

In vitro propagation and conservation of the European endangered liverwort *Riella helicophylla*



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Abstract



Riella helicophylla

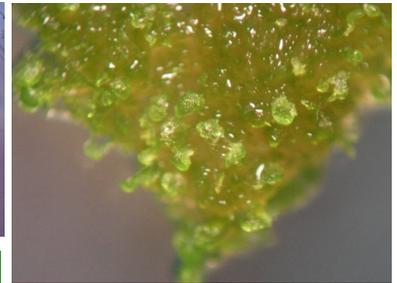
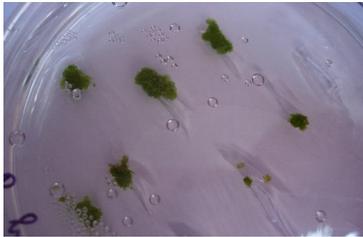
The liverwort genus *Riella* Mont. (Riellaceae, Sphaerocarpaceae), includes ca. 23 taxa growing commonly submerged in clean, shallow, fresh or brackish waters of seasonal ponds, streams and more rarely in permanent waters of arid and semiarid regions. The *Riella* species inhabit all continents except Antarctica and have disjunct distributions and scattered populations. The *Riella* species are rare and/or underrecorded due to its specific habitat types, ecology and biology. Most of the time, they survive in the spore bank. When grown in the laboratory from pond sediments, the cultures are often contaminated with algae and other organisms which prevent their use in experiments depending on the purity of the starting materials. In this study, establishment and propagation, as well as conservation issues of the *Riella* model species (namely *R. helicophylla* (Mont.) Hook. Spanish genotype) are given. The two phase system (solid and liquid) axenic cultures are developed for the purpose of achieving fully developed gametophytes. The disposal of xenic organism and the germinability of spores are some of the problems that were solved out. Spores were able to germinate on solid medium, and developed some kind of callous tissue that after being transferred to the two phase culture developed into green plants - gametophores. This method allows for obtaining large amounts of gametophytes free from algal contamination which will be used in subsequent high through output sequencing for the study of genetics and genomics of *Riella*.



Riella noronhai



Riella echinata



Thallose protonema and spore germination of *Riella helicophylla* (upper left) in axenic Condition and the bud formation (upper right) Two phase test culture (down pics)

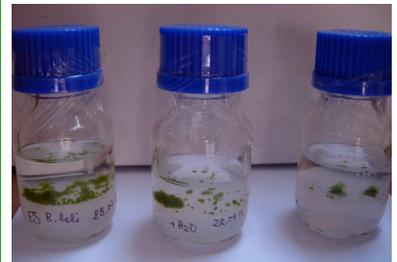


Riella helicophylla female (upper pic) and male (left pic) and in situ (down)

The genus *Riella* Mont. (Riellaceae, Sphaerocarpaceae) includes some 22 species of aquatic thallose liverworts and is distributed in areas of Mediterranean or subdesertic climate types on all continents except Antarctica. Their small size, ephemeral aquatic habitat, and dependence on the nature of the flooding season makes finding populations somewhat difficult. Populations are generally demographically fluctuant, with some years experiencing sudden demographic blooms and other of complete absence, as population growth may be inhibited both by too high or too low water levels. Furthermore, their specialised aquatic habitat offers very little of interest to bryologists, apart from *Riella*, and so they are often overlooked.

In Europe, 6 taxa are known to grow but some are doubtful and new species could arise. However, all species from the genus are rare and threatened and in urge for conservation due to habitat destruction.

The sporophyte of *Riella helicophylla* was collected and dried for two months. Spores seems to have some kind of dormancy and has to pass a certain time of complete drought. Afterwards, the sporophyte or spores were surface sterilized and placed to solid medium for germination. After achieving the germination to small thallose protonema and the thalli germs were noted it was placed to another fresh solid medium and the liquid solution enriched with salts imitating natural brackish water were added. The fully developed plants were achieved. At present male and female plants are unified in the same axenic flask with aim to get sporophyte development.



Fully developed plants of *Riella helicophylla* in axenic condition

