Arctoa (2013) **22**: *51-60*

AREOANA ANALYSIS OF MOSS LEAF CELL STRUCTURE OF TWO *CYRTOMNIUM* SPECIES (MNIACEAE, BRYOPHYTA)

AREOANA-АНАЛИЗ КЛЕТОЧНОЙ СТРУКТРУРЫ ЛИСТА ДВУХ ВИДОВ МХОВ РОДА *CYRTOMNIUM* (MNIACEAE, BRYOPHYTA)

OLEG V. IVANOV[1] , ALEKSEY M. PYATNITSKIY[2], MICHAEL S. IGNATOV[3] & ELENA V. MASLOVA[4]

ОЛЕГ В. ИВАНОВ[1], АЛЕКСЕЙ М. ПЯТНИЦКИЙ[2], МИХАИЛ С. ИГНАТОВ[3], ЕЛЕНА В. МАСЛОВА[4]

Abstract

Two species of the moss genus *Cyrtomnium* were studied for the parameters of their leaf cells. The computer program AREOANA, specially designed for this kind of studies, allows involving large datasets in the analysis. In this study, it processed 81 leaves with altogether 140 000 cells. Quite a bit different in their basic characteristics (area, length, width, length to width ratio, angle between the cell length and costa, number of cell corners), both species leaves showed considerable and stable differences in area distributions of cells taken separately with 4, 5, 6, 7 and 8 corneres, which allows discrimination by statistical methods with 6% accuracy. A possible relation of useful quantitative parameters with leaf morphogenesis is discussed.

Резюме

Для двух видов мхов рода *Cyrtomnium* проведено исследование количественных характеристик клеток листовой пластинки, распознанных с помощью компьютерной программы AREOANA, позволившей на 81 листе произвести обсчет более 140 000 клеток. При том, что основные характеристики клеток (площадь, длина, ширина, отношение длины к ширине, угол наклона к средней жилке, количество углов) очень похожи у обоих видов, распределения по отдельно взятым 4-х, 5-и, 6-и, 7-и и 8-и угольным клеткам устойчиво различаются, что позволяет разделять эти виды аналитическими методами только по распределениям площадей клеток с 6% вероятностью ошибок. Обсуждается возможная связь используемых статистических параметров с особенностями морфогенеза листа.

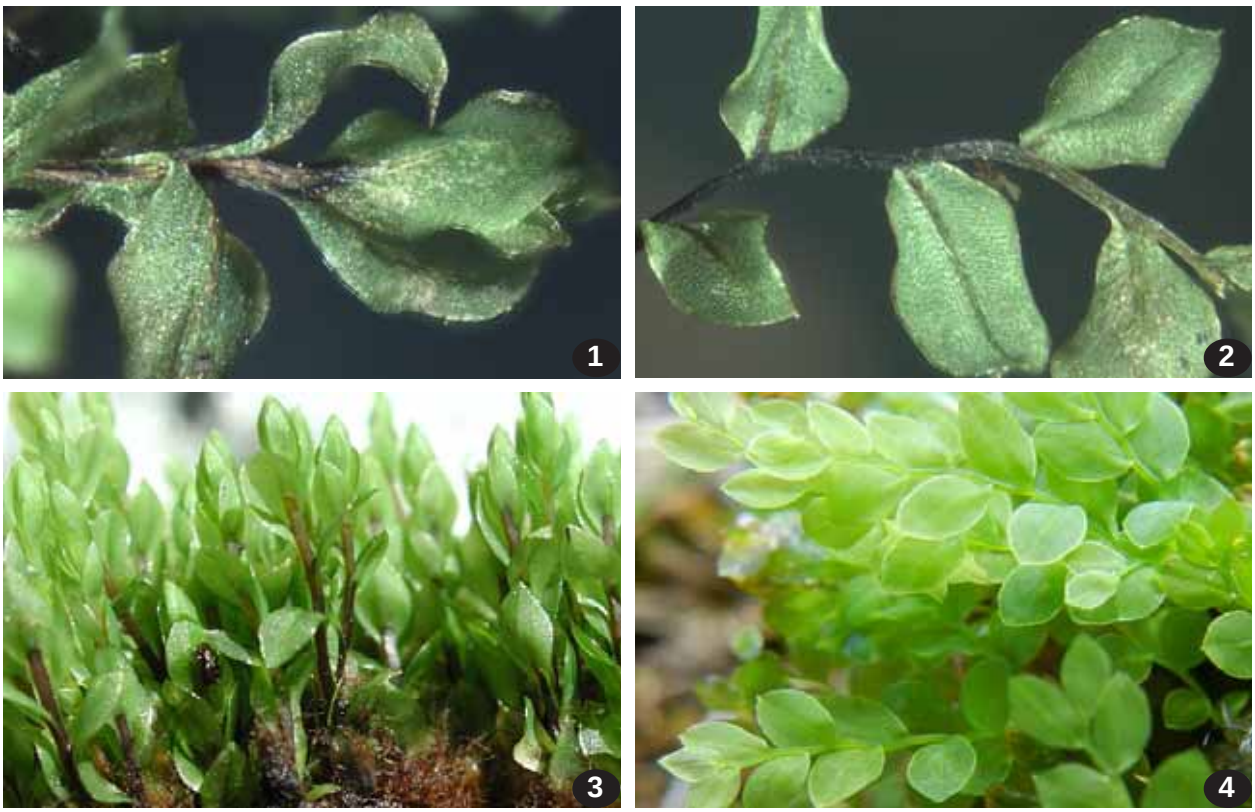
KEYWORDS: cellular structure, leaf morphogenesis, mosses, *Cyrtomnium*, digital image processing, pattern recognition

INTRODUCTION

Leaf cell length and width are important characteristics of moss species and they are widely used in species circumscriptions. The accuracy of data on cell dimentional characters was briefly discussed by Ivanov & Ignatov (2011), on the example of *Mnium spinosum* and *M. spinulosum*. Basing on the measurement of more than 7000 cells with the computer program, we showed only a moderate congruence between the published data and results of digital image analyses. Further comparison of two *Plagiomnium* species, *P. medium* and *P. elatum*, showed a considerable potential of this method also in rectifying the meaning of some characters used in morphological descriptions, *e.g.* cell arrangement in oblique rows (Ivanov & Ignatov, 2012/2013). In the present paper we continue the exploration of the possibilities of the digitized areolation analysis by mathematical methods.

Two species selected for this study are *Cyrtomnium hymenophyllum* (Bruch *et al*.) Holmen and *C. hymenophylloides* (Huebener) T.J. Kop. (Mniaceae). These are the only species known in this small and well-defined genus, widely distributed in Arctic, Subarctic, and relatively cold mountain areas of Holarctic. These two species are usually rather easy to distinguish, as the former has a wide and decurrent leaf base, while the leaves are strongly narrowed to the base in *C. hymenophylloides*, which obviously correlates with the ability of the latter species to turn leaf perpendicularly to light source. The latter is important, as it often grows in shaded environments (Figs. 1-4). Cell shapes, mostly quadrate-ovate in *C. hymenophyllum* versus hexagonal-ovate in *C. Hymeno-*

[1] – P.N. Lebedev' Institue of Physics of Russ. Acad. Sci., Leninsky 53, Moscow 119991 Russia – Россия 119991, Москва, Ленинский проспект, 53, ФИАН; e-mail: ivanov@td.lpi.ru
[2] – Medical Computer Systems (MECOS) Company, Ugreshskaya 2 , Moscow, 115088, Russia – Россия 115088 Москва, Уграшская 2, ЗАО Медицинские компьютерные системы (МЕКОС); e-mall: alpyat@list.ru
[3] – Main Botanical Garden of the Russ. Acad. Sci., Botanicheskaya 4, Moscow, 127276 Russia – Россия 127276, Москва, Ботаническая, 4, Главный ботанический сад РАН; e-mail: misha_ignatov@list.ru
[4] – Belgorod State University, Pobedy square, 85, Belgorod, 308015 Russia – Россия 308015, Белгород, пл. Победы, 85, Белгородский государственный университет; e-mail: e_maslova@list.ru



Figs. 1-4. *Cyrtomnium* species: 1 & 3: *C. hymenophyllum*; 2 & 4: *C. hymenophylloides*; 1&2 : from dry herbarium collections; 3&4 – *in situ* photos, the #3 courtesy of Michael Lüth (www.milueth.de).

phylloides, were underlined by Limpricht (1890-95) and even used in key for identification by Savicz-Lyubitskaya & Smirnova (1970), but our preliminary tests (Table 1) found out that (a) the average number of corners per cell; (b) cell length to width ratio; and (c) ratio of cell area to the area of minimal rectangle enclosing the cell, i.e. the characteristics of similarity to rectangle, – all the three are nearly the same in these two species. Therefore, we suspected that this visual impression about different cell areolation is inappropriately explained, and decided to test it with AREOANA-program (http://www.arctoa.ru/areoana).

Table 1. Morphometric data on two studied *Cyrtomnium* species: means and ranges of variation (in parenthesis) after cutting off 10% of marginal values.

Character	*C. hymenophyllum*	*C. hymenophylloides*
N shoots	7	9
N leaves	38	43
N cells	70366	74006
Cell area, μm^2	737 (147-1509)	793 (174-1608)
Cell length, μm	37.9 (18.8.-64.7)	39.0 (20.4-64.3)
Cell width, μm	25.7 (12.0-39.9)	26.8 (13.0-41.1)
Cell l:w ratio	1.59 (1.05-3.00)	1.55 (1.05-2.86)
Mean number of corners	5.576	5.563
Area/box area[1]	0.731 (0.371–0.893)	0.725 (0.393–0.877)

[1] – Area/box area is the ratio of cell area to area of minimal rectanlge enclosing the cell; it shows how much the cell is similar to rectangle, ranging from 0.5 (triangle) to 1.0 (rectanlge).

MATERIAL AND METHODS

Leaves from MHA herbarium collections were used, representing different populations from Eurasia (cf. Table 3). Leaves were photographed under Carl Zeiss NU2 light microscope, using the Nikon D70 camera (2000 x 3008 pixel). Three frames with polarized filters at 0°, 30° and 60° angles were taken for each image, and their combined image provided a polarized light "staining" of all cell walls, following the algorithm developed before (Ivanov & Ignatov, 2011; 2012/2013). In total, 81 leaves from 16 shoots were studied (Table 3). Small leaves fit one frame, however, many leaves were larger and thus several images of one leaf taken with a certain overlap were assembled by internal tool of AREOANA program (http://arctoa.ru/areoana/) after the cell outline recognition. A number of conflicts in recognitions coming from neighboring frames were corrected manually with an editor of this program. Conflicting situations with incomplete outlining of cells at the leaf edges and along the costa remained (cf. Figs. 6-9); however, their noise did not affect the cell statistics and they were left underedined.

After preliminary tests that had showed very close values for the basic cell parameters of two species (Table 1), a more complex study was conducted, with a separate study of cells with 4, 5, 6, 7 and 8 angles (or sides), as the AREOANA algorithm approximates cells as polygons, with a number of cell vertexes (*i.e.* points where

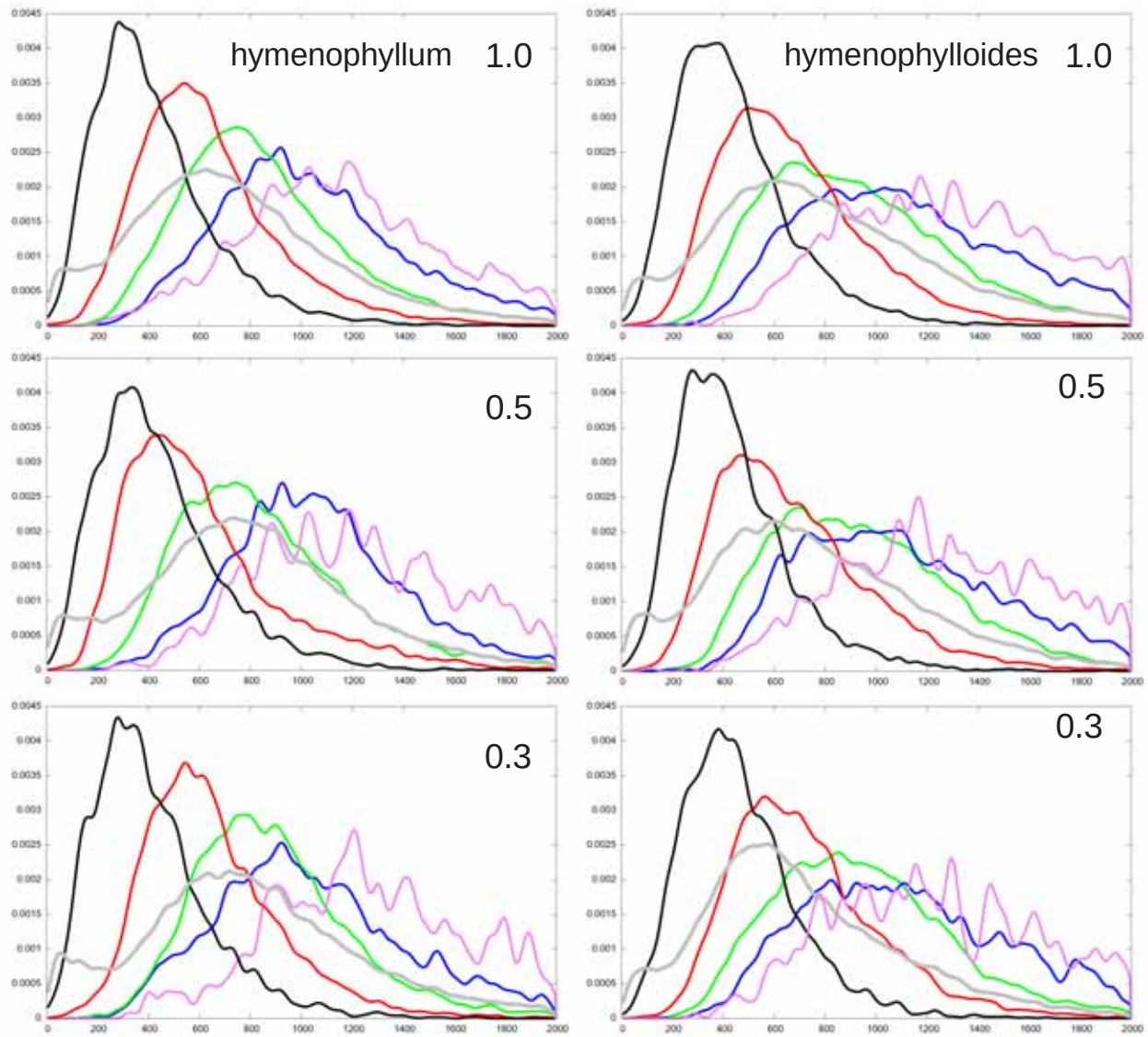


Fig. 5. Distribution of leaf cell number in arbitrary units (Y axis) vs. cell size (X axis), smoothed by Gaussian fuction[1] (cf. page 000) for cells in *Cyrtomnium hymenophyllum* (left) and *C. hymenophylloides* (right) for cell groups: black – 4-angled, red – 5-angled; green – 6-angled; deep blue – 7-angled; pink – 8-angled, grey – all cells. The upper graphs show data for the whole sample (1.0), the middle ones for dataset with half leaves stochastically cut off (0.5), and the lower graph for 30% leaves (0.3). The graphs demonstrate a relative stability: for example 6-angled cells (green) have the peak right from 5-angled cell (red) peak in *C. hymenophyllum*, but in *C. hymenophylloides* green peak is under or almost under the red peak.

three of four cell walls are joining), connected by straight lines. Note that the areas with actively dividing cells have many quadrangular cells, while a 'maximally developed' area is composed of hexagonal cells. This fact comes from Euler theorem saying that when three cells join at the vertex, the average number of corners equals 6, so if a 7-angled cell appears somewhere at least one of neighboring cells must be 5-angled.

The motivation for using this approach appeared from another preliminary test that showed stable difference in distribution curves built separately for cells with 4,5,6,7,8 corners (Fig. 5). Cutting off 50 and even 70% of leaves did not affect these differences, thus the further analysis was conducted using two estimators described below. For both of them we started with the analysis of identified specimens, then found delimiting cri-

Table 2. Portion of cells with respecive number of angles in two *Cyrtomnium* species.

Species	Number of cell corners				
	4	5	6	7	8
C. hymenophyllum	0.11359	0.31304	0.41225	0.12883	0.03229
C. hymenophylloides	0.10643	0.29327	0.45284	0.12351	0.02395

[1] – Gaussian function, where σ=5 mm for cell width and length, and σ=50 mm^2 for cell squares.

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

Table 3 (opposite page). Specimens used for AREOANA analysis and their identifications by computer algorithm. Correct identifications are marked by «+», misidentified leaves are marked by «–». D6 is share of hexagonal cells in a leaf, as7 shows area skewness for 7-angled cells, ex8 is curtosis for 8-angled cells.

teria, and finally checked if these criteria work for any individual leaf.

Estimator #1 used only portions of cells D with N=4,5,6,7,8 corners: after the portions of cells with N corners each were found, then a leaf in question was compared by distances between its individual D and mean D, calculated for the whole dataset of each species.

Estimator #2 used a rather complicated probability model for classification. Its dataset includes: (1) D, portion of cells; (2) ckewnes, $CV=(x-x_{mean})^3/\sigma^3$, where x is cell area, σ - average square deviation; (3) curtosis, $EX=(x-x_{mean})^4/\sigma^4$; (4) A, mean of cell area, μm^2; (5) coefficient of variation $CV=\sigma/A$. For each of five parameters (1 to 5), the data were obtained or calculated separately for cells with 4,5,6,7,8 corners. Two additional columns include the number of cells in leaf and total leaf area (as sum of cell areas). The resultant datamatrix with 27 columns (see supplenmentary material http://arctoa.ru/ru/Archive-ru/22/Cyrtomnium-supplement1.pdf) was analyzed to find the maximally efficient dividing line between the species in 27-dimension space, given earlier found mean values for both species.

The formal explanation of the procedure is as follows:

The probability model for classification fits a logistic distribution using maximum likelihood to the decision values of all binary classifiers, and computes the *a posteriori* class probabilities for the multi-class problem using quadratic optimization. The probabilistic regression model assumes (zero-mean) laplace-distributed errors for the predictions, and estimates the scale parameter using maximum likelihood. The method follows Chang Chih-Chung & Lin Chih-Jen: LIBSVM: a library for Support Vector Machines (http://www.csie.ntu.edu.tw/~cjlin/libsvm), the formulations of models, algorithms, *etc*. are available at http://www.csie.ntu.edu.tw/~cjlin/papers/libsvm.ps.gz. More implementation details and speed benchmarks can be found on: Rong-En Fan, Hsune Chen & Chih-Jen Lin: Working Set Selection Using the Second Order Information for Training SVM (http://www.csie.ntu.edu.tw/~cjlin/papers/quadworkset.pdf).

RESULTS

The variation of cell parameters was found to be broad and strongly overlapping (Table 1). However, distributions of cells with different number of corners reveal a surprisingly stable pattern shown in Fig. 5: hexagonal cells have the peak right from pentagonal cell peak in *C. hymenophyllum*, but in *C. hymenophylloides* the former peak is under or almost under the latter peak. The difference in distribution curves of 5- and 6-angular cells retains after stochasticall cutting off from consideration 50% and even 70% of leaves. Note also that distribution curves for 6-angled cells and for all cells go differently in the right part of the graph: in *C. hymenophylloides,* large 6-anged cells prevail above the relative portion of cells of this size among all leaves. Similarly, stable differences in 7- and 8-angled cells are given in supplementary material in on-line version (http://arctoa.ru/ru/Archive-ru/22/Cyrtomnium-supplement2.pdf).

The accuracy of the last two estimators for 81 leaves is given in Table 3. The simple Estimator #1 made 23 mistakes out of 81 leaves (28.3%). However, the Estimator #2 gave a much more satisfactory error probability (6.2%) assuming that leaves used in the analysis were not specially selected and included smaller, undeveloped and partly damaged ones (Figs. 6-9). Also, all five mistakes were done for one leaf among several others from the same shoot where majority of leaves were correctly recognized, *i.e.* if we judge by majority of correctly recognized leaves, then the algorithm correctly identified all 16 plants.

DISCUSSION

The attempt to find criteria for species separation by cell characters can be considered as successful, taking into account 6% of mistakes for individual leaves, and no mistake for the whole shoot. However, the main achievement seems to be in highlighting an interesting and neglected character that opens up a great potential for further analysis of moss leaf areolation.

The number of corners is a character that is difficult to evaluate by eye under microscope in the course of the ordinary study. On the contrary,, in the digitized areolation images, this is one of the simplest and straightforward characters, not requiring complicated methods of calculation as is needed even for the cell length and width (Ivanov & Ignatov, 2011; 2012/2013).

The stability of this character is amazing as the distinction between the species involves not only fully developed leaves, but also ones of much smaller size. Although the accuracy of the estimator #1 is not great, it is still a fascinating result that more than 70% of leaves were correctly recognized even by such a simple criteria as a portion of cells with a given number of corners in leaf.

The difference between distribution curves shown in Fig. 5 indicates that there are many large hexagonal cells in *C. hymenophylloides* whereas in *C. hymenophyllum,* they are proportionally fewer. This can be interpreted as if the cell divisions in mid-leaf in *C. hymenophylloides* are more regular, whereas 5- and 7-angled cells are more common in juxtacostal area of mid-leaf of *C. hymenophyllum*, and after the late elongation hexagonal cells are not that numerous as in the former species. The present material rather poses this problem for further study than gives a definite answer. However, the stability of this result (Fig. 5) makes it definitely not an artefact.

The new character of distribution of cells with a different number of corners can be perceived to a certain extend through visualization, the examples are given in Figs. 6-9. Red dots of 5-angled cells are obviously concentrated towards leaf margin in *C. hymenophylloides,* being more scattered throughout lamina in *C. hymenophyllum*. This is seen in both smaller (Figs. 6, 8) and

project	name	estimator #1	estimator #2	d6	as7	ex8
1108	hymenophylloides_Ignatov_altaj_02_07_89_1_1_	+	+	0,4752	0,6095	-0,616
1116	hymenophylloides_Ignatov_altaj_02_07_89_1_2_	+	+	0,4423	0,752	-0,4845
1125	hymenophylloides_Ignatov_altaj_02_07_89_1_3_	+	+	0,4752	0,7093	-1,1131
1134	hymenophylloides_Ignatov_altaj_02_07_89_1_4_	-	-	0,4277	0,6502	-0,5079
1142	hymenophylloides_Ignatov_altaj_02_07_89_1_5_	-	+	0,4204	0,6365	-0,8815
1150	hymenophylloides_Ignatov_altaj_02_07_89_1_6_	+	+	0,4208	0,6943	-0,5965
322	hymenophylloides_Ignatov_altaj_03_07_91_1_4_	-	+	0,3796	0,7929	1,6054
327	hymenophylloides_Ignatov_altaj_03_07_91_1_5_	+	+	0,4856	0,636	1,498
331	hymenophylloides_Ignatov_altaj_03_07_91_1_6_	+	+	0,5025	0,5601	-0,0939
338	hymenophylloides_Ignatov_altaj_03_07_91_1_7_	+	+	0,4835	0,768	-0,4645
346	hymenophylloides_Ignatov_altaj_03_07_91_1_8_	+	+	0,4771	0,6787	-0,6985
354	hymenophylloides_Ignatov_altaj_03_07_91_1_9_	+	+	0,4528	0,6818	-0,999
992	hymenophylloides_Ignatov_orulgan_11_3982_1_1_	+	+	0,4488	0,7453	-0,3235
1002	hymenophylloides_Ignatov_orulgan_11_3982_1_2_	+	+	0,4876	0,5414	-0,8316
1012	hymenophylloides_Ignatov_orulgan_11_3982_1_3_	+	+	0,4911	0,5211	-0,9644
1022	hymenophylloides_Ignatov_orulgan_11_3982_1_4_	+	+	0,4936	0,6524	-0,2327
1033	hymenophylloides_Ignatov_orulgan_11_3982_1_5_	+	+	0,4943	0,7141	-0,8089
1039	hymenophylloides_Ignatov_orulgan_11_3982_1_6_	+	+	0,4613	0,6219	-0,7161
1047	hymenophylloides_Ignatov_orulgan_11_3982_1_7_	+	+	0,4386	0,5511	-0,3796
1051	hymenophylloides_Ignatov_orulgan_11_3982_1_8_	-	+	0,3929	0,8263	-0,3784
1158	hymenophylloides_Ignatov_sahalin_06_917_1_1_	-	+	0,3596	0,5146	0,078
1170	hymenophylloides_Ignatov_sahalin_06_917_1_3_	-	-	0,4059	0,5539	-0,1283
1179	hymenophylloides_Ignatov_sahalin_06_917_1_4_	-	+	0,4235	0,5221	-0,6482
1187	hymenophylloides_Ignatov_sahalin_06_917_1_5_	+	+	0,4362	0,4922	-0,693
1201	hymenophylloides_Ignatov_sahalin_06_917_1_7_	-	+	0,4328	0,5753	-0,5475
1054	hymenophylloides_Ignatov_ustmaia_00-94_1_1_	+	+	0,4741	0,6145	-0,8124
1062	hymenophylloides_Ignatov_ustmaia_00-94_1_2_	+	+	0,4885	0,5868	-1,0579
1071	hymenophylloides_Ignatov_ustmaia_00-94_1_3_	+	+	0,4499	0,5615	-0,9088
1080	hymenophylloides_Ignatov_ustmaia_00-94_1_4_	+	+	0,4446	0,7427	-0,6298
1089	hymenophylloides_Ignatov_ustmaia_00-94_1_5_	+	+	0,4329	0,6994	-1,0031
1097	hymenophylloides_Ignatov_ustmaia_00-94_1_6_	+	+	0,4459	0,6634	-0,6474
1105	hymenophylloides_Ignatov_ustmaia_00-94_1_7_	+	+	0,4535	0,5996	-1,1321
629	hymenophylloides_Krasnoiarsk_Lunina_31_08_89_1_1_	+	+	0,5107	0,942	-0,8792
647	hymenophylloides_Krasnoiarsk_Lunina_31_08_89_1_4_	+	+	0,4421	0,7434	-0,2222
655	hymenophylloides_Krasnoiarsk_Lunina_31_08_89_1_5_	+	+	0,457	0,7667	-0,8595
665	hymenophylloides_Krasnoiarsk_Lunina_31_08_89_1_6_	+	+	0,4717	0,7005	-0,7867
673	hymenophylloides_Krasnoiarsk_Lunina_31_08_89_1_7_	+	+	0,4757	0,953	-0,7831
681	hymenophylloides_Krasnoiarsk_Lunina_31_08_89_1_8_	+	+	0,4722	0,7691	-0,2537
688	hymenophylloides_Krasnoiarsk_Lunina_31_08_89_1_9_	-	+	0,4144	0,7775	-2
78	hymenophylloides_Pisarenko_12_09_09_1_4_	-	+	0,4247	0,7042	0,4197
84	hymenophylloides_Pisarenko_12_09_09_1_5_	-	-	0,395	0,4865	-0,1809
90	hymenophylloides_Pisarenko_12_09_09_1_6_	-	+	0,3723	0,499	0,0833
27	hymenophylloides_Pisarenko_12_09_09_1_7_	+	+	0,4571	0,4061	0,7795
1491	hymenophyllum_AAFedosov_08_78_1_6_	+	+	0,3802	0,3437	-1,9715
1497	hymenophyllum_AAFedosov_08_78_1_7_	-	+	0,4654	0,5471	-0,58
1507	hymenophyllum_AAFedosov_08_78_1_8_	-	+	0,4459	0,536	0,2431
1519	hymenophyllum_AAFedosov_08_78_1_9_	-	-	0,4769	0,5466	-0,7763
1376	hymenophyllum_Afonina_29_08_83_1_2_	-	+	0,4648	0,2962	-0,5288
1384	hymenophyllum_Afonina_29_08_83_1_3_	+	+	0,4098	0,6372	-1,0288
1390	hymenophyllum_Afonina_29_08_83_1_4_	-	+	0,4504	0,4904	-0,6537
1400	hymenophyllum_Afonina_29_08_83_1_5_	-	+	0,4404	0,5252	0,4805
1412	hymenophyllum_Afonina_29_08_83_1_6_	-	+	0,4421	0,6441	1,6692
1444	hymenophyllum_Afonina_29_08_83_1_9_	+	+	0,4158	0,5988	0,1289
691	hymenophyllum_Cukotka_Afonina_30_08_74_1_1_	+	+	0,4287	0,6667	0,0808
701	hymenophyllum_Cukotka_Afonina_30_08_74_1_2_	+	+	0,3447	0,8445	0,3768
711	hymenophyllum_Cukotka_Afonina_30_08_74_1_3_	+	+	0,4239	0,5897	-0,3572
721	hymenophyllum_Cukotka_Afonina_30_08_74_1_4_	+	+	0,4193	0,6584	3,0446
727	hymenophyllum_Cukotka_Afonina_30_08_74_1_5_	+	+	0,4033	0,8941	-0,5445
733	hymenophyllum_Cukotka_Afonina_30_08_74_1_6_	+	+	0,4011	0,8317	-0,803
739	hymenophyllum_Cukotka_Afonina_30_08_74_1_7_	+	+	0,4009	0,8708	-0,556
31	hymenophyllum_Fedosov_5_103_1_4_	-	+	0,4451	0,5561	0,0005
21	hymenophyllum_Fedosov_5_103_1_5_	+	+	0,4103	0,6528	-0,0914
23	hymenophyllum_Fedosov_5_103_1_6_	+	+	0,4363	0,7073	1,5724
363	hymenophyllum_Ignatov_11_3575_1_1_	+	+	0,3628	0,7303	2,6035
369	hymenophyllum_Ignatov_11_3575_1_2_	+	+	0,3595	0,626	-0,409
376	hymenophyllum_Ignatov_11_3575_1_3_	+	+	0,3973	0,5408	0,4573
382	hymenophyllum_Ignatov_11_3575_1_4_	+	+	0,3854	0,6167	-0,2016
388	hymenophyllum_Ignatov_11_3575_1_5_	+	+	0,3674	0,7492	0,2113
394	hymenophyllum_Ignatov_11_3575_1_6_	+	+	0,3168	0,9519	0,1006
400	hymenophyllum_Ignatov_11_3575_1_7_	+	+	0,4017	0,6654	0,837
406	hymenophyllum_Ignatov_11_3575_1_8_	+	+	0,3596	0,6129	0,6733
412	hymenophyllum_Ignatov_11_3575_1_9_	+	+	0,4116	0,5268	-0,4788
119	hymenophyllum_Malashkina_08_08_11_1_6_	+	+	0,3906	0,6137	1,8185
953	hymenophyllum_Malashkina_08_08_11_2_1_	+	+	0,3205	1,0952	-0,9536
957	hymenophyllum_Malashkina_08_08_11_2_2_	+	+	0,359	0,4821	6,977
961	hymenophyllum_Malashkina_08_08_11_2_3_	-	-	0,4352	0,7	-0,0647
965	hymenophyllum_Malashkina_08_08_11_2_4_	-	+	0,4394	0,5426	1,199
972	hymenophyllum_Malashkina_08_08_11_2_5_	+	+	0,4098	0,5734	1,2842
979	hymenophyllum_Malashkina_08_08_11_2_6_	+	+	0,4006	0,5553	0,4263
985	hymenophyllum_Malashkina_08_08_11_2_7_	-	+	0,461	0,5265	-0,0745

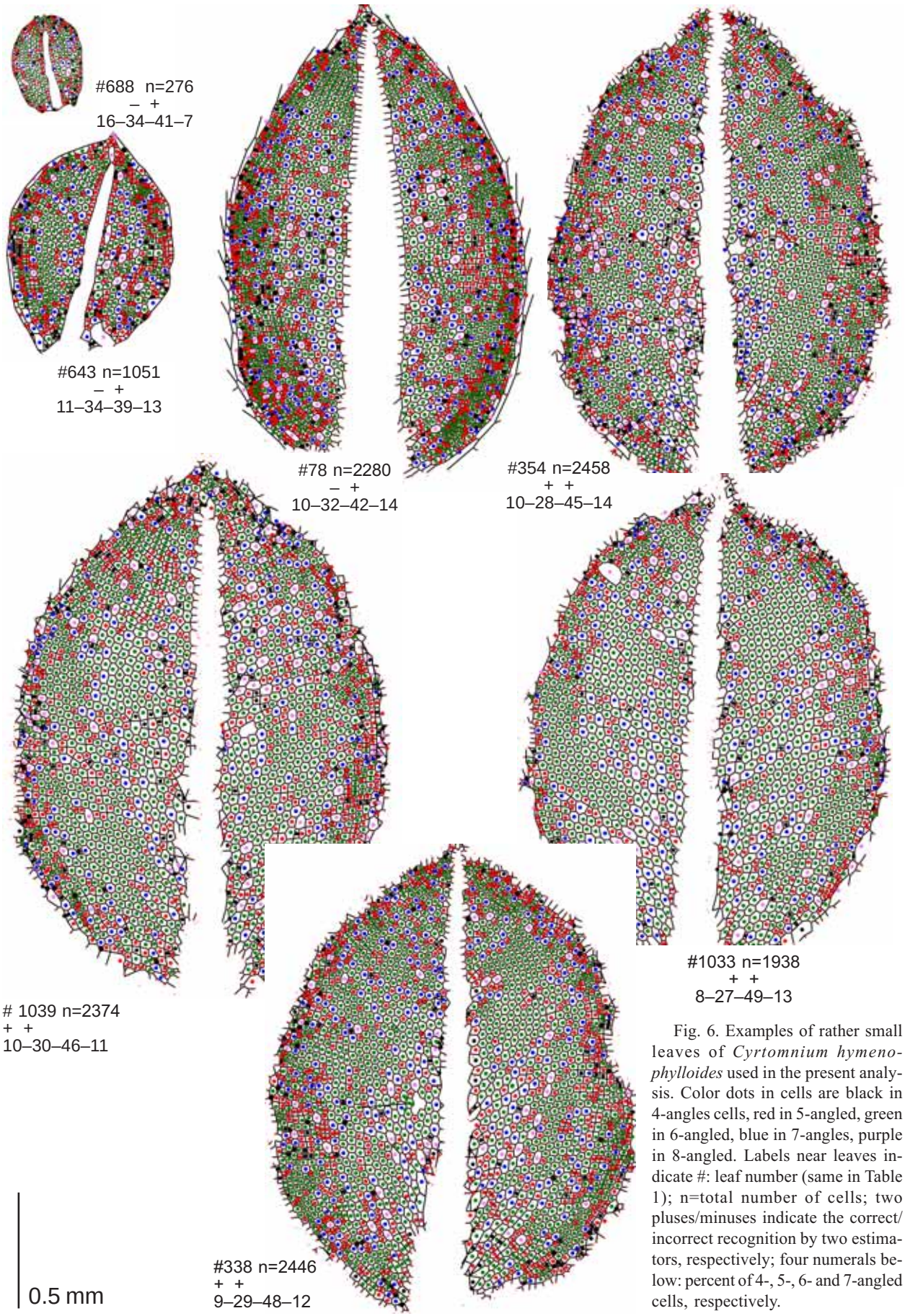


Fig. 6. Examples of rather small leaves of *Cyrtomnium hymenophylloides* used in the present analysis. Color dots in cells are black in 4-angles cells, red in 5-angled, green in 6-angled, blue in 7-angles, purple in 8-angled. Labels near leaves indicate #: leaf number (same in Table 1); n=total number of cells; two pluses/minuses indicate the correct/incorrect recognition by two estimators, respectively; four numerals below: percent of 4-, 5-, 6- and 7-angled cells, respectively.

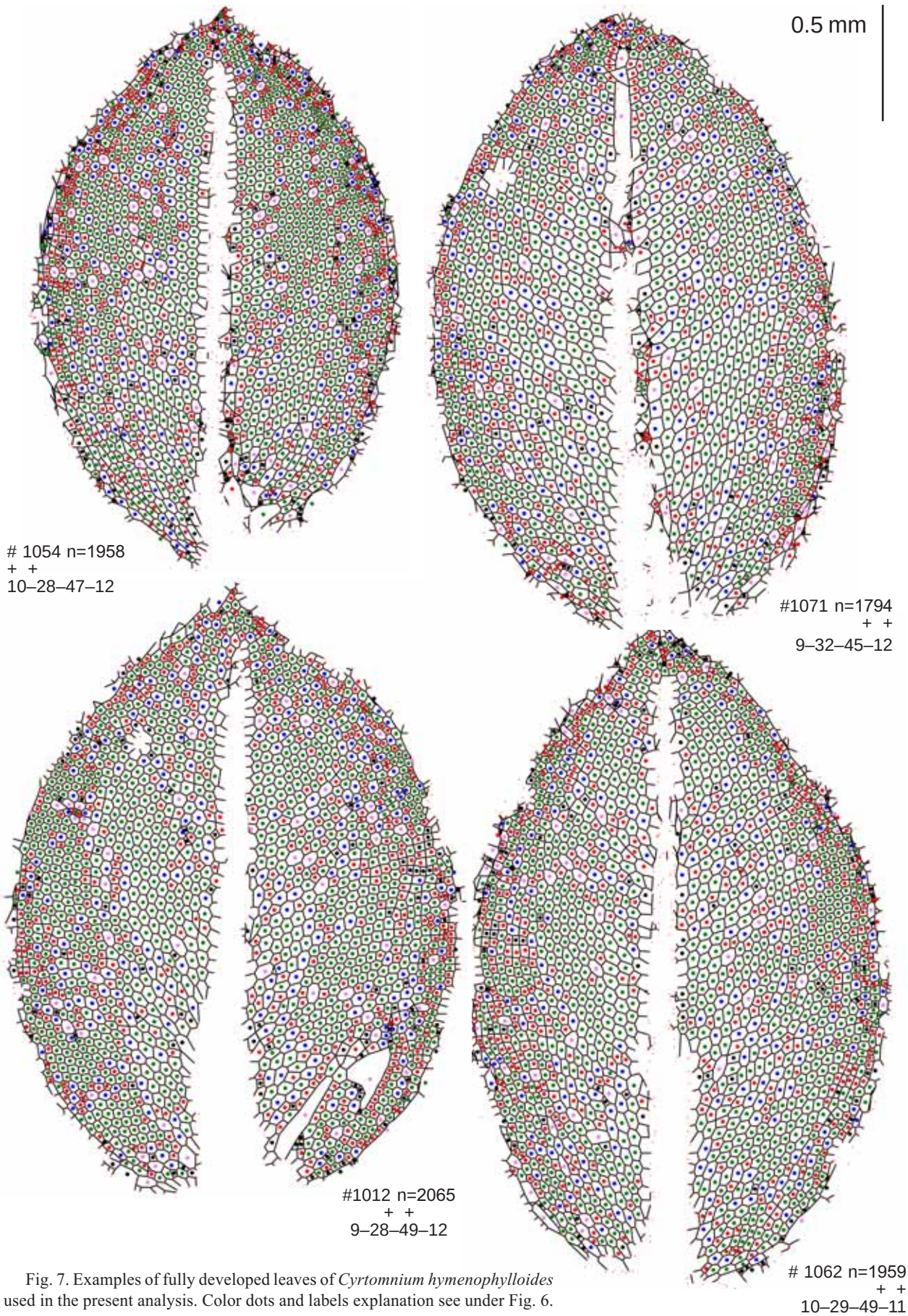


Fig. 7. Examples of fully developed leaves of *Cyrtomnium hymenophylloides* used in the present analysis. Color dots and labels explanation see under Fig. 6.

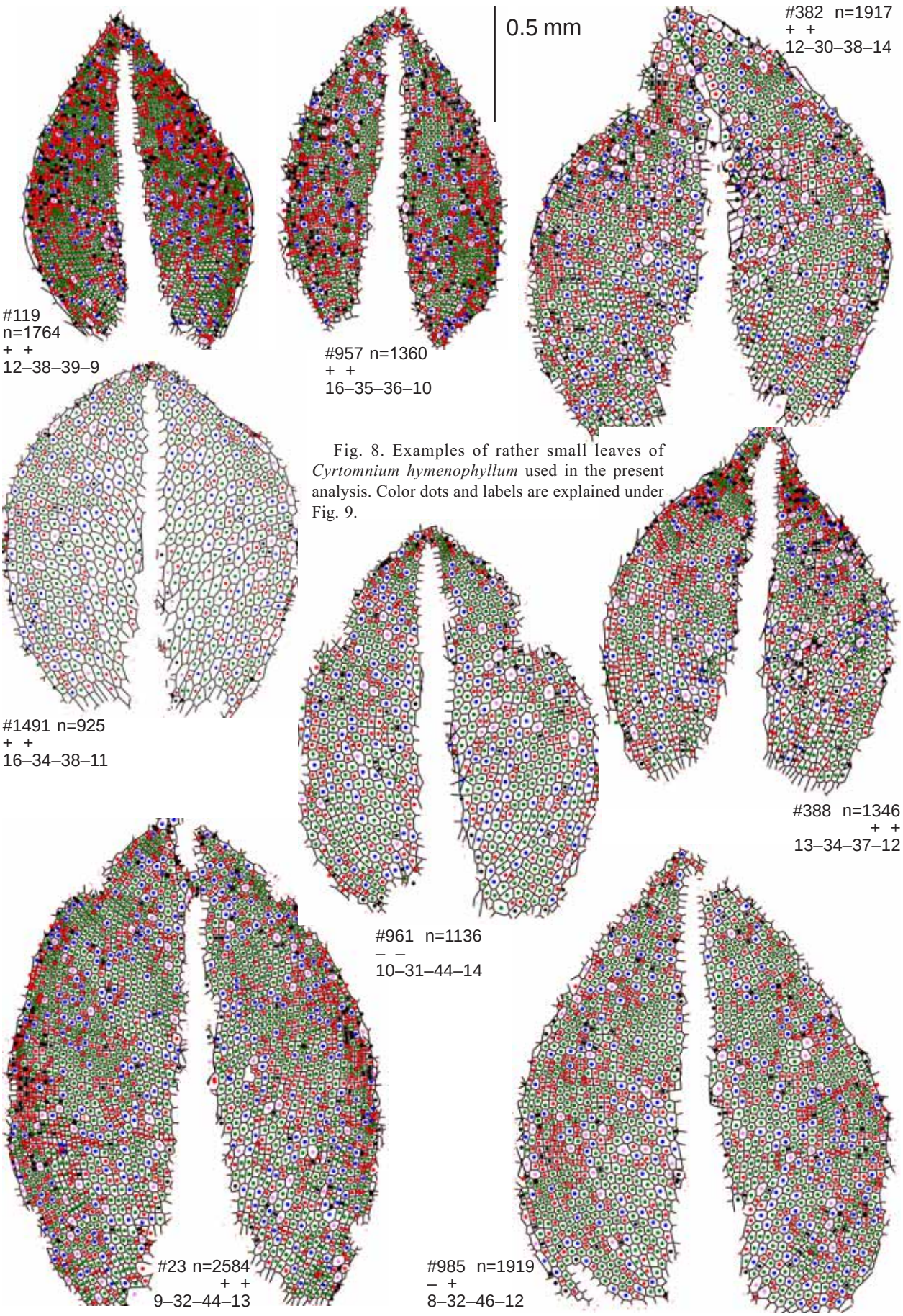


Fig. 8. Examples of rather small leaves of *Cyrtomnium hymenophyllum* used in the present analysis. Color dots and labels are explained under Fig. 9.

1281

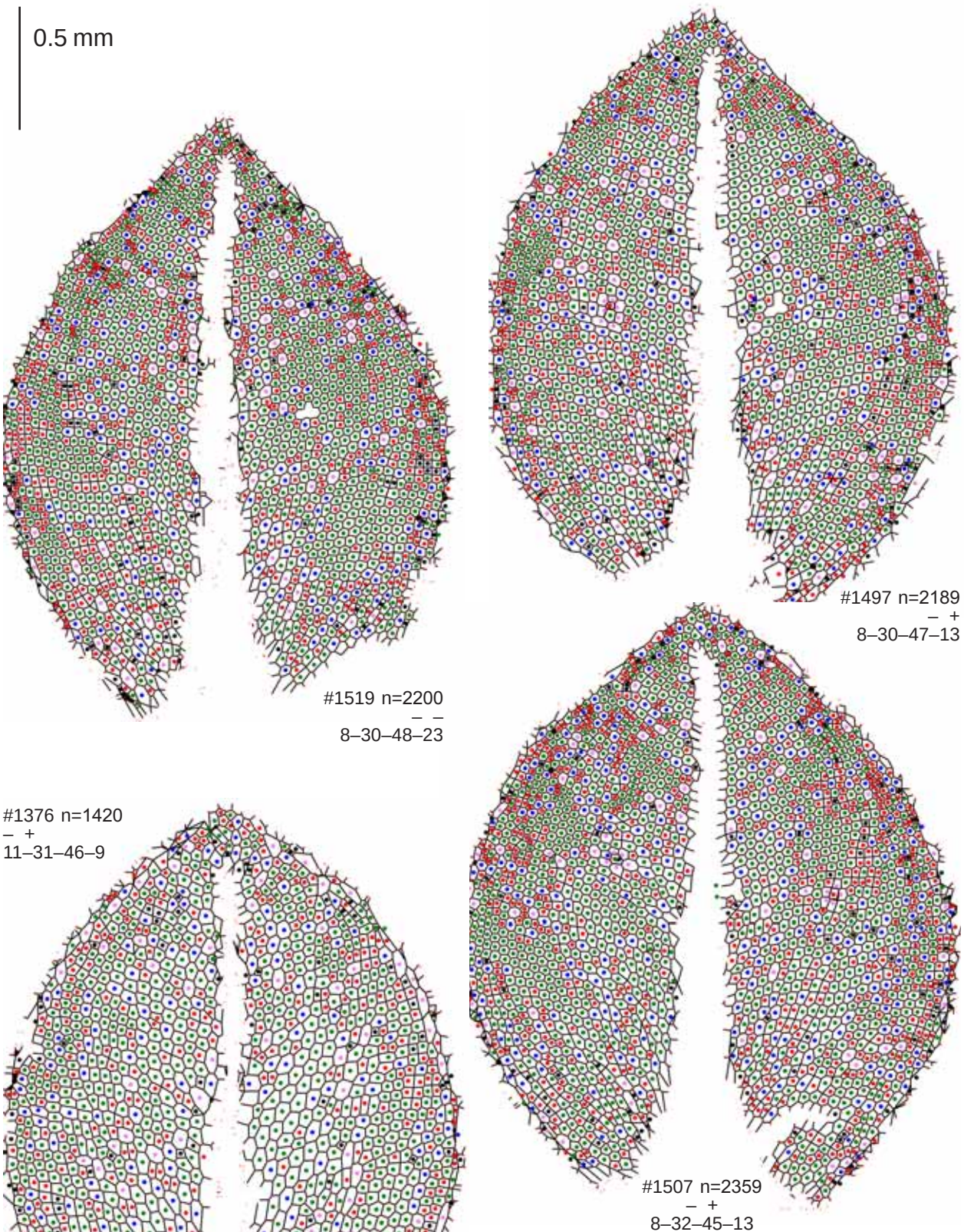


Fig. 9. Examples of fully developed leaves of *Cyrtomnium hymenophyllum* used in the present analysis. Color dots in cells are black in 4-angles cells, red in 5-angled, green in 6-angled, blue in 7-angles, purple in 8-angled. Labels near leaves indicate #: leaf number (same in Table 1); n=total number of cells; two pluses/minuses indicate the correct/incorrect recognition by two estimators, respectively; four numerals below: percent of 4-, 5-, 6- and 7-angles cells, respectively.

larger (Figs. 7, 9) leaves. It looks that being more firmly restricted by stronger border [2-3(-5)-rowed in *C. hymenophylloides vs.* 1(-2)-rowed in *C. hymenophyllum*] submarginal cells do not achieve their full development into hexagons, getting stuck in their differentiation at 5-angle stage. At the same time, the difference in size between 5- and 6-angled cells in *C. hymenophylloides* is smaller than in *C. hymenophyllum,* where this parameter is well pronounced (Fig. 5).

Leaves of *C. hymenophyllum* are very variable, small-sized and with small cells in the lower part of plants (Fig. 8: #119, #957), while in the upper part, they are small but with large cells (Fig. 8: #1491). Looking considerably different from the fully developed large leaves, these small leaves appear to be well recognizable by both estimators. Both types of leaves demonstrate well the relatively even distribution of pentagonal cells throughout leaf.

ACKNOWLEDGENENTS

We are grateful for help in image processing to M.A. Kolesnikova, discussions on the method of analysis to V.A. Nechitailo and B.S. Sokilinsky, M. Lüth for providing us his in situ photographs, A.B. Ivanova for English correction. The work was partly supported by RFBR 13-04-01592 and RFBR 11-02-00615.

LITERATURE CITED

IVANOV, O.V. & M.S. IGNATOV 2011. On the leaf cell measurements in mosses. – *Arctoa* **20**: *87-98.*

[ИВАНОВ, О.В., М.С. ИГНАТОВ 2012. Двухмерное цифровое представление клеточной сети растений с помощью оптической поляризационной микроскопии. – *Цитология* **54**(11): *862-869.*] / **English translation**: IVANOV, O.V. & M.S. IGNATOV 2013. 2D Digitization of plant cell areolation by polarized light microscopy. – *Cell and Tissue Biology* **7**(1): *103-112.*

LIMPRICHT, K.G. 1890–1895. Die Laubmoose Deutschlands, Oesterreichs und der Schweitz. II. Abtheilung: Bryineae (Stegocarpae [Acrocarpae, Pleurocarpae excl. Hypnaceae]). – *In Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweitz. Verlag von Eduard Kummer Leipzig. Ed. 2. iv, 853 pp.*

[SAVICZ-LYUBITSKAYA, L.I. & Z.N. SMIRNOVA] САВИЧ-ЛЮБИЦКАЯ Л.И., З.Н. СМИРНОВА 1970. Определитель листостебельных мхов СССР. Верхоплодные мхи. – [Handbook of mosses of USSR. The acrocarpous mosses] *Л., Наука* [*Leningrad, Nauka*]. *822 pp.*