

Morphological and anatomical differentiation within the *Aneura pinguis* complex (Metzgeriales, Hepaticae)

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Abstract: Three cryptic species of the *Aneura pinguis* complex (*A. pinguis* species A, B and C) were biometrically studied with respect to 18 morphological and anatomical quantitative traits. All 231 gametophytes used in this study were earlier identified by isozyme analysis. The cryptic species of *A. pinguis* differed statistically significantly in respect of 13 quantitative traits, most strongly in the area of dorsal epidermal cells, thickness and number of cells in the thallus cross section, size of inner cells in the thallus cross section, and thallus width. The greatest differences occurred between species A and C (Mahalanobis distance $D = 4.48$, $P \leq 0.001$), and the smallest between species B and C ($D = 3.05$, $P \leq 0.05$). In all species, biometric differences between male and female plants were found. Sex ratio differed among species: in species A females and males were equally frequent, in species B male plants prevailed, while in species C female plants were more numerous.

Key words: liverworts, *Aneura pinguis*, cryptic species, phenotypic variation, biometric analysis, sexual dimorphism

INTRODUCTION

The genus *Aneura* Dumort. comprises several species, most of which have been described recently, and some are endemic (FURUKI 1991, FURUKI 1994, FURUKI & LONG 1994, SCHUSTER 1992). *A. pinguis* (L.) Dumort. is a widespread, almost cosmopolitan species occurring on all continents and shows a very wide scale of ecological tolerance (MÜLLER 1951–1958, SCHUSTER 1992, PATON 1999, DAMSHOLT 2002). It is the oldest species of the genus *Aneura* described by Carl Linnaeus in 1753. *A. pinguis* is treated as a taxonomically uniform species by MÜLLER (1951–1958), FURUKI (1991), and PATON (1999). SCHUSTER (1992) and DAMSHOLT (2002) distinguished within *A. pinguis* one variety *A. pinguis* var. *angustior* (Hook.) Schust.

Moreover, DAMSHOLT (2002) recognized *A. pinguis* var. *denticulata* Nees and *A. pinguis* var. *fuscovirens* (Lindb.) Damsh. However, the last taxon is usually treated as a synonym of *A. pinguis* (MÜLLER 1951–1958, SCHUSTER 1992).

A. pinguis is a thallose, dioecious species, with a very simple morphological structure, without clearly differentiated lamina from costa, and with straight, simple or weakly crisped or undulate 1–3-stratose margins. Thalli smooth, turgid and brittle, varying in size, 2–6 (–10) mm wide and 20–50 mm long, lingulate to sublinear, simple to occasionally irregularly branched, often fertile, sporophytes frequent, vegetative propagation lacking (MÜLLER 1951–1958, FURUKI 1991, SCHUSTER 1992, PATON 1999, DAMSHOLT 2002). It differs from other species of the genus *Aneura* in respect of narrow unistratose wings of thallus, shape of thallus margin, its smooth dorsal surface, and lack of vegetative propagation (FURUKI 1991, SCHUSTER 1992, PATON 1999, DAMSHOLT 2002).

A. pinguis is an example of liverwort species that express considerable morphological variation in respect of shape and size of thallus and sexual branches. SHOWALTER (1926, 1928) distinguished 4 partly intersterile races from Europe, which differed in season of sexual activity. Some of the races were free from endophytic fungi (SHOWALTER 1926, LIGNORE et al. 1993). Differences in structure, size and number of oil bodies were also noted by SCHUSTER & DAMSHOLT (1974), SCHUSTER (1992), and PATON (1999). Nevertheless, SCHUSTER (1992) pointed out that the extremely simple gametophytic structure offers virtually no criteria even for segregating subspecies. That author also emphasized that the simplicity in organization of *A. pinguis* clearly makes it difficult to understand the differentiation of this taxon basing on classic morphological-anatomical approaches.

Separation of phenotypic plasticity from genetically fixed morphological differences is very important for bryophyte taxonomy. Biometric studies together with genetic methods have given an opportunity to find new reliable anatomical and morphological features for some critical species, e.g. *Lophozia* (Dumort.) Dumort. (BISANG 1991), *Porella* L. (BOISSELIER-DUBAYLE & BISCHLER 1994), *Calypogeia* Raddi (BUCZKOWSKA 2004), or even for species previously regarded as cryptic, like *Conocephalum conicum* (L.) Dumort. and *C. salebrosum* Szweyk., Buczk. & Odrzyk. (SZWEYKOWSKI et al. 2005). At first, isozyme studies have revealed differentiation of *A. pinguis* into 3 genetically distinct groups in Poland (SZWEYKOWSKI & ODRZYKOWSKI 1990, ANDRZEJEWSKA 2000) and recently yet another group was identified from the British Isles (BĄCZKIEWICZ & BUCZKOWSKA 2005). A preliminary biometric study of 8 morphological and anatomical traits on genetically identified material showed some differences in cell size between the cryptic species described in Poland (ANDRZEJEWSKA 2000). This biometric study was undertaken in order to describe morphological and anatomical variation of 3 cryptic species of *A. pinguis* from Poland. We intended to check whether some morphological or anatomical diagnostic traits could be useful in their separation.

MATERIAL AND METHODS

Plant material

Plants used in biometric analyses were collected from different parts of Poland: the Bieszczady Mts., Tatry Mts., Małe Pieniny Mts., Pomerania, and the

Białowieża National Park (for collection sites, see Appendix). Samples were initially identified as the *A. pinguis* complex according to their anatomical and morphological traits, including oil bodies (MÜLLER 1951–1958, SCHUSTER 1992). Afterwards, one part of each sample was dried and deposited as a voucher at LLH (Liverworts Laboratory and Herbarium). The other part was used for isoenzymatic investigations (BĄCZKIEWICZ et al. in preparation) and a study of oil bodies (BUCZKOWSKA et al. 2005).

Biometric analysis

A total of 231 isoenzymatically identified gametophytes from 77 samples (colonies) of Polish populations belonging to 3 cryptic species of the *A. pinguis* complex were studied: 40 samples of species A, 22 of species B, and 15 of species C. Unequal sample size of particular cryptic species was caused by different frequencies of the species. In Poland the most frequent is species A, but species B and C occur rarely (BĄCZKIEWICZ et al. in preparation).

Morphometry

Three thalli were randomly taken from each sample to be measured. The 18 quantitative traits were selected on the basis of MÜLLER's (1951–1958), SCHUSTER's (1992), ANDRZEJEWSKA's (2000) and our original observations (Table 1). For the 3 thalli taken from each sample, 10 measurements were done for traits 5–12 and 14–18. Measurements of traits 3–13 were made in the thallus cross section, while traits 1, 2 and 14–18 were studied in plane view. Cross sections were made about 5–8 mm from the apex, depending on thallus size, and measurements were made under a stereoscopic or a light microscope. Each cross section was photographed by a microscope

Table 1. List of traits used in biometric analyses of 3 cryptic species of *A. pinguis*

No.	Trait	Method of observation
1	Length of thallus (mm)	in plane view
2	Width of thallus (mm)	“
3	Thickness of thallus (μm)	in cross section
4	Number of cells in cross section of median part of thallus	“
5	Depth of dorsal epidermal cells of median part of thallus (μm)	“
6	Width of dorsal subepidermal cells (μm)	“
7	Length of dorsal subepidermal cells (μm)	“
8	Width of ventral epidermal cells (μm)	“
9	Length of ventral epidermal cells (μm)	“
10	Width of inner cells in median part of thallus (μm)	“
11	Length of inner cells in median part of thallus (μm)	“
12	Cross-sectional area of inner cells in median part of thallus (μm^2)	“
13	Number of strata of cells at thallus margin	“
14	Width of dorsal epidermal cells at thallus margin (μm)	in plane view
15	Length of dorsal epidermal cells at thallus margin (μm)	“
16	Width of dorsal epidermal cells in median part of thallus (μm)	“
17	Length of dorsal epidermal cells in median part of thallus (μm)	“
18	Area of epidermal cells in median part of thallus (μm^2)	“

camera; measurements were made on the micrographs by using UTHSCSA Image Tool program (developed at the University of Texas Health Science Center at San Antonio, Texas and available from the Internet by anonymous FTP from maxrad6.uthscsa.edu). The remains of the thalli after cross section were packed into small bags and attached to vouchers. The method of taking measurements is shown in Fig. 1.

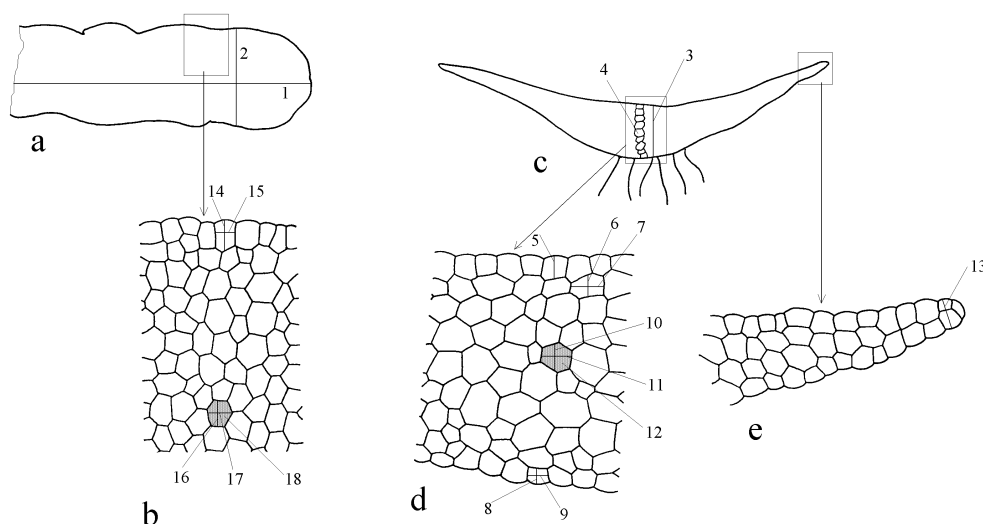


Fig. 1. Diagram showing the methods of measurement: a, b = plane view; c, d, e = thallus cross section

Statistical analysis

Descriptive statistics and coefficients of variation were computed to evaluate morphological and anatomical variation of plants of 3 cryptic species of *A. pinguis*. Differentiation in quantitative morphological and anatomical traits was tested by 2-way analysis of variance (ANOVA) with species and gametophyte gender specified as main effects. Species \times gender interaction was also specified in the analysis. The assumption of homogenous variances was tested by the Levene test. Normality of the data was checked by the Kolomogorov-Smirnov test for normality. Since trait 13 did not fit the assumption of normal distribution, its statistical significance was tested by the Kruskal-Wallis nonparametric test and it was excluded from discriminant analysis. The standard method of discriminant analysis was used in order to compute Mahalanobis distances between cryptic species and to design the best diagnostic features. The significance of Mahalanobis distances was tested by the F statistic, and Bonferroni correction was applied to evaluate statistical significance (SOKAL & ROHLF 1997). Complete linkage method of cluster analysis based on Euclidean distances, were performed to examine relationships between the cryptic species of *A. pinguis*. Four data matrices obtained by successive computing of mean values from original measurements were used in the analyses: (1) matrix of original data ($N = 2310$, i.e.

77 colonies \times 3 thalli \times 10 cells); (2) matrix of means for gametophytes (N = 231); (3) matrix of means for colonies (N = 77); and (4) matrix of means for cryptic species (N = 3). The analyses were performed by STATISTICA 7.1 for Windows.

RESULTS

The descriptive statistics for 3 cryptic species of *A. pinguis* are presented in Table 2. The biggest plants were found in species A (traits 1–4), the plants had also the biggest of all examined cells (Table 2). The smallest thalli were in species C, but plants of species B had the smallest epidermal and subepidermal cells in the median part of the thallus cross section (Fig. 2.1–2.3) and in plane view (traits 7–12, 15, 17). Thallus margin was built of 1–2 strata (trait 13) in species C (1.14 on average), whereas of 1–4 strata in species A and B (1.34 and 1.41 on average, respectively). In all species, differences between male and female plants were found. In cryptic species A and C, female plants had the highest mean value for almost all examined traits, whereas in cryptic species B the highest value was found in male plants. Biometric analysis of 231 gametophytes of *A. pinguis* shows relatively high levels of phenotypic variation within the cryptic species. The lowest variability coefficient was observed in plants of species A, the highest in species C for several of the studied traits (Table 2). Variability coefficients show that all the species are most variable in the number of strata of cells in the thallus margin (trait 13). Plants of species A and B are also highly variable in thallus size (traits 1, 2), while plants of species C, in thallus thickness (trait 3), in the cross-sectional area of inner cells in the median part of thallus (trait 12), and in number of cells in the thallus cross section (trait 4).

Two-way analysis of variance (ANOVA) showed statistically significant differences between the studied cryptic species in respect of means of 13 from 17 examined traits. The highest statistically significant differences between the cryptic species were found in size of the dorsal epidermal cells measured in plane view (traits 16–18), thallus thickness and number of cells in the thallus cross section (traits 3, 4), and in size of dorsal epidermal and inner cells in the thallus cross section (traits 10–12). Statistically significant differences between female and male plants were also found in traits 3, 4, and 10 (Table 3). The significant interaction between species and gender in traits 3, 12 and 16–18 implies that differences between the studied species in these traits depend on gender (Table 3, Fig. 3). Multiple comparisons (Scheffé test) showed that females and males were statistically different only in species C in respect of thickness of the thallus cross section (trait 3, Table 4). Highly significant differences were found between species A and B as well as between species A and C in respect of 11 and 9 traits, respectively (Table 4). The smallest differences occurred between species B and C, as those species differ only in respect of 4 traits (Table 4). In the case of species A and B, greater differences were noted between the females than the males of species A and both sexes of species B (Table 4). It is interesting that in the case of species pair A and C, male plants of species C differed highly significantly from both sexes of species A in respect of traits 3 and 4, but female plants of species C in traits 16–18 and additionally in 14–15 from male plants of species A (Table 4). Kruskal-Wallis test showed that there was no statistically significant difference in mean number of strata of cells at the thallus margin (trait 13).

Table 2. Means and variability coefficients for studied traits from 3 cryptic species of *A. pinguis* (A, B, C) broken down by gametophyte gender. V% = coefficient of variability. Units as in Table 1

Trait	<i>A. pinguis</i> species A (N = 120)						<i>A. pinguis</i> species B (N = 66)						<i>A. pinguis</i> species C (N = 4)			
	♀, N = 33		♂, N = 33		Sterile, N = 54		♀, N = 15		♂, N = 39		Sterile, N = 12		♀, N = 21		♂, N = 9	
	Mean	V%	Mean	V%	Mean	V%	Mean	V%	Mean	V%	Mean	V%	Mean	V%	Mean	V%
1	18.5	32.2	15.8	23.4	17.1	29.0	14.5	13.0	16.6	25.4	13.2	16.9	14.2	9.1	12.9	11.9
2	3.6	32.1	3.4	40.3	3.9	36.67	2.4	19.6	3.0	31.1	2.3	31.4	2.3	20.2	1.9	23.7
3	460	22.7	414	19.0	387	17.1	356	28.2	355	23.2	333	26.3	362	21.5	229	28.7
4	9.5	18.2	9.1	18.5	8.4	19.6	8.9	29.2	8.4	21.8	7.8	29.7	7.9	25.3	5.8	27.1
5	32.6	18.5	30.5	15.3	29.8	14.0	28.4	11.1	28.3	16.7	26.7	14.2	28.6	10.5	26.8	17.4
6	39.2	11.6	37.7	18.0	40.7	14.3	35.7	11.7	36.7	17.0	36.4	11.9	34.5	17.5	37.6	16.3
7	58.3	14.6	56.6	16.9	57.8	12.98	48.8	13.8	52.5	19.2	51.5	17.9	56.5	20.5	54.9	26.0
8	34.6	11.8	34.2	14.5	35.1	14.56	31.3	7.6	34.6	14.9	35.1	10.5	34.7	13.25	33.8	18.2
9	47.6	11.3	48.1	14.1	48.9	13.7	42.7	15.2	46.9	15.3	49.6	12.6	47.4	17.7	48.4	20.3
10	49.5	13.4	46.3	10.8	47.2	12.0	40.9	9.8	42.2	15.6	45.6	14.9	45.1	14.1	39.8	16.6
11	62.3	14.2	59.5	13.9	60.8	13.89	50.8	9.4	53.5	15.3	59.8	14.2	61.5	15.6	55.9	14.6
12	3,040	27.1	2,687	19.9	2,690	21.9	1,937	18.1	2,206	29.8	2,598	26.0	2,812	33.49	2,313	23.9
13	1.2	45.0	1.2	33.1	1.5	54.8	1.3	36.1	1.5	51.7	1.4	36.4	1.2	33.8	1.0	0.0
14	36.5	16.4	37.7	13.2	36.9	11.7	33.6	7.9	35.2	13.6	35.6	9.7	33.0	5.9	33.7	12.8
15	41.7	18.1	43.0	17.0	44.8	17.3	36.0	12.9	37.4	13.9	35.4	17.2	36.8	14.35	38.6	16.3
16	43.2	16.1	42.1	17.0	42.0	18.2	32.2	13.2	37.3	14.3	37.4	15.5	34.7	12.4	34.6	22.3
17	80.4	13.3	72.6	16.9	76.7	15.7	60.7	24.9	59.8	21.0	58.3	16.6	59.5	17.4	74.5	17.0
18	3,029	19.6	2,754	21.3	2,890	23.9	1,964	29.0	1,957	26.6	1,977	24.0	1,830	26.76	2,028	31.2

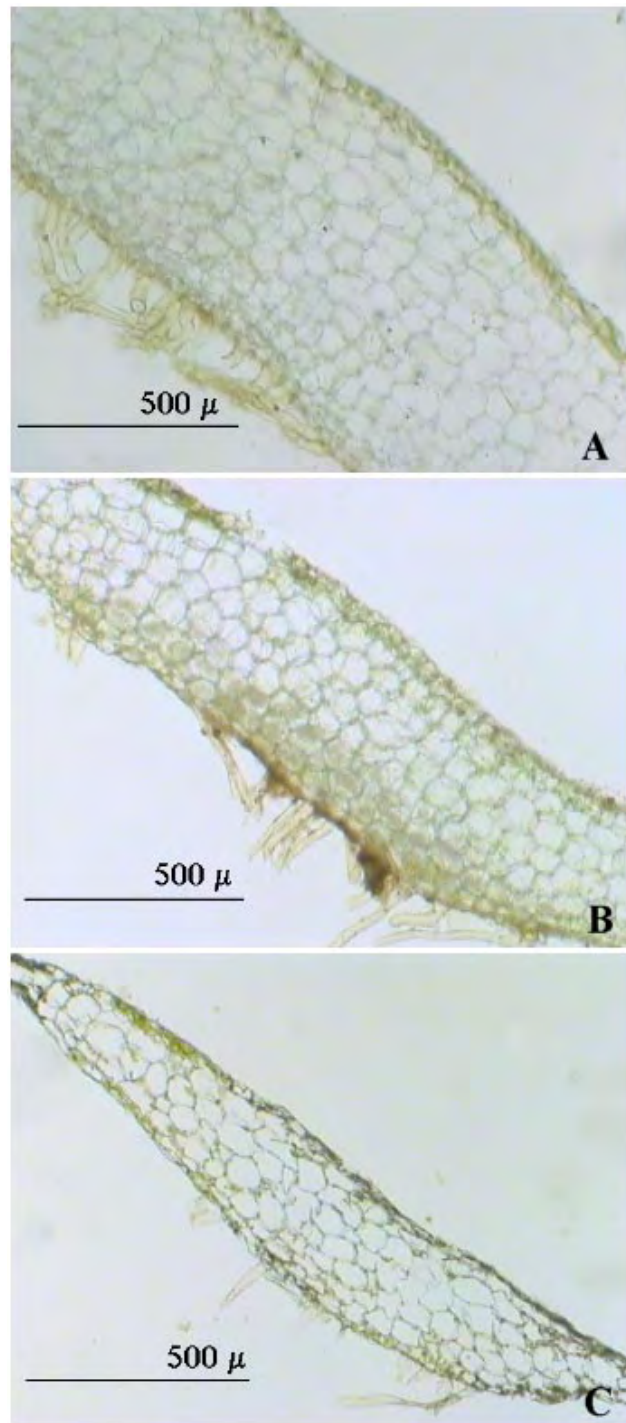


Fig. 2.1. Micrographs of thallus transverse sections of *A. pinguis* species A, B, and C

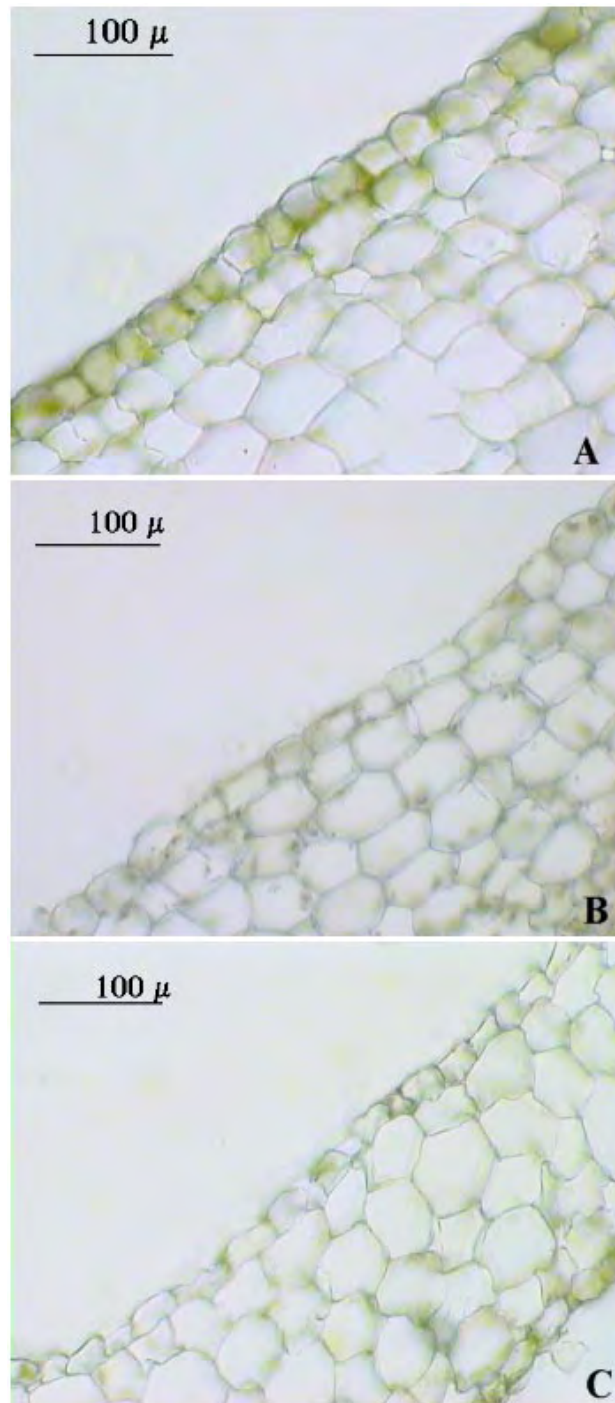


Fig. 2.2. Micrographs of dorsal epidermal and median cells in transverse sections of thalli of *A. pinguis* species A, B, and C

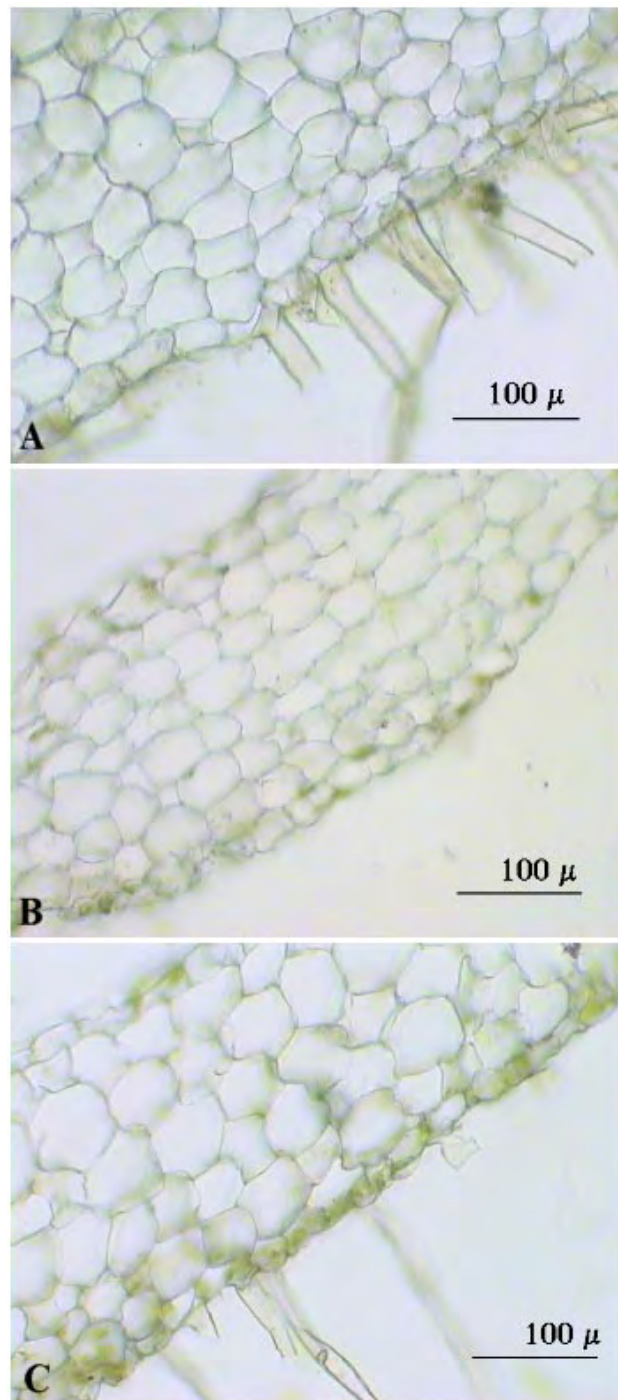


Fig. 2.3. Micrographs of ventral epidermal cells in transverse sections of thalli of *A. pinguis* species A, B, and C

Table 3. Analysis of variance (ANOVA) for 17 studied traits in 3 cryptic species of *A. pinguis* (A, B, C); *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$

Traits	F		
	Species df = 2, 144	Gender df = 1, 144	Species \times gender df = 2, 144
1	4.23	0.36	2.53
2	13.18***	0.17	2.76
3	26.94***	13.53***	4.61*
4	15.20***	8.12**	2.14
5	9.10***	2.41	0.63
6	2.61	0.62	1.60
7	6.33*	0.01	1.06
8	1.35	0.63	2.43
9	2.81	2.12	1.00
10	16.99***	4.57*	2.88
11	14.88***	1.52	2.30
12	17.14***	2.27	3.45*
14	7.75***	1.68	0.06
15	12.06***	1.66	0.01
16	28.19***	1.25	3.42*
17	24.00***	0.87	8.10***
18	40.75***	1.49	6.42**

Table 4. Analysis of variance (ANOVA), results of multiple comparisons (Scheffé test) for each pair of *A. pinguis* cryptic species and gender; *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$

Traits	A♀- A♂	B♀- B♂	C♀- C♂	A♀- B♂	A♀- B♀	A♂- B♂	A♂- B♀	A♀- C♂	A♀- C♀	A♂- C♂	A♂- C♀	B♀- C♂	B♀- C♀	B♂- C♂	B♂- C♀
2				*				*	*						
3			*	***	*			***	*	***		*		*	
4								***		***		*		*	
5				*											
7					*										
10				***	**			**							
11				**	**		*						*		*
12				***	***		*						*		
14												*			
15						*	*					*			
16				**	***			*	***	***	**				
17				***	***	**			***		*				
18				***	***	***	*		***		***				

The distribution of samples in the plane of the first 2 discriminant axes U_1 , U_2 indicates distinctness of species A along the first axis (Fig. 4). A partial separation was also observed between species B and C along the second axis. Samples of species A spread out along both axes, indicating their greater within-species variation concerning traits correlated with these axes than variation of species B and C. Traits 18, 17, 15, 16 and 3 were most strongly correlated with the first discriminant axis,

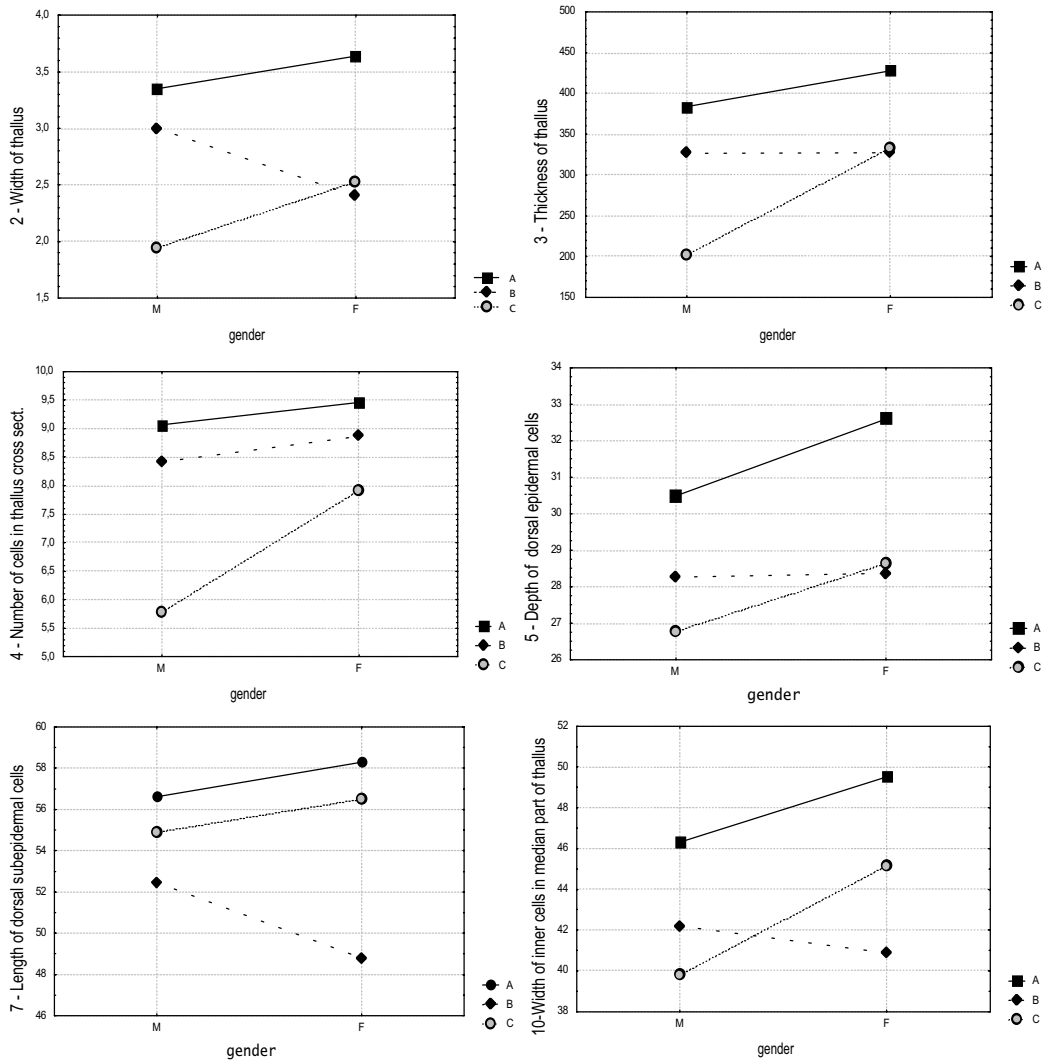


Fig. 3. Means of statistically significant traits in a 2-way ANOVA, showing effects of cryptic species and gametophyte gender and their interaction for 13 selected traits, in which statistically significant differences between the cryptic species of *A. pinguis* were noted

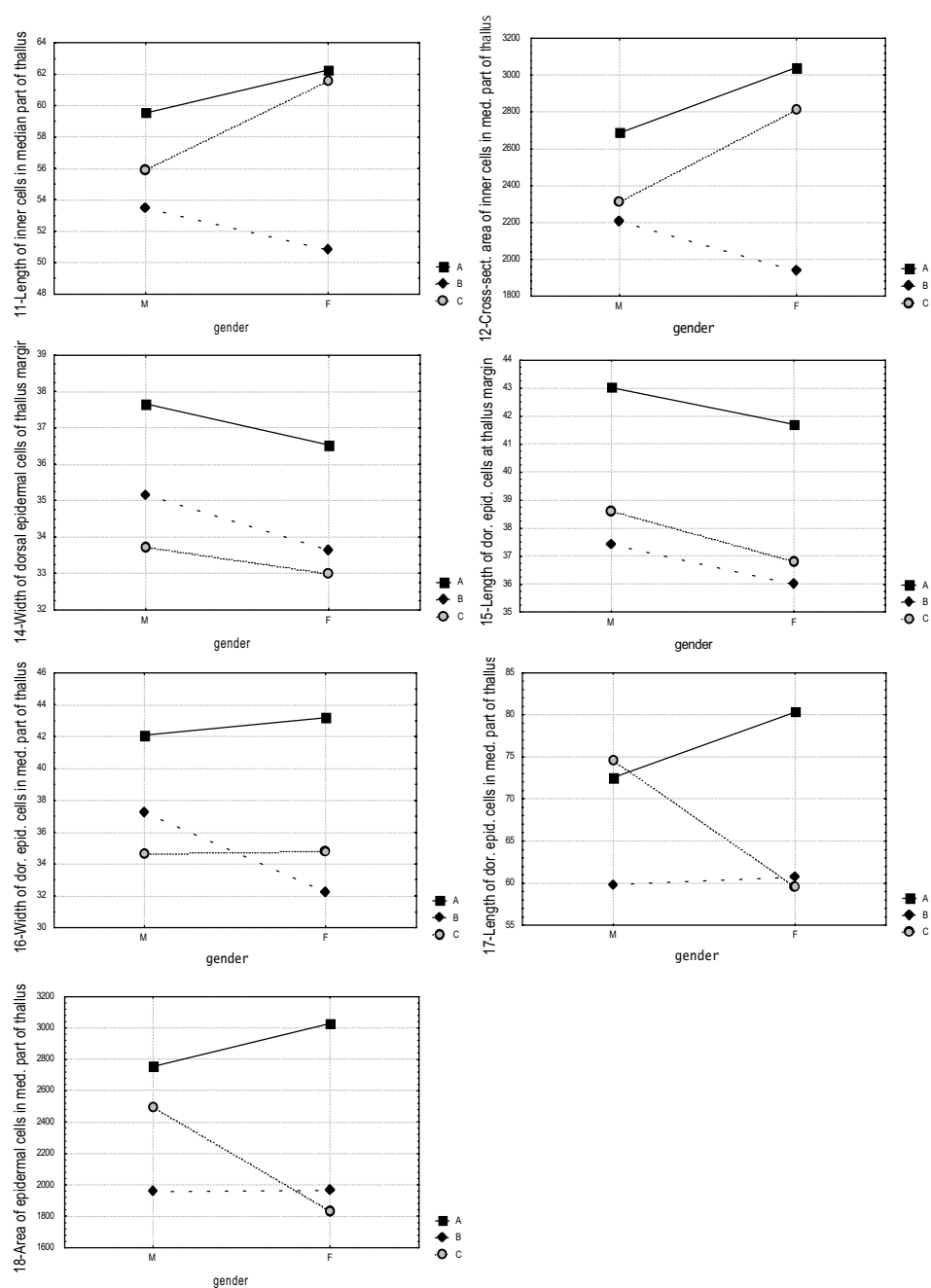


Fig. 3 continued. Means of statistically significant traits in a 2-way ANOVA, showing effects of cryptic species and gametophyte gender and their interaction for 13 selected traits, in which statistically significant differences between the cryptic species of *A. pinguis* were noted

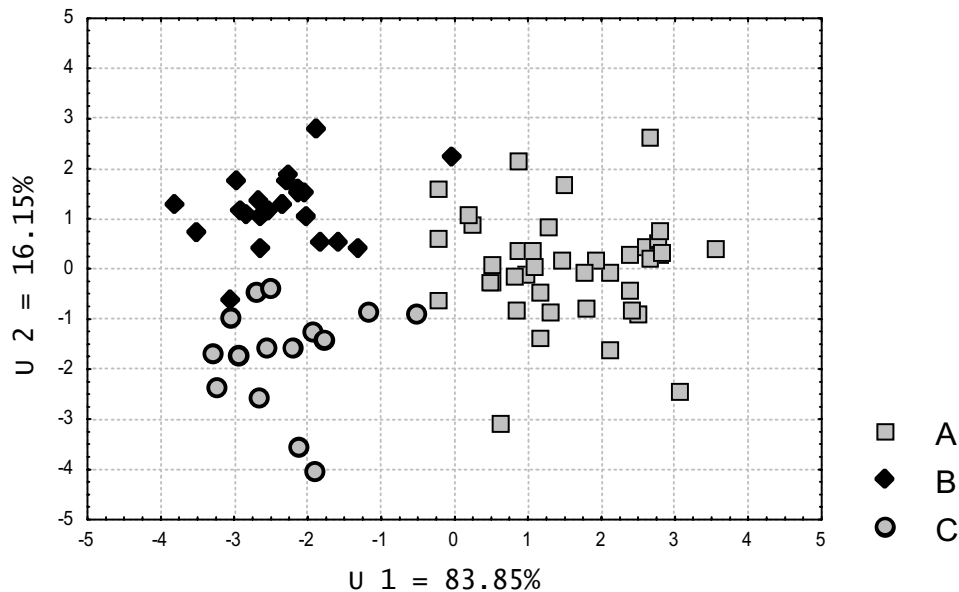


Fig. 4. Scatter diagram of all samples of 3 cryptic species of *A. pinguis* in the plane of the first 2 discriminant axes (U_1 , U_2)

while traits 17 and 4 were correlated with the second axis, so these traits were the most important in separating the examined species. The greatest Mahalanobis' distances, equal to $D = 4.48$ and 4.40 (both statistically significant at $P \leq 0.001$ with Bonferroni correction), were found between species A–C, and A–B, respectively, whereas the shortest $D = 3.04$ ($P \leq 0.05$) between species B–C. The cluster analysis based on the shortest Euclidean distance showed separateness of species A, and the most similar in respect of the examined morphological and anatomical traits are species B and C (Fig. 5). Since traits 3 and 4 (which were included to the set of traits the most strongly correlated with discriminant axes) displayed statistically significant differences between sexes, the analysis was repeated without these traits. Exclusion of these traits had not any important effect on the result of the analysis.

The studied cryptic species of the *A. pinguis* complex differed also in sex ratio: in species A females and males were equally frequent, in species B male plants prevailed ($\delta = 59.1\%$, $\delta = 22.7\%$ of gametophytes), while in species C female plants dominated ($\text{♀} = 20.0\%$, $\text{♀} = 46.7\%$ of gametophytes) (Table 2).

DISCUSSION

Liverworts are generally regarded as plants that are easily affected by the modifying influence of the environment and characterized by a large morphological plasticity (SCHUSTER 1966, SZWEYKOWSKI 1984, BISCHLER & BOISSELIER-DUBAYLE 1997), which can mask differences between closely related species and make it impossible to recognize cryptic species. It is difficult to separate environmentally induced phenotypic plasticity from genetically fixed morphological differences. There-

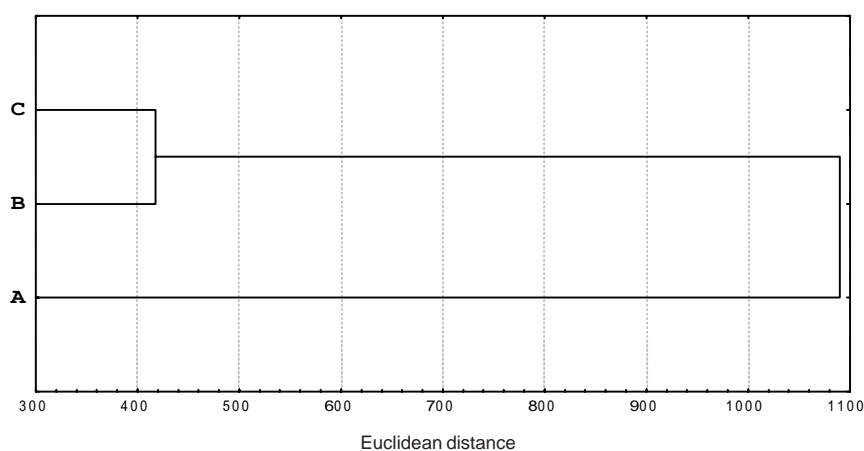


Fig. 5. Dendrogram of 3 cryptic species of *A. pinguis* constructed on the basis of the shortest Euclidean distances according to the complete linkage method, using the set of 18 quantitative traits

fore cryptic species in liverworts are usually detected by application of isozymatic and molecular studies and frequently they can be determined only by means of genetic markers. However, morphological and anatomical studies of genetically identified plants in some cases give the opportunity to find new morphological features characteristic for cryptic species, like in the *Conocephalum conicum* complex (SZWEYKOWSKI & BOBOWICZ 1978, 1979, AKIYAMA & HIRAOKA 1994). Detection of morphological and anatomical traits correlated with genetic markers and preserved in herbarium material allowed to identify the type specimen and gave the possibility to describe one of the cryptic species of *C. conicum* as a new species according to the ICBN rules as *C. salebrosum* (SZWEYKOWSKI et al. 2005). Morphological differences were also detected in some other cryptic species. In the complex of *Riccia dictyospora* Howe, morphological differences between cryptic species were noted in their overall size (DEWEY 1989). Cryptic species of *Corsinia coriandria* (Spreng.) Lindb. differ in sex distribution: haploids are dioecious, while polyploids are monoecious (BOISSELIER-DUBAYLE & BISCHLER 1998).

The present biometric study of the *A. pinguis* complex based on genetically determined plants showed that, in spite of within-species morphological and anatomical variation, the cryptic species differ statistically significantly in respect of means of several quantitative traits. The study showed significantly greater morphological differences between species A and C as well as A and B than between species B and C and confirmed the conclusions of a preliminary morphological and anatomical study of ANDRZEJEWSKA (2000). Our results of this morphometric study correspond also with results obtained on the basis of an isoenzymatic analysis (BĄCZKIEWICZ & BUCZKOWSKA 2005, BĄCZKIEWICZ et al. 2005, BĄCZKIEWICZ et al. in preparation) and a study of oil bodies (BUCZKOWSKA et al. 2005). The accordance of results obtained by different techniques gives additional evidence that the genetically detected groups of *A. pinguis* represent real biological species. However, the final taxonomic treatment of the cryptic species demands further studies. Since thalli of

A. pinguis have a very simple morphological structure (SCHUSTER 1992), the most significant differences between the cryptic species of *A. pinguis* concern mainly cell size. Epidermal cells have a higher diagnostic value, while subepidermal cells appeared to be less diagnostic. Similar morphological differences in size of epidermal cells, rib thickness, and number of cells in the rib have been also detected between cryptic species of the *Pellia epiphylla* complex (ZIELIŃSKI 1987), another liverwort genus of very simple morphological structure. In the case of *Pellia epiphylla*, like in cryptic species of *A. pinguis*, results of morphological and isozymatic analyses were consistent (ZIELIŃSKI 1987). Size and some traits observed in the thallus cross section are diagnostic also for 2 other species of *Aneura*, i.e. *A. gigantea* Schust. and *A. subaquatica* Schust. (SCHUSTER 1985).

The dioecious species of the genus *Aneura* express sexual dimorphism, as the male thalli are smaller and less branched than female thalli (MÜLLER 1951–1958, FURUKI 1991, SCHUSTER 1992). Sexual dimorphism was observed in all examined cryptic species of *A. pinguis*: in species A and C female plants are bigger and had bigger cells than the male ones, while in species B the female plants are smaller. Sexual dimorphism complicated here the variation pattern; in the case of traits 3, 12 and 16–18, a statistically significant interaction occurred, indicating that differences between species in these traits depend on sex (Table 4). Bigger male plants in species B, inversely than in species A and C, could result from unequal sample size of both sexes in particular species or due to habitat differences. Maybe in species B, the habitat for the male populations was not as suitable as for the female populations. These suggestions demand further observations. In spite of almost the same time of collection of all samples (July, August) there was variation among the cryptic species of *A. pinguis* in proportion of fertile to sterile gametophytes: in species A only 55% gametophytes were fertile, compared to 80% in species B and almost 70% in species C. Colonies usually consisted of one sex; only few colonies were mixed. Examined species differed also in sex ratio: only in species A the female and the male plants were equally frequent, in species B the male plants prevailed, while in species C the female plants were the most frequent. Deviation from the expected 1:1 sex ratio in bryophytes is frequent. In unisexual mosses a female-biased sex ratio is commonly observed, so it can be considered characteristic for them, but in some species a male-biased sex ratio has been also reported, e.g. in *Racomitrium* Brid., *Atrichum* P. Beauv., and *Bryum* Hedw. (SHAW 2000). In *Ceratodon purpurascens* Hedw. Jenn., many populations have more female than male plants, but male-biased populations have also been observed (SHAW & BEER 1999). In liverworts female-biased sex ratios have been noted for *Cryptothallus mirabilis* Malmb. and *Sphaerocarpos texanus* Austin (SHAW 2000). It demands detailed observations, however, to verify if the time of gametangium formation as well as the sex ratio between cryptic species of *A. pinguis* differ among species, or among populations. Some observations suggest that the ratio of females to males can be modified by environmental factors (SHAW 2000). For example, in the unisexual *S. sphaericum* H. Lindb. under unfavourable conditions mainly the male sex is expressed (CRONBERG & NATCHEVA 2002). On the other hand, male plants in *Bryum argenteum* Hedw. appear to be restricted to protected habitats, whereas female plants are widespread (LONGTON 1988).

CONCLUSIONS

1. The studied cryptic species of *A. pinguis* (A, B and C) differed statistically significantly in respect of 13 quantitative traits and the most distinct was cryptic species A.
2. The most useful diagnostic traits were:
 - a. width, length and area of dorsal epidermal cells measured in plane view (traits 16–18), which were the highest in species A and the lowest in species C;
 - b. thallus size, thallus thickness and number of cells in the thallus cross section (traits 2–4), which were the highest in species A and the lowest in species C; and
 - c. both width and area of inner cells in the thallus cross section (traits 10–12), which were the highest in species A and the lowest in species B.
3. We have not found so far any qualitative morphological or anatomical features, which could be useful in determination of the cryptic species.

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APPENDIX

Collection sites of specimens used for biometric studies

A. pinguis species A

PODLASKIE PROV.: 39868 Białowieża National Park, European bison reserve; MAŁOPOLSKIE PROV.: TATRA MTS.: 40161, 40162, 40443 Biały Potok valley, 40160, 40441 Kraków gully in Kościeliska valley, 40171, 40423, 40424, 40426, 40430, 40435 NE slope of Skupniów Uplaz Mt., 40165, 40166, 40156, 40419, 40440, 40445, 40446, 40452 Wielka Sucha Woda stream in Chochołowska valley, 40420, 40447, 40448, 40449, 40450, 40451 Koziniecki gully in Chochołowska valley, 40170 Sucha Woda valley, 40130, 40169, 40172 Pańszczyca valley; MAŁE PIENINY MTS.: 40190, 40199 small stream on N slope of Repowa Mt., 40193, 40195, 40196, 40202 Skalskie stream, 40203, 40207, 40208 small stream near Skalskie reserve, 40209 morass near source of Skalskie stream.

A. pinguis species B

ZACHODNIOPOMORSKIE PROV.: 40133, 40266, 40454 Diabli Skok reserve near Wałcz; WARMIŃSKO-MAZURSKIE PROV.: 40261 Redykajny reserve near Olsztyn; PODLASKIE PROV.: 39864, 39870, 39874 Białowieża National Park, European bison reserve; WIELKOPOLSKIE PROV.: 40325 Poznań, Kopanina; MAŁOPOLSKIE PROV.: TATRA MTS. 40439 Biały Potok valley; PODKARPACKIE PROV.: BIESZCZADY MTS. 40144, 40146, 40150, 40181, 40211 Moczarne in valley of Górna Solinka stream, 40180, 40223 old quarry near Brzegi Górne, 40174, 40177, 40179, 40220, 40458 Sianki, Niedźwiedzi stream, 40175 S slope of Ryczywół Mt. near Cisna village.

A. pinguis species C

ZACHODNIOPOMORSKIE PROV.: 40152, 40154 Garczyn lake near Kościerzyna; WARMIŃSKO-MAZURSKIE PROV.: 40251, 40260, 40267 Ruskie lake near Ostruda; PODLASKIE PROV.: 39869 Białowieża National Park, European bison reserve; MAŁOPOLSKIE PROV.: TATRA MTS.: 40299, 40432, 40433, 40438 NE slope of Skupniów Uplaz, 40252, 40254, 40332, 40335 Sucha Woda valley, 40256 Pańszczyca valley.