GEOGRAPHIC DISTRIBUTION OF *PELLIA* SPP. (*HEPATICA*E, *METZGERIA*LES) IN POLAND BASED ON ELECTROPHORETIC IDENTIFICATION

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ABSTRACT

Many species of liverworts (*Hepaticae*) are morphologically plastic to such an extent that their proper recognition may cause difficulties even for a specialist. Central European species of the genus *Pellia* are notorious in this respect. Searching for less malleable diagnostic characters (than morphological and/or anatomical ones) we showed recently that the electrophoretic phenotypes (obtained by means of separation of enzyme proteins on starch gels) can be used for reliable identification of *Pellia* species with much reliability. During the last 20 years we performed electrophoretic identification of over 500 (exactly 541) colonies and discovered that at least two of the "classic" species of the genus *Pellia* are in fact species complexes comprising taxa having the character of sibling species. In this paper we have mapped all stations of electrophoretically identified *Pellia* species, including the sibling species mentioned; marked differences in their geographical distributions are discussed. Our work clearly shows that using more natural taxa (i.e. genetically defined sibling species instead of weakly characterised species complexes) in bryogeography, one can obtain meaningful results even in a geographically restricted area as, for instance in Poland.

KEY WORDS: Bryogeography, electrophoretic identification, *Hepaticae*, *Pellia*.

INTRODUCTION

In spite of their big – for liverworts – size and their frequently copious occurrence, the geographical distribution of particular species of the genus *Pellia* is known only very generally. This strange situation is due to the notorious difficulty in identifying the plants. Not only sterile plants are practically impossible to name, but also identification of fertile material in some groups, esp. in the *Pellia epiphylla* complex is – sometimes – a troublesome problem. Thus, frequently, only those stations, where typical, fertile plants were found, are published in floristic lists. Starting our work on genetic structure of *Pellia* populations we realised that we would not succeed in our work unless we found methods allowing identification of each individual thallus irrespective of where it is growing (i.e. on soil or in water) or whether it is fertile or not. Among others, we tried the electrophoretic separation of enzyme proteins in crude extracts of living plants either brought directly from the field or grown for longer or shorter period of time in a glasshouse. We discovered that some enzyme proteins give distinct band pattern phenotypes on gels (we used exclusively starch for this purpose) allowing immediate and safe identification of all *Pellia* species growing in Poland. At the beginning we used peroxi-

dases, which were easy to separate, and their detection on starch gels was relatively cheap. We used this (and some other enzymatic systems) in our work, achieving – periodically – quite interesting results.

One of our results was that some species as generally accepted by bryologists (e.g. *Pellia epiphylla* s. str., i.e. n=9 plants, *Pellia endiviifolia*) turned out to be genetically heterogeneous and differentiated into groups of populations, which could be separated on the basis of their zymogram patterns. The differences between these groups were (i) complex, i.e. had stable multienzyme phenotypes (Zieliński 1987, Szweykowski, Odrzykoski 1990), and (ii) were of a similar magnitude as is observed in generally accepted species (Szweykowski, Odrzykoski 1990). Thus, we propose that these groups form new, undescribed species, which – because of a seeming lack of good, i.e. reliable, diagnostic morphological characters – fall into the category of sibling species. We are trying now to formalize their taxonomic status.

In the present paper we will show the geographic distribution of all species (sibling species included) as discussed below. Only locations of plants that have been identified electrophoretically were taken into account and entered on the distributional maps.

Problems connected with the geographical distribution of particular species of the genus *Pellia* will be clear after a short discussion of the intricate history of particular taxa.

Pellia epiphylla (L.) Corda.

This is one of the "classic" species of the genus described as early as 1753 by Linnaeus (as *Jungermannia epiphylla*). In 1927 Heitz showed that the species harbours plants with two different chromosome numbers: $n=9$ and $n=18$; in 1934 Lorbeer recognized the $n=18$ plant as belonging to a different species, which he named *Pellia borealis*. This species is not generally accepted, but the number of bryologists agreeing with the specific status of the plant with 18 chromosomes seems to be growing (see e.g. Schuster 1992, pages 467-471). Quite recently (Odrzykoski et al. in press) it has been shown rather unambiguously that *Pellia borealis* is an amphiploid formed by two distinct sibling species still undescribed formally; we call them – for the time being – *Pellia epiphylla* species *S* and *Pellia epiphylla* species *N*. After exclusion of *Pellia borealis* (i.e. of $n=18$ plants) *Pellia epiphylla* s.str. consists – at least in Poland – of two different sibling species, which were differentiated on the basis of their dissimilar multienzyme phenotypes (Zieliński 1987, Szwejkowski, Odrzykoski 1990). The formal taxonomic description is still lacking.

Thus, *Pellia epiphylla* in the classic meaning forms a species complex; some of the species await formal taxonomic description. The whole complex differs from the rest of *Pellia* species by bisexuality (paroeious).

Pellia neesiana (Gottsche) Limpricht

This is also one of the "classic" species of the genus. The plant was discovered by Gottsche (1867) and treated as a mere form of *Pellia epiphylla* (*Pellia epiphylla* fo. *neesiana*). Limpricht recognized the specific status of the plant in 1876. The only taxonomic change since, was the description of a *Pellia columbiana* by Krajina and Bradshaw in 1951. This taxon found little acceptance and is treated by Schuster (1992, p. 459) as subspecies of *Pellia neesiana* [as *Pellia neesiana* ssp. *columbiana* (Krajina and Bradshaw) Schuster]. *Pellia neesiana* ssp. *neesiana* – as the sole subspecies occurring in Europe (ssp. *columbiana* is restricted in its occurrence to the north-western part of the North American continent) – does not form, seemingly, any distinct sibling species, at least in the territory of Poland.

Recently, R. M. Schuster (1991) described a new species named *Pellia appalachiana*. It shows a strange mixture of *Pellia epiphylla* and *Pellia neesiana* characters and is dioecious.

According to Schuster (1991, 1992), it is a "partially stabilized hybrid between *P. epiphylla* and *P. neesiana*". The new species is known till now only from a few stations in eastern North America.

Pellia endiviifolia (Dicks.) Dum.

The species has been taxonomically recognized by Dickson as early as in 1801 (as *Jungermannia endiviifolia*). Only recently, a new species from North America – *Pellia megaspora* Schuster – was segregated by Schuster in 1981; it seems to be restricted in its occurrence to the north-eastern part of the North American continent. Our studies (Zieliński 1987) disclosed that Polish plants of *Pellia endiviifolia* comprise two different sibling species characterized electrophoretically; we

call them – for the time being – *Pellia endiviifolia* species *A* and *Pellia endiviifolia* species *B*; the latter, i.e. the species *B* is – most probably – the typical form of *Pellia endiviifolia* s.l. Thus, *Pellia endiviifolia* in the classic sense forms a species complex.

This complex (including *Pellia megaspora*) differs from the two preceding ones (i.e. *Pellia epiphylla* and *Pellia neesiana* complexes) to such an extent that it has been segregated as a different subgenus (*Apopellia* Grolle 1983) or section (*Pellia* – Schuster 1992).

Describing the geographical distribution of *Pellia* taxa, one has to remember their complex and intricate taxonomic character. Usually, in nearly all floras, discussion is restricted to the three "classic" species, ignoring the changes discussed above; only distributions of *Pellia borealis* (Schuster 1992, De Sloover & Messe 1982, Zieliński, Szwejkowski, Rutkowska 1985, Zieliński 1987), *Pellia neesiana* ssp. *columbiana* (Schuster 1992) and *Pellia megaspora* (ibidem) are discussed incidentally. Preliminary studies on the geographical distribution of the sibling species mentioned above (Zieliński, Szwejkowski, Rutkowska 1985, Zieliński 1987) showed differences in their geographical areas. The present paper summarizes all known data on this problem.

MATERIAL AND METHODS

For twenty years (earliest collections were made in 1974) we collected all *Pellias* found in various parts of Poland, irrespective of whether they could be identified (i.e. when they were fertile and typical) or not. After bringing the live material to the laboratory in Poznań, several thalli – one at a time – were analysed for peroxidase phenotype as described in Szwejkowski et al. 1981, and later also for diaphorase phenotype (Odrzykoski et al. in press); the analyses were performed either immediately on plants which were brought from the field, or on plants grown in a glasshouse. To check the stability of electrophoretic patterns, we electrophoresed thalli from one sample several times, immediately after collection and after various periods of cultivation. A part of each sample was dried prior to the electrophoresis and preserved as voucher specimens in POZW. As in the case of *Pellia epiphylla*-*Pellia borealis* complex the determination of chromosome number is critical for identification of particular species; this number has been checked in most (but not all!) cases. Every identified sample was put into a file, which formed the basis for construction of distributional maps.

List of stations can be obtained from Jerzy Szwejkowski on request.

RESULTS

A. Monoecious species (*Pellia epiphylla*-*Pellia borealis* complex).

1. *Pellia epiphylla* s. str. Only plants with a haploid chromosome number (i.e. $n = 9$) belong here.

1.1. *Pellia epiphylla* species *N*. This species was differentiated at the beginning on the basis of a different peroxidase phenotype PX3 (Zieliński, Szwejkowski, Rutkowska 1985). The species grows only in the northern part of the country, where it is rather widespread, although by no means common. The most southern stations reach the neighbourhood of Warsaw city (Fig. 1).

1.2. *Pellia epiphylla* species *S* (= PX1 and PX2 in Zieliński 1984). Populations belonging to this species occur mainly in the southern part of Poland, most frequently in hill or low

Fig. 1. General geographical distribution of two sibling species: *Pellia epiphylla* species N and *Pellia epiphylla* species S in Poland. Detailed distribution in some special regions (numbered rectangles) is shown in the next figure.

mountain regions; only one population is known to occur in the northern part of N.-W Poland. Thus, both sibling species of *Pellia epiphylla* s. str. (i.e. N and S) are practically allopatric in this country (Figs 1, 2 and 3). This species shows variation of the peroxidase phenotype with two allelic variants: PX1 and PX2 (Zieliński 1987).

2. *Pellia borealis* Lorbeer. This critical taxon has been interpreted differently (see K. Müller 1954; Schuster 1992; Newton 1986). Recently, we delivered proof (Odrzykoski, Chudzińska, Szweykowski in press) that it is of an amphiploid origin. Namely, it is a doubled hybrid between *Pellia epiphylla* species S and *Pellia epiphylla* species N. *Pellia*

borealis is by far the commonest *Pellia* in Poland, particularly in the lowlands (see Figs 4, 5 and 6), but its geographic distribution in the western part of Europe seems to be more restricted. At least in the Ardennes (de Sloover & Messe 1982) it seems to grow only in higher elevations; recently it has been discovered in one locality in the British Isles (Newton 1986).

B. Dioecious species.

3. *Pellia neesiana*. This is a mountain species reaching elevation 1990 m. in the Tatra ridge. It shows also restricted occurrence in the lowlands (Figs 7 and 8).

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Fig. 2. Detailed geographical distribution of two sibling species: *Pellia epiphylla* species N and *Pellia epiphylla* species S in some more interesting regions of Poland;
1: Sudetes;
2: S-Central Poland, Tatra Mts. excluded.

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Fig. 3. Detailed geographical distribution of two sibling species: *Pellia epiphylla* species *N* and *Pellia epiphylla* species *S* in some more interesting regions of Poland;
3: Tatra Mts.;
4: Polish part of the East Carpathians ridge.

Fig. 4. General geographical distribution of *Pellia borealis* in Poland. Detailed distribution in some regions (numbered rectangles) is shown in the next figures.

4. *Pellia endiviifolia*. Populations of this taxon are differentiated into two sibling species: *Pellia endiviifolia* species A and *Pellia endiviifolia* species B. Plants belonging to the second one (B) form characteristic outgrowths at the thallus apex (fo. *furcigera*) in the autumn. The plants belonging to the species A are – apparently – unable to do so. There is also an ecological – although not very sharp – difference. *Pellia endiviifolia* species B (forming outgrowths) prefers basic sites with lime, whereas the new form (species A) is more neutrophytic. Species B occurs, first of all, on moderately dry substrates and grows frequently in the lowlands. Species A is often bound to water – pure water running on mineral ground, then in shallow water and where the water is still; it can be found also frequently along mountain springs (on mineral detritus or on rocks) close to the water surface or even in the re-

gions of watersprays. Its occurrence in lowlands is very restricted (Figs 9 and 10).

DISCUSSION

Bryogeography – as biogeography in general – is description and interpretation of geographical distribution of the existing taxa. Obviously, the concept of particular taxa is crucial for biogeography. Moreover, the more natural the taxa in question, the more meaningful their geographical distributions.

The situation in bryogeography is – of course – exactly the same. As we have shown (Odrzykoski, Szweykowski 1991) some taxa of holarctic distribution are, in fact, complexes of sibling species, which show much more restricted geographical areas. A typical example is *Conocephalum conicum*. This

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Fig. 5. Detailed geographical distribution of *Pellia borealis* in some more interesting regions of Poland;
 1: NW Poland (Pomerania);
 2: Sudetes.

Fig. 6. Detailed geographical distribution of *Pellia borealis* in some more interesting regions of Poland;
3: S Central Poland, Tatra Mts. excluded;
4: Tatra Mts.

Fig. 7. General geographical distribution of *Pellia neesiana* in Poland. Detailed geographical distribution in the Tatra Mts. and its foothills (rectangle no.1) is shown in the next figure.

holarctic taxon is a species collectiva, comprising at least 5 different species, which differ profoundly as far as their genetic structure (shown by electrophoretic patterns of over 30 enzyme systems – Odrzykoski, Szweykowski 1991) is concerned. The particular species show also differences – although slight – in their morphology (Schuster 1992, Szweykowski, Buczkowska in preparation). They show also more restricted geographical distributions: species *L*, for instance, grows only in Europe, whereas species *A* is known, so far, only from the eastern part of the North American continent; the differences in geographical distribution concern not only the intercontinental scale, they are also realized in details of their distributions in more restricted areas. For example *Conocephalum conicum* species *L* does not occur in higher elevations, where only species *S* grows (in the Tatra Mts., for instance, the highest sites, where species *L* grows, all lie at

elevations not higher than 1000 m., whereas species *S* is known to be widespread in limerich areas to the elevation of ca. 1800 m., and reaches over 2000 m. (Szweykowski, unpublished).

Results of our studies on *Pellia* – although by no means as complete as in the case of *Conocephalum conicum* – seem to show that the situation in this genus is very similar. We know very little about the general (i.e. intercontinental) geographical distribution of the new taxa we have discovered. We do know, however, that their geographical ranges in Poland differ in some cases considerably from those in other regions. For instance the diploid species ($n=18$) *Pellia borealis* is widespread in Poland from the lowlands (the northern 2/3 of the country) to the foothills of the Tatra Mts. and of the Sudetes, and it seems to be absent in the higher elevations (Figs 4, 5 and 6). At the same time it is apparently more re-

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Fig. 10. Detailed geographical distribution of two sibling species: *Pellia endiviifolia* species A and *Pellia endiviifolia* species B in the Polish part of the Tatra Mts and its foothills (1) and in the SE part of Poland (2).

stricted in Western Europe: it was found in the British Isles only recently in one locality, moreover, it grows in the Ardennes in the higher elevations only (de Sloover & Messe 1982). It is also not known beyond Europe.

In the case of both sibling species of *Pellia epiphylla* s.str. (i.e. $n=9$), which differ markedly in their geographical ranges in Poland (see Figs 1, 2 and 3), there are some interesting extensions of their geographical ranges: *Pellia epiphylla* species *N* grows also in the northern part of Europe (Zieliński 1987) and in North America (Odrzykoski, unpublished data). We can be reasonably sure, then, that the geographical ranges of the new taxa in Poland are only a part – might be a small one – of their general distribution.

One should, however, remember that both *Conocephalum conicum* and species of the genus *Pellia* produce spores that germinate still in the closed sporangium; thus, diaspores of these liverworts are many-celled when leaving the capsule and their ability to disperse to longer distances might be low. It would be very important to perform similar studies on species having one-celled spores, to check whether this character plays a role in genetic differentiation.

Hence, it seems, that the electrophoretic studies of liverwort species can give bryogeographically valuable data even when performed in a restricted area.

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LITERATURE CITED

- de SLOOVER J.R., MESSE V., 1982. Spatial distribution of the autodiploid *Pellia borealis* in the geographic range of the related haploid taxon *Pellia epiphylla*. Journ. Hattori Bot. Lab. 53: 153-155.
DICKSON J., 1801. Plantarum Cryptogamicum Britanniae Fasc. IV.: 19.
GOTTSCHE C.H., 1867. Einige Bemerkungen zu Thom. Jensen Consp. Hepaticarum Daniae. Hedwigia 6: 65-77.
HEITZ E., 1927. Geschlechtchromosomen bei *Pellia Fabbronia* (diöcisch) und *Pellia epiphylla* (monöcisch). Ber. Deutsch. Bot. Ges. 45: 607-610.

- KRAJINA V., BRADSHAW T.C., 1951. A new species of *Pellia*. Bryologist 54: 59-67.
LIMPRICHT G., 1876. Lebermoose. In: Kryptogamenflora von Schlesien, Cohn F. (ed.). Breslau, pp. 225-352.
LINNAEUS C., 1753. Species Plantarum, Holmiae, pp. 1200.
LORBEER G., 1934. Die Zytologie der Lebermoose mit besonderer Berücksichtigung allgemeiner Chromosomenfragen. I. Teil. Jahrb. Wiss. Bot. 80: 565-818.
MÜLLER K., 1954. Die Lebermoose Europas, In: Kryptogamenflora von Deutschland, Österreich und der Schweiz. Bd. VI. I. Abt. (III. Auflage). Rabenhorst L. (ed.). Leipzig, pp. 756.
NEWTON M.E., 1986. *Pellia borealis* Lorbeer: its cytological status and discovery in Britain. Journ. Bryol. 14: 215-230.
ODRZYKOSKI I.J., CHUDZIŃSKA E., SZWEYKOWSKI J., The hybrid origin of the polyploid liverwort, *Pellia borealis*. (in press)
ODRZYKOSKI I.J., SZWEYKOWSKI J., 1991. Genetic differentiation without concordant morphological divergence in the thallose liverwort *Conocephalum conicum*. Pl. Syst. Evol. 178: 135-151.
SCHUSTER R.M., 1981. Evolution and speciation in *Pellia*, with special reference to the *Pellia megaspora-endiviifolia* complex (Metzgeriales). I. Taxonomy and distribution. Journ. Bryol. 11(3): 411-432.
SCHUSTER R.M., 1991. Diagnoses of new taxa of *Hepaticae*; I. *Jungermanniidae*. Journ. Hattori Bot. Lab. 70: 143-150.
SCHUSTER R.M., 1992. The *Hepaticae* and *Anthocerotae* of North America, vol. V. Field Museum of Nat. Hist. Chicago, pp. 937.
SZWEYKOWSKI J., ODRZYKOSKI I.J., 1990. Chemical differentiation of *Aneura pinguis* (L.) Dum. (*Hepaticae*, *Aneuraceae*) in Poland and some comments on application of enzymatic markers in bryology. In: Zinsmeister, H. D., Mues, R. (eds.) Bryophytes, their chemistry and chemical taxonomy. Clarendon Press, Oxford, pp. 437-448.
SZWEYKOWSKI J., ZIELIŃSKI R., MENDELAK M., 1981. Variation of peroxidase isoenzymes in Central European taxa of the liverwort genus *Pellia*. Bull. Pol. Acad. Sci., Sci. Biol. 29(1-2): 9-19.
ZIELIŃSKI R., 1987. Genetic variation of the liverwort genus *Pellia* with special reference to Central European territory. Rozprawy i Studia Uniw. Szcz. XCVIII(24): 1-297.
ZIELIŃSKI R., SZWEYKOWSKI J., RUTKOWSKA E., 1985. A further electrophoretic study of peroxidase isozyme variation in *Pellia epiphylla* (L.) Dum. from Poland, with special reference to the status of *Pellia borealis* Lorbeer. Monogr. Syst. Bot. Missouri Bot. Gard. 11: 199-209.

ROZMIESZCZENIE GEOGRAFICZNE GATUNKÓW RODZAJU PELLIA (HEPATICAЕ, METZGERIALES) W POLSCE NA PODSTAWIE OZNACZEŃ ELEKTROFORETYCZNYCH

STRESZCZENIE

Liczne gatunki wątrobowców wykazują tak wysoki stopień zmienności morfologiczno-anatomicznej o charakterze modyfikacji, że sprawiają trudności w oznaczaniu nawet specjalistom. Przykładem mogą być gatunki rodzaju *Pellia*. Poszukując – w związku z prowadzonymi pracami genetyczno-populacyjnymi – bardziej pewnych cech diagnostycznych niż wspomniane morfologiczno-anatomiczne, użyliśmy w tym charakterze fenotypów elektroforetycznych otrzymanych na drodze elektroforetycznego rozdzielania białek enzymatycznych na żelu skrobiowym. Wykazaliśmy w szeregu prac, że fenotypy takie mogą być użyte jako zupełnie pewne cechy diagnostyczne. W czasie 20 ostatnich lat przeprowadziliśmy ponad 500 (dokładnie 541) oznaczeń elektroforetycznych roślin pochodzących z różnych populacji gatunków rodzaju *Pellia* z terenu Polski. Dostępnym niespodziewanym wynikiem tych badań było stwierdzenie, że niektóre gatunki tego rodzaju są gatunkami zbiorowymi; w rezultacie wyróżniliśmy kilka taksonów o charakterze gatunków bliźniaczych. W niniejszej pracy prezentujemy mapy obrazujące rozmieszczenie w Polsce wszystkich gatunków oznaczonych elektroforetycznie (wraz z nowo wyróżnionymi gatunkami bliźniaczymi); stwierdzamy wyraźne różnice w charakterze zasięgów poszczególnych taksonów. Wyniki te wskazują niedwuznacznie, że użycie bardziej naturalnych taksonów w briogeografii – tj. taksonów określonych na podstawie kryteriów genetycznych zamiast niezbyt precyzyjnie określonych gatunków zbiorowych, jak to się czyniło często dotychczas – prowadzi do znaczących wyników nawet na podstawie materiałów z tak małego regionu briogeograficznego, jakim jest Polska.

SŁOWA KLUCZOWE: briogeografia, oznaczanie elektroforetyczne, wątrobowce, *Hepaticae*, *Pellia*.