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Coelomycetous fungi in human disease. A review: Clinical entities, pathogenesis, identification and therapy

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Summary

Coelomycetes are being recovered with increasing frequency in human disease. They are frequently acquired by traumatic implantation and are of concern in profoundly immunosuppressed individuals. A definition of this group of organisms is provided, along with their clinical manifestations, methods for laboratory diagnosis, criteria for identification, *in vitro* susceptibility data, and guidelines for antifungal therapy and management.

Key words Coelomycetes, Human mycoses, Antifungal therapy, *In vitro* susceptibility

Coelomicetos y enfermedad humana. Una revisión: entidades clínicas, patogenia, identificación y tratamiento

Resumen

Cada vez se aislan con mayor frecuencia coelomicetos asociados a enfermedades humanas. A menudo se adquieren por implantación traumática y afectan a personas profundamente inmunosuprimidas. Esta revisión ofrece una definición de este grupo de microorganismos y sus manifestaciones clínicas, los métodos para el diagnóstico de laboratorio, los criterios para su identificación, los datos de sensibilidad *in vitro* a los antifúngicos y las orientaciones para el tratamiento de estas micosis.

Palabras clave Coelomicetos, Micosis humanas, Terapia antifúngica, Sensibilidad *in vitro*

Although the majority of opportunistic mycoses due to filamentous fungi are caused by hyphomycetous organisms, i.e., fungi that bear their conidia free such as aspergilli, fusaria, and numerous other genera, there are increasing reports of both cutaneous/subcutaneous and invasive disease due to the Coelomycetes. These asexual fungi, unlike the Hyphomycetes, produce their conidia within some type of enclosed or semi-enclosed structure [1,2]. Although these categories Coelomycetes and Hyphomycetes have been rejected as formal taxonomic ranks, they are retained as general descriptors of major morphological characteristics. Bearing conidia within structures, they are not ubiquitous airborne organisms, and are therefore not as likely to be acquired by inhalation. More frequently they are implanted through some type of traumatic event. Immune competent hosts are generally more able to resist these organism than are those immunosuppressed due to either immunocompromising disease entitites, or suppression as a result of aggressive therapeutic modalities. This review will attempt to alert clinicians and laboratorians to consider these organisms in contemporary medicine, to enumerate clinical entities attributed to Coelomycetes, to provide morphologic and microscopic characteristics useful for their identification, and to offer some guidelines relative to management and antifungal therapy.

DEFINITION OF COELOMYCETES

Coelomycetous fungi are parasites and saprobes of terrestrial vascular plants inhabiting twigs, branches and leaves of various plant hosts. These organisms may also be parasites of other fungi, and are ubiquitous in soil, salt and freshwater environments, and in sewage [1]. They are mitosporic, asexual fungi that produce their reproductive propagules (conidia = mitospores) in fruiting bodies known as conidiomata. These fruiting bodies are in contrast to those produced by the sexual, meiotic Ascomycetes, known as ascomata and containing sexual ascospores enclosed in asci [2,3]. Species of Coelomycetes that have been determined to have sexual states are usually connected to various genera of

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Ascomycetes, although more recent data suggests basidiomycetous connections as well [4].

Coelomycetes have traditionally been grouped into the Orders Melanconiales and Sphaeropsidales depending upon their production of either acervuli or pycnidia, respectively. This distinction is loosing favor, however, as many overlapping intermediate forms create a continuum between these traditional categories [1]. Pycnidia are spherical to subspherical structures that usually have an opening (ostiole) in the top portion of the conidioma (Figure 3c). Their outer walls are multicellular and are composed of various types of coverings defined by the shape of the cells, i.e., textura angularis (Figure 4b), textura imbricata, textura prismatica, etc. Pycnidia may be separate (Figures 4a, 4b) or aggregated, superficial or immersed (Figure 5b). In acervuli, the basal stroma lacks lateral and upper walls, and no specialized method of dehiscense (opening of the fruit body) exists. Acervuli are immersed, and may be separate or confluent (Figure 2c). Stromata are in the intermediate classification, and are usually immersed in host tissue. They may be convoluted, uni- or multilocular (locule defined as a cavity enclosed by fungal, host or host/fungal tissue, within which conidia are produced) (Figure 5c), superficial or immersed, of various shapes, thick, multicellular walls, pigmented yellow, orange, brown or black, separate or aggregated, with one or more dehiscent ostioles. Conidiogenous cells formed on the inner surface of the walls of fruiting bodies may be thallic (formed from preexisting hyphae) or blastic (blown out). Blastic conidia are produced primarily by either phialides or annellides, and are characterized by their size, shape, appendages, septations, color, and ornamentation.

Many Coelomycetes are further characterized by their host-specificity, and species that do not vary morphologically do so in their pathogenicity for various hosts. A further significant dilemma in characterizing Coelomycetes grown in the laboratory has to do with the diversity seen in artifical cultures. Although the genera *Phomopsis* and *Pestaloptiopsis* appear to exhibit limited cultural deviation [1], other genera show considerable variability in their cultural and morphological features, ability and extent of conidiation, formation of sclerotia, chlamydoconidia and/or appressoria.

CLINICAL MANIFESTATIONS

Coelomycetes incite a variety of clinical entities. As mentioned, the method of acquisition is frequently by implantation of the fungus from plant/woody material or the soil through abrasions, lacerations, puncture wounds or other traumas into cutaneous/subcutaneous tissue, rather than by inhalation of conidia. Initial presentations in this setting are often superficial or ocular, with subsequent progression to invasive, subcutaneous disease. Of concern in the immunocompromised host is the potential for dissemination to other sites. Those compromised patients most at risk for mycoses due to these organisms (as well as for other non-coelomycetous agents) include bone marrow transplant recipients, solid organ transplant recipients, cancer patients, diabetics, and those with other immunosuppressions due to long term steroid use, and other immunocompromising conditions. Coelomycetes are documented etiologic agents in the clinical entities cited in Table 1. They include eumycotic black grain mycetomas by *Pyrenochaeta* (*Phoma*) *romeroi* [5], *P. mackinno-*

Table 1. Mycoses caused by Coelomycetes.

Clinical Entities	Organism(s)[a]	Reference(s)[c]
Eumycotic black grain mycetoma	*Pyrenochaeta* (*Phoma*) *romeroi*	5
	Pyrenochaeta mackinnonii	5
	Pseudochaetosphaeronema larense	6
Onychomycosis	*Nattrassia mangiferae* [b]	7,8,9,10
	Botryodiplodia theobromae	5
Cutaneous phaeohyphomycosis	*Nattrassia mangiferae* [b]	9,10
Subcutaneous phaeohyphomycosis	*Pyrenochaeta* (*Phoma*) *romeroi*	11
	Nattrassia mangiferae [b]	12,13,14
	Pleurophoma species	15
	Pleurophomopsis lignicola	16
	Botryodiplodia theobromae	17
	Phoma hibernica	18
	Phoma cruris-hominis	5
	Phoma glomerata	5
	Phoma cava	19
	Phoma minutella	20
	Phoma eupyrena	21
	Phoma minutispora	3
	Phoma sorghina	22
	Phoma species	15,23
Keratitis/Keratomycosis	*Colletotrichum dematium*	24
	Colletotrichum gloeosporioides	25,26
	Colletotrichum graminicola	27
	Colletotrichum coccodes	28
	Botryodiplodia theobromae	29,30
	Phoma oculo-hominis	5
	Sphaeropsis subglobosa	31
Endophthalmitis	*Nattrassia mangiferae* [b]	32
Systemic/Invasive disease	*Coniothyrium fuckelii*	35,36
	Pleurophoma pleurospora	42
	Phoma species	43
	Nattrassia mangiferae	37,38,39,40,41
Sinusitus (allergic, non-invasive)	*Pleurophomopsis lignicola*	33
Osteomyelitis	*Phomopsis* species	34
Fungemia	*Nattrassia mangiferae* [b]	37,38

a: Organisms recovered from humans but not documented as etiologic agents include *Pestalotiopsis* and *Libertella* species, as well as other unnamed, difficult-to-characterize isolates.
b: Formerly *Hendersonula toruloidea*; includes isolates reported as *Scytalidium dimidiatum* and *Scytalidium hyalinum.*
c: This list is not all inclusive.

nii [5], and *Pseudochaetosphaeronema larense* [6], onychomycosis by *Nattrassia mangiferae* [7-10] and *Botryodiplodia theobromae* [5], cutaneous/subcutaneous phaeohyphomycosis due to *Pyrenochaeta* (*Phoma*) *romeri* [11], *N. mangiferae* [8,9,10,12-14], *Pleurophoma* species [15], *Pleurophomopsis lignicola* [16], *B. theobromae* [17], and several *Phoma* species [3,5,15,18-23], keratitis/keratomycosis by *Colletotrichum* species [24-28] *B. theobromae* [29,30], *P. oculo-hominis* [5], and *Sphaeropsis subglobosa* [31], endophthalmitis by *N. mangiferae* [32], allergic, non-invasive sinusitus by *Pleurophomopsis lignicola* [33], osteomyelitis by a *Phomopsis* species [34], and various presentations of systemic/invasive disease by *Coniothyrium fuckelii* [35,36], *N. mangiferae* [37-41], *Pleurophoma pluerospora* [42], and *Phoma* species [43]. They are now also incriminated in brain abscess formation [37]. Table 2 provides an overview of the numbers, types of organisms, and patient history (when available) for coelomycetous fungi submitted to Fungus Testing Laboratory over the past 13 years. Histopathological documentation of hyphal elements in tissue, as for other mycoses, is necessary to authenticate an organism as an etiologic

Table 2. Prevalence of coelomycetous clinical isolates[a].

Organism	No	Source (No)	Patient History
Colletotrichum species	19	Eye (6)	Corneal ulcers
		Maxillary sinus (2)	Malignancy, liver transplant recipient
		Wounds/pustules (8)	Septic shock
			Bone marrow transplant recipient
		Synovial fluid (1)	
		Abdominal fluid (1)	Peritonitis
		Oral swab (1)	
Coniothyrium fuckelii	6	Skin nodules (6)	
Botryodiplodia theobromae	4	Cornea (4)	Corneal ulcers
Libertella species[b]	19	Bronchial washing (12)	
		Ethmoid sinus (1)	Immunocompromised patient
		Blood (1)	
		Sputum (2)	Hypersensitivity pneumonitis
		Skin (1)	Skin from foot and hand
		Vitreous fluid (1)	Endophthalmitis
		Elbow (1)	Chronic infection
Microsphaeropsis olivacea	2	Right vitreous (1)	
		Toe (2)	
Nattrassia mangiferae[c]	21	Joint (1)	
		Bronchial washing (1)	
		Nails (3)	
		Arm lesion(1)	Invasive
		Foot wounds (8)	
		Leg lesions (1)	
		Toes (2)	
		Skin biopsy finger (1)	
		Bronchial washing (1)	
		Blood (1)	(In press)
		Brain abscess (1)	(In press)
Pestalotiopsis species	7	Sinus (1)	
		Fingernail (1)	
		Bronchial biopsy (1)	
		Eye (2)	Corneal abrasions
		Scalp (1)	
		Foot (1)	
Phoma species	33	Cornea (1)	Corneal ulcer
		Bronchial washing (2)	Leukemia, fever (1)
		Sputum (6)	Hemoptysis (1)
		Maxillary sinus (2)	
		Scalp (4)	
		Nails (4)	
		Foot (5)	AIDS patient (1)
		Bone marrow (1)	
		Synovial fluid (1)	
		Dialysis fluid (1)	
		Hip tissue (1)	Chronic septic arthritis
		Superficial wounds (4)	
		Stump wound (1)	
Phomopsis species	5	Shin lesion (1)	
		Cornea (1)	
		Sputum (1)	
		Skin scraping scalp (1)	
		Right distal finger (1)	Osteomyelitis
Pleurophoma species	5	Leg wound (1)	Heart transplant patient
		Forearm wound (1)	Heart transplant patient
		Forearm wound (1)	Repeat culture, patient above
		Dialysis fluid (1)	Peritoneal dialysis
		Finger wound (1)	
Pyrenochaeta species	6	Nail (1)	
		Tibial wound (1)	
		Breast debridement (1)	
		Sinus drainage (1)	Sinusitis
		Fluid aspirate hand (1)	Lymphoma
		Knee wound (1)	

a: Clinical isolates submitted to the Fungus Testing Laboratory for the years 1987 through 1999.
b: Probable anamorphs of diatrypaceous genera *Diatrype* and *Diatrypella.*
c: Include organisms submitted is *Scytalidium dimidiatum* and *Scytalidium hyalinum* [49].

agent. Hyphal elements may exhibit considerable pleomophism in human tissue ranging from moniliform, bead-like yeast forms, to short branched or unbranched hyphae (Figure 3a), to arthroconidial-like forms in skin scrapings and nail clippings.

LABORATORY DIAGNOSIS

Coelomycetous fungi usually exhibit a moderate to rapid growth rate on a variety of routine fungal media, may appear moniliaceous or dematiaceous [44,45], and are not particularly difficult to recover from excised material. The problem lies in promoting diagnostic reproductive structures necessary for characterization of the isolates. Not only is a considerable amount of time required, particularly for the pycnidial species (up to months in some strains), but it is also necessary to utilize a medium upon which these pycnidia will develop. Various coelomycete authorities have suggested that the culture of Coelomycetes on sterilized plant tissue produces conidiomata more representative of those in nature, and that culture on nutrient-rich synthetic media often results in atypical characteristics [1,46]. These anomalies may include acervuli in culture versus pycnidia in nature, as well as several transitional forms in the continuum between Hyphomycetes and Coelomycetes. Although many laboratories use some formulation of potato dextrose agar for filamentous fungi [47], the addition of plant tissue on water agar may facilitate the production of conidiomata. Our experience with carnation leaf agar used for species

Table 3. Features of selected coelomycetes recovered in culture from humans.

Genus/Species	Culture[a]	Conidiomata	Conidiogenesis	Conidia
Nattrassia mangiferae[b]	Black, woolly	Unilocular/multilocular Eustromatic Ostiole in each locule Immersed/erumpent Thick-walled Globose, to 2 mm	Conidiophores absent Phialidic	Hyaline to versicolored 10-16 x 3.5-6.5 µm
Lasiodiplodia theobromae	Black, woolly	Unilocular/multilocular Eustromatic Ostioles absent Immersed/superficial Thick-walled Globose, to 5 mm	Conidiophores absent Phialidic	Hyaline to dark 1-septate Longitudinal striations 20-30 x 10-15 µm
Colletotrichum species (many host-specific)	Variable, woolly Gray-brown Sometimes sclerotia Honey-colored Masses of conidia	Acervular Separate or confluent Sometimes setae Brown appressoria, sometimes complex	Conidiophores present Hyaline to brown, Septate Phialidic	Hyaline, aseptate Straight or curved Sometimes medianly constricted 10-27 x 3-6.5 µm
Phomopsis species (many host-specific)	Variable, woolly Gray-brown	Unilocular/multilocular Eustromatic Separate or aggregated Ostioles single to several Immersed Thick-walled Subglobose	Conidiophores present Septate, branched, hyaline Phialidic	Hyaline *alpha* short, ellipsoidal 2-4 x 5-8 µm *beta* long, filamentous 0.4-0.5 x 18-22 µm
Phoma species[d] (many host-specific)	Variable, woolly Gray-brown	Unilocular Pycnidial Separate or aggregated Ostioles single to several Immersed/semi-immersed Mostly thin-walled	Conidiophores absent[c] Phialidic, hyaline	Hyaline Mostly aseptate Often guttulate[e] Variable size, small, mostly 1.5-4 x 3-6 µm
Pleurophomopsis lignicola	Variable, woolly Gray-brown Reddish-brown	Unilocular/bilocular Pycnidial Prominent necks Superficial or immersed Thick-walled	Conidiophores present Septate with branches below septa Openings not below transverse septa Phialidic	Hyaline, short cylindrical, aseptate, 2.5-3 x 1.5 µm
Pleurophoma species	Variable Olivaceous Brown-gray	Unilocular Pycnidial Separate Ostioles single Superficial Thick-walled With or without short necks	Conidiophores present Septate with branches below septa Openings on branches below transverse septa	Hyaline Short cylindrical Without guttules Variable size, small, mostly 0.5-1.5 x 2-4 µm
Microsphaeropsis species[f]	Variable Brown-gray	Uniloculor Pycnidial Separate Ostioles single Immersed Thin-walled Globose	Conidiophores absent Phialidic	Brown Aseptate Thin or thick-walled Smooth or ornamented Small to larger 1.5-5.5 x 4-9.5 µm
Coniothyrium species	Variable Brown-gray	Unilocular Pycnidial Separate Ostiole central Immersed Thin or thick-walled Globose	Conidiophores absent Annellidic Hyaline or pale brown	Brown 0-1 euseptate 2.5-7 x 4-8.5 µm

a: Potato flakes agar.
b: Prominent dematiaceous arthroconidial *Scytalidium dimidiatum* synanamorph. Recent molecular evidence indicates *Scytalidium hyalinum* is also identical to *N. mangiferae,* and may just be a melanin-deficient cultural mutant of *S. dimidiatum* (49).
c: Conidiophores present in two species, *P. cava* and *P. tracheiphila.*
d: Many species with alternarioid chlamydoconidia.
e: Guttule = small oil droplet; may occur singly or at both ends.
f: Species which cannot be retained in the genus *Coniothyrium* [1].

level identification of fusaria has shown it to be a useful addition to promote conidiomata in some genera, namely the pycnidial species [48] (Figure 5a). Another medium frequently cited as useful in coelomycete studies is oatmeal agar [1].

Upon the macroscopic observation of apparent fruiting structures, examination by a stereoscopic microscopic may reveal conidia being extruded from ostioles. Less mature pycnidia may need to be crushed to determine conidial formation inside. Another more permanent and less disruptive method for observing internal contents in pycnidia is to cut them out of the agar and embed them in paraffin. They may then be sectioned with a microtome and examined utilizing various stains such as the hematoxylin and eosin, periodic acid and Schiff's reagent, or Grocott's methenamine silver nitrate stains (Figures 4d, 5c). The observation of conidia that appear free within the cavity (locule) of the conidioma rather than being enclosed in structures such as asci indicates one is dealing with a coelomycete rather than an ascomycete.

IDENTIFICATION OF ISOLATES

Although some genera of Coelomycetes are quite easily identified, with others there is considerable overlap in the diagnostic characteristics. Additionally, some are in need of revision taxonomically. For example, *Botryodiplodia theobromae* is easily identified on the basis of its large, striate, two-celled conidia (Figures 2a, 2b), and acervular *Colletotrichum* species usually display characteristic appressoria (Figures 1a,1b, 1c, 1d). *Phomopsis* species, when mature, generally display both their *alpha* and *beta* conidia (Figure 5d). *Nattrassia mangiferae*, although very slow to form mature, versicolored (darkened middle cell and pale end cells) pycnidial conidia (Figure 3d), is easily recognized by its distinctive *Scytalidium dimidiatum* and *Scytalidium hyalinum* synanamorphs [49,50] (Figure 3b). *Pyrenochaeta* species usually exhibit setae around their ostioles. *Pestalotiopsis* species have apical and basal appendages (Figures 2c, 2d). The distinction between *Phoma*, *Pleurophoma*, *Pleurophomopsis*, *Coniothyrium* and some species of *Microsphaeropsis* is less clear, however, and differentiation is often based on difficult-to-assess characteristics such as the composition of pycnidial walls, conidiophores versus no conidiophores, features of the conidiophores when present, the type of conidiogenous cells (phialidic versus annellidic), openings in conidiogenous cells, and the color, size, and shape of conidia [51,52]. An example of the dilemma encountered in coelomycete identification is illustrated in the case of *Coniothyrium fuckelii*. Although the original description of the genus *Coniothyrium* described the conidiogenous cells as annellidic, those in *C. fuckelii* appear phialidic, and most authorities now believe this species should more properly be placed in the genus *Microsphaeropsis* [1,53]. The separation of genera based upon the type of conidiogenous cell, i.e., annellidic (percurrently proliferating) versus phialidic, is often extremely problematic without the aid of scanning electron microscopy. For these reasons, until more definitive work is done on the organism that is known variously as *Coniothyrium fuckelii*, a *Pleurophoma* species, or possibly a *Microsphaeropsis* species, some prefer to place this organism in the *Coniothyrium-Microsphaeropsis* Complex [53,54]. In a like manner, many similar-looking genera with small, unicellular, hyaline conidia isolated in routine mycology laboratories are lumped into the genus *Phoma*, or referred to as *Phoma*-like species. Without examination by a coelomycete authority, identification of similar species is extremely difficult. Table 3 provides features useful for identification of selected coelomycetes recovered in culture from humans.

IN VITRO ANTIFUNGAL SUSCEPTIBILITY DATA AND THERAPY

In vitro susceptibility data generated from the FTL is presented in Table 4. Isolates tested before 1997 were evaluated by a macrobroth dilution method previously described and utilized for several years in this facility [55]. Testing from 1997 forward utilized the

Table 4. *In vitro* antifungal susceptibility data generated from the Fungus Testing Laboratory[a].

Organism	AMB[b]		5-FC		FLU		ITRA		MON		KETO		NAT	
	S[c]	R[d]	S	R	S	R	S	R	S	R	S	R	S	R
Colletotrichum species	8[e]		3		1	5	4	3	2		1		3	
Coniothyrium Microsphaeropsis Complex	1				1		1				1			
Botryodiplodia theobromae	2					1		1					2	
Nattrassia mangiferae	2				2	1	1	2			2	1		
Pestalotiopsis species	1		1			1	1						1	
Phoma species	5	2	2		2	2	9				5			
Phomopsis species	3				3		2	1					1	
Pleurophoma species	2		1		2		2		1		2			
Pyrenochaeta species	2					2	2				1			

a: Susceptibility based upon 48 hour minimum inhibitory concentrations from isolates submitted to the FTL for antifungal susceptibility testing.
b: Breakpoints in µg/ml: AMB (amphotericin B, < 1 = S, > 2 = R); 5-FC (5-flurocytosine, < 16 = S, > 32 = R); FLU (fluconazole, < 32 = S, > 64 = R); ITRA (itraconazole, < 0.5 = S, > 1 = R); MON (miconazole, < 8 = S, > 16 = R); KETO (ketoconazole, < 8 = S, > 16 = R); NAT (natamycin/pimaracin, values < 32 presumed susceptible).
c: S = susceptible.
d: R = resistant.
e: Number of isolates.

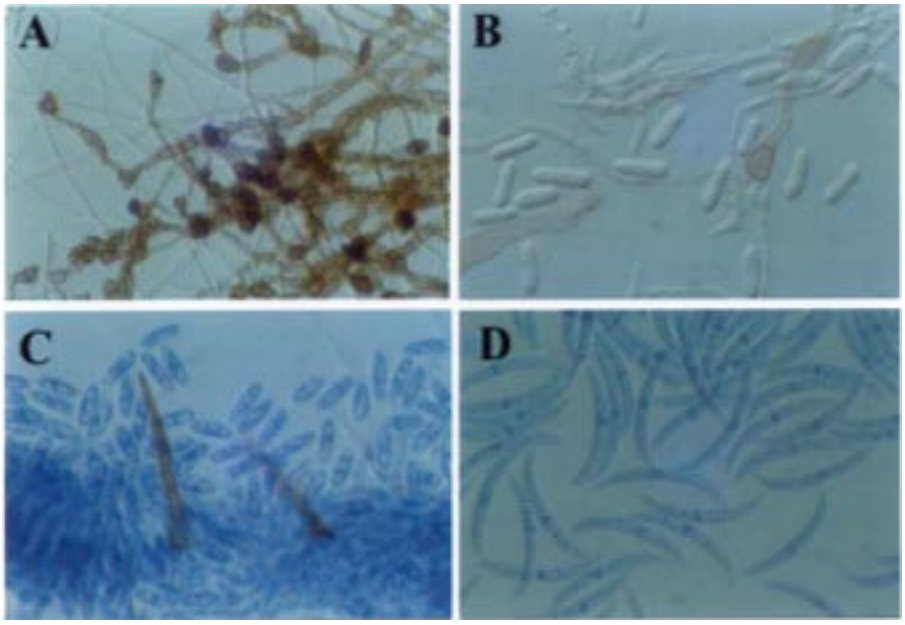

Figure 1. 1a. Complex appressoria of *Colletotrichum gloeosporioides* Group, PFA slide culture, 25°C, 7 days, 230x. 1b. Single, brown appresorium and conidia of *Colletotrichum gloeosporioides* Group, PFA slide culture, 25°C, 7 days, 460x. 1c. Setae (brown) and conidia of *Colletotrichum coccodes,* PFA, 25°C, 7 days, 460x. 1d. Curved conidia of *Colletotrichum dematium,* PFA slide culture, 25°C, 7 days, 920x.

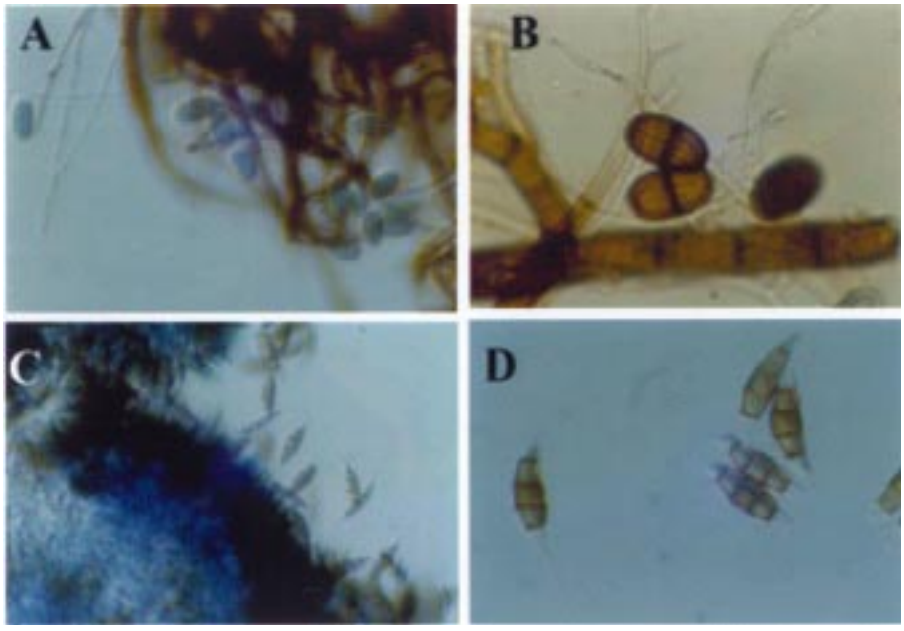

Figure 2. 2a. Young, hyaline conidia of *Botryodiplodia theobromae,* PFA tease mount, 25° C, 10 weeks, 460x. 2b. Mature, brown, septate, longitudinally striated conidia of *Botryodiplodia theobromae,* PFA tease mount, 14 weeks, 920x. 2c. The edge of the conidioma and conidia of a *Pestalotiopsis* species, PFA tease mount, 25°C, 10 days, 460x. 2d. Conidia of a *Pestalotiopsis* species: 4-euseptate, 5-celled, and bearing apical and distal appendages, PFA tease mount, 25°C, 10 days, 920x.

National Committee for Clinical Laboratory Standards M27-A macrobroth dilution method for yeast antifungal susceptibility testing modified for mould testing [56]. Although standardization in antifungal susceptibility testing for filamentous fungi is only commencing and there is difficulty in establishing clear correlates between *In vitro* data and clinical efficacy [57], these data may provide guidelines for antifungal therapy. It is important to realize however, as Rex *et al*. have pointed out, that numerous other factors such as pharmacokinetics of the drug, general host factors, site of infection, and virulence of the pathogen also influence the outcome [58]. Similarly, concise breakpoints are also not available. For the purposes of these data, however, the following minimum inhibitory concentrations, in µg/ml, were chosen to approximate *in vitro* susceptibility or resistance with these systemic antifungal agents:

Amphotericin B (<1 = S, > 2 = R), 5-fluorocytosine (< 16 = S, > 32 = R), ketoconazole (< 8 = S, > 16 = R), itraconazole (< 0.5 = S, > 1 = R), fluconazole (< 32 = S,

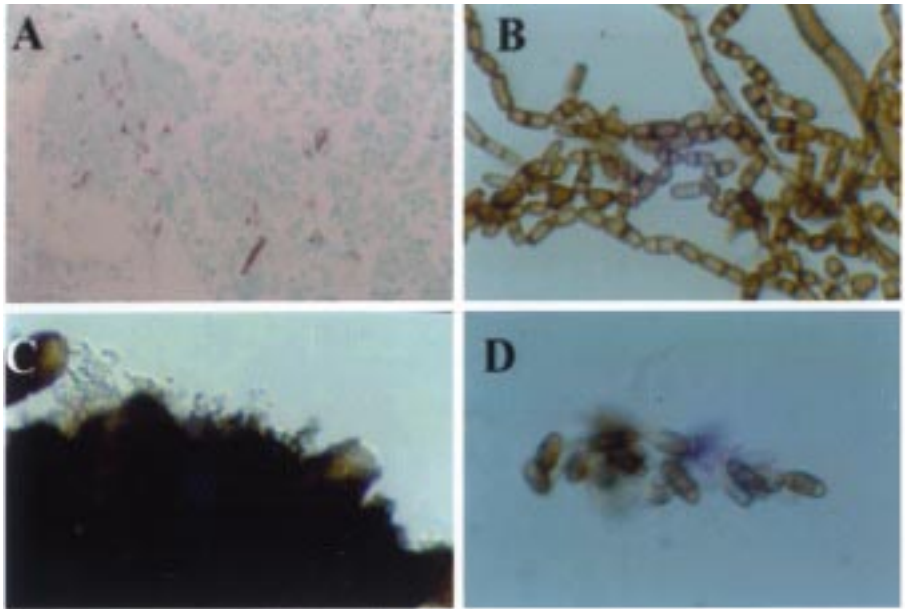

Figure 3. 3a. Hyphal elements of *Nattrassia mangiferae* in tissue, GMS stain, 230x. 3b. *Scytalidium dimidiatum* arthroconidial synanamorph of *Nattrassia mangiferae,* PFA slide culture, 25°C, 6 days, 920x. 3c. The edge of a multilocular pycnidium of *Nattrassia mangiferae* demonstrating two ostioles and immature conidia, PFA tease mount, 25°C, 10 weeks, 230x. 3d. Mature, versicolored conidia of *Nattrassia mangiferae,* PFA tease mount, 25°C, 12 weeks, 920x.

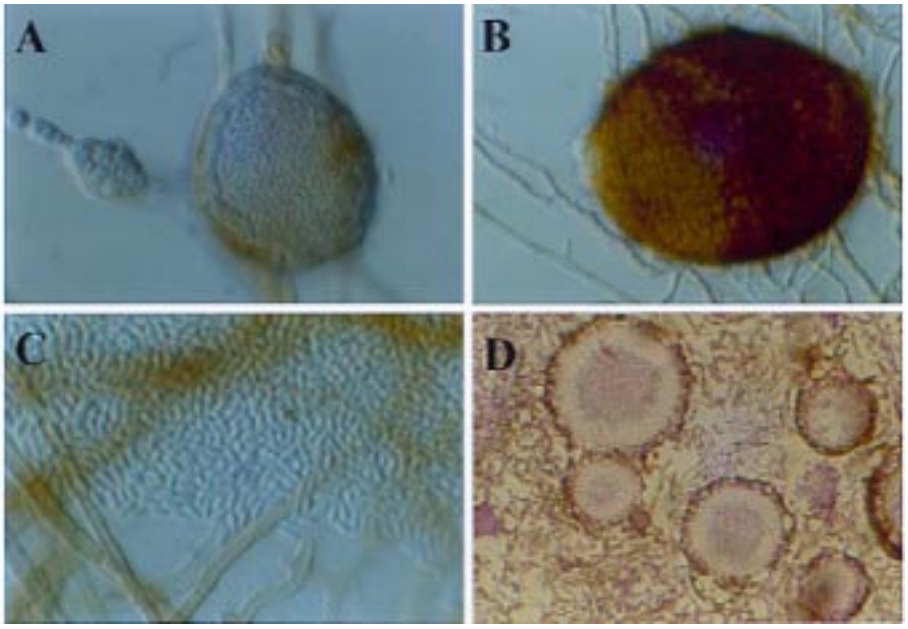

Figure 4. 4a. Thin-walled pycnidium and alternarioid chlamydoconidium of a *Phoma* species, PFA slide culture, 25ºC, 8 days, 460x. 4b. Texture angularis outer covering of a *Phoma* species, PFA tease mount, 25ºC, 14 days, 460x. 4c. Small, hyaline conidia of a *Phoma* species, PFA slide culture, 25ºC, 7 days, 920x. 4d. Immersed pycnidia of *Coniothyrium fuckelii* in PFA agar, 25ºC, 4 weeks, H&E stains, 230x.

> 64 = R) and miconazole (<8 = S, > 16 = R). Natamycin or pimaricin, a polyene administered topically to the eye, is difficult to asses, as drug concentrations evaluated *in vitro* (up to 32 µg/ml) may be lower than those actually achieved in the eye. The *in vitro* data generated indicates most isolates appeared susceptible to amphoterin B, with only two *Phoma* species appearing resistant. With regard to the triazoles itrazonazole and fluconazole, results were mixed. Some dematiaceous species appeared resistant to itraconazole, a drug frequently touted for this group of organisms, while fluconazole showed quite low MICs for several species. The low MICs for fluconazole are somewhat surprising, in that filamentous fungi in general frequently appear resistant *in vitro*. One needs to bear in mind the following caveat, however, with regard to *Nattrassia mangiferae* and the dermatomycoses caused by this organism (and its *Scytalidium dimidiatum / Scytalidium hyalinum* synanamorphs): despite *in vitro*

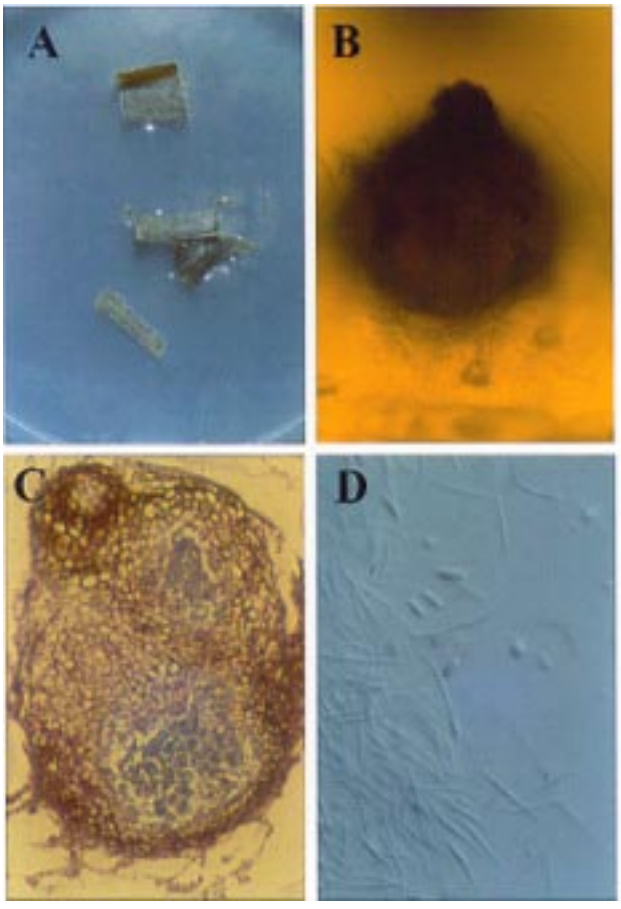

Figure 5. 5a. Pycnidia (small black dots) forming on carnation leaf agar, 25°C, two weeks. 5b. Immersed pycnidium of a *Phomopsis* species on carnation leaf agar, 25°C, two weeks, 230x. 5c. Cross-section of a multilocular pycnidium of a *Phomopsis* species on carnation leaf agar, 25°C, two weeks, periodic acid Shiff's fungal stain, 230x. 5d. Hyaline alpha (short, ellipsoidal) and beta (long, filiform) conidia of a *Phomopsis* species, carnation leaf agar, 25°C, two weeks, 920x.

data suggesting otherwise, these organisms are frequently very refractory to antifungal therapy and require long-term dosing regimens for eradication.

CONCLUSION

Coelomycetous fungi appear to be increasing in incidence in human disease. They are frequently acquired through some type of traumatic implantation, and are of particular concern in patients being maintained on long term immunosuppressive therapy. While their recovery in the laboratory is not particularly difficult, identification for some species remains arduous due to poorly defined, difficult to assess criteria and atypical characteristics displayed under artificial growth conditions. *In vitro* antifungal susceptibility data coupled with case reports suggests that empiric therapy with amphotericin B in deep, invasive disease is warranted. Topical antifungals as well as oral triazole agents appear efficacious in superficial /subcutaneous settings.

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