CHAPTER V-1

Fungi: Identification

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1 INTRODUCTION

Most scientists who find and try to identify entomopathogenic fungi have little mycological background. This chapter presents the basic skills and information needed to allow non-mycologists to identify the major genera and, in some instances, most common species of fungal entomopathogens to the generic or, in many instances, to the specific level with a degree of confidence.

Although many major species of fungal entomopathogens have basic diagnostic characters making them quickly identifiable, it must be remembered that species such as *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Sorok.) Metsch, and *Verticillium lecanii* (Zimm.) Viégas are widely agreed to be species complexes whose resolutions will depend on correlating molecular, morphological, pathobiological and other characters (Soper *et al.*, 1988; Humber, 1996). The keys in this chapter cannot treat the total variation known for these common genera and species, but the information given is a detailed guide to the diagnostic characters of many important fungal entomopathogens.

This chapter also discusses the preparation of mounts for microscopic examination. Similar points are covered in other chapters, but good slide mounts and simple issues of microscopy are indispensable skills for facilitating the observation of key taxonomic characters. Many publications discuss the principles of microscopy, but a manual by Smith (1994) is easy to understand and notable for its many micrographs showing the practical effects of the proper and improper use of a light microscope.

The recording of images presents a wholly new set of options and challenges in increasingly computerized laboratories. Until this century, the only visual means to record microscopic observations was with drawings; such artwork, whether rendered freehand or with the aid of a camera lucida, still remains an important means of illustrating many characters. The photographs in this chapter were acquired directly as digital files and then adjusted, composed into plates and labelled with photographic software, and printed with a dye sublimation printer. Such a

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non-traditional approach to scientific illustration will, undoubtedly, become much more common in the near future.

2 PREPARATION AND OBSERVATION OF MICROSCOPE SLIDES

The identification of most entomopathogenic fungi necessarily depends on the observation of microscopic characters. Fortunately, however, many common entomopathogens can, with relatively little experience, be easily identified to the genus or, in some instances, the species by observation with either the unaided eye or low magnifications from hand lenses or stereo microscopes. Species identifications usually require confirmation of essential microscopic characters.

The ease with which key microscopic characters can be seen is directly affected by the quality of one's microscopy and slide preparative techniques. The following sections outline the few major skills needed to use a microscope well or to make good slide preparations.

A Köhler illumination: the first and most important step

The key to observing fine details in a microscope is not magnification; it is optical resolution, the ability to distinguish two adjacent objects. Many factors can affect image resolution, but the first and most important is to maintain Köhler illumination when using bright field or differential interference optics. Phase-contrast images are much less sensitive to the physical settings of a microscope, but it is always a good idea to maintain Köhler illumination at all times.

The following steps to achieve Köhler illumination should be repeated for each objective used. Focus sharply on any object in a slide and then:

1. Close down the field diaphragm (at the light source) and adjust the height of the condenser so that both the inner edge of this iris diaphragm and the object in the slide are sharply focused when seen through the eyepieces.
2. Open the field diaphragm until its image nearly fills the field of view and then centre the field diaphragm image in the field of view with the condenser's centring screws.
3. Adjust the opening of the condenser diaphragm. The image of this diaphragm is seen by removing an eyepiece and looking down the inside of the microscope body; a focusing telescope can be useful but is not truly necessary for this step. The condenser diaphragm should be adjusted so that its opening fills some 80–90% of the diameter of the image in this back focal plane.

The condenser diaphragm should never be opened wider than the full diameter of the back focal plane; the resulting 'glare' of too much uncollimated light in the system severely degrades the image resolution. A frequent error in light microscopy is to close down the condenser diaphragm too far to increase the image contrast, but the resulting interference effects (seen as increasing graininess and darkening of object edges) also dramatically reduces image resolution.

B Coverslips

Microscopic image resolution is also affected by the type and thickness of coverslips used in slide preparations. The optics of microscope lenses are calculated to allow maximal resolution with no. 1½ coverslips (0.16–0.19 mm thick); maximal resolution is lower with either no. 1 and no. 2 coverslips (with thicknesses of 0.13–0.17 and 0.17–0.25 mm, respectively). Use glass coverslips for diagnostic work. Plastic coverslips are too thick and cause intolerable image degradation; they should be reserved for specialized experiments and avoided for general microscopic observations.

Full-sized 18 or 22 mm square or round coverslips may not be the most practical size for diagnostic purposes or whenever one must make large numbers of mounts in a short time. The total amount of glass and mounting medium to be used can be greatly reduced by scribing square coverslips into quarters with a diamond or carbide pencil and a slide edge as a straightedge, and then gently breaking those coverslips along the scratches if they do not break during the scribing. Ten or twelve such miniature coverslips can fit on a standard slide. Not only is less material consumed in this process, but the smaller area under each coverslip makes it easier to locate the fungus to be observed.

C Mounting media

Regardless of the mounting medium used, it is important to use no more than is needed to fill the volume under the coverslip. It is alright to use too little mounting medium, but using too *much* floats the coverslip, does not flatten the material to be examined, and prevents any later sealing with nail polish or other slide sealants. Mounting medium can be removed and a preparation further flattened without spreading mounting medium all over the slide (or microscope) and without lateral movement on the specimen by placing the slide into a pad of bibulous paper and applying whatever pressure is needed.

The choice of mounting medium and the means of preparing slide mounts can profoundly affect the apparent sizes of taxonomically important structures (Humber, 1976).

Recipes for some useful mounting media are given in the Appendix to this chapter. These include pure lactic acid (to which acidic stains such as aniline blue or aceto-orcein may be added), lactophenol (which is more useful for semi-permanent mounts than is lactic acid, and is also compatible with acidic stains), and aceto-orcein (a very useful general mount for diagnostic purposes that can hydrate even dried specimens and is nearly required for identifying entomophthoralean fungi).

D Handling of the material to be observed

Novice slide-makers often include too much material in a slide with the mistaken belief that 'more is better'. In fact, the most useful slides usually include the very little amount of material that has been carefully teased apart and spread in the mounting medium. Using only small amounts of material in mounts may force repeated preparations to see specific structures, but the effort required is often distinctly rewarded by the results. In all practicality, most preparations for diagnostic uses can be prepared fairly quickly since the most critical characters may be readily seen regardless of the care in preparation. Mounts intended for photography and or archival preservation, however, do benefit greatly from the most fastidious possible preparative attention.

The most useful tools for preparing slides of many fungi are not standard dissecting needle probes. The points of such probes are much too large to tease apart delicate fungal structures. The best tools may be '0' and 'minuten' insect pins mounted in soft wood sticks (e.g. the thick wooden match sticks available in the US or wooden chopsticks). The blunt ends of stainless steel '0' (whose heads have been cut off) or 'minuten' insect pins should be pushed into the sticks. The points of both of these types of pins remain small and distinctly pointed even when viewed at high magnification (see Figure 1). The '0' pins are superb for coarse operations or teasing apart leathery or hard structures; 'minuten' pins are excellent for manipulating hyphae, conidiophores, or other delicate structures. These insect pins are also versatile tools for manipulating cultures. The points of '0' pins can be pounded out into very useful microspatulas. Standard or flattened points of '0' needles can be flame sterilized but the points of 'minuten' pins may melt and even burn if flamed; autoclaving in glass Petri dishes or in groups in folded foil packets is a convenient way to sterilize these pins.

The art of making good slides consistently is, once again, mostly a matter of practice and common sense. Most taxonomically important structures can be detected well enough at magnifications of 50–75× to know if a slide merits examination on the compound microscope. Virtually all microscopic examination of entomopathogenic fungi for diagnostic purposes can be done at a magnification of 400–450×; oil immersion is only rarely needed.

E Semi-permanent slide mounts

Most slide mounts are made strictly for immediate observation rather than for long-term storage for

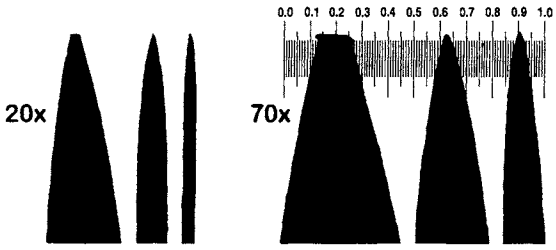

Figure 1 Comparative appearances at low magnification of dissecting needle tips (left to right: standard dissecting needle, 0' insect pin, and 'minuten' insect pin). The higher magnification set is superimposed over an ocular micrometer scale (total length, 1.0 mm).

later reference. Many differing techniques can be used to make semi-permanent slides, but those most useful for invertebrate pathogens involve means to seal slides prepared with aqueous mounting media.

A very short-term seal may be obtained by painting a melted mixture of roughly equal amounts of paraffin and petroleum jelly around the coverslip. Extreme caution must be used if melting this mixture over an open flame (alcohol burner, etc.) since paraffin vapour is highly flammable.

Coverslips are most often sealed by ringing them with fingernail polish, Canada balsam or another slide-making resin. Apply a relatively narrow and thin first layer; once the sealant is dried, a thicker and more secure seal can be built up by later applications of the sealant, but always be sure that the edges of the subsequent layer(s) cover the inner and outer edges of earlier layer(s). Such preparations may remain sealed for several months but should not be relied on to last for years. No sealing method is likely to work unless only a *minimal* amount of mounting medium is included under the coverslip; slides on which any amount of mounting medium protrudes from under the coverslip will probably fail to seal.

More secure, longer-lasting aqueous mounts can be prepared with methods using two coverslips of unequal sizes (Kohlmeyer & Kohlmeyer, 1972). The basic method shown in Figure 2 is simple: The material is spread in a minimal drop of mounting medium on the small coverslip; the large coverslip is then lowered onto the small one; the smaller coverslip of this sandwich is then attached to the standard microscope slide by a drop of glycerol, immersion oil or resin; and the space under the edge of the large coverslip is filled with a permanent sealant. Kohlmeyer & Kohlmeyer (1972) modified this basic procedure with a preliminary sealing of the small coverslip onto the large one and allowing this first ring to dry before attaching the sandwich to the slide. Such a procedure is easier to describe than to execute flawlessly. Several points should be heeded to increase the likelihood of success:

- The relative size differences of the coverslips should be small. Pairing 18 mm and 22 mm square coverslips is suitable; mixing square and round coverslips should be avoided.
- It takes practice to get the sizes of the drops of fluids small enough.
- It is easiest to use a small paint brush to apply the sealant.
- Adjusting the viscosity and solvent concentration in the sealant is the most difficult problem in this technique. Too much solvent tends to create bubbles in the sealing ring and may destroy the longevity of the mount. Inadequately thinned sealant may be too viscous to fill the space under the large coverslip.
- Excess (hardened) sealant can be cut away with a razor blade to improve the cosmetic appearance of the preparation.

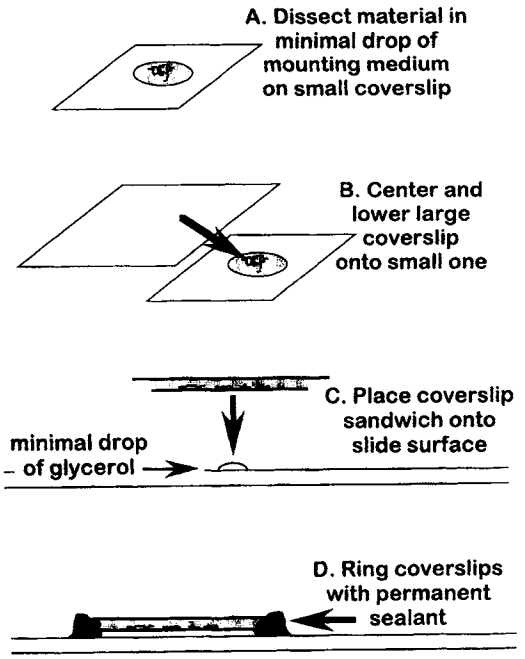

Figure 2 Outline of the procedure to make coverslip 'sandwiches' and semi-permanent slides.

3 KEY TO MAJOR GENERA OF FUNGAL ENTOMOPATHOGENS

This key should be used together with the taxonomic treatments and photos in Section 4. The key includes all fertile (spore-bearing) states most likely to be found for the genera treated. A greater number of entomopathogenic fungal genera are illustrated and keyed (although in less detail) by Samson *et al.* (1988).

Those with access to the World Wide Web may find a glimpse of the possible future of taxonomic mycology there in the form of an interactive key to

Fusarium species (Seifert, 1995; <http://res.agr.ca/brd/fusarium/>). Few species of this complex genus affect insects but this interactive key offers a significant model for future similar on-line keys to pathogens of invertebrates that could become important and highly accessible tools for a broad spectrum of scientists, regardless of their academic backgrounds and specialties.

Vegetative states of most fungi have little taxonomic value and are not characterized in the key. If no spores are seen in a collection, specimens (or cultures) should be incubated for a further time in room conditions of temperature, humidity and light and, if reasonable, part of any fresh collection of infected specimens should be incubated in a humid chamber at 100% RH for 24–48 h but watch closely for fast-growing fungal and bacterial saprobes that may soon overwhelm a real pathogen.

It is assumed that this key will be used primarily with infected specimens but most of the included fungi should also be identifiable from sporulating cultures so long as the user is aware of the host's identity and has a general idea about the appearance of the fungus on that host.

A brief glossary of terms used in the key and generic discussions is presented at the end of this chapter and should help to clarify many potential questions. More detailed definitions of terms can be found in many mycological textbooks or in *Ainsworth & Bisby's Dictionary of the Fungi* (Hawksworth *et al.*, 1995).

1. Spores and hyphae or other fungal structures visible on exterior of host or host body is obscured by fungus; few or no spores form inside host cadaver 2
1a. Fungal growth and sporulation wholly (or nearly wholly) confined to *interior* of host body 30

2. Elongated macroscopic structures (synnemata or club-like to columnar stromata) project from host 3
2a. Fungal growth may cover all or part of the host and may spread onto the substrate but large, projecting structures are absent 10

3. Conidia form on synnemata and/or on mycelium on the host body 4
3a. Flask-like to laterally flattened fruiting structures (perithecia) present whether on or submersed in an erect, dense to fleshy, club-like to columnar stroma or on body of host; if mature, containing elongated asci with thickened apical caps 9

4. Conidia formed in short to long chains 5
4a. Conidia produced singly on many separate denticles on each conidiogenous cell or, if in some sort of slime, singly (slime sometimes not evident) or in small groups in a slime droplet 7

5. Conidiogenous cells flask-like, with swollen base and a distinct neck, borne singly or in loose clusters; chains of conidia often long and divergent (when borne on clusters of conidiogenous cells) ..... *Paecilomyces*
5a. Conidiogenous cells short, with rounded to broadly conical apices (not having a distinctly narrowed and extended neck) 6

6. Conidiogenous cells clustered on more or less swollen vesicle on short to long, conidiophores projecting laterally from synnemata and/or the hyphal mat covering the host; conidia pale to yellow or violet in mass; affecting spiders ..... *Gibellula*
6a. Conidiogenous cells borne at apices of broadly branched, densely intertwined conidiophores that form a compact hymenium; conidia borne in parallel chains and usually green in mass ..... *Metarhizium*

7. Conidiogenous cell with swollen base and elongated, narrow to spine-like neck; conidia formed singly (usually with a distinct slime coating) or small groups in a slime droplet ..... *Hirsutella*
7a. Conidiogenous cells producing several to many conidia, each formed singly on separate denticles 8

8. Conidiogenous cell with an extended, denticulate apex (growing apex repeatedly forms a conidium and regrows [rebranches] just below the new conidium) ..... *Beauveria*

8a. Conidiogenous cell short and compact, cylindrical to broadly clavate, with apex studded by many denticles, each of which bears a single conidium ..... *Hymenostilbe*

9. Erect stroma bears perithecia superficial to partially or fully immersed (with only small circular opening raised above stromatic surface); perithecia scattered or aggregated into more or less differentiated, apical or lateral fertile part; asci (if present) with thickened apical cap perforated by narrow canal and filiform ascospores (that usually dissociate into one-celled part spores); conidia, if simultaneously present, being formed on host body, on lower portion of stroma, or on separate synnemata ..... *Cordyceps*

9a. Perithecia occur *only* on or partially immersed in a cottony to woolly hyphal layer covering host ..... *Torrubiella*

10. Fungus covering host is a stroma (fleshy to hard mass of intertwined hyphae); sporulation occurs in cavities below the stromatic surface . 11

10a. Host partially to completely covered by wispy, cottony, woolly, or felt-like growth or by a dark-coloured, extensive patch having columns and chambers below its surface but *not* forming a dense stroma 12

11. Spores are fusoid, one-celled conidia discharged in a slime mass from fertile chambers immersed in the stroma but not set off by a differentiated wall ..... *Aschersonia*

11a. Globose to flask-like perithecia delimited by a distinct wall are immersed in stroma and contain elongated asci with thickened apices or, at maturity, a (non-slimy) mass of globose, ovoid or rod-like spores formed by dissociation of multiseptate ascospores; *Aschersonia* conidial state often present on same stroma ..... *Hypocrella*

12. Fungus a dark brown to black, sometimes extensive patch on woody plant parts; upper surface dense to felt-like, with elongated or clavate thick-walled cells (teleutospores) remaining attached; open chambers and vertical fungal columns underlie the more or less solid upper surface and shelter living scale insects, some of which contain prominently coiled haustorial hyphae ..... *Septobasidium* (see Couch, 1938; not treated here)

12a. Fungal hyphae emerging from or covering host are colourless to light coloured, wispy to cottony, woolly, felt-like or waxy-looking mat 13

13. Flask-like to laterally compressed perithecia present, superficial to partially immersed in fungus covering the host; asci elongate, with thickened apex; when mature, filiform multiseptate ascospores tend to dissociate into 1-celled part-spores; conidial state(s) may occur simultaneously on host body or synnemata; especially on spiders or homopterans ..... *Torrubiella*

13a. Spores form on external surfaces of the fungus; no sexual structures (perithecia) are present 14

14. Conidia form on cells with elongated denticulate necks bearing multiple conidia on awl- to flask-shaped or short blocky conidiogenous cells; conidia form singly or successively in dry chains or slime drops (Hyphomycetes) 15

14a. Conidia forcibly discharged and may rapidly form forcibly or passively dispersed secondary conidia (Entomophthorales) 22

15. Conidiogenous cell with an extended,

denticulate apex (growing apex repeatedly forms an conidium and regrows (rebranches) just below the new conidium) ..... *Beauveria*

15a. Conidiogenous cells are awl- to flask-shaped, with or without an obvious neck; conidia borne singly, in chains, or in slime drops 16

16. Conidia single or in chains on apices of conidiogenous cells 17

16a. Conidia aggregate in slime drops at apices of conidiogenous cells 20

17. Conidia borne singly on conidiogenous cell with swollen base and one or more narrow, elongated necks; conidia globose or, if not, usually having an obvious slime coat; especially on mites ..... *Hirsutella*

17a. Conidia borne in chains, not covered by any obvious slime 18

18. Conidiophores much branched in a candelabrum-like manner but very densely intertwined, and forming nearly wax-like fertile areas; conidiogenous cells short, blocky, without apical necks; conidial chains long and, usually, laterally adherent in prismatic columns or continuous plates ..... *Metarhizium*

18a. Conidiophores individually distinct and unbranched or with a main axis and short side branches bearing single or clustered conidiogenous cells 19

19. Conidiogenous cells flask-like, with swollen base and a distinct neck, borne singly or in loose clusters; chains of conidia often long and divergent (when borne on clusters of conidiogenous cells) ..... *Paecilomyces*

19a. Conidiogenous cells short and blocky with little obvious neck, borne in small clusters on short branches grouped in dense whorls on (otherwise unbranched) conidiophores; conidial chains short; especially on Noctuidae (Lepidoptera) ..... *Nomuraea*

20. Conidia aggregating in slime droplets with morphology either (1) macroconidia, elongated, gently to strongly curved with somewhat pointed ends, one or more transverse septa and usually a short (basal) bulge or bend ('foot') and/or (2) microconidia aseptate, with variable morphology; conidiogenous cells often distinctly thicker than vegetative hyphae; hyphae often with terminal or intercalary chlamydospores (thick-walled spore-like swellings of vegetative cells; surface smooth or decorated) ..... *Fusarium*

20a. Conidiogenous cells little thicker than hyphae, occurring singly or grouped into regular clusters and/or whorls; conidia one-celled; mycelium highly uniform in diameter 21

21. Conidiogenous cells usually tapering uniformly from base to truncate apex, usually without a swollen base or distinct neck; occurring singly, in pairs or whorled along hyphae or in terminal clusters ..... *Verticillium*

21a. Conidiogenous cells with a swollen to flask-like base and a (usually short) neck often bent out of axis of the conidiogenous cell; conidiogenous cells borne singly, clustered, or in whorls aggregating in loose 'heads' on erect apically branching conidiophores poorly differentiated from vegetative hyphae ..... *Tolypocladium*

22. In aceto-orcein, primary conidia obviously uninucleate and sometimes seen to be bitunicate (with outer wall layer lifting partially off of spores in liquid mounts) 23

22a. In aceto-orcein, primary conidia obviously multinucleate or nuclei not readily seen 26

23. Conidia long clavate to obviously elongated (length/width ratio usually ≥2.5), papilla broadly conical, often

with a slight flaring or ridge at junction with basal papilla 24

23a. Conidia ovoid to clavate; papilla rounded and frequently laterally displaced from axis of conidium ... 25

24. Conidia readily forming elongate secondary capilliconidia attached laterally to and passively dispersed from capillary conidiophores; rhizoids and cystidia not thicker than hyphae; rhizoids numerous, often fasciculate or in columns ..... *Zoophthora*

24a. Conidia never forming secondary capilliconidia; conidia often strongly curved and/or markedly elongated; rhizoids and/or cystidia 2–3× thicker than hyphae; especially on dipterans (or other insects) in wet habitats (on wetted rocks, in or near streams, etc.) *Erynia*

25. Conidia never producing secondary capilliconidia; rhizoids 2–3× thicker than hyphae, terminating with prominent discoid holdfast; cystidia at base 2–3× thicker than hyphae, tapering towards apex ..... *Pandora*

25a. Conidia never producing secondary capilliconidia; rhizoids not thicker than hyphae, numerous, solitary to fasciculate, with weak terminal branching system or sucker-like holdfasts; cystidia as thick as hyphae, often only weakly tapered ..... *Furia*

26. In aceto-orcein, nuclei staining readily, with obviously granular contents 27

26a. In aceto-orcein, nuclei not readily visible or not staining 29

27. Conidia with apical point and broad flat papilla; discharged by cannon-like expulsion of fluid from conidiogenous cell forming halo-like zone around conidia after discharge ..... *Entomophthora*

27a. Conidia without apical projection and discharged by eversion of a rounded (not flat) papilla 28

28. Conidia pyriform with papilla merging smoothly into spore outline; formed by direct expansion of tip of conidiogenous cell (with no narrower connection between conidiogenous cell and conidium); rhizoids never formed ..... *Entomophaga*

28a. Conidia globose with papilla emerging abruptly from spore outline; formed on conidiogenous cells with a narrowed neck below the conidium; if present, rhizoids 2–3× thicker than hyphae, with discoid terminal holdfast ..... *Batkoa*

29. Conidia globose to pyriform, papilla rounded, with many (inconspicuous) nuclei; secondary conidia: (a) single, forcibly discharged and resembling primaries; (b) single, passively dispersed capilliconidia formed in axis of capillary conidiophore or (c) numerous on a primary conidium, small, forcibly discharged (microconidia) ..... *Conidiobolus*

29a. Conidia globose to pyriform, papilla flattened, usually 4–nucleate; secondary conidia (a) forcibly discharged, resembling primary or (b) almond- to drop-shaped, laterally attached to a capillary conidiophore with a sharp subapical bend; especially on aphids or mites .... *Neozygites*

30. Affecting larval bees (Apidae and Megachilidae), causing chalkbrood; fungus in cadavers is white or black, organized as large spheres (spore cysts) containing smaller-walled spherical groups (asci) of (asco)spores ..... *Ascosphaera*

30a. Affecting insects other than bees; spores formed individually rather than in spherical groups of inside larger spheres 31

31. Spores formed *inside* a fungal cell, in a more or less loosely fitted outer (sporangial) wall 32

31a. Spores forming directly at apices of hyphae or hyphal bodies by budding

or intercalary (thick-walled but not confined loosely inside remnant of another cell) 33

32. Spores (oospores) thick-walled, smooth walled, colourless; formed inside irregularly shaped cell (oogonia); some cells in thick mycelium producing narrow tube through cuticle with evanescent terminal vesicle from which motile, biflagellate zoospores are released; affecting mosquitoes ..... *Lagenidium*

32a. Spores (resistant sporangia) globose or subglobose, golden-brown with hexagonally reticulated surface; formed inside close fitting thin (but evanescent) outer wall ..... *Myiophagus*

33. Affecting gregarious cicadas (Homoptera: Cicadidae); terminal segments of abdominal exoskeleton drop off to expose loose to compact, colourless to coloured fungal mass; spores thin-walled or, if thick-walled, with strongly sculptured surface *Massospora*

33a. Not affecting cicadas, with spores occurring throughout body (not confined to terminal abdominal segments) 34

34. Spores (zygospores or azygospores) with outer surfaces smooth or with surface irregularly roughened, warted, or spinose; colourless to pale or deeply coloured (various colours possible), brown, grey, or black 35

34a. Spores (thick-walled resistant sporangia) with surface regularly decorated with ridges, pits, punctations, striations, reticulations; yellow–brown to golden-brown 37

35. Resting spores grey, brown or black (outer wall is coloured; inner wall is hyaline), with smooth or rough surface; binucleate but nuclei often not staining strongly in aceto-orcein if spore wall is cracked; infected hosts from which conidia were discharged and then produced almond- to drop-shaped secondary capilliconidia should be evident in the infected population; affecting aphids, scales, or mites ..... *Neozygites*

35a. Resting spores colourless, coloured, or dark, surfaces smooth or rough; infected host population may or may not include cadavers producing conidia but, if present, conidia not as above 36

36. When spores are gently crushed in aceto-orcein (to crack walls and partially extrude cytoplasm), nuclei are poorly stained (or unstained) and, if seen, do not have obviously granular contents (Ancylistaceae) *Conidiobolus*

36a. When spores are gently crushed in aceto-orcein (to crack walls and partially extrude cytoplasm), nuclei stain well and have obviously granular contents ..... *Entomophthoraceae* (genus undetermined)

37. Sporangia ellipsoid (not globose), with a preformed dehiscence slit (may not be obvious); wall very thick, golden-brown, pitted to elaborately sculptured; affecting larvae/pupae of mosquitoes (or midges) ..... *Coelomomyces*

37a. Sporangia globose or subglobose, with no visible dehiscence slit; wall relatively thin; surface with low (hexagonally) reticulated ridges; affecting terrestrial insects ..... *Myiophagus*

4 DIAGNOSES AND CRITICAL CHARACTERS OF MAJOR ENTOMOPATHOGENS

This section is organized by fungal classes, starting with the conidial fungi that are the most commonly encountered fungal entomopathogens and moving through the ascomycetes and basidiomycetes, zygomycetes, oomycetes and chytridiomycetes that

are progressively less common and may have narrower host ranges. Generic treatments include a brief diagnosis, and lists of major (but not all) diagnostic characters, characterizations of some common and important species, references to taxonomic literature useful for species identification, and, in some instances, further comments.

Labels on the figures correspond to the lettered diagnostic characters of the genera and species.

A Deuteromycota: Hyphomycetes

These conidial fungi produce their spores on exposed hyphae rather than in some sort of closed fruiting structure; *Aschersonia* is the only major entomopathogenic genus *seeming* to be an exception to this generalization. Even on the relatively uncommon occasions when conidial fungi (anamorphs) occur together with their sexual states (teleomorphs), both morphs have different scientific names. The hyphae of Hyphomycetes and their teleomorphs are frequently septate. Most entomopathogenic Hyphomycetes grow readily on many common cutlure media; surprisingly few of these fungi are difficult to grow *in vitro* or have specialized nutritional requirements.

Species in nearly every genus of entomopathogenic Hyphomycetes are distinguished by the morphologies of their conidia and conidiogenous cells and by the identity of their hosts. Other distinctive characters used in these genera are specifically noted in the generic treatments.

Important general reference works for identifying many more genera of Hyphomycetes than treated here are Carmichael *et al.* (1980), Samson (1981) and Samson *et al.* (1988).

1. Aschersonia *Montagne and* Hypocrella *Saccardo (Figure 3)*

Conidial state: *Aschersonia*, with stroma hemispherical or cushion-shaped (sometimes indistinct), superficial, usually light to brightly coloured (yellow, orange, red, etc.), covering host insect, with one or more conidia-forming zones (locules) sunken into stroma and opening by wide pore or irregular crack; conidia hyaline, one-celled, spindle-shaped, extruded onto stromatic surface from locules in slime masses. **Sexual state:** *Hypocrella*, with perithecia (walled structures containing asci and ascospores) globose to pyriform, immersed in stroma with opening protruding from stroma; asci cylindrical, with prominent hemispherical apical thickening penetrated by a narrow canal; ascospores filiform, with numerous transverse septa, dissociating at maturity to produce numerous cylindrical part-spores but sometimes remaining intact. **Hosts:** coccids and aleyrodids.

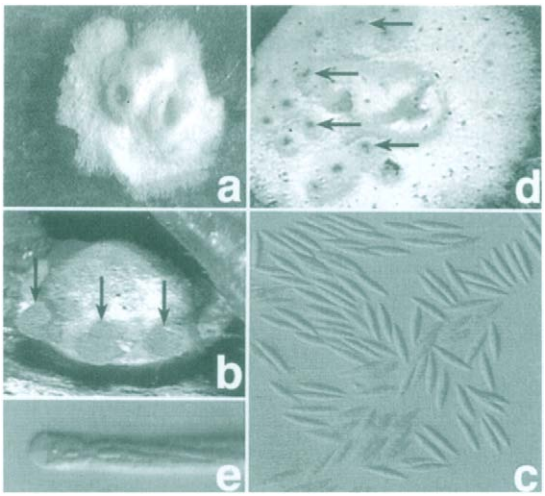

Figure 3 *Aschersonia* (a–c) and *Hypocrella* (d–e). (a) Stroma with three depressed conidiogenous areas. (b) Slimy masses of spores (arrows) on stromatic surface. (c) Conidia. (d) Stroma bearing *Hypocrella* perithecia (arrows indicate perithecial ostioles) and two conidiogenous zones of the *Aschersonia* state. (e) Thickened apex of ascus.

a. Key diagnostic characters

(a) Stroma: presence, size, cross-sectional profile, colour. **(b)** (*Aschersonia*) Conidiogenous locules: sunken in stroma, arrangement on stroma, release of conidia in slime. **(c)** (*Aschersonia*) Conidia elongated, fusoid, aseptate. **(d)** (*Hypocrella*) Perithecia: embedded in stroma. **(e)** (*Hypocrella*) Asci: long, with apical thickening penetrated by a narrow channel.

b. Major species

Aschersonia aleyrodis Webber – stromata ca. 2 mm diam. × 2 mm high, orange to pink or cream-coloured, surrounded by thin halo of hyphae spreading on leaf surface. Conidia bright orange in mass, 9–12 × 2 μm.

c. Main taxonomic literature

Petch (1914, 1921); Mains (1959a,b).

d. General comments

Aschersonia species are the conidial states of the less frequently found *Hypocrella* states; both genera are widespread in the tropics and subtropics. *Hypocrella* perithecia are immersed in the surface of stromata on which *Aschersonia* state may also occur. The taxon-

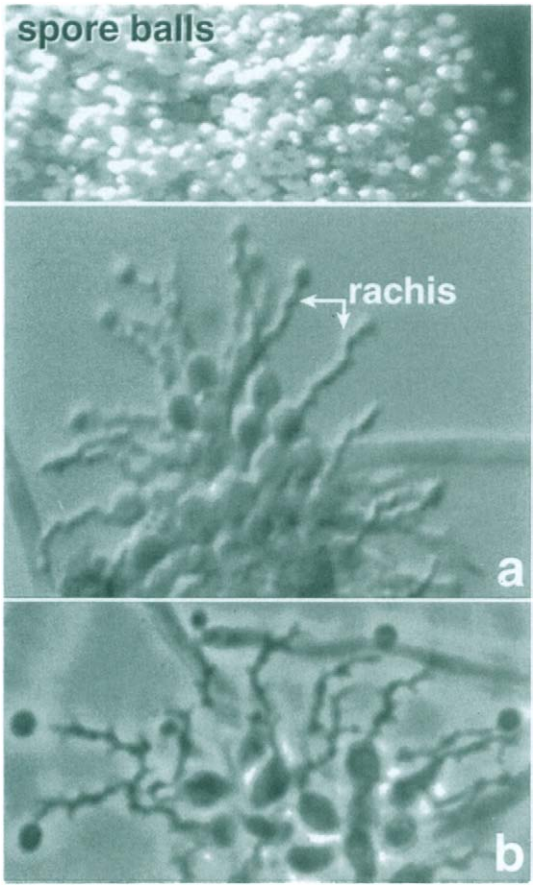

Figure 4 *Beauveria bassiana*. (Upper) Spore balls representing dense clusters of large numbers of conidiogenous cells and conidia. (a,b) Conidiogenous cells with globose bases and extended, denticulate raches.

omy for both genera is that of Petch (1914, 1921) but needs thorough revision.

2. Beauveria *Vuillemin (Figure 4)*

Forming a dense white covering on host exoskeleton, occasionally synnematous (forming erect fascicles of hyphae); conidiogenous cells usually densely clustered (or whorled or solitary), colourless, with globose or flask-like base and denticulate (toothed) apical extension (rachis) bearing one conidium per denticle; conidia aseptate. **Sexual state:** *Cordyceps* (for *B. brongniartii*; Shimazu *et al.*, 1988). **Hosts:** extremely numerous and diverse.

a. Key diagnostic characters

(a) Conidiogenous cells: extending apically into sympodial rachis. **(b)** Rachis: denticulate, with one conidium per denticle. (Note: rachis ***must*** be denticulate to be identified as *Beauveria*). **(c)** Conidia: size, shape, and surface characteristics.

b. Major species

B. bassiana (Balsamo) Vuillemin: conidia nearly globose, ≤3.5 μm diam. *B. brongniartii* (Saccardo) Petch: conidia long ovoid to cylindrical, 2.5–4.5 (6) μm long; mostly on Scarabaeidae (Coleoptera). *B. amorpha* Samson & Evans: conidia short cylindrical, flattened on one side or curved, 3.5–5 × 1.5–2.0 μm.

c. Main taxonomic literature

Hoog (1972); Samson & Evans (1982).

3. Fusarium *Link (Figure 5)*

Fruiting body (if present) a stromatic pad (sporodochium) with conidial hymenium on its surface, pale tan to yellow to orange or red. Conidiophores solitary or aggregated, simple or branched, bearing apical conidiogenous cells. Conidiogenous cells (phialides) short, cylindrical to much elongated, awl-like; forming one or two conidial types: *macroconidia* curved to canoe-shaped with sometimes prominent foot-like appendage on basal cell, with one or more transverse septa, usually released in slime heads or spore masses, and/or *microconidia* aseptate, small, ovoid to cylindrical, produced in slime or dry and in chains from elongate,

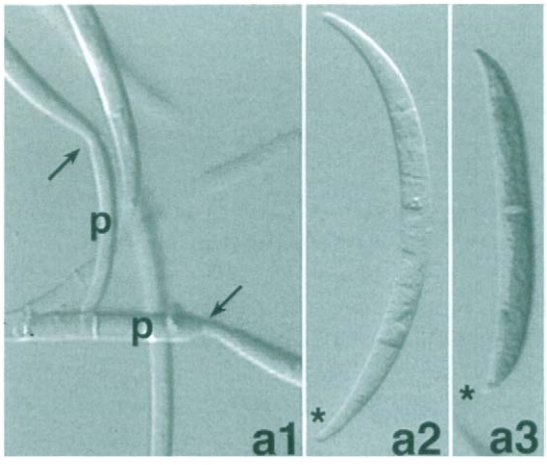

Figure 5 *Fusarium coccophilum*. (a1) Phialides (p; conidiogenous cells) bearing developing macroconidia; arrows indicate the slight bend ('foot') at the base of a new conidium. (a2, a3) Macroconidia with transverse septa and poorly differentiated feet (*).

awl-shaped conidiogenous cells. **Sexual state:** *Nectria.* **Hosts:** scales and many other insects.

a. Key diagnostic characters

(a) Macroconidia are the most important diagnostic character for the genus. These spores are highly variable in morphology, but usually are elongated, more or less curved, frequently described as boat- or canoe-shaped, and include one to several transverse septa. **(b)** Macroconidia: morphology, number of septa, colour. **(c)** Colours of mycelium and/or exudations into culture media.

b. Main taxonomic literature

Booth (1971); Nelson *et al.* (1983).

c. General comments

Few of the many *Fusarium* species seem to be entomopathogens (Booth, 1971; Hajek *et al.*, 1993). Specialists in *Fusarium* taxonomy disagree strongly about how to define or identify taxa, and no authoritative revision of *Fusarium* taxonomy synthesizing molecular and more traditional characters is available. Seifert (1995) provides an illustrated key to *Fusarium* species (FusKey: *Fusarium* Interactive Key) on the World Wide Web at <http://res.agr.ca/brd/fusarium/>.

4. Gibellula *Cavara (Figure 6)*

Synnemata (if present) white, yellowish, greyish to distinctly violet when fresh; becoming brownish with age, with conidial heads in a compact hymenium or more or less isolated on synnema; conidiophores septate, rough-walled, with a small apical swelling (vesicle); conidiogenous cells (phialides) clustered on short swollen cells on vesicle, without obvious necks, with apical wall becoming progressively thickened; conidia aseptate, smooth, single or in short chains. **Sexual state:** *Torrubiella.* **Hosts:** spiders.

a. Key diagnostic characters

(a) Synnemata: presence, colour, arrangement of conidiophores on synnema. **(b)** Wall texture on hyphae or conidiophores. **(c)** Conidiophores: size, surface texture, presence/absence of narrow isthmus below terminal vesicle.

b. Major species

G. pulchra (Saccardo) Cavara: conidiophores long, projecting from surface of white, lilac, yellow or orange synnemata. *G. leiopus* (Vuillemin) Mains: conidiophores short, crowded, forming dense fertile areas on lilac to purple synnemata.

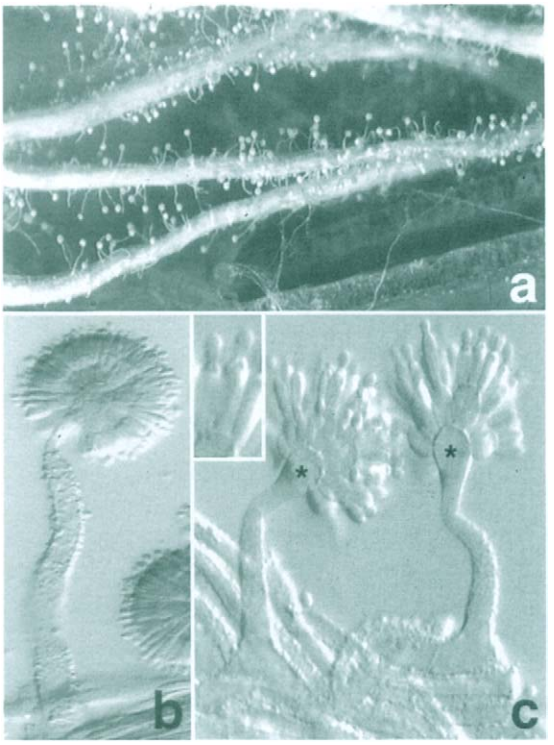

Figure 6 *Gibellula pulchra.* (a) Synnemata with laterally projecting conidiophores. (b) Rough-surfaced conidiophore narrowing apically to a vesicle bearing densely clustered conidiogenous cells. (c) Apical vesicles (*) of conidiophores bearing layer of 'metulae' and clusters of conidiogenous cells (phialides) whose apices thicken progressively (inset) with each conidium formed.

c. Main taxonomic literature

Samson & Evans (1992).

d. General comments

The apical vesicles of *Gibellula* conidiophores resemble those of *Aspergillus* but *Aspergillus* has (usually) globose conidia borne on phialides with a short neck. *Pseudogibellula formicarum* (Mains) Samson & Evans closely resembles *Gibellula pulchra* but is an insect rather than spider pathogen and has conidiogenous cells whose apices multiply denticulate (Samson & Evans, 1973). A second conidial state of *Gibellula/Torrubiella* species, *Granulomanus* sp. (Hoog, 1978), forms elongated

cylindrical conidia on polyphialidic conidiogenous cells.

5. Hirsutella *Patouillard (Figure 7)*

Synnemata (if present) erect, often prominent, thin, compact, hard or leathery; conidiogenous cells (phialides) scattered to crowded, projecting laterally from synnema or from hyphae on host body, swollen basally and narrowing into one or more slender necks; conidia aseptate (rarely 2-celled), hyaline, round, rhombic, elongate or like segments of citrus fruit, covered by persistent mucus, borne singly or 2 or more in droplets of mucus. **Sexual state:** *Cordyceps* or *Torrubiella*. **Hosts:** many diverse insects (one species affecting nematodes).

a. Key diagnostic characters

(a) Conidiogenous cell (generic character): with swollen to flask-like base and one or more elongated, narrow necks. **(b)** Conidia: borne singly or in small groups, covered by persistent slime (slime absent from some species). **(c)** Synnemata: present in most species but some species not forming synnemata. **(d)** Conidiogenous cell (specific characters): shape, size, with a single neck or polyphialidic.

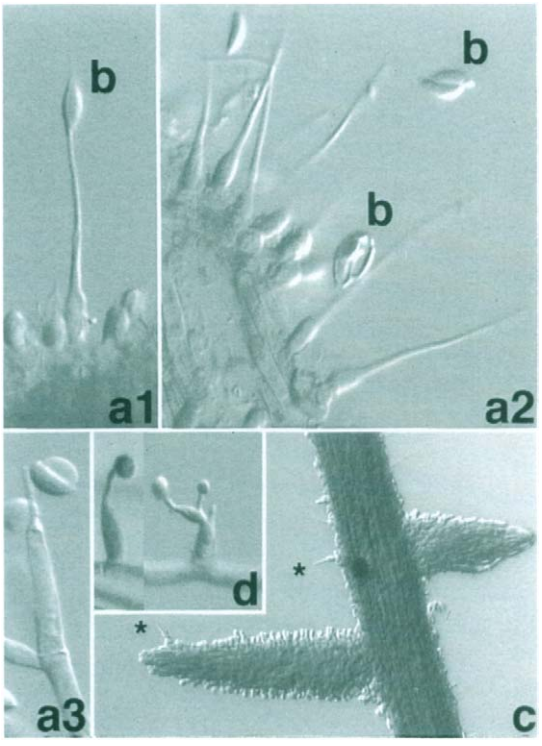

Figure 7 *Hirsutella*. (a1–a3) Conidiogenous cells with swollen bases and narrow, extended necks forming conidia with a slime coating (esp. the conidial clusters, (b) in a2 and the conidium in a3). (c) Synnema with lateral branches and conidiogenous cells (*). (d) Mono- (left) and polyphialidic (right) conidiogenous cells.

b. Major species

H. citriformis Speare: on leaf- and planthoppers (Homoptera: Cercopidae, Delphacidae); synnemata long, numerous, grey or brown, with many short lateral branches. *H. rhossiliensis* Minter & Brady: on nematodes and mites; not forming synnemata; conidiogenous cells with a single short, narrow neck; conidia resemble orange segments (straight on one side, curved on the other) or ellipsoid. *H. thompsonii* Fisher: on mites; conidiogenous cells with a distinctly swollen base and one short, narrow neck or polyphialidic; conidia globose with a smooth or wrinkled surface and no obvious slime layer.

c. Main taxonomic literature

Mains (1951); Minter & Brady (1980); Samson *et al.* (1980); Minter *et al.* (1983); Evans & Samson (1982); Rombach & Roberts (1989).

d. General comments

Despite the importance of this genus, *Hirsutella* has never been monographed, and its literature is dispersed. Identifying *Hirsutella* species can be difficult due to this lack of a monograph and also because the morphologies of *Hirsutella* species intergrade with species of *Verticillium*, *Tolypocladium*, and other genera (Humber & Rombach, 1987).

6. Hymenostilbe *Petch emend. Samson & Evans (Figure 8)*

Synnemata cylindrical or slightly tapered apically, covered by compact layer of conidiogenous cells; conidiogenous cells polyblastic, bearing solitary conidia on short denticles; conidia aseptate, hyaline, smooth or roughened. **Sexual state:** *Cordyceps*. **Hosts:** diverse insects.

a. Key diagnostic characters

(a) Synnemata present. **(b)** Conidiogenous cells form compact hymenium. **(c)** Conidiogenous cells polyblastic. **(d)** Teleomorphic connections with

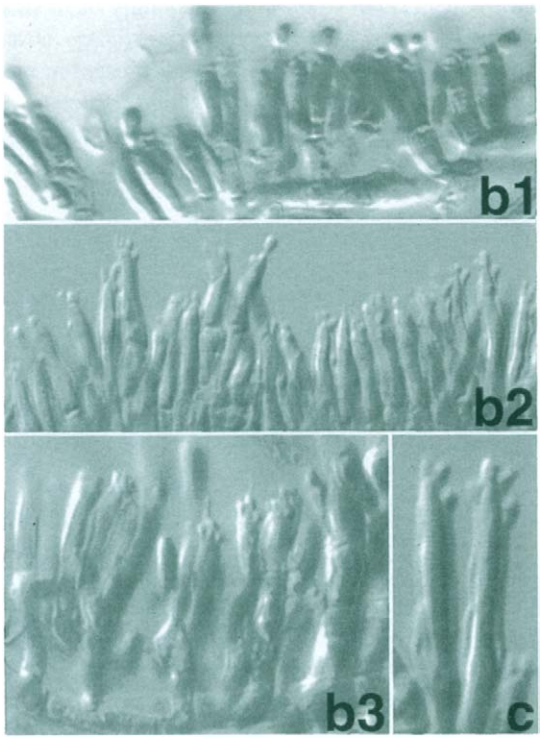

Figure 8 *Hymenostilbe*. (b1–b3) Hyphae bearing lateral conidiogenous cells with denticulate apices. (c) Apical denticles (conidia have been dislodged).

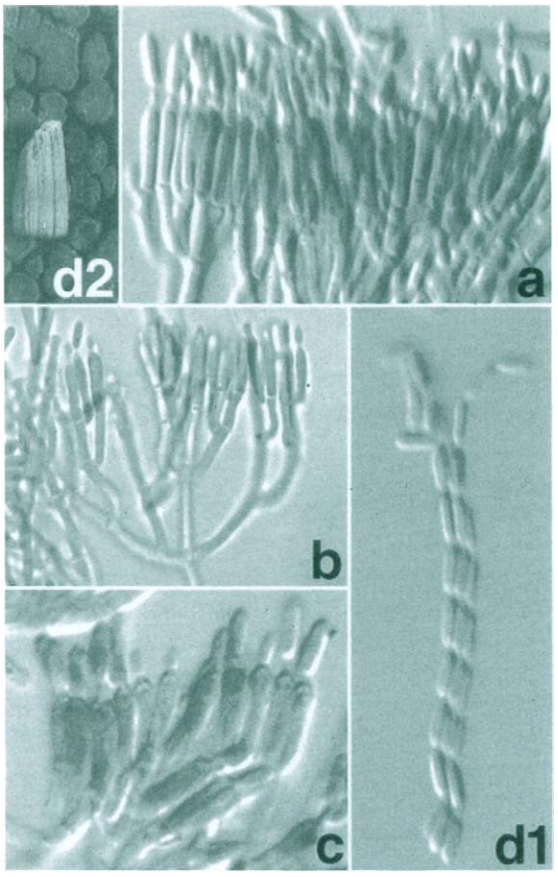

Figure 9 *Metarhizium anisopliae*. (a) Conidiogenous cells (phialides) forming a dense layer (hymenium). (b) Branched conidiophore; note that conidiogenous cells develop in a common plane. (c) Thickened, blunt tips of conidiogenous cells. (d1) Laterally adherent conidial chains. (d2) Lateral and top views of conidial columns.

Cordyceps spp. (which may occur together with *Hymenostilbe* synnemata).

b. Major species
H. dipterigena Petch: on flies; associated with *Cordyceps dipterigena;* synnemata brown, long.

c. Main taxonomic literature
Samson & Evans (1975).

7. Metarhizium *Sorokin (Figure 9)*

Mycelium often wholly covering affected hosts; conidiophores in compact patches; individual conidiophores broadly branched (candelabrum-like), densely intertwined; conidiogenous cells with rounded to conical apices, arranged in dense hymenium; conidia aseptate, cylindrical or ovoid, forming chains usually aggregated into prismatic or cylindrical columns or a solid mass of parallel chains, pale to bright green to yellow–green, olivaceous, sepia or white in mass. **Sexual state:** *Cordyceps* (Liang *et al.*, 1991). **Hosts:** extremely numerous and diverse.

a. Key diagnostic characters
(a) Conidiogenesis occurring in dense hymenia. **(b)** Conidiophores branching repeatedly at broad angles and resembling a candleholder (although observation of individual conidiophores is difficult). **(c)** Conidiogenous cells clavate or cylindrical, with a rounded to conical apex, no obvious neck; apical wall progressively thickening as conidia are produced. **(d)** Conidia produced in long chains; chains often adhere laterally to form prismatic columns or solid plates.

b. Major species

M. anisopliae (Metschnikoff) Sorokin var. *anisopliae*: conidiogenous cell cylindrical; conidia ≥9 μm long, cylindrical and often with a slight central narrowing, forming very long, laterally adherent chains, usually some shade of green.

M. anisopliae (Metsch.) Sorok. var. *majus* (Johnston) Tulloch: morphology as for *M.a.* var. *anisopliae* but conidia ≤11 μm; host usually a scarabaeid. *M. flavoviride* Gams & Rozsypal: conidiogenous cells clavate to broadly ellipsoid; conidia light grey–green in mass, ovoid (not cylindrical), 7–11 μm long; relatively slow to develop. (two varieties; see Rombach *et al.*, 1986.)

c. Main taxonomic literature

Rombach *et al.* (1986, 1987).

8. Nomuraea *Maublanc (Figure 10)*

Mycelium septate, white, with flocculent overgrowth, sparse in culture to dense on insects (often completely covering the host), usually becoming green, or purple–grey to purple as sporulation proceeds; conidiophores single or (rarely) synnematous (if synnematous, with a sterile base and distal fertile zone), erect, bearing whorls of short and blocky branches (metulae) with clusters of short phialides on metulae; conidiogenous cells short, with blunt apices and little if any distinct neck; conidia aseptate, smooth, round to ovoid or elongate and slightly curved, in short, divergent chains, pale to dark green, purple–grey to purple, or (rarely) white in mass. **Sexual state:** *Cordyceps* (for *N. atypicola*). **Hosts:** Noctuidae (Lepidoptera) or spiders.

a. Key diagnostic characters

(a) Conidiophores: with conigenous cells in dense, individually distinct whorls. **(b)** Conidiogenous cells: short, blocky, with no distinct neck (but seemingly papillate at apex). **(c)** Conidia: one-celled, in short, divergent chains.

b. Major species

N. rileyi (Farlow) Samson: on Noctuidae (especially larvae); conidial mass light (grey–green), covering host; conidia ovoid, in short chains. *N. atypicola* Yasuda: on spiders; conidial mass lavender-grey to purple.

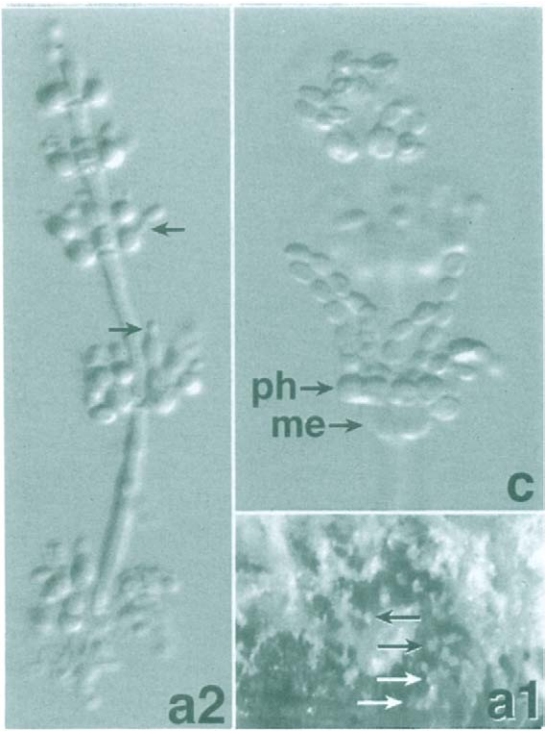

Figure 10 *Nomuraea rileyi.* (a1) Note beaded appearance of conidiogenous whorls (arrows) on conidiophores. (a2) Whorls of blocky conidiogenous cells with newly forming conidia (arrow). (c) Short, divergent chains of conidia produced on phialides (ph) clustered on short sterile cells (metulae; me).

c. Main taxonomic literature

Samson (1974).

9. Paecilomyces *Bainier (Figure 11)*

Conidiophores usually well developed, synnematous in many species, bearing whorls of divergent branches and conidiogenous cells (phialides), colourless to pigmented (but not black, brown or olive); conidiogenous cells with a distinct neck and base flask- to narrowly awl-shaped or nearly globose, borne singly or in groups in whorls on conidiophores, on short side branches or in apical whorls; conidia aseptate, hyaline to coloured, in dry divergent chains. **Sexual state:** *Cordyceps* or *Torrubiella.* **Hosts:** numerous, diverse insects.

a. Key diagnostic characters

(a) Conidiophores often synnematous. **(b)** Conidiogenous cells (phialides) single or whorled, with

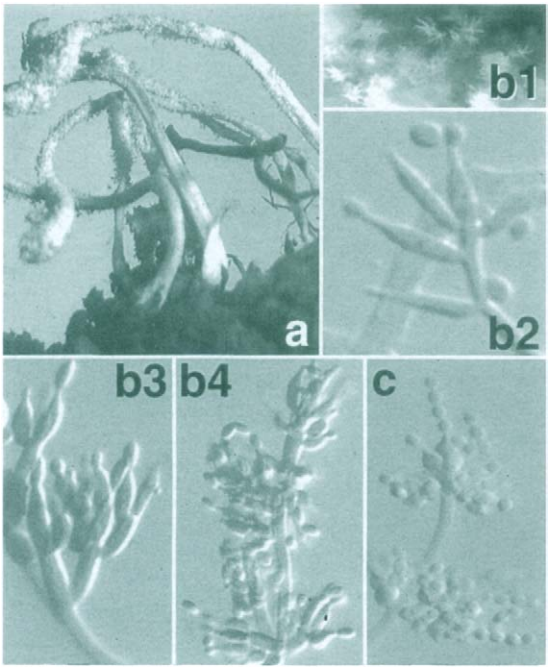

Figure 11 *Paecilomyces*. (a) Synnemata. (b1) Clusters of divergent conidial chains. (b2–b4) Conidiogenous cells (phialides) with swollen bases and prominent necks. (c) Conidial chains elongate from the base as new conidia form. (a, b1, b2, c = *P. farinosus*; b3 = *P. javanicus*; b4 = *P. fumosoroseus*.)

swollen (flask-like, clavate to globose) base and a distinct neck; orientation of phialides gives cluster of spore chains a feathery to cottony appearance. **(c)** Conidia: in long chains, one-celled, ovoid to elongate (rarely globose).

b. Major species

P. farinosus (Holm ex S.F. Gray) Brown & Smith – synnemata often present; conidia short fusoid to lemon-shaped, ≤3 µm long, smooth walled, white to cream-coloured in mass. *P. fumosoroseus* (Wize) Brown & Smith: synnemata usually present; conidiophores and phialides with smooth uncoloured walls; conidia long ovoid, ≤4 µm long, rosy-tan to smoky-pink (or grey) in mass. *P. lilacinus* (Thom) Samson: conidial mass grey to tan with indistinct lavender shading; conidiophores slightly coloured (in comparison to conidia), with roughened walls; conidia ellipsoid to fusoid, 2–3 µm long.

c. Main taxonomic literature

Samson (1974).

d. General comments

There are many entomopathogenic species of *Paecilomyces* but few have been connected to sexual states (which are always *Cordyceps* spp.). Some *Paecilomyces* species are difficult to identify; such common and important species (for biocontrol) as *P. fumosoroseus* and *P. lilacinus* can be difficult to distinguish unless one keeps the morphologies of the conidia and conidiophores firmly in mind. As with many entomopathogens, the morphologies of critical characters may vary depending on whether the observations are made from specimens or cultures.

10. Tolypocladium *W. Gams (Figure 12)*

Conidiophores irregularly branched; conidiogenous cells (phialides) single or clustered, often forming terminal 'heads' with aggregated clusters of conidiogenous cells; conidiogenous cells with globose to flask-like base, narrowing abruptly to distinct neck that often bends away from axis of conidiogenous cell; conidia globose to cylindrical, aseptate, colourless, in slime heads; especially from nematoceran dipteran hosts. **Sexual state:** *Cordyceps* (for *T. inflatum* [=*T. niveum*]; Hodge *et al.*, 1996). **Hosts:** mostly small dipterans.

a. Key diagnostic characters

(a) Conidiogenous cells (phialides): with flask-like to subglobose base, short narrow necks often bent out of the axis of the base; occurring singly or in whorls on vegetative cells. **(b)** Conidia: aseptate, released in slime drops.

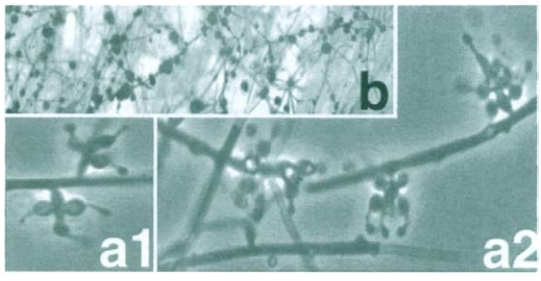

Figure 12 *Tolypocladium inflatum*. (a1,a2) Conidiogenous cells with swollen bases and thin, often bent necks. (b) Mucoid conidial balls formed atop clusters of conidiogenous cells.

b. Major species
T. cylindrosporum W. Gams: conidia cylindrical, straight or slightly curved. *T. extinguens* Samson & Soares: conidia subglobose, short ovoid or slightly curved (bean-shaped).

c. Main taxonomic literature
Bissett (1983); Samson & Soares (1984).

d. General comments
This genus has conidiogenous cells (phialides) that, may resemble those of species of *Verticillium*, *Hirsutella* and other genera forming conidia in slime drops. The shapes of *Tolypocladium* conidia tend to be distinct from those usually seen in *Hirsutella* but strongly resemble those of *Verticillium*; *Tolypocladium* phialides differ from those in *Verticillium* by having a distinct, occasionally bent neck. Except for releasing conidia into slime droplets, *Tolypocladium* phialides could be mistaken for young conidiogenous cells of *Beauveria* that had not yet formed an elongated, denticulate rachis. *Tolypocladium* remains a poorly circumscribed genus despite its use for the commercial production of the antibiotic cyclosporin.

11. Verticillium *Nees per Link (Figure 13)*

Conidiophores little differentiated from vegetative hyphae; conidiogenous cells (phialides) in whorls (verticils) of 2–6, paired, or solitary on hyphae or apically on short side branches; conidia hyaline, aseptate, borne in slime droplets or dry chains. **Sexual state:** *Cordyceps*, *Torrubiella* for entomopathogens. **Hosts:** scales, aphids, other insects or nematodes.

a. Key diagnostic characters
(a) Conidiogenous cells (phialides): occurring in whorls or pairs (or singly); elongated and usually tapering uniformly from the base. **(b)** Conidia: released into slime drops at apices of phialides.

b. Major species
V. lecanii (Zimmermann) Viégas [species complex]: conidia short ovoid to obviously elongate and cylindrical with rounded apices, 2–10 µm long, usually 1–1.7 µm wide. *V. fusisporum* W. Gams: conidia fusoid (spindle-shaped), 4–6 µm long.

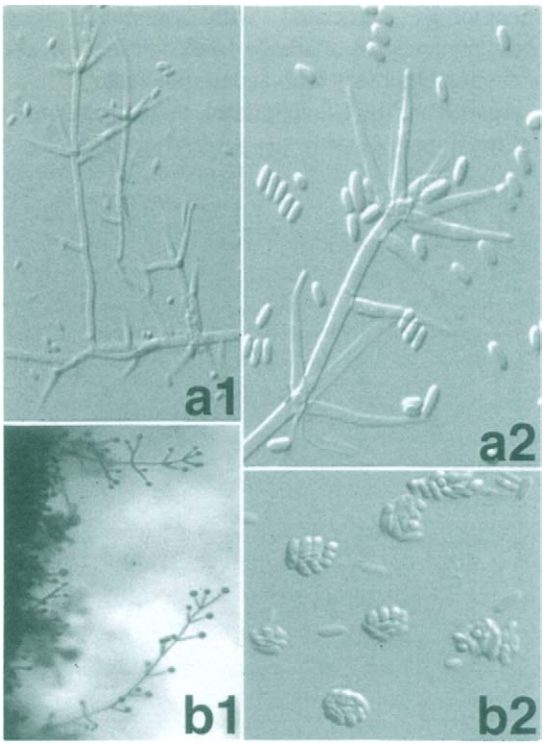

Figure 13 *Verticillium lecanii*. (a1, a2) Tapered conidiogenous cells (phialides) occur singly or in whorls. (b1) Mucoid conidial balls formed apically on individual phialides. (b2) Conidial balls and individual conidia.

c. Main taxonomic literature
Gams (1971, 1988).

B Ascomycota and Basidiomycota

The ascomycetous sexual states (teleomorphs) of the conidial states (anamorphs) discussed above (and, indeed, the great majority of entomopathogenic hyphomycetes in general) belong to a very narrow range of genera in the Clavicipitales (Pyrenomycetes). The Clavicipitales – the order containing *Claviceps purpurea*, which causes ergot in rye and other grasses – is characterized by having long asci with a prominent apical thickening (cap) traversed by a thin channel through which the ascospores are discharged. Clavicipitalean ascospores tend to be thread-like and multiseptate; while still in the asci, however, these ascospores frequently dissociate at the septa to form one-celled 'part spores'. The

teleomorphs associated with entomopathogenic hyphomycetes are relatively rare or difficult to connect unambiguously with anamorphic taxa.

Apart from the Clavicipitales, there are relatively limited examples of insect-associated ascomycetes. A few *Nectria* species (Pyrenomycetes: Hypocreales) with *Fusarium* conidial states affecting mostly scales. *Ascosphaera* spp. are the remarkable causative agents of chalk-brood, a serious disease affecting bee larvae. Entomogenous Loculoascomycetes belong mostly to the genera *Myriangium* and *Podonectria* and are not treated here. The most mystifying and, perhaps diverse entomogenous ascomycetes may be the minute ectoparasites of the order Laboulbeniales (Tavares, 1985).

The Septobasidiales (Teliomycetes), mainly *Septobasidium* spp., comprise the only entomogenous basidiomycetes. These fungi are, effectively, zoophilic rusts whose nourishment derives wholly from partial parasitism of scale insect populations underlying crust-like fungal thalli. The global knowledge of these fungi depends heavily on a classic monograph by Couch (1938). *Septobasidium* is included in the key in Section 3 but is not discussed separately.

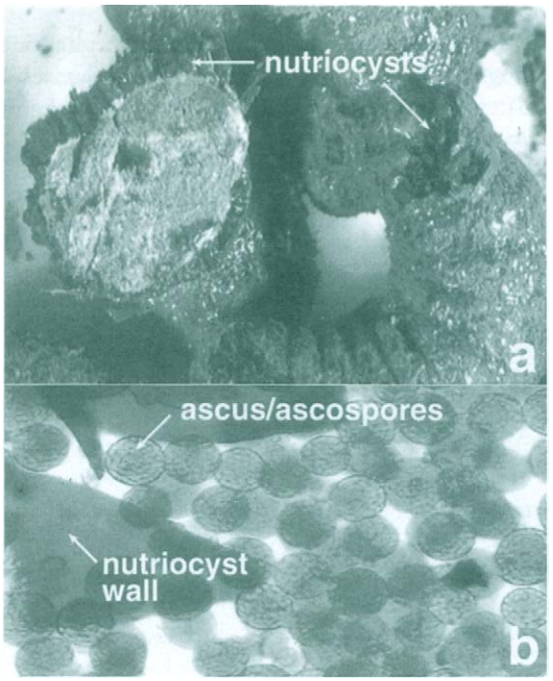

Figure 14 *Ascosphaera aggregata*. (a) Melanized nutriocysts under cuticle of bee larva. (b) Ruptured nutriocyst walls and globose asci containing large numbers of ovoid ascospores.

1. Ascosphaera *Spiltoir & Olive (Figure 14)*

[Ascosphaeromycetes: Ascosphaerales] Affecting larval bees (Hymenoptera: Apoidea); mycelium septate, with sex organs (globose ascogonia and papillate trichogynes) formed on separate mycelia; fertilized ascogonia swell to form large 'nutriocyst' (spore cyst) containing many individual asci; each ascus internally producing numerous ascospores. Appearance of mature infection is a blackened (or white) larva filled with a dense mass of balls (nutriocysts) containing balls (asci) containing ovoid to elongate spores.

a. Key diagnostic characters

(a) Pathogenic to bee larvae. **(b)** Mature infection is notable for the presence of balls (ascospores) within balls (asci) within balls (nutriocysts).

b. Major species

A. aggregata Skou: affecting leaf-cutting bees (Megachilidae) in North America; nutriocysts 140–550 × 100–400 μm; ascospores long ovoid to cylindrical, 4–7 μm long. *A. apis* (Maassen ex Claussen) Olive & Spiltoir: affecting honeybees (*Apis mellifera*) worldwide; nutriocysts 50–120 μm diam.; ascospores short ovoid or allantoid (bean-shaped), 2–3.5 μm long.

c. Main taxonomic literature

Skou (1972, 1988); Rose *et al.* (1984).

2. Cordyceps *Fries (Figure 15)*

[Pyrenomycetes: Clavicipitales] Forming one or more erect stromata on a host, with perithecia confined to an apical (or subapical) fertile portion or with scattered on stromatic surface; perithecia flask-shaped, superficial to fully immersed in stroma; asci elongated, with thickened apical cap penetrated by a fine pore, with eight filiform, multiseptate ascospores which usually fragment to form 1-celled part-spores. **Asexual states:** *Beauveria, Hirsutella, Hymenostilbe, Metarhizium, Nomuraea, Paecilomyces, Verticillium* and other genera. **Hosts:** numerous, diverse insects.

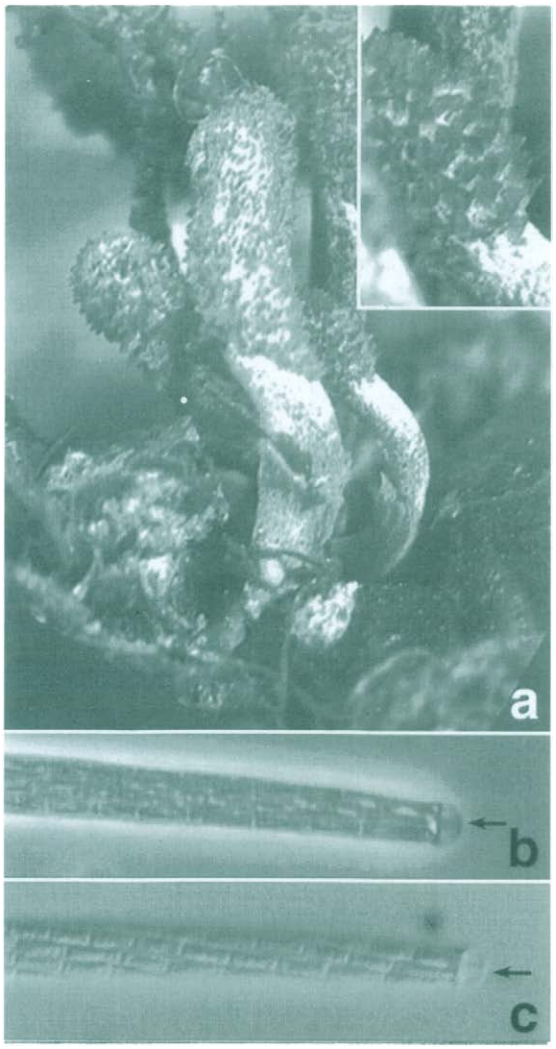

Figure 15 *Cordyceps*. (a) Stromata bearing superficial perithecia (esp. in inset). (b,c) Asci with thickened tips and a distinct central canal (arrows); in (c), threadlike ascospores that, at maturity. dissociate to form part-spores. (a, inset = *Cordyceps* sp. from lepidopteran; b,c = *C. corallomyces*.)

a. Key diagnostic characters

(a) Erect stroma(ta) bearing asci in perithecia. **(b)** Asci: filiform, with a thickened apical cap having a central channel. **(c)** Ascospores: at first 8 filiform spores per ascus with many transverse septa; in most species, dissociating at maturity to yield 1-celled part-spores.

b. Major species

C. lloydii Fawcett: on ants; stromata white to cream-coloured, ≤1 cm high, with discoid apical fertile part; perithecia partially immersed. *C. militaris* Link:Fries: on lepidopterans; stromata single (rarely multiple) per host, <10 cm high, thickly clavate and unbranched, orange, with swollen fertile part at apex. *C. sinensis* (Berkeley) Saccardo: on larval lepidopterans; stromata dark brown to black, <8 cm high, with elongated and little swollen apical fertile portion; used in Chinese herbal medicine. *C. tuberculata* (Lebert) Mains: on lepidopterans; stromata off-white, several per host, with partially immersed, sulphur to bright yellow perithecia scattered toward apices. *C. unilateralis* (Tulasne) Saccardo: on ants; fertile portion a swollen pad borne below apex of stroma; ascospores remain filiform (not dissociating to part-spores).

c. Main taxonomic literature

Kobayasi (1941, 1982); Mains (1958); Kobayasi & Shimizu (1983).

d. General comments

The taxonomy of *Cordyceps* and *Torrubiella* is problematic because so many of the species (ca. 280 in *Cordyceps* and more than 50 in *Torrubiella*) are poorly described, little is known of their anamorphs, and the types for many of these fungi cannot be borrowed from the National Science Museum in Tokyo, the major repository for these types. Kobayasi's (1941, 1982) extensive keys to *Cordyceps* and *Torrubiella* are unusable unless one is already completely familiar with the full spectrum of their species. The easiest way to identify many of these fungi is to compare specimens with the exquisite watercolour illustrations in Kobayasi & Shimizu (1983, whose text is in Japanese) and then to work backwards from a tentative identification using the (English) keys and descriptions in Kobayasi (1941, 1982). Mains (1958) provides a good key to North American *Cordyceps* species.

3. Torrubiella *Boudier (Figure 16)*

[Pyrenomycetes: Clavicipitales] Stroma absent or poorly developed as a light- to brightly-coloured mycelial mat (subiculum) on the host; perithecia elongate, white to yellow to orange or red, superficial to immersed; asci elongated, with thickened apical cap penetrated by a fine pore, with 8 filiform, multiseptate ascospores filiform, multiseptate, fragmenting at maturity to form 1-celled part-spores;

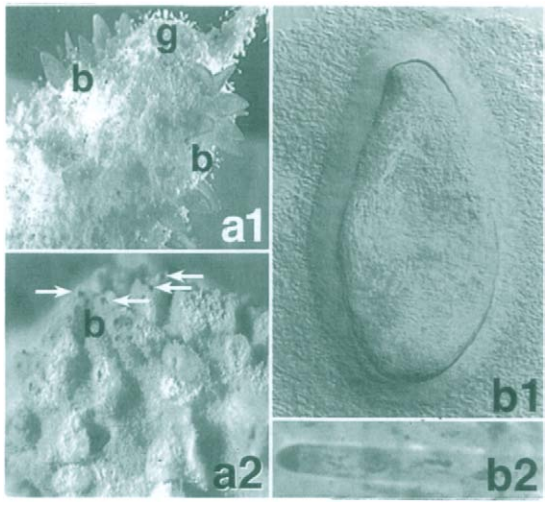

Figure 16 *Torrubiella.* (a1,a2) Perithecia on flocculent (a1) to stromatic (a2) mass covering host; perithecia (b) either superficial (a1) or clad by mycelium (a2); conidiophores of a *Gibellula* anamorph (g) are visible in a1. (b1) Perithecium (from a2) showing thick covering of loose hyphae; interior is filled by a sharply delineated bubble. (b2) Thickened apical cap of developing ascus from (b1).

especially on spiders or scale insects. **Asexual states:** *Gibellula, Granulomanus, Hirsutella, Verticillium* and other genera. **Hosts:** spiders, Homoptera.

a. Key diagnostic characters

(a) Subiculum (mycelium covering host) compact to woolly, *not* organized into distinct stroma (either erect as in *Cordyceps* or covering host). **(b)** Perithecia: immersed to superficial. **(c)** Host: generally spiders (or Homoptera).

b. Major species

None of the more than 50 species and varieties in this genus is especially common. See the general comments above for *Cordyceps*. Most species are found on spiders and are often occur together with an anamorphic (conidial) state; most non-araneous species of *Torrubiella* affect scale insects but are easily distinguished from *Hypocrella* by the absence of a compact, dense stroma.

c. Main taxonomic literature

Kobayasi (1982); Kobayasi & Shimizu (1982, 1983); Humber & Rombach (1987).

C Zygomycota, Entomophthorales

The most significant entomopathogens in the Zygomycota belong in the order Entomophthorales (Roberts & Humber, 1981). These fungi produce thick-walled resting spores (zygospores or azygospores) as their overwintering states but are more often found producing numerous forcibly dispersed conidia that serve as dispersive and infective units. Conidia that do not contact a suitable host upon discharge have a nearly unique ability to produce one or more types of secondary conidia which may also forcibly dispersed.

Outside the Entomophthorales, the only other significant associations with arthropods are found in the Trichomycetes, a diverse group of fungi that are mainly endocommensal in the guts of insects or crustaceans (Lichtwardt, 1986).

Entomopathogenic entomophthoraleans generally develop vegetatively in a host haemocoel by producing small, readily circulated, rod-like to irregularly hyphal bodies rather than thread-like hyphae. In many genera, these vegetative states are naturally wall-less and may be distinctly mobile and ever-changing in shape; many of these cells closely resemble the host's haemocytes. The thick-walled spores of these fungi can be referred to as 'resting spores' although in strict mycological terms they are zygospores or azygospores (terms that denote *only* the presence or absence of gametangial conjugations prior to their formation).

'Rhizoids' and 'cystidia' are terms applied to many different sorts of structures in a wide spectrum of cryptogamic organisms but whose meanings for the Entomophthorales are noted in the brief glossary (184*f*). Cystidia but not rhizoids may be produced *in vitro*. Rhizoids and cystidia are taxonomically significant structures for several of the genera discussed below.

Despite the widely varying taxonomies used in them, the best general works for identifying a broad range of entomopathogenic entomophthoralean species include MacLeod & Müller-Kögler (1970, 1973), Waterhouse & Brady (1982), Keller (1987, 1991) and Balazy (1993). Several approaches to the familial and generic taxonomy of this order have appeared since the 1960s when the use of *Entomophthora* as the sole genus for nearly all the order's entomopathogens came under question. A revised phenetic classification considering only these

entomopathogens (Remaudière & Hennebert, 1980; Remaudière & Keller, 1980) provoked detailed reanalyses of the taxonomic characters of the Entomophthorales (Humber, 1981) and the counter-proposition of a comprehensive, evolutionarily based classification (Ben-Ze'ev & Kenneth, 1982a,b) that, as further refined by Humber (1989), is used below.

1. Batkoa *Humber (Figure 17)*

[Entomophthoraceae] Hyphal bodies elongated, walled (not protoplastic); conidiophores simple with a narrow 'neck' between conidium and conidiogenous cell; primary conidia globose, multinucleate, discharged by papillar eversion; rhizoids (if present) 2–3 times diameter of vegetative hyphae or conidiogenous cells, with prominent terminal discoid holdfast; resting spores bud laterally from parental hypha; unfixed nuclei have granular contents staining in aceto-orcein.

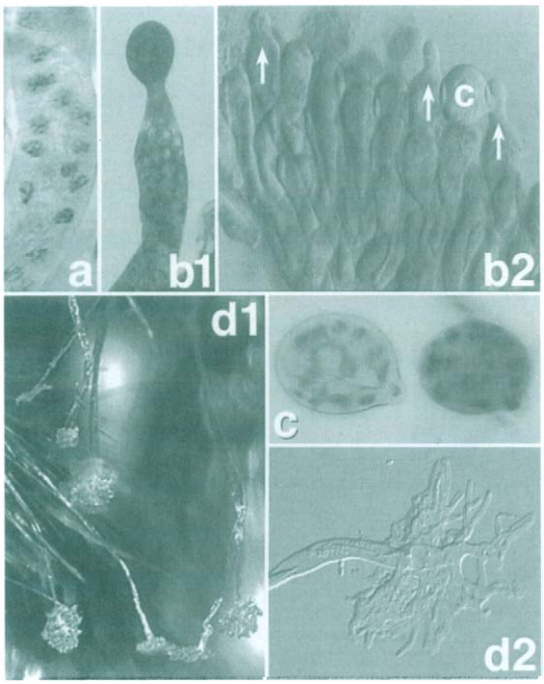

Figure 17 *Batkoa*. (a) Nuclei in hypha stained by aceto-orcein. (b1) Developing conidium with narrow neck between conidiogenous cell and conidium. (b2) Extended tips of conidiogenous cells (arrows) before conidia develop, and a discharged conidium (c). (c) Multinucleate conidia stained in aceto-orcein. (d1,d2) Rhizoids with discoid terminal holdfasts.

a. Key diagnostic characters

(a) Nuclei: contents granular and stain in aceto-orcein. **(b)** Conidiogenous cells: with distinct apical narrowing below conidium. **(c)** Conidia: globose, multinucleate. **(d)** Rhizoids (if present): thick, with discoid terminal holdfast

b. Major species

B. apiculata (Thaxter) Humber: conidia ca. 30–40 μm diam.; papilla often with pointed extension (apiculus); on homopterans and flies. *B. major* (Thaxter) Humber: conidia ca. 40–50 μm diam.; papilla often with pointed extension; on diverse insects.

c. Main taxonomic literature

MacLeod & Müller-Kögler (1973) (as *Entomophthora* spp); Humber (1989); Keller (1987), 1991 (as *Entomophaga* spp.).

2. Conidiobolus *Brefeld (Figure 18)*

[Ancylistaceae] Mycelium initially coenocytic but becoming septate, often forming walled, elongate hyphal bodies; conidiophores unbranched; primary conidia globose to pyriform with rounded apex and prominent papilla, multinucleate, forcibly discharged by papillar eversion; secondary conidia on primary conidia form: (1) singly, resembling primaries, forcibly discharged; (2) multiply, forcibly discharged (microconidia; in subgenus *Delacroixia*) or (3) as cylindrical capilliconidia passively dispersed from capillary conidiophore (in subgenus *Capillidium*); resting spores (zygospores, rarely azygospores) forming in or little displaced from hyphal axis (not budding laterally), conjugations are usually between adjacent cells in a hypha; unfixed nuclei unstained (or poorly stained and without coarsely granular contents) in aceto-orcein.

a. Key diagnostic characters

(a) Nuclei: not staining in aceto-orcein; contents not obviously granular. **(b)** Conidiophores: simple (or rarely, basally bifurcate). **(c)** Conidia: globose to pyriform, multinucleate. **(d)** Resting spores: formed in axis of host hypha, mostly as zygospores. **(e)** Vegetative cells: walled (not protoplastic). **(f)** Subgenera are defined by types of secondary conidia formed (Ben-Ze'ev & Kenneth, 1982a).

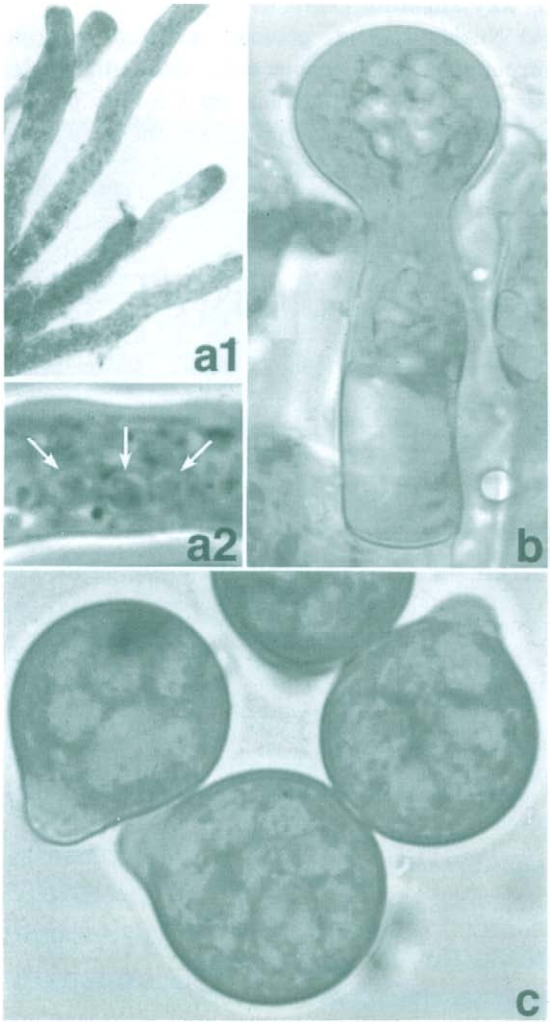

Figure 18 *Conidiobolus*. (a1) Hypha in aceto-orcein showing lack of nuclear differentiation. (a2) Nuclei (arrows) with clear nucleoplasm and dense central nucleolus (living hypha; phase contrast). (b) Conidium developing at apex of conidiogenous cell. (c) Globose conidia with rounded papillae. (a1, a2, b = *C. thromboides*; c = *C. obscurus*.)

b. Major species

C. coronatus (Costantin) Batko: older conidia become covered with villose spines (1–2 up to many μm long); often forming secondary microconidia; a weak pathogen of insects or vertebrates. *[Note: positive identification* requires *presence of villose conidia]. C. obscurus* (Hall & Dunn) Remaudière & Keller: conidia globose, hemispherical papilla emerges abruptly from spore outline, 30–40 μm diam.; nuclei may showing faint (finely granular) peripheral staining in aceto-orcein; no capilliconidia or microconidia formed; especially on aphids. *C. thromboides* Drechsler: conidia mostly pyriform, papilla merges gradually into spore outline, 17–30 μm diam.; no capilliconidia or microconidia formed; especially on aphids.

c. Main taxonomic literature

King (1976, 1977); Keller (1987).

3. Entomophaga *Batko (Figure 19)*

[Entomophthoraceae] Hyphal bodies fusoid to beaded, amoeboid protoplasts, later rod-like to spherical; conidiophores simple; primary conidia pyriform to ovoid, multinucleate, discharged by papillar eversion; rhizoids and cystidia not formed; resting spores bud laterally from parental hypha; unfixed nuclei have granular contents staining in aceto-orcein.

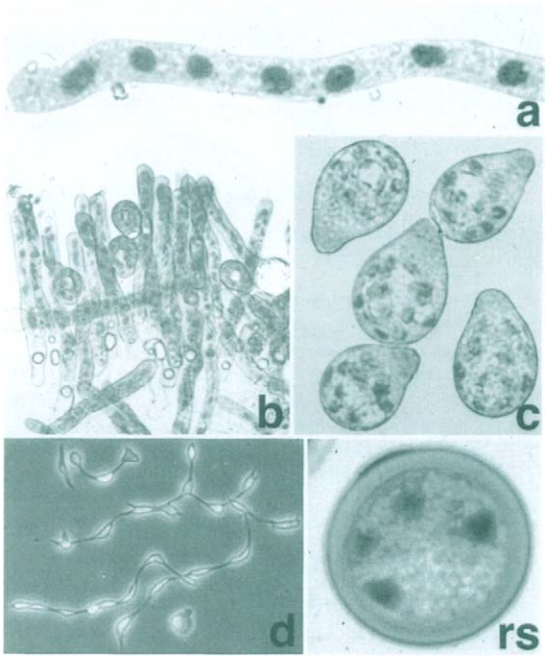

Figure 19 *Entomophaga*. (a) Nuclei stained by aceto-orcein. (b) Unbranched conidiogenous cells and conidia. (c) Pear-shaped, multinucleate conidia. (d) Amoeboid vegetative protoplasts (in culture). (e) Immature, 4-nucleate resting spore; resting spores of many *Entomophaga* species are binucleate when fully mature.

a. Key diagnostic characters
(a) Nuclei: with coarsely granular contents stained in aceto-orcein. **(b)** Conidiogenous cells: no elongated, narrow neck subtending conidium. **(c)** Conidia: pyriform, multinucleate. **(d)** Vegetative growth: protoplastic, bead-like to fusoid or irregularly hyphoid; walled only late in development in host.

b. Major species
E. aulicae (Hoffman in Bail) Batko [species complex]: affecting many lepidopterans; *E. maimaiga* Humber, Soper & Shimazu (Soper *et al.*, 1988) specifically affects gypsy moths, *Lymantria dispar*, in Japan in North America. *E. grylli* (Fresenius) Batko [species complex]: affecting diverse acridids (Orthopteran); *E. calopteni* (Bessey) Humber (Humber, 1989) specifically affects melanopline (spur-throated) grasshoppers and forms resting spores but not primary conidia.

c. Main taxonomic literature
Keller (1987); Soper *et al.* (1988); Balazy (1993).

4. Entomophthora *Fresenius (Figure 20)*

[Entomophthoraceae] Vegetative cells short, rod-like (with or without cell walls); conidiophores simple; conidiogenous cells club-shaped; primary conidia with prominent apical point and broad, flat basal papilla, with 2–12 (to ca. 40) nuclei, forcibly discharged by cannon-like mechanism with discharged conidia attached to substrate in a droplet of discharged cytoplasm; rhizoids (if present) ca. diameter of hyphae, numerous, isolated or fasciculate; resting spores bud laterally from parental hypha; unfixed nuclei have granular contents staining in aceto-orcein.

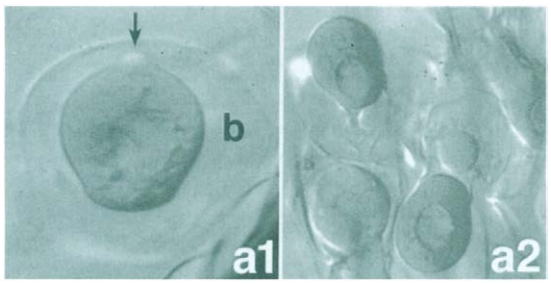

Figure 20 *Entomophthora.* (a1) Discharged primary conidium with apiculus (arrow) and a broad, nearly flat basal papilla, surrounded by a halo-like droplet of cytoplasm (b). (a2) Secondary conidia are broadly obovoid, not prominently apiculate, and form rapidly on discharged primary conidia (ghosts of which are visible below the secondary conidia).

a. Key diagnostic characters
(a) Primary conidia: with a broad, flat papilla and a pointed apical projection; at specific level: conidial size, number and size of nuclei. **(b)** Primary conidial discharge: cannon-like; discharged conidia surrounded by halo-like droplet of cellular material. **(c)** Secondary conidia more broadly clavate, non-apiculate. **(d)** Rhizoids: present or absent; location, number, morphology if present.

b. Major species
E. culicis (Braun) Fresenius: affecting mosquitoes and blackflies; conidia binucleate. *E. muscae* (Cohn) Fresenius [species complex]: affecting muscoid flies; individual species in complex distinguished by size, conidial nuclei (number and size), and hosts affected. *E. planchoniana* Cornu – affecting aphids (especially in Europe).

c. Main taxonomic literature
MacLeod *et al.* (1976); Keller (1987).

5. Erynia *Nowakowski [sensu Humber (1989)] (Figure 21)*

[Entomophthoraceae] Hyphal bodies rod-like to filamentous, walled; conidiophores digitately branched (rarely simple); primary conidia pyriform (clavate) to elongate, curved or straight with rounded to acute apices, uninucleate, basal papilla conical (often with slight flare at junction with spore) or rounded, bitunicate (outer wall layer *may* separate in liquid mounts), discharged by papillar eversion; secondary conidia resemble primaries or more globose, discharged by papillar eversion; rhizoids 2–3× diameter of hyphae, without differentiated terminal holdfast; cystidia columnar (some may branch), 2–3× diameter of hyphae; resting spores bud laterally from parental hypha; unfixed nuclei have granular contents staining in aceto-orcein.

a. Key diagnostic characters
(a) Conidiophores: branching close to conidiogenous cells; it is often difficult to separate individual

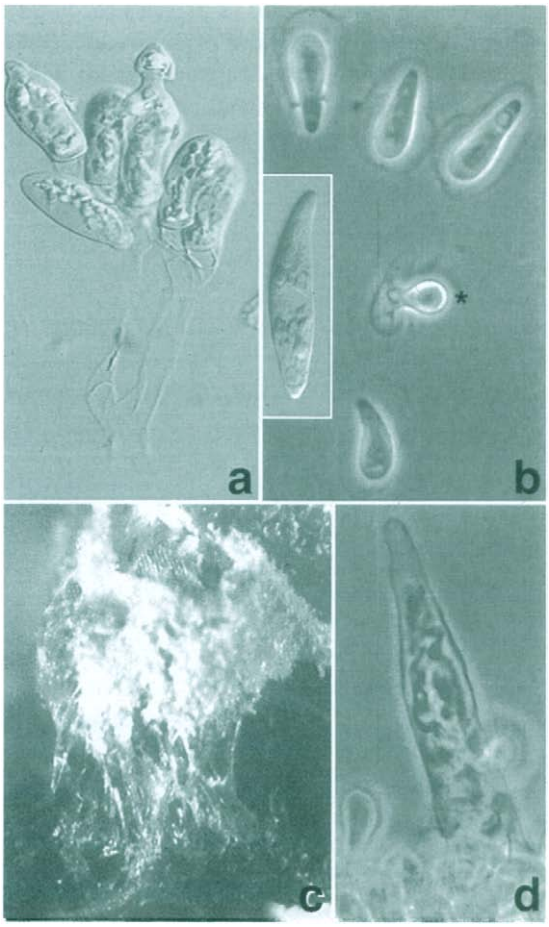

Figure 21 *Erynia*. (a) Branched conidiophore with short, blocky conidiogenous cells. (b) Bitunicate primary conidia with outer wall layer completely separated; nearly globose secondary conidium (*) is forming one spore; inset: conidium with conical papilla. (c) Rhizoids emerging from head of simuliid fly. (d) Cystidium with primary conidium (out of focus at left indicating relative size). (a, b, d = *E. aquatica*; b inset, c = *E. conica*.)

conidiophores. **(b)** Conidia: uninucleate, bitunicate, clavate to elongate, apices rounded or tapered to a blunt point; papillae broadly conical (and flared at junction with conidium) or rounded. **(c)** Rhizoids: 2–3× thicker than hyphae, without discoid terminal holdfast. **(d)** Cystidia: 2–3× thicker than hyphae, not strongly tapered.

b. Major species

E. aquatica (Anderson & Anagnostakis) Humber: on Culicidae and Chironomidae (Diptera), esp. in temporary, cold snow-melt pools; conidia long clavate 30–40 × 15–18 µm. *E. conica* (Nowakowski) Remaudière & Hennebert: on Culicidae and Chironomidae (Diptera); conidia 30–80 × 12–15 µm, often gently curved, tapering to subacute apex. *E. ovispora* (Nowakowski) Remaudière & Hennebert: on nematoceran dipterans; conidia broadly ovoid to ellipsoid, 23–30 × 12–14 µm. *E. rhizospora* (Thaxter) Remaudière & Hennebert: on Trichoptera; conidia lunate to straight, 30–40 × 8–10 µm, broad in middle; tapering strongly to a blunt apex; resting spores wrapped within layer of fine brown hyphae, often on surface of host rather than within.

c. Main taxonomic literature

Keller (1991) (*Erynia* as defined by Remaudiere & Hennebert, 1980); Balazy, 1993 (as *Zoophthora* subgenus *Erynia*).

6. Furia *(Batko) Humber (Figure 22)*

[Entomophthoraceae] Hyphal bodies hypha-like, walled; conidiophores branched (rarely simple); primary conidia clavate to obovoid, uninucleate, basal papilla rounded, bitunicate (outer wall layer *may* separate in liquid mounts), discharged by papillar eversion; rhizoids numerous, may be fasciculate, with diameter of vegetative hyphae or conidiogenous cells, no discoid terminal holdfast; cystidia with diameter of hyphae or conidiogenous cells; resting spores bud laterally from parental hypha; unfixed nuclei have granular contents staining in aceto-orcein.

a. Key diagnostic characters

(a) Conidiophores: branching close to conidiogenous cells. **(b)** Conidia: uninucleate, bitunicate, obovoid to clavate; apices and papillae rounded. **(c)** Rhizoids: as thick as hyphae, with sucker-like attachments or weak terminal branching systems but no discoid terminal holdfast; numerous, single, fasciculate, or in pseudorhizomorphs. **(d)** Cystidia: as thick as hyphae.

b. Major species

F. americana (Thaxter) Humber: conidia obovoid, 28–35 × 14–16 µm; on cyclorrhaphan (muscoid) flies. *F. virescens* (Thaxter) Humber: conidia broadly clavate to obovoid, 20–30 × 9–13 µm; especially on Noctuidae (Lepidoptera).

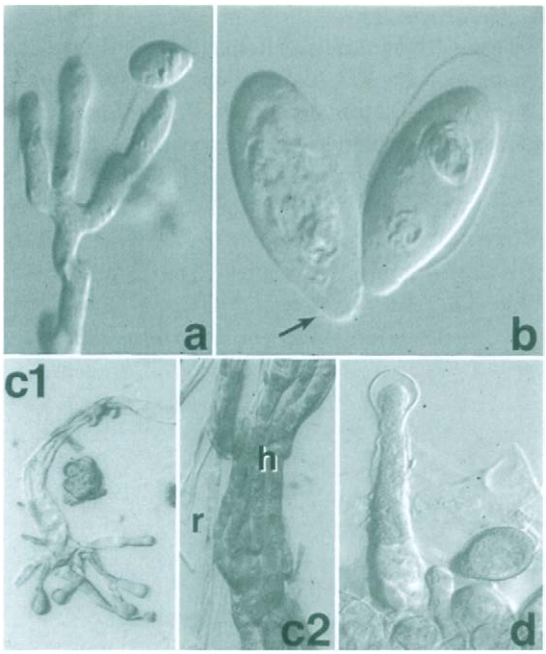

Figure 22 *Furia.* (a) Branched conidiophore; most are more richly branched than this. (b) Bitunicate primary conidia showing line of separation (arrow) between papilla and spore body, with outer wall layer separating (at right). (c1) Rhizoid with sparsely branched apical holdfast. (c2) Comparative diameters of rhizoids (r, devoid of cytoplasm) and hyphae (h). (d) Cystidium projecting from hymenium (note relative size of conidium at right).

c. Main taxonomic literature

Li & Humber (1984); Keller (1991) (as *Erynia* spp.); Balazy (1993) (as *Zoophthora* subgenus *Furia* spp.).

7. Massospora *Peck (Figure 23)*

[Entomophthoraceae] Hyphal bodies spherical to elongate, initially wall-less; development confined to and filling terminal abdominal segments of host; conidiophores lining small cavities in host abdomen; conidia 1–6 (mostly 2) nucleate, passively dispersed upon disarticulation of abdominal exoskeleton of living cicada; resting spores thick-walled, surface deeply reticulate, budded off from parental hyphal bodies; conidia and resting spores not usually formed within same host individual; restricted to emergences of gregarious cicadas (Homoptera: Cicadidae), especially in North and South America; unfixed nuclei have granular contents staining in aceto-orcein.

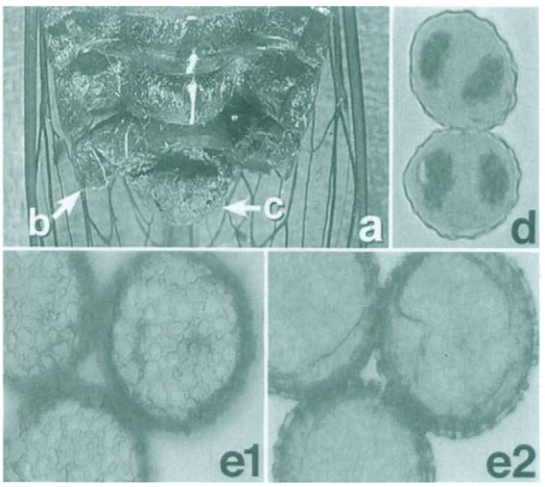

Figure 23 *Massospora.* (a) Abdomen of cicada with terminal segments fallen away (at b) to expose a dense mass of conidia (c) below. (d) Primary conidia of *M. cicadina* have two nuclei and a warted surface. (e1, e2). Surface (e1) and optical section (e2) of *M. cicadina* resting spores showing reticulate, deeply sculptured surface.

a. Key diagnostic characters

(a) Affecting gregarious cicadas (with a high specificity for individual cicada species). **(b)** Fungal growth largely confined to interior of terminal 3–4 abdominal segments. **(c)** Spore dispersal from living cicadas with internal spore mass exposed by disarticulation and sloughing away of terminal abdominal exoskeleton. **(d)** Conidia: size, shape, number and position of nuclei. **(e)** Resting spores: size, morphology of decorated surface.

b. Major species

M. cicadina Peck: affecting 17-year cicada (*Magicicada septemdecim*).

c. Main taxonomic literature

Soper (1974, 1981).

8. Neozygites *Witlaczil (Figure 24)*

[Neozygitaceae] Hyphal bodies irregularly shaped, rod-shaped or spherical, usually 3–5 nucleate; conidiophores simple; primary conidia round, ovoid or broadly fusoid, with relatively flattened basal papilla, mostly 4-nucleate, forcibly discharged a short distance by papillar eversion; secondary conidia usually (more or less almond-shaped) capilliconidia

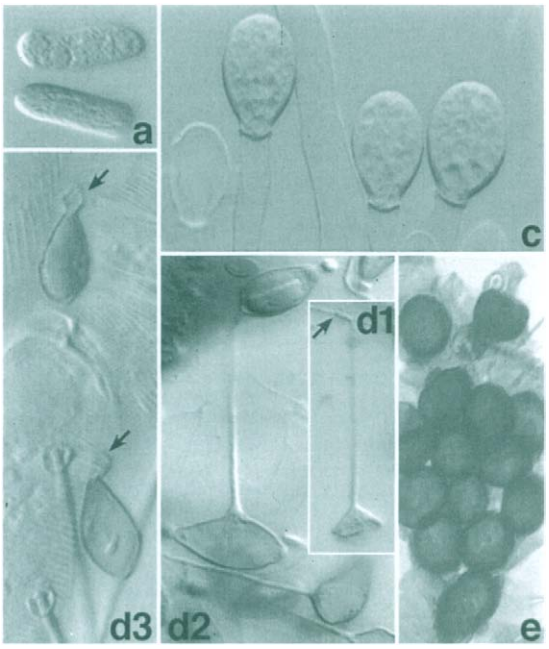

Figure 24 *Neozygites* (from mites). (a) Rod-like hyphal bodies. (c) Pyriform/ovoid conidia; note the nearly flat papilla. (d1–d3). Secondary capilliconidia produced atop capillary conidiophores (d1, arrow notes usual apical bend of conidiophore; d2). (d3) Capilliconidia attached to mite leg by apical slime drop (haptor; arrows). (e) Darkly melanized resting spores.

passively dispersed from capillary conidiophores; resting spores bud laterally from conjugation bridge between gametangia (hyphal bodies), black to smoke-grey, binucleate; nuclei in unfixed material staining poorly in aceto-orcein (except during mitosis); especially on Homoptera, thrips, and mites.

a. Key diagnostic characters

(a) Hyphal bodies: rod-like to spherical, routinely 4-nucleate. **(b)** Nuclei: not staining well in aceto-orcein during interphase, but with vermiform chromosomes and central metaphase plate staining well during mitosis. **(c)** Conidia: mostly 4-nucleate (3–5 overall), with flattened papilla. **(d)** Secondary conidia: like primaries (and forcibly discharged) or passively dispersed, drop- to almond-shaped capilliconidia, on capillary conidiophores, with a mucoid apical droplet (haptor). **(e)** Resting spores: zygospores with conspicuously darkened outer wall layer, arising from conjugation bridge between gametangia, ovoid and smooth or round (and usually roughened), binucleate.

b. Major species

N. fresenii (Nowakowski) Remaudière & Keller: conidia subglobose, 17–20 μm diam.; zygospores ovoid, 25–50 × 15–30 μm; on aphids. *N. floridana* (Weiser & Muma) Remaudière & Keller: conidia 10–14 μm diam.; zygospores subglobose, dark brown, outer wall roughened, 14–25 μm in long dimension; on tetranychid mites. [*Note: N. floridana is the taxon most often identified from tetranychid mites, but descriptions of Neozygites spp. from mites are incomplete and overlap too much to distinguish meaningfully among these species.*] *N. parvispora* (MacLeod, Tyrrell & Carl) Remaudière & Keller: conidia small, 9–15 μm diam.; zygospores black, spherical to flattened, 15–20 μm diam.; especially on thrips.

c. Main taxonomic literature

Keller (1991); Balazy (1993).

9. Pandora *Humber (Figure 25)*

[Entomophthoraceae] Hyphal bodies filamentous, protoplastic or walled; conidiophores digitately branched; primary conidia clavate to obovoid, uninucleate, basal papilla rounded, bitunicate (outer wall layer *may* separate in liquid mounts), discharged by papillar eversion; secondary conidia similar to primary or more nearly globose; rhizoids 2–3× diameter of hyphae or conidiogenous cells, with discoid terminal holdfast; cystidia taper, at base, 2–3× diameter of hyphae or conidiogenous cells; resting spores bud laterally from parental hypha; unfixed nuclei have granular contents staining in aceto-orcein.

a. Key diagnostic characters

(a) Conidiophores: branching digitately. **(b)** Conidia: uninucleate, bitunicate, obovoid to clavate; apices and papillae rounded. **(c)** Rhizoids: 2–3× thicker than hyphae, relatively sparse, with prominent terminal discoid holdfast. **(d)** Cystidia: 2–3× thicker than hyphae at base, tapering toward bluntly pointed apex. **(e)** Vegetative growth: hyphoid, protoplastic or walled in host.

b. Major species

P. blunckii (Bose & Mehta) Batko: on lepidopterans (esp. diamondback moth, *Plutella xylostella*); conidia pyriform, 15–20 × 7–11 μm. *P. delphacis* (Hori) Humber: especially on planthoppers (Homoptera: Delphacidae); conidia broadly clavate, 30–35 ×

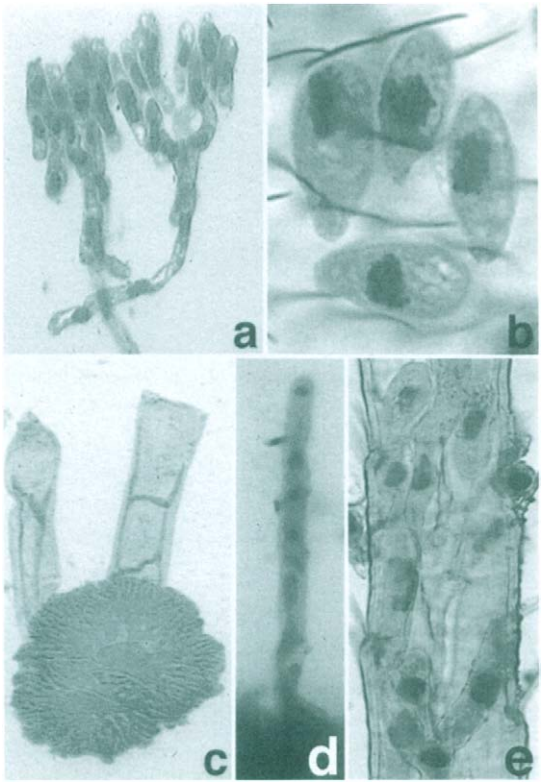

Figure 25 *Pandora.* (a) Branched conidiophores. (b) Uninucleate primary conidia (not showing bitunicate nature). (c) Discoid terminal holdfasts of rhizoids. (d) Cystidium projecting from hymenium, with individual nuclei stained in aceto-orcein. (e) Hyphal bodies with deeply stained nuclei in leg of aphid.

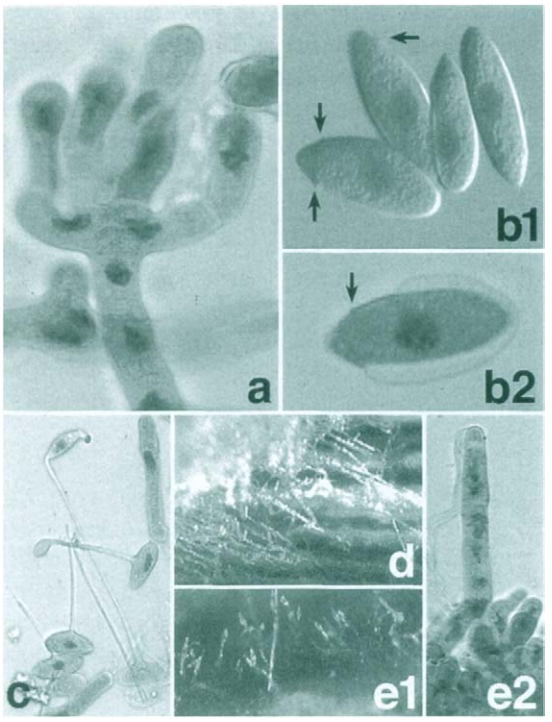

Figure 26 *Zoophthora.* (a) Branched conidiophore with prominently stained nuclei. (b1) Primary conidia showing conidal papilla and slight flaring at junctions (arrows) between papilla and spore body. (b2) Conidium showing flared junction and bitunicate nature. (c) Secondary capilliconidia. (d) Numerous thin rhizoids attaching host to substrate. (e1) Low magnification of rhizoids projecting from hymenium. (e2) Cystidium is about as thick as conidiogenous cells.

12–18 μm; growing and sporulating well *in vitro* on diverse media. *P. neoaphidis* (Remaudière & Hennebert) Humber: on aphids; conidia broadly clavate, 15–40 × 9–16 μm, often with papilla laterally displaced; often difficult to isolate or reluctant to grow and sporulate well *in vitro.*

c. Main taxonomic literature

Humber (1989); Keller (1991) (as *Erynia* spp.); Balazy (1993) (as *Zoophthora* subgenus *Neopandora* spp.).

10. Zoophthora *Batko [sensu Humber (1989)] (Figure 26)*

[Entomophthoraceae] Hyphal bodies rod-like to hyphoid, walled; conidiophores digitately branched (rarely simple); primary conidia clavate to obovoid, uninucleate, basal papilla rounded, bitunicate (outer wall layer *may* separate in liquid mounts), discharged by papillar eversion; secondary conidia (1) resembling primaries or more globose and discharged by papillar eversion or (2) elongate capilliconidia passively dispersed from capillary conidiophore; rhizoids as thick as vegetative hyphae, numerous, individual or fasciculate, discoid terminal holdfast absent; cystidia as thick as hyphae; resting spores bud laterally from parental hypha; unfixed nuclei have granular contents staining in aceto-orcein.

a. Key diagnostic characters

(a) Conidiophores: branching digitately close to conidiogenous cells. **(b)** Conidia: papilla broadly

conical (with slight flare at junction with spore), rarely rounded, elongate (L/D usually 2.5), straight or curved, apex rounded or tapering to point. **(c)** Secondary conidia: frequently forming elongated capilliconidia passively dispersed from capillary conidiophore or forming forcibly discharged conidia generally resembling primaries. **(d)** Rhizoids: as thick as hyphae, holdfasts (if present) weakly differentiated, sparse terminal branching systems, and/or small and sucker-like; usually numerous, single or fasciculate or forming thick pseudorhizomorphs. **(e)** Cystidia: as thick as hyphae, untapered or weakly tapered, toward apex

b. Major species

Z. phalloides Batko: conidia elongated, curved, with bluntly rounded apices; on aphids. *Z. phytonomi* (Arthur) Batko: conidia cylindrical, straight; resting spores colourless (or, possibly, darkened with roughened outer wall); especially affecting *Hypera* spp. (Coleoptera: Curculionidae) on alfalfa. *Z. radicans* (Brefeld) Batko [species complex; see Balazy, 1993]: conidia more or less bullet-shaped, tapering to bluntly pointed apex, 15–30 μm long, with L/D ratio of ca. 2.5–3.5.

c. Main taxonomic literature

Keller (1991); Balazy (1993).

D Watermolds: Chytridiomycetes and Oomycetes

These fungi produce uni- or biflagellate zoospores that are both dispersive and infective units. Flagellate zoospores may be released from two possible sorts of sporangia, those with either thin or thick walls (usually propagative sporangia versus meiosporangia in which meiosis occurs, respectively). It would be unusual to detect hosts infected by these fungi during their vegetative states; these fungi are usually only detected when sporangia have been formed or are releasing zoospores.

1. Coelomomyces *Keilin (Figure 27)*

[Chytridiomycetes: Blastocladiales] Mycelium parasitic in haemocoel of aquatic dipteran larvae, coarse, wall-less in early development, budding off thick-walled resistant meiosporangia with thick deep golden or yellow-brown walls decorated by folds, ridges, warts, pits, etc.; sporangia releasing posteriorly uniflagellate zoospores on germination; zoospores infecting copepods or other aquatic crustaceans; haploid mycelium in crustacean haemocoel is wall-less, cleaving off posteriorly uniflagellate gametes that fuse in pairs; biflagellate zygotes encysting on and infecting dipteran hosts. (See life-cycle diagram and other figures in Chapter V-4.)

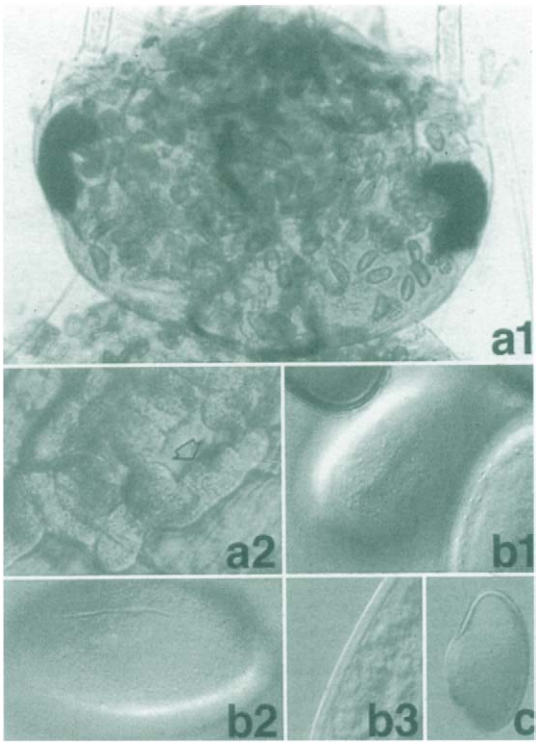

Figure 27 *Coelomomyces.* (a1) Resistant sporangia in mosquito head (photo: D.W. Roberts). (a2) Wall-less hyphal bodies in mosquito haemocoel (from Padua *et al.*, 1986, *J. Invertebr. Pathol.* **48**, 286). (b1–b3) Resistant sporangia with punctate surface texture (in b1), preformed germination slit (b2), and bilayered wall structure (b3). (c) Early stage of resistant sporangium germination with gelatinous plug bulging through the germination slit. (a2–c = *C. stegomyiae.*)

a. Key diagnostic characters

(a) Mosquito (or other aquatic dipteran) larvae becoming filled with golden-brown resistant sporangia; before sporangiogenesis, body filled with wall-less hyphae. **(b)** Resistant sporangia: ovoid, thick-walled, golden-brown, usually with obvious

sculpturing or punctation on surface and a preformed germination slit. **(c)** Zoospores: posteriorly uniflagellate when released from germinating resistant sporangia or (as gametes) from haploid thalli in copepod or cladocerans. Note: Gametes fuse in pairs; biflagellate zygote swims to and encysts on dipteran host.

b. Major species

C. indicus Iyengar: resistant sporangia 25–65 × 30–40 μm, with longitudinal ridges frequently anastomosing; affecting *Anopheles* spp. in Africa, India, Australasia and Philippines. *C. dodgei* Couch & Dodge: resistant sporangia 37–60 × 27–42 μm with longitudinal slits (striae) 3–4 μm apart (giving banded appearance to sporangia); affecting *Anopheles* spp. in North America. *C. psorophorae* Couch: resistant sporangia 55–165 × 40–80 μm, surface with closely spaced punctation (seen in optical section as vertical channels through wall 2–10 μm thick); affecting aedine and culicine mosquito larvae in the Northern Hemisphere.

c. Main taxonomic literature

Bland *et al.* (1981); Couch & Bland (1985).

2. Myiophagus Thaxter (Figure 28)

[Chytridiomycetes: Blastocladiales] Monotypic genus. Vegetative thallus endozoic, coenocytic, branched with frequent constrictions becoming devoid of cytoplasm during formation of sporangia, and dissociating to produce free sporangia at maturity; sporangia globose (oval, ellipsoidal, or fusiform), with slightly thickened wall, forming 1–5 exit papillae; zoospores posteriorly uniflagellate, uninucleate, oval to elongated (4–5 × 7–7.5 μm), with yellow to orange granules in anterior; resting sporangia mostly spherical, with golden-coloured outer wall decorated with polygonal reticulation, cracking open upon germination to extrude globose sporangium and releasing zoospores; affecting scales, weevils and lepidopterans (*not* known from mosquitoes or insects with aquatic stages).

a. Key diagnostic characters

(a) Resistant sporangia: globose, thick-walled, golden-brown, with prominent hexagonal reticulation of surface. **(b)** Zoospores (*rarely* observed): posteriorly uniflagellate.

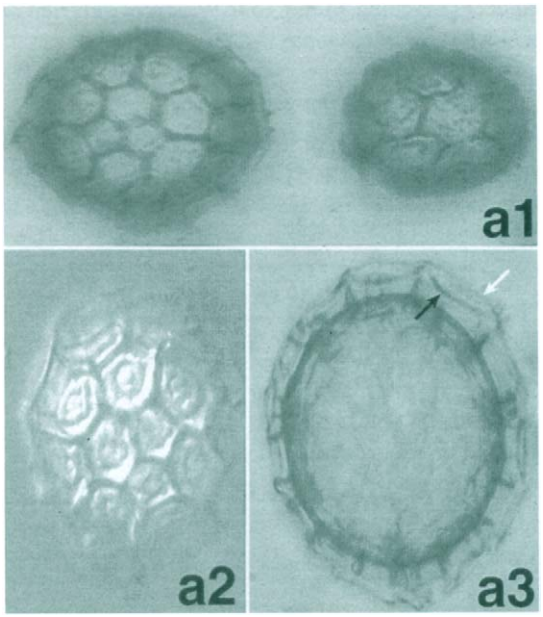

Figure 28 *Myiophagus ucrainicus.* (a1–a3) Reticulate surface of resistant sporangia. (a3) The reticulate wall is distinctly bilayered; the outer (white arrow) is the parental sporangium and the inner (black arrow) is the spore.

b. Only species

M. ucrainicus (Wize) Sparrow: resistant sporangia golden-brown with hexagonally reticulated surface, 20–30 μm diameter; zoospores (rarely observed) posteriorly uniflagellate, released on germination of resistant sporangia or from globose thin-walled sporangia.

c. Main taxonomic literature

Sparrow (1939); Karling (1948).

3. Lagenidium *Schenk (See figures in Chapter V-4)*

[Oomycetes: Lagenidiales] Mycelium parasitic in haemocoel of mosquito larvae, coarse and thick, coenocytic at first, becoming septate and forming oval to spherical segments that serve as zoosporangia or sex organs; partially differentiated contents of zoosporangia extruding through thin evacuation tube (7–10 × 50–300 μm) to thin-walled vesicle, formed outside host body; zoospores laterally biflagellate and reniform, cleaving in vesicle and dispersing on dissolution of vesicle wall; oospores (zygotes) thick-walled forming between segments of same or

adjacent mycelial strand. (*See life-cycle diagram and other figures in Chapter V-4.*)

a. Key diagnostic characters

(a) Mycelium initially coenocytic, becoming cellular with each cell then acting as a zoosporangium or gametangium. **(b)** Zoosporogenesis: individual cells (zoosporangia) develop a thin exit tube penetrating host cuticle; partially cleaved cytoplasmic blocks are transferred through this tube into a growing vesicle at apex of the tube; zoospores complete differentiation in the vesicle and are dispersed on the disappearance of the evanescent gelatinous vesicle wall. **(c)** Zoospores: laterally biflagellate (with anterior tinsel flagellum and posterior whiplash flagellum). **(d)** Resistant spores: thick-walled oospores, globose, formed by conjugations of adjacent cells (gametangia).

b. Major species

L. giganteum Couch: affecting mosquitoes (only entomopathogenic species).

c. Main taxonomic literature

Sparrow (1960); Bland *et al.* (1981).

d. General comments

Pythium species (Oomycetes: Peronosporales), rarely affect insects, but when they do resemble *L. giganteu* since the zoospores of both genera are dispersed from external vesicles in the same manner. *Pythium*, however, produces only thin hyphae (≤3–4 μm diam.) in which cytoplasm remains readily visible after the production and release of zoospores whereas the hyphae of *L. giganteum* are coarse (generally ≥5 μm diam.) and their contents convert entirely into zoosporangia or oospores.

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BRIEF GLOSSARY OF MYCOLOGICAL TERMS

Italicized terms appearing in the definitions are separately defined in this section. Irregular plurals of terms appear in parentheses at the start of definitions. Terms are applicable within the context of this chapter to fungi listed in brackets at the end of definitions.

Ascus (*asci*). Cell in which a single nucleus undergoes meiosis, after which one or more (usually eight) *ascospores* are cleaved out of the cytoplasm. [Ascomycota]

Capilliconidium (*capilliconidia*). A passively dispersed conidium produced apically on a long, slender (capillary) *conidiophore* arising from another conidium. [Entomophthorales, e.g. *Neozygites*, *Zoophthora*]

Conidiogenous cell. The cell on which a conidium forms, usually with only a single place (locus) on which a conidium forms; some conidiogenous cells have two or more conidiogenous loci. [Hyphomycetes, Entomophthorales]

Conidiophore. A simple or branched hypha or hyphal system bearing conidiogenous cells and their conidia. [Hyphomycetes, Entomophthorales]

Conidium (*conidia*). Fungal mitospore formed externally on a *conidiogenous cell*; conidia are not formed wholly inside any other cell (*ascus*, *sporangium*, etc.) nor as external meiospores (basidiospores on a basidium, the cell in basidiomycetes in which both karyogamy and meiosis occurs prior to basidiosporogenesis). [Hyphomycetes, Entomophthorales]

Cystidium (*cystidia*). In the Entomophthorales, more or less differentiated hyphae that precede and facilitate the emergence of the developing *conidiophores* through the host cuticle; cystidia usually project above the *hymenium*, but soon lose their turgor and collapse. Cystidia are rarely seen on any but very fresh specimens. [Entomophthorales; e.g. *Pandora* spp.]

Denticle. One of several to many small, conical to spike- or thorn-like or truncate projections on a conidiogenous cell, each of which bears a single conidium. [Hyphomycetes; e.g. *Beauveria* or *Hymenostilbe* spp.]

Hymenium (*hymenia*). A compact palisade layer of sporulating cells (conidiogenous cells, asci, etc.). [Hyphomycetes; Ascomycota; Entomophthorales]

Papilla (*papillae*). The basal portion of an entomophthoralean conidium by which conidia are attached to conidiogenous cells and which is usually involved in forcible discharge of conidia. [Entomophthorales]

Perithecium (*perithecia*). A globose, ovoid or pear-shaped walled structure in which *asci* and *ascospores* form; perithecia may be superficial or partially to fully immersed in the fruiting body. Each

perithecium has an apical opening (ostiole) through which the *ascospores* are discharged. [Ascomycota: Pyrenomycetes]

Polyphialide. A conidiogenous cell having more than one neck, each of which produces one or more conidia; relatively common in *Hirsutella* species that do not form synnemata. [Hyphomycetes]

Rachis (*raches*). A geniculate (or sometimes zig-zag) apical extension of a conidiogenous cell produced by sympodial branching of the elongating extension beneath each successive conidium formed. [*Beauveria* spp.]

Rhizoid. In the Entomophthorales, more or less differentiated hyphae that contact and anchor a host to the substrate; they may or may not have differentiated terminal holdfasts. [Entomophthorales]

Sporangium (*sporangia*). A cell or 'spore sac' inside of which (mitotic or meiotic) spores form; this is a very general term that can be correctly applied to diverse structures in nearly every class of fungi.

Stroma (*stromata*). A loose to fleshy or dense mass of vegetative hyphae on or in which spores (conidia or ascospores) are produced. Conidial stromata are usually very dense and compact, not extending very far above the host or substrate) (e.g. *Aschersonia* spp.); ascomycetous stromata bearing *perithecia* may be either low and compact (e.g. *Hypocrella* spp.) or upright and club- to column-like (e.g. *Cordyceps* spp.). [Hyphomycetes; Ascomycota]

Synnema (*synnemata*). An erect, branched or simple (unbranched) aggregation of hyphae; loosely fasciculate to compact, leathery or brittle in consistency, bearing conidiogenous cells and conidia. [Hyphomycetes; e.g. *Hirsutella*]

Zoospore. A uni- or biflagellate motile spore produced in a *zoosporangium*. [Chytridiomycetes; Oomycetes]

Zoosporangium (*zoosporangia*). The *sporangium* in which flagellate *zoospores* develop; *zoospores* and *zoosporangia* are formed only by watermolds. [Chytridiomycetes; Oomycetes]

APPENDIX: RECIPES OF STAINS AND REAGENTS

Aceto-orcein (nuclear stain/mounting medium)

Orcein (natural or synthetic)	1.0 g
Acetic acid, glacial	45.0 ml

Dissolve the orcein in hot glacial acetic acid, dilute 1 : 1 with distilled water and reflux or boil for at least 5 min. If boiled, replace lost volume with 50% glacial acetic acid. Filter at least twice to remove undissolved particulates. This stain continues to throw a precipitate over time and requires periodic clarification by filtration. There are many other ways of preparing aceto-orcein, most of which recommend refluxing; this simplified procedure yields a very satisfactory stain.

Lactophenol (mounting medium)

Phenol (crystals)	20 g
Lactic acid	20 g
Glycerol	40 g
Distilled water	20 ml

Lactic acid (mounting medium)

Anhydrous lactic acid with or without the addition of stains such as acid fuchsin, aniline blue or other acidic dyes can be used as an outstanding temporary to semi-permanent mounting medium.