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A COMPARISON OF EARLY STAGES OF BRANCH DEVELOPMENT IN *BRACHYTHECIUM* AND *CALLIERGON* (BRYOPHYTA)

СРАВНЕНИЕ РАННИХ СТАДИЙ РАЗВИТИЯ ВЕТОЧЕК *BRACHYTHECIUM* И *CALLIERGON* (BRYOPHYTA)

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Abstract

Early stages of branch development in *Brachythecium* and *Calliergon* are studied, using serial sections through the shoot apex. Our data suggest that the specific arrangement of pseudoparaphyllia (or, in alternative terminology, proximal branch leaves) of *Brachythecium* are likely caused by reduction of the two outermost pseudoparaphyllia that are developed in most of other pleurocarpous families, including C*alliergon*.

Резюме

Ранние стадии развития веточек у мхов *Brachythecium* и *Calliergon* изучены на сериях анатомических срезов верхушки побега. Выявлено, что заложение первых веточных листьев *Brachythecium*, специфичное только для семейства Brachytheciaceae, формируется на самых ранних стадиях развития и отличия наблюдаемой у этого семейства картины от всех прочих бокоплодных мхов связано, по-видимому, с редукцией двух латерально расположенных псевдопарафиллиев, развитых у большинства бокоплодных мхов.

INTRODUCTION

The fact that the branches in pleurocarpous mosses are surrounded by specific structures at early stages of their development was first described by Warnstorf (1914), who termed these structures “pseudoparaphyllia.” Iwatsuki (1963) was probably the first bryologist who employed the characters of pseudoparaphyllia in taxonomy. Morphological circumscription of structures around young branches and dormant buds have recently seen a number of alternative interpretations (Allen, 1987; Akiyama, 1990a,b; Akiyama & Nishimura, 1993; Ignatov, 1999, etc.). Ignatov & Hedenäs (2006) reviewed this problem, showing that the present understanding of different stem structures is rather imperfect, and definitions of paraphyllia and pseudoparaphyllia are vague.

The purpose of this paper is to compare early stages of development of *Brachythecium* and *Calliergon*, two genera that appear to have contrastingly different patterns of pseudoparaphyllia development. As was shown by Ignatov (1999), the families Brachytheciaceae and Meteoriaceae are unique among the pleurocarps in having a distinctive arrangement of pseudoparaphyllia (or, in other terminologies scaly, or juvenile, or proximal branch leaves). According to Ignatov (l.c.), the first pseudoparaphyllium in Brachytheciaceae and Meteoriaceae is pointed downwards, whereas the second and third ones are situated at the angle of ca. 120° and 240° angles to the first one, respectively (Fig. 1). Contrary to that, most pleurocarpous mosses, including *Calliergon*, have the first pseudoparaphyllium in a lateral position, designated by Ignatov & Hedenäs (2006) as the “four o’clock” position (Fig. 1). The development of the latter pattern was described by Leitgeb (1868) and expanded upon by Berthier (1971); see also Fig. 2.

The immediate questions which we address in this study are: (1) do branches in *Calliergon*

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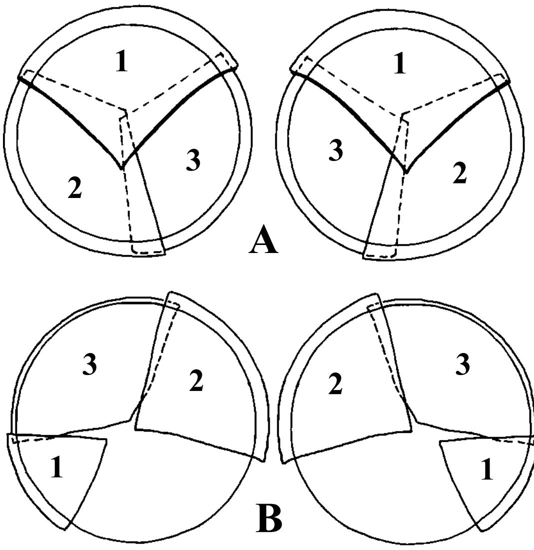
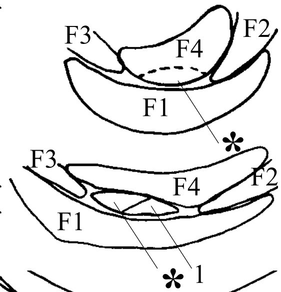


Fig. 1. Scheme of the arrangement of proximal branch leaves (pseudoparaphyllia) in *Brachythecium* (A) and *Calliergon* (B), expanded from Ignatov (1999).



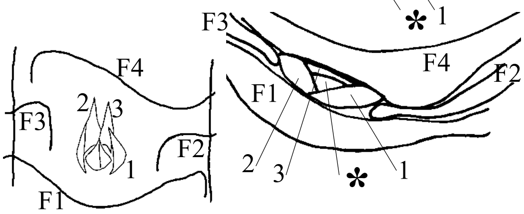


Fig. 2. Eearly stages of branch development in most of mosses: a schematic summary of Leitgeb (1868) and Berthier (1971).

F1–4 – order of stem leaves; * – branch initial cell (BIC), later – tetrahedral cell of branch apex, 1–3 – order of branch leaves.

and *Brachythecium* differ from the earliest stages of their development or does the difference in arrangement of pseudoparaphyllia (=outermost leafy structures around branch primordia) appear later due to, for example, a displacement of pseudoparaphyllia at the subsequent stages of branch development; (2) if the difference between *Calliergon* and *Brachythecium* occurs even at the earliest stages, with what do these differences correlate.

METHODS AND MATERIALS

Brachythecium rivulare B.S.G. (Moscow, Fili–Kunzevo Park, coll. Ignatov 25.III.2005, MHA); *B. rutabulum* (Hedw.) B.S.G. (Tver Province, Udomlya Distr., coll. Notov VII-2005, TVER) and *Calliergon giganteum* (Schimp.) Kindb. (Tver Province, Vyshnij Volochek Distr., VII-2005, TVER) were studied.

Both freshly collected plants and herbarium collections less than 1 year old were used; the latter were wetted before preparation for 24 h in water before preparation. Stem apical parts of ca. 5 mm lenght, in which most of the leaves had been detached, were used.

Samples were fixed in 4% paraformaldehyde for 12 h, dehydrated in 70% ethanol, stained in uranil-acetate (2% solution in 70% ethanol for 10 hours), and then dehydrated through a graded ethanol/acetone series to 100% acetone. After that samples were embedded in araldite 6005 medium, according to the protocol of manufacturer.

Some fresh specimens of *Brachythecium rivulare* were fixed in 2.5% glutaraldehyde for one week, stained with osmium tetroxide (1% in water, 10 h), and mounted in Epon 812, according to the protocol of the manufacturer [section from them shown in Figs. 3-7].

Sections were cut 4-6 μm thick, put on glass slides without mounting medium, and sometimes stained with methylene blue.

Sections were photographed under Axioplan optical microscope with Canon F35 camera (jpg files of 1.7-1.9 Mb in rgb mode).

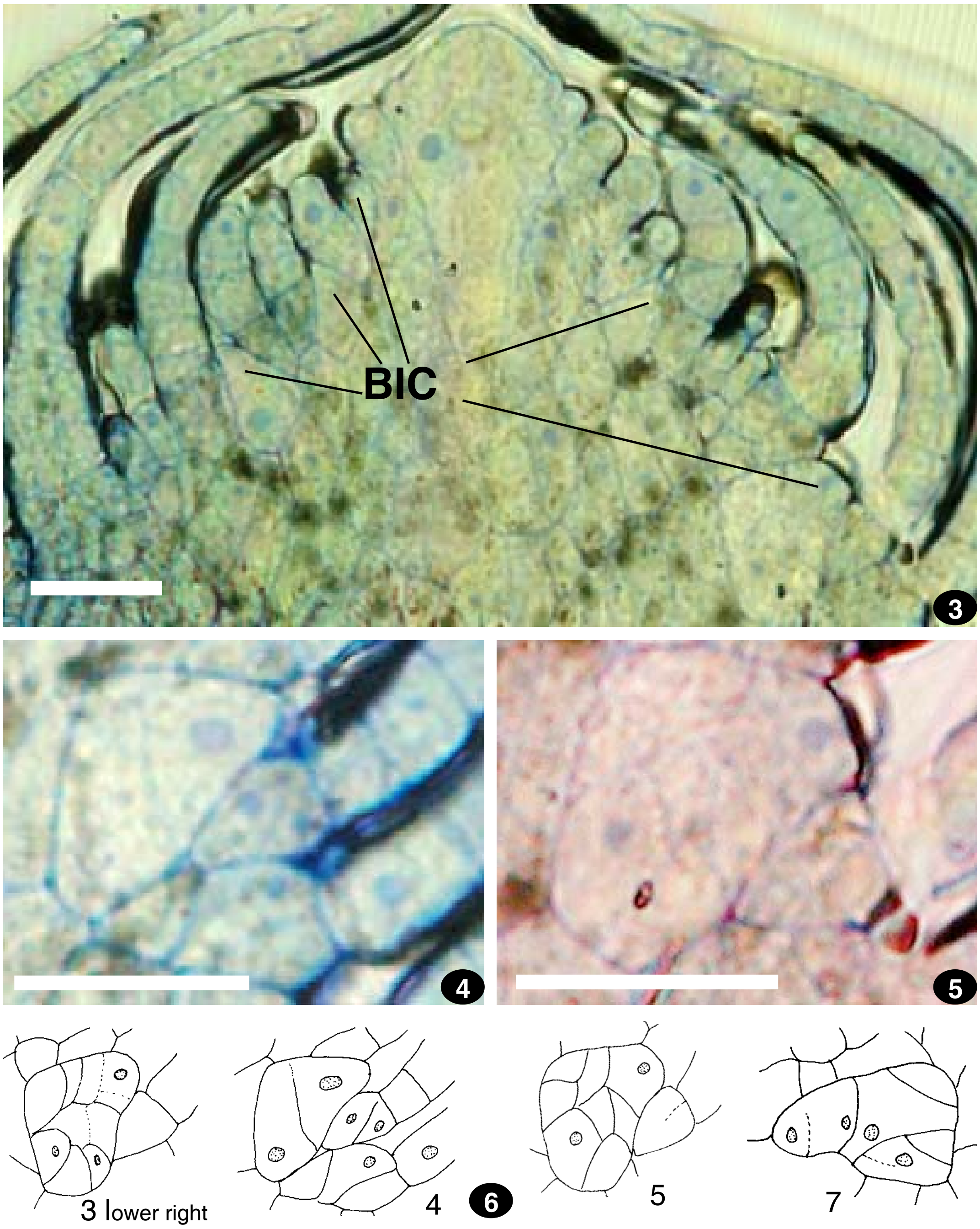
Since the main target of the study was the arrangement of the foliose structures around the branch primordia, we tried to obtain oblique cross sections that were perpendicular to the axis of the branch primordia. Longitudinal and transverse sections were studied as well.

TERMINOLOGY

Since the homology of foliose structures around branch primordia are not clear a priori, we will call all of them proximal branch leaves, including their earliest stages (from 1 cell) and including also what is commonly called pseudoparaphyllia.

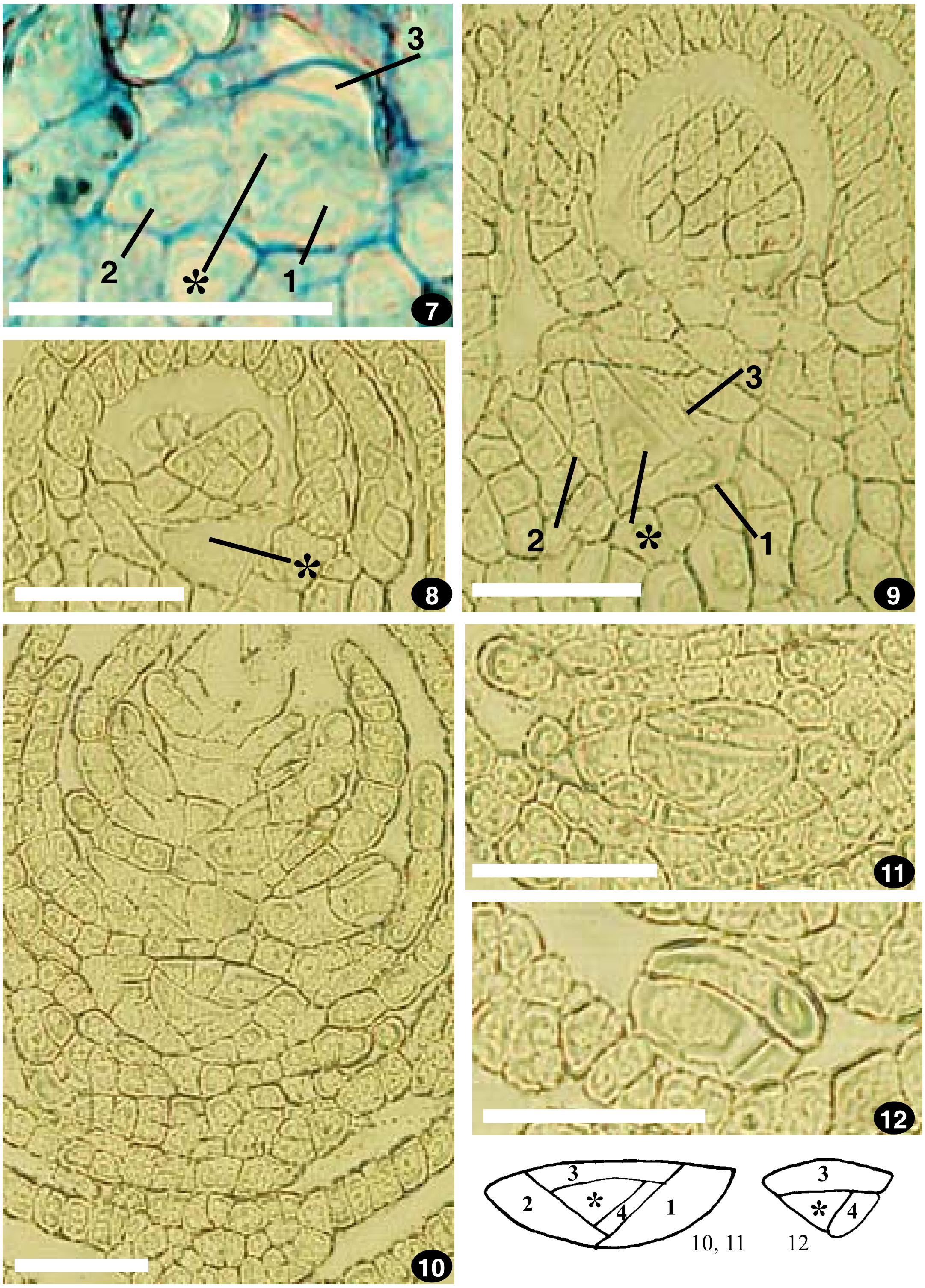
RESULTS

Two species of *Brachythecium* were found very similar in all essential characters, thus they both are discussed as one. However, quite different organizations were found in the shoot apices of *Brachythecium* (Figs. 3-20) and *Calliergon* (Figs. 21-33) in respect to the following:

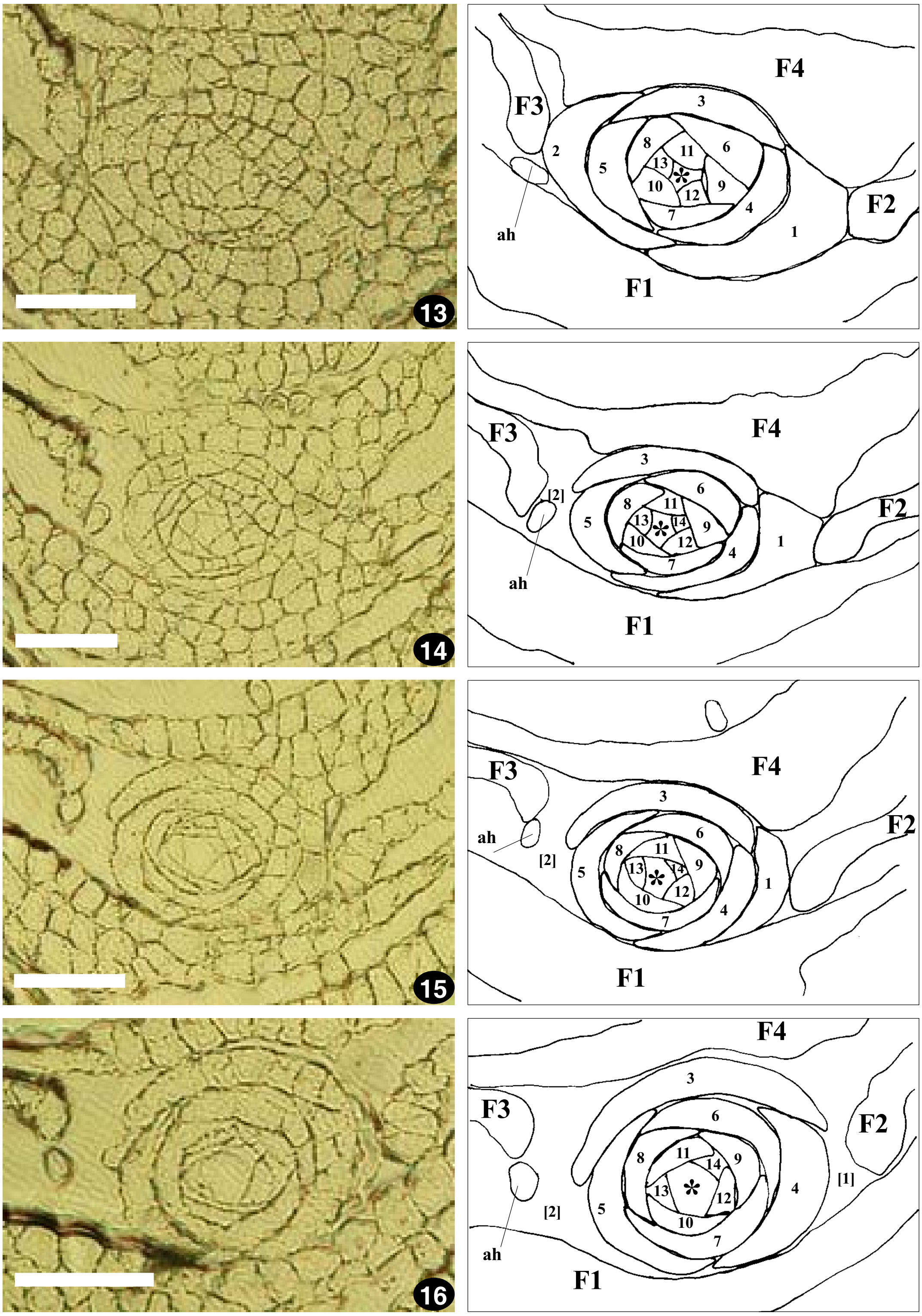


Figs. 3-6. *Brachythecium rivulare*. 3 – Longitudinal section of the apical part of stem; 4-5 – Longitudinal sections of BIC with several cells cut off from it by first divisions. 6 – sketches of BIC with several cells cut off from it by first divisions): '3-5' longitudinal sections shown in photographs 3, 4, 5; '7' – transverse section shown in photograph 7. Scale bars 25 µm.

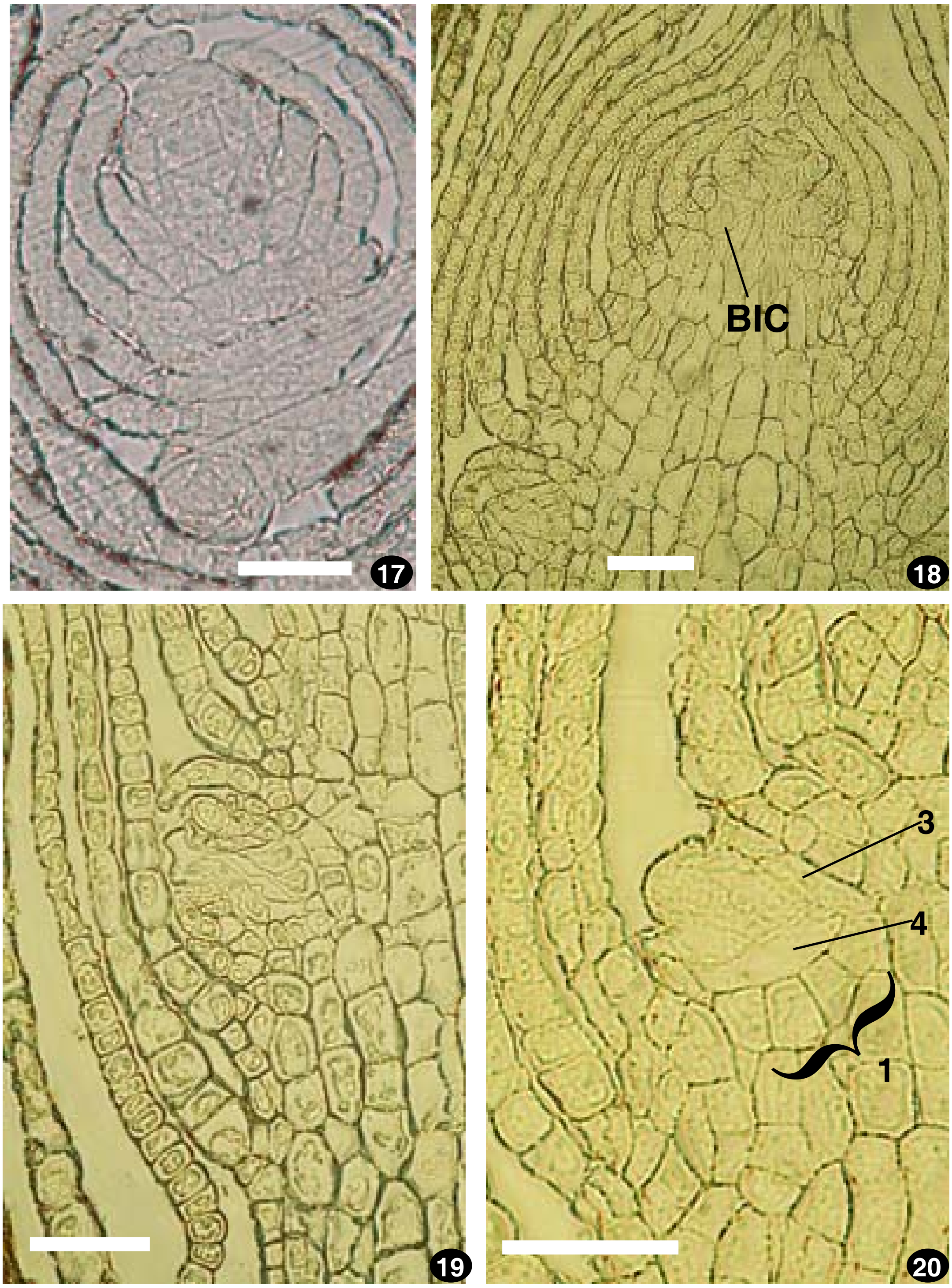
1) Young leaves of *Brachythecium* are arranged more tightly, with no space between them, except those around the branch initial cell (BIC), cf. Figs. 3, 17-18. In contrast the leaves of *Calliergon* are more separated at the same stage of development (Fig. 21-22). Correspondingly, neighboring leaves are tightly pressed against the young branches in *Brachythecium* (Figs. 12, 17,



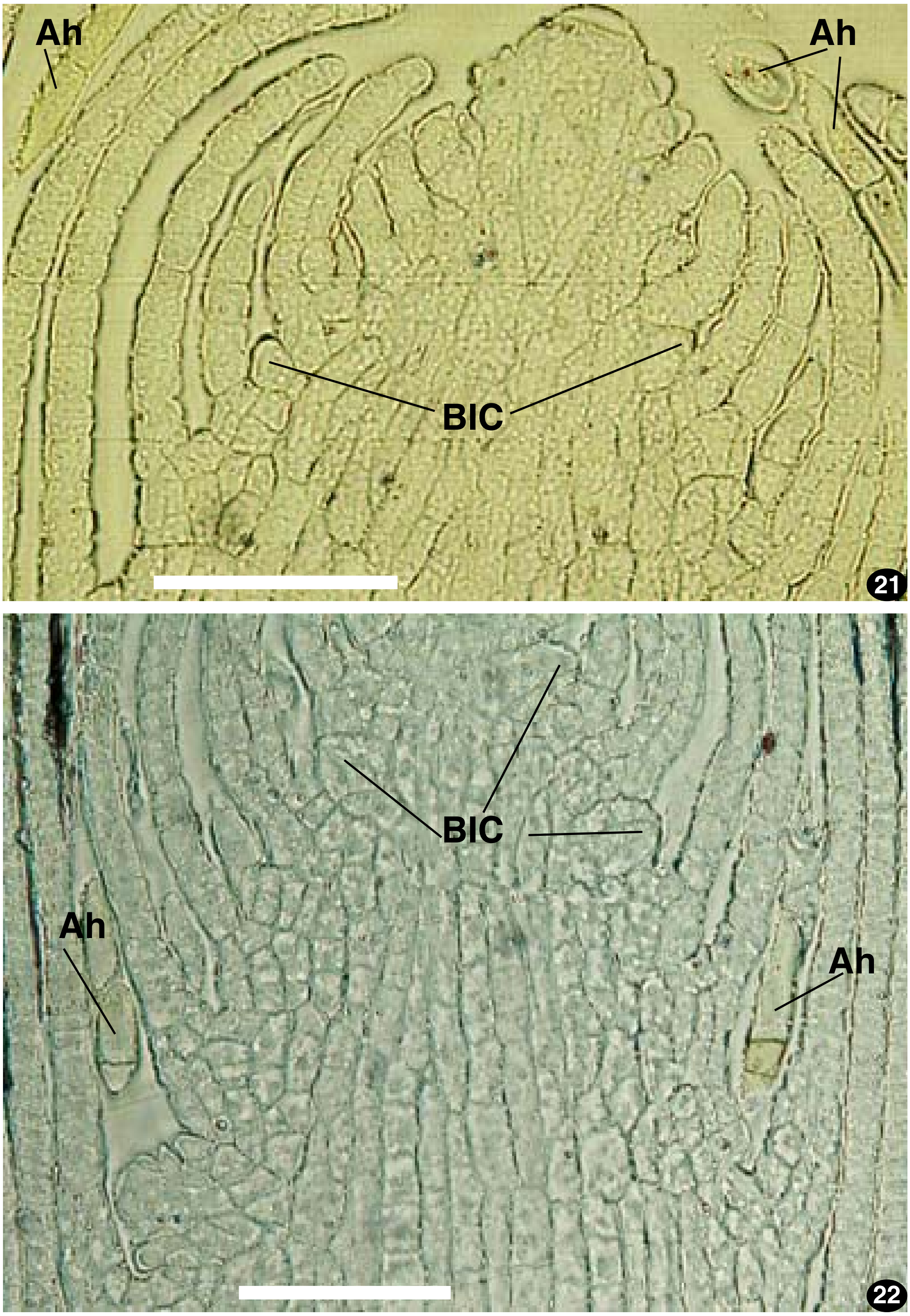
Figs. 7-12. *Brachythecium rivulare* (7) and *B. rutabulum* (8-12). 7 – Section of BIC with three first cut off cells below stem surface and ±parallel to it; 8-12 – Transverse sections of branch primordium at the early stage; 10-12 – from the same primordium cut at different levels from base (10) to top (12); see also schematic explanation. * – branch initial cell. Scale bars 50 µm.



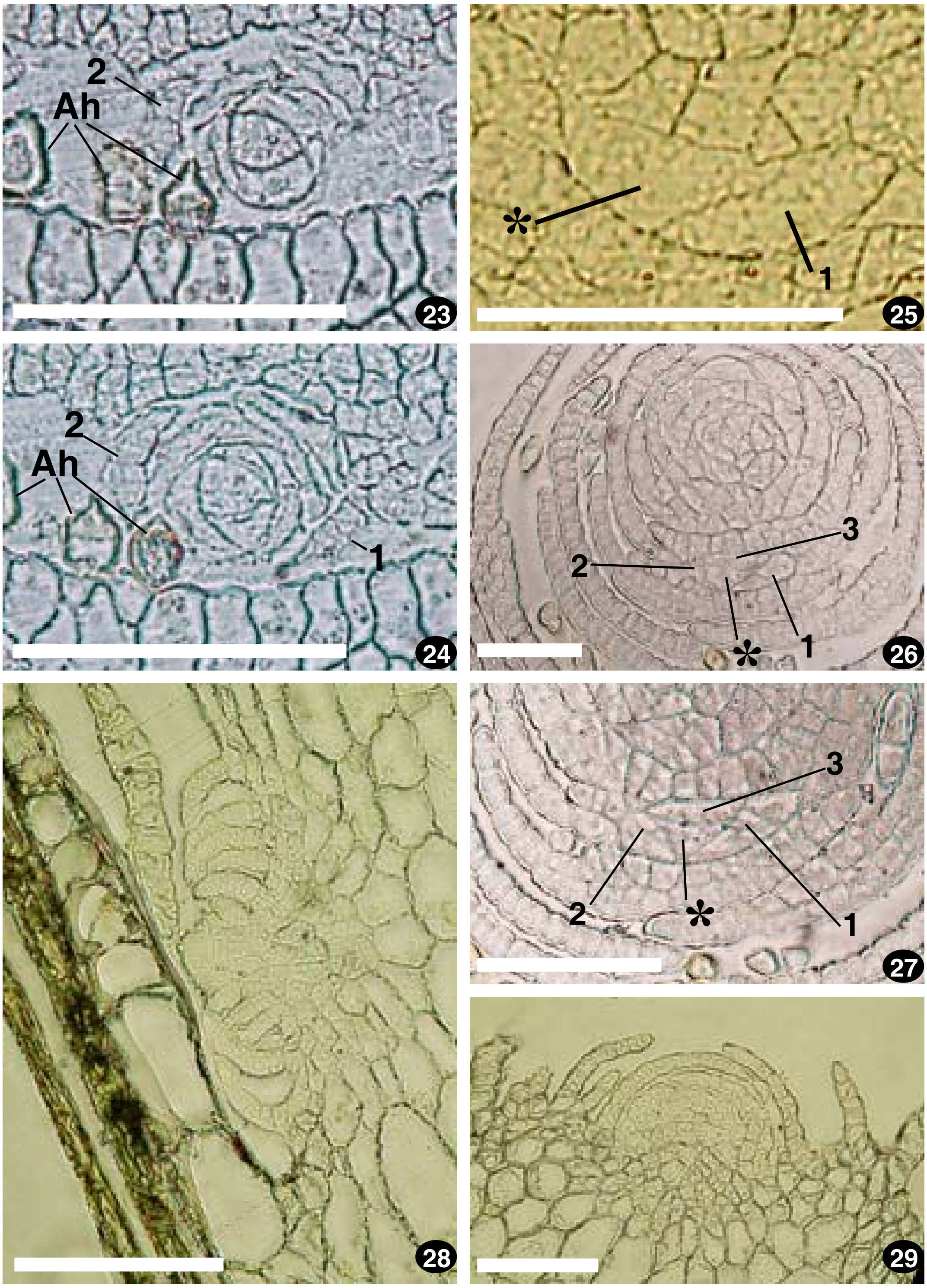
Figs. 13-16. *Brachythecium rutabulum*. Sections of one branch primordium at different levels from base to middle. F1-F4 – stem leaf sequence, numbers – branch leaf sequence; ah – axillary hairs; * – branch initial cell. Scale bars 50 µm.



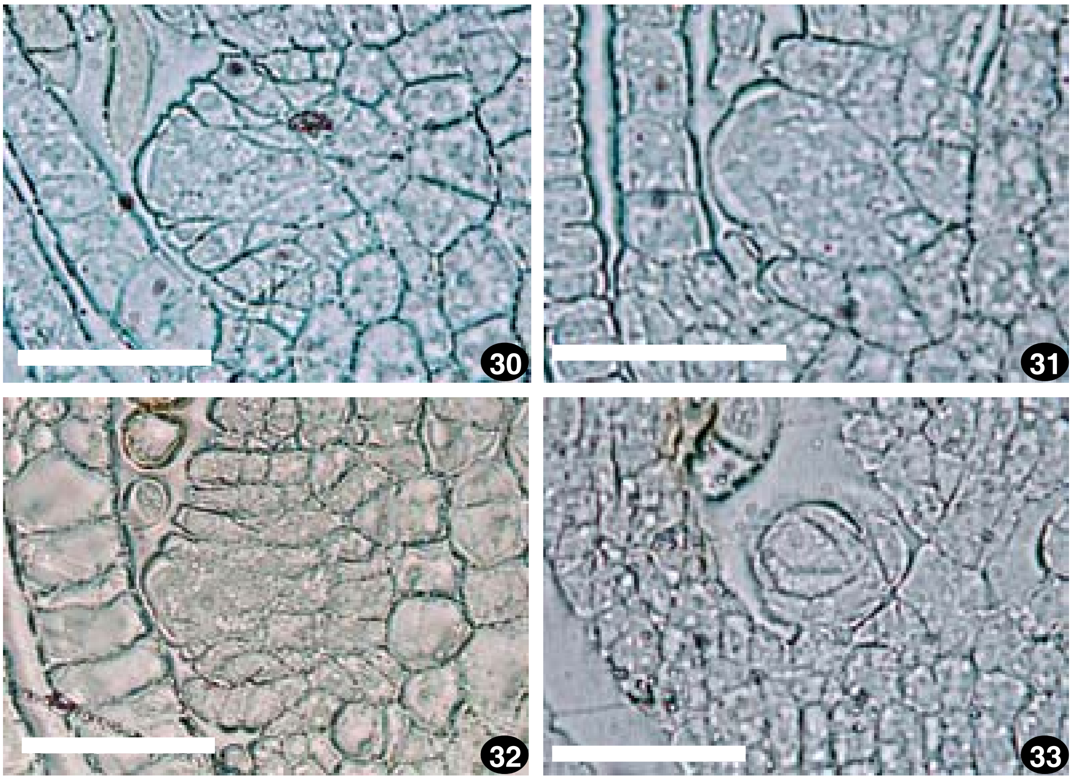
Figs. 17-20. *Brachythecium rutabulum*. 17-18 – Oblique (±parallel to stem surface) sections of upper shoot, showing young branch primordia (note that at the distance ca. 10 leaves from apex branch primordia are protected by young branch leaves); 19-20 – Longitudinal sections of stem, including branch primordium, numbers – our interpretetion of descendents of first divisions of the BIC (cf. scheme at Fig. 10-12). Scale bars 50 µm.



Figs. 21-22. *Calliergon cordifolium*. Longitudinal section of the apical (21) and subapical (22) part of shoot. Scale bars 50 µm.



Figs. 23-29. *Calliergon cordifolium*. 23-24 – transverse sections of one branch primordium at different levels, showing lateral position of first branch leaves; 25-27 – transverse sections of one another branch primordium at different levels (cf. Fig. 2); 28 – longitudinal section of branch primorium ca. 1 mm from apex; 29 – oblique (between longitudinal and transverse) section of branch primordium. * – branch initial cell. Scale bars 50 μm.



Figs. 30-33. *Calliergon cordifolium*. 30 – longitudinal, 31 – oblique, 32 – transverse sections of stem, showing early stages of branch development [30, 32 – ca. 15 leaves from apex, 31 – between 5 and 10]; 33 – section parallel to stem surface, showing branch primordium at early stage of development. Scale bars 25 μm.

19), whereas there is space between the young branches and the surrounding leaves in *Calliergon* (Figs. 23-24, 33).

2) The BIC of *Brachythecium* is recognizable sometimes already between the second and third leaves from the apex (counting on the longitudinal section, cf. Fig. 3), and shortly thereafter exceeds the diameter of the basal leaf cells. The apical cell of the primordial branch in *Brachythecium* is larger than that in *Calliergon* and starts to produce leaves earlier. At the distance of 9-10th leaves from the apex, the branch leaves cover most of the branch primordium (Figs. 18-19). The BIC in *Calliergon* is smaller, develops more slowly and is never so conspicuous. The branch primordium in *Calliergon* remains 'naked' for a longer time (Figs. 21-22, 30-33) and even buds about 1 mm below the shoot apex (e. g. Fig. 28) are not covered totally by the branch leaves.

3) Axillary hairs are visible in *Calliergon* early in development (Figs. 21-24), whereas in Brachytheciaceae they were never observed at the early stage of shoot development (3, 10, 17-20), appearing no earlier than the tenth leaf from apex, counting along a longitudinal section (Figs. 13-16).

4) The sequence of cell divisions in the BIC is basically similar in *Brachythecium* (Figs. 10-11) and *Calliergon* (Figs. 25-27), and it is typical for mosses, following the classical scheme (Figs. 2) discovered by Leitgeb (1868) and detailed by Berthier (1971). The early stages of differentiation demonstrate that the first cell is cut from the BIC in a lower-lateral position, or 'four o'clock position', and the next is formed 'theoretically' at 120° from the first one. However, due to transverse pressing of the BIC by the surrounded leaves in most of pleurocarps, which results in the lens-like shape of the BIC, cf. Fig. 25, this angle is usually larger, and further displacement makes it up to 180° and sometimes even more.

In transverse section the BIC appears to be differentiated between the costa and the leaf margin of the leaf lying acroscopic to it (Figs.2, 10,

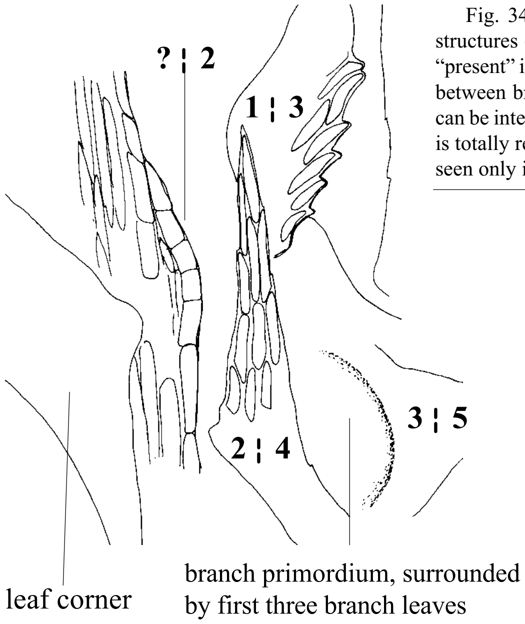


Fig. 34. *Kindbergia praelonga*, branch primordium and nearby stem structures (from Ignatov & Huttunen, 2002). Two numbers mean "past" ¦ "present" interpretations of sequence of youngest branch leaf. Note the ridge between branch primordium and leaf corner: in view of present results, it can be interpreted as a slightly developed second branch leaf, which usually is totally reduced in Brachytheciaceae. Note that in *Kindbergia* this case is seen only in lagre, very well developed palnts.

27). The first division is oblique, with the upper end of the first division cell wall being directed toward the costa of the acroscopic (ematernali) leaf.

This classical pattern (cf. Fig. 2) is discernible in *Calliergon* from the early stages of development, whereas in *Brachythecium* it is obvious at a moderately early stage (Figs. 10-11), while the situation at the earliest stage is somewhat different. This means that the sequence of cell divisions are the same (otherwise it is impossible to understand the similarity – cf. Figs. 10-11 and 25-27), but this sequence is not as obvious in *Brachythecium* at the earliest stages (Figs. 7-9).

The main difference is that the first divisions of BIC in *Brachythecium* occur very early, are obliquely periclinal to the stem surface, and do not extend to the stem surface (Figs. 4-7). These first-formed cells do not form leaves but participate instead in building the stem surface (Figs. 13-16). This has also been confirmed by ordinary microscopic observation, cf. e. g. Ignatov (1999). Only the descendants of the third cell reach the stem surface to form the first branch leaf (called the 'first pseudoparaphyllium' by Ignatov, 1999). In contrast, the first divisions in *Calliergon* are about perpendicular to the stem surface, cutting off cells that extend to the stem surface (Fig. 22) – at least the position of the first two branch leaves seems to be the same as those of the first two cells cut off from the BIC and the whole space between leaves in oblique section is covered by proximal branch leaves (Fig. 27). Sections of young buds of *Calliergon* (Figs. 30-33) confirm additionally, that all cells cut off from the apical cell develop into leaves.

DISCUSSION

The earliest stages of development in *Brachythecium* (Figs. 10-12) and *Calliergon* (Figs. 25-27) look similar, and thus we conclude that the sequence of early divisions of the BIC is basically the same as that described by Berthier (Fig. 2). Differences exist in the level of the stem at which this pattern occurs. In *Calliergon* the divisions are obviously anticlinal and occur after the BIC extends beyond the stem surface (Fig. 26), whereas in *Brachythecium* the divisions occur while the BIC is still embedded so that the first two cells cut off from the BIC never appear above (or even on?) the stem surface. At least, all the obtained transverse sections of the branch primordium beyond the stem surface have no foliose structures that could be attributed to the two first cells derived from BIC.

Thus the anomalous position of first branch leaves in *Brachythecium* can be explained by reduction or lack of development of leaves from the first two merophytes. Additional evidence for that can be seen in Figs. 13-16: below the stem surface (Fig. 13) the structures (#[1] and [2]) obviously developed from two earliest cells cut off the BIC as seen, but they do not extend above the stem surface.

This reduction is probably dependent on the fact, that the BIC is much submerged into stem and its first divisions cut off cells that are developing first under the stem surface (Figs. 3-7).

This latter fact corresponds also with the late development of axillary hairs in *Brachythecium* – if the protective role of mucilage produced by axillary hairs is assumed, then the 'internal' stage of branch development can be assumed as an adaptation – the spacing of young leaves by additional structure might be harmful for young branches. Again, having no protective substance around, branches must develop very quickly and cover themselves by foliose protective structures, and that is what they actually do.

When the BIC divides at an early stage, its first division occurs close to the apex, where the stem did not undergone much growth. Thus, the first division occurs in *Brachythecium* at the stage when the BIC is not much wider than long (Figs. 7-9). In *Calliergon* (Fig. 25) and some other mosses described by Berthier (1971) the BIC at the stage of first division is much wider.

The first division of the BIC is oblique, but the angle is somewhat different. Some (obviously not enough) observations show that the line of the division is directed upwards approximately to the costa of the leaf acroscopic to it ('maternal' leaf). Thus, the relatively wider is the BIC, the wider is the angle of the first division with the stem axis (cf. Figs. 10-11 and Figs. 25-27).

Several anomalous cases in Brachytheciaceae can be interpreted as a result of that second leaf, which does not develop in most of Brachytheciaceae, having a chance to develop. For example, several cases when the 'first pseudoparaphyllium' in Brachytheciaceae was the second – i. e. when its corner is covered by lateral 'pseudoparaphyllium', were such cases were observed in *Rhynchostegium* and *Pseudoscleropodium* (Ignatov, 1999), and were considered previously as an error of development. Note that these two genera belong to the most basal subfamily of Brachytheciaceae (Ignatov & Huttunen, 2002). Interestingly, in Meteoriaceae, one of the phylogenetically most basal genera, *Trachypus*, always has two 'first pseudoparaphyllia' in lateral position typical for most of pleurocarps (Huttunen & Ignatov, 2004).

In *Kindbergia*, low ridge(s) is (are) found in lateral position(s) to the branch primordium, between the primordium and decurrency of the laterally situated leaf(ves) – see Fig. 34.

CONCLUSIONS

If the above interpretation is correct, our data suggest that two other pseudoparaphyllia in Brachytheciaceae are reduced, and the "first pseudoparaphyllium" of Brachytheciaceae is homologous to the third branch leaf in *Calliergon* (as well as most other pleurocarps, except those where pseudoparaphyllia are filamentous or otherwise narrow). Thus, we confirm here the conclusions of Akiyama (1990b), Ireland (1971) and Allen (1987), who concluded that Brachytheciaceae have no pseudoparaphyllia, whereas Amblystegiaceae s. l. do, if pseudoparaphyllia are considered as the two outermost foliose structures in lateral position to branch primordium.

The term pseudoparaphyllia should possible be restricted to these two first branch leaves (especially because of their special role in forming paraphyllia-like structures – see Ignatov & Hedenäs, 2006).

It is important to note, however, that the present volume of observations allow us to interpret the situation only with a certain degree of probability. Additional studies of other species may lead to modification of the suggested scheme considerably. The present conclusion is, in part, a hypothesis for further testing. The hypothesis implies that there are several scenarios of early branch development, and each of them presents a complex of cross-correlated items.

Figuratively speaking, the difference between *Brachythecium* and *Calliergon* can be described as follows:

– in *Brachythecium* all the processes of branch differentiation lead to modification of the suggested scheme considerably than in *Calliergon*. The BIC develops in a short subapical zone, in a very limited space, resulting in its overall submergence into the stem tissue and loss of outermost foliar structure. This submergence also allows for a delay in axillary hair development.

– *Calliergon* first builds space for young branch development. This space is not much affected by drought because of numerous axillary hairs, which (according to some suggestions) produce mucilage, allowing the BIC to develop, not hurrying for covering by outer branch leaves, and thus allowing all merophytes to realize their potentials to form leaves.

Interestingly, in some angiosperms, e. g. in Rosaceae, active growth may also result in the loss of some of the most proximal foliose structures, sometimes recognized as 'prophyllia' (Kostina, 1997).

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