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## A large-scale assessment of *Isoetes histrix* s.l. swards in the Mediterranean basin

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### Abstract

One of the most vulnerable community types in the Mediterranean basin are the *Isoetes histrix* swards. The present study, based on a literature review, was aimed to define patterns of plant communities with *I. histrix* and to identify the key factors driving these patterns at large spatial scale. Data from 7 areas (Corsica, Crete, Italian peninsula, Portugal, Sardinia, Spain, and Tunisia) were assembled into a composite matrix including 255 surveys and 314 species. To each survey the following abiotic variables were associated: geographic position, elevation, distance from the sea, annual precipitation, average annual temperature, and substrate type. Exploratory analysis reveals extremely high beta diversity among and within the localities, which calls for protection measures to be taken individually in each area. The most influential abiotic factor explaining this diversity is precipitation, which necessitates special management plans to maintain the hydrological status of each site. The fact that precipitation, a relatively unpredictable and unstable climatic variable is most influential, confirms that the isoetid communities of the Mediterranean region are vulnerable to climatic changes.

Key words: abiotic factors, beta diversity, Habitat Directive, ponds, temporary wetlands.

### Introduction

*Isoetes histrix* Bory (land quillwort) is an amphibian lycopod with a circum-Mediterranean distribution, also occurring in Atlantic Europe, the Channel Islands, and SW Britain (Prada, 1983; Quézel, 1998). Similarly to other species of the genus, it shows conspicuous morphological variability in length of phyllopodium prickles, leaf length and orientation, and megaspore ornamentation that have been interpreted differently in systematics (Bagella *et al.*, 2011). By comparing spore ornamentation and microornamentation and taking into account chromosome numbers, Bagella *et al.* (2016) circumscribed 3 taxa within the *I. histrix* complex: *I. histrix*, *I. gymnocarpa* Gennari and *I. sicula* Tod. On the contrary, Troia & Greuter (2014) considered *I. sicula* as a synonym of *I. gymnocarpa*. Further interpretations were provided earlier by other authors (Cesca & Peruzzi, 2001; Bolin *et al.*, 2008; Carta *et al.*, 2009; Ernandes, 2011) as well. From these issues raises the opportunity to consider *I. histrix* as a species complex and to refer to it as *I. histrix* s.l. (hereafter mentioned as *I. histrix* in the present assessment). It is considered a diagnostic species of the *Isoetion durieui* Br.-Bl. 1936 alliance which includes ephemeral pioneer annual and dwarf perennial ephemeral isoetid communities confined to warm Mediterranean climate, which colonize small areas and grow on soils that dry up in early spring (Biondi *et al.*, 2012; Biondi *et al.*, 2014; Deil, 2005; Rivas-Martínez *et al.*, 2002). The

conservation value of *Isoetion* was recognized nearly a century ago (Braun-Blanquet, 1936). More recently, European legislation has assigned a key role to this alliance in biodiversity conservation and the Habitat Directive (Commission of the European Community, 1992) referred the constituting communities to habitat type 3170\*- Mediterranean temporary ponds, which is considered for protection with high priority. This habitat type is characterised by oligotrophic waters containing low concentrations of minerals, generally on sandy soils of the West Mediterranean. The dominating plants are *Isoetes* spp., which are characteristic of very shallow water (few centimetres) with a flooding period no longer than 2-3 months (Commission of the European Community, 2013). Despite the increased awareness of the public on the vulnerability of such Mediterranean temporary ponds, their conservation value is frequently overlooked and their importance largely unappreciated and misunderstood. Besides the threats resulting from inadequate management, or even from partial or total destruction, climate change in the Mediterranean region might modify the hydrological regimes of freshwater inland water bodies and Mediterranean temporary ponds are particularly sensitive to this alteration (Ruiz, 2008).

*I. histrix* swards have been investigated by several authors but the available information is mostly fragmented and dispersed. However, a general summary of this vegetation type would be useful to recognize the main ecological drivers of its distribution at large

spatial scale and to better address conservation issues. Therefore, the present study was aimed i) to define patterns of plant communities with *I. hystrix* found in different areas of the Mediterranean basin and ii) to identify the key factors driving these patterns at large spatial scale. Better knowledge of vegetation structure and composition in temporary ponds is absolutely necessary for establishing habitat conservation priorities and strategies (Pinto-Cruz *et al.*, 2009) and the results of this study are hoped to provide a benchmark for their management.

## Material and methods

### Data collection

A literature survey was conducted to find papers including phytosociological studies on communities with *I. hystrix*. First, journal articles were searched in Google Scholar using the key words *Isoetion* and Braun-Blanquet 1936. Then, the bibliographies of the articles thus found were used to search for additional sources. All published tables used Braun-Blanquet's (1951) classical abundance-dominance (A-D) scale. In the present study, all relevés in which *I. hystrix* had an A-D value no less than 2, which largely means a cover  $\geq 5\%$ , were included, thus excluding sample sites where this species was probably more accidental than typical. In the more than 200 papers examined only 16 contained relevés in which this criterion was satisfied (Bagella *et al.*, 2009; Biondi & Bagella, 2005; Braun-Blanquet, 1936; Daoud-Bouattour *et al.*, 2009; Gradstein & Smittenberg, 1977; Lorenzoni & Paradis, 1997; Paradis *et al.*, 2002; Gigante *et al.*, 2013; Paradis *et al.*, 2005; Paradis *et al.*, 2008; Pottier-Alapetite, 1952; Rivas Goday, 1970; Rivas-Martínez *et al.*, 1980; Téllez & Franzi, 1987; Rudner, 2004; Rudner, 2005).

The relevé data were assembled into a composite matrix such that plant nomenclature was standardized according to the Catalogue of life online database (2016 edition). The species considered characteristic of temporary wetlands, identified according to Grillas *et al.* (2004) and Bagella & Caria (2012) (abbreviated here as *tw* species), represent the most relevant group in *I. hystrix* swards. The final matrix includes 255 relevés and 314 species. According to their geographical distribution, the sites in which the relevés were taken were clustered in 7 areas: Corsica (54 surveys), Crete (6), Italian peninsula (34), Portugal (87), Sardinia (28), Spain incl. Gibraltar (39), Tunisia (7). In addition to sites, the following abiotic variables were associated to each relevé: geographic position (latitude and longitude), elevation above sea level, distance from the sea, annual precipitation, average annual temperature, and parent material. Latitude, longitude, elevation, and distance from the sea were extracted from Google Earth. For the other variables,

the primary sources were the papers from which the relevé data were extracted. Whenever reported, they were referred to online databases and geological maps. Parent material was classified into two categories: acid and basic, coded by 0 and 1, respectively.

### Data analysis

Given that the data originate from various sources so that conditions of data collection and sampling effort were by no means uniform, and that the Braun-Blanquet scores are not readily suitable to numerical analysis, only the presence-absence scores of species were used. This data type is the less prone to sampling bias than any other, quantitative data type. Some simple descriptive statistics were first derived from the raw data. A dissimilarity matrix of sites was calculated using the Jaccard coefficient, which was then subjected to UPGMA clustering and principal coordinates analysis (PCoA). On the ordination diagram, the geographic classification of sites was superimposed using convex hulls drawn around points representing sites from the same area. For details of these multivariate analyses, see Podani (2000, 2001). To examine the relationship between pairwise floristic dissimilarity and environmental (geographic, climatic and geological) differences of site pairs, matrix correlations for each abiotic factor were calculated. Environmental differences among survey sites were expressed using the Euclidean distance. Without testing for significance, these correlations allow us to find an order of importance of the abiotic variables.

In order to evaluate beta diversity relationships in the studied sites, the SDR simplex approach suggested by Podani & Schmera (2011) was adopted. This involves decomposition of the Jaccard dissimilarity between sites into two additive components, richness difference (D) and species replacement (R) which reflect quite different ecological drivers. Together with the complement of dissimilarity, i.e. Jaccard similarity (S), these three quantities may be used to illustrate each pair of sites as a point in an equilateral triangle, the so-called simplex diagram. The shape of the point cloud is informative about the structure of the data matrix (for noted examples, see the paper cited above). In addition to graphical display, numerical results also assist the user to evaluate diversity relationships in the data. Percentage contributions of S, D and R to the total, as well as combinations of these, such as S+D which corresponds to nestedness and S+R which is richness agreement were used.

## Results

### General statistics

The number of species in the seven geographic areas greatly varies, from 25 to 144. The total number of



*tw* species was 43, ranging between 3 and 29 per area (Fig. 1). These figures appear understandable given the very different sample sizes used. However, there was no significant correlation between the number of sites and the number of species listed for each area ( $r = 0.191$ ) and between the number of sites and the number of *tw* species listed for each area ( $r = 0.067$ ) suggesting that for overall analysis sample size effects may not always matter. On the contrary, there was a positive correlation between the total number of species and the number of *tw* species ( $r = 0.894$ ). Around half of the *tw* species (21) were unique to an area, while just one species (*Juncus capitatus* Weigel) was found everywhere (Table 1).

**Correlations with abiotic factors**

Matrix correlations between the Jaccard dissimilarity and environmental distance for each abiotic factor are presented in Table 2. These are used here primarily to assess the relative importance of different geographic, climatic and geological variables upon floristic dissimilarity. In this regard, the most influential is annual precipitation, which has a correlation of 0.466 with floristic dissimilarity. Thus, the availability of water is the most influential environmental variable in the studied communities. This is immediately followed by geographic distance ( $r = 0.435$ ) so that the distributional ranges of constituting species also affect greatly the actual species composition in a given site. Elevation, distance from the sea, temperature and the parent material exhibit lower correlations with floristic dissimilarity, in that order, and are thus less influential on floristic differences. Correlations among all pairs of abiotic variables are given for completeness; these are usually very low as expected. A noted exception is the high correlation (0.686) between temperature and parent material, which is due primarily to the occur-

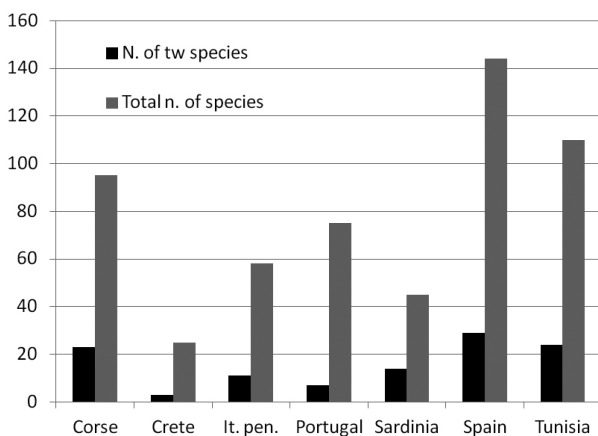


Fig. 1 - The number of species and characteristic (*tw*) species for the major study areas.

Tab. 1 - Characteristic (*tw*) species and their distribution in the study areas (\* = present).

|   | Corse | Crete | Italian peninsula | Portugal | Sardinia | Spain | Tunisia |
|---|-------|-------|-------------------|----------|----------|-------|---------|
| <i>Agrostis salmantica</i> (Lag.) Kunth                                   | *     |       |                   |          |          | *     | *       |
| <i>Anagallis arvensis</i> L. subsp. <i>parviflora</i> (Hoff. & Link) Arc. | *     |       | *                 |          |          |       | *       |
| <i>Anagallis minima</i> (L.) E.H.L. Krause                                |       |       | *                 |          |          |       |         |
| <i>Antinoria agrostidea</i> (DC.) Parl.                                   |       |       |                   |          | *        | *     |         |
| <i>Centaurium pulchellum</i> (Swartz) Druce                               |       |       |                   |          |          |       | *       |
| <i>Cicendia filiformis</i> (L.) Delarbre                                  | *     |       | *                 | *        | *        | *     | *       |
| <i>Crassula vallantii</i> (Willd.) Roth                                   |       |       |                   |          | *        | *     |         |
| <i>Eryngium galioides</i> Lam.  |       |       |                   |          |          | *     |         |
| <i>Eryngium pusillum</i> Desf.  |       |       |                   |          |          |       | *       |
| <i>Exaculum pusillum</i> (Lam.) Caruel                                    |       |       |                   |          |          | *     |         |
| <i>Helosciadium crassipes</i> W.D.J. Koch ex Rehb.                        | *     |       |                   |          | *        | *     | *       |
| <i>Hypericum humifusum</i> L.   |       |       | *                 |          |          | *     |         |
| <i>Illecebrum verticillatum</i> L.  | *     |       |                   |          |          | *     | *       |
| <i>Isoetes longissima</i> Bory subsp. <i>tegulensis</i> Genn.             |       |       |                   |          | *        |       |         |
| <i>Isoetes longissima</i> Bory subsp. <i>longissima</i>                   | *     |       |                   |          | *        | *     | *       |
| <i>Isolepis cernua</i> (Vahl) Roem. & Schult.                             | *     | *     |                   | *        | *        | *     | *       |
| <i>Isolepis pseudosetacea</i> (Daveau) Gand.                              |       |       |                   |          |          | *     |         |
| <i>Isolepis setacea</i> (L.) R.Br.  | *     |       |                   |          |          | *     |         |
| <i>Juncus bufonius</i> L.   | *     | *     | *                 | *        | *        | *     | *       |
| <i>Juncus capitatus</i> Weigel  | *     | *     | *                 | *        | *        | *     | *       |
| <i>Juncus hybridus</i> Brot.  |       |       |                   |          | *        | *     |         |
| <i>Juncus pygmaeus</i> Rich. ex Thuill.                                   | *     | *     | *                 | *        | *        | *     | *       |
| <i>Juncus tenajeya</i> Ehrh.  | *     | *     | *                 | *        | *        | *     | *       |
| <i>Kickxia cirrhosa</i> (L.) Fritsch                                      |       |       |                   | *        | *        | *     |         |
| <i>Lotus angustissimus</i> L.   |       | *     |                   |          |          |       |         |
| <i>Lotus parviflorus</i> Desf.  | *     |       | *                 |          | *        | *     |         |
| <i>Lythrum borysthenicum</i> (Schränk) Litv.                              | *     |       |                   | *        | *        | *     | *       |
| <i>Lythrum hyssopifolia</i> L.  | *     |       |                   | *        | *        | *     | *       |
| <i>Lythrum portula</i> (L.) D. A. Webb                                    | *     |       |                   | *        | *        | *     | *       |
| <i>Lythrum tribracteatum</i> Sprengel                                     |       |       |                   |          | *        | *     |         |
| <i>Marsilea strigosa</i> Willd.   |       |       |                   |          | *        | *     |         |
| <i>Mentha pulegium</i> L.   | *     |       |                   |          | *        | *     | *       |
| <i>Myosotis sicula</i> Guss.  |       |       |                   |          | *        | *     |         |
| <i>Myosurus minimus</i> L.  |       |       |                   |          | *        | *     |         |
| <i>Ophioglossum lusitanicum</i> L.  | *     |       | *                 | *        | *        | *     | *       |
| <i>Pilularia minuta</i> Dur.  |       |       |                   |          | *        | *     | *       |
| <i>Pulicaria sicula</i> (L.) Moris  | *     |       |                   |          | *        | *     | *       |
| <i>Pulicaria vulgaris</i> Gaertn.   |       |       |                   |          | *        | *     | *       |
| <i>Radiola linoides</i> Roth  | *     | *     | *                 | *        | *        | *     | *       |
| <i>Ranunculus lateriflorus</i> DC.  |       |       |                   |          | *        | *     | *       |
| <i>Ranunculus longipes</i> Cutanda  |       |       |                   |          | *        | *     | *       |
| <i>Ranunculus ophioglossifolius</i> Vill.                                 | *     |       |                   |          | *        | *     | *       |
| <i>Ranunculus revelierei</i> Boreau                                       | *     |       |                   |          | *        | *     | *       |
| <i>Romulea revelieri</i> Jord. & Fourr.                                   | *     | *     | *                 | *        | *        | *     | *       |
| <i>Trifolium michelianum</i> Savi   |       |       |                   | *        | *        | *     | *       |
| <i>Trifolium micranthum</i> Viv.  |       |       |                   |          |          | *     | *       |

rence of limestone bedrock in the relatively warm area of Crete.

**Clustering and ordination**

The UPGMA dendrogram (Appendix I) groups the relevés according to their geographical distribution, with a few outliers and exceptions. Three relevés from the south-central Spain (24, 29, 30) are separated first from the rest, followed by four obvious outliers, two from Sardinia and other two from Corsica. Then, there is a division between two major groups, sites from Portugal and the rest. Within the latter, the groups from Italy and Sardinia are clear cut and are separated from

Tab. 2 - Matrix correlations among floristic dissimilarity and distances in abiotic factors between the study sites. Only the lower semimatrix is presented.

|                         | Floristic dissimilarity | Geographic position | Elevation | Distance from sea | Temperature | Annual precipitation | Parent material |
|-------------------------|-------------------------|---------------------|-----------|-------------------|-------------|----------------------|-----------------|
| Floristic dissimilarity | 1                       |                     |           |                   |             |                      |                 |
| Geographic position     | 0.435                   | 1                   |           |                   |             |                      |                 |
| Elevation               | 0.322                   | 0.372               | 1         |                   |             |                      |                 |
| Distance from sea       | 0.236                   | 0.139               | 0.153     | 1                 |             |                      |                 |
| Temperature             | 0.159                   | 0.374               | 0.128     | 0.037             | 1           |                      |                 |
| Annual precipitation    | 0.466                   | 0.544               | 0.366     | 0.083             | 0.051       | 1                    |                 |
| Parent material         | 0.097                   | 0.218               | 0.152     | -0.047            | 0.686       | 0.024                | 1               |

each other as well. Most of the relevés from Corsica are also in the same cluster, with two Tunisian sites intermingled. Finally, the relevés from southwestern Spain combine with some relevés either from Tunisia or from Crete.

The PCoA ordination (Fig. 2) largely confirms these findings even though the first two dimensions account only for 12% of the total variation. On this plane, relevés from Portugal and Sardinia completely separate from the others, while relevés from Italy and Corsica overlap to some extent with the others. Note that the other geographic groups of relevés, all of which with much lower sample size, are located around the origin.

#### SDR simplex analysis

The SDR simplex diagrams are seen as insets in Fig.

2, one calculated for the entire data set, and those superimposed on the ordination for the separate areas. In the entire data set, matrix fill is only 4.88%, i.e. the data matrix is very sparse. As a result, there is an extremely high beta diversity (90%) dominated by contributions from species replacement (67%) while richness differences are responsible for 23% of beta diversity. Thus, similarity accounts for only 10%. Community structure within major geographic areas is fairly similar to this general picture in many cases, as shown by the ternary diagrams for Portugal, Spain and these two taken together (Iberia), as well as for Italy and Corsica. Samples from Sardinia exhibit somewhat lower beta diversity, just like the few sample sites from Tunisia. Sites of Crete are fairly similar to each other, and have similar species richness, so the points lie close to the

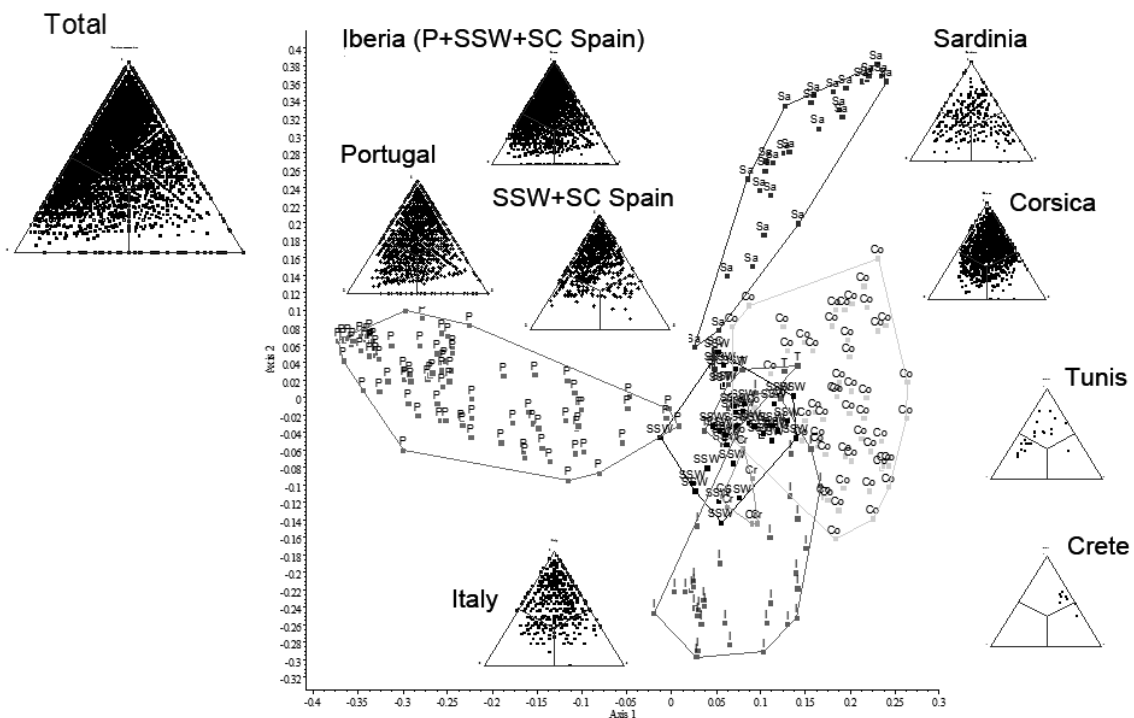


Fig. 2 - PCoA ordination of *I. histrix* swards in the Mediterranean, with simplex diagrams for the entire data set (upper left) and major geographic areas separately (insets on the ordination). Symbols: Co – Corsica, Cr – Crete, I – peninsular Italy, P – Portugal, Sa – Sardinia, SC – south-central Spain, SSW – southwestern Spain, T – Tunisia.

right edge of the triangle. Nestedness is relatively low, and only very few sample sites are completely nested (those represented by points on the bottom edge).

## Discussion

*Isoetes histris* swards characterized by a significant ( $\geq 5\%$ ) cover of this species represent just an aspect of the habitat type 3170\*. Indeed this habitat also includes other types of the swards where *I. histris* has lower cover or is totally absent (Biondi *et al.*, 2012). Nevertheless, in the context of Mediterranean temporary ponds, the *I. histris*-rich swards are considered highly relevant in terms of biodiversity and to which conservation efforts must be oriented primarily (Médail, 2004). Therefore, a detailed study was sought to reveal the floristic and diversity conditions in different stands of the communities and to evaluate the effect of major abiotic factors on these conditions. Our present survey is an attempt to satisfy this goal by analyzing a large presence-absence data set derived from the literature.

*I. histris* swards are well differentiated in space within the Mediterranean basin and it was possible to identify factors that control their large scale pattern. The great variation of the number of species/area with the lower values found in the eastern Mediterranean (i.e. Crete) mirrors the general observation that floristic assemblages are richer in the ponds located in the western Mediterranean (Médail, 2004). Whereas this conclusion was apparently unaffected by sample size, high correlation between the total floristic richness and the number of species characteristic of temporary wetlands (*tw* species) per area was detected. Thus, the use of this subset of species, as a surrogate of vascular plant biodiversity, provides a good opportunity for simplifying the monitoring of the flora of Mediterranean temporary ponds. Trained practitioners can easily adopt the procedure in the field as an efficient alternative to other, more time-consuming inventory designs (Lumbreras *et al.*, 2016).

The absence of some *tw* species in the *I. histris* swards in some areas and, on the contrary, their presence in other areas must be interpreted in different way considering their general chorology, their rarity at local scale and their capacity to grow under different ecological conditions. Some species are endemic to or present in a relatively small area. For instance *Eryngium galioides* Lam. is endemic to few localities of the Iberian Peninsula (Jury, 1996) while *Romulea revelieri* Jord. & Fourr. is endemic to Sardinia and Corsica (Bagella *et al.*, 2013). *Pilularia minuta* Dur. and *Marsilea strigosa* Willd., two aquatic flagship ferns, were found respectively only in Tunisia and Spain, even though they are also distributed in other Mediterranean areas. Normally, they grow in the deeper parts of temporary ponds where *I. histris* swards are replaced by other ve-

getation types (Caria *et al.*, 2015; Rhazi *et al.*, 2006;). *P. minuta* and *M. strigosa* are two target species for conservation, both listed in Annex I of the Bern Convention. *P. minuta* is assessed as critically endangered in Northern Africa (García *et al.*, 2010) and as endangered in Europe; *M. strigosa* is also listed in Annex II of the Habitat Directive and is assessed as vulnerable at the European scale (Bilz *et al.*, 2011). Their presence in *I. histris* swards is of particular interest because these habitats can serve as refugia to ensure the survival of these two species.

It was shown that there is an extremely high beta diversity of *Isoetes* assemblages all over the Mediterranean. The dominating process explaining this structure is species replacement, while richness difference is less substantial. These two components together are responsible for around 90% of the total relative gamma diversity of the study area. The high proportion of species replacement is due to the fact that the 314 taxa in the species pool of the region are very unevenly distributed (each area can have no more than c. 140 species) and the species rich areas have relatively few species in common. The reason behind the richness difference component is not simply the different sample size because more than 100 species occur in Tunisia which is represented only by 7 sites, whereas in the 28 sites from Sardinia only 44 species were found.

It is interesting that within-area community structure is fairly similar to the overall structure (i.e., high beta diversity) in most cases, with the exception of Sardinia, Tunisia and especially Crete. We should not draw far-reaching conclusions from the latter case, however, because sample size was too small to exhaust all possibilities of floristic diversity. Even though beta diversity is high within most areas, they are separated from each other quite sharply – in the classification there were very few outliers and the groups of sites reproduced the geographic distribution.

The consequence of high beta diversity, and very low similarity and nestedness is straightforward from the viewpoint of nature conservation: there are no specific sites, nor particular areas which could be given priority against the others. That is, as many sites as possible deserve protection in every study area, which confirms the decision by policy makers that these communities and their habitats deserve particular attention.

The major underlying ecological factor explaining the high beta diversity among the sites is annual precipitation; it has higher correlation with between-site dissimilarity than geographical distance. Not surprisingly then, these two are also highly correlated with each other ( $r = 0.544$ ), suggesting the existence of a precipitation gradient in the Mediterranean basin. Moreover, swards with *I. histris* occupy generally the peripheral area of Mediterranean temporary ponds which represent a critical position on direct upland

contact. This position makes plants particularly sensitive to variations in hydrological periods (Bagella & Caria, 2013). Another geographical factor, elevation, has lower effect although its influence has been reported on pond plant communities by Oertli *et al.* (2002) and Rolon & Maltchik (2006). Note that temperature is even less correlated with floristic dissimilarity, and the parent material was even more neutral in this sense.

The fact that precipitation, a relatively unpredictable and unstable climatic variable is most influential confirms that the *I. hystrix* communities of the Mediterranean region are vulnerable to climatic changes both at the local and global scales. Reclamation still in progress in several areas causes irreparable damage which may be further deteriorated with the obvious interaction with climate change effects (Cízková *et al.*, 2016). Mitigation actions should be performed within proper water management plans, including special protection during drought periods when these habitats are more sensitive to changes in the environment (Serrano & Zunzunegui, 2008).

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**Appendix I: UPGMA classification of survey sites with *I. hystrix* swards.**

Symbols as in Fig. 2. The symbols become more eligible if the picture is enlarged in the online version, hosted at the journal's website [www.scienzadellavegetazione.it/sisv/rivista/rivista\\_elenco.jsp](http://www.scienzadellavegetazione.it/sisv/rivista/rivista_elenco.jsp).

