

Molecular Detection of *Histoplasma* in Bat-Inhabited Tunnels of Camino de Hierro Tourist Route, Spain

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We detected *Histoplasma capsulatum* in 2 bat-inhabited tunnels of a tourist route in northern Spain. This finding confirms that the geographic distribution of this fungal pathogen is wider than previously thought. Our results highlight the need for surveillance and assessment of the potential infection risk for workers and visitors.

Histoplasmosis is a primary pulmonary infection caused by inhalation of aerosolized spores of *Histoplasma* fungi, naturally present in soils, after disruption of soil aggregates (1). This fungal disease is endemic to tropical, subtropical, and temperate regions, and most cases occur in Latin America (e.g., Brazil, Guyanas), North America (mainly in the Ohio and Mississippi River Valleys), and Western and Central Africa. Most cases occurring in Latin America are attributed to *H. capsulatum* sensu stricto (formerly *H. capsulatum* Panama or H81 lineage) and *H. suramericanum* (formerly *H. capsulatum* LAm A lineage). In North America, *H. mississippiense* (formerly *H. capsulatum* NAM 1) and *H. ohiense* (formerly *H. capsulatum* NAM 2) are the predominant etiologic agents of chronic pulmonary histoplasmosis; *H. ohiense* is more virulent (2). Cases in Africa, often with skin and bone involvement, are associated with *Histoplasma* varietas *duboisii*, also referred to as *H. duboisii* or *H. capsulatum* H88 lineage (2).

Different species of the genus *Histoplasma* are found in soils enriched with bird and bat guano, which contribute to the accumulation of nitrogen and phosphorus in soils, favoring fungal growth. In particular, bats (order Chiroptera) act as natural

reservoirs and dispersers of *Histoplasma*, which is often isolated from their organs and guano collected from natural and artificial roosts, including abandoned caves and tunnels, mines, cellars, or basements (3). In addition, numerous histoplasmosis outbreaks linked to bat guano exposure have been reported; for example, a severe histoplasmosis outbreak with fatal outcomes occurred among workers in abandoned tunnels contaminated with spore-bearing dust in the Dominican Republic (4).

In nonendemic regions, such as Europe, few imported cases of histoplasmosis and even fewer apparently autochthonous cases have been reported (5). Regarding the presence of *Histoplasma* spp. in the environment, reports are related to isolations by traditional culture methods from guano and soil samples collected in caves inhabited by bats in Romania and in a chicken farm in Italy (6,7). In addition, in northern Italy, some persons tested positive for histoplasmin skin tests, suggesting that environmental conditions may have enabled establishment and spread of *Histoplasma* in certain areas of Europe (8). In Spain, *Histoplasma* has not been isolated from bat organs or guano, and most clinical cases of histoplasmosis have been considered imported, resulting from international travel and migration (9). Numerous cases of histoplasmosis related to bat exposure have been reported in Africa (10), Central America (4), South America (11), and North America (12). We describe detection of *H. capsulatum* in 2 bat-inhabited tunnels of a tourist route in northern Spain.

The Study

To shed light on the distribution of *Histoplasma* beyond traditional known endemic areas, we conducted a study in 2 bat-inhabited tunnels of the Camino de Hierro in Salamanca, northern Spain, a pedestrian

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route receiving >60,000 visitors since its opening as an ecotourism attraction in 2021 (Appendix, <https://wwwnc.cdc.gov/EID/article/31/5/24-1117-App1.pdf>). We collected 101 guano samples in the tunnels (Appendix Table 1) and found almost 42% were positive for *Histoplasma* by nested PCR, using previously published primers (Appendix Table 2). Specifically, the *Hcp100* gene sequences we isolated (submitted to GenBank under accession nos. PP887829–78) (Table) shared high homology with GenBank sequences corresponding to *H. capsulatum* s.s., *H. suramericanum*, *H. capsulatum* LAm B2, and *H. capsulatum* var. *duboisii*.

Moreover, our phylogenetic analyses (Appendix) indicated that the newly obtained sequences form a fully supported monophyletic group with multiple GenBank sequences of *Histoplasma* (posterior probability = 1; bootstrap support = 100%), without a clear geographic or host-related pattern (Figure).

To determine species of bats roosting in this area, we used BLAST (<https://blast.ncbi.nlm.nih.gov>) to identify cytochrome c oxidase subunit I sequences obtained from *Histoplasma*-positive samples. Results corresponded to different species that are mainly distributed in Europe: *Myotis blythii* was

Table. Identities of *Histoplasma* isolates obtained from guano samples collected in the tunnels of the Camino de Hierro tourist route in study of molecular detection of *Histoplasma*, Spain*

Isolate code	Isolate BLAST identity (sublineage)†	Isolate accession no.	Source species for guano samples	Source accession no.
H1	<i>H. suramericanum</i> (LAm A1)	PP887829	<i>Rhinolophus ferrumequinum</i>	PP919660
H2	<i>H. suramericanum</i> (LAm A1)	PP887830	<i>Myotis blythii</i>	PP919661
H3	<i>H. suramericanum</i> (LAm A1)	PP887831	<i>Myotis</i> sp.	PP919662
H4	<i>H. capsulatum</i> variant <i>duboisii</i> / <i>H. capsulatum</i> sensu lato (LAm B2)	PP887832	<i>M. blythii</i>	PP919663
H5	<i>H. suramericanum</i> (LAm A1)	PP887833	<i>Myotis</i> sp.	PP919664
H6	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887834	<i>M. blythii</i>	PP919665
H7	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887835	<i>M. blythii</i>	PP919666
H8	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887836	<i>Myotis</i> sp.	PP919667
H9	<i>H. suramericanum</i> (LAm A1)	PP887837	<i>M. blythii</i>	PP919668
H10	<i>H. suramericanum</i> (LAm A1)	PP887838	NA	NA
H11	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887839	<i>Miniopterus schreibersii</i>	PP919669
H12	<i>H. suramericanum</i> (LAm A1)	PP887840	<i>M. blythii</i>	PP919670
H13	<i>H. suramericanum</i> (LAm A1)	PP887841	<i>M. blythii</i>	PP919671
H14	<i>H. suramericanum</i> (LAm A1)	PP887842	<i>M. blythii</i>	PP919672
H15	<i>H. suramericanum</i> (LAm A1)	PP887843	<i>M. blythii</i>	PP919673
H16	<i>H. suramericanum</i> (LAm A1)	PP887844	<i>M. blythii</i>	PP919674
H17	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887845	<i>Myotis</i> sp.	PP919675
H18	<i>H. suramericanum</i> (LAm A1)	PP887846	<i>M. blythii</i>	PP919676
H19	<i>H. suramericanum</i> (LAm A1)	PP887847	<i>M. blythii</i>	PP919677
H21	<i>H. suramericanum</i> (LAm A1)	PP887849	<i>Myotis</i> sp.	PP919679
H22	<i>H. suramericanum</i> (LAm A1)	PP887850	<i>Myotis</i> sp.	PP919680
H23	<i>H. suramericanum</i> (LAm A1)	PP887851	<i>M. blythii</i>	PP919681
H26	<i>H. suramericanum</i> (LAm A1)	PP887854	<i>Myotis</i> sp.	PP919684
H27	<i>H. suramericanum</i> (LAm A1)	PP887855	<i>M. blythii</i>	PP919685
H28	<i>H. suramericanum</i> (LAm A1)	PP887856	<i>M. blythii</i>	PP919686
H29	<i>H. suramericanum</i> (LAm A1)	PP887857	<i>R. ferrumequinum</i>	PP919687
H30	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887858	<i>M. blythii</i>	PP919688
H31	<i>H. suramericanum</i> (LAm A1)	PP887859	NA	NA
H32	<i>H. suramericanum</i> (LAm A1)	PP887860	<i>M. blythii</i>	PP919689
H33	<i>H. suramericanum</i> (LAm A1)	PP887861	<i>M. blythii</i>	PP919690
H34	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887862	<i>Myotis</i> sp.	PP919691
H35	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887863	<i>R. ferrumequinum</i>	PP919692
H36	<i>H. suramericanum</i> (LAm A1)	PP887864	<i>Myotis</i> sp.	PP919693
H37	<i>H. capsulatum</i> sensu stricto	PP887865	<i>M. blythii</i>	PP919694
H38	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887866	<i>Myotis</i> sp.	PP919695
H39	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887867	<i>M. blythii</i>	PP919696
H40	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887868	<i>M. schreibersii</i>	PP919697
H41	<i>H. suramericanum</i> (LAm A1)	PP887869	<i>M. blythii</i>	PP919698
H42	<i>H. capsulatum</i> var. <i>duboisii</i> / <i>H. capsulatum</i> s.l. (LAm B2)	PP887870	<i>M. blythii</i>	PP919699
H43	<i>H. capsulatum</i> s.s.	PP887871	<i>M. blythii</i>	PP919700
H49	<i>H. suramericanum</i> (LAm A1)	PP887877	<i>M. blythii</i>	PP919706
H50	<i>H. suramericanum</i> (LAm A1)	PP887878	<i>M. blythii</i>	PP919707

*GenBank accession numbers are shown; further details are provided in Appendix Table 4 (<https://wwwnc.cdc.gov/EID/article/31/5/24-1117-App1.pdf>). NA, not available.

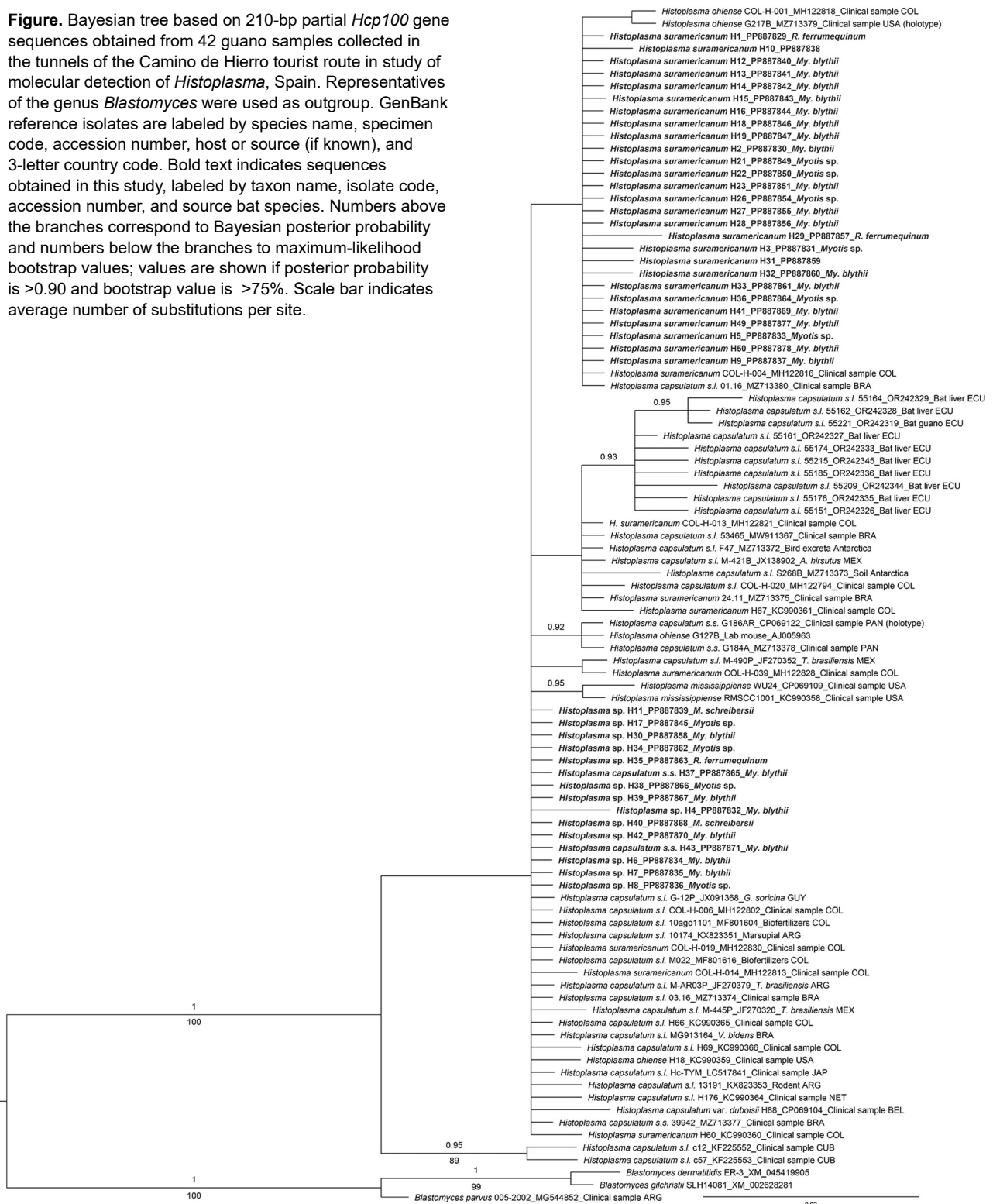
†Species names, according to (2), followed by sublineage when applicable. *H. capsulatum* s.s. = *H. capsulatum* Panama or H81 lineage; *H. capsulatum* var. *duboisii* = *H. capsulatum* African lineage or *H. duboisii*; *H. suramericanum* = *H. capsulatum* LAm A. Note that the identity of some isolates could not be determined at species level because the corresponding Hcp100 sequences shared high similarity values with several GenBank isolates representing different *Histoplasma* species or lineages (separated by a slash).

the most common, followed by *Rhinolophus ferrumequinum* and *Miniopterus schreibersii* (all sequences were submitted to GenBank under accession nos. PP919660–707) (Table). That finding increases the

number of possible hosts and dispersers associated with this human pathogen.

The degree of positivity we observed suggests a substantial presence of *Histoplasma* in the tunnels

Figure. Bayesian tree based on 210-bp partial *Hcp100* gene sequences obtained from 42 guano samples collected in the tunnels of the Camino de Hierro tourist route in study of molecular detection of *Histoplasma*, Spain. Representatives of the genus *Blastomyces* were used as outgroup. GenBank reference isolates are labeled by species name, specimen code, accession number, host or source (if known), and 3-letter country code. Bold text indicates sequences obtained in this study, labeled by taxon name, isolate code, accession number, and source bat species. Numbers above the branches correspond to Bayesian posterior probability and numbers below the branches to maximum-likelihood bootstrap values; values are shown if posterior probability is >0.90 and bootstrap value is >75%. Scale bar indicates average number of substitutions per site.



studied, which seems reasonable because the moderate temperature, constant humidity, and darkness of both tunnels investigated are suitable environmental conditions for fungal growth. Of note, outside known areas of endemicity, *Histoplasma* has been isolated from soils contaminated with bat guano in Romania (6).

A total of 728 cases of human histoplasmosis have been diagnosed in 17 countries in Europe, of which Spain accounts for up to 60% (5). Most cases were imported from Central and South America (9), except 4 cases: 1 autochthonous laboratory-acquired case and 3 cases with no epidemiologic history in patients who had never visited endemic areas. One case was in a drug user with HIV/AIDS, another was in a patient who had disseminated histoplasmosis develop after renal transplantation, and the third was in a patient who had occasionally traveled to France, Italy, and the United Kingdom and had previously been treated with an immunosuppressant, suggesting reactivation of a latent infection (13). However, to date, *Histoplasma* has not been isolated from environmental samples in Spain, and no autochthonous clinical case of histoplasmosis has been associated with exposure to bats.

Conclusions

The results of this study indicate that *Histoplasma* is present in bat-inhabited tunnels at Camino de Hierro in northern Spain. That finding evidences that the geographic distribution of this genus is wider than previously thought and also reinforces the known association between *Histoplasma* and bats.

The risk for histoplasmosis increases with contact with guano deposited in bat roosts. Exploring caves and similar environments is a well-documented source of *Histoplasma* exposure; the first outbreaks of histoplasmosis were related to bat-inhabited locations dating back to the 1930s (14). For that reason, the need to assess the presence of *Histoplasma* in bat-inhabited places before opening them to public access has been emphasized (11). Therefore, even though no autochthonous cases of histoplasmosis have been reported in Spain, the detection of *Histoplasma* in such a popular tourist attraction as Camino de Hierro makes it advisable to alert local clinicians about the importance of considering histoplasmosis in the differential diagnosis of patients with community-acquired pneumonia. Clinicians can refer to available clinical diagnostic algorithms for histoplasmosis for evidenced-based testing guidance (15).

Our results provide a warning about the presence of *Histoplasma* in Camino de Hierro but also could stimulate further research on bat populations in Spain, opening lines of research into their role in

the transmission of histoplasmosis and other airborne infectious diseases. Research on the effects *Histoplasma* and other pathogens on the health of bats should also be considered, and we advise carrying out a serologic study to assess possible exposure to *Histoplasma* among workers and susceptible persons who have visited the tunnels. Given the tourism value and high number of visitors to Camino de Hierro, health authorities should consider our findings and implement measures to prevent potential cases of histoplasmosis, in both visitors and workers of this tourist route.

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About the Author

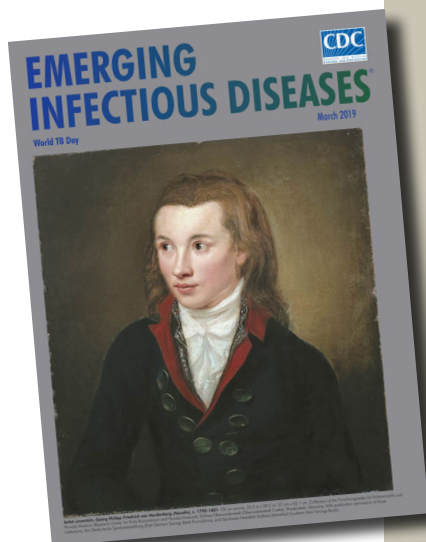
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References

- Emmons CW. Isolation of *Histoplasma capsulatum* from soil. Public Health Rep. 1949;64:892–6. <https://doi.org/10.2307/4587021>
- Sepúlveda VE, Márquez R, Turissini DA, Goldman WE, Matute DR. Genome sequences reveal cryptic speciation

- in the human pathogen *Histoplasma capsulatum*. MBio. 2017;8:e01339–17. <https://doi.org/10.1128/mBio.01339-17>
3. Gugnani HC, Denning DW. Infection of bats with *Histoplasma* species. Med Mycol. 2023;61:myad080. <https://doi.org/10.1093/mmy/myad080>
 4. Armstrong PA, Beard JD, Bonilla L, Arboleda N, Lindsley MD, Chae S-R, et al. Outbreak of severe histoplasmosis among tunnel workers – Dominican Republic, 2015. Clin Infect Dis. 2018;66:1550–7. <https://doi.org/10.1093/cid/cix1067>
 5. Antinori S, Giacomelli A, Corbellino M, Torre A, Schiuma M, Casalini G, et al. Histoplasmosis diagnosed in Europe and Israel: a case report and systematic review of the literature from 2005 to 2020. J Fungi (Basel). 2021;7:481. <https://doi.org/10.3390/jof7060481>
 6. Alteras I. First Romanian isolation of *Histoplasma capsulatum* from the soil. Dermatol Int. 1966;5:69–71. <https://doi.org/10.1111/j.1365-4362.1966.tb05188.x>
 7. Sotgiu G, Mazzoni A, Mantovani A, Ajello L, Palmer J. *Histoplasma capsulatum*: occurrence in soil from the Emilia-Romagna region of Italy. Science. 1965;147:624.
 8. Taylor ML, Reyes-Montes MDR, Estrada-Bárceñas DA, Zancopé-Oliveira RM, Rodríguez-Arellanes G, Ramírez JA. Considerations about the geographic distribution of *Histoplasma* species. Appl Environ Microbiol. 2022;88:e0201021. <https://doi.org/10.1128/aem.02010-21>
 9. Molina-Morant D, Sánchez-Montalvá A, Salvador F, Sao-Avilés A, Molina I. Imported endemic mycoses in Spain: evolution of hospitalized cases, clinical characteristics and correlation with migratory movements, 1997–2014. PLoS Negl Trop Dis. 2018;12:e0006245. <https://doi.org/10.1371/journal.pntd.0006245>
 10. Pakasa N, Biber A, Nsiangana S, Imposo D, Sumaili E, Muhindo H, et al. African histoplasmosis in HIV-negative patients, Kimpese, Democratic Republic of the Congo. Emerg Infect Dis. 2018;24:2068–70.
 11. Rocha-Silva F, Figueiredo SM, Silveira TT, Assunção CB, Campolina SS, Pena-Barbosa JP, et al. Histoplasmosis outbreak in Tamboril cave – Minas Gerais state, Brazil. Med Mycol Case Rep. 2013;4:1–4.
 12. Velasco-Castrejón O, González-Ochoa A. Primary pulmonary epidemic histoplasmosis in an abandoned mine. Mykosen. 1977;20:393–9.
 13. Buitrago MJ, Cuenca-Estrella M. Current epidemiology and laboratory diagnosis of endemic mycoses in Spain [in Spanish]. Enferm Infecc Microbiol Clin. 2012;30:407–13.
 14. Benedict K, Mody RK. Epidemiology of histoplasmosis outbreaks, United States, 1938–2013. Emerg Infect Dis. 2016;22:370–8. <https://doi.org/10.3201/eid2203.151117>
 15. Smith DJ, Free RJ, Thompson GR III, Baddley JW, Pappas PG, Benedict K, et al.; Endemic Mycoses Diagnostic Algorithm Subject Matter Expert Group. Clinical testing guidance for coccidioidomycosis, histoplasmosis, and blastomycosis in patients with community-acquired pneumonia for primary and urgent care providers. Clin Infect Dis. 2024;78:1559–63. <https://doi.org/10.1093/cid/ciad619>

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etymologia revisited

Streptomycin

strep'to-mi'sin

In the late 1930s, Selman Waksman, a soil microbiologist working at the New Jersey Agricultural Station of Rutgers University, began a large-scale program to screen soil bacteria for antimicrobial activity. By 1943, Albert Schatz, a PhD student working in Waksman's laboratory, had isolated streptomycin from *Streptomyces griseus* (from the Greek *strepto-* ["twisted"] + *mykēs* ["fungus"] and the Latin *griseus*, "gray").

In 1944, William H. Feldman and H. Corwin Hinshaw at the Mayo Clinic showed its efficacy against *Mycobacterium tuberculosis*. Waksman was awarded a Nobel Prize in 1952 for his discovery of streptomycin, although much of the credit for the discovery has since been ascribed to Schatz. Schatz later successfully sued to be legally recognized as a co-discoverer of streptomycin.

References:

1. Comroe JH Jr. Pay dirt: the story of streptomycin. Part I. From Waksman to Waksman. Am Rev Respir Dis. 1978;117:773–81.
2. Wainwright M. Streptomycin: discovery and resultant controversy. Hist Philos Life Sci. 1991;13:97–124.

https://wwwnc.cdc.gov/eid/article/25/3/et-2503_article

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Appendix

Materials and Methods

Sampling location

The study was conducted in Camino de Hierro, a pedestrian route of 17 km (with 20 tunnels and 10 bridges) that runs along a section of a closed railway line built to facilitate the cross-border connection between Spain and Portugal at the end of the 19th century. After almost 40 years of abandonment, the Camino de Hierro was opened as an ecotourism attraction in 2021, receiving since then more than 60,000 visitors (1).

The two longest tunnels of Camino de Hierro (tunnel 1, or “La Carretera tunnel,” 40°59′08.4″N 6°50′19.5″W, 1500 m long; and tunnel 3, or “Morgado tunnel,” 40°57′10.9″N 6°51′06.5″W; 423 m long) are home to one of the most important colonies of hibernating and breeding cave bats in the Iberian Peninsula (Appendix Figure 1).

Only in the tunnel 3, there are more than 12,000 bats of different species (Appendix Figure 2), most listed as vulnerable and/or protected in the Spanish Catalogue of Threatened Species (2).

Guano sample collection

A set of 101 guano samples was collected in the tunnels 1 and 3. Samples were collected using sterile scoops from the topsoil under roosting bats after natural defecation, without interacting with the animals at any time to minimize stress (Appendix Figure 1, panel D). They were placed in 50 mL Falcon tubes without preservatives and kept at 4°C during transportation

within 2 h of collection to the laboratory of the Center for Research on Tropical Diseases of the Universidad de Salamanca, CIETUS (Spain).

Sample collection was approved by the Bioethics Committee of the Universidad de Salamanca (ref. RD 53/013, registration no. 965/2023), and authorized by the Servicio Territorial de Medio Ambiente (Delegación Territorial de Salamanca, Junta Castilla y León, Spain), under license AUES_SA_12 (JC)_23.

Genomic DNA extraction

DNA was immediately extracted from guano samples using commercial kits following the manufacturer's instructions, adjusting the elution volume to 50 µL. DNA concentration was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, United States). DNA extractions were stored at −20°C until use (Appendix Table 1).

PCR amplification and amplicon purification

To detect the presence of *Histoplasma* DNA in guano samples, we amplified by nested PCR a 210-bp fragment of Hcp100, a nuclear gene encoding for a regulatory protein involved in fungal adaptation and survival within macrophages, following methodology previously described (3). Each primary reaction mixture contained 12.5 µL of MyTaq Red Mix (Bioline, United Kingdom), 1 µL of each primer (10 nM), 3 µL of DNA template, and Milli-Q water to a final volume of 25 µL, as recommended by the manufacturer. The master mix for nested-PCR reactions was identical, except that 1 µL of the first reaction product was used as DNA template. Additionally, a bat-specific primer pair targeting the mitochondrial cytochrome c oxidase subunit I (COI) gene was used to identify the bat species corresponding to each guano sample positive for *Histoplasma* (Appendix Table 2).

PCR reactions were carried out in a GeneExplorer thermal cycler (Bioer, People's Republic of China). PCR products were run on 1.5% agarose gels under an UVP Biodoc-It® 2 imaging system (Analytik Jena, Germany) and purified using a Gel and PCR Clean up kit (Macherey-Nagel GmbH & Co. KG, Germany). Purified amplicons were Sanger sequenced in both directions by the Nucleus sequencing service of the Universidad de Salamanca, on a 3500 Genetic Analyzer sequencer (Applied Biosystems, USA), with the same primers used for amplification.

Sequences edition, alignment and phylogenetic analyses

Sequence edition and consensus assembly were performed in Geneious v. 7.1.9 (4). After trimming low quality ends, BLAST similarity searches were performed against the NCBI database to identify the source of the sequences. To place our Hcp100 sequences in a phylogenetic context, we downloaded all 301 homologous sequences, obtained from different *Histoplasma* species, available in GenBank on 1st June 2024.

There were many identical sequences so only 51 unique sequences were finally used. Additionally, three sequences generated from representatives of the genus *Blastomyces*, closely related to *Histoplasma* (5), were used as outgroup (Appendix Table 3).

The final dataset consisted of 104 sequences, which were automatically aligned with MAFFT (6) using the G-INS-I strategy, as implemented in Geneious (4).

Maximum likelihood (ML) and Bayesian inference (BI) analyses were conducted. The ML phylogenetic tree was built using IQ-TREE v. 2.2.2.7 (7), with the optimal partitioning scheme and the corresponding best-fit model of nucleotide substitution being selected by the integrated version of ModelFinder (8). Branch support (BS) was assessed using the “complete bootstrap” option with 1,000 non-parametric replicates.

The BI analysis was carried out using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) method, as implemented in MrBayes v. 3.2.7a (9). To estimate the best-fit substitution model for the single partition identified by ModelFinder, the reversible jumping model choice was used (10), allowing a gamma distributed rate heterogeneity across sites, and a proportion of invariant sites. Four independent runs, each with six chains, were executed for 50 million generations, sampling every 1,000, with the first 25% discarded as burn-in and the posterior probabilities (PP) being calculated from the remaining ones. To avoid unnecessary computational overload, the analysis was automatically stopped when the average standard deviation of split frequencies (σ) fell below 0.01.

The convergence of the runs was additionally assessed by checking that the value of the effective sample size (ESS) for each parameter was higher than 200, using Tracer v. 1.7.2 (11). Both ML and BI trees were visualized and compared in FigTree v. 1.4.4 (12). Significant support was assumed for nodes with BS values $\geq 70\%$ and PP values ≥ 0.95 . Since both analyses yielded

similar topologies, and no incongruences were detected, only the Bayesian tree showing PP and BS support values is provided in the main text.

Results

Among the 101 DNA samples analyzed, 42 (41.6%) were positive for the Hcp100 gene. BLAST results showed that our sequences share high homology with Hcp100 GenBank sequences corresponding to *H. capsulatum* s.s., *H. capsulatum* LAm A1 (= *H. suramericanum*), *H. capsulatum* LAm B2, and *H. capsulatum* var. *duboisii* (BLAST similarity values >98.6%; Appendix Table 4).

BLAST-based identification of the 40 corresponding COI sequences (see Table in main text) indicated that they represent bat species mainly distributed in Europe from, at least, three genera: *Myotis blythii*, also known as “lesser mouse-eared bat,” and congeneric species (83.3%), *Rhinolophus ferrumequinum* or “greater horseshoe bat” (7.14%), and *Miniopterus schreibersii* or “Schreiber's bent-winged bat” (4.76%).

Histoplasma has previously been detected in guano samples of *M. schreibersii* from caves in New South Wales and Australia (13). However, this is the first time that *R. ferrumequinum* is associated with *Histoplasma*, previously isolated from the guano of a single species of the same genus, *R. luctus*, in a Malaysian cave (14). It is also associated for the first time with *M. blythii*, previously isolated from guano samples of two congeneric bat species, *M. chinensis* and *M. muricola* (15). For two *Histoplasma*-positive samples (H10 and H31) we were unable to determine the identity of the source bat species, as we could not amplify the COI gene (Table).

References

1. La_Gaceta_de_Salamanca. Éxito confirmado del Camino de Hierro. 2024 [cited 2024 Oct 12]. <https://www.lagacetadesalamanca.es/provincia/exito-confirmado-camino-hierro-20240508122802-nt.html>
2. Revilla-Martín N, Alonso-Alonso P, Sereno-Cadierno J, Llanos-Guerrero C, Hernández-Tabernero L, Lizana-Avia M. Bat conservation and ecotourism: the case of two abandoned tunnels in Salamanca, Western Iberia. *Barbastella* (Alcala Hen). 2021;14:63–8. <https://doi.org/10.14709/BarbJ.14.1.2021.09>

3. Bialek R, Feucht A, Aepinus C, Just-Nübling G, Robertson VJ, Knobloch J, et al. Evaluation of two nested PCR assays for detection of *Histoplasma capsulatum* DNA in human tissue. J Clin Microbiol. 2002;40:1644–7. [PubMed](https://pubmed.ncbi.nlm.nih.gov/12288442/) <https://doi.org/10.1128/JCM.40.5.1644-1647.2002>
4. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28:1647–9. [PubMed](https://pubmed.ncbi.nlm.nih.gov/22172222/) <https://doi.org/10.1093/bioinformatics/bts199>
5. Kandemir H, Dukik K, de Melo Teixeira M, Stielow JB, Delma FZ, Al-Hatmi A, et al. Phylogenetic and ecological reevaluation of the order Onygenales. Fungal Divers. 2022;115:1–72. <https://doi.org/10.1007/s13225-022-00506-z>
6. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. [PubMed](https://pubmed.ncbi.nlm.nih.gov/23794206/) <https://doi.org/10.1093/molbev/mst010>
7. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32:268–74. [PubMed](https://pubmed.ncbi.nlm.nih.gov/25345332/) <https://doi.org/10.1093/molbev/msu300>
8. Kalyanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermin LS. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods. 2017;14:587–9. [PubMed](https://pubmed.ncbi.nlm.nih.gov/28758468/) <https://doi.org/10.1038/nmeth.4285>
9. Huelsenbeck JP, Ronquist F. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 2001;17:754–5. [PubMed](https://pubmed.ncbi.nlm.nih.gov/11869474/) <https://doi.org/10.1093/bioinformatics/17.8.754>
10. Huelsenbeck JP, Larget B, Alfaro ME. Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. Mol Biol Evol. 2004;21:1123–33. [PubMed](https://pubmed.ncbi.nlm.nih.gov/15259232/) <https://doi.org/10.1093/molbev/msh123>
11. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol. 2018;67:901–4. [PubMed](https://pubmed.ncbi.nlm.nih.gov/30149272/) <https://doi.org/10.1093/sysbio/syy032>
12. Rambaut A. FigTree v 1.4.4. 2018 [cited 2024 Jan 1]. <https://github.com/rambaut/figtree/releases/tag/v1.4.4>

13. Hunt PJ, Harden TJ, Hibbins M, Pritchard RC, Muir DB, Gardner FJ. *Histoplasma capsulatum*. Isolation from an Australian cave environment and from a patient. Med J Aust. 1984;141:280–3. PubMed <https://doi.org/10.5694/j.1326-5377.1984.tb113099.x>
14. Ponnampalam J. Isolation of *Histoplasma capsulatum* from the soil of a cave in central Malaysia. Am J Trop Med Hyg. 1963;12:775–6. PubMed <https://doi.org/10.4269/ajtmh.1963.12.775>
15. Gugnani HC, Denning DW. Infection of bats with *Histoplasma* species. Med Mycol. 2023;61:myad080. PubMed <https://doi.org/10.1093/mmy/myad080>
16. Walker FM, Williamson CH, Sanchez DE, Sobek CJ, Chambers CL. Species from feces: order-wide identification of Chiroptera from guano and other non-invasive genetic samples. PLoS One. 2016;11:e0162342. PubMed <https://doi.org/10.1371/journal.pone.0162342>
17. Sepúlveda VE, Márquez R, Turissini DA, Goldman WE, Matute DR. Genome sequences reveal cryptic speciation in the human pathogen *Histoplasma capsulatum*. mBio. 2017;8:e01339–17. PubMed <https://doi.org/10.1128/mBio.01339-17>

Appendix Table 1. Data on the 101 DNA extractions from guano samples collected in Camino de Hierro.

DNA extraction number	Collection date	DNA extraction kit	A260/A280 ratio	A260/A230 ratio	Concentration (ng/μL)	Isolate code
1	2023 Feb 27	NZY _s	1.88	1.51	33.5	
2	2023 Feb 27	NZY _s	1.945	1.005	14.9	
3	2023 Feb 27	NZY _s	2	2.015	36	
4	2023 Feb 27	NZY _s	1.93	1.97	45.55	
5	2023 Mar 27	NZY _s	1.8	1.985	48.65	
6	2023 Mar 27	NZY _s	1.835	0.98	14.6	
7	2023 Feb 27	NZY _s	1.805	0.195	10.4	
8	2023 Jan 23	PCI + NZY _t	1.82	1.72	34.6	
9	2023 Jan 23	PCI + NZY _t	1.825	1.525	12.5	
10	2023 Jan 23	PCI + NZY _t	1.81	1.835	13.6	
11	2024 Apr 18	QIAGEN _{PS}	1.885	1.675	192.65	
12*	2023 Feb 27	QIAGEN _{PS}	1.87	1.295	45.65	H1
13*	2023 Oct 27	QIAGEN _{PS}	2.165	0.06	11.65	H2
14	2024 Apr 18	QIAGEN _{PS}	1.82	0.568	48	
15	2024 Apr 18	QIAGEN _{PS}	1.935	1.045	21.3	
16	2024 Apr 18	QIAGEN _{PS}	1.945	0.725	24.55	
17	2024 Apr 18	QIAGEN _{PS}	1.91	0.815	23.95	
18	2024 Apr 18	QIAGEN _{PS}	1.85	0.93	17	
19	2024 Apr 18	QIAGEN _{PS}	2.04	0.96	28.9	
20	2024 Apr 18	QIAGEN _{PS}	2.045	0.445	19.4	
21	2024 Apr 18	QIAGEN _{PS}	1.935	1.9	100.45	
22*	2024 Apr 18	QIAGEN _{PS}	2	0.695	10.35	H3
23	2024 Apr 18	QIAGEN _{PS}	1.86	2.09	221.15	
24	2024 Apr 18	QIAGEN _{PS}	1.185	0.61	18.55	
25*	2024 Apr 18	QIAGEN _{PS}	1.83	1.455	117.65	H4
26	2024 Apr 18	QIAGEN _{PS}	1.87	0.705	173.1	
27	2024 Apr 18	QIAGEN _{PS}	1.865	1.2	139.5	
28	2024 Apr 18	QIAGEN _{PS}	1.95	1.935	101.5	
29	2024 Apr 18	QIAGEN _{PS}	1.92	2.03	91.7	
30*	2024 Apr 18	QIAGEN _{PS}	1.9	2.325	146.5	H5
31*	2024 Apr 18	QIAGEN _{PS}	1.895	1.48	99.95	H6
32	2024 Apr 18	QIAGEN _{PS}	1.865	2.22	160.05	
33*	2024 Apr 18	QIAGEN _{PS}	2.07	0.84	58.15	H7
34*	2024 Apr 18	QIAGEN _{PS}	1.9	0.81	146.3	H8
35*	2024 Apr 18	QIAGEN _{PS}	1.895	2.005	76.85	H9
36*	2024 Apr 18	QIAGEN _{PS}	1.91	1.545	77.8	H10
37	2024 Apr 18	QIAGEN _{PS}	1.855	1.275	101.55	
38*	2024 Apr 18	QIAGEN _{PS}	1.89	1.12	62.35	H11

DNA extraction number	Collection date	DNA extraction kit	A260/A280 ratio	A260/A230 ratio	Concentration (ng/μL)	Isolate code
39*	2024 Apr 18	QIAGEN _{PS}	1.83	1.48	62.5	H12
40	2024 Apr 18	QIAGEN _{PS}	1.86	1.275	64	
41*	2024 Apr 18	QIAGEN _{PS}	1.93	1.29	134.45	H13
42	2024 Apr 18	QIAGEN _{PS}	1.915	0.35	21.4	
43*	2024 Apr 18	QIAGEN _{PS}	1.905	0.29	110.2	H14
44*	2024 Apr 18	QIAGEN _{PS}	1.89	1.76	123.05	H15
45*	2024 Apr 18	QIAGEN _{PS}	1.885	1.965	95.95	H16
46*	2024 Apr 18	QIAGEN _{PS}	1.975	0.71	48.55	H17
47*	2024 Apr 18	QIAGEN _{PS}	1.96	0.695	139.95	H18
48*	2023 Feb 27	QIAGEN _{PS}	1.635	0.405	6.7	H19
49*	2023 Apr 27	QIAGEN _{PS}	1.48	0.27	29.15	H21
50*	2023 Feb 27	QIAGEN _{PS}	1.68	0.575	25.9	H22
51*	2023 Feb 27	QIAGEN _{PS}	1.74	0.585	20.85	H23
52	2024 Apr 18	QIAGEN _{PS}	1.775	0.44	36.25	
53*	2024 Apr 18	QIAGEN _{PS}	1.325	0.1	12.25	H26
54	2024 Apr 18	QIAGEN _{PS}	1.61	0.695	27.75	
55	2024 Apr 18	QIAGEN _{PS}	1.755	0.81	24.75	
56	2024 Apr 18	QIAGEN _{PS}	1.4	0.055	6.35	
57	2024 Apr 18	QIAGEN _{PS}	1.285	0.295	16.95	
58	2024 Apr 18	QIAGEN _{PS}	1.76	0.665	31.65	
59	2024 Apr 18	QIAGEN _{PS}	1.495	0.285	22.6	
60	2024 Apr 18	QIAGEN _{PS}	0.905	0.135	4.8	
61	2024 Apr 18	QIAGEN _{PS}	2.56	0.115	2.9	
62*	2024 Apr 18	QIAGEN _{PS}	1.76	1.085	37.5	H27
63	2024 Apr 18	QIAGEN _{PS}	1.775	1.17	61.95	
64	2024 Apr 18	QIAGEN _{PS}	2.27	0.72	23.45	
65	2024 Apr 18	QIAGEN _{PS}	2.315	0.205	6.9	
66	2024 Apr 18	QIAGEN _{PS}	2.445	0.44	10.25	
67	2024 Apr 18	QIAGEN _{PS}	2.13	0.775	20.3	
68	2024 Apr 18	QIAGEN _{PS}	1.77	1.045	26.05	
69	2024 Apr 18	QIAGEN _{PS}	2.375	0.09	4.85	
70	2024 Apr 18	QIAGEN _{PS}	0.7	0.7	8.85	
71	2024 Apr 18	QIAGEN _{PS}	1.47	1.105	65.5	
72	2024 Apr 18	QIAGEN _{PS}	1.695	1.33	69	
73	2024 Apr 18	QIAGEN _{PS}	1.645	0.19	18.15	
74	2024 Apr 18	QIAGEN _{PS}	1.635	1.37	82.5	
75	2024 Apr 18	QIAGEN _{PS}	1.79	1.945	154.9	
76	2024 Apr 18	QIAGEN _{PS}	1.505	1.04	52.45	
77	2024 Apr 18	QIAGEN _{PS}	1.765	1.75	38.7	
78	2024 Apr 18	QIAGEN _{PS}	1.32	0.205	24.6	
79	2024 Apr 18	QIAGEN _{PS}	1.56	0.86	47.3	
80	2024 Apr 18	QIAGEN _{PS}	1.7	0.835	88.35	
81	2024 Apr 18	QIAGEN _{PS}	1.72	0.65	20.7	
82	2024 Apr 18	QIAGEN _{PS}	1.215	0.035	3.55	
83*	2024 Apr 18	QIAGEN _{PS}	1.37	0.895	13.95	H28
84*	2024 Apr 18	QIAGEN _{PS}	1.175	0.45	6.75	H29
85*	2024 Apr 18	QIAGEN _{PS}	1.35	0.91	8.15	H30
86*	2024 Apr 18	QIAGEN _{PS}	0.755	0.605	2	H31
87*	2024 Apr 18	QIAGEN _{PS}	2.32	0.28	6.5	H32
88*	2024 Apr 18	QIAGEN _{PS}	1.575	0.28	11.7	H33
89*	2024 Apr 18	QIAGEN _{PS}	1.99	0.735	15.15	H34
90*	2024 Apr 18	QIAGEN _{PS}	1.515	0.355	6.35	H35
91*	2024 Apr 18	QIAGEN _{PS}	1.595	0.29	16.15	H36
92*	2024 Apr 18	QIAGEN _{PS}	0.61	0.24	1.95	H37
93*	2024 Apr 18	QIAGEN _{PS}	0.525	0.025	0.9	H38
94*	2024 Apr 18	QIAGEN _{PS}	2.64	1.365	9.45	H39
95*	2024 Apr 18	QIAGEN _{PS}	1.56	0.835	17.75	H40
96*	2024 Apr 18	QIAGEN _{PS}	1.76	0.21	9.8	H41
97*	2024 Apr 18	QIAGEN _{PS}	1.62	0.29	9.9	H42
98*	2024 Apr 18	QIAGEN _{PS}	1.815	0.44	3.05	H43
99*	2024 Apr 18	QIAGEN _{PS}	1.79	0.52	13.05	H49
100*	2024 Apr 18	QIAGEN _{PS}	1.39	0.46	8.8	H50
101	2024 Apr 18	QIAGEN _{PS}	2.155	1.225	18.05	

*Positive for Hcp100 are marked with an asterisk. NZY_s, NZY Soil gDNA Isolation kit; NZY_t, NZY Tissue gDNA Isolation kit; PCI, phenol chloroform isoamyl alcohol; QIAGEN_{PS}, Qiagen DNeasy Powersoil Pro kit.

Appendix Table 2. Primers and parameters for PCR amplification of Hcp100 and COI

Primer name and sequence (5'–3')	Amplicon size (bp)	Cycling parameters			Reference	
		Step	Temp.	Time		
<i>Histoplasma</i> : first PCR (Hcp100)						
Hcl:	391	Initial denaturation		95°C	2 min	(3)
GCGTTCCGAGCCTTCCACCTCAAC		x35	Denaturation	95°C	1 min	
Hcll:			Annealing	65°C	1 min	
ATGTCCCATCGGGCGCCGTGTAGT			Extension	72°C	2 min	
			Final extension	72°C	10 min	
<i>Histoplasma</i> : nested PCR (Hcp100)						
HcIII:	210	Initial denaturation		95°C	5 min	(3)
GAGATCTAGTCGCGGCCAGGTTCA		x30	Denaturation	95°C	30 s	
HcIV:			Annealing/ext.	72°C	1 min	
AGGAGAGAACTGTATCGGTGGCTTG		Final extension	72°C	5 min		
Bat identification (COI)						
SFF-145f:	202	Initial denaturation		95°C	2 min	(16)
GTHACHGICYCAYGCHTTYGTAATAAT		x35	Denaturation	95°C	1 min	
			Annealing	65°C	1 min	
SFF-351r:			Extension	72°C	2 min	
TCCWGCRTGDGCWAGRTTCC			Final extension	72°C	10 min	

Appendix Table 3. Set of 304 Hcp100 sequences originally downloaded from GenBank.

Species name* (genetic group)†	Isolate or strain code	GenBank accession no.‡	Source or host§	Geographic origin
<i>Blastomyces dermatitidis</i>	ER-3	XM_045419905‡	NA	NA
<i>Blastomyces gilchristii</i>	SLH14081	XM_002628281‡	NA	NA
<i>Blastomyces parvus</i>	005–2002	MG544852‡	Clinical sample	Argentina
<i>H. ohienne</i> *	G127B	AJ005963‡	Lab mouse (R)	NA
<i>H. capsulatum</i> s.l.	DMic 02426	KX823346	Clinical sample	Argentina
<i>H. capsulatum</i> s.l.	13123	KX823347	<i>Calomys musculinus</i> (R)	Argentina
<i>H. capsulatum</i> s.l.	17726	KX823348	<i>Calomys musculinus</i> (R)	Argentina
<i>H. capsulatum</i> s.l.	16978	KX823349	<i>Monodelphis dimidiata</i> (M)	Argentina
<i>H. capsulatum</i> s.l.	17679	KX823350	<i>Akodon azarae</i> (R)	Argentina
<i>H. capsulatum</i> s.l.	10174	KX823351‡	<i>Didelphis albiventris</i> (M)	Argentina
<i>H. capsulatum</i> s.l.	13647	KX823352	<i>Calomys laucha</i> (R)	Argentina
<i>H. capsulatum</i> s.l.	13191	KX823353‡	<i>Calomys laucha</i> (R)	Argentina
<i>H. mississippiense</i> *	NAm1	XM_001543550	NA	NA
<i>H. capsulatum</i> s.s.*	G186AR ^H	XM_045433674	NA	Panama
<i>H. capsulatum</i> s.l.	NA	MG913164‡	<i>Vampyriscus bidens</i> (Ch)	Brazil
<i>H. capsulatum</i> s.l.	53465	MW911367‡	Clinical sample	Brazil
<i>H. capsulatum</i> s.l.	53505	MW911368	Clinical sample	Brazil
<i>H. capsulatum</i> s.l.	10ago1101	MF801604‡	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	M027	MF801605	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	CUEVA100	MF801606	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	M025	MF801607	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	18nov1006	MF801608	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	M014	MF801609	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	8nov1101	MF801610	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	28feb1203	MF801611	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	M030	MF801612	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	nn6. FacM	MF801613	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	13525	MF801614	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	21jul1105	MF801615	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	M022	MF801616‡	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	R3. 10cm	MF801617	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	R3. sup	MF801618	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	R3. 15cm	MF801619	Biofertilizers	Colombia
<i>H. capsulatum</i> s.l.	COL_H_020	MH122794‡	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_037	MH122795	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_043	MH122796	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_066	MH122797	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_036	MH122798	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_007	MH122799	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_057	MH122800	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_008	MH122801	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_006	MH122802‡	Clinical sample	Colombia
<i>H. capsulatum</i> s.l. (LAm B1)	COL_H_012	MH122803	Clinical sample	Colombia

Species name* (genetic group)†	Isolate or strain code	GenBank accession no.‡	Source or host§	Geographic origin
H. capsulatum s.l. (LAm B1)	COL_H_016	MH122804	Clinical sample	Colombia
H. capsulatum s.l. (LAm B1)	COL_H_017	MH122805	Clinical sample	Colombia
H. capsulatum s.l. (LAm B1)	COL_H_018	MH122806	Clinical sample	Colombia
H. capsulatum s.l. (LAm B1)	COL_H_029	MH122807	Clinical sample	Colombia
H. capsulatum s.l. (LAm B1)	COL_H_032	MH122808	Clinical sample	Colombia
H. capsulatum s.l. (LAm B1)	COL_H_035	MH122809	Clinical sample	Colombia
H. capsulatum s.l. (LAm B1)	COL_H_040	MH122810	Clinical sample	Colombia
H. capsulatum s.l. (LAm B1)	COL_H_056	MH122811	Clinical sample	Colombia
H. capsulatum s.l. (LAm B1)	COL_H_042	MH122812	Clinical sample	Colombia
H. suramericanum* (LAm A2)	COL_H_014	MH122813‡	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_H_015	MH122814	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_H_024	MH122815	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_H_004	MH122816‡	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_H_048	MH122817	Clinical sample	Colombia
H. ohienae*	COL_H_001	MH122818‡	Clinical sample	Colombia
H. ohienae*	COL_H_005	MH122819	Clinical sample	Colombia
H. ohienae*	COL_H_038	MH122820	Clinical sample	Colombia
H. suramericanum* (LAm A2)	COL_H_013	MH122821‡	Clinical sample	Colombia
H. suramericanum* (LAm A2)	COL_H_025	MH122822	Clinical sample	Colombia
H. capsulatum s.l.	COL_H_033	MH122823	Clinical sample	Colombia
H. capsulatum s.l.	COL_H_034	MH122824	Clinical sample	Colombia
H. suramericanum* (LAm A2)	COL_H_041	MH122825	Clinical sample	Colombia
H. suramericanum* (LAm A2)	COL_H_055	MH122826	Clinical sample	Colombia
H. suramericanum* (LAm A2)	COL_H_062	MH122827	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_H_039	MH122828‡	Clinical sample	Colombia
H. suramericanum* (LAm A2)	COL_H_053	MH122829	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_H_019	MH122830‡	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_H_021	MH122831	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_H_044	MH122832	Clinical sample	Colombia
H. capsulatum (LAm B1)	COL_H_047	MH122833	Clinical sample	Colombia
H. capsulatum (LAm B1)	COL_H_064	MH122834	Clinical sample	Colombia
H. suramericanum* (LAm A1)	COL_S1	MH122835	Chicken manure	Colombia
H. suramericanum* (LAm A1)	COL_S2	MH122836	Chicken manure	Colombia
H. suramericanum* (LAm A1)	COL_S3	MH122837	Chicken manure	Colombia
H. suramericanum* (LAm A2)	COL_H_068	MH122838	Clinical sample	Colombia
H. capsulatum s.l.	M-431P	JF270313	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-433P	JF270314	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-434P	JF270315	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-435P	JF270316	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-436P	JF270317	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-441P	JF270318	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-444P	JF270319	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-445P	JF270320‡	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-446P	JF270321	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-447P	JF270322	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-448P	JF270323	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-453P	JF270324	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-455P	JF270325	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-456P	JF270326	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-457P	JF270327	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-458P	JF270328	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-459P	JF270329	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-460P	JF270330	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-462P	JF270331	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-463P	JF270332	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-468P	JF270333	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-469P	JF270334	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-470P	JF270335	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-471P	JF270336	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-473P	JF270337	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-474P	JF270338	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-475P	JF270339	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-476P	JF270340	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-477P	JF270341	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-478P	JF270342	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-479P	JF270343	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-480P	JF270344	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-481P	JF270345	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-482P	JF270346	Tadarida brasiliensis (Ch)	Mexico

Species name* (genetic group)†	Isolate or strain code	GenBank accession no.‡	Source or host§	Geographic origin
H. capsulatum s.l.	M-483P	JF270347	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-485P	JF270348	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-486P	JF270349	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-487P	JF270350	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-489P	JF270351	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-490P	JF270352‡	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-491P	JF270353	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-492P	JF270354	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-493P	JF270355	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-494P	JF270356	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-495P	JF270357	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-497P	JF270358	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-502P	JF270359	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-503P	JF270360	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-507P	JF270361	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-510P	JF270362	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-511P	JF270363	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-512P	JF270364	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-513P	JF270365	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-514P	JF270366	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-515P	JF270367	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-516P	JF270368	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-517P	JF270369	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-518P	JF270370	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-519P	JF270371	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-520P	JF270372	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-521P	JF270373	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-522P	JF270374	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-523P	JF270375	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-524P	JF270376	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-525P	JF270377	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-AR01P	JF270378	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-AR03P	JF270379‡	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-AR05P	JF270380	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-484P	HM921044	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-501P	HM921045	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-506P	HM921046	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-527P	HM921047	Glossophaga soricina (Ch)	Mexico
H. capsulatum s.l.	N014	HM921048	Nyctalus noctula (Ch)	France
H. capsulatum s.l.	M-420	JX091346	Artibeus hirsutus (Ch)	Mexico
H. capsulatum s.l.	M-421	JX091347	Artibeus hirsutus (Ch)	Mexico
H. capsulatum s.l.	M-422	JX091348	Artibeus hirsutus (Ch)	Mexico
H. capsulatum s.l.	M-425	JX091349	Artibeus hirsutus (Ch)	Mexico
H. capsulatum s.l.	M-426	JX091350	Artibeus hirsutus (Ch)	Mexico
H. capsulatum s.l.	M-466P	JX091351	Mormoops megalophylla (Ch)	Mexico
H. capsulatum s.l.	M-467P	JX091352	Myotis californicus (Ch)	Mexico
H. capsulatum s.l.	M-526P	JX091353	Glossophaga sp. (Ch)	Mexico
H. capsulatum s.l.	M-527P	JX091354	Glossophaga sp. (Ch)	Mexico
H. capsulatum s.l.	M-528P	JX091355	Glossophaga sp. (Ch)	Mexico
H. capsulatum s.l.	M-529P	JX091356	Glossophaga sp. (Ch)	Mexico
H. capsulatum s.l.	M-530P	JX091357	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-531P	JX091358	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-535P	JX091359	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-536P	JX091360	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-537P	JX091361	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-538P	JX091362	Mormoops megalophylla (Ch)	Mexico
H. capsulatum s.l.	M-539P	JX091363	Mormoops megalophylla (Ch)	Mexico
H. capsulatum s.l.	M-540P	JX091364	Pteronotus davyi (Ch)	Mexico
H. capsulatum s.l.	M-541P	JX091365	Pteronotus parnellii (Ch)	Mexico
H. capsulatum s.l.	M-542P	JX091366	Pteronotus parnellii (Ch)	Mexico
H. capsulatum s.l.	G-8P	JX091367	Glossophaga soricina (Ch)	French Guiana
H. capsulatum s.l.	G-12P	JX091368‡	Glossophaga soricina (Ch)	French Guiana
H. capsulatum s.l.	G-13P	JX091369	Carollia perspicillata (Ch)	French Guiana
H. capsulatum s.l.	G-18P	JX091370	Glossophaga soricina (Ch)	French Guiana
H. capsulatum s.l.	M-421B	JX138902‡	Artibeus hirsutus (Ch)	Mexico
H. capsulatum s.l.	M-422B	JX138903	Artibeus hirsutus (Ch)	Mexico

Species name* (genetic group)†	Isolate or strain code	GenBank accession no.‡	Source or host§	Geographic origin
H. capsulatum s.l.	M-425B	JX138904	Artibeus hirsutus (Ch)	Mexico
H. capsulatum s.l.	M-426B	JX138905	Artibeus hirsutus (Ch)	Mexico
H. capsulatum s.l.	M-444B	JX138906	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-445B	JX138907	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-446B	JX138908	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-450B	JX138909	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-453B	JX138910	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-454B	JX138911	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-455B	JX138912	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-456B	JX138913	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-458B	JX138914	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-459B	JX138915	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-460B	JX138916	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-468B	JX138917	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-470B	JX138918	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-471B	JX138919	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-473B	JX138920	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-474B	JX138921	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-476B	JX138922	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-478B	JX138923	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-480B	JX138924	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-481B	JX138925	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-483B	JX138926	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-484B	JX138927	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-485B	JX138928	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-487B	JX138929	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-501B	JX138930	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-506B	JX138931	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-507B	JX138932	Tadarida brasiliensis (Ch)	Mexico
H. capsulatum s.l.	M-510B	JX138933	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-511B	JX138934	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-512B	JX138935	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-513B	JX138936	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-514B	JX138937	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-515B	JX138938	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-516B	JX138939	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-517B	JX138940	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-518B	JX138941	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-519B	JX138942	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-520B	JX138943	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-521B	JX138944	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-522B	JX138945	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-523B	JX138946	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-524B	JX138947	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-525B	JX138948	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-526B	JX138949	Glossophaga sp. (Ch)	Mexico
H. capsulatum s.l.	M-527B	JX138950	Glossophaga sp. (Ch)	Mexico
H. capsulatum s.l.	M-528B	JX138951	Glossophaga sp. (Ch)	Mexico
H. capsulatum s.l.	M-529B	JX138952	Glossophaga sp. (Ch)	Mexico
H. capsulatum s.l.	M-530B	JX138953	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-531B	JX138954	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-532B	JX138955	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-535B	JX138956	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-536B	JX138957	Natalus stramineus (Ch)	Mexico
H. capsulatum s.l.	M-538B	JX138958	Mormoops megalophylla (Ch)	Mexico
H. capsulatum s.l.	M-539B	JX138959	Mormoops megalophylla (Ch)	Mexico
H. capsulatum s.l.	M-541B	JX138960	Pteronotus parnellii (Ch)	Mexico
H. capsulatum s.l.	M-542B	JX138961	Pteronotus parnellii (Ch)	Mexico
H. capsulatum s.l.	M-AR01	JX138962	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	M-AR03	JX138963	Tadarida brasiliensis (Ch)	Argentina
H. capsulatum s.l.	Hc-TYM	LC517841‡	Clinical sample	Japan
H. capsulatum s.l.	c12	KF225552‡	Clinical sample	Cuba
H. capsulatum s.l.	c57	KF225553‡	Clinical sample	Cuba
H. capsulatum s.l.	F49	MZ713369	Bird excreta	Antarctica
H. capsulatum s.l.	F54	MZ713370	Bird excreta	Antarctica
H. capsulatum s.l.	S269	MZ713371	Soil	Antarctica
H. capsulatum s.l.	F47	MZ713372‡	Bird excreta	Antarctica

Species name* (genetic group)†	Isolate or strain code	GenBank accession no.‡	Source or host§	Geographic origin
<i>H. capsulatum</i> s.l.	S268B	MZ713373‡	Soil	Antarctica
<i>H. capsulatum</i> s.l.	03.16	MZ713374‡	Clinical sample	Brazil
<i>H. suramericanum</i> * (RJ)	24.11	MZ713375‡	Clinical sample	Brazil
<i>H. capsulatum</i> s.l.	20231	MZ713376	Clinical sample	Brazil
<i>H. capsulatum</i> s.s.*	39942	MZ713377‡	Clinical sample	Brazil
<i>H. capsulatum</i> s.s.*	G184A	MZ713378‡	Clinical sample	Panama
<i>H. ohienne</i> *	G217B ^H	MZ713379‡	Clinical sample	USA
<i>H. capsulatum</i> s.l.	01.16	MZ713380‡	Clinical sample	Brazil
<i>H. capsulatum</i> s.l.	55205	OR242318	Bat guano	Ecuador
<i>H. capsulatum</i> s.l.	55221	OR242319‡	Bat guano	Ecuador
<i>H. capsulatum</i> s.l.	55225	OR242320	Bat guano	Ecuador
<i>H. capsulatum</i> s.l.	55267	OR242321	Bat guano	Ecuador
<i>H. capsulatum</i> s.l.	U003	OR242322	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	U007	OR242323	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	U008	OR242324	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	U009	OR242325	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55151	OR242326‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55161	OR242327‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55162	OR242328‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55164	OR242329‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55165	OR242330	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55166	OR242331	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55170	OR242332	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55174	OR242333‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55175	OR242334	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55176	OR242335‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55185	OR242336‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55186	OR242337	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55187	OR242338	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55189	OR242339	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55194	OR242340	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55195	OR242341	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55202	OR242342	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55204	OR242343	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55209	OR242344‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55215	OR242345‡	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55216	OR242346	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55219	OR242347	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55225	OR242348	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55233	OR242349	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55239	OR242350	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55242	OR242351	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55252	OR242352	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55255	OR242353	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55264	OR242354	Undetermined species (Ch)	Ecuador
<i>H. capsulatum</i> s.l.	55281	OR242355	Undetermined species (Ch)	Ecuador
<i>H. mississippiense</i> *	RMSCC1001	KC990358‡	Clinical sample	USA
<i>H. ohienne</i> *	H18	KC990359‡	Clinical sample	USA
<i>H. suramericanum</i> * (LAm A1)	H60	KC990360‡	Clinical sample	Colombia
<i>H. suramericanum</i> * (LAm A2)	H67	KC990361‡	Clinical sample	Colombia
<i>H. capsulatum</i> (LAm B1)	H59	KC990362	Clinical sample	Colombia
<i>H. capsulatum</i> var. <i>duboisii</i> *	H91	KC990363	Clinical sample	Liberia
<i>H. capsulatum</i> s.l.	H176	KC990364‡	Clinical sample	Netherlands
<i>H. capsulatum</i> (LAm B2)	H66	KC990365‡	Clinical sample	Colombia
<i>H. capsulatum</i> (LAm B2)	H69	KC990366‡	Clinical sample	Colombia
<i>H. capsulatum</i> s.s.*	H81	KC990367	Clinical sample	Panama
<i>H. capsulatum</i> var. <i>duboisii</i>	H88	CP069104‡	Clinical sample	Belgium
<i>H. mississippiense</i> *	WU24	CP069109‡	Clinical sample	USA
<i>H. capsulatum</i> s.s.*	G186AR ^H	CP069122‡	Clinical sample	Panama

*Species name according to Sepúlveda *et al.* (17). Based on the information available in the literature or GenBank for that specific isolate: *H. capsulatum* s.s. (= *H. capsulatum* Panama or H81 lineage); *H. mississippiense* (= *H. capsulatum* NAm 1); *H. ohienne* (= *H. capsulatum* NAm 2); *H. suramericanum* (= *H. capsulatum* LAm A); and *H. capsulatum* var. *duboisii* (= *H. capsulatum* African lineage or *H. duboisii*). H, holotype; NA, not available.

†Group or sublineage, indicated only when available in previous molecular studies.

‡Unique GenBank sequence included for reference purposes in our phylogenetic analyses.

§Host: (Ch), chiropters; (M), marsupials; (R), rodents.

Appendix Table 4. Hcp100 sequences obtained from guano samples collected in Camino de Hierro and putative identity based on BLAST results.

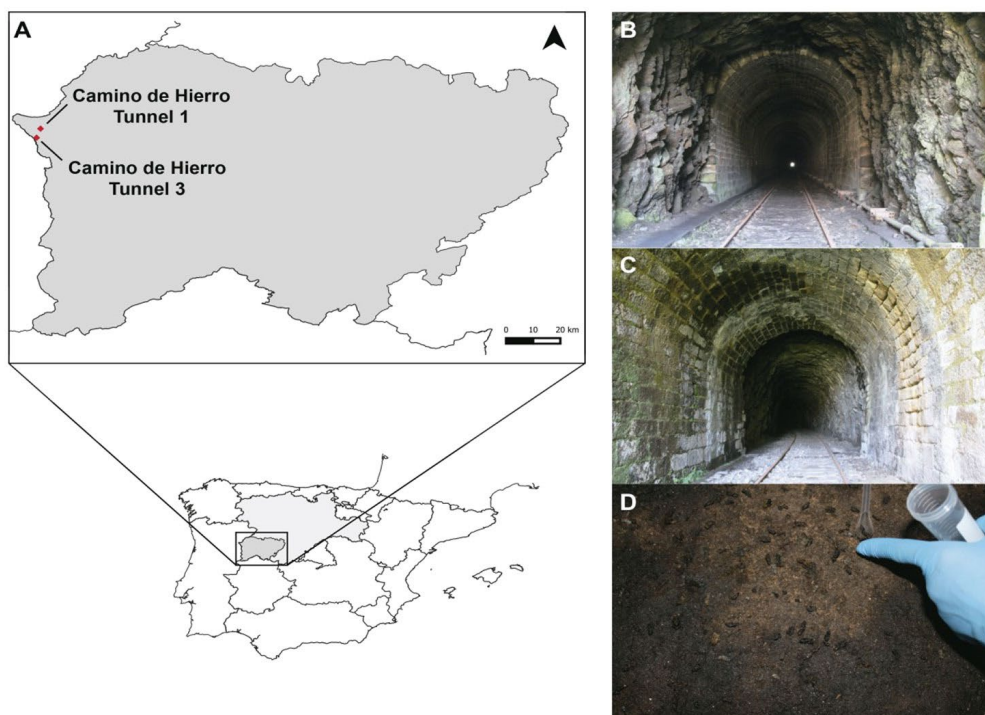
Seq. ID (accession no.)	Most similar GenBank accessions*	Identity (%)	Species† (genetic sublineage)‡	Country
H1 (PP887829)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H2 (PP887830)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H3 (PP887831)	MH122817 MH122816	99.49 99.49	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H4 (PP887832)	KC990365 KC990363	98.57 98.57	<i>H. capsulatum</i> s.l. (LAm B2) <i>H. capsulatum</i> var. <i>duboisii</i>	Colombia Liberia
H5 (PP887833)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H6 (PP887834)	KC990365 KC990363	99.52 99.52	<i>H. capsulatum</i> s.l. (LAm B2) <i>H. capsulatum</i> var. <i>duboisii</i>	Colombia Liberia
H7 (PP887835)	KC990365 KC990363	99.52 99.52	<i>H. capsulatum</i> s.l. (LAm B2) <i>H. capsulatum</i> var. <i>duboisii</i>	Colombia Liberia
H8 (PP887836)	KC990365 KC990363	99.52 99.52	<i>H. capsulatum</i> s.l. (LAm B2) <i>H. capsulatum</i> var. <i>duboisii</i>	Colombia Liberia
H9 (PP887837)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H10 (PP887838)	MH122817 MH122816	99.49 99.49	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H11 (PP887839)	KC990365 KC990363	99.52 99.52	<i>H. capsulatum</i> s.l. (LAm B2) <i>H. capsulatum</i> var. <i>duboisii</i>	Colombia Liberia
H12 (PP887840)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H13 (PP887841)	MH122817 MH122816	99.49 99.49	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H14 (PP887842)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H15 (PP887843)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H16 (PP887844)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H17 (PP887845)	KC990365 KC990363	99.52 99.52	<i>H. capsulatum</i> s.l. (LAm B2) <i>H. capsulatum</i> var. <i>duboisii</i>	Colombia Liberia
H18 (PP887846)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H19 (PP887847)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H21 (PP887849)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H22 (PP887850)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H23 (PP887851)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H26 (PP887854)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H27 (PP887855)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H28 (PP887856)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H29 (PP887857)	MH122817 MH122816	98.97 98.97	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H30 (PP887858)	KC990365 KC990363	99.52 99.52	<i>H. capsulatum</i> s.l. (LAm B2) <i>H. capsulatum</i> var. <i>duboisii</i>	Colombia Liberia
H31 (PP887859)	MH122817 MH122816	99.49 99.49	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H32 (PP887860)	MH122817 MH122816	99.49 99.49	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H33 (PP887861)	MH122817 MH122816	100.00 100.00	<i>H. suramericanum</i> (LAm A1) <i>H. suramericanum</i> (LAm A1)	Colombia Colombia
H34 (PP887862)	KC990365 KC990363	99.52 99.52	<i>H. capsulatum</i> s.l. (LAm B2) <i>H. capsulatum</i> var. <i>duboisii</i>	Colombia Liberia
H35	KC990365	99.52	<i>H. capsulatum</i> s.l. (LAm B2)	Colombia

Seq. ID (accession no.)	Most similar GenBank accessions*	Identity (%)	Species† (genetic sublineage)‡	Country
(PP887863)	KC990363	99.52	<i>H. capsulatum</i> var. <i>duboisii</i>	Liberia
H36	MH122817	99.49	<i>H. suramericanum</i> (LAm A1)	Colombia
(PP887864)	MH122816	99.49	<i>H. suramericanum</i> (LAm A1)	Colombia
H37	MZ713377	99.47	<i>H. capsulatum</i> s.s.	Brazil
(PP887865)	KC990363	99.06	<i>H. capsulatum</i> var. <i>duboisii</i>	Liberia
H38	KC990365	99.52	<i>H. capsulatum</i> s.l. (LAm B2)	Colombia
(PP887866)	KC990363	99.52	<i>H. capsulatum</i> var. <i>duboisii</i>	Liberia
H39	KC990365	99.52	<i>H. capsulatum</i> s.l. (LAm B2)	Colombia
(PP887867)	KC990363	99.52	<i>H. capsulatum</i> var. <i>duboisii</i>	Liberia
H40	KC990365	99.52	<i>H. capsulatum</i> s.l. (LAm B2)	Colombia
(PP887868)	KC990363	99.52	<i>H. capsulatum</i> var. <i>duboisii</i>	Liberia
H41	MH122817	100.00	<i>H. suramericanum</i> (LAm A1)	Colombia
(PP887869)	MH122816	100.00	<i>H. suramericanum</i> (LAm A1)	Colombia
H42	KC990365	100.00	<i>H. capsulatum</i> s.l. (LAm B2)	Colombia
(PP887870)	KC990363	100.00	<i>H. capsulatum</i> var. <i>duboisii</i>	Liberia
H43	MZ713377	100.00	<i>H. capsulatum</i> s.s.	Brazil
(PP887871)	KC990365	99.52	<i>H. capsulatum</i> s.l. (LAm B2)	Colombia
	KC990363	99.52	<i>H. capsulatum</i> var. <i>duboisii</i>	Liberia
H49	MH122817	100.00	<i>H. suramericanum</i> (LAm A1)	Colombia
(PP887877)	MH122816	100.00	<i>H. suramericanum</i> (LAm A1)	Colombia
H50	MH122817	100.00	<i>H. suramericanum</i> (LAm A1)	Colombia
(PP887878)	MH122816	100.00	<i>H. suramericanum</i> (LAm A1)	Colombia

*For each isolate, at least, two hits with identical or almost identical sequence identity values were found.

†Species name according to Sepúlveda *et al.* (17): *H. capsulatum* s.s. (= *H. capsulatum* Panama or H81 lineage); *H. capsulatum* var. *duboisii* (= *H. capsulatum* African lineage or *H. duboisii*); and *H. suramericanum* (= *H. capsulatum* LAm A).

‡ Genetic subgroup or sublineage, indicated only when this information is available in previous molecular studies.



Appendix Figure 1. Sampling sites. A) Map of the Iberian Peninsula showing the approximate geographic location of the sampling points. The province of Salamanca (Castilla y León, Spain) is shaded in dark grey. B) Exit of La Carretera tunnel (tunnel 1). C) Exit of Morgado tunnel (tunnel 3). D) Appearance of the bat feces collected. Note the almost intact rod shape of the excrements, suggesting recent defecation. Photos by Laura Noelly Niño Puerto.



Appendix Figure 2. Small group of bats roosting together in the roof of Morgado tunnel. Photo by Laura Noelly Niño Puerto.