

Report for the Doctoral thesis entitled “**Taxonomic and genetic investigation on sect. *Ricciella* genus *Riccia* in Europe with emphasis on *R. fluitans* and *R. rhenana*” presented by Galin Gospodinov.**

The Doctoral thesis presented by Galin Gospodinov deals with the clarification of the taxonomic and systematic relationships of a conflictive group of taxa included in subgenus *Ricciella* of the liverwort genus *Riccia* (Ricciaceae, Marchantiophyta). The thesis is geographically centered in Europe and an emphasis has been paid to the species' complex of *Riccia fluitans*/*R. rhenana* and also *Riccia canaliculata*/*R. duplex*. The thesis is organized in six chapters and final additional sections including conclusions, bibliography, supplementary data, etc.

Overall remarks. Figure captions generally should be written in a self-explanatory manner so that there is no need to search additional information in the text for their interpretation. In these regard, I have noticed many of them have not been sufficiently detailed. Especially the phylogenetic trees (i.e. Fig 4.4 do not indicate if values above branches indicate bootstrap values, there is not a distance scale of the tree, etc.)

Chapter 1. This chapter provides a detailed introduction to the taxonomic and systematic problematics of this group of species. It identifies morphological constraints that hinder reliable identification of the species and consequently have been considered historically differently by a variety of authors. It is a necessary chapter to introduce the reader into the back-and-forth taxonomy of the group. The information is well organised, and the arguments are easy to follow. I have noticed two references that have not been cited in this chapter and also in any of the introductory parts of the other chapters of the thesis. One deals with overall general diversity of liverworts and also provides updated numbers of taxa within Ricciaceae (see Söderstrom et al. 2016. World checklist of hornworts and liverworts. *PhytoKeys* 59: 1-828). The other reference which is more related to the research presented in this Doctoral Thesis is a recent study published by Levins et al. 2025 Evolution of sexual systems and regressive evolution in *Riccia*. *New Phytologist* 246: 769-784. <https://doi.org/10.1111/nph.20454>, that deals with molecular phylogeny of *Riccia* and includes information on reproductive systems, ploidy levels, etc, that is totally aligned with contents in chapters 4, 5, and 6 I guess that at the time of publication of that study the analyses and writing of the thesis may have had been at an advanced stage for its inclusion however, it would be necessary checking before the eventual publication of any of the chapters to avoid redundancy and potential overlap.

Chapter 2. This chapter provides insights into the survival during the unfavourable conditions coupled with resting periods of thalli of *Riccia fluitans* and *R. rhenana*. These taxa are widely sterile across their distribution range and the survival of apical meristems of thalli allows population recovery after unfavourable/resting periods. This mechanism is apparently highly efficient given the absence of production of spores. The chapter is basically based on observational data and could have strengthened their conclusions if experiments could have been designed in order to

investigate, if possible, the proportion of apical meristems finally contributing to the restart of the populations.

Chapter 3. This chapter presents a compilation of records of *Riccia fluitans*/*R. rhenana*, extracted from public databases. It identifies the most widespread distribution of *R. fluitans* compared to *R. rhenana* across Europe. Then it performs chromosome counting, as a means of distinguishing between the two taxa, on the existing Bulgarian populations of both species. The results obtained from such identification challenges the distribution pattern observed across Europe, as *R. rhenana* now emerges as more widespread than *R. fluitans* in Bulgaria. It also finds some correlation of the two taxa with different microhabitat occurrence. These results are interesting and would be desirable to be investigated at a broader geographical scale as they challenge the current knowledge on the distribution of the two taxa which may have implications for biodiversity assessment and conservation, especially at the edge areas of distribution where records are scarce.

Chapter 4. This chapter uses cytogenetic and sequence data to examine differentiation between taxa in two haploid/diploid species pairs of *Riccia*. This study provides for the first time C-values from several *Riccia* populations and finds a correspondence with chromosome counts, c-values and phylogenetic clustering. Although the number of populations investigated is to some extent limited, it still includes sampling from different countries. The results are promising as it identifies c-values as a potential, cheap and fast means of distinguishing between *R. fluitans*/*R. rhenana* and *R. canaliculata*/*R. duplex*, respectively. It also shows that genome sizes of diploids do not exactly double those of haploids indicating some degree of genome downsizing during their diversification process. It would be desirable to investigate if this downsize occurs at the time of the formation of diploids or is a progressive process across generation. To do this it would be worth producing diploids artificially (the colchicine-induced polyploids mentioned in the introduction of the chapter) and then obtain C-values from them to see if genome doubling is decoupled from genome downsizing. Caution should be taken to writing, in page 58 a reference to *rbcl* primers is specified, while this region is not used in the phylogenetic analysis, as indicated two lines before that.

Chapter 5. In this chapter evidence of allopolyploidy is obtained for *Riccia rhenana* using a combination of allozymes, plastid sequence and nuclear *gpd* sequence data thereby resolving the taxonomic problem of this species being considered a cytotype of *R. fluitans*. Overall I find sufficient empirical evidence to support this conclusion however other interpretations have not been sufficiently discussed. In the discussion section (page 81), the polyphyletic topology of *R. fluitans* in the plastid tree (page 77) is discussed in terms on cryptic speciation however, given the relatively restricted sampling the actual plastid diversity of the species is not sufficiently known and thus it could just indicate intraspecific diversity. It is also surprising that given the apparent rarity (or absence) of males in *Riccia fluitans* across its distribution range it acted as the paternal donor in the genome of *R. rhenana* which would deserve further exploration. On methodological grounds it is indicated that new primers were designed for *gpd* amplification but the sequence source from which these have

designed is not indicated. The separation of the two *gpd* sequences in the *R. rhenana* samples has been done by statistical methods (using mixed sequence reader). This method has some limitation which have not been specified and the actual base calling from the two genomes can only be validated by cloning and sequencing afterwards. This validating by cloning has not been done and thus, the diversity of sequences classified in A and B could have been overestimated. This has potential implications for the number of clades obtained afterwards in the phylogenetic analysis (Fig. 5.6 in page 78). It could have been interesting to obtain some reference sequences from cloning the amplicons of *R. rhenana* to assist (and confirm) the phasing analysis.

Chapter 6. This chapter makes a preliminary account to the taxonomy and systematics of section *Ricciella* using DNA sequences generated for three plastid regions. Sequences are generated de novo for seven taxa. Two phylogenetic analyses are conducted, one including sequences of *TrnL-F* and *rps4* with taxa of sect. *Ricciella* only and another one for *rbcl* including these sequences and additional sequences retrieved from GenBank from other *Riccia* species basically derived from data published in Cargill et al. 2016. Please note some inconsistency in labelling *Riccia stricta* is labelled to have an origin from Africa in Table 6.1 while it is labelled with the code AU (Australia) in Figure 6.1 page 90. While the conclusions support previous results from Cargill et al. 2016 and the recently published paper of Levins et al. (2025) it is surprising why the overall taxonomic conclusions are based solely on a gene tree obtained from *rbcl* region. Since the study of Cargill et al 2016 from which these sequences were obtained also considers sequences of *TrnL-F* and *rps4* it would have been desirable to combine all the molecular information at hand to obtain a more robust phylogenetic tree based on the three plastid regions. The selection of only *rbcl* for the phylogenetic analysis has not been justified in the chapter. This chapter may require substantial work to include the aforementioned published and available data and to account for possible overlap in aims and results already published in the paper of Levins et al. (2025).

In my opinion the Doctoral Thesis presented contains sufficient amount of work and substantial scientific advances (except for my comments in chapter 6) in the knowledge of a group of controversial taxa and therefore it should merit a **positive assessment** as it fully meets the requirements set out in Article 187(1-2) of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended).

Burjassot, 06/02/2026

Dr. José Gabriel Segarra Moragues

Reviewer

University of Valencia

Dept. of Botany and Geology

Burjassot (Valencia)

Spain