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Allegrezza M., Biondi E. & Felici S., 2006. A phytosociological analysis of the vegetation of the central sector of the Adriatic aspect of the Italian peninsula. *Hacquetia* 5 (2): 5-45

Beguín C. & Theurillat J.P., 1984. Quelques aspects du complexe des falaises rocheuses sur silice dans le Haut-Valais (Alpes, Suisse). *Candollea* 39 (2): 647-673.

Meriaux J.-L., 1983. La classe des *Potametea* dans le nord-ouest de la France. *Coll. Phytosoc.* 10 (1981): 115-129.

Westhoff V. & Maarel Van der E., 1978. The Braun-Blanquet approach. In Whittaker R.H. (Ed.), *Classification of Plant Communities*: 287-399. W. Junk, the Hague

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In the cover:

Mountain garrigues with *Genista corsica* (Loisel.) DC. and *Genista sulcitana* Vals. on the mountaintop of M. Linas. (Picture by Giuseppe Fenu)

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Journal of the Italian Society for Vegetation Science

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Guest Editors

Centre for the Conservation of Biodiversity (CCB), Department of Life and Environmental Sciences, University of Cagliari, Italy.

Biological diversity is hardly threatened at global level and this is causing an increasing and constantly loss of wild plant diversity. This biodiversity crisis, mainly due to human actions (e.g. fragmentation of habitats, invasive alien species, pollution, climate change), leads to drastic increase of species extinction rate. Aiming to halt the continuing loss of plant diversity, international conventions such as the Convention on Biological Diversity (CBD), the Global Strategy for Plant Conservation (GSPC) and the European Strategy for Plant Conservation (ESPC) have been underwritten.

Understanding, documenting and developing conservation strategies are key issues that need to be urgently faced. In particular, conservation studies represent a crucial issue in the Mediterranean context, which represents a key area for the conservation of plant due to high rates of overall and regional endemism and the elevated species richness. Nowadays, this diversity is subjected to both natural and anthropogenic factors, particularly in insular contexts where endemic plants therefore deserve particular attention.

Convention on Biological Diversity defined *in situ* and *ex situ* conservation as two distinct approaches to the protection of wild species. *In situ* conservation is needed to ensure the survival of species as a key element of biodiversity and it is particularly required for species considered of priority importance because they are endangered. It is considered the most appropriate way of preserve biodiversity, while *ex situ* conservation approaches should be applied as critical components of an integrated conservation programme.

In this special issue of Plant Sociology, we assembled selected papers issued from the "X International Meeting Biodiversity Conservation and Management: Conservation studies on Mediterranean threatened flora and vegetation" that was held in Villacidro (Sardinia, Italy) and organised by the Centre for the Conservation of Biodiversity (CCB) and *Hortus Botanicus Karalitanus* (HBK) of the University of Cagliari on June 13-18, 2016; it ended with a post-congress excursion in Sulcis-Iglesiente biogeographic sector, carried out on 18th June.



Participants pose for group photo during the field trip of the "X International Meeting Biodiversity Conservation and Management", Villacidro (Sardinia, Italy). (Picture by M. Porceddu).

The congress saw the participation of students and researchers from different countries of the Mediterranean area, such as Spain, Italy, Lebanon, France and Egypt. The selected papers covered the following main topics:

- General aspects of European and national legislation related to the flora/vegetation conservation;
- Evaluation of plant and habitat conservation status: international procedures;
- Monitoring and conservation actions (*in situ* and *ex situ*) on threatened flora and habitat;
- Concrete conservation actions: plant reintroduction, reinforcement and translocations, habitat restoration and rehabilitation;
- Study cases of conservation and management of flora and habitats diversity.

Specifically, papers on conservation and management of the flora and habitats diversity in the Mediterranean area, phytosociology as plant synecology and towards an ecological characterization of Mediterranean landscapes and related themes were selected for this special issue.

The organization of the International Meeting was possible thanks to the hard work of the local Organizing and Scientific Committees; the conference organizers thank the SISV, Forestas Agency, Cagliari Province, the AUSER Association and all the other scientific associations for their significant support.

A phytosociological review of siliceous sedges in C-W Spain and their state of conservation based on diversity indices

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Abstract

A study was made of waterlogged areas in C-W Spain, and revealed the presence of the alliance *Genistion micrantho-anglicae*, and a series of associations belonging to *Isoeto-Nanojuncetea* included in habitat 3170*. This work describes the new association *Ericetum scopario-lusitanicae* in *Genistion micrantho-anglicae*, and assigns it to habitat 4020*. Due to the importance of these areas, we study their state of conservation and analyse their diversity by applying Shannon's index and establishing a relationship between the characteristic and companion species abundance in the community. The analysis of the diversity and state of conservation of the sampled plots at a global scale over the whole territory shows a conservation level of VmCa-VmCo > 0. However the study of individual plots reveals a trend towards the transformation of heathland into plant communities of *Molinia caerulea*, *Juncus acutiflorus*, *Pteridium aquilinum* and *Rubus ulmifolius*.

Key words: conservation, diversity, habitat, vegetation.

Introduction

We analyse environments undergoing temporary and permanent waterlogging in central-western Spain. Their high phytocenotic diversity is conditioned by the moisture gradient. In small areas with temporary waterlogging of an ephemeral nature there is a predominance of Habitat 3170* (temporary Mediterranean lakes and pools), represented by the following associations: *Pulicario uliginosae-Agrostietum salmanticae*; *Junco pygmaei-Isoetum velati*; *Hyperico humifusi-Cicendietum filiformis*; *Periballio laevis-Illecebretrum verticillati*; *Sibthorpio-Pinguiculetum lusitanicae*. If the waterlogging persists for longer periods, and only the upper soil horizon dries out, the dominant communities belong to Habitat 6410 (meadows with *Molinia caerulea* on moist soils most of the year). This is a non-priority habitat for the EU, which we propose should be made priority due to its transitional character between 3170* and 4020*. These communities belong to the associations *Hyperico undulati-Juncetum acutiflori* and *Lobelio urentis-Lotetum pedunculati*.

A study is made of wetland vegetation in Sites of Community Interest (SCI): Sierra Morena, Almadén-Chillón-Guadalmaz, Sierra de Canalizos and Guadiana-Laderas Vertientes in central-western Spain.

Numerous authors have conducted research in these areas; Rivas Goday *et al.* (1954) spoke of these permanently and temporarily flooded areas in his work on the Sierra Madrona (Finca de Aulagas), in which he studied the siliceous sedges of *Erica tetralix* and *Genista anglica*. Subsequently this same author again discussed these waterlogged sites in his work on the vegetation and flora of the province of Badajoz (Rivas Goday, 1964). Velasco Negueruela (1980) described the communities of *Erica tetralix* in the eastern areas of the Portuguese-Extremaduran biogeographic unit, *Erico tetralicis-Myricetum galeae* Ladero & A. Velasco in A. Velasco 1980, an association that Cano (1988) located sporadically in the Sierra Morena in Jaén, and which was later investigated by Cano *et al.* (1996) in the Finca de Aulagas (Sierra Madrona, C. Real). Rivas-Martínez (1979) conducted a review of western European heathlands and sedges and proposed the association *Cisto psilosepali-Ericetum lusitanicae* Ladero ex Rivas-Martínez 1979, which includes the alliance *Ericion umbellatae* Br.-Bl., P. Silva, Rozeira & Fontes 1952, based on the relevé taken in the location of Granadilla (Cáceres), and created for the first time the alliance *Genistion micrantho-anglicae* Rivas-Martínez 1979. Studies on the Guadiamar basin (Seville) by Pérez Latorre *et al.* (2002) revealed the presence

of this association. Valdes Francis (1984) gave a table of seven relevés in the Sierra de Cabo Gata (Cáceres) and included them in *Cisto psilosepali-Ericetum lusitanicae*, while Castro (2005) cited this association in westernmost Portuguese territories in the Sierra de Gata (Sierra de San Mamede, Portugal). Belmonte (1998) published four relevés in *Cistus psilosepalus* and *Erica lusitanica* in the Monfrague National Park. Other researchers such as Quesada (2010) have cited this association in Sierra Morena in Jaén, but without the presence of *Cistus psilosepalus*. Siliceous sedges are characterised by growing in permanently waterlogged environments, and consequently on gley soils, where the moisture gradient and the gleyic character are the factors that condition the presence of a particular type of sedge. The permanently waterlogged areas with *Sphagnum* are essentially located towards the interior of the area, and this is where the association *Erico tetralicis-Myricetum galeae* grows, whereas communities of *Erica lusitanica* are found in the outer zones. These types of permanently waterlogged sites contain habitats of community interest 3170* and 4020*. That are also present in Italy (Gigante et al., 2013) and we have interpreted them according to the European Directive (Biondi et al., 2012); furthermore, they are covered by Act 9/1999 of the Castile-La Mancha regional legislation. For this reason, these communities are of great importance, both from the standpoint of phytosociological knowledge and to determine their current state of conservation. Due to the degradation of some of these wetlands, we took phytosociological relevés and mapped different sites of community interest to study their diversity and state of conservation.

Material and methods

Several SCIs were mapped in the province of Ciudad Real (Spain) and phytosociological relevés were taken of the different associations in two types of habitats: 3170* and 6410. A total of 64 relevés were analysed, of which 19 correspond to *Cicendion* (Rivas Goday in Rivas Goday & Borja 1961) Br.-Bl. 1967 (C), 13 to *Hyperico-Juncetum acutiflori* Teles 1970 (HJ), 19 to a community of *Molinia caeruleae* (CoMc), 6 to *Preslion cervinae* Br.-Bl. ex Moor 1937 (Pc), and 7 to *Verbenion supinae* Slavnic 1951 (Vs). Siliceous sedges belong to habitat 4020*, with 12 samplings corresponding to the association *Erico tetralicis-Myricetum galeae* (EM), 20 to the new association we describe in this work, *Ericetum scopario-lusitanicae* (Esl) – of which 10 samplings were made by ourselves and the other 10 were taken from Quesada (2010) and Pérez Latorre et al. (2002) –, and 16 included by several authors in *Cisto psilosepali-Ericetum lusitanicae* (C-E). Finally, we studied 9 relevés belonging to the association *Lavandulo-Ericetum scopariae* Rivas-Martínez

& Cano 2011, recently published by Rivas-Martínez (2011), making a total of 57 sedge relevés (Tab. 1). Data from 58 meteorological stations were used for the bioclimatic analysis, and the bioclimatic indices were determined at www.globalbioclimatics.org by Professor Rivas-Martínez (Tab. 2). An ordination analysis was performed using Ward's agglomerative cluster analysis, Euclidean distance, and a DECORANA and RA with the CAP3 community analysis package. The first cluster analysis includes 4 associations – EM, Esl, C-E, L-E – to separate the associations in the alliance *Genisthion micrantho-anglicae* (EM, Esl, C-E); successive ordination analyses were done between them, as association L-E is located at the outer edge of *Genisthion micrantho-anglicae* and is not included in the alliance (Rivas-Martínez, 2011). A phytosociological study was made of the different communities by preparing phytosociological tables and a synthetic table of sedges. The table of syntaxa was compiled with 4 associations – EM, Esl, C-E, L-E – following the studies of Biondi (2011) and Ernandes & Marchiori (2012), which give an ecology of *Marsilea strigosa* Willd. in Italy that is equal to the ecology for this species in Spain and similar to the ecology of *Marsilea batardae* Launert in Sierra Morena, and includes both species in habitat 3170*.

The floristic diversity was determined by applying Shannon's index to the total species present in the association, the characteristic species of the association and higher syntaxonomic units, and the companion species belonging to other neighbouring associations. The diversity of both species groups shows the trend towards a greater or lesser conservation rate. The degree of conservation was determined by finding the average values for the abundance indices of the characteristic species in the association and higher syntaxonomic units (Vmca) and the companion species (Vmco); the difference between Vmca-Vmco indicates the state of conservation of the association *in situ*. An association can be assumed to present its highest degree of conservation when all the species present are characteristic of the association and higher syntaxonomic units. All plant associations are formed by two groups of species: those that give the association its character, and companion species belonging to other neighbouring associations. This situation is stable on a spatial-temporal scale while the environmental parameters that generated it persist. If the environmental conditions change, the companion species behave opportunistically and displace the community's characteristic species, leading to an imbalance between the two species groups (characteristic and companion) in a community, and thus a change in species abundance values. The new method we propose indicates the trend and state of conservation of the plant association.

Tab. 1 - Source of the relevés used.

Source of the relevés	n° rel.	References
<i>Cisto psilosepali-Ericetum lusitanicae</i>	16	Rivas-Martínez (1979)
<i>Ericetum scopario-lusitanicae</i>	5	Pérez Latorre et al. (2002)
<i>Ericetum scopario-lusitanicae</i>	5	Quesada (2010)
<i>Ericetum scopario-lusitanicae</i>	10	Rels. Own
<i>Erico tetralicis-Myricetum galeae</i>	12	Rels. Own
<i>Lavandulo-Ericetum scopariae</i>	1	Rivas-Martínez (2011)
<i>Lavandulo-Ericetum scopariae</i>	8	Rels. Own

Tab. 2 - Bioclimatic data for the study area.

Weather Station	Altitude	UTM	T°m	P	It/Itc	Io	Ic
ALÁJAR	577	29 S 706711 4193625	15.1	1151	323	6.34	15
ALMADÉN DE LA PLATA	450	29 S 757600 4186952	14	831	259	4.94	18
ALMADÉN MINAS	535	30 S 340722 4292480	16.2	625	316	3.22	20
ALMODOVAR DEL CAMPO	670	30 S 398550 4284132	14.9	489	279	2.74	20
ALMODOVAR DEL RIO	150	30 S 322312 4186846	17	598	345	2.93	18
ANDUJAR	212	30 S 407453 4210724	18.1	464	372	2.14	19
ARACENA	731	29 S 715460 4195700	14.5	1026	281	5.88	18
AROCHE EL VINCULO	426	29 S 681704 4196733	15.7	838	340	4.43	15
AZUEL	662	30 S 383775 4242782	15.5	636	298	3.42	19
BAILÉN	343	30 S 432128 4216955	17.9	582	369	2.71	20
BERLANGA	573	30 S 253635 4241006	15.2	493	294	2.7	18
CABEZA DEL BUEY	550	30 S 307281 4287668	16.3	586	320	2.99	19
CABEZA LA VACA	759	29 S 726571 4218213	14.9	878	289	4.92	18
CABEZAS RUBIAS CUMBRES DE EN-	565	29 S 670133 4189084	14.8	993	309	5.61	15
CALZADA DE CALATRAVA	685	30 S 431852 4280078	14.5	445	268	2.57	21
CASTELLAR DE SANTISTEBAN LA	790	30 S 488662 4234248	14.8	740	299	4.16	19
COZAR	860	30 S 494199 4277940	13.6	478	241	2.93	21
EL CENTENILLO	824	30 S 436237 4243739	14.7	679	270	3.84	20
EL CENTENILLO T (51-63) P (51-88)	824	30 S 436237 4243739	14.7	679	272	3.86	19
ENCINASOLA	433	29 S 686972 4222759	16.5	778	338	3.94	19
FONTANOSAS	570	30 S 366757 4290151	14.9	672	285	3.76	19
FREGENAL DE LA SIERRA	580	29 S 705870 4226917	14.4	750	287	4.33	16
GRANJA DE TORREHERMOSA	593	30 S 274099 4242259	15	541	284	3.01	19
GUADALCANAL	907	30 S 256475 4215686	13.8	610	254	3.67	19
HINOJOSA DEL DUQUE	540	30 S 312592 4263615	14.8	477	284	2.68	18
HORNACHUELOS	184	30 S 302670 4189144	19.3	745	407	3.21	18
HORNACHUELOS (59-74, 51-87)	184	30 S 302670 4189144	19.5	701	406	2.99	19
JEREZ DE LOS CABALLEROS	492	29 S 695246 4243311	15.6	665	314	3.56	17
LAS NAVAS DE LA CONCEPCION	434	30 S 283540 4201349	14.3	720	284	4.19	16
MESTANZA PRIMERA CENTRAL	549	30 S 405578 4265548	15.2	426	298	2.33	18
MINAS DE RIOTINTO	421	29 S 711585 4175241	16.4	713	355	3.62	16
MONTEMOLÍN	640	29 S 751768 4207844	16.6	744	358	3.74	16
MONTIZÓN	700	30 S 493532 4246110	13.6	612	254	3.75	19
MONTORO	195	30 S 378543 4209562	17.5	572	361	2.72	18
PANTANO DE CALA	220	29 S 757143 4178612	16.8	744	352	3.69	17
PANTANO DE ENCINAREJO	280	30 S 411981 4223621	16.5	560	333	2.82	18
PANTANO DE GUADALÉN	310	30 S 457617 4223563	16.6	578	343	2.9	19
PANTANO DE GUADALMELLATO	200	30 S 353334 4211518	17	698	349	3.43	18
PANTANO DE PUENTE NUEVO	460	30 S 329498 4220462	16.3	604	328	3.09	18
PANTANO DEL JÁNDULA	360	30 S 414983 4231296	16.8	505	340	2.5	20
PANTANO DEL PINTADO	300	30 S 240759 4207913	16.2	647	330	3.33	17
PANTANO DEL RUMBLAR	300	30 S 429144 4223916	16.7	657	343	3.28	19
PEÑARROYA-PUEBLONUEVO	550	30 S 301395 4242075	17.6	524	358	2.49	20
POSADAS	88	30 S 314459 4185786	17.7	687	359	3.24	18
POZOBLANCO	649	30 S 337997 4249430	15.6	477	295	2.55	20
POZOBLANCO (54-91, 61-90)	649	30 S 337997 4249430	16.1	515	311	2.67	20
PUERTOLLANO	700	30 S 408652 4280310	14.5	448	280	2.57	19
SANTA CRUZ DE MUDELA	721	30 S 459383 4276192	15.2	370	286	2.03	21
SANTELMO	350	29 S 677547 4185541	17.8	727	378	3.4	17
TORRE DE JUAN ABAD LA GRANJA	790	30 S 482605 4281656	14.6	415	273	2.37	19
TORRECAMPO	572	30 S 352275 4260275	16.7	539	334	2.69	20
USAGRE	566	29 S 747596 4248450	15.3	602	295	3.28	19
VALDEZUFRE	611	29 S 721424 4192157	17.2	921	366	4.46	17
VILLANUEVA DE CORDOBA	725	30 S 357663 4242738	15.7	600	318	3.18	18
VILLANUEVA DE LOS INFANTES	875	30 S 500000 4287185	13.8	468	251	2.84	20
VILLANUEVA DE SAN CARLOS SE-	690	30 S 430338 4272693	15	401	288	2.23	20
ZAFRA	508	29 S 725486 4257057	15.4	574	296	3.11	18
ZUFRE	480	29 S 729066 4181261	16.3	904	348	4.62	16

The study area

These sites, dominated by Palaeozoic quartzite rocks and slates, are characterised by their high rainfall produced by the barrier effect of the mountains against Atlantic squalls, and have a continental influence due to their proximity to the La Mancha plateau. All these factors condition the presence of forests of *Quercus pyrenaica*, *Quercus canariensis* and *Quercus broteroi*. The territory is in the easternmost areas of the Portuguese-Extremaduran biogeographical unit (Mariánico-Monchiquense unit), with a predominantly subhumid-humid ombrotype.

Results and discussion

Phytosociological analysis

The analysis of siliceous sedges in the more continentalised areas of the Mariánico-Monchiquense biogeographic sector (Rivas-Martínez, 2011) reveals the presence of three types of associations: *Erico tetralicis-Myricetum galeae* (EM) and *Ericetum scopario-lusitanicae* (Esl), which occupy more permanently waterlogged areas with highly gleyic soils, with high rainfall and a subhumid and humid ombroclimate. These are areas dominated by forests of *Arbutus-Quercetum pyrenaicae* (Rivas Goday in Rivas Goday, Esteve, Galiano, Rigual & Rivas-Martínez 1960) Rivas-Martínez 1987, *Doronico plantaginei-Quercetum canariensis* Rivas-Martínez & Cano 2011, and *Pistacio terebinthi-Quercetum broteroi* Rivas Goday in Rivas Goday, Esteve, Galiano, Rigual & Rivas-Martínez 1960, while the temporarily waterlogged areas dominated by forests of *Pyro bourgaeanae-Quercetum broteroi* Cano, García Fuentes, Torres, Pinto, Cano-Ortiz, Montilla, Muñoz, Ruiz & Rodríguez 2004 are home to the sedge *Lavandulo luisieri-Ericetum scopariae* (L-E), which represents the first dynamic stage of these forests. Forests developed in rainy environments with subhumid-humid ombrotype (see Tab. 2). These types of communities are usually located in the central part of the Mariánica range with values of $I_0 > 4$. The association EM always occupies the part of the tesela where there is a high degree of permanent flooding, a lack of oxidation-reduction phenomena and thus highly gleyic soils, with such emblematic species as *Erica tetralix*, *Myrica gale* and *Genista anglica*. The spaces left by this type of vegetation are occupied by species such as *Sibthorpia europea*, *Pinguicula lusitanica* and *Drosera rotundifolia*. The outer edge of EM is colonised by Esl, a community dominated by *Erica scoparia* and *Erica lusitanica*, with an absence of *Cistus psilosepalus* and *Erica australis* subsp. *aragonensis*. Permanently waterlogged areas are home to communities of *Genistion micrantho-anglicae* (Rivas-Martínez, 1979), with the presence of habitat 4020* in this alliance, and including the association *Erico tetralicis-Myricetum gale*,

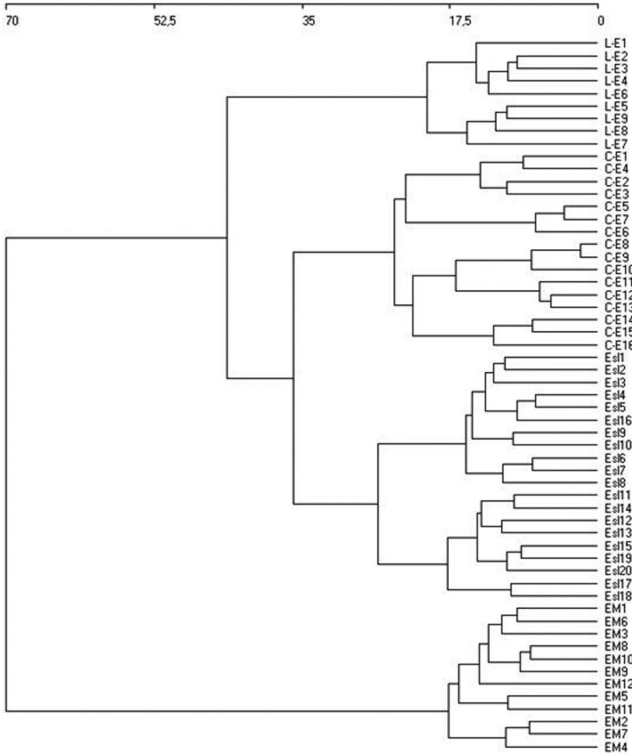


Fig. 1 - Cluster analysis for the four groups studied (L-E, C-E, Esl and EM).

and on its outer edges on less waterlogged soils, a community of *Erica lusitanica*, and outside this a community of *Erica scoparia*. The statistical study thus perfectly separates the four groups between the different sedge communities (EM, Esl, C-E, L-E) (Fig. 1). The communities Esl and C-E are close (Figs. 2, 3, 4) in the cluster analyses, DECORANA and RA. As the difference between both associations is the absence of *Cistus psilosepalus* and *Erica australis* subsp. *aragonensis* in Esl, it is also located in more continental en-

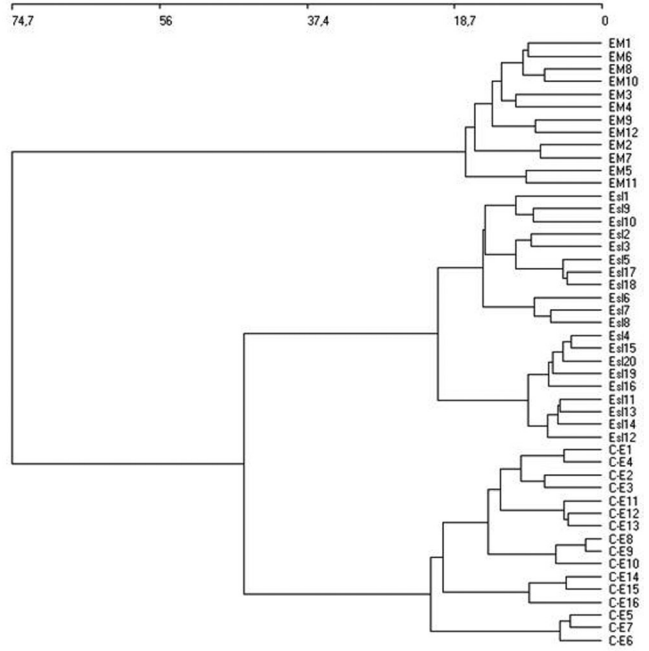


Fig. 2 - Cluster analysis for the three groups studied (EM, Esl and C-E).

vironments than C-E, which is more oceanic. In view of this, we propose *Ericetum scopario-lusitanicae* ass. nova (Tab. 3, relevés 1-20, *typus* inv. 6) as a vicarious association of C-E for the easternmost areas of the Mariánico-Monchiquense sector. This is corroborated in the synthetic table for the four previous associations (Tab. 3).

Analysis of the diversity and state of conservation

The association *Erico tetralicis-Myricaetum galeae* is always located in the most siliceous sites occupied by the alliance *Genistion micrantho-anglicae*. In this case, we studied 12 samplings from different locali-

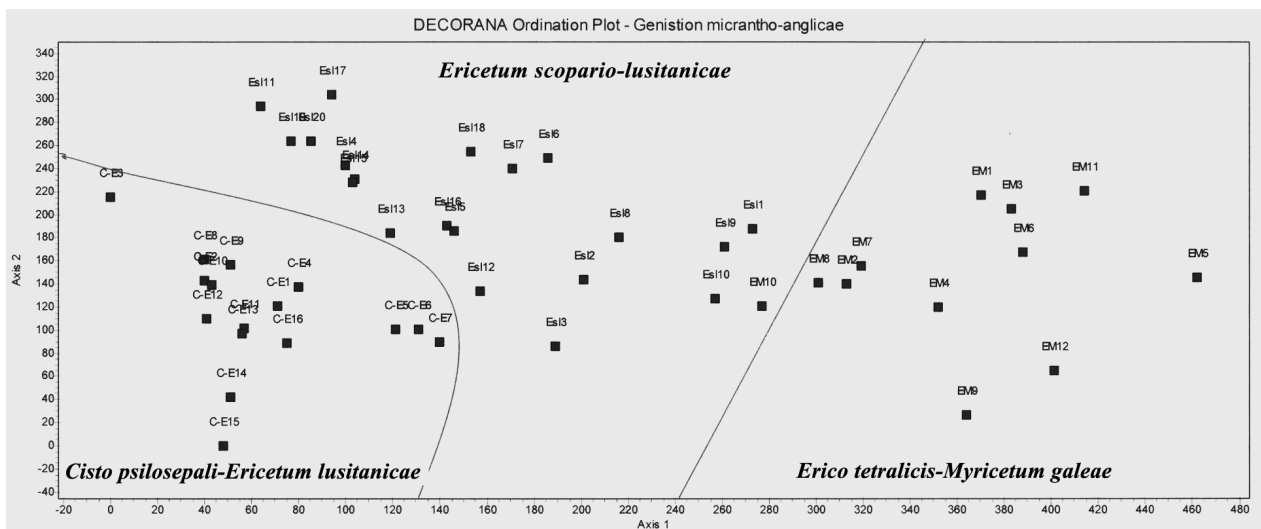


Fig. 3 - DECORANA ordination analysis of the communities EM, Esl and C-E in the alliance *Genistion micrantho-anglicae*.

Tab. 4 - EM: *Erico tetralix-Myricaetum galeae*. Esl: *Ericetum scopario-lusitanicae*. C-E: *Cisto psilosepali-Ericetum lusitanicae*. L-E: *Lavandulo luisieri-Ericetum scopariae*.

	EM	Esl	C-E	L-E	Presences
Characteristic					
<i>Erica scoparia</i> subsp. <i>scoparia</i> L.	II	III	III	V	4
<i>Calluna vulgaris</i> (L.) Hull	II	I	III	II	4
<i>Erica lusitanica</i> Rudolphi	II	V	III	.	3
<i>Erica tetralix</i> L.	V	I	.	I	3
<i>Halimium ocymoides</i> (Lam.) Willk.	I	I	.	I	3
<i>Genista anglica</i> L.	II	.	.	I	2
<i>Erica erigena</i> R. Ross	II	I	.	.	2
<i>Myrica gale</i> L.	I	.	.	.	1
<i>Lavandula stoechas</i> L. subsp. <i>luisieri</i> Rozeira	.	I	I	II	3
<i>Erica australis</i> subsp. <i>australis</i>	.	I	.	II	2
<i>Frangula alnus</i> subsp. <i>alnus</i> Mill.	.	I	.	.	1
<i>Erica umbellata</i> Loeffl. ex L.	.	I	.	.	1
<i>Cisto psilosepalus</i> Sweet	.	.	V	.	1
<i>Erica australis</i> L. subsp. <i>aragonensis</i>	.	.	II	.	1
<i>Halimium halimifolium</i> subsp. <i>halimifolium</i> (L.) Willk.	.	.	.	I	1
Companions					
<i>Molinia caerulea</i> (L.) Moench subsp. <i>altissima</i> (Link) Domin	V	II	I	II	4
<i>Rubus ulmifolius</i> Schott	I	III	V	II	4
<i>Juncus acutiflorus</i> Ehrh. ex Hoffm.	II	I	I	I	4
<i>Scirpoides holoschoenus</i> (L.) Soják	I	II	III	I	4
<i>Carum verticillatum</i> (L.) W. D. J. Koch	II	I	.	II	3
<i>Sphagnum</i> sp.	III	I	.	I	3
<i>Potentilla reptans</i> L.	II	I	.	I	3
<i>Potentilla erecta</i> (L.) Raeusch.	I	I	.	I	3
<i>Radiola linoides</i> Roth	I	I	.	I	3
<i>Salix atrocinerea</i> Brot.	I	I	I	.	3
<i>Lotus pedunculatus</i> Cav.	I	I	I	.	3
<i>Eleocharis multicaulis</i> (Sm.) Desv.	II	.	.	I	2
<i>Pinguicula lusitanica</i> L.	I	.	.	I	2
<i>Pteridium aquilinum</i> (L.) Kuhn var. <i>aquilinum</i>	II	I	.	.	2
<i>Anagallis tenella</i> (L.) L.	II	I	.	.	2
<i>Sibthorpia europaea</i> L.	II	I	.	.	2
<i>Carex elata</i> subsp. <i>reuteriana</i> (Boiss.) Luceño & Aedo	I	I	.	.	2
<i>Carex pendula</i> Huds.	I	I	.	.	2
<i>Holcus lanatus</i> L.	I	I	.	.	2
<i>Eleocharis palustris</i> subsp. <i>palustris</i> (L.) Roem. & Schult.	I	I	.	.	2
<i>Ranunculus bulbosus</i> L. subsp. <i>aleae</i> (Willk.) Rouy & Fouc.	I	I	.	.	2
<i>Lobelia urens</i> L.	I	I	.	.	2
<i>Mentha pulegium</i> L.	I	I	.	.	2
<i>Dactylorhiza elata</i> (Poir.) Soó	I	.	.	.	1
<i>Danthonia decumbens</i> (L.) DC.	I	.	.	.	1
<i>Carex demissa</i> Hornem.	I	.	.	.	1
<i>Carex echinata</i> Murray	I	.	.	.	1
<i>Cicendia filiformis</i> (L.) Delarbre	I	.	.	.	1
<i>Drosera rotundifolia</i> L.	I	.	.	.	1
<i>Hypericum elodes</i> L.	I	.	.	.	1
<i>Hypericum humifusum</i> L.	I	.	.	.	1
<i>Hypochaeris radicata</i> L.	I	.	.	.	1
<i>Isoetes velatum</i> A. Braun subsp. <i>velatum</i>	I	.	.	.	1
<i>Juncus tenageia</i> Ehrh. ex L. fil.	I	.	.	.	1
<i>Rosa canina</i> L.	.	I	I	II	3
<i>Hypericum undulatum</i> Schousb. ex Willd.	.	I	.	I	2
<i>Pteridium aquilinum</i> (L.) Kuhn in Kerst.	.	I	III	.	2
<i>Genista triacanthos</i> Brot.	.	I	I	.	2
<i>Lythrum salicaria</i> L.	.	I	I	.	2
<i>Alnus glutinosa</i> (L.) Gaertn.	.	I	.	.	1
<i>Blechnum spicant</i> (L.) Roth subsp. <i>Spicant</i>	.	I	.	.	1
<i>Callitriche brutia</i> Petagna	.	I	.	.	1
<i>Carex elata</i> All. subsp. <i>tartessiana</i> Luceño & Aedo	.	I	.	.	1
<i>Cruciata glabra</i> subsp. <i>glabra</i> (L.) Ehrend.	.	I	.	.	1
<i>Dorycnium rectum</i> (L.) Ser. in DC.	.	I	.	.	1
<i>Epilobium obscurum</i> Schreb.	.	I	.	.	1
<i>Equisetum telmateia</i> Ehrh.	.	I	.	.	1
<i>Hypericum tomentosum</i> L.	.	I	.	.	1
<i>Juncus subnodulosus</i> Schrank	.	I	.	.	1
<i>Myosotis debilis</i> Pomel	.	I	.	.	1
<i>Narcissus pseudonarcissus</i> subsp. <i>munozii-garmendiae</i> (Fern. Casas) Fern. Casas	.	I	.	.	1
<i>Narcissus triandrus</i> subsp. <i>pallidulus</i> (Graells) Rivas Goday	.	I	.	.	1
<i>Pulicaria paludosa</i> Link	.	I	.	.	1
<i>Ranunculus hederaceus</i> L.	.	I	.	.	1
<i>Rosa pouzinii</i> Tratt.	.	I	.	.	1
<i>Rosa corymbifera</i> Borkh.	.	.	I	I	2
<i>Carex flava</i> L. subsp. <i>oedocarpa</i> (Andersson) O. Bolòs & Vigo [sub]	.	.	I	.	1

<i>Galium broterianum</i> Boiss. & Reut.	I	.	1
<i>Genista falcata</i> Brot.	II	.	1
<i>Holcus annuus</i> C.A. Mey subsp. <i>setigulumis</i> (Boiss. & Reut.) M. Seq. & Castrov. [sub]	I	.	1
<i>Lithodora diffusa</i> (Lag.) I. M. Johnston	I	.	1
<i>Mentha suaveolens</i> Ehrh.	I	.	1
<i>Origanum vires</i> Hoffmanns. & Link	I	.	1
<i>Prunella vulgaris</i> L.	I	.	1
<i>Ulex minor</i> Roth	I	.	1
<i>Thymus mastichina</i> L.	II	.	1
<i>Rosmarinus officinalis</i> L.	II	.	1
<i>Celtica gigantea</i> (Link) F. M. Vázquez & Barkworth	I	.	1
<i>Fluggea tinctoria</i> (L.) G. L. Webster	I	.	1
<i>Glyceria declinata</i> Bréb.	I	.	1
<i>Juncus bufonius</i> L.	I	.	1
<i>Juncus pygmaeus</i> Rich. ex Thuill.	I	.	1
<i>Juniperus oxycedrus</i> subsp. <i>lagunae</i> (Pau ex C. Vicioso) Rivas Mart.	I	.	1
<i>Pulicaria odora</i> (L.) Rchb.	I	.	1
<i>Ranunculus ollisiponensis</i> subsp. <i>ollisiponensis</i> Pers.	I	.	1

ties by applying Shannon's index to the total species (Shannon_H_T), and separately to the characteristic species (Shannon_H_Ca) and (Shannon_H_Co). In table the relevés EM4, EM5 and EM12 have a Shannon's index value of = 0, due to the fact that *Erica tetralix* is only present in these samplings as a characteristic species with an abundance-dominance value of 2 in EM4 and EM5, and 1 in EM12, with companion species exceeding the diversity of characteristic species. Within the companion species there is a dominance of *Pteridium aquilinum* and *Molinia caeruleae*; in this case the value of Shannon_H_Co = 1.92, 2.46 and 2.28, which is very similar to the total diversity of Shannon_H_T > 2.05. In the rest of the samplings, the diversity of companion species far exceeds that of characteristic species, and from the overall point of view the value of Shannon_H_Co = 1.96 > Shannon_H_Ca = 0.76, compared to a total diversity of 2.27. This reveals that the sedges of *Erico tetralix-Myricaetum galeae*, present in the 12 localities studied, have a tendency to transform into other communities, fundamentally *Molinia caeruleae* and *Juncus acutiflori*. This phenomenon has been observed in the locality of Finca de Aulagas (Sierra Morena), where Rivas Goday (1953) and Cano *et al.* (1996) incorporated *Genista anglica*, *Erica tetralix*, *Erica scoparia*, *Carum verticillatum*, *Sibthorpia europaea* and *Pinguicula lusitanica* in their samplings. However, these plant communities have disappeared in recent samplings in the same locality, and been replaced by a fragmented community of *Hyperico-Juncetum acutiflori*.

Based on the relationship between the abundance of characteristic and companion species in each sampling in EM4, EM5 and EM12 VmCa-VmCo can be observed to have negative values of -0.21, -0.55 and -0.52 respectively, indicating that these three areas in the sampling are at high risk of disappearing and becoming transformed into other plant communities, as VmCa < VmCo.

In the case of the sedge *Cisto psilosepali-Ericetum lusitanicae*, we include 16 samplings published by

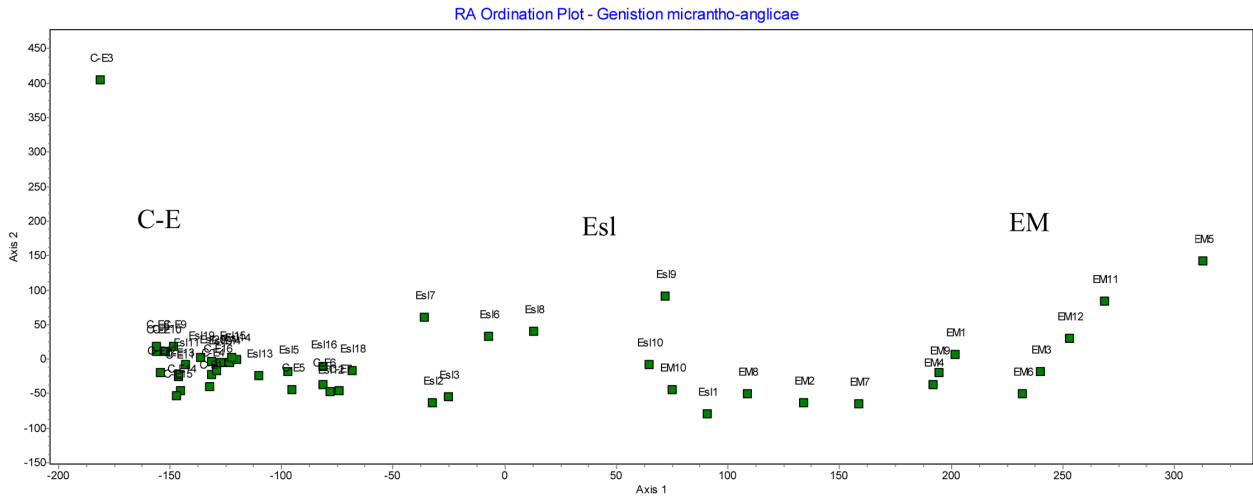


Fig. 4 - RA ordination analysis of the communities EM, EsI and C-E in the alliance *Genistion micrantho-anglicae*.

several authors for Portugal and the westernmost areas of Spain. The total diversity ranges between 2.16 for C-E15 and 3.05 for C-E2. In all the areas sampled –C-E1 to C-E16, with the exception of C-E13– the value of Shannon_H_Ca < Shannon_H_Co. The diversity of characteristic species ranges from 0.99 for C-E4 to 1.54 for C-E11, while the diversity of companion species is between 1.77 for C-E11 and 2.86 for C-E3. In all cases indicating a dominance of the diversity of companion species compared to the characteristic species in the community, while C-E13 complies with Shannon_H_Ca > Shannon_H_Co, with values of 1.71 and 1.36 respectively. In general terms, it can be seen (Fig. 5) that the mean value of the diversity of companion species far exceeds the diversity of characteristic species.

Applying the mean species abundance values, VmCa can be seen to exceed Vmco in all areas due to a low diversity in characteristic species but high abundance values. *Erica lusitanica* presents abundance-dominance values of 4 and 5, except in C-E14, C-E15 and C-E16, in which this species does not exist, and *Cistus psilosepalus* is present in all these samplings. This fact, together with the high diversity in companion species and low abundance-dominance values, is the cause of the low VmCo values. All this indicates that despite a high diversity of companion species, the degree of conservation is good, as it is always the case that VmCa > VmCo.

The total maximum diversity for the new association proposed, *Ericetum scopario-lusitanicae*, corresponds to EsI10 with a value of 3.08 and a minimum value of

Tab. 5 - Values for Shannon’s index and VmCa, VmCo and VmCa-VmCo by association.

Ass.	EsI	Es2	Es3	Es4	Es5	Es6	Es7	Es8	Es9	Es10	Es11	Es12	Es13	Es14	Es15	Es16	Es17	Es18	Es19	Es20	Vm
Shannon_H_T	2.31	2.5	2.89	2.03	2.25	2.75	2.42	2.84	2.89	3.08	2.24	2.25	2.79	1.98	2.33	2.26	2.86	2.36	2.25	2.58	2.49
Shannon_H_Ca	1.01	1.54	2.1	1.03	1.08	0.69	0.69	0.69	0.95	1.52	0	0	0	0.67	0	1.04	0	0	0	0.64	0.68
Shannon_H_Co	2	2.04	2.28	1.57	1.93	2.68	2.27	2.74	2.75	2.87	2.14	2.14	2.73	1.68	2.25	1.92	2.82	2.26	2.15	2.46	2.28
Vmca	1.2	1.8	2.3	1	1.2	1.2	1	0.9	1	1.7	0.2	0.3	0.3	0.5	0.5	1.2	0.5	0.4	0.5	0.9	0.93
Vmco	0.22	0.19	0.25	0.13	0.15	0.36	0.24	0.39	0.37	0.41	0.26	0.26	0.44	0.19	0.26	0.19	0.43	0.26	0.25	0.31	0.28
Vmca-Vmco	0.98	1.61	2.05	0.87	1.05	0.84	0.76	0.51	0.63	1.29	0	0.04	0	0.31	0.24	1.01	0.07	0.14	0.25	0.59	0.65
Ass.	C-E1	C-E2	C-E3	C-E4	C-E5	C-E6	C-E7	C-E8	C-E9	C-E10	C-E11	C-E12	C-E13	C-E14	C-E15	C-E16	Vm				
Shannon_H_T	2.76	3.05	2.98	2.76	2.57	2.35	2.58	2.49	2.49	2.321	2.33	2.294	2.22	2.37	2.16	2.93	2.54				
Shannon_H_Ca	1.07	1.37	1.03	0.99	1.37	1.37	1.38	1.28	1.28	1.318	1.55	1.268	1.71	1.34	0.68	1.37	1.27				
Shannon_H_Co	2.57	2.86	2.83	2.59	2.26	1.9	2.261	2.04	2.15	1.882	1.77	1.934	1.37	1.89	1.92	2.69	2.18				
Vmca	2	2	1.83	1.83	3	3	3	2.6	2.6	2.83	3.16	2.50	3	1.83	1.16	1.83	2.66				
Vmco	0.83	1.04	0.95	0.79	0.55	0.44	0.59	0.53	0.57	0.44	0.30	0.30	0.18	0.40	0.34	0.91	0.57				
Vmca-Vmco	1.17	0.96	0.88	1.04	2.45	2.56	2.41	2.07	2.03	2.39	2.86	2.20	2.82	1.43	0.82	0.92	2.09				
Ass.	EM1	EM2	EM3	EM4	EM5	EM6	EM7	EM8	EM9	EM10	EM11	EM12	Vm								
Shannon_H_T	2.1	2.38	2.18	2.06	2.55	2.32	2.33	2.21	2.43	2.01	2.36	2.38	2.28								
Shannon_H_Ca	1.06	1.26	0.69	0	0	1.31	0.64	1.33	0.67	1.52	0.66	0	0.76								
Shannon_H_Co	1.67	2	1.93	1.92	2.47	1.87	2.15	1.75	2.28	1.06	2.15	2.29	1.96								
VmCa	1.25	2	1.12	0.5	0.5	1.62	1.12	2.25	1.15	2.12	1	0.37	1.12								
VmCo	0.55	0.65	0.68	0.71	1.05	0.52	0.71	0.39	0.71	0.23	0.81	0.89	0.65								
VmCa-VmCo	0.7	1.38	0.44	-0.21	-0.55	1.1	0.41	1.86	0.44	1.89	0.19	-0.52	0.47								

1.98 in Esl14. In the 20 sampling plots Shannon_H_Co > Shannon_H_Ca and Shannon's diversity index is =0, specifically in plots Esl11, Esl12, Esl13, Esl15, Esl17, Esl18 and Esl19, due to the sole presence of *Erica lusitanica* with low abundance-dominance values (Tab. 5). The relation between the characteristic and companion species abundance highlights the low values for VmCa-VmCo for the areas previously mentioned, with negative values for Esl11 and Esl13, indicating that these seven sampled areas have a poor state of conservation (Tab. 5).

The causes of the transformation of some communities into others is due to improper management of the territory, such as the partial drainage of waterlogged areas and excessive grazing pressure.

Conclusions

The study of permanently and temporarily waterlogged areas in the central and western Iberian Peninsula reveals the presence of EU priority habitats 3170* and 4020* and the non-priority habitat 6410, which acts as a transition between the two previous ones. Habitat 3170* includes several plant associations such as *Pulicario uliginosae-Agrostietum salmanticae*, *Juncus pygmaei-Isoetum velati*, *Hyperico humifusii-Cicendietum filiformis*, *Periballio laevis-Illecebreium verticillati*, *Sibthorpio-Pinguiculetum lusitanicae*. Habitat 4020* is represented by three sedge associations included in the alliance *Genistion micrantho-anglica*, within which we propose the new association *Ericetum scopario-lusitanicae* for less oceanic and more continentalised Portuguese-Extremaduran territories.

The analysis of the diversity and state of conservation of the sampled plots on a global scale for the

whole territory shows a positive state of conservation with values of VmCa-VmCo > 0 (Figure 5). However, the study of individual plots points to a tendency for sedge communities to become transformed into plant communities of *Molinia caerulea*, *Juncus acutiflorus*, *Pteridium aquilinum* and *Rubus ulmifolius*.

The analysis of characteristic and companion species diversity reveals the trend in the state of conservation of the plant association, as the characteristic species diversity for some sampling plots is zero, and the diversity of companion species is the same as the total community diversity. This is similar to the assessment of the state of conservation obtained from the relation between VmCa-VmCo, which for these cases is also zero, close to zero, or takes negative values, as in the case of the samples Esl11, Esl13, Esl15, Esl17, Esl18, Esl19, EM4, EM5 and EM12, for example. However, the average Shannon and VmCa values are high, so the state of conservation of the three associations in the sampled territory is acceptable.

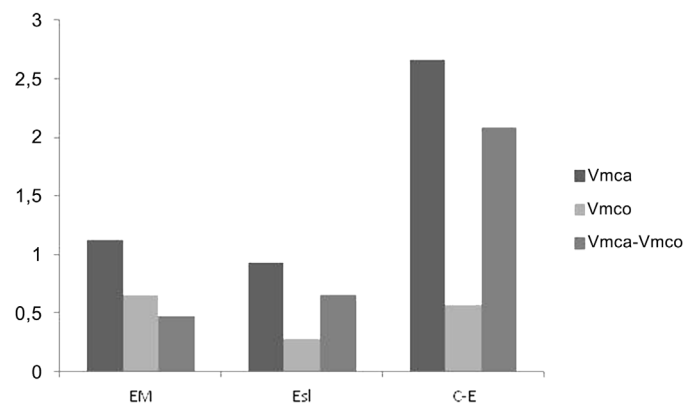


Fig. 5 - Mean values for the three associations studied (Vmca and Vmco).

Syntaxonomic scheme

CALLUNO-ULICETEA Br.-Bl. & Tüxen ex Klika & Hadac 1944

ULICETALIA MINORIS Quantin 1935

Genistion micrantho-anglica Rivas-Martínez 1979

Cisto psilosepali-Ericetum lusitanicae Ladero ex Rivas-Martínez 1979

Erico tetralicis-Myricetum galeae Ladero & A. Velasco in A. Velasco 1980

Ericetum scopario-lusitanicae ass. nova hoc loco

Ericion umbellatae Br.-Bl., P. Silva, Rozeira & Fontes 1952

Lavandulo-Ericetum scopariae Rivas-Martínez & Cano 2011

Other syntaxa quoted in the text

Arbuto unedonis-Quercetum pyrenaicae (Rivas Goday, in Rivas Goday, Esteve, Galiano, Rigual & Rivas-Martínez 1960) Rivas-Martínez 1987; *Cicendion* (Rivas Goday in Rivas Goday & Borja 1961) Br.-Bl. 1967; Community of *Molinia caeruleae*; *Doronico plantaginei-Quercetum canariensis* Rivas-Martínez & Cano 2011; *Hyperico humifusii-Cicendietum filiformis* Rivas Goday (1964) 1970; *Hyperico undulati-Juncetum acutiflori* Teles 1970; *Isoeto-Nanojuncetea* Br.-Bl. & Tüxen ex Westhoff, Dijk & Passchier 1946; *Juncion acutiflori* Br.-Bl. in Br.-Bl. & Tüxen 1952; *Juncus pygmaei-Isoetum velati* Rivas Goday 1956; *Lobelio urentis-Lotetum pedunculati* Rivas Goday 1964; *Molinion*

caeruleae Koch 1926; *Periballio laevis-Illecebretum verticillati* Rivas Goday 1954; *Pistacio terebinthi-Quercetum broteroi* Rivas Goday, in Rivas Goday, Esteve, Galiano, Rigual & Rivas-Martínez 1960; *Preslion cervinae* Br.-Bl. ex Moor 1937; *Pulicario uliginosae-Agrostietum salmanticae* Rivas Goday 1956; *Pyro bourgaeanae-Quercetum broteroi* Cano, García Fuentes, Torres, Pinto, Cano-Ortiz, Montilla, Muñoz, Ruiz & Rodríguez 2004; *Sibthorpio europeae-Pinguiculetum lusitanicae* Ladero & A. Velasco in A. Velasco 1980; *Verbenion supinae* Slavnic 1951.

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Appendix I: Sites in the relevés

Tab. 3 - *Ericetum scopario-lusitanicae* (Esl). Esl1: Bonal de la Teresas, SCI Sierra Morena; Esl2, Esl3: SCI Guadiana and Laderas Vertientes; Esl4, Esl5, Esl6, Esl7, Esl8: SCI Sierra Morena; Esl9, Esl10: Cortijo Robledillo, SCI Sierra Morena. Esl11-Esl15 (Pérez la Torre et al., 2002, Tab. 2, relevés 1-5, in *Acta Botánica Malacitana* 27: 198); Esl16-Esl20 (Quesada, 2010, doctoral thesis “estudio y análisis de la flora, vegetación y paisaje vegetal de las riberas de la provincia de Jaén (S.España): Propuestas para su gestión”).

Understanding common reed die-back: a phytocoenotic approach to explore the decline of palustrine ecosystems

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Abstract

It is well known that since more than half a century, in Europe, *Phragmites australis* is suffering a process of decline, known in literature as ‘common reed die-back’. Several hypotheses have been formulated but the actual causes of the phenomenon have been only partially understood. The several studies produced on this topic generally focused on the population approach and took seldom into account the floristic and vegetational features of the reed-dominated plant communities involved in die-back processes. The present study tries to fill this knowledge gap. Starting from a phytosociological approach, supplemented by the results of a recent three-year-long research project focused on morphological and ecological traits of dying-back reed beds, we analyzed the floristic and vegetational differences between declining and non-declining stands, based on a data set constituted by 80 relevés. Data refer to reed-dominated stands along the shores of five freshwater ecosystems in central Italy: the Lakes Trasimeno, Chiusi and Vico, the Fucecchio and Colfiorito Marshes. The statistical process, including cluster analysis and PCA, allowed to refer all the relevés to the association *Phragmitetum australis* Savič 1926, with eight variants differentiated from an ecological and floristic point of view. The indicator species analysis pointed out the *taxa* playing a diagnostic and/or differential role in each group, and provided useful information to understand pattern and processes occurring in the declining and non-declining reed-dominated phytocoenoses. As a general outcome, a clear inverse relation between number of species per relevé and intensity of the die-back process was showed. This supports the idea that the aquatic monospecific reed-beds are the most suffering ones, while the nitrophilous species-rich phytocoenoses, colonizing drier sediments and often in contact with disturbed areas, are the ones where common reed grows most healthily.

Key words: central Italy, common reed decline, indicator species, phytosociology, vegetation, wetlands.

Introduction

Since more than six decades ago, when the retreat of stands of *Phragmites australis* (Cav.) Steud. around several Swiss lakes was first reported by Hürlimann (1951), conspicuous phenomena of common reed decline have been observed in several areas of Europe and became the topic of a large scientific production (e.g. Den Hartog *et al.*, 1989; Van Der Putten, 1997; Brix 1999; Ostendorp 1999; Rücker *et al.*, 1999; Armstrong & Armstrong, 2001). In the last 15 years also several Italian wetlands have been the scene of drastic processes of reed die-back (Fogli *et al.*, 2002; Gigante *et al.*, 2011; Gigante & Venanzoni, 2012; Angelini *et al.*, 2012; Reale *et al.*, 2012). In particular, the recent detection of reed decline in three wetlands of conservation importance in central Italy, the lakes Trasimeno, Chiusi and Montepulciano (Gigante *et al.*, 2011, 2014; Lastrucci *et al.*, 2016), stimulated further surveys in other lakes to estimate the actual occurrence of this phenomenon in the Mediterranean Basin.

Although generally considered a strong and tolerant plant species, even invasive in some areas of the world, such as N-America (Chambers *et al.*, 1999; Saltonstall,

2002; Kettenring *et al.*, 2011), and occasionally also in its native range (Foggi *et al.*, 2011), *P. australis* displays evident signs of suffering and decline in particular environmental conditions, bringing to wide-scale disappearance of palustrine ecosystems. Several hypotheses have been formulated, from chemical traits of the sediments to eutrophication, artificially stabilized water table, litter accumulation, parasitic attacks, mechanical damage, grazing and many others (e.g. Boar & Crook, 1985; Weisner & Graneli, 1989; Cízková *et al.*, 1996; Hellings & Gallagher, 1992; Armstrong *et al.*, 1996a, 1996b; Weisner, 1996; Clevering, 1998), however the ecological reasons behind such processes of reed decline remain hard to disentangle. Emphasis has been given to the role of artificial changes in the hydrologic regime (Ostendorp, 1989; Rea, 1996) and prolonged flooding has been repeatedly related to reed die-back (Gigante *et al.*, 2011, 2014; Lastrucci *et al.*, 2016). Recently, a clear correlation between permanent submersion, water depth and reed die-back occurrence has been proved (Lastrucci *et al.*, 2017).

In the huge literature about common reed decline, the large majority focused on the population level and only few studies took into account the floristic features of

the reed-dominated ecosystems and the involved plant communities. Few authors investigated the effects on floristic diversity caused by the dynamic processes of reed expansion and decline (e.g. Lenssen *et al.*, 1999; Greco & Patocchi, 2003; Mäemets & Freiberg, 2004; Van Geest *et al.*, 2005), with the limit of no specific focus on die-back. In a recent paper based on a phytosociological approach, Gigante *et al.* (2013) reported about conditions of extreme floristic poverty in declining reed stands.

The present study is part of a three-years research project funded by the Italian Ministry of University and Scientific Research (“FIRB” 2013, grant number RBFR13P7PR), which took into account a wide set of morphological, ecological and physiological parameters with the aim to clarify and better understand the common reed die-back phenomenon, providing useful knowledge to be used as early warning monitoring tools. Some results of the project have already been published by Lastrucci *et al.* (2017) and Cerri *et al.* (2017a, 2017b). Here we discuss the floristic and vegetational features of the reed-dominated plant communities involved in die-back processes. Aims of the study were i) to point out the floristic differences between declining and non-declining stands based on a representative data set and ii) to give these floristic differences a phytosociological and, consequently, ecological interpretation, using species and communities as environmental indicators.

Materials and Methods

Study areas and data sampling

The vegetation of the reed-dominated stands along the shores of five freshwater ecosystems in central Italy has been sampled in September 2014. The five study areas were: the Lakes Trasimeno, Chiusi and Vico, the Fucecchio and Colfiorito Marshes (Fig. 1). All the sites are included in the Natura 2000 Network (SAC IT5210034, SPA IT5210072, SAC/SPA IT5130007, SAC/SPA IT5190009, SAC IT5210018, SPA IT5210070, SAC IT6010024, SPA IT6010057). Basic geographic, morphologic and ecological information about the sites is reported in Tab. 1.

The vegetation survey has been carried out applying the phytosociological methodology (Braun-Blanquet, 1979) in 80 plots (size: 3 m × 3 m), 18 per each wetland, located in correspondence of the sampling sites used for the study of the reed die-back symptoms in the above-mentioned “FIRB” project (for more details see Lastrucci *et al.*, 2017). Each plot was characterized by flat slope. Each relevé consisted of the complete list of vascular species and the relative cover values, recorded by adopting Braun-Blanquet’s cover scale, modified in order to include the values 2m, 2a and 2b proposed by Barkman *et al.* (1964), better specifying

the cover range referred to the value “2” and corresponding to ranges of: 5% with many individuals of small size (2m), 5-12,5% (2a), 12,5-25% (2b). Based on Lastrucci *et al.* (2017), declining and non-declining plots have been distinguished on the ground of several diagnostic traits, among which a key role was played by the clumping habit, *i.e.* the occurrence of an abnormal growth form caused by loss of apical dominance and development of dormant lateral buds, leading to the formation of clumps of culms (Armstrong *et al.*, 1996b; Van Der Putten, 1997; Dinka & Szeglet, 2001; Gigante *et al.*, 2011, 2014). This diagnostic trait was quantitatively measured by Lastrucci *et al.* (2017), and the reported values could be used to evaluate the level of decline of each plot.

Data processing

After a numerical transformation according to the conversion scale proposed by Westhoff & Van Der Maarel (1978), the 80 relevés were used to build a “species × relevés” matrix. A distance matrix was produced based on the Euclidean distance, by applying the function *vegdist* from the “vegan” package (Oksanen *et al.*, 2017) in R environment (R Core Team, 2017). The distance matrix was then subjected to cluster analysis using the *hclust* function and applying the Ward method. For each resulting group, Pearson’s *phi* coefficient was calculated (Chytrý *et al.*, 2002) by applying the *multipatt* function from the “indicpecies” package (De Caceres & Legendre, 2009). Based on the results, the indicator species for each cluster have been pointed out. We considered a species as diagnostic of each group when $\phi \geq 0.40$ and $p < 0.05$. In accordance with Illyés *et al.* (2007), we adopted as threshold value $\phi = 0.40$, which results particularly suitable since it

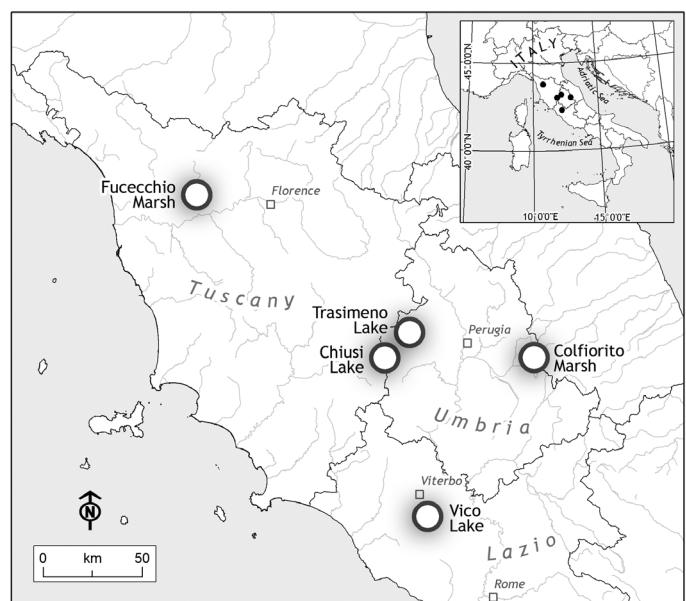


Fig. 1 - Location of the five study areas.

Tab. 1 - General information about the study sites (SAC: Natura 2000 Special Area of Conservation; SPA: Natura 2000 Special Protection Area). Maximum water depth sources are according to Lastrucci *et al.* (2017); geology derives from the geological map of Italy (source: <http://www.pcn.minambiente.it/viewer/>).

Site	Province, Region	Lat Long	Surface (ha)	Protected Areas	Altitude (m a.s.l.)	Max. water depth (m)	Bioclimate	Geology
Colfiorito Marsh (C)	Perugia, Umbria	43°01'23.00" 12°52'36".00"	135	Wetland of International Importance (Ramsar Site); Regional Park of Colfiorito; SAC IT5210034 "Palude di Colfiorito"; SPA IT5210072 "Palude di Colfiorito"	756	1.6	Temperate Oceanic Submediterranean, lower supratemperate, lower humid	Limestone and pelagic marly limestone; Marl and marly limestone of pelagic facies; Micritic limestone and pelagic clay
Fucecchio Marsh, Le Morette (F)	Pistoia, Tuscany	43°48'30.38" 10°48'20.14"	102	Wetland of International Importance (Ramsar Site); Natural Reserve "Padule di Fucecchio"; SAC/SPA IT5130007 "Padule di Fucecchio"	13	1.7	Temperate Oceanic Submediterranean, lower mesotemperate, upper subhumid	Debris, alluvial and fluvial-lacustrine deposits, actual beaches
Chiusi Lake (H)	Siena, Tuscany	43°03'22.11" 11°57'55.79"	360	SAC/SPA IT5190009 "Lago di Chiusi"	252	5.7	Temperate Oceanic Submediterranean, upper mesotemperate, lower subhumid [Mediterranean Pluviseasonal oceanic, upper mesomediterranean, lower subhumid]	Debris, alluvial and fluvial-lacustrine deposits, actual beaches; Sands and conglomerates
Trasimeno Lake (T)	Perugia, Umbria	43°08'05.50" 12°06'04.60"	12150	Regional Park of Lago Trasimeno; SAC IT5210018 "Lago Trasimeno"; SPA IT5210070 "Lago Trasimeno"	257	6.3	Temperate Oceanic Submediterranean, upper mesotemperate, upper subhumid	Debris, alluvial and fluvial-lacustrine deposits, actual beaches; Debris, alluvial terraces, fluvial-lacustrine and fluvial-glacial deposits; Lacustrine and fluvial-lacustrine deposits; Sandstone and arenaceous-marly turbiditic units; Clay and clay-calcareous turbiditic units
Vico Lake (V)	Viterbo, Latium	42°18'58.40" 12°10'05.89"	1209	Natural Regional Reserve "Lago di Vico"; SAC IT6010024 "Lago di Vico"; SPA IT6010057 "Lago di Vico-Monte Fogliano e Monte Venere"	507	50	Mediterranean Pluviseasonal oceanic, upper mesomediterranean / lower supramediterranean, lower Subhumid [Temperate Oceanic Submediterranean, lower supratemperate / upper mesotemperate, upper Subhumid]	Debris, alluvial and fluvial-lacustrine deposits, actual beaches; Latites, trachytes, phonolites (lavas, ignimbrites, pyroclastic deposits); Phoidites, tephrites (lavas, pyroclastic deposits and ignimbrites)

produces neither too long nor too short lists of diagnostic species for each vegetation unit. The number of relevés of each cluster was virtually standardised to an equal size (Tichý & Chytrý, 2006) in order to eliminate dependency of the *phi* coefficient for presence/absence data on the relative size of groups within the data set.

A principal component analysis (PCA) was carried out in order to explore the groups with reference to three quantitative ecological variables: 1) average water depth, measured for each relevé at the moment of the sampling, that coincides both with the end of the vegetative season and the end of the dry period, 2) number of species per relevé, and 3) clumping rate per relevé, measured in a 1 m × 1 m located at the centre of the relevé plot. The values of the clumping rate, intended as the ratio between the number of stems in each clump and the total number of stems per square meter, were derived from Lastrucci *et al.* (2017) and refer to the same plots where the phytosociological relevés have been performed. This parameter has been proved to be a robust proxy to detect and quantify the occurrence of die-back, with a diagnostic role (Lastrucci *et al.*, 2017). Finally, the geographical location was considered as an additional qualitative parameter. The PCA analysis was performed by using the *PCAmix* function from the package "PCAmixdata" (Chavent *et al.*, 2014).

The species nomenclature is updated according to the most recent reviews and matches with the database

AnArchive (Lucarini *et al.*, 2015). For the syntaxonomic framing, we followed the standards proposed by Biondi & Blasi (2013) and Biondi *et al.* (2014).

Results

The cluster analysis allowed to point out six main clusters, the second of which has been further subdivided in three groups due to prominent floristic differences which, being the relevés extremely species-poor, could not be detected by the indicator species analysis. The resulting dendrogram is reported in Fig. 2 and the indicator species per group are listed in Tab. 2. The phytosociological tables of the single groups are reported in the Tabs. 3-8, while a synoptic table is showed in Tab. 9. Based on their floristic, physiognomical and structural traits, all the groups have been referred to the association *Phragmitetum australis* Savič 1926. The different groups show clear differences which are hereafter described.

The results of the PCA analysis are illustrated in Fig. 3. The role of the three quantitative ecological variables is evident in the vectorial space with the relevés (Fig. 3c). The number of species ("num_spe") positively effects on the Groups V, VI and VIII, while the water depth ("wat_dep") and the clumping rate ("clu_rat") strongly influence the distribution of the Groups I, IV and VII (Figs. 3c, 3d). The Groups II and III ap-

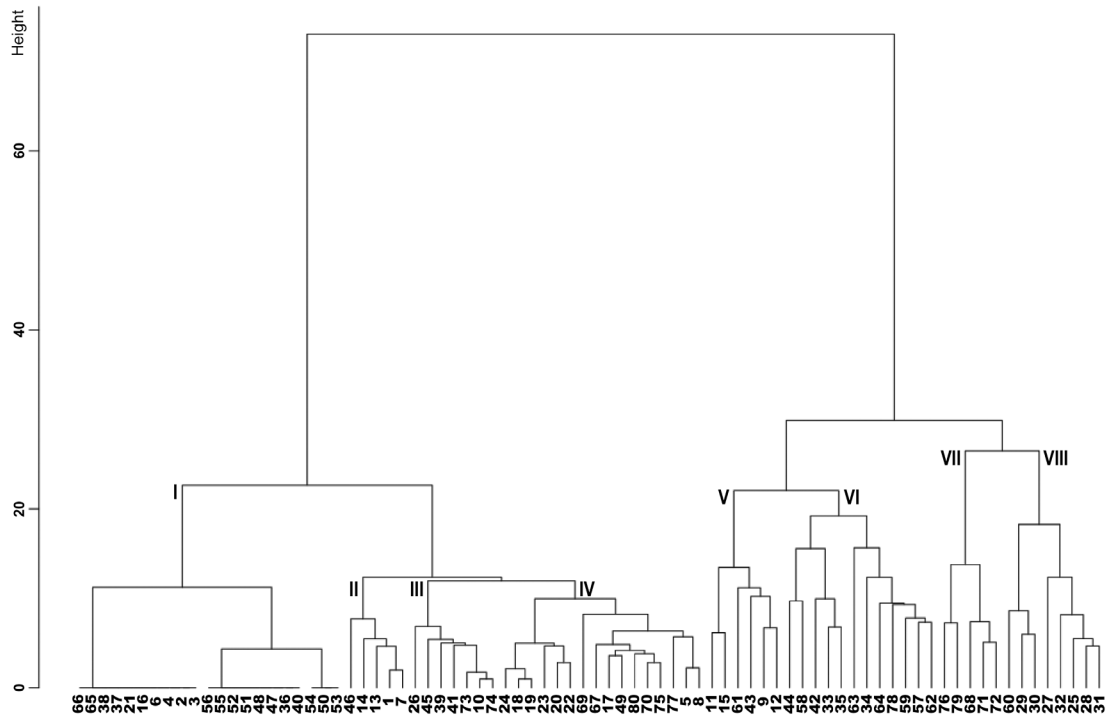


Fig. 2 - Dendrogram of the 80 relevés (*hclust* function, Ward method); the eight groups are indicated with roman numerals.

pear rather not related to those parameters.

In Fig. 4, the correlation between the average number of species per relevé in the eight groups and the average clumping rate per group (accounting for the level of die-back) is indicated, showing a robust inverted relation between the two variables (Spearman's R: -0.952, $p < 0.001$).

Group I: *PHRAGMITETUM AUSTRALIS* “*nudum*”

The Group I (Tab. 3) includes relevés from all the five study sites. It groups together all the plots referring to monospecific stands, where *P. australis* is the only plant species in the community, with changeable cover values ranging from 3 to 5. Due to the extreme floristic poverty, a floristic-based classification was not possible. From the phytosociological and ecological point of view, the only feasible classification had to be grounded on the dominant role performed by *P. australis*, allowing the framing of these amphibian stands in the alliance *Phragmition communis* Koch 1926. The sampled plant communities should be considered as a basal phytocoenon, or as an extremely impoverished aspect of the association *Phragmitetum australis* Savič 1926, already typically species-poor (Landucci et al., 2013). These monospecific stands are well known in literature and sometimes referred to as *Phragmitetum “nudum”*, e.g. by Burian & Sieghardt (1979) and Sieghardt (1990). As pointed out by the PCA results (Fig. 3), this group is composed almost exclusively by permanently submerged stands. It includes the plots where the water depth at the end of the dry period is

Tab. 2 - Indicator species for the clusters of relevés produced by the dendrogram, and related phi coefficients with statistical significance. The groups 1-3, including from monospecific to extremely species-poor relevés, do not own any indicator species.

	phi coefficient	p value	
Group 4			
<i>Myriophyllum spicatum</i> L.	0.587	0.005	**
<i>Najas marina</i> L.	0.475	0.027	*
Group 5			
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	0.743	0.001	***
<i>Eclipta prostrata</i> (L.) L.	0.683	0.001	***
<i>Persicaria lapathifolia</i> (L.) Delarbre	0.621	0.002	**
<i>Bidens connatus</i> Muhl. ex Willd.	0.552	0.009	**
<i>Amorpha fruticosa</i> L.	0.521	0.006	**
<i>Xanthium orientale</i> L. subsp. <i>italicum</i> (Moretti) Greuter	0.511	0.007	**
Group 6			
<i>Urtica dioica</i> L.	0.810	0.001	***
<i>Galium aparine</i> L.	0.620	0.001	***
<i>Eupatorium cannabinum</i> L.	0.552	0.001	***
<i>Scutellaria galericulata</i> L.	0.475	0.030	*
<i>Limniris pseudacorus</i> (L.) Fuss	0.425	0.048	*
Group 7			
<i>Schoenoplectus lacustris</i> (L.) Palla	0.777	0.001	***
<i>Nymphaea alba</i> L.	0.607	0.007	**
<i>Phalaris arundinacea</i> L.	0.497	0.019	*
Group 8			
<i>Juncus effusus</i> L.	0.927	0.001	***
<i>Galium palustre</i> L.	0.683	0.001	***
<i>Persicaria hydropiper</i> (L.) Delarbre	0.587	0.003	**
<i>Lycopus europaeus</i> L.	0.573	0.003	**
<i>Ranunculus repens</i> L.	0.517	0.018	*

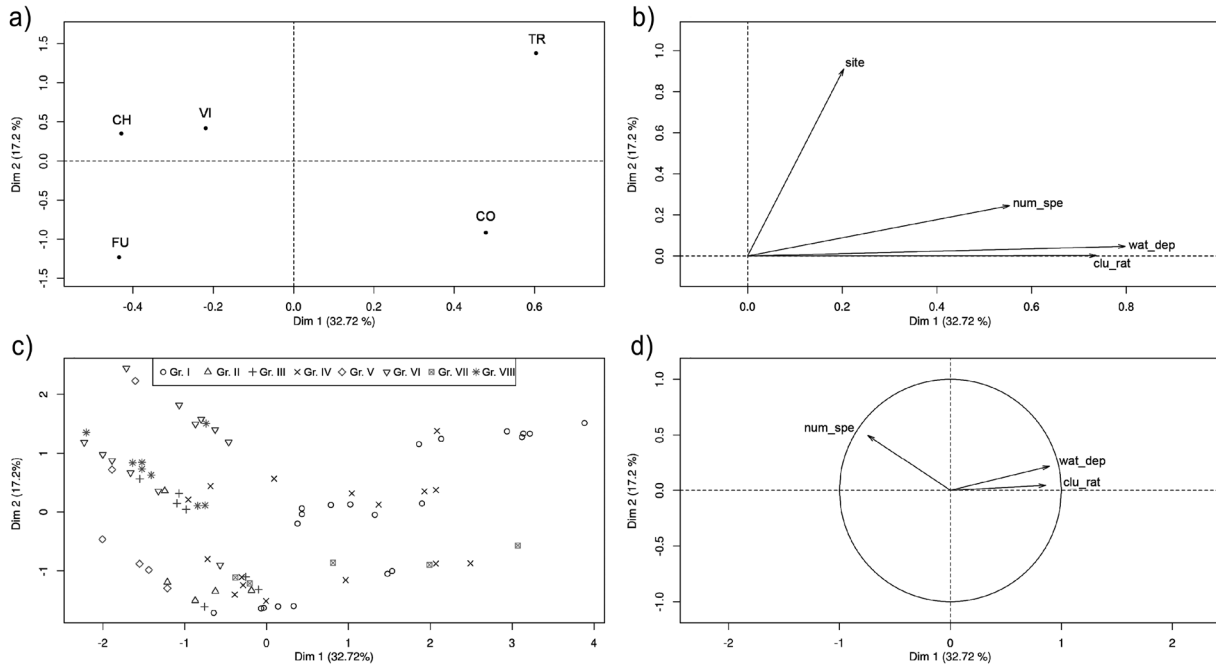


Fig. 3 - Results of the PCA analysis: a) levels component map; b) squared loadings; c) scattergram; d) correlation circle. CH = Lake Chiusi, CO = Colfiorito Marsh, FU = Fucecchio Marsh, TR = Lake Trasimeno, VI = Lake Vico; num_spe = number of species per relevé, wat_dep = water depth at the end of the dry season, clu_rat = clumping rate; the latter, expressed as ratio between the number of stems participating in clumps and the total number of stems per square meter, derives from Lastrucci *et al.* (2017).

the highest, with average values around 59.9 cm ± 8.9 (SE). The vector representing the clumping habit (Fig. 3), has also a prominent role for these relevés and indicates the occurrence of a condition of evident decline.

Group II: *PHRAGMITETUM AUSTRALIS* - species-poor variant with *Lythrum salicaria*

The Group II (Tab. 4) includes a small cluster of relevés, almost all carried out in Fucecchio Marsh, rather poor and including only 5 species per relevé on average ± 0.8 (SE). Besides *P. australis*, the only *taxon* in

common is *Lythrum salicaria*, a frequent occurrence in the palustrine vegetation belonging to the class *Phragmito-Magnocaricetea*. Since this species was observed in several stands in all the study areas, it cannot be considered as an indicator species for Group II but only as a differential *taxon*, with respect to the other species-poor groups (Groups I, III and IV). The reed stands included in the Group II grow in areas where in summer the bottom sediment generally emerges, due to the lowering of the water depth (average values around 6.9 cm ± 5.5). The condition of decline is scarce or absent. From the phytosociological point of view, these plant communities are characterized by a co-occurrence of hygrophilous and nitrophilous *taxa* and represent a transition stage between typical and sub-nitrophilous reed beds. A certain level of disturbance is indicated by the presence of the alien *Cyperus odoratus*.

Group III: *PHRAGMITETUM AUSTRALIS* - species-poor variant with *Calystegia sepium*

The Group III (Tab. 4) also puts together relevés very poor in species (5 on average ± 0.9), carried out in several study sites. It includes only reed beds in healthy status, without any sign of die-back, colonizing backward sites completely emerging in summer (average values of the water depth = 0.0 cm ± 0.0). Several hygrophilous species are present, although sporadically, such as *Mentha aquatica*, *Limniris pseudacorus*, *Lycopus europaeus*, *Carex riparia*. The only constant presence, although not suitable as an indicator species being also present in other groups, is *Calystegia sepium*,

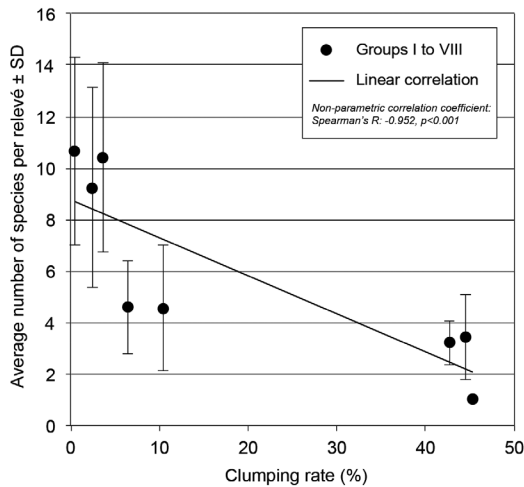


Fig. 4 - Correlation between average number of species per relevé in the eight groups vs. average clumping rate per group; for the latter parameter, values derive from Lastrucci *et al.* (2017).

Tab. 3 - Group I: *Phragmitetum australis* Savič 1926 “*nudum*”.

Rel. N.	66	65	38	37	21	16	6	4	2	3	56	55	52	51	48	47	36	40	54	50	53	
Plot ID	14EC002	14EC001	14ECH06	14ECH05	14EV05	14EFu16	14EFu06	14EFu04	14EFu02	14EFu03	14ET08	14ET07	14ET04	14ET03	14ECH16	14ECH15	14ECH04	14ECH07	14ET06	14ET02	14ET05	
Group N.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Water depth (cm)	70.7	61.0	59.3	58.0	53.3	0.0	9.3	7.7	12.0	10.3	114.7	101.0	103.0	89.3	18.7	53.3	63.7	59.9	98.7	82.7	131.7	
Clumping rate (%)	24.2	30.5	5.2	6.5	24.3	0.0	27.6	27.3	35.4	48.7	59.8	90.4	83.6	96.4	100.0	100.0	36.6	0.0	27.3	27.0	100.0	
Number of species per relevé	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
Dominant species																						
<i>Phragmites australis</i> (Cav.) Steud.	5	5	5	5	5	5	5	5	5	5	4	4	4	4	4	4	4	4	3	3	3	21

Tab. 4 - Group II: *Phragmitetum australis* Savič 1926, species-poor variant with *Lythrum salicaria*. Group III: *Phragmitetum australis* Savič 1926, species-poor variant with *Calystegia sepium*. Group IV: *Phragmitetum australis* Savič 1926, aquatic variant with *Myriophyllum spicatum*.

Rel. N.	46	14	13	1	7	26	45	39	41	73	10	74	24	18	19	23	20	22	69	67	17	49	80	70	75	77	5	8				
Plot ID	14ECH14	14EFu14	14EFu13	14EFu01	14EFu07	14EV10	14ECH13	14ECH08	14ECH09	14ECH09	14EFu10	14ECO10	14EV08	14EV02	14EV03	14EV07	14EV04	14EV06	14ECO05	14ECO03	14EV01	14ET01	14ECO16	14ECO06	14ECO11	14ECO13	14EFu05	14EFu08				
Group N.	2	2	2	2	2	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4				
Water depth (cm)	0.0	0.0	0.0	28.3	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	68.7	65.0	22.3	20.0	38.7	19.3	40.0	62.3	79.0	0.0	59.7	0.0	0.0	15.3	9.7				
Clumping rate (%)	3.6	0.0	0.0	13.1	15.4	0.0	0.0	61.7	0.0	7.8	0.0	3.4	0.0	31.1	97.0	0.8	100.0	31.3	48.4	100.0	91.2	57.7	4.7	100.0	0.0	0.0	13.8	35.8				
Number of species per relevé	7	6	3	3	4	6	9	4	5	4	2	2	5	2	2	6	3	6	2	3	2	3	4	2	3	7	3	2				
Dominant species																																
<i>Phragmites australis</i> (Cav.) Steud.	5	5	5	5	5	5	5	5	5	5	5	5	4	4	5	5	5	5	3	3	5	4	5	3	5	5	5	5	5	28		
Differential species Group II																																
<i>Lythrum salicaria</i> L.	1	+	1	+	+	.	r	.	.	r	7		
Differential species Group III																																
<i>Calystegia sepium</i> (L.) R. Br.	+	+	+	1	+	+	1	7		
Indicator and differential (d) species Group IV																																
<i>Myriophyllum spicatum</i> L.	+	+	+	+	1	+	6		
<i>Persicaria amphibia</i> (L.) Delarbre (d)	1	.	.	.	+	r	+	1	5		
<i>Najas marina</i> L.	r	.	.	+	.	r	.	.	.	+	4		
<i>Potamogeton lucens</i> L. (d)	r	.	.	+	.	r	3		
<i>Ceratophyllum demersum</i> L. (d)	+	+	3		
<i>Ricciocarpos natans</i> (L.) Corda (d)	2b	+	+	.	.	3			
<i>Potamogeton nodosus</i> Poir. (d)	+	1	2		
<i>Phragmito-Magnocaricetea</i>																																
<i>Mentha aquatica</i> L.	+	+	1	+	.	4		
<i>Schoenoplectus lacustris</i> (L.) Palla	+	.	.	+	.	+	.	.	3		
<i>Limniris pseudacorus</i> (L.) Fuss	+	+	2		
<i>Lycopus europaeus</i> L.	+	+	.	2		
<i>Galio-Urticetea</i>																																
<i>Barbarea vulgaris</i> W.T. Aiton	+	+	2		
<i>Bidentetea tripartitae</i>																																
<i>Cyperus odoratus</i> L.	.	1	.	1	1	3		
Other species																																
<i>Solanum dulcamara</i> L.	1	r	.	+	+	4		
<i>Amorpha fruticosa</i> L.	.	.	+	+	2		
Sporadic species	1	3	0	0	0	1	4	1	2	1	0	0	1	0	0	0	0	0	0	0	0	1	0	2	0	0	3	0	0			

which has an important ecological role indicating a slight nitrophilous character for this plant community. From the phytosociological point of view, this vegetation type can be interpreted as a typical aspect of the association *Phragmitetum australis*, where *Calystegia sepium* is generally frequent (Landucci et al., 2013).

Group IV: *PHRAGMITETUM AUSTRALIS* - aquatic variant with *Myriophyllum spicatum*

The Group IV (Tab. 4) includes 16 relevés from the Lakes Vico and Trasimeno and Fucecchio Marsh. It refers to reed beds with a prolonged submersion, with average values of the water depth at the end of the dry season around 33.3 cm ± 7.1. The sampled stands are generally species-poor (3 species per relevé on average

± 0.4), however the analysis of the Pearson’s *phi* coefficient points out two indicator species: *Myriophyllum spicatum* and *Najas marina* (Tab. 2). Besides these two taxa, a relevant number of other hydrophytes can be sporadically found in the relevés of Group IV, such as *Persicaria amphibia*, *Potamogeton lucens*, *P. perfoliatus*, *P. nodosus*, *Ceratophyllum demersum*, *Najas minor* and the aquatic bryophyte *Ricciocarpos natans* (Tab. 4). Their presence is a clear indication of the prolonged condition of submersion for this vegetation type. Few other hygrophilous species are sporadically present, such as *Mentha aquatica* and *Schoenoplectus lacustris*. The reed population generally shows clear symptoms of decline, the more prominent as the longer is the submersion period. From the phytosociological

point of view, they can be considered as a variant of the association *Phragmitetum australis*, representing the contact with the aquatic communities of the classes *Potametea* and *Lemnetea*.

Group V: *PHRAGMITETUM AUSTRALIS* - hygro-subnitrophilous variant with *Echinochloa crus-galli*

The Group V (Tab. 5) is composed by relevés rather rich in species (11 on average \pm 1.5). As pointed out by the analysis of Pearson's *phi* coefficient, especially the annual hygro-subnitrophilous species are diagnostic for this cluster, e.g. *Echinochloa crus-galli* and *Persicaria lapathifolia*, together with several alien taxa such as *Xanthium orientale* subsp. *italicum*, *Bidens connatus*, *Eclipta prostrata* and the perennial *Amorpha fruticosa*. *Calystegia sepium* is also very frequent and performs a differential role, although it cannot be considered a diagnostic species, being present also in other groups (e.g. Groups III, VI, VIII). The relevés of the Group 5 refer to healthy stands, not showing any symptom of die-back. They have been performed along the shores of Fucecchio Marsh, Lake Chiusi and Lake Trasimeno, in stands flooded only temporarily, which in summer appear totally emerged (average values of the water depth = 0.0 cm \pm 0.0). The reed individuals do not show any sign of decline. The annual hygro-nitrophilous component of the vegetation in the floristic spectrum is typical of the amphibian environments affected at the end of summer by natural disturbance, due to the accumulation of sediment and vegetal rests, with a consequent increase of nutrients. From the phytosociological point

Tab. 5 - Group V: *Phragmitetum australis* Savič 1926, hygro-subnitrophilous variant with *Echinochloa crus-galli*.

Rel. N.	11	15	61	43	9	12	
Plot ID	14EFu11	14EFu15	14ETr13	14ECh11	14EFu09	14EFu12	
Group N.	5	5	5	5	5	5	
Water depth (cm)	0.0	0.0	0.0	0.0	0.0	0.0	
Clumping rate (%)	0.0	0.0	3.3	0.0	0.0	0.0	
Number of species per relevé	13	6	16	12	8	9	Presences
Dominant species							
<i>Phragmites australis</i> (Cav.) Steud.	5	5	5	5	5	5	6
Indicator and differential (d) species Group V							
<i>Calystegia sepium</i> (L.) R. Br. (d)	1	.	1	1	1	1	5
<i>Echinochloa crus-galli</i> (L.) P. Beauv.	1	1	+	.	1	.	4
<i>Xanthium orientale</i> L. subsp. <i>italicum</i> (Moretti) Greuter	1	+	.	.	+	.	
<i>Persicaria lapathifolia</i> (L.) Delarbre	1	+	.	.	.	+	3
<i>Eclipta prostrata</i> (L.) L.	+	.	.	.	1	+	3
<i>Amorpha fruticosa</i> L.	+	.	.	.	1	+	3
<i>Bidens connatus</i> Muhl. ex Willd.	1	+	2
<i>Phragmito-Magnocaricetea</i>							
<i>Lythrum salicaria</i> L.	3	3	+	r	.	1	5
<i>Oenanthe aquatica</i> (L.) Poir.	+	1	2
<i>Bidentetea tripartitae</i>							
<i>Bidens frondosus</i> L.	.	.	.	+	+	+	3
<i>Cyperus odoratus</i> L.	r	.	.	.	+	.	2
<i>Potametea pectinati</i>							
<i>Persicaria amphibia</i> (L.) Delarbre	1	+	2
Sporadic species	1	0	12	8	0	0	

of view this community represents the contact with the annual pioneer hygro-subnitrophilous vegetation of the class *Bidentetea* and can be considered as a variant of the association *Phragmitetum australis*.

Group VI: *PHRAGMITETUM AUSTRALIS* - nitrophilous variant with *Urtica dioica*

The Group VI (Tab. 6) mainly refers to the Lakes Chiusi and Trasimeno. The stands are very rich in species (10 per relevé on average \pm 1.1). The Pearson's *phi* coefficient points out a statistically significant presence of five species: two perennial nitrophilous (*Urtica dioica*, *Galium aparine*), one hygro-subnitrophilous (*Eupatorium cannabinum*) and two hygrophilous taxa typical from palustrine vegetation (*Limniris pseudacorus*, *Scutellaria galericulata*). Also in this case, like for the Groups III and V, the environmental conditions are featured by a period of emersion of the bottom sediment in summer (average values of the water depth = 0.0 cm \pm 0.0) and the general status of the reeds is very good,

Tab. 6 - Group VI: *Phragmitetum australis* Savič 1926, nitrophilous variant with *Urtica dioica*.

Rel. N.	44	58	42	33	35	63	34	64	78	59	57	62	
Plot ID	14ECh12	14ETr10	14ECh10	14ECh01	14ECh03	14ETr15	14ECh02	14ETr16	14ECo14	14ETr11	14ETr09	14ETr14	
Group N.	6	6	6	6	6	6	6	6	6	6	6	6	
Water depth (cm)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Clumping rate (%)	0.0	5.6	0.0	0.0	0.0	10.4	0.0	8.3	2.5	5.7	3.8	7.5	
Number of species per relevé	10	9	7	13	15	18	12	12	6	9	6	8	Presences
Dominant species													
<i>Phragmites australis</i> (Cav.) Steud.	4	5	5	5	5	5	5	5	5	5	5	5	12
Indicator species Group VI													
<i>Calystegia sepium</i> (L.) R. Br.	1	1	1	2	1	2b	3	+	.	1	+	2b	11
<i>Urtica dioica</i> L.	.	+	.	1	+	2a	2	2a	2a	2a	3	3	10
<i>Limniris pseudacorus</i> (L.) Fuss	1	+	1	1	+	.	.	+	6
<i>Galium aparine</i> L.	.	+	1	+	+	.	.	+	5
<i>Eupatorium cannabinum</i> L.	.	.	.	+	1	.	1	1	4
<i>Scutellaria galericulata</i> L.	+	.	.	+	2	3
<i>Phragmito-Magnocaricetea</i>													
<i>Lythrum salicaria</i> L.	+	+	.	.	+	1	5
<i>Mentha aquatica</i> L.	.	.	1	2	+	r	4
<i>Carex pseudocyperus</i> L.	.	.	.	1	1	.	+	3
<i>Galium palustre</i> L. subsp. <i>elongatum</i> (C. Presl) Lange	.	.	.	+	1	.	r	3
<i>Carex riparia</i> Curtis	3	2b	2
<i>Lycopus europaeus</i> L.	.	.	.	+	+	2
<i>Galio-Urticetea</i>													
<i>Stachys palustris</i> L.	.	.	.	1	+	.	.	+	3
<i>Cirsium creticum</i> (Lam.) d'Urv. subsp. <i>triumfettii</i> (Lacaita) K. Werner	+	r	2
<i>Agrostiotea stoloniferae</i>													
<i>Agrostis stolonifera</i> L.	.	2b	1	+	1	2a	.	1	.	.	2	+	8
<i>Lycopus exaltatus</i> Ehrh. ex L. fil.	.	.	2	.	+	2
<i>Pulicaria dysenterica</i> (L.) Bernh.	.	.	r	.	.	+	2
Other species													
<i>Cirsium arvense</i> (L.) Scop.	.	+	+	+	3
<i>Rubus ulmifolius</i> Schott	1	1	1	3
<i>Salix cinerea</i> L.	r	+	2
<i>Elymus repens</i> (L.) Gould	r	.	.	2a	.	.	.	2
<i>Sambucus ebulus</i> L.	1	1	2
<i>Artemisia vulgaris</i> L.	1	.	.	+	.	2
Sporadic species	3	0	0	1	1	8	3	1	2	4	0	1	

without any symptom of die-back. From the phytosociological point of view these stands are interpreted as a contact phytocoenosis with the perennial subhygro-nitrophilous vegetation of the class *Galio-Urticetea* Passarge ex Kopecký 1969, and can be considered as a variant of the association *Phragmitetum australis*. Similar plant communities dominated by common reed, developed in disturbed habitats, sometimes in contact with anthropized areas and marginal to the palustrine ecosystems, are well known from literature. They have been considered as “pseudo” reed beds (Greco & Patocchi, 2003; Gigante et al., 2013) and sometimes framed quite in the class *Galio-Urticetea* (Mucina et al., 1993; Pellizzari et al., 2005). In the considered areas, from a floristic point of view there is a strong affinity with the vegetation type described by Gigante et al. (2013) as *Phragmitetum australis* var. with *Rubus ulmifolius* Schott, although in the present study the occurrence of the latter is rather sporadic. Some relevés of this group (Tab. 6, Rels. N. 33, 34 and 35), performed at Lake Chiusi, refer to vegetation growing in contact with the association *Thelypterido palustris-Phragmitetum australis* Kuiper ex van Donselaar 1961 which represents a peculiar type of reed bed living on floating mats, as reported by Lastrucci et al. (2014).

Group VII: *PHRAGMITETUM AUSTRALIS* - species-poor variant with *Schoenoplectus lacustris*

The Group VII (Tab. 7) includes a heterogeneous set of relevés carried out in the reed beds of Colfiorito, some of which in permanently submerged stands and some in drier areas (average values of the water depth = 38.7 cm ± 21.8). They are extremely species-poor (3 species per relevé on average ± 0.4) and are differentiated by the presence of *Schoenoplectus lacustris*, a species widely distributed in the area in the deeper waters along the reed bed waterfront. The presence of *Nymphaea alba* is also an indication of the prolonged period of submersion. Condition of decline have been detected in the permanently submerged plots (Rels. N. 68, 71, 72). From the phytosociological point of view, they are interpreted as a transitional variant towards the association *Schoenoplectetum lacustris* Chouard 1924, observed in the site. The reed-dominated community developed on emerging sediment (Rels. N. 76, 79) shows a better health status and is differentiated also by the presence of *Phalaris arundinacea*. This species is widely represented in the surrounding areas (Pedrotti, 1982; Orsomando & Raponi, 2002) with the association *Phalaridetum arundinaceae* Libbert 1931.

Group VIII: *PHRAGMITETUM AUSTRALIS* - dry variant with *Juncus effusus*

The Group VIII (Tab. 8) refers to eight relevés mainly performed at the Lake Vico, in areas with a top soil from drenched to dry at the end of the dry season,

when the surface water is completely absent (average values of the water depth = 0.0 cm ± 0.0). The reed beds in this site occupy a large muddy area in the N-W sector of the lake, extensively grazed by cattle. The general condition of the reed individuals is good, without any symptom of die-back. The relevés are rather rich in species (9 per relevé on average ± 1.4) and are differentiated by a combination of *taxa* from transition meadows (*Ranunculus repens*), grazed meadows

Tab. 7 - Group VII: *Phragmitetum australis* Savič 1926, species-poor variant with *Schoenoplectus lacustris*.

Rel. N.	76	79	68	71	72	
Plot ID	14EC012	14EC015	14EC004	14EC007	14EC008	
Group N.	7	7	7	7	7	
Water depth (cm)	0.0	0.0	37.7	36.3	119.3	
Clumping rate (%)	3.8	0.0	34.3	100.0	75.0	
Number of species per relevé	3	4	4	3	2	Presences
Dominant species						
Phragmites australis (Cav.) Steud.	4	5	3	3	3	5
Indicator species Group VII						
Schoenoplectus lacustris (L.) Palla	.	2	+	4	3	4
Phalaris arundinacea L.	4	2	.	.	.	2
Nymphaea alba L.	.	.	2	2a	.	2
Sporadic species						
	1	1	1	0	0	

Tab. 8 - Group VIII: *Phragmitetum australis* Savič 1926, dry variant with *Juncus effusus*.

Rel. N.	60	29	30	27	32	25	28	31	
Plot ID	14ETr12	14EV113	14EV114	14EV111	14EV116	14EV109	14EV112	14EV115	
Group N.	8	8	8	8	8	8	8	8	
Water depth (cm)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Clumping rate (%)	7.7	0.0	0.0	0.0	6.9	0.0	0.0	5.6	
Number of species per relevé	9	10	11	16	11	9	4	4	Presences
Dominant species									
Phragmites australis (Cav.) Steud.	5	5	5	5	5	5	4	5	8
Indicator and differential (d) species Group VIII									
Juncus effusus L.	.	+	1	+	2b	2	3	3	7
Agrostis stolonifera L. (d)	+	+	+	1	1	+	.	.	6
Lycopus europaeus L.	.	.	+	1	+	1	.	1	5
Galium palustre L.	.	.	+	1	1	r	.	.	4
Ranunculus repens L.	1	+	+	3
Persicaria hydropiper (L.) Delarbre	.	+	+	1	3
<i>Phragmito-Magnocaricetea</i>									
Calystegia sepium (L.) R. Br.	+	.	+	1	.	+	.	.	4
Carex riparia Curtis	.	+	1	1	3
Oenanthe aquatica (L.) Poir.	.	.	.	+	+	+	.	.	3
<i>Bidentetea tripartitae</i>									
Bidens frondosus L.	.	.	.	+	+	+	+	.	4
<i>Potametea pectinati</i> , <i>Lemneteae minoris</i>									
Persicaria amphibia (L.) Delarbre	+	+	+	.	3
Other species									
Solanum dulcamara L.	.	+	.	2	2a	.	.	+	4
Rubus ulmifolius Schott	3	2b	1	3
Equisetum arvense L.	.	+	+	2
Sporadic species									
	4	1	0	5	2	0	0	0	

Tab. 9 - Synoptic Table; the indicator species of the clusters of relevés produced by the dendrogram, and some additional differential (d) species, are indicated. The sporadic species of each single group have been removed (this group, although indicated as “dry” on average, includes the floating mats).

Group N.	1	2	3	4	5	6	7	8
Number of relevés	21	5	7	16	6	12	5	8
Average water depth (cm)	59.9	6.93	0.0	33.3	0.0	0.0	38.7	0.0
Average clumping rate (%)	45.3	6.4	10.4	44.5	0.6	3.7	42.6	2.5
Average number of species per relevé	1	5	5	3	11	10	3	9
Indicator species Group 4								
Myriophyllum spicatum L.	.	.	.	II
Najas marina L.	.	.	.	II
Indicator species Group 5								
Echinochloa crus-galli (L.) P. Beauv.	IV	.	.	.
Eclipta prostrata (L.) L.	III	.	.	.
Persicaria lapathifolia (L.) Delarbre	III	.	.	.
Amorpha fruticosa L.	.	I	.	.	III	.	.	.
Xanthium orientale L. subsp. italicum (Moretti) Greuter	.	I	.	.	III	.	.	.
Bidens connatus Muhl. ex Willd.	II	.	.	.
Indicator species Group 6								
Urtica dioica L.	I	V	.	.
Galium aparine L.	III	.	.
Limniris pseudacorus (L.) Fuss	.	I	I	.	I	III	.	.
Eupatorium cannabinum L.	II	.	.
Scutellaria galericulata L.	II	.	.
Indicator species Group 7								
Schoenoplectus lacustris (L.) Palla	.	.	.	I	.	.	IV	.
Nymphaea alba L.	II	.
Phalaris arundinacea L.	II	.
Indicator species Group 8								
Juncus effusus L.	V
Lycopus europaeus L.	.	.	I	.	.	I	.	IV
Galium palustre L.	III
Ranunculus repens L.	II
Persicaria hydropiper (L.) Delarbre	II
<i>Phragmito-Magnocaricetea</i>								
Phragmites australis (Cav.) Steud.	V	V	V	V	V	V	V	V
Mentha aquatica L.	.	I	I	I	I	II	I	.
Lythrum salicaria L.	.	V	II	.	V	III	.	.
Calystegia sepium (L.) R. Br.	.	.	V	.	V	V	I	III
Oenanthe aquatica (L.) Poir.	.	I	.	.	II	.	.	II
Carex riparia Curtis	.	.	I	.	.	I	.	II
Lysimachia vulgaris L.	.	I	.	.	I	.	.	.
Galium palustre L. subsp. elongatum (C. Presl) Lange	I	II	.	.
Glyceria sp.	.	.	I
Bolboschoenus sp.	I	.	.	.
Bolboschoenus glaucus (Lam.) S.G. Sm.	I	.	.	.
Veronica anagallis-aquatica L.	I	.	.	.
Carex pseudocyperus L.	II	.	.
<i>Galio-Urticetea</i>								
Barbarea vulgaris W.T. Aiton	.	I	I
Stachys palustris L.	.	.	I	.	.	II	.	.
Galega officinalis L.	.	.	I
Thalictrum lucidum L.	.	.	I
Epilobium hirsutum L.	I	.	.	.
Cirsium creticum (Lam.) d'Urv. subsp. triumfettii (Lacaita) K. Werner	I	.	.
<i>Bidentetea tripartitae</i>								
Cyperus odoratus L.	.	III	.	.	II	.	.	.
Bidens frondosus L.	III	.	.	III
Atriplex prostrata Boucher ex DC.	.	.	I
Bidens tripartitus L.	I	.	.	.
Lipandra polysperma (L.) S. Fuentes, Uotila et Borsch	I	.	.	.
Oxybasis urbica (L.) S. Fuentes, Uotila et Borsch	I	.	.	.
<i>Agrostietea stoloniferae</i>								
Agrostis stolonifera L.	IV	.	IV
Rumex obtusifolius L.	.	I

Pulicaria dysenterica (L.) Bernh.	I	.	.	
Lycopus exaltatus Ehrh. ex L. fil.	I	.	.	
<i>Potametea pectinati</i> , <i>Lemnetea minoris</i>											
Persicaria amphibia (L.) Delarbre	.	II	I	II	.	II	
Ceratophyllum demersum L.	I	.	.	.	
Potamogeton lucens L.	I	.	.	.	
Potamogeton nodosus Poir.	I	.	.	.	
Ricciocarpos natans (L.) Corda	I	.	.	.	
Other species											
Solanum dulcamara L.	III	.	I	I	III
Rubus ulmifolius Schott	I	II	.	II
Plantago major L.	I	.	I	.	.
Cirsium arvense (L.) Scop.	I	II	.	.
Salix cinerea L.	I	I	.	.
Cornus sanguinea L.	I
Juncus conglomeratus L.	I
Stellaria media (L.) Vill.	I	.	.
Erigeron bonariensis L.	I	.	.
Samolus valerandi L.	I	.	.
Symphotrichum squamatum (Spreng.) G.L. Nesom	I	.	.
Artemisia vulgaris L.	I	.
Elymus repens (L.) Gould	I	.
Salix purpurea L.	I	.
Sambucus ebulus L.	I	.
Solanum nigrum L.	I	.
Equisetum palustre L.	I	.
Equisetum arvense L.	II

(*Juncus effusus*), hygro-subnitrophilous (*Persicaria hydropiper* and palustrine phytocoenoses (*Lycopus europaeus*, *Galium palustre*). *Agrostis stolonifera* also plays a differential role. From the phytosociological point of view, these mixed reed beds can be considered as a transitional variant towards the meadows of the class *Molinio-Arrhenatheretea* Tüxen 1937.

Discussion and conclusive remarks

The present study provides an overview of the floristic-vegetational features of a variety of reed beds from different wetlands in central Italy, part of which have been formerly diagnosed as affected by die-back (Lastrucci *et al.*, 2017). The results show that the floristic and vegetational features, neglected by the large majority of the scientific production, play a clear role in the die-back phenomenology.

Although the common reed-dominated vegetation is a typically species-poor phytocoenosis (see, *e.g.*, Philippi, 1977; Gerdol, 1987; Balátová-Tulácková *et al.*, 1993), our results show a certain differentiation among types and, on average, between healthy and declining stands. The phytosociological analysis allowed to point out eight major types, which differ between each other for species number, floristic composition and levels of nitrophily and hydrophily, as supported by the indicator species. In particular, when we compare the healthy and the declining stands, it is evident that, as already suggested by Gigante *et al.* (2013), there is a clear correlation between number of species per relevé and intensity of the reed decline expressed as clumping rate (Fig. 4), which has been proved to be a robust quantitative diagnostic symptom of die-back

(Lastrucci et al., 2017).

It is acknowledged that reed decline most heavily affects the reed stands growing in permanent submergence with deep water levels (Hellings & Gallagher, 1992; Weisner et al., 1993; Rea, 1996; Mauchamp et al., 2001), and that prolonged submergence is strongly related to incidence and severity of die-back (Lastrucci et al., 2017). Results of our study show that the permanently submerged stands are also the ones with the lowest floristic variety, with the extreme situation represented by the monospecific vegetation referred to *Phragmitetum vulgaris* “*nudum*” (Tab. 3).

It has been reported that the reed-dominated vegetation tends to be monospecific when growing in permanently flooded areas (Sieghardt, 1990; Cízková et al., 1996; Schmieder et al., 2002) and that, in general, prolonged submergence or lack of drying up can often co-occur with low values of species richness, especially with reference to macrophytes (see, e.g., Van Geest et al., 2005). Indeed, periods of drying up are needed for seed germination and survival of several macrophytic species (Keddy & Constabel, 1986; Coops & Van Der Velde, 1995; Bonis & Grillas, 2002). Additionally, the litter generated by *Phragmites australis*, slowly decomposing especially in submerged conditions, can inhibit the growth of wetland species (Van Der Putten, 1993; Van Der Putten et al., 1997).

On the other side, studies on the reed productivity reported that the highest aboveground dry matter production of *Phragmites australis* could be found in the landward zone (Sieghardt, 1990). These results match with the observed preferential occurrence of non-declining stands in drier locations, only temporarily submerged, generally on the land-facing border of the reed beds, often in contact with agricultural areas, as reported both in the present study and in former investigations (e.g. Gigante et al., 2014; Lastrucci et al., 2016).

The stands where reed does not show symptoms of decline, developing in terrestrial areas, are also the richest in species. It has been indicated in literature that recurring periods of low water level tend to increase plant biodiversity (Riis & Hawes, 2002). However, as already noticed by Gigante et al. (2013), this floristic richness is often due to the increase of nitrophilous species, favoured by the terrestrial environment and by the nutrient income from the agricultural areas in the surroundings.

Overall it can be stated that, in the study sites, only the pauci-specific stands including hygrophilous *taxa* (Groups II and III) seem to better correspond to typical, wet reed beds where reeds grow healthily and the floristic spectrum includes typical wetland species. It should be emphasized that the aquatic stands of reed often represent a refugium for little floating or rooted aquatic *taxa* (see e.g. Group IV) and their disruption and retreat implies the disappearance of suitable mi-

cro-habitats for these vulnerable species.

Studies on patterns and processes of common reed die-back appear extremely important for conservation purposes. This phenomenon affects not only the reed populations themselves, but also general aspects of wetland ecosystems, due to the key role played by *Phragmites australis* in providing habitat for other flora and fauna elements, filtering a wide range of pollutants, maintaining shore stability, only to mention some of the most prominent ecosystem services provided by this very common species (Ostendorp, 1993; Kiviat, 2013). Additionally, reed decline might also have social and economic impacts, given the importance of reed beds for eco-tourism and for several traditional human activities (Kiviat, 2013). For these reasons a deeper understanding of the phenomenon of reed decline is more and more urgent.

Syntaxonomic scheme

PHRAGMITO-MAGNOCARICETEA Klika in Klika & Novák 1941

PHRAGMITETALIA Koch 1926

Phragmiton communis Koch 1926

Phragmitetum australis Savič 1926

“*nudum*”

var. with *Lythrum salicaria*

var. with *Calystegia sepium*

var. with *Myriophyllum spicatum*

var. with *Echinochloa crus-galli*

var. with *Urtica dioica*

var. with *Schoenoplectus lacustris*

var. with *Juncus effusus*

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Appendix I: Localities, dates and sporadic species of the relevés.

[the sequence is as follows: Relevé Number, Plot ID, locality, date (gg/mm/aaaa), Lat/Long (degrees, minutes), sporadic species with cover values].

Tab. 3 - Group I: *Phragmitetum australis* Savič 1926 "nudum": Rel. 66, 14ECo02, Colfiorito Marsh, 29/09/2014, N43°1.51' E12°52.532; Rel. 65, 14ECo01, Colfiorito Marsh, 29/09/2014, N43°1.598' E12°52.575; Rel. 38, 14ECh06, Lake Chiusi, 15/09/2014, N43°3.401' E11°58.67; Rel. 37, 14ECh05, Lake Chiusi, 15/09/2014, N43°3.341' E11°58.659; Rel. 21, 14EVi05, Lake Vico, 09/09/2014, N42°19.923' E12°9.275; Rel. 16, 14EFu16, Fucecchio Marsh, 03/09/2014, N43°48.74' E10°48.49; Rel. 6, 14EFu06, Fucecchio Marsh, 03/09/2014, N43°48.308' E10°48.254; Rel. 4, 14EFu04, Fucecchio Marsh, 03/09/2014, N43°48.247' E10°48.072; Rel. 2, 14EFu02, Fucecchio Marsh, 03/09/2014, N43°48.346' E10°48.372; Rel. 3, 14EFu03, Fucecchio Marsh, 03/09/2014, N43°48.279' E10°48.175; Rel. 56, 14ETr08, Lake Trasimeno, 26/09/2014, N43°6.223' E12°11.066; Rel. 55, 14ETr07, Lake Trasimeno, 26/09/2014, N43°6.019' E12°11.178; Rel. 52, 14ETr04, Lake Trasimeno, 26/09/2014, N43°6.04' E12°11.196; Rel. 51, 14ETr03, Lake Trasimeno, 26/09/2014, N43°6.169' E12°11.171; Rel. 48, 14ECh16, Lake Chiusi, 19/09/2014, N43°2.645' E11°58.477; Rel. 47, 14ECh15, Lake Chiusi, 19/09/2014, N43°2.613' E11°58.341; Rel. 36, 14ECh04, Lake Chiusi, 15/09/2014, N43°3.207' E11°58.634; Rel. 40, 14ECh07, Lake Chiusi, 19/09/2014, N43°2.717' E11°58.472; Rel. 54, 14ETr06, Lake Trasimeno, 26/09/2014, N43°5.842' E12°11.169; Rel. 50, 14ETr02, Lake Trasimeno, 25/09/2014, N43°5.691' E12°11.087; Rel. 53, 14ETr05, Lake Trasimeno, 26/09/2014, N43°5.791' E12°11.049.

Tab. 4 - Group II: *Phragmitetum australis* Savič 1926, species-poor variant with *Lythrum salicaria*: Rel. 46, 14ECh14, Lake Chiusi, 15/09/2014, N43°3.159' E11°57.012, *Lysimachia vulgaris* L. 1; Rel. 14, 14EFu14, Fucecchio Marsh, 03/09/2014, N43°48.751' E10°48.522, *Oenanthe aquatica* (L.) Poir. +, *Xanthium orientale* L. subsp. *italicum* (Moretti) Greuter 1, *Rumex obtusifolius* L. +; Rel. 13, 14EFu13, Fucecchio Marsh, 03/09/2014, N43°48.764' E10°48.565; Rel. 1, 14EFu01, Fucecchio Marsh, 03/09/2014, N43°48.39' E10°48.501; Rel. 7, 14EFu07, Fucecchio Marsh, 03/09/2014, N43°48.374' E10°48.435; Group III: *Phragmitetum australis* Savič 1926, species-poor variant with *Calystegia sepium*: Rel. 26, 14EVi10, Lake Vico, 09/09/2014, N42°19.981' E12°9.225, *Juncus conglomeratus* L. 1; Rel. 45, 14ECh13, Lake Chiusi, 15/09/2014, N43°3.545' E11°57.104, *Stachys palustris* L. +, *Galega officinalis* L. +, *Thalictrum lucidum* L. r, *Plantago major* L. r; Rel. 39, 14ECh08, Lake Chiusi, 15/09/2014, N43°4.012' E11°57.717, *Carex riparia* Curtis 1; Rel. 41, 14ECh09, Lake Chiusi, 15/09/2014, N43°4.049' E11°57.392, *Atriplex prostrata* Boucher ex

DC. +, *Cornus sanguinea* L. +; Rel. 73, 14ECo09, Colfiorito Marsh, 29/09/2014, N43°1.563' E12°52.749, *Glyceria* sp. r; Rel. 10, 14EFu10, Fucecchio Marsh, 03/09/2014, N43°48.689' E10°48.703; Rel. 74, 14ECo10, Colfiorito Marsh, 29/09/2014, N43°1.221' E12°52.732; Group IV: *Phragmitetum australis* Savič 1926, aquatic variant with *Myriophyllum spicatum*: Rel. 24, 14EVi08, Lake Vico, 09/09/2014, N42°18.11' E12°10.663, *Potamogeton perfoliatus* L. r; Rel. 18, 14EVi02, Lake Vico, 09/09/2014, N42°19.218' E12°11.643; Rel. 19, 14EVi03, Lake Vico, 09/09/2014, N42°19.295' E12°11.688; Rel. 23, 14EVi07, Lake Vico, 09/09/2014, N42°20.194' E12°10.084; Rel. 20, 14EVi04, Lake Vico, 09/09/2014, N42°19.473' E12°11.815; Rel. 22, 14EVi06, Lake Vico, 09/09/2014, N42°20.37' E12°9.498; Rel. 69, 14ECo05, Colfiorito Marsh, 30/09/2014, N43°1.364' E12°52.155; Rel. 67, 14ECo03, Colfiorito Marsh, 29/09/2014, N43°1.696' E12°52.58; Rel. 17, 14EVi01, Lake Vico, 09/09/2014, N42°19.149' E12°11.737, *Najas minor* All. +; Rel. 49, 14ETr01, Lake Trasimeno, 25/09/2014, N43°5.502' E12°10.676; Rel. 80, 14ECo16, Colfiorito Marsh, 29/09/2014, N43°1.674' E12°52.416, *Phalaris arundinacea* L. r, *Sorghum* sp. +; Rel. 70, 14ECo06, Colfiorito Marsh, 30/09/2014, N43°1.398' E12°52.165; Rel. 75, 14ECo11, Colfiorito Marsh, 29/09/2014, N43°1.417' E12°52.162; Rel. 77, 14ECo13, Colfiorito Marsh, 29/09/2014, N43°1.152' E12°52.437, *Agrostis stolonifera* L. r, *Equisetum palustre* L. r, *Poa trivialis* L. 1; Rel. 5, 14EFu05, Fucecchio Marsh, 03/09/2014, N43°48.234' E10°48.041; Rel. 8, 14EFu08, Fucecchio Marsh, 03/09/2014, N43°48.439' E10°48.629.

Tab. 5 - Group V: *Phragmitetum australis* Savič 1926, hygro-subnitrophilous variant with *Echinochloa crus-galli*: Rel. 11, 14EFu11, Fucecchio Marsh, 03/09/2014, N43°48.758' E10°48.658, *Lipandra polysperma* (L.) S. Fuentes, Uotila et Borsch +; Rel. 15, 14EFu15, Fucecchio Marsh, 03/09/2014, N43°48.721' E10°48.483; Rel. 61, 14ETr13, Lake Trasimeno, 25/09/2014, N43°11.425' E12°6.68, *Bolboschoenus* sp. +, *Mentha aquatica* L. +, *Veronica anagallis-aquatica* L. r, *Bidens tripartitus* L. 1, *Oxybasis urtica* (L.) S. Fuentes, Uotila et Borsch +, *Urtica dioica* L. 1, *Epilobium hirsutum* L. +, *Erigeron bonariensis* L. +, *Plantago major* L. r, *Rubus ulmifolius* Schott 1, *Samolus valerandi* L. +, *Symphotrichum squamatum* (Spreng.) G.L. Nesom 1; Rel. 43, 14ECh11, Lake Chiusi, 15/09/2014, N43°3.402' E11°58.738, *Bolboschoenus glaucus* (Lam.) S.G. Sm. 2, *Galium palustre* L. subsp. *elongatum* (C. Presl) Lange 1, *Lysimachia vulgaris* L. +, *Limniris pseudacorus* (L.) Fuss +, *Cirsium arvense* (L.) Scop. +, *Salix cinerea* L. +, *Solanum dulcamara* L. +, *Stellaria media* (L.) Vill. r; Rel. 9, 14EFu09, Fucecchio Marsh, 03/09/2014, N43°48.701' E10°48.718; Rel. 12, 14EFu12, Fucecchio Marsh, 03/09/2014, N43°48.778' E10°48.627.

Tab. 6 - Group VI: *Phragmitetum australis* Savič 1926, nitrophilous variant with *Urtica dioica*: Rel. 44, 14ECh12,

Lake Chiusi, 15/09/2014, N43°3.289' E11°56.803, *Lysimachia vulgaris* L. 1, *Populus nigra* L. 2, *Salix purpurea* L. +; Rel. 58, 14ETr10, Lake Trasimeno, 25/09/2014, N43°5.377' E12°4.909; Rel. 42, 14ECh10, Lake Chiusi, 15/09/2014, N43°3.891' E11°57.968; Rel. 33, 14ECh01, Lake Chiusi, 15/09/2014, N43°3.241' E11°57.031, *Teucrium scordium* L. 1; Rel. 35, 14ECh03, Lake Chiusi, 15/09/2014, N43°3.401' E11°57.022, *Bidens frondosus* L. r; Rel. 63, 14ETr15, Lake Trasimeno, 25/09/2014, N43°5.453' E12°4.729, *Phalaris arundinacea* L. +, *Galega officinalis* L. 2b, *Xanthium orientale* L. subsp. *italicum* (Moretti) Greuter 1, *Atriplex* cfr. *patula* L. +, *Bidens tripartitus* L. +, *Ranunculus repens* L. 2a, *Echinochloa crus-galli* (L.) P. Beauv. +, *Solanum nigrum* L. r; Rel. 34, 14ECh02, Lake Chiusi, 15/09/2014, N43°3.33' E11°56.97, *Thelypteris palustris* Schott 2, *Scrophularia auriculata* L. +, *Persicaria lapathifolia* (L.) Delarbre +; Rel. 64, 14ETr16, Lake Trasimeno, 25/09/2014, N43°5.898' E12°3.931, *Cyperus longus* L. +; Rel. 78, 14ECo14, Colfiorito Marsh, 29/09/2014, N43°1.086' E12°52.61, *Daucus carota* L. r, *Equisetum arvense* L. +; Rel. 59, 14ETr11, Lake Trasimeno, 25/09/2014, N43°4.934' E12°6.147, *Brachypodium rupestre* (Host) Roem. et Schult. 1, *Brachypodium sylvaticum* (Huds.) P. Beauv. +, *Convolvulus arvensis* L. +, *Solanum dulcamara* L. +; Rel. 57, 14ETr09, Lake Trasimeno, 25/09/2014, N43°5.865' E12°4.038; Rel. 62, 14ETr14, Lake Trasimeno, 25/09/2014, N43°4.906' E12°6.192, *Artemisia verlotiorum* Lamotte 1.

Tab. 7 - Group VII: *Phragmitetum australis* Savič 1926, species-poor variant with *Schoenoplectus lacustris*: Rel. 76, 14ECo12, Colfiorito Marsh, 29/09/2014, N43°1.127' E12°52.365, *Equisetum palustre* L. 1; Rel. 79, 14ECo15, Colfiorito Marsh, 29/09/2014, N43°1.72' E12°52.625, *Calystegia sepium* (L.) R. Br. 1; Rel. 68, 14ECo04, Colfiorito Marsh, 29/09/2014, N43°1.654' E12°52.637, *Mentha aquatica* L. +; Rel. 71, 14ECo07, Colfiorito Marsh, 30/09/2014, N43°1.658' E12°52.635; Rel. 72, 14ECo08, Colfiorito Marsh, 30/09/2014, N43°1.632' E12°52.398;

Tab. 8 - Group VIII: *Phragmitetum australis* Savič 1926, dry variant with *Juncus effusus*: Rel. 60, 14ETr12, Lake Trasimeno, 25/09/2014, N43°11.466' E12°6.498, *Cyperus longus* L. +, *Lemna minuta* Kunth 1, *Pulicaria dysenterica* (L.) Bernh. +, *Epilobium hirsutum* L. +; Rel. 29, 14EVi13, Lake Vico, 09/09/2014, N42°20.395' E12°8.965, *Stachys palustris* L. 1; Rel. 30, 14EVi14, Lake Vico, 09/09/2014, N42°20.375' E12°8.993; Rel. 27, 14EVi11, Lake Vico, 09/09/2014, N42°20.022' E12°9.136, *Mentha aquatica* L. 1, *Bidens tripartitus* L. r, *Juncus bufonius* L. +, *Ranunculus* cfr. *sardous* Crantz +, *Stellaria media* (L.) Vill. +; Rel. 32, 14EVi16, Lake Vico, 09/09/2014, N42°20.389' E12°9.728, *Alisma plantago-aquatica* L. +, *Carex pseudocyperus* L. +; Rel. 25, 14EVi09, Lake Vico, 09/09/2014, N42°20.398' E12°9.772; Rel. 28, 14EVi12, Lake Vico, 09/09/2014, N42°19.968' E12°9.149; Rel. 31, 14EVi15, Lake Vico, 09/09/2014, N42°20.397' E12°9.693.

Rupicolous habitats of interest for conservation in the central-southern Iberian peninsula

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Abstract

We studied the *Quercus rotundifolia* Lam. formations in the central-southern Iberian Peninsula, working particularly in areas in the Rondeño and Subbético biogeographical sectors. As a result we propose two new plant associations with an edaphoxeric character: *Bupleuro gibraltari-Quercetum rotundifoliae*; and *Junipero phoeniceae-Quercetum rotundifoliae* included in habitat 9340. In both formations there is a high number of endemic plants often found in habitat 8210 "Calcareous rocky slopes with chasmophytic vegetation" like *Antirrhinum graniticum*, *Antirrhinum onubense*, *Saxifraga reuteriana*, *Cerastium gibraltarium*.

Key words: conservation, endemics, phytosociology, *Quercus*, scree, woodlands.

Introduction

The study focuses on the central-southern Iberian Peninsula, characterised by its steep orography comprising large rocky crests, and exemplifying the typical character of the Betic and Sierra Morena mountain ranges. This orography, along with the increase in rainfall in mountain areas, causes certain areas to serve as refugia for endemic flora and for distinctive plant communities. In view of this, our aim is to highlight the important botanical and ecological value of these territories, which have been considered by some authors and government agencies as microreservations. In previous studies, Cano *et al.* (2015, 2016a, 2016b) highlighted the significance of these areas due to their high rate of endemics, and proposed formulas to establish the level of conservation. Cano-Ortiz *et al.* (2015) also noted the importance of these wild territories based on their endemic species and habitats, focusing the study fundamentally on the formations of *Juniperus oxycedrus* L. subsp. *badia* (H. Gay) Debeaux, and attributed the spread of these areas to anthropogenic action. Piñar Fuentes *et al.* (2012, 2013) and del Río *et al.* (2011) studied the diversity of geological substrates and climate trends, and found that rainfall is becoming increasingly concentrated in shorter periods of time, with most rain falling in September and October, and in only March and April in spring, which subjects the vegetation to water stress over long periods and threat-

ens the future survival of these edaphoxerophilous communities.

Other authors have recently carried out comparative analyses between the formations of *Quercus ilex* L. subsp. *ilex* in southern Italy and *Quercus rotundifolia* = *Quercus ilex* L. subsp. *ballota* (IK) in southern Spain (Musarella *et al.* 2012, 2013). A large number of relevés have previously been published on the woodlands in the southern Iberian Peninsula, and particularly holm oak woodlands (Rivas Goday *et al.*, 1959; Rivas-Martínez, 1975; Costa *et al.*, 1982; Costa *et al.*, 1987; Rivas-Martínez, 1987; Nieto *et al.*, 1988; Navarro, 1989; Cano & Valle, 1990; Galán de Mera, 1993; Madrona, 1994; García Fuentes, 1996; Gómez Mercado *et al.*, 2000; Alonso, 2002; Pinto Gomes & Paiva Ferreira, 2007; Molina *et al.*, 2008; Gómez Mercado, 2011), but always following the criterion of not separating the holm oak woodlands on rocky substrates and shallow soils with low water-retention capacity (edaphoxerophilous) from strictly climatophilous woodlands. Subsequently Rivas-Martínez (2011) established a climatophilous and edaphoxerophilous diagnosis for *Quercus rotundifolia* woodlands; and more recently Quinto Canas *et al.* (2012) and Pérez Latorre *et al.* (2015) mooted the possibility of considering these woodlands on scree. As a result of this, our aim is to justify the separation of the climatophilous and edaphoxerophilous aspects of these woodland formations.

The edaphoxerophilous woodland formations of

Quercus rotundifolia are well represented in several biogeographical units, and appear both in the central and more continentalised eastern zones, and even in more oceanic and siliceous territories. The study area focuses essentially on the Betic, Western Iberian Mediterranean and Central Iberian Mediterranean biogeographical provinces. All these zones share the fact that they contain small mountain chains formed by quartzite, granite, pre-Cambrian slate, limestone and limestone-dolomites with altitudes ranging between 280-1500 m. A study was made of 100 meteorological stations in the central-southern Iberian Peninsula, 29 of which have an ombrothermic index (I_o) between 3.6 and 6.3; meaning that this territory has a humid-humid ombrotype; while the 71 remaining stations have an I_o between 2.02 and 3.6, implying a dry ombrotype prevailing throughout the whole territory. The continentality values range between 10.8 for Santiago Do Cacem (Portugal) to 21.7 in Vianos (Albacete, Spain). All this explains the presence of a Mediterranean-pluviseasonal-oceanic macrobioclimate in the westernmost areas of the territory in the study, and a Mediterranean-pluviseasonal-continental macrobioclimate in the easternmost territories. The thermotype ranges from thermomediterranean belt in the warmer territories near the Guadalquivir river valley, and suprasediterranean belt on the rocky crests of the Subbética range. However the mean values for I_o (3.89), I_c (18.54) and I_{tc} (284) clearly signal the territorial dominance of the dry-subhumid ombrotype, the mesomediterranean thermotype and the Mediterranean-pluviseasonal-oceanic macrobioclimate; with strong evidence of the continental influence of the plateau in the easternmost mountain areas (Jaén, Ciudad Real and Toledo), where there are also indications of the Mediterranean-pluviseasonal-continental macrobioclimate (Cano-Ortiz et al., 2015).

Material and methods

In this work we used 209 relevés from the 13 associations described, including the *typus*, and the two new communities (McQr1-13: *Myrto communis-Quercetum rotundifoliae* Rivas Goday in Rivas Goday, Borja, Esteve, Rigual & Rivas-Martínez 1959; RlQr1-16: *Rubio longifoliae-Quercetum rotundifoliae* Costa, Peris & Figuerola 1982; PbQr1-14: *Pyro bourgaeanae-Quercetum rotundifoliae* Rivas-Martínez 1987; HhQr1-14: *Hedero helici-Quercetum rotundifoliae* Costa, Peris et Stübing 1987; BhQr1-12: *Berberido hispanicae-Quercetum rotundifoliae* Rivas-Martínez 1987; AdQr1-12: *Adenocarpus decorticans-Quercetum rotundifoliae* Rivas-Martínez 1987; AaQr1-20: *Asparago acutifoliae-Quercetum rotundifoliae* Rivas-Martínez, Cantó, Fernández-González & Sánchez-Mata in Rivas-Martínez et al. 2002; Qr1-15: *Quercetum*

rotundifoliae Br.-Bl. & O. Bolòs (1956) 1957; RoQr1-38: *Rhamno oleoidis-Quercetum rotundifoliae* Rivas-Martínez 2002; PcQr1-26: *Paeonio coriaceae-Quercetum rotundifoliae* Rivas-Martínez 1964; JlQr1-11: *Junipero lagunae-Quercetum rotundifoliae* Rivas Goday ex 1965 corr. Rivas-Martínez in Rivas-Martínez et al. 2011; BgQr1-8: *Bupleuro gibraltarici-Quercetum rotundifoliae* ass. nova hoc loco; JpQr1-13: *Junipero phoeniceae-Quercetum rotundifoliae* ass. nova hoc loco; RmQr1-7: *Rhamno myrtifoliae-Quercetum rotundifoliae* Pérez-Latorre, Soriguier-Solanas & Cabezudo 2015). The sampling was made over a broad territory in Spain, covering a variety of areas and taking relevés in *Quercus rotundifolia* formations.

These relevés were prepared following the phytosociological methodology of Braun-Blanquet as described in works such as Braun-Blanquet (1979), Géhu & Rivas-Martínez (1981) and Biondi (2011). A variety of statistical treatments were applied to establish a separation between *Quercus* communities. An Excel table was created with 209 relevés x 512 species, and an ordination cluster analysis was applied using the Bray-Curtis distance with Ward's agglomerative method. A DECORANA, RA and DCA multivariate ordination analysis was also applied. To explain the presence of tree communities of *Quercus rotundifolia* on scree and lithosoils in rainy environments we referred to the new ombro-edaphoxeric index proposed by Cano et al. (in press). To obtain information on the diversity of the different plant associations we applied Simpson's dominance indexes and Margalef's diversity index to the characteristic, companion and endemic species. Statistical analyses were made to establish the differences between Margalef's index of the different associations studied by ANOVA analysis of variance.

For the authorship and homogenisation of the taxa obtained from the bibliography we used the works of Flora Ibérica: Castroviejo et al. (eds.) (1986, 1990, 1993a, 1993b, 1997a, 1997b); Muñoz-Garmendia & Navarro (eds.) (1998); Talavera & Castroviejo (eds.) (1999, 2000) and Paiva et al. (eds.) (2001); Flora de Andalucía Occidental: Valdés et al. (eds.) (1987), Flora Europea: Tutin et al. (eds.) (1964-80).

For a better visualisation of how these communities of climatophilous holm oak woodlands are related to the edaphoxerophilous formations, we built a network of phytosociological placement for the associations in the study using free software (Pajek 4.10 <http://vlado.fmf.uni-lj.si/pub%20/networks/pajek/default.htm>). This allows us to see more clearly the floristic affinities between the relevés used. All the taxa present in fewer than 20% of the relevés in each community were eliminated from the table of floristic composition, in addition to taxa that were insignificant in the communities studied.

Results

Phytosociological analysis

In this study we analysed 12 plant associations from holm oak woodlands described previously by their authors (McQr1-13: *Myrto-Quercetum rotundifoliae*; RIQr1-16: *Rubio-Quercetum rotundifoliae*; PbQr1-14: *Pyro-Quercetum rotundifoliae*; HhQr1-14: *Hedero-Quercetum rotundifoliae*; BhQr1-12: *Berberido-Quercetum rotundifoliae*; AdQr1-12: *Adenocarpus-Quercetum rotundifoliae*; AsQr1-20: *Asparago-Quercetum rotundifoliae*; Qr1-15: *Quercetum rotundifoliae*; RoQr1-38: *Rhamno-Quercetum rotundifoliae*; PcQr1-26: *Paeonio-Quercetum rotundifoliae*; JIQr1-11: *Junipero-Quercetum rotundifoliae*), and two new communities of edaphoxerophilous stands (BgQr1-8: *Bupleuro gibraltari-Quercetum rotundifoliae*; JpQr1-13: *Junipero phoeniceae-Quercetum rotundifoliae*). The 12 holm oak woodland associations have been described by their authors as climatophilous woodlands, corresponding to thermo and supramedi-terranean thermotypes in dry-subhumid environments.

The formations in Grazalema and Cazorla grow in rainy environments on rocky limestone and limestone-dolomitic substrates. In the case of Grazalema, the edaphoxerophilous holm oak woodland is located in the thermomediterranean thermotype, and may extend to the lower mesomediterranean with high rainfall; it therefore has I_0 values of 10.68. In Cazorla the holm oak woodland is located in an I_0 of 8.42; between the lower humid to upper humid ombrotype according to Rivas-Martínez *et al.* (2002). These two edaphoxerophilous communities have a different floristic composition from the rest of the climatophilous woodlands of *Quercus rotundifolia*, and do not share the same ecological aspects and catenal contacts. These two communities are therefore perfectly separated in the different statistical analyses (Figs. 1, 2, 3). The new association *Bupleuro gibraltari-Quercetum rotundifoliae* ass. nova (Tab. 1, rels. 1-8, *typus* rel. 3) is characterised by the constant presence of *Bupleurum gibraltarium*, *Rhamnus myrtifolia* subsp. *iranzi*, *Hedera hibernica*, *Hedera maderensis* subsp. *iberica*, *Aristolochia baetica* and *Rhamnus oleoides* subsp. *oleoides*. From a catenal standpoint, these edaphoxerophilous holm oak woodlands contact with the pinsapo fir formations of *Paeonio broteroi-Abietetum pinsapo*. This community grows on marble limestone and compact limestone screes in a humid ombrotype in the westernmost territories of the Ronda biogeographic sector. This association is differentiated from *Rhamno myrtifoliae-Quercetum rotundifoliae*, described by Pérez Latorre *et al.* (2015) in the Almajarensis unit, which has greater continentality and a subhumid ombroclimate, and occupies the easternmost part of the Ronda sector on limestone dolomites and kakirites. The new lower thermo-mesomedi-

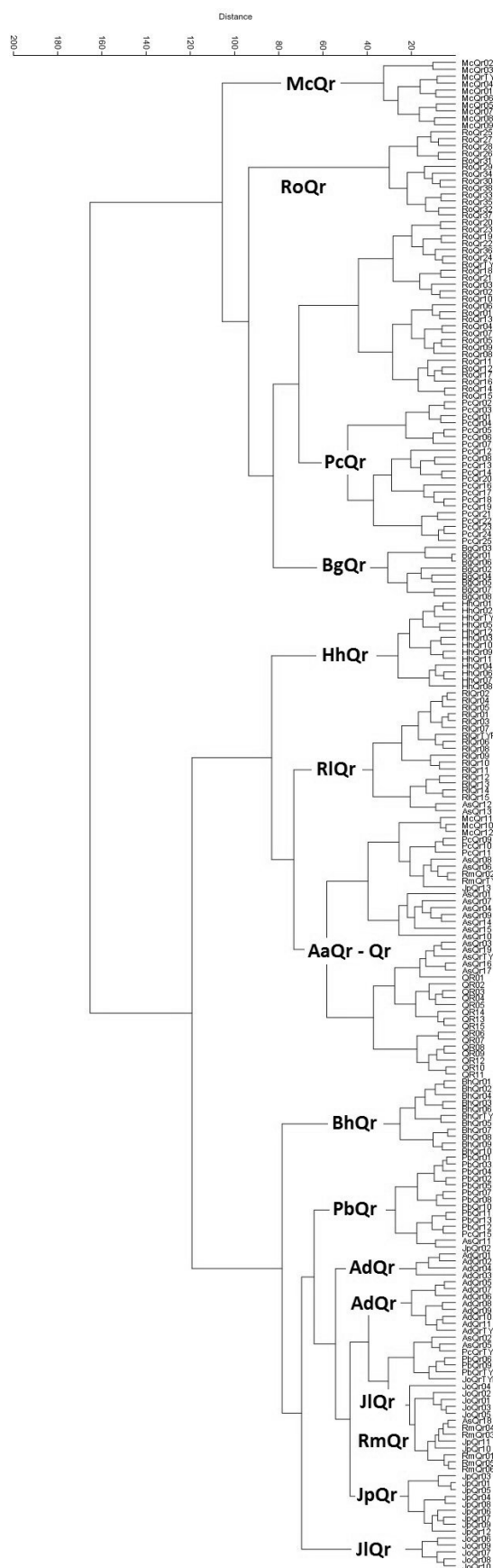


Fig. 1 - Cluster analysis of *Quercus rotundifolia* woodlands in the southern-central Iberian Peninsula.

Tab. 1 - Ass. *Junipero phoeniciae-Quercetum rotundifoliae* and *Bupleuro gibraltari-ci-Quercetum rotundifoliae*.

Order	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
Nº cluster	BgQr01	BgQr02	BgQr03*	BgQr04	BgQr05	BgQr06	BgQr07	BgQr08	JpQr01	JpQr02	JpQr03*	JpQr04	JpQr05	JpQr06	JpQr07	JpQr08	JpQr09	JpQr10	JpQr11	JpQr12	JpQr13	
Area in m ² l=100 m ²	10	6	6	6	6	6	6	6	6	6	5	6	6	6	4	10	10	4	1.6	10	1	
Altitude in m l=10 m	140	75	120	90	60	90	70	100	110	100	130	100	150	120	90	150	156	91	120	132	142	
Covering in %	70	75	60	60	60	70	80	75	75	75	75	70	65	60	75	75	75	75	80	55	80	
Orientation	E	E	SE	SW	E	NE	W	S	SW	SW	SE	N	NW	SE	NW	W	W	SE	S	SE	W	
Slope in %	60	70	70	50	80	80	15	80	30	25	50	60	45	30	15	60	20	45	18	70	80	
Average height of vegetation in m.	7	4	3.5	5	3	5	6	3.5	2.5	2.5	3	5	5	3	4	7	6	6.5	7	3	2	
Char. species																						
<i>Quercus rotundifolia</i> Lam.	3	4	3	3	3	3	4	4	4	5	5	5	4	4	5	5	5	3	4	3	4	
<i>Juniperus phoenicea</i> subsp. <i>phoenicea</i> L.	1	-	1	-	-	1	-	1	+	1	1	1	1	1	1	1	1	-	-	-	-	
<i>Juniperus oxycedrus</i> L. subsp. <i>badia</i> (H. Gay) Debeaux	2	-	1	-	-	2	+	1	1	2	2	-	2	3	2	2	2	-	-	-	-	
<i>Hedera hibernica</i> (G. Kirchn.) Bean	1	2	3	2	3	3	2	1	-	-	1	1	-	-	1	1	1	-	-	-	-	
<i>Rhamnus myrtifolia</i> subsp. <i>iranzoi</i> Rivas Mart. & J.M. Pizarro	1	1	2	1	-	1	-	1	-	-	-	-	-	2	-	-	-	-	+	2	+	
<i>Rubia peregrina</i> subsp. <i>pererina</i> L.	1	-	+	1	-	1	1	+	+	1	-	2	1	1	-	-	-	1	+	+	2	
<i>Paeonia broteri</i> Boiss. & Reut.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	
Diff. species of <i>Bupleuro gibraltari-ci-Quercetum Rotundifoliae</i>																						
<i>Bupleurum gibraltarium</i> Lam.	+	1	2	1	2	1	+	2	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Smilax aspera</i> var. <i>aspera</i> L.	-	-	1	1	+	-	2	1	-	-	-	2	-	-	-	-	-	-	-	-	-	
<i>Rhamnus oleoides</i> subsp. <i>oleoides</i> L.	-	+	-	1	1	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Arbutus unedo</i> L.	+	-	1	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Olea europea</i> var. <i>sylvestris</i> (Mill.) Lehr	-	-	-	2	3	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Aristolochia baetica</i> L.	-	1	1	2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Rhamnus velutinus</i> subsp. <i>velutinus</i> Boiss.	-	1	-	-	1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Asparagus albus</i> L.	-	-	-	+	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Hedera maderensis</i> subsp. <i>iberica</i> McAllister	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Doronicum plantagineum</i> L.	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Diff. species of <i>Junipero phoeniciae-Quercetum Rotundifoliae</i>																						
<i>Phillyrea latifolia</i> L.	-	-	-	-	-	-	+	-	-	-	3	2	2	-	3	-	-	1	-	-	-	
<i>Jasminum fruticans</i> L.	-	-	2	-	-	-	-	-	-	-	3	-	2	-	3	-	-	+	-	-	-	
<i>Acer monspessulanum</i> L.	-	-	-	-	-	-	-	-	-	1	-	1	-	1	1	-	-	+	-	-	-	
<i>Buxus sempervirens</i> L.	-	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	1	-	-	3	-	
Char. species of <i>Quercetalia ilicis</i>																						
<i>Viburnum tinus</i> L.	-	-	2	-	-	-	1	-	-	1	1	-	-	1	-	-	-	-	-	-	-	
<i>Ruscus aculeatus</i> L.	-	-	+	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	
<i>Juniperus oxycedrus</i> subsp. <i>oxycedrus</i> L.	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	+	-	-	
<i>Piptatherum paradoxum</i> (L.) P. Beauv.	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Char. species of <i>Pistacio lentiscis-Rhamnetalia alaterni</i>																						
<i>Euphorbia characias</i> L.	+	-	-	1	+	+	-	-	-	1	-	1	-	1	-	-	-	-	-	-	-	
<i>Pistacia terebinthus</i> L.	-	1	+	+	1	-	1	+	-	-	-	-	-	-	-	-	-	1	-	-	-	
<i>Ceratonia siliqua</i> L.	-	1	-	1	-	-	+	+	-	-	-	-	-	-	-	1	-	-	-	-	-	
<i>Pistacia lentiscus</i> L.	+	-	+	-	-	-	1	1	-	-	1	-	-	-	-	-	-	-	-	-	-	
<i>Bupleurum fruticosum</i> L.	-	-	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
<i>Quercus coccifera</i> L.	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	
Char. species of <i>Quercetalia ilicis</i>																						
<i>Lonicera implexa</i> Aiton	+	-	+	-	-	+	1	-	-	-	-	-	-	-	-	-	-	+	+	-	2	
<i>Carex halleriana</i> Asso	2	-	-	-	-	+	-	-	1	2	-	-	-	-	-	-	-	-	1	2	-	
<i>Daphne gnidium</i> L.	-	-	-	1	+	-	+	+	-	-	-	-	-	-	-	-	-	1	+	-	-	
<i>Asparagus acutifolius</i> L.	-	-	-	-	1	-	+	-	-	-	1	-	-	-	-	-	-	+	-	-	-	
<i>Rhamnus alaternus</i> L.	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	+	
Transgressive and mesophytic species of <i>Quercus-Fagetea</i> and <i>Salici-Populetea</i>																						
<i>Tamus communis</i> L.	-	-	+	+	-	-	+	+	-	-	-	1	-	-	-	-	-	-	-	-	-	
<i>Daphne laureola</i> L.	1	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	
<i>Hedera helix</i> L.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	+	-	-	
<i>Sorbus aria</i> (L.) Crantz	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Quercus faginea</i> subsp. <i>faginea</i> Lam.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	+	-	-	
<i>Helleborus foetidus</i> L. (Diff.)	-	-	-	-	-	-	-	-	-	1	-	1	-	1	-	-	-	+	1	-	-	
<i>Abies pinsapo</i> Boiss. (Terr.)	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Companions species																						
<i>Ceterach officinarum</i> subsp. <i>officinarum</i> Willd.	-	1	+	+	+	-	+	+	-	1	-	1	-	1	-	-	-	-	-	+	-	
<i>Aphyllanthus monspeliensis</i> L.	1	-	+	-	-	-	-	+	+	1	1	1	1	1	-	-	-	-	-	-	2	
<i>Ptilostemon hispanicus</i> (Lam.) Greuter	+	-	+	1	-	+	-	-	-	-	-	-	-	-	-	-	1	+	-	+	-	
<i>Thymus orospedanus</i> Villar	1	-	-	-	-	-	-	-	+	1	-	1	2	-	1	2	-	-	1	-	-	
<i>Echinopartum boissieri</i> (Spach) Rothm.	1	-	-	-	-	-	-	-	+	1	-	-	2	-	1	-	-	-	+	2	+	
<i>Chaenorhinum villosum</i> subsp. <i>granatense</i> (Willk.) Valdés	+	-	1	+	1	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	
<i>Berberis hispanica</i> Boiss. & Reut.	2	-	-	-	-	-	-	-	1	-	1	-	1	1	1	1	-	-	-	-	-	
<i>Asplenium trichomanes</i> L.	+	+	+	-	-	+	+	+	-	-	-	1	-	-	-	-	-	-	-	-	-	
<i>Linum suffruticosum</i> L.	+	1	+	-	-	+	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	
<i>Crataegus laciniata</i> Ucria	1	-	-	-	-	-	-	-	1	-	-	1	1	1	-	-	-	-	-	+	-	
<i>Lithodora fruticosa</i> (L.) Griseb.	1	-	-	-	-	-	-	-	+	1	-	-	1	-	-	1	-	-	-	-	-	
<i>Pinus nigra</i> subsp. <i>salzmannii</i> (Dunal) Franco	1	-	-	-	-	-	-	-	+	-	-	-	1	-	-	1	-	-	-	-	-	
<i>Biscutella valentina</i> (Loefl. ex L.) Heywood	1	-	-	-	-	-	-	-	+	1	-	-	2	-	-	-	-	-	-	-	-	
<i>Erinacea anthyllis</i> Link.	1	-	-	-	-	-	-	-	+	1	-	-	-	-	-	1	-	-	-	-	-	
<i>Rosa micrantha</i> Borrer ex Sm. in Sowerby	1	-	-	-	-	-	-	-	+	-	-	1	1	-	-	-	-	-	-	-	-	
<i>Cistus albidus</i> L.	-	-	+	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	+	
<i>Thapsia villosa</i> L.	-	-	+	+	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	
<i>Dactylis glomerata</i> L. subsp. <i>his</i>																						

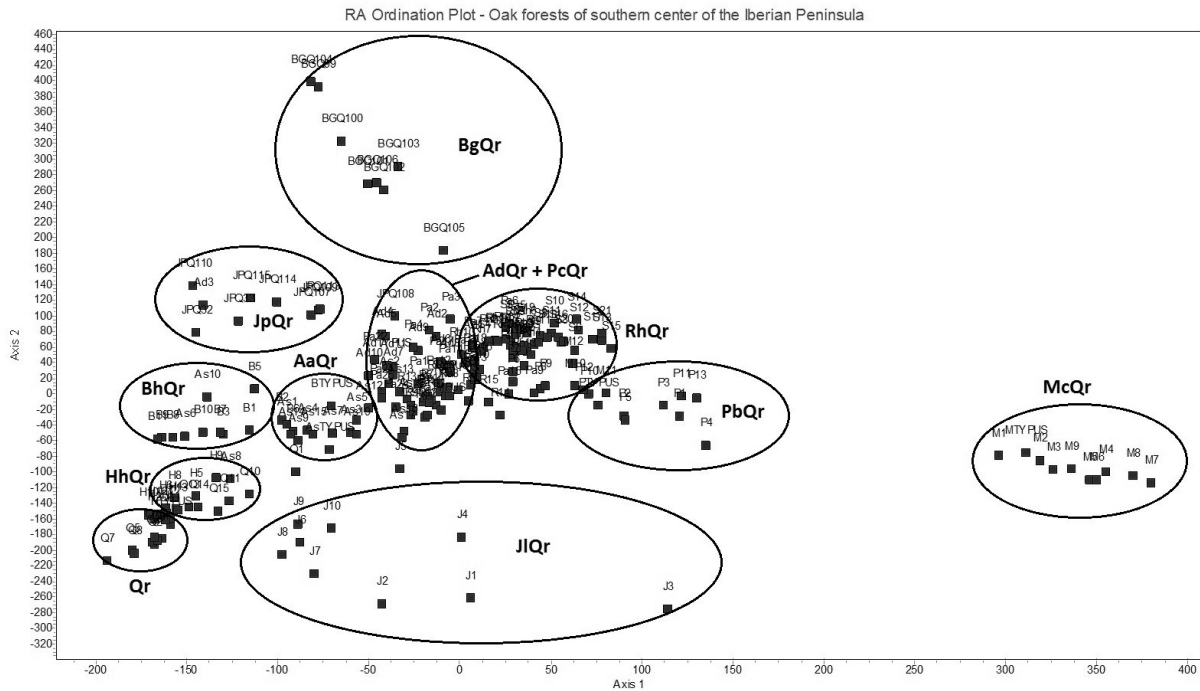


Fig. 3 - RA analysis of *Quercus rotundifolia* woodlands in the southern-central Iberian Peninsula.

terrestrial association *Bupleuro gibraltari-ci-Quercetum rotundifoliae* has significant floristic differences with *Rhamno myrtifoliae-Quercetum rotundifoliae*, due to the presence of *Bupleurum gibraltari-cum*, *Rhamnus myrtifolia* subsp. *iranzoi* (Rivas-Martínez & Pizarro, 2012), *Hedera maderensis* subsp. *iberica*, *Hedera iberica*, *Viburnum tinus* and *Aristolochia baetica*; and the absence of species such as *Pinus halepensis*, *Chamaerops humilis*, *Ephedra fragilis* and *Rhamnus myrtifolia* subsp. *myrtifolia*.

The high rainfall in the central part of the Subbética mountain ranges of Cazorla, Segura, Las Villas, Má-gina and Pandera caused by the funnel effect of the Guadalquivir valley and the screening effect from the Atlantic squalls, together with the steep orography and limestone and limestone-dolomitic substrates, leads to the growth of an edaphoxerophilous holm oak community which occupies all the rocky crests with a humid ombrotype in the meso and supramediterranean thermotype in the Subbético sector. These edaphoxerophilous formations, *Junipero phoeniceae-Quercetum rotundifoliae* (Tab. 1, rels. 9-21, *typus* rel. 11), are constituted and characterized by *Quercus rotundifolia*, *Juniperus oxycedrus* subsp. *badia*, *Hedera hibernica*, *Juniperus phoenicea*, *Acer monspessulanum*, *Buxus sempervirens* and *Helleborus foetius* like differential of *Quercus robur-Fagetea sylvatica* undergrowth, and are the basis for our proposal of the new association. It is differentiated from the previous one floristically, biogeographically and catenally, as in this case it contacts with the Baetic oak woodlands of *Viburno*

tini-Quercetum alpestris and *Berberido hispanicae-Quercetum alpestris*, and the maple woodlands of *Daphno latifoliae-Aceretum granatensis* (Rivas-Martínez et al., 2011). The floristic differentiation of these two new edaphoxerophilous associations from the rest of the climatophilous associations of *Quercus rotundifolia* can be clearly seen in Tab. 1.

The network analysis of phytosociological placement highlights how these associations are related to each other through their floristic composition. As can be seen in Fig. 4, the associations JpQr (*Junipero phoeniceae-Quercetum rotundifoliae*) and BgQr (*Bupleuro gibraltari-ci-Quercetum rotundifoliae*) are well characterised by their floristic composition, which is not found in any of the other associations; and there is a greater affinity between them than between the rest of the climatophilous communities. The connection with all the other climatophilous associations is established by means of the central nucleus of characteristic species common to all the communities belonging to *Quercetum ilicis*.

Diversity analysis

The analysis of total diversity (Fig. 5) shows a high value for both the relevés of McQr1-13: *Myrto communis-Quercetum rotundifoliae*, and the relevés of BgQr: *Bupleuro gibraltari-ci-Quercetum rotundifoliae*. The itemised application of Margalef's diversity index to characteristic, endemic and companion species (Tab. 2) reveals clear differences that do not agree with the total diversity values.

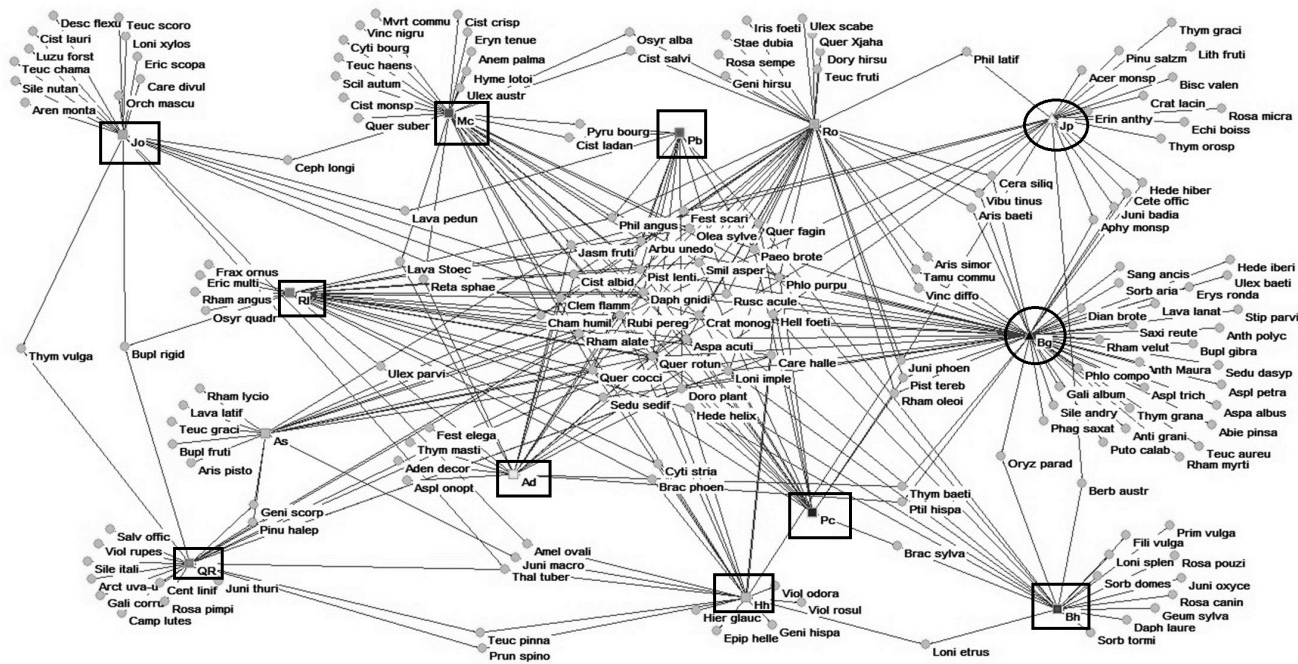


Fig. 4 - Network of the phytosociological placement of the associations studied.

In the case of the diversity of characteristic species, some associations stand out above the mean (McQr, RIQr, PbQr, AaQr, RoQr, PcQr, BgQr, JpQr), whereas all the other associations present low diversity values for characteristic species (Fig. 7). These differences are evident in the analysis of variance of Margalef's index, which shows significant differences between the associations as can be seen in Fig. 6.

The diversity analysis points to significant differences between the values for endemic species in the communities of AdQr, BgQr and JIQR. It is worth noting that the associations with a lower diversity of characteristic species have a greater diversity of companion species. This decline in the number of characteristic species and rise in companion species can be interpreted

as an association having a poor state of conservation, and if this process were to continue these associations would be substituted by others. There are six types of associations with zero diversity (Fig. 7) of endemic species; the two new types of edaphoxerophilous formations (BgQr, JpQr) have the maximum diversity, along with AdQr: *Adenocarpo decorticans-Quercetum rotundifoliae*, which is not an edaphoxerophilous

Tab. 2 - Diversity values for the associations studied considering characteristic, companion and endemic species.

	McQr	RIQr	PbQr	HhQr	BhQr	AdQr	AaQr	Qr	RoQr	PcQr	Rmr	JIQR	BgQr	JpQr
Margalef_C	5.53	6.28	5.14	3.10	2.78	2.98	5.79	3.31	6.96	6.07	1.95	3.71	6.04	4.70
Margalef_Co	3.35	2.66	1.89	2.73	1.46	2.73	5.64	3.13	1.14	3.80	2.62	2.98	3.24	3.38
Margalef_E	0.00	0.00	0.51	0.00	0.91	1.36	0.91	0.00	0.48	0.91	0.00	0.00	1.06	1.08

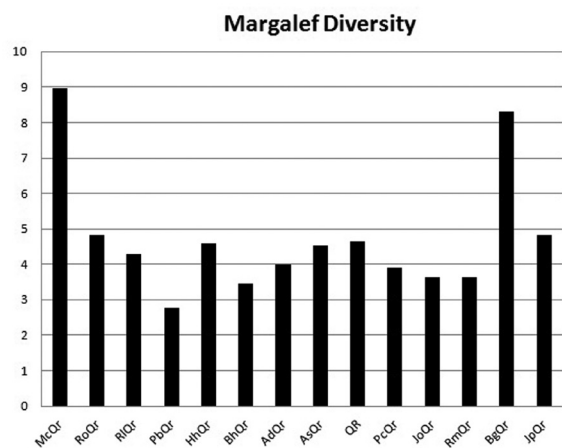


Fig. 5 - Total diversity for the associations studied.

Association	Value	Standard		
		Error	t	P value
AdQr	0,145	0,065	2,222	0,027
AsQr	0,063	0,069	0,919	0,359
BgQr	0,313	0,063	4,954	< 0,0001
BhQr	0,138	0,065	2,140	0,034
HhQr	-0,020	0,066	-0,300	0,764
JoQr	-0,018	0,065	-0,282	0,778
JpQr	0,424	0,066	6,453	< 0,0001
McQr	-0,020	0,066	-0,300	0,764
PbQr	-0,020	0,066	-0,309	0,758
PcQr	-0,027	0,071	-0,380	0,704
QR	-0,021	0,067	-0,317	0,752
RIQr	-0,022	0,067	-0,324	0,746
RmQr	-0,015	0,063	-0,234	0,815
RoQr	0,018	0,031	0,601	0,549

Fig. 6 - Analysis of the variance of Margalef diversity of endemic species, bold, significant associations to 95% significance.

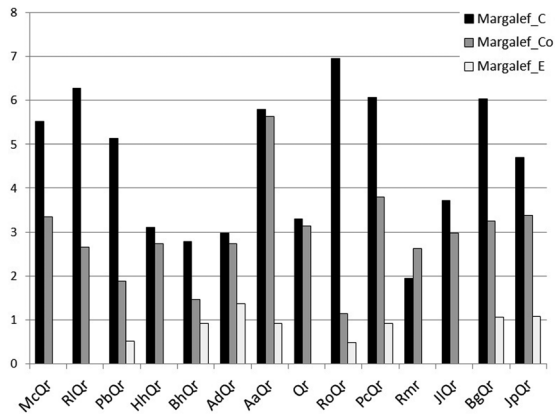


Fig. 7 - Diversity of companion, endemic and characteristics species.

community, but is in the Nevadense sector, one of the Spanish biogeographic units with the greatest rate of endemics. The association BgQr: *Bupleuro gibraltari-ci-Quercetum rotundifoliae* described for the Rondeño sector and JpQr: *Junipero phoeniceae-Quercetum rotundifoliae* for the Subbético sector have a high rate of endemic taxa due to the particular orography of the territories, which act as a species refugium. These sites are therefore of great interest for conservation. This conservation should be enacted through the Habitats Directive 92/43/EEC, whose Habitat 9340 includes all the *Quercus rotundifolia* associations in this study. In the case of holm oak woodlands on scree there are other habitats of interest such as 8210, which may be

located sporadically or not at all in climatophilous holm oak woodlands.

Discussion

Territories behave differently in response to the general climate, the type of substrate and the topography of the terrain. For this reason areas on rocky crests, even though they may be located in rainy environments and surrounded by climactic forests, behave differently from the territories around them due to their reduced capacity for retaining groundwater in the soils. In these circumstances islands evolve that may potentially contain edaphoserries, minoriserries and permaserries (Cano et al., 2016). All plant communities growing on rocky crests, steeply sloping areas with extreme gradients and similar environments are very significantly influenced by the soil, which conditions their existence. The whole territory has a particular type of substrate and an orography which determines its greater or lesser capacity to retain water. For this reason areas with high rainfall behave differently from the rest of the territory, which leads to the installation of edaphoxeric communities that contain a high number of endemic species. These associations should therefore not be considered as climatophilous and edaphoxerophilous concurrently (Rivas-Martínez et al., 2011), as they have different catenal contacts, ecology and flora (See Tab. 3); furthermore, these edaphoserries comprise a high number of endemisms, which are mostly included in EU priority habitats.

Tab. 3 - Analysis of the synthetic table of the different associations of holm oak woodlands in the center and south of the Iberian peninsula, which takes into account the characteristic species of class, endemic companions of interest and other companions with less interest.

Quercetea ilicis class species

- Asparagus acutifolius L.
- Daphne gnidium L.
- Rubia peregrina subsp. peregrina L.
- Smilax aspera L. var. aspera
- Rhamnus alaternus subsp. alaternus L.
- Carex halleriana Asso
- Lonicera implexa Aiton
- Clematis flammula L.
- Olea europaea L. var. sylvestris (Mill.) Lehr
- Lonicera etrusca G. Santi
- Vincetoxicum nigrum (L.) Moench
- Rubia peregrina subsp. longifolia (Poir.) O. Bolós
- Arisarum simorhinum Durieu.
- Colutea hispanica Talavera & Arista

Quercetalia ilicis order species

- Quercus rotundifolia Lam.
- Quercus coccifera L.
- Ruscus aculeatus L.
- Viburnum tinus L.
- Doronicum plantagineum L.
- Quercus suber L.
- Carex distachya Desf.
- Juniperus oxycedrus L. subsp. badia (H. Gay) Debeaux
- Bupleurum rigidum L.

	RmQr	McQr	PcQr	AdQr	HhQr	JpQr	RoQr	PbQr	AaQr	BgQr	BhQr	RlQr	JIQr	Qr
Asparagus acutifolius L.	.	IV	IV	III	I	I	IV	I	III	II	II	IV	I	.
Daphne gnidium L.	I	V	III	III	.	.	V	IV	IV	III	I	III	I	.
Rubia peregrina subsp. peregrina L.	III	.	III	IV	V	III	.	I	III	IV	.	.	III	V
Smilax aspera L. var. aspera	II	IV	III	.	.	I	V	II	I	IV	.	IV	.	.
Rhamnus alaternus subsp. alaternus L.	.	II	I	.	II	I	IV	I	II	.	.	IV	.	III
Carex halleriana Asso	.	.	I	.	V	II	I	.	II	I	I	II	.	V
Lonicera implexa Aiton	II	II	II	.	.	.	III	.	I	III	.	II	.	III
Clematis flammula L.	.	II	II	II	.	I	I	.	I	.	I	III	.	.
Olea europaea L. var. sylvestris (Mill.) Lehr	.	III	I	.	.	.	IV	II	.	III	.	III	.	.
Lonicera etrusca G. Santi	.	.	I	.	III	.	.	.	I	.	II	.	I	.
Vincetoxicum nigrum (L.) Moench	.	II	I	I	I	.
Rubia peregrina subsp. longifolia (Poir.) O. Bolós	.	IV	V	V	.	.
Arisarum simorhinum Durieu.	.	.	I	.	.	.	IV	.	.	II
Colutea hispanica Talavera & Arista	.	.	I	I
Quercus rotundifolia Lam.	V	V	IV	V	V	V	V	V	V	V	V	V	V	V
Quercus coccifera L.	III	III	II	.	II	II	IV	III	IV	.	.	V	.	V
Ruscus aculeatus L.	I	.	I	.	.	I	II	II	I	II	I	II	I	.
Viburnum tinus L.	.	I	I	.	.	II	I	I	I	II	.	I	.	.
Doronicum plantagineum L.	.	.	.	II	.	.	.	III	.	II	.	.	I	.
Quercus suber L.	.	IV	I
Carex distachya Desf.	I	I	.
Juniperus oxycedrus L. subsp. badia (H. Gay) Debeaux	V	.	.	.	IV
Bupleurum rigidum L.	I	I	I	II	II	III

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Appendix I: Sporadic species

Tab. 1 - rel. 2: 1 *Dianthus boissieri* Willk., + *Rumex scutatus* L., + *Allium roseum* L., + *Ferula communis* subsp. *catalaunica* (C. Vicioso) Sánchez Cuxart & Bernal, + *Centranthus macrosiphon* Boiss; rel. 3: + *Quercus faginea* subsp. *alpestris* (Boiss.) Maire, 1 *Thymus mastichina* subsp. *mastichina* (L.) L., + *Conopodium marianum* Lange, + *Centaurea pullata* subsp. *pullata* L., + *Sedum acre* L.; rel. 4: 1 *Brachypodium sylvaticum* var. *gaditanum* (Talavera) A. Galán de Mera, 1 *Brachypodium phoenicoides* (L.) Roem. & Schult., + *Ruta angustifolia* Pers., + *Brachypodium retusum* subsp. *boissieri*

(Nyman) Romero García, + *Linaria aeruginea* (Gouan) Cav.; rel. 5: + *Bryonia dioica* Jacq., + *Celtis australis* L., + *Campanula mollis* L.; rel. 7: 2 *Chamaerops humilis* L., + *Polypodium cambricum* L. subsp. *cambricum*; rel. 8: + *Euphorbia nicaeensis* subsp. *nicaeensis* All., + *Fumana thymifolia* (L.) Webb, + *Polygala rupestris* Pourr., + *Globularia alypum*, + *Cheilanthes maderensis* Lowe; rel. 12: 1 *Clematis flammula* L., 1 *Lonicera splendida* Boiss., 1 *Anarrhinum laxiflorum* Boiss., 1 *Antirrhinum tortuosum* Bosc ex Vent; rel. 14: 1 *Argyrolobium zanonii* (Turra) P.W. Ball; rel. 16: 1 *Rubus canescens* DC., 1 *Saxifraga erioblasta* Boiss. & Reut. in Boiss., 1 *Leopoldia comosa* (L.) Parl.; rel. 19: + *Digitalis obscura* L., + *Urginea maritima* (L.) Baker; rel. 20: 1 *Osyris alba* L., + *Phillyrea angustifolia* L., + *Salvia lavandulifolia* Vahl subsp. *lavandulifolia*, + *Asphodelus albus* subsp. *Albus* Mill., + *Cytisus scoparius* subsp. *reverchonii* (Degen & Hervier) Rivas Goday & Rivas Mart.; rel. 21: 2 *Bupleurum rigidum* L. subsp. *paniculatum* (Brot.) H. Wolff in Engl., + *Coronilla juncea* L., 1 *Festuca capillifolia* L.M. Dufour in Roem. & Schult., 1 *Thymus membranaceus* Boiss., 1 *Ulex parviflorus* Pourr.

Appendix II: Location of the relevés

Tab. 1 - Rels. 1 and 6, Sierra de Grazalema; rel. 2, Sendero el Santo (Mirador de Grazalema) (30S 278852/2927980); rel. 3, Sierra de Grazalema (30S 301232/4036702); rel. 4, Prox. Grazalema; rel. 5, Route "Molinos Harineros" (30S 301232/4036702); rel. 7, Prox Benamahoma-Grazalema (30S 303894/4035575); rel. 8, Benamahoma-Grazalema to 5 km of Grazalema (30S 302779/4035301); rels. 9 and 10, Pico Cabañas-Quesada (Cazorla, Jaén); rel. 11, Sierra Valdepeñas de Jaén; rel. 12, Burunchel (Cazorla, Jaén); rels. 13 and 14, "Cerrada del Utrero" (Cazorla, Jaén); rel. 15, Towards Pico Cabañas-Quesada; rel. 16, Descent from Pico Cabañas (30S 8499706/4188275) (Cazorla, Jaén); rel. 17, Ascent to the Cabañas from Tiscar (30S 0503102/4188033) (Cazorla, Jaén); rel. 18, Lower part "Cerrada de Utrero"; near the ponds of the Hydroelectric Power Plant (Cazorla, Jaén); rel. 19, Intersection Fuentasanta de Martos-Valdepeñas de Jaén; rel. 20, Sierra de Valdepeñas de Jaén (Jaén); rel. 21, Alto del Calabrial, Sierra de Gádor (Almería), taken from Giménez Luque (2000), Tab. 105, rel. 2, pag 302.

Anthropogenic impacts on riparian trees and shrubs in an eastern Mediterranean stream

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Abstract

This study contributes in listing and understanding the distribution of riparian species according to environmental and anthropogenic factors, recognizing the impact of these factors on biodiversity and tree growth and conducting an easy method for the assessment of habitat quality in a typical riparian ecosystem in the Eastern Mediterranean Basin. The methodology involved field assessment and the evaluation of riparian habitat quality by giving scores to different criteria, composing the habitat quality index. The results showed that river channel deviation is the most significant factor affecting riparian habitat quality. Non disturbed sites have significant higher scores, yet they are not classified as in natural conditions due to the effect of intrinsic environmental factors on habitat quality, namely bioclimatic conditions and river flow regime. An increase of biodiversity was recorded when habitat quality improved. Higher riparian habitat quality resulted in the presence of old growth trees, and climax species. This study allowed us to assess the requirements of major riparian species in terms of habitat quality, and to classify them based on their functional adaptation, in order to adopt appropriate ecosystem restoration and conservation plans.

Key words: anthropogenic impact, habitat quality index, Lebanon, riparian trees and shrubs.

Introduction

Riparian biotas are one of the most complex and diverse ecosystems; they constitute a transitional zone between aquatic and terrestrial biotas and include biotic and abiotic elements found near flowing water (Lowrance *et al.*, 1985; Klapproth, 1999). Based on flow characteristics, rivers are grouped into “perennial” with a permanent flow, “intermittent” rivers with temporary flow in the stream channel and “ephemeral” rivers that flow for short periods after rainfall or snowmelt (Zaimes, 2007).

Nonetheless, these riparian ecosystems are of the most fragile ecotones (Camporeale & Ridolfi, 2006); declines in biodiversity are far greater in riparian ecosystems than in terrestrial ecosystems (Sala *et al.*, 2000). The causes of disturbances vary from natural, such as floods and drought, or anthropogenic activities such as change of land use, water pollution, flow regulation, and dams construction. These disturbances can produce large-scale changes in the plant community and represent a persistent risk on the biodiversity and conservation of riparian ecosystems (Klapproth, 1999; Allan, 2004; Miserendino *et al.*, 2011). The Red List of European habitats cites that “temperate and boreal hardwood riparian woodland” habitat is endangered, while the “Mediterranean and Macaronesian riparian woodland” is vulnerable according to the IUCN red listing assessment categories. Natural systems modification (i.e. hydrology) and climate change are listed amongst the main threats (European Commission, 2016).

Flooding can influence a riparian habitat; during inundation, soil becomes anoxic. Floods also affect species composition by removing pre-existent seedlings and creating bare spaces for more adapted species (Hook 1984; Naiman & Décamps, 1997; Bendix & Hupp, 2000).

When drought occurs for a long period, river flow is hindered; the moistened areas of the channel bed are limited to a series of ponds leading to the encroachment of the riparian vegetation into the stream channel (Zaimes *et al.*, 2010). The removal of vegetation can modify flow characteristics, decrease infiltration and increase surface runoff (Walling & Fang, 2003; Miserendino *et al.*, 2011). It can also alter the functioning of river ecosystems by increasing river sediment loads that can lead to shoreline erosion (Dudgeon *et al.*, 2006) and thus an increase in nutrients leading to the overgrowth of algae, which alters habitat suitability for endemic species (Hall *et al.*, 2001; Miserendino *et al.*, 2011). Dams and channelization cause hydrologic regime alteration, disrupting riparian vegetation species composition and distribution, soil biogeochemistry, and sediment moisture retention (Naiman *et al.*, 1998). Since food, nutrients, and shelter for aquatic life are no longer available in the same quantity as before some vulnerable riparian species will be eliminated downstream of the dam (Griggs, 2009). Water quality is also affected by the construction of dams as purification process will break off (Govorushko, 2007).

Many researchers rely on field assessment which allows not only conducting an inventory of species and

estimating their growth and vitality, but also examines human disturbances in order to evaluate the habitat quality (Leonard *et al.*, 1992; Baker *et al.*, 2006; Barbour *et al.*, 1999; Tharme, 2003).

Munnee *et al.*, (2001) introduced the QBR index (“Qualitat del Bosc de Ribera” in English, “Riparian Forest Quality”) a mean to assess the riparian habitat quality. This index is divided into four sections: total vegetation cover, vegetation cover structure, cover quality, and river channel alteration. Each section has a series of criteria to be assessed. Further, the values of scores for all criteria in each section are summed. The total of the four sections gives the final QBR index for each plot ranging between zero and 100. The plots are distributed in five quality classes according to their QBR score (Tab. 1).

Although riparian zones require continuous assessment and monitoring and even though water is a limiting factor in semi-arid regions, Mediterranean rivers are among the most impounded in the World (Grantham *et al.*, 2010). Moreover, these areas are rarely studied in the Eastern Mediterranean Basin.

Lebanon has a typical Mediterranean climate with four dry months, during which the availability of water is limited. Therefore, riparian areas represent crucial ecosystems frequently affected by anthropogenic activities in addition to environmental factors. Until now, the ecological status of most rivers in Lebanon remains unstudied (Abboud *et al.*, 2012). Abi Saleh *et al.*, (1996) described some of the riparian vegetation series of Lebanon, and their distribution according to vegetation levels and the type of bedrock: the vegetation on limestone formed basically by *Platanus orientalis* L. and divided into lower level (near the riverbanks in the coastal areas where *Platanus orientalis* is usually accompanied by *Vitex agnus-castus* L., *Laurus nobilis* L., *Nerium oleander* L., *Salix alba* L.) and medium and upper level (where *Alnus orientalis* Decne., *Salix libani* Bornm. coexist).

The vegetation on sandstone is represented by *Rhododendron ponticum* var. *brachycarpum* Boiss. accompanied by *Alnus orientalis*, *Salix libani*, *Equisetum telmateia* Ehrh. and *Drosera rotundifolia* L.. Finally, the vegetation on talwegs is dominated by *Ostrya carpiniifolia* Scop. and *Fraxinus ornus* L.

However, habitat quality as affected by both the physical environment and anthropogenic activities was never assessed.

This investigation aims at understanding the effect of the environmental factors and human interventions on the riparian ecosystems woody species distribution, diversity and vitality in an East Mediterranean stream. The study contributes in inventorying the tree and shrub riparian species along Nahr Ibrahim River, understanding their distribution and response to environmental and anthropogenic factors. A simple meth-

Tab. 1 - Habitat quality classes according to QBR index.

Riparian habitat quality class	QBR
Riparian habitat in natural condition	>= 95
Some disturbance, good quality	75- 90
Important disturbance, fair quality	55-70
Strong alteration, poor quality	30- 50
Extreme degradation, bad quality	<= 25

odology is tested for the assessment of habitat quality in a typical riparian ecosystem in the Eastern Mediterranean Basin. These objectives aim to prioritize areas of intervention for biodiversity conservation and ecosystem restoration as well as provide solutions for water management policies.

Materials and methods

Study area

Nahr Ibrahim represents an important perennial stream flowing westward on the western slopes of Mount Lebanon, with a length of 30 Km and a basin surface of 330 km². The river watershed is mostly karstic, with few sandstone and basalt protuberance (Papazian, 1981). Nahr Ibrahim covers an altitudinal range from sea level to 1,980 m (Thermo Mediterranean to Mediterranean Montane) and has several tributaries, with many drying out in summer.

Nahr Ibrahim is renowned for its many cultural and historical values. With diversity in fauna and flora, this river was declared as an important natural site by the Ministry of Environment (MoE, 2010). Despite its cultural, historical and ecological values, Nahr Ibrahim River is threatened by different anthropogenic activities (industry, waste dumping, tourism, dam construction and agriculture expansion). Assessment and management of the river’s riparian zone, which demonstrates a co-evolution between natural and anthropogenic characteristics, should be taken into consideration (Abboud *et al.*, 2012).

Vegetation sampling

Twenty one plots were selected, covering all bioclimatic zones, soil and rock types, slope and flow regimes of the main river and its tributaries while taking into account accessibility to the plot, due to the steep slopes and dense vegetation of the valley. Ten sites out of twenty-one cross a perennial stream, five cross an intermittent stream, and six are located on ephemeral effluents.

The geographical coordinates of the sites are listed in the supplementary material table.

In order to assess the characteristics of the physical environment and to have an acceptable sampling size for trees (400 m²), we used plots of 40 m length along the river, with 10 m width from each river bank, on both side of the streams to cover all the riparian zone

in width.

The field survey was conducted between August 2014 and September 2015. In each plot, the physical environment characteristics; altitude, aspect and slope for both right and left side of the stream, soil and bedrock type and the number of dry months (NDM) were recorded. All trees and shrubs were identified and counted, including the non-riparian species. The canopy cover was estimated as well as shrub land coverage. The diameter at breast height (DBH) of trees was measured when DBH is superior to 10 cm. The type of disturbance (if present) was also described for each plot. The QBR index (Munnee *et al.*, 2001) was applied to all plots in order to evaluate the riparian habitat quality, and habitat quality was classified accordingly (Tab. 1).

Data analysis

We calculated the equivalent Hill number (H) of Shannon-Wiener diversity index (Burton *et al.*, 2005; Jost, 2006) to assess tree and shrub diversity in each plot, according to the following equation:

$$(H): \text{Exp}(-\sum p_i \log p_i)$$

Where, p_i is the proportion of individuals found in species i ($p_i = n_i/N$, where n_i is the number of individuals in species i and N is the total number of individuals in the community).

Statistical analysis allowed us to estimate the significance of Pearson’s correlation between QBR from one hand and DBH of trees and biodiversity indices from another hand. Analysis of Variance (using Tuckey’s test) was conducted to study the effect of vegetation level and river flow regime on the riparian habitat quality, and to study the relation between the type disturbance and QBR index, while chi-square test

enabled us to study the effect of river flow regime and vegetation level on the density of the canopy cover. These combined analyses revealed the effect of both environmental and anthropogenic factors on the quality of the riparian habitat.

Results

According to the QBR index, the quality of the riparian habitat in all plots was not satisfying: only 3 plots were in good condition with some disturbances and none in natural condition. Three plots exhibited extreme degradation, and almost half of them showed important disturbance or fair riparian habitat quality. The physical characteristics of the plots are resumed in table 2. Plots were almost evenly distributed amongst vegetation belts. Canopy cover varied between dense forests (> 70%) to scrubland (< 10%).

The analysis denoted a weak correlation between the QBR and Shannon equivalent number of woody species ($r = 0.491$, with P-value = 0.024), and an increase of Shannon equivalent number when QBR augmented (Fig. 1). The QBR index values increased in the perennial stream, when compared to intermittent and ephemeral tributaries; however the differences were not statistically significant (Tab. 3).

Chi-square tests showed that canopy cover varied significantly according to vegetation levels: discontinued canopy cover with less than 10% is noticed in Mediterranean Montane sites. Oppositely, all plots within the thermo-Mediterranean level have significantly higher canopy cover (>70%) than in other vegetation levels. Plots located in the Mesomediterranean and Supra-Mediterranean levels have intermediate canopy cover (Tab. 4).

Oppositely, canopy cover was not significantly af-

Tab. 2 - Sites characteristics.

Sites	Vegetation level	NDM	Water regime	Canopy cover (%)	QBR	Shannon index	Diversity (Hill)
Nahr Ibrahim 1	Thermo- Mediterranean	0	Perennial	>70	25	0	1
Nahr Ibrahim 2	Thermo-Mediterranean	0	Perennial	>70	60	0.605	1.831
Nahr Ibrahim 3	Thermo-Mediterranean	0	Perennial	>70	75	0.784	2.190
Amez	Montane Mediterranean	2	Intermittent	<10	35	0.401	1.493
Ain El Ghwaybe	Mesomediterranean	0	Perennial	40-70	65	0.663	1.941
Afka	Supra- Mediterranean	0	Perennial	40-70	70	0.614	1.848
Nabeh El Rouwes	Supra- Mediterranean	1	Intermittent	40-70	55	0.534	1.706
Mghayre	Supra- Mediterranean	2	Intermittent	40-70	50	0.832	2.298
Bir El Het	Thermo-Mediterranean	0	Perennial	>70	70	0.686	1.986
Ain Aalaa	Montane Mediterranean	3	Ephemeral	<10	20	0.215	1.240
Artaba Charbine	Montane Mediterranean	2	Intermittent	<10	25	0.674	1.962
Mazraat El Siyad	Supra- Mediterranean	2	Intermittent	>70	55	0.456	1.578
Abboud	Supra- Mediterranean	3	Ephemeral	40-70	65	0.383	1.467
Hdayne	Mesomediterranean	0	Perennial	40-70	75	0.735	2.085
Yanouh	Mesomediterranean	0	Perennial	10 40	85	0.231	1.260
Jannet Artaba	Mesomediterranean	0	Perennial	40-70	35	0.703	2.020
Chouwen	Thermo-Mediterranean	0	Perennial	>70	70	0.786	2.195
Yahchouch	Mesomediterranean	4	Ephemeral	40-70	65	0.92	2.509
Ain El Lebne	Montane Mediterranean	3	Ephemeral	<10	50	0.613	1.846
Akoura-Jord	Montane Mediterranean	3	Ephemeral	<10	35	0.566	1.761
Mchete	Supra- Mediterranean	4	Ephemeral	40-70	55	0.91	2.484

Tab. 3 - Effect of flow regime on QBR index (One-way ANOVA test).

River flow regime	N	Average QBR	Standard deviation
Perennial	10	63	18.738
Intermittent	5	44	13.416
Ephemeral	6	48.33	17.795

Tab. 4 - Effect of vegetation level and flow regime on canopy cover (Chi square test).

Vegetation Level	River Flow	Canopy cover				Exact P	Chi 2
		<10%	10-40%	40-70%	>70%		
Thermomed	Perennial	0	0	0	5	0.000***	37.33
	Mesomed	0	1	4	0		
	Supramed	0	1	4	1		
	Montanemed	5	0	0	0		
River Flow	Perennial	0	1	4	5	0.072	9.92
	Intermittent	2	1	1	1		
	Ephemeral	3	0	3	0		

ected by river flow regime, even if higher canopy cover was observed along perennial rivers, when compared to sites with ephemeral regime (Tab. 4).

However, when we associated the type of disturbances present in each plot to the respective QBR index values, we found that QBR values varied significantly with the main types of anthropogenic activities found along the river; channel modification is the principal disturbance affecting the QBR index negatively, followed by change of land use, waste dumping, embankments, and finally tree cutting, or grazing (Tab. 5).

Analysis of variance showed that *Salix libani* and *Tamarix smyrnensis* grow in sites with the lowest riparian habitat quality (respective QBR values 32.37 and 35.65) while *Salix acmophylla* and *Salix alba* are found in distorted sites (respective QBR values 40.74 and 53.93) and *Platanus orientalis* is found in fair quality classes (QBR value 62.6). *Nerium oleander* is found in a single site that is relatively undisturbed (QBR value 75) as shown in Table 6.

Tab. 5 - Effect of anthropogenic activities on QBR index (One-way ANOVA Duncan test).

Disturbance	N	QBR average values
Channel modification	3	36.67 ^a
Change of land use	8	46.25 ^{ab}
Solid and liquid waste dumping	5	55.00 ^{ab}
Embankments	4	57.50 ^{ab}
Tree cutting and grazing	2	62.50 ^{ab}
No disturbance	5	72.00 ^b

Tab. 6 - Species distribution according to QBR (One-way ANOVA Tuckey test).

Species	N	QBR average values
<i>Salix libani</i>	76	32.37 ^a
<i>Tamarix smyrnensis</i>	23	35.65 ^{ab}
<i>Salix acmophylla</i>	54	40.74 ^{abc}
<i>Salix alba</i>	248	53.93 ^{abcd}
<i>Platanus orientalis</i>	365	62.60 ^{bc}
<i>Nerium oleander</i>	10	75 ^c

Figure 2 shows that DBH increased with a higher QBR index values exhibiting a weak correlation ($r = 0.134$; $p\text{-value} = 0.001$). Moreover, DBH is highly correlated to canopy cover density, and negatively correlated to NDM (Tab. 7).

Discussion

The influence of the bioclimatic conditions on vegetation cover is pertinent; higher altitude exhibit a diminishing canopy cover, while lower altitudes have a denser canopy. The presence of optimal temperature and humidity are reflected in lush dense tree canopy. In fact, the Montane Mediterranean level is occupied by ephemeral streams bordered by shrubs dominated

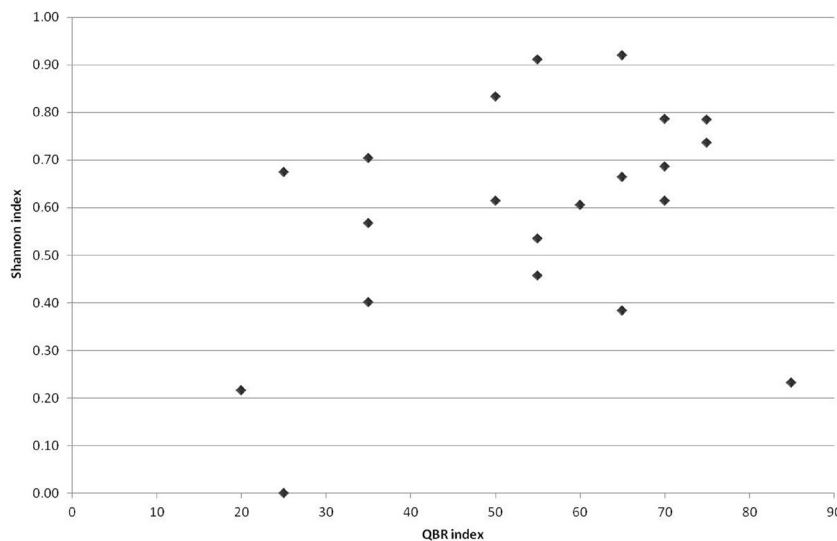


Fig. 1 - Shannon index distribution according to QBR index.

Tab. 7 - Effect of Canopy cover, QBR and number of dry month on diameter.

			NDM	Canopy cover	QBR
DBH	Pearson Correlation	1	-.088*	.152**	.134**
	Sig. (2-tailed)		0.023	0	0.001
	N	666	666	666	666

by *Salix libani* and *Rhododendron ponticum*, which explains the low percentage of canopy cover obtained in this level (Abi Saleh *et al.*, 1996). In addition, the lower section of the river crosses a canyon with steep slopes which favors the development of a dense canopy cover that is a continuum to the adjacent non riparian woods (Angiolini *et al.*, 2016). Hence, the riparian habitat quality is widely shaped by both canopy density and structure and by flow regime as expressed by Munnee *et al.* (2001), which explains why QBR values are significantly more affected by canopy cover that is part of the QBR test calculation rather than river flow regime.

Nonetheless, when we downscaled the analysis to the type of disturbances, it was pertinent that river channel deviation is the most significant factor affecting riparian habitat quality. In fact, channel modification may lead to river metamorphosis (a complete change of river's morphology) which explains its ponderous effect on riparian health (Gregory, 2006; Stella *et al.*, 2012). As a result, non-riparian species may replace riparian species, leading to habitat fragmentation or loss. Such diagnosis could not be captured by the QBR values as the canopy cover was estimated for all trees combined, regardless of the dominance or not of riparian tree species.

Tree cutting and grazing lead to localized bank and channel erosion and decrease in vegetation cover. This effect is reversible once the source of disturbance has

stopped, which explains its limited effect. Non disturbed sites have significant higher scores, yet they are not classified as in natural conditions due to the effect of intrinsic environmental factors on habitat quality (especially in high mountains with ephemeral streams).

Biodiversity of riparian species, as expressed by Shannon equivalent number is weakly correlated to riparian habitat quality. Although there was an evident increase of biodiversity when habitat quality improved, plots with lowest biodiversity are present in both plots with degraded or good habitat quality. The weak correlation of diversity and habitat quality was similarly denoted by Angiolini *et al.* (2016) who stressed on the effect of geomorphology and land use types on riparian plant communities assemblage. The bioclimatic conditions, geomorphology of the river, and the type of bedrock and the presence of alluvial soils largely affect spatial distribution of plant species in complex relation along with anthropogenic activities which rarely override natural factors (Engelhardt *et al.*, 2012; Nucci *et al.*, 2012; Gumiero *et al.*, 2015). Another explanation is that perennial species such as trees and shrubs are not promptly affected by habitat degradation and disturbances as herbaceous species, and if some tree species are vulnerable to disturbances, they are soon replaced by shrubs. The spatial and temporal variability of the water regime induces a greater adaptation of the riparian species. In such habitat, species that require near permanent moisture and those that adapt to greater drought cohabit, thus increasing the biodiversity of this habitat type (Nilsson & Svedmark, 2002; Gumiero *et al.*, 2015). Moreover, the presence of substantial contiguous forests within the river watershed increase the resilience of riparian vegetation to biodiversity degradation (Von Behren *et al.*, 2013).

For instance, *Salix acmophylla* and *Salix alba* were found in strongly distorted sites, this could be attrib-

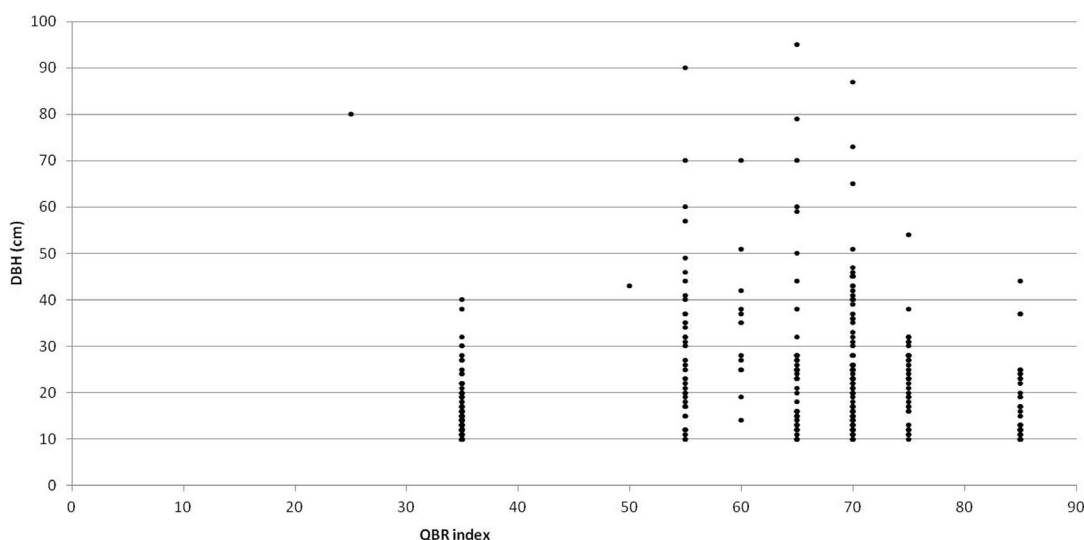


Fig. 2 - Diameter distribution according to QBR index.

uted to the fact that willow trees are pioneer species tolerating flood and low debris and colonizing remarkably affected sites through sexual and vegetative reproduction, which explains their presence in such sites (Friedman *et al.*, 2006). *Platanus orientalis* being a climax species in eastern Mediterranean streams of low and moderate altitude (Abi Saleh *et al.*, 1996) is more likely to be found in sites with lower disturbances and higher canopy cover. *Tamarix smyrnensis* is also known for its tolerance to drought (Bond *et al.*, 2008) which explains its presence in sites with low QBR.

When we investigated the effect of riparian habitat quality on vitality aspects for each species, such as DBH of tree species, and their regeneration rate, only weak correlation values were found. This could be explained by the low regeneration count (in nested plots of 20 m² in each plot) and the heterogeneous distribution of tree species in our sampled plots (for instance, species are not found in all plots). However, when we combined all species together, it was obvious that tree growth (illustrated by DBH) is related to habitat quality, canopy cover and negatively affected by river flow regime (Gumiero *et al.*, 2015). Higher riparian habitat quality resulted in the presence of old growth. Correspondently, this also explains that in such dense groves with old growth, regeneration rates are low, as observed during the survey (results not shown here).

Based on all results, we were able to classify riparian species according to their functional adaptations as per Naiman *et al.* (1998) (Tab. 8).

Conclusions

Our assessment proved that this riparian ecosystem is shaped by different environmental characteristics and anthropogenic activities. Channel modification has the strongest negative effect on riparian habitat quality. Nonetheless, tree and shrub riparian species show a relative resilience to the degradation of habitat quality, due to their intrinsic traits (like longevity), and the possibility to reiterate and reproduce asexually. This study allowed us to assess the requirements of major riparian species in terms of habitat quality, and to classify them based on their functional adaptation, in order to adopt appropriate ecosystem restoration and conservation plans.

The combined effect of anthropogenic impacts and natural distribution of riparian species along environmental gradients should be assessed in the future. Such investigation should be coupled with satellite imagery and remote sensing tools to assess riparian forest structure and composition and its degree of fragmentation. At a second stage, the riparian vegetation successions after disturbance should be considered in order to simulate the effect of climate change on riparian tree and shrub species distribution.

Tab. 8 - Riparian species classification according to environmental adaptations.

Species	Disturbance		
	Anthropogenic	Drought	Shade
<i>Salix libani</i>	Avoider	Resister	Avoider
<i>Tamarix smyrnensis</i>	Resister	Resister	Avoider
<i>Salix alba</i>	Invader, endurer, resister	Avoider	Resister
<i>Salix acmophylla</i>	Invader, endurer, resister	Avoider	Avoider
<i>Platanus orientalis</i>	Avoider	Resister	Resister

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Appendix I: Geographical coordinates (degree, decimal) and altitudes of the study sites.

Sites	X	Y	Altitude (m)
Nahr Ibrahim 1	34.059.167	35.639.500	10
Nahr Ibrahim 2	34.066.333	35.657.000	27
Nahr Ibrahim 3	34.082.833	35.683.500	109
Amez	34.051.333	35.797.500	1506
Ain el ghwaybe	34.085.167	35.878.500	962
Afka	34.072.167	35.887.333	1098
Akoura- nabe el rouwes	34.109.167	35.907.000	1256
Mghayre	34.114.000	35.884.833	1245
Bir el het	34.077.667	35.724.500	284
Ain aalaa	34.134.167	35.877.333	1705
Artaba charbine-nabee el jered	34.122.667	35.858.000	1710
Mazraat el siyad	34.113.333	35.863.333	1386
Abboud	34.101.000	35.860.333	1234
Hdayne	34.088.167	35.867.333	805
Yanouh	34.095.500	35.896.167	987
Jannet artaba	34.078.667	35.830.500	756
Chouwen	34.081.167	35.775.833	407
Yahchouch	34.063.000	35.741.500	594
Akoura- ain el lebne	34.146.500	35.934.833	1766
Akoura	34.119.000	35.925.000	1521
Mchete	34.047.667	35.753.167	973

Monitoring biodiversity patterns in three Mediterranean mountain pastures in the Pollino National Park (S-Italy)

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Abstract

We are monitoring three mountain herbaceous communities (a snow-bed community, a mesophile meadow and a xeric pasture) in the Pollino National Park (S-Apennines, Italy), in order to investigate relationships among climate, soil properties, vegetation structure (composition and spatial heterogeneity) and dynamics (seasonal patterns of species richness). To date, field data revealed striking differences of climate, soil and vegetation traits among the communities. The mesophile and the xeric community show a higher species richness than the snow-bed one, while the mesophile and the snow-bed community have a higher floristic relatedness. Species-area relationships evidence a substantial spatial heterogeneity in all sites. In two cases local plant diversity patterns appear significantly related to soil traits. Differences in seasonal biodiversity patterns are also evident, suggesting that contrasting mountain communities may be differently affected by climate warming components (*i.e.* summer drought vs. spring warming).

Key words: biodiversity, climate change, seasonal dynamics, soil analyses, species-area relationships, vegetation.

Introduction

Mountain areas had a central role in improving plant diversity in the Mediterranean global biodiversity hotspot (Thompson, 2005; Cañadas *et al.*, 2014). Currently, increasing global temperatures are promoting significant floristic rearrangement in European mountain floras (Gottfried *et al.*, 2012). The consequences of such changes on regional biodiversity vary across mountain ranges. The available data suggest that lower-latitude European mountains are subjected to pronounced biodiversity loss and turnover (Pauli *et al.*, 2012). Because the ecosystem functioning and services depend on biodiversity (Vogel *et al.*, 2012; Isbell *et al.*, 2015), understanding plant diversity patterns in these areas is crucial to preserve an unrepeatable biodiversity heritage under ongoing environmental changes.

An array of historical and contemporary drivers contributed to current plant diversity patterns in Mediterranean mountains. Over time, the Mediterranean rangelands acted as key refuge areas, corridors facilitating floristic exchanges, and sites for active plant speciation (Quézel, 1985). The plant migrations promoted by geological or climatic events strongly influenced the current Mediterranean mountain flora (Thompson, 2005). On the Apennine range, in the Italian Peninsula, this is proved by the numerous species shared with the eastern European regions and the alpine and northern European areas. Floristic components that represent

the legacy of plant migrations occurred respectively at the end of the Tertiary Age and during the ice ages of the Pleistocene (Pignatti, 1978). The long-history of human impact also promoted floristic diversification on Mediterranean mountains, due to the fragmentation of forest ecosystems and their replacement by species-rich open communities (Thompson, 2005).

The contemporary drivers of plant diversity on the Mediterranean mountains include regional and local variations of topography (*i.e.* elevation; ground surface), soil (*i.e.* bedrock, texture, drainage, and further physical and chemical features), and climate (*i.e.* temperature, rainfall, snow, and their seasonal patterns) (Thompson, 2005). Accordingly, variations of geomorphology and soil features were often related to mountain vegetation patterns at regional (Sebastià, 2004; Garcia-Palacios, 2012) and local (Miller & Alpert, 1984; Tillman & Olff, 1991; Gough *et al.* 2000; Gargano *et al.*, 2010) scales. Such relationships between abiotic ecosystem components and vegetation depend on the influence that the spatial heterogeneity of resource patterns exert on composition, productivity and functioning of plant assemblages (Bliss *et al.*, 2002; Maestre *et al.*, 2005). In addition, in Mediterranean climatic areas, vegetation shows also a substantial temporal heterogeneity. Because climate variations challenge the maintenance of species homeostasis (Bozinovic *et al.*, 2011), inter-seasonal climate differences are recognized as a major driver of plant selec-

tion in the Mediterranean region, where they induces contrasting limitations to plant growth around the year (*i.e.* winter cold stress vs. summer aridity and heat stress) (Mittrakos, 1980, 1982; Prentice *et al.*, 1992). As a consequence, Mediterranean plant communities reveal consistent biodiversity variations across seasons (Výlamazalová *et al.*, 2012).

Recent works showed that spatial ecosystem complexity can buffer the impact of anomalous environmental conditions as those promoted by the climate change (Fridley *et al.*, 2011; Godfree *et al.*, 2011). On the contrary, the possible relationships between ecosystem seasonal patterns and responsiveness to climate change remain rather unexplored. Yet, the seasonal timing of biodiversity expression may have important implications under the ongoing climate warming. Several components of climate warming, (*e.g.* increasing temperature, decreasing rainfall), may alter seasonal biological dynamics (*i.e.* plant growth and reproduction) which are crucial for the maintenance of community composition and structure (Parmesan & Yohe, 2003; Cleland *et al.*, 2007). The raising winter temperature induces an overall anticipation of spring phenological events (Fu *et al.*, 2014), with major effects on early flowering taxa (Fitter & Fitter, 2002). In the late growing season, climate warming increases the likelihood of events of unusually high temperature and drought (Orsenigo *et al.*, 2014), which may affect extent and timing of plant flowering, fruiting and recruitment (*e.g.* Abeli *et al.*, 2012; Mondoni *et al.*, 2012). Overall, in Mediterranean mountain ecosystems, increasing temperature may exacerbate the typical regime of summer stress, causing a higher risk of biodiversity loss (Pauli *et al.*, 2012). Then, the temporal timing of biodiversity expression in oro-mediterranean communities might make them differently exposed to early- or late- acting components of climate change.

We are investigating patterns of species richness and dynamics in high-mountain herbaceous communities of the Pollino Massif (Southern Apennine), in the heart of Mediterranean Basin. In this paper we characterize three different plant communities based on a) climate and soil properties, b) floristic composition and, finally c) spatial and seasonal biodiversity variations. The obtained results are used to address the following questions: 1) how do abiotic and biotic ecological components differ among the study communities? 2) how do spatial and temporal diversity patterns vary among ecologically different herbaceous communities? 3) do such different seasonal biodiversity patterns reflect a different sensitiveness to climate warming components?

Materials and methods

Study area and definition of sampling units

We carried out field work over two seasons (2012

and 2013) in three sites located in the Pollino National Park, a large protected area in the Southern Italy (Fig. 1). The landscape complexity of this territory allows the maintenance of an extraordinary plant diversity which is facing significant environmental variations due to land use (Gargano *et al.*, 2012) and climate changes (Federico *et al.*, 2009, 2010).

The first study site (Grande Porta del Pollino, hereafter GPP: N 39.92508°, E 16. 20968°; elevation: 1,900 m a.s.l) occurred in a large doline where prevalent vegetation was dominated by *Alopecurus alpinus* Vill., *Poa alpina* L., along with *Bellis pusilla* (N. Terracc.) Pignatti, *Crepis aurea* (L.) Cass. subsp. *glabrescens* (Caruel) Arcang. Such species are typical of the snow-bed fragments of the Southern Apennine, which are communities constituted by small herbs adapted to low temperature and longstanding snow cover (Tomasselli *et al.*, 2003; Gargano *et al.*, 2010). The second site (Piano di Ruggio, hereafter PR: N 39.91197°, E 16.13053°; elevation: 1570 m a.s.l) was mainly covered by mesophile grasslands characterized by numerous grasses (*e.g.* *Festuca microphylla* (St.-Yves ex Coste) Patzke, *Dactylis glomerata* L. subsp. *hispanica* (Roth.) Nyman, *Alopecurus pratensis* L., *Cynosurus cristatus* L.), together with rare endemic taxa (*i.e.* *Plantago media* L. subsp. *brutia* (Ten.) Arcang.), and species close to their range border (*i.e.* *Gentiana lutea* L.). On the calcareous Apennine such communities are



Fig. 1 - Location of the Pollino National Park (on the left in gray) with respect to the Italian peninsula, and position of the study sites within the protected area. GPP, Grande Porta del Pollino. PR, Piano di Ruggio. MS, Monte Serra.

confined to flat surfaces which allow the formation of rich loamy soils. The third study site (Mt. Serra, hereafter MS: N 39.84804°, E 16.09311°; elevation: 1,400 m a.s.l.) was a rocky pasture dominated by *Festuca circummediterranea* Patzke, *Poa bulbosa* L., *Bromus erectus* Huds., *Koeleria lobata* (M. Bieb.) Roem. & Schult., *Armeria canescens* (Host) Ebel. This vegetation type is very frequent on Apennines, especially on rocky surfaces showing little developed soils and high drainage. Due to the rocky nature of the topsoil, such pastures are structurally characterized by a discontinuous vegetation cover. All species names cited above agree with Conti *et al.* (2005).

To carry out field observations in absence of grazing, in each study stand we delimited a 10 x 10 m area by a fence. Within the fenced area we permanently defined 8 sampling units of 3 m² for investigating abiotic (*i.e.* climate and soil) and biotic (*i.e.* species occurrence and cover) traits.

Climate and soil sampling

We monitored soil temperature by using data-loggers (Hobo® Pendant UA-001-64) with a logging interval of 1 h. Data-loggers were positioned at a depth of 4 cm in the soil of each sampling unit. Since snow cover has an insulating effect which stabilizes soil temperature around 0.5 °C, we used temperature data to evaluate occurrence and length of periods with persistent snow cover. To this scope we considered the no. of consecutive days (at least 10 consecutive days) with 0 < average temperature < 1 °C.

To determine soil traits, we took small soil samples (1-2 Kg) representing all soil horizons in each sampling unit by using an Edelman-type manual auger with penetration capacity of 100-120 cm. We stored samples collected in the field in polyethylene bags for subsequent laboratory analyses. Here, we measured a set of variables accounting for textural and chemical soil traits. Soil texture (% of sand, silt, and clay) was analyzed by the Boyocous method. Organic matter [OM (g/Kg)] was derived from the content in organic carbon determined by the Walkley-Black method. Soil pH was determined by potentiometric measurement in soil-water suspension. Cation exchanged capacity [CEC (meq/g)] was calculated on the basis of the compulsive exchange between a barium-soil and magnesium sulfate solution; the amount of exchanged magnesium was measured by EDTA titration. The atomic spectrometric methods were used to determine base saturation: Magnesium [Mg (meq/g)] and Calcium [Ca (meq/g)] by atomic absorption; Potassium [K (meq/g)] and Sodium [Na (meq/g)] by atomic emission. All soil analyses were carried out according to the official guidelines MUACS (D.M. 11/05/1992 e D.M. 13/09/1999). Such analyses supported soil classification based on the WRB (FAO 2006) model. Soil

traits were evaluated for each soil horizon (A1, A2, Bw), and then averaged to have a unique value per sampling unit.

Vegetation sampling

To minimize interference with plant growth, we studied patterns of species occurrence and cover in each sampling unit by using the phytosociological approach. Hence, over two years, we recorded plant cover data according to the scale: r = rare species; += cover < 1%; 1 = 1 < cover < 20%; 2 = 20 < cover < 40%; 3 = 40 < cover < 60; 4 = 60 < cover < 80%; 5 = 80 < cover < 100%.

To investigate temporal variation of community composition and structure, we repeated field surveys at 10 days-intervals in each stand. Overall, we performed 408 surveys (GPP=104, PR=152, MS=152), which allowed to follow vegetation dynamics over the whole vegetative and blooming period.

Data analyses

Before performing statistical analyses, we transformed plant cover data following van der Maarel (1979). Therefore: r=1, +=2, 1=3, 2=5, 3=7, 4=8, 5=9. For each relevés, we calculated the Shannon diversity index by using the transformed cover values as a proxy of relative species importance (Mendes *et al.*, 2008; Hejda *et al.*, 2009). To explore the floristic relationships among the study sites we performed a cluster analysis on the relevés matrix (118 species x 408 relevés), by using the incremental sum of squares as classification method and the chord distance as similarity measure.

We evaluated spatial and temporal variations of floristic structure based on two biodiversity estimators: species richness (*SR*) and the Shannon diversity index (*H*). We studied spatial accumulation patterns of plant diversity by means of species-area relationships, using a linear regression model to check the strength of the relationships between surface sampled and *SR*. Local soil effects on plant diversity and abundance were evaluated by using the Pearson correlation to test relationships between soil traits, and between soil parameters and *H*.

Finally, to evaluate relationships between climate and community seasonal dynamics we derived the following three variables from hourly temperature data: the average of maximum daily temperatures of the decade preceding each survey (MT_{10}), the average of minimum daily temperatures of the decade preceding each survey (mT_{10}), and the average of mean daily temperatures of the decade preceding each survey (T_{10}). For each studied community we analyzed the strength of the effects of such temperature parameters on the seasonal variations of *H* by a multiple linear regression model. To avoid problems with the collinearity among predictors resulted from a first regression run, we followed a factor analysis approach to create a new set of

independent and uncorrelated variables fitting the response variable as well as the original predictors. This was done by performing principal component analysis (PCA) on standardized values of MT_{10} , mT_{10} and T_{10} . The standardization of each variable was done by subtracting mean from each value and then dividing it for standard deviation. In the last step, we ran a regression model in which the values of H were regressed against the factor scores produced by the factor analysis. In this analysis we applied a stepwise regression method to evaluate the relative contribution of the three original predictors in influencing the response variable.

Results

Climate and soil features

The average temperature measured over the period January 2012 - December 2013 clearly varied across the study sites (Tab. 1). We recorded a marked thermophily of MS site compared to PR and GPP. The differences in average temperature were also accompanied by diverse patterns of temperature dynamics across seasons. PR and, especially, GPP showed a long phase with stable temperature corresponding to a durable snow cover. This was followed by an abrupt temperature increase at the spring. In contrast, at MS the soil temperature varied greatly during the whole year, long periods with persistent snow cover were almost absent, and the temperature increased gradually from spring to the summer.

Field observations and subsequent laboratory analyses revealed also substantial pedological differences among the study sites (Tab. 1). MS showed a rendic soil where the thin litter layer had overlain directly the calcareous bedrock (this caused the extremely high value of organic matter reported in Tab. 1). We classified this soil as a Hyperumi-Rendzic-Leptosol, where the coarse texture was accompanied by high pH and calcium content. Instead, at PR and GPP the soil showed well structured horizons. The PR soil qualified as Haplic Phaeozem, and revealed a loamy texture with sub-neutral pH values, the amount of calcium resulted to be almost halved than MS. Finally, the GPP soil resulted to be a Haplic Cambisol (Humic Dystic), it was also characterized by a loamy texture, but organic matter, pH and calcium content were much lower than PR (Tab. 1). Textural traits appeared to be strongly related to all other soil features (Tab. 2), suggesting that soil structure is a relevant driver of other nutritional properties. As revealed by textural soil properties, the study sites defined a gradient whose extremes were GPP and MS (Fig. 2). At GPP the soil structure appeared to be unbalanced due to the large fraction of silt and clay and the poorness of sand (Fig. 2). Contrariwise, the MS soil was very poor in clay content (Fig. 2). Instead, due to the balanced soil texture, PR constituted

Tab. 1 - Average values \pm standard deviation of climate, soil, floristic traits and vegetation characterization of the three study sites.

Parameter	GPP	PR	MS
Altitude (a.s.l.)	1,900	1,570	1,400
Average temperature (°C)*	6.7 \pm 7.1	8.2 \pm 7.0	11.8 \pm 8.9
Minimum temperature (°C)*	4.5 \pm 4.6	5.8 \pm 5.0	8.0 \pm 6.5
Persistent snow cover (days)*, **	310	216	38
Sand (%)	7.2 \pm 2.4	27.4 \pm 3.7	37.5 \pm 18.2
Silt (%)	70.3 \pm 2.3	59.7 \pm 3.5	59.0 \pm 19.0
Clay (%)	22.5 \pm 1.8	13.0 \pm 1.5	3.5 \pm 2.3
pH	4.8 \pm 0.1	6.9 \pm 0.4	7.2 \pm 0.1
Organicmatter (g/Kg)	93.2 \pm 8.6	122.1 \pm 13.1	262.1 \pm 6.3
C/N	10.7 \pm 0.5	11.6 \pm 0.4	10.6 \pm 2.8
Ca (meq/g)	1.8 \pm 1.0	23 \pm 5.8	43.8 \pm 0.7
Speciesrichness	9.9 \pm 2.1	29.7 \pm 4.3	29.3 \pm 4.1
Shannon index (H)	2.153 \pm 0.2	3.289 \pm 0.2	3.269 \pm 0.1
Community physiognomy	Snow-bed	Mesophile meadow	Xeric pasture
Vegetation type	<i>Bellidi pusillae-Alopecuretum gerardii</i>	<i>Meo-Asphodeletum</i>	<i>Bromion erecti</i>

*Reference period 01 January 2012 - 31 December 2013. **Sum over 2 years.

Tab. 2 - Pearson's correlations among the soil traits evaluated in the study.

	SAND	SILT	CLAY	pH	OM	C/N	Ca
SAND	1.000	-0.884**	-0.726**	0.753**	0.669**	0.326	0.756**
SILT		1.000	0.320	-0.438*	-0.307	-0.478	-0.391
CLAY			1.000	-0.881**	-0.905**	0.043	-0.957**

**, the correlation is significant at the 0.01 level. *, the correlation is significant at the 0.05 level.

the central portion of the gradient (Fig. 2). Also, MS showed greater soil texture variations among sampled plots, while at GPP and PR soil structure showed less spatial heterogeneity (Fig. 2).

Floristic relationships and biodiversity patterns

Overall, the floristic surveys allowed us to identify 118 taxa. The three communities showed evident differences in terms of species richness, and were floristically well differentiated. Indeed, 84% of the recorded species resulted limited to a single site. As a consequence, the numerical classification assigned the relevés to three well defined clusters, each accounting for one of the study sites (Fig. 3). However, the cluster analysis evidenced that the PR and GPP communities had a higher floristic relatedness compared to MS (Fig. 3).

As far as biodiversity comparisons among stands are concerned, MS and PR showed similar levels of species richness, average amount of species per sampling unit, and H (Tab. 1), while GPP showed much lower values for all the biodiversity proxies (Tab. 1).

Spatial biodiversity patterns

Based on SAR models, the amount of species recorded significantly increased with the sampled area in all the study sites (Fig. 4). At GPP the levels of H were positively related to the percent of sand in the soil ($r = 0.78$; $P = 0.02$; $N = 8$), while at MS, H increased

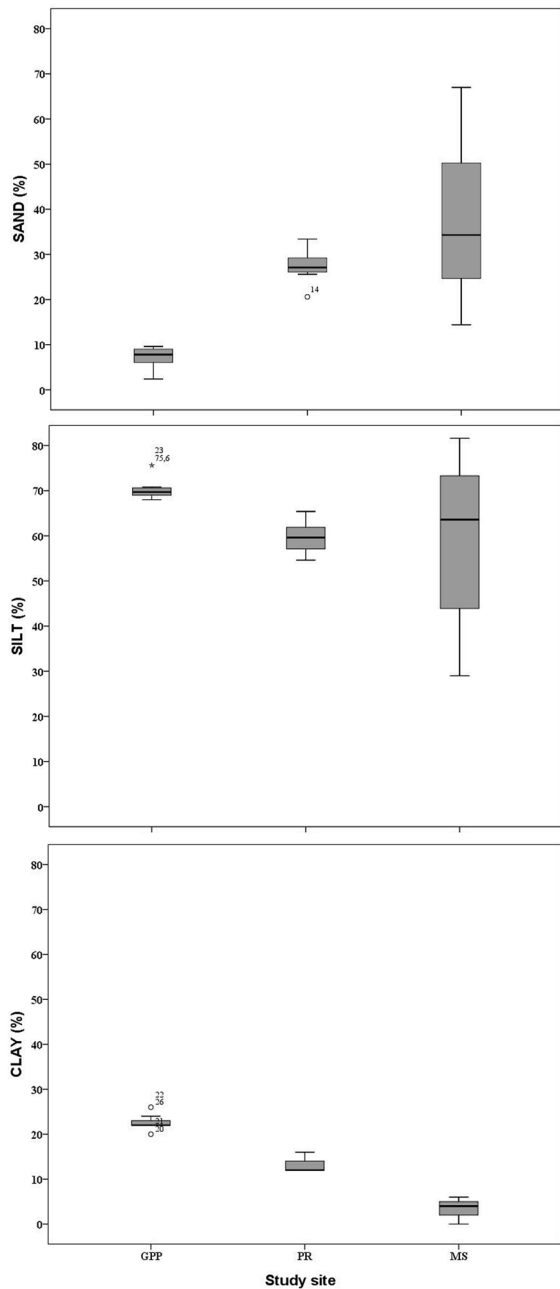


Fig. 2 - Boxplots showing soil texture features (%) of the three study sites. Vertical bars represent 95% of confidence interval.

with the fraction of clay ($r = 0.72$; $P = 0.04$; $N = 8$). In contrast, at PR we did not find relationships between biodiversity proxies and soil traits.

Seasonal biodiversity patterns

As far as the effects of the temperature on seasonal biodiversity variations are concerned (Fig. 4), the stepwise linear regression model (Tab. 3) showed no relationships at GPP ($R^2 = 0.013$; $P = 0.639$; $N = 104$). Instead, at both PR and MS sites we have found significant effects of temperature variations on the level of H (respectively $R^2 = 0.240$, $P < 0.001$, $N = 152$, and $R^2 =$

0.164, $P < 0.001$, $N = 152$). Based on the regression coefficients the highest effects were due to the second component score produced by the PCA carried out on the original temperature variables (Tab. 3). Since most of the variance observed on this component was due to mT_{10} (Tab. 4), the variation of minimum temperature during the days preceding our surveys resulted to have major consequences on biodiversity. Also, the regression coefficients showed in Tab. 3 indicated that such temperature variations induced contrasting effects on biodiversity in the two communities (positive at PR and negative at MS).

Discussion

Ecological relationships among communities

The spatial heterogeneity of the Mediterranean landscape contributes substantially in increasing local and overall plant diversity (Thompson, 2005). At regional scale the complexity of Mediterranean landscapes produces marked ecological differences which have a major role in structuring vegetation patterns. As evidenced by Sebastiá (2004), most of such differences are related to abiotic variations depending on topography and soil features. Accordingly, our study sites revealed striking differences in soil traits (*i.e.* texture, pH) which affect the nutritional status of the soil and, then, composition and structure of plant communities. Especially, soil texture appeared a powerful ecological predictor due to its relationships with other soil properties. Indeed, in spite of a comparable species

Tab. 3 - Stepwise regression coefficients for the factor scores obtained by the PCA carried out on standardized MT_{10} , mT_{10} and T_{10} variables. Dependent variable = H .

Site	Model	B	SDE	β	t	Sig.
GPP	1 (Constant)	0.3315	0.0048		69.382	0.000
	REGR factor score 1	-0.0037	0.0046	-0.0868	-0.799	0.426
	REGR factor score 2	0.0013	0.0061	0.0217	0.217	0.829
	REGR factor score 3	0.0067	0.0055	0.1328	1.230	0.222
PR	1 (Constant)	0.5174	0.0015		353.642	0.000
	REGR factor score 2	0.0115	0.0017	0.4739	6.591	0.000
	2 (Constant)	0.5164	0.0015		338.839	0.000
	REGR factor score 2	0.0110	0.0017	0.4536	6.340	0.000
	REGR factor score 3	0.0060	0.0027	0.1599	2.235	0.027
	MS	1 (Constant)	0.5166	0.0015		340.483
REGR factor score 2	-0.0063	0.0012	-0.3886	-5.166	0.000	
2 (Constant)	0.5174	0.0016		332.517	0.000	
REGR factor score 2	-0.0065	0.0012	-0.4062	-5.423	0.000	
REGR factor score 3	0.0022	0.0011	-0.1548	2.067	0.040	

Tab. 4 - Regression scores of the standardized variables on the three components extracted by PCA.

Variable	1	2	3
(z)MT ₁₀	0.895	0.443	0.051
(z)mT ₁₀	0.446	0.894	0.055
(z)T ₁₀	0.694	0.678	0.241

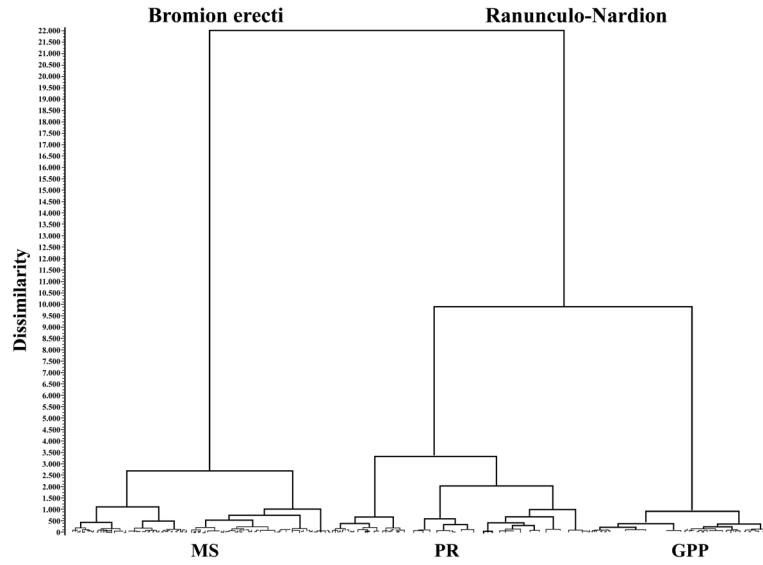


Fig. 3 - Numerical classification of the relevés taken in the three different communities.

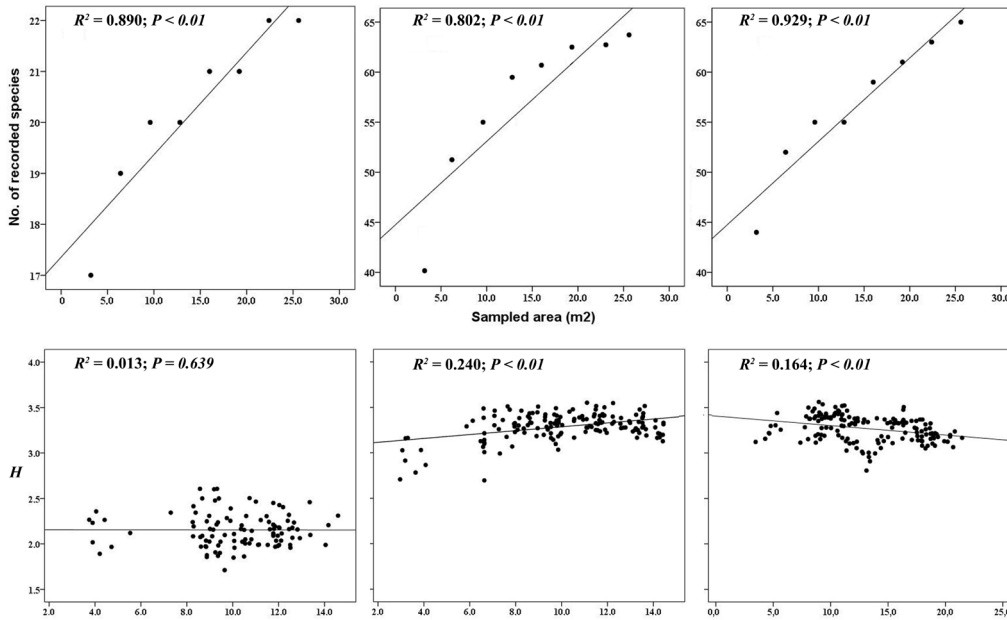


Fig. 4 - Species-area relationships (top) and seasonal biodiversity patterns (bottom) found in the three study communities. H , Shannon diversity index. mT_{10} , average of daily minimum temperatures recorded in the decade preceding each floristic survey.

richness (MS = 63 taxa, GPP+PR = 69 taxa), the classification of floristic data indicated a major differentiation (shared species < 12%) between the community established on soil with coarse texture (MS) and those found on loamy soils (PR and GPP). Since soil texture greatly influences the drainage rate of water provided by rainfall and snowmelt (Ellenberg, 1986), our findings fit the expectation that the shift from xeric to mesic conditions is the most relevant ecological transition in differentiating Mediterranean mountain grasslands (Sebastiá, 2004). In addition, the snow-bed community, floristically related to the PR mesic veg-

etation by the cluster analysis, resulted established on soil with a very low pH. Soil pH is a further important driver of soil nutrients availability (Tillmann & Olf, 1991; Pärter, 2002) and, in cold environments, its decrease is often paralleled by a loss of plant richness (e.g. Gough *et al.*, 2000). This was evident for the GPP community, and supports the hypothesis that the snowbed communities of the southern Apennine are an ecological variant of mesophile meadows, specialized to extreme mountain environments (Tomaselli *et al.*, 2003), where plant productivity is limited by severe climatic and soil constraints.

Spatial and seasonal ecological patterns within communities

The relative contribution of the different drivers of plant assemblage may depend on the considered scale (Rahel, 1990). Abiotic (climate, geomorphology, soil) and biotic (disturbance, biotic interactions) drivers may be more relevant at landscape and local scale, respectively (Sebastiá, 2004). Nonetheless, consistent relationships between soil and vegetation patterns may occur also at small spatial scales, depending on local geomorphology (*e.g.* Miller & Alpert, 1984; Gargano *et al.*, 2010), as well as on disturbance (Gigon & Leutert, 1996). All the plant communities considered in our work revealed considerable local variations, fitting the general expectation that the species encountered increase with the sampled area (Crawley & Harrald, 2001). This may result from the influence of soil heterogeneity on local plant patterns (Garcia-Palacios *et al.*, 2012). However, the importance of soil properties in differentiating plant communities may vary among phytocoenosis types (Critchley *et al.*, 2002). In our study the effects of local soil variations on biodiversity depended on the ecological context. The mesophile PR community revealed no relationships between local patterns of soil and plant diversity. Instead, according to a minimum law, small variations in the rarest soil component (% of clay and sand at MS and GPP respectively) promoted a significant rise of local plant diversity in the two ‘extreme sites’.

Climate variations may alter community composition because they induce physiological limitations on species (Bozinovic *et al.*, 2011). Mediterranean climate constrains vegetation by different patterns of seasonal stress (Mitrakos, 1980, 1982; Prentice *et al.*, 1992), which promote striking intra-annual variations of composition and structure in plant communities (Vylamazalová *et al.*, 2012). Our models indicated that extent and fashion of such variations vary substantially among ecologically different communities. As a probable consequence of the short season suitable to plant growth, we did not find significant dynamics in the coolest site. In contrast, species diversity increased with temperature in the mesic site, indicating that in this context winter and spring low temperatures may exert major constraints than summer aridity stress. Finally, in the site showing higher thermophily and soil poorness, the vegetation appeared to be more severely constrained by summer drought, as suggested by the declining diversity from spring to summer.

Conservation implications under the ongoing environmental changes

As known for the whole Mediterranean region (Bates *et al.*, 2008), precipitations are significantly decreasing in our study area (Federico *et al.*, 2009, 2010). The consequent sharpening of summer drought stress

can threaten ecological systems as the Mediterranean grasslands, which are very sensitive to rainfall variations (Figuroa & Davy, 1991). Under this scenario, the differences in biodiversity patterns described above have relevant implications. The maintenance of the community homeostasis under new climate scenarios requires the ability of the species to cope with a wide range of ecological conditions (Bozinovic *et al.*, 2011). The range of species tolerance is affected by high-level interactions, and it often augments in (functionally)-diverse communities (Urbina *et al.*, 2015). This furnishes a functional justification for the higher resilience of species-rich communities (Reich *et al.*, 2001; Tillman *et al.*, 2006). Hence, the scarce dynamics of the species-poor GPP vegetation provides a further evidence that highly specialized snow-bed communities tend to adopt a resistant behavior against novel environmental conditions (Baptist *et al.*, 2009). However, because high diversity is thought to increase productivity while it reduces resistance to drought stress (Vogel *et al.*, 2012), ongoing climate variations can severely constrain also species-rich communities as MS and PR. Especially, fertile grasslands can experience more severe drought constraints (Grime *et al.*, 2000). Therefore, a more pronounced productivity loss may occur in mesic communities like PR, which exhibit their maximum diversity in the summer. According to (Vogel *et al.*, 2012), the impact of increasing aridity on high-mountain grasslands may be further exacerbated by the Mediterranean pastoral systems, which favor an intensification of summer grazing pressure in high-mountain belts (Gargano *et al.*, 2012). Instead, xerophitic plant communities as MS appear less sensitive to increasing summer aridity, because in this period most of species have concluded their growth cycle. In this case, major concerns may originate from spring climate variations. Indeed, the anticipation of plant growth promoted by warmer spring temperature may increase the risk to undergo unfavorable climate events, especially in early flowering taxa (Fitter & Fitter, 2002). This may challenge the maintenance of composition and structure in communities characterized by early seasonal biodiversity peaks.

Our work suggests that oro-mediterranean herbaceous communities are suitable to investigate relationships among abiotic ecological traits, (seasonal) biodiversity patterns, and possible effects of climate change. A complex interplay of abiotic vegetation drivers (*i.e.* climate, soil) originates biodiversity patterns which can confer to communities a different sensitivity versus climate change components.

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Towards a global checklist of the world gypsophytes: a qualitative approach

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Abstract

Interest in plants growing on special substrates has increased considerably in recent years. The studies on halophytes (plants restricted to saline soils) and serpentinophytes (those restricted to ultramafic rocks) are good evidence of this trend. Research on the phenomenon of gypsophily has not been developed as widely as the other two before-mentioned fields, but important progress has been reached. The existence of a global database about gypsophytes and territories with gypsum substrates would imply a big leap in quality. The bibliographical criterium was selected in order to build this compilation as the only preliminary way to face the problem. According to the research about reviewing of distribution and ecology patterns of 209 *taxa*, it is possible to assure that there are gypsum outcrops in 112 countries. In 71 of those countries some clues point to the existence of a flora on gypsum, in which clear and undoubted cases of plant species directly related to gypsum soils in 53 countries have been found. These results show, on the one hand, the need of a deep correction to increase the data contained in previous reviews on gypsum outcrops distribution and, on the other hand, the diffusion of gypsophily phenomenon in plant species. Although the presence of genuinely gypsophyte *taxa* is much higher in dry climates, gypsum outcrops also show floristic peculiarities in wet climates, such as a refuge for xerothermophilic *taxa*, which clearly fits within the phenomenon of gypsum edaphism.

Key words: biogeography, gypsophily, gypsophile, gypsum, flora, soil.

Introduction

Having pointed out that early humans moved across the primordial landscape, they must have been keenly aware of spatial variation in the natural world (Lomolino, 2001); among those variations the fact that different types of soil can give different types of vegetation was probably perceived. Nevertheless, it was not until Theophrastus (371-287 BC), that the first explicit statement on this subject was delivered: “For it is the differences of soil which give a special character to the vegetation. (However the word “special” is used here in a somewhat wide sense)” (Theophrastus, 1999).

Since then, scientists have begun to figure out and explain the existence of peculiar floras associated with different sorts of soils (the “special characters” of Theophrastus), so much so that a number of fruitful studies have been carried out on saline and serpentine soils. Although to a lesser extent, the study about plant ecology on gypsum environments has acquired great significance, especially in recent years. The study of this edaphism is not a small incentive (Mota *et al.*, 2016): gypsum is a stressful environment that imposes severe restrictions on plants, where *taxa* are restricted to this type of substrate, with unique ecophysiological processes, some of them endemic of a region, or even rigorously local distributed species. Some of the gypsicolous *taxa* are endangered, thus they must also

be considered from the perspective of Conservation Biology. In addition, gypsum is an industrial mineral; this fact could jeopardise the conservation of biodiversity, whose preservation and exploitation interest need to be harmonised. This poses a serious challenge (Mota *et al.*, 2004, 2011).

In order to delve into this exciting topic and to be able to carry out studies that reach the gypsum outcrops worldwide, the global network of researchers GYPNET was constituted (<http://gypnet.weebly.com>). The first meeting took place in Aranjuez (Madrid, Spain), in 2016 March, and was conducted by Sara Palacio (Instituto Pirenaico de Ecología, Jaca) and Adrián Escudero (Rey Juan Carlos University, Madrid). This article, complemented by other published study – Mota *et al.* (2016) – are an attempt to reach that goal.

The development of this checklist is essential to understand the gypsophily phenomenon. It is true that previous lists elaborated by expert criteria cannot provide explanations about the mechanisms that make gypsophily possible, still they can be useful to put forward new hypotheses and try to verify (or not) the existing ones (Mota *et al.*, 2016).

The main aim of the present study is to show the preliminary results stemming from the elaboration of the global checklist of gypsophytes, beginning with the review, on the one hand, of the flora in those countries with gypsum substrates, and on the other, providing the examples of *taxa* restricted to such substrates.

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Results and Discussion

The results of the performed qualitative analysis are shown at the end of this section. Altogether, information about 112 countries (see Fig. 1), 208 cited *taxa* (145 gypsophytes, 146 gypsoclines and 10 suspicious species) has been sought. There are 71 countries with proven presence of gypsophyte species, without gypsophytes but with presence of gypsoclines, 53 countries are counted. There are three countries without presence of gypsophytes or gypsoclines, although with distinguishable traces in flora and vegetation. A total of 36 countries were able to catalogue as “with gypsum deposits but with no data about floristic or vegetation linked to gypsum”. This latter fact confirms that the articles from Scopus database directly referring to gypsophily or gypsophytes include ten countries (Mota *et al.*, 2016).

The first outcome, which can be extracted from the collected information, is that gypsum is an extremely abundant mineral, and that gypsum substrates are widespread in all continents, and under a number of different climatic conditions, and also that they occur in many geological and edaphic variants (soils with crusts, sands with no crusts, in gravel pits (“haswa”), mixed with clay, sands, marl, etc.). The general idea that gypsum soils are only present in arid and semi-arid climates, does not fit with reality, since there are outcrops in very rainy areas too.

A fact beyond all discussion is the wider presence of confined *taxa* to gypsum environments in arid climates than in humid climates. Undoubtedly, gypsophily is a more extended phenomenon than was believed until now. In this sense, the world map of gypsum soils and gypsum habitats published by Escudero *et al.* (2015) – based on the previous map by Verheyne & Boyadgiev (1997) – is a good starting point, but it could be increased with numerous regional additions. This is one of the basic tasks that GYPNET should encourage.

Moreover, according to the classic definition of edaphism collected in the botanical dictionary of Font Quer (1982), and adding in brackets the necessary hints to fit the case of gypsum, gypsum edaphism can be defined as: ‘the set of geobotanical phenomena dependent on the [gypsum] nature of soil’. Therefore, geobotanical phenomena linked to the gypsum nature of the soil can be described, which do not entail the confinement of endemic *taxa* to gypsum substrates. An example could be the fact that in humid zones gypsum substrates act as a refuge for xerophthermophilic *taxa*, which are absent (or almost) around gypsum outcrops. This occurs in areas as humid as the Alps, Germany, Poland, Nova Scotia (Canada) or the slopes of Sierra de Líbar (Spain). Another preconceived idea about gypsum outcrops, which should be delved into, is that such stressful conditions make gypsum soils largely

unsuitable for the growth of trees (Rivas-Martínez & Costa, 1970; Palacio *et al.*, 2007). According to this thesis, trees are absent or are very rare, and therefore, forests might not develop on gypsum. However, there are beech forests on gypsum soils in Germany (Schmid & Leuschner, 1998), holm-oaks and pinewoods in Sicily (Italy) on gypsum too (G. Spampinato, Mediterranean University of Reggio Calabria, com. pers.), etc. Denying the existence of forests on gypsum is difficult where there are gypsophyte trees (Poppendieck, 1981; Prado, 1998). Even in Spanish gypsum outcrops such assertion does not conform to reality (cf. Garrido-Becerra *et al.*, 2016). What is certain is that vegetation on gypsum is more scattered and sparse than that on other types of neighbouring substrates, less stressful environments for vegetation. That lower productivity in gypsum soils could be a generalizable feature at the global level. As an example, the formations of *Acacia-Commiphora* woodland in East Africa and the Horn of Africa: the description of the facies in this biome on gypsum includes a reference to a more sparse distribution, especially when the presence of endemic gypsophytes is mentioned (Friis *et al.*, 2016). In this regard, considering the lower competition with trees and shrubland, a greater insolation as one of the drivers of the presence of certain species in gypsum (Palacio *et al.*, 2007) is logical, although with the mentioned nuances.

The countries analyzed are detailed below.

Countries with proven presence of gypsophyte species

AFGHANISTAN. Numerous gypsophyte species can be found here, such as *Mattiastrum sessiliflorum* Rech.f. & Riedl, *Ferula oopoda* (Boiss. & Buhse) Boiss. or *Acanthophyllum spinosum* (Desf.) C.A. Mey. Numerous gypsoclines as *Atraphaxis spinosa* L. or *Ferula foetida* (Bunge) Regel, can also be cited (Sadat, 1989; Podlech, 2012).

ALGERIA. This country has important gypsum deposits, and gypsum soils take up 7,966 km² (FAO, 1990). Two components of the flora with gypsum affinity can be recognized in this country. On the one hand, in the north under Mediterranean climate, there are outcrops that share floristic elements with the Iberian Peninsula (e.g. *Helianthemum squamatum* (L.) Dum. Cours.), while the southern outcrops, in the desert climate of the Sahara, can host other gypsophytes such as *Echium suffruticosum* Baratte (Quézel & Santa 1962-1963).

ARGENTINA. This country has remarkable gypsiferous resources, widely distributed in the north. Outcrops with an associated gypsophile flora are found in the western part of the country, in the so-called Monte Desert biome – inner basins at the Andes foothills – where severe aridity conditions exist (Abraham *et al.* 2009, Devillers & Devillers-Terschuren 1996). As gypsophyte plants, *Halophytum ameghinoi* Speg., *Polygala*

hieronymi Chodat or *Atriplex argentina* Speg. have been cited (Devillers & Devillers-Terschuren 1996). In addition, the gypsophyte tree *Cochlospermum tetraporum* Hallier has been cited in the provinces of Salta and Jujuy, in the north, in the Chaco Serrano biome (Poppendieck 1981, Prado 1998). Knowing more data about the plant communities which this tree inhabits would be useful, since they probably contain other gypsophyte or gypsocline species.

ARMENIA. This country has several gypsum outcrops located on the slopes of its numerous mountain ranges. *Gypsophila aretioides* Boiss., *G. bicolor* (Freyn. & Sint.) Grossh. or *Lactuca takhtadzhianii* Sosn. can be cited as gypsophytes (Komarov, 1934-1964; Chemonics International Inc., 2000).

AUSTRALIA. The island-continent has significant deposits of gypsum in Victoria, South Australia, Western Australia, Northern Territory and New South Wales. Still, not in all of them is a characteristic flora and vegetation recognizable. This occurs mainly in the southwestern area, under a Mediterranean climate. In Western Australia, gypsum dunes around saline lakes are significant (Mattiske, 1995a,b; FloraBase, 2015); here, *taxa* such as *Goodenia gypsicola* Symon or *Conostephium pungens* G.J. Keighery are present. The state of Southern Australia also has gypsiferous deposits, with gypsophytes such as *Austrostipa geoffreyi* S.W.L. Jacobs & J. Everett or *Melaleuca nanophylla* Carrick. (Symon, 2007). In the limit of their range, gypsocline *taxa* can be found in New South Wales, as is the case of *Kippistia suaedifolia* F. Muell. (PlantNET, 2015).

AZERBAIJAN. Gypsum outcrops have been located both in Nakhchivan exclave and in the rest of the country. Azeri flora has gypsophytes as *Scrophularia thesioides* Boiss. & Buhse, or several species of *Astragalus* genus as *A. argyroides* G. Beck, *A. onobrychioides* M. Bieb. and *A. sanguinolentus* M. Bieb. (Komarov, 1934-1964).

BAHRAIN. This small island has much of its territory occupied by sabkha (see Khan *et al.*, 2006), some of which have a large gypsum presence (Bridges & Burhan, 1980). In these environments, gypsophytes species such as *Erodium glaucophyllum* (L.) L'Hér.; or halogypsophytes as *Panicum turgidum* Forssk. can be found (Al-Eisawi, 2003).

BOLIVIA. In the south of the country the “árbol del papel” is found (*Cochlospermum tetraporum*), a gypsophyte species already mentioned in the section dedicated to neighbouring Argentina (Poppendieck 1981, Prado 1998).

BOTSWANA. In the eastern and southeastern areas of the country, the presence of stenochoric *taxa* linked to gypsum soils has been reported, such as *Euphorbia venterii* L.C. Leach ex R.H. Archer & S. Carter or *Blepharis bainesii* S. Moore ex C.B. Clarke, and, therefore, they can be considered as gypsophytes (Setshogo, 2005).

CHAD. The north of the country is occupied by the Sahara desert, where gypsophytes as *Fagonia latifolia* Delile or *Helianthemum lippii* (L.) Dum. Cours. have been found, together with gypsoclines as *Zilla spinosa* (L.) Prantl or *Stipagrostis obtusa* (Delile) Nees (Le Houérou 1995; African Plant Database, 2015). In southern Bahr el Gazhal, deposits are located (Van Straaten, 2002), of which no floristic data are available. All these outcrops are low on gypsum purity; so that most of the consumed gypsum is imported (USGS, 2016).

CHINA. This country is the world's leading producer of gypsum (132,000 kt per year [Crangle, 2016]). Outcrops with an associated peculiar flora are located especially in the arid region of Xinjiang, where Wu *et al.* (1994-2013) reported the presence of three *taxa* linked to gypsum substrates: *Astragalus arpilobus* Kar. & Kir., *A. oxyglottis* M. Bieb. and *Lachnoloma lehmannii* Bunge. This assessment is likely to be short, since in Chinese territory some *taxa* are present which are referred to as gypsophytes in neighboring countries (*Sal-sola affinis* C.A. Mey. ex Schrenk and *Seseli aemulans* Popov) or, at least, as gypsoclines, *Nitraria sphaerocarpa* Maxim., *Ferula canescens* (Ledeb.) Ledeb., etc. (Komarov, 1934-1964).

CYPRUS. This island, along with Spain, are the only countries in Europe where gypsophilous vegetation is specifically protected (Anon., 2015). Some gypsophytes shared with other Middle East countries may be found, such as *Gypsophila linearifolia* (Fisch. & C.A. Mey.) Boiss. and *Herniaria hemistemon* J. Gay, or endemic elements as *Allium cypricum* subsp. *lefkarensis* (Brullo, Pavone & Salmeri) Christodolus & Hand or *Onobrychis venosa* Desv. (Euro+Med, 2006-2015; Hand, 2009).

EGYPT. The three deserts of the country have large gypsum outcrops (although often mixed with other materials such as sand and salts). In the Western Desert, the communities of *Resedeceae Randonia africana* Coss. monotypic genus (Abdallah, 1967; El Ghani & Marei, 2003) are remarkable. In the Eastern Desert there are also gypsophytes. Some of them show a rare biogeographic pattern, as *Moricandia sinaica* (Boiss.) Boiss., which reaches Somalia across the Red Sea coastline (African Plant Database, 2015). Some gypsophytes shared with Israel and neighbouring territories can be found in the Sinai Desert gypsum outcrops, such as *Haloxylon negevensis* (Iljin & Zohary) L. Boulou (Euro+Med, 2006-2015; Danin, 2015).

ETHIOPIA. This country has numerous deposits, totalling 1,423.4 km² of gypsum soils (FAO 1990). Outcrops with an associated peculiar flora are found mainly in the southeast of the country, in the Harerghe province, with gypsophytes as *Blepharis gypsophila* Thulin & Vollesen, *Kleinia gypsophila* J.-P. Lebrun & Stork, etc. (Thulin & Vollesen, 2015; Lebrun & Stork,

1989; African Plant Database, 2015). Yet, Ethiopian gypsum does not cease to amaze researchers, and recently two new *Nyctaginaceae* gypsophytes, endemic to Lele Hill (Bale province), have been discovered: *Commicarpus macrothamnus* Friis & O. Weber and *C. lelensis* Friis & Sebsebe (Friis *et al.*, 2016).

GEORGIA. This transcaucasian republic has gypsum outcrops where several gypsophyte species have been cited, such as *Scabiosa meskhetica* Schchian or *Tragopogon marginatus* Boiss. & Buhse (Komarov, 1934-1964). Some of these *taxa* are endangered, as is the case of *Salvia compar* (Wissjul.) Trautv. ex Sosn (Erstavii *et al.*, 2001). Curiously, despite having described this gypsophyte flora, gypsum production is scarce, only 0.13 kt per year (USGS, 2016).

IRAN. This country has numerous gypsum outcrops and is the second producer of this material with 22,000 kt per year (Crangle, 2016). In addition, it has a rich associated flora widely distributed in almost all its geography. The south-western zone is worth noting, especially Ilam and Lorestan provinces – e.g. *Euphorbia acanthodes* Akhani or *Ferula behboudiana* (Rech. f. & Esfand.) D.F.Chamb.– (Akhani, 2004); western portion of Semnan province – e.g. *Centaurea lachnopus* Rech.f. or *Acantholimon cymosum* Bunge – (Eftekhari & Asadi, 2001); and Yazd province – *Astragalus myrianthus* Beck or *Acanthophyllum sordidum* Bunge ex Boiss.– (Tilaki *et al.*, 2011); as well as the northeastern area of the country, *i.e.* Khorasan province and surrounding areas – e.g. *Limonium sogdianum* (Pop.) Ikonn.-Gal. or *Onobrychis meshhedensis* (Širj. & Rech.) Ranjbar – (Eftekhari & Asadi, 2001). In addition, numerous gypsocline *taxa* from various typologies have been reported, such as halogypsophytes (e.g. *Hypocylix kernerii* Wol), calcareousgypsophytes (e.g. *Paracaryum luristanicum* Nábělek), gypsoserpentinophytes (e.g. *Astragalus assadii* Maassoumi & Podl.), etc. (Akhani & Ghorbanli, 1993; Akhani, 2004; Podlech, 1988).

IRAQ. The area of gypsiferous soils in Iraq was estimated at 12,503,000 ha or 28.6% of all the agricultural soils in the country, (or 6.7% of all gypsiferous soils in the world). Gypsiferous soils are well represented in the Euphrates river basin in Iraq (Jaradat, 2002) and especially in the area of Mosul (Guest, 1966), whose "Mosul Marble" has been well-known since Assyrian times. Among the Iraqi gypsophytes, *Kaviria azaurena* (Mouterde) Sukhor., *Astragalus akhanii* Podlech, *A. baba-alliar* Parsa, etc. can be mentioned (Guest, 1966; Townsend & Guest, 1974).

ISRAEL. The Flora Palaestina (Zohary & Feinbrum-Dothan, 1966-1986) mentions ten plants considered gypsophytes growing in Israeli territories (e.g. *Haloxylon negevensis* (Iljin & Zohary) L. Boullos, *Fagonia mollis* Delile, *Nasturtiopsis coronopifolia* subsp. *arabica* (Boiss.) Greuter & Burdet). Probably, this is

a conservative estimation and the Israeli gypsophyte flora might be larger, given the presence in Israel of *taxa* recognized as gypsophytes in other territories, such as *Echium suffruticosum*, *Helianthemum kahiricum* Delile, etc. (Danin, 2015).

ITALY. It is the second European producer and the tenth one in the world, with 4,100 kt per year (USGS, 2016). Although there are small outcrops across almost the whole country (Antolini, 1984), large deposits are located in Emilia Romagna, Sicily and, to a lesser extent, in Calabria. A number of gypsophytes grow on the Sicilian outcrops, such as *Brassica villosa* subsp. *tinei* (Lojac.) Raimondo & Mazzola, *Gypsophila arrostii* Guss., etc. (Gianguzzi *et al.*, 2010). Emilia Romagna gypsum outcrops are very interesting from the bryophytes point of view (Aleffi *et al.*, 2014), but as far as vascular plants are concerned, there is no component of gypsophytes, with the exception of *Allosorus persicus* (Bory) Christenh. This rupicolous fern has its only Italian population on gypsum rocks, thus it can be considered as a gypsophyte at national level (Pignatti, 1982). Recently, a group of researchers has developed a project to elaborate and analyse the checklist of Italian gypsophytes (Musarella *et al.*, 2016).

JORDAN. Some gypsiferous outcrops can be found in this country's deserts, although gypsum soils only occupy 0.8% of the national territory (FAO, 1990). In these zones, gypsophyte *taxa* such as *Herniaria hemistemon* or *Erodium glaucophyllum* can be found, as well as gypsocline species as *Nitraria retusa* (Forsk.) Asch. or *Limonium pruinosum* Kuntze (Zohary & Feinbrum-Dothan 1966-1986; Al-Eisawi, 1996; Muselman, 2007).

KAZAKHSTAN. The country that occupies most of the Aral-Caspian depression, host in their deserts and steppes numerous gypsophytes, such as *Anabasis gypsicola* Iljin, *Ferula eremophila* Korovin, *Gypsophila aulieatensis* B. Fedtsch., etc. (Komarov, 1934-1964).

KUWAIT. This small emirate has 354 km² of gypsum soils (FAO, 1990). In this country, some gypsophytes as *Diplotaxis harra* (Forsk.) Boiss. subsp. *harra* or *Herniaria hemistemon* have been mentioned, as well as several gypsocline *taxa* as *Haloxylon salicornicum* (Moq.) Bunge ex Boiss (Daoud & Al-Rawi 1985).

KYRGYZSTAN. This country is largely occupied by the great mountain range of the Tian Shan ("Heaven mountains") and, along with others (Turkmenistan, Uzbekistan and Tajikistan), shows the presence of gypsum and gypsophytes not only in the lowlands, but also in the middle mountains, under very severe climatic conditions (especially in winter). Among the Kyrgyz gypsophyte flora, *Ferula gypsacea* Korovin, *Haplophyllum leptomerum* Lincz.& Vved., *Centaurea lasiopoda* Popov & Kult., etc. can be cited (Komarov, 1934-1964). Curiously, mining production is scarce:

0.113 kt per year (USGS, 2016).

LEBANON. Compared to the rest of the Middle East countries, the Lebanese gypsum substrates are scarce, and their production is only 0.11 kt per year (USGS, 2016). In this territory, *Astragalus guttatus* Banks & Sol. and *Prosopis farcta* (Banks & Sol.) J.F. Macbr. have been cited, which are *taxa* respectively considered as gypsophyte and gypsocline in other countries (Lebanon FLORA, 2016).

LIBYA. This country has deposits where gypsophytes as *Henophyton deserti* (Coss. & Durieu) Coss. & Durieu, *Diploptaxis harra* subsp. *harra*, *Helianthemum lippii*, etc. can be found (Euro+Med, 2006-2015, African Plant Database, 2015).

MAURITANIA. The deposits of this country are located in sabkha (Van Straaten, 2002). In such outcrops, gypsophytes as *Randonia africana*, *Fagonia latifolia* can be found; or halogypsophytes as *Frankenia thymifolia* Desf. (Le Houérou, 1995; African Plant Database, 2015).

MEXICO. This country has large deposits and is the seventh producer in the world, 5,300 kt per year (USGS, 2016). Floristically, the most interesting outcrops are located in the north of the country, in several states under desert climate as Baja California – e.g. *Fagonia palmeri* Vasey & Rose – (Felger et al., 2012); Coahuila, – e.g. *Marshalljohnstonia gypsophila* Henrickson, or *Dyssodia gypsophila* B.L. Turner – (Henrickson, 1976; Powell & Turner, 1977); Chihuahua – e.g. *Tiquilia hispidissima* (Torr. & A. Gray) A.T. Richardson, or *Machaeranthera gypsophila* B.L. Turner – (Moore & Jansen, 2006; Anon., 1993–2015); Durango – e.g. *Dicranocarpus parviflorus* (A. Gray) A. Gray, or *Xanthisma gypsophilum* (B.L. Turner) D.R. Morgan & R.L. Hartm. – (Moore & Jansen, 2006); Nuevo León – e.g. *Erigeron gypsoverus* G.L. Nesom, or *Verbisina hintoniarum* B.L. Turner – (Nesom, 2007; Hinton & Turner, 2007) and San Luís Potosí – e.g. *Pellea ribae* A. Mend. & Windham, or *Sisyrinchium zamudioi* Espejo, López-Ferr. & Ceja – (Mendoza et al., 2001; Espejo et al., 1998). In the south, on the Pacific coast, frequently in rupicolous or subrupicolous positions among tropical deciduous forests, the outcrops of Colima are worth mentioning, with species such as *Graptopetalum glasii* Acevedo-Rosas & Cházaro or *Pinguicula colimensis* McVaugh & Mickel – which are also in Michoacán and Guerrero – (Acevedo-Rosas & Cházaro, 2003; Mc Vaugh & Mickel, 1963); Jalisco, with the gypsocline *Agave gypsophila* Gentry – which is also present in Colima and Guerrero – (García-Mendoza, 2003); and Oaxaca, with species as *Pinguicula medusina* Zamudio & Studnička (Zamudio & Studnicka, 2000). In the state of Campeche, in the middle of a tropical forest landscape, the outcrop of the Zohlaguna plateau is found, where there are some *taxa* listed as gypsophytes – e.g. *Holographis websteri* T.F. Daniels or *Lantana dwyeriana* Moldenke – even, *taxa*

that could be called ‘hyrogypsophytes’ in “bajos” communities—e.g. *Fuirena stephani* Ramos & Diego – (Martínez & Galindo-Leal, 2002). The Mexican gypsum flora characterization began with the work of J. Valdés and H. Flores-Olvera (UNAM, México D.F.) (see Sánchez del Pino, 1999). Currently, H. Flores-Olvera, H. Otorena (also from UNAM) and M.J. Moore (Oberlin College, OH, USA) are elaborating a checklist of Mexican gypsophytes.

MOLDOVA. This small country has gypsum areas on its border with Ukraine, where kastification phenomena exist (Klimchouk, 1996). The presence of the gypsophyte *Gypsophila collina* Ser. and the gypsocline *Astragalus exscapus* L. subsp. *exscapus* have been cited (Euro+Med, 2006-2015).

MONGOLIA. In the Gobi desert, there are considerable gypsum deposits, where several gypsocline *taxa* are present, some of them endemic, such as *Allium mongolicum* Regel, and *Cleome gobica* Grub. (Virtual Flora of Mongolia, 2015; Chimed-Ochir et al., 2010).

MOROCCO (including Western Sahara). Like its neighbor Algeria, there are two components in the flora with gypsum affinity: on the one hand, a northern-Mediterranean part, where there are outcrops with either endemic elements (e.g. *Perralderia pau* Font Quer), or species in common with the Iberian Peninsula (e.g. *Lepidium subulatum* L.) (Deil, 2005; Fennane & Ibn Tattou, 2005). On the other, a Saharan component, with a desert climate where gypsophytes as *Fagonia latifolia* – or, according to Le Houérou (1995) halogypsophytes as *Suaeda vermiculata* Forssk. ex J.F. Gmel. – can be found (African Plant Database, 2015).

NAMIBIA. In the Namib desert, there is abundant gypsum mainly on the surface, which is presented as gypsum-enriched sands and gravels; these gypsum-bearing sands grading between 30-90% gypsum (Van Straaten, 2002). The *Arthraerua leubnitziae* (Kuntze) Schinz endemism can be cited as a gypsophyte; and *Tetraena stapfii* (Schinz) Beier & Thulin, and *Salsola tuberculata* (Fenzl ex Moq.) Schinz (Van Rooyen, 2010) as halogypsophytes. Furthermore, the communities on shallow soils (partly quartz covered) above gypsum crusts, which are integrated by *Brownanthus pubescens* (N.E. Br. ex C. A. Maass) Bullock, *Ruschia inconspicua* L. Bolus and *Portulacaria pygmaea* Pillans, deserve being mentioned (Jürgens, 2004).

NIGER. In the country of the Ténéré Desert, considerable gypsum reserves can be found, especially in the I-n-Aridal area (Van Straaten, 2002), which are not significantly exploited (USGS, 2016). In this republic there are typically Saharan gypsophytes, like *Fagonia latifolia* or *Stipagrostis ciliata* (Desf.) De Winter, as well as gypsoclines as *Panicum turgidum* (Le Houérou, 1995; African Plant Database, 2015).

NORWAY. Within the Svalbards archipelago, in the western part of the Spitsbergen island, there is a region

with great gypsum outcrops dating from the lower Permian period (Lauritzen, 1981). This is precisely the region is called Gipsdalen ('Land of Gypsum' in Norwegian) and is a part of a natural protected area, the Sassen-Bünsow Land National Park (Brekke & Hansson, 1990). At archipelago scale, *Carex marina* subsp. *pseudolagopina* (Sørensen) Böcher, *Juncus castaneus* Sm. and *Kobresia simpliciuscula* (Wahlenb.) Mack. can be considered as gypsophytes (Brekke & Hansson, 1990; Engelskjøn *et al.*, 2003).

OMAN. Although much of the Sultanate's geology is ultramafic, with Semail Ophiolite (Searle & Cox 1999), there are also gypsum materials (FAO, 1990) and the country is a major producer of this mineral (USGS, 2013). Gypsophytes as *Cleome glaucescens* DC., or *Physorhynchus chamaerapistrum* Boiss. inhabit its outcrops, along with some gypsoclines as *Panicum turgidum* (Ghazanfar, 2007-2010).

PALESTINE (state of). With a one-off exception, all the gypsophyte flora present in southern Israel is also present in Cisjordanian territory, with gypsophyte *taxa* as *Halothamnus lancifolius* (Boiss.) Kothe-Heinr., *Herniaria hemistemon*, *Reseda muricata* C. Presl (Zohary & Feinbrum-Dothan, 1966-1986).

PAKISTAN. In this country, there are considerable gypsum deposits (USGS, 2013). Some *taxa* that have been cited as gypsophytes in neighboring countries are present, such as *Acanthophyllum sordidum* Bunge ex Boiss., or *Ferula oopoda* (eFloras, 2008; Komarov, 1934-1964). The ecological behaviour of this sort of *taxa* should be further explored.

QATAR. In this little Emirate, a gypsum desert appears in the west and north-west, particularly in the Dukhan area. There are elements shared with other parts of the Middle East, such as *Bassia muricata* (L.) Asch., or *Reseda muricata* (Norton *et al.*, 2009).

ROMANIA. The gypsum deposits of Transilvanian Basin are very important (especially those from the Cluj region). From the floristic point of view, several gypsoclines as *Centaurea phrygia* subsp. *razgradensis* (Velen.) Greuter, *Krascheninnikovia ceratoides* (L.) Gueldenst., etc. can be mentioned. *Gypsophila collina*, can be cited as gypsophyte (Kovács, 2008).

RUSSIA. It is the ninth gypsum producer in the world (4,500 kt per year [USGS, 2016]). The cases of a peculiar flora and vegetation associated with gypsum occur in three clearly defined zones: Pinega river basin, beside the city of Arkhangelsk, in the Arctic, with a dry and very cold climate, where the gypsophyte *Gypsophila uralensis* subsp. *pinensis* (Perf.) R. Kam – a local endemism – occurs, (Goryachkin *et al.*, 2005). Secondly, Dagestan in the North Caucasus, where species such as *Astragalus onobrychioides* or *Thymus pulchellus* C.A. Mey are present, along with Lower Volga where *taxa* as *Astragalus amarus* Pall. or *Bienertia cycloptera* Bunge exist. In the last two mentioned areas,

there are also some stepparic gypsoclines as *Krascheninnikovia ceratoides* among many others (Komarov, 1934-1964; Euro+Med, 2006-2015).

SAUDI ARABIA. Gypsum outcrops exist in the deserts of this country, where gypsophytes such as *Moricandia sinaica*, *Diploaxis acris* (Forssk.) Boiss., *Salvia deserti* Decne., etc. are present (Anon., 2014).

SOMALIA (including Somaliland). This country is one with the largest gypsum deposits in the world. In fact, gypsum soils extend 10,161 km², which is 16.2 % of the total national surface. A rich, peculiar and specific flora is associated with these large outcrops. The northern part of the country is home to several species, particularly in the regions of Sannag – e.g. *Helianthemum somalense* Gillett, *Otostegia ericoidea* Ryding, *Atriplex erigavoensis* Thulin; Bari region – e.g. *Helianthemum speciosum* Thulin, *Fagonia gypsophila* Beier & Thulin – and, especially, Nugal region – e.g. *Dorstenia gypsophila* Lavranos, *Euphorbia columnaris* P.R.O. Bally, *Aloe nugalensis* Thulin. In more southern areas of the country it is also possible to find species linked to gypsum substrates such as *Indigofera gypsacea* Thulin, or *Polygala gypsophila* Thulin (Thulin, 1993-2006, 2002, 2007; Ryding, 2005; African Plant Database, 2015).

SOUTH AFRICA. The country has a great geological variety, which includes gypsum. In fact, it produces 559.44 kt per year of this mentioned mineral (USGS, 2016). From a floristic point of view, the most outstanding outcrops are located in the southwest of the country, in arid conditions, in the Succulent Karoo and the Desert biomes. *Euphorbia melanohydrata* Nel has been reported as a gypsophyte taxon related to gypsum crusts (Jürgens, 2004), while *Stipagrostis subacaulis* (Ness) De Winter would match the gypsocline behaviour (Fish *et al.*, 2015). Gypsum-related communities have been described, whose leading species deserve to be studied. This would be the case of *Tetraena clavata* (Schltr. & Diels) Beier & Thulin, *Euphorbia brachiata* E. Mey ex Boiss., etc. (Nußbaum, 2003). In addition, it would be worth studying other cases, such as Sekhukhuneland locality, where a vegetation associated with a mixture of gypsum-ultramafic materials has been recognized (Siebert *et al.*, 2003).

SPAIN. Is the first gypsum producer in the European Union and the sixth worldwide, with a production of 6,400 kt per year (USGS, 2016). Spanish gypsum outcrops (or aljezares) are the most extended and hold peculiar associated flora and vegetation that has been recognized since ancient times (see Mota *et al.*, 2011, for the historical review), and are now enjoying protection at European level, as the Iberian gypsum steppes *Gypsophiletalia* (*1520), a priority habitat in the Directive Habitat (92/43/ECC) (Anon., 2013). Mota *et al.* (2011) listed 41 gypsophyte and 41 gypsocline *taxa*. Still, this question cannot be considered closed, as the advances

in taxonomy and chorology add new *taxa* to the gypsophyte list, e. g. *Chaenorhinum gamezii* Marchal & Güemes, *Linum castroviejoii* Mart. Labarga, Pedrol & Muñoz Garm., etc. (Güemes et al., 2014; Martínez Labarga & Muñoz Garmendia, 2015).

SUDAN. This country has gypsosous soils (FAO, 1990) and outcrops that produce 132 kt per year. In this country there are typically Saharan gypsophytes such as *Stipagrostis ciliata*, as well as gypsoclines *taxa* such as *Limoniastrum guyonianum* Boiss. or *Echionilon fruticosum* Desf. (Le Houérou, 1995; African Plant Database, 2015).

SYRIA. In relative terms, this country possesses one of the largest areas of gypsiferous soils in the world: 3,966 km², or 21.6 % of its extension is gypsum soil (FAO, 1990). Gypsophyte *taxa* such as *Campanula fastigiata* Dufour ex Schult. or *Suaeda asphaltica* Boiss. are present in these soils, as well as many gypsocline *taxa* such as *Nitraria retusa* or *Salsola orientalis* S.G. Gmel (Euro+Med, 2006-2015).

TAJIKISTAN. This country has a lot of gypsum outcrops with a number of gypsophyte elements such as *Ferula kelifi* Korovin, *Lachnoloma lehmannii*, *Phlomidoides gypsacea* (Popov) Adylov, Kamelin & Makhm (Komarov, 1934-1964)

TUNISIA. This Maghreb country presents many gypsum outcrops, as they represent 9.3 % of its soils (FAO, 1990). It is possible to find endemic gypsophytes such as *Anarrhinum brevifolium* (Coss. & Kralik) D.A. Sutton and *Sixalix thysdrusiana* (Le Houér.) Greuter & Burdet. There are also many halogypsophytes and marsh-gypsophytes, like the species *Lavatera flava* Desf. (Le Houérou, 1995; Pottier-Alapetite, 1981).

TURKEY. This country has a lot of gypsum deposits, being the fifth mundial producer, with 10,000 kt per year (USGS, 2016). Peculiar flora and vegetation associated with the gypsic deposits has been documented. The floristically interesting outcrops are distributed throughout the country. It is specially worth to mention localities such as Sivas – e.g. *Campanula sivasica* Kit Tan & Yıldız or *Elymus nodosus* (Nevski) Melderis subsp. *gypsicola* Melderis–; Eskişehir – e.g. *Gypsophila simonii* Hub.-Mor. or *Achillea gypsicola* Hub.-Mor.–; Erzincan – e.g. *Scrophularia lepidota* Boiss. or *Thymus spathulifolius* Hausskn. & Velen.–; as well as Ankara – e.g. *Verbascum gypsicola* Vural & Aydoğdu or *Acantholimon anatolicum* Dogan & Akaydin – (Davis, 1965-1988; Ketenoglu et al., 2000; Akpulat & Celik, 2005; Yildirim, 2012).

TURKMENISTAN. This country presents many gypsum outcrops both in the region of Karakum Dessert and in Kopet Dag Ranges, and specially in Köytendag Range, in Uzbekistan frontier. Rich gypsicolous flora grows on these substrates, with many endemic elements, such as *Cleome turkmena* Bobrov, *Mattiastrum turcomanicum* Brand, *Muretia oeroilanica* Korovin,

etc. (Komarov, 1934-1964).

UKRAINE. Gypsum outcrops are concentrated in two areas: Crimea (specially in Kerch Peninsula, Mindat, 1993-2016) and the westernmost part of the country, belonging to the Badenian Basin (Klimchouk, 1996; Peryt et al., 1998). *Gypsophila collina*, a rare pontic gypsophyte endemism, can be found on Crimean gypsum. In the continental part of the country different stepparian character gypsoclines have been cited, such as *Krascheninnikovia ceratoides* (Euro+Med, 2006-2015).

UNITED ARAB EMIRATES. In this country gypsum deposits are poorly represented and yield an insignificant production (USGS, 2016). Gypsophytes such as *Herniaria hemistemon* or gypsoclines like *Deverra tortuosa* (Desf.) DC. have been cited (Brown & Sakkir, 2004).

UNITED STATES OF AMERICA. This country is the second largest gypsum producer worldwide, with 11,500 kt per year (USGS, 2016). It has numerous deposits distributed almost throughout all the country, a fact that has been documented in the past (Adams et al., 1904). Those outcrops possess a peculiar flora associated and are located in the SW of the country, in states such as Arizona – e.g. *Tetranneuris verdiensis* R. A. Denham & B. L. Turner or *Gaillardia multiceps* Greene (Anon., 1993-2015) –; Colorado – e.g. *Cryptantha gypsophila* Reveal & C.R. Broome – (Reveal & Broome, 2006); New Mexico – e.g. *Nerisyrenia hypercorax* P.J. Alexander & M.J. Moore or *Townsendia gypsophila* Lowrey & Knight – (Alexander et al., 2014; Lowrey & Knight, 1994); Oklahoma – e.g. *Nama stevensii* C. L. Hitchc.– (Buckallew & Caddell, 2003); Texas – e.g. *Tiquilia hispidissima* (Torr. & A. Gray) A.T. Richardson or *Senecio warnockii* Shinnery – (Moore & Jansen, 2006) and Wyoming – e.g. *Townsendia grandiflora* Nuttall or *Physaria macrocarpa* (A. Nelson) O’Kane & Al-Shehbaz – (Anon., 1993-2015). Furthermore, in other states *taxa* with certain preference for gypsum is present, as in the cases of California (e.g. *Eriogonum gossypinum* Curran), Kansas (e.g. *Psilostrophe villosa* Rydberg ex Britton), Nevada (e.g. *Artemisia pygmaea* A. Gray) and Utah (e.g. *Arctomecon humilis* Coville) (Anon., 1993-2015). Currently, M.J. Moore (Oberlin College, OH, USA) are preparing a checklist of USA gypsophytes.

UZBEKISTAN. Among all those countries that conform Central Asia, this is the one with the richest gypsophile flora. It is present both in lowlands (Kyzyl Kum Desert, and specially, Fergana Valley) and in the mountain side of Pamir-Alay (specially in Gissar Range). The number of Gypsophytes is probably around half hundred, with the presence of a large number of endemisms such as *Astragalus namanganicus* Popov, *Calligonum santoanum* Korovin, *Ferula primaeva* Korovin, *Hedysarum jaxarticum* Popov, etc. (Komarov, 1934-1964; Kasputina, 2001). Curiously, gypsum production only reaches 50 kt per year (USGS, 2016).

YEMEN. This country holds large gypsum deposits, as gypsum soils sum up to 2,931 km², being 8.8 % of the territory (FAO, 1990), although its production is only of 100 kt per year (USGS, 2016). This republic is a biogeographical crossroad, also in the case of gypsum environments, as gypsophytes linked to the Saharo-arabic and Mediterranean flora – e.g. *Diploaxis harra* subsp. *harra* – and other tropical elements linked to the Africa Horn – e.g. *Commicarpus reniformis* (Chiov.) Cuf.– (Al Khulaidi, 2013) can be recognized here.

Countries without gypsophytes but with presence of gypsoclines

ANGOLA. Gypsocline plant *Stipagrostis subacaulis* (Ness) De Winter (Fish *et al.*, 2015) has been cited in the southwest of the country, in the northernmost of Namib dessert biome although the largest deposits are located more to the north of the country, in the Dombe Grande deposit (Van Straaten, 2002).

AUSTRIA. This country presents gypsum deposits that could be exploited (USGS, 2016). Furthermore, it is possible to find communities of *Astragalus exscapus* L. subsp. *exscapus* and *Crambe tatarica* L. that are included in the habitat 6250 Pannonic loess steppic grassland (sensu Habitat Directive) (Anon, 2013). Some of the characteristic *taxa* of this habitat show a gypsocline behaviour in other countries, therefore, it would be necessary to evaluate if they are present on gypsum in addition to loess.

CHILE. This country holds important gypsum deposits and produce 129,000 kt per year of such mineral (USGS, 2016). However, there are no references of flora linked to gypsum deposits, plant communities of *Eriocyce* (that are detailed in Peru section) could be an exception to this, whose peripheral distribution reaches the northernmost part of the country in Arica Region (Cáceres *et al.*, 2013).

CZECH REPUBLIC. The Badenian Basin deposits are peripherally present in this country (Peryt *et al.* 1998). The presence of gypsocline steppe *taxa* such as *Astragalus exscapus* subsp. *exscapus* and *Crambe tatarica* (Euro+Med, 2006-2015) have also been reported.

DJIBOUTI. This small country possesses different endorheric basins, such as lake Assal, where different kinds of salts are present, including gypsum (Van Straaten, 2002). These environments are inhabited by halogypsophyte *taxa* such as *Dracaena ombet* Heuglin ex Kotschy & Peyr. (African Plant Database, 2015).

DOMINICAN REPUBLIC. From a floristic point of view, it is important to emphasize the Enriquillo Valley basin (“the Caribbean Dead Sea”). In Enriquillo Basin some halogypsophytes have been cited, such as the *Cactaceae* species *Leptocereus paniculatus* (Lam.) D.R. Hunt and *Consolea moniliformis* (L.) A. Berger (Oldfield, 1997).

ERITREA. In the Danakil basin, there are deposits of

late Tertiary to Pleistocene evaporites including halites, gypsum and potassium salts. This is an area located mainly in Ethiopia with a small portion reaching into Eritrea, along with the coastal area of eastern. Gypsoclines such as *Dracaena ombet* Heuglin ex Kotschy & Peyr. have been cited in this country (African Plant Database, 2015).

FRANCE. There are not very numerous gypsum outcrops here. From a floristic point of view, deposits of the alpine area are remarkable (some of them reach noteworthy heights). There, the gypsocline *Onosma alpina* (A. DC.) Boiss. can be found, as well as *Festuca rupicola* Heuff. (Aeschmann *et al.*, 2004). These Alpine gypsum outcrops (and neighbouring Switzerland) serve as shelter for missing or scarce xerophilic elements in the surrounding vegetation (Gensac, 1968; Biedermann *et al.*, 2014).

GERMANY. There are large gypsum outcrops here, specially in Thuringia and Saxony Anhalt. The presence of gypsum is linked to thermophile communities, such as beech woods (Schmid & Leuschner, 1998). However, it is not possible to find gypsocline flora, with the significant exception of *Astragalus exscapus* subsp. *exscapus* (Becker T. & Voß., 2003; Brekke & Hansson, 1990; Podlech, 1988).

GREECE. In Crete, the mining prospection of the Altsi deposits in the eastern portion of the island (USGS, 2016) has been mentioned. There, the gypsocline *Viola scorpiuroides* Coss. grows, in addition, it is presented on dolomite and, perhaps, phyllites at the western end of the island (Turland *et al.*, 1993).

HAITI. As in the case of Dominican Republic, it is possible to find gypsum outcrops in the Enriquillo lake basin, and a halogypsicolous behaviour has been observed in *Leptocereus paniculatus* and *Consolea moniliformis* (Oldfield, 1997).

HUNGARY. In this country there are no important gypsum outcrops; in fact, there has been no production of gypsum since 2010 (USGS, 2016). By way of compensation, steppe gypsoclines have been cited such as *Astragalus exscapus* subsp. *exscapus*, *Krascheninnikovia ceratoides* (L.) Gueldenst., etc.; but surely these species grow on loess or alkali soils, not on gypsum (Euro+Med, 2006-2015).

INDIA. In the Thar desert there are gypsum deposits; however, there is no confirmation of clearly defined gypsophilous flora (cf. Rawat, 2008), even though halogypsophytes such as *Haloxylon salicornicum* (Hooker, 1872-1897) have been cited. Additionally, there is information about vegetation dynamics, in the case of abandoned quarries that are colonized by xenophytes such as *Prosopis juliflora* (Sw.) DC. (Sharma *et al.*, 2001). It would be of interest to research further into the edaphic behaviour of the autocton flora of the Thar desert.

KENYA. There are important gypsum outcrops here,

in the Garissa area and on the Somalian border (Van Straaten, 2002). There is no distinguishable gypsophyte flora, although gypsocline elements have been described, which are also shared with Somalia and Ethiopia, such as *Microcharis gyrata* (Thulin) Schrire, *Gossypium bricchettii* (Ulbr.) Vollesen, etc. (African Plant Database, 2015).

MALI. Its main outcrops are located in the north of this country, in the heart of the Sahara, in the Tessalit and Taoudenni areas (Van Straaten, 2002). Therefore, it shares gypsoclines of the Saharan floristic catalogue such as *Cornulaca monacantha* Delile or *Stipagrostis pungens* (Le Houérou, 1995; African Plant Database, 2015).

PERU. In the southern coast of the country it is possible to find the communities of the Garua desert with gypsum soils colonized by beds of spherical cacti of genus *Eriosyce* (subgen. *Islaya*), such as *E. islayensis* Backeb (Devillers & Devillers-Terschuren, 1996; Cáceres et al., 2013). However, it would be advisable to carry out newer botanical prospections.

SLOVAKIA. This country presents some small gypsum deposits where steppe gypsocline have been cited, such as *Astragalus exscapus* subsp. *exscapus*, *Krascheninnikovia ceratoides*, etc.; but probably these populations are growing on other types of substrates (Euro+Med, 2006-2015).

SWITZERLAND. This country shares the gypso-alpine floristic entourage with France (see above in section dedicated to France).

Countries without presence of gypsophytes or gypsoclines, although there are distinguishable traces in flora and vegetation.

Those countries where a clear influence of gypsum on vegetation has been documented are included here, although there is no statement of presence of special flora linked to gypsum.

BRAZIL. Gypsum material can be found in numerous areas of the country, although it is important to mention the northeast of the country, with a semiarid climate, where it is possible to find a type of vegetation called caatinga. In this area, in the municipality of Araripina (state of Pernambuco), there are large gypsum outcrops from which most of the Brazilian gypsum is obtained. There are no clear mentions of flora linked to gypsum outcrops; but there are studies of microflora that conform mycorrhiza (Mergulhão, 2010). These studies have described the presence on gypsiferous substrate of stenocorous vascular plants such as *Spondias tuberosa* Arruda, *Aspidosperma pyriforme* Mart. and *Parapiptadenia zehntneri* (Harms) M.P. Lima, even with the possibility to colonize abandoned gypsum quarries (*Ruellia paniculata* L., *Alternanthera tenella* Colla and *Ziziphus joazeiro* Mart.). On these taxa and communities, it would be interesting to con-

tinue researching.

CANADA. It is possible to find gypsum deposits in the Atlantic area of the country, in Ontario and Western Canada (Kogel et al., 2006). However, gypsophile flora has not been described, although there are some rare and uncommon local plant species associated with gypsum. Among these taxa *Anemone parviflora* Michx. or *Viola canadensis* L., among others (Maze-rolle et al., 2015) can be cited.

POLAND. Badenian Basin materials can be found in the southernmost area of the country, which are shared with Ukraine and Czech Republic (Peryt et al., 1998). Outcrops can be locally relevant, being reflected in the place names (e.g. Mount Gipsowa) and yielding a gypsum production of 1,085 kt per year (USGS, 2016). There are references that gypsum outcrops can act as refuge for xerothermic elements like *Campanula bononiensis* L. or *Verbascum phoeniceum* L. (Keilholz, 1927).

Countries with gypsum deposits but without floristic or vegetation data linked to gypsum.

Those countries where there are references on the existence of gypsum deposits, but not on the existence of a flora associated to gypsum soils, or the existence of biases on flora and vegetation belong to this list. This question is open to oncoming studies.

ALBANIA; BELARUS; BHUTAN; BOSNIA AND HERZEGOVINA; BULGARIA; CAPE VERDE (with deposits in Maiao island); COLOMBIA (there are deposits in the Cordillera Oriental, coast of Guajira peninsula and part of the Cordillera Central [Ponce & Torres Dunggan, 2006]); CROATIA; CUBA (deposits belong to the upper Jurassic, and are exploited in three populations: Canasí, Punta Alegre and Baitiquirí [Ponce & Torres Dunggan, 2006]); ECUADOR (Ponce & Torres Dunggan [2006] cite gypsum exploitation in the south, province of Loja, in Malacatos and Bramaderos); GHANA (small amounts of gypsum and gypsiferous clays were reported from near Accra and localities in the Western Region, and from the Keta region [Van Straaten, 2002]); GREENLAND (gypsum materials have been located outside the ice sheet, especially on the east coast of the island, where there is a geological formation called Gipsdalen, “gypsum valley” in Danish [Clemmens et al., 1985; Kent & Clemmens, 1996]); INDONESIA; IRELAND (in two sites in southern County Monaghan); JAMAICA; KOREA (Republic of); LAOS; LATVIA; MACEDONIA; MAGADASCAR (exploited deposits are present mainly in Antsahampano); MALAWI (small gypsum occurrences are known in several seasonally flooded shallow valleys (dambos) in the northern part of the country and the Kasangadzi Dambo. Malawi imports most of its gypsum needs); MOZAMBIQUE. There are several gypsum and anhydrite occurrences in oil and gas exploration boreholes in the coastal zone of Mozambique.

The most extensive gypsum and anhydrite deposits are date back to the Oligocene/Miocene age and occur in the evaporite sequence of the Temane Formation [Van Straaten, 2002]); NICARAGUA (deposits were originated in tertiary age, and are located in the Central Province [Ponce & Torres Duggan, 2006]); NIGERIA; PARAGUAY; PORTUGAL; PUERTO RICO (despite the fact that there is no mining production, gypsum outcrops have been cited, especially in Isla Mona (Kaye, 1959); SERBIA; TAIWAN; TANZANIA (the major rock gypsum and anhydrite resource is located in a remote area, at Pindiro and Mandawa in southeastern Tanzania [Van Straaten, 2002]); THAILAND (the fourth worldwide producer, and recently, with the highest increase in production (from 0.86 kt in 2008 to 12,500 in 2015 [Crangle, 2016; USGS, 2016]); UGANDA (The best known source of natural gypsum is at Kibuku, in the southwestern area of Lake Albert in Bundibugyo District); UNITED KINGDOM; URUGUAY (known deposits are associated with Santa Lucía and La Laguna Merín basins in lands from cretaceous age [Ponce & Torres Duggan, 2006]); VENEZUELA (presents deposits in the Cordillera de la Costa which is the main gypsum district of the country, located in Páramo Peninsula. Also, northern sedimentary formations contain gypsum deposits [Ponce & Torres Duggan, 2006]); VIETNAM; ZAMBIA (gypsum clays occur in surficial environments of the Kafue Rats and the Siloana Plain, close to hot springs. Furthermore, there are gypsiferous clays of Lochinvar, on the edges of the alluvial plain of the Kafue River. Gypsum content in these clays reaches 40% with crystals up to 4 cm in size [Van Straaten, 2002]).

To conclude, it is important to mention that perhaps the ANTARCTIC TERRITORIES should be included among the countries of the previous paragraph, as gypsum outcrops have been found in the areas that are not covered by ice. This is the case of Seymour Island (Tartar *et al.*, 1993) or the Dry Valleys in McMurdo region (Keys, 1979). Studies of biota present on these deposits are of interest as they are analogues of Mars (Losiak, 2016). The main objective of their citation here is to encourage the study of gypsophily, or at least, gypsophyte flora in these territories.

Conclusions

Gypsum outcrops are widely distributed worldwide, being present in 112 countries. The phenomenon of Gypsophily is widespread in 71 countries, in which there are unquestionable references to gypsophyte *taxa*; while in 53 countries *taxa* with a certain preference for gypsum are mentioned. These data contrast with previous works that directly allude to gypsophily, which is only circumscribed to ten countries. This indicates very clearly the need to undertake further research in additional geographical areas. The main ob-

jective of those citations of countries here is to encourage the study of gypsophily, or at least, gypsophyte flora in these territories.

The existence of gypsophyte *taxa* mainly occurs in dry climates. Nevertheless, in higher humidity conditions the presence of gypsum still has a visible effect on flora and vegetation, since the outcrops serve as a refuge for xerothermophilic *taxa* absent (or almost) on the surrounding vegetation of the outcrop.

The vegetation on gypsum is more sparse and scattered than that existing on other sorts of substrates adjacent to gypsum outcrops, although depending on the climate, some forests might thrive on this material.

Due to the great mismatch between countries with gypsum outcrops and the available information about them, uniting the scientific community in the effort to characterize the edaphism of gypsum phenomenon around the planet would be worthwhile.

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The species-specific monitoring protocols for plant species of Community interest in Italy

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Abstract

The results of a project for the identification of species-specific monitoring protocols for the Italian plant species protected under the Habitats Directive (Annexes II/IV/V) are presented. The project led to the development of 118 monitoring factsheets, providing an operational guidance for 107 vascular *taxa*, 10 bryophytes and 1 lichen *taxon*. Each factsheet includes information on the species (distribution, biology, ecology, conservation status, threats, *etc.*) and the description of field methodologies for the detection of the two main reporting parameters, *i.e.* population size and habitat quality. Practical information to plan field activities are also given. Protocols were designed to address the requirements of the European reporting system with the aim to standardize future monitoring activities, optimize efforts at national scale and overcome some current problems related to data heterogeneity and discrepancies from the EC standards. More than 60 botanists collaborated to identify the best practices and to design an operational field survey format through several stages of discussion and sharing. The protocols, developed by ISPRA and Scientific Societies and shared with the Italian institutions responsible for the Directive application, were published in a dedicated National handbook. The work provides a first uniform technical basis for future national monitoring plans.

Key words: conservation, EC-Habitats Directive, field methodologies, plant species monitoring, population size.

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Introduction

The reference framework of the project presented in this paper is the Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, known as the Habitats Directive (HD hereafter), and its implementation in Italy. The HD and the Natura 2000 network with more than 27,000 sites, covering about 18% of the terrestrial surface of the EU (EC, 2015a; EEA, 2015), represent the core strategy of nature conservation and the most important tools aiming at protecting biodiversity in Europe (*e.g.* Balmford *et al.*, 2003; Maiorano *et al.*, 2007). In order to evaluate the effects of the conservation policies and the effectiveness of HD, the European Commission (EC) requires the assessment of the conservation status of species and habitats at national and biogeographical level. Monitoring habitats and species listed in the Annexes of the HD is a key step in the HD implementation, but also a mandatory action for Member States, arising from Article 11. Moreover, according to Article 17, Member States are required to report every 6 years on the main results of this survey. HD monitoring and reporting are not restricted to Natura 2000 sites, therefore data need to be collected both in and outside the network (Evans & Arvela, 2011).

Reporting under Article 17 follows a standard methodology and uses a format proposed by the EC and approved by Member States after discussion and consultation. The use of common standards and formats is necessary to harmonize the content of the National reports developed by Member States, and to allow the aggregation of data at European level (DG Environment, 2017). On the basis of the National reports indeed, the EC in collaboration with EEA (European Environment Agency) produces composite reports and makes them accessible to other EU institutions and to the public (EC, 2015b). These composite reports provide an overview of the status of the European biodiversity and represent a component for evaluating EU policy, in particular for measuring progress towards the 2020 targets set under the Biodiversity Strategy (EEA, 2015; EC, 2015b).

In the third Italian National Report, covering the period 2007–2012, the status of the species and habitats of Community interest was assessed using available knowledge and expert judgment (Genovesi *et al.*, 2014). The Italian Report reveals an increase in the quality of data and assessments compared to the past, but despite these significant advances, there are still some data gaps due to heterogeneity, inconsistency in collection methods, discrepancies from the HD reporting requests, lack of data for some parameters and a low percentage of information derived from *ad hoc* field surveys and long-term monitoring (Ercole & Giacanelli, 2014). A major cause of these problems is

the absence of a standardized monitoring and data collection program at a national level, both for vascular and non vascular flora (Ravera *et al.*, 2016; Fenu *et al.*, 2017).

To overcome several of these deficiencies and to improve monitoring systems for HD in Italy, an *ad hoc* project was set up in 2015, aimed to standardize future monitoring activities and to optimize efforts at national scale. A network of institutions (Italian Ministry for the Environment, Regions and Autonomous Provinces) and scientific societies with the coordination of the Italian National Institute for Environmental Protection and Research (ISPRA) has been organized with the main goal to identify nationwide shared monitoring protocols for habitats and species listed in the annexes of the HD.

In particular, for plant species of Community interest all activities were carried out through a scientific collaboration between ISPRA and SBI (Italian Botanical Society) with a further contribution of SLI (Italian Lichen Society), and led to the identification of species-specific monitoring protocols for all plant species listed in the HD. Protocols were designed to address the requirements of the European reporting system. Standardized data collection methods should lead to achieve comparable results and to overcome some current problems related to data heterogeneity and discrepancies from the EC standards and should allow greater repeatability and comparability over time. At the end of the project (October 2016) all protocols have been collected in a dedicated National handbook (Ercole *et al.*, 2016).

Main goals and organization of the project for plant species

The main aim of the project was to define nationwide shared monitoring protocols for Italian plant species of Community interest, consistent to the HD reporting requests and based on the best current scientific knowledge. The protocols were strongly focused on species-specific methodologies to measure/estimate the species population size and status.

Species monitoring is the regular observation and recording of changes in status and trend of a *taxon* in a certain territory and time interval. Beyond a purely scientific interest, the primary purpose of monitoring is to collect information useful in developing conservation policy, to examine the outcomes of management actions and guide management decisions (*e.g.* Fenu *et al.*, 2015). Moreover, monitoring should have further goals, such as detection of significant changes in resource abundance, assessment of the effects of management on population/community dynamics, and providing suggestions for applied research questions. Finally, monitoring data can be used to assess the conservation

status or to predict the effects of various management practices on population size, condition, stage distribution (including seed production and/or seedlings development) and demographic processes (e.g. survivorship, growth, seedling recruitment; Menges & Gordon, 1996; Barni *et al.*, 2013; Fenu *et al.*, 2015). The parameters selected and the frequency of measurement will depend on specific management or conservation objectives, on initial assessment of threat or need, on the biology of the species and on available resources (Menges & Gordon, 1996; Fenu *et al.*, 2015; Giovino *et al.*, 2015). However, because time and resources for monitoring activities are generally limited also for HD plants, only few plant populations are involved with an adequate monitoring program and generally hasty estimation of abundance or extent of populations prevails. As a consequence, few experiences, always at the regional level and often focused on narrow endemic plant species (e.g., Fenu *et al.*, 2011, 2015; Campisi

et al., 2014; Plume *et al.*, 2015; Troia & Lansdown, 2016), were carried out so far.

The project activities began in April 2015; more than 60 botanists pertaining to different universities and research institutes were involved. The network was headed by an ISPRA-SBI coordination group and organized into 12 units: 10 units for vascular plants and two for non-vascular plants (Fig. 1). The project was carried out through several stages of discussion and sharing, making progressive improvement steps to the identification of the best practice and to provide complete “monitoring factsheets”, concise and operational.

Firstly, the checklist of the Italian HD plant *taxa* has been defined and updated following the current knowledge and the latest taxonomic and systematic studies (Rossi *et al.*, 2016). Then, monitoring factsheet format and contents have been carefully chosen in order to have a first draft common framework for the involved researchers. In addition, a “survey format” to test in the field the monitoring techniques was elaborated and each working group began to work on the assigned *taxa*.

The monitoring protocols were identified for all the *taxa* and tested in the field for a set of target species, selected on the basis of their representativeness of different reproductive/propagative strategies and distribution patterns.

The protocols were submitted to the Italian institutions responsible for the HD implementation and application to be reviewed: in early 2016 all draft factsheets were shared for comments and suggestions, first with the Italian Ministry for the Environment and then with Regions and Autonomous Provinces, as responsible institutions for monitoring under HD. After an extensive review and implementation work, the retail version of the factsheets was completed.

Project findings

Update of the list of taxa

Plant species to be monitored under the HD are all those listed in Annexes II, IV and V distributed on the Italian territory. For the purpose of the project the list of Italian plant *taxa* to be monitored under HD has been revised and updated on the basis of recent studies, also by a taxonomic point of view.

In addition to the 109 *taxa* considered in the third Italian Report (Ercole & Giacanelli, 2014; Fenu *et al.*, 2017), five other vascular species, listed in Annexes II/IV, were also included in the project: *Botrychium simplex* E.Hitchc., *Centranthus amazonum* Fridl. & A.Raynal, *Elatine gussonei* (Sommier) Brullo, Lanfr., Pavone & Ronsisv., *Klasea lycopifolia* (Vill.) Á.Löve & D.Löve [HD name: *Serratula lycopifolia* (Vill.) A.Kern], *Jacobaea vulgaris* Gaertn. subsp. *gotlandica* (Neuman) B.Nord [HD name: *Senecio jacobea* L.

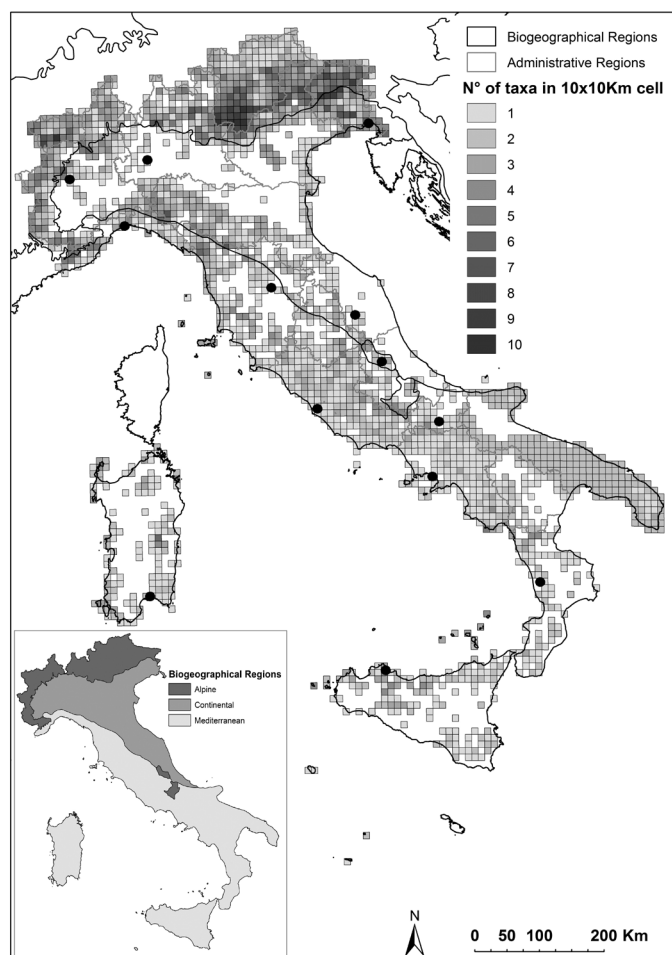


Fig. 1 - Geographic location of the main working groups involved in the project (black dots), overlapping the density map of the Italian plant species of Community interest (Annexes II, IV, V; distribution data from the III Italian Report). The boundaries of the Biogeographical and administrative Italian Regions were also indicated.

subsp. *gotlandicus* (Neuman) Sterner]. Although their presence in Italy is certain, they had not been previously included in the checklist ex Art.17.

Four HD plants previously reported for Italy but currently considered probably extinct (Rossi et al., 2016) have also been included in the project. This choice was made according to a precautionary principle and in view of a possible future rediscovery of these plants (*Bromus grossus* Desf. ex DC., *Coleanthus subtilis* (Tratt.) Seidl, *Mandragora officinarum* L., *Thesium ebracteatum* Hayne).

The updated list includes a total of 118 *taxa*: 107 vascular plants, 10 bryophytes and one lichen *taxon*; 115 of these *taxa* are protected by the HD at the species level and 3 at the genus or subgenus level (*i.e.* *Cladonia* L. subgenus *Cladina* (Nyl.) Vain., *Sphagnum* L. sp. pl., *Lycopodium* L. sp. pl.).

According to the latest updates of the Italian endemic flora (Peruzzi et al., 2014, 2015), about half of the Italian HD *taxa* are endemic. Among the vascular plants, 54 species are endemic to Italy, two are endemic to Sardinia and Corsica, one to Sicily and Malta and three *taxa* have subspecies endemic to Italy. Among bryophytes *Riccia breidleri* Jur. ex Steph. is an alpine endemism, the only one among non-vascular plants (Aleffi et al., 2008).

Field survey format and species-specific monitoring protocols

The published handbook (Ercole et al., 2016) contains the species-specific protocols and specific field survey formats (for vascular plants, bryophytes and lichens) developed in the project. The use of standardized protocols and survey formats is essential in order to ensure uniformity in spatial terms (data recorded in the same way in different areas of the territory, such as Regions), and in temporal terms (data recorded with the same methods in different years and from different detectors). The survey formats have been designed to be in compliance with the requirements of HD reporting, scientifically rigorous, applicable both at local and national level, suitable to ensure collection of homogeneous data and repeatable over time.

The monitoring protocols developed for each of the 118 *taxa* were tested in the field on a set of 12 target species, identified according to representativeness criteria for life forms, phenology, ecology and biogeography (Tab. 1). Among them, *taxa* with very limited/narrow distribution and *taxa* with wide distribution were included. Field surveys allowed to adjust the methodology across the different groups of botanists involved in the project, to verify applicability of the protocols and to improve the survey formats.

For each *taxon* a specific monitoring factsheet has been filled. A single factsheet has been realized even for *taxa* protected at the *genus* level, and in the cases of

species represented in Italy by two or more subspecies (*Anchusa crispa* Viv., *Asplenium adulterinum* Milde, *Dianthus rupicola* Biv., *Gentiana lutea* L. and *Stipa austroitalica* Martinovsky), since in both cases a comprehensive HD reporting is required.

The factsheet format includes two sections, a first one with information on the species (descriptive section) and a second one dedicated to the monitoring. The former contains relevant information such as: conservation status and trend *sensu* HD derived from the third Italian Report (Genovesi et al., 2014), European (Bilz et al., 2011) and National (Rossi et al., 2016) IUCN assessments, chorotype, distribution in Italy, major biological characteristics, ecological requirements, plant communities and threats. In particular, plant communities where each plant species grows and their syntaxonomical attribution (for vascular plants mainly according to Biondi et al., 2014) have been briefly described, as relevant information for plant species, although HD reporting does not require it. Since many of the species are threatened by specific and relevant pressures, even the description of the identified threats for each *taxon* has been reported.

Each factsheet also contains the distribution map of the *taxon*: 98 maps are the ones already produced in the third Report (standard European grid 10×10 km), 5 maps are derived from the third Report with meaningful updates, 7 maps are brand-new elaborated on the same standard grid, and 8 are regionally based maps (Administrative Region of presence/extinction). Moreover, original photos contribute to illustrate morphology and habitat of the *taxon*.

The monitoring section includes the description of field methodologies for the detection of the two main HD parameters, *i.e.* population size and habitat for the species. Concerning population size, for each species the methodologies and the following information are provided: minimum percentage of populations to be monitored; number, size, and criteria for *plot* placement; specific parameters to be detected (counts or estimates) including number of individuals, *i.e.* *genet* and/or *ramet*, which is not always easy to determine. There are several plants that use vegetative reproduction and therefore generate clonal populations, in which only the number of *ramet* can be estimated (*e.g.* *Lamyropsis microcephala* (Moris) Dittrich & Greuter, *Arnica montana* L., *Gentiana lutea* L., *Ribes sardoum* Martelli). In these cases it is extremely difficult, or impossible, to establish the exact number of individuals or *genet*.

Other parameters detected are the number of colonies/clumps (*e.g.* for ferns as *Asplenium adulterinum* Milde and *Vandenboschia speciosa* (Willd.) G.Kunkel [HD name: *Trichomanes speciosum* Willd.]) and the extent of covered area (for bryophytes, lichens, hydrophytes and plants with high clonal propagation, *e.g.*

Tab. 1 - Target plant species selected for the field test.

<i>Taxon</i>	<i>Distribution</i>	<i>Life form</i>	<i>Habitat</i>	<i>No. populations monitored</i>	<i>Regions involved</i>
<i>Androsace mathildae</i> Levier	Endemic to Central Apennines	Chamaephyte	Montane limestone cliffs	1 (representative population)	Abruzzo
<i>Armeria helodes</i> F.Martini & Poldini	Narrow endemic to Friuli Venezia Giulia	Hemicryptophyte	Alkaline fens of peaty soil that are saturated in water and rich in calcium and magnesium	7 (all populations)	Friuli Venezia Giulia
<i>Eo Kochia saxicola</i> (Guss.) Freitag & G.Kadereit [HD name: <i>Bassia saxicola</i> (Guss.) A.J.Scott]	Endemic to South Italy (Tyrrhenian coast)	Chamaephyte	Maritime rocks and calcareous or volcanic cliffs	5 (all populations)	Campania, Sicilia
<i>Cypripedium calceolus</i> L.	Eurasian	Geophyte	Open woodland, scrub under conifers, forest margins, grasslands	3 (representative populations)	Valle d'Aosta, Piemonte, Lombardia, Trentino-Alto Adige, Veneto, Friuli Venezia Giulia, Abruzzo
<i>Gentiana lutea</i> L.	South Europe	Geophyte	Mountain grasslands and meadows	11 (representative populations covering the entire distribution in Italy)	Peninsular Italy, Sardegna
<i>Isoetes malinverniana</i> Ces. & De Not.	Endemic to North Italy (Po plain)	Hydrophyte	Freshwater: clear, fresh and running spring water	14 (all actual populations) + 5 (extinct)	Piemonte, Lombardia
<i>Lamyropsis microcephala</i> (Moris) Dittrich & Greuter	Narrow endemic to Sardegna	Geophyte	Montane dwarf shrub and steppe vegetation	4 (only known populations)	Sardegna
<i>Acis nicaeensis</i> (Ardoino) Lledó, A.P.Davis & M.B. Crespo [HD name: <i>Leucojum nicaeense</i> Ardoino]	Endemic to Maritime Alps (France and in the northwest of Italy's border)	Geophyte	Rocky areas and barren slopes on stony soils	1 (only known population)	Liguria
<i>Marsilea quadrifolia</i> L.	Eurasian	Hydrophyte	Freshwater: ponds, rice fields and ditches	6 (representative populations)	Piemonte, Lombardia, Veneto, Trentino-Alto Adige, Emilia-Romagna, Toscana
<i>Primula palinuri</i> Petagna	Endemic to South Italy (Tyrrhenian coast)	Geophyte	Northern, northwestern or northeastern slopes on calcareous substrate with neutral pH	2 (all populations)	Campania, Basilicata, Calabria
<i>Ribes sardoum</i> Martelli	Narrow endemic to Sardegna	Nano-Phanerophyte	Mountain areas on calcareous substrates	1 (only known population)	Sardegna
<i>Silene hicesiae</i> Brullo & Signor.	Endemic to the Aeolian Islands	Chamaephyte	Rocky slopes of small volcanic islets	1 (main population)	Sicilia

Marsilea quadrifolia L.). In some cases, the number of flowering/fruited individuals or other specific data can also be required.

The protocols may differ greatly depending on both the extent of the populations of each species and their biological and ecological characteristics. In Italy, many HD plant species have a restricted distribution area and small populations. In these cases (which are usually the better known and investigated) it is recommended to count all the individuals. For populations with a high number of individuals the methodologies usually involve counts on a representative number of plots, in order to sample a significant portion of the population (at least 10%) and to obtain subsequently the total estimate. In the case of more widespread species (e.g. *Ruscus aculeatus* L.), the population size can be estimated calculating the number of 1×1 km grid cells, in conformity to what has been recently specified by the European Commission for Annex V species (DG Environment, 2017).

The factsheet also includes some practical informa-

tion to plan field activities, specifically: monitoring frequency requested in a year and in the six years between two reporting cycles, best period for sampling (months of flowering, fruiting, etc.), number of requested working days per year, minimum number of people to be employed in field surveys.

Concluding remarks

Despite monitoring activities are the basis for many national and international conventions and directives and represent a fundamental tool for natural resource management and conservation, currently an Italian national monitoring system does not exist yet. Recommendations on how to monitor biodiversity are numerous (e.g. Nimis *et al.*, 2002; Balmford *et al.*, 2003; Mace, 2005; Baiamonte *et al.*, 2015; Fenu *et al.*, 2015; Evangelista *et al.*, 2016), but are still insufficiently taken into account at a National scale. Although several experiences have been realized at the local level, long-term monitoring data on individual taxa are available

only in few cases (Fenu *et al.*, 2017).

Italy hosts a high number of plant *taxa* of Community interest (exceeded only by Spain and Portugal among the Member States), therefore monitoring efforts are particularly demanding. Additionally, the high endemism rate of the Italian plant species of Community interest, about 50% of the total, determines an extraordinary national responsibility in conservation.

The last Italian Report *ex Art.17* showed a negative situation for our HD plant species with nearly half of them declared in an unfavourable conservation status (Ercole & Giacanelli, 2014; Fenu *et al.*, 2017), confirming the results of the Italian IUCN assessments of policy species (Rossi *et al.*, 2014, 2016). Moreover the trend of several *taxa* suggests that many of these species might move to categories of higher extinction risk in the coming years (Fenu *et al.*, 2017). Such evidences demonstrate that the efforts undertaken in the last 20 years are still insufficient to maintain many *taxa* at a favourable conservation status. Significant work still remains to achieve the HD conservation targets and monitoring activities are crucial to reach the goal.

In conclusion, this project provides for the first time uniform technical bases for future national HD monitoring plans. The species-specific protocols identified for Italian plant species of Community interest can help to overcome the lack of shared standards and some current problems related to data heterogeneity, allowing greater replicability and comparability of data collection over time. It is also interesting to note that the requirements of the HD monitoring (*e.g.* for demographic data) may partly overlap and coincide with the needs related to research projects and specific studies. At the same time further scientific research will contribute to the improvement of species-specific monitoring protocols that will gradually become more accurate and useful for HD monitoring purposes.

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Ecology and conservation status of *Muscari gussonei* (Parl.) Nyman in Sicily: a narrow endemic species threatened by habitat reduction

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Abstract

Muscari gussonei (Parl.) Nyman (*Hyacinthaceae*) is a rare endemic psammophyte occurring in southern Sicily (Italy). It is listed as Endangered species (EN) in the Red Book of Italian plants, included in the Annex I of Berne Convention (1979), and Annex II of the Habitat Directive 92/43/EEC as a priority species. This species characterizes the psammophilous plant communities ascribed to the *Vulpio-Leopoldietum gussonei*, together with many therophytes of the *Cutandietalia maritimae* order. Aims of this work were to examine the structure and floristic composition of the *M. gussonei* community, assess its conservation status and propose conservation measures. This study is part of a LIFE project (LIFE11 NAT/IT/000232 - Action D.1). Distribution and population structure were investigated through many field surveys carried out between 2013 and 2015. In order to define the ecological requirements of *M. gussonei*, 10 permanent plots were designed, while for characterizing the habitat of *M. gussonei* 41 phytosociological relevés were randomly carried out. Classification of relevés by using cluster analysis revealed three plant communities with *M. gussonei*, each with specific key species and linked to different environments. Three vegetation types (white dunes, grey dunes, inland or fossil dunes) were confirmed by canonical component analysis (10 plots) and they are correlated to a gradient of ecological features ranging from coastal to inland areas. After a census of *M. gussonei* populations joined to their mapping, the conservation status, according to IUCN guidelines, was assessed confirming the EN category. Finally, this study provides some relevant issues for the implementation of conservation measures.

Key words: conservation status monitoring, Leopoldia LIFE project, Mediterranean coastal conservation, plant distribution, population structure, psammophilous vegetation.

Introduction

Coastal dunal environments are one of the most threatened habitats of the Mediterranean area, chiefly as a result of anthropogenic destruction, habitat fragmentation and alteration (Médail & Verlaque, 1997; Acosta *et al.*, 2007, 2009; De Luca *et al.*, 2011; Fenu *et al.*, 2012, 2013; Sciandrello *et al.*, 2015; Pinna *et al.*, 2015). These habitats are characterized by a very specialized flora, sometimes with endemic elements. One of these is *Muscari gussonei* (Parl.) Nyman (= *Leopoldia gussonei* Parl.), a small *Hyacinthaceae* species growing on sandy substrates more or less close to the coast. This species is a narrow endemic of southern Sicily (Garbari & Di Martino, 1972; Giardina *et al.*, 2007; Brullo *et al.*, 2011) and characterizes the psammophilous plant community named *Vulpio-Leopoldietum gussonei*, together with many other annual plants of the *Cutandietalia maritimae* order (Brullo & Marcenò, 1974; Minissale & Sciandrello, 2015). *Muscari gussonei* is an endangered species, mentioned by the 1979 Berne Convention (Annex I), included in the Annex II of the Habitat Directive 92/43/EEC as a priority conservation species (Fenu *et al.*, 2017a), and listed in the Red Book of Italian plants (Conti *et al.*, 1997; Rossi *et al.*, 2016). Currently, *Muscari gussonei* is severely threatened by human activities, which are one

of main causes of its distribution range reduction up to few and scattered populations (Vandepitte *et al.*, 2012). Gussone (1827) and Lojacono-Pojero (1908-1909) recorded *Muscari gussonei* from Terranova (nowadays Gela) up to Capo Passero (SE Sicily). Afterward, Albo (1919) confirmed its occurrence in some localities of south-eastern Sicily (*e.g.* Sampieri, Marzamemi, Portopalo, etc.). Garbari & Di Martino (1972) found it at the so-called “Macconi di Gela” (Mignechi). Finally, Brullo & Marcenò (1974), while examining the survived populations of *Muscari gussonei*, were able to find the species just in few stands along the coast of Ragusa (Cammara, Passo Marinaro, Branco Piccolo, Randello, Refriscolaro and Mignechi), while some of the sites reported by Albo (1919) disappeared likely as a consequence of anthropogenic changes (Marina della Marza, S. Maria del Focallo, Capo Isola delle Correnti, Macchitella di Gela).

The present study is part of the LIFE-Leopoldia project (LIFE11 NAT/IT/000232) (action D.1 – Monitoring of species of greater importance for conservation). In particular, one of the project aims is the restoration of degraded coastal dunes, as well as the reinforcement of the *Muscari gussonei* populations.

The aim of our research is the assessment and monitoring of the scattered surviving populations and identification of its ecological requirements both in natural

conditions and degraded habitats.

Material and methods

Study area

The surveyed area (Fig. 1) includes the coastal belt of the southern part of Sicily, from Gela to Capo Passero, an area featured by dunes and wetlands. It is mainly characterized by Pleistocene substrates, such as calcarenites and sand deposits that often extend inland (Lentini *et al.*, 1984).

This area is also very important from the phyto-geographical viewpoint. Indeed, several endemic or rare species are found, such as *Helianthemum sicianorum*, *Reaumuria vermiculata*, *Hormuzakia aggregata*, *Rhus tripartita*, *Nonea vesicaria*, *Helianthemum lippii*, *Lobularia lybica*, *Retama raetam* subsp. *gussonei*, *Serapias orientalis* subsp. *siciliensis*, *Tuberaria villosissima* var. *sicula* and *Muscari gussonei* (Brullo *et al.*, 2007; Brullo & Sciandrello, 2006; Brullo *et al.*, 2011; Brullo *et al.*, 2013). According to the phytogeographic classification of Sicily (Brullo *et al.*, 2011), this area belongs to the Camarino-Pachinense district included in the southern Sicilian subsector together with the Hyblaean district. According to the bioclimatic classification proposed by Rivas-Martínez (1993, 2004), the study area is referred to the Mediterranean pluviseasonal oceanic bioclimate, with thermotypes ranging from

the lower thermomediterranean to upper thermomediterranean and ombrotypes from the lower semiarid to upper semiarid (Brullo *et al.*, 1996; Bazan *et al.*, 2015).

The study area includes five Sites of Community Interest (SCIs) of European Union Network “Natura 2000”, *i.e.* ITA050001 “Biviere e Macconi di Gela”, ITA050011 “Torre Manfredia”, ITA080003 “Vallata del F. Ippari (Pineta di Vittoria)”, ITA080004 “Punta Braccetto, Contrada Cammarana”, ITA080006 “Cava Randello, Passo Marinaro” and one Special Protection Area (SPA) ITA050012 “Torre Manfredia, Biviere e Piana di Gela”.

Sampling and statistical analysis

Literature focusing on the *M. gussonei* distribution range was reviewed. In addition, further dried specimens from the herbaria of Catania (CAT) and Palermo (PAL) were examined. Basing on these data, all the known sites with *M. gussonei* were visited over the period 2013-2015, and more potentially suitable sites for this species were searched. For the risk assessment, the IUCN protocol (2001) was followed according to the most recent guidelines. In particular for the extinction risk, the criterion B was applied by estimating trends in the Area of Occupancy (AOO) using a 2×2 km grid (IUCN, 2017a).

The field work, for examining structure, floristic composition and catenal contact of the surveyed plant communities, was carried out from 2013-2015, during

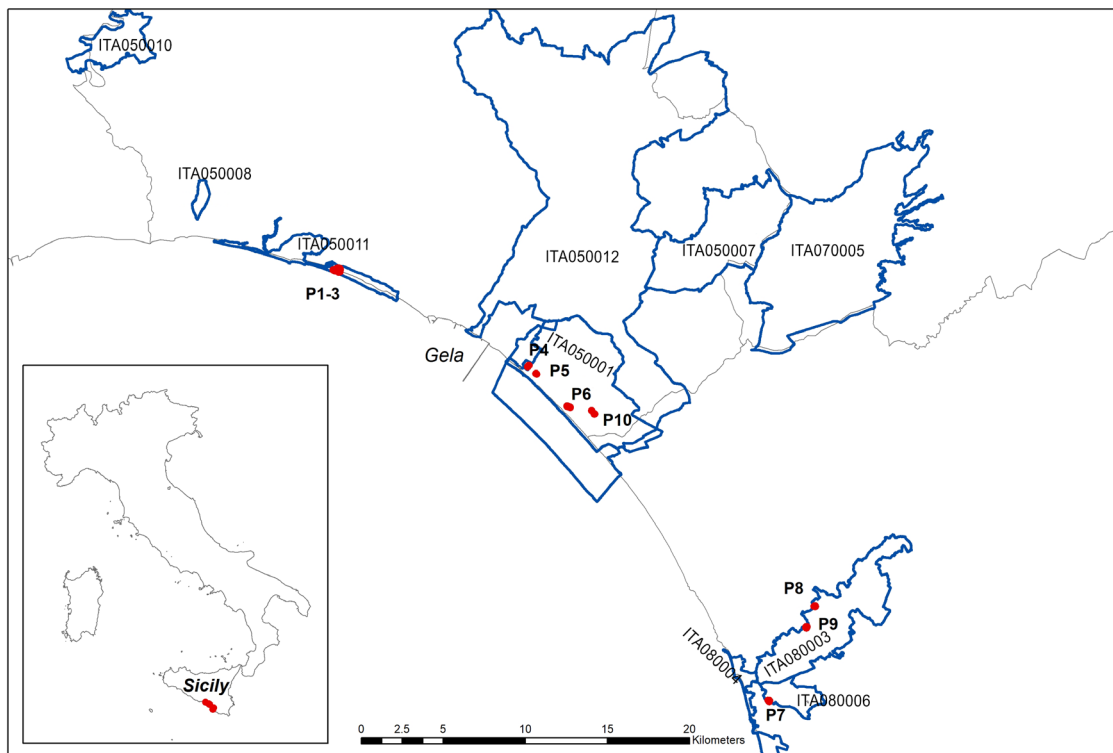


Fig. 1 - Study area and *Muscari gussonei* distribution (red dots) within Natura 2000 sites. Plots 1-3 Poggio Arena (ITA050011); Plot 4 Santa Lucia, Plot 5 Cava Cammarata, Plot 6 C.da Mignechi, Plot 10 Biviere di Gela (ITA050001); Plot 7 Passo Marinaro (ITA080006); Plot 8 C.da Gerbe, Plot 9 C.da Brancato (ITA080003).

which 41 randomly chosen relevés were performed (25-100 m²), including woody vegetation, if any. Moreover, 10 permanent plots (2 x 2 m), featured by the therophytic plant community *Vulpio-Leopoldietum gussonei*, were identified, geo-referred and phytosociologically characterized (Braun-Blanquet, 1964; Biondi, 2011). Ten soil samples (about 2 kg each) from each plot (between 0 and 15 cm depth) so as to represent a single sedimentation event, were dried at 110 °C for 24 hours, before being analyzed. The grain size analyses were performed by dry sieving for 10 min, using a set of 32 sieves with mesh sizes ranging from 4 to 0.06 mm (Wentworth, 1922).

Numerical analysis (Cluster Analysis - UPGMA method, Euclidean coefficient) was performed using the program package SYN-TAX 2000 (Podani, 2001). Environmental gradients influencing plant communities were examined with canonical component analysis (CCA), using PC-ORD (v4.34) software. Original Braun-Blanquet sampling scale has been transformed into the ordinal scale according to Van der Maarel (1979). Species nomenclature follows Giardina *et al.* (2007) and Raimondo & Spadaro (2009), while phytosociological nomenclature is based on Biondi *et al.* (2014). For the correlation between vegetation types and habitats we referred to the Italian Interpretation Manual for the Habitats of Directive 92/43/EEC (Biondi *et al.*, 2009).

Two indices were calculated for estimating the plant community diversity: (1) species richness of each vegetation cluster (SR), and (2) the Shannon-Wiener Diversity Index (H). The latter one takes into account the degree of equitability (J) of the species distribution (Morris *et al.*, 2014). Spearman rank correlation coefficients (r) were used to evaluate the importance of environmental factors in the distribution of the plant diversity (De Luca *et al.*, 2011; Hettenbergerová *et al.*, 2013). A p-value of 0.05 was taken as indicating a statistically significant difference.

Results

M. gussonei plant community

The floristic diversity of the randomly chosen 41 relevés account for 63 species of vascular plants (Tab. 1). Most species belong to Mediterranean chorological elements (60%), with the dominance of therophytes (46%), followed by hemicryptophytes (21%), chamaephytes (13%) and geophytes (11%). As one may expect, a lower floristic diversity characterizes the permanent plots; in fact, a total of 48 vascular species (x 10 permanent plots) were recorded. Whereas, structure and floristic composition of the permanent plots is more or less stable during the three years of monitoring (Tab. 2).

In its optimal habitat, *M. gussonei* is associated with

several annual psammophytes occurring on soils with high percentage of sand (83%) with a pH 7.63 (mean values) and low presence of organic matter (Tab. 3).

The results of the cluster analysis (41 random relevés) show two main vegetation groups, each supported by specific indicator species, because structural-dominant species of each plant community (Fig. 2). The first group (A) gathers the woody coastal plant communities dominated by *Retama raetam* subsp. *gussonei* and *Ephedra fragilis*, belonging to the *Asparago horridi-Retametum gussonei* (5330 “Thermo-Mediterranean and pre-desert scrub”), while the second group (B) is split into 2 sub-clusters: the first (B1) is characterized by a chamaephytic vegetation ascribed to the *Crucianellion maritimae* dominated by *Ononis hispanica* subsp. *ramosissima* on disturbed retrodunes (B1.1) or *Helianthemum lippii* on fossil/inner dunes (B1.2) (2210 “*Crucianellion maritimae* fixed beach dunes”), while the second (B2) is featured by annual plant communities dominated by *Muscari gussonei* (2230 “*Malcolmietalia* dune grasslands”).

The values of species richness and diversity index (Tab. 1; 63 species/41 relevés) indicate that the woody plant communities (cluster A) have a moderate diversity with an average species richness of 14 and an average Shannon-Wiener index (H) of 2.5 (J=0.95). Both these values slightly decrease in the chamaephytic vegetation with *Ononis hispanica* subsp. *ramosissima* (cluster B1.1) with an average of 12 species and H=2.36 (J=0.95). The values slightly increase both in the chamaephytic vegetation with *Helianthemum lippii* (cluster B1.2) with an average of 16 species and H=2.65 (J=0.96) and in the annual plant communities dominated by *M. gussonei* (B2) with an average of 16 species and H=2.66 (J=0.97).

As concerns our survey exclusively focused on the therophytic plant community dominated by *M. gusso-*

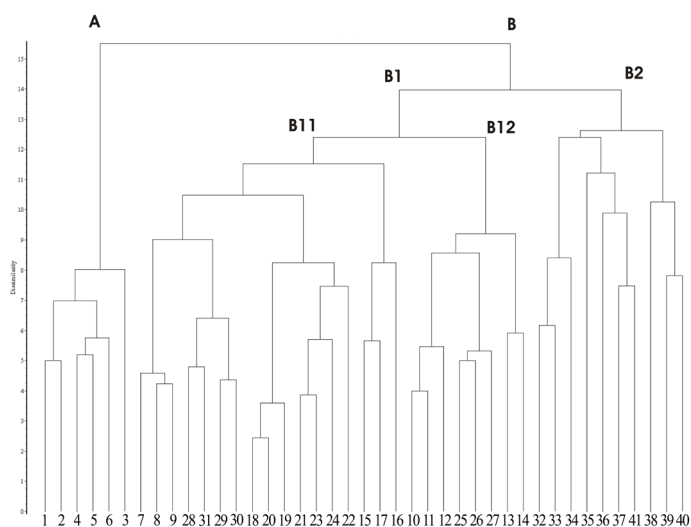


Fig. 2 - Cluster analysis (UPGMA euclidean) (41 random relevés).

Tab. 1 - Phytosociological surveys (41 relevés/63 species). Localities: PA (Poggio Arena), CC (Cava Cammarata), PV (Pineta di

		Localities													
		PA	PA	PA	PA	PA	PA	CC	CC	CC	CC	CC	CC	CC	
		1	2	3	4	5	6	10	11	12	13	14	15	16	
		100	100	100	50	50	50	50	50	50	50	50	50	50	
		90	90	90	90	95	90	85	85	80	85	80	85	80	
		25	10	15	15	20	20	35	30	25	30	30	30	30	
		S	S	S	S	S	S	SO	SO	SO	SO	SO	SO	SO	
		12	16	14	15	17	14	21	22	23	22	22	23	23	
		132	151	135	140	130	133	473	484	488	480	480	481	481	
		15	14	15	16	12	12	13	15	12	18	21	21	21	
		3	3	3	3	3	3	40	40	45	40	40	40	40	
		2.582	2.513	2.565	2.657	2.354	2.354	2.441	2.579	2.569	2.793	2.961	2.961	2.961	
		0.8819	0.8813	0.8668	0.891	0.877	0.877	0.8835	0.8788	0.8905	0.9072	0.9197	0.9197	0.9197	
		0.9536	0.9521	0.9472	0.9584	0.9472	0.9472	0.9517	0.9523	0.9534	0.9663	0.9725	0.9725	0.9725	
T scap	Med.	Euphorbia terracina L.	+	+	1	+	1	+	1	2	2	1	2	2	
T scap	Med.	Erodium laciniatum (Cav.) Willd.	.	.	+	+	+	1	1	+	1	+	1	+	
G bulb	End. cam.-pach.	Muscari gussonei (Parl.) Nyman	+	+	+	+	+	+	+	1	+	2	+	+	
H caesp	Med.	Ononis hispanica L. subsp. ramosissima (Desf.) Förther & Podlech	+	1	+	1	1	
H scap	Med.	Centauria sphaerocephala L.	1	1	2	1	+	+	+	+	+	1	+	+	
T scap	SO-Med.	Cutandia divaricata (Desf.) Asch. ex Barbey	+	+	+	+	+	
H bien	O Med.	Daucus carota L. subsp. maritimus (Lam.) Batt. in Batt. & Trab.	+	+	1	+	+	
Ch suffr	O Med.	Rhodalsine geniculata (Poir.) F. N. Williams	2	1	1	1	2	1	1	+	+	.	.	.	
T scap	Med.	Anisantha rigida (Roth) Hyl.	+	+	.	.	.	
H scap	Med.	Lobularia maritima (L.) Desv.	+	+	+	+	+	.	.	+	1	1	1	1	
Ch frut	S Med.	Launaea fragilis (Asso) Pau	+	1	1	1	
T scap	Med.	Rumex bucephalophorus L.	+	+	+	+	+	
H scap	Med.	Alkanna tinctoria Tausch	
P caesp	End. cam.-pach.	Retama raetana (Forsk.) Webb & Berth. subsp. gussonei (Webb) Greuter in Greuter & Raus	4	4	3	4	4	4	
T caesp	Med.	Vulpia fasciculata (Forssk.) Fritsch	
T scap	Med.	Lagurus ovatus L.	.	.	.	+	+	+	1	
T scap	Med.	Silene colorata Poir.	
H scap	O Med.	Cachrys libanotis L.	
Ch suffr	S Med.	Helianthemum lippii (L.) Dum.-Cours.	4	4	3	4	3	
H bien	Med.	Scolymus hispanicus L.	
T scap	Med.	Corynephorus divaricatus (Pourr.) Breistr.	
T scap	S Med.	Hormuzakia aggregata (Lehm.) Guşul.	+	+	+	
T scap	Med.-Iran.-Tur.	Senecio glaucus L. subsp. coronopifolius (Maire) C. Alexander	
NP	Med.	Asparagus acutifolius L.	+	+	+	+	
G rhiz	Med.	Asphodelus ramosus L.	
T scap	Med.-Iran.-Tur.	Brassica tournefortii Gouan	
T scap	Med.	Ononis diffusa Ten.	
H bien	C Med.	Seseli tortuosum L. subsp. maritimum (Guss.) Brullo C, Brullo, Giusso & Sciandrello	
NP	Med.	Asparagus horridus L.	1	+	2	1	+	2	
Ch suffr	Nat. (Sudafri.)	Carobrotus edulis (L.) N. E. Br.	
H caesp	Med.-Trop.	Hyparrhenia hirta (L.) Stapf in Prain	
H caesp	Nat.	Saccharum spontaneum L. subsp. aegyptiacum (Willd.) Hack.	
T scap	Med.	Stipa capensis Thunb.	
G bulb	Med.	Charybdis paneracion (Steinh.) Speta	
G rhiz	Med.	Cyperus capitatus Vandel.	
G rhiz	Boreo-Trop.	Imperata cylindrica (L.) Raeusch.	
T scap	O Med.	Medicago italica (Mill.) Grande subsp. tornata (L.) Emb. & Maire	
T scap	Med.	Andryala integrifolia L.	
T scap	Cosmop.	Avena barbata Pott ex Link	
H ros	S Med.	Carlina gummifera (L.) Less.	
T scap	O Med.	Coronilla repanda (Poir.) Guss.	
NP caesp	Med.	Ephedra fragilis Desf.	2	1	4	2	1	1	
Ch suffr	Med.	Lotus creticus L.	
NP	Med.-Iran.-Tur.	Lycium intricatum Boiss.	1	2	2	1	1	+	
T scap	Med.	Maresia nana (DC.) Batt.	
T scap	End. sic.-pel.	Plantago afra L. subsp. zwierleinii (Nicotra) Brullo	
H scap	Med.	Bituminaria bituminosa (L.) E. H. Stirton	
Ch suffr	Med.	Crucianella maritima L.	
T scap	C Med.	Echium sabulicolum Pomel	
T scap	E Med.	Lotus halophilus Boiss. & Spruner	
G bulb	E Med.	Ornithogalum gussonei Ten.	
T scap	Med.	Polycarpon tetraphyllum (L.) L. subsp. diphyllum (Cav.) O. Bolòs & Font Quer	
T scap	Med.	Urtica membranacea Poir. in Lam.	+	+	
Ch suffr	Med.	Ajuga iva (L.) Schreb.	
H bien	Med.	Asphodelus fistulosus L.	
T scap	Med.	Centaurium tenuiflorum (Hoffmanns. & Link) Fritsch in Mitt.	
T scap	Med.-Iran.-Tur.	Dasyphyllum villosum (L.) P. Candargy	
T scap	Med.	Medicago littoralis Rohde ex Loisel.	
G rhiz	Cosmop.	Phragmites australis (Cav.) Trin. ex Steud.	
Ch frut	Med.	Prasium majus L.	
T scap	Med.	Pseudorhiza pumila (L.) Grande	
NP	Nat.	Rumex lunaria L.	+	
T scap	End. cam.-pach.	Torilis nemoralis (Brullo) Brullo & Giusso	

nei, it was possible to highlight some relevant floristic variations strictly linked to the ecological gradients, thus confirming the high variability detected for the psam-mophilous therophytic communities from the whole Mediterranean area (Diez Garretas et al., 2003; Costa et al., 2011; Tomaselli et al., 2011; Conti et al., 2017).

Our assumption of differentiation of the *M. gusso-nei* ephemeral plant community (*Vulpio-Leopoldietum*

gussonei), along a gradient of ecological conditions ranging from the coast to inner areas, highlighted by the floristic diversity, is better supported by inserting some ecological parameters and performing a canonical component analysis (CCA).

The result of the CCA performed on the permanent plots (48 taxa/10 plots) (Tab. 2), shows a main gradient of distance of the sea, floristic richness and altitude on

Tab. 3 - Soil analysis of the permanent plots.

Plot	Site	Clay (%)	Silt (%)	Sand (%)	pH	CE (mS/cm)	Nitrogen (%)	Organic matter	Total limestone (%)	Mg (ppm)	Na (ppm)	K (ppm)	Ca (ppm)
1	Poggio Arena	3.45	7.85	88.7	7.32	65	0.08	1.5	18	0.01	0.02	0.09	0.49
2	Poggio Arena	9.15	5.85	85	7.28	56.4	0.04	0.8	18	0.01	0.01	0.11	0.3
3	Poggio Arena	6.8	9.85	83.35	7.31	62	0.04	0.7	18	0.01	0.01	0.13	0.3
4	Santa Lucia	12.6	5.2	82.2	7.88	70.7	0.06	1.1	19	0.01	0.01	0.14	0.39
5	Cava Cammarata	10.5	15	74.5	7.9	74.1	0.06	1.2	18	0.01	0.01	0.15	0.36
6	Mighechi	11.65	4.95	83.4	7.88	65.8	0.02	0.4	16	0.01	0.01	0.16	0.26
7	Passo Marinaro	11.2	5.65	83.15	7.83	72.7	0.07	1.5	16	0.01	0.05	0.18	0.74
8	Pineta Vittoria	7.2	9.85	82.95	7.92	58.8	0.06	1.1	10	0.01	0.01	0.18	0.34
9	Pineta Vittoria	4.3	2.7	93	7.91	60	0.04	0.8	14	0.01	0.01	0.19	0.42
10	Biviere Gela	7.05	18.85	74.1	7.1	62.3	0.04	0.7	18	0.01	0.01	0.19	0.49

fied by the number of the third column in Tab. 2). Thus, some species, as *Muscari gussonei*, *Vulpia fasciculata*, *Erodium laciniatum*, *Cutandia divaricata*, *Anisantha rigida*, occupy a central position revealing a wider ecological amplitude and they are common in the entire macro-community; on the contrary, at the extremities of the axes we can see several species with a narrow ecological niche characterizing the three different habitats, such as *Maresia nana*, *Brassica tournefortii* and *Hormuzakia aggregata* for the white dunes, *Plantago afra* subsp. *zwierleinii*, *Coronilla repanda* and *Alkanna tinctoria* for inner dunes.

The Spearman correlation shows a significant positive correlation between species richness and altitude

($r = 0.78$; $p < 0.05$), as well as for the sea distance ($r = 0.66$). In addition, the numerical abundance of *Muscari gussonei* individuals is negatively correlated to altitude ($r = -0.76$; $p < 0.05$) and distance to the sea ($r = 0.93$; $p < 0.05$).

Distribution range, population structure and size

M. gussonei disappeared from many sites where it was recorded in the past, such as Sampieri, Marzame-mi, Portopalo, Marina della Marza, S. Maria del Focallo, Capo Isola delle Correnti, Macchitella di Gela, etc.

Our investigations allowed to confirm its occurrence just in 4 macro-sites (Fig. 4): 1. Poggio Arena (Gela); 2. Macconi di Gela (a. Santa Lucia, b. Cava Cammara-

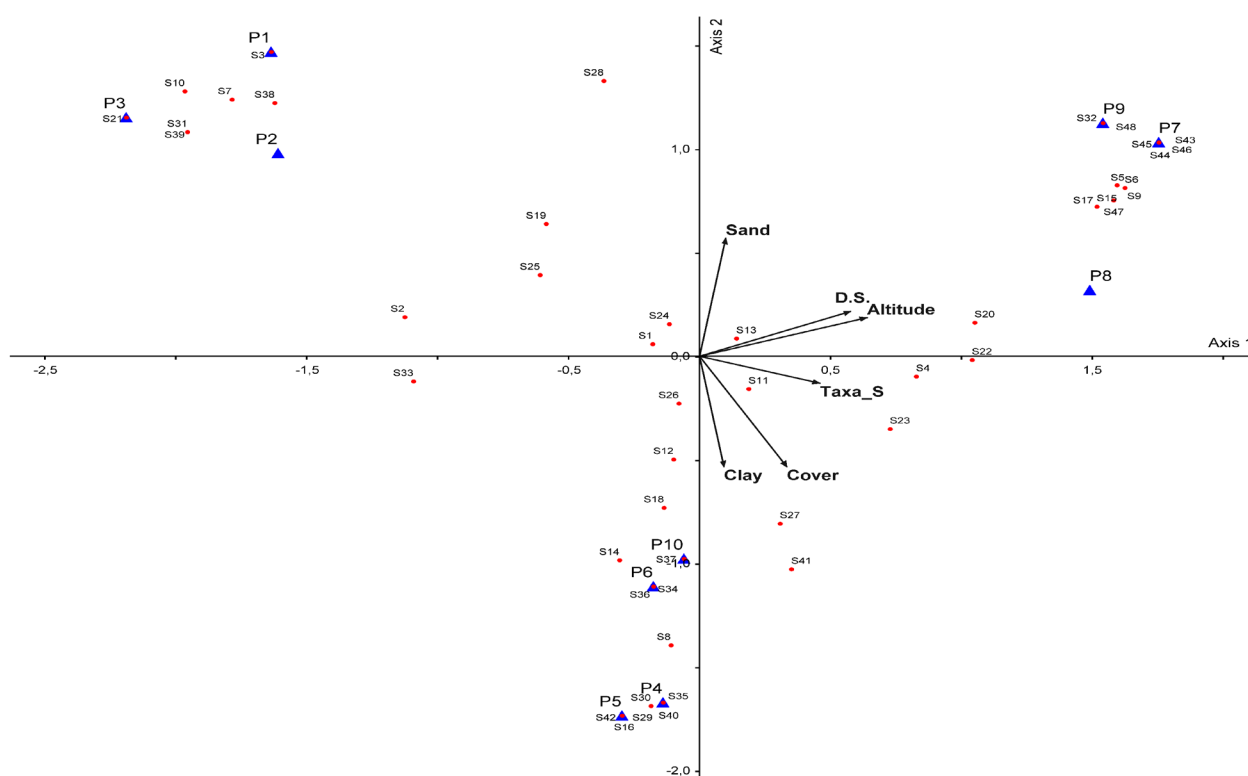


Fig. 3 - CCA (10 plots). Total variance ("inertia") in the species data: 1.8094. Eigen-value Axis 1, 0.42; Axis 2, 0.34; Axis 3, 0.25. Variance in species data % of variance explained Axis 1, 23.4; Axis 2, 19.1; Axis 3, 14.2. Cumulative % explained Axis 1, 23.4; Axis 2, 42.5; Axis 3, 56.7. Each species is identified with the same number of Tab. 2. Acronyms: P= Plot (1-10, see Fig. 1), D.S. = distance of the sea; Taxa_S = floristic richness; Cover = vegetation cover.

ta, c. Industrial site, d. Biviere di Gela, e. C.da Mignechi); 3. Ragusa (a. Passo Marinaro, b. Cava Randello, c. Cammarana, d. Refriscolaro, e. Branco Piccolo); 4. Pineta di Vittoria (a. C.da Brancato, b. C.da Gerbe).

The site hosting the largest population (about 1,500 mature individuals spread over an area of 5,000 m², is Poggio Arena (site 1), a coastal dunal system (about 45 m high) close to the sea rather well preserved. The population from Macconi di Gela (site 2) is really fragmented mainly due to the intensive agricultural activities. Currently, a total of five sub-populations were identified within site 2: a. Santa Lucia with about 120 plants in an area of about 800 m²; b. Cava Cammarata with approximately 340 plants in a small area of about 500 m² whose survival is severely threatened by quarrying activities; c. Industrial site with approximately 50 plants in an area of about 810 m²; d. Biviere di Gela with about 50 plants in an area of about 500 m²; e. C.da Mignechi with about 420 plants in an area of 690 m². The just mentioned areas were, in the past, the most important sites with *M. gussonei*, as highlighted by Garbari & Di Martino (1972). In addition to the above mentioned threats, site 2 is seriously threatened by two invasive plants, namely *Carpobrotus edulis* (L.) N.E. Br. and *Saccharum spontaneum* L. subsp. *aegyptiacum* (Willd.) Hack. The Cava Randello-Punta Braccetto population (site 3) is also very disturbed, particularly for the intensive agricultural activities and overgrazing. A total of five sub-populations were surveyed within site 3: a. Passo Marinaro, 80 mature individuals spread over an area of about 710 m², severely disturbed by rabbits; b. Cava Randello, less than 70 plants were

recorded in an area of about 800 m²; c. Cammarana, 60 plants were found in 800 m²; d. Refriscolaro, 50 plants were surveyed in 700 m²; e. Branco Piccolo, 30 plants spread over an area of about 480 m².

Lastly, the Pineta di Vittoria population (site 4) is the innermost stand, localized at 50 m a.s.l., about 6 km far from the coast. This is the only population which seems to be in a phase of recovery, likely due to the crop reduction. In particular, less than 820 mature individuals were recorded in C.da Brancato (820 m²), but the abandonment of cultivated fields is favouring the expansion of *M. gussonei* populations, while in C.da Gerbe about 80 plants were surveyed in an area of 500 m², interspersed with the bushes of *Retama raetam* subsp. *gussonei*.

Considering the data about the current distribution of *M. gussonei*, more than 50% of the current population can be found at Poggio Arena (Fig. 4, site 1). In the absence of disturbing factors, the number of plants growing at Poggio Arena is destined to increase, especially for the optimal conditions of the site and the number of mature individuals. Whereas, the Macconi di Gela population is declining. The main disturbance is the continuous physical modification of the dunes chiefly made for creating new areas suitable for cultivations or service roads, all activities that lead to the a drastic reduction and fragmentation of the natural habitat of *M. gussonei*. Furthermore, the overuse of nitrates favours some invasive species, as *Saccharum spontaneum* subsp. *aegyptiacum* and *Carpobrotus edulis*, thus representing another serious threat. The population from Cava Randello-Punta Braccetto is also strongly altered. In fact, this population is also declining due to overgrazing, fires, and rabbits. Only for the for the Pineta di Vittoria population a positive trend has been detected, being the general ecological conditions of the area slightly improving (Tab. 4).

According to the IUCN Red List categories and criteria (2001), the population of *M. gussonei* occupies an area (AOO) of about 40 km² (4 location, Fig. 4). Thus, considering the small population size, the distance among different populations, as well as the several threats, we may conclude that the distribution area of *M. gussonei* is highly fragmented and at risk of further reduction.

The current conservation status of *M. gussonei*, according to Conti *et al.* (1997) is endangered (EN). Based on the field investigations carried out in the present survey, we confirm the IUCN rank proposed by Brullo *et al.* (2010), EN B2 ab (ii, iii, iv).

Discussion

Almost 2 centuries ago, Gussone (1827) recorded *M. gussonei* along the sandy coast of Sicily, from Gela to Capo Passero (southern-easternmost point of Sicily). During the last decades, southern Sicily has been dra-

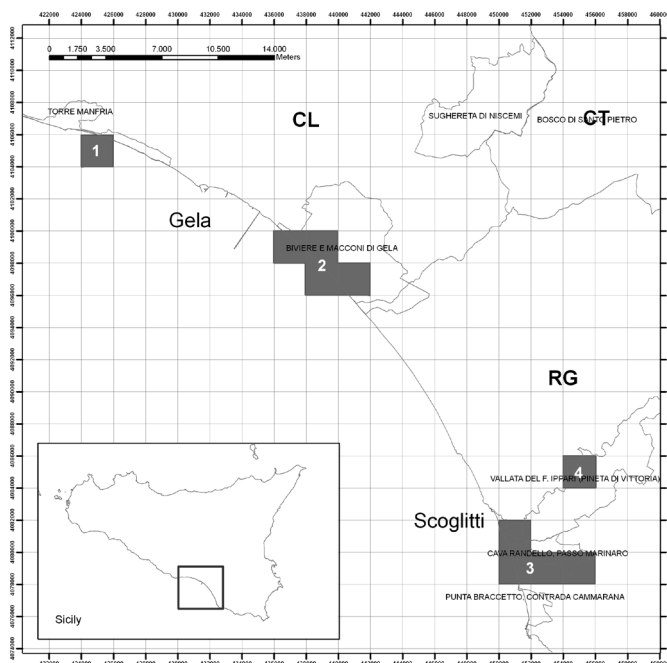


Fig. 4 - Geographical distribution (AOO according to IUCN 2017a) of *Muscari gussonei* (grid of 2 x 2 km).

Tab. 4 - The four locations where *M. gussonei* has been located, with number of individuals, area, altitude range and identified threats (IUCN, 2017b).

Locality	No. <i>M. gussonei</i>	Area m ²	Altitude (m)	Threats
1 Poggio Arena (Manfria, Gela)	1,500	5,000	6-10	1.3 Tourism & recreation areas; 2.3.1 Nomadic grazing; 6.1 Recreational activities; 7.1.1 Increase in fire frequency/intensity; 8.1 Invasive non-native/alien species (<i>Saccharum spontaneum</i> , <i>Carpobrotus edulis</i> , <i>Acacia saligna</i>)
2 Macconi Gela	980	3,300	10-22	2.1.3 Agro-industry farming (greenhouse cultivation); 8.1 Invasive non-native/alien species (<i>Saccharum spontaneum</i> , <i>Carpobrotus edulis</i> , <i>Acacia saligna</i>)
3 Passo Marinaro (Ragusa)	290	3,490	32	2.1.3 Agro-industry farming (greenhouse cultivation); 8.1 Invasive non-native/alien species (<i>Saccharum spontaneum</i> , <i>Carpobrotus edulis</i>)
4 Pineta Vittoria	900	1,300	52-58	2.3.1 Nomadic grazing; 7.1.3 Fire; 8.1 Invasive non-native/alien species (<i>Saccharum spontaneum</i>)

matically modified, especially due to agricultural over-exploitation (e.g. glasshouses) and urban sprawling. Unfortunately, we have not enough and reliable historical data to precisely quantify the population declining for the species, but the high fragmentation of the small and few-numbered populations suggests that *M. gussonei* has suffered a drastic reduction, especially over the past 50 years; probably the currently existing sites may constitute a remnant of a once larger population.

Our surveys allowed to record the presence of about 3,700 mature individuals of *M. gussonei* that are still surviving, despite the above-mentioned threats. Currently, they are unevenly distributed on areas ranging from 200 to 5,000 m², at altitudes of 10-85 m a.s.l., on south- to north-facing slopes (0-30°), close to the sea up to 6,000 m towards the inner stands (Tab. 1).

Our study analyses the floristic composition of the *M. gussonei* plant community and clarifies some ecological requirements which are relevant issues for future conservation measures to be implemented in order to preserve such an endangered species.

From the sinecological point of view, according to Brullo & Marcenò (1974), Brullo & Grillo (1985), *Muscari gussonei* chiefly grows in a plant community, named *Vulpio-Leopoldietum gussonei*, typical of semi-fixed or fixed dunes. This association, dominated by annual species, belongs to the *Laguro ovati-Vulpion fasciculatae* Géhu and Biondi 1994, alliance of order *Cutandietalia maritimae* Rivas-Martínez, Diez-Garretas & Asensi 2002 (Minissale & Sciandrello, 2015). As displayed by our cluster analysis the association grows in different closely related sandy habitats represented by the clearings within the shrubby vegetation (*Asparago horridi-Retametum gussonei* Brullo, Guarino & Ronsisvalle 2000) or woody communities (*Ephedro fragilis-Juniperetum macrocarpae* Bartolo, Brullo & Marcenò 1982). It is also found in the clearings of the *Centaureo-Ononidietum ramosissimae* Br.-Bl. & Frei in Frei 1937, chamaephytic association characterized by *Ononis hispanica* subsp. *ramosissima*.

A more in-depth analysis within the association by the classification of plots (10), using CCA, allowed to highlight three clear groups within the *M. gusso-*

nei community (*Vulpio-Leopoldietum gussonei*), each one with typical species useful to characterize the community as a result of small changes in ecological conditions, as proposed by Biondi (2011). The indicator species for each sub-community are the following: *Maresia nana*, *Brassica tournefortii* and *Hormuzakia aggregata* (instable dunes), *Anisantha rigida* and *Cutandia divaricata* (stable dunes), and *Plantago afra* subsp. *zwierleinii*, *Coronilla repanda* and *Alkanna tinctoria* (inland o fossil dunes).

Moreover, the classification of relevés (wider plots), using cluster analysis (41), displayed three vegetation types where *M. gussonei* grows. The annual plant communities dominated by *Muscari gussonei* (*Vulpio-Leopoldietum gussonei* - 2230 *Malcolmietalia* dune grasslands), woody psammophilous plant communities dominated by *Retama raetam* subsp. *gussonei* (*Asparago-Retametum gussonei* - 5330 Thermo-Mediterranean and pre-desert scrub), and, finally, chamaephytic vegetation with *Ononis hispanica* subsp. *ramosissima* or *Helianthemum lippii* (*Centaureo-Ononidietum ramosissimae*, *Helianthemum lippii* comm. - 2210 *Crucianellion maritimae* fixed beach dunes). These plant communities are related to the vegetation series outlined for the south-eastern Sicily by Bazan *et al.* (2010) and Minissale & Sciandrello (2013).

Concluding, the dune ecosystem of southern Sicily, despite falling within several SCIs, is severely threatened by intensive agriculture, urban sprawl and invasion of alien plants that often lead to the full destruction of these habitats (Campos *et al.*, 2004; Sciandrello *et al.*, 2015). On the basis of these considerations, it is evident that the conservation measures taken so far have been insufficient and it is therefore necessary to act more effectively. Some potential actions that could be implemented in order to preserve the surviving populations of *M. gussonei* are: 1) to enable a better management of SCIs sites; 2) to start an intensive monitoring plan of the populations; 3) to eradicate invasive plants, such as *Carpobrotus edulis*, *Saccharum spontaneum* subsp. *aegyptiacum*, or *Arundo donax*; 4) to create ecological corridors among different populations (Vandepitte *et al.*, 2012); 5) to seed sampling

to reinforce the natural populations or translocate *M. gussonei* to other suitable growing sites. Some of these actions have been implemented during the LIFE project (LIFE11 NAT/IT/000232) at the SCI “Biviere e Macconi di Gela” and SCI “Punta Braccetto, Contrada Cammarana” or during the CARE-MEDIFLORA pro-

ject (Fenu *et al.*, 2017b).

In any case, the presence of protected areas is crucial for ensuring the survival and numerical growth of target species and habitats (Fois *et al.*, 2018), as also Prisco *et al.* (2016) highlighted for the Italian sandy coastal environments.

Syntaxonomic scheme

QUERCETEA ILICIS Br.-Bl. ex A. & O. Bolòs 1947

PISTACIO LENTISCI-RHAMNETALIA ALATERNI Rivas-Martínez 1975

Oleo-Ceratonion Br.Bl.1936 em. Rivas-Martínez 1975

Asparago horridi-Retametum gussonei Brullo, Guarino & Ronsisvalle 2000

Juniperion turbinatae Rivas-Martínez 1975 corr. 1987

Ephedro fragilis-Juniperetum macrocarpae Bartolo, Brullo & Marcenò 1982

HELICHRYSO-CRUCIANELLETEA MARITIMAE (Sissingh 1974) Géhu, Rivas-Martínez & Tüxen in Géhu 1975 em. Biondi & Géhu in Géhu & Biondi 1994

CRUCIANELLETTALIA MARITIMAE Sissingh 1974

Crucianellion maritimae Rivas Goday & Rivas-Martínez 1958

Centaureo-Ononidietum ramosissimae Br.-Bl. & Frei in Frei 1937

Helianthemum lippi comm.

TUBERARIETEA GUTTATAE (Br.-Bl. in Br.-Bl. & al. 1952) Rivas Goday & Rivas-Martínez 1963

CUTANDIETALIA MARITIMAE Rivas-Martínez, Díez-Garretas & Asensi 2002

Laguro ovati-Vulpion fasciculatae Géhu & Biondi 1994

Vulpio fasciculatae-Leopoldietum gussonei Brullo & Marcenò 1974

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Revealing the taxonomy of an endemic oak of Lebanon

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Abstract

The taxonomy of oaks in Lebanon relies on the works of Mouterde (1966). Since half a century, there were no taxonomic revision for these species, namely for *Quercus pinnatifida* Gmelin, which is no more in use worldwide after successive nomenclature revisions. This name was replaced by *Quercus pubescens* Willd. subsp. *pubescens* which has never been recorded in Lebanon. However, *Quercus kotschyana* O. Schwarz is cited as an endemic species of Lebanon, but it was never mentioned by Mouterde works, nor by the literature that followed (i.e. Abi Saleh, 1976, 1996; Menitsky, 2005; Tohme & Tohme, 2014). This work aims at revealing the taxonomy of an endemic oak of Lebanon, and clarify the existing confusion in the nomenclature of species. The comparison of collected plant material from different sites, with the holotype of *Quercus kotschyana*, and the botanical description and drawings found in the literature, allowed us to conclude that *Quercus pinnatifida* Gmel. was wrongly attributed to the taxon found in Lebanon, and that the actual present species is *Quercus kotschyana* O. Schwarz.

Key words: Lebanon, *Quercus kotschyana*, *Quercus pubescens*, *Quercus pinnatifida*, taxonomy.

Introduction

The flora of Lebanon relies on the works of Mouterde who elaborated an exhaustive inventory of flora for Lebanon and Syria (Mouterde, 1966). Amongst the cited oak species, the author mentions *Quercus pinnatifida* Gmelin and provides the synonym *Quercus lanuginosa* Willd. var. *pinnatifida* (C.C. Gmel.). However, this taxon, was initially described in Germany from a single individual, bearing no fruits, and considered as a variety of *Quercus lanuginosa* (Mouterde, 1966; Gmelin, 1826). Moreover, Abi Saleh *et al.* (1996), then Tohme & Tohme (2014), do not go in-depth in the taxonomy of the species, and follow the same nomenclature and describe the presence of *Quercus pinnatifida* in Lebanon, on the western slopes of northern Mount Lebanon (in the following locations: Danniyeh, Ehden, cedars of Bsherre, Tannourine cedars, and Khan Sannine). The habitat corresponds to the Mediterranean montane and Supra Mediterranean vegetation stages. The species is associated to the cedar vegetation series (Abi Saleh *et al.* 1976).

Nonetheless, *Quercus pinnatifida* was forgotten due to several recombination and nomenclature revision as noted by Bussotti & Grossoni (1998). According to Govaerts & Frodin (1998), *Quercus pinnatifida* is no more in use, and is considered as a synonym of *Quercus pubescens* Willd. subsp. *pubescens*, a subspecies of *Quercus pubescens* (Willdenow, 1805) present in Europe and Turkey but never mentioned in Lebanon (Roskov *et al.*, 2015).

In a different context, the IPNI cites *Quercus kotschyana* O. Schwarz as an oak species of Lebanon. It was

initially named by Kotschy *Quercus tauzin*, a synonym of *Quercus pyrenaica* Willd. (Willdenow, 1805; Kotschy, 1862; Schwarz, 1935). Govaerts & Frodin (1998) quote it as a probable hybrid of *Quercus pubescens* and *Quercus cerris* L. with a certain similitude with *Quercus vulcanica* Boiss. & Heldr. ex Kotschy. The red list of oaks cites *Quercus kotschyana* as an endemic species to Lebanon that requires assessment, and stresses out the lack of information about this species (Eastwood & Oldfield, 2007). Nonetheless, *Quercus kotschyana* is not mentioned by Mouterde (1966), Tohme & Tohme (2014) or Menitsky (2005). According to Bussotti & Grossoni (1998), it was cited by Camus (1930–1936) under his list of European and Mediterranean oaks, under the *Quercus* L. subgenus, *Quercus* section (*Lepidobalanus* Endl.), as a species endemic to Lebanon.

Subsequently, the objective of this communication is to clarify the following questions:

- i) Is *Quercus pubescens* subsp. *pubescens* present in Lebanon, or is it *Quercus kotschyana*?
- ii) Are there two different species, or is there a certain confusion in the taxonomy?

Material and methods

The analysis relied on both taxonomic revision using the botanical description of species and the respective provided drawings, from different references namely:

The scanned holotype of the material of *Quercus kotschyana* (i.e. the holotype [Schwarz, 1935] —LEBANON, Bsherre: near cedars, 28 July, 1855, S-G-5164 (S!)) that was provided by the Natural History Museum

of Sweden and (Schwartz (1935) the botanical description and illustrations of *Quercus kotschyana* from Schwarz (1935).

The botanical description and illustrations of *Quercus pinnatifida* from Mouterde (1966).

Assuming that *Quercus pinnatifida* is a synonym of *Quercus pubescens* subsp. *pubescens*, as previously mentioned, we compared the botanical description of both *taxa* with each other and further with *Quercus kotschyana*.

In October 2015 we conducted a field survey to sample leaves and fruits of this *taxon* (under both nominations of *Quercus pinnatifida* and *Quercus kotschyana*) in order to compare them with the holotype and drawings, and with their area of distribution with the literature, including the sites mentioned by Schwartz (1935), Mouterde (1966) and Tohme & Tohme (2014).

In Figures 1 and 2 we show respectively, the holotype scanned leaves and the leaves of the collected material.

Results and discussion

In regard to *Quercus pinnatifida* found in Lebanon, Mouterde (1966) described it as close to *Quercus cerris* and *Quercus cedrorum* Kotschy: “very close to *Quercus cedrorum*, leaf lobes often slightly sharp and very shortly mucronate, often themselves lobules-dentate, separated from each other by deep sinuses, which often enter until very near the midrib itself (remininding those of *Quercus cerris*). Leaves are polymorph, hairless or glabrescent at maturity, but strongly pubes-

cent when young. Branches are often fairly bright red. Acorns very similar to those of *Quercus cedrorum* that can also achieve strong growth”. Moreover, the author described the acorns of *Quercus cedrorum*: “Cupule with appressed scales, gray, strongly exceeding the acorns which can be very large”.

In the Flora of Turkey (Hedge & Yaltirik, 2008), *Quercus pubescens* leaves are described as “most variable but generally oblong-obovate, greyish green above, brownish gray beneath, asymmetrically subcordate or rounded, thick-textured, with 3-6 strongly undulate forwardly-pointing irregular acute lobes with revolute margins; indumentum densely to thinly stellate-tomentose beneath, with many scattered minute stellate hairs above”. As for the fruits, they are described as “cupule shallow to 15 mm diameter; scales appressed, lanceolate, pubescent, brownish-grey; acorn 2/3 exerted”. The authors cited that the typical form of this *taxon* is characterized by “short petioles, and undulate-margined, lobed, greyish leaves. These persist on the trees in a dried state over winter”.

Quercus pubescens is part of the Mediterranean evergreen coppice in Europe, while in Turkey it makes part of steppic or semi-steppic vegetation of Anatolia, at an elevation reaching 1700 m (Hedge & Yaltirik, 2008). However, this species and its associated vegetation series are not found in the Lebanese flora.

Drawings of leaves of both *taxa*, in those two respective references show large discrepancy.

In respect to *Quercus kotschyana*, the leaves are “moderate or small, with remotely inserted stipules,



Fig. 1 - The scanned leaves of *Quercus kotschyana* holotype from the National History Museum, Sweden.



Fig. 2 - The scanned leaves of *Quercus kotschyana* collected from Bsherre in October 2015.

glabrous, soon deciduous, petiolate or sub-sessile". Petioles are "slender, 1.5-2.7 cm long, non-channeled and with stellate hairs". The blade is "strongly leathery, 6-12 cm long, 3.2-6.5 cm wide, round-oval to oblong lanceolate, a little above the base, sinuses are narrow acute cutting almost down to the to the mid-rib. Lobes are broadly linear, acuminate, with distinct sinuate margins". Fruits are "in peduncles 1.5-5 cm long, thick, tomentose, crammed in short racemes 2-6, at the axis of the apical leaves. The cupule is hemi-ellipsoidal with frequent scales dense gray-tomentose linear-lanceolate" (Schwarz, 1935). Additional locations where the *taxon* is found, were also cited: "Danniye above Floa valley, above Ehden, at an elevation of 5,000 feet" (above 1,500 m).

The description and illustrations of *Quercus kotschyana* as provided by Schwarz (1935) as well as the scanned holotype kindly provided by the Natural History Museum of Sweden match with *Quercus pinnatifida* as described and drawn by Mouterde (1966), while showing a large discrepancy with those of *Quercus pubescens*.

We compared the botanical description and drawings of *Quercus kotschyana* with those of *Quercus vulcanica*, and found that they are different. This conclusion converges with Jablonski (2016) who cites that the latter species is different from *Quercus kotschyana* found in Lebanon.

Moreover, the locations of the collected material as mentioned in the consulted holotype and by Schwarz (1935) converge with those mentioned by Mouterde (1966) and Tohme & Tohme (2014), in sites where only one oak *taxon* is present (i.e. in Bsharre, near the cedars, where the holotype is collected from).

Additional sites were identified (Arz Jaj, Jord Aqoura, and Jord Afqa). Initial results confirmed the botanical description, the area of distribution as well as the presence of one single species that is *Quercus kotschyana*, namely from Bsharre where the holotype was collected from (Schwarz, 1935).

Conclusions

Based on the above, the *taxon* found in Lebanon cannot be attributed to *Quercus pubescens* found in Turkey and Europe, as their area of distribution are not the same, their habitat is different, as it occurs in Lebanon between 1,500 and 2,000 m in more sub-humid conditions, and in association with a different set of species that are more adapted to sub-humid conditions (Stephan *et al.*, 2016; Abi Saleh *et al.*, 1996; Mouterde, 1966). Whereas *Quercus pubescens* is more distributed into different bioclimatic zones. Moreover, leaf and cupule description show a certain discrepancy between both species, namely the deep sinuses and feathery upper blade of the Lebanese *taxon*, and the

thick-textured blade and hairy beneath with forwardly-pointing irregular acute lobes of *Quercus pubescens* (Govaerts & Frodin, 1998; Menitsky, 2005; Hedge & Yaltirik, 2008).

These results show with clearness that *Quercus pinnatifida* Gmel. was wrongly attributed to the *taxon* found in Lebanon, and that the actual present species is *Quercus kotschyana* O. Schwarz. Further in-depth morphometric and molecular analysis will be conducted to characterize this endemic oak of Lebanon, and conduct the IUCN red listing assessment and a sound action plan for its conservation.

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Active management actions for the conservation of the endangered Mediterranean island flora: the CARE-MEDIFLORA project

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Abstract

The Mediterranean Basin is one of the world's most biodiverse regions and it roughly counts 30,000 different plant *taxa*, of which approximately 50% are endemic *taxa* to the region. Thus, this area has been recognized among the world's 34 biodiversity hotspots. Furthermore, the rate of endemism of the big Mediterranean islands is higher than that usually recorded in the neighbouring mainland areas. Plants are vulnerable to many threats mainly represented by physical factors, such as climate change, extreme weather events, recurrent fires, agriculture, as well as by biological factors, such as invasive species and pests. All these factors are particularly worrying in island ecosystems where urban sprawl and human activities may represent a major source of threat hampering the preservation of important habitats and plant species, especially when circumscribed to small areas. In addition, less than 10% of these areas is protected (*e.g.* nature reserves, regional or national parks, *etc.*) and, likely most worrying, their management is not always based on the specific scientifically based plant needs. Given these circumstances, many plant species of the Mediterranean area are facing the risk of a severe decline and require urgent protection measures. While *in-situ* conservation is the fundamental approach to biodiversity conservation, *ex-situ* conservation is an alternative and effective way to prevent immediate extinction. The CARE-MEDIFLORA project, an initiative of eight institutions all having a long experience in plant conservation, will make a step forward by using *ex situ* collections to experiment with *in situ* active management actions and measures for some *taxa* within the period of three years of the project. The involved institutions will jointly work to address both short-term and long-term needs, including: (1) *in situ* conservation for some of the most endangered plant species of the Mediterranean islands through active management actions (*e.g.* reintroduction, reinforcement, fencing, *etc.*), in collaboration with the most relevant local authorities to ensure the sustainability of the results; (2) *ex situ* conservation of the most endangered plant species of the Mediterranean islands through the collection and seed banking of accessions that will be representative of the overall diversity of the selected *taxa*; (3) establishing a network connecting scientific institutions from the Mediterranean islands in order to ensure the circulation of information, knowledge and project results sustainability. In addition, great efforts will be devoted to the training of conservation plant specialists, in order to increase collaboration among institutions dealing with *in situ* and *ex situ* conservation and to increase awareness about the vulnerability of the native flora through the involvement of local stakeholders and environment-related agencies.

Key words: alien species eradication, *ex situ* conservation, fence erection, *in situ* conservation, Mediterranean islands, plant translocations, seed banking, threatened Mediterranean flora.

Introduction

The Mediterranean Basin is an important center of plant diversity since, in only 1.6% of the Earth's surface, it hosts almost 10% of the world's plants; for this reason, it has been identified as one of the 34 global biodiversity hotspots (Mittermeier *et al.*, 2005). The extremely high rate of regional endemism is likely the most striking feature of the Mediterranean flora, with approximately 60% of all native *taxa* being Mediterranean endemics, half of which corresponding

to narrow endemic species (Thompson, 2005). This outstanding biodiversity is chiefly due to the unique paleogeographical, geological, and climatic history of the Mediterranean (*e.g.* Nieto Feliner, 2014). Actually, it lies at the intersection of the Eurasian and African landmasses, and is characterized by a noteworthy geomorphological and pedological variability. Furthermore, the Mediterranean sea shows some other peculiar conditions, being a semi-enclosed basin surrounded by a complex orography, which strongly affects the local climate and causes relevant interac-

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tions and feed-backs among ocean-atmosphere-land processes; all these factors, together with other characteristics of the sea (e.g. high water temperature and salinity, very limited tides, waves and meteorological phenomena) make the Mediterranean climatology really peculiar, also for its relevant annual and seasonal variability.

Plant diversity is, however, not evenly distributed. Actually, in areas featured by a high biodiversity, as the insular mountain ranges, the endemic-plant richness largely depends on environmental conditions and it was possible to identify hierarchically arranged hotspots within hotspots (Cañadas *et al.*, 2014). In fact, within the Mediterranean Basin, 12 main meso-hotspots, accounting for roughly 44% of the endemic flora, have been recognized (Médail & Quézel, 1997, 1999); among these, all the large Mediterranean islands (i.e. Sicily, Sardinia, Cyprus, Corsica and Crete) and Balearic Archipelago play a relevant role, reaching more than 40% of endemism.

Mediterranean islands are defined as continental and oceanic islands, respectively, based on their different geological history. The first ones are the result of the fragmentation of continental plates (Rosenbaum *et al.*, 2002), while the second ones have originated as a consequence of a volcanic activity (Guillou *et al.*, 2004). Also for this reason, they are floristically rather diverse being, at least partly, the result of different processes occurred during their evolution. Despite their isolation and the crucial role played by the Mediterranean islands as climatic *refugia* (Médail & Diadema, 2009; Gentili *et al.*, 2015), some similarities are still shared with the floras of the surrounding mainland areas.

Nowadays, the Mediterranean plant diversity is severely threatened both by natural and anthropogenic factors, and it deserves particular attention from a conservation viewpoint; this phenomenon is particularly relevant in the insular context. The Mediterranean basin was the cradle of some of the greatest civilizations with the foundation of many human settlements across the whole hotspot for more than four millennia, while the subsequent soil over-exploitation and the conversion of much of the pristine vegetation to agricultural lands went together (Tucker & Evans, 1997; Vogiatzakis *et al.*, 2016); the population of the Mediterranean basin has recently exceeded 300 million and it is still increasing, especially in the North African countries.

The Mediterranean islands encompass a wide range of habitats within a small and restricted range. Among these, coastlines are particularly connotative of insular systems. They comprise a variety of valuable and fragile habitats such as sandy or rocky shores, dunes, cliffs, lagoons, salt marshes, estuaries and deltas. Those habitats are particularly prone to several severe threats for the conservation of biodiversity. Moreover, Mediterranean islands are featured by important and

exclusive mountain habitats with a remarkable flora which might be severely affected as for example by global warming. As one may expect, the geomorphological features of the Mediterranean usually limit the chance for plants to overcome the acting threatening factors by migrating upwards or sideways in neighbouring areas. For this reason, insular ecosystems are considered more fragile than continental ones, while the uncertainty regarding the conservation of valuable native flora is much more exacerbated in insular habitats than in their mainland counterparts.

The preservation of biodiversity, a well-established priority in the global environmental policies, is a key component of the UN 2030 Agenda for Sustainable Development and is a global commitment under the Strategic Plan for Biodiversity 2020, as well as under the EU Biodiversity Strategy to 2020 (i.e. Target 6). Nowadays, biological diversity faces several threats and the loss of biodiversity is constantly increasing (Pimm *et al.*, 1995; Butchart *et al.*, 2010; Ceballos *et al.*, 2015). Actually, several international conventions set ambitious targets to reduce biodiversity loss. The Global Strategy for Plant Conservation (GSPC), adopted by the Convention on Biological Diversity in 2002 and updated in 2010, provides the overall framework for plant conservation at global and national level. Plant conservation has been embedded within target 5 of the Global Strategy for Plant Conservation (GSPC) (2008), that was updated at the Conference of the Parties to the Convention on Biological Diversity (CBD, 2010) to “At least 75 per cent of the most important areas for plant diversity of each ecological region protected, with effective management in place for conserving plants and their genetic diversity” together with target 7 that concerns *in situ* protection of threatened plant species “At least 75 per cent of known threatened plant species conserved *in situ*” and target 8 being related to *ex situ* conservation “At least 75 per cent of threatened plant species in *ex situ* collections, preferably in the country of origin, and at least 20 per cent available for recovery and restoration programs”.

However, despite the efforts made, the loss of biodiversity is constantly increasing worldwide mainly by the continuous and growing human-related impacts (i.e. pollution, global warming, industrialization, urbanization and consequent “waste of land”). In fact, despite the adoption of several shared directives aiming at the protection of plant species and areas featured by a remarkable biodiversity, such instruments do not seem to be as effective as needed and expected.

A similar general trend is observed at European level. In Europe, the Habitat Directive (HD) and the Natura 2000 network with more than 27,000 sites covering about 18% of the terrestrial surface of the EU (European Commission, 2015), represent the core strategy of nature conservation in the EU countries and the

most important tool aiming at halting, or at least significantly reducing, biodiversity loss (e.g. Balmford *et al.*, 2005; Maiorano *et al.*, 2007; Pullin *et al.*, 2009). Habitat Directive promotes the conservation of biodiversity by maintaining, or restoring, natural habitats and species in a favourable conservation status by means of cogent protection policies (European Commission, 1992). However, the last European Reports of HD reveals negative conservation status for a fairly good number of plant species protected by the Directive (EEA, 2015). At the same time, a high percentage of “unknown” conservation status was reported for 13 States (>20% in Portugal, Italy, Spain, Denmark and UK), highlighting the urgency of promoting in-depth researches on species listed in the Habitat Directive at continental level (Fenu *et al.*, 2017).

In recent years, great efforts have been made to take concrete conservation actions and, in particular, a great development covered the structures dealing with *ex situ* conservation. *Ex situ* strategies (*i.e.* conservation of species outside their natural habitats) represent alternative ways to preserve plant diversity. Germplasm conservation, including seed banking, pollen/tissue storage and vegetative cloning, allows to preserve most of the genetic material in a small space. Several germplasm banks are particularly interested in preserving the flora of the Mediterranean islands such as BG-SAR in Sardinia (Fenu *et al.*, 2015), BGS-CAT in Sicily, MPCU of MAICH in Crete, or BGVIB in Sóller, Mallorca (Spain). Despite several objective constraints (e.g. plants that produce few viable seeds, plants growing in peculiar habitats or unorthodox seeds, *etc.*), main purpose of these structures is to ensure any possible effort for the long-term conservation of the highest number of plant *taxa*. Optimistically, *ex situ* conservation could reach significant levels in the forthcoming years (at least in some territories worldwide) and with accessions representative of the natural variability. However, the main question still remains: how these accessions will be used for future conservation activities?

Conversely, despite the strategic importance of the *in situ* measures highlighted by all most important international conventions (e.g. Target 7 of the GSPC for 2020), their full application remain far from being widely achieved. *In situ* strategies (*i.e.* conservation of species in their natural habitats) is considered the most appropriate way of conserving biodiversity and the preservation of the areas where natural populations of species exist is an underlying condition for their proper and effective conservation. Thus, according to Article 8 of the GSPC, *in situ* conservation is generally considered the primary approach for species conservation as it ensures that species are maintained in their natural environments, allowing evolutionary processes to continue, while *ex situ* conservation plays

a complementary role to *in situ* conservation, providing a “safety back-up” and an insurance policy against extinction in the wild. In this context, plant translocation is a relatively recent development and a potentially important tool for reducing the extinction risk of threatened species and improving their conservation status. Translocation, or the controlled placement of plant material into a natural or managed area (e.g. Godefroid *et al.*, 2011; Abeli *et al.*, 2016; Volis, 2016), includes population reinforcement, reintroduction and introduction aiming at enhancing population viability, for instance by increasing population size and/or genetic diversity. Given that, any translocation ideally requires a thorough understanding of the biology of the species involved (e.g. life form, reproductive biology, symbiotic relationships, *etc.*), a well-arranged translocation plan should take into account: the selection of the planting area(s), the origin of the genetic material, the type of propagative material (seeds or cuttings), planting methods, and lastly the monitoring actions to be undertaken (IUCN-SSC, 2013, Godefroid *et al.*, 2010). Though some examples are available worldwide, very few translocations have been carried out in the Mediterranean territories compared to the true need of reducing the extinction risk for many plant species, most of which narrow endemics (e.g. Cogoni *et al.*, 2013; Rita & Cursach, 2013, Piazza *et al.*, 2011).

The importance of translocations, aiming to contribute to the recovery of a given threatened species, is particularly relevant when it is part of an integrated *ex situ* and *in situ* conservation approach. In particular, the tight connection between *in situ* and *ex situ* conservation strategies is the emerging tool in the conservation of plant diversity (e.g. Cogoni *et al.*, 2013; Volis, 2016). However, several constraints may hamper the implementation of these conservation actions, such as the high economic and time costs, the availability of optimal sites, the difficulties (or impossibility) of implementing these actions on private areas, and the high uncertainty of success chiefly connected to natural stochastic events (e.g. Godefroid *et al.*, 2011; Fenu *et al.*, 2015; Volis, 2016). Considering these limitations, it is often necessary and useful to identify other active management measures, such as fencing (to prevent grazing and to protect the most critical life-cycle stage for the population survival), removing/eradicating alien invasive plants or controlling pest plants.

Study area

The Mediterranean Basin, with about 10,000 islands and islets and 244 of which are inhabited, encompasses one of the largest “archipelagos” in the world (Pons *et al.*, 2013). Some eastern Mediterranean countries, such as Croatia and Greece (Nikolic *et al.*, 2008; Kougioumoutzis *et al.*, 2016), include a remarkable

number of these islands; however, the largest Mediterranean islands (Sicily and Sardinia), as well as around 1100 islets, are located in the western side (Pons *et al.*, 2013). For historical and geographical reasons, but also due to the particular biotic interactions among species, Mediterranean insular conditions determine specific plant diversity and assemblages (Pons *et al.*, 2013). Accordingly, the rate of plant endemism reaches very high levels in the Mediterranean islands, generally comprising between 10-12% of the total vascular flora (*e.g.* Pons *et al.*, 2013; Fenu *et al.*, 2014). In particular, plant endemism rate is considerably higher in mountain ranges and in satellite uninhabited islets, where endemics represent about 35-40% of the vascular flora (*e.g.* Brullo *et al.*, 2005a, 2005b; Guarino *et al.*, 2005; Trigas *et al.* 2013; Kougioumoutzis *et al.*, 2016; Fois *et al.*, 2016).

The largest Mediterranean islands are six and can be divided into Tyrrhenian (*i.e.* Balears, Corsica, Sardinia and Sicily) and the East Mediterranean (*i.e.* Crete and Cyprus). The Tyrrhenian islands belong to the Protoligurian massif, the Hercynian formation – corresponding more or less to the actual Balearic Islands, Corsica, Sardinia and Sicily – that underwent fragmentation during the Oligo-Miocene (Rosenbaum *et al.*, 2002). The Balearic Islands form an archipelago of five major islands and about 100 small islets, covering a surface of 4,992 km². Only the main island (Mallorca) is characterized by the presence of true mountain ranges, *i.e.* Serra de Llevant and Serra de Tramuntana whose highest peak is the Puig Major (1,445 m a.s.l.); the Balearic flora displays 1551 *taxa* of which 140 narrow endemics (Sáez *et al.*, 2013). Corsica, covering a surface of 8,748 km², is mostly mountainous with several peaks above 2,500 m, of which the highest is the Monte Cinto (2,710 m a.s.l.). The peculiarity of Corsica is his alpine zone; furthermore, the great altitudinal range generates its endemic plants richness. The Corsican flora amounts to 2,798 *taxa*, of which 302 are endemic (13.49%), including 132 exclusive *taxa*, 78 Corso-Sardinian *taxa*, 19 *taxa* which belong to the Italian-Tyrrhenian superprovince, and 7 *taxa* can be found in both Corsica and the Balearic Islands, while other endemics belongs also to other adjacent areas (Jeanmonod & Gamisans, 2013).

Sardinia, placed at the center of the Thyrrenian sea, covers a surface of 24,089 km² and is the second largest island in the whole basin after Sicily. The high mountain of Sardinia is represented by the Gennargentu massif whose highest peak is Punta la Marmora 1,834 m a.s.l. Sardinian flora, after the latest floristic researches, counts more than 3,000 *taxa*, of which 347 are endemic (*e.g.* narrow endemics, Sardinian endemics, Corso-Sardinian endemics, Corso-Sardinian-Balearic endemics) with 45.8% (183 *taxa*) being exclusive endemics (Fenu *et al.*, 2014).

Sicily is the largest Mediterranean island with an area of 25,711 km² and, being placed in the center of the Mediterranean basin, it acted, and still acts, as a crossroad for plant westward and eastward migrations. Its highest peak is represented by Mt. Etna (currently 3,340 m a.s.l.), the highest active volcano of Europe. As concerns its floristic richness, the vascular flora is currently estimated to consist of about 3,200 *taxa* (Giardina *et al.*, 2007; Raimondo *et al.*, 2010) with about 370 narrow endemics (*i.e.* exclusively occurring in Sicily).

The East Mediterranean group includes Crete and Cyprus; Crete is the fifth largest island in the Mediterranean Basin and is located in the southernmost part of Greece. It has a total area of 8,700 km² including 200 satellite small islands and islets around it. The island is generally characterized as mountainous and the proximity of the high mountains to the sea is characterized by large deep gorges, accommodating unique habitats. According to the latest update reports (Dimopoulos *et al.* 2013, 2016; Strid 2016), the flora of Crete comprises about 2,100 native *taxa* with ca. 11% of them being endemic to the island (228 *taxa*). The floristic region of Crete - Karpathos is the most important center of endemism in Greece and it is characterized by the highest rates of vascular plant endemism and range-restrictedness (Georghiou & Delipetrou 2010; Dimopoulos *et al.*, 2016).

Cyprus is the third largest island in the Mediterranean with an area of 9,251 km². The island is divided into three main geomorphological zones, the Troodos Range, the Pentadaktylos Range and the Mesaoria plain. The geology and geomorphology of the island, the climatic conditions, its location between the three continents (Europe, Africa and Asia), along with more than 10,000 years of history and civilization, yielded a flora of great diversity and richness (Tsintides *et al.*, 2007). The flora of Cyprus comprises 1,640 indigenous *taxa* (species and subspecies). The endemic flora of Cyprus includes 142 endemic *taxa* which consists account for 8.66% of the native flora of the island (Hand *et al.*, 2011).

The CARE-MEDIFLORA approach

Due to the overall situation, in which extraordinary rates of endemism are associated with an exceptional degree of environmental and human-related threats, some not secondary features are shared by the Mediterranean insular territories. Such similarities and differences represent a great opportunity to join and harmonize methods and methodologies focused on endangered plant conservation in such a peculiar and unique natural laboratories. With this vision and under this light, no project aiming to develop knowledge and methodologies in plant conservation islands has been

developed and implemented so far.

The project CARE-MEDIFLORA, 80% funded by the MAVA Foundation, is an initiative led by institutions of six Mediterranean islands and the IUCN/SSC Mediterranean Plant Specialist Group (more details at <http://www.care-mediflora.eu/>), that have long experience in plant conservation activities. The protection of threatened flora towards the targets of GSPC (Global Strategy for Plant Conservation) constitutes the main focus of the project partners (institutions and Gene Banks from six Mediterranean islands) which, among others, have already successfully collaborated in a previous project named “*Ensuring the survival of endangered plants in the Mediterranean*” (Gil *et al.*, 2013).

The approach agreed by all partners is mainly based on using *ex situ* activities as a tool to improve *in situ* conservation of threatened plant species, *i.e.* by using genetic material (seeds) and know-how from previous *ex situ* actions (*e.g.* seed collections, germination experiments, living plant collections, *etc.*) for implementing studies and field works aiming at a true *in situ* conservation of the species.

The project strategy combines different methodologies for prioritizing endangered plant species occurring in each Mediterranean island (six partner island). In this light, the project aims at tackling the issue of conservation urgency towards endangered plant species of the Mediterranean through an innovative multi-level approach that encompasses *in situ* and *ex situ* methodologies. Actually, the CARE-MEDIFLORA project is arranged into four main objectives: (1) elaboration of conservation priorities and selection of target species in each island; (2) *in situ* conservation actions; (3) *ex situ* conservation actions and, lastly, (4) networking and communication activities.

1. Elaboration of conservation priorities (Comparison and harmonization of the criteria for establishing the conservation priorities) and selection of target species in the different islands.

In a preliminary stage the partners will cooperate in order to select the species that will be targeted during the project, using common criteria to prioritize their conservation actions. This phase has been identified as a crucial starting point in order to develop knowledge and common methodologies among islands. As a consequence of this shared approach, four different main criteria were established and followed:

Threat degree: it refers to the inclusion of a plant species in a threat category of global IUCN Red List (IUCN, 2012a); additionally also the plants listed in the national or regional (IUCN, 2012b) catalogues was been considered (*e.g.* Delage & Hugot, 2015). In the framework of the CARE-MEDIFLORA project, it was agreed that the plant species should be at least “threatened” (thus corresponding to the CR, EN and VU cat-

egories); additionally, also DD plant species could be included as a precautionary principle.

Regional Responsibility: it indicates the highest relevance given to those species whose distribution is circumscribed to a specific area and represents the first order of priority at local level (Martin *et al.*, 2010; Bacchetta *et al.*, 2012; Gauthier *et al.*, 2010; Fenu *et al.*, 2015). Actually, given the aforesaid peculiarities of the Mediterranean area, plant conservation priority settings at finer-scales should be preferred due to biogeographic and cultural diversity and regional threats. Accordingly, a special priority will be given to the endemic plant species and plants deserving a conservation interest for a given island (*e.g.* peripheral and isolated plant populations, PIPPs, *etc.*).

Policy plant species: it refers to those species listed in the annexes of Habitat Directive (Annexes II, IV and V). The conservation of plant species of community interest in a favourable conservation status by means of cogent protection policies is mandatory for all EU member states (*e.g.* Fenu *et al.*, 2017). In addition, each partner may consider those plants listed in other specific national or regional regulations: for example in Crete about 80 *taxa* are protected by Greek Presidential Decree 67/81 “On the protection of native flora and wild fauna and the determination of coordination procedures and control of research on these” or the Balearic List of Threatened species (Sáez & Roselló, 2001; CAIB, 2005).

Finally, *wetlands and plants* eco-physiologically linked to such peculiar habitats have a particular interest for conservationists. This is particularly true for those species unable to migrate to other sites which, in a context of climate change or climatic instability, could act as *refugial* stands.

Main result of this preliminary phase will be a checklist including the whole pool of species selected by each island. Furthermore, always considering these four criteria, each partner will decide which are the plant populations needing urgent *in situ* conservation measures (such as translocation, alien species eradication, fencing, *etc.*) and in which populations seed collection could be performed for germplasm conservation. For the selected plants/populations, each partner will plan its own *in situ* and *ex situ* activities. Of course, changes to the targeted species list will be always possible, respecting the four criteria, during the project thus making the list flexible, open and continuously upgradable (*i.e.* dynamic list).

2. In situ conservation actions

It includes all the *in situ* measures aiming at improving the conservation status of the selected species/populations. The optimal action will consist in the plant translocations (including reintroduction and/or reinforcement), but also complementary active manage-

ment measures (as passive defence measures which may consist in fencing the area where the threatened species/populations occur, eradicating or controlling pest plants, or restoring the natural vegetation within or around the area, thus reconnecting isolated remnants) will be adopted.

In order to define whether the translocation of the target species is possible and feasible, it will be necessary to perform a preliminary survey chiefly based on historical data, current distribution range of the species, distance from the nearest natural population(s) and availability of the potential growing sites. In addition, researches on the life cycle, reproductive biology, population biology and ecological requirements of the particular species or plant group are needed and crucial for having a reliable *in situ* action (Falk et al., 1996; Valee et al., 2004; Menges, 2008; IUCN/SSC, 2013; Cogoni et al., 2013; Fenu et al., 2016; Volis, 2016); a translocation plan will be created for each target species taking into account the guidelines of IUCN/SSC (2013). Where relevant, in cases of target species selected for *in situ* actions and already sampled and stored in the partners' seed banks during previous projects, such as the above mentioned "*Ensuring the survival of endangered plants in the Mediterranean islands*", propagative material (mainly seeds) may be obtained from these collections. A similar approach will be adopted in order to plan the passive or other management actions. In addition, all partners are committed for the periodical monitoring of all *in situ* activities, as well as for the maintenance of the protections (e.g. fences) in order to verify if and to which degree the initial objectives have been accomplished. Finally, in order to make the *in situ* activities more effective, they will be implemented in collaboration with the local authorities, and consequently regional authorities and local stakeholders will be actively involved in the monitoring process.

3. Ex situ conservation actions

Ex situ measures, such seed collection, curation and storage for germplasm conservation, are a relevant part of the project. Germplasm collection and curation will be carried out considering the national and international regulations and standards (such as those ones developed by the international networks of Genmeda, Ensconet, etc.). The germplasm will be collected following criteria aiming at maximizing the representativeness of the genetic diversity of the populations in each island (Bacchetta et al., 2006). To achieve this goal, collections of the same *taxon* will be carried out in more than one location and, for those *taxa* occurring in two or more islands, they will be sampled considering their multiple occurrence.

As a precautionary measure, aiming at ensuring the conservation of the collected seed material, accessions

will be duplicated in the seed banks of other project's partners or, if necessary and appropriate, in other public institutions.

Moreover, data concerning the germination eco-physiology of the collected germplasm will be obtained through seed germination tests. The species to be tested will be selected on the basis of their availability, particularly in terms of number of seeds per accession. Therefore, germination tests will be carried out only for those species whose distribution allow the collection of adequate quantity of seeds to be used both for *ex situ* conservation and seed germination tests. All info concerning the collection of accessions and germinations outcomes will be published in the project's website, as well as in the database of the Mediterranean network Genmeda.

In addition to the long term seed conservation, each institution will guarantee seeds availability for future recovery or restoration programmes and, at the same time, seed collection will be partly dedicated to an "active collection" to be used for producing plants.

In cases of extremely endangered perennial plants, a collection of cuttings could be useful for having an *ex situ* "copy" of the wild population. Therefore, such "living duplicates" will be cultivated in the partners' botanical gardens, and they will enable their use in recovery plans but also for disseminating the project targets and results.

In the framework of this activity, joint field trips will be carried out by the members of the partner institutions involved in the project during the whole collecting seasons. Seed sampling will be performed by the personnel of the partner institutions in collaborations with regional authorities and local stakeholders.

4. Networking and communication activities

Networking among the project partners is considered as a priority: local institutions/authorities in each island will be in regular contact with related international initiatives/projects at Mediterranean level, aiming at exchanging experiences so as to improve collaboration and effectiveness on plant conservation, as well as building a wider and more sustainable network of Mediterranean Plant Conservation Centres. All island partners of this project have already successfully collaborated in a previous MAVA funded project, while project partners from four out of the six islands had also collaborated in past Interreg projects and are already members of the network Genmeda (Network of Mediterranean Plant Conservation Centres) that funded in 2010, counts so far in total 13 members from 7 different countries. Special actions will be devoted for the enlargement of the members of this network from both Northern and Southern Mediterranean countries and for the improvement of its functioning: this will enable to share the knowledge with botanical gardens,

seed banks (and other conservationists) from other parts of the Mediterranean, especially from Northern African and Near East countries.

The autonomy of each partner for the activities in their island will be respected. Sharing knowledge with the staff of local authorities will be provided by project partners, aiming at the long-term continuation of *in situ* plant monitoring, as well as for the maintenance needs of infrastructure provided by the project. Furthermore, there will be the possibility for the partners' members to visit each other institutions during the annual project meeting, so as to share know-how and problems solutions. In this framework, the elaboration of further common lines of research between two or more partners will be strongly encouraged.

Communication in its various forms will be considered one major point and a project communication team will be established; then each partner will present in detail the project in the social networks and in its own website, taking care to constantly update the dedicated web page(s) whenever there will be significant news or key outcomes, by a project information leaflet in all languages of the partners, technical reports and scientific papers.

In addition, non-electronic communication will have a prominent importance: each partner will organize some local event to disseminate the aims of the project and the key issues related to the theme of native flora conservation. These events will be open to both local people and private stakeholders and actors, as well as to anyone who might have an interest in the topics. A final international workshop involving all the partners will be held at the end of the project. This event will be aimed at presenting the results of the project and at intensifying the collaboration between the Mediterranean partners, as well as laying the foundations to draw up new cooperation among institutions involved in flora conservation. The participation of the partners in scientific meetings with posters and oral communications will be greatly supported, while other kinds of communication will see the creation of a project leaflet.

Expected results and project benefits

Given the current situation of the native vascular flora and its conservation status, there is a serious need for changing the management of the natural habitats in the whole Mediterranean basin. This project will concretely contribute to alleviate the lack of dedicated conservation management plans by providing effective measures both for *in situ* and *ex situ* protection. In particular, at the end of the project the following goals will be achieved: (a) a list of plants needing urgent *in situ* and *ex situ* conservation measures based on scientific criteria; this list could be useful also for future conservation programs both at local and national level;

(b) 60 *in situ* conservation actions (10 per island) addressing at least 30 different threatened plant *taxa* (at least 5 per island); (c) a total of 600 accessions for *ex situ* actions related to at least 120 target *taxa* (at least 100 accessions for min. 20 *taxa* per island).

Furthermore, the project will strengthen the existing network of Mediterranean institutions involved in native plant conservation, including both *in situ* and *ex situ* plant conservation specialists. In addition, the project will provide exchange opportunities for the partners staff.

On the one hand, this project promote a management strategy for following similar directions in different territories in the framework of the commitments deriving from the international conventions (such as the Convention on Biological Diversity), the common European and the Euro-Mediterranean Partnership policies, and so on. Actually, cross-border cooperation projects appear as one of the most effective tool contributing to the joint development of an indispensable management plan. In this light, this kind of cooperative project display strong points, such as exchange of experiences, good practices implementation, collaboration on the setting up of common methodologies and, not less relevant, the adaptation of the tools to the specific peculiarities of each territory.

Concluding remarks

As far as we know, the CARE-MEDIFLORA project represents the first attempt to develop a common approach and methodology for plant conservation in the Mediterranean insular context, where a high level of endemism is associated with a remarkable degree of environmental and human influences. In fact, there are very few successful projects of translocation of threatened plant species in the Mediterranean area aiming to promote the long term conservation of threatened plant species in their natural habitats. In this sense, CARE-MEDIFLORA also constitutes a unique opportunity to unify and coordinate methods and methodologies on endangered plant conservation in such a peculiar natural laboratories. The experimental conservation actions, particularly the plant translocations, may act as a model for other threatened species occurring in the Mediterranean islands and in the whole Mediterranean area. In fact, the project actions can be replicated in the partners countries (at a larger scale), as well as in other Mediterranean countries with similar environmental conditions.

In addition, further outcomes may emerge from the project that will be useful for conservation policies both at local and national level, particularly for contributing to the national reporting to the relevant Multilateral Environmental Agreements (MEAs, such as CBD and GSPC). In fact, this project is focused on the

conservation of priority plants selected according to the regional responsibility criterion and those included in the main international regulations (e.g. Habitat Directive) attaining also to the GSPC and European Plant guidelines. Additionally, these priority lists may support at local level the identification of the Important Plant Areas (IPAs) and the Key Biodiversity Areas (KBAs) in the Mediterranean islands. The results that will be achieved through the CARE-MEDIFLORA project may contribute to reach the targets of the EU Biodiversity Strategy to 2020 (i.e. Target 6) and several Aichi Targets (e.g. 11, 12 and 19); in particular, the implementation of the *in situ* conservation measures can significantly contribute to the achievement of the Aichi Target 12 that, although these actions are the best way to conserve natural plant populations, very little has been done in the Mediterranean area compared to what is necessary to prevent the risk of extinction of many plant species.

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Seed conservation actions for the preservation of plant diversity: the case of the Sardinian Germplasm Bank (BG-SAR)

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Abstract

The Sardinian Germplasm Bank (BG-SAR) is a facility of the *Hortus Botanicus Karalitanus* (HBK), which belongs to the University of Cagliari (Italy). Its main objective is the conservation, study and management of the germplasm of Sardinian endemic, threatened and policy species (i.e., species inserted in the Habitat Directive 92/43/EEC, CITES and Bern convention), *taxa* from insular territories of the Mediterranean region, as well as Crop Wild Relatives (CWR), landraces, useful plants and plant remains. A summary of the procedures implemented by BG-SAR for the *ex situ* conservation, some international scientific results achieved, and some research projects at regional, national and international level in which the bank is involved, are reported in this work, with the main aim to highlight how a germplasm bank can be considered an important tool for the preservation of plant biodiversity. This paper allows to make a reflection about the importance of the germplasm banks, as well as their staff members, who constantly and daily work in order to preserve and conserve the planet's biodiversity.

Key words: archaeobotany, endangered species, *ex situ* conservation, invasive species, research activity, seed germination.

Introduction

The Mediterranean Basin, with ca. 11,700 endemic plant species, has been recognized as the second largest hotspot of the 36 hotspots in the world and the largest of the world's five Mediterranean-climate regions (CEPF, 2016). Mediterranean islands and islets are singular for their species richness and high endemism rates. Among them, Sardinia (and its ca. 300 circum-Sardinian islets, including four archipelagos; Fenu *et al.*, 2014), situated in the western Mediterranean Basin and covering 24,090 km², is the second largest island in the Mediterranean Sea, and it could be classified as a meso-hotspot within the Tyrrhenian macro-hotspot and the Mediterranean mega-hotspot of biodiversity (Cañadas *et al.*, 2014). The particular geological and human history of Sardinia has been a determining factor in the development of its floristic peculiarities (Fenu *et al.*, 2014).

The Sardinian flora consists of 2,494 *taxa* (Conti *et al.*, 2007), and 290 of them are considered as Sardinian endemic (e.g., narrow endemics, Sardinian endemics, Corso-Sardinian endemics, Corso-Sardinian-Balearic endemics; Bacchetta *et al.*, 2012; Fenu *et al.*, 2014), and 183 as exclusive Sardinian plant species; moreover, Sardinia is a priority region for the biology conser-

vation due to its high number of endemic plant species (Bacchetta *et al.*, 2012; Fenu *et al.*, 2015), and most of them facing several threatening factors, e.g. land-use and land-cover change, habitat fragmentation, climate warming and introduction of alien species.

In this context, from 1997, the research center named Sardinian Germplasm Bank (BG-SAR) plays a central role in the conservation of Sardinian plant diversity and in the protection of the most sensitive ecosystems of the island. Since 2015, BG-SAR is a facility of the *Hortus Botanicus Karalitanus* (HBK), which belongs to the University of Cagliari. The Bank preserves, studies and manages the germplasm of Sardinian endemic, threatened and policy species inserted in the Habitat Directive 92/43/EEC, CITES and Bern convention, *taxa* from insular territories of the Mediterranean region, as well as Crop Wild Relatives (CWR), landraces, useful plants and archaeological plant remains (Porceddu *et al.*, 2015; Uccesu *et al.*, 2016a).

The main research activities of BG-SAR mainly concern the *ex situ* conservation, seed germination ecology and seedling development studies, archaeobotany and studies on the potential invasiveness of the "Invasive Alien Species" (IAS), mainly dangerous in Sardinian coastal habitats. The staff and researchers belonging to BG-SAR are constantly involved

in several initiatives with the aim to promote local awareness, involving schools at all levels, to spread the results at local, national and international levels. In addition, BG-SAR offers free learning courses and/or practical activities in the field of plant biodiversity characterization, management and conservation (e.g., HEI-PLADI Erasmus+ project; <https://dibt.unimol.it/HEI-PLADI/elearning/>), as well as practical training for *ex situ* conservation techniques (IPAMed project by CARE-MEDIFLORA; <http://www.medplantsnetwork.net/training-for-ex-situ-conservation-by-care-mediflora-partners-in-collaboration-with-iucn-med-in-progress/>).

The research center participates in international seed conservation consortia, such as the European Native Seed Conservation Network (ENSCONET), the Network of Mediterranean plant conservation centres (GENMEDA) and, at national level, it is a founding member of the Italian Network of Germplasm Banks for the *Ex situ* Conservation of Native Flora (RIBES).

A summary of the procedures implemented by BG-SAR for the *ex situ* conservation, some international scientific results achieved during the last years, and some research projects at regional, national and international level, are reported in this work, with the main aim to highlight how a germplasm bank can be considered an important tool for the preservation of plant biodiversity.

Seed conservation and preservation at BG-SAR

The seed conservation processes carried out at BG-SAR follow internationally recognized protocols and guidelines for the gene bank standards (Bacchetta *et al.* 2006, 2008a). All information are recorded and managed in IrisBG (Botanical Software Ltd © 2016), a suitably designed database to manage botanical and seed bank collections.

The collected germplasm is subject to a quarantine and post-maturation period, and only afterwards, the seeds are introduced in the bank. Subsequently, each accession is cleaned, quantified, selected and processed. The seed lots are gradually dried at 15°C and 15% of relative humidity (RH), in order to reach ca. 3-5% of internal seed moisture content, and stored at -25°C (as base collections under long-term conservation) and/or at +5°C (as active collections under medium-term conservation). Actually, BG-SAR preserves approximately 2,800 seed lots, many of which endemics of Sardinia. In particular, the bank preserves the germplasm referable to 47% of policy species and to 41% of exclusive Sardinian endemics (Fenu *et al.*, 2015). It is important to highlight that the bank preserves most of the *taxa* included in the Annexes of the Habitats Directive [e.g., *Astragalus maritimus* Moris, *A. verrucosus* Moris, *Brassica insularis* Moris, *Gen-*

tiana lutea L. subsp. *lutea*, *Helianthemum caput-felis* Boiss., *Lamyropsis microcephala* (Moris) Dittrich & Greuter, *Linum muelleri* Moris, *Ribes sardoum* Martelli, Rouya polygama (Desf.) Coincy and *Silene velutina* Pourr. ex Loisel.], species considered, *sensu* Bacchetta *et al.* (2012), the ten most threatened exclusive endemic species of Sardinia (*Anchusa littorea* Moris, *Aquilegia barbaricina* Arrigoni & E.Nardi, *Aquilegia nuragica* Arrigoni & E.Nardi, *A. maritimus*, *A. verrucosus*, *Centranthus amazonum* Fridl. & A.Raynal, *Dianthus morisianus* Vals., *L. microcephala*, *Polygala sinisica* Arrigoni and *R. sardoum*), and several Sardinian *taxa* listed in the Italian national and regional red lists resulting threatened according to the criteria of the IUCN (Conti *et al.*, 1992, 1997; Rossi *et al.*, 2013) or inserted in attention list as the IUCN Top50 species of the Mediterranean islands (Montmollin de et Strahm, 2005; Pasta *et al.*, 2017). In addition, BG-SAR preserves several accessions referable to Crop Wild Relatives (Ucchesu *et al.*, 2016a).

Seed germination ecology studies

Studies on the germination ecophysiology are constantly carried out to increase the biology knowledge and define the optimum germination protocol for each of the preserved *taxa*. For example, specific studies on seed germination ecology of the Sardinian endemic *Ribes multiflorum* Kit. ex Roem. & Shult. subsp. *sandalioticum* Arrigoni (Mattana *et al.*, 2012) and *R. sardoum* (Porceddu *et al.*, 2017), the only two members of *Ribes* genus present in Sardinia, were carried out, demonstrating that these *taxa* needed (after dormancy release) low temperatures for seed germination, highlighting an increasing risk from global warming for both *taxa*. Recently, it was demonstrated that seeds of *Paeonia corsica* Sieber ex Tausch exhibited differential temperature sensitivity for the different sequential steps in the removal of dormancy and germination processes, that resulted in the precise and optimal timing of seedling emergence (Porceddu *et al.*, 2016). More recently, Cuenca Lombraña *et al.* (2016, 2017), in their studies on *Gentiana lutea* L. subsp. *lutea*, underscored the importance of studying the germination behaviour in the field and identified the type of seed dormancy in order to define the real thermal requirements of a species with high conservation value. In addition, studies on thermal time, niche modeling and the effect of global warming on the seed germination of Mediterranean species were carried out (e.g., Orrù *et al.*, 2012a; Porceddu *et al.*, 2013; Picciau, 2016).

Moreover, germination tests for some endemic and endangered *taxa* [e.g., *Phleum sardoum* (Hackel) Hackel, *Rouya polygama* (Desf.) Coincy, *B. insularis*, *Lavatera triloba* L. subsp. *triloba*, *L. triloba* subsp. *pallescens* (Moris) Nyman (Santo *et al.*, 2014a,b,

2015a,b)], and IAS species [*Acacia saligna* (Labill.) Wendl., *Carpobrotus edulis* (L.) N.E.Br., *Lycium ferocissimum* Miers (Meloni *et al.*, 2015; Podda *et al.*, 2015a,b)] belonging to coastal habitats were conducted to detect the effect of environmental abiotic stresses such as salinity (e.g. NaCl), or the nutrient availability (e.g. KNO₃) on the seed germination behaviour.

Germplasm identification by image analysis

Image analysis technique carried out at BG-SAR is developed in order to identify and characterize the autochthonous germplasm in entry to the bank. Basically, digital images of germplasm are acquired by a flatbed scanner and successively processed and elaborated with specific software as KS-400 V. 3.0 (Carl Zeiss Vision, Oberkochen, Germany) and ImageJ v.1.9 producing macros and plugins able to detect morphometric and colorimetric measurements on seeds (Bacchetta *et al.*, 2008b; Bacchetta *et al.*, 2010; Grillo *et al.*, 2010; Ucchesu *et al.*, 2016b).

Applying this technique, Bacchetta *et al.* (2008b) and Grillo *et al.* (2010) characterized and discriminated seeds of wild plants typical of the Mediterranean vascular flora, referable to the *Apiaceae*, *Asteraceae*, *Boraginaceae*, *Brassicaceae*, *Caryophyllaceae*, *Cistaceae*, *Fabaceae*, *Lamiaceae*, *Poaceae* and *Scrophulariaceae* families. These studies stimulated further researches in many areas, including agronomical field (e.g., Grillo *et al.*, 2011; Orrù *et al.*, 2012b, 2015; Lo Bianco *et al.*, 2015; Sarigu *et al.*, 2017). In recent years, the morphometric analyses were applied to archaeological plants remains. These materials, such as seeds and fruits, were studied with the aim to investigate the evolution of crops and wild plants, explore agrarian practices harvesting, crop processing and determining the role of species with high cultural and economic value, such as cereals and fruits [e.g., *Cucumis melo* L., *Ficus carica* L., *Malus domestica* Borkh., *Olea europaea* L., *Prunus domestica* L., *Pyrus communis* L. and *Vitis* sp. (Orrù *et al.*, 2013; Sabato *et al.*, 2015; Ucchesu *et al.*, 2014, 2016a,b)]. For example, in the case of the *Vitis* genus, it was possible to establish that the seeds found in the archaeological site of Sa Osa (Cabras, Central-Eastern of Sardinia) in a Nuragic well dated to Bronze Age (about 3000 Before Present “BP”; Orrù *et al.*, 2013) belonged to grape cultivar (Ucchesu *et al.*, 2014) allowing to establish that in Sardinia viticulture was present from to Bronze Age. Recently, archaeological samples of *Prunus* sp. of Santa Giusta (Oristano, Central-Eastern of Sardinia) were compared through morphometric analysis with different traditional varieties and wild species from Sardinia. This study allowed to identify correctly the archaeological samples referable to *P. spinosa* and *P. domestica* (Ucchesu *et al.*, 2017).

Projects

An important part of financing for BG-SAR comes from regional, national and international projects. Below, some of them are presented. For example, at regional scale, the project denominated “Morpho-colorimetric, ecophysiology and omic analysis of *Vitis vinifera* and *Vitis sylvestris* in Sardegna” funded in September 2015 through the “Legge Regionale 7 agosto 2007 RAS”. The expected results will consist to draw phyletic relationships between Sardinian wild grapes, cultivated grapes, and the archaeological seeds (found in archaeological contexts).

The “Pilot project of *Gentiana lutea* L. *in situ* and *ex situ* conservation, genetic characterization, populational reinforcement and reintroduction of *Gentiana lutea* L.” was supported by the Autonomous Region of Sardinia. The project allowed to assess the actual distribution range of *G. lutea* in Sardinia, to investigate the species phenology and the reproductive biology, and to identify the main threatening factors for this *taxon*; in addition, it allowed the implementation of the *ex situ* and *in situ* conservation measures, the identification of the optimal requirements for seed dormancy release and germination, and the investigation of the genetic diversity.

At international level, the LIFE+ PROVIDUNE project “Conservation and restoration of habitats dune in five Site of Community Importance (SCI) areas of the Provinces of Cagliari, Matera and Caserta” (2009-2014; <http://www.lifeprovidune.it>), financed by the European Union, was aimed to protect and restore the priority coastal dunes habitat with *Juniperus* ssp. and other related habitats characterizing the Mediterranean sandy coasts.

The LIFE+ project RES MARIS “Recovering Endangered Habitats in the Capo Carbonara MARIne Area, Sardinia” (2014-2018; <http://www.resmaris.eu>) aims at the conservation and recovery of marine and terrestrial ecosystems of the emerged and submerged beach system, in particular of priority habitats (DIR. 92/43/EEC) included in the marine SCI ITB040020 “Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu” (Acunto *et al.*, 2017). Within the terrestrial conservation actions, the germplasm of the structural species of each habitat was collected and the best protocols for germination were investigated to produce plants for the restoration actions.

The ENPI CBC MED project ECOPLANTMED “ECOLOGICAL use of native PLANTs for environmental restoration and sustainable development in the MEDITERRANEAN region” (2014-2015; <http://www.ecoplantmed.eu>) was aimed to the *ex situ* conservation, duplication and germination experiments of the target plant species, the publishing of the “Manual for the propagation of the target native plant species” (Bal-

lesteros *et al.*, 2015) and the “Guide of good restoration practices in Mediterranean habitat” (Marzo *et al.*, 2015), the creation of a germplasm bank in Tunisia and finally, two pilot restoration actions of Mediterranean habitats in Lebanon and Tunisia.

Since April 2016, BG-SAR is responsible of the scientific management of the project CARE-MEDIFLORA “Conservation Actions for Threatened Mediterranean Island Flora: *ex situ* and *in situ* joint actions”, funded by MAVA Foundation (<http://www.care-mediflora.eu/>). The project aims to improve the conservation status of threatened Mediterranean plant species. It is implemented by institutions of six Mediterranean islands and the IUCN/SSC Mediterranean Plant Specialist Group.

Conclusions

In the latest years, good progress has been made at BG-SAR relating to understanding the mechanisms of seed behaviour, dormancy, germination, viability and longevity of several *taxa*, as well as ensuring the preservation of plant biodiversity. In addition, through the experimental researches carried out, further knowledges are provided on the possibility of efficient preservation of seeds over long periods. All these approaches allow BG-SAR, not only to attain an effective *ex situ* conservation and to deepen the knowledge of the endangered Mediterranean species, but also to face actual and important problems such as global warming and invasiveness of alien species. The evaluation of salt stress allows us, in particular for endemic and rare species, to acquire information regarding their germination ecology that might be useful in the case of population reintroduction. The knowledges obtained for IAS, for example, give an important contribution in developing a wide management strategy for the control of the biological invasions; in particular, we could predict the potential ability of each species to successfully colonize via seeds some coastal habitats.

Concerning the image analysis, it has proved to be a repeatable, reliable and non-destructive method. It is fully accepted and utilized, not only for archaeological studies and/or taxonomic investigations of wild *taxa*, but also for studies on cultivated plants; this method can be useful to compare different varieties, contributing to the cataloguing and conservation in germplasm banks, or allowing the definition of objective parameters for the typifying of particular landraces in the attribution of European trademarks such as protected designation of origin (PDO) and protected geographical indication (PGI). In addition, it can be a valid helpful tool to discover false attributions (synonyms/homononyms) and origin of cultivars in different areas. The study of plant remains provides the opportunity to explore agrarian practices and crop domestication processing, enabling, therefore, a deeper insight into the way farming com-

munities manage their crops and use plants for their subsistence going beyond the mere identification of plant species. Furthermore, scientific archaeobotanical works are encouraged, which may have wider implications, as the identification of wild plants and to facilitate the study of the role of wild resources in human subsistence as a complement to the diet.

Consistently with these observations, BG-SAR continues to promote further researches and projects that are of fundamental importance for diversity plant conservation in the Mediterranean area, due to the position of the island in the middle of the Mediterranean Sea, the inclusion in the Mediterranean mega-hotspot of biodiversity and the high concentration of endemism.

Future researches may be focused, for example, on nursery and plant production systems for wild plant species that maximize genetic diversity, so that introduced seeds and plant materials have the resilience to cope with future environmental stresses (Hay and Probert, 2013). Regarding future perspectives, the bank is going to expand the working area to genetic studies. A laboratory dedicated to genetic analysis is in the planning, design and construction phase; it will allow BG-SAR to make a survey on genetic diversity of the studied *taxa* (e.g., endemic and threatened ones), thus reconstructing the best estimates of the historical and evolutionary relationships among the various taxonomic entities.

As well, concerns about the costs of *ex situ* conservation compared with *in situ* conservation have been allayed, the *ex situ* conservation revealed to be excellent value for money. Scientists, government departments and non-governmental organisations increasingly appreciate seed banking as an effective and economic conservation tool because of its complementarity to *in situ* approaches (Li and Pritchard, 2009). Especially in this time of financial crisis where the most scientific works are self-funded, the regional, national and international projects provide the resources necessary to continue in this important direction for the seed banking conservation. Regarding the critical aspects, as already mentioned in a previous paper regarding the BG-SAR (Atzeri *et al.*, 2012), the bank has no special management problems but it is necessary to highlight the difficulties due to the precarious work of most of the staff. Even the high management costs are not covered by a safe and consistent revenue. According to this vision, we argue that BG-SAR can be considered an important tool for the preservation of plant diversity, as well as the several germplasm banks that constantly and daily work to preserve and conserve the planet’s biodiversity.

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The LIFE Nature and Biodiversity project “WetFlyAmphibia” - Conservation of amphibians and butterflies of open wet areas and their habitats at the Foreste Casentinesi National Park (Italy)

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Abstract

The LIFE project “Conservation of amphibians and butterflies of open wet areas and their habitats at the Foreste Casentinesi National Park” aims at improving the conservation status of three endangered amphibia (*Bombina pachypus*, *Salamandrina perspicillata* and *Triturus carnifex*) and two butterflies (*Euplagia quadripunctaria* and *Eriogaster catax*) of EC interest, inhabiting open wet areas. The project consists of different actions in 156 planned areas. Actions include restoration of existing wetlands, creation of new wetlands, removal of trees and shrubs and the reintroduction of *B. pachypus* and *S. perspicillata* in some sites. The restoration of wetland habitats includes the creation of patches of the EC habitat interest H6430 Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels. The project started in 2015 and has a duration of 6 years. Here the challenge represented by different ecological requirements of target animals and plants is described as well as the solutions found to achieve the project aims.

Key words: amphibia reintroduction, endangered butterflies, EC H6430 habitat, habitat restoration, priority species, wetlands.

Introduction

The LIFE project “Conservation of amphibians and butterflies of open wet areas and their habitats at the Foreste Casentinesi National Park” (LIFE14 NAT/IT/000759; acronym: WetFlyAmphibia) is funded with 1,596,342.00 € and has the Foreste Casentinesi National Park as coordinator. Five other partners are involved: D.R.E.Am. Italia Ltd., the University of Pavia, the University of Bologna, Reparto Carabinieri Biodiversità of Pratovecchio and Centro Nazionale Carabinieri Biodiversità of Pieve Santo Stefano (formerly Corpo Forestale dello Stato, “forestry rangers”) and the local municipal authority “Unione dei Comuni Montani del Casentino”. The project started in 2015 and has a planned duration of 6 years (2015-2021). WetFlyAmphibia aims at improving the populations of three endangered European Community interest amphibia (*Bombina pachypus* Bonaparte, *Salamandrina perspicillata* Savi and *Triturus carnifex* Laurenti) and two butterflies (*Euplagia quadripunctaria* Poda and *Eriogaster catax* L.) of open wet areas of the considered national park, which is situated between Tuscany and Emilia-Romagna regions (N-Apennines, N-Italy; Fig. 1). The analysis of the population dynamics of the target species shows different conservation concerns. *Bombina pachypus* is in sharp decline at a national

level and in the project area, the other target species have an irregular distribution due to several factors of disturbance and threat, mainly because of the alteration and reduction of their habitats and breeding sites. The aim is achieved through several conservation actions aimed to stop the threats for the populations of amphibians and butterflies in open wet areas and to favour the establishment of safe populations.

Planned conservation actions include population reinforcement for the target amphibia, the restoration of existing wetlands and the creation of new wetlands. The restoration of these environments also involves restoring of the aquatic and hygrophilous vegetation, including the habitat of EC interest H6430 “Hydrophilous tall herb fringe communities of plains and of the montane to alpine levels”, a process that is expected to favour the natural return of some of the target species, without any artificial release. Moreover, the restoration of the vegetation involves the population reinforcement or reintroduction of endangered plant species important for the area, specifically *Hottonia palustris* L. and *Tozzia alpina* L.

Further specific objectives of the project are: 1) improvement of the conservation status of *B. pachypus*, *S. perspicillata* and *T. carnifex* populations, through the reinforcement of populations, the improvement of the conservation status of their habitats and the



Fig. 1 - Study area. The location of the Parco Nazionale delle Foreste Casentinesi, Monte Falterona e Campigna where the LIFE project WetFlyAmphibia is implemented.

creation of new breeding areas; 2) amelioration of the conservation status of *E. quadripunctaria* and *E. catax* populations, through the improvement of the conservation status of their habitats; 3) improvement of the conservation status of open wetlands (habitat 6430) and rare or unique plant species related to these habitats; 4) increment of the awareness of local people about the need of conservation of amphibians and butterflies species.

Expected results are: 1) detailed distribution maps of the target species including an assessment of their population size; 2) protocol of the *ex situ* breeding of *B. pachypus*; 3) protocol of the *ex situ* reproduction of selected plant species belonging to the habitat 6430; 4) improved conservation status of the target species; 5) increased local people awareness about the conservation of amphibians and butterflies in the area.

WetFlyAmphibia has just started and the first results on the effectiveness of the project will be available in the next years, as it will continue until 2021. Here, as a very preliminary outcome, only some information on the process of habitat restoration for H6430 is provided.

Methods

Study area

The area where the project is carried out is the Foreste Casentinesi, Monte Falterona e Campigna Na-

tional Park. The area hosts one of the most valuable mountain forest in Europe, the heart of which is the “Foreste Demaniali Casentinesi” (Casentinesi state-owned Forests) that include the “Riserva Naturale Integrale di Sasso Fratino” (Integral Natural Reserve of Sasso Fratino), established in 1959. Small wetlands (ponds, lakes, streams) are interspersed in pastures and forests clears and represent the main habitats for the target amphibia. Here some valuable plant species are found such as *Tozzia alpina* L., *Hottonia palustris* L., *Peplis portula* L. and *Chara globularis* Thuill. Other species locally rare, like *Trollius europaeus* L., *Carex hirta* L. and *Filipendula ulmaria* (L.) Maxim., grow near the ponds and in relict patches of habitat 6430.

It is also a territory consisting of residential areas with a rich historical, artistic and architectural background. The Foreste Casentinesi National Park represents one of the oldest forests in Europe, which is composed of centuries old fir woods, beech and mountain-maple woods.

The vertebrate fauna is represented by large mammals, particularly ungulates (Deer, Fallow Deer, Roe Deer, Wild Boar and Mouflon) and Wolves, these latter are the largest predators currently present in the Park. With regard to amphibians, there are 12 species living in the Park including the species target of the project and other interesting species such as the Spotted salamander (*Ambystoma maculatum* Shaw) and the Alpine newt (*Ichthyosaura alpestris* Laurenti).

General methods

Project aims are achieved through direct actions in 156 areas within the Foreste Casentinesi National Park. These actions include the creation of new wetlands in seven areas, the restoration of the typical vegetation of habitat 6430 in 15 areas, cutting vegetation and trees in 24 wetlands, fence installation in nine areas, installation of structures to facilitates the access of the target amphibia in 46 areas, restoration or creation of fountains in 48 areas, deepening puddles in 15 areas. Additionally, it is planned to reintroduce at least 10-15 (each year) small metamorphosed individuals of *Bombina pachypus* for each area, and at least 1,000-1,500 *Salmandrina terdigitata* eggs each year. Monitoring will include the assessment of the population status of target amphibians and butterflies, the success of habitat restoration, the impact of the project on ecosystem functions and the socioeconomic effects of the project.

Restoration of H6430 and other riparian and aquatic vegetation

The restoration of the vegetation in the new or restored ponds implies the creation of three different vegetation patches encompassing the submerged part of a pond, where the amphibia will reproduce and live during their initial life stage, and the terrestrial neigh-

bours used by the amphibia as corridors between the ponds and the forest. The three vegetation types here reconstructed represent the aquatic vegetation, the hygrophilous vegetation (*Magnocaricion*) and the tall herb fringes of H6430.

The restoration of the aquatic vegetation (*Potamogeton* spp., *Myriophyllum* spp., *Chara globularis*) will be done by relocating small ramets of the target species from current areas of occurrence within the project area to the new or restored ponds. This approach will reduce the cost of production and transportation. Special attention will be paid to avoid the accidental release of non-native fauna connected to the aquatic plants. For instance, some lakes and ponds within the project area are known to host the Louisianan Crayfish (*Procambarus clarkii* Girard). These sites will be disregarded as potential source of aquatic plant material to avoid the unwanted relocation of crayfish larvae. Because the target amphibia have different requirements in terms of aquatic vegetation, the choice of plant species to be relocated in each site accounts for the target species a pond is destined to. For instance, eggs of *B. pachypus* require high amount of light, so ponds destined to this species will receive *Chara* or *Myriophyllum*. On the other hand, *T. carnifex* requires deeper ponds and broad-leaved vegetation, so *Potamogeton* spp. will be established in new ponds (Kinne, 2004).

The initial project objective is the restoration of the riparian vegetation (*Magnocaricion*) and habitat 6430 in the new or restored ponds, achieved through the production and planting of not less than 15 herbaceous species and not less than 10,000 individual plants. A planting scheme based on the species ecological requirements has been developed. Specifically, at each planting sites the position of each species will be based on soil humidity and light requirements determined according to Ellenberg's ecological indicators (Ellenberg, 1974) modified by Pignatti *et al.* (2005). Figure 2 illustrates a general planting scheme.

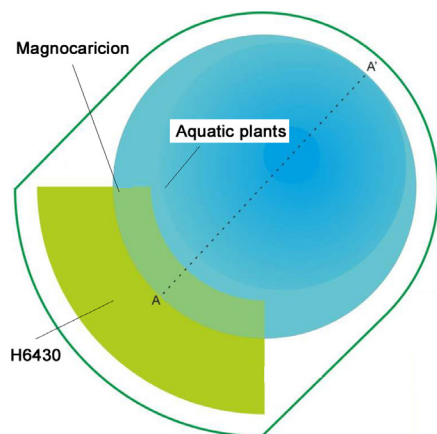


Fig. 2 - General scheme of the three vegetation belts to be created in new or restored wetlands.

Development of seed germination protocols

Ex situ reproduction of plant species belonging to the *Magnocaricion* and the H6430 is an important part of the project because large numbers of species and plant individuals will be required for restoration (Tab. 1). However, germination and cultivation requirements for most of the species selected for restoration were unknown. Thus, germination protocols for some of these species were developed at the University of Pavia to increase the success of *ex situ* propagation.

Seed germination tests were performed at the Germplasm Seed Bank of the University of Pavia sowing

Tab. 1 - List of the plant species reproduced *ex situ* for habitat restoration.

<i>Aconitum lycoctonum</i> L. emend. Koelle
<i>Adenostyles australis</i> (Ten.) Nyman
<i>Aegopodium podagraria</i> L.
<i>Alchemilla xanthochlora</i> Rothm.
<i>Alliaria petiolata</i> (M.Bieb.) Cavara & Grande
<i>Angelica sylvestris</i> L.
<i>Arabis alpina</i> (Willd.) Briq.
<i>Arctium lappa</i> L.
<i>Caltha palustris</i> L.
<i>Carduus personata</i> (L.) Jacq.
<i>Carex cuprina</i> (Heuff.) A.Chem.
<i>Carex hirta</i> L.
<i>Carex leporina</i> L.
<i>Carex pendula</i> L.
<i>Carex pseudocyperus</i> L.
<i>Carex riparia</i> Curtis
<i>Carex strigosa</i> Huds.
<i>Chaerophyllum aureum</i> L.
<i>Chaerophyllum temulum</i> L.
<i>Circaea lutetiana</i> L.
<i>Cynoglossis borellieri</i> (All.) Vural
<i>Digitalis lutea</i> L.
<i>Epilobium hirsutum</i> L.
<i>Epilobium montanum</i> L.
<i>Epilobium parviflorum</i> Scribn.
<i>Eupatorium cannabinum</i> L.
<i>Filipendula ulmaria</i> (L.) Maxim.
<i>Heracleum sphondylium</i> L.
<i>Hypericum tetrapterum</i> Fries
<i>Juncus articulatus</i> L.
<i>Juncus conglomeratus</i> L.
<i>Juncus effusus</i> L.
<i>Juncus inflexus</i> L.
<i>Lunaria rediviva</i> L.
<i>Myosotis scorpioides</i> L.
<i>Peplis portula</i> L.
<i>Petasites hybridus</i> (L.) Gaertn.
<i>Pimpinella major</i> (L.) Huds.
<i>Podospermum canum</i> C.A. Mey
<i>Rubus idaeus</i> L.
<i>Salvia glutinosa</i> L.
<i>Sambucus ebulus</i> L.
<i>Saxifraga rotundifolia</i> L.
<i>Senecio ovatus</i> Willd.
<i>Silene dioica</i> (L.) Clairv.
<i>Sparganium erectum</i> L.
<i>Thalictrum aquilegifolium</i> L.
<i>Trollius europaeus</i> L.

seeds in Petri dishes filled with 1% agar, with three replicates per tests. Seeds were then incubated in temperature- and light-controlled incubators for four weeks. Treatments differed across species and included stable temperature, alternate temperatures, cold stratification and/or the addition of 250 mg/l of Gibberellic Acid (GA₃) to stimulate germination in dormant species (Tab. 2; Baskin & Baskin, 2014). Cold stratification was performed by incubating seeds for two months at 0 °C in dark conditions.

Germination treatments were selected based on data available in the Seed Information Database (SID) of the Millennium Seed Bank of the Royal Botanic Garden, Kew (<http://data.kew.org/sid/>). For species not included in this database several tests were planned based on information available on other species of the same genus or on previous tests performed at the Seed Bank. Seeds were scored for germination at weekly intervals with germination defined as visible radicle emergence. At the end of each test non-germinated seeds were cut to check their viability. Seeds with fresh embryos were considered as viable seeds, while seeds with dark embryos or fungus infected seeds were considered non-viable. The final germination percentage were computed excluding non-viable seeds.

Preliminary results and discussion

The objective of the project in terms of species for the restoration of wet habitats has been largely exceeded in spring 2017. In fact, at the time of the proposal presentation it was not considered that both mountain and plain subtypes of H6430 (Biondi & Blasi, 2010) were present in the study area, so further additional species had to be produced to restore the two subtypes of H6430 depending on the elevation of each site. So far, 52 species have been produced for a total of about 12,000 individual (Tab. 1), which are ready to be released in the intervention sites. Plant production has been partially done in the premises of Carabinieri Forestali (formerly Ufficio Territoriale per la Biodiversità, UTB of Pieve Santo Stefano of the Corpo Forestale dello Stato) and at the Botanical Garden of the University of Pavia. However, most of the production has been entrusted to an external private company (Flora Conservation, Pavia), specialized in the production of the native flora. The University of Pavia provided the expertise for the production of plants, in particular seed germination and cultivation tests were performed to identify best germination protocols for the selected species.

Tab. 2 - Results of the germination tests performed on some of the species selected for habitat restoration.

Species	Temperature	GA ₃	Stratification	Viability (%)	Germination (%)
<i>Caltha palustris</i> L.	25/15°C	no	No	78.3	29.7
	25/15°C	Yes	No	83.3	20
	25/15°C	No	2 months	73.3	52.3
<i>Carex leporina</i> L.	30/20°C	No	No	98.3	98.3
	25/15°C	No	2 months	96.7	100
<i>Carex otrubae</i> Podp.	25/15°C	Yes	No	33.3	0
	30/20°C	No	No	20	8.3
	30/20°C	No	2 months	28.3	5.8
<i>Carex pendula</i> L.	30/20°C	No	No	93.3	62.5
	30/20°C	Yes	No	81.6	100
<i>Carex strigosa</i> Huds.	30/20°C	No	No	91.6	78.1
	30/20°C	Yes	No	90	77.7
<i>Chaerophyllum aureum</i> L.	25/15°C	Yes	No	85	0
	5°C	No	2 months	90	11.1
<i>Chaerophyllum temulum</i> L.	25/15°C	Yes	No	83.3	24
	25/15°C	No	2 months	90	61.1
<i>Digitalis lutea</i> L.	20°C	No	2 months	100	96.6
	20°C	No	2 months	100	18.3
<i>Juncus articulatus</i> L.	25/15°C	No	2 months	100	65
	20°C	Yes	No	100	1.6
	20°C	No	2 months	96.6	10.3
<i>Juncus conglomeratus</i> L.	30/20°C	No	2 months	93.3	94.6
	20°C	Yes	No	96.6	0
	20°C	No	2 months	100	63.3
<i>Juncus effusus</i> L.	30/20°C	No	2 months	100	63.3
	20°C	Yes	No	100	0
<i>Silene dioica</i> (L.) Clairv.	20°C	Yes	No	93.3	82.1
<i>Lunaria rediviva</i> L.	5°C	No	2 months	97.8	0
	15/5°C	No	2 months	100	2.2
<i>Pimpinella major</i> (L.) Huds.	20/10°C	No	2 months	88.3	90.6
	20°C	No	2 months	88.3	79.2
<i>Trollius europaeus</i> L.	20°C	Yes	No	83.3	92
	20°C	No	2 months	75	4.4
<i>Peucedanum ostrutium</i> (L.) Koch	25/15°C	Yes	No	50	93.3
	25/15°C	No	2 months	85	50.9

Results of the germination tests are reported in Table 2. Seed viability was generally high, except for *Carex otrubae*, in which germination was very low. Viability was high, but germination very low in *Lunaria rediviva*. This species will require additional tests to identify its germination requirements. It is likely that the cold stratification has induced dormancy in the seeds instead of increasing their germination potential (Mondoni *et al.*, 2017).

Best germination results were obtained at high temperatures (above 25 °C) in most *Cyperaceae* and *Juncaceae* (Tab. 2). GA₃ was essential to stimulate germination in *Peucedanum ostrutium*, *Carex pendula* and *Trollius europaeus*, but was detrimental in other species, like *Caltha palustris*, *Chaerophyllum aureum* and the genus *Juncus*. In the latter group of species, cold stratification was more effective in releasing seed dormancy (Tab. 2).

Despite the development of these protocols, the germination and consequently the production of some species (*e.g.* *Chamaenerion angustifolium* (L.) Scop., *Carex otrubae* Podp., *Caltha palustris* L.) resulted particularly difficult. A reason is probably the low quality of produced seeds by the very small local populations of these species in the area. So far, about 12,000 plants ready to be released in the project sites have been produced (Tab. 1).

In June 2017, a first planting trial has been carried out at the Metaletto Lake (Camaldoli, AR) in the premises of Carabinieri Forestali. This site was the first restored through a reduction of the reed community that formerly occupied about 80% of the small lake up to about 60%. Moreover, three small ponds were excavated to create a suitable habitat for the reproduction of *B. pachypus*. Here, about 500 plants of different species has been planted, according to the planting scheme described above. A recent survey (July 2017) revealed that, though the summer in the area was very dry, most plants survived and only little damages were done by ungulates. Other wetlands will be restored or created in 2017 and 2018.

WetFlyAmphibia is a highly demonstrative project because it highlights the complexity of making actions matching the ecological requirement of target animals and plants. Plants are often neglected in large conservation projects, but, on the other hand, they are essential to provide suitable habitat requirements for target animals. The restoration of existing wetlands and the creation of new ponds had to be planned according both plant and animal requirements in terms of pond depth, inclination of the shores, position within the general context, elevation and light requirements of the species.

Other interesting challenges will come from the

population reinforcement of *Hottonia palustris* and *Tozzia alpina*. The first is a distylous plant, in which the frequency of the two morphs may strongly affect the long-term reproductive effort of a population and in turn the success of the reinforcement (Brys *et al.*, 2007). The second is a hemiparasitic plant that requires a complete understanding of the relationships between the species and the host (*e.g.* Holzapfel *et al.*, 2016).

Interestingly, the project highlighted the synergy between a private native plant company and two public institutions. The production of the target species was possible thanks to the reciprocal know-how exchange between the three actors. The seed/plant native market is becoming always more important and may also represent an important economic opportunity for biologists and naturalists.

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