Preslia 97: 191–216, 2025
doi: 10.23855/preslia.2025.191

Preslia

Striking diversity in Alpine lakes: genetic variation, hybridization and taxonomy of the aquatic *Ranunculus trichophyllus* group

Petr Koutecký[1,*], Jan Prančl[2], Magdalena Lučanová[1,2], Viktorie Brožová[1], Michaela Nagy Nejedlá[2], Jiří Košnar[1], Eva Koutecká[1], Peter Englmaier[3,4] & Zdeněk Kaplan[2,5]

[1]Faculty of Science, University of South Bohemia, Branišovská 1760, CZ-37005 České Budějovice, Czech Republic; [2]Institute of Botany, Czech Academy of Sciences, Zámek 1, CZ-25243 Průhonice, Czech Republic; [3]Faculty of Life Sciences, University of Vienna, Djerassiplatz 1, A-1030 Vienna, Austria; [4]OECONSULT, Expert consultancy for scientific ecology, Einsiedlergasse 23/8, A-1050 Vienna, Austria; [5]Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-12801 Prague, Czech Republic
*corresponding author: kouta@jcu.cz

Abstract: The *Ranunculus trichophyllus* group has a complex evolutionary history and remains taxonomically unresolved. In central Europe outside the Alps, two tetraploid cytotypes distinguished as *R. trichophyllus* A and B have been recognized in previous studies. Another ecologically specialized taxon, *R. confervoides* (*R. trichophyllus* subsp. *eradicatus*), is reported to have a disjunct distribution in northern Europe, the Alps and Pyrenees, and mountains and northern regions of Asia and North America. However, the classification of the populations outside northern Europe as *R. confervoides* has recently been questioned. We studied the *R. trichophyllus* group in 35 lakes in the Alps and five in northern Europe, involving genome size determination, sequencing the nuclear ITS region and two non-coding plastid DNA regions, and recording 10 morphological characters. The populations referred to as *R. confervoides* in the Alps did not substantially differ from the northern European ones morphologically, had identical genome size and shared the plastid haplotypes and ITS genotypes with them. Consequently, we consider the northern and the Alpine populations to belong to the same species *R. confervoides*. Interestingly, we also identified plants that combine morphological characters of *R. confervoides* and *R. trichophyllus* A, even though the latter was not found in the Alps during our study. Their status remains unclear; they may represent hybrids, but partly they can be a manifestation of morphological plasticity of these taxa. Nine plastid haplotypes were observed in *R. confervoides* and the "intermediate" populations, which contrasts with only two haplotypes identified in each of the two *R. trichophyllus* cytotypes outside the Alps. This surprising haplotype diversity may result from faster fixation of mutations in the Alpine populations due to prevailing isolation and clonality. Alternatively and not exclusively, this diversity may indicate higher age of the Alpine *R. confervoides*. Three hybrids unknown so far were recorded in lakes at the foothills of the Alps: triploid *R. circinatus* × *R. confervoides*, pentaploid hybrids of *R. confervoides* with an unknown species, and hybrids between *R. trichophyllus* B and the same unknown species. In addition, one putative allohexaploid population of *R. confervoides* and *R. trichophyllus* parentage was found. Given ecological distinctness and prevailing cleistogamy in *R. confervoides*, its involvement in multiple hybridizations is rather surprising. However, this species can produce open flowers when growing on exposed shores of lakes. We hypothesize that in such conditions hybridization can happen and the hybrids may survive by vegetative growth for a very long time.

Received: 16 Oct 2024; Revised: 16 Jan 2025; Accepted: 22 Jan 2025; Published: 24 Jun 2025

Keywords: Alps, *Batrachium*, chromosome count, flow cytometry, genome size, hybridization, nuclear ITS, morphology, plastid DNA, taxonomy

Introduction

Ranunculus sect. *Batrachium* (henceforth abbreviated as *Batrachium*) ranks among the taxonomically most intricate groups of aquatic plants in Europe. This monophyletic group (Hörandl & Emadzade 2012) includes mostly white-flowering aquatic *Ranunculus* species with submerged leaves divided into a number of capillary segments and in some species also with floating laminar leaves; rarely the capillary leaves are absent (Cook 1966, Wiegleb et al. 2017). About 30 species are recognized worldwide (Wiegleb et al. 2017), but recent studies show that this number might be underestimated due to the existence of morphologically cryptic taxa (e.g. Prančl et al. 2018, 2025, Koutecký et al. 2022), and some new species have been described recently (Wiegleb et al. 2022, Jopek et al. 2023). In addition, *Batrachium* also comprises numerous interspecific hybrids (Wiegleb et al. 2017, Bobrov et al. 2022, Koutecký et al. 2022, Zalewska-Gałosz et al. 2023).

In central Europe outside the Alps, eight or nine *Batrachium* species are recognized in the majority of floras and determination keys (e.g. Englmaier 2008, 2016, Prančl & Kaplan 2019), with a few additional taxa of an Atlantic distribution reaching westernmost Germany (Wiegleb 2021). The delimitation of the central European species has recently been studied using genome size and molecular data (Prančl et al. 2018, Koutecký et al. 2022), with some of them revealed to comprise several cytotypes and/or distinct genotypes. In addition, at least 10 hybrids of distinct parental combinations, and about 10 additional hybrid lineages of unknown parentage have been recorded in this area (Zalewska-Gałosz et al. 2015, 2023, Koutecký et al. 2022).

Ranunculus trichophyllus Chaix sensu lato (thread-leaved water-crowfoot) is a small-flowered plant with capillary leaves only (Fig. 1). It is distinguished by the following characters: stem up to 1.5 (–2.5) m tall; leaves capillary, on generative stems up to 6 cm long, with flaccid segments (sometimes subrigid, especially if incrusted by calcium carbonate), as long as or shorter than internodes; flowers often opening under water or cleistogamous (i.e. with unopened buds only, petals do not fully develop in such flowers); petals 2–5 (–7) mm long, with lunate nectar pit; pedicels 1–5 cm, slightly recurved; receptacles hairy, globose (not elongating) in fruit; carpels up to 40; achenes hairy at least when young (Wiegleb et al. 2017, Koutecký et al. 2022). In central Europe this species is tetraploid ($2n = 32$) but two cytotypes with different genome size, marked as cytotypes A and B, were revealed by Prančl et al. (2018). The ranges of quantitative morphological characters broadly overlap between the cytotypes, although the cytotype A tends to have smaller quantitative values of vegetative parts (e.g. stem diameter, leaf length, number of segments) and flowers (e.g. petal length) than the cytotype B, and with some experience, numerous populations can be identified morphologically. In addition, the cytotypes have partly different habitat preferences (the cytotype A prefers acidic while the cytotype B carbonate-rich waters, Kaplan et al. 2019) and are well differentiated based on molecular data, especially the internal transcribed spaces (ITS) of the nuclear ribosomal DNA (Koutecký et al. 2022). In the Alps, one additional taxon is recognized: *R. confervoides* (Fr.) Fr. (Englmaier 2008, 2016, Wiegleb 2011), often treated at the subspecific rank as

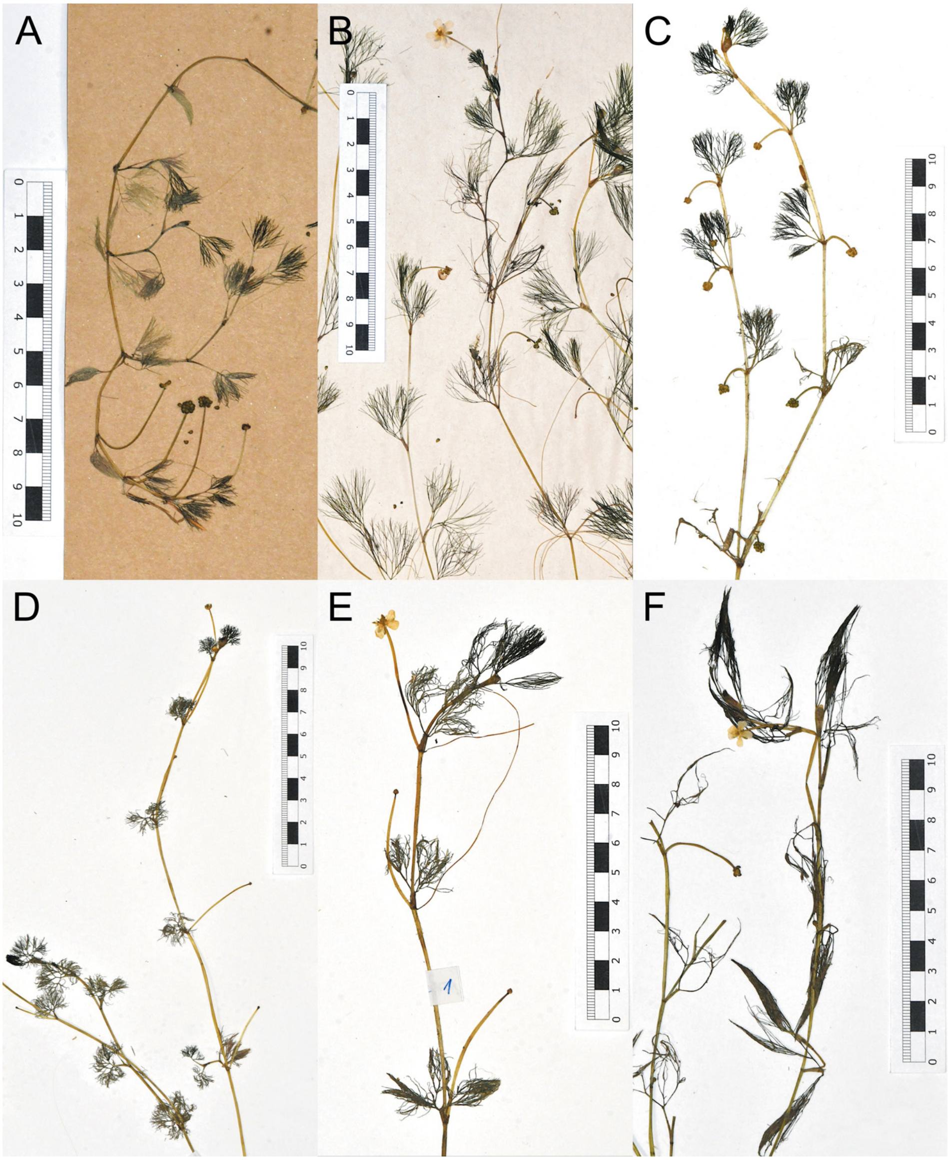

Fig. 1. Variability of the *Ranunculus trichophyllus* group in the Alps. (A) *R. confervoides*, AT-08-1; the stem was originally prostrate. (B) *R. confervoides/R. trichophyllus* A "intermediate", AT-05-5. (C) *R. trichophyllus* B, AT-16-5. (D) *R. circinatus* × *R. confervoides*, AT-17-5. (E) *R. trichophyllus* hybrid, AT-17-1. (F) *R. trichophyllus* s.l. 6x, SW-13-1. See Supplementary Table S1 for locality details. Note long thin pedicels in hybrids with no achenes (D, E) or only a few achenes (F) developed, and long adventive roots on the upper nodes (A, B, E).

R. trichophyllus subsp. *eradicatus* (Laest.) C. D. K. Cook (e.g. Cook 1993, Aeschimann et al. 2004, Tison & de Foucault 2014, Pignatti 2017, Lauber et al. 2018), or earlier in the monograph of Cook (1966) as *R. trichophyllus* subsp. *lutulentus* (Songeon et E. P. Perrier) P. Fourn. (subsequently replaced by the earlier epithet *R. trichophyllus* subsp. *eradicatus* in Cook 1967). This taxon is characterized by stems rooting at almost every node and often being prostrate, while stems are erect and rooting only at the lowermost nodes in *R. trichophyllus* cytotypes A and B (henceforth abbreviated as *R. trichophyllus* A and B, respectively). Quantitative characters of *R. confervoides* are generally at the lower end of *R. trichophyllus* group variation: leaves less than 4 cm long with very fine segments, very small flowers with petals less than 4.5 mm long, and developing only 10–15 (–25) achenes per flower. It usually flowers underwater and is mostly cleistogamous (Cook 1966, Englmaier 2008, 2016, Wiegleb 2011), while chasmogamous (open) flowers generally prevail in *R. trichophyllus* (Wiegleb et al. 2017).

Ranunculus confervoides has been generally considered an arcto-alpine taxon. In the Alps it has been reported from oligo- to mesotrophic mountain lakes mainly on calcium-rich bedrocks, mostly in the subalpine and alpine belts (e.g. Cook 1993, Aeschimann et al. 2004, Englmaier 2008, 2016, Pignatti 2017). It has also been reported from the Pyrenees and the Portuguese part of the Sistema Central Mts (Cook 1986), although the latter is considered erroneous by Pizzaro (1995). It has also occasionally been reported from outside Europe, namely from Asian mountains and the northern territories of Asia and North America (Cook 1966, Luferov 2004). However, the occurrence of *R. confervoides* in the Alps, the Pyrenees, Asia and North America has recently been challenged by Wiegleb et al. (2017) and Wiegleb (2021). Under their view, *R. confervoides* is restricted only to northern Europe, while the other records belong to the polymorphic *R. trichophyllus*; Wiegleb et al. (2017) mention that the Alpine plants are more robust than the northern ones, without further details. This treatment is also accepted in the recent monographic book on European aquatic plants (Schou et al. 2023) and Plants of the World Online database (POWO 2024; occurrence in Greenland and the Asian mountains retained).

Until recently, genetic variation of *R. confervoides* used to be virtually unknown. Prančl et al. (2018) showed that genome size of *R. confervoides* (treated as *R. trichophyllus* subsp. *eradicatus* in that paper) is similar to central European *R. trichophyllus* A, while it is markedly different from *R. trichophyllus* B. Interestingly, *R. confervoides* is mostly reported from alkaline-rich waters, so it seems to be a distinct taxon from *R. trichophyllus* A, which prefers acidic waters. Thus, the genome size data thus might be useful to distinguish between *R. confervoides* and *R. trichophyllus* B in alkaline waters. DNA data are very scarce for *R. confervoides*. There are no population genetic studies available for that taxon. In the GenBank database, there are no sequences from the Alps and only one specimen from northern Europe (Finland) is sequenced for the markers used in the recent phylogenetic studies of *Ranunculus* sect. *Batrachium*, such as the ITS region and *rpl*32–*trn*L$^{\mathrm{UAG}}$, *psb*E–*pet*L non-coding plastid regions (Bobrov et al. 2015, Jopek et al. 2023); for the 3’*rps*16–5’*trn*K plastid region used on central European *Batrachium* by Koutecký et al. (2022) no sequence is available. The available sequences show that this single accession is closely related to but not completely identical with some *R. trichophyllus* accessions and with some other taxa (*R. kauffmannii* Clerc and *R. schmalhausenii* Luferov among the European ones) in the ITS, while the chloroplast haplotype is identical with these taxa and also with some accessions determined as *R. aquatilis* L. (Bobrov et al. 2015, Jopek et al. 2023).

As a part of taxonomic revisions of the European *Batrachium*, we aim at exploring the diversity of *R. trichophyllus* group in the Alps and clarifying the status of the ecologically specialized and long-time recognized but recently challenged *R. confervoides*. Using genome size estimation by flow cytometry and sequencing diagnostic DNA regions, we ask which taxa/cytotypes/genotypes are present in the Alps. For the lakes in high elevations, we specifically ask whether (i) these Alpine populations are identical with the northern European ones and different from the lowland and peri-Alpine *R. trichophyllus*, or (ii) the Alpine populations are only a subset of variation of central European *R. trichophyllus* and are different from the northern European *R. confervoides*. If the latter is true, the Alpine populations are expected to comprise only a limited number of genotypes fixed by autogamy, identical or closely related to central European *R. trichophyllus* genotypes.

Methods

Plant material

We collected *R. trichophyllus* s.l. samples in 35 Alpine lakes situated mostly in the Eastern Alps (Fig. 2), including both high-elevation lakes, where *R. confervoides* is generally reported to occur, and lakes in the Alpine valleys and the foothills where "lowland" *R. trichophyllus* is expected. A part of the *R. confervoides* samples comes from our previous study (Prančl et al. 2018). For comparison, we sampled five *R. confervoides* populations in northern Europe (Sweden, Finland, Estonia); one of these populations comes from the same lake as the plant sequenced by Bobrov et al. (2015). Usually, we collected five individuals per population and taxon (if several morphological taxa co-occurred); however, for some sites from the previous study collected by our collaborators fewer or a single plant are available. As an individual, we consider a distinct cluster of stems; in dense and continuous populations, we sampled plants at least 5 m apart. For each sampled plant we collected material for a herbarium voucher and performed flow cytometry and DNA extraction from the same ramet. Voucher specimens are preserved in herbaria CBFS and PRA, and locality details are presented in Supplementary Table S1. The map of localities (Fig. 2) was prepared using QGIS 3.10.3 (QGIS Development Team 2020) with the elevation map of Europe as a background (EEA 2022).

Flow cytometry

Genome size was estimated using flow cytometry following methods described in Prančl et al. (2018). Briefly, we used propidium iodide as a stain and *Bellis perennis* L. as the internal standard (2C = 3.38 pg, Schönswetter et al. 2007). Where available, at least three plants per population and taxon were measured individually, whereas the rest of the population was measured in bulked samples to verify population homogeneity; if multiple cytotypes were revealed in a bulked sample, all individuals were reanalysed separately.

Chromosome counting

Chromosome counting was performed for selected populations to calibrate the genome size data, using the methodology from the previous study (Prančl et al. 2018). Briefly, chromosomes were counted from root tips of adventive roots of cultivated plants or from seedlings. Roots were pre-treated with p-dichlorobenzene for 3 h at room temperature, fixed in a 3:1 mixture of 96% ethanol and acetic acid, macerated in a 1:1 mixture of ethanol and hydrochloric acid for 10 s and stained with lacto-propionic orcein. Preparations were made using the squash method.

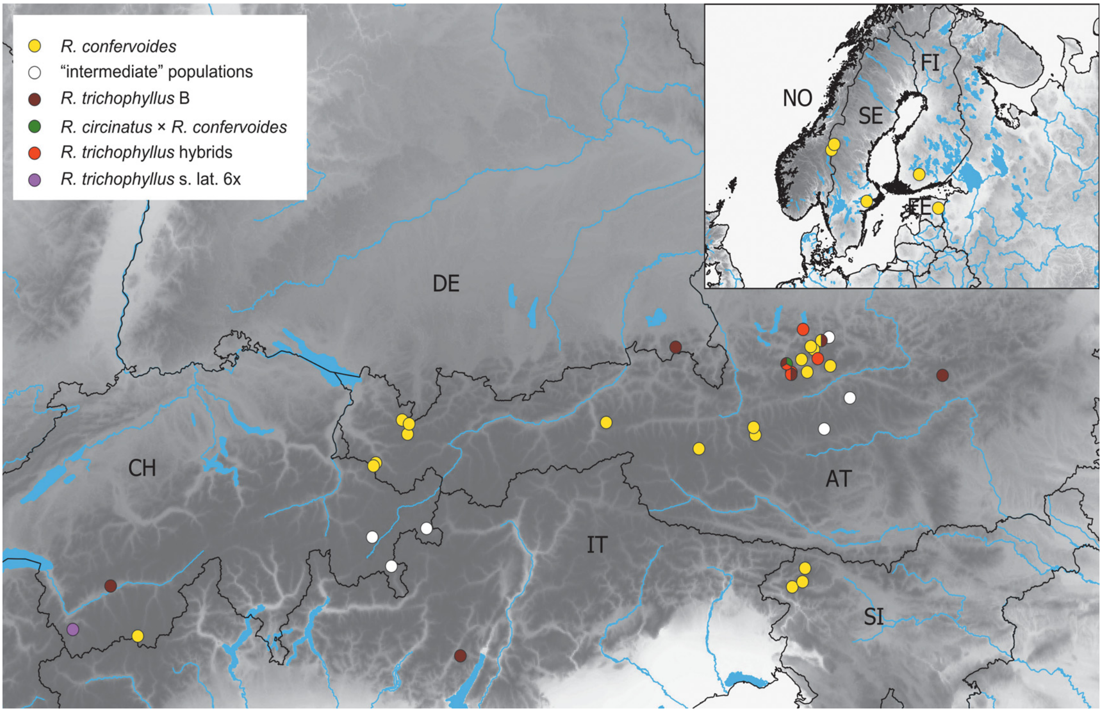

Fig. 2. Map of studied localities. Genome size groups are marked using different colours; the *Ranunculus confervoides*/*R. trichophyllus* A group is split into typical *R. confervoides* and "intermediate" populations (see the text for details).

DNA sequencing

DNA was extracted from silica-dried leaves of plants analysed by flow cytometry. The methods were the same as in our previous study (Koutecký et al. 2022). Briefly, DNA was extracted using a CTAB protocol (Doyle & Doyle 1987) or sorbitol extraction protocol (Štorchová et al. 2000), with minor modifications. Three regions that have been found to provide good resolution at the species level in *Batrachium* were amplified: the ITS region of the nuclear ribosomal DNA (using primers ITS-F and ITS4; King et al. 2001 and White et al. 1990, respectively) and two non-coding plastid spacers, *rpl*32–*trn*L^{UAG} and 3'*rps*16–5'*trn*K (Shaw et al. 2007; for the former, we used internal primers described in Koutecký et al. 2022). Sanger sequencing was performed by Eurofins Genomics. The ITS region was sequenced from both directions, the *rpl*32–*trn*L^{UAG} spacer from the internal primers, and the 3'*rps*16–5'*trn*K from the reverse primer and re-sequenced in the opposite direction when needed. Usually we sequenced 1–2 individuals per population and taxon; the number of sequenced individuals is shown in Supplementary Table S1.

Intra-individual polymorphisms in the ITS sequences were coded using IUPAC ambiguity codes; because we have not separated the individual ITS copies using molecular cloning or NGS technologies, we refer to these sequences as "genotypes" instead of "ribotypes". Sequences were aligned manually using BioEdit 7.2.0 (Hall 1999). Data from the two plastid regions were concatenated. For comparison with central European taxa, the dataset was supplemented with sequences of the 17 most frequent genotypes of all taxa (ITS) and all 11 haplotypes (plastid DNA) present in central Europe from Koutecký et al. (2022). The two alignments (ITS and plastid DNA) were trimmed according to the shortest sequence and collapsed into unique genotypes using FaBox 1.5 (Villesen 2007). If individuals within one population showed the same genotypes (ITS or plastid), only one was retained in the dataset. The plastid data were analysed in PopART (Leigh & Bryant 2015) using a TCS network (Clement et al. 2002); informative gaps were coded as unique substitutions to count as one mutation step. The ITS data were visualized as a phylogenetic network in SplitsTree 6 (Huson & Bryant 2006) using a NeighborNet algorithm based on P distance and handling of ambiguous states set to average. The sequence data are deposited in GenBank. Sequences from one individual for each combination of the cytotype, geographic region (the Alps and northern Europe), plastid haplotype and ITS genotype are included (accession numbers PQ661805–PQ661841, PQ661842–PQ661878 and PQ644841–PQ644877 for the *rpl*32–*trn*L^{UAG}, 3'*rps*16–5'*trn*K and ITS regions, respectively; see Supplementary Table S2). To allow comparison with previous data, individual genotypes/haplotypes were named according to our other studies (Koutecký et al. 2022, Prančl et al. 2025), i.e. identical haplotypes from these studies share identical names.

Morphology

We recorded 10 morphological characters that are used in determination keys to distinguish *R. confervoides* from *R. trichophyllus* and/or can be used as a measure of plants "robustness" (Table 1). All measurement were performed from the herbarium material. In most of the material, we preserved petals or whole flowers in separate paper envelopes to prevent shape distortion during drying. The summary data for populations are presented in Supplementary Table S3; the matrix for individuals is available from the corresponding author on request. Populations were classified into groups based on genome sizes and fertility (Fig. 3). For the purpose of the morphological analysis, one of the groups, the *R. confervoides/R. trichophyllus* A genome size group, was divided into three subgroups, based on geographical origin and presence of the two categorical characters defining *R. confervoides* (plants rooting at the upper nodes and stems prostrate to ascending): (i) *R. confervoides* from northern Europe, (ii) *R. confervoides* from the Alps, (iii) "intermediate group" from the Alps with one or both diagnostic characters missing (these plants thus look intermediate between *R. confervoides* and non-Alpine *R. trichophyllus* A; note that we did not find any sample that unequivocally fits the typical habitus of non-Alpine *R. trichophyllus* A, see below). Differences between the groups for individual characters were tested using one-way ANOVA and Tukey HSD test, if the ANOVA test was significant. The putative hexaploids were excluded from ANOVA of generative characters due to the very low number of observations (only two samples available); for achene number, sterile hybrids were omitted, too. Statistical analyses were performed and graphs plotted in R version 4.1.2 (R Core Team 2021).

Table 1. Morphological characters of the studied species.

Growth form	Prostrate (growing horizontally at the bottom surface or in the sediment, often with branched rhizomes and short vertical stems) / ascending (horizontal section shorter than the erect part of a stem) / erect (± no horizontal growth)
Stem length	Recorded approximately with a precision of 5 cm; only vertical parts of stems were considered. ">" (i.e. stem longer than) marks incomplete plants with basal parts missing (for example floating plants and plants from very deep waters)
Rooting on the upper nodes	Yes / no; evaluated in the upper 1/3 of the stem
Stem diameter	Measured using a stereomicroscope with a precision 0.05 mm; recorded in the middle of the internode below the uppermost measured leaf (see leaf length)
Leaf length	Including the petiole, precision 1 mm, mean of three leaves. In fruiting plants, three successive leaves downwards starting from the leaf that supports the uppermost fruiting pedicel. If only flowers were present on a plant, three leaves starting from the lowermost flower. In non-flowering plants, three fully developed leaves from the middle part of a stem; young leaves on the tops of stems were avoided
Flower type	Cleistogamous (permanently closed, pollination takes place in a flower bud stage) / open (frequently under water) / mixed (both types present) / flowers absent
Petal length	Measured using a stereomicroscope with a precision 0.1 mm; one petal per flower, mean of three flowers if available; only petals from open (chasmogamic) flowers included
Fruiting pedicel length	Length of the uppermost fruiting pedicel, precision 1 mm
Number of achenes	Number of mature achenes per one etaerio
Fertility	Full (all pistils developed into achenes) / partial (% of pistils developed into achenes) / sterile (no achenes)

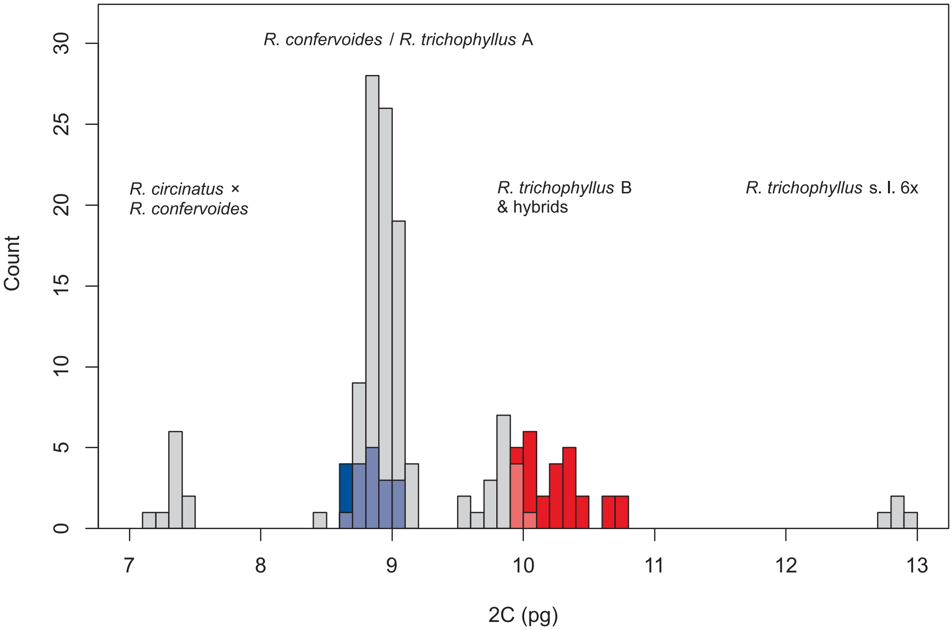

Fig. 3. Genome size distribution of the *Ranunculus trichophyllus* group. Samples from the Eastern Alps are depicted in grey, except for sterile hybrids with genome sizes between 2C = 9.9 and 10.8 pg (red). Samples from the northern Europe are in blue. The groups are overlaid if two of them are present in the same interval.

Results

Genome size and ploidy levels

Four main genome size groups can be recognized in the studied material (Fig. 3 for data on individuals, Supplementary Table S1 for population means): (i) The first group, median 2C = 7.36 pg, comprises two populations from Hallstättersee (Austria) of morphologically conspicuous plants with rather rigid leaves with short segments, no flowers or only with unopened buds, and not developing any fruits (i.e. sterile; we use the word "sterile" in this sense throughout the manuscript and distinguish such plants from non-flowering but potentially fertile ones). Based on genome size ranges from the previous study (Prančl et al. 2018; the same locality was sampled under collection no. B14-118), these plants are triploid and both the genome size and morphology correspond to a hybrid of diploid *R. circinatus* and tetraploid *R. trichophyllus* A or *R. confervoides.* The ploidy was confirmed by direct chromosome counting (2n = 3x = 24; Fig. 4A). (ii) The second group, median 2C = 8.90 pg, corresponds to *R. trichophyllus* A or *R. confervoides*, which cannot be distinguished based on the genome size (see Prančl et al. 2018). From the morphological point of view, this cytotype contains populations of a characteristic *R. confervoides* habitus as well as populations that are morphologically more or less intermediate between *R. confervoides* and non-Alpine *R. trichophyllus* A ("intermediate group"). All

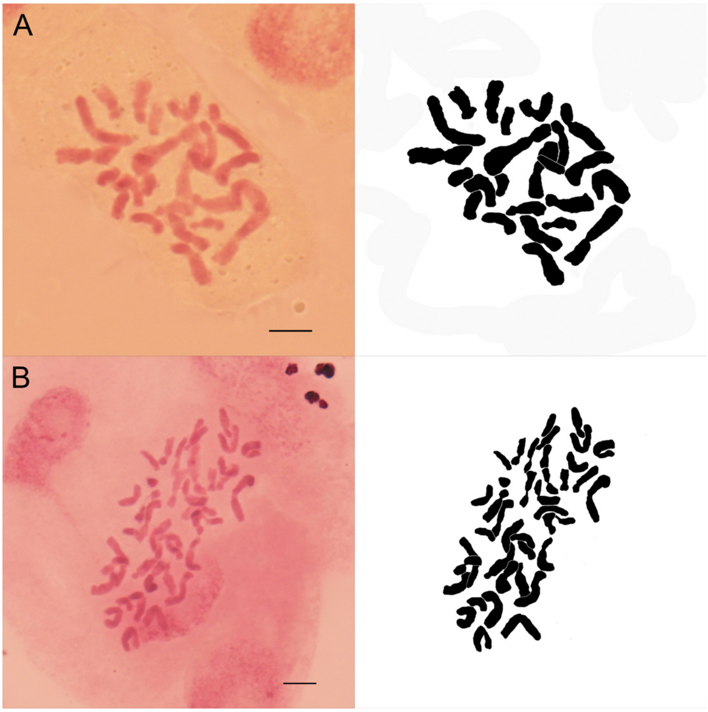

Fig. 4. Chromosomes (photograph of cytological preparation on the left with its interpretation on the right): A – *Ranunculus circinatus* × *R. confervoides*, AT-17b, 2n = 3x = 24; B – *R. trichophyllus* s.l. hybrid, AT-15b, 2n = 5x = 40. Scale bar: 10 µm.

R. confervoides samples from northern Europe also fell into this group. For two populations chromosomes were counted: K17-82 (Austria) and SK-16 (Finland). Both populations were tetraploid, 2n = 4x = 32. (iii) The third group, median 2C = 10.01 pg, contains fertile plants identified as *R. trichophyllus* B (tetraploid in central Europe; Prančl et al. 2018, Koutecký et al. 2022), and generally more robust plants that are sterile or do not develop flowers at all. There is a slight genome size difference between the fertile and sterile plants with certain overlap. While the fertile plants have genome sizes in the lower part of this group's range below 2C = 10.10 pg (median 9.84 pg), the sterile plants have genome sizes above 2C = 9.90 pg (median 10.24 pg). Chromosomes were successfully counted for one population of rather robust sterile plants from the Hallstättersee lake, Austria (population AT-15b, in the previous study sampled under no. B14-119; mean 2C = 10.13 pg). This population was pentaploid, 2n = 5x = 40 (Fig. 4B), and is thus most likely of hybrid origin. (iv) The fourth group was formed by a single population from Switzerland (SW-13), which has by far the highest genome sizes among the studied samples, median 2C = 12.85 pg. The genome size of these plants is closest to values expected for a hexaploid, but we failed to confirm the ploidy by chromosome counting due to the lack of suitable material.

Plastid haplotypes

Alignment of the two concatenated plastid regions (*rpl*32–*trn*L^{UAG} and 3'*rps*16–5'*trn*K) was 1,506 bp long and contained 49 polymorphic sites, from which 28 sites are specific for the rather distant species *R. circinatus* and *R. rionii*, which together form the well-separated *R. circinatus* haplotype group (Fig. 5). No samples from the Alps belonged to this group. Three other haplotype groups can be distinguished in the studied material. (i) The *R. peltatus* haplotype group includes the most frequent central European haplotype "pelt" and several minority haplotypes differing by one or two mutations steps, only one of them present in the Alps. (ii) The *R. trichophyllus* haplotype group includes two haplotypes ("trich-A" and "trich-B") that are also known outside the Alps (Koutecký et al. 2022), and two additional haplotypes unknown so far, one of which is frequent. Both these haplotypes were found in samples of the *R. confervoides*/*R. trichophyllus* A genome size group and three hybrid populations. (iii) Surprisingly, another haplotype group was revealed: the *R. confervoides* haplotype group, which contains five haplotypes differing by one or two mutation steps ("conf-3" to "conf-7"). All these haplotypes were specific for the *R. confervoides*/*R. trichophyllus* A genome size group. None of them was found outside the Alps during the previous study (Koutecký et al. 2022), which covered part of central Europe including southern Germany, northern and eastern Austria, Hungary, Czechia, Slovakia, and southernmost Poland and involved 258 populations in total, among them 89 from the *R. trichophyllus* group.

The *R. confervoides*/*R. trichophyllus* A genome size group had five haplotypes from the *R. confervoides* haplotype group and four haplotypes from the *R. trichophyllus* haplotype group. Among the later, two haplotypes ("conf-1" and "conf-2") are specific for *R. confervoides*/*R. trichophyllus* A group, while the "trich-A" haplotype (found in one northern European and one Alpine population) is frequent in *R. trichophyllus* A outside the Alps and the "trich-B" haplotype (one Alpine population, SW-30) is frequent in *R. trichophyllus* B (Koutecký et al. 2022). Importantly, there were no haplotypes specific

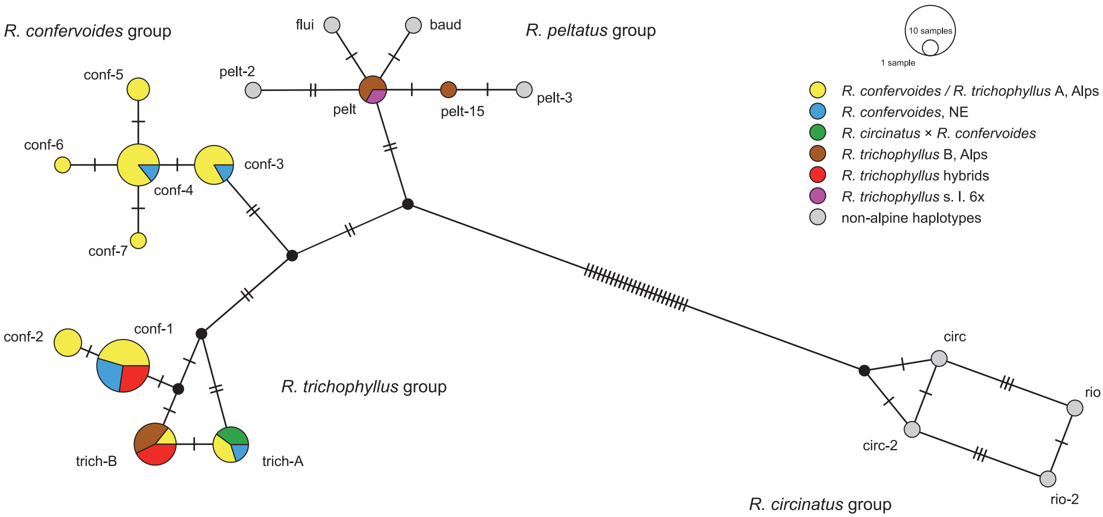

Fig. 5. Haplotype network (TCS-type) of concatenated *rpl*32–*trn*L$^{\text{UAG}}$ and 3'*rps*16–5'*trn*K sequences of the *Ranunculus trichophyllus* group; for comparison, sequences of all haplotypes found in central Europe outside the Alps (Koutecký et al. 2022) are included (one sequence per haplotype). The frequencies of haplotypes in the dataset are represented by circle sizes, and the frequencies of cytotypes defined by genome sizes and fertility are indicated by colours. Abbreviations: NE – northern Europe. The haplotypes are named after the species in which the particular haplotype is the most frequent: baud – *R. baudotii*, circ – *R. circinatus*, conf – *R. confervoides*, flui – *R. fluitans*, pelt – *R. peltatus*, rio – *R. rionii*, trich-A, trich-B – *R. trichophyllus* cytotypes A and B, respectively.

for northern European *R. confervoides*; all haplotypes found in northern Europe were found also in the Alps. The distribution of the haplotypes revealed in the *R. confervoides*/*R. trichophyllus* A group does not show any clear pattern (Supplementary Figure S1). *Ranunculus trichophyllus* B comprised three haplotypes: two from the *R. peltatus* group ("pelt" and "pelt-15") and one from from the *R. trichophyllus* haplotype group ("trich-B"). There were no haplotypes specific for the hybrids. Both populations of the triploid hybrid *R. circinatus* × *R. trichophyllus* s.l. from the Hallstättersee lake had the "trich-A" haplotype from the *R. trichophyllus* haplotype group. The sterile hybrids with genome size similar to *R. trichophyllus* B had "trich-B" and "conf-1" haplotypes from the *R. trichophyllus* group. The unique putatively hexaploid population from Switzerland (SW-13) had the "pelt" haplotype, similarly as two samples of *R. trichophyllus* B.

ITS genotypes

The alignment of the ITS region was 696 bp long and contained 57 polymorphic positions, including three 1 bp indels. The structure of the NeighborNet network (Fig. 6) is mainly defined by non-Alpine central European genotypes; all samples from the Alps were similar to one of these genotypes or were additive combinations of them, with one exception (see below). All samples with genome size of *R. confervoides*/*R. trichophyllus* A clustered in the same part of the network and were close to but not identical with one of the main genotypes of *R. trichophyllus* A outside the Alps (CE82). There was no obvious

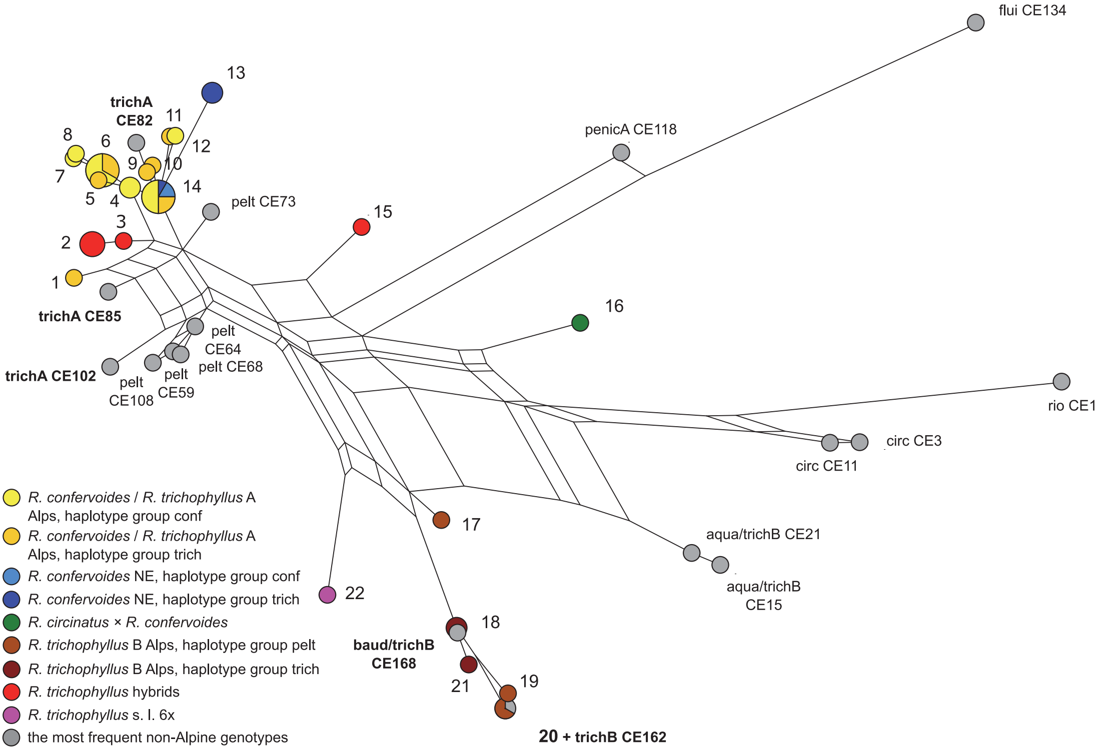

Fig. 6. NeighborNet of the ITS sequences of the *Ranunculus trichophyllus* group; for comparison, the most frequent genotypes in central Europe outside the Alps (Koutecký et al. 2022) are included; the genotypes found in the *R. trichophyllus* group in that study are in bold. The frequencies of the Alpine genotypes in the dataset are represented by circle sizes, and the frequencies of cytotypes defined by genome sizes and fertility are indicated by colours. Abbreviations: NE – northern Europe; conf, pelt and trich (in the legend) – *R. confervoides*, *R. peltatus* and *R. trichophyllus* plastid haplotype groups, respectively; CE – central European genotypes; the species' abbreviations: aqua – *R. aquatilis*, baud – *R. baudotii*, circ – *R. circinatus*, flui – *R. fluitans*, pelt – *R. peltatus*, penic A – *R. penicillatus* cytotype A, rio – *R. rionii*, trichA, trichB – *R. trichophyllus* cytotype A, B, respectively.

difference between samples having *R. trichophyllus* or *R. confervoides* plastid haplotype groups nor between samples from the Alps and northern Europe. Importantly, the most frequent ITS genotype no. 14 was found in samples having haplotypes from both *R. confervoides* and *R. trichophyllus* haplotype groups and from both geographical regions. The only slightly different genotype no. 1 is found in a single population from Switzerland (SW-27) and might be influenced by hybridization, as it additively combines the *R. confervoides*-like genotypes with the rather distant central European *R. trichophyllus* A genotype CE102. Samples classified as *R. trichophyllus* B clustered close to genotypes known for this cytotype outside the Alps, and are markedly different from *R. confervoides* and *R. trichophyllus* A. From the two ITS variants known in *R. trichophyllus* B outside the Alps and marked as groups B1 and B2 in Koutecký et al. (2022) (i.e. B1: genotypes with a few or no intra-individual polymorphisms, in Fig. 6 represented by genotypes CE162 and CE 168, and B2: genotypes that additively combine the previous with *R. circinatus*-like sequences, CE15 and CE21), only the B1 group was found in the Alpine lakes, except for one sample. Genotype no. 17 showed several polymorphic positions combining the ITS sequence of *R. trichophyllus* B1 and B2 groups. The putative hexaploids showed a specific genotype (no. 22) that additively combined sequences of *R. trichophyllus* B1 with *R. confervoides*/*R. trichophyllus* A. The triploid hybrids additively combined sequences of *R. circinatus* with *R. confervoides*/*R. trichophyllus* A. Last, the sterile hybrids with genome size slightly higher than *R. trichophyllus* B combined a specific sequence unknown so far (several unique polymorphic positions, including one 1 bp insertion) with either *R. confervoides/R. trichophyllus* A (genotypes no. 2 and 3) or *R. trichophyllus* B (the B2 variant; genotype no. 15).

Morphology and its correlation with molecular data

We analysed differences between the genome size groups in several quantitative morphological characters that can be considered as a measure of plant "robustness" or are used to distinguish *R. confervoides* from *R. trichophyllus*. We also recorded several categorical characters (growth form, rooting on the upper node, flower type, fertility) (Table 1, Supplementary Table S3). The *R. confervoides*/*R. trichophyllus* A genome size group showed conspicuous variation and was therefore for the purpose of this analysis divided into three subgroups based on geographical origin and two categorical characters specific for *R. confervoides* (plants rooting at the upper nodes and stems prostrate to ascending): two *R. confervoides* groups (the Alps and northern Europe) and a group of plants "intermediate" between *R. confervoides* and *R. trichophyllus* A. The latter group is characterized by a mixture of characters of these two taxa. In most populations one of the specific characters was missing (i.e. plants were not rooting on upper nodes or had erect stems); in one population both characters were missing, but these plants differed from *R. trichophyllus* A by more slender stems and leaves and cleistogamy, while *R. trichophyllus* A has usually open flowers. We did not find any populations that could be unequivocally classified as *R. trichophyllus* A.

The results of the analysis of quantitative characters are depicted in Fig. 7. The triploid hybrids *R. circinatus* × *R. confervoides* were excluded from this analysis because of conspicuously different leaf morphology (segments relatively rigid, short and more or less oriented in one direction, though not completely in one plane as in *R. circinatus*) and absence of floral and fruit characters. All characters except for achene number showed significant differences between the groups ($P < 0.05$ in the ANOVA). Plants of *R. confervoides* from the Alps had slightly higher values of all characters than plants from northern Europe, but variation ranges broadly to completely overlapped. The only marked and statistically significant difference was the fruiting pedicel length, although also in this character the broad variation range of the Alpine plants included values observed in the northern plants. The Alpine plants classified as "intermediate" showed slightly higher values of all characters than the Alpine *R. confervoides* with a broad overlap or were very variable with the variation exceeding both previous groups. The other three genome size groups showed higher values of stem diameter and erect growth form with stems longer than ~30 cm (except for some hybrids). Leaf length broadly overlapped between all three *R. confervoides*/*R. trichophyllus* A subgroups and *R. trichophyllus* B, while hybrids and the putative hexaploids had significantly longer leaves. All six groups

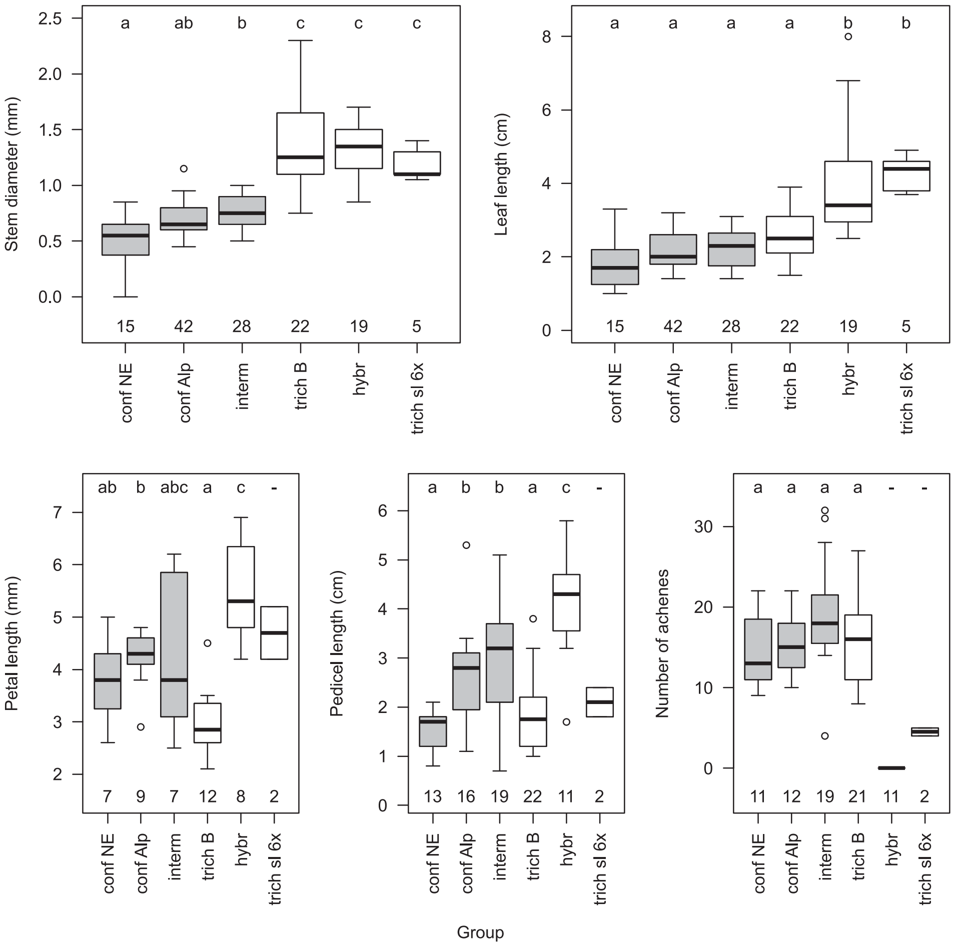

Fig. 7. Quantitative morphological characters of the *R. trichophyllus* group (top – vegetative, bottom – generative). Median, quartiles and non-outlier range are shown. The number of analysed individuals is shown below boxes; letters above boxes indicate groups that significantly differ in Tukey HSD test ($p < 0.05$). The *R. confervoides/R. trichophyllus* A genome size group is depicted in grey. Abbreviations: conf NE – *R. confervoides*, northern Europe; conf Alp – *R. confervoides*, the Alps; interm – the "intermediate" group; trich B – *R. trichophyllus* B; hybr – *R. trichophyllus* sterile hybrids; trich sl 6x – the putative hexaploid population (SW-13).

had both cleistogamous and open flowers with variable frequency; the sterile hybrids, if flowering, had mostly open flowers. *Ranunculus trichophyllus* B had the smallest open flowers, while the sterile hybrids and the putative hexaploids had larger flowers (i.e. with longer petals). The *R. confervoides/R. trichophyllus* A group had medium flower size, the "intermediate" subgroup being markedly variable. The sterile hybrids had the longest pedicels (in this case, pedicels bearing bare receptacles, with no fruits developed). The number of achenes did not differ between *R. confervoides* (both geographic groups), "intermediate" plants and *R. trichophyllus* B, while it was reduced in putative hexaploids and achenes were absent in the sterile hybrids.

While the *R. confervoides*/*R. trichophyllus* A group as a whole is supported by morphological and genetic data, its ad hoc defined morphological subgroups are not. The "typical" *R. confervoides* plants (i.e. prostrate and rooting on the upper nodes) both from northern Europe and the Alps had the same plastid haplotypes from both the *R. confervoides* and the *R. trichophyllus* haplotype groups (Fig. 5) and partly also the same ITS genotypes (the most frequent genotype no. 14, Fig. 6). The "intermediate" group (lacking one or both determination characters of *R. confervoides*, see above) did not substantially genetically differ from the Alpine *R. confervoides*. Three out of six populations had haplotypes from the *R. confervoides* haplotype group, while the other three from the *R. trichophyllus* group, one having the haplotype "trich-B" and two the most frequent haplotype "conf-1", which is found also in northern European samples of *R. confervoides*. No "intermediate" sample had the "trich-A" haplotype, despite a certain morphological similarity to *R. trichophyllus* A. The ITS genotypes of "intermediate" plants were either identical to *R. confervoides* (genotypes no. 6 and 14, the latter found also in samples from northern Europe) or very similar to them, except for the genotype no. 1, which seems to be of hybrid origin (Supplementary Table S1).

Discussion

Variation of morphological characters

We observed significant variation in the studied characters that partly reflects the genetic differences, but partly seems to be related to environmental conditions or breeding system. This plasticity must be taken into account in determination and taxonomic classification.

The studied vegetative characters are quite variable in *Batrachium*. The stem length showed large variation (from less than 10 cm to 1–2 m), which can be interpreted as a common manifestation of phenotypic plasticity in aquatic plants. However, the stem length is also obviously correlated with the growth form (prostrate vs erect), which is an important taxonomic character. Stem diameter, which well separates the *R. confervoides*/*R. trichophyllus* A group from the others, is correlated with the stem length and therefore the growth form, too. Variability of leaf length seems to correspond to taxonomic differences and/or ploidy. While *R. confervoides/R. trichophyllus* A group and *R. trichophyllus* B (both tetraploid) had rather short leaves less than 4 cm long, the pentaploid hybrids and the putative hexaploids had longer leaves; in hybrids, the longer leaves were likely inherited from the second, yet unknown parent.

Floral characters showed significant variation, too. Despite that *R. trichophyllus* is reported to form mostly chasmogamous (open) flowers and *R. confervoides* mostly cleistogamous flowers (Wiegleb et al. 2017), we observed prevailing cleistogamy in most of the genome size groups (see below). Petal length of open flowers ranged from very small (~2 mm) to medium-sized (~7 mm) (Fig. 7). Although part of this variation can be related to genetic differences (the largest flowers were found in the sterile hybrids and might be inherited from their unknown parent), the proportion of cleistogamy as well as petal length can be mainly attributed to the environment. Most of the studied plants grow submerged, which leads to more frequent autogamy/cleistogamy (as outcrossing is impossible in submerged *Batrachium* that does not have specialized hydrophilous pollen). Even if open flowers develop under water, they tend to be smaller than flowers of the same

Fig. 8. Terrestrial form of *Ranunculus confervoides* with open showy flowers growing on the emerged shore of a mountain lake with lowered water level (population K18-32, Slovenia, Krnsko jezero lake).

plant above water (personal observation). This is clearly demonstrated in *R. trichophyllus* B, which surprisingly had the smallest flowers in our dataset, while outside the Alps it has a petal length on the upper end of the *R. trichophyllus* group variation (Koutecký et al. 2022). Outside the Alps this cytotype often grows in rather shallow ponds or alluvial pools and mostly flowers above water, while in the cold and deep Alpine lakes it usually does not reach water surface and flowers mostly submerged, with cleistogamous or (more rarely) small open flowers. Similarly, when submerged open flowers develop in *R. confervoides*, they are small with petal length ~2.5–5.0 mm, but terrestrial forms growing on emerged shores of lakes with lowered water levels may develop larger showy flowers with petals up to 7 mm (Fig. 8).

Conspicuous variation was observed in fruiting pedicel length. Part of the variation can be due to taxonomic or geographic differences (*R. confervoides* from the northern Europe, the Alps, and the "intermediate" group, Fig. 7), while the long thin pedicels observed in *Batrachium* hybrids seem to be connected to their sterility regardless their parentage. This feature was observed also in various unrelated central European hybrids (Koutecký et al. 2022).

Taxonomy: Ranunculus confervoides in the Alps or not?

Wiegleb et al. (2017) state that *R. confervoides* is restricted to northern Europe while the Alpine plants traditionally classified as *R. confervoides* or *R. trichophyllus* subsp. *eradicatus* belong to variable *R. trichophyllus* s. str. Our data support the traditional classification, but also show presence of yet unknown diversity.

Five groups were distinguished within *R. trichophyllus* s.l. in the studied area based on genome size and fertility (Fig. 3). They were quite well supported also by molecular data (Fig. 5, 6). Among them, all *R. confervoides* populations (having prostrate stems and rooting at upper nodes) belong to a single genome size group, together with populations that lack one or both diagnostic characters. We marked the later plants as "intermediate", because they are morphologically more or less intermediate between *R. confervoides* and non-Alpine *R. trichophyllus* A, and we marked the whole group as *R. confervoides*/*R. trichophyllus* A. Compared to the other genome size groups, it has on average more slender (shorter and thinner) stems and shorter leaves, although variation ranges of the morphological traits partly overlap among the individual genome size groups (Fig. 7, Supplementary Table S3).

The *R. confervoides*/*R. trichophyllus* A genome size group as a whole is a quite variable assemblage in the Alps. Despite that Wiegleb et al. (2017) state that plants from Alpine lakes are more robust than the northern European ones, none of the studied quantitative characters fully discriminated the Alpine and northern *R. confervoides* nor the "intermediate" populations. Among these three subgroups, the northern European *R. confervoides* generally show the lowest, while the "intermediate" plants the highest values of the quantitative characters, but the variation ranges broadly overlap and differences are not statistically significant (Fig. 7). The subgroups partly differ only in the stem width (the "intermediate" plants being significantly different from the northern European *R. confervoides*, but the Alpine *R. confervoides* having values between the two extremes and not different from either of them) and in fruiting pedicel length (significantly shorter in the northern European plants). However, the variation of the later character seems to be underestimated because only a limited number of populations from northern Europe was available to us. Schou et al. (2023) report pedicel length 1–5 cm for the northern European *R. confervoides*, which is comparable with the range observed by us in the Alps.

Presence/absence of cleistogamous flowers, which is deemed to distinguish *R. confervoides* from *R. trichophyllus*, cannot be used to separate these taxa. Cleistogamous flowers prevailed in all three subgroups of the *R. confervoides*/*R. trichophyllus* A group. Only one population from the Alps with two flowering individuals, classified as *R. confervoides*, had only chasmogamous flowers, while in all other flowering populations and ~80% of individuals cleistogamous flowers strongly prevailed or were the only flower type present. Similarly, all populations and ~80% of individuals of *R. trichophyllus* B had only or mostly cleistogamous flowers. Chasmogamous flowers prevailed only in hybrids (but one out of six flowering populations was fully cleistogamous) and in the single population of the putative hexaploids (Supplementary Table S3).

Genetic data well discriminated the genome size groups, but did not discriminate any of the three morphological and geographical subgroups within the *R. confervoides*/*R. trichophyllus* A group. We found the same plastid haplotypes and ITS genotypes in the Alps and northern Europe, despite the limited sampling of the latter. There were no plastid haplotypes specific for the northern European *R. confervoides*. The northern European ITS genotypes did not form any separate cluster in the ITS network and the most frequent genotype no. 14 was shared between the Alps and northern Europe. The northern European and Alpine *R. confervoides* appear genetically identical. The plants morphologically "intermediate" towards *R. trichophyllus* cytotype A had plastid haplotypes identical with *R. confervoides* or very similar (haplotypes "conf-5" and "conf-7" differing in one mutation step), with one exception. The population from the Swiss Alps (SW-30) had haplotype "trich-B", which is most often found in unrelated *R. trichophyllus* cytotype B. Although it had an ITS genotype, which is the most frequent one in *R. confervoides* (no. 14), presence of a different plastid haplotype may indicate past hybridization; such incongruence between plastid DNA and the nuclear ITS region has been observed also in earlier studies (Bobrov et al. 2015, Koutecký et al. 2022). Alternatively, the "trich-B" haplotype might originate de novo from the haplotype "trich-A", most common in *R. trichophyllus* A and found also in *R. confervoides*, as these two differ only in the length of one polyA/T repeat. The ITS genotypes of the "intermediate" plants were identical to those found in *R. confervoides* (no. 6 and 14) or very similar to them (no. 12). The only specific genotype 1 that was found in one population (SW-27 from the Swiss Alps) additively combines the common *R. confervoides* genotype no. 6 with one of the central European *R. trichophyllus* A genotypes (CE-102) not found in the Alps, and is likely of hybrid origin.

In conclusion, our data do not support the view of Wiegleb et al. (2017) that *R. confervoides* is confined to northern Europe and does not occur in the Alps. We support the traditional view that *R. confervoides* has a widely disjunct distribution and occurs both in northern Europe and the Alps. We have shown that at least a part of the Alpine populations is identical to those from northern Europe in terms of genome size, chromosome count, genetic variation and morphology. Importantly, one of the sampled *R. confervoides* populations from Finland (SK-16) comes from the same lake as the plants sequenced by Bobrov et al. (2015), a paper coauthored by the same authors as the world *Batrachium* checklist (Wiegleb et al. 2017). This sample corroborates that we use the name *R. confervoides* for the same taxon.

Plants of the typical *R. confervoides* appearance, genome size and genotypes occupy a much wider range of elevations in the Alps than the usually reported subalpine and alpine zones (e.g. Aeschimann et al. 2004, Englmaier 2016, Pignatti 2017). We have found them also in the montane zone, mostly above 1,400 m a.s.l., but sometimes even below 1,000 m a.s.l., the elevational range of the sampled localities being 589–2,570 m a.s.l. Occurrence in lower elevations has already been reported by Drozdowski et al. (2015) from a part of the northern Austrian Alps; our data confirm these records, some localities being identical. The plants "intermediate" to *R. trichophyllus* A were found at a similar elevational range, 692–2,304 m a.s.l. (Supplementary Table S1).

The northern Europe-Alpine disjunction is rather frequent among terrestrial plants, while rare among the aquatic ones. Apart from *R. confervoides*, the other examples in Europe include *Stuckenia filiformis* (Pers.) Börner, *Sparganium hyperboreum* Beurl., *S. angustifolium* Michx. or *Isoëtes lacustris* L., although most of these species rarely occur also in lower European mountains and/or in clear oligotrophic waters in lower elevation in northern part of western and central Europe (Hultén & Fries 1986). Some other species are found central European mountains, but have more extensive distribution in western Europe and/or northern part of central and eastern Europe (e.g. *Myriophyllum alternifolium* DC., some *Potamogeton* species, *Isoëtes echinospora* Durieu).

Two questions remain open: (i) What is the taxonomic status of the "intermediate" populations? (ii) What are the relationships between *R. confervoides* and other taxa of the *R. trichophyllus* group?

There are two views on the classification of the “intermediate” populations. One possible view is that they should be treated within *R. confervoides*, whose morphological definition should be expanded. These populations do not substantially differ from *R. confervoides* genetically and in genome size and grow in the same habitats and elevational range. Aquatic plants are known for high level of phenotypic plasticity. Indeed, Cook (1966) mentioned morphological stability of some populations of *R. confervoides* in cultivation, while others developed forms similar to *R. trichophyllus* (“subspecies *trichophyllus*”) and conversely, some *R. trichophyllus* populations produced *R. confervoides*-like plants (“generally indistinguishable”) when cultivated in cold water. It is impossible to link Cook’s results with currently known cytotypes/genotypes of *R. trichophyllus* s.l., but it is likely that at least part of the “intermediate” populations represents this type of variation. Alternatively, the “intermediate” populations could be hybrids between *R. confervoides* and *R. trichophyllus* A or some other lineage of the *R. trichophyllus* group. The presence of hybridization is indicated by the SW-27 and SW-30 populations (see above). Although the absence of the “trich-A” haplotype in the “intermediate” populations may seemingly speak against hybridization with *R. trichophyllus* A, the pattern cannot be interpreted in this way. First, its complete absence is possible in hybrids if *R. confervoides* is always the maternal parent. Second, this haplotype is rarely found in typical *R. confervoides*, too, so even presence of the “trich-A” haplotype would not prove hybridization. The hypotheses above are not mutually exclusive and the “intermediate” morphology could result from a combination of both plasticity and hybridization. The current data do not provide enough resolution to answer the origin of the “intermediate” populations and more variable genetic markers are needed.

The phylogenetic relationships of *R. confervoides* with other *Batrachium* species remain unclear. The previous studies (Bobrov et al. 2015, Jopek et al. 2023) show that *R. confervoides* is close to some genotypes of *R. trichophyllus* (s.l.) as well as the other species – namely Eurasian *R. kauffmannii*, Asian *R. mongolicus* (Krylov) Serg. and *R. ashibetsuensis* Wiegleb – but is rather distant from other *R. trichophyllus* genotypes. This is confirmed by our study. All *R. confervoides* samples clustered in the same part of the ITS network and are close to one of the central European *R. trichophyllus* A genotypes (genotype no. 82 from Koutecký et al. 2022). The apparent similarity of *R. confervoides* with this genotype as well as identical genome size and existence of “intermediate” populations may even suggest that they could be merged into one taxon. However, we do not support this view, especially because of a certain differentiation manifested by the existence of *R. confervoides*-specific haplotypes and existence of well differentiated ITS genotypes specific for *R. trichophyllus* A (genotypes no. 102 and 85, the latter being the additive combination of genotypes no. 82 and 102). *Ranunculus trichophyllus* B is clearly different in genome size and DNA sequences and less related to *R. confervoides* than some other, morphologically very distinct species, such as the heterophyllous, large-flowered *R. peltatus* Schrank. However, due to limited variation of the markers used, the current knowledge does not allow full understanding of the phylogeny of the *Batrachium* group. More variable markers and analytical approaches taking possible reticulations through hybridization and allopolyploidy into account are needed.

Due to the unresolved phylogeny, we refrain from proposing any taxonomic and nomenclatural changes at the moment. At the present level of knowledge we suggest keeping recognizing *R. confervoides* (or *R. trichophyllus* subsp. *eradicatus* if subspecific rank is preferred) as a separate taxon occurring both in northern Europe and the Alps, owing to its specific morphology, distribution and habitat. However, once the phylogeny is resolved, the whole *R. trichophyllus* group requires taxonomic revision, typification of numerous previously published names and subsequent nomenclature revision.

Unexpected plastid DNA variation

Ranunculus confervoides shows surprisingly high haplotype diversity compared to the non-Alpine populations of the *R. trichophyllus* group. *Ranunculus confervoides* includes seven plastid haplotypes in the Alps belonging to two distinct, albeit close haplotype groups. Five of these haplotypes have not been found in the previous study in central Europe (Koutecký et al. 2022); two additional specific haplotypes were found in the "intermediate" populations. This is a conspicuous difference from the rather uniform non-Alpine populations of *R. trichophyllus* cytotypes A and B with only two haplotypes each, despite a much more extensive sampling including 89 populations (we omit the northern European *R. confervoides* from this comparison due to limited data). We hypothesize that this unexpected diversity of the Alpine plants reflects faster fixation of mutations due to small populations sizes and clonality. *Ranunculus confervoides* is predominately autogamous and confined to individual lakes, while the non-Alpine *R. trichophyllus* s.l. may form more extensive populations spreading over adjacent fishponds or alluvial pools. Certain level of clonality might be expected in all *R. trichophyllus* s.l. populations (similarly to other aquatic plants), but in *R. confervoides* with prostrate stems rooting at nodes the clonality seems to be more pronounced. Alternatively and not exclusively, the higher haplotype diversity of *R. confervoides* may result from higher age of its genotypes, which are preserved ("frozen") by frequent clonal reproduction and autogamy, while the non-alpine populations may belong to limited number of more recent genotypes that spread more effectively (see below).

The possible faster fixation of haplotypes and maintaining differences between populations of *R. confervoides* can be reinforced by isolation of the individual lakes. *Batrachium* plants can be transferred by birds between lakes (mostly seeds in this case, as transfer of vegetative fragments is negligible in most of aquatic plants; Li 2014). Within one watershed, transport is also possible by water (only downstream) or water animals such as fish (only lakes connected by suitable streams). However, especially the high-elevation lakes seem to be quite isolated due to their rather small size, rugged mountain landscape between them, and generally low productivity, making them little attractive as a food source for birds. In opposite, the non-Alpine populations of *R. trichophyllus* are much better connected both in terms of landscape structure and migration of fish and birds and certain gene flow between them is thus more likely.

Nevertheless, isolation is not complete and besides clonal reproduction, sexual reproduction including outcrossing must be present in *R. confervoides* in the Alps. The occurrence of interspecific hybrids (see below) indicates that *R. confervoides* is occasionally capable of cross-pollination. Genetic similarity between northern European and Alpine populations and the number of localities in the Alps suggest that some dispersal between the two areas and between the lakes must occasionally occur or have occurred in the past. Population genetic studies with variable markers (such as microsatellites or RAD-seq) would be needed to confirm the proposed hypotheses and to estimate the level of the current gene flow.

Hybridization of the submerged cleistogamous species?

Despite that hybridization is frequent in *Batrachium*, the species *R. confervoides* seems to be rarely involved. There are 29 interspecific hybrids reported in the world-wide checklist of *Batrachium* (Wiegleb et al. 2017). In central Europe, Koutecký et al. (2022) found nine *Batrachium* hybrids with known parentage among 12 putatively non-hybrid species and their cytotypes, and 12 additional hybrids of unclear parentage or back-crosses. Prančl et al. (2018) estimated using flow cytometry that ~15% of samples from central Europe were hybrids. Frequent occurrence or even dominance of hybrids is reported from rivers in Poland, Lithuania, and the Volga Basin (Zalewska-Gałosz et al. 2015, 2023, Butkuvienė et al. 2020, Bobrov et al. 2022). However, for the mostly cleistogamous and ecologically specialized *R. confervoides* hybridization is rather unexpected. Only one hybrid has been reported for this species so far: *R. confervoides* × *R. schmalhausenii* from Finland and Russia, based on morphology of examined herbarium specimens without any genome size or genetic data available (Bobrov et al. 2015).

Our data revealed at least three different hybrids of *R. confervoides* in the Alps. (i) Triploid plants were found at two sites in the Hallstättersee lake, Austria (populations AT-15c and AT17b; Fig. 1D). These plants were previously published as *R. circinatus* × *R. trichophyllus* A (Prančl et al. 2018; under collection no. B14-118) based on genome size only, because *R. trichophyllus* A does not differ from *R. confervoides* in the genome size and participation of the latter in hybridization was not considered. Nevertheless, the DNA sequences and new collections that allowed us to examine morphology more in detail show that *R. confervoides* is the tetraploid parent. Both populations are genetically identical. The plastid DNA does not determine the parents: both populations have a "trich-A" haplotype, which is typical for *R. trichophyllus* A, but is also rarely found in *R. confervoides* both in the Alps and northern Europe. The ITS genotype (no. 16) is an additive combination of *R. circinatus* and *R. confervoides*, with two positions specific for the latter and not found in any sample of *R. trichophyllus* A (Koutecký et al. 2022). Last, some individuals have roots on upper nodes, a character typical for *R. confervoides*. (ii) At two sites in the Hallstättersee lake, we recorded plants that have a genome size slightly higher than *R. trichophyllus* B and they were sterile if flowering (no achenes developed). Both populations (AT-16a, AT-17c) showed some characters of *R. confervoides* (prostrate growth, one individual also rooting on upper nodes) but were more robust with significantly thicker stems and longer leaves (Fig. 7, Supplementary Table S3). One of them was pentaploid. The ITS genotype (no. 2) showed an additive combination of *R. confervoides* with yet unknown sequence, which has several positions unique within the whole *R. trichophyllus* group, including a 1 bp insertion. The plastid haplotype "trich-B" is probably inherited from this unknown parent. It might be some yet unrecognized Alpine taxon/genotype inhabiting sites or habitats not sampled for the present study, such as rivers, or it might be extinct. (iii) In the Toplitzsee lake and two sites in the Hallstättersee lake, Austria, we recorded plants with similar genome size and the same or very similar ITS genotypes (no. 2 and 3) as the pentaploid hybrid described above, but with "conf-1" plastid haplotype. These plants were morphologically very similar to the hybrid described above, but had erect stems only (i.e. no prostrate growth typical of *R. confervoides*). We assume that both these hybrids have the same parental combination, while they differ in which taxon was maternal, i.e. they come from two independent hybridization events.

In addition to *R. confervoides* hybrids, we recorded two other cytotypes of hybrid origin unknown so far. The putative hexaploid population SW-13 has *R. trichophyllus* B and *R. confervoides/R. trichophyllus* A parentage (ITS genotype no. 22) and is probably a recent allohexaploid that originated from two tetraploids due to occurrence of an unreduced gamete. Another hybrid (ITS genotype no. 16) was found in the Traunsee lake, Austria (B20-011). Its parents are *R. trichophyllus* B and the unknown taxon contributing the specific ITS sequence, as mentioned above.

How can mostly submerged and cleistogamous *R. confervoides* from a specific habitat, where other *Batrachium* species usually do not co-occur, repeatedly hybridize? It might be indeed a very rare event. However, *R. confervoides* is able to grow on the wet substrate after the drop of water level in a lake. This is described from northern Europe (Schou et al. 2023) and we observed terrestrial plants rarely in the Alps. These terrestrial forms are able to produce relatively large (petals up to 7.2 mm) showy flowers (Fig. 8), which most likely allow allogamy. If more species co-occur at a site, hybridization is likely in such terrestrial conditions.

Large and deep lakes provide a unique combination of conditions that allows *Batrachium* hybridization and hybrid survival. All three *R. confervoides* hybrids were recorded in a single lake, the Hallstättersee lake; one of the hybrids was also collected in the Toplitzsee lake, which is only about 25 km upstream in the same watershed. The Hallstättersee lake is a large (8.5 km^2) and deep (mean depth 65 m, maximum 125 m) glacial lake in the Northern Limestone Alps, located at the relatively low elevation (508 m a.s.l.). It is relatively cold (mean annual surface water temperature 11.7 °C), oligotrophic, with large transparency (mean 6.5 m) (Land Oberösterreich 2024). The lake is flowed through by the Traun River and several smaller streams and has an extensive watershed over 600 km^2. During our study, we found only hybrids and *R. trichophyllus* cytotype B growing in the Hallstättersee lake, while *R. circinatus* was recorded only as floating fragments near the lake outlet (Prančl et al. 2018), i.e. brought from other parts of the lake or from the watershed upstream. The Traunsee lake, where a hybrid between the unknown taxon and *R. trichophyllus* B was recorded as floating fragments, is even larger (24 km^2) and deeper lake (mean 95 m, maximum 191 m) located downstream along the Traun river (Land Oberösterreich 2024). Neither *R. confervoides* nor the unknown second parental species were observed in any of the two lakes, but we cannot exclude their presence, as we explored only a minor fraction of the lakes. *Ranunculus confervoides* is known from numerous sites in the watershed (e.g. Drozdowski et al. 2015, and this study). Large and deep lakes like Hallstättersee and Traunsee provide a combination of habitats suitable for various *Batrachium* species, long-term stability (the conditions being similar hundreds or even thousands of years), and extensive watersheds, from which fruits and vegetative fragments can be brought. Despite that hybridization of *R. confervoides* is probably a very rare event, we hypothesize that at such sites and over long time, the temporal co-occurrence of different species and their hybridization are likely. The stable conditions then allow long-term survival and spread of the hybrids by vegetative growth, even after one or both parents disappeared from a site. Cases of such old and "orphan" hybrid clones were already documented among aquatic plants (e.g. Preston et al. 1999, Kaplan & Fehrer 2013, Kaplan et al. 2018). Alternatively (but not exclusively), the hybrids may have originated elsewhere in the watershed and have been transferred to the lakes, where they survive. Whether each of the hybrids originated once or repeatedly (i.e. how many different clones are present), and whether they are present in other large Alpine lakes, remains an open question.

Supplementary materials

Fig. S1. Geographic distribution of the plastid haplotypes.
Table S1. Locality details, genome sizes, ITS genotypes and plastid haplotypes.
Table S2. GenBank accession numbers.
Table S3. Morphological characters.

Supplementary materials are available at https://www.preslia.cz

Acknowledgements

We are grateful to all colleagues who helped us in the field and contributed with additional samples. Vojtěch Dolejšek and Alžběta Manukjanová assisted with laboratory work. We thank the Swiss database Info Flora – the National Data and Information Center on the Swiss Flora (https://www.infoflora.ch) for the *Batrachium* locality list. We also thank the reviewers for their comments and suggestions. The research was supported by grant no. 22-10464S from the Czech Science Foundation. JP, ML, MNN and ZK were also supported by long-term research development project no. RVO 67985939 of the Czech Academy of Sciences.

References

Aeschimann D., Lauber K., Moser D. M. & Theurillat J.-P. (2004) Flora Alpina. Vols. 1–3. – Haupt, Bern.

Bobrov A. A., Butkuvienė J., Chemeris E. V., Patamsytė J., Lambertini C., Aučina A., Sinkevičienė Z. & Naugžemys D. (2022) Extensive hybridization in *Ranunculus* section *Batrachium* (*Ranunculaceae*) in rivers of two postglacial landscapes of East Europe. – Scientific Reports 12: 12088.

Bobrov A. A., Zalewska-Gałosz J., Jopek M. & Movergoz E. A. (2015) *Ranunculus schmalhausenii* (section *Batrachium*, *Ranunculaceae*), a neglected water crowfoot endemic to Fennoscandia – a case of rapid hybrid speciation in postglacial environment of North Europe. – Phytotaxa 233: 101–138.

Butkuvienė J., Sinkevičienė Z., Naugžemys D., Žvingila D., Skridaila A. & Bobrov A. A. (2020) Genetic diversity of aquatic *Ranunculus* (*Batrachium*, *Ranunculaceae*) in one river basin caused by hybridization. – Plants 9: 1–17.

Clement M., Snell Q., Walke P., Posada D. & Crandall K. (2002) TCS: estimating gene genealogies. – In: Proceedings 16th International Parallel and Distributed Processing Symposium (IPDPS), 15–19 April 2002, Fort Lauderdale, FL, USA, p. 184, IEEE Computer Society, Los Alamitos.

Cook C. D. K. (1966) A monographic study of *Ranunculus* subgenus *Batrachium* (DC.) A. Gray. – Mitteilungen der Botanischen Staatssammlung München 6: 47–237.

Cook C. D. K. (1967) Emendations to A monographic study of *Ranunculus* subgenus *Batrachium* (DC.) A. Gray. – Mitteilungen der Botanischen Staatssammlung München 6: 621–622.

Cook C. D. K. (1986) *Ranunculus* subgen. *Batrachium* (DC.) A. Gray. – In: Castroviejo S., Laínz M., López-González G., Montserrat P., Muñoz Garmendia F., Paiva J. & Villar L. (eds), Flora Iberica 1: 285–298, Real Jardín Botánico, Madrid.

Cook C. D. K. (1993) *Ranunculus* subgen. *Batrachium* (DC.) A. Gray. – In: Tutin T. G., Burges N. A., Chater A. O., Edmondson J. R., Heywood V. H., Moore D. M., Valentine D. H., Walters S. M. & Webb D. A. (eds), Flora Europaea, ed. 2, 1: 285–286, Cambridge University Press, Cambridge.

Doyle J. J. & Doyle J. L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. – Phytochemical Bulletin 19: 11–15.

Drozdowski I., Mrkvicka A. C. & Mrkvicka G. F. (2015) Die Wasserpflanzenflora stehender Gewässer des steirischen Salzkammergutes (Österreich) sowie Anmerkungen zum Vorkommen von Großmuscheln, Krebsen und Amphibien. – Biodiversität und Naturschutz Österreich–BCBEA 1: 233–251.

EEA (2022) Elevation map of Europe based on GTOPO30. – European Environment Agency. URL: https://www.eea.europa.eu/data-and-maps/data/digital-elevation-model-of-europe.

Englmaier P. (2008) *Ranunculus* subg. *Batrachium*. – In: Fischer M. A., Oswald K. & Adler W. (eds), Exkursionsflora für Österreich, Liechtenstein und Südtirol, ed. 3, p. 279–282, Land Oberösterreich, OÖ Landesmuseen, Linz.

Englmaier P. (2016) *Ranunculus* sect. *Batrachium* (*Ranunculaceae*): Contribution to an excursion flora of Austria and the Eastern Alps. – Neilreichia 8: 97–125.

Hall T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucleic Acids Symposium Series 41: 95–98.

Hörandl E. & Emadzade K. (2012) Evolutionary classification: a case study on the diverse plant genus *Ranunculus* L. (*Ranunculaceae*). – Perspectives in Plant Ecology, Evolution and Systematics 14: 310–324.

Hultén E. & Fries M. (1986) Atlas of North European vascular plants north of the Tropic of Cancer. Vols. 1–3. – Koelz Scientific Books, Königstein.

Huson D. H. & Bryant D. (2006) Application of phylogenetic networks in evolutionary studies. – Molecular Biology and Evolution 23: 254–267.

Jopek M., Wiegleb G., Babik W. & Zalewska-Gałosz J. (2023) *Ranunculus dahlgreniae* (section *Batrachium*, *Ranunculaceae*), a new species from Crete, Greece, with remarks on taxonomy and phylogenetic relations within the section. – Acta Societatis Botanicorum Poloniae 92: 167462.

Kaplan Z., Danihelka J., Chrtek J., Zázvorka J., Koutecký P., Ekrt L., Řepka R., Štěpánková J., Jelínek B., Grulich V., Prančl J. & Wild J. (2019) Distributions of vascular plants in the Czech Republic. Part 8. – Preslia 91: 257–368.

Kaplan Z. & Fehrer J. (2013) Molecular identification of hybrids from a former hot spot of *Potamogeton* hybrid diversity. – Aquatic Botany 105: 34–40.

Kaplan Z., Fehrer J., Bambasová V. & Hellquist C. B. (2018) The endangered Florida pondweed (*Potamogeton floridanus*) is a hybrid: why we need to understand biodiversity thoroughly. – PLoS ONE 13: 1–20.

King R. A., Gornall R. J., Preston C. D. & Croft J. M. (2001) Molecular confirmation of *Potamogeton* × *bottnicus* (*P. pectinatus* × *P. vaginatus*, *Potamogetonaceae*) in Britain. – Botanical Journal of the Linnean Society 135: 67–70.

Koutecký P., Prančl J., Košnar J., Koutecká E., Hanzlíčková J., Lučanová M., Nejedlá M. & Kaplan Z. (2022) Waking up from a taxonomist's nightmare: emerging structure of *Ranunculus* section *Batrachium* (*Ranunculaceae*) in central Europe based on molecular data and genome sizes. – Botanical Journal of the Linnean Society 198: 417–437.

Land Oberösterreich (2024) Land Oberösterreich. Umwelt und Natur. Kurzprofile oberösterreichischer Seen. – URL: https://www.land-oberoesterreich.gv.at/139145.htm.

Lauber K., Wagner G. & Gygax A. (2018) Flora Helvetica – Illustrierte Flora der Schweiz. Ed. 6. – Haupt, Bern.

Leigh J. W. & Bryant D. (2015) POPART: full-feature software for haplotype network construction. – Methods in Ecology and Evolution 6: 1110–1116.

Li W. (2014) Environmental opportunities and constraints in the reproduction and dispersal of aquatic plants. – Aquatic Botany 118: 62–70.

Luferov A. N. (2004) Taksonomicheskii konspekt lyutikovykh (*Ranunculaceae*) Dal'nego Vostoka Rossii [A taxonomic synopsis of *Ranunculaceae* of the Far East of Russia]. – Turczaninowia 7: 5–85.

Pignatti S. (ed.) (2017) Flora d'Italia. Vols. 1–4. Ed. 2. – Edagricole, Milano.

Pizzaro J. (1995) Contribución al estudio taxonómico de *Ranunculus* L. subgen. *Batrachium* (DC.) A. Gray (*Ranunculaceae*). – Lazaroa 15: 21–113.

POWO (2024) Plants of the World Online. – Royal Botanic Gardens, Kew. URL: https://powo.science.kew.org.

Prančl J. & Kaplan Z. (2019) *Ranunculus* sect. *Batrachium* – lakušníky. – In: Kaplan Z., Danihelka J., Chrtek J., Kirschner J., Kubát K., Štech M. & Štěpánek J. (eds), Klíč ke květeně České republiky [Key to the flora of the Czech republic], Ed. 2, p. 363–367, Academia, Praha.

Prančl J., Koutecký P., Lučanová M., Nagy Nejedlá M., Brožová V., Dolejšek V., Košnar J., Trávníček P. & Kaplan Z. (2025) Identifying the richness and evolutionary relationships of *Ranunculus* sect. *Batrachium* in its diversity centre in south-western Europe. – Scientific Reports 15: 14008.

Prančl J., Koutecký P., Trávníček P., Jarolímová V., Lučanová M., Koutecká E. & Kaplan Z. (2018) Cytotype variation, cryptic diversity and hybridization in *Ranunculus* sect. *Batrachium* revealed by flow cytometry and chromosome numbers. – Preslia 90: 195–223.

Preston C. D., Hollingsworth P. M. & Gornall R. J. (1999) The distribution and habitat of *Potamogeton* ×*suecicus* K. Richt. (*P. filiformis* Pers. × *P. pectinatus* L.) in the British Isles. – Watsonia 22: 329–342.

QGIS Development Team (2020) QGIS Geographic Information System. – Open Source Geospatial Foundation Project. URL: https://qgis.org.

R Core Team (2021) R: a language and environment for statistical computing. – R Foundation for Statistical Computing, Vienna. URL: https://www.R-project.org.

Schönswetter P., Suda J., Popp M., Weiss-Schneeweiss H. & Brochmann C. (2007) Circumpolar phylogeography of *Juncus biglumis* (*Juncaceae*) inferred from AFLP fingerprints, cpDNA sequences, nuclear DNA content and chromosome numbers. – Molecular Phylogenetics and Evolution 42: 92–103.

Schou J. C., Bjarne M., van de Weyer K., Lansdown R., Wiegleb G., Holm P., Baastrup-Spohr L. & Sand-Jensen K. (2023) Aquatic plants of northern and central Europe including Britain and Ireland. – Princeton University Press, Princeton & Oxford.

Shaw J., Lickey E. B., Schilling E. E. & Small R. L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. – American Journal of Botany 94: 275–288.

Štorchová H., Hrdličková R., Chrtek J., Tetera M., Fitze D. & Fehrer J. (2000) An improved method of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution. – Taxon 49: 79–84.

Tison J.-M. & de Foucault B. (2014) Flora Gallica – Flore de France. – Biotope Editions, Mèze.

Villesen P. (2007) FaBox: an online toolbox for FASTA sequences. – Molecular Ecology Notes 7: 965–968.

White T. J., Bruns T. D., Lee S. & Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis M. A., Gelfand D. H., Sninsky J. J. & White T. J. (eds), PCR protocols: a guide to methods and applications, p. 315–322, Academic Press, New York.

Wiegleb G. (2011) Wasserhahnenfuß – *Ranunculus* subgen. *Batrachium*. – In: Jäger E. J. (ed.), Rothmaler – Exkursionsflora von Deutschland. Gefäßpflanzen: Grundband, ed. 20, p. 317–321, Spektrum Akademischer Verlag, Heidelberg.

Wiegleb G. (2021) *Ranunculus* sect. *Batrachium*. – In: Müller F., Ritz C. M., Welk E. & Wesche K. (eds), Rothmaler – Exkursionsflora von Deutschland. Gefäßpflanzen: Grundband, ed. 22, p. 328–332, Springer Spektrum, Berlin & Heidelberg.

Wiegleb G., Bobrov A. A. & Zalewska-Gałosz J. (2017) A taxonomic account of *Ranunculus* section *Batrachium* (*Ranunculaceae*). – Phytotaxa 319: 1–55.

Wiegleb G., Desfayes M. & Bobrov A. A. (2022) *Ranunculus oblitus* (section *Batrachium*, *Ranunculaceae*), a new species from South America. – Phytotaxa 559: 132–144.

Zalewska-Gałosz J., Jopek M. & Ilnicki T. (2015) Hybridization in *Batrachium* group: controversial delimitation between heterophyllous *Ranunculus penicillatus* and the hybrid *Ranunculus fluitans* × *R. peltatus*. – Aquatic Botany 120: 160–168.

Zalewska-Gałosz J., Kwiatkowska M., Prančl J., Skubała K., Lučanová M., Gebler D. & Szoszkiewicz K. (2023) Origin, genetic structure and evolutionary potential of the natural hybrid *Ranunculus circinatus* × *R. fluitans*. – Scientific Reports 13: 1–15.

Neočekávaná diverzita v alpských jezerech: genetická variabilita, hybridizace a taxonomie skupiny lakušníku niťolistého (*Ranunculus trichophyllus*)

Skupina lakušníku niťolistého (*Ranunculus trichophyllus* s.l.) má složitou evoluční historii a zahrnuje několik genotypů, jejichž taxonomické zhodnocení není dosud dořešeno. Ve střední Evropě mimo Alpy se podle předchozích studií vyskytují dva tetraploidní cytotypy, označované jako *R. trichophyllus* A a B, které se liší ve velikosti genomu a v sekvencích DNA. Další ekologicky specializovaný tetraploidní taxon náležící do této skupiny, *R. confervoides* (*R. trichophyllus* subsp. *eradicatus*), je udáván ze severní Evropy, Alp a Pyrenejí a z hor a severních oblastí Asie a Severní Ameriky. Příslušnost populací k tomuto taxonu byla ale nedávno mimo severní Evropu zpochybněna. Studovali jsme variabilitu skupiny *R. trichophyllus* ve 35 jezerech v Alpách a 5 jezerech v severní Evropě. Měřili jsme velikost genomu, sekvenovali ITS úsek jaderné DNA a dva nekódující úseky plastidové DNA a zaznamenávali 10 morfologických znaků. V Alpách jsme nalezli populace odpovídající *R. confervoides*, které se od severoevropských morfologicky nelišily, měly stejnou velikost genomu a sdílely s nimi plastidové haplotypy i genotypy v ITS úseku. Na základě toho usuzujeme, že severoevropské i alpské populace patří ke stejnému druhu, *R. confervoides*. Zároveň jsme objevili populace, které kombinují morfologické znaky *R. confervoides* a *R. trichophyllus* A (druhý jmenovaný jsme ale v Alpách nenalezli). Identita těchto rostlin je nejasná. Může jít o křížence, ale částečně také o projev morfologické plasticity některého z uvedených taxonů. Celkem jsme u *R. confervoides* a „přechodných“ populací nalezli 9 plastidových haplotypů, zatímco u obou cytotypů *R. trichophyllus* mimo Alpy byly nalezeny pouze dva u každého. Tento překvapivě vysoký počet haplotypů mohl vzniknout díky rychlejší fixaci mutací v alpských populacích, které jsou navzájem izolované a převládá v nich klonální rozmnožování. Mohl by také indikovat větší stáří druhu *R. confervoides* ve srovnání s *R. trichophyllus* s.l. mimo Alpy. V jezerech na úpatí Alp jsme zaznamenali tři dosud neznámé křížence: triploidního *R. circinatus* × *R. confervoides*, pentaploidního křížence *R. confervoides* s dosud neznámým druhem a křížence *R. trichophyllus* B se stejným neznámým druhem. Dále jsme nalezli jednu pravděpodobně alohexaploidní populaci vzniklou z *R. confervoides* a *R. trichophyllus* B. Vzhledem k ekologické specializovanosti a převažující kleistogamii je opakovaná hybridizace *R. confervoides* překvapivá. Tento druh je ale schopen na obnažených březích jezer vytvářet terestrické formy, které mají nápadné otevřené (chasmogamické) květy. Domníváme se, že za takových podmínek a při společném výskytu s dalšími lakušníky může docházet k hybridizaci. Kříženci pak mohou vegetativně přežívat velmi dlouhou dobu, případně se i dále šířit v rámci povodí.

How to cite: Koutecký P., Prančl J., Lučanová M., Brožová V., Nagy Nejedlá M., Košnar J., Koutecká E., Englmaier P. & Kaplan Z. (2025) Striking diversity in Alpine lakes: genetic variation, hybridization and taxonomy of the aquatic *Ranunculus trichophyllus* group. – Preslia 97: 191–216.

Preslia, a journal of the Czech Botanical Society

https://www.preslia.cz