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Two morphologically distinct groups of the *Calypogeia fissa* complex were found in Europe

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Abstract: Two genetically distinct groups (P_S and P_B) detected previously within the *C. fissa* complex in Europe were studied with respect to 47 morphometric characters. The two examined groups differed statistically significantly with respect to 34 morphological traits. The forward stepwise method of discriminant analysis showed that the set of diagnostic characters could be limited to nine. The best diagnostic features were morphological characters describing the shape of leaf: length and width of leaf, height of dorsal part and distance from the apex to the ventral base of the leaf, length of the 3rd coordinate of the leaf, and underleaf width as well as characters of the stem: length of internodes and size of internode cells. Plants of the P_S group were smaller (shoot width range from 922-1780 μm) than plants of the P_B group (1600-3900 μm). Based on genetically identified samples, classification functions for each group were computed and the derived functions were used for the classification of samples from the herbarium collections. The principal component analysis and dendrogram constructed on the basis of Euclidean distance, using the set of diagnostic characters, divided the examined samples into two groups that correlated with groups detected by isozyme markers. Results of multivariable analysis showed that it is possible to satisfactorily characterise morphologically both genetically distinct groups of the *C. fissa* complex.

Key words: Bryophyta, liverworts, *Calypogeia*, morphology, biometry, classification functions

1. Introduction

A limited number of good diagnostic qualitative traits resulting from reduced, as compared to angiosperms, morphology and high phenotypic plasticity of the gametophyte is a serious problem in bryophyte taxonomy. Application of different molecular methods in bryophyte taxonomy revealed that many liverwort species described on the basis of morphological traits, in fact, are only species complexes composed of several cryptic or semicryptic species (Bischler & Boisselier-Dubayle 2000; Shaw 2001; Szweykowski *et al*. 2005; Wachowiak *et al*. 2007; Bączkiewicz *et al*. 2008; Heinrichs *et al*. 2010; Kreier *et al*. 2010; Sawicki *et al*. 2010). Thus, it is expected that some liverwort species can still be discovered, even in Europe.

Genetic differences between cryptic species are of the same rank as between morphologically well-characterised species, although, due to great morphological similarity, they cannot be described as taxonomically distinct species, e.g., *Pellia epiphylla* (Zieliński 1987), *Conocephalum conicum* (Szweykowski & Krzakowa 1979), *Aneura pinguis* (Bączkiewicz & Buczkowska 2005; Bączkiewicz *et al*. 2008), *Reboulia* (Boisselier-Dubayle *et al*. 1998). A formal taxonomic treatment of these species is difficult. Morphological differences, if they exist, are slight, and concern, mostly, quantitative traits which have continuous phenotypic range. It is practically impossible to say whether the observed differences have a genetic basis or not (Szweykowski 1984). One way to resolve this problem is to precisely characterise morphologically plants that have been determined genetically. Morphological and anatomical studies based on genetically identified plants led to description of one of cryptic species detected in *Conocephalum conicum* complex as a new *C. salebrosum* species (Szweykowski *et al*. 2005). Similarly, biometric studies based on genetically identified material allowed

determination of morphological diagnostic characters for a new taxon of the *Calypogeia* genus (Buczkowska 2010) previously detected by isozyme and molecular markers (Buczkowska & Bączkiewicz 2011; Buczkowska & Dabert 2011).

Calypogeia fissa (L.) Raddi is regarded as a suboceanic-mediterrean, amphiatlantic species (Müller 1951-1958; Damsholt 2002). In Europe, it occurs west of Ireland, Great Britain, Spain, Portugal and France across the Balkans to Central Russia in the east as well as in southern parts of the Nordic countries and Iceland. The species is also known from Asia (Turkey and East Himalayas) and North Africa (Marocco, Tunesia), Mediterranean islands and Macaronesia (Paton 1999; Damsholt 2002). *C. fissa* in Poland occurs mainly in the western regions of the country (Koła 1989; Szweykowski 2006). This species seems to be morphologically well characterised and typically developed plants are readily distinguished from other *Calypogeia* species by their characteristic bidentate leaves and by underleaves armed with teeth on the lateral margin (Müller 1951-1958). However, some less typical forms can be occasionally found, which create problems for taxonomists (Schuster 1969; Damsholt 2002; Paton 1999). Currently within *C. fissa*, two subspecies are formally recognized: *C. fissa* subsp. *fissa* occurring in Europe (Grolle & Long 2000) and *C. fissa* subsp. *neogea* Schust. known only from North America (Schuster 1969). In Europe, one form – *C. fissa* f. *subxeropholia* Schiffn. was distinguished by Bischler (1957) and two varieties (var. *fissa*, var. *intermedia*) and two forms (f. *fissa*, f. *subintegrifolia*) by Damsholt (2002). Moreover, several other varieties and forms were described by Warnstorf (1917); however, none of them was accepted. *C. fissa* is a very variable species with regard to morphological traits like size, shape of underleaf lateral margin and leaf apex as well as ecological preferences (Schuster 1969; Paton 1999; Damsholt 2002).

The isoenzyme studies have shown that the species is genetically differentiated and composed of genetically distinct groups which have been tentatively called as P_S, P_B and G (Buczkowska 2004a). The groups P_S, P_B were found in Poland and comprised, respectively, small and bigger plants, the group G was noted in Germany. The P_S group was genetically most distinct, whereas genetic distance between groups P_B and G were lower. These groups detected by genetic methods also differed in oil body characters and habitat requirements (Buczkowska 2004a).

The main purpose of the present study was: (*i*) to check whether any stable morphological differences existed between genetically detected groups; (*ii*) to examine what was the range of morphological and anatomical variation of the groups, and (*iii*) to find the diagnostic characters facilitating their identification.

2. Material and methods

2.1. Plant material

Plants used in biometrical studies originated from different regions of Europe. The studied plants were initially determined on the basis of morphological traits according to Müller (1951-1958) and Damsholt (2002). In the living plants, just after collection, the oil body characters were studied. In general, 53 samples of *C. fissa* complex were examined (Appendix 1). The samples were divided into two parts. Part one contained 40 samples identified on the basis of isozyme markers and oil body characters as *C. fissa* group P_S and P_B according to Buczkowska (2004a). Part two comprised 11 samples from herbarium collections which were determined based on morphological criteria only. Additionally, 2 samples determined in the previous study as group G (Buczkowska 2004a) were included in the biometrical analysis. Five stems from each sample were measured with respect to 47 quantitative traits according to the method described by Buczkowska (2004b).

2.2. Statistical analysis

Statistical analyses were performed in two steps: the analyses in the first step were based on a matrix of 40 samples genetically identified as group P_S and P_B (Appendix 1). Descriptive statistics: means, ±95% confidence intervals for the mean, standard deviations, minimums, maximums and coefficients of variation were computed for each group. The significance of the difference between means of the examined groups was tested by one-way analysis of variance (ANOVA). The forward stepwise method of discriminant analysis and Wilks' lambda value was used in order to design the best diagnostic features that facilitate discriminating between the examined groups. The Wilks' Lambda value fit the range from 0 (perfect discrimination) to 1 (lack of discrimination). The classification functions for the studied groups were also computed and the derived functions were used to classify the samples from herbarium collections which were not genetically determined. The functions can be used for classifying new cases into different groups with a better than chance accuracy. A new case can be classified to the group for which it has the highest classification score (Krzyśko 1990).

In the second step, all samples were included in analyses. Principal component analysis and cluster analysis (agglomeration and k-means clustering methods) were performed to examine differences between the investigated groups. Normality of the data was checked by the Shapiro-Wilk test. For cluster analysis, standardized data were used. Statistical analyses were performed using STATISTICA 8.0 for Windows.

3. Results

3.1. Morphological variation

Descriptive statistics and the coefficient of variation of all examined characters were calculated for the two examined groups (P_S and P_B) distinguished on the basis of isozyme markers (Appendix 2). The P_S group had lower values of means of almost all studied characters than the P_B group. In general, plants of the P_S group were smaller, had shorter internodes of stem and smaller underleaf and stem cells (Appendix 2). Analysis of variance (ANOVA) showed that the studied groups differed statistically significantly with respect to the means of 34 analyzed traits. The greatest differences between the studied groups were found in the features connected with the leaf (23-29), underleaf (1, 2) and stem (34-46). The examined groups did not differ statistically significantly with regard to the following traits: 3, 7, 12, 14-17, 19, 21 and 30-33 (Appendix 2).

The computed coefficients for both groups showed that the P_B group exhibited a bigger range of morphological variation than the P_S group. The coefficient of variation of 47 studied characters in the P_S group ranged from 4.98% to 20.83% and in P_B – from 7.36% to 25.73%. The P_S group had a higher coefficient of variation than P_B only for few characters (Appendix 2). The lowest values of the coefficient of variation in both groups were noted in the characters describing the shape of the underleaf (12-13) and of the leaf (30-33). The P_S group was more variable with respect to the morphological characters of the leaf and underleaf, whereas group P_B had higher values of this coefficient for morphological characters of the stem and size of the leaf and underleaf cells (Appendix 2).

3.2. Differences between groups

From the initial set of 47 characters, the set of the diagnostic ones was selected using Wilks' lambda criterion in the forward stepwise method of discriminant analysis based on a matrix of 40 samples identified genetically as group P_S and P_B. In the analysis, nine characters were included in the model: 23, 26, 8, 25, 2, 44, 36, 29 and 24. A highly significant value of the Wilks' Lambda of 0.104 ($F=24.86$, $p<0.0001$) calculated for the 9 traits included in the model indicated good discrimination between the analyzed groups. Thus, these characters can be regarded as diagnostic features, which morphologically best discriminate groups P_S and P_B of *C. fissa* detected in isozyme analysis. Based on the set of the 9 diagnostic traits, classification functions were computed (Table 1). The functions were used for the classification of samples from the herbarium collections. Almost all samples were classified by the functions derived based on genetically identified samples as belonging to the P_S or P_B group with very high probability ($p=0.996$ to $p=1.00$). Only one sample 50 was classified as group P_B with lower probability $p=0.820$. Two samples (41 and 42) distinguished by isozyme analyses as group G of *C. fissa* (Buczkowska 2004a) were classified by the functions as the P_B group (Table 2).

Table 2. Classification of samples from herbarium collections based on the computed functions for the two studied groups of the C. *fissa* complex

Sample No.	Probability of classification for group	
	P_S	P_B
41	0.000	1.000
42	0.002	0.998
43	1.000	0.000
44	1.000	0.000
45	1.000	0.000
46	1.000	0.000
47	0.998	0.002
48	0.996	0.004
49	0.000	1.000
50	0.180	0.820
51	0.000	1.000
52	0.000	1.000
53	0.000	1.000

The principal component analysis based on the 9 diagnostic characters and including 53 samples of *C. fissa* complex proved the existence of morphological hiatus between the studied groups. The examined samples of the *C. fissa* complex in the space of PCA

Table 1. Classification functions for the two studied groups of the C. *fissa* complex computed based on a matrix of 40 samples genetically identified for 9 diagnostic characters

No.	Character	Group	
		P_S	P_B
23	length of leaf	-0.019	-0.034
26	distance from apex (B) to ventral base of leaf (C)	-0.008	0.029
8	width of underleaf	-0.099	-0.189
25	height of dorsal part of leaf	0.153	0.256
2	length of cells of rhizoid initial field in underleaf	2.392	3.369
44	length of the 5th internode	-0.048	-0.076
36	width of stem cells in the 5th internode	5.801	7.186
29	length of the 3rd coordinate	0.129	0.183
24	width of leaf	-0.014	-0.035
Constant		-97.362	-174.291

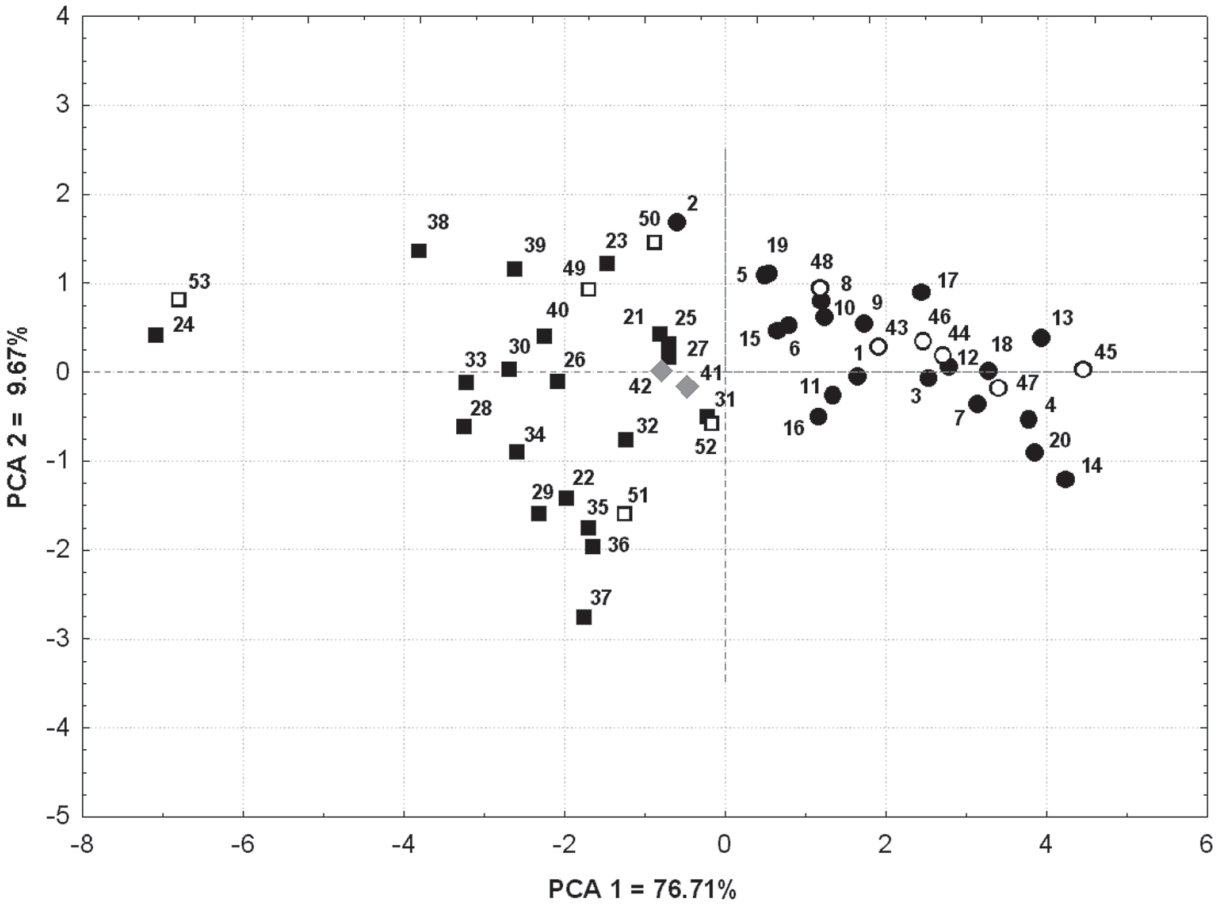

Fig. 1. Scatter plot of 53 samples of C. *fissa* complex in the PCA axes
Explanations: Circles – P_S group, squares – P_B group. Panes – G group. Full or empty symbols denote respectively samples identified genetically or morphologically only

axes were divided into two sets of samples separated along the 1st PCA axis; the sets are correlated with groups P_S and P_B detected by genetic markers (Fig. 1). Samples identified as the P_S group were located at the right side of the plot and made a relatively compact set with the exception of sample 2, which approached group P_B. Samples classified as the P_B group were located at the left side of the plot with two very distinct samples (24, 53). Plants of these two samples were larger than plants from the other samples of this group. Two samples of group G (41, 42) from Germany were located in the range of morphological variation of the P_B group. The position of samples which were not genetically identified in the PCA space was in accordance with the determination performed by classification functions, samples 43-45 and 46-48 were placed within the range of morphological variation of the P_S group, whereas samples 49-53 - in the range of the P_B group (Fig. 1). The 2nd PCA axis failed to differentiate the studied groups and revealed higher morphological variation of the P_B group (Fig. 1). The first two principal components explain 86.38% of the total variation included in the 9 analysed characters. The most strongly correlated with the 1st PCA axis were morphological

Table 3. Correlation coefficient between the 9 diagnostic characters and PCA axes based on all studied samples of the C. *fissa* complex

No.	Character	PCA 1	PCA 2
2	length of cells of rhizoid initial field in underleaf	-0.81	-0.28
8	width of underleaf	-0.64	0.71
23	length of leaf	-0.95	-0.08
24	width of leaf	-0.95	0.12
25	height of dorsal part of leaf	-0.91	0.18
26	distance from apex (B) to ventral base of leaf (C)	-0.98	-0.03
29	length of the 3rd coordinate	-0.92	0.12
36	width of stem cells in the 5th internode	-0.83	-0.18
44	length of the 5th internode	-0.83	-0.43
% of total variance		76.71	9.67
% cumulative variance		76.71	86.38

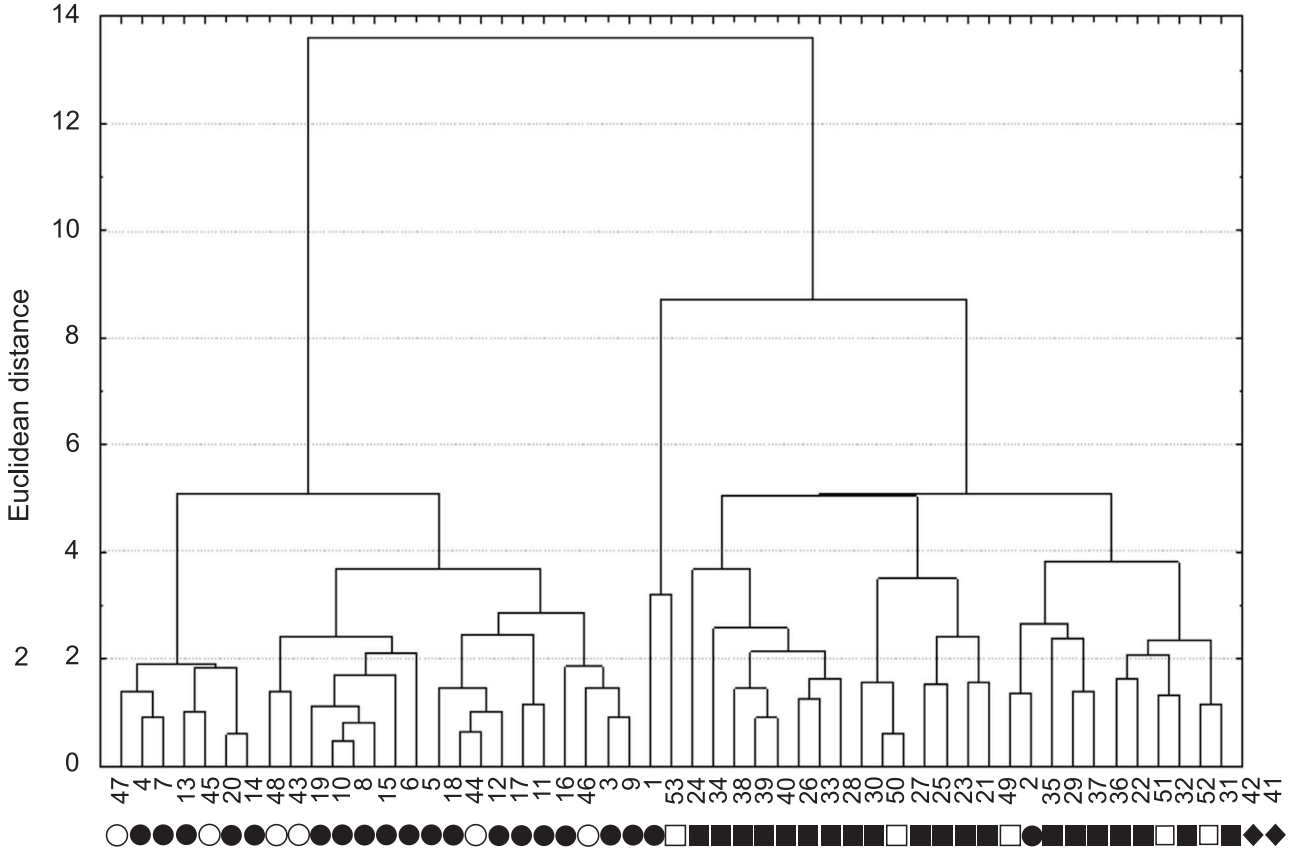

Fig. 2. A dendrogram of 53 studied samples of C. *fissa* complex constructed on the basis of the Euclidean distance according to the complete linkage method, using the set of nine diagnostic characters
Explanations: Circles – P_S group, squares – P_B group. Panes – G group. Full or empty symbols denote respectively samples identified genetically or morphologically only

characters of the leaf (26, 23 and 24); thus, these characters played the most important role in the separation of the examined groups of *C. fissa*, whereas the width of underleaf (8) and length of the 5th internode (44) (most strongly correlated with the 2nd PCA axis) were responsible for higher variation of the P_B group (Table 3).

Similar results of grouping were obtained in the cluster analysis. The dendrogram constructed on the basis of the Euclidean distance divided the examined samples into two groups: P_S and P_B, which correlated with the groups detected by genetic markers. The 2 sample 2 determined by isozyme markers as P_S and two samples of group G were included in the cluster of the

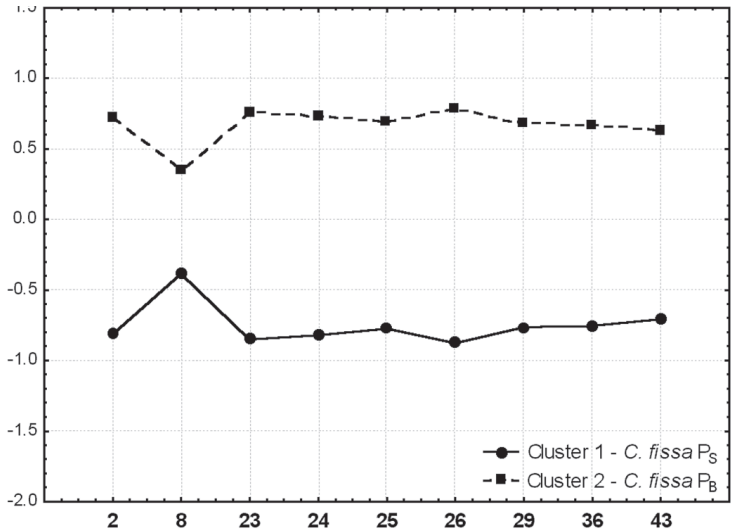

Fig. 3. Plot of means of nine diagnostic characters for each cluster obtained in the k-means clustering method

P_B group (Fig. 2). 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 the 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, the results of k-means clustering entirely match the clusters found in the joining analysis, all samples detected as the P_S group with the exception of sample 2 were included to cluster 1, whereas all samples of the P_B group (with group G) – to cluster 2. All the 9 diagnostic traits differentiated the two clusters very well (Fig. 3). Results of multivariate analyses showed that nine morphological traits (2, 8, 23-26, 29, 36, 44) were sufficient for proper classification of *C. fissa* into these two genetically distinct groups.

3.3. Shape of the leaf apex and lateral margin of the underleaf

In both groups, leaves with an acute apex prevailed and comprised 71% in P_S group and 82% in P_B of all the observed leaves, while leaves with a bidentate apex were less frequent – 22% and 11%, respectively (Fig. 4). Two remaining types of the leaf apex known in European species of the *Calypogeia* genus (truncated and rounded; Buczkowska 2004b) were very rare in the examined plants. Underleaves armed with teeth were more frequent in the P_S than in the P_B group, in the P_B underleaves with angulation prevailed (Fig. 5). These two groups did not differ with respect to numbers of cells between the sinus and the base of the underleaf (trait 7) and almost insignificantly in underleaf sinus depth (trait 11).

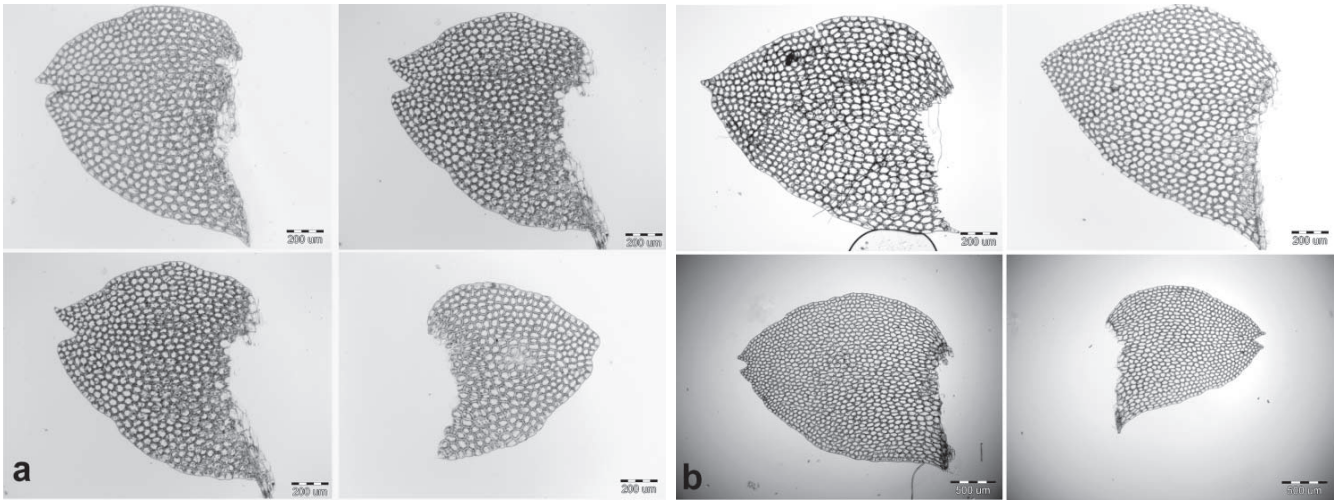

Fig. 4. Leaves of C. *fissa* complex
Explanations: a – group P_S, b – group P_B

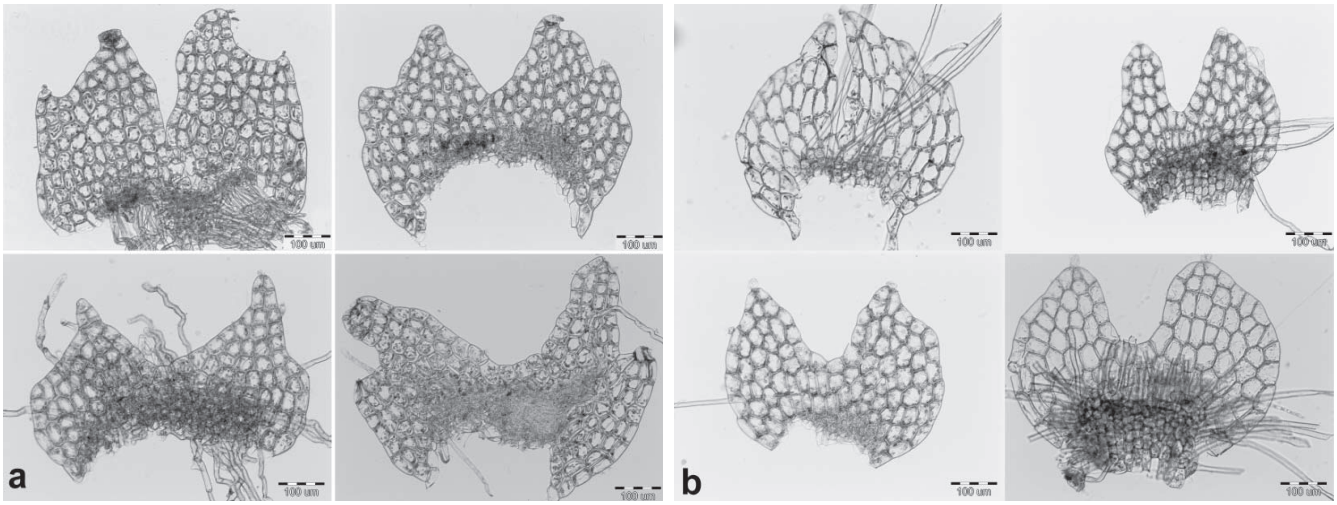

Fig. 5. Underleavs of C. *fissa* complex
Explanations: a – group P_S, b – group P_B

4. Discussion

Isozyme analyses revealed that *C. fissa* in Poland was differentiated into two genetically distinct groups, tentatively called P_S and P_B, which differed also in oil body characters (Buczkowska 2004a). The present biometrical study showed that, in spite of a relatively high level of variability, these groups could be recognised also morphologically. The two types of plants were also found in different parts of Europe. Among the 47 quantitative characters, 34 showed statistically significant differences in arithmetical means between the examined groups. Because the quantitative traits were less reliable, therefore, a combination of characters was needed for their separation. Nevertheless, from among the studied characters, it was possible to find a set of 9 diagnostic ones which allowed proper classification of the majority of samples belonging to the *C. fissa* complex to a particular group. This makes it possible to apply such an analysis in daily taxonomic practice. The best diagnostic features are morphological characters describing the shape of the leaf: 26 – distance from apex to ventral base of leaf, 29 – length of the 3rd coordinate of the leaf, 23 – length of leaf, 24 – width of leaf, 25 – height of dorsal part of leaf as well as characters of the stem: 44 – length of the 5th internode and size of internode cells and 8 – underleaf width. Plants of the P_B group were bigger (width of shoots ranged from 1600-3900 µm, on average 2383 µm), had usually longer than wider leaves and longer internodes (segment between underleaves), whereas plants of P_S were smaller (922-1780 µm, on average 1443 µm) with leaves almost as long as wide or wider than long and internodes were short. The two groups also differed with respect to habitat preferences, the P_B group grew in wet places, mainly on organic substrate like plant litter, *Carex* tussocks on lakeshore, wet tree roots in *Carici elongatae-Alnetum*, while group P_S occurred mainly on clay, sandy soil on ditch banks or roadsides.

Determination of difficult samples can be easier and more reliable using classification functions of discriminant analysis (Krzyśko 1990). The classification functions computed on the basis of genetically identified plants offer the possibility to compare herbarium specimens, including nomenclatural types, with respect to their morphological and anatomical features, with plants for which experimental research was done. Such functions were applied in case of the type specimen of *C. muelleriana* Schiffn.) Müll. Frib. and *C. integristipula* Steph. (Buczkowska 2004b). In the present study, the classification functions computed on the basis of 40 genetically identified samples were used for the determination of the new specimens from herbarium collections. Almost all analysed herbarium specimens were classified with very high probability (p=0.996-1.000) indicating that the two groups of *C. fissa* (P_S and P_B), detected previously by genetic methods, were well characterized also morphologically. Determination of the samples based on classification functions was consistent with the identification by molecular markers: *trn*G and *trn*L (Buczkowska *et al.* unpubl.). In future, the functions can be helpful for the determination of other samples and the atypically developed plants.

The studied samples of the *C. fissa* complex characterised by the set of the diagnostic characters were separated in the space of the PCA axes into two distinct groups (P_S and P_B) corresponding with the groups detected on the basis of the isoenzyme analyses. The same classification was obtained by cluster analysis. It is interesting to note that the two samples of group G were morphologically similar to samples of the P_B group. Their morphological similarity was confirmed by all biometrical analyses. The results of morphological studies wholly reflected the genetic differences revealed within the *C. fissa* complex by isozyme markers and oil body features (Buczkowska 2004a). The genetic distance between G and P_B groups was over 3 times lower (D=0.235) than that between G and Polish P_S group (0.710) or between groups P_S and P_B (0.700) in Poland (Buczkowska 2004a). The magnitude of morphological hiatus between the examined groups indicates that there is a correlation between their morphological features, including the characters of oil bodies and genetic distance. The correlations between isozyme phenotypes and oil body traits have been observed in other species of the *Calypogeia* genus (Szweykowski & Krzakowa 1990; Buczkowska *et al.* 2004). Thus, the recognition of the two genetically distinct groups of *C. fissa* on the basis of morphological criteria is possible in most instances. It is of great importance in the case of the older herbarium samples, where no experimental analyses have been done.

The results obtained in the present studies of the *C. fissa* complex are similar to other morphometric analysis of bryophytes that have been carried out on genetically detected material (Boisselier-Dubayle & Bischler 1994; Buczkowska 2010; Sawicki *et al.* 2010) and revealed that most of the morphological variation observed in bryopytes is genetically fixed; they do not support the Schuster's (1966) point of view that most of the morphological variation observed in liverworts is of modificatory character. Although such correlations have not always been noted, for example, in the *Porella platyphylla – P. platyphylloidea* complex, no correlation between three different genotypes and morphological features have been recorded (Therrien *et al.* 1998); similarly, no correlation has been found between genotype and the presence or absence of a hairy vaginula in *Orthotrichum speciosum* (Plášek & Sawicki 2010). Precise biometrical studies carried out on distinct genetic

units provide an opportunity to check the existence of correlations between genetic and morphological features case-by-case in order to estimate how wide this phenomenon is present in liverworts. Such studies also allow the delimitation of the range of phenotypic variability and provide reliable diagnostic features offering the possibility of proper classification of a great part of samples even of herbarium material, as was proved in the case of other *Calypogiea* species (Szweykowski & Buczkowska 1998; Buczkowska 2010), *Odontoschisma* (Szweykowski & Buczkowska 1999), or *Conocephalum* (Szweykowski *et al*. 2005). However, it should be pointed out firmly that in currently collected material of the *Calypogiea* species at least, oil bodies have to be checked, to make classification as reliable as possible.

It is interesting to note that, in the set of diagnostic characters for groups of the *C. fissa* complex, the morphological characters of the stem were also included, besides those of the leaf and underleaf. These characters, i.e. length of internodes and size of internodes cells exhibited a relatively high level of coefficient of variation, especially in the P_B group. These characters, according to Buch (1935), were regarded as susceptible to environmental conditions and, therefore, little attention was paid to the traits so far. Despite their relatively high coefficient of variability (V), the ± 95% confidence limits for means of both groups did not overlap, so the characters can be accepted as diagnostic for the studied groups. However, their diagnostic value should be verified on wider material genetically identified.

Bidentate leaves and underleaves armed with teeth on the lateral margin have been frequently regarded as the main diagnostic features of the *C. fissa* (Müller 1951-1958; Bischler 1957; Schuster 1969). The present study showed that leaves acute at the apex were more frequent in the P_B, but bidentate in the P_S group. Similarly, underleaves with angulation on the lateral margin were frequently found in P_B, but with teeth – in the P_S group. Bidentate leaves are also found in other *Calypogeia* species, but less frequently (Paton 1999; Buczkowska 2004b). Paton (1999) noted that the shape of the leaf apex was variable and had limited taxonomic value. The present study suggests that high variability of this feature in *C. fissa* could have resulted from its genetic heterogeneity and further studies are needed to verify stability of this trait. To classify the *Calypogeia* species properly, besides these features, a set of quantitative morphological and anatomical characters has to be applied. Paton (1999) suggested that in *C. fissa*, there is no correlation between lateral leaves that are entire at the apex and underleaves with lateral lobes above the middle, which is characteristic for North America *C. fissa* subsp. *neogea* R. M. Schust. The present study revealed that such a correlation did occur in the *C. fissa* complex in Poland.

The results of the present biometrical study showed that morphological differences of the genetically distinct P_S and P_B groups of the *C. fissa* complex appear to be satisfactory. The presence of diagnostic morphological characters available to analysis in dried herbarium specimens will give, in future, an opportunity to accomplish formal taxonomic descriptions of these genetically distinct groups of *C. fissa*. However, further studies are needed to finish the taxonomic research. Investigations should be conducted on the type specimens of: *C. fissa* ssp. *fissa* – the typical subspecies known from western Europe, *C. fissa* var. *fissa* and var. *intermedia* – the varieties distinguished by Damsholt (2002) and *C. fissa* var. *macrophylla* and var. *microphylla* – the varieties described by Warnstorf (1917) as well as *C. fissa* ssp. *neogea* – the subspecies described in North America by Schuster (1969) to verify if any of the several already described taxa fit the investigated groups.

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References

Bączkiewicz A. & Buczkowska K. 2005. Genetic variability of the *Aneura pinguis* complex (Hepaticae) in central and western Europe. Biological Lett. 42: 61-72.

Bączkiewicz A., Sawicki J., Buczkowska K., Polok K. & Zieliński R. 2008. Application of different DNA markers in studies on cryptic species of *Aneura pinguis* (Jungermanniopsida, Metzgeriales). Cryptogamie Bryol. 29: 3-21.

Bischler H. 1957. Revision des espéces suisses de Calypogeja. Candollea 16: 9-76.

Bischler H. & Boisselier-Dubayle M. C. 2000. New approaches to the systematics of liverworts. Nova Hedwigia 70: 37-44.

Boisselier-Dubayle M. C. & Bischler H. 1994. A combination of molecular amd morphological characters for delimitation of taxa in European *Porella*. J. Bryol. 18: 1-11.

Boisselier-Dubayle M. C., Lambourdiere J. & Bischler H. 1998. Taxa delimitation in *Reboulia* investigated with morphological, cytological, and isozyme markers. Bryologist 101: 61-69.

Buch H. 1935. Vorarbeiten zu einer Lebermoosflora Fenno-Scandias III. Die Gattung *Calypogeia* Raddi. Mem. Soc. F. Fl. Fenn. 11: 197-214.

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

Buczkowska K. 2004b. The genus *Calypogeia* Raddi (Jungermanniales, Hepaticae) in Poland, biometrical analysis of morphological and anatomical variation. Nova Hedwigia 78: 121-146.

Buczkowska K. 2010. Morphological differentiation of *Calypogeia muelleriana* (Jungermanniales, Hepaticae) in Poland. Biodiv. Res. Conserv. 17: 23-32.

Buczkowska K. & Bączkiewicz A. 2011. New taxon of the genus *Calypogeia* (Jungermanniales, Hepaticae) in Poland. Acta Soc. Bot. Pol. 80(4): 327-333. DOI 10.5586/asbp.2011.039

Buczkowska K. & Dabert M. 2011. The development of species-specific SCAR markers for delimitation of *Calypogeia* species. J. Bryol. 33(4): 291-299. DOI 10.1179/1743282011Y.0000000029

Buczkowska K., Odrzykoski I. J. & Chudzińska E. 2004. Delimitation of some European species of *Calypogeia* Raddi (Jungermanniales, Hepaticae) based on cytological characters and multienzyme phenotype. Nova Hedwigia 78: 147-163

Damsholt K. 2002. Illustrated Flora of Nordic Liverworts and Hornworts. Nordic Bryological Society, Lund.

Grolle R. & Long D. G. 2000. An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia. J. Bryol. 22: 103-140

Heinrichs J., Hentschel J., Bombosch A., Fiebig A., Reise J., Edelmann M., Kreier H. P., Schäfer-Verwimp A., Caspari S., Schmidt A. R., Zhu R. L., Konrat M., Shaw B. & Shaw A. J. 2010. One species or at least eight? Delimitation and distribution of *Frullania tamarisci* (L.) Dumort. s. l. (Jungermanniopsida, Porellales) inferred from nuclear and chloroplast DNA markers. Mol. Phylog. Evol. 56: 1105-1114. DOI 10.1016/j.ympev.2010.05.004

Koła W. 1989. Analiza florystyczno-ekologiczna wątrobowców Wzgórz Trzebnicko-Ostrzeszowskich. In: I. Kuczyńska (ed.). Zmiany szaty roślinnej Dolnego Śląska pod wpływem działalności człowieka. Acta Univ. Wratislaviensis 1156, Prace Botaniczne 44: 305-355.

Kreier H. P., Feldberg K., Mahr F., Bombosch A., Schmidt A. R., Zhu R. L., Konrat M., Shaw B., Shaw A. J. & Heinrichs J. 2010. Phylogeny of the leafy liverwort *Ptilidium*: Cryptic speciation and shared haplotypes between Northern and Southern Hemispheres. Mol. Phylog. Evol. 57: 1260-1267. DOI 10.1016/j.ympev.2010.10.002

Krzyśko M. 1990. Analiza dyskryminacyjna. 155 pp. Wyd. Nauk.-Tech., Warszawa.

Müller K. 1951-1958. Die Lebermoose Europas. Rabenhorst's Kryptogamen Flora von Deutschland, Oesterreich u. d. Schweiz. Ed. 3. Akademische Verlagsgesellschaft Geest & Portig K.-G., Leipzig.

Paton J. A. 1999. The Livervort Flora of the British Isles. Harley Books, Martins.

Plášek V. & Sawicki J. 2010. Is the Hairy Vaginula an Diagnostic Feature in the Taxonomy of the Genus *Orthotrichum*? Acta Soc. Bot. Pol. 79: 73-80.

Sawicki J., Plášek V. & Szczecińska M. 2010. Molecular Studies Resolve *Nyholmiella* (Orthotrichaceae) as a Separate Genus. J. Syst. Evol. 48: 183-94. DOI 10.1111/j.1759-6831.2010.00076.x

Shaw A. J. 2001. Biogeographic patterns and cryptic speciation in bryophytes. J. Biogeogr. 28: 253-261.

Schuster R. M. 1966. The *Hepaticae* and *Anthocerotae* of North America. Vol. 1. Columbia University Press, New York and London.

Schuster R. M. 1969. The *Hepaticae* and *Anthocerotae* of North America. Vol. 2. Columbia University Press, New York and London.

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

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

Szweykowski J. & Buczkowska K. 1998. The rhizoid-initial field – a valuable taxonomic character in species of the genus *Calypogeia* Raddi (Hepaticae, Jungermanniales). Nova Hedwigia 67: 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., 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

Szweykowski J. & Krzakowa M. 1979. Variation of four enzyme systems in Polish populations of *Conocephalum conicum* (L) Dumort (Hepaticae, Marchantiales). Bull. Acad. Polon. Sci. Ser. Sci. Boil. 27: 37-41.

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

Therrien J. P., Crandall-Stotler J. & Stotler R. E. 1998. Morphological and genetic variation in *Porella platyphylla* and *P. platyphylloidea* and their systematic implications. Bryologist 101: 1-19.

Wachowiak W., Bączkiewicz A., Chudzińska E. & Buczkowska K. 2007. Cryptic speciation in liverworts – a case study in the *Aneura pinguis* complex. Bot. J. Linn. Soc. 155: 273-282.

Warnstorf C. 1917. Die europäischen Artgruppen der Gattung *Calypogeia* Raddi (1820). Bryolog. Zeitschr. 1: 97-114.

Zieliński R. 1987. Genetic variation of the liverwort genus *Pellia* with special reference to Central European territory. 297 pp. Uniwersytet Szczeciński, Szczecin.

Appendix 1. Collection sites of the studied populations of *Calypogeia fissa* complex

Sample No.	Locality	Collector	Herbarium	Group[1]
Samples identified genetically based on isozyme markers				
1	W Poland, Lubuskie Province, Bogumiłów near Żary, sandy soil on ditch banks	KB, JS	POZW 39191	P_S
2	W Poland, Lubuskie Province, Bogumiłów near Żary, sandy soil on ditch banks	KB, JS	POZW 39199	P_S
3	W Poland, Lubuskie Province, Bogumiłów near Żary, sandy soil on ditch banks	KB, JS	POZW 39194	P_S
4	W Poland, Lubuskie Province, Tuplice near Lubsko, sandy soil on ditch banks	KB, JS	POZW 39203	P_S
5	Central Poland, Wielkopolska Province, Antonin near Ostrów Wlkp., sandy soil on ditch banks	KB	POZW 39087	P_S
6	Central Poland, Wielkopolska Province, Antonin near Ostrów Wlkp., sandy soil on ditch banks	KB	POZW 39090	P_S
7	Central Poland, Wielkopolska Province, Antonin near Ostrów Wlkp., sandy soil on ditch banks	KB	POZW 39103	P_S
8	Central Poland, Wielkopolska Province, Antonin near Ostrów Wlkp., sandy soil on ditch banks	KB	POZW 39105	P_S
9	Central Poland, Wielkopolska Province, Antonin near Ostrów Wlkp., sandy soil on ditch banks	KB	POZW 39089	P_S
12	Central Poland, Wielkopolska Province, Antonin near Ostrów Wlkp., sandy soil on ditch banks	JS, WK	POZW 31435	P_S
13	Central Poland, Wielkopolska Province, Antonin near Ostrów Wlkp. sandy soil on ditch banks	JS, WK	POZW 31436	P_S
10	NW Poland, Pomerania Province, small tributaryof Słupia river near Czarna Dąbrówka, sandy bank	KB, AB	POZW 42427	P_S
11	NW Poland, Pomerania Province, small tributary of Słupia river near Czarna Dąbrówka, sandy bank	KB, HB, JS	POZW 35649	P_S
14	NW Poland, Pomerania Province, small tributary of Słupia river near Czarna Dąbrówka, sandy bank	KB, HB, JS	POZW 35648	P_S
15	NW Poland, Pomerania Province, Bytów, forest N of Dąbrówka Bytowska, humus soil at the roadside	KB, JS, HB	POZW 35676	P_S
16	Germany, Nadrenia, Westerwald, forest road from Kasbachtals to Erleler Ley, on clay	RD	POZW 36718	P_S
17	Holland, Einde Goor near Hilversum, N of Utrecht, sandy soil in *Quercetum*	JS, RG, HG	POZW 34161	P_S
18	Holland, Einde Goor near Hilversum, N of Utrecht, sandy soil in *Quercetum*	JS, RG, HG	POZW 34162	P_S
19	Belgium, Liege, Hautesfagnes ridge, Helle stream near Binster, 320 m a.s.l., soil on bank	JS	POZW 34138	P_S
20	Germany, Schwarzwald, sandy soil in forest	IM	POZW 42428	P_S
21	W Poland, Lubuskie Province, Tuplice near Lubsko, sandy soil on ditch banks	KB, JS	POZW 39206	P_B
22	W Poland, Lubuskie Province, Tuplice near Lubsko, sandy soil on ditch banks	KB, JS	POZW 39207	P_B
23	W Poland, Lubuskie Province, Tuplice near Lubsko, sandy soil on ditch banks	KB, JS	POZW 39208	P_B
24	W Poland, Lubuskie Province, Lake Płytkie near Brody, *Carex* tussocks at lakeshore	KB, JS	POZW 39210	P_B
25	SW Poland, Lower Silesia Province, Sobieszów near Jelenia Góra, in *Molinietum*	JS	POZW 32160	P_B
26	SW Poland, Lower Silesia Province, Sobieszów near Jelenia Góra, in *Molinietum*	JS	POZW 31115	P_B
27	Central Poland, Wielkopolska Province, Poznań, Lake Strzeszynek, *Carex* tussocks at lakeshore	KB	POZW 35608	P_B
28	NW Poland, Pomerania Province, Lake Małe Sitno near Czarna Dąbrówka, *Carex* tussocks at lakeshore	KB, JS	POZW 35641	P_B
29	NW Poland, Pomerania Province, Lake Małe Sitno near Czarna Dąbrówka, *Carex* tussocks at lakeshore	KB, AB	POZW 42341	P_B
30	NW Poland, Pomerania Province, Lake Duże Katarzynki near Lipnica, sandy bank	KB, JS, HB	POZW 35694	P_B
31	NW Poland, Pomerania Province, Lake Duże Katarzynki near Lipnica, sandy bank	KB, JS, HB	POZW 35690	P_B
32	NW Poland, Pomerania Province, Lake Duże Katarzynki near Lipnica, on wet sand at lakeshore	KB, JS, HB	POZW 35692	P_B
33	NW Poland, Pomerania Province, Lake Duże Katarzynki	KB, JS, HB	POZW 35691	P_B

34	NW Poland, Pomerania Province, Lake Kamień near Miastko, peaty soil at lakeshore	KB, AB	POZW 42205	P_B
35	W Poland, Lubuskie Province, Mierków forestry, humusin *Carici elongate-Alnetum*	SR, KB	POZW 42298	P_B
36	W Poland, Lubuskie Province, Starosiedle forest division, *Carex* tussocks at lakeshore	SR, KB	POZW 42273	P_B
37	W Poland, Lubuskie Province, Biecz forestry, humus and plant litter in *Carici elongate-Alnetum*	SR, KB	POZW 42309	P_B
38	Holland, Maarssen, 2 km North of Utrecht, Molenpolder, on wet peat	JS, RG, HG	POZW 34147	P_B
39	Holland, Maarssen, 2 km North of Utrecht, Molenpolder, on wet peat	JS	POZW 34148	P_B
40	Holland, Maarssen, 2 km North of Utrecht, Molenpolder, on wet peat	JS	POZW 34149	P_B
41	Germany, Bonn – Bad Godesberg, Annaberger Bachtal, pine forest	AS, DQ	POZW 39074	G
42	Germany, pine forest near Bonn,	AS, DQ	POZW 39076	G
	Samples identified morphologically by classification functions			
43	SW Poland, Lower Silesia Province, Sobótka near Wrocław, on soil	WK	POZW 02258	P_S
44	SW Poland, Sudetes, Kaczawskie Mts, Kamienica stream, 300 m a.s.l., on soil	JS	POZW 02259	P_S
45	SW Poland, Sudetes, Kaczawskie Mts, valley of Bobrawa river near Lwówek Śląski, 290 m a.s.l., on soil	JS	POZW 02260	P_S
46	Portugal, Beira Litoral, Luso, National Park of Mata de Buçaco, Cruz Alta, 433 m a.s.l.	AS-V, IV	S-V 31461	P_S
47	Azores Archipelago, São Miguel Island, east part of island, Furnas, 280 m a.s.l.	AS-V, IV	S-V 29323	P_S
48	Greece, Thessaly, Distr. East Central, Prov. Magnisia, Pilion-Halbinsel, road between Chania and Zagora, 820-830 m a.s.l.	AS-V, IV	S-V 29826	P_S
49	Georgia, Ajaria, Batumi, in Horto Botanico, pars Australiae.	AA	OP 110668	P_B
50	Czech Republic, Krnov, Hájek in Květnice Mts. 450 m a.s.l.	JD	OP 150890	P_B
51	Germany, Bayern, Alpenvorland, between Rieden-Beckstetten and Frankenhofener lake, 652 m a.s.l.	AS-V, IV	S-V 31656	P_B
52	Germany, Baden-Württemberg, western Bodenseegebiet, Schienerberg-Nordabhang above Bankholzen, Öde Halde north from Langenmoos, 620 m a.s.l.	AS-V, IV	S-V 25448	P_B
53	Atlantic Islands, Madeira, road from Encumeada-Pass to Pico Ruivo, 1150 m a.s.l.	AS-V, IV	S-V 25699	P_B

Explanations: [1] – Groups detected by isozyme markers (Buczkowska 2004a); samples 1-42 identified genetically, 43-53 by classification functions. Collectors: AA – A. L. et I. I. Abramovi, AB – A. Bączkiewicz, AS – A. Sloga, AS-V – A. Schäfer-Verwimp, DQ – D. Quandt, HB – H. Barczak, HG – H. Greven, IM – I. Melosik, IV – Inge Verwimp, JD – J. Duda, JS – J. Szweykowski, KB – K. Buczkowska, RD – R. Düll, RG – R. Gradstein, SR – S. Rosadziński, WK – W. Koła. Herbaria: S-V – Herb. Schäfer-Verwimp., OP – Musei Silesiensis Opava, POZW – Herbarium of Adam Mickiewicz University

Appendix 2. Descriptive statistics for 47 quantitative characters for the two studied groups of the *C. fissa* complex and F statistics

No.		*C. fissa* P_S							
		Mean	- 95%	+ 95%	Min.	Max.	SD	v %	Mean
1	width of cells of rhizoid initial field in underleaf	17.6	16.7	18.5	13.8	21.9	2.3	12.9	22.9
2	length of cells of rhizoid initial field in underleaf	20.5	19.5	21.5	15.7	26.8	2.5	12.3	27.8
3	width of cells in underleaf lobe	28.9	27.1	30.6	19.2	38.6	4.3	14.9	32.2
4	length of cells in underleaf lobe	39.9	38.0	41.9	30.5	50.9	4.9	12.2	51.6
5	width of cells in underleaf middle	29.2	27.4	30.9	20.7	40.4	4.4	15.0	33.2
6	length of cells in underleaf middle	35.3	33.4	37.1	25.8	43.8	4.6	13.2	47.6
7	number of cells between the sinus and base of underl.	2.2	2.1	2.4	1.6	3.9	0.5	20.7	2.4
8	width of underleaf	503.3	462.5	544.1	281.1	700.0	101.0	20.1	578.6
9	length of the whole underleaf	420.4	388.8	452.0	254.4	528.3	78.2	18.6	484.2
10	length of underleaf to the base of rhizoid initial field	327.7	302.3	353.2	193.3	445.0	63.1	19.2	395.5
11	underleaf sinus depth	174.5	159.9	189.2	109.0	266.7	36.4	20.8	200.8
12	ratio of width to height of underleaf - 8/9	1.2	1.1	1.2	1.0	1.5	0.1	9.6	1.2
13	'measure' of underleaf decurrence - 9/10	1.3	1.3	1.3	1.1	1.4	0.1	6.0	1.2
14	ratio of width of underleaf to width of stem - 8/47	2.3	2.2	2.4	1.7	2.7	0.3	11.7	2.2
15	width of marginal cells in dorsal part of leaf	35.2	34.0	36.4	29.6	40.9	2.9	8.3	35.9
16	length of marginal cells in dorsal part of leaf	27.9	26.6	29.2	22.7	36.6	3.2	11.6	29.7
17	width of median cells in leaf	36.3	34.5	38.1	29.2	45.4	4.4	12.1	39.0
18	length of median cells in leaf	48.2	45.5	50.8	38.2	60.5	6.6	13.6	58.0
19	width of cells at ventral leaf base	36.4	35.1	37.7	30.4	42.4	3.3	9.0	38.5
20	length of cells at ventral leaf base	54.2	51.6	56.9	42.0	64.3	6.5	12.1	66.6
21	width of 2nd row marginal cells in dorsal part of leaf	34.0	32.5	35.6	27.9	43.4	3.9	11.3	35.0
22	length of 2nd row marginal cells in dorsal part of leaf	32.8	31.5	34.2	25.3	39.3	3.4	10.4	36.6
23	length of leaf	876.9	823.3	930.4	663.3	1188.3	132.5	15.1	1366.2
24	width of leaf	907.0	843.5	970.4	641.1	1271.7	157.2	17.3	1340.9
25	height of dorsal part of leaf	282.0	262.0	302.0	194.4	380.0	49.5	17.6	416.1
26	distance from apex to ventral base of leaf	1021.3	953.7	1088.8	756.7	1395.0	167.2	16.4	1592.1
27	length of the 1st coordinate	685.3	632.2	738.5	482.2	1045.0	131.6	19.2	1054.1
28	length of the 2nd coordinate	650.5	597.7	703.3	445.6	1021.7	130.7	20.1	989.4
29	length of the 3rd coordinate	628.3	576.0	680.6	418.9	988.3	129.5	20.6	949.4
30	ratio of length to width of leaf - 23/24	1.0	1.0	1.0	0.9	1.1	0.0	4.9	1.0
31	ratio of leaf length to distance from apex to ventral leaf base - 23/26	0.9	0.8	0.9	0.8	0.9	0.0	5.0	0.9
32	ratio of distance A-C to the 1st coordinate - 26/27	1.5	1.5	1.6	1.3	1.7	0.1	7.1	1.5
33	ratio of length of leaf dorsal part to width of leaf 25/24	0.3	0.3	0.3	0.2	0.4	0.0	11.2	0.3
34	width of stem cells in the 4th internode	23.1	22.0	24.2	18.0	26.6	2.7	11.7	28.6
35	length of stem cells in the 4th internode	54.5	50.9	58.0	39.7	79.7	8.8	16.1	75.8
36	width of stem cells in the 5th internode	23.1	22.2	24.1	19.3	27.0	2.3	10.1	28.6
37	length of stem cells in the 5th internode	56.0	52.9	59.1	41.2	76.5	7.6	13.7	77.8
38	width of stem cells in the 6th internode	22.9	21.9	23.9	19.3	27.6	2.5	10.8	28.3
39	length of stem cells in the 6th internode	55.5	52.2	58.8	42.5	70.1	8.2	14.8	76.1
40	number of stem cells in the 4th internode	7.6	7.3	8.0	6.5	9.7	0.8	10.7	10.1
41	number of stem cells in the 5th internode	7.6	7.2	7.9	5.7	9.7	0.9	11.3	10.0
42	number of stem cells in the 6th internode	7.4	7.1	7.7	5.7	9.1	0.8	10.5	10.1
43	length of the 4th internode	527.1	486.8	567.4	328.5	701.7	99.7	18.9	905.4
44	length of the 5th internode	527.6	485.5	569.6	328.5	762.8	104.2	19.7	921.0
45	length of the 6th internode	522.2	483.7	560.7	328.5	701.7	95.3	18.2	918.3
46	width of the whole plant	1443.7	1343.8	1543.6	922.6	1780.6	247.4	17.1	2383.5
47	width of stem (without leaves)	230.1	212.7	247.4	136.7	335.6	42.9	18.7	268.7

Explanations: ±95% – confidence intervals, SD – standard deviation, V% – coefficient of variation, * – p Ł 0.05, ** – p Ł 0.01

C. fissa P_B						F
- 95%	+ 95%	Min.	Max.	SD	v %	
22.1	23.8	16.2	26.7	2.0	8.9	84.54**
26.5	29.1	17.6	30.9	3.1	11.0	83.57**
29.7	34.6	19.7	49.5	6.0	18.6	3.67
47.2	55.9	30.9	77.1	10.6	20.6	27.77**
30.9	35.5	17.6	45.2	5.5	16.6	7.76*
43.9	51.4	21.4	69.7	9.1	19.1	42.20**
2.2	2.6	1.3	3.1	0.5	21.3	0.97
531.3	625.9	393.9	775.5	114.6	19.8	6.77*
446.3	522.2	322.0	778.6	92.0	19.0	6.74*
366.4	424.6	242.4	538.9	70.6	17.8	13.21**
183.7	217.9	118.7	283.3	41.5	20.6	4.86*
1.1	1.3	0.9	1.6	0.1	11.9	0.15
1.2	1.3	1.1	1.5	0.1	8.2	7.53*
2.1	2.3	1.5	2.8	0.3	15.8	0.02
33.6	38.3	27.1	55.2	5.8	16.1	0.02
27.9	31.4	19.7	36.4	4.3	14.4	3.91
37.1	40.8	31.0	50.0	4.4	11.4	3.23
54.4	61.7	39.4	80.7	8.9	15.4	8.51*
36.4	40.6	30.4	50.0	5.1	13.2	2.31
63.0	70.1	48.8	82.3	8.6	12.9	25.33**
33.2	36.8	24.8	45.2	4.3	12.2	0.70
35.3	37.9	30.3	43.5	3.2	8.7	14.43**
1269.6	1462.8	1073.3	2102.4	234.0	17.1	61.77**
1252.6	1429.2	1005.1	1878.3	214.0	16.0	54.38**
387.2	444.9	329.7	620.2	69.8	16.8	56.49**
1498.6	1685.5	1268.8	2259.3	226.5	14.2	85.22**
971.7	1136.5	813.3	1695.0	199.6	18.9	51.00**
907.9	1070.9	768.9	1621.7	197.4	20.0	49.12**
869.3	1029.4	738.8	1595.0	193.9	20.4	46.78**
1.0	1.1	0.9	1.3	0.1	12.3	1.37
0.8	0.9	0.8	1.1	0.1	7.4	0.35
1.5	1.6	1.2	1.9	0.1	9.2	0.44
0.3	0.3	0.2	0.4	0.0	13.0	0.01
27.3	30.0	21.2	36.7	3.3	11.6	39.35**
70.8	80.8	56.3	107.3	12.1	16.0	50.77**
27.3	29.8	25.5	36.7	3.1	10.7	47.85**
72.9	82.8	55.2	102.0	12.1	15.5	57.36**
26.9	29.6	22.3	36.7	3.3	11.7	42.94**
71.4	80.7	58.4	102.0	11.2	14.8	44.33**
9.5	10.8	6.0	12.5	1.6	15.6	54.04**
9.3	10.7	6.0	12.4	1.7	16.9	46.78**
9.2	10.9	6.0	13.7	2.0	19.9	41.95**
812.6	998.1	537.7	1373.0	224.6	24.8	63.38**
827.6	1014.4	537.7	1373.0	226.3	24.6	66.78**
820.8	1015.8	537.7	1372.2	236.3	25.7	64.55**
2186.4	2580.5	1603.6	3898.4	477.3	20.0	60.62**
249.7	287.6	216.1	389.6	46.0	17.1	7.47*