Morphological differentiation of *Calypogeia muelleriana* (Jungermanniales, Hepaticae) in Poland

Katarzyna Buczkowska

Department of Genetics, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland, e-mail: androsac@amu.edu.pl

Abstract: Morphological and anatomical characters and oil bodies were studied in 52 samples from Poland: 31 samples of typical *Calypogeia muelleriana* and 21 of a newly detected taxon with the use of isozyme markers. Plants of the new taxon morphologically resemble *C. muelleriana*, but differ from it significantly in 16 morphological traits as well as oil body characters. The greatest morphometric differences were found in the features connected with underleaves. The Mahalanobis distance based on the 47 quantitative traits, between the newly detected taxon and *C. muelleriana* was larger than that between *C. muelleriana* and *C. azurea* — a generally accepted species. The forward stepwise method of discriminant analysis showed that the set of diagnostic characters could be limited to five. A dendrogram constructed on the basis of the Euclidean distance, using the set of diagnostic characters, divided the examined samples into two groups that correlated with groups detected by genetic markers. Results of a multivariate analysis showed that five morphological traits are sufficient for a proper classification of plants to these two taxa.

Key words: Bryophyta, liverworts, *Calypogeia*, biometry, oil bodies, new taxon

1. Introduction

*Calypogeia* Raddi is considered as one of the most difficult genera among liverworts (Schuster 1969; Szweykowski 2006). The simple morphological structure of the gametophyte together with high, environmentally induced, phenotypic plasticity resulted in a situation when valid distinction between species may be masked (Schuster 1969; Szweykowski 1984). On the one hand, environmentally induced modifications were sometimes described as distinct species (Warnstorf 1917); on the other hand, truly distinct species may be still unrecognized. Application of experimental methods, such as isozyme and molecular markers combined with biometry, to bryophyte taxonomy in many cases helped to recognize hidden taxa and to determine species taxonomic boundaries and, subsequently, to find good diagnostic characters, e.g. for *Porella baueri* (Schiffn.) C. Jens. (Boisselier-Dubayle *et al.* 1998), *Conocephalum salebrosum* Szweyk., Buczk. & Odrzyk. (Szweykowski *et al.* 2005) or for those regarded by many authors, e.g. Schuster (1969), Shlyakov (1979) and Müller (1951-1958), as difficult species pairs of the *Calypogeia* genus, i.e., *C. muelleriana* (Schiffn.) Müll. Frib. – *C. azurea* Stotler & Crotz, *C. muelleriana* – *C. sphagnicola* (Arnell & J. Perss.) Warnst. & Loeske or *C. neesiana* (C. Massal. & Carestia) Müll. Frib. – *C. integristipula* Steph. (Buczkowska 2004a). Morphological and anatomical studies based on genetically identified plants in some cases make it possible to find new morphological features, facilitating identification of newly distinguished taxa, as in the *Conocephalum conicum* complex (Szweykowski *et al.* 2005).

Recent isozyme studies of the Polish species of *Calypogeia* indicate that some new taxa, unrecognized to date, may occur in Poland apart from the well known and accepted species, such as: *C. integristipula*, *C. neesiana*, *C. azurea*, *C. muelleriana*, *C. suecica* (Arnell & J. Perss.) Müll. Frib. and *C. sphagnicola* (Szweykowski & Krzakowa 1990; Buczkowska *et al.* 2004; Szweykowski 2006). Two genetically distinct groups of plants, which differ in terms of oil body features and morphology, were recognized within the complex of *C. fissa* (L.) Raddi (Buczkowska 2004b) and *C. sphagnicola* (Buczkowska *et al.* 2009). Another taxon detected in this way is the taxon that morphologically
resembles *Calypogeia muelleriana*. For this reason, it has been previously overlooked (Buczkowska 1999). Morphological differences between *C. muelleriana* and the newly detected unit, in view of a high morphological plasticity of liverworts, could be misinterpreted by most taxonomists as environmentally induced variation of *C. muelleriana*. The newly detected unit corresponds with an atypical form of *C. muelleriana* reported by Szweykowski (2005). This author noted morphological diversification of the species in Poland and distinguished two forms, i.e. typical and atypical, and suggested that the taxonomical status of the atypical form demands further clarification. According to Szweykowski (2006), the typical form is widespread in the south-western part of Poland, whereas the atypical form occurs in the western part of lowlands. Isozyme studies have proved that the above forms of *C. muelleriana* are genetically distinct taxa (Buczkowska & Bączkiewicz submitted). The genetic distance among typical *C. muelleriana* and the newly detected group was higher (D = 1.093) than between the two well-known and accepted species: *C. muelleriana* and *C. azurea* (D = 0.628). Genetic differences between the examined taxa were also higher than between different liverwort species belonging to the same genus, e.g. *Marchantia polymorpha* L. and *M. aquatica* (Nees) Burgeff I = 0.562 (D = 0.576) (Boisselier-Dubayle et al. 1995), *Porella platyphylla* (L.) Pfeiff. and *P. cordeana* (Hüb.) Moore I = 0.486 (D = 0.722) (Boisselier-Dubayle et al. 1998) or *Ptilidium pulcherrimum* (Web.) Vain. and *P. ciliare* (L.) Hampe (D = 0.739) (Adamczak et al. 2005).

The aim of my studies was to answer the following questions: (i) is the taxon recently distinguished in Poland on the basis of isozyme studies morphologically distinct from the typical form of *C. muelleriana*, and (ii) what is the range of variation of morphological and anatomical characters?

2. Material and methods

2.1. Plant material

Plants used in biometrical studies were collected from different regions of Poland (Appendix). Plants were initially determined on the basis of morphological traits according to Müller (1951-1958), Schuster (1969) and the author’s observations (Buczkowska 2004a). In living plants, directly after collection, oil body characters were studied. After identification, each sample was divided into two parts: one was deposited as a voucher in the POZW herbarium, while the other was used for isozyme analysis and biometrical studies. A total of 52 samples of *C. muelleriana* s.l. from 16 populations were examined: 31 samples of the typical form and 21 of the atypical form (the genetically detected new taxon). The Discriminant Analysis was also conducted on 35 samples of *C. azurea* used in the previous biometrical study by Buczkowska (2004a) for comparative purposes. Since *C. azurea* is the widespread, genetically and morphologically distinct, well-known and generally accepted species of the genus *Calypogeia* (Müller 1951-1958; Schuster 1969; Buczkowska et al. 2004), it was the best species to be designated as a reference group.

2.2. Biometrical analysis

Each sample used in biometrical studies was genetically identified on the basis of 11 isozyme loci. Five stems from each sample were measured and biometrical analysis of 47 morphometric traits was performed according to the method described by Buczkowska (2004a). Descriptive statistics, i.e. means, ±95% confidence intervals for the mean, standard deviations, minimums, maximums and coefficients of variation, were computed to evaluate the range of variation of anatomical and morphological traits. Normality of the data was verified by the Shapiro-Wilk test. The significance of the difference between two means was tested by one-way analysis of variance (ANOVA). Multivariate analyses, i.e. discriminant analysis and cluster analysis (agglomeration and k-means clustering methods), were performed to examine relationships between the investigated groups and to find the best characters that facilitate discrimination between the groups. For cluster analysis standardized data were used. Biometrical data were analysed statistically using STATISTICA 8.0 for Windows.

3. Results

3.1. Morphometry

Descriptive statistics and the coefficient of variation of all examined characters were calculated for the two groups distinguished on the basis of isozyme markers: the newly detected taxon and the typical form of *C. muelleriana* (Table 1). Analysis of variance (ANOVA) showed that the two groups differ significantly in 16 analysed traits. The greatest differences between the studied groups were found in the features connected with underleaves: traits 13, 7, 11, 4, 5 and 2 had the highest values of F statistics (Table 1, Figs. 1-2). The newly detected taxon differs significantly also in the most of the features concerns with stem (Table 1). The Discriminant Analysis was also conducted on 35 samples of *C. azurea* used in the previous biometrical study by Buczkowska (2004a) for comparative purposes. Since *C. azurea* is the widespread, genetically and morphologically distinct, well-known and generally accepted species of the genus *Calypogeia* (Müller 1951-1958; Schuster 1969; Buczkowska et al. 2004), it was the best species to be designated as a reference group.

The discriminant analysis based on the 47 quantitative morphological and anatomical characters revealed a distinct morpho-anatomical gap between the studied forms. The scatterplot for the two discriminant functions (canonical roots) shows that the most significant
Table 1. Descriptive statistics for 47 quantitative characters of *C. muelleriana* s.s. and the new taxon and *F* statistics

| No. | Mean | -95% | +95% | Min. | Max. | SD | F | Mean | -95% | +95% | Min. | Max. | SD | V% |
|-----|------|------|------|------|------|----|---|------|------|------|------|------|----|-----|
| 1   | 21.9 | 21.5 | 22.3 | 19.2 | 24.1 | 1.1 | 5.1 | 21.9 | 21.3 | 22.5 | 19.6 | 24.3 | 1.3 | 5.9 |
| 2   | 23.3 | 22.8 | 23.8 | 20.9 | 26.2 | 1.3 | 5.5 | 27.0 | 26.0 | 28.0 | 23.4 | 31.7 | 2.2 | 8.3 |
| 3   | 35.5 | 32.6 | 34.5 | 27.0 | 37.7 | 2.7 | 7.9 | 33.7 | 32.8 | 34.6 | 29.1 | 36.4 | 1.9 | 5.7 |
| 4   | 45.6 | 44.2 | 47.0 | 39.3 | 53.5 | 3.7 | 8.2 | 55.2 | 53.4 | 57.0 | 44.9 | 59.9 | 3.9 | 7.1 |
| 5   | 28.6 | 27.9 | 29.3 | 24.1 | 32.1 | 1.9 | 6.6 | 33.3 | 32.4 | 34.2 | 29.2 | 37.8 | 1.9 | 5.8 |
| 6   | 46.1 | 44.5 | 47.7 | 37.8 | 52.4 | 4.3 | 9.3 | 47.0 | 44.8 | 49.3 | 36.2 | 53.1 | 4.9 | 10.5 |
| 7   | 6.5  | 6.2  | 6.7  | 4.3  | 8.1  | 0.8 | 12.3| 2.8  | 2.6  | 3.0  | 2.3  | 4.1  | 0.5 | 16.2 |
| 8   | 32.1 | 31.7 | 32.5 | 28.8 | 38.2 | 2.5 | 7.5 | 36.5 | 35.2 | 37.3 | 32.0 | 41.3 | 3.0 | 8.1 |
| 9   | 41.2 | 39.9 | 42.4 | 35.0 | 46.6 | 3.3 | 8.1 | 39.6 | 38.2 | 40.9 | 34.8 | 43.4 | 2.9 | 7.3 |
| 10  | 50.1 | 48.6 | 51.6 | 42.8 | 58.8 | 4.2 | 8.4 | 55.6 | 52.9 | 58.2 | 44.9 | 64.2 | 5.9 | 10.5 |
| 11  | 34.9 | 38.6 | 42.2 | 33.5 | 44.9 | 3.2 | 8.2 | 39.5 | 37.3 | 40.4 | 34.0 | 42.4 | 3.1 | 7.9 |
| 12  | 6.4  | 6.2  | 6.5  | 4.6  | 8.3  | 0.9 | 13.2| 3.6  | 3.3  | 3.8  | 3.0  | 4.3  | 0.9 | 2.0 |
| 13  | 8.5  | 8.3  | 8.8  | 6.3  | 9.9  | 0.7 | 15.9| 3.9  | 3.6  | 4.2  | 3.0  | 4.7  | 0.9 | 2.8 |

Explanations: ±95% = confidence intervals, SD = standard deviation, V% = coefficient of variation. * ñ p ≤ 0.05, ** ñ p ≤ 0.01, *** ñ p ≤ 0.001

1 – width of cells of rhizoid initial field in underleaf, 2 – length of cells of rhizoid initial field in underleaf, 3 – width of cells in underleaf lobe, 4 – length of cells in underleaf lobe, 5 – width of cells in underleaf middle, 6 – length of cells in underleaf middle, 7 – number of cells between the sinus and base of underleaf, 8 – width of underleaf, 9 – length of the whole underleaf, 10 – length of underleaf to the base of rhizoid initial field, 11 – underleaf sinus depth, 12 – ratio of width to length of underleaf, 13 – ‘measure’ of underleaf decurrence – 9/10, 14 – ratio of width of underleaf to width of stem – 8/47, 15 – width of marginal cells in dorsal part of leaf, 16 – length of marginal cells in dorsal part of leaf, 17 – width of median cells in leaf, 18 – length of median cells in leaf, 19 – width of cells at ventral leaf base, 20 – length of cells at ventral leaf base, 21 – ratio of length to width of cells in dorsal part, 22 – ratio of distance A-C to the 1st coordinate – 26/27, 23 – ratio of length of leaf dorsal part to width of leaf ñ 25/24, 24 – ratio of distance from apex to ventral leaf base – 23/26, 25 – ratio of length of leaf dorsal part to width of leaf – 25/24, 26 – number of stem cells in the 4th internode, 27 – length of stem cells in the 4th internode – 38 – number of cells in the 4th internode, 39 – length of stem cells in the 6th internode, 40 – number of stem cells in the 6th internode, 41 – number of stem cells in the 5th internode, 42 – number of stem cells in the 3rd internode, 43 – length of the 4th internode, 44 – length of the 5th internode, 45 – length of the 6th internode, 46 – width of the whole plant, 47 – width of stem (without leaves).

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and clear discrimination is provided for the two studied groups by the first discriminant function (Fig. 3). The second function provides discrimination between *C. muelleriana* s.s. and the new taxon, (they both show negative values for the second canonical function) and the reference species, *C. azurea*, which shows positive values. Mahalanobis distance between the new taxon and *C. muelleriana* s.s. was bigger (D = 16.49) than between *C. azurea* and the typical form of *C. muelleriana* (D = 13.95), as well as between *C. azurea* and the newly detected taxon (D = 10.72). All Mahalanobis distances were statistically significant at p ≤ 0.001. Characters most strongly correlated with the 1st discriminant function were 13, 7, 11, 20 and 2, thus these characters play the most important role in separating the examined groups of *C. muelleriana*. Characters that were responsible for the separation of both groups of *C. muelleriana* and *C. azurea*, included 32, 16, 20, 12 and 7. These characters are most strongly correlated with the 2nd discriminant function.
In order to verify how many characters are necessary for the correct classification of plants of the studied groups, the forward stepwise method of discriminant analysis was used. Wilks’ lambda criterion was the basis for the creation of a set of characters best discriminating the studied taxa. The calculated Wilks’ Lambda value fits the range from 0 (perfect discrimination) to 1 (a lack of discrimination). In the 5th step, discrimination between the analysed taxa was highly significant (Wilks’ Lambda = 0.036; $F = 247.11$,

Fig. 2. Underleaves of the studied taxa: a, b – the new taxon; c, d – *C. muelleriana* s.s.; a, c – on the stem, b, d – separated from the stem

Fig. 3. Distribution of samples of *C. muelleriana* s.s., the new taxon and *C. azurea* in the two discriminant functions (canonical roots)
p<0.0001), thus the set of diagnostic characters could be limited to five traits having the biggest discriminative power: 7, 13, 2, 11, 20.

The diagnostic value of 5 traits selected above was tested by performing cluster analysis. The dendrogram constructed on the basis of the Euclidean distance divided the examined samples into two groups, which correlated with groups detected by genetic markers: \textit{C. muelleriana} s.s. and the new taxon (Fig. 4). Correctness of the partitioning was verified by the k-means clustering method of cluster analysis. In this method the number of clusters is established a priori and investigated samples are allocated into these clusters with the goal of minimizing the within-cluster variance and maximizing the between-cluster variance. In this case results of k-means clustering entirely matched the clusters found in the joining analysis, all samples of the new taxon were included in the cluster 1, whereas all samples of \textit{C. muelleriana} s.s. in the cluster 2 (Fig. 5). The results of multivariate analysis showed that five

![Fig. 4. A dendrogram of the studied taxa: \textit{C. muelleriana} s.s. (full circles) and the new taxon (full squares) constructed on the basis of the Euclidean distance according to the complete linkage method, using the set of five diagnostic characters](image)

![Fig. 5. Plot of means of five diagnostic characters for each cluster obtained in the k-means clustering method](image)
morphological traits (7, 13, 2, 20, 11) are sufficient for a proper classification of plants into these two taxa.

3.2. Oil bodies

The taxa distinguished within *C. muelleriana* s.l. on the basis of isozyme patterns also differed in the characters of oil bodies. In both taxa, oil bodies are colourless, present in all cells of leaves and underleaves, usually divided into segments, rarely spherical (undivided). However, oil bodies in the plants of typical *C. muelleriana* are smaller (3-) 5-6 x 8-10(-15) µm, (3-) 4-6 (-8) per cell, while in plants of the newly detected taxon oil bodies are bigger (4-8 x 10-17 µm), (3-) 6 (-9) per cell and more distinctly segmented than these of the typical form (Fig. 6).

4. Discussion

Isozyme studies have proved that plants named by Szweykowski (2006) as the atypical form of *C. muelleriana* in fact represent the new taxon, which is genetically distinct from all *Calypogeia* species occurring currently in Poland, including the groups recently detected within *C. fissa* and *C. sphagnicola* (Buczewska & Baczkiewicz submitted). The present biometrical analyses also revealed its morphological distinctness. Because the taxon under consideration most strongly resembles *C. muelleriana* s.s. and, in addition, the two taxa co-occur in the northern part of the country and sometimes form mixed patches, particular attention was paid to the morphological comparison.

![Fig. 6. Oil bodies in cells of leaves: a, c, e – the new taxon; b, d, f – *C. muelleriana* s.s.; a, b, c, d – of median cells; e, f cells at ventral leaf base; bar for a, b = 50 mm, for c, d, e, f = 25 mm](image-url)
between the typical *C. muelleriana* and the newly detected taxon. The typical form of *C. muelleriana* in respect of morphological characters exactly corresponds with its type specimen, with which the plants growing in Poland were compared in detail (Buczkowska 2004a). The new taxon differs from *C. muelleriana* s.s. in oil bodies features, therefore, the classification of fresh material should not be difficult. In *Calypogeia* oil body characters are regarded as highly diagnostic, which offers a possibility of a quick and accurate identification of particular species (Buch 1935; Szweykowski & Krzakowa 1990; Buczkowska 2004a; Buczkowska et al. 2004). Moreover, statistically significant differences in 16 other morphological traits facilitate identification of herbarium material without data on oil bodies. The studied taxa do not differ in their overall size (trait 46) and the shape of leaves (traits 23-33). The most useful morphological features separating the two taxa are characters describing the shape of underleaves, i.e. the measure of underleaf decurrence (trait 13), underleaf sinus depth (trait 11), the number of cells between the sinus and the base of underleaf (trait 7) as well as cell size (traits 2, 4, 5). The new taxon, in contrast to *C. muelleriana* s.s., has underleaves that are not decurrent, with a distinct sinuses, most often with angulations or obtuse teeth on lobe external margins. The number of cells between the sinus and the base of the underleaf is lower (average 2.8) in the new taxon than in *C. muelleriana* s.s. (average 6.5). Some statistically significant differences were also found in stem structure (traits 35, 41, 47). In the new taxon, narrower stems with more numerous, but smaller cells were observed. Biometrical analyses showed that the newly detected taxon differs from the other species of the *Calypogeia* genus occurring in Poland, including the newly detected groups in *C. fissa* and *C. sphagnicola* (Buczkowska 2004a, 2004b; Buczkowska et al. 2009). The obtained results make it possible to describe the studied group as a separate species. However, abundant herbarium material needs to be examined in detail to establish finally the taxonomic status of the analysed taxon. So far, many new species were described within the *Calypogeia* genus, with some of them being subsequently reduced to synonyms of particular species (Müller 1951-1958; Schuster 1969), therefore it has to be verified if any of the several already published names fits the taxon discussed above.

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**References**

ADAMCZAK M., BUCZKOWSKA K., BĄCZKIEWICZ A., & WACHOWIAK W. 2005. Comparison of allozyme variability in Polish populations of two species of *Ptilidium* Nees (Hepaticae) with contrasting degrees of sexual reproduction. Cryptogamie, Bryology 26: 151-165.

BOISSELIER-DUBAYLE M. C., JUBIER M. F., LEJEUNE B. & BISCHLER H. 1995. Genetic variability in the three subspecies of *Marchantia polymorpha* (Hepaticae): isozymes, RFLP and RAPD markers. Taxon 44: 363-376.

BOISSELIER-DUBAYLE M. C., LAMBOURDIERE J. & BISCHLER H. 1998. The leafy liverwort *Porella baueri* (Porellaceae) is an allopolyploid. Plant Syst. Evol. 210: 175-197.

BUCH H. 1935. Vorarbeiten zu einer Lebermoosflora Fennoscandias III. DieGattung *Calypogeia* Raddi. Mem. Soc. F. Fl. Fenn. 41: 197-214.

BUCZKOWSKA K. 1999. Plastyczność morfologiczno-anatomiczna i problem gatunku u mszaków, na przykładzie pary krytycznych gatunków rodzaju *Calypogeia* Raddi: *C. neesiana* i *C. integrisipula*. Ph. D Thesis, Department of Genetics, Adam Mickiewicz University, Poznan, Poland.

BUCZKOWSKA K. 2004a. The genus *Calypogeia* Raddi (Hepaticae, Jungermanniales) in Poland, biometrical analysis of morphological and anatomical variation. Nova Hedwigia 78: 121-146. DOI 10.1127/0029-5035/2004/0078-0121.

BUCZKOWSKA K. 2004b. Genetic differentiation of *Calypogeia fissa* Raddi (Hepaticae, Jungermanniales) in Poland. Plant Syst. Evol. 247: 187-201. DOI 10.1007/s00606-003-0156-9.

BUCZKOWSKA K. & BĄCZKIEWICZ A. New taxon of the genus *Calypogeia* (Jungermanniales, Hepaticae) in Poland. Acta Soc. Bot. Pol. (submitted).

BUCZKOWSKA K., BĄCZKIEWICZ A., SAWICKI J. & SZCZĘŚNIAK M. 2009. Genetic differentiation within *Calypogeia sphagnicola* (Jungermanniales, Hepaticae) in Poland. Nowellia bryologica, special issue: 34-42.

BUCZKOWSKA K., ODRZYKOSKI I. & CHUDZIŃSKA E. 2004. Delimitation of some European species of *Calypogeia* Raddi (Hepaticae, Jungermanniales) based on cytological characters of oil bodies and multienzyme phenotype. Nova Hedwigia 78: 147-163. DOI 10.1127/0029-5035/2004/0078-0147.

MULLER K. 1951-1958. Die Lebermoosflora Europas. In: Dr. L. Rabenhorst’s Kryptogamen Flora von Deutschland, Österreich und der Schweiz, pp. 1161-1187. Akademische Verlagsgesellschaft Geest & Portig K.-G., Leipzig.
SCHUSTER R. M. 1969. The Hepaticae and Anthocerotae of North America east of the hundredth meridian, 2, pp. 98-215. Columbia University Press, New York-London.

SHILYAKOV R. N. 1979. Pieczenocznyje mchi sjewiera S.S.S.R. Vol. 2 Lofozievije (Hepatics of the northern territories of the USSR, Vol. 2, Lophoziaceae). Nauka, Leningrad.

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

SZWEYKOWSKI J. 1984. Species problems and taxonomic methods in Bryophytes. In: R. M. SCHUSTER (ed.). The New Manual of Bryology, 2, pp. 1130-1171. The Hattori Bot. Lab. Nichinan, Miyazaki.

SZWEYKOWSKI J. 2006. An annotated checklist of Polish liverworts and hornworts. In: Z. MIREK (ed.). Biodiversity of Poland, 4, 18-20 pp. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

SZWEYKOWSKI J., BUCZKOWSKA K. & ODRZYKOSKI I. J. 2005. Conocephalum salebrosum (Marchantiopsida, Conocephalaceae) a new Holarctic liverwort species. Plant Syst. Evol. 253: 133-158. DOI 10.1007/s00606-005-0301-0.

WARNSTORF C. 1917. Die europäische Artgruppen der Gattung Calypogeia Raddi (1820). Bryolog. Zeitschr. 1: 97-114.
Appendix. Collection sites of the studied populations of *Calypogeia muelleriana* s.s. and the new taxon. The POZW No. denotes voucher number in the herbarium. Mixed samples were marked with an asterisk.

| Population No. | Locality | Geographic coordinates | POZW No. | Collector |
|----------------|----------|------------------------|----------|-----------|
| 1              | NW Poland, Pomorskie Province, Lake Orle near Miastko | N54°01'05'', E17°04'04'' | 41346, 41182, 42208, 42210, 41152*, 41153* | KB, AB |
| 2              | NE Poland, Warmińsko-Mazurskie Province, Lake Godle near Elk | N53°52'00'', E22°31'48'' | 41708, 41709* | KB, AB |
| 3              | Central Poland, Wielkopolskie Province, Antonin near Ostrów Wlkp. | N51°30'31'', E17°50'43'' | 42234 | KB |
| 4              | NW Poland, Pomorskie Province, Lake Książę near Kościeryczyna | N54°04'50'', E17°58'04'' | 35610 | KB, AB |
| 5              | NW Poland, Pomorskie Province, Lake Lubygosć near Kartuzy | N54°24'33'', E17°59'24'' | 42214, 42215, 42217, 42218, 42213*, 42216* | KB, AB |
| 6              | NW Poland, Pomorskie Province, Lake Głęboczko near Bytów | N54°11'45'', E17°33'26'' | 35629, 42222* | KB, AB |
| 7              | W Poland, Lubuskie Province, Nowogród Bobrzański | N51°48'05'', E15°10'08'' | 42304, 42314, 42315, 42319 | SR, KB |
| 8              | W Poland, Lubuskie Province, Starosiele forest division | N51°44'12'', E15°13'50'' | 42320, 42321, 42322, 42323, 42324 | SR, KB |
| 9              | SW Poland, Dolnośląskie Province, Izerskie Mts, peat bog called ‘Na Izerze’ | N50°50'45'', E15°21'40'' | 35108, 36669, 36668, 36675 | KB, AB |
| 10             | NW Poland, Pomorskie Province, Lake Orle near Miastko | N54°01'05'', E17°04'04'' | 41151, 42152*, 42153* | KB, AB |
| 11             | NE Poland, Lake Godle near Elk | N53°52'00'', E22°31'48'' | 41706, 41707, 41714, 41709* | KB, AB |
| 12             | NW Poland, Pomorskie Province, Lake Głęboczko near Bytów | N54°11'45'', E17°33'26'' | 35625, 42222* | KB, AB |
| 13             | NE Poland, Wigierski National Park, Lake Sucharek near Suwałki | N54°01'48'', E23°03'40'' | 32295, 32296, 35596 | KB, AB |
| 14             | NW Poland, Pomorskie Province, Lake Smolowe near Miastko | N54°01'40'', E17°04'30'' | 36789, 36786, 36790, 42285a, 42285b | KB, AB |
| 15             | NW Poland, Pomorskie Province, Lake Lubygosć near Kartuzy | N54°24'33'', E17°59'24'' | 42220, 42213*, 42216* | KB, AB |
| 16             | NW Poland, Pomorskie Province, Lake Jeleń near Bytów | N54°11'04'', E17°31'05'' | 42418 | KB, AB |

Explanations: Collectors, AB – Alina Bączkiewicz, KB – Katarzyna Buczkowska, SR – Stanisław Rosadziński