Basidiospores of two *Ganoderma* species and others of two related genera under the scanning electron microscope

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Summary. Basidiospores of *Ganoderma eminii*, *G. alluaudii*, *Magoderna subresinosum*, and *Haddowia longipes* are compared. Holes in the ecto-perisporium are considered to be artefacts.

All the species concerned in this paper are or were once included in the genus *Ganoderma* although all have basidiospores very much at variance with those of the type species, *Ganoderma lucidum* ([W. Curtis] Fr.) Karst.

Of the four species investigated, two have already been segregated from *Ganoderma* (Steyaert, 1972). The other two have been retained in the genus because of similarities in their anatomical and microscopical features, other than those of the basidiospores, as compared with those of the type species.

Pegler & Young (1973) published their researches on the basidiospores of the British *Ganoderma* species, as seen under the scanning electron microscope, with the result that further features were discovered. In extension of these studies, researches on the basidiospores of *G. eminii* P. Henn., *G. alluaudii* Pat. & Har., *Magoderna subresinosum* (Murrill) Stey. and *Haddowia longipes* (Lév.) Stey. were carried out.

It has long been known that the *Ganoderma* and *Amauroderma* basidiospores are bitunicate. Yet there is some variability as to what separates the ecto-perisporium from the epi-endosporium. This function is mostly carried out by echinules, but in a few cases there are crests or costae, which leads one to query whether, for the latter cases, distinct genera should be considered or not. The segregation of *M. subresinosum* and *H. longipes* does not seem controversial. The first because of a basidiospore structure which recalls that of *Amauroderma* and a basidiomal anatomy at variance with that of *Ganoderma*. The second is separated because of the peculiar costate ornamentation of the basidiospores, which is quite distinct from the echinules of those of *Ganoderma*.

By means of the scanning electron microscope, Pegler & Young (1973) have revealed the quite unsuspected structure of the basidiospore ecto-sporidia in *G. lucidum*, *G. valesiacum* and *G. pfeifferi*, which are dotted over by more less circular holes. This is indeed a revelation, as these holes are quite undetectable under the light microscope and causes surprise to one who has examined thousands of *Ganoderma* basidiospores using former conventional techniques. With respect to these three species the author holds the opinion that *G. lucidum* and *G. valesiacum* are synonyms, to which can be added *G. tsugae* Murill. The basidiospore structure is identical in both and, if there is apparently a difference in the context coloration, one must note that this may vary considerably. Steyaert (1972: 95) has pointed out that context coloration changes from nearly pure white, in Karsten's Finnish

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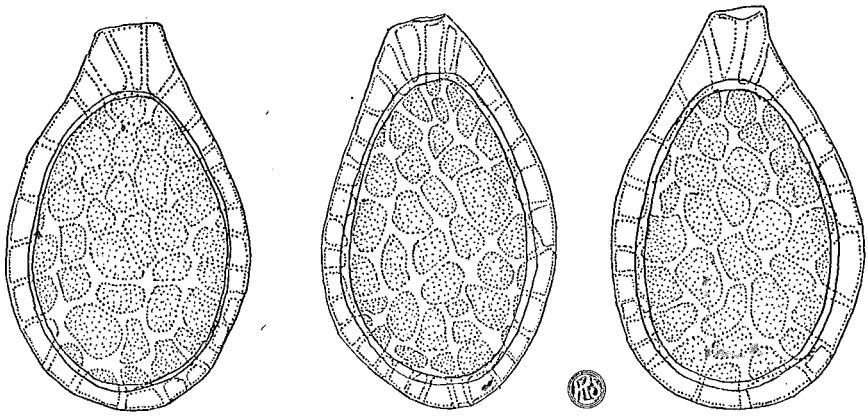

FIG. 1. *Ganoderma eminii*. Basidiospores, Zaïre, *Van Meel* 850, × 2000.

specimen of *G. lucidum*, to variegated light brown in Portuguese specimens, with a variety of intermediate colorations in the intermediate latitudes.

Comparison of *G. lucidum* basidiospores with those of *G. resinaceum* shows, in contrast, distinctive differences in their structures. Whereas *G. lucidum* has ovate basidiospores with relatively few and longer echinules, those of *G. resinaceum* are broadly ellipsoidal with thin, shorter but more densely crowded echinules. Pegler & Young have demonstrated that the ecto-perisporium of *G. resinaceum* basidiospores is not dotted over with holes whereas that of *G. pfeifferi* is. The basidioma of *G. pfeifferi* is morphologically and anatomically quite distinct from that of *G. lucidum*, yet it has basidiospores of the same type as those of the latter, likewise dotted with holes.

A point to be kept in mind, when one tries to find an explanation of this difference, is that the ecto-perisporium of the basidiospore of *G. resinaceum* is much more closely supported than in those of either *G. lucidum* or *G. pfeifferi*. One therefore wonders whether these holes are not artifacts following implosion or explosion of the ecto-perisporium between the echinules during the preparation for microscopical observation, whether under the light or scanning electron microscope. A point that must be remembered is that in freshly formed basidiospores the ecto-perisporium keeps a prominent rounded apex devoid of echinules. This apex soon deflates, usually some time after the basidiospore has been expelled. It can be postulated that the holes develop at this moment permitting thereby the deflation of the apex. Steyaert (1967) has observed that the ecto-perisporium of *G. adspersum* (Schulzer) Donk (syn. *G. europaeum* Stey.) is formed first and the epi-endosporium slowly appears inside it, beginning as a central globule which gradually expands and fills the ecto-perisporium. It is hardly conceivable that at this stage the outer envelope of the basidiospore is pierced by the holes that are revealed in the scanning electron microscope pictures of the mature basidiospores. There are reasons to believe that the basidiospores of all *Ganoderma* species follow the same procedure in their formation.

Ganoderma eminii *P. Henn.* in Engler, Bot. Jahrb. 17: 24 (1893).

The basidiospores of *G. eminii* are the biggest so far recorded for the genus; ranging from 20 to 35 μm in length. Not only are they the biggest but they

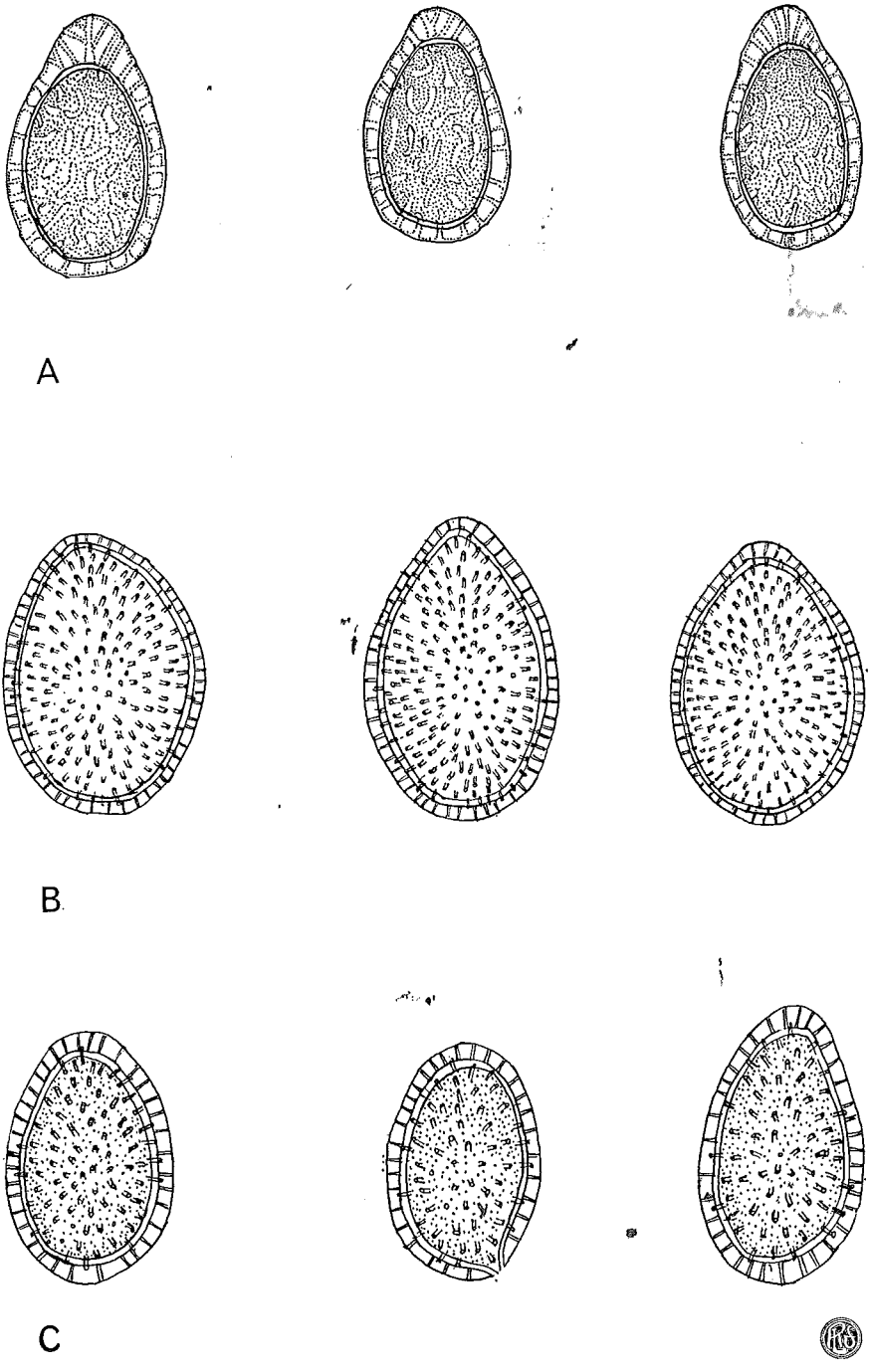

FIG. 2. **A,** *Ganoderma alluaudii*. Basidiospores, Kenya, *Cairns* 5956, × 2000. **B,** *Magoderna subresinosum*. Basidiospores, Philippine Islands, *Merrill* s.n. × 2000. **C,** *Magoderna subresinosum*. Basidiospores, Kenya, *Brown* 1020, × 2000.

also differ the most from those of all other species. Instead of echinules separating the epi-endosporium from the ecto-perisporium, there is a reticulum of ridges, sometimes interrupted by crests 2–4 μm high (Fig. 1). Examined under the S.E.M. the basidiospores have very apparent holes in the ecto-perisporium (Plate 15 A & B). However, not all are so provided, as Fig. 7 shows, where only dimples can be seen.

This seems to lend support to the hypothesis that the holes are due solely to an artifact: either an implosion or an explosion of what is contained within the region delimited by the ridges circling the holes.

Material examined: ZAÏRE, Exploration Hydrobiologique du lac Tanganyika 1946–47, Station 112, Tembwe, 17 Nov. 1947, *Van Meel* 850.

Ganoderma alluaudii *Pat. & Har.* in Bull. Soc. Mycol. Fr. 22: 117 (1906).

Ganoderma alluaudii has much in common with *G. eminii* in that both species are usually mesopodially stipitate. If *G. alluaudii* is usually of a darker brown or even blackish brown colour *G. eminii* is more often of a reddish brown or even yellow, the latter indicating that the cutis hymenioderm is yet imperfectly formed. But assurance of the distinction of the two species can only be obtained by an examination of the basidiospores. The latter are of a distinctly smaller size, 11 to 17 μm in length, and closer in wall structure to the majority of the *Ganoderma* species. The ecto-perisporium is supported by a mixture of crests and echinules (Fig. 2A). The scanning electron microscope pictures show a mixture of dimples and holes (Plate 15).

Material examined: KENYA, Naromuru, s. hosp., June 1951, *Cairns* 5958 (RLS.55.K.91).

From the examination of the basidiospores of the two above mentioned species it appears that the holes that have been demonstrated by the S.E.M. are more an indication that the ecto-perisporium is not fully supported by echinules, crests or ridges, rather than a natural occurrence and a distinctive feature of the ecto-perisporium.

Magoderna subresinosum (*Murrill*) *Stey.* in Persoonia 7: 112 (1972).

Magoderma subresinosum, with its dark fuliginous or black, usually shiny cutis, much resembles a *Ganoderma*, either pleuropodially stipitate, sometimes with branches bearing several pilei, or dimidiate with a narrow, decurrent base. Divergencies from *Ganoderma* appear when the basidiomata are cut open (preferably down the middle). Context and tube layer are either concolorous in 'warm buff' (Ridgway) or with the tube layer frequently 'buckthorn brown'. This latter dark colour is due to the tubes being enveloped by a deposit, probably of melanoid substances, which appears, on sectioning the tube layer, as an amber or dark brown ring around each tube. This ring, it must be noted, does not reach the rim of the tube but is some 10 to 20 μm away from it. The deposit of 'melanoid substances' may vary in intensity. A heavy deposit shows up as a relatively broad and dark encircling zone, contrasting with the surrounding tissues. With a lighter deposit the ring is usually narrower and visible only as a waxy deposit concolorous with

the surrounding tissues and obliterating all details of the hyphal tissue. In the latter case the tube layer is practically concolorous with the context.

On the sections of the basidiomata cut into halves, are dark brown, fine striae or dots of melanoid substances contrasting sharply with the context and never seen in *Ganoderma*.

With regard to the basidiospores, although they are ovate they recall, rather, those of *Amauroderma* in that they are echinulate right up to the apex. In addition there is a difference between the eastern Asiatic basidiospores and the east African ones. In the Philippine specimens (Fig. 2B) the basidiospore echinules are more densely crowded than in those of the eastern African specimen (Fig. 2C), as seen under the light microscope. Under the S.E.M. the Philippine basidiospores (Plate 15E) retain their shape better than those of the east African specimen (Plate 15G) which has wider spaced echinules.

It is noticeable, also, that the Philippine basidiospore is more ellipsoidal than the east African, which is ovoid. It should be noticed too that holes also occur in the ecto-perisporium of the latter. (Plate 15G.)

Material examined: PHILIPPINE ISLANDS, Prov. Rizal, s. hosp., 1908, *Merrill* s.n., (RLS.62.K.52); KENYA, Rabongo forest, s. hosp., 25 Apr. 1964, *H. E. Brown* 1020.) (RLS.65.K.106).

Haddowia longipes (*Lév.*) *Steyaert* in Persoonia 7: 109 (1972).

In describing the new genus *Haddowia* Steyaert (1972: 108) wrote of the basidiospores: 'no outer wall to the spores which appear unitunicate'. This was written with a mental reservation and was the principal incentive for carrying out the present investigation.

It is a fact that when the basidiospores are examined under the light microscope with the long axis of the spore pointing towards the eye no wall is visible between the costae. But since the advent of the scanning electron microscope the limits of vision of the light microscope have been put very much in evidence.

As the S.E.M. photographs in Plate 15 H–K show there is unmistakably an ecto-perisporium but it has ruptured and disappeared between the costae. There remains, however, a fragment that hangs close to the hilum as shown in Plate 15K. It is clear also that fragments of the ecto-perisporium cover the costae (Plate 15 H & J).

It is evident now that the basidiospores are bitunicate. This feature and the fact that the species has a hymeniodermic type of cutis puts it very close to *Ganoderma* but it should nevertheless be segregated from the latter by the very peculiar costae of the basidiospores.

Material examined: KENYA, Mazeras, Mwashi river area, growing up from dead roots, Mar. 1921, *T. D. Maitland* 556, (RLS.55.K.68).

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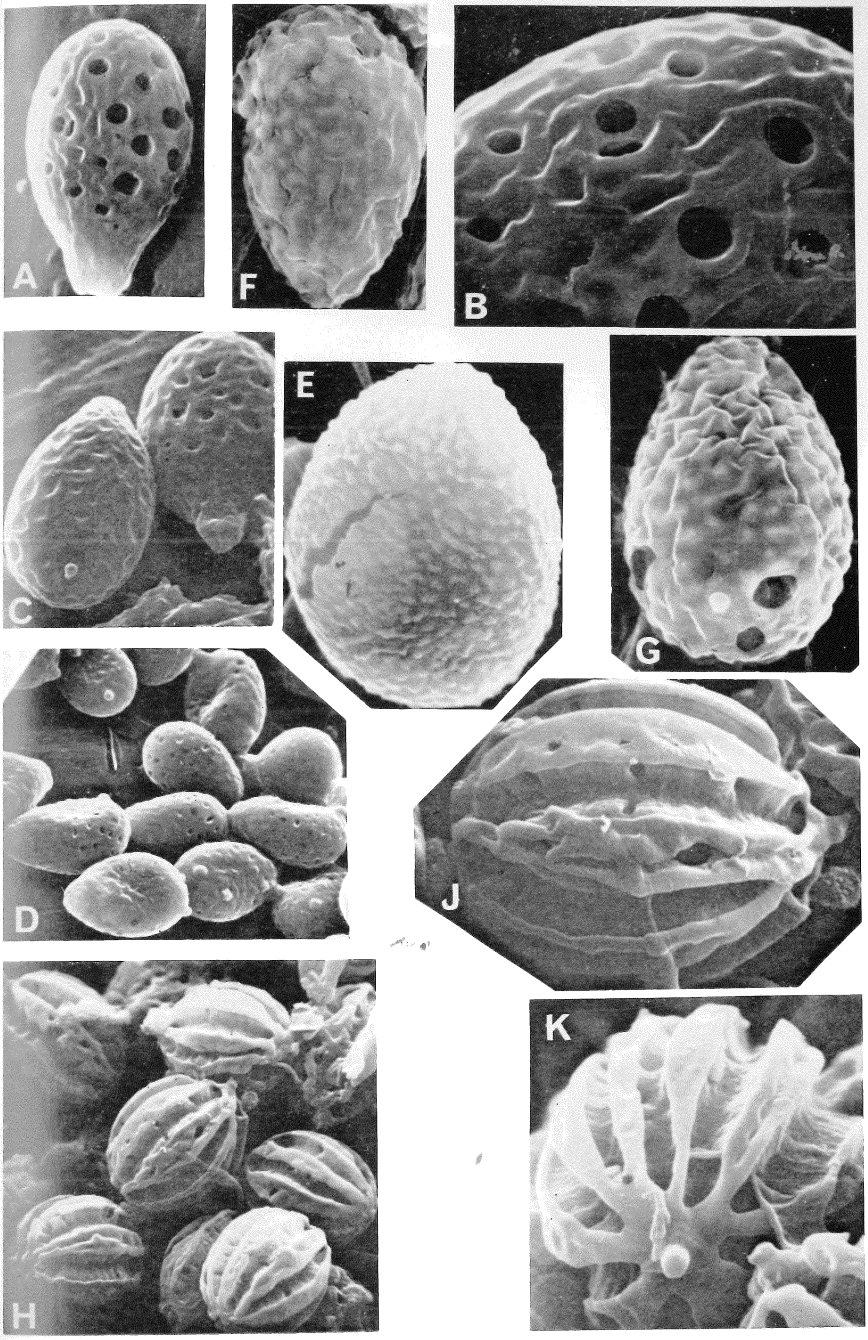
References

Pegler, D. N. & Young, T. W. K. (1973). Basidiospore form in the British species of *Ganoderma* Karst. Kew Bull. 28(3): 351–364, Pl. 20–25, Figs. 1–2.

Steyaert, R. L. (1967). Considérations générales sur le genre *Ganoderma* et plus spécialement sur les espèces européennes. Bull. Soc. Roy. Bot. Belg. 100: 189–211.

—— (1972). Species of *Ganoderma* and related genera mainly of the Bogor and Leiden Herbaria, Persoonia 7(1): 55–118.

PLATE 15



Basidiospores. **A–C,** *Ganoderma eminii*. **A, C,** *Van Meel* 850, × 1300; **B,** *Van Meel* 850, × 3300. **D,** *G. alluaudii*, Kenya, *Mrs. Cairns* 5956, × 1000. **E–G,** *Magoderna subresinosum*. **E,** *Merrill* (ex Herb. *Bresadola*) RLS. 62.K.52 × 3600; **F,** *H. E. Brown* 1020, × 2850; **G,** *H. E. Brown* 1020 × 3000; **H–K,** *Haddowia longipes*. **H,** *Maitland* 556 × 1300; **J, K,** *Maitland* 556 × 3250.