ISOENZYME MARKERS OF TWO HEPATIC SPECIES: *BARBILOPHOZIA LYCOPODIOIDES* (WALLR.) LOESKE, AND *B. HATCHERI* (A. EVANS) LOESKE

ALINA BĄCZKIEWICZ[1], KATARZYNA BUCZKOWSKA[1], MARLENA LEMBICZ[2]

[1] Adam Mickiewicz University
Institute of Experimental Biology, Department of Genetics
Międzychodzka 5, 60-371 Poznan, Poland
e-mail: A.B.: alinbacz@main.amu.edu.pl
e-mail: K.B.: androsac@main.amu.edu.pl

[2] Adam Mickiewicz University
Institute of Environmental Biology, Department of Plant Taxonomy
Al. Niepodległości 14, 61-713 Poznan, Poland
e-mail: lembicz@main.amu.edu.pl

(Received: April 15, 2002. Accepted: January 16, 2003)

ABSTRACT

Two closely related species of the genus *Barbilophozia*: *B. lycopodioides* and *B. hatcheri* were studied in populations from the Tatra Range (S Poland), where they are frequent and widely distributed. Both species play an important role in plant communities and grow here very often side by side. Typically developed plants are quite easy to distinguish (even in the field), however morphologically intermediate forms, difficult to recognize by using of classical taxonomic methods, sometimes are found. We found enzymatic markers, that allow to recognize the critical forms. Both studied species are different in enzymatic patterns of glutamate oxaloacetate transaminase (GOT) and peroxidases (PX). In GOT four different phenotypes were detected. The first two (GOT 1 and GOT 2) were characteristic for *B. hatcheri* and next two (GOT 3 and GOT 4) for *B. lycopodioides*. Peroxidase patterns, that were monomorphic and specific for each species, exhibit different mobility in anodal and cathodal parts of gel. Results of the studies allowed us to draw the conclusion, that PX and GOT are good isoenzymatic markers and they can have practical application for identification of *Barbilophozia* species.

KEY WORDS: *Barbilophozia lycopodioides*, *B. hatcheri*, liverworts, isoenzyme markers.

INTRODUCTION

The species of the genus *Barbilophozia* Loeske are frequent, widely distributed and are a very important element of hepatic flora in the Tatra Mts. (S Poland). In Europe the genus is currently recognized as consisting of three species: *Barbilophozia barbata* (Schmidel ex Schreb.) Loeske, *B. lycopodioides* (Wallr.) Loeske, *B. hatcheri* (A. Evans) Loeske. *B. barbata* shows narrow phenotypes diversity and is quite easy to recognize. Two other species: *B. lycopodioides* and *B. hatcheri* are considered as a critical species complex and their taxonomy has been the subject of a debate between bryologists. The geographic distribution of these two species is similar, but *B. lycopodioides* has a holarctic, almost strictly subarctic-subalpine distribution, whereas *B. hatcheri* – bipolar species has a wider range extending from subarctic-subalpine to arctic-alpine regions (Schuster 1969). Both species have a large ecological scale of tolerance, occupy similar sites preferring places of moderate moisture. *B. lycopodioides*, which is more tolerant for pH occurs on calcareous as well as on granite rocks, whereas *B. hatcheri* grows mainly on granite rocks. The species of the genus *Barbilophozia*, likewise other liverworts, often occur as pioneer plants occupying mainly places with lower competition of flower plants and playing an important role in the first stages of succession (Szweykowski 1996).

An ability to inhabit various ecological niches by these species is directly connected with a wide range of morphological variation, which gives those simple organisms a large adaptative capability that, according to Schuster (1966), compensates the low level of genetic variation observed in liverworts. The morphological plasticity, simple gametophyte structure, which often occurs in sterile stage and the small size of liverworts are the main reasons of difficulties with taxa determination by means of classical taxonomy methods. In spite of intensive studies (Schuster 1969; Müller 1951-1958; Smith 1990; Schljakov 1980) the

taxonomic status of *B. lycopodioides* and *B. hatcheri* remains still unclear. Besides typically developed plants, which can be easy distinguished on the ground of morphological features (Schuster 1969; Müller 1951-1958), and can scarcely be confused, there are some untypically developed forms with a modification of main diagnostic characters used to separate *B. lycopodioides* from *B. hatcheri*. The diagnostic characters include the presence of gemmae, the shape of margin cells of leaf lobes or the size of plants (Müller 1951-1958; Schuster and Damsholt 1974). The modifications of the above mentioned features and the occurrence of so-called 'intermediate' forms caused that *B. hatcheri* was treated as a variety of *B. lycopodioides* (Schljakov 1980). Some doubts about the taxonomic status of *B. hatcheri* has also Smith (1990), Schuster (1988), however pointed out that *B. hatcheri*, even if sometimes difficult to separate from *B. lycopodioides*, is a clearly distinct species.

The purpose of the present study is to investigate isozyme polymorphism within two species *B. lycopodioides* and *B. hatcheri* in Poland in respect to two enzyme systems: peroxidases and glutamate oxaloacetate transaminase and check whether their phenotypes can be used as diagnostic markers.

MATERIAL AND METHODS

Plant material

The material for electrophoretical studies was collected from natural populations in the mountains of the Tatras National Park. We sampled a total of 22 populations: 13 of *B. lycopodioides* and 9 of *B. hatcheri*. The names of the species are in accordance with Grolle and Long (2000). The samples from each discrete population measured approximately 10 cm × 10 cm. Each of them was divided into two parts: one of them was put into pots filled with peat mixed with soil and kept in a glasshouse until they were studied (for several weeks), the second part was dried and deposited as voucher specimens in the POZW herbarium (Table 1).

The electrophoretic analyses were performed for 10 gametophytes from every sample. Plants were identified according to morphological features (Schuster 1969) by Prof. dr Jerzy Szweykowski. The analyses were repeated 3 times.

Electrophoretic technique

A single stem was homogenized in 40 µl of extraction buffer (Wyatt et al. 1989). Paper wicks (3 mm × 8 mm) Whatmann were soaked with the crude extract and next put into 10% starch-gel (Starch Art) prepared with lithum-boric buffer system (Wendel and Weeden 1989).

Gels were specifically stained for peroxidases (PX – E.C.1.11.1.7.) and glutamate oxaloacetate transaminase (GOT – E.C.2.6.1.1). These enzymes were detected by standard staining methods (Wendel and Weeden 1989).

RESULTS

Both species presented specific banding patterns for peroxidases (PX) and glutamate oxaloacetate transaminase (GOT) enzyme systems. The electrophoretic phenotypes of PX were different for *B. lycopodioides* and *B. hatcheri*, but inside each species they were monomorphic. Three zones of activity were revealed for PX system (Fig. 1). Two of

TABLE 1. Collection sites for all populations included in the isozyme analyses. All studied populations came from Tatra Mts. in Poland.

Sample no.	POZW no.	Stations	m a.s.l.	Date	Collectors
Barbilophozia lycopodioides					
1.	38902	Mała Pańszczycka Młaka peat bog	1260	01.08.1999	ML, JS
2.	38882	Toporowe Stawy peat bog	1100	30.08.1999	AB, JS,
3.	38949	Dol. Pyszniańska valley	1700	31.07.1999	KB, AB
4.	38961	Pyszniańska Przełęcz saddle	1720	31.07.1999	ML, KB
5.	38964	Las Gąsienicowy forest	1450	02.08.1999	KB, AB
6.	38879	Brzeziny village	1050	30.07.1999	JS, AB
7.	39286	Dol. Pańszczycy valley	1230	18.08.2000	AB, KB
8.	39287	Dol. Suchej Wody valley	1250	18.08.2000	AB, KB
9	39242	Dol. Jaworzynka valley, Żleb pod Czerwienicą gully	1450	15.08.2000	AB, KB
10.	39284	Dol. Suchej Wody valley, Psia Trawka forest	1200	18.08.2000	AB, KB
11.	39266	Las Capowski forest	975	16.08.2000	AB, KB
12.	39268	Las Capowski forest	975	16.08.2000	KB, AB
13.	39245	Dol. Jaworzynki valley, Żleb pod Czerwienicą gully	1460	15.08.2000	AB, KB
Barbilophozia hatcheri					
1.	38830	Las Capowski forest	950	30.07.1999	JS, ML
2.	38832	Las Capowski forest	970	30.07.1999	KB, JS
3.	38789	Dol. Spadowiec valley	1100	25.07.1999	KB, JS
4.	38905	Ornak Mt.	1700	29.07.1999	KB, AB
5.	38852	Pośredni Goryczkowy Wierch Mt.	1750	21.07.1999	KB, AB
6.	39318	Żółta Turnia Mt.	1690	20.08.2000	KB, AB
7.	39319	Żółta Turnia Mt.	1700	20.08.2000	AB, KB
8.	39314	Dol. Gąsienicowa valley, place called Dubrawiska	1490	20.08.2000	AB, KB
9.	39343	Dol. Suchej Wody valley	1200	23.08.2000	KB, AB

Collectors: AB – Alina Bączkiewicz; KB – Katarzyna Buczkowska; ML – Marlena Lembicz; JS – Jerzy Szweykowski.

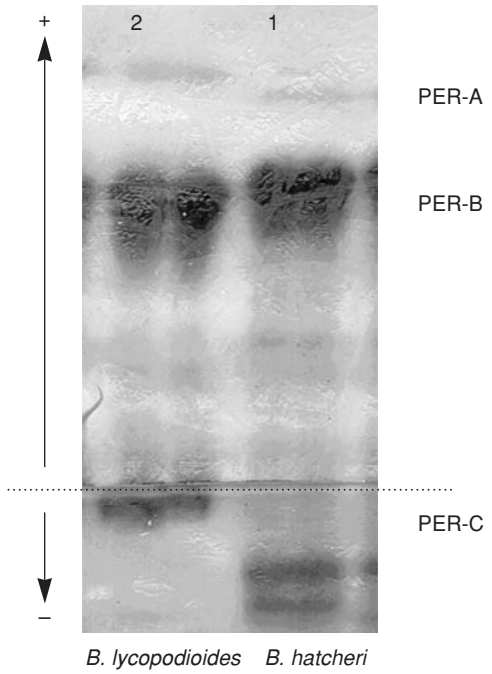

Fig. 1. The phenotypes of peroxidase isoenzymes in *Barbilophozia hatcheri* (1) and *B. lycopodioides* (2).

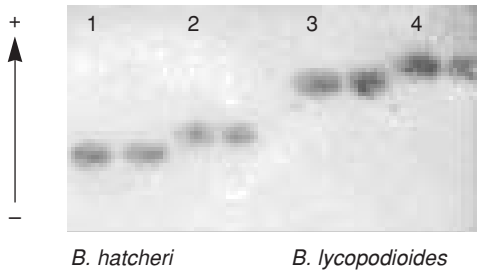

Fig. 2. The phenotypes of GOT isoenzyme in *Barbilophozia hatcheri* (1, 2) and *B. lycopodioides* (3, 4).

them (PX-A, PX-B) were found in the anodal and one (PX-C) in the cathodal part of gel. The first zone included the fastest migrating bands: PX-A1 – specific for *B. hatcheri*, and PX-A2 for *B. lycopodioides*. The second zone (PX-B), slower migrating, but more active than the first one, was formed by two bands: PX-B1 occurring only in *B. hatcheri* and PX-B2 occurring only in *B. lycopodioides*. Cathodal peroxidases (PX-C) were composed of two fenotypes. The first of them in Figer 1 (PX-C2) specific for *B. lycopodioides* included one band, but the second one (PX-C1) specific for *B. hatcheri* included a few connected bands.

As it is illustrated in Figer 2, four different alleles of GOT enzyme system were detected in the two taxa. The first two slower bands (GOT 1 and GOT 2) were characteristic for *B. hatcheri* and the next two (GOT 3 and GOT 4) for *B. lycopodioides*.

DISCUSSION

Both investigated species have relatively large, partly overlapping, range of morphological variation (Schuster 1988; Schuster and Damsholt 1974; Schljakov 1980), therefore their classification only on the ground of morphological features can sometimes be difficult. However, isozyme research, which has recently played an important role in solving some taxonomic problems in liverworts provide isoenzyme markers that allow to classify the critical forms correctly. These studies have given the solution to such problems as for example in the genus of *Pellia* Raddi (Krzakowa 1981; Odrzykoski et al. 1996) *Plagiochila* (Dumort.) Dumort. (Krzakowa and Szweykowski 1977), *Calypogeia* Raddi (Szweykowski and Krzakowa 1990), *Lophozia* (Dumort.) Dumort. (Krzakowa et al. 1991) or *Porella* Dumort. (Boisselier-Dubayle et al. 1994).

Our studies reveal that the phenotypes of PX and GOT are useful for identification of *B. lycopodioides* and *B. hatcheri*. A lot of information about the difference between the species was provided by peroxidase complex that is composed of PX-A, PX-B and PX-C (Fig. 1). The distance between bands PX-A and PX-B in *B. lycopodioides* is always longer than the distance between the same bands in *B. hatcheri*. The cathodal part of gel is remarkable where the peroxidase electrophoretic pattern shows two different phenotypes. Both phenotypes were identical inside the species in all investigated material. Consequently, the cathodal pattern can serve as the diagnostic marker for these species. Peroxidase isoenzymes in the cathodal part have been known as good taxa markers in bryology for a long time (Krzakowa 1991; Krzakowa 1993). They were detected in other critical species of liverworts, for example in *Lophozia incisa* (Schrad.) Dumort. and *L. opacifolia* Culm. ex. Meyl. (Krzakowa et al. 1991). *B. lycopodioides* and *B. hatcheri* were different in the anodal part in GOT too (Fig. 2). The GOT system, which is also species-specific, was often used as a control (Odrzykoski 1995). Our study has confirmed this opinion.

As it is well known, the electrophoretic data reveal that genetic differences very often correspond with morphological traits (Boisselier-Dubayle and Bischler 1994; Bischler and Boisselier-Dubayle 1999; Wyatt et al. 1997). In liverworts complexes of critical, closely related species are known. Moreover, the high plasticity of morphological characters, induced by environmental conditions, cause that they are difficult to recognize only on the basis of morphological characters. The material identified by the electrophoretic method can be used for searching new, reliable morphological and anatomical diagnostic features, that allow to classify untypically developed plants in the field as well as in the herbarium, as has been shown in studies of the genus *Calypogeia* (Szweykowski and Buczkowska 1998; Buczkowska 1999) or *Odontoschisma* (Dumort.) Dumort. (Szweykowski and Buczkowska 1999).

The results of our studies show genetic differences between *B. lycopodioides* and *B. hatcheri* and support the Schuster's (1988) opinion that they must be treated as two species. However, evidence of the genetic distinctness between these species demands wider genetic studies and detailed morphological analyses.

ACKNOWLEDGMENTS

We thank Professor Dr. Jerzy Szweykowski (Dept. of Genetics of the Adam Mickiewicz University) for field assistance, verification of plant material and valuable comments on the manuscript. We would like to express our thanks to the Management of the Tatra National Park for their interest in our studies and many facilities provided during the field work.

LITERATURE CITED

BISCHLER H., BOISSELIER-DUBAYLE M.C. 1999. Polyploidy in liverworts – a case study of tetraploid *Sauteria*. Hausknechtia Beih. 9: 41-44.

BOISSELIER-DUBAYLE M.C., BISCHLER H. 1994. A combination of molecular and morphological characters for delimitation of taxa in European *Porella*. J. Bryol. 18: 1-11.

BUCZKOWSKA K. 1999. Plastyczność anatomiczno-morfologiczna a problem gatunku u mszaków na przykładzie pary krytycznych gatunków rodzaju *Calypogeia* Raddi: *C. neesiana* i *C. integristipula*. [Morphological – anatomical plasticity and the species problem in Bryophytes, as evidence by the critical species pair belonging to the genus *Calypogiea* Raddi: *C. neesiana* and *C. integristipula*.] Tezy pracy doktorskiej, Uniwersytet im. A. Mickiewicza, Poznań. (in Polish).

GROLLE R., LONG D.G. 2000. An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia. J. Bryol. 22: 103-140.

KRZAKOWA M. 1981. Evolution and speciation in *Pellia*, with special reference to the *Pellia megaspora-endiviifolia* complex (Metzgeriales), IV. Isozyme investigation. J. Bryol. 11: 447-450.

KRZAKOWA M. 1991. Peroxidases in genetics and taxonomic investigations. In: Biochemical, molecular and physiological aspects of plant peroxidases. Lobarzewski J., Greppin H., Penel C.I., Gaspar T.H. (eds). University of Geneva, pp. 15-20.

KRZAKOWA M. 1993. The significance of cathodic peroxidases in the taxonomy of bryophytes. In: Plant peroxidases: Biochemistry and physiology. Welinder K.G., Rasmussen S.K., Penel C.I., Greppin H. (eds). University of Geneva, pp. 213-219.

KRZAKOWA M., SZWEYKOWSKI J. 1977. Peroxidases as taxonomic characters. II. *Plagiochila asplenioides* (*B.*) Dum. sensu Grolle (= *P. maior* S.Arnell) and *Plagiochila porelloides* (= *P. asplenioides*, non Grolle; Hepaticae, Plagiochilaceae). Bull. Soc. Am. Sci. Poznań. 17: 33-36.

KRZAKOWA M., SZWEYKOWSKI J., BISANG I., KOŹLICKA M. 1991. Peroxidases as taxonomic character in *Lophozia incisa* (Schrad.) Dum. and *Lophozia opacifolia* (Culm.) Meyl. J. Bryol. 16: 551-559.

MÜLLER K. 1951-1958. Die Lebermoose Europas. Rabenhorst's Kryptogamen Flora von Deutschland, Oesterreich u. d. Schweiz. Akademische Verlagsgesellschaft Geest & Portig K.-G. Leipzig, pp. 631-635.

ODRZYKOSKI I.J. 1995. Sibling species of the *Conocephalum conicum* complex (Hepaticae, Conocephalaceae) in Poland: distribution maps and description of an identification method based on isozyme markers. Fragm. Flor. Geobot. 400: 393-404.

ODRZYKOSKI I. J., CHUDZIŃSKA E., SZWEYKOWSKI J. 1996. The hybrid origin of the polyploid liverwort *Pellia borealis*. Genetica 98: 75-86.

SCHLJAKOV R.N. 1980. Pieczenocznyje mchi Sjewiera S.S.S.R. [Hepatics from the northern regions of the USRR]. Nauka, Leningrad. vol. 3, pp. 29-34. (in Russian)

SCHUSTER R.M. 1966. The Hepaticae and Anthocerotae of North America. Columbia University Press, New York and London. vol. 1, pp. 311.

SCHUSTER R.M. 1969. The Hepaticae and Anthocerotae of North America. Columbia University Press, New York and London. vol. 2, pp. 332-360.

SCHUSTER R.M. 1988. The Hepaticae of South Greenland. Nova Hedwigia. 92: 49-51.

SCHUSTER R.M., DAMSHOLT K. 1974. The Hepaticae of West Greenland from ca. 66°N to 72°N. Meddelser om Groenland Kobenhavn 199: 62-66.

SMITH A.J.E. 1990. The Liverworts of Britain and Ireland. Cambridge Univ. Press: 96-98.

SZWEYKOWSKI J. 1996. Wątrobowce. In: Przyroda Tatrzańskiego Parku Narodowego. [Liverworts. In: Nature of the Tatra National Park.] Mirek Z., Głowaciński Z., Klimek K., Piękoś-Mirkowa H. (eds). Zakopane–Kraków, pp. 335-346. (in Polish)

SZWEYKOWSKI J., BUCZKOWSKA K. 1998. The rhizoid-initial field – a valuable taxonomic character in species of the genus *Calypogiea* Raddi (Hapaticae, Jungermanniales). Nova Hedwigia 67 (1-2): 23-43.

SZWEYKOWSKI J., BUCZKOWSKA K. 1999. Cell walls as taxonomic markers in Polish species of the genus *Odontoschisma* (Dum.) Dum. (Hepaticae, Cephaloziaceae). Acta Soc. Bot. Pol. 68: 39-45.

SZWEYKOWSKI J., KRZAKOWA M. 1990. Peroxidases as taxonomic markers for some *Calypogeia* species collected in Poland. Nova Hedwigia 51: 241-255.

WENDEL J.F., WEEDEN N. 1989. Visualisation and interpretation of plant isozymes. In: Isozymes in plant biology. Soltis D.E. and Soltis P.S. (eds). Dioscoroides Press, Portland, Oregon, pp. 5-45.

WYATT R., ODRZYKOSKI I.J., STONEBURNER A. 1989. High levels of genetic variability in the haploid moss *Plagiomnium ciliare*. Evolution 43 (5): 1085-1096.

WYATT R., ODRZYKOSKI I.J., KOPONEN T. 1997. *Mnium orientale sp. nov.* from Japan is Morphologically and Genetically Distinct from *M. hornum* in Europe and North America. Bryologist, 100: 226-236.

IZOENZYMATYCZNE MARKERY DWÓCH GATUNKÓW WĄTROBOWCÓW: *BARBILOPHOZIA LYCOPODIOIDES* (WALLR.) LOESKE I *B. HATCHERI* (A. EVANS) LOESKE

STRESZCZENIE

Dwa blisko spokrewnione gatunki z rodzaju *Barbilophozia*: *B. lycopodioides* i *B. hatcheri* są częstym elementem flory wątrobowców Tatr. Oba gatunki charakteryzują się szeroką tolerancją ekologiczną i zajmują podobne siedliska, a co za tym idzie, często spotyka się je rosnące razem. Duża plastyczność morfologiczna tych roślin powoduje powstanie nietypowych form, często trudnych do oznaczenia za pomocą klasycznych metod taksonomicznch. Rozwiązaniem tego problemu może być znalezienie izoenzymatycznych markerów, pozwalających na prawidłową identyfikację wątpliwych form. Oba taksony zbadano pod względem dwóch układów enzymatycznych: transaminazy glutaminianowo-octanowej (GOT) i peroksydaz (PX). U obu gatunków zaobserwowano inną ruchliwość prążków peroksydaz, i to zarówno w części anodowej jak i katodowej żelu. Elektroforetyczne fenotypy peroksydaz były monomorficzne, stałe i specyficzne dla każdego gatunku. U obu taksonów znaleziono cztery różne fenotypy GOT. Pierwsze dwa (GOT 1 i GOT 2) były charakterystyczne dla *B. hatcheri* a następne dwa (GOT 3 i GOT 4) dla *B. lycopodioides*. Powyższe wyniki wskazują, że isoenzymy PX, jak i GOT są dobrymi markerami i mogą mieć praktyczne zastosowanie do oznaczania gatunków z rodzaju *Barbilophozia*.

SŁOWA KLUCZOWE: *Barbilophozia lycopodioides*, *B. hatcheri*, wątrobowce, izoenzymatyczne markery.