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Freshwater lichens in springs of the eastern Italian Alps: floristics, ecology and potential for bioindication

J. Nascimbene[1]*, H. Thüs[2], L. Marini[3], P.L. Nimis[1]

[1] Department of Biology, University of Trieste, via Giorgieri 10 – 34100 Trieste, Italy.
[2] Department of Plant Ecology and Systematics, Technical University of Kaiserslautern, Erwin Schrödinger-Str., 67653 Kaiserslautern, Germany.
[3] Department of Environmental Agronomy and Crop Production, University of Padova, viale dell'Università 16 – 35020 Legnaro, Padova, Italy.

Freshwater habitats of the Italian Alps are largely unexplored and further floristic-ecological surveys are needed to clarify the role of freshwater lichens in these environments. This applies especially to springs, since they seem to be suitable for a relatively high number of aquatic species due to their ecological stability. The present work is focused on springs in an alpine region, and is centered on: (a) floristics of freshwater lichens of the Italian Alps, (b) ecological and morphological information on potential indicator species for calcareous and siliceous springs. The study was carried out in the eastern Italian Alps on 36 perennial springs. Single springs proved to host a surprisingly low number of species, while at regional level the entire pool of investigated springs host a relevant lichen flora representing 45% of the freshwater lichens of the Italian Alps. One species is new to Italy and 3 are new to Trentino-Alto Adige. Biodiversity conservation measures should therefore be planned at regional level, including a whole network of sites in different altitudinal belts and with different substrates. *Verrucaria elaeomelaena* and *V. funckii* are the most frequent species on calcareous and siliceous springs respectively. They are suggested as potential indicator species, since they proved to be indicative of the main physical, ecological and hydrochemical features of their habitat. A detailed morphological description of these two species is also provided in order to enhance their identification by environmentalists.

Keywords: aquatic lichens, biomonitoring, conservation, CRENODAT, indicator species.

Introduction

Springs are a complex habitat, highly sensitive to disturbance (primarily water abstraction) and therefore in urgent need of conservation. They have a limited seasonal fluctuation in physiochemistry (Cantonati et al. 2006), and host several groups of specialized organisms, including freshwater lichens. In their extensive review on the hydrobiology of Alpine springs, Cantonati et al. (2006) summarised the main chemical-physical and biotic features of this habitat, focusing on its importance for biodiversity conservation.

Lichens mostly colonise terrestrial habitats, but a few species are restricted to submerged or partially inundated rocks, such as in springs, rivers, and lakes (Thüs 2002). Freshwater lichens belong to a few genera, the most representative being *Verrucaria*. In spite of their limited number, they are often difficult to identify by non-specialists. The taxonomical position of several species still awaits further research, and often the morphological characters used in the identification keys are not clear, or deserve a critical review (e.g. Aptroot & Seaward 2003). Taxonomical problems are a constraint for advances in ecological studies and for the use of freshwater lichens as biomonitors. Nascimbene & Nimis (2006) provided a review on freshwater lichens of the Italian Alps, underlining that further floristic and ecological surveys are needed to clarify the role of this poorly known guilt in the freshwater habitats of the Alps (e.g. Nascimbene 2006, Thor & Nascimbene 2007). This applies especially to springs, which are suspected to host a relatively high number of aquatic species due to their ecological stability. However, most of the information on freshwater lichens in the Alps is in the form of floristic surveys (e.g. Keller 2000), mainly focused on streams, while springs are only marginally considered (Cantonati et al. 2006).

In freshwater habitats, species distribution is known to be affected by several ecological factors related to the length of submergence, shading, substrate (lithology, stability), water chemistry, speed and transportation (Aptroot & Seaward 2003, Gilbert 1996, Gilbert & Giavarini 1997, 2000, Mühlenhoff & Büdel 1995, Thüs 2002). However, information on their effects is still scanty (e.g. Davis et al. 2000, 2003, Gilbert & Giavarini 1997, Pentecost

* Corresponding author: E-mail: junasc@libero.it

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1977, Ried 1960a, b), especially in the alpine environment (e.g. Keller & Scheidegger 1994). Nascimbene & Nimis (2006) suggested to concentrate further research in those sites where chemical-physical and ecological data related to water and habitat quality are available, in order to relate the occurrence of freshwater species to physical and biological parameters. A recent multidisciplinary project in the north-estern Italian Alps (CRENODAT, see Cantonati et al. 2005) was focused on springs, providing a complex database in which the values of several ecological parameters are available for c. 100 springs. The present work, which relates biological and CRENODAT data, is focused on springs in an alpine region, and is centered on: (a) floristics of freshwater lichens of the Italian Alps, (b) ecological and morphological information on potential indicator species for calcareous and siliceous springs.

Materials and methods

Study sites and lichen survey

The study was carried out in the province of Trento (NE Italy) on 36 perennial springs not impacted by artificial structures for water abstraction and homogenously distributed in the study area. Their main features are summarized in Table 1. The altitudinal distribution ranges from the submediterranean (3 sites) to the montane (24 sites) and subalpine/alpine (9 sites) belts. Seventeen springs are on siliceous rocks and 19 on calcareous rocks. According to the ecomorphological classification of springs by Steinmann (1915) and Thienemann (1922), all of the selected sites are rheocrene, while according to the geomorphological classification by Howein & Schroeder (2006), based on the granulometric features of the substrate, they belong to different types, mainly cobble (21 sites) and bolder (9 sites) springs. Light availability and water speed were estimated on a five classes ordinal scale. Water transportation is expressed on a three level ordinal scale (Table 1). Most of the springs are in partially shaded conditions (shading 25-75%), have a low water speed (<50 cm s^{-1}), and water transportation (<1-5 l s^{-1}). The percentage of inorganic and organic sediments, water temperature, percentage of O_2, water pH measured at 20°C, conductivity, alkalinity, N-NO_3, N-tot, P-PO_4, P-tot, SiO_2, SO_4, Cl, Ca, Mg, Na, K, D.O.C. were directly measured according to standard protocols (APAT, 2004; APHA, 2000; Cantonati et al., in press.) and the values of two series of data were averaged.

In each spring, lichens were collected both in perennially and periodically inundated micro-sites in the first 5 meters from the headwater. Species nomenclature follows Nimis & Martellos (2003), except for *Verrucaria funckii* (Thüs 2002).

Indicator species

An Indicator Species Analysis (Dufrêne & Legendre 1997) was used to describe differences in species composition and frequency between calcareous and siliceous springs, and to determine how strongly each species was associated with its substrate. For each species, the Indicator Value (IV) ranges from 0 (no indication) to 100 (maximum indication). Statistical significance of IV was tested by a Montecarlo test, based on 1000 randomizations. This analysis was performed by PC-ORD (McCune & Mefford 1997). Significant indicator species were subjected to a morphological and ecological description.

Results

Floristics

Thirty-four species were found (Table 2). *Verrucaria funckii* is new to Italy. According to Orange (2004) and Thüs (2006), it is not a synonym of *V. pachyderma* (Nimis 2003), which is known from a single locality in Trentino-Alto Adige (Nimis 1993). *Thelidium inundatum*, a poorly known species that deserves further study, *Staurothele solvens*, and *Hymenelia cyanocarpa* are new to Trentino-Alto Adige. Following Nimis (1993, 2003), the species belong to three main ecological guilds on the basis of their water requirements (Table 2):

1) Species of perennially inundated habitats and therefore most sensitive to water features. Six species belong to this guild, five of which are *Verrucaria*. The most frequent (*V. aquatilis, V. rheitrophila, V. elaeomelaena, V. funckii*) are associated with highly specialised algae: either *Dilabifilum* sp. a filamentous green alga or *Heterococcus caespitosus* (Tschermak-Woess 1988, Thüs 2002) a representative of the Xanthophyta. At higher altitude, the diversity of associated green algal phcyobionts increases: *Verrucaria latebrosa* is associated with *Stichococcus* sp. (Thüs unpublished) and *Dermatocarpon rivulorum* with *Diplosphaera chodatii* (Reháková 1968). All of them reproduce by ascospores. *Verrucaria elaeomelaena* and *V. funckii* are the most frequent species on calcareous and siliceous springs, respectively (14 and 12 sites). The former was found in all altitudinal belts, while the latter is lacking at low altitudes (submediterranean belt). *Verrucaria aquatilis* was found both in calcareous and in siliceous springs;

2) Semi-aquatic species of periodically inundated habitats. Thirteen lichens belong to this guild, mostly species of *Ionaspis, Staurothele,* and *Verrucaria*. Two species have a trentepohlioid photobiont, and two reproduce by lichenised propagules (*Hymenelia*

Table 1. Main features of the 36 investigated springs. [a]ID: identification code of the spring according to the CRENODAT project; [b] Frwl: number of freshwater lichens in each site; [c]Rock: represent the rock type of the spring. C=carbonatic; S=siliceous; [d]Alt.: altitude (m); [e]Esp.: esposition (°); [f]Spring typ.: spring typology according to the geomorphological classification of springs by Howein & Schroeder (2006); [g]Shading: shading conditions of the habitat expressed on a five level ordinal scale (1) completely exposed; (2) shading reaching 25%; (3) shading reaching 50%; (4) shading reaching 75%; (5) shading > 75%; [h]Speed: water speed expressed on a five level ordinal scale (1) 0-<10 cm s^{-1}; (2) <30 cm s^{-1}; (3) <50 cm s^{-1}; (4) 50 - 100 cm s^{-1}; (5) > 100 cm s^{-1}; [i]W trans.: water transportation expressed on a three level ordinal scale. (1): < 1 l/s; (2): 1-5 l/s; (3): >5 l/s.

ID[a]	Locality	Frwl [b]	Rock [c]	Alt.[d]	Esp.[e]	Spring typ.[f]	Shading[g]	Speed[h]	W transp.[i]
CV0992	Val Tamburli	1	C	992	90	bolder spring	4	2	2
MB1439	Toghe	1	C	1440	215	cobble spring	2	2	1
BR0470	Maso Gori	2	C	470	135	bolder spring	4	5	3
LD1400	Cortelì	1	C	1400	180	bolder spring	3	4	1
BC0564	Laurel	1	C	565	315	cobble spring	2	3	2
LD1501	Tormendos	1	C	1502	135	cobble spring	2	3	1
AD0905	Vermongo	1	C	905	45	bolder spring	3	3	3
MD1871	Fedaia	1	C	1871	315	cobble spring	2	4	2
PS1250	Val Canali	2	C	1250	180	cobble spring	4	3	2
BR1764	Corna Rossa	1	C	1765	270	cobble spring	3	3	2
BS1526	Viotte	1	C	1527	0	cobble spring	2	2	1
BR1358	Nambi	3	C	1358	315	bolder spring	3	4	2
BR1378	Rislà	3	C	1379	315	cobble spring	2	3	2
BR2239	Vallazza	1	C	2240	180	bolder spring	4	3	2
AT0971	Masere	1	C	972	90	cobble spring	5	2	1
BR1315	Valagola	3	C	1315	315	cobble spring	3	2	2
BR1436	Scala di Brenta	2	C	1436	45	rock spring	3	5	1
LD0927	Del Graì	1	C	928	0	gravel spring	3	2	1
MP0656	Vallarsa	1	C	656	315	cobble spring	5	3	2
CV1200	Perengola	1	S	1200	270	rock spring	3	4	2
CV0962	Pian Gran	1	S	962	315	bolder spring	5	2	1
CV1215	Peterlazet	1	S	1215	270	cobble spring	4	3	2
CV2051	Aia dei Sorgati	3	S	2051	45	cobble spring	2	3	2
CV1280	Val Calamento	1	S	1280	0	cobble spring	5	3	2
AN1685	Malga Gavazzi	1	S	1685	45	cobble spring	2	2	1
CV1684	Campigol dei Solai	1	S	1685	180	bolder spring	4	3	2
AD2153	Conca delle Levade	9	S	2153	135	cobble spring	1	3	2
AD1990	Palone-Siniciaga	1	S	1990	315	cobble spring	2	3	2
AD1853	Malga Val di Fumo	2	S	1853	215	gravel spring	1	2	2
AD1300	Borzago	2	S	1300	180	cobble spring	4	3	3
AD2739	Adamello	1	S	2739	215	bolder spring	1	3	3
AD1234	Ponte Prese	2	S	1235	180	cobble spring	4	3	1
AD1653	Val d'Arnò	1	S	1654	90	cobble spring	2	2	1
BR0804	Pissidrina sud	1	S	804	0	gravel spring	3	2	1
LT1239	Daiano	1	S	1240	225	cobble spring	4	2	1
OC2278	Pian Venezia	5	S	2278	90	gravel spring	2	2	1

ochrolemma, and *Koerberiella wimmeriana*). Six species form apothecia and three perithecia. Most of them were found in alpine/subalpine springs. Only *Verrucaria margacea* is present from the submediterranean to the alpine belt.

3) Terrestrial species growing on sheltered and humid rocks. Fifteen lichens belong to this guild, mostly species of *Polyblastia, Porpidia*, and *Verrucaria*. Two species have a trentepohlioid photobiont, four produce apothecia and nine perithecia. They are mainly montane, and grow on humid calcareous rocks. These species grow in the peripheral part of the springs and mark a transition to other rocky habitats.

Table 2. Species list ordered according to 3 ecological groups on the basis of water requirement. Species: nomenclature follows (Nimis & Martellos, 2003), except for *Verrucaria funckii* (Thüs, 2002). Ecological groups: In = species of perennially inundated habitats; Sa = semi-aquatic species of periodically inundated habitats; Ter = terrestrial species growing on sheltered and humid rocks. Altitudinal belt: Sub = submediterranean; Mont = montane; Sub/Alp = subalpine and alpine. Substrate: Ca = carbonatic rocks; Si = siliceous rocks. IV: observed Indicator Value '**' Marks p<0.001; '°°' Marks new species to Italy; '°' Marks new species to Trentino-Alto Adige.

Species	N° of sites	Ecological groups			Altitudinal belt			Substrate		IV
		In	Sa	Ter	Sub	Mont	Sub/Alp	Ca	Si	
Dermatocarpon rivulorum	1	+					+		+	5.9
Verrucaria aquatilis	3	+			+	+		+	+	6.8
Verrucaria elaeomelaena	14	+			+	+	+	+		73.7**
Verrucaria funckii°°	12	+				+	+		+	64.7**
Verrucaria latebrosa	1	+					+		+	5.9
Verrucaria rheitrophila	1	+				+		+		5.3
Aspicilia aquatica	2		+				+		+	11.8
Bacidina inundata	1		+				+		+	5.9
Hymenelia cyanocarpa°	1		+				+		+	5.9
Ionaspis lacustris	1		+				+		+	5.9
Ionaspis odora	1		+				+		+	5.9
Koerberiella wimmeriana	2		+				+		+	11.8
Rhizocarpon lavatum	3		+				+		+	17.6
Staurothele fuscocuprea	1		+				+		+	5.9
Staurothele solvens°	1		+			+		+		5.3
Staurothele succedens	3		+			+		+		15.8
Thelidium inundatum°	1		+			+		+		5.3
Verrucaria hydrela	5		+			+		+	+	19.2
Verrucaria margacea	6		+		+	+	+	+	+	9.3
Gyalecta jenensis	1			+		+		+		5.3
Hymenelia ochrolemma	1			+			+		+	5.9
Petractis clausa	1			+		+		+		5.3
Polyblastia albida	1			+		+		+		5.3
Polyblastia sepulta	1			+		+		+		5.3
Polyblastia cfr. *ventosa*	1			+		+		+		5.3
Porpidia glaucophaea	1			+			+		+	5.9
Porpidia macrocarpa	2			+			+		+	11.8
Protoblastenia calva	1			+		+		+		5.3
Rhizocarpon badioatrum	1			+			+		+	5.9
Staurothele rupifraga	1			+		+		+		5.3
Thelidium minutulum	1			+		+		+		5.3
Thelidium papulare	1			+		+		+		5.3
Verrucaria dufourii	1			+		+		+		5.3
Verrucaria cfr. *muralis*	1			+		+		+		5.3

Table 3. Results of the morphological analysis on *Verrucaria elaeomelaena* (six specimens from different sites) and *V. funckii* (10 specimens from different sites). Mean values and standard error are expressed in μ. SL = spore length; SW = spore width; SL/SW = ratio between spore length and spore width; TT = thallus thickness; EW = excipulum width; EH = excipulum height; IT = involucrellum thickness; IW = involucrellum width; IH = involucrellum height.

	Morphological parameters								
	SL	SW	SL/SW	TT	EW	EH	IT	IW	IH
Verrucaria elaeomelaena	24.4±1.6	12.3±1	2.0±0.2	75.0±39.4	243.3±44.1	170±33.5	64.2±19.6	436.7±83.8	200±26.1
n° of measurements	60	60	60	6	6	6	6	6	6
Verrucaria funckii	21.4±1.7	9.0±1	2.4±0.3	140.2±39.5	217.2±45.6	180.3±40.1	46.6±13.5	391.8±82.7	234.8±52.2
n° of measurements	95	95	95	10	10	10	10	10	10

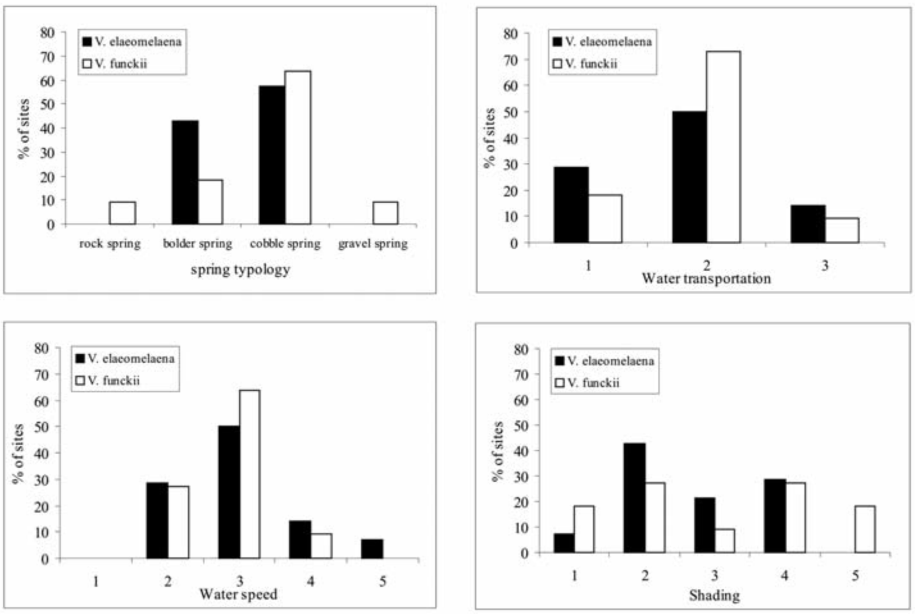

Fig. 1. Occurrence of *Verrucaria elaeomelaena* and *V. funckii* in different springs types and in different conditions of water transportation, water speed, and shading. See notes to Table 1 for additional remarks on the values used in the X axis.

Both the species of guild 1 and 2, which represent 62% and 45% of the freshwater lichens occurring in Trentino Alto Adige and in the Italian Alps, respectively, properly belong to the spring habitat. Their total richness decreases from the alpine/subalpine (13) to the montane (9) and submediterranean belt (3), and from siliceous (14) to calcareous (8) springs (Table 2). Considering species richness at spring level (Table 1), this is surprisingly low: 83% of the sites host 1-2 species, with no significant difference between calcareous and siliceous springs.

Indicator species

In the last column of Table 2 the results of the Indicator Species Analysis are reported. Due to the low frequency of the species in the springs, the Indicator Values are generally low. However, two significant indicator species were found: *Verrucaria elaeomelaena* for calcareous springs (IV=73.7, P=0.001), and *Verrucaria funckii* for siliceous springs (IV=64.7, P=0.001).

Morphological and ecological description of the two indicator species

The indicator species *Verrucaria elaeomelaena* and Verrucaria funckii are very similar and were often confused (see discussion). Therefore, we present a detailed description of their main morphological features (Table 3). The two species significantly differ (t-test) in the dimensions of the spores (SL, SW; $P<0.01$) and in the ratio between spores length and width ($P<0.01$), those of *V. elaeomelaena* being typically "egg-shaped" and larger than those of *V. funckii*. The thallus is green, brown-olive-greenish to greyish, well developed and normally without fissures in both species. However, its thickness is significantly higher ($P<0.01$) in *V. funckii*. The other morphological features do not significantly differ between the two species. Spore dimensions and the ratio length/width have a relatively restricted range of variation, indicating a considerable stability of this character in the study area. The other characters have a high variability in both species.

The occurrence of the two species seems to be related to some physical, ecological and hydrochemical features of the sites (Fig.1; Tables 4 and 5). They generally prefer bolder and cobble springs with intermediate conditions of water transportation (1-5 l s^{-1}) and speed (30-50 cm s^{-1}). Both species are tolerant to shading, growing also in densely forested sites where shading reaches or exceeds 75%. Some hydrochemical parameters (conductivity, and Mg and Na contents) proved to significantly differ between sites in which *Verrucaria elaeomelaena* occurs and sites in which the species was not found. Analogously, springs with *Verrucaria funckii* significantly differ in pH, conductivity, alkalinity, and Cl, Mg, and SiO_2 contents from those in which the species was not found.

Table 4. Comparison of chemical-physical variables in calcareous springs classified on the basis of the occurence of *Verrucaria elaeomelaena*. * Marks parameters that significantly differ ($p<0.05$) between springs where the species is present and absent.

	Verrucaria elaeomelaena							
	Present				Absent			
	mean	ds	min	max	mean	ds	min	max
% inororganic sediments	49.29	22.69	10	90	52.00	21.68	30	80
% organic sediments	50.71	22.69	10	90	38.00	28.64	0	70
T°	6.48	1.70	3	8.57	7.21	1.70	5.35	9.5
O2%	85.97	8.63	71.9	100	85.83	6.48	80	94
pH	7.92	0.19	7.47	8.14	7.97	0.19	7.73	8.26
Conductivity (µS/cm)	314.71*	299.65	144	1338	273.6*	129.11	79	413
Alkalinity (mg/l)	129.65	33.60	82.3	210	161.00	58.05	98	231
N-NO_3 (µg/l)	773.79	471.36	229.3	1823.4	831.40	229.83	658.5	1230
Ntot (µg/l)	833.57	502.48	259	1940	903.00	200.10	710	1230
P-PO_4 (µg/l)	3.34	3.56	0.00	13.00	2.32	1.29	1.10	4.2
Ptot (µg/l)	8.02	9.91	1.40	36.60	6.10	2.46	2.70	8.8
SiO_2 (mg/l)	2.71	1.57	0.55	5.44	1.79	0.31	1.34	2.06
SO_4 (mg/l)	59.65	206.80	1.20	778.00	11.13	13.78	3.04	35.49
Cl (mg/l)	1.10	1.32	0.19	4.60	2.23	3.63	0.32	8.714
Ca (mg/l)	61.79	67.31	18.21	288.19	43.09	10.45	31.77	59.76
Mg (mg/l)	9.48*	13.21	1.28	54.00	16.69*	7.79	8.75	26.08
Na (mg/l)	0.95*	1.21	0.12	4.43	1.26*	1.76	0.26	4.40
K (mg/l)	0.45	0.49	0.00	1.71	0.67	0.51	0.27	1.291
D.O.C. (mg/l)	1.10	0.74	0.40	3.10	1.60	1.31	0.56	3.9

Table 5. Comparison of chemical-physical variables in siliceous springs classified on the basis of the occurence of *Verrucaria funckii*. * Marks parameters that significantly differ ($p<0.05$) between springs where the species is present and absent ** Marks $p<0.01$.

	Verrucaria funckii							
	Present				Absent			
	mean	ds	min	max	mean	ds	min	max
% inororganic sediments	54.54	28.00	10	100	61.50	32.89	30	100
% organic sediments	45.45	28.00	0	90	42.67	40.01	1	100
T°	5.98	2.32	2.9	11.7	7.35	1.06	5.75	8.1
O_2%	85.43	9.16	65	97.7	85.04	17.18	59.2	105
pH	6.72*	0.52	5.9	7.68	7.4*	0.81	6.05	8.04
Conductivity (µS/cm)	53*	39.72	11	157	204.5*	200.90	14	568
Alkalinity (mg/l)	18.6*	15.15	3.5	56.7	84.33*	61.43	5	150
N-NO_3 (µg/l)	390.38	303.70	107.5	1224.4	447.77	296.85	135.20	972.80
Ntot (µg/l)	476.00	306.74	215.00	1272.00	519.00	355.52	134.00	1134
P-PO_4 (µg/l)	3.80	3.00	0.90	10.80	3.57	3.37	0.00	9.80
Ptot (µg/l)	8.39	3.85	2.40	13.90	5.88	3.77	0.30	11.10
SiO_2 (mg/l)	7.30**	2.56	4.30	12.50	3.26**	1.82	2.09	6.40
SO_4 (mg/l)	6.69	7.10	1.07	25.18	29.09	61.23	0.82	153.91
Cl (mg/l)	0.40	0.39	0.12	1.50	0.83	0.81	0.14	2.08
Ca (mg/l)	8.40	8.31	1.44	30.99	31.99	31.34	2.51	87.65
Mg (mg/l)	0.66**	0.33	0.19	1.01	8.33**	7.95	0.20	21.36
Na (mg/l)	1.56	1.00	0.35	3.58	1.68	1.88	0.27	5.15
K (mg/l)	0.53	0.20	0.25	0.84	0.55	0.38	0.00	1.04
D.O.C. (mg/l)	1.15	1.02	0.43	4.00	0.88	0.34	0.37	1.30

Discussion

Springs of the Alps proved to be a suitable habitat for several aquatic lichens, and some species have a promising potential for bioindication, since they are indicative of the main physical and hydrochemical features of this habitat.

Most of the aquatic species are known to occur in the Italian Alps only from old records (Nimis 1993, 2003), confirming the urgent need of further studies on this poorly known guild whose richness and conservation status were so far overlooked. Analogously to other freshwater habitats, the occurrence and richness of aquatic species in springs seems to be related to altitude and substrate. The altitudinal distribution of aquatic species belonging to the spring habitat follows the pattern described by Nascimbene & Nimis (2006) for the freshwater lichen flora of the Italian Alps, with a higher diversity in the subalpine/alpine belt. It is however remarkable that *Verrucaria margacea* was found also at low altitudes (600 m), since it was always regarded as a montane to alpine taxon (Zschacke 1934, Thüs 2002, Orange 2000). Cantonati et al. (2006) considered springs as azonal or extrazonal habitats, due to the constant and low temperatures that enhance the presence of stable vegetation and of glacial relicts. Therefore, the unusual occurrence of *V. margacea* at low altitudes could be considered dependent on these special thermal conditions. On the contrary, the species does not occur in submediterranean creeks, or rivers where temperatures vary to a larger degree, depending on the surrounding air temperature. The lichen flora of siliceous springs is richer in species than that on calcareous springs, indicating a higher suitability of siliceous rocks for aquatic lichens in the Alps (Nascimbene & Nimis 2006). Most of the species are restricted to different rock types, while only a few (*Verrucaria aquatilis, V. hydrela* s.l., and *V. margacea*) are able to establish both on calcareous and on siliceous substrates. The number of species occurring on both substrata becomes even smaller, if the separation of *V. hydrela* and *V. calcaria* is applied. We followed Nimis (2003) treating *Verrucaria calcaria* Zsch. as a synonym to *V. hydrela*, although apart from its different ecology (calcareous instead of siliceous substrata) a new character was observed that might help to distinguish the two taxa. In *V. hydrela* the angle between the involucrellum and the lower part of the exciple is transparent and the cellwalls in this area are colourless, while in *V. calcaria* this area is filled by cells with distinctly brown cell walls. These differences are also visible in the type specimens of *V. hydrela* and *V. calcaria*, but if this single character is sufficient for the separation on a species level remains open to debate. A revision of the *V. hydrela*-group including molecular data is urgently needed and currently in progress (Thüs unpublished).

Verrucaria funckii and *V. elaeomelaena* are the most frequent species of siliceous and calcareous springs, respectively, confirming the ecological remarks by Zschacke (1934), and Orange (2000) who reported these species as typical of springs in Central Europe and in the British Isles.

Despite the fact that they are similar in the colour of the thallus and often confused with each other (Orange 2000, but see also Swinscow 1968, Hawksworth 1989, Keller 2000), they proved to be different in the thickness of the thallus and in the morphology of spores. The morphological characters of both species are in the range of those available in literature (Orange 2000, Purvis et al. 1993, Thüs 2002, Wirth 1995), which however indicate that they could be highly variable, especially in spore size. For example Orange (2000) indicates different spores sizes than Purvis et al. (1993) in the British Isles. In the study area spore size proved to be relatively stable and the values measured in *V. funckii* correspond to the detailed description by Thüs (2002) based on Central European material.

The potential for bioindication of these two species is corroborated by the ecological description, which revealed that they are indicative of some important features of their habitat. The high suitability of bolder and cobble springs is probably related to substrate stability which is indispensable for freshwater lichens (Thüs 2002). Water transportation and speed could influence both the submersion of the thalli and their establishment, being related to the inundation period, to the formation of splash water and to the probability of erosion effects. An intermediate condition proved to be an important habitat quality for the two *Verrucaria*, which seems to be hindered by both low (desiccation) and high (erosion) water transportation and speed values. The effects of these factors are partially related to shading, which enhances the tolerance of the species to desiccation by improving air humidity. *Verrucaria funckii* is very desiccation-sensitive if air humidity is low, while it can survive above the water level if air humidity is constantly high (Ried 1960a, 1960b). Shady conditions may therefore compensate for shorter submersion periods. According to the results of Pentecost (1977) and Gilbert & Giavarini (1997), some chemical factors related to spring lithology such as pH, conductivity, alkalinity, silica and Mg values (Cantonati et al. 2006) seem to influence the occurrence of the two *Verrucaria* species, suggesting their relation to water chemistry. *Verrucaria elaeomelaena* and *V. funckii* are therefore suggested as potential indicator species for rapid monitoring purposes on the basis of four main reasons: (a) they are widespread in a wide range of environments and altitudinal belts and can be considered as typical spring lichens, (b) they are strictly bound to different lithology, (c) they are clearly visible, having a well developed thallus, and can be identified by spore size and shape, and (d) their occurrence is indicative for certain physical and hydrochemical factors related to springs ecology.

Our study gives new insights into an overlooked lichen habitat revealing that the potential of springs for aquatic lichen richness seems to be highly dependent on the scale. Single springs proved to host a surprisingly low number of species, while at regional level the entire pool of investigated springs host a relevant aquatic lichen flora. This pattern has important implications for conservation, suggesting that the protection of single sites is not an effective measure, and that a biodiversity conservation plan for spring habitats should be necessarily developed at regional level, including a whole network of sites in different altitudinal belts and with different substrates.

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