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PRP8 intein in cryptic species of *Histoplasma capsulatum*: Evolution and phylogeny

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ABSTRACT

The PRP8 intein is the most widespread intein among the Kingdom Fungi. This genetic element occurs within the *prp8* gene, and is transcribed and translated simultaneously with the gene. After translation, the intein excises itself from the Prp8 protein by an autocatalytic splicing reaction, subsequently joining the N and C terminals of the host protein, which retains its functional conformation. Besides the splicing domain, some PRP8 inteins also have a homing endonuclease (HE) domain which, if functional, makes the intein a mobile element capable of becoming fixed in a population. This work aimed to study (1) The occurrence of this intein in *Histoplasma capsulatum* isolates ($n = 99$) belonging to different cryptic species collected in diverse geographical locations, and (2) The functionality of the endonuclease domains of *H. capsulatum* PRP8 inteins and their phylogenetic relationship among the cryptic species. Our results suggest that the PRP8 intein is fixed in *H. capsulatum* populations and that an admixture or a probable ancestral polymorphism of the PRP8 intein sequences is responsible for the apparent paraphyletic pattern of the LAmA clade which, in the intein phylogeny, also encompasses sequences from LAmB isolates. The PRP8 intein sequences clearly separate the different cryptic species, and may serve as an additional molecular typing tool, as previously proposed for other fungi genus, such as *Cryptococcus* and *Paracoccidioides*.

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1. Introduction

Inteins, or "internal proteins", are coding sequences that occur in eukaryotes, bacteria, archaea (Perler, 2000), and some viruses (Pietrokovski, 1998). Inteins are transcribed and translated with flanking sequences (exteins), and are subsequently excised by an autocatalytic process allowing the host protein to assume its normal conformation and function (Xu et al., 1993; Chong et al., 1996). Intein host genes are usually those that encode for house-keeping proteins, such as metabolic enzymes, polymerases and others. Although inteins do not improve the fitness of the host organism, their localization at important and thus conserved sites may be the reason for their "survival", since they recognize specific and conserved genetic niches in the genome by the homing process (Liu, 2000).

Three types of inteins occur in nature: the bi-functional inteins, whose splicing (Spl) domain is interrupted by a homing endonuclease (HE) domain; the mini-inteins that have a continuous splicing domain; and the split-inteins, whose N and C-terminal portions of the Spl domain can perform protein trans-splicing after translation (Perler, 2000). The Spl and HE intein domains present conserved amino acid motifs related to their functionality, known as blocks. The Spl domain allows for precise excision of the intein from the host protein, and is comprised of blocks A and B that facilitate N-Splicing, and blocks F and G that facilitate C-Splicing. The central HE domain is comprised of blocks C, D, E and H that are responsible for the mobility of the intein (Fig. 1). Mobility of this parasitic element may result in occupation of empty alleles, subsequent duplication, and fixation in sexual populations (Koufopanou and Burt, 2005).

The population dynamics of the HEs seems to follow a model of invasion, fixation, degeneration, loss and reinvasion. Once the intein is established in the population, there is no selection for endonuclease functionality, since this domain can degenerate by mutations, deletions and genetic drift. Once "empty", HE sites will eventually be reoccupied again, according to the cyclic model proposed by Koufopanou and Burt (2005). However, a recent analysis of HE inteins suggested that the HE domain can persist over long evolutionary times for several reasons, including its higher recombination frequency, complex host population structures that prevent the fixation of HE, and changes in host fitness as a result of HE replication and fixation (Gogarten and Hilario, 2006).

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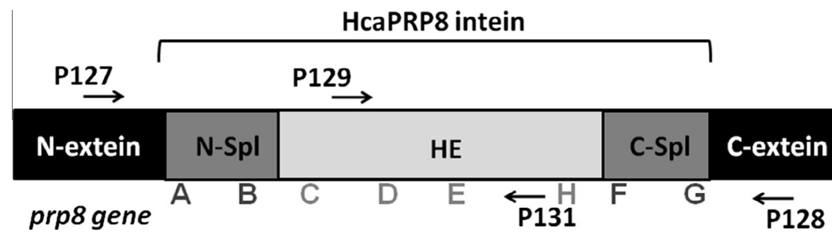

Fig. 1. PCR scheme of the amplification of the HcaPRP8 intein. Extein, Spl and HE refer to the host gene, splicing domain and homing endonucleases domain, respectively. N is for amino and C is for carboxyl terminals. A–E, H, F and G refer to the conserved motifs (blocks) of the Splicing and HE domains. P127/P128 and P129/P131 are the primer pairs used for the amplification of the 5′and 3′ ends and the core of the PRP8 intein, respectively, for all isolates of *Histoplasma capsulatum* listed on Table 1.

Most of the inteins described in eukaryotes occur in fungi, and one of the most studied is the PRP8 intein (Perler, 2000), which occurs as either a mini or a bi-functional intein within the *prp8* gene that encodes for the highly conserved Prp8 nuclear protein. Prp8 is critical to spliceosome activity in eukaryotic cells because it is required for pre-mRNA splicing prior to protein translation (Grainger and Beggs, 2005). The PRP8 intein occurs intermittently in all fungal phyla, yet is best described among the Ascomycota, including the important human pathogens *Aspergillus fumigatus* (Order Eurotiales), *Paracoccidioides brasiliensis*, *Histoplasma capsulatum* (Butler et al., 2006), *Emmonsia parva* and *Blastomyces dermatitidis* (Theodoro et al., 2011) (Order Onygenales), as well as in the phytopathogen *Botrytis cinerea* (Butler et al., 2006).

PRP8 inteins are thought to have functional HE domains because they are saturated with synonymous base substitutions and thus may be constrained by selection (Butler et al., 2006), yet the only one proven to be functional is *Bci*PRP8, found in *B. cinerea* (Bokor et al., 2010). PRP8 HE domains are very diverse, possibly due to their long evolutionary history (Butler et al., 2006), and thus have the potential for inferring phylogenetic relationships within fungal species. Butler and Poulter (2005) studied PRP8 intein diversity and successfully distinguished strains of two varieties of *Cryptococcus neoformans* (*neoformans* and *grubii*) and *C. gattii*., and in 2008, Theodoro et al., 2008 used PRP8 intein sequences to differentiate isolates belonging to the four cryptic species of *Paracoccidioides* genus: S1, PS2, PS3 (Matute et al., 2006) and Pb01-like (Teixeira et al., 2009).

In this work, we examine the distribution and phylogenetic relationships of PRP8 inteins among isolates belonging to the *H. capsulatum* complex. *H. capsulatum* (teleomorph, *Ajellomyces capsulatus*) is a thermally dimorphic fungus of the Ajellomycetaceae family (Untereiner et al., 2004) that occurs world-wide and causes the disease histoplasmosis in humans (Rippon, 1988; Kwon-Chung and Bennett, 1992). The fungus grows as a saprobe in nature, often in soil containing accumulated guano. If this soil is disturbed, microconidia may be inhaled, where conditions prompt conversion of the fungi to yeasts that can persist in host tissues. Histoplasmosis is primarily a disease of the lungs, and the vast majority of infections are asymptomatic. However acute respiratory symptoms may occur and *H. capsulatum* infection can become disseminated in immunocompromised persons (Kwon-Chung and Bennett, 1992).

H. capsulatum was formerly divided in three varieties: *capsulatum*, *farciminosum* and *duboisii* (Rippon, 1988). In 2003, Kasuga et al. (2003) used multi-locus sequencing to define phylogenetic relationships among *Histoplasma* and led to the identification of cryptic species that fell within delineated geographic clades. Here, we compare PRP8 intein sequences among these cryptic species in order to further our understanding of (1) the presence of this genetic element in different populations of *H. capsulatum*; (2) intein functionality in *Histoplasma*, suggested by *in silico* analysis; (3) the phylogenetic relationships among these inteins sequences, since the PRP8 intein has proven to be suitable to differentiate among cryptic species from other fungal pathogens and (4) the evolutionary pattern of the PRP8 intein in *Histoplasma* genus, concerning the divergence among the cryptic species proposed previously by Kasuga et al. (2003).

2. Material and methods

2.1. H. capsulatum isolates and culture

A total of 99 isolates were used in this work, 33 of which were previously identified by phylogenetic analysis (Kasuga et al., 2003). These belonged to the following geographic clades; Latin American A (LAmA, 9), Latin American B (LAmB, 5), North American 1 (NAm1, 3), North American 2 (NAm2, 7), Africa (2), Eurasia (2), Netherlands (1), H66 lineage (1), H69 lineage (1), H81 lineage (2). The remaining (66) (*H. capsulatum* isolates had no previous identification (Table 1). All isolates were revived from frozen stock on Brain Heart Infusion (BHI) agar slants and maintained as mycelia at 27 °C until growth was sufficient for harvesting and DNA extraction, which ranged from 2 to 4 weeks.

2.2. DNA extraction

DNA was isolated using the DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA) with slight modifications to the manufacturer's instructions. For each isolate, two to three colonies of *H. capsulatum* mycelia were picked from the BHI slants and placed in 5 ml polypropylene tubes containing 800 μl Qiagen ATL buffer and 60 U of proteinase K. The mycelia were homogenized using the Omni TH Mixer (Omni Intl., Kennesaw, GA) at slow speed for 30 s and then high speed for 30 s, using a clean probe between each isolate. Homogenates were capped and incubated at 55 °C for 1 h with frequent vortexing and then cooled at room temperature (RT). RNase A (Sigma–Aldrich Corp., St. Louis, MO) was then added to a final concentration of 1 mg/ml and incubated for 5 min at RT, followed by the addition of 900 μl Qiagen buffer AL and vortexing. Homogenates were incubated at 70 °C for 10 min, then transferred to 1.7 ml microcentrifuge tubes and centrifuged at 10,000×*g* for 10 min. Clear supernatants (1 ml each) were transferred to clean microcentrifuge tubes and a 50% volume of ethanol (Sigma–Aldrich Corp.) was added. The suspensions were vortexed and transferred to Qiagen DNeasy columns, and manufacturer's instructions were followed throughout the remainder of the procedure, and DNA was eluted in 200 μL of Tris HCl 10 mM, pH 8.0, and maintained at −20 °C.

2.3. Multi-locus sequence typing

Multi-locus sequencing was performed to determine geographic clade identities of 66 unknown *H. capsulatum* isolates using the phylogeny schema developed by Kasuga et al. (2003). DNA was

Table 1
H. capsulatum isolates used in this work.

Species	Variety	Isolate identification*				Location	Sender of isolate	Reference for species identification
		RMSCC	Kasuga et al., 2003	ATCC	Others			
NAm2	*capsulatum*	1003	H11		848	Missouri/USA	G. Kobayashi	Kasuga et al., 2003
	capsulatum	1019	H18	4745	5-1MD	Missouri/USA	G. Kobayashi	
	capsulatum	2404	H77	10886	C.W. Emmons 6613	Virginia/USA		
	capsulatum	2434	H84	26320	C.W. Emmons 6623	Georgia/USA		
	capsulatum	2436	H86	32682	A.F. DiSalvo SC74	S.Carolina/USA		
	capsulatum	2472	H97		0001	Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2767	H130		15	Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	1005				Washington/USA	D. Carter	This work
	capsulatum	1006				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	1008				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	1015				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	1017				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2304				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2305				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2306				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2308				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2309				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2310				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2311				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2312				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2313				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2314				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2315				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2316				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2317				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2318				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2319				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2320				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2321				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2322				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2323				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2324				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2325				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2326				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2473				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2474				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2475				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2476				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2477				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2478				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2479				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2480				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2481				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2753				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2754				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2755				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2756				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2757				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2758				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2759				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2760				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2762				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2764				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2765				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2766				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2768				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2769				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2770				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2772				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2773				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2774				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2775				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2776				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2777				Indiana/USA	P. Connolly and J. Wheat	
	capsulatum	2778				Indiana/USA	P. Connolly and J. Wheat	
LAmA	*capsulatum*	2350	H60		H-0057-I-11	Bogota/Colombia	A. Restrepo, E. Castaneda and J. McEwen	Kasuga et al., 2003
	capsulatum	2351	H61		H-0057-I-14	Bogota/Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	2352	H62		H-0057-I-15	Bogota/Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	2353	H63		H-0057-I-18	Bogota/Colombia	A. Restrepo, E. Castaneda and J. McEwen	

	capsulatum	2355	H73		H-0057-I-24	Bogota/Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	2358	H67		30177, JE	Medellin/ Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	2364	H71		21337, JJM	Medellin/ Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	2367	H76		T29302, GC	Medellin/ Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	4704	EH391			Mexico	Maria Lúcia Taylor	
	capsulatum	2134				Texas/USA	J. Taylor	This work
	capsulatum	2761				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum				CDC B6206			
LAmB	*capsulatum*	2349	H59		H-0057-I-10	Bogota/Colombia	A. Restrepo, E. Castaneda and J. McEwen	Kasuga et al., 2003
	capsulatum	2359	H68		30318, CH	Medellin/ Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	2363	H70		30956, WS	Medellin/ Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	2365	H75		14056, HC	Medellin/ Colombia	A. Restrepo, E. Castaneda and J. McEwen	
	capsulatum	2435	H85	28308	CDC B923	Argentina		
	capsulatum	2368				Medellin/ Colombia	A. Restrepo, E. Castaneda and J. McEwen	This work
Africa	*duboisii*	2444	H91	24295	D. Grigoriu 8123	Guinea-Liberian Border		Kasuga et al., 2003
	capsulatum	4719	H143		CBS 287.54	South Africa		
NAm1	*capsulatum*		H9	38904	Downs	Missouri/USA	E. Keath and G. Kobayashi	Kasuga et al., 2003
	capsulatum		H126			Missouri/USA	G. Kobayashi	
	capsulatum		H127			Missouri/USA	G. Kobayashi	
	capsulatum	1001				Washington/USA	D. Carter	This work
	capsulatum	2212				Texas/USA	J. Taylor	
	capsulatum	2763				Alabama/USA	W. Dismukes, S. Moser and B. Hines	
	capsulatum	2771				Indiana/USA	P. Connolly and J. Wheat	
Eurasia	*capsulatum*		H205		HP4, NIH 37-384-23	Thailand	N. Poonwan and Y. Mikami	Kasuga et al., 2003
	farciminosum		H212		IFM 5418, 848.63 IP	Algeria	Institut Pasteur	
	Lineage H66		*capsulatum*	2357	H66		13594, GH	
	Medellin/ Colombia	A.	Restrepo, E. Castaneda and J. McEwen					
	Lineage H69		*capsulatum*	2360	H69		21402, JVM	
	Medellin/ Colombia	A.	Restrepo, E. Castaneda and J. McEwen					
	Lineage H81		*capsulatum* Panama	2431	H81	26028	M.D. Berliner G184B	
	capsulatum	2433	H83	26030	M.D. Berliner G186B	Panama		
	Netherlands		*capsulatum*	4741	H176		CBS 243.69	Netherlands

* ATCC, American Type Culture Collection, Rockville, MD, USA; RMSCC, Roche Molecular Systems Culture Collection, Alameda, CA, USA; CDC, Centers for Disease Control and Prevention, Atlanta, GA, USA; CBS, Centraalburaeu voor Schimmelcultures, Baarn, The Netherlands.

amplified using previously described primers for four genetic loci (Kasuga et al., 1999), ADP-ribosylation factor, H antigen precursor, delta-9 fatty acid desaturase, and alpha-tubulin. PCR was performed in 25 μL reactions containing 0.2 μM each forward and reverse primer, 2.5 mM $MgCl_2$, 0.2 mM each dNTP, and 0.625 U Taq DNA Polymerase (Roche Carolina Inc., Florence, SC) in a buffer of 10 mM Tris pH 8.3/50 mM KCl. Thermal cycling was performed in MicroAmp 96-well optical reaction plates using the GeneAmp PCR System 9700 (Applied Biosystems, Inc., Foster City, CA) as follows: 1 cycle of 94 °C for 5 min; 12 cycles of denaturation at 94 °C for 15 s, annealing at 65 °C for 30 s (decreasing 0.7 °C each cycle), and extension at 72 °C for 1 min. Twenty more cycles were run using the same parameters, but the annealing temperature was held constant at 56 °C, with a final extension at 72 °C for 5 min.

Amplicons were sized on 1.75% agarose and visualized with ethidium bromide under ultraviolet light and were treated with Exo-SAP-IT (USB Corp., Cleveland, OH) according to manufacturer's instructions for primer digestion and nucleotide dephosphorylation. Sequencing with locus-specific primers was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc.) in a 3730 DNA Analyzer (Applied Biosystems, Inc.). Consensus sequences were generated from raw data using Sequencher 4.9 software (Gene Codes Corp., Ann Arbor, MI), aligned using MUSCLE algorithm (Edgar, 2004), implemented in MEGA v5.0 (Tamura et al., 2011). *ARF* consensus sequences were manually concatenated with those of *ANTI-H*, *OLE* and *TUB* in the BioEdit Sequence Alignment Editor (Hall, 1999). The alignment file was converted to Phylip format in the software DAMBE (Xia and Xie, 2001) and a Maximum Likelihood (ML) phylogenetic analysis was performed using the program PhyML 3.0 (Guindon et al., 2010). The Kimura-2-parameters model (Kimura, 1980), pointed as the best fit substitution method by MEGA v.5.0 (Tamura et al., 2011), was used as evolutionary model. The Ti/Tv ratio, gamma shape parameter, and proportion of non-variant sites were estimated by maximum likelihood from a Neighbor-Joining tree (BIONJ). Bootstrap re-sampling (Felsenstein, 1985) was applied to assess support for

individual nodes using 1000 replicates with random additions and NNI (Nearest Neighbor Interchange) was used as tree topology search operation. FigTree v.1.3.1 software (Rambaut, 2006).

2.4. Primer design and PCR for amplification and sequencing of HcaPRP8 inteins

Two primer pairs were designed for the complete sequencing of the PRP8 intein from *H. capsulatum* isolates. The primers P127 (5′ TCAACTGATGAAGAGCAATCC 3′) and P128 (5′ TGGATCAATGAAATCTTCAAGG 3′) were designed based on the *prp8* gene sequences flaking the HcaPRP8 intein (Fig. 1), from the Broad Institute website database (http://www.broadinstitute.org/annotation/genome/histoplasma_capsulatum) in HCBG_00494.2 locus of *H. capsulatum* G186AR strain.

Based on the sequencing of the 5′ and 3′ ends, the primers P129 (5′ CAGAGAGTTTATCATCAACC 3′) and P131 (5′ ATTCTGAGGTTGACAGTACC 3′) were designed in conserved regions to amplify and sequence the internal region of the PRP8 intein (Fig. 1). PCR was carried out in a 25 μL reactions containing ~20 ng of the extracted DNA, 10 mM Tris–HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM dNTP (Roche), 0.8 μM of each primer and 0.625 unit of Taq Polymerase (Roche), in a Veriti thermal cycler (Applied Biosystems). The thermal cycling conditions were: 95 °C for 4 min followed by 40 cycles of 95 °C for 1 min, 54 °C for 1 min and 72 °C for 2 min, and a final cycle of 72 °C for 10 min. The PCR products were identified by 1.0% agarose gel electrophoresis stained with ethidium bromide. The amplicons were purified by using Exosap-IT according to manufacturer's instruction and PCR primers were used for sequencing in a 3730 DNA Analyzer (Applied Biosystems) as described for MLST.

2.5. Sequence and phylogenetic analyses

The sequences of each isolate were initially analyzed with the software MEGA version 5.0 (Tamura et al., 2011), where the sequences from 3′ and 5′ ends and from the core of the PRP8 intein were joined and a consensus sequence of the entire PRP8 intein was obtained for each isolate. The codon sequences were aligned by using the MUSCLE. After that, the unique sequences (those that were identical to each other, representing a haplotype) were identified and the alignment converted to Phylip format using the software DAMBE (Xia and Xie, 2001). Nucleotide variation, substitution patterns, and genetic distances were examined using MEGA version 5.0 (Tamura et al., 2011).

The translated sequences were compared to the VMA Intein from *Saccharomyces cerevisiae* (GenBank access number: Q874G3) in order to observe the presence or absence of the two aspartic acid residues (Asp-218 and Asp-326) that are involved in the activity of the homing endonuclease (Posey et al., 2004; Koufopanou and Burt, 2005).

The deduced amino acid sequences of the HcaPRP8 inteins haplotypes were aligned together with the amino acid sequence of the *Bci*PRP8 (PRP8 intein from *B. cinerea*, locus number BC1G_06754.1 – Broad Institute database), the only PRP8 intein whose functionality of HE domain was proved by *in vivo* experiments (Bokor et al., 2010), in order to address whether the HE domain from HcaPRP8 might be functional.

Maximum Likelihood (ML) phylogenetic analysis was performed using the program PhyML 3.0 (Felseistein, 1993), with the same parameters used for the MLST phylogeny. The best evolutionary model, calculated in MEGA v5.0 (Tamura et al., 2011) was also the Kimura-2-parameters.

3. Results

3.1. Multilocus sequence typing and phylogenetic analysis

The 66 *H. capsulatum* isolates were predominantly identified as belonging to the NAm2 clade (58/66, 88%, Fig. 2). Of the remaining isolates, four belonged to NAm1, three to LAmA, and one to LAmB. All of the NAm 1 isolates showed 100% sequence identity to H9 (Kasuga et al., 2003). The LAmA isolates were the most diverse, with none showing sequence identity with each other or isolates previously identified to that clade. The LAmB isolate 2368 showed 100% identity with H68, H70, and H75 from prior studies. Of the 58 NAm2 isolates, 9 were unique (1005, 1017, 2473, 2480, 2759, 2764, 2772, 2775, 2778), as were three isolates delineated in prior studies (H84, H86, and H130). The remaining 46 NAm2 isolates shared identity with at least one other isolate. The NJ consensus tree (Fig. 2) was highly compatible with ML and MP trees generated using the same *H. capsulatum* four-locus MLST sequence alignment (data not shown).

3.2. PCR and sequencing of HcaPRP8 inteins

The PCR with the primers P127 and P128 amplified fragments of 1995 bp (part of the *prp8* host gene or extein plus 1605 bp of the entire intein), whereas the PCR with the primers P129 and P131 amplified fragments of 625 bp from the core region of the PRP8 intein (data not shown).

Among the 99 complete PRP8 intein sequences analyzed, 44 haplotypes (GenBank access numbers: JX274602-JX274645) were found. Genetic distances calculated by employing the Kimura 2-parameter model (Kimura, 1980) ranged from $d = 0.001$ to $d = 0.046$ among the haplotypes. The greatest genetic distances were observed between the isolate H176 from Netherlands clade (Kasuga et al., 2003) and the remaining haplotypes (Supplementary material 1).

Deduced translation of the intein nucleotide sequences the 99 amino acids sequences resulted in identification of 10 unique sequences, showing that most of the substitutions observed in the nucleotide alignment were synonymous mutations. Both substitutions and *indels* were observed. The NAm2 isolate 2766 showed a deletion of nucleotides from intein positions 529-531, (the amino acid residue Gln from position 177) and intein sequences of H91, H143 (Africa clade), H9 haplotype, H127, and 2763 (NAm1 clade) had a GGG insertion in the positions 1144-1146 (the amino acid residue Gly, Supplementary material 2). Both *indels* occur between the blocks C and D from the HE domain.

The alignment of the conserved amino acid blocks, corresponding to the Spl and HE domains, including the PRP8 intein from *B. cinerea* (*Bci*PRP8) shows a high conservation of the blocks, mainly those from Spl domain. All HcaPRP8 sequences, presented both essential aspartates (D) in blocks C and E, whereas the LAmA isolate 2761 presented an asparagine (N) in the place of the second essential aspartate (D) (Fig. 3).

3.3. Phylogenetic analysis of the PRP8 intein sequences from Histoplasma isolates

According to the ML tree constructed, most isolates (58) fall within the NAm2 clade, while only 4 (isolates 2212, 1001, 2771 from H9 haplotype and 2763) fall within the NAm1 clade, one isolate (2368), from H68 haplotype, was identified as LAmB, and three (isolates CDC6206, 2134 and 2761) were identified as belonging to LAmA. The clades NAm1, NAm2, Africa, Netherlands and LAmB are monophyletic and very well resolved, with high bootstrap values. However, in the PRP8 intein phylogeny, the LAmA clade, which also

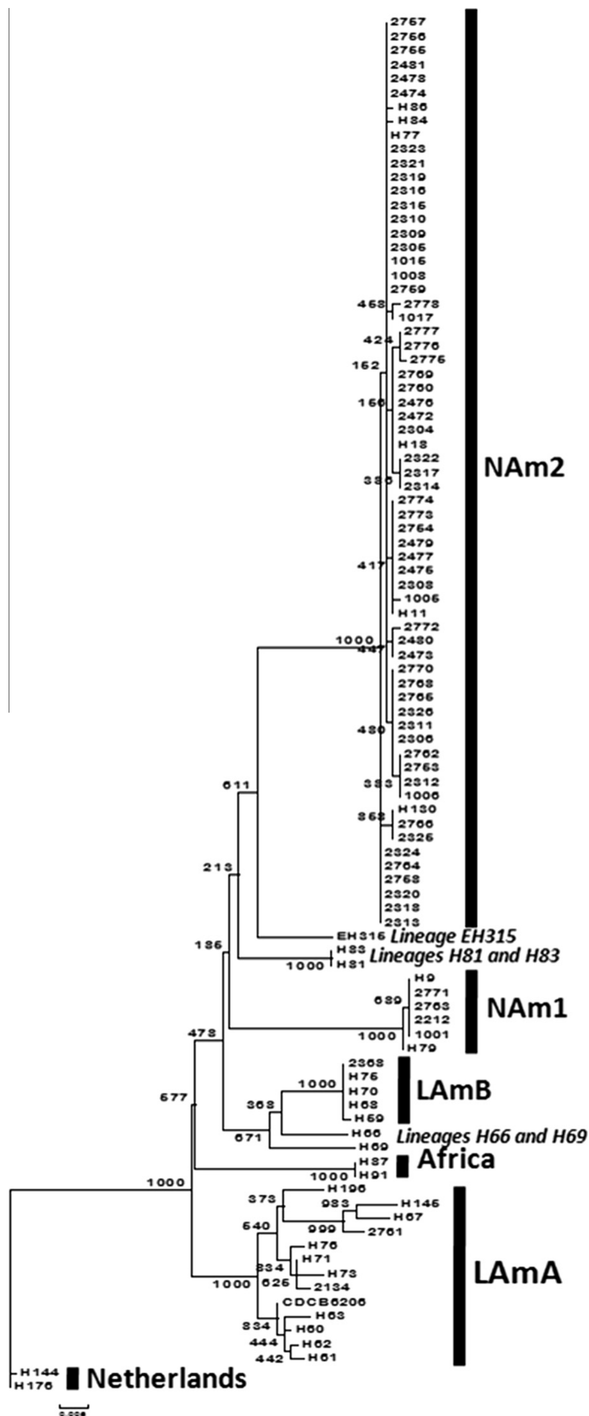

Fig. 2. Neighbor Joining (NJ) phylogenetic tree of *H. capsulatum* study isolates grouped into geographic clades as deduced by multilocus sequence typing. The NJ tree is representative of 1000 iterations and is limited to those that appeared in greater than 70% of those iterations, and branches with bootstrap values of less than 100% are shown. All isolates are described by their respective number (Table 1) and may be followed by the name designated by Kasuga et al. (2003) in parentheses with "L" preceding if noted as a lineage. Taxa with no study number indicate *H. capsulatum* sequences of known geographic clade (Kasuga et al., 2003) that were included in phylogenetic analysis as reference sequences Respective geographic clades are shown at right of taxonomic groups. Genbank Accession numbers for unique DNA sequences generated at the four MLST loci used to determine phylogeny are as follows: alpha-tubulin, JX431893-JX431908; ADP-ribosylation factor JX443624-JX443640; H antigen precursor, JX458483-JX458498; delta-9 fatty acid desaturase, JX458499-JX458514.

encompasses the Eurasia isolates, as previously noticed by Kasuga et al. (2003), also includes PRP8 intein sequences from LAmB and H81/H83 lineage isolates (Fig. 4, in which the LAmA does not refer to a clade but indicates isolates which belong to LAmA clade identified in MLST analysis performed herein and by Kasuga et al., 2003).

4. Discussion

Until now, *Histoplasma* PRP8 intein-encoding sequences had been sequenced for only the strains G217B, G186AR, WU24, H143 and JER2004 available in GenBank and Inbase databases. This is the first study in which a large, and therefore significant, number of *Histoplasma* samples were used in order to study the presence of the PRP8 intein as well as its polymorphisms in isolates belonging to the different cryptic species, previously proposed by Kasuga et al. (2003).

By analyzing the HcaPRP8 sequences available online and performing a synonymous/non-synonymous analysis (dS/dN), by Nei-Gojobori method (Nei and Gojobori, 1986), comparing PRP8 intein sequences from different fungal species, Poulter et al. (2007) and Butler et al. (2006) deduced that the HE from HcaPRP8 is active. In a population with active HE inteins it is expected to observe some individuals without the intein, which means that the HE is in the invasion and not in the degeneration phase of the homing cycle, therefore some empty alleles are likely in population. This scenario occurs for *B. cinerea* population, which has an active HE domain. So, that was our first question: whether we could find individuals without intein in *Histoplasma* population. Curiously, despite of the high dS/dN values found previously by Butler et al. (2006); the presence of the two essential aspartates (one in block C and the other in block E, of the HE domain) in almost all samples and the high conservation of residues from HE blocks (when the HcaPRP8 amino acid sequences are compared to the *Bci*PRP8 in Fig. 1), all the isolates herein evaluated presented a bi-functional PRP8 intein. This might indicate that the intein is fixed in this population and, because of the observation of one isolate with a substitution in the second aspartate (D was replaced with N), we could speculate that the degeneration process of the HE might have begun in some *Histoplasma* populations.

While the HE function is still unsure, the splicing activity of HcaPRP8 is very well known. Besides the presence of essential residues, such as the first Cys in block A, the motif TxxH in block B and the last His and Asn in block G, as well as the high conservation of all Spl domain, the splicing of HcaPRP8 was already experimentally observed by Liu and Yang (2004) in a non native extein (between a maltose binding protein and a thioredoxin), in *Escherichia coli* model. The precursor protein, spliced protein and excised intein were identified by their predicted sizes, by western blotting. The splicing efficiency at 37 °C was only 25%, while at 25 °C it was 100% (no band for the precursor protein was observed by western blotting).

It is interesting to note that, as a dimorphic fungus, *H. capsulatum* must convert the mycelia (M) to yeast (Y) form during infection under the host high temperature (37 °C). If the splicing efficiency at 37 °C is as low, the M-Y transition may be disturbed because many different mRNA would not be correctly spliced, which would decrease the fitness of the pathogen. Thus, our initial hypothesis was that there would be a balance between decreased fitness of the host and active spread of the HcaPRP8 inteins, leading to a long persistence of the homing endonuclease, especially if the selective disadvantage to the carrier and the mobility of the intein are not the same in different subpopulations of *H. capsulatum* (Gogarten and Hilario, 2006; Theodoro and Bagagli, 2009; Theodoro et al., 2011). However, to confirm this balance we should have observed at least few empty alleles as an indicative of active spread (invasion phase). Since this was not the case, we cannot rule out the possibility of the HcaPRP8 intein is not efficient in non native

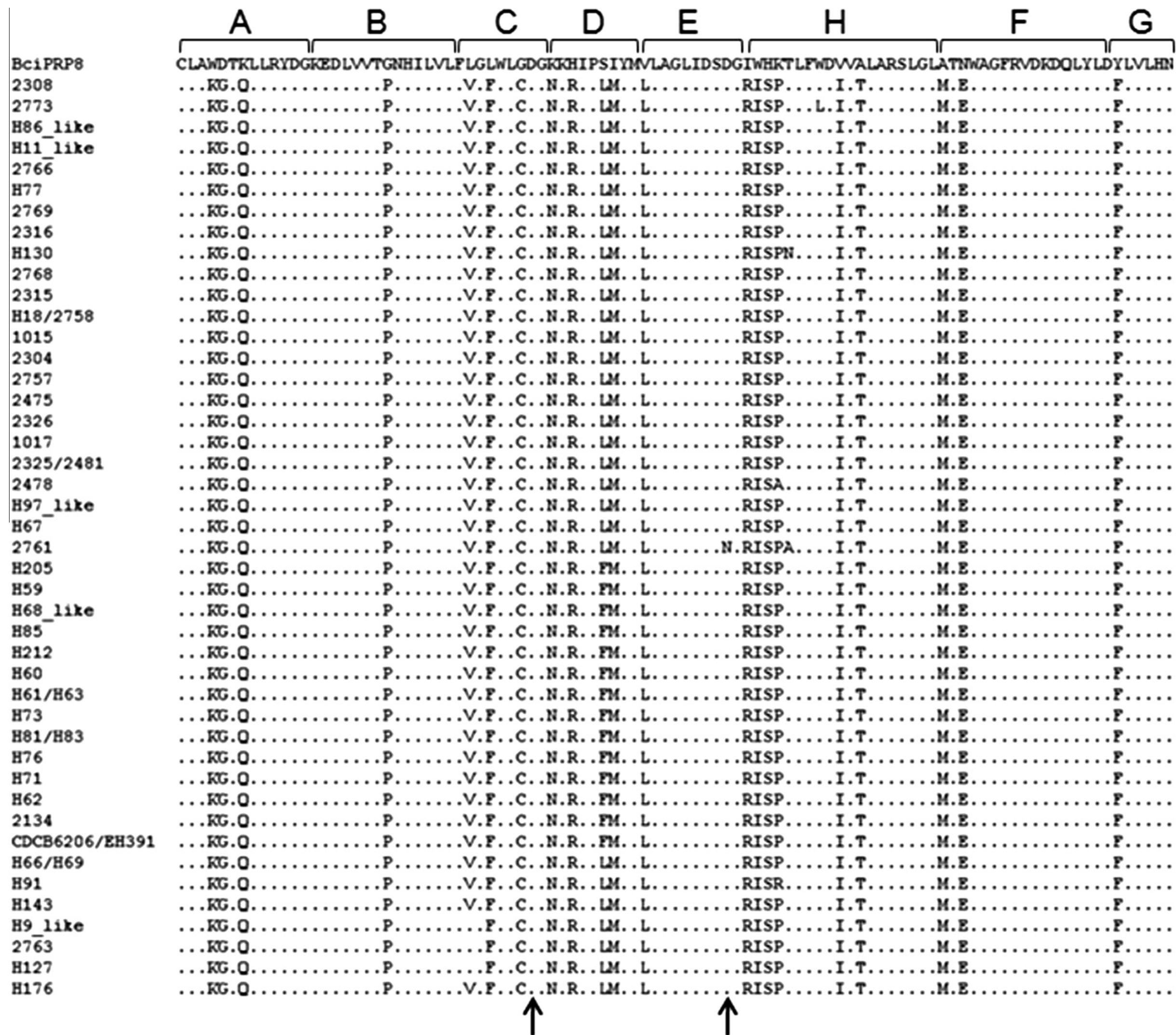

Fig. 3. Alignment of the deduced amino acid sequences of the conserved blocks from the Splicing and HE domains of HcaPRP8 and *Bci*PRP8 inteins. Deduced amino acid sequences of blocks A, B, F and G from the Splicing domain and C–E and H from the HE domain 44 haplotypes of HcaPRP8 intein, identified in this work, aligned with the amino acid sequence of the BciPRP8. The arrows indicate the position of the two essential aspartic acids according to VMA intein of *S. cerevisiae*. The "." indicates amino acid identity.

extein models or that maybe this was a characteristic exclusive from that HcaPRP8 used by Liu and Yang (2004).

The observation that all individuals from *H. capsulatum* population have the PRP8 intein, although going against our expectations as explained above, is actually a valuable new finding because it strengthens the idea of using this intein as a therapeutic target. The therapeutic potential of the PRP8 intein has been pointed out because it is located in an essential and most highly conserved eukaryotic gene, namely, *prp*8, which encodes for the Prp8 protein involved in mRNA splicing. Furthermore, the absence of this intein in the vertebrate hosts makes it a safe target for drugs that may inhibit its splicing function (Paulus, 2003; Liu and Yang, 2004; Zhang et al., 2010). But, of course, a good therapeutic target should be present in all pathogen population and the HcaPRP8 probably fills this requirement.

Concerning the phylogenetic analysis of the PRP8 intein in *Histoplasma* population, most of the clades found previously by multilocus sequencing type (Kasuga et al., 2003) were here observed and in most of the cases the DNA polymorphisms were associated to the geographic location, with only one exception: the isolate 2761, from North America (Alabama, US) that shares a common ancestor with H67, a LAmA isolate. This is also observed in the MLST tree (Fig. 2). This finding may be explained by the host (bats or human) migration from Latin America introducing this genotype in North America.

In the PRP8 intein phylogeny obtained in this study, the LAmA/Eurasia clade also includes isolates from the LAmB and H81 lineage clades, an observation not present in the MLST performed by Kasuga et al. (2003) and herein repeated with additional samples. The LAmA clade is not considered a phylogenetic species under the criteria proposed by Dettman et al. (2003a,b) because Kasuga et al. (2003) found some discordance in the tree topologies for two (*arf* and *tub1*) of the four genes analyzed. The authors maintained it as the most diverse clade, including samples from Mexico to Brazil and also from Eurasia, probably due a recent migration from South America to Europe. In our analysis the unresolved LAmA branch, which also encompass LAmB and H81 lineages may be the result of lateral transfer events if we consider LAmA is indeed a different species: haplotypes of LAmB form a small group within the Latin American super clade, which indicates LAmB and LAmA have been genetically isolated for long time after an ancient lateral gene transfer. The mechanism by which this lateral transfer occurs is as yet unknown.

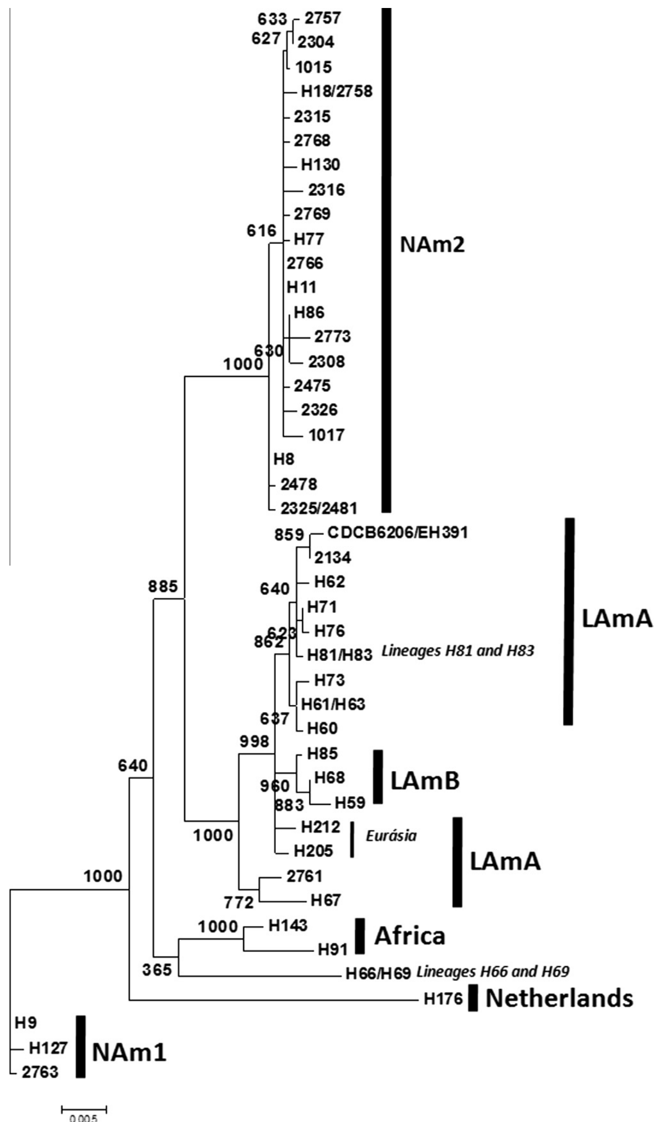

Fig. 4. Molecular Phylogenetic anaylsis by Maximum Likelihood method. Bootstrap consensus tree inferred from 1000 replicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 44 nucleotide sequences (number of unique sequences from a total of 100 sequences). All ambiguous positions were removed for each sequence pair. The black bars indicate isolates belonging to each clade already described by Kasuga et al. (2003).

Lateral gene transfer seems to be, indeed, a characteristic of some inteins. For instance, in a study of HE activity of the VMA intein from species of *Saccharomyces*, Posey et al. (2004) observed that the endonucleases PI-ScaI (from *S. cariocanus*) is more efficient to recognize and cut the *vma* gene of *S. cerevisiae* than its own *vma* gene being, according the authors, adapted to horizontal transfer. The PRP8 intein is also known to have been horizontally transferred from ascomycetes to basidiomycetes from *Cryptococcus* genus (species *C. gatii* and *C. neoformans*) (Butler et al., 2006).

However, lateral gene transfer among *Histoplasma* phylogenetic species complex species does not appear to have occurred by intein invasion from an intein- containing species to one without (the likely scenario that occurred between Ascomycetes and Basidiomycetes), since this parasitic element is, apparently, fixed in this genus. The most parsimonious idea is that the common ancestor of the genus *Histoplasma* genus already had the intein, whose phylogeny matches closely with the MLST phylogeny obtained by Kasuga et al. (2003) and also with the isolates used in this work. Indeed, lateral gene transfer by intein invasion does not appear to have happened in the PRP8 intein history within Ascomycetes. Recent findings focusing on the evolutionary diversification of the PRP8 intein among *Botrytis* species and other ascomycetes have shown that this element has been vertically transferred in Ascomycota. During that process, intein losses may have occurred, as evidenced by a characteristic genetic footprint left by past intein invasion (the AGY codon in the C-extein +1 residue) (Bokor et al., 2012).

Thus, the observation of LAmB isolates inside the LAmA clade could be explained by eventual hybridization or admixture between populations with overlapped geographical areas, leading to the introgression of a gene (PRP8 intein, in this case) from one species to other or, another very reasonable hypothesis is the presence of shared ancestral polymorphism (Taylor et al., 2000), due to a slow divergence of intein sequences, compared to the genes used in MLST, as the *H. capsulatum* populations diverged.

Despite of the paraphyletic status of the LAmA/Eurasia clade in the PRP8 intein phylogeny, this parasitic element showed to be suitable to distinguish among the remaining cryptic species of *Histoplasma*, NAm1 and 2, LAmB, Netherlands and Africa with exception of Australian isolates, whose cultures or DNAs were not accessible to perform this study, being therefore an additional molecular marker for species recognition in *Histoplasma* complex.

Author's note

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2013.05.001.

References

Bokor, A.A.M., Kan, J.A.L.V., Poulter, R.T.M., 2010. Sexual mating of *Botrytis cinerea* illustrates PRP8 intein HEG activity. Fungal Genet. Biol. 47, 392–398.
Bokor, A.A.M., Kohn, L.M., Poulter, R.T.M., Kan, J.A.L.V., Burt, A., 2012. PRP8 inteins in species of the genus Botrytis and other ascomycetes. Fungal Genet. Biol. 49, 250–261.
Koufopanou, V., 2004. Homing endonuclease genes: the rise and fall and rise again of a selfish element. Curr. Opin. Gen. Dev. 14, 609–615.
Butler, M.I., Gray, J., Goodwin, T.J., Poulter, R.T.M., 2006. The distribution and evolutionary history of the PRP8 intein. BMC Evol. Biol. 6, 1–26.
Butler, M.I., Poulter, R.T., 2005. The PRP8 inteins in *Cryptococcus* are a source of phylogenetic and epidemiological information. Fungal Genet. Biol. 42, 452–463.
Chong, S., Shao, Y., Paulus, H., Benner, J., Perler, F.B., Xu, M.Q., 1996. Protein splicing involving the *Saccharomyces cerevisiae* VMA intein. The steps in the splicing pathway, side reactions leading to protein cleavage, and establishment of an in vitro splicing system. J. Biol. Chem. 271, 22159–22168.
Dettman, J., Jacobson, D., Taylor, J.W., 2003a. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote Neurospora. Evolution 57, 2703–2720.

Dettman, J., Jacobson, D., Turner, E., Pringle, A., Taylor, J.W., 2003b. Reproductive isolation and phylogenetic divergence in Neurospora: comparing methods of species recognition in model eukaryote. Evolution 57, 2721–2741.
Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32 (5), 1792–1797.
Felsenstein, J., 1993. PHYLIP (Phylogeny Inference Package) version 3.69. Distributed by the author. Seattle: Department of Genetics, University of Washington. Available from: <http://evolution.genetics.washington.edu/phylip.html>.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Gogarten, J.P., Hilario, E., 2006. Inteins, introns, and homing endonucleases: recent revelations about the life cycle of parasitic genetic elements. BMC Evol. Biol. 6, 1–5.
Grainger, R.J., Beggs, J.D., 2005. Prp8 protein: at the heart of the spliceosome. RNA 11, 533–557.
Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst. Biol. 59 (3), 307–321.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. 41, 95–98.
Kasuga, T., Taylor, J.W., White, T.J., 1999. Phylogenetics relationships of varieties and geographical groups of the human pathogenic fungus *Histoplasma capsulatum* darling. J. Clin. Microbiol. 37, 653–663.
Kasuga, T., White, T.J., Koenig, G., McEwen, J., Restrepo, A., Castañeda, E., Lacaz, C.S., Heins-Vaccari, E.M., Freitas, R.S., Zancopé-Oliveira, R.M., Qin, Z., Negroni, R., Carter, D.A., Mikami, Y., Tamura, M., Taylor, M.L., Miller, G.F., Poonwan, N., Taylor, J.W., 2003. Phylogeography of the fungal pathogen *Histoplasma capsulatum*. Mol. Ecol. 12, 3383–3401.
Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Koufopanou, V., Burt, A., 2005. Degeneration and domestication of a selfish gene in yeast: molecular evolution versus site-directed mutagenesis. Mol. Biol. Evol. 22, 1535–1538.
Kwon-Chung, K.J., Bennett, J.E., 1992. Medical Mycology, 1st ed. Lea and Febiger, Philadelphia.
Liu, X.Q., 2000. Protein-splicing intein: genetic mobility, origin and evolution. Annu. Rev. Genet. 34, 61–76.
Liu, X.Q., Yang, J., 2004. Prp8 intein in fungal pathogens: target for potential antifungal drugs. FEBS Lett. 572, 46–50.
Matute, D.R., McEwen, J.G., Montes, B.A., San-Blas, G., Bagagli, E., Rauscher, J.T., Restrepo, A., Morais, F., Nino-Veja, G., Taylor, J.W., 2006. Cryptic speciation and recombination in the fungus Paracoccidioides brasiliensis as revealed by gene genealogies. Mol. Biol. Evol. 23, 65–73.
Nei, M., Gojobori, T., 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. 3, 418–426.
Paulus, H., 2003. Inteins as targets for potential antimycobacterial drugs. Front. Biosci. 8, 1157–1165.
Perler, F.B., 2000. InBase, the intein database. Nucleic Acids Res. 28, 344–345.
Pietrokovski, S., 1998. Identification of a virus intein and a possible variation in the protein-splicing reaction. Curr. Biol. 8, 634–635.
Posey, K.L., Koufopanou, V., Burt, A., Gimble, F.S., 2004. Evolution of divergent DNA recognition specificities in VDE homing endonucleases from two yeast species. Nucleic Acids Res. 32, 3947–3956.
Poulter, R.T., Goodwin, T.J., Butler, M.I., 2007. The nuclear-encoded inteins of fungi. Fungal Genet. Biol. 44, 153–179.
Rambaut, A., 2006. Figtree: Tree figure drawing tool, version 1.3.1. Distributed by the author. Edinburgh, Scotland, United Kingdom: Institute of Evolutionary Biology, University of Edinburgh; 2006–2009. Available from: <http://tree.bio.ed.ac.uk/software/figtree/>.
Rippon, J.W., 1988. Medical Mycology, 3rd ed. Saunders, Philadelphia.
Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.
Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Gêiser, D.M., Hibbett, D.S., Fisher, M.C., 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. 31 (1), 21–32.
Teixeira, M.M., Theodoro, R.C., de Carvalho, M.J., Fernandes, L., Paes, H.C., Hahn, R.C., Mendoza, L., Bagagli, E., San-Blas, G., Felipe, M.S., 2009. Phylogenetic analysis reveals a high level of speciation in the Paracoccidioides genus. Mol. Phylogenet. Evol. 52, 273–283.
Theodoro, R.C., Bagagli, E., 2009. Inteins in pathogenic fungi: a phylogenetic tool and perspectives for therapeutic applications. Mem. Inst. Oswaldo Cruz 104, 497–504.
Theodoro, R.C., Bagagli, E., Oliveira, C., 2008. Phylogenetic analysis of PRP8 intein in *Paracoccidioides brasiliensis* species complex. Fungal Genet. Biol. 45, 1284–1291.
Theodoro, R.C., Volkmann, G., Liu, X.Q., Bagagli, E., 2011. PRP8 intein in Ajellomycetaceae family pathogens: sequence analysis, splicing evaluation and homing endonuclease activity. Fungal Genet. Biol. 48, 80–91.
Untereiner, W.A., Scott, J.A., Naveau, F.A., Sigler, L., Bachewish, J., Angus, A., 2004. The Ajellomycetaceae, a new family of vertebrate-associate Onygenales. Mycologia 96, 812–821.
Xia, X., Xie, Z., 2001. DAMBE: Data analysis in molecular biology and evolution. J. Hered. 92, 371–373.
Xu, M., Southworth, M.W., Mersha, F.B., Hornstra, L.J., Perler, F.B., 1993. In vitro protein splicing of purified precursor and the identification of a branched intermediate. Cell 75, 1371–1377.
Zhang, L., Zheng, Y., Callahan, B., Belfort, M., Liu, Y., 2010. Cisplatin inhibits protein splicing, suggesting inteins as therapeutic targets in mycobacteria. J. Biol. Chem. 286 (2), 1277–1282.