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# 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

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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*.

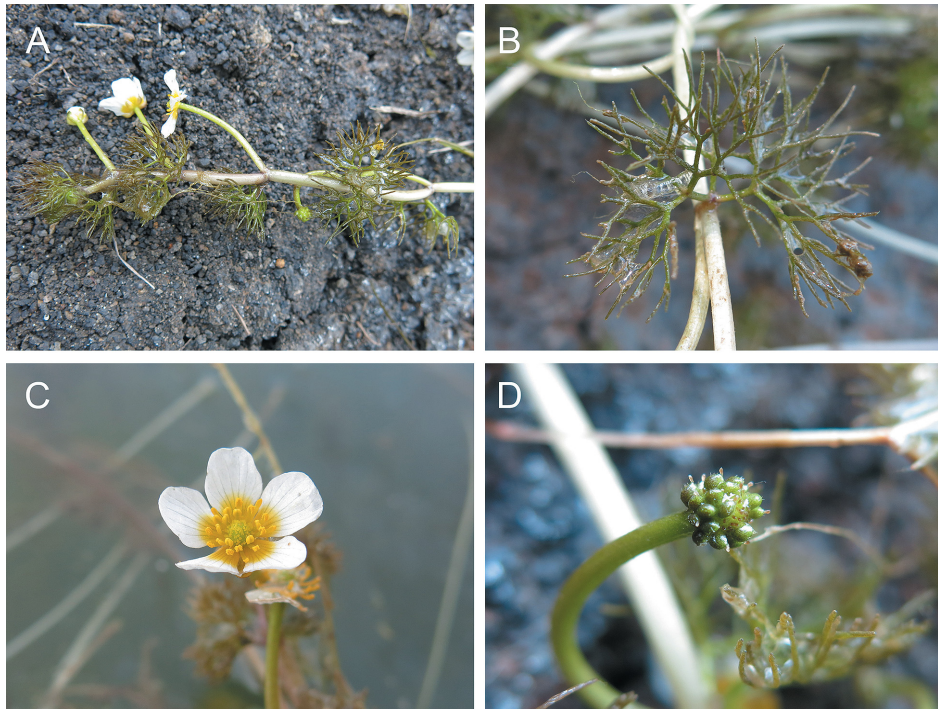



**Fig. 4.** Details of *Ranunculus* ×*limnophilus*: (A) apical part of a flowering branch, (B) leaf, (C) flower and (D) receptacle of a post-anthesis flower with undeveloped achenes.

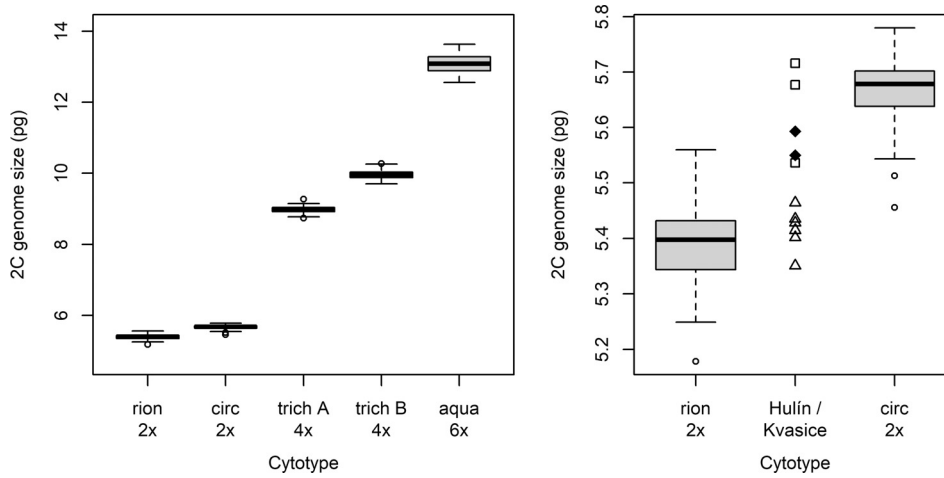



**Fig. 5.** Boxplots showing ranges in the variation in holoploid genome sizes (2C-values) of *Ranunculus aquatilis*, *R. circinatus*, *R. rionii*, *R. trichophyllus* A and *R. trichophyllus* B in central Europe as identified in previous studies (Prančl et al. 2018, Koutecký et al. 2022), compared with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




**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).

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## 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.

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