

Small-scale heterogeneity of *Ranunculus trichophyllus* in Lake Tovel (microhabitat, morphology, phenolic compounds and molecular taxonomy)

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SUMMARY - *Small-scale heterogeneity of Ranunculus trichophyllus in Lake Tovel (microhabitat, morphology, phenolic compounds and molecular taxonomy)* - A water buttercup (*Ranunculus trichophyllus* Chaix) and a stonewort (*Chara aspera* Detharding ex Willdenow) are forming the actual macrophyte vegetation in Lake Tovel (Prov. Trento, Italy). Both elements and their similar distribution pattern have already been recorded more than 60 years ago. The two taxa were found in patches near shore and in deep layers not falling dry at all and showed striking morphological differences between the microhabitats. The focus was set on two populations of *Ranunculus*: one prostrate, vivid green with many flowers opening above the water surface, common for the shorelines around the lake; the other one, mostly soft, prostrate dark-green to brownish and sterile, found regularly at 3-4 m depth in the shallow SE basin. Potential reasons for this variability are discussed, mainly: (1) extreme environmental gradients leading to a high phenotypic plasticity of one taxon over longer periods in time and (2) hybridisation of two closely related (infraspecific) taxa occurring within the larger lake catchment leading to a specific littoral ecotype, respectively. The approach to solve this question combines classical biometry with modern chemotaxonomic and genetic analyses (ITS1 sequences documented in this paper).

RIASSUNTO - *Eterogeneità a piccola scala del Ranunculus trichophyllus nel Lago di Tovel (microhabitat, morfologia, composti fenolici e tassonomia molecolare)* - Il ranuncolo a foglie capillari (*Ranunculus trichophyllus* Chaix) e la chara (*Chara aspera* Detharding ex Willdenow) costituiscono la vegetazione di macrofite del Lago di Tovel (Trentino, Nord Italia). Entrambe le specie e il loro simile modello di distribuzione furono già segnalati e descritti più di 60 anni fa. I due taxa sono stati rilevati a chiazze sia in prossimità delle rive sia a livelli più profondi mai soggetti ad andare a secco e hanno evidenziato elevate differenze morfologiche fra i due microhabitat. L'attenzione è stata indirizzata su due popolazioni di *Ranunculus*: una prostrata, di vivace colore verde e con molti fiori che si schiudono sopra la superficie, comune nelle zone riparie; l'altra più soffice, prostrata e di colore dal verde scuro al marrone, presente di regola a 3-4 m di profondità nel bacino poco profondo di SE. Vengono discusse le potenziali ragioni di questa variabilità, principalmente: (1) gradienti ambientali estremi che avrebbero portato a una elevata plasticità fenotipica di un taxon su più lunghi periodi nel tempo e (2) l'ibridazione di due taxa strettamente correlati (infraspecifici) occorsa all'interno del bacino lacustre più ampio, che avrebbe portato a uno specifico ecotipo litorale. L'approccio per rispondere al quesito combina la biometria classica con analisi chemiotassonomiche e genetiche (sequenze ITS1 riportate in questo scritto).

Key words: *Ranunculus trichophyllus*, phenology, ITS sequences, biometry, chemotaxonomy

Parole chiave: *Ranunculus trichophyllus*, fenologia, sequenze ITS, biometria, chemiotassonomia

1. INTRODUCTION

As a contribution to the project SALTO (2001-2004) (Borghi *et al.* 2006), a large interdisciplinary study of Lake Tovel to identify potential changes of lake characters since the end of the red blooms in the 1960s, the actual distribution pattern of macrophytes were investigated and compared to earlier investiga-

tions. A small number of submerged macrophytes with an uneven distribution pattern was already recorded and documented by Baldi (1941). A later study after the end of the red blooms by Pedrotti & Contini-Pedrotti (1996), integrating samples from the 1980s to the 1990s, discussed typical characters of the littoral and submerged vegetation types. Beside the type ssp. (*Ranunculus trichophyllus* Chaix ssp. *trichophyllus*)

these authors were putting a second infra-specific taxon *ssp. eradicatus* (Laest.) Cook (= *ssp. lutulentus* (Perrier & Songon) Vierhapper) into discussion. However, the authors mentioned that the latter could only be found occasionally on the shores of Lake Tovel, but they found it constantly at ponds from much higher altitude within the wider catchment of the lake. The morphological details of the aquatic *Ranunculus* type within Lake Tovel have not been documented. The morphological variations of other taxa of *Ranunculus* subgen. *Batrachium*, although frequently mentioned in literature (Casper & Krausch 1981), have rarely been documented (Zander & Wiegand 1987). Although *R. trichophyllus* is widely distributed within the boreal and temperate zones of the northern hemisphere, its phenotypic variability has been mentioned several times (Wiegand in press), but rarely studied (e.g., Cook 1966). The cultivation experiments by Cook (1966) made with *Ranunculus trichophyllus* *ssp. trichophyllus* at temperature between 6 and 8 °C lead to drastic morphological changes resulting finally into delicate, procumbent specimens with roots at most nodes, i.e. a phenotype finally not distinguishable from *ssp. eradicatus*. Beside temperature other reasons need to be considered for *R. trichophyllus* variability: (1) strong environmental gradients (temperature, water level, nutrients), allowing expression of high phenotypic plasticity by development of different ecotypes of one species over a longer period in time, or (2) the potential hybridisation of closely related (infraspecific) taxa occurring within the catchment (*ssp. trichophyllus* and *ssp. eradicatus*) what would lead to a specific near-shore population. This is a first approach to identify potential reasons for niche inherent differences by combining results from classical morphological details found under different microhabitat conditions within Lake Tovel with analyses from novel taxonomic techniques (ITS sequencing and chemotaxonomic analyses).

2. MATERIALS AND METHODS

Fieldwork - In June 2002 a first screening of the macrophytes was made by wading along the shores and with a viewing box from the boat. End of July 2002, the near shore areas (mainly close to Albergo Lago Rosso and at the SE shore of the Red Bay from surface down to 20 cm) were mapped in detail and sampled (Area A samples, Fig. 1). In August 2002, a sample from a small patch of a few long specimens over 4 m depth was taken with a rake (Area B sample). In July 2002, divers took photographs and samples from larger stands at 3-4.5 m in the extreme SW part of the lake (Area C samples). In early July 2003, divers sampled these deeper areas again. The samples were forwarded immediately under cool and dark conditions to the lab for chemotaxonomic and genetic analyses. As a chemotaxonomic and genetic reference *R. tricho-*

phyllus was sampled from a mesotrophic, canalised calcareous open spring creek near Völs / Innsbruck in N-Tyrol (Völser Giessen) in July 2003.

Morphology - Colour photographs were made from several samples and all areas. Dried specimens were measured with a ruler and/or using a string to follow uneven shoots. Prostrate basal parts (normally with short rooted internodes but without leaves) and erect parts were separately analysed. From branched specimens the main axis (central, strongest and longest part) was measured. Due to the low number of samples and the problems in sampling complete specimens during diving, only the ranges are given for total specimen length.

Chemotaxonomy - For genetic / biochemical analyses brown (dead) plant parts mainly found in samples from area C were eliminated before the analysis. The plant material was extracted with 50% acetone (v/v in water); 10 mg dry mass per ml solvent, for 2 hrs at room temperature with constant shaking. Total phenolic content was determined using tannic acid as standard and the method by Folin-Ciocalteu (Box 1983), modified after Choi *et al.* (2002). Detection of individual phenolic compounds was performed by RP-HPLC (C18, 250 x 4 mm, Kromasil 5 µm), method modified after Gross *et al.* (1996). Solvent A was 1% acetic acid, solvent B methanol. Gradient 0-40 min 5-60% B, 40-45 min 60-100% B, 45-60 min 100% B. Peak area was determined for each signal based on the wavelength at which the UV spectrum exhibited a prominent maximum using photodiode array detection (underlined in Fig. 3).

Molecular taxonomy - Plant DNA was extracted using the Qiagen DNeasy Plant Mini Kit. We amplified the nuclear ribosomal DNA (ITS region using primers ITS1 and ITS 4 (White *et al.* 1990). PCR products were purified using the QIAquick PCR Purification Kit and sequenced by GATC, Konstanz on an ABI-377HT-Sequencer. Our sequences (Genbank, RT_Tov_4.0 = DQ311656; RT_Tov_3.0 = DQ311657; RT_Tov_0.2 = DQ311658) and a partial ITS1 sequence of *R. trichophyllus* (Genbank AY680067) were aligned using the software BioEdit.

3. RESULTS

The habitat

Lake Tovel is a clear, dimictic, calcareous lake situated at 1177 m a.s.l., surface area 38 ha, maximum depth 39 m. The lake basin comprises a large deep central part with steep shores and a larger shallow bay toward SW. The low water retention time allows normally only a weak temperature stratification of the

epilimnion in summer. High water level fluctuations are common, showing every year a decrease during winter which is ending by a fast refill in spring (end of April, beginning of May). Ferretti & Borsato (2004) recorded a decline of water level by 4.7 m in winter 2001/2002 and 3 m in winter 2002/2003. The decrease of water level between May and July was less than 50 cm in both years (Tab. 1), whereas in the dry and hot summer 2003 a considerable water level loss started in August already. Beside of some small year to year variations the waters are normally well oxygenated, alkaline and have low nutrient concentrations. Reduced water exchange rate and warm temperatures during warm summers can lead to temporal oxygen oversaturation and an increase in pH.

Ranunculus was found in patches under three different microhabitat conditions (Tab. 1, Fig. 1):

- (A) The near shore banks and water retreat areas on shallow gradient shore around the lake with fine calcareous gravels and coarse sands down to a maximum depth of 50 cm in July, sometimes even over bedrock with little gravel. Highest water temperatures reached were 12.6 °C in 2002 and 17.1 °C in 2003. Tadpoles are accumulating in masses within some stands, especially those close to Albergo Lago Rosso.
- (B) A small area along the opening of the small bay (part of the SW bay) close to Albergo Lago Rosso in vicinity of larger stonewort stands. This patch is small and can not be found every year. The water depth was around 4 m and water temperatures over ground between 6-7 °C. The substratum is formed by fine calcareous sand. Some eutrophication influence by surface runoff from the road (milky waters after rainfalls) is possible.
- (C) The central, deepest part of the extreme SW basin. Very fine sands and white soft mud, both

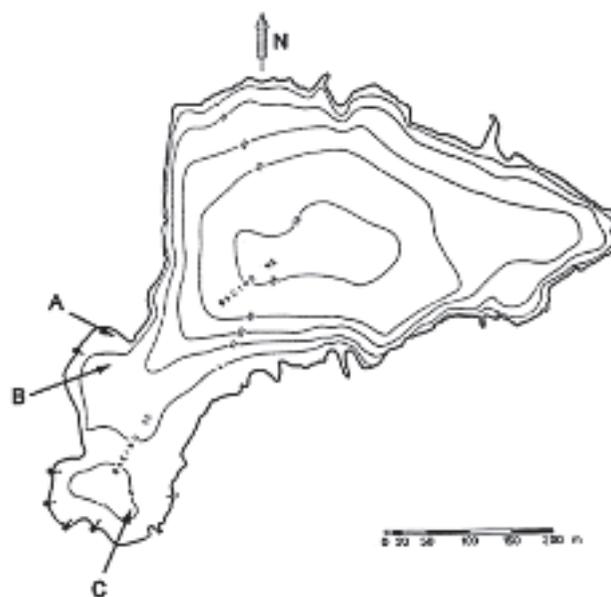


Fig. 1 - Sampling sites for *Ranunculus* indicated by the letters in the map of Lake Tovel for details see text.

Fig. 1 - Aree di campionamento del *Ranunculus* indicate dalle lettere nella mappa del Lago di Tovel, per dettagli vedere il testo.

easily stirred up, are forming the substratum. By the strong influence of cold seepage springs the temperature often stays below 6 °C at 4 m depth during all summer.

Reference specimens are deposited under IB 26509-26513 (Area C), IB 26514, 26515 (Area B) and IB 26516-26519 (Area A) at the registered herbarium of the Institute of Botany, LFU University Innsbruck, Austria.
Morphological characterisation

Tab. 1 - Environmental data (key factors and seasons) for site A and site C (4 m depth) in the Red Bay.

Tab. 1 - Dati ambientali (parametri chiave e stagioni) per il sito A e il sito C (profondità 4 m) nella Baia Rossa.

	A		C	
	2002	2003	2002	2003
Temperature spring (°C)	8.0	14.7	4.6	5.6
Temperature summer (°C)	15.2	21.1	5.4	7.3
Water depth spring (m)	0.6	0.5	4.6	4.5
Water depth summer (m)	0.4	0.1	4.4	4.1
O ₂ peak spring (%)	103	115	91	102
O ₂ peak summer (%)	108	116	100	127
pH spring	8.0	8.3	8.0	8.2
pH summer	8.2	8.3	8.1	8.2
PO ₄ -Ptot summer peak (µg l ⁻¹)	6.5	5.5	8.5	5
NO ₃ -N summer peak (µg l ⁻¹)	410	370	490	390

Ranunculus showed a high variability in growth form and size (Fig. 2), from very short to long ascending and / or floating morphotypes to prostrate creeping shoots with short ascending side branches (Fig. 2C). Whereas all ascending and floating types were fertile the creeping deep water specimens (central SW basin) were sterile. The petals of the near-shore type were white with yellow moon-shaped nectaries and kept separately in open emergent flowers. The number of carpels varied between 20 and 30. The specific morphological features of ecotypes from the different areas are the following:

- (A) In area A (near shore) a very high variability in respect to branching, internode length, leaf length and total length was found (Tab. 2). With the exception of the rooted, compressed basal internodes and basal side-branch nodes, all nodes were found with flower buds but had no roots (Fig. 2A). Normally the longer specimens were loosely floating and extended their flowers over the water surface. All specimens were lightly calcified. The length of the flower stalks did not vary much (Tab. 2) and showed no constant relationship to the internode length.
- (B) In area B the two specimens showed the typical characters of *R. trichophyllus* as found in many streams and lakes. Long extended nodes with one flower bud, one stalked leaf and 2-3 long roots extended into the water were typical as well as cleistogamic flowers. The specimens were only lightly calcified.
- (C) All specimens in area C were soft non-calcified, sometimes covered with thin green filaments of algae (*Zygnemales*). The majority was prostrate with variable basal internode length and short green ascending side-branches. The majority of basal nodes showed both one green leaf or a side-branch and several roots. High size variability of leaf length but normally shorter internodes of ascending branches (10-20 cm long) with non-rooted nodes was recorded. The leaf length-to-internode ratio of the upward parts is significantly lower than for the specimens from areas A and B (Tab. 2). In several samples from area C in July 2002 and 2003 brown (dead?) remnants of side-branches and brown (dead) leaves were found together with green leaves on the same basal node.

Chemotaxonomy and molecular taxonomy

Total phenolic content of all samples ranged from 1.5 to 2.2 % of dry mass and differed significantly between sampling depth and site (one-way ANOVA, $df = 2$, $F = 36.48$, $p = 0.003$). The results for samples from site A (0.2 m depth) and site C (3 m) did almost not differ (2.3 and 2.4% of dry matter (dm)),

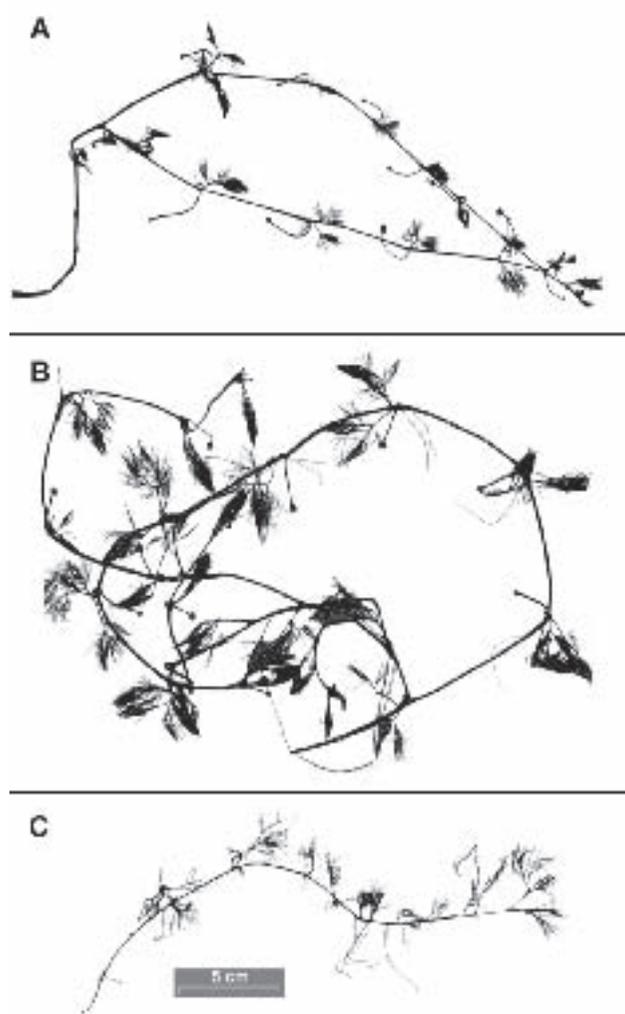


Fig. 2 - Morphological variations of *Ranunculus trichophyllus* from Lake Tovel in 2002/2003. (A) specimen from near shore area A with short basal part; (B) section of the erect main shoot of a specimen from area B arranged in a circle to fit the herbarium sheet; (C) prostrate specimen with long roots, short side branches and small leaves from area C (cold spring zone) (all sizes adjusted to scale in C).

Fig. 2 - Variazioni morfologiche del *Ranunculus trichophyllus* dal Lago di Tovel nel 2002/2003. (A) morfotipo della zona riparia dell'area A con la parte basale corta; (B) sezione del germoglio principale eretto del morfotipo dell'area B, collocato in cerchio per adattarlo al foglio dell'erbario; (C) morfotipo prostrato con germogli lunghi, diramazioni laterali corte e piccole foglie dall'area C (zona delle sorgenti fredde) (tutte le dimensioni sono riferite alla scala in C).

but were higher than those from samples from site C (4 m, 1.9% dm) and the reference sample from Völser Giessen (1.6% dm).

Tab. 2 - Morphometric features for specimens of *Ranunculus trichophyllus* from the three microhabitats as in figure 1 (measures in mm except for total length; total number of measurements in brackets; bold print numbers indicate a statistically highly significant difference of data-pairs using Student's t-test).

Tab. 2 - Caratteristiche morfometriche per le forme di *Ranunculus trichophyllus* dai tre microhabitat come in figura 1 (misure in mm eccetto per la lunghezza totale; in parentesi il numero totale di misure; i numeri in grassetto indicano differenze altamente significative rilevate con il test t di Student).

	A		B		C	
Total length						
range (cm)	3.5 – 51	(7)	65 – 104	(2)	4.3 – 29	(10)
Basal internode length						
mean	8.7 ± 6.2	(20)	no data		18.1 ± 12.8	(41)
range	1 – 23				5 – 46	
Leaf length						
mean	21.1 ± 4.3	(39)	41.1 ± 2.4	(17)	29.0 ± 7.9	(34)
range	15 – 26		36 – 44		14 – 41	
Ascending internode length						
mean	30.3 ± 17.2	(41)	72.0 ± 13.4	(18)	12.3 ± 4.7	(38)
range	2 – 63		52 – 100		5 – 23	
Internode to leaf length ratio						
mean	1.50 ± 0.81	(38)	1.76 ± 0.31	(17)	0.48 ± 0.18	(33)
range	0.11 – 2.74		1.50 – 2.32		0.20 – 0.95	
Flower stalk length						
mean	20 ± 5.5	(29)	22.7 ± 2.2	(15)	no flowers	
range	8 – 25		18 – 27			

Eight major signals were detected in HPLC separations of the samples and could be used as preliminary chemotaxonomic markers. They exhibited distinct retention times and UV spectra. Using these signals, distinct differences among the samples became apparent (Fig. 3).

In general, samples of the same site and depth display similar patterns. Nevertheless, some differences are also found between replicate samples from one site, e.g., peak no. 4 from Lake Tovel, 4.0 m depth. The sample from Völser Giessen showed only parallel peaks to the samples from 0.2 m depth in Lake Tovel. Samples from 3 and 4 m depth in Lake Tovel show a high similarity, with samples from 3 m depth exhibiting higher signals.

ITS1 sequences of *Ranunculus* samples from Lake Tovel (Tab. 3) matched largely with a sequence published for *R. trichophyllus* by Hörandl *et al.* (2005). The sequences from 3 m and 4 m depth in Lake Tovel are completely identical, whereas the one from the 0.2 m sample differs the most both from the deeper samples and from the GenBank sequence.

4. DISCUSSION

Lake Tovel has a limited number of macrophytes for several reasons: high water level fluctuation, high water renewal rate, low temperatures and low nutrient concentrations. However, two submerged macrophytes seem to be stable elements of the phytobenthos of the lake since many years, indicated by the comparison of the study by Baldi (1941) and our results. A moderate increase in the areas settled by *Ranunculus* in recent years could be possible (Rott *et al.* 2003) as a consequence of higher water temperatures in summer due to global warming in recent years. It is evident that changes of the macrophytes are not the main reason for changes in phytoplankton composition, although competition for nutrients between the two major primary producers might not be excluded.

It seems as if in Lake Tovel not all potential space for macrophytes is occupied although *Ranunculus* covers more than one niche in the lake, the near shore shallow waters and the deeper waters in the SW bay. Near shore the water level changes from May until August are normally small and thus offer sufficiently

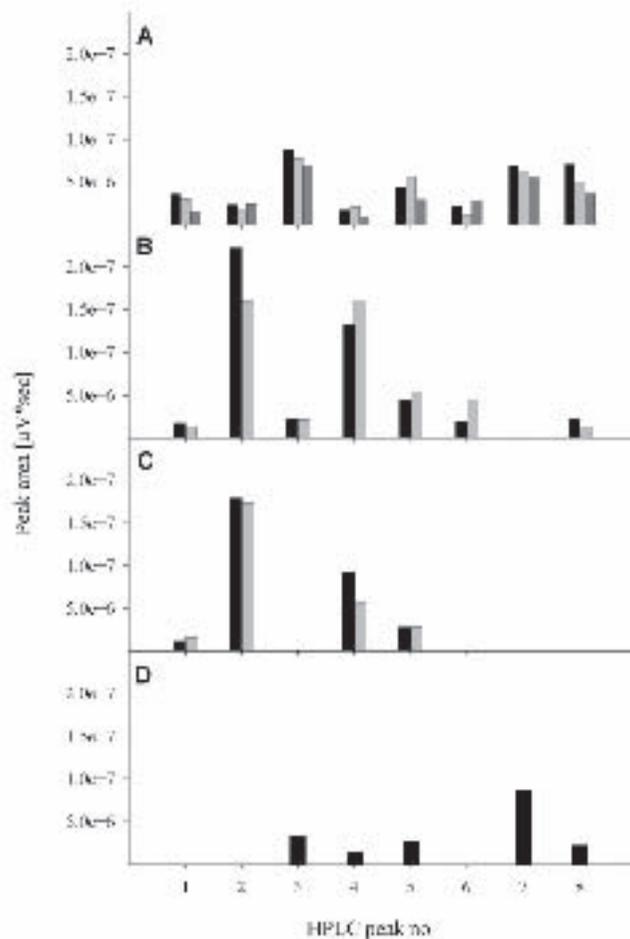


Fig. 3 - Chemotaxonomic fingerprint of *R. trichophyllus* samples from different locations (A-C) Lake Tovel, where A: area A, B: area C, depth 3 m, C: area C, depth 4 m, and (D): reference sample from Völser Giessen. Different shading denote different replicate samples from one area. Three samples were available for depth 0.2 m, two samples each for the depths 3 and 4 m and only one from Völser Giessen. Note: same y-axis used for all samples. Details for peaks investigated: (No./Retention time/UV-maxima (maximum used for peak area calculation is underlined): 1/10.8/227, 279; 2/13.8/219, 235, 331; 3/14.7/219, 239, 331; 4/16.7/255, 351; 5/17.2/251, 33; 6/19.5/251; 7/22.3/243, 327; 8/22.7/255, 343.

Fig. 3 - Impronta digitale chemiotassonomica di campioni di *R. trichophyllus* da diverse aree (A-C) del Lago di Tovel, dove A: area A, B: area C, profondità 3 m, C: area C, profondità 4 m, e (D): campione di riferimento da Völser Giessen. Le differenti ombreggiature denotano differenti campioni replicati della stessa area. Erano disponibili tre campioni per la profondità di 0,2 m, due campioni per le profondità 3 e 4 metri e solo 1 da Völser Giessen. Nota: stesso asse delle y per tutti i campioni. Dettagli per i picchi studiati: (No./tempo di ritenzione/UV-massimo (il massimo usato per il calcolo dell'area del picco è sottolineato): 1/10.8/227, 279; 2/13.8/219, 235, 331; 3/14.7/219, 239, 331; 4/16.7/255, 351; 5/17.2/251, 33; 6/19.5/251; 7/22.3/243, 327; 8/22.7/255, 343.

stable conditions for growth, flowering and fruiting. Why is *Ranunculus* so variable in this area? Is it a consequence of subsequent germination of seeds during spring, an effect of specific overwintering strategies or a result of interbreeding of infraspecific taxa in the catchment? The deep water area C type shows characters of slow clonal growth what indicates a specific stressful situation in this microhabitat (see Santamaria 2002) given by low temperature and very soft, unstable sediments. The statistically significant lower internode to leaf length ratio and the mainly prostrate sterile growth in area C separate the growth form types from A and B areas clearly from the C type. However, the robust specimens with cleistogamous flowers from B area differ again considerably from the small prostrate near-shore type, where the many roots on the nodes of the B type and the few on the nodes of the A type are to some extent also contradicting the classical view. However, the flower and fruit morphology and the internode-to-leaf length ratio of the area A and area B type did not differ considerably.

Why is the area C type mainly sterile? Light limitation, a well know reason for flower reductions (Barko & Smart 1981), can not be the case, but it could be the slow growth at constantly low temperatures (less than 6 °C) and the persistence of overwintering creeping shoots as shown by a morphological model investigation of running water specimens of *Ranunculus* subgenus *Batrachium* from Saxonia (Zander & Wiegler 1987).

The molecular and chemical markers suggest differences between the deeper growing plants (C type) and those of the shallow areas (three replicates from 0.2 m depth). When comparing the ITS1 region of our samples to that of a *R. trichophyllus* ITS1 sequence published in Hörandl *et al.* (2005), GenBank (AY680067), those for the ecomorph from the deeper site (C type, 3 and 4 m depth) exhibit the same base pair polymorphisms, while the sequence for the 0.2 m (A-type) sample differ from both the GenBank and the C-type specimens (Tab. 3). The ITS region is a popular species-specific molecular marker, but its value for phylogenetic studies was questioned recently because substantial intraspecific polymorphism may occur (Alvarez & Wendel 2003), which might not correlate with phenotypic plasticity. Due to the limited number of sequences analysed in our study, we cannot say whether our results reflect small variations within high morphological plasticity of the typical *R. trichophyllus* ssp. *trichophyllus* or indicate that different (sub)species are involved (see also Hörandl *et al.* 2005 for a discussion of the use of ITS sequences in buttercups). At present it cannot be excluded that according to the ITS1 sequence the samples from area A might be another *Ranunculus* (sub)species than those from area C.

The chemotaxonomic fingerprint largely confirmed the molecular differences. Peak pattern of samples

Tab. 3 - Alignment of ITS1 sequence of *R. trichophyllus* (GenBank AY680067) compared to ITS1 sequences of *R. trichophyllus* samples from different depth in Lake Tovel (RT_TOV_4.0 = DQ311656 and RT_TOV_3.0 = DQ311657 from area C, RT_Tov_0.2 = DQ311658 from area A1). Differences in our samples compared to the GenBank sequence AY680067 are highlighted in grey.

Tab. 3 - Allineamento di sequenze ITS1 di *R. trichophyllus* (GenBank AY680067) a confronto con quelle di campioni da differenti profondità del Lago di Tovel (RT_TOV_4.0 = DQ311656 e RT_TOV_3.0 = DQ311657 dall'area C, RT_Tov_0.2 = DQ311658 dall'area A1). Le differenze fra le sequenze dei nostri campioni e quelle della GenBank AY680067 sono evidenziate in grigio.

	5	15	25	35	45	55	
AY680067	-----	-AAAGCAGAC	GACCCGCGAA	CATGTTAAAA	AGTACCTTGC	GGA--GGTGA	
RT_Tov_4.0	TGTCGAACCT	GCAAGCAGAC	GACCCGCGAA	CATGTTAAAA	AGTACCTTGC	GGATTGGTGA	
RT_Tov_3.0	TGTCGAACCT	GCAAGCAGAC	GACCCGCGAA	CATGTTAAAA	AGTACCTTGC	GGATTGGTGA	
RT_Tov_0.2	-----	---AGCAGAC	GACCCGCGAA	CATGTTAAAA	AGTACCTTGC	GGATTGGTGA	
	65	75	85	95	105	115	
AY680067	AGGGTGCAG	CCCCGATCCA	ATCCGCTTGT	CGGGTCGCGG	AGTCGGTCGC	ATCCCACGTG	
RT_Tov_4.0	AGGGTGCAG	CCCCGATCCA	ATCCGCTTGT	CGGGTCGCGG	AGTCGGTCGC	ATCCCTTGTG	
RT_Tov_3.0	AGGGTGCAG	CCCCGATCCA	ATCCGCTTGT	CGGGTCGCGG	AGTCGGTCGC	ATCCCTTGTG	
RT_Tov_0.2	AGGGTGCAG	CCCCGATCCA	ATTCGTTTGT	CGGGTCGCGG	AGTTGGTCGC	ATCCCTTGTG	
	125	135	145	155	165	175	
AY680067	GTTGTGTCCG	CTTTGCGTTC	CCGCACAACA	TCAAAATCCG	GCGCGATTGG	CGTCAAGGAA	
RT_Tov_4.0	GTTGTGTCCG	CTTTCGCTTC	CCACACAACA	TCAAAATCCG	GCGCGATTGG	CGTCAAGGAA	
RT_Tov_3.0	GTTGTGTCCG	CTTTCGCTTC	CCACACAACA	TCAAAATCCG	GCGCGATTGG	CGTCAAGGAA	
RT_Tov_0.2	GTTGTGTCCG	CTTTCGCTTC	CCGCACAACA	TCAAAATCCG	GCGCGATTGG	CGTCAAGGAA	
	185	195	205	215	225	235	
AY680067	ATCTTAGCGG	AAACAAAGCG	TTGTGCCTTT	ACCGGTACAG	CGCCAAGAAT	CCCAATACTC AA	
RT_Tov_4.0	ATCTTAGCGG	AAACAAAGCG	TTGTGCCTTC	ACCGGTACAG	TGCCAAGAAT	CCCAATACTC AA	
RT_Tov_3.0	ATCTTAGCGG	AAACAAAGCG	TTGTGCCTTC	ACCGGTACAG	TGCCAAGAAT	CCCAATACTC AA	
RT_Tov_0.2	ATCTTAGCGG	AAACAAAGCG	TTGTGCCTTC	ACCGGTACAG	TGCCAAGAAT	CCCAATACTC AA	

taken in area C at both depths (3 and 4 m) were highly similar but distinct to that of samples from area A (shallow depth, 0.2 m). Remarkably, area A samples had a higher similarity to the reference sample from Völser Giessen (Fig. 2).

Based on the UV spectra, some of the HPCL peaks are possibly flavonoid glycosides. Quercetin glycosides have been described earlier from *R. trichophyllus* and related species (Gluchoff-Fiasson *et al.* 1994; Hennion *et al.* 1994; Fiasson *et al.* 1997; Gluchoff-Fiasson *et al.* 1997). The observed differences in flavonoid-like compounds between samples might result from different light regimes at the different sampling depth, but might also reflect phenotypic variability or species-specific differences. Flavonoids are frequently used as chemotaxonomic markers. We used successfully the relative concentration of luteolin-, apigenin- and chrysoeriol glycosides to unmistakably distinguish between puzzling *Elodea* specimens (*E. canadensis* and *E. nuttallii*; Erhard *et al.* in prep., Erhard & Gross 2005).

In spite of the clear differences between the ecomorphs from the near shore area A and area C found by both the molecular and chemical taxo-

nomic approach, taxonomic implications can not be drawn yet. Although *Ranunculus* shows a high morphological plasticity within the littoral zone of lake Tovel, specimens closely related to ssp. *eradicatus* as put into discussion by Pedrotti & Contini-Pedrotti (1996) could not be found and confirmed. The phenotypic plasticity of the different morphotypes sampled at the selected areas might result from an adaptive response to unpredictable and extreme environmental changes in these microhabitats. Such adaptive responses are much easier to study with heterophyllous aquatic plants (Wells & Pugliucci 2000). Clonal growth of the different specimens might prevent genetic exchange between near shore and deeper areas and strengthen the observed morphological differences.

It is possible that the *Ranunculus trichophyllus* ssp. *eradicatus* reaches occasionally its lower limit in altitude in the highly variable Lake Tovel shores and interbreeds with ssp. *trichophyllus* specimens, although more morphological criteria for the type species than for the subspecies were found. Hybridisation of (sub)species could only be excluded if additional chloroplast molecular markers would be

analysed besides the nuclear ITS gene. Remarkably, classic taxonomy placed the sample from area A closer to that from area B and distinguished sample C, but unfortunately not enough material from area B was available to perform these alternative taxonomic methods. Yet, as stated above, phenotypic variation might not be related to molecular difference. The combined analysis of highly variable buttercups by classical and molecular analyses has just begun. To answer these questions, additional intense seasonal sampling at the lake and in its catchment, and more genetic studies of alpine populations from this and other sites would be necessary. Transplantation and/or cultivation experiments would also be helpful to solve the taxonomic questions concerning the *Ranunculus* types found here.

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