SVENSK BOTANISK TIDSKRIFT. 1945. BD 39, H. 2.

CONSTRUCTION OF THE FLORAL ORGANS IN THE GENUS LANGSDORFFIA.

BY

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Only a few of the genera of the Balanophoraceae family have to some extent been fully investigated with regard to the inflorescence, flower and gametophyte structure. Through some work, I (FAGERLIND 1938 a, b, 1945 a, b) have contributed to expanding our knowledge of the genera *Helosis*, *Ditepalanthus* and *Balanophora*, which are now among the best known members of the family in this respect. One of the most imperfectly researched is the small genus *Langsdorffia*. In 1935 HARMS provided a summary of the construction at *Langsdorffia hypogaea*, which he considers the only species in the genus. He apparently relies for the most part on the information provided by his predecessors (HOOKER 1856, HOFMEISTER 1859, EICHLER 1869, ENGLER 1889), and in part on his own observations. His description deviates in several points from the earlier statements of VAN TIEGHEM (1896, 1907), which is also emphasized by HARMS.

The collections of the Natural History Museum in Stockholm contain material from *Langsdorffia hypogaea* preserved in alcohol. In the hope of being able to decide how the circumstances actually exist in the cases where the information from HARMS and VAN TIEGHEM diverge, and of being able to fill in the gaps in my descriptions, I subjected the material mentioned to a microscopic examination. The material turned out to be well preserved. Some specimens were left unstained. Others were only treated with fast green, which stains the cell walls. Others were also treated with the nuclear dyes. The same applies to *Langsdorffia* as what VAN STEENIS (1931) stated for *Exorhopala* and most of the Balanophoraceae, namely that the material, when preserved in alcohol, has a brown dye, which causes the cell nuclei to emerge without staining. It was shown that the unstained and fast green-treated preparations were the best.

The material preserved in alcohol consisted of two numbers. One, hereinafter referred to as No. 1, was collected by MALME in Matto Grosso (Brazil) in 1894. For the other, hereinafter referred to as No. 2, information on the place of collection, date and collector was missing. Herbarium material of the type can be found in the Reichsmuseum, also collected by MOSÉN in Minas Geraes in 1876 (N: o 4404). Since this material had obvious similarities to No. 2, I suspect that it came from the same collection.

Initially, I thought No. 1 and 2 were representatives of two closely related species. I noticed certain differences that I initially considered essential. However, further investigation showed that the properties that were present at No. 1 were also present at low frequency at No. 2, and that on the other hand the characteristic properties of these latter could also be demonstrated as exceptional phenomena at No. 1. An examination of the herbarium material of the Reichsmuseum showed that the members of the property pairs were represented in very different frequencies in different individuals. It is clear from this that the observed differences have nothing to do with species differences.

Langsdorffia has been depicted several times. The pictures are so different from each other that one has to assume that they refer to different species of the genus. My material No. 1 shows great agreement with EICHLERS Figure I: III. Material No. 2 shows significantly shorter

"stolons". It is more in line with EICHLER's Fig. I: II. At the apex of the stolon the female and then the male spadices are endogenously formed and have the appearance as in EICHLER's illustration.

The female spadices contain an at least apparently simple axis. It is basally provided with a rather large number of tight bracts. Otherwise, it bears a very large number of densely clustered female flowers. In the previous literature it was stated that the female flowers, or at least their upper parts, have fused together. This impression is indeed obtained when studying longitudinal sections in a microscope. The flowers, however, fall apart slightly when the spadices are dissected using needles. The impression that adhesions exist is only an apparent one.

With several Balanophoraceae genera - e.g. *Helosis, Ditepalanthus, Balanophora* (cf. FAGERLIND 1945 b) - there are facts that clearly indicate that the female spadix is not simple, although it appears so on a superficial examination. The secondary axes have been reduced, more or less, to insignificant formations, which in the most extreme cases represent only low bulges, which rise only insignificantly above the surface of the primary axis. For *Langsdorffia* such bulges are not detectable. However, it is not out of the question that these "bulges" have "collapsed" here until they have completely disappeared. The peculiar order in which the female flowers develop indicates that this may be the case. The basal flowers are often late developed. Otherwise, the development takes place in an acropetal direction, but not in an uninterrupted sequence. Rather, one gets the impression that the female flower mass is composed of individual, regular areas [Areen], within which the development occurs in the centripetal direction. The areas, in turn, do not develop in an acropetal sequence. Each such area can represent a "heavily flattened" inflorescence of the second order.

The female flowers are insignificant formations, the size and shape of which are shown in Fig. 1 a and c. The ones from No. 1 are usually narrower and longer than those from No. 2. However, quite large variations occur in different parts of the same spadix and from spadix to spadix. The female flower has a funnel-shaped depression on its upper surface. It is often difficult to detect because the inner funnel's epidermis is often in close contact with the style that emanates from the bottom of the funnel. HARMS (and formerly HOFMEISTER) apparently underestimated the size of the "funnel" because of this fact. He writes about the ovary: "Starting at the apex in a short irregular border." In reality, the "funnel" should be a little weaker than in the closely related genus *Thonningia,* of which HARMS writes: "Flowers similar to *Langsdorffia,* but the apex of the ovary extended into a longer tube that surrounds the lower part of the style like a perigone." VAN TIEGHEM regards the funnel as a reduced perigone in both *Langsdorffia* and *Thonningia*. He is undoubtedly right. The female flowers in *Balanophora* have no perigone. In contrast, the *Helosis* group has an epigynic, greatly reduced perigone (cf. FAGERLIND 1938 a, b).

The perigone consists only of outer and inner epidermis. Cross sections through the same show that locally one and the same cell can represent these two layers (Fig. 1 f). Apically, it also consists of a single cell layer (Fig. 1 b, d). The longitudinal sections show that the perigone is made up of 4-6 cell junctions [Zelletagen]. From VAN TIEGHEM'specification, I cannot confirm that there are three indistinct corners on the perigone. The top edge of the perigone is uneven. Thanks to the size of the four corner cells (see Fig. 1 f), you often get the impression of quadrangularity. It remains to be seen whether this is an expression of the fact that the perigone is made up of four leaves or whether it is a result of the spatial conditions.

Adjacent to the perigone cuticle is a layer with rod-like formations as shown in Fig. 1 d (see also HOFMEISTER 1859, Fig. XII: II).

The cross section of the single style shows 3-12 central cells in addition to an epidermis. The central cells gradually disappear higher up (Fig. 1 f-h). In the weakly swollen club-shaped part of the stigma (Fig. 1 e) there are few or no central cells except the epidermis. The style is at least basally stronger than in *Balanophora elongata* (FAGERLIND 1945 b), but apart from that it has the same structure as the stigma as in the latter type. VAN TIEGHEM's and HARMS' description suggests that the conditions are the same as in the genus *Thonningia*. In the young stage, the style in *Langsdorffia* is angled just outside the mouth of the perigone. It is therefore pressed against the greater part of the surface formed by the different flower tips. The angles align later.

The information about the construction of the ovary is very different. The older methodology was not sufficient to supply satisfactory specimens from these small objects. HOOKER (1856) states that MARTIUS and RICHARD searched in vain for the cavity of the ovary in 1818 and 1822, respectively. At first he himself was inclined to deny their occurrence. In a note, however, he adds that new, well-preserved material convinced him of the presence of the cavity. He states that a very small, few-celled ovule hung down into it. HOFMEISTER (1859) believed to have found a cavity that continued through a clear stylar canal. According to him, it is almost completely filled by a drooping, unicellular ovule. An extension from one of the upper wall cells of the cavity served as the "funiculus". EICHLER (1869) found the pistil to be constructed from two marginally fused carpels. It was simple and provided with a stylar channel. In his view, the cavity was completely filled by a basal-central, upright, multicellular ovule. This view was apparently accepted by ENGLER (1889).

VAN TIEGHEM (1907), on the other hand, completely denies the occurrence of an ovule and a cavity. The embryo sac mother cell is said to be constituted by a cell in the center of the massive ovary.

Despite VAN TIEGHEM's statements, HARMS (1935) agrees with EICHLER-ENGLER. He says: "A single ovule, without integument, elongated, grown around the wall of the ovary."

My own investigation shows that VAN TIEGHEM's view is correct. Even during the youngest stage that was available to me (unfortunately it was not younger than when the embryo sac reached the 4-nucleus stage), I was unable to perceive a stylar canal, an ovary cavity or a differentiated ovule. Around the tip of the young embryo sac is a small cell tissue made of vacuole-free cells, the cytoplasm of which is remarkably stainable (Fig. 2 a). Judging from EICHLER's text and pictures, it is precisely this tissue that he interpreted as an ovule. The border between the above-mentioned tissue and the surrounding one, interpreted by EICHLER as the pericarp, runs in such a way that it is impossible that the compact pistil would be the result of an intergrowth process. The pistil therefore clearly shows the same basic construction as in *Balanophora*. I recently explained how the phylogenetic origin of such a compact pistil can be explained (FAGERLIND 1945 b). In connection with this evidence that HARMS et al. incorrectly understood the ovary structure in *Langsdorffia*, it should be noted that he also interpreted the conditions in *Balanophora* the same way, which must now be proven to be wrong (FAGERLIND 1945 b). VAN TIEGHEM's and HARMS'descriptions of the genus *Thonningia* suggests that this also has a completely compact ovary of the *Balanophora* type.

The ovary in *Langsdorffia* and even more in *Thonningia* is more strongly built than in *Balanophora*. It was not possible to decide whether the cell layers that surround the embryo sac in the first-mentioned genus (Fig. 2a) are completely epidermal in origin. This is the case with *Balanophora* (FAGERLIND 1945 b).

The youngest embryo sacs that I have observed are four-nucleate. At the base of the embryo sac are three small, degenerate cells (Fig. 2 a-f). They are undoubtedly sister megaspores. The embryo sac is therefore monosporic, originating from the apical cell of a tetrad. The situation is similar for *Balanophora* (FAGERLIND 1945 a). The young 4-nucleate embryo sacs are equipped with vacuoles. Two different nucleus locations have been observed:

1) The nuclear pairs are clearly separated from each other; usually a vacuole is found between them (Fig. 2 a-b).

2) The nuclear pairs are more or less close together in "one and the same cytoplasm mass" in the upper half of the embryo sac (Fig. 2 c-d). If the nuclei are in the latter position, a more or less clearly marked bulge can often be seen on the periphery of the embryo sac next to the nuclei (Fig. 2 d). It forms the beginning of the expulsion of a tubular arm, which will soon take up the basal nuclei pair (Fig. 2 e - f). I suspect that nuclear position of no. 1 precedes the nuclear position of no. 2 and that the apparent nuclear shift is related to the expulsion of the tube mentioned.

The tube-shaped appendage grows upwards, far above the original embryo sac apex. It digs a path forward between the somatic cells that are displaced and degenerate. The nuclear pair remaining in the "original embryo sac body" remains in the upper part of the same. The other nuclear pair is taken up in the tube tip. This is followed by the last nuclear division step of the embryo sac (Fig. 2 g). It often happens a little later at the lower than at the upper pole, which is not unusual when it comes to elongated embryo sacs. The four nuclei in the tube tip form the egg apparatus and the upper polar nuclei (Fig. 2 h). The four nuclei in the "original embryo sac body" never develop into cells. Rather, the nuclei merge exactly as in *Balanophora* according to EKAMBARAM and PANJE (1935) - cf. also FAGERLIND 1945a – gradually to a lobed degenerative nucleus with several nucleoli (Fig. 2 h - m). These processes often take place before endosperm formation, but often take a significantly longer time (Fig. 2 q). I have not observed a migration of the "lower polar nuclei" upwards and a merging with the upper one. If such a course occurs, it is in any case a rarity. I have observed such a deviation from the normal in *Balanophora* (FAGERLIND 1945 a).

The embryo sac of *Langsdorffia* is therefore not the simple cylindrical formation, as EICHLER, VAN TIEGHEM and HARMS have claimed. Rather, it shows a not insignificant resemblance to the horseshoe-shaped embryo sacs of *Balanophora*. There the bend was caused by the embryo sac base growing out sideways and then upwards. The process took place during the two-nucleate phase. Apparently, this is the same appearance as that described in *Langsdorffia*. The only difference is that the side tube is expelled at somewhat different times and also basally – in *Balanophora* and subapically at *Langsdorffia*. It is certainly the process of embryo sac penetration that is so characteristic of most Lorantales representatives (cf. FAGERLIND 1945 b) that we have here before us. The fact that the processes have different localizations in different cases can be related to which places offer the least resistance. As I have noted earlier, certain circumstances suggest that tubular bulges on megaspores or young embryo sacs are related to their volume increase and the resistance that the environment offers (*Galium* and related genera - FAGERLIND 1937, *Balsamita* and related genera - FAGERLIND 1939).

Endosperm development begins before the first division of the embryo occurs. The first endosperm nucleus division and the immediately following stage are missing in the material. Later stages show that the endosperm consists of a large, mononuclear basal cell, the upper end of which is more or less enclosed by a group of smaller endosperm cells, between which the embryo is inserted (Fig. 2 n-p). In *Balanophora* (LOTSY 1899, EKAMBARAM and PANJE

1935, ZWEIFEL 1939), the first endosperm nucleus division leads to the formation of an apical and a basal cell. The latter no longer shares. The former divides further and develops into a cellular tissue enclosing the embryo. Apparently exactly the same processes are taking place in *Langsdorffia*. The endosperm is therefore "helobial". However, the basal cell is significantly more voluminous than that of *Balanophora*. In most cases, the "original embryo sac body" with its fused or fusing nuclei can be seen underneath and often somewhat next to the large basal cell.

The first division of the zygote leads to the formation of two cells that lie side by side (Fig. 2 o-p). The wall that divides them coincides with the longitudinal axis of the embryo. The same is the case with *Balanophora* (EKAMBARAM and PANJE 1939, ZWEIFEL 1939).

The construction of the male inflorescences, apart from one point, corresponds to the description that HARMS has provided. He states that small, stunted, conical female flowers are found between the male flowers. Conical formations are now actually found between the male flowers belonging to material no. 1 (Fig. 1 i). But they have nothing to do with rudiments of female flowers. The formations start in pairs (Fig. 1 p) from the edge of insignificantly deepened pits (Fig. 1 r). In the center of each such pit is a male flower. These pits are also found in Material No. 2. The edge of the pit here is drawn out under the flower to a scaly formation, which in certain cases is more or less forked (Fig. 1 l-o). The cleavage can go so far that the scale is replaced by two formations that correspond to the "cones" mentioned above. A closer examination of material No. 1 shows that the "cones" at the inflorescence base are often replaced by scales that are more or less split (Fig. 1 j-k). An examination of the herbarium material of the Reichsmuseum shows that all variations of scales with a more or less clear indication of splitting up to "cone pairs" occur very often in one and the same individual.

In at least most of the *Balanophora* species (cf. HARMS 1935 and FAGERLIND 1945 b), every male flower is inserted into its cell in a "honeycomb system". Following each cell there is a scale-like formation, which has mostly been interpreted as the cover of the male flower, an interpretation that appears appealing, although others are also conceivable (cf. FAGERLIND 1945 b). Cross-sectional images of the scales at *Balanophora elongata* show that the middle part of these scales is thinned (FAGERLIND 1945 b). The "cone pairs" of *Langsdorffia* are apparently homologous to these scales. The pit system, like the "honeycomb system" in *Balanophora*, is probably due to the fact that the basal parts of the scales have fused.

The male flower consists of a stem part, three or rarely two petals and three or two superposed, extrorse stamens. The latter have basally fused together. The stamens are short, completely combined to form a cylindrical structure, as has also been stated by HARMS. HARMS writes of the anthers that they are "connected at the back, forming a hollow synandrium in the middle". Such a construction would be a real paradox. The anthers are only connected to each other in the most basal areas (Fig. 1 h - ö). Otherwise they are completely free from each other. Each anther is provided with a vascular strand that can be traced down into the stem part of the flower. The three (two) strands contained in one and the same stem converge into a single one, centrally located in the basal section.

HARMS states that the *Langsdorffia* anther is dithetic and contains 4 pollen sacs. According to VAN TIEGHEM, however, each anther contains two horseshoe-shaped pollen sacks, the bend of which is directed upwards. My series of sections (Fig. 1 v – ö) show that VAN TIEGHEM's specification is correct. Nevertheless, the anthers are dithecal. The relationships with those in *Balanophora* section *Balanophorotypus* (and in *Balania*?) – cf. HARMS 1935 and FAGERLIND 1945 b – have their phylogenetic origin in normal 4-loculate anthers. Parts that otherwise remain

somatic in the apical tissue that lies between corresponding loculi in the two thecae have apparently been converted into sporogenic tissue.

The present work shows that the correspondences between the genera *Balanophora* and *Langsdorffia* are significantly greater than was previously imagined. It is already clear that there are obvious similarities between these and the genus *Thonningia*, which is still poorly known in many respects. The two first-mentioned genera also have similarities in terms of properties that otherwise do not or only rarely occur in the plant world. I compile them below:

1) Compact ovaries.

2) Emergence of the embryo sac from the apical cell of the megaspore tetrad.

3) Horseshoe-shaped embryo sacs.

4) Failure of polar nucleus fusion.

5) Absence of antipodal cell formation. Fusion of the 4 basal nuclei of the embryo sac.

6) Helobial endosperm formation. The basal cell of the endosperm has no division ability.

7) Longitudinally oriented primary wall in the embryo.

8) "Honeycomb system" in the male inflorescence. "Covering bracts" with a tendency to split.

9) Synandrium formation.

10) "Merging" of equivalent pollen sacs.

Agreement on such a wide range of unusual properties cannot lead to any other conclusion than that the two genera are very close to each other. In addition to the matches listed here, there are others that have been pointed out by previous researchers. I just want to remind you that the two genera are distinguished by the presence of balanophorin. In the past, closer relationships between the genera *Langsdorffia* and *Thonningia* were assumed than between one of these and *Balanophora*. The result was (cf. HARMS 1935) that the latter genus was allowed to represent its own "Tribe", Balanophoroideae-Balanophoreae, while the first two genera were assigned to the tribe Balanophoroideae-Langsdorffieae. VAN TIEGHEM, who wanted to split the Balanophoraceae into a number of smaller families, had *Balanophora* and *Langsdorffia-Thonningia* representing different families. It appears clear that this division is unjustified. The three genera *Balanophora*, *Langsdorffia* and *Thonningia* form a closed unit within the family Balanophoraceae.

Summary.

1) The female flower of *Langsdorffia* consists of a very compact ovary of the *Balanophora* type, of a reduced epigynous perigone and of a pistil with a stigma swollen in the apical part. (ovule, stylar canal and ovary cavity are completely absent.)

2) The embryo sac is monosporic. It develops from the apical cell of the tetrad.

3) A subapically placed lateral tube grows out of the 4-nucleate embryo sac, which receives the basal nuclear pair. The tube grows far beyond the original embryo sac tip. The egg apparatus is formed in the tube tip.

4) Polar nucleus fusion does not take place. The "lower polar nucleus" and the antipodal nuclei merge to form a degenerative, strongly lobed nucleus.

5) Endosperm formation is "helobial". The basal cell has no division.

6) The primary wall of the embryo is oriented longitudinally.

7) The stamens and the basal part of the anthers are united.

8) Each anther contains two horseshoe-shaped pollen sacs.

9) The supposed rudimentary female flowers between the male flowers consist of split scales, which are probably the bracts of the male flowers.

10) A whole series of similarities with regard to peculiar properties are found between the genera *Balanophora* and *Langsdorffia*, agreements which indicate that there is an intimate phylogenetic connection between the two genera.

LITERATURE CITED

EICHLER, A. G., 1869: *Balanophoreae.* C. F. PH. VON MARTIUS: Flora Brasiliensis, Vol. IV, Pars 2. Monachii.

EKAMBARAM, T. und PANJE, R. R., 1935: Contributions to our knowledge of *Balanophora.* II. Life-history of *B. dioica.* — Proc. Ind. Ac. Sc., Vol. 1. Bangalore.

ENGLER, A., 1889: *Balanophoraceae.* — A. ENGLER und K. PRANTL: Die natürlichen Pflanzenfamilien, Teil III, Abt. 1. Leipzig.

ERNST, A., 1913: Embryobildung bei *Balanophora.* Flora, Bd 106. Jena.

FAGERLIND, F., 1937: Embryologische, zytologische und bestäubungsexperimentelle Studien in der Familie *Rubiaceae* nebst Bemerkungen über einige Polyploiditätsprobleme. — Acta Horti Bergiani, Bd 11. Uppsala.

FAGERLIND, F., 1938 a: Bau und Entwicklung der floralen Organe von *Helosis cayennensis.* — Svensk Bot. Tidskr., Bd 32. Uppsala.

FAGERLIND, F., 1938 b: *Ditepalanthus,* eine neue Balanophoraceen-Gattung aus Madagaskar. — Ark. f. Bot., Bd 29 A. Stockholm.

FAGERLIND, F., 1939: Kritische und revidierende Untersuchungen über das Vorkommen des *Adoxa*-(„*Lilium*“)-Typs. — Acta Horti Bergiani, Bd 13. Uppsala.

FAGERLIND, F., 1945 a: Bildung und Entwicklung des Embryosacks bei sexuellen und agamospermischen *Balanophora-Arten.* Svensk Bot. Tidskr., Bd 39. Uppsala.

FAGERLIND, F., 1945 b: Blüte und Blütenstand der Gattung *Balanophora.* — Bot. Not. 1945. Lund. (Im Druck.)

HARMS, H., 1935: *Balanophoraceae.* — A. ENGLER und K. PRANTL: Die natürlichen Pflanzenfamilien, Bd 16 b. Leipzig.

HOFMEISTER, W., 1859: Neue Beiträge zur Kenntnis der Embryobildung der Phanerogamen I. — Abh. Mat.-Phys. Cl. Sächs. Ges. Wiss., Bd 6. Leipzig.

HOOKER, J. D., 1856: On the structure and affinities of *Balanophoreae.* —Trans. Linn. Soc., Vol. 22. London.

LOTSY, J. P., 1899: *Balanophora globosa,* eine wenigstens örtlich verwitwete Pflanze. — Ann. Jard. Bot. Buitenzorg, Vol. 16. Leide.

VAN STEENIS, C. G. G. J., 1931: Some remarks an the genus *Rhopalocnemis.* — Handl. 6. Nederl. Ind. Natuurwet. Congr. Bandoeng.

VAN TIEGHEM, M. P., 1896: Sur l'organisation florale des Balanophoracees et sur la place de cette famille dans sous-classe des dicoeydones involuldes ou Loranthinées. — Bull. Soc. Bot. France, 3. sér., Tome 43. Paris.

VAN TIEGHEM, M. P., 1907: Sur les inovulées. — Ann. Sc. Nat. Bot., 9. sér., Tome 6. Paris.

TREUB, M., 1898: L'organe femelle et l'apogamie du *Balanophora elongata.* — Ann. Jard. Bot. Buitenzorg, Vol. 15. Leide.

ZWEIFEL, R., 1939: Cytologisch-embryologische Untersuchungen an *Balanophora abbreviata* und *B. indica.* — Vierteljahrsschr. Nat.forsch. Ges. Zürich, Jahrg. 84. Zürich.

Fig. 1. *a*. Longitudinal section through young female flowers (material no. 1). *b*. The same, the perigone part at a larger magnification. *c*. Longitudinal section through older female flowers (Material No. 2). *d*. The same, the perigone part at a larger magnification. *e*. The stigma and the apical part of the style. *f*. Cross section through perigone and stylar base. *g*. Cross section through stylar base. *h*. Cross section through the medial part of the style. *i*. "Cone" from a male inflorescence (Material No. 1). *j – k*. In between things between "scale" and "cone pair" (Material No. 1). *l--o*. Likewise (Material No. 2). *p*. Cross-section through male flowers (stem part) and intermediate "cone" (Material No. 1). *q*. Longitudinal section through a male flower. *r–ö*. Cross sections of a male flower at different levels. (The position of the sectional planes corresponds to the lines in Fig. *q*. In Fig. *r* is indicated the pit into which the male flower is inserted. In Fig. *s*. The "cone pair" "belonging to" the male flower is shown.). - Vascular bundle hatched, pollen compartments dotted.

Fig. 2. *a*. Longitudinal section through the ovary part of the female flower with a 4-nucleate embryo sac. *b–c*. 4-core embryo sacs with different nuclear positions. *d–f*. Different stages of the formation of the "tube". *g*. The last nuclear division in the embryo sac. *h*. Fertilization in the mature embryo sac. *i–m*. The nuclear fusion processes in the basal part of the embryo sac. The earlier development of the embryo and endosperm. *q*. Basal nuclei left behind under the endosperm.

Figure 1

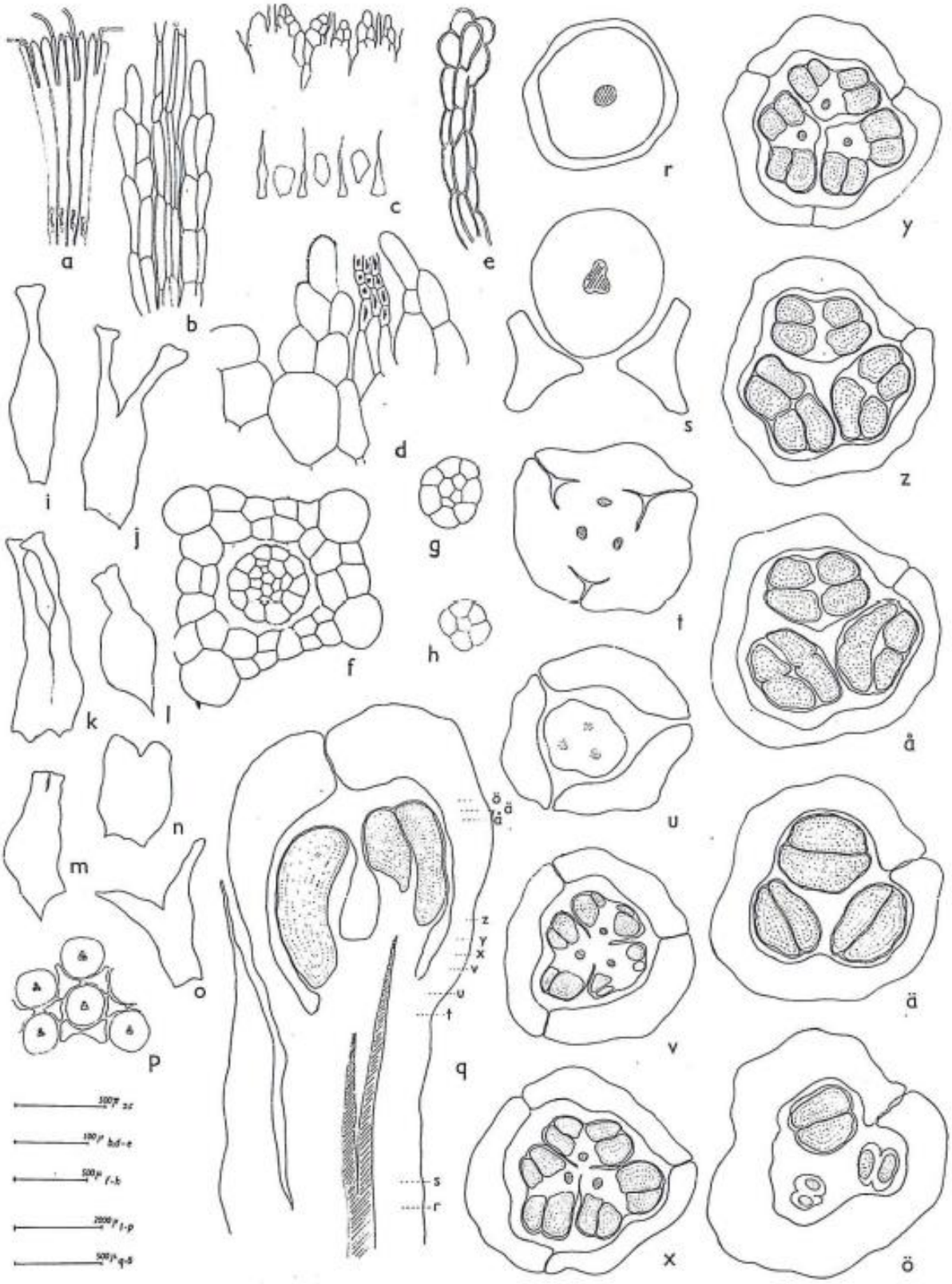

Figure 2