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Phylogenetic and evolutionary aspects of *Paracoccidioides brasiliensis* reveal a long coexistence with animal hosts that explain several biological features of the pathogen

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Abstract

The habitat of the mycelial saprobic form of *Paracoccidioides brasiliensis*, which produces the infectious propagula, has not been determined and has proven difficult for mycologists to describe. The fungus has been rarely isolated from the environment, the disease has a prolonged latency period and no outbreaks have been reported. These facts have precluded the adoption of preventive measures to avoid infection.

The confirmation of natural infections in nine-banded armadillos (*Dasypus novemcinctus*) with *P. brasiliensis*, in high frequency and wide geographic distribution, has opened new avenues for the study and understanding of its ecology. Armadillos belong to the order Xenarthra, which has existed in South America ever since the Paleocene Era (65 million years ago), when the South American subcontinent was still a detached land, before the consolidation of what is now known as the American continent. On the other hand, strong molecular evidence suggests that *P. brasiliensis* and other dimorphic pathogenic fungi – such as *Blastomyces dermatitidis*, *Coccidioides immitis* and *Histoplasma capsulatum* – belong to the family Onygenaceae *sensu lato* (order Onygenales, Ascomycota), which appeared around 150 million years ago.

P. brasiliensis ecology and relation to its human host are probably linked to the fungal evolutionary past, especially its long coexistence with and adaptation to animal hosts other than *Homo sapiens*, of earlier origin. Instead of being a blind alley, the meaning of parasitism for dimorphic pathogenic fungi should be considered as an open two-way avenue, in which the fungus may return to the environment, therefore contributing to preserve its teleomorphic (sexual) and anamorphic (asexual) forms in a defined and protected natural habitat.

Keywords: *Paracoccidioides brasiliensis*; Dimorphic pathogenic fungi; Infection; Systemic mycoses; Evolution; Ecology

1. *Paracoccidioides brasiliensis* and its elusive biology

Paracoccidioides brasiliensis is the etiological agent of paracoccidioidomycosis (PCM), one of the most important systemic mycoses in Latin America. This fungus is thermo-dimorphic, that is, it grows as a yeast like structure in the host tissue or when cultured at 35–37 °C, and as mycelium in the saprobic condition or when cultured at room temperature (18–23 °C). The teleomorphic or sexual (meiospores) form is still unknown (Lacaz, 1994).

The exact places and conditions where the fungus occurs in nature are also not yet determined. Several indirect evidences indicate that the saprobic form may occur in restricted soil conditions, producing infective propagula such as arthoconidia and aleuroconidia that cause primary infection in the lung by the airborne route (Restrepo, 1985; Restrepo-Moreno, 1994). The possibility of infection by traumatic route cannot be completely ruled out. Natural infection in some wild and domestic animals has been observed by intradermal and serological tests, the fungus has been repeatedly isolated from armadillos *Dasypus novemcinctus* (Costa et al., 1995a,b; Bagagli et al., 1998, 2003; Ono et al., 2001; Restrepo et al., 2000), and clinical manifestation of the disease has been recently reported in dogs (Ricci et al., 2004; Farias et al., 2005).

The incidence of PCM infection, without symptoms of the disease, seems to be high in the endemic areas, as detected by intradermal tests, varying from 2 to 60% (Restrepo-Moreno, 1994; Fava and Fava Neto, 1998). Instead, the development of the disease, with uni- or multifocal lesions, decreases with rate around 1–3 cases per 100 thousand inhabitants in many

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Brazilian regions, where it occurs more frequently (Wanke and Londero, 1994). PCM is a life-threatening disease, representing the eighth most common cause of death from chronic or recurrent types of infectious and parasitic diseases in Brazil (Coutinho et al., 2002). Two distinct forms of PCM disease can be observed: (a) an acute or juvenile form that occurs in young people of either sex and seems to evolve more rapidly once the primary infection occurs, and (b) a chronic or adult form, that occurs mainly in male individuals, with a characteristic prolonged period of latency, that in some well documented cases, can reach as long as 30–40 years (Franco et al., 1994). Gender association in PCM disease seems to be related to the inhibition of transition from the mycelial to the yeast form by the action of the 17-beta-estradiol female hormone (Loose et al., 1983; Salazar et al., 1988; Aristizabal et al., 2002).

PCM causes a significant social impact in most Latin American countries, since it affects mainly rural workers in their productive period of life. Its treatment is frequently difficult and long, and patients often need hospitalization, with increasingly high treatment costs. Occurrence of sequelae is also an important aspect of PCM, frequently causing pulmonary dysfunction, serious dermatological deformations or other disabilities. Relapses, either by activation of endogenous quiescent focus or by new infections, also occur frequently (Franco et al., 1994).

While *P. brasiliensis* can be cultured quite easily from clinical samples, including the armadillo's tissues, the same does not hold true for suspected environmental samples. The fungus has been isolated only sporadically from soil or related materials, and hence previous findings that have proven very difficult to reproduce (Franco et al., 2000). It is possible to mimic the fungal saprobic form in vitro conditions, using artificial culture medium or some natural substrate, such as sterilized soil. Growth of the mycelial phase of *P. brasiliensis* is relatively slow in comparison with other decomposer fungi and seems to have a restricted ability to compete or survive in the presence of the normal soil microbiota (Restrepo, 1985; Lacaz, 1994). The production of infective propagula, such as arthro- and aleuroconidia, as well as resting spores (chlamidospores), has been occasionally demonstrated in the laboratory, though in some isolates only and in very low amounts (Restrepo, 1985; Franco et al., 1989).

2. Phylogenetic aspects of *P. brasiliensis*

Great advances have been made over the last decade in the understanding of the phylogenetic and evolutionary relationships of several fungal groups (Bowman et al., 1992, 1996; Taylor, 1995; Taylor et al., 2000). This knowledge about evolutionary biology is particularly valuable in the area of medical mycology. The correct classification of ''problematic'' pathogenic species according to their location in the tree of life, such as *Pneumocystis carinii*, now classified as a fungus, and *Rhinosporidium seeberi* and *Pythium insidiosum*, now classified as non-fungal species, provides a more adequate basis for the treatment of the corresponding infections (Edman et al., 1988; Stringer et al., 1989; Fredricks et al., 2000; Herr et al., 2001; Mendoza et al., 2002).

Morphological and molecular evidence has suggested that the main dimorphic pathogenic fungi causing systemic mycoses (*Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum* and *P. brasiliensis*) belong to the family Onygenaceae *sensu lato*, order Onygenales, phylum Ascomycota (Table 1), although the sexual forms of some of these species have yet to be discovered (Leclerc et al., 1994; Taylor, 1995; Peterson and Sigler, 1998; Bialek et al., 2000; Herr et al., 2001; San-Blas et al., 2002; Untereiner et al., 2004). In a study with an ample number of species of this group, using molecular and traditional mycological tools, it was demonstrated a monophyletic clade distinct from the Onygenaceae *sensu lato* to encompass *Ajellomyces* and its corresponding anamorph genera (*Blastomyces*, *Emmonsia* and *Histoplasma*) and *P. brasiliensis* (Untereiner et al., 2004). The new family Ajellomycetaceae was proposed to accommodate a member of this lineage of saprobic and pathogenic vertebrate-associated fungus. The correct molecular taxonomic position of these fungi has opened new possibilities for the study and understanding of their ecoepidemiological relationships with their respective hosts.

One of the most important and exclusive mycological characteristics of many fungal species within the order Onygenales is their ability to degrade keratin, a quite insoluble protein of the skin, hair and nails, and hence their frequent association with animals and their products, including dung, feathers and bones (Currah, 1985; Alexopoulos et al., 1996). In some members of the family Ajellomycetaceae, such as in the

Table 1
Classification of dimorphic pathogenic Ascomycetes, Order Onygenales, based on morphology and molecular data

Class	Order	Family	Mitosporic species (teleomorphic form)
Plectomycetes	Onygenales	Onygenaceae *sensu lato*[a]	*Blastomyces dermatitidis* (*Ajellomyces dermatitidis*) *Coccidioides immitis* (ND) *Coccidioides posadasii* (ND) *Emmonsia crescens* (*Ajellomyces crescens*) *Emmonsia parva* (ND) *Histoplasma capsulatum* (*Ajellomyces capsulatus*) *Lacazia loboi* (ND) *Paracoccidioides brasiliensis* (ND)

ND, not discovered yet.

[a] A new family (Ajellomycetaceae) was recently proposed for a distinct monophyletic clade that includes all the above mentioned members of Onygenaceae, except *Coccidioides* spp.