Phyletic Considerations of the Harpellales and Asellariales (Trichomycetes, Zygomycotina) and the Kickxellales (Zygomycetes, Zygomycotina)
Author(s): Stephen T. Moss and Tom W. K. Young
Source: *Mycologia*, Vol. 70, No. 5 (Sep. - Oct., 1978), pp. 944-963
Published by: Mycological Society of America
Stable URL: http://www.jstor.org/stable/3759130
Accessed: 22/08/2008 15:58

---

---

# PHYLETIC CONSIDERATIONS OF THE HARPELLALES AND ASELLARIALES (TRICHOMYCETES, ZYGOMYCOTINA) AND THE KICKXELLALES (ZYGOMYCETES, ZYGOMYCOTINA)

STEPHEN T. MOSS

*Department of Biological Sciences, Portsmouth Polytechnic,*
*Portsmouth, Hampshire, PO1 2DY U.K.*

AND

TOM W. K. YOUNG

*Department of Applied Biology, Chelsea College,*
*University of London, London, SW10 0QX U.K.*

## SUMMARY

The Harpellales, Asellariales (Trichomycetes) and Kickxellaceae (Zygomycetes) share several characters which separate them from the other trichomycete orders, Amoebidiales and Eccrinales, as well as all other members of the Zygomycotina. The relatively complex septal apparatus comprising a crosswall with a central pore occluded by a biumbonate plug, the fundamentally coemansioid form of the asexual reproductive apparatus, similar wall structure, serological affinity and the labyrinthiform organelle (Kickxellaceae) or trichospore appendage(s) (Harpellales) could indicate a close phyletic relationship. A putative phyletic series based essentially upon the morphology of these fungi is proposed and the evidence for their relationship assessed.

The Harpellales and Asellariales together with the Amoebidiales and Eccrinales are orders of thallial, arthropodophilic fungi comprising the Trichomycetes, Zygomycotina. Members of these orders are recognized by extracellular holdfasts associated with, although not penetrating, the cuticle lining the digestive tracts of marine, freshwater and terrestrial arthropods or, in *Amoebidium parasiticum* Cienkowski, Amoebidiales, the external surfaces of freshwater arthropods. Characteristically the Trichomycetes develop nonseptate, Amoebidiales and Eccrinales, or irregularly septate, Harpellales and Asellariales, vegetative mycelia and asexual sporangia. Further, most genera of the Harpellales produce zygospores, a character used to include them in the Zygomycotina (Lichtwardt, 1973a). Recently, however, several workers have speculated on the phyletic affinities of these orders with each other and other fungal taxa (Sangar, Lichtwardt, Kirsch and Lester, 1972; Sangar and Dugan, 1973; Lichtwardt, 1973b; Moss, 1975; Moss and Lichtwardt, 1976).

The Harpellales, comprising the unbranched Harpellaceae and the branched Genistellaceae (Légeriomycetaceae Pouzar, 1972) are endocommensals of the midguts, in the Harpellaceae, or hindguts, in the Genistellaceae, of freshwater larvae and nymphs of certain Insecta. Possibly division of the Harpellales into two families, based on the presence or absence of branching and position within the host gut, is unnatural since such characters as zygospore type (Moss, Lichtwardt and Manier, 1975), number and arrangement of spore appendages and the presence or absence of a collar on the released spore, which may be considered to be of prime importance taxonomically, tend to indicate that certain genera of the Harpellaceae, for example *Stachylina,* are more closely related to specific genera of the Genistellaceae, for example *Smittium* (FIG. 18), and taxonomic revision of these families may, perhaps, become necessary. The Asellariales, with the single family Asellariaceae, occur within the fore or hindguts of marine, freshwater, and terrestrial Isopoda (Crustacea) or terrestrial Collembola and Diptera (Insecta). Members of the Kickxellaceae (sensu Linder, 1943) are typically saprophytic, occurring on the dung of herbivorous mammals and in soils throughout the world although *Coemansia reversa* van Tieghem & Le Monier, reported to grow in association with species of *Isaria,* and *Martensella corticii* Thaxter ex Linder in constant association with *Corticium radiosum* Fries (Linder, 1943), are possibly hyperparasitic. Kreisel (1969) established the Kickxellales to accommodate the Dimargaritaceae and the Kickxellaceae. The Kickxellales, the trichomycete orders Amoebidiales, Eccrinales and Harpellales, and the Endomycetales, Spermophthorales, Taphrinales, and Ustilaginales were placed together in the Endomycetes. No reference was made to the Asellariales. Apart from this taxonomic revision of Kreisel, the Kickxellaceae and Dimargaritaceae (Mucorales) (Linder, 1943; Benjamin, 1958, 1959; Zycha and Siepmann, 1969; Ellis and Hesseltine, 1974) and the Harpellales (Duboscq et al., 1948; Tuzet and Manier, 1950; Lichtwardt, 1954, 1960, 1973a, 1973b) have been classified, essentially on gross morphological grounds, in the Zygomycetes and Trichomycetes respectively. We propose, however, to retain the Kickxellales for the Kickxellaceae alone.

The possibility of a closer phyletic affinity between the Kickxellaceae (Zygomycetes) and the Harpellales and Asellariales (Trichomycetes) than generally accepted taxonomic schemes suggest, was initially indicated by the immunological research of Sangar et al. (**1972**). The fine-structural studies of Farr and Lichtwardt (**1967**), Young (**1969**), Reichle and Lichtwardt (**1972**), Manier (**1973a**, **1973b**), Moss (**1975**) and Moss and Lichtwardt (**1976**) have tended to lend support to this supposition. Sangar et al. (**1972**) demonstrated serologically that *Linderina pennispora* Raper & Fennell and *Dipsacomyces acuminosporus* Benjamin (Kickxellaceae) appear to be more closely related to isolates of *Smittium* spp. (Harpellales) than to *Amoebidium parasiticum* (Amoebidiales), the only other axenically cultured trichomycete genus, or to the other Zygomycetes tested. Ultrastructurally members of the Kickxellaceae (Young, **1969**; Benny and Aldrich, **1975**) Harpellales (Farr and Lichtwardt, **1967**; Manier and Coste-Mathiez, **1968**; Reichle and Lichtwardt, **1972**; Lichtwardt, **1973b**; Manier, **1973a**; Moss, **1975**, **1976**; Moss and Lichtwardt, **1976**, **1977**), and Asellariales (Manier, **1973b**; Moss, **1975**) display certain essentially similar micromorphological characters, for example, the septum and associated structures, which nowadays are considered taxonomically important. The Amoebidiales are nonseptate, and may be excluded from further consideration both on this ground, the absence of zygospores, the presence of multispored sporangia and the probable absence of chitin, a constituent of the cell wall of the Harpellales (Whisler, **1963**; Sangar and Dugan, **1973**), and cellulose from the cell wall (Trotter and Whisler, **1965**). Exclusion of the Eccrinales hinges upon the presence of cellulose and the probable absence of chitin (Robin, **1853**; Lichtwardt, **1954**) from the cell wall and the structure of the septum of *Astreptonema gammari* Léger & Duboscq ex Manier, the only species investigated ultrastructurally (Moss, **1975**), which appears to differ in fundamental structure from that recorded for members of the Kickxellaceae, Harpellales and Asellariales. Further, *Parataeniella* spp. (Eccrinales) may produce multispored sporangia, unlike the unisporous sporangia of the Harpellales and arthrospores of the Asellariales. Lichtwardt (**1973b**) reviewed and speculated upon the possible interfamily relations of the Trichomycetes and latterly, Lichtwardt (**1976**), and Moss and Lichtwardt (**1976**) emphasized the need to examine the evidence which could possibly augur for close common ancestry of the Kickxellaceae and Harpellales. It is our aim in this paper to assess the evidence which could be taken to relate the Kickxellaceae (Zygomycetes) with the Harpellales and Asellariales (Trichomycetes).

## COMPARATIVE ASPECTS

1. *General morphology of the asexual apparatus.*—Basically, the asexual apparatus of members of the Kickxellaceae consists of a septate, aerial sporangiophore with sporocladial branches, each bearing several pseudophialides on which are developed unisporous sporangiola. The merosporangia are usually elongate and associated with fluid droplets at maturity, although *Spirodactylon aureum* Benjamin and *Spiromyces minutus* Benjamin are dry-spored and their merosporangia tend to be ellipsoidal to spherical. Harpellales, on the other hand, produce branched (Genistellaceae) or unbranched (Harpellaceae) thalli with either the entire thallus (Harpellaceae) or the distal regions of the lateral branches (Genistellaceae) becoming regularly septate at maturity to form a series of uninucleate generative cells. From the termino-lateral region of each generative cell a single unisporous, elongate-ellipsoidal merosporangium (= trichospore, Manier and Lichtwardt, **1968**) is produced. In many genera each merosporangium is borne upon a short lateral branch, termed the collar region of the generative cell, which in some genera remains attached to the base of the released sporangium as a collar. Members of the Asellariales do not produce exogenous, deciduous merosporangia but the regularly septate branches fragment into single-celled arthrospores. In *Asellaria ligiae* Tuzet & Manier ex Manier and possibly other species of Asellariales the arthrospores germinate by the production of a single termino-lateral branch, similar in position and form to a trichospore produced from its generative cell. Lichtwardt (**1973b**) noted the morphological similarity of the germinated arthrospore of *A. ligiae* to the asexual reproductive spore of *Carouxella scalaris* Manier, Rioux & Whisler ex Manier & Lichtwardt (Harpellaceae) which comprises a generative cell with a termino-lateral, nondeciduous merosporangium; this entire unit, i.e., generative cell plus "spore" separates from the other generative cells of the thallus to form the diaspore of Manier et al. (**1961**). Neither the diaspores of *C. scalaris* nor the germinated arthrospores of *A. ligiae* have been examined ultrastructurally to determine whether the gross morphological similarities are supported at the fine-structural level. Considerable information is available on the asexual apparatus of the Kickxellaceae and Harpellales and although in these groups adaptive radiation has doubtless accounted for the present morphology of individual genera, it seems that there could be a basic, common pattern of morphology, which we have termed the "coemansioid pattern," in which these structures can be compared directly and are, perhaps, homologous (FIG. 1).

Thus, the unisporous, characteristically elongate merosporangium of

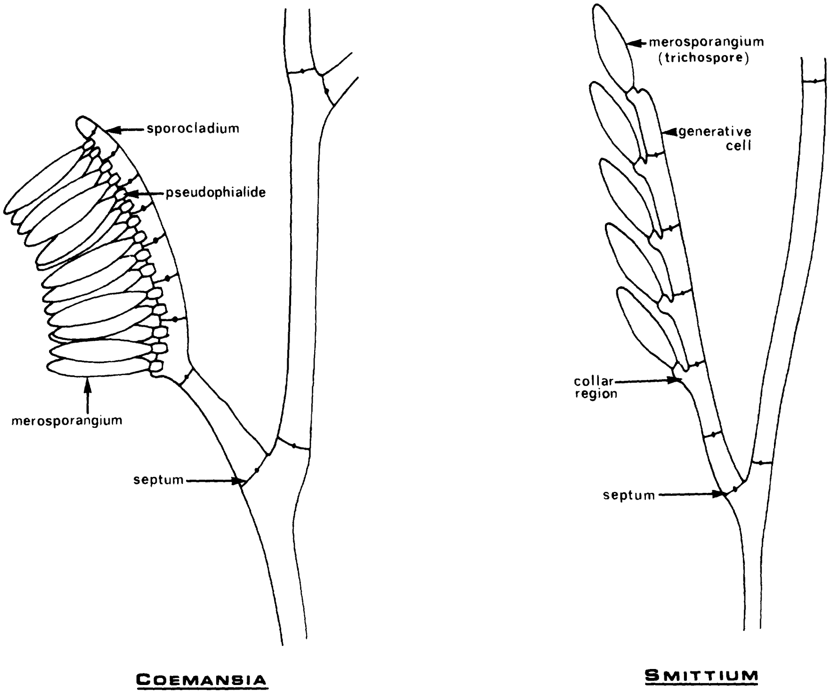


FIG. 1. The 'coemansioid pattern' of the asexual apparatus of *Coemansia* (Kickxellales) and *Smittium* (Harpellales).

the Kickxellaceae could be the homologue of the unisporous, characteristically elongate trichospore of the Harpellales, the pseudophialide the homologue of the collar region of the generative cell and the sporocladium that of the generative cell(s).

2. *Wall structure.*—The wall of the vegetative, sporocladial and generative cells of the Kickxellaceae and Harpellales examined is composed of an outer electron-opaque amorphous layer, which seems to be readily separable from the inner electron-lucent fibrillar layer by boiling in potassium hydroxide, or, in addition in the Kickxellaceae, by mechanical disintegration with Ballotini beads (FIG. 2).

The septal form common to the Kickxellaceae, Harpellales and Asellariales appears to be unique in comprising a crosswall with a central pore occluded by a biumbonate, electron-opaque plug, resembling in general form the bordered pit of a pine tracheid. The septum is essentially dolipore in form and continuity of the cytoplasm is maintained by the plasmalemma which passes through the pore around the plug.

Occasional septa have been observed in *Coemansia aciculifera* Linder which appear to possess micropores (FIG. 5), in addition to the central pore, indicating the possibility of cytoplasmic continuity through these also. Such micropores have not been detected in Harpellales. However, wherever septa occur, they are characteristically doliform and bear a central, plugged pore. Structurally the crosswall is mainly fibrillar and the appearance of chemically treated septa is very similar in the two groups. The plug does not normally seem to survive the chemical digestion treatment (boiling in KOH followed by autoclaving in a mixture of $H_2O_2/CH_3COOH$) by remaining in the pore, although it may not actually be dissolved. The crosswall is continuous with the inner fibrillar layer of the cell wall (FIGS. 2–9).

From the region of the septum which subtends the spore in *Linderina pennispora, L. macrospora* Chang and *Kickxella alabastrina* Coemans a labyrinthiform structure develops basipetally, external to the plasmalemma and extending into the pseudophialide (FIG. 11). This has been termed a "labyrinthiform organelle" (Young, **1974**) or "abscission vacuole" (Benny and Aldrich, **1975**). The labyrinthiform organelle appears, initially, to be contiguous with the inner, fibrillar layer of the pseudophialide wall and it seems to correspond in position and initial appearance, at least, with the extraplasmalemmal appendage(s) characteristic of the spores of the Harpellales. Later, in *Linderina,* the material of the labyrinthiform structure appears to degenerate, possibly then contributing to the fluid associated with the released merosporangia, as suggested by Benny and Aldrich (**1975**), whilst in the Harpellales it differentiates into a spore appendage(s) embedded in a matrix (FIGS. 10, 12) which is possibly mucilaginous (Moss, **1976**; Moss and Lichtwardt, **1976**). Thus the Harpellales and slime-spored members of the Kickxellaceae share extraplasmalemmal structures, the appendage(s) and labyrinthiform organelle respectively, which subtend the plugged merosporangial septum and could be interpreted as homologous. Further, in some observations of *L. pennispora* the material comprising the labyrinthiform organelle furthest from the merosporangial septum was found differentiated into a more dense core and a less dense matrix (FIG. 13), resembling in sectioned material the appendage of, for example, *Stachylina grandispora* Lichtwardt. Sporangial appendages in the Harpellales vary in number from one to seven, being constant for individual genera, are contiguous with the septal region, as in *Linderina,* extraplasmalemmal and also develop basipetally to extend into the collar region of the generative cell, when present, or the generative cell itself. Two features serve to distinguish the extraplasmalemmal

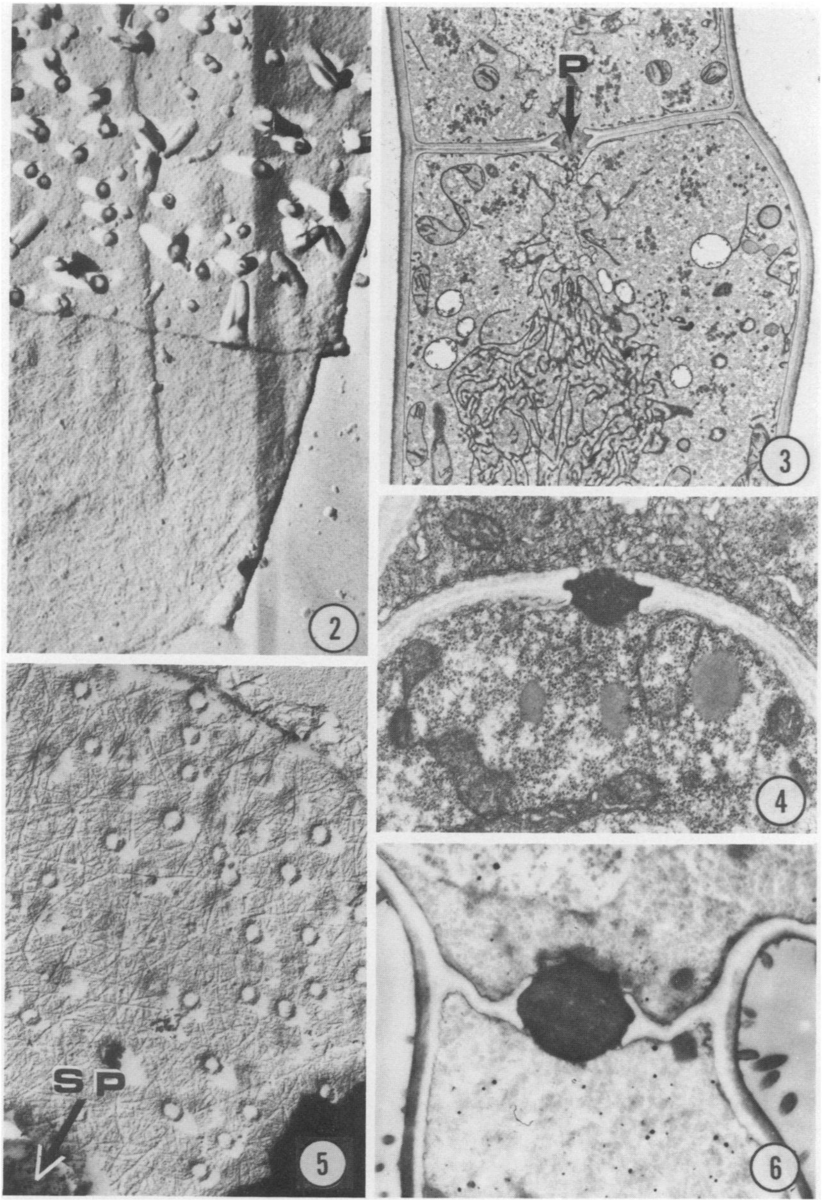


FIG. 2–6. 2. *Martensiomyces pterosporus* (Kickxellales). An aerial hypha, disintegrated with Ballotini beads, showing the spiny outer and fibrillar inner layer of the wall; carbon replica, × 10,500. 3, 4. *Genistellospora homothallica* (Harpellales). 3. A median section through a hyphal crosswall showing the electron-opaque, biumbonate plug (P) in the septal pore; potassium permanganate

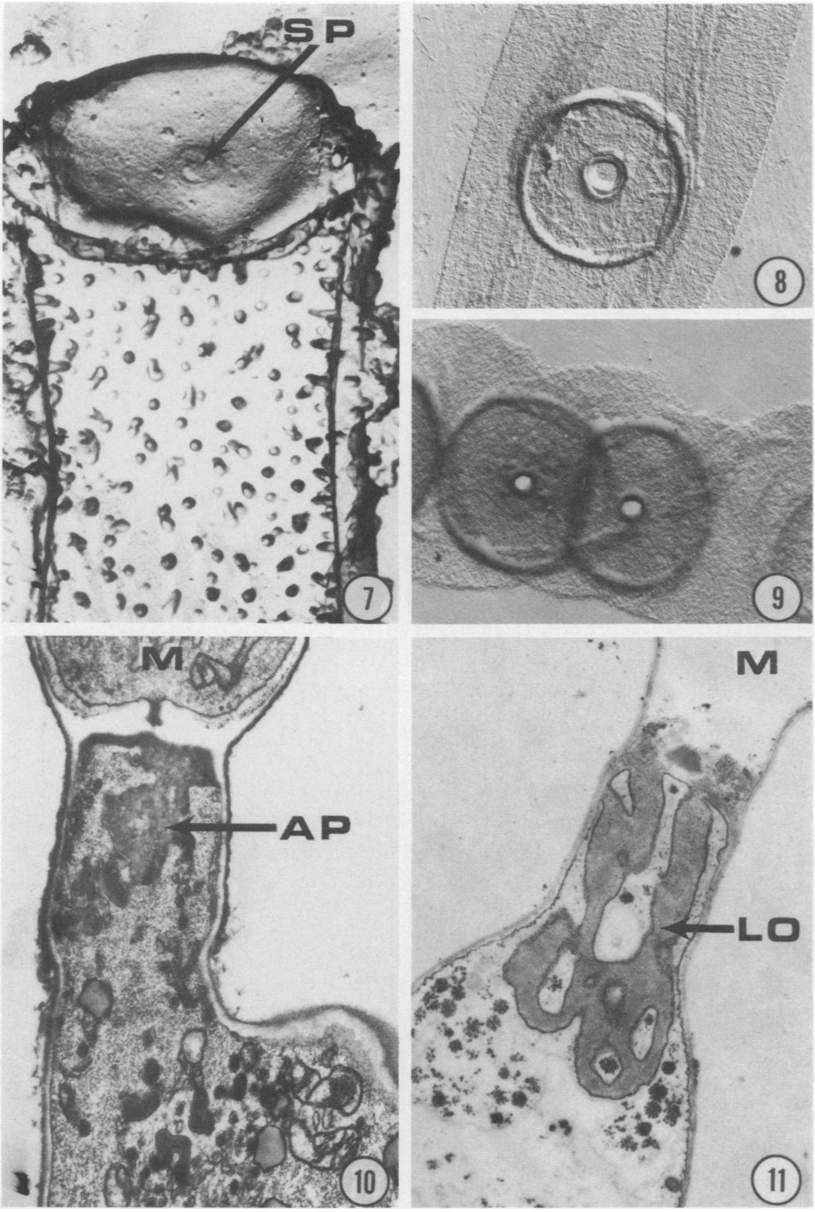
labyrinthiform organelles and appendage initials of the Kickxellaceae and Harpellales from the plasmalemmasomes and lomasomes known to be associated with the phialidic septum subtending the developing conidium in species of *Penicillium* (Zachariah and Fitz-James, **1967**; Fletcher, **1971**) and *Aspergillus* (Oliver, **1972**). First, these structures, although superficially resembling the extraplasmalemmal organelles of the Kickxellaceae and Harpellales are clearly membranous in nature and, secondly, they are not restricted to development within the apical region of the phialide but may also develop in association with the wall of the conidium. Such phialidic structures should not, on the present evidence, be considered to be homologous with the extraplasmalemmal developments from the septal region subtending the merosporangia of the Kickxellaceae and Harpellales.

Merosporangia in the Kickxellaceae and Harpellales seem also to share certain morphological and structural features in common. They are essentially tubular, smooth externally, constricted basally, and appear, like the cell wall elsewhere, to comprise an amorphous layer which covers and/or impregnates the largely fibrillar part of the wall. With the exception of the two dry-spored kickxellaceous genera *Spirodactylon* and *Spiromyces,* merosporangia in the Kickxellaceae are immersed in droplets of fluid at maturity and it is not uncommon to find in spore preparations entire merosporangia liberated by means of circumscissile rupture of the apex of the pseudophialide. Such propagules are then highly reminiscent of the liberated merosporangia of the Harpellales which are detached by circumscissile rupture either at the apex of the collar region, when present, or generative cell or, as in *Smittium* spp., *Trichozygospora chironomidarum* Lichtwardt and *Stachylina* spp., partway down the collar region, the latter remaining attached to the spore as a basal collar.

Characteristically the sporangiospore in the Kickxellaceae and the Harpellales develops acropetally, and the base is always morphologically distinct from the apex, a condition not usually associated with sporangiospores. The sporangiospores also have a fundamentally two-layered wall although in the Kickxellaceae, with the exception of *Coemansia reversa,*

---

fixation, × 6,200. 4. A median section through a hyphal crosswall, as in FIG. 3; acrolein/glutaraldehyde: osmium tetroxide fixation, × 9,700. 5. *Coemansia aciculifera* (Kickxellales). Part of a septum with septal pore (SP) from an aerial hypha, disintegrated with Ballotini beads. Micropores are present in the fibrillar layer of the crosswall; shadowed with gold/palladium, × 21,600. 6. *Linderina pennispora* (Kickxellales). A median section through a sporangiophore septum. For comparison with FIGS. 3 and 4; glutaraldehyde fixation, × 9,000.



FIGS. 7–11. 7. *Martensiomyces pterosporus* (Kickxellales). A hyphal cross-wall in surface view showing the typical central septal pore (SP); carbon replica, × 10,500. 8. *Coemansia mojavensis* (Kickxellales). A hyphal septum showing the well-defined fibrillar rim of the septal pore; digested by boiling in 5% potassium

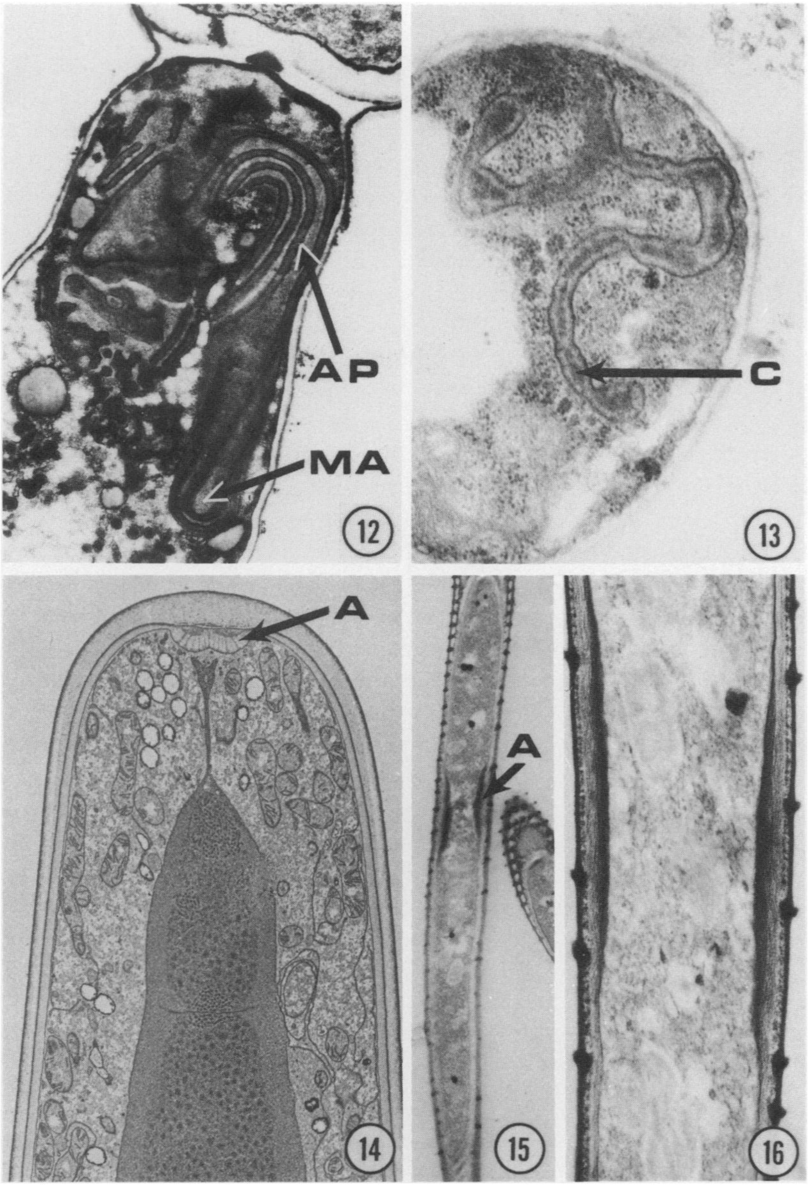
the outer essentially amorphous layer is ornamented. The inner fibrillar layer of the sporangiospore wall in each group, however, is basically similar. The apex of the sporangiospore of *Genistellospora homothallica* Lichtwardt, and other species (Manier, **1973b**; Moss, unpublished; Moss and Lichtwardt, **1976**), shows an annular thickening of this fibrillar layer (FIG. 14) in the region of germination believed to be related to holdfast production (Moss and Lichtwardt, **1976**). Although no apical annulus has been observed in a kickxellaceous spore, those of *Kickxella alabastrina* and *Coemansia aciculifera* do seem to possess an annular thickening of the inner layer of the wall, again in the region in which the spore germinates (FIGS. 15, 16). These specialized regions of the wall have not been reported for other types of spore and it is tempting to suggest them to be homologous.

*3. Wall composition.*—Although no comprehensive analyses of the walls of the Kickxellaceae and Harpellales have been performed, the fibrillar layer of the wall appears to contain chitin (Whisler, **1963**; Benny, **1972**; Sangar and Dugan, **1973**). As neither chitin nor cellulose has been detected in *Amoebidium parasiticum* (Trotter and Whisler, **1965**) and the presence of cellulose only has been indicated in *Enterobryus* spp. (Lichtwardt, **1954**), the probability of a fundamental difference in wall composition between the Kickxellaceae and Harpellales on one hand and the Amoebidiales and Eccrinales on the other, could be high. No information is available on the wall structure and composition of any species of the Asellariales.

*4. Sexual reproduction.*—No confirmed sexual stage has been reported for any member of the Asellariales, Eccrinales or Amoebidiales, although Lichtwardt (**1973b**) has reported conjugation between cells of adjacent branches in *Asellaria ligiae,* an arrangement which resembles the early stages of zygospore formation in the Harpellales. In the Harpellales,

---

hydroxide followed by autoclaving in an equivolume mixture of glacial acetic acid and hydrogen peroxide (40 vols.), shadowed with gold/palladium, × 7,000. 9. *Smittium culisetae* (Harpellales). Hyphal septa. For comparison with FIG. 8; treatment as for FIG. 8, × 5,900. 10. *Stachylina grandispora* (Harpellales). L.S. collar region. An early stage in development of the extraplasmalemmal appendage (AP) in the region of the septum subtending the merosporangium (M); potassium permanganate fixation, × 16,000. 11. *Linderina pennispora* (Kickxellales). L.S. apex of a pseudophialide. The extraplasmalemmal labyrinthiform organelle (LO) has developed in the region of the crosswall which subtends the merosporangium (M). For comparison with FIG. 10; glutaraldehyde: osmium tetroxide fixation, × 18,000.

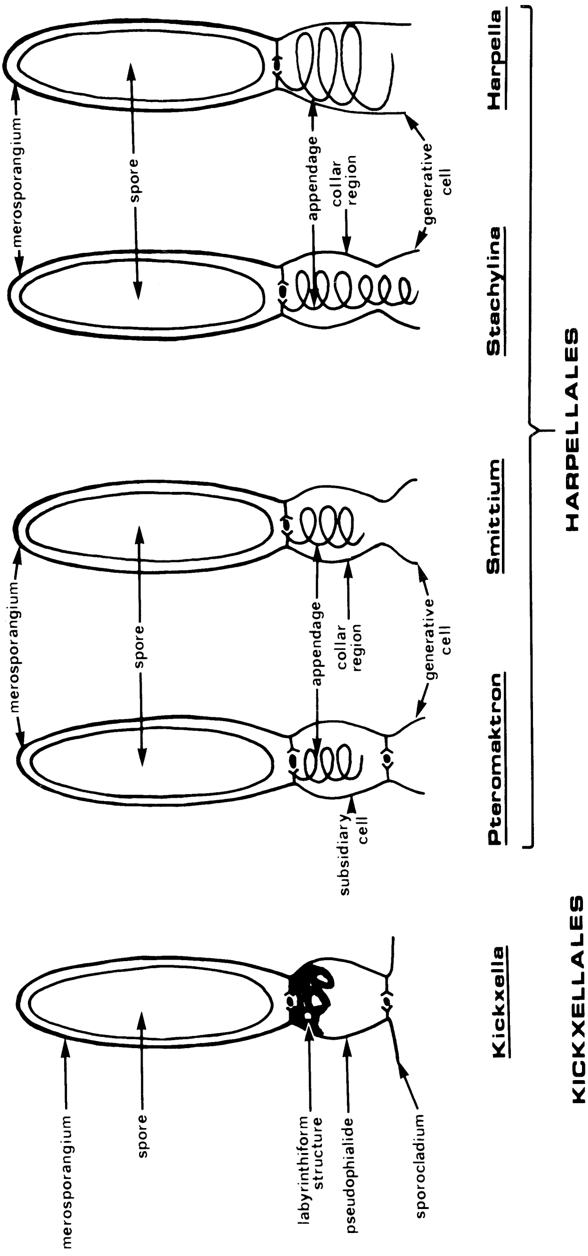


FIGS. 12–16. 12. *Stachylina grandispora* (Harpellales). L.S. collar region showing the electron-opaque appendage (AP) developing in a matrix (MA); potassium permanganate fixation, × 12,500. 13. *Linderina pennispora* (Kickxellales). T.S. of a labyrinthiform organelle in which the electron-opaque core

the zygospores form following conjugation between cells of the same, or different thalli, are supported apically upon zygosporophores which arise from the conjugation tube (*Glotzia* spp.) or one of the conjugants (other genera), and are biconical and apically thickened when mature. In *Trichozygospora chironomidarum* the zygospore bears appendages similar in structure and extraplasmalemmal position to those of the sporangiospores (Moss and Lichtwardt, 1977). Mature zygospores of members of the Kickxellaceae are spherical and lack appendages. Such dissimilarity in form of a major stage of development in the life cycle of these organisms could be taken to argue strongly against a close phyletic relationship. It is, however, generally accepted that the form of an organism or structure is likely to have resulted from selective pressures over a long period of time and zygospore types are unlikely to be exceptional in this respect. Initially, zygospores of the Harpellales are spherical and the mature biconical form, or tendency towards elongation, could be an adaptation which aids ingestion by the host, passage through the gut of an arthropod or enables the protoplast within the zygospore to develop into a small, often folded, thallus (Whisler, 1963) with a holdfast capable of immediate growth and attachment following germination, both of which are prerequisites for successful endocommensalism. The simultaneous occurrence of zygospore and sporangiospore appendages, which it has been suggested (Whisler, 1963; Lichtwardt, 1967, 1976; Reichle and Lichtwardt, 1972; Moss, 1976; Moss and Lichtwardt, 1976), function in attachment to debris in fast flowing water and thus retention of spores within the vicinity of the host population can, perhaps, be more readily explained than might at first sight seem possible. Although the nuclear events in zygospore development have not been fully investigated, the potential for appendage formation must inevitably constitute part of the haploid genome which initially enters the developing zygospore and which, in *T. chironomidarum,* is expressed in the production of extraplasmalemmal appendages similar to those of the sporangiospore (Moss and Lichtwardt, 1976). Naturally it would be of considerable interest to know whether rudi-

---

(C), resembling the appendage of *Stachylina grandispora,* has differentiated. For comparison with FIG. 12; glutaraldehyde: osmium tetroxide fixation, × 29,400. 14. *Genistellospora homothallica* (Harpellales). Median L.S. apex of sporangiospore within the merosporangium. The annulus (A) is terminal; potassium permanganate fixation, × 4,000. 15. *Coemansia aciculifera* (Kickxellales). An oblique L.S. sporangiospore showing the approximately median position of the annulus (A). For comparison with FIG. 14; potassium permanganate, × 7,500. 16. *C. aciculifera.* L.S. annulus; potassium permanganate fixation, × 28,000.

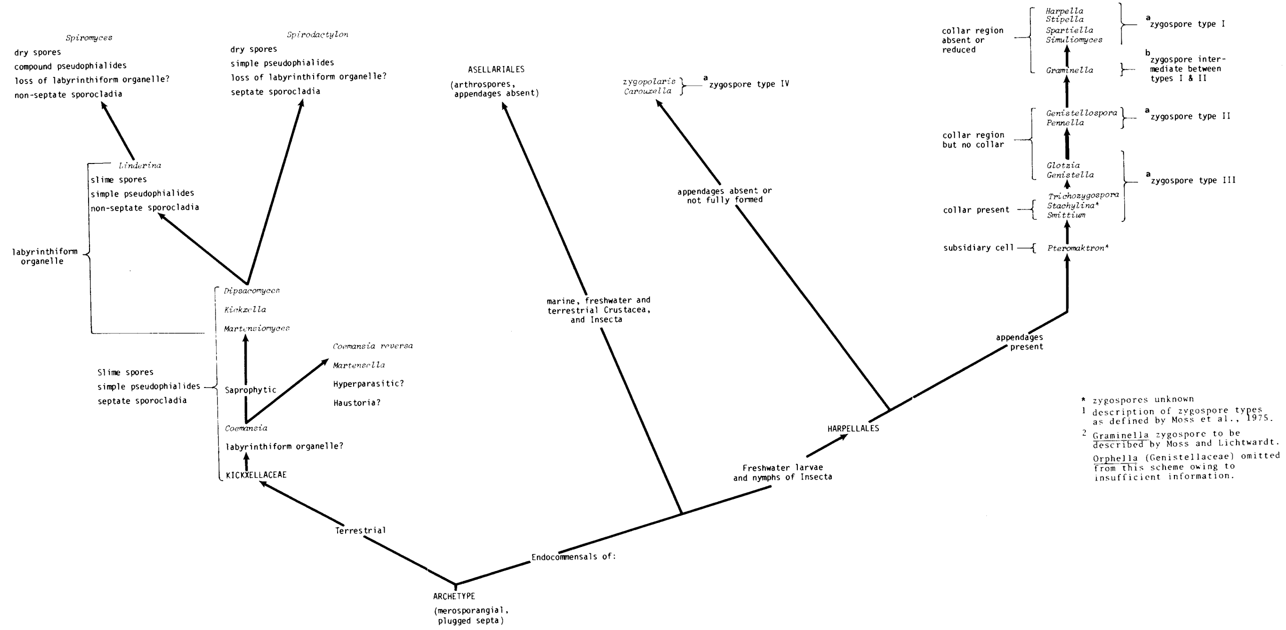

Harpella
Stachylina
Smittium
Pteromaktron
Kickxella
HARPELLALES
KICKXELLALES
merosporangium
spore
appendage
collar region
generative cell
merosporangium
spore
appendage
collar region
generative cell
subsidiary cell
merosporangium
spore
labyrinthiform structure
pseudophialide
sporocladium

mentary appendages are formed during zygospore formation in the Kickxellaceae. No ultrastructural study of the development of a kickxellaceous zygospore has been published and this is clearly an area in which investigation is required.

## DISCUSSION

As no fossil record exists any attempt to assess the degree of relationship between the Kickxellales and Trichomycetes must be somewhat speculative. Further, natural relationships tend to be obscured by the phenomenon of adaptive radiation, in the Kickxellales adaptation to the terrestrial life and in the Harpellales and most Asellariales, adaptation to life as endocommensal aquatic fungi. Thus the problem of assessing the value to be placed upon the evidence available, which is itself incomplete, is exacerbated. However, on the basis of the present evidence the arguments for removing the Dimargaritaceae from the Kickxellales (sensu Kreisel, **1969**), and allying Kickxellaceae (Kickxellales—new sense) with the Harpellales and Asellariales, would seem to be quite strong. Apart from the production of regularly septate mycelia, reduced merosporangia and spherical zygospores, the Dimargaritaceae and the Kickxellaceae share few characters in common. To consider the key characters, Dimargaritaceae are essentially parasites or facultative parasites of other Mucorales and Ascomycetes; septa, although flared, regularly bear biconvex plugs usually with a globose enlargement on each side of the septum, and the plugs are soluble in dilute alkali; the merosporangia, borne upon deciduous sporiferous branchlets, are regularly two-spored and the apex and base of the spore apparently lack differentiation when mature; zygospores appear punctate. Kickxellaceae, on the other hand are essentially saprophytic; the flared septa bear biconvex plugs insoluble in dilute alkali, lacking globose enlargements; merosporangia are unisporous, borne upon nondeciduous pseudophialides, and the spores are differentiated into an apical zone and a basal attachment region; zygospores appear smooth or pitted. Biochemically, however, the outer layer of the hyphal wall and the septal plugs in the Dimargaritaceae and Kickxellaceae appear to contain lipofuscin pigment (Benny, **1972**). Whether lipofuscin pigment is restricted in occurrence to members of these two families is not known at the present time and the significance of this observation remains to be determined.

FIG. 17. Comparative morphology of merosporangia and subtending cells in the Kickxellales and Harpellales.



* zygospores unknown

[1] description of zygospore types as defined by Moss et al., 1975.

[2] Graminella zygospore to be described by Moss and Lichtwardt.

Orphella (Genistellaceae) omitted from this scheme owing to insufficient information.

The Trichomycetes, comprising the Harpellales, Asellariales, Eccrinales, and Amoebidiales may not constitute a natural class (Sangar et al., **1972**; Sangar and Dugan, **1973**; Lichtwardt, **1973b**; Moss, **1975**) and the available evidence tends to indicate that the Harpellales and Asellariales are closely related, the Eccrinales and Amoebidiales sharing no natural affinity with any of the other orders. The question of the natural systematic position of the Eccrinales and Amoebidiales remains unsolved at present. A unifying feature of the thallus structure in the Kickxellaceae, Harpellales and Asellariales is the common form of the crosswall. This morphological character is likely to be less mutable, than, for example, the form of the asexual propagule in which adaptive radiation is presumed to have occurred in relation to habitat and method of dispersal and is probably, therefore, a fundamental character deserving emphasis from the taxonomic viewpoint. Ecologically, adaptation of the Kickxellaceae to the terrestrial environment and of the Harpellales and most Asellariales to the aquatic/endocommensal environment has presumably lead to the present-day divergence in form from the archetype and it is in this connection that the pseudophialide/labyrinthiform organelle/collar-region/appendage complex merits a more detailed analysis.

Assuming the merosporangium, pseudophialide, labyrinthiform organelle, sporocladium complex to be the homologues of the trichospore, collar region, appendage(s), generative cell(s), respectively, it becomes necessary to seek phylogenetic links and explain the morphological exceptions. Any phyletic series, based upon the structure of present-day derivatives of primitive stock, must inevitably be highly speculative. Possibly, a strong filial clue could lie with such genera as *Kickxella* (Kickxellaceae) and *Pteromaktron* (Genistellaceae) in which the full complement of cells and septa are present in the asexual apparatus and the putative homologies are reasonably clear.

The elongate merosporangium is subtended by a pseudophialide (*Kickxella*) or subsidiary cell (*Pteromaktron*) (FIG. 17). These cells are delimited apically from the merosporangium and basally from the sporocladium or generative cell by the characteristic septa. In *Pteromaktron protrudens* Whisler the appendage is confined to the subsidiary cell (Whisler, **1963**). It is possible to envisage a sequence of development in the Harpellales from the *Pteromaktron* state, through some

FIG. 18. A putative phyletic scheme of the Kickxellales, Harpellales, and Asellariales.

species of *Smittium* (e.g. *S. culicis* (Tuzet & Manier) Manier and *S. mucronatum* Manier & Mathiez ex Manier), involving loss of the septum delimiting the generative cell from the subsidiary cell, with retention of the appendage within the collar region of the generative cell, derived from the subsidiary cell, to the *Stachylina* type in which the appendage extends through the collar region into the generative cell (Moss, 1976), to *Harpella* in which the collar region has been lost during the course of evolution (FIG. 17). Naturally any linear sequence can be read in either direction equally well although, assuming that the coemansioid pattern does not represent a case of parallel evolution, the coemansioid form could represent the morphological status of these fungi at the time of divergence into terrestrial saprophytes (Kickxellaceae) and aquatic endocommensals (Harpellales and most Asellariales). Although at present, the labyrinthiform organelle is known to occur only in *Linderina* species and *Kickxella alabastrina* (also in *Martensiomyces pterosporous* Meyer and *Dipsacomyces acuminosporous,* personal communication, Benny and Aldrich), all slime-spored species, it might be predicted that the organelle will be found in *Coemansia,* also slime-spored, but is perhaps either absent from or vestigial in *Spirodactylon* and *Spiromyces,* dry-spored species with ovoid to globose merosporangia. The loss of septa, which parallels that in the Harpellales, can also be envisaged in the Kickxellaceae. Thus from the *Coemansia* form, could be derived the *Linderina* type with a multipseudophialidic, nonseptate sporocladium, to *Spiromyces* in which the sporocladium is nonseptate and the merosporangia arise from a single, nonseptate enlargement of the sporocladium (compound pseudophialide), the final stage in the reduction of pseudophialides to a single cell through the loss of septa during evolution. These ideas are summarized in FIG. 18.

A striking feature of the germinating spore in the Harpellales and Asellariales is the development of a holdfast which serves to attach the thallus to the chitinous lining of the arthropod gut. In the Kickxellaceae, the reported, possibly hyperparasitic, association of *Coemansia reversa* with *Isaria* species and *Martensella* species with *Corticium* could indicate perhaps, a basic biochemical relationship in the Kickxellaceae and Harpellales involving chitin which, in the former, may have led to parasitism or attempted infection of the potential host whereas in the latter, the association has led to the endocommensal state.

Although, based on the evidence presented, the thesis that the Kickxellaceae, Harpellales and Asellariales are closely related can be supported, it is clear that several key areas require intensive investigation before more than tentative conclusions can be drawn. Biochemically, detailed analyses of wall composition and appendage and labyrinthiform organelle composition, ontogeny and function should be rewarding. Morphologically, the cytology of zygospore development largely at the light microscopic and certainly at the ultrastructural level remains to be elucidated (Moss and Lichtwardt, **1977**). In addition, the immunological approach of Sangar et al. (**1972**) could be considerably extended to include reciprocal reactions against the antibodies of the species used originally and a wider range of species from the Kickxellaceae, such as *Coemansia reversa,* a more primitive form on the basis of the postulate and *Spiromyces minutus* Benjamin, a more advanced form (FIG. 18); together with members of the Dimargaritaceae and the Piptocephalidaceae. Unfortunately, only one genus of the Harpellales (Clark et al., **1963**; Lichtwardt, **1964**) and none of the Asellariales have been maintained successfully in axenic culture, an essential requirement for detailed biochemical studies.

It is obvious that for accurate taxonomic conclusions to be drawn many gaps in current knowledge of the Kickxellales and Trichomycetes will have to be filled.

## ACKNOWLEDGMENTS

We wish to thank Mr. Alan Ginman for assistance in preparation of the photographs.

## LITERATURE CITED

**Benjamin, R. K.** 1958. Sexuality in the Kickxellaceae. *Aliso* **4**: 149–169.

——. 1959. The merosporangiferous Mucorales. *Aliso* **4**: 321–433.

**Benny, G. L.** 1972. Histochemistry of the cell wall and septum of vegetative cells of selected species of Dimargaritaceae and Kickxellaceae. *Mycologia* **64**: 854–862.

——, **and H. C. Aldrich.** 1975. Ultrastructural observations on septal and merosporangial ontogeny in *Linderina pennispora* (Kickxellales; Zygomycetes). *Canad. J. Bot.* **53**: 2325–2335.

**Clark, T. B., W. R. Kellen, and J. E. Lindegren.** 1963. Axenic culture of two Trichomycetes from Californian mosquitoes. *Nature* **197**: 208–209.

**Duboscq, O., L. Léger, and O. Tuzet.** 1948. Contribution à la connaissance des Eccrinides: les Trichomycètes. *Arch. Zool. Exp. Gen.* **86**: 29–144.

**Ellis, J. J. and C. W. Hesseltine.** 1974. Two new families of Mucorales. *Mycologia* **66**: 87–95.

**Farr, D. F., and R. W. Lichtwardt.** 1967. Some cultural and ultrastructural aspects of *Smittium culisetae* (Trichomycetes) from mosquito larvae. *Mycologia* **59**: 172–182.

**Fletcher, J.** 1971. Conidium ontogeny in *Penicillium*. *J. Gen. Microbiol.* **67**: 207–214.

**Kreisel, H.** 1969. *Grundzüge eines natürlichen Systems der Pilze.* J. Cramer, Lehre. 246 p.

**Lichtwardt, R. W.** 1954. Three species of Eccrinales inhabiting the hindguts of millipeds with comments on the Eccrinids as a group. *Mycologia* **46**: 564–585.

——. 1960. Taxonomic position of the Eccrinales and related fungi. *Mycologia* **52**: 410–428.

——. 1964. Axenic culture of two new species of branched Trichomycetes. *Amer. J. Bot.* **51**: 836–842.

——. 1967. Zygospores and spore appendages of *Harpella* (Trichomycetes) from larvae of Simuliidae. *Mycologia* **59**: 482–491.

——. 1973a. Trichomycetes. Pp. 237–243. *In: The fungi, Vol. IVB.* Eds., G. C. Ainsworth, F. K. Sparrow and A. S. Sussman. Academic Press, London and New York.

——. 1973b. The Trichomycetes: what are their relationships? *Mycologia* **65**: 1–20.

——. 1976. Trichomycetes. Pp. 651–671. *In: Recent advances in aquatic mycology.* Ed. E. B. Gareth Jones. Elek Science, London.

**Linder, D. H.** 1943. The genera *Kickxella, Martensella* and *Coemansia. Farlowia* **1**: 49–77.

**Manier, J.-F.** 1973a. L'ultrastructure de la trichospore de *Genistella ramosa* Léger et Gauthier. Trichomycète Harpellale parasite du rectum des larves de *Baetis rhodani* Pict. *Compt. Rend. Hebd. Séances Acad. Sci.* **276**: 2159–2162.

——. 1973b. Quelques aspects ultrastructuraux du Trichomycète Asellariale. *Asellaria ligae* Tuzet et Manier, 1950 ex Manier, 1968. *Compt. Rend. Hebd. Séances Acad. Sci.* **276**: 3429–3431.

——, **and F. Coste-Mathiez.** 1968. L'ultrastruture du filament de la spore de *Smittium mucronatum* Manier, Mathiez 1965 (Trichomycète, Harpellale). *Compt. Rend. Hebd. Séances Acad. Sci.* **266**: 341–342.

——, **and R. W. Lichtwardt.** 1968. Révision de la systematique des Trichomycètes. *Ann. Sci. Nat. Bot.* **9**: 519–532.

——, **J.-A. Rioux, and H. C. Whisler.** 1961. *Rubetella inopinata* n. sp. et. *Carouxella scalaris* n. g., n. sp., Trichomycètes parasites de *Dasyhelea lithotelmatica* Strenzke, 1951 (Diptera Ceratopogonidae). *Nat. Monspeliensia, Sér. Bot.* **13**: 25–58.

**Moss, S. T.** 1975. Septal structure in the Trichomycetes with special reference to *Astreptonema gammari* (Eccrinales). *Trans. Brit. Mycol. Soc.* **65**: 115–127.

——. 1976. Formation of the trichospore appendage in *Stachylina grandispora* (Trichomycetes). Pp. 279–294. *In: Microbial ultrastructure.* Annual Technical series of the Society for Applied Bacteriology. Eds. R. Fuller and D. Lovelock. Academic Press, London.

——, **and R. W. Lichtwardt.** 1976. Development of trichospores and their appendages in *Genistellospora homothallica* and other Harpellales and fine-structural evidence for the sporangial nature of trichospores. *Canad. J. Bot.* **54**: 2346–2364.

——, **and** ——. 1977. Zygospores of the Harpellales: an ultrastructural study. *Canad. J. Bot.* **55**: 3099–3110.

——, ——, **and J.-F. Manier.** 1975. *Zygopolaris,* a new genus of Trichomycetes producing zygospores with polar attachment. *Mycologia* **67**: 120–127.

**Oliver, P. T. P.** 1972. Conidiophore and spore development in *Aspergillus nidulans*. *J. Gen. Microbiol.* **73**: 45–54.

**Pouzar, Z.** 1972. *Genistella* Léger et Gauthier vs. *Genistella* Ortega; a nomenclatural note. *Folia Geobot. Phytotax., Praha* **7**: 319–320.

**Reichle, R. E. and R. W. Lichtwardt.** 1972. Fine structure of the trichomycete *Harpella melusinae* from black-fly guts. *Arch. Mikrobiol.* **81**: 103–125.

**Robin, C.** 1853. Histoire naturelle des végétaux parasites qui croissent sur les animaux vivants. Baillère, Paris. 45 p.

**Sangar, V. K., and P. R. Dugan.** 1973. Chemical composition of the cell wall of *Smittium culisetae* (Trichomycetes). *Mycologia* **65**: 421–431.

——, **R. W. Lichtwardt, J. A. W. Kirsch, and R. N. Lester.** 1972. Immunological studies on the fungal genus *Smittium* (Trichomycetes). *Mycologia* **64**: 342–358.

**Trotter, M. J., and H. C. Whisler.** 1965. Chemical composition of the cell wall of *Amoebidium parasiticum*. *Canad. J. Bot.* **43**: 869–876.

**Tuzet, O., and J.-F. Manier.** 1950. Les Trichomycètes. Révision de leur diagnoses. Raisons qui nous font y joindre les Asellariées. *Ann. Sci. Nat. Bot., Sér. 11,* **12**: 15–23.

**Whisler, H. C.** 1963. Observations on some new and unusual enterophilous Phycomycetes. *Canad. J. Bot.* **41**: 887–900.

**Young, T. W. K.** 1969. Ultrastructure of aerial hyphae in *Linderina pennispora*. *Ann. Bot. (London)* **33**: 211–216.

——. 1974. Ultrastructure of the sporangiospore of *Kickxella alabastrina* (Mucorales). *Ann. Bot. (London)* **38**: 873–876.

**Zachariah, K., and P. C. Fitz-James.** 1967. The structure of phialides in *Penicillium claviforme*. *Canad. J. Microbiol.* **13**: 249–256.

**Zycha, H. and R. Siepmann.** 1969. *Mucorales*. J. Cramer, Lehre. 355 p.

Accepted for publication March 20, 1978