II}+1^{I}$); D. Pyrenean-Cantabrian plant-11A, 2n = 62 (31^{II}). The arrow in C indicates a monovalent. Scale bar = 10 μm

Lisbon area (2n = 71). *Carex lepidocarpa* displayed both regular (2n = 70) and irregular configurations (2n = 67, 69). The Pyrenean-Cantabrian morphotype samples displayed great cytogenetic variability with multiple regular and irregular configurations (2n = 61, 62, 64, 68, 70).

The Norwegian *C. flava* showed the most common chromosome number reported for this species, 2n = 60 (Schmid 1982; Halkka et al. 1992). *Carex castroviejoi* was counted for the first time, displaying 2n = 62, an unexpected number given its former taxonomic ascription to *C. lepidocarpa* (Hartvig 1991).

Discussion

SYSTEMATIC IMPLICATIONS AND EVOLUTIONARY PATTERNS

The sampled members of *Carex* sect. *Ceratocystis* form a monophyletic group if *C. durieui* is excluded, which is in agreement with previous works that suggested the taxonomic identity of the section based on morphological (Chater 1980; Egorova 1999; Crins 2002; Luceño and Jiménez-Mejías 2008) and molecular data (Hendrichs et al. 2004b; Escudero et al. 2008). Limited phylogenetic resolution and the non-monophyly of most species within *C. viridula* aggregate (results not shown) may be interpreted as the result of extensive gene flow among taxonomical units (Nieto Feliner et al. 2001; Suárez-Santiago et al. 2007). However, incomplete lineage sorting of ancestral ITS polymorphisms due to recent divergence may not be ruled out as an alternative explanation (Gernandt et al. 2001; Albadalejo et al. 2005; Martín-Bravo et al. 2010), especially in view of the high frequency and internal position of the species-independent R1 in the parsimony network (Fig. 3; Pleines et al. 2009). Reticulate evolution has hindered the elucidation of systematic relationships in molecular studies of different plant groups (Comes and Abbott 2001; Fuertes Aguilar and Nieto Feliner 2003; Albaladejo et al. 2005; among others). Reticulation may be reflected as low molecular differentiation between different taxa (Suárez-Santiago et al. 2007), as observed in our data set (0.07-0.65% pairwise distance among the plastid haplotypes; a minimum of 0.16% among ITS ribotypes). Morphological and allozyme studies have previously supported the recognition of several taxa within the *C. viridula* aggregate (Crins and Ball 1989a; Hedrén 2002). Thus, the lack of resolution of many taxa (Fig. 2A) should

not be directly interpreted as evidence of non-monophyly, since the absence of genetic differentiation is not always related to morphological divergence (Fuertes Aguilar and Nieto Feliner 2003; Suárez-Santiago et al. 2007; Martín-Bravo et al. 2010).

Carex durieui is a narrow endemic from the NW Iberian Peninsula whose placement within section *Ceratocystis* has not been a matter of discussion (Kükenthal 1909; Chater 1980; Crins and Ball 1988; Luceño 1994; Luceño and Jiménez-Mejías 2008). It has frequently been considered the ancestral species of the section due to its low chromosome number (2n = 52, 53; Crins and Ball 1988; Luceño and Castroviejo 1993). However, our phylogenetic reconstructions are congruent with its exclusion from section *Ceratocystis* (Figs. 2A, 2B). Although *C. durieui* shows certain morphological affinities with some members of section *Ceratocystis* (subglobose spikes, deflexed utricles with bifid beaks; Luceño and Jiménez Mejías 2008), the winged utricles and the narrow rigid leaves are very distinctive characteristics not displayed by other species in section *Ceratocystis*. An increased taxon sampling is required to investigate the taxonomic placement and the phylogenetic relationships of *C. durieui*.

Our plastid phylogeny could provide new insights about the cytogenetic evolution within section *Ceratocystis* (Fig. 2A), although our sampling is centred in Europe (Appendix 1), and most chromosome numbers were obtained from Iberian samples (Table 2). *Carex hostiana*, with the lowest chromosome number in the section (2n = 56), appears as the sister taxon of the remaining core *Ceratocystis* (except for *C. hostiana*-2 sample, see below). This could indicate an evolutionary trend towards an increase in the chromosome number within the section (agmatoploid series; Crins and Ball 1988). However, in view of the plastid phylogeny, the cytogenetic evolution appears to have been complex since taxa with both low and high chromosome numbers are placed within clades B and C (Fig. 2A). This may indicate that several independent increases (and/or decreases) in chromosome number have occurred, and thus that the overall increase in the chromosome number has not been linear.

The geographical limitations of our molecular sampling (especially for the American taxa) prevent us from evaluating a whole taxonomic treatment of section *Ceratocystis*. Nonetheless, concerning the two major taxonomic approaches within *C. flava* group (two biological species *vs.* several morphological species for the amphi-Atlantic taxa; see Introduction, Table 1), the phylogenetic relationships retrieved from the plastid data seem to reject the synthetic proposal (Schmid 1983; Crins and Ball 1989b), since *C. flava* s.s. is nested within *C. viridula* aggregate (Fig. 2A). On the other

hand, the general congruence of haplotypes and ribotypes with well-defined taxa or problematic morphotypes (76.5% and 78.1% specificity, respectively) leads us to consider analytical views (e.g. Chater 1980; Egorova 1999) as more appropriate for species circumscription in section *Ceratocystis*. Therefore, our results tentatively suggest that the four amphi-Atlantic taxa in *C. flava* group should be rather treated as distinct species (*C. demissa*, *C. flava*, *C. lepidocarpa* and *C. viridula* s.s.), although future systematic studies with an expanded geographic sampling and more molecular markers would be desirable. The *rps16-5'trnK* phylogeny is mainly in agreement with the morphological character of deflected *vs.* patent utricles. This has been one of the most important diagnostic features in *C. flava* group (e.g., Egorova 1999; Crins 2002; Luceño and Jiménez Mejías 2008). Taxa with patent utricles (*C. demissa*, *C. viridula* s.s., and their allies) are grouped together in clade B, whereas those with deflected ones (*C. flava* s.s., *C. lepidocarpa* and their allies) are placed in clade C, sister to the former (Fig. 2). Interestingly, the recently described *C. castroviejoi* (Jiménez-Mejías and Luceño 2009) and the problematic Atlantic-Iberian morphotype (Table 1), were included in clade B (patent utricles), in spite of having deflected utricles, which suggests at least two independent origins for such character.

Carex castroviejoi, although unresolved in the plastid phylogeny (Fig. 2A), forms a well-supported monophyletic group in the ITS phylogeny (Fig. 2B). This fact is due to a highly divergent ribotype (R28, Figs 2B, 4) separated by eight mutational steps from the widespread R1 and clearly isolated from the ribotypes found in the other European members of section *Ceratocystis*. These results, together with the morphological distinctiveness of this species (Jiménez-Mejías and Luceño 2009), support its taxonomic identity.

In the light of our results, western *C. lepidocarpa s.l.* is closely related with *C. nevadensis*, and the Atlasic and Pyrenean-Cantabrian plants. These samples are placed in a distinct lineage within clade C in the plastid phylogeny (Fig. 2A), whereas the remaining *C. lepidocarpa* samples and *C. flava* are unresolved. This topology is partially congruent with previous allozyme data that supported the taxonomic distinctiveness of subsp. *lepidocarpa* and subsp. *jemtlandica* (Hedrén and Prentice 1996; Hedrén 2002). In addition, the taxonomic ascription of two of the problematic morphotypes (*C. flava* var. *alpina* and *C. tumidicarpa* subsp. *cedercreutzii*) is suggested by the sharing of haplotypes / ribotypes with well-defined species,(*C. flava* s.s and *C.*

demissa, respectively; Fig. 2A, Appendixes 2-3). This matches previous treatments proposed for these populations (Schmid 1983; Crins and Ball 1989b).

The disjunct New Zealand *C. flaviformis* and the South-African *C. monotropa* displayed taxon-specific divergent ribotypes (Fig. 4), which support the taxonomic identity of both species and may point to two different long-dispersal events and subsequent allopatric divergence. The derived positions of both species with respect to Northern Hemisphere taxa in the ribotype network (Fig. 4) suggest a north-to-south migration.

ITS EVOLUTION

The presence of PS in direct ITS sequences has been considered the result of the co-occurrence of different ITS copies in the same genome (Sang et al. 1999; Fuertes Aguilar and Nieto Feliner 2003; among others). ITS intraindividual polymorphisms can be explained by both hybridization (Calonje et al. 2009; Poczai and Hyvönen 2010) or by lineage sorting (Albadalejo et al. 2005; Martín-Bravo et al. 2010). Ribotype 1, shared between different taxa, displayed a high frequency (57.9% of all included samples; Fig. 4, Appendix 3) and amount of mutational connections, and an internal placement in the ribotype network (Fig. 4). These features suggest that it is an ancestral ribotype and that it probably predates the speciation in the core *C. flava* (Schaal et al. 1998; Pleines et al. 2009). Thus, lineage sorting could be cause of at least part of the taxonomic incongruence of R1.

Within section *Ceratocystis,* several divergent, direct ribotypes are species-specific (Fig. 4): *Carex castroviejoi* (R28), *C. lutea* (R31), *C. sp*. (R32), *C. monotropa* (R10) and *C. flava* s.s. (R12-R13). These sequences showed the highest genetic distance values (0.8-1.9%) with respect to the central R1. Most of the detected variability in these divergent ribotypes was concentrated in the ITS2 spacer (Appendix 3), in contrast with the generally higher variability of the ITS1 spacer in angiosperms (Baldwin et al. 1995). These taxa were placed into two different groups using the SP analysis of the ITS dataset (Fig. 4): *Carex castroviejoi*, *C. lutea* and *C. sp*. were included within *C. hostiana* allies, whereas *C. monotropa* and *C. flava* s.s. were placed in the core *C. flava*. These results are in agreement with the distant relationship of *C. lutea* and *C. sp.* with respect to the remaining *C. flava* group, as proposed by Derieg et al. (2008). This strong ITS differentiation pattern appears to be linked to a certain degree of isolation between

the divergent ITS ribotypes and the remaining section *Ceratocystis* members. Two types of barriers may have been involved in this differentiation process: a) geographical barriers as the result of long-term isolation in putative glacial refuges (e.g., *Carex lutea*, *C. sp.*; Derieg et al. 2008) or disjunct ranges (*C. monotropa*), or b) pre-zygotic reproductive barriers, exemplified by the significantly lower chromosome numbers in *C. flava* s.s. (Schmid 1983) and *C. castroviejoi* (2n = 58, 60, 62) with respect to other members of the *C. viridula* aggregate (2n = 66, 68 ,70, 72). However, this latter cytogenetic barrier may sometimes not be as effective at preventing gene flow as geographic isolation, since *C. hostiana* has the lowest number within section *Ceratocystis* (2n = 56), but its ribotypes showed a lower divergence with respect to R1 (0.03-0.05 %).

RETICULATE EVOLUTION: UNMASKING PROBLEMATIC MORPHOTYPES WITHIN EXTENSIVE HYBRIDIZATION AREAS

Despite the frequent hybridization reports in *Carex* sect. *Ceratocystis* members (Chater 1980; Schmid 1982; Stoeva and Štěpánková 1990; Halkka et al. 1992; Hedrén 2002), the phylogeny did not allow us to trace back clear hybridization events for most samples included. Only two samples, *C. demissa*-14 and *C. hostiana*-2, showed incongruent placements within the nuclear and plastid networks and phylogenies (Figs. 2-4), the latter being a probable case of plastid capture (Schaal et al. 1998).

Nevertheless, in view of our data (haplotype and ribotype diversity and chromosome counts), several extensive hybridization areas in South Europe may be suggested, each of them characterized by the presence of a problematic morphotype. In these areas, genetic exchange between two different taxa occurs (Fuertes Aguilar et al. 1999a; Albadalejo et al. 2005; Suárez-Santiago et al. 2007; among others), the "pure" parental morphological extremes are frequently absent, and the problematic morphotype is the most frequent phenotype. We propose at least three possible extensive hybridization areas: Corsican mountains, the Atlantic-Iberian strip and the northern ranges of the Iberian Peninsula (the Pyrenees and Cantabrian Mountains).

The Corsican and the Atlantic-Iberian plants probably involve *C. demissa* and *C. viridula* s.s. as parentals, as indicated by the incongruent ribotype-haplotype sharing and intermediate ribotypes found in the studied populations. Within Corsica, both *C. demissa* and *C. viridula* s.s. most frequent haplotypes (H1-H2, Fig. 2A, Appendix 2)

were found. Moreover, some of the ribotypes (R4-R5) were intermediate between the most frequent *C. viridula* s.s. ribotype R3 and the *C. demissa* one R1 (Fig. 4, Appendix 3). In the Atlantic-Iberian plants, ribotypes were related to both *C. viridula* s.s. and *C. demissa*, whereas haplotypes were to *C. viridula* s.s. ones (R3, R4, and R10, Fig. 4, Appendix 3; H2-H3, Fig. 2A, Appendix 2). In addition to the molecular evidence, both groups of populations in the Corsican and Atlantic-Iberian regions show ambiguous morphological characteristics and lack clear morphological limits with respect to the corresponding well-defined species (Lambinon et al. 1992; Luceño and Jiménez-Mejías 2008; Jiménez-Mejías 2011). The introgression process has apparently resulted in two different morphological outcomes in each hybridization area; whereas the Atlantic-Iberian problematic morphotype usually resembles *C. flava* s.s. (Coutinho 1939; Sampaio 1947; Chater 1980; Luceño 1994), the Corsican plants have been identified as *C. nevadensis* (Chater 1980) or as *C. viridula* s.s. (Lambinon et al. 1992; Piquemal and Gamisans 2007).

Carex lepidocarpa and *C. demissa* could have been involved in the origin of the Pyrenean-Cantabrian problematic morphotypes. Although the haplotypes and the ribotypes found in this group are included within *C. lepidocarpa* variability (Figs. 2-4, Appendixes 2-3), non-molecular insights support the putative hybrid origin of these populations: intermediate morphology (Luceño and Jiménez-Mejías 2008; Jiménez-Mejías 2011) and an irregular chromosomal arrangement (Fig. 5, Table 2). Although both putative parental taxa can be found in the Pyrenees and Cantabrian Mountains as "pure" morphological forms, the majority of the individuals show the Pyrenean-Cantabrian problematic morphotype, which is characterized by intermediate morphological features (Luceño and Jiménez-Mejías 2008). Chromosomal irregularities reported from *C. flava* group hybrids (Schmid 1982; Halkka et al. 1992) have also been found in the Pyrenean-Cantabrian plants, where up to six different regular and irregular diploid numbers were inferred (Table 2). The frequent mixture of siliceous and limestone substrates in these mountains systems (Villar et al. 1997) would allow for the coexistence and eventual hybridization between the acidophilous *C. demissa* and the basophilous *C. lepidocarpa* (Chater 1980; Crins and Ball 1989b; Luceño and Jiménez-Mejías 2008; among others). Most chromosome numbers detected in those populations were included within *C. demissa* and *C. lepidocarpa* variability; however, the presence of relatively low numbers (2n = 61, 62, Table 2, Fig. 5) may also represent *C. flava* s.s.

residual specimens included within the hybridization area (e.g., samples Pyrenean-Cantabrian plant-4A and 11A; cf. Luceño and Jiménez-Mejías 2008).

CONCLUDING REMARKS

In the light of the systematic structure inferred from our mainly Euro-North African sampling, the analytic approach seems to fit better with the taxonomy of section *Ceratocystis*. Therefore, the amphi-Atlantic taxa within the *C. flava* group might be considered at species rank. *Carex durieui* should be excluded from section *Ceratocystis*. Some of the morphologically problematic specimens from southern European populations may be an outcome of extensive hybridization, as in the Atlantic-Iberian strip, Corsica and the Pyrenean-Cantabrian Mountains populations. However, other problematic morphotypes were included within the molecular variability of well-defined species (*C. flava* var. *alpina*, *C. tumidicarpa* subsp. *cedercreutzii*, *C. nevadensis* and Atlasic plants). Therefore, despite some useful taxonomic hints derived from our molecular and cytogenetic data, further morphological studies are necessary to re-evaluate some taxonomical boundaries.

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Appendix 1

Studied material and GenBank accession numbers. Accesions numbers are provided in the following order: ITS, *5'trnK* and *rps16* (— = sequence no obtained); sequences obtained from GenBank database are marked with an asterisk. In populations with more than one individual sampled for cytogenetic studies, the sequenced one is underlined.

Section Ceratocystis.

Well-defined taxa. ***Carex barrosii***— **1.** Chile, Llanquihue, Vega de Cayutué, *C. Rudolph* (67790 CONC), JN634673, —,—. ***Carex cryptolepis***— **1.** Canada, Ontario, EU247866*, —, —; **2.** USA, Ohio, EU247867*, —, —; **3.** USA, Wisconsin, EU247868*, —, —; **4.** USA, Minnesota, EU257869*, —, —; **5.** USA, New York, EU247870*, —, —. ***Carex demissa***— **1.** Austria, Wien, Bezirk, *B. Wallnöfer* (691776 MA), JN634653, JN627690, JN627757; **2.** Great Britain, Scotland, Highlands, Glencoe, *S. Martín-Bravo et al. 141SMB07* (UPOS), JN634654, JN627691, JN627758; **3.** Madeira island, Bica da Casa-Lomo do Mouro, *C. Navarro* (654698 MA), JN634655, JN627692, JN627759; **4.** Morocco, Rif Range, Tighidin mount, *P. Jiménez-Mejías et al. 93PJM07(1)* (UPOS), JN634656, JN627693, JN627760; **5.** Sardinia, Fonni, Gemargentu, *S. Castroviejo* (3465 UPOS), JN634657, JN627694, JN627761; **6.** Sicily, Palermo, Geraci Siculo, *M. A. García et al.* (645612 MA), JN634658, JN627695, JN627762; **7.** Spain, Asturias, Cantabrian Mountains, Lago Ercina, *J. M. Marín et al. 15504JMM(1)* (UPOS), JN634659, JN627696, JN627763; **8.** Spain, Asturias, Cantabrian Mountains, Puerto de Somiedo, *P. Jiménez-Mejías et al. 449PJM05(10)* (UPOS), JN634660, JN627697, JN627764; **9.** Spain, Asturias,Monte Deva, *S. Martín-Bravo et al. 599SMB05(10)* (UPOS), JN634661, JN627698, JN627765; **10.** Spain, Ávila, Sierra de Gredos, Laguna Grande, *P. Jiménez-Mejías et al. 201PJM04* (UPOS), JN634662, JN627699, JN627766; **11.** Spain, Burgos, Iberian Range, Laguna Larga de Neila, *J. M. Marín et al. 12504JMM* (UPOS), JN634663, JN627700, JN627767. **12A,B,C,D.** Spain, Cádiz, Alcalá de los Gazules, Aljibe, *M. Portillo and M. Luceño 1105MPE* (UPOS), not sequenced; **13A,B,C.** Spain, Cádiz, Tarifa, Sierra del Bujeo, *P. Jiménez-Mejías et al. 87PJM04* (UPOS), JN634664, JN627701, JN627768; **14.** Spain, Gerona, Pyrenees, Prat Fondal, *A. Pérez-Haase* (BCC), JN634665, JN627702, JN627769. ***Carex durieui***— **1.** Portugal, Beira Litoral, between Águeda and Travasso,

P. Jiménez-Mejías et al. 139PJM05 (UPOS), JN634718, JN627703, JN627770; **2.** Spain, Pontevedra, Las Gándaras de Budiño, *J. M. Marín et al. 1302JMM* (UPOS), JN634719, JN627704, JN627771. ***Carex flava* s.s.**— **1.** Belgium, Luzembourg, Bellefontaine, *M. Leten* (RNG), JN634682, JN627705, JN627772; **2.** Bulgaria, Rhodopes, *C. Navarro et al.* (726921 MA), JN634683, JN627706, JN627773; **3.** Italy, L'Aquila, Paso di Campanelle, *C. Navarro et al.* (700011 MA), JN634684, JN627707, JN627774; **4.** Italy, L'Aquila, Paso di Campanelle, *C. Navarro et al.* (700131 MA), JN634685, JN627708, JN627775; **5.** Norway, Troms, Skjervoy, *M. Luceño 4005ML(1)*, (UPOS), JN634688, JN627709, JN627776; **6.** Norway, Troms, Hamneidet, *M. Luceño 4305ML* (UPOS), JN634689, JN627710, JN627777; **7.** Russia, Mursmank, Kandalaksha, *T. Filimonova* (722954 MA), JN634690, JN627711, JN627778; **8.** Canada, New Foundland, EU247873*, —, —. ***Carex flaviformis***— New Zealand, South Island, Pukaki Scientific Reserve, *P. J. de Lange 5937*, EU247873*, —, —. ***Carex hostiana***— **1.** Germany, Bavaria, Kreis Weilheim, *W. Lippert* (M 0127453), JN634715, JN627712, JN627779; **2.** Great Britain, Scotland, Pass of Drumochter, *S. Martín-Bravo et al. 102SMB07* (UPOS), JN634717, JN627713, JN627780; **3.** Spain, Navarra, Baztan, *A. Balda* (3502 UPOS), JN634716, JN627714, JN627781. ***Carex lepidocarpa* subsp. *lepidocarpa***— **1.** Belgium, Luxembourg between Virton and Arlon, *K. Camelbeke* (589951 MA), JN634650, JN627715, JN627782; **2.** Ireland, Mannin peninsula, *C. Breen* (462751 MA), JN634651, —, —; **3.** Slovenia, Nr. L. Bled, *H. J. M. Bowen 9571* (RNG), JN634707, JN627716, JN627783; **4.** Spain, Albacete, Sierra de Segura, Riopar, *P. Jiménez-Mejías et. al. 53PJM04(10)*, JN634703, JN627717, JN627784; **5A,B.** Spain, Asturias, Cantabrian Mountains, Puerto de Somiedo, *P. Jiménez-Mejías et al. 450PJM05*, JN634699, JN627718, JN627785; **6.** Spain, Guadalajara, Póveda de la Sierra, *A. Martínez* (302694 JACA), JN634704, —, —; **7.** Spain, Jaén, Sierra de Cazorla, Hoya del Cambrón, *P. Jiménez-Mejías et al. 70PJM04(4)*, JN634705, JN627719, JN627786; **8.** Spain, Valladolid, Encinas de Esgueva, *J. L. Fernández* (342466 MA), JN634706, JN627720, JN627787; **9.** Sweden, Gotland, *O. Thulin 2004* (O), JN634652, JN627721, JN627788; **10.** Switzerland, St. Gallen, Rheintal, *W. Koch* (462808 MA), JN634708, JN627722, JN627789. ***Carex lepidocarpa* subsp. *jemtlandica***— **1.** Norway, Hedmark, Furuberget, *K. Lye and T. Berg 19437* (O), JN634710, JN627723, JN627790; **2.** Norway, Hedmark, Furuberget, *K. Lye 18373* (O), JN634711, JN627724, JN627791; **3.** Norway, Hedmark, Stormysa, *L. Galten and F. Wischmann* (O), JN634709, JN627725, JN627792. ***Carex lutea***— **1.**

USA, North Carolina, EU247878*, —,—; **2.** USA, North Carolina, EU247879*, —, —; **3.** USA, North Carolina, EU247880*, —, —; **4.** USA, North Carolina, EU247881*, —, —. ***Carex monotropa***— Lesotho, Sani Pass, *O. M. Hilliard and B. L. Burtt* (250970 E), JN634679, —, —. ***Carex sp.*** — **1.** USA, Ohio, EU247876*, —, —; **2.** USA, Ohio, EU247877*, —, —. ***Carex viridula*** **s.s.**— **1.** Finland, Inari Lapland, Utsjoki, *H. Toivonen* (321048 MA), JN634666, JN627726, JN627793; **2.** France, Allier, Vernusse, *R. Deschâtres* (562440 MA), JN634667, —, —; **3.** Greece, Pellas, Voras Mount, *M. Luceño and M. Guzmán 1504ML(2)* (UPOS), JN634668, JN627727, JN627794; **4.** Greenland, Moist, *N. Jacobsen* (446898 MA), JN634669, —, —; **5.** Portugal, Beira Litoral, Coimbra, Lagoa das Braças, *M. Luceño* (342693 MA), JN634670, JN627728, JN627795; **6.** Portugal, Beira Litoral, Lagoa da Vela, *P. Jiménez-Mejías et al. 9PJM05(9)* (UPOS), not sequenced; **7.** Spain, Burgos, Iberian Range, Laguna Larga de Neila, *J. M. Marín and M. Luceño 12604JMM(3)*, not sequenced; **8.** Spain, Asturias, Cantabrian Range, Lago Ercina, *J. M. Marín and M. Luceño 15604JMM(3)*, not sequenced.

Problematic morphotypes. ***Carex castroviejoi***— **1A,B.** Greece, Pindhos Range, Epiro, Konitsa, *P. Vargas and M. Luceño 281PV04* (UPOS), individual A sequences JN634712, JN627730, JN627797, individual B sequences JN634713, JN627731, JN627798; **2.** Greece, N Pindhos, Katara Pass, *A. J. Richards* (RNG), JN634714, —, —. ***Carex flava*** **var.** ***alpina***— **1.** Germany, Baviera, Oberbayern, Regierungsbezirk, *H. Löffelmann* (642657 MA), JN634686, —, —; **2.** Switzerland, Valais, *P. Vargas* (342461 MA), JN634687, JN627732, JN627799. ***Carex nevadensis***— **1.** Spain, Almería, Sierra de los Filabres, Gergal, *A. Pallarés* (578965 MA), JN634645, —, —; **2.** Spain, Granada, Sierra Nevada, Laguna de la Caldera, *M. Luceño et al. 6900ML* (UPOS), JN634691, —, —; **3.** Spain, Granada, Sierra Nevada, Laguna de la Mosca, *P. Jiménez-Mejías et al. 219PJM04(1)*, JN634646, JN627733, JN627800; **4.** Spain, Granada, Sierra Nevada, Chorreras de Siete Lagunas, *P. Jiménez.-Mejías et al. 220PJM04(1)*, JN634647, JN627734, JN627801. ***Carex tumidicarpa*** **subsp.** ***cedercreutzii***— **1.** Azores, S. Miguel, *A. Hansen* (RNG), JN634677, JN627735, JN627802; **2.** Azores, Faial, Caldeira, *A. Hansen 264* (RNG), JN634678, —, —. **Atlasic Plants**— **1.** Morocco, High Atlas, Oukaimedem, *S. L. Jury et al.* (RNG), JN634696, —, —; **2.** Morocco, High Atlas, Oukaimedem, *A. Herrero et al.* (746566 MA), JN634697, JN627736, JN627803. **Corsican Plants**— **1.** Corsica, Col Saint-Jean, *J. Lambinon* (694625 MA), JN634672,

JN627737, JN627804; **2.** Corsica, Ghisoni, *M. Escudero and M. Luceño 90ME07(2)*, JN634674, JN627738, JN627805; **3.** Corsica, Asco, *M. Escudero and M. Luceño 109ME07(2)*, JN634676, JN627739, JN627806. **Atlantic-Iberian Plants— 1A,B.** Portugal, Baixo Alentejo, Alcocer do Sal, *P. Jiménez-Mejías et al. 117PJM05* (UPOS), JN634671, JN627740, JN627807; **2A,B,C.** Portugal, Beira Litoral, Louriçal, *P. Jiménez-Mejías et al. 14PJM05* (UPOS), JN634675, JN627741, JN627808; **3A,B,C,D.** Portugal, Estremadura, Serra da Sintra, *P. Jiménez-Mejías et al. 4PJM05* (UPOS), individual B sequences JN634680, JN627742, JN627809, individual C sequences JN634681, JN627743, JN627810; **4.** Spain, Huelva, Andévalo, *P. Jiménez-Mejías et al. 25PJM07* (UPOS), not sequenced. **Pyrenean-Cantabrian Plants— 1.** Andorra, Pyrenees, Lagos de Pessosn, *J. M. Marín et al. 11804JMM* (UPOS), JN634700, JN627744, JN627811; **2.** Francia, Hauter Pyrénées, lac de Badet, *C. Aedo and J. Pedrol* (647393 MA), JN634695, JN627745, JN627812; **3.** Spain, Asturias, Cantabrian Mountains, Somiedo, *P. Jiménez-Mejías et al. 451PJM05(1)* (UPOS), JN634648, JN627746, JN627813. **4A,B,C,D,E,F.** Spain, Cantabria, Cantabrian Mountains, Fuente De, *J. M. Marín et al. 14804JMM* (UPOS), JN634698, JN627747, JN627814; **5.** Spain, Gerona, Pyrenees, Prat Fondal, *A. Pérez-Haase* (BCC), JN634701, JN627748, JN627815; **6A.** Spain, Gerona, Pyrenees, Vall de Ribes, Núria, *S. Castroviejo* (529383 MA), JN634702, JN627749, JN627816; **6B.** *M. Luceño et al. 116BIS05ML* (UPOS), not sequenced; **7.** Spain, Huesca, Pyrenees, Benasque, Aigualluts, *S. Martín-Bravo 546SMB05* (UPOS), JN634692, JN627750, JN627817; **8A,B,C,D.** Spain, Huesca, Pyrenees, Panticosa, Balneario, *P. Jiménez-Mejías et al. 348PJM05* (UPOS), JN634693, JN627751, JN627818; **9.** Spain, Huesca, Pyrenees, Monte Perdido *J. Fernández et al. 69JFA03* (UPOS), JN634694, JN627752, JN627819. **10.** Spain, Huesca, Pyrenees, Aneyet, *P. Jiménez-Mejías et al. 433PJM05* (UPOS), not sequenced; **11A.** Spain, Lérida, Pyrenees, Val d'Aran, Baños de Tredós, *J. M. Marín et al. 7704JMM* (UPOS), JN634649, JN627753, JN627820; **11B.** *M. Luceño et al. 9205ML* (UPOS), not sequenced.

Section *Spirostachyae. Carex distans*— France, Pyrénées Atlantiques, Cambo les Bains, *M. Escudero and P. Jiménez-mejías 1ME06(1)* (UPOS), EU483663, JN627754, JN627821.

Section *Sylvaticae*. *Carex cretica*— Crete, Kissamos, Koutsomatados, *S. Martín-Bravo et al. 389SMB05(2)* (UPOS), DQ384118, JN627755, JN627822.

Section *Tumidae*. *Carex melanostachya*— Austria, Neusiedler See, *O. Angerer* (M 127402), JN634644, JN627756, JN627823.

Appendix 2

Nucleotide variation in plastid 5´*trnK*/*rps16* sequences. Countries are termed following TDWG geographical codes (Brummit 2001) at level 3 "Botanical countries." Nucleotide position refers to the aligned matrix only with core *Ceratocystis* sequences.

Sample	Country	**Haplotype**	*5'trnK*																*rps16*							
			0 3 3	0 4 4	0 4 5	0 5 6	1 2 8	1 4 6	3 0 8	3 2 2	4 1 0	4 5 6	5 3 1	5 4 9	5 7 1	5 9 0	6 4 4	0 6 4	1 5 0	2 4 6	5 6 4	5 2 4	6 2 0	7 2 5	7 3 7
C.castroviejoi-1A,1B	GRC																									
C.tumidicarpa cedercreutzii-1	AZO																									
Corsican-1	COR																									
C.demissa-1	AUT																									
C.demissa-2	GRB	H1	T	G	C	C	T	A	G	T	G	G	C	G	-	G	C	C	T	T	C	C	C	T	G	
C.demissa-3	MDR																									
C.demissa-4	MOR																									
C.demissa-5	SAR																									
C.demissa-6	SIC																									
C.demissa-7,8,9,10,11,13B	SPA																									
Corsican-2,3	COR																									
C.demissa-14	SPA																									
Atlantic-Iberian-2C	POR	H2	T	G	C	C	T	A	G	T	G	G	C	G	**G**	G	C	C	T	T	C	C	C	T	G	
C.viridula-1	FIN																									
C.viridula-3	GRC																									
Atlantic-Iberian-1A,3B,3C	POR	H3	T	G	C	**G**	T	A	G	T	G	G	C	G	**G**	G	C	C	T	T	C	C	C	T	G	
C.viridula-5	POR	H4	T	G	C	C	T	A	G	T	G	G	C	G	**G**	G	C	C	**G**	T	C	C	C	T	G	
C. flava-2	BUL	H5	T	G	C	C	T	A	**A**	**C**	**A**	**T**	C	G	-	G	C	C	T	T	C	C	C	**A**	G	
C. flava-3A	ITA																									
C. flava alpina-2	SWI																									
C. flava-5,6	NOR	H6	T	G	C	C	T	A	**A**	**C**	**A**	G	C	G	-	G	C	C	T	T	C	C	C	**A**	G	
C. flava-7	RUN																									
C. flava-1	BGM	H7	T	G	C	C	T	A	**A**	**C**	**A**	G	C	G	-	G	C	C	T	T	C	C	C	T	**C**	
C. lepidocarpa s.s.-1	BGM																									
C. lepidocarpa s.s.-5A,8	SPA																									
C. lepidocarpa s.s.-9	SWE																									
C. nevadensis-3	SPA	H8	T	G	C	C	T	A	**A**	**C**	**A**	G	C	G	-	G	C	**A**	T	T	C	C	C	T	G	
Atlasic plant-2	MOR																									
Pyrenean-Cant.-2	FRA																									
Pyrenean-Cant.-3,4E,7,8A	SPA																									
C.lepidocarpa s.s.-4,7	SPA	H9	T	G	C	C	T	A	**A**	**C**	**A**	G	C	G	-	G	C	**A**	T	T	C	**A**	C	T	G	
Pyrenean-Cant.-9	SPA	H10	T	G	C	C	T	A	**A**	**C**	**A**	G	C	G	-	**T**	C	**A**	T	T	**A**	C	C	T	G	

(Continued)

(Continued)

Sample	Country	**Haplotype**	*5'trnK*															*rps16*							
			0	0	0	0	1	1	3	3	4	4	5	5	5	5	6	0	1	2	5	5	6	7	7
			3	4	4	5	2	4	0	2	1	5	3	4	7	9	4	6	5	4	6	2	2	2	3
			3	4	5	6	8	6	8	2	0	6	1	9	1	0	4	4	0	6	4	4	0	5	7
Pyrenean-Cant.-1[1],5,6A,11A	SPA	H11	T	G	C	C	T	A	**A**	**C**	**A**	G	**A**	G	-	G	C	**A**	T	T	C	C	C	T	G
C.nevadensis-4	SPA	H12	T	G	C	C	T	A	**A**	**C**	**A**	G	C	G	-	G	**T**	**A**	T	T	C	C	C	T	G
C.lepidocarpa jemtlandica-1,2,3	NOR	H13	T	**A**	C	C	T	A	**A**	**C**	**A**	G	C	G	-	G	C	C	T	T	C	C	C	T	**C**
C. lepidocarpa s.s.-3[2]	YUG																								
C. lepidocarpa s.s.-10	SWI	H14	T	**A**	C	C	T	A	**A**	**C**	**A**	G	C	G	-	G	C	C	T	T	C	C	C	T	G
C. hostiana-3	SPA	H15	T	G	C	C	**G**	A	G	T	G	G	C	**A**	-	G	C	C	T	**A**	C	C	**A**	T	G
C. hostiana-1	GER	H16	**G**	G	**T**	C	**G**	A	G	T	G	G	C	**A**	-	G	C	C	T	T	C	C	**A**	T	G
C. hostiana-2	GRB	H17	T	G	C	C	T	**T**	G	T	G	G	C	G	-	G	C	C	T	T	C	C	C	T	G

[1] Andorra; [2] Slovenia

Appendix 3

Nucleotide variation in nuclear ITS sequences. Nucleotide positions differing with respect to the widespread R1 are marked in bold. Countries are termed following TDWG geographical codes (Brummit 2001) at level 3 "Botanical countries." Nucleotide position refers to the aligned matrix only with core *Ceratocystis* sequences.

Sample	Country	**Ribotypes**	ITS1																										5.8s				ITS2									
			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	2	2	2	2	3	4	4	4	4	5	5	5	5	5	5	5	6
			0	0	1	2	2	3	4	4	4	5	6	7	7	8	0	2	2	5	5	6	8	9	9	0	2	2	2	7	0	1	2	3	3	1	4	5	6	8	8	9	0
			1	2	5	7	8	3	3	4	8	0	4	2	6	7	9	4	7	3	8	0	9	6	8	7	0	5	8	8	7	8	1	4	5	5	3	0	7	3	6	8	2
C.demissa-1	AUT																																									
C.demissa-2	GRB																																									
C.demissa-3	MAD																																									
C.demissa-4	MOR																																									
C.demissa-5	SIC																																									
C.demissa-7	SPA																																									
C.demissa-9	SPA																																									
C.demissa-10	SPA																																									
C.demissa-11	SPA																																									
C.demissa-13	SPA																																									
C.lepidocarpa s.s.-1	BGM	R1	C	C	A	T	C	A	C	C	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.lepidocarpa s.s.-2	IRE																																										
C.lepidocarpa s.s.-9	SWE																																										
C. nevadensis-1,3,4	SPA																																										
Pyrenean-Cant.-3,11A	SPA																																										

(Continued)

(Continued)

Sample	Country	**Ribotypes**	ITS1																									5.8s				ITS2									
			001	002	015	027	028	033	043	044	048	050	064	072	076	087	109	124	127	153	158	160	189	196	198	207	220	225	228	378	407	418	421	435	515	543	550	567	583	586	588	592	602
		R1	*C*	*C*	*A*	*T*	*C*	*A*	*C*	*C*	*G*	*C*	*C*	*T*	*C*	*T*	*C*	*T*	*G*	*A*	*G*	*T*	*A*	*T*	*T*	*A*	*A*	*A*	*C*	*T*	*T*	*C*	*C*	*C*	*A*	*A*	*C*	*T*	*A*	*C*	*C*	*C*	*T*	*C*
C.viridula-2	FRA	R3	C	C	A	T	C	**G**	C	**T**	**A**	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.demissa-14 *C.viridula*-1 *C.viridula*-3 *C.viridula*-4 *C.viridula*-5 Atlantic-Iberian-1A	SPA FIN GRC GNL POR POR	R1R3	C	C	A	T	C	**R**	C	**Y**	**R**	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
Corsican-1	COR	R2R3	C	C	A	T	C	**R**	C	**Y**	**R**	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	**R**	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.barrosii-1 *C.cryptolepis*-5	CLS NWY	R4	C	C	A	T	C	A	C	**T**	**A**	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
Corsican -2 Atlantic-Iberian-2C	COR POR	R1R4	C	C	A	T	C	A	C	**Y**	**R**	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
Corsican -3	COR	R2R5	C	C	A	T	C	**R**	C	**Y**	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	**R**	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.tumidicarpa cedercreutzii-1,2	AZO	R1R6	C	C	A	T	C	**R**	C	C	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.cryptolepis-1	ONT	R7	C	C	A	T	C	A	C	**T**	**A**	**T**	C	T	**T**	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C

(Continued)

(Continued)

Sample	Country	**Ribotypes**	ITS1																										5.8s				ITS2													
			001	002	015	027	028	033	043	044	048	050	064	072	076	087	089	102	124	127	153	158	160	189	196	198	207	220	225	228	378	407	418	421	435	515	543	550	567	583	586	588	598	602
		R1	*C*	*C*	*A*	*T*	*C*	*A*	*C*	*C*	*G*	*C*	*C*	*T*	*C*	*T*	*C*	*T*	*C*	*T*	*G*	*A*	*G*	*T*	*A*	*T*	*T*	*A*	*A*	*A*	*C*	*T*	*T*	*C*	*C*	*C*	*A*	*A*	*C*	*T*	*A*	*C*	*C*	*C*	*T*	*C*
C.cryptolepis-2 *C.cryptolepis*-3 *C.cryptolepis*-4	OHI WIS MIN	R4R8	C	C	A	T	C	A	C	**T**	**A**	C	C	T	**Y**	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.flaviformis-1	NZS	R9	C	**T**	A	T	C	A	C	**T**	**A**	T	C	T	**T**	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.monotropa-1	LES	R10	C	C	A	T	C	A	C	**T**	**A**	C	**G**	T	C	T	C	T	**A**	A	G	T	A	T	T	A	A	A	C	**C**	**C**	C	C	**T**	A	A	**T**	**G**	A	**T**	C	C	T	**T**
Atlantic-Iberian-3B,3C	POR	R1R11	C	C	A	T	C	A	C	C	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	**Y**	C	T	C
C.flava-1	BGM	R12	C	C	A	T	**T**	A	C	**T**	G	C	C	**A**	C	T	**T**	T	G	A	G	T	A	T	T	A	A	A	**G**	T	T	C	C	C	A	A	C	**G**	A	C	**T**	C	T	C
C.flava-8	NFL	R13	C	C	A	T	**T**	A	C	**T**	G	C	C	**A**	C	T	**T**	T	G	A	G	T	A	T	T	A	A	A	**T**	T	T	C	C	C	A	A	C	**G**	A	C	**T**	C	T	C
C.flava-2	BUL	R13R14	C	C	A	T	**T**	A	C	**T**	G	C	C	**W**	C	T	**Y**	T	G	A	G	T	A	T	T	A	A	A	**W**	T	T	C	C	C	A	A	C	**K**	A	C	**T**	C	T	C
C.flava-3,4	ITA	R1R15	C	C	A	T	**Y**	A	C	**Y**	G	C	C	**W**	C	T	**Y**	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	**K**	A	C	**Y**	C	T	C
C.flava alpina-1	GER	R1R16	C	C	A	T	**Y**	A	C	**Y**	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.flava alpina-2	SWI	R16R17	C	C	A	T	**Y**	A	C	**T**	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.flava-5,6 *C.flava*-7	NOR RUN	R1R18	C	C	A	T	**Y**	A	C	C	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C

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(Continued)

Sample	Country	**Ribotypes**	ITS1																									5.8s				ITS2									
			001	002	015	027	028	033	043	044	048	050	064	072	076	078	079	102	124	127	153	158	160	189	196	198	207	220	225	228	378	407	418	423	435	515	543	550	567	583	586	588	598	602
		R1	*C*	*C*	*A*	*T*	*C*	*A*	*C*	*C*	*G*	*C*	*C*	*T*	*C*	*T*	*C*	*T*	*G*	*A*	*G*	*T*	*A*	*T*	*T*	*A*	*A*	*A*	*C*	*T*	*T*	*C*	*C*	*C*	*A*	*A*	*C*	*T*	*A*	*C*	*C*	*C*	*T*	*C*
C.nevadensis-2 Pyrenean-Cant.-7	SPA	R1R19	C	C	A	T	C	A	C	C	G	C	**Y**	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
Pyrenean-Cant.-8A	SPA	R19R20	**Y**	C	A	T	**Y**	A	C	C	G	C	**T**	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
Pyrenean-Cant.-9	SPA	R19R21	**Y**	C	A	T	C	A	C	C	G	C	**T**	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
Pyrenean-Cant.-2	FRA	R21R22	**T**	C	A	T	C	A	C	C	G	C	**Y**	T	C	T	C	T	G	A	G	T	**R**	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
Atlasic-1,2 Pyrenean-Cant.-4E	MOR SPA	R23	C	C	A	T	C	A	C	C	G	C	C	T	C	T	C	T	G	A	G	T	**G**	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.lepidocarpa s.s.-5A Pyrenean-Cant.-1[1],5,6A	SPA	R1R23	C	C	A	T	C	A	C	C	G	C	C	T	C	T	C	T	G	A	G	T	**R**	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.lepidocarpa s.s.-4,6,7,8	SPA	R1R24	C	C	A	T	**Y**	A	C	C	G	C	**Y**	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.lepidocarpa s.s.-3[2]	YUG	R25R26	C	C	A	T	**Y**	A	C	C	G	C	**Y**	T	C	T	C	T	G	A	**A**	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.lepidocarpa jemtlandica-3	NOR	R1R26	C	C	A	T	C	A	C	C	G	C	C	T	C	T	C	T	G	A	**R**	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C. lepidocarpa s.s.-10	SWI																																										

(Continued)

(Continued)

Sample	Country	**Ribotypes**	ITS1																								5.8s				ITS2									
			001	002	015	027	028	033	043	044	048	050	064	072	076	087	109	124	127	153	158	160	189	196	198	207	220	225	228	378	407	418	423	435	514	530	567	613	683	686	688	689	902
		R1	*C*	*C*	*A*	*T*	*C*	*A*	*C*	*C*	*G*	*C*	*C*	*T*	*C*	*T*	*C*	*T*	*G*	*A*	*G*	*T*	*A*	*T*	*T*	*A*	*A*	*A*	*C*	*T*	*T*	*C*	*C*	*C*	*A*	*A*	*C*	*T*	*A*	*C*	*C*	*C*	*T*	*C*
C.lepidocarpa jemtlandica-2	NOR	R19R26	C	C	A	T	C	A	C	C	G	C	**Y**	T	C	T	C	T	G	A	**R**	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.lepidocarpa jemtlandica-1	NOR	R27	C	C	A	**C**	C	A	C	C	G	C	**T**	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	T	A	C	C	C	T	C
C.castroviejoi-1A,1B,2	GRC	R28	C	C	**G**	T	C	A	**T**	C	G	C	C	T	C	T	C	**C**	G	A	G	T	A	**C**	T	**G**	A	**G**	C	T	T	C	C	C	A	A	C	T	**C**	C	C	C	T	C
C.hostiana-1 *C.hostiana*-3	GER SPA	R29	C	C	**G**	T	C	A	C	C	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	C	C	C	A	A	C	**G**	**C**	C	C	C	T	C
C.hostiana-2	GRB	R29R30	C	C	**G**	T	C	A	C	C	G	C	C	T	C	T	C	T	G	A	G	T	A	T	T	A	A	A	C	T	T	**Y**	C	C	A	A	C	**K**	**M**	C	C	C	T	C
C.lutea-1,2,3,4	NCA	R31	C	C	**T**	T	C	A	C	C	G	C	C	T	C	**A**	C	T	G	**T**	G	T	A	T	T	**G**	A	A	C	T	**C**	C	**T**	C	**G**	**G**	C	**G**	A	C	C	**T**	T	C
C.sp.-1,2	OHI	R33	C	C	**T**	T	C	A	C	C	G	C	C	T	C	T	C	T	G	A	G	**A**	A	T	**C**	**G**	**G**	A	C	T	T	**T**	C	C	**G**	A	C	**G**	**C**	C	C	C	**A**	C

[1] Andorra; [2] Slovenia

CAPÍTULO 3

Morphological boundaries reassessed within *Carex flava* group in the Iberian Peninsula, North Africa and Macaronesia

P. Jiménez-Mejías, S. Martín-Bravo & M. Luceño

Abstract

Statistical studies of morphological variation within plant complexes facilitate the unravelling of their intricate taxonomy, avoiding subjectivity in taxonomic partitions. The *Carex flava* group is a species complex within which hybridization phenomena are common, obscuring the taxonomic boundaries between taxa. The Iberian Peninsula, North Africa and Macaronesia constitute the SW limit of the Old World distribution of the group. Despite being a peripheral area, it is also one of the most problematic from a taxonomic point of view due to the presence of several problematic morphotypes of disputed status: Atlasic populations, Atlantic Iberian plants, Pyrenean-Cantabrian plants, *C. nevadensis* from Sierra Nevada and *C. tumidicarpa* ssp. *cedercreutzii* from Azores. Using quantitative characters, we performed a biometric study using multivariate and univariate techniques. A standardized analytical approach revealed the occurrence of five well-defined morphogroups: *C. demissa*, *C. flava*, *C. lepidocarpa*, *C. viridula* and a high mountain morphotype from the Sierra Nevada and Atlas. Internal heterogeneity was revealed within *C. demissa* when the Azorean populations were tested for taxonomic significance. Populations from the Pyrenees and Cantabrian mountains were found to have an intermediate morphology between *C. demissa* and *C. lepidocarpa*. In addition some individuals showed affinities to *C. flava*. The Atlantic Iberian morphotype, despite having been reported as a *C. demissa* × *C. viridula* hybrid, displayed a disparate morphology that sometimes matched *C. lepidocarpa*.

Keywords. Clinal variation, discriminant analysis, MANOVA, principal component analysis.

Introduction

Taxonomic problems within plant complexes are one of the main causes of disagreement between floristic treatments, and thus of instability in the classification system. Different authors recognize different taxonomic partitions, often at different taxonomic ranks. Faced with this problem, empirical measurement of morphological variation is highly desirable, and may help to unify taxonomic opinions and to establish a standardized starting point for further treatments of the studied group.

Carex sect. *Ceratocystis* Dumort is a small group of sedges (4–18 species depending on the treatment (Jiménez-Mejías *et al.*, under review (Chapter 2)) that is mainly distributed in temperate Eurasia, North Africa and North America (Schmid, 1983; Crins, 2002). The main morphological characteristics of this group are obovate achenes, utricles attenuated in a long bifid beak and short subcylindrical to elliptical or globose female spikes (Crins and Ball, 1988; Crins, 2002; Luceño and Jiménez Mejías, 2008). Hybridization processes (Schmid, 1982, 1984; Hedrén and Prentice, 1996) and scarcely marked distinctive features have greatly hindered the taxonomy of the group (Schmid, 1983; Crins and Ball, 1989a,b; Egorova, 1999; Luceño and Jiménez-Mejías, 2008). As a consequence, botanists generically refer to most of the members (with the exception of *C. hostiana* DC.) as "*Carex flava* group" (Schmid, 1983; Crins and Ball, 1988; Stoeva and Štěpánková, 1990; Pikälä and Toivonen, 1994; Hedrén, 2002; Luceño and Jiménez-Mejías, 2008).

Leaving aside matters of taxonomic rank, most authors recognize four widely distributed taxa within the aggregate, namely *C. flava* L., *C. demissa* Hornem., *C. lepidocarpa* Tausch. and *C. viridula* Michx. In addition, there are some more or less restricted endemics, such as the American *C. cryptolepis* Mack. and *C. lutea* Le Blond, or the recently described European *C. castroviejoi* Jim.-Mejías & Luceño. The taxonomic independence of these taxa is supported by morphometric (Schmid, 1983; Crins and Ball, 1989a; Pykälä and Toivonen, 1994; Blackstock and Ashton, 2001), chemical (Derieg *et al.*, 2008; Hedrén, 2002, 2004; Blackstock and Ashton, 2010) and molecular (Derieg *et al.*, 2008; Jiménez-Mejías *et al.*, under review (Chapter 2)) data.

Cytogenetically, *Carex flava* gr. can be roughly divided into two categories: plants with low chromosome numbers ($2n \leq 61$) such as *C. flava* ($2n = 58,60$), and those with relatively high chromosome numbers ($2n = 66–72$), including *C. demissa*, *C.*

lepidocarpa and *C. viridula* (Schmid, 1982; Halkka *et al.*, 1992; Luceño and Castroviejo, 1993; Roalson, 2008). This cytogenetic pattern lends support to the hypothesis that *C. flava* gr. constitutes an aneuploid series with two biological species (Schmid, 1982, 1983; Crins and Ball, 1989b): *C. flava*, and a wide *C. viridula* concept including the remaining taxa. Our recent molecular approach (Jiménez-Mejías *et al.*, under review) showed that *C. flava* gr. is monophyletic and is arranged in two clades, one containing plants mainly with patent utricles (*C. demissa*, *C. viridula* and allies; herein referred to as the patent-utricles phylogroup) and the other grouping species with deflexed utricles (*C. flava*, *C. lepidocarpa* and allies; herein, deflexed-utricles phylogroup). In addition, the identified haplotype sequences showed clear taxon-specificity (76.5%), which further increased if morphologically equivocal populations were included within the most closely related well-defined taxon (88.2%).

Definitive clarification of the taxonomy of the *Carex flava* gr. is not yet in sight, despite the quantity of studies on the subject. Together with more broadly circumscribed studies, local studies remain necessary to unravel the evolutionary behaviour and taxonomic arrangement of the group. This will ensure that underlying diversity is not neglected when analysed in broader contexts. For example, a regional study on Swedish *C. lepidocarpa s.l.* (Hedrén and Prentice, 1996) shed light on the existence of two races within this taxon (ssp. *lepidocarpa* and ssp. *jemtlandica* Palmgr.), and locally focused researches refused the taxonomic value of *C. bergrothii* Palmgr. (Hedrén, 1998) and clarified the situation with regard to *C. flava* in the British Isles (Blackstock and Ashton, 2001, 2010). Most of these investigations have been performed in the northern part of the *C. flava* gr. distribution range, whereas the situation in southern areas remains relatively unclear. It is known that, in comparison with northern populations, southernmost areas display different levels of genetic variation (Taberlet *et al.*, 1998; Hewitt, 1999; among others) and differentiation (Hampe and Petit, 2005). Phenomena such as isolation and long-standing Pleistocene glacial refuges, or peripheral differentiation during warmer periods (Hampe and Petit, 2005), are behind these differences. Thus, a different situation could be found in the southern extreme of the *C. flava* gr. distributional range.

Taxonomic treatments of *C. flava* gr. in the Iberian Peninsula differ considerably in the number, rank and distribution of the taxa considered (Table 1). The only biosystematic study specifically focused in this area was the cytogenetic study of Luceño and Castroviejo (1993). On the other hand, Macaronesian and North African

populations have been obliquely treated in systematic revisions (Schmid, 1983; Crins and Ball, 1989a,b), or revised within the context of local floras and checklists (Maire, 1957; Press and Short, 1997; Valdés *et al.*, 2002; Acebes-Ginovés *et al.*, 2004; Silva *et al.*, 2005; Jardim and Sequeira, 2008) with no regard for more comprehensive points of view.

Multivariate morphometric analyses have been widely used to unravel the taxonomic structure of the group. These techniques help to summarize large datasets, synthesizing the problem in exploratory studies. Studies on *C. flava* gr. have established widely accepted key characters for taxa identification and delimited the morphological boundaries between them. The present study provides the statistical background for the already published *Flora Iberica* treatment (Luceño and Jiménez-Mejías, 2008), which was indeed based on preliminary results from our first genetic and morphometric explorations. The prospected area was enlarged to cover the neighbouring areas of Macaronesia and North Africa. Thus, the main aims of this study were to contribute to consolidating the taxonomy of the group by: 1) morphometric delimitation of those well-defined taxa that occur in the studied area; 2) testing the cohesiveness of the problematic morphogroups within the already established phyletic contexts, but also considering clinal variation between the different genetic groups; 3) considering those phenomena that shape the inferred morphological structure of the group in the Iberian Peninsula, North Africa and Macaronesia.

Materials and methods

STUDY GROUP

Nine categories were defined: four well-defined species (*C. demissa*, *C. flava*, *C. lepidocarpa* and *C. viridula*), which are broadly accepted to have been delimited in previous studies; and five problematic morphotypes (Atlantic-Iberian, Pyrenean-Cantabrian and Atlasic plants, *C. nevadensis* and *C. tumidicarpa* ssp. *cedercreutzii*), a set of populations of uncertain taxonomic assignation.

Atlantic-Iberian populations were previously reported as *C. flava* (Coutinho, 1939; Sampaio, 1946; Luceño, 1994). Even B. Schmid, a monographer, identified vouchers as *C. flava* in revision tags. Luceño and Jiménez-Mejías (2008) described

Table 1. *Carex flava* gr. treatments for North Africa and the Iberian Peninsula considered in the main floristic works and systematic revisions of *Carex*. The first column indicates the tentative classification followed in this study, mainly following that in Luceño and Jiménez-Mejías (2008). that in Luceño and Jiménez-Mejías (2008).

		Maire, 1957	Vicioso 1959	Luceño 1994	Franco and Afonso 1994	Bolòs and Vigo 2001
Well-defined taxa	***C. demissa*** Hornem**.**	*C. flava* ssp. *oederi* (Retz.) Syme	*C. flava* p.p. *C. oederi* var. *demissa* (Hornem.) C.Vicioso	*C. demissa*	*C. demissa*	*C. flava* ssp. *oedocarpa* (Andersson) P.D.Sell
	C. flava L.	-	-	*C. flava*	-	*C. flava* ssp. *lepidocarpa* (Tausch) Nyman
	C. lepidocarpa Tausch.	*C. flava* ssp. *eu-flava* Maire	*C. flava* p.p. *C. lepidocarpa*	*C. lepidocarpa*	-	*C. flava* ssp. *lepidocarpa*
	C. viridula Michx.	-	-	*C. viridula*	*C. viridula* ssp. *viridula*	*C. flava* ssp. *viridula* (Michx.) O.Bolòs & Vigo
Problematic morphotypes	**Atlantic-Iberian plants**	-	-	*C. flava*	*C. demissa*	-
	Pyrenean-Cantabrian plants	-	*C. oederi* var. *alpestris* Asch. & Graebn.	*C. flava* p.p. *C. lepidocarpa* p.p.	-	*C. flava* ssp. *alpina* (Kneuck.) O.Bolòs, Masalles & Vigo
	C. nevadensis Boiss & Reuter (s.l.)	*C. flava* var. *nevadensis* (Boiss. & Reuter) Briq.	*C. lepidocarpa* var. *nevadensis* (Boiss &. Reut.) Kük. *C. oederi* var. *alpestris*.	*C. lepidocarpa*	-	-

them as probable hybrids between *C. demissa* and *C. viridula* due to the ambiguous molecular attribution observed using nuclear and plastid markers (Jiménez-Mejías *et al.*, under review (Chapter 2).

Pyrenean-Cantabrian populations were also characterized as hybrid populations (Jiménez-Mejías *et al.* under review), since remarkable cytogenetic instability was detected despite molecular homogeneity. In this case, the proposed parental taxa were *C. demissa* and *C. lepidocarpa*, as hybrids showed intermediate features of these taxa. Additionally, cytotypes related to *C. flava* were found in some populations with plants morphologically similar to this taxon. This led us to suspect that this species could also take part in the hybrid pool. Some of the reports of *C. flava* from North Spain (Luceño, 1994; Bolòs and Vigo, 2001) were based on this problematic morphotype.

Carex tumidicarpa ssp. *cedercreutzii* was described from the Azores (Fagerström, 1967). This taxon is accepted by Macaronesian authors in an expanded sense, including populations from Madeira and the Canary Islands (León *et al.*, 1990; Press and Short, 1994). However, its taxonomic status has been disputed by monographers. Schmid (1980) considered that Azorean and Madeiran plants deserved taxonomic recognition (*C. viridula* ssp. *cedercreutzii* (Fagerstr.) B. Schmid), whereas Crins and Ball (1989a,b) observed no significant differences and regarded it as *C. demissa*. Chater (1980) briefly described Azorean populations as taxonomically doubtful in *Flora Europaea*.

Lastly, Atlasic plants and *C. nevadensis* are small plants from the high mountains of the High Atlas (SW Morocco) and Sierra Nevada-Filabres range (SE Spain, Andalusia) respectively. They are geographically isolated from other taxa of the group. Lack of differentiation between high mountain morphs from S Europe makes these plants and related populations truly problematic. This led Chater (1980) and Schmid (1983) to consider many of the southern European mountain populations under a widened *C. nevadensis* concept, and North African authors (Maire, 1957; Valdés *et al.*, 2002) to regard Atlasic plants as conspecific with the SE Spanish taxon. In contrast, Luceño (1994) proposed that such plants were extreme variations (dwarf specimens) within a clinal continuum, refusing to attribute any taxonomic value and considering them to be *C. lepidocarpa* mountain ecotypes.

SAMPLING

Dried specimens from the following herbaria were examined for this study: ARAN, COI, FI, JACA, K, LEB, LISE, LISI, LISU, M, MA, MSB, O, RNG and TFC, as well as field collections that were deposited in UPOS.

A total of 194 Iberian, North African and Macaronesian populations were measured (Appendix 1): 111 of well-defined taxa (55 *C. demissa*, 39 *C. lepidocarpa*, 17 *C. viridula*) and 83 of problematic morphotypes (6 Atlasic plants, 10 Atlantic-Iberian plants, 43 Pyrenean-Cantabrian plants, 11 *C. nevadensis* and 13 Azorean *C. tumidicarpa* ssp. *cedercreutzii*). In addition, 13 populations of non-Iberian *C. flava* were included as a control in order to identify possible *C. flava* individuals among Pyrenean-Cantabrian plants. The amount of studied material was greatly limited by the condition of the specimens. The inclusion of curvature characters of the fruit (see Table 2) forced us to choose only those materials that included complete female spikes that had not become deformed after pressing. This limitation was particularly problematic for Atlantic-Iberian plants, since little recent material was available in the herbaria and most of the utricles had become detached.

The measured characters were selected from those previously reported in taxonomic studies and our own observations. Seventeen taxonomically useful quantitative characters were selected (Table 2) from a previous study of 24 characters. In contrast to previous works, we focused on the utricle-beak curvature and the relative position of the utricle with respect to the axis of the spike, in the upper, middle and lower part of each spike.

Measurements were taken using an ocular micrometer, with the exception of the largest characters (more than 10 mm), which were measured using a standard 30 cm rule. Angles were measured using an angular encoder. Where possible, up to three ripe stems were measured on each sheet; the values used in the analyses were the averages of these measurements.

ANALYTICAL APPROACH

All statistical analyses were performed using the software PASW statistics v.18 (SPSS, Chicago, Illinois, USA).

- Step 1: Preliminary exploration

In Step 1 we explored the structure of the dataset and checked whether different groups could be obtained, depicting the main morphological relationships among the defined categories. A PCA was performed over the whole dataset using all measured characters. Kaiser's measure of sampling adequacy and Barlett's test of sphericity were performed to evaluate the suitability of the data for finding structure. Only principal components with eigenvalues greater than 1 were retained.

We evaluated the homogeneity of the well-defined taxa and, subsequently, morphological relationships between those phylogroups found by Jiménez-Mejías *et al.* (under review (Chapter 2)).

- Analyses of datasets included in Steps 2 and 3

In Steps 2 and 3 the dataset was split in order to analyse only part of the overall variation. We statistically tested the robustness of the morphogroups obtained in Step 1, and whether subjacent morphological relationships could be found when only part of the overall variation was analysed. Multivariate exploratory analyses are sensitive to heterogeneity in the datasets (cf. Jiménez-Mejías and Cabezas, 2007). Thus, restricting datasets to more closely allied plants and considering only the more variable characters, boundaries between groups can become clearer or the underlying structure can be revealed.

The analytical approach followed in Steps 2 and 3 was based on that suggested for *Hedera* by Valcárcel and Vargas (2010), which can be summarized in three stages: 1) First, the structure of the given dataset was explored by PCA. In order to achieve the best split among homogeneous morphogroups, only those characters with the highest scores in a preliminar correlation matrix were selected to explore each subset. As in Step 1, Kaiser's measure of sampling adequacy and Barlett's test of sphericity were performed and only principal components with eigenvalues greater than 1 were retained. 2) Second, following the identification of homogeneous morphogroups, DFA was used to test the accuracy of our *a priori* identification. Analyses were performed using a within-group covariation matrix. For each subset, the same variables as included in the PCA were included. To evaluate the discriminant function, 30% of the samples were randomly excluded from the analyses and used as a confirmatory blind control.

Table 2. Variables included in the different analyses performed and scores obtained in the first plotted components of PCA analyses (PCI, PCII and PCIII). Those variables that most contributed to each component are in bold. Analyses and subsets are abbreviated as follows: preliminary exploration (PE), patent utricles phylogroup (PUP), *C. demissa* plus *C. tumidicarpa* ssp. *cedercreutzii* (DC), deflexed utricles phylogroup (DUP) and morphologically continuous subsets 1 (s1), 2 (s2) and 3 (s3).

[1] Internode length between the two uppermost female spikes; [2] The angle measured was considered from 180º (erect) to 0º (deflexed)

Variable	Description	Analyses / datasets															
		PE		**PUP**		**DC**			**DUP**		**s1**		**s2**		**s3**		
		PCI	PCII	PCI	PCII	PCI	PCII	PCIII	PCI	PCII	PCI	PCII	PCI	PCII	PCI	PCII	PCIII
STLN	Stem length (cm)	-0.34	0.34	0.51	0.44	0.47	0.35	**0.68**	0.55	0.60	-0.59	0.39	0.62	0.53	0.23	0.26	0.58
LFWD	Upper leaf width (mm)	-0.85	0.65	0.58	0.22	0.66	-0.37	0.37	-	-	-	-	-	-	0.09	**0.89**	0.08
ST/LF	Stem / upper leaf length ratio	-0.14	0.01	-	-	0.16	0.17	**0.84**	-	-	-0.60	0.05	0.60	0.49	0.60	-0.31	0.46
BRLN	Lowest bract length (cm)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	0.10	-0.04
BRWD	Lowest bract width (mm)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	**0.82**	0.17
BRSH	Lowest bract sheath length (mm)	0.07	0.01	0.11	**0.92**	**0.86**	-0.54	-0.15	-	-	0.22	-0.10	-0.30	**0.73**	-	-	-
UPIN[1]	Inflorescense uppermost internode length (mm)	-	-	-	-	-	-	-	0.68	-0.32	-	-	-	-	-	-	-
LWIN	Inflorescense lowest internode length (mm)	0.14	0.03	0.16	**0.86**	**0.83**	-0.50	0.20	-	-	0.25	-0.04	-0.29	0.77	-0.03	0.14	**0.91**

(Continued)

Table 2 *(Continued)*

Variable	Description	Analyses / datasets															
		PE		**PUP**		**DC**			**DUP**		**s1**		**s2**		**s3**		
		PCI	PCII	PCI	PCII	PCI	PCII	PCIII	PCI	PCII	PCI	PCII	PCI	PCII	PCI	PCII	PCIII
MSLN	Male spike length (mm)	-0.18	0.37	0.61	0.57	0.63	0.42	0.21	**0.75**	0.36	-0.31	0.17	0.37	**0.71**	0.42	0.35	0.32
MSPD	Male spike peduncle lenght (mm)	-	-	-	-	-	-	-	**0.82**	-0.09	-	-	-	-	-	-	-
UTLN	Utricle total length (mm)	-0.26	**0.86**	**0.90**	0.13	0.10	**0.91**	0.02	0.20	**0.92**	-0.26	**0.90**	0.55	0.58	0.58	0.63	0.18
UTWD	Utricle width (mm)	-0.29	0.69	-	-	0.28	0.49	-0.56	0.14	0.76	-0.14	0.67	-	-	0.50	0.44	0.14
UTBK	Utricle beak length (mm)	-0.06	**0.85**	**0.92**	0.13	0.01	**0.86**	0.24	-0.10	**0.90**	-0.06	**0.91**	0.37	0.67	**0.69**	0.49	0.16
UTCV	Utricle beak curvature (degrees)	**-0.83**	0.18	0.22	0.04	-	-	-	0.61	0.22	**-0.80**	0.20	**0.84**	0.04	0.39	0.29	0.55
UTUP[2]	Upper utricles arrangement (degrees)	0.74	-0.06	-	-	-	-	-	-	-	**0.77**	-0.03	**-0.80**	0.01	-	-	-
UTMD[2]	Middle utricles arrangement (degrees)	**0.83**	-0.17	-	-	0.01	-0.13	-0.02	-0.71	-0.32	**0.80**	-0.20	**-0.86**	-0.03	**-0.71**	-0.02	-0.05
UTLW[2]	Lower utricles arrangement (degrees)	**0.79**	-0.23	-	-	-0.18	-0.03	-0.09	-0.58	-050	**0.79**	-0.19	**-0.85**	-0.02	**-0.80**	-0.13	-0.01

Classifications provided by DFA (considering included and excluded samples) were evaluated using Cohen's kappa statistic (Titus *et al.*, 1984). If after Steps 2 and 3 an underlying structure was suspected for any recovered morphogroup, the dataset was split and reanalysed. 3) Lastly, MANOVA and the Scheffé post-hoc test were used to statistically validate the most discriminant characters for each detected morphogroup (categories not recovered as morphogroups were not included). All morphogroups were tested together, considering data up to species level in one analysis (see below) and up to subspecies level in another. The characters were plotted for each morphogroup at species level as box-plot graphs. In addition, the quartile distribution was calculated for each variable and morphogroup to check the degree of overlap.

Valcárcel and Vargas' criteria for taxonomic significance (2010) were slightly simplified for the purposes of our study. Tested groups were considered taxonomically significant when DFA retrieved more than 70% correctly classified excluded cases, and a combination of at least two characters with less than 25% overlap in pairwise comparisons was validated by MANOVA post-hoc groupings. When DFA reported more than 70% correctly classified excluded cases, but only a single variable with less than 25% overlap, supported by MANOVA post-hoc groupings, was found, taxonomic significance was considered if there was a clear geographical split among the compared groups. Species and subspecies level were conditioned by the phyletic structuring recovered in our previous molecular study (Jiménez-Mejías *et al.*, under review (Chapter 2)). Taxa recovered in independent clades or with identified taxa-specific sequences were unequivocally considered at the species level. Otherwise, the taxon was considered at the subspecies level.

- Datasets of step 2: homogeneous phylogroups

Two subsets were analysed separately, corresponding to the two main clades (phylogroups) found by Jiménez-Mejías *et al.* (under review (Chapter 2)) within the *C. flava* group. These showed slight overlap in Step 1 (see *Results*): the patent-utricles phylogroup (*C. demissa*, *C. viridula*, and *C. tumidicarpa* ssp. *cedercreutzii*), and the deflexed-utricles phylogroup (*C. flava*, *C. lepidocarpa*, *C. nevadensis* and Atlasic plants).

Atlantic-Iberian and Pyrenean-Cantabrian plants, despite molecular assignation to the patent and deflexed phylogroups respectively, were excluded from the analyses

due to the extensive intermediacy observed in Step 1 (see *Results*). They were analysed separately as problem groups in Step 3.

- Datasets of step 3: morphologically continuous subsets

This set of analyses explored the boundaries between phylogroups. In each subset we included a problematic morphotype together with the overlapping morphogroups detected in Step 1 (see *Results*).

Three subsets were analysed separately: subset 1 (Pyrenean-Cantabrian plants, *C. demissa*, *C. flava* and *C. lepidocarpa*), subset 2 (Atlantic-Iberian plant, *C. demissa*, *C. lepidocarpa* and *C. viridula*) and subset 3 (*C. nevadensis*, Atlasic Plants, Pyrenean-Cantabrian plants and *C. viridula*). In this latter subset, *C. nevadensis* and Atlantic plants were considered as a single morphogroup (see Deflexed-utricles phylogroup, *Results*).

Regarding subsets 1 and 2, those problematic morphotypes included were previously reported as hybrid-origin plants (Luceño and Jiménez-Mejías, 2008), so the analytical approach was designed to clarify morphological relationships with the putative parental species. Firstly, a PCA was performed to contrast possible structural rearrangement after reducing the analysed data. Refused this (see *Results*), DFA was used to validate differences among the well-defined morphogroups and to check the taxonomic assignation of the problematic morphotypes.

For subset 3, PCA and DFA were carried out to establish and validate the recovered morphogroups.

Results

In all delimited datasets, Kaiser's measure of sampling adequacy was >0.5, and Barlett's test of sphericity was significant. Both positive evaluations support the suitability of the datasets to be explored using PCA (cf. SPSS user guide, 2009).

PRELIMINARY EXPLORATION

Thirteen variables were included in the preliminary exploration (Table 2).

The first four components accounted for 72.64% of the total variance (36.42%, 17.48%, 10.70% and 8.05% respectively). The characters that most contributed to the first two components were those related to utricle curvature and arrangement (UTCV, UTMD, UTLW) in component 1, and to utricle size and shape (UTLN, UTBK) in component 2 (Table 2). The scatter-plot using PC-I and PC-II revealed clear separation among the four well-defined species (Fig. 1A,B). Two of the problematic morphotypes were intermingled with several taxa (Fig. 1C): 1) Pyrenean-Cantabrian plants were found among *C. demissa*, *C. flava* and *C. lepidocarpa*; 2) Atlantic-Iberian plants were found among *C. demissa*, *C. lepidocarpa* and *C. viridula*. Atlasic plants and *C. nevadensis* were merged with each other and intermingled mainly with *C. viridula* (Fig. 1A,B). *Carex tumidicarpa* ssp. *cedercreutzii* partly overlapped with *C. demissa*, and to a lesser extent with *C. viridula* (Fig. 1A).

It should be noted that the resolution of the scatter-plot decreases towards the lowest PC-II scores. This is due to the strong contribution of utricle size to PC-II, which makes populations with the smallest utricles overlap in this area of the plot (Fig. 1A,B).

HOMOGENEOUS PHYLOGROUPS

- Patent utricles phylogroup

Eight variables were chosen as those that most contributed to separating *C. demissa* and *C. viridula* as well-defined species (Table 2).

The first three components accounted for 47.04%, 16.50% and 13.26% of the variance respectively, and 76.80% of the total variance (Table 3). The variables that most contributed to each component were related to utricle size in PC-I (UTLN, UTBK), and to inflorescence shape in PC-II (BRSH, LWIN) (Table 2). PCA showed that *C. demissa* and *C. viridula* split almost perfectly (Fig. 2A). However, *C. tumidicarpa* ssp. *cedercreutzii* completely overlapped with *C. demissa*. When considering *C. demissa* and *C. tumidicarpa* ssp. *cedercreutzii* as the same morphogroup (*C. demissa s.l.*), DFA yielded UTBK and UTLN as the main characters that allow distinction between *C. demissa* and *C. viridula* (Table 3), with a significant value for well-classified excluded cases and Cohen's *k*. In addition, MANOVA revealed significant differences between two characters with an overlap of less than 25% (LFWD, UTBK); Fig. 3, Table 4).

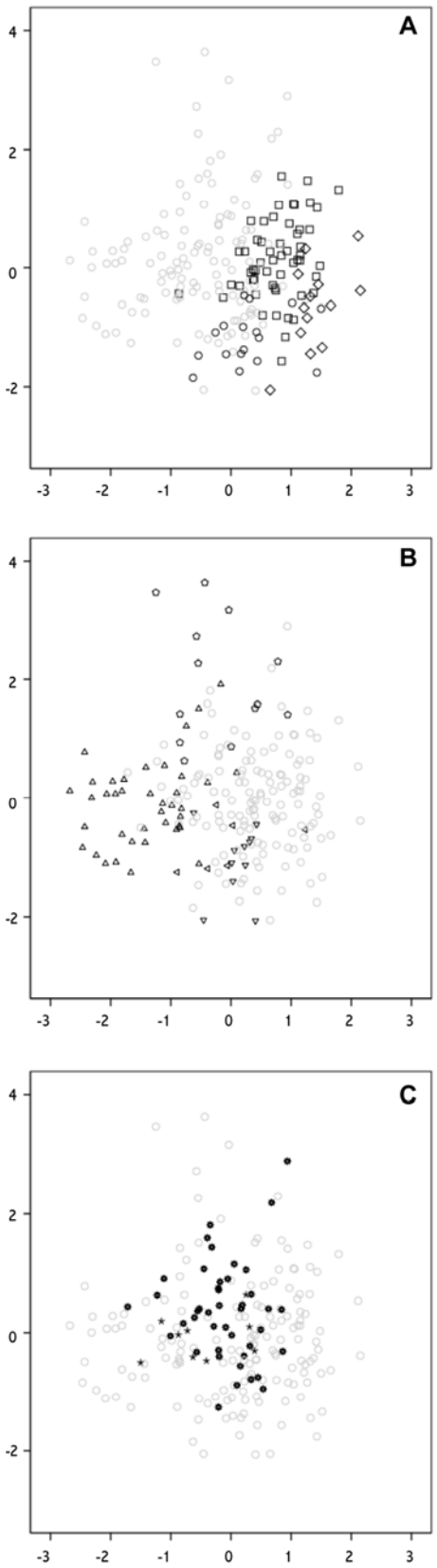

Fig. 1: Scatter plot of the first two principal components extracted in the preliminary exploration. To ease interpretation, different categories are plotted in each figure: **A** Patent utricles phylogroup (□ *C. demissa*; ○ *C. viridula*; ◇ *C. tumidicarpa* ssp. *cedercreutzii*); **B** Deflexed utricles phylogroup (⬠ *C. flava*; △ *C. lepidocarpa*; ▽ *C. nevadensis*; ◁ Atlasic plants); **C.** Intermediate problematic morphotypes (✶ Atlantic-Iberian plants; ❋ Pyrenean-Cantabrian plants).

When *C. demissa* and Azorean *C. tumidicarpa* ssp. *cedercreutzii* were tested separately, taxonomic significance was recovered for the latter. The first four components accounted for 32.95%, 15.78%, 14.15% and 10.66% of the variance respectively, and a total of 73.53% (Table 3). A scatter plot using the first three components (Fig. 2F) showed that they were slightly split. The variables that contributed most were BRSH and LWIN for PC-I, UTLN and UTBK for PC-II, and ST/LF for PC-III. DFA retrieved UTWD as the main character that allows discrimination between *C. demissa* and *C. tumidicarpa* ssp. *cedercreutzii*, with significant values for correctly classified excluded cases and Cohen's *k* (Table 3). In addition, although significant mean differences were found for UTWD, UTLW and ST/LF, only the first character did show less than 25% overlap. If the Canary and Madeiran plants were included under *C. tumidicarpa* ssp. *cedercreutzii* (results not shown), DFA validation and Cohen's *k* were not significant.

- Deflexed utricles phylogroup

Ten variables were analysed to evaluate the morphological patterns within the deflexed utricles phylogroup (Table 2). The first two components of PCA explained 65.10% of the total variance (44.18% and 20.92% respectively (Table 3)). As found in the patent utricles phylogroup, the variables that contributed most to each component were related to inflorescence shape in PC-I (MSLN, MSPD) and to utricle size in PC-II (UTLN, UTBK) (Table 3; Fig. 2B). The plot revealed three homogeneous clusters: *Carex flava*, *C. lepidocarpa* and a third cluster in which *C. nevadensis* and Atlasic plants overlapped. DFA based on these three morphogroups retrieved utricle characters (UTLN, UTBK, UTLW) as the most discriminant (Table 3). Validation using excluded cases was significant for the three morphogroups, whereas Cohen's *k* was not significant for *C. flava* (Table 3). MANOVA found significant differences in up to six characters with less than 25% overlap for each pairwise comparison between morphogroups (Fig. 3; Table 4): UTLN, UTBK, UTCV, UTLW between *C. flava* and *C. lepidocarpa*; LFWD, BRLN, MSLN, UTLN and UTBK between *C. flava* and *C. nevadensis* plus Atlasic plants; and BRLN, MSLN, UTLN, UTCV, UTUP and UTMD between *C. lepidocarpa* and *C. nevadensis* plus Atlasic plants.

When PCA, DFA and MANOVA were performed considering *C. nevadensis* and Atlasic plants separately, no split or taxonomic significance was recovered (results not shown).

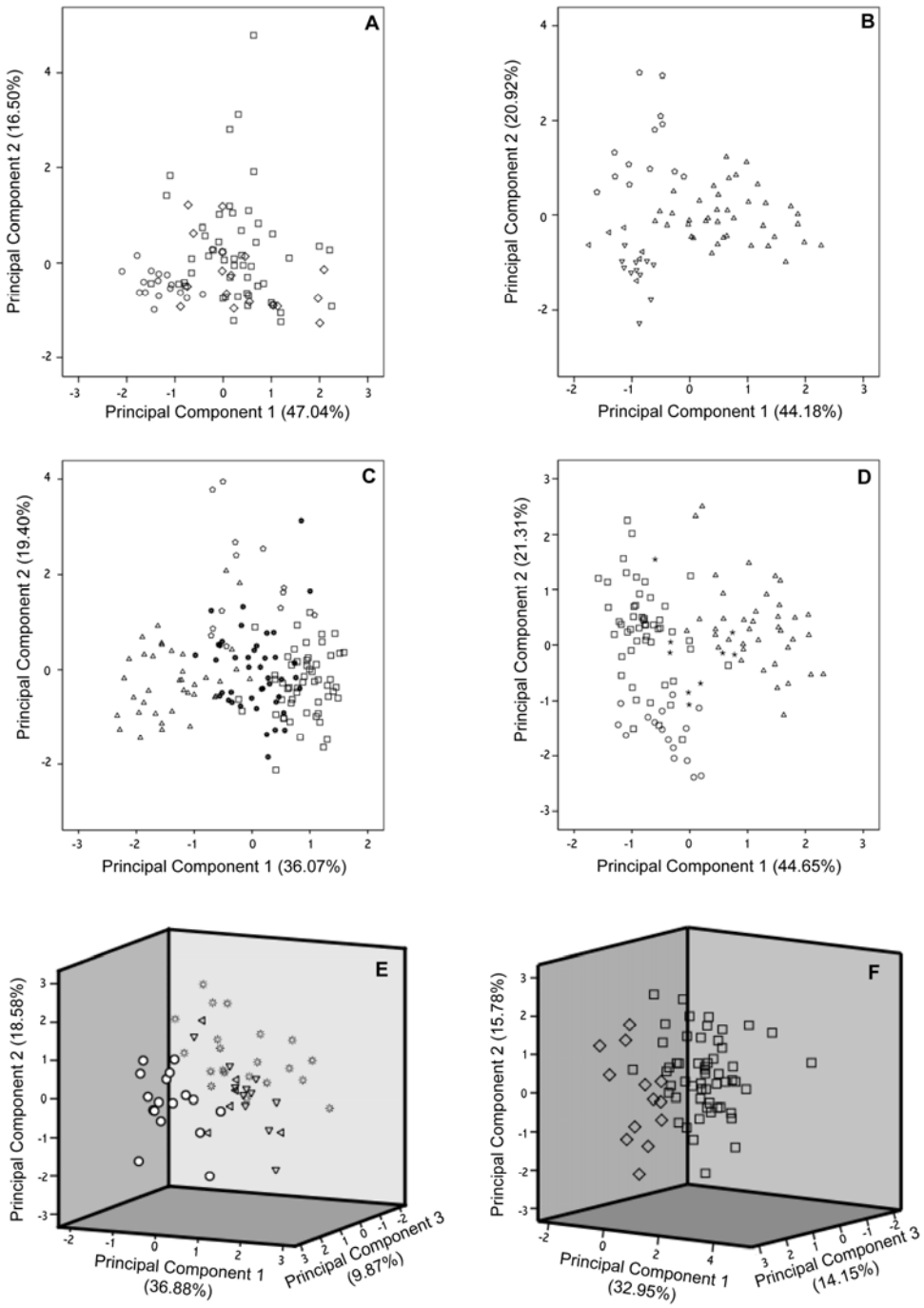

Fig. 2: Scatter plot of the first two (**A,B,C,D**) and three (**E,F**) principal components extracted in each set of analyses: **A** Patent utricles phylogroup; **B** Deflexed utricles phylogroup; **C** subset 1; **D** subset 2; **E** subset 3; **F.** *C. demissa* plus *C. tumidicarpa* ssp. *cedercreutzii*. Symbols depict the different categories considered: □ *C. demissa*; ⬠ *C. flava*; △ *C. lepidocarpa*; ○ *C. viridula*; ✶ Atlantic-Iberian plants; ◁ Atlasic plants; ▽ *C. nevadensis*; ✱ Pyrenean-Cantabrian plants; ◇ *C. tumidicarpa* ssp. *cedercreutzii*.

Table 3. Main results of the statistical analyses. Three first columns indicate the analyzed dataset, number of specimens included (N_I; those selected for DFA within brackets) and number of variables included (N_V) in each analyses. Two next columns summarize main PCA results: number of principal components with eigenvalue > 1 (N_{PC}) and accumulated variance accounted, and number of groups found. The five last columns display results of DFA: Wilks' λ, percentage of correctly classified not-included cases, Cohen's *k*, percentage of categories retrieved when problematic morphotypes are tested, and significant variables (factor loading ≥ 0.5) recovered by the analyses (variables abbreviations specified in Table 2). Well-defined taxa and problematic morphotypes are abbreviated as follow: atn (Atlantic-Iberian plants), ats (Atlasic plants), ced (Azorean *C. tumidicarpa* ssp. *cedercreutzii*), dem (*C. demissa*), fla (*C. flava*), lep (*C. lepidocarpa*); nev (*C. nevadensis*), pyr (Pyrenean-Cantabrian plants) vir (*C. viridula*). Asterisks represent statistical significance at the following levels: **0.00<p<0.01; *0.01<p<0.05.

			Principal components analysis		Discriminant analysis				
Dataset	**N_I**	**N_V**	**N_{PCs} (%Var)**	**Groups**	**Wilks' λ**	**%Corr. class.**	**Cohen's *k***	**% class. prob. morph.**	**Significant variables**
Homogeneous phylogroups									
Patent utricles phylogroup (dem ss, vir, ced)	85(58)	8	3 (76.80)	2 (ced+dem, vir)	0.33**	ced+dem: 95.7 vir: 100	ced+dem: 0.8** vir: 1**	-	UTBK UTLN
dem, ced	68(54)	11	4 (73.53)	2 (dem, ced)	0.33**	dem: 100 ced: 100	dem: 0.8** ced: 1**	-	UTWD
Deflected utricles phylogroup (lep, fla, ats, nev)	69(51)	10	2 (65.10)	3 (lep, fla, ats+nev)	0.075** 0.291**	lep: 100 fla: 100 nev+ats: 100	fla: 0.7^{ns} lep: 1** nev+ats: 1**	-	UTBK UTLN UTLW
Morphologically continuous subsets									
Subset 1 (dem, fla, lep, pyr)	148(77)	12	3 (68.90)	3 homogeneous (dem, fla, lep) pyr intermingled and intermediate among all them	0.058** 0.397**	dem: 100 fla: 100 lep: 87.5	dem: 1** fla: 0.6^{ns} lep: 0.9**	pyr: dem (41.9) fla (23.3) lep (34.9)	UTCV UTLW UTBK UTLN
Subset 2 (dem, lep, vir, atn)	116(64)	11	2 (66.97)	3 homogeneous (dem, lep, vir) atn scattered, intermingled	0.053** 0.424**	dem: 94.4 lep: 93.8 vir: 100	dem: 0.9** lep: 0.9** vir: 1*	atn: dem (50.0) lep (37.5) vir(12.5)	UTCV UTMD UTLW UTBK MSLN
Subset 3 (ats, nev, vir, pyr)	79(60)	13	4 (70.10)	3 (vir, ats+nev, pyr), partly intermingled	0.127**	vir: 100 pyr: 80 ats+nev: 100	vir: 1** pyr: 0.7** ats+nev: 0.8**	-	UTLN UTBK BRWD

MORPHOLOGICALLY CONTINUOUS SUBSETS

- Subset 1 (Pyrenean-Cantabrian plants, *C. demissa*, *C. flava* and *C. lepidocarpa*)

Twelve variables were analysed (Table 2).

In PCA, the first three variables accounted for 36.07%, 19.40% and 13.43% of the variance respectively, making a total of 68.90% (Table 3). For PC-I, the variables that contributed most were those related to utricle curvature and arrangement (UTCV, UTUP, UTMD, UTLW) whereas for PC-II they were related to utricle size (UTLN, UTBK) (Table 2). The scatter plot of the first two components revealed that *C. demissa*, *C. flava* and *C. lepidocarpa* constituted well-defined homogeneous clusters. Pyrenean-Cantabrian plants occupied an intermediate position and were intermingled among the three other morphogroups (Fig. 2C; Table 3).

DFA considering only the well-defined taxa reported similar results to those found by PCA: UTCV, UTLW, UTBK and UTLN were those characters that most contributed to discriminating among the morphogroups (Table 3). Validation using excluded cases yielded significant results for all morphogroups, whereas Cohen's *k* was non-significant only for *C. flava* (Table 3). MANOVA found significant differences in up to four characters, with less than 25% overlap between the pairs *C. demissa–C. lepidocarpa* (UTCV, UTUP, UTMD, UTLW) and *C. demissa–C. flava* (UTLN, UTBK, UTCV, UTLW) (Fig. 3, Table 4), giving additional support to the distinctiveness of the three groups.

When assignation of the Pyrenean-Cantabrian plants was tested using DFA, different samples were assigned to each of the three included morphogroups (Table 3): 41.9% to *C. demissa* (18 samples), 34.9% to *C. lepidocarpa* (15 samples), and 23.3% to *C. flava* (ten samples). In addition, some of the problematic samples (11.62%) displayed similar scores for two of the morphogroups.

- Subset 2 (Atlantic-Iberian plants, *C. demissa*, *C. lepidocarpa* and *C. viridula*)

Eleven variables were included in the analysis of this subset (Table 2).

In PCA, the first two components accounted for 44.65% and 21.31% of the variance, making a total of 65.96% (Table 3). In PC-I the variables that contributed most were those related to utricle curvature and arrangement (UTCV, UTUP, UTMD, UTLW). PC-II was mainly constituted by variables related to inflorescence shape (LWIN, BRSH, MSLN) (Table 2). As in Subset A, the scatter plot of the first two components showed *C. demissa*, *C. lepidocarpa* and *C. viridula* to be relatively well-defined and homogeneous clusters, whereas Atlantic-Iberian plants were scattered, and overlapped with the other morphogroups (Fig. 2D; Table 3).

DFA of only the well-defined taxa yielded utricle curvature and arrangement (UTCV, UTMD, UTLW) as the most discriminant variables, along with utricle beak and male spike length (UTBK, MSLN) (Table 3). Validation using excluded cases and Cohen's *k* statistic yielded significant values (Table 3). MANOVA found significant differences and less than 25% overlap between up to nine characters of *C. lepidocarpa* and *C. viridula* (Fig. 3; Table 4).

When taxonomic assignation of Atlantic-Iberian plants was tested by DFA, the samples were mainly assigned to *C. demissa* (5 samples; 50%) and *C. lepidocarpa* (4 samples; 37.5%) with high scores, whereas only one sample (12.5%) was classified as *C. viridula* (Table 3).

- Subset 3 (*C. nevadensis*, Atlasic plants, Pyrenean-Cantabrian plants and *C. viridula*)

Thirteen variables were chosen for the PCA (Table 2). The first four components accounted for 22.90%, 20.98%, 14.63% and 11.58% of the variance respectively, making a total of 70.10% (Table 3). The variables that contributed the most were UTLW, UTMD and UTBK for PC-I, LFWD and BRWD for PC-II, and LWIN for PC-III (Table 2). In the scatter plot, the first three components were required to recover some separation between the included categories, although they remained partially intermingled (Fig. 2E; Table 3).

When three morphogroups were considered in the DFA (*C. nevadensis* and Atlasic plants as a single morphogroup), the analysis yielded UTLN and UTBK as the main characters that allow their differentiation. Validation with excluded cases and Cohen's *k* yielded significant results (Table 3).

Comparison between *C. viridula* and Atlasic plants plus *C. nevadensis* as a single morphogroup yielded ST/LF, BRLN, UTBK and UTCV as characters with less than 25% overlap that were also significantly different according to the MANOVA post-hoc test (Fig. 3; Table 4).

Discussion

PREVIOUSLY WELL-DEFINED TAXA WERE UNEQUIVOCALLY RECOVERED

Earlier morphometric analyses revealed the existence of at least four well-defined taxa within the *C. flava* gr. in Europe and North America (Schmid, 1983; Crins and Ball, 1989a): *C. demissa*, *C. flava*, *C. lepidocarpa* and *C. viridula*. As expected, our analyses recovered these four taxa. In addition, those characters found to be discriminant by DFA, significantly different by MANOVA, and/or with less than 25% overlap, mostly matched those reported in previous treatments (cf. Schmid, 1983; Crins and Ball, 1989b; Chater, 1980; Egorova, 1999): utricle size and curvature, lowest internode on inflorescence (presence of sub-basilar spikes) and male spike penduncle length (see Tables 3–4). In addition, the arrangement of utricles within the female spike was revealed as a taxonomically useful character.

The only well-defined taxon that did not produce a statistically significant Cohen's *k* in the DFA grouping was *C. flava* (Table 3). This may be attributed to the small sample size (only 13 populations) and relatively wide variation (Figs. 1A, 2B, 2C). However, the characters identified by DFA (UTBK, UTLN) also showed less than 25% overlap and were found to be significantly different from all other morphogroups according to MANOVA post-hoc tests (Fig. 3, Tables 3–4). These results support a clear morphological split and validate the taxon within our morphometric approach. All previous morphometric approaches, together with biosystematic evidence, strongly support the independent character of *C. flava* and its morphological distinctiveness (Schmid, 1983; Crins and Ball, 1989a; Pykälä and Toivonen, 1994; among others).

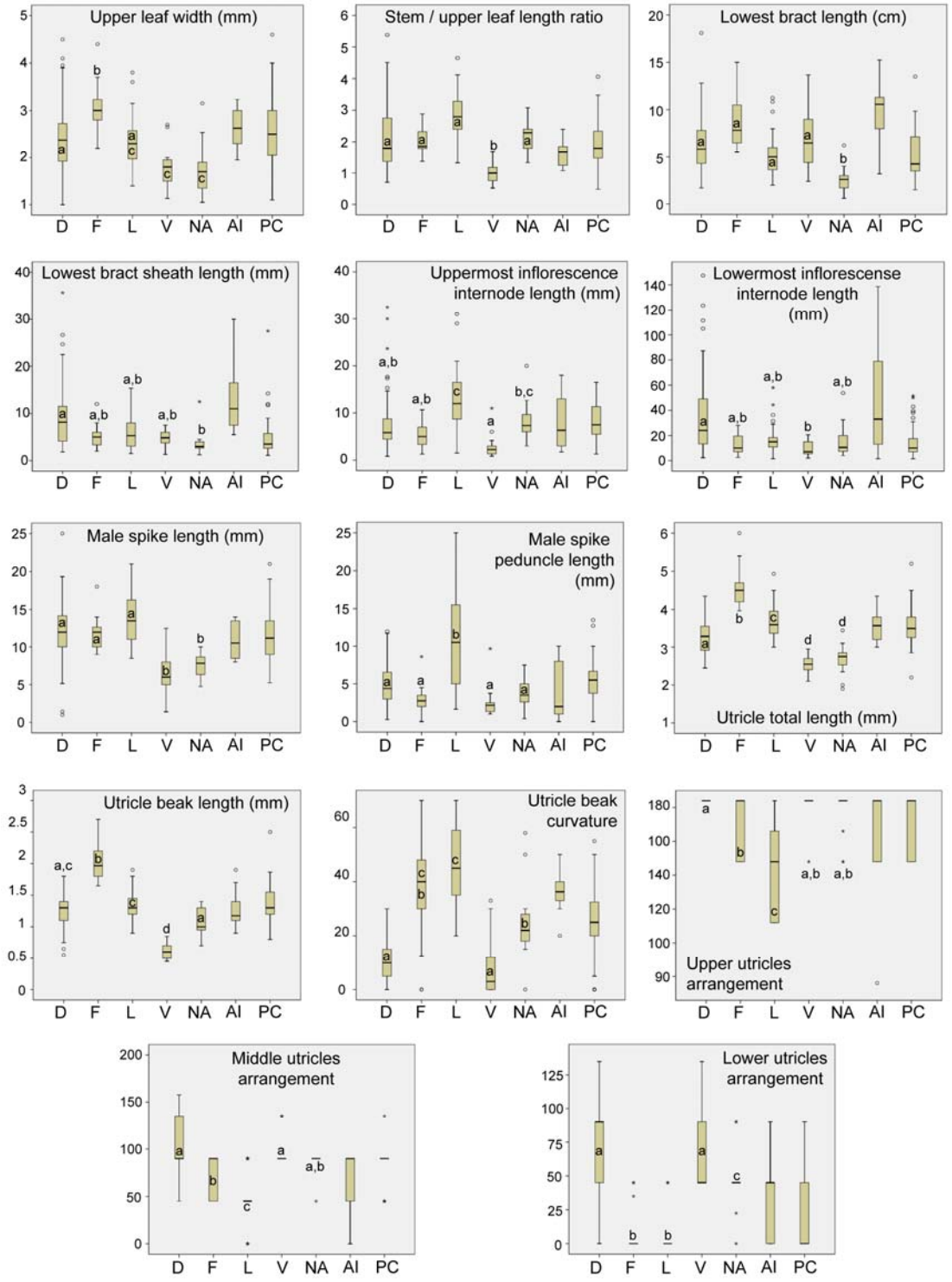

Fig. 3: Box-plots of the most discriminant characters retrieved by DFAs or with less than 25% overlap. The x-axis represents the different categories considered; the y-axis represents the measurements. Homogeneous morphogroups and problematic morphotypes are labelled as follows: D (*C. demissa*), F (*C. flava*), L (*C. lepidocarpa*), V (*C. viridula*), NA (*C. nevadensis* and Atlasic plants together), PC (Pyrenean-Cantabrian plants) and AI (Atlantic Iberian plants). Small letters within or next to boxes of definite morphogroups indicate groups with similar mean values according to the MANOVA Scheffé post-hoc test. The boxes cover 50% of the data values ranging between the 25th and 75th percentiles, and the lines show 95% of the values between the 5th and 95th percentiles. The line within the box shows the median. Dots represent outlying values.

Table 4. Characters that showed less than 25% overlap in pairwise comparisons at species level (abbreviations specified in Table 2). Those characters found to be significantly different by MANOVA post-hoc Scheffé test are marked with an asterisk.

	C. demissa sl	*C. flava*	*C. lepidocarpa*	*C. viridula*
C. flava	UTLN* UTBK* UTCV* UTLW*	-		
C. lepidocarpa	UTCV* UTUP* UTMD* UTLW*	LFWD* UPIN* MSPD* UTLN* UTBK*	-	
C. viridula	LFWD* UPIN MSPD UTBK*	ST/LF* LFWD* UPIN MSLN* UTLN* UTBK* UTCV* UTLW*	ST/LF* UPIN* MSLN* MSPD* UTLN* UTBK* UTCV* UTUP* UTMD*	-
C. nevadensis + Atlasic platns	LFWD BRLN* BRSH* MSLN* UTLN* UTCV*	LFWD* BRLN* MSLN* UTLN* UTBK*	BRLN* MSLN* UTLN* UTCV* UTUP* UTMD*	ST/LF* BRLN* UTBK* UTCV*

MORPHOLOGICAL EVIDENCE OF HYBRIDIZATION: INTERMEDIACY OF PYRENEAN-CANTABRIAN PLANTS AND DISPARATE MORPHOLOGY IN PROBLEMATIC ATLANTIC-IBERIAN MORPHOTYPE

Two extensive hybridization areas have been proposed to explain the existence of problematic morphotypes in the Iberian Peninsula (Luceño and Jiménez-Mejías, 2008): the Pyrenees and Cantabrian mountains (*C. demissa*, *C. flava* and *C. lepidocarpa*) on the one hand, and the Atlantic-Iberian strip (*C. demissa*, *C. viridula*) on the other. These hypotheses were supported by additional molecular and cytogenetic insights (Jiménez-Mejías *et al.* under review). Pyrenean-Cantabrian plants were found to be molecularly homogeneous, with unequivocal assignation in a plastid phylogeny to the W-European *C. lepidocarpa* clade. However, remarkable cytological instability was also detected, involving many irregular configurations, as well as more than a diploid number of chromosomes within the same population. Inferred numbers ranged from *2n*=61, fairly close to those reported for *C. flava* (*2n*=58,60), to *2n*=70, the most common number for *C. demissa*. In terms of the Iberian-Atlantic plants, they were karyologically more homogeneous, but molecular data showed incongruities between expected plastid and nuclear sequences.

Our dataset revealed that Pyrenean-Cantabrian plants are also clearly intermediate among the three well-defined species proposed as parents (Figs. 1C,2C), supporting the hypothesis of hybrid amalgamation according to our previous cytological observations. DFA assigned different samples to different taxa (Table 3), mostly to *C.*

demissa and *C. lepidocarpa*. In the case of plants classified as *C. flava*, although DFA cannot be considered conclusive, a visual check of the discriminant characters unequivocally placed them within the variation thresholds of *C. flava*. Moreover, the most closely related cytotypes to *C. flava* were found in the Picos de Europa (Cantabrian Mountains) and Val d'Arán (Pyrenees), the geographical origin of the samples recovered as *C. flava* in the present morphological study.

However, Atlantic-Iberian plants displayed a disparate morphology. They partially matched one of the parents (*C. demissa*) but clearly did not fit with the other potential parent (*C. viridula*). Atlantic-Iberian plants and *C. viridula* barely overlapped in PCA (Fig. 2D), and when morphological assignation was tested using DFA, only a single Atlantic-Iberian sample was recovered as *C. viridula* (Table 3). In contrast, some of the plants overlapped in PCA and were assigned by DFA to *C. lepidocarpa* (Table 3). Rieseberg (1995) stated that morphological intermediacy cannot be expected as the most common result of the hybridization processes, and remarked that expression of extreme or novel characters is a possible outcome. Plants that were truly similar to the Atlantic-Iberian morphotype were also detected in Catalonia, growing together with *C. demissa* and *C. viridula* (obs. pers.; cf. Pérez-Haase *et al.*, 2008). The strong similarity of some of the Portuguese samples to *C. lepidocarpa* misled the *Flora Iberica* treatment (Luceño and Jiménez-Mejías, 2008) to consider *C. lepidocarpa* as belonging to the Portuguese flora (in the Estremadura region), an error that should be taken into account in order to delete this species from the plant catalogue of Portugal.

AMALGAMATION OF DWARF PLANTS

The preliminary exploration showed an amalgamation of plants with the lowest scores on PC-II. In this area, the PCA scatter-plot failed to recover a clear split between Pyrenean-Cantabrian, Andalusian and Moroccan mountain plants and *C. viridula* (Fig. 1A,B). When analysed separately, it was possible to recover at least *C. viridula* and Pyrenean-Cantabrian plants as taxonomically significant independent morphogroups, although no clear split was found when PCA results were plotted (Fig. 2E; Table 3). However, the analyses failed to distinguish Atlasic plants from *C. nevadensis* (Fig. 2E).

The strong contribution of utricle size to PC-II (Table 2) led to the merger of samples with the smallest utricles in the preliminary exploration. Dwarfism is a phenomenon that makes taxonomic distinction difficult for such plants: their small size

hides taxonomically useful characters, making these plants truly similar not only to the naked eye, but also to morphometric exploration. This apparent indistinctiveness explains the incongruence and disparateness between treatments that included such populations (Maire, 1957; Chater, 1980; Schmid, 1983; Luceño, 1994), and also explains the confusion of *C. nevadensis* in N Spain with the Pyrenean-Cantabrian hybrid complex (e.g. Molina *et al.*, 2006). Our analyses were unable to distinguish the high mountain populations from the Sierra Nevada and Atlas, despite their strong isolation from other members of sect. *Ceratocystis*, and a distance of more than 600 km between them. The small size of plants from these populations may explain the inability to find differences using our analytical approach.

IMPLICATIONS FOR THE TAXONOMY OF THE C. FLAVA *GROUP*

According to the *Flora Iberica*, three well-defined taxa are found throughout most of the study area: *C. demissa*, *C. lepidocarpa* and *C. viridula*. These three species as also confirmed for North Africa (see Appendix 1). Additionally, our data support the existence of residual *C. flava* individuals in Val d'Aran and the Picos de Europa.

Azorean populations of *C. tumidicarpa* ssp. *cedercreutzii* deserve taxonomic recognition as a subspecies of *C. demissa* (see Appendix 2), according to our criteria for taxonomic significance, since distinction is possible even though morphological split is not complete. The geographical disjunction between the two subspecies suggests a process of incipient divergence. Populations from the Madeira and Canary archipelagos must be regarded as ssp. *demissa*. The morphological divergence of Azorean populations could indicate an earlier colonization event compared to those that took place on Madeira or the Canary Isles, or different genetic processes affecting populations from each archipelago.

With regard to the Atlantic-Iberian and Pyrenean-Cantabrian plants, morphological intermediacy and the lack of morphological discontinuities preclude precise taxonomic partitions. Given such variation, it appears accurate to refer to this set of plants as hybrid amalgamations.

Following our criterion regarding genetically defined species, the recovery of Atlasic plants and *C. nevadensis* as an independent morphogroup from *C. lepidocarpa* supports its recognition as a subspecies (*C. lepidocarpa* ssp. *nevadensis*). However, our analysis was unable to distinguish between them even though they are strongly

geographically isolated. As explained above, dwarfism could be behind the morphological indistinctiveness, hiding any morphological sign of incipient divergence. It will be necessary to test the underlying diversity in these populations to ensure a stable taxonomic status for both Sierra Nevada and Atlas plants.

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Appendix 1

List of studied materials included in morphometrical analyses.

***C. DEMISSA*- CANARY ISLANDS. Tenerife.** Cañadas del Teide, Fuente Guadajara, 2400 m, 28.V.1983, *J.S. Socorro & W. Wildpret* (TFC); Barranco del Río, 2200 m, 13.VII.1983, *J.S. Socorro* (TFC); Cañadas del Teide, Fuente Guadajara, 2400 m, 13.VII.1983, *J.S. Socorro* (TFC). **MADEIRA.** Madeira, E of Ribeira da Jonela ,Curral Falso, 650 m, 27.VI.1978, *O.H. Rustan 538* (O); Madeira, camino Bica da Cana a Casa do Lombo do Mouro, 1445 m, 28.VI.2000, *C. Navarro* (MA); Madiera, 3000 ft, *D1837, .C.M. Lemann* (K). **MOROCCO**. Tanger, Bou Hassim, bosques claros de *Quercus pyrenaica*, 1350-1450 m, 9.IV.1995, *A. Boratynski & A. Romo* (RNG); Djebel Tidighine, suelos higroturbosos en bosque de *Cedrus atlantica*, V.2007, *P. Jiménez-Mejías et al. 93PJM07* (UPOS); Atlas rifain, Ketama à Telata, 1450 m., 5.VII.1932, *Sennen & Mauricio 8546* (RNG). **PORTUGAL.** Serra do Gerês, Lagoa do Marinho, 8.VI.1977, *Malato-Beliz & J.A. Guerra* (MA); .Cova de Ouro, na estrada Brga-Chaves, 26.VI.1968, *A. Fernandes et al.* (COI); Pouca de Aguiar, Lixa do Alvão, 5.VII.1960, *A.R. Pinto da Silva et al.* (MA); Viseu, Cota, 3.VII.1980, *J. Franco & M. Lousa 7213* (LISI); Ramego, Montemouro, Ponte de Recongos, 900 m, 11.VIII.1948, *F. Fontes et al.* (LISE). **SPAIN**. **Almería**. Fiñana, umbría de Sierra Nevada, 1800 m, VI.1995, *A. Pallarés* (MA). **Asturias.** Lago Enol, Cangas de Onís, 25.VI.1993, *C. Aedo* (MA); Gijón, Carbainos, 3.VI.1980, *N.S. Nava* (MA). **Ávila**. Barco de Ábila, 1750-1800 m, 1.VII.1999, *Montserrat et al.* (JACA). **Bilbao.** Peña de Alluitz, Macizo de San Antonio de Urquiola, *E. Guinea*, 21.VIII.1946 (MA); Butron, 28.V.1906, *Sennen & Elías* (MA); Macizo del Gorbea, Campo de Árraba, VII.1946, *E. Guinea* (MA); de Olaeta al Alto de Cruceta, 680 m, 9.VII.1992, *J. Loidi et al.* (MA). **Burgos**. Neila, Laguna Larga de Neila, 1890 m, 19.VII.2000, *M. Luceño & J. Martín* (MA). **Cádiz.** Tarifa, Sierra del Bujeo, 400 m, 23.V.2004, *P. Jiménez-Mejías et al. 87PJM04* (UPOS). **Cantabria**. La Vilga, *Salcedo* (MA); Liencres, Piélagos, 20.VII.1969, *E. Loriente* (MA; with *C. viridula*); Reinosa, pantano del Ebro, 840 m, 15.VII.1984, *G. Morante & J.A. Alejandre* (MA); Arija, 860 m, 25.VI.1988, *P. Urrutia* (MA). **(A) Coruña**. Brandoñas de Arriba, 440 m, 22.VI.1981, *S. Castroviejo et al.* (MA); Melide, entre Furelos y Leboreiro, 470 m, 19.VI.1992, *G. Nieto Feliner et al.* (MA); Curtis, Teixeiro, 18.VIII.1982, *E. Valdés-*

Bermejo et al. (MA). **Huesca.** Macizo de la Maladeta, lago junto al refugio de la Renclusa, 2.VII.1985, *P. Vargas & M. Luceño* (MA); Benasque, Valle de Lliterola, bajo el Perdigueret, 2465 m, 12.VIII.1997, *J.V. Ferrandez* (JACA); Torla, Ordesa, Gradas de Soaso, 1650-1700 m, 7.VIII.1971, *A. Gallego & H. Pipio* (JACA); Los Lecherines, 23.VIII.1979, *Amich et al.* (MA); Bielsa, Barranco de Fuensanta, 2230 m, 23.VIII.1991, *P. Montserrat et al.* (JACA); Turbón-Laspaules, subida al Valle de las Aras, 1580-1720 m, 29.,VI.1992, *J.A. Sesé* (JACA); Valle de Tena, *A. Zubilla* (MA). **Gerona**. Puigcerdá, subida al puerto de Tosas, 1450 m, 20.VII.1986. *García Ada et al.* (MA). **León**. Macizo occidental de Picos de Europa, travesía del Puerto de Panderrueda al Refugio Vegarredonda, 2040 m., 1.VIII.2005, *S.Martín-Bravo 65SMB04* (UPOS); San Feliz de las Lavanderas, 25.VII.1982, *J. Andrés* (LEB); La Vid, 10.VII.1983, *C. Pérez Morales* (LEB). **Lugo.** Viveiró, 860 m, 16.VII.1983, *E. Valdés Bermejo & M. Castroviejo* (MA); Abadir, 750 m, 16.VII.1982, *E. Valdés-Bermejo et al.* (MA). **Madrid.** La Padriza, 21.VI.1980, *D. Belmonte* (MA); Somosierra, 1400 m, 26.VIII.1933, *Cuatrecasas* (MA); Sierra de Guadarrama, Canencia, VI.1916, *C. Vicioso* (MA). **Navarra**. Ataun, 23.VI.1978, *Equipo Pinos* (ARAN). **Orense**. Lovios, Sierra del Xeres, 14.VIII.1981, *E. Valdés-Bermejo et al.* (MA). **Pontevedra**. Lalia, 8.VI.1944, *Vieiter* (MA); Vilanova de Arousa, 27.VI.1983, *E. Valdés-Bermejo et al.* (MA). **(La) Rioja.** San Millán de la Cogolla, 29.VII.1987, *M. Luceño & P. Vargas* (MA). **Segovia.** Puerto de Somosierra, 1450 m, 28.VII.1987. *M. Luceño & P. Vargas* (MA). **Soria**. Pinar Grande, 11.VIII.1935, *L. Ceballos & C. Vicioso* (MA); Quintana Redonda, 1050 m, 6.VI.1976, *A. Segura Zubizarreta* (MA). **Zamora**. Rivadelago, Sierra Alta, VI.1941, *M. Lora* (MA).

***C. FLAVA*- BELGIUM**. Luxembourg, Bellefontaine, bois de Maîtrejíbois, *M. Leten*, 14.VI.1989 (JACA). **BULGARIA**. Rila, lake Suhoto, 1885 m, 7.VII.2004, *C. Aedo et la.* (MA); Rhodopes centrales, 1324 m, 2.VII.2004, *C. Navarro et al.* (MA). **ITALY.** Abruzzo, L'Aquila, Paso di Campanelle, *C. Navarro et al.* (MA); Aosta, Lago Combal, Val Veny, 2.IX.1990, *P. Montserrat* (JACA); Appennino Tosco-Emiliano, Modena, Dintorni di Piandelagotti, *A. Lunardi* (FI). **FINLAND**. Kainuu, Paltamo, Melalahti, 27.VI.1984, S. Leinonen (JACA); Tervola, Peura, prope praedium Mäki-Peura, 26.VII.1953, *P.S. Jokela* (JACA). **NORWAY**. Troms, Skjervoy, *M. Luceño 4005ML(1)* (UPOS); Troms, Hamneidet, *M. Luceño 4305ML* (UPOS). **RUSSIA**. Mursmank, Kandalaksha, *T. Filimonova* (MA). **SLOVENIA.** Flushes by path wood

above road, south east side of Bohinjsko Jezero, 10.VII.1972. *G. Halliday 323/72* (RNG). **SWITZERLAND**. Pass de Simplon, 2000 m, 20.VIII.1990, *M. Luceño & P. Vargas* (JACA).

***C. LEPIDOCARPA*- MOROCCO**. Middle Atlas, 7.5 km along minor road to Senuai from main Azrou-Midelt road, 1995 m, 10.VII.2002, *S.L. Jury & M. Ait Lafkih 19756* (RNG). **SPAIN**. **Albacete.** Nacimiento del río Mundo, 1000 m, 20.VII.1984, *M. Luceño* (MA). **Asturias**. Arvás, *Lagasca* (MA). **Bilbao.** Montoria, barranco de la Mina, 820 m, 31.VII.1986, *P. Urrutia & J.A. Alejandre* (MA); ad fluvium Ansa prope Bilbao, V.1850, *Willkomm* (MA). **Burgos.** Salguero de Villasuso en Mena, *Salcedo* (MA); Aranda de Duero, VI.1942, *Caballero López* (MA). **Cantabria**. Abiada, Hermandad de Campoó de Suso, 1100 m, 19.VI.1988, *C. Aedo* (MA). **Cuenca**. Laguna del Marquesado, 9.VI.1974, *A. González et al.* (MA). **Guadalajara.** Río Salado, *F. Ferreras* (MA); Taravilla, Laguna de la Parra, 1120 m, 20.VI.1995, *M.A. Carrasco et al.* (MA). **Huesca.** Sallent, colina de Pourtalet, 1700 m, 10.VIII.1980, *J. Vivant* (ARAN); Torla, Ordesa, sendero al Circo de Soaso, paredes rezumantes, 27.VII.2004, *M.L. Buide & J.M. Marín 16MBR04* (UPOS); Torla, Ordesa, Circo de Soaso, 30.VII.2004, *M.L. Buide & J.M. Marín 52MBR04* (UPOS); Biescas, 1250 m, *P. Montserrat*, 23.VII.1969 (JACA); Jaca, El Boalar de Atarés, 730 m, 1982, *P. Montserrat* (MA). Seira, Barbaruens, 980 m, 3.VII.1979, *P. Montserrat et al.* (JACA); Astún-Las Torrullas, camino al Ibón de Escalar, 1780-2090 m, 18.VI.1986, *P. Montserrat & J. L. Remon* (JACA); Benasque, *C. Pau* (MA); Aisa, El Bozo, 1700 m, 20.VII.1967, *P. Montserrat & G. Rodríguez* (JACA). **Jaén.** La Hoya del Cambrón, 1000 m, 21.VII.1984, *M. Luceño* (MA). **León.** Cistierna, 9.IX.1979, *Carbó et al.* (LEB); Puerto de la Magdalena, 11.VII.1978, *Araceli* (LEB); Rucayo, VII.1978, *E. Hernández* (LEB). **Lleida**. Valle de Arán, Circo de Colomers, 2000 m, VII.1995, *A. Pallarés* (MA); Valle de Arán, Tredós, 1900 m, VII.1995, *A. Pallarés* (MA). **Navarra.** Otsagabia, Irati, 1000 m, 13.VII.1994, *I. Aizpuru* (ARAN). **Soria**. La Póveda, 6.VII.1959, *A. Segura Zubizarreta* (MA); Santa Inés, Gargantillas, 30.VIII.1969, *A. Segura Zubizarreta* (MA); Neguilla, 16.V.1935, *C. Vicioso* (MA); Fuentepinilla, 28.VI.1967, *A. Segura Zubizarreta* (MA); Quintana Redonda, 1050 m, 6.VI.1970, *A. Segura Zubizarreta* (MA). **Palencia**. Entre Camasobres y Piedrasluengas, 1200 m, 15.VII.1985, *M. Luceño & P. Vargas* (MA); Espigüete, 17.VIII.1995, *Aldasoro* (MA). **Teruel.** Valdelinares, 1900 m, 18.VI.1983, *G. Mateo & A. Aguilella* (MA); Sierra del

Tremedal, 18.VI.1907, *C. Pau* (MA). **Vitoria**. Lasarte, *Gredillo* (MA); Entzia, 1030 m, 20.VI.1998, *A. Darnistaque* (ARAN). **Zamora**. Alcañices, 4.VI.1981, *F. Navarro & C.J. del Valle* (MA).

***C. viridula*- MOROCCO**. Tétouan, 71 km. from Chefchaouèn on road to Ketama, 13 km W from Bab Berret, 1450 m, 15.VI.2001, *A. Acchal et al.* (RNG). **PORTUGAL**. Beira Litoral, Lagoa Ervedeira, 10.VIII.1987, *M. Guzmán et al.* (MA); proximo de São Jacinto, 22.VII.1961, *J. Paiva et al.* (MA); Coimbra, Lagoa das Braças, 21.IX.1984, *M. Luceño* (MA); Pinhal do Urso, Lagoa dos Linhos, 17.VI.1926, *Carrisso & Mendoça* (COI); Ponte da Murcella, Moura Morta, VI.1892, *M. Ferreira* (COI). **SPAIN**. **Asturias.** Lagos de Covadonga, Lago Ercina, 2.IX.2004, *J.M. Marín 156JMM04* (UPOS). **Burgos**. Sierra de Neila, Laguna de las Pardillas, 1869 m, 19.VII.2000, *M. Luceño 3600ML* (UPOS); Miranda de Ebro, 27.VIII.1912, *Hno. Elías* (MA; with *C. demissa*). **Cantabria**. Liencres, Piélagos, 20.VII.1969, *E. Loriente* (MA). **Huesca**. Monzón, rivera del Cinca, 250 m, *J.V. Ferrández*, 18.V.1989 (JACA); Bielsa, Pineta, 1100 m, 29.VIII.1990, *R. Jiménez* (JACA). **León**. Turcia, 7.IX.1982, *M. Ladero et al.* (MA); La Balastrera, 14.VII.1982, *Fernández Alaez & Fernández Alaez* (LEB). **Segovia.** Cantalejo, la laguna, 19.IX.1982, *T. Romero* (MA); Cantalejo, laguna de Navahornos, 900 m, 18.VII.1987, *García Adá et al.* (MA). **Soria.** Cubo de la Solana, lagunas de La Dehesa, 1070 m, 4.VI.1995, *J.L. Benito* (JACA).

ATLASIC PLANTS- **MOROCCO.** 72 Km S from Marrakech, Oukaïmedem, 2619 m, 4.VII.1987, *S.L. Jury et al.* (RNG); Oukaïmedem, Jbel Angour, 2820 m, 15.VI.2001, *S.L. Jury et al.* (RNG); Umgebung von Oukaimedem und Berge S des Ortes, 2500-3000 m, 10.VIII.1999, *D. Podlech 55321* (MSB); N. side of J. Angour, near Oukaimedem, ca. 3000 m., 19.VII.1975, *D. Podlech 37806* (MSB); Toubkal, Réfugé Nalbus, 3100 m., 9.VIII.1951, *W. Rauh 275* (M); Adrar-n-Oukaïmeden, vertiente N, 3000 m, 29.VII.2006, *A. Herrero* & *al. AH3090* (MA).

ATLANTIC IBERIAN PLANTS- **PORTUGAL.** Entre Fernan-Ferro e Apostiça, 4.V.1886, *J. Daveau* (LISU); Vendas-Novas, Vale do Falagueiro, 12.V.1947, *A. Fernandes & Sousa 2142* (COI); Aveiro, Águeda, Fermentelos, entre Vale Coutinho e Monte Grande, 19.V.1977, *A. Marques & A. Pereira* (COI); Serra de Sintra, VIII.1885, *J. Daveae* (LISU); Pinhal de Foja, VII.1880, *A. Moller* (COI); Alcácer do Sal, Montevil,

30.IV.2005 *P. Jiménez-Mejías et al. 117PJM05* (UPOS); Saião, Aguas Boas, 7.VIII.1958, *A. Teles & B. Rainza* (MA); Cortegaça, 15.VI.1964, *J. Matos & A. Dinis* (COI); Sintra, carretera al convento de los Capuchos, 2.V.05, *P. Jiménez-Mejías et al. 174PJM05* (UPOS). **SPAIN**. Huelva, Villanueva de los Castillejos, Los Huertecillos, 17.IV.2004, *P. Weickert & E. Sánchez Gullón* (UPOS).

C. TUMIDICARPA SSP. ***CEDERCREUTZII***- **AZORES**. São Miguel, Pico do Carvão, VIII.1894, *B.T. Carreiro 55* (COI); Faial, caldera, 1000 m., 3.VII.1973, *A. Hansen 106* (RNG); Faial, caldera, 9.VII.1973, *A. Hansen 264* (RNG); São Miguel, Lameiro, VII.1903, *B.T. Carreiro 485A* (COI); São Miguel, slope of the road through the wood ca. 6 Km S of Laje Furmas, 1.IX.1970, *A. Hansen* (RNG); São Miguel, Caldeiras Velhas da Ribeira Grande, 28.VIII.1968, *J. Ormonde 404* (COI); São Miguel, Lagoa do Fogo, VII.1879, *F. Carr* (COI); São Miguel, Serra da Água de Pau, Pico da Barrosa, pastured Calluna heath, 900 m, 23.VI.1964, *P. Dansereau et al. 819* (LISE); Terceira, Caldeira de Agualva, 7.VI.1937; *R.T. Palhinha & L.G. Sobrinho* (LISU, paratypus); São Miguel, Sete Cidades, Lagoa Verde, 7.IX.1938, *A.G. da Cunha & L.G. Sobrinho* (LISU, paratypus); Pico, Serra da Madalena, 670 m, 15.VI.1964, *P. Dansereau et al. 675* (LISE); São Jorge, Pico da Serra, 22.VIII.1937, *A.G. da Cunha & L.G. Sobrinho* (LISU; paratypus); Terceira, Lagoa do Pico da Falca, 7.VIII.1966, *J. Ormonde 204* (COI)

C. NEVADENSIS- **SPAIN. Almería**. Bayarcal, Puerto de la Ragua, 2000 m, VI.1995, *A. Pallarés* (MA); Bayarcal, Fuente Mosquera, El Chullo, 2200 m, VI.1995, *A. Pallarés* (MA); Sierra de los Filabres, Gergal, Prado de la Nava, 2100 m, VI.1995, *A. Pallarés* (MA). **Granada**. Laguna de las Yeguas, 2900 m, 11.VIII.1962, *H. Merxmüller & W. Wiedmann 696/62* (M); Pico de Veleta, Baches de San Juan, 2600 m, VII.1959, *S. Dürr* (M); Moanchil, 3000 m, 23.VII.1984, *M. Luceño* (MA); Prados de Otero, 7.VII.1980, *M. Ladero et al.* (MA); Barranco del Goterón, 3.IX.1972, *Fernández-Casas* (MA); Hoya de la Mora, 2100 m, 7.VII.1971, *Fernández-Casas* (MA); Corral del Veleta, 2950 m, 23.VIII.1985, *M. Guzmán et al.* (MA); Laguna Grande de Siete lagunas, 2910 m, 20.VII.2004, *P. Jiménez-Mejías et al. 220PJM04* (UPOS).

PYRENEAN-CANTABRIAN PLANTS- **ANDORRA**. Lagos de Pesons, 5.VIII.2004, *J.M. Marín et al. 9404JMM* (UPOS); Puerto de Envalira, 2540 m, 31.VIII.1976, *S. Castroviejo & E. Valdés-Bermejo*; Molleres d'Arcalis, 2190 m, 17.VII.2004, *E. Carrillo*

(BCN); Lagos de El Serat, 2200 m, 6.VIII.1990, *M. Luceño & P. Vargas* (MA). **SPAIN**. **Asturias.** Sotres, 1150-1800 m, 7.VII.1978, *S. Castroviejo et al.* (MA); Puerto de Somiedo, 1550 m, 20.VI.1981, *N. Mayor et al.* (MA). **Cantabria**. Fuente Dé, estación superior del teleférico, 1.IX.2005, *J.M. Marín 14904JMM* (UPOS); Fuente Dé, M. Luceño, 11.VIII.1984 (MA); macizo central de los Picos de Europa, El Cable, Las Pozas, 11.VIII.1984, *M. Luceño* (MA). **Girona**. Ger, Prat Fondal, Molleres, 2300 m, VII.2004, *A. Pérez-Haase* (BCN). **Huesca**. Balneario de Panticosa, 1600 m, 13.VIII.1984, *M. Luceño* (MA); Sallent de Gállego, 1840-1850 m, 21.VII.1982, *P. Montserrat et al.* (LEB); Panticosa, 1650-1700 m, 12.VII.1995, *P. Montserrat & J. A. Sesé* (JACA); Candanchú, Tobazo, 25.VII.1991, *F. Gómiz* (LEB); Bielsa, sobre el lago Urdiceto, 2415 m, 28.VII.1998, *J.V. Ferrandez* (JACA); Circo de Soaso, 1700 m, VIII.1983, M. Luceño (MA), 2560 m, 19.VIII.1993, *D. Gómez* (JACA); San Juan de Plan, Viadós-Millares, 1760-2000 m, 19.VIII.1993, *J. V. Ferrandez & J.A. Sesé* (JACA); Benasque, Portillón de la Picada, 2320 m, 3.VIII.1995, *V.J. Arán & M.J. Tohá* (MA); Bielsa, refugio de la Estiba, 2100-2400 m, 27.VII.1991, *J.A. Sesé & R. Jiménez* (JACA); Sahún, Aigüeta la Vall, 19.VIII.1992, *J.A. Sesé & J.V. Fernández* (JACA); Castejón de Sos, Liri, 1200 m, 1.VIII.1958, *Tornero* (JACA); Fanlo, Monte Perdido, 2560 m, 19.VIII.1993, *D. Gómez* (JACA). **Lleida.** Val d'Arán, carretera a Baños de Tredós, 1740 m, 2.VIII.2004, *J.M. Marín & M. Luceño 7704JMM* (UPOS); Puerto de la Bonaigua, 1780 m, 6.VII.1993, *C. Aedo et al.* (MA); Valle de Arán, Las Artigas, supra Les Bordes, 1800 m, VII.1995, *A. Pallarés* (MA); Valle de Arán, Baqueira, 2.VIII.2004, *J.M. Marín et al. 7304JMM* (UPOS). **León**. Valdelugueros, 6.VII.1986, *J. Andrés* (LEB); Puerto de Leitariegos, 2.VIII.1981, *E. Puente* (LEB); Puerto de Vergarada, 7.VII.1973, *La Blanca* (LEB); Puerto de San Glorio, 29.VI.1986, *J. Andrés & R. Carbó* (LEB); Burón, valle de Mirva, 6.VII.1988, *J. Andrés* (LEB); Burón, Valle de San Pelayo, 8.VII.1988, *J. Andrés* (LEB); Aralla, 16.VII.1983, *C. Pérez Morales* (LEB); Campohermoso, 12.VII.1977, *M.J. López Pacheco* (LEB); Monte de Tejedo, 27.VI.1986, *J. Andrés & R. Carbó* (LEB); Puerto de Pontón, 11.VIII.1978, *J. Andrés* (LEB); Polvoredo, 7.VII.1983, *J. Andrés* (LEB); Puerto de Vergarada, Pico Huevo, 12.VII.1987, *J. Andrés et al.* (LEB); Riega de Mampodre, Maraña, 23.VI.1997, *E. Puente et al.* (LEB). **Navarra.** Yesa, Fuente de Canes, 6.V.1982, *C. Fernández León* (ARAN). **Palencia**. Curavacas, 7.VII.1991, *J. Andrés* (LEB); Panderrueda, 15.VIII.1978, *C. García Gonzáles* (JACA); Laguna de las Lomas, Cardaño de Arriba, 1950 m, 11.VII.1995, *C. Aedo et al.* (MA).

Appendix 2

Based on our results, *C. tumidicarpa* ssp. *cedercreutzii* is formally combined under the priority name *C. demissa* Hornem., retaining its subspecies status. A brief description and a key to distinguish it from *C. demissa* ssp. *demissa* and *C. viridula* are provided.

Carex demissa ssp. ***cedercreutzii*** (Fagerstr.) Jim.-Mejías, Martín-Bravo and Luceño **comb. nov.**

Carex tumidicarpa ssp. *cedercreutzii* Fagerstr. in Acta Soc. Fauna Fl. Fenn. 79(3): 3 (1967). **[basionym.]**

Typus: Achada, in gehautem Wald, 4.VI.1938, *C.Cedercreutz* (**holo-** H, photo!; **iso-** H, photo!).

≡ *C. viridula* ssp. *cedercreutzii* (Fagerstr.) B.Schmid in Watsonia 14: 317 (1983).

Plant tufted. **Stems** 8–45(50) cm long, trigonous, straight or slightly curved. **Leaves** 4–13(15) cm × 1–2.8(3) mm, 1.5–5 times shorter than stems, flat; basal sheaths grey-brown, becoming fibrous. **Inflorescence** with the uppermost spikes typically crowded at the top of the inflorescence, rarely not overlapping, the lowermost often distant from the others or sub-basilar; lowest bract leaf-like, 3–11.5 cm × 1.2–3(3.4) mm, generally much longer than the inflorescence. **Male spike** 1, 8–14(18) mm long, fusiform; sessile or with an up to 6 mm-long peduncle. **Female spikes** 2–3(4), the lowermost 5.5–13 mm long and with 30–56(65) utricles, short cylindrical to elliptical, sessile. **Male glumes** 0.7–1.3(1.4) mm long, oblong to elliptical, obtuse to rounded, brown with a paler midrib. **Female glumes** ca. 3 mm long, subacute, brown to almost hyaline, with green midrib. **Utricles** 2.5–4.7 × 0.8–1.2 mm, obovate, green to yellowish, conspicuously nerved, with a 0.8–1.7 mm beak, short bifid, straight or slightly curved (up to 10(25)º with respect to the utricle body), the lowest of each spike patent to erect-patent, the middle ones erect-patent and the uppermost ones erect. **Stigmas** 3. **Achene** obovoid, trigonous.

Ecology: wet soils on clay or volcanic sand, in meadows, stream shores, heaths or woods, up to 1000 m.

Distribution: Endemic from the Azores archipelago, on the islands of Flores, Pico, São Jorge, São Miguel and Terceira.

Phenology: May to August

Observations: The previous reports from the other Macaronesian archipelagos (Madeira and Tenerife in the Canary Isles) should be considered as belonging to *C. demissa* ssp. *demissa*.

Key for straight-beaked species of *C. flava* group in the Iberian Peninsula, North Africa and Macaronesia

1. Utricles 2–3 mm long, with a 0.5–0.9 beak, often slightly inflated; male spike sessile or with a 1–6.6(9) mm peduncle; female spikes crowded at the top of the inflorescence, the two uppermost ones separated from each other by 1–8(11) mm, although the lowermost is often distant from the others; leaves flat or channelled, up to 2.6(3) mm wide; stems frequently straight ..... ***C. viridula***

- Utricles 2.5–4(4.4) mm long, with a (0.6)0.7–1.7 beak, not inflated; male spike with a 1–10(12) mm peduncle; female spikes frequently separated along the top of the inflorescence, the two uppermost ones separated from each other by 1.5–20(32) mm, the lowermost generally sub-basilar; leaves flat, up to 3.7(5) mm wide; stems frequently curved..... (*C. demissa*) **2.**

2. Utricles 0.9–1.5 mm wide; lower utricles of each spike patent to slightly deflexed; leaves equal in size to the stems or up to 3(4) times shorter .... ***C. demissa*** ssp. ***demissa***

- Utricles 0.8–1.2 mm wide; lower utricles of each spike patent to erect-patent; leaves 1.5–5 times shorter than stems ..... ***C. demissa*** ssp. ***cedercreutzii***

CAPÍTULO 4

Contrasting patterns of molecular and morphological plant differentiation in circum-Mediterranean mountains (*Carex* sect. *Ceratocystis*, Cyperaceae)

P. Jiménez-Mejías, M. Escudero, S. Martín-Bravo & M. Luceño

Abstract

Bent-beaked taxa of *Carex* sect. *Ceratocystis* are found throughout many circum-Mediterranean mountain ranges (Atlas, Sierra Nevada, Cantabrian range, Pyrenees, Alps, Pindhos range). The populations from different ranges constitute a set of plants with a low level of morphological differentiation between them and other sect. *Ceratocystis* taxa. As a result, the number of recognized taxa in these high mountains ranges from one (*C. nevadensis*) to four depending on the treatment. Previous molecular data showed that populations from different ranges belong to different genetic groups, each associated with a well-defined lowland species. We performed a multivariate analysis of morphological data, including microscopic characters from the achene surface, to study the pattern of variation between mountain ranges. Phylogenetic analyses of plastid *rps*16 and 5'*trn*K sequences were used to correlate the morphological variation with the main haplotype groups. Three different genetic groups were identified among mountain plants of sect. *Ceratocystis*. Plants from the Atlas, Sierra Nevada, Pyrenees and Cantabrian range were included within the western *C. lepidocarpa* group; samples from the Alps were assigned to the *C. flava*–eastern *C. lepidocarpa* complex; *Carex castroviejoi* from the Greek Pindhos range formed a third group. Contrasting patterns of molecular and morphological variation were found between ranges. In the case of plants from the Atlas, Sierra Nevada and Pyrenean-Cantabrian system, the different sets of populations belonged to the same genetic group, but were morphologically distinct according to multivariate analysis. On the contrary, plants from the Pyrenean-Cantabrian system and the Alps differed little in terms of morphology even though they belonged to different genetic groups. Hybridization, convergence induced by similar environmental conditions, ecological specialization, and incipient divergence are discussed as processes that may be involved in this pattern.

Keywords: *Carex flava*, circum-Mediterranean mountains, cryptic taxa, multivariate analysis, micromorphology, SEM, taxonomic boundaries

Introduction

Circum-Mediterranean mountains have been considered as a model in the study of plant evolution in the historical biogeographical context of the last Quaternary glaciations (Vargas, 2003). Cyclic contraction–expansion of these ranges gave rise to disjunction and isolation, leading to genetic divergence, whereas population contact prevented divergence (Comes and Kadereit, 1998, 2003; Kadereit *et al.,* 2004). As a consequence, the combination of recent diversification processes and the effect of multiple secondary contacts has led to intricate taxonomic patterns (see Dixon *et al.*, 2007).

Carex sect. *Ceratocystis* Dumort. is a small group (5–19 species depending on the treatment) of tussock-forming sedges mainly distributed in temperate Eurasia and North America (Schmid, 1983; Crins and Ball, 1989a,b), and characterized by short life-cycles and hybridization processes (Schmid, 1982; Hedrén and Prentice, 1996). The high taxonomic complexity of sect. *Ceratocystis* has led authors to place many of the taxa within the so-called "*C. flava* group" (Schmid, 1983; Crins and Ball, 1989a,b; Stoeva and Štěpánková, 1990; Luceño and Castroviejo, 1993; Pikälä and Toivonen, 1994; Blackstock and Ashton, 2001; Hedrén, 2002; Luceño and Jiménez Mejías, 2008). Previous morphological, cytogenetic and biochemical data have demonstrated the existence of five well-defined species: *C. demissa* Hornem., *C. hostiana* DC., *C. flava* L., *C. lepidocarpa* Tausch. and *C. viridula* Michx. (Schmid, 1983; Crins and Ball, 1989a; Hedrén, 2002). However, several problematic forms from the circum-Mediterranean mountains (Atlas, Sierra Nevada, Cantabrian range, Pyrenees, Alps and Pindhos; Fig. 1) continue to have a disputed taxonomic status (Table 1). These correspond to medium to small-size plants – usually no more than 10 cm high – with bent utricle beaks, the lowest on each spike being deflexed. These forms grow in mountain peat bogs and wet meadows, on different kinds of rocks. The close morphological affinities of these plants are reflected by conflicting taxonomic treatments (Table 1), ranging from analytical to synthetic views. For example, in the *Flora Europaea* (Chater, 1980) most of these morphotypes were united under a morphologically and geographically wide *C. nevadensis* concept. On the other hand, Schmid (1983) considered the plants from the Alps as a distinct form (*C. flava* var. *alpina*) that is not closely related to the Sierra Nevada taxon.

Table 1 Taxonomic treatments of the problematic mountain morphotypes within *C. flava* gr. plants in Europe and Northwest Africa.

Tentative classification used for this work	Geographical range	Previous treatments
C. castroviejoi	Pindhos Range (NW Greece)	*C. lepidocarpa* (Chater 1980; Strid and Tan 1991)
C. nevadensis	Sierra Nevada and Filabres Range (SE Spain)	*C. lepidocarpa s.s.* (Luceño 1994) *C. lepidocarpa* subsp. *nevadensis* (Luceño 1999) *C. lepidocarpa* var. *nevadensis* (Kükenthal 1909; Vicioso, 1959) *C. nevadensis* (Chater 1980; Luceño and Jiménez Mejías 2008)
Alpine plants	Alps	*C. flava s.s.* (Crins and Ball 1989b) *C. flava* var. *alpina* (Schmid 1983) *C. nevadensis* (Chater 1980)
Atlasic plants	High Atlas	*C. flava* var. *nevadensis* (Maire 1957) *C. nevadensis* (Valdés *et al.* 2002)
Pyrenean-Cantabrian plants	Pyrenees and Cantabrian Mountains	*C. demissa* × *C. lepidocarpa* (Luceño and Jiménez-Mejías 2008) *C. flava* var. *alpina* (Luceño 1994 p.p.; Bolòs and Vigo 2001) *C. lepidocarpa* var. *nevadensis* (Vicioso 1959) *C. nevadensis* (Molina *et al.* 2006)

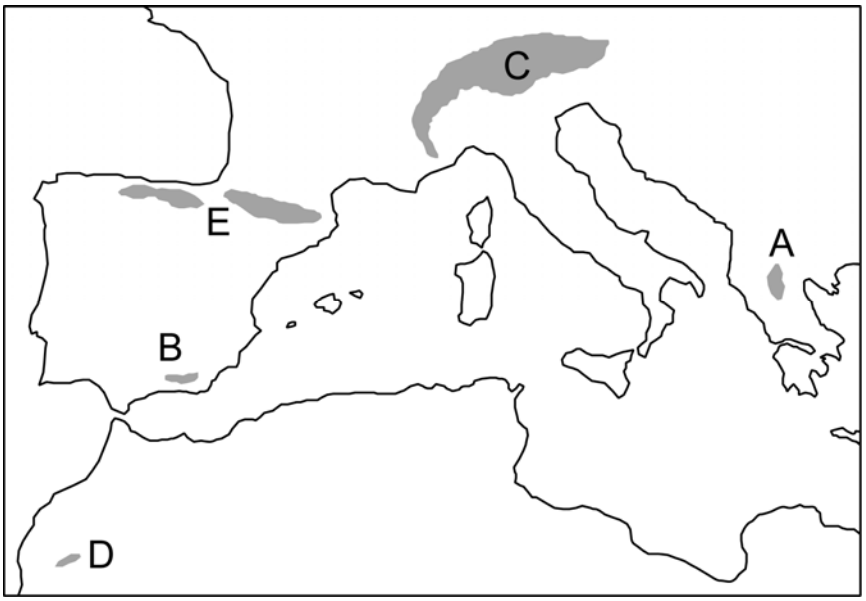

Fig. 1 Distribution map showing the approximate range of the problematic mountain morphotypes of the *C. flava* group in the Mediterranean Basin: A) *C. castroviejoi* (Pindhos range), B) *C. nevadensis* (Sierra Nevada, Sierra de los Filabres), C) Alpine plants (Alps), D) Atlasic plants (SW High Atlas), and E) Pyrenean-Cantabrian plants (Pyrenees-Cantabrian range).

Phylogenetic analyses of 60 samples of sect. *Ceratocystis* using the plastid markers *rps16* and *5'trnK* (Jiménez-Mejías *et al.*, under review (Chapter 2)) revealed that the European members of the *C. flava* group are arranged in two main clades, one including the straight-beaked taxa (*C. demissa* and *C. viridula*), and the other grouping the bent-beaked taxa (*C. flava* and *C. lepidocarpa*). Among the latter, *C. flava* and eastern *C. lepidocarpa* constituted an unresolved complex, whereas western *C. lepidocarpa* formed a well-supported clade. The heterogeneous phylogenetic placement of the mountain haplotypes was revealed by the preliminary sampling carried out in this study: most included samples were placed within the two different bent-baked taxa subgroups (western *C. lepidocarpa* lineage and *C. flava*-eastern *C. lepidocarpa* complex), whereas *C. castroviejoi* was recovered together with the straight-beaked taxa.

Multivariate statistical analyses based on morphological and molecular data have been widely used to successfully unravel systematic relationships within taxonomic complexes in Cyperaceae and specifically in *Carex* sect. *Ceratocystis* (e.g. Crins and Ball, 1989a; Stoeva and Štěpánková, 1990; Blackstock and Ashton, 2001; among others). However, mountain morphotypes of the *C. flava* group were not included in these studies. Principal Component Analysis (PCA) is among the most widely used multivariate analyses. It allows the overall morphological variation within the dataset to be explored without *a priori* taxonomic assumptions. On the other hand, MANOVA supports morphological characters that allow the discrimination of different groups (see Jiménez-Mejías and Cabezas (2007) and Valcárcel and Vargas (2010)).

Taxonomy is mainly based on macromorphological features, since the human brain processes most sensory information as visual (Bickford *et al.*, 2007). In the search for additional morphological characters, micromorphology and anatomy have been used successfully to discriminate macromorphologically similar taxa (Stuessy, 1990). Micromorphological characters of the achene epidermis have been studied in the taxonomy of the Cyperaceae tribe Cariceae, which includes *Carex*, with diverse results. They have facilitated the differentiation of almost identical species complexes (Menapace and Bujek, 1987; Standley, 1987a; Starr and Ford, 2001; Waterway, 1990; Zhang, 2009). However, they have been reported as taxonomically useless for other groups of closely related plants (Standley, 1987b**;** Rothrock *et al.*, 1997). Within *Carex* sect. *Ceratocystis*, the achene epidermis has been studied by Crins and Ball (1989a) and Salo *et al.* (1994). They found that a more or less developed central silica body with

Table 2 Macro- and micromorphological characters studied and loadings for the two principal components (PC-I and PC-II) from MPCA and mPCA. The highest loadings for each component are marked in bold.

Macromorphology

Character	Label	MPCA		
		PC-I	**PC-II**	**PC-III**
Lowest bract length	LBL	0.204	**0.769**	-0.132
Lowest bract width	LBW	0.243	**0.785**	0.075
Male spike width (mm)	MSW	0.184	0.155	**0.906**
Male spike length / width ratio	MSR	0.395	0.342	**-0.746**
Female glume mucro (mm)	FGM	-0.385	0.412	0.026
Utricle length (mm)	UTL	**0.865**	0.333	-0.054
Utricle width (mm)	UTW	**0.716**	0.120	-0.070
Utricle beak length (mm)	UTB	**0.892**	0.084	0.074

Micromorphology

Character	Label	Calculation (see Fig. 2)	mPCA	
			PC-I	**PC-II**
Central body height	cbh	A/B	-0.362	**0.377**
Central body area	cba	B/C	**0.482**	-0.017
Satellite bodies height	sbh	$\frac{\left(\frac{(D+E)}{2}-F\right)}{B}$	0.028	0.211
Central body shape 1	cbs1	A/C	0.016	**0.563**
Central body shape 2	cbs2	G/C	**0.381**	0.298
Inter-platforms gap width	igw	$\frac{(H_1+H_2)}{2}$	0.097	0.264

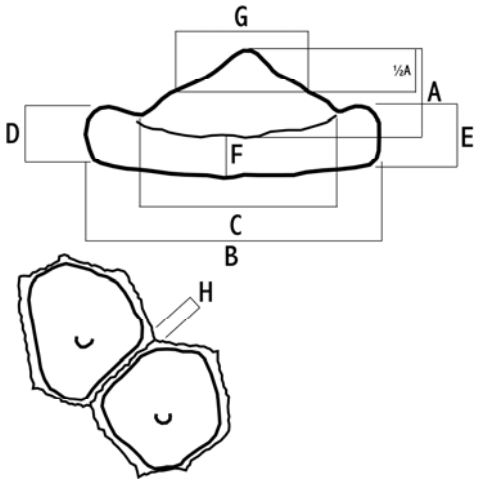

Fig. 2 Schematic drawing of the measurements of the inner anticlinal wall and silica bodies of the achene epidermis cells (A–H) taken from SEM pictures. A) Lateral view, B) overhead view.

several peripheral satellite bodies is located within the epidermis cell, protruding from the inner anticlinal wall. The considerable intraspecific variation between the studied taxa of sect. *Ceratocystis* did not allow precise species circumscription. However, a correlation between the number and size of silica bodies with chromosome number was found, which allowed discrimination of taxa with a lower chromosome number (*C.*

flava; *2n*=58–60) from those with a higher number and size (*C. demissa*, *C. lepidocarpa* and *C. viridula*; *2n*=66–72).

The development of DNA-based barcoding methods has helped taxonomists to detect and morphologically differentiate similar species, including cryptic taxa and species complexes (i.e. Hebert *et al.*, 2004; Bickford *et al.*, 2007; Muellner *et al.*, 2009; Lu *et al.*, 2010). Molecular phylogenies have sometimes acted as preliminary barcoding systems, allowing the identification of new taxa before their formal description (Gurushidze *et al.*, 2008; Pillon *et al.*, 2009; Derieg *et al.*, 2008). The phylogeny of sect. *Ceratocystis*, which is based on *rps*16 and 5'*trn*K plastid sequences (Jiménez-Mejías *et al.*, under review (Chapter 2)), showed that haplotypes have a high taxon-specificity (88.2% of the haplotypes were taxon-specific). These results suggest that plastid sequencing may be used as a barcoding system to elucidate the taxonomic circumscription within *Carex* sect. *Ceratocystis*.

In this paper, we use macro-, micromorphological and molecular data in an effort to clarify the systematic relationships of *Carex* sect. *Ceratocystis* mountain morphotypes from circum-Mediterranean ranges. The main aims of our study were to: 1) explore taxonomic affinities between morphotypes from different mountain ranges and well-defined species on the basis of a plastid-based barcoding method; 2) look for diagnostic characters at the macro- and micromorphological (achene surface) levels, to assess the degree of differentiation between populations from different mountain ranges; and 3) discuss the possible evolutionary processes involved in the patterns of molecular and morphological differentiation found.

Materials and methods

CIRCUMSCRIPTION OF STUDY GROUP

We considered five different groups of problematic mountain bent-beaked morphotypes (Table 1; Fig. 1): *C. castroviejoi* (Pindhos range), *C. nevadensis s.s.* (Sierra Nevada and Sierra de los Filabres, SE Spain), Alpine plants (Alps), Atlasic plants (High Atlas) and Pyrenean-Cantabrian plants (Pyrenees and Cantabrian Mountains).

MOLECULAR STUDY

A total of 31 *rps*16 and 5'*trn*K sequences were included (Appendix 1): 20 populations from the five groups of problematic morphotypes of sect. *Ceratocytis* and eleven populations of the remaining taxa (*C. hostiana*, *C. demissa*, *C. flava*, *C. lepidocarpa* subsp. *lepidocarpa*, *C. lepidocarpa* subsp. *jemtlandica* Palmgr., *C. viridula*, and the introgressive *C. demissa* × *C. viridula*). These latter were chosen in order to represent the haplotype variation of sect. *Ceratocytis* in Europe and North Africa, including all the different haplotypes found in Jiménez-Mejías *et al.* (under review (Chapter 2)). Most sequences (24 rps16 and 5´trnK) were taken from this previous phylogenetic study. Sampling was expanded by obtaining seven additional rps16 and 5´trnK sequences, mainly of populations from the Alps (Appendix 1). Herbarium specimens (M, MA) and silica-dried materials collected in the field (vouchers deposited at UPOS) were included. Total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, California). The PCR conditions and primers followed those described in Schönswetter *et al*. (2006) for *rps16* and Escudero and Luceño (2009) for *5'trnK*. Sequencing was carried out by Stab Vida (Caparica, Lisboa, Portugal). Sequences were edited using Seqed (Applied Biosystems, California). Informative indels were codified as additional characters. Statistical parsimony analysis of the haplotype matrix was performed using TCS (Clement *et al*., 2000).

MACROMORPHOLOGICAL STUDY

One hundred and eleven specimens from herbarium (BM, JACA, LEB, M, MA, MSB, NEU, RNG) and field collections (deposited at UPOS) from the five groups of mountain morphotypes were included in the macromorphological morphometric study: eleven specimens of *C. castroviejoi*, 17 of *C. nevadensis s.s.*, 21 Alpine plants, six Atlasic plants and 56 Pyrenean-Cantabrian plants (Appendix 1). From a previous PCA exploration using 24 characters, we selected eight macromorphological quantitative characters (Table 2) as those that provided the best resolution between the five considered groups. Glume and utricle colour were annotated as qualitative characters.

PCA was conducted to study the macromorphological variation and test morphological relationships (MPCA). Only principal components with eigenvalues greater than 1 were retained. Euclidean distances among group centroids were

calculated and weighted according to the components' relative contribution. MANOVA and the Scheffé post-hoc test were used to statistically test character differences between groups. The quartile distribution was calculated for each variable and morphotype to check the degree of overlap. Characters were considered discriminant either on the basis of MANOVA results or when an overlapping threshold ≤ 25% was found (Valcárcel and Vargas, 2010).

Measurements were made using an ocular micrometer, taking into account differences up to a tenth of a millimetre; all observations were performed under the magnification of a stereoscopic binocular Nikon SMZ645 microscope.

Six specimens of Atlasic plants from the K and MPU herbaria were additionally studied after the analyses were concluded. Despite they were not included in the morphometric analyses, they were taken into account for the taxonomical characterization of Atlasic populations as a separate *C. lepidocarpa* subspecies (see Appendix 2).

MICROMORPHOLOGICAL STUDY

Fifty-one achenes from the five groups of mountain morphotypes were studied: seven from *C. castroviejoi*, ten from *C. nevadensis s.s.*, 17 from Alpine plants, three from Atlasic plants and 14 from Pyrenean-Cantabrian plants (Appendix 1). With the exception of three of these samples (Table 2), all were also included in the macromorphological study. Although most achenes were taken from different vouchers, the lack of mature fruits forced us to include several achenes from the same voucher in a few cases. Achenes were treated (modified from Salo *et al*., 1994) to remove the periclinal and outer anticlinal walls of the epidermis cells, in order to allow the observation of silica bodies. Achenes were digested in a solution of acetic anhydride and sulphuric acid (9:1) for 24 hours at room temperature, washed with distilled water, and then placed in an ultrasonic bath in a Nahita 621/2 sonicator for 10 minutes. Finally, achenes were placed in Petri dishes and air-dried at room temperature. Sonication was repeated when the periclinal and outer anticlinal walls were not totally removed by this treatment. Micromorphology was examined under a Hitachi S3000-N scanning electron microscope at ×1200 magnification after gold coating. Eight different measurements were taken directly from the images (Fig. 2). Two different pictures were taken from each sample: a lateral shot, in order to get the cell anticlinal axis placed perpendicular to

the eye, and thus obtaining a view of the body shape that was as clear as possible; and an overhead shot, with the central body axis aligned with the eye. In order to minimize perspective and scale effects in lateral pictures, micromorphological characters were included as five different ratios (Table 2), which codified different shape parameters. Only igw (see Table 2) was directly included as an average measurement, since it was obtained from the overhead shot and scale was expected to be constant. The resulting micromorphological dataset was analysed using PCA (mPCA), MANOVA and quartile distribution as described above for the macromorphological study.

Results

Molecular variation

All plastid-combined 5´*trnK-rps16* sequences obtained for the mountain morphotypes were 1401 bp long (5'*trn*K: 650-651 bp, *rps*16: 749–750 bp). Nineteen different haplotypes were detected, nine of which were found in mountain morphotypes (Fig. 3). The SP analysis yielded a single network (Fig. 3), in which the nine haplotypes were placed in four different groups: *C. castroviejoi* grouped with the straight-beaked plants (*C. demissa* and *C. viridula*); Alpine plants within the *C. flava*–eastern *C. lepidocarpa* complex; and *C. nevadensis*, Pyrenean-Cantabrian and Atlasic plants in the western-*C. lepidocarpa* group.

Macromorphological variation

The first three components showed eigenvalues higher than one. They accounted for 69.50% of the variance (37.67% for PC-I; 17.39% for PC-II; 14.44 for PC-III). Examination of the scatter-plots from the first three principal components (Fig. 4) revealed *C. castroviejoi* as a clearly distinct group, split from all others with the highest scores of PC-II and PC-III (Figs. 4B–C). All remaining groups overlapped slightly. Alpine plants were displaced toward the highest scores of PC-I (Figs. 4A–B), whereas Atlasic plants and *C. nevadensis* were displaced toward the lowest values. Morphological characters with the highest loadings were, in descending order, UTB, UTL and UTW for PC-I, LBW and LBL for PC-II, and MSW and MSR for PC-III. On the basis of weighted Euclidean distances, Atlasic plants and *C. nevadensis* were the

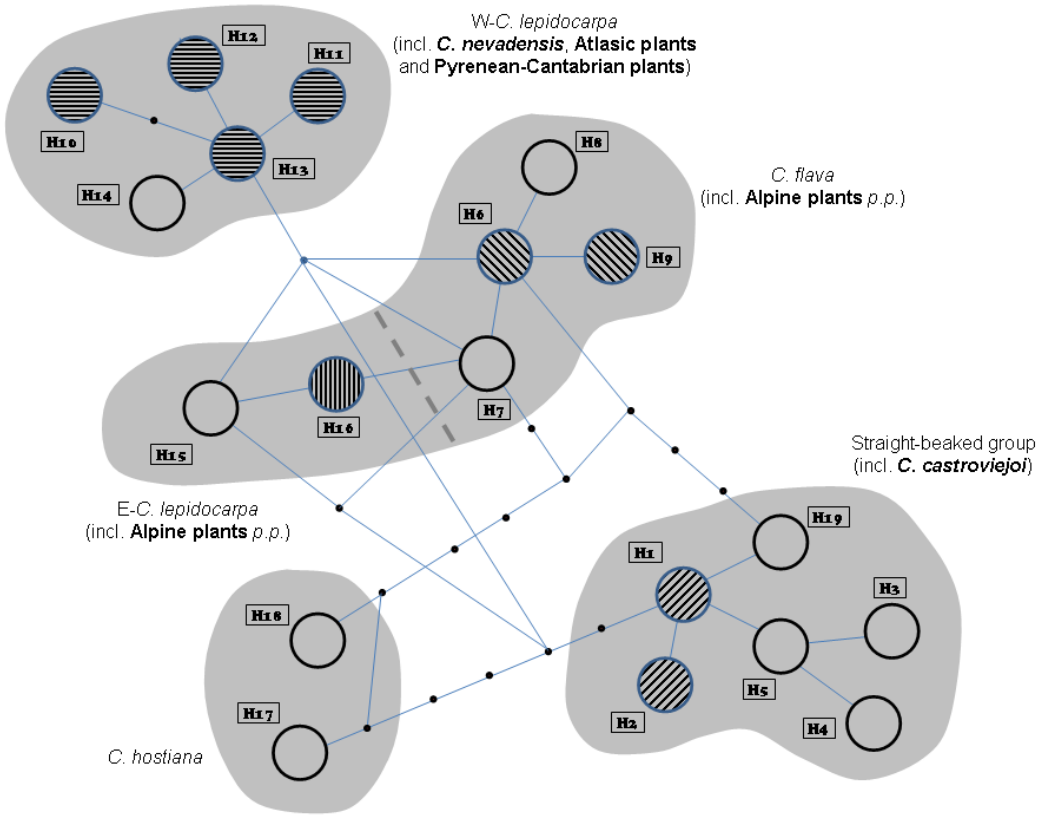

Fig. 3 Statistical parsimony network of the 19 haplotypes (H1-H19) found. Dots represent haplotypes that are extinct or not sampled, and lines between haplotypes represent a sequence mutation step. Full circles depict haplotypes displayed by the problematic mountain morphotypes; empty circles indicate haplotypes found in other taxa of sect. *Ceratocystis*.

closest groups (0.151), whereas Atlasic and Alpine plants were the most different (2.245). MANOVA retrieved significant differences for the eight characters analysed. According to the Scheffe post-hoc test, a unique combination of characters can be found for each group. In addition, when a group was individually compared against all others, the overlap in the majority of such characters was less than 25%. The main macromorphological characters that allowed distinction between populations of the different groups of mountain morphotypes are summarized in Table 4.

MICROMORPHOLOGICAL VARIATION

SEM photographs revealed slight differences in the morphology of silica bodies in the different mountain morphotypes (Fig. 5). Principal component analysis of the micromorphological dataset (mPCA; Fig. 4D) showed no clear split between populations from different mountain ranges. However, the observed grouping trends

contrasted with those observed in MPCA. *Carex castroviejoi* and Atlasic plants were retrieved as peripheral, displaced towards the lowest values of both components, and slightly overlapping with other samples. Alpine plants were placed along PC-II, showing relatively low PC-I scores, whereas Pyrenean-Cantabrian samples were found at medium values of PC-I and PC-II. *Carex nevadensis* was completely intermingled with Alpine and Pyrenean-Cantabrian plants, and partially with *C. castroviejoi*. The first two components accounted for a variance of 67.60% (35.15% for PC-I; 32.45% for PC-II). Weighted Euclidean distances revealed that micromorphological similarity among Atlasic plants and *C. nevadensis* was higher than between *C. nevadensis* and Pyrenean-Cantabrian or Alpine plants. The micromorphological characters with the highest absolute loadings for each component were cbs1 and igw for PC-I and cba and cbs2 for PC-II (Table 2). Despite the poor resolution yielded by PCA, MANOVA found significant differences in all the characters analysed except for igw. When compared in pairs, the Scheffe post-hoc test retrieved morphogroups in different clusters for at least one character, except for *C. nevadensis*, which could not be differentiated from Pyrenean-Cantabrian and Alpine plants, and Atlasic plants, which were not distinct from Pyrenean-Cantabrian plants. Additionally, character overlap between mountain morphotype groups equal to or below 25% was observed in most of the characters distinguished by MANOVA. The main micromorphological characters are summarized in Table 4.

Discussion

Incongruent patterns of systematic relationships revealed by molecular and morphological data

Molecular, macro and micromorphological data reveal that mountain populations of the *C. flava* group are more heterogeneous than could be expected on the basis of the reported taxonomic homogeneity (Chater, 1980; Luceño, 1994). Different degrees of differentiation, as well as patterns of systematic relationships, can be inferred from our results: 1) A genetically and morphologically distinct unit, *C. castroviejoi*, whose taxonomic identity is clearly supported by molecular (Fig. 3) and

macromorphological (Fig. 4B,C) data, and partially by micromorphology (Fig. 4D). The main diagnostic character of this taxon is the male spike width (MSW; Tables 3–4), in congruence with the original species description (Jiménez-Mejías and Luceño, 2009); 2) Genetically indistinguishable but morphologically distinct units, in the case of *C. nevadensis* and Atlasic plants with respect to Pyrenean-Cantabrian plants. Qualitative features and quantitative macro and micromorphological characters (Fig. 4A,B,D; Tables 3–4) allow the recognition of two divergent groups – the Sierra Nevada and Atlas populations – within the western-*C. lepidocarpa* genetic group (Fig. 3). Previous morphological explorations reported the taxonomic distinctiveness of these southern populations in comparison with the clinal variation found between lowland *C. lepidocarpa* morphs and Pyrenean-Cantabrian plants (Chapter 3). The low level of genetic differentiation (Fig. 3) together with the morphogeographic compartmentalization highlight the taxonomic unity of such populations within a single species (Stuessy, 1990). Therefore the subspecies rank could be accurate for at least these two sets of populations: *C. lepidocarpa* subsp. *nevadensis* in SE Spain (Table 1) and a new subspecies in the High Atlas, which is herein described (*C. lepidocarpa* subsp. *atlasica,* Appendix 2). 3) Genetically isolated but morphologically similar units, as in the case of Alpine and Pyrenean-Cantabrian populations. Despite genetic (Fig. 3) and geographical differences, these two groups of populations are macromorphologically close, and the distance between their PCA cluster centroids (1.062; Table 3) is lower than that between the Pyrenean-Cantabrian plants and the most closely genetically related Atlasic plants (1.574) and *C. nevadensis* (1.150). Additionally, Pyrenean-Cantabrian and Alpine populations had no character with less than 25% overlap (Table 3). For these populations, *a priori* knowledge of their geographical origin and genetic barcoding appears to constitute the most straightforward way to achieve reliable identification. Overall, these incongruent patterns of genetic and morphological differentiation represent the systematic complexity of these sets of mountain plants within the *C. flava* group, and suggest the requirement for caution when considering taxonomic boundaries.

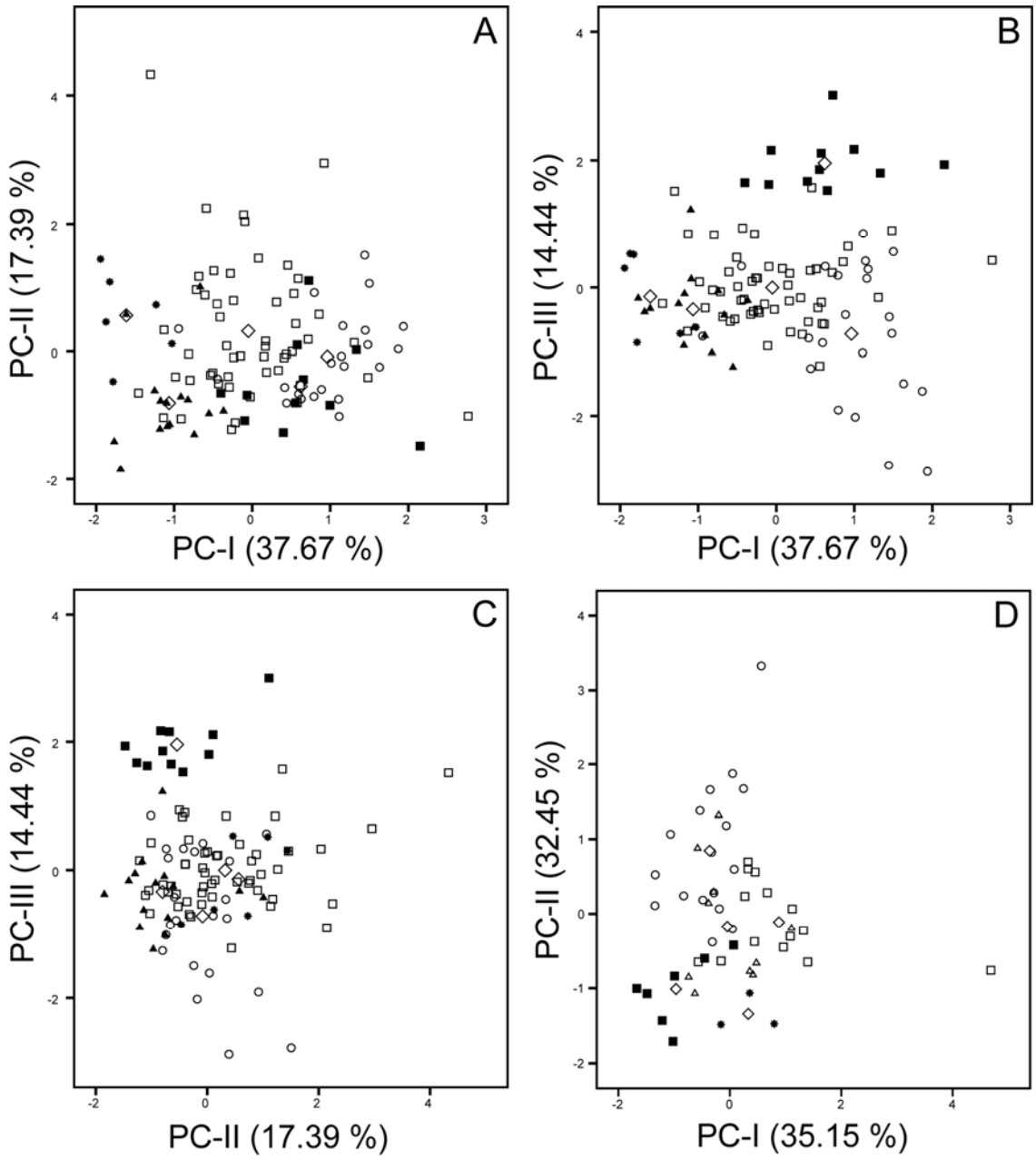

Fig. 4 Scatter plots from the multivariate analyses based on macromorphological (**A–C**) and micromorphological (**D**) data. The different problematic mountain morphotypes are depicted with different symbols: *C. castroviejoi* (■), *C. nevadensis* (▲), Pyrenean-Cantabrian plants (□), Alpine plants (○) and Atlasic plants (✹). Group centroids are represented by diamonds (◇).

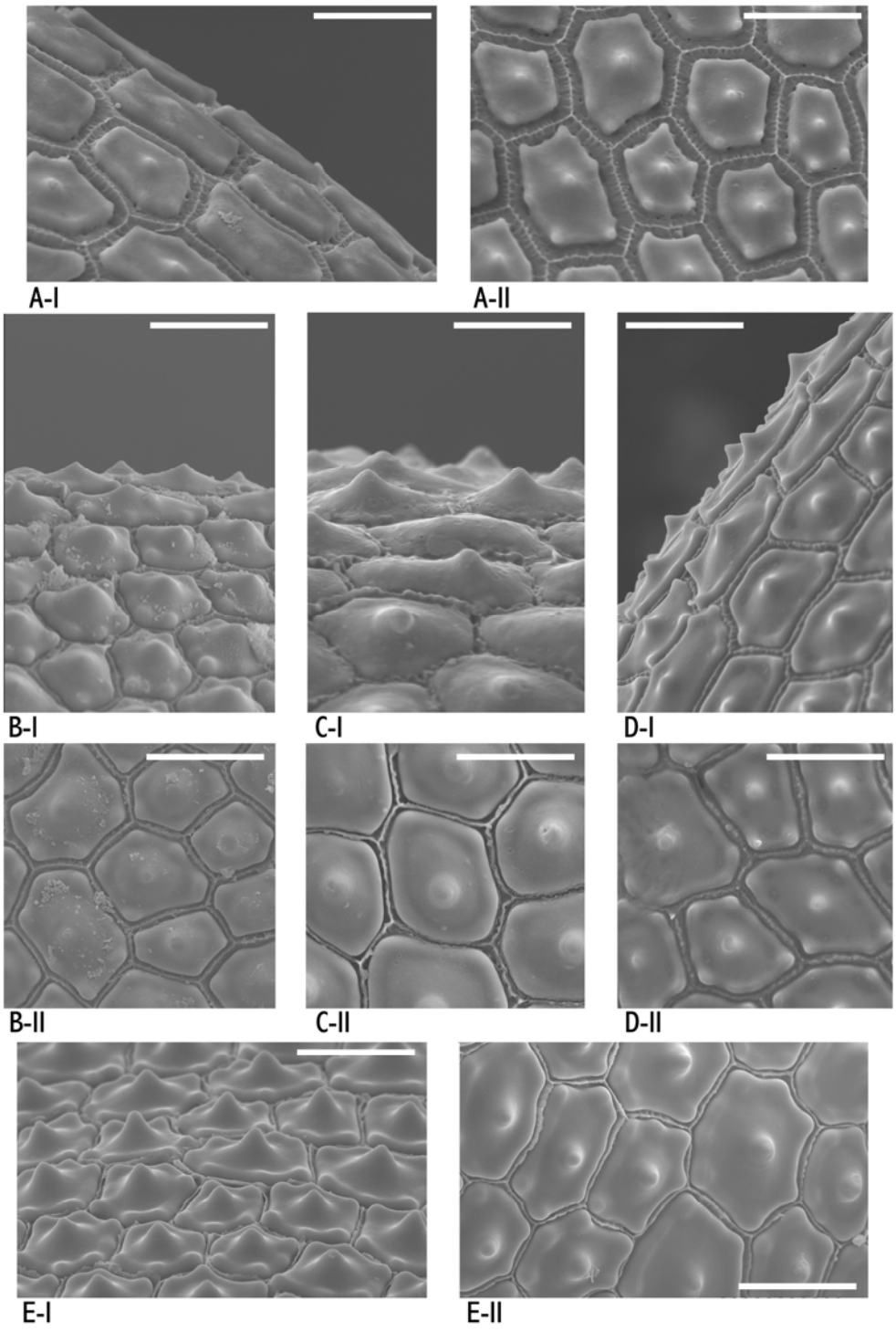

Fig. 5 SEM photographs (A, lateral shot; B, overhead shot) of the wall of the achene of *C. flava* gr. mountain plants: **A,** Alpine plant (Switzerland, Appenzell, RNG); **B,** Atlasic plant (Morocco, Jbel Angour, MSB2497); **C,** *C. castroviejoi* (Greece, Epirus, Milia, UPOS); **D,** *C. nevadensis* (Spain, Granada, Laguna de la Caldera, UPOS); **E,** Pyrenean-Cantabrian plant (Spain, Cantabria, Áliva, UPOS).

CONVERGENCE, INCIPIENT DIVERGENCE OR HOMOGENIZATION?

Despite the relatively similar macromorphological characteristics, which have resulted in a complex taxonomy (Table 1), molecular data allows the linkage of different mountains morphotypes to different genetic groups. Thus, mountain plants from the *C. flava* group might be interpreted as biological entities that are partially isolated from other members of the section *Ceratocystis*, but strongly isolated from each other, with different evolutionary histories that led to the sharing of similar macromorphological features by different phenomena. At least three biological mechanisms could be invoked to explain this morphological homogeneity: convergence, recent divergence and hybridization.

Firstly, morphological convergence between plants from different genetic groups can be induced by similar environmental conditions, as reported for other mountain plant groups (Valcárcel *et al.*, 2006; Lu *et al.*, 2010). The ecological pressure in mountain habitats would modify the well-developed lowland habit to achieve similar adaptations through morphological reduction.

Secondly, isolation during warmer interglacial cycles appears to be insufficient to force strong divergence in mountain populations. Remarkably, the degree of morphological differentiation detected between the mountain populations belonging to the western-*C. lepidocarpa* group follows a pattern of increasing differentiation from the Atlas to the Pyrenees: Atlasic plants are more dissimilar to Pyrenean-Cantabrian populations (1.574), than the latter are to the nearer Sierra Nevada plants (1.150) (Table 3). Successive disruption of genetic exchange, following south-to-north climate warming (Kropf *et al.*, 2006), accurately explains such a pattern of recent divergence.

Third, hybridization has frequently been reported for taxa of sect. *Ceratocystis* (Schmid, 1982; Hedrén and Prentice, 1996; Blackstock and Ashton, 2001, 2010), enabling genetic exchange and therefore contributing to the homogenization of morphological discontinuities. Possible secondary contacts during glacial periods between mountain populations and/or with lowland counterparts could have prevented complete speciation (Comes and Kadereit, 1998; Kadereit *et al.*, 2004; Martín-Bravo *et al.*, 2010). Previous biosystematic evidence (Jiménez-Mejías *et al*., under review (Chapter 2); Chapter 3) supports the hybrid origin of Pyrenean-Cantabrian plants within an extensive area of hybridization between *C. demissa* and *C. lepidocarpa.*

Table 3. Statistical comparisons among morphotypes based on morphological data. Above the diagonal, each cell displays the weighted Euclidean distances between cluster centroids in macro (bolds) and micromorphological (italics) analyses. Below the diagonal, characters that showed significant differences among pairs of morphotypes are marked with an M superscript if retrieved by the MANOVA post hoc test and/or with an O if less than 25% overlap threshold was found.

	Alpine plants	**Atlasic plants**	**Pyrenean-Cantabrian plants**	***C. castroviejoi***	***C. nevadensis***
Alpine plants		**2.604** *1.985*	**1.062** *1.487*	**1.094** *1.691*	**2.061** *0.924*
Atlasic plants	MSR^{M} FGM^{MO} UTL^{MO} UTB^{MO} UTW^{O}cbh^{MO} cba^{MO} cbs2^{O}		**1.574** *1.178*	**2.426** *1.327*	**0.836** *1.061*
Pyrenean-Cantabrian plants	UTL^{M} UTB^{M} cbh^{MO} cba^{O} cbs1^{O}	LBW^{M} FGM^{MO} UTL^{MO} cba^{O} cbs1^{O} cbs2^{O}		**1.072** *1.997*	**1.150** *0.928*
C. castroviejoi	MSW^{MO} MSR^{MO} UTL^{M} cbh^{O} cba^{MO} sbh^{O} igw^{O} cbs2^{MO}	MSW^{MO} FGM^{MO} UTL^{MO} UTB^{MO} cba^{O} sbh^{MO} igw^{O}	MSW^{MO} MSR^{MO} sbh^{MO} cbs1^{MO} igw^{O}		**1.902** *1.162*
C. nevadensis	LBL^{O} LBW^{O} MSR^{M} UTL^{MO} UTW^{MO} UTB^{MO}	FGM^{MO} cbh^{MO} cba^{MO}	LBL^{O} LBW^{MO} UTL^{MO} UTW^{O} UTB^{O}	MSW^{MO} UTL^{MO} UTW^{O} UTB^{MO} sbh^{MO}	

Table 4 Summary of the most important macro and micromorphological characteristics within *Carex flava* gr. mountain morphotypes.

Morphotype	Macromorphology		Micromorphology	
	Inflorescence and spikes	Utricles	Central body	Satellite bodies
C. castroviejoi	Lowest bract 17–75×0.8–2.5 mm, leaf-like. Male spike 3.0–4.0(4.3) mm wide, widely fusiform to elliptical. Female glumes dark brown, muticous.	3.3–4.4×0.9–1.6 mm, with a 1.2–2.5 beak, greenish to yellowish or light brownish.	Well-developed, prominent and rounded, filling an area of at least half of the platform surface.	Absent to inconspicuous and forming a low ridge around the platform borders.
C. nevadensis	Lowest bract 1–5.1(13) mm, setaceous, exceptionally leaf-like if the spike is basilar. Male spike 1.5–2.5(3) mm wide, fusiform to narrowly elliptical. Female glumes dark brown, muticous.	2–3×1 mm, with a 0.9–1.2 mm beak, dark brownish at least in the distal part.	Flattened but sharp, filling an area no more than a quarter of the platform surface.	Well-developed, prominent, rounded.
Alpine plants	Lowest bract 30.0–91.8(110.0)×1–2.5(3), leaf-like. Male spike 1–2.7(3.0) mm wide, fusiform. Female glumes brown, muticous.	(3.0)3.1–5×1–1.7(2) mm, with a (1)1.2–2.2 beak, generally yellowish.	Flattened, rarely sharp, filling an area no more than a quarter of the platform surface; sometimes inconspicuous.	Poorly developed, flattened, sometimes slightly prominent and sharp.
Atlasic plants	Lowest bract (10)22.0–62.5×0.8–1.8 mm, leaf-like to setaceous. Male spike 1.5–2.2 mm wide, fusiform to narrowly elliptical. Female glumes light brown to almost hyaline, with a 0.1–0.3 mm mucro.	2.3–3.4×0.8–1.8 mm, with a 0.9–1.3 beak, greenish to yellowish.	Well-developed, sharp to rounded, filling an area of at least half of the platform surface.	More or less well-developed, not too prominent, rounded.
Pyrenean-Cantabrian plants	Lowest bract 1.5–9.9(13.5)×0.9–3.7(4), leaf-like, rarely setaceous. Male spike 1.5–2.9(3) mm wide, fusiform. Female glumes light to dark brown, muticous, rarely with an up to 0.3 mm mucro.	(2.2)2.4–4.5(5.2)×0.8–1.5(1.6) mm, with a 0.8–1.9(2.5) beak, greenish to yellowish or light brownish.	Well-developed, sharp to rounded, filling an area of approximately half the platform surface.	Well-developed, prominent, rounded.

Conclusions

The incongruent patterns found between genetic and morphological data in most mountain morphotypes illustrate the difficulty of taxonomic decisions within this complex in *Carex* sect. *Ceratocystis*. This work demonstrates that in a taxonomically difficult group, the use of information from different sources (macro- and micromorphology, DNA sequences, geography…) is desirable as it may provide more support to the evolutionary hypotheses inferred, and taxonomic decisions therein derived. This is because systematic data from different sources can reveal both congruent and incongruent aspects.

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Appendix 1

List of studied materials. Symbols within brackets are indicated if samples were included in macromorphological study (*M*), micromorphologycal study (*m*) and molecular study (haplotype number H1-H19, followed by *5'trnK* and *rps16* GenBank accession numbers); symbol # indicates new sequences obtained in this study; ×*n* indicates the number of samples included from the same population, if more than one was included.

ALPINE PLANTS — **Austria 1.** Tal des Faltbaches S von Neuhaus am Zeilerrain *J. Poelt et al.*, BM (*M*); **2.** Feuchten, Felsschutt, *M. Moore*, NEU (*M; m*); **3.** Oberosterreich, S of Bad Ischl Rosmoor, *E.V. Watson*, RNG (*M; m*); **4.** Tirol, Kufstein SE Kaiser. Hintersteiner Tal Wegrans, *J. Höller,* M (*M; m*; H6#, JN627829, JN627836); **5.** Tirol, Bezirk Reutte, Allgäuter Alpen *F. Schuhwerk 94/124*, M (*M; m*; H6#, JN627830, JN627837). **France 6.** Savoie, *Wallace*, RNG (*M*; *m*); **7.** Savoie, Etang de Crosany, *C. Pin*, RNG (*M*). **Italy 8.** Bormio, Val Pettin, *D.W. Tormaz*, NEU (*M*); **9.** Bormio, sentien de la Pliniana, *D.W. Tormaz,* NEU (*M*); **10.** Bormio, sur la rive ganche du Trodolfo, *D.W. Tormaz*, NEU (*M*; *m*); **11.** Bormio, marécauges au defrus de la rive ganche du Trodolfo, *D.W. Tormaz*, NEU (*M*); **12.** Dolomiten, Plan de Gralba, *G. Langer*, M (*M; m*; H16#, JN627826, JN627833). **Germany 13.** Bayern, *A. Hemp & D. Podlech*, M (*m*); **14.** Bayern, S. Bächental, Lärchkogel, *J. Höller*, M (*M*; *m*; H16#, JN627827, JN627834); **15.** Bayern, Karwendelgebirge, *W. Lippert 26592*, M (*M*; *m*; H9#, JN627828, JN627835); **16.** Bayern, München, *H. Förther 4817*, M (*M; m*); **17.** Bayern, Unterhalp Fereinsalm, H. Löffelmann, RNG (*M*; *m*×2); **18.** Bayern, Enzianhütte, *J.Höller*, M (*m*). **Switzerland 19.** Kanton Uri, *A. Kneucker*, RNG, *C. flava* var. *alpina* isolectotypus (*M; m*); **20.** Canton d'Appenzell, nord du Hirschberg, *A. Charpin & P. Geissler*, RNG (*M*; *m*); **21.** Valais, *P. Vargas*, MA (H6, JN627732, JN627799); **22.** Emosson, *C. Oberson*, NEU (*M; m*); **23.** Canton de Neuchâtel, Vallée de la Brévine, *J.P. Brandt*, NEU (*M*); **24.** Lignières, NEU (*M*; *m*).

ATLASIC PLANTS — **Morocco 1.** Jbel Angour, *S.L.Jury et al.*, RNG (*M*); **2.** Oukaïmedem, *S.L.Jury et al.*, RNG (*M*; *m*); **3.** Adrar-n-Oukaïmedem, *A.Herrero et al.*, MA (*M*; *m*; H13, JN627736, JN627803); **4.** Marrakech, Oukaimeden, *D.Podlech*, MSB

(*M*); **5.** Jbel Angour, *Davis*, MSB (*M*); **6.** Jbel Toubkal, Réfugé Nalbus, *W.Rauh*, M (*M*; *m*).

PYRENEAN-CANTABRIAN PLANTS — **Andorra 1.** Puerto de Envalira, *S.Castroviejo & B.Valdés-Bermejo*, MA (*M*); **2.** Lagos de El Serat, *M.Luceño & P. Vargas*, MA (*M*); **3.** Noguera de Ruda, *J.M.Marín et al.*, UPOS (*M*); **4.** Lagos de Pesons, *J.M.Marín et al.*, UPOS (*M*; *m*; H11, JN62774, JN627811). **France 5.** Refugio de Valaz, *S.Muñoz*, MA (*M*); **6.** Hautes Pyrénées, Le Plan-Piau Engaly, *C.Aedo et al.*, ARAN (*M*); **7.** Hautes Pyrénées, Lac de Badet, *C.Aedo &. J. Pedro*, MA (*M*; *m*; H13, JN627745, JN627812). **Spain 8.** Asturias, Cantabrian Mountains, Puerto de Somiedo, *P.Jiménez-Mejías et al.,* UPOS (*M*; *m*; H13, JN627746, JN627813); **9.** Cantabria, Cantabrian Mountains, Brañavieja, *C.Aedo*, MA (*M*); **10.** Cantabria, Cantabrian Mountains, Picos de Europa, El Cable, *M.Luceño*, MA (*M*); **11.** Cantabria, Cantabrian Mountains, Picos de Europa, Fuente Dé, *M.Luceño*, MA (*M*); **12.** Cantabria, Cantabrian Mountains, Fuente Dé, Áliva, *J.M.Marín et al*, UPOS (*M*×2; *m*; H13, JN627747, JN627814); **13.** Girona, Pyrenees, Vall de Ribes, Núria, *M.Luceño et al.*, UPOS (*M*; *m*); **14.** Huesca, Pyrenees, Los Lecherines, *Amich et al.*, MA (*M*, *m*); **15.** Huesca, Pyrenees, Panticosa, *M. Luceño*, MA (*M*); **16.** Huesca, Pyrenees, Candanchú, *F.Gómiz*, LEB (*M*); **17.** Huesca, Pyrenees, Circo de Soaso, *M. Luceño*, MA (*M*); **18.** Huesca, Pyrenees, Benasque, *V.J.Arán & M.J.Tohá,* JACA (*M*); **19.** Huesca, Pyrenees, Canfranc, *L.Villar et al.*, JACA (*M*); **20.** Huesca, Sallent de Gállego, *P.Montserrat et al.*, JACA (*M*); **21.** Huesca, Pyrenees, Benasque, refugio de Ángel Orús, *P.Jiménez-Mejías et al.*, UPOS (*M*); **22.** Huesca, Pyrenees, Panticosa, Balneario, *P.Jiménez-Mejías et al.*, UPOS, (*M*; *m*; H13, JN627751, JN627818); **23.** Huesca, Pyrenees, Panticosa, lagos, *P.Jiménez-Mejías et al.*, UPOS, (*M*; *m*×2); **24.** Huesca, Pyrenees, Ordesa, Góriz, *P.Jiménez-Mejías et al.*, UPOS (*M*; *m*); **25.** Huesca, Pyrenees, Ordesa, *J.Fernández-Arroyo et al.*, UPOS (H10, JN627752, JN627819); **26.** Huesca, Pyrenees, Formigal *M. Portillo.*, UPOS (*M*; *m*); **27.** Huesca, Pyrenees, Ibón de Anayet, *P.Jiménez-Mejías et al.*, UPOS (*M*; *m*); **28.** Huesca, Pyrenees, Sahún, *J.A.Sesé & J.V. Ferrández*, JACA (*M*); **29.** Huesca, Pyrenees, Bielsa, *J.V.Ferrández*, JACA (*M*); **30.** Huesca, Pyrenees, Panticosa, *P.Montserrat & J.A. Sesé*, JACA (*M*); **31.** Huesca, Pyrenees, Refugio de la Estiba, *J.A.Sesé & R. Jiménez*, JACA (*M*); **32.** Huesca, Pyrenees, San Juan de Plan, *J.V.Ferrández & J.A. Sesé*, JACA (*M*); **33.** Huesca, Pyrenees, Fanlo, Monte Perdido, *D.Gómez*, JACA (*M*);

34. Huesca, Pyrenees, Castejón de Sos, *Tornero*, JACA (*M*); **35.** León, Cantabrian Mountains, Puerto de San Glorio, *J.Andrés & R. Carbó*, LEB (*M*); **36.** León, Cantabrian Mountains, Burón, *J.Andrés*, LEB (*M*); **37.** León, Cantabrian Mountains, Puerto de San Glorio, *J.Andrés et al*, LEB (*M*); **38.** León, Cantabrian Mountains, Puerto de Somiedo, *M.Mayor et al.*, MA (*M*); **39.** León, Cantabrian Mountains, Peña Santa de Castilla, *S.Martín-Bravo*, UPOS (*M*); **40.** León, Cantabrian Mountains, Maraña, *E.Puente et al.*, LEB (*M*; *m*); **41.** León, Cantabrian Mountains, Puerto del Pontón, *J.Andrés*, LEB (*M*); **42.** León, Cantabrian Mountains, Campohermoso, *M.J.López-Pacheco*, LEB (*M*); **43.** León, Cantabrian Mountains, Puerto de Leitariegos, *E.Fuentes*, LEB (*M*); **44.** León, Cantabrian Mountains, Aralla, *C.Pérez-Morales*, LEB (*M*); **45.** León, Cantabrian Mountains, Valdelugueros, *J.Andrés*, LEB (*M*); **46.** León, Cantabrian Mountains, Burón, *J.Andrés*, LEB (*M*); **47.** León, Cantabrian Mountains, Puerto de Vergarada, *Blanca*, LEB (*M*); **48.** León, Cantabrian Mountains, Monte de Tejedo, *J.Andrés & R.Carbó*, LEB (*M*); **49.** León, Cantabrian Mountains, Polvoredo, *J.Andrés*, LEB (*M*); **50.** León, Cantabrian Mountains, Pico Huevo, *J.Andrés et al*, LEB (*M*); **51.** Lleida, Pyrenees, Val d'Aran, Tredós, *J.M.Marín et al.*, UPOS (*M*; *m*); **52.** Lleida, Pyrenees, Val d'Aran, Tredós, *M.Luceño et al.*, UPOS (*M*; *m*); **53.** Lleida, Pyrenees, Val d'Aran, Les Bordes, *A.Pallarés*, MA (*M*); **54.** Lleida, Pyrenees, Val d'Aran, Baños de Tredós, *J.M.Marín et. al.*, UPOS (*M*; H11, JN627753, JN627820); **55.** Palencia, Cantabrian Mountains, Curavacas peak, *J.Andrés*, LEB (*M*); **56.** Palencia, Cantabrian Mountains, Cardaño de Arriba, *C.Aedo et al.,* MA (*M*).

C. CASTROVIEJOI — **Greece 1.** Macedonia, Greneva, Pindhos, *K.H.Rechinger*, K (*M*); **2.** Pindhos, Mt. Smolikas, *Cambridge University Expedition,* K (*M*); **3.** Pindhos, Katara Pass, *A.J.Richards,* BM (*M*; *m*); **4.** Pindhos, Mt. Smolikas, *A.J.Richards*, RNG (*M*); **5.** Pindhos, Grevena, *D.Podlech*, MSB (*m*); **6.** Ioannina, *A.J.Richards* (*M*; *m*); **7.** Epirus, Konitsa, *P.Vargas & M.Luceño*, UPOS (*M*; *m*; H1, JN627730, JN627797); **8.** Epirus, Valia Kalda, *M.Luceño et al.*, UPOS, *C. castroviejoi* holotypus (*M*; *m*; H2#, JN627824, JN627831); **9.** Epirus, Milia, *M.Luceño et al.*, UPOS (*M*×4; *m*×2); **10.** Central Pindhos, Ipi, pr. Mt. Smolikas, *R. Gonzalo et al.*, MA (H1#, JN627825, JN627832).

C. NEVADENSIS — **Spain 1.** Almería, Sierra de los Filabres, Gergal, *A.Pallarés*, MA (*M*; *m*); **2.** Almería, Sierra Nevada, Fiñana, *A.Pallarés*, MA (*M*; *m*); **3.** Almería,

Sierra Nevada, El Chullo, , *A.Pallarés*, MA (*M*; *m*); **4.** Almería, Sierra Nevada, Puerto de la Ragua, , *A.Pallarés*, MA (*M*; *m*); **5.** Granada, Sierra Nevada, Corral del Veleta, *M.Luceño et al.*, MA (*M*); **6.** Granada, Sierra Nevada, Monachil, *M.Luceño*, MA (*M*); **7.** Granada, Sierra Nevada, Hoya de la Mora, *J.Fernández-Casas*, MA (*M*); **8.** Granada, Sierra Nevada, Barranco del Goterón, *J.Fernández-Casas*, MA (*M*); **9.** Granada, Sierra Nevada, Prados de Otero, *M.Ladero et al.,* MA (*M*); **10.** Granada, Sierra Nevada, Pico Veleta, *H.Merxmüller & W.Wiedmann,* M (*M*); **11.** Granada, Sierra Nevada, Pico Veleta, Barranco de San Juan, *S.Dürr*, M (*M*; *m*); **12.** Granada, Sierra Nevada, Laguna de las Aguas Verdes, *P.Jiménez-Mejías & M.Escudero*, UPOS (*M*; *m*); **13.** Granada, Sierra Nevada, Laguna de la Caldera, *M.Luceño et al.*, UPOS (*M*×2*; m*); **14.** Granada, Sierra Nevada, Laguna de la Caldera, *M.Luceño et al.*, UPOS (*M*; *m*×3); **15.** Granada, Sierra Nevada, Siete Lagunas, *P.Jiménez-Mejías et al.*, UPOS (*M*; H12, JN627734, JN627801); **16.** Granada, Sierra Nevada, Laguna de la Mosca, *P.Jimenez-Mejías et al.*, UPOS (*M*; H13, JN634646, JN627733).

C. DEMISSA — **Austria 1.** Wien, Bezirk, *B.Wallnöfer*, MA (H1, JN634653, JN627757).

C. FLAVA — **Belgium 1.** Luxembourg, Bellefontaine, *M.Leten*, RNG (H7, JN627705, JN627772); **Italy 2.** L'Aquila, Paso di Campanelle, *C.Navarro et al*, MA (H6, JN627707, JN627774).

C. HOSTIANA — **Germany 1.** Bayern, Kreis Weilheim, *W.Lippert*, M (H17, JN627712, JN627779). **Great Britain 2.** Scotland, Pass of Drumochter, *S.Martín-Bravo et al.*, UPOS (H18, JN627713, JN627780). **Spain 3.** Navarra, Baztan*, A.Balda* (H19, JN627714, JN627781).

C. LEPIDOCARPA SUBSP. LEPIDOCARPA — **Spain 1.** Valladolid, Encinas de Esgueva, *J.L.Fernández* MA (H13, JN627720, JN627787); **2.** Jaén, Cazorla, Hoya del Cambrón, *P.Jiménez-Mejías et al.*, UPOS (H14, JN627719, JN627786). **Switzerland 3.** St. Gallen, Rheintal, *W. Koch*, MA (H16, JN627722, JN627789).

C. LEPIDOCARPA SUBSP. JEMTLANDICA — **Norway 1.** Hedmark, Furuberget, *K.Lye*, O (H15, JN627724, JN627791)

C. VIRIDULA — **Portugal 1.** Coimbra, Lagoa das Braças, *M.Luceño*, MA (H4, JN627728, JN627795). **Greece 2.** Pellas, Voras Mount, *M.Luceño & M.Guzmán*, UPOS (H5, JN627727, JN627794).

C. DEMISSA × VIRIDULA — **Portugal 1.** Estremadura, Serra da Sintra, *P.Jiménez-Mejías et al.*, UPOS (H3, JN627742, JN627809).

Appendix 2

High Atlas *C. lepidocarpa* populations, a new subspecies.

Carex lepidocarpa Tausch. subsp. ***atlasica*** Jim.-Mejías, Martín-Bravo & Luceño **subsp. nova**

Typus: Morocco. High Atlas: Adrar-n-Oukaïmeden, vertiente N, borde pedregoso de arroyo, granitos, 31º11'09"N 7º51'19''W, 3000 m, 29.VII.2006, *A. Herrero & al. AH3090* (holo-MA [746566], fig. 6).

A subspecie sat simili, Carex lepidocarpa subsp. *nevadensis* (Boiss. & Reut.) Luceño*, nova nostra praesertim squamis foemineis mucronatis pallidioribusque atque utriculis pallide viridibus differt.*

Plant tufted. **Fertile stems** 1.5–14(20) cm long, trigonous, smooth, erect or slightly curved. **Leaves** 1–3 mm wide, shorter than or as long as stems, flat; ligule short, rounded to subacute, scarious; basal leaf sheaths inconspicuous, weak, light brown to purplish. **Inflorescence** with the uppermost spikes clustered at top of inflorescence, sometimes with a short peduncled basilar spike; lowest bract 2.2–6.2 cm × 0.8–1.8 mm, generally longer than inflorescence, short leaf-like, rarely bristle-like. **Male spike** 1, 4–9 × 1.5–2.2 mm, narrowly fusiform, with a 2–7 mm peduncle. **Female spikes** 1–3, the lowermost 5–7 mm long. **Male glumes** oval, subacute to obtuse, light brown, with a lighter middle nerve and a conspicuous scarious margin. **Female glumes** oval, subacute to obtuse, mucronate, with a mucro 0.1–0.3 mm long, light brown to almost hyaline, with a hyaline lighter single middle nerve. **Utricles** 2.3–3.4 × 0.8–1.8 mm, greenish to yellowish, those from the lower half of each spike deflexed to almost patent, those from the upper half patent, and the apical ones erect-patent to erect, elliptical, trigonous, plurinerved, abruptly attenuated to a 0.9–1.3(1.5) mm beak, straight to deflexed (0–50º with respect to utricle body), bidentate or bifid, smooth. **Stigmas** 3. **Achenes** obovoid, trigonous.

Phenology: June-July
Ecology: Wet soils in high mountains, over igneous rocks and schist. 2000-3200 m.

Distribution: Morocco, SW High Atlas, known from Jbel Oukaïmedem, Jbel Angour, Jbel Gourza, Jbel Toubkal and Reraya valley

Paratypi: Morocco: High Atlas: in Atlantis Majoris monte Gourza supra oppidum *Amismiz*, 2700-2800 m, 29.VII.1925, R. Maire (MPU); in Atlantis Majoris Valle Reraya, environs d'Arround 2000 m, 19.VII.1926, *R. Maire* (K, MPU); in Atlantis Majoris Valle Reraya, convallis Ouenkrim, 2700-3100 m, 20.VII.1924 (MPU); in Atlantis Majoris Valle Reraya, convallis Ouenkrim, 2780 m, 20.VII.1924 (MPU); Reraya, hte vallée de l'acif Ouenkrim, 3000-3180 m, 24.VII.1923 (MPU); Reraya, hte vallée de l'acif Ouenkrim, 2850-3180 m, 24.VII.1923 (MPU); 72 Km S from Marrakech, Oukaïmedem, 31º12'N, 7º 51'W, 2619 m, 4.VII.1987, *S.L. Jury et al.* (MA, RNG); Marrakech, Hoher Atlas, Umgebung von Oukaimedem und Berge S des Ortes, 31º11'N, 7º51'W, 2500-3000 m, 10.VIII.1999, *D. Podlech* 55321 (MSB); Marrakech, Oukaïmedem, Jbel Angour, 31º10'28''N, 7º50'43''W, 2820 m, 15.VI.2001*, S.L. Jury et al.* (RNG); N. side of J. Angour, near Oukaimedem, ca. 3000 m., wet igneous rocks by stream, 19.VII.1975, *D. Podlech* 37806 (MSB); Toubkal, Réfugé Nalbus, 3100 m., 9.VIII.1951, *W. Rauh* 275 (M).

Fig. 6 Holotype of *C. lepidocarpa* subsp. *atlasica* Jim. -Mejías, Martín-Bravo & Luceño (*A. Herrero et al. AH3090*, MA 746566).

CAPÍTULO 5

Taxonomical delimitation and drivers of speciation in the Ibero-North African *Carex* sect. *Phacocystis* river-shore group (Cyperaceae)*

P. Jiménez-Mejías, M. Escudero, S. Guerra-Cárdenas, K. A. Lye & Modesto Luceño

*Modified from Jiménez-Mejías, P., M. Escudero, S. Guerra-Cárdenas, K. A. Lye & Modesto Luceño. In press. Taxonomical delimitation and drivers of speciation in the Ibero-North African Carex sect. Phacocystis river-shore group (Cyperaceae). *American Journal of Botany*

Abstract

***Premise of the study*:** The Ibero- North African *Carex* sect. *Phacocystis* river-shore group is a set of perennial helophytic species for which taxonomical boundaries are not well-defined. In the present study, we delimitate the different taxonomic units, address the phylogeographic history, and evaluate the drivers of differentiation that have promoted diversification of these plants.

***Methods*:** We analyzed molecular data using statistical parsimony for plastid sequences (26 samples from 26 populations) and principal coordinate analysis, Neighbor Joining and Bayesian Analysis of Population Structure for AFLPs (186 samples from 26 populations). Chromosome numbers from 14 samples (9 populations) are newly reported.

***Key results*:** Three species can be distinguished (*C. acuta*, *C. elata* and *C. reuteriana*). Unexpectedly for rhizome-growing helophytes, the vegetative reproduction detected was incidental. The widespread *C. elata* was found to be a genetically poorly differentiated taxon, whereas the local *C. reuteriana* displayed geographical structuring. Geographical factors seem to be the main driver of differentiation in both taxa.

***Conclusions*:** Despite apparent morphological and ecological similarities, *C. elata* and *C. reuteriana* display disparate genetic structure and evolutionary histories, which may have originated from small ecological differences. *Carex elata* is broadly distributed throughout Europe, and its northern populations were recently founded, probably after the last glacial maximum. In contrast, *C. reuteriana* is an Ibero – North African endemic, with long-standing populations affected by isolation and limited gene flow. It is likely that high-density blocking effects and different gene-flow barriers act together to delimitate its distribution and promote its relatively high population differentiation.

Keywords. AFLP; high density blocking effect; Strait of Gibraltar; cytogenetics; Guadalquivir; haplotypes; helophyte; isolation by distance; *rpl32-trnL*^{UAG}; *ycf6-psbM*.

Introduction

Aquatic plants display a huge diversity of biological and morphological features. However, the relative homogeneity imposed by the environment drives them to share a common set of ecological opportunities and limitations, which have led botanists to assume general patterns for certain groups, such as wide morphological variation, extensive clonal propagation and animal or water-dispersed diaspores (Margalef, 1983; Barrett et al., 1993; Santamaría, 2002). Helophytes are aquatic plants that are emergent and rooted below the water surface. They are both ecologically and physically a bridge between terrestrial and aquatic environments. The unlimited water availability and nutrient richness found in such habitats led helophytes to achieve record primary productivity (4-7 g C m^{-2} day^{-1}; Margalef, 1983). In addition to their ecological importance, their behavior as invasive weedy plants (Madsen, 1997; Chambers et al., 1999, Ash et al., 2004; Hussner et al., 2010) and potential as a food (NAS, 1976; Jiang and Cao, 2008) or energy source (Granéli, 1984; Mariani et al., 2010) has attracted the interest of researchers. Many recent works have reported on the population-structure and phylogeographic aspects of various helophytes. Even among taxonomically close helophyte species, a total lack of genetic patterns has been demonstrated, as shown in the following examples: 1) *Sagittaria sp.* (Dorken and Barret, 2004; Chen et al., 2008) and reeds such as *Phragmites australis* (Lambertini et al., 2006; Lambertini et al., 2008) or *Arundo donax* (Mariani et al., 2010) display relatively low variation over enormous areas; 2) in *Typha sp.* (Na et al., 2010; Till-Bottraud et al., 2010), both geographical distance-dependent differentiation and a large-scale lack of structure have been reported; 3) in the salt-marsh plants *Triglochin maritima* (Lambratch et al., 2006) and *Carex extensa* (Escudero et al., 2010a), large-scale structuring has been detected; and 4) in the freshwater species *Iris pseudacorus* (Lamote et al., 2010) and *Sparganium emersum* (Pollux et al., 2009), fine-scale differentiation was found. This heterogeneity matches the underlying variation among aquatics habitats (Santamaría, 2002), which causes different plants to be affected by different evolutionary drivers and to adapt accordingly.

The section *Phacocystis* Dumort. is one of the largest section of the genus *Carex* (ca. 90 spp; Dragon and Barrington, 2008) and taxonomically one of the most difficult. First, although high species diversification in this group has engendered wide

morphological variation, weak taxonomical boundaries have strongly complicated species discrimination. Second, hybridization phenomena, including hybrid-origin species, have obscured species delimitation in this section (Chater, 1980; Cayouette and Morisset, 1986; Egorova, 1999; Nakamatte and Lye, 2007; Dragon and Barrington, 2008). In the Iberian Peninsula, the so-called river-shore *Carex* group comprises a set of big-sized, tussock-forming helophytic sedges, which grow in aquatic habitats with apparently minor differences regarding ecological preferences (Table 1). Some group members are widespread throughout Europe, as well as in the Iberian Peninsula and North Africa, where they co-exist with other closely related taxa (Fig. 1; Luceño and Aedo, 1994; Luceño and Jiménez-Mejías, 2008). As a result, different publications that have focused on this area differ in both the number and rank of the investigated taxa (Table 2), sometimes including all of them within widespread species, and sometimes splitting some morphs as local endemics. The karyological approach conducted by Luceño and Aedo (1994) on the Iberian Peninsula stated that two species and four taxa were found in Spain and Portugal, a view later followed in Flora Iberica (Luceño and Jiménez-Mejías, 2008): 1) *C. acuta* L.; 2) *C. elata* All. ssp. *elata*; 3) *C. elata* ssp. *reuteriana* (Boiss.) Luceño & Aedo; and 4) *C. elata* ssp. *tartessiana* Luceño & Aedo. In addition, the North African *C. mauritanica* Boiss. & Reut. was considered to be a different species (Table 2). Based on chromosome counts, Luceño and Aedo proposed a new biosystematic arrangement of the group. The higher chromosome numbers found in *C. acuta* (2n = 84 - 86) established a strong chromosome discontinuity versus the continuity found between *C. elata* (2n = 76 - 77) and *C. reuteriana* (2n = 73 – 75, exceptionally 76); which, together with some morphological features, suggested to the authors that they should recognize *C. reuteriana* and consider it under *C. elata*. In addition to the lack of morphological differentiation among river-shore *Carex*, putative zones of hybridization were reported from several localities (cf. Luceño and Aedo, 1994; Luceño, 1994; Luceño and Jiménez-Mejías, 2008). Intermediate individuals were reported in distribution contact zones between *C. reuteriana* ssp. *reuteriana* and *C. elata* (northeastern Iberian Peninsula, Sierra de Urbión) and among the two *C. reuteriana* subspecies (southeastern Spain, northeastern Sierra Morena). Also a punctual contact between *C. acuta* and *C. reuteriana* ssp. *tartessiana* has been suggested (southwestern Iberian Peninsula, Doñana). Although the first of the problem areas may be a consequence of misidentification (the specimens studied were clearly identified as

Table 1. Comparison of the main taxonomic treatments of the Ibero-North African river-shore *Carex* sect. *Phacocystis*.

Present work		**Kükenthal** (1909)	**Vicioso** (1959)	**Chater** (1980)	**Luceño and Aedo** (1994), **Luceño and Jiménez-Mejías** (2008)
C. acuta L.		*C. gracilis* Curtis	*C. gracilis* var. *gracilis*	*C. acuta*	*C. acuta*
C. elata All.		*C. hudsonii* A.Benn.	*C. elata*	*C. elata*	*C. elata* ssp. *elata*
C. reuteriana s.l.	*C. reuteriana* Boiss. ssp. *reuteriana*	*C. reuteriana*	*C. gracilis* var. *gracilis* *C. nigra* var. *broteriana* (Samp.) Vicioso *C. nigra* var. *reuteriana* (Boiss.) Vicioso	*C. nigra* (L.) Reichard	*C. elata* ssp. *reuteriana* (Boiss.) Luceño & Aedo
	C. reuteriana ssp. *tartessiana* (Luceño & Aedo) Rivas Mart.	Not explicit, probably treated under *C. gracilis*	*C. gracilis* var. *mauritanica* (Boiss. & Reut.) Vicioso	*C. acuta*	*C. elata* ssp. *tartessiana* Luceño & Aedo
	C. mauritanica Boiss. & Reut.	*C. gracilis*			*C. mauritanica* Boiss.

Table 2. Taxonomical scheme of the Iberian and North African river-shore *Carex* sect. *Phacocystis*.

Taxon	**Worldwide distribution**	**Distribution in the Iberian Peninsula and N Africa**	**Ecological preferences**
C. acuta L.	Eurasia, eastwards to central Siberia southwards to the Mediterranean	Local occurrence in northwestern Iberian Peninsula: river Duero basin and northern Cantabrian Mountains	Rivers, lakeshores and wetlands in general; indifferent to the soil
C. elata All.	Europe, northwestern Africa and Middle East eastward to Iran	Eastern Iberian Peninsula, Atlantic Iberian strip, Middle Atlas and northeastern Algerian coastal wetlands	Rivers, lakeshores and wetlands in general; preferably on basic soils, but also on sands
C. reuteriana Boiss. ssp. ***reuteriana***	Iberian Peninsula	Northwestern and central Iberian Peninsula	Streams; strictly acidophilous
C. reuteriana ssp. ***tartessiana*** (Luceño & Aedo) Rivas Mart.	Iberian Peninsula	Southern Iberian Peninsula: Sierra Morena and Betic Ranges	Streams; indifferent to the soil
C. mauritanica Boiss. & Reut.	Northwestern Africa	Rif and Tellian Atlas	Streams; indifferent to the soil

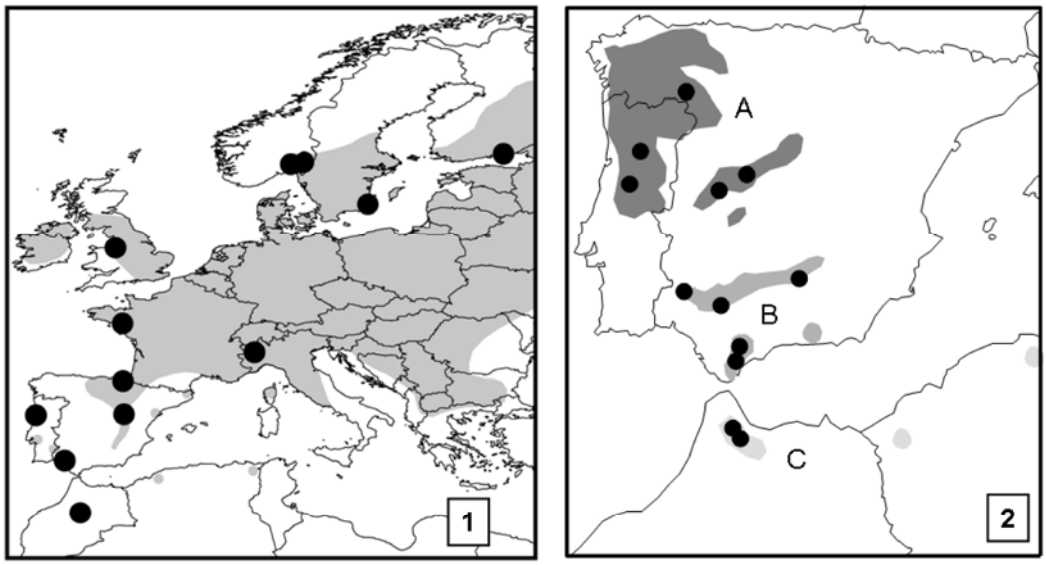


Fig. 1 Sampling localities of **1)** *C. elata*; and **2)** *C. reuteriana* s.l.; shaded areas depict the approximate distribution range in different grey tones: **2A.** *C. reuteriana* ssp. *reuteriana*, **2B.** *C. reuteriana* ssp. *tartessiana*, and **2C.** *C. mauritanica*.

tussocky *C. nigra*, pers. obs.), in the other zones it remains unknown whether the problem is due to hybridization, intra-specific morphological variation or simply misidentification.

AFLPs have been successfully used to clarify taxonomic relationships within sect. *Phacocystis*, and to address some phylogeographical questions (Nakamatte and Lye, 2007; Schönswetter et al., 2008; Volkova et al., 2008). In the present study, we developed a new approach to the analysis of the taxonomy and phylogeographic structure of the river-shore *Carex* group, using new chromosome counts, AFLPs and plastid sequences. The main aims of the present study are: 1) to genetically delimitate the different units of the *Carex* sect. *Phacocystis* river-shore group in the Iberian Peninsula and northwestern Africa, as well as evaluate possible inter-specific gene flow events; 2) to assess the phylogeographic structure of this species group, focusing on the Iberian Peninsula and North Africa; and 3) to test which factors have been drivers of differentiation and speciation.

Material and methods

A PRIORI TAXONOMIC CIRCUMSCRIPTION

In order to ease the interpretation of results, a consensus treatment based on Luceño and Jiménez-Mejías (2008) was followed: *Carex acuta*, *C. elata*, *C. mauritanica* and *C. reuteriana* are presented along the work as independent, distinct species. Within *C. reuteriana*, two subspecies are recognized, ssp. *reuteriana* and ssp. *tartessiana* (Rivas-Martínez et al., 2002). This taxonomic arrange account the morphological and geographical variation of the group. For more details, see Tables 1 and 2.

SAMPLING

A total of 186 samples from 26 populations (2 of *C. acuta*, 12 of *C. elata*, 2 of *C. mauritanica*, 5 of *C. reuteriana ssp. reuteriana* and 5 of *C. reuteriana* ssp. *tartessiana*) were included in the AFLP study (Table 3). Collected fresh leaf material was inmediately stored in silica gel; voucher specimens were deposited at LIV, O and UPOS herbarium. Sample size varied from 3 individuals (1 population) to 10 individuals (6 populations). The chosen sampling covers the distribution of *C. elata* and *C. reuteriana*. However, we only managed to obtain materials from the western half of the *C. mauritanica* distribution (Rif mountains, Morocco). Remarkably, the type parish of *C. reuteriana* ssp. *tartessiana* was sampled, and for *C. elata* a population from Piedmont was included where the type material of this binomen is from. Since *C. acuta* is a rare taxon in the Iberian Peninsula, only two populations were included, 1 from north Spain and 1 from Great Britain, in order to test hybridization or introgressive processes with the other taxa. Plastid sequences were obtained from a single individual per each of the populations.

Fourteen samples from nine populations were included in the cytogenetic study (Table 3): 4 populations from *C. elata*, 3 from *C. reuteriana* ssp. *tartessiana* and 2 from *C. mauritanica*. This is the first study reporting chromosome counts for the northern African *C. mauritanica*. With this sampling, we covered the main gaps in our

Table 3. Geographic location, average gene diversity, BAPS clustering and haplotype, considered chromosome number and voucher of the included populations. Labelling of the populations includes taxon (Acu=*C. acuta*; Ela=*C. elata*; Mau=*C. mauritanica*; Reu=*C. elata* ssp. *reuteriana*; Tar=*C. elata* ssp. *tartessiana*), country [following TDWG botanical countries nomenclature (Brummit, 2001): FIN = Finland, FRA = France, GRB = Great Britain, ITA = Italy, MOR = Morocco, POR = Portugal, SPA = Spain, SWE = Sweden]. N_I, number of individuals. Gene diversity is only shown for populations with at least five individuals. Number of individuals regarding BAPS group in each population is indicated with Arabic numbers. The two GeneBank accessions given for each sample (within brackets) are *rpl32-trnL*^{UAG} and *ycf6-psbM* in the order provided.Chromosome numbers marked with an asterisk (*) are counts from the included populations, bibliographic data are indicated with superscripts ([1] = Luceño and Aedo, 1994; [2] = Roalson, 2008; [3] = original counts), number of individuals counted from this population is indicated with brackets; if the configuration found was irregular it is specified after the inferred diploid number. Herbarium acronyms follow Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

Taxon /Population	Locality	Longitude /Latitude	N_I	Average gene diversity ± SD	BAPS Groups	Plastid Haplotype (GenBank accession)	Considered chromosome number (2n)		*Voucher* / herbarium
C. acuta									
Acu_GRB	Merseyside, Formby	-3.07/53.56	4	-	I:4	H1 (JN222841, JN222815)	≥ 80 [1,2]		*M.Dean* (cultivated material) / LIV
Acu_SPA	Zamora, Carrascal	-5.82/41.49	6	-	I:6	H1 (JN222841, JN222815)			*S.Martín-Bravo 36SMB09* / UPOS
C. elata									
Ela_FIN	Karelia Australis, Kymen Iääni	26.87/60.47	5	0.140 ± 0.087	II-A:5	H2 (JN222842, JN222816)	76 [2]		*K.A.Lye 560* / O
Ela_FRA	Loire Atlantique, St. Gildas des Bois	-2.08/47.51	8	0.106 ± 0.061	II-A:3 II-B:5	H2 (JN222843, JN222817)	76 *,[3](1)	38^{II} $36^{II} + 1^{IV}$	*P.Jiménez-Mejías and M.Escudero 20PJM06* / UPOS
Ela_GRB	Hatchmere, Cheshire	-2.67/53.24	6	0.107 ± 0.065	II-A:3 II-B:3	H2 (JN222844, JN222818)	76 [2]		*M.Dean* (cultivated material) / LIV
Ela_ITA	Piemonte, Lago di Candia	7.91/45.32	8	0.147 ± 0.085	II-A:3 II-B:5	H2 (JN222845, JN222819)	76 [2]		*P.Jiménez-Mejías 74PJM09* / UPOS
Ela_MOR	Middle Atlas, Lac de Wiwane	-5.34/33.13	9	0.090 ± 0.052	II-B:9	H2 (JN222846, JN222820)	76 *,[1]		*P.Jiménez-Mejías et al. 66PJM07* / UPOS
Ela_NOR_1	Oslo	10.86/59.87	4	-	II-A:4	H2 (JN222852, JN222826)	76 [2]		*K.A.Lye 249* / O
Ela_NOR_2	Akershus, Auskog-Høland	11.62/59.87	8	0.090 ± 0.053	II-A:8	H2 (JN222853, JN222827)	76 [2]		*K.A.Lye 73* / O
Ela_POR	Aveiro, Vagor	-8.70/40.57	3	-	II-B:3	H2 (JN222847, JN222821)	76 *,[3](1)		*M.Escudero et al. 51ME07* / UPOS
Ela_SPA_1	Guadalajara, Laguna de Taravilla	-1.98/40.65	10	0.079 ± 0.044	II-B:10	H2 (JN222848, JN222822)	76 *,[1](1)		*P.Jiménez-Mejías and F.Cabezas 31PJM09* / UPOS

(Continued)

Table 3. ***(Continued)***

Taxon /Population	Locality	Longitude /Latitude	N_I	Average gene diversity ± SD	BAPS Groups	Plastid Haplotype (GenBank accession)	Considered chromosome number (2n)	*Voucher* / herbarium
Ela_SPA_2	Huelva, Doñana National Park, La Rocina	-6.52/37.13	10	0.094 ± 0.052	II-B:10	H2 (JN222849, JN222823)	76 *[,3] (1)	*P.Jiménez-Mejías and F.J.Fernández 1PJM07* / UPOS
Ela_SPA_3	Guipúzcoa, Tolosa	-2.07/43.14	9	0.084 ± 0.048	II-B:9	H2 (JN222850, JN222824)	76 *[,3](2)	*P.Jiménez-Mejías et al. 104PJM06* / UPOS
Ela_SWE	Öland	16.55/56.62	4	-	II-A:4	H2 (JN222851, JN222825)	76 [2]	*K.A.Lye 1059* / O
***C. reuteriana* s.l.**								
C. reuteriana* ssp. *reuteriana								
Reu_POR_1	Coimbra, Lousã	-8.23/40.10	5	0.052 ± 0.034	III:5	H4 (JN222856, JN222830)	≤ 75 [1]	*M.Escudero et al. 60ME07* / UPOS
Reu_POR_2	Lamego, Bigorne, Petrarouca	-7.88/41.03	7	0.090 ± 0.053	III:7	H4 (JN222857, JN222831)	≤ 75 [1]	*M.Escudero et al. 37ME07* / UPOS
Reu_SPA_1	Ávila, Hoyos del Espino, Río Tormes	-5.16/40.34	8	0.106 ± 0.061	III:8	H4 (JN222858, JN222832)	≤ 75 [1]	*J.M.Marín and M.Luceño 5504JMM* / UPOS 1004
Reu_SPA_2	Cáceres, Jerte	-5.76/40.22	6	0.115 ± 0.069	III:6	H4 (JN222859, JN222833)	≤ 75 [1]	*P.Jiménez-Mejías et al. 57PJM07* / UPOS
Reu_SPA_3	Zamora, Lago de Sanabria	-6.72/42.12	5	0.069 ± 0.045	III:5	H4 (JN222840, JN222814)	≤ 75 [1]	*S. Martín Bravo et al. 165SMB07* / UPOS
C. reuteriana* ssp. *tartessiana								
Tar_SPA_1	Cádiz, El Gastor, Río Guadalete	-5.45/36.88	10	0.089 ± 0.050	IV-A:10	H5 (JN222860, JN222834)	72 (2), 74 (2) *[,3]	*P.Jiménez-Mejías and I.Pulgar 34PJM07* / UPOS
Tar_SPA_2	Málaga, La Sauceda	-5.57/36.56	10	0.064 ± 0.038	IV-A:10	H5 (JN222861, JN222835)	74 *[,3] (2)	*P.Jiménez-Mejías and I.Pulgar 17PJM07* / UPOS
Tar_SPA_3	Huelva, San Telmo	-6.97/37.79	10	0.090 ± 0.050	IV-B:10	H4 (JN222862, JN222836)	74 *[,3] (1)	*J.M.Marín and M.Luceño 804JMM* / UPOS
Tar_SPA_4	Jaén, Despeñaperros	-3.06/38.39	10	0.067 ± 0.038	IV-B:10	H3 (JN222863, JN222837)	≤ 75 [1]	*P.Jiménez-Mejías and L.Reina 67PJM09* / UPOS
Tar_SPA_5	Sevilla, El Ronquillo, Rivera de Huelva	-6.17/37.67	8	0.091 ± 0.052	IV-B:8	H3 (JN222864, JN222838)	74 *[,1]	*P.Jiménez-Mejías et al. 35PJM07* / UPOS
C. mauritanica								
Mau_MOR_1	Rif, Oued Laou	-5.30/35.14	7	0.093 ± 0.055	IV-C:7	H3 (JN222854, JN222828)	74*[,3] (1)	*A.J.Chaparro et al. 8AJC05* / UPOS
Mau_MOR_2	Rif, Oued Loukos	-5.44/35.03	9	0.074 ± 0.042	IV-C:9	H3 (JN222855, JN222829)	74*[,3] (1)	*A.J.Chaparro et al. 3AJC05* / UPOS

knowledge regarding karyological information in the Ibero - North African distribution of these taxa (cf. Luceño and Aedo, 1994).

DNA ISOLATION, PCR AMPLIFICATION AND AFLP PROCEDURE

Total DNA was extracted from dried tissue using a DNeasy plant extraction minikit (Qiagen, California).

The variability of 10 plastid regions was tested (*5'trnK* intron, *matK*, *psbA-trnH*, *rpl16*, *rpl32-trnL*^{UAG}, *rps16*, *trnL-F*, *trnS-G*, *trnT-L* and *ycf6-psbM*). The most variable plastid regions were *ycf6-psbM* and *rpl32-trnL*^{UAG}, which were amplified and sequenced using primers and protocols as previously described (Shaw et al. 2005, Shaw et al. 2007).

AFLP procedure followed Gaudeul et al. (2000) with modifications of Escudero et al. (2008a). The following primer combinations were used: *Eco*RI AGA (6-FAM) - *Mse*I CAT and *Eco*RI AGG (VIC) - *Mse*I CAA. Selective PCR products were run on a capillary sequencer (ABI Prism 3700 DNA analyzer; Applied Biosystems, Foster City, California, USA) with the internal size standard GeneScan 500 LIZ (Applied Biosystems). Replicates were included to estimate reproducibility. Seventeen replicate samples (9 % of the sampling) were used to calculate the average proportion of correctly replicated bands (Bonin et al., 2004). Markers with low reproducibility were excluded. Rare presences and absences below the frequency of the error rate were removed from the matrix.

CHROMOSOME COUNTS

Meiotic plates were prepared following the protocol described by Luceño (1988) and later observed under a microscope (Nikon eclipse E400) and photographed using a digital camera (Nikon DxM1200 F). Diploid numbers were inferred from meiotic configurations in pollen mother cells at metaphase I, following the formula (1 × each univalents) + (2 × each bivalents) +·…·+ (n × n-valents), where n indicates the number of homologous chromosomes associated with one another at metaphase I.

DATA ANALYSIS

- Plastid sequences

Plastid sequence chromatograms were visualized and edited using SeqEd ver. 1.0.3 (Applied Biosystems). Matrices were manually aligned using a text editor. Informative indels were codified as binary characters (A / T). Statistical Parsimony analysis (SP) was conducted using TCS ver. 1.21 (Clement et al., 2000), in order to identify identical haplotypes and establish the genealogical relationships among them.

- AFLP

Fragments in the range of 50-500 bp were automatically scored using GeneMapper 3.7 and manually revised. The results were exported as a presence/absence (1/0) matrix.

In order to obtain a clear understanding of the analyses, we defined sub-data sets based on our results. Our results provide a clear genetic partition which fits with the existence of three species (*C. acuta*, *C. elata* and *C. reuteriana*; see Discussion and Appendix 1). Based on this fact, three matrices were edited for analyses: 1) a matrix containing the whole dataset; 2) a matrix containing only *C. elata* samples; and 3) a matrix containing *C. reuteriana* ssp. *reuteriana*, *C. reuteriana* ssp. *tartessiana* and *C. mauritanica* (*C. reuteriana* s.l. in the remainder of the text).

Principal coordinate analysis (PCoA) with Jaccard's coefficient was used to analyze the whole dataset in NTSYS ver. 2.10t (Rohlf, 1997). Neighbor Joining (NJ) analysis of tree topology was performed using Nei-Li distances (Nei and Li, 1979) as implemented in PAUP* ver. 4.0b10 (Swofford, 2002). Branch reliability was assessed by bootstrapping (10,000 replicates). Bayesian Analysis of Population Structure (BAPS version 4.14; Corander et al., 2003) was used to estimate the population structure by clustering individuals and populations into panmictic groups (mixture clustering). We ran 10 replicates from each of the nine simulations from K=2 to K=10. Analysis of molecular variance (AMOVA) to evaluate genetic differentiation was computed using ARLEQUIN ver. 3.5 (Excoffier and Lischer, 2009) for the whole dataset, considering different groups: the three species (*C. acuta*, *C. elata* and *C. mauritanica*) considered by

Luceño and Aedo (1994); the three species (*C. acuta*, *C. elata* and *C. reuteriana*) proposed from this study (see results, see Appendix 1); and the four BAPS groups (see results). For AMOVA, only populations with more than five individuals were taken into account.

BAPS, PCoA and AMOVA analyses were performed on the *C. elata* and *C. reuteriana* s.l. datasets. Phenotype diversity was evaluated using R-script AFLP-dat ver. 2008 (Ehrich, 2006, updated in 23rd January 2008); clones were identified as those phenotypes that differed in a number of bands below the frequency of the error rate. Gene diversity according to Nei's diversity (Nei, 1987) was computed using ARLEQUIN ver. 3.5 (Excoffier and Lischer, 2009). A Mantel test was performed to estimate the correlation between genetic (Nei, 1972) and Euclidean geographic distances, as implemented in GENALEX ver. 6 (Peakall and Smouse, 2006). Different subpartitions within each dataset were considered in order to evaluate geographical units: for *C. elata*, a subpartition following the assignment of individuals to the two BAPS groups (see results); and for *C. reuteriana* s.l. four different groupings and subpartitions (1 - *C. reuteriana* ssp. *reuteriana*, 2- *C. reuteriana* ssp. *tartessiana*, 3- *C. reuteriana* ssp. *reuteriana* + *C. reuteriana* ssp. *tartessiana*, and 4- *C. reuteriana* ssp. *tartessiana* + *C. mauritanica*) considering both taxonomic partition and geographical discontinuities.

Different biological or ecological features previously reported as important factors in the evolution of the genus *Carex* were evaluated over the three data sets as possible drivers of the differentiation and speciation processes. The different populations were grouped following different criteria, such as the best given mixture partition in each case (see results). The tested features were: ecological preferences (calcareous *vs*. siliceous substrate; Choler et al., 2004; Escudero et al., 2008b), biogeographical areas (Escudero et al., 2009), diploid chromosome numbers (Luceño and Aedo, 1994; reviewed in Hipp et al., 2009) and the haplotypes reported in the present study (Table 3). Haplotype partitions was not tested in the *C. elata* dataset, because all of the populations were homogeneous. Three chromosome-based partitions were performed in the dataset: 1) $2n \geq 80$ (*C. acuta*); 2) $2n = 76$ (*C. elata*); 3) $2n \leq 75$ (*C. reuteriana* s.l.). Chromosome data were taken from Luceño and Aedo (1994), Roalson (2008) and our own counts. It should be noted that for the 12 populations, chromosomal information came from the exact sampling sites used in this study (Table

3). Geographical partitions chosen were: 1) for the whole dataset: non-Mediterranean Europe, Iberian Peninsula, Italian Peninsula, and North Africa (considering the main putative glacial refugia in the Mediterranean, post-glacial re-colonized areas and the North African disjunction; Taberlet et al., 1998, Escudero et al., 2008a); 2) for *C. elata*: southern Europe + North Africa and central + northern Europe (considering putative glacial refuges and postglacial colonized areas; Taberlet et al., 1998); and 3) for *C. reuteriana* s.l.: the northwestern Iberian Peninsula, Central Range, Sierra Morena, Penibetic Range and northwestern Africa (following the main regional patches in which the distribution is split; see Fig. 1).

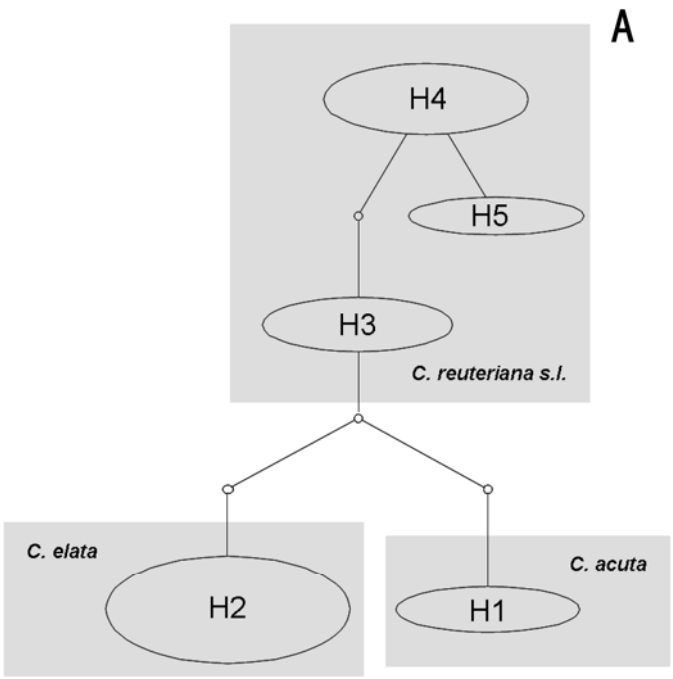
- Morphological study

Our molecular results regarding systematic relationships of *C. reuteriana* ssp. *reuteriana*, *C. reuteriana* ssp. *tartessiana* and *C. mauritanica* (see Results) leaded us to re-evaluate morphological boundaries. Herbarium specimens from 69 different collections of these taxa were examined. In addition to the vouchers of the 26 populations included in the molecular and cytogenetic studies, materials from BCN, COI, FI, G, LISE, MA, MGC, MPU, PO, SALA, SANT, SEV and UPOS herbaria were studied (Appendix 2). Diagnostic characters provided in previous taxonomical revisions of the group (Luceño and Aedo, 1994; Luceño and Jiménez-Mejías, 2008) were examined in the studied specimens: basal sheaths, lowest bract length, sex distribution, spikes size, and utricle size, surface and color.

Results

PLASTID SEQUENCES

The complete aligned length of the plastid matrix was 1123 bp (*rpl32-trnL*^{UAG} 710 bp; *ycf6-psbM*, 413 bp), and a total of six nucleotide substitutions were found. In addition, two indels were coded: one of 7 bases (*rpl32-trnL*^{UAG}) and the other of 17 bases (*ycf6-psbM*). Five different haplotypes (labeled H1-H5; Fig. 2A, Table 3) were identified. Two taxa displayed a single, specific haplotype: *Carex acuta* (H1) and *C.*



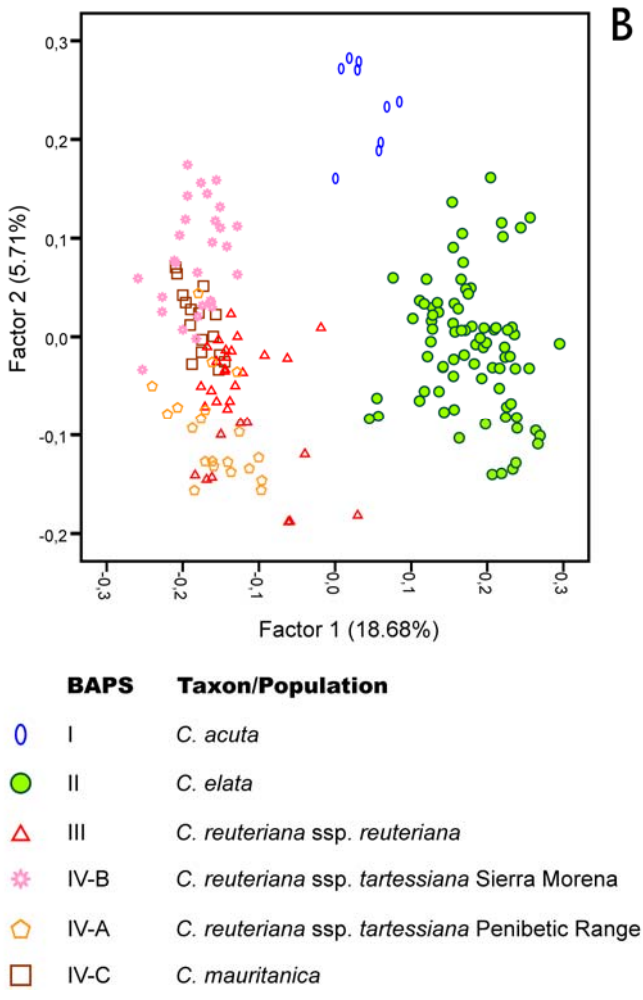


Fig. 2 A) Statistical parsimony network of the five *ycf6-psbM* and *rpl32-trnL*^{UAG} haplotypes found among all of the sampled populations; small black circles represent haplotypes extinct or not sampled; shape sizes are proportional to the number of samples included; each line between haplotypes represents a mutation step. **B)** PCoA scatter plot of AFLP data from the whole dataset; genetic groups identified by the BAPS software are depicted by different shapes.

elata (H2); another two taxa showed a single haplotype, but shared with *C. reuteriana* ssp. *tartessiana*: *C. mauritanica* (H3) and *C. reuteriana* ssp. *reuteriana* (H4); *C. reuteriana* ssp. *tartessiana* showed three different haplotypes: H3, H4 and H5, from which only the last one was taxon-specific. A single network was yielded by the SP analysis (Fig. 2A).

AFLPs

- Whole dataset

A resulting matrix of 140 AFLP fragments with a 98.2% of reproducibility was analyzed.

PCoA performed over the whole dataset revealed three well-defined clusters (Fig. 2B): one containing *C. acuta*; another with *C. elata*; and the last combining *C. reuteriana* ssp. *reuteriana*, *C. reuteriana* ssp. *tartessiana* and *C. mauritanica*. The three first factors of the PCoA accounted for 18.68%, 5.71% and 5.27% of the variation, respectively. NJ analysis retrieved a similar structure, with three homogeneous clusters each containing one of these three groups (Fig. 3). Only branches containing *C. acuta* showed significant bootstrap support (> 75 %) which was taxonomically relevant; within the remaining clades, only groups of 2-3 samples from the same populations were significantly supported. However, BAPS analyses suggested that K=4 is the most accurate number of groups: *C. acuta*, *C. elata*, *C. reuteriana* ssp. *reuteriana*, and a fourth cluster containing *C. reuteriana* ssp. *tartessiana* plus *C. mauritanica* (groups I-IV respectively; Table 3). AMOVA analysis (Table 4) revealed up to 41.62 % of the variation among the populations and 58.38 % within them. When they were merged into the species put forth by Luceño and Aedo (1994), or the three species proposed in this study (which match clusters yielded by PCoA), the variation among populations within the groups was higher in the former (34.38 % *vs* 20.82 %, respectively); and among groups higher in the latter (13.52 % vs. 28.76 %). Following the best BAPS partition (K = 4), values of variation among populations within groups (17.94 %) and among groups (29.20 %) were similar to those found for the three proposed species.

After BAPS partition, the best scoring *a priori* evaluated grouping (Table 5) was the chromosome-based partition (-6271.32), followed by haplotypes (-6336.63). The

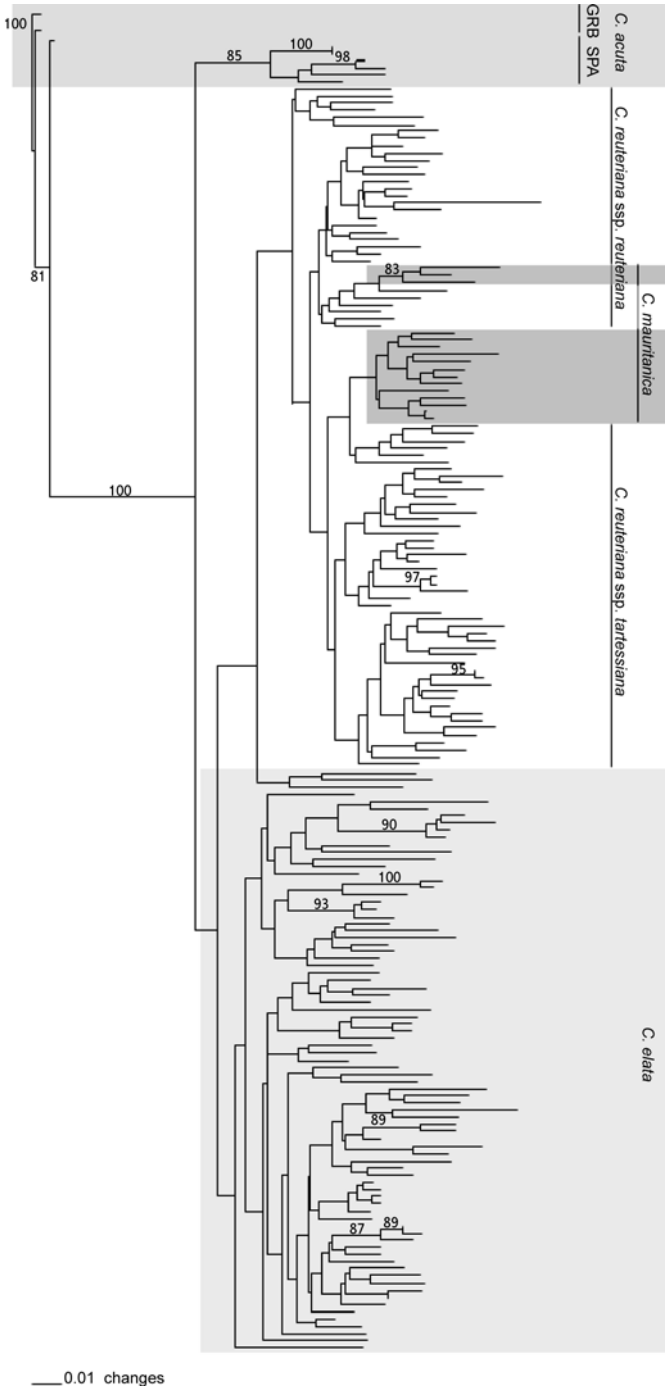


Fig. 3 Neighbor-joining tree constructed with the AFLP data from the whole dataset using Nei and Li (1979) genetic distance coefficients; numbers above branches indicate bootstrap values > 50% (10,000 replicates). Shading and lines indicate groups of samples from the same species and / or subspecies.

worst scoring *a priori* evaluated grouping was achieved for populations sorted by ecological preference (-7230.94). In any case, the marginal likelihood of the optimal partition suggested by blind BAPS analysis displayed a significantly better fit (-6193.38; Table 5).

Table 4 AMOVA analyses for AFLP genotypes.

Grouping compared and source of variation	d.f.	Sum of Squares	Variance components	Percentage of variation
Whole dataset				
Populations				
Among pops.	21	845.142	4.48868	41.62%
Within pops	145	912.954	6.29623	58.38%
Three species from Luceño and Aedo (1994)				
Among groups	2	108.129	1.63395	13.52%
Among pops.	19	737.013	4.15430	34.38%
Within pops.	145	912.954	6.29623	52.10%
Three species proposed in this work				
Among groups	2	339.460	3.59036	28.76%
Among pops.	19	505.682	2.59902	20.82%
Within pops.	145	912.954	12.48561	50.43%
BAPS partition K=4				
Among groups	3	430.040	3.47866	29.20%
Among pops.	18	415.101	2.13740	17.94%
Within pops.	145	912.954	6.29623	52.85%
C. elata				
Populations				
Among pops.	8	171.461	1.86953	21.15%
Within pops	61	425.196	6.97043	78.85%
***C. reuteriana* s.l.**				
Populations				
Among pops.	11	334.411	3.12117	34.93%
Within pops	145	482.558	5.81395	65.07%
Two subpartitions (*C. reuteriana* ssp. *reuteriana*, *C. reuteriana* ssp. *tartessiana* + *C. mauritanica*)				
Among groups	1	90.602	1.61778	16.55%
Among pops.	10	243.808	2.34137	23.96%
Within pops.	83	482.558	5.81395	59.49%
Two subpartitions (*C. reuteriana* ssp. *reuteriana* + *C. reuteriana* ssp. *tartessiana*, *C. mauritanica*)				
Among groups	1	56.890	1.06118	10.97%
Among pops.	10	277.521	2.79529	28.91%
Within pops.	83	482.558	5.81395	60.12%
Three subpartitions (*C. reuteriana* ssp. *reuteriana.*, *C. reuteriana* ssp. *tartessiana*, *C. mauritanica*)				
Among groups	2	144.884	1.76933	18.58%
Among pops.	9	189.527	1.93818	20.36%
Within pops.	83	482.558	5.81395	61.06%

- *Carex elata*

Carex elata was found to be subdivided into two groups (groups IIA, IIB; Table 3), as suggested by BAPS: one containing mainly Iberian and Moroccan individuals, and another with North European samples (populations from France, Italy and Great Britain showed individuals assigned to both groups). Although PCoA did not show any clear split within *C. elata*, when the two BAPS groups were plotted they were almost perfectly separated (Fig. 4A). The first three factors accounted for 9.22 %, 7.61 % and 6.15 % of the variation, respectively. The distribution of pairwise differences among the sampled plants was unimodal, refuting the notion of clonal reproductive behavior in *C. elata* (cf. Ehrich et al., 2008): only two clones were found among the 81 samples included. The average gene diversity was 0.124 (SD = 0.062). No clear geographical structuring was found for the gene diversity, although the poorest populations were both from the Iberian Peninsula (ElaSPA1, ElaSPA3; Table 3); the most diverse populations were those from Italy (Ela_ITA; Table 3) and Finland (Ela_FIN; Table 3). AMOVA analysis found 21.15 % variation among the populations and 78.85% variation contained within the populations (Table 4). Results from the Mantel test for the twelve populations were not significant (R^2 = 0.020; P = 0.180). When subpartitions were tested, the Mantel test yielded statistical significance for group IIB (R^2 = 0.1941; P = 0.030) but not for group IIA (R^2 = 0.015; P = 0.350).

In a comparison of the two partitions (biogeographical regions *vs.* ecological preferences) with the blind one given by BAPS (Table 5), the geography-based partition was the best (-2667.75), although the best partition from mixture analysis was significantly better (-2632.78).

Table 5 Probabilities obtained by the different proposed clustering over the datasets. The two best partitions are indicated in bold.

Grouping	**Log. Likelihood**		
	Whole dataset	***C. elata***	***C. reuteriana* s.l.**
Mixture analysis	**-6193.38**	**-2632.78**	**-2703.78**
Ecological preference	-7230.94	-2742.97	-2927.43
Geographical areas	-7163.90	**-2667.75**	**-2763.83**
Haplotype	-6336.63	-	-2855.81
Chromosome numbers	**-6271.32**	-	-

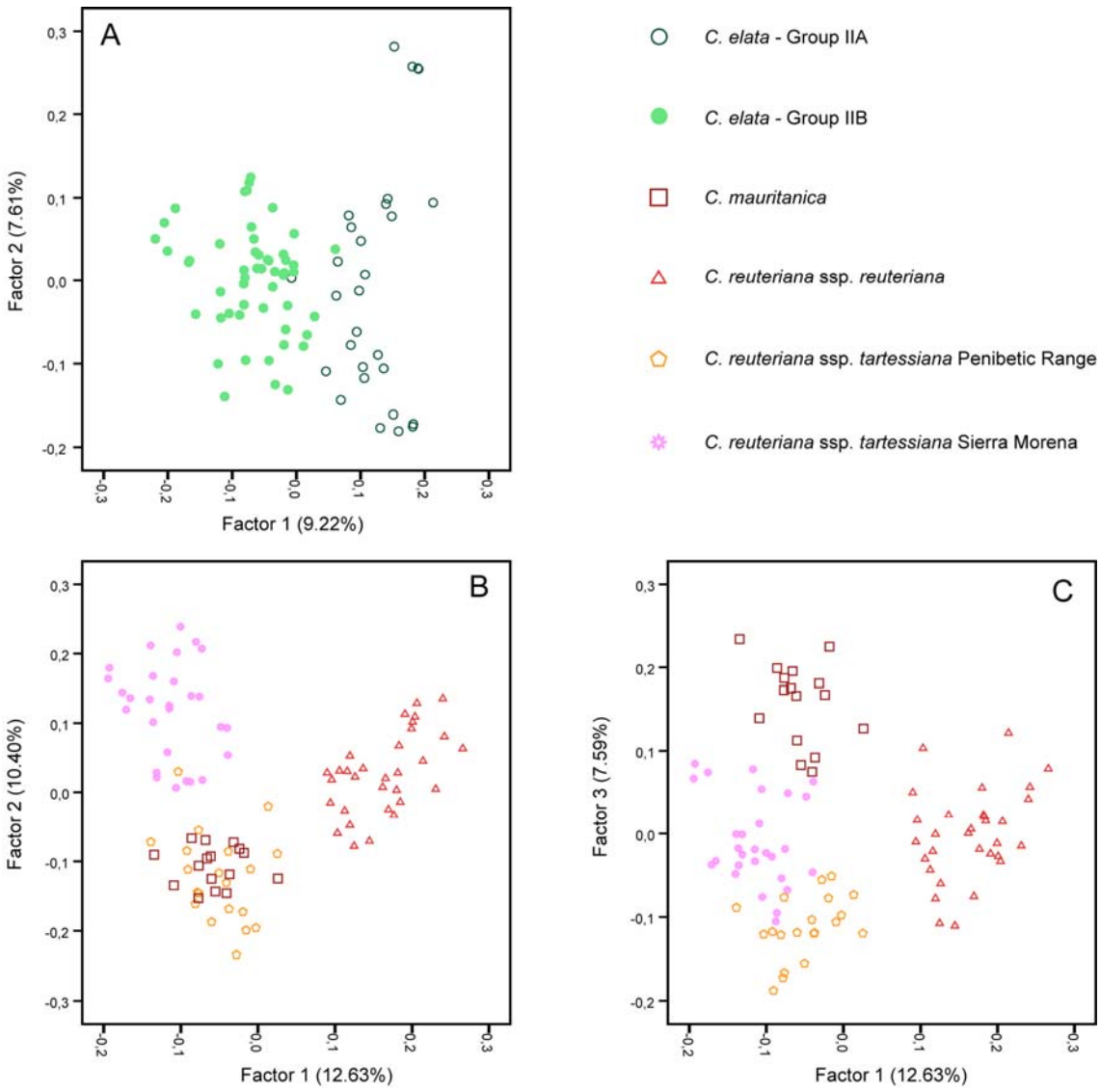


Fig. 4 PCoA scatter plots of AFLP data from the partial datasets: **A)** *C. elata*; **B, C)** *C. reuteriana* s.l. Genetic groups identified by the BAPS software are depicted by different shapes in each of the plots.

- *Carex reuteriana s.l.*

Carex reuteriana s.l. was subdivided by BAPS into four groups, with clear geographical meaning which match groups III and IV from the whole dataset analysis: the northern and central Iberian Peninsula (*C. reuteriana* ssp. *reuteriana*), which matches group III and three clusters from group IV, the first from the southwestern Penibetic Range (*C. reuteriana* ssp. *tartessiana* populations TarSPA_1 and TarSPA_2, labelled IV-A), another from the Sierra Morena (*C. reuteriana* ssp. *tartessiana*

populations from TarSPA_3 to TarSPA_5, labeled IV-B) and the last one containing North African samples (*C. mauritanica* populations, labeled IV-C). PCoA (Fig. 4B, C) displayed two clear groups, which match those retrieved by BAPS when the whole matrix was analyzed: one group containing samples of *C. reuteriana* ssp. *reuteriana*, and another group which combined *C. reuteriana* ssp. *tartessiana* and *C. mauritanica*. The scatter plot of factors 1 and 3 (Fig. 4C) revealed that this latter cluster is subdivided into three subclusters, matching the BAPS result when *C. reuteriana* s.l. is analyzed alone: *C. reuteriana* ssp. *tartessiana* from the southwestern Penibetic Range (group IV-A), *C. reuteriana* ssp. *tartessiana* from the Sierra Morena (group IV-B) and the North African *C. mauritanica* (group IV-C). In this case, the three first factors accounted for 12.63 %, 10.48 % and 7.59 % of the variation. The distribution of pairwise differences was unimodal as in *C. elata* (see above); only three clones among 93 samples were found. The average gene diversity was 0.124 (SD = 0.062). Again, no geographical structure was found for diversity; the first quartile (the most diverse populations) contained *C. reuteriana* ssp. *reuteriana* populations from central Spain (ReuSPA_1, ReuSPA_2) plus one *C. mauritanica* population (MauMOR_1), whereas in the last quartile a Portuguese *C. reuteriana* ssp. *reuteriana* population (ReuPOR_1) plus two *C. reuteriana* ssp. *tartessiana* populations (TarSPA_2, TarSPA_4) were found. AMOVA analyses (Table 4) revealed that 34.93 % of the variation was found among the populations and 65.07 % within the populations. Analyses considering three subpartitions (1 - *C. reuteriana* ssp. *reuteriana*, 2 - *C. reuteriana* ssp. *tartessiana*, 3 - *C. mauritanica*) or considering *C. reuteriana* ssp. *tartessiana* and *C. mauritanica* as a single group (1 - *C. reuteriana* ssp. *reuteriana*, 2 - *C. reuteriana* ssp. *tartessiana* + *C. mauritanica*) did not yield large differences among the results (16.55 % and 18.58 % of the variation found among the groups, respectively). However, if both subspecies of *C. reuteriana* were merged together into the same subpartition (1- *C. reuteriana* ssp. reuteriana + *C. reuteriana* ssp. *tartessiana*, 2- *C. mauritanica*) variation between groups was much lower (10.97%). The Mantel test for the whole *C. reuteriana* s.l. was insignificant ($R^2 = 0.005$; $P = 0.33$). When subpartitions were tested, ssp. *reuteriana* (5 populations; $R^2 = 0.174$; $P = 0.03$) and ssp. *tartessiana* plus *C. mauritanica* (7 populations; $R^2 = 0.436$; $P = 0.01$) were statistically significant. In contrast, if only ssp. *tartessiana* was tested, the statistical significance was marginal (5 populations; $R^2 = 0.195$; $P = 0.09$).

Evaluated groupings (Table 5) revealed that the second best score was yielded by geographical areas (-2763.83), while the worst score was obtained with ecological preference (-2927.43). Again, the marginal likelihood of the optimal BAPS partition was significantly better (-2703.78).

CHROMOSOME COUNTS

Carex elata counted samples displayed a diploid number 2n = 76, in agreement with the most frequently reported number for this taxon (Faulkner, 1972; Luceño and Aedo, 1994). A single *C. elata* individual from France displayed a multivalent, which was a chain tetravalent. *Carex reuteriana* ssp. *tartessiana* counts revealed 2n = 72 and 74, increasing the lower range of chromosome numbers known from this taxon. Eventually, the first counts for *C. mauritanica* revealed that the chromosome number of these plants is 2n = 74, showing that these populations are karyologically closer to *C. reuteriana* than to *C. acuta* or *C. elata*.

MORPHOLOGICAL STUDY

Detailed examination of the Iberian and North African samples (Table 6, Appendix 1) did not revealed any clear morphological split in the quantitative characters considered between the three taxa. However, utricles tend to be larger, and inflorescence to have higher male proportion (with more male and androgynous spikes) in *C. reuteriana* ssp. *tartessiana* and *C. mauritanica* than in *C. reuteriana* ssp. *reuteriana*. Such morphological features were previously reported for *C. reuteriana* ssp. *tartessiana* (Luceño and Jiménez-Mejías, 2008).

Luceño and Aedo (1994), who examined *C. mauritanica* type material, proposed that the higher ratio of utricle length / width that was found in this voucher might be enough to distinguish *C. mauritanica* from Iberian taxa. However, the results show that with a slightly increased sampling, the utricle length / width ratio threshold found in *C. mauritanica* overlaps with *C. reuteriana* subspecies.

Table 6 Character comparison between population groups of *C. reuteriana* s.l.

Character	*C. reuteriana* ssp. *reuteriana*	*C. reuteriana* ssp. *tartessiana*	*C. mauritanica*
Basal sheaths	Orange-brownish, reddish or purplish	Orange-brownish to reddish, rarely purplish	Orange-brownish to reddish
Lowest bract length	2.4-19(20) cm × 0.3-3(3.5) mm	7.5-19 cm × 0.5-3.2 mm	5.5-34 cm × 1.2-4.5 mm
Sex distribution	1 male spike, rarely with an additional small spike at its base, 0-3(6) androgynous spikes and 0-4 female spikes	(1)2-4 male spikes, (0)1-5 androgynous spikes, rarely the lowest spike completely female	(1)2-3 male spikes, (0)1-4 androgynous spikes, rarely the lowest spike completely female
Male spikes size	15-60(70) × 2-4 mm	10-70 × 1-4 mm	22-78 × 1.5-4 mm
Lowest (female or androgynous) spike size	13-65(90) × 2.5-4.6 mm	18-80(115) × 2.7-5.3 mm	32-85(100) × 2-6 mm
Utricle	1.8-3(3.5) × 1-1.6 mm (1.2-2.7 length/width ratio), greenish to yellowish, smooth, with a 0.1-0.3 mm beak	(1.8)2.3-4.5 × 1.1-1.9 mm (1.2-2.6 length/width ratio), greenish to yellowish, smooth or with few papillae towards the apex, with a 0.1-0.3 mm beak	2.2-3.7 × 1-1.8 mm (1.8-3.3 length/width ratio), greenish to yellowish, smooth or with few papillae towards the apex, with a 0.2-0.4 mm beak

Discussion

TAXONOMICAL IDENTITY: THREE SPECIES, FOUR TAXA, NO HYBRIDS, NO CLONES

In the present study, we attempted to evaluate the taxonomical status of a species group using molecular techniques. AFLP and plastid sequence analyses (Figs. 2A, B) reveal a clear genetic partition in the river-shore *Carex* sect. *Phacocystis*: *C. acuta*, *C. elata*, and *C. reuteriana* s.l. Alternatively, BAPS analysis suggests a partition in four groups, since *C. reuteriana* s.l. were split into two groups: *C. reuteriana* ssp. *reuteriana* and *C. reuteriana* ssp. *tartessiana* + *C. mauritanica*. Although NJ is mostly congruent to the yielded partitions, only *C. acuta* clusters are statistically supported (Fig. 3). AMOVA results indicate that partition of the three taxa (*C. acuta*, *C. elata.* and

C. reuteriana s.l.) provide a similar level of differentiation as the best BAPS partition (K = 4) (28.76% vs. 29.20%; Table 4). In addition, Bayesian analyses detected that chromosome number ranges, followed by haplotypes, displayed the best partition after the one given by BAPS individual clustering. The resulting congruence between AFLPs and these partitions highlight the independent species status of *C. acuta*, *C. elata* and *C. reuteriana*. Based on these results the species status of *C. acuta*, *C. elata* and *C. reuteriana* is strongly supported. Variation within *C. reuteriana* should be regarded as two subspecies (ssp. *reuteriana* and a second subspecies combining ssp. *tartessiana* and *C. mauritanica*; see Appendix 1) on basis to genetic and morphological results. Little consensus among previous treatments was observed for Iberian taxa (see Table 1). The main confusion was the split of *C. reuteriana* s.l. among disparate species (Vicioso, 1959; Chater, 1980; Table 1). The first approach that merged all Iberian populations (excluding *C. acuta*) within a single taxon was that presented in Luceño and Aedo (1994), and followed in Flora Iberica (Luceño and Jiménez-Mejías, 2008). Our results represent a new step toward a more natural taxonomical arrangement of this group: 1) considering *C. reuteriana* s.l. as a separate species from *C. elata*; and 2) combining *C. mauritanica* within *C. reuteriana* s.l. (Appendix 1).

Based on our results, chromosomal data appears to be a fairly accurate taxonomic tool for *Carex* sect. *Phacocystis* river-shore species. Despite the fact that karyological information misled Luceño and Aedo (1994) to consider how the systematic relationships were organized within the group, these authors found it very useful for distinguishing between taxonomical units. Moreover, our results suggest that chromosomal rearrangements and transitions could have been a driver of speciation in this species group, which is in accordance with previous studies in *Carex* (Hipp, 2007; Escudero et al., 2010b).

Unexpectedly, based on our results, extensive clonal propagation is refuted for both *C. elata* and *C. reuteriana* s.l., contrary to what is expected for an aquatic rhizomatous plant (Barret et al., 1993; Santamaría, 2002). Studies in related rhizome-creeping taxa have shown the importance of clonal propagation in their populations (*C. bigelowii* Schonswetter et al., 2008; *C. nigra* Jiménez-Mejías et al., in preparation). In this sense, the traditional division of *Carex* sect. *Phacocystis* taxa into tussock-forming and rhizome-creeping forms (cf. Kükenthal's (1909) and Egorova's (1999) treatments), mostly appears to fit two different kinds of life-history and ecological strategies.

Although hybridization has been regarded as one of the main problems in taxonomic delimitation within *Carex* sect. *Phacocystis* (Chater, 1980; Cayouette and Morisset, 1986; Egorova, 1999), the homogeneous grouping provided by BAPS prevents us from proposing extensive hybridization or ingressive processes within our sampling. Additionally, it appears that, at least in the studied taxa, hybridization does not contribute too much to the overall genetic variation. Thus, at this point, problematic taxonomic populations previously regarded as putative contact zones (cf. Luceño and Aedo, 1994; Luceño and Jiménez-Mejías, 2008) should be understood as a lack of morphological differentiation due to wider than expected overlapping variation, rather than as a result of hybridization events.

PHYLOGEOGRAPHIC PATTERNS IN THE RIVER-SHORE CAREX GROUP AND DRIVERS OF THE GENETIC DIFFERENTIATION

The present study reveals that phylogeographic history and the importance of drivers of genetic differentiation of ecologically and genetically closely related species may be disparate.

On one hand, in *C. elata*, genetic differentiation among populations (21.15 % in AMOVA) is moderate-low, which illustrates the rough geographical split between the northern and southern range shown by the BAPS grouping and PCoA (Tables 3, 5, Fig. 4A), which fit Pleistocene refugia with posterior post-glacial expansion. This is a common pattern found in phylogeography (Taberlet et al., 1998), and has also been reported in aquatic plants (Lambracht et al., 2007; Escudero et al., 2010a). Accordingly, the southern group displays isolation by distance, which may fit with a long-standing status may characterize the southern sampled areas as putative refugia for *C. elata* (cf. Brochmann et al., 2003). However, for the northern group, Mantel test results were not significant, suggesting lack of geographical structure, which may indicate recent expansion. In any case, this weak geographical structure and differentiation degree within *C. elata* can be interpreted as the result of relatively low general limitations in gene-flow and / or recent origin of the northern populations.

On the other hand, overall differentiation values were higher in *C. reuteriana* s.l. than in the more widespread *C. elata* (34.93 % in AMOVA and 3 haplotypes), and a strong step-descent north-south geographical structure was found (see Figs. 2B, 4B,C).

The stronger genetic partition would be result from the split between the two major geographical groups, with no contact zones: with the more northern group containing *C. reuteriana* ssp. *reuteriana*, and the more southern group containing *C. reuteriana* ssp. *tartessiana* plus *C. mauritanica*. It should be noted that isolation by distance in the whole *C. reuteriana* s.l. data set was not supported by results from the Mantel test, which entails that the divergence among *C. reuteriana* s.l. populations has been complex enough to cause differences that cannot be simply explained by distance. The greater plastid and AFLP diversity detected in the Iberian Peninsula (Table 3) could suggest a European origin for this taxon and north-to-south migration to northern Africa, although our limited North African sampling does not allow us to state it definitively. Mantel test results within the two major groups clearly support the strong influence of geographical distance on gene flow (see results). Different kinds of barriers, unequivocally placed between the regional distribution patches, could help to promote this divergence: 1) the Strait of Gibraltar, between Andalusian and North African populations; 2) the Northern Plateau, between NW Iberian and the Central Range populations; and 3) the Guadalquivir valley between the Sierra Morena and Penibetic Range, although this was only marginally supported. In *C. helodes* (Escudero et al., 2008a), the Strait of Gibraltar was found to be an effective barrier. Guadalquivir valley has been reported to be a barrier in other families (*Erophaca,* Leguminosae*,* Casimiro-Soriguer et al., 2010; *Hypochaeris*, Compositae, Ortiz et al., 2007). Nevertheless, the effectiveness of these barriers may have varied from Pleistocene age to nowadays. Overall, this regional structure of *C. reuteriana* s.l. strongly supports a long-standing scenario, with gene-flow limitations and subsequent differentiation.

The remaining questions are: Which mechanisms or factors can explain the detected genetic and phylogeographical differences between taxa as genetically and ecologically similar as *C. elata* and *C. reuteriana*? Why has *C. reuteriana* never colonized northern Europe, while *C. elata* has done so?

Summarizing, *C. elata* displays greater intra-population variation and weaker geographical partition than *C. reuteriana* s.l. (Table 3; Figs. 3, 4). In agreement with historical reasons, small ecological differences between both species might help to explain these genetic and phylogeographical differences. While *C. reuteriana* is restricted to streams, *C. elata* grows in more diverse types of wetlands (see Table 2), suggesting a more generalist ecological strategy. Dispersal of propagules by migratory

waterbirds is assumed to be common in aquatic plants (Santamaría, 2002), and it has been suggested in Cyperaceae in particular (Schmid 1986; Mueller and van der Valk, 2002). The ability of *C. elata* to colonize extensive marshes, peat-bogs and lakesides, allows for better interaction with potential waterbirds dispersors than the streams where *C. reuteriana* s.l. grows. This may enable higher seed-mediated gene flow between populations, avoiding fine-scale intra-regional differentiation. In general, *C. elata* could have *a priori* advantages in the colonization of northern lands after last glacial maximum (see the tabula rasa hypothesis in Brochmann et al., 2003). In *C. reuteriana* s.l., according to the long-standing populations scenario proposed, ecological differences and dispersal limitations in comparison to *C. elata* would have prevented northern habitat colonization. In addition, the possibility of northern European colonization by *C. reuteriana* decreased following the *C. elata* colonization, because this more generalist species could occupy the *C. reuteriana* niche ("high density blocking" hypothesis; Hewitt, 1993). Reciprocally, extensive propagation of *C. elata* in western Iberia could be prevented by the presence of *C. reuteriana*. Climate and ecological factors may be also important, although their role would remain unclear. For example, climatic limitation restricting *C. elata* in its southern limit may explain, at least partially, its disjunct distribution in southern Iberian Peninsula and northern Morocco. However, in comparison with the Spanish southernmost *C. reuteriana s.l.* populations in the Penibetic Range, the Iberian southernmost populations of *C. elata* (Doñana National Park) are in an area where summer average temperatures are clearly higher and precipitations lower (cf. AEMET, 2011). Additionally, in North Africa, populations of *C. elata* can be found further South from the *C. mauritanica* area (Fig. 1).

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Appendix 1

Reviewed treatment, identification key and proposed nomenclatural adjustments for the Ibero-North African *Carex* sect. *Phacocystis* river-shore sedges.

REVIEWED TREATMENT

Based on the results of the present study, we consider that three species and two subspecies should be taken into account among *Carex* sect. *Phacocystis* river-shore sedges present in the Iberian Peninsula and North Africa.

- *Carex acuta* L.
- *Carex elata* All.
- *Carex reuteriana* Boiss.
  - ssp. *reuteriana*
  - ssp. *mauritanica* (incl. ssp. *tartessiana*)

The following key based in our observations and previous treatments (Luceño and Aedo, 1994; Luceño and Jiménez-Mejías, 2008) enables identification of Iberian and North African river-shore *Carex* sect. *Phacocystis* species.

1. Lowest bract clearly longer than the inflorescence; basal sheaths bearing leaf-blades, brownish towards the base; utricles broadly biconvex, greenish to yellowish, minutely covered by papillae of the same color ..... *C. acuta*
- Lowest bract as long as or shorter than the inflorescence, rarely longer; basal sheaths scale-like, sometimes elongated, straw coloured, yellowish, orange-brownish, reddish or purplish; utricles biconvex to plano-convex, grey-greenish with whitish papillae or yellowish to greenish, smooth or with few papillae towards the apex 2
2. Utricles grey-greenish, covered by whitish papillae, at least on the upper half, with the inner side purplish; lowest bract generally shorter than the inflorescence; basal sheaths yellowish to straw-colored..... *C. elata*

- Utricles yellowish to greenish, smooth or with few papillae towards the apex, with the inner side whitish; lowest bract as long as the inflorescence; basal sheaths orange-brownish, reddish or purplish..... 3 (*C. reuteriana*)

3. 1(2) upper spikes male, lower spikes generally completely female or 0-3(6) androgynous; utricles 1.8-3(-3.5) mm long. ..... *C. reuteriana* ssp. *reuteriana*
- (1)2-4 upper spikes male, generally all lower spikes androgynous; utricles (1.8)2.3-4.5 mm long..... *C. reuteriana* ssp. *mauritanica*

NOMENCLATURE

Carex reuteriana Boiss. subsp. *mauritanica* (Boiss. & Reut.) Jim.-Mejías & Luceño, comb. nov.

≡ *C. mauritanica* Boiss. & Reut., Pugill. Pl. Afr. Bor. Hispan.: 116 (1852) [basionym]
C. gracilis var. *mauritanica* (Boiss. & Reut.) C.Vicioso, Bol. Inst. Forest. Invest. Exp. 30(79): 82 (1959)
C. acuta subsp. *mauritanica* (Boiss. & Reut.) Asensi & Díez Garretas, in Lazaroa: 331 (1986)
C. elata subsp. *mauritanica* (Boiss. & Reut.) A.Galán, Lagascalia 18: 57 (1995)

= *C. elata* subsp. *tartessiana* Luceño & Aedo, Bot. J. Linn. Soc. 114: 205 (1994).
C. reuteriana subsp. *tartessiana* (Luceño & Aedo) Rivas Mart., Itinera Geobot. 15: 699 (2002)

– *C. gracilis* sensu Maire, Fl. Afrique N. 4: 127 (1957) p.p. non Curtis

Appendix 2

List of additional *C. reuteriana* s.l. materials included in the morphological study. Herbarium acronyms follow Index Herbariorum (http://sciweb.nybg.org/science2/IndexHerbariorum.asp).

TAXON. **COUNTRY. Region.** Origin, *Voucher*, (Herbarium).

C. REUTERIANA SSP. *REUTERIANA*. **PORTUGAL. Aveiro.** A saída de Castelo de Paiva, *A. Reis Moura s.n. 1966* (COI); S. João da Madeira, estrada para vale de Camara, junto do rio, *A. Rozeira et al. s.n. 1962* (PO). **Castelo Branco.** Serra da Estrela, Pedinha, no cruzamento das ribeiras de Bandoira e ribeira de Passos da Serra, *J. Matos et al. 7875* (COI). **Porto.** Valongo, próximo a Carvoeira, na margem da ribeira de Valongo, *G. Costa and J. Araújo s.n.* (PO). **Viana do Castelo.** Caminha, Freixeiro de Soutelo, *Honrado et al.* (SANT). **Vila Real.** Serra do Gerez, Ponte de Maceira, *A. Moller 1281* (COI). **Viseu.** Santa Cruz da Trapa vale do rio Vonga, prope Paredes, *P. Silva et al. 1663* (LISE). **SPAIN. Ávila.** Sierra de Gredos, Garganta de Los Conventos, *J. M. Marín 5304JMM* (UPOS). **Cáceres.** Bajadas de Puerto Viejo hacia Valverde del Fresno, *M. Ladero and A. Valdés s.n. 1982* (SALA); Serradilla, *D. Belmonte s.n.* (SANT). **Coruña, La**. Dumbría, Santa Uxía, por debajo del embalse del Xallas, *R. I. Louzán 2513* (SANT). **León.** Castrocontrigo, entre Torneros y Morla, junto al río Eria, *Alamillo et al. s.n. 1981* (SALA); Valdesamario, *M. de Godos s.n. 1993* (SANT). **Lugo.** Puente de Ferreirós, *J. Izco et al. s.n. 1980* (SANT). **Madrid.** Torrents près La Granja, *Reuter s.n. 1841* (G, lectotypus *C. reuteriana*); La Pedriza de Manzanares, *D. Gómez et al. s.n. 1984* (JACA); Jarama river, Montejo de la Sierra, *S. Rivas Martínez s.n. 1962* (BCN). **Orense.** Lobios, Serra de Santa Eufemia, afluente del río Caldo, *I. Pulgar s.n. 1996* (SANT). **Oviedo**. La Coba, Grandas de Salime, *C. Aedo et al. s.n. 1994* (SANT). **Salamanca.** Miranda del Castañar, *F. J. Fernández Díez s.n. 1972* (SALA); Viellavieja de Yeltes, *F. Amich s.n. 1977* (SALA).

C. REUTERIANA SSP. *TARTESSIANA*. **SPAIN**. **Cádiz.** San Carlos del Tiradero, Sierra de Ojén, *J. M. Nieto and T. E. Díaz s.n. 1981* (MGC); Los Barrios, Sierra de Luna, *Sierra Cotta et al. s.n.* (MGC); Los Barrios, Ahojiz river, *Brinton-Lee s.n. 1970* (SEV). Tarifa, Cerro de los Morrones, Cabeza del Gallego, *Arroyo and Gil s.n. 1981* (SEV). **Córdoba.** Santa María de

Trasierra, Guadiato river, *Devesa s.n.* (SEV); Villanueva del Rey, Molinos river, *Arenas et al. s.n. 1979* (SEV); Villaviciosa, Guadiato river, *Luceño and Vargas s.n.* (MA); Guadalora river, road to artificial lake of Retortillo *s.n. 1981* (SEV). **Granada**. Sierra Nevada, Lanjarón, *Vicioso and Ceballos s.n. 1930* (MA); **Huelva**. Sierra de Aracena, between Alajar and Santa Ana la Real, *Rivera s.n. 1978* (SEV); Sierra de Aracena, between Zufre and Santa Olalla, Rivera de Huelva, *Cabezudo and Valdés s.n. 1976* (SEV); Gibraleón, Belmonte, *E. Sánchez-Gullón and P. Weickert s.n.* (UPOS); Sierra de Aracena, La Granada de Río Tinto, *Cabezudo and Valdés s.n. 1976* (SEV). **Málaga**. 14 km W of Marbella, along road to Benahavís. Río Guadalmina, *E.A. Leadlay et al. 337* (BM); Ronda, *Luceño and Vargas s.n. 1985* (MA). **Sevilla**. Gerena, ctra. Genera-Aznalcóllar, río Guadiamar, cerca de las ruinas del Molino del Pino, *B. Cabezudo et al. s.n. 2001* (MGC). El Ronquillo, *Guzmán et al. s.n. 1989* (MA, holotypus *C. elata* ssp. *tartessiana*); Cazalla de la Sierra, *García and Bañez s.n. 1982* (SEV); Villaverde del Río, *Morales et al. s.n. 1982* (SEV).

C. MAURITANICA. **ALGERIA.** Oran, circa Tlemcem, *Boissier and Reuter s.n. 1849* (G, photo!, holotypus *C. mauritanica*); Bords de la Safsaf, près de Tlemcem, *A. Warion s.n. 1871* (FI, G); Gorges de Keddara, *Maire s.n. 1913* (MPU). **MOROCCO**. Prope Souk-et-Tleta de Ketama, *Maire s.n. 1929* (MPU); montibus Zaianicis, Hareba, *Maire s.n. 1926* (MPU).

CAPÍTULO 6

Genetically diverse but with surprisingly little geographic structure: the complex history of the widespread herb *Carex nigra* (Cyperaceae)

P. Jiménez-Mejías, M. Luceño, K. A. Lye, C. Brochmann & G. Gussarova

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Abstract

Aim. Historical and geographical factors leave characteristic genomic footprints that let us infer the main shapers of the current genetic structure of a species. Given sufficient divergence time and wide distribution, the different range edges and the central parts of the distribution area should display different features. Here we address the history of one of the most widespread and taxonomically complex sedges in Europe, *Carex nigra* s.lat.

Location. Amphi-Atlantic, Central and Northern Europe, Circum-Mediterranean mountain ranges, Central Siberia, Himalayas.

Methods. A total of 469 samples from 83 populations, covering most of the species range, were analyzed for Amplified Fragment Length Polymorphism (AFLP) and cpDNA markers. Bayesian clustering, Principal Coordinates Analysis, and estimates of diversity and differentiation were used for the analysis of AFLP data. Plastid data were analyzed with statistical parsimony, maximum parsimony network and Bayesian phylogenetic inference.

Results. Overall genetic diversity was high, but differentiation among population was rather limited. Main glacial refugia were inferred in the Mediterranean basin and in western Russia; in addition, minor refugia may have been located in the North Atlantic region. In the southern part of the range, we found high, but geographically quite poorly structured genetic diversity, whereas the patterns differed between different areas in the north. The North American populations were genetically very similar to European ones.

Main conclusions. The data are consistent with extensive gene flow, which has obscured the recent history of the taxon. The limited differentiation in the south probably results from mixing of lineages expanding from different local refugia. Northward postglacial colonization resulted in a leading-edge pattern of low diversity in the Netherlands, Belgium, Scotland and Iceland, whereas in Fennoscandia, the high levels of diversity observed suggest broadfronted colonization from the south as well as from the east. The pattern found in American populations is consistent with postglacial

colonization, possibly even anthropogenic introduction from Europe. The data suggest that the tussock-forming populations of *C. nigra*, often referred to as a distinct species (*C. juncella*), represent an ecotype that has originated repeatedly from different rhizome-creeping populations.

Keywords. Centrifugal differentiation, glacial refugia, *rpl32-trnL*^{UAG}, boreal-temperate herb, trans-Atlantic dispersal, vicariance, *ycf6-psbM*

Introduction

Only three studies have explored the phylogeography of widespread temperate herbs across their entire geographic range in Europe (*Melica nutans* and *Carex digitata*, Tyler, 2002a,b; *Carex pilosa*, Rejzková *et al.*, 2008). High levels of genetic variation, high dispersal ability and, in general, lack of strong geographical structuring were inferred, but more species of this element should be examined to establish whether this represents a general pattern. In widely distributed species, different parts of the range can be affected by different historical events and resulting in distinct genetic patterns. The Pliocene-Pleistocene climate oscillations strongly affected the geographic ranges of species and ecosystems. Unsuitable habitat conditions caused species to contract and split their ranges, leading to isolation and vicariant divergence (Kropf *et al.*, 2006), whereas secondary contacts during colonization/recolonization in warmer periods often led to re-establishment of gene flow, counteracting complete speciation (Comes & Kadereit, 1998; Kadereit *et al.*, 2004). In refugial populations, long-term isolation drives genetic differentiation, whereas in the southernmost ones –the rear edge– reduction of suitable habitats can force population decrease and loss of genetic diversity (Hampe & Petit, 2005). In recently colonized territories loss of diversity may be induced by successive founder effects in the colonization front (Hewitt, 1996; Hewitt 1999; Schönswetter *et al.*, 2003; Puşcaş *et al.* 2008). However, increased genetic variation in recently colonized areas can be found in central parts of the distribution range, due to contact zones where different colonization fronts meet (Konnert & Bergmann 1995; Taberlet *et al.*, 1998; Hewitt. 1999; Petit *et al.*, 2003; Bylebyl *et al.*, 2008).

Early phylogeographic studies showed that western Asia and South Europe, and particularly, the Mediterranean Peninsulas (Iberia, Italy, Balkan and Anatolia), were the main places where temperate and boreal taxa survived the glaciations and from where they re-colonized central and northern Europe (Comes & Kadereit, 1998, 2003). However, several more recent studies draw a more complex picture, with persistence of forest ecosystems demonstrated in Central Europe (Willis *et al.*, 2000; Willis & Andel, 2004).

Carex nigra (L.) Reichard is one of the most widespread sedges in Europe (Fig. 1A). It occurs almost continuously in peat-bogs and lakesides in lowlands across

Central and North Europe. Southwards it occurs scattered in the high mountains of the four Mediterranean Peninsulas and in Corsica, Sicily and northwestern Africa. Eastwards, *C. nigra* is found scattered in Central Siberia, the Himalayas and Caucasus (Egorova, 1999; Noltie, 1994). Westwards, it shows a typical amphi-Atlantic distribution with occurrences in Iceland, Greenland and northeastern North America. *Carex nigra* is wind-pollinated and reported as more or less self-incompatible (Faulkner, 1973). It belongs to section *Phacocystis* Dumort., a taxonomically complex group with many species characterized by clonal growth through creeping rhizomes and widespread interspecific hybridization (Faulkner, 1973). Hybrids have been reported between *C. nigra* and almost all co-occurring taxa in the section (Sylvén, 1963; Chater, 1980; Jermy *et al.*, 2007). The fruits and utricles (perigynia) of *C. nigra* lack dispersal adaptations such as special devices found in other *Carex* species (Allessio Leck & Schütz, 2005). However, viable seeds have been recovered from bird gut contents (Schmid, 1986; Mueller & van der Valk, 2002), suggesting endozoochory as a possible main dispersal mechanism in *C. nigra*. It has variable somatic chromosome numbers (*2n* = 80-88; Roalson, 2008) with no geographic structuring.

The taxonomy of *C. nigra* s. lat. is highly problematic and there is little consensus among different treatments. Wide variation in vegetative characters has led to the description of numerous taxa at the rank of species and below. The main intraspecific division accepted by most botanists is between the most common, rhizome-creeping plants (var. *nigra*) and plants forming conspicuous tussocks (*C. nigra* var. *juncea* (Fr.) Hyl.), which also have been recognized as a distinct subspecies (*C. nigra* subsp. *juncella* (Fr.) Lemke) or even species (*C. juncella* Fr.). These two growth forms occur sympatrically in North Europe and western Siberia (Sylvén, 1963; Chater, 1980; Egorova, 1999), var. *juncea* typically in seasonally flooded places, and var. *nigra* mostly in year-round humid habitats or in habitats appearing dry, but with water available from below. *Carex nigra* subsp. *intricata* (Tin.) Rivas Mart. (*Carex intricata* Tin.) is a name applied in local floristic treatments to the dwarfed, densely-tufted plants with wide leaves that grow in high mountains of Corsica, Sicily, Sierra Nevada, and North Africa (Maire, 1957; Vicioso, 1959; Pignatti, 1982; Chater, 1980). Other alpine forms more similar to typical *C. nigra* have been reported as *C. nigra* subsp. *alpina* (Gaudin) Lemke from northern and central European ranges (Chater, 1980). The name *C. nigra* subsp. *dacica* (Heuff.) Soó has been erroneously used (belongs to *C. bigelowii*;

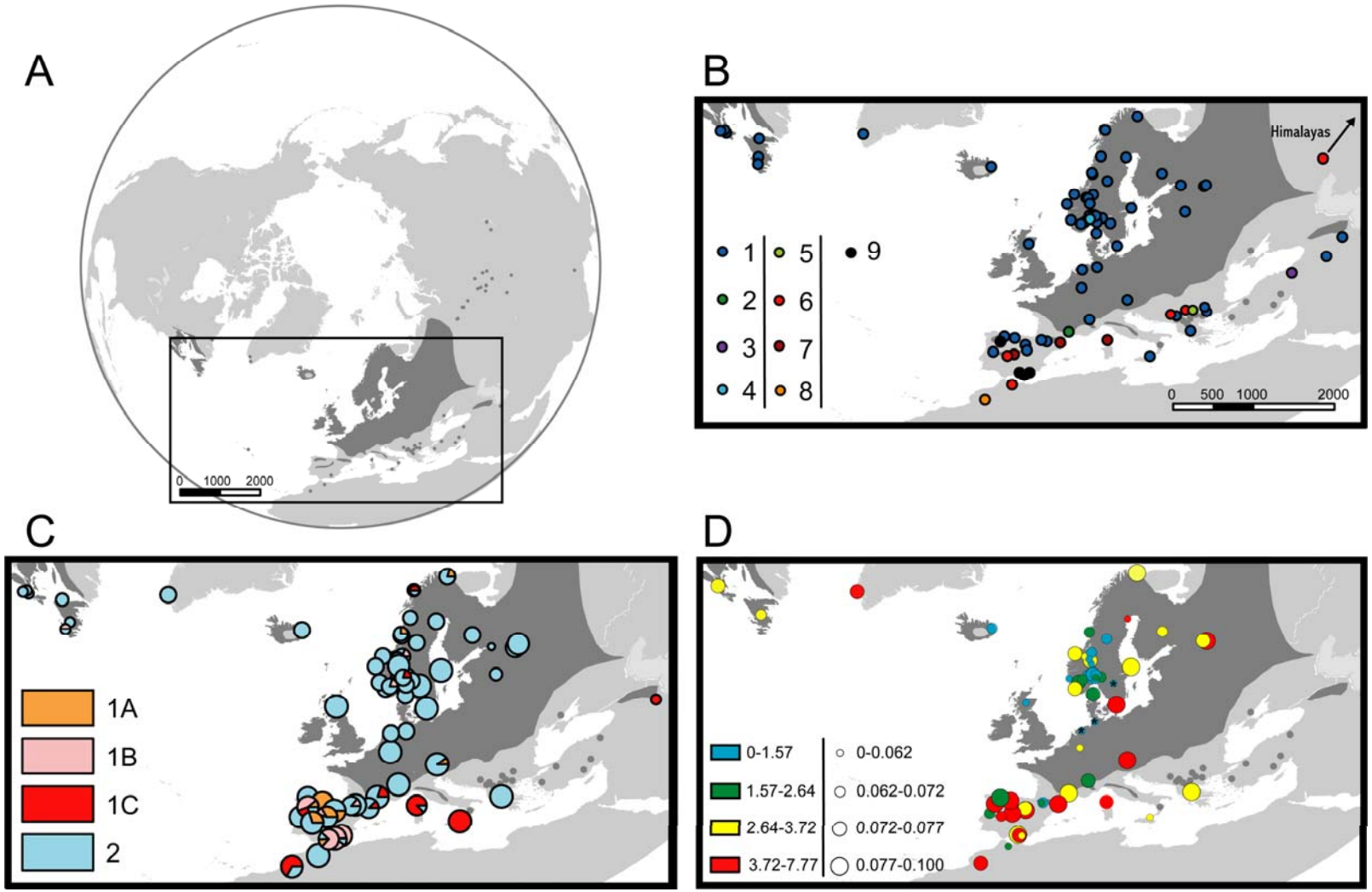


Fig. 1. A. Global distribution range (shaded) of *Carex nigra* (modified from Hultén 1958). **B.** Geographic distribution of cpDNA haplotypes; **C.** Geographic distribution of genetic (AFLP) groups as identified by the software STRUCTURE; **D.** Geographic distribution of AFLP gene diversity and rarity (DW); circle size depicts gene diversity within the population, colours represent DW values, partitions match interquartile intervals. Three circles with stars represent populations consisting of a single clone, for which DW could not be calculated.

cf. Egorova, 1999) to emphasize the distinctiveness of the plants from the Balkan Peninsula and Turkey (Chater, 1980; Nilsson, 1985), mainly based on differences in the colour of the basal sheaths. Finally, *C. transcaucasica* T.V. Egorova and *C. nigra* subsp. *drukyuliensis* Noltie are *C. nigra*-like taxa described from Caucasus and Bhutan, respectively (Noltie, 1994; Egorova, 1999). This complex taxonomy reflects the huge morphological and ecological variation encountered in *C. nigra* s. lat. across its geographic range.

Based on extensive range-wide sampling, we here address the phylogeographic history of *C. nigra* s. lat. based on AFLP markers and plastid DNA sequences. In particular, because this species occurs more or less continuously in Northern and Central Europe but only scattered, seemingly relictual, in the Mediterranean, we address whether its genetic structure differs between the recently colonized northern areas (front edge) and the long-standing zones (rear edge) in the south. We also aim to contribute to the taxonomy of this complicated species, especially to assess whether the tussock-forming plants form a separate genetic group, representing a distinct taxon, or whether they group with rhizomatous plants from different geographic areas.

Materials and methods

SAMPLING

Leaf material was collected in the field and stored in silica gel. In addition, a few samples were taken from herbarium material (E, UPOS) and used for cpDNA sequencing (see below). A total of 469 samples from 83 different populations were included in the study (Table 1). The sample size in each population varied from 1 (12 populations) to 10 individuals (16 populations). Our sampling covered well the American, European and North African parts of the species range, and also included populations from many of the Circum-Mediterranean mountain ranges where isolated populations of *C. nigra* are found. A special effort was made to cover the morphological variation reported in *C. nigra* s. lat., especially to include populations described as distinct taxa. We sampled the type localities of *C. intricata*, *C. nigra* var. *juncea* and *C. nigra s.str.*, plants from Bulgaria and Greece assignable to *C. nigra* "subsp. *dacica*", plants from Bhutan (Himalayas) assignable to *C. nigra* subsp.

Table 1. Geographic location, average gene diversity, AFLP-based clustering assignment and haplotype of the 83 studied populations of *C. nigra* plus the *C. bigelowii* ssp. *rigida* included population as outgroup. Labelling of the populations include growing habit (Jun = tussock-forming "var. *juncea*"**;** Nig = rhizome-creeping "var. *nigra*"), country [following TDWG botanical countries nomenclature (Brummit, 2001): AUT = Austria, BGM = Belgium, BUL = Bulgaria; COR = Corsica, DEN = Denmark, EHM = Eastern Himalayas, FIN = Finland, FRA = France, GER = Germany, GRB = Great Britain, GRC = Greece, GRL = Greenland, ICE = Iceland, IRN = Iran, ITA = Italy, MOR = Morocco, NET = Netherlands, NFL = Newfoundland, NOR = Norway, NSC = Nova Scotia, POR = Portugal, RUW = Northwest European Russia, SIC = Sicily, SPA = Spain, SWE = Sweden, TRC = Transcaucasus; TUR = Turkey; YUG-CN = Montenegro; YUG-SE: Serbia]. N_I, number of individuals; N_P, number of AFLP phenotypes found. Gene diversity is showed only for populations with at least five individuals. The two GeneBank accessions given for each sample in cpDNA Haplotypes column (within brackets) are rpl32-trnLUAG and ycf6-psbM in the order provided.

Pop.	Locality	Longitude /Latitude	N_I	N_P	Average gene diversity ± SD	STRUCTURE Groups	DW	cpDNA Haplotype (GenBank accessions)	Collector
Jun_NOR_1	Troms, Tromsø, Prestvatnet	18.93/69.67	3	3	-	2:3	1.20	1 (JN627606, JN627521)	K.A.Lye
Jun_NOR_2	Akershus, Oppegård	10.83/59.80	10	8	0.058 ± 0.034	2:10	1.72	1 (JN627607, JN627522)	K.A.Lye
Jun_NOR_3	Hedmark, Os, Narbuvoll	11.33/62.47	5	5	0.072 ± 0.046	1B:1, 2:4	1.53	1 (JN627608, JN627523)	K.A.Lye
Jun_NOR_4	Buskerud, Kongsberg	9.64/59.97	5	5	0.0656 ± 0.042	2:5	1.32	1 (JN627609, JN627524)	K.A.Lye
Jun_NOR_5	Sør Trøndelag, Oppdal	9.58/62.27	5	5	0.062 ± 0.040	2:5	2.68	1 (JN627610, JN627525)	K.A.Lye
Jun_NOR_6	Vestfold, Hof, Rønneberg	10.07/59.48	5	4	0.066 ± 0.045	2:5	1.31	1 (JN627611, JN627526)	K.A.Lye
Jun_NOR_7	Hedmark, Folldal	10.05/62.13	5	4	0.061 ± 0.042	2:5	0.96	1 (JN627612, JN627527)	K.A.Lye
Jun_NOR_8	Finnmark, Sør-Varanger	30.08/69.05	5	5	0.083 ± 0.052	1A:1, 2:4	2.93	1 (JN627613, JN627528)	K.A.Lye
Jun_NOR_9	Oslo, Bogstad	10.62/59.97	5	5	0.055 ± 0.036	2:5	1.57	1 (JN627614, JN627529)	K.A.Lye
Jun_NOR_10	Oppland, Ringebu, Elstad	10.16/61.50	5	5	0.058 ± 0.038	2:5	1.18	1 (JN627615, JN627530)	K.A.Lye
Jun_NOR_11	Nordland, Saltdal, Junkerdal	15.58/66.78	4	4	-	2:4	1.77	1 (JN627616, JN627531)	K.A.Lye
Jun_NOR_12	Telemark, Tinn, Frøystaul	8.43/59.32	5	5	0.070 ± 0.045	2:5	2.64	1 (JN627617, JN627532)	K.A.Lye

(Continued)

Table 1. *(Continued)*

Pop.	Locality	Longitude /Latitude	N_I	N_P	Average gene diversity ± SD	STRUCTURE Groups	DW	cpDNA Haplotype (GenBank accessions)	Collector
Jun_NOR_13	Aust-Agder, S of Valle church	7.52/59.13	5	5	0.077 ± 0.049	1B:1, 2:4	2.39	1 (JN627618, JN627533)	K.A.Lye
Jun_SWE_1	Värmland, Årjäng, Sanda	12.27/59.37	5	4	0.063 ± 0.044	2:5	2.53	1 (JN627619, JN627534)	K.A.Lye
Jun_SWE_2	Jämtland, Strömsund	15.45/63.70	5	5	0.068 ± 0.043	2:5	1.57	1 (JN627620, JN627535)	K.A.Lye
Jun_SWE_3	Västergötland, Sparreseter (var. *juncea* type parish)	13.70/58.51	10	1	0	2:10	-	1 (JN627621, JN627536)	K.A.Lye
Nig_AUT	Abtenau, Salzburg	13.43/47.65	10	10	0.095 ± 0.053	1A:1, 2:9	7.77	1 (JN627622, JN627537)	P.Jiménez-Mejías
Nig_BGM	Hautes Fagnes, Liège	6.17/50.55	10	2	0.040 ± 0.042	2:10	3.02	1 (JN627623, JN627538)	S.Martín-Bravo & M.Escudero
Nig_BUL_1	Pirin National Park, Bangsko	23.42/41.74	1	-	-	-	-	1 (JN627624, JN627539)	P. Jiménez-Mejías *et al.*
Nig_BUL_2	Vitoša Natural Park, Sofia	23.28/42.56	1	-	-	-	-	1 (JN627625, JN627540)	P. Jiménez-Mejías & F. Madroñal
Nig_BUL_3	Rila National Park, Seven Rila Lakes	23.58/42.12	1	-	-	-	-	1 (JN627626, JN627541)	P. Jiménez-Mejías *et al.*
Nig_COR	Campanelle	9.15/42.08	8	8	0.077 ± 0.044	1C:7, 2:1	5.12	8 (JN627627, JN627542)	M.Escudero & M.Luceño
Nig_DEN	Nordjylland, Skagen	10.42/57.65	5	4	0.073 ± 0.050	2:5	2.60	1 (JN627628, JN627543)	K.A.Lye
Nig_EHM	Bhutan, N of Thimphu Dzong	89.39/27.32	1	-	-	-	-	6 (JN627629, JN627544)	A.J.C.Grierson & D.G.Long (E257027)

(Continued)

Table 1. *(Continued)*

Pop.	Locality	Longitude /Latitude	N_I	N_P	Average gene diversity ± SD	STRUCTURE Groups	DW	cpDNA Haplotype (GenBank accessions)	Collector
Nig_FIN	Rantasalmi, Southern Savonia	28.37/62.05	5	4	0.067 ± 0.046	2:5	3.07	1 (JN627630, JN627545	K.A.Lye
Nig_FRA	Cevennes, Mont Aigoual	3.54/44.08	10	8	0.083 ± 0.048	1C:2, 2:8	3.72	2 (JN627631, JN627546)	P.Jiménez-Mejías
Nig_GER	Rotenburg, Niedersachsen	9.41/53.06	5	1	0	2:5	-	1 (JN627632, JN627547)	K.A.Lye
Nig_GRB	Scotland, Highlands	-3.46/56.78	10	5	0.049 ± 0.032	2:10	1.33	1 (JN627633, JN627548)	S.Martín-Bravo *et al.*
Nig_GRC	Epirus, Valia Kalnta	21.20/39.85	10	5	0.081 ± 0.052	2:10	3.66	1 (JN627634, JN627549)	M.Luceño *et al.*
Nig_GRL	Narsaq, Kujalleq	-46.05/60.92	5	5	0.076 ± 0.048	2:5	4.79	1 (JN627635, JN627550)	K.B.Westergaard & K.I.Flatberg
Nig_ICE	East Iceland, Egilstadir valley	-14.37/65.27	5	4	0.063 ± 0.044	2:5	1.51	1 (JN627636, JN627551)	K.A.Lye
Nig_IRN	Azerbaian, Arasbaran	46.82/38.77	2	2	-	1C:2	1.37	1 (id. a: JN627637, JN627552; id. b: JN627638, JN627553)	M.Amini-Rad
Nig_ITA	Valle d'Aosta, La Thuile	6.88/45.69	10	7	0.074 ± 0.043	2:10	2.25	1 (JN627639, JN627554)	A.J.Chaparro
Nig_MOR_1	High Atlas, Djebel Oukaïmedem	-7.84/31.21	9	4	0.073 ± 0.050	1C:6, 2:3	4.29	7 (JN627640, JN627555)	A.J.Chaparro *et al.*
Nig_MOR_2	Rif, Djebel Tidighin	-4.55/34.85	10	2	0.057 ± 0.060	2:10	2.19	6 (JN627641, JN627556)	P.Jiménez-Mejías *et al.*
Nig_NET	Drenthe, Assen	6.64/53.04	5	1	0	2:5	-	1 (JN627642, JN627557)	K.A.Lye

(Continued)

Table 1. *(Continued)*

Pop.	Locality	Longitude /Latitude	N_I	N_P	Average gene diversity ± SD	STRUCTURE Groups	DW	cpDNA Haplotype (GenBank accessions)	Collector
Nig_NFL_1	Norris Point	-57.79/49.50	3	2		2:3		1 (JN627643, JN627558)	J.A.Dragon
Nig_NFL_2	Glovertown	-54.03/48.63	2	1	0.070 ± 0.045	2:2	2.53	1 (JN627644, JN627559)	K.A.Lye
Nig_NFL_3	Avalon Peninsula	-53.55/47.52	2	2		1B:1, 2:1		1 (JN627645, JN627560)	K.A.Lye
Nig_NOR_1	Oppland, Ringebu	10.12/61.45	9	8	0.074 ± 0.043	2:9	3.42	1 (JN627646, JN627561)	K.A.Lye
Nig_NOR_2	Møre og Romsdal, Fræna	6.83/62.88	5	5	0.075 ± 0.047	2:5	2.42	1 (JN627647, JN627562)	K.A.Lye
Nig_NOR_3	Troms, Tromsø, Tromsdalen	19.07/69.60	2	2	-	1C:1, 2:1	3.51	1 (JN627648, JN627563)	K.A.Lye
Nig_NOR_4	Hordaland, Stord, Tveitavatn	5.47/59.77	5	3	0.049 ± 0.039	2:5	1.23	1 (JN627649, JN627564)	K.A.Lye
Nig_NOR_5	Nordland, Bindal, Holm	12.12/65.18	4	3	-	1A:1, 2:3	1.89	1 (JN627650, JN627565)	K.A.Lye
Nig_NOR_6	Østfold, Hvaler, Asmaløy	10.95/59.03	5	4	0.060 ± 0.042	2:5	0.99	1 (JN627651, JN627566)	K.A.Lye
Nig_NOR_7	Rogaland, Gjesdal, Hunnedal	6.60/58.88	5	3	0.075 ± 0.059	2:5	2.91	1 (JN627652, JN627567)	K.A.Lye
Nig_NOR_8	Sogn & Fjordane, Bremanger	4.95/61.78	5	2	0.023 ± 0.025	2:5	0.39	1 (JN627653, JN627568)	K.A.Lye
Nig_NOR_9	Akershus, Enebakk, Mosjoen	10.99/59.80	5	3	0.064 ± 0.050	2:5	1.08	1 (JN627654, JN627569)	K.A.Lye
Nig_NOR_10	Akershus, Oppegård	12.17/64.97	5	4	0.063 ± 0.044	2:5	2.11	1 (JN627655, JN627570)	K.A.Lye
Nig_NOR_11	Akershus, Asker	10.39/59.81	1	1	-	2:1	-	1 (JN627656, JN627571)	K.A.Lye
Nig_NOR_12	Buskerud, Kongsberg, Sagvollen	9.64/59.56	4	4	-	2:4	1.23	4 (JN627657, JN627572)	K.A.Lye

(Continued)

Table 1. *(Continued)*

Pop.	Locality	Longitude /Latitude	N_I	N_P	Average gene diversity ± SD	STRUCTURE Groups	DW	cpDNA Haplotype (GenBank accessions)	Collector
Nig_NOR_13	Oslo, Bogstad	10.63/59.97	5	4	0.073 ± 0.050	1C:1, 2:4	1.54	1 (JN627658, JN627573)	K.A.Lye
Nig_NSC_1	Inverness	-61.35/45.72	2	2		2:2		1 (JN627659, JN627574)	K.A.Lye
Nig_NSC_2	Guysborough	-61.88/45.67	2	2	0.074 ± 0.045	2:2	1.94	1 (JN627660, JN627575)	K.A.Lye
Nig_NSC_3	Antigonish	-62.00/45.08	2	2		2:2		1 (JN627661, JN627576)	K.A.Lye
Nig_POR	Beira Alta, Serra da Estrela	-7.62/40.37	9	5	0.066 ± 0.042	2:9	2.55	1 (JN627662, JN627577)	S.Martín-Bravo
Nig_RUW_1	Leningrad Region, Tosno	30.77/59.60	1	1	-	2:1	-	1 (JN627663, JN627578)	G. Konechnaya
Nig_RUW_2	Pskov Region, Sebezhskiy district, River Nische	28.81/56.33	3	3	-	2:3	2.91	1 (JN627664, JN627579)	G. Konechnaya
Nig_RUW_3	Tver Region, Udomelskij, district, Lake Bolshaya	34.79/57.88	7	6	0.077 ± 0.047	2:7	3.15	1 (JN627665, JN627580)	P.Volkova
Nig_RUW_4	Tver Region, Udomelskij, district, Lake Eremkovo	35.27/57.85	9	9	0.086 ± 0.048	2:9	3.81	1 (JN627666, JN627581)	P.Volkova
Nig_SIC	Nebrodi, Monte Soro (*C. intricata* type parish)	14.64/37.94	10	2	0.045 ± 0.048	1C:10	3.14	1 (JN627667, JN627582)	P.Jiménez-Mejías
Nig_SPA_1	Sierra Nevada, Almería, El Chullo	-3.01/37.10	9	5	0.062 ± 0.040	1B:9	3.03	9 (JN627668, JN627583)	P.Jiménez-Mejías

(Continued)

Table 1. *(Continued)*

Pop.	Locality	Longitude /Latitude	N_I	N_P	Average gene diversity ± SD	STRUCTURE Groups	DW	cpDNA Haplotype (GenBank accessions)	Collector
Nig_SPA_2	Sierra Nevada, Granada, Laguna de las Aguas Verdes	-3.37/37.05	8	2	0.090 ± 0.093	1A:1, 1B:6, 2:1	2.75	9 (JN627669, JN627584)	P.Jiménez-Mejías
Nig_SPA_3	Sierra Nevada, Granada, Laguna de la Yegua	-3.38/37.06	10	7	0.073 ± 0.043	1B:9, 2:1	4.10	9 (JN627670, JN627585)	P.Jiménez-Mejías
Nig_SPA_4	Zamora, Iberian NW Massif, Peña Trevinca	-6.74/42.18	9	7	0.100 ± 0.058	1A:1, 1B:4, 1C:2, 2:2	6.82	9 (JN627671, JN627586)	S.Martín-Bravo & P.Jiménez-Mejías
Nig_SPA_5	Pyrenees, Huesca, Panticosa	-0.24/42.76	9	6	0.038 ± 0.024	2:9	1.66	1 (JN627672, JN627587)	P.Jiménez-Mejías *et al.*
Nig_SPA_6	Pyrenees, Huesca, Anayet	-0.41/42.77	7	6	0.065 ± 0.040	1B:6, 2:4	1.17	1 (JN627673, JN627588)	P.Jiménez-Mejías *et al.*
Nig_SPA_7	Pyrenees, Girona, Núria	2.14/42.40	8	5	0.090 ± 0.057	1C:1, 2:7	5.73	8 (JN627674, JN627589)	P.Jiménez-Mejías *et al.*
Nig_SPA_8	Cantabrian Mountains, León, Somiedo	-6.23/43.07	8	3	0.087 ± 0.067	2:8	1.80	1 (JN627675, JN627590)	S.Martín-Bravo & P.Jiménez-Mejías
Nig_SPA_9	Cantabrian Mountains, Palencia, Curavacas	-4.68/42.99	10	4	0.099 ± 0.067	1A:10	4.15	1 (JN627676, JN627591)	S.Martín-Bravo & P.Jiménez-Mejías
Nig_SPA_10	Iberian System, Soria, High Duero River	-2.86/41.92	10	7	0.088 ± 0.052	1A:6, 2:4	4.66	1 (JN627677, JN627592)	P.Jiménez-Mejías *et al.*
Nig_SPA_11	Iberian System, Burgos, Sierra de Neila	-3.05/42.04	9	6	0.075 ± 0.046	1A:4, 1B:1	3.49	1 (JN627678, JN627593)	P.Jiménez-Mejías *et al.*

(Continued)

Table 1. *(Continued)*

Pop.	Locality	Longitude /Latitude	N_I	N_P	Average gene diversity ± SD	STRUCTURE Groups	DW	cpDNA Haplotype (GenBank accessions)	Collector
Nig_SPA_12	Central Range, Ávila, Hoyocasero	-4.98/40.40	9	7	0.096 ± 0.056	1A:3, 1B:1, 2:5	4.07	8 (JN627679, JN627594)	P.Jiménez-Mejías *et al.*
Nig_SPA_13	Central Range, Ávila, Gredos Cirque	-5.27/40.26	10	5	0.069 ± 0.044	1A:3, 2:7	3.77	6 (JN627680, JN627595)	P.Jiménez-Mejías *et al.*
Nig_SWE_1	Scania, Malmö,	13.92/55.44	10	9	0.084 ± 0.047	2:10	5.36	1 (JN627681, JN627596)	S.Martín-Bravo *et al.*
Nig_SWE_2	Uppland, Blidö, (*C. nigra* type parish)	18.89/59.60	10	4	0.079 ± 0.054	2:10	2.98	1 (JN627682, JN627597)	P.Jiménez-Mejías & K.A.Lye
Nig_SWE_3	Norrbotten, Luleå, Trolltjern	22.11/65.57	5	5	0.061 ± 0.039	2:5	3.80	1 (JN627683, JN627598)	K.A.Lye
Nig_TRC	Armenia, Kotayk, Tehenis	44.24/40.19	1	-	-	-	-	1 (JN627684, JN627599)	J.Aldasoro *et al.* (UPOS)
Nig_TUR	Erzincam, Sakaltutan Geçidi	39.04/39.31	1	-	-	-	-	3 (JN627685, JN627600)	C.Aedo *et al.* (UPOS)
Nig_YUG-CN_1	Crna Gora, Durmitor National Park, Barno Jezero	19.09/43.16	1	-	-	-	-	1 (JN627686, JN627601)	P. Jiménez-Mejías
Nig_YUG-CN_2	Crna Gora, Durmitor National Park, Crno Jezero	19.09/43.15	1	-	-	-	-	6 (JN627687, JN627602)	P. Jiménez-Mejías
Nig_YUG-SE_1	Serbia, Vlasinsko Jezero, W *Sphagnum* bogs	22.33/42.71	1	-	-	-	-	5 (JN627688, JN627603)	P. Jiménez-Mejías
Nig_YUG-SE_2	Serbia, Vlasinsko Jezero, S shores	23.28/42.56	1	-	-	-	-	6 (JN627689, JN627604)	P. Jiménez-Mejías
C. bigelowii ssp. *rigida*	Norway, Hedmark, Alvdal, Tronden	-	-	-	-	-	-	outgroup (JN627605, JN627520)	K.A. Lye

drukyuliensis, and plants from Armenia and Iran assignable to *C. transcaucasica*. As *C. nigra* typically can spread vegetatively by creeping rhizomes, samples were taken as far from each other as possible to minimize clone sampling. Voucher specimens from populations sampled in the field were deposited in the herbaria of the Botanical Museum, Oslo (O, Norway) and Pablo de Olavide University in Seville (UPOS, Spain). *Carex bigelowii* subsp. *rigida* was included as an outgroup (cf. Dragon & Barrington, 2009).

DNA ISOLATION, PCR AMPLIFICATION AND AFLP FINGERPRINTING

Total DNA was extracted from dried tissue using DNeasy plant extraction kit (Qiagen, California). The variability in 10 different plastid regions was tested using a subset of plants, and *ycf6-psbM* and *rpl32-trnL*^{UAG} were the most variable ones and therefore chosen for full analysis. Primers and protocols described in Shaw *et al.* (2005, 2007) were used. A single plastid sequence per population was obtained, except for the Nig_IRN population, from which two samples were sequenced, a total of 84 sequences.

459 samples from 73 of the populations were successfully analysed for AFLPs (Table 1). The laboratory procedure followed Gaudeul *et al.* (2000) with the modifications of Schönswetter *et al.* (2006a). A pilot study was performed on eight samples from four populations, including one replicate of each. This pilot study included a selection of AFLP primers successfully used in *Carex* (Schönswetter *et al.* 2006b; Nakamatte & Lye, 2007; Schönswetter *et al.* 2008; Jiménez-Mejías *et al.*, in press (Chapter 5)). We chose primer combinations that retrieved the highest number of informative characters after reproducibility was tested: *Eco*RI AGT (6-FAM)/*Mse*I AGC, *Eco*RI ACC (NED)/*Mse*I ACC and *Eco*RI AGG (VIC)/*Mse*I CA. Selective PCR products were run on a capillary sequencer (ABI PRISM 3100, Applied Biosystems) with the internal size standard GeneScan ROX 500 (Applied Biosystems, Foster City, California, USA). Data collection and fragment sizing were done using the program GeneMapper version 3.7 (Applied Biosystems). Fragments in the range 50-500 bp were automatically scored with GeneMapper 3.7 and manually revised. The results were exported as a presence/absence (1/0) matrix.

Negative controls and replicates were included throughout the process. Reproducibility was estimated based on 69 replicated samples (15% of the sampling) as the average proportion of correctly replicated bands (Bonin *et al.*, 2004). Markers with

low reproducibility were excluded. Rare presences and absences below the frequency of error rate were carefully checked and used only if they were unambiguous. Linked alleles were removed from the matrix.

DATA ANALYSES

Plastid sequence chromatograms were visualized and edited using SeqEd (Applied Biosystems, California). Due to the few variable characters observed the sequence matrix was easily aligned manually in a text editor. Gap coding was applied using the ‘‘Simple coding” method by Simmons & Ochoterena (2000) implemented in the SeqState software (Müller, 2005). A Statistical parsimony analysis (SP) was conducted using TCS (Clement *et al.*, 2000); this software also identifies which samples display identical sequences (haplotypes). Branch support for the TCS network was obtained using SplitsTree4 (Huson & Bryant, 2006). Maximum Parsimony Analyses were conducted in PAUP*4.0b10 (Swofford, 2002). Random trees (not just addition sequence) were used as starting point, with 100 replicates. The tree-bisection-reconnection (TBR) algorithm was used for branch-swapping. Steepest descent option was not in effect. No more than 20 trees of score (length) greater than or equal to 1 were set to be saved in each of the replicates. Branches were collapsed if maximum branch length was zero. The ‘‘MulTrees” option was in effect and no topological constraints were enforced. In order to obtain corrected values of the CI, the index was recalculated with uninformative characters excluded. Parsimony bootstrap support values were obtained from 1000 pseudoreplicates with 4 random sequence additions using heuristic search options of PAUP including both informative and uninformative characters. Bayesian phylogenetic analyses were conducted in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The Akaike information criterion with an empirical correction for small sample sizes (AICc), as implemented in MrAIC (Nylander, 2004) together with PHYML (Guindon & Gascuel, 2003) where used for substitution model selection. Bayesian analyses included sampling for 6,000,000 generations during 2 simultaneous runs under F81 model; sample frequency 1000; burn-in 1000 generations taken to reach stationarity. The priors were set according to the output of MrAIC. Coded indels were included as a separate data partition using F81-like model implemented in MrBayes for binary data. The results of the analysis were summarized as 50%-majority rule consensus trees. All

Bayesian analyses were run 2 times to confirm convergence. The repeated Bayesian analyses converged to identical topologies and similar posterior probability values.

For the AFLP data, a Bayesian clustering analysis was performed in STRUCTURE 2.2. (Pritchard *et al.*, 2000) using the Bioportal computer service of the University of Oslo (http://www.bioportal.uio.no). STRUCTURE estimates the number of genetically homogeneous groups (*K*) found in the data set. The admixture model was considered most accurate for our dataset, as recommended by Ehrich *et al.* (2008), in which shared group membership is allowed for an individual. In order to assess hierarchical structure, each identified group was analyzed separately. Similarity among the runs and stabilization of *K* likelihood scores were the main criteria used to choose the optimal number of groups; they were calculated using the R script, Structure-sum (Ehrich, 2006; updated version 2009).

The variation among AFLP genotypes was also evaluated by running a Principal Coordinate Analysis (PCoA) based on Jaccard's coeficient as implemented in NTSYS (Rohlf, 1997). "Frequency-down-weighted marker value" (DW) was calculated as a rarity measure (Schönswetter & Tribsch, 2005) using the R-script AFLPdat (Ehrich, 2006; updated version 2009), which accounts for differences in sample size as explained in Ehrich *et al.* (2008). Gene diversity of the populations (after removing clones) was evaluated according Nei's formula for haplotype diversity (Nei, 1978) also as implemented in AFLPdat. Analyses of molecular variance (AMOVA) to assess the level of genetic differentiation among populations and groups, and Nei's gene diversity *H* (1973) as a diversity measure, were computed using ARLEQUIN 3.5.1.2 (Excoffier & Lischer, 2009). For AMOVA, eight different groupings were tested for the whole dataset: populations, STRUCTURE groups from K=2 and K=4 (see results), Old *vs.* New World, plastid haplotypes, comparison of haplotype H9 *vs.* all other haplotypes together (see results), and taxonomic assignment to varieties *juncella* or *nigra*; in addition, seven partial datasets were analyzed: plants from glaciated areas, plants from unglaciated areas, and five different plastid haplotype partitions (H1 alone, H9 alone, H1-H5, H6-H8, H1-H8). For DW, gene diversity and AMOVA analyses, the populations from Newfoundland and Nova Scotia were merged in two single populations, one from each area, in order to minimize sampling size effect.

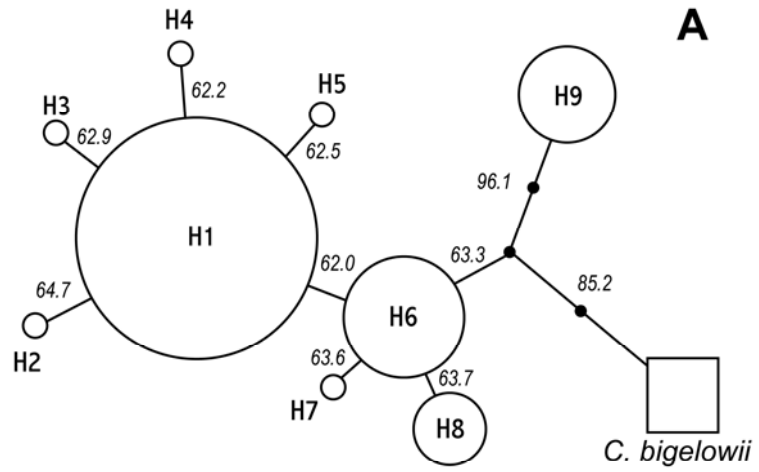
Results

cpDNA sequences

The complete plastid matrix contained 1129 bp *(rpl32-trnL*^{UAG} 715 bp; *ycf6-psbM*, 414 bp). Three indels of seven, five and one base, respectively, were coded as additional characters, the last one being autoapomorphic. Nine different haplotypes (labelled H1-H9; Fig. 1B; Table 1) were identified in *C. nigra*. The SP analysis retrieved a single network (Fig. 2A). Rooting with *C. bigelowii* suggested that the ancestral haplotype of *C. nigra* was unsampled or extinct (Schaal *et al.* 1998). Among the identified *C. nigra* haplotypes, H6 and H9 appeared closest to the ancestral sequence. A split of two mutational steps divided the *C. nigra* haplotypes into two groups: one with the Iberian haplotype H9, and one with all other haplotypes. The most widespread haplotype was H1, which occupied a central position within its group together with haplotype H6. The latter occurred scattered in the Mediterranean and also showed a remarkably disjunct occurrence in the Himalayas. The other phylogenetic analyses revealed two main clades (Fig. 2B) from wich four cpDNA haplotype groups can be defined. One of the clades only contained haplotype H9 (NW-SE Iberian group; 94% bootstrap support (bs) and 1.00 posterior probability (pp)). All the remaining sequences were placed in the other clade (64% bs, 0.99 pp). This latter showed a polytomy consisting of the unresolved haplotypes H6 and H7 (southern/eastern group), the western Mediterranean haplotype H8 (western Mediterranean group; 61% bs, 1 pp), and one subclade with haplotypes H1-H5 (widespread group; 65% bs; not recovered in the Bayesian analysis).

AFLPs

The geographic coverage of the AFLP analysis was somewhat more limited than in the cpDNA analysis, since herbarium material could not be included and since some samples were obtained after the AFLP study was finished. The final AFLP matrix consisted of 180 polymorphic markers in 459 individual plants. The final error rate was 1.43%. The STRUCTURE analysis with admixture model (Fig. 1C) suggested K=2 as the optimal number of groups in the total dataset. The results were identical among the 10 replicate runs and only a few individuals showed mixed ancestry. Group 1



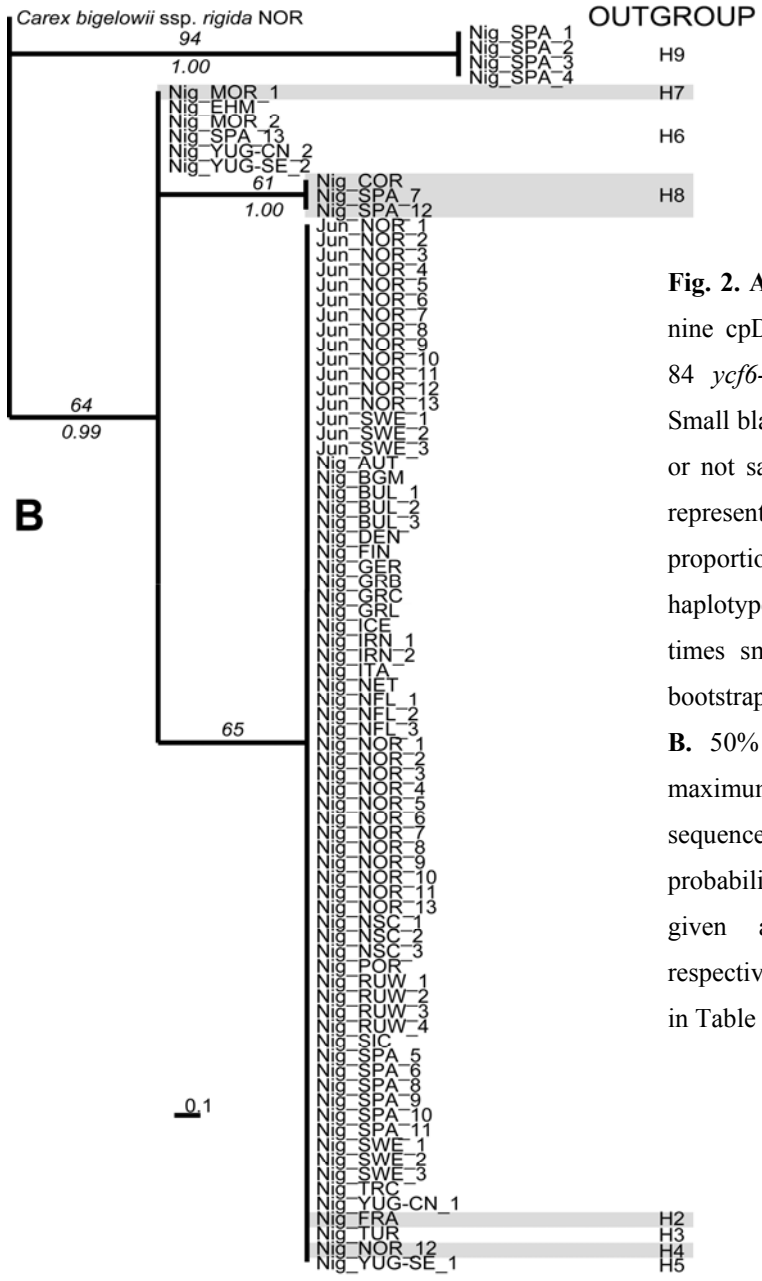


Fig. 2. A. Statistical parsimony network of the nine cpDNA haplotypes identified among the 84 *ycf6-psbM* and *rpl32-trnL*^{UAG} sequences. Small black circles represent haplotypes extinct or not sampled; each line between haplotypes represents a mutation step. Circle size is proportional to the number of samples of each haplotype, except for H1 which is shown ten times smaller. Numbers near branches show bootstrap support values obtained in Splitstree. **B.** 50% majority rule consensus tree from maximum parsimony analysis of the cpDNA sequences. Bootstrap support (%) and posterior probability values (obtained in MrBayes) are given above and below the branches respectively. Samples are labelled as explained in Table 1.

comprised populations from W and C Mediterranean and Caucasus, while Group 2 covered most of the distribution area of *C. nigra*. Some populations contained a single individual assigned to another group, while five populations from Spain and Morocco showed more or less equal numbers of individuals assigned to different groups (see Table 1). In separate STRUCTURE analyses for each group, no internal structure was revealed in Group 2. Group 1 was further divided into four subgroups. One of these was excluded since only low scores from a few individuals were assigned to it; the other three subgroups mostly kept together plants from the same geographic areas. Subgroup 1A comprised populations from Sierra Nevada and NW Spain, subgroup 1B comprised C-N Iberian populations, and subgroup 1C comprised populations from Atlas, Corsica, Sicily and Caucasus. Most of the Group 1 populations had the plastid haplotypes H6-H9, whereas most of the Group 2 populations had the plastid haplotypes H1-H5 (Figs 1B & 1C).

PCoA revealed no clear splits within the dataset, and the two first axes accounted only for 9.97% of the variation (Fig. 3). The two main STRUCTURE groups were separated along axis 1, but with wide overlap (Fig. 3A). The four main cpDNA haplotype groups defined as H1-H5 (widespread group), H6-H7 (souther/eastern group), H8 (western Mediterranean group) and H9 (NW-SE Iberian group) could not be distinguished based on the AFLP data (Fig. 3B). The samples belonging to "var. *juncea*" and "var. *nigra*" were fully intermixed in the PCoA plot (Fig. 3C). Only some Atlantic European populations (Belgium and the Netherlands) appeared somewhat distinct (Fig. 3D). Individuals from some southern populations were placed peripherally in the plot (Sierra Nevada, Sicily, Rif and Atlas; Fig. 3E). The N American, Greenlandic and Icelandic populations were found in different parts of the plot (Fig. 3F).

The distribution of pairwise AFLP differences within populations was distinctly bimodal (Fig. 4), suggesting the existence of clones in the dataset. Taking into account the error rate (1.43%), individuals differing by up to 2.6 markers (rounded up to 3) were considered to represent the same clone. Although precautions had been taken during sampling, putative clones were found in 46 of the 71 populations analysed. Putative clones were even identified within populations of tussock-forming plants ("var. *juncea"*).

The average gene diversity after removing the 146 identified clones was 0.089 (SD=0.044). The populations from the Netherlands, Belgium and Germany (NW

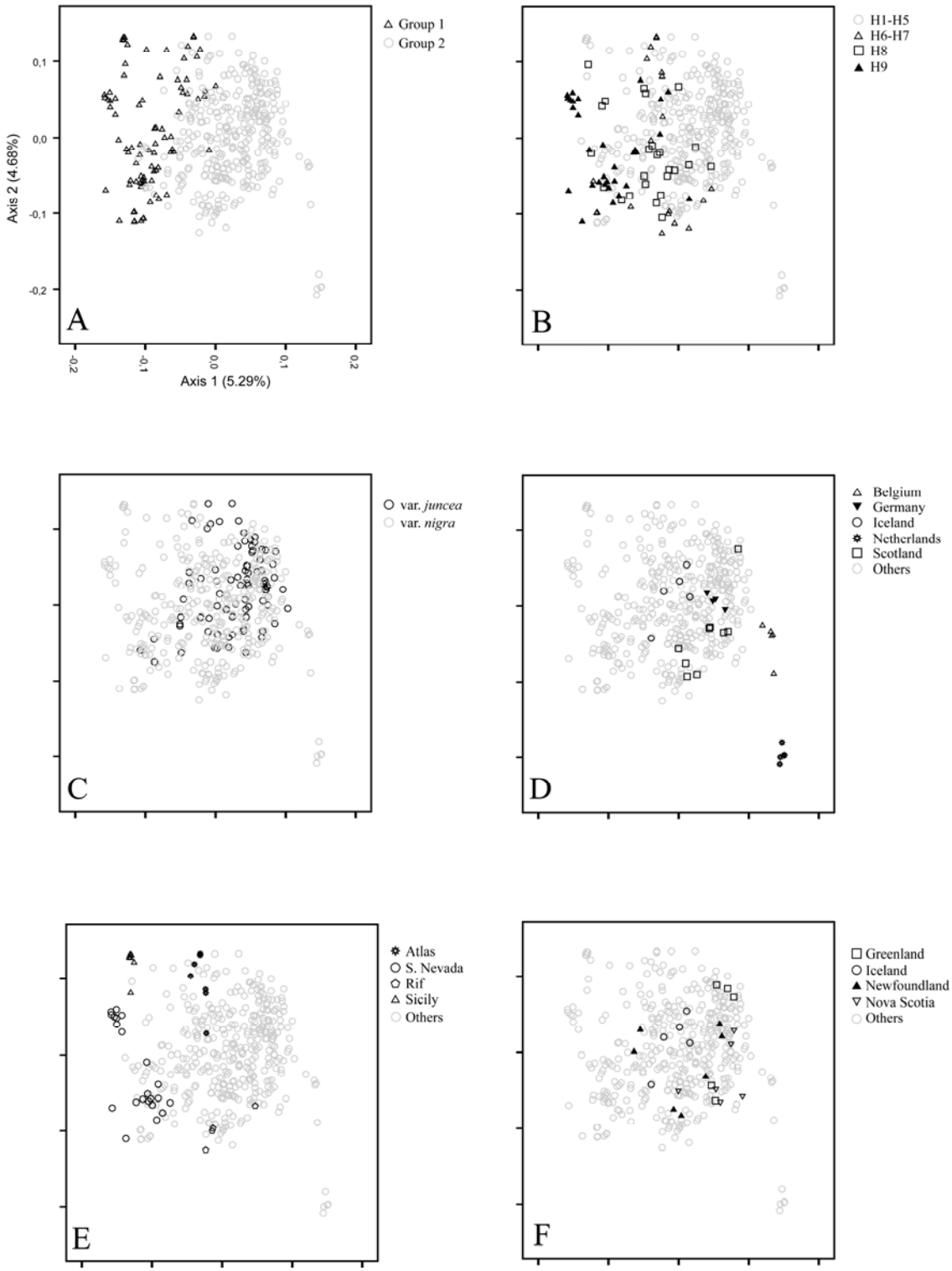


Fig. 3. PCoA of AFLP data for *C. nigra*. The same data are shown in each plot. **A.** Genetic groups identified by the software STRUCTURE. **B.** Main inferred cpDNA haplotype groups. **C.** Rhizome-creeping growth form (var. *nigra*) vs tussock-forming growth form (var. *juncea*). **D.** Placement of populations from NW Europe (Belgium, Germany, Netherlands, Scotland and Iceland; remaining populations displayed in light grey). **E.** Placement of the southern peripheral populations (Atlas, Rif, Sicily and Sierra Nevada; remaining populations displayed in light grey). **F.** Placement of the populations from Iceland, Greenland and North America (remaining populations displayed in light grey).

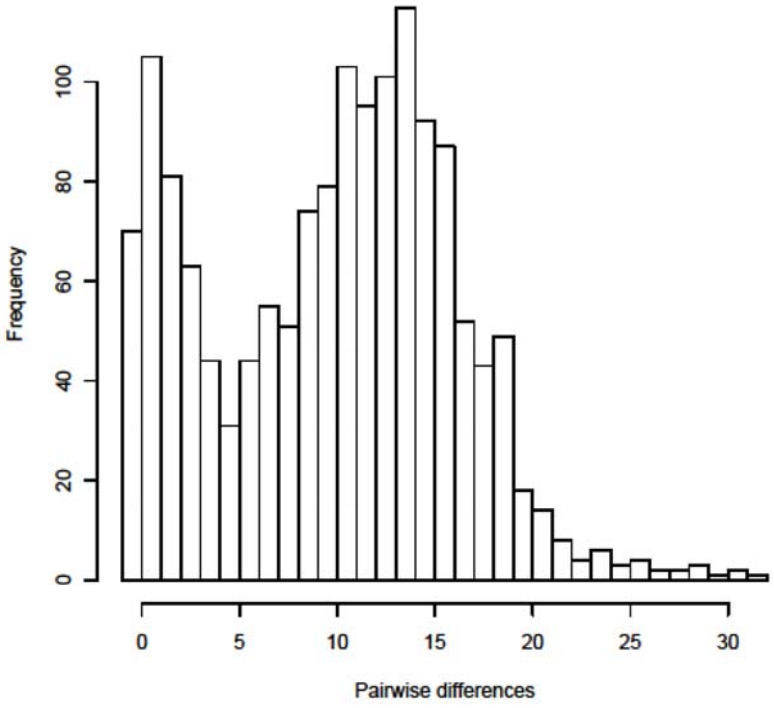


Fig. 4. Distribution of the number of pairwise differences among *C. nigra* samples within populations.

Europe), Moroccan Rif and Sicily were among the least diverse ones (Fig. 1D; Table 1). There was little difference in average diversity between tussock-forming and rhizome-creeping populations (0.062 *vs.* 0.058) and between glaciated and unglaciated areas (0.061 *vs.* 0.056). Surprisingly, the Old World populations contained less average diversity (0.058) than the New World populations (0.071).

Most of the genetically most distinctive populations (i.e., with high DW; Fig. 1D; Table 1) originated from the southern part of the range (e.g. from the Austrian Alps, Spain, Corsica, Atlas, France, and Greece), but some populations from Scandinavia, Russia and Greenland also had high DW values. The lowest DW values were observed in northern European populations, and in a single Central Pyrenean population.

In the AMOVA analyses of the total dataset (Table 2), most of the variation was found within populations, and 34.53% of the variation was found among populations, 8.52% between the two main STRUCTURE groups, and 15.44% among the four STRUCTURE groups/subgroups. Only 6.26% of the variation was found among cpDNA haplotypes, and when the populations with the divergent haplotype H9 were

Table 2 AMOVA analyses for AFLP genotypes of *C. nigra* based on the entire dataset

Grouping compared and source of variation	d.f.	Sum of Squares	Variance components	Percentage of variation
Populations				
Among pops.	66	1592.070	2.76305	34.53
Within pops	392	2053.817	5.23933	65.47
STRUCTURE K=2				
Among groups	1	117.701	0.71882	8.52
Within groups.	457	3528.186	7.72032	91.48
STRUCTURE K=4				
Among groups	3	249.616	1.36341	15.44
Within groups	455	3396.271	7.46433	84.56
Old World *vs.* New World				
Among groups	1	16.475	-0.17169	-2.19
Among pops.	65	1575.595	2.77622	35.39
Within pops.	392	2053.817	5.23933	66.80
Haplotypes				
Among groups	6	252.718	0.52054	6.26
Among pops.	60	1339.352	2.55653	30.74
Within pops.	392	2053.817	8.31640	63.00
H9 *vs.* all other haplotypes				
Among groups	1	79.237	0.75709	8.75
Among pops.	65	1512.833	2.65168	30.66
Within pops.	392	2053.817	5.23933	60.58
C. nigra "var. *nigra*" *vs.* "var. *juncea*"				
Among groups	1	39.188	0.09287	1.15
Among pops.	65	1552.882	2.73402	33.89
Within pops.	392	2053.817	5.23933	64.95

Table 3 AMOVA analyses for AFLP genotypes of *C. nigra* based on partial datasets.

Dataset, grouping compared and source of variation	d.f.	Sum of Squares	Variance components	Percentage of variation
Glaciated areas				
Among pops.	39	682.569	2.13365	29.80
Within pops.	195	979.942	5.02534	70.20
Unglaciated areas				
Among pops.	26	843.438	3.26376	37.45
Within pops.	197	1073.875	5.45114	62.55
H1				
Among pops.	54	1131.703	2.44905	32.00
Within pops.	300	1561.294	5.20431	68.00
H1-H5 (widespread group)				
Among pops.	56	1156.068	2.38493	31.21
Within pops.	312	1639.894	5.25607	68.79
H6-H8 (southern/eastern group plus western Mediterranean group)				
Among pops.	5	198.469	3.84068	42.55
Within pops.	48	248.864	5.18466	57.45
H1-H8				
Among pops.	62	1413.975	2.62243	33.33
Within pops.	360	1888.758	5.24655	66.67
H9 (NW-SE Iberian group)				
Among pops.	3	98.858	3.09467	37.50
Within pops.	32	165.058	5.15807	62.50

compared with all the remaining populations, differentiation was only 8.75%. The variation between the rhizomatous ("var. *nigra"*) and the tussock-forming ("var. *juncea"*) groups was only 1.15%. The Old and New World populations were not overall differentiated (-2.19%). In the AMOVA analyses of partial datasets (Table 3), the populations containing the southern/eastern haplotypes H6-H8 were more differentiated (42.55%) than those with haplotypes H1-H5 (31.21%). However, when populations with haplotypes H1-H8 were all tested together, differentiation was 33.33%. The populations with the most divergent haplotype H9 were also quite differentiated for AFLPs (37.50%)

Discussion

POOR DIFFERENTIATION SUGGESTS EXTENSIVE GENE FLOW

Our plastid and AFLP diversity data clearly reveals that the Mediterranean is a diversity center for *C. nigra*. Both markers show however poor geographical structure and high overall genetic diversity, implying a historical scenario with vicariance and multiple secondary contacts as the main drivers that have shaped the current genetic structure. Low levels of differentiation have also been reported in the closely related *C. bigelowii* (Schönswetter *et al.*, 2008), in line with the expectation of high intrapopulational variation and low differentiation in wind-pollinated outcrossers (Hamrick & Godt, 1990). The continuity of the *C. nigra* range north of the Mediterranean has clearly promoted extensive gene flow, preventing differentiation and increasing genetic variation (cf. O'Brien & Freshwater, 1999; Stenström *et al*. 2001). Interestingly, some Scandinavian populations show admixture of different Mediterranean AFLP groups, suggesting gene flow due to long-distance dispersal. This is not unexpected as ability to colonize at enormous geographic scales previously have been reported in *Carex*; dispersal events have even been suggested between continents (Schönswetter *et al.*, 2008; Escudero & Luceño, 2009) and poles (Vollan *et al.* 2006; Escudero *et al.*, 2010). The extensive gene flow inferred in *C. nigra* has obviously obscured the history of this taxon.

GLACIAL REFUGIA AND POSTGLACIAL COLONIZATION

In contrast to what is expected in regions colonized postglacially, glacial refugia are assumed to typically be characterized by high genetic distinctiveness (DW) (Tribsch *et al.*, 2002; Schönswetter & Tribsch, 2005) as well as high genetic diversity (Taberlet *et al.*, 1998; Hewitt, 1999). The high distinctiveness and diversity that we observed in the *C. nigra* populations from southern Europe (mainly Mediterranean Basin) and western Russia (Tver Oblast) suggest that these regions served as refugia during the last glaciation, as proposed for other plants (e.g. Konnert & Bergman, 1995; Taberlet *et al.*, 1998; Petit *et al.*, 2003; Schönswetter *et al.*, 2003; Magri *et al.*, 2006; Schönswetter *et al.*, 2006a; Skrede *et al.*, 2006). Our data also suggest possible minor refugia in the northern part of the distribution range: the populations from Sweden and Greenland have unexpectedly high DW values, and a rare cpDNA haplotype (H4) was only detected in southern Norway. *In situ* glacial survival in northern Europe and/or in North Atlantic areas has recently been proposed for some tree species (Willis *et al.*, 2000; Palmé *et al.*, 2003; Magri *et al.*, 2006) and also for few herbs (Tyler 2002a,b; Rejzková *et al.*, 2008; Schönswetter *et al.*, 2008; Westergaard *et al.*, 2011). However, the above-mentioned northern populations of *C. nigra* were overall poorly differentiated (see Figs 1C & 3), suggesting that the rare markers enhancing their DW values are of recent (postglacial) origin (eg. local introgession events; Jonsson & Prentice, 2000). A recent mutation may explain the occurrence of the rare haplotype H4 in *C. nigra* in Norway, as suggested for *Betula* by Palmé *et al.* (2003).

In *Carex nigra*, our results suggest that postglacial colonization fits the "southern richness *vs.* northern purity" paradigm (Hewitt, 2001). The Mediterranean region harbours most of the genetic diversity, in contrast to northern areas (Figs 1B & 1C). The among-population variation is also higher (37.45%) in unglaciated than in formerly glaciated areas (29.80%) (Table 3). In the Mediterranean, vicariance has been proposed as the main process that has driven differentiation (Zhang *et al.*, 2001; Kropf *et al.*, 2006; Martín-Bravo *et al.*, 2010), promoted by populations being split among different mountains during warmer periods and subsequent isolation. In *C. nigra*, it appears that some populations remained quite isolated and differentiated genetically, whereas others expanded and met during colder periods, leading to extensive mixing of more or less differentiated gene pools. It is possible that two main range shifts have shaped the overall phylogeographic structure of *C. nigra* (Figs 1B, 2A & 2B): One early expansion reaching Himalayas, and second more recent expansion of the

currrently widespread lineage, reaching the Caucasus and North America and establishing secondary contacts with all other groups.

Effect of isolation on rear edge populations can be observed as so-called centrifugal differentiation. The most isolated peripheral populations of *C. nigra*, i.e. those from Atlas, Rif, Sicily and Sierra Nevada, most likely were little influenced by the secondary contacts (Figs 1 & 3E). Genetic drift may have led to loss of genetic diversity in the populations from Rif and Sicily. In these areas *C. nigra* forms tiny patches, and may have experienced peripheral depauperation in marginal habitats (Lönn & Prentice, 2002; Johannesson & André, 2006). Climatic marginality, such as very dry summers in low-altitude mountains (less than 2500 m in the Rif, and 1850 m in Sicily) could play a role (cf. Puşcaş *et al.* 2008). In contrast, populations from Sierra Nevada and Atlas showed genetic diversity around average, which can be explained by the availability of suitable environments at altitudes over 3000 m, allowing *C. nigra* to form huge populations.

Northward migration of *C. nigra* appears to have had different genetic consequences at different sides of the colonization front. The diversity loss in Fennoscandia appears to be quite moderate whereas other parts of NW Europe are genetically more depauperate (Fig. 1D; Table 1). Repeated bottlenecks during a rapid expansion process (leading edge model) could explain the low diversity found in the populations from Belgium, N Germany, Netherlands and Scotland (Hewitt, 1999; Wróblewska & Brzosko, 2006), resulting in reduced genetic diversity and inter-populational genetic homogenization (Hewitt, 1996; Petit *et al.*, 2003). Genetic drift appears to have operated so strongly in such cases that it has resulted in differentiation of some populations (Fig. 3D), as suggested for *Saxifraga cernua* in Scotland (Westergaard *et al.*, 2008). In contrast, the higher diversity found in Fennoscandia can be explained by accumulation of variation in a suture zone between westwards and northwards colonization fronts (Konnert & Bergmann, 1995; Hewitt, 1999; Walter & Epperson, 2001; Petit *et al.*, 2003). This suture zone may also have contributed to gene flow among source areas (Tyler, 2002a,b). It is also possible that the colonization process in Fennoscandia may have been broad-fronted enough to maintain genetic variability (Eidesen *et al.*, 2007).

Our results suggest that the trans-Atlantic distribution pattern of *C. nigra* can be explained by recent cross-oceanic dispersal from Europe to North America, probably occurring more than once. *Carex nigra* is found in the New World along the northeastern Atlantic coast, from Greenland southward to New York, where it is generally considered native (Cayouette & Morisset, 1986; Standley *et al.*, 2002). The North American populations included in our analyses were very similar to the European ones, but represented large parts of

the European variation, suggesting multiple colonizations (Fig. 3; Table 2). This finding is in line with other recent studies suggesting that the North Atlantic Ocean represents a less severe barrier to plant migration than traditionally envisioned (Abbott & Brochmann, 2003), which also has been demonstrated for other *Carex* species (Schönswetter *et al.*, 2008; Westergaard *et al.*, in press). In *C. nigra*, colonization of North America from Europe was earlier suggested by Dragon & Barrington (2008). However, we cannot exclude the possibility that the species was at least partly introduced into North America by humans. The American presence of genetic group 1B (otherwise spread through the Iberian Peninsula and Norway; Fig. 1C) and the negative AMOVA values obtained in the New World - Old World comparison (Table 2) reveal that some American plants are genetically more similar to Old World plants than to other American plants, even those from the same location.

ROLE OF INTERSPECIFIC HYBRIDIZATION

All specimens included in this study were examined to exclude possible interspecific hybrids. In agrement with our morphological observations, genetic variation patterns detected in selected "pure" *C. nigra*, did not indicate presence of extensive interspecific introgression. In particular, genetic depauperation revealed by AFLPs in populations from Belgium, N Germany, Netherlands and Scotland could imply that outcrossing with other sympatric taxa is not enough to increase the overall genetic diversity of *C. nigra* against the negative effect of the successive bottlenecks during postglacial colonization. Other areas where high levels of genetic diversity were found, as the Mediterranean Basin and western Russia, were proposed as glacial refugia. In the closely related halophile *Carex* group (*C. aquatilis*, *C. paleacea*, and allies) hybridization has been proposed as a mechanism for maintaining genetic variability (Volkova *et al.* 2009). However, in NW Europe *C. nigra* broadly co-exists with six taxa from the same section (*C. acuta* L.*, C. aquatilis*, *C. bigelowii* Schwein., *C. cespitosa* L., *C. elata* All. and *C. trinervis* Degl.). Although all possible hybrids with *C. nigra* have been reported (cf. Schultze-Motel, 1968-1969; Jermy *et al.*, 2007), they do not seem to contribute much to the total genetic variation within *C. nigra*.

In an expanded sampling including eleven additional European and Mediterranean *Carex* species from sect. *Phacocystis* (Jiménez-Mejías, 2011), H9 was found to be shared with three allopatric taxa *C. buekii* Wimm. (E Europe), *C. randalpina* B.Walln. (C Europe) and *C. trinervis* Degl. (W Europe). This haplotype sharing can be explained by hybridization or incomplete lineage sorting. However, external position of H9 in the network together with

the absence of H9 progenitor alleles in *C. nigra* as well as in *C. buekii*, *C. randalpina* and *C. trinervis* (Jiménez-Mejías, 2011) provide strong indication for a past hybridization event (e.g Bänfer, 2006; Pleines et al., 2009).

TAXONOMIC IMPLICATIONS

Our genetic data have several important taxonomic implications. We found no support for taxonomic distinction between the typical rhizome-creeping "var. *nigra*" and the tussock-forming "var. *juncea*". The latter was formerly considered a distinct species (*C. juncella*; e.g. Sylvén, 1963), but lately, with a notable exception of Egorova (1999), regarded as conspecific with *C. nigra*. The different growth forms are however maintained in cultivation, suggesting a genetic basis, although regular chromosome pairing has been observed in artificial hybrids between them (Faulkner, 1973). As the two growth forms appeared completely intermingled in our 'genome-wide' AFLP analysis (Fig. 3C), it is therefore likely that they only differ at one or a few loci or have transriptome-level differences not detectable by AFLP, and that "var. *juncea*" can be considered to represent an ecotype that has originated repeatedly from different rhizome-creeping populations.

Carex nigra subsp. *intricata* was described from the southernmost part of the *C. nigra* distribution area. Our sampling covered all the mountain ranges from which *C. intricata* has been reported: Atlas, Corsica, Sicily and Sierra Nevada. The results clearly show that the populations from these areas are genetically heterogeneous (Figs 1B, 1C, 2B & 3E). In our total material, the populations from Atlas, Sicily and Sierra Nevada are genetically most divergent, providing a basis for being treated as two or three separate taxa within *C. nigra*. However, the close morphological similarity between these populations and typical *C. nigra s.str.*, as well as our detection of introgression among the different genetic groups, suggest that further studies must be carried out before taxonomic decisions are made. In particular, additional morphological comparisons are necessary to re-evaluate taxonomical boundaries in the circum-Mediterranean material before assigning taxonomic ranks.

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CAPÍTULO 7

Systematics and evolution of *Carex* sect. *Phacocystis* Dumort. s.s. in Europe, Mediterranean basin and Irano-Turanian region. Preliminary results.

P. Jiménez-Mejías, S. Martín-Bravo & M. Luceño

Abstract

Carex sect. *Phacocystis* is a taxonomically problematic group mainly distributed in northern Hemisphere. Hybridization has been widely reported between the members of the section. In a restricted circumscription it is composed by 16 taxa in Europe, the Mediterranean basin and Irano-Turanian region. Section *Phacocystis* taxa with a mainly Eurasian distribution have been tentatively proposed as a monophyletic group in the basis of phylogenetic analyses including some of the species. Nuclear (ITS and ETS1f) and plastid (*rpl32-trnL*^{UAG} and *ycf6-psbM*) have been used to asses the phylogenetic relationships among these mainly Eurasian members of the section. Incongruent phylogenetic reconstructions were obtained when the markers were analyzed separately or together. When samples involved in significant phylogenetic conflicts were removed from analysis, a structure in three clades was recovered: 1- *Carex bigelowii* ssp. *dacica* clade (independent from ssp. *bigelowii*), 2- Mediterranean clade, and 3- a core *Phacocystis* clade that contained most of the members of our tentative ingroup. ITS recombination was detected in several of those instances of phylogenetic conflict. regarding to these cases of polyphyly detected in *C. nigra s.l.* and *C. orbicularis*, hybridization is proposed for the former, whereas morphological convergence seems to be behind the second case.

Keywords. introgression, paraphyly, polyphyly, reticulation, topological incongruence.

Introduction

Carex L. is one of the largest genera among the non-agamospermic angiosperms, with about 2000 species across all the continents except the Antarctic, and with an ecological radiation that ranges from polar wetlands to subtropical semi-arid environments (Reznicek, 1990). It is included in the tribe Cariceae together with the genus *Schoenoxiphium* Nees, *Kobresia* Willd. and *Uncinia* Pers. *Carex* has been traditionally subdivided in four subgenera: *Psyllophora* (Degl.) Peterm., *Vignea* (T.Lestib.) Peterm., *Vigneastra* (Tuck.) Kük. and *Carex* (Kükenthal, 1909; nomenclature updated following Egorova, 1999). A systematic review of most sections within subgenus *Carex* has been well established by Waterway *et al.* (2010). The mainly aquatic section *Phacocystis* Dumort. was revealed as closely related to other sections *Glaucescentes* Reznicek, *Limosae* (Heuff.) Meinsh. and *Squarrosae* J.Carey also composed of mostly wetland plants.

The section *Phacocystis* is morphologically distinguished from other sections by 1- well-developed robust rhizomes (creeping or tussock-forming); 2- unsheathing lowestmost bract, frequently with two membranous pieces in its base (auricles); 3- cylindric female spikes; 4- two stigmata; 5- biconvex, papillose (rarely smooth) utricles, with a short truncate beak or beakless; and 6- lenticular achenes (Chater, 1980; Standley *et al*., 2002; Luceño and Jiménez-Mejías, 2008).

The taxonomic circumscription and systematic arrangement of sect. *Phacocystis*, and, therefore, monophyly, are still being debating. It has been traditionally related to sect. *Bicolores* (Fr.) Rouy (Kükenthal, 1909), since both groups share the following features: biconvex, frequently papillose utricles, with a scarcely developed beak (sometimes absent) and two stigmata. However, ITS sequences revealed that *Bicolores* and *Phacocystis* were not closely related, and that the sect. *Temnemis* Raf. (= *Cryptocarpae* (Bailey) Mack.) is the most closely allied to *Phacocystis* (Hendrichs *et al.*, 2004). These findings supported an enlarged concept of sect. *Phacocystis* which has been already proposed by several authors (Faulkner, 1973; Chater, 1980) who merged sections *Phacocystis* and *Temnemis*. The studies of Dragon and Barrington (2008, 2009) focused on the internal systematic arrangement of the section, showing that that it is a paraphyletic group with sect. *Temnemis*, but also with the morphologically unrelated sect. *Scitae* Kük. In addition, the sect. *Praelongae* (Kük.) Nelmes, included also within

Table 1. Main taxonomical treatments of *Carex* sect. *Phacocystis* (halophile species excluded) in Europe, N Africa and Middle East, indicating their geographic scopus. The recently described *C. randalpina* B.Wall. is not included, which was formerly considered as *C. acuta* (Wallnöfer, 1993).

Kükenthal (1909) / *World revision*	**Chater** (1980) / *Europe*	**Luceño and Jiménez Mejías** (2008) *Iberian Peninsula*	**Egorova** (1999) / *Former USSR* (*Eastern Europe, Transcaucasus*)	**Nilsson** (1986) , **Kukkonen** (1996) / *Turkey & Iran, respectively*
C. panormitana Guss.	-	-	-	-
C. gracilis Curtis	*C. acuta* L.	*C. acuta* *C. mauritanica* Boiss.[4,5] *C. elata* ssp. *tartessiana* Luceño & Aedo[5]	*C. acuta* - -	*C. acuta* - -
C. reuteriana Boiss.	*C. nigra* (L.) Reichard	*C. elata* ssp. *reuteriana* (Boiss.) Luceño[4]	-	-
C. goodenoughii Gay		*C. nigra*	*C. nigra*	*C. nigra*
	ssp. *intricata* (Tineo) Maire[2]	-	ssp. *intricata*	-
	ssp. *alpina* (Gaudin) Lemke[2]	-	ssp. *alpina*	ssp. *alpina*
	"ssp. *dacica*" (Heuff.) Soó[2,3]	-	-	"ssp. *dacica*"[3]
	var. *juncea* (Fries) Hyl.	-	*C. juncella* (Fries) Th.Fries	-
	-	-	*C. transcaucasica* Egor.	-
C. aquatilis Wahlenb.	*C. aquatilis*	-	*C. aquatilis*	-
	ssp. *stans* (Drej.) Hult.	-	ssp. *stans*	-
C. hudsonii A.Bennett	*C. elata* All.	*C. elata* ssp. *elata*	*C. elata*	*C. elata*
C. omskiana Meinsh.	ssp. *omskiana* (Meinsh.) Jalas	-	ssp. *omskiana*	ssp. *omskiana*
C. buekii Wimm.	*C. buekii*	-	*C. buekii*	-
C. cespitosa L.	*C. cespitosa*	*C. cespitosa*	*C. cespitosa*	*C. cespitosa*
C. rigida Good.	*C. bigelowii* Torr. ex Schwein	-	*C. bigelowii*	-
	ssp. *rigida* (Good.) Schultze.Motel	-	ssp. *dacica* (Heuff.) Egor.	-
	ssp. *ensifolia* (Gorodk.) Holub	-	ssp. *ensifolia*	-
	ssp. *arctisibirica* (Jurtz.)A&D.Löve	-	ssp. *arctisibirica*	-
C. orbicularis Boott	-	-	*C. orbicularis* ssp. *kotschyana* (Boiss & Hohen) Kukkonen	*C. orbicularis* ssp. *kotschyana*
C. rufina Drejer	*C. rufina*	-	*C. rufina*[3]	-
C. trinervis Degl.	*C. trinervis*	*C. trinervis*	-	-
C. kurdica Hand.Mazz.[1]	-	-	*C. kurdica*[2]	*C. kurdica*

[1]Described in 1914 as "*Kükenthal ex Handel Mazzetti*"; [2]listed as *C. nigra* varieties, but without a definitive taxonomical statement; [3]until Egorova's typification (1999), the name *C. dacica* was erroneously applied to eastern European and Turkish populations of *C. nigra*; [4]not included in the treatments, but explicitly accepted; [5]Recently re-evaluated as *C. reuteriana* ssp. *reuteriana* and *C. reuteriana* ssp. *mauritanica* (including *C. mauritanica* and *C. elata* ssp. *tartessiana*) (Jiménez-Mejías *et al.*, in press).

Phacocystis by some American authors (Standley *et al.*, 2002), seemed to be associated to this clade, despite its relationships are still uncertain. Regardless such problems of sectional delimitation, they found that the taxa traditionally included in the sect. *Phacocystis* were arranged in four more or less supported main groups: 1- a first clade (American-*C. aquatilis* clade) which groups most American species together with the Circumboreal *C. aquatilis* Wahlenb., the amphi-Atlantic *C. rufina* Drej., and the halophile taxa formerly constituting sect. *Temnemis* (*C. paleacea* Wahlenb. and allies); 2- a second one (*C. bigelowii* clade) that contains *C. bigelowii* Schwein. and its allies; 3- a third clade (Asian-Australasian clade) that comprises species from Australasia and Asia Far East; and, finally, 4- a clade where those predominantly Eurasian species were recovered (Eurasian clade, sect. *Phacocystis s.s.*).

Chater (1980) considered sect. *Phacocystis* in Europe (excluding the highly problematic halophile taxa) composed by 9 species (Table 1). However, other authors considered up to 5 additional taxa (species and subspecies; see below), plus 2-3 additional species if Middle East, North Africa and the Caucasus are taken into account (Table 1). Most of the incongruities between treatments are due to disagreements regarding to the *C. nigra* complex and/or *C. acuta*-like plants, especially the southernmost populations (i.e. Mediterranean).

Carex nigra is widely distributed through Central and Northern Europe, reaching southwards the Mediterranean mountain ranges, eastwards Siberia and Hymalayas. In addition, it displays a typical amphi-Atlantic disjunction with populations in Iceland, Greenland and E North America (Chater, 1980; Noltie, 1994; Egorova, 1999; Standley *et al.*, 2002). North European tussock-forming populations have been regarded as a distinct form in subspecies or variety rank (*C. nigra* ssp. *juncea* (Fries) Soó; *C. nigra* var. *juncea* Fries), sometimes, even as an independent species (*C. juncella* Fries; Sylvén, 1963; Egorova, 1999). In spite of its apparent distinctiveness in the field of the typical var. *nigra* rhizome-creeping forms, its taxonomical distinctiveness has been refused by recent molecular results (see Chapter 6). On the other hand dwarf *C. nigra* mountain forms of Sierra Nevada, Atlas, Corsica and Sicily have been considered in local treatments as a separate species (*C. intricata* Tin.; Chater, 1980; Pignatti, 1982) or a distinct race (*C. nigra* ssp. *intricata* (Tineo) Rivas Mart.) (Maire, 1957; Vicioso, 1959). Additionally, Egorova (1999) considered the populations from the Caucasus belonged to a different species, *C. transcaucasica* Egor.

Table 2. Materials included in this study. Taxonomic treatment followed is a consensus from Chater (1980), Nilsson (1986), Kukkonen (1996), Egorova (1999) and Jiménez-Mejías *et al.* (in press, see Chapter 5). Botanical countries are expressed in the code following TDWG nomenclature (Brummit, 2001).

Taxon		**Global distribution**	
Sample code	Locality (herbarium voucher and laboratory code if applicable)		GenBank accessions: ITS, ETS1f, *rpl32-trnL*^{UAG} and *ycf6-psbM*
C. acuta		**Europe and Western Asia**	
Acu_GRB	England, Merseyside, Formby		forthcoming, forthcoming, forthcoming, forthcoming
Acu_FIN	Ostrobottnia, southwest of Tervola (O; 391-Lye)		forthcoming, forthcoming, forthcoming, forthcoming
Acu_ITA	Calabria, La Sila, Lago Cecita		forthcoming, forthcoming, forthcoming, forthcoming
Acu_NOR	Oppland, Ringebu, Elstad (O; 300-Lye)		forthcoming, forthcoming, forthcoming, forthcoming
Acu_SPA	Zamora, Carrascal, Duero river		forthcoming, forthcoming, forthcoming, forthcoming
Acu_TUR	Bolu, Abant lake		forthcoming, forthcoming, forthcoming, forthcoming
C. aquatilis		**Circumboreal**	
Aqu_FIN	Inari Lappland, Enontekio (O; 1024A-Lye)		forthcoming, forthcoming, forthcoming, forthcoming
C. bigelowii* ssp. *bigelowii		**Eastern Boreal America, Iceland and North Scandinavia**	
Big_big_GRL	Western Greenland, between Qassiarsuk and Tassiussaq		forthcoming, forthcoming, forthcoming, forthcoming
C. bigelowii* ssp. *dacica		**Alps, Carpathians, British Isles, Scandinavia and Iceland**	
Big_dac_GER	Seetaler Alps, Rothaide (GJO)		forthcoming, forthcoming, forthcoming, forthcoming
Big_dac_ICE	Gelfoss, Geysir		forthcoming, forthcoming, forthcoming, forthcoming
Big_dac_NOR	Hedmark, Alvdal, Tronden (O; 321A-Lye)		forthcoming, forthcoming, forthcoming, forthcoming
C. buekii		**Eastern Europe eastward Kazakhstan, Transcaucasus and Anatolia**	
Bue_BUL	Sofia		not obtained, forthcoming, forthcoming, forthcoming
Bue_GER	Regensburg, Regen river		forthcoming, forthcoming, forthcoming, forthcoming
C. cespitosa		**Europe to Central Asia**	
Ces_FIN	Ostrobottnia, Tervola (O; 390-Lye)		forthcoming, forthcoming, forthcoming, forthcoming
Ces_NOR	Akershus, Ås, Pollevatn (O, 66A-Lye)		forthcoming, forthcoming, forthcoming, forthcoming
Ces_SPA	Navarra, Lesaka, Bidasoa river		forthcoming, forthcoming, forthcoming, forthcoming
C. elata* ssp. *elata		**Europe, North Africa and Anatolia; rare in Iran**	
Ela_ela_ITA	Piemonte, Lago di Candia		forthcoming, forthcoming, forthcoming, forthcoming
Ela_ela_NOR	Aurskog, Aakershus (O; Lye-73)		forthcoming, forthcoming, forthcoming, forthcoming
Ela_ela_SPA	Huelva, Doñana National Park, La Rocina		forthcoming, forthcoming, forthcoming, forthcoming
C. elata* ssp. *omskiana		**NE Europe, Russia to C Siberia**	
Ela_oms_BLT	Estonia, Voru district. Viitinaku, Kogreküla (TU)		forthcoming, forthcoming, forthcoming, forthcoming
Ela_oms_FIN	South Häme, Hämeenlinna, Keinusaari (SEV)		forthcoming, forthcoming, forthcoming, forthcoming
C. kurdica		**Middle East and Eastern Mediterranean**	
Kur_GRC	Epirus, Acheron river sources		forthcoming, forthcoming, forthcoming, forthcoming
Kur_IRN_1	Kurdestan, 45-50 km from Sanandaj to Tangi-Sar village (IRAN)		forthcoming, forthcoming, forthcoming, forthcoming
Kur_IRN_2	Kurdestan, 18-20 km from Marivan to Paveh (IRAN)		forthcoming, forthcoming, forthcoming, forthcoming

(Continued)

(Continued)

Taxon		**Global distribution**	
Sample code	Locality (herbarium voucher and laboratory code if applicable)		GenBank accessions: ITS, ETS1f, *rpl32-trnL*^{UAG} and *ycf6-psbM*
***C. nigra* s.s.**		**Europe, North Africa, Siberia, Anatolia, Eastern North America; rare in Iran**	
Nig_BUL	Rila National Park, Seven Rila Lakes		forthcoming, forthcoming, forthcoming, forthcoming
Nig_GRL	South Greenland, Narsaq		forthcoming, forthcoming, forthcoming, forthcoming
Nig_MOR	Rif, Djebel Tidighin		forthcoming, forthcoming, forthcoming, forthcoming
Nig_NET	Drenthe, Assen		forthcoming, forthcoming, forthcoming, forthcoming
Nig_RUW	Tver Region, Udomelskij district, Lake Bolshaya		forthcoming, forthcoming, forthcoming, forthcoming
Nig_SWE	Scania, Malmö		forthcoming, forthcoming, forthcoming, forthcoming
Nig_SPA	Palencia, Cantabrian Mountains, Curavacas		forthcoming, forthcoming, forthcoming, forthcoming
Nig_TUR	Erzincam, Sakaltutan Geçidi		forthcoming, forthcoming, forthcoming, forthcoming
C. nigra* var. *juncea		**North Europe to Central Siberia**	
Nig_jun_NOR	Akershus, Oppegård (O)		forthcoming, forthcoming, forthcoming, forthcoming
C. nigra* ssp. *drukyuliensis		**Hymalayas (Buthan)**	
Nig_dru_EHM	Bhutan, N of Thimphu Dzong (E)		forthcoming, forthcoming, forthcoming, forthcoming
C. nigra* ssp. *intricata		**Sierra Nevada, Atlas, Corsica and Sicily**	
Nig_int_COR	Campanelle		forthcoming, forthcoming, forthcoming, forthcoming
Nig_int_MOR	High Atlas, Oukaïmedem		forthcoming, forthcoming, forthcoming, forthcoming
Nig_int_SIC	Nebrodi, Monte Soro (type parish)		forthcoming, forthcoming, forthcoming, forthcoming
Nig_int_SPA	Granada, Sierra Nevada		forthcoming, forthcoming, forthcoming, forthcoming
C. orbicularis* ssp. *altaica		**C Asia (Altai)**	
Orb_alt_ALT	Between Surijza and Artishtu river basins (MA)		forthcoming, forthcoming, forthcoming, forthcoming
C. orbicularis* ssp. *kotschyana		**Caucasus, Iran**	
Orb_kot_IRN_1	Azerbaijan, Meshkin Shahr, Qotur Sou, Shabil (IRAN)		forthcoming, forthcoming, forthcoming, forthcoming
Orb_kot_IRN_2	Kurdestan, Sanandaj (IRAN)		forthcoming, forthcoming, forthcoming, forthcoming
C. orbicularis* ssp. *orbicularis		**Hymalayas and Karakoram**	
Orb_orb_NEP	Dhawagiri, Mustang (E)		forthcoming, forthcoming, forthcoming, forthcoming
C. panormitana		**Sardinia, Sicily and Tunisia**	
Pan_SIC	Palermo, Oreto river		forthcoming, forthcoming, forthcoming, forthcoming
C. randapina		**Regions sourrounding eastern Alps**	
Ran_AUT	Oberösterreich, Attergau (type parish)		forthcoming, forthcoming, forthcoming, forthcoming
Ran_ITA_1	Trentino, Grigno (FI)		forthcoming, forthcoming, forthcoming, forthcoming
Ran_ITA_2	Friuli-Venetia Giulia, Polcenigo (FI)		forthcoming, forthcoming, forthcoming, forthcoming
C. reuteriana* ssp. *reuteriana		**NW and C Iberian Peninsula**	
Reu_reu_SPA	Cáceres, Jerte		forthcoming, forthcoming, forthcoming, forthcoming

(Continued)

(Continued)

Taxon	**Global distribution**	
Sample code	Locality (herbarium voucher and laboratory code if applicable)	GenBank accessions: ITS, ETS1f, *rpl32-trnL*^{UAG} and *ycf6-psbM*
C. reuteriana* ssp. *mauritanica	**S Iberian Peninsula and N Africa**	
Reu_mau_MOR_1	Rif, Laou river	forthcoming, forthcoming, forthcoming, forthcoming
Reu_mau_MOR_2	Rif, Loukos river	forthcoming, forthcoming, forthcoming, forthcoming
Reu_mau_SPA	Sevilla, El Ronquillo, Rivera de Huelva (type parish)	forthcoming, forthcoming, forthcoming, forthcoming
C. transcaucasica	**Transcaucasus**	
Tra_IRN_1	Azerbaijan, Meshkin Shahr, Ghotour Soui, Shabil (IRAN)	forthcoming, forthcoming, forthcoming, forthcoming
Tra_IRN_2	Azerbaijan, Arasbaran area, Doghroon mountain (IRAN)	forthcoming, forthcoming, forthcoming, forthcoming
Tra_TRC	Armenia, Kotayk, Tehenis (UPOS)	forthcoming, forthcoming, forthcoming, forthcoming
C. thunbergii	**Russian Far East, NE China, Japan**	
Thu_JAP	Honshu, Niigata (UPOS)	forthcoming, forthcoming, forthcoming, forthcoming
C. trinervis	**Atlantic Europe from SW Spain to Denmark**	
Tri_DEN	Jylland, Riebe, Fanø (O; 590-Lye))	forthcoming, forthcoming, forthcoming, forthcoming
Tri_FRA	Landes, Mimizan Plage	forthcoming, forthcoming, forthcoming, forthcoming
Tri_POR	Beira Litoral, Figueira-da-Foz, Lagoa da Vela	forthcoming, forthcoming, forthcoming, forthcoming
Tri_SPA	Huelva, Doñana National Park, finca El Gato	forthcoming, forthcoming, forthcoming, forthcoming

Table 3. Summary of sequence characteristics (excluding the outgroup *C. acutiformis*): aligned length, coded indels, and number of constant, variable and informative characters (including coded indels).

Region	**Aligned length**	**Coded indels**	**Number of constant characters**	**No. of uninformative characters**	**No. of parsimony informative characters**
ITS1	219	1	199	14	7
5.8S	166	0	266	0	0
ITS2	217	0	198	5	14
ETS1f	544	0	482	33	29
rpl32-trnL^{UAG}	734	2	725	6	5
ycf6-psbM	414	2	401	10	5
Combined complete matrix (58 accessions)	2294	5	2172	68	59
Combined final analysis* (49 accessions)	2294	5	2188	70	41

*After removing samples involved in supported phylogenetic conflicts

The circumscription of *C. acuta* has also been controversial. *Carex acuta* is a mainly European taxa that extends eastward to Siberia, Caucasus and SW Asia (Luceño and Jiménez-Mejías, 2008). Other medium-sized *Carex* sect. *Phacocystis* members plants with lowermost bract as long as or longer than the inflorescence have been amalgamated under a wide *C. acuta* concept (eg. Maire, 1957; Vicioso, 1959; Hartvig, 1991). The morphological heterogeneity of such set of plants is remarkable, and more recent works recognize some populations as distinct taxa: *C. kurdica* Hand.-Mazz from SW Asia and E Mediterranean (Nilsson, 1986; Kukkonen, 1996; see also Chapter 8), *C. panormitana* from Sardinia, Sicily and Tunisia (Pignatti, 1982; see also Chapter 8), *C. randalpina* B.Wall. from C Europe (Wallnöfer 1992, 1993) and *C. reuteriana* Boiss. from Iberian Peninsula and NW Africa (Luceño and Aedo, 1994; Jiménez-Mejías *et al.*, in press (see Chapter 5)).

Interspecific hybridization is widely known to occur among European taxa of sect. *Phacocystis* species (Sylvén, 1963; Schultze-Motel, 1968-1969; Faulkner, 1973; Chater, 1980; Jermy *et al.*, 2007). In the related complex of halophile sedges, interspecific hybridization has been proposed as a mechanism to maintain genetic diversity (Volkova *et al*., 2008). However, in *C. nigra* it appears that introgression does not contribute extensively to *C. nigra* gene pool (see Chapter 6). There is a rainsing evidence about the evolutionary role of hybridization in *Carex* and related to Cariceae genus. Recombination and concerted evolution are processes that can operate after hybridization, sometimes contributing to fix genetic hybrid features, but sometimes also obscuring such phenomena of horizontal gene transfer. For instance, in *Carex* sect. *Ovales* (Hipp *et al.*, 2006) incongruent phylogenetic signals were retrieved between the nuclear regions ITS and ETS. In addition, in the related genus *Schoenoxiphium*, evidences of hybridization and ITS recombination have been found to be related to a cladogenetic event (*S. rufum* clade), leading to the rarely reported phenomenon of independent inheritance of ITS1 and ITS2 (Gehrke *et al.*, 2010).

Karyologically, the genus *Carex*, as other Cyperaceae, is characterized by some cytogenetic characteristics of evolutionary interest, mainly the holocentric chromosomes, which allow to form aneuploid series (via fission or fusion of chromosomes), as well as chromosomal rearrangements (Malheiros and Gardé, 1950; Luceño and Guerra, 1996; Greilhuber, 1995). Within sect. *Phacocytis*, the first cytogenetic approach was made by Faulkner (1972). The inferred euploid numbers allowed the grouping of the different studied taxa in five categories: *C. bigelowii* (2n = 68-70), *C. paleacea* (2n = 72), *C. aquatilis*-*C. elata* (2n = 76), *C. cespitosa* (2n = 78-80)

and *C. acuta*-*C. nigra s.l.* (2n = 84). However, in the basis of the observed differences in the chromosome size, similar numbers found in different species were considered homoplasious, thus not indicating close systematic relationships (Faulkner, 1972). A low fertility degree in interspecific hybrids and irregularities of chromosome pairing during meiosis were interpreted as evidences of divergence between the involved species (Faulkner, 1973). In this sense, despite the high fertility degree found in *C. acuta* × *C. nigra* hybrids, the usually abnormal meiosis supported their distinct taxonomic status, whereas the regular pairing in the *C. nigra* × *C. juncella* crossbreed was taken as a sign of conspecifity (Faulkner, 1973). The taxonomical value of the cytogenetic data in sect. *Phacocytis* was confirmed in a later study in Iberian taxa of sect. *Phacocystis* (Luceño and Aedo, 1994). The close relationships traditionally assumed between the Iberian *C. reuteriana* and *C. nigra*-*C. acuta* (Vicioso, 1959; Chater, 1980) was rejected due to the observed chromosome numbers of *C. reuteriana* (2n = 74-75(76)), much closer to the euploid number of *C. elata* (2n = 76) than to that of *C. acuta*-*C. nigra* (2n = 84). As a result, *C. reuteriana* was subordinated under *C. elata* (*C. elata* ssp. *reuteriana* (Boiss.) Luceño; Luceño and Aedo, 1994). The taxonomic status of *C. reuteriana* as an independent species was recently supported using chromosome data, AFLPs and sequences (Jiménez-Mejías *et al.*, in press (see Chapter 5)).

In this work, we perform phylogenetic analyses of two nuclear (ITS, ETS1f) and two plastid regions (*rpl32-trnL*^{UAG} and *ycf6-psbM*) focusing on Eurasian taxa of sect. *Phacocystis s.s.* to: 1- clarify their phylogenetic relationships; 2- evaluate the relative role of hybridization in the observed phylogenetic pattern; and 3- asses the current taxonomic treatments in light of our systematic study.

Materials and Methods

TENTATIVE INGROUP DEFINITION

The scopus of this work covers those taxa putatively belonging to the sect. *Phacocystis* s.s. as defined by Egorova (1999), with subsequent modifications regarding to the circumscription of the Eurasian clade (Dragon and Barrington, 2008). Our geographical scopus lays mainly in Europe, North Africa and W Asia (Table 2), with additional members in Central Asia (e.g. *C. orbicularis*). The circum-boreal *Carex aquatilis*, and the amphi-Atlantic *C. bigelowii* ssp. *bigelowii*, since they have been

placed in other lineages (Dragon and Barrington, 2008), were included as outgroups. Similarly, as *C. rufina* belonged to other clade was not included. On the contrary, we included of *C. bigelowii* ssp. *dacica* since this taxon has been regarded as possibly different from the type subspecies (Nakamatte and Lye, 2007), and it could be related to members of the Eurasian clade as shown by micromorphological data (Nakamatte, 2009).

SAMPLING

Fifty-eight samples from 24 taxa were included in the study (Table 2): fifty-five samples represented the 17 taxa found in Europe, North Africa and W Asia presumably belonging to the sect. *Phacocystis s.s.* clade (including *C. bigelowii* ssp. *dacica*, and two additional samples of the Central Asian subespecies of *C. orbicularis*); a sample of *C. aquatilis*, *C. bigelowii* ssp. *bigelowii*, and *C. thunbergii* each as outgroups representing the other three sect. *Phacocytis* clades (see Introduction; Dragon and Barrington, 2008), and a sample of *C. acutiformis* Ehrh. as a not closely related outgroup.

PCR AMPLIFICATION AND *SEQUENCING*

Total DNA was extracted from silica-dried material collected in the field, as well as from herbarium specimens (FI, IRAN, E, GJO, MA, SEV, TU, and UPOS) using DNeasy plant Mini Kit (Qiagen, CA). Two nuclear and two plastid regions were amplified for all the samples, except for a *C. buekii* population for which ITS failed. The nuclear regions chosen were ITS and ETS1f; for primers and protocols see Escudero *et al.* (2008) and Starr *et al.* (2003) respectively. The variability of 10 plastid regions was tested (*5'trnK* intron, *matK*, *psbA-trnH*, *rpl16*, *rpl32-trnL*^{UAG}, *rps16*, *trnL-F*, *trnS-G*, *trnT-L* and *ycf6-psbM*), of which *ycf6-psbM* and *rpl32-trnL*^{UAG} were found as the most variable ones; for primers and protocols see Shaw *et al.*(2005, 2007) respectively. Sequences were edited using Seqed 1.3.0 (Applied Biosystems, CA, USA), and IUPAC symbols were applied to represent nucleotide ambiguities. Limits of the ITS and ETS1f were determined as specified by Starr *et al.* (1999) and Starr *et al.* (2003) respectively. Limits considered for the sequences of *ycf6-psbM* and *rpl32-trnL*^{UAG} regions were established from the clean fragments found in the chromatograms. Single nucleotide polymorphism was considered only when the two peaks showed equal (or almost equal) height.

SEQUENCE ANALYSES

Matrices were manually aligned using a text editor. Informative indels were codified as binary characters (A / T). Maximum parsimony (MP) and Bayesian inference (BI) analyses were performed for each nuclear region separately (ITS1, 5.8S, ITS2 and ETS1f) and combined ITS. cpDNA regions were analyzed together, separately and combined with nrDNA. It allowed to identify instances of phylogenetic incongruence between different regions and genomes. To evaluate the significance of phylogenetic conflicts, we followed a modified criterion from Gerkhe *et al.* (2010). Conflicting significant nodes were identified as those supported by posterior probability (pp) >0.90 and/or bootstrap support (bs) >75 and whose circumscription varied between any of the phylogenies. The sequences involved in the identified conflicts and in recombination (see below) were removed from final combined analysis in order to avoid their disruptive effect on the phylogenetic reconstructions (Fuertes Aguilar and Nieto Feliner 2003).

Parsimony analyses were conducted under Fitch parsimony, as implemented in TNT 1.1 (Goloboff *et al.* 2008) with equal weighting of all characters, and gap treated as missing-data (the maximum number of trees retained in TNT RAM memory options was set to 500,000). Heuristic searches were replicated 10,000 times retaining a maximum of two trees in each replicate, with tree bisection–reconnection (TBR) branch swapping. A second heuristic search set was made based on the trees retained in RAM memory in the first heuristic search set. The trees were obtained from the strict consensus of all trees obtained in the heuristic searches. Clade supports were assessed by bootstrapping with 10,000 re-samplings based on the conditions of the first heuristic search set. Only bootstrap supports equal or exceeding 75% were considered significant (Hillis and Bull 1993).

For BI analyses, the simplest model of sequence evolution that best fits the data under the Akaike Information Criterion was determined for each marker as implemented in JModeltest (Posada, 2008). Bayesian analyses were performed with the

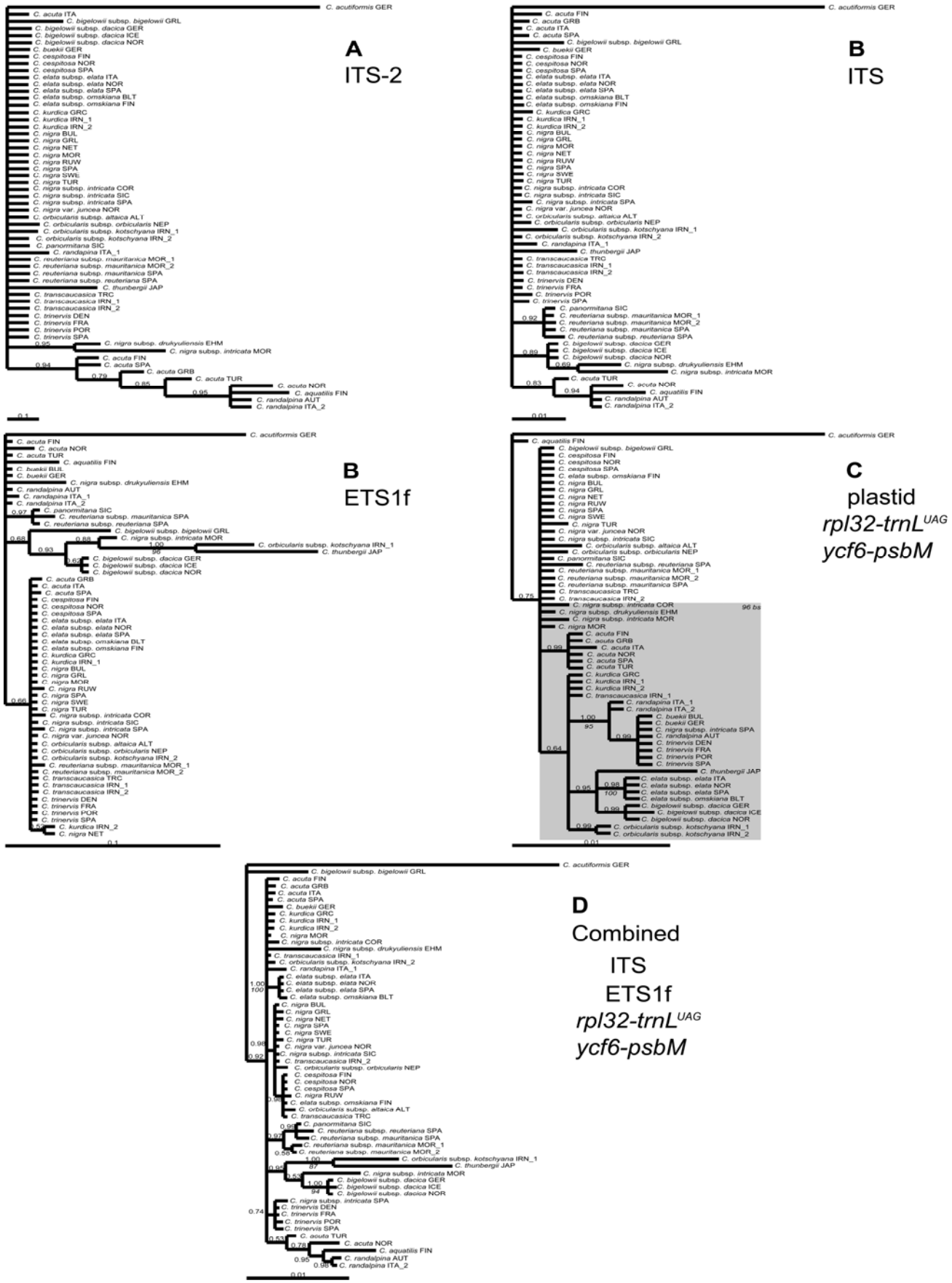

Fig. 1. 50% Bayesian majority rule DNA trees based on: **A.** ITS-2; **B.** complete ITS sequence; **C.** ETS1f; **D.** combined plastid data (*rpl32-trnL*^{UAG} and *ycf6-psbM*); and **E.** combined nuclear and plastid data (ITS, ETS1f, *rpl32-trnL*^{UAG} and *ycf6-psbM*); Bayesian posterior probability values above 0.50 p.p. are given in regular character, whereas bootstrap support values above 75% are given in italics. The grey square in **C.** represent a clade recovered by MP analyses (96 bs) but not in the BI reconstruction. Botanical countries are expressed following TDWG nomenclature (Brummit, 2001).

Table 4 Summary of changes in clade support used for the detection of significant topological incongruencies.

	ITS combined	**ITS2**	**ETS1f**	**plastid**	**Combined**
C. acuta	Partially recovered in *C. aquatilis* clade (0.94); rest of samples in politomy	Forming a clade with *C. aquatilis* and *C. randalpina* (0.94); partially recovered asclosely allied to *C. aquatilis* and *C. randalpina* (0.95)	Unresolved	Monophyletic (0.99)	Unresolved
C. elata (except ssp. *omskiana* from Finland)	Unresolved	Unresolved	Unresolved	Monophyletic (0.98) Nested in *C. bigelowii* ssp. *dacica* clade (0.95)	Monophyletic (1.00), out from *C. bigelowii* ssp. *dacica* clade
C. nigra ssp. *intricata* Atlas	Unresolved	Recovered with *C. nigra* Hymalayas (0.95)	Recovered in *C. bigelowii* ssp. *dacica* clade (0.93)	Recovered in a well-suported clade (96%), out from *C. bigelowii* ssp. *dacica* clade (0.95).	Recovered in *C.bigelowii* ssp. *dacica* clade (0.95)
C. randalpina	Partially recovered in *C. aquatilis* clade (0.94); a sample from Italy in politomy	Nested in a clade with *C. acuta* (0.94); partially recovered closely allied to *C. aquatilis* and *C. acuta* p.p. (0.95); a sample from Italy in politomy	Unresolved	Samples from Italy recovered as sister to *C. buekii*-*C. trinervis* clade (1.00) the sample from Austria recovered in *C. buekii*-*C.trinervis* clade (0.99)	Partially recovered in *C. aquatilis* clade (0.95); samples and well-supported (0.98); a sample from Italy in politomy.
C.bigelowii ssp. *dacica* clade	Marginally well-supported (0.89); including *C. nigra* from Atlas and Hymalayas	Unresolved	Well supported (0.93); including *C. nigra* from Atlas, *C. orbicularis* p.p. (Orb_IRN1) and *C. thunbergii*	Well supported (0.95); including *C. thunbergii* and *C. elata*; *C. bigelowii* ssp. *dacica* monophyletic (0.99)	Well supported (0.95); including *C. nigra* from Atlas, *C. orbicularis* p.p. and *C. thunbergii*; *C. bigelowii* ssp. *dacica* monophyletic (1.00)

selected models using MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003). Four Markov Chain Monte Carlo runs were performed simultaneously in each analysis for 5,000,000 generations, with a sampling interval of 100 generations. The first 12,500 generated trees (25%) were discarded before calculating the majority rule consensus tree, thus ensuring likelihood stationary. Clade posterior probabilities were considered significant when equal or exceeding 0.90.

For those taxa/samples whose phylogenetic placement was involved in significant conflicts between phylogenies (*C. acuta* from Norway, *C. elata* (except a ssp. *omskiana* sample) and *C. randalpina* p.p.; see results), ITS sequences were assessed for recombination using SimPlot version 3.5.1 (Lole *et al.*, 1999). This program examines how the phylogenetic relationships of a sequence changes along its entire length. It requires, at least, two reference taxa as possible "parents" and an outgroup. Similarity was calculated using the Kimura 2-parameter model with the transversion/transition default value of 2.0. Breakpoints of the recombinants were identified using bootscanning analyses with 100 replicates. For each of the incongruent instances, we selected those phylogenetically most closely related to taxa as putative parentals (Robertson *et al.*, 1995). Firstly, for the *C. acuta* sample from Norway, the chosen taxa were *C. aquatilis* and non-incongruent samples of *C. acuta* (see Fig. 1A, 1B and 2). Secondly, for incongruent *C. randalpina* samples, we tested *C. aquatilis* and *C. buekii* on one hand, and *C. aquatilis* and *C. trinervis* on the other one (see Fig. 1A, 1B, 1C and 2). Remarkably, as the *C. randalpina* samples involved in conflict did not displayed recombination (see results), recombination was subsequently tested for the not incongruent sample from Italy (Ran_ITA_1). Finally, the lack of phylogenetic resolution prevented us to determine one of the putative parental taxa for *C. elata* (the other was *C. bigelowii* ssp. *dacica*), so we had to compare all possible taxa (considering those samples not involved in significant conflicts) as alternative parents (*C. acuta*, *C. buekii*, *C. cespitosa*, *C. kurdica*, *C. nigra*, *C. panormitana*, *C. randalpina*, *C. reuteriana* and *C. trinervis*; see Fig. 1D). We used *C. bigelowii* ssp. *dacica* for *C. acuta* from Norway and *C. randalpina* tests, and *C. aquatilis* for *C. elata*.

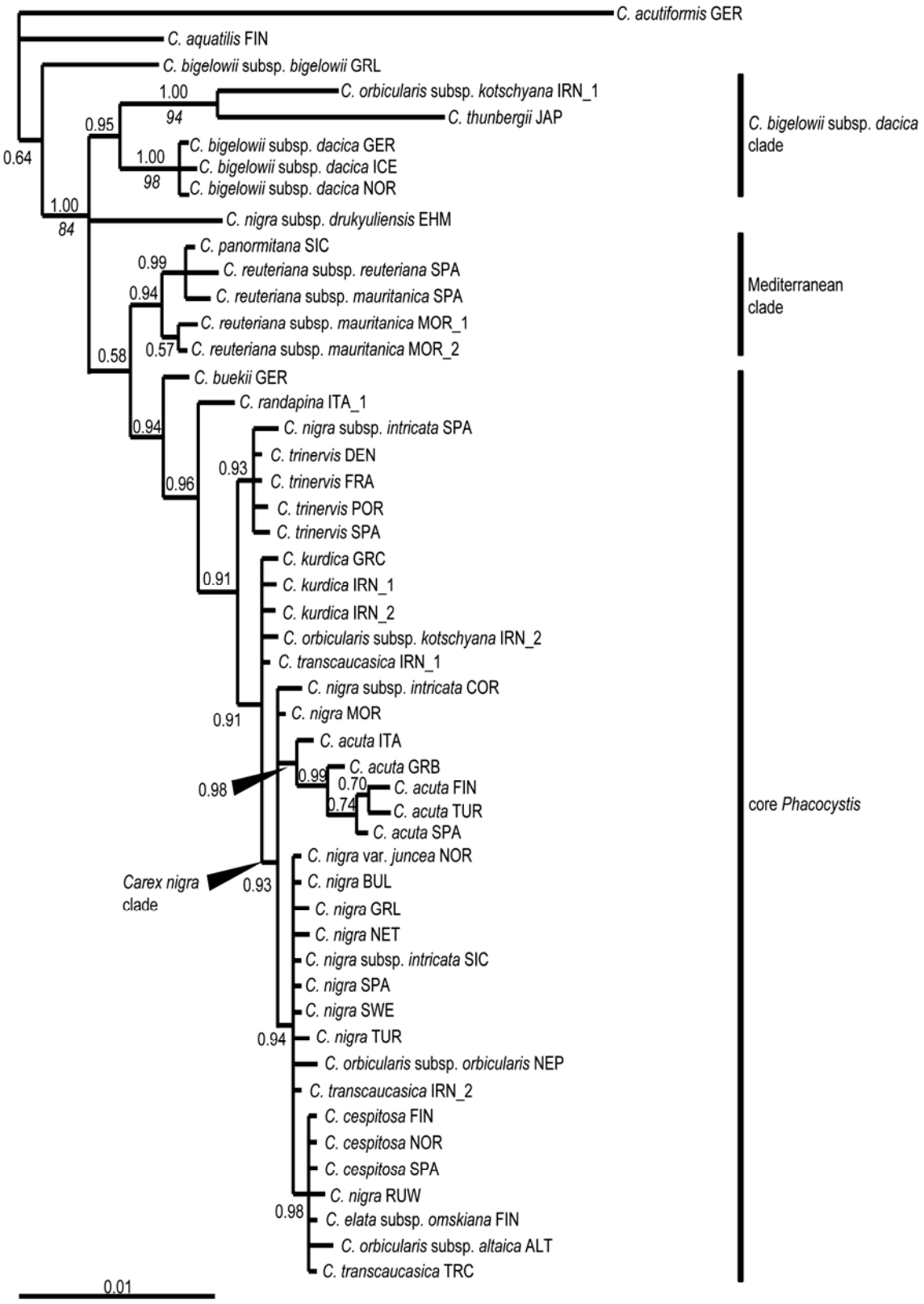

Fig. 2. 50% majority rule DNA trees based on combined nuclear and plastid data (ITS, ETS1f, *rpl32-trnL*^{UAG} and *ycf6-psbM*) excluding the samples involved in supported phylogenetic conflicts; Bayesian posterior probability values above 0.50 p.p. are given above in regular, whereas bootstrap support values above 75% are given in italics.

Results

PHYLOGENETIC INCONGRUENCE BETWEEN MOLECULAR MARKERS

Sequence characteristics are displayed in table 3. The phylogenetic trees from the analysis of the ITS2, complete ITS sequence, ETS1f, plastid and combined nuclear-plastid data are showed in Fig. 1A, 1B, 1C, 1D and 1E respectively. Analyses of ITS1 and 5.8s regions did not retrieve any well-supported clade (data not shown).

Comparison of the tree topologies inferred from each marker and the combined data revealed several supported instances of phylogenetic conflict. Three major instances can be identified (table 4): 1) A sample of *C. acuta* was recovered as allied to *C. aquatilis* and *C. randalpina* in ITS (0.94 pp; Fig 1B), whereas *C. acuta* was monophyletic (0.99 pp) and unrelated to *C. aquatilis* in the plastid phylogeny (Fig 1C); 2) *C. randalpina* was also partly clustered with *C. aquatilis* in ITS (0.94 pp; Fig. 1B), but clearly associated to *C. buekii* and *C. trinervis* in the plastid phylogeny (1.00 pp; Fig. 1C); and 3) *C. elata*, almost monophyletic in both plastid and nuclear-plastid analysis (0.98 and 1.00 pp respectively; Figs. 1C-D), but associated to *C. bigelowii* ssp. *dacica* clade (0.95 pp; Fig. 1C) in the plastid phylogeny but not in the combined (Fig. 1D). Two samples of *C. nigra* (ssp. *intricata* from Atlas, and ssp. *drukyuliensis* from the Hymalayas), and one of *C. orbicularis* ssp. *kotschyana* (from Iran) also showed incongruities between individual gene trees, but only the placement of *C. nigra* ssp. *intricata* from Atlas was found to be significantly incongruent (see Figs. 1C-D).

PHYLOGENETIC RELATIONSHIPS FROM COMBINED ANALYSIS

Exclusion of samples/taxa with significant topological conflicts between phylogenies from the combined analysis resulted in a better resolved phylogeny (Fig. 2). The phylogeny displayed a well-supported clade (1.00 pp, 84 bs) where three major subclades were identified: 1- the "*C. bigelowii* ssp. *dacica* clade" (0.95 pp), joining the European *C. bigelowii* ssp. *dacica*, with a sample of the Iranian *C. orbicularis* ssp. *kotschyana* and the Japanese *C. thunbergii*; 2- the "Mediterranean clade" (0.94), grouping *C. panormitana* and the *C. reuteriana* subspecies (*reuteriana* and

mauritanica); and 3- the "core *Phacocystis*", containing all the remaining samples (0.93 pp). The taxa considered under our tentative ingroup split between these two latter clades. *Carex orbicularis* and *C. nigra* are recovered as highly polyphyletic, although most *C. nigra* samples were grouped in a well supported clade (*C. nigra* clade; 0.93 p.p.) together with *C. acuta* and *C. cespitosa*, while *C. orbicularis* was scattered through four different clades.

ITS RECOMBINATION

Our analyses detected several apparent instances of recombination in the ITS region in the studied instances. In *C. acuta* from Norway, ITS1 was *C. acuta*-like whereas ITS2 was more similar to *C. aquatilis* than to the remaining *C. acuta* samples (Fig. 3A).

For *C. elata*, recombination was found when the reference taxa compared were *C. acuta* and *C. bigelowii* ssp. *dacica*, with an ITS-1 more similar to the former, and an ITS-2 closer to the later (Fig. 3B).

No evidence for recombination was found for the two incongruent samples of *C. randalpina* (Fig. 3C, 3D) either compared with *C. buekii* of *C. trinervis*. However, the sample from Italy (Ran_ITA_1), which did not show an incongruent placement, displayed an ITS recombinant pattern when tested with *C. aquatilis* and both *C. buekii* and *C. trinervis* as putative parentals (Fig. 3E, 3F).

Discussion

SYSTEMATIC ARRANGEMENT OF EURASIAN MEMBERS OF CAREX *SECT.* PHACOCYSTIS *S.S.*

Due to the hybridization and recombination processes detected, main systematic relationships within the section are discussed on the basis to Fig. 2, the tree retrieved from the combined analysis after samples affected by significant phylogenetic conflicts were removed. Our phylogenetic reconstruction displays a similar pattern to the one found by Dragon and Barrington (2008): The core *Phacocystis* matches the Eurasian clade; placement of *C. bigelowii* ssp. *dacica* together with *C. thunbergii* (included in

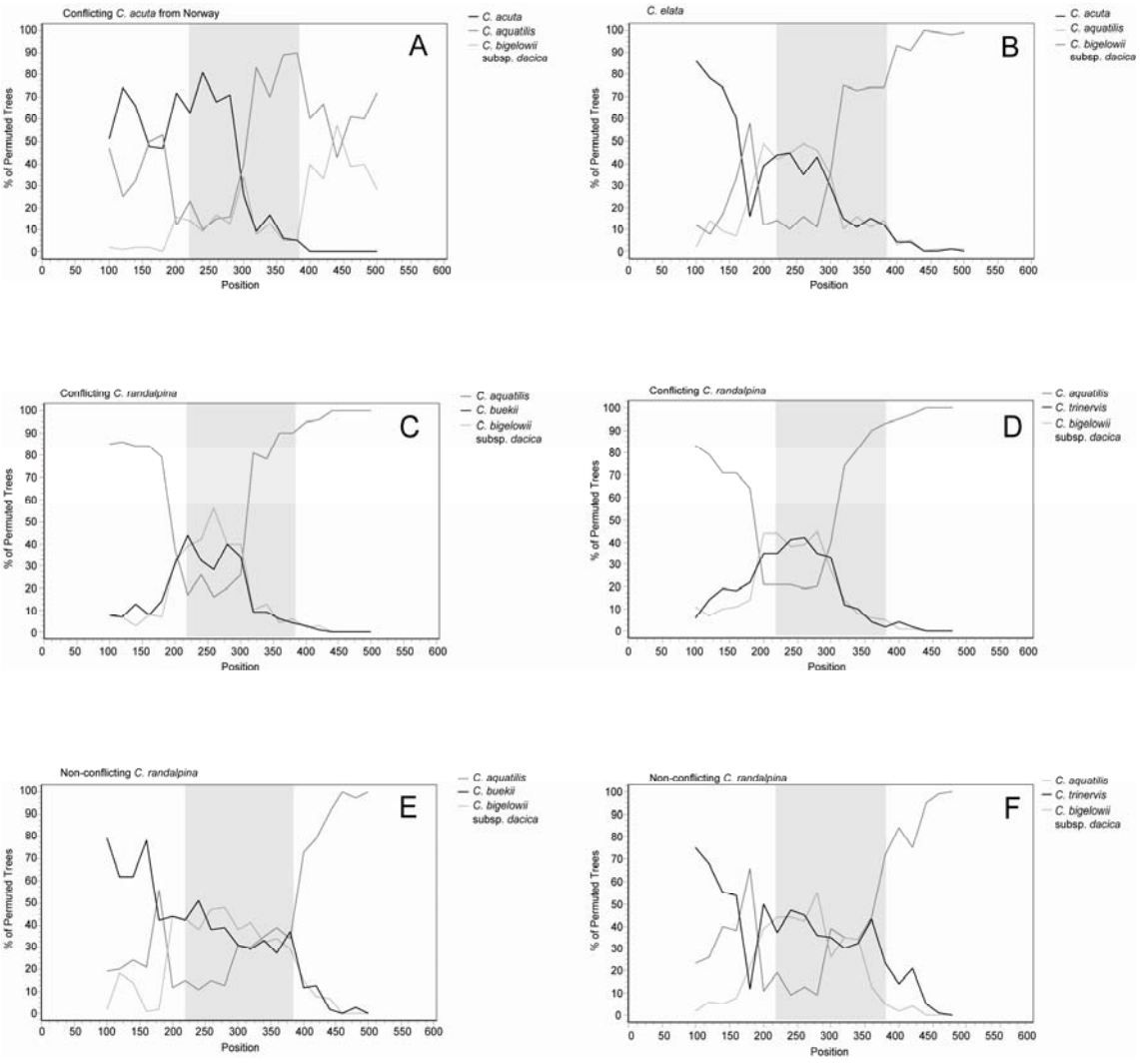

Fig. 3. Simplot bootscaning analyses using a window of a 200 bp, a step of 20 bp and the Kimura 2-parameter model. The sequences tested for recombination in each analysis are: **A.** Incongruent *C. acuta* (Acu_NOR); **B.** Incongruent *C. elata* tested using *C. acuta* as reference. **C-D.** incongruent *C. randalpina* (Ran_AUT, Ran_ITA_2) using *C. buekii* (**C**) and *C. trinervis* (**D**) as one of the reference sequences;
E-F. non-incongruent *C. randalpina* (Ran_ITA_1) using *C. buekii* (**E**) and *C. trinervis* (**F**) as one of the reference sequences. Vertical axis indicates the degree of phylogenetic similarity with the tested sequence. The 5.8S region is located in the shaded area.

Dragon and Barrington (2008) phylogeny). It suggests that *C. bigelowii* ssp. *dacica* clade would be equivalent to the Asian-Australasian clade; and *Carex aquatilis* and *C. bigelowii* ssp. *bigelowii*, representing respectively American-*C. aquatilis* and *C. bigelowii* clades did not displayed close relatives among the members of our tentative ingroup. Remarkably, we retrieved an additional group, the Mediterranean clade, formed by *C. panormitana* and *C. reuteriana*. Most of the sampled European members

of the section were placed within the core *Phacocystis* (Fig. 2), together with the mostly Asiatic *C. kurdica* and *C. orbicularis*. *Carex buekii* was found to be sister to the remaining members of the clade. Interestingly, Stoeva *et al.* (2005) considered *C. buekii* as "the eldest" taxon among the European sect. *Phacocystis* members in the basis of allegedly primitive features, such as hypostomatal leaves and its low chromosome number (2n = 68). *Carex trinervis* and *C. kurdica* sample sets kept clustered, although the monophyly of none of both species was supported due to the placement of a *C. nigra* ssp. *intricata* sample in *C. trinervis* clade, and to the lack of resolution among *C. kurdica*, embedded in a polytomy.

The split of the two subspecies of *C. bigelowii* in different branches of the phylogeny (Figs. 1C-D,2) suggests morphological convergence between two different phylogenetic species: *C. bigelowii s.s.* and *C. dacica*. Taxonomic independence of ssp. *dacica* was already suggested by Nakamatte and Lye (2007), who tested the systematic relationships among subspecies *bigelowii* and *"rigida"* (= *dacica*) with AFLP. A clear genetic structure within *C. bigelowii* was also found by Schönswetter *et al.* (2008), who showed that three genetic groups with geographical congruence matched three subspecies: Amphi-Atlantic (ssp. *bigelowii*), European (ssp. *dacica*), and Amphi-Beringian (ssp. *lugens*). As *C. bigelowii* ssp. *dacica* clade could be equivalent to the Asian-Australasian clade reported by Dragon and Barrington (2009), ssp. *dacica* would be the single European member of a clade mainly diversified in the W Pacific.

Carex acuta and *C. cespitosa* were nested within the *C. nigra* clade revealing their close relationships. *Carex acuta* and *C. nigra* were believed to be closely allied on the basis of chromosomal and morphological features by Faulkner (1973). The nesting of *C. acuta* and *C. cespitosa* in *C. nigra* clade arises the question of paraphyly of the "core *C. nigra*". The acceptance of paraphyletic taxa is inherent to certain evolutionary processes, such as peripatric speciation or allopolyploidy, which indirectly implies the paraphyly of the direct ancestors (reviewed in Hörandl and Stuessy, 2010). Despite the evolutionary forces that have enabled the differentiation of *C. acuta* and *C. cespitosa* remain unclear, they are clearly morphological species distinct from *C. nigra* on the basis of morphology. In addition, they display certain cytogenetic features that may support a relative reproductive isolation with *C. nigra*: on one hand, *C. cespitosa* and *C. cespitosa* has different euploid numbers (2n = 78-80 and 2n = 84 respectively (Faulkner, 1972)); on the other hand, hybrids between *C. acuta* and *C. nigra* show irregular chromosome pairing in meiosis (Faulkner, 1973).

Two of the *C. transcaucasica* populations and two of the *C. nigra* ssp. *intricata* (including the type parish) have been placed within core *C. nigra* (Fig. 2). The remaining populations (*C. nigra* ssp. *intricata* from Morocco and Sierra Nevada (Spain) and a *C. transcaucasica* from Iran could be regarded as hybrids (see below). Prior to its formal description, *C. transcaucasica* was previously considered under *C. nigra* (Egorova, 1999). AFLP-based results showing extensive gene flow between *C. nigra s.l.* (including populations of *C. transcaucasica*) and the lack of differentiation between the analyzed populations (see Chapter 6). It advices against the taxonomic identity of *C. transcaucasica* and argue in favour of its inclusion in a wider *C. nigra* concept.

Morphological convergence and biased taxonomic decisions derived could be partly the case of the rampant polyphyly detected in *C. orbicularis*, with its 3 different subspecies in different clades (Fig 2). However, the polyphyly of ssp. *kotschyana* in the combined phylogeny (Figs. 1D, 2), but the monophyly when only plastid data is analyzed (Fig. 1C), could be due to hybridization. An extended sampling of this Asian mountain species is required to get insights into its systematic and possible hybridization processes.

CYTOGENETIC EVOLUTION

Cytogenetic variation in *Carex* is remarkable, and evolution has been characterized as rapid and diverse. Contrasting trends have been inferred from different groups, such as sect. *Ovales* (chromosome number decreasing; Hipp, 2007) and sect. *Spirostachyae* (both decrease and increase of chromosome number; Escudero *et al.* 2010). A trend to increase the chromosome number towards terminal branches may be inferred for sect. *Phacocystis* from the consensus tree (Fig. 2). Trend to increase chromosome number has been also proposed in *Carex* sect. *Ceratocystis* (Crins and Ball, 1988; see also Chapter 2). Those taxa with lower numbers are placed in the outermost branches of the combined tree (*C. bigelowii* ssp. *dacica*, 2n = 68-70; *C. buekii*, 2n = 64; *C. reuteriana*, 2n = 72-75(76); *C. kurdica*, 2n = 74) (see Faulkner (1972), Stoeva *et al.* (2005), Jiménez-Mejías *et al.* (in press; see Chapter 5); and Chapter 8, respectively) whereas numbers 2n > 80 appear in the core *Phacocystis*. *Carex cespitosa*, with a relatively low number (2n = 78-80) is nested within *C. nigra* (2n = 84); it could be related with a decrease in chromosome number following fusion processes.

HYBRIDIZATION IN SECT. PHACOCYSTIS*: HYBRID ORIGIN SPECIES* VS. *SPECIFIC HYBRIDIZATION EVENTS*

Incongruent phylogenetic placement (Fig. 1B-D) and recombinant ITS sequences found in *C. elata* (Fig. 3B) and *C. randalpina* (Fig. E-F) are identified as a possible sign of the hybrid origin of these species. Hybridization seems to be a common source of variation and a driver of speciation within sect. *Phacocystis*. Several studies have demonstrated hybrid origin species in the closely related halophilic group of *Carex* (Volkova *et al.* 2008; Korpelainen *et al.* 2010). However, in these set of taxa, morphological intermediates are frequently found in mixed populations where two parental taxa occur (Cayouette and Morisset, 1985). Our outcomes would depict hybrid origin stabilized species with characteristic morphology.

In addition, several instances of specific hybridization can be identified within our sampling. The phylogenetic grouping displayed between *C. aquatilis* and a *C. acuta* sample from Norway, and the recombinant ITS found in this latter, allow to identify this plant as an hybrid. Hybrid populations between *C. aquatilis* and *C. acuta* are known from different areas in Norway (K. Lye, pers. comm.). *Carex nigra s.l.* was found polyphyletic due to the phylogenetic placement of populations from Atlas, Hymalayas and Sierra Nevada, plus a *C. transcaucasica* sample. AFLP analysis of Atlas and Sierra Nevada populations (see Chapter 6) did not found a clear split between Sierra Nevada and Atlas populations and the vast majority of the European samples analyzed. Everything mentioned before, together with the congruent grouping of most samples forming a *C. nigra* core within the *C. nigra* clade (Fig. 2) could lead to interpret the polyphyletic samples as a result of specific hybridization. In addition, hybridization could be also involved in the incongruent placement displayed by the Finnish sample of *C. elata* ssp. *omskiana* (Ela_oms_FIN) given the congruent grouping of most *C .elata* samples in the plastid and nuclear-plastid combined phylogenies (Fig. 1C-D).

Future research prospects in *Carex* sect. *Phacocystis*

Despite our research contributes interesting data to the study of the phylogeny and evolution of section *Phacocystis*, we are concerned that our results are preliminary, and that an expanded sampling is clearly needed to address some questions with more

confidence. In our opinion, the main points that deserve further attention in the next future are:

- **Outgroups**: an enlarged sampling from other *Carex* sect. *Phacocystis* lineages is needed to ensure the correspondence of our results with the main clades obtained by Dragon and Barrington (2008), and to shed light on the origin of putatively recombinant ITS sequences.

- **Mediterranean clade**: based on AFLP data, populations of *C. reuteriana* displayed a strongly structured genetic diversity, clearly influenced by geography (Jiménez-Mejías *et al.*, in press; see Chapter 5). However, an increased population sampling would be key to clarify the phylogenetic relationships between *C. reuteriana* and *C. panormitana*. A special effort should be done to sample populations of this latter from Sardinia..

- ***Carex orbicularis*** and ***C. buekii***: additional samples could help to elucidate the evolutionary processes involved in the rampant polyphyly detected within *C. orbicularis*, and to test the monohyly of *C. buekii*.

- **Estimation of divergence times:** Molecular dating could provide a temporal context that allows relating important processes during the evolutionary history of section *Phacocystis* (speciation, extinction, radiation), with historical geologic/climatic events. It could also clarify whether hybridizations processes inferred in the group are recent or ancient. The taxonomic adscription of a c. 4 million years old fossil of a *C. aquatilis*-like utricle (E. Martinetto, pers. comm.) would be critical to reliably calibrate the molecular dating analyses.

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CAPÍTULO 8

Heterogeneity of *Carex acuta* s.l. in its southernmost area: re-evaluation of *C. panormitana* Guss. and *C. kurdica* Kük. ex Hand.-Mazz., and new records for Europe and the Mediterranean Basin

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Abstract

Carex sect. *Phacocystis* is one of the taxonomically most complicated. Despite it has been intensively studied by Central and North European authors, the taxonomic situation in southern Europe and southwestern Asia remains unresolved. In this sense, the widely distributed *C. acuta* has been found heterogeneous in those areas. Previous taxonomic work has recognized several taxa as independent from *C. acuta*, as *C. reuteriana* from Iberian Peninsula and North Africa, and *C. randalpina* from eastern Alps. After extensive herbarium revision, we re-valorized two additional taxa from Central and Eastern Mediterranean Basin: the Tyrrhenian *C. panormitana* and the Eastern Mediterranean and Irano-Turanian *C. kurdica*. Detailed description of both taxa and a key for the recognition of South Europe, North African and SW Asian species of *Carex* sect. *Phacocystis* are provided. An epitype is designated for *C. kurdica* in order to prevent nomenclatural problems, since the lectotype material was found to be inmature.

Keywords. Cryptic taxa, neglected species, taxonomy.

Introduction

Carex sect. *Phacocystis* Dumort is the biggest section of the genus, with approximately 90 spp. (Dragon and Barrington, 2009). It is morphologically characterized by oblong to cylindrical or rarely orbicular female spikes, two stigmas and lenticular, frequently papillose, and short-beaked utricles (Chater, 1980; Egorova, 1999; Luceño and Jiménez-Mejías, 2008).

The taxonomy of *Carex* sect. *Phacocystis* is highly complicated due to hybridization and the faint morphological boundaries among species. Seven taxa have been traditionally reported in S Europe and N Africa: *C. acuta* L., *C. bigelowii* subsp. *dacica* (Heuff.) Egor. (= subsp. *rigida* (Gooden) W.Schultze-Motel), *C. buekii* Wimm., *C. cespitosa* L., *C. elata* All. subsp. *elata*, *C. nigra* (L.) Reichard (including *C. intricata* Tin.) and *C. trinervis* Degl. (Maire, 1957; Chater, 1980). In the neighboring SW Asia, five taxa of the sect. *Phacocystis* have been reported: the already mentioned *C. acuta*, *C. buekii*, *C. elata* subsp. *elata* and *C. nigra* (including *C. transcaucasica* Egor.), plus C. *orbicularis* subsp. *kotschyana* (Boiss. & Hohen) Kukkonen, and *C. kurdica* Hand.-Mazz. (Nilsson, 1985; Kukkonen, 1998; Jiménez-Mejías and Luceño, 2011).

Cytogenetically, south European members of *Carex* sect. *Phacocystis* were arranged in four fairly well-defined classes regarding chromosome number (Faulkner, 1972): 1- a first one only containing *C. bigelowii* (2n = 68-70); 2- a second one including plants with 2n = 74-76 (eg. *C. elata*); 3- a third one formed only by *C. cespitosa* (2n = 78-79); and 4- a last one from 2n ≥ 80. In this sense, *Carex acuta* s.s. with 2n = 82 – 86 is unequivocally located within the group with higher chromosome numbers (Faulkner, 1972; Luceño and Aedo, 1994).

Although *Carex* sect. *Phacocystis* has been intensively studied by north and central European authors (Sylvén, 1963; Faulkner, 1972; Stenström *et al.*, 2001; Stoeva *et al.*, 2005; Wallnöfer, 2006; Dean and Ashton, 2008; Nakamatte and Lye, 2008, 2010; Schönswetter *et al.*, 2008; among others), taxonomic situation in the SW of the Eurasian distribution remains quite unresolved. Among all the taxa, *C. acuta* s.l. is probably the less homogeneous set, working as a hotchpotch in the taxonomic sense. Under *C. acuta* s.l. it has been named a set of plants characterized by medium-size (around 50 cm. high) and lowest-bract as long as or longer than the inflorescence. Several previous studies had emphasized the heterogeneity of circunmediterranean plants considered as *C. acuta*,

even demonstrating that some populations are indeed independent taxa. Luceño and Aedo (1994) noted that Ibero-North African plants referred as *C. reuteriana* Boiss. and *C. mauritanica* Boiss. & Reuter, partly considered *C. acuta* by some authors (cf. Kükenthal, 1909; Vicioso, 1959; Chater, 1980; among others), were fairly distinct and grouped them under *C. elata* All. Later, Jiménez-Mejías *et al.* (in press.) arranged them as a distinct species with two subspecies: *C. reuteriana* subsp. *reuteriana* from NW and C Iberian Peninsula, and *C. reuteriana* subsp. *mauritanica* (Boiss. & Reuter) Jim.Mejías & Luceño, from S Iberian Peninsula and NW Africa. Chromosomal data was critical in the taxonomic revalorization of *C. reuteriana*, since the diploid numbers found by Luceño & Aedo (1994) 2n = 73-75(76) was lower than *C. acuta* diploid number. On the other hand, Wallnöfer (1992, 1993) noted that some *C. acuta*-like Austrian populations actually belonged to an additional undescribed species, *C. randalpina* B. Wall., from the Alps eastern boundaries. Originally reported from Austria, Germany and Slovenia, it has been later found in NE Italy (Prosser, 1998), Croatia (Stančić, 2009) and Hungary (Mesterházy, 2010). By contrast, *C. kurdica* is also a *C. acuta*-like plant described from Turkish Mesopotamia by Handel-Mazzetti (1914) and remained nearly unnoticed until Nilsson's treatment for *Flora of Turkey* (1986). Later, Kukkonen (1998) considered this species also occurring in Iraq, Iran and Afghanistan. Additionally, *C. acuta s.s.* is also known from this area (Amini Rad, 2006). Eventually, *C. panormitana* Guss. is a taxon which stays still controversial. It has been only studied in local contexts, unconnectedly from more comprehensive views. Subsequently, *C. panormitana* has been considered in regional treatments (Pignatti, 1982; Pignatti *et al.*, 2001), but it was synonimized to *C. acuta* in *Flora Europaea* (Chater, 1980). It would be a Tyrrhenian taxon, found in Sicily only in the type parish, and more extensively in Sardinia (Pignatti *et al.*, 2001).

During an extensive revision of herbarium materials, it was revealed that the heterogeneity found within putative materials of *C. acuta* s.l. and related forms was much wider than expected. Some Balkan, Turkish and North African (Tunisian) materials did not totally fit the characters of those taxa previously reported in such areas. The aim of this study is to unravel the taxonomical status of problematic *Carex acuta*-like forms from the central and eastern Mediterranean and the Middle East, and to provide a revalorized status, complementing our previous taxonomic results from Iberian Peninsula and N Africa (Luceño and Aedo, 1994; Jiménez-Mejías *et al.*, in press.).

Materials and methods

A total of 70 specimens from BEO, BEOU, BM, E, GDOR, FI, K, IRAN, M, MPU, RNG, SO and SOM herbaria have been studied, as well as field collections deposited in UPOS herbarium (Pablo de Olavide University, Seville, Spain) (Appendix 1). All selected materials were *C. acuta*-like materials plants characterized by medium-size (around 50 cm. high) and lowest-bract as long as or longer than the inflorescence. The geographical area studied was delimited from West to East as follow: Sardinia, Tunisia, Sicily, Italian peninsula (south of Po river), Gulf of Venice region, Balkan peninsula (South of Danube and Sava rivers), Turkey, and the Middle East. In this way, all the central and eastern regions which surround the Mediterranean shores are representatively covered, plus the area of *Carex* sect. *Phacocystis* in southwestern Asia. Traditional characters used in taxonomic delimitation of *Carex* sect. *Phacocystis* species (Kükenthal, 1909; Chater, 1980; Egorova, 1999; Luceño and Jiménez-Mejías, 2008) were examined, emphasizing on those reported in the regional studies that cover the included areas (Maire, 1959; Pignatti, 1980; Nilsson, 1986; Strid and Kit Tan, 1991; Kukkonen, 1998; Jiménez-Mejías *et al.*, in press.). Measurements were taken with an ocular micrometer up to a tenth of millimeter, except the larger ones (>10 mm) that were taken with a standard 30cm rule.

For a Greek population of *C. acuta*-like plants, chromosome counts from mother pollen cells were made. Meiotic plates were prepared following the protocol described by Luceño (1988) and later observed under a microscope (Nikon eclipse E400) and photographed using a digital camera too (Nikon DxM1200 F). Diploid numbers were inferred from meiotic configurations in pollen mother cells at metaphase I.

Results

Materials from *C. acuta s.s.* were found in mainland Italy, most of the Balkan Peninsula, northern Turkey and Syria. Such populations showed utricle morphology as the main key character for distinguishing them from the other detected taxa: shorter or as long as the glumes, biconvex, a bit inflated, typically yellowish or greenish-brown and minutely papillose on most of the surface, always abruptly contracted into a short beak. Absence of entire leaf-sheath in fertile stems was another characters shared by all

the materials. The materials that differed from typical *C. acuta* populations displayed utricles as long as or longer than the glumes, plano-convex or biconvex, but not inflated, and without papillae in most of the body, abruptly contracted or gradually attenuated into a beak.

Populations from Sicily and Sardinia, assignable to *C. panormitana*, showed as the most distinct characters a broad hyaline margin in male and female glumes and utricles without papillae, contracted into a beak. These characters were also observed in the Tunisian materials.

On the other hand, *C. kurdica* materials from eastern Turkey and Iran revealed as species-characteristic feature the utricles attenuated into a whitish beak, frequently papillose. Additionally, they displayed straw-colored elongated but leafless sheaths. Although those characters partly match some of those reported for *C. elata* subsp. *elata* (Egorova, 1999; Luceño and Jiménez-Mejías, 2008), *C. kurdica* materials contrasted with it in having utricles light green, whitish inside, and papillae only in the uppermost part of the utricle, whereas *C. elata* has greyish-green utricles, frequently purplish inside, and papillae on almost all the upper half of the utricle. Studied *C. acuta*-like materials from Mediterranean areas of the Balkans (Albania and Greece) distinctly shared those characters with the Asian *C. kurdica* plants. Additionally, plants from Maraş and Adana (Turkey), previously reported as *C. acuta* (Nilsson, 1985) were also reclassified as *C. kurdica*.

All distinct features are summarized in table 1.

The chromosomal number found in the studied Greek population was 2n = 74 (Fig. 1), far distinct from those reported from *C. acuta* (2n = 82 – 86).

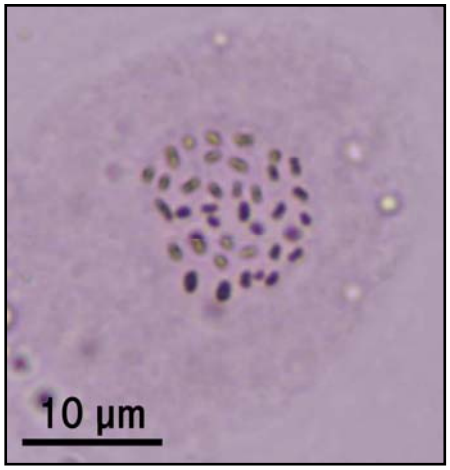

Fig. 1. Meiotic plate from the studied *C. kurdica* plant 264PV04 from Greece.

Discussion

TAXONOMIC REARRANGEMENT

On the light of the herbarium revision results, Mediterranean *C. acuta*-like materials are clearly revealed as a heterogeneous set of plants. In addition, the chromosomal number found in the studied Greek population was different from those reported from *C. acuta s.s.* Three distinct units can be identified: a broadly distributed, morphologically well-defined *C. acuta*, and two more geographically restricted taxa: *C. panormitana* in Sardinia, Sicily, and Tunisia; and *C. kurdica* in Albania, Greece and southern Turkey, extending towards the Middle East.

Carex kurdica is reported new for Europe, which means a widening of more than 2,000 kilometers to the west. *Carex parnomitana* is first cited from N Africa.

TYPIFICATION PROBLEMS IN C. KURDICA

Carex kurdica was described by Kükenthal from Turkish Kurdistan materials in a Handel-Mazzetti's catalogue of Middle East plants. Two different collector numbers were cited in the protologue: 2991 and 3007, but only the second one is kept at WU herbarium in Wien (Fig. 2). It also seems to be the single material studied by Kükenthal, as it can be deducted from a letter contained in the sheet: "Of the two forwarded Carices from Kurdistan nr. 2821 belongs to *Carex cilicica* Boiss. (...) Nr. 3007, which I have retained with your kind permission (...) has caused me much confusion (...) So I am forced to establish a new species: *Carex kurdistanica* Kük.". The other two authors that have studied this species, Nilsson and Kukkonen, neither found the sheet number 2991. Nilsson (1985) lectotypified over material number 3007 without any further reference to the another collection. Later, Kukkonen (1998) noted that problem and emphasized it despite two materials were cited in the protologue, he only found number 3007. The lectotype displays two not mature flowering stems. In spite of it, it was the material photographed and drawn together with the protologue. Additionally, the Latin diagnosis emphasized in the unripe character of the studied material: "*Utriculi squamas superantes, suberecti, sessiles, membranacei, plerumque steriles*". Seen the importance reported to ripe utricles for a correct *C. kurdica* determination against related plants, we

considered that an epitype is desirable to prevent problems with the identity of the species and to precise the application of the name (ICBN, Art. 9.7; McNeill et al. 2006). We propose the specimen IRAN 43618 from Iranian Kurdistan (45-50 km from Sanandaj to Tangi-Sar village, 1300 m, 31-5-2006, *Amini Rad*) as the epitype. (isoepitype UPOS 4737; Fig. 3) Specimens from this exsiccate show all the morphological characters to allow us to discriminate *C. kurdica*: from the basal leaf-sheaths to ripe utricles. In addition, its identity has been confirmed by molecular analysis (unpublished data).

Table 1. Main diagnosis morphological characters to distinguish among *C. acuta*, *C. kurdica* and *C. panormitana*.

Character	***C. acuta***	***C. kurdica***	***C. panormitana***
Basal sheaths	Fertile stem with basal sheaths with lamina, brown; leafless scale-like basal sheaths only in sterile shoots, brown, more or less coriaceous.	Fertile and sterile stem with weak basal sheaths straw-coloured to brownish, the outermost leafless, scale-like, enlarged, sometimes disintegrating and absent.	Fertile stems without basal sheaths, but covered with the remains of old leaves; leafless scale-like basal sheaths only in sterile shoots; brown or reddish-brown, papiraceous, easily disintegrating.
Female glumes	Dark-brown to purplish-black, obtuse, sometimes enrolled at the top and apparently acute, without hialine margin.	Dark-brown to purplish-black, acute to subacute, without hialine margin.	Dark to purplish-brown, acute to rounded, with a conspicuous wide hyaline margin.
Utricle	Apparently shorter or as long as the glumes, biconvex, a bit inflated, yellowish to greenish-brown, minutely papillose on most of the surface, abruptly contracted into a short beak.	Apparently longer than the glumes, plano-convex, ligth greenish, whitish above, smooth, papillose towards the appex, rarely on the whole upper half, gradually attenuated into a whitish beak.	Apparently longer than the glumes, narrowly binconvex, greenish to yellowish, smooth, sometimes with few papillae toward the apex, abruptly contacted into a short beak.

Fig. 2. Lectotype of *C. kurdica* Hand.-Mazz. (*Handel-Mazzetti 3007*) kept in WU.

Fig. 3. Isoepitype of *C. kurdica* Hand.-Mazz. (UPOS 4737).

IDENTIFICATION KEY, DESCRIPTIONS AND NOMENCLATURAL SURVEY

The following identification key lets us distinguish among the species of *Carex* sect. *Phacocystis* in S Europe (Mediterranean basin, Iberian and Balkan peninsulas), N Africa and SW Asia (north to Anatolia and South Caucasus)

1. Utricles nerveless or faintly nerved 2

1. Utricles conspicuously nerved 5

2. Basal sheaths scale-like, elongated, reddish-brown, keeled, splitting into a ladder-fibrosille structure; female spikes (2.5)4-8(10) cm long ..... ***C. buekii***
(E & C Europe, east to W Kazakhstan, Caucasus; absent from most of the S Balkan peninsula; local in SW Anatolia)

2. Basal sheaths different, if scale-like and reddish then female spikes generally shorter than 2.5(3) cm 3

3. Plants tussock-forming; basal sheaths scale-like, dark-purplish to blackish ..... ***C. cespitosa***
(Eurasia; scattered through C and N Europe, rare towards the S: N Spain, France Massif Central and SE Bulgaria, doubtful in the SE Alps; NE Anatolia)

3. Plants with creeping rhizomes or sometimes forming loose tussocks; basal sheaths scale-like or with lamina, reddish, brownish or straw-coloured 4

4. Utricles orbicular to suborbicular, abruptly contracted into a beak, sometimes slightly inflated-biconvex and then horizontally patent; leaves not rigid; basal scale-like sheaths papyraceous, sometimes absent ..... ***C. orbicularis*** subsp. ***kotschyana***
(Mountains of SW Asia, from E Turkey to W Afghanistan)

4. Utricles ovate to elliptical, gradually contracted into a beak, never inflated-biconvex, obliquely patent; leaves rigid; basal scale-like sheaths strongly coriaceou ..... ***C. bigelowii*** subsp. ***dacica***
(N Europe including British Isles; Carpathians; E Alps)

5. Plants tussock-forming; basal sheaths leafless, keeled, yellowish to light-brownish; utricles greyish-green, elliptical to ovate, sometimes with the inner side conspicuously purplish, more or less attenuated into a beak; lowest bract generally shorter than the inflorescence ..... ***C. elata*** subsp. ***elata***

(Europe, eastward to 30º E; NW Africa; Anatolia, W Caucasus; local in W Iran, Marivan)

5. Without the above combination of characters 6

6. Leaves rigid, canaliculate, involute; utricles greyish-green; plants with long creeping rhizomes ..... ***C. trinervis***

(European Atlantic coasts, from Denmark to SW Spain; extinct in the British Isles)

6. Leaves flat or plicate, not rigid; utricles never greyish-green; plants tussock-forming or with creeping-rhizomes 7

7. Leaves epi- or amphistomatic; utricles plano-convex, papillose at least on the upper half; lowest bract shorter or as long as the inflorescence ..... ***C. nigra***

(N & C Europe, also in mountain ranges from S Europe, Corsica and Sicily; NW Africa; Anatolia; Caucasus and adjacent mountains; NE North America; scattered through Siberia)

7. Leaves hipostomatic, utricles different; lowest bract usually longer than the inflorescence 8

8. Utricles biconvex, yellowish to greenish-brown, abruptly contracted into a 0.1-0.3 beak, shorter or as long as the glumes, papillose over most of the surface; scale-like basal sheaths absent from flowering stems, those present brownish and with a well-developed lamina ..... ***C. acuta***

(Most of Europe except E and S Iberia, Mediterranean islands and Greece; N Anatolia; Syria, SE Caucasus; scattered through central and eastern Russian Federation)

8. Utricles plano-convex, generally greenish or yellowish-green, gradually attenuated or abruptly contracted into a (0.1)0.2-0.5 beak, generally longer than the glumes, not papillose or only in the upper half; scale-like sheaths present or not in flowering stems 9

9. Leaves 6-11 mm wide; lowest female spike pendent, 8.5-17.3 cm × 3.5-6 mm; utricles papillose on the upper half ..... ***C. randalpina***

(Regions surrounding E Alps: Austria, Croatia, Germany, Hungary, Italy and Slovenia)

9. Leaves up to 5 mm wide; lowest female spike erect or pendent, typically smaller; utricles smooth or papillose only on the beak 10

10. Male and female glumes with a broad hyaline margin; utricles smooth ..... ***C. panormitana***

(Sardinia, Sicily and N Tunisia)

10. Glumes without hyaline margin; utricles smooth or papillose at the top 11

11. Utricle beak whitish, frequently papillose; scale-like basal sheaths straw-coloured to brownish, sometimes elongated.....***C. kurdica***
(S Albania, N Greece, SE Anatolia, Mesopotamia, NW Iran and NW Afghanistan; probably also in mountains of Lebanon and Palestine)

11. Utricle beak not whitish, smooth, rarely whitish and papillose; scale-like basal sheaths orange-brownish to reddish-brown.....(*C. reuteriana*) 12.

12. 1(2) upper spikes male, lower spikes generally completely female or 0-3(6) androgynous; utricles 1.8-3(-3.5) mm long..... ***C. reuteriana*** subsp. ***reuteriana***
(NW and C Iberian Peninsula)

12. (1)2-4 upper spikes male, all lower spikes androgynous; utricles (1.8)2.3-4.5 mm long.....***C. reuteriana*** subsp. ***mauritanica***
(S Iberian Peninsula, Morocco, Algeria)

1. Carex kurdica Kük. ex Hand.-Mazz., Ann. K. K. Naturhist. Hofmus. 28: 23 (1914).
Ind. Loc.: "Am Ufer des Bohtan unterhalb Sert (Nr. 2991) und des Tigris von dort abwärts (Nr. 3007) bis Finik ober Dschesiret-ibm-Omar sehr verbreitet, grossem in den Fluss hineinwachsende Horste bildend, 500-650 m, 17.u.18./VIII.1910. [On the banks of Bohtan below Sert (Nr. 2991) and from there down the Tigris (Nr. 3007) to Finik upper Dschesiret-ibm-Omar, very common, large, forming tussocks growing into the river].
Lectotype: designated by Nilsson in Davies (ed.) Fl. of Turkey, 9: 155 (1985): "Kurdistania media: ad ripam tigridis in saltu inter Sert (Siirt) et Drcheriset-ibn-Omar (Cizre), ca. 500 m, 18 viii 1910, Handel-Mazzetti 3007" (WU!; Fig. 2)
Epitype: (**designated here**): Iran, Kurdestan, 45-50km from Sanandaj to Tangi-Sar village, 1300 m, 31-5-2006, *Amini Rad* (IRAN 43618); isoepitype: UPOS 47347 (Fig. 3).

Tussock-forming plant. **Stems** 40-100 cm long, 0.9-1.6 mm wide below the inflorescence, sharply trigonous, smooth or scabrous above. **Basal sheaths** 2-5 cm, leafless, the outermost scale-like, enlarged, from straw-colored to brownish, weak and easily disintegrated, sometimes totally absent from flowering stems. **Leaves** green, as

long as or a bit shorter than the stems, 2.8-5.4(6) mm wide, hypostomatic, flat, smooth or antrorsely scabrous towards the apex; ligule 2-3 cm, twice as long as wide. **Inflorescence** 6.5-23(26) cm long, with 1-3 upper male spikes and 2-4 lower female spikes, rarely with an intermediate androgynous spike between the male and female ones, overlapping at the top of the stem, sometimes the lowest separated, shortly pedunculated, erect. **Lowest bract** 4.5-22(25) cm × 1-4 mm wide, frequently equalling the inflorescence, sometimes a bit shorter, sheathless, with hyaline or light-brown auricles, 1.1-3(4) mm long, at the base; the second bract 0.3-4.5 cm × 0.2-1.8 mm, generally as long as the spike, leaf-like or setaceous, also with well-developed auricles at the base, generally hyaline or light-brown. **Male spikes** 1.5-5 cm × 2.5-6 mm, fusiform to cylindrical, with a 0.4-2.5(5) cm peduncle. **Female spikes** 2-7.2 cm × 3-5 mm, with approximately 90-200 flowers, cylindrical, dense or attenuated towards the base, the lowermost with a 0.5-3 cm peduncle, sometimes sessile. **Male glumes** (2.7)3-5.1 × 0.7-1.4 mm, narrowly obovate to oblong, truncate to rounded at apex, light or dark brown or reddish-brown, with the middle nerve lighter. **Female glumes** 2.2-3.5 ×0.8-1.5(1.6) mm, ovate to elliptical, acute to subacute, with a single middle nerve, frequently the lowermost 3-nerved, dark-brown to purplish-black. **Utricles** 2.3-3.2 × 1.3-2.2 mm, exceeding the glumes, ovate to elliptical, plano-convex, with 2-7 nerves, light greenish, becoming whitish above, smooth, with papillae towards the apex, sometimes in the whole upper half, gradually attenuated into a 0.2-0.5 mm beak, cylindrical, truncate, papillose, whitish, stipeless or with an inconspicuous 0.1 mm stipe. Stigmata 2. Nut 1.3-2.3 × 1-2 mm, ovate to broadly elliptical, narrowly biconvex.

Distribution: AFG, ALB, GRC, IRQ, IRN, LBS?, PAL?, TUR. Irano-Turanian element, also present in coastal regions of the NE Mediterranean.
Ecology: Streamsides and swampy meadows. 500-1600 m.
Observations: *Carex acuta* materials cited from Lebanon (Kükenthal, 1909; sub "*C. gracilis* var. *libanotica* Kük") and Palestine (Post, 1933; sub "*C. rufa* (L.) Simonk") were not revised. Further revisions would be desirable to confirm the identity of such plants.

2. Carex panormitana Guss., Fl. Sicul. Syn. 2: 575 (1844).

Ind. Loc.: "Ad fluviorum ripas; *Palermo al fiume Oreto*."

Lectotype: designated by Arrigoni in Bollettino della Societa. Sarda di Scienze Naturali (1984): "18. *Carex panormitana*, Martio, Aprili; Marzo, Fiume Oreto" (NAP, photo!)

Tussock-forming plant. **Stems** up to 1 m long, 1-2 mm wide below the inflorescence, trigonous, smooth, rarely scabrous above. **Basal sheaths** mostly absent from flowering stems, which are covered by the remains of old leaves; leafless sheaths in sterile shoots, scale-like, brown or reddish-brown, papiraceous, weak, easily disintegrating and disappearing. **Leaves** green, as long as or a bit shorter than the stems, 2.5-5 mm wide, hypostomatic, flat, generally smooth; ligule up to 2 cm, generally as long as wide, rounded to truncate. **Inflorescence** 8-25 cm long, with 1-2 upper male spikes, 0-1 intermediate androgynous spikes and 2-4(5) lower female spikes, generally not or scarcely overlapped, erect, rarely the lowest patent. **Lowest bract** 6-30 cm × 1.5-4.5 mm, as long as or longer than the inflorescence, sheathless, without auricles or with hyaline or light-brown scarcely developed auricles at the base, rarely with dark-brown auricles; the second bract (3)4.7-15 cm × 0.5-3.5(4) mm, leaf-like or setaceous, generally not reaching the top of the inflorescence, sometimes with well-developed dark-brown auricles. **Male spikes** (1)2-7 cm × 2-5 mm, cylindrical to fusiform, with a 1-3(3.3) cm peduncle. **Female spikes** 2.5-10(11.5) cm × 3-6 mm, with approximately 50-200 flowers, cylindrical, dense or attenuated towards the base, the lowermost with a 5-20 mm peduncle. **Male glumes** 3-6.5 × 1-1.4 mm, elliptical to oblong, rounded at apex, brown to reddish-brown, with the middle nerve lighter and a conspicuous hyaline margin, wider towards the apex. **Female glumes** 3-4 × 0.7-1 mm, elliptical to oblong, acute to rounded at apex, with a single middle nerve, sometimes the lowermost 3-nerved, dark-brown to purplish-brown, with the middle nerve lighter and a wide hyaline margin. **Utricles** 2.3-3.6 × 1.3-1.7, exceeding the glumes, ovate to elliptical, narrowly biconvex, with (2)4-8 nerves scarcely marked, greenish to yellowish, smooth, sometimes with few papillae towards the apex, abruptly contracted into a 0.1-0.4 mm beak, cylindrical, truncate, smooth, with a up to 0.3 mm stipe, sometimes stipeless. Stigmata 2. Nut 1.4-2.2 × 1.3-1.4 mm, broadly elliptical, narrowly biconvex.

Distribution: SAR, SIC, TUN. Tyrrhenian sub-endemic, with a local occurrence in N Africa (N Tunisia).

Ecology: Streamsides. 0-100 m.

Observations: Extremely local in Sicily, where it is only known from the type parish at Oreto river, nowadays within an urban sector of Palermo city; listed as CR by IUCN and recorded in the Italian Red List (Pignatti *et al.*, 2001).

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Appendix 1

List of materials studied.

***CAREX ACUTA*: ALBANIA**. Çajup, Mali Lumxheriës, 4000 ft. 12.7.1933, *A.H.G. Alston & N.Y. Sandwith 2249* (BM, K). **BOSNIA-HERZEGOVINA**. Prope opp. Tuzla, VI.1940, *N. Muravjov* (BEO) **BULGARIA.** Mündung des Kamtschia-Flusses, am Flussufer, 21.V.1966, *G.Langer* (M); Zapadni Rodopi: pokraĭ blattsata ya Longurlii, 9.VII.1930, *D. Ĭordanov & D. Ahtarov* (SO); Močurpivi livadi nado t gr. Devin. Centralin Rodopi, 1.VIII.1940, *D. Ĭordanov* (SO); Zablatenite mesta pokraĭ ž.p. linija meždu Sofija i gara Iekŭr, 11.VI.1930, *D. Ĭordanov* (SO); vlažini livadi kraĭ rekata v mest. Kastrakliĭski livadi, Devinsko; 3.VIII.2006 (SOM); Palakarija, Potočeta v Livada yuzno ot s. Široki dol, 29.VI.2002, *A. Petrova* (SOM); Vlažna livada v dolinata na r. Iskŭp, severno ot gr. Samokov, 15.VI.2002, A. Petrova (SOM); Tabachusko ezero, Kraĭ ezeroto, planinski stedi, 31.VIII.1960, V. Velčev & I. Boldev (SOM); NW of Dospat, on the western side of the dam, hay meadows in openings of coniferous forest, c. 1200 m., 41º42' N 24º02' E, 24.VII.1997, *E. Bergmeier et al.* (SOM); in fossis uliginosis agri Samokovensi, Tatarsky Brod ad lacum Kalkovo, 5.VI.1912, *D. Davidoff* (SOM). **IRAN.** North of Sanandaj, 1.5 km after Sarab-ghamish village, 1750 m, 26.V.2004, *Amini Rad* (IRAN). **ITALY**. **Liguria.** Apennino di Praggia, VI.1843, *G. De Notaris* (GDOR). **Veneto.** Padova, Venezia, *A. Fiori* (FI); Battaglia, Venezia, 14.V.1896, *L. Vaccari* (FI); Estuario Veneto, Cavallino, Treporti, Liso, Torcello, 26.IV.1940, *M. Minio* (FI). **Friuli-Venezia-Giulia.** Istria, Lamischie (FI). **Abruzzo.** Rivisondoli, L'Aquila, località Pantaniello, in un acquitrino, 1300 m, *G. Pirone* (FI). **Lazio.** Viterbo, Centrale di Traponzo, depositi aluviali di sponda del F. Marta, 300-350 m, *A. Ottoveggio* (FI). **Calabria.** Cosenza, Sila Grande, c. 6 km ENE of Camigliatello Silano, southern end of Lago Cecita, close to bridge of SS 177, 1140 m., 13.VI.1997, *Optima Iter VIII 1452* (RNG); Parque Nacional della Sila, lago Cecita, puente de la SS177 desde Camigliatello Silano, 1140 m., orillas arenosas, 24.VII.2009, *P. Jiménez Mejías & B. Peceño 122PJM09* (UPOS); Sila, 20 km W of San Giovanni in Fiore, Lagos Arvo, to the W of Lorica, 1280 m, *D. Davis & S. Sutton D65452* (BM); La Sila, Calabria, La Scurca (SS107 km 91), 1300 m (FI); La Sila, Piana di Cecita, 1050 m, 15.V.1950, *G. Sarfatti & R. Conradi* (FI); La Sila, Molarotta, Stazione Alpeggio piana del Mucone, 1130 m, *G.*

Negri et al. (FI). **MONTENEGRO**. Durmitor, Zmijmije jezero, in turfosis, VIII.1937, *V. Blečić* (BEO); Vražje jezero, 3.VIII.1912, *N. Ko*šanin (BEOU); Durmitor, Pašina voda, 1400 m., 23.VII.1992, *D.L. 389.92* (BEOU); Durmitor Žugića Bare, 1400 m., 16.VII.1994, *V. & N. Stevanović* (BEOU); Durmitor, Riblje jezero, 2.VII.1989, *V. Stevanović & S. Jovanović* (BEOU); Biogradska gora, mrtva šuma, 6.VII.1989, *V. Stevanović & S. Jovanović* (BEOU). **SERBIA**. Vlasina, 23.VI.1947, *D. Milovanović* (BEO); Vlasinsko Jezero, S shores, 1200 m aprox., 20.VI.2010, *P. Jiménez-Mejías 67PJM10* (BEO, UPOS); Derdapska Klisura, Pecka Bara-Trajanov put, pored Dunava, 20.X.1965, *N. Diklić* (BEO); Obrenovac, močvarna mesta u lokalitetu Zabran, aluvium reke Save, 12.VIII.1953, *Ž. Adamović* (BEO); Dunav, Ada Capljinac kod Velikog Sela močvare, 7.X.1996, *V. Stevanović* (BEOU). **SYRIA**. inter Aleppo et Acutab, 1865, *C. Haussknecht* (BM, K). **TURKEY**. **Erzurum**. Palandöken Da., 25 km from Çat to Erzurum, 2350 m, 27.VII.1966, *Davis 47392* (E); Kop Dag Pass, alpine meadows, mashy places, 8000 ft., 9.VIII.1962, *P. Furse 3747* (K). **Ağri**. 15 km from Eleşkirt to Horasan, E of Tahir pass, 2200 m, 24.VII.1966, *Davis 47119* (E); 3 km E of Taşliçay, 1700 m, water meadows of Murat Çay, 24.V.1966, *Davis 43532* (E); d.Suluçem, Musun, S end of Balik G. 2300 m, 23.VII.1966, *Davis 47064* (E K). **Bolu**. Abant, 1350 m, 27.VI.1975, *A. Baytop* (K). **Kars**. Haçuvan (Kars-Ardahan), 1900 m, 30.VI.1957 (BM E K); 10 km from Kağizman on the Kars-Iğdir road, 43º 8' E 40º 10` N, 2.VII.1963, *N. Jardine 550B* (E); Susuz, 1750 m, 5.VII.1957, *Davis & Hedge D30644* (BM E K); Sarikamiş, 2100 m, 7.VII.1957, *Davis & Hedge D30774* (BM E K); Mountains E of Kağizman, N side of bass between Akçay and Cumaçay, 2300 m, 17.VII.1966 (E).

***CAREX KURDICA*: ALBANIA**. Tepalenë, Uji, vid floden Drino, 6.VI.1983, *C.E. Sonck* (RNG). **GREECE**. Epirus, fuentes del Aqueronte, 11.VII.2004, *P. Vargas 264PV04* (UPOS); Pisoderion, 4200 ft., wet upland meadow in valley below the village, 12.VI.1932, *A.H.G. Alston & N.Y. Sandwith 809* (K). **IRAQ**. Gali'Ali Bey, 18.VI.1961, *R.W. Haines* (E); Gali Ali Bey region, 6.VII.1971, *S. Omar et al. 38336* (K). **IRAN.** 15 miles SE of Mahabad, damp hay meadow, 5000 ft., 21.V.1962, *P. Furse 2178* (IRAN); Kurdestan, 45-50 km from Sanandaj to Tangi-Sar village, 1300 m, 31.V.2006, *Amini Rad* (epitype materials; IRAN UPOS); Kurdestan, Sanandaj, 5 km after Sarab-ghamish village, 28.V.2006, *Amini Rad* (IRAN UPOS); Kurdestan, Sanandaj, 32 km after Sarab-ghamish village, 28.V.2006, *Amini Rad* (IRAN UPOS); Kurdestan, 18-20 km from

Marivan to Paveh, 30.V.2006, *Amini Rad* (IRAN UPOS); Kurdistan, 75 km WNW of Sanandaj towards Marivan, valley of the Gul-i-Chida, 1830 m, 18.V.1966, *J.C. Archibald 1988* (E K). **TURKEY. Adana**. Feke, Goksu gorge below Himmetli, 700-800 m, 9.VII.1952, *Davis 19860* (E K); **Hakkari**. 40 km from Yüksekova to Semdinli, 1600 m, 15.VI.1966, *Davis 45108* (E K); **Maraş**. Güksun, Çardak, near Findik. 1300 m, 6.V.1957, *Davis & Hedge 27615* (BM E).

***CAREX PANORMITANA*: SICILY.** Ad fluviorum ripas, Palermo al fiume Oreto. Martio, *Todaro 1114* (BEOU, BM, FI, K); Palermo al fiume Oreto, "*from Gussone, 1846*" [F Boot's handwritting] (K); ad ripas huminis Orethi prope Panormun, "*from Parlatore, 1847*" [F. Boot's handwritting] (K); Ponte della Gratia ad flumen Oreto, 20.V.1855, *E. & A. Huet du Pavillion* (BM FI K); Ad ripas Fl. Orethi prope Panormum, 1844, *Herbarium Parlatoreanum* (FI); Palermo, *Erbario O. Beccario* (FI). **SARDINIA.** Sasfari à Scala di Giocca, V.V.1895, *U. Marteilli* (FI). **TUNISIA.** Lit de l'oued Bata prés El-Fedja, 11.V.1888, *Cosson* (FI, G, MPU).

CAPÍTULO 9

Carex cespitosa L. distribution reappraised in W Europe (South of Scandinavia)

P. Jiménez-Mejías, K. A. Lye, S. Martín-Bravo & M. Luceño

Abstract

Carex cespitosa L. is a sect. *Phacocystis* member distributed across the Palearctic from Iberian Peninsula to Japan. In Europe, it has been widely reported from most of the countries of the central and northern parts of the continent. Previous works have already stated that some reports were confusions. Here, the distribution of *C. cespitosa* is reappraised based in reliable literature records and herbarium specimens. As a result, the plant has been found to be more scattered and rare than supposed. Its presence in Italy is discussed. The hybrids with other related species are briefly commented.

Keywords. Chorology, herbarium revision, scattered distribution, taxonomic confusion.

Introduction

Carex cespitosa L. is a sect. *Phacocystis* Dumort member, one of the biggest sections of *Carex* with approximately 90 spp. (Dragon and Barrington, 2009). Taxa belonging to sect. *Phacocystis* are morphologically characterized by oblong to cylindrical female spikes, two stigmas and lenticular, frequently papillose, and short-beaked utricles (Chater, 1980; Egorova, 1999; Luceño and Jiménez-Mejías, 2008).
Many biosystematic works have contributed to unravel the intricate relationships among the members within this group (Sylvén, 1963; Faulkner, 1972; Cayouette and Morisset, 1985; Standley, 1987; Luceño and Aedo, 1994; Stoeva *et al.*, 2005; Wallnöfer, 2006; Nakamatte and Lye, 2007; Dean and Ashton, 2008; Dragon and Barrintong, 2008; among others). Despite it is one of the most intensively studied *Carex* groups, it is also among the most taxonomically complicated ones due to the ability of hybridize of the different taxa and the faint morphological boundaries among them.

The typus of the sect. *Phacocystis* is just *C. cespitosa* L. (Egorova, 1999). This species is widely distributed across the Palearctic, from N Iberian Peninsula (Jiménez-Mejías *et al.*, 2007) to Japan [subsp. *minuta* (Franch.) Worosch; Ohwi, 1965]. Distribution of *Carex cespitosa* L. in Europe has been strongly obscured mainly due to: 1- the early use of the Linnaean binomen to widely refer tussock-forming sect. *Phacocystis* plants, and 2- the intricate taxonomy of the section, which has leaded to many taxonomical rearrangements, nomenclatural changes, and misidentifications through the history of botany. After a wide herbarium revision work and an extensive bibliographic research, distribution of *Carex cespitosa* L. in Western Europe is reappraised and its conservation status briefly commented.

Results and discussion

DISTRIBUTION

Distribution of *C. cespitosa* in Europe based on herbarium records and reliable bibliographic data is showed in Fig. 1.

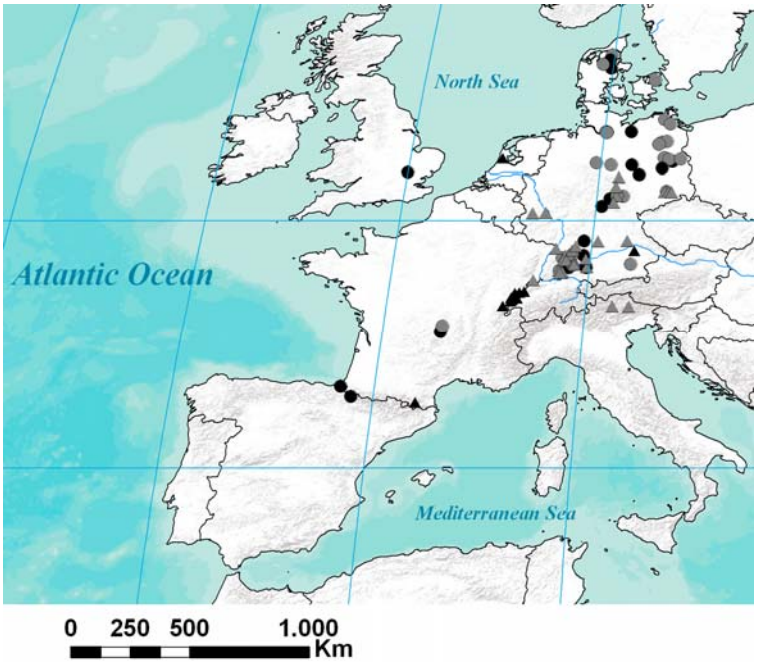

Figure 1. *Carex cespitosa* distribution in western Europe south of Scandinavia (west of Poland, Czech Republic, Austria and Slovenia). Triangles represent reliable bibliographic references, circles revised herbarium vouchers; grey shapes indicate data before 1969, black shapes indicate data from and after 1970.

- Denmark

Carex cespitosa was known from numerous localities through most of Danish mainland (including North Jutlandic Island) and Baltic territories (Funen, Zealand and Bornholm) (Winstedt 1943, Hultén, 1950, but due to agricultural expansion it is now becoming rare (Løjtnant & Worsøe 1993). Nowadays, there are only 6 remaining localities in Zealand and 7 in Jutland (cf. *Naturbasen*, at http://www.fugleognatur.dk, Naturhistorisk Museum Århus, last visited 23-Aug-2011). *Carex cespitosa* is listed as LC (least concern) in the Danish Red List (DMU, 2007).

Specimens studied: **Central Denmark**: 1 km s of Pøt Mølle, near Hammel, 18.VI.1979, *I.Nielsen & al. 831* (L, MA, SANT). **North Denmark**: Ilsø NE of Arden, S of Aalborg, 9.VI.1967, *K.Larsen & S.Laegaard 296* (L, MA, SANT); meadow near Bramslev east of Hobro. 2.VI.1980, *J.C.Schou* (BASBG); east of Hobro, 20 m, 56º 37' 59'' N 9º 49' 18'' E, 14.IX.2005, *K. A. Lye 29306* (O). **Southern Denmark:** Sønderso**,** 9.VI.1967, *T. Sørensen* (LISU). **Zealand**: Selandia bor. in palude silvae Jonstrupvang, 28.VIII.1903, *Lorenten* (G)

- France

Carex cespitosa has attracted the attention of French botanists due to its scarceness, and its distribution and conservation status is really well-studied, documented and brought it up to date. It is distributed through the mountain systems of the Jura, Massif Central and Pyrenees, in the regions of Alsace, Franche-Comté, Auvergne and Eastern Pyrenees (reviewed in Olivier *et al*., 1995; Duhamel, 2004). It appears to be relatively frequent at least in the Massif Central (Tort, 1988; Olivier *et al.*, 1995; CHLORIS, 2010) and not too rare in Franche-Comté (Gillet *et al.*, 1980; Prost, 1992; Cosson and Morcrette, 1999; Ferrez and Guyonneau, 2005). However, it is known a single location in both Eastern Pyrenees (Villeneuve; cf. Terrisse, 1994) and Alsace (étang d'Hirsingue; cf. Issler *et al*. 1965; Schultze-Motel, 1968).

It is protected in French territory as VU (Vulnerable)

Specimens studied: **Auvergne**: Besse, megaphorbie, bords du chemin carrossable condusant au lac de Montcineyre, 11.VII.1971, *G.Bosc* (BBF); Puy-de-Dôme, lac de Montcineyre, 3.VII.1962, *Gaulle* (MA); Puy-de-Dôme, Narse d'Espinasse, près Randanne, 999 m., 10.VII.1934, *d'Alleizette 7340* (B, G, MA); Puy-de-Dôme, dans le marais dit "Narse d'Espinasse", commune de Saulzet-le-Froid, 1000 m., 11.VI.1994, *E.Grenier* (B).

- Germany

Despite *C. cespitosa* is widely scattered through all the German territory, Schultze-Motel (1968) clearly noted that it is only relatively common in the N and the SW (Baden-Württemberg). In the northern half of the country, *C. cespitosa* is more or less scattered in peat-bogs from Schleswig-Holstein and Mecklenburg-West Pomerania. From Central Germany to the South, this plant can be found mainly in the upper Rhine and Danube basins (Black Forest, Eastern Jura, SW Bavaria), middle Elbe river and Harz mountains.

Specimens studied: **Baden-Württemberg.** Alpenvorland. Ummendorfer Ried, Westrand, mindestens 10 Horste in Feuchtwiese zwischen Randweg und BachBaar, 4.VI.1996, *O.Sebald 12737*; Naturschutzgebiet Birkenried zwischen Geisingen und

Pfohren, nasse Wiese am Rand, 2.VI.1967, *O.Sebald 1620* (STU); Bärenthal, bei Sportplatz, zahlreich, 10.VII.1980, *O.Sebald 7521* (STU); auf Torfwiesen im Juragebiet des Hochplateaus der badischen Baar zw. Kirchen und Aulfingen, 675 m, 47º55' N 26º18'E, 4.VI.1895, *Schatz 16* (B, BASBG, BREM, STU, W); 2 km W Wiesensteig, an der Fils, 5 Horste, 29.6.1984, *O.Sebald 8771* (STU); Schönbuch, nasse Waldwiese nördlich Breitenholz, Brentenhau, 480 m, 18.V.1965, *O.Sebald* (STU); Torfgrube bei Schopfloch, 1932, *K.Müller* (STU); Schwäbische Alb, Blatt Nendingen, zahlreich in nassen Wiesen im Bäratal oberhalb Ensisheim, 690 m, 16.V.1967, *O.Sebald 1550* (STU); Wiese 1 km östl. Mittelsteinbach, 14.V.1970, *O.Sebald 3186* (STU); Schopflocher Torfgrube, 6.V.1951, *Leidolf* (STU). **Bavaria**. Wet meadow 1 km southwest of the Fitzendorf, 6.VI.1998, *K.Kiffe* (B); Isental, Wasentegernbach W, Talsohle, 435 m., 20.V.1961 (M); Königsberg, 12.VI.1873 (BREM). **Berlin**. Forsthaus Fahlenberg am Gosener Kanal, 8.VI.1975, *D.Benkert* (B); Jungfernheide, 25.V.1901, *W.Lackowitz* (B); Jungfernheide, 19.VI.1901, *F.Woller* (MA); Karlshorst, in Walde, 8.VI.1910, *R.Gross* (B); Möchernitz sudlich von Spandauer Schiffahrlskanal 28.IV.1895, *R.Schulz* (B); Spandauer Stadtheide, 15.VI.1901, *P.F.F.Schulz* (B). **Brandenburg.** Fürstenberg, Boberow-See, 1960, *H.Freitag* (JE); Lange-Damm-Wiesen nahe Hennickendorf, Sumpfwiese zwischen Hügel 1 und 4, 10.V.1974, *G.Stohr* (B); Lychen, in allen Wiesen, 25.V.1889, *Heiland* (B); Maxsee bei Müncheberg, in feuchter Wiese, 6.VI.1936 *G.Krumbholz 1936/19* (B); Rheinsberg, auf einer sumpfwiese nördlich von Möckern, 19.V.1902, *R.Schulz* (B); **Hamburg**. Bramfeld, VI.1891 *Fritsche* (L); Farmsen, 10.VI.1891, *J.Schmidt* (JE); Wandsbeck, VI.1892, *Pram* (JE). **Lower Saxony**. Braunschweig, 1.VI.1891, *H.Kuehn* (CHE); Hannover, auf der Döhrener Masch, *Jahn* (JE); Verden, Westen, 18.V.1933, *Th.König* (BREM) **Mecklenburg-West Pomerania**. Grimmen, 6.VI.1917, *E. Schenz* (L; Kreis Lübz, Nasse ungepflegte Wiese vor dem Quassliner Moor bei der Quassliner Mühle, 24.VI.1980, *I.Heinrich* (B)); Peenetal, Gützkow, VI.1956, *J.Bissé* (JE). **North Rhine-Westfalia**. Dusseldorff, 1893? (BREM). **Saxony**. Erfurt, Neu-Schmidtsleds, V.1884, *Reimerkes* (JE); Hohenstein, Am Pechgraben, VI.1894 (JE); Meiningen, zwischen Herpf un Bettenhausen, 28.VI.1972, *L.Meinunger* (JE); Weimar, 24.VI.1924, *W.Rothmaler* (JE). **Saxony-Anhalt**. Magdeburg, Aken, feuchter Erlen-Birken-Bruch, ca. 2 km nordöstlich Aken, 27.V.1984, *H.J.Zündorf 4573* (JE); Wolmirstedt, Rogätz, wiese von dem NSG "Rogätzer Hang", 26.VI.1979, *W.Westhus* (JE). **Thuringia**. Gotha,

nasswiese in einer schleife des Kleinen Leinakanals ca. 800 m nördlich Wipperoda, 12.V.1992, *H.J.Zündorf 10635* (JE).

- Great Britain

Carex cespitosa was definitely excluded from the account of Cyperaceae for the British Isles by Jermy *et al.* (2007), who attributed all previous British records to misidentifications. However, herbarium revision work in the Natural History Museum of London (BM) revealed a reliable herbarium record of this species for the British Isles: "England, Hertfordshire, near West Mill". The voucher studied specimen contained a typical flowering stem and two vegetative shoots of *C. cespitosa*, together with other two young flowering stems. These two latter seem to be more similar to *C. elata* All., a relatively common species in the British Isles. This fact would have made the correct identification of the *C. cespitosa* samples even more difficult.

The locality was visited during 2010 and the plant was unsuccessfully searched. However, a new population of about 300 individuals was found around 2.5 kilometres to the South from the original record in a spring-fed mire (T. James, pers. comm.). A research work performed by J.G. Dony (1977) about changes in the flora of the neighbor county of Bedfordshire early informed about the decrease of marsh and water meadows environments; in this paper, he considered up to eleven Cyperaceae species as currently extinc, mainly due to land drainage. The re-discovering of living specimens of *C. cespitosa* is a lucky chance and great news for the British Flora.

Specimens studied: **England**, Hertfordshire, near West Mill, near Buntingford; 26.V.1960, *J.G. Dony* 3815 (BM 939135); Hertfordshire, Braughing (Hamel's) Meads, spring-fed mire beneaht a steep, wooded hill overlooking the flood plain of the River Rib, 10.V.2011, *T.J. James* (UPOS).

- Italy

Carex cespitosa was recorded from Italy in Flora Europaea (Chater, 1980) and Flora d'Italia (Pignatti, 1982). In the most recent Checklist of Italian plants (Conti *et al*., 2005) *C. cespitosa* is reported from the regions of Piedmont, Lombardy, Friuli-Venezia Giulia and Liguria, although its presence in Piedmont has been recently refused

(Jiménez-Mejías and Selvaggi, 2011). Despite this alleged distribution, no herbarium specimens from Italian herbaria could be found in spite of the intensive herbarium search. The full collections of FI (Florence), RO (Roma) and TO (Torino) were checked in situ, and materials from BERG (Bergamo), BOLO (Bologna) and PA (Palermo) were studied too, with no positive results. Personal communications from the curators and staff from BOZ (Bolzano), GDOR, GE (Genova), MSNM (Milano), ROV (Rovereto) and UVV (Venezia) were also negative related to the finding of Italian voucher materials from *C. cespitosa* in their herbaria

The single reliable reference known from *C. cespitosa* in Italy is an old sheet from South Tyrol in W herbarium cited by Dr. B. Wallnöfer (2004): "Bolzano, com. P. Gredler", although he did not discard the possibility of label misplacement. Also Schultze-Motel (1968) in Flora von Mitteleuropa listed the Italian Tyrol for *C. cespitosa*: "Cadore, Ampezzo, Unter-Friaul".

Pignatti (1982) had already noted that the wrong early use of the binomen *C. cespitosa* could be a source of confusion and warned about some of the Italian records could be indeed misidentifications. In this sense, all the materials labeled as *C. cespitosa* that we had the chance of studying from the above cited Italian herbaria were other related sect. *Phacocystis* species. As Dr. Wallnöfer (2004) proposed for South Tyrol, we kindly suggest that newer vouchers would be needed to confirm the presence of this taxon in Italy, especially in the NE regions, since the populations closest to Italian territory are the stations from Austria and Slovenia (Schultze-Motel, 1968; Martinčič and Sušnik, 1984), the single countries where *C. cespitosa* inhabits the Alps.

This plant is not recorded in the Red and Blue List of the Italian Flora (Pignatti *et al.* 2001).

- Netherlands

The single current location known for *C. cespitosa* in the Netherlands are the meanders of river Hunze in North Holland, near to Spijkerboor (De Boer, 1974).

In the Red List of the Vascular plants of the Netherlands (Minister van Landbouw, Natuur en Voedselkwaliteit, 2004) *C. cespitosa* is listed under the category of *Gevoelig* ("sensitive").

Specimens studied: Spijkerboor, 8.VI.1973 *D. Bakker & D.T.E. van der Ploeg* (L).

- Spain

In the Iberian Peninsula, the presence of *C. cespitosa* was recently reported by Jiménez-Mejías & al. (2007) and recorded in *Flora Iberica* (Luceño & Jiménez Mejías, 2008) for a single location in the Pyrenees in the north-facing Bidasoa river, in Navarra region close to the French border. During herbarium revision, a second population has been found in Sarries, in Navarra region as well. This population is located in a south-facing valley from the river Ebro basin, placed 65 km from the river Bidasoa populations. Regarding the global distribution of *C. cespitosa*, Spanish populations are critically important since they constitute de westernmost limit of the distribution of this taxon.

Carex cespitosa is listed in the Red List of Spanish Vascular Flora (Moreno *et al*., 2008) as critically endangered (CR).

Specimens studied: **Navarra.** Lesaka, Zalain, orilla del Bidasoa, 20 msm, 43º 16' 25'' N 1º 43' 6'' W, 20.VI.1981, *I.Aizpuru & P.Catalán* (MA); Lesaka, Zalain, orilla del Bidasoa, 30 msm, 18.V.1983, *I.Aizpuru & P.Catalán* (ARAN, MA); Lesaka, Zalain, orilla del Bidasoa, 30 msm, 16.II.2006, 43º 16' 33'' N 1º 41' 49'' W, *P. Jiménez-Mejías & al.* (UPOS); Sarries, sauceda-aliseda, 1.VI.1986, *I.Aizpuru & P.Catalán* (SALA).

- Switzerland

Carex cespitosa has been recently found in the Jura in the cantons of Vaud and Neuchâtel (Cosson & Morcrette, 1999; Morcrette & al. 2002; Druart, 2004). As the authors of the re-discovering noted, *C. cespitosa* was previously recorded from Neuchâtel in the 19th century (Godet, 1853), but it was not finally considered within the Swiss flora (Welten and Sutter, 1982; Moser *et al.*, 2002). Populations from the Swiss Jura are associated to those from Franche-Comté (see France epigraph; Cosson and Morcrette, 1999), being all in the Upper Doubs basin and proximities.

During herbarium revision, we detected additional materials from Bern and Schaffhausen cantons, some of them from the late 20th century. Presence of *C. cespitosa* in these regions could be probable, since other populations from Swiss and German Jura are not remote. Given its rarity, it should be desirable to check the persistence of such populations.

The conservation status considered for *C. cespitosa* in Switzerland is VU (Druart, 2004).

Specimens studied: **Bern**. Fäggen, IV.1845, *Schatch* (ZT). **Schaffhausen**: Herblingen, 25.V.1971, *K. Isler-Hübscher* (ZT); Fulachtal, Herblingen, 11.VII.1979, *Kummer* (ZT).

HYBRIDS

In the area of study, the hybrids of *C. cespitosa* with other three species of the section have been detected:

- *C. cespitosa* × *C. acuta*
  Carex allolepis Rchb., Icon. Fl. Germ. Helv. 8: 233, f. 586 (1846)

Recorded doubtfully by Schultze-Motel (1968) from North Germany, and confirmed by Kiffe (2001a) from the South-West of the country. No specimens have been studied.

- *C. cespitosa* × *C. elata*
  Carex × *strictiformis* Almq. in C.J.Hartman, Handb. Skand. Fl., ed. 11: 469 (1879).
  = *Carex* × *frankii* Podp., Vestn. Klubu Prír. v Prostejove 10: 6 (1907 publ. 1908), *nom. illeg.*

A hybrid easily distinguishable from the parental taxa. It has been previously recorded from Germany (Schultze-Motel, 1968; Kiffe, 2001a,2001b). It is intermediate between the two parents, in both quantitative and qualitative characters: leaves

hipostomatic; basal sheaths enlarged, reddish, brown-reddish or yellowish-brown, sometimes with a ladder-fibrosille structure; male and female spikes shows intermediate length, generally longer than in *C. cespitosa* (20(30) mm); and utricles faintly nerved o nerveless, with the inner side reddish.

Specimens studied: *DENMARK*: **Zealand**. in palude silva Tievilde, VI.1887, *Th.Holm* (CHE). *FRANCE*: **Franche-Comté.** Marais du Sour paist Besançon, VI.1846, *Grenier* (K). *GERMANY*: **Bavaria**. Freising SW, 12.V.1947, *J.Höller* (M). **Berlin**. in der Möchernitz, 14.VI.1901, *M.Conrad* (B). **Mecklenburg-West Pomerania**. Kreis Nordwestmecklenburg, Neukloster, Fechtwiesenbrache am Ortsausgang südöstl. der Strasse nach Wismar ("Am Düsterberg"), 9.VI.1997, *K.Kiffe* (B). **Saxony-Anhalt**. Krs. Stendal, bei Ferchels nördlich des Biologischen Station “Untere Havel”. **Thuringia**. Weimar, Ettersberg, 16.VI.1894, *J.Bormüller* (B)

- *C. cespitosa* × *C. nigra*

  Carex × *bolina* O.Lang, Linnaea 24: 551 (1851)

  = *Carex* × *sororia* Meinsh., Fl. Ingr.: 404 (1878), *nom. illeg.*

  = *Carex* × *peraffinis* Appel, Jahresber. Schles. Ges. Vaterl. Cult. 1891(2): 158 (1892).

It was also previously recorded from Germany (Schultze-Motel, 1968; Kiffe, 2001b). This hybrid is more variable than the former, since *C. nigra* morphology is a quite variable taxon.

The main diagnosis characters are the amphistomatic leaves. It is an intermediate feature among the parental taxa, the hipostomatic *C. cespitosa* and the epistomatic *C. nigra*. Rhizomes vary between a densely caespitose plant to rhizomes with more or less enlarged internodes. Basal sheaths are mainly reddish, more labile than in *C. cespitosa*, and sometimes break up like in *C. nigra*. Utricles are suborbicular, faintly nerved or nerveless.

Specimens studied: *GERMANY*: **Bavaria**. Oberbayern, Pöcking, Kr. Starnberg, Moorgraben in Luss, 8.VI.1958, *J. Poelt* (M). **Brandenburg**. Biesenthaler Becken südl. Biesenthal, Feuchtwiese am Südrand des Streesees, 21.V.1998, *K.Kiffe* (B); Lkr. Oberhavel, Feuchwiese westlich des Nehmitz sees, 11.VI.2001, *K.Kiffe* (B); Westende

des Dretzsees nördl. Teschendorf, "Kleine Herrenwiese", Feuchtwiese, 21.V.1998, *K.Kiffe* (B); Südwestlich Lychen, im gestörten Rand-bereich im Erlenbruch des Mellenseemoores südlich Mellensee, 14.V.1999, *K.Kiffe* (B). **Hesse**. Vogelsbergkreis, NSG "Mühlwiesen bei Niedermoos", Feuchtwiese am Abfluss des Niedermooser Teiches, 18.V.2001, *K.Kiffe* (B). **Lower Saxony**. Stade, Schiwingetal nordöstl, Mulsum, in einer ca. 10 Jahre alten Erlenpflanzung auf einer Feuchtwiese an einem Bach nördl. der Schwinge, 29.V.1997 *K.Kiffe* (B); Niedersachsen, Lkr. Lüchow-Dannenberg, SE Jiggel (bei Clenze), Jiggeler Moor, 14.VI.1998 (B) . **Mecklenburg-West Pomerania**. Kreis Parchim, Sternberg, südöstl. Dabel, Feuchtwiese am weg auf die Halbinsen am Klein-Pritzer See, 10.V.1997, *K.Kiffe* (B). **Schleswig-Holstein**. Kreis Hezogtum Lauenburg, Hellbachtal bei Mölln, 17.V.1997, *K.Kiffe* (B); Kreis Hezogtum Lauenburg, nordöstl. Lauenburg, Feuchtwiese bei Stötebrück westlich des Elbe-Lübeck-Kanals, 12.VI.1999, *K.Kiffe* (B).

Conclusions

Carex cespitosa occurs mostly scattered in South and West Europe. In the British Isles, it has a single occurrence in southern England, as well as in Netherlands. In Spain only two populations are known in the western Pyrenees. In Switzerland it is mostly restricted to Jura. It is probably absent in Italy, and there are no records from Belgium. *Carex cespitosa* become less rare in France where it inhabits in three mountain systems: Jura, Massif Central, and eastern Pyrenees, being really scarce in this latter with a single station. In Scandinavia and Germany, its distribution becomes extensive, although in Denmark it could be more scarce and scattered than expected.

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CAPÍTULO 10

Discusión y conclusiones

Discusión

TAXONOMIC DECISIONS IN THE LIGHT OF MOLECULAR PHYLOGENETIC AND MORPHOLOGICAL DATA

From the evolutionary theories settled by Darwin (1837), a general concern when trying to order biodiversity has been that classifications reflect the evolutionary history of the organisms. To achieve this aim, different kind of data sources have been used when performing taxonomic arrangements (macro and micromorphological, biochemical, reproductive, karyological and genetic evidences, among others; Stuessy, 1990). One of the most debated issues in biology has been the species concept, which constitutes the basic unit considered in taxonomy: a set of metapopulations that evolves together more or less independently from other metapopulations sets (de Queiroz, 2007). Several species concepts have been formulated to delimitate what groups of populations might be considered as forming a single species. Biological, morphological or phylogenetic species concepts emphasize in different biological properties (e.g. reproductive isolation for the biological species, diagnosability for the morphological species, or monophyly for the phylogenetic species; reviewed in Stuessy (1990) and de Queiroz, (2007)). There is a general consensus to expect that populations forming a plant species must be diagnosable through a specific morphological character or combination of characters (Castroviejo, 2004; Wiens, 2007). The great variety of evolutionary histories, biogeographical scenarios and reproductive relationships between taxa has resulted in the application of different species concepts to particular taxonomic groups, to accurately fit its most important biological properties (de Queiroz, 2007) and relevant historical events (O'Hara, 1993). In this way, when treating groups of low morphological differentiation, additional evidences may be helpful to establish species limits. Thus, Valcárcel & Vargas (2010), when treating *Hedera* L., consider ploidy or geographical isolation degree as supporting "characters" to split between some morphologically poorly defined populations at species rank or below. On the contrary, in *Androsace vitaliana* (L.) Lapeyr., the weak morphological features reported for recognizing several infraspecific taxa in different mountain ranges, were refused by Dixon *et al*. (2007), on the basis of the recent biogeographical history of the species: They argued that multiple interglacial contacts among genetic groups have been key

processes in the Plio-Pleistocene history of *A. vitaliana*. So given the predominant role of such processes, that prevented divergence through mixing genomes, advises against performing exhaustive subdivisions.

The development of phylogenetic systematics since Hennig (1950) brought an intense debate about whether to accept or not paraphyletic groups in natural classifications (reviewed in Podani, 2010). Apart from higher taxonomic ranks, it appears that at least species, as mutually exclusive reticulate metapopulational systems, can be defined as paraphyletic: from a practical synchronic point of view, they do not have to include all descendants of a single common ancestor (Rieppel, 2010). Hörandl & Stuessy (2010) proposed two main mechanisms that let paraphyletic species co-exist with new derived taxa: 1- budding from small stocks of parental taxa populations (e.g. peripatric speciation) and, 2- hybridization processes. They concluded that paraphyly helps to more accurately compile the evolutionary history of taxa than exclusive monophyly (for which they coined the term holophyletic). Monophyly is the main property of the phylogenetic species concept (de Queiroz, 2007). If other biological properties better define partitions within the "diffuse" reticulating sets of individuals, avoiding paraphyletic groups do not have to be the primary criterion to define species (Brummitt, 2008).

On the contrary, there is a wide consensus among systematists about the rejection of polyphyly, as it is a grouping artifact that weakens the predictive quality of the considered groups (Hörandl & Stuessy, 2010). Polyphyly arises when the taxonomical partitions include a set of phylogenetically heterogeneous units. In this way, cladistic reconstructions result in several independent origins for the considered single taxon. Despite the frequently invoked "cryptic" character of polyphyletic taxa, diagnostic characters usually become apparent after a morphological re-evaluation. In this sense, Muellner *et al*. (2009) proposed tentative characters to define each of the "cryptic" taxa in *Cedrela* (Meliaceae) revealed by their phylogenetic analyses; on the other hand, Lu *et al.* (2010) invoked unavailability of suitable herbarium materials of East Asian *Gaultheria* (Ericaceae) as the reason that avoided an earlier discovering of the cryptic species. It describes such taxa as poorly characterized rather than "cryptic". Actually, polyphyly uses to be nothing else than human errors: The taxonomist is misled by morphology, relying on homoplasic rather than apomorphic characters to circumscribe taxa. Polyphyly as an artifact could be also a result of hybridization phenomena (cf. discussion about *Armeria villosa* in Fuertes-Aguilar *et al.* 1999).

- The *C. flava* group

In the *C. flava* group, systematic relationships inferred from morphological data led to establish a synthetic treatment in Europe considering two species: *C. flava* and an enlarged *C. viridula* (including *C. demissa*, *C. lepidocarpa*, *C. nevadensis* and *C. viridula*; Schmid, 1983; Crins & Ball, 1989). Evidences from cytogenetics (chromosome pairing in meiosis) and reproductive biology (hybrid fertility degree) revealed that both groups mainly behave as biological species (Schmid, 1982). However our phylogenetic results depict a more complex situation: they constitute two sister clades (Chapter 2), one containing *C. demissa*, *C. viridula s.s.* and their relatives (mostly taxa with straight utricles; clade A), and another one with *C. flava*, *C. lepidocarpa*, *C. nevadensis* and their allies (taxa with bent utricles; clade B) (Fig. 1). This fits better those analytical approaches that consider each of the *C. viridula* aggregate. taxa as distinct species (eg. Chater, 1980). This view also agrees with results from allozyme data (Hedrén, 2004). In addition, it would avoid the paraphyly of *C. viridula s.l.*, and the resulting taxonomic treatment would provide a clearer picture of the evolutionary history of the group.

The Iberian endemic *C. nevadensis s.s.* (Sierra Nevada) and the North African dwarf forms could be considered as two relatively well-defined taxa from a morphological point of view, different from *C. lepidocarpa* (Chapters 3-4). Nevertheless, the phylogenetic analyses displayed that these populations are included in the western *C. lepidocarpa* lineage of the clade B, the (Chapter 2). As a result, we propose to consider them as distinct subspecies within *C. lepidocarpa*: *C. lepidocarpa* subsp. *nevadensis* and *C. lepidocarpa* subsp. *atlasica*. This view agrees with the morphogeographical but not genetic compartimentalization of these populations.

Taxonomic distinction of some of the *C. flava* Circunmediterranean problematic morphotypes from mountains cannot be easily performed based in morphology (Chapter 4). However they displayed disparate phylogenetic relationships with well defined lowlands forms from different species (Pyrenean-Cantabrian plants and *C. nevadensis* are closer to the Western *C. lepidocarpa* lineage, whereas Alpine plants are to Eastern-Central *C. lepidocarpa* and/or *C. flava*) (Chapters 2, 4). In the case of *C. nevadensis* and other Circunmediterranean mountains plants, morphological similarity between

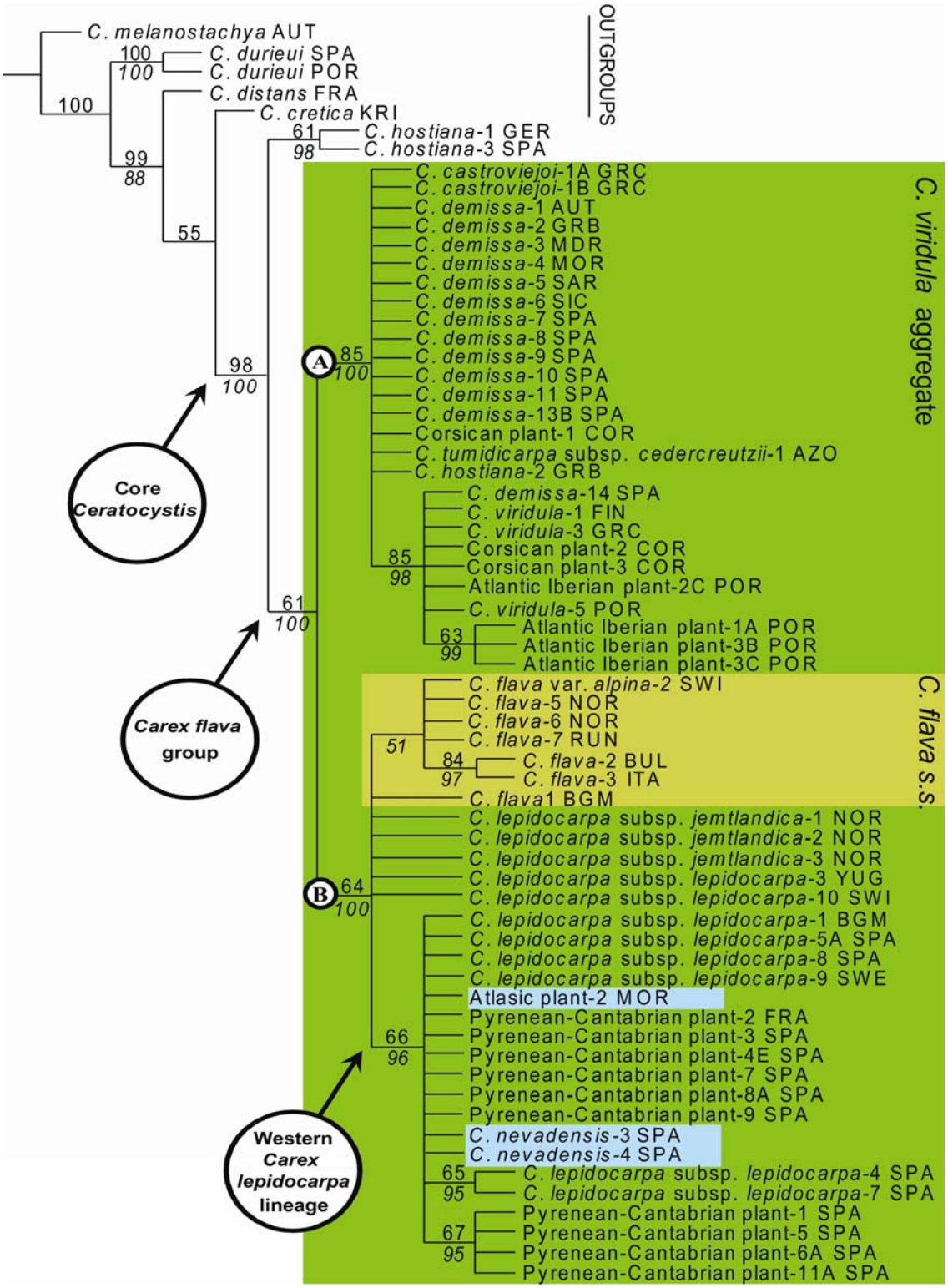

Fig. 1 Phylogenetic tree of *Carex* sect. *Ceratocystis* modified from Chapter 2, showing the paraphyly of *C. viridula* aggregate (green) due to nesting of *C. flava* (yellow), and the nested placement of *C. nevadensis* and Atlasic plants within western *C. lepidocarpa* lineage (blue).

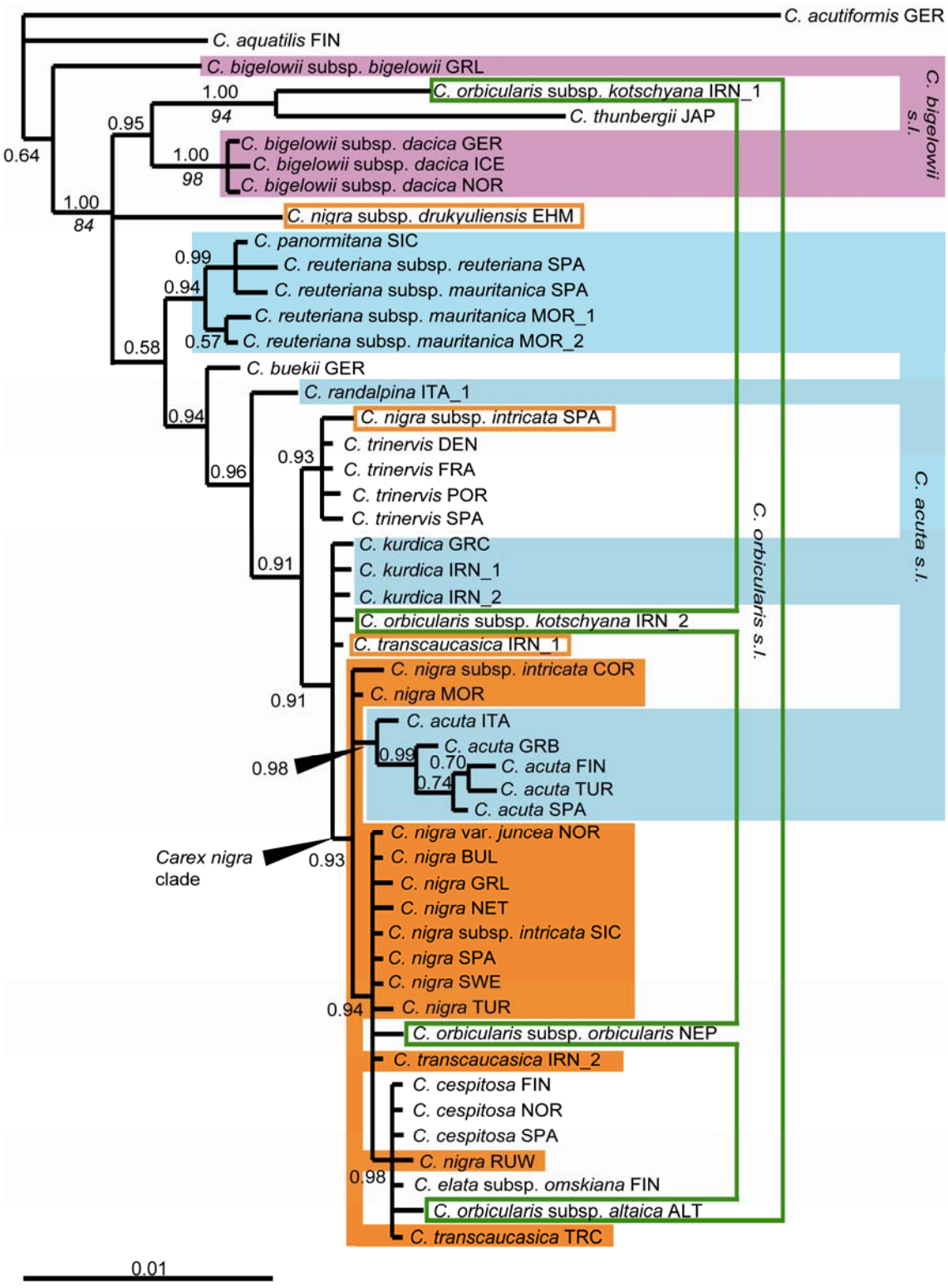

Fig. 2. Phylogenetic tree of sect. *Phacocystis* modified from Chapter 7, showing the major instances of polyphyly (purple and cyan) and paraphyly (orange). Putative hybrid polyphyletic populations of *C. nigra* are depicted within empty orange squares.

morphotypes leaded Chater (1980) to consider an enlarged *C. nevadensis* for all these populations, a taxon that have been revealed polyphyletic by our phylogenetic analyses.

- *Carex* sect. *Phacocystis*

In *Carex* sect. *Phacocystis*, the relative low value of quantitative characters (cf. Chater, 1980; Egorova, 1999; Standely *et al.*, 2002; Luceño & Jiménez-Mejías, 2008), together with the poorly developed qualitative diagnostic features, have strongly obscured the taxonomic structure of the group. When our genetic results are contrasted with the previous taxonomic treatments, a mosaic of bizarre situations appears (Fig. 2). On one hand, apparently distinctive *C. nigra* tussock-forming populations, previously considered as distinct species (*C. juncella*; Sylvén, 1963; Egorova, 1999) were revealed as genetically identical to typical rhizome-creeping plants (see Chapter 6). On the other hand, taxa regarded as conspecific displayed disparate phylogenetic placements, (Fig. 2; e.g. *C. panormitana*, merged under *C. acuta* (Chater, 1980) and *C. reuteriana*, treated as a subspecies of *C. elata* (Luceño & Aedo, 1994); Chapter 7). Perhaps, the most paradigmatic of the studied cases is that of *C. nigra*. It is a morphologically well defined species (regardless of the tussock-forming plants, see above) which have been mostly recovered in a single clade ("core *C. nigra*", see Chapter 7) in the phylogenetic reconstructions (*C. nigra* clade; fig. 2). However *C. acuta* and *C. cespitosa* were nested within this *C. nigra* clade. Intra-specific variation has been previously noted within *C. nigra* and many infraspecific taxa have been described (cf. Vicioso, 1959; Chater, 1980; Nilsson, 1985). However, morphological species circumscription in section *Phacocystis* (e.g. Egorova, 1999; Jermy *et al*. 2008) would clearly reject the inclusion of well-defined species as *C. acuta* or *C. cespitosa* within *C. nigra*. *Carex cespitosa* even displays a distinct chromosome number ($2n = 78$) when compared with *C. acuta* and *C. nigra* ($2n \geq 80$) (Faulkner, 1972; see Roalson (2008) for a review). In addition, extensive gene flow between *C. nigra* populations as well as admixture of the different genetic groups have been detected (Chapter 6). Given such situation, the relative morphological heterogeneity of *C. nigra* can be partly explained by a geographical cline (eg. populations from Caucasus (*C. transcaucasica*) or the Mediterranean (*C. intricata*)) and partly by phenotypic plasticity (the tussock-forming *C. juncella*). All these evidences prevent us to perform an infraspecific treatment within *C. nigra*. Thus, it

appears appropriate to consider *C. nigra* as a non-monophyletic, genetically variable species, in which two additional species, *C. acuta* and *C. cespitosa*, are nested.

Carex orbicularis have been also misinterpreted as a single species: The three geographical subspecies traditionally considered (*orbicularis* from Hymalayas and Karakoram, *kotschyana* from Caucasus and Zagros, and *altaica* from Altai) were retrieved in different clades in the phylogenetic reconstructions (Fig. 2). This could reveal a subjacent molecular and morphological heterogeneity not previously detected. However, as only four samples were included, a wider sampling is required before considering further taxonomic decisions.

EVOLUTIONARY PATTERNS

- Role of hybridization

Hybridization is a process of huge evolutionary importance: allopolyploidy -and other chromosome mutations- has been largely documented as a speciation mechanism (stasipatric speciation; White, 1978) across many groups of angiosperms, whereas instances of hybridization not involving polyploidy are also not rare behind the origin of some species (Rieseberg, 1997; Soltis & Soltis, 2009).

In the sections *Ceratocytis* and *Phacocystis* hybridization is widely known (Chater, 1980; Cayouette & Morisset, 1986; Egorova, 1999; Nakamatte & Lye, 2007; Dragon & Barrington, 2008; Luceño & Jiménez Mejías, 2008). Isolated instances of hybridization have been found in both sections, as the plastid capture event detected in *C. hostiana* (Chapter 2) or the introgression produced by *C. aquatilis* in some *C. acuta* populations (Chapter 7). However, the consequences of hybridization on the evolutionary history of each of the sections could be far distinct: whereas in sect. *Phacocystis* hybridization may have promoted several speciation events, in sect. *Ceratocystis* hybridization just appears to be behind instances of lack of morphological definition (see below).

1- *Carex* sect. *Phacocystis*

At least two taxa within the European sect. *Phacocystis* are probably hybrid species: *C. elata* and *C. randalpina* (Chapter 7). Phylogenetic incongruities between the

topology yielded when nuclear (ITS, ETS) and plastid (*rpl32-trnL*^{UAG}, *ycf6-psbM*) markers are analyzed separately, characterize them as stabilized hybrids between lineages. However, such hybridization promoting speciation could be more common than suspected, since intra-lineage reticulation events cannot be identified comparing phylogenetic signals.

Additionally, three of the *C. nigra* populations were not recovered in the same clade with the remaining *C. nigra* samples: Atlas, Sierra Nevada and Hymalayas (Chapter 7; see also Fig. 2). As explained above, mixture of genetic groups detected with AFLP and analyses of molecular variation with AMOVA did not retrieve a clear genetic discontinuity for the studied populations from Atlas and Sierra Nevada (Chapter 6). The contrasting patterns obtained with AFLP data on one hand and nuclear / plastid sequences on the other could indicate that polyphyly of these populations is a result of hybridization.

2- *Carex* sect. *Ceratocystis*

In *Carex* sect. *Ceratocystis* no instances of hybridization promoting speciation were detected (Chapter 2). Plants from this group have short-life cycles (Schmid, 1984). On the contrary, in sect. *Phacocystis* longevity of individuals via rhizomes may reach hundreds of years (Jónsdóttir *et al.*, 2000). It has been proposed that somatic mutations can be an important source of genetic variation in long-lived plants (D'Amato, 1997). Such mutations in vegetative tissues could play their role predisposing the hybrids to solve fitness problems. On the contrary, plants from sect. *Ceratocystis* could be more sensitive to problems derived from hybridization phenomena. Extensive genetic interchange at population level in few generations would allow backcrossing with the parental taxa as an easy way to stabilize the hybrid population (Soltis & Soltis, 2009). It matches with our proposal of wide hybridization areas where extensive gene flow have been established between taxa (Luceño & Jiménez-Mejías, 2008; Chapter 2). Such pools of hybrid plants act as genetic bridges between parentals, thus the absence of any kind of strong reproductive isolation would not allow the divergence and final taxonomic split of hybrid populations.

The morphological study focused on the Iberian Peninsula (Chapter 3) helps to explain the phenotypic affinities between the well-defined taxa and the problematic morphotypes from the two inferred extensive hybridization areas (Chapter 2): Pyrenees

and Cantabrian Mountains (*C. demissa* × *C. lepidocarpa*) and Atlantic Iberian strip (*C. demissa* × *C. viridula*). Morphological intermediacy is frequently expected in hybrids, although the expression of extreme or novel characters upon the genome mixture is another of the possible outcomes (transgressive segregation; Rieseberg, 1995; Rieseberg *et al.*, 1999; Soltis & Soltis, 2009). In this sense, plants from each of those zones has developed a different appearance in regard to the putative parental taxa: whereas Pyrenean-Cantabrian plants were morphologically intermediate between *C. demissa* and *C. lepidocarpa*, Atlantic Iberian ones showed a disparate morphology that strongly resembles *C. lepidocarpa*, far from the expected intermediacy between *C. demissa* and *C. viridula*.

ALLOPATRIC DIVERGENCE

As explained above, gene flow between different taxonomic units can be established via introgression, preventing genetic divergence among units when reproductive barriers are not developed between taxa. Thus, allopatry may foster divergence by preventing gene flow.

Two kind of barriers are suggested as promoting divergence in *C. flava* group: 1- different chromosome numbers, a pre-zygotic barrier that difficults hybridization between different species (Schmid, 1982), and 2- geographical isolation. In sect. *Ceratocystis* geographical barriers appear to be more efficient as barrier than cytogenetic differences. ITS sequence divergence between northern hemisphere *C. flava* group taxa was much lower than among widely allopatric southern hemisphere taxa (*C. flaviformis*, *C. monotropa*) and between them and their northern counteparts.. At a lower taxonomic level, allopatric divergence could also explain the morphological divergence found in *C. lepidocarpa* subsp. *nevadensis* and subsp. *atlasica* (both isolated in high mountains at warm latitudes) with regard to typical *C. lepidocarpa*, and the incipient morphological split of *C. demissa* subsp. *cedercreutzii* (an endemic from Azores archipelago) from the type subspecies.

The role of geographical isolation as a barrier to gene flow varies between different sect. *Phacocystis* species. For the widely distributed *C. nigra* and *C. elata*, it does not appear to be important geographical barriers affecting the overall genetic variation of these taxa. Extensive gene flow explains such lack of intraspecific genetic structure, with probably key contribution of water-bird mediated dispersal, as suggested

for other aquatic plants (Mueller and van der Valk, 2002; Santamaría, 2002). Only those marginal southern populations of *C. nigra* displayed some genetic features of peripheral isolation due to gene flow disruption as postglacial warming proceeded from south to north (Kropf *et al.*, 2006). On the contrary, the genetic structure of the Ibero-North African *C. reuteriana* is strongly affected by geography (Chapter 5). Effective barriers are found between each of the patches in which its distribution is subdivided: 1- the Southern Iberian Plateau, which avoids the contact between the central-northern subsp. *reuteriana* and the southern subsp. *mauritanica*, and 2- several minor barriers between the patches of each subspecies: Northern Iberian Plateau for subsp. *reuteriana,* Guadalquivir valley and Strait of Gibraltar for subsp. *mauritanica* (see references in Chapter 5). Disparate patterns within sect. *Phacocystis* taxa reflect the underlying variation among aquatics habitats (Santamaría, 2002), which causes that different taxa are differently affected by environment: marshes and lakes, where *C. elata* mostly inhabits, allow a better interaction with potential disperser waterbirds than mountain streams where *C. reuteriana* is found.

POST GLACIAL (RE)COLONIZATION

Phylogeographic studies have accumulated a wealth of data during the last decade, showing that western Asia and South Europe, and particularly the Mediterranean Peninsulas (Iberia, Italy, Balkan and Anatolia), were the main places where temperate and boreal taxa survived the glaciations and re-colonized central and northern Europe (Comes & Kadereit, 1998, 2003). This common pattern is confirmed for *C. nigra* (Chapter 6), and partially retrieved for *C. elata* (Chapter 5). The different colonization fronts have contacted forming a suture zone at different latitudes in each of the cases: in *C. nigra* probably in Scandinavia, whereas in *C. elata* it is centered along Great Britain-France and North Italy. Contrasting patterns of post-glacial colonization of Europe from refugia have been found when comparing different organisms (Taberlet *et al.*, 1998).

Lack of differentiation of American *C. nigra* populations also implies that colonization of such continent has been probably a recent process. North Atlantic Ocean was not a strong barrier for plant colonization during and at the end of the last glaciation (Abbott & Brochmann, 2003). This may be specially true in *Carex*, resulting in the typical Amphi-Atlantic distribution pattern displayed by some species (*C. bigelowii* ssp.

bigelowii, Schönswetter *et al.*, 2008; *C. rufina*, Westergaard *et al.*, in press),. However, whereas in the closely related *C. bigelowii* (Schönswetter *et al.*, 2008) migration was inferred from North America to Europe, the migration in *C. nigra* would be the opposite as inferred from the pattern of genetic diversity (Chapter 6). However, the genetic similarity between American and some European populations does not allow to discard, at least in part, an anthropogenic introduction.

CHROMOSOME EVOLUTION

Cytogenetic variation in *Carex* is remarkable, and evolution has been characterized as rapid and diverse. Thus, contrasting trends have been inferred from different groups (punctuational or continuous chromosome number variation; sect. *Ovales*, Hipp (2007); sect. *Spyrostachyae*, Escudero *et al.* (2010); respectively).

Carex sect. *Ceratocystis* has been interpreted as an aneuploid series with an evolutionary increase in the chromosome number (Schmid 1982; Crins & Ball 1988). However, such pattern does not fit the phylogenetic structure of the group inferred from plastid data. A general increase in the chromosome number could be proposed for the section, since *C. hostiana* is placed sister to *C. flava* group, and it displays the lowest chromosome number among the section members (*2n* = 56; Roalson, 2008). However this increase would not be in a linear fashion, since *C. flava* group is arranged in two clades, each one displaying relatively high and low numbers.

In *Carex* sect. *Phacocystis* a trend to increase the chromosome number towards terminal branches may be also inferred for the Eurasian clade (Chapter 7). Those taxa with lower numbers are placed in the outermost branches (2n = 64-74; *C. buekii*, *C. kurdica*) whereas numbers 2n > 80 appear towards terminal tips. However, at least a clear event of chromosome decrease can be retrieved, as *C. cespitosa* (2n = 78-80) is nested within *C. nigra* (2n = 84), probably through fusion processes.

Conclusiones / Conclussions

1- La sección *Ceratocystis* del género *Carex* es un grupo monofilético si se excluye *C. durieui*. *Carex hostiana* es el táxon hermano al grupo de *C. flava*. Este último se subdivide en dos clados principales, cada uno caracterizado por caracteres del utrículo: por un lado se agrupan principalmente especies con picos rectos (*C. demissa*, *C. viridula* y afines) y por el otro táxones con picos curvos (*C. flava*, *C. lepidcarpa* y afines). Dentro del clado de las especies de pico curvo se encuentra un subclado bien definido compuesto por muestras occidentales de *C. lepidocarpa* y formas asociadas, anidado entre las *C. lepidocarpa* centro-orientales y *C. flava*.

1- *Carex* sect. *Ceratocystis* is a monophyletic group if *C. durieui* is excluded. *Carex hostiana* is the sister of *C. flava* group. This latter is subdivided in two main clades characterized by certain morphological features of the utricle: on one hand, plants mainly with straight beaks (*C. demissa*, *C. viridula* and allies), and on the other hand plants with bent beaks (*C. flava*, *C. lepidocarpa* and relatives). Within the bent-beaked clade there is a well defined subclade mainly composed by western *C. lepidocarpa* samples and allied forms, nested among eastern-central *C. lepidocarpa* and *C. flava*.

2- La evolución cromosomática inferida en *Carex* sect. *Ceratocystis* no concuerda con la hipótesis previamente propuesta de una serie aneuploide linear. A pesar de ello, parece existir una tendencia general a aumentar el número cromosómico, pero no de un modo linear.

2- Chromosome evolution of *Carex* sect. *Ceratocystis* does not fit the proposed hypothesis of increasing linear aneuploid series. Despite it seems to be a general trend to increase in chromosome number, it would not be in a linear fashion.

3- Los morfotipos problemáticos del grupo de *C. flava* que se encuentran en los Pirineos y la Cordillera Cantábrica, los de la fachada atlántica ibérica, y los de Córcega, tiene un origen probablemente híbrido: las plantas pirenaico-cantábricas principalmente entre *C. demissa* y *C. lepidocarpa*, mientras que las atlánticas ibéricas y las corsas lo serían entre *C. demissa* y *C. viridula*. Estas tres regiones constituirían áreas de hibridación extensiva. En lo que a tales morfotipos ibéricos se

refiere, las plantas de Pirineos y Cordillera Cantábrica son intermedias entre los parentales, mientras que los híbridos atlánticos muestran una morfología totalmente dispar a sus parentales, y tienden a asemejarse a *C. lepidocarpa*.

3- The problematic *C. flava* morphotypes found in the Pyrenees and Cantabrian mountains, Atlantic Iberian Strip, and Corsica, have probably a hybrid origin: Pyrenean-Cantabrian plants mainly between *C. demissa* and *C. lepidocarpa*, and Atlantic Iberian and Corsican between *C. demissa* and *C. viridula*. Those three regions would constitute extensive hybridization areas. Regarding the Iberian hybridization areas, the hybrid morphotype from Pyrenees and Cantabrian mountains displays intermediate morphological features between the parental taxa *C. demissa* and *C. lepidocarpa*. However, the hybrid morphotype *C. demissa* × *C. viridula* from the Atlantic Iberian strip displayed a disparate morphology with affinities to *C. lepidocarpa*.

4- Los morfotipos enanos de las montañas circunmediterráneas muestran afinidades genéticas diferentes. Las plantas pirenaico-cantábricas y de los Alpes muestran un amplio solapamiento de caracteres a pesar de que las primeras se relacionan a las *C. lepidocarpa* occidentales y las segundas al grupo que forma *C. flava* junto con las *C. lepidocarpa* centro-orientales. Dentro del linaje de las *C. lepidocarpa* occidentales, se detecta una considerable variación morfológica que apoya la distinción de al menos dos subespecies adicionales en las montañas más meridionales: subsp. *nevadensis* de Sierra Nevada, y subsp. *atlasica* del Atlas.

4- Dwarf *C. flava* group morphotypes from Circunmediterranean mountains show disparate genetic affinities. Pyrenean-Cantabrian and Alpine plants displayed a wide morphological overlapping despite the first are related to the western *C. lepidocarpa* lineage and the second to the *C. flava*- eastern-central *C. lepidocarpa* aggregate. Additionally, within the western *Carex lepidocarpa* lineage a considerable morphological variation is detected, supporting the recognition of at least two mountain subspecies: subsp. *nevadensis* from Sierra Nevada, and subsp. *atlasica* from Atlas.

5- La recientemenete descrita *C. castroviejoi* de las montañas de Grecia y Albania, hasta entonces incluida en *C. lepidocarpa*, es una especie bien definida genética y morfológicamente que merece reconocimiento taxonómico.

5- *Carex castroviejoi*, a recently described species from Greece and Albania mountains, hiterto included under *C. lepidocarpa*, is a genetically and morphologically well-defined species which deserves taxonomic recognition.

6- *Carex elata*, una planta ampliamente distribuida, presenta una estructura geográfica escasamente diferenciada en relación con la similar *C. reuteriana*. En esta última, el aislamiento por distancia, probablemente mediado por varias barreras geográficas, habría promovido la diferenciación de los diferentes núcleos de población. La distribución alopátrica mostrada por los dos táxones, a pesar de sus similares requerimientos ecológicos, podría ser explicada por el efecto de bloqueo por densidad, que habría prevenido la hibridación entre ellas.

6- The extensively distributed *C. elata* is weakly geographically structured in comparison with *C. reuteriana*. In the latter, isolation by distance, probably mediated by several geographical barriers, would have promoted the differentiation of the populations of the different distribution patches. The allopatric distribution displayed by both taxa, despite similar ecological requirements, could be explained by high density blocking effect, which could have prevented hybridization between them.

7- *Carex nigra* muestra una escasa estructura geográfica en su diversidad genética, probablemente debido a flujo genético extensivo, que habría promovido la mezcla de diferentes grupos genéticos. La colonización hacia el norte y el oeste desde los refugios del sur y el este habría resultado en una enorme pérdida de diversidad hacia los Países Bajos, Escocia e Islandia, mientras que el encuentro de diferentes frentes de colonización en Fennoscandia habría disparado la variación. En las áreas más meridionales de su distribución se detectó una alta diversidad genética. Procesos de diferenciación centrífuga en podrían estar afectando a alguna de las poblaciones más sureñas (Atlas, Sicilia y Sierra Nevada).

7- *Carex nigra* displays a scarcely structured pattern of genetic diversity, probably due to extensive gene flow, which has promoted genetic admixture. North and westward colonization from south and eastern putative glacial refugia resulted in a strong loss of diversity towards Netherlands, Scotland and Iceland, whereas the meeting of postglacial colonization fronts in Fennoscandia resulted in a strong increase of genetic variation. A high genetic diversity was detected in the southern parts of the range. Processes of centrifugal

allopatric differentiation could be affecting some southernmost populations (Atlas, Sicily and Sierra Nevada).

8- La forma amacollada de *C. nigra* conocida como var. *juncea* se habría originado varias veces desde las poblaciones de rizomas reptantes de la típica var. *nigra*.

8- Multiple origin of the distinctive tussock forming *C. nigra* var *juncea* from rhizome-creeping populations of typical var *nigra* are supported by genetic analyses.

9- Los miembros europeos de la sección *Phacocystis* (excepto *C. aquatilis*, *C. rufina* y los táxones halófilos) se organizan en tres clados principales: *C. bigelowii* subsp. *dacica* por un lado, un clado mediterráneo que agrupa a *C. panormitana* y *C. reuteriana*, y un amplio clado euroasiático.

9- European members of *Carex* sect. *Phacocystis* (except. *C. aquatilis*, *C. rufina*, and the halophile taxa) are arranged in three main clades: *C. bigelowii* subsp. *dacica* clade, Mediterranean clade (*C. panormitana* and *C. reuteriana*) and a wide Eurasian clade, where the mainly Asiatic *C. kurdica* and *C. orbicularis* are nested.

10- La hibridación es una fuerza evolutiva importante en *Carex* sect. *Phacocystis*. Especies bien definidas como *C. elata* y *C. randalpina* son de origen probablemente híbrido. La hibridación también podría tener importancia local en algunas de las poblaciones más meridionales de *C. nigra* (Atlas y Sierra Nevada).

10- Hybridization is an important evolutionary force in *Carex* sect. *Phacocystis*, and well-defined species as *C elata* and *C. randalpina* arose probably from hybrid origin. Also, hybridization could have local importance in some of the southernmost peripheral populations of *C. nigra* (Atlas and Sierra Nevada).

11- Algunas de las poblaciones mediterráneas asignadas a *C. acuta* son morfológica y genéticamente heterogéneas y realmente incluyen otras entidades biológicas. Se pueden reconocer tres táxones: *C. acuta* (ampliamente distribuída), *C. kurdica* (Albania, Grecia y sur de Anatolia) y *C. panormitana* (Cerdeña, Sicilia y Túnez).

11- The putative *C. acuta* Mediterranean population are morphologically and genetically heterogeneous, and three taxa can be recognized: *C. acuta* (widely distributed), *C. kurdica* (Albania, Greece and southern Turkey) and *C. panormitana* (Sardinia, Sicily and Tunisia).

12- La distribución de *C. cespitosa* es mucho más restringida de lo que se ha dicho, debido a numerosas confusiones taxonómica. Esta especie crece de modo está muy dispersa en Europa occidental, con solo dos poblaciones en España (Pirineo Navarro), una en Gran Bretaña (Inglaterra) y unas pocas disyuntas en Francia (Pirineo Catalán, Macizo Central y Jura) en Suiza (Jura). Las citas de Italia podrían ser debidas a errores de identificación y deben ser confirmadas.

12- *Carex cespitosa* distribution is much more restricted than it has been stated due to frequent taxonomical confusions. It is strongly scattered in W Europe, with only two populations in Spain (western Pyrenees), one in Great Britain (England), and a few disjunct populations in France (eastern Pyrenees, Massif Central and Jura) and Switzerland (Jura). Reports from Italy could be misidentifications and need further confirmation.

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APÉNDICE 1

Carex sects. *Ceratocystis* y *Phacocystis* (Cyperaceae). *Flora Iberica**

M. Luceño & P. Jiménez-Mejías

*Modificado de Luceño M. & Jiménez-Mejías, P. 2008. *Carex* sects. *Ceratocystis* y Phacocystis. In S. Castroviejo *et al.* (eds.). *Flora Iberica*, 18: 191-204, 327-246.

Carex L.

Sect. 28. **Ceratocystis** Dumort.

Plantas cespitosas o con rizomas de entrenudos ± cortos. Tallos trígonos, lisos o escábridos. Bráctea inferior generalmente foliácea y de mayor longitud que la inflorescencia, excepcionalmente setácea y de menor longitud que la inflorescencia. Espiga masculina solitaria, fusiforme o cilíndrica; espigas femeninas erectas, ovoides, densifloras. Glumas femeninas de ápice variable, nunca largamente aristadas. Estigmas 3. Utrículos de erectos a reflejos, trígonos o plano-convexos, glabros, lisos, sin inclusiones cristalinas de color rojo en las células epidérmicas. Aquenios de contorno obovado, raramente elíptico.

50. C. durieui Steudel ex Kunze, Suppl. Riedgräs. 4: 149 (1844) [Duriéui]

Ind. loc.: "Auf sumpfigen Abhägen der niederen Berge in Asturia, namentlich in der Sierra del Chorro und der Sierra del Aguitero bei Grado" *Ic.:* Lám. 56

Laxa o densamente cespitosa. Tallos fértiles 30-50 cm, delgados, lisos, trígonos. Hojas 0,5-0,8 mm de anchura, de menor longitud que los tallos, setáceas, enrolladas, ásperas; lígula muy corta; generalmente sin antelígula; vainas basales enteras, de color pardo a pardo-púrpura obscuro. Bráctea inferior 8-45 mm, setácea. Espiga masculina solitaria, de 16-20 × 1,5-3 mm, fusiforme; espigas femeninas 1-2, de 8-11 × 9-10 mm, agrupadas bajo la masculina, globosas, erectas. Glumas masculinas ovales, de color pardo-púrpura obscuro o negruzcas, de ápice variable; glumas femeninas anchamente ovales, agudas, obtusas o subagudas, de color negruzco o púrpura muy obscuro, generalmente con estrecho margen escarioso. Utrículos 5,5-6,2 × 2,1-2,5(3,2) mm, patentes o reflejos, de contorno anchamente oval a suborbicular, plano-convexos, alados, plurinervios, aunque los nervios son poco perceptibles, teñidos de color púrpura, bruscamente estrechados en un pico de 1,8-2,3 mm, bífido, escábrido, curvado. Aquenios 1,9-2,1 × 1-1,3 mm, de contorno obovado, trígonos. 2*n* = 52, 53.

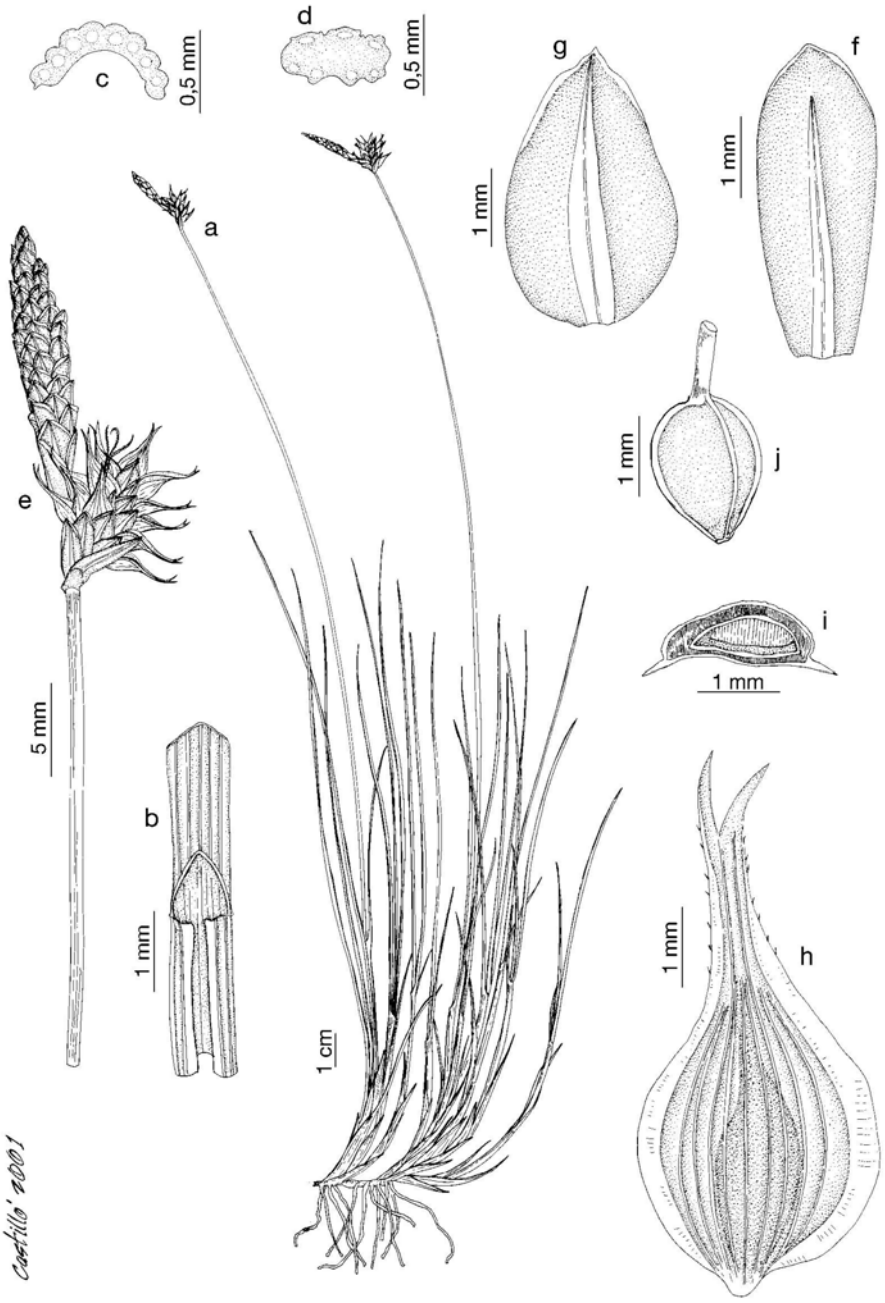
Turberas y prados muy húmedos en substratos ácidos; 10-900 m. IV-VII(VIII). ◆ NW de la Península Ibérica. **Esp.:** C Lu O Po. **Port.:** BL DL Mi.

51. C. hostiana DC., Cat. Pl. Horti Monsp.: 88 (1813) [Hostiána]

C. hornschuchiana Hoppe in Flora 7: 595 (1824)
Ind. loc.: "Hab. in Austria" [lectótipo designado por A. Charpin & M. Luceño in Candollea 48: 465 (1993): lámina in Host, Icon. Descr. Gram. Austriac. 4, tab. 95 (1809)] *Ic.:* Boott, Ill. Carex, tab. 441-443 (1867); Jermy & Tutin, Brit. Sedges ed. 2: 153 (1982)

Cespitosa, en ocasiones laxamente, con rizoma de entrenudos ± largos. Tallos fértiles (15)20-50(60) cm, lisos o ligeramente escábridos, obtusamente trígonos. Hojas 2-5 mm de anchura, de menor longitud que los tallos, planas o ligeramente aquilladas, de color verde glauco cuando vivas, sobre todo por el envés, estrechadas bruscamente en una punta setácea; lígula generalmente hasta 1 mm, de ápice redondeado; antelígula hasta 4 mm, de ápice redondeado o truncado; vainas basales bastante fibrosas, de color pardo claro. Bráctea inferior foliácea, de mayor longitud que la espiga pero mucho menor que la inflorescencia. Espiga masculina solitaria, raramente 2, de 10-20 × 2-4 mm, fusiforme; espigas femeninas (1)2-3(4), de 8-20 × 4-6 mm, ovoides o cortamente cilíndricas, distantes, erectas. Glumas masculinas obovadas o elípticas, de color pardo obscuro, con margen escarioso ancho y ápice ± obtuso; glumas femeninas anchamente ovales, agudas o mucronadas, de color pardo-púrpura obscuro, con margen escarioso ancho. Utrículos 3,5-5 × 1,5-2,5 mm, patentes, de contorno oval, ± trígonos, plurinervios, estrechados bruscamente o atenuados en un pico de 0,8-1,2 mm, bífido, escábrido. Aquenios 2-3 × 1-1,4 mm, de contorno obovado o elíptico, trígonos. 2*n* = 56.

Prados húmedos y turberas ligeramente ácidas, con un rango de pH entre 5,5 y 6,5, aunque puede vivir en substratos calcáreos; 200-1200 m. (IV)V-VII. C y W de Europa hasta el C de Suecia, muy escasa en la Península Ibérica y en Turquía; NE de Norteamérica. En la Península crece, cada vez menos abundante, en puntos aislados del N. **Esp.:** Lu Na O S So SS.



Lám. 56.–*Carex durieui*, a-e) playa del Cabalar, Ortigueira, La Coruña (MA 519275); f-j) Gestoso, La Coruña (MA 314877): a) hábito; b) lígula; c) sección transversal de una hoja a nivel de la lígula; d) sección transversal de una hoja a media altura; e) inflorescencia; f) gluma ó; g) gluma '; h) utrículo; i) sección transversal de un utrículo; j) aquenio.

52-56. gr. **C. flava**

Observaciones.–El grupo de *C. flava*, es –junto con la sección *Phacocystis*– el mejor estudiado de todo el género, al tiempo que uno de los más complejos. Se trata de un grupo atípico dentro de *Carex*, cuyos táxones son estrategas *r* y presentan ciclos de vida cortos y una evolución caracterizada por los fenómenos de hibridación.

La inmensa mayoría de las poblaciones ibéricas están formadas por un único taxon, por lo que su determinación resulta relativamente sencilla; sin embargo, en algunas zonas de la Península crecen poblaciones híbridas en las que se desarrollan numerosas formas intermedias y los progenitores llegan incluso a desaparecer. Las dos áreas de hibridación extensiva son Cordillera Cantábrica, Pirineos y Sistema Ibérico septentrional por un lado, y la fachada atlántica peninsular por otro.

En las zonas montañosas del norte (**And. Esp.:** Bu Ge Hu L Le Na O P S Vi) aparecen formas introgresivas en cuyo origen están implicadas *C. flava*, *C. lepidocarpa* y *C. demissa*. *C. flava* es una

planta boreoalpina que está desapareciendo de nuestro territorio por reticulación con las otras especies. Las formas introgresivas tienden a asemejarse a individuos pequeños de *C. flava*, y han sido citados en ocasiones como *C. flava* var. *alpina* Kneuck. in Allg. Bot. Z. Syst. 5: 8 (1899) [cf. J. Vigo in Acta Bot. Barcinon. 35: 740 (1983)], y otras –que habitualmente crecen en alta montaña– como *C. nevadensis*. Los morfotipos asignables a *C. flava* s.str. son extremadamente raros y parecen quedar restringidos al Valle de Arán.

En la fachada atlántica peninsular (Esp.: H. Port.: BA BAl BL E Mi R) las formas introgresivas tienen su origen en C. demissa y C. viridula. Estos individuos presentan espigas subglobosas, con los utrículos inferiores con el pico algo curvado, por lo que también han sido reiteradamente confundidos con C. flava [cf. A.O. Chater in Tutin & al., Fl. Eur. 5: 310 (1980); M. Luceño in Ruizia 14: 87 (1994)]. Los procesos de introgresión recientes no parecen afectar a C. nevadensis.

52. C. flava L., Sp. Pl.: 975 (1753) [fláva]

C. flava var. *alpina* Kneuck. in Allg. Bot. Z. Syst. 5: 8 (1899)
C. flava subsp. *alpina* (Kneuck.) O. Bolòs, Masalles & Vigo in Collect. Bot. (Barcelona) 17: 95 (1988), p.p
Ind. loc.: "Habitat in Europae paludibus" [lectótipo designado por B. Schmid in Watsonia 14: 312 (1983): LINN 1100.40] Ic.: Lám. 57.

Cespitosa. Tallos fértiles 10-20 cm, obtusamente trígonos, lisos o ligeramente escábridos bajo la inflorescencia. Hojas 3-4 mm de anchura, de menor longitud que los tallos, planas, de color verde pálido, ± lisas, poco rígidas; lígula hasta 3 mm, de ápice truncado, de menor anchura que el limbo; antelígula hasta 2 mm, de ápice truncado o redondeado, a veces inexistente; vainas basales enteras, de color pardo claro. Bráctea inferior foliácea, de c. 5 cm × 2,5-3 mm, de longitud mayor que la inflorescencia. Espiga masculina solitaria, de 1-1,8 cm × 2 mm, ± fusiforme, sésil o con un pedúnculo hasta de 5 mm; espigas femeninas 2-3, de 7-8 × 8-9 mm, aproximadas, erectas, subglobosas, subsésiles. Glumas masculinas ovales, de ápice obtuso, de color pardo-rojizo claro u obscuro; glumas femeninas ovales, obtusas, agudas o acuminadas, de color pardo rojizo con estrecho margen escarioso, uninervias. Utrículos 3,8-4,5 × 1-1,8 mm, los inferiores reflejos, los de la mitad superior de patentes a erectos-patentes y los apicales generalmente erectos, de contorno oval, trígonos, inflados, plurinerviados, estrechados bruscamente en un pico de 1,5-1,8 mm, reflejo en los utrículos de la mitad inferior de la espiga (20-60º respecto del cuerpo del utrículo), recto o casi en los de la mitad superior, bífido, liso. Aquenios 1,8 × 0,9 mm, de contorno obovado, trígonos. $2n = 58^*, 60^*, 62$.

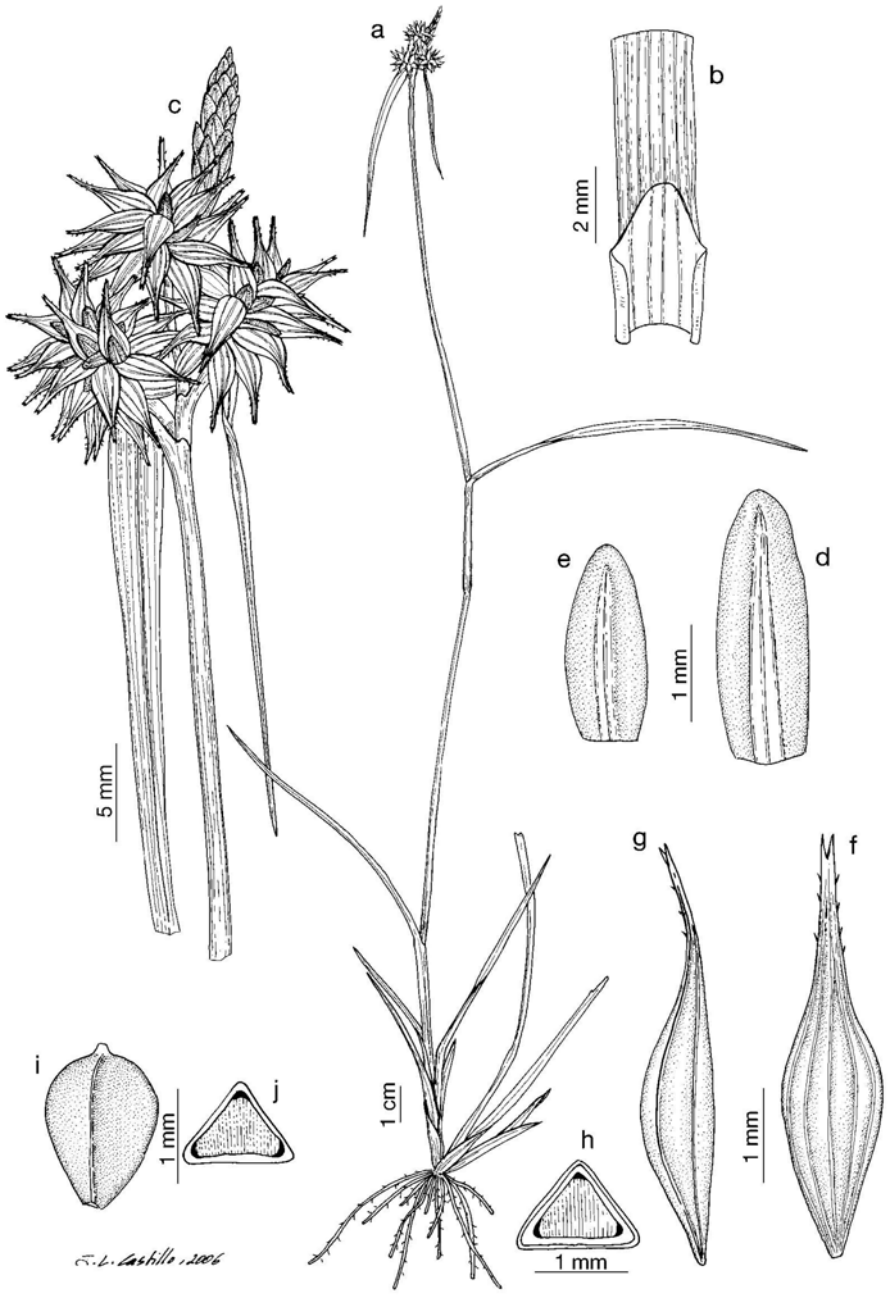
Suelos húmedos, silíceos (también sobre otros substratos fuera de la Península); 1700-1800 m. VI-VIII. Europa, C y W de Asia y Norteamérica. Valle de Arán. **Esp.:** L.

Observaciones.–Como hemos señalado en las observaciones al gr. de *C. flava,* esta especie participa de formas híbridas con *C. demissa* y *C. lepidocarpa*, plantas que tienden a asemejarse a individuos pequeños de *C. flava*. El proceso de reticulación que está sufriendo este taxon hace que los ejemplares puros sean bastante raros. Del abundante material pirenaico y cantábrico estudiado únicamente podemos asegurar que pertenecen a *C. flava* algunos individuos del Valle de Arán. Uno de ellos, cuyo estudio cariológico hemos llevado a cabo, ha mostrado uno de los números característicos de la especie ($2n = 31^{II}$).
Las plantas citadas bajo *C. flava* en Portugal corresponden a ejemplares introgresivos entre *C. demissa* y *C. viridula*, como también se ha explicado en las observaciones al grupo.

53. C. nevadensis Boiss. & Reut., Pugill. Pl. Afr. [nevadénsis]
Bor. Hispan.: 118 (1852)

C. lepidocarpa var. *nevadensis* (Boiss. & Reut.) Kük. in Engl., Pflanzenr. 38[IV.20]: 673 (1909)
C. lepidocarpa subsp. *nevadensis* (Boiss. & Reut.) Luceño in Anales Jard. Bot. Madrid 57: 176 (1999)
Ind. loc.: "In pratis turfosis regionis alpinae et nivalis Sierra Nevada" [lectótipo designado por W.J. Crins & P.W. Ball in Canad. J. Bot. 67: 1060 (1988): Reuter (G)]
Ic.: Lám. 58

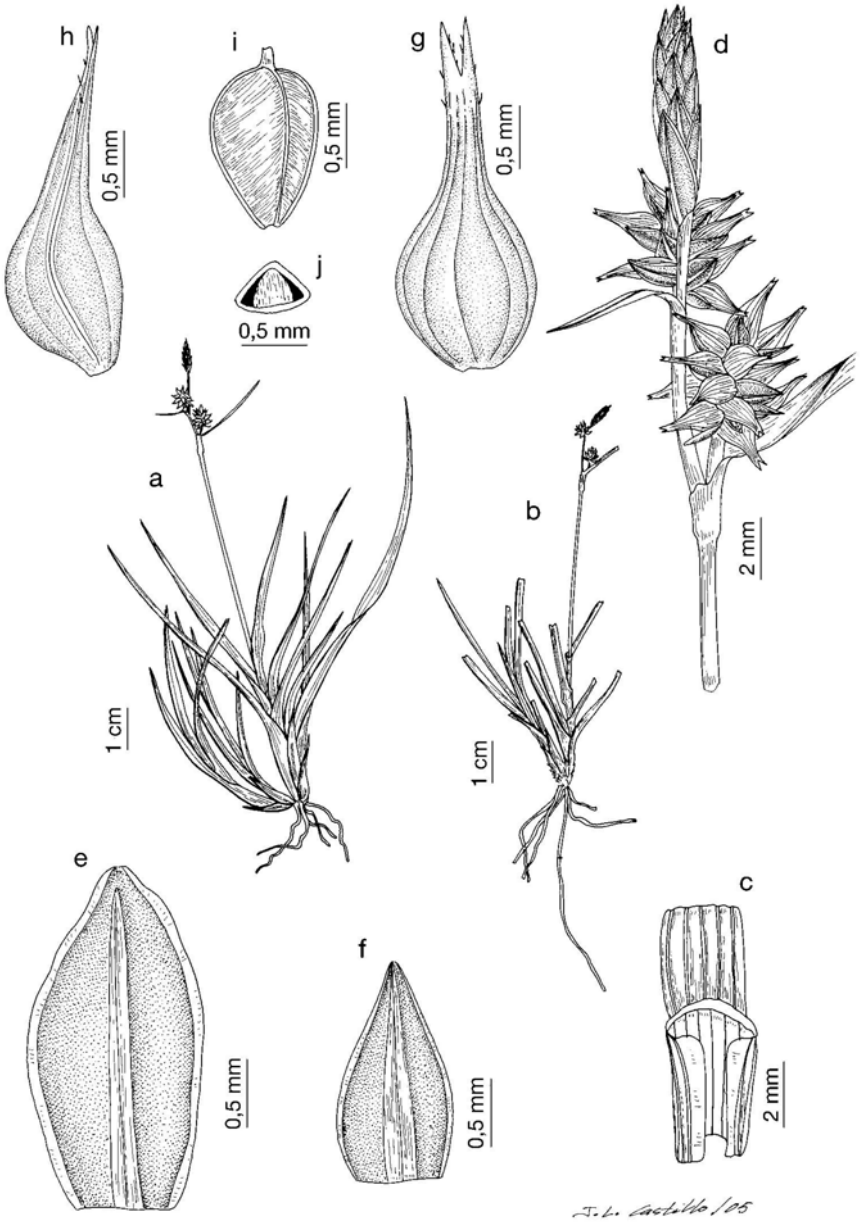
Cespitosa. Tallos fértiles (1)3-12(20) cm, obtusamente trígonos, lisos. Hojas 1-3,1 mm de anchura, de longitud menor o igual que los tallos, planas, algo rígidas, de color verde claro, ± lisas; lígula 1,5-2 mm, de ápice ± obtuso; sin antelígula; vainas basales ± enteras, de color pardo claro. Bráctea inferior 1,5-3,2 cm × 0,9-1,5 mm, generalmente de longitud menor o igual que la inflorescencia, muy raramente algo mayor, setácea o muy cortamente foliácea. Espiga masculina solitaria, muy rara vez 2, de (5)6-10(12) × 1,2-2,5(2,8) mm, fusiforme, sésil o casi; espigas femeninas (1)2-3(5), de 4-10 × 3-6 mm, ± agrupadas junto a la masculina, a veces la inferior subbasilar, subglobosas, la(s) superior(es) en ocasiones andrógina(s). Glumas masculinas ovales, obtusas, de color pardo, con estrecho margen escarioso; glumas femeninas ovales, agudas, de color pardo-rojizo muy obscuro, con una banda central más clara y margen escarioso estrecho, uninervias. Utrículos (1,5)2,2-3(3,1) × 0,7-1,7 mm, los inferiores reflejos, los de la



Lám. 57.–*Carex flava*, carretera a Baños de Tredós Lérida (UPOS 1922): a) hábito; b) lígula; c) espigas; d) gluma ó; e) gluma '; f) utrículo, vista dorsal; g) utrículo, vista lateral; h) sección transversal de un utrículo; i) aquenio; j) sección transversal de un aquenio.

mitad superior de patentes a erectos-patentes y los apicales generalmente erectos, de contorno elíptico, trígonos, plurinerviados, de color pardo obscuro al menos en la mitad apical, más raramente pardo claro, bruscamente contraídos en un pico de 0,8-1,5 mm, ± reflejo en los utrículos de la mitad inferior de la espiga (15-60º respecto del cuerpo del utrículo), recto o casi en los de la mitad superior, bífido, liso o con acúleos dispersos. Aquenios 1,1-1,5 × 0,6-1,1 mm, de contorno obovado, trígonos. 2*n* = 68.

Turberas, cervunales húmedos y bordes de arroyos y lagunas, sobre micaesquistos; 2000-3085 m. (V)VI-VIII. ◆ Sierra Nevada y Sierra de los Filabres. Esp.: Al Gr.



Lám. 58.–*Carex nevadensis*, a) chorreras de Siete Lagunas, Sierra Nevada, Granada (MA 422353); b-j) Corral del Veleta, Sierra Nevada, Granada (MA 342440): a, b) hábito; c) lígula; d) inflorescencia; e) gluma ó; f) gluma '; g) utrículo, vista dorsal; h) utrículo, vista lateral; i) aquenio; j) sección transversal de un aquenio.

Observaciones.–Los caracteres que definen este taxon parecen suficientemente consistentes como para considerar el rango específico, a pesar de nuestras opiniones pasadas [M. Luceño in Ruizia 14: 88 (1994)]. *C. nevadensis* es un taxon de morfología poco variable, intermedia entre *C. lepidocarpa* y *C. demissa*. En contra de la subordinación bajo *C. lepidocarpa*, ha de valorarse la ecología de la planta: suelos ácidos en *C. nevadensis* frente a básicos en *C. lepidocarpa*. Además, la distribución de esta especie, restringida a las altas montañas del SE peninsular, hace muy difícil la hibridación con otros táxones del grupo; únicamente es simpátrica con una población almeriense de *C. demissa*, sin que se hayan encontrado intermedios introgresivos.

Los ejemplares citados como *C. nevadensis* de la Cordillera Cantábrica y Pirineos corresponden a formas introgresivas de *C. flava*, *C. lepidocarpa* y *C. demissa*; estas plantas presentan una morfología muy variable, intermedia entre la de los parentales, y se pueden diferenciar bien de *C. nevadensis* por su bráctea de anchura mayor y sus utrículos mayores. La distinción de las formas enanas de cumbre es más compleja, aunque frecuentemente posible: presentan utrículos de color verde o amarillento, excepcionalmente pardo obscuro, y con el pico generalmente recto.

Respecto de la presencia de *C. nevadensis* en otras montañas europeas, parte de la confusión viene dada por la sinonimización que a ella hace A.O. Chater [in Tutin & al. (eds.), Fl. Eur. 5: 310] de *C. flava* var. *alpina*, que es una forma de alta montaña de *C. flava*, descrita de C Europa. Finalmente, la presencia de *C. nevadensis* en Córcega quedó descartada por J. Lambinon & al. [in Candollea 47: 306-311 (1992)], quienes hacen corresponder estas plantas a formas de *C. viridula*.

54. C. lepidocarpa Tausch in Flora 17: 179 (1834) [lepidocárpa]

C. flava auct. iber., p.p., non L., Sp. Pl.: 975 (1753)
C. flava var. *lepidocarpa* (Tausch) Godr., Fl. Lorraine 3: 118 (1844)
C. flava subsp. *lepidocarpa* (Tausch) Nyman, Consp. Fl. Eur.: 771 (1882)
C. flava subsp. *alpina* sensu O. Bolòs & Vigo, Fl. Països Catalans: 306 (2001), p.p., non (Kneuck.) O. Bolòs, Masalles & Vigo in Collect. Bot. (Barcelona) 17: 95 (1988)
Ind. loc.: "In aquis stagnantibus, turfosis Austriae, Bohemiae, Pannoniae" [lectótipo designado por W.J. Crins & P.W. Ball in Canad. J. Bot. 67: 1058 (1989): Czechoslovakia, Praha, No. 1636 (PRC)]
Ic.: Lám. 59

Cespitosa. Tallos fértiles 10-47(85) cm, obtusamente trígonos, lisos, rectos o algo arqueados. Hojas 1,4-3,6(5) mm de anchura, generalmente de menor longitud que los tallos, planas, poco rígidas, de color verde claro u obscuro, ± lisas; lígula hasta 2,7 mm, obtusa o truncada; antelígula hasta 1,5(4,2) mm, de ápice redondeado, a veces inexistente; vainas basales ± enteras, de color pardo. Bráctea inferior 2,9-10(11) cm × 0,6-2,9(3,6) mm, generalmente de mayor longitud que la inflorescencia, foliácea. Espiga masculina solitaria, de (5)6,5-20(26) × 1,3-3 mm, cilíndrica o fusiforme, con un pedúnculo hasta de 4 cm; espigas femeninas (1)2-4, de 4-15 × 4-11 mm, aproximadas o ligeramente separadas, elípticas o subglobosas, subsésiles o cortamente pedunculadas, erectas. Glumas masculinas ovales, obtusas, de color pardo, con estrecho margen escarioso; glumas femeninas ovales, agudas u obtusas, de color pardo-rojizo claro u obscuro, con margen escarioso estrecho, uninervias. Utrículos 3-4,2(5) × 0,9-1,7(2) mm, los de la mitad inferior reflejos, los de la mitad superior de reflejos a patentes y los apicales generalmente patentes a suberectos, de contorno elíptico, inflado-trígonos, plurinerviados, bruscamente estrechados en un pico de 0,9-1,7(2,1) mm, reflejo (25-90º respecto del cuerpo del utrículo), bidentado o bífido, liso o ligeramente escábrido. Aquenios 1,2-1,8 × 0,9-1,4 mm, de contorno obovado, trígonos. 2*n* = 66, 68, 70.

Turberas y prados muy húmedos en suelos básicos; 50-2500 m. (IV)V-VII(IX). Europa, Marruecos (Alto Atlas) y E de Norteamérica. En la Península habita sobre todo en la mitad E y zonas montañosas del Cantábrico. **And**. **Esp.:** Ab B Bi Bu CR Cu Ge Gu Hu J L Le Lo Na O P S Sg So SS Te Va Vi Z Za. **Port.:** E.

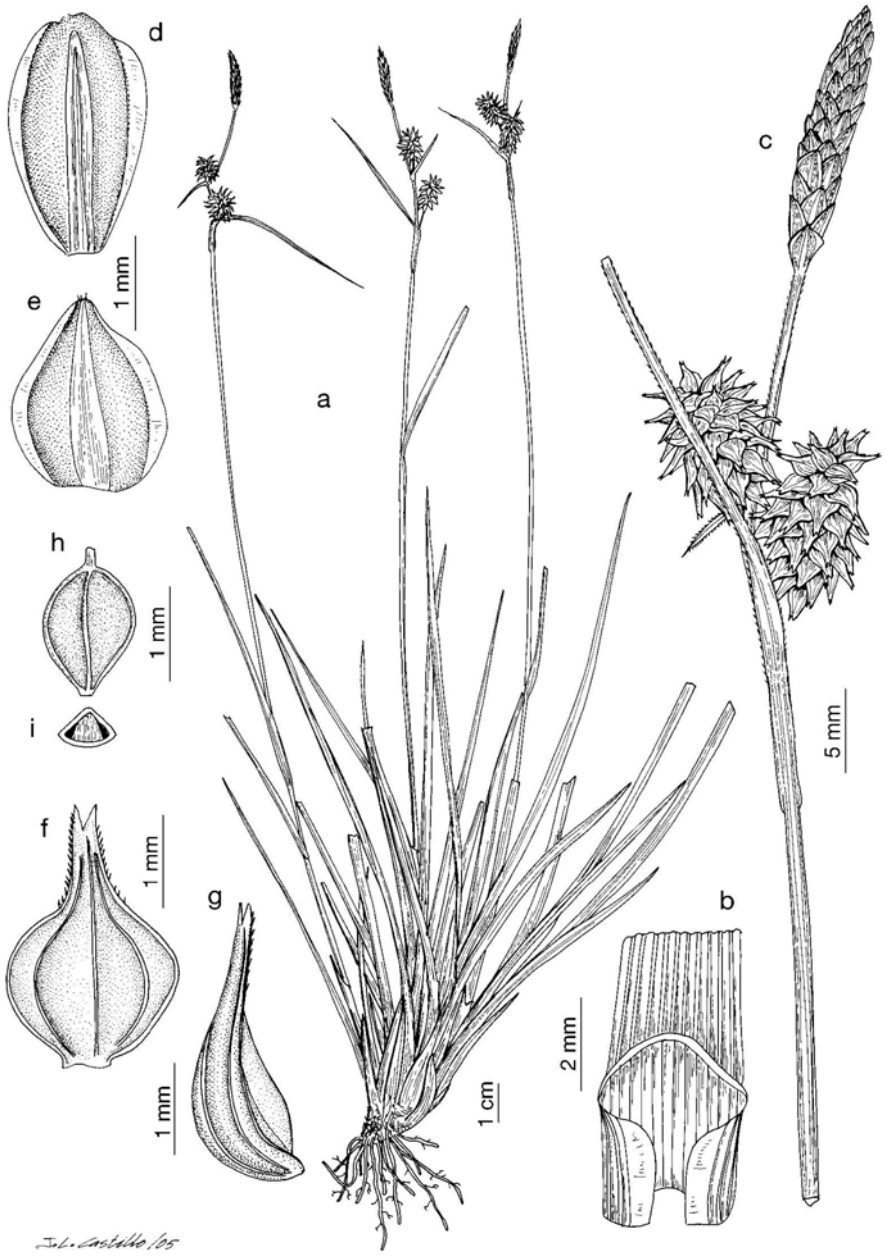
55. C. demissa Hornem., Fl. Dan. 8(23): 4 (1808) [demíssa]

C. oederi var. *oedocarpa* Andersson, Pl. Scand. 1: 25 (1849)
C. flava subsp. *oedocarpa* (Andersson) P.D. Sell in P.D. Sell & G. Murrell (eds.), Fl. Great Britain Ireland 5: 363 (1996)
C. flava auct. iber., p.p., non L. Sp. Pl.: 975 (1753)
C. oederi auct. iber., p.p., non Retz., Fl. Scand. Prodr.: 179 (1779)
Ind. loc.: "In alpinis Tellemarken Norvegiae invenit Celeb." [lectótipo designado por K. Wiinstedt in Bot. Tidsskr. 48: 192 (1948): *Vahl* (C)]
Ic.: Lám. 60

Cespitosa. Tallos fértiles 3,5-35(45) cm, lisos, obtusamente trígonos, frecuentemente arqueados. Hojas 1,8-4(9) mm de anchura, de menor longitud que los tallos, lisas, planas, algo rígidas; lígula hasta 2,5 mm, de menor anchura que el limbo, de ápice redondeado o truncado; generalmente sin antelígula; vainas basales enteras, de color pardo muy claro o verde pálido. Bráctea inferior foliácea, de 2,2-12(18) cm × (1)1,3-3,2 mm, generalmente de longitud mucho mayor que la inflorescencia. Espiga masculina solitaria, de (1)4,5-20(25) × 1,3-3,3 mm, cilíndrica o fusiforme, con un pedúnculo de 2-11(28) mm; espigas femeninas (1)2-5, de 5,5-16 × 5,3-8,5 mm, cortamente oblongas o elípticas, erectas, las superiores aproximadas, la inferior casi siempre distante, basilar o casi, largamente pedunculada. Glumas masculinas ± ovales, agudas u obtusas, generalmente de color pardo-rojizo claro, excepcionalmente obscuro; glumas femeninas ovales, agudas u obtusas, generalmente de color pardo rojizo claro, con 1-3 nervios. Utrículos 2,5-4,1(4,3) × 1-1,5(1,6) mm, los inferiores patentes, raramente algunos reflejos, los de la mitad superior de patentes a erectos-patentes y los apicales generalmente erectos, inflado-trígonos, con todos los nervios resaltados, estrechados bruscamente en un pico de (0,7)0,8-1,7(1,8) mm, liso o ligeramente escábrido, recto o ligeramente curvado (0-30º respecto del cuerpo del utrículo). Aquenios 1,1-1,5 × 0,9-1,2 mm, de contorno obovado, trígonos. 2*n* = 68, 70.

Turberas y bordes de arroyos sobre substratos ácidos, aunque en ocasiones aparece sobre substratos calizos en regiones con precipitaciones elevadas; 100-2400 m. (IV)V-VIII. Eurasia, hasta Asia Central, Marruecos (Rif y Atlas), Canarias, Azores y Madeira; naturalizada en el NE de Norteamérica, Tasmania y Nueva Zelanda. Pirineos, comarca de La Selva, cornisa Cantábrica, Galicia, N y C de Portugal, Montes de León, Sistemas Central e Ibérico, Sierra Nevada almeriense (muy poco frecuente), Parque Natural de Los Alcornocales (Cádiz) y comarca del Andévalo (Huelva). **Esp.:** Al Av B Bi Bu C Ca Cc Ge Gu H Hu L Le Lo Lu M Na O Or Po S Sa Sg So SS T Te Vi Za. **Port.:** BA BAl BB BL DL E Mi TM.

Observaciones.–Como hemos señalado en las observaciones al gr. de *C. flava*, algunas poblaciones de la franja atlántica peninsular muestran introgresión con *C. viridula* y tienden a presentar utrículos inferiores reflejos y con el pico algo curvado, por lo que han sido reiteradamente confundidas con *C. flava*.

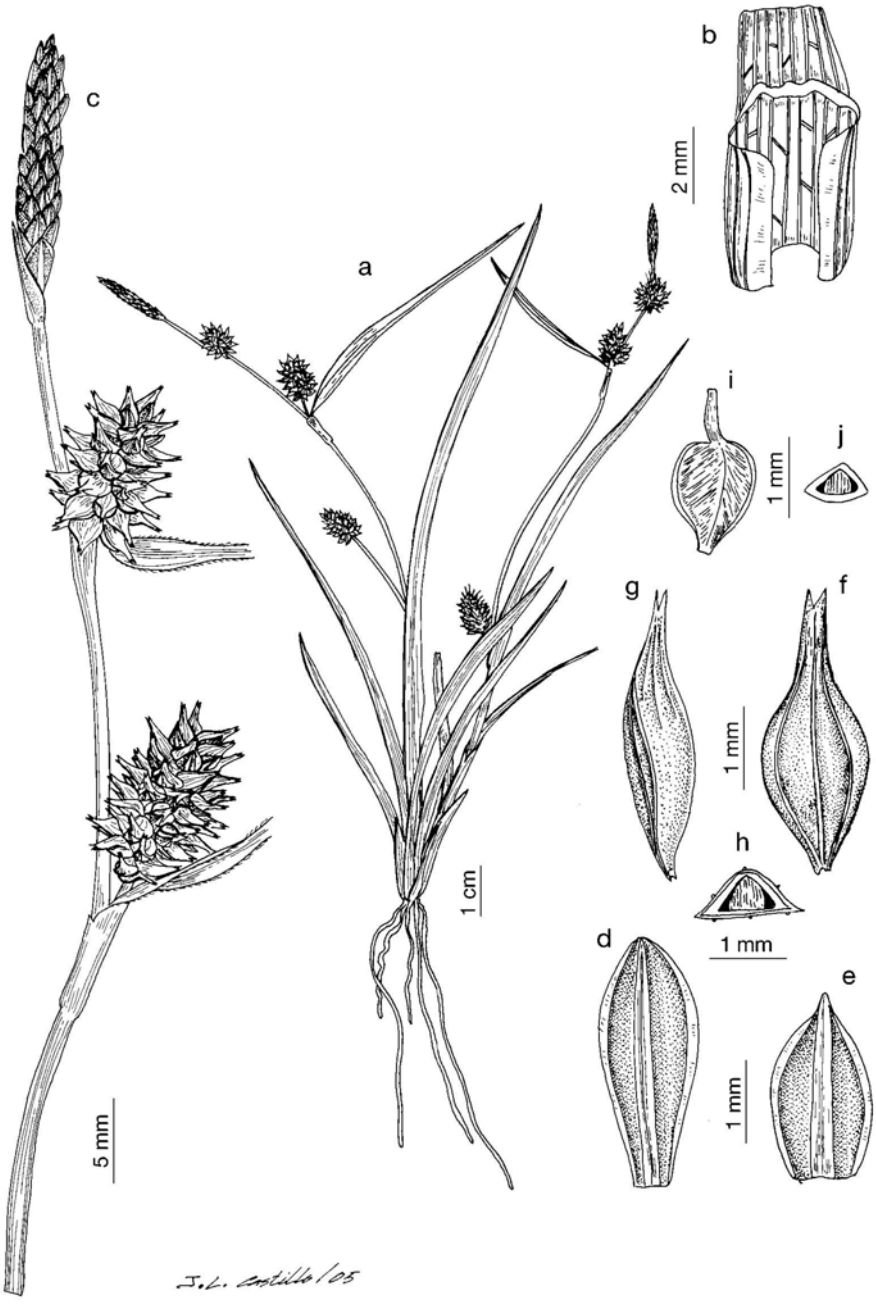


Lám. 59.–*Carex lepidocarpa*, Velilla de Medinaceli, Soria (MA 386925): a) hábito; b) lígula; c) inflorescencia; d) gluma ó; e) gluma '; f) utrículo, cara abaxial; g) utrículo, vista lateral; h) aquenio; i) sección transversal de un aquenio.

56. C. viridula Michx., Fl. Bor.-Amer. 2: 170 (1803) [virídula]

C. oederi Retz., Fl. Scand. Prodr.: 179 (1779)
C. oederi Ehrh. in Beitr. Naturk. 6: 83 (1791), nom. illeg.
C. flava subsp. *viridula* (Michx.) O. Bolòs & Vigo, Fl. Països Catalans 4: 305 (2001)
C. serotina Mérat in Nouv. Fl. Env. Paris ed. 2, 2: 54 (1821)
Ind. loc.: "Hab. in Canada" [lectótipo designado por T.V. Egorova, Sedges Russia: 281 (1999): *Michaux* (P)]
Ic.: Jermy & Tutin, Brit. Sedges ed. 2: 163 (1982) [sub *C. serotina*]; lám. 61

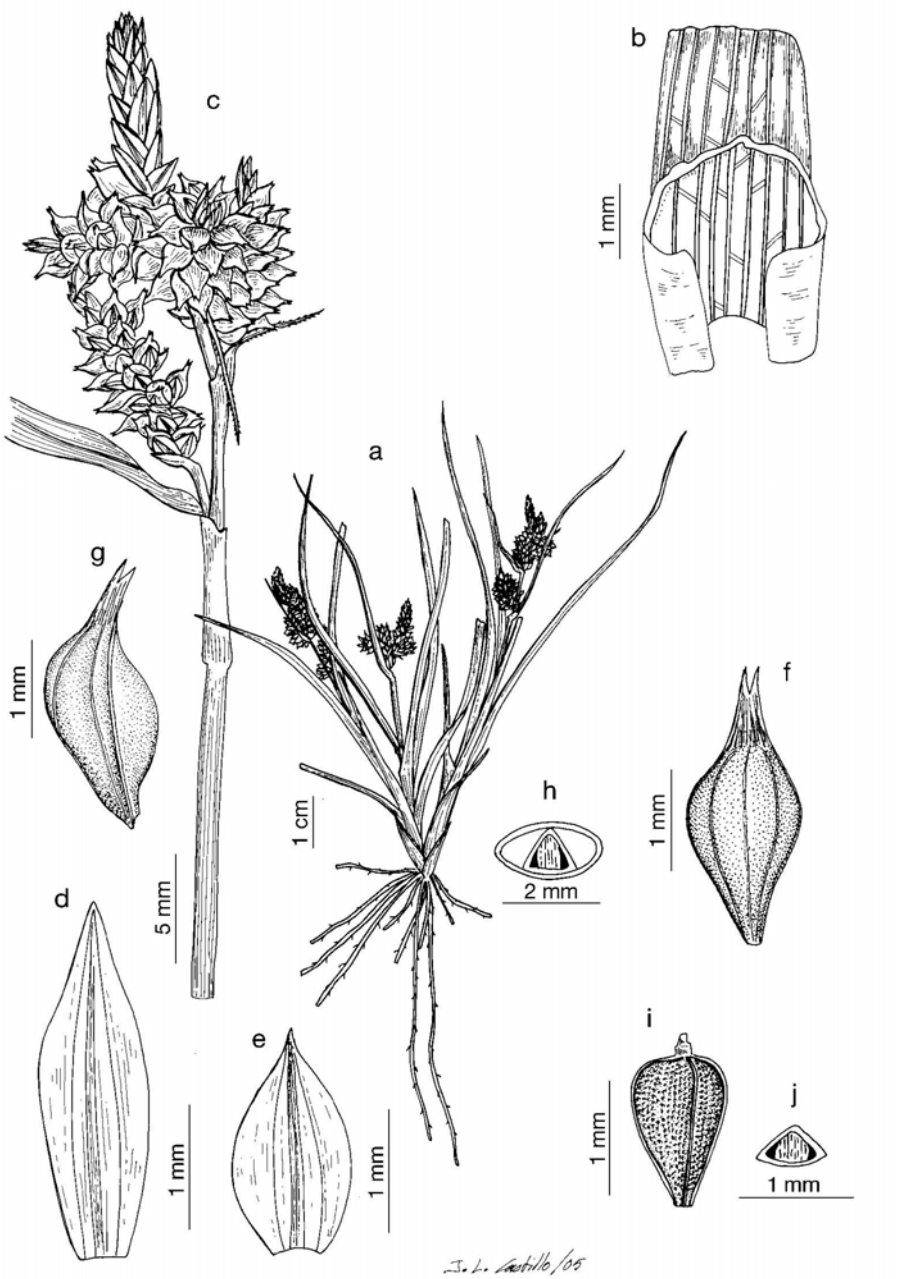
Cespitosa. Tallos fértiles 2-20(40) cm, obtusamente trígonos, lisos, completamente rectos. Hojas 1-2,6(3,1) mm de anchura, de menor longitud que los tallos, lisas, de color verde claro, planas o algo canaliculadas, poco rígidas; lígula muy corta, con ápice frecuentemente oblicuo y truncado; antelígula hasta 0,5 mm, de ápice redondeado, muchas veces inexistente; vainas basales enteras, de color pardo. Bráctea inferior foliácea, de 2,4-13 cm × 0,9-2,2 mm, de mayor longitud que la inflorescencia. Espiga masculina solitaria, de 4-12(20) × 1-3,3 mm, cilíndrica o fusiforme, sésil o con un pedúnculo hasta de 20 mm; espigas femeninas 1-8, de 5-17 × 3-8 mm, cortamente cilíndricas a subglobosas, generalmente



Lám. 60.–*Carex demissa*, majada de los Nietos, Vinuesa, Soria (MA 386927): a) hábito; b) lígula; c) inflorescencia; d) gluma ó; e) gluma '; f) utrículo, vista dorsal; g) utrículo, vista lateral; h) sección transversal de un utrículo; i) aquenio; j) sección transversal de un aquenio.

agrupadas bajo la masculina, muy rara vez la inferior separada e incluso subbasilar, sésiles o cortamente pedunculadas, erectas. Glumas masculinas lanceoladas o estrechamente ovales, agudas, obtusas o acuminadas, de color pardo rojizo claro u obscuro; glumas femeninas ± ovales, agudas u obtusas, de color pardo a pardo-rojizo claro, con 3 nervios. Utrículos (1,8)2,2-3 × 0,8-1,6 mm, los inferiores patentes o ligeramente reflejos, los de la mitad superior de patentes a erectos-patentes y los apicales con frecuencia erectos, subglobosos, de contorno obovado, plurinerviados, bruscamente estrechados en un pico de 0,3-0,8(1,3) mm, recto, bidentado, liso. Aquenios 1-1,6 × 0,7-1,3 mm, de contorno obovado, trígonos. 2*n* = 68*, 70, 72*.

Turberas y otros suelos húmedos; indiferente edáfica, aunque en la Península habita frecuentemente en humedales con cierto grado de salinidad; 5-1900 m. (V)VI-XII(I). Europa, W de Asia, Japón y Norteamérica. Muy dispersa en la Península. **Esp.:** Ab B Bi Bu C Ge Gu Hu Le Lu O Po S Sa Sg So Vi. **Port.:** BL DL E.



Lám. 61.–*Carex viridula*, lagunas de Ruidera, Albacete (MA 237708): a) hábito; b) lígula; c) inflorescencia; d) gluma ó; e) gluma '; f) utrículo, vista dorsal; g) utrículo, vista lateral; h) sección transversal de un utrículo; i) aquenio; j) sección transversal de un aquenio.

Observaciones.–La problemática nomenclatural de este taxon ha sido abordada en profundidad por varios especialistas del grupo de *C. flava*. En Europa los nombres que se han aplicado con mayor frecuencia son *C. oederi* Retz. y *C. serotina* Mérat; por otro lado, en Norteamérica el nombre utilizado ha sido *C. viridula* Michx. El nombre prioritario que debiera aplicarse a esta planta sería. *oederi* Retz., pero las discusiones acerca de este binomen aconsejan ser prudentes en su uso.

B. Schmid [in Watsonia 14: 309-319 (1983)] lo rechaza en favor de *C. viridula* debido a los problemas que surgen en torno a su tipificación: entre el material de A. Retzius no existe ningún pliego que corresponda a *C. oederi*, y el anteriormente considerado como tipo está corregido por el propio Retzius como *C. pilulifera*. M. Hedrén [in Nordic J. Bot. 22: 257-301 (2002)] lectotipifica *C. oederi* sobre la lámina 371 de *Flora Danica* (Oeder, 1768), pero B. Schmid (l.c., 1989) ya había señalado que se trata de una lámina de escaso detalle que no puede ser identificada inequívocamente. Por ello, M. Hedrén (l.c., 2002) designa un epítipo, un pliego cuya etiqueta parece corresponder con la escritura de Retzius. Sin embargo, E. Nelmes [in J. Bot. 77: 301-304 (1939)] había identificado con anterioridad este material de herbario como un individuo poco desarrollado de *C. lepidocarpa*. Por todo lo expresado, preferimos no usar el binomio de Retzius y seguir con el uso de *C. viridula* Michx., prioritario sobre *C. serotina* Mérat.

Sect. 42. **Phacocystis** Dumort.

Plantas cespitosas o no. Tallos trígonos, lisos o escábridos. Bráctea inferior foliácea, no o apenas envainante. Espigas masculinas 1-varias, terminales, cilíndricas o fusiformes, raramente ovoides, o bien la terminal ginecandra; espigas laterales femeninas y/o andróginas, ovoides o cilíndricas, generalmente densifloras. Glumas femeninas de ápice variable. Estigmas 2. Utrículos de erectos a erecto-patentes, ± comprimidos, biconvexos o plano-convexos, glabros, frecuentemente papilosos.

Observaciones.–La sección *Phacocystis* es una de las más complicadas desde el punto de vista taxonómico de todo el género. Sus mecanismos de evolución y el comportamiento reproductivo de las especies han sido objeto de profundos estudios [J. Cayouette & P. Morisset in Canad. J. Bot. 63: 1957-1982 (1985); J.S. Faulkner in Bot. J. Linn. Soc. 65: 271-300 (1972); in Bot. J. Linn. Soc. 67: 233-253 (1973); M. Luceño & C. Aedo in Bot. J. Linn. Soc. 114: 183214 (1994)]. Se trata de plantas que siguen una estrategia ecológica de tipo *k*, con un importante porcentaje de reproducción vegetativa. En la meiosis estas plantas presentan frecuentes irregularidades en el apareamiento de los cromosomas que conllevan la inviabilidad de los aquenios: una misma especie exhibe varios números de cromosomas y la presencia de trivalentes y univalentes es un fenómeno común. Además, son frecuentes los fenómenos de hibridación.

84. C. elata All., Fl. Pedem. 2: 272 (1785) [eláta]

Ind. loc.: "In spongiosis vallis Segusinae et Ulciensis non infrequens"

Densamente cespitosa, de cepa robusta que forma grandes macollas, aunque puede desarrollar rizomas con entrenudos de varios centímetros. Tallos 30-170 cm, escábridos a lisos en la zona superior, agudamente trígonos. Hojas 3-6(10) mm de anchura, generalmente de menor longitud que los tallos, planas, ásperas en los bordes y el nervio medio por el envés, con frecuencia también hacia el ápice por el haz, de blandas a medianamente rígidas, un poco glaucas; lígula hasta 5 mm, de ápice subagudo a redondeado; sin antelígula; vainas basales escuamiformes, de color pardo amarillento a pardo rojizo obscuro, raramente purpúreas, enteras. Bráctea inferior foliácea, de longitud de mucho menor a algo mayor que la inflorescencia, no o apenas envainante. Espigas masculinas 1-4, de (10)20-80 mm, ± cilíndricas; espigas andróginas 0-5(6) y/o espigas femeninas 0-3(4), de 1380(115) mm, cilíndricas, densas, distribuidas a lo largo de la parte superior del tallo, generalmente cercanas las unas de las otras, erectas o colgantes. Glumas masculinas estrechamente oblongas, de ápice redondeado a subagudo, pardo-rojizas a negras, con la quilla verde, sin margen escarioso; glumas femeninas de longitud igual o algo menor que los utrículos, ovales, oblongo-lanceoladas u oval-lanceoladas, de ápice agudo, de pardas a negruzcas con quilla central verde. Utrículos 1,8-4,5 × 1-2,5 mm, suberectos o erecto-patentes, de contorno ± elíptico, compri¬midos, ligeramente biconvexos o planoconvexos, lisos o papilosos, con los ner¬vios perceptibles, bruscamente estrechados en un corto pico hasta de 0,2 mm, truncado o raramente emarginado, papiloso. Aquenios 1,5-2 × 1,1-1,5 mm, de contorno anchamente obovado a suborbicular, lenticulares, pardos.

Bordes y lechos de ríos, gargantas y lagunas; 10-1900 m. (II)IV-VII(VIII). Gran parte de Europa, Cáucaso, W de Irán y Marruecos. Distribuida por la mayor parte de la Península. Esp.: Ab Av Bi Bu C Ca Cc Co CR Cu (Ge) Gr Gu H (Hu) J Le Lo Lu M Ma Na O Or P Po Sa Se Sg So SS (T) Te To V Vi Z Za. Port.: AAl BA BB BL DL E Mi R TM. N.v.: masiega.

Observaciones.–El grupo de *C. elata* muestra una acusada variabilidad, lo que entraña serias dificultades para su clasificación. En el presente tratamiento hemos admitido 3 subespecies: la subsp. *elata*, del CN y E de la Península, así como de puntos aislados de la franja atlántica, la subsp. *reuteriana*, del NW y C-CW de la Península, con su límite S en Sierra Morena, en España, y la sierra de São Mamede en Portugal, y la subsp. *tartessiana*, de distribución exclusivamente andaluza.

En ciertas zonas, la identificación de estas 3 subespecies se hace problemática. Ello ocurre en el Sistema Ibérico septentrional (Burgos, Soria), donde pueden verse formas intermedias entre las subespecies *elata* y *reuteriana*, y en Sierra Morena oriental (Ciudad Real, Jaén), zona de contacto de las subespecies *reuteriana* y *tartessiana*.

En no pocas localidades de Burgos y Soria los individuos analizados muestran papilas ± conspicuas, de la misma coloración que el utrículo; del mismo modo, la bráctea inferior de los tallos presenta una longitud muy variable, desde menor que la espiga que axila hasta algo mayor que la inflorescencia. Podemos considerar estos individuos como formas introgresivas entre las subespecies *elata* y *reuteriana*.

En Sierra Morena oriental podemos considerar la vertiente andaluza como el límite NE de la distribución de la subsp. *tartessiana* s.str. Sin embargo en Ciudad Real no pocos individuos de la subsp. *reuteriana* se asemejan a la subsp. *tartessiana*; estos individuos también pueden ser encontrados en algunos ríos jienenses. La proximidad entre ambas subespecies aconseja ser prudentes en la interpretación de estos ejemplares; probablemente se estén produciendo fenómenos de hibridación entre ambos táxones.

1. Utrículos de color verdoso-grisáceo, con papilas blanquecinas altas hacia el ápice; bráctea inferior generalmente de menor longitud que la inflorescencia, con frecuencia del tamaño de la espiga inferior ..... **a.** subsp. **elata**
– Utrículos verdes, a veces teñidos de negro, lisos o con alguna papila poco elevada de la misma coloración que el utrículo; bráctea inferior de longitud ± igual que la inflorescencia 2
2. Espiga masculina generalmente solitaria; espigas inferiores generalmente femeninas . ..... **b.** subsp. **reuteriana**
– Espigas masculinas (1)2-4; espigas inferiores generalmente andróginas ..... **c.** subsp. **tartessiana**

a. subsp. **elata**

C. stricta sensu Gooden. in Trans. Linn. Soc. 2: 196, 210 tab. 21 fig. 9 (1794), non Lam., Encycl. 3: 387 (1792)
Ic.: Boott, Ill. Carex, tab. 584, 585 (1867) [sub *C. stricta*]; Jermy & Tutin, Brit. Sedges ed. 2: 205 (1982) [sub *C. elata*]

Tallos generalmente muy escábridos hacia el ápice. Hojas hasta de 6(7) mm de anchura, ± rígidas, generalmente ásperas por el haz; vainas basales pardo amarillentas a pardo anaranjadas, brillantes. Bráctea inferior generalmente de menor longitud que la inflorescencia, con frecuencia igual o algo menor que su espiga. Espigas masculinas 1-2, la superior de 25-80 × 3-6 mm; espigas andróginas 0-3(4) y espigas femeninas 0-2(3), de 17-71(73) × 3,5-6,5 mm, anchamente cilíndricas a oblongas. Glumas femeninas generalmente oblongas u oblongo-lanceoladas, de color pardo obscuro o negruzcas. Utrículos 2,5-4,5 × 1,5-2,5 mm, de color verdoso-grisáceo, con papilas blanquecinas altas al menos hacia el ápice, con los nervios claramente perceptibles. 2*n* = 76, 77, 74-78*, 80*.

Bordes de ríos y lagunas; en la Península Ibérica más frecuente en substratos calizos; 10-1750 m. (III)IV-VII(VIII). Gran parte de Europa aunque más rara en el S; Balcanes, Cáucaso, W de Irán y algunas localidades en Marruecos. En la Península habita en el CN y NE, y puntos aislados del SE y de la franja atlántica. **Esp.:** Ab Bi Bu Cu (Ge) Gu H (Hu) J Lo Na P Sg So SS T Te V Vi Z. **Port.:** AAl BAl BL.

b. subsp. **reuteriana** (Boiss.) Luceño [Reuteriána]
in Anales Jard. Bot. Madrid 47: 143 (1990)
C. reuteriana Boiss. in Boiss. & Reut., Pugill. Pl. Afr. Bor. Hispan.: 116 (1852) [basión.]
C. stricta var. *microstachya* Merino, Fl. Galicia 3: 173 (1909)
C. broteriana Samp. in Ann. Sci. Acad. Polytecn. Porto 19(2): 68 (1934)
Ind. loc.: "Hab. ad torrentes Castellae veteris propè la Granja (Reut. Jul. 1841), habeo quoque ex Lusitaniâ in herb. Pavon" [lectótipo designado por H.M. Burdet et al. in Candollea 36: 551: (1981): *Reuter s.n.*, Herb. Boissier (G)]
Ic.: Lám. 71

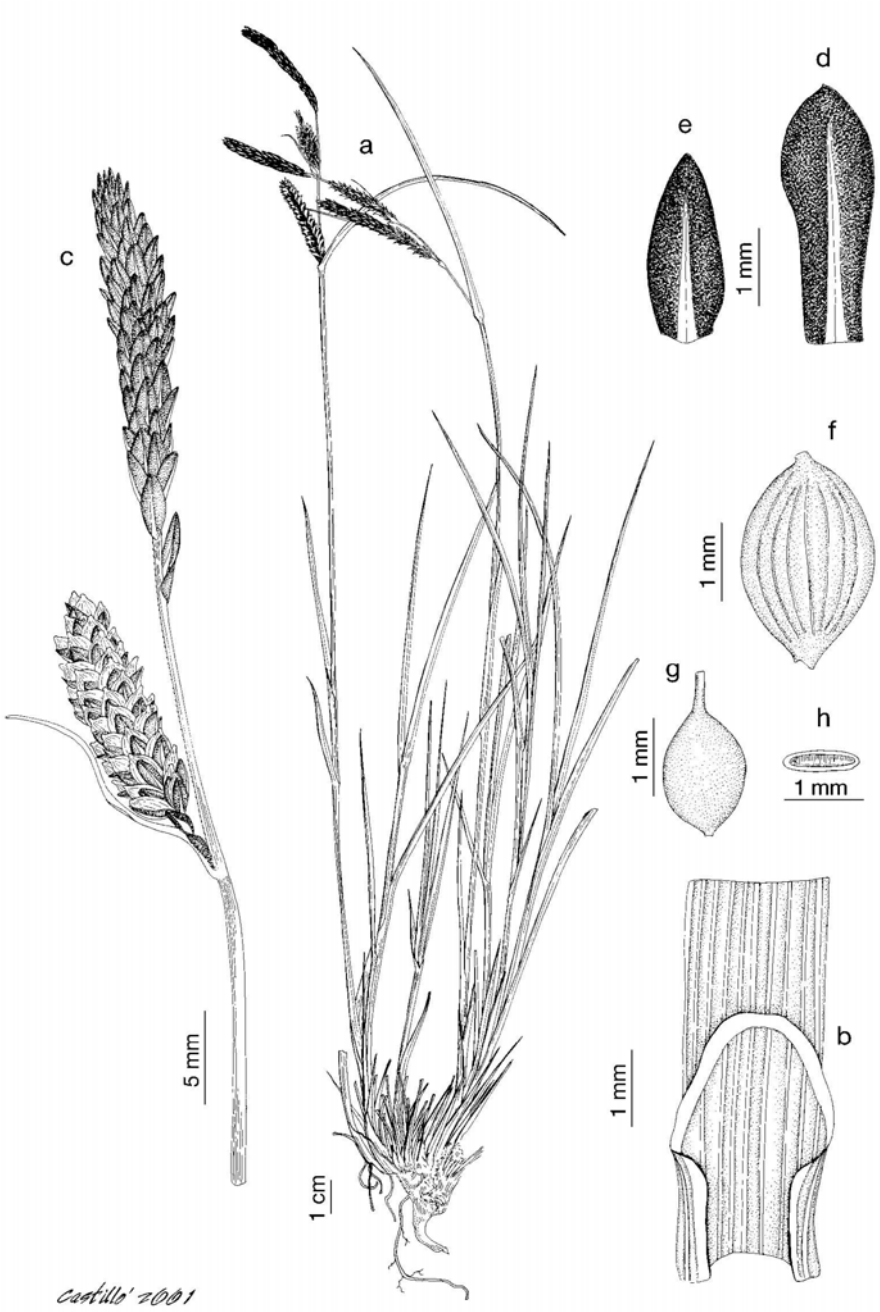
Tallos escábridos hacia el ápice a casi lisos. Hojas hasta de 6 mm de anchura, de algo rígidas a ± blandas, generalmente lisas por el haz; vainas basales de pardo-anaranjadas a pardo-rojizas, excepcionalmente purpúreas, fibrosas. Bráctea inferior de longitud poco menor, igual o algo mayor que la inflorescencia, pero siempre mucho mayor que su espiga. Espiga masculina solitaria, de (15)2060(70) × 2-4 mm, raramente con otra más pequeña en su base; espigas andróginas 0-3(6) y espigas femeninas 0-3(4), de 13-65(90) × 2,5-4,6 mm, estrechamente cilíndricas. Glumas femeninas de ovales a lanceoladas, de color pardo a negruzcas, con la quilla verde. Utrículos 1,8-3(3,5) × 1-1,6 mm, verdes, a veces teñidos de negro, lisos, con los nervios ± perceptibles. 2*n* = 73, 74, 75, 76.

Bordes y lechos de ríos y gargantas en las montañas y sus proximidades, sobre suelos silíceos; 300-1900 m. III-VII. ◆ NW, C-CW de la Península Ibérica, Montes de Toledo y Sierra Morena. **Esp.:** Av C Cc CR Gu Le Lu M O Or P Po Sa Sg So To Za. **Port.:** AAl BA BB BL DL Mi R TM.

c. subsp. **tartessiana** Luceño & Aedo in Bot. J. [tartessiána]
Linn. Soc. 114: 205 (1994)
C. acuta auct. hisp., non L., Sp. Pl.: 978 (1753)
C. mauritanica auct. hisp., non Boiss. & Reut., Pugill. Pl. Afr. Bor. Hispan.: 116 (1852) *Ind. loc.:* "Sevilla, El Ronquillo, 29SQB4978, 350 m, 29-III-1989, en borde de arroyo, Guzmán, Luceño & Vargas" [holótipo MA 505836; isótipos G, SEV]
Ic.: Lám. 72

Tallos escábridos hacia el ápice a casi lisos. Hojas (3)4-8(10) mm de anchura, ± rígidas, lisas o ásperas por el haz; vainas basales de pardo-anaranjadas a pardo-rojizas, excepcionalmente purpúreas, fibrosas. Bráctea inferior de longi¬tud de menor a algo mayor que la inflorescencia, pero siempre mucho mayor que su espiga. Espigas masculinas (1)2-4, la superior de (10)20-70 × 1,2-3,5 mm; espigas andróginas (0)1-5, generalmente ninguna femenina, de (18)25¬80(115) × 2,3-5,3 mm, estrecha o anchamente cilíndricas. Glumas femeninas de ovales a lanceoladas, pardas o negras con la quilla verde. Utrículos (1,8)2,3-4,5 × 1-2 mm, verdes, lisos o con alguna papila poco elevada hacia el ápice, con los nervios ± perceptibles. 2n = 74, 75, 76.

Bordes y lechos de ríos y gargantas, indiferente edáfica; 20-500 m. (II)III-VI(VII). ◆ Andalucía; el límite N de distribución se encuentra en la vertiente S de Sierra Morena y el W en el Andévalo (Huelva). **Esp.:** Ca Co Gr H J Ma Se.

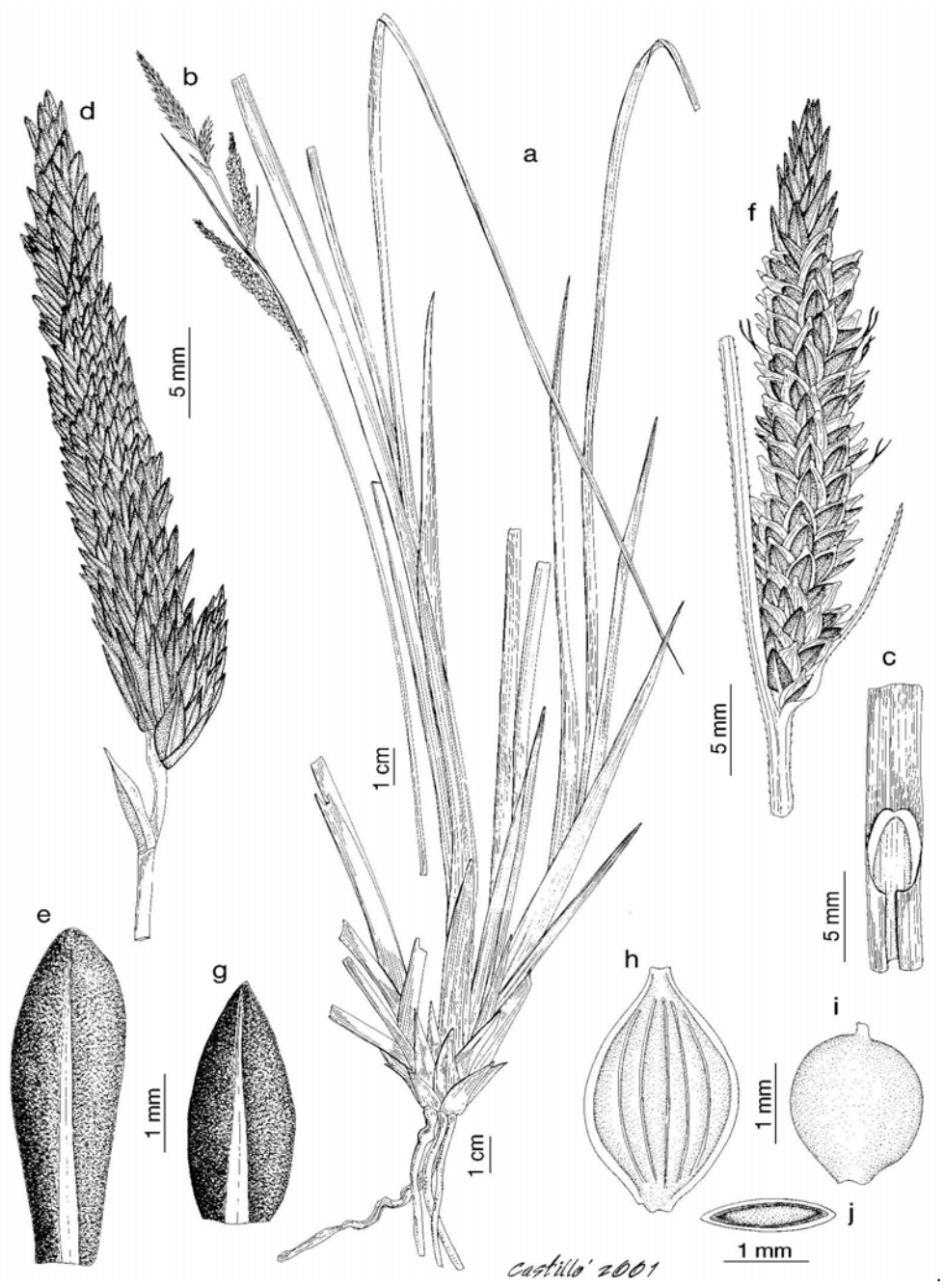


Lám. 71.–*Carex elata* subsp. *reuteriana*, entre Vadillo y Navaleno, Soria (MA 342553): a) hábito; b) lígula; c) inflorescencia ó y '; d) gluma ó; e) gluma '; f) utrículo; g) aquenio; h) sección transversal de un aquenio

85. C. cespitosa L., Sp. Pl.: 978 (1753) [cespitósa]

Ind. loc.: "Habitat in Europae paludibus turfosis" [lectótipo designado por E.S. Marshall in J. Bot. 45: 366 (1907): LINN 1100.69]
Ic.: Mossberg & Stenberg, Suuri Pohjolan Kasvio: 814 (2005)

Densamente cespitosa, de cepa robusta que forma grandes macollas. Tallos 23-150 cm, escábridos en la zona superior, agudamente trígonos. Hojas 2-4 mm de anchura, de longitud generalmente igual a los tallos, planas, ásperas en los bordes y el nervio medio por el envés y por el haz hacia el ápice, ± rígidas; lígula 1,5-2,5 mm, de ápice agudo a obtuso; sin antelígula; vainas basales escuamiformes, de color púrpura, enteras. Bráctea inferior setácea a cortamente foliácea, de menor longitud que la inflorescencia, no envainante. Espiga masculina solitaria, de (12)15-30 mm, de fusiforme a subcilíndrica; espigas femeninas 12(3), de (8)12-25(30) mm, cortamente cilíndricas, densas, situadas en la parte superior del tallo junto a la masculina, ± solapadas, erectas. Glumas masculinas estrechamente oblongas u obovadas, de ápice redondeado, pardo-rojizas con estrecho margen escarioso; glumas femeninas de menor longitud



Lám. 72.–*Carex elata* subsp. *tartessiana*, puerto La Asperilla, río Guadalete, El Gastor, Cádiz (MA 469327): a) parte inferior de la planta; b) parte terminal de un tallo florífero; c) lígula; d) espiga ó; e) gluma ó; f) espiga andrógina; g) gluma '; h) utrículo; i) aquenio; j) sección transversal de un aquenio.

que los utrículos, de estrechamente oblongas a elípticas, de ápice redondeado a obtuso, pardo-rojizas ± obscuras, con quilla verde a pajiza y margen escarioso muy estrecho. Utrículos 2-2,7 × 1,2-1,7 mm, erectos, obovados a elípticos, ± comprimidos, plano-convexos, con papilas blanquecinas al menos hacia el ápice, sin nervios, sin pico o bruscamente estrechados en un pico hasta de 0,1 mm, truncado o raramente emarginado, generalmente papiloso. Aquenios ca. 0,7 × 1,5 mm, de contorno obovado, lenticulares, de color pardusco. 2*n* = 78*, 79*, 80?*.

Bordes y lechos de ríos y gargantas; 10-40 m. IV-VII. Europa, principalmente C y N, Rusia, Turquía, Kazajstán, China y Japón. En la Península Ibérica solo conocemos poblaciones del río Bidasoa. **Esp.:** Na SS.

86. C. nigra (L.) Reichard, Fl. Moeno-Francof. 2: 96 (1778) [nígra]

C. acuta var. *nigra* L., Sp. Pl.: 978 (1753) [basión.]
C. fusca All., Fl. Pedem. 2: 269 (1785)
C. goodenowii J. Gay in Ann. Sci. Nat., Bot. ser. 2, 11: 191 (1839), nom. illeg.
C. intricata Tineo ex Guss., Fl. Sicul. Syn. 2: 574 (1844)
C. vulgaris Fr., Novit. Fl. Suec. Mant. 3: 153 (1845), nom. illeg.
C. nigra subsp. *carpetana* C. Vicioso ex Rivas Mart. in Publ. Inst. Biol. Aplicada 42: 110 (1967), nom. inval., sine ind. holotypus
Ind. loc.: "Habitat in Europa ubique: in siccioribus" [neótipo designado por M. Luceño & C. Aedo in Bot. J. Linn. Soc. 114: 207 (1994): MA 194334]
Ic.: Jermy & Tutin, Brit. Sedges ed. 2: 207 (1982)

Rizoma de entrenudos largos, a veces laxamente cespitoso. Tallos 5-50 cm, escábridos en la zona superior, trígonos. Hojas 3-6(10) mm de anchura, de longitud igual o menor que los tallos, planas, ásperas en los bordes, ± blandas, glaucas; lígula hasta 4 mm, de ápice subagudo a redondeado; sin antelígula; vainas basales generalmente con limbo desarrollado, de color pardo claro u obscuro con frecuencia brillantes, enteras. Bráctea inferior foliácea, igual, algo menor o rara vez mayor que la inflorescencia, no envainante. Espiga masculina solitaria, de (5)10-30 mm, ocasionalmente con otra de longitud mucho menor en su base, ± fusiforme o cilíndrica; espigas femeninas 2-4, de (7)10-40(50) mm, ocasionalmente alguna andrógina, cilíndricas, densas, distribuidas a lo largo de la parte superior del tallo, generalmente cercanas las unas de las otras, erectas. Glumas masculinas estrechamente oblongas u obovadas, de ápice obtuso o redondeado, raramente agudo, negras con una banda central verde y sin margen escarioso o con uno muy estrecho hacia el ápice; glumas femeninas de menor longitud que los utrículos, ovales, de ápice agudo u obtuso, negruzcas con quilla central verde y generalmente margen escarioso estrecho. Utrículos (1,8)2-3(3,6) × 1-2 mm, erectos o suberectos, de contorno elíptico o suborbicular, comprimidos, papilosos, con los nervios ± perceptibles, aunque en ningún caso muy prominentes, de verdes a negruzcos, ± bruscamente estrechados en un corto pico hasta de 0,3 mm, truncado, papiloso. Aquenios 1,3-1,8 × 0,9-1,6 mm, de contorno elíptico, obovado o suborbicular, lenticulares. 2*n* = 84, 85, 82-85*, 86*.

Prados húmedos, turberas, pozas temporalmente inundadas y bordes de arroyos; 1000-3300 m.(IV)V-VIII(IX). Gran parte de Europa, W de Asia, N de África (Rif y Atlas), Groenlandia y NE de América del Norte. Pirineos, Cordillera Cantábrica, Sistema Ibérico septentrional, Montes de León, Sistema Central y Sierra Nevada. **And. Esp.:** Al Av Bi Bu Cc Cu Ge Gr Gu Hu L Le Lo Lu M Na O Or P S Sa Sg So SS Te Vi Za. **Port.:** BA BB Mi.

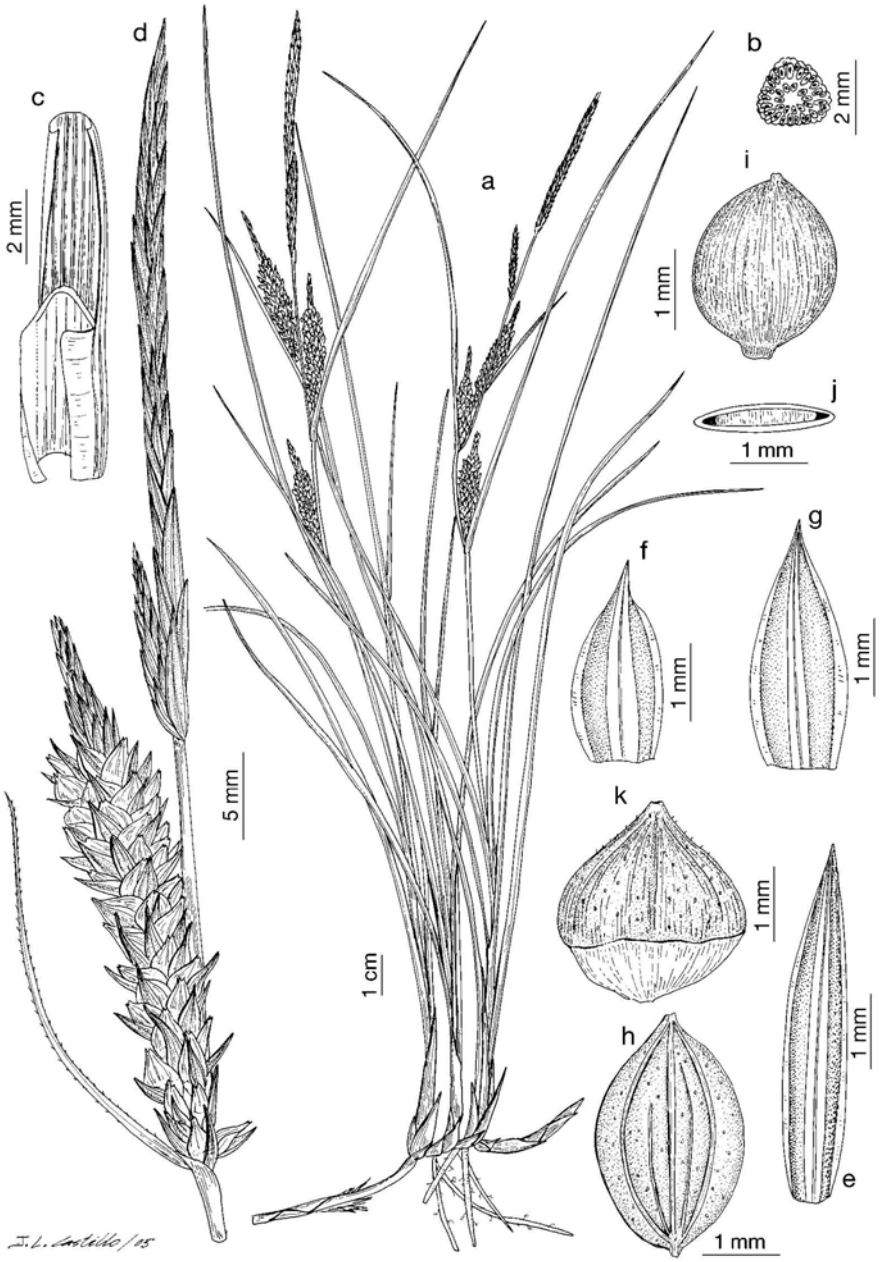
Observaciones.–En su concepción actual, *C. nigra* parece ser un taxon polifilético. La escasa diferenciación de las distintas formas no permite llevar a cabo una identificación certera, por lo que optamos por una concepción sintética del taxon y nos abstenemos, por el momento, de ensayar un tratamiento infraespecífico.

87. C. acuta L., Sp. Pl.: 978 (1753) [acúta]

C. gracilis Curtis, Fl. Londin. 2, tab. 282 (1798)
Ind. loc.: "Habitat in Europae ubique" [lectótipo designado por T.V. Egorova, Sedges Russia: 446 (1999): LINN 1100.71]
Ic.: Boott, Ill. Carex, tab. 548-550 (1867); Jermy & Tutin, Brit. Sedges ed. 2: 209 (1982)

Densamente cespitosa, de cepa robusta que forma grandes macollas, aunque puede desarrollar rizomas con entrenudos de varios centímetros. Tallos 40-100 cm, escábridos en la zona superior, agudamente trígonos. Hojas 3-5(7) mm de anchura, de longitud igual o mayor que los tallos, planas, ásperas en los bordes, ± blandas, de color verde obscuro; lígula hasta 5 mm, de ápice redondeado o emarginado; sin antelígula; vainas basales con limbo desarrollado, de color pardo, ± enteras. Bráctea inferior foliácea, de longitud mucho mayor que la inflorescencia, no envainante. Espigas masculinas 2-4, de (15)20-50(60) mm, ± cilíndricas; espigas andróginas 1-2 y espigas femeninas 2-4, de (30)40-90 mm, cilíndricas, densifloras, distribuidas a lo largo de la parte superior del tallo, generalmente cercanas las unas de las otras, erectas o colgantes. Glumas masculinas ± oblongas, negruzcas, de ápice generalmente obtuso, sin margen escarioso o con él muy estrecho en el ápice; glumas femeninas de longitud menor, igual o mayor que los utrículos, lanceoladas u ovales, de ápice agudo, obtuso o apiculado, negruzcas con quilla central verde, sin margen escarioso. Utrículos (2)2,5-4 × 1-1,7(2) mm, erectos o suberectos, de contorno elíptico, fuertemente biconvexos, papilosos, con los nervios perceptibles pero no muy prominentes, amarillentos, bruscamente estrechados en un pico hasta de 0,3 mm, truncado, papiloso. Aquenios 1,3-2 × 0,9-1,6 mm, de contorno obovado o anchamente elíptico, lenticulares, pardos. 2*n* = 84, 84+f, 85, 86.

Bordes de ríos y arroyos; 0-1300 m. IV-VII. Gran parte de Europa, aunque rara en el S; W de Asia (Líbano, Siria, región del Cáucaso y Turquía) y regiones occidentales de Siberia. Ha sido citada del NW de África, donde debe ser confirmada a causa de las numerosas confusiones que se han producido entre esta especie y las del grupo de *C. elata*. En la Península crece en puntos del N y el valle del Duero. **Esp.:** Le (Lo) O? P S So Za. **Port.:** BA DL TM.



Lám. 73.–*Carex trinervis*, a-j) cruce de Costa de Lavos, Figueira da Foz, Beira Litoral (MA 342679); k) Port Lagoa das Braças, Beira Litoral (MA 515535): a) hábito; b) sección transversal de una hoja; c) lígula; d) inflorescencia; e) gluma ó; f, g) glumas '; h) utrículo; i) aquenio; j) sección transversal de un aquenio; k) utrículo teratogénico.

88. C. trinervis Degland. in Loisel., Fl. Gall. 2: 731 (1807) [trinérvis]

Ind. loc.: "In sabuletis uliginosis circa Baionam" [SW France] [lectótipo designado por M.J.Y. Foley in Candollea 56: 135 (2001): Herb. Loiseleur (AV)] Ic.: Lám. 73

Rizoma de entrenudos largos. Tallos 10-40 cm, lisos o algo escábridos en la zona superior, obtusamente trígonos. Hojas (0,5)1,5-2,5(3) mm de anchura, de longitud igual o mayor que los tallos, canaliculadas, lisas o ásperas en los bordes, rígidas, glaucas; lígula hasta de 1,5 mm, de ápice obtuso; sin antelígula; vainas basales con limbo desarrollado, de color pardo, enteras o algo rotas. Bráctea inferior foliácea, de mayor longitud que la inflorescencia, no envainante. Espigas superiores masculinas (1)2-4, de (15)25-40 mm, ± fusiformes; espigas laterales 2-4, femeninas o andróginas, de (7)20-40 mm, cilíndricas, densas, distribuidas a lo largo de la parte superior del tallo, generalmente cercanas las unas de las otras, erectas. Glumas masculinas estrechamente oblongas u obovadas, de ápice agudo, pardo-rojizas, con estrecho margen escarioso; glumas femeninas de longitud ± igual que los utrículos, ovales, de ápice obtuso, subagudo o mucronado, de color pardo rojizo obscuro, con margen escarioso y quilla verde.

Utrículos (2)2,5-4,5 × 1,3-2,5 mm, erectos o suberectos, de contorno elíptico, comprimidos, ± biconvexos, con papilas bajas y los nervios perceptibles, de color verde ceniciento, sin pico o bruscamente estrechados o gradualmente atenuados en un pico hasta de 0,2 mm, truncado, papiloso. Aquenios 1,6-2,3 × 1-1,5 mm, de contorno ± obovado, biconvexos, de color pardo obscuro. 2*n* = 83*, 84, 85.

Arenas litorales húmedas y bordes de lagunas cercanas a la costa; 0-30 m. V-VIII(IX). Endemismo de las costas del W de Europa: Alemania, Bélgica, Dinamarca, España, Francia, Holanda y Portugal, conocida de Gran Bretaña, donde se la considera extinta. En la Península Ibérica habita en una estrecha franja costera entre Oporto y Leiria, y Parque Nacional de Doñana. **Esp.:** H. **Port.:** BL DL E.

Observaciones.–Numerosos individuos de esta especie presentan deformaciones importantes en la forma del utrículo debidas al parasitismo por himenópteros, de modo que su mitad inferior está bruscamente estrechada.

APÉNDICE 2

Carex castroviejoi Luceño & Jiménez-Mejías (Cyperaceae), a new species from North Greek mountains*

P. Jiménez-Mejías & M Luceño

*Modificado de Jiménez-Mejías P. & M. Luceño. 2009. *Carex castroviejoi* Luceño & Jiménez-Mejías, a new species from North Greek mountains. *Acta Botanica Malacitana*, 34: 231-233.

Carex castroviejoi Luceño & Jiménez Mejías, **sp. nov.** (fig. 1-2)

- *C. lepidocarpa* sub Chater (1980), Flora Europaea 5: 310, p.p., non Tausch
- *C. lepidocarpa* sensu Strid & Kit Tan (1991), Mountain Flora of Greece, 2: 853-854, non Tausch

Holotypus: **Greece, Epirus**, Ioannina, Parque Valia Kalnta, 1698 m, 39º 52' N 21º 11' E, taludes pedregosos en dominio del *Pinus heldreichii* y hayedo, 29.VI.2008, *M. Luceño* (3108ML), *P. Vargas* & *F.J. Fernández* [UPOS (3442)]. **Isotypus**: UPOS.

Speciei Carex lepidocarpa Tausch. *similis a qua praesertim, spica mascula late fusiformis, differt*.

Perennial tufted-forming. Fertile stems 7-40 cm length, trigonous, smooth, erect or slightly curved. Leaves 0.9-3 mm width, frequently shorter than stems, flat, light green; ligule short, hardly appearing over the sheath mouth, truncate to rounded, scarious, absent from the cauline leaves; anteligule 1-2 mm, rounded; basal leaf sheaths unconspicuous, weak, light brown. Lowest bract 1-6 cm × 0.5-3 mm, as long as or a bit longer than inflorescence, shortly leaf-like, sometimes bristle-like. Male spike 1, 7-20 × 3-3.5(4,2) mm, widely fusiform to elliptical, with a 1-15(20) mm peduncle; female spikes 1-3, the lower 7-10 mm length, generally clustered at stem tops, sessile or short-peduncled, erect, sometimes with a long-peduncled basilar spike. Male glumes oval, subacute to obtuse, entirely brown, with a lighter middle nerve; female glumes oval, subacute to obtuse, brown, with a ligher unique middle nerve and sometimes an inconspicuous scarious margin. Stigmata 3. Utricles 3.2-4.5(4.8) × 0.9-1.5 mm, green to dark brown, those from the lower half of each spike strongly deflexed, those from the upper half deflexed to patent, and apical ones patent to erect-patent, elliptical, trigonous, plurinerved, gradually attenuated to a 1.2-2.2(2.5) mm beak, deflexed (30-40º in reference to utricle body), bidentate or bifid, smooth. Achenes 1,5-1,8 × 1 mm, narrowly obovate, trigonous.

Etymology: The new species is dedicated to our dear friend and master on botany Santiago Castroviejo Bolívar, main author of *Flora Iberica*, the most important work of the Spanish plant science history.

Figure 1. Holotype of *Carex castroviejoi* Luceño &Jiménez Mejías

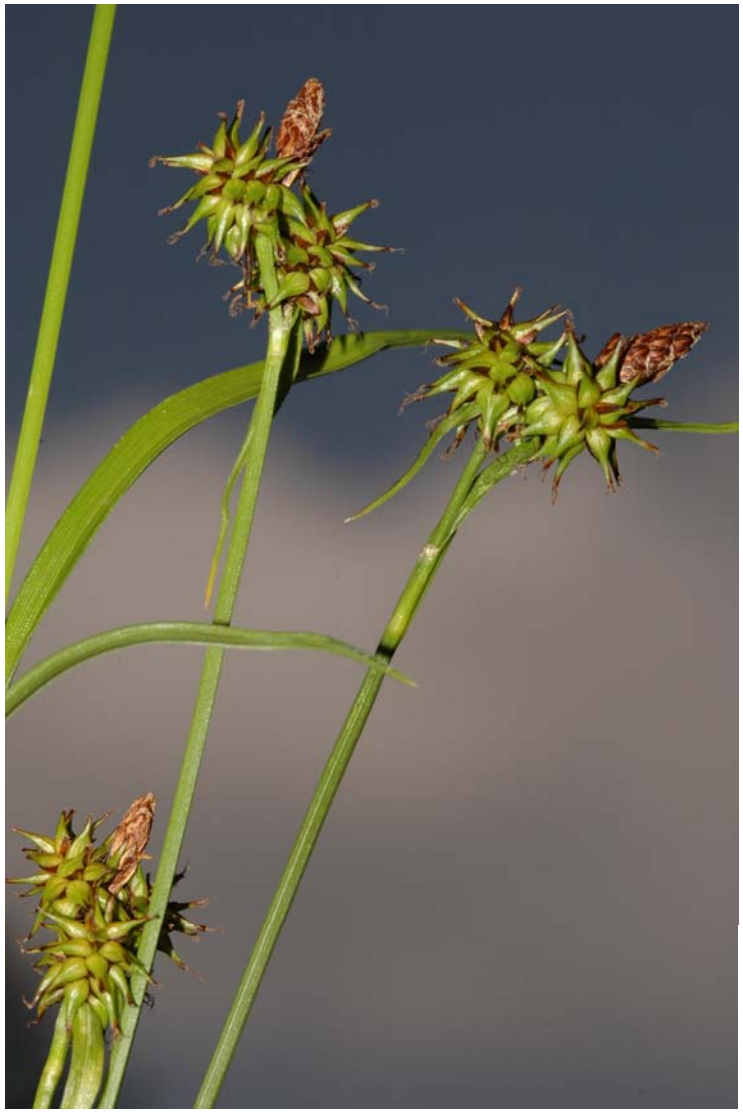


Figure 2. *Carex castroviejoi* specimen from type locality

Figure 3. Distribution map including studied populations (represented by star symbols).

Ecology: Boggy soils over ophiolitic rocks, in montane forests of Pindus range, N Grecce.

Distribución: Epirus and West Macedonia provinces (fig. 3). Apart from type materials, we also studied plants from the following stations:

Paratypi: **Greece. Epirus**, Ioannina, Milia, Valia Kalnta Nacional Park, 1513 m, 39º 51' N 21º 12' E, arroyos, prados turbosos sobre serpentinas, 28.VI.2008, *M. Luceño* (2008ML), *P. Vargas* & *F.J. Fernández* (ATH, E, M, MA, MGC, NY, TAU, UPOS); N Pindhos, Katara Pass, glushes on the N side at 1600 m with *Blysmus* and *C. echinata*, on serpentine substrate, 5-VII-1988, *A.J. Richards* (BM, RNG); Pindhus, Katara Pass 8 km east of Metsovon, 1650 m, flushes on serpentine, N of top of pass, 30-VI-1985, *A.J. Richards* & *G. Chaytor* (BM, con *C. viridula s.str.*); Pindhus Mts, W slopes below Mt. Smolikas, 6000 ft., 26-VII-1961, *Cambridge University Expedition* (K); Konitsa, Vasilitsa ski resort, 1580 m, turberas sobre serpentinas, 12-VII-2004, *P. Vargas* (281PV04). **West Macedonia,** Distr. Grevena, Montes Pindhus, in declivibus boreali orientalibus montis Aphtia, in valle Arkudolaka (Valea Kalda) ditiones pagi Perivoli, substr. serpent., 1700-2100 m., 30,31-VI-1957, *K.H. Rechinger* 18442 (K)

Carex castroviejoi is a *C. flava* group member from sect. *Ceratocystis*, species complex of difficult taxonomy and tend to hybridization. Traditionally, four species have been widely accepted in Europe (*C. demissa* Hornem., *C. flava* L. *s.str.*, *C. lepidocarpa* Tausch. y *C. viridula* Michx. *s.str.*); the high mountain dwarf forms were included in *Flora Europaea* under *C. nevadensis* Boiss. & Reuter epigraph (cf. Chater, 1980, Flora Europaea 5: 310), although it is now known that this group is a heterogeneous set of different taxa (manuscript in preparation). It can be easily distinguished two morphological groups of plants within *C. flava* group: plants with

straight and patent utricles (*C. demissa* and *C. viridula s.str.*) and plants with bent utricles, the lower from each spike reflected (*C. flava s.str.*, *C. lepidocarpa* and most of the high mountains forms). *Carex castroviejoi* would be within this latter group and, in fact Chater cited these plants under *C. lepidocarpa* epigraph. Morphological, karyological and molecular studies (manuscript in preparation) strongly supported the taxonomic autonomy *C. castroviejoi* from the remaining members of *Carex* sect. *Ceratocystis*. It is a plant from ophilitic soils, ecologically isolated from the other *C. flava* group taxa. Morphological affinity to *C. lepidocarpa* seems to be a convergence phenomenon, since our phylogenetic studies show *C. castroviejoi* as an indenpendient and isolated lineage, not closely related to *C. lepidocarpa*.

Key to distinguish the *C. flava* group plants with bent-beaked utricles from the Mediterranean Basin

1. Male spike at least 3 mm width, widely fusiform to elliptical; utricle beak smooth..... *C. castroviejoi*
- Male spike up to 3 mm width, terete, linear or narrowly lanceolated; utricle beak smooth or scabrid.....2
2. Utricles dark brown, at least in the upper half, (1,5)2,2-3(3,1) mm length; lowest bract setaceous or shortly leaf-like, up to 1,5 mm wide on middle part; apical utricles of female spikes erect..... *C. lepidocarpa* subsp. *nevadensis*
- Utricles green, yellow or light brown, longer than 3 mm; lowest bract leaf-like, wider than 1,5 mm on middle part; apical utricles of female spikes erect to patent 3
3. At least some utricles from the upper half of the spike with deflexed beak; female spikes oblong to subglobose; male spike usually pedunculate .... *C. lepidocarpa* subsp. *lepidocarpa*
- Utricles form the upper half of the spike with erect-patent beaks; female spikes subglobose; male spike sessile..... *C. flava* s.str.

Acknowledgements

The authors wish to thank curators of BM, K and RNG for facilities in study materials in their herbaria, F.J. Fernández for technical support, and Dr. P. Vargas for kindly collecting materials.

APÉNDICE 3

Contribuciones corológicas / Chorological contributions

1-

Carex cespitosa L., novedad para la Península Ibérica

***Carex cespitosa* L., first record for Iberian Peninsula**

Modified from: Jiménez-Mejías, P., M. Escudero, A.J. Chaparro & M. Luceño. 2007. Novedades corológicas del género *Carex* para la Península Ibérica. *Acta Botanica Malacitana*, 305-312.

Materiales: ESPAÑA: Navarra: Lesaka, Zalain, orilla del Bidasoa, 20 msm, 43º 16’ 25’’ N 1º 43’ 6’’ W, 20-VI-1981, I. Aizpuru & P. Catalán. MA; Ídem, 30 msm, 18-V-1983, P. Catalán. ARAN, MA; Ídem, 30 msm, 16-VII-2006, 43º 16’ 33’’ N 1º 41’ 49’’ W, P. Jiménez, M. Luceño, M. Escudero & I. Aizpuru. UPOS.

Carex cespitosa L. pertenece a la sección *Phacocystis* Dumort. Presenta un amplio área de distribución dentro de la Región Paleártica, tradicionalmente considerada desde el CS de Francia (Chater, 1980) hasta Japón (Ohwi, 1965). Debido a la dificultad taxonómica que entraña la sección *Phacocystis*, ha sido no pocas veces confundida con otras especies afines, como *C. elata* All. o *C. nigra* (L.) Reichard. Las principales características que distinguen a *C. cespitosa* frente a otros representantes europeos de su sección son: utrículos sin nervios, con papilas blanquecinas al menos hacia el ápice; espigas, de pequeño tamaño, la masculina de 10-20 mm de longitud, las femeninas de 10-20(30) mm; bráctea inferior frecuentemente setácea, más corta o de la misma longitud que la inflorescencia; vainas basales, enteras, de color púrpura; y hábito densamente cespitoso, de modo que la planta crece formando robustas macollas en el lecho de ríos y en turberas (Chater, 1980; Egorova, 1999). Los números cromosomáticos conocidos para esta especie son 2n = 78, 79, 80? (Faulkner, 1972).

Se trata de una novedad para el catálogo de Ciperáceas ibéricas, no considerada, por tanto, en la revisión taxonómica de la sect. *Phacocystis* para la Península (Luceño & Aedo, 1994), ni en la monografía ibérica del género *Carex* (Luceño, 1994). Las localidades más próximas se encuentran en Formiguères, vertiente N del Pirineo catalán francés (Terrisse, 1994), y son citadas en la Flora dels Països Catalans (Bolòs & Vigo, 2001).

La importancia biogeográfica de esta localidad, extremo SW de la distribución de *C. cespitosa*, unido a que es la única conocida en la península Ibérica, aconsejan llevar a cabo estudios para evaluar su estado de conservación y considerar la posible catalogación como especie protegida.

2-

Carex elata All. subsp. *elata*, nuevas localidades: ampliación de su área a la mitad W de la península Ibérica.

***Carex elata* All. subsp. *elata*, new localities: distribution range expanded to the western half of the Iberian Peninsula**

Modified from: Jiménez-Mejías, P., M. Escudero, A.J. Chaparro & M. Luceño. 2007. Novedades corológicas del género *Carex* para la Península Ibérica. *Acta Botanica Malacitana*, 305-309.

Materiales: ESPAÑA: Huelva: Almonte, Doñana, El Rocío, Finca La Rocina, Magnocaricion, 28-VI-1977, S. Castroviejo. MA; Ídem, arroyo, 16-IV-1978, S. Castroviejo, M. Costa & E. Valdés-Bermejo. MA; Ídem, próximo a la casa de Bernabé, zona encharcada, 29-VI-1988, S. Castroviejo, M. Luceño & P. Vargas. MA; Mazagón, en las turberas de Magnocaricion, 1-V-1978, S. Castroviejo & E. Valdés-Bermejo, MA; Moguer, Laguna de las Madres, 4-IV-1980, C. Romero & P. Romero, SEV; Palos de la Frontera, Paraje Natural Laguna de las Madres, arroyo Madre del Avitor, suelos arenosos, con *C. paniculata* subsp. *lusitanica*, 21-III-2006, P. Jiménez & E. Narbona. UPOS. Jaén: Chilluevar, carretera al Tranco km. 23, 21-IV-1984, J. Cobos. MA. Palencia: Torquemada, bordes del río Pisuerga, 21-V-1988, C. López & A. Romero Abelló. MA; Cordovilla la Real, Puente de Cordovilla, bordes del río Pisuerga, 15-V-1987, C. López & A. Romero Abelló. MA; Villalba de Guardo, Laguna de En medio, 25-7-2001, F. Cabezas & C. Aedo. MA. PORTUGAL: Alto Alentejo: Elvas, freguesia da Ajuda, Hacendade de S. Rafael, margem do Guadiana, 8-IV-1954, Beliz & al. MA; Ídem, S. Rafael, pequenas charcas secas da margem do Guadiana, 1-V-1975, Malato-Beliz & J.A. Guerra MA. Bajo Alentejo: Entre S. Cristóvão e Santa Susana, motas duma ribeira, 2-IV-1963, J. Paiva, J. Matos & A. Marques. COI. Beira Litoral: Aveiro, ponte de Azurva, na estrada para Aveiro, 20-IV-1965, A. Fernandes, R. Fernandes & J. Matos. COI; Ídem, ao longo da margem (esquerda) pantanosa do Ribeiro do Pano (entre Barreira Branca e Olhos da Azenha), 24-V-1977, A. Marques & Angelo Pereira. COI; Ídem, Águeda, Fermentelos, entre Porto d'Asna, Sorgaçal e Porto da Minhoteira, ao longo da margem da Pateira, 18-IV-1977, A. Marques. COI; Ídem, Águeda, entre a ponte de Perrães e Espinhel, margem direita do rio Cértima, 14-II-1978, A. Marques & Angelo Pereira. COI; Ídem, Águeda, Ois da Ribeira, próximo do Hotel, margem da Pateira de Fermentelos, 10-V-1978, A. Margues. COI.

Carex elata, al igual que *C. cespitosa*, pertenece a la sect. *Phacocystis*. Se trata de una planta con una morfología muy variable que presenta varias razas geográficas repartidas a lo largo de su amplio área de distribución: N de África, Europa, Siberia Occidental y Cáucaso (Kukkonen, 1998). La taxonomía del grupo en la península Ibérica es bastante problemática y en el estudio monográfico llevado a cabo por Luceño & Aedo (1994) se revela la existencia de 3 entidades dentro del complejo de *C. elata*: la subsp. *elata*, que los autores califican de calcícola y circunscriben a la mitad E de la Península, la subsp. *reuteriana* (Boiss.) Luceño & Aedo, silícícola, endémica del cuadrante NW de la Península, y la subsp. *tartessiana* Luceño & Aedo, indiferente edáfica, endémica del S de la Península.

La subespecie nominal de *C. elata* fue relegada por Luceño (1994) a la mitad E de la península, asociada a los sustratos básicos de las formaciones calizas españolas en las provincias de Álava, Albacete, Burgos, Cuenca, Guadalajara, Guipúzcoa, Navarra, Soria, Rioja y Zaragoza. Bolòs & Vigo (2001) la citan además de Gerona y Tarragona y Peralta & al. (1992) para Huesca.

Los ejemplares del Parque Nacional de Doñana fueron considerados como pertenecientes a la subsp. *tartessiana*, por la presencia de más de una espiga masculina y ser las inferiores andróginas, así como por la tendencia de muchos ejemplares a presentar la bráctea inferior de longitud similar a la de la inflorescencia. La escasez de vainas basales afilas fue interpretada por Luceño (1994) como un posible signo de hibridación antigua con *C. acuta* L., barajando la posibilidad de que ésta última estuviera siendo eliminada de Andalucía por procesos de hibridación. Sin embargo, la observación detallada de estos materiales confirma la existencia de papilas blanquecinas en los utrículos, así como algunos ejemplares con la bráctea inferior de longitud similar a la espiga inferior, caracteres ambos propios de la subsp. *elata*. Además, el número cromosomático conocido para esta población, 2n = 76 (Luceño & Aedo, 1994), entra en el rango dado para *C. elata* s.l. y no llega al de *C. acuta* (2n = 84, 84 + f, 85, 86). Por todo ello, consideramos estas poblaciones como *C. elata* subsp. *elata*.

Otra modificación corológica sustancial es el hallazgo de materiales portugueses. Se confirma que algunos ejemplares de la franja atlántica portuguesa pertenecen a la subsp. *elata*. Del mismo modo, las poblaciones del bajo Guadiana, consideradas por Luceño & Aedo (1994) como las más occidentales de la subsp. *tartessiana*, las desestimamos como tales y adscribimos a la subsp. *elata*.

En lo que respecta a las preferencias edáficas de la subsp. *elata*, a raíz de estos nuevos hallazgos debe dejar de considerarse estrictamente calcícola y entender esta tendencia ecológica como una preferencia.

La configuración actual de la distribución de *C. elata* subsp. *elata* en la península Ibérica se muestra en la figura 1.

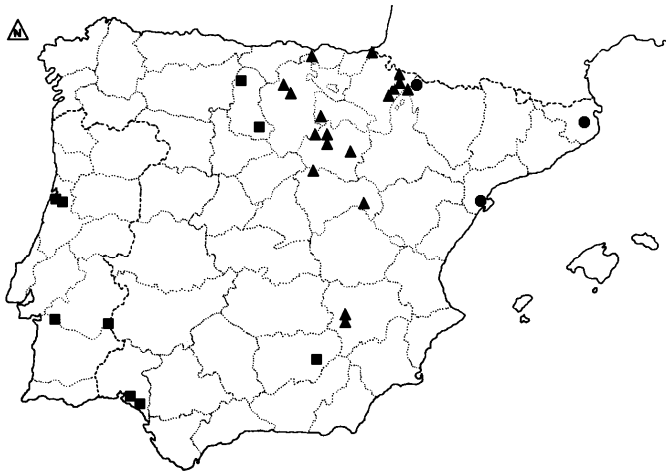


Figura 1.
Distribución de *C. elata* subsp. *elata*: citas aportadas por Luceño & Aedo (▲), localidades originales señaladas en el presente trabajo (■), otras referencias bibliográficas (●).
C. elata subsp. *elata* distribution: stations indicated by Luceño & Aedo (▲), original stations indicated in this work (■), other bibliographic references (●).

El cambio de estatus taxonómico de las poblaciones onubenses tiene repercusión sobre la Lista Roja de la Flora Vascular de Andalucía (Cabezudo & Talavera, 2005); en ésta se incluye *C. acuta* L. bajo la categoría de Datos Insuficientes (DD) al tomar como base las poblaciones de Doñana y su entorno, pues, como ya se ha dicho, se pensó que eran formas de *C. acuta* que estaban siendo eliminadas por introgresión con *C. elata* subsp. *tartessiana*. De este modo, debe considerarse que la referencia del catálogo a las poblaciones de *C. acuta* de la "depresión del Guadalquivir occidental (Huelva)" [sic] es en realidad a *C. elata* subsp. *elata*, igualmente escasa en Andalucía y necesitada de protección.

3-

Carex trinervis Degl., novedad para la flora española.

***Carex trinervis* Degl., first record for Spain**

Modified from: Luceño, M. & P. Jiménez-Mejías. 2007. *Carex trinervis* Degl., novedad para la flora española. *Acta Botanica Malacitana*, 309-310.

Carex trinervis Degl. es un endemismo europeo que crece sobre arenas en los márgenes de lagunas y cursos de agua dulce litorales. Su distribución se extiende a lo largo de las costas atlánticas continentales europeas desde Dinamarca hasta la península Ibérica (Chater, 1980), aunque también se conocía de la isla de Gran Bretaña, donde parece haberse extinguido (Jermy & al., 1982).

Esta planta pertene a la sect. *Phacocystis* Dumort., la cual es fácilmente reconocible entre la mayoría de las secciones del subgénero *Carex* por sus dos estigmas. *C. trinervis* se diferencia de los demás representantes ibéricos de su sección por sus largos rizomas reptantes, sus hojas canaliculadas, marcadamente glaucas y rígidas, y sus utrículos sin papilas.

Su presencia en nuestro país fue ya puesta en duda por Vicioso (1959), quién contrastó que la única exsiccata española repartida por Sennen (Exs. pl. Esp., n. 5192) no correspondía a esta planta. Luceño & Aedo (1994) en su revisión de la sect. *Phacocysti*s para la península Ibérica la descartaron para España y limitaron su distribución ibérica a Portugal, en la franja costera entre Oporto y Leiría; esta última localidad, donde no se colecta desde 1917, era considerada hasta ahora como la localidad más meridional conocida hasta el momento. Aunque Chater (1980) cita en la distribución de *C. trinervis* nuestro país, lo hace con un interrogante, con toda seguridad por la ausencia de pliegos testigo, pero quizá animado por la presencia de la planta en Portugal y en localidades francesas tan cercanas a España como Bayona.

En recientes campañas encuadradas en el proyecto 17/2006 para la recolección de ciperáceas en el Parque Nacional de Doñana, autorizado por la Estación Biológica de Doñana, hemos localizado una población de esta planta en la zona de reserva del arroyo de La Rocina:

C. trinervis Degl. **Huelva**: Parque Nacional de Doñana, arroyo de La Rocina, finca de El Gato, camino de Moguer, arenas húmedas, con *C. hispida*. *M. Luceño* & *P. Jiménez Mejías*, 27-4-2007 2205UPOS

Esta nueva localidad es, por tanto, la más meridional de la especie y amplía el rango de distribución de la planta en más de 500 km. hacia el sur. Curiosamente, pese a crecer en una zona tan visitada por los botánicos como el Parque Nacional de Doñana, donde se han llevado a cabo multitud de estudios florísticos (Fernández Galiano & Cabezudo, 1976; Cabezudo, 1979; Castroviejo & al., 1979; Rivas-Martínez & al., 1980), se trata de la primera recolección de esta planta en nuestro país. Ello podría ser debido a su confusión de esta planta con formas juveniles de *C. hispida*, especie con la que crece mezclada y con la que muestra cierto parecido superficial, pese a ser de un porte mucho menor.

La aparición de *C. trinervis* en España aconseja su inclusión en la Lista Roja. La disyunción con la localidad más próxima (más de 500 km), su interés biogeográfico (extremo meridional de su distribución), y su limitada distribución mundial son factores que deberían ser tenidos en cuenta para considerar alguna categoría de protección para esta planta.

4-

Carex cespitosa L. (Cyperaceae) excluded from Piedmont flora (Italy)

Modified from: Jiménez-Mejías P. & A. Selvaggi. 2011. *Carex cespitosa* L. –PIE. In Selvaggi A., A. Soldano & M. Pascale (eds.). Note floristiche piemontesi n. 309-392. *Rivista piemontese di Storia naturale*, 32: 376-377

Carex cespitosa L. (= "*Carex caespitosa*") è un'entità eurasiatica, distribuita in Europa per lo più nelle regioni centrali e settentrionali e assente in quelle meridionali e occidentali (Chater, 1980). Nella regione alpina *C. cespitosa* è stata segnalata nelle alpi orientali in Germania e Austria (Schultze-Motel, 1968), mentre non è presente nelle alpi svizzere e francesi (Cosson & Morcrette, 1999; Olivier *et al.,* 1995). In Italia *C. cespitosa* è indicata come presente nella regione alpina in generale (Pignatti, 1982; Conti *et al.*, 2005). La presenza in Piemonte, contrariamente a quanto indicato in Pignatti (1982), Aeschimann *et al.* (2004) e Conti *et al.* (2005), è da ritenersi erronea in base alle argomentazioni apportate nella presente nota. La verifica dei campioni conservati in *hb.* TO ha escluso la presenza di esemplari riconducibili alla specie. Tutte le revisioni hanno ricondotto le raccolte *sub C. cespitosa* L. a *C. nigra* (L.) Reichard (= *C. fusca* All. *p.p.*), *C. elata* All. subsp. *elata* o *C. acuta* L. L'entità è stata confusa in

particolare con le congeneri *C. elata* e *C. nigra*, considerate da alcuni autori, in particolare nella "Nuova Flora Analitica d'Italia" (Fiori, 1923-1929), come varietà di *C. cespitosa*. I dati *sub C. cespitosa* antecedenti alla pubblicazione della "Flora d'Italia" di Pignatti (1982) sono da considerare per questo motivo ambigui in quanto, in mancanza di indicazione della varietà, possono essere associati a entità differenti. Numerose sono le bibliografie della prima metà del 1900 che segnalano la presenza della specie in Piemonte (Negri, 1905; Ferrari, 1912; Vaccaneo, 1929; Gola, 1932-1933; 376 Note floristiche piemontesi n. 309-392 Sappa & Piovano, 1950; Bertolani Marchetti, 1954) ma nessun campione d'erbario ne certifica la corretta identificazione. E' da escludere inoltre la presenza di *C. cespitosa* in Valchiusella (Fiori, 1923-1929; Pignatti, 1982), dove la specie era segnalata sulla base di una raccolta di Vaccari e Wilczek conservata in *hb.* FI (vedi Peyronel *et al.*, 1988) e proveniente da "entre Pasquere et la Bocchetta delle Oche , 1600- 2600 m, 30 août 1906", che deve essere attribuita a *C. nigra* (*rev.* P. Jiménez-Mejías, 2010). Il dato di Borgofranco d'Ivrea (Peyronel *et al.*, 1988) relativo a due raccolte di L. Vaccari conservate in *hb.* FI, di cui una datata 6 maggio 1900, è anch'esso da ricondurre ad altra entità ovvero, nel caso specifico, a *C. elata* in base alla revisione di P. Jiménez-Mejías del 2010. La segnalazione di presenza nell'Appennino piemontese, che origina da una segnalazione di De Notaris (1844) per la "Val d'Olba" (=Val d'Orba), successivamente riportata da Gola (1912), Fiori (1923-1929) e Pignatti (1982), non supportata da campioni d'erbario, per i motivi precedentemente esposti è da escludersi anch'essa. In tempi più recenti la specie è stata segnalata per i Laghi di Ivrea (Minuzzo *et al.*, 2005); il ritrovamento, non confermato da campioni d'erbario (C. Minuzzo, *in verbis*), è stato considerato non attendibile. Soldano & Sella (2000) e Antonietti (2005) hanno escluso la presenza della specie rispettivamente nelle province di Biella e del Verbano-Cusio-Ossola. In base a quanto evidenziato, in assenza di dati certi e segnatamente di campioni d'erbario, coerentemente con i risultati della revisione di *C. cespitosa* L. in Sud Europa e Italia da parte di P. Jiménez-Mejías, si esclude *C. cespitosa* L. dalla flora del Piemonte.

5-

***Carex buekii* Wimm. new for Turkey.**

Modified from: P. Jiménez-Mejías & M. Luceño. In press. *Carex buekii* +Tu, *Carex elata* ssp. *omskiana* – Tu. In Greuter W. & T. Raus (eds.). Med-Checklist Notulae, 30. *Willdenowia*.

Turkey, Anatolia, B6 Kayseri: Pinarbaşi, river marsh, with *Salix*, 18.6.1954, *Davis* 2192/3 (BM, K) C6 Adana: d. Simbeyli, Anti-taurus, 600 m., river sides, 12.4.1957, *Davis & Hedge* D.26647 (BM, K).

Both localities are separated by only 50 km. and they were cited by Nilsson (1986) in flora of Turkey under *C. elata* subsp. *omskiana* (Meinsh.) Jalas. Despite both vouchers are incomplete, and the second is even immature, the observed characters do not match those traditionally reported for *C. elata* subsp. *omskiana*. In view of the reddish scale-like, reticulate splitting basal sheaths of Adana materials, and suborbicular to broadly-obovate, faintly nerved or nervelees, yellowish-green reddish-potted, ca. 2 mm utricles of Kayseri voucher, both collections matches fairly well the variability of *C. buekii* rather than *C. elata* subsp. *omskiana* (cf. Chater, 1980; Egorova, 1999). In addition, *C. buekii* has been cited from neighbouring countries. Egorova (1999) confirmed *C. buekii* from Transcaucasus, including Georgia, and Stoeva & al. (2005) reported it from Bulgaria. However, *C. elata* subsp. *omskiana* is considered to reach southward NW Ukraine in Europe (Chater, 1980), whereas Egorova (1999) did not found Caucasian materials, and cited doubtfully Pre-Caucasus and northern Caucasus after Grossheim reports (1940, 1949), not considering Transcaucasus as part of the distribution. Materials from Maraş (Andirin, 13 Km S. of Andirin at Çatak, 800 m, Coode & Jones 1137!) cited by Nilsson (1986) were not revised; it would be desirable to confirm the taxonomical identity of this collection.

6-

***Carex buekii* Wimm., new for the Republic of Macedonia**

Modificado de Jiménez-Mejías, P., S. Martín-Bravo, M. Rat, G. Anakov & M. Luceño. In press. Interesting new records in SE European *Carex* L. *Biologica Serbica*.

REPUBLIC OF MACEDONIA: Porec, Kapina; 30.III.1926, *H. Oehm* (BEO).

This species is included in sect. *Phacocystis* Dumort., a group of complex taxonomy due to unclear morphological limits and frequent hybridization processes (Luceño & Jiménez-Mejías, 2008). This section is easily distinguished from their relatives of subgenus *Carex* by the cylindrical to oblong-ovoid female spikes and lenticular utricles with two stigmata (Chater, 1980; Egorova, 1999; Luceño & Jiménez-Mejías, 2008). The main morphological features which distinguish *C. buekii* are the coriaceous carinate reddish basal sheaths, that split in a ladder-fibrosille structure, faintly nerved or nerveless papillose utricles, and female spikes 4-10 cm long (Chater, 1980; Egorova, 1999). The most reliable chromosome number report for this species is $2n = 64$ (Stoeva & al. 2005).

Carex buekii is distributed through Central and Eastern Europe, eastwards to Kazakhstan. Previous reports for this taxon from the former Yugoslavia refers to Croatia (Alegro & Marković, 1999) and Slovenia (Schultze-Motel, 1980; Martinčič & Sušnik, 1984). It has been also cited from Bosnia-Herzegovina and Serbia (Ascherson & Graebner, 1902–1904; Hayek, 1933), but these records are in need of confirmation (no vouchers in BEO, BEOU and BUNS herbaria). The closest known populations to this reported here are in Bulgaria, where it is considered a scattered taxon (Stoeva & al. 2005; Assyov & Petrova, 2006).

7-

***Carex castroviejoi* Luceño & Jim.Mejías, new for Albania**

Modificado de Jiménez-Mejías, P., S. Martín-Bravo, M. Rat, G. Anakov & M. Luceño. In press. Interesting new records in SE European *Carex* L. *Biologica Serbica*.

ALBANIA: Gramsh, Guri i Topit, 2000 m. serpentine; 23.VII.1956, *Muzeumi I Shkencave te Natyrës 453* (SOM). Fushë e Qerit, 1700 m. serpentine; 28.VII.1956, *Muzeumi I Shkencave te Natyrës 459* (SOM).

This constitutes the first reference to the presence of this recently described taxon (Jiménez-Mejías & Luceño, 2009) out of Greece. *Carex castroviejoi* was firstly reported to grow on ultrabasic substrates (ophiolitic rocks) in Pindhos range (Northern Greece). In congruence, the two new populations found in Albania, located around 80 km north of the previously known Greek localities, are also growing on serpentines.

This species is included in sect. *Ceratocytis* Dumort., one of the most intensively studied groups within genus *Carex*, but also one of the most taxonomically complex. Within the section, the systematics of the so-called *C. flava* group (to which *C. castroviejoi* belongs) are highly controversial, due to the faint morphological limits and hybridization processes among its taxa (Schmid 1982, 1984; Hedrén & Prentice 1996; Luceño & Jiménez-Mejías 2008). Plants belonging to this group are small to medium-size sedges with subglobose female spikes, mainly distributed across temperate areas of the Northern Hemisphere (Schmid, 1983; Crins & Ball, 1989). Mature specimens of *C. castroviejoi* may be distinguished from other related species by its deflexed utricles with bent and smooth beaks, and the widely fusiform male spike, at least 3 mm wide (Jiménez-Mejías & Luceño, 2009).

Another species of the *C. flava* group (*C. lepidocarpa* Tausch.) was newly recorded from Albania recently (Guri i Topit; Barina & Pifkó, 2008). In our opinion, the new record of *C. castroviejoi* from Albania suggests that it would desirable to study those specimens to discard possible misidentifications.

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