

ON THE BRANCH PRIMORDIA STRUCTURE IN THE BASAL PLEUROCARPOUS
MOSESSES (BRYOPHYTA)

ОСОБЕННОСТИ СТРОЕНИЯ ЗАЧАТКОВ ВЕТОЧЕК В БАЗАЛЬНЫХ ГРУППАХ
БОКОПЛОДНЫХ МХОВ

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Abstract

The development of leaves on branch primordia is studied in Ptychomniales, Hookeriales, and basal families of the Hypnales, including the Trachylomataceae, Plagiotheciaceae, Acrocladiaceae, etc. Many of them are characterized by “lacking pseudoparaphyllia”. However, the definition of this character remains vague. In order to avoid misleading terminology, we suggest distinguishing, with certain refinements, the *Bryum*-type and *Climacium*-type of branch primordia. Their main difference concerns the origin of the most proximal branch leaves: in the *Climacium*-type, they are derived from cells that are the first merophytes produced by the branch apical cell, while in the *Bryum*-type, the first merophytes do not produce leaves and the first branch leaves appear on branch primordia from cells that are later descendants of the branch apical cell. The *Bryum*-type is often associated with a leaf deep splitting to its base into separate segments, and appearing as independent structures (and sometimes referred to “pseudoparaphyllia”) although originating from a single merophyte as a compound leaf. *Bryum*-type branch primordia are characteristic of basal groups of pleurocarpous mosses, while *Climacium*-type is represented in most of advanced families.

Резюме

Рассматривается развитие листьев в в базальных группах бокоплодных мхов (Ptychomniales, Hookeriales и базальных семействах порядка Hypnales), которые часто описываются как не имеющие псевдопарафиллий. Вместе с тем последний термин понимается разными авторами очень неоднозначно, и более информативным, по-видимому, следует считать подразделение зачатков веточек на *Bryum*- и *Climacium*-типы, с некоторыми уточнениями. Во втором из них первые листья веточки развиваются из мерофитов, отделившихся первыми от апикальной клетки веточки и располагаются по краю зачатка веточки, тогда так в *Bryum*-типе первые листья формируются с задержкой, на некотором расстоянии конутри от края примордия, а первые мерофиты веточки не производят листьев. У зачатков веточек *Bryum*-типа первые листья часто рассечены до основания на узкие и иногда не связанные между собой доли (которые часто трактовали как псевдопарафиллии), образуя так называемый составной лист, образованный из потомков одной клетки. На филогенетическом древе распределение *Bryum*- и *Climacium*-типов весьма показательно: первые характерны для базальных групп, тогда как большинство продвинутых семейств порядка имеет зачатки веточек *Climacium*-типа.

KEYWORDS: branch primordia, bryophytes, DAPI, evolution, fluorescence microscopy, LSCM, pleurocarpous mosses, pseudoparaphyllia, SEM

INTRODUCTION

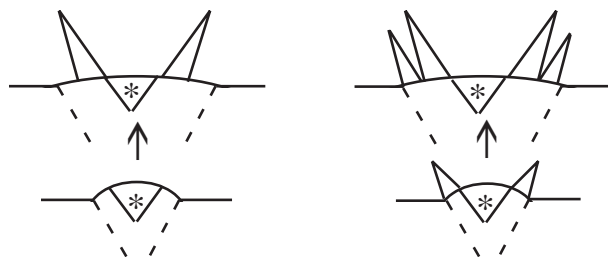
Molecular phylogenetic analyses of the pleurocarpous mosses have overturned the classical systematic interpretations of the group, which dominated throughout the 20th century (Fleischer, 1904-1923; Brotherus, 1925). At the same time, phylogenetic trees for pleurocarps mostly have rather poor resolutions precluding the development of a universally accepted system. In spite of this, the topologies in basal parts of molecular phylogenetic trees found in various analyses are relatively similar. Two points that

are important for the following discussion include: (1) Ptychomniales are found in a position sister to the Hypnales plus Hookeriales (Buck *et al.*, 2004; Cox *et al.*, 2010; Huttunen *et al.*, 2012), and (2) Fontinalaceae, Habrodontaceae, Plagiotheciaceae, Acrocladiaceae, Catagoniaceae, Fabroniaceae, Lepydontaceae, Rutenbergiaceae, Stereophyllaceae, and Trachylomataceae form a basal grade of the Hypnales (Tsubota *et al.*, 2004; Buck *et al.* 2004; Gardiner *et al.*, 2005; Ignatov *et al.*, 2007; Cox *et al.*, 2010; Huttunen *et al.*, 2012, 2013).

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Scheme 1: *Bryum* (left) and *Climacium* (right) types of branch primordia (summarized from Akiyama, 1990). Branch apical cell is marked by asterisk (*).

The present paper considers these basal families of the Hypnales, and also Hookeriales and Ptychomniales. The order Hypnodendrales, the basal group of the super-order of Hypnanae (Goffinet *et al.*, 2009), is not included in the scope of this study.

Morphological characters associated with these groups were discussed in a number of papers (*e.g.* Pedersen & Newton, 2007; Huttunen *et al.*, 2013; Hedenäs, 1996). Among other characters, the protective leafy structures of branch primordia were widely discussed. However, their terminology was rather confusing. First branch leaves were called either scaly, or embryonic, or juvenile, or proximal branch leaves, or sometimes pseudoparaphyllia. The latter term was controversial since its origin, as it was first proposed for *Rhynchostegium* by Warnstorf (1904–1906), although Ireland (1971), in his comprehensive overview, found this genus lacking pseudoparaphyllia. Subsequent attempts to clarify its meaning brought other controversies, partly overviewed by Ignatov & Hedenäs (2007).

In order to avoid misleading terminology, we suggest that cases sometimes described as “pseudoparaphyllia absent or present” (Budyakova *et al.*, 2003) refer to the *Bryum*- and *Climacium*-types of branch primordia developed by Akiyama (1990) and Akiyama & Nishimura (1993) based on the original distinction of these types by Lorch (1931). Accepting this, we suggest a slightly different terminology. ‘Primordium’ will be applied to any young branches at very early stages of their development, irrespectively whether or not it has leaf-like structures, *i.e.* buds will be not considered different from primordia.

The difference between the two types is where the first branch leaves appear: (1) in the *Climacium*-type, **at the edge** of branch primordium, derived from cells that are the first merophytes produced by branch apical cell, or (2) in the *Bryum*-type, **within** the area formed by branch apical cell, derived from cells that are later descendants of the branch apical cell, while its first merophytes do not produce leaves. Scheme 1 explains this situation.

Although the definitions of *Bryum*-type and *Climacium*-type are relatively easy to understand, it sometimes less easy to apply due to the following subtleties in definition.

1. Positional criterion. In the *Bryum*-type, there is a leafless area formed by branch cells, but situated along

its periphery, outside the outer leaf-like structures, while in the *Climacium*-type such leaf-like structures arise from the outermost cells produced from branch apical cell.

2. Temporal criterion. The *Bryum*-type is characterized by a delay in the development of leaves derived from early merophytes of branch apical cell, so for a certain time the branch primordium remains totally naked. In the *Climacium*-type, all merophytes develop into leaves without delay.

Although positional and temporal criteria are not congruent in some cases, *e.g.* delay in development may occur after differentiation of the first merophytes into leaves (in some Neckeraceae), we found them to be consistent for the groups discussed in this paper.

It is worthy noting that proximal branch leaves in basal pleurocarps are often split to base, and their segments, sometimes referred to ‘filamentose pseudoparaphyllia’, are parts of such compound leaves (Ignatov & Spirina, 2012). Their homology can be understood from series of sections at sequential stages of development, shown for example for *Hypnum cupressiforme* (Spirina & Ignatov, 2008). A compound leaf can be recognized by arrangement of all its segments in position where one leaf should be according to phyllotaxis, *cf.* arrowed ones in Figs. 6, 9, 10, 16, 46, 48.

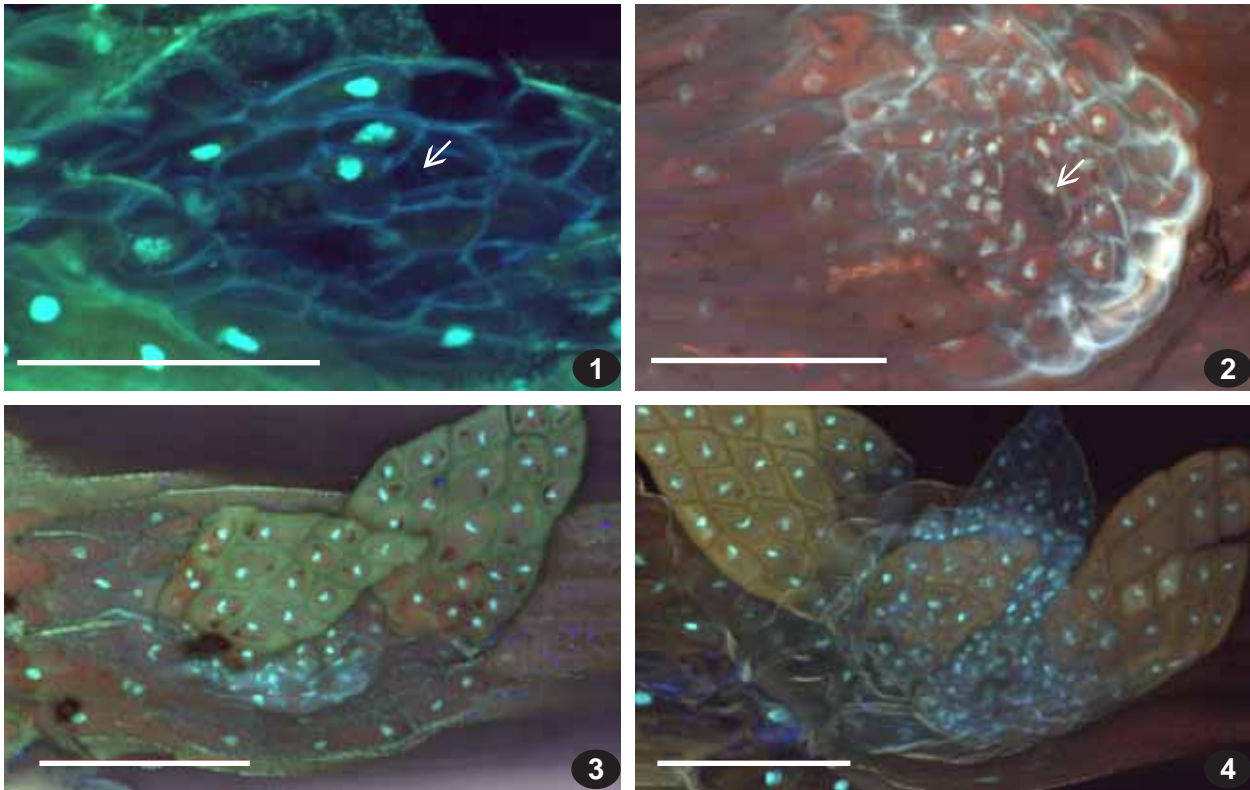
MATERIAL AND METHODS

Taxa sampling

This study included representatives of seven genera of the Ptychomniales and 26 genera of Hookeriales, from all of the families recognized in the Hookeriales by Goffinet *et al.* (2009). Only few specimens were studied and illustrated from the Hypnales, mostly from the Plagiotechaceae (as defined by Huttunen *et al.*, 2013), for comparison with the Hookeriales and Ptychomniales. Data on the structure of branch primordia from previous publications (Ignatov, 1999; Budyakova *et al.*, 2003; Spirina & Ignatov, 2011; Ignatov & Spirina, 2012) were considered for discussion. A species list is provided in Appendix.

Material for SEM was prepared in two ways. In some cases, shoots from herbarium were placed in water for 30 min, and after removing leaves were transferred directly onto Peltier cooled sample holder and analyzed in low-vacuum mode at -30°C . Otherwise, shoots were fixed in 4% glutaraldehyde for 7 days, post-fixed with 1% osmium tetroxide in Na-phosphate buffer pH 6.8 for 6 hours, washed in water, dehydrated through an ascending alcohol-acetone series, dried at critical point, covered by gold, and observed under LEO-430.

Material for LSCM was taken both from living plants and from dried herbarium material. Fresh samples were deaerated and fixed in 4% paraformaldehyde in 0.05M phosphate buffer pH 7.0 with 0.1% Triton-X, 0.1% Nonidet P-40 and 0.01% Fluorescence Brightener28 for 3 hours, washed in 0.05M phosphate buffer and replaced in 100% cold methanol at -20°C for 12 hours, washed in deionized water, and replaced in 0.1% Fluorescence



Figs. 1-4. Branch primordia of *Orthorrhynchium elegans*: 1-2 – leafless stages; 3-4 branch primordia with first branch leaves sitting on hummocks of inflated cells of young branch (LSCM, DAPI staining). Scale bars: 50 μm for all. Apical cells arrowed.

Brightener28 for 3 hours, and observed in TDE-Tris mountain media under Olympus FV-1000. Herbarium samples were prepared in two ways: 1) shoots were fixed in 4% paraformaldehyde with 0.1% Triton-X and 0.01% Fluorescence Brightener28 for 3 hours, then stained by 0,1mM DAPI for 15 min; 2) shoots without fixation were stained by 0,1mM DAPI for 15 min, then both types of samples were investigated under Olympus FV-1000, with 405 and 473 nm lasers.

In all the photographs, the stem apex is either on the top or on the right.

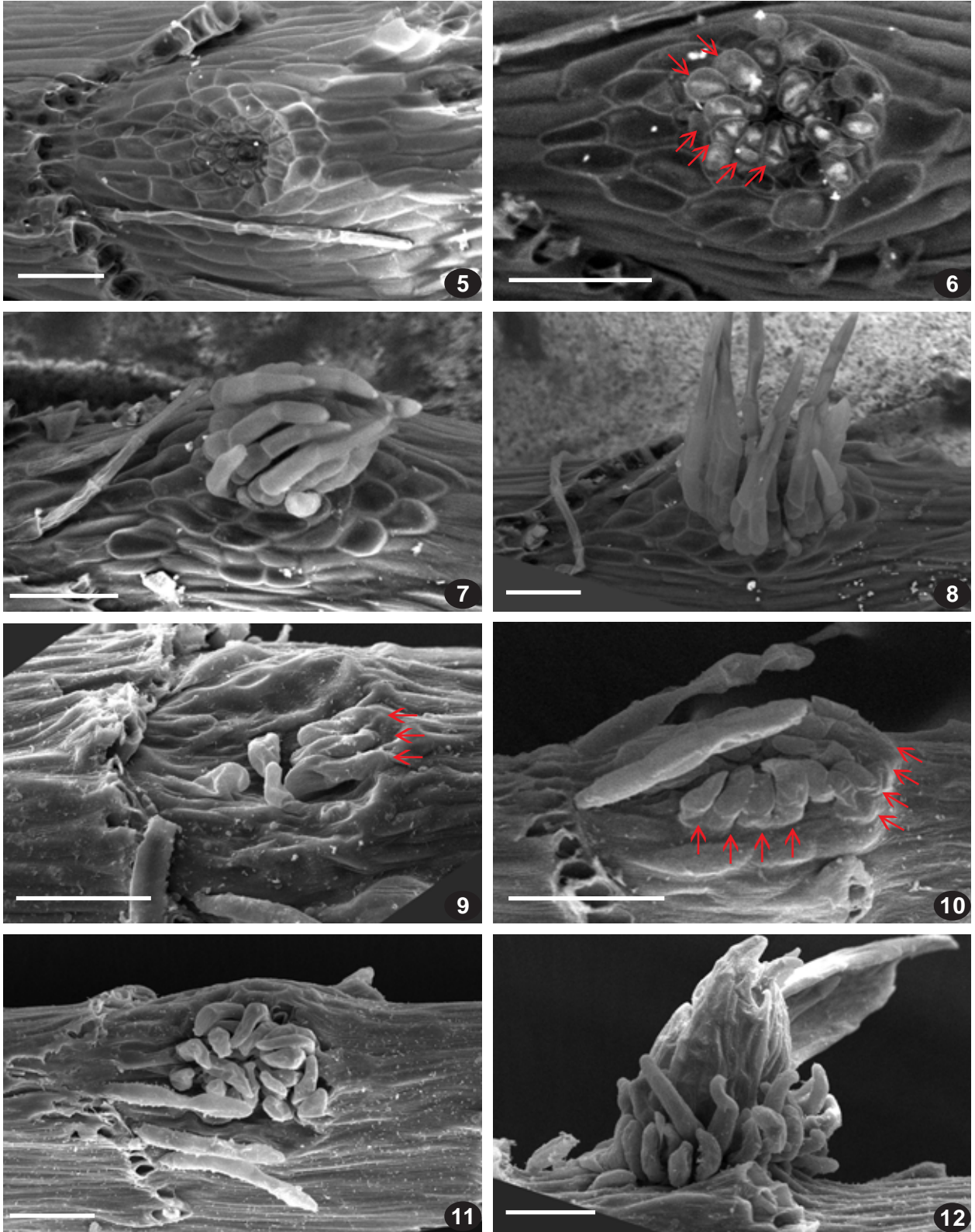
OBSERVATIONS PTYCHOMNIALES

Orthorrhynchium. At its earliest stage, a branch primordium is a group of large, soft cells slightly elevated above the stem surface (Figs. 1-2), and lack leaf-like structures around it. The primordia closer to the shoot apex often have small ovate-lanceolate to ovate leaves (Figs. 3-4), although in many shoots the branch primordia are naked throughout. When leaves start to develop, sometimes they are apparently sitting at the sides of cell hummock (Fig. 4), but if the latter is less conspicuous and looks flat, the first developed leaves might appear to be on the stem around the branch primordium. This case is easy to misinterpret as the *Climacium*-type. However, tracing where basal leaf cells adjoin primordium ensures that, as in the case shown in Fig. 3-4, leaves are within, not around the group of short enlarged cells, *i.e.* the primordium is in fact of *Bryum*-type.

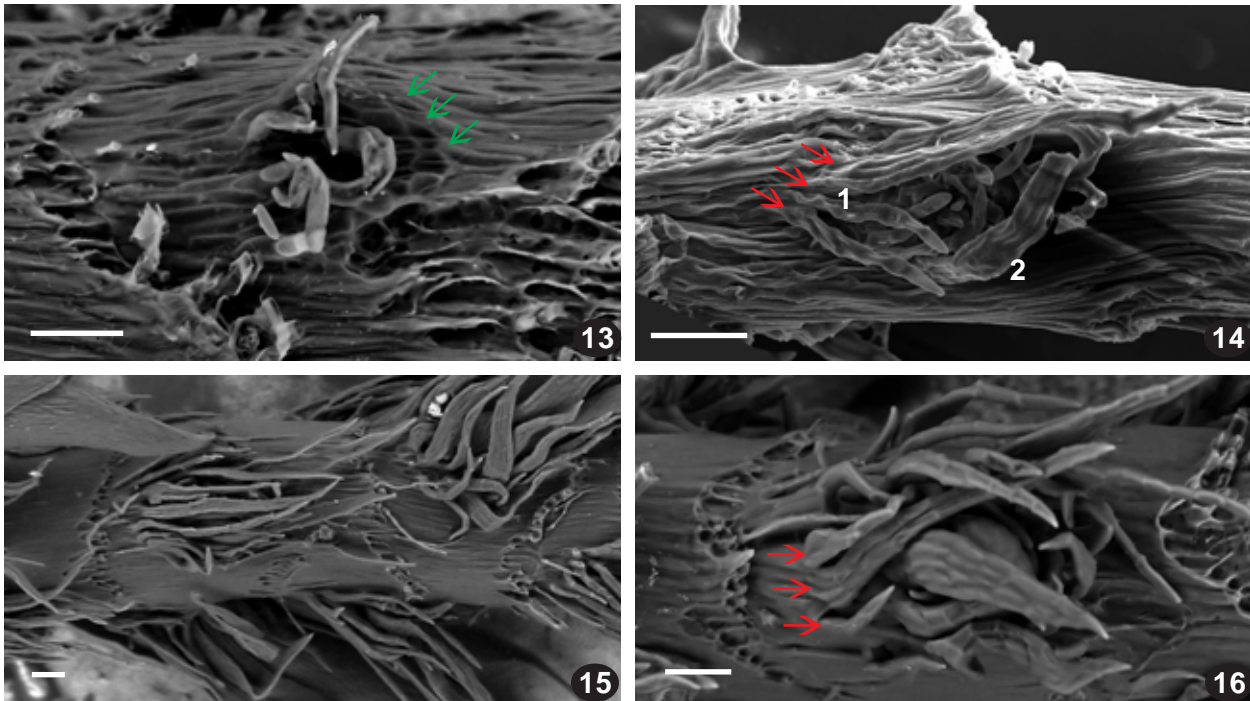
The position of *Orthorrhynchium* at the base of clade of the Ptychomniales was found by Huttunen *et al.* (2012), earlier this recently established family with few species in one genus was treated among basal Hypnales near the Phyllogoniaceae (Goffinet *et al.*, 2009).

Hampeella has branch primordia as indistinct (Fig. 9) or shallow, somewhat funnel-shaped depressions (Fig. 5). As only herbarium material was used, artifacts are difficult to exclude. However, the photographs in Figs. 5-8 were made from wetted herbarium material, frozen on Peltier cooled stub simultaneously with applied low vacuum, thus likely more similar to fresh material. No leaf-like structures are observed at the stage when the branch apical cell remains in the bottom centre of the funnel-shaped depression (Fig. 5), but shortly after the primordium starts to rise to stem level, many cells almost simultaneously produce peg-like or subulate outgrowths (Fig. 6), in many cases arranged in a series (Figs. 9-10), thus interpreted as a compound leaf (cf. Ignatov & Spirina, 2012). After the hollow-shaped primordium transforms to a hummock-shaped projection, its leaf-like structures gradually become more similar to leaves of a mature shoot (Figs. 8 & 12), leaving subulate proximal branch leaves only near the branch base. Being pressed by larger leaves, subulate leaves mask the leafless zone at the branch base, which is better seen at earlier stages (*e.g.* Figs. 7-8, 10).

Cladomniopsis (not shown) has somewhat inflated and leafless primordia, similar to *Orthorrhynchium*, *i.e.*,



Figs. 5-12. Branch primordia of *Hampeella*. 5-8: *Hampeella pallens* (SEM, frozen); 9-12: *Hampeella alaris* (SEM, gold coated): 5 – leafless stages, the primordium is of shallow funnel shape; 6 – shortly after start of raising, leaves are formed by series of subulate structures, likely constituted compound leaves (arrowed); 7-8 – subsequent stages of branch leaf development; note a considerable leafless part of branch primordium; 9-11 – stages of branch leaf development, note a serial arrangement of subulate structures (#9-10) likely representing compound leaves; 12 – later stage of branch primordium development, after ovate leaves start to appear, leaving subulate appendages only at branch bases. Scale bars: 50 μ m for all.



Figs. 13-16. Branch primordia of Ptychomniaceae taxa (SEM, gold coated): #13: *Garovaglia elegans*; #14: *Ptychomnion aciculare*; #15-16: *Glyphothecium sciuroides*; note that linear first branch leaves in *Garovaglia* are situated within the hollow outline (its upper border arrowed), while the proximal branch leaves of *Ptychomnion* are much resembling those in Hypnales, being arranged around (not within) the area formed by branch apical cell; #15-16: numerous foliose structures (likely compound proximal branch leaves) around branch primordia of *Glyphothecium*; note extensive area without any foliose structures. Scale bars: 50 μ m for all. In #14 & #16 arrows point segments of a probably compound leaf.

the *Bryum*-type, but usually on the early stages, bearing two small leaves, similar to the next genus.

Garovaglia (Fig. 13) has lanceolate leaf-like structures within the former depression, if considering its outline by analogy with *Hampeella* (Fig. 5).

Ptychomnion (Fig. 14) and **Cladomnion** (not shown) have lanceolate leaf-like structures around branch primordia. Sometimes the outermost occurs in the standard ‘4 o’clock’ position (note that this position can be mirrored and be ‘8 o’clock’, cf. Ignatov & Hedenäs, 2007), and is compound (Fig. 14, arrowed). As the arrangement of the most proximal branch leaves is obviously around the branch primordium, this case should be referred to *Climacium*-type.

Glyphothecium (Figs. 15-16) has been described as having paraphyllia (Pedersen & Newton, 2007), with a note that they are distributed along the stem in patches or continuously. Their photographs show rather abundant narrowly lanceolate structures around branch primordia in leaf axils. Their huge number suggests that the first branch leaves are compound, composed of a number of lanceolate “parts” (Fig. 16, arrowed), although direct evidence for that is not available at present.

Thus, the Ptychomniales show different variants of protective structures development, and they are parallel to those observed in the Hypnales. Interestingly, their position in phylogenetic trees (e.g. Pedersen & Newton,

2007; Huttunen *et al.*, 2012) corresponds well to the structure of branch primordia. The *Orthorrhynchium*- and *Hampeella*-variants through *Cladomniopsis*-variant evolve to a *Glyphothecium*-variant.

Pedersen & Newton (2007) in the morphomatrix for the Ptychomniales, indicate paraphyllia presence in *Cladomnion*, *Glyphothecium* and *Ptychomnion*. Apparently, deeply dissected and compound proximal branch leaves were treated as paraphyllia (Fig. 14-16). We disagree with this interpretation even for *Glyphothecium*, although superficially its stem looks quite ‘paraphyllose’. The topography of foliose structures definitely indicates their origin from branches.

HOOKERIALES

The Hookeriales is much larger than the Ptychomniales, and more diverse. Its species are classified into seven families and 52 genera (Goffinet *et al.*, 2009), with ca. 400 currently accepted species, according to Tropicos (<http://www.tropicos.org>). However, the branch primordial structure in the order is apparently more homogeneous than in the Ptychomniales, based on our study of representatives of 24 genera from 7 families. The quite apparent *Bryum*-type of branch primordia is represented in *Hookeria* (Figs. 17, 25-28, 31, 33) despite it has been treated as having “foliose pseudoparaphyllia” (Hedenäs, 1996; Ignatov, 1999), “pseudoparaphyllia filamentose or absent” (Goffinet *et al.*, 2009), “pseudoparaphyllia present” (Huttunen *et al.*, 2013).

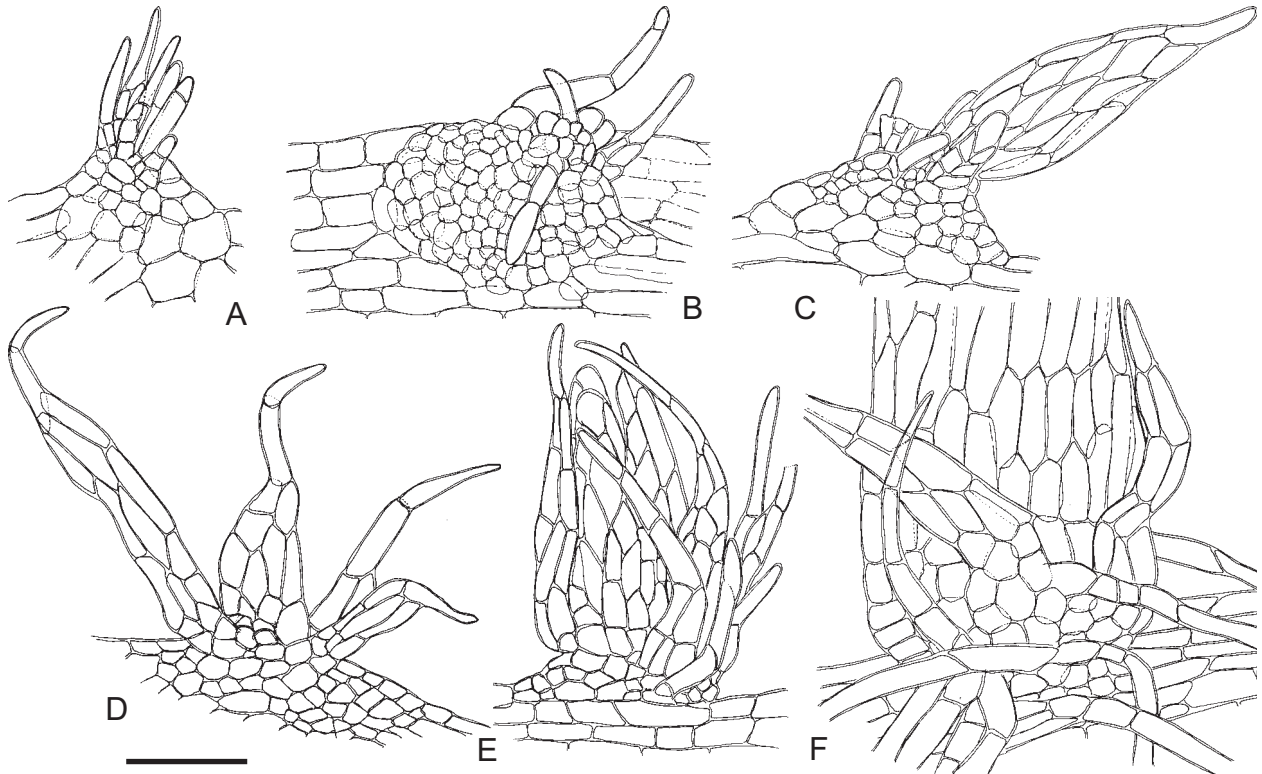


Fig. 17. Branch primordia of *Hookeria lucens* (U.S.A., California, 7 Aug. 1989, M. Ignatov, MHA): A-F – stages of branch development. Note that some primordia (e.g. B and C) have much more cells and raise first leaves much higher above stem than others (e.g. E). Compare with #25-28, 31. Scale bar: 50 μ m for all.

The hummock of small cells first appears and remains totally leafless for some time (Fig. 31). Then subulate leaves appear near its apex (Figs. 17A,B, 28) and each subsequent leaf has a broader base, so leaf shapes are graded from subulate through lanceolate to ovate (Figs. 17C,D). Well-developed branches have a basal portion with scattered subulate to narrowly lanceolate leaves (Figs. 17E, F; 25, 33).

Note, however, that in the case where the hummock of inflated cells is small (which is not rare in distal portions of shoots, cf. Figs. 26-27 and Fig. 17D), it is not very conspicuous under a light microscope or even SEM, and collapses during preparation of specimens for SEM observation. It is nothing surprising that such branch primordia were interpreted as having ‘foliose pseudoparaphyllia’. In uncertain cases, the recommendation would be to check branch primordia further down on the stem.

The leafless bases of branches formed by inflated rather homogeneous cells can be interpreted as represented totally by merophytes #1-3 (or #4-6, if considering that the three first outermost merophytes are forming the stem surface), although sometimes they are so numerous, that it is difficult to make any estimations (Figs. 17B, 28).

Another genus which may develop extensive sac-like leafless young branches at early stages is *Hypopterygium*. This genus has a diverse structure of young branches: in distal parts of shoots, branch primordia are flat and sometimes totally leafless, although readily recognizable by autofluorescence (Fig. 30). Later, narrowly lanceolate

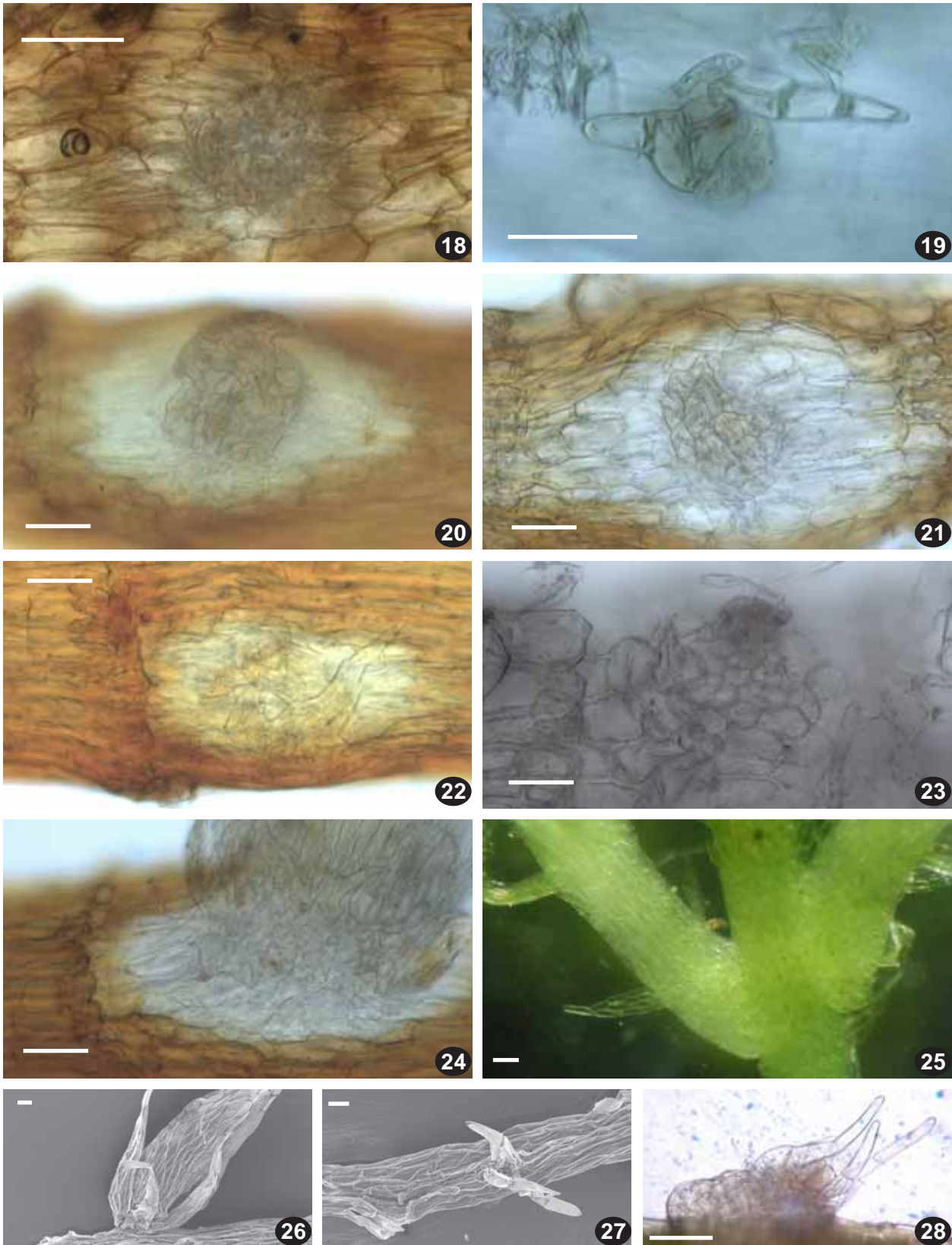
proximal branch leaves appear and they are easily referred to “foliose pseudoparaphyllia”, especially from the side (Fig. 41). A careful tracing of their outlines, as well as observation from above, especially at somewhat earlier stage, clearly indicate their origin well within the area of cells derived from a branch apical cell (Fig. 43).

In the proximal part of *Hypopterygium* shoots, the branch primordia are much larger, sac-like (Fig. 32), and, initially, leafless. They often change their growth direction from more or less perpendicular to stem (Fig. 32) to rather parallel to stem, comparable to that shown for *Hookeria* in Fig. 28. In the latter case, most of the young branch is naked and leaves are crowded near its apex only.

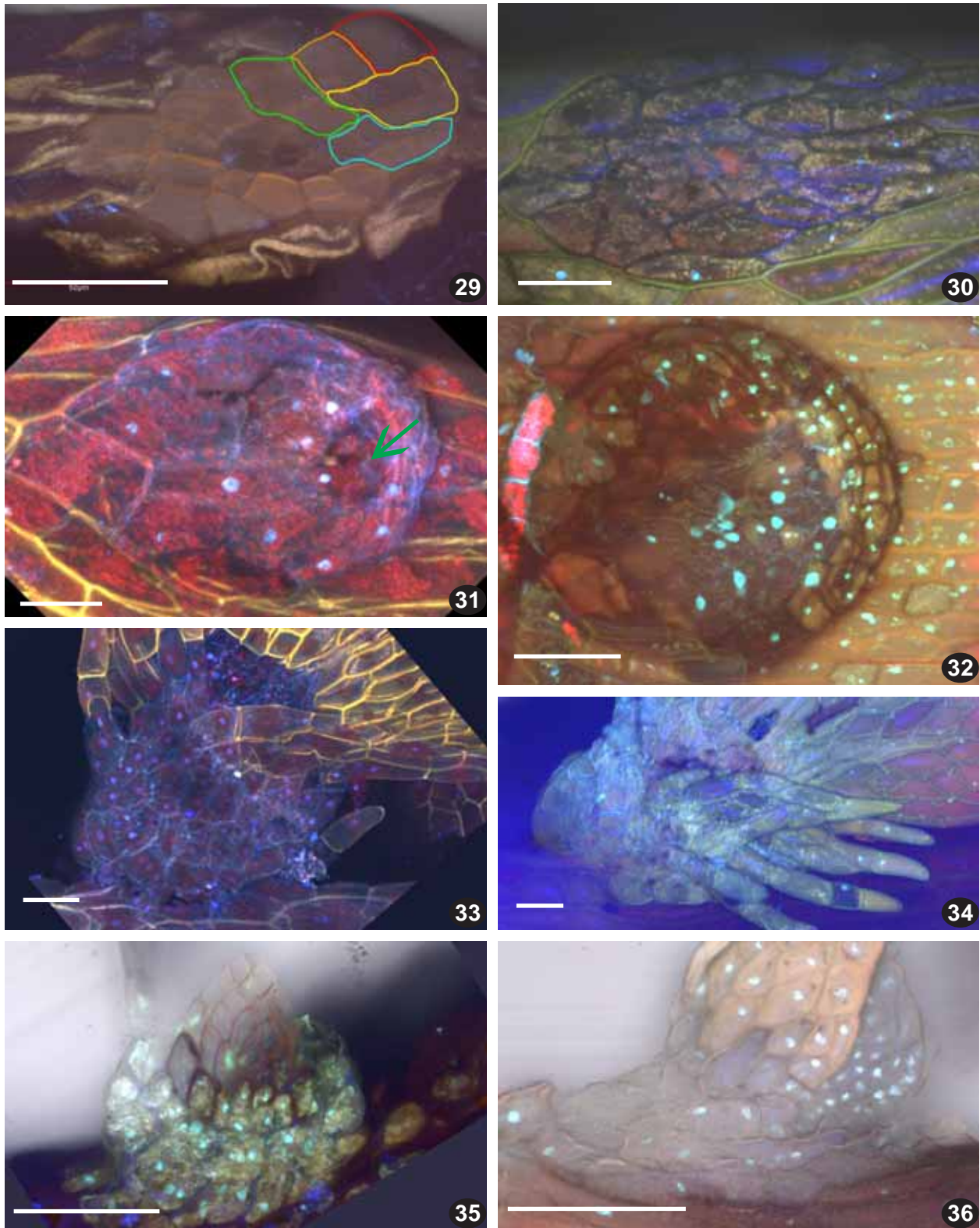
It seems that in the Hookerales, variation within different parts of one collection and sometimes even within a single shoot is great, exceeding those that can be found by comparing different genera and even families. However, all the variants seem to fall within the *Bryum*-type, contrary to the Ptychomniales, where advanced members of the order have the *Climacium*-type.

Diversity of branch primordia in Hookeriales is partly shown in Figs. 18-41 and 43. In most of the genera that were examined, the area around branch apical cells is not especially contrasting under the light microscope (Fig. 18), and more apparent are cases of the next developing stage, when growth of branch primordium results in a pellucid area (Figs. 20-22). The cells originating from branch apical cell are usually paler, with cell walls much thinner than in surrounding cortical cells (Figs.

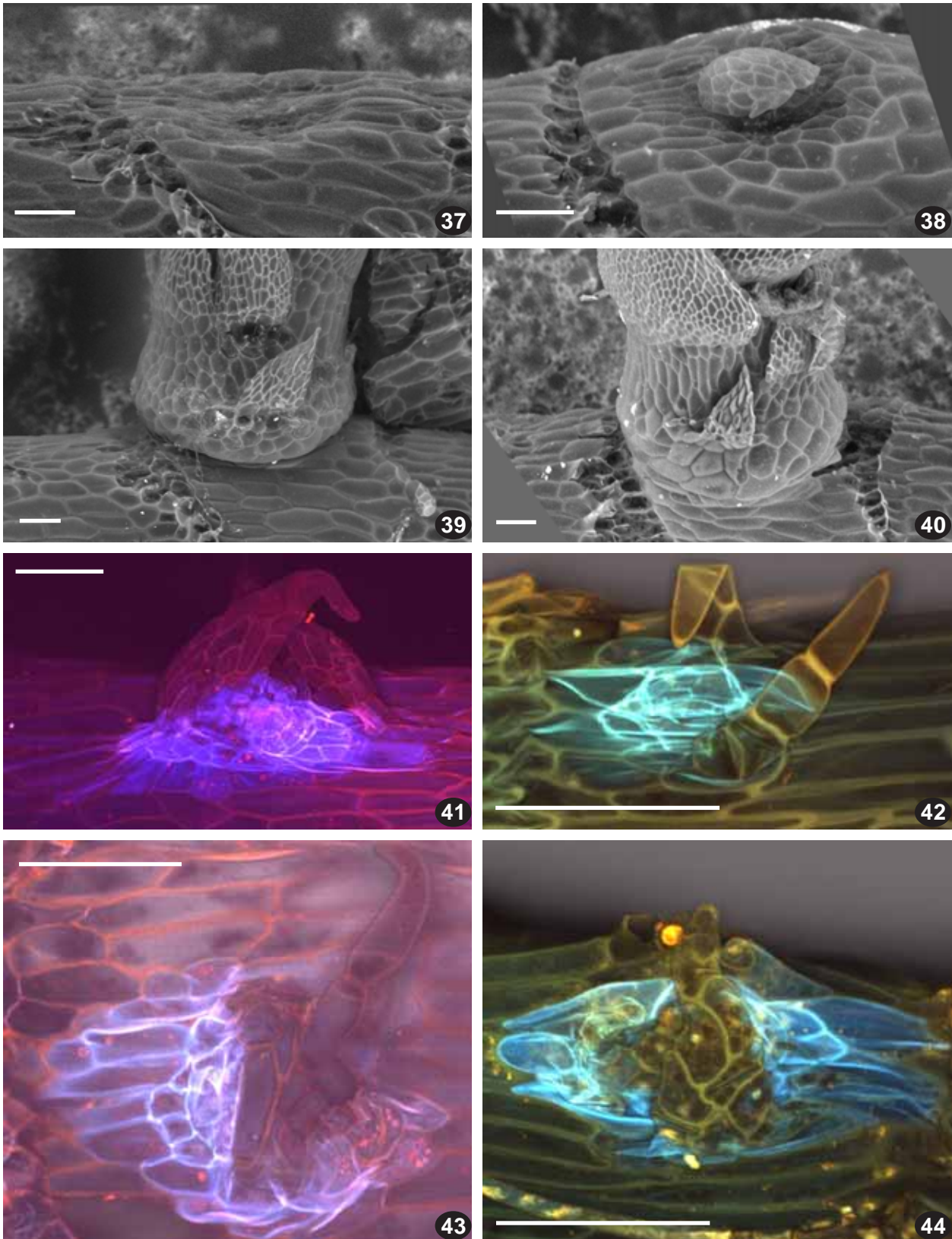
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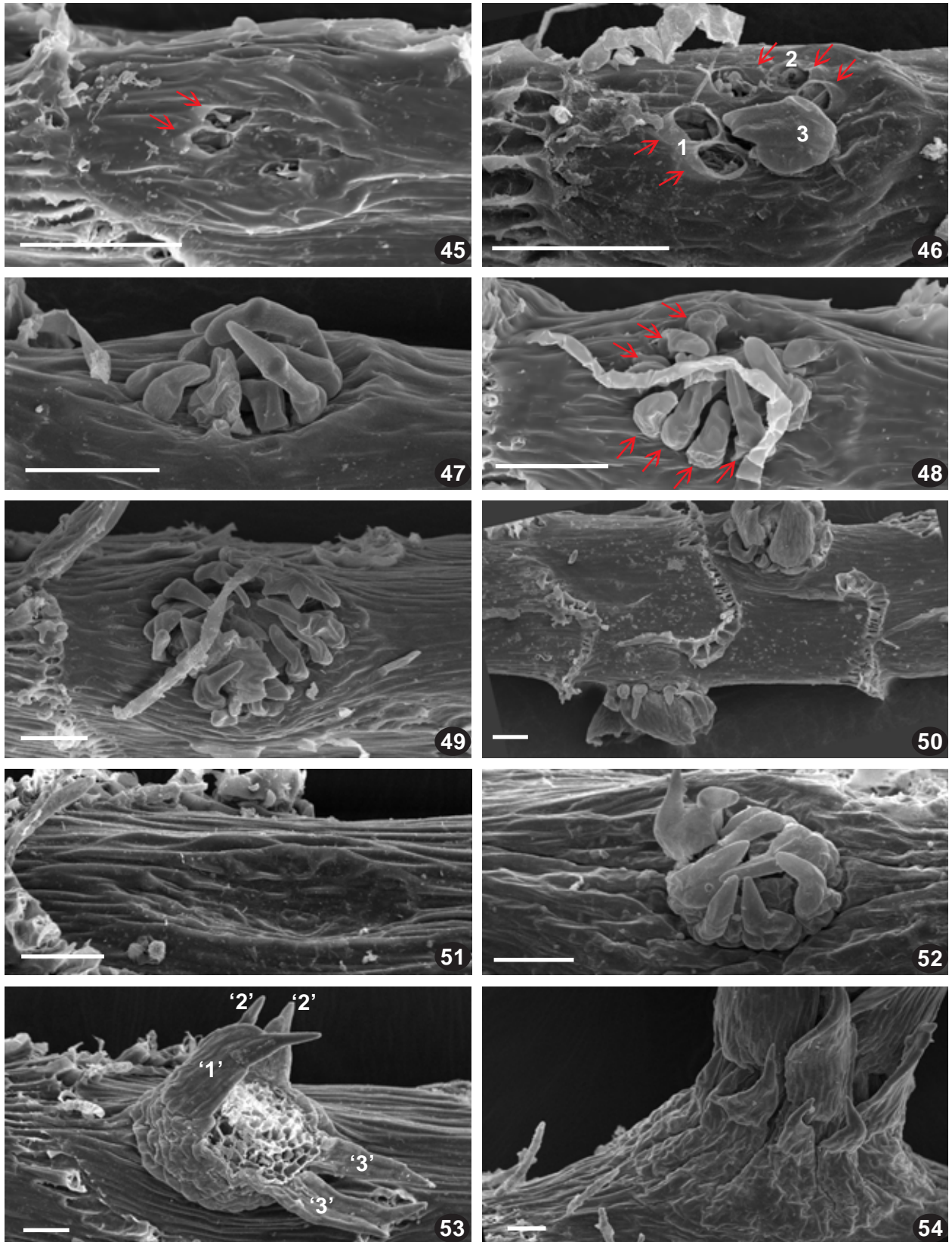
Figs. 18-28. Branch primordia of Hookeriales. *Leskeodon philippinensis* (#18), *Lepidopilidium portoricens* (#19), *Hypnella diversifolia* (#20, 22, 24), *Hookeriopsis acicularis* (#21), *Sauloma tenella* (#23), *Hookeria lucens* (#25, 28) and *Hookeria acutifolia* (#26-27). All from light microscopy, except 26-27, SEM, gold coated. Pictures show variation within *Bryum*-type: #18 – hollow stage; #19-21 & 23 – various raisings of young branches with leaves that are apparently sitting on branch tissue; #22 provides a view that can be interpreted as proximal branch leaves sitting around pale area, formed from the apical branch cell; comparing, however with #43, indicates a possibility of alternative interpretation. Scale bars: 50 μ m for all.



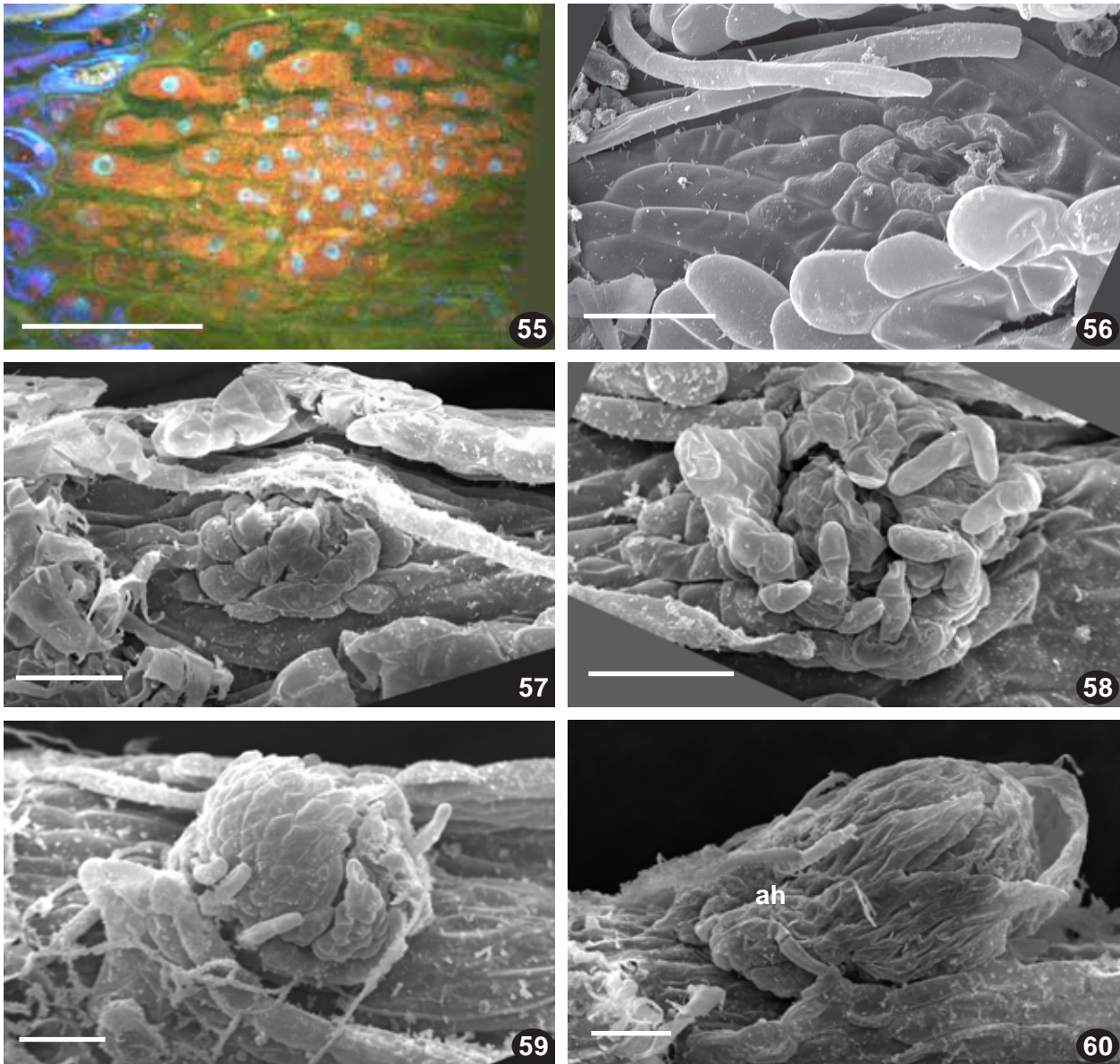
Figs. 29-36. Branch primordia of Hookeriales: *Cyathophorum bulbosum* (#29), *Hypopterygium flavolimbatum* (#30, 32), *Hookeria lucens* (#31, 33), *Calyptrochaeta japonica* (#34), *Daltonia splachnoides* (#35), *Lopidium concinnum* (#36), LSCM, DAPI staining. #29 – hollow-stage, note cell arrangement as in a leaf, showing sequence in a rainbow color order and that leaf is laying on the stem on its dorsal side; #30 – almost flat stage in *Hypopterygium*; #31 – totally leafless young branch; apical cell arrowed; #32 – sac-like and almost leafless stage of large branch primordium of *Hypopterygium*; #33-34 – bases of developed branches, showing subulate proximal and much broader older leaves; #35 – muff-life leafless zone at branch base of *Daltonia*; #36 – young branch base of *Lopidium*; note, that in this genus and in *Daltonia* proximal branch leaves are broad. Scale bars: 50 μ m for all.



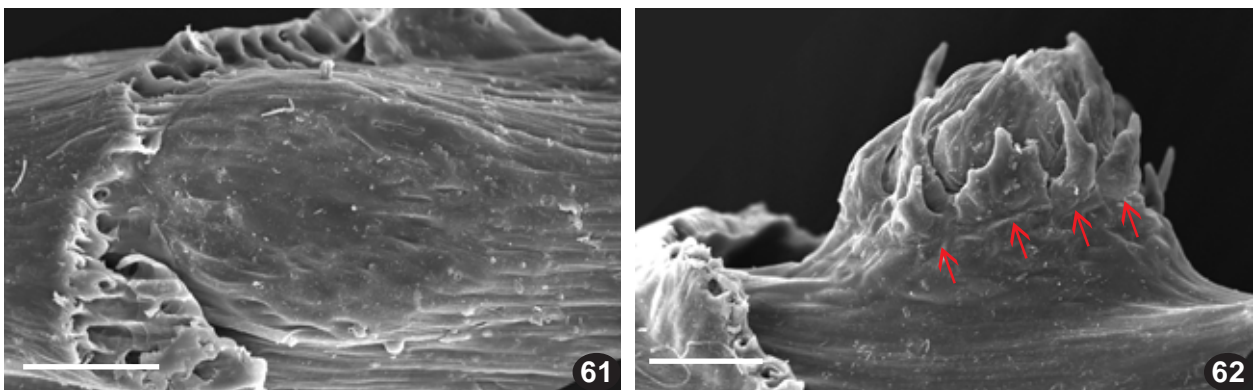
Figs. 37-44. Branch primordia of *Callicostella papillata* (#37-40, SEM, gold coated), *Hypopterygium flavolimbatum* (#41 & 43) and *Amblystegium serpens* (#41-44, LSCM, DAPI staining). #37-38 – starting from the hollow, *Callicostella* develop quite broad most proximal branch leaf, which likely does not correspond to first merophytes that form leafless branch base (#39-40). #41 & 43 – blue fluorescence of younger cell walls (cf. with pellucide zones in #20-22) indicate *Bryum*-type of branch primordia with leaves appearing upon branch tissue, in contrast to #42 & 44, *Climacium*-type, where proximal branch leaves appear “outside” [more precisely at the edge, see scheme 1 on page 000] area formed by branch apical cell. Scale bars: 50 μ m for all.



Figs. 45-54. Branch primordia of *Acrocladium chlamydophyllum*, #45-50, and *Trachyloma indicum*, #51-54 (SEM, gold coated). Stages from hollow (#45 & #51) to branch with ovate-lanceolate leaves (#50 & #54) are performed. Some most proximal branch leaves (#45 & 46, arrowed) are broken from their bases, allowing to see "transverse section" of compound leaves at stage of two-three cells wide. When 'mature' ovate leaves appear (#50 and #54), compound proximal leaves with filamentous segments may retain (#50) or disappear, likely fallen off (#53-54). Note compound '2d' and '3d' branch leaves in #53, in quotation marks because the filamentose structures (cf. #52) seems to be not retained. Scale bars: 50 μ m for all.



Figs. 55-62. Branch primordia of *Plagiothecium denticulatum*, #55-60, and *Orthothecium chryseon*, #61-62 (SEM, gold coated, except #55. LSCM, DAPI). Primordium at first can be flat (#55), or shallow hollow (#61), or as a small hole-like (#56, cf. 63-1). First stages of exvagination are still leafless (Fig. 57). Later on, filamentous segments of compound proximal branch leaves appear (Fig. 58), and then broad leaves start to develop (Figs 59-60). Most proximal branch leaves can be strongly lacinate and up to compound (arrowed), likely due to position upon branch base that strongly enlarging during the time of these leaves growth. Scale bars: 50 μ m for all.



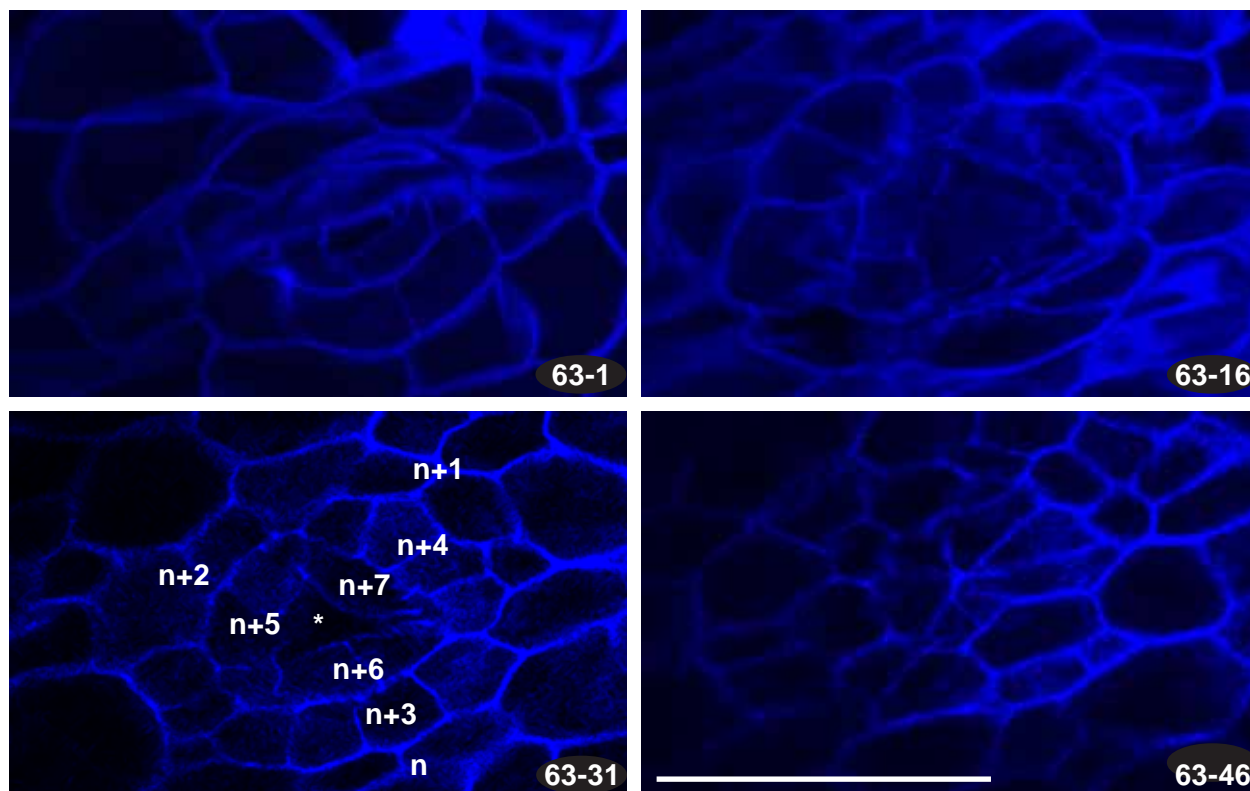


Fig. 63. Scanning through branch primordium of *Plagiothecium cavifolium* (LSCM, fluorescence brightener #28). Second number is that of scanning that is done at 0.5 μm one from another, thus the distance from upper (63-1) and lower (63-46) is 23 μm . Note that the branch apical cell at the stem surface is exposed only partly comparing with its size 8 μm below surface (63-16), being partly covered by surrounding surface cells (cf. #56 and Fig. 1 in Akiyama, 1990). Merophytes are numbered in #63-31 assuming indefinite number of first merophytes at the periphery of hollow; apical cells marked by asterisk (*). Scale bars: 50 μm for all.

18, 20-22, 24). The position of most proximal branch leaves within these areas indicates the *Bryum*-type of branch primordia: first branch merophytes do not produce leaves. This pattern is even better seen through the fluorescence microscopy (cf. 41 & 43).

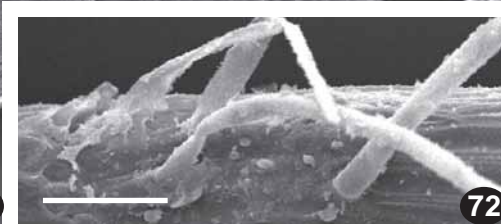
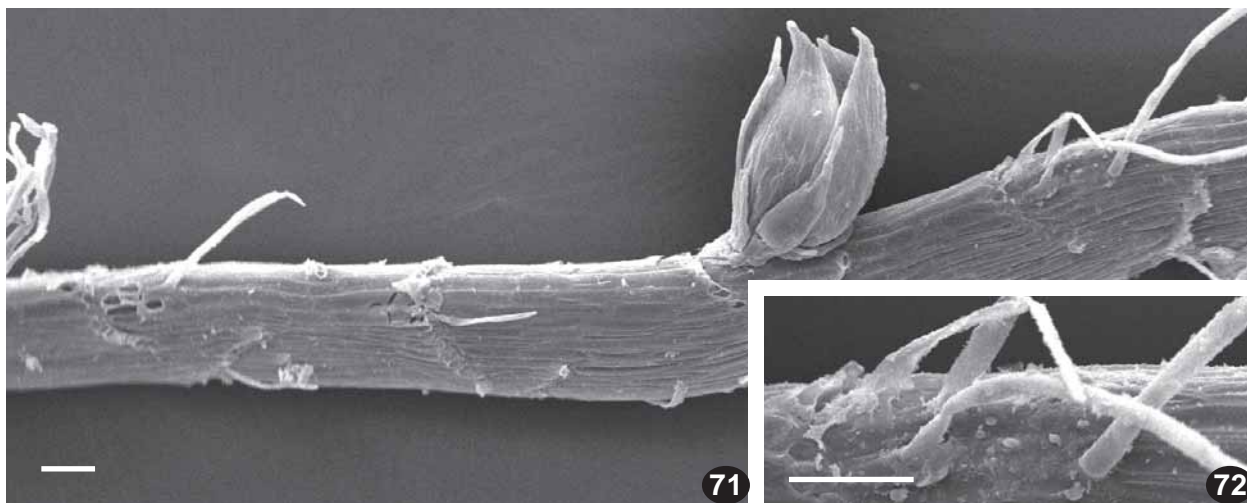
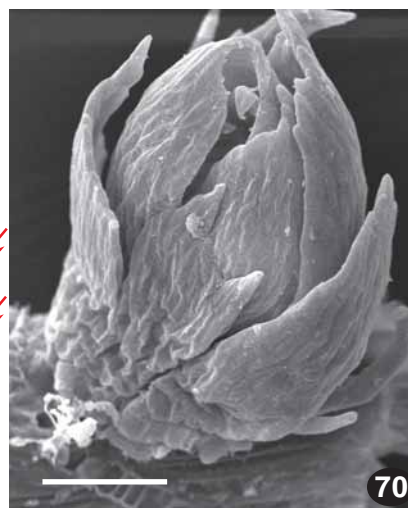
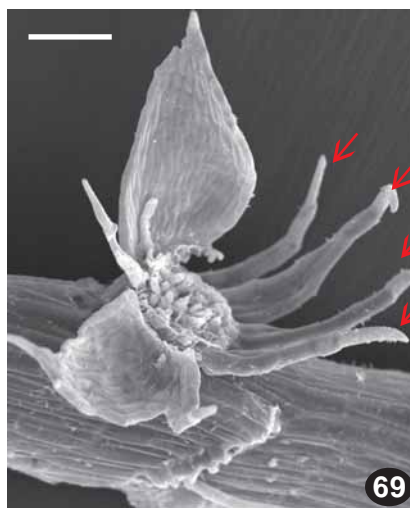
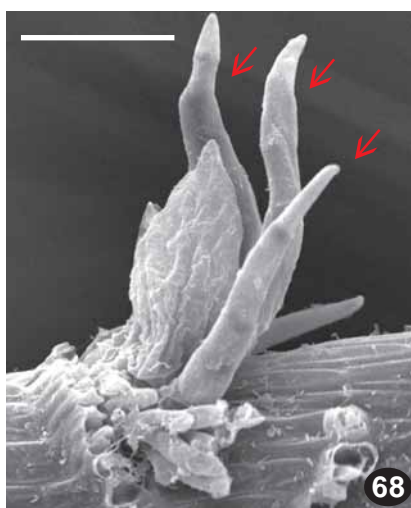
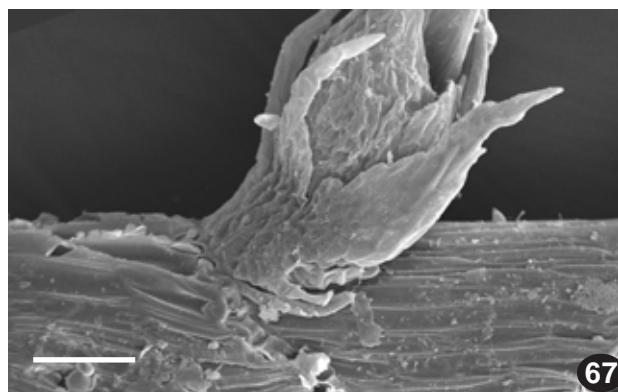
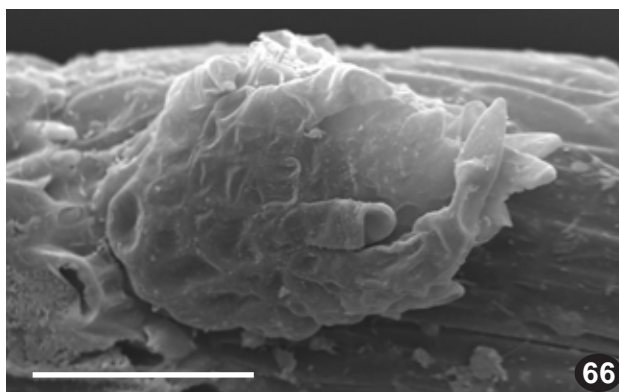
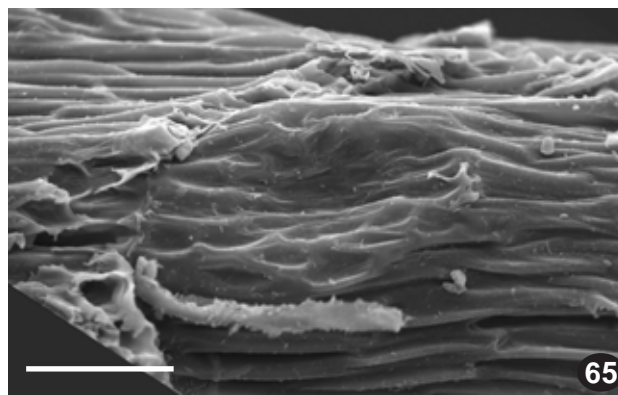
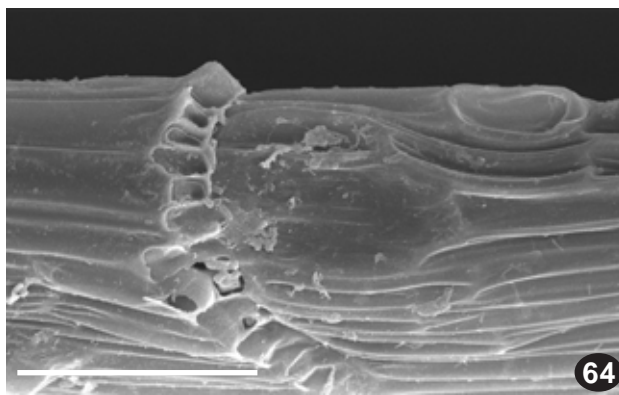
Narrow and subulate, or more broad, leaf-like structures appear usually near or on the mound of inflated cells (Figs. 17, 19, 43). According to our limited observations, this happens more commonly in distal parts of shoots, while in their proximal parts, where sympodial branching occur (i.e. resulting in primary modules), the branch primordia may remain leafless longer (Figs. 31, 32). This is especially apparent in *Hypopterygium* and *Hookeria*, and similar cases were seen in *Benitotania*, *Bryobrothera*, *Sauloma*, etc.

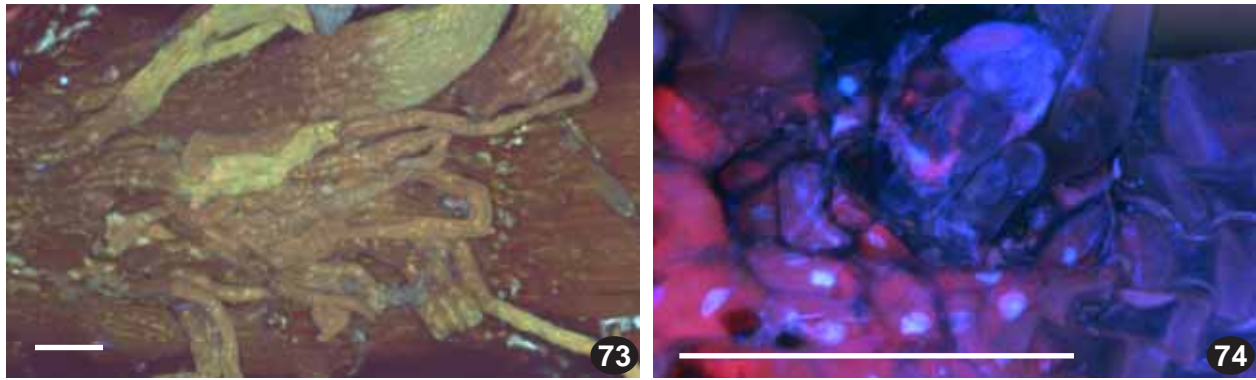
Narrow leaves are especially characteristic of *Calypstrochaeta* (Fig. 34), *Hookeria* (Figs. 17, 25, 28, 33), *Lepidopilidium* (Fig. 19), and *Sauloma*. Broader proximal branch leaves occur in *Daltonia* (Fig. 35); *Lopidium* (Fig. 36), and *Callicostella* (#37-38). A general correlation that

can be seen in the Ptychomniales and basal Hypnales where narrow foliose structures are associated with the hollow-like primordium is not relevant for the Hookeriales. A possible explanation is that in two former orders, these most proximal foliose structures are in fact a segments of compound leaves (e.g. Figs. 5-12 for *Hampeella* and 45-50 for *Acrocladium*) whereas in Hookeriales, even narrow leaves are homologous to the whole leaf, and not its part, as e.g. in *Hampeella* (Figs. 7-8) and *Acrocladium* (Figs. 45-50).

Deductively, the leafless branch base surface is composed by first branch merophytes (e.g. Fig. 31, 32, 39-40), so the most proximally developed branch leaves are formed by somewhat later merophytes. This interpretation explains the absence of an exact position of the first proximal branch leaf, which commonly occurs in the Hypnales (cf. Ignatov & Hedenäs, 2007), and is represented in most groups as the '4 o'clock position' pattern. The absence of regularity is often treated as an evidence that these structures are not homologous to leaves, but that

Figs. 64-72. Branch primordia of *Isopterygiopsis muelleriana* (SEM, gold coated): #64-65 – stage of leafless hollow; #66 – stage of small raising, with still poorly differentiated leaves; #67-69 – young branches, showing deeply dissected and up to compound 'third' leaf, arrowed in Figs. 68 & 69 (cf. comment to Fig. 53). #70 more developed branch with ovate-lanceolate leaves and narrow leaves and lobes of the compound leaves only near branch bases; #71 (partly magnified on #72) shoot showing position of axillary rhizoids, sometimes (#72) surrounded area similar in shape and position to hollow (cf. Fig. 65). In other cases (#71, left) rhizoids are more clearly axillary, however still likely related to branch primordia that are sometimes much reduced and closely adjoining to leaf axil (#64). In #68 the 3-4-celled uniseriate axillary brood bodies are seen at brach base. Scale bars: 50 μm for all.





Figs. 73-74. Branch primordia of *Rutenbergia prionodon*, #73 and *Rhizofabronia perpilosa*, #74 (LSCM, DAPI staining). In *Rutenbergia*, the foliose structures around branch primordia are strongly lacinate, having long uniseriate “ciliae” along their margin. In *Rhizofabronia* branch primordia is very small, hole-like, and most proximal branch leaves are very narrow, 7-10 μm wide to be able appear from such ‘hole’. Scale bars: 50 μm for all.

they need a special recognition as a specific structure, for example ‘pseudoparaphyllia’. However, an alternative explanation of irregularity by an indefinite number of first merophytes which do not develop leaves seems no less relevant.

Reduction of some proximal leaves is not a rare event in mosses (Spirina & Ignatov, 2011; Ignatov & Spirina, 2012), and in the case of basal pleurocarpous mosses a number of first branch merophytes do not produce leaves, in a way as young stems of *Schistostega pennata* (Hedw.) F. Weber & D. Morh do (Leitgeb, 1874). The number of reduced leaves in basal pleurocarps seems to be rather indefinite (Ignatov & Spirina, 2012) compared with, for example, the invariable two proximal branch leaf reduction in the Brachytheciaceae (Spirina & Ignatov, 2005), and commonly, although not always, one proximal branch leaf reduction in the Fontinalaceae (Spirina & Ignatov, 2011). Sometimes a fixed position of most proximal developed branch leaves may be observed in the Plagiotheciaceae (*Isopterygiopsis*, see below).

BASAL HYPNALES

Previous studies on the Habrodontaceae (Budyakova *et al.*, 2003) and current observations on *Acrocaldium*, (Figs. 45-50), *Trachyloma* (Figs. 51-54), *Plagiothecium* (Figs. 55-60 & 63), *Orthothecium* (Figs. 61-62), *Isopterygiopsis* (Figs. 64-72) and *Rhizofabronia* (Fig. 74) illustrate the *Bryum*-type of branch primordia in most of basal families of the Hypnales. An exception is provided in *Rutenbergia* (Figs. 73), which is discussed below.

Compound leaves of *Hampeella*-type are common in the basal Hypnales, obviously correlated to a quite deep position of the branch apical cell that has no protective structures around and often is deeply submerged and partly covered by surrounding surface cells (Figs. 56, 63 and cf. also Figs. 1 & 4 in Akiyama, 1990). The *Hampeella*-type of narrow proximal branch leaves (Ignatov & Spirina, 2012) occurs in *Plagiothecium*, *Acrocaldium*, *Trachyloma*, etc., although they are not always seen at the base of developed branches.

Orthothecium sometimes has been treated as an exception in the Plagiotheciaceae, as a genus that some-

times has ‘pseudoparaphyllia’, contrary to most other genera of the family. This likely relates to a rather large and, conclusively, rapidly growing branch primordium, resulting in deeply lacinate proximal developed branch leaves (Fig. 62) situated on the basal part of branch.

In *Isopterygiopsis muelleriana*, the most proximal developed branch leaf is interesting in that it is (1) in a ‘12 o’clock’ position and (2) is deeply dissected, almost to the base. This can be paralleled to *Hampeella* (Fig. 8) or maybe to *Hypnum cupressiforme* (Spirina & Ignatov, 2008). The ‘12 o’clock’ position had been interpreted as the reduction morphologically of the first branch leaves, *i.e.*, produced by the first two branch merophytes, in the Brachytheciaceae and Leucodontaceae (Spirina & Ignatov, 2005, 2010). *Isopterygiopsis* suggests a similar case; moreover, the first merophytes sometimes seem to be used for developing 3-4-celled and uniseriate axillary brood bodies (Fig. 68).

Branch primordia of *Isopterygiopsis muelleriana* show interesting details concerning the position of axillary rhizoids. In some views (Fig. 72), they are in the surrounding area similar in shape and position to the depression-stage of the primordium. Moreover, these depressions are sometimes as small as only two cells and situated immediately (looking ‘appressed’) in leaf axils (cf. Figs. 64-65). Thus, we can conclude that rhizoids surround branch primordia that is well-known for *Cinclidium* (Koponen, 1968) where rhizoid topography follows an elongate branch primordium outline.

For a long time, *Trachyloma* has been treated within the Pterobryaceae, before molecular phylogenetic analyses put it in an isolated position in the basal grade (Tsubota *et al.*, 2004; Huttunen *et al.*, 2012), which is supported by the presence of *Bryum*-type branch primordia (Figs. 51-54).

Rhizofabronia is a small plant and position of apical cell in small depressions/ hole imposes limits on width of its first leaves, which are narrow and filamentose in order to be able to grow through the bottleneck of the hole (Fig. 74, and compare with Figs. 18 and 56).

The foliose structures around branch primordia in *Rutenbergia* (Fig. 73) are reminiscent of those of *Glyphothecium* (Figs. 15-16). Apparently, they should be treated as proximal branch leaves, which are strongly dissected and partly compound. Note also their “ciliate” margins, similar to ‘cilia-like teeth’ on leaves of *Rhizofabronia*.

CONCLUSIONS

The *Bryum*-type branch primordium is the common case in basal groups of pleurocarpous mosses and it significantly delimits basal Hypnales from advanced ones that mostly have *Climacium*-type branch primordia (Ignatov, 1999; Ignatov & Spirina, 2012). However, even within the small order Ptychomniales, *Bryum*- to *Climacium*-type transition occurs.

The *Bryum*-type sometimes is described in terms of ‘pseudoparaphyllia lacking’, e.g. in Plagiotheciaceae. However, due to an inconsistent application of this term, e.g. *Hampeela* and *Plagiothecium* are described differently, despite their structure being highly similar.

Some proximal branch leaves are reduced in the *Bryum*-type of branch primordium, and in many groups the most proximally developed branch leaves are dissected to the base. Compound leaves, composed of several laminae not connected to each other above the stem surface, however are homologous to one leaf, originating from a single branch merophyte.

The term ‘paraphyllia’ is not an appropriate term in the Ptychomniales. Numerous foliose structures around branch bases are strongly dissected in this order and represent compound proximal branch leaves.

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Appendix: Specimen studies (all from MHA), and index to illustrations in the paper	Figs.
Ptychomniales	
Ptychomniaceae	
<i>Cladomnion ericoides</i> (Hook.) Wilson	New Zealand, South Island, 16 Aug. 1994, B.C. Tan, A. Fife —
<i>Cladomniopsis crenato-obtusa</i> M. Fleisch.	Argentina, Terra Fuego, 20 Feb. 1984, C.M. Matteri —
<i>Glyphothecium sciuroides</i> (Hook.) Hampe	New Zealand, South Island, Lake Rotoiti, H. Streimann, 58179 15,16
<i>Hampeella alaris</i> (Dixon & Sainsbury) Sainsbury	Australia, Tasmania, H. Streimann, 59704 5-8
<i>Hampeella pallens</i> (Sande Lac.) M. Fleisch.	Australia, Queensland, 19 Aug. 1999, H. Streimann & T. Pocs 9-12
<i>Orthorrhynchium elegans</i> (Hook. f. & Wilson) Reichardt	Papua New Guinea, D.H. Norris, 63985. 1-4
<i>Garovaglia elegans</i> (Dozy & Molk.) Hampe ex Bosch & Sande Lac.	Papua New Guinea, H. Streimann, 17372, 24899. 13
<i>Ptychomnion aciculare</i> (Brid.) Mitt.	Australia, 18 June 1999, H. Streimann 14
Hookeriales	
Hypopterygiaceae	
<i>Cyathophorum bulbosum</i> (Hedw.) Müll. Hal.	New Zealand, South Island, 6 Feb. 1993, H. Streimann, 51426 29
<i>Dendroclythium decolyi</i> (Broth. ex M. Fleisch.) Kruijer	Papua New Guinea, Morobe Prov., 23 July 1981, D.H. Norris, 64060 —
<i>Hypopterygium flavolimbatum</i> Müll. Hal.	Russian Far East, Primorsky Territory, 16 Oct. 2008, M. Ignatov; 30, 32 25 June 2012, E.V. Malashkina 41, 43
<i>Lopidium concinnum</i> (Hook.) Wilson	Australia, Victoria, 4 Dec. 1996, H. Streimann, 58671 36
Saulomataceae	
<i>Sauloma tenella</i> (Hook. f. & Wilson) Mitt.	Australia, Western Australia, 12 Oct. 1973, D.H. Norris 23
Daltoniaceae	
<i>Achrophyllum dentatum</i> (Hook. f. & Wilson) Vitt & Crosby	Australia, New South Wales, 24 Apr. 1998, H. Streimann, 61075 —
<i>Benitotania elimbata</i> H. Akiy., T. Yamag. & Suleiman	Malaysia, 20 Sept. 2002, H. Akiyama, M. Suleiman —
<i>Bryobrothera crenulata</i> (Broth. & Paris) Thér.	Papua New Guinea, Morobe Prov., 14 June 1981, D.H. Norris, 61964; — Australia, Queensland, 25 May 1974, D.H. Norris —
<i>Calyptrochaeta japonica</i> (Cardot & Thér.) Z. Iwats. & Nog.	Japan, Kushu, 14 Sept. 2009, T. Yamaguchi, 31308 34
<i>Daltonia splachnoides</i> (Sm.) Hook. & Taylor	New Zealand, South Is., 16 Aug. 1994, B.C. Tan & A. Fife, 94-335 35
<i>Distichophyllum crispulum</i> (Hook. f. & Wilson) Mitt.	Australia, Queensland, 19 Aug. 1999, H. Streimann 64189 —
<i>Distichophyllidium nymanianum</i> M. Fleisch.	Papua New Guinea, Morobe Prov., 29 May 1981, D.H. Norris, 60455 —
<i>Leskeodon philippinensis</i> Broth.	Papua New Guinea, Morobe Prov., 27 Aug. 1981, D.H. Norris, 66020 18
Schimperobryaceae	
<i>Schimperobryum splendidissimum</i> (Mont.) Margad.	Chile, Valdivia, 18 Apr. 1982, L.R. Landrum —
Hookeriaceae	
<i>Crossomitrium patrisiae</i> (Brid.) Müll. Hal.	Suriname, 6 Feb. 1965, P.A. Florschütz & P.J.M. Maas —
<i>Hookeria acutifolia</i> Hook. & Grev.	U.S.A., North Carolina, 25 Oct. 1958, D.H. Norris 26,27
<i>Hookeria lucens</i> (Hedw.) Sm.	Russia, Caucasus, 1 Febr. 2012 M. Ignatov & E. Ignatova 31,33
<i>Hookeria lucens</i> (Hedw.) Sm.	Russia, Caucasus, 18 Aug. 1996, T. Akatova; 17 U.S.A., Northern California, 7 Aug. 1989, M. Ignatov 25,28
Leucomiaceae	
<i>Leucomium strumosum</i> (Hornsch.) Mitt.	Suriname, 21 Nov. 1981, J. Florschütz-de Waard & R. Zielman, 5718 —
Pilotrichaceae	
<i>Actinodontium raphidostegum</i> (Müll. Hal.) Bosch & Sande Lac.	Papua New Guinea, 26 Aug. 1981, D.H. Norris, 65958 —
<i>Brymela tutezona</i> Crosby & B.H. Allen	Panama, Cocle, 22 Apr. 1992, N. Salazar Allen <i>et al.</i> , 13656 —
<i>Callicostella papillata</i> (Mont.) Mitt.	Vanuatu, Espiritu Santo, 20 Oct. 1998, H. Streimann & P. Ala, 62549 37-40
<i>Cyclodictyon albicans</i> (Hedw.) Kuntze	Equador, Napu, 25 Sept. 1980, L.Holm-Nielsen <i>et al.</i> —
<i>Hemiragis aurea</i> (Lam. ex Brid.) Kindb.	Costa Rica, 14 May 1983, R.Liesner, 15634 —
<i>Hookeriopsis acicularis</i> (Mitt.) A. Jaeger	Puerto Rico, 9 Jan. 1940, W.C. Steere 21
<i>Hypnella diversifolia</i> (Mitt.) A. Jaeger	Colombia, Antioquia, 11 July 1992, S.P. Churchill <i>et al.</i> , 18335 20,22,24
<i>Lepidopilum polytrichoides</i> (Hedw.) Brid.	Costa Rica, Limon, 5 Nov. 1988, G. Herrera, 2295 —
<i>Lepidopilidium portoricense</i> (Müll. Hal.) H.A. Crum & Steere	Suriname, Sipalwini, 8 July 2001, B. Allen 23690 19
Hypnales	
Rutenbergiaceae	
<i>Rutenbergia prionodon</i> (Besch.) Renaud	Mauritius, 5 Dec. 1972, M. & C. Crosby 73
Trachylomataceae	
<i>Trachyloma indicum</i> Mitt.	Philippines, Mindanao, 9 Aug. 1999, Schumm, Schwarz, #4428; 51-54 Malaysia, Pahang State, 29 July 2007, M. Ignatov —
Fabroniaceae	
<i>Rhizofabronia perpilosa</i> (Broth.) Broth.	Tanzania, Moshi district, 17 June 1988, T. Pocs, R. Ochyra & H. Bednarek-Ochyra, 88133/B 74
Plagiotheciaceae	
<i>Isopterygiopsis muelleriana</i> (Schimp.) Z. Iwats.	Russian Far East, Primorsky Territory, M. Ignatov <i>et al.</i> , #06-2623 64-72
<i>Orthothecium chryseum</i> (Schwägr.) Schimp.	Russia, Sakhalin, 20 Aug. 2006, M. Ignatov, V. Teleganova 61-62
<i>Plagiothecium cavifolium</i> (Brid.) Z. Iwats.	Japan, Honshu, 24 July 2012, U. Spirina 63
<i>Plagiothecium denticulatum</i> (Hedw.) Schimp.	Russia, Tatarstan Rep., 18 Aug. 2003, M. Ignatov, E. Ignatova, 03-53 55-60
Acrocladiaceae	
<i>Acrocladium chlamytophyllum</i> (Hook. f. & Wilson) Müll. Hal. & Broth.	Australia, Victoria, 27 Feb. 1997, H. Streimann 59467 45-50
<i>Acrocladium auriculatum</i> (Mont.) Mitt.	Argentina, Terra Fuego, 14 Feb. 1984, C. Matteri & M. Schiavone —
Amblystegiaceae	
<i>Amblystegium serpens</i> (Hedw.) Bruch <i>et al.</i>	Russia, Moscow, 20 Nov. 2012, M Kostina 42, 44