

Original Scientific Paper

Riccia fluitans and *Riccia rhenana* (Marchantiales) in Bulgaria, with special emphasis on their cytogenetics and European distribution

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ABSTRACT:

Riccia fluitans and *R. rhenana* are widely distributed aquatic liverworts with challenging species delimitation. The insufficient identification causes uncertainty in the species distribution. In this study, we summarize the occurrence data for Bulgaria, and compare it with the records available in GBIF database for the territory of Europe. Based on the Bulgarian occurrence and the European occurrence, comparison of the frequencies of both species is made. The results of the detailed studies of *Riccia fluitans* complex in Bulgaria show the opposite pattern as compared to the European occurrence in GBIF. This is a consequence of hard morphological delimitation between the two taxa, which are cytogenetically clearly distinctive. The striking prevalence of *R. fluitans* over *R. rhenana* from GBIF data shows the need for careful species determination and highlights the risks of using such databases. The field data from Bulgaria indicated species-specific trends with respect to habitat origin and microhabitat preferences.

Keywords: aquatic, diploid, GBIF, haploid, liverworts, *Riccia fluitans* complex, spread

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INTRODUCTION

Accurate species identification is crucial for understanding species distribution, ecology and biogeography. In the case of closely related species, occupying similar or almost identical ecological niches, this step has a profound role. Misidentification can lead to conclusions based on observations of multiple competing species rather than a single species. This issue is additionally compounded when analyzing data from diverse sources without critical evaluation, further distorting our understanding and conclusions.

Riccia fluitans L. and *R. rhenana* Lorb. ex Müll. Frib. (Ricciaceae, Marchantiales) are species of liverworts, sharing similar morphology and similar ecological niches (SCHUSTER 1992; DAMSHOLT 2009). They are the most common aquatic representatives of genus *Riccia* L. in Europe, but being morphologically almost indistinguishable in their aquatic form, their distribution remains poorly known. The lack of stable morphological discriminative traits, and the existence of other morphologically similar members of the section, caused the treatment of this group under the generalized name “*Fluitans Complex*” (BERRIE 1964). The morphological similarity between *R. fluitans* and *R. rhenana* is due to the general lack of stable traits (EVANS 1922), a large phenotypic plasticity, and their proposed genetic relationship. It has been hy-

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pothesized that *R. rhenana* is an autodiploid deriving from *R. fluitans* (BERRIE 1964). Since both species inhabit similar ecological niches, ancestor displacing events might be speculated (ALIX *et al.* 2017).

Currently, there are no studies dealing with the distribution of the complex in Europe. Also, there is no detailed data, based on chromosome counts for the species distribution in Bulgaria. The species determination based on morphology is questionable, and the only reliable method for delimitation, especially of aquatic forms, is based on the chromosome counts, where *R. fluitans* is a haploid $n=8$ plant, while *R. rhenana* is a diploid $n=16$ (MÜLLER 1941). The aim of this work is to present the current knowledge on the distribution of *R. fluitans* and *R. rhenana* in Bulgaria and to draw attention to the importance of correct species determination. Thus, we summarize the distribution of *R. fluitans* and *R. rhenana* in Bulgaria and compare it with the reports generated from GBIF (<https://www.gbif.org/>) for Europe. For the purposes of this work, the GBIF datasets were intentionally not revised, so they fully represent the common awareness on both species.

MATERIALS AND METHODS

For the purposes of this study, two different datasets were compiled. One dataset represents the haploid-diploid distribution in Bulgaria (Table 1) and the second dataset represents the distribution of *R. fluitans* and *R. rhenana* in Europe. The dataset with Bulgarian samples contains 63 entries of published (IORDANOFF 1931; TZONEV 2006; NATCHEVA *et al.* 2007, 2008; ELLIS *et al.* 2021) and herbarium data (Table 1). Attempt was made to re-visit all known locations and collect live material for chromosome counting. The dataset of the general distribution of *R. fluitans* and *R. rhenana* across Europe is based on 13,566 entries, acquired from GBIF. Out of them, 13,458 entries represented *R. fluitans* (<https://www.gbif.org/occurrence/download/0010210-250325103851331>), while small portion of just 108 entries represented *R. rhenana* (<https://www.gbif.org/occurrence/download/0003928-250402121839773>). No GBIF data was used for the Bulgarian distribution, since more accurate data are available. The geographic parameters specified for both GBIF sets are as follows: Continent: Europe; Country area: Albania, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Montenegro, Netherlands (Kingdom of the), Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, United Kingdom of Great Britain, and Northern Ireland. For both sets, only entries with geographic coordinates were selected. The entries from GBIF were taken the way they were provided by the platform. No revision of the identifications of the entries was made.

Two habitat factors for the Bulgarian samples were scored: microhabitat (aquatic vs. terrestrial) and origin of the site. The aquatic habitat means that the given sample was collected floating in water, while the terrestrial habitat means that the sample was collected growing on mud. The origin of the habitat was treated in three categories: 1) natural – a natural wetland with no or minor human impact, where the hydrology did not suffer significant impact; 2) semi-natural – a water body that is strongly affected by man and is a remnant of an older natural wetland, possibly harboring remnants of an older local population; 3) man-made – entirely artificial water body constructed over non-wetland habitat, where the populations of *Riccia* are most likely newly established.

Table 1. Data on records of *Riccia fluitans* and *R. rhenana* in Bulgaria. Abbreviations: S - substrate, f - floating, t - terrestrial, HO-habitat origin, n - natural, sn - semi-natural, mm - man-made, dash (-) - no data.

Species	Voucher number	Site name	Longitude	Latitude	S	HO	Date of collection	Coll.	Det.
<i>R. fluitans</i>	6544	Znepole region, Stefanovo village near the town of Radomir. In a channel of microdam eastwards of the village	23.039843	42.48568	f	n	30.10.2005	V. Georgiev	A. Ganeva
<i>R. fluitans</i>	-	Lozenska Mt., Sua Gabra river	23.595718	42.563935	f	sn	2019	G. Gospodinov, R. Natcheva	G. Gospodinov, R. Natcheva
<i>R. fluitans</i>	-	Iskar Dam	23.626043	42.439938	f	sn	2020	G. Gospodinov, R. Natcheva	G. Gospodinov, R. Natcheva
<i>R. fluitans</i>	-	Kalimok	26.51591	44.03681	-	sn	17.07.2021	B. Gyosheva	R. Natcheva
<i>R. fluitans</i>	11298	Forebalkan: N of town Botevgrad, loc. Somalia, swamp at the foothill of the landfill of Botevgrad, with <i>Typha latifolia</i> and <i>Juncus effusus</i> .	23.80273	42.93452	f	sn	15.09.2022	K. Vasilev	R. Natcheva
<i>R. fluitans</i>	11316	Forebalkan: N of town Botevgrad, loc. Somalia, ditch at the foothill of the landfill of Botevgrad, with <i>Typha latifolia</i> and <i>Juncus effusus</i> .	23.803647	42.933978	f	sn	23.03.2023	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11389	SE of Slavovitsa village, in swamp Selskia gyol, among <i>Schoenoplectus</i> sp., with <i>Riccia rhenana</i> (n=16), n=8	24.059118	42.312024	f	n	27.04.2023	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11590	Persin Island: S edge of Pischansko blato, along a canal along the road, on periodically dry mud under the shade of willows.	25.210693	43.667163	t	n	13.09.2023	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11959	Kalimok marsh: in pools on the bottom of a temporary drying canal, shaded by shrubs, with <i>Ricciocarpos natans</i>	26.515838	44.0369	f	sn	15.06.2022	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11960	Kalimok marsh: on soil on the bottom of a temporary drying canal, shaded by shrubs	26.515838	44.0369	t	sn	15.06.2022	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11961	Kalimok marsh: in a canal, shaded by trees	26.522205	44.025891	t	sn	15.06.2022	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11962	Kalimok marsh: at the bank of a canal, shaded by trees	26.522205	44.025891	t	sn	15.06.2022	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	9402	Znepole: Choklyovo marsh, floating among rushes	22.831972	42.396861	f	n	28.07.2008	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	12443	In a slow flowing river E of village Stefanovo, interwoven among <i>Veronica beccabunga</i> , on silicate bedrock	23.039843	42.48568	f	n	01.10.2018	G. Gospodinov, R. Natcheva	G. Gospodinov, R. Natcheva
<i>R. fluitans</i>	11537	Znepole region: E of village Stefanovo, loc. Izvoro, in the a carstic spring	23.040283	42.48683	f	n	01.05.2024	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11538	Znepole region: E of village Stefanovo, loc. Izvoro, in the outlet of a carstic sprin	23.040512	42.486773	f	n	01.05.2024	R. Natcheva	R. Natcheva

<i>R. fluitans</i>	11556	Znepole region: E of village Stefanovo, loc. Izvoro, on wet soil at the edge a carstic spring	23.040512	42.486773	f	n	05.01.2024	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11536	Znepole region: E of village Stefanovo, loc. Trichkov izvor, in the outlet of a carstic spring	23.041445	42.486073	f	n	05.01.2024	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	8868	Forebalkan: at Lukovitski Karst Park south of the town of Lukovit, on the sandy bank of river Panega	24.056278	43.186444	t	n	31.10.2006	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	11520	Persin Island: S edge of Pischansko blato, on periodically dry mud under the shade of willows.	25.212023	43.66694	t	n	13.09.2023	R. Natcheva	R. Natcheva
<i>R. fluitans</i>	12455	Persin Island: on the shore of a canal, S of Pischansko blato, on periodically dry mud under the shade of willows.	25.212729	43.666707	t	sn	13.09.2023	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	9406	Znepole: Znepole Region: Choklyovo marsh, on the bank of <i>Salix</i> sp., sterile, 866 m	22.831972	42.396861	f	n	28.07.2008	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11294	Sofia city: between neighbourhoods Lyulin 5/6 and Suhodol, loc. Braniste, W of hill Malka Konyovitsa, in swamp, shaded by shrubs.	23.239854	42.703737	f	mm	29.12.2022	A. Tanev	A. Tanev
<i>R. rhenana</i>	-	Lozenska Mt. Above German monastery	23.446447	42.587126	-	n	2019	G. Gospodinov	G. Gospodinov
<i>R. rhenana</i>	9775	Lozen Mt.: small pond at the saddle W of peak Polovrak	23.509557	42.580081	t	sn	16.03.2019	A. Tanev	R. Natcheva
<i>R. rhenana</i>	10781	Iskar dam, loc. Shavarite, in a temporary dry former fishery pond, at the base of <i>Typha</i> , terrestrial form	23.543663	42.441961	t	mm	05.12.2021	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11880	Lozenska Mt.: N of Gabra village, in small lakes along river Suha Gabra.	23.599141	42.562499	f	sn	26.02.2020	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11389	SE of Slavovitsa village, in swamp Selskia gyol, among <i>Schoenoplectus</i> sp., with <i>Riccia rhenana</i> (n=16), n=8	24.059118	42.312024	f	n		R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11968	Forebalkan: NE of Nanovitsa neighborhood, at the pot-hole lake Blatoto, on mud and plant debris at the base of <i>Typha angustifolia</i> shaded by <i>Salix</i> sp.	24.151631	43.042051	t	n		R. Natcheva B. Gyosheva	R. Natcheva
<i>R. rhenana</i>	-	Belene town, "Antichnite garla"	25.109342	43.659936	t	mm	-		R. Natcheva
<i>R. rhenana</i>	-	Srebarna	27.06145	44.11678	-	n	2021	B. Gyosheva	R. Natcheva
<i>R. rhenana</i>	-	Srebarna	27.06636	44.08954	-	n	2021	B. Gyosheva	R. Natcheva
<i>R. rhenana</i>	11949	Srebarna marsh: S of Srebarna village, on soil among grasses in temporary dry <i>Salix</i> grove.	27.066722	44.089941	t	n	16.06.2022	R. Natcheva	R. Natcheva

<i>R. rhenana</i>	11954	Srebarna marsh: NE part of the marsh, on temporary dry plant debris along a fishermen's track among <i>Phragmites australis</i> .	27.079491	44.120281	t	n	16.06.2022	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11955	Srebarna marsh: NE part of the marsh, on temporary dry plant debris along a fishermen's track among <i>Phragmites australis</i> .	27.079491	44.120281	t	n	16.06.2022	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11951	Srebarna marsh: NE part of the marsh, on temporary dry soil along a fishermen's track among <i>Phragmites australis</i> .	27.080486	44.120849	t	n	16.06.2022	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11953	Srebarna marsh: NE part of the marsh, on temporary dry soil along a fishermen's track among <i>Phragmites australis</i> .	27.080771	44.120761	t	n	16.06.2022	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11954	Srebarna marsh: NE part of the marsh, on temporary dry plant debris along a fishermen's track among <i>Phragmites australis</i> .	27.080771	44.120761	t	n	16.06.2022	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	11955	Srebarna marsh: NE part of the marsh, on temporary dry plant debris along a fishermen's track among <i>Phragmites australis</i> .	27.080771	44.120761	t	n	16.06.2022	R. Natcheva	R. Natcheva
<i>R. rhenana</i>		Srebarna	27.08103	44.12092	-	n	2021	B. Gyosheva	R. Natcheva
<i>R. rhenana</i>	9769	Ropotamo Nature Reserve: Arkutino lake	27.725887	42.331815	f	n	05.11.2018	S. Tsoneva, V. Georgiev, Ch. Gushev	G. Gospodinov, R. Natcheva
<i>R. rhenana</i>	12439	Dragichevo marsh, on damp mud along the shore, intermediate terrestrial form, on silicate sandstones, argillite and conglomerate bedrock	23.156738	42.631653	t	n	09.01.2018	G. Gospodinov	G. Gospodinov
<i>R. rhenana</i>	12440	Dragichevo marsh, on damp mud along the shore, intermediate terrestrial form, on silicate sandstones, argillite and conglomerate bedrock	23.156738	42.631653	t	n	09.01.2018	G. Gospodinov	G. Gospodinov
<i>R. rhenana</i>	-	Dragichevo marsh, on wet mud along the shore, terrestrial form, on silicate sandstones, argillite and conglomerate bedrock	23.156738	42.631653	t	n	09.01.2018	G. Gospodinov	G. Gospodinov
<i>R. rhenana</i>	12441	Dragichevo marsh, floating among <i>Typha</i> , <i>Lemna trisulca</i> , <i>minor</i> , <i>Potamogeton</i> sp., on silicate bedrock	23.156738	42.631653	f	n	09.01.2018	G. Gospodinov	G. Gospodinov
<i>R. rhenana</i>	11447	Sofia, the South park, lake Panorama, near the feeding pipe, floating among old <i>Typha</i> stems.	23.308	42.6593	f	mm	18.03.2023	A. Tanev	A. Tanev
<i>R. rhenana</i>	9775	Lozen Mt.: small pond at the saddle W of peak Polovrak	23.509557	42.580081	f	sn	16.03.2019	A. Tanev	R. Natcheva
<i>R. rhenana</i>	12446	W of town Belene in the ancient canals "Garla" (the Throats), on mud on the periodically dry bottom of the canal	25.109415	43.659771	t	mm	10.09.2018	G. Gospodinov, R. Natcheva	G. Gospodinov, R. Natcheva

<i>R. rhenana</i>	11590	Persin Island: S edge of Pischansko blato, along a canal along the road, on periodically dry mud under the shade of willows.	25.210693	43.667163	t	n	13.09.2023	R. Natcheva	R. Natcheva
<i>R. rhenana</i>	9769	Ropotamo Nature Reserve: Arkutino lake	27.725887	42.331815	f	n	05.11.2018	S. Tsoneva, V. Georgiev, Ch. Gushev	G. Gospodinov, R. Natcheva
<i>R. rhenana</i>	11963	Dyavolsko marsh, SW of the town of Primorsko, on mud with <i>Typha latifolia</i> , <i>Typha angustifolia</i> , <i>Sparganium erectum</i> , <i>Salvinia natans</i> .	27.7306	42.24766	t	n	24.9.2022	B. Gyosheva	R. Natcheva
<i>R. rhenana</i>	10682	NE of village Bystritsa, in a temporary pond, on mud at the edge of <i>Typha latifolia</i> belt towards the inner part of the pond	23.367868	42.595414	t	n	17.10.2022	R. Natcheva	R. Natcheva

For the chromosome counts, plant material was fixed directly in the field in Clarke's solution (3:1 ethyl alcohol/glacial acetic acid) or plants were collected and kept alive in a greenhouse. After a period of adaptation, a modified Gomori's haematoxylin staining method was used (MELANDER & WINGSTRAND 1953). Fresh actively growing thallus tips were placed in 0.01% solution of colchicine for 90 min. After washing in distilled H₂O they were fixed in Clarke's solution for 2 h at room temperature or in a fridge for 24 h. After washing in distilled H₂O the thalli were placed in 1M hydrochloric acid for 40 min at 60°C followed by washing in distilled H₂O. The samples were incubated in hydrochloric acid /ether in ratio 1:1 for 15 min at 60°C, washed in distilled H₂O and stained in Gomori's Hematoxylin for 1.45/2 h at 60°C. Samples were squashed in 45% acetic acid and observed under a light microscope. At least five thalli per populations were checked.

RESULTS

We were able to confirm the presence in 53, out of the 63 known Bulgarian sites for *Riccia fluitans* complex (Table 1) among them, 31 turned out to be diploid *R. rhenana* and 22 were haploid *R. fluitans* (Fig. 1). Ten entries could not be refound and chromosome count could not be obtained. These were treated as *R. fluitans* complex. The distribution of the *R. fluitans* complex in Bulgaria clearly shows the prevalence of the diploid *R. rhenana* over the haploid *R. fluitans* (Fig. 2). The European distribution (Fig. 3) shows the opposite pattern - strong prevalence of *R. fluitans* over *R. rhenana*. In both occasions mixed populations have been observed.

In Bulgaria, *R. fluitans* was found as aquatic in 13 populations, and as terrestrial in eight populations (Fig. 4A). *Riccia rhenana* was collected as terrestrial from 18 populations and from nine aquatic accessions. With respect to habitat origin, the diploid *R. rhenana* was present in all habitat types (Fig. 4B). It was the only species of the two studied in man-made habitats (four populations), but it was also rather common in natural habitats (23 populations), and with low number of records (three populations) in semi-natural habitats. The haploid *R. fluitans* occurred both in semi-natural (11 populations) and natural locations (11 populations), but it was not reported from entirely man-made habitats.

Figure 1. *Riccia fluitans* $n=8$ (left) and *R. rhenana* $n=16$ (right) chromosome sets.

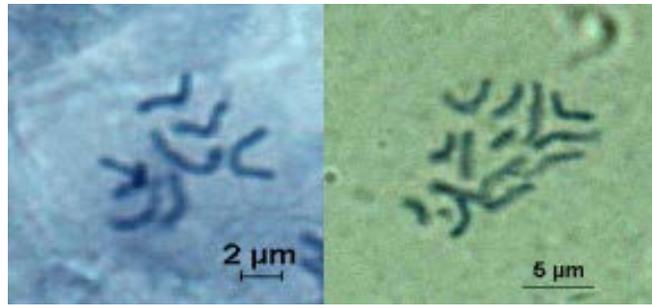


Figure 2. Distribution of *Riccia fluitans* (yellow dots) and *R. rhenana* (red dots) in Bulgaria, green dots– not confirmed cytologically i.e. *R. fluitans* complex. Black circles indicate the sites where both species co-occur.

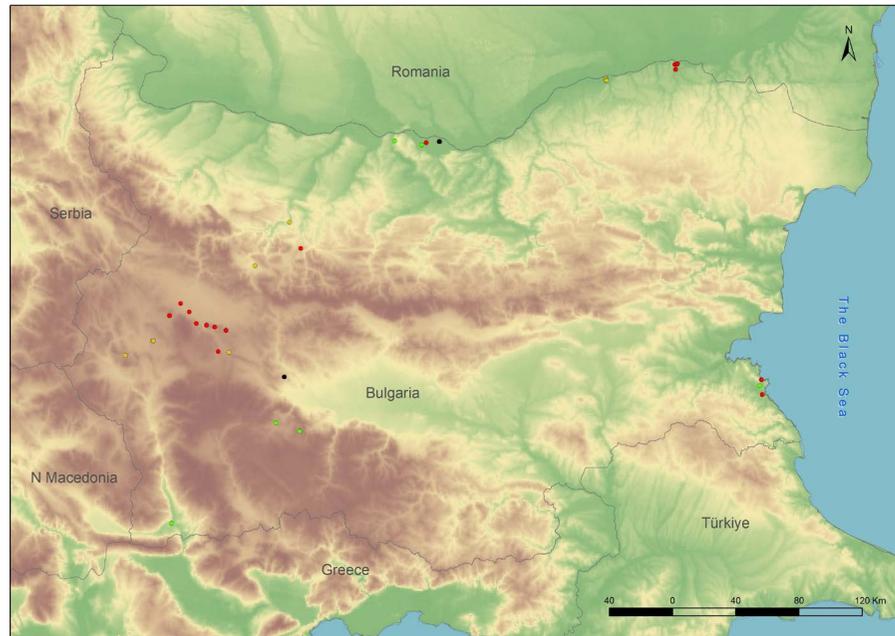


Figure 3. Distribution of *Riccia fluitans* (yellow dots) and *R. rhenana* (red dots) in Europe, based on data acquired from GBIF (state of June 2025).

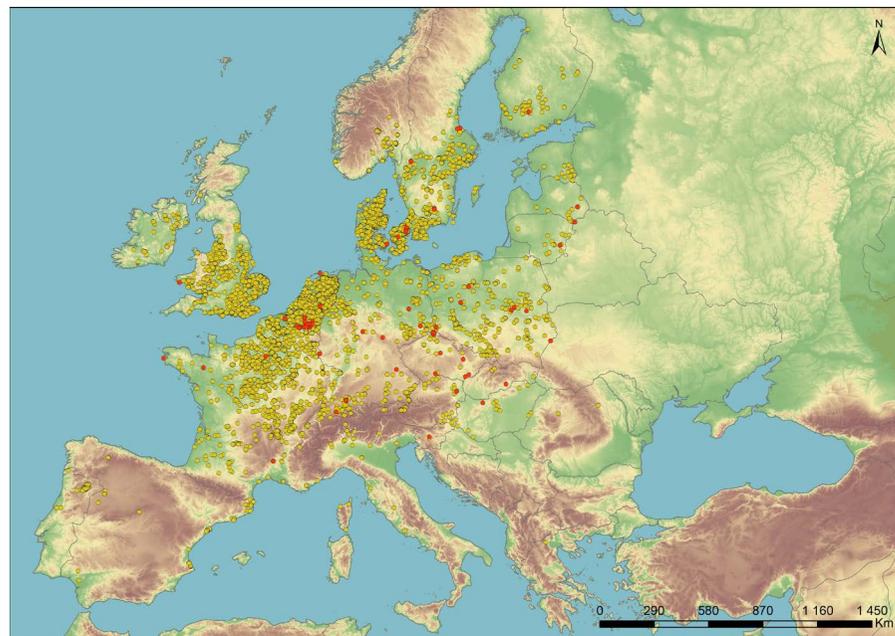
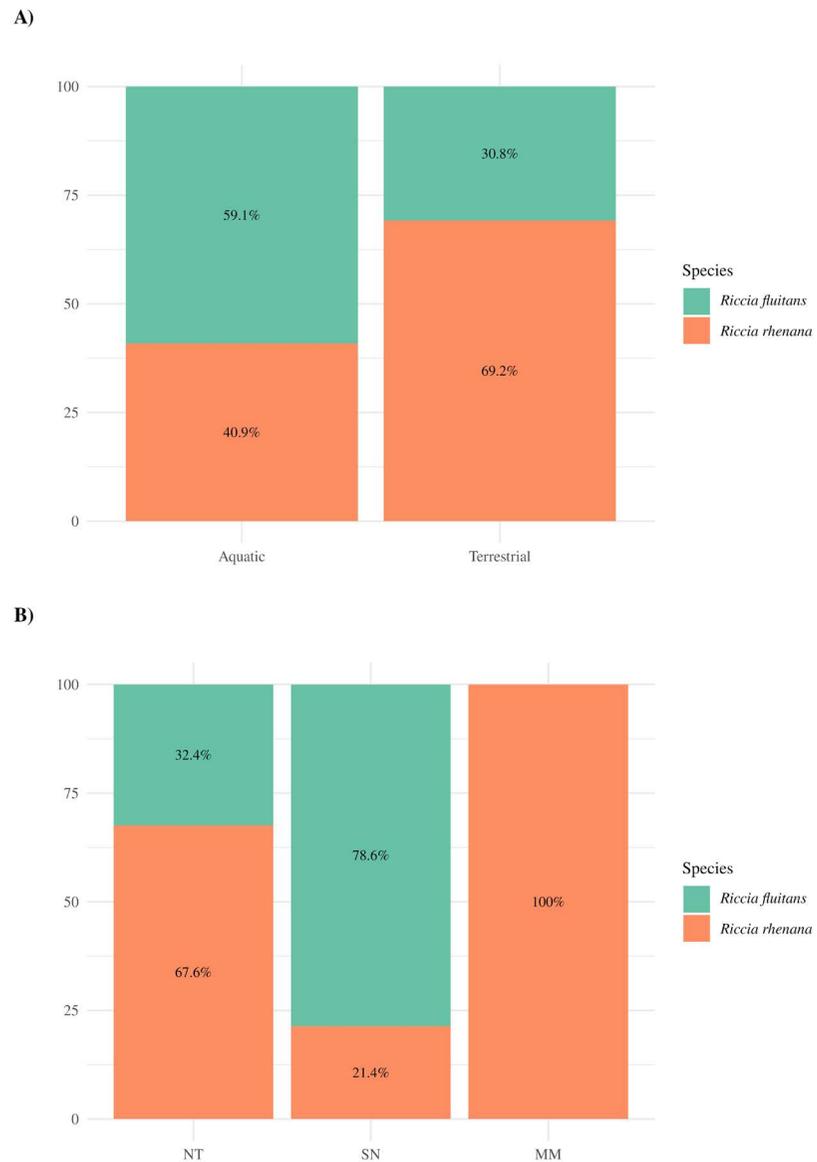


Figure 4. A) Microhabitat (aquatic or terrestrial records) occurrence (%) of *Riccia fluitans* and *R. rhenana* in Bulgaria. B) Occurrence of *Riccia fluitans* and *R. rhenana* in Bulgaria, based on records made by site origin. NT - natural, SN - semi-natural, MM - man-made.



DISCUSSION

Even being a cosmopolitan group of plants, bryophytes are restricted to specific niches (SHAW & GOFFINED 2000). Thus, their real distribution can be huge but rather limited and restricted to particular conditions. These can be found only in very specific types of habitats (SLACK 1990). Given that speciation can also occur through evolutionary drive of polyploidization (RIESBERG & WILLIS 2007), contributing to increased species diversity within the community (ROSS 1972), both species are likely to compete for establishment and coexistence. It seems they have huge niche overlap and their ecological preferences are not clear. Despite being potentially genetically related, they should be reproductively isolated and sexual reproduction between the original and derived chromosome number should not be expected (NEWTON 1984), while this isolation would serve for the establishment of two discrete gene pools (NEWTON 1990). We have observed a case of coexistence of *R. fluitans*

and *R. rhenana* at two localities, without evidence of prevalence of the one to another. There might be other cases of coexistence that have remained unnoticed, especially when only aquatic forms were present. This might suggest the possibility of sympatric evolution, for which polyploidization is believed to be the single most important mechanism (OTTO & WHITTON 2000). In general, polyploidization has the potential to alter plant morphology, physiology, phenology, and ecology within just one or few generations (LEVIN 2002; TE BEEST *et al.* 2012), providing the newly formed polyploid with features superior to the ancestral progenitor. In this sense, a polyploid can potentially outcompete its progenitor or/and exploit new niches (LEITCH & LEITCH 2008; TE BEEST *et al.* 2012). Such advantage can be considered as higher spread potential, and suggest *R. rhenana* to be an invasive species worth of further investigations. Its population trends in Europe remain unknown (CAMPISI & COGONI 2019). Newly formed polyploids often exhibit rapid range expansion (LEVIN 1983; HULL-SANDERS *et al.* 2009; TREIER *et al.* 2009) covering various habitats. The origin of *R. rhenana* remains uncertain, and if it is native to Europe (SÖDERSTRÖM 1992). It is included in “DAISIE –Inventory of alien invasive species in Europe” (available at <https://www.gbif.org/dataset/39f36f10-559b-427f-8c86-2d28aff68ca>) as “Native to some parts of Europe”. PRESTON *et al.* (2011), listed it as a “recent introduction” to the British and Irish liverwort flora. In the work of WYATT & DAVIDSON (2013), *R. rhenana* is concluded to be restricted to calcareous seepage fens, at least in Georgia (United States). However, no cytological confirmations of the cited materials are mentioned. In Bulgaria, *R. rhenana* occupies various habitats and such restricted occurrence as the mentioned above is not supported by our results. Although it was not possible to re-find a few historical reports and to verify their species identity, the prevalence of *R. rhenana* over *R. fluitans* at a significantly smaller territory as Bulgaria suggests that the abundance of *R. rhenana* is much higher in Europe, than the GBIF data suggests.

Riccia rhenana has been found in its terrestrial form much more often, than *R. fluitans* (Fig. 4A). This could partly be a sampling bias, as terrestrial forms are often easier to detect and collect. However, our observations indicate that the diploid seems to be much more tolerant and freely growing as a terrestrial plant, while the haploid occurs as a terrestrial form in cases where it is forced by some unexpected and sudden event. Thus, it could be speculated that the terrestrial haploids (i.e. *R. fluitans*) might be more short-living as compared to the terrestrial diploids (i.e. *R. rhenana*), and are likely more sensitive to prolonged periods of drying.

Despite the limited data, the habitat origin seems to play a role in the species occurrence (Fig. 4B). The semi-natural habitats are of significant interest since they most likely harbor old species establishments and the haploid *R. fluitans* is found relatively more often, than the diploid *R. rhenana* in such sites. Multiple factors should be considered for the proper understanding of the competing nature of the haploid-diploid occurrence. For example, drastic changes in the water level, could affect negatively a haploid population, since its response to such change should be much slower, and not as effective as the response of the diploid. Another factor is the depth of the given water body. Both the haploid and the diploid rest as fragments at the bottom of the inhabited pond or lake during winter, and rely on the increased light period in the beginning of spring to start growing again (SCHUSTER 1992). In this period, they are competing with the rest of the inhabitants to grow and reemerge to the surface, where they should establish and maintain their population. Both species are found usually co-occurring with species of *Lemna* L. (most often *L. minor* L. and *L. trisulca* L.), species of *Potamogeton* L., and *Ricciocarpos natans* (L.) Corda. In such competing environment, the haploid seems in a disadvantage position, since all the floating competitors shade the bottom and

if the early start is not efficient enough, the population establishment will be negatively affected. Possessing two functional genomes, the diploid is expected to respond faster to the conditions of the environment and to its competitors. Here, we should also mention the temperature sensitivity of both species can differ and the habitat type and origin can significantly alter the thermal regime as well. By this, we could expect that the population establishment and population maintenance of *R. rhenana* is more effective, and favored by genetic advantage. In the course of our fieldwork, two populations of *R. fluitans* were found to be fertile, comprised only by female individuals (spores have never been observed), while *R. rhenana* has never been observed with reproductive structures. Even not evidenced to reproduce sexually in Bulgaria, the haploid *R. fluitans* invests resources in the production of sexual structures, which indicates that not all resources are focused on the clonal reproduction by fragmentation. Presumably, the diploid should be monoicous, and by this, freely reproducing sexually, which is the case of *R. duplex* Lorb. ex Müll. Frib. (MÜLLER 1941). Unlike it, *R. rhenana* is always sterile and reproducing exclusively via thallus fragmentation (DAMSHOLT 2009).

In conclusion, based on the current data, *R. rhenana* is more abundant than *R. fluitans* in Bulgaria and the chromosome number remains the only reliable character for species delimitation. Thus, *R. rhenana* is likely much more common in Europe but routinely misidentified for its haploid relative or simply under-recorded and overlooked. Growing in a wider range of habitats and being more often found, gives *R. rhenana* the polyploid invasive features, but this remain obscured and needs further investigations. At this stage, we are not able to discuss further the invasive potential of the diploid *R. rhenana*, since no distinctive habitat types have been identified for the haploid *R. fluitans*. However, one feature of the preferred habitat of *R. fluitans* seems to be more stable water levels over the year, while *R. rhenana* seems to be much more tolerant to significant water fluctuations, which might be a result of its polyploid origin. The results obtained clearly suggest that further studies are needed to elucidate the true European distribution of both species, their detailed niche preferences, and the competitive relationships.

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REFERENCES

- ALIX K, GÉRARD PR, SCHWARZACHER T & HESLOP-HARRISON JS. 2017. Polyploidy and interspecific hybridization: partners for adaptation, speciation and evolution in plants. *Annals of Botany* **120**: 183–194. <https://doi.org/10.1093/aob/mcx079>
- BERRIE GK. 1964. Experimental studies on polyploidy in liverworts I. The *Riccia fluitans* complex. *Bryologist* **67**: 146–152. <https://doi.org/10.2307/3240799>
- CAMPISI P & COGONI A. 2019. *Riccia rhenana* (Europe assessment). The IUCN Red List of Threatened Species 2019: e.T87538748A87773194 [Accessed on 18.12.2025]
- DAMSHOLT K. 2009. *Illustrated Flora of Nordic Liverworts and Hornworts* (2nd edn). Nordic Bryological Society, Lund.
- ELLIS LT, AH-PENG C, ASLAN G, BAKALIN VA, BERGAMINI A, CALLAGHAN DA, CAMPISI P, RAIMONDO FM, CHOI SS, CSIKY J, CSIKYNÉ RADNAI E, CYKOWSKA-MARZENCKA B, CZERNYADJEVA IV, KALININA YM, AFONINA OM, DOMINA G, DRAPELA P, FEDOSOV VE, FUERTES E, GABRIEL R, KUBOVÁ M, SOARES ALBERGARIA I, GOSPODINOV G,

- NATCHEVA R, GRAULICH A, HEDDERSON T, HERNÁNDEZ-RODRÍGUEZ E, HUGONNOT V, HYUN CW, KIRMACI M, ÇATAK U, KUBEŠOVÁ S, KUČERA J, LA FARGE C, LARRAÍN J, MARTIN P, MUFEED B, MANJU CN, RAJESH KP, NÉMETH C, NAGY J, NORHAZRINA N, SYAZWANA N, O'LEARY SV, PARK SJ, PEÑA-RETES AP, RIMAC A, ALEGRO A, ŠEGOTA V, KOLETIĆ N, VUKOVIĆ N, ROSADZIŃSKI S, ROSSELLÓ JA, SABOVLJEVIĆ MS, SABOVLJEVIĆ AD, SCHÄFER-VERWIMP A, SÉRGIO C, SHKURKO AV, SHYRIAIEVA D, VIRCHENKO VM, SMOCZYK M, SPITALE D, SRIVASTAVA P, OMAR I, ASTHANA AK, STANIASZEK-KIK M, CIENKOWSKA A, ŞTEFĂNUŢ MM, ŞTEFĂNUŢ S, TAMAS G, BÎRSAN CC, NICOARĂ GR, ION MC, PÓCS T, KUNEV G, TROEVA EI, VAN ROOY J, WIETRZYK-PEŁKA P, WĘGRZYN MH, WOLSKI GJ, BOŻYK D & CIENKOWSKA A. 2021. New national and regional bryophyte records, 65. *Journal of Bryology* **43**: 67–91. <https://doi.org/10.1080/03736687.2021.1878804>
- EVANS AW. 1922. Recent studies on certain species of *Riccia*. *Bryologist* **25**: 81–86. <https://doi.org/10.2307/3238237>
- HULL-SANDERS HM, JOHNSON RH, OWEN HA & MEYER GA. 2009. Effects of polyploidy on secondary chemistry, physiology, and performance of native and invasive genotypes of *Solidago gigantea* (Asteraceae). *American Journal of Botany* **96**: 762–770. <https://doi.org/10.3732/ajb.0800200>
- JORDANOFF D. 1931. Pflanzengeographische Studien der Sümpfe Bulgariens in ihrer Beziehung zur höheren Vegetation I. Binnensümpfe. – Annuaire de l'Université de Sofia. Faculté Physico-mathématique. Livre 3 - Sciences Naturelles, **27**: 75–156.
- LEITCH AR & LEITCH IJ. 2008. Genomic plasticity and the diversity of polyploid plants. *Science* **320**: 481–483. <https://doi.org/10.1126/science.1153585>
- LEVIN DA. 1983. Polyploidy and novelty in flowering plants. *American Naturalist* **122**: 1–25.
- LEVIN DA. 2002. *The role of chromosomal change in plant evolution*. Oxford University Press, USA.
- MELANDER Y & WINGSTRAND KG. 1953. Gomori's hematoxylin as a chromosome stain. *Stain Technology* **28**: 217–223. <https://doi.org/10.3109/10520295309105236>
- MÜLLER K. 1941. Beiträge zur Systematik der Lebermoose II. *Hedwigia* **80**: 90–118.
- NATCHEVA R. 2008. New bryophyte records in the Balkans: 5. *Phytologia Balcanica* **14**: 426–428.
- NATCHEVA R, TSAKIRI E & DIHORU G. 2007. New bryophyte records in the Balkans: 1. *Phytologia Balcanica* **13**: 101–106.
- NEWTON ME. 1984. The cytogenetics of bryophytes. In DYER AF & DUCKETT JG (eds.), *The Experimental Biology of Bryophytes*, pp. 65–96, Academic Press, London.
- NEWTON ME. 1990. Genetic structure of hepatic species. *Botanical Journal of the Linnean Society* **104**: 215–229. <https://doi.org/10.1111/j.1095-8339.1990.tb02219.x>
- OTTO SP & WHITTON J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* **34**: 401–437. <https://doi.org/10.1146/annurev.genet.34.1.401>
- PRESTON CD, HARROWER CA & HILL MO. 2011. Distribution patterns in British and Irish liverworts and hornworts. *Journal of Bryology* **33**: 3–17. <https://doi.org/10.1179/1743282010Y.0000000001>
- RIESEBERG LH & WILLIS JH. 2007. Plant speciation. *Science* **317**: 910–914.
- ROSS HH. 1972. The origin of species diversity in ecological communities. *Taxon* **21**: 253–259. <https://doi.org/10.2307/1218192>
- SCHUSTER RM. 1992. *The Hepaticae and Anthocerotae of North America East of the Hundredth Meridian*, Vol. VI. Field Museum of Natural History, Chicago, IL.
- SHAW AJ & GOFFINET B (eds.). 2000. *Bryophyte biology*. Cambridge University Press.
- SLACK NG. 1990. Bryophytes and ecological niche theory. *Botanical Journal of the Linnean Society* **104**: 187–213. <https://doi.org/10.1111/j.1095-8339.1990.tb02218.x>
- SÖDERSTRÖM L. 1992. Invasions and range expansions and contractions of bryophytes. In: BATES JW & FARMER AM (eds.), *Bryophytes and lichens in a changing environment*, pp. 131–158, Oxford Academic.
- TE BEEST M, LE ROUX JJ, RICHARDSON DM, BRYSTING AK, SUDA J, KUBEŠOVÁ M & PÝŠEK P. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* **109**: 19–45. <https://doi.org/10.1093/aob/mcr277>

- TREIER UA, BROENNIMANN O, NORMAND S, GUI SAN A, SCHAFFNER U, STEINGER T & MÜLLER-SCHÄRER H. 2009. Shift in cytotype frequency and niche space in the invasive plant *Centaurea maculosa*. *Ecology* **90**: 1366–1377. <https://doi.org/10.1890/08-0420.1>
- TZONEV P. 2006. Plant communities, habitats and ecological changes in the vegetation. Proceedings of the *Botanical Congress*, Sofia, pp. 323–333.
- WYATT R & DAVISON PG. 2013. Notes on the Distribution and Ecology of *Riccia rhenana* in Georgia. *Evansia* **30**: 130–134. <https://doi.org/10.1639/079.030.0405>



REZIME

***Riccia fluitans* i *Riccia rhenana* (Marchantiales) u Bugarskoj, sa posebnim osvrtom na njihovu citogenetiku i distribuciju u Evropi**

Galin GOSPODINOV i Rayna NATCHEVA

Riccia fluitans i *R. rhenana* su široko rasprostranjene vodene jetrenjače, zahtevne za taksonomsko razgraničenje. Nedovoljna identifikacija dovodi do nedovoljnog poznavanja distribucije vrsta. U ovoj studiji sumirani su podaci o rasprostranjenosti za Bugarsku i komparirani sa zapisima dostupnim u GBIF bazi podataka za teritoriju Evrope. Na osnovu njihove distribucije u Bugarskoj i generalno u Evropi, komparirana je frekvencija njihovog pojavljivanja. Rezultati detaljnih studija kompleksa *Riccia fluitans* u Bugarskoj pokazuju suprotan obrazac u poređenju sa podacima o distribuciji dostupnim u GBIF bazi. Ovo je posledica problema u morfološkom razgraničenju dva taksona, koji su citogenetski jasno različiti. Upečatljiva prevalencija *R. fluitans* nad *R. rhenana* očigledna na osnovu GBIF podataka pokazuje potrebu za pažljivim određivanjem vrsta i ističe rizike korišćenja takvih baza podataka. Terenski podaci iz Bugarske ukazuju na specifične trendove za vrstu u pogledu porekla staništa i preferencija mikrostaništa.

Ključne reči: vodene vrste, diploidi, GBIF, haploidi, jetrenjače, *Riccia fluitans* complex, rasprostranjenje