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Sphagnum centrale and *S. palustre* from Mediterranean basin: a comparison with conspecific North American populations by microsatellite analysis

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Abstract – Twenty nine South European specimens of *Sphagnum centrale*, *S. palustre*, *S. papillosum* and *S. magellanicum* were studied with 15 microsatellite markers. In contrast with eastern North American populations, our analysis showed a genetic overlapping between *S. centrale* and *S. palustre* in mixed populations. Moreover, Mediterranean species showed a genetic richness (total number of alleles) higher than that calculated in conspecific American samples. As Mediterranean *Sphagnum* bogs are remnant populations, microsatellites could well work for selecting source populations in order to recover Mediterranean peatlands.

Genetic richness / Mediterranean peatland conservation / peat mosses / relict populations

INTRODUCTION

Peatlands are ecosystems with a great importance in the global climate since they fix large amounts of carbon. These systems cover about 3% of the Earth land surface (Yu *et al*., 2011). Even if peatlands are distributed worldwide, the largest areas are in North America and North Eurasia, whereas in South Europe they are in regression (Vasander *et al*., 2003; Yu *et al*., 2011). Indeed peatlands are protected by the European Council Habitat Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora).

One primary objective of nature conservation is the maintenance of genetic diversity, which implies the need to acquire information about intraspecific genetic variation in populations of endangered/vulnerable species (Reed & Frankham, 2003). The outcomes of population studies based on molecular markers should be taken into account for planning strategies aimed at conservation strategies. The usefulness of population genetics in biodiversity conservation, in particular for plants, has been indeed recognized since the end of ‘80s (Avise, 1994).

At present, many projects aim to restore degraded peatlands due to habitat loss and/or exploitation; the efforts are mainly focused on the establishment of the best environmental conditions (see for instance Robroek *et al*., 2009). Nevertheless, in most cases genetic imprint of the species and/or populations are not taken in account, although the use of molecular methods (see Crespo Pardo *et al.*, 2014, for a review) can provide data for the application of different recovery procedures.

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The scarceness of *Sphagnum* in South Europe makes unrealistic the use of close populations to recover the relict peatlands. Some authors have criticized reintroductions because they often involve genotype translocations across different geographical areas (Edmands, 2006). In fact, especially in predominantly selfing populations, organisms tend to be highly locally adapted, with a strong linkage disequilibrium, which would be broken by introducing new non-adapted genotypes, extraneous to local genetic pools, with the risk of outbreeding depression (Fischer & Matthies, 1997; Edmands, 2006; Johnson & Shaw, 2015). However, Krishnamurthy & Francis (2012), suggest the calculation of genetic distances between source and receiving populations, in order to overcome this problem.

Sphagnum occupies the most basal position in moss phylogenies, holding a highly conserved genome; accordingly, sequences for the internal transcribed spacers of the nuclear ribosomal DNA can be easily aligned across its species (e.g. Shaw *et al.*, 2003). Furthermore, microsatellite primers that were developed for one group of closely related *Sphagnum* species amplify homologous *loci* across the whole genus (Shaw *et al.*, 2008). By contrast, systematic studies *sensu lato*, mainly carried out by microsatellites, highlight the occurrence of interspecific hybridization, molecular divergence between disjunct populations, cryptic speciation and introgression, all phenomena demonstrating an ongoing molecular evolution in peat mosses, in striking contrast to the traditional idea of living fossils, frequently used to tag these plants (e.g. Shaw *et al.*, 2003, 2014; Karlin *et al.*, 2008, 2009; Ricca *et al.*, 2008, 2011).

Genetic approach in conservation biology is directed towards assisting in resolution of taxonomic uncertainties. This effort is seriously compromised by the lack of an agreed definition for species in biodiversity conservation. For instance the relationship between *Sphagnum centrale* C. Jens. and *S. palustre* L. has been extensively considered in the past years, since the morphological identification between these taxa is often difficult (Daniels & Eddy, 1985; Anderson & Ammann, 1991), and both taxa from North American populations were differentiated by a microsatellite analysis (Karlin *et al.*, 2010).

South European population of *Sphagnum* have been poorly studied so far, in relation to biodiversity and conservation aspects. Terracciano *et al.* (2012) studied the genetic variability in Italian populations of *S. palustre* with inter simple sequence repeat (ISSR) markers. They found a higher genetic variation in the northern populations, which have greater possibility of gene exchange with larger Northern-European populations. However, they suggested local extinction risk for all the Italian populations due to their very small size and the vulnerability of those peatlands.

This study aims to: i) investigate the genetic structure of populations of *Sphagnum centrale* and *S. palustre* in South Europe by microsatellites; ii) compare them to conspecific eastern North American populations; iii) clarify the relationships between the two taxa in the studied populations. The results are discussed in relation to management and conservation implications.

MATERIALS AND METHODS

Sampling

A total of 29 samples were analysed: 9 samples of *S. palustre* from Croatia, Italy and Spain, 11 of *S. centrale* from Bulgaria, Italy, Serbia and Turkey, 5 of

S. papillosum Lindb. from Greece, Italy and Spain, and 4 of *S. magellanicum* Brid. from Italy and Spain. All species, (the three first of which are allopolyploids and the last one haploid), are included in the Section Sphagnum. Although the present study aimed to investigate on a molecular ground the relationships between *S. centrale* and *S. palustre* from Mediterranean basin, some samples of *S. magellanicum* and *S. papillosum* were also included in the study, in order to validate the analysis. *Sphagnum palustre* and *S. centrale* were regarded as distinct species, according to Crum (1984) and Karlin *et al*. (2010). Species names and their acronyms used in this work, origin information, and Herbarium data are reported in Table 1.

All the specimens were herbarium samples from AYDN, BP, NAP, SANT, Herbarium Orto Botanico Universitá della Calabria (Table 1).

Table 1. Provenance and herbarium information of the samples analysed of the four *Sphagnum* species studied. Acronym legend: CN = *S. centrale*; MG = *S. magellanicum*; PL = *S. palustre*; PP = *S. papillosum*; BG = Bulgaria; ES = Spain: GR = Greece, HR = Croatia; IT = Italy; RS = Serbia; TR = Turkey)

Sphagnum species (acronym sample used)	Provenance	Herbarium and accession code
S. centrale (CN-BG)	Bulgaria, Vitosha Mt.	NAP 30/2000
S. centrale (CN-IT1)	Italy, Piemonte, Lagoni di Mercurago	NAP 12/2009
S. centrale (CN-IT2)	Italy, Veneto, Vigo di Cadore	NAP 103/2009
S. centrale (CN-IT3)	Italy, Piemonte, Val d' Ala	NAP 154/1999
S. centrale (CN-IT4)	Italy, Piemonte, Val d' Ala	NAP 155/1999
S. centrale (CN-IT5)	Italy, Piemonte, Valle di Viù	NAP 170/1999
S. centrale (CN-IT6)	Italy, Piemonte, Valdieri	NAP 132/2010
S. centrale (CN-IT7)	Italy, Piemonte, Valdieri	NAP 133/2010
S. centrale (CN-RS1)	Serbia, Mt Vrtop	BP 9536/S
S. centrale (CN-RS2)	Serbia, Okruglica, Vlasina lake	BP 9474/S
S. centrale (CN-TR)	Turkey, Trabzon, Ağaçbaşı Yayla	AYDN 6074-S
S. magellanicum (MG-ES1)	Spain, Vall de Conangles (Lleida)	SANT, Bryo 2407-A
S. magellanicum (MG-ES2)	Spain, Xistral (Lugo)	SANT, Bryo 2408-A
S. magellanicum (MG-ES3)	Spain, P.to del Tremedal (Avila)	SANT, Bryo 2409-A
S. magellanicum (MG-IT)	Italy, Piemonte, Valdieri	NAP 49/2010
S. palustre (PL-ES1)	Spain, Galicia, Abadin	SANT, Col. Carlos Real 155
S. palustre (PL- ES2	Spain, Galicia, Muras	SANT, Col. Carlos Real 296
S. palustre (PL- ES3)	Spain, Galicia, Ourol	SANT, Col. Carlos Real 383
S. palustre (PL-HR1)	Croatia, Gorski kotar Mts	BP 9534/S
S. palustre (PL-HR2)	Croatia, Gorski kotar Mts	BP 9531/S
S. palustre (PL-IT1)	Italy, Lazio, Posta Fibreno	NAP 179/2010
S. palustre (PL-IT2)	Italy, Toscana, Lago di Sibolla	NAP 57/2006
S. palustre (PL-IT3)	Italy, Piemonte, Lagoni di Mercurago	NAP 128/2006
S. palustre (PL-IT4)	Italy, Cosenza, Parco Monte Caloria	Herbarium Orto Botanico Universitá della Calabria 1202
S. papillosum (PP-ES1)	Spain, Galicia, Cervo	SANT, Col. Carlos Real 379
S. papillosum (PP-ES2)	Spain, Galicia, Muras	SANT, Col. Carlos Real 162
S. papillosum (PP-ES3)	Spain, Galicia, Abadin	SANT, Col. Carlos Real 154
S. papillosum (PP-GR)	Greece, Voras Mts.	BP 9523/S
S. papillosum (PP-IT)	Italy, Piemonte, Lagoni di Mercurago	NAP 129/2006

Microsatellite analysis

DNA was extracted following the protocols described in Terracciano *et al.* (2012). Primer sequences and microsatellite characteristics for the 15 markers analysed in this study are described by Shaw *et al.* (2008). The 15 microsatellite markers, numbered as in Shaw *et al.* (2008), are: 1, 3, 4, 5, 7, 9, 10, 12, 14, 16, 17, 18, 19, 20, 22, 28, 29, 30.

Microsatellites were amplified in 8 µl multiplexed reactions, each targeting a set of three *loci*. Primer sets were arrayed for multiplexing according to expected fragment sizes (for non overlapping amplification products) and alternating fluorophores. Each primer pair included a forward primer fluorescently labeled with HEX or 6-FAM (Integrated DNA Technologies, Coralville, IA). Multiplexing was accomplished using a Qiagen Multiplex PCR kit (Valencia, CA), scaled for smaller reactions, but otherwise used according to the manufacturer's recommendations. Five to 20 ng of genomic DNA in 3 µl H_2O served as template in each reaction. A standard thermocycling regime was implemented for all primer sets, with no additional optimization. This consisted of an initial denaturation and hot-start activation at 95°C for 15 min, then 30 cycles of 94°C for 30 sec, 54°C for 90 sec and 72°C for 60 seconds. A final extension at 60°C for 30 min was performed. PCR products were diluted in sterile water, and 1.2 µl of the dilution was mixed with GS500 size standard and Hi-Di TM Formamide (Applied Biosystems, Foster City, CA) for electrophoresis on an ABI 3730 sequencer. Size determinations and genotype assignments were made using GeneMarker 1.30 software (Softgenetics, State College, PA).

Genetic analyses

Fragment sizes were coded as alleles. All statistical calculations were performed using GenAlEx v 6.1 (Peakall & Smouse, 2006). The Analysis of Molecular Variance (AMOVA, Excoffier *et al.*, 1992) was calculated using the Codominant-Genotypic genetic distance option.

The proportion of shared alleles (POSA; Bowcock *et al.*, 1994) was calculated using Microsatellite Analyzer 4.05 (Dieringer & Schlötterer, 2003). POSA ranges from 0 to 1, with 1 indicating that the two species are genetically identical. The Principal Coordinates Analysis (PCoA) was performed as described in Smouse & Peakall (1999). Nei's Genetic Identity (Nei, 1972, 1978) among populations was calculated as well.

Scanning Electron Microscope (SEM)

SEM observations were performed to rule out any possible morphological misidentification between *Sphagnum centrale* and *S. palustre* (Daniels & Eddy, 1985; Anderson & Ammann, 1991). Moss samples (see Table 1, acronyms PL-IT3 and CN-IT1) from Lagoni di Mercurago (Northern Italy, 45° 733' 900''N; 8° 550' 220'' E) were chosen because that was the only site where the two species are sympatric. Shoots of *Sphagnum centrale* and *S. palustre* were fixed with 3% glutaraldehyde for 24 h at 4°C, post-fixed in 2% OsO4 in 0.1 M phosphate buffer (pH 6.8) at 4°C for other 24 h; afterwards, shoots were thoroughly washed in phosphate buffer, cut into small pieces (3-5 mm), mounted on stubs and observed humid under an environmental scanning electron microscope FEI QUANTA 200 working in low vacuum conditions.

RESULTS

Microsatellite analyses

The number of amplicons *per* markers varied from 4 (marker number 3) to 24 (marker 10). Allele frequencies are shown in Table 2. The proportion of shared alleles between *Sphagnum centrale* and *S. palustre* ranged from 0.06 (marker 22) to 0.70 (marker 29). The mean value for the 15 markers was 0.33.

The expected heterozygosity for *Sphagnum centrale*, *S. palustre*, *S. papillosum* and *S. magellanicum* was 0.697, 0.605, 0.320 and 0.351 respectively, whereas the observed heterozygosity was 0.800, 0.706, 0.200, 0.000. The heterozygosity levels for each marker for *S. centrale* and *S. palustre* are given in Table 3.

PCoA clearly separates the four species, with the exception of *Sphagnum centrale* and *S. palustre*, slightly overlapping; in particular, three samples of *S. palustre* -those from Italy, (PL-IT1, Pl-IT2, PL-IT3) – grouped with *S. centrale* from Bulgaria, Italy and Serbia (CN-BG, CN-IT1, CN-RS1, CN-RS2) (Fig. 1). An AMOVA for the four species showed a percentage of variation within species of 68% and 32% among species (PhiPT=0.324; p=0.001). An AMOVA between *S. centrale* and *S. palustre* was performed as well. The percentage of variation within species was 82% and 18% between species (PhiPT=0.178; p=0.0002). The genetic distances (Fst) between the four species, all significant ($p<0.05$), and the Nei's Genetic Identity are given in Table 4; as expected, the lowest divergence (lowest Fst) and the highest Nei's Genetic Identity were observed between *S. central* and *palustre*.

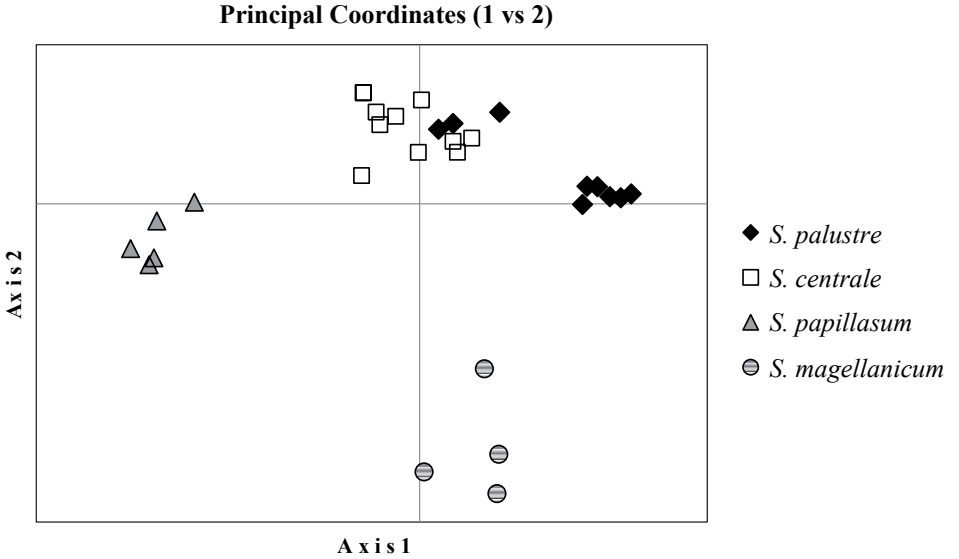


Fig. 1. Principal Coordinates Analysis of *Sphagnum centrale*, *S. magellanicum*, *S. palustre* and *S. papillosum* genotypes. The first two principal coordinates comprise the 26.47% and 22.37% of the variation, for a cumulative total of 48.84%.

Table 2. Allele frequency and proportion of shared alleles (POSA) for 15 microsatellite markers in *Sphagnum centrale* and *S. palustre*. Marker numbers are those used by Shaw *et al.* (2008)

Marker	Allele	*S. centrale*	*S. palustre*	POSA	Marker	Allele	*S. centrale*	*S. palustre*	POSA
1	242	–	0.20	0.39	**14**	227	0.08	–	0.21
	243	0.04	0.25			229	–	0.13	
	244	0.25	–			231	0.13	–	
	245	0.08	0.05			238	0.04	–	
	250	0.17	0.20			245	0.08	–	
	251	0.13	0.30		**17**	153	0.08	–	0.33
	252	0.04	–			155	0.58	–	
	254	0.04	–			156	–	0.20	
	255	0.08	–			158	0.17	0.30	
	256	0.04	–			160	–	0.15	
	261	0.13	–			161	0.08	0.20	
3	165	0.88	0.40	0.53		162	0.08	0.15	
	167	–	0.10		**18**	96	0.22	0.57	0.51
	168	0.13	0.40			126	–	0.14	
	169	–	0.10			138	0.11	–	
4	175	–	0.33	0.34		139	0.11	–	
	176	0.11	–			153	0.56	0.29	
	177	0.06	–		**19**	243	0.05	–	0.15
	180	0.17	0.25			245	0.05	–	
	182	0.11	–			253	0.20	–	
	183	0.06	0.17			254	–	0.06	
	186	0.11	0.25			255	0.10		
	189	0.11	–			256	–	0.06	
	195	0.22	–			260	0.15	–	
	198	0.06	–			261	0.05	0.11	
5	184	–	0.10	0.42		262	0.05	–	
	185	–	0.05			263	0.05	0.44	
	186	–	0.05			264	0.05	0.33	
	187	0.13	0.05			265	0.10	–	
	188	–	0.10			266	0.10	–	
	189	0.08	0.10			267	0.05	–	
	190	0.08	0.10		**20**	272	0.17	–	0.16
	191	0.13	0.15			273	0.17	–	
	192	–	0.05			278	–	0.06	
	193	0.04	–			282	0.17	–	
	194	0.08	0.10			284	0.04	–	
	196	0.08	–			287	–	0.22	
	197	–	0.10			288	0.08	0.11	
	199	0.08	–			289	–	0.33	
	200	0.04	–			290	–	0.06	
	201	0.17	–			291	0.08	0.17	
	202	0.04	–			292	–	0.06	
	203	–	0.05			293	0.17	–	
	205	0.04	–			298	0.08	–	
9	153		0.11	0.06		303	0.04	–	
	170	0.09	–		**22**	87	–	0.06	0.49

Table 2. Allele frequency and proportion of shared alleles (POSA) for 15 microsatellite markers in *Sphagnum centrale* and *S. palustre*. Marker numbers are those used by Shaw *et al.* (2008) (*continued*)

Marker	Allele	*S. centrale*	*S. palustre*	POSA	Marker	Allele	*S. centrale*	*S. palustre*	POSA
	174	–	0.56			90	0.04	–	
	175	0.09	–			91	0.08	–	
	178	0.09	–			96	0.04	–	
	180	0.05	–			98	0.13	0.31	
	181	–	0.22			99	0.04	–	
	182	0.32	–			100	0.04	–	
	183	0.05	–			101	0.17	0.13	
	185	0.09	–			102	0.04	–	
	186	0.05	–			103	0.08	–	
	189	0.09	–			104	0.29	0.19	
	190	0.09	0.06			106	0.04	0.19	
	192	–	0.06			110	–	0.13	
10	179		0.05	0.10	**28**	221	0.13	0.10	0.27
	209	–	0.05			223	0.08	0.20	
	210	0.09	–			224	0.04	0.20	
	212	0.09	–			226	0.08	–	
	213	0.09	–			228	0.08	–	
	214	0.05	–			231	–	0.05	
	215	0.05	0.10			233	–	0.20	
	216	0.05	0.05			234	–	0.10	
	217	0.05	–			235	–	0.10	
	218	0.09	–			237	0.13	–	
	219	0.05	–			239	0.25	–	
	220	0.05	–			241	0.08	–	
	222	–	0.10			242	0.04	–	
	224	–	0.10			244	0.08	0.05	
	227	–	0.05		**29**	181	0.05	–	0.70
	229	–	0.10			189	0.05	–	
	230	0.18	–			190	0.20	0.15	
	231	–	0.05			192	0.05	0.10	
	232	–	0.10			193	0.30	0.25	
	233	–	0.05			195	0.25	0.50	
	234	–	0.15			199	0.10	–	
	239	0.09	–		**30**	131	0.09	–	0.25
	244	0.09	–			135	0.05	–	
	258	–	0.05			137	–	0.06	
14	192	0.08	–	0.21		138	0.41	0.25	
	206	0.08	–			139	0.14	–	
	211	0.04	–			140	–	0.19	
	212	0.04	0.25			141	–	0.25	
	214	0.13	0.50			143	0.09	–	
	217	0.04	0.13			144	–	0.25	
	219	0.17	–			147	0.09	–	
	220	0.04	–			153	0.05	–	
	225	0.04	–			154	0.09	–	

Table 3. Number of samples (N), number of alleles (Na) and observed heterozygosity (Ho) at each locus for *Sphagnum centrale* and *S. palustre* for 15 microsatellite markers, numbered as in Shaw *et al.* (2008)

		Markers														
		1	**3**	**4**	**5**	**9**	**10**	**14**	**17**	**18**	**19**	**20**	**22**	**28**	**29**	**30**
S. centrale	N	11	7	9	11	10	10	11	11	8	9	11	11	11	9	10
	Na	10	2	9	12	10	13	13	5	4	12	9	11	10	7	8
	Ho	0.55	0.00	0.44	0.64	0.30	0.50	0.55	0.00	0.00	0.56	0.36	0.55	0.64	0.11	0.60
S. palustre	N	9	9	5	9	8	9	7	9	7	8	8	7	9	9	7
	Na	5	4	4	12	5	13	4	5	3	5	7	6	8	4	5
	Ho	1.00	0.00	0.40	0.89	0.13	0.78	0.00	0.11	0.00	0.00	0.50	0.71	0.67	0.44	0.86
Total	N	20	16	14	20	18	19	18	20	15	17	19	18	20	18	17
	Na	11	4	10	19	14	24	14	7	5	14	14	13	14	7	12

Table 4. Pairwise population Fst values/Nei's Genetic Identity (GI) matrix between *Sphagnum centrale*, *S. magellanicum*, S. *palustre* and *S. papillosum*

Fst/GI	*S. centrale*	*S. magellanicum*	*S. palustre*	*S. papillosum*
S. centrale	0.000/1.000			
S. magellanicum	0.310/0.145	0.000/1.000		
S. palustre	0.151/0.398	0.323/0.184	0.000/1.000	
S. papillosum	0.306/0.196	0.509/0.095	0.373/0.078	0.000/1.000

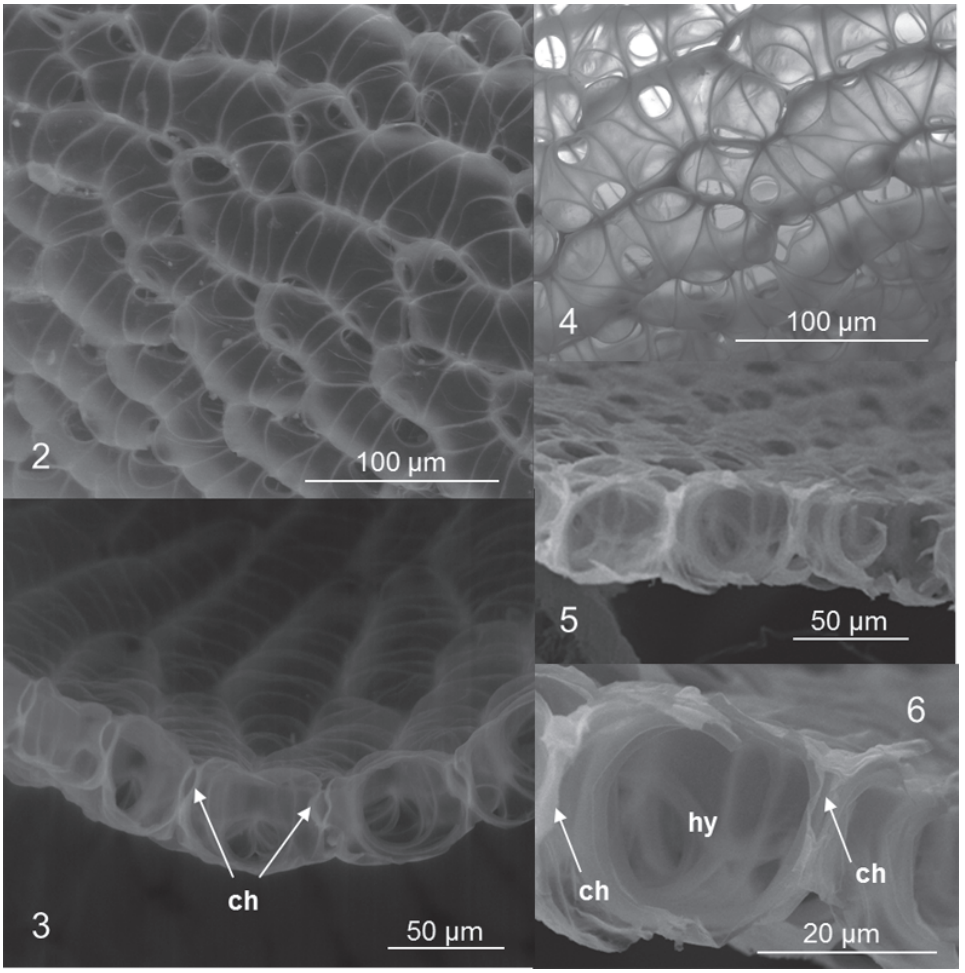
SEM observations

SEM observations indicate that no morphological misidentification occurred in the mosses sampled at Lagoni di Mercurago, where *Sphagnum centrale* and *S. palustre* are sympatric. Abaxial phyllidium surface (Figs 2 and 4) is not diagnostic, showing in both species hyalocysts of similar size, with numerous pores, mainly located between V-shaped wall thickenings. However, the two species can be distinguished according to the different shape of their chlorocysts, very narrow and barrel-shaped in *S. centrale* (Figs 5 and 6), triangular-shaped and protruding towards the adaxial surface of the phyllidium in *S. palustre* (Fig. 3).

DISCUSSION

Conservation of the biodiversity is one of the main focus in the framework of the environmental biology; knowledge and classification of the organisms are fundamental aspects to promote conservation policy. Research attention is mainly converged on vulnerable organisms and habitats that are exposed to a concrete extinction risk.

Bogs and fens are relict habitats in Southern Europe, and therefore are worthy of attention, especially because they may keep a noticeable fraction of the



Figs 2-6. SEM micrographs of *Sphagnum palustre* (NAP 128/2006) and *S. centrale* (NAP 12/2009). **2.** *S. palustre* abaxial phyllidium surface. **3.** *S. palustre* phyllidium cross section showing triangular chlorocysts. **4.** *S. centrale* abaxial phyllidium surface. **5.** *S. centrale* phyllidium cross section. **6.** Detail of the *S. centrale* phyllidium cross section showing narrow, barrel-shaped chlorocysts (ch = chlorocyst; hy = hyalocyst).

biodiversity, as shown in the present study. However, the identification of peat mosses, and particularly *Sphagnum* species, possesses some problems due to their high phenotypic plasticity (Stenøien *et al*., 2014; Szurdoki *et al*., 2014); mosses growing in wet habitats exhibit high morphological variability in response to water level fluctuations and quality changes, among other environmental conditions (Hedenäs, 1996). Another factor affecting morphological variability and confusing the species classification of peat mosses is hybridization (Cronberg & Natcheva, 2002; Flatberg *et al.*, 2006; Natcheva & Cronberg, 2007). *Sphagnum centrale* and *S. palustre* are regarded as allopolyploid taxa, having one parental species in common (Karlin *et al.*, 2010).

Previous studies in other *Sphagnum* sections (i.e. within the *S. subsecundum* Nees *in* Sturm complex) have pointed out the differences between American and European populations (Shaw *et al.*, 2008). In line with this study, our results contrast with those reported by Karlin *et al*. (2010), who found that, in eastern North American populations, *S. centrale* and *S. palustre* formed two distinct entities on the basis of microsatellite analysis. They found a between-species molecular variance of 29% and a between-species Nei's Genetic Identity of 0.608. These values remarkably contrast with ours (18% of the total variance between species and Nei's Genetic Identity of 0.398); despite the lower genetic identity, in our case the two taxa are less divergent, and this is in agreement with their partial overlapping in the PCoA. However, in agreement with the results by Karlin *et al*. (2010), taxa other than *S. centrale* and *S. palustre*, formed distinct clusters in American as well as in Mediterranean populations. The pattern of heterozygosity is more complex than previously reported (Karlin *et al.*, 2010). *Sphagnum centrale* and *S. palustre* from eastern North America very frequently showed fixed heterozygosity, which support hybrid origin for these diploid species; in Mediterranean samples fixed heterozygosity is only observed once (at locus 1 for *S. palustre*). Fixed heterozygosity occurs in allopolyploid taxa, and indicates a disomic inheritance for *S. centrale* and *S. palustre* from eastern North America; a predominance of disomic inheritance was also calculated in our conspecific samples (heterozigosity observed in *S. centrale* and *S. palustre* 0.800 and 0.706, respectively). American samples showed higher levels of heterozygosity than Mediterranean ones; this result could be related to the small population size, or to ongoing genetic drift in Mediterranean populations. However, this conclusion is not consistent with the results from POSA analysis, which indicate a higher genetic richness of Mediterranean populations according to the higher number of alleles over all *loci* found in these populations, despite the lower number of *loci* analysed (fifteen *versus* seventeen). Even comparing the *loci* in common showing very low levels of variability (*loci* 3 and 17), Mediterranean populations seem to have a higher genetic richness; in fact, *S. centrale* and *S. palustre* from eastern North America have a single allele in common at marker 3, *versus* four (one American allele plus other three) alleles found in Mediterranean conspecific taxa. Similarly, at locus 17, only two alleles were observed in American samples *versus* seven (two American alleles plus other five) alleles found in South European ones. These results indicate an overall higher genetic richness in the Mediterranean populations, and suggest that, after the last glaciations, the eastern North America populations have recruited from Mediterranean populations. Mediterranean basin is considered a hot spot of biodiversity, including genetic diversity in mosses, e.g., *Pleurochaete squarrosa* (Brid.) Lindb. (Grundmann *et al.*, 2008; Spagnuolo *et al.*, 2007, 2009); this finding is probably related to the role of refuge area for animals and plants of the Mediterranean region during the last ice ages. The retention of an ancient polymorphism, reflecting the ancient larger population sizes, could provide a feasible explanation (Van der Velde & Bijlsma, 2003; Spagnuolo *et al.*, 2009), despite the lower size of these relict populations compared to those from Northern Europe and eastern North America. Even if in some cases the number of shared alleles (Table 2) between both taxa was very low (see *loci* 9, 10 and 30), the percentage of shared alleles in relation to their total number was overall comparable to that observed in eastern North American populations (23% *versus* 25%).

According to the lower divergence and the partial overlapping between the diploid mosses *Sphagnum centrale* and *S. palustre* from Mediterranean area, and to the absence of fixed heterozygosity in both taxa, a between-species cross scenario may be hypothesized in the past in the study area. It is possible that the two taxa,

able to hybridize in the past, lost this capacity while diverging in time. Indeed, the partial overlapping between the two species in sympatric Italian population, coupled to the difficult morphological identification (Daniels & Eddy, 1985; Anderson & Ammann, 1991) pushed us to observe morphological characters for ruling out any possible misidentification. A comparison of sympatric Italian samples by SEM showed triangular chlorocysts projecting on adaxial surface in *S. palustre* phyllidium, and barrel shaped chlorocysts in *S. centrale*, indicating a correct identification of mixed Italian populations (Figs 3, 5 and 6).

To our knowledge this is the first contribution addressing the management and conservation of Mediterranean *Sphagnum* populations by microsatellite analysis; the high genetic richness and the role as genetic reservoir are good reasons for their conservation. The management of the endangered South-European *Sphagnum* populations should take in account two critical aspects: i) a proper *in situ* conservation of the endangered wetlands; and ii) the increment of the population size and genetic diversity. Whereas the application of the legislation devoted to conservation is a key issue in the first, the choice of the closest population as a source for the transplant is decisive for the success of reintroduction programs, in order to reduce the risk of outbreeding depression. To maximize the accomplishment of the reintroduction programs in plants, Krishnamurthy & Francis (2012), have suggested to calculate the genetic distances with barcoding tools, but the highly conserved nature of the *Sphagnum* genome makes impossible the sequence approach in practice. Instead, microsatellites, used in plants for conservation purposes (Reunova *et al.*, 2014), have proved their suitability to assess the distinction between *Sphagnum* populations of different origin, and therefore, could be potentially used to calculate between-population genetic distances in order to individuate source populations for recovery of Mediterranean peatlands.

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