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The importance of molecular analyses for understanding the genetic diversity of *Histoplasma capsulatum*: An overview

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ABSTRACT

Advances in the classification of the human pathogen *Histoplasma capsulatum* (*H. capsulatum*) (ascomycete) are sustained by the results of several genetic analyses that support the high diversity of this dimorphic fungus. The present mini-review highlights the great genetic plasticity of *H. capsulatum*. Important records with different molecular tools, mainly single- or multi-locus sequence analyses developed with this fungus, are discussed.

Recent phylogenetic data with a multi-locus sequence analysis using 5 polymorphic loci support a new clade and/or phylogenetic species of *H. capsulatum* for the Americas, which was associated with fungal isolates obtained from the migratory bat *Tadarida brasiliensis*.

This manuscript is part of the series of works presented at the "V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi" (Oaxaca, Mexico, 2012).

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Importancia de los análisis moleculares en la comprensión de la diversidad genética de *Histoplasma capsulatum*: revisión

Palabras clave:
Histoplasma capsulatum
Instrumentos moleculares
Análisis multi-*loci*
Diversidad genética

RESUMEN

Los resultados de diversos análisis genéticos que respaldan la alta diversidad de este hongo dimorfo confirman los progresos en la clasificación del patógeno humano *Histoplasma capsulatum* (*H. capsulatum*) (un ascomiceto). La presente revisión destaca la importante plasticidad genética de *H. capsulatum*. Se describen los datos importantes con los diferentes instrumentos moleculares, sobre todo, los análisis de las secuencias individuales o multi-*loci* establecidos con este hongo.

Datos filogenéticos recientes con un análisis multi-*loci* de secuencias utilizando 5 *loci* polimorfos respaldan un nuevo clado y/o especie filogenética de *H. capsulatum* del continente americano, asociado a aislamientos fúngicos obtenidos del murciélago migratorio *Tadarida brasiliensis*.

Este artículo forma parte de una serie de estudios presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

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The scientific history of the pathogenic fungus *Histoplasma capsulatum* began with the histopathological findings, published by Samuel Taylor Darling in 1906, in tissues of a patient from Martinique, who was working on the construction of the Panama Channel. Darling observed intracellular parasites with 1–6 μm in diameter surrounded by a translucent halo.[5] Due to its resemblance to *Leishmania*, it was described as a protozoan, and was named "*H. capsulatum*" because of the similarity of its halo with a capsule. Later, in 1912, Henrique da Rocha Lima inferred the mycotic nature of this pathogen, characterizing it as a yeast.[6] The fungus is a saprobe-geophilic organism that utilizes two nomenclatures depending on its sexual state: *H. capsulatum* (anamorph or asexual state) and *Ajellomyces capsulatus* (teleomorph or sexual state); both constitute the same holomorph organism, which is the causative agent of histoplasmosis, a systemic mycosis with primary respiratory compromise.

Before the description of the sexual state of *H. capsulatum* by Kown-Chung,[18,19] this pathogen was classified into the

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Division Deuteromycota, Order Moniliales, and Family Moniliaceae, based on the morphological criteria proposed in 1899 by Saccardo.[24] Currently, *H. capsulatum* is classified into the Kingdom Fungi, Subkingdom Dikarya, Phylum Ascomycota, Class Eurotiomycetes, Order Onygenales, and Family Onygenaceae and/or Ajellomycetaceae,[10–12] according to the analyses of six loci: *18S*, *5.8S* and *28S* of the *rRNA* genes; *EF1α* (Elongation factor-1α); *RPB1*and *RPB2* (RNA polymerase II subunits 1 and 2).

In the environment, this organism grows preferentially in bat and bird guano that contains high concentrations of nitrogen and phosphorus in addition to other micronutrients. These conditions, together with optimal air and soil temperatures (18–28 °C), humidity (>60%), and darkness (fosters sporulation), characterize the ideal ecological niche of *H. capsulatum* , which favors the development of its multicellular infective mycelial phase (M-phase).[27,29,34–36]

Classification of *Histoplasma capsulatum*

The biological species *H. capsulatum* comprised three taxonomic varieties: *H. capsulatum* var. *capsulatum* Darling, 1906; *H. capsulatum* var. *duboisii* (Vanbreuseghem, 1957) – Ciferri, 1960; and *H. capsulatum* var. *farciminosum* (Rivolta, 1873) – Weeks, Padhye, et Ajello, 1985. These varieties were identified by their micromorphologies, geographic distribution, host-association, and clinical forms of the disease. Currently, with the advent of molecular techniques to classify fungal species, these taxonomic varieties have been included in a molecular taxonomy based on the phylogenetic species concept.[15]

Taylor et al.[33] indicated that the concepts and/or criteria of species recognition often used in mycology are the biological and morphological ones, and most of the described species have been identified with phenotypic characters. However, some pathogenic fungi show few informative characters thereby leading to skewed, controversial, and erroneous classifications.[8,9] To overcome these inconveniences, most authors have promoted genetic and molecular statements for genotypic and phylogenetic classifications of pathogenic fungi.

Genotyping of *Histoplasma capsulatum*

H. capsulatum isolates have been grouped based on their genotype patterns using different molecular assays (Table 1). First, Vincent et al.[37] grouped clinical strains in three classes according to their Restriction Fragment Length Polymorphism (RFLP) profiles and hybridization with mtDNA and rDNA probes. Class 1 included only the Downs strain of *H. capsulatum* var. *capsulatum* from North America; Class 2 was formed by 14 *H. capsulatum* var. *capsulatum* strains from North America and two *H. capsulatum* var. *duboisii* strains from Africa; finally, Class 3 grouped four *H. capsulatum* var. *capsulatum* strains from Central America and two *H. capsulatum* var. *capsulatum* strains from South America. Spitzer et al.,[25] using the same methodology, proposed a new Class 4; one year later, a Class 5 based on hybridization with a probe of the YPS-3 gene was recorded.[26]

Keath et al.,[16] in accordance with Spitzer et al.,[26] increased the number of strains studied and expanded the fungus genotyping to six classes, including four subclasses within Class 5. It is noteworthy that, from the initial proposal of Vincent et al.[37] until the classification of Keath et al.,[16] most of the studied strains came from restricted geographic areas of North America, and very few strains were isolated in Central (Panama) and South America (Colombia).

Later, Poonwan et al.,[22] analyzed 13 clinical isolates of *H. capsulatum* from Thailand with Random Amplification of Polymorphic DNA (RAPD-PCR), using three oligonucleotides separately, and found that isolates from Thailand formed two to four homogeneous groups that were clearly separated from the G-217B reference strain from North America.

Jiang et al.[13] genotyped 24 fungal isolates from the United States of America through the nucleotide sequence analysis of the ITS1-5.8S-ITS2 region of the rDNA, and found 10 different *H. capsulatum* sequence patterns. As a result, they suggested that this method could be useful for reorganizing isolates from other classifications.

Based on a RAPD-PCR assay with the single random primer 1281, Reyes-Montes et al.[23] distinguished four groups (I–IV) and two subgroups (Ia and Ib) of *H. capsulatum* from different origins in Latin America (Mexico, Guatemala, Panama, and Colombia), which were isolated from clinical and environmental sources. In this study, the reference strain G-186B from Panama (Class 3, according to Vincent et al.[37]) formed a single group. The latter authors suggested that RAPD-PCR profiles with suitable random primers could be used for classifying fungal isolates in accordance with their source and geographic distribution. The RAPD-PCR method with four independent random primers was used by Muniz et al.[21] to analyze 48 *H. capsulatum* samples from Rio de Janeiro State (Brazil) isolated from different sources (soil, animal, and human clinical samples), which were grouped according to the genetic polymorphism generated for each primer. The RAPD-PCR profiles were able to separate the Brazilian isolates from the North American (United States of America) isolates in accordance with their percentage of similarity. Afterwards, Zancopé-Oliveira et al.,[38] using a similar procedure, analyzed 22 Brazilian *H. capsulatum* isolates, mostly from human clinical samples, and identified three clusters: cluster I, with isolates from the Brazilian north-eastern region; a major cluster II, with isolates from the Brazilian south-eastern and south regions; and cluster III (48% similarity), with isolates from Goias State in the central region of Brazil.

Additional molecular studies by Carter et al.,[2–4] Kasuga et al.,[14] and Taylor et al.[31] have referred differences in the population structures of *H. capsulatum* (clonal and recombinant). The analyses of *H. capsulatum* genetic populations, mainly with the $(GA)_n$, $(GT)_n$ and $GT(A)_n$ multiallelic markers (microsatellites), enabled the possibility of distinguishing *H. capsulatum* isolates from the United States of America and Colombia, suggesting the separation of these fungal populations in distinct phylogenetic species.[2,4] Recently, Taylor et al.,[32] based on the sequences of a 240-nt fragment of the $(GA)_n$ microsatellite and its flanking regions, found

Table 1
Relevant molecular classifications of *Histoplasma capsulatum* isolates.

H. capsulatum classifications	No. of isolates	Sources	Assays
3 Classes[37]	20	Human and naturally infected animal	RFLP, hybridization with mtDNA and rDNA probes
5 Classes[26]	9	Human	RFLP, hybridization with mtDNA, rDNA and YPS-3 probes
6 Classes[16] and 4 subclasses	76	Human and soil	*Idem* above
2–4 Groups[22]	13	Human	RAPD-PCR using three primers
10 Patterns[13]	24	Human	RFLP with ITS region
4 Groups[23]	14	Human and soil	RAPD-PCR using the 1281 primer
8 Clades[15] (7 phylogenetic species)	137	Human, soil and naturally infected animal	MLS analysis with four markers (*arf*, *H-anti*, *ole1*, and *tub1*)
3–4 Clades[20]	51	Human, soil and naturally infected animal	*Idem* above and ITS region

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