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CHLOROGASTER DIPTEROCARPI
A new peristomate gasteroid taxon of the Sclerodermataceae

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Chlorogaster dipterocarpi, a striking gasteroid fungus, collected in a dipterocarp forest in Sabah, is described here as new. It is characterized by epigeous, slenderly pyriform basidiomes with a dark green exoperidium consisting of dehiscent, conical warts and a pale green circular peristome, large, dark brown spores with crested ornamentation and a thin-walled hyaline paracapillitium. It clearly belongs to the Sclerodermataceae, but the unusual combination of characters demands for a new genus. A similar but probably unripe fungus from Papua New Guinea might represent a second species belonging to this new genus.

During mycological fieldwork by the first author in eastern Sabah, Borneo (Malaysia) in 1999 a striking dark green gasteromycete was found twice in lowland dipterocarp forest near the Danum Valley Field Centre. Most obvious is the dark green overall colour, combined with prominent dehiscent conical warts (almost as in *Lycoperdon perlatum* Pers.: Pers.) and a pale green, very distinct annular peristome with a simple aperture. Microscopically the huge, strongly crested-spinulose, dark spores stand out. On account of the morphological structures the taxon belongs to the Sclerodermataceae, but, despite an extensive literature search, no specific or generic name could be found. A picture was published in the journal Svampe (Læssøe, 1999) in order to attract attention, and several experts in tropical and/or gasteroid fungi have been contacted, but in vain. We therefore conclude that this find represents an undescribed genus.

All microscopical structures were measured in water with some added detergent. Colours are indicated according to Munsell Soil Color Charts (1988) and Kornerup & Wanscher (1978).

Chlorogaster Læssøe & Jalink, *gen. nov.*

Gasterocarpus epigaeus, tenuiter pyriformis vel late clavatus, pseudostipitatus, atrovirentibus vel denigricantibus conspicuis et conicis verrucis omnino tectus. Peristomium praesens. Exoperidium ex elementis irregularibus vel angularibus vel isodiametricis vel rectangularibus compositum, destincte ab endoperidio hyphoide separatum. Capillitium nullum, paracapillitium praesens. Sporae globosae, badiae, ornamentum altissimum et crestatum praebentes, usque ad 30 μm diametro ornamentum incluso.

Typus: *Chlorogaster dipterocarpi* Læssøe & Jalink.

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Gasterocarp epigeous, slenderly pyriform to broadly clavate, pseudostipitate, entirely covered with dark green to blackish conspicuous conical warts. Peristome present. Exoperidium consisting of irregular (rounded) angular, almost isodiametric to rectangular elements, clearly delimited from the hyphal endoperidium. True capillitium lacking, paracapillitium present. Spores globose, dark brown, with very high, crested ornament, up to 30 μm in diameter including ornamentation.

Chlorogaster dipterocarpi Læssøe & Jalink, *spec. nov.* — Figs. 1–3; Colour plate 3 (p. 447)

Gasterocarpus epigaeus, tenuiter pyriformis, pseudostipite longo, initio verrucis conicis atroviridibus, ad maturitatem verrucis dehiscentibus et peristomio destincto, viridi, aperturam parvam habente. Exoperidium ex elementis irregularibus vel angularibus vel subisodiametricis vel rectangularibus compositum, 10–22 × 5–16 μm destincte ab endoperidio hyphoide seperatum. Sporae globosae, 18–25(–28) μm diametro ornamentum incluso, badiae, ornamentum altissimum et crestatum praebentes. Paracapillitium e hyphis gracilibus hyalinis parietibus tenuibus septis numerosis constans. Fibulae desunt.

Holotypus: Malaysia, Sabah, Danum Valley, 30.I.1999, *T. Læssøe & J. Omar*, TL-6029 (holotype C, isotype UMS).

Gasterocarps (Colour plate 3) 45–60 mm high, pyriform with a broad head and a rather slender pseudostipe, epigeous except for the lower 5 to 10 mm of the pseudostipe which is immersed in the soil, at first entirely dark green (K. & W. 30F5) and covered all over with dehiscent, truncate to conical angular warts up to 1.5 mm long that leave a reticulate pattern (particularly on the head) of slightly angular to almost round scars surrounded by low warts or ridges (reminding of *Lycoperdon perlatum*), when mature with a pale green (K. & W. 30B5) peristome. Head up to 28 mm across and 10 mm high. Peristome in young specimens covered with the exoperidium and invisible, at maturity well demarcated, circular, flat, fimbriate, pale green, 4–5 mm in diameter. Pseudostipe 35–50 mm high (higher in material not collected) and 5–6 mm thick to 8 mm immediately below head, base subbulbous to somewhat tapering, rhizoids not observed. Gleba when submature dark grey, non-gelatinous, with white veins and very firm, later more fluffy and dull olive (Munsell 2.5 Y 6/4). Gleba attached to the endoperidium. Exoperidium in section dark olive green; endoperidium vivid yellow green (c. K. &W. 30B7), firm, 1.5 mm thick, slightly thinner near the apex. Tissue in the pseudostipe firm, yellow green (concolorous with the endoperidium), near the base darker and more olive green (like the exoperidium). Smell absent. Taste not tested.

Spores (13.5–)14.0–17.5(–23.5) μm (excl. ornamentation), (18.0–)19.5–24.5(–28.5) μm (incl. ornamentation), Q = 1.00–1.03(–1.18), globose, some subglobose, dark brown, not congophilous nor cyanophilous, when very young surrounded by solid wide sheath. Ornamentation consisting of prominent straight to undulating or slightly curved crests or spines, (2.8–)4.3–6.6(–9.0) μm high. As observed with SEM the ornamentation appears highly complex with more or less distorted plates (Fig. 1d), in some cases showing almost a reticulate ornamentation. Some spores show a short conical appendix, which is presumably an apiculus (Fig. 1c). No trophocysts could be observed but the ornamentation of many (submature) spores is imbedded in amorphous, congophilous and cyanophilous matter. Paracapillitium (Fig. 2) fairly elastic, 3.0–5.5 μm wide, thin-walled (0.1–0.2 μm), sparsely branched, hyaline, with adhering, slightly yellowish amorphous

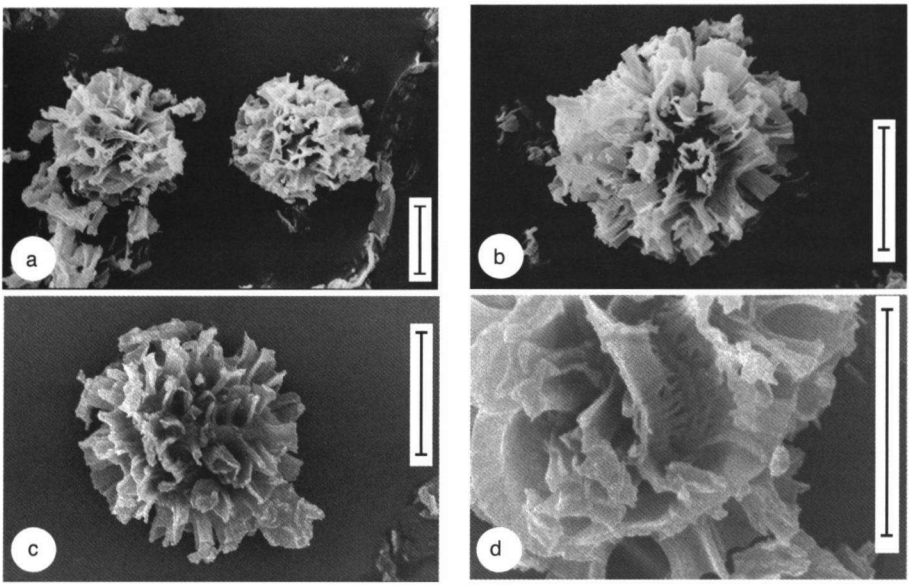

Fig. 1. *Chlorogaster dipterocarpi*. a–c. Basidiospores as observed with SEM. d. Detail of the spore ornamentation (with SEM). Scale bars = 10 μm.

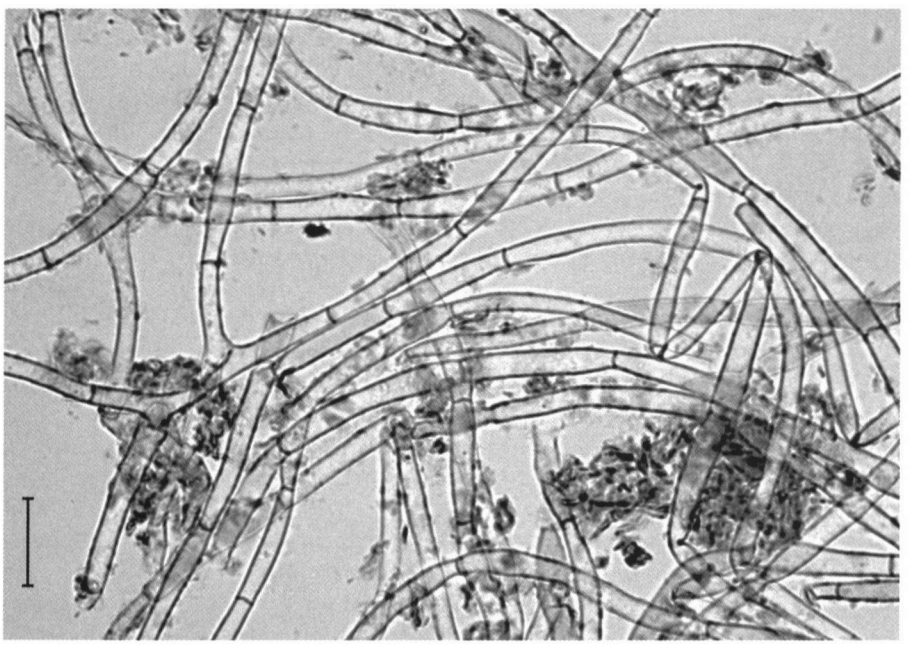

Fig. 2. *Chlorogaster dipterocarpi*. Paracapillitium. Scale bar = 20 μm.

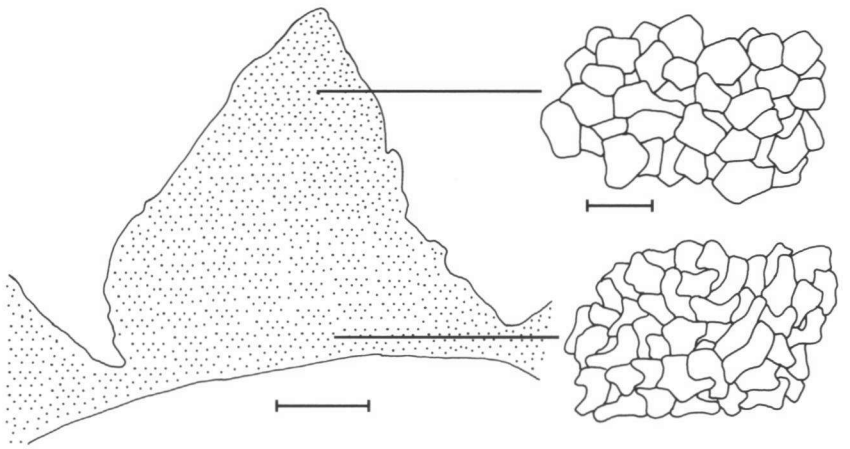

Fig. 3. *Chlorogaster dipterocarpi*. Exoperidial warts, longitudinal section and details of structure of tissue. Scale bar = 200 μm & 20 μm.

matter, with numerous ring-like (secondary) septa, not or slightly constricted at septa, with strongly cyanophilous content especially next to the septa, moderately congophilous. Basidia not observed, but based on spore grouping in the youngest material the basidia are probably (4–)5–8-spored. The presence of single very large spores indicates that some 1–2–3-spored basidia may occur. Exoperidium clearly delimited from the hyphal endoperidium, consisting of slightly thick-walled (up to 0.35 μm) irregular (rounded) angular, almost isodiametrical to oblong or lobed elements of 10–22 × 5–16 μm (Q = 1.0–5.0), with brown parietal pigment, arranged in a textura angularis near the top of the exoperidial warts with a gradual transition into a textura epidermioidea close to the endoperidium (Fig. 3). Endoperidium changing gradually from outside to inside. The outer layer of about 150 μm thick is composed of densely interwoven, long, thin-walled, moderately branched, 2–3 μm wide hyphae, irregularly inflated up to 4.5 μm in diameter, with olive yellow content, yellowish parietal pigment and with abundant amorphous clots of olive yellow intercellular pigment. The inner layer consists of interwoven, non-inflated, sparsely branched, septate, slender hyphae (1.5–2.5 μm in diameter), which are tightly packed close to the outer layer and gradually become more loosely interwoven toward the gleba. Towards the gleba the abundance and size of pigment clots decreases significantly. The pigment clots do not dissolve in water, but do rapidly so in alkaline solutions: then a prominent olive pigment is exuded by the peridium into the medium. Tissue in the pseudostipe consisting of interwoven hyphae, a mixture of broad, up to 6.5 μm wide hyphae resembling the paracapillitium and much thinner, up to 2.2 μm wide hyphae. No clamp-connections have been observed in any tissue.

Ecology — Probably ectotrophic, in heavy soil under mature dipterocarps amongst specimens of a tiny, pinkish, stipitate polypore probably *Microporellus inusitatus* var. *lateritius* Corner, a species apparently hitherto only known from the type. In close proximity, on well-drained soil with a thick ectotrophic root zone, several typical ectotrophic

fungi were found including several *Russula* species, *Craterellus* aff. *cornucopioides* and *Cantharellus* sp. Another collection of the above described taxon was seen near the Bornean Jungle Lodge c. 10 km from the type site, but could not be preserved. It grew on level terrain, near a river, in a younger, denser dipterocarp stand. No associated ectotrophic fungi, nor the characteristic polypore, were seen in the immediate vicinity.

Material studied. Malaysia, Sabah, Danum Valley, East Trail near main Danum Valley field centre road, under dipterocarps, 30.I.1999, *T. Læssøe & J. Omar*, TL-6029 (holotype C; isotype UMS).

DISCUSSION

Chlorogaster belongs to the family Sclerodermataceae on account of the structure of the immature gleba, the presence of paracapillitium, the morphology of the spores and the tough peridium. The current edition of the Dictionary of Fungi (Kirk et al., 2001) accepts the Sclerodermataceae within Boletales and includes besides *Scleroderma* also *Pisolithus*, *Calostoma*, *Astraeus*, *Favillea*, *Horakiella* and *Tremellogaster*. Within this family *Chlorogaster* is (based on micro- and macromorphology) most closely related to *Scleroderma*, from which it differs in the presence of a peristome, the more differentiated structure of the peridium (even in comparison to the few *Scleroderma* species with a marked peridial differentiation as *Scleroderma echinatum*, S. *tenerum* and *S. leptopodium* (Petri, 1900; Demoulin & Dring, 1975; Guzman & Ovrebo, 2000)), the occurrence of bright green and blue pigments and the strong and complex ornamentation of the spores. None of the known species of *Scleroderma* forms a peristome (Guzmán, 1970, 1971).

Extensive efforts by Hibbett & Binder to obtain usable DNA through PCR by SDS extraction, CTAB extraction, gel purification, ethanol precipitation, geneclean and Qiagen DNeasy extraction kit; and also at various temperatures, with touchdown methods, gradient ramping methods, high magnesium, low magnesium, did not succeed. Although we failed to obtain molecular data it is likely that *Chlorogaster* belongs in the *Calostoma*/*Pisolithus*/*Scleroderma* lineage (Hughey et al., 2000) within the Boletales clade (Hibbett et al., 1997) or Sclerodermatineae (Binder & Bresinsky, 2002). The green pigmentation would indicate a possible linkage with *Pisolithus* from which it differs in the absence of peridioles. A well-formed peristome is also found in *Calostoma* which, however, in other respects seems more distantly related to *Chlorogaster*. *Tremellogaster*, another genus considered to be closely related to *Scleroderma* (Guzmán, 1971), is characterized by globose, sessile basidiomes with a thick and strongly gelatinous peridium (Fischer, 1924). *Veligaster*, which is considered synonymous with *Scleroderma* by many authors (Demoulin & Dring, 1975; Sims et al., 1995), has pseudostipitate basidiomes like *Chlorogaster* but differs in having a different peridium structure with a subgelatinous exoperidium (Guzmán, 1969; Guzmán & Tapia, 1995). *Veligaster columnaris* is nested within *Scleroderma* in the molecular phylogenetic study by Binder & Bresinsky (2002). Another genus included in the Sclerodermataceae is *Horakiella* (Castellano & Trappe, 1992), which is a hypogeous genus with more or less globose basidiomes with an undifferentiated peridium and pale brown to hyaline spores with a low reticulate ornamentation.

In 1997 a blackish gasteromycete, which shows great resemblance to the Bornean material, was collected in Papua New Guinea by A. Verbeken. This material was studied by Derous and Demoulin and a short description with line drawings of the microscopical details is given by Derous (2000), who suggested that it might be a new genus, related to the collection depicted by Læssøe (1999). They refrained from formally describing it as a new species since all basidiomes were still unopened and therefore probably not mature. The material was carefully re-examined by Jalink and indeed there is no trace of an aperture nor peristome. The gross morphology and the striking similarity in size and ornamentation of the spores, the lack of a true capillitium and both the macroscopical and microscopical details of the peridium indicate that the New Guinean collection might represent a second species belonging to *Chlorogaster*. However, differences in other characters summarized in Table I indicate that the Papuan collection is certainly not conspecific with *Chlorogaster dipterocarpi*.

Since it is not known whether the species from Papua New Guinea will develop a peristome or not, the taxonomic position of this species remains uncertain. It is still possible that the species from Papua New Guinea will develop a peristome at maturity. Apparently the peristome is formed in one of the last stages of the maturation of the basidiome since in the young specimens of *Chlorogaster dipterocarpi* there is no trace of a peristome either.

Material studied. Papua New Guinea, Madang, hills behind Ramu Sugar Guesthouse, narrow creek of Waris river, 05° 57' S 145° 53' E, alt. c. 450 m, narrow gallery-like forest along creek, on soil, 03.III.1997, *A. Verbeken 97-460* (GENT).

Table I. Differences of *Chlorogaster dipterocarpi* and the collection from Papua New Guinea.

	Chlorogaster dipterocarpi (TL-6029) from Malaysia	*Verbeken 97-460* from Papua New Guinea
shape and size basidiome	slenderly pyriform; 45–60 × –28 mm	broadly clavate to broadly pyriform; 18–22 × 11–12 mm
pseudostipe	35–50 mm long	9–12 mm long
peristome	present in mature specimens	not present (yet?)
colour exoperidium	dark green	greenish black
colour endoperidium and tissue of pseudostipe	vividly yellow green	vividly yellow green with (almost blackish) blue veins in base of pseudostipe
gleba	submature: firm and very dark grey with white veins; mature: fluffy and dull olive	submature: firm and dark purplish grey, then dark purplish brown
paracapillitium	elastic slender more or less cylindrical hyphae with numerous (secondary) septa, width 3.0–5.5 μm	less well-developed hyphae with few septa (no secondary septa), varying in width, undulated, width 2.0–4.5 μm

Scleroderma and *Pisolithus* are considered to be ectotrophic. Miller & Miller (1988) have claimed species of *Calostoma* to be saprotrophic – a statement that should be tested. We strongly suspect *Chlorogaster* and, indeed, *Calostoma* spp. to be ectotrophic as well. This further illustrates the predicted and already documented richness of ectotrophs within the dipterocarp forest type in South-East Asia (Lee, 1990; Watling, 1994; Watling & Lee, 1995; Sims et al., 1997).

The mycorrhizal partner of *Chlorogaster* could well be a dipterocarp. Most genera of the Dipterocarpaceae are known to form ectomycorrhizal associations (Lee, 1990). In the field mycorrhizal connections have been observed between dipterocarps and basidiomata of *Scleroderma sinnamariense* (Watling, 1994; Sims et al., 1997) and *Pisolithus aurantioscabrosus* (Watling et al., 1995). Several other Sclerodermatales, such as *Pisolithus abditus, Scleroderma columnare, S. dyctiosporum, S. echinatum, S. leptopodium, S. verrucosum* and *Astraeus hygrometicus*, are known from dipterocarp forests and members of the Sclerodermatales can be rather common in these forests (Rifai, 1987; Smits, 1994; Watling & Lee, 1995; Sims et al., 1997, 1999; Watling et al., 1999; Binder & Bresinsky, 2002; Kanchanaprayudh et al., 2003).

Although exciting it is not surprising to find such a distinctive new genus in the tropical rain forest. As for example Guzmán & Ovrebo (2000) already noted: "our knowledge of the biodiversity [fungi] of all regions, not just the tropics, remains poorly documented".

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