ENTOMOPATHOGENIC FUNGAL IDENTIFICATION

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RICHARD A. HUMBER

USDA-ARS Plant Protection Research Unit
US Plant, Soil & Nutrition Laboratory
Tower Road
Ithaca, NY 14853-2901

Phone: 607-255-1276 / Fax: 607-255-1132
Email: *Richard Humber@ars.usda.gov* or *rah3@cornell.edu*
http://arsef.fpsnl.cornell.edu

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FOREWORD

Both Don Steinkraus and I are glad to have the opportunity to work with you today at this workshop on the identification of entomopathogenic fungi. This is a wonderful opportunity for the two of us to work with a diverse group of participants coming from a mixture of backgrounds with major training that may or may not include any formal training in either mycology or entomology. Don and I come from a similarly diverse pair of backgrounds: My graduate training was as a mycologist while Don's was as an entomologist/invertebrate pathologist. However, both of us have spent our entire careers focussing on various aspects of the organismal biology of entomopathogenic fungi and their use for biocontrol and other purposes.

We hope that no matter what your own background may be as you come into this workshop, that you will leave with a greater sense of assurance that you can, indeed, identify a wide range of the most common fungal species (not just the genera!) that cause diseases in invertebrates.

Most of the material in this syllabus is drawn from a chapter on fungal identification published in 1997 in the *Manual of Methods in Insect Pathology* (L. Lacey, editor; Academic Press, London). That chapter and a companion chapter on the preservation of fungi are in the public domain and free of copyright restrictions. For sake of brevity I have not tried to include much illustrative material in this syllabus, but have taken a different tack in regard to the identification of the major species of the genera included here that should be a material aid whether you are an old hand at these fungi or coming to them for the first time today: The charts for each genus are laid out in a key-like manner that reproduces the priority in which the characters of each genus should be viewed when identifying species. The species included in these charts are restricted to those most commonly encountered in North America; no attempt is made to provide comprehensive 'keys' to all species of the included genera.

In addition to the illustrated lecture time during the day, you will have an opportunity to work with cultures and specimens of these fungi and to become acquainted with the real appearance of some of the most important fungal pathogens of insects.

Time is too limited during this workshop to make anything more than a preliminary introduction and survey of the major fungi affecting insects. We both hope that you will take the initiative to look for sick insects during the course of your own work in the laboratory and field, or anywhere you may find yourself with a moment to take a look for any insects that might look altogether healthy of 'normal.' Fungus-infected insects are around us throughout much of the year; finding them is merely a matter of adjusting your search image. Have fun looking for and working with them!

Rich Humber
USDA-ARS
Ithaca, NY

Don Steinkraus
University of Arkasas
Fayetteville, AR

IT IS TIME FOR AN UPDATE…

This brief manual, originally prepared in 1998, has proven its usefulness for many purposes but the progress in the ongoing (and usually molecularly based) revision of the taxonomy of several important genera of insect-pathogenic fungi needs to be recognized and incorporated to improve the longevity and utility of this overview. As before, the generic treatments and keys to species presented here include all of the fungi most commonly encountered throughout the world. No such abbreviated a document can be comprehensive, but this manual should provide a basis for a reasonably confident identification of about 90-95% of the fungi likely to be found affecting agriculturally important arthropod pests.

Richard A. Humber
November 2005

IMPORTANT TECHNIQUES FOR OBSERVING ENTOMOPATHOGENIC FUNGI

Compound microscopes and Köhler illumination

The key to observing small structures in a microscope is not magnification; it is optical resolution – the ability to distinguish two adjacent objects. Many factors affect image resolution, but the most important step anybody can take to obtain the highest quality images from any compound microscope is to set 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. Once you are familiar with this process, it should only take a few seconds to adjust the microscope, but the time spent doing so it more than worth the effort.

The following steps to achieve Köhler illumination *should be repeated for each objective used* and rechecked regularly during use of a light microscope:

- Focus on *any* object in a slide (so you know where the image plane is).
- Close the field diaphragm (at the light source) and adjust the condenser height so that *both* the edge of field diaphragm and the object appear to be sharply focused.
- Open the field diaphragm until its image nearly fills the field of view, and
- Use the condenser centering screws to center the field diaphragm image in the field of view
- Adjust the opening of the condenser diaphragm: The image of this diaphragm is seen by removing an eyepiece and looking down the microscope body (a focusing telescope is useful but not truly necessary for this step). The condenser diaphragm should be adjusted so that its opening fills some 80-90% of the diameter the image in this back focal plane.

The condenser diaphragm should *never* be opened wider than the full dimaeter of the back focal plane; the resulting 'glare' of too much light entering the system severely degrades the overall resolution. The most common errors in adjusting light microscopes are to close the condenser diaphragm too far to increase the image contrast or to lower the condenser too far (again to increase contrast or to decrease the lighting level. Both of these errors result introduce diffraction artifacts (with an increasing graininess and darkening of object edges) and substantially decrease the overall resolution within the image.

Slide mounts

Coverslips

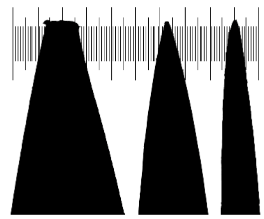
- Use #1½ coverslips (0.16-0.19 mm thick)
- Do ***not*** use plastic coverslips!
- There are many advantages in using small coverslips obtained by scoring 18- or 22-mm square coverslips into quarters (or even into sixths!) with a diamond or carbide pencil. Always handle the coverslips by the edges; avoid getting fingerprints on them!

Mounting Media

- Use a minimal amount, no more than required to fill the volume under the coverslip. (This skill takes some practice!)
- Commonly used mounting media:
  lactic acid – with or without added stains (cotton blue, etc.)
  lactophenol – 20 g lactic acid + 20 g phenol + 40 g glycerol + 20 ml dist. Water
  aceto-orcein – 1.0 g orcein 45.0 ml glacial acetic acid. Dissolve orcein in acid, dilute 1:1 with distilled water and reflux or boil or at least 5 minutes. If boiled, replace volume with 50% acetic acid. Clarify by centrifugation and/or filtration and reclarify periodically (this stain throws a lot of precipitate).

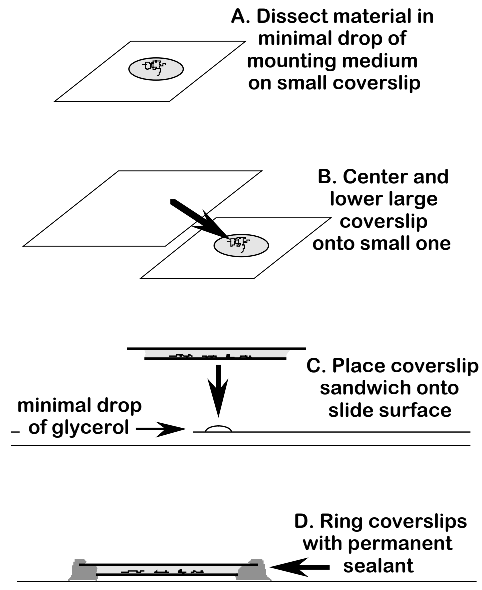
Handling of the Material to be Observed

- Use a *small* amount of material
- Tease apart and spread the material to be viewed as efficiently as you can
  (*Hint:* The best tools for this are 'minuten' or '0' insect pins [see the adjacent drawing] whose blunt ends are pushed into standard wooden matchsticks)
- Preparing slides is easiest is using a stereo microscope with bottom illumination



Semi-Permanent Slide Mounts

- You will not be able to seal slides unless the *minimal* amount of mounting medium is wholly contained under the coverslip.
- The most convenient short-term preservation of slides can be obtained by ringing a slide with either fingernail polish, Canada balsam, or another slide-making resin. The most effective approach with this method is to apply a thin layer, let it dry, and then repeat the process. This sort of seal may be good for several months but cannot be counted to last for many years.
- The most durable slide mounts for material in aqueous mounting media involves sandwiches of two unequal sized coverslips. The basic method shown here: 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 and Kohlmeyer[1] describe a modified version of this technique in which thesmall coverslip is sealed to the large one before attaching the sandwich to the slide and then sealing the large coverslip onto the slide with the mounting resin.

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 lots of 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 a difficult problem. 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**.**

[1] Kohlmeyer J, Kohlmeyer E. 1972 Permanent microscopic mounts. *Mycologia* 64: 666-669

KEY TO MAJOR GENERA OF FUNGAL ENTOMOPATHOGENS

This key is a slightly updated version of that originally published in an illustrated chapter by Humber[2] in L. Lacey's (1997) *Manual of Techniques in Insect Pathology*. The key includes all fertile (spore-bearing) states most likely to be foundfor the genera treated but has been modified to reflect some of the major taxonomic changes published since 1997. Genera whose names appear here in **BOLD CAPS** are treated further in this manual; all other named genera (unless footnoted with a reference) are discussed and illustrated in the Humber chapter in *Manual of Techniques in Insect Pathology*.

Although many entomopathogenic fungi are illustrated and keyed in the *Atlas of Entomopathogenic Fungi*[3] it is important to note that this book's key includes only the *one* most characteristic state in which a fungus might occur; it does not treat multiple spore types produced by various fungi such as the conidia and resting spores of the Entomophthorales, or the various alternative conidial states of many Hyphomycetes. Also, the illustrations in this *Atlas* are completely unmarked and provided with only very rudimentary captions; the principle diagnostic features in the photographs included in this *Atlas* are often obscure or unobvious unless one is already familiar with the taxa being illustrated.

An extraordinary interactive key to *Fusarium* species by K. Seifert was mounted on the web in 1995 but in 2005 appears to be available only in a non-interactive (but still highly useful) Acrobat-formatted version the full material of that website at <*www.ctu.edu.vn/colleges/agri/gtrinh/fuskey.pdf*>. Few species of this complex genus affect insects, 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 hours 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 after the key should help clarify many potential questions. More detailed definitions of terms can be found in many mycological textbooks or in the any edition of the *Ainsworth & Bisby's Dictionary of the Fungi*[4] published by CABI Bioscience.

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

   Fungal growth and sporulation wholly (or nearly wholly) confined to *interior* of host body 32

2. Elongated macroscopic structures (synnemata or club-like to columnar stromata) project from host 3

   Fungal growth may cover all or part of the host and may spread onto the substrate but large, projecting structures are absent 11

3. Conidia form on synnemata and/or on mycelium on the host body 4

[2] Humber RA. 1997. Fungi: Identification. In *Manual of Techniques in Insect Pathology* (L.A. Lacey, ed.), pp. 153-185. Academic Press: London.

[3] Samson RA, Evans HC, Latgé JP. 1988. *Atlas of Entomopathogenic Fungi*. Springer-Verlag, Berlin.

[4] Current edition: Kirk PM, Cannon PF, David JC, Stalpers JA (eds.). 2001. *Ainsworth & Bisby's Dictionary of the Fungi*, 9th edition. CABI Publishing: Wallingford.

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 10

4. Conidia formed in short to long chains 5

   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, pairs, whorls, or loose clusters; chains of conidia often long and divergent (when borne on clusters of conidiogenous cells) 6

   Conidiogenous cells short, with rounded to broadly conical apices (*not* having a distinctly narrowed and extended neck) 7

6. Conidia formed in axis of conidiogenous cell, arranged end-to-end in chains .... **PAECILOMYCES/ISARIA**

   Conidia forming obviously at an angle out of axis of conidiogenous cell, with successive spores forming to opposite sides of conidiogenous cell apex resulting in flat, ribbon-like chains with conidia in a zipper-like, arrangement .... **EVLACHOVAEA**

7. 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

   Conidiogenous cells borne at apices of broadly branched,densely intertwined conidiophores forming a compact hymenium; conidia borne in parallel chains and usually green in mass .... **METARHIZIUM**

8. 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**

   Conidiogenous cells producing several to many conidia, each formed singly on separate denticles 9

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

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

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

    Perithecia occur *only* on or emerging from a cottony to woolly layer covering host .... Torrubiella

11. Fungus covering host is a stroma (fleshy to hard mass of intertwined hyphae); sporulation occurs in cavities below the stromatic surface 12

    Host partially to completely covered by wispy, cottony, woolly, or felt-like growth or by a dark-colored, extensive patch having columns and chambers below its surface but *not* forming a stroma 13

12. 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**

    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

13. 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[5]

Fungal hyphae emerging from or covering host are colorless to light colored, wispy to cottony, woolly, felt-like or waxy-looking mat 14

14. 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

Spores form on external surfaces of the fungus; no sexual structures (perithecia) are present 15

15. Conidia form on cells with elongated denticulate necks bearing multiple conidia or on awl- to flask-shaped or short blocky conidiogenous cells; conidia form singly or successively in dry chains or slime drops (Hyphomycetes) 16

Conidia forcibly discharged and may rapidly form forcibly or passively dispersed secondary conidia (Entomophthorales) 24

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

Conidiogenous cells are awl- to flask-shaped, with or without an obvious neck; conidia borne singly, in chains, or in slime drops 17

17. Conidia single or in chains on apices of conidiogenous cells 18

Conidia aggregate in slime drops at apices of conidiogenous cells 22

18. 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**

Conidia borne in chains, not covered by any obvious slime 19

19. 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 columns or continuous plates .... **METARHIZIUM**

Conidiophores individually distinct and unbranched or with a main axis and short side branches bearing single or clustered conidiogenous cells 20

20. 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**

Conidiogenous cells elongated and awl- or flask-shaped borne singly or in loose clusters but not arranged in dense whorls; chains of conidia often long 21

21. Conidiogenous cells with a distinct neck, and more or less swollen bases; conidia formed in axis of conidiogenous cell, arranged end-to-end in chains ....**PAECILOMYCES/ISARIA**

Conidiogenous cells awl-like or flask-shaped and with disting neck; conidia formed obviously out of axis of conidiogenous cell, with successive spores forming to opposite sides of conidiogenous cell apex resulting in flat, ribbon-like chains with conidia in a zipper-like, arrangement .... **EVLACHOVAEA**

22. 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

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[5] See Couch JN. 1938. *The genus* Septobasidium. University of North Carolina Press: Chapel Hill.

Conidiogenous cells little thicker than hyphae, occurring singly or grouped into regular clusters and/or whorls; conidia one-celled; mycelium highly uniform in diameter 23

23. 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 (formerly *Verticillium* spp) .... **LECANICILLIUM (and other segregate genera)**

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 singl, clustered, or in whorls aggregating in loose 'heads' on erect apically branching conidiophores poorly differentiated from vegetative hyphae .... Tolypocladium

24. 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) 25

In aceto-orcein, primary conidia obviously multinucleate or nuclei not readily seen 28

25. Conidia long clavate to obviously elongated (length/width ratio usually $\geq$2.5), papilla broadly conical, often with a slight flaring or ridge at junction with basal papilla 24

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

26. 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**

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

27. Conidia never producing secondary capilliconidia; rhizoids 2-3x thicker than hyphae, ending with a discoid holdfast; cystidia at base 2-3x thicker than hyphae, tapering towards apex .... **PANDORA**

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

28. In aceto-orcein, nuclei staining readily, with obviously granular contents 29

In aceto-orcein, nuclei not readily visible or not staining 31

29. 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**

Conidia without apical projection and discharged by eversion of a rounded (not flat) papilla 30

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

Conidia globose (rarely pyriform) with papilla emerging abruptly from spore outline; formed on conidiogenous cells with a narrowed neck below the conidium; rhizoids often present, 2-3x thicker than hyphae, with terminal holdfast discoid or flat plate of short branches .... **BATKOA**

31. 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**

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**

32. 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

Affecting insects other than bees; spores formed individually rather than in spherical groups of inside larger spheres 33

33. Spores formed *inside* a fungal cell, in a more or less loosely fitted outer (sporangial) wall 34

    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) 35

34. Spores (oospores) thick-walled, smooth walled, colorless; 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

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

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

    Not on cicadas; spores throughout body, not confined to terminal abdominal segments) 36

36. Spores (zygospores or azygospores) with outer surfaces smooth or with surface irregularly roughened, warted, or spinose; colorless to pale or deeply colored (various colors possible), brown, gray, or black 37

    Spores (thick-walled resistant sporangia) with surface regularly decorated with ridges, pits, punctations, striations, reticulations; yellow-brown to golden-brown 39

37. Resting spores gray, brown or black (outer wall is colored; 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**

    Resting spores colorless, colored, or dark, surfaces smooth or rough; infected host population may or may not include cadavers producing conidia but, if present, conidia not as above 38

38. 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**

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

39. 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

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

BRIEF GLOSSARY OF MYCOLOGICAL TERMS

Irregular plurals of terms appear in parentheses at the start of definitions. The fungal groups given in brackets at the end of each definition are the ones for which the definition is applicable. This glossary is extracted with only slight modification from Humber (1997; op. cit).

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): 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): 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 loose 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 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 spores attach to conidiogenous cells and which is involved in forcible discharge of conidia. [Entomophthorales]

perithecium (perithecia): a globose, ovoid or pear-shaped walled structure in which *asci* and *ascospores* form; perithecia are superficial or partially to fully immersed in a fruiting body. Each perithecium has an apical hole (ostiole) through which the **ascospores** are dispersed. [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 below each successive conidium formed. [*Beauveria*]

rhizoid: more or less differentiated hyphae that contact and anchor a host to the substrate; they may or may not have differentiated terminal holdfasts. [Entomophthorales, *e.g.*, *Pandora*, *Zoophthora*]

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 formed in a **zoosporangium**. [Chytridiomycetes; Oomycetes]

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

BATKOA Humber

Zygomycota: Entomophthorales: Entomophthoraceae

Mycelium coenocytic, often forming hyphal bodies; conidiophores unbranched but forming a distinctly narrower extension at apex before initiation of conidium; primary conidia globose to pyriform, multinucleate, forcibly discharged by papillar eversion; secondary conidia form singly, resembling primaries and forcibly discharged; resting spores thick-walled with two distinct wall layers, budded off laterally from parental hypha; rhizoids, if present, monohyphal, thicker than vegetative hyphae, usually terminating in discoid holdfast; cystidia absent; nuclei large and stainins (with coarsely granular contents) in aceto-orcein.

Differences from other similar genera:

Conidiobolus*:** conidia globose to *pyriform*; *apices of conidiogenous cells show no narrowing below the conidium; and hosts are not attached to the substrate by rhizoids.* **NOTE: *B. apiculata* and *Conidiobolus obscurus* can be easily confused on basis of overall morphology of conidia *except for sstaining of nuclei and probable presence of rhizoids for B. apiculata

***Entomophaga*:** conidia pyriform and multinucleate with nuclei large (often ≥ 4µm long) and nuclear contents staining and distinctly granular when mounted in aceto-orcein vs. *Conidiobolus in which nuclei are smaller (usually 3.5 µm long) contents never show prominent granular staining in aceto-orcein*

Priority of characters for species identification:

conidial size > conidial nuclear number > rhizoids ±/if +, morphology > host

Key Literature:

Humber RA. 1989. Synopsis of a revised classification for the Entomophthorales (Zygomycotina). *Mycotaxon* 34: 441-460.

Balazy S. 1993. Entomophthorales. Flora of Poland (Flora Polska), Fungi (Mycota) 24: 1-356. Polish Acad. Sci., W. Szafer Inst. Botany, Kraków.

CONIDIA size/morphology	**NUCLEAR NUMBER** in primary conidia	**RHIZOIDS**	**HOST**	**SPECIES**
29-35 x 30-40 µm; globose; papills ± hemispherical an often apiculate (with small point)	comparatively few, ≤ 25 ?	Usually present, thick (≥2x diam of hyphae), with terminal holdfast discoid or a flat plate of short radiating branches	aphids, other hemipterans, small flies, many other insects	***apiculata***
38-45 x 55-60 µm; globose; papilla	15-56		Diverse flies, lepidopterans, coleopterans	***major***
30-40- x 40-60 µm; pyriform to long ovoid	numerous, 50-80	may be present but often absent	small flies	***papillata***

CONIDIOBOLUS Brefeld

Zygomycota: Entomophthorales: Ancylistaceae

Mycelium coenocytic but becoming septate, often forming hyphal bodies; conidiophores usually unbranched; primary conidia globose to pyriform, multinucleate, forcibly discharged by papillar eversion; secondary conidia form: (1) singly, resembling primaries and forcibly discharged, (2) multiply, forcibly discharged (microconidia), or (3) as cylindrical capillicondiia passively dispersed from capillary conidiophore; resting spores zygospores, thick-walled with two distinct wall layers, formed in the hyphal axis after copulations usually between adjacent cells or, if azygospores (not arising from gametangial conjugations) as apical or intercalary swellings of hyphae, but ***never budding off laterally***; nuclei unstained (or poorly stained and without coarsely granular contents) in aceto-orcein.

Differences from other similar genera:

Batkoa*:** conidia globose, formed on conidiogenous cells with distinct apical narrowing below spore and infected insects often attached by thick rhizoids with discoid holdfasts *vs. Conidiobolus in which apices of conidiogenous cells show no narrowing' below the conidium and hosts are not attached to the substrate by rhizoids.* **NOTE: *Conidiobolus obscurus* and *Batkoa apiculata* can be easily confused on basis of overall morphology of conidia except for sstaining of nuclei and probable presence of rhizoids for *B. apiculata

***Entomophaga*:** conidia pyriform and multinucleate with nuclei large (often ≥ 4µm long) and nuclear contents staining and distinctly granular when mounted in aceto-orcein vs. *Conidiobolus in which nuclei are smaller (usually 3.5 µm long) contents never show prominent granular staining in aceto-orcein*

Major taxonomic subdivisions:

subgenus *Conidiobolus* —2° conidia produced singly, resembling and forcibly discharged from 1° conidium

subgenus *Capillidium* — (few rare spp) 2° conidium like primary and forcibly discharged OR cylindrical and passively dispersed from apex of a long, narrow (capillary) secondary conidiophore

subgenus *Delacroixia* — single secondary conidium resembles primary and is forcibly discharged OR many small (subglobose to drop-shaped) microconidia, each of which is forcibly discharged from a short sterigma.

Priority of characters for species identification:

2° conidial types > conidial shape > conidial size > host

Key Literature:

King DS. 1976a. Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy. I. Biology and cluster analysis. *Canad. J. Bot*. 54: 45-65.

King DS. 1976b. Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy. II. Taxonomic considerations. *Canad. J. Bot*. 54: 1285-1296.

King DS. 1977. Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy. III. Descriptions of recognized species. *Canad. J. Bot*. 55: 718-729.

Keller S. 1987. Arthropod-pathogenic Entomophthorales of Switzerland. I. *Conidiobolus*, *Entomophaga*, and *Entomophthora. Sydowia* 40: 122-167.

Balazy S. 1993. Entomophthorales. Flora of Poland (Flora Polska), Fungi (Mycota) 24: 1-356. Polish Acad. Sci., W. Szafer Inst. Botany, Kraków.

2° CONIDIA types	**SHAPE** of conidia	**SIZE** of conidia	**HOST**	**SPECIES**
forming only a single 2° conidium similar in size to the primary	globose; papilla ± hemispherical and emerging abruptly from spore outline	30—40 µm diam.	aphids (or other Homoptera)	***obscurus***
	pear-shaped (pyriform)	17—30 µm diam.	aphids (or other Homoptera)	***thromboides***
forming numerous small microconidia as well as single 2° like the primary	globose,, papilla often elongated,; old conidia becoming villose ('hairy') *(villose conidia **MUST** be present to ID as his species!)*	highly variable, especially if 2° microconidia are confused with primaries	diverse insects (but a weak pathogen)	***coronatus***

ENTOMOPHAGA Batko

Zygomycota: Entomophthorales: Entomophthoraceae

Hyphal bodies fusoid to beaded, amoeboid progoplasts later becoming rod-like ro spherical and walled only in moribund host; conidiophores unbranched; primary conidia pyriform to ovoid, multinucleate, discharged by papillar eversion; rhizoids and cystidia not formed; resting spores thick-walled (with two ± distinct layers) bud laterally from parental hypha; unfixed nuclei stain and reveal granular contents in aceto-orcein.

Differences from other similar genera:

Conidiobolus: globose to pyriform conidia with nuclei failing to stain (or staining only weakly and without obvious granularity) in aceto-orcein *vs. Entomophaga in which nuclei stain prominently and appear granular in aceto-orcein*

Batkoa: globose, multinucleate conidia formed on conidiogenous cells with distinct apical narrowing below conidium and with hosts often attached by stout rhizoids having discoid terminal holdfasts *vs. Entomophaga in which conidia are always pyriform (never globose) and no rhizoids attach affected hosts to the substrate*

Priority of characters for species identification:

host > conidial characters > other characters

Key Literature:

Balazy S. 1993. Entomophthorales. Flora of Poland (Flora Polska), Fungi (Mycota) 24: 1-356. Polish Acad. Sci., W. Szafer Inst. Botany, Kraków.

Keller S. 1987. Arthropod-pathogenic Entomophthorales of Switzerland. I. *Conidiobolus*, *Entomophaga*, and *Entomophthora*. *Sydowia* 40: 122-167.

Soper RS, Shimazu M, Humber RA, Ramos ME, Hajek AE. 1988. Isolation and characterization of *Entomophaga maimaiga* sp. nov., a fungal pathogen of gypsy moth, *Lymantria dispar*, from Japan. *J. Invertebr. Pathol.* 51: 229-241.

HOST	SIZE of conidia	OTHER CHARACTERS	SPECIES
Lepidoptera (diverse; but not Lymantriida)	Variable among unresolved members of species complex but conidia generally ca 30-40 µm long	apparently an unresolved species complex including several entities with distictive genotypes resolvable by molecular tests	***aulicae***
gypsy moth larvae (Lymantriidae)	20-36 x 16-28 µ; (20-) 26 (-35) nuclei	molecular markers completely distinguish this species from other lepidopteran pathogens	***maimaiga***
Orthoptera (Melanoplinae; spur-throated grasshoppers)	[standard primary conidia not formed]	infected insects produce only resting spores; typical conidia are not produced	***calopteni***
(non-melanopline grasshoppers)	variable among unresolved members of species complex	unresolved species complex (≥5 species), all of which routinely produce conidia except for *E. calopteni*	***'grylli'***

ENTOMOPHTHORA Fresenius Zygomycota: Entomophthorales: Entomophthoraceae

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

Differences from other similar genera:

This genus is now restricted to a few species, all of which have conidia with an apical projection and a broad, relatively flat basal papilla; a discharged primary conidium attaches to the substrate in a distinctive (cytoplasmic?) droplet and quickly forms and discharges a secondary conidium. The immediately recognizable conidia of *Entomophthora* species are completely unique to and diagnostic for their genus.

Priority of characters for species identification:

host > number/size of conidial nuclei > conidial size > other characters

Key Literature:

MacLeod DM, Müller-Kögler E, Wilding N. 1976. *Entomophthora* species with *E. muscae*-like conidia. *Mycologia* 68: 1-29.

Keller S. 1984. *Entomophthora muscae* als Artenkomplex. *Mitt. Schweiz. Entomol. Ges.* 57: 131-132.

Balazy S. 1993. *Entomophthorales*. Flora of Poland (Flora Polska), Fungi (Mycota) 24: 1-356. Polish Acad. Sci., W. Szafer Inst. Botany, Kraków.

Keller S, Kalsbeek V, Eilenberg J. 1999. Redescriptiion of *Entomophthora muscae* (Cohn) Fresenius. *Sydowia* 51: 197-209.

Keller S. 2003. The genus *Entomophthora* (Zygomycetes, Entomophthorales) with a description of five new species. *Sydowia* 54: 157-197.

HOST	NUCLEI in conidia	CONIDIA size	OTHER CHARACTERS	SPECIES
Diptera (muscoid flies)	4-10 per spore, 3-3.5 µm diam.	17-25 x 12-22 µm	rhizoids (without apical holdfasts) emerge only where mouthparts contact substrate	***schizophorae*** (a member of the partially resolved *E. muscae* species complex)
(mosquitoes, midges or blackflies)	2 (rarely 3)	11-16 x 7-12 µm	rhizoids numerous, fomring a stout fascicle under the host thorax	***culicis***
Homoptera (aphids)	4-6 per spore; 2.5-3.5 µm diam.	13-23 µm long (mostly 16-20 µm long)	no cultures currently available despite repeated attempts	***planchoniana*** (primarily European)
	4-6 per spore; 3.5-5 µm diam.	11-15 µm long	can be cultured *in vivo* (but cultures do poorly at temperatures >20°C)	***chromaphidis*** (primarily N. America and Australia)

NEOZYGITES Witlaczil

Zygomycota: Entomophthorales: 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, 4- (rarely 5-) nucleate, forcibly discharged a short distance by papillar eversion; secondary conidia usually (more or less almond-shaped) capilliconidia passively dispersed from capillary conidiophores; resting spores bud laterally from conjugation bridge between gametangia (hyphal bodies), black to smoke-gray, binucleate; nuclei in unfixed material staining poorly in aceto-orcein (*except* during mitosis); especially on Homoptera, thrips, and mites.

Differences from other similar genera:

No other entomophthoralean genera are really similar to *Neozygites*. Other genera (e.g., *Zoophthora* and *Conidiobolus*) produce secondary capilliconidia, but none of the capillaries have the sort of single or double bend at the apex characteristic of *Neozygites*. No conidia in any other genus of the order have the same tendency to be slightly (but still obviously) melanized and smoke-grey found in *Neozygites* species. Except for *Basidiobolus* (in which all cells are uninucleate), *Neozygites* is the only other genus showing strong control over the number of nuclei in vegetative cells (averaging 4, and only occasionally being 3 or 5 except immediately after mitosis and before cell division).

Major taxonomic subdivisions:

No taxonomic subdivision has been recognized, but there seems to be a distinct difference between homopteran pathogens in this genus, most of which have ovoid zygospores with smooth surfaces, and the pathogens of mites and thrips, all of which have globose or subglobose resting spores with roughened surfaces.

Priority of characters for species identification:

host > conidial characters > zygospore characters

Key Literature:

Balazy S. 1993. Entomophthorales. Flora of Poland (Flora Polska), Fungi (Mycota) 24: 1-356. Polish Acad. Sci., W. Szafer Inst. Botany, Kraków.

Keller S. 1991. Arthropod-pathogenic Entomophthorales of Switzerland. II. *Erynia*, *Eryniopsis*, *Neozygites*, *Zoophthora*, and *Tarichium*. *Sydowia* 43: 39-122.

Keller S. 1997. The genus *Neozygites* (Zygomycetes, Entomophthorales) with special reference to species found in tropical regions. *Sydowia* 49: 118-146.

HOST	CONIDIA	RESTING SPORES	SPECIES
Homoptera (Aphididae)z	globose to pyriform (extended at papilla), 18-22 z 14-18 µm	Ovoid (or slightly flattened to one side), black to dark smoke-grey, surface smooth	***fresenii***
Thysanoptera	globose, 13-16 x 11-14 µm	globose to subglobose. surface grooved or ridged, dark brown to black	***parvispora***
Acarina	globose, 11-18 x 9-15 µm	Globose to subglobose, surface granular and roughened, dark brown to black	***floridana***

PANDORA Humber

Zygomycota: Entomophthorales: 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 2-3x diameter of hyphae or conidiogenous cells, with discoid terminal holdfast; cystidia taper, at base, 2-3x diameter of hyphae or conidiogenous cells; resting spores bud laterally from parental hypha; unfixed nuclei have granular contents staining in aceto-orcein.

Differences from other similar genera:

***Furia*:** Conidia similar in shape but rhizoids and cystidia no thicker than vegetative hyphae (and rhizoids numerous, often fasciculate, and with no terminal discoid holdfast *vs. Pandora in which rhizoids are less numerous, isolated rather than fasciculate, and both rhizoids and cystidia at least 1.5x thicker than vegetative hyphae*

***Erynia*:** Conidia of most species with conical papillae and often markedly elongated, rhizoids and cystidia also thicker than vegetative hyphae but rhizoids with no differentiated terminal holdfasts *vs. Pandora in which conidial papillae are alwas rounded (never conical) and rhizoids have discoid terminal holdfasts*

Major taxonomic subdivisions:

The genus as treated here is not subdivided, but represents one result of raising the four subgenera of *Zoophthora* Batko (1966) to generic rank (Humber, 1989). Other current classifications for these fungi continue to treat *Pandora* species as a subgenus of *Zoophthora* (Balazy, 1993) or as belonging to *Erynia* (as a broadly defined genus incorporating three of the four Batkoan subgenera; Keller, 1991, uses this classification).

Priority of characters for species identification:

host > conidialcharacters > resting spore characters > other characters

Key Literature:

Balazy S. 1993. Entomophthorales. Flora of Poland (Flora Polska), Fungi (Mycota) 24: 1-356. Polish Acad. Sci., W. Szafer Inst. Botany, Kraków.

Batko A. 1966. On the subgenera of the fungus genus *Zoophthora* Batko 1964 (Entomophthoraceae). *Acta Mycol.* 2: 15-21.

Humber RA. 1989. Synopsis of a new classification of the Entomophthorales (Zygomycotina). *Mycotaxon* 34: 441-460.

Keller S. 1991. Arthropod-pathogenic Entomophthorales of Switzerland. II. *Erynia*, *Eryniopsis*, *Neozygites*, *Zoophthora*, and *Tarichium*. *Sydowia* 43: 39-122.

HOST	CONIDIA	RESTING SPORES	OTHER CHARACTERS	SPECIES
Homoptera (Aphididae)	obovoid to clavate, 15-40 x 9-16	none ever observed despite common occurrence on many diverse aphid hosts	grows fairly well in vitro	***neoaphidis***
(Delphacidae, Cicadellidae)	same shape as *P. neoaphidis*, 30-35 x 12-18 µm	none known	on same media, grows faster in vitro than *P. neoaphidis*	***delphacis***
Lepidoptera	pyriform to ovoid, 15-18 x 7-9 µm	none known	occurs primarily on larvae, especially of *Plutella xylostella*	***blunckii***
Diptera	subglobose to angular	Yellow to tan, 37-62 µm diam, surface densely covered by rounded warts (bullations)	apparently specific for adult sarcophagid flies	***bullata***

ZOOPHTHORA Batko

Zygomycota: Entomophthorales: 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.

Differences from other similar genera:

Erynia: rhizoids and cystidia markedly thicker than vegetative hyphae, conidia of most species with conical papilla and never forming secondary capilliconidia vs. *Zoophthora in which rhizoids/cystidia are not thicker than vegetative hyphae and all species can form secondary capilliconidia*

Furia: conidia not markedly elongated, always with rounded papilla, and never producing secondary capilli-conidia vs. *Zoophthora with conical papillae on conidia that routinely produce secondary capilliconidia*

Major taxonomic subdivisions:

Zoophthora is variously treated in the current classifications: Balazy (1993) follows Batko's (1966) concepts of a broadly defined genus only some of whose species can produce capilliconidia. In the taxonomy followed by Keller (1991) and Humber (1989) *Zoophthora* is restricted to those species of Batko's *Zoophthora* subgenus *Zoophthora*, all of which form secondary capilliconidia.

Priority of characters for species identification:

host > conidial characters > capilliconidia characters > other characters

Key Literature:

Balazy S. 1993. Entomophthorales. Flora of Poland (Flora Polska), Fungi (Mycota) 24: 1-356. Polish Acad. Sci., W. Szafer Inst. Botany, Kraków.

Batko A. 1966. On the subgenera of the fungus genus *Zoophthora* Batko 1964 (Entomophthoraceae). *Acta Mycol.* 2: 15-21.

Humber RA. 1989. Synopsis of a new classification of the Entomophthorales (Zygomycotina). *Mycotaxon* 34: 441-460.

Keller S. 1991. Arthropod-pathogenic Entomophthorales of Switzerland. II. *Erynia*, *Eryniopsis*, *Neozygites*, *Zoophthora*, and *Tarichium*. *Sydowia* 43: 39-122.

HOST	CONIDIA	CAPILLICONIDIA	OTHER CHARACTERS	SPECIES
MANY, diverse	bullet-shaped to long ovoid, 15-30 µm long	elongated, gently swollen in center, 17-22 x 5-6 µm	*extremely* common on a very wide range of hosts; no adequate resolution of this species complex exists)	***radicans*** (species complex)
Homoptera (Aphididae)	cylindrical, curved (± phalloid), 32-40 x 11-14 µm	almond-shaped to hooked, 17-25 x 6-8 µm	relatively uncommon aphid pathogen, primarily known from N. America	***occidentalis*** (mainly N. America)
	Clavate to nearly cylindric, 24-35 x 9-12 µm	stongly lunate with on side nearly flat, 18-28 x 9-13 µm	forms dark, rough-walled resting spores; appears to be very specific for *Anoecia* spp	***aphidis*** (*rare*; only European)
Coleoptera (Curculionidae)	long ellipsoid, with blunt ends, 23-26 x 6.5-7.5 µm	Nearly fusoid, thickest in middle, 20-25 x 6.5-8 µm	resting spores either smooth and colorless or roughened/dark; specific for *Hypera* spp	***phytonomi***

CORDYCEPS Fries

Sordariomycetes: Hypocreales: Clavicipitaceae

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 8 filiform, multiseptate ascospores which usually fragment to form 1-celled part-spores. **Asexual states:** *Beauveria, Hirsutella, Hymenostilbe, Metarhizium, Nomuraea, Paecilomyces, Verticillium* and many other genera.

Differences from other similar genera:

***Torrubiella*:** perithecia are superficial or partially embedded in a loose hyphal mat covering the host body rather than an erect stroma *vs. Cordyceps produces erect stromata bearing perithecia (perithecia are* not *formed on the host's body)*

Major taxonomic subdivisions:

Cordyceps is a huge and complex genus whose only practical (but hardly user-friendly) taxonomy was devised by Kobayasi (1941, 1982; Kobayasi and Shimizu, 1983). Kobayasi (1941) did recognize several subgenera within this highly diverse assemblage, although the real usefulness and correctness of these subgenera remains debatable:

- subgenus *Eucordyceps* – perithecia arranged ± perpendicular to surface of stroma; ascospores filiform, multicellular, dissociating to yield 1-celled cylindrical partspores
- subgenus *Neocordyceps* – perithecia embedded in stroma and mostly at oblique to acute angles to surface of stroma; ascospores filamentous, multicellular, dissociating to yield 1-celled fusoid partspores
- subgenus *Ophiocordyceps* – ascospores relatively short and thick, with only relatively few cells that do not dissociate to form partspores

Priority of characters for species identification:

host > stromatic characters > perithecial characters > ascospore characters

Key Literature:

Kobayasi Y. 1941. The genus *Cordyceps* and its allies. *Sci. Rep. Tokyo Bunrika Daig., Sect. B* 5: 53-260.
Kobayasi Y. 1982. Keys to the taxa of the genera *Cordyceps* and *Torrubiella. Trans. Mycol. Soc. Jpn.* 23: 329-364.
Kobayasi Y, Shimizu D. 1983. *Iconography of vegetable wasps and plant worms.* Hoikusha Publ. Co., Osaka.

HOST	STROMATA	PERITHECIA	ASCOSPORES	SPECIES
Lepidoptera	club-like, chrome-yellow to orange; peritheciain swollen distal fertile part	completely embedded in stroma except for ostiole, perpendicular to stromatic surface	filamentous, dissociating to form 1-celled *cylindrical* partspores	***militaris***
Hymenoptera (wasps)	long, thin, light tan to pale yellow; fertile part slightly swollen, cylindrical, and apically located	embedded obliquely in stroma, with bulbous base tapering uniformly towards apex (ostiole)	filamentous with cells somewhat swollen and dissociating to form *fusoid* partspores	***sphecocephala***
(ants)	light-colored (tan to brown) bearing one or more subapical pad-like fertile parts (with embedded perithecia)	ovoid, completely embedded in stroma, oriented perpendicular to surface of fertile part	filamentous, dissociating to form *cylindrical* partspores	***unilateralis***
Diptera	sterile brown stipe with apical fertile part lighter in color, discoid to bulbous	Narrow ovoid, strongly tapering, perpendicular to surface of fertile part	filamentous, dissociateg to form *cylindrical* partspores	***dipterigena***

ASCHERSONIA Montagne

Sordariomycetes: Hypocreales: anamorphic Clavicipitaceae
[Deuteromycota: Hyphomycetes]

Stroma variable in shape (sometimes indistinct), superficial, white to brightly colored or dull, covering host scale insect; one or more fertile chambers (pycnidia) are submerged in stroma and open by wide pores or irregular cracks and are not differentiated from stromatic tissues by any distinct wall; conidiophores slender, branched, bearing apical phialides and line the pycnidial chambers; conidia hyaline, 1-celled, spindle-shaped; paraphyses (sterile hyphae projecting above conidiophores) present or absent; conidia discharged into a common slime mass that exudes from pycnidium onto stromatic surface.

Differences from other similar genera:

***Hypocrella*:** the sexual state of *Aschersonia* spp, with similar stromatic morphologies, but with distinctly walled. flask-shaped fruiting bodies (perithecia) containing asci and ascospores embedded in or partially emergent from the stroms *vs. Aschersonia with its one-celled conidia produced in wall-less chambers in the stroma*

Podonectria: pseudothecia (perithecium-like fruiting structures) are emergent from a loose hyphal covering of affected scale insects; asci are *bitunicate* (dehiscing by inner wall emerging through the outer wall) *and have a prominently thickened wall and a clear indentation into the inner (bottom) surface of the flattened apex* (rather than a central canal as in Clavicipitaceae); *conidia tetraradiate* (with multiseptate arms radiating from small, basal cell)

Major taxonomic subdivisions:

subgenus *Leprieuria* — host in Coccidae; long sterile hyphae (paraphyses) are *present* in the conidial hymenium
subgenus *Aschersonia* — host in Aleyrodidae; long sterile hyphae (paraphyses) are *absent* in the conidial hymenium

NOTE: PhD research by Miao Liu at Cornell Univ. being prepared in late 2005 for publication indicates that Petch's subgeneric distinctions are not supported by genomic evidence

Priority of characters for species identification:

stromatic morphology (size, shape, color, etc.) > conidial morphology (size, shape) > host

Key Literature:

Petch T. 1914. The genera *Hypocrella* and *Aschersonia*. *Ann. Roy. Bot. Gard. Peradeniya* 5: 521-537.

Petch T. 1921a. Studies in entomogenous fungi. II. The genera of *Hypocrella* and *Aschersonia*. *Ann. Roy. Bot. Gard. Peradeniya* 7: 167-278.

Mains EB. 1959a. North American species of *Aschersonia* parasitic on Aleyrodidae. *J. Insect Pathol.* 1: 43-47.

Mains EB. 1959b. Species of *Hypocrella*. *Mycopathol. Mycol. Appl.* 11: 311-326.

Mains EB. 1959c. Species of *Aschersonia* (Sphaeropsidales). *Lloydia* 22: 215-221.

[Taxonomic revisions for some taxa will be published by Miao Liu with Kathie T. Hodge and Priscila Chaverri]

STROMA	CONIDIA	HOST	SPECIES
flattened or centrally thickened, ± round, 1.5-5 mm diam., white to cream, with several (often radially arranged) fertile chambers releasing slimy masses of yellow to carrot-orange conidia	9-16 x 1.5-2 µm, fusoid to needle-like	whitefly (Aleyrodidae)	***aleyrodis*** syn:: ***A. goldiana*** teleo: *H. libera*
hemispherical to subcylindric, 1-4 mm diam, brown to orange-brown with a thin white layer of hyphae surrounding stroma and extending a short distance, with a single chamber in which red-brown conidia form	11-14 x 3-5 µm, broad and fusoid	scale (Coccidae)	***cubensis***
irregularly shaped, columnar masses 1 to several mm in diam., white (becoming dark with age) bearing one or more distinct cup-like outgrowths on surface with one fertile conidial chamber in base of each cup producing slime mass of yellow to red conidia	10-13 x 3.5-4 µm, fusoid with tips slightly drawn out (like a short spicule or spine)		***turbinata*** teleo: *H. turbinata*

BEAUVERIA Vuillemin

Sordariomycetes: Hypocreales: anamorphic Clavicipitaceae
[Deuteromycota: Hyphomycetes]

On an host, mycelium growing emerging through host exoskeleton to form dense white covering on surface, occasionally forming synnemata; conidiogenous cells densely clustered or in whorls or solitary, colorless, short, with base globose or flask-like and extending apically and repeatedly branching a short distance below each of several apically-formed conidia (forming a denticulate); conidia 1-celled, hyaline.

Differences from other similar genera:

Tritirachium: conidiogenous cells with elongated rachis, but conidia separate from scars not raised on denticles *vs. Beauveria with rachis always denticulate (conidia borne on small projections from rachis)*

Hirsutella: phialides strongly similar to *Beauveria* conidiogenous cells that have formed only a single conidium, but *Hirsutella* polyphialidic or producing several conidia in slime droplet *vs. Beauveria in which neck always elongates and bears multiple spores on separate denticles with conidia never borne in slime and conidiogenous cells usually strongly clustered rather than single.*

Priority of characters for species identification:

conidial size > conidial shape > conidial color > other characters

SPECIAL NOTES: The continuing molecular studies by SA Rehner and colleagues will become the basis for an entirely new classification for this genus and will necessarily depend heavily on genomic characters but will attempt to reconcile traditional taxonomic characters with the molecular evidence.

Although they are only very rarely produced on specimens or in culture, synnemata can be formed by some isolates.

Key Literature:

Hoog GS de. 1972. The genera *Beauveria, Isaria, Tritirachium,* and *Acrodontium* gen. nov.. *Stud. Mycol.* 1: 1-41.

Samson RA, Evans HC. 1982. Two new *Beauveria* spp. from South America. *J. Inverr. Pathol.* 39: 93-97.

Rehner SA. 2005. Phylogenetics of the insect pathogenic genus *Beauveria.* In *Insect-Fungal Associations: Ecology and Evolution* (FE Vega, M Blackwell, eds): 3-27. Oxford Univ. Press: Oxford.

Rehner SA, Buckley E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84-98.

LENGTH of conidia	**SHAPE** of conidia	**COLOR** of conidia or colony	**CONIDIOPHORE** and other key characters	**SPECIES**
1.5-3.5 µm	globose or subglobose; in globose clusters	White to cream spores [pink-gray, pink-tan, tan, gray]; colony reverse pale to yellow	***c'phore*** smooth and colorless; conidial chains often long and conidial heads diffuse	***bassiana*** species complex with ≥3 components
3-6 µm	(distinctly) ovoid; in gluobose clusters	White to cream spores; colony reverse may be colorless to slightly yellow	***c'phore*** may be roughened and slightly colored; conidial heads often compact with short conidial chains	***brongniartii*** species complex with ≥2 components [not in N America??]
	± ovoid, many bulging on one side or irregular			***amorpha*** [S America, Asia]
	Long ovoid to cylindric, many spores gently curved		Conidiogenous cells arising singly or in clusters, often on short side branches; forming compact spore balls	***caledonica*** [Europe

EVLACHOVAEA Borisov & Tarasov

Sordariomycetes: Hypocreales: anamorphic Clavicipitaceae
[Deuteromycota: Hyphomycetes]

Colonies in culture white, sometimes becoming pink to orange with age, with reverse sometimes showing exudation of red pigments or colorless to slightly yellow; conidiophores not markedly differentiated from vegetative hyphae, mononematous or synnematous; conidiogenous cells variable in shape either awl-like and without distinct neck or flask-like with distinct neck, borne singly, in pairs, in whorls or in *Paecilomyces*-like clusters; conidia aseptate, smooth, variable in shape from ovoid or elongate/cylindrical to more or less wedge-shaped, formed out of axis of conidiogenous cells with successive conidia formed to opposite side resulting in flat, ribbon-like chains of conidia with a zipper-like (or herringbone or chevron-like) appearance; chlamydospores, if present, broadly clavate and curved, one-celled or as series of 2-4 cells. Species may have wide distributions and host ranges affecting insects from multiple orders or families but the taxonomy of this genus remains largely unaddressed with several new species from Asia and South America still awaiting formal description. Teleomorphs (where known): species of *Cordyceps*.

Differences from other similar genera:

Paecilomyces: conidiogenous cells and conidial shapes like those of many *Paecilomyces* species, but differing in end-to-end arrangement of conidia in chains of Paecilomyces versus *the alternating angles of conidia in Evlachovaea and flat, ribbon-like chains*

***Lecanicillium* and other *Verticillium*-like genera:** conidiogenous cells of some *Evlachovaea* spp awl-like and arranged as in verticillioid genera and conidial shapes can resemble those of verticilliod fungi but conidial chains of *Evlachovaea spp never slime down to form mucoid spore balls*

Mariannaea: As in the anamorph of *Cordyceps militaris*, *Mariannaea* species procude elongate conidia at an angle from the apex of conidiogenous cells and accumulate in ribbon-like flat imbricate chains in which conidia attach side-by-side and in parallel manner (all in the same direction) but then slime down into mucoid spore balls whereas *conidial arrangement in Evlachovaea chains alternates from one side to the other to yield zipper-like chains*

Priority of characters for species identification:

conidial morphology > chlamydospore presence/morphology > geographical origin > host

Key Literature:

Borisov BA, Tarasov KL. 1999. Notes on biodiversity of causal agents of invertebrate mycoses in Adjaria (south-western Georgia). 1. *Evlachovaea kintrischica* gen. et sp. nov. (Hyphomycetes) from Kintrishi Reservation. *Mikol. Fitopatol.* 33: 248-256.

Humber RA, Tanzini MR, Alves SB. 2002. *Evlachovaea*: First reports of an unusual and little known entomopathogenic fungal genus from the New World. Abstracts, 8th Internat. Colloq. Invertebr. Pathol. and Microb. Control, Foz do Iguassu, Brazil, p. 74-75.

Luz C, Rocha LFN, Humber RA. 2003. Record of *Evlachovaea* sp. (Hyphomycetes) on *Triatoma sordida* in the State of Goiás, Brazil, and its activity against *Triatoma infestans* (Reduviidae, Triatominae). *J. Med. Entomol.* 40: 451-454.

Sung G-H, Spatafora JW. 2004. *Cordyceps cardinalis*, a new species of *Cordyceps* with an east Asian-eastern North American distribution. *Mycologia* 96: 658-666.

CONIDIA	GEOGRAPHICAL ORIGIN	SPECIES
triangular (wedge-shaped) to ovoid or subglobose, ca 3-4.5 x 2.5-3.5 µm, in irregular chains; chlamydospores lateral, irregular in shape but tending to be curved and expanding at apex	Georgia (Adjaria, near Black Sea)	***kintrischica***
± cylindrical with rounded ends, 4-6 x 1.5-2.5 µm; chlamydospores absent	Japan, US Brazil, Colombia	Anamorph of ***Cordyceps cardinalis***

HIRSUTELLA Patouillard

Sordariomycetes: Hypocreales: anamorphic Clavicipitaceae
[Deuteromycota: Hyphomycetes]

Synnemata (if present) erect, cylindrical or slightly tapered, varying from short and verrucose to long and hair-like, unbranched to sparingly branched or with many short side branches; conidiogenous cells (phialides) scattered to crowded, projecting laterally from synnema or from nonsynnematous hyphae emerging from host body, with swollen (flask-like to subglobose) base and narrowing into a slender, elongated neck with some species producing one or more shorter secondary necks (polyphialides); conidia 1-celled, hyaline, oblong to subcylindric, round, rhombic, or like the segment of a citrus fruit, and usually covered by persistent mucus, borne singly or in small groups in mucoid droplets.

Differences from other similar genera:

Tolypocladium: phialides often in clustered heads (and rarely on synnemata) whose necks often bend out of axis of the phialides and bear multispored slime droplets vs. *Hirsutella often synnematous and with conidia formed singly or only in small slime drops with few (usually <5) conidia.*

Beauveria: conidiogenous cells resembling Hirsutella after formation of only first conidium but distinctly different as the rachis elongates and multiple conidia are formed on separate denticles vs. *Hirsutella in which the necks are never denticulate (and shorter secondary necks, if formed) usually emerge from the base of the phialide and never form dense clusters resulting in the characteristic Beauveria spore balls*

Priority of characters for species identification:

host > synnemal characters > conidial morphology

SPECIAL NOTE: This genus has been one of the most difficult for identifications among all major genera of fungal entomopathogens largely because of the very large number of species, high variability among these species, and (sadly!) the lack of any monographic treatment of the genus of any comprehensive key to its species.

Key Literature:

The literature for this genus is spread throughout the scientific literature; no formal monograph of *Hirsutella* has yt been published, although some (unpublished) summaries of key taxonomic characters are available; characters for this chart are drawn from a recent PhD dissertation by Kathie Hodge. The following are some essential references.

Speare AT. 1920. On certain entomogenous fungi. *Mycologia* 12: 62-76.

Mains EB. 1951. Entomogenous species of *Hirsutella*, *Tilachlidium*, and *Synnematium*. *Mycologia* 43: 691-718.

Minter DW, Brady BL. 1980. Mononematous species of *Hirsutella. Trans. Brit. Mycol. Soc.* 74: 271-282.

Minter DW, Brady BL, Hall RA. 1983. Five hyphomycetes isolated from eriophyid mites. *Trans. Brit. Mycol. Soc.* 81: 455-471.

Samson RA, McCoy CW, O'Donnell KL. 1980. Taxonomy of the acarine parasite *Hirsutella thompsonii*. *Mycologia* 72: 359-377.

Hodge KT. 1998. Revisionary studies in *Hirsutella* (Anamorphic Hypocreales: Clavicipitaceae). PhD dissertation, Cornell University.

Hirsutella (continued)

HOST	SYNNEMATA	CONIDIAL MORPHOLOGY	SPECIES
nematode or mite	nonsynnematous	ovoid to ellipsoid, 5 -8.5 x 2.3-4.5 µm (up to 3 per mucoid drop)	***rhossilisnsis***
Acarida	synnemata rare (and *only* in culture)	globose, surface smooth or warty, often with no obvious mucoid coat, 3-4 µm diameter	***thompsonii***
Lepidoptera	1-10 x ca. 0.5 mm (var. *longicolla*) 20-25 x ca. 0.5 mm (var. *cornuta*), dark olivaceous brown to black, and lighter toward tips	Boat-shaped (naviculoid) to cylindrical, usually flattened laterally, 6-11 x 2.5-5.0 µm (up to 3 per mucoid drop)	***longicolla***
	20-100 x 0.5-1 µm, dark olivaceous but lighter in color towards apices	ellipsoid, 5-8 x 2-2.5 µm	***gigantea***
Hymenoptera	long (20-30 mm), thin, leathery, rarely branched, dark brown to black	bean-shaped (allantoid), 9-11 x 1-1.5 µm	***saussurei***
Hemiptera	numerous on individual hosts, long (0.3–15 mm), with very many easily dislodged, short synnemata borne perpendicularly to main axis)	Long fusoid to slightly curved, 5.0-8.5 x 1.5-2.5 µm (up to 3 per mucoid drop)	***citriformis*** global but most common in SE Asia

LECANICILLIUM Gams & Zare
[*VERTICILLIUM* Nees]

Sordariomycetes: Hypocreales: anamorphic Clavicipitaceae
[Deuteromycota: Hyphomycetes]

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. Teleomorphs (where known): *Torrubiella.*

Differences from other similar genera:

***Hirsutella*:** phialides with swollen bases but necks sometimes indistinct (especially on nonsynnematous species), but when phialides of this type, spores are borne only singly or in very small groups (2-5) vs. *Verticillium in which clusters of conidia tend to contain larger numbers of spores and conidiogenous do not have distinct necks*

***Tolypocladium*:** phialides often in dense groups or clusters, but not necessarily whorled, with prominent necks frequently seen to be bent out of axis of conidiogenous cells, with mucoid slime droplets containing numerous conidia vs. *Verticillium in which arrangement of conidiogenous cells is never so strongly clustered and conidiogenous cells do not have distinct necks*

Evlachovaea*:** confusion occurs only for species forming dry conidial chains that later slime down (see ***Special Note below). Conidiogenous cells of *Evlachovaea* spp can be awl-like and in pairs or whorls, and the conidia are always formed at an angle to the axis of the conidiogenous cell, while alternating their direction (resulting in zipper-like chains of conidia) that *do no slime down* as will *Lecanicillium* spp (and whose chains are never zipper- or chevron-like because of the alternating orientations of conidia).

Major taxonomic subdivisions and changes:

Verticillium Section *Prostrata* (Gams 1971)— phialides on vegetative hyphae with no erect conidiophores formed; this section including all entomopathogenic spp; teleomorphs in Clavicipitaceae (Pyrenomycetes: Hypocreales) and especially with *Cordyceps* spp for entomopathogens

Beginning in 2000, a necessary revision reclassified all species of *Verticillium* sect. *Prostrata* to other genera. The great majority of entomopathogenic *Verticillium*s were put in the new genus *Lecanicillium*, typified by *L. lecanii* (Zimmermann) Zare & Gams, and *Verticillium lecanii* species complex [▮▮▮▮▮▮] was resolved into three named species as noted in the key below.

Priority of characters for species identification:

conidial characters > conidiogenous cell

SPECIAL NOTE: Identifications can be complicated in species where conidia are at first produced in chains (either end-to-end OR side-to-side and at an angle to the axis of the conidiogenous cells, as in *Mariannaea* spp) but that then slime down into typical spore balls; the otherwise unnamed *Lecanicillium* anamorph of *Cordyceps militaris* is in this group of species.

Key Literature:

Gams W. 1971. Cephalosporium-*artige Schimmelpilze (Hyphomycetes)*. Gustav Fischer Verlag, Stuttgart. 262 pp.

Gams W. 1988. A contribution to the knowledge of nematophagous species of *Verticillium*. *Neth. J. Plant Path.* 94: 123-148.

Zare R, Gams W, Culham A. 2000. A revision of *Verticillium* sect. *Prostrata* I. Phylogenetic studies using ITS sequences. *Nova Hedwigia* 71: 465-480.

Sung GH, Spatafora JW, Zare R, Hodge KT, Gams W. 2001. A revision of *Verticillium* sect. *Prostrata*. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. *Nova Hedwigia* 72: 311-328.

Gams W, Zare R. 2001. A revision of *Verticillium* sect. *Prostrata*. III. Generic classification. *Nova Hedwigia* 72: 329-337.

Zare R, Gams W. 2001. A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium*. *Nova Hedwigia* 73: 1-50.

Zare R, Gams W, Evans HC. 2001. A revision of *Verticillium* section *Prostrata*. V. The genus *Pochonia*, with notes on *Rotiferophthora*. Nova Hedwigia 73: 51-86.

Zare R, Gams W. 2001. A revision of *Verticillium* section *Prostrata*. VI. The genus *Haptocillium*. *Nova Hedwigia* 73: 271-292.

Lecanicillium (continued)

CONIDIA	CONIDIOGENOUS CELLS	SPECIES
2.5-3.5 x 1-1.5 µm usually very uniform in shape	11-20 x 1.3-1.8 µm, strongly tapered Frequently forming short secondary necks projecting from apical part of phialide	***L. lecanii***
2.5-6 x 1-1.8 µm distinctly variable in size in any specimen or culture	20-35 x 1.1-1.7 µm, secondary necks very uncommon	***L. muscarium***
5.0-10.5 x 1.5-2.5 µm	20-40 x 1.2-2.7 µm. secondary necks uncommon	***L. longisporum***
3-5 x 1.3-2.0 µm, broadly fusoid (with blunt apices)	awl-like, tapering uniformly from base 16-26 x 1-1.5 µm	***L. fusisporum***
macroconidia: falcate (curved with ± pointed ends), 5-10 x 1.2-1,7 µm; *microconidia* (if present): oval-ellipsoid, 2.7-3.7 x 1-1.5 µm	Long, tapering uniformly, 25-35 x 1-1.5 µm	***L. psalliotae***
2.7-4 x 1.5-2.2 µm ovoid to subglobose, produced singly	Single, paired, whorled or clustered; flask-shaped at first but desiccating to fine denticle	***L. aphanocladii***

METARHIZIUM Sorokin

Sordariomycetes: Hypocreales: anamorphic Clavicipitaceae
[Deuteromycota: Hyphomycetes]

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.

Differences from other similar genera:

This genus is highly distinctive and not likely to be confused with any other fungus affecting insects. The characteristic branching pattern of the conidiophores and alignment of conidiogenous cells in dense, plate-like hymenial zones is a major diagnostic feature of this genus. Although they are only very rarely produced, synnemata can be observed. A nonentomogenous genus (*Myrothecium*) has a morphology much like that of *Metarhizium* except that its conidia are released in copious numbers into a slime mass whereas *Metarhizium* conidia are always produced in dry chains.

Priority of characters for species identification:

conidial characters > conidiogenous cell (phialide) morphology > conidial color > other characters

SPECIAL NOTE: The 'classical' taxonomy of this genus has been upheld but expanded by the wholly sequence-driven classification proposed by Driver et al. (2000). No proper identification of *Metarhizium* isolates can now be done except in comparison to the Driver classification that recognized a total of four varieties within *M. anisopliae*, and four varieties plus one other distinct but still unnamed additional variety under *M. flavoviride* as well as *M. album* (a rare fungus known only from the Philippines).

Key Literature:

Rombach MC, Humber RA, Roberts DW. 1986. *Metarhizium flavoviride* var. *minus* var. nov., a pathogen of plant- and leafhoppers of rice in the Philippines and Solomon Islands. *Mycotaxon* 27, 87-92.

Rombach MC, Humber RA, Evans HC. 1987. *Metarhizium album* Petch, a fungal pathogen of leaf- and planthoppers of rice. *Trans. Br. Mycol. Soc.* 88, 451-459.

Driver F, Milner RJ, Trueman JWH. 2000. A taxonomic revision of *Metarhizium* based on a phylogenetic analysis of rDNA sequence data. *Mycol. Res.* 104: 134-150.

CONIDIA	PHIALIDE	COLOR of conidia	OTHER CHARACTERS	SPECIES
9 µm long, cylindrical, often with middle ± constricted	cylindrical	green (many possible shades), yellow-green, brown (often with green tones) to yellow	ubiquitous, with extremely wide host range, more often affecting hosts in soil than on aerial plant parts	***anisopliae*** var. ***anisopliae***
11 µm long (shape same as for *M.a. anisopliae*)			widely distributed; possibly stable diploid form of *M.a.* var. *anisopliae*	***anisopliae*** var. ***majus***
7-9 µm long, ± ovoid		Dull grey-green; often comparatively slow to begin to sporulate on cultures	affecting Orthoptera; best known from Africa; previously identified as *M. flavoviride*	***anisopliae*** var. ***acridum***
7-9 µm long ovoid	clavate (wider at apex)	dull yellowish green or grey-green	Grows more slowly and sporulates later in culture than *M. anisopliae*; widely distributed	***flavoviride*** var. ***flavoviride***
7 µm long ovoid				***flavoviride*** var. ***minus***
4-6 x 1.5-2.5 µm ovoid	Clavate, with apex sometimes nearly conical	white to pale brown	Conidia in powdery masses rather than compact columns	***album***

NOMURAEA Maublanc

Sordariomycetes: Hypocreales: anamorphic Clavicipitaceae
[Deuteromycota: Hyphomycetes]

Mycelium septate, white, with flocculent overgrowth, sparse in culture to dense on insects (often completely covering the host), usually becoming green, or purple-gray 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-gray to purple, or (rarely) white in mass.

Differences from other similar genera:

***Paecilomyces*:** phialides with distinct necks bearing long chains of conidia vs. *Nomuraea with conidia only in short chains and conidiogenous cells in tightly clustered whorls spaced along erect conidiophores*

***Penicillium*:** phialides cylindrical (somewhat elongated, not short and blocky) appearing singly or in small groups but usually at apices of conidiophores and producing globose conidia in long dry (and often columnar) chains vs. *Nomuraea with short, nearly cuboid phialides in whorled clusters spaced along conidiophore and forming only short chains of nonglobose conidia*

Priority of characters for species identification:

host > conidial morphology

Key Literature:

Samson RA. 1974. *Paecilomyces* and some allied Hyphomycetes. *Stud. Mycol.* 6: 1-119.

Tzean SS, Hsieh LS, Chen JL, Wu WJ. 1992. *Nomuraea viridulus* a new entomogenous fungus from Taiwan. *Mycologia* 84: 781-786.

Tzean SS, Hsieh LS, Chen JL, Wu WJ. 1993. *Nomuraea cylindrospora* comb. nov. *Mycologia* 85: 514-519.

HOST	CONIDIA	SPECIES
Lepidoptera (Noctuidae)	ovoid dull grey-green (seafoam green), rarely white 3.5-4.5 x 1-2 µm	***rileyi***
spider	ovoid to cylindrical or fusoid dull lavender to (pinkish to purplish) grey 3-8 x 2-4 µm	***atypicola***

PAECILOMYCES **Bainier**
ISARIA **Persoon : Fries**

Sordariomycetes: Hypocreales: anamorphic Clavicipitaceae
[Deuteromycota: Hyphomycetes]

Conidiophores usually well developed, synnematous in many species, septate, bearing whorls of divergent branches and conidiogenous cells (phialides), hyaline to pigmented (not black, brown, or olive); conidiogenous cells flask- to awl-shaped or subglobose with a distinct neck, borne singly or in whorls; conidia 1-celled, hyaline to light colored (shades of yellow through red, tan, lavender, or grass-green), in dry divergent chains.

Differences from other similar genera:

***Penicillium*:** phialides rarely have narrower, elongated apices (necks); conidia mostly globose; colors mostly 'dark' and various shades of green (but not grassy green) vs. Paecilomyces phialides with distinct necks, conidia almost never globose, colors white to 'bright' (shades of yellow, pink, red, orange, tan, lavender)

***Nomuraea*:** phialides short, blocky, with no distinct necks, in small clusters on (conidiophores with distinct 'muffs' of phialides and conidial chains spaced on conidiophores); conidia in short chains (3-5 conidia) vs. Paecilomyces phialides usually elongated and with distinct necks, not in distinctly 'bead-like' clusters along conidiophore, and conidial chains usually relatively long

Evlachovaea*:** Conidiogenous cells of *Evlachovaea* spp can be awl-like and in pairs or whorls, but they can also be in the sorts of clusters typical of *Paecilomyces*. Where all *Paecilomyces conidia are produced in the axis and of conidiogenous cells and connect in end-to-end in chains*; *Evlachovaea* conidia form at an angle to the axis of the conidiogenous cell and alternating their direction (resulting in zipper-like conidial chains that do no slime down (but also see the notes in the discussion of ***Lecanicillium/Verticillium).

Major taxonomic subdivisions:

Paecilomyces Section *Paecilomyces* — no entomopathogens; teleomorphs in Plectomycetes (Byssochlamys, etc.)
Paecilomyces Section *Isarioidea* — all entomopathogenic spp; teleomorphs in Pyrenomycetes (Hypocreales, Clavicipitaceae, and especially with *Cordyceps* spp for entomopathogens

SPECIAL NOTES: A taxonomic revision of *Paecilomyces* similar to that of *Verticillium* from 2000 is just now beginning: Hodge *et al.* (2005) and Gams *et al.* (2005) have published the first steps to reclassify all clavicipitaceous taxa. Samson (1974) placed all of these fungi in question in *Paecilomyces* sect. *Isarioidea*; they will have to be removed from *Paecilomyces*, with nearly all being moved to (or again to be used in) *Isaria*. So far, **only** *P. farinosus* that has been moved formally to *Isaria*; this generic name has been formally proposed to be conserved with *I. farinosa* as its type species to be conserved against two other Persoon species of *Isaria*.

Priority of characters for species identification:

conidial size > conidial shape > conidial color > other characters

SPECIAL NOTE: Along with the shift of species of *Isaria,* a major genomically based molecular revision of entomopathogenic *Paecilomyces* species is overdue and can be expected to be completed in the next several years.

Key Literature:

Brown AHS, Smith G. 1957. The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. *Trans. Brit. Mycol. Soc.* 40: 17-89.
Samson RA. 1974. *Paecilomyces* and some allied Hyphomycetes. *Stud. Mycol.* 6: 1-119.
Hodge KT, Gams W, Samson RA, Korf RP. 2005. Lectotypification and status of *Isaria* Pers. : Fr. *Taxon* 54: 485-489.
Gams W, Hodge KT, Samson RA, Korf RP, Seifert KA. 2005. (1684) Proposal to conserve the name *Isaria* (anamorphic fungi) with a conserved type. *Taxon* 54: 537.

Paecilomyces / Isaria (continued)

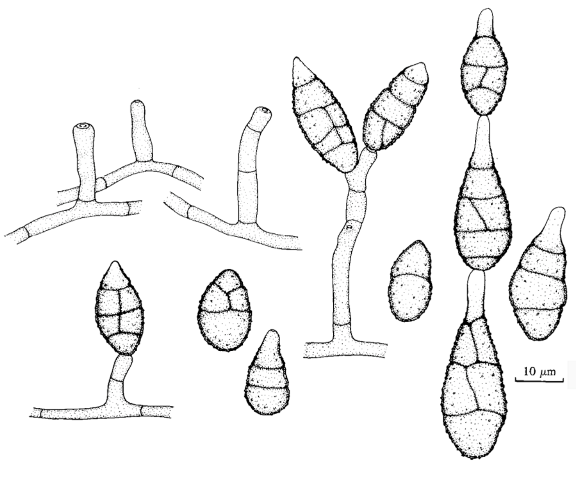
LENGTH of conidia	**SHAPE** of conidia	**COLOR** of conidia or colony	**CONIDIOPHORE** and other key characters	**SPECIES**
≤3.5-4.0 µm	long ovoid	pink-gray, pink-tan, tan, gray; reverse pale to yellow	***c'phore*** smooth and colorless; conidial chains often long and conidial heads diffuse	***fumosoroseus***
	short fusoid, lemon-shaped	purple-gray, tan, gray; colony reverse may be dark	***c'phore*** often roughened and slightly colored; conidial heads often compact with short conidial chains	***lilacinus***
	lemon-shaped, short ovoid	white, cream to yellowish; reverse may be yellow	***c'phore*** smoth and colorless	***farinosus***
	subglobose to angular	pink, red or wine-colored		***amoenoroseus***
≥5 µm	long ovoid or long fusoid	white/cream		***javanicus***

COMMON FUNGAL CONTAMINANTS OF INSECT CADAVERS

Careful testing is required to determine whether any fungi in these genera are primary pathogens, facultative pathogens, or contaminant saprobes.

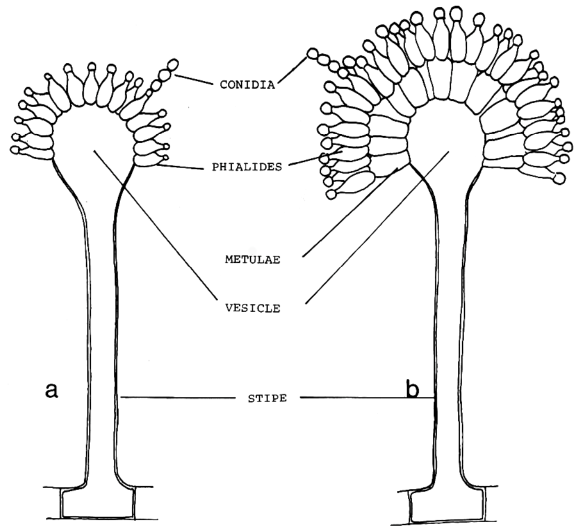
Alternaria

Conidia dark colored, drop-shaped, flat, multicellular (with longitudinal, oblique *and* transverse septa), in chains arising from pores on conidiophores that are often melanized.



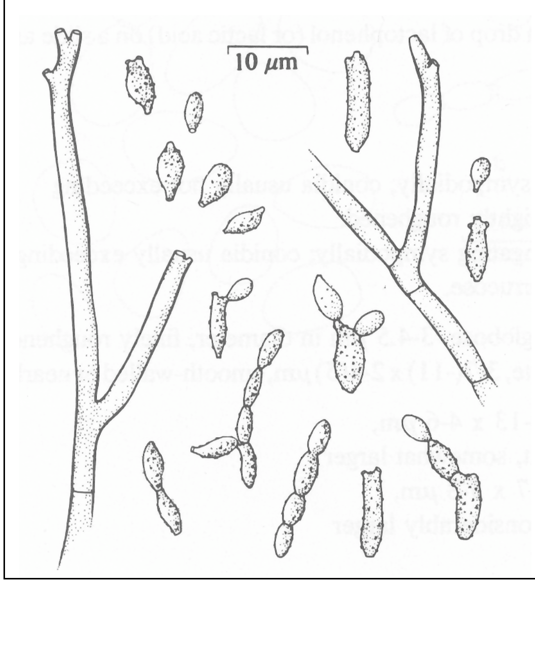
Aspergillus

Conidia generally globose with smooth or decorated surfaces in chains on flask-shaped phialides with a short, broad neck (or no distinct neck) on one or two layers of sterile cells (metulae) on apical vesicle atop differentiated conidiophores; conidiophore base is an inverted 'T' shape.



Cladosporium

Conidial shapes highly variable but always showing prominent small, flat attachment scars; branched chains easily disrupted, arising from pores on dark (to pale) conidiophores; new conidia form at apices (rather than at bases) of chains.



Penicillium

Conidiophores prominent, erect, bearing brush-like clusters of flask-shaped, short-necked (or neckless) phialides forming long chains of globose (to ovoid) conidia with smooth or rough surfaces; conidia in blue-green to ochre or dark colors (rarely white or shades of yellow, red or orange).