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

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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

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resembles *C. 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 (2006). 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 parts 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 izozyme 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 Discriminat 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. discriminat 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 four leaf and three stem characters; however, from these traits only one (No. 20) had a high value of F statistics (Table 1).

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.	*C. muelleriana*							New taxon							F
	Mean	-95%	+95%	Min.	Max.	SD	V%	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	0.01
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	58.17**
3	33.5	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	0.05
4	45.6	44.2	47.0	39.3	55.3	3.7	8.2	55.2	53.4	57.0	44.9	59.9	3.9	7.1	80.04**
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	75.28**
6	46.1	44.5	47.7	37.8	52.8	4.3	9.3	47.0	44.8	49.3	36.2	53.1	4.9	10.5	0.52
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	352.97***
8	828.9	787.2	870.5	623.3	1156.7	113.5	13.7	684.6	645.8	723.4	555.6	783.3	85.3	12.5	24.47**
9	654.0	624.6	683.4	511.1	873.3	80.2	12.3	544.9	515.8	574.0	432.2	652.2	63.9	11.7	27.13**
10	468.1	447.3	489.0	351.1	605.6	56.8	12.1	474.2	448.6	499.8	365.6	582.2	56.2	11.9	0.14
11	110.8	101.9	119.6	66.7	166.7	24.2	21.9	235.4	219.7	251.1	143.3	294.2	34.5	14.6	234.98***
12	1.3	1.2	1.3	1.1	1.4	0.1	6.6	1.3	1.2	1.3	1.2	1.4	0.1	5.1	0.56
13	1.4	1.4	1.4	1.3	1.5	0.1	4.4	1.1	1.1	1.1	1.1	1.2	0.0	3.0	336.95***
14	2.4	2.4	2.5	2.1	2.8	0.2	7.3	2.4	2.3	2.5	2.1	2.7	0.2	7.3	0.72
15	36.2	35.1	37.4	30.9	44.8	3.1	8.6	35.0	34.0	36.1	31.8	38.3	2.3	6.4	2.23
16	32.6	31.7	33.5	28.2	38.8	2.5	7.5	36.5	35.2	37.9	32.0	41.3	3.0	8.1	26.31**
17	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	3.18
18	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	15.41*
19	39.9	38.6	41.2	34.2	46.2	3.4	8.6	38.6	37.1	40.1	32.5	44.5	3.3	8.5	1.79
20	81.4	78.7	84.1	70.0	102.1	7.4	9.0	67.7	64.9	70.5	53.4	82.9	6.2	9.1	49.58**
21	34.9	33.8	35.9	28.5	42.1	2.9	8.3	35.1	34.0	36.2	30.9	39.4	2.5	7.1	0.10
22	37.6	36.8	38.5	33.1	42.8	2.4	6.4	41.2	39.7	42.8	36.2	47.2	3.4	8.3	19.91*
23	1179.4	1124.4	1234.4	882.2	1456.7	149.9	12.7	1203.6	1152.0	1255.2	952.2	1331.1	113.4	9.4	0.39
24	1259.3	1181.6	1337.0	940.0	1847.8	211.8	16.8	1254.7	1197.9	1311.5	1072.2	1420.0	124.7	9.9	0.01
25	448.9	423.0	474.8	331.1	636.7	70.7	15.8	450.7	432.0	469.3	386.7	515.6	41.0	9.1	0.01
26	1494.1	1412.9	1575.3	1093.3	2030.0	221.5	14.8	1490.7	1405.2	1576.3	1153.3	1740.0	187.9	12.6	0.00
27	931.7	881.1	982.3	710.0	1293.3	138.0	14.8	933.7	881.5	986.0	777.8	1064.4	114.8	12.3	0.00
28	865.5	814.1	916.9	663.3	1262.2	140.1	16.2	866.4	818.4	914.5	703.3	986.7	105.6	12.2	0.00
29	825.0	772.8	877.3	626.7	1252.2	142.4	17.3	818.6	774.6	862.6	667.8	938.9	96.6	11.8	0.03
30	0.9	0.9	1.0	0.8	1.1	0.1	5.9	1.0	0.9	1.0	0.9	1.1	0.0	5.1	1.33
31	0.8	0.8	0.8	0.7	0.8	0.0	3.5	0.8	0.8	0.8	0.8	0.9	0.0	4.0	5.92*
32	1.6	1.6	1.6	1.5	1.8	0.1	4.3	1.6	1.6	1.6	1.5	1.7	0.1	5.3	0.00
33	0.4	0.4	0.4	0.3	0.4	0.0	4.6	0.4	0.4	0.4	0.3	0.4	0.0	4.8	0.30
34	25.4	24.5	26.2	20.6	31.0	2.2	8.9	26.4	25.6	27.1	24.4	31.2	1.6	6.0	3.08
35	69.8	64.7	74.9	52.5	95.8	13.8	19.8	62.3	57.7	66.9	43.8	74.1	10.1	16.2	4.52*
36	25.5	24.7	26.3	22.5	31.4	2.1	8.2	26.0	25.2	26.8	23.2	28.9	1.7	6.5	0.90
37	70.5	65.7	75.3	52.5	99.8	13.0	18.4	68.4	64.3	72.5	49.5	84.8	9.1	13.3	0.42
38	25.5	24.9	26.2	22.9	30.8	1.8	7.0	26.1	25.4	26.8	23.6	29.3	1.6	6.1	1.36
39	71.0	66.8	75.3	54.6	92.6	11.6	16.4	67.1	62.9	71.3	54.2	90.1	9.2	13.7	1.67
40	8.2	7.8	8.6	6.4	11.9	1.2	14.3	8.8	8.2	9.5	6.9	10.9	1.4	15.9	3.24
41	8.1	7.7	8.4	6.3	9.9	1.0	11.8	8.9	8.3	9.6	7.5	11.1	1.4	15.8	7.05*
42	8.1	7.8	8.5	6.3	10.7	1.1	13.0	8.5	7.9	9.1	7.3	11.4	1.3	15.4	1.46
43	642.1	585.9	698.3	439.3	1092.3	153.2	23.9	717.4	645.9	789.0	494.3	945.8	157.2	21.9	2.97
44	665.1	613.6	716.6	494.3	1067.9	140.4	21.1	747.1	677.3	816.8	506.5	945.8	153.2	20.5	3.96
45	660.8	616.5	705.1	488.2	1049.5	120.8	18.3	731.4	664.8	798.0	549.2	976.3	146.3	20.0	3.60
46	1984.6	1888.3	2080.9	1473.0	2439.6	262.6	13.2	1946.2	1856.1	2036.4	1538.9	2158.9	198.1	10.2	0.32
47	343.8	330.3	357.4	279.5	425.9	36.9	10.7	301.3	289.7	312.8	257.5	339.3	25.4	8.4	21.08**

Explanations: ±95% – confidence intervals, SD – standard deviation, V% – coefficient of variation. * – p ≤ 0.05, ** – p ≤ 0.01, *** – 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 height of underleaf – 8/9, 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 – width of 2nd row marginal cells in dorsal part of leaf, 22 – length of 2nd row marginal cells in dorsal part of leaf, 23 – length of leaf, 24 – width of leaf, 25 – height of dorsal part of leaf, 26 – distance from apex to ventral base of leaf, 27 – length of the 1st coordinate, 28 – length of the 2nd coordinate, 29 – length of the 3rd coordinate, 30 – ratio of length to width of leaf – 23/24, 31 – ratio of leaf length to distance from apex to ventral leaf base – 23/26, 32 – ratio of distance A-C to the 1st coordinate – 26/27, 33 – ratio of length of leaf dorsal part to width of leaf – 25/24, 34 – width of stem cells in the 4th internode, 35 – length of stem cells in the 4th internode, 36 – width of stem cells in the 5th internode, 37 – length of stem cells in the 5th internode, 38 – width of stem cells in the 6th internode, 39 – length of stem cells in the 6th internode, 40 – number of stem cells in the 4th internode, 41 – number of stem cells in the 5th internode, 42 – number of stem cells in the 6th 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)
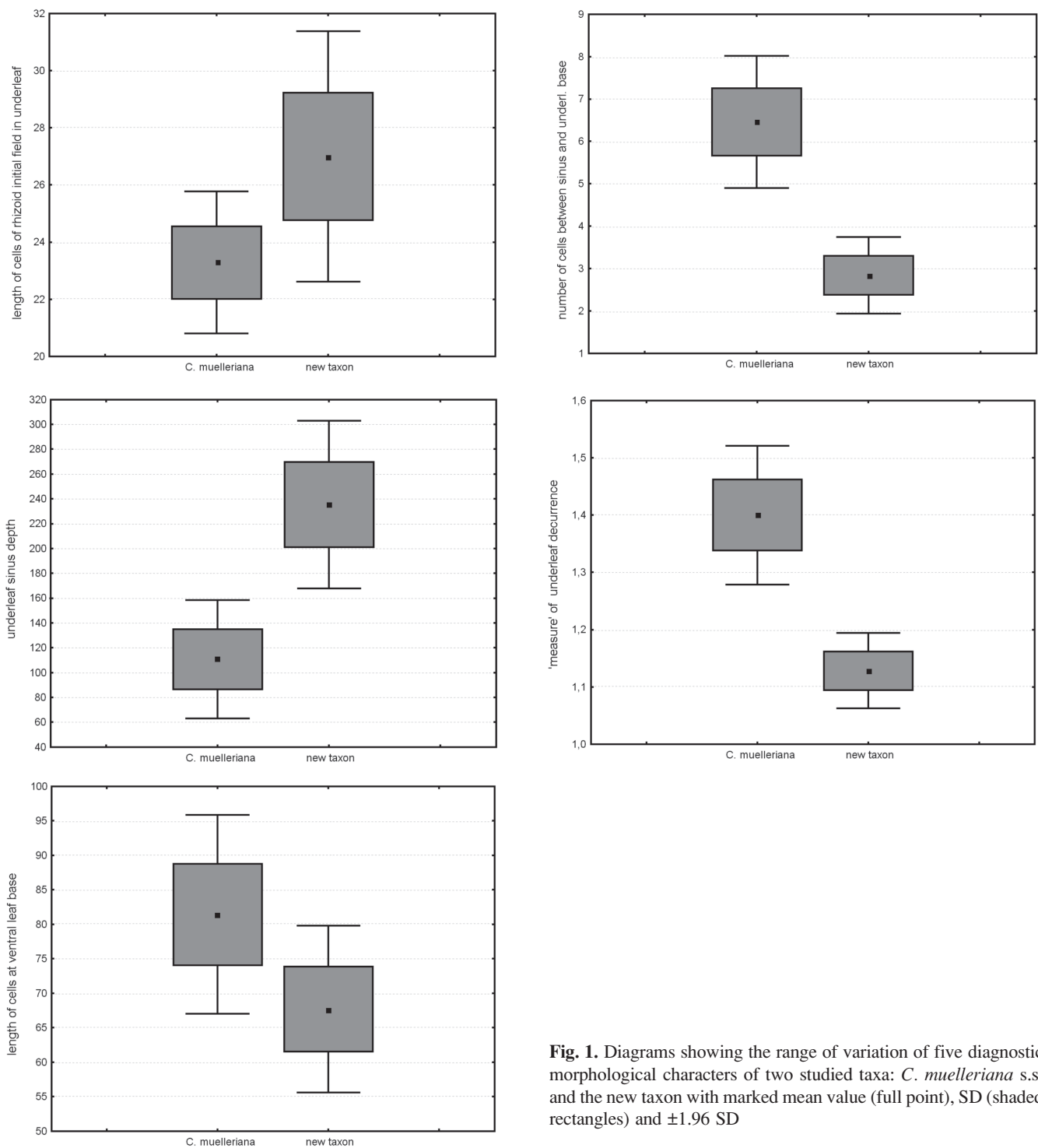
Fig. 1. Diagrams showing the range of variation of five diagnostic morphological characters of two studied taxa: *C. muelleriana* s.s. and the new taxon with marked mean value (full point), SD (shaded rectangles) and ±1.96 SD

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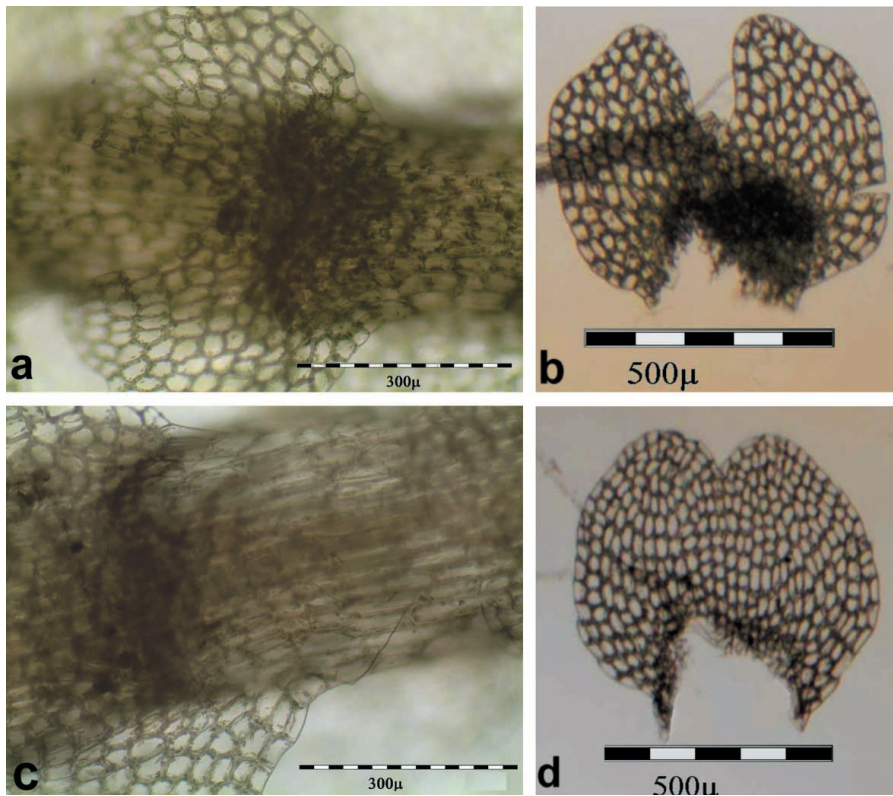
Fig. 2. Underleaves of the studied taxa: a, b – the new taxon; c, d – *C. muelleriana* s.s.; a, c – on the stem, b, d – separated from the stem

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,
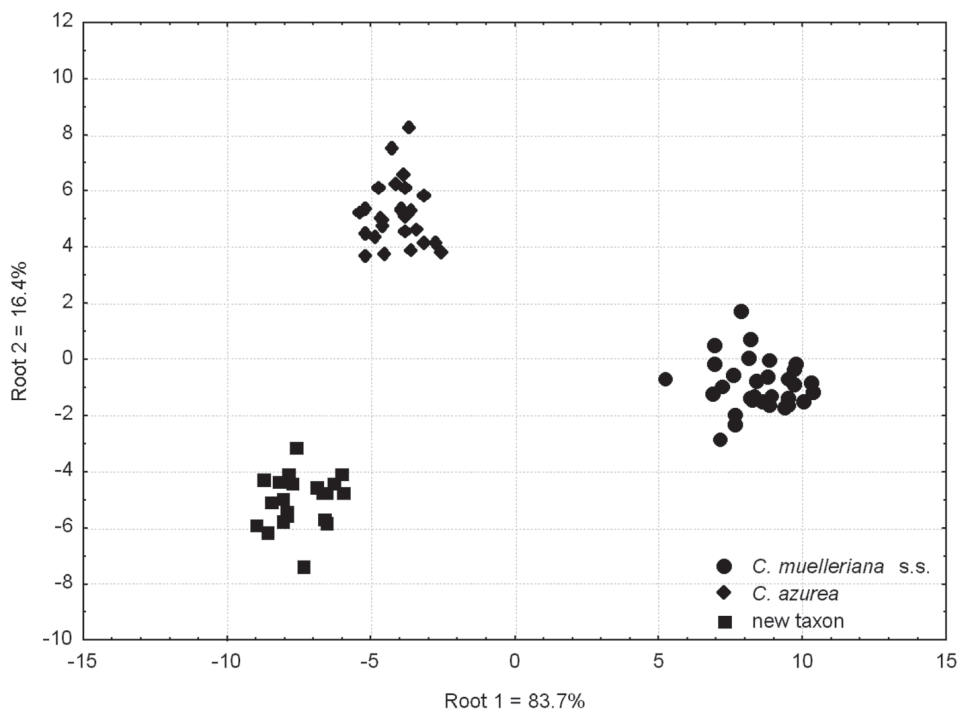
Fig. 3. Distribution of samples of *C. muelleriana* s.s., the new taxon and *C. azurea* in the two discriminant functions (canonical roots)
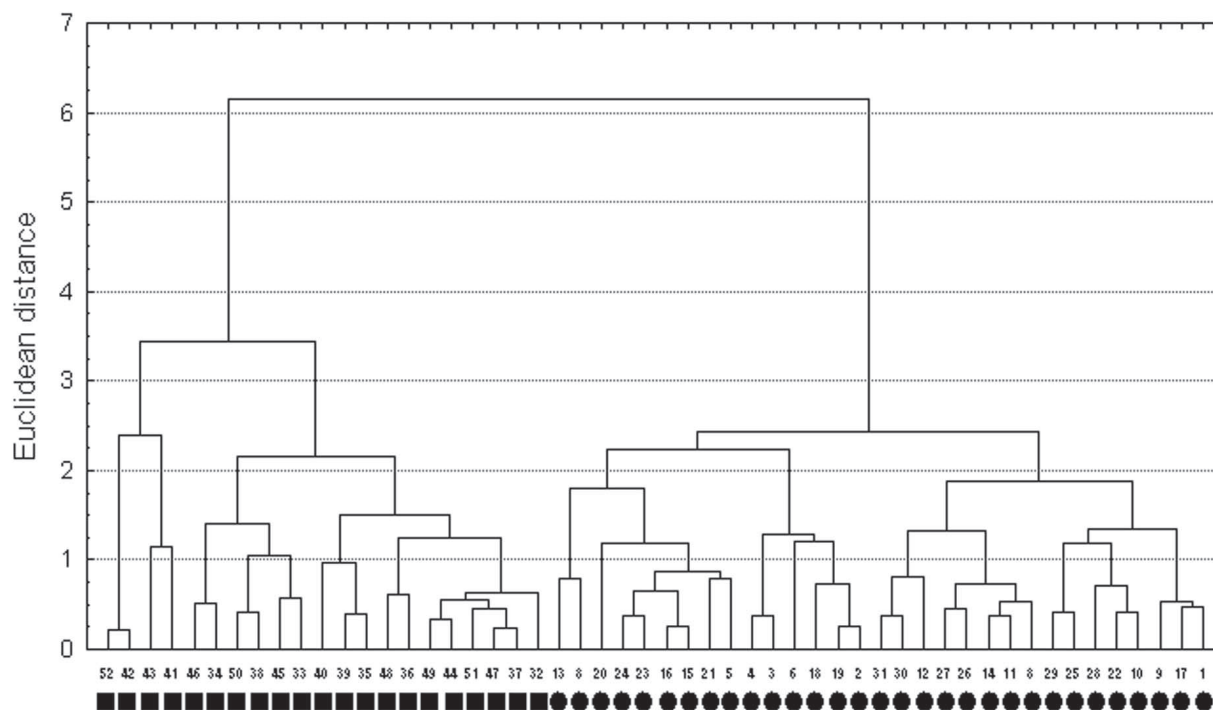
Fig. 4. A dendrogram of the studied taxa: *C. muelleriana* s.s. (full circles) and the new taxon (full squares) constructed on the basis of the Euclidean distance according to the complete linkage method, using the set of five diagnostic characters

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: *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 *C. muelleriana* s.s. in the cluster 2 (Fig. 5). The results of multivariate analysis showed that five
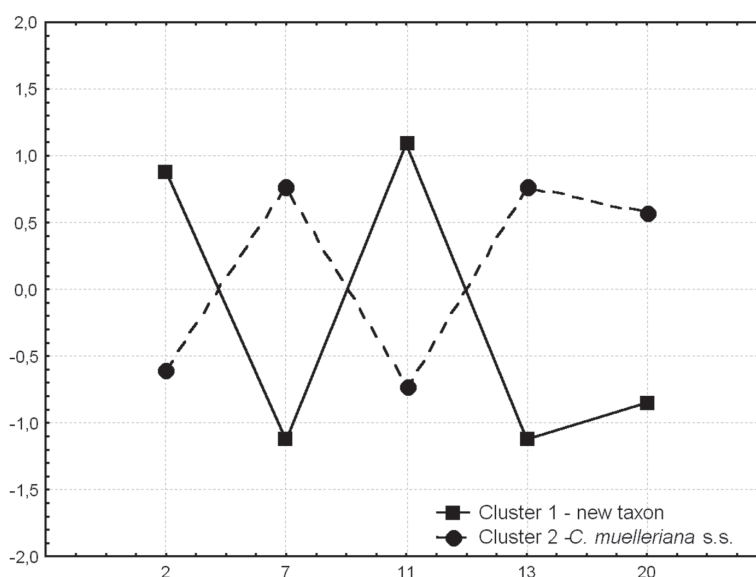
Fig. 5. Plot of means of five diagnostic characters for each cluster obtained in the k-means clustering method
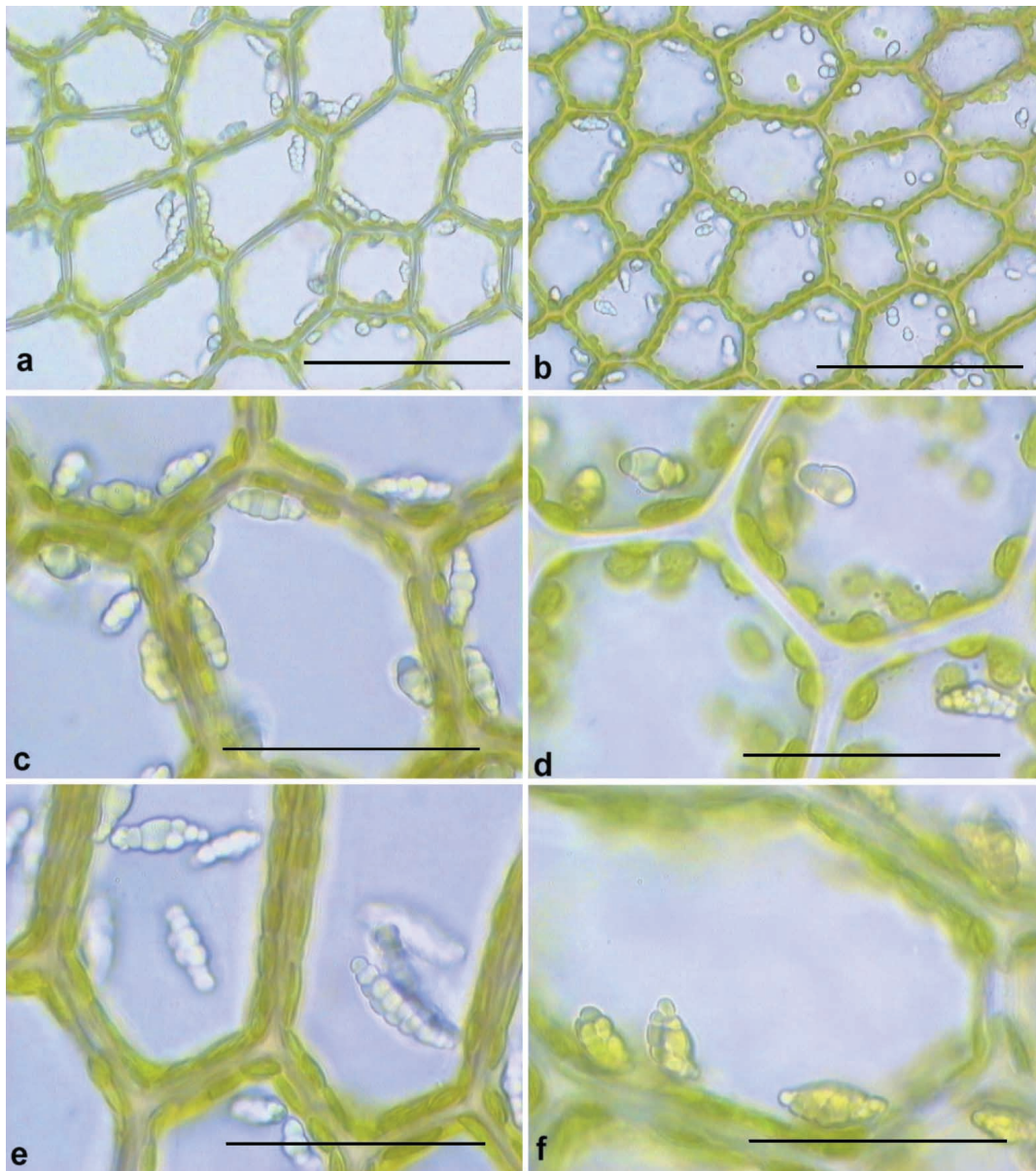
Fig. 6. Oil bodies in cells of leaves: a, c, e – the new taxon; b, d, f – *C. muelleriana* s.s.; a, b, c, d – of median cells; e, f cells at ventral leaf base; bar for a, b = 50 mm, for c, d, e, f = 25 mm

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* (Buczkowska & 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

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. muellerina* 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. muellerina* 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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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
		C. muelleriana s.s.		
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 Ełk	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 Książe near Kościerzyna	N54°04'50'' E17°58'04''	35610	KB, AB
5	NW Poland, Pomorskie Province, Lake Lubygość 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, Starosiedle 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
		New taxon		
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 Ełk	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 Smołowe near Miastko	N54°01'40'' E17°04'30''	36789, 36786, 36790, 42285a, 42285b	KB, AB
15	NW Poland, Pomorskie Province, Lake Lubygość 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