Hydropisphaera angelicae (*Bionectriaceae*), a new species from Spain

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Abstract: A detailed description of *Hydropisphaera angelicae* sp. nov. is presented, based on a collection on dead stems of *Angelica sylvestris* in Spain. The fungus was sequenced and its placement in the genus *Hydropisphaera* is confirmed by analysis of LSU sequences. This species is characterized by ascomata bearing a stellate apical fringe of fasciculate hairs, blackening upon drying, and narrowly fusiform, faintly striate ascospores.
Keywords: Ascomycota, *Hypocreales*, ribosomal DNA, taxonomy.

Résumé : une description détaillée de *Hydropisphaera angelicae* sp. nov. est présentée à partir d'une récolte sur tiges mortes d'*Angelica sylvestris* en Espagne. Le champignon a été séquencé, et son placement dans le genre *Hydropisphaera* est confirmé par l'analyse des séquences LSU. Cette espèce est caractérisée par ses ascomes porteurs d'une frange apicale de poils fasciculés, noircissant en séchant et ses ascospores étroitement fusiformes et faiblement striées.
Mots-clés : ADN ribosomal, Ascomycota, Hypocréales, taxinomie.

Resumen: Basada en una recolección española en tallos muertos de *Angelica sylvestris*, se presenta una descripción detallada de *Hydropisphaera angelicae* sp. nov. Su situación en el género *Hydropisphaera* fue confirmada mediante el análisis de la secuencia LSU del hongo. Esta especie se caracteriza por sus ascomas rodeados por una franja esteliforme apical de pelos fasciculados, su ennegrecimiento tras la desecación y por sus ascosporas estrechamente fusiformes finamente estriadas.
Palabras clave: ADN ribosómico, Ascomycota, *Hypocreales*, taxonomía.

Introduction

In the continuity of a worldwide survey of *Hydropisphaera* Dumort. (Lechat & Fournier, 2016; 2017a; 2017b; 2020; Lechat & Gardiennet, 2009; Lechat *et al*., 2010), we had the opportunity to examine a bionectriaceous fungus collected in Spain, which turned out to be an undescribed species of *Hydropisphaera*. Known species of *Hydropisphaera* are morphologically divided in two groups: one comprising species having fasciculate or solitary hairs around the apex or scattered on the ascomatal wall, and the other comprising glabrous ascomata (Lechat & Fournier, 2020). Based on morphological characteristics, phylogenetic analysis and comparison with known species in the genus, the specimen described herein is considered to represent a previously undescribed species of *Hydropisphaera*.

Material and methods

Dry specimens were rehydrated and examined using the method described by Rossman *et al.* (1999). Microscopic observations and measurements were made in water. The holotype specimen was deposited in LIP herbarium (University of Lille). Cultures of the living specimen were made on PDA (Potato Dextrose Agar) with 5 mg/L of streptomycin in Petri dishes 5 cm diam. incubated at 25°C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Oviedo, Spain): Total DNA was extracted from pure culture, blending a portion using a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65ºC. A similar volume of chloroform:isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13.000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL ddH_2O. PCR amplification was performed with the primers LR0R and LR5 (Vilgalys & Hester, 1990) to amplify the 28S nLSU region. PCR reactions were performed under a program consisting of a hot start at 95ºC for 5 min, followed by 35 cycles at 94ºC, 54ºC and 72ºC (45, 30 and 45 s respectively) and a final 72ºC step 10 min. Chromatograms were checked searching for putative reading errors, and these were corrected.

Analyses were performed online at www.phylogeny.lirmm.fr (Dereeper *et al*., 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (Zwickl, 2006), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric version of the approximate likelihood-ratio test, implemented in PhyML SH-aLRT (Anisimova & Gascuel, 2006). Nomenclature follows MycoBank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

Taxonomy

Hydropisphaera angelicae Lechat, J. Fourn. & Rubio, *sp. nov*. – Mycobank MB 836042 – Fig. 2

Diagnosis: Differs from the most similar species *H. suffulta* (Berk. & M.A. Curtis) Rossman & Samuels in having ascomata becoming blackish brown to nearly black when dry and significantly larger, narrowly fusiform ascospores 34–38 × 5.5–6.5 µm vs. 12–17 × 4–5 µm.

Holotype: Spain, Asturias, Soto del Barco, Riberas de Pravia, carretera a Los Veneros, 43º 29′ 58″ N; 6º 3′ 39″ W; 83 m asl, on dead twigs of *Angelica sylvestris* L. (*Apiaceae*), 22 Oct. 2019, *leg*. E. Rubio ERD-8205 (LIP). GenBank LSU sequence MT681191.

Etymology: The epithet refers to the herbaceous host *Angelica sylvestris* on which this species was collected.

Ascomata perithecial, solitary, scattered to gregarious, occasionally coalescent in small groups, superficial, without a stroma, with basal hyphae spreading over the substratum, globose (300–)360–400(–430) µm diam., (Me = 380 µm, n = 20), pale to dark orange, becoming dark brownish-orange to nearly black, collapsing in a cupulate fashion when dry, not changing color in 3% KOH or lactic acid. **Basal hyphae** densely radiating from the ascomatal base and extending over the substratum, hyaline to pale yellow, 2–3 µm diam., smooth. **Ascomatal apex** conical, with a short, acute papilla composed of a palisade of narrowly clavate cells, margin with fasciculate hairs up to 90 µm long, 3–3.5 µm wide, hyaline to pale yellow, cylindrical, slightly flexuous, rounded at tips, septate, arising from cells of ascomatal wall, agglutinated to form sparse, triangular teeth

25–40 µm wide at base, arranged in an irregular, stellate fringe around upper part of ascomata. **Ascomatal wall** 80–100 µm thick in upper part, containing some orange oily droplets, composed of two regions: outer region 60–80 µm wide, of subglobose to angular cells up to 25 µm in greatest dimension, with pale orange walls 1–1.2 µm thick; inner region 10–20 µm wide, of elongate, flattened cells 5–14 × 3–4 µm, with hyaline walls 1.5–2 µm thick. **Asci** (80–)100(–110) × (13–)15–18(–20) µm (Me = 100 × 17 µm, n=30), short-stipitate, clavate, apex simple, containing 8 irregularly biseriate or multiseriate ascospores. **Ascospores** (32–)34–38(–41) × 5.5–6.5(–7) µm (Me = 36 × 6 µm, n=30), narrowly fusiform, slightly curved in side view, rounded at ends, equally to subequally 1-septate, slightly constricted at septum, hyaline to orange en masse, faintly striate with inconspicuous striae only visible in lactic cotton blue.

Cultural characteristics: After two weeks on PDA at 25°C, colony 25–30 mm diam., white to tan in centre, white at margin. Aerial mycelium white composed of smooth, hyaline, septate hyphae 2.5–3.5 µm diam., not sporulating after four weeks.

Discussion

Based on the morphological features of its sexual and asexual morphs, along with phylogenetic analysis of its LSU sequences, the new species described above is placed in *Hydropisphaera*. The species of this genus are characterized by pale yellow, orange, brownish orange, brown to nearly black ascomata not changing colour in 3% KOH or lactic acid, glabrous or with an apical crown of fasciculate hairs, and with an outer ascomatal wall of thin-walled cells that result in the ascomata collapsing and becoming cupulate upon drying (Lechat & Fournier, 2020; Rossman *et al.*, 1999). Our fungus fits this concept well and comparison of morphological characteristics as well as phylogenetic analysis of LSU sequences with

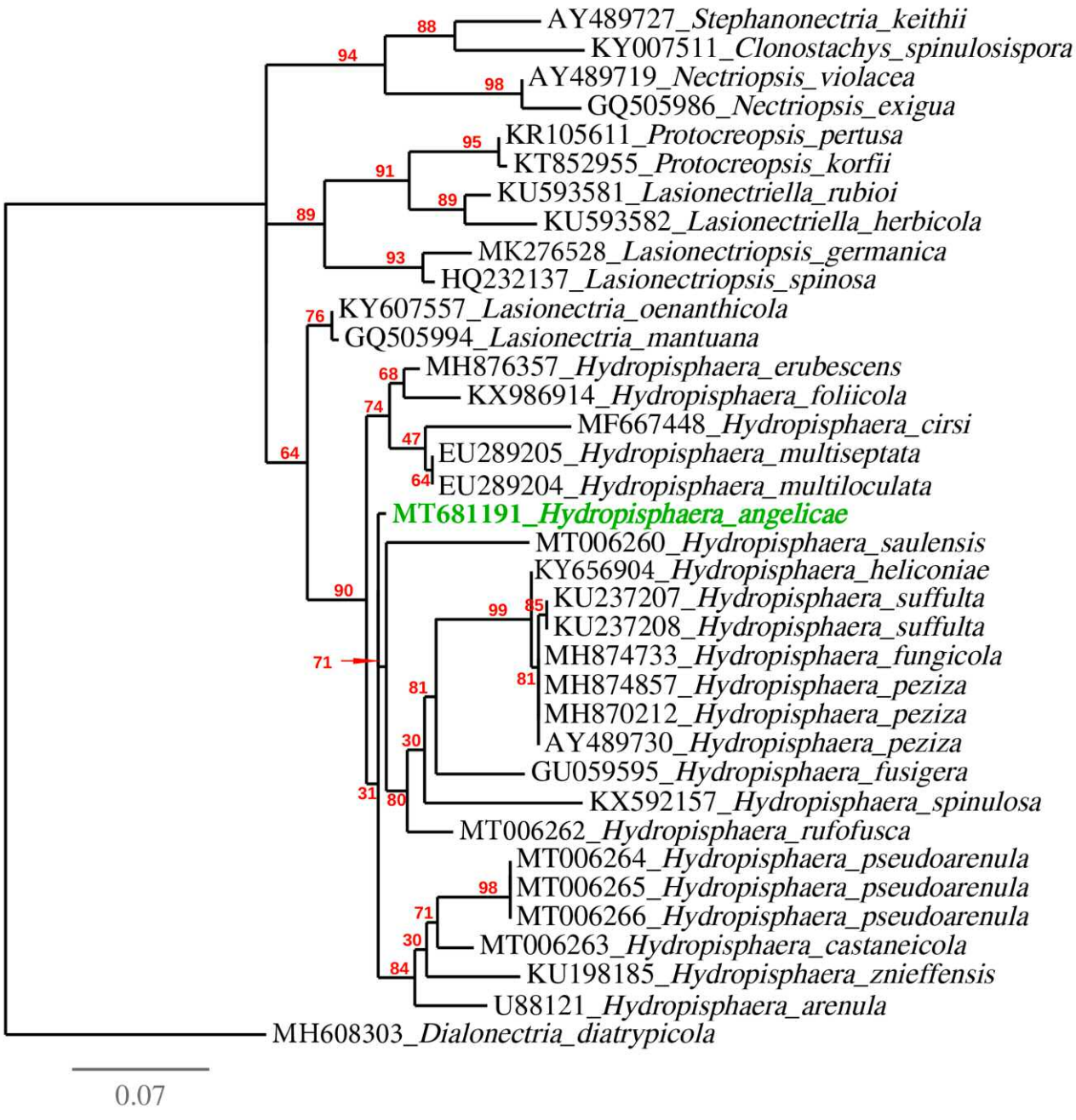

Fig. 1 – Maximum likelihood phylogeny (-lnL = 3009.08500) of *Hydropisphaera* spp. inferred by PhyML 3.0, model HKY85 from a 790 bp matrix of 28S rRNA sequence, rooted with *Dialonectria diatrypicola*.

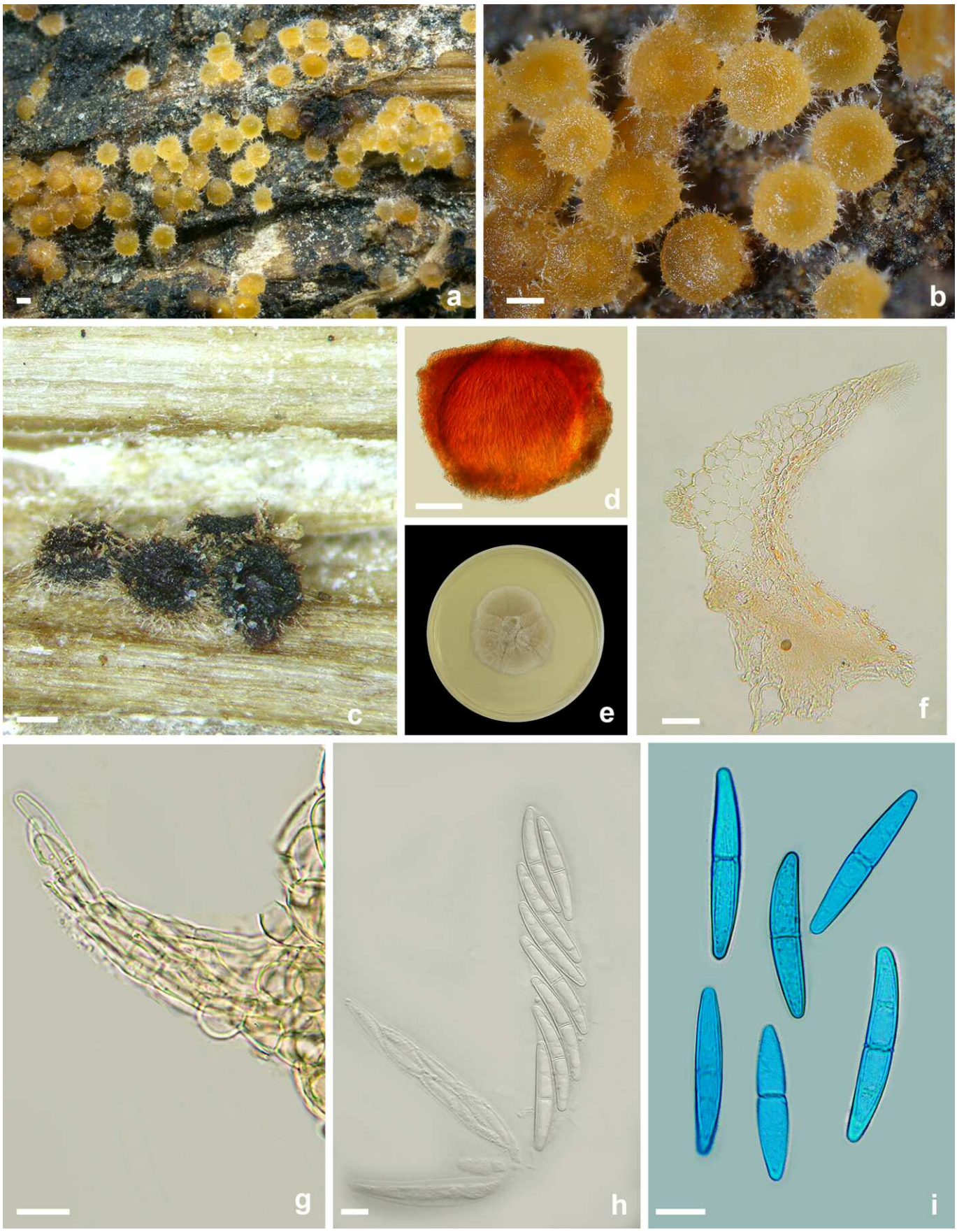

Fig. 2 – a-g: *Hydropisphaera angelicae* (Holotype); a, b: Ascomata in fresh state on the substratum; c: Dried ascomata; d: Close-up of rehydrated ascoma in side view in water; e: Culture after two weeks; f: Lateral ascomatal wall in vertical section; g: Fasciculate hairs arising from ascomatal wall; h: Asci and ascospores in water; i: Ascospores in lactic cotton blue. Scale bars: a, b, c = 200 µm; d = 100 µm; f = 20 µm; g-i = 10 µm.

known species leads us to introduce *H. angelicae* as a new species. Our phylogenetic analysis (Fig. 1) shows that *H. angelicae* is nested in the *Hydropisphaera* clade on an isolated branch between *H. arenula* (Berk. & Broome) Rossman & Samuels and *H. saulensis* Lechat & J. Fourn. *Hydropisphaera saulensis* somewhat resembles *H. angelicae* by having ascomata becoming blackish brown when dry, but differs in having verrucose, significantly smaller ascospores (9–)10–11(–12) × 3.5–4 µm vs. (32–)34–38(–41) × 5.5–6.5(–7) µm, and its occurrence in tropical areas. *Hydropisphaera arenula* differs from our fungus primarily in having glabrous ascomata and smaller ascospores 14–16 × 3.5–4 µm. Morphologically, the most similar species is *H. suffulta* having ascomata of the same colour when fresh and a stellate fringe of fasciculate hairs around the upper part of the ascomata. However, *H. suffulta* primarily differs from *H. angelicae* in having ascomata that do not become blackish upon drying, significantly smaller ascospores 12–17 × 4–5 µm vs. (32–)34–38(–41) × 5.5–6.5(–7) µm, and a tropical to subtropical distribution.

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References

Anisimova M. & Gascuel O. 2006. — Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Systematic Biology*, 55 (4): 539–552. doi: 10.1080/10635150600755453

Dereeper A., Guignon V., Blanc G., Audic S., Buffet S., Chevenet F., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M. & Gascuel O. 2008. — Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, 2008 Jul 1;36 (Web Server issue): W465–469. doi: 10.1093/nar/gkn180

Lechat C., Farr D.F., Hirooka Y., Minnis A.M. & Rossman A.Y. 2010. — A new species of *Hydropisphaera*, *H. bambusicola*, is the sexual state of *Gliomastix fusigera*. *Mycotaxon*, 111: 95–102. doi: 10.5248/111.95

Lechat C. & Fournier J. 2016. — *Hydropisphaera znieffensis*, a new species from Martinique. *Ascomycete.org*, 8 (2): 55–58. doi: 10.25664/art-0170

Lechat C. & Fournier J. 2017a. — *Hydropisphaera foliicola*, a new species from Martinique. *Ascomycete.org*, 9 (1): 6–8. doi: 10.25664/art-0193

Lechat C. & Fournier J. 2017b. — *Hydropisphaera heliconiae*, a new species from Martinique (French West Indies). *Ascomycete.org*, 9 (3): 59–62. doi: 10.25664/art-0200

Lechat C. & Fournier J. 2020. — Three new species of *Hydropisphaera* (*Bionectriaceae*) from Europe and French Guiana. *Ascomycete.org*, 12 (2): 39–46. doi: 10.25664/art-0296

Lechat C. & Gardiennet A. 2009. — *Hydropisphaera castaneicola* sp. nov. *Bulletin mycologique et botanique Dauphiné-Savoie*, 192: 5–8.

Rossman A.Y., Samuels G.J., Rogerson C.T. & Lowen R. 1999. — Genera of *Bionectriaceae*, *Hypocreaceae* and *Nectriaceae* (*Hypocreales*, Ascomycetes). *Studies in Mycology*, 42: 1–248.

Vilgalys R. & Hester M. 1990. — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172 (8): 4238–4246. doi: 10.1128/jb.172.8.4238-4246.1990

Zwickl D.J. 2006. — *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. Dissertation. Austin, The University of Texas.


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