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ON THE LEAF CELL MEASUREMENTS IN MOSSES

ОБ ИЗМЕРЕНИЯХ КЛЕТОК ЛИСТА У МХОВ

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Abstract

The newly developed method of cell net digitizing is applied to illustrate variation in leaf cell length, width and square on the example of two moss species, *Mnium spinosum* and *M. spinulosum*. The accuracy of the literature data is tested by use of a large array of computerized measurements.

Резюме

Новый разработанный авторами метод оцифровки клеточной сети использован для измерения длины, ширины и площади клеток листовой пластинки мхов, на примере *Mnium spinosum* и *M. spinulosum*. Точность литературных указаний проверяется на больших массивах компьютеризированных измерений.

KEYWORDS: mosses, leaf cells, digitizing, measurements, *Mnium*, morphology, polarized microscopy

INTRODUCTION

Since the early days of bryology, the leaf cell nets were in the focus of studies. The areolation-patterns are reflected in such moss species epithets as “angustirete”, “brevirete”, “densirete”, laxirete”, etc. The outlines of laminal cells became a standard element of species descriptions in the middle of the XIX century, although the areolation in some species is given already in the colored illustrations in ‘Species Muscorum’ (Hedwig , 1801). Since the end of the XIX century (e.g. Lindberg & Arnell, 1890; Limpricht, 1885-1904, etc.) cell size has become an ordinary and important part of species description.

The length and width of cells are a common element of morphological description nowadays (Crum & Anderson, 1981; Smith, 2004; Ignatov & Ignatova, 2003-2004; Noguchi, 1989, 1992, etc.), however values provided by different authors for the same species sometimes vary greatly, cf. Table 1. Some authors report values for cell width consistently lower than others, likely because of measuring only the cell lumen width or otherwise measuring a fixed distance, say 50 or 100 μm, and then dividing it by a number of cells crossing it.

Most publications provide laminal cell length and width without any special definition as a self-evident one. Only few papers and manuals explain explicitly what and where has to be measured to obtain the data comparable with those of other authors (Hedenäs, 1993; 2003; Ignatov & Ignatova, 2003-2004).

A recently developed method of cell net digitizing (Ivanov & Ignatov, to e published) has opened new possibilities for measurements on large massifs of data, and its possible usefulness for bryology will be illustrated here by some examples.

MATERIAL AND METHODS

Microscopy: The study is conducted with a polarized microscope (modified ordinary light microscope) where two polarizing filters are rotatable, while a specimen is immobile. The first

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Table 1. Comparison of published data on cell width in five pleurocarpous moss species, in μm.

	Noguchi (1992)	Smith (2004)	Latwon (1971)	Limpricht (1885-1904)	Ignatov & Ignatova (2004)
Ptilium crista-castrensis	2	4-5	3-5	5	4-6
Callicaldium haldanianum	4-4.5	-	5-7	6	5-8
Calliergonella lindbergii	3-4	5.0-6.5	4-7	6-7	5-7
Isopterygiopsis muelleriana	4-4.5	4-6	-	5-6	-
Hylocomium splendens	3-4	5-7	4-6	5	5-6

filter (called polarizer) is placed in front of the condenser. The second one (called analyzer) stands after the objective These filters are rotated in such a way as to ensure a maximally dark field when the specimen is absent. The polarized light induced in this way allows the "staining" of cell walls (Fig. 1). The complete 'staining' of all cell walls is achieved through a combination of three digital photographs by simultaneous rotation of both filters at angles of 30°, 60° and 90° (Fig. 1B-D).

Digitizing: The algorithm for finding cell walls searches maximally bright places between darker inner parts of cells and then it fixes the cell corners (join points of 3 or more cells); the next step involves digitized nets where corners of each cell are connected by straight lines (Fig. 1E). This simplification does not affect much the accuracy in such analyses of images with 600-1300 cells per picture (to be published), at least in cases where the cell length to width ratio is less than 3-4:1.

The piloted program generally failed to recognize only a trace amount of cells, especially those damaged by fungi, or broken in the course of specimen preparation. Figs. 1-3, 5 have a few areas where not all cells are recognized, although the program often provides one hundred percent recognition (Fig. 4). This program require UNIX operation system; is can be obtained for free upon agreement from the authors (inquire by e-mail). Its detailed description is given in Ivanov & Ignatov (submitted for publication).

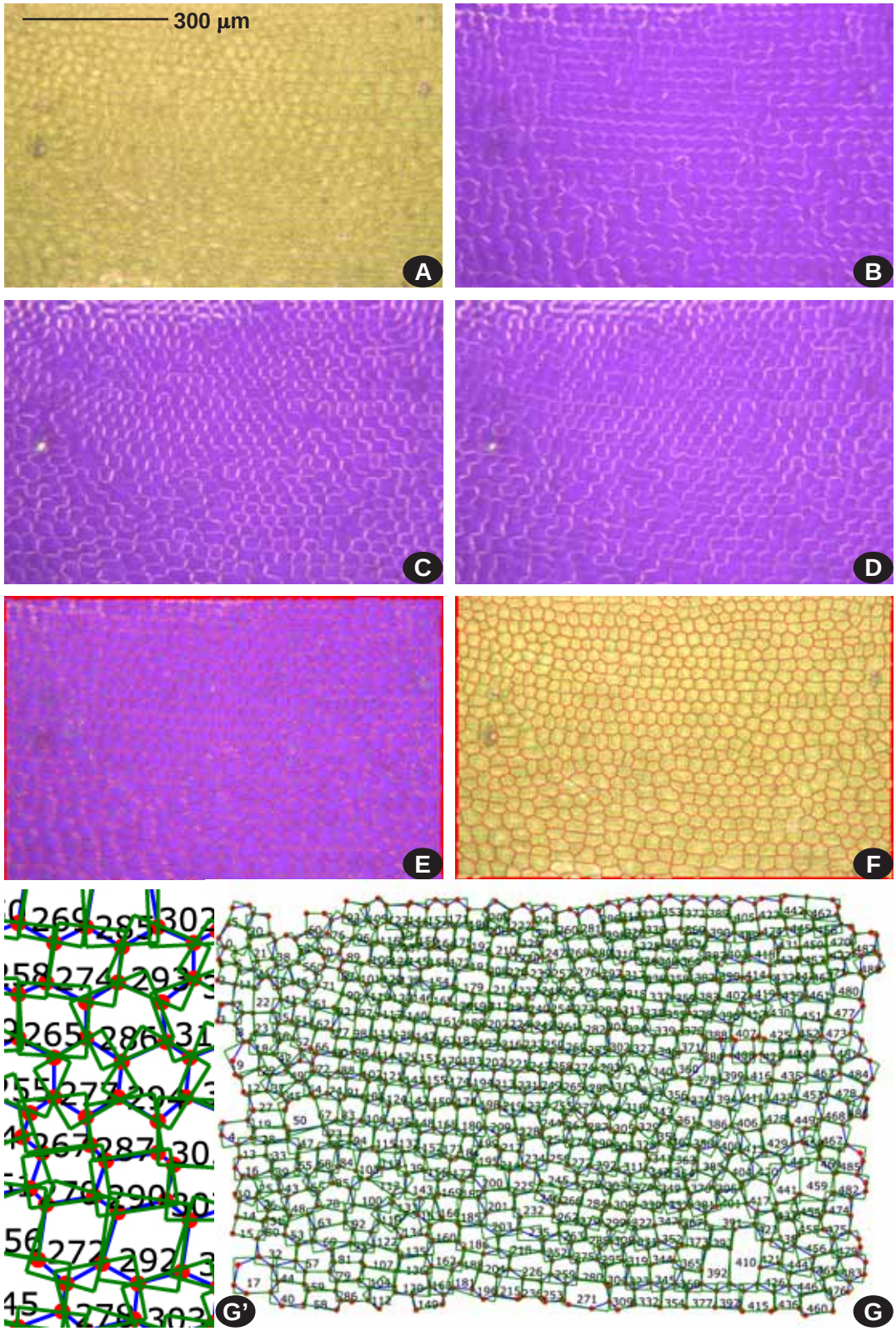
Leaf study: Individual photographs (example in Figs. 2-4) were taken in order from lower part of leaf (however excluding basal part with obviously elongate cells) towards leaf apex considering minimal overlapping with previous picture, and in broad leaves by two (closer to costa and then closer to margin) at the same distance from base. Examples of raw data shown in Tables 3-4.

Width and length are trivial dimensions in a quadrate-rectangular cell net, e.g., in a well-known cosmopolitan *Ceratodon purpureus*, while already in *Tortula mucronifolia*, which has cells 'more or less' close to a quadrate shape, the measurement procedure is not so straightforward (Fig. 1). There exists a temptation to provide a calculation of length as a difference between coordinates of the upper and lower points, and similarly of width for coordinates in the direction perpendicular to length. This method however fails to work for cells in oblique rows like in, e.g., many Mniaceae, where a diagonal could be measured instead of length, and all this occurs due to the unsolved problem of length definition.

To avoid these ambiguities, we defined the cell length and width as the length of, correspondingly, a longer and a shorter side of the minimal rectangular outlining the cell contour. The program of finding this minimal rectangular is simple enough and can be applied to a cell of any shape in any part of a leaf. An illustration of how it works is given in Fig. 1G.

For further testing if this method can be used for bryological purposes, we compare two species of *Mnium, M. spinosum* and *M. spinulosum*, because several authors have published quite different dimensional characteristics for their cells (Table 2). They can be clearly distinguished from each other by sexual condition, dioicous and synoicous

Fig. 1. *Tortula mucronifolia* (from: Yakutia, Ignatov, 00-368, MHA). A – cell net in transmitted light; B, C, D – photographps in polarized light at 30°, 60° and 90°, E – sum of B+C+D, with digitized cell outlines; F – digitized cell net over photo in transmitted light (cf. A); G – digitized cell net with rectangulars outlining each cells (G' – magnified part of G). Scale bar 300 μm for A-E.


300 µm
A
B
C
D
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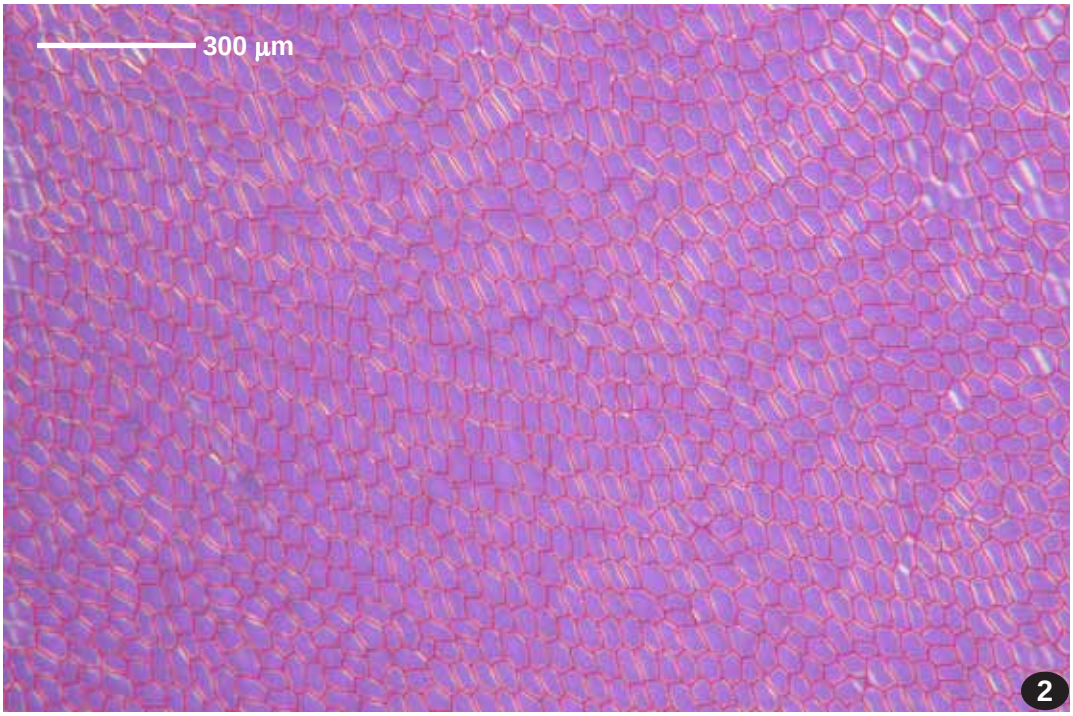
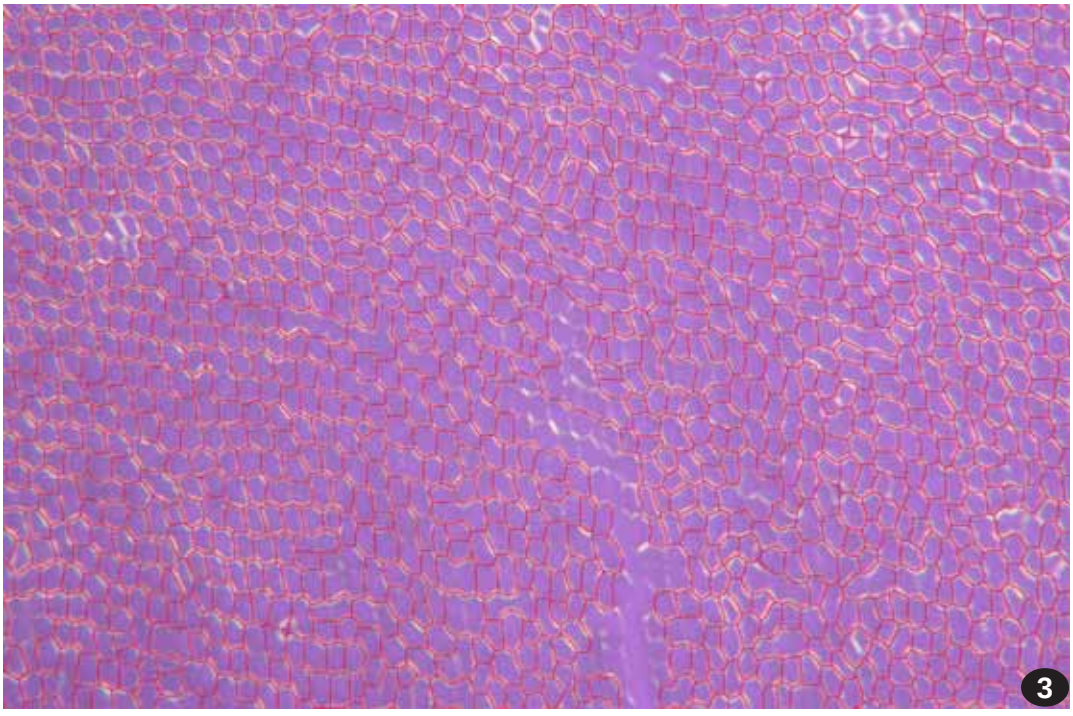
F
G'
G





Figs. 2-3. (2) Cells of *Mnium spinosum* Altai, Ignatov 0/1534 (MHA); and (3) *Mnium spnulosum* Altai, Ignatov, 0/1535 (MHA): cell net in polarized light with digitized cell outlines.

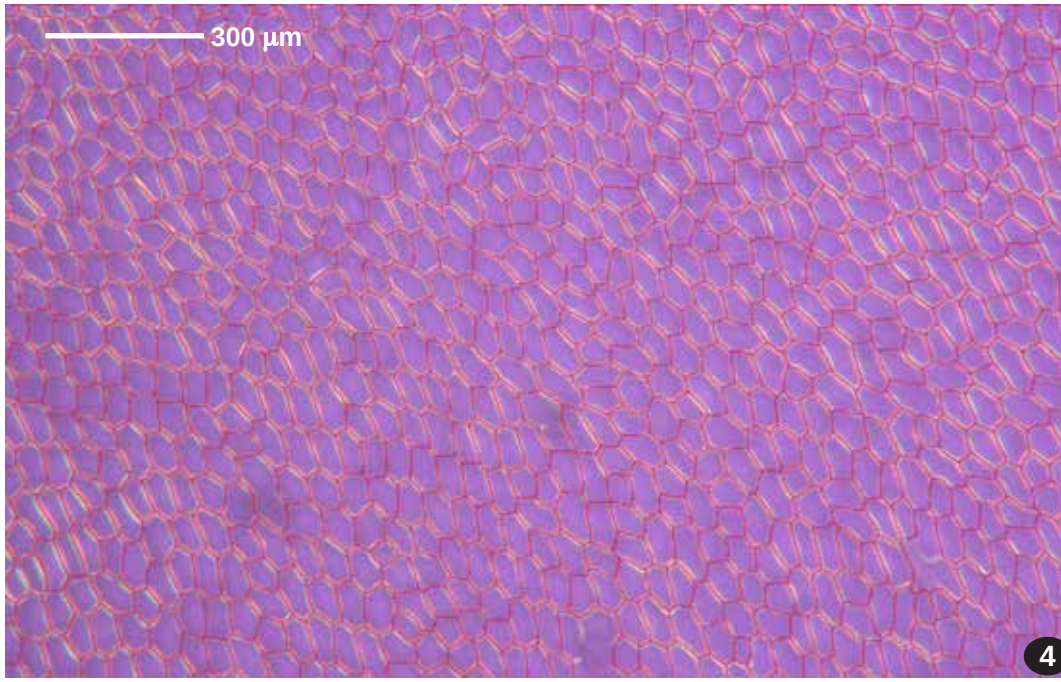


Fig. 4. Cells of *Mnium spinosum* Karelia (Maximov & Maksimova, 29.V.2002, PTZ, MHA): cell net in polarized light with digitized cell outlines.

correspondingly. There are also many characters that allow separation of these species from other superficially similar members of the genus, so any identification mistakes are ruled out in our study.

Comparison of *Mnium spinosum* and *M. spinulosum*

Despite a certain difference in cell dimensions in these species (Table 2), three of the four authors who provided concrete data on it agree that cells in *M. spinosum* are larger than in *M. spinulosum,* while Limpricht' measurements found them subidentical.

The measurement involves three specimens of each species, from geographically separated regions (the Caucasus, the Altai and Russian Far East).

At least two leaves from each of at least two shoots were used for photography and measurements, so 12-30 pictures (cf. Figs. 2-4) were taken for width and length calculation.

Graphs for these three pairs of specimens are shown in Fig. 5, depicting distribution curves smoothened by Gaussian function, where σ=5 µm for cell width and length, and σ=50 µm² for cell squares.

$$F(x)=\sum_{n=1}^{n_{MAX}}\left[\frac{1}{\sigma\cdot\sqrt{2\pi}}\cdot e^{\frac{-(x-t_n)}{2\cdot\sigma^2}}\right]$$

The results from Fig. 5 indicate that the laminal cells of *M. spinosum* is larger in specimens from Far east, but equal in Caucasus and even smaller in Altai.

Table 2. Comparison of published data on cell dimensions in *Mnium spinosum* and *M. spinulosum*, in µm.

	M. spinosum	*M. spinulosum*
Smith, 2004	16-40 wide	—
Crum & Anderson, 1981	—	21-25(-35)
Lawton, 1971	—	near costa 30-40, towards the margin 18-25
Limpricht, 1885-1904	0.022-0.028 mm	0.02-0.03 mm
Noguchi 1989	15-22 x 30-35	15-22 x 20-30
Hallingback et al., 2008	12-22 wide	—
Ignatov & Ignatova, 2003	21-27 x 35-50	17-25 x 20-30
Koponen, 1980	14-19 x 28-43 *	19-35 * * – measured from pictures

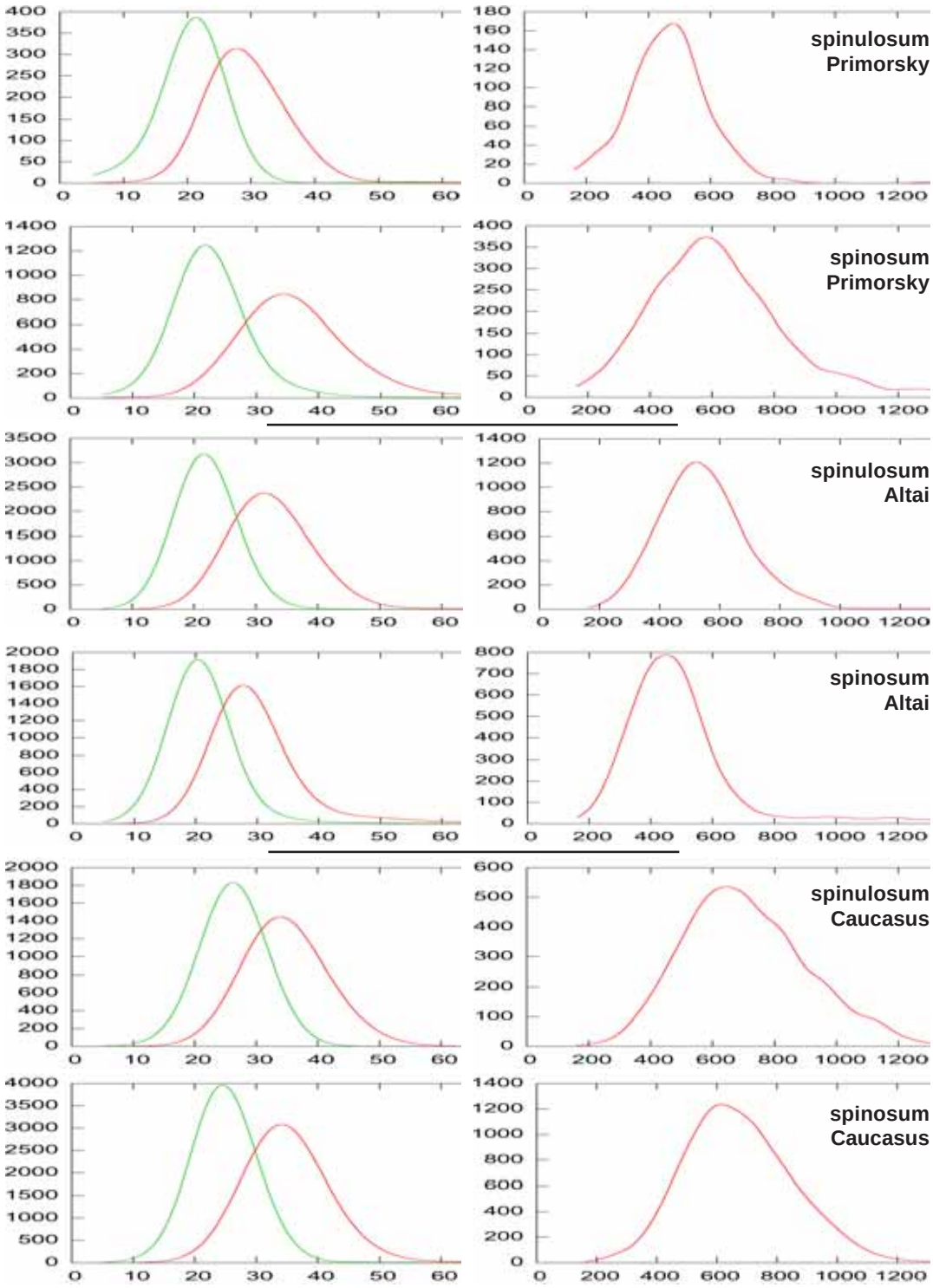


Fig. 5. Three pairwise comparisons of *Mnium spinulosum* and *M. spinosum* from Primorsky Territory (Russian Far East), Altai (southern West Siberia) and Caucasus. Left column pictures show distribution of leaf cell width (left peak/green) and length (right peak/red), in μm; right column gives distributions of square (in sq. μm). Axe Y is number of measured cells. Distribution curves smoothened by Gaussian function (cf. page 91).

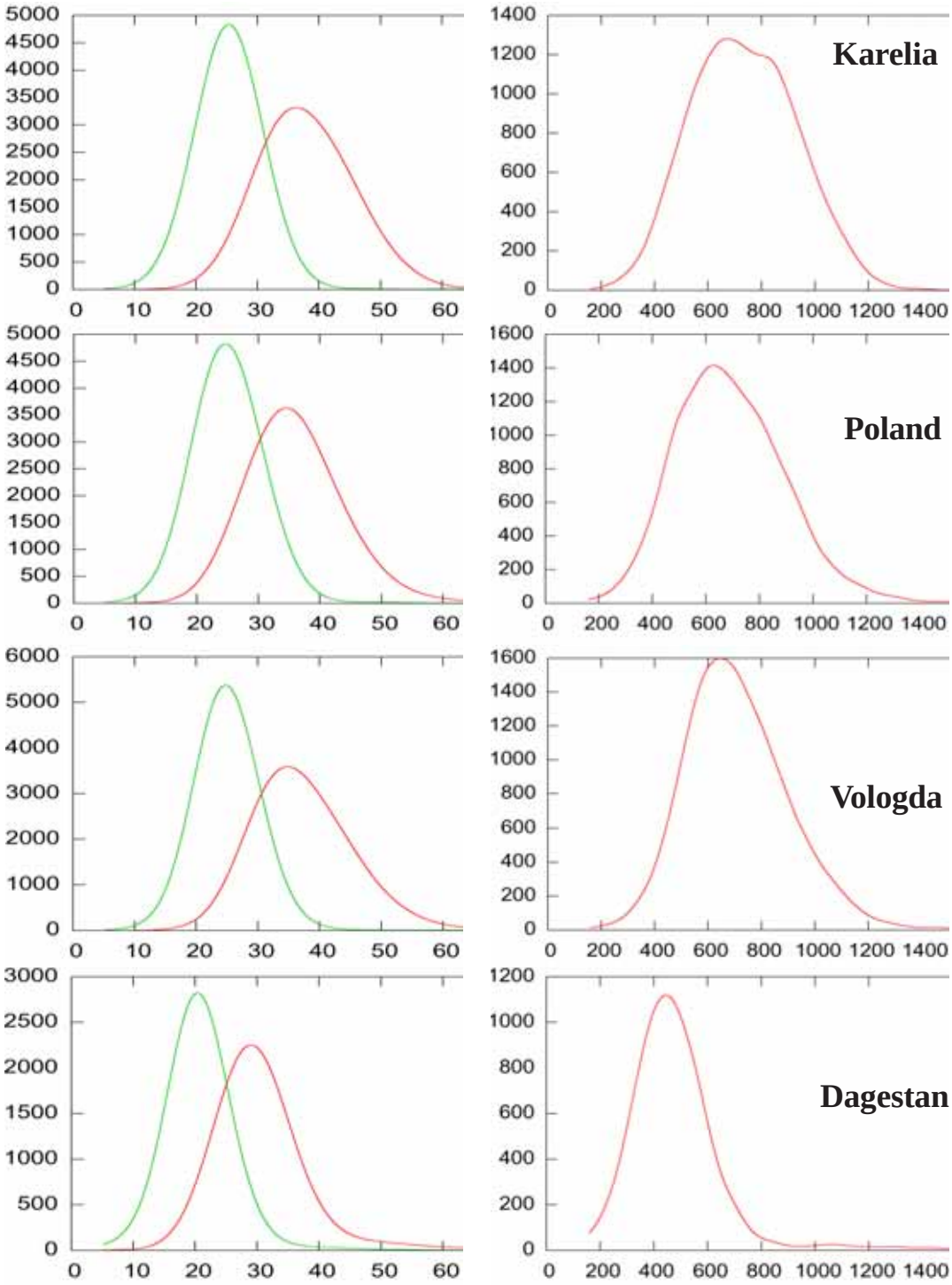


Fig. 6. A comparison of *Mnium spinulosum* from four localities. Left column pictures show distribution of leaf cell width (left peak/green) and length (right peak/red), in μm; right column gives distributions of square (in sq. μm). Axe Y is number of cells. Distribution curves smoothened by Gaussian function (cf. page 91).

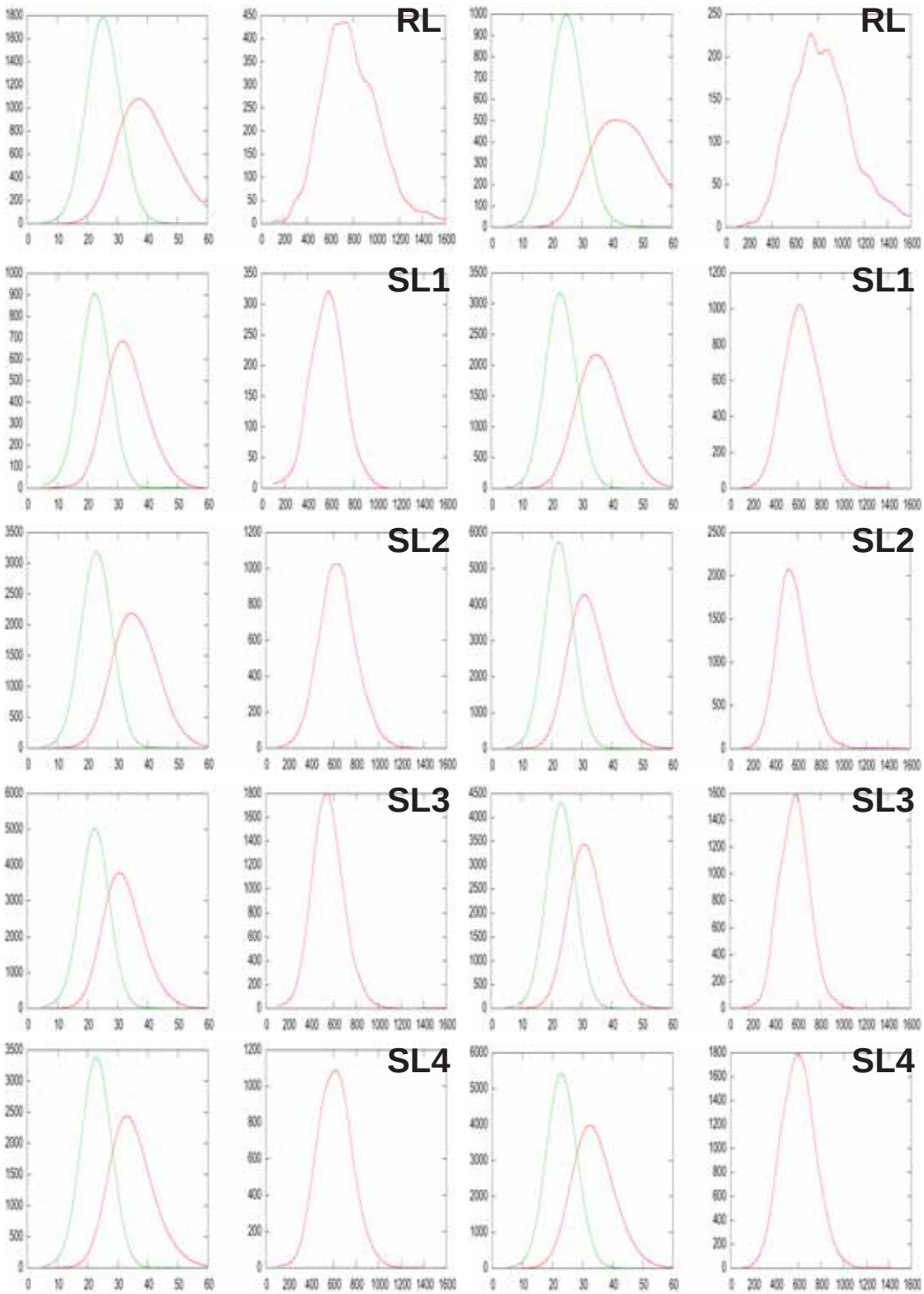


Fig. 7. *Mnium spinosum* (Archangelsk). Distribution of cell width (left peak/green) and length (right peak/red) and square (graphs with single peak), for five leaves (one from upper rosette RL, and four stem leaves SL1-4, from rosette to mid-stem) of two shoots from the same specimen. Data for these graphs are shown in Tables 3-4.

Tables. 3-4. Cell measurements in leaves of two shoots of *Mnium spinosum* (Archangelsk). Data for two shoots is presented in two tables, in the left and right sides of page. Lines provide data for individual pictures for rosette leaves (RL) and four stem leaves (SL1-4, in order from top to middle part of stem). Individual photographs (example in Fig. 4) are marked by letters; they were taken in order from lower part of leaf (however excluding basal part with obviously elongate cells) towards leaf apex considering minimal overlapping with previous picture, and in broad leaves by two (closer to costa and then closer to margin) at the same distance from base. Column abbreviations are as follow: n – number of cells per picture; x – length, y – width, s – square, d – dispersion. The data are presented as graphs in Fig. 7. Note the considerably larger cells in rosette leaves.

	n	x	d	y	d	s	d
RLA	325	44.87	9.098	27.12	4.653	929.0	259.83
RLB	553	40.10	7.186	25.97	4.668	807.1	210.61
RLC	550	38.10	7.347	25.79	4.196	754.1	191.67
RLD	376	38.11	6.740	23.88	4.473	696.2	175.06
RLE	339	44.54	8.386	28.60	4.863	983.3	263.40
RLF	301	40.29	7.746	27.58	4.551	857.1	232.60
RLG	363	40.08	8.310	25.77	4.579	795.5	230.00
SL1 A	676	39.67	7.023	24.72	4.154	755.2	192.27
SL1 B	667	38.44	6.938	24.85	4.094	741.4	181.58
SL1 C	650	39.45	7.200	23.53	3.750	727.1	168.07
SL1 D	654	35.89	6.331	24.18	3.564	666.6	155.64
SL1 E	592	37.52	7.063	22.47	4.677	656.4	163.66
SL1 F	474	34.73	5.182	24.37	3.885	646.6	148.93
SL1 G	536	35.70	6.226	22.49	3.908	616.3	147.35
SL1 H	445	34.89	5.818	23.16	3.788	616.6	142.70
SL2 A	757	35.78	6.888	23.21	3.631	634.0	162.03
SL2 B	644	32.96	6.032	22.97	3.368	584.7	141.17
SL2 C	685	35.74	6.652	24.39	4.081	657.4	157.97
SL2 D	600	32.82	5.527	23.17	4.325	588.3	153.13
SL2 E	641	33.72	6.089	24.00	3.526	629.1	142.14
SL2 F	780	31.49	4.818	23.10	3.566	566.6	131.45
SL2 G	604	33.21	5.985	22.63	4.006	582.1	144.11
SL2 H	656	32.65	4.800	22.93	3.781	581.3	139.01
SL2 I	376	32.64	6.141	22.09	4.292	562.2	141.43
SL2 J	496	30.58	4.368	23.13	3.405	545.7	117.41
SL2 K	679	32.01	4.930	22.13	3.554	548.4	125.94
SL3 A	780	35.42	5.575	23.96	3.834	654.8	153.31
SL3 B	665	38.25	6.765	24.25	4.495	716.8	157.37
SL3 C	744	34.09	5.549	23.94	4.348	627.9	147.89
SL3 D	627	37.35	7.144	22.83	3.467	671.2	155.45
SL3 E	733	34.22	5.779	23.65	3.362	625.7	151.16
SL3 F	542	33.64	5.573	23.12	3.999	595.2	150.90
SL3 G	641	35.96	6.890	23.26	3.932	628.1	162.18
SL4 A	518	34.36	6.258	23.28	3.555	603.2	150.51
SL4 B	734	34.43	6.408	23.57	3.688	630.3	159.81
SL4 C	621	32.93	5.296	24.70	3.375	631.8	145.71
SL4 D	664	34.11	5.858	24.13	3.759	643.6	152.14
SL4 E	653	32.56	4.853	23.52	4.157	601.8	145.01
SL4 F	658	32.16	4.916	24.01	3.773	599.0	139.45
SL4 G	633	33.27	5.193	24.61	3.568	632.0	146.20

	n	x	d	y	d	s	d
RLA	186	46.97	7.754	25.38	5.861	906.1	272.83
RLB	227	45.99	7.594	28.18	4.630	961.5	235.61
RLC	353	41.11	7.800	23.97	4.450	758.3	205.58
RLD	249	48.60	7.829	26.09	4.935	984.2	261.56
RLE	268	45.82	7.364	26.90	4.606	956.5	252.58
RLF	331	39.49	7.470	26.14	4.480	788.4	197.57
SL1 A	719	39.51	7.218	22.88	3.666	708.9	182.73
SL1 B	669	36.43	6.574	24.37	3.839	683.4	170.12
SL1 C	648	40.32	6.967	22.58	4.397	713.0	181.16
SL1 D	707	35.67	5.772	24.25	3.680	661.0	155.86
SL1 E	730	37.19	6.699	24.56	4.074	700.8	164.81
SL1 F	633	35.06	5.782	25.19	3.973	671.7	155.18
SL1 G	532	35.22	6.440	23.16	3.864	624.0	159.61
SL2 A	763	36.25	6.702	22.94	4.149	618.9	173.39
SL2 B	732	32.64	5.970	23.35	3.733	582.1	150.86
SL2 C	771	35.59	6.728	23.41	3.446	635.7	161.79
SL2 D	869	31.65	5.182	23.70	3.389	582.9	141.88
SL2 E	731	36.25	6.486	22.54	3.715	646.2	162.28
SL2 F	835	32.15	4.949	23.25	3.314	586.6	127.68
SL2 G	534	33.14	5.552	23.06	3.420	597.3	134.08
SL2 H	617	30.78	4.358	23.71	3.739	563.6	128.19
SL2 I	739	33.78	5.387	22.71	3.814	602.5	150.52
SL2 J	567	31.68	4.732	23.55	3.529	572.1	130.15
SL2 L	624	32.80	5.708	23.51	3.987	588.8	150.29
SL3 A	686	34.89	6.446	23.36	3.505	612.5	141.24
SL3 B	850	34.25	5.582	24.16	3.741	643.4	142.06
SL3 C	722	32.95	4.945	24.46	3.514	623.8	135.15
SL3 D	787	34.17	5.233	24.06	3.655	642.5	139.91
SL3 E	761	31.79	4.536	23.67	3.533	586.6	133.79
SL3 F	796	32.41	5.127	24.35	3.586	619.8	138.37
SL3 G	612	31.35	4.527	23.65	3.413	574.5	129.71
SL3 H	654	32.22	4.910	24.08	3.184	603.9	128.01
SL4 A	793	35.30	7.091	22.15	3.069	597.7	153.46
SL4 B	587	36.46	6.229	24.31	3.994	677.1	157.86
SL4 C	613	35.47	6.062	23.86	3.463	645.7	142.51
SL4 D	722	35.42	6.157	24.77	3.709	681.7	162.02
SL4 E	634	32.29	5.165	23.98	3.437	600.9	141.07
SL4 F	594	35.87	6.186	24.92	4.143	684.7	181.92
SL4 G	474	35.24	6.416	24.09	4.049	661.5	179.19
SL4 H	733	35.01	6.160	23.68	3.777	623.6	152.04
SL4 I	690	35.93	6.155	24.14	3.986	680.1	152.42
SL4 J	626	34.12	5.503	24.04	3.854	636.2	138.86
SL4 K	718	34.48	6.559	24.22	3.720	650.5	164.69

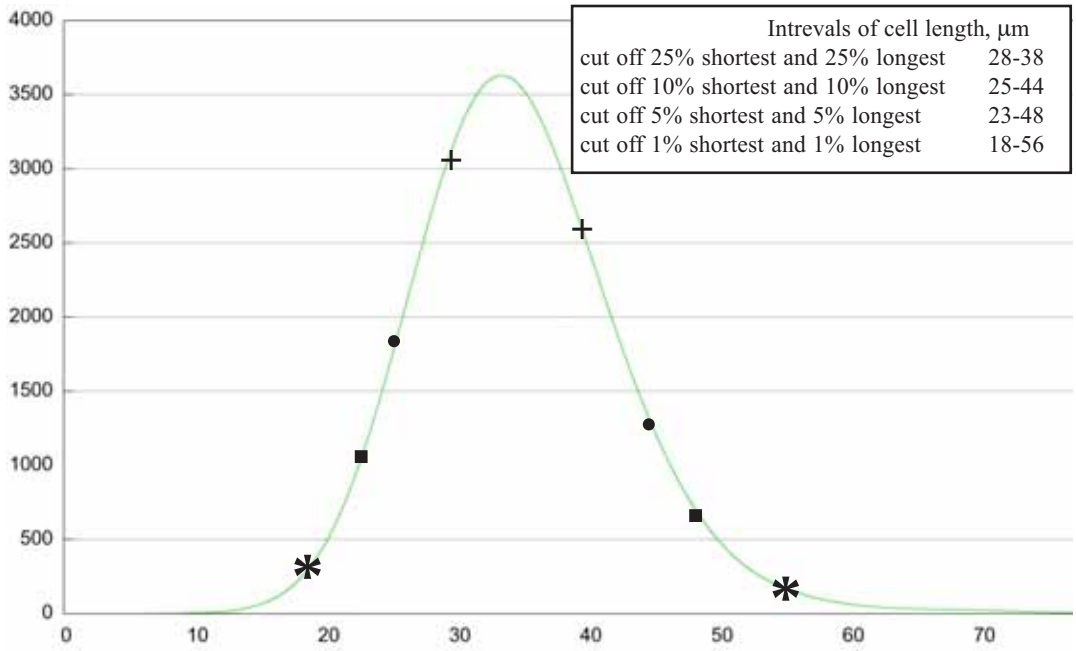
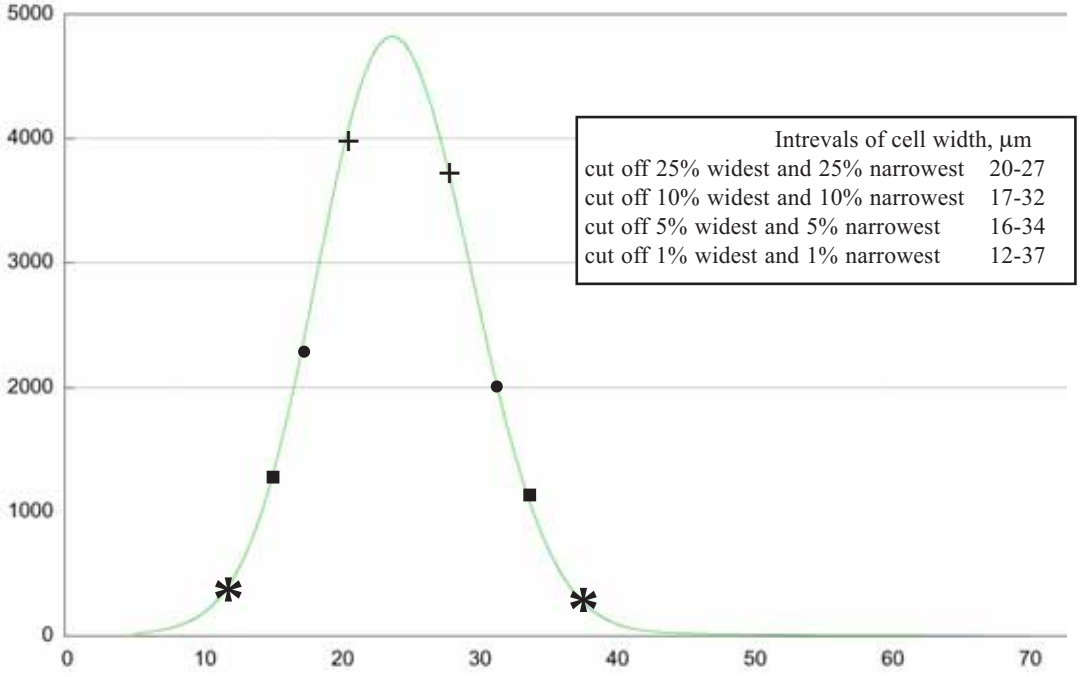


Fig. 8. Distribution of leaf cell lengths (above) and widths (below) of the Karelian specimen of *Mnium spinosum* (7326 cells measured, one of picture in Fig. 4). Axe X – μm; Axe Y – number of cells.Graphs show distribution curves smoothened by Gaussian function (cf. page 91), with intervals of most common length and width, after cut off 25% shortest/narrowest and 25% longest/widest cells (crosses), 10% (solid circles), 5% (solid rectangulars) and 1% (asterisk). Intervals of the most common lengths and widths in μm shown within graphs.



LEAF CELL VARIATION IN *MNIUM SPINOSUM*

Measurements of additional specimens of *M. spinosum* from different parts of Russia and Central Europe (from where *M. spinulosum* was not readily available) reveal an even greater variation (Fig. 6).

Plants from a wet boreal conifer forest from the middle elevation in Central Europe (Poland) and from northern part of Russia (Karelia and Vologda) have much bigger cells comparatively with plants from a relatively dry *Betula* forest in Dagestan.

The latter may be correlated with the quite xeric climate of the latter area. However, another explanation is also possible. Plants from Dagestan collection have only male shoots, and the subapical leaf rosette was not formed in them.

The comparison of cells in leaves of subapical rosette and of those from the middle part of stem was done using plants from Arkhangelsk Province in Northern European Russia.

Measurements in two shoots (Fig. 7, Tables 3-4) show that cells in rosette leaves are larger, thus confirming ordinary observations from the routine practice of mass identification of collections (likely familiar to any bryologist who always need to know which leaves to detach and measure for proper determination in the course of a routine identification process).

The variation of cell length and width in different specimens appeared to be greater than expected, at least greater than reported in handbooks and floras (cf. Table 2). Thus the question arises which values should be used for standard morphological descriptions. A possible approach can be obtained from cutting off 10, 5 and 1 percent of cells with maximal and minimal values of their width and length (Fig. 8).

The data were obtained from the Karelian specimen (cf. Figs. 4, 6) which had one of the maximal number of measureed cells, 7326, and represented likely the most optimally developed plant, at least its cells were one of the largest. Cutting off 2% of cells with marginal values of width and length, and even 10% of such cells, provided an interval that looked too broad to be practical, at least for ordinary purposes, like routine identification. It looks that some of the published intervals correspond more or less to the most common cell size (between 50 and 80 percent of cells).

It should be kept in mind, of course, that the graphs in Fig. 8 illustrate variation in only one specimen, and the adding of plants from other regions, as well as involving in examination not fully developed plants will expand the variation intervals with respective cuttings off marginal values.

* * *

Summing up, it seems that the computerized mass measurements of moss laminal cells supply data considerably different from those obtained by an ordinary measurements of 'typical' cells. The computrized measurements open a path to better understanding of variation within single leaf, different leaves of individual plants, as well as within and among populations.

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Appendix. Specimen information

Mnium spinosum

Altai Ignatov #0/1534 (MHA)
Arkhangelsk Ignatov 2.VIII.1988 (MHA)
Caucasus (Karachaevo-Cherkessiya, Arkhyz) Ivanov 2149 (MHA)
Dagestan Ignatov&Ignatova #09617 (MHA)
Karelia Maksimov & Maksimova, 29.V.2002 (MHA ex PTZ)
Poland Ignatov&Ochyra 10.III.1995 (MHA)
Primorsky Ignatov #07-226 (MHA)
Vologda Ignatov&Ignatova 19.VIII.2001 (MHA)

Mnium spinulosum

Altai Ignatov #0/1535 (MHA)
Caucasus (Karachaevo-Cherkessiya, Arkhyz) Ivanov 2253 (MHA)
Primorsky Ignatov #07-549 (MHA)