Preslia 97: 637–658, 2025
doi: 10.23855/preslia.2025.637

Preslia

Rare interspecific hybridization at the diploid level in *Ranunculus* sect. *Batrachium*

Zdeněk Kaplan[1,2,*], Bohumil Trávníček[3], Jan Prančl[1] & Petr Koutecký[4]

[1]Czech Academy of Sciences, Institute of Botany, Zámek 1, CZ-25243 Průhonice, Czech Republic; [2]Department of Botany, Faculty of Science, Charles University, Benátská 2, CZ-12800 Prague, Czech Republic; [3]Department of Botany, Palacký University in Olomouc, Faculty of Science, Šlechtitelů 27, CZ-78371 Olomouc, Czech Republic; [4]Faculty of Science, University of South Bohemia, Branišovská 1760, CZ-37005 České Budějovice, Czech Republic
*corresponding author: kaplan@ibot.cas.cz

Abstract: Hybridization and polyploidization are significant evolutionary mechanisms. Allopolyploidy, the formation of polyploids through hybridization between distinct species followed by whole-genome duplication, is common mainly in plants, where it facilitates rapid speciation and ecological diversification. Hybridization between diploid species often results in F_1 hybrids with reduced fertility due to irregular meiosis. However, subsequent genome duplication can restore fertility by providing homologous chromosome pairs. Understanding the initial hybridization between diploids is therefore crucial for elucidating the early stages of allopolyploid formation. *Ranunculus* sect. *Batrachium* is known for the frequent occurrence of hybrids and polyploids, many of which are allopolyploid. Surprisingly, no unequivocal natural hybrid between diploid species has been discovered until recently in spite of the large body of evidence on interspecific hybridization in this group. This study provides evidence for hybridization at the diploid level, based on an integrated morphological, cytological and molecular approach. A new interspecific hybrid *R. circinatus* × *R. rionii* was detected in two flooded sand pits in the Czech Republic and described as *R.* ×*limnophilus*. Maternal inheritance patterns indicated independent, reciprocal origin of the hybrids at each locality. The ecological and historical context of the hybrid's emergence is discussed, including colonization of a newly formed habitat that allowed coexistence of the two parental species, their hybridization and the subsequent vegetative persistence of the hybrid despite its sterility. Similar conditions may have prevailed during the early postglacial period, facilitating the formation of allopolyploids, which today constitute the majority of species diversity in this group. A review and categorization of the biotypes that constitute the complex diversity in *Ranunculus* sect. *Batrachium* is also provided.

Keywords: *Batrachium*, Czech Republic, flow cytometry, genome size, hybridization, molecular identification, morphology, taxonomy

Introduction

Hybridization has an important role in plant evolution (Barton 2001, Rieseberg et al. 2003, Abbott et al. 2016). Although many hybrids are sterile and short-lived, the consequences of hybridization vary greatly and may result in various effects on the involved

Received: 9 Jun 2025; Revised: 30 Sep 2025; Accepted: 3 Oct 2025; Published: 11 Dec 2025

populations. On one hand, introgressive hybridization can lead to a reduction or loss of differentiation between independent evolutionary lineages (Mallet 2005, Abbott et al. 2013, Harrison & Larson 2014) as well as genetic erosion of the parental gene pools (Linder & Rieseberg 2004). This can be a serious threat to rare species with small, isolated populations, which may be introgressed by widespread species (Wolf et al. 2001, Čertner et al. 2020) or even by invasive species (Huxel 1999, Guo 2014).

In other cases, hybridization can produce evolutionary innovations (Anderson & Stebbins 1954, Arnold 1997). It increases genetic variation by producing new combinations of alleles (Leal-Bertioli et al. 2018) and the incorporated alleles may offer opportunities for adaptation and enhance the fitness of hybrids (Rieseberg & Wendel 1993, Barton 2001, Seehausen 2004, Suarez-Gonzalez et al. 2018, Wong et al. 2022). Hybridization can also generate new species, a process known as hybrid speciation (Arnold 1992, Rieseberg et al. 2003, Abbott et al. 2010, Schumer et al. 2014). If hybridization is followed by polyploidization (i.e. allopolyploidization), instant reproductive isolation between different ploidies is established (Soltis & Soltis 2009, Soltis et al. 2014). At the same time, hybrid fertility is ensured due to the presence of two complete chromosome sets, which enables the pairing of homologous chromosomes during meiosis (Stebbins 1958). Reproductive isolation coupled with restored fertility facilitates the establishment of new species (Soltis & Soltis 2009).

Hybridization and polyploidization have shaped the evolutionary history of numerous aquatic plants (Les & Philbrick 1993). Hybrids are recorded in e.g. *Callitriche* (Prančl et al. 2020), *Myriophyllum* (Moody & Les 2002, Sturtevant et al. 2009), *Najas* (Les et al. 2010), *Nuphar* (Arrigo et al. 2016), *Nymphaea* (Kabátová et al. 2014, Dąbrowska et al. 2015), *Potamogeton* (Kaplan & Fehrer 2009, 2013, Zalewska-Gałosz et al. 2009, 2010), *Stuckenia* (Fehrer et al. 2022) and *Utricularia* (Bobrov et al. 2022b). The frequency of hybridization and its effect on speciation vary considerably between groups of aquatic plants. For example, in *Callitriche*, hybrids are relatively rare (Prančl et al. 2020), whereas in *Potamogeton*, the number of hybrids substantially exceeds the number of species (Kaplan et al. 2018) and the hybrid diversity even includes a triple hybrid (Kaplan & Fehrer 2007). While all the hybrids in *Callitriche* and *Stuckenia* are sterile and can succeed only locally by long-term vegetative persistence at the site of origin or within a watercourse (Prančl et al. 2020, Fehrer et al. 2022), *Utricularia ×neglecta*, a hybrid between *U. tenuicaulis* and *U. vulgaris*, has become an evolutionarily successful taxon that has colonized a large part of Europe and also occurs in Western Siberia (Bobrov et al. 2022b).

Hybridization is also frequent in *Ranunculus* section *Batrachium* (hereafter referred to as *Batrachium*). The occurrence of interspecific hybrids in this group has long been recognized based on morphology and experimental crossing (Félix 1912, 1915, 1927, Cook 1966, Dahlgren 1991, Hong 1991). However, only recent molecular studies (Bobrov et al. 2015, 2022a, Zalewska-Gałosz et al. 2015, 2023, Butkuvienė et al. 2020, Gebler et al. 2022, Koutecký et al. 2022, 2025, Prančl et al. 2025) have revealed the actual extent of hybridization. The diversity in *Batrachium* is further complicated by polyploidy. Several ploidy levels have been documented (Cook 1962, 1966, Prančl et al. 2018), and recent findings revealed a total of eight distinct ploidy levels (Prančl et al. 2025), most of which are of allopolyploid origin (Koutecký et al. 2022, Prančl et al. 2025).

In addition, some of the currently recognized species include cryptic lineages that differ in their genetic profiles and genome sizes (Prančl et al. 2018, 2025, Koutecký et al. 2022, 2025). The overall diversity in *Batrachium* thus exhibits a very complex pattern that requires operational categorization. Any recognizable group of individuals that share identical or very similar genotypes, cytotypes and phenotypes, and differ from other recognizable groups by characteristic sets of traits, may be referred to as a 'biotype'.

Based on the evolutionary history of individual lineages, their ploidy levels, viability, fertility and frequency of hybridization, the following groups of biotypes may be distinguished in *Batrachium*: (i) basic diploid species (e.g. *R. circinatus*, *R. rionii*, diploid cytotype of *R. fluitans*, Cook 1962, 1966, Prančl et al. 2018), (ii) autopolyploids (e.g. autotriploid cytotype of *R. fluitans*, Prančl et al. 2018, Koutecký et al. 2022, Zalewska-Gałosz et al. 2023), (iii) primary hybrids between diploid species (e.g. *R. circinatus* × *R. fluitans*, Bobrov et al. 2022a, Gebler et al. 2022, Zalewska-Gałosz et al. 2023), (iv) old, stabilized allopolyploids (e.g. *R. penicillatus*, Cook 1966; *R. schmalhausenii*, Bobrov et al. 2015), (v) primary homoploid hybrids between polyploid species (e.g. *Ranunculus peltatus* × *R. trichophyllus*, Prančl et al. 2018), (vi) primary heteroploid hybrids (e.g. *R. aquatilis* × *R. peltatus*, Prančl et al. 2018; *R. aquatilis* × *R. circinatus*, Bobrov et al. 2022a), and (vii) backcross hybrids and introgressants (e.g. *Ranunculus peltatus* × *R. trichophyllus*, *R. penicillatus* × *R. peltatus*, Prančl et al. 2018, Koutecký et al. 2022).

The different types of hybrids vary greatly in their fertility and subsequent development, and consequently they have significantly different roles in evolution (Koutecký et al. 2022, Prančl et al. 2025). The most striking contrast is between true interspecific hybrids (biotype groups iii and v–vii) and species of hybrid origin (hybridogenous species, mainly allopolyploids, biotype group iv). The hybrids are recurrently produced in mixed populations of two or more species and usually exhibit intermediate morphology and reduced fertility to complete sterility. Their genome sizes combine those of the gametes (either reduced or unreduced) from their parents, and their ITS regions contain both parental copies (at least in primary hybrids). In addition, the hybrid individuals are mostly restricted to the water bodies where they originated, and their existence is often only temporary (although in rivers and large lakes they may persist vegetatively for long periods and may also disperse downstream in rivers; Prančl et al. 2018, Kaplan et al. 2019b, Koutecký et al. 2025). In contrast, the hybridogenous species mostly originated long ago, are fertile and sexually self-reproducing, often have developed specific morphology and genome size, concerted evolution of ITS has (at least partly) homogenized their parental copies, and these species have spread and established their own geographical ranges. Following taxonomic tradition in *Batrachium* (Cook 1966, Wiegleb et al. 2017), also adopted in our previous studies (Prančl et al. 2018, 2025, Koutecký et al. 2022, 2025), the former are referred to as hybrids and designated using hybrid formulae or the multiplication sign in their binomials, whereas the latter are treated as species.

The complexity of diversity and hybrid evolution in *Batrachium* may be further increased by multiple hybridization (Bobrov et al. 2022a). Many hybrids are at least partially fertile (Cook 1966, 1970, Dahlgren 1991) and may hybridize with other species, resulting in complex hybrids such as polyhybrids (hybrids resulting from crosses between three or more distinct parental lineages) and double hybrids (hybrids formed by crossing two different hybrids, thereby integrating genomes from up to four different species). When hybridization is followed by polyploidization, new allopolyploids can arise, which may subsequently participate in additional hybridization events, giving rise to evolutionary entities of exceptionally complex origin. These phenomena underscore the intricate evolutionary history of *Batrachium*. Deciphering the origins of such complex hybrids and identifying all parental genotypes remains an extremely challenging task.

Cook (1966, 1970) assumed that all *Batrachium* species are potentially capable of hybridization. However, based on crossing experiments and field observations, Cook (1966) and Hong (1991) concluded that diploid taxa hybridize only rarely. Wiegleb et al. (2017) listed hybrids for most European species, but only one between the (generally) diploid species (*R. circinatus* × *R. fluitans*; however, this hybrid is actually triploid, Koutecký et al. 2022, Zalewska-Gałosz et al. 2023), and, for example, no hybrid involving the diploid *R. rionii* was known. Only recently, two intermediate plants randomly collected from mixed populations of *R. circinatus* and *R. rionii* were detected and identified as hybrids between these species (Prančl et al. 2018, Koutecký et al. 2022).

Given the growing body of evidence on interspecific hybridization in recent *Batrachium* studies, it is somewhat surprising that among the detected hybrids, diploids have generally not been found. Yet it is hybridization between diploid species that plays a crucial role in the evolution of *Batrachium*, which has resulted in complex hybridization networks and numerous allopolyploid species. Hybridization followed by genome duplication is more common in the emergence of allopolyploids than when genome duplication precedes hybridization. This is because F_1 hybrids between species often produce unreduced gametes at relatively high rates. This sequence effectively overcomes the reproductive barriers inherent in interspecific hybrids (Ramsey & Schemske 1998). It is therefore of great importance to investigate hybridization between diploid species in *Batrachium* in detail. The aims of this study are: (i) to explore mixed populations of *R. circinatus* and *R. rionii* and document their diversity, (ii) to identify the samples using flow cytometry and DNA sequencing, (iii) to compare the samples with all similar central-European species, (iv) to characterize the hybrids morphologically, and (v) to provide their formal taxonomic description.

Methods

Plant material

The occurrence of mixed populations of *R. circinatus*, *R. rionii* and morphologically intermediate plants are reported at two sites in the Morava river basin in central Moravia, Czech Republic. These sites are former sand pits, now filled with groundwater and forming large permanent lakes. One is the Hulínská pískovna sand pit near the town of Hulín (Fig. 1), the other is the Kvasická pískovna sand pit, located between the villages of Kvasice and Tlumačov (Fig. 2). Plants suspected to be hybrids were first observed at these sites in 2016. We surveyed the aquatic vegetation at both lakes in 2019 and collected samples representing the morphological variation of the local *Batrachium* diversity. Additional samples of the parental species, as well as of the similar species *R. trichophyllus* and *R. aquatilis*, were collected during previous studies in various parts of central Europe (Prančl et al. 2018, Koutecký et al. 2022), and a representative selection was used here for comparison. The samples of *R. trichophyllus* included both previously distinguished cytotypes A and B (Prančl et al. 2018, Koutecký et al. 2022, 2025). Locality details are

Fig. 1. Mixed *Ranunculus* sect. *Batrachium* population in the Hulínská pískovna sand pit.

Fig. 2. Stands of *Potamogeton nodosus*, *Ranunculus* ×*limnophilus* and its parental species in the Kvasická pískovna sand pit.

Table 1. List of samples, with locality details, vouchers, genome sizes, ITS genotypes, plastid haplotypes and GenBank accession numbers. Ratio – ratio of flow cytometry fluorescence intensity of the sample to that of the internal standard *Bellis perennis*. 2C – genome size calculated based on *Bellis perennis* 2C = 3.38 pg. In the last three columns, GenBank accession numbers are listed. * – adopted from Koutecký et al. (2022). Continued on page 643; for correct alignment, view in two-page mode (with cover page displayed).

Taxon	Ref. no.	Locality	Coordinates (WGS 84)	Collected
R. aquatilis	K16-24-1	CZ, distr. Kroměříž, Hulín: lake in Hulínská pískovna sand pit, E bank	49°17'59"N, 17°28'09"E	30 V 2016, B. Trávníček
R. circinatus	ZK19/83	CZ, distr. Kroměříž, Kvasice: lake in Kvasická pískovna sand pit	49°14'56.7"N, 17°29'03.5"E	19 VI 2019, Z. Kaplan & B. Trávníček
	ZK19/84	CZ, distr. Kroměříž, Kvasice: lake in Kvasická pískovna sand pit	49°14'56.7"N, 17°29'03.5"E	19 VI 2019, Z. Kaplan & B. Trávníček
	ZK19/86	CZ, distr. Kroměříž, Hulín: lake in Hulínská pískovna sand pit, at S bank	49°17'47.2"N, 17°26'58.8"E	19 VI 2019, Z. Kaplan & B. Trávníček
R. rionii	B16-035	CZ, distr. Kroměříž, Hulín: lake in Hulínská pískovna sand pit, at S bank	49°17'47"N, 17°26'56"E	18 VI 2016, B. Trávníček
	ZK19/81	CZ, distr. Kroměříž, Kvasice: lake in Kvasická pískovna sand pit	49°14'56.7"N, 17°29'03.5"E	19 VI 2019, Z. Kaplan & B. Trávníček
	ZK19/85	CZ, distr. Kroměříž, Kvasice: lake in Kvasická pískovna sand pit	49°14'56.7"N, 17°29'03.5"E	19 VI 2019, Z. Kaplan & B. Trávníček
R. ×limnophilus = *R. circinatus* × *R. rionii*	B16-036-1	CZ, distr. Kroměříž, Hulín: lake in Hulínská pískovna sand pit, at S bank	49°17'47"N, 17°26'56"E	18 VI 2016, B. Trávníček
	B16-036-2	CZ, distr. Kroměříž, Kvasice: lake in Kvasická pískovna sand pit	49°14'57"N, 17°29'03"E	7 VIII 2016, B. Trávníček
	ZK19/82	CZ, distr. Kroměříž, Kvasice: lake in Kvasická pískovna sand pit	49°14'56.7"N, 17°29'03.5"E	19 VI 2019, Z. Kaplan & B. Trávníček

summarized in Table 1. Voucher specimens are deposited in the herbaria PRA and OL. Nomenclature, taxonomic concepts and species delimitations in *Batrachium* follow Koutecký et al. (2022) and for the other vascular plants Kaplan et al. (2019a).

Morphology

All main characters used to distinguish *R. aquatilis*, *R. circinatus*, *R. rionii* and *R. trichophyllus* (Englmaier 2016, Prančl et al. 2018, Prančl & Kaplan 2019, Hanzlíčková 2021, Koutecký et al. 2022) were recorded for the plant samples collected in the Hulínská pískovna and Kvasická pískovna sand pits. All measurements were done on herbarium material. The variation in individual characters assessed across pooled individuals of each recognized taxon is presented in Table 2 and compared with character values obtained from previous morphological studies. Preliminary morphological identifications of the samples were subsequently verified by flow cytometry and DNA sequencing.

Flow cytometry

Genome size was estimated using flow cytometry, following the methods described in Prančl et al. (2018). Propidium iodide was used as the stain, and *Bellis perennis* served as the internal standard (2C = 3.38 pg; Schönswetter et al. 2007).

Voucher	Ratio	2C (pg)	ITS genotype	cp haplotype	ITS	*rpl*32–*trn*L^{UAG}	3'*rps*16–5'*trn*K
OL	–	–	21	pelt	PV875564	PV932428	PV932429
PRA	1.68	5.68	3	circ	PV873166	PV932416	PV932422
PRA	1.64	5.54	3	circ	PV873167	PV932417	PV932423
PRA	1.69	5.72	3	circ	PV873168	PV932418	PV932424
PRA	1.59	5.39	–	–	–	–	–
PRA	1.60	5.40	1	rion	PV873169	PV932419	PV932425
PRA	1.62	5.46	1	rion	PV873170	PV932420	PV932426
PRA	1.63	5.51	3+1	rion	PV875563	MW448114*	–
OL	–	–	3+1	circ	MW430775*	MW448113*	MW448079*
PRA	1.66	5.59	3+1	circ	PV873171	PV932421	PV932427

DNA sequencing

DNA was extracted from silica-dried leaves or from herbarium specimens of the same plants that were analysed by flow cytometry. The methods were the same as reported in Koutecký et al. (2022). Briefly, DNA was extracted using a CTAB protocol. Three DNA regions were amplified: the ITS region of the nuclear ribosomal DNA and two non-coding plastid spacers, *rpl*32–*trn*L^{UAG} and 3'*rps*16–5'*trn*K. Sanger sequencing was done by Eurofins Genomics. The ITS region was sequenced in one direction in all samples and in both directions in the samples showing intra-individual polymorphisms and/or genotypes not previously recorded; consensus of both sequences was then used. The *rpl*32–*trn*L^{UAG} spacer was sequenced in two parts from the internal primers, while the 3'*rps*16–5'*trn*K in only one direction. Intra-individual polymorphisms in the ITS sequences were coded using IUPAC ambiguity codes; because the individual ITS copies were not sequenced using molecular cloning or NGS technologies, these sequences are referred to as ‘genotypes’ instead of ‘ribotypes’. Designations of individual ITS genotypes and plastid haplotypes follow those reported in previous studies (Koutecký et al. 2022, 2025, Prančl et al. 2025).

Table 2. Variation in key diagnostic morphological characters of *Ranunculus circinatus*, *R. rionii* and their hybrid collected in this study (rows 1–3), compared with values from selected central-European morphological studies (rows 4–21). Continued on page 645; for correct alignment, view in two-page mode (with cover page displayed).

Taxon	Origin	Data source	Characters			
			Capillary leaves			
			firmness	shape	length (cm)	spatial arrangement of segments
R. circinatus	Kvasice and Hulín	this study	rigid	circular to semicircular	0.8–1.7	lying in one plane
R. rionii	Kvasice and Hulín	this study	± flaccid	suborbicular to obconical	1.4–3.9	divergent, not lying in one plane
R. circinatus × *R. rionii*	Kvasice	this study	± rigid	suborbicular to obconical	1.0–2.2	divergent, not lying in one plane
R. aquatilis	Czech Republic	Prančl & Kaplan 2019	± flaccid	suborbicular to obconical		spreading in various directions
	central Europe	Prančl et al. 2018, Koutecký et al. 2022	mostly flaccid		2–6	
	central Europe	Hanzlíčková 2021			1.2–4.2(–6.7)	
	Eastern Alps	Englmaier 2016	flaccid		2–5	
R. circinatus	Czech Republic	Prančl & Kaplan 2019	rigid	circular to semicircular	0.8–2(–3.2)	arranged in one plane
	central Europe	Prančl et al. 2018, Koutecký et al. 2022	rigid	circular to semicircular	1–3	lying in one plane
	Eastern Alps	Englmaier 2016	rigid		(1–)1.5–2.5	
R. rionii	Czech Republic	Prančl & Kaplan 2019	± flaccid	suborbicular to obconical		spreading in various directions
	central Europe	Prančl et al. 2018, Koutecký et al. 2022	mostly flaccid		1–3	
	central Europe	Hanzlíčková 2021			(1.0–)1.9–2.5 (–6.5)	
	Eastern Alps	Englmaier 2016	flaccid		< 2	
R. trichophyllus A	Czech Republic	Prančl & Kaplan 2019	± flaccid	suborbicular to obconical		spreading in various directions
	central Europe	Prančl et al. 2018, Koutecký et al. 2022	flaccid		1–3	
	central Europe	Hanzlíčková 2021			(0.9–)1.9–2.8 (–3.7)	
R. trichophyllus B	Czech Republic	Prančl & Kaplan 2019	± flaccid	suborbicular to obconical		spreading in various directions
	central Europe	Prančl et al. 2018, Koutecký et al. 2022	mostly flaccid		1–5	
	central Europe	Hanzlíčková 2021			(1.0–)2.1–3.2 (–5.0)	
R. trichophyllus	Eastern Alps	Englmaier 2016	flaccid		(2–)3–5	

	Pedicels	Petal	Nectar pit	Receptacle	Achenes		
relative length to the adjacent internode	length (cm) at fruit time / after flowering	length (mm)	shape	elongation after flowering	number per receptacle after anthesis	indumentum	length (mm)
0.1–0.8	3.3–13.2	6.5–8.5	lunate	not elongating	24–37	sparsely hairy	1.1–1.2
0.3–1.0	1.1–2.5	3.7–4.3	lunate	somewhat elongating	48–85	glabrous	1.0–1.1
0.1–0.6	2.0–6.6	4.1–6.3	lunate	not elongating	0–32	sparsely hairy	1.1–1.2
usually shorter	usually < 5	4–10	circular				1.3–2
usually shorter	1–6	4–10	circular	not elongating	~20–50	hairy (at least when young)	
usually shorter	(1.9–)2.6–4.4 (–11.8)	(3.5–)4.7–6.7 (–9.0)	circular or lunate		(8–)13–44 (–52)	hairy	(1.4–)1.5–1.8 (–2.3)
shorter			circular	not elongating	30–40	± hairy	1.4–1.7
usually 0.2–0.5		(5–)6–10	lunate, rarely circular				
markedly shorter	2–10	6–10	lunate	not elongating	~20–50	hairy (at least when young)	
markedly shorter			lunate	not elongating	40–50	hairy	1.4–1.7
shorter			lunate, rarely pyriform	somewhat elongating	~25–70 (–100)	glabrous	0.8–1.3
shorter	1–3	2–5	lunate, rarely pyriform	somewhat elongating	~30–80	glabrous	
	(1.1–)1.9–2.5 (–3.6)	(1.7–)3.2–4.1 (–5.5)	lunate or pyriform		(12–)38–68 (–85)	glabrous	0.9–1.1
shorter			lunate	elongating	60–80(–100)	± glabrous	1–1.4
shorter	1–3	2–4	lunate	not elongating	~10–30	hairy	1–1.5
shorter	1–3	2–4	lunate	not elongating	~10–30	hairy	
mostly shorter	(1.0–)2.0–3.0 (–4.6)	(1.7–)2.9–4.2 (–5.8)	lunate		(10–)19–27 (–37)	hairy	(1.1–)1.3–1.5 (–1.7)
shorter	1.4–4.5	3.5–6.5(–7.5)	lunate	not elongating	~10–50	hairy	1–1.8
shorter	1.5–5	3.5–7	lunate	not elongating	~15–40	hairy	
	(0.9–)1.7–2.9 (–5.2)	(2.6–)3.6–5.0 (–6.6)	lunate, rarely circular		(8–)16–29 (–63)	hairy	(1.3–)1.4–1.6 (–2.0)
shorter			lunate	not elongating	15–30	hairy	1.5–1.7

Results

Morphological assessment

Only two *Batrachium* taxa, *R. circinatus* and *R. rionii*, were found near Hulín in 2019. The intermediate plants sampled there in 2016 were not rediscovered. Near Kvasice, three morphological groups of *Batrachium* plants were recorded and distinguished: *R. circinatus*, *R. rionii* and plants with ± intermediate morphology, both in 2016 and 2019.

The two species differed mainly in the shape and size of their leaves, length of pedicels, size of petals and number and size of achenes in the aggregate fruit (Table 2). Although there is considerable overlap in leaf length and shapes, and flexibility of the leaves was difficult to assess precisely, pedicel length, petal length and number of achenes clearly distinguish the plants of the two species.

The putative hybrid (Figs 3–4) occupies mostly intermediate positions in these characters (Table 2), particularly in flower size (Fig. 4C). However, in some features the hybrid resembles one parental species more than the other. Leaf shape, with segments spreading in various directions and forming a three-dimensional structure (Fig. 4B) is more similar to that of *R. rionii*. In contrast, the ratio of leaf length to the length of the adjacent internode greatly overlaps that of *R. circinatus* (Table 2).

Plants of all three groups flowered abundantly near Kvasice. While those of the putative parental species produced numerous well-developed achenes indicating a high fruit set, flowers of the intermediate plants appeared to be sterile. Their carpels were mostly aborted shortly after anthesis; only in a few flowers up to 32 young carpels were produced per receptacle (Fig. 4D). However, these presumably did not develop into mature achenes containing seeds, as no fully developed achenes were recorded for any of the hybrid plants. A closer inspection of several undeveloped achenes revealed that they were hollow, lacking any seed tissue.

Of the two other similar species, *R. aquatilis* and *R. trichophyllus*, only the former was found in these lakes, specifically near Hulín. However, it did not grow directly in the lake, where *R. circinatus* and *R. rionii* occurred, but at a single site, on a wet muddy bank. The other species, *R. trichophyllus*, used to be very rare in this part of central Moravia and was last recorded there in 1957 (Kaplan et al. 2019b). It was not found during the current fieldwork. These two species are best distinguished from *R. circinatus* and *R. rionii* by floral and fruit characters (Table 2). Nonetheless, their involvement in the origin of the putative hybrids cannot be ruled out based solely on morphology.

Genome size variation and differentiation

The genome sizes of all the plants from both lakes indicate a diploid level (Table 1, Fig. 5). The values identified in samples of *R. circinatus* and *R. rionii* fall within the previously identified ranges of variation in these species. The entire variation ranges of these species in Central Europe are similar and partly overlapping. The genome size of the morphologically intermediate plants from Hulín and Kvasice are consistent with the hypothesis that they are hybrids between *R. circinatus* and *R. rionii* and are diploids. Importantly, the involvement of *R. trichophyllus* and *R. aquatilis* is ruled out, as these species are tetraploid and hexaploid, respectively, with much higher genome sizes than

Fig. 3. A single large, richly branched plant from the Kvasická pískovna sand pit, which was used to prepare the type collection of the name *Ranunculus* ×*limnophilus*.

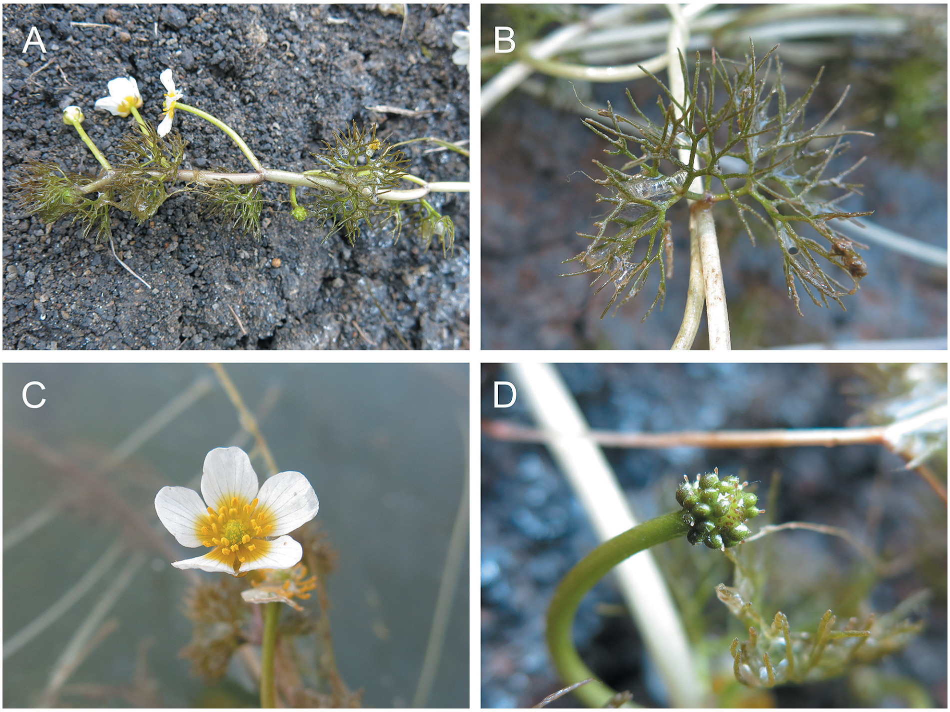

Fig. 4. Details of *Ranunculus* ×*limnophilus*: (A) apical part of a flowering branch, (B) leaf, (C) flower and (D) receptacle of a post-anthesis flower with undeveloped achenes.

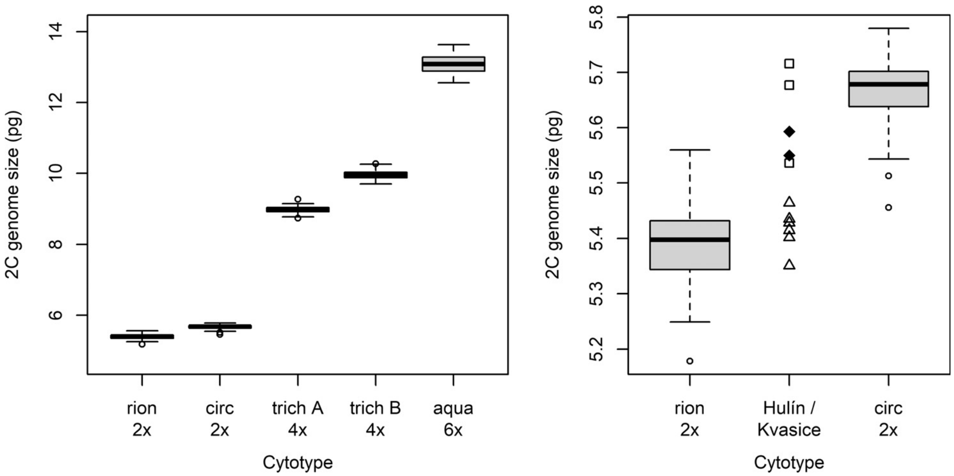

Fig. 5. Boxplots showing ranges in the variation in holoploid genome sizes (2C-values) of *Ranunculus aquatilis*, *R. circinatus*, *R. rionii*, *R. trichophyllus* A and *R. trichophyllus* B in central Europe as identified in previous studies (Prančl et al. 2018, Koutecký et al. 2022), compared with values of plants sampled near Hulín and Kvasice (□ *R. circinatus*, △ *R. rionii*, ◆ *R. circinatus* × *R. rionii*).

those identified in the putative hybrids. However, due to the partial overlap in genome sizes between the parental species, genome size data alone cannot unequivocally confirm the hybrid origin of the morphologically intermediate plants.

Variation in ITS and molecular identification

The alignment of the ITS region prepared for the central-European samples of *R. aquatilis*, *R. circinatus*, *R. rionii* and *R. trichophyllus* and all samples from the two sand pits was 670 bp long and contained 30 polymorphic positions (Table 3). All the *Batrachium* species were clearly differentiated based on species-specific polymorphisms and combinations of polymorphisms. Comparison of the ITS sequences obtained from all three samples of the putative hybrids with those of all potential parental species allowed unambiguous identification of *R. circinatus* and *R. rionii* as the parental species. The parental genotypes involved in hybridization correspond to the most common central-European variants of these species (genotypes no. 3 and 1 in Koutecký et al. 2022). Specifically, the diagnostic positions 114, 130, 256, 264, 483 and 574 consistently showed additive sequence patterns of these two species, while the sequences displayed no polymorphisms at other sites. In contrast, the morphologically similar *R. aquatilis*, *R. trichophyllus* A and *R. trichophyllus* B can all be excluded as parents, particularly due to diagnostic positions 162, 163, 248, 279, 509 and 572 in the alignment, which consistently contained bases absent from the hybrids.

Variation in the cpDNA region and identification of the maternal parent

Two plastid regions were sequenced and analysed. The alignment of 3'*rps*16–5'*trn*K was 593 bp long and contained eight polymorphic sites, including four single-nucleotide substitutions and four indels (Table 4). However, the parental species *R. circinatus* and

Table 3. Variable positions in the alignment of ITS sequences from samples of *Ranunculus* sect. *Batrachium* collected in this study (rows 1–9), compared with central-European genotypes of the four most similar species (rows 10–18) as distinguished in Koutecký et al. (2022). Polymorphic nucleotide sites are coded using the IUPAC code.

Taxon	Sample ref. no.	Genotype	Position in alignment																													
			71	97	114	119	121	128	130	132	150	162	163	168	189	234	256	264	266	267	478	479	483	509	510	525	541	561	572	574	587	595
R. rionii	ZK19/81		C	G	A	C	T	T	T	C	C	A	C	T	G	C	C	G	G	C	A	T	T	T	T	C	G	C	A	A	C	C
R. rionii	ZK19/85		C	G	A	C	T	T	T	C	C	A	C	T	G	C	C	G	G	C	A	T	T	T	T	C	G	C	A	A	C	C
R. circinatus × *R. rionii*	B16-036-2		C	G	R	C	K	T	Y	C	C	A	C	T	G	C	Y	S	G	C	A	T	K	T	T	C	G	C	A	M	C	C
R. circinatus × *R. rionii*	B16-036-1		C	G	R	C	K	T	Y	C	C	A	C	T	G	C	Y	S	G	C	A	T	K	T	T	C	G	C	A	M	C	C
R. circinatus × *R. rionii*	ZK19/82		C	G	R	C	K	T	Y	C	C	A	C	T	G	C	Y	S	G	C	A	T	K	T	T	C	G	C	A	M	C	C
R. circinatus	ZK19/83		C	G	G	C	G	T	C	C	C	A	C	T	G	C	T	C	G	C	A	T	G	T	T	C	G	C	A	C	C	C
R. circinatus	ZK19/84		C	G	G	C	G	T	C	C	C	A	C	T	G	C	T	C	G	C	A	T	G	T	T	C	G	C	A	C	C	C
R. circinatus	ZK19/86		C	G	G	C	G	T	C	C	C	A	C	T	G	C	T	C	G	C	A	T	G	T	T	C	G	C	A	C	C	C
R. aquatilis	K16-24-1		C	G	G	C	G	T	Y	Y	Y	W	Y	Y	G	C	Y	C	R	Y	R	Y	G	C	T	Y	G	Y	W	C	C	C
R. circinatus		3	C	G	G	C	G	T	C	C	C	A	C	T	G	C	T	C	G	C	A	T	G	T	T	C	G	C	A	C	C	C
R. circinatus		11	C	G	G	C	G	T	C	C	C	A	C	T	G	C	T	C	G	Y	A	T	G	T	T	C	G	C	A	C	C	C
R. rionii		1	C	G	A	C	T	T	T	C	C	A	C	T	G	C	C	G	G	C	A	T	T	T	T	C	G	C	A	A	C	C
R. trichophyllus A		82	T	G	A	C	G	C	C	C	C	T	T	T	A	C	C	C	G	T	G	C	G	C	C	C	A	C	T	C	C	C
R. trichophyllus A		85	Y	R	A	C	G	Y	C	C	C	T	T	T	R	Y	C	C	G	T	G	C	G	C	C	C	A	C	T	C	C	C
R. trichophyllus A		102	C	A	A	C	G	T	C	C	C	T	T	T	G	T	C	C	G	T	G	C	G	C	C	C	A	C	T	C	C	C
R. trichophyllus B		162	C	G	G	Y	G	T	T	T	T	T	T	T	G	C	C	C	G	T	G	C	G	C	T	T	G	C	T	C	Y	Y
R. aquatilis		15	C	G	G	C	G	T	Y	Y	Y	W	Y	Y	G	C	Y	C	R	Y	R	Y	G	C	T	Y	G	C	W	C	C	C
R. aquatilis		21	C	G	G	C	G	T	Y	Y	Y	W	Y	Y	G	C	Y	C	R	Y	R	Y	G	C	T	Y	G	Y	W	C	C	C

Table 4. Variable positions in the plastid alignment of 3'*rps*16–5'*trn*K sequences from samples of *Ranunculus* sect. *Batrachium* collected in this study (rows 1–8), compared with central-European haplotypes of the four most similar species (rows 9–15) as distinguished in Koutecký et al. (2022). In mononucleotide repeat regions, the present base and repeat length are indicated.

Taxon	Sample ref. no.	Haplotype	Position in alignment							
			175	259–269	270	275	422	498–505	512–517	518
R. rionii	ZK19/81		T	T(11)	G	C	C	A(8)	– – – – – –	T
R. rionii	ZK19/85		T	T(11)	G	C	C	A(8)	– – – – – –	T
R. circinatus × *R. rionii*	B16-036-2		T	T(11)	G	C	C	A(8)	– – – – – –	T
R. circinatus × *R. rionii*	ZK19/82		T	T(11)	G	C	C	A(8)	– – – – – –	T
R. circinatus	ZK19/83		T	T(11)	G	C	C	A(8)	– – – – – –	T
R. circinatus	ZK19/84		T	T(11)	G	C	C	A(8)	– – – – – –	T
R. circinatus	ZK19/86		T	T(11)	G	C	C	A(8)	– – – – – –	T
R. aquatilis	K16-24-1		C	T(9)	G	C	A	A(7)	AATTTT	A
R. circinatus		circ	T	T(11)	G	C	C	A(8)	– – – – – –	T
R. circinatus		circ-2	T	T(10)	G	C	C	A(8)	– – – – – –	T
R. rionii		rion	T	T(11)	G	C	C	A(8)	– – – – – –	T
R. rionii		rion-2	T	T(10)	G	C	C	A(8)	– – – – – –	T
R. aquatilis		pelt	C	T(9)	G	C	A	A(7)	AATTTT	A
R. trichophyllus A		trich-A	C	T(11)	–	T	A	A(7)	– – – – – –	A
R. trichophyllus B		trich-B	C	T(10)	–	T	A	A(7)	– – – – – –	A

R. rionii do not differ in this region, and it therefore cannot be used to identify the maternal parent. Nevertheless, it provides additional support for the exclusion of *R. aquatilis*, *R. trichophyllus* A and *R. trichophyllus* B, as these have haplotypes that consistently differ from those found in all the hybrid individuals.

The alignment of sequences of the *rpl*32–*trn*L^{UAG} intergenic spacer was 877 base pairs long and highly variable, including 28 polymorphic sites, four of which were indels (Table 5). The two parental species of the hybrids consistently differ by two single-nucleotide polymorphisms at positions 103 and 664, and by the length of the polyA repeat at positions 109–118. These differences allowed identification of the maternal species of the two hybrid plants from Kvasice as *R. circinatus*, whereas that of the hybrid from Hulín as *R. rionii* (haplotypes 'circ' and 'rion' from Koutecký et al. 2022, respectively).

Table 5. Variable positions in the plastid alignment of *rpl*32–*trn*L^{UAG} sequences from samples of *Ranunculus* sect. *Batrachium* collected in this study (rows 1–9), compared with central-European haplotypes of the four most similar species (rows 10–16) as distinguished in Koutecký et al. (2022). In mononucleotide repeat regions, the present base and repeat length are indicated.

Taxon	Sample ref. no.	Haplotype	Position in alignment																											
			103	109–118	158	253	306–309	430	458	479	480–486	487	513	525	550	584	595	603	663	664	675	686	728	740	750	751	790	800	810	866
R. rionii	ZK19/81		G	T(10)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	C	C	A	C	A	A	T	A	A	A	T
R. rionii	ZK19/85		G	T(10)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	C	C	A	C	A	A	T	A	A	A	T
R. circinatus × *R. rionii*	B16-036-2		T	T(9)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	A	C	A	C	A	A	T	A	A	A	T
R. circinatus × *R. rionii*	B16-036-1		G	T(10)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	C	C	A	C	A	A	T	A	A	A	T
R. circinatus × *R. rionii*	ZK19/82		T	T(9)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	A	C	A	C	A	A	T	A	A	A	T
R. circinatus	ZK19/83		T	T(9)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	A	C	A	C	A	A	T	A	A	A	T
R. circinatus	ZK19/84		T	T(9)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	A	C	A	C	A	A	T	A	A	A	T
R. circinatus	ZK19/86		T	T(9)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	A	C	A	C	A	A	T	A	A	A	T
R. aquatilis	K16-24-1		T	T(9)	C	C	ATAT	C	A	A	T(6)	G	G	C	G	G	C	C	G	A	A	C	C	G	G	G	G	G	G	C
R. circinatus		circ	T	T(9)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	A	C	A	C	A	A	T	A	A	A	T
R. circinatus		circ-2	T	T(9)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	A	C	A	C	A	A	T	A	A	A	T
R. rionii		rion	G	T(10)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	C	C	A	C	A	A	T	A	A	A	T
R. rionii		rion-2	G	T(10)	A	T	----	T	A	T	T(7)	–	A	G	T	G	A	T	T	C	C	A	C	A	A	T	A	A	A	T
R. aquatilis		pelt	T	T(9)	C	C	ATAT	C	A	A	T(6)	G	G	C	G	G	C	C	G	A	A	C	C	G	G	G	G	G	G	C
R. trichophyllus A		trich-A	T	T(9)	C	C	ATAT	C	C	A	T(6)	G	G	C	G	A	A	C	G	A	A	C	G	G	G	G	G	G	G	C
R. trichophyllus B		trich-B	T	T(9)	C	C	ATAT	C	C	A	T(6)	G	G	C	G	A	A	C	G	A	A	C	G	G	G	G	G	G	G	C

Discussion

A combination of morphological, cytometric and molecular data demonstrated that the morphologically intermediate plants from the Hulínská pískovna and Kvasická pískovna sand pits are a previously unknown hybrid *R. circinatus* × *R. rionii*. All other *Batrachium* species can be excluded as potential parents as they differ in morphology, DNA sequences and genome size. Both the parental species were present at both localities together with the hybrid plants. Although numerous hybrids have already been identified in *Batrachium*, either through traditional methods involving field observations and morphological comparison or through modern analytical approaches such as flow cytometry and DNA sequencing, this represents the first unequivocally confirmed diploid hybrid in *Batrachium* in central Europe. While hybrids between *R. circinatus* (diploid) and *R. fluitans* (diploid or triploid) were recently confirmed using DNA sequencing in Germany (Koutecký et al. 2022), Poland (Gebler et al. 2022) and Lithuania (Bobrov et al. 2022a), the German and Polish plants were identified as triploid (Koutecký et al. 2022, Zalewska-Gałosz et al. 2023) and the ploidy level of the Lithuanian plants remains unknown. Only recently, a diploid hybrid was discovered in a single mixed population of two newly recognized cryptic species, tentatively designated *R. peltatus* B and *R. peltatus* C, in southern Spain (Prančl et al. 2025).

Batrachium diversity exhibits a complex pattern composed of well-defined diploid species, autopolyploids, allopolyploids, cryptic species, primary hybrids and introgressants (Koutecký et al. 2022). During interspecific hybridization, the probability that a hybrid individual will arise through the involvement of an unreduced gamete is significantly higher compared to intraspecific crossing (Kato et al. 2008, Mason & Pires 2015). This is due to reproductive barriers encountered during interspecific hybridization, which may be bypassed through unreduced gametes. The frequent production of unreduced gametes contributes substantially to the formation of allopolyploids, which is recognized as a major mechanism of plant speciation. Most *Batrachium* species are polyploid (Prančl et al. 2018, 2025, Koutecký et al. 2022), and recent molecular studies (Bobrov et al. 2015, Zalewska-Gałosz et al. 2015, Koutecký et al. 2022, Prančl et al. 2025) indicate their hybridogenous origin. Allopolyploidization therefore appears to be the predominant mode of speciation in *Batrachium*. Within this context, interspecific crossing resulting in a diploid hybrid is a notable exception.

The identified haplotypes in the two hybrid clones from the two lakes revealed different donors of plastid DNA: *R. circinatus* near Kvasice and *R. rionii* near Hulín. This demonstrates that hybridization occurred independently in the two lakes via reciprocal crosses. The occurrence of at least two successful hybridization events within a small geographic area over a short period of time raises the question of whether this interspecific hybrid is truly rare, or whether it has simply remained undetected until now.

The parental species have relatively wide distributions. *Ranunculus circinatus* is found mainly in the temperate zone of Eurasia, from Ireland and France in the west to easternmost Siberia in the east (Cook 1966). *Ranunculus rionii* is distributed in Europe (primarily central and south-eastern regions) and extends into western and central Asia as far as China (Cook 1966, Wiegleb et al. 2017). Although their ranges overlap, frequent co-occurrence may be restricted to a few areas such as central Europe. Elsewhere, these species tend to be ecologically differentiated: the perennial *R. circinatus* typically occurs in deep, permanent water bodies, whereas the annual *R. rionii* is more often found in warmer regions and frequently inhabits shallow-water habitats that may dry out in summer. Even in central Europe, their co-occurrence is very rare: only five out of 640 sites sampled in our previous studies (Prančl et al. 2018, Koutecký et al. 2022). Besides the Hulín and Kvasice sites, these include two other flooded sand pits (one in Moravia near the village of Spytihněv, ~12 km south of Kvasice; one in south-western Hungary near the village of Tótszerdahely) and one shallow fishpond (near the village of Třebařov, Czech Republic). At none of the three additional sites were intermediate plants recorded; morphology and genome size of all samples were consistent with the two species. Flooded sand pits may provide a suitable combination of ecological factors that enable the co-occurrence of both species and promote hybrid formation and persistence. Given the morphological similarity to the parental species, it is possible that the hybrids may be more frequent in mixed populations than previously recognized. In fact, several hybrids reported in our studies (Prančl et al. 2018, 2025, Koutecký et al. 2022, 2025) were only discovered by large-scale population screenings. Morphological identification of hybrids of aquatic plants is often difficult and can be misleading (e.g. Kaplan & Fehrer 2011, Kaplan et al. 2018, 2019c, Zalewska-Gałosz et al. 2018, Prančl et al. 2020). Therefore, any suspected hybrid should be verified using molecular analysis.

Sand and gravel extraction near Kvasice began in the early 1960s and ended in the 1970s, when the Hulínská pískovna sand pit was established. Aerial photographs in the mapping portal mapy.com show that the size of the Kvasice lake has remained unchanged since 2001, while the Hulín lake continues to increase in area due to ongoing extraction. The Kvasická pískovna sand pit is an important ornithological site. Water birds have gradually introduced numerous aquatic species of plants to both lakes. In addition to the *Batrachium* taxa discussed, the following aquatic plants are recorded there: *Lemna minor*, *L. trisulca*, *Persicaria amphibia*, *Potamogeton crispus*, *P. nodosus*, *P. pusillus*, *Spirodela polyrhiza*, *Stuckenia pectinata*, *Sparganium erectum*, *Typha latifolia*, *Rorippa amphibia*, *Ranunculus sceleratus*, *Rumex hydrolapathum* and *Zannichellia palustris*. Both parental species of the new *Batrachium* hybrid are known to occur in this part of the Morava river basin and are known to inhabit flooded sand and gravel pits in early stages of terrestrialization (Kaplan et al. 2019b). It is likely that these conditions facilitated colonization of the new lakes.

In addition, one more ecological factor may have favoured hybrid formation. As previously shown for other aquatic plants (Kaplan et al. 2002, Kaplan & Fehrer 2004), hybridization is more likely to occur in new water bodies than in old ponds. First, recently flooded habitats often offer freely available nutrients conducive to seedling establishment, unlike old water bodies where thick layers of sapropelic sediment, often toxic due to anaerobic conditions, may inhibit growth. Second, clear water in new aquatic environments promotes germination of hybrid seeds lying on the bottom, a process impeded in old eutrophic ponds with turbid water. Third, small seedlings in newly formed habitats face less competition from fast growing perennials. Fourth, young hybrid plants are less likely to be eaten by herbivores in a young artificial lake than in a commercial pond stocked with herbivorous fish.

The favourable conditions in both lakes support not only the formation of hybrids but also their long-term persistence. While *R. rionii* appears to be an annual that dies shortly after flowering, *R. circinatus* prefers permanent aquatic habitats where, particularly in deep water, it often persists vegetatively for a long time. The hybrids seem to have inherited this trait. Despite being sterile, the hybrid clone has persisted in the Kvasice sand pit for several years, spreading by shoot fragments along the lake edge. This vegetative propagation compensates for the reduced fitness of the hybrids due to their sterility.

Similar conditions may have occurred during the early postglacial period, when numerous new aquatic habitats emerged, the ranges of species shifted, and previously isolated lineages occasionally came into contact. Such circumstances could have facilitated interspecific hybridization and the subsequent formation of allopolyploids, which today constitute the majority of European *Batrachium* diversity.

FLORA OF THE CZECH REPUBLIC

Ranunculus* ×*limnophilus
Z. KAPLAN, TRÁVN., P. KOUTECKÝ et PRANČL
[= ***R. circinatus*** × ***R. rionii***]

HOLOTYPE

Moravia: Distr. Kroměříž: shallow edge of lake in Kvasická pískovna sand pit 1 km NE of Kvasice; alt. 181 m.
Coordinates (WGS 84): 49°14'56.7"N, 17°29'03.5"E

Coll. Z. Kaplan & B. Trávníček 19/82 19 VI 2019

Fig. 6. Holotype of *Ranunculus* ×*limnophilus*.

Taxonomic conclusions

***Ranunculus ×limnophilus* Z. Kaplan, Trávn., P. Koutecký et Prančl, nothosp. nova**
= *R. circinatus* Sibth. × *R. rionii* Lagger

Ranunculus ×limnophilus is intermediate between its parental species, *R. circinatus* and *R. rionii*, in most of its characters. It can be distinguished from *R. circinatus* mainly by shorter petals (4.1–6.3 mm long) and the leaf segments not lying in one plane, spreading in different directions and forming a three-dimensional structure. From *R. rionii* it differs by longer pedicels (2.0–6.6 cm long after flowering) and larger flowers. From both its parents it differs in its sterility. Its carpels mostly abort shortly after anthesis, or a small proportion may initiate development but apparently do not mature into achenes containing seeds.

Etymology: The epithet limnophilus is derived from the Greek noun limnē (lake) and adjective philos (loving), which refers to the occurrence of the hybrid in sand pit lakes.

Type: Czech Republic, Moravia, distr. Kroměříž: shallow edge of lake in Kvasická pískovna sand pit 1 km NE of Kvasice, 49°14'56.7"N, 17°29'03.5"E (WGS 84), alt. 181 m, 19 VI 2019, coll. Z. Kaplan & B. Trávníček no. 19/82 (holotype: PRA 24022, Fig. 6; isotypes: PR 1000001, PRA 24021).

Additional specimens (paratypes): Czech Republic, Moravia, distr. Kroměříž: southern edge of lake in Hulínská pískovna sand pit 1.9 km SW of Hulín, emerged on wet substrate, 49°17'47"N, 17°26'56"E (WGS 84), alt. 185 m, 18 VI 2016, coll. B. Trávníček, in herb. J. Prančl no. B16-036 (PRA); Czech Republic, Moravia, distr. Kroměříž: southeastern edge of lake in Kvasická pískovna sand pit 1 km NE of Kvasice, 49°14'57"N, 17°29'03"E (WGS 84), 7 VIII 2016, coll. B. Trávníček (OL); Czech Republic, Moravia, distr. Kroměříž: southern edge of lake in Kvasická pískovna sand pit 0.8 km NE of Kvasice, 49°14'52"N, 17°28'58"E (WGS 84), 7 VIII 2016, coll. B. Trávníček (OL).

Acknowledgements

We are grateful to Michaela Nagy Nejedlá and Vojtěch Dolejšek for assistance with molecular analyses and Pavel Trávníček for help with flow cytometric analyses. This research was supported by grant no. 22-10464S from the Czech Science Foundation. ZK and JP were also supported by long-term research development project no. RVO 67985939 of the Czech Academy of Sciences.

References

Abbott R., Albach D., Ansell S., Arntzen J. W., Baird S. J. E., Bierne N., Boughman J. W., Brelsford A., Buerkle C. A., Buggs R., Butlin R. K., Dieckmann U., Eroukhmanoff F., Grill A., Cahan S. H., Hermansen J. S., Hewitt G., Hudson A. G., Jiggins C., Jones J., Keller B., Marczewski T., Mallet J., Martinez-Rodriguez P., Möst M., Mullen S., Nichols R., Nolte A. W., Parisod C., Pfennig K., Rice A. M., Ritchie M. G., Seifert B., Smadja C. M., Stelkens R., Szymura J. M., Väinölä R., Wolf J. B. W. & Zinner D. (2013) Hybridization and speciation. – Journal of Evolutionary Biology 26: 229–246.

Abbott R. J., Barton N. H. & Good J. M. (2016) Genomics of hybridization and its evolutionary consequences. – Molecular Ecology 25: 2325–2332.

Abbott R. J., Hegarty M. J., Hiscock S. J. & Brennan A. C. (2010) Homoploid hybrid speciation in action. – Taxon 59: 1375–1386.

Anderson E. & Stebbins G. L. (1954) Hybridization as an evolutionary stimulus. – Evolution 8: 378–388.

Arnold M. L. (1992) Natural hybridization as an evolutionary process. – Annual Review of Ecology and Systematics 23: 237–261.

Arnold M. L. (1997) Natural hybridization and evolution. – Oxford University Press, Oxford.

Arrigo N., Bétrisey S., Graf L., Bilat J., Gerber E. & Kozlowski G. (2016) Hybridization as a threat in climate relict *Nuphar pumila* (*Nymphaeaceae*). – Biodiversity and Conservation 25: 1863–1877.

Barton N. H. (2001) The role of hybridization in evolution. – Molecular Ecology 10: 551–568.

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

Bobrov A. A., Volkova P. A., Kopylov-Guskov Y. O., Mochalova O. A., Kravchuk A. E. & Nekrasova D. M. (2022b) Unknown sides of *Utricularia* (*Lentibulariaceae*) diversity in East Europe and North Asia or how hybridization explained old taxonomical puzzles. – Perspectives in Plant Ecology, Evolution and Systematics 54: 125649.

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.

Čertner M., Kolář F., Frajman B., Winkler M. & Schönswetter P. (2020) Massive introgression weakens boundaries between a regionally endemic allopolyploid and a widespread congener. – Perspectives in Plant Ecology, Evolution and Systematics 42: 125502.

Cook C. D. K. (1962) Studies on *Ranunculus* L. subgenus *Batrachium* (DC.) A. Gray. I. Chromosome numbers. – Watsonia 5: 123–126.

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. (1970) Hybridization in the evolution of *Batrachium*. – Taxon 19: 161–166.

Dąbrowska M. A., Rola K., Volkova P., Suda J. & Zalewska-Gałosz J. (2015) Genome size and phenotypic variation of *Nymphaea* (*Nymphaeaceae*) species from Eastern Europe and temperate Asia. – Acta Societatis Botanicorum Poloniae 84: 277–286.

Dahlgren G. (1991) Karyological investigations in *Ranunculus* subg. *Batrachium* (*Ranunculaceae*) on the Aegean Islands. – Plant Systematics and Evolution 177: 193–211.

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

Fehrer J., Nagy Nejedlá M., Hellquist C. B., Bobrov A. A. & Kaplan Z. (2022) Evolutionary history and patterns of geographical variation, fertility, and hybridization in *Stuckenia* (*Potamogetonaceae*). – Frontiers in Plant Science 13: 1042517.

Félix A. (1912) Études monographiques sur les Renoncules françaises de la section *Batrachium*. IV. – Bulletin de la Société Botanique de France 59: 112–120.

Félix A. (1915) Études monographiques sur les Renoncules françaises de la section *Batrachium*. V. – Bulletin de la Société Botanique de France 61 (‘1914’): 352–355, plates VII–VIII.

Félix A. (1927) Etudes monographiques sur les Renoncules françaises de la section *Batrachium* (suite). – Bulletin de la Société Botanique de France 74: 277–280.

Gebler D., Zalewska-Gałosz J., Jopek M. & Szoszkiewicz K. (2022) Molecular identification and habitat requirements of the hybrid *Ranunculus circinatus* × *R. fluitans* and its parental taxa *R. circinatus* and *R. fluitans* in running waters. – Hydrobiologia 849: 2999–3014.

Guo Q. (2014) Plant hybridization: the role of human disturbance and biological invasion. – Diversity and Distributions 20: 1345–1354.

Hanzlíčková J. (2021) Zhodnocení kryptické diverzity ve skupině lakušníku niťolistého (*Ranunculus trichophyllus* agg.) [Evaluation of cryptic diversity in the group of thread-leaved water-crowfoot (*Ranunculus trichophyllus* agg.)]. – Master thesis, Charles University, Praha.

Harrison R. G. & Larson E. L. (2014) Hybridization, introgression, and the nature of species boundaries. – Journal of Heredity 105: 795–809.

Hong D.-Y. (1991) A biosystematic study of *Ranunculus* subgenus *Batrachium* in S Sweden. – Nordic Journal of Botany 11: 41–59.

Huxel G. R. (1999) Rapid displacement of native species by invasive species: effects of hybridization. – Biological Conservation 89: 143–152.

Kabátová K., Vít P. & Suda J. (2014) Species boundaries and hybridization in central-European *Nymphaea* species inferred from genome size and morphometric data. – Preslia 86: 131–154.

Kaplan Z., Danihelka J., Chrtek J. jun., Kirschner J., Kubát K., Štech M. & Štěpánek J. (eds) (2019a) Klíč ke květeně České republiky [Key to the flora of the Czech Republic]. Ed. 2. – Academia, Praha.

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

Kaplan Z. & Fehrer J. (2004) Evidence for the hybrid origin of *Potamogeton* ×*cooperi* (*Potamogetonaceae*): traditional morphology-based taxonomy and molecular techniques in concert. – Folia Geobotanica 39: 431–453.

Kaplan Z. & Fehrer J. (2007) Molecular evidence for a natural primary triple hybrid in plants revealed from direct sequencing. – Annals of Botany 99: 1213–1222.

Kaplan Z. & Fehrer J. (2009) An orphaned clone of *Potamogeton* ×*schreberi* in the Czech Republic. – Preslia 81: 387–397.

Kaplan Z. & Fehrer J. (2011) Erroneous identities of *Potamogeton* hybrids corrected by molecular analysis of plants from type clones. – Taxon 60: 758–766.

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: e0195241.

Kaplan Z., Fehrer J. & Jobson R. W. (2019c) Discovery of the Northern Hemisphere hybrid *Potamogeton* ×*salicifolius* in the Pilbara region of Western Australia. – Telopea 22: 141–151.

Kaplan Z., Plačková I. & Štěpánek J. (2002) *Potamogeton* ×*fluitans* (*P. natans* × *P. lucens*) in the Czech Republic. II. Isozyme analysis. – Preslia 74: 187–195.

Kato J., Ohashi H., Ikeda M., Fujii N., Ishikawa R., Horaguchi H., Amano J., Hayashi M. & Mii M. (2008) Unreduced gametes are the major causal factor for the production of polyploid interspecific hybrids in *Primula*. – Plant Biotechnology 25: 521–528.

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.

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 (*Ranunculaceae*). – Preslia 97: 191–216.

Leal-Bertioli S. C. M., Godoy I. J., Santos J. F., Doyle J. J., Guimarães P. M., Abernathy B. L., Jackson S. A., Moretzsohn M. C. & Bertioli D. J. (2018) Segmental allopolyploidy in action: increasing diversity through polyploid hybridization and homoeologous recombination. – American Journal of Botany 105: 1053–1066.

Les D. H. & Philbrick C. T. (1993) Studies of hybridization and chromosome number variation in aquatic angiosperms: evolutionary implications. – Aquatic Botany 44: 181–228.

Les D. H., Sheldon S. P. & Tippery N. P. (2010) Hybridization of hydrophiles: natural interspecific hybrids in *Najas* (*Hydrocharitaceae*). – Systematic Botany 35: 736–744.

Linder C. R. & Rieseberg L. H. (2004) Reconstructing patterns of reticulate evolution in plants. – American Journal of Botany 91: 1700–1708.

Mallet J. (2005) Hybridization as an invasion of the genome. – Trends in Ecology & Evolution 20: 229–237.

Mason A. S. & Pires J. C. (2015) Unreduced gametes: meiotic mishap or evolutionary mechanism? – Trends in Genetics 31: 5–10.

Moody M. L. & Les D. H. (2002) Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. – Proceedings of the National Academy of Sciences of the United States of America 99: 14867–14871.

Prančl J., Fehrer J., Caklová P., Bambasová V., Lučanová M. & Kaplan Z. (2020) Intricate evolutionary history of *Callitriche* (*Plantaginaceae*) taxa elucidated by a combination of DNA sequencing and genome size. – Taxon 69: 1016–1041.

Prančl J. & Kaplan Z. (2019) *Ranunculus* sect. *Batrachium* – lakušník. – In: Kaplan Z., Danihelka J., Chrtek J. jun., 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.

Ramsey J. & Schemske D. W. (1998) Pathways, mechanisms, and rates of polyploid formation in flowering plants. – Annual Review of Ecology and Systematics 29: 467–501.

Rieseberg L. H., Raymond O., Rosenthal D. M., Lai Z., Livingstone K., Nakazato T., Durphy J. L., Schwarzbach A. E., Donovan L. A. & Lexer C. (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. – Science 301: 1211–1216.

Rieseberg L. H. & Wendel J. (1993) Introgression and its consequences in plants. – In: Harrison R. G. (ed.), Hybrid zones and the evolutionary process, p. 70–109, Oxford University Press, Oxford.

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.

Schumer M., Rosenthal G. G. & Andolfatto P. (2014) How common is homoploid hybrid speciation? – Evolution 68: 1553–1560.

Seehausen O. (2004) Hybridization and adaptive radiation. – Trends in Ecology & Evolution 19: 198–207.

Soltis P. S. & Soltis D. E. (2009) The role of hybridization in plant speciation. – Annual Review of Plant Biology 60: 561–588.

Soltis D. E., Visger C. J. & Soltis P. S. (2014) The polyploidy revolution then…and now: Stebbins revisited. – American Journal of Botany 101: 1057–1078.

Stebbins G. L. (1958) The inviability, weakness, and sterility of interspecific hybrids. – Advances in Genetics 9: 147–215.

Sturtevant A. P., Hatley N., Pullman G. D., Sheick R., Shorez D., Bordine A., Mausolf E., Lewis A., Sutter R. & Mortimer A. (2009) Molecular characterization of Eurasian watermilfoil, Northern milfoil, and the invasive interspecific hybrid in Michigan lakes. – Journal of Aquatic Plant Management 47: 128–135.

Suarez-Gonzalez A., Lexer C. & Cronk Q. C. B. (2018) Adaptive introgression: a plant perspective. – Biology Letters 14: 20170688.

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

Wolf D., Takebayashi N. & Rieseberg L. (2001) Predicting the risk of extinction through hybridization. – Conservation Biology 15: 1039–1053.

Wong E. L. Y., Hiscock S. J. & Filatov D. A. (2022) The role of interspecific hybridisation in adaptation and speciation: insights from studies in *Senecio*. – Frontiers in Plant Science 13: 907363.

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., Kaplan Z. & Kwolek D. (2018) Reinterpretation of *Potamogeton* ×*nerviger*: solving a taxonomic puzzle after two centuries. – Preslia 90: 135–149.

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.

Zalewska-Gałosz J., Ronikier M. & Kaplan Z. (2009) The first European record of *Potamogeton* ×*subobtusus* identified using ITS and cpDNA sequence data. – Preslia 81: 281–292.

Zalewska-Gałosz J., Ronikier M. & Kaplan Z. (2010) Discovery of a new, recurrently formed *Potamogeton* hybrid in Europe and Africa: molecular evidence and morphological comparison of different clones. – Taxon 59: 559–566.

Vzácný případ hybridizace mezi diploidními lakušníky (*Ranunculus* sect. *Batrachium*)

Hybridizace a polyploidizace představují klíčové mechanismy evolučních změn u rostlin. Allopolyploidizace, tedy vznik polyploidních organismů hybridizací mezi různými druhy následovaný celogenomovou duplikací, je častým fenoménem zejména u cévnatých rostlin. Tento proces umožňuje rychlou speciaci a ekologickou diverzifikaci. Křížení mezi diploidními druhy často vede ke vzniku F_1 hybridů se sníženou plodností v důsledku nepravidelné meiózy. Následná duplikace genomu však může plodnost obnovit vytvořením homologních párů chromozomů. Pochopení prvotních fází hybridizace mezi diploidy je proto zásadní pro objasnění vzniku allopolyploidních komplexů. Lakušníky (*Ranunculus* sect. *Batrachium*) jsou vodní rostliny známé častým výskytem kříženců a polyploidů, z nichž mnohé vznikly právě allopolyploidizací. Přestože existuje už velké množství molekulárních důkazů o hybridizaci v této skupině, překvapivě nebyl až donedávna zaznamenán jediný případ existence diploidního křížence. Předkládaná studie přináší důkazy o hybridizaci na diploidní úrovni prostřednictvím integrativního morfologického, cytologického a molekulárního přístupu. Nový mezidruhový hybrid *R. circinatus* × *R. rionii* byl nalezen na dvou lokalitách v zaplavených pískovnách na Kroměřížsku na střední Moravě a popsán jako *R.* ×*limnophilus*. Analýza chloroplastové DNA ukázala, že tam vznikl nejméně dvakrát, protože přenos pylu při křížení proběhl oběma směry. K hybridizaci došlo na relativně nových, uměle vytvořených vodních stanovištích, která umožnila kolonizaci a společný výskyt obou rodičovských druhů, jakož i vegetativní přežívání a množení vzniklých sterilních kříženců. Součástí práce je rovněž přehled a kategorizace biotypů, které tvoří celou komplikovanou diverzitu lakušníků.

How to cite: Kaplan Z., Trávníček B., Prančl J. & Koutecký P. (2025) Rare interspecific hybridization at the diploid level in *Ranunculus* sect. *Batrachium.* – Preslia 97: 637–658.

Preslia, a journal of the Czech Botanical Society

https://www.preslia.cz