MOSS LEAF MORPHOGENESIS AND ITS CORRELATION WITH THE LEAF SHAPE IN FUNARIACEAE (BRYOPHYTA)

МОРФОГЕНЕЗ ЛИСТА МХОВ И ЕГО СВЯЗЬ С ФОРМОЙ ЛИСТА У FUNARIACEAE (BRYOPHYTA)

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Abstract

A method of cell coding is suggested, which allows transforming the sequence of reconstructed cell divisions in moss leaves into a datamatrix ready for statistical analysis. The comparison is done for *Physcomitrium pyriforme*, *P. sphaericum* and *Physcomitrella patens*, showing a contrasing difference of *P. sphaericum*, but revealing certain differences between *Physcomitrium pyriforme* and *Physcomitrella* as well. Assuming that the cell divisions perform alternation of longitudinal and transverse divisions, *P. sphaericum* is characterised by minimal omissions of divisions. In *Physcomitrium pyriforme* divisions in costal halves of leaf are more numerous than in marginal halves, whereas in *Physcomitrella* the number of transverse divisions in these parts of leaf is about equal. The correlation between the number of missed cell divisions with shape of the distal leaf part is discussed.

Резюме

Приводится описание формализованного метода записи последовательности клеточных делений при реконструкции развития листовой пластинки мха с точностью до отдельной клетки и представления ее в цифровой матрице для дальнейшей математической обработки. Сравнение трех модельных видов, *Physcomitrium pyriforme*, *P. sphaericum* и *Physcomitrella patens,* показало резкие отличия *P. sphaericum* от двух других видов. Если считать правилом чередование продольных и поперечных делений, то у этого вида имеется наименьшее число пропусков делений. Отличия между *Physcomitrium pyriforme* and *Physcomitrella* более сложные и выявляются только при сравнительном анализе количества делений в областях листа ближе к краю и ближе к жилке: поперечных делений у первого вида ближе к краю меньше, чем ближе к жилке, тогда как у второго их число равно. Обсуждается также корреляция числа пропущенных делений со степенью заостренности листьев.

KEYWORDS: bryophyte, leaf, cell division, *Physcomitrium, Physcomitrella*

INTRODUCTION

The leaf morphogenes in general was characterized in XIX century (Lorentz, 1864; Leitgeb, 1874), and later its rather detailed description has been published by Pottier (1925) and Frey (1970, 1972). According to this, the moss leaf is building from a several pairs of of descenders of the leaf apical cell. First cells appeared from the apical cell form proximal part of leaf, while later divisions give cells composing upper sectors through the number of successive divisions.

The understanding of the principal order of cell divisions and identification of structural homologies, however, did not serve to subsequent development of methods allowing comparison of any two or more leaves by difference in their cell division order. In the present paper we suggest one of the possible approches to this. In particular, we present the method of cell division coding for subsequent computer treatment. An example of subsequent analysis of data coded in this way is provided.

The models of the cell division order were developed moslty for roots of Angiosperms (Bell & Tomlinson, 1980; Barlow *et al.*, 1982, 1991; Barlow & Lück, 2004; Lück *et al.*, 1997). The ideas of denoting divisions were fruitful, but difficult to apply to divisions in moss leaves and not easy transform to format suitable for computer analysis.

The main idea of the present coding is based on that of Corona (1978, 1987), whose coding principles include several *a priori* set rules and format of data suitable for subsequent numerical treatment. The coded division is receiving a unique number, identifying also its position within the cell net massif of given leaf, and appearing homologous with the same number in any other leaf.

THE CELL DIVISION CODING

The principle mechanics of sector formation from the single apical cell is illustrated in Fig. 1. It is somewhat simplified by ignorance the (sub)bilateral simmetry of the leaf and its attachmet to stem, as well as costa. In addition, all the divisions are considered as either longitudinal or transversal, and the first division in the sector is always longitudinal (*e.g.* pointed towards apical cell,
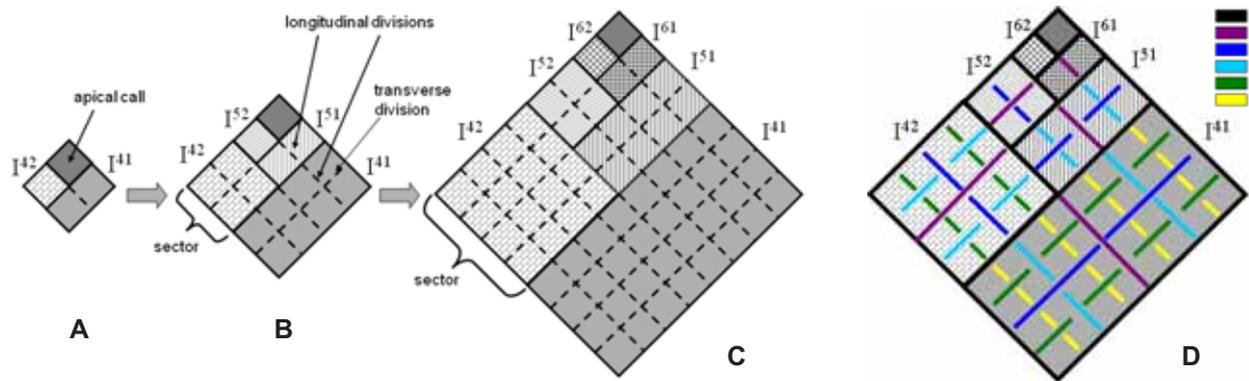
Fig.1. Principle scheme of sector formation in moss leaves. Abbreviations I^{41}, I^{42}, I^{51}, I^{52}, I^{61}, I^{62} are according to Frey (1970).

as explained in Fig. 1, where all divisions are performed as oblique). After these assumptions, the 'ideal scheme' allows definition of criteria for unequivocal retrospective reconstruction of the cell division order. Some restrictions related to possibly too heavy assumption will be discussed below.

The sequence of cell divisions, as it is appears from the 'ideal scheme', is rather straightforward, as in each sector divisions are following the exponential progression: 2, 4, 8, 16, 32, 64, *etc.*, *e.g.* 2 in power 1, 2, 3, 4, 5, 6, *etc*. These power numbers can be visualized easily in a sequence of 'rainbow colors' as shown in Fig. 1 (and cf. subsequent Figs. 3-4). Thus the color applied in this way unambiguously denotes the order of divisions.

Considering the 'ideal scheme', the number of leaves of *Physcomitrium pyriforme, P. sphaericum* and *Physcomitrella patens* were 'painted' for their upper six sectors, as upper parts of their leaves have almost no problematic places difficult for understanding in a way similar to 'ideal scheme' (cf. Figs. 3-4). In case if an alternative variants of reconstruction were possible for the same area, the acceptance of one of them based on the minimal differences from the 'ideal scheme'.

These leaves served for adjusting numerical coding procedure to the moss leaves, which was finally formulated as follow.

1. Each division is a combination of four numbers. Three first numbers characterize the division position, while the fourth number indicates if it really performed or not, and in the former case if it was longitudinal or transversal.

2. **The first number** is the number of sector, counting from the apical cell. The numeration is consecutive, *i.e.* the sectors I^{62}, I^{61}, I^{52}, I^{51} in Fig. 1C will be 1, 2, 3 and 4, correspondingly. The apical cell itself is not counted. In our examples first number was 1 to 6, while in more complete amalysis of *Physcomitrium* it can be up to 10, and in other mosses up to 14(-20).

3. **The second number** is the order of cell divisions inside a given sector, *i.e.* 'the color of line'. The black line in Figs. 1D and 3-4, which subdivides leaf into sectors is not considered (or considered as '0'), but not in-

cluded in datamatrix. In the present analysis the second number was 1 to 8 (*i.e.* some sectors may include $2^8 = 256$ cells, though in fact the real number was much smaller, because some divisions were omitted, cf. Fig. 5).

3. **The third number** indicates the position of the division in relation to previous divisions. It requires more attention, as its finding is not that straightforward in comparison with other numbers. It cannot be found before all is known about the order of previous divisions in the given sector. As soon as the latter is known the vacant places for division of this order are receiving their third number as follow (see Fig. 2):

– if previous division was longitudinal, *e.g.* 'violet' in Fig. 2A, the next 'deep-blue' divisions are numbered in the order from lateral (1) to axial (2);

– if previous division was transverse, *e.g.* 'deep-blue in Fig. 2B, the numbering is going from the distal to proximal and in the following order of the subsequent 'light-blue' divisions will be: 1: distal lateral; 2: proximal lateral; 3: distal axial; 4 proximal axial.

– the next 'green' level requires already 8 numbers, (Fig. 2C) reardless how many divisions really happened.

– in case if some divisions are absent, their numbers are still applying (but in this case the fourth number will be 0, showing that division potentally possible in this position was not performed).

– in case if division happened in 'wrong' direction, *i.e.* not exhibiting a regular alternation 'longitudinal–transvere–longitudinal–transverse and so on', numbering retain the same priority: from distal to proximal, from lateral to axial (Fig. 2C).

Potentially the third number may be up to 256 or even more in large leaves, although in our case it does not exceed 32.

4. **The fourth number** indicates if division is absent (0) or longitudinal (1) or transverse (2). It can have only one of these three states.

The example in Fig. 3 illustrates how 22 divisions in a small leaf of *Physcomitrium pyriforme* (Fig. 3A) can be coded and then from its codes (*i.e.* the 22 combinations of four numbers in each) be perfomed in a schematic way (Fig. 3B). The translation of numbers into

Fig. 2. Explanation of the third number principle (see text for explanation). Costa is on the left.
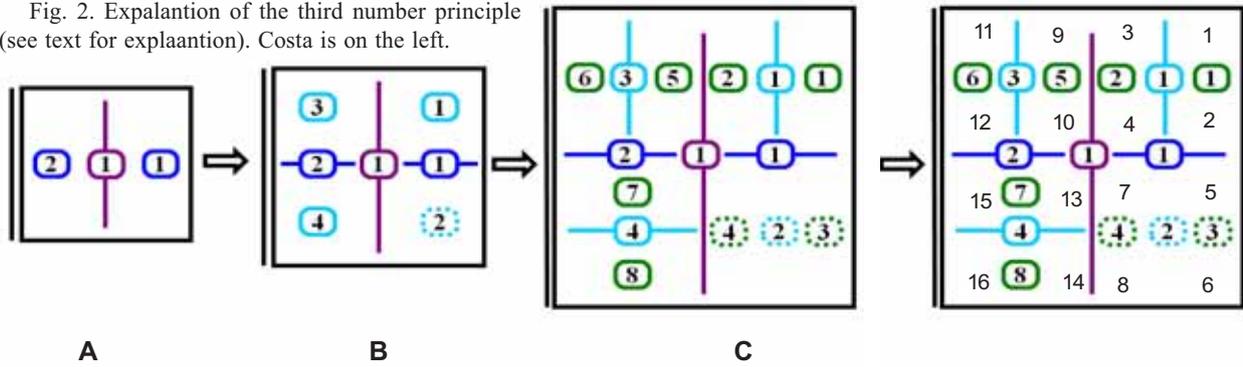
such a scheme is unambigous, at least in room of the present assumptions, including among others only two possible positions of division: longitudinal or transverse.

After this coding is done, the information about cell divisions in the leaf can be performed in a datamatrix format where the column names are first three numbers of all cell divisions, while data in a line for each specimen contian the fourth number (Fig. 3C).

<center>COMPARISON OF LEAVES OF THREE SPECIES OF FUNARICAEAE BY CELL DIVISION SEQUENCE.</center>

In order to check if this way of coding may be used for leaf comparison, the three species were chosen: *Physcomitrella patens* (Hedw.) Bruch et al*., Physcomitrium pyriforme* (Hedw.) Hampe and *P. sphaericum* (C.F.Ludw. ex Schkuhr) Brid. The choise depended on the rather simple structure of these leaves, *i.e.* a relatively short costa vanishing well below apex, large and therefore not so numerous cells, and also absence of distinct border. Both herbarium and fresh material (from nature and, for

Physcomitrella, also from laboratory culture) was used.

Leaves were mounted on slides in Moveol medium and then photographed with Nikon D70 attached to light microscope, the 2000x3008 pixel, and after that cell walls were marked in the graphic editor.

Divisions were reconstructed in 6 or 5 upper sectors (given in two numbers) for 140 leaves (83 and 57) of *Physcomitrella patens*, 88 (51 and 37) of *Physcomitrium pyriforme*, and 35 (25 and 10) of *Physcomitrium sphaericum*. Some examples are shown in Fig. 4.

According to the 'ideal scheme', the divisions change the direction, so the next one is performed at 90° with the previous one. However, the second ('deep-blue') division in most 5th and 6th sectors is longitudinal, as the acceptance of any transversal division as a second one leads to much more deviations from the 'ideal scheme' (Fig.1). So the second division in these sectors is accepted as longitudinal for *Physcomitrella patens* in 92% of cases, for *Physcomitrium pyriforme* in 85%, while in *P.*
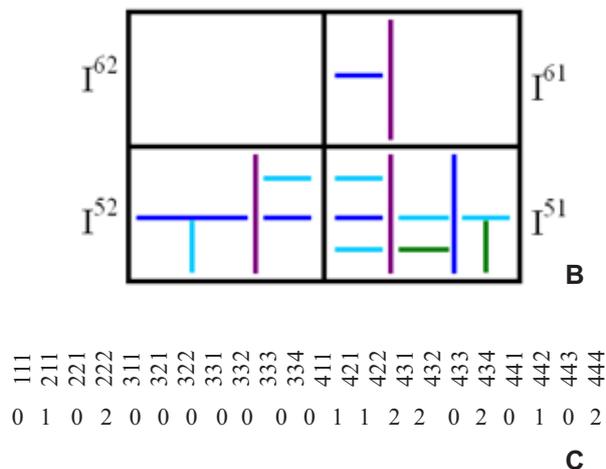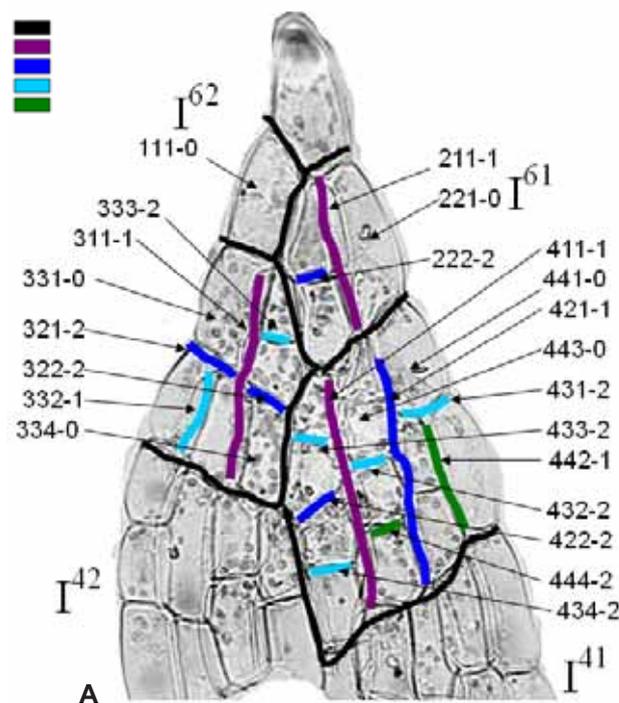

111	211	221	222	311	321	322	331	332	333	334	411	421	422	431	432	433	434	441	442	443	444
0	1	0	2	0	0	0	0	0	0	0	1	1	2	2	0	2	0	1	0	2

C

Fig. 3. Example of cell division coding. A: small leaf of *Physcomitrium pyriforme,* showing cell divisions coded by combinations of four numbers; B – scheme of these division, built from the corresponging datamatrix, showin in C. I^{51}, I^{52}, I^{61}, I^{62} – sectors, denoted according to Frey (1970).
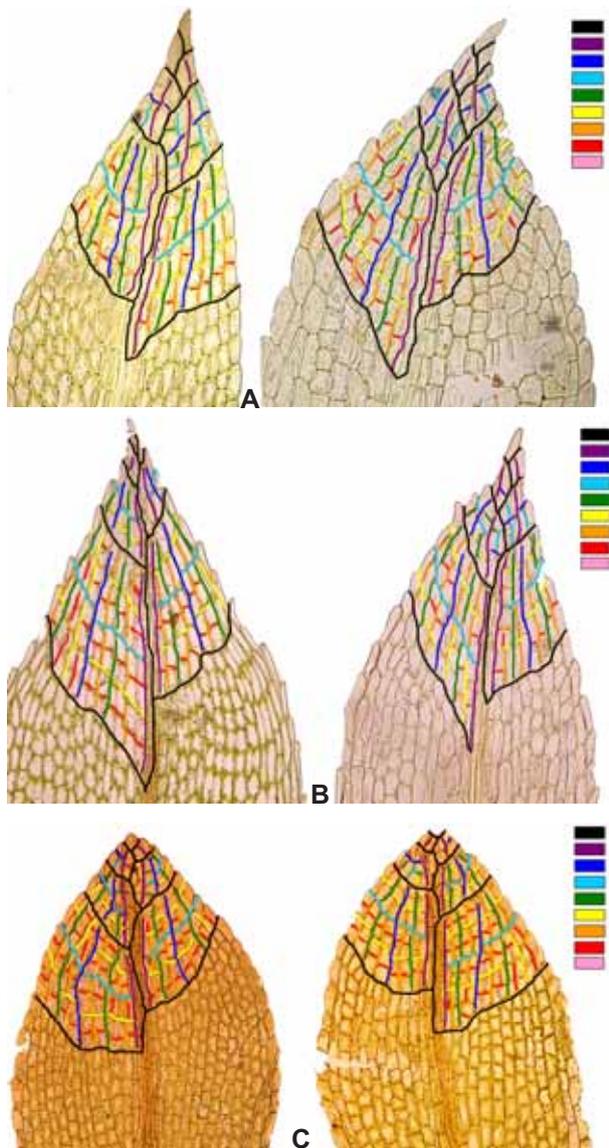
Fig. 4. Retrospective reconstruction of cell divisions in six upper sectors in *Physcomitrium pyriforme* (A)*, Physcomitrella patens* (B) and *Physcomitrium sphaericum* (C)*.*

Table 1. Percent of leaves with the deviations from 'ideal scheme' in 5 & 6th sectors in *Physcomitrium pyriforme* and *Physcomitrella patens* in relation to leaf width.

Number of cells from costa to margin	*Physcomitrium pyriforme*	*Physcomitrella patens*
5–8	60%	75%
9–12	90%	90%
13–16	88%	100%
17–24	82%	100%

In 4th sector the second, 'deep-blue' division is longitudinal in *Physcomitrium pyriforme* in 80% of cases, in *Physcomitrella patens* in 75%, in *Physcomitrium sphaericum* in 100%.

In 3d sector the second division is already mostly transverse in *Physcomitrium pyriforme* and *Physcomitrella patens,* but not in *Physcomitrium sphaericum*. The percent of leaves deviated from 'ideal scheme' is much smaller: in *Physcomitrium pyriforme* – 18%, in *Physcomitrella patens* – 30%, in *Physcomitrium sphaericum* – 80%.

In the first and second sectors the 'deep-blue' division is not always performed and have no obvious rules.

THE RELATION BETWEEN CELL DIVISION ORDER AND LEAF SHAPE

A comparison of a number of distributions of various coded parameters of three studied species shows a quite distinct position of *Physcomitrium sphaericum*, while *Physcomitrium pyriforme* and *Physcomitrella patens* are rather similar to each other. The most obvious differences between the latter species were found when the laminal cells were subdivided into two parts: closer to costa (called "costal halves of leaves" and closer to margin ("marginal halves of leaves"). The demarcation line between them was the oldest longitudinal division in the corresponding sectors.

The differences in a number of divisions between species are shown in Fig. 5.
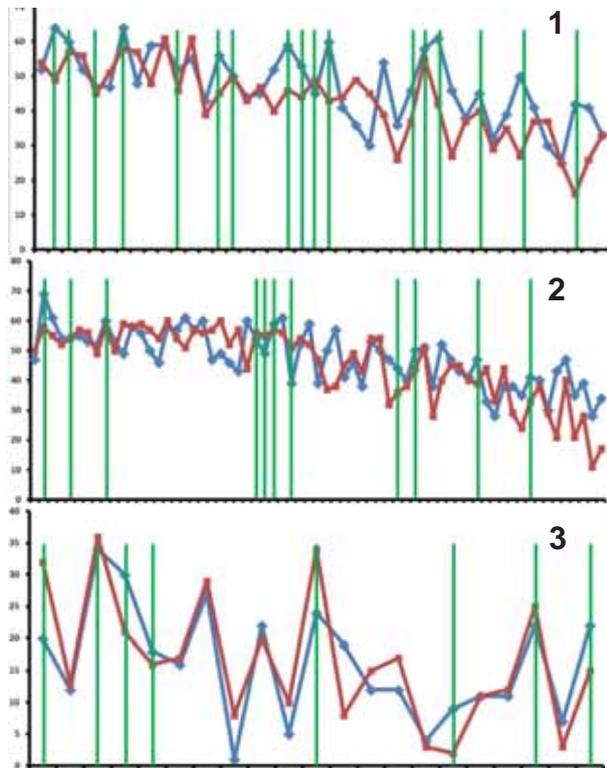
In *Physcomitrium pyriforme* the number of longitudinal divisions in the costal part (red kinked curve) is rather equal in average to that in marginal part (monotonous blue curve), as seen in Fig. 5-1. Contrary to this, transverse divisions in costal part (red curve) are more numerous in comparison with marginal part (blue curve) (Fig. 5-3).

In *Physcomitrella patens* longitudinal (Fig. 5-5) and transverse (Fig. 5-7) graphs are rather similar, both of them comprising a relatively equal number of divisions in costal and marginal parts of leaf (in a way similar to

sphaericum in 100% of the achived reconstructions. The deviations (*i.e*. if division direction changes from longitudinal to transvere or opposite) occur in most leaves, excepting only very small ones from the lower part of stem (Table 1). The latter case obviously relates to the absence of costa in such leaves and to the width of leaf base equal to stem width, thus not restricting the growth from sides. The larger leaves must have additional longitudinal divisions to become ovate or broadly ovate and achieve the leaf shape a common in Funariaceae.

Fig. 5 (opposite page). The differences in number of the laminal cell divisions in *Physcomitrium pyriforme* (1-4), *Physcomitrella patens* (5-8) and *Physcomitrium sphaericum* (9-12). Pairs 1 & 3, 5 & 7 and 9 & 11 show comparative number of divisions in marginal halves of leaf [blue line] and costal halves of leaf [red line]. Figs. 1, 5, 9 show longitudinal divisions, 3, 7, 11 – transverse divisions. Leaves are arranged along axis X in order of increasing number of divisions in marginal halves of leaf.

Graphs in the right column are based on the same data as those in the left one, correspondingly, but leaves are arranged along the axis X by the leaf width (in number of cells). Green vertical lines show more acute leaves (see text for explanation).
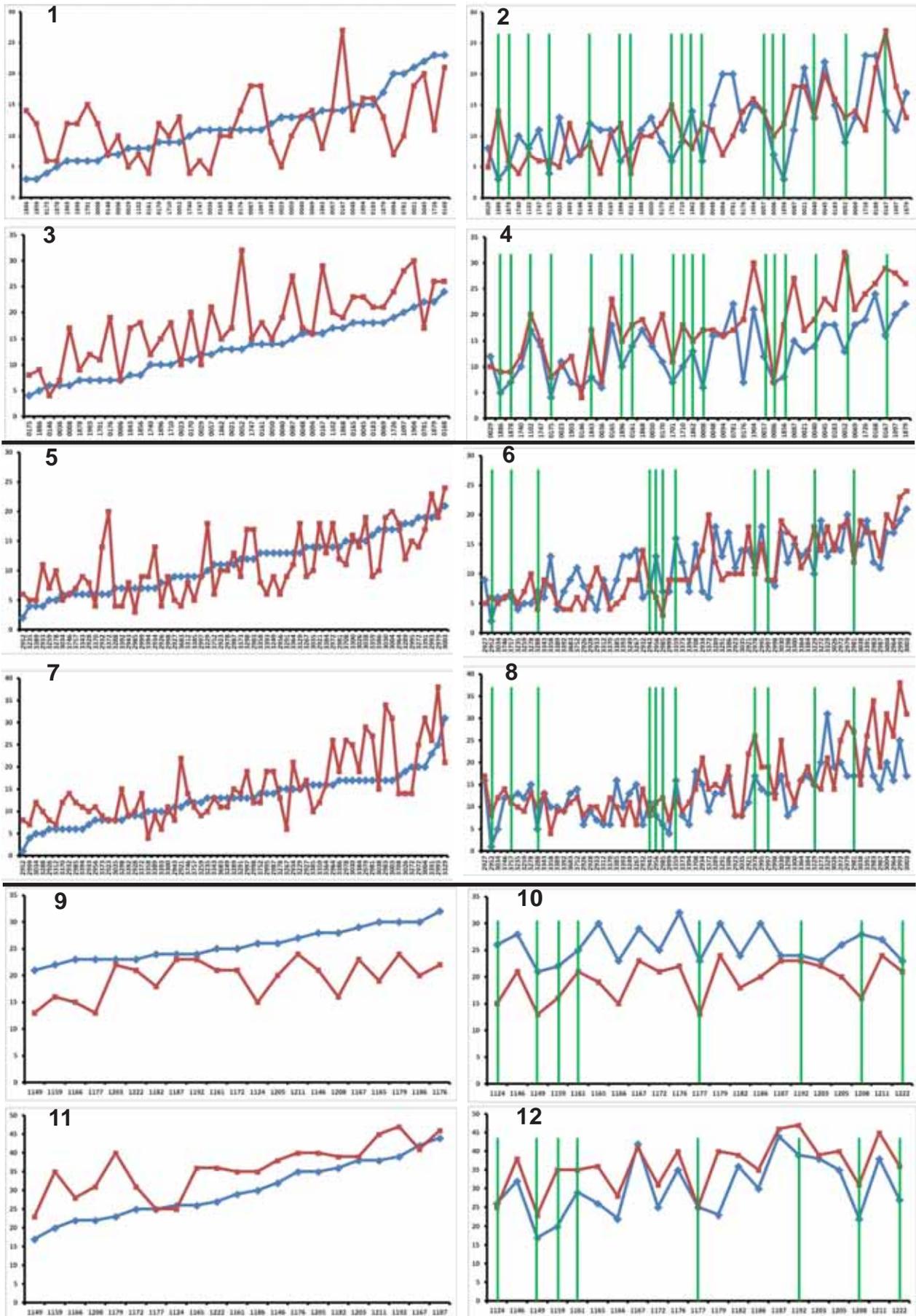
longitudinal divisions in *Physcomitrium pyriforme*).

The pattern of *Physcomitrium sphaericum* is distinct from both these species: the number of longitudinal divisions in the costal part is lower than in marginal part (Fig. 5-9), while the corresponding number for transverse divisions is higher (Fig. 5-11), being more similar in this respect to *Physcomitrium pyriforme* (Fig. 5-1).

In general, in all three species transverse divisions of cells prevail in costal part of lamina, although in *Physcomitrella* this is not that distinct as in two other species.

The comparison of cell divisions with shape of the distal leaf parts are performed in Figs. 5 and 6, where more acute leaves are marked by green lines. First of all, leaves in each species were sorted into more acute and less acute as follow: (1) in *Physcomitrium pyriforme* leaves were sorted among acuminate vs. acute; 2) in *Physcomitrella*: between leaves tapered to the apex at <40° vs. >40° angle; (3) in *Physcomitrium sphaericum*: between leaves tapered to the apex at <90° vs. >90° angle. Although rather few examples do not allow statistical suport, the following tendency is apparent: in *Physcomitrium pyriforme* acuminate character state is associated with leaves where missing divisions are more numerous in marginal (m) halves of leaf than in costal (c) ones: m>c=13, m=c=2, m<c=1, while in other species no apparent difference was found.

It is also quite expected that the number of missing divisions is declining in broader leaves. This tendency is apparent in all species, but the most distinct it is in *Physcomitrella* (Fig.6-2). Note also that in broader leaves the missing divisions in coastal halves are more numerous than in costal: red line in Figs 6-1 and 6-2 towards the right end is below the blue line.

Fig. 6 (opposite page). The number of missed divisions, both longitudinal and transversal, in *Physcomitrium pyriforme* (1), *Physcmitrella patens* (2) and *Physcomitrium sphaericum* (3) in marginal halves of leaves [blue line] and costal halves of leaves [red line]. Leaves are arranged along axe X in order of leaf width (in number of cells). Green vertical lines show more acute leaves (see text for explanation).

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