Habitat differentiation in the threatened aquatic plant genus *Baldellia* (L.) Parl. (Alismataceae): Implications for conservation

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ARTICLE INFO

ABSTRACT

A comparative analysis of the habitats of *Baldellia ranunculoides* (subsp. *ranunculoides* and subsp. *repens*) and *Baldellia alpestris* (Alismataceae) was carried out across central and western Europe. Soil samples from 43 populations were analyzed and the composition of accompanying vegetation was analyzed by canonical correspondence and indicator species analysis. Significant differences in the habitat conditions and the accompanying vegetation were found between the three taxa. *B. ranunculoides* subsp. *repens* is growing in lowland water bodies on acidic substrates (pH 5–6.3) which are markedly richer in organic matter and poorer in cations, especially in Ca^{2+}. *B. ranunculoides* s. str. is also a lowland taxon, but grows on more mineral and basic substrates (pH 7–9), usually rich in Ca^{2+}. The endemic *B. alpestris* grows in the most distinct habitats, on substrates with low pH and with very low Ca^{2+} concentrations, at much higher altitudes than the other taxa and in very specific plant communities that have almost no affinities with those of the two lowland *Baldellia*-taxa. The *Baldellia*-taxa are threatened by eutrophication of their habitats. Moreover, many of the extant populations, in particular those of *B. ranunculoides* s. str., are very small and will be threatened by environmental stochasticity. Reintroduction and the creation of new populations might reduce the risk of extinction. Information given on the detailed habitat preferences of the *Baldellia*-taxa could help conservation efforts for these endangered taxa and identify suitable sites for (re-) introductions.

1. Introduction

Aquatic ecosystems and in consequence aquatic organisms have been affected by multiple anthropogenic pressures (Egertson et al., 2004). Rivers have been strongly regulated, many ponds have been destroyed and ponds as well as lakes have been affected by nutrient enrichment (Roelofs, 1983; Cronk and Fennessy, 2001; Preston and Croft, 2001). As a consequence, in many countries aquatic plants are among the most threatened groups of organisms (Moser et al., 2002). The species of the genus *Baldellia* are no exception, having strongly declined throughout their area of distribution due to habitat destruction, fragmentation and other anthropogenic changes (Preston and Croft, 2001; Jones, 2006) and they are now threatened in many European countries (e.g. Korneck et al., 1996; Aronsson, 1999; Wigginton, 1999; Moser et al., 2002).

Baldellia is one of several small genera of the exclusively aquatic family Alismataceae (Cook, 1990). There are only two generally recognized species in the genus: *B. ranunculoides* (L.) Parl. (=*B.*

ranunculoides s.str.), native to Europe and the Mediterranean and *B. alpestris* (Coss.) Vasc., endemic to the mountains of northern Portugal and northwestern Spain (Vasconcellos, 1970; Cook, 1983; Moreno Saiz and Sainz Ollero, 1992). Many studies have recognized, however, a third taxon, either called *B. ranunculoides* subsp. *repens* (Lam.) A. Löve & D. Löve or *B. repens* (Lam.) van Ooststroom, with a more Atlantic distribution (e.g. Lawalrée, 1959; Van Rompaey and Delvosalle, 1972; Cools, 1989; Ingelög et al., 1991; Lid and Lid, 2005). Numerous differences in the morphology, genetics, vegetative growth, resource allocation and phenology support the taxonomic separation of subsp. *ranunculoides* and subsp. *repens* (Glück, 1905; Casper and Krausch, 1980; Vuille, 1988; Triest and Vuille, 1991; Preston and Croft, 2001; Jacobson, 2003).

To help the conservation of a taxon, information on its distribution, habitat preferences and biology is needed. Very little, however, is known about the ecology of the *Baldellia*-taxa. This is particularly true for *B. alpestris*, but also for the subsp. *repens*, which was often not differentiated from *B. ranunculoides* s.l. in the past. The anatomy and morphology of the genus has been described by Glück (1905, 1906), the reproductive biology of all three *Baldellia*-taxa has been studied by Vuille (1988) and their

genetics by Triest and Vuille (1991) and Jacobson (2003). More detailed studies on the biology of the subsp. repens have been carried out only on a local scale, e.g. in Belgium (Lawalrée, 1959), in Scandinavia (Lindblad and Stahl, 1989, 1990) and in the United Kingdom (Jones, 2006).

We carried out a detailed comparative study of the habitat preferences of the three Baldellia-taxa in western and central Europe and the plant communities in which they occur. Our aims were to: (1) analyze differences in the ecological niche of the three taxa, and (2) provide a basis for more effective conservation measures and priorities.

2. Methods

2.1. Study species

All Baldellia-taxa are stoloniferous, perennial (rarely annual), rooted water plants, usually 5–30 cm tall (Vuille, 1988; Preston and Croft, 2001) that grow in lakes, ponds and slow streams. The three Baldellia-taxa can be differentiated by their morphology (Lawalrée, 1959; Casper and Krausch, 1980; Vuille, 1988; Triest and Vuille, 1991; Franco and Afonso, 1994). B. alpestris differs from B. ranunculoides s.l. in having smaller and more elliptical leaves (maximum size of leaf blades of B. ranunculoides s.l.: 2×10 cm; B. alpestris: 1×5 cm) with a blunt apex; it has smaller petals (maximum length 6 mm; B. ranunculoides s.l.: 6–15 mm) and fewer nutlets (maximum 20; B. ranunculoides s.l.: 20–45). The most important characteristics for the differentiation of B. ranunculoides subsp. ranunculoides and subsp. repens in the field are: (1) The general habit and vegetative modus of reproduction. Subspecies ranunculoides is an erect plant without runners, whereas subsp. repens is a creeping plant with runners and has inflorescences that are rooting at the nodes. (2) The number of flowers per pseudowhorl, which is mostly 10–20 for subsp. ranunculoides and only 2–3 for subsp. repens. (3) The surface of nutlets is smooth in subsp. ranunculoides and papillous in subsp. repens.

B. ranunculoides s.str. and B. alpestris are outcrossing but self-compatible, whereas subsp. repens is strongly self-incompatible. B. ranunculoides subsp. repens has the typical characteristics of an insect-pollinated plant with high-contrast floral elements (showy pink or purple petals and yellow anthers). B. ranunculoides s.str. and B. alpestris have smaller and less contrasted flowers (Vuille, 1988).

B. ranunculoides s.str. is an Atlantic/west Mediterranean taxon (Markgraf, 1981) growing mainly in coastal regions of western Europe and the Mediterranean (Meusel et al., 1992; de Bolos and Vigo, 2003). The exact distribution of B. ranunculoides subsp. repens is not known. It appears to be restricted to the more Atlantic regions of western and northern Europe and to some fairly isolated sites in the western Mediterranean (Lawalrée, 1959). Its main centre of distribution, where it appears to be more frequent than subsp. ranunculoides, is probably in western and central France (e.g. Sologne, Forêt d'Orléans), Belgium and The Netherlands (e.g. Limburg; Casper and Krausch, 1980; Dupont, 1990).

B. alpestris is an endemic of northern Portugal and northwest Spain. It is occurring in mountainous regions of Asturia and Leon in Spain and of Tras-os-Montes and Minho in Portugal, as well as in some coastal regions of Beira and Alto Alentejo (Portugal; Casper and Krausch, 1980; Cook, 1983; Franco and Afonso, 1994). Romero et al. (2003) reports it also from coastal regions of Galicia and northern parts of the Sistema Iberico mountains between Burgos and Soria.

In central and western Europe B. ranunculoides s.str. is a characteristic species of the class Littorelletea uniflorae, and it can be found in all its principal alliances (Pott, 1995; Schubert et al.,

1995). B. ranunculoides subsp. repens was described as a characteristic species of the Eleocharitetum multicaulis (alliance Eleocharition multicaulis, class Littorelletea; Casper and Krausch, 1980) or of the Eleocharitetum acicularis (alliance Eleocharition acicularis, class Littorelletea) (Schoof-van Pelt, 1973). B. alpestris is usually classified as an element of the Spargania-Glycerion (class Phragmitetea) (Sardinero Roscales, 1994) or as a species of the class Isoëto-Nanojuncetea (Casper and Krausch, 1980).

2.2. Data collection

The survey was carried out in the habitats of all three Baldellia-taxa in the years 2005–2006 at the height of the growing season (June/August). We have inspected more than 100 localities where one of the Baldellia-taxa was indicated in the available literature or by herbarium data. We found Baldellia in less than half of the visited sites. Altogether, 43 populations in nine European countries were sampled and analyzed: 19 of B. ranunculoides s. str., 15 of subsp. repens and 9 of B. alpestris (see Fig. 1 and Appendix A). In a few large water bodies more than one population was sampled if they were separated by a distance of at least 2 km. The species and subspecies of Baldellia were determined according to the detailed keys of Triest and Vuille (1991) and Jones (2006). At each Baldellia site we recorded the altitude, measured the depth of the water, recorded all accompanying species, estimated population size, and took samples of the substrate for chemical analyses. Because at many of the sites the Baldellia plants grew under semi-dry conditions, a physico-chemical analysis of the water was not carried out. Nomenclature for Baldellia-taxa follows Casper and Krausch (1980), for co-occurring plants Flora Europaea (Tutin et al., 1980). The schematic distribution map in Fig. 1 (inset) is based on Cook (1983), Hulten and Fries (1986), Meusel et al. (1992), Moreno Saiz and Sainz Ollero (1992), Franco and Afonso (1994), de Bolos and Vigo (2003), and Romero et al. (2003).

2.3. Substrate analysis

The substrate samples were collected from the root layer (c. 15 cm below the surface). From each site six randomly taken subsamples were pooled (final mass: c. 500 g), mixed and stored at 4 °C for 5–10 days until they were analyzed. The analyses were carried out using the standard methods developed by the Swiss Federal Research Station of Agroecology and Agriculture (FAL, 2003). The substrate samples were first homogenized manually and grained with a SK-100 crusher (Retsch Technology, Haan, Germany). The dry matter content of the samples was determined by drying the samples at 105 °C to constant weight. Organic matter content was assessed by ignition of dry matter at 500 °C to constant weight for 1.5–3 h. Total N was measured by Kjeldahl's method. The carbon-to-nitrogen (C/N) ratio was calculated as follows: organic matter content $\times 0.58$/total N (FAL, 2003). For further analysis the samples were dissolved in 20 ml of 1:1 HCl. After 2 h, 80 ml of hot demineralized water was added and the solution was placed for 20 min into a boiling water bath. After cooling to ambient temperature the quantity of extracted solution was adjusted to 200 ml and filtered. The content of P was determined spectrophotometrically (spectrophotometer UV/VIS 916 AA, GBC Scientific Equipment, Dandenong, Australia) by the ammonium molybdate method (wavelength 436 nm) and represented as P_2O_5 content (conversion factor: $P \times 2.294 = P_2O_5$). The K-content was assessed by flame spectrophotometry and represented as K_2O (conversion factor: $K \times 1.205 = K_2O$). Ca^{2+} and Mg^{2+} contents were determined with an atomic adsorption photometer (UV/VIS 906 AA, GBC Scientific Equipment). The contents are expressed in g/kg of dry matter. The pH was measured in a water/
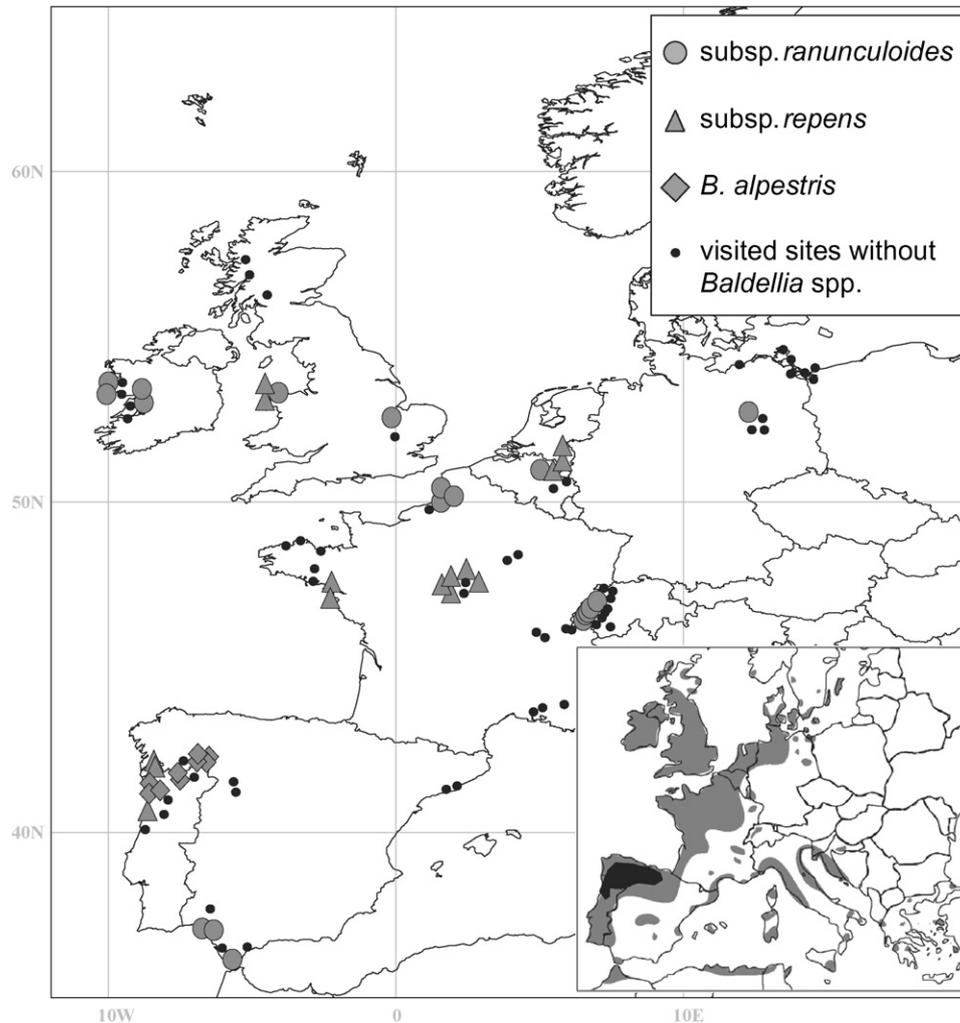
Fig. 1. Geographical location of the *Baldellia*-sites investigated in this study (see also Appendix A). Inset: schematic representation of the global distribution of *B. ranunculoides* s. str. (grey) and the narrow endemic *B. alpestris* (black). The detailed distribution of *B. ranunculoides* subsp. *repens* is unknown (probably very similar to subsp. *ranunculoides*, but the subspecies is absent in the eastern Mediterranean).

substrate suspension (1:2.5, w/v) using a glass electrode pH-meter (CG 842, Schott Instruments, Mainz, Germany). Electrolytic conductivity in water/substrate suspensions (1:5, w/v) was measured using a Metrohm 712 conductimeter (Metrohm, Buckingham, UK) with a platinum electrode and platinum temperature Pt100 sensor (platinum sensor with $100\,\Omega$ nominal resistance at $0\,^{\circ}C$).

2.4. Statistical analysis

The environmental conditions at the sites of the three *Baldellia*-taxa were compared by Kruskal–Wallis one-way analyses of variance, with p-values calculated by the Monte-Carlo permutation test available in SPSS 12.0 (10,000 runs). If the Kruskal–Wallis test was significant, pairwise comparisons (post hoc tests) were carried out using the Tukey method, which compares U-values obtained in pairwise U-tests with critical values based on the studentized range (Sokal and Rohlf, 1995). To study whether not only the mean values for the environmental variables differed between the *Baldellia*-taxa, but also their variances, we tested the homogeneity of variances with the Levene Statistic.

A preliminary detrended correspondence analysis (DCA) indicated long gradients in the vegetation at the habitats of the three *Baldellia*-taxa (1st axis: 6.1 S.D.), and following Lepš and Šmilauer (2003) the vegetation data were therefore analyzed using unimodal instead of linear models. The vegetation was analyzed by correspondence analysis (CA), and the differences among the vegetation accompanying the *Baldellia*-taxa were analyzed by means of a canonical correspondence analysis (CCA) with the presence of the *Baldellia*-taxa as explanatory variables. The significance of the differences in vegetational composition was tested with Monte-Carlo tests (9999 permutations; CANOCO for Windows 4.5, Ter Braak and Šmilauer, 2002). Cover values were $\log - (x + 1)$transformed prior to analysis, as were most environmental variables. Downweighting of rare species was used to avoid outliers, but results of analyses without downweighting were qualitatively the same.

The explanatory power of the environmental variables recorded for the differences in the vegetation of the sites was studied by a CCA. Forward selection, using partial Monte Carlo permutation tests ($p < 0.05$) as available in CANOCO for Windows 4.5 (Ter Braak and Šmilauer, 2002), was used to identify the environmental variables in the final model. The cover values for the *Baldellia*-taxa were excluded from all analyses of the vegetation.

To find plant species that are typical for sites with individual *Baldellia*-taxa, an indicator species analysis (Dufrêne and Legendre,

1997) was carried out. The indicator values obtained combine measures of the relative abundance and relative frequency of species (Dufrêne and Legendre, 1997). The significance of the indicator species was tested using the Monte-Carlo test available in PC-ORD (McCune and Mefford, 1999), using 10,000 permutations.

3. Results

3.1. Environmental conditions

There were clear differences in the habitat preferences of the three *Baldellia*-taxa (Fig. 2). Moreover, the taxa also differed in the width of their ecological niche with respect to several habitat variables, as indicated by significantly different variances (Table 1). The sites with *B. ranunculoides* s.str. differed from those of the other two taxa in that they had on average a higher pH and a higher Ca^{2+} and Mg^{2+} content (Fig. 2c, k and l). However, the range of Ca^{2+} concentrations found at sites with *B. ranunculoides* s.str. was wide and the taxon also occurred at sites with hardly any calcium (sites 4, 6–8, 13, 19; see Appendix A). Sites with *B. ranunculoides* s.str. also had a higher dry matter but a lower organic matter content

(Fig. 2e and f), and lower total N concentrations (Fig. 2g) than those with the other two taxa.

While *B. ranunculoides* s.str. and subsp. *repens* were observed exclusively in the lowlands; *B. alpestris* appears to be a species of mountains (Fig. 2a). However, it has a wider altitudinal range than the other taxa (see Table 1) and can also occur at low altitudes. While *B. ranunculoides* s.str. and subsp. *repens* were exclusively taxa of shallow water (<20 cm deep), *B. alpestris* grew in a much wider range of water depths (Fig. 2b and Table 1) and occurred in water up to 60 cm deep. A lower Mg^{2+} content (Fig. 2l) was the only site characteristic that was special for *B. ranunculoides* subsp. *repens*. The substrate in which the three taxa were growing did not differ significantly with respect to conductivity, C/N ratio, and phosphate content (Fig. 2d, h and i).

The size of the populations of the three taxa differed (see population sizes in Appendix A). At some sites both *B. alpestris* and the subsp. *repens* were found to form almost monospecific stands of more than 10,000 individuals. In the case of the subsp. *repens* c. 40% of the studied populations were larger than that number. In contrast, most populations of *B. ranunculoides* s.str. were small or medium-sized and only two populations consisted of more than 1000 individuals.
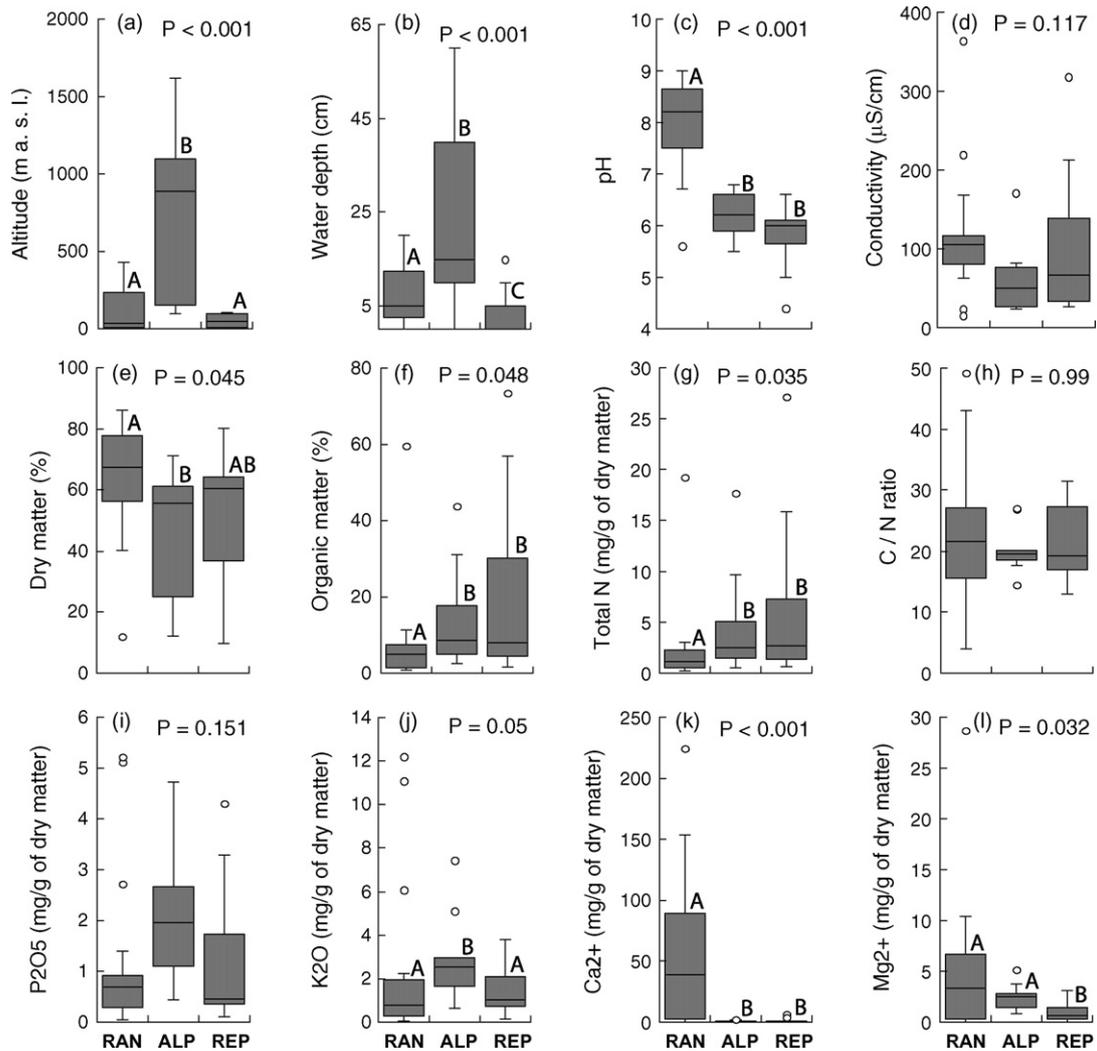

Fig. 2. Habitat conditions of the three *Baldellia*-taxa. Box plots indicate the median and the 25th and 75th percentiles of the observed distribution of measurements of 12 environmental variables in the habitats of each taxa. Whiskers indicate maximum and minimum values, circles are outliers. *P*-values for the differences among the taxa are results of Kruskal–Wallis one-way-ANOVAs with Monte-Carlo permutation tests (10,000 runs). Bars with different capital letters on top indicate individual taxa that are significantly different at the 0.05 level (Tukey method). RAN: *Baldellia ranunculoides* s. str.; ALP: *B. alpestris*; REP: *B. ranunculoides* subsp. *repens*.

Table 1
Test of homogeneity of variances (Levene statistic) among the three *Baldellia*-taxa of the distribution of the environmental and substrate variables used for further analysis

Variable	Levene statistic	*P*-value
Altitude	22.201	<0.001
Water depth	21.291	<0.001
pH	1.881	0.166
Conductivity	1.126	0.334
Dry matter	0.497	0.612
Organic matter	5.476	0.008
Total N	3.091	0.056
C/N ratio	3.756	0.032
P_2O_5	0.001	0.999
K_2O	2.626	0.085
Ca^{2+}	23.580	<0.001
Mg^{2+}	5.205	0.010

Degrees of freedom for all variables were 2 and 40.

3.2. Vegetation analysis and co-occurring species

A correspondence analysis (CA) indicated that there were strong gradients in the vegetation ($\lambda_1 = 0.75$, $\lambda_2 = 0.71$; proportion of total inertia 6.0% and 5.8%) and that the three *Baldellia*-taxa differed in the vegetation they were growing with (Fig. 3). The overall differences in the vegetation were highly significant (CCA with *Baldellia*-taxa as explanatory variables: trace = 0.91, $p < 0.0001$). The differences between the vegetation of the highland taxon *B. alpestris* and that of the two lowland taxa were particularly strong (trace = 0.58, $p < 0.0001$), but the vegetation at sites with *B. ranunculoides* s.str. and subsp. *repens* differed also significantly (trace = 0.35, $p = 0.0017$).
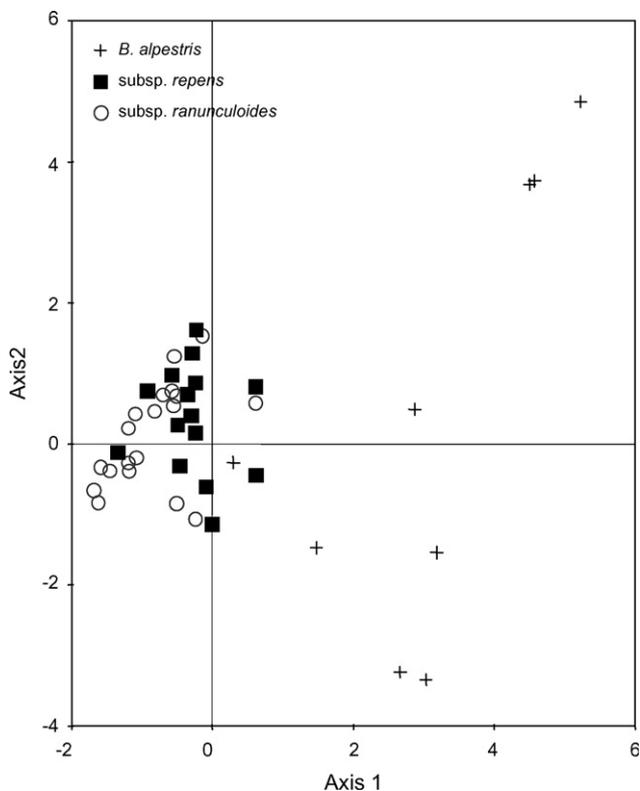

Fig. 3. CA-ordination diagram of the vegetation in which *Baldellia ranunculoides* s.str., subsp. *repens* and *B. alpestris* occur. Downweighting of rare species was carried out. Only site scores are shown to avoid clutter.

Table 2
Indicator species for sites with *Baldellia* sp.: percentage of perfect indication

	Indicator value (%)	*P*-value
Indicators for subsp. *ranunculoides*:		
Potamogeton gramineus	26	0.0330
Samolus valerandi	26	0.0352
Chara sp.	26	0.0429
Indicators for subsp. *repens*:		
Galium palustre	27	0.0296
Salix sp.	20	0.0415
Indicators for *B. alpestris*:		
Ranunculus ophioglossifolius	67	0.0001
Isoetes sp.	33	0.0060
Juncus heterophyllus	33	0.0072
Callitriche stagnalis	32	0.0049
Corrigiola litoralis	22	0.0391
Gentiana pneumonanthe	22	0.0403
Juncus acutiflorus	22	0.0402
Oenanthe crocata	22	0.0407
Potamogeton nodosus	22	0.0397
Ranunculus ololeucos	22	0.0403
Carum verticillatum	22	0.0403

The indicator values combine relative abundance and relative frequency, see Dufrêne and Legendre (1997). *P*-values were derived from Monte-Carlo tests of significance with 10,000 permutations.

The distinctiveness of the vegetation at sites with *B. alpestris* is also shown by the results of the indicator species analysis: 11 species were significantly associated with *B. alpestris*, but only three species with *B. ranunculoides* s.str. and only two species with subsp. *repens* (Table 2). Most of the indicator species for *B. alpestris* are Atlantic/west-Mediterranean phytogeographical elements (e.g. *Carum verticillatum*, *Corrigiola litoralis*, *Juncus heterophyllus*, *Oenanthe crocata*, and *Ranunculus ololeucos*, Table 2). The species that most frequently were associated with all three *Baldellia*-taxa, and not with a particular taxon, were common species in aquatic communities like *Mentha aquatica*, *Hydrocotyle vulgaris* and *Eleocharis palustris* (Table 3).

In a CCA-ordination of the vegetation together with the recorded environmental variables only altitude and pH had a significant effect on the composition of the vegetation ($p < 0.05$). The eigenvalues of the CCA-ordination ($\lambda_1 = 0.47$, $\lambda_2 = 0.33$; proportion of total inertia 5.0% and 3.5%) were lower than those of the CA-ordination, but still high and highly significant (trace = 0.799, $p < 0.0001$), indicating that pH and altitude explained a substantial part of the variation in vegetational composition among the sites with *Baldellia*. The CCA-ordination restricted by altitude and pH separated clearly the sites with the different *Baldellia*-taxa (Fig. 4). Sites with *B. ranunculoides* s.str. were separated from those with the other two taxa by their higher pH, and sites with *B. alpestris* were separated, although somewhat less clearly, from the other taxa by their higher altitude.

4. Discussion

We found significant differences between the habitat conditions and accompanying species of the three *Baldellia*-taxa in central and western Europe. *B. ranunculoides* s.str. is a lowland species, growing on mineral and basic substrates (pH 7–9), mainly rich in Ca^{2+}. Such calcareous habitats occur for example next to the sea shore close to Ca^{2+}-rich dunes (e.g. sites 10–12, see Appendix A), in lakes and ponds on limestone outcrops (e.g. sites 5 and 9) or on Ca^{2+}-rich substrates in pre-alpine regions (sites 14–18). In regions close to the Atlantic or Mediterranean coast *B. ranunculoides* s.str. can also grow in brackish waters with salinities of 150–1850 μS/cm (in comparison *Cladium mariscus*: up to

Table 3
Species occurring most frequently with taxa of the genus *Baldellia*

Species	Constancy (%)
Mentha aquatica	48.8
Hydrocotyle vulgaris	37.2
Eleocharis palustris	30.2
Phragmites australis	30.2
Lysimachia vulgaris	27.9
Ranunculus flammula	27.9
Hypericum elodes	23.3
Lycopus europaeus	23.3
Alisma plantago-aquatica	20.9
Molinia caerulea	18.6
Agrostis stolonifera	14.0
Juncus bulbosus	14.0
Apium indundatum	11.6
Carex rostrata	11.6
Eleocharis acicularis	11.6
Juncus articulatus	11.6
Juncus maritimus	11.6
Potamogeton gramineus	11.6

Constancy is given as the proportion of all 43 *Baldellia* sites investigated in which a given species occurred (only species with constancy >10% are listed).

15,500 μS/cm; *Iris pseudacorus*: up to 10,400 μS/cm; Fullana Montoro, 2001), which is not the case for subsp. *repens* (Preston and Croft, 2001).

B. ranunculoides subsp. *repens* is also a taxon of lowland water bodies, but grows on acidic substrates, which are markedly richer in organic matter. Acidic peat substrates (pH 5.0–6.5), with an organic matter content of more than 50% of dry matter, are colonized only by subsp. *repens*. It is growing on substrates, which are clearly poorer in Ca^{2+}. Its habitats are thus similar to those of other typical *Littorelletea*-species of northern and eastern Europe like *Luronium natans*, *Lobelia dortmana*, *Littorella uniflora*, *Juncus bulbosus*, and *Eleocharis acicularis*, that grow on substrates poor in Ca^{2+} (0.0–3.0 mg/g; Szankowski and Klosowski, 2001; Arts, 2002; Murphy, 2002). The habitat differences are also reflected in our vegetation analysis. Schoof-van Pelt (1973), who did the most detailed vegetation analysis of all Littorelletean associations in western Europe so far, was not able to detect any differentiating species for the subspecies. Our study that covered a larger geographical area and analyzed a broader spectrum of habitats

demonstrates for the first time a significant differentiation in the composition of the vegetation accompanying the two subspecies of *B. ranunculoides* s.l.

Our observations on habitat differentiation between the two subspecies of *B. ranunculoides* s.l. confirm and extend local observations from western Europe (Roelofs, 1983; Roelofs et al., 1984; Vuille, 1988; Arts and den Hartog, 1990; Brouwer et al., 1999; Brouwer and Roelofs, 2001; Roelofs, 2002) and the Mediterranean (Maremmani et al., 2003; Dimopoulos et al., 2005). The habitat differentiation between the taxa may also explain why in certain regions where subsp. *repens* is common, *B. ranunculoides* s.str. is very rare. This is the case, for example, in central France (Dupont, 1990) and in Wales (Jones, 2006) where acidic and Ca^{2+}-poor soils predominate. However, the two subspecies of *B. ranunculoides* s.l. have not always been distinguished in the general literature (Tutin et al., 1980). Even publications devoted exclusively to aquatic plants (Cook, 1983) as well as more detailed ecological studies (Loucougaray et al., 2004), did not differentiate between the subspecies. Our results show that apart from evident morphological (Vuille, 1988) and genetic differences (Triest and Vuille, 1991; Jacobson, 2003), there are clear ecological differences between the two subspecies, suggesting that future studies and conservation efforts need to differentiate between the subspecies of *B. ranunculoides* s.l.

Vuille (1988) proposed that the Iberian endemic species, *B. alpestris*, is ecologically an intermediate between subsp. *ranunculoides* and subsp. *repens*. However, our study – which is the first detailed analysis of its habitat preferences – shows clearly, that it is the most distinct taxon of the genus with respect to its habitat, as indicated by both the environmental and vegetation data (in order to facilitate the comparison we have placed *B. alpestris* in Fig. 2 between both subspecies of *B. ranunculoides*). *B. alpestris* grows, similarly to *B. ranunculoides* subsp. *repens*, in habitats with low pH and on substrates with low Ca^{2+} concentrations, but it occurs at much higher altitudes (up to 1800 m) and in plant communities that are very different from those supporting the two other *Baldellia*-taxa. *B. alpestris* occurs mainly in glacial lakes (sites 42–43) of the Iberian mountains where it is forming large, almost monospecific stands, along gently sloping and shallow shores. It can be found also, however, in deep artificial village ponds and in mountain streams with very steep slopes and a
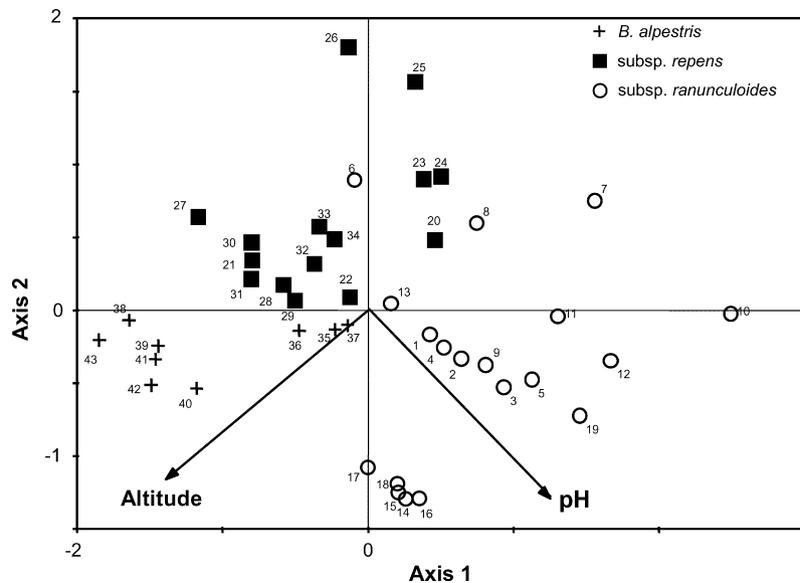

Fig. 4. CCA-Ordination diagram of the vegetation in which *Baldellia ranunculoides* s.str., subsp. *repens* and *B. alpestris* occur, with altitude and pH as environmental variables. Downweighting of rare species was activated. Only site scores are shown to avoid clutter. Numbers refer to the sites studied (see Appendix A).

depth of ca. 50–100 cm (sites 35–37). Most of the indicator and co-occurring species of *B. alpestris* belong to the west-Mediterranean phytogeographical element. These species have become extremely rare or are extinct in central and northern Europe, which explains the very isolated position of *B. alpestris* in our vegetation analysis. According to Cook (1983), *B. alpestris* is a neo-endemic species. However, in a genetic study of Triest and Vuille (1991) *B. alpestris* was strongly differentiated from *B. ranunculoides* s.l., which together with our results might suggest that *B. alpestris* is rather a paleo-endemic taxon.

There were strong differences between the three *Baldellia*-taxa in the structure of their populations which may be related to their mode of propagation. Subspecies *repens* and *B. alpestris* multiply clonally by inflorescence stolons of up to 0.5 m length, whereas *B. ranunculoides* s.str. mainly reproduces sexually, but may also exhibit some short-distance clonal growth by means of corm-buds (Vuille, 1988; Jones, 2006). Well established populations of the two clonal taxa subsp. *repens* and *B. alpestris* frequently form large, locally dominant (or even monospecific) stands. In contrast, the mainly sexual taxon *B. ranunculoides* s.str. does so only rarely and shoot density can be very low (Jones, 2006). It has been suggested that the reproductive strategy of subsp. *ranunculoides*, which is self-compatible, is similar to that of many annual plants (Vuille, 1988), since it invests more resources in sexual reproduction and flowers profusely.

4.1. Implications for conservation

The *Baldellia*-taxa probably are weak competitors (Cook, 1983; Preston and Croft, 2001) that grow in specific aquatic habitats, which combine oligotrophy and disturbance resulting in rather open vegetation with low biomass. The related *L. natans* has a similar habitat preference (Willby and Eaton, 1993). Eutrophication favouring fast-growing species (Casper and Krausch, 1980; Markgraf, 1981) has therefore been suggested as the most important factor for the decline of *Baldellia*-species and other water plants (Roelofs, 1983; Brouwer and Roelofs, 2001; Arts, 2002; Murphy, 2002; Szankowski and Klosowski, 2006). Other factors that have been suggested as possible causes of the decline of many plants of Littorelletea-communities include both acidification and alkalinization (Roelofs, 1983; Szankowski and Klosowski, 2001, 2006; Arts, 2002).

Acidification, caused mainly by anthropogenic acid rain, should be most harmful to *B. ranunculoides* s.str., since this taxon is growing mostly on substrates with a pH > 7.0, whereas the two other *Baldellia*-taxa were found frequently in acidic habitats. However, in The Netherlands negative effects of acidification on communities with both *B. ranunculoides* s.str. and subsp. *repens* have been observed (Roelofs, 1983). An increase in alkalinity (e.g. due to the addition of alkaline stream water used to compensate for water losses) has often been cited as a cause of the decline of Littorelletean and Isoetid species (Brouwer et al., 1999; Brouwer and Roelofs, 2001; Murphy, 2002). Our results suggest that *B. ranunculoides* subsp. *repens* and *B. alpestris* are restricted to habitats with a very low pH. *B. ranunculoides* s.str., in contrast, has a much broader amplitude with respect to the alkalinity of substrate and water, and could potentially be much more resistant to alkalinization. Because of this difference it is important that conservationists take the ecological and taxonomic differentiation of the two subspecies of *B. ranunculoides* into account. To date this has only been the case in Scandinavia and the Benelux countries (Lawalrée, 1959; Van Rompaey and Delvosalle, 1972; Cools, 1989; Ingelög et al., 1991; Lid and Lid, 2005).

The analysis of the size of the existing populations showed that many of the *Baldellia*-populations are very small. This is particularly true for *B. ranunculoides* s. str. Both theoretical considerations (Menges, 1991) and empirical evidence (Matthies et al., 2004) indicate that small populations of plants are threatened not only by habitat deterioration, but also by environmental, demographic and genetic stochasticity. Because the number of extant populations is small and in many regions no populations exist any longer, it is unlikely that suitable habitats will be colonized by *Baldellia*. The creation of new populations at suitable sites should therefore be considered (cf. Vittoz et al., 2006). The list of specific indicator species for sites with one of the taxa (Table 2) can be helpful in identifying new sites specifically suited for each taxon.

Acknowledgements

Appendix A

Location and short characterization of *Baldellia*-sites investigated in this study. Population size classes: 1: 1–100 plants; 2: 101–1000; 3: more than 1000 individuals; 4: more than 10,000 individuals.

Site Nr.	Site name	Country/region	Geographical coordinates	Alt. (m)	Habitat type	Population size
B. ranunculoides subsp. *ranunculoides*						
1	El Acebuche 1	Spain/W Andalucia	37°02′59.9″ N–6°34′11.9″ W	37	Lagoon	1
2	El Acebuche 2	Spain/W Andalucia	37°02′54.9″ N–6°34′10.2″ W	36	Lagoon	1
3	Facinas	Spain/E Andalucia	36°09′19.4″ N–5°41′16.2″ W	33	Road channel	2
4	Loch Cutry	Ireland/County Clare	53°02′14.3″ N–8°46′09.6″ W	38	Lake shore	3
5	Loch Blackloon	Ireland/County Clare	53°09′41.7″ N–8°50′31.5″ W	22	Lake shore	3
6	South of Clifden	Ireland/County Galway	53°27′45.0″ N–10°00′42.8″ W	11	Stream shore	2
7	Ballyconneely	Ireland/County Galway	53°25′27.2″ N–10°04′25.9″ W	1	Lagoon	2
8	Cors Erddreiniog	United Kingdom/Wales	53°18′12.0″ N–4°17′48.0″ W	5	Fen border	2

Appendix A (Continued)

Site Nr.	Site name	Country/region	Geographical coordinates	Alt. (m)	Habitat type	Population size
9	Peterborough	United Kingdom/England	52°33′08.1″ N–0°09′13.7″ W	30	Pond shore	1
10	Ornithological Parc	France/Marquenterre	50°15′51.5″ N–1°33′30.9″ E	1	Dune slack	1
11	Fort-Mahon-Plage 1	France/Marquenterre	50°20′58.0″ N–1°33′45.0″ E	7	Dune slack	1
12	Fort-Mahon-Plage 2	France/Marquenterre	50°20′54.5″ N–1°33′52.1″ E	7	Dune slack	1
13	Platwijers	Belgium/Limburg	50°58′15.6″ N–5°20′14.7″ E	38	Fish pond bottom	1
14	Lac de Neuchâtel 1	Switzerland/Canton Vaud	46°47′09.1″ N–6°40′05.8″ E	433	Road channel	2
15	Lac de Neuchâtel 2	Switzerland/Canton Vaud	46°47′12.1″ N–6°40′03.2″ E	432	Lake shore	1
16	Lac de Neuchâtel 3	Switzerland/Canton Vaud	46°47′47.9″ N–6°41′36.5″ E	432	Lake shore	1
17	Lac de Neuchâtel 4	Switzerland/Canton Vaud	46°47′25.8″ N–6°40′46.9″ E	432	Road channel	1
18	Lac de Neuchâtel 5	Switzerland/Canton Fribourg	46°54′34.7″ N–6°56′20.6″ E	432	Lake shore	1
19	Gülpersee	Germany/Havelland	52°43′56.1″ N–12°15′28.9″ E	21	Lake shore	2
B. ranunculoides subsp. *repens*						
20	Paramos	Portugal/Douro Littoral	40°58′10.7″ N–8°38′38.0″ W	10	Fen border	1
21	Furgeira	Portugal/Minho	41°46′04.1″ N–8°38′31.9″ W	100	Peatland	2
22	Rio Lima	Portugal/Minho	41°49′31.6″ N–8°19′31.6″ W	55	River shore	1
23	Llanfairynghornwyn	United Kingdom/Wales	53°23′54.0″ N–4°33′12.0″ W	5	Dune slack	4
24	Fydlyn	United Kingdom/Wales	53°23′42.0″ N–4°34′00.0″ W	5	Dune slack	4
25	La Chap.-des-Marais	France/Brière	47°26′31.9″ N–2°14′59.8″ W	1.5	Peatland channel	1
26	La Pierre Fendue	France/Brière	47°23′18.6″ N–2°17′44.6″ W	2	Peatland pond	2
27	Etang des Brosses	France/Sologne	47°30′16.5″ N–1°54′44.2″ E	103	Pond shore	1
28	Etang de Bièvre	France/Sologne	47°30′00.9″ N–1°50′57.1″ E	98	Shore of small pond	4
29	E. la Grand Corbois	France/Sologne	47°30′57.2″ N–1°54′01.4″ E	101	Shore of small pond	1
30	La Noue Mazone	France/Foret d'Orleans	47°54′42.3″ N–2°26′20.3″ E	88	Lake shore	1
31	E. de la Grande Rue	France/Foret d'Orleans	47°41′40.7″ N–2°52′42.4″ E	105	Lake shore	4
32	De Maten	Belgium/Limburg	50°57′22.4″ N–5°27′38.2″ E	52	Fish pond shore	1
33	Lake Banen 1	The Netherlands/Limburg	51°15′57.4″ N–5°47′58.2″ E	30	Pond shore	4
34	Lake Banen 2	The Netherlands/Limburg	51°15′50.1″ N–5°47′60.3″ E	30	Pond shore	4
B. alpestris						
35	Silva Escura	Portugal/Douro Littoral	41°15′14.4″ N–8°34′37.2″ W	100	Ancient village pond	1
36	Devesa	Portugal/Douro Littoral	41°14′49.5″ N–8°35′11.5″ W	150	Ancient village pond	1
37	Aqualangua	Portugal/Douro Littoral	41°16′04.3″ N–8°29′43.7″ W	100	Stream shore	1
38	Lake Rabagao	Portugal/Tras os Montes	41°45′48.6″ N–7°44′48.2″ W	862	Artificial lake shore	2
39	Lake Alto Cavado	Portugal/Tras os Montes	41°48′05.6″ N–7°52′31.7″ W	888	Artificial lake shore	3
40	Rio Tera	Spain/Sanabria	42°07′12.1″ N–6°41′55.1″ W	1030	River shore	1
41	Rio Caradena	Spain/Sanabria	42°07′13.0″ N–6°45′43.4″ W	1100	Stream shore	1
42	Laguna Roya	Spain/Segundera	42°08′11.4″ N–6°47′38.5″ W	1615	Glacial lake	4
43	Laguna del Pavon	Spain/Segundera	42°07′56.1″ N–6°46′33.9″ W	1595	Glacial lake	4

References

Aronsson, M. (Ed.), 1999. Rödlistade kärlväxter i Sverige. vol. 1. ArtDatabanken SLU, Uppsala.

Arts, G.H.P., 2002. Deterioration of atlantic soft water macrophyte communities by acidification, eutrophication and alkalinization. Aquat. Bot. 73, 373–393.

Arts, G.H.P., den Hartog, C., 1990. Phytogeographical aspects of the West European soft-water macrophyte flora. Acta Bot. Neerl. 39, 365–370.

Brouwer, E., Roelofs, J.G.M., 2001. Degraded softwater lakes: possibilities for restoration. Restor. Ecol. 9, 155–166.

Brouwer, E., Soontiëns, J., Bobbink, R., Roelofs, J.G.M., 1999. Sulphate and bicarbonate as key factors in sediment degradation and restoration of Lake Banen. Aquat. Conserv.: Mar. Freshwat. Ecol. 9, 121–132.

Casper, S.J., Krausch, H.-D., 1980. Pteridophyta und Anthophyta. Teil 1. In: Ettl, H., Gerloff, J., Heynig, H. (Eds.), Süsswasserflora von Mitteleuropa. Band 23. VEB Gustav Fischer, Jena, pp. 157–184.

Cook, C.D.K., 1983. Aquatic plants endemic to Europe and the Mediterranean. Bot. Jahrb. 103, 539–582.

Cook, C.D.K., 1990. Aquatic Plant Book. SPB Academic Publishing, The Hague.

Cools, J.M.A., 1989. Atlas van de Noordbrabantse Flora. Stichting Uitgeverij van de Koninklijke Natuurhistorische Vereniging, Tilburg.

Cronk, J.K., Fennessy, M.S., 2001. Water Plants: Biology and Ecology. Lewis Publishers, Boca Raton.

de Bolos, O., Vigo, J., 2003. Flora dels Paisos Catalans. IV. Barcino S.A., Barcelona.

Dimopoulos, P., Sykora, K., Gilissen, C., Wiecherink, D., Georgiadis, Th., 2005. Vegetation ecology of Kalodiki Fen (NW Greece). Biologia (Bratislava) 60, 69–82.

Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67, 345–366.

Dupont, P., 1990. Atlas partiel de la flore de France. Collection Patrimoines Naturelles. vol. 3. Série Patrimoine Génétique. Muséum d'Histoire Naturelle, Paris.

Egertson, C.J., Kopaska, J.A., Downing, J.A., 2004. A century of change in macrophyte abundance and composition in response to agricultural eutrophication. Hydrobiologia 524, 145–156.

FAL (Eidgenossische Forschungsanstallt für Agrarökologie und Landbau), 2003. Referenzmethoden der eidgenössischen landwirtschaftlichen Forschungsan-

stalten. Band 2. Bodenuntersuchungen zur Standort-Charakterisierung. FAL Reckenholz, Zürich.

Franco, J.A., Afonso, M.L.R., 1994. Nova Flora de Portugal. Fasciculo I., vol. 3. Cambridge University Press, Lisboa.

Fullana Montoro, J., 2001. Plan de protection de recursos hidricos de la zona humeda de Almenara. Varios (Castellon). Ministerio de Medio Ambiente. Oficina de planification hidrologica, Varios.

Glück, H., 1905. Biologische und morphologische Untersuchungen über Wasser und Sumpfgewächse. Erster Teil: Die Lebensgeschichte der europäischen Alismataceen. Gustav Fischer, Jena.

Glück, H., 1906. Alismataceae. In: Kirchner, O., Loew E., Schröter, C. (Eds.), Lebensgeschichte der Blütenpflanzen Mitteleuropas. Band I, Abteilung I. Eugen Ulmer, Stuttgart, pp. 584–648.

Hulten, E., Fries, M., 1986. Atlas of North European vascular plants: north of the tropic of cancer. Koeltz Scientific Books, Königstein.

Ingelög, T., Thor, G., Hallingbäck, T., Andersson, R., Aronsson, M., 1991. Floravard i jordbrukslandskapet. Skyddsvärda växter. SBT, Lund.

Jacobson, A., 2003. Diversity and phylogeography in Alisma (Alismataceae), with emphasis on Northern European taxa. Scripta Academica Lundensia. Ph.D. Thesis. University of Lund, Sweden.

Jones, R.A., 2006. Creeping water-plantain (Dyfr lyriad ymlusgawl), *Baldellia ranunculoides* subsp. *repens* (Lam.) A. Löve & D. Löve in Wales. In: Leach, S.J. et al. (Eds.), Botanical Links in The Atlantic Arc. BSBI Conference Report No. 24, pp. 311–319.

Korneck, D., Schnittler, M., Vollmer, I., 1996. Rote Liste der Farn und Blütenpflanzen (Pteridophyta et Spermathophyta) Deutschlands. Schriftenreihe für Vegetationskunde 28, 21–187.

Lawalrée, A., 1959. *Baldellia repens* (Lamk.) van Oostsroom en Belgique. Bull. Jard. Bot. Etat Brux. 29, 7–14.

Lepš, J., Šmilauer, P., 2003. Multivariate Analysis of Ecological Data Using CANOCO. Cambridge University Press, Cambridge.

Lid, J., Lid, D.T., 2005. In: Elven, R. (Ed.) Norsk flora. 7th ed., Det Norske Samlaget, Oslo.

Lindblad, R., Stahl, B., 1989. Krypfloka (*Apium inundatum*) och flocksvalting (*Baldellia ranunculoides*) i norra Halland (SW Sweden). Svensk Bot. Tidskr. 83, 211–217.

Lindblad, R., Stahl, B., 1990. Revsvalting, *Baldellia repens*, i Norden. Svensk Bot. Tidskr. 84, 253–258.

Loucougaray, G., Bonis, A., Bouzillé, J.B., 2004. Effects of grazing by horses and/or cattle on the diversity of coastal grasslands in western France. Biol. Conserv. 116, 59–71.

Maremmani, A., Bedini, S., Matosevic, I., Tomei, P.E., Giovannetti, M., 2003. Type of mycorrhizal associations in two coastal nature reserves of the Mediterranean basin. Mycorrhiza 13, 33–40.

Markgraf, F., 1981. Ordnung Helobiae. In: Hegi, G. (Ed.) Illustrierte Flora von Mitteleuropa. Band I, Teil 2, Dritte, völlig neubearbeitete Auflage. Paul Parey, Berlin, pp. 154–171.

Matthies, D., Bräuer, I., Maibom, W., Tscharntke, T., 2004. Population size and the risk of local extinction: empirical evidence from rare plants. Oikos 105, 481–488.

McCune, B., Mefford, M.J., 1999. PC-ORD for Windows. MjM Software Design, Gleneden Beach, OR, USA.

Menges, E.S., 1991. The application of minimum viable population theory to plants. In: Falk, D.A., Holsinger, K.E. (Eds.), Genetics and Conservation of Rare Plants. Oxford University Press, Oxford, pp. 47–61.

Meusel, H., Jäger, E., Weinert, E., 1992. Vergleichende Chorologie der Zentraleuropäischen Flora. vol. 1–3. Gustav Fischer, Jena.

Moreno Saiz, J.C., Sainz Ollero, H., 1992. Atlas corologico de las Monocotiledoneas endemicas de la poninsila Iberica y Baleares. Bases para una politica de conservacion. Colleccion Tecnica. Icona, Madrid.

Moser, D.M., Gygax, A., Bäumler, B., Wyler, N., Palese, R., 2002. Liste Rouge des fougères et plantes à fleurs menacées de Suisse. BUWAL, Bern.

Murphy, K.J., 2002. Plant communities and plant diversity in softwater lakes of northern Europe. Aquat. Bot. 73, 287–324.

Pott, R., 1995. Die Pflanzengesellschaften Deutschlands. 2nd ed. Eugen Ulmer, Stuttgart.

Preston, C.D., Croft, J.M., 2001. Aquatic plants in Britain and Ireland. Harley Books, Colchester, Essex.

Roelofs, J.G.M., 1983. Impact of acidification and eutrophication on macrophyte communities in soft waters in The Netherlands. I. Field observations. Aquat. Bot. 17, 139–155.

Roelofs, J.G.M., 2002. Soft-water macrophytes and ecosystems: why are these so vulnerable to environmental changes? Aquat. Bot. 73, 285–286.

Roelofs, J.G.M., Schuurkes, J.A.A.R., Smits, A.J.M., 1984. Impact of acidification and eutrophication on macrophyte communities in soft waters in The Netherlands. II. Experimental studies. Aquat. Bot. 18, 389–411.

Romero, M.I., Rubinos, M., Ramil, P., 2003. Luronium natans, a rare species in the Iberian peninsula. Belg. J. Bot. 137, 85–90.

Sardinero Roscales, S., 1994. Estudio de la vegetation y de la flora del Macizo Occidental de la Sierra de Gredos (Sistema Central, Espana). Ph.D. Thesis. Faculty of Pharmacy, Departement of Botany II, University of Madrid.

Schoof-van Pelt, M.M., 1973. Littorelletea—a study of the vegetation of some amphiphytic communities of Western Europe. Stichting Studentenpers, Nijmegen.

Schubert, R., Hilbig, W., Klotz, S., 1995. Bestimmungsbuch der Pflanzengesellschaften Mittel und Nordostdeutschlands. Gustav Fischer, Stuttgart.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd ed. Freeman and Co., New York.

Szankowski, M., Klosowski, S., 2001. Habitat conditions of the phytocenoses dominated by Luronium natans (L.) Rafin. in Poland. Hydrobiologia 455, 213–222.

Szankowski, M., Klosowski, S., 2006. Habitat variability of the Littorelletea uniflorae plant communities in Polish Lobelia lakes. Hydrobiologia 570, 117–126.

Ter Braak, C.J.F., Šmilauer, P., 2002. CANOCO reference manual and CanoDraw for Windows. User's Guide: Software for Canonical Community Ordination. (Version 4.5). Microcomputer Power, Ithaca NY, USA.

Triest, L., Vuille, F.-L., 1991. Isozyme variation in several seed collections and hybrids of Baldellia (Alismataceae). In: Triest, L. (Ed.), Isozymes in Water Plants. Opera Bot. Belg. 4, 37–48.

Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), 1980. Flora Europaea, vol. 1–5. Cambridge University Press, Cambridge.

Van Rompaey, E., Delvosalle, L., 1972. Atlas de la Flore Belge et Luxembourgeoise. Jardin Botanique National de Belgique, Bruxelles.

Vasconcellos, J.C., 1970. Novas combinaçoes nomenclaturais de plantas da flora Portuguesa. Bol. Soc. Broteriana, Ser. 2. 44, 81–83.

Vittoz, P., Wyss, T., Gobat, J.-M., 2006. Ecological conditions for Saxifraga hirculus in Central Europe: a better understanding for a good protection. Biol. Conserv. 131, 594–608.

Vuille, F.-L., 1988. The reproductive biology of the genus Baldellia (Alismataceae). Plant Syst. Evol. 159, 173–183.

Wigginton, M.J. (Ed.), 1999. British Red Data Books. 1. Vascular plants. Joint Nature Conservation Committee, Totnes, Devon.

Willby, N.J., Eaton, J.W., 1993. The distribution, ecology and conservation of Luronium natans (L.) Raf. in Britain. J. Aquat. Plant Manage. 31, 70–76.