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ADDITIONAL OBSERVATIONS ON PROTONEMATA OF *SCHISTOSTEGA PENNATA* (BRYOPHYTA)

ДОПОЛНИТЕЛЬНЫЕ НАБЛЮДЕНИЯ ПРОТОНЕМЫ *SCHISTOSTEGA PENNATA* (BRYOPHYTA)

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Abstract

The protonema of *Schistostega pennata* is well known due to its specific structure, making it luminous in a cat-eye manner. The growth patterns of protonemata are overviewed, including a filiform type that was not previously described for this species. The latter, however, seems to be important for the species, forming "bridges" between soil pieces and thus building its own habitat. The sticky surface of propaguliferous protonemata is also capable to stabilize surfaces where the species grows. Growth of protonemata is studied both in nature and in cultivation, showing outstanding plasticity and ground-cementing capacity.

Резюме

Протонема *Schistostega pennata* хорошо известна благодаря ее уникальным пластинчатым структурам из линзовидных клеток, благодаря которым она светится наподобие кошачьего глаза. Изучены особенности роста протонемы, включая ее нитевидную форму, которая ранее не была описана. В то же время нитевидная протонема позволяет строить "мосты" между фрагментами субстрата, создавая условия для дальнейшего роста пластинок. Клейкая поверхность выводковых тел протонемы также способствует закреплению субстрата. Рост протонемы изучался как в природе, так и в культуре, что позволило подтвердить ее выдающуюся пластичность и способность "цементировать" поверхности, на которых она растет.

KEYWORDS: brood bodies, cell divisions, chloroplasts, ecology, morphogenesis, protonemata, *Schistostega*

INTRODUCTION

Schistostega pennata (Hedw.) F. Weber & D. Mohr, the only representative of the family Schistostegaceae, is a Circum-Holarctic moss species, in general infrequent in most regions of the world, being found in most areas in caves. Recently it became rather common in mesic to humid conifer forests in Moscow Province (Ignatov & Ignatova, 2001) and neighboring areas, where it quickly spread on the soil walls under upturned roots of fallen trunks (Fig. 1). This increase of occurrence is obviously a result of protection in forest reserves.

The luminous protonema of *Schistostega pennata* is very well known, so it became a part of folk stories of the elf, goblins or dragon gold (mentioned, *e.g.,* by Berqvist, 1991; Crum & Anderson, 1981; Glime, 2009) and one of few mosses that deserved protection as a Nature Monument, in Hokkaido, Japan (Iwatsuki, 1977; Kanda, 1988).

Morphology of *Schistostega* and ability of its protonema to shine like a cat eye was elaborately examined in a number of special publications (Vuillemin, 1887; Noll, 1888; Toda, 1918; Gistl, 1926).

The main structure of the protonema of *Schistostega* was carefully described by Vuillemin (1887); however, the subsequent illustration of Noll (1888), reproduced by Goebel (1930) and some other handbooks (*e.g.* Savicz-Lyubitskaya & Smirnova, 1970), became more well known, despite providing only a general appearance of "plane" protonemata. From this picture, as well as from a number of recent illustrations (e.g. Harpel, 2007), and from the description of protonemata as producing plates (Lazarenko, 1955), one may understand this protonema as having a solid structure, which in fact never occurs in this species.

Nehira (1967) studied *Schistostega* in caves in Japan and stated that he did not observe any "plates" described by Goebel (1930). In fact Goebel's description "...Linsenzellen bestehenden Aste breiten sich alle in einer Ebene aus..." [branches formed by lens-shaped cells broadened, forming plane structure] does not really imply a plate. But in any case, any appropriate terminology of *Schistostega* protonema did not appear. Toda (1918) called structures formed by numerous spherical cells on

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long and strong filamentous “stalk” as a “tadpole-shaped protonema,” Vuillemin (1887) described this structure as “buisson lumineux” [luminous thornbush], Gaisberg & Finckh (1926) called it “Palmellaform”, referring to palmella stage in some algae. In the Kerner vor Marilaun’ “Pflanzenleben,” the following description of *Schistostega* protonemata has been given: “From the much branched threads ... numerous twigs rise up vertically, bearing groups of spherical cells arranged like bunches of grapes. ...All the cells of a group lie in one plane, and each of these plants is at right angles to the rays of light...” (translation of F.W. Oliver, cited by Glime, 2009). Ignatov & Ignatova (2001) describing numerous hanging protonemas on soil faces under uprutned roots called it “umbrellas.”

The incongruence is obviously related to the enormous variation in protonemata structure. As it will be discussed below, the shape of aerial part of protonemata is much affected by light intensity and variation of its direction. Discussing this matter we will call its umbrella-like or plate-like or tadpole-shaped or thornbush-like or botryoid-cluster or grape-like structures as simply protonema outgrowth, subdividing it into flat, botryoid and thornbush variants.

Yet, Vuillemin (1887), Correns (1899), Toda (1918), Nehira (1967), Kanda (1971), Edwards (1978) and others, who illustrated protomenata of *Schistostega*, displayed a rather small part of it, not showing its structure in full view and in all its interesting details. So, our additional observations on protonema morphology is one point of this paper. Another point is the study of growth rate in nature and in culture on soil taken from the natural environment. Previous data on this are quite comprehensive, especially those of Toda (1918), but in nature mostly cave populations were collected, not forest ones like in Middle European Russia. Thus, the present paper addresses these items, touching on some related aspects of biology and ecology of the species.

METHODS AND MATERIALS

Most of our observations were conducted in Zvenigorod Biological Station of Moscow State University, ca. 50 km W of Moscow, mainly in spruce, pine-spruce, and spruce-birch forest. Protonema of *Schistostega* was observed also in cultivation in the Main Botanical Garden of Russain Academy of Sciences in Moscow.

In situ protonemata were studied with a 10x handlens. In the biological station, soil pieces with protonemata were also carefully transported to a laboratory where they were studied and photographed under MBS-9 stereomicroscope.

In the botanical garden protonemata were additionally photographed using Olympus SZX16 with the Infinity 4 digital camera. Nuclei positions were detected by DAPI (4',6-diamidino-2-phenylindole) staining of living plants with LCSM Olympus FV1000. DAPI was applied in phosphate buffer (pH=7,1), without any additional treatment, as some compounds commonly used with DAPI for its better penetration into cells greatly affected the chloroplasts. Usually 2 mkl of 0,001%. solution was applied to amount of buffer under 24×24 mm cover glass where some protonemata were placed. Most of big sand grains were removed, though perfect clearing affects protonemata too much, thus some sand grains were left; moreover, they protect the large spherical cells from deformation caused by cover glass pressure. Observations were possible 5 minutes after DAPI addition.

Growth in nature has been a special focus from 8 to 20 July, 2012, when it rained three days a week and the temperature was ca. 25°C daytime, ca. 17°C nighttime, which according to observation in previous years could be assumed as an optimal condition, as rare rain and dry weather reduce the amount of “shiny” areas of protonemata, whereas numerous rains erode the surface, so many populations flush down from the soil walls.

Growth in cultivation was studied in Petri dishes. *Schistostega* grew on soil lumps taken from the native habitats. Several places in every dish were photographed every two days. After photography about half the soil pieces were seeped, so the humidity was about as in the forest, with water condensation every morning on the upper plate. Petri dishes were kept at the windows with diffuse light, at the temperature of a summer house without heating (17–25°C).

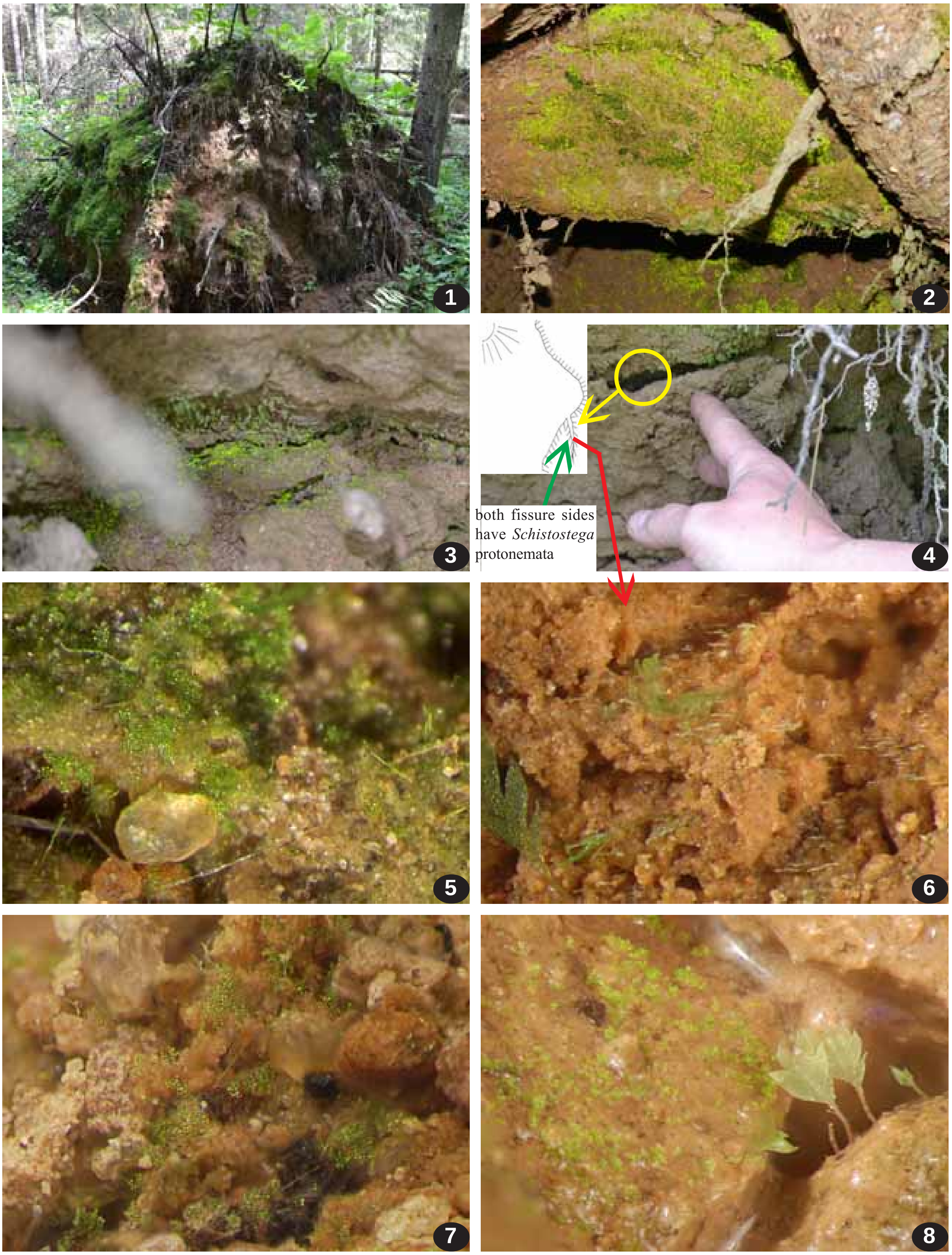
Cultivation continued in the second half of September, with plants collected in the same place on 15 September. The temperature in this case was 17–20°C in both day and night.

OBSERVATIONS

1. **Type of growth regarding direction to the light source**

The known fact on *Schistostega* is that its flat protonema outgrowths develop perpendicularly to the light source (*e.g.* Noll, 1888; Goebel, 1930). Published photographs (*e.g.* Ignatov & Ignatova, 2001) show that they are growing in parallel planes. Toda (1918) found that once formed the protonema of *Schistostega* does not change its direction towards light, being nevertheless positively heliotropous, as new filaments appear and grow mostly towards the light source.

We compared protonemata from different parts of soil walls under upturned roots of fallen trunks (Fig. 1), finding the following patterns. The protonema is rather variable in shape in places of abundant growth on relatively recently appeared and at the same time rather open soil faces. Although partial shade from neighboring trees makes *Schistostega* habitats moderately shaded during most times of the day, most populations receive direct light several minutes to tens of minutes in a sunny day. Although most populations were not faced to the south, the latter position is not totally impossible and occurs



Figs. 1-8. Habitat and growth of *Schistostega pennata*: #1: upturned roots of fallen trunk, a typical habitat of *Schistostega* in Moscow Province; #2: *Schistostega*, photo with flashlight; #3: protonemata near soil fissures and mature gametophores on drier places; #4: flaking off soil clumps, with a fissure suitable for *Schistostega* protonemata growth; #5 & 7: variable shape and orientation of protonemata of *Schistostega* on a relatively open place; #6 & 8: uniformly perpendicular to light source protonemata of *Schistostega* when growing in deep shade (#8 at a different angle would look like #6), cf. #6 also with #24.

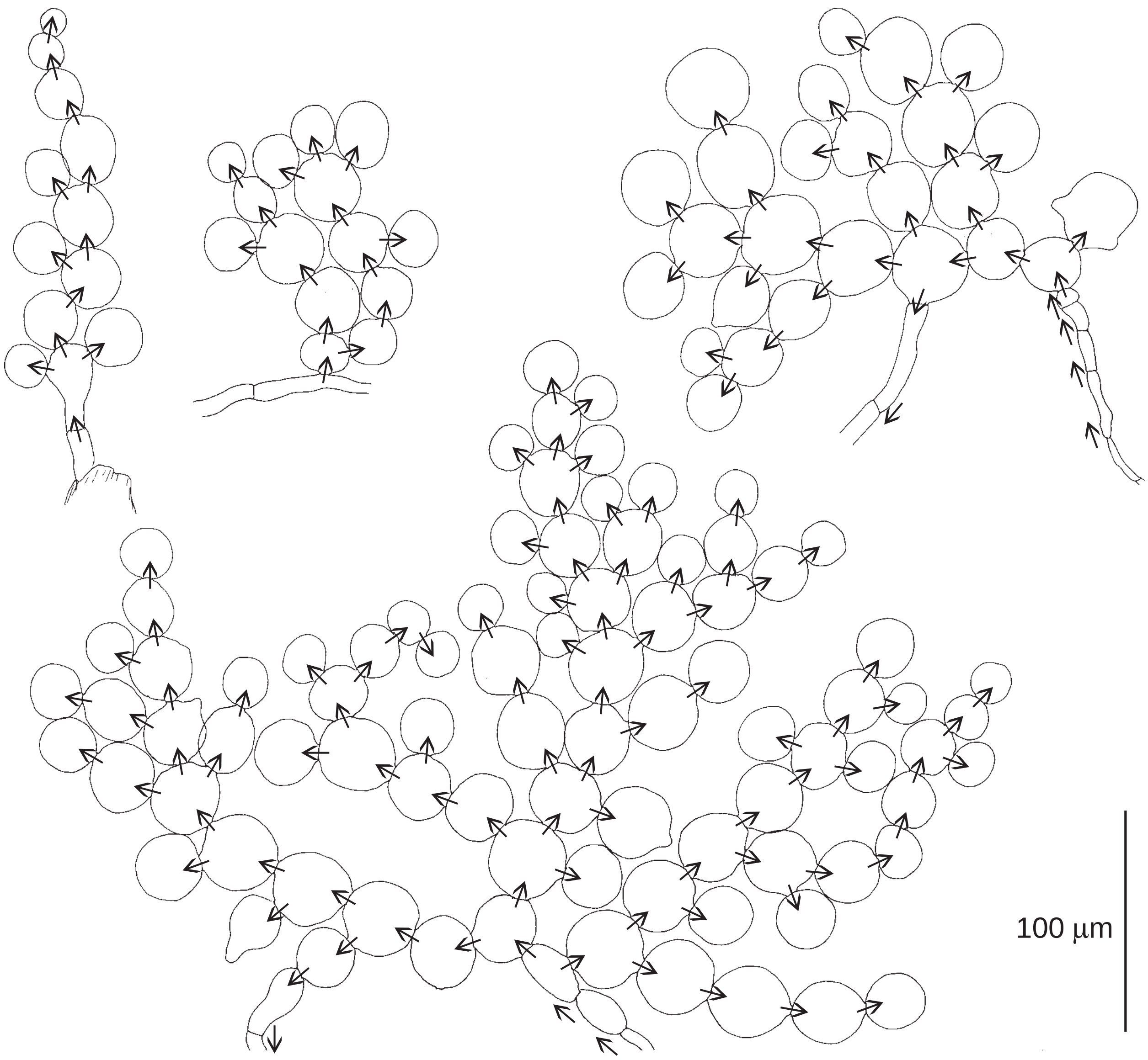


Fig. 9. Average shape of flat protonema outgrowths of *Schistostega pennata*, showing connections between cells, that allows understanding of its growth as shown in Fig. 10. Arrows are only upon cell joinings and show sequence of cell origin.

from time to time on otherwise appropriately shaded upturned roots.

In these "moderately sunny" places protonemata are usually quite abundant (Fig. 2), especially in more wet depths in the distal surfaces of "microcaves" and also strips along soil cracks (Fig. 3) where the wetter and "deeper" areas often have protonemata, whereas drier areas around them often have well-developed gametophores. In such places of abundant growth, the shape of the protonema is quite variable, including both filaments and protonema outgrowth structures of various shape (Figs. 5, 7).

Study of soil cracks surprisingly revealed that *Schistostega* occurs also in narrow fissures behind flaking off soil clumps. Careful removal of soil pieces (Fig. 4) allows discovering plants in such fissures that are only a few millimeters wide and a few centimeters deep. Especially surprising was seeing protonemata not only on the side of a main soil mass (*e.g.* from side of tree trunks), but also on opposite faces that seem never to receive any direct light, and can be illuminated only from the protonema from the wall opposite to it! In such fissures protonema units look much more uniform: they appear as low lamellae, strictly perpendicular to the "entrance" to a fissure, and usually looking quite pale (cf. Fig. 6) in a side view. Scattered gametophores may occur in such places as well, but they are strongly underdeveloped, being represented by sterile individuals, usually with just a few leaf pairs.

Similar growth as low and more or less regular lamellae (Fig. 8) seems to be correlated with deeper shade, as far as we can understand from the comparison on a num-

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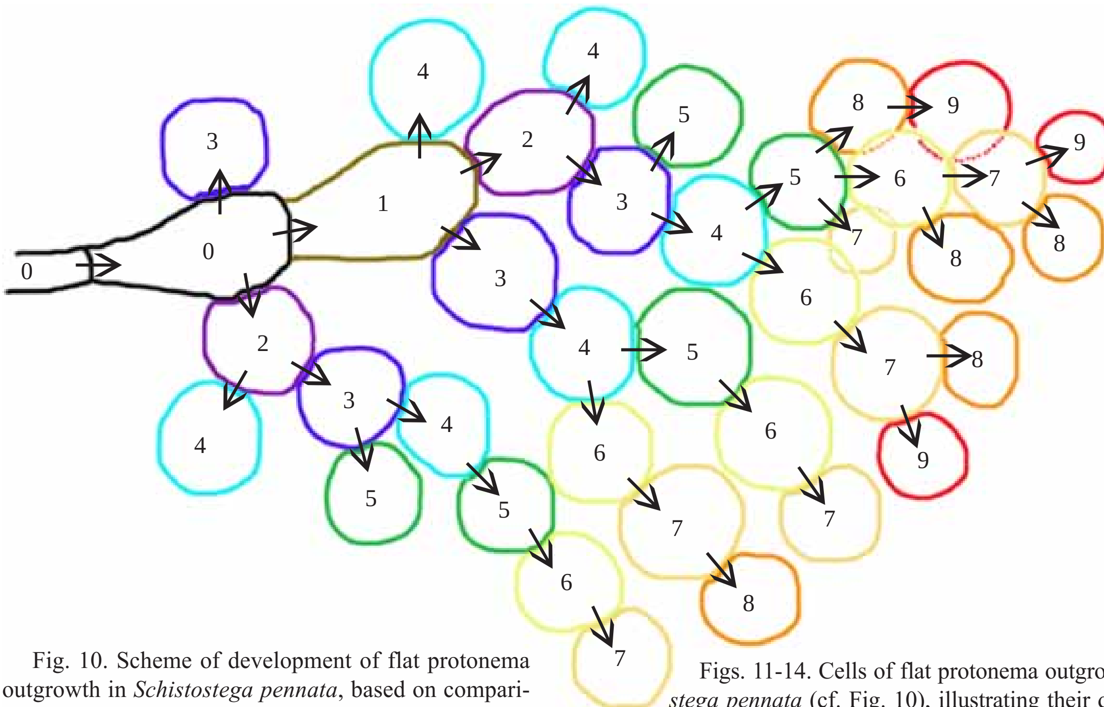
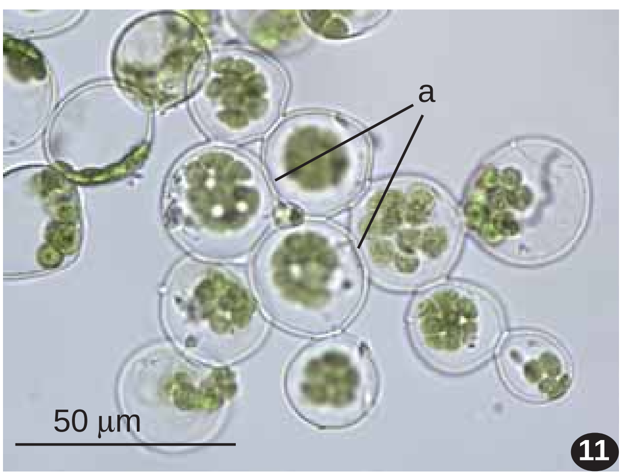
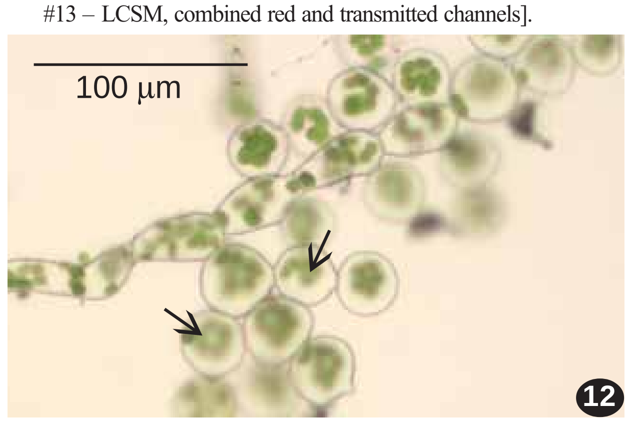
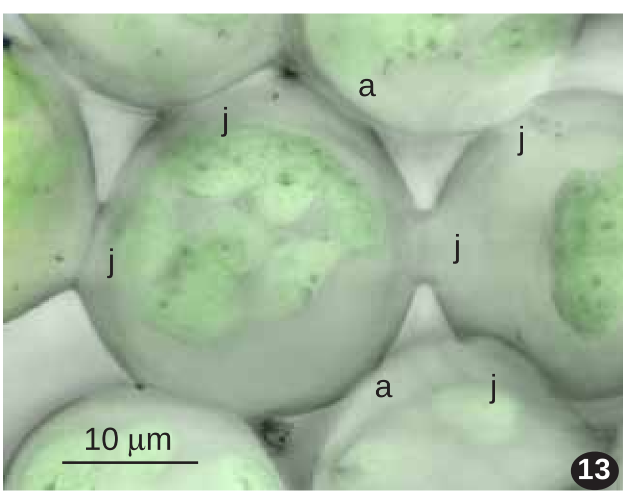


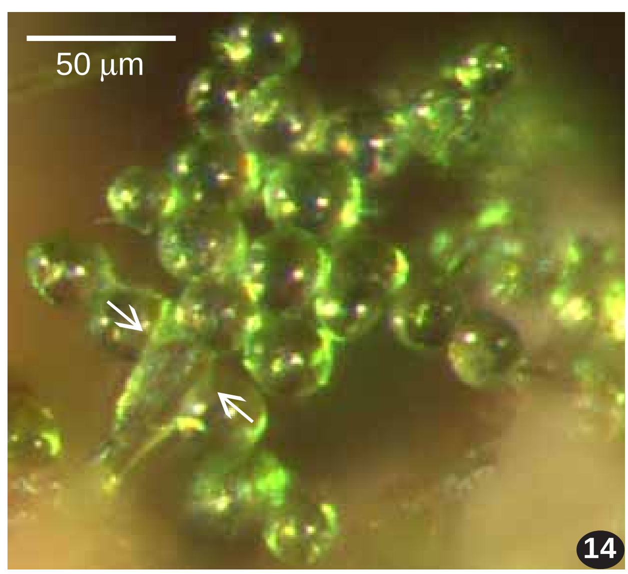
Fig. 10. Scheme of development of flat protonema outgrowth in *Schistostega pennata*, based on comparison of different stages (cf. Fig. 9). Colors (in a rainbow order) and numbers approximate the age of cells, which in the case of optimal growth condition (see text) may be equal to one day. Arrows are only upon cell joinings and show sequence of cell origin.

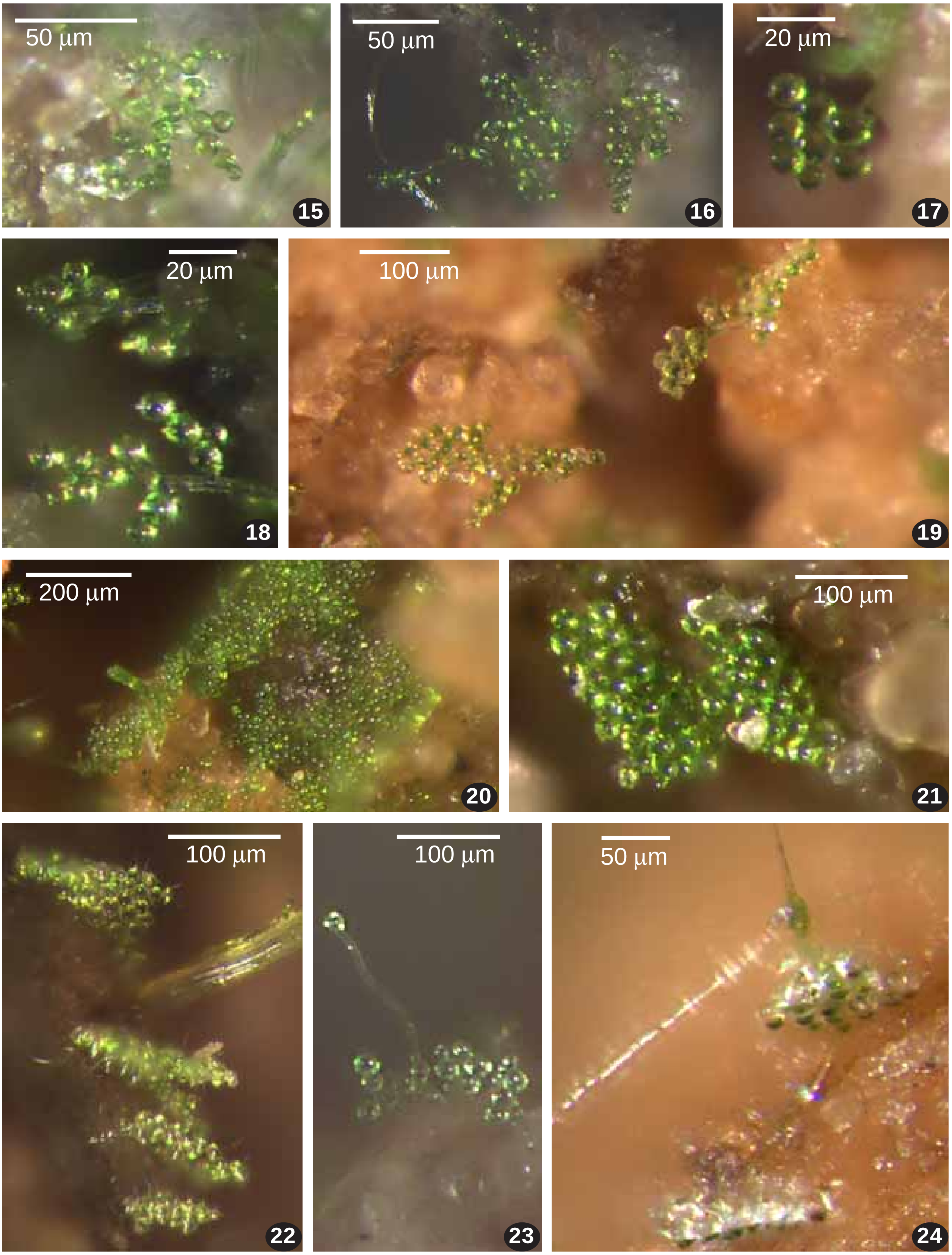
Figs. 11-14. Cells of flat protonema outgrowth of *Schistostega pennata* (cf. Fig. 10), illustrating their development on clavate-elongate cells (#12 & 14) and showing cell joinings (j) and adjacencies (a) (#11 & 13). Note fairly symmetrical positions of spherical cells in case of trichotomous branching (#12 & 14, arrowed, and cf. Fig. 27). [##11, 12, 14 – light microscopy; #13 – LCSM, combined red and transmitted channels].



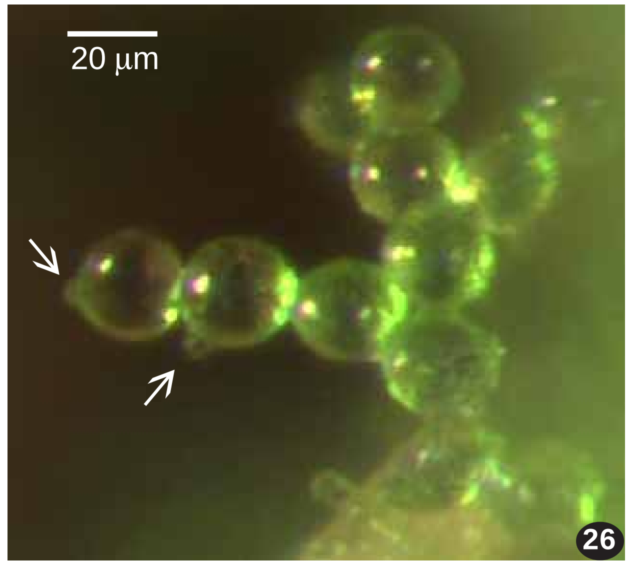


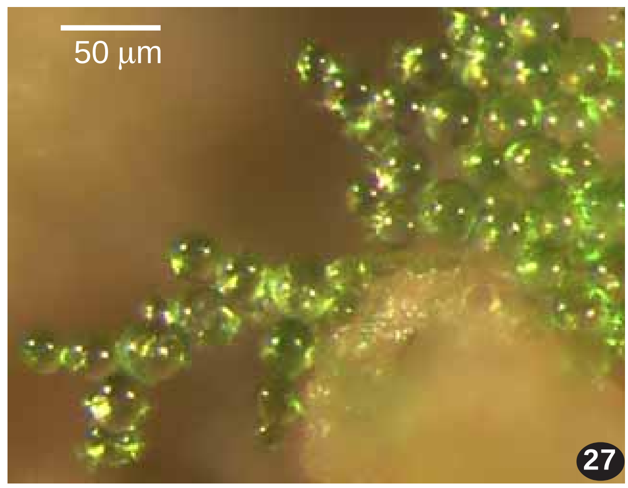






Figs. 15-24. Variation in shape of protonema outgrowths of *Schistostega pennata.* More or less flat protonema outgrowth can be more loose (#15, 16, 18, 19), or dense (#20, 21, 22), have conspicuously parallel position (#20, 21, 22, 24) or not especially so (#19). In most cases flat "peak-like" protonema outgrowths are sitting on procumbent filaments (#16 right), although not rarely erect "stalks" can be seen as well (#18, 19). Distal cells of flat protonema outgrowths sometimes produce filamentous structures (#16 left, & 23), and brood bodies (#24). At many angles cells look rather uniformly green, and only side view (#24) allows seeing chloroplasts grouped on one side of cells (cf. Fig. 6).





Figs. 25-27. Protonema of *Schistostega pennata.* #25: trichotomously branched cell at base of flask-shaped cell terminated with gemma, cf. Fig. 24; #26-27: upright thornbush-shaped protonema outgrowths composed of spherical cells only; note new cell initials, a yeast-mode evagination (arrowed), cf. Figs. 37, 38, 40.

ber of soil pieces under the stereomicroscope in the laboratory.

2. **Development of flat protonema outgrowths**

Noll's picture illustrates the *Schistostega* protonema rather as a solid plate structure (Noll, 1888; Goebel, 1930; Savicz-Lyubitskaya & Smirnova, 1970) and therefore quite exceptional among mosses. Some superficial observation with handlens and stereomicroscope may be interpreted in the same way (Figs. 20-23). However, the study of joints between cells shows that the branching of *Schistostega* protonemata is not principally distinct from that in other mosses.

In most cases flat protonema outgrowth forms as follows. The filamentous caulonemata of *Schistostega* at first modify into inflated clavate cells (Figs. 10, 14, 37) that continue a chain of 1-3 cells of the same shape and then change to a spherical cell.

The clavate cell produces at its distal end laterally two spherical cells in precisely opposite positions, although cells appear one by one (Figs.10, 12, 14).

Further divisions of spherical cells, both terminal and lateral, can occur in three ways: it can be monotomous, dichotomous and, in fewer cases, trichotomous.

The trichotomous branching seems to be essential for protonema positioning in more or less one plane (Figs. 12, 14). Chloroplasts and nuclei (see below) grouping at the cell wall distal from the light source seems to facilitate fixation of the position where lateral spherical cells should appear. Note that such chloroplast arrangement is a characteristic not only of spherical cells of the protonema outgrowth, but also of the elongate cell, including the "stalk" of flat protonema outgrowth (Fig. 34), as well as of some other cells of the *Schistostega* protonema (Fig. 33). The exact identification of the new cell position seems possible in *Schistostega* because of cell division by a yeast-mode evagination (cf. Figs. 26, 37, 38, 40). At least, a start from a fairly small area allows a new cell to find a "right" position. A relative angularity of protonema cells (Figs. 37, 44, *etc.*) also likely participates in marking out the new cell position.

In addition, the plane structure of flat protonema outgrowth is dependent on a somewhat sticky cell surface (which can be observed also for gemmae by touching plants with the needle), thus two neighboring cells touching each other by their sides appear to be somewhat fixed one against another (Figs. 11, 13, *etc.*). In addition, spherical cells of protonemata are able to reach their final size rather gradually, increasing from 15-25 µm near the flat protonema outgrowth margin to larger cells, 25-35(-40) µm in its central part (cf. Figs. 9, 11, 14, *etc.*).

However, both sticky surface and continuous growth are not specific for flat protonema outgrowth and take

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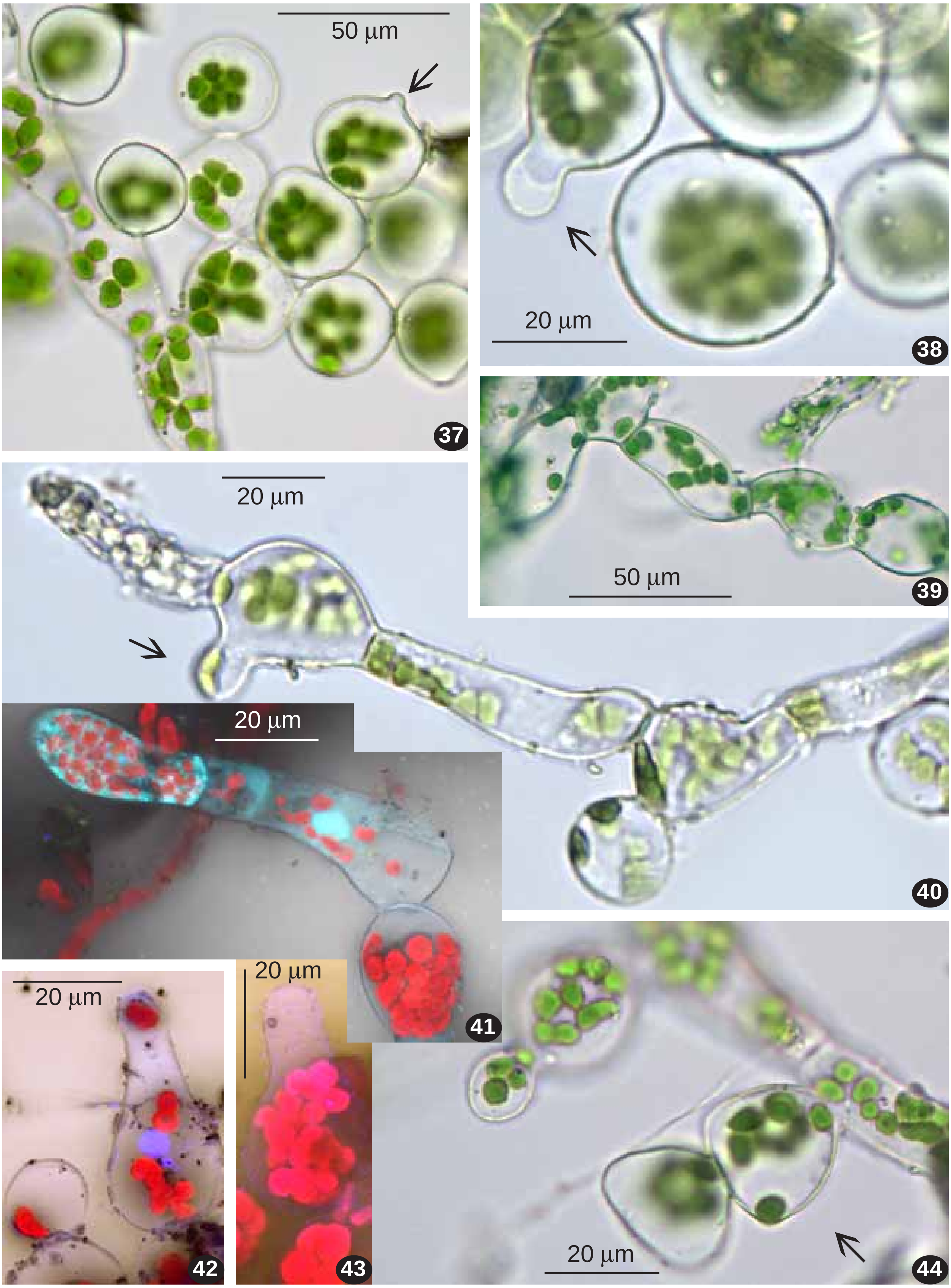
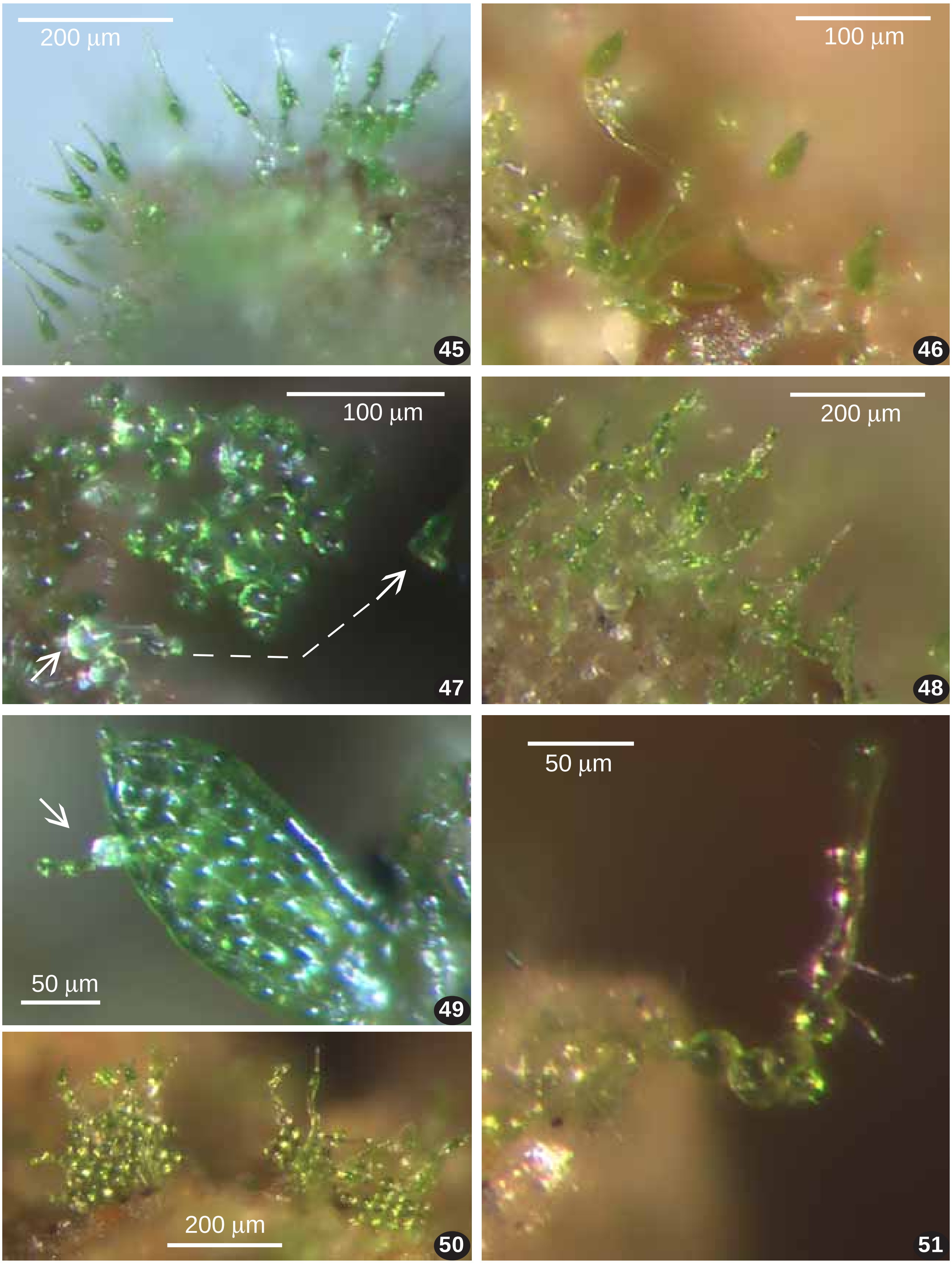
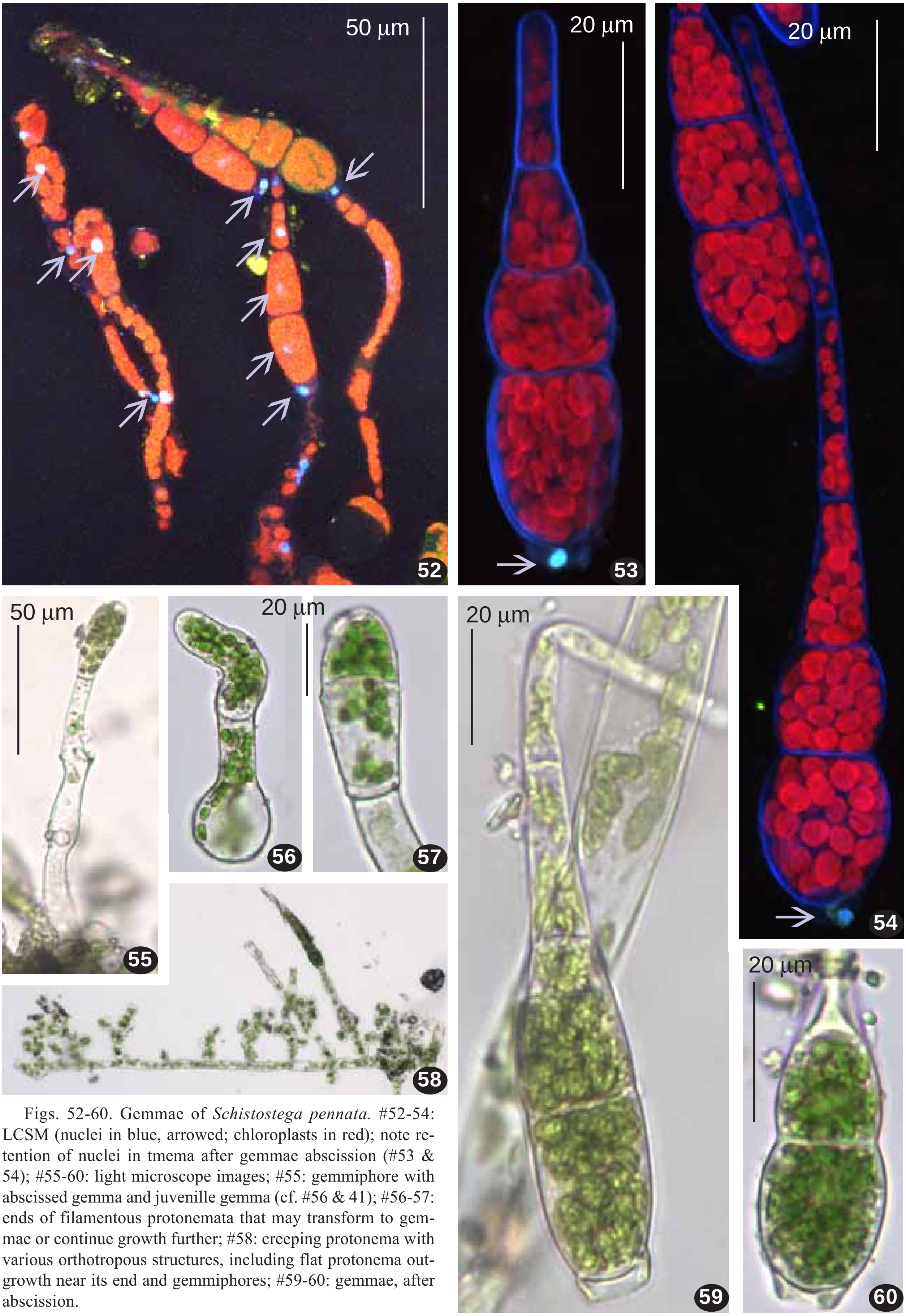
Figs. 28-36. Chloroplasts and nuclei arrangement in flat protonema outgrowth cells of *Schistostega pennata*, except #33: filamentous protonema at base of gemmae; #34: “stalk” of flat protonema outgrowth. #29 & 32: light microscope, the rest: LCSM, with DAPI-stained living protonemata, showing nuclei in blue and chloroplast autofluorescence in red. Chloroplasts are grouped at cell wall opposite to light source (#28, arrow). At front view, chloroplasts are arranged in “rosettes” with “hole” in the middle, as seen under light miscroscope, and “hole” is filled by nuclei, as appeared from the DAPI staining. #33-34 show chloroplasts concentrated at the distal cell wall not only in spherical cells. Scale bars are 20 µm for all pictures.



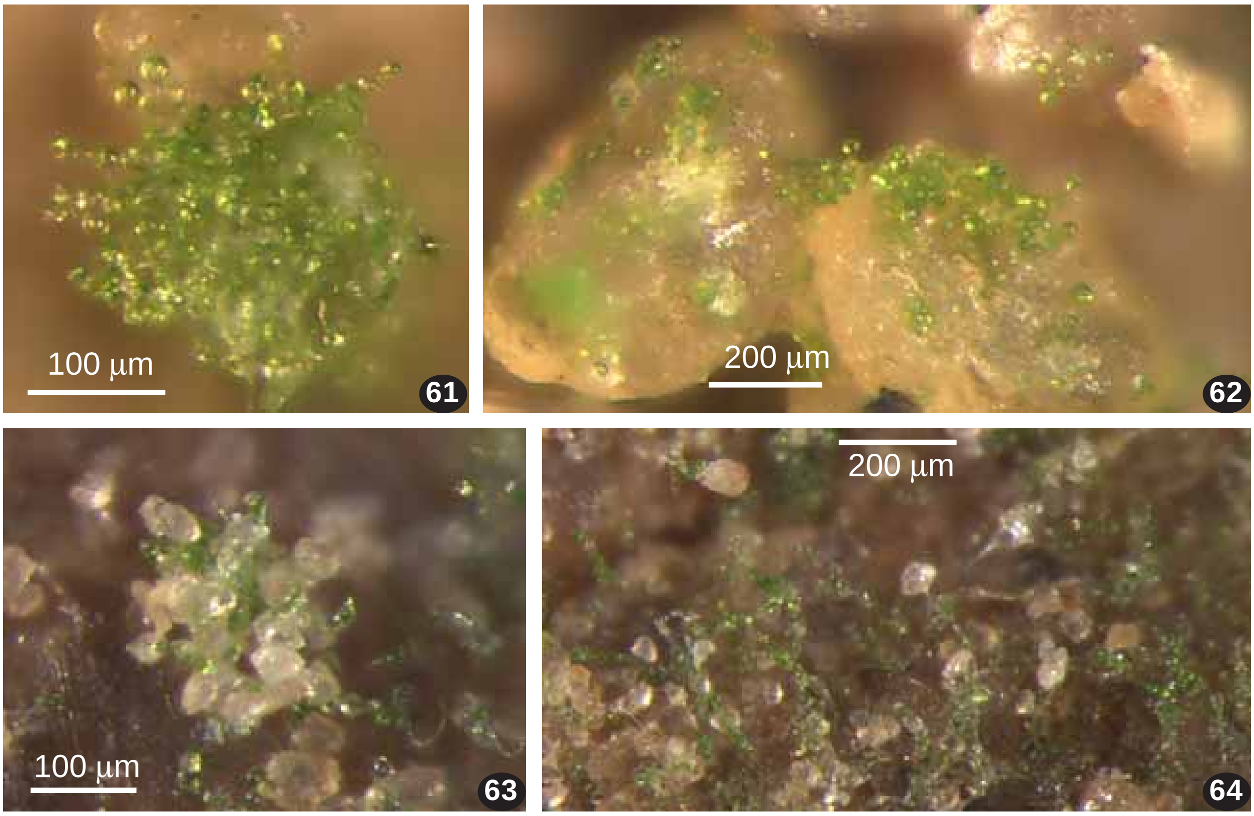
Figs. 37-44, light microscope, except #41-43 (LCSM). Cell variation within protonema outgrowths of *Schistostega pennata*, showing spherical, #37-38, obtriangular, #44, inflated-tortuous, #39, and bottle-shaped, #42-43, variants. #40 and 41 illustrate ends of filamentous and potentially gemmiferous protonemata, with young cells with pellucid preplastids (that have red autofluorescence of chloroplasts). #37, 38, 40 display sequence of new cell formation on flat protonema outgrowth (arrowed).



Figs. 45-51. Gemmiferous protonemata of *Schistostega pennata*: #45 shows gemmae in dense growth; #46 & 48: gemmae at various stages of development; #47: gemmiphores, with gemmae fallen off and lying somewhat aside (arrowed); #49: young leaf of *Atrichum undulatum*, a species commonly associated with *Schistostega*, with flask-shaped gemmiphores on it, already without gemmae; #50: flat protonema outgrowth with gemmae and potential gemmiphores at their edges; #51: filamentous orthotropous protonemata (cf. Fig. 79-80).



Figs. 52-60. Gemmae of *Schistostega pennata*. #52-54: LCSM (nuclei in blue, arrowed; chloroplasts in red); note retention of nuclei in tmema after gemmae abscission (#53 & 54); #55-60: light microscope images; #55: gemmiphore with abscissed gemma and juvenille gemma (cf. #56 & 41); #56-57: ends of filamentous protonemata that may transform to gemmae or continue growth further; #58: creeping protonema with various orthotropous structures, including flat protonema outgrowth near its end and gemmiphores; #59-60: gemmae, after abscission.



Figs. 61-64: Various growth of *Schistostega pennata* protonema: #61-62: regeneration from the protonema outgrowth fallen on soil: spherical cells start to produce upright chains of lens-shaped cells or filamentous protonema (cf. #56); #63-64: gemmiferous protonemata gluing up sand grains.

place in botryoid and thornbush-shaped protonema outgrowths of *Schistostega* as well.

Thus we consider trichotomous branching to be the most important constructive element of flat protonema outgrowth. Similarly, the bases of gemmiferous structures that are not rarely developed at the distal side of flat protonema outgrowth always have the trichotomous structures that look absolutely necessary to hold (and keep in a correct direction) this rather heavy group of flask-shaped cells continued in tmema and then several cells of gemmae (Figs. 24, 25, cf. Figs. in page 11).

This structure of protonemata shown in Figs. 10, 11 and 14 seemingly can be assumed as the most "developed" in terms of structural complexity in this species, although not the most common. More commonly flat protonema outgrowths sit immediately on soil (Figs. 6, 8, 21) and in this case flat structures can develop from the only slightly elongate cells (Fig. 9, above left) or perfectly spherical ones (Fig. 9, above right).

However, the upright-growing protonema is not necessarily growing in one plane. Some indivuduals are composed of spherical cells only and they branch freely (Fig. 26), which is more common in more open places in better-lighted habitats (Fig. 27). This agrees with the field observation that the shadier the habitat, the more flat protonema outgrowth is.

In general, the protonema of *Schistostega* is formed by a great variety of cell types: linear, rectangular, spherical, flask-shaped, tmemas, clavate, bottle-shaped, inflated tortuous, *etc*. (see Figs. in pages 5, 7-9 & 11). The transitional variation between them seems immense: spherical cells may appear on linear, and sometimes even as thin as 3 µm wide filaments of protonemata. The flat protonema outgrowths that appear on such thin threads are able to grow in a specific direction, being arranged in the same plane (*e.g.* Fig. 74), and hanging even on the thinnest of observable filaments, strictly keeping their orientation, apparently perpendicular to the light source.

3. **Cell divisions and its modifictaions**

Rather unusual is the pattern of cell divisions in spherical cells of protonemata, reminiscent of yeast (*Saccharomyces*) cell division. Small evagination of the cell wall first appears (Figs. 29, 37), then it enlarges, being at first without chloroplasts (Fig. 38). After a certain time one or two or occasionally more chloroplasts migrate and then the cell wall between new and mother cell appears (Figs. 31, 42). Rarely the chloroplast fails to penetrate the young cell (Fig. 29), which probably is not long-living, although it can exist at least several days (interestingly, the chloroplastless cells "normally" appear in *Schistostega* at the base of gemmae).

In some cases a single chloroplast was observed outside of the assemblage of the remaining chloroplasts (*e.g.*

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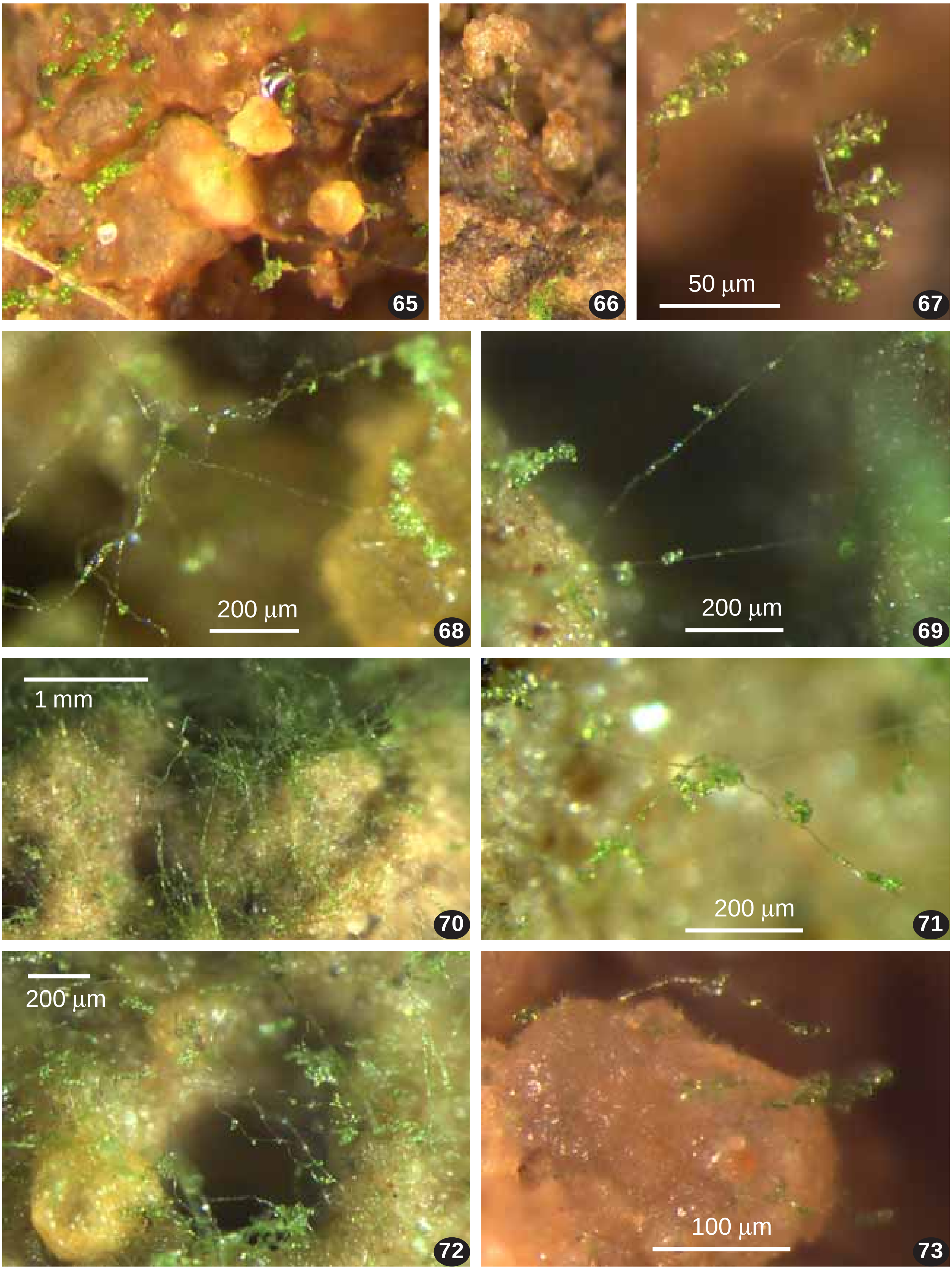
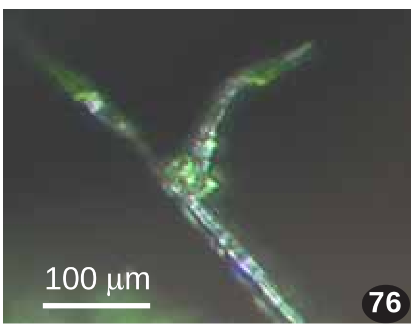
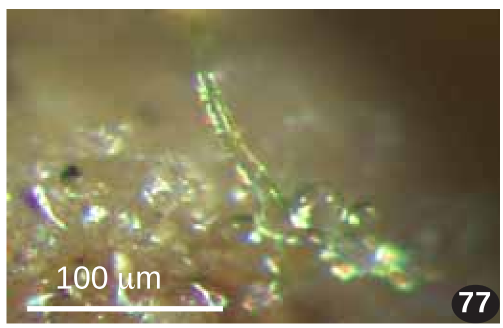
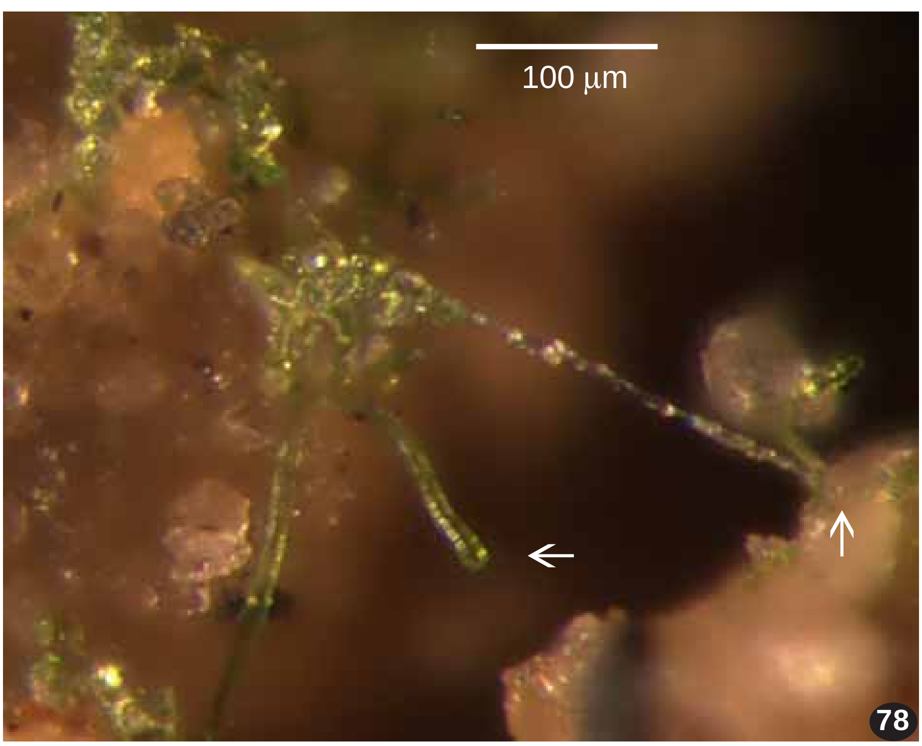


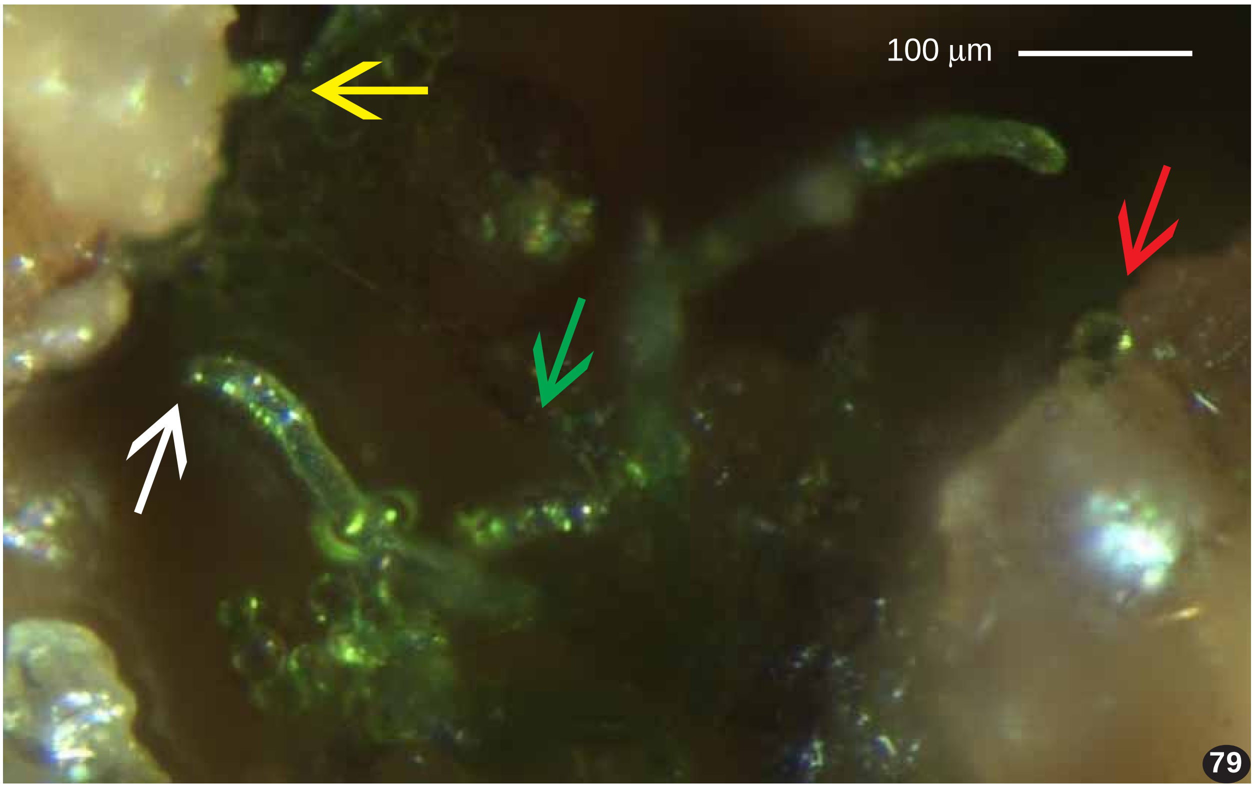
Figs. 65-73. Thin filamentous protonemata of *Schistostega pennata*, the thinnest being ca. 3 mm thick. Note flat protonema outgrowth development both on soil surface (from hardly seen creeping filamentous protonemata) and on thin, hanging filaments; sometimes flat protonema outgrowths are formed of only a few cells, but in other cases composed of no less than 10 cells (# 65), however, at places almost without any spherical cells (# 70). Pictures #65 & #66 are taken immediately from nature, others from plants grown in Petri dishes.

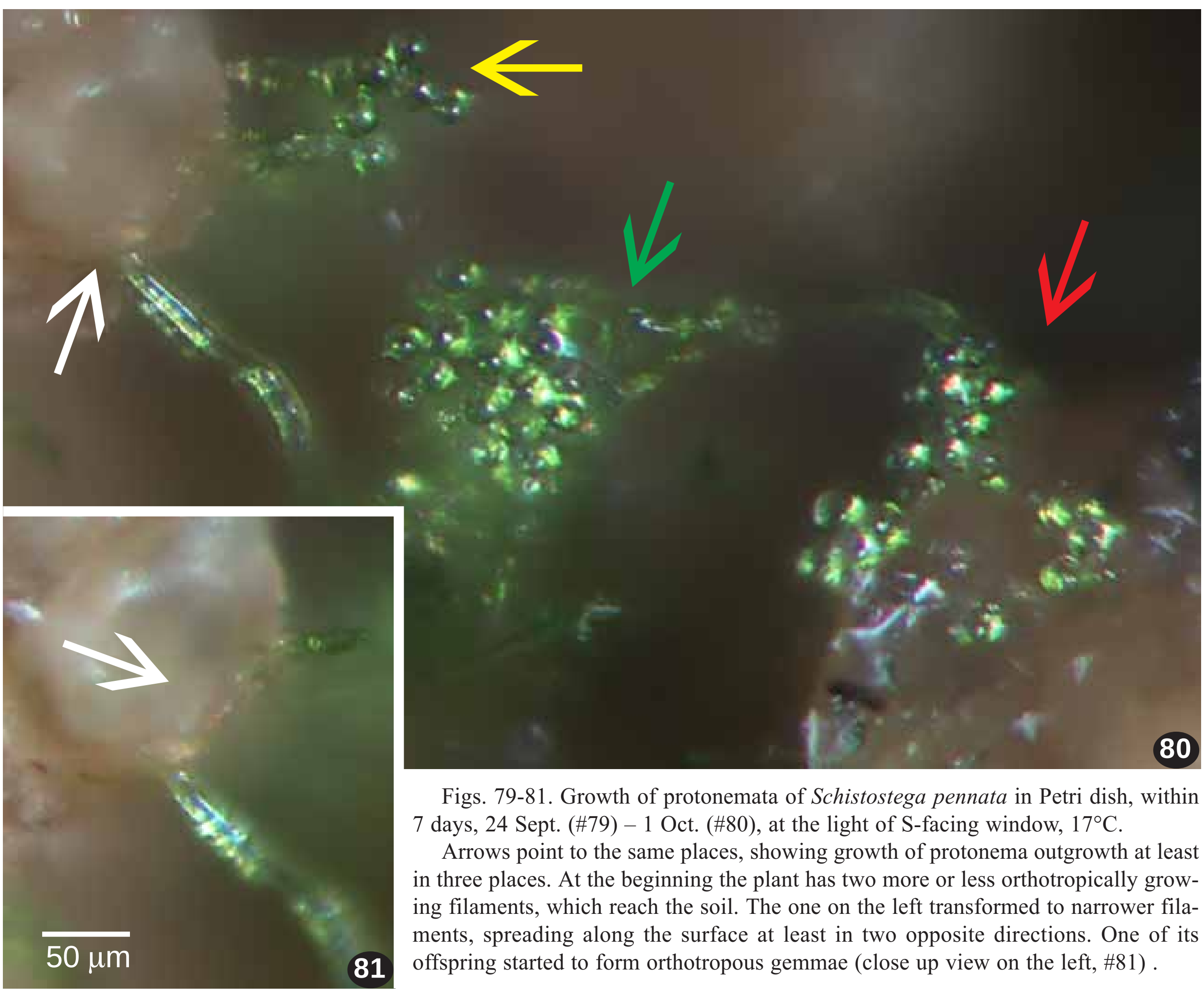






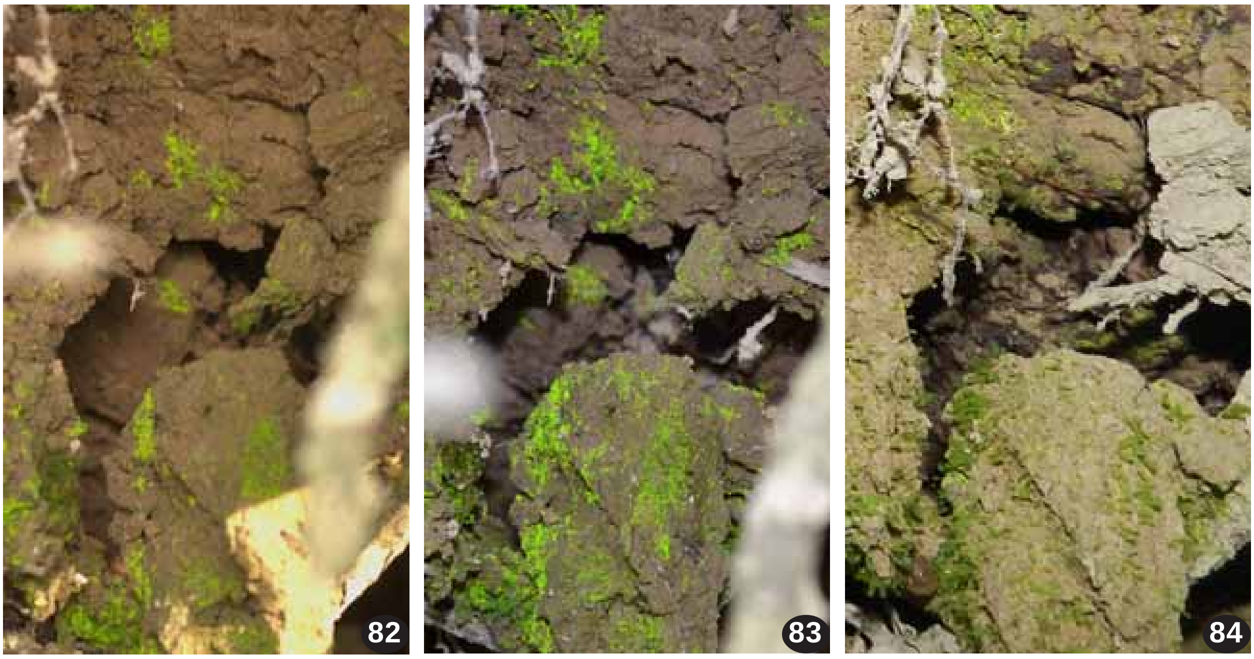
Figs. 74-78. Protonemata of *Schistostega pennata,* forming bridges between soil pieces. #74 shows ca. 2.5 mm filamentous protonemata with ca. 14 flat protonema outgrowths of different shapes; #75 illustrates a rather massive soil piece, which does not flex the filament much; # 76 displays flask-like cell with young gemmae upon filamentous protonemata, >2 mm long, hanging across depression; #77: fallen flat protonema outgrowth; it was normally standing, but after 3 minutes of drying in open Petri dish it fell, reaching the neighboring soil grain; #78: protonemata crossing a fissure on soil surface; note central filament (horizontal arrow) that directly grows to the closest "land" and compare with filaments in #79-80. Vertical arrow points to protonema spread on surface in several directions and producing gemmiphores after reaching of "land" across fissure: compare with #81.





Figs. 79-81. Growth of protonemata of *Schistostega pennata* in Petri dish, within 7 days, 24 Sept. (#79) – 1 Oct. (#80), at the light of S-facing window, 17°C.

Arrows point to the same places, showing growth of protonema outgrowth at least in three places. At the beginning the plant has two more or less orthotropically growing filaments, which reach the soil. The one on the left transformed to narrower filaments, spreading along the surface at least in two opposite directions. One of its offspring started to form orthotropous gemmae (close up view on the left, #81) .



Figs. 82-84. Part of *Schistostega* population on soil bank under upturned roots of fallen trunk, showing growth rate in 2012: #82: 8 July, #83: 18 July, and #84: 15 September. Note considerable expansion of protonemata in ten days in mid-summer, and developing potonemata into mature gametophores in about two months, on a much drier soil.

Figs. 85, 87-89. Male plants of *Schistostega* in mid-September. Many populations have extensive "whitish" areas due to numerous perigonia (#87-88). Otherwise among well-developed pinnate shoots there are smaller shoots at their bases terminated by perigonia (#85, arrowed). In about 10 days in Petri dish, many areas like that in #87 became reddish due to many just-fertilized female plants (#89) that were not seen shortly before that (compare with #87).

Fig. 86. One area of 3× 8 cm^2, where *Schistostega* looked quite dense, which is not a rare appearance of its growth. In this rectangle we counted 1075 large sterile plants and 301 smaller pinnate shoots; no plants with sporophytes (in average 58 plants per cm^2). Another plot 3×5 cm^2 (not shown) included 966 large sterile plants, 249 smaller pinnate shoots, and 275 developed plants with sporophytes (in average 99 plants per cm^2).

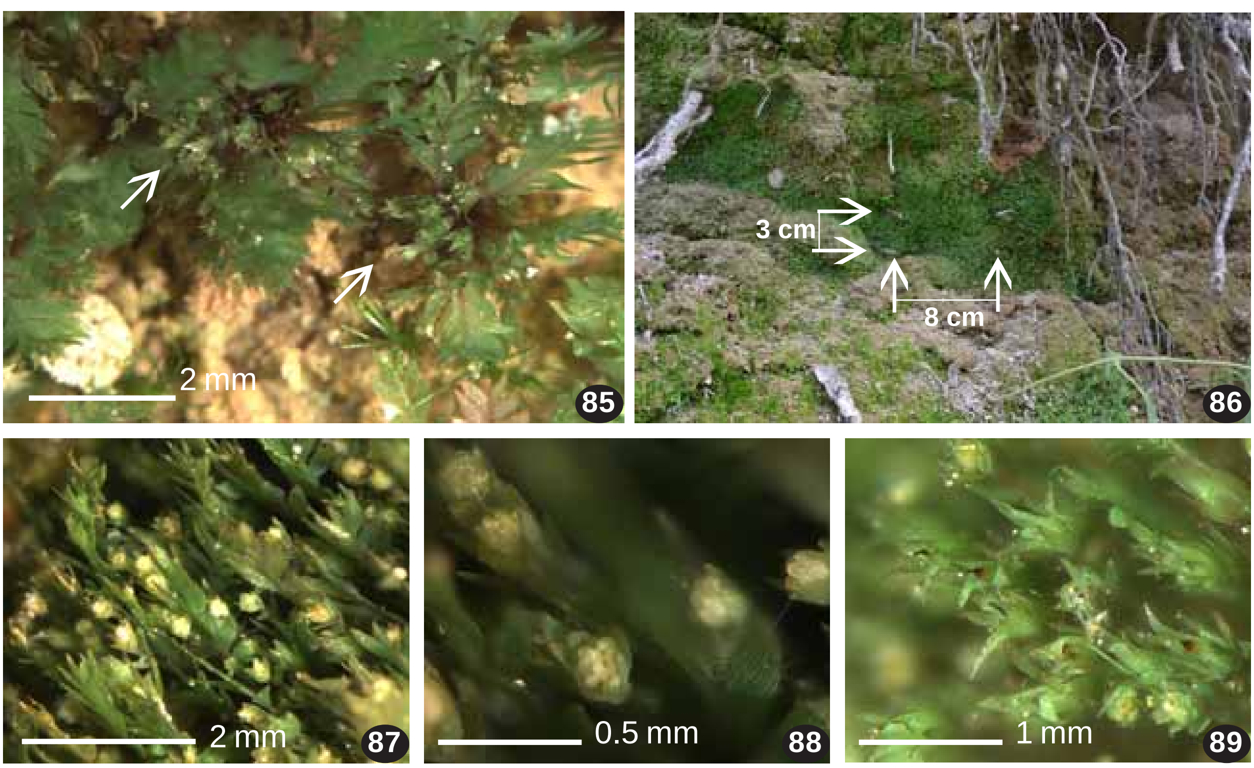


Fig. 44, arrow), and an indistinct evagination sometimes was seen near it. Otherwise, chloroplast assemblage was observed close to the evagination, so one or more chloroplasts were "waiting at the enterance" (Fig. 29, arrow).

Spherical cells, especially those situated at the edge of flat protonema outgrowth, often elongate at one end (Figs. 42-43), producing filamentous structures that are mostly negatively geotropic (Figs. 48, 51, 79-80), but often growing inclined to horizontal (Fig. 78), being after certain elongation just too heavy to maintain the original straight position. The apical part of such filaments sometimes is densely filled by colorless proplastids (cf. Figs. 40 and 41).

4. **Chloroplast position**

General observation of protonemata under stereomicroscope from above usually does not allow one to see its chloroplast position (Figs. 15-23), which, however, is quite apparent from the side view, or even better from the side and a little below (Fig. 24). The strict position of chloroplasts at the distal end in relation to light makes the whole flat protonema outgrowth whitish (cf. Figs. 24 and 6).

Front view under light microscope on flat protonema outgrowth cells, *i.e.* from above or from below, shows chloroplasts in regular rosettes, often with a "hole" in the center (Figs. 28 and 32). These holes, as can be shown from fluorescence microscopy, are occupied by nuclei (Figs. 29, 31, 35, 36).

5. **Gemmae**

The protonemal gemmae of *Schistostega* were exhaustively described by Vuillemin (1887), Correns (1899), Edwards (1978), and others. In our experiments they were especially abundant after the Petri dish was kept in a rather shady place.

Gemmae are often developed at the distal part of flat protonema outgrowths (Figs. 24-25, 50) or appear in compact groups (Figs. 45), usually on somewhat elevated places, or form an "open lawn" (Fig. 48). The developed gemma is sitting on a tmema, a small cell without chloroplasts, and with a fragile cell wall. After breakage, the gemma retains two horn-like projections, remnants of cell walls of the tmema (Figs. 59-60). Nuclei in tmemae are easily visible (Figs. 52-54), whereas in chloroplast-rich cells of gemmae they are obscured by densely arranged chloroplasts. It is rather unexpected to see nuclei in many (Figs. 52-53, 54 below right), although not in all, broken tmemae, that looks like totally empty broken cells (Figs. 59-60).

Flask-shaped gemmiphores may appear on thin filamentous protonemata, found occasionally in fairly unexpected places – in the middle of a "filamentous bridge" or epiphyllous on other mosses.

Edwards (1978) noted that gemmae are sticky and thus can serve for dispersion by mites, Collembola, etc. We, however, observed various Arthropoda with adhering gemmae without a long beak, described by Edwards as premature gemmae. At the same time, the attenuate "beak" of fully developed gemmae can easily work as an anchor, hooking gemmae to *e.g.* protonemata (Fig. 59).

6. **Growth in cultivation**

The possibility to cultivate *Schistostega* has been reported already by Miyoshi (1912), and Toda (1918) has much expanded data on temperature, humidity, light and media properties that affect its growth. According to the latter author "the more damp the air is, the better the growth of the moss [protonema implied – MI], but that too much dampness of the soil is injurious to its development."

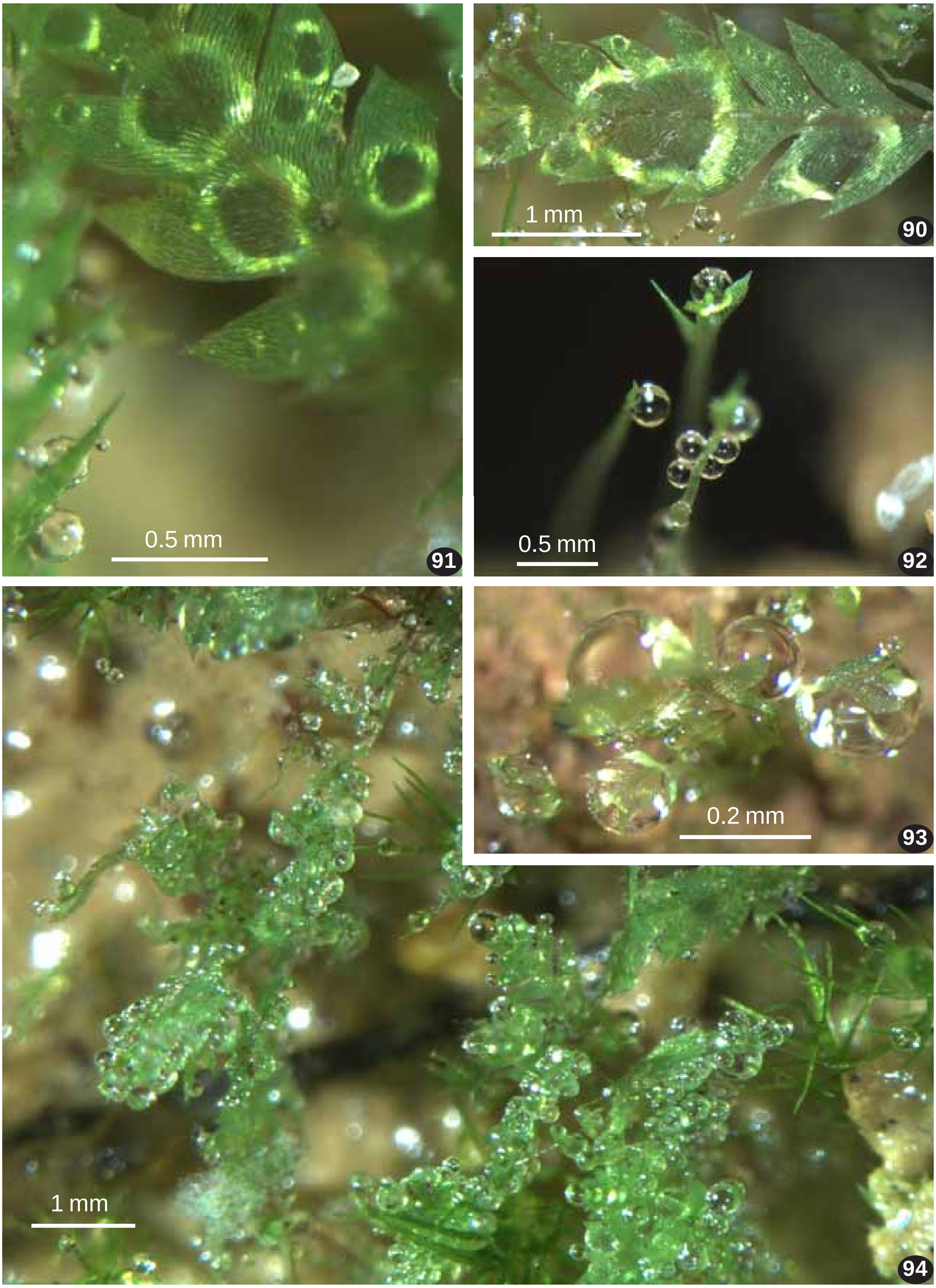
This is accurately the same as we observed. High humidity was necessary to achieve any growth. However, seeping soil surface directly with water drops is harmful, as the sand grains flush off from the surface, damaging both tiny filamentous and flat protonemata. On the other hand, the maintenance of moisture by wetting some soil pieces that lack protonemata or pouring some water on the dish bottom, that subsequently is absorbed by soil pieces with protonemata, maintains environments that are fairly suitable and allow protonema growth for a long time.

Most of our experiments kept soil damp, but not wet, which maintains protonemata in good shape, but observation within 10 days with the temperature 20-25°C did not promote sufficient growth. Wet soil has been more successful: flat protonema outgrowths have been increased by one cell at the edge of protonemata in ten days.

Keeping protonemata in wet condition at 17° at the N-facing window in September resulted in a maximal growth rate: protonemata developed full-sized spherical cells in 1-2 days (Figs. 79-81).

It was very difficult to monitor its growth, as one place got changes due to erosion and invertebrate activity, causing rapid decline and reappearance of new umbellate protonemata. One successful example is shown in Figs. 79-80. Note two rather thicker filaments that grew originally orthotropously (Fig. 79), but then changed direction of growth towards the closest "land," reached it (Fig. 80), divided into several threads and crept along the surface, producing gemmiphores (Fig. 81). Note that 24 hours was found as a period of new cell appearance also in *Physcomitrella patens* (Hedw.) Bruch et al. (Brockman, 2010). Interestingly, we observed a number of cases where thick filamentous protonemata growing across fissures were directed to a closest point (cf. Fig. 78).

Growth in culture in the temperature interval 25-30°C resulted in the development of numerous tiny protonema filaments (Figs. 65-77), some being only 3 μm wide. The presence of spherical cells on some of them ensures that they belong to *Schistostega*; otherwise they superficially look more like mold that also sometimes appears in these Petri dishes. In the natural populations, such pattern was not frequently observed, although in some cases filaments



Figs. 90-94. Dew on gametophores of *Schistostega.* Note that water drops are much more abundant on waxy surface of *Schistostega* than on *Dicranella heteromalla* (narrow green leaves). #90-91 shows double lighting of leaves, also by light reflected from drop hanging on the lower leaf surface.

forming bridges between soil pieces were seen in the field; photographs 65 and 66 represent such cases.

7. **Growth in nature**

Growth in nature has been studied by comparisons of photographs of the same upturned roots taken every two days, which demonstrated a sufficient expansion within 10 days (Figs. 82-83) in midsummer, at ca. 25°C daytime, ca. 17°C nighttime. Assuming that a developed protonema, like that in Fig. 10, can appear within ten days, the result indicates that the weather conditions should be considered close to most auspicous, allowing many new protonemal individuals to appear.

8. **Soil surface stabilizing and forming its own environment**

Both field observation and study of culture reveal "bridges" formed by filamentous protonemata, sometimes covering distances of several millimeters long. They can be formed by a rather stout, up to 18 µm thick, thread with oblique cell walls, or be less than 5 µm thick, easily quivering even by an slight wind.

The most typical way of forming bridges happens just because of drying. A fallen down flat protonema outgrowth (Fig. 77) often reaches an adjoining soil grain, thus its "stalk" covers a distance that may occur between its base and the place that the fallen protonema outgrowth reaches. In some cases a big spherical cell is developed at the end of a quite elongated orthotropous filament (Fig. 23), and such "stalks" could be up to half a millimeter. Such a structure appears to be rather unstable, providing an ability to reach neighboring soil pieces after either drying or partly also affected by wind (which may be efficient if the protonema partly loses its rigidity due to some water loss). The narrower the filamentous protonema, the longer it could be, although the thinner it is, the more strongly it is inclined, up to horizontal and somewhat hanging. Being especially flexible, these narrower filaments are easily moved by wind, and finally anchor to another side of a cave, fissure or depression.

The rigidity of the protonema outgrowth "stalks" seems to be rather high: at least some mites "walking" upon the protonema outgrowth "canopies" (cf. Fig. 5) and "forest" of gemmae (cf. Fig. 45, 48) did not break plants a lot, neither trampling flat protonema outgrowths down, nor destroying fragile gemmae, although some parts of them adhering to Arthopoda bodies were seen, especially on hairy spiders. In one case during the observation a filament between two soil grains was broken near one of its ends by a fast-moving insect, but the filament did not fall, but remained in about a horizontal position, holding on by only one of its ends.

In this respect it is interesting to consider the role of protonemata in stabilizing the soil surface. Wiring by rigid filaments seems to be one of those aspects. Another option is its protonema outgrowth. When flat protonema outgrowth falls, it cannot stand up again and remains attached to the ground; moreover, its surface, as well as those of brood bodies, is somewhat sticky. Figures 61-62 represent such fallen "plates" that are cementing to the surface. Separate cells of this mass lying on the surface produce upright filamentous structures, or chains of spherical cells, or sometimes even generate gametophores.

Gemmae production on a protonema and its sticky surface also can be assumed as a capability to glue sand grains and create its own environment (Fig. 63-64). The latter becomes more long-lasting, increasing the probability of a plant to successfully reach the next "stepping stone" of upturned roots of a fallen trunk.

9. **Supplementary observations**

Within the period of 1-2 months the areas covered by protonemata on a soil bank may almost completely change into "gametophore forest" (Figs. 82-84). In the latter, the protonema is lacking or difficult to observe only in places of rather dense growth, which may be as high as 100 plants on one square centimeter, which means that every square millimeter has in average of one plant (Fig. 86). In moderately dense populations with several millimeters between gametophores, low "peak-like" flat protonema outgrowths were frequently observed in mid-summer. In this respect it is interesting to consider a protection of protonemata by adult plants. *Schistostega* was found to be extremely capable to collect dew in the morning, perhaps needing a special designation as a "roriphilous (dew-loving) plant." The waxed surface of pinnate gametophores, which makes them glaucous in color, seems to be highly hygrophobous and dew drops last on it sometimes up to mid-day. In Petri dishes and plastic container cultivations the same phenomenon may be observed, and those cases are shown in Figs. 90-94. Note that the horizontally growing pinnate compound leaf of *Schistostega* allows it to hold on its lower surface fairly big drops, providing reflective light back to the plant. Similar effect is well known in *Rhizomnium* and *Plagiomnium* species, where water drops are arranged and shining under many leaves in a dewy morning and after rain. It seems this can be used by plants for increasing photosynthesis, which is important for sciophytic mosses like *Schistostega*.

Studying sporophyte production in mid-summer 2012 we saw sporophytes at different stages of development. Some were fully raised on a seta, others had setae still elongating, and finally in a certain number of plants capsules remained perfectly sessile, albeit even in this case the operculum usually had fallen off. Very rarely female shoots with unfertilized archegonia were found. This was quite congruent with our previous observations largely confined to mid-summertime (when student summer classes took place). However, retrieving several tens of populations we failed to find any apparent male plants. Visiting the same area in September 2012, however, we found male plants not only in most populations, but also in abundant and conspicuous state. Sometimes they appeared among well-developed pinnate shoots on much shorter stems terminated by perigonia (Fig. 85, arrowed), while more commonly they were met as extensive "whitish" areas due to numerous exposed antheridia on tops of rather high pinnate shoots in dense growth (Fig. 87-88). In about 10 days in Petri dishes and plastic containers, the latter whitish areas turned to reddish due to many just-fertilized female plants (Fig. 89) that were not seen shortly before that, cf. Fig. 87. It can be deduced, that females accelerated their growth after fertilization; however, reaching the stage of "just pregnant archegonia" (Fig. 89) they did not change for at least several weeks. It is still an open question, however, if the season of fertilization is strictly restricted to autumn, or it occasionally happens in other periods of the year. The ubiquitous presence of perigonial plants in September supports the former, but in this case sporophyte-bearing plants with sessile capsules should be considered as having an ability to conserve the process of sporophyte development for a long time, awaiting suitable conditions, as obviously during summertime new sporophytes are continuously appearing.

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