Arctoa (2015) **24**: *431-451*
doi: 10.15298/arctoa.24.35

ON THE LEAF DEVELOPMENT IN *OEDIPODIUM* (OEDIPODIALES, BRYOPHYTA)

О РАЗВИТИИ ЛИСТА *OEDIPODIUM* (OEDIPODIALES, BRYOPHYTA)

MICHAEL S. IGNATOV[1], ULYANA N. SPIRINA [1, 2], ELENA V. MASLOVA[3], OLEG V. IVANOV[4] & ELENA A. IGNATOVA[4]

МИХАИЛ С. ИГНАТОВ[1], УЛЬЯНА Н. СПИРИНА[1, 2], ЕЛЕНА В. МАСЛОВА[3], ОЛЕГ В. ИВАНОВ[4], ЕЛЕНА А. ИГНАТОВА[5]

Abstract

Leaf development in *Oedipodium griffithianum* was studied based on herbarium and living material, using microscopic observations of plants at different stages of development and series of sections. It turned out that the apical cell may lose its bifacial structure, thus the leaves develop the bilaterally symmetric areolation pattern, similar to that seen in *Oedipodium* protonemata. Young leaves never have zones of small, actively dividing cells in their basal parts, similar to those seen in leaves of most other mosses. Contrary to the common pattern of leaf forming by means of groups of 4×4, 4×8, 8×8 cells (descending from a single cell), the leaf development in *Oedipodium* has an opportunistic model of growth, where the cell divisions proceed randomly throughout the lamina, being not obviously correlated one with another in time, nor having a definite direction and position where it is performed. The leaves in *Oedipodium* are bi- to multistratose at very early stages of growth, not overlapping each other by their corners and only later develop the basal decurrency. The similarity and possible affinity of *Oedipodium* with the Upper Permian fossil mosses of Angaraland are discussed.

Резюме

Развитие листа *Oedipodium griffithianum* изучалось на живом и гербарном материале, с использованием световой и флуоресцентной микроскопии, а также серий анатомических срезов. Как выясняется, апикальная клетка листа легко теряет свою обратнотреугольную форму и переходит к делениям, приводящим к образованию билатереально симметричной клеточной сети, сходной с таковой пластинчатой протонемы этого вида. В молодых листьях никогда не наблюдается зоны, образованной мелкими, тонкостенными, активно делящимися клетками, характерной для ранних стадий развития листьев большинства мхов. В противоположность нормальному развитию листа, с образованием блоков 4×4, 4×8, 8×8 клеток (потомков одной клетки, поделившихся несколько раз продольно и поперечно), в листьях *Oedipodium* реализуется совершенно другой механизм роста, при котором отдельные деления клеток не согласованы между собой во времени, направлении деления и положении друг относительно друга. Наиболее молодые листья *Oedipodium* многослойны в своем основании, не налегают друг на друга углами основания и образуют низбегания только на поздних стадиях развития. Обсуждается сходство и возможные родственные связи *Oedipodium* с некоторыми образцами мхов, найденными в верхней перми Ангариды.

KEYWORDS: *Oedipodium*, mosses, leaf development, areolation, Protosphagnales

INTRODUCTION

"No one can have an idea of the beauty and delicacy of texture of this plant who has not seen it growing" – such a described characteristic of *Oedipodium griffithianum* appeared in the "Muscologia Brittanica" (Hooker & Taylor, 1827). This statement was considered so relevant to this moss that it has been fully rewritten in "Bryologia Europaea" (Bruch & Schimper, 1844). Indeed, the light green color and juicy body seen in the twilight in rock niches where *Oedipodium* grows provide an extraordinary impression: it doesn't look like any other moss. As we found that the species was rather easy to cultivate, we were able to observe its development which appeared to have many unusual characters still incompletely or inexactly described in literature. The leaf development of *Oedipodium* is the focus of the present paper.

[1] – Tsitsin Main Botanical Garden of Russian Academy of Sciences, Botanicheskaya 4, Moscow 127276 Russia; e-mail: misha_ignatov@list.ru
[2] – Biological Faculty, Tver State University, Zhelyabova 33, Tver 170100 Russia; e-mail: ulayspirina@mail.ru
[3] – Belgorod State University, Pobedy square, 85, Belgorod, 308015 Russia – Россия 308015, Белгород, пл. Победы, 85, Белгородский государственный университет; e-mail: e_maslova@list.ru
[4] – P.N. Lebedev' Institute of Physics of Russ. Acad. Sci., Leninsky 53, Moscow 119991 Russia – Россия 119991, Москва, Ленинский проспект, 53, ФИАН; e-mail: ivanov@td.lpi.ru
[5] – Biological Faculty, Moscow State University, Moscow 119991 Russia; e-mail: arctoa@list.ru

Until recently, *Oedipodium* was not classified among basal mosses and it was not evaluated as a "living fossil". Like in many other cases, the lack of peristome has precluded the exact definition of its systematic position. This position was resolved by molecular phylogenic methods, which placed the genus among nematodontous lineages (Hyvönen *et al*., 2004; Cox *et al*., 2010). Other topologies, *i.e.* a topology summarized from the recent molecular analyses (Shaw *et al*., 2011), and another one based on 14-17 chloroplastic regions (Chang & Graham, 2011), indicate its even more basal position between *Andreaea* and nematodontuous groups. Recent studies of sporoderm ultrastructure of *Oedipodium* support the latter conclusion, *i.e*., close to basal lineages (Brown *et al*., 2015; Polevova, 2015). Contrary to this, the study of placenta and water-conducting cells of *Oedipodium griffithianum* by Ligrone & Duckett (2011) has shown that *Oedipodium* is more similar with *Tetraphis, Buxbaumia* and arthrodontous mosses in this respect. These authors admitted the loss of peristome in *Oedipodium*. On the other hand, Shimamura & Deguchi (2008) studied a series of sporophyte sections and found nothing that may reject the idea that *Oedipodium* is a primarily eperistomate moss. However, all these discrepancies shift the position of *Oedipodium* in phylogenetic trees to one or two nodes only, so its placement among "living fossils" remains unchallenged.

Our interest to study the leaf development in *Oedipodium* originally arose from the attempts to undertake a retrospective reconstruction of cell divisions which result in the type of areolation of the mature leaf. In most other moss leaves the apical cell is apparent and the sectors cut off from it (see Scheme 1) are fairly well delimited.

The principal way of moss leaf development in *Oedipodium* was described in the classical publications of Schimper (1860) and Lorentz (1864), and also in the subsequent comprehensive studies of Pottier (1925), and Frey (1970, 1972). A synoptic picture of it is given in Scheme 1. Apical cell in a series of divisions (usually 4-7 to each side) produces the 'mother cells' of corresponding sectors. The cells appeared earlier are situated at leaf base, and the sectors formed by them are usually composed of the largest number of cells. Being the earliest in time of origin, the cells of the basal sector are the latest in time of their differentiation (Scheme 1C). There are only few exceptions from this pattern of leaf development in the basal bryophyte lineages, including *Takakia*, which has no entire leaves (Spence & Schofield, 2007), and *Andreaea* (Pottier, 1925), and in fossil Protosphagnales (Maslova *et al*., 2012).

However in many leaves of *Oedipodium* we observed an areolation pattern where the position of apical cell was unclear and even the presence of a single apical cell was questionable.

The problem of the apical cell in leaves and protonematal plates (= Protonemablättern by Correns, 1899 and Geobel, 1930) of *Oedipodium* is not new. It was in a focus of study of Correns (1899) who found that both stem leaves and protonematal plates grow by means of the division of bifacial obtriangular or obtrapezoid apical cell. At the later stages of leaf development, cells in *Oedipodium* become lingulate in shape. Although the observations of Correns (1899) are excellent and accurate, and his illustrations are precise, his studies were limited by herbarium material. Thus, some additional studies of growth of fresh plants in culture may provide a better understanding of growth pattern in this interesting moss.

A comprehensive bulk of information on protonema developmental stages was published by Duckett *et al*. (2004) for a number of species with unusual protonema structures. In this paper photographs of all stages of *Oedipodium* protonemata development were presented, although their structure was only briefly explained in the figure legends. The present study is aimed to better understand the developmental pathway of protonema in *Oedipodium* by a comparison with the development of stem leaves, and to determine their homology and features peculiar among mosses.

MATERIAL AND METHODS

Cultivation

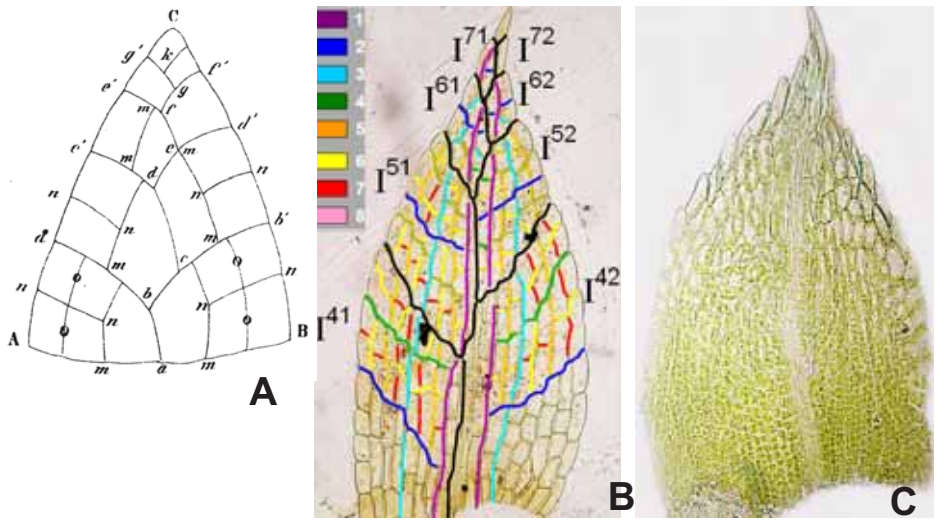
Living plants were delivered to us by V.E. Fedosov, who collected them in September 2014 on Olkhovaya Mountain, Primorsky Territory (voucher specimens in MW). This locality was briefly described by Ignatov *et al*. (2006). Plants with small amount of their original substrate were deposited in Petri dishes on wet filter paper, and cultivated with 10 hours light a day, with 12°C associated with light time, and +7° in the "night". No additional nutrients were added.

Microscopic studies

Cultivated plants were photographed under stereomicroscope Olympus SZX16, the latter equipped with an Infinity 4 digital camera. Some micrographs obtained from several optical sections were composed using the software package HeliconFocus 4.50 (Kozub *et al.,* 2008).

For anatomy observations, material was taken from cultivated plants. Apical parts of shoots were isolated, leaves were removed. Prepared stems were de-aerated and fixed in 2.5% glutaraldehyde in 0.05M PBS for 3 hours, post-fixed with 1% osmium tetroxide in PBS, pH 6.8, for 6 hours. Then material was dehydrated through an ascending ethanol-acetone series to 100% acetone. After that samples were embedded in araldite 6005 medium, according to the protocol of manufacturer.

Sections were cut 2 ìm thick with glass knives, put on glass slides without mounting medium, stained by 0.01% Berberin and photographed under Olympus FV-1000 with 473 nm laser.



Scheme 1. Moss leaf development. A: from Schimper (1860), with order of cell divisions according to alphabet; B: from Donskov (unpubl.), with order of cell divisions marked by colors and abbreviations of sectors according to Frey (1970); C: young leaf of *Physcomitrium pyriforme*, showing still undifferentiated cells of earlier formed proximal sectors and differentiated cells of distal sectors.

Material for LSCM was taken both from dried herbarium specimens and cultivating plants and was prepared in two ways: 1) samples were fixed in 4% paraformaldehyde in 0.05M PBS pH 7.0 with 0.01% Triton-X, 0.01% Nonidet P-40 and 0.01% FB28 for 3 hours, then stained by 0.1 mM DAPI for 15 min and replaced in DMSO; 2) shoots without fixation were stained by 0.1 mM DAPI or 0.01% Berberin for 15 min, then both types of samples were investigated under Olympus FV-1000 with 407 nm and 473 nm lasers.

Areoana analysis

Leaves of *Oedipodium griffithianum* from MHA herbarium collections were used. They were photographed under Carl Zeiss NU2 light microscope, using the Nikon D70 camera (2000×3008 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 and analyzed in AREOANA program (Ivanov & Ignatov, 2011; 2013).

Comparison with other mosses

Leaves most similar in shape and lamina areolation to *Oedipodium* leaves were found among fossil mosses of the order Protosphagnales. Illustrations and descriptions of latter were partly published by Maslova & Ignatov (2013), but some newly obtained fossil material is described here for the first time. Its origin and preparation are described in Maslova *et. al.* (2012).

Observation

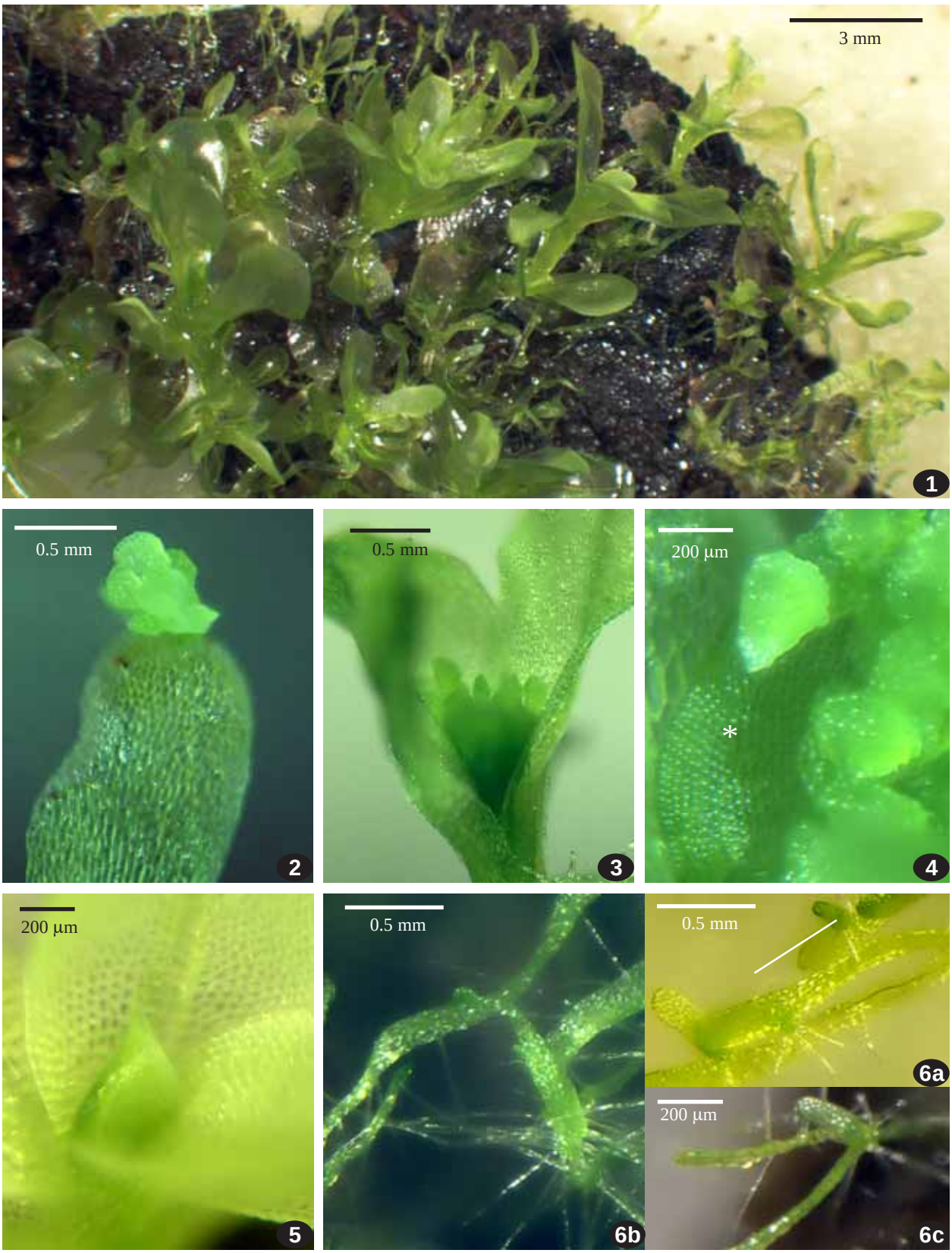
Overall growth

Plants grown in Petri dishes were partly well-developed, with broad leaves similar to those characteristic for the species seen in nature (Figs. 1, 3, 5). On the other hand, on the same piece of soil many plants had only very narrow leaves (Figs. 1, 6, 23), growing in delicate rosettes. Leaf-like protonematal plates also appeared in abundance. They looked like single leaves, growing individually and producing laterally either another plates or rosette plants with more or less narrow leaves (Figs. 20, 22-24). Protonematal plates developed rhizoids at their bases, which looked exceedingly stiff and rigid, thus the plants were seen standing on them, as on stilts, and keeping the base of the rosettes or individual leaves above the ground (Fig. 6b). Occasionally young plants originated on leaves of narrow-leaved plants were seen as well. Lenticular gemmae were produced abundantly in axils of upper leaves (Figs. 3-4), or occasionally upon different parts of leaf body, for example, on tips of narrow leaves (Fig. 2) or leaf margin (Fig. 38). We also observed the rather easy propagation of the gemmae. Being placed in Petri dishes on wet filter paper without any nutrients, they started to propagate in 2-4 weeks in the same environments as the adult plants.

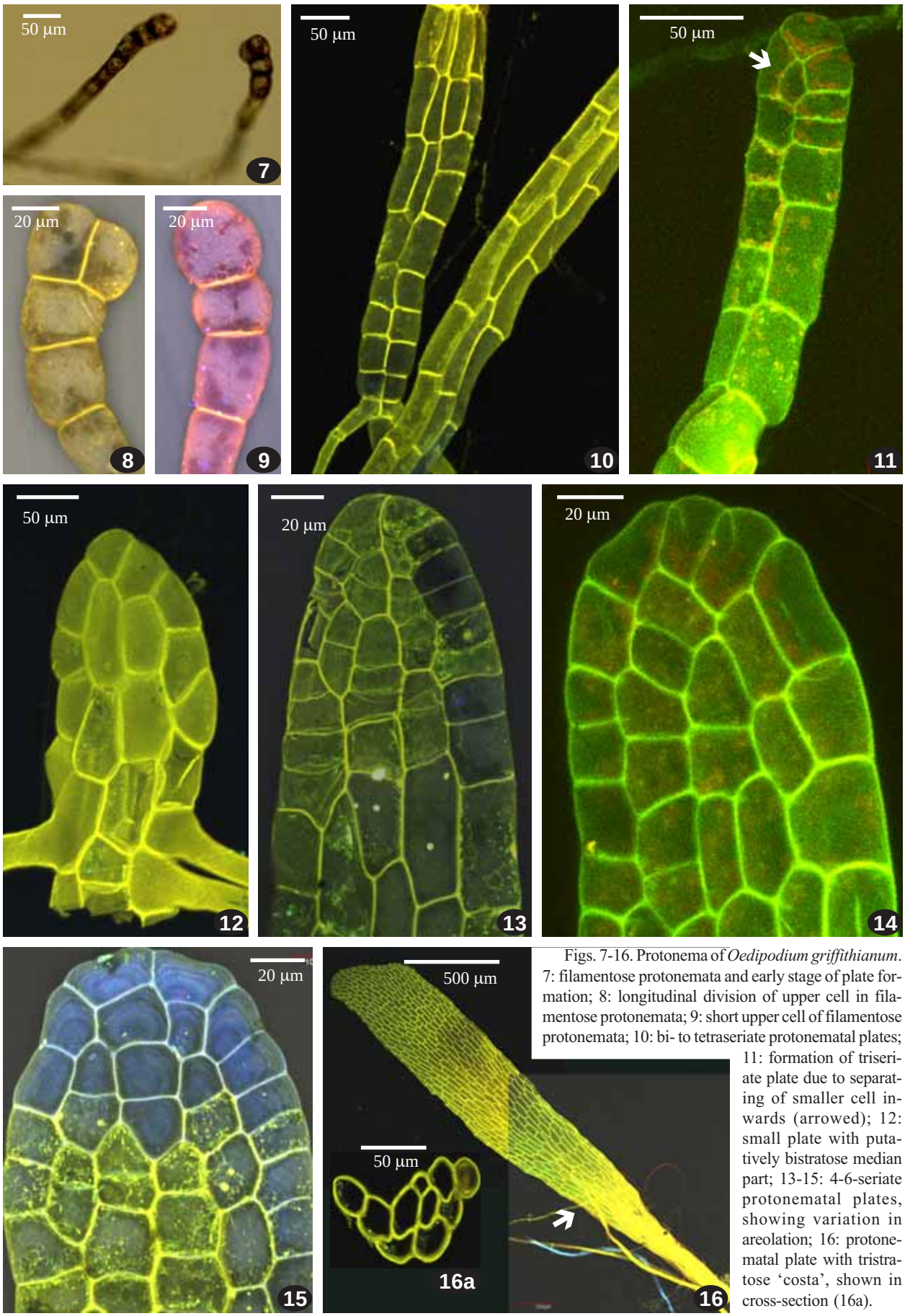
Protonematal plates

Many publications describe this genus as having thallose protonema (Ligrone & Duckett, 2011; Crum, 2007, *etc.*). However, contrary to the case of *Sphagnum* where protomena becomes thallose almost immediately after spore propagation, the ordinary filamentose protonema exists in *Oedipodium* as well. Its cells often are unusually short and sometimes illustrated as a bead-like threads (Goebel, 1930; Crum, 2001), but we observed also longer cells of ca. 8:1 as well (Fig. 24). The cases where cells are getting shorter (Figs. 7-9) indicate a tendency towards the plate formation. The short upper cell (Fig. 9) may divide longitudinally (Fig. 8) to become the biseriate plate form (Fig. 10). In the course of elongation, cells may divide as shown in Fig 11, separating a smaller cell inwards. It seems that, by means of such divisions, the plate becomes 4-6- or more seriate in form (Figs. 13-16).

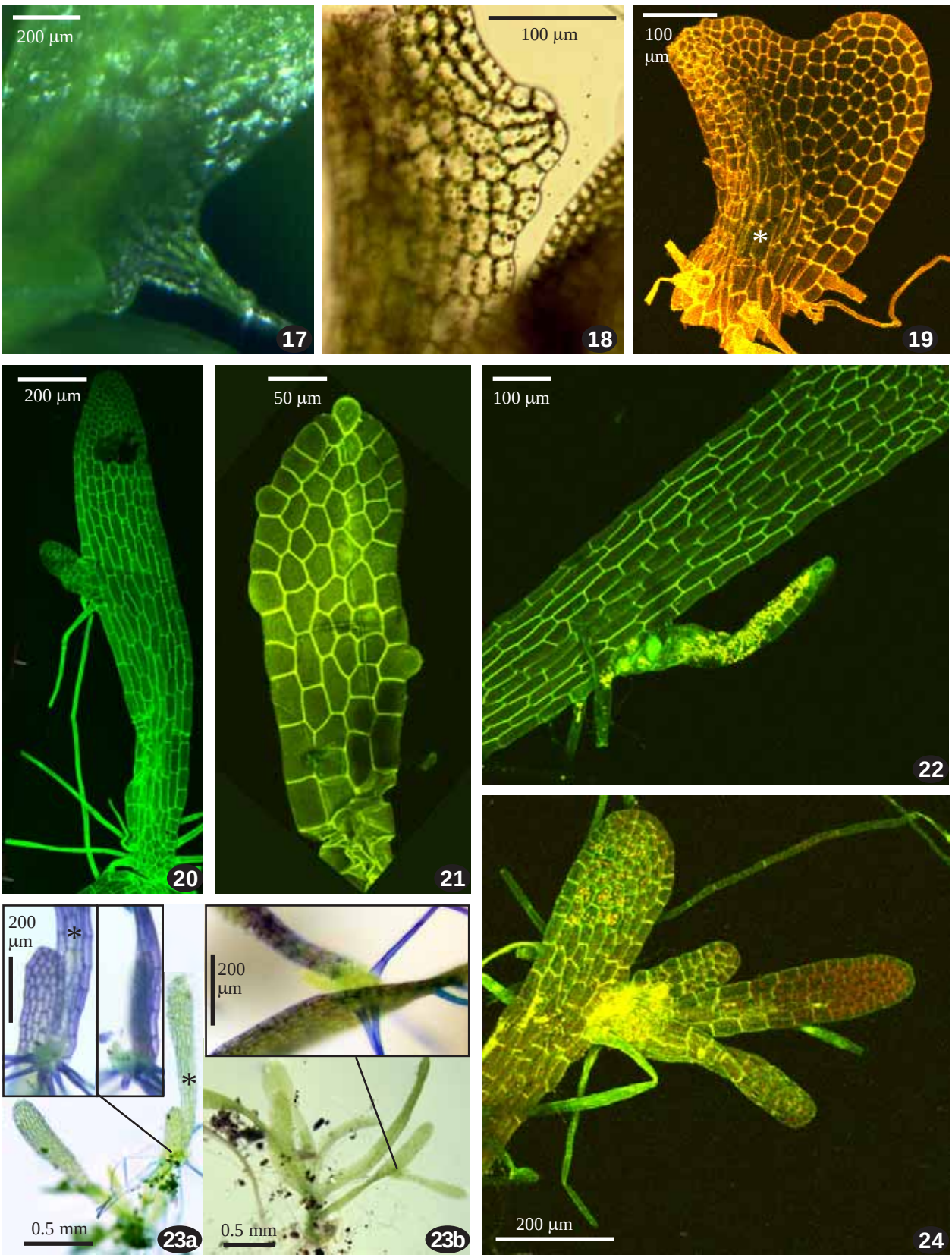
Periclinal divisions were observed in such protonematal plates as well. One example is shown in Fig. 12: central part of this tetraseriate plate looks turgid, likely due to bistratose structure in the median part. In a number of protonematal plates of 10-20 cells wide we found bi- to tristratose areas near their bases (cf. Fig. 16). This costa-like median part is formed of homogeneous cells, as well as costa in leaves (cf. Fig. 42).



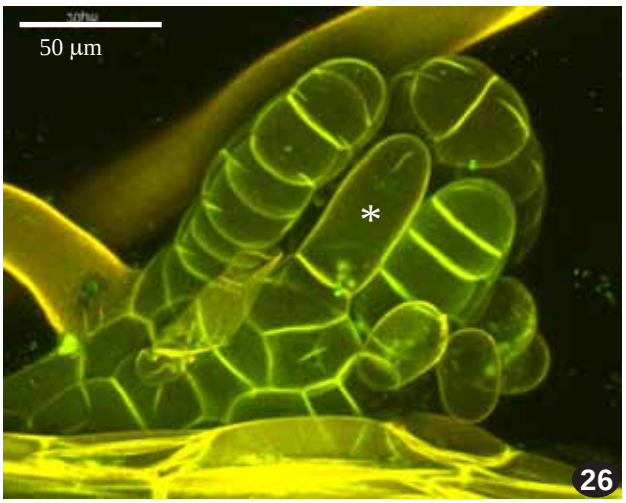
Figs. 1-6. Cultivated plants of *Oedipodium griffithianum*. 1: plants on wet soil; 2: narrow leaf with the cluster of lenticular gemmae near its apex; 3: upper leaves with abundant lenticular gemmae in their axils; 4: lenticular gemmae in axil of conduplicate leaf at stage comparaba to that in Fig. 5; asterisk marks areolation that look regular, but in fact ut usn't (see discussion in Fig. 444)s; 5: apical part of stem covered by conduplicate leaf; 6: narrow protonematal plates with young gametophores, appearing laterally, as in 6a (cf. Figs. 22-24) or unbranched (6b); rosette in 6c is composed by three leaf-like structure, two narrower, similar to protonematal plates and one shorter, having shorter cells and lacking inflated cells in the marginal row of cells.



Figs. 7-16. Protonema of *Oedipodium griffithianum*. 7: filamentose protonemata and early stage of plate formation; 8: longitudinal division of upper cell in filamentose protonemata; 9: short upper cell of filamentose protonemata; 10: bi- to tetraseriate protonematal plates; 11: formation of triseriate plate due to separating of smaller cell inwards (arrowed); 12: small plate with putatively bistratose median part; 13-15: 4-6-seriate protonematal plates, showing variation in areolation; 16: protonematal plate with tristratose 'costa', shown in cross-section (16a).



Figs. 17-24. Branching and outgrowths in *Oedipodium griffithianum*. 17-18: lobes at leaf margins; 19: bilobate leaf with multistratose costa, marked by asterisk [see also discussion in the text]; 20, 22, 24: narrow protonematal plates with young shoots developing from their margins; 21: protonematal plate with some cells at margin enlarged and inflated: those are putatively gametophore initials. 23: two rosettes of narrow-leaved plants with young shoots upon their leaves; close up of the latter are in frames, showing that in case of 23a the leaf marked by asterisk is developed on leaf margin (close up presents frontal and lateral views), while in 23b it is originated on leaf surface at a certain distance from the leaf margin.



Figs. 25-26. Early stages of development of lateral innovation on stem in *Oedipodium griffithianum*. 25: bud with one leaf larger than other leaves; 26: 'pro-gemma' filament formation among young leaves (marked by asterisk).

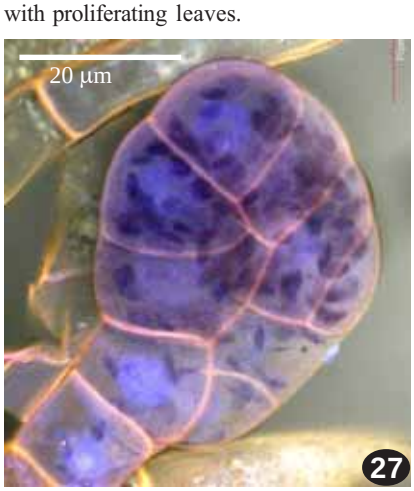
Along with entire and elongate protonematal plates, Duckett *et al.* (2004) illustrated a few deeply lobate ones (cf. Figs. 5f, h in their publication). Similar structures were found in our specimens as well. One of them is shown in Fig. 19. However, it differs from plates given by Duckett *et al.* (l.c.) in cell outlines indicating the presence of multistratose area (marked by asterisk in Fig. 19), which is very similar to costa in leaves. Various outgrowths on sides of leaves, both narrow and fully developed, occasionally appear (Figs. 17-18). Similar structures were observed in herbarium collections from nature as well.

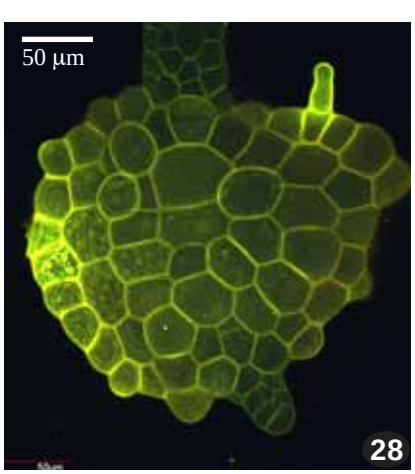
Conspicuously inflated cells are often seen at margin of some plates (Figs. 21). These cells may proliferate into foliose structures. Such secondary structure may comprise an individual leaf (Fig. 22) or form small plants with a rosette of narrow leaves (Figs. 20, 24). In fact, a careful study of the former case, where the leaf looks like a solitary one (Fig. 22) almost always allows finding of inflated cells at its base and/or additional very small leaves (Fig. 6a, pointed by thin line). It suggests that all such single leaves are, in fact, juvenile plants that produce so far only one leaf, while the formation of the next leaf is strongly delayed. Observation of the development of 'protonemata' from the lenticular gemmae supports this proposition.

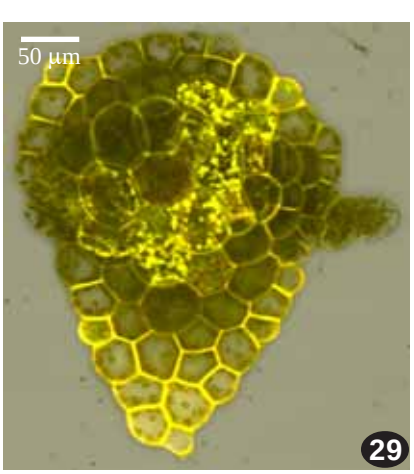
In some plants, the innovations appear from narrow plates. They are very narrow and posess numerous inflated cells along margin, similar to those shown in Fig. 21. Such cases comprise the most certain variant of the branched protonemata. Thus, the main distinction between protonematal plates and narrow leaves can be assumed as the presence of such inflated marginal cells, easily producing small plants. However, exceptions were observed: some narrow leaves (in plants growing as rosettes) also may develop small plants on their leaf surface and leaf margin (Fig. 23), making the distiction between given foliose structures quite vague in some cases.

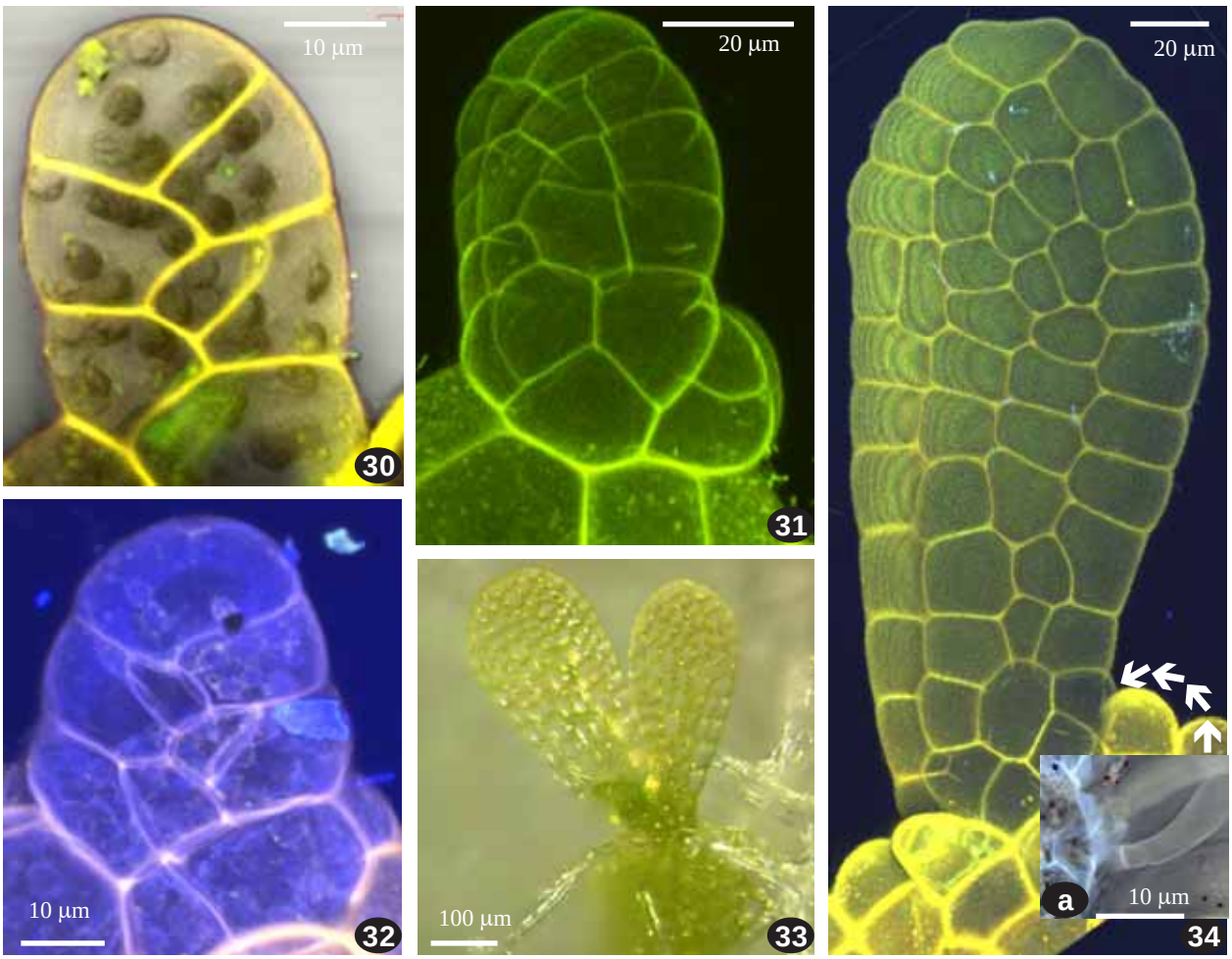
Another case, where the problem of distinguishing protonemata and leaves appears is as follow. Duckett *et al.* (2004) indicated that the mature protonematal plates in *Oedipodium* are lobate, similar to that in Fig. 19. In our case, however a similar lobate structures were found only at the

Figs. 27-29. Lenticular gemmae of *Oedipodium griffithianum*. 27: young gemma with bifacial apical cell; 28-29: isolated gemmae with proliferating leaves.









Figs. 30-34. Leaf-like proliferation of lenticular gemmae of *Oedipodium griffithianum*. 30: first cutting of inner upper cell angle; 31-34: different stages of development of juvenile leaves with multiseriate laminae; 33: gemmae propagating into shoot with two larger leaves and bud with very small leaves at their bases; Fig 34a shows axillary hairs behind the base of leaf in Fig. 34.

lowermost part of small shoots (*i.e.* in place where ordinary leaves occur). The upper leaves from the same shoot are shown in Figs. 51-53.

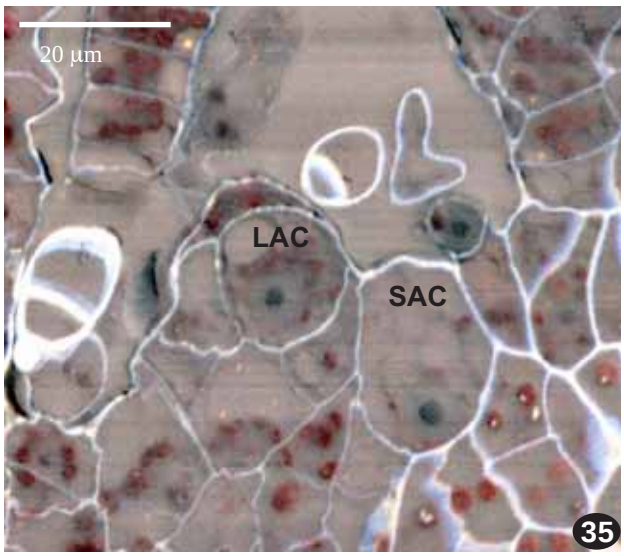
Lenticular gemmae and their propagation

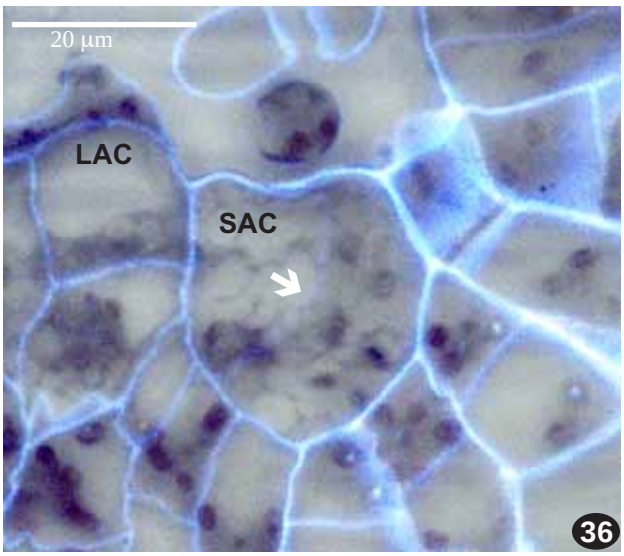
The leaves propagated from gemmae are, indeed, the easiest to observe, as they are quite flat and not covered by surrounding leaves, contrary to upper stem leaves. Both Correns (1899) and Duckett *et al*. (2004) illustrated such leaves grown from gemmae. They were illustrated as biseriate by Correns (1899), while the light microscope photograph given by Duckett *et al*. (2004) showed a broader structure with a larger number of cells in width.

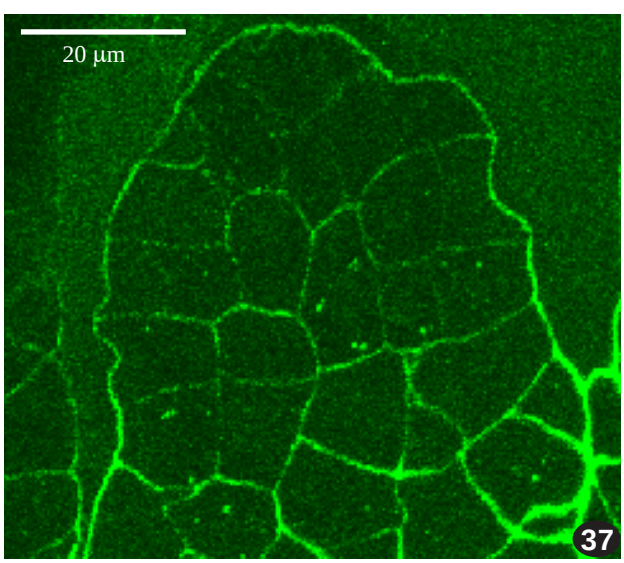
The gemmae themselves are usually developed near stem apex (Figs. 3-4). In the course of their formation, they pass through the two-celled stage (similar to the early stage of thallose protonema development shown in Fig. 8) and illustrated by Correns (1899, Fig. 86) and Goebel (1930, Fig. 1048). Both Correns and Goebel described young gemmae of *Oedipodium* as essentially symmetric structures, with two equal or subequal cells situated side by side at their tops. We found however that at least in some cases they have a bifacial apical cell similar to the leaf apical cell (Fig. 27). A putatively earlier stage of gemmae formation is shown in Fig. 26: among leaves of a young lateral bud there is a filamentose stricture. We are inclined to interpret this filament as a progemma by two reasons. First, no other plant organs are filamentose, except axillary hairs (which cannot be two-celled at base like here) and rhizoids (which have oblique cell walls). Second, we observed gemmae clusters in a similar position on some other plants.

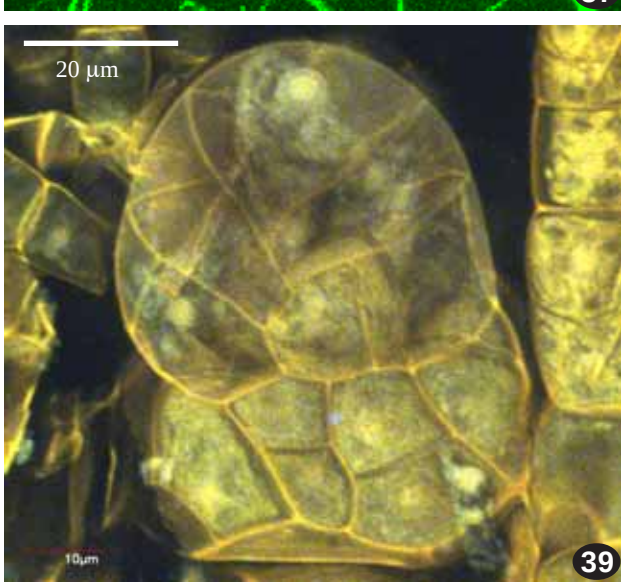
The leaf-like structures growing out of gemmae are shown in Figs. 30-34. They have obvious bifacial apical cell as in leaves, and also possess a peculiar pattern of cell divisions. In young moss leaves cell divisions are usually subequal. More rarely a division may cut off one angle of subquadrate cell by an arching cell wall, but in this case it is an inner lower angle (according to our observations in other mosses). In *Oedipodium* the inner upper cell angles are cutting off at early stages of such leaf development (Figs. 30, 32), resulting in its widening to a tetraseraite or wider structure (Fig. 34).

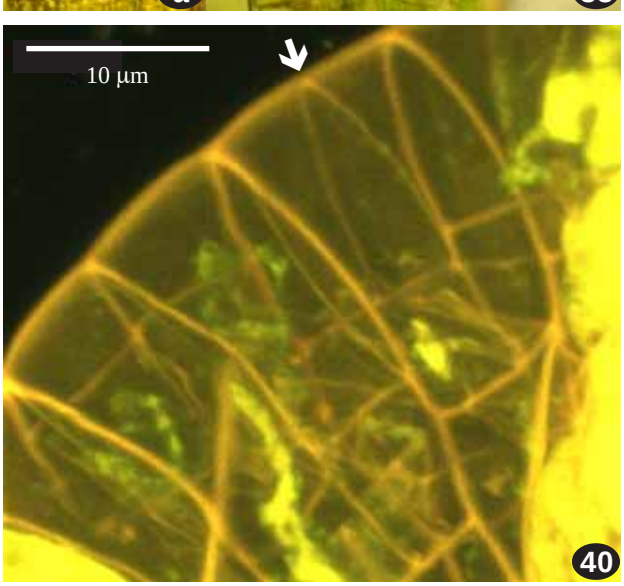
Correns (1899), Goebel (1930) and Duckett *et al*. (2004) considered foliose structure developed from *Oedi-*











Figs. 35-40. Early stages of leaf development in *Oedipodium griffithianum*. 35-36 (two sections of 2 µm with one between them not shown): first leaf that is moslty bistratose (cf. Fig. 41) [LAC – leaf apical cell; SAC – stem apical cells]; Fig. 36 shows the most recent division of stem apical cell (arrowed); 37: stem leaf at relatively early stage of development, comparable with that in Fig. 39; 38a: apical part of stem with two young leaves shown in Fig. 38 and 40, and small hardly seen leaf magnified in Fig. 39; 39: leaf with bifacial apical cell and three first merophytes at each side; 40: recently formed wall in leaf border cell (arrowed).

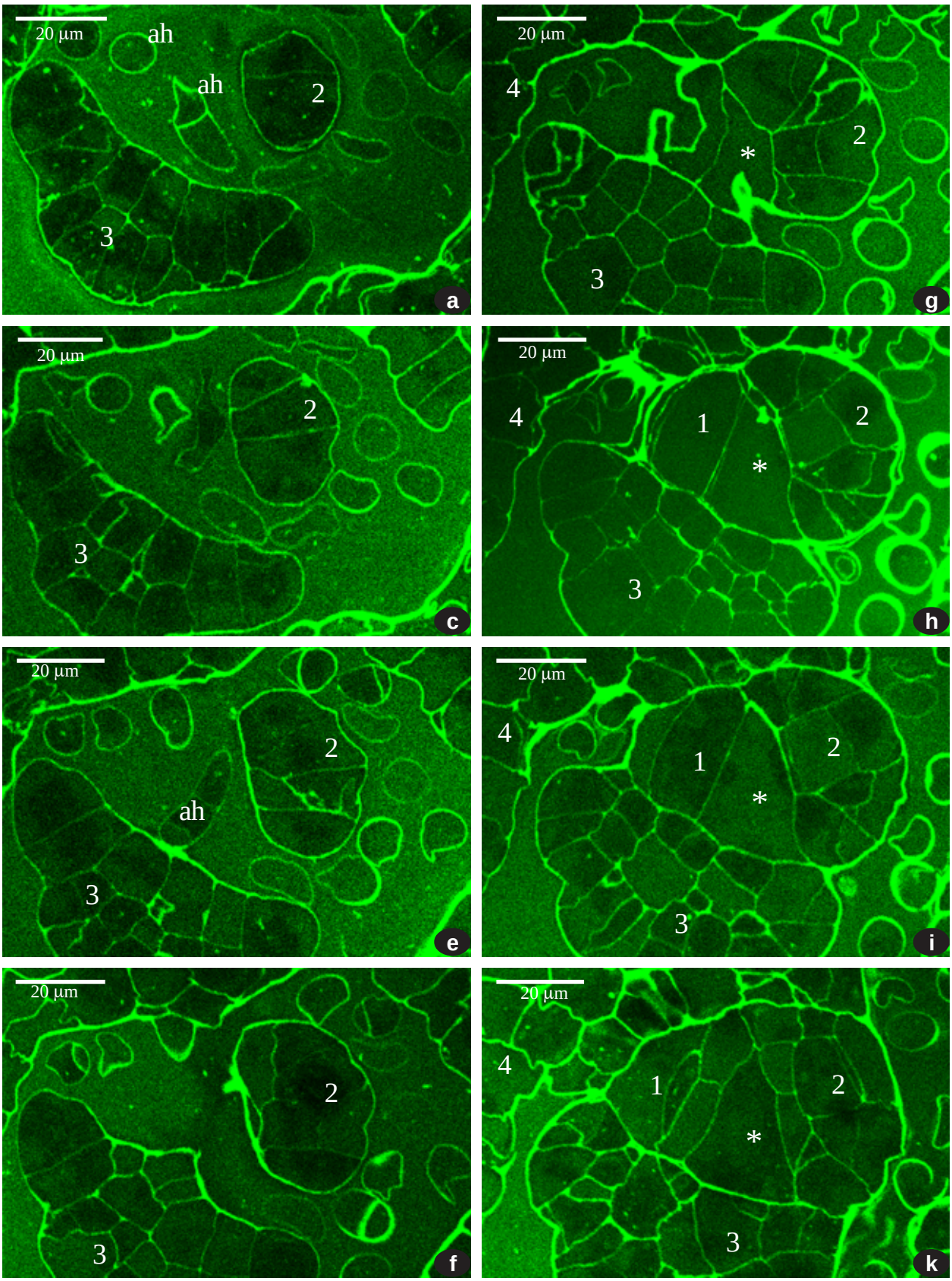
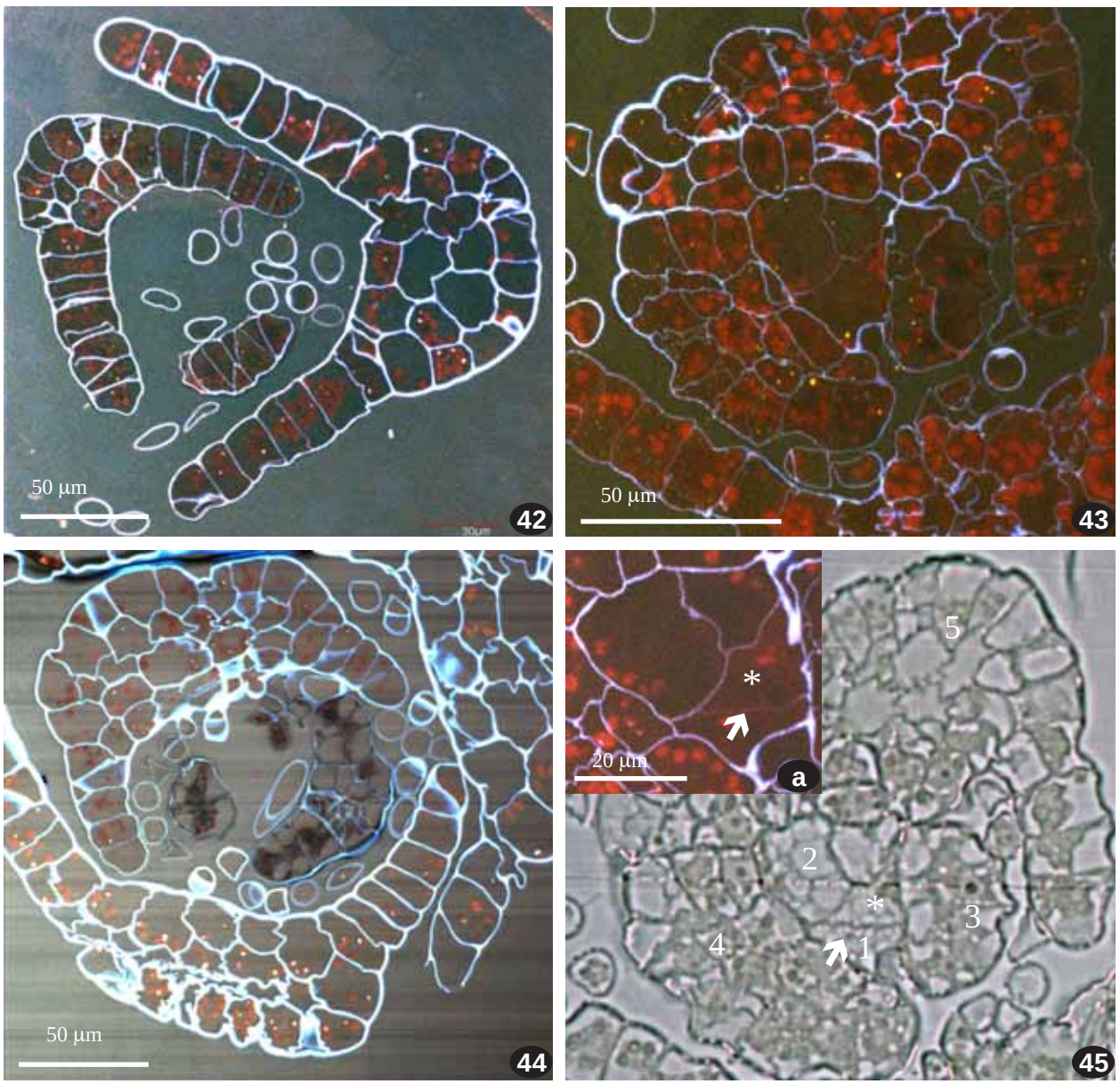


Fig. 41. Transverse sections of stem apical zone of *Oedipodium griffithianum*. The shown part of series is 22 µm long (sections are 2 µm thick; letters are sequential, and lacking letter indicates that the corresponded section is not shown). Numerals indicate leaf numbers, apical cell is marked by asterisk, ah – axillary hairs. Note that even 3d leaf has almost no unistratose part (cf. Figs. 46 and 53).



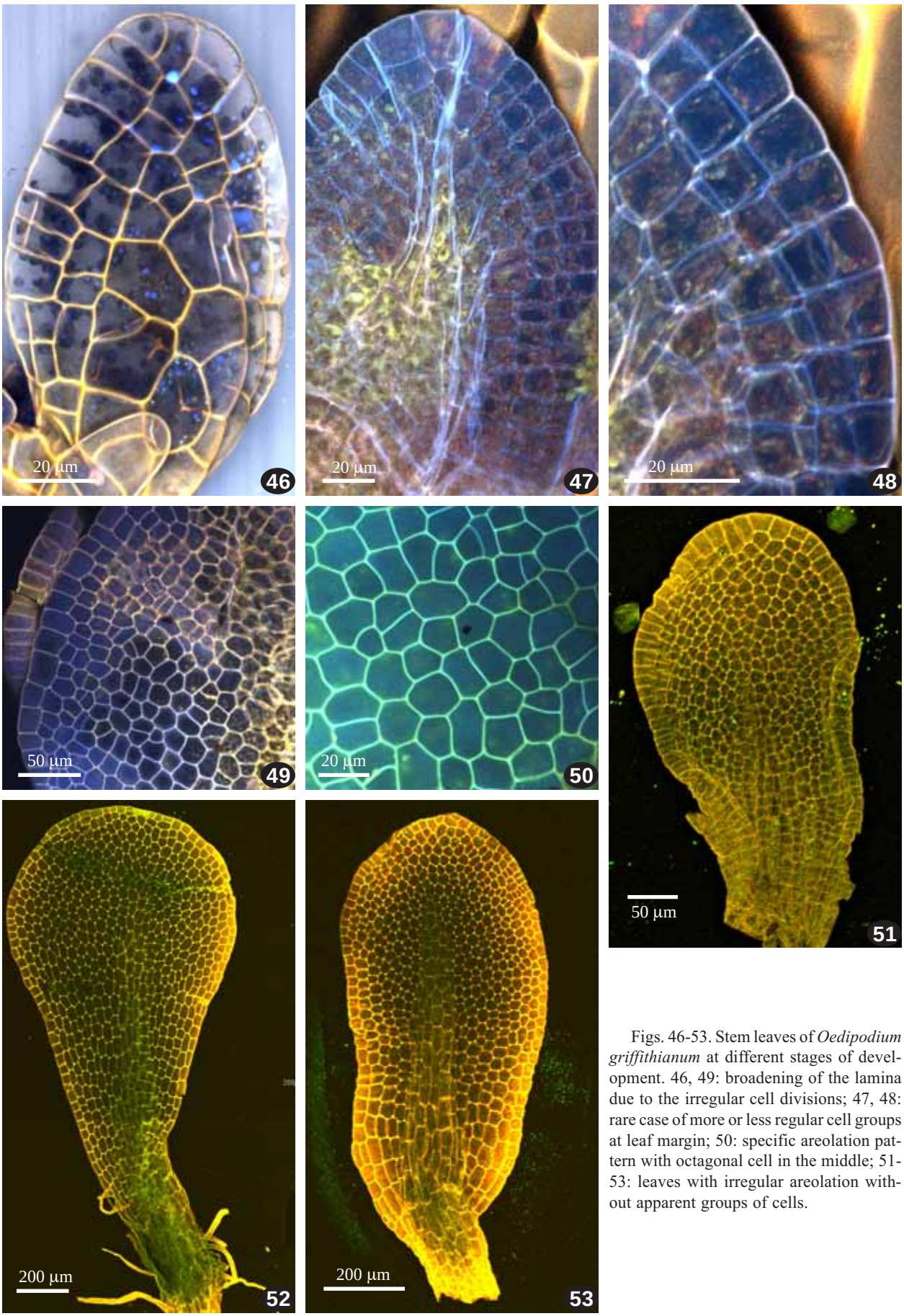
Figs. 42-45. Selected transverse sections of *Oedipodium griffithianum:* at 50 µm above apical cell (Fig. 42), at 20 µm above apical cell (Fig. 44), and at 2-4 µm below its top (Figs. 43, 45). Fig. 42 shows conduplicate leaf structure (cf. Fig. 5) that provides an efficient protection for the apical cell. Figs. 45 and 45a show subequal division of the stem apical cell (arrowed).

podium gemmae to be protonemata. However, we saw small other juvenille leaves (cf. Fig. 31) and axillary hairs (Fig. 34) developing near their bases. They surround a conspicuously differentiated (albeit small) cell that can be assumed as a stem apical cell. And finally, the propagated gemmae often have more than one leaf (Fig. 33). Thus, these 'protonemata' can be interpreted rather as leaves of young and very short shoots.

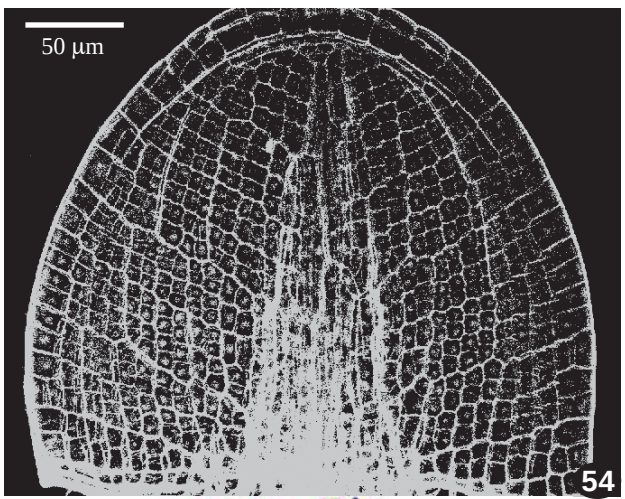
Stem leaves

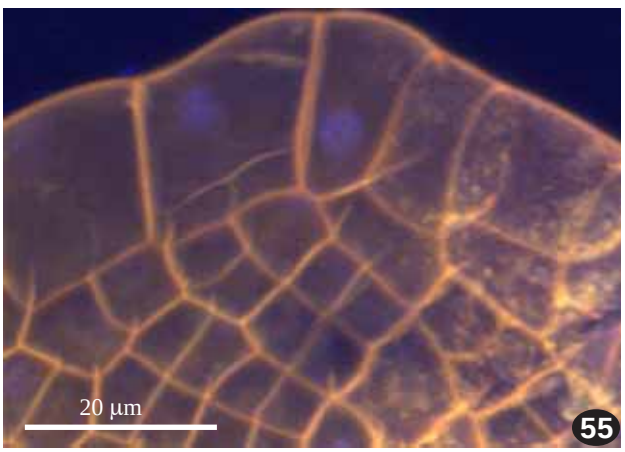
Direct observations of young leaves were made at the stage of 40-50 µm long when three cells to each side of the leaf apical cell were cut off. Small leaves were detached and examined by means of LCSM. Three sectors on each side of leaf can be recognized (Fig. 39). Earlier stages of leaf development were observed in longitudinal sections, partially represented in Figs. 35-37, and in three complete series of transverse sections, partially shown in Fig. 41 and Figs. 42-45.

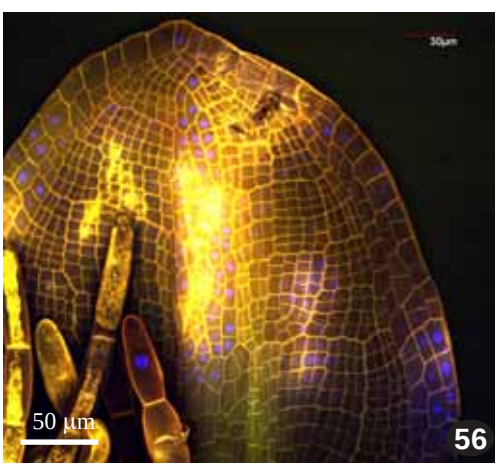
In general, the observed arrangement of cells in young leaves of *Oedipodium* fits the classical scheme of leaf development in mosses (Scheme 1 in page 433). However, in transverse sections, unistratose lamina is present only in the uppermost part of leaf primordium, and already since 15 µm from its apex, the young leaf becomes partially bistratose and at the level of apical cell it is completely bistratose (Fig. 41). Thus, in *Oedipodium* unistratose lamina cannot develop from the cells of leaf base corners as it happens in most other mosses (Frey, 1970), and its formation starts from the marginal cells where divisions produce cells inwards as can be deduced from *e.g.* Figs. 41 and 46.



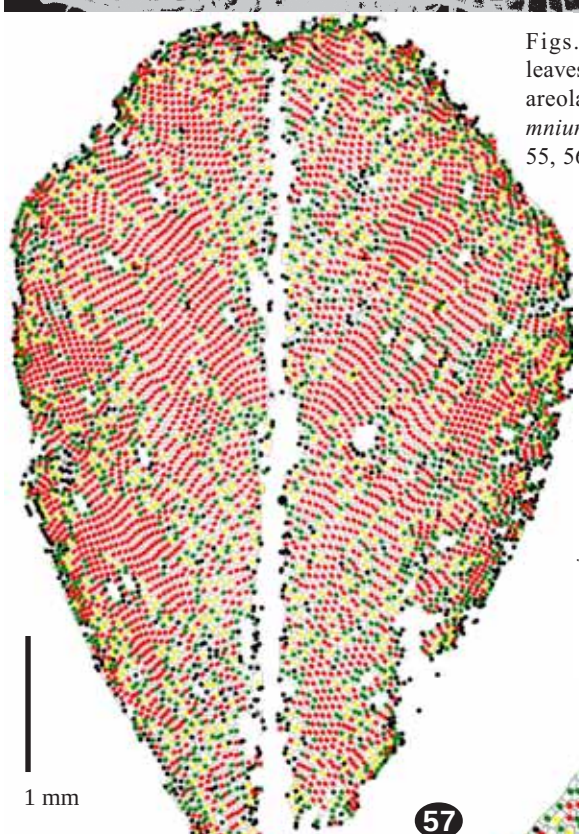
Figs. 46-53. Stem leaves of *Oedipodium griffithianum* at different stages of development. 46, 49: broadening of the lamina due to the irregular cell divisions; 47, 48: rare case of more or less regular cell groups at leaf margin; 50: specific areolation pattern with octagonal cell in the middle; 51-53: leaves with irregular areolation without apparent groups of cells.



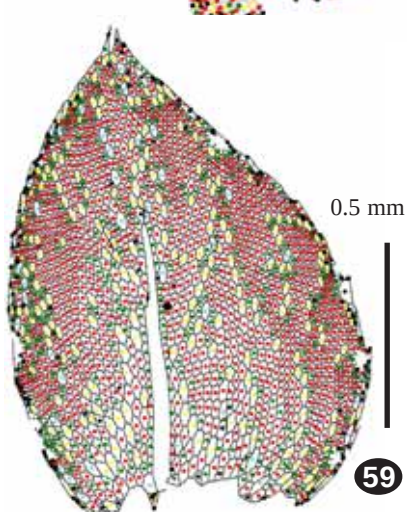


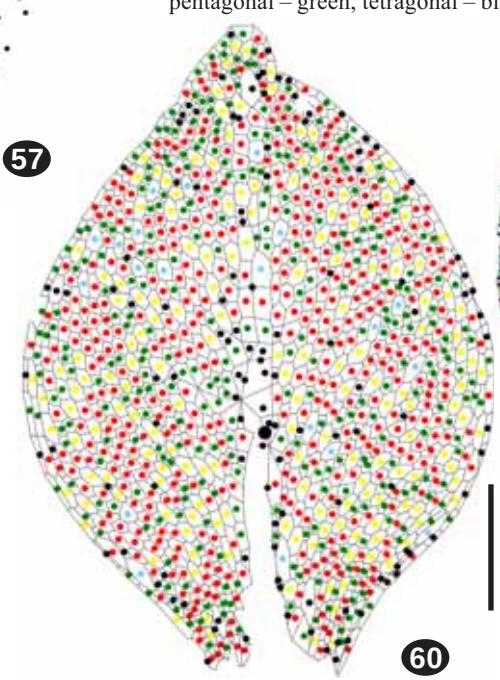


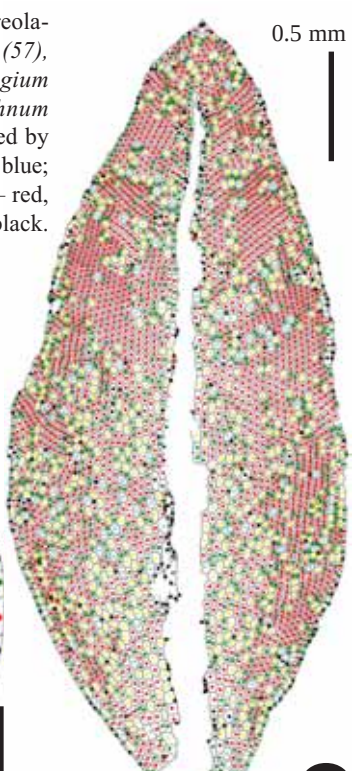
Figs. 54-56. Young leaves with regular cell areolation. 54: *Rhizomnium andrewsianum*; 55, 56: *R. punctatum*.



Figs. 57-60. Digitized leaf areolation of *Rhizomnium tuomikoski (57), Mnium stellare (58), Hypopterygium flavolimbatum (59)* and *Splachnum vasculosum (60)* with cells marked by dots of different color: octagonal – blue; heptagonal – yellow; hexagonal – red, pentagonal – green, tetragonal – black.







The leaf border in *Oedipodium* is peculiar in cell division pattern: its large, ca. 25 μm wide cells undergo division into 'slices' 5 μm wide (Fig. 40). It is especially interesting because 5 μm is the size of young, actively dividing cells in growing leaves of all other mosses which we studied. Usually a zone of such 'mashed cells' with still unclear outlines occurs in basal leaf corners, while the upper leaf portion has much thicker cell walls at the same stage (Scheme 1C, page 433). However in *Oedipodium* the absence of unistratose basal leaf corners at the early stage of leaf development leaves no room for such a meristematic zone.

Observations on series of sections (Fig. 41) demonstrate the stages of young leaf formation. The costal area is strong and the lamina is developing at narrow angle, resulting in almost a conduplicate leaf structure (cf. Figs. 5 and 42). Such shape seems to be quite functional for *Oedipodium*, providing efficient protection to the stem apical cell, gametangia and developing gemmae.

The subsequent broadening of the lamina results from the cell divisions between the border and costa. *Oedipodium* does not have any regularity in time and direction of cell divisions in the main part of lamina (Figs. 49-53). Only in few leaves in their distal parts more or less regular groups of 4×2 cells were observed at margins (Figs. 47-48). A regular areolation might be assumed from the observations under stereomicroscope (Fig. 4: asterisk), however cells of the same leaves under compound microscope look irregular (cf. Figs. 49-50).

Fig. 51 illustrates the position of the most recent divisions, which are marked by much lighter cell walls in comparison with more firm walls of older cells. They are fairly randomly spread throughout the lamina, contrary to examples of *Rhizomnium* leaves shown in Figs. 54-56. The latter pattern is characteristic for most other modern moss species; more illustrations are published by, *e.g*., Frey (1970, 1972), Donskov (2015), *etc*.

The broadening of the leaf itself includes also the broadening of leaf base and expansion of basal decurrency, so the development approaches the state common for most mosses.

Areoana-analysis

The principal scheme of leaf development (Scheme 1 in page 433) is universal for all mosses, with apparently the only exceptions of *Andreaea* and *Takakia*. The implementation of this pattern of cell divisions results in leaf lamina composed of sectors of cells, described in details by Frey (1970). Each sector originates from a single cell and often is recognizable as clearly outlined groups of 4×4, 4×8, 8×8, *etc*. In many cases the digitized images of the lamina areolation with the use of Areoana-program (Ivanov & Ignatov, 2011, 2013) allow the visualization of such groups simply by marking the numbers of angles seen in each cell. The method of digitizing in Areoana analysis approximates the cell as a polygon where angles are the points of joining of three (more rarely of four) cell walls. Thus, the number of angles (or, what is the same, the number of sides) is a straightforward procedure. Then, if all cells with 4, 5, 6, 7, and 8 angles are marked by different colors, one may see the picture like Figs. 57-60. The mentioned groups originated from a single cell (*i.e*. a rather homogeneous groups of 4×4, 4×8, 8×8 cells) appear to be enriched by hexagonal cells, while borders between them have higher percentage of penta- and heptagonal cells. This rule will be discussed in details elsewhere, while here we just provide examples of how it looks. At the same time, it is easily seen that *Oedipodium* has a strikingly different areolation (Figs. 61-65). Groups of hexagonal cells are absent, or at best, they are small and arranged near the leaf margin. This structure corresponds with the 'opportunistic' growth pattern illustrated above (Figs. 49-50) where the cell divisions are random in time and space, unlike, *e.g.*, those observed in *Rhizomnium* (Figs. 54-56).

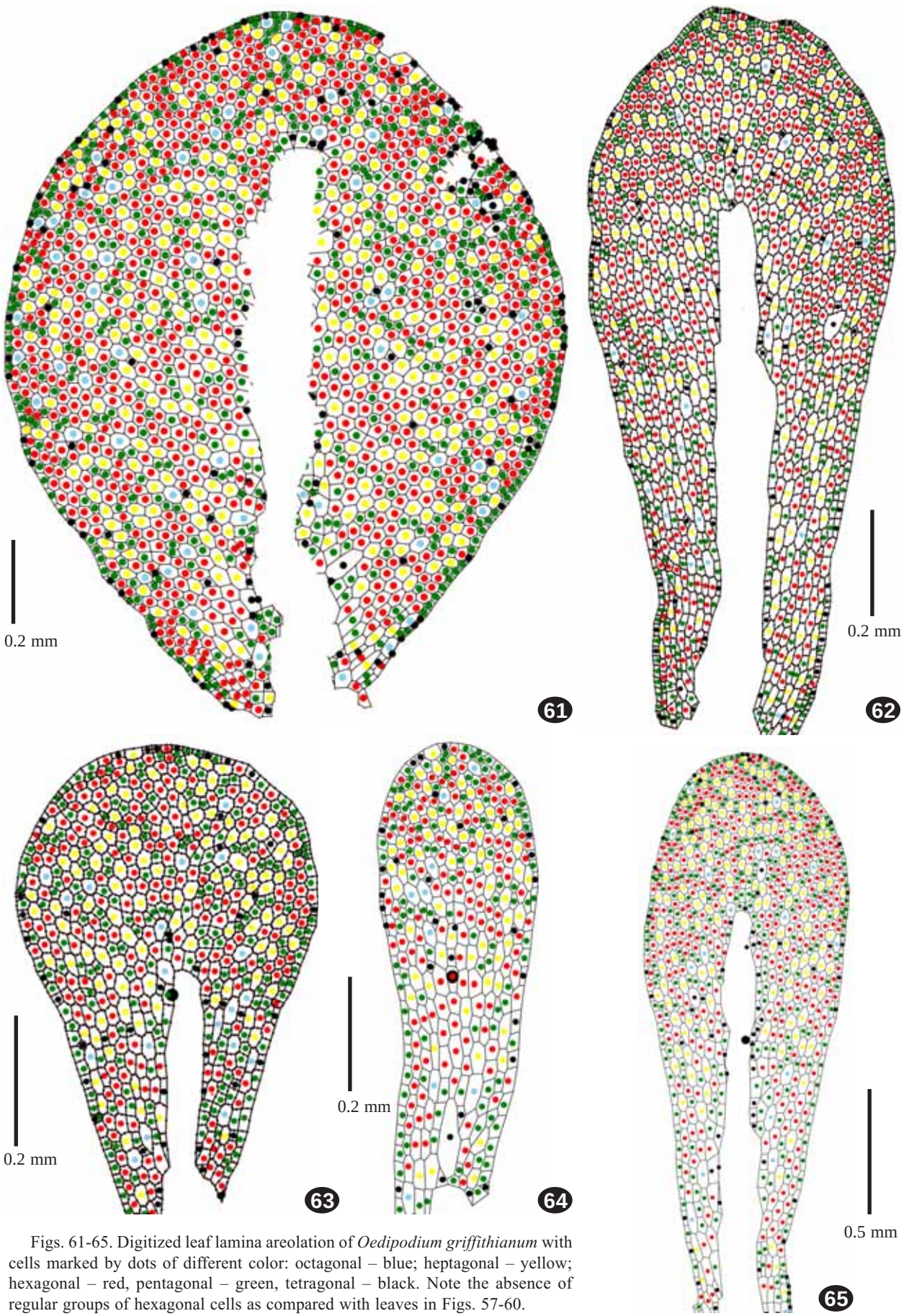
Distinction between protonematal plates and leaves

The description of protonematal plates given above already pointed the problem of differentiation of these two structures. Although the ultimate difference would be that protomenata never bears gametangia, this character in not practical for sorting out small innovations. Some additional aspects of this problem are discussed here.

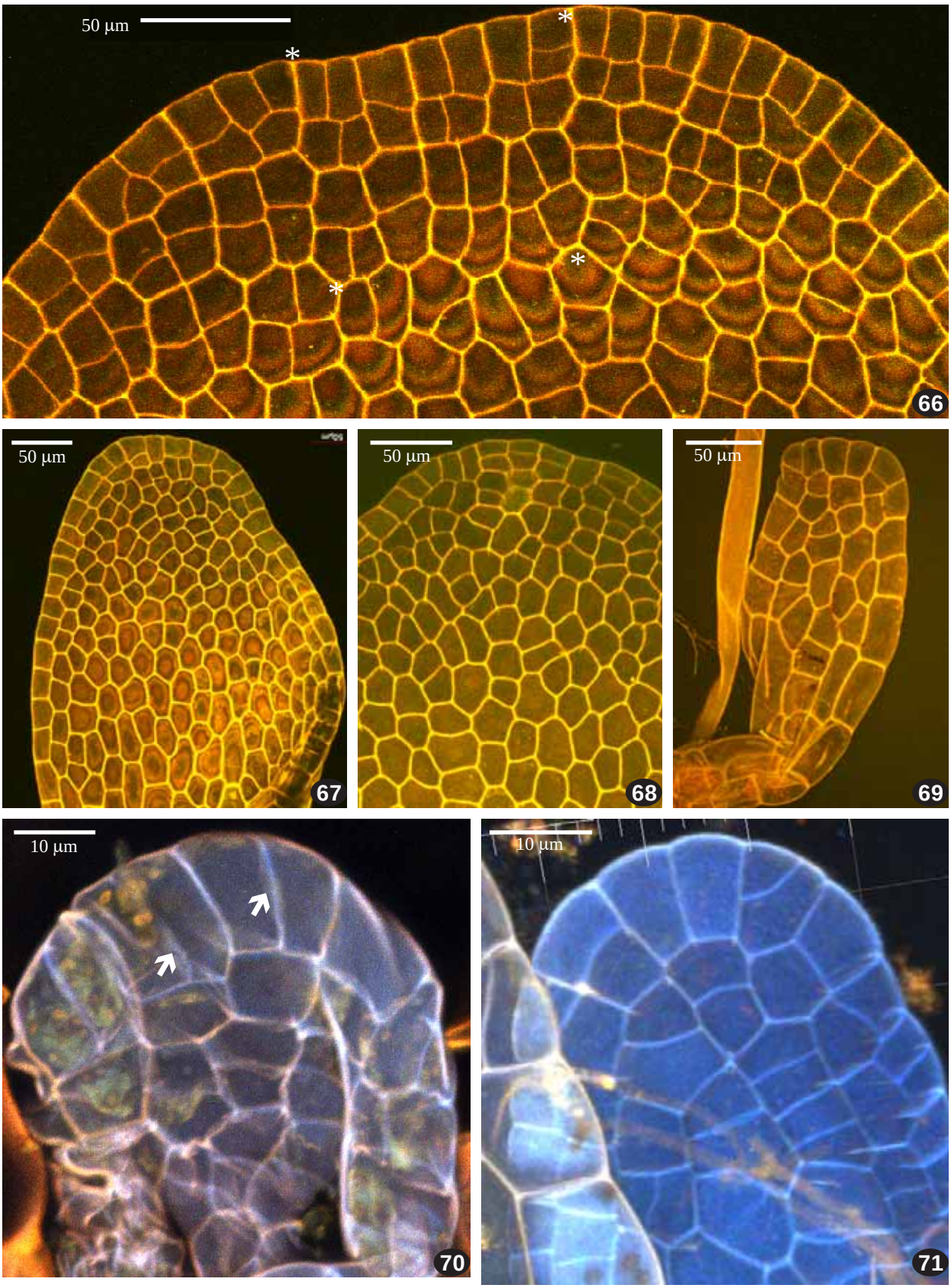
Correns (1899) pointed that both protonematal plates and leaves develop by means of division of bifacial apical cell. He also admitted that at later stages of development the protonematal leaves may have distal ends without apparent bifacial apical cell. However, the only one unequivocal bifacial apical cell was illustrated by him for the 'protonemata' deriving from the lenticular gemma (Correns, 1899, Fig. 85). We consider such structures as young leaves, as discussed above.

Correns (1899, Fig. 87) illustrated one obtrapezoid cell at the apex of protonematal plate similar to those shown in the present paper in Figs. 11-15. Althought this cell is fairly oblique and has an aspect of the leaf apical cell, the areolation produced by it does not fit cell arrangement typically developed in moss leaves (Scheme 1). The areolation in Fig. 87 (Correns, l.c.) is essentially bilaterally symmetric (reminiscenting the areolation described for *Andreaea*, *e.g*. by Goebel (1930) and Crum (2001).

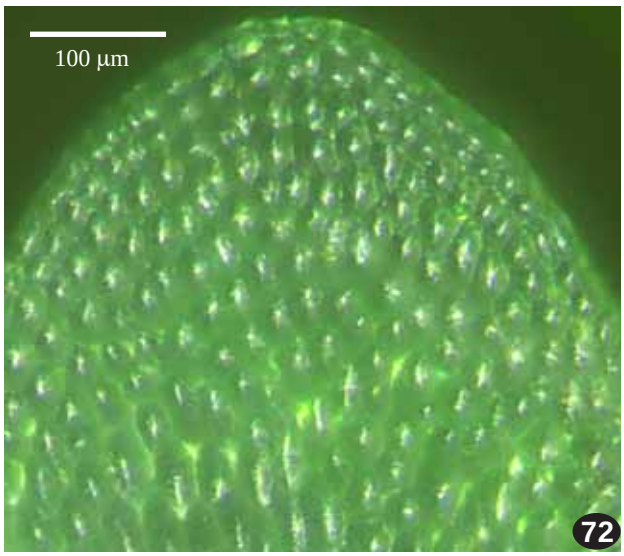
Correns did not specifically discussed if bifacial apical cell is universally present in stem leaves, which is usually consided as a 'default' character state in all mosses. The present study reveals that this is not always so. Some old well-developed leaves have distal part like that shown in Fig. 66, in which the place where the apical cell would be expected (with corners marked by asterisks) is occupied by a sector of regularly differentiated cells. Similar cases, however, occur in smaller leaves and sometimes even very young leaves near stem apex have no bifacial apical cell, but cells, which were called by Correns as "lingulate" (Fig. 70 and cf. with

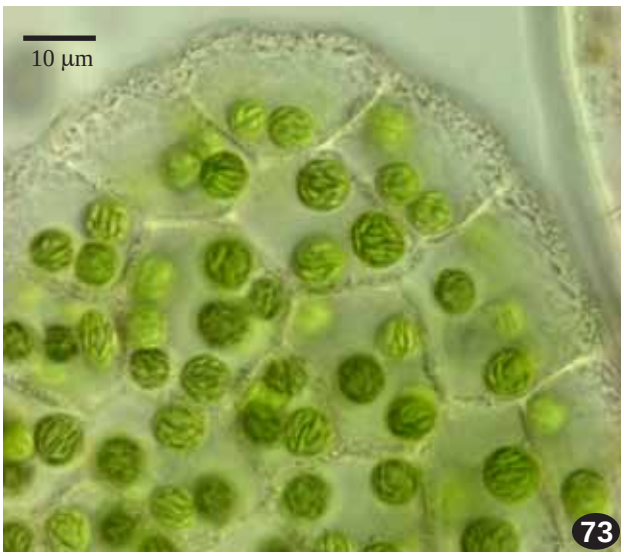


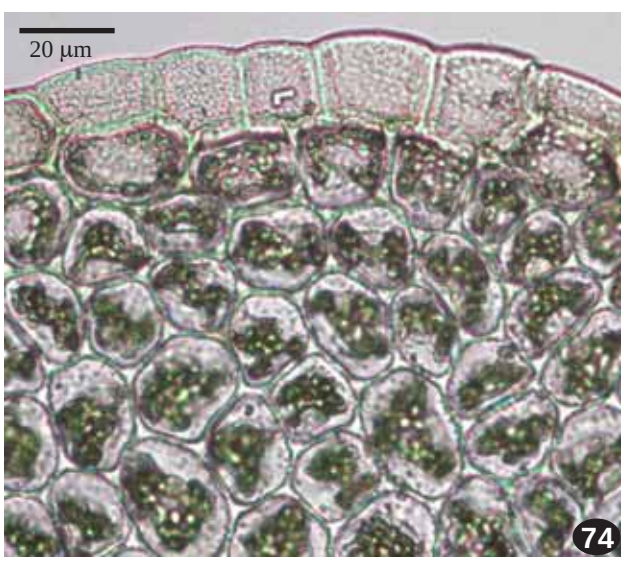
Figs. 61-65. Digitized leaf lamina areolation of *Oedipodium griffithianum* with cells marked by dots of different color: octagonal – blue; heptagonal – yellow; hexagonal – red, pentagonal – green, tetragonal – black. Note the absence of regular groups of hexagonal cells as compared with leaves in Figs. 57-60.

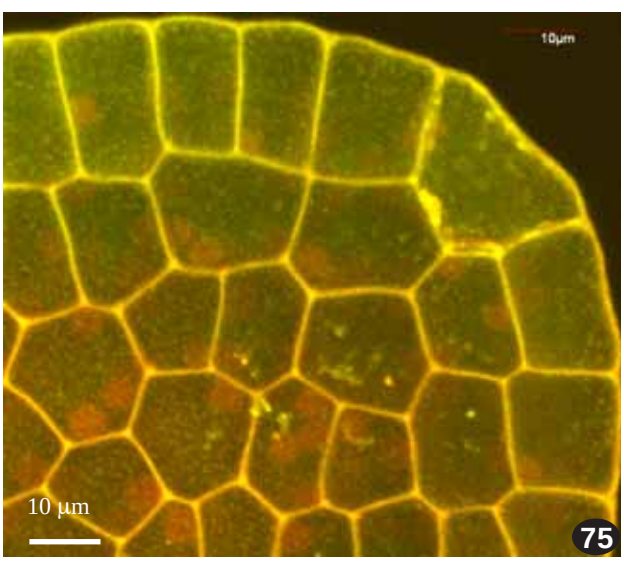


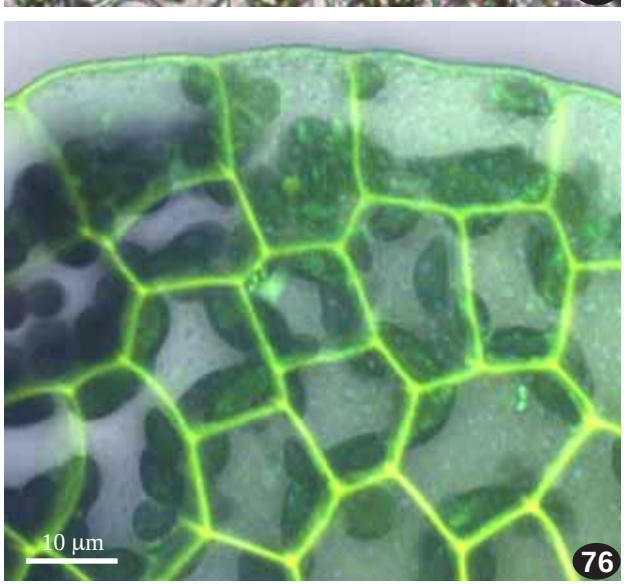
Figs. 66-71. Stem leaves of *Oedipodium griffithianum* showing apical part without apparent bifacial apical cell. Leaves 70-71 were taken from apical part of well developed broad-leaved plants, however no apparent bifacial cells were found. In Fig. 70 two equal uppermost cells recently underwent equal divisions (arrowed).



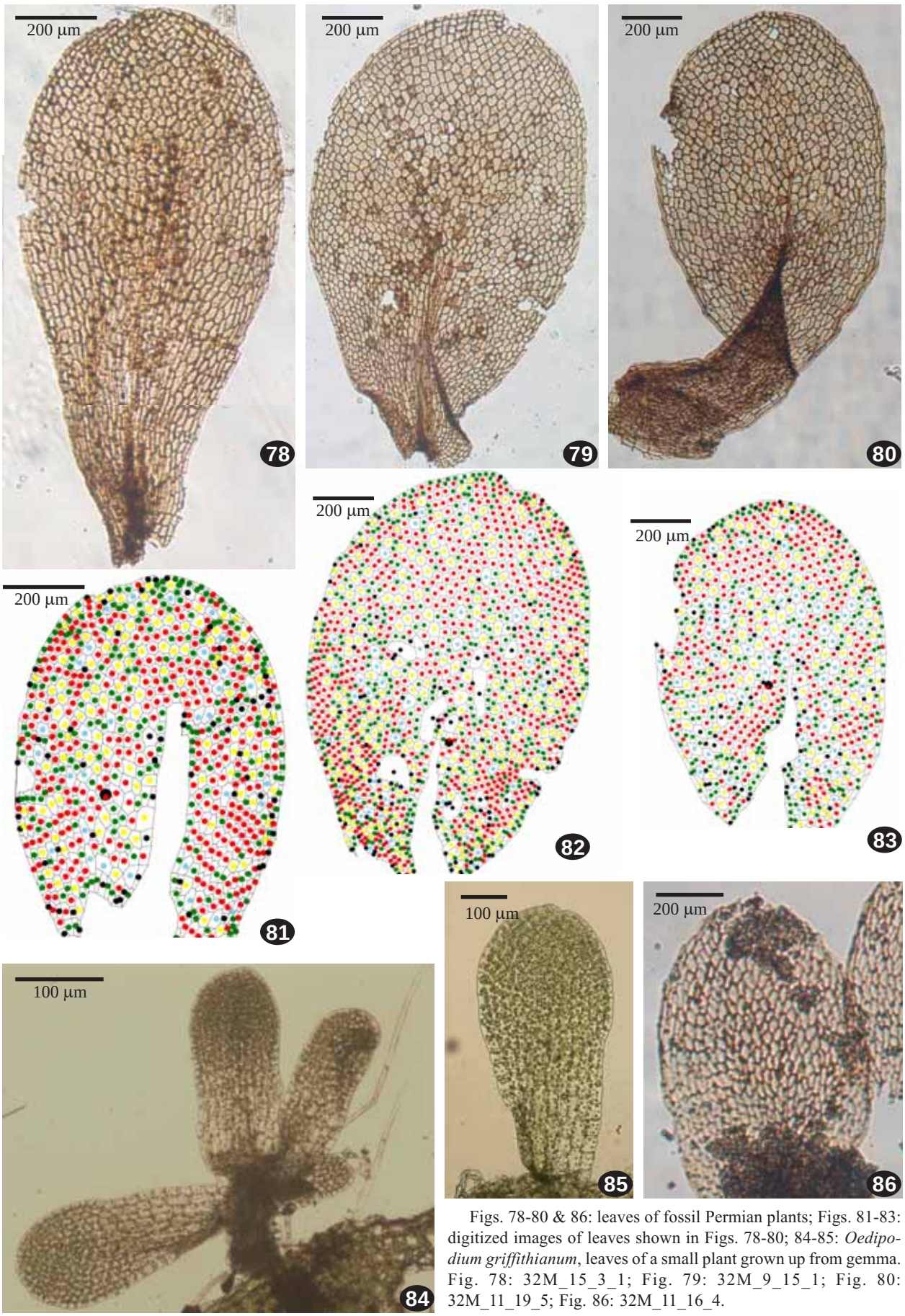








Figs. 72-77. Leaves of *Oedipodium griffithianum* showing areolation in subapical part; pictures were made: 72 – under stereomicroscope, 73 – under compound light microscope, from living plant; 74 – under compound light microscope, from herbarium material; 75 – under LCSM, from living material, with berberin staining. Figs. 76-77 illustrate apparent apical cell surrounded by 5 and 6 cells correspondingly.



Figs. 78-80 & 86: leaves of fossil Permian plants; Figs. 81-83: digitized images of leaves shown in Figs. 78-80; 84-85: *Oedipodium griffithianum*, leaves of a small plant grown up from gemma. Fig. 78: 32M_15_3_1; Fig. 79: 32M_9_15_1; Fig. 80: 32M_11_19_5; Fig. 86: 32M_11_16_4.

Fig. 69). We cannot totally reject the artifact from the cultivation, but, at least sometimes, such 'wrong' stem leaves did appear on broad-leaved plants and they looked rather similar to those found in nature. This is certainly not always so and in some leaves the apical cells is apparent, albeit usually surrounded by 4-6 cells (Figs. 76-77).

Summing up, the structural difference between protonematal plates and leaves in *Oedipodium* is extremely subtile. The more observations are made, the more similarities appear. Contrary to that, in *Sphagnum* and *Tetraphis* there is little in common between the leaf lamina areolation and the cell areolation of thallose protonemata.

Gemmae are especially enigmatic in *Oedipodium*, combining properties of leaf (the presence, albeit occasional, of bifaciual apical cell, Fig. 27) and of protonemata, as they are able to produce leafy shoots. Having such potentials, gemmae might serve as a good model object, considering that from the developmentalistic approach, the stem, leaf and protonema are developed from 'the stem cells' of diffenret types (Kofuji & Hasebe, 2014).

Cell walls

There is a temptation to correlate extreme plasticity of leaf development in *Oedipodium* with its cell surface structure. The granulose cellular surface is well-known in *Oedipodium*, however, it probably was never shown that such granulose structure exists also on internal cell walls (Fig. 73). Cell walls can be assumed as rather soft, easily modifiable by pressure from neigboring cells, causing dents at places of their contacts (Fig. 74). If this proposition is true, it may explain that in living state cells look quite inflated (Fig. 72), provinding plants with 'juicy' (Hooker & Taylor, 1827) or 'fleshy' (Crum, 2007) texture. Further investigations are needed for better understanding of *Oedipodium* cuticle properties.

Comparison with other mosses

As it was shown above, *Oedipodium* has a number of unique features in its leaf development. The unusual growth pattern results in the development of leaves with very narrow base. Among other mosses, a more or less narrowly attenuate base occurs in Splachnaceae and Mniaceae, but both these groups are characterized by distinct sectors of their leaf areolation (Figs. 57-58 & 60) observed during the development.

Maximal similarity with *Oedipodium* can be demonstrated in some Protosphagnalean fossils dated from the Upper Permian. The latter was recently a subject of publication of Maslova *et al.* (2012) and Maslova & Ignatov (2013). The problematic taxonomy of Protosphagnalean fossils at the generic level precludes their exact naming so far, as discussed by Maslova & Ignatov (2013), while the position among the order Protosphagnales is unequivocal.

Being fragmentary, the Protosphagnalean fossils provide only a limited number of characters for a comparison, but some of them are conspicuously similar to that observed in *Oedipodium*:

1) leaf shape (Figs. 78-80), spatulate, with gradually attenuate long base (cf. leaf shape of *Oedipodium* from Figs. 61-65);

2) subconduplicate young leaves (Figs. 87, 89), similar to young leaves of *Oedipodium* (Figs. 5, 42);

3) leaf apical cell is unapparent, and its place is occupied by a pair of elongate rectangular cells (Fig. 87) [compare with Figs. 68-69] or apical cell is surrounded by five cells (Fig. 88);

4) cuticle surface is finely granulose (Fig. 88), compare with Figs. 73-74 and 76-77; this character requires additional studies, as fossil material may posses numerous artifacts.

5) one ovoid body found in the same deposits with the leaves (Fig. 90) of Protosphagnalean fossils is quite similar to lencticular gemmae of *Oedipodium* in size and shape and has a number of somewhat enlarged cells at margin, a reminiscence of the *Oedipodium* case (Figs. 28-29).

The differences in leaf morphology observed between the Protosphagnalean fossil material and *Oedipodium* include:

1) more apparent groups of the hexagonal cells (Figs. 81-83);

2) leaves of Protosphagnalean mosses have a very narrow insertion, not overlapping each other by their decurrent bases (Figs. 92-94). Although mature leaves of *Oedipodium* have normally a decurrent base, the unusually narrow base of young leaves requires a detailed study of this character.

At the moment, no final conclusion is possible for the putative affinity of *Oedipodium* with Protosphagnalean mosses, and their similarity requires additional attention. Characters mentioned above in the comparison of *Oedipodium* with the Permian plants are not widely used and accepted in modern day moss systematics, and their potential usefulness remains unknown.

Finally, a certain caution is needed in this comparison because it is difficult to exclude some anomalous growth pattern caused by cultivation. However out attempts to find any difference between living and herbarium material for the leaf apical cell and the lamina areaolation gave nothing.

Acknowledgements

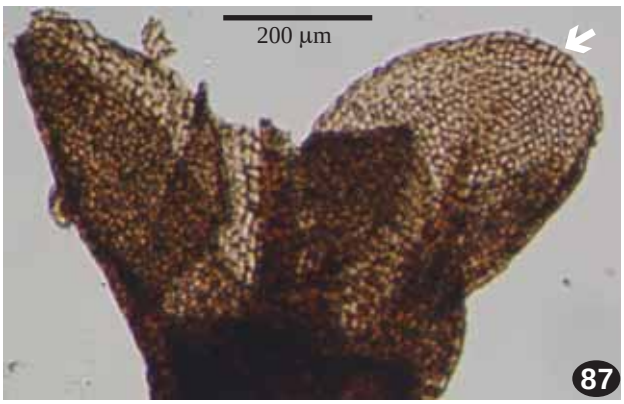
We are grateful to Benito Tan for helpful discussion and English correction. The work was partly supported by RFBR 13-04-01592.

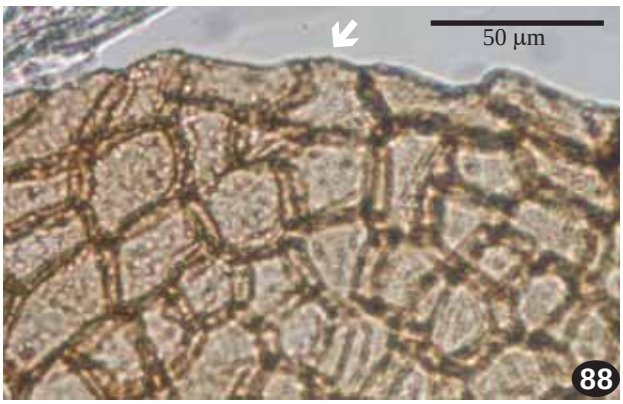
Literature Cited

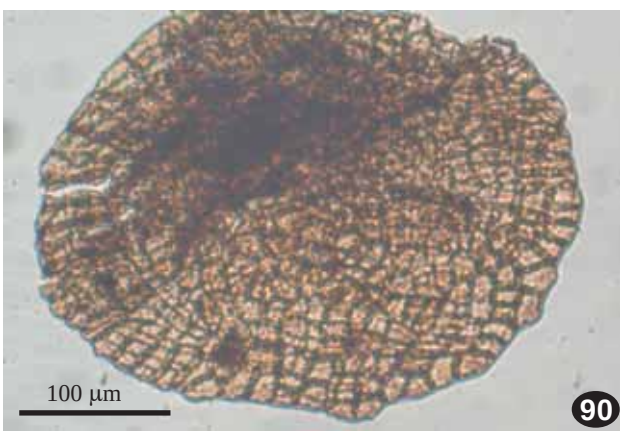
BROWN, R.C., B.E. LEMMON, M. SHIMAMURA, J.C. VILLARREAL & K.S. RENZAGLIA. 2015. Spores of relictual bryophytes: Diverse adaptations to life on land. – *Review of Palaeobotany and Palynology* **216**: *1–17.*

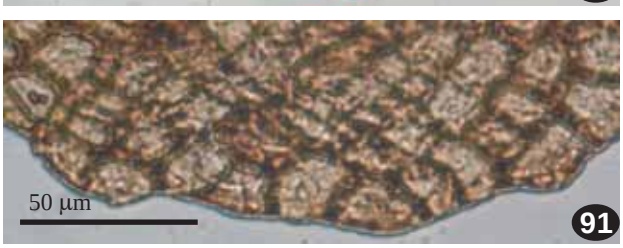
BRUCH, P. & W. P. SCHIMPER. 1844. Fasc. 23–24. *Oedipodium, Tayloria, Dissodon, Tetraplodon, Splachnum, Orthodontium, Mielichhoferia. – In: Bryologia Europaea. E. Schweizerbart, Stuttgart*.

CHANG, Y. & S.W. GRAHAM. 2011. Inferring the higher order phylogeny of mosses (Bryophyta) and relatives using a large, multigene plastid data set. – *American Journal of Botany* **98**(5): *839–849*.









Figs. 87-94. Fragments of Permian fossil mosses, showing similarities with *Oedipodium*: 87 & 89: small, more or less conduplicate leaves; pair of cells in the position of leaf apical cell is arrowed in Fig. 87; 88: leaf apical cell surronded by five cells; 90-91: a putative lenticular gemma, with some marginal cells looking bifacial; 92-94: stem fragments showing narrow leaf bases or their traces.

87: 32M_1_51_1;
88: 32M_14_9_3;
89: 32M_9_19_1;
90 & 91: 32M_6_20_1;
92: 32M_12_11_1;
93: 32M_A1_1;
94: 32M_1_12_8.

CORRENS, C. 1899. Untersuchungen über die Vermehrung der Laubmoose. – *Gustav Fischer, Jena, xxiv + 472 pp.*

COX, C.J., B. GOFFINET, N.J. WICKETT, S.B. BOLES & A.J. SHAW. 2010. Moss diversity: a molecular phylogenetic analysis of genera. – *Phytotaxa* **9**: *175–195*.

CRUM, H.A. 2001. Structural diversity of bryophytes.– *The University of Michigan Herbarium, Ann Arbor., 379 pp.*

CRUM, H.A. 2007. Oedipodiaceae.– *In: Flora of North America Editorial Committee (eds.) Flora of North America North of Mexico. Vol*. ***27****: 116–117*.

DONSKOV, D.G. 2015. Moss leaf morphogenesis and its correlation with the leaf shape in Funariaceae (Bryophyta). – *Arctoa* **24**: *503–508.*

DUCKETT, J.G., J.B.W. FLETCHER, H.W. MATCHAM, D.J. REED, A.J. RUSSELL & S. PRESSEL. 2004. In vitro cultivation of bryophytes: a review of practicalities, problems, progress and promise. – *Journal of Bryology* **26**: *3–20*.

FREY, W. 1970. Blattentwicklung bei Laubmoosen. – *Nova Hedwigia* **20:** *463-556.*

FREY, W. 1972. Entwicklungsgeschichte des Blattzellnetzes bei Leskeaceen und Thuidiaceen (Musci). – *Nova Hedwigia* **23**(1): *159-170*.

GOEBEL, K. 1930. Organographie der Pflanzen. 2 Teil. – *Jena, Verlag von Gustav Fischer. 643-1378 s.*

HOOKER, W. J. & T. TAYLOR. 1827. Muscologia Britannica (ed. 2). – *Longman, Rees, Orme, Brown & Green, London, xxxvii + 272 pp.*

HYVÖNEN, J., S. KOSKINEN, G.L. SMITH MERRILL, T.A. HEDDERSON & S. STENROOSE. 2004. Phylogeny of the Polytrichales (Bryophyta) based on simultaneous analysis of molecular and morphological data. – *Molecular Phylogenetics and Evolution* **31**: *915–928.*

IGNATOV, M.S., E.A. IGNATOVA & V.YA. CHERDANTSEVA. 2006. *Oedipodium griffithianum* (Oedipodiopsida, Bryophyta) – new species and new class for Russian flora. – *Arctoa* **15**: *211–214*.

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

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.*

KOFUJI, R. & M. HASEBE 2014. Eight types of stem cells in the life cycle of the moss *Physcomitrella patens*. – *Current Opinion in Plant Biology* **17**: *13–21.*

KOZUB, D., V. KHMELIK, Yu. SHAPOVAL, V. CHENTSOV, S. YATSENKO, B. LITOVCHENKO & V. STARYKH. 2008. Helicon Focus Software. – *http://www.heliconsoft.com*

LIGRONE, R. & J.G. DUCKETT. 2011. Morphology versus molecules in moss phylogeny: new insights (or controversies) from placental and vascular anatomy in *Oedipodium griffithianum*. – *Plant Systematics and Evolution* **296**: *275–282.*

LORENTZ, P.G. 1864. Studien über Bau und Entwicklungsgeschichte der Laubmoose. – *Lorentz P.G., Moosstudien*: *1–36*.

MASLOVA, E.V., YU.V. MOSSEICHIK, I.A. IGNATIEV, O.V. IVANOV & M.S. IGNATOV. 2012. On the leaf development in Palaeozoic mosses of the order Protosphagnales. – *Arctoa* **21**: *241–264*.

MASLOVA, E.V. & M.S. IGNATOV. 2013. On costa variation in leaves of fossil Permian Protosphagnalean mosses. – *Arctoa* **22**: *61–68*.

NEWTON, A. E., C. J. COX, J. G. DUCKETT, J. A. WHEELER, B. GOFFINET, T. A. J. HEDDERSON & B. D. MISHLER. 2000. Evolution of the major moss lineages: phylogenetic analyses based on multiple gene sequences and morphology. – *Bryologist* **103**: *187–211.*

POLEVOVA, S.V. 2015. Sporoderm ultrastructure of *Oedipodium griffithianum* (Oedipodiopsida, Bryophyta). – *Arctoa* **24**: *419-430.*

POTTIER, J. 1925. Nouvelles recherches sur le dáveloppement de la feuille des Muscinées. – *Bulletin de la Société Botanique de France* **72**: *629–689, Pl. 11–14*.

SHAW, A.J., P. SZÖVÉNYI & B. SHAW. 2011. Bryophyte diversity and evolution: windows into the early evolution of land plants. – *American Journal of Botany* **98**(3): *352–369*.

SHIMAMURA, M. & H. DEGUCHI. 2008. Sporophyte anatomy of *Oedipodium griffithianum* (Oedipodiaceae). – *In: Mohamed, H., B.B. Baki, A. Nasrulhaq-Boyce et al. (eds.) Bryology in the New Millennium, Kuala-Lumpur*, *319–325.*

SCHIMPER, W.P. 1860. Synopsis Muscorum Europaeorum. – *E. Schweizerbart, Stuttgart., clix + v + 733 pp.*

SPENCE, J.R. & W.B. SCHOFIELD. 2007. Takakiaceae. – *In: Flora of North America Editorial Committee (eds.) Flora of North America North of Mexico. Vol*. ***27****: 42–44.*