A NECTRIA PARASITIC ON A LIVERWORT: WITH FURTHER NOTES ON NEOTIELLA CROZALSIANA

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In Pahang, four years ago, when scanning with a lens a gingerleaf covered with liverworts, I descried upon a certain species the tiniest red fruit-bodies. Before returning to Singapore I collected as many leaves with the liverwort as possible and keeping them alive in the laboratory for a few days I drew up this account of a new species of Nectria. It resembled so closely the discomycete, *Neotiella crozalsiana,* that I determined to compare them, the more particularly as doubts had been expressed on my interpretation of that species, whether it were not truly a pyrenomycete. In England, in 1933, I collected fresh material of *N. crozalsiana,* from the wood where I discovered it, and having checked my previous account I reaffirm its correctness and may now, with a bryophilous pyrenomycete for comparison, vindicate my standpoint. I have for distribution abundant dried material of both species and a little of *N. crozalsiana* in formalin-alcohol. To Dr. Fr. Verdoorn, of Leiden, I am indebted for the identification of the liverwort.

Nectria (Dialonectria) **egens** n. sp.

Mycelium superficiale, ex hyphis hyalinis separatis, non contextis, reticulato-ramosis, haud raro in ramis brevibus inter se conjunctis, 2.5—4μ latis, in valleculis inter parietes convexos superficiales cellularum foliorum et stipitum hospitalium appressis, compositum: hypharum cellulæ 6—20μ longæ, membranis 1—1.5μ crassis, quæque hyphopodio uno, parvo, subgloboso, subsessili, tenuiter tunicato, 3—4μ longo x 2—3μ lato, præditæ.

Perithecia 95—150μ alta, 65—105μ lata, *sparsa,* solitaria vel raro bina, amphigena ad folia hospitis marginem versus disposita, *minutissima,* pyriformia, ostiolata, *glabra,* tenera, *aurantio-rubra,* collo brevi plus minus distincto 20—34μ longo x 18—25μ lato prædita. *Paries perithecii* 12—20μ crassus, dense plectenchymaticus, hyphis contiguis, e stratis duobus compositus: *strato externo* 1—2 cellulas crasso, luminibus cellularum 5—12 x 1—3μ, ex hyphis crasse tunicatis pigmento plus minus deficientibus contexto: *strato interno* 2—3 cellulas crasso, luminibus cellularum 6—14 x 4—10μ, ex hyphis tenuiter tunicatis granulis aurantiacis plenis contexto. *Periphyses numerosi,* in collum projicientes, haud emergentes, e cellula una aut cellulis duobus granulis aurantiacis plenis compositi: cellulæ terminales periphysium inferiorum cylindricæ, 12—16 x 3—4μ; periphysium superiorum minores 6—8 x 2—3.5μ, pigmentoque plus minus deficientes. *Paraphyses nulli.*

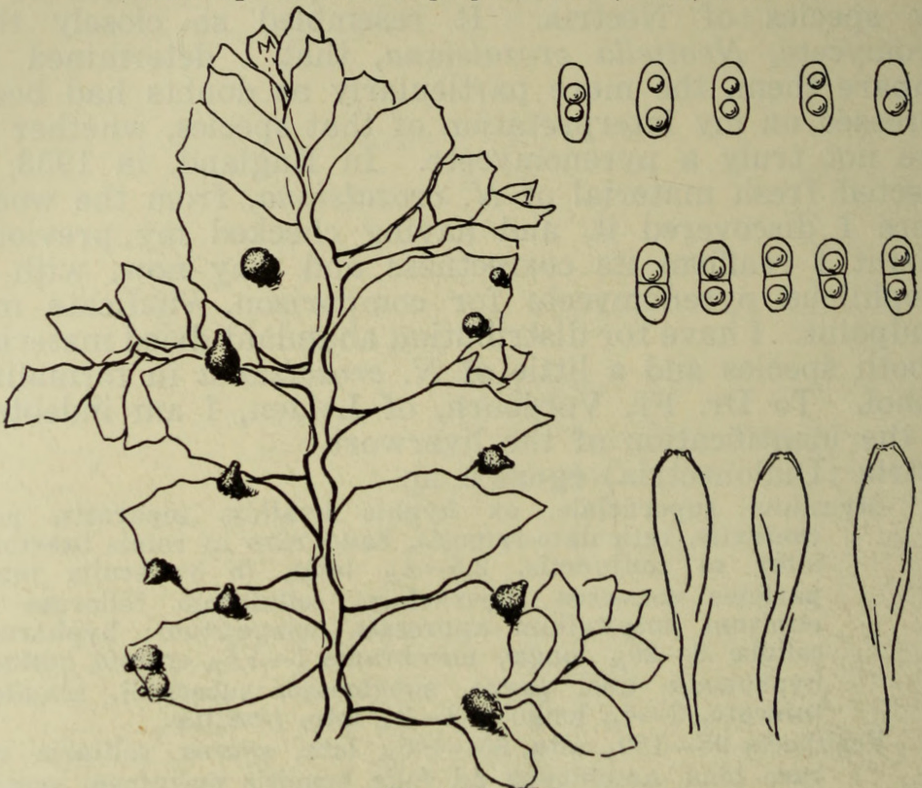
Asci 40—46 x 9—10μ, 8-sporati, subventricosi, apicibus obtusis, tenuiter tunicati, inoperculati, ostiolo simplici iodo non cœrulescenti dehiscentes.

Sporæ 8—9.5 x 3.5—4μ, irregulariter instructæ, *albae, hyalinae, ellipsoideae,* leves, tenuiter tunicatæ, intus nebulosæ, guttis duobus raro singulis hyalinis aut leniter flavidis, 2—2.5μ latis præditæ, *tandem saepe septum transversum plus minus medium inter guttas duas deponentes sed haud vel leniter ad septum constringentes.*

Conidia non visa.

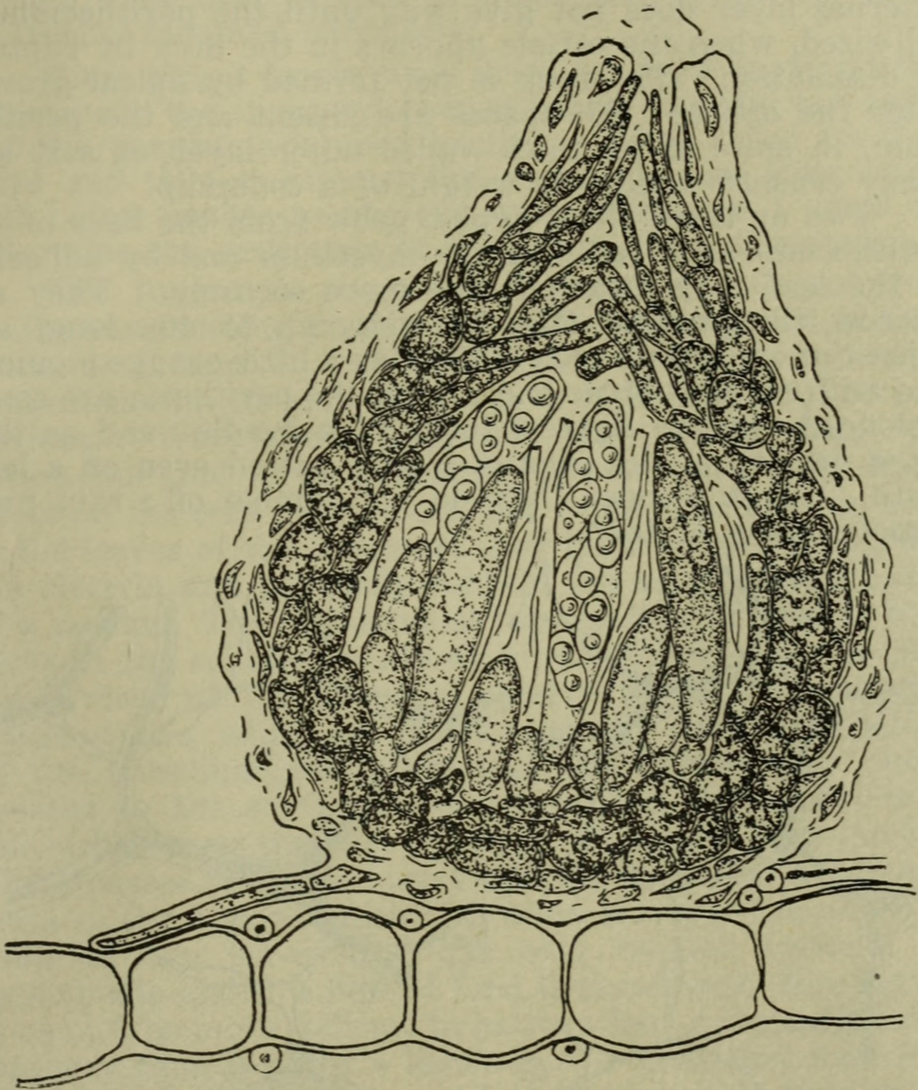
Hab. in foliis stipitibusque *Leptolejeuneae corynephorae* (Nees) Schiffn., ad folia zingiberis crescentis, parasitica, in silvis paludosis prope Tembeling, Pahang, peninsulæ malayanæ, Nov—Dec. 1930.

Species habitu, mycelio parasitico sparso superficiali hyphopodiato atque peritheciis glabris solitariis valde distincta: forma sporarum *N. episphaeriae* (Tode) similis.



Text-Fig. 1.
Leptolejeunea corynephora with the perithecia of *Nectria egens*, × ca. 50: ascospores and apices of dehisced asci of *N. egens*, × 1000.

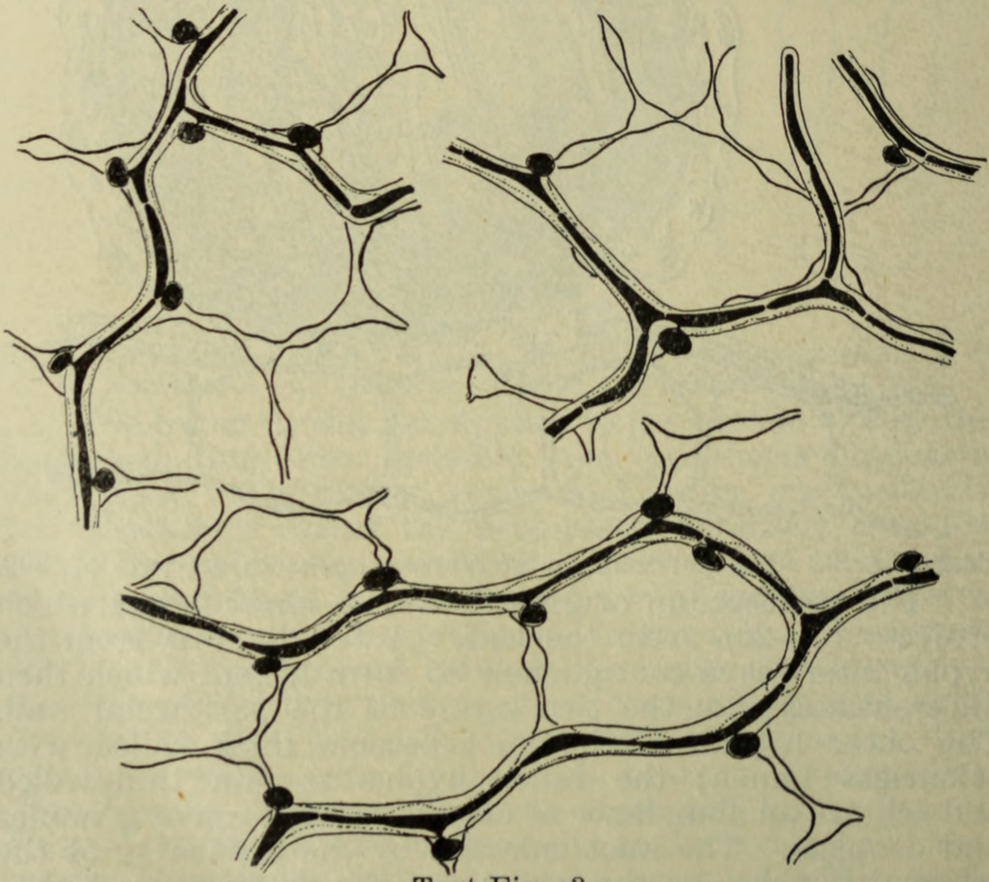
The living hyphæ are applied to the host so closely that unstained they are invisible, the perithecia appearing unattached. Saffranine, eosine and iodine are ineffective but cotton-blue stains the protoplasm intensely by which means the hyphæ can be traced. They have a habit which distinguishes them at once from those of moulds and stray spores which sprout in profusion in the felt of liverworts. They lie in the grooves between the outer convex walls of the cells of the liverwort, in most cases singly, and at the corners of the host-cells, over the junctions of their anticlinal walls, each hyphal cell bears a hyphopodium, Text-Fig. 3. The mycelium, on stem and leaves, has the form of a network with hyphopodia at the nodes, and almost every intercellular groove may be occupied. The hyphæ rarely traverse the face of the host-cells: they never grow into the air vegetatively: and I did not succeed in detaching them. At the edge of a leaf they follow round the grooves to the other side and they progress from leaf to leaf along the stem. They may reach young leaves which are half-grown but they do not penetrate the growing-point, and they die off from old leaves which are browning. There are no haustoria and the cells of the host suffer no obvious harm.



Text-Fig. 2. The perithecium of *Nectria egens* in section: × 800.

The perithecium originates from a short hypha which projects 10—20μ from the leaf. A few laterals from the hypha interweave contiguously to form a knot which then differentiates into the two layers of the perithecial wall. The outer hyphæ of the knot become thick-walled with colourless lumen; the inner hyphæ remain thin-walled and acquire an abundance of carotin in the form of granules and droplets. The knot enlarges by the branching of the internal hyphae, by the growth of the ascogenous hyphae and by the elongation of the cells of the outer layer. It remains globose until some 60μ wide when the neck is formed. The internal tissue in expanding presses upon the external layer and forces it out as the neck on the side away from the leaf: this weakened area probably corresponds with the apex of the primordium opposite the original hypha over which the laterals merely closed by arching and interweaving of their extremities, the remainder of the wall being compounded of the body of the hyphæ. But the external layer does not give way until the perithecium is full-sized, when the ostiole appears in the neck by rupture or dissolution. The neck is not formed by apical growth after the ostiole. Air-spaces are absent and the perithecium, in spite of its thick-walled outer layer, is soft and easily crushed under the weight of a coverslip.

Two or three short hyphæ grow from the base of the perithecium as a rudimentary mycelium and by adhering to the leaf may attach it the more securely. They are narrow, thick-walled, 2.5—4μ wide, up to 40μ long, and consist of one or two cells containing a little orange pigment, and with simple obtuse ends. But the perithecia are easily dislodged—by the lightest touch of a needle—and as they often develop at the edge of a leaf, poised even on a leaf-tooth, it is a wonder they do not tumble off; rain must remove many of them.



Text-Fig. 3.
Hyphæ of *N. egens* above the cells of the leaf of *L. corynephora*: the hyphæ stained with cotton-blue, the shrunken protoplasm being shown black: hyphopodia at the junctions of the anticlinal walls; × 800.

It seems that the asci are feeble and discharge their spores with little vigour so that they barely escape the ostiole. I placed coverslips over patches of the liverwort with fresh perithecia, hoping that some would be directed towards them, but no spores arrived even when scarcely .5 mm. away and left for 2—3 days. Neither do the spores emerge in tendrils nor collect in a powder round the neck, though some may be seen at times just within the ostiole of freshly mounted perithecia.

Some ginger-leaves were dried for 2—4 days and then moistened: the liverwort turned brown and did not revive but the perithecia seemed healthy. When dried for 7—8 days and then moistened the perithecia also discoloured. But being unable to observe spore-discharge I could not determine the resistance of the perithecia to desiccation.

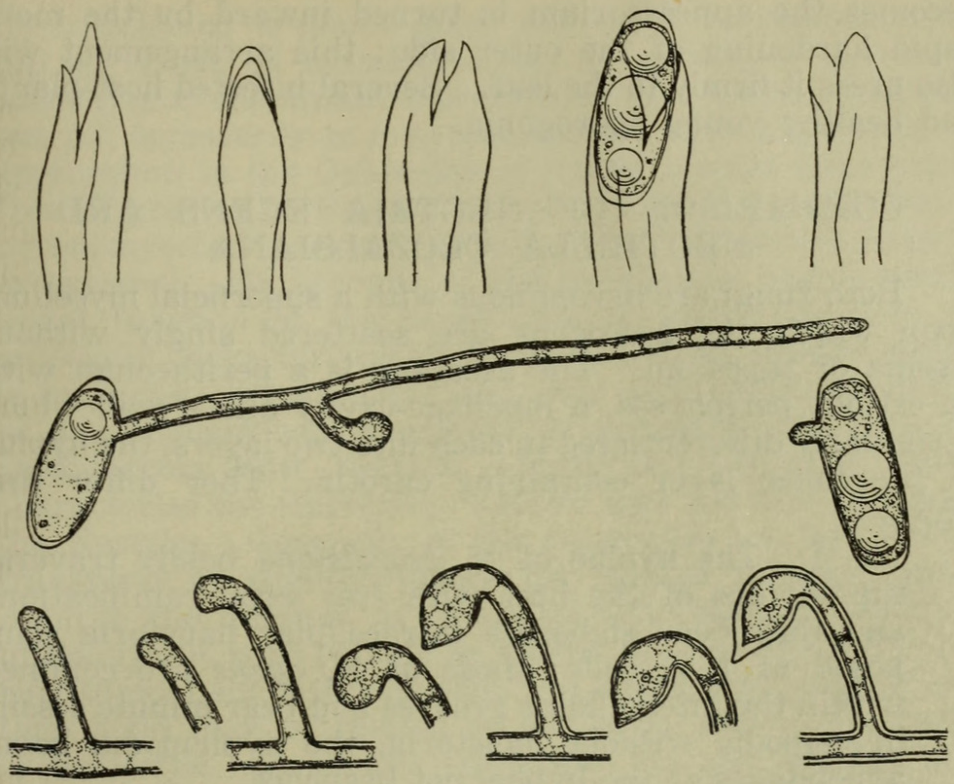
The fungus is a parasite because it does not grow on the surrounding ginger-leaf or on other species of liverworts or lichens on the same leaves and it is intimately connected with its host. Its source of food is not, however, obvious. Were there haustoria they would stain with cotton-blue and in all probability would have some visible effect on the cells of the host, but while dead cells do occur in the leaves of the liverwort they have no connection with the Nectria and generally contain the very narrow hyphæ of a mould. The hyphæ must tap the flow of food in those parts of the cellulose walls which are immediately beneath the surface of the liverwort, and with their hyphopodia. These organs, which are borne regularly over the junctions of the anticlinal walls, are like the hyphæ very closely pressed to the surface of the host and they seem to be thin-walled over the part which is in contact. All the food which passes from the leaves into the stem and whatsoever flows in the opposite direction must traverse the anticlinal walls of the leaves and the only barrier between the hyphopodia and this flow of food is exceedingly thin cuticle. It cannot be more difficult to extract food from a liverwort this way than it is for a lichen-hypha to extract food from a thick-walled gonidium: and the alternative that in the grooves on the liverwort collects some matter to the special liking of the fungus is improbable. The curious distribution of the perithecia follows also the parasitic habit. The perithecia develop only on the leaves and mostly on their distal halves which are the areas of greatest photosynthesis because the cells of the leaves have many more chloroplasts than those of the stem and the distal halves of the leaves are better illuminated as they do not overlap.

The fungus does not interfere with the growth of its host. Infected plants, dotted with perithecia, bore numerous sporogonia in all stages of development and they appeared as large and vigorous as uninfected plants. At most it is a weak parasite as one would expect from its powers of attack.

SYSTEMATIC POSITION

At the instance of *Neotiella crozalsiana,* the affinity of specialised, reduced ascomycetes cannot be inferred directly from the form of the ascocarp. In this case, however, the fungus is certainly a pyrenomycete and the perithecium a normal body. The asci are inoperculate and though this is a property of inoperculate discomycetes few of them possess carotin, and the structure and texture of the perithecium, the form of the spores and the absence of paraphyses are typical of Nectria. Several Nectriæ are bryophilous but none of the bryophilous Inoperculeæ, or indeed any genus of the family, has any close connection with *N. egens.* The other bryophilous Nectriæ, *e.g. N. muscivora* (B. et Br.) v. Hœhn.,*Calonectria Brongniartii* (Cr.) Sacc., *C. duplicella* (Nyl.) Karst., and *Pseudonectria Metzgeriae* v. Hœhn., have larger perithecia seated on a white floccose subiculum, representing a less degraded state of the stroma, and larger spores than *N. egens,* which in its minuteness and meagre mycelium is the most depauperate: in general appearance and spore-characters it approaches *N. episphaeria.* Yet in one respect it seems aberrant. The asci of *N. egens* discharge through a distinct, if immarginate, foramen. In Nectria typically, according to Tulasne, de Bary and Pethybridge, though there seem to have been no exact observations in recent years, the walls of the asci deliquesce at maturity and the mass of spores liberated inside the perithecium is squeezed through the ostiole in a slimy tendril. But to what extent are these bryophilous species really cogeneric with other Nectriæ which may be saprophytes or parasites of most diverse order preferring stems, leaves or fruits, ferns, fungi, algæ, lichens or even insects? This large group of such uniform construction appears but formally classified at present at levels of convergence and without regard for sequence of environment or or biological trend. Not merely as Fusaria do they appeal for physiological enquiry.

The hyphopodia resemble those of such Perisporiaceæ as Balladyna or the appressoria of Erysiphaceæ. The intracellular haustorium may have evolved from such a rudiment.



Text-Fig. 4.
Neotiella crozalsiana: apices of dehisced asci, germinating spores, and stages in the development of the haustorial branch: × 500.

NEOTIELLA CROZALSIANA Grelet

A few details may be added to my previous account, c.f. Text-Fig. 4. The asci dehisce through a bilabiate slit which is generally more or less median and longitudinal but which is occasionally so oblique as to appear as an elongate lid. Several germinating spores were found on the leaves of the host. A single germ-tube arises near one end of the spore and as it grows the oil-drops in the spore dwindle and disintegrate. When the germ-tube is about 100μ long, the first cell is cut off near the spore and from this cell a haustorial branch grows out: as in mildews and other ectoparasites the germ-tube sinks a haustorium at the first opportunity. The haustorial branch is formed by apical growth and acropetal thickening of the wall. It starts obliquely forward, in the direction of the apex of the parent hypha, for a distance of 10—50μ, then by unilateral expansion it turns sharply at right angles towards the parent hypha: the apex dilates into the body of the appressorium and contracts again into the beak. The wall thickens more rapidly and extensively on the exposed parts and hence it seems that the thin-walled expansion which becomes the appressorium is turned inward by the more rapid hardening of the outer side: this arrangement will also press it firmly to the leaf. Several infected host-plants had healthy young sporogonia.

COMPARISON OF NECTRIA EGENS AND NEOTIELLA CROZALSIANA

Both fungi are bryophilous with a superficial mycelium upon which the ascocarps are scattered singly without stroma or tapesium. The ascocarp is a perithecium with an ostiole, periphyses, a mucilage-cavity and a wall which is similarly differentiated in each into two layers, the hyphæ of the inner layer containing carotin. They differ this way:—

1. The hyphæ of *N. crozalsiana* boldly traverse the surface of the host with free ærial ramifications and they bear elaborate intracellular haustoria composed of 1—2 cells: those of *N. egens* are confined within the intercellular grooves and bear minute sessile hyphopodia without haustoria, the hyphopodia being appendages of the hyphæ not branches.
2. The ascocarp of *N. crozalsiana* is hairy, that of *N. egens* glabrous.
3. The ostiole in *N. crozalsiana* is bounded by a palisade of clavate hyphal ends, which is lacking from *N. egens.*
4. The paraphyses of *N. crozalsiana* contain carotin at least in the proximal cells: paraphyses are lacking from *N. egens.*
5. The ascus of *N. crozalsiana* is 5-times longer and 3-times broader than in *N. egens*: it opens by slit in *N. crozalsiana* and a pore in *N. egens.*
6. The spores of *N. crozalsiana* are four or five times longer and broader than those of *N. egens* and they are triguttate and continuous: those of *N. egens* are biguttate and uniseptate.
7. The asci of *N. crozalsiana* discharge the spores forcibly for a distance of 3 cm. or more: those of *N. egens* are so feeble that the spores barely clear the ostiole and do not travel a distance of even one millimetre.

In points 2—7 *N. crozalsiana* is an operculate discomycete and *N. egens* a pyrenomycete. In the Operculeæ a secondary mycelium always develops copiously from the base of the ascocarp, with which part the fruit-body of

N. crozalsiana is homologous, but rarely or scantily from the perithecia of pyrenomycetes: in a reduced operculate discomycete one would therefore expect a hairy ascocarp but not necessarily in a pyrenomycete. The margin of the apothecium in the Operculeæ is nearly always determinate from a greater or less amount of marginal growth, but in pyrenomycetes marginal growth from the ostiole appears exceptional. On account of the mechanical and constructional function of the paraphyses in apothecia, they are especially characteristic of discomycetes and always persist to maturity: in pyrenomycetes they are commonly lacking or are diffluent and disappear at maturity, and they are absent from most Nectriæ. Wide asci and wide spores characterise the Operculeæ, narrow asci and narrow spores the Nectriæ. Generally in the Operculeæ the asci have a transverse or slightly oblique lid, though in some typical Aleuriæ and Pezizeæ, as Phillipsia and Cookeina according to Bœdijn, it is so oblique as almost to be a slit, and it is a longitudinal slit in Boudierella and Ascozonus; a slit rather than a lid may be related to the large size of the spores or projectile. In all the Operculeæ the asci dehisce with great vigour: in the Nectriæ they dehisce either very feebly or liberate the spores by dissolution of the walls. And if it still can be argued that *N. crozalsiana* is a pyrenomycete, either it belongs in the Hypocreales in which case the first point must be considered whether in the same family two bryophilous pyrenomycetes could have evolved with such radically different modes of parasitism and spore-dispersal, c.f. the uniformity of the Erysiphales and Uredinales, or it represents an unknown order. My interpretation of the fruit-body, as a persistently juvenile form of cleistocarpic apothecium, clearly stands the test and with the abundance of information which it supplies concerning the evolution of the ascocarp it seems also the most natural. Given, in fact, a cleistocarpic apothecium maturing at so early a stage, it could but be perithecioid. The resemblance between *N. crozalsiana* and *N. egens* is one of convergence, enhanced by their reduced and simplified construction, and I do not doubt that *Nectriella Lophocoleae* Massal. is another such humariaceous discomycete, if it is not indeed the same species as *N. crozalsiana*.

As for the general relation of fungi to bryophytes, whether a phylum of such primitive repute supports a fungus-flora of a primitive stamp, there is yet insufficient knowledge to declare, but if, as in this case, the fungi are degenerate that opinion of the Bryophyta must also be revised.

SUMMARY

Nectria (Dialonectria) *egens,* found in Malaya, is described as a new species parasitic on the liverwort *Leptolejeunea corynephora.* It is ectoparasitic with the hyphæ in the grooves between the convex superficial walls of the host. Haustoria are absent but food appears to be absorbed from the host through hyphopodia placed over the anticlinal walls. The asci dehisce feebly through an immarginate pore.

Supplementary details are given on the dehiscence of the ascus, the germination of the spores and the development of the haustorial branch in *Neotiella crozalsiana.* My former account of *N. crozalsiana* is confirmed and a comparison with the true pyrenomycete, *N. egens,* supports the contention that the perithecioid fruit-body of *N. crozalsiana* is but a persistently juvenile form of cleistocarpic operculate apothecium.

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