Experimental investigations on the variability of *Riccia gougetiana* and *Riccia ciliifera* from Czechoslovakia

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During the XII. International Phytogeographical Excursion through Czechoslovakia the senior author collected a big *Riccia* in South Slovakia which was determined by him as *Riccia gougetiana* Mont. The reasons for such a determination were: the big thalli measuring till 2.5 mm in width and very long cells in the ventral scales: till 180 μ ; These two characters distinguish the plant from the rather similar *Riccia ciliifera* Link, a widespread xerothermic species. Unfortunately, no spores were found (and these would have been crucial for determination).

The determination aroused a discussion between the senior author and two eminent central european bryologists: Dr. Boros Adam from Hungary and Dr. Jan Šmarda from Czechoslovakia who also collected this plant. In a common paper (Boros, Šmarda, Szweykowski, Vajda 1961) there is: ... „Die *Riccia* „*ciliifera*“ der beiden Standorte hielten J. Šmarda, J. Jovet-Ast and A. Boros für *R. ciliifera*. Dagegen meint J. Szweykowski, dass es kein *R. ciliifera* ist, weil das Moos sehr gross ist und die Zellen der Bauchschuppen in der Länge bis 180 Mikronen messen. Die Pflanze gehört demnach nach Szw (i.e. Szweykowski — J.S.!) zu *Riccia gougetiana* Mont., die für ČSR neu wäre. A. Boros bemerkt dazu, dass in Ungarn solche Formen oft vorkommen die nach seinen Erfahrung nur Modifikationen der *Riccia ciliifera* sind“ (end of quotation).

In this situation we decided to investigate this plant more thoroughly by biometrical, cytological, and experimental methods.

MATERIAL

The realization of the project was delayed from various reasons until the spring 1963. On the 11th of April 1963 two groups of plants were taken from the herbarium; these were:

a. "*Riccia gougetiana*" collected by the senior author in Czechoslovakia (naked soil on a steppe slope of the hill Tarbucka by Velky Kamenec, S. Slovakia) on the 8th of Juli 1958, and

b. *Riccia ciliifera* Link also from Czechoslovakia (on soil between

serpentine rocks at the river Jihlavka by Mohelno, Central Moravia) collected by J. Szweykowski on the 24th of Juli 1958.

About twenty thalli of each species were put on wet soil into Petri dishes (the soil consisted of 50%:50% mixture of quartz sand and clay). In spite of being preserved in herbarium for 5 years in an exsiccated state they began to grow in a short time. After about five weeks they were transplanted to a plastic box 20 cm $\times$ 20 cm, each species taking a half of the soil surface. The box was covered with glass and put into a glasshouse. The plants were sprayed daily with distilled water using a plastic atomizer. They were grown, therefore, under identical conditions. The differences observed may be considered as based on genetical constitution of the plant and not as modifications caused by environment. Such method permits us to distinguish between phenotypical and genotypical variability. For comparison both plants from herbarium (not cultivated) and living ones (after cultivation) were taken.

Biometrical characteristic

Method: The width of thalli, the length and the width of cells in ventral scales and their mean surface were measured. The width of the thalli was determined on single (not branched) plants near the apex, in the place where the thallus is normally wide.

The ventral scales in both *Riccia ciliifera* and *Riccia gougetiana* consist of various cells: the marginal, very elongated ones, the isodiametric cells at scale basis, and moderately elongated cells (c. 2—3 times as long as wide). Only the last category of cells was taken into consideration (Fig. 1).

Fig. 1. A piece of a ventral scale of *Riccia gougetiana*. The broken line shows where the measurements were carried out.

The mean surfaces of cells were determined according to Ammann's method (J. Ammann 1932): a brass diaphragm with a rectangular opening 5 $\times$ 7 mm was put into the eyepiece. Then the cells of a ventral scale were drawn with an Abbe apparatus. The surface of the rectan-

gular field was measured and the number of cells in it was counted. Then, by dividing the surface by the cell number the mean cell surface was calculated (Fig. 2).

Fig. 2. Showing the amounts of cells in equal areas in *Riccia ciliifera* (A) and *R. gougetiana* (B).

In every case but one the calculations were based on 50 measurements. The mean cell surface in the cultivated *Riccia ciliifera* was measured in 10 plants only because the ventral scales were so small and delicate that it was not possible to get 50 of them big enough for measuring.

RESULTS

1. The thallus width (Fig. 3 and table 1). The thallus in *Riccia gougetiana* is much wider than that of *Riccia ciliifera*. It is interesting that this character does not change in the culture at all in *Riccia ciliifera* whereas cultivated *Riccia gougetiana* became about twice as wide as specimens collected in the field.

Table 1
Thallus width

		Amplitude	$m \pm M$	$\pm \sigma$	V
<i>Riccia ciliifera</i>	cultivated	0.7–1.6 mm	0.9 ± 0.03 mm	± 0.22 mm	24.4%
	herbarium	0.5–1.3 mm	0.9 ± 0.03 mm	± 0.22 mm	24.4%
<i>Riccia gougetiana</i>	cultivated	2.5–4.3 mm	3.3 ± 0.07 mm	± 0.48 mm	14.5%
	herbarium	1.2–2.4 mm	1.7 ± 0.03 mm	± 0.27 mm	15.9%

Explanation of symbols in biometrical tables: $m \pm M$ — statistical mean and standard error; $\pm \sigma$ — standard deviation; v — coefficient of variation.

Fig. 3. Diagram illustrating the variability of the thallus width.

1 — *Riccia ciliifera*: 2 — cultivated, 3 — herbarium specimens. 4 — *Riccia gougetiana*: 5 — cultivated, 6 — herbarium specimens.

2. The length of ventral scale cells (Fig. 4 and table 2). *Riccia ciliifera* has shorter cells in the ventral scales than *Riccia gougetiana* — this fact was already stated by K. Müller (1950—1958). In culture the cells became a little shorter both in *Riccia ciliifera* and in *Riccia gougetiana*. The effects of cultivations were in both species rather small.

Fig. 4. Diagram illustrating the variability of the ventral scales cell length.

1 — *Riccia ciliifera*: 2 — cultivated, 3 — herbarium specimens. 4 — *Riccia gougetiana*: 5 — cultivated, 6 — herbarium specimens.

Table 2
Ventral scales cell length

		Amplitude	$m \pm M$	$\pm \sigma$	V
<i>Riccia ciliifera</i>	cultivated	50—90 μ	$72 \pm 1 \mu$	$\pm 9 \mu$	11.0%
	herbarium	70—140 μ	$90 \pm 2 \mu$	$\pm 15 \mu$	16.5%
<i>Riccia gougetiana</i>	cultivated	80—140 μ	$108 \pm 2 \mu$	$\pm 13 \mu$	11.6%
	herbarium	80—160 μ	$121 \pm 2 \mu$	$\pm 17 \mu$	14.1%

3. The width of ventral scale cells (Fig. 5 and table 3). Both species are rather similar in this respect. In the culture the cells of *Riccia ciliifera* became a little narrower whereas in *R. gougetiana* they practically did not change.

Fig. 5. Diagram illustrating the variability of the ventral scales cell width.

1 — *Riccia ciliifera*: 2 — cultivated, 3 — herbarium specimens. 4 — *Riccia gougetiana*: 5 — cultivated, 6 — herbarium specimens.

Table 3
Ventral scales cell width

		Amplitude	$m \pm M$	$\pm \sigma$	V
<i>Riccia ciliifera</i>	cultivated	20–48 μ	$39 \pm 1 \mu$	$\pm 6 \mu$	15.7%
	herbarium	20–68 μ	$42 \pm 1 \mu$	$\pm 4 \mu$	10.4%
<i>Riccia gougetiana</i>	cultivated	36–56 μ	$49 \pm 1 \mu$	$\pm 5 \mu$	10.0%
	herbarium	28–56 μ	$38 \pm 1 \mu$	$\pm 8 \mu$	28.3%

4. Ventral scales, mean cell surface (Fig. 6 and table 4). Both plants are very variable in this respect but the cells of *Riccia gougetiana* are, in general, bigger than those of *Riccia ciliifera*. In the culture the cells of the first became larger; unfortunately, the ventral scales in cultivated *R. ciliifera* were so poorly developed that it was impossible to find 50 scales big enough for calculating the mean cell surface.

Fig. 6. Diagram illustrating the variability of the ventral scales mean cell surface.

1 — *Riccia ciliifera*: 2 — cultivated, 3 — herbarium specimens. 4 — *Riccia gougetiana*: 5 — cultivated, 6 — herbarium specimens.

Table 4
Ventral scales, mean cell surface

		Amplitude	$m \pm M$	$\pm \sigma$	V
<i>Riccia ciliifera</i>	cultivated	1700—2500 μ^2	not calculated		
	herbarium	1200—4200 μ^2	2340—85 μ^2	$\pm 600 \mu^2$	25.7%
<i>Riccia gougetiana</i>	cultivated	2600—5200 μ^2	3616 $\pm 70 \mu^2$	$\pm 492 \mu^2$	13.6%
	herbarium	2200—4400 μ^2	3202 $\pm 88 \mu^2$	$\pm 620 \mu^2$	19.1%

Cytology

Both plants have the same chromosome number $n=8$. They are therefore diploids. Unfortunately, we met with big difficulties in making good preparations (acetic-orcein and acetic-carmin squashes) of thallus apices and we were unable to investigate the morphology of the chromosomes.

DISCUSSION

Both herbarium specimens we investigated show distinct differences in thallus width and in the length of the ventral scale cells (Figs. 7, 8). These differences not only did not disappear in our cultures but, on the

Fig. 7. *Riccia gougetiana* (to the left) and *Riccia ciliifera* (to the right) cultivated under identical conditions.

Fig. 8. *Riccia gougetiana* from southern Slovakia cultivated in the glasshouse.

contrary, became even more distinct. Moreover, the reactions of the plants to the culture conditions were different: *Riccia gougetiana* was just thriving under the glass (it became twice as large there as in natural habitat) whereas *Riccia ciliifera* did not change its external dimensions, diminishing at the same time the ventral scale cells. It is not possible therefore to consider the big plant merely as a modification of *Riccia ciliifera*.

The variability of both plants is of such a kind that it does not permit to keep them apart on the basis of a single character when sterile. We

Fig. 9. A-B. Scatter diagram for *Riccia ciliifera* and *R. gougetiana* based on the measurements of 50 thalli from the herbarium (A) and after cultivation (B). The characters used were: the thallus width (y-axes) and ventral scales cell length (x-axes).

put, therefore, two characters simultaneously into a scatterdiagram (Fig. 9). When using the thallus width and ventral scale cell length for constructing the scatter diagram it is evident that both populations do not overlap practically. The differences in cultivated material are quite obvious, too.

The method of constructing a scatter diagram may be used for practical determination of sterile plants. But it should be kept in mind that there exists a third similar species of *Riccia*, namely *Riccia zachariae*

Lorbeer ex K. Müller Leberm Eur. Ergzbd. 1940, which being as big as *Riccia gougetiana* has smaller ventral scale cells. It may be very difficult then to recognize that species preserved in herbarium. It can be determined probably only in vivo being a tetraploid with a chromosome number $n=16$.

The comparison of the scatter diagrams (Fig. 9, A and B) shows very clearly the usefulness of culture method for solving critical taxonomical problems.

Fig. 10. *Riccia gougetiana* from southern Slovakia (A-F, L-O) and *Riccia ciliifera* from Mohelno (G-K, P-S): A-D and G-I epidermal cells, E, F, J, K — marginal cilia, L-S — cross sections of thalli.

SUMMARY AND CONCLUSIONS

1. Two populations belonging to two different species of genus *Riccia* (*Hepaticae*, *Marchantiales*) namely *Riccia ciliifera* and *Riccia gougetiana* from two stations in Czechoslovakia were compared by biometrical and cytological methods using material both from herbarium and cultivated in a glasshouse.
2. They show constant differences in at least two characters: the thallus width and the ventral scale cell length.
3. The differences between these two plants became more striking in the culture.
4. A scatter diagram method is advised for determination of sterile plants of *Riccia gougetiana-ciliifera* complex.
5. The chromosome number in gametophytic tissue was investigated and $n=8$ in both cases was found.
6. The usefulness of the method of cultivations of critical plants for solving taxonomical problems is emphasized.

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*Doświadczalne badania nad zmiennością dwóch gatunków
rodzaju Riccia (Hepaticae)*

Streszczenie

Autorzy zbadali zmienność czterech cech (szerokość plechy, długość i szerokość komórek w łuskach brzusznych oraz średnie pole tych komórek) u dwóch populacji rodzaju *Riccia* z południowej Słowacji. Jedna z populacji należała do typowej *Riccia ciliifera*, druga zaś, obejmująca bardzo duże rośliny, oznaczana była bądź jako *R. ciliifera*, bądź jako *R. gougetiana*. 50 roślin z każdej populacji hodowano w identycznych warunkach i zmienność ich porównano biometrycznie. Wyniki hodowli dowodzą, iż duże rośliny różnią się od roślin *R. ciliifera* szeregiem cech oraz typem reakcji na warunki środowiska. Autorzy wyciągają z tego wniosek, że nie mogą one być uważane za formy *Riccia ciliifera* i zaliczają je do *R. gougetiana*.