

Systematics and management of natural resources: the case of *Spartina* species on European shores

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Abstract: Discrepancies in the identification of some plants and, in consequence on their autochthonous or allochthonous character, can lead to the adoption of inappropriate habit management strategies such as conservation, control, elimination, etc. A clear illustration of this is the case of a plant with an evident expansive behaviour located on the North Atlantic coast of the Iberian Peninsula, which has been considered by some authors as *Spartina versicolor*, a native of the European coasts. However, other authors have identified this plant as *Spartina patens*, originally from the North American Atlantic coasts, but introduced on both the Atlantic and Mediterranean coasts of Europe. This species shows an invading behaviour, playing a clear and evident role in the transformation of the habitats that it colonizes. In this work, results based on the use of sequences of the internal transcribed spacer (ITS) region of nrDNA, widely used in taxonomy and molecular phylogeny between closely related species, is reported. These data indicate that the identity of those plants growing on the European littoral is similar to those native to the North American Atlantic coasts.

Key words: *Spartina*; coastal habitats; conservation; management; phylogeny Poaceae; rDNA sequences

Introduction

Spartina Schreb. is a genus composed of 15–17 species, most of them growing in moist to wet saline habitats, both coastal and inland (Barkworth 2007). The species of this genus are distributed along the Atlantic and Mediterranean coast of Europe and Africa, where they may have been introduced (Barkworth 2007) and are also located in the Atlantic and Pacific coastlines and inland of America (Baumel et al. 2002a). Its ability to colonize new territories is well-known, and is not confined to the majority of the individual species of this genus, but is also demonstrated by its frequent hybrids. Thus it often acts as an invader, with high capacity for transforming the habitat (e.g. Daehler et al. 1996; Gouletquer 2002; Ayres et al. 2004; Campos et al. 2004; Bortolus 2006; Strong & Ayres 2009).

According to Tutin (1980), the genus *Spartina* is represented by two native species on the European coasts: *S. maritima* (Curtis) Fernald and *S. versicolor* Fabre. Additionally, there are two non-native species located on these coasts: *S. alterniflora* Loisel., from North American coasts, and *S. densiflora* Brongn., from South America. Furthermore, the hybrid *S. x townsendii* H. Groves & J. Groves (*S. alterniflora* x *S. maritima*)

has been reported in Europe, as well as the species *S. anglica* C.E. Hubb., presumably an allopolyploid species derived from the aforementioned hybrid (Ayres & Strong 2001; Baumel et al. 2002b; Ainouche et al. 2004; Fortune et al. 2008).

However, the origin, systematics and nomenclature of those plants identified as *Spartina versicolor* is not completely clear. This taxon was described from the French Mediterranean coast (Fabre 1849) and its presence was also noted on the central Tyrrhenian coast of Italy (Fiori & Paleoti 1896), on the Portuguese coast (Daveau 1897) and on the French Atlantic coast (Coste 1906). Other closely related species, belonging to the same systematic group, have been described in various coastal areas: Parlato (1848–1850) described *S. duriaei* from materials collected by Durieu de Maisonneuve on the Algerian coast. Additionally two other species, originally from the North American Atlantic coast, were described in the late eighteenth and early nineteenth centuries: *S. patens* (Aiton) Muhl. (\equiv *Dactylis patens* Aiton) (Aiton 1789; Muhlenberg 1813), cultivated in England from seeds from New York; and *S. juncea* (Michx.) Willd. (\equiv *Trachynotia juncea* Michx.) (Michaux 1803; Willdenow 1809), which grows in Carolina and Georgia in the USA.

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The identification of this systematic group of plants from European and North African coastal territories has been controversial. On the Mediterranean coast, the same *Spartina* species reported has been treated under different names (*Spartina versicolor*, *S. juncea*, *S. patens*) with different management models proposed depending on its systematization: in cases where it has been considered a native species (Coste 1906; Guinochet & Vilmorin 1978; Tutin 1980; Gamisans et al. 1989; Bolòs & Vigo 2001; Fraga et al. 2003; Fenu & Bacchetta 2008), it is seen as indicative of a good state of conservation of the ecosystem (Ferrendis Pomés 2002). According to this approach, in areas where it is a threatened species it would thus be targeted for protection (Ballesta 1996) and even perhaps considered as a potential species for use in the restoration of coastal habitats (e.g. Montoya & Galofré i Saumell 1997; Vizcaino et al. 2000; Society for Ecological Restoration International 2008). Conversely, other authors have questioned the origin and identity of these Mediterranean plants (Pignatti 1982), or even consider that it is a widely distributed species in America and Europe, with different geographical races in diverse areas (Saint-Yves 1932; Maire 1953). Finally, a different approach taken is its consideration as a non-native species originally from the North American Atlantic coast (Fournier 1977), therefore identifying it as an invader that must be removed (Sanz Elorza et al. 2001, 2004).

Similar disagreements occur in relation to the examples found on the European Atlantic coast, with some authors considering *Spartina versicolor* as a native species (Daveau 1897; Coutinho 1913; Vicioso 1946; Franco & Afonso 1998; Sánchez Gullón 2001), while other authors suggest different origins: 1) a native species from the Mediterranean coast (Bueno et al. 1993; van der Maarel & van der Maarel-Versluys 1996; Campos & Silván Beraza 2001; Torre Fernández 2003; Bedia 2004; Almagro Bonmatí et al. 2006); 2) a species of ambiguous origin (González Costales 2008); 3) an expanding species reaching more northern coasts as a result of climate change (Braña Vigil et al. 2009); or 4) even a species from the North American Atlantic coast exhibiting invasive behaviour on the European Atlantic coast (SanLeón et al. 1999; Campos et al. 2004; Fagúndez Díaz & Barrada Beiras 2007; Campos & Herrera 2009a, 2009b; Sánchez Gullón & Verloove 2009), which needs to be controlled and eliminated.

Considering the different systematic treatments and management models presented above, it is clear why the interpretation of the origin and phytogeographical significance of populations of these plants in each territory is still a controversial topic. Indeed taxonomic errors usually remain unnoticed, and transcend to the limits of ecology and environmental management, so misidentifications could generate worldwide loss of time, knowledge and money (Bortolus 2008). That is to say, there is a need to ascertain adequate criteria for the plants' identification and systematization in order to establish appropriate strategies for the proper man-

agement of the populations in their distribution areas.

The specific aims of this study are: (1) to infer phylogenetic relationships among 14 species of the genus *Spartina* (Table 1); (2) to evaluate the consistency of the existing systematic treatments in the previous studied species; and (3) to investigate the current status and conservation/elimination strategies for the species treated as *Spartina versicolor* and *S. patens*. The results may help us to understand the systematics used by different authors, and to provide basic information to enable the development of a scientific strategy for the efficient conservation or removal of these species.

Material and methods

For these investigations, sequencing of the internal transcribed spacer (ITS) region of nrDNA was performed. The ITS is intercalated in the 16S-5.8S-26S region separating the elements of the rDNA locus. The ITS region consists of three parts: the ITS1 and ITS2 and the highly conserved 5.8S rDNA exon located in between (Wheeler & Honeycutt 1988). The advantages of this region are: (1) biparental inheritance, in comparison to the maternally inherited chloroplast and mitochondrial markers; (2) easy PCR amplification, with several universal primers available for various kinds of organisms; (3) the occurrence of multiple copies distributed throughout the genome; (4) its moderate size, allowing easy sequencing; and (5) the fact that based on published studies it shows variation that makes it suitable for evolutionary studies at the species or generic level (e.g. Baldwin et al. 1995; Liston et al. 1996; Álvarez Fernández & Wendel 2003). In addition, the ITS region has been widely used to reconstruct phylogenetic relationships at various taxonomic levels within Poaceae (e.g. Hsiao et al. 1995, 1999; Barker et al. 2001) and has also been successfully used to identify different closely species within the genus *Spartina* (e.g. Baumel et al. 2002a; Ainouche et al. 2003; Fortune et al. 2008). In total, 40 accessions from 14 species were included in the ITS data set to investigate the phylogenetic relationships among species of the genus *Spartina* (Table 1), with four samples from the genus *Spartina* collected on the coast of Asturias (Northern Spain): three of *S. versicolor* (Asturias 1, 2 and 3) and one of *S. maritima* (Asturias). Voucher specimens were collected, dried by pressing in absorbent paper, stored at room temperature, and kept in the Herbarium of the University of Oviedo (FCO). In addition, a sample of *Cynodon dactylon* (L.) Pers. from Brittany (France) was used as an outgroup in the phylogenetic analyses because it is closely related to *Spartina* (Hsiao et al. 1999). Chromosome numbers were taken from Baumel et al. (2002a), Barkworth (2007) and the "Index to Plant Chromosome Numbers Data Base" (<http://www.tropicos.org/Project/IPCN>).

Total DNA was extracted from leaf tissue using the CTAB method (Doyle & Doyle 1987) with slight modifications. Double-stranded nrDNA were amplified using a gradient thermal cycler (MyCycler™, Bio-Rad). The PCR parameters were as follows: 5 min pre-treatment at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 56°C, 1 min at 72°C, plus a final extension of 10 min at 72°C. Standard primers were used for amplification of the ITS region (Sun et al. 1994). PCR products were run on a 1.5% agarose gel stained with ethidium bromide, in order to evaluate the quality and quantity of the amplified templates, and then purified using ExoSAP-IT (USB Corporation, Ohio) following the manufacturers' protocols. Both strands were

Table 1. List of taxa, chromosome number, origin of the analyzed taxa, identification and GenBank accession numbers. Sequences directly retrieved from GenBank (ITS) are in italics.

Taxa	2n	Source	Id.	Accession no.
<i>Spartina alterniflora</i> Loisel.	62	Florida (USA)	1	<i>AJ489595</i>
		Hampshire (United Kingdom)	2	<i>AJ489598</i>
		[var. <i>glabra</i> (Muhl. ex Elliott) Fernald]		
		Marchwood (United Kingdom)	3	<i>AJ489594</i>
		Maryland (USA)	4	<i>AJ489597</i>
		Massachusetts (USA)	5	<i>AJ489596</i>
		South England (United Kingdom)	6	<i>AF272775</i>
<i>S. argentinensis</i> Parodi	40	Santa Fe (Argentina)	7	<i>AF372642</i>
<i>S. arundinacea</i> (Thouars) Carmich.	40	Crozet Islands (France)	8	<i>AF372634</i>
<i>S. bakeri</i> Merr.	40	Florida 1 (USA)	9	<i>AJ489599</i>
		Florida 2 (USA)	10	<i>AF372639</i>
<i>S. ciliata</i> Brongn.	?	Buenos Aires (Argentina)	11	<i>AF372636</i>
<i>S. cynosuroides</i> (L.) Roth	40	New Jersey (USA)	12	<i>AF372637</i>
		Texas (USA)	13	<i>AJ489600</i>
<i>S. densiflora</i> Brongn.	60*	California 1 (USA)	14	<i>AJ489785</i>
		California 2 (USA)	15	<i>AJ489786</i>
		California 3 (USA)	16	<i>AF372635</i>
<i>S. foliosa</i> Trin.	60	California 1 (USA)	17	<i>AJ489787</i>
		California 2 (USA)	18	<i>AF372641</i>
<i>S. gracilis</i> Trin.	40	California (USA)	19	<i>AJ489788</i>
		Hsiao et al., 1999 [voucher: UTC-194828]	20	<i>AF019844</i>
		Washington (USA)	21	<i>AJ489792</i>
		Wyoming 1 (USA)	22	<i>AJ489789</i>
		Wyoming 2 (USA)	23	<i>AJ489790</i>
		Wyoming 3, Yellowstone (USA)	24	<i>AJ489791</i>
<i>S. maritima</i> (Curtis) Fernald	60	Asturias, Rodiles (Spain)	25	JN133289
		Brittany (France)	26	<i>AF272776</i>
		Suffolk (United Kingdom)	27	<i>AJ489793</i>
<i>S. patens</i> (Aiton) Muhl.	40	Louisiana (USA)	28	<i>AJ489794</i>
		New Jersey (USA)	29	<i>AF372638</i>
		Texas (USA)	30	<i>AJ489795</i>
<i>S. pectinata</i> Link	40	Hsiao et al., 1999 [voucher: Kew1975–938]	31	<i>AF019843</i>
		Missouri 1 (USA)	32	<i>EF153082</i>
		Missouri 2 (USA)	33	<i>AF372640</i>
		Vermont (USA)	34	<i>AJ489796</i>
<i>S. spartinae</i> (Trin.) Merr. ex Hitchc.	40	Texas 1 (USA)	35	<i>AJ489797</i>
		Texas 2 (USA)	36	<i>AJ489798</i>
<i>S. versicolor</i> Fabre	?	Asturias 1, Rodiles (Spain)	37	JN133290
		Asturias 2, El Espartal (Spain)	38	JN133291
		Asturias 3, Los Quebrantos (Spain)	39	JN133292
		Valencia (Spain)	40	<i>AJ489784</i>
<i>Cynodon dactylon</i> (L.) Pers.	36	Brittany (France)	41	<i>AF372643</i>

* This chromosome count was obtained by Gerish (1979), who reported it for *Spartina foliosa*, but Spicher & Josselyn (1985) demonstrated that the plants he worked with were almost certainly *S. densiflora* (for more details see Barkworth 2007).

sequenced to check the reliability of the detected differences. All chromatograms were visually examined to correct possible misinterpretations of the computational routine. Sequenced data were assembled and edited using Muscle 4.0 within Geneious Pro 4.8.5 (Biomatters, Auckland, New Zealand). The limits of the regions were determined

by the position of flanking primers. IUPAC (International Union of Pure and Applied Chemistry) symbols were used to represent nucleotide ambiguities.

A maximum parsimony (MP) analysis was conducted by heuristic search using the MEGA 4 (Kumar et al. 2008). The maximum parsimonious tree was performed using the

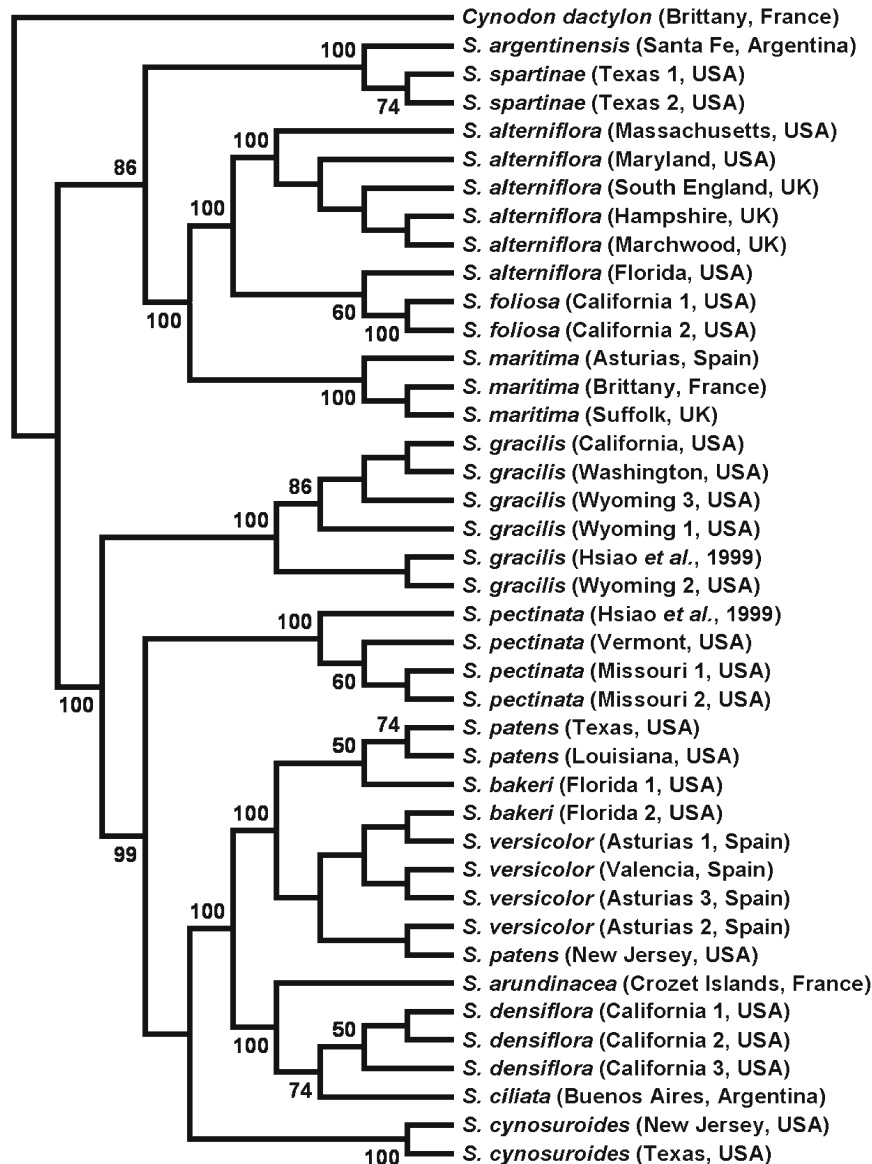


Fig. 1. Consensus tree of 41 samples of the genus *Spartina* and *Cynodon*, inferred from the 3779 most parsimonious trees (the numbers at nodes are bootstrap values, 2000 replicates). The consistency index is 0.74038, the retention index is 0.92904 and the composite index is 0.76668 for 118 parsimony informative characters.

close neighbor interchange (CNI; level = 3) algorithm in which the initial trees were obtained with random addition of sequences (10 replicates). The robustness of nodes was inferred from a bootstrap analysis of 2000 replicates. In order to display conflicts in data, SplitsTree 4.10 (Huson & Bryant 2006) was used to construct an unrooted network (uncorrected P, NeighborNet distance) of relationships between individuals. All alignment gaps were treated as missing data. Such a network, compared to a phylogenetic tree, potentially offers a more realistic visual presentation of possible reticulate phylogenetic patterns by depicting the incompatible signals in a net-like scheme (Moulton & Huber 2009). Relationships among ribotypes in the clade of *Spartina bakeri*, *S. patens* and *S. versicolor* were inferred using TCS software (Clement et al. 2000), which uses a statistical parsimony method to estimate ancestral or intermediate ribotypes. The accession numbers of the sequences submitted to GenBank are listed in Table 1.

Results

Firstly, an exploratory data analysis of the studied sequences of *Spartina* was performed, and summarized in Table 2. The alignment of 40 ITS sequences produced a matrix of 606 characters with 19.47% of these being parsimony informative.

In the maximum parsimony analysis (Fig. 1) the distribution of the nrDNA ribotypes shows that *Spartina* genus is a monophyletic group, with bootstrap values exceeding 50%. *Spartina* is split in two main clades; the first one (consisting primarily of hexaploid species) includes *S. alterniflora*, *S. foliosa*, *S. maritima*, *S. spartinae* and *S. argentinensis*, and is supported by an 86% bootstrap value. The second clade (consisting primarily of tetraploid species) is well-supported (100% bootstrap) and contains nine species: *S. arundinacea*, *S. bakeri*, *S. ciliata*, *S. cynosuroides*, *S. densiflora*, *S. gra-*

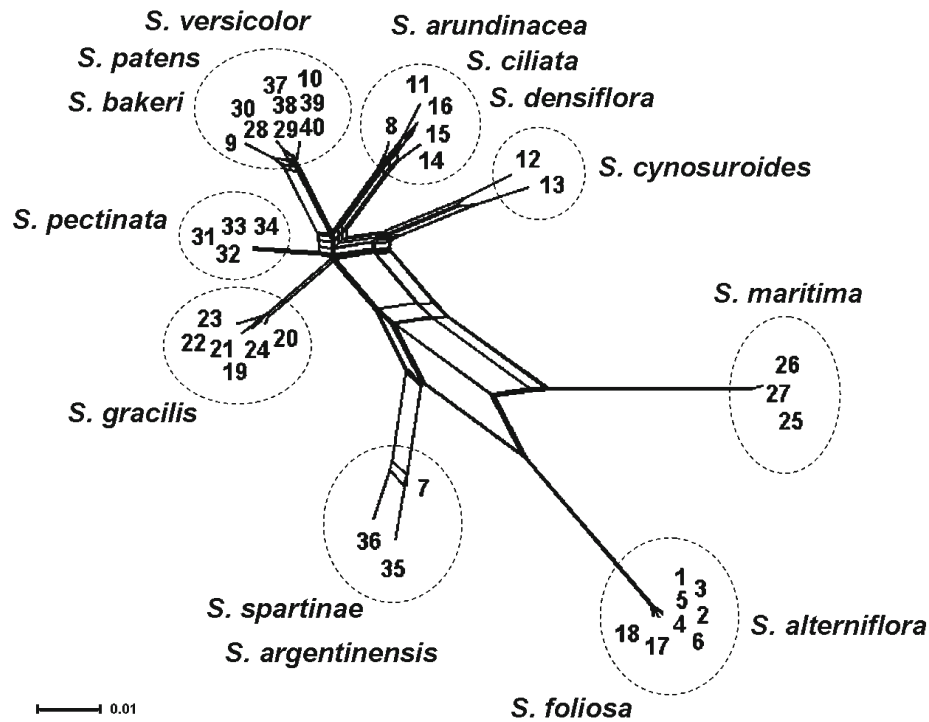


Fig. 2. NeighbourNet network for ITS sequences from 40 accessions of the *Spartina* group. The least squares fit index for the split network has a value of 99.97%. Groups have been circled and labeled to correspond with the distinct groups indicated in the phylogenetic tree (Fig. 1). The numbers correspond to the arrangement of the populations of Table 1.

Table 2. Alignment and sequence characteristics of the nrDNA region within *Spartina*.

Sequence characteristics	ITS data set
Sequence length range (bp)	589–602
Sequence length mean (bp)	596.42
G+C content (range%)	51.42–54.11
G+C content (mean%)	52.42
Aligned sequence length (bp)	606
Parsimony informative characters	118
Constant characters	464
Uninformative characters	24

cilis, *S. patens*, *S. pectinata* and *S. versicolor*. Within this clade, *S. bakeri*, *S. patens* and *S. versicolor* appear closely related, forming a well-supported monophyletic sister group (100% bootstrap) to the species: *S. arundinacea*, *S. ciliata* and *S. densiflora* which form another monophyletic clade (100% bootstrap).

The unrooted SplitsTree NeighbourNet network in Fig. 2 provides a graphical representation of ribotypes which is not purely dichotomous where reticulation indicates alternative mutational pathways (i.e. homoplasy) within groups. The characteristics of ITS sequences for the clade composed by the species *Spartina bakeri*, *S. patens* and *S. versicolor* are summarised in Table 3. In addition, the topology of genealogy generated by TCS software with a 95% cutoff value to construct the significant connection is shown in Fig. 3. These results were consistent with the maximum parsimony analysis (Fig. 1) and NeighbourNet network

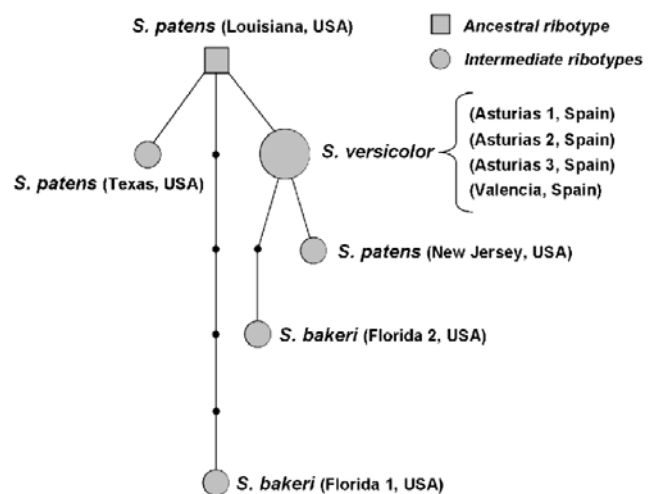


Fig. 3. Statistical parsimony analysis of *S. bakeri*, *S. patens* and *S. versicolor*. Black dots represent intermediate ribotypes missing in the data set.

(Fig. 2). The sample of *S. patens* from Louisiana (USA) was identified as an ancestral ribotype. In addition, it should be noted that regardless of the origin of the samples (Atlantic or Mediterranean region), *S. versicolor* presented the same ribotype.

Discussion

The molecular phylogeny of various *Spartina* species presented here, and based on ITS regions (Fig. 1), shows a consensus tree highly consistent with those ob-

Table 3. Summary of the nucleotide site variation of the Internal Transcribed Spacers (ITS1 and ITS2) in *Spartina bakeri*, *S. patens* and *S. versicolor*.

Taxon	Nucleotide position ITS region									
	0	0	0	0	1	2	3	4	4	4
	5	8	8	9	9	8	9	4	4	9
	9	6	8	2	0	6	3	0	5	8
<i>S. bakeri</i> Florida 1 (USA)	C	C	C	T	A	A	A	C	A	–
<i>S. bakeri</i> Florida 2 (USA)	G	T	T	T	G	C	C	G	T	–
<i>S. patens</i> Louisiana (USA)	C	C	T	T	G	C	C	C	T	–
<i>S. patens</i> New Jersey (USA)	C	T	T	T	G	C	C	C	T	A
<i>S. patens</i> Texas (USA)	C	C	T	C	G	C	C	C	T	–
<i>S. versicolor</i> Rodiles (Spain)	C	T	T	T	G	C	C	C	T	–
<i>S. versicolor</i> El Espartal (Spain)	C	T	T	T	G	C	C	C	T	–
<i>S. versicolor</i> Los Quebrantos (Spain)	C	T	T	T	G	C	C	C	T	–
<i>S. versicolor</i> Valencia (Spain)	C	T	T	T	G	C	C	C	T	–

tained by other authors (Baumel et al. 2002a; Fortune et al. 2007), as well as with the phylogenetic relationships and evolutionary dynamics in polyploid species of *Spartina* indicated by Ainouche et al. (2009). Diversification of the genus *Spartina* raises interesting questions regarding biogeography, history and evolution of species. Our results provide strong evidence for existence of two main clades in the genus: the first one consisting of hexaploid native American plants [*S. alterniflora* Loisel. ($2n = 62$) and *S. foliosa* Trin. ($2n = 60$)], a hexaploid native European species [*S. maritima* (Curtis) Fernald ($2n = 60$)], and two other tetraploid native American plants [*S. argentinensis* Parodi and *S. spartinae* (Trin.) Merr., both ($2n = 40$)]. The second well-supported main clade within *Spartina* revealed by our study was mainly constituted by tetraploid species ($2n = 40$), most of them native to America [*S. arundinacea* (Thouars) Carmich., *S. bakeri* Merr., *S. cynosuroides* (L.) Roth, *S. gracilis* Trin., *S. patens* (Aiton) Muhl. and *S. pectinata* Link]. This second clade also included *S. versicolor* Fabre (considered native to European coasts and with unknown chromosome count), as well as *S. densiflora* Brongn. ($2n = 60?$) and *S. ciliata* Brongn. ($2n = ?$). The clades obtained in this study are supported by recently published articles (see Baumel et al. 2002a; Ainouche et al. 2009), which also considered two main lineages in the genus, meanwhile our data markedly differ from the two “stirps” (lineages) recognized by Saint-Yves (1932) based on the anatomy of the leaf blade.

With regard to the Cantabrian coast (Northern Spain), the sample identified as *Spartina maritima* can be seen to constitute a clade with the samples belonging to the same species from Brittany and Britain (Fig. 1). The history of this Euro-African species is still an unsolved question, with several hypotheses proposed: current populations of *S. maritima* could actually represent a relic from a wider distribution of an ancestral hexaploid species, or alternatively, *S. maritima* was introduced from North America to the Eastern Atlantic coast and subsequently vanished from the

New World. Comparative analyses of various gene histories in this clade should therefore help exploring this question and estimating the divergence time between *S. maritima* and its sister lineage *S. alterniflora* / *S. foliosa* (see Ainouche et al. 2003). Furthermore, the three Cantabrian samples identified as *S. versicolor* were integrated into the second clade, mainly constituted by tetraploid species (Fig. 1). Indeed, these samples together with other European *S. versicolor* from Valencia (Spain) formed a well-defined subclade with the North American samples of *S. patens* and *S. bakeri*, the close relationships between these latter two species having previously been highlighted by Baumel et al. (2002a), and also reported by Saint-Yves (1932) and Barkworth (2007). The NeighbourNet analysis (Fig. 2) was consistent with the abovementioned consensus tree, strongly supporting the groupings set out in Fig. 1. The statistical parsimony analysis of *S. patens*, *S. bakeri* and *S. versicolor* (Fig. 3) indicated that the ribotype of *S. versicolor* is intermediate between that of the American samples of *S. patens* from Louisiana and New Jersey (USA), and that of *S. bakeri* (sample 2) from Florida. However, the other *S. bakeri* (sample 1) from Florida displayed the highest number of intermediate ribotypes. Finally, *S. patens* (Louisiana) also showed similarities to *S. patens* from Texas.

As a result of the data presented herein, it should be concluded that the European plant identified as *Spartina versicolor* is identical to the North American accession *S. patens* (= *S. juncea*), and therefore they should be treated as the same species. This is a clear example showing the need for constant reliable taxonomic checking through updated methods and perspectives (Bortolus 2008) and the importance of genealogical approaches to better understand the historical and evolutionary context of species expansion (Fortune et al. 2008). Besides, the presence of *S. patens* on the Iberian coasts is fully accepted (e.g. Ainouche et al. 2003; Ayres et al. 2004; Page et al. 2010), and consequently, it has already been included in numerous catalogues of invasive plants of the Iberian Peninsula coast (e.g. Sanz

Elorza et al. 2004; Campos & Herrera 2009a, 2009b).

Within this assumption, the management of European coastal natural resources should consider that *Spartina patens* strongly modifies and disturbs such habitats (Sanz Elorza et al. 2004; Campos & Herrera 2009a; Page et al. 2010). The goals of the management of *S. patens* on European shores should target to prevent the establishment and spread of any *Spartina* populations. Outreach and education about the threat and management of *Spartina* and measures to prevent new introductions are also important elements. Given the difficulty of working in estuarine environments and the high cost of the management methods, early detection and controls are critical to a successful protection of estuaries from infestation by *Spartina*. The main efforts to achieve this goal should include (1) preventing the movement of *Spartina* propagules to other areas suitable for invasion; (2) establishing clear procedures, authorities, and responsibilities for action, and a framework for implementation of the management and (3) education and information of the general public about *Spartina* and the need to control it. Potential management options may be considered according to the size of populations, location, and species of *Spartina*. Actually this kind of actions have already started within this genus (e.g. Daehler et al. 1996; Howard Morgan & Sytsma 2010), where deciding whether the control or eradication approach is the key for the subsequent management decisions.

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