

A species concept in the genus *Pleurocladula* (Hepaticae)

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Abstract

On the basis of comparative cultures, biometric studies, variability of isozymes and phenolic compounds, the authors come to conclusion that two genetically different taxa are in evidence.

INTRODUCTION

The two critical taxa *Pleurocladula albescens* (Hook.) Grolle and *Pleurocladula islandica* (Nees) Grolle (Grolle 1979) are common-found growing in the alpine belts of most European mountain ranges. Their taxonomic status has been frequently, debated, but still there is no agreement among hepatologists on this issue. They are treated as different species (Nees 1838, Pearson 1902, Krzakowa 1972), as varieties (Spruce 1882, Schuster 1950, Muller 1954, Arnell 1956 and others), or even as modifications of only one species (Jones 1958, Schuster 1974, Schuster and Damsholt 1974, Paton 1976). The main reason for these differences in opinions in the existence, according to many authors, of intermediate forms of the two taxa in question.

MATERIAL AND METHODS

Both *Pleurocladula* taxa are widespread in the Tatry Mountains of southern Poland and are often found together. A biometrical study of 47 herbarium specimens collected in these mountains has shown that although they can be generally separated without difficulty, some few intermediate forms do exist (Szweykowski and Krzakowa 1966). However, as has been shown earlier (Szweykowski 1958), the

discovery of such forms among herbarium specimens can be misleading, as two genetically and taxonomically different forms can form similar phenotypes when grown under different ecological conditions. In such doubtful cases, proper interpretation of transitional forms can be done only in simultaneous culture under identical conditions.

This method was applied to *Pleurocladula* plants collected in the Tatry Mts. (Krzakowa 1972). The results of these studies so far not seem to have been properly understood or have been disregarded (Schuster and Damsholt 1974). Additional studies have been performed and some old observations have been revised with new, more precise mathematical methods.

RESULTS

THE PROBLEM OF INTERMEDIATE FORMS

It follows from observations (Krzakowa 1972) that the recognition of such intermediate forms depends largely, at last in *Pleurocladula*, on the number and kind of traits considered in the study. From the midst of 12 populations investigated, population no. 4 shows evidence of a transitional character when put on a scatter diagram constructed on the basis of amphigastrium width and the relation of leaf width to the leaf incision depth (Fig. 1) or amphigastrium width and the relation of leaf incision depth to the leaf length (Fig. 2). The same population and — which is more important — the same sample behaves

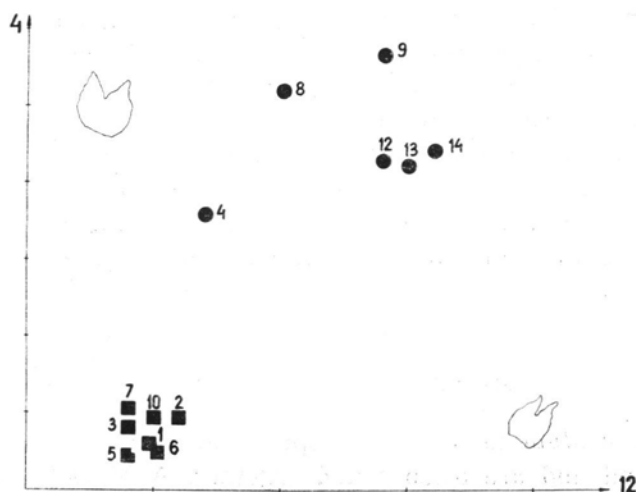


Fig. 1. Scatter diagram constructed on the basis of the mean values of singular characters

4 — the relation of leaf width to the leaf incision depth, 12 — amphigastrium width

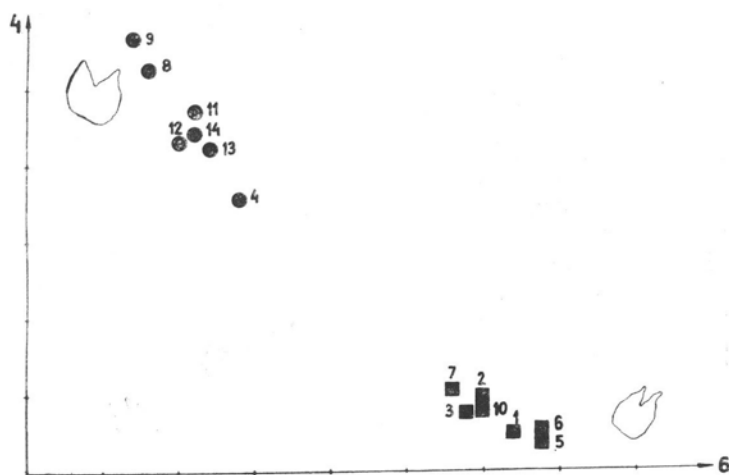


Fig. 2. Scatter diagram constructed on the basis of the mean values of singular characters

4 — the relation of leaf width to the leaf incision depth, 6 — the relation of leaf incision depth to the leaf length

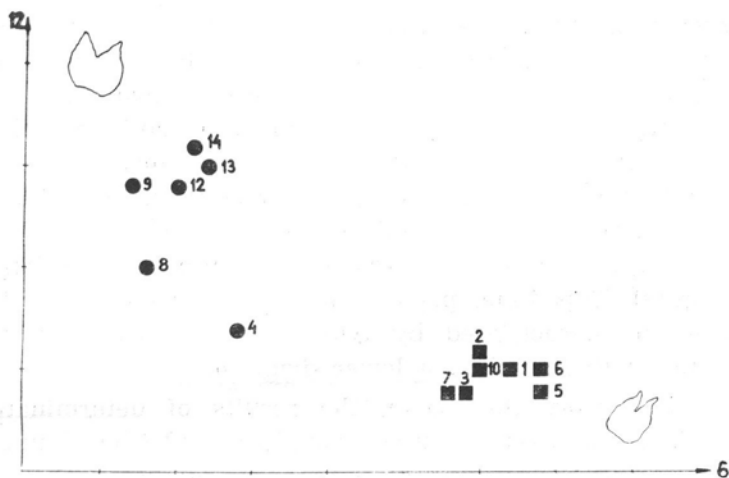


Fig. 3. Scatter diagram constructed on the basis of the mean values of singular characters

12 — amphigastrium width, 6 — the relation of leaf incision depth to the leaf length

as typical *P. albescens* on the diagrams when characterised by the relations of the leaf width to the leaf incision depth and of leaf incision depth to the leaf length (Fig. 3) or the leaf width to the relation of the leaf incision depth to the leaf length, respectively (Fig. 4).

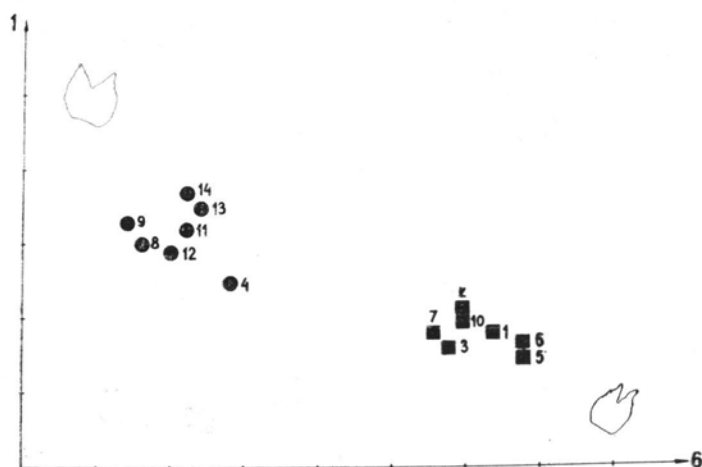


Fig. 4. Scatter diagram constructed on the basis of the mean values of singular characters

1 — leaf width, 6 — the relation of leaf incision depth to the leaf length

Especially instructive in this respect are the diagrams of 12 populations cultured under similar conditions (Fig. 5). On the upper diagram of Fig. 5 the population no. 12 clusters with *P. islandica* when traits taken under consideration were leaf width and amphigastrium width. When the sample was described in terms of two traits (relations leaf width to the incision depth and leaf incision depth to leaf length), however, it is situated inside of the *P. albescens* cluster (Fig. 6 upper diagram). That this second clustering (i.e. with *P. albescens*) is proper shown by the similarly constructed scatter diagram for the same populations after a period of 7 months of cultivation (lower diagram of the same figure). This time, population no. 12 clusters with *P. albescens* even when characterised by two single traits (leaf width and amphigastrium width) see Fig. 5 lower diagram.

As is evident from the above, the results of determination are highly dependent on the traits used: sample no. 12 (Fig. 5 upper diagram) being a *P. albescens* could be termed *P. islandica* if characterised by leaf and amphigastrium width. The only means of avoiding such situation is to use as many characters as possible and treat them simultaneously. For this we use multivariate biometrical methods.

One of these methods, that of ordination according to Euclidean distances, was applied to the *Pleurocladula* material (Krzakowa 1972). The ordination of 12 population samples taken in the field (i.e. herbarium samples) shows sample no. 12 as an intermediate form (Fig. 7). The samples were characterised according to 14 different traits (for details consult the original paper by Krzakowa l.c.). The den-

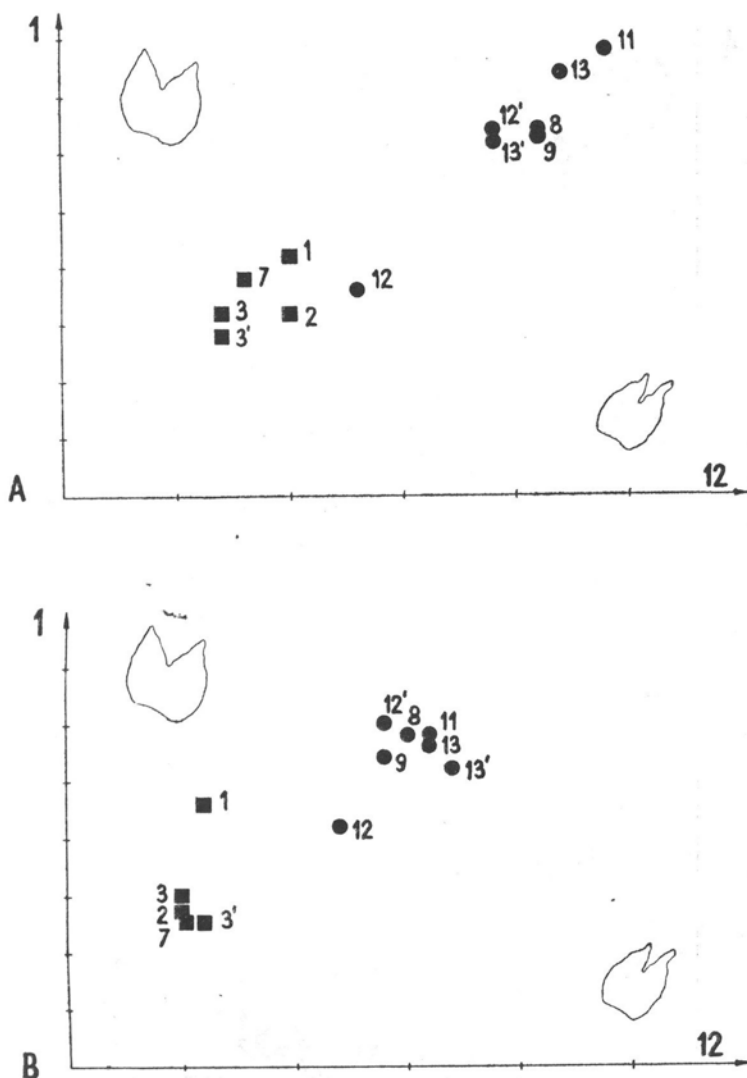


Fig. 5. Scatter diagrams constructed on the basis of the mean values of characters
1 — leaf width, 12 — amphigastrium width for herbarium (A) and cultivated material (B)

drite (Fig. 7.2) shows samples of the same populations after 7 months of culture in a glasshouse. This time, sample no. 12 clusters with *P. albescens*; the dendrite shows four replications of each sample separately.

When we apply principal components analysis (Blackith and Reyment 1971) to the results of the same experiment, the picture

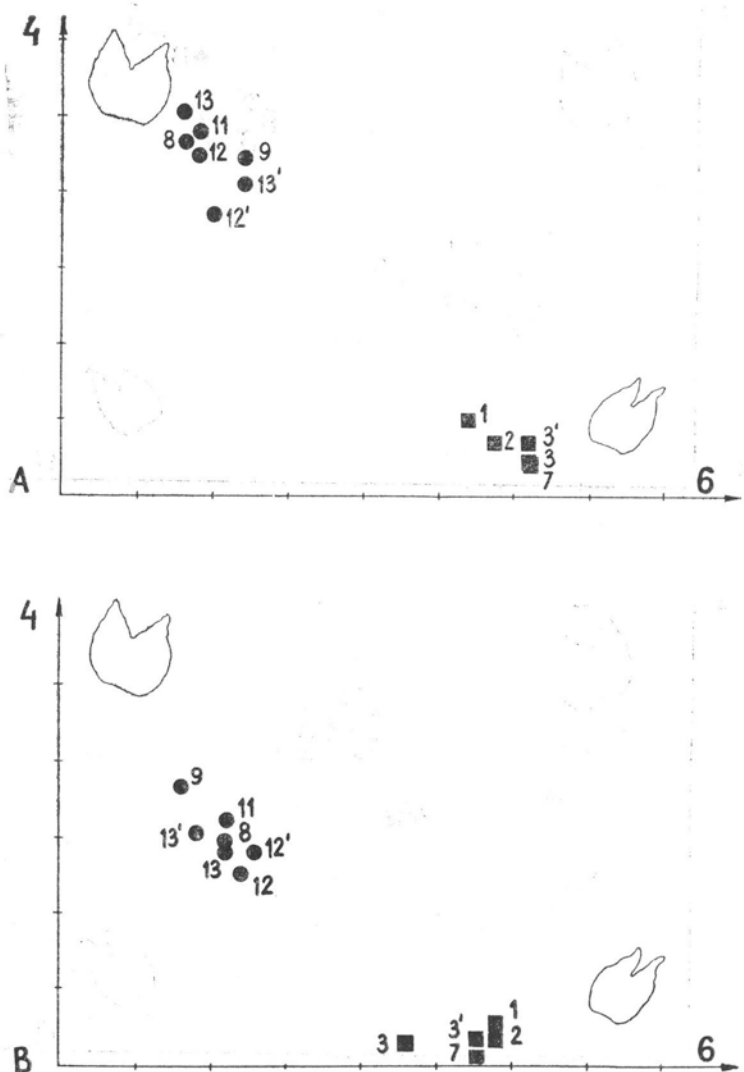


Fig. 6. Scatter diagrams constructed on the basis of the mean values of characters

For herbarium (A) and cultivated material (B)

For explanation see Fig. 2

is even more clear (Fig. 8). The hiatus between *P. islandica* and *P. albescens* is very distinct. At the same time one can see that population no. 12 is not exactly intermediate; it is only genetically more different from the rest.

By multivariate methods which give us the overall similarity or dissimilarity of the taxa studied, the information on the importance

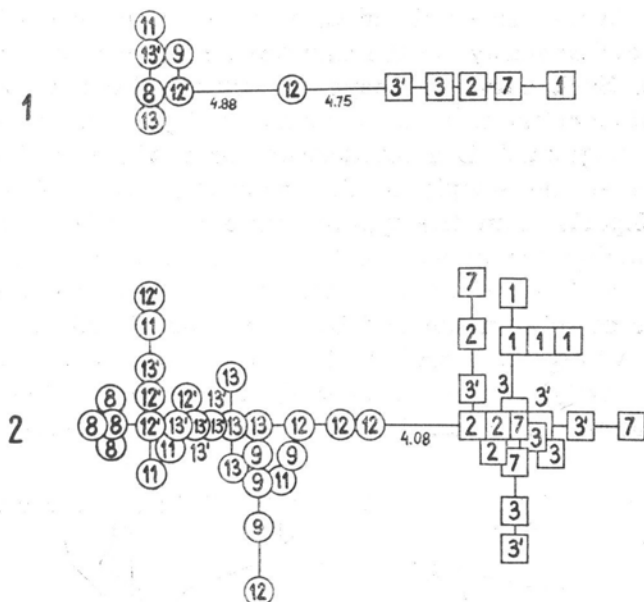


Fig. 7. Dendrite constructed on the basis of 14 characters for initial (1) and cultivated material (2)

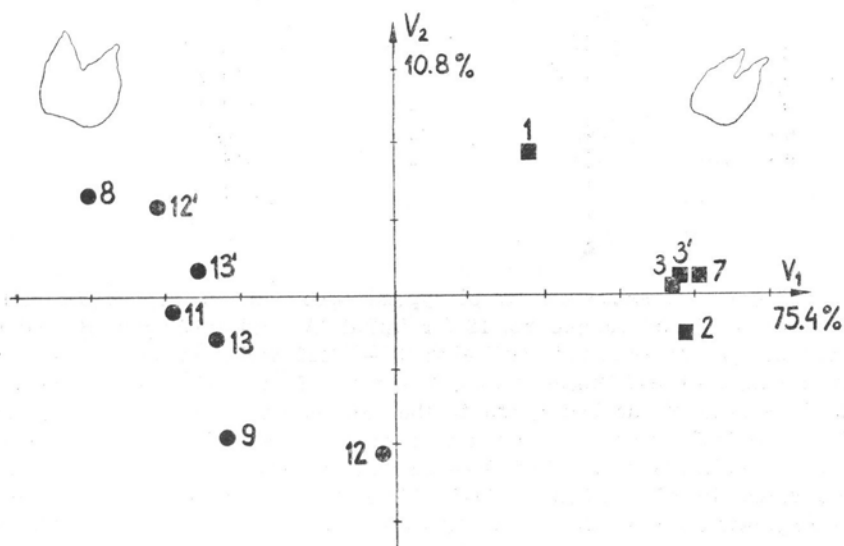


Fig. 8. Scatter diagram constructed on the basis of two principal components

of particular traits is usually lost and this information cannot be easily recovered. This is a serious drawback of the methods and therefore there are several additional techniques to avoid this inconvenience. One of these techniques is the method of shape and variability curves by Jentys-Szaferowa (1959). The curves, which are not easily

Technically, is hardly possible to give direct proof of this hypothesis. But the fact that intermediate samples lost this intermediacy after a period of culture speaks in favour of such an explanation.

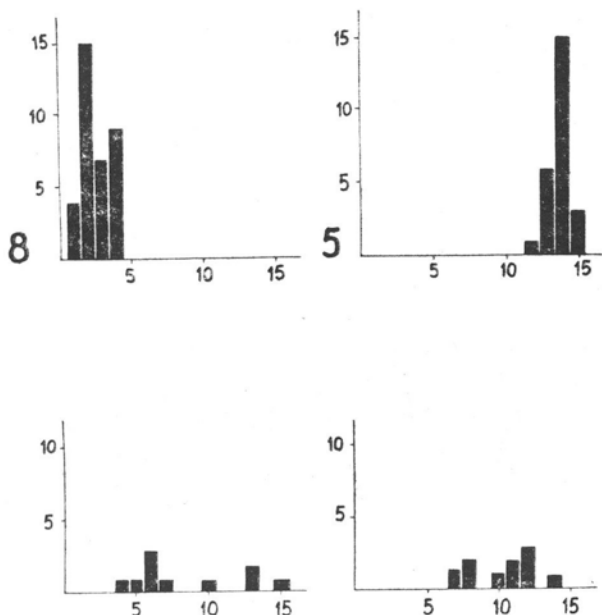


Fig. 10. Diagrams of Anderson's coefficient for typical populations of *P. albescens* (8) and *P. islandica* (5) and individual samples of intermediate value

In conclusion, forms of intermediate character between *Pleurocladula albescens* and *P. islandica* found in the Tatry Mts., are either environmentally induced modifications or plants genetically distinct from the typical form. In the later case they are simply different, their "intermediacy" is to some extent "artificial" and depends on traits used in describing the material.

DISTINGUISHING CHARACTERISTICS

It has been said above that the problem of using appropriate characteristics in describing taxa is a crucial one. Taking into account the notorious morphological plasticity of liverworts, there is little hope of finding singular characteristics which could be used for safe separation of closely related and similar (i.e. "critical") taxa. In the case of *Pleurocladula* we could not find any morphological character of this kind (see the polygons of variability of particular traits (Fig. 11).

The situation changes drastically when one uses more than one characteristics at a time (i.e. simultaneously). And as is well known

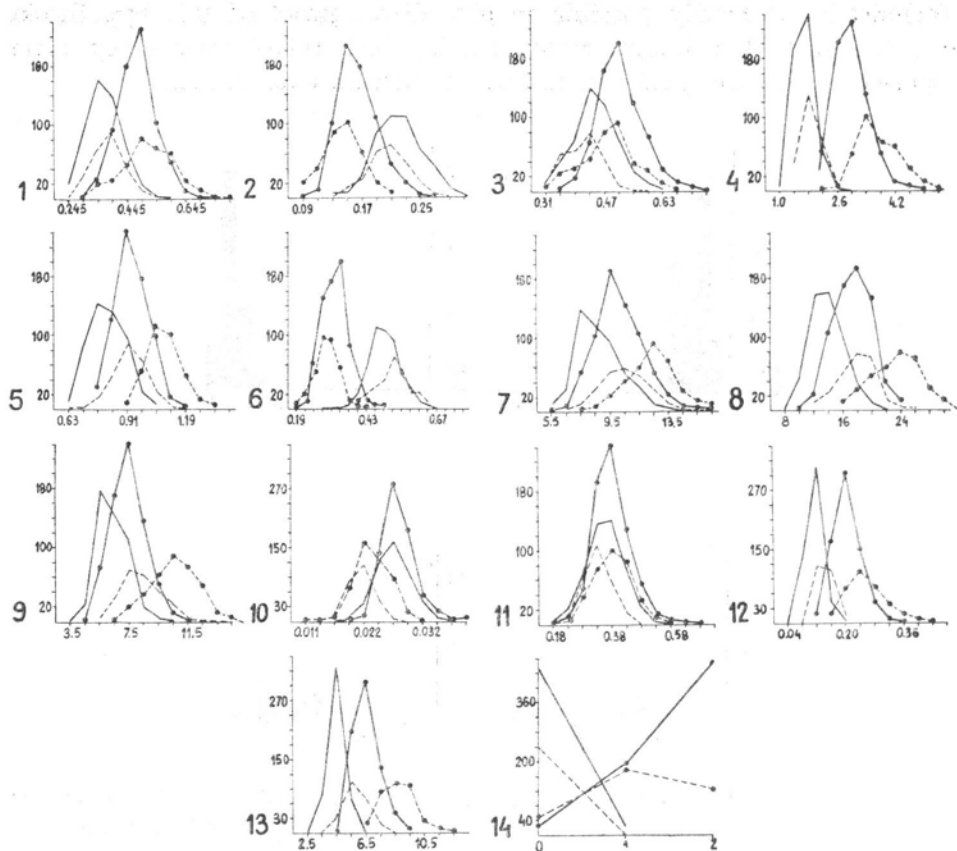


Fig. 11. Variability polygons of 14 characters for herbarium (discontinuous lines) and cultivated material (continuous lines)

P. albescens — lines with solid circles, *P. islandica* — lines without circles

from the numerical taxonomy, our results are dependent on the number of traits used. The more traits one takes into consideration the more proper — more precise — results one gets. The problem with liverworts, however, is that having a comparatively simple structure they do not allow taking very numerous characteristics, at least as far as their morphology and anatomy is concerned. Therefore, it is very important for the future evolution of bryophyte taxonomy to find new categories of characters that could be used as taxonomical markers.

As far as *Pleurocladula* is concerned, the cytological characters are of no use: both taxa show the same number of chromosomes $n=9$ (Krzakowa 1969) and the chromosomes are very small, not allowing for checking their morphology to apply the new methods of differential staining. On the other hand, chemical characteristics turned out to be very promising. We studied two kinds of these: phenolic compounds and some enzyme systems.

As far as phenolic compounds are concerned (Krzakowa 1980), 14 populations from various parts of the Tatry Mts., were studied. At least 100 mg of dry plants (aprox. 1000 shoots from an absolutely clean population sample) were taken for analysis of each population. The results are as follows: there are 40 various phenolic compounds present in both *Pleurocladula* taxa. Plants that we call *P. islandica* are rather homogenous: their chromatograms are very similar as far as number and character of spots are concerned. In addition, there are three spots never occurring in *P. albescens* i.e. they can serve as taxonomic markers (Fig. 12). Plants that we call *P. albescens* are more variable: their chromatograms vary in regard to both the number and kind of spots. In spite of this, however, the plants show also three different spots which never occurred in *P. islandica*. It follows that both taxa can be positively characterised, i.e., both have their characteristic phenolic components which can be used for distinguishing them. This discovery is especially important because phenolics are fairly stable substances and can be found in herbarium samples too. Analyses of phenolic compounds were performed on dry plants collected about 10 years before the analyses were made.



Fig. 12. Chromatographic profiles of phenolic compounds of *P. albescens* (A) and *P. islandica* (B)

Still more difficult technically are enzyme analyses in *Pleurocladula*. One must, obviously, perform these analyses not only on living plants, but the plants have to be grown at least some months under similar conditions because of possible environmental influence on isoenzyme patterns. This is very cumbersome and very seriously limits the application of these methods to taxonomical problems. We assayed

6 different populations of three localities where both species grew together. From each locality one population of *P. islandica* and one population of *P. albescens* was taken for analysis. As is well known, both *Pleurocladula* species are very small plants, and individual stems cannot be examined as easily as we could do in our study on *Plagiochila* (Krzakowa and Szweykowski 1979). Therefore, each analysis was made of 60 leafy stems crushed together. Three different enzyme systems were assayed: peroxidase (PX), esterase (EST) and acid phosphatase (AP). Two of them — PX and EST — show visible differences between both taxa (Fig. 13).

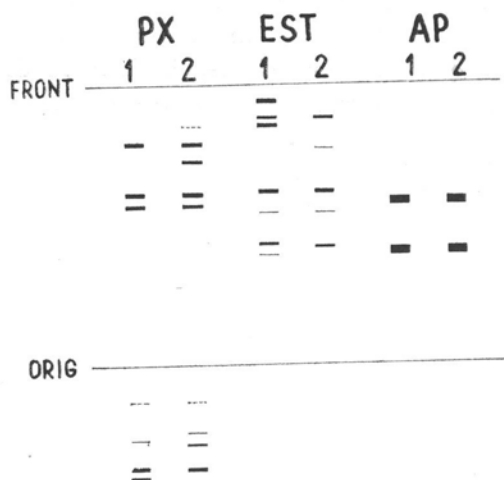


Fig. 13. Isozymes patterns after starch gel electrophoresis for three enzyme systems: Peroxidase (PX), Esterase (EST) and Acid Phosphatase (AP)
1 — *P. albescens*, 2 — *P. islandica*

As isozyme patterns are under direct genetic control, consistent and stable differences exhibited in these patterns should be not overlooked in discussing the taxonomic status of both *Pleurocladulas*.

DISCUSSION

When discussing problems of taxonomic distinction, two different points must be taken into consideration. Firstly, as the differences between two groups of organisms may be of two different kinds, modificatory or genetically conditioned, one has to realize that their importance for the taxonomist is by no means the same. Only the second category is valied as a basis for taxa recognition. It follows that the possibility of distinguishing between these two kinds of varia-

bility is of primary importance to the taxonomist. Secondly, because such genetically different groups of organisms do exist in nature, they should be taxonomically recognized as separate taxa. But their taxonomical rank is to some extent arbitrary and depends on many circumstances such as degree of difficulty in recognition and on the presence of reliable diagnostic characteristics.

Among the various taxonomic categories, that of species is of utmost importance not only because it is the basic taxonomical unit but also because it can be described quite strictly in biological and not only in classificatory terms. On other words, a species is, in a sense, a biological reality that should be discovered by a taxonomist (Szwedkowski 1978). Unfortunately, the biological definition of species is non-operational in the sense that, usually, there is no possibility to check the existence of gene flow between populations. Therefore, we have to add some additional concepts, and the concept of morphological hiatus — morphological in the broadest sense of the word, i.e., including anatomy, embryology, cytology, ultrastructure, chemistry and so on — is a most useful one. Obviously, a hiatus must be of a complex nature: many different characteristics should show a break in their variability to serve at the basis for species delimitation. The complex nature of taxonomical hiatus is often seen in situations when no one characteristic taken alone shows a hiatus, but combinations of characteristics do.

Returning to the problem of taxonomic status of *Pleurocladula albescens* and *Pleurocladula islandica*, we can summarize the results of our work in the respect as follows.

1. There is little doubt that there are two genetically different groups of plants belonging to the genus *Pleurocladula* and corresponding to two taxa called *P. albescens* and *P. islandica*. They behave differently in comparative cultures and they show different isoenzyme patterns.

2. Morphological hiatus between the two groups does exist: however, it can be sometimes difficult to observe in dried herbarium plants. In such cases a detailed biometrical study and phenolic compounds spectrum can help. The intermediate forms reported by many authors, at least in the Tatry Mts., have turned out to be either environmentally induced modifications of typical forms or genetical variants that are morphologically different. They are by no means intermediate. It should be stressed, however, that the ultimate judgment whether a form is intermediate or not can be achieved only after a proper combination of characteristics is studied. To be safe, one should investigate as many characteristics as possible. One or two haphazardly taken traits can fail — and this is frequently the case — to distinguish even well characterised entities. For example two "good" species of *Pellia epiphylla* and *Pellia endiviifolia* cannot be separated on the basis of cell size. Therefore, the results of Schuster and Damsholt (1974)

cannot be taken as arguments against taxonomic separation of *Pleurocladula albescens* and *Pleurocladula islandica*. Similarly, superficial studies on relatively few herbarium samples (Paton 1976) cannot be relevant here. The study of *Pleurocladula* (Krzakowa 1972) was based on examination of a minimum of 100 randomly collected shoots per population, the average being 250 per population. From each shoot one leaf was taken for measurements. To obtain comparable data, the third leaf under the shoot apex was constantly taken. Only in cases when the third leaf was not properly developed, that is, damaged by insects or nematodes, the next one was detached. This is the meaning of the expression "well formed leaf" (Krzakowa 1972). Therefore, the supposition that "only the typical selected leaves" were studied (Paton 1976) is simply not true. Altogether 3570 herbarium leaves and 3900 leaves from cultures were measured. A total of 64,890 measurements was performed.

3. Distinguishing characteristics are, unfortunately, rather scarce. To study this problem in more detail, we performed the analysis of discrimination power (Rao 1965, Caliński and Kaczmarek 1977) of characteristics used in Krzakowa's study. Six characteristics turned out to be highly significant in distinguishing between the taxa. The sequence of characteristics in order of diminishing discriminatory power is as follow: a) the presence or absence of lateral outgrowth on amphigastria (Fig. 11, char. no. 14); b) relation of leaf incision depth to the leaf length (Fig. 11, char. no. 6); c) width of amphigastrium base measured in number of cells (Fig. 11, char. no. 4); d) amphigastrium width (Fig. 11, char. no. 12); e) relation of leaf width to leaf length (Fig. 11, char. no. 13); f) width of the leaf lobe measured in number of cells at its base. All cell size characteristics failed to distinguish between *P. albescens* and *P. islandica*. This is specially interesting in respect to the results of Schuster and Damsholt (1974). In addition, the phenolic compounds spectra are also different (Krzakowa 1980). It follows that only through analyses of ample material involving these characteristics can be taken as serious arguments in the evaluation of the taxonomic value of the two taxa in question.

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Koncepcja gatunku u wątrobowców na przykładzie rodzaju *Pleurocladula*

Streszczenie

Na podstawie analizy biometrycznej materiałów zielnikowych i kultur porównawczych jak również badań biochemicznych (zmienność izozymów i związków fenolowych) wykazano, że dwa gatunki rodzaju *Pleurocladula* (*P. albescens* (Hook.) Grolle i *P. islandica* (Nees) Grolle) są genetycznie odmienne.