Fundación Miguel Lillo
Tucumán
Argentina

Two new stipitate species of *Phylloporia* (Basidiomycota, Hymenochaetaceae) from Chamela Biology Station, U.N.A.M. in Jalisco, Mexico

Dos nuevas especies estipitadas de *Phylloporia* (Basidiomycota, Hymenochaetaceae) de la Estación de Biología Chamela, U.N.A.M. en Jalisco, México

Chamorro-Martínez, Hannya A.[1]; Tania Raymundo[2]; César Ramiro Martínez-González[3]; Elvira Aguirre Acosta[4]; Ricardo Valenzuela[2]*

[1] Maestría en Biociencias, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prolongación de Carpio y Plan de Ayala s/n, Santo Tomás, Azcapotzalco, 11340, Ciudad de México, México.
[2] Laboratorio de Micología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prolongación de Carpio y Plan de Ayala s/n, Santo Tomás, Azcapotzalco, 11340, Ciudad de México, México.
[3] Departamento de Fitotecnia, Instituto de Horticultura, Universidad Autónoma Chapingo, Km 38.5 Carretera Federal México-Texcoco, Texcoco, Estado de México, 56230, México.
[4] Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México. Ciudad Universitaria, Apartado postal 70-233, Coyoacán, 04510 Ciudad de México, México.
* Corresponding author: <rvalenzg@ipn.mx>

ABSTRACT

Phylloporia species are characterized by having annual to perennial, with resupinate, pileate-sessile to pileate-stipitate basidiomata, and duplex context. They have monomitic to dimitic hyphal system, basidiospores are globose to cylindrical, thick-walled, yellowish to pale brown in KOH. The genus includes 72 species described worldwide, and only eight taxa have been recorded in Mexico, five of which have been molecularly characterized. The objective of this study is to describe and illustrate two new stipitate species of *Phylloporia* from the Chamela Biology Station located in the municipality of La Huerta in the state of Jalisco, Mexico: *P. rajchenbergii* and *P. ryvardenii*. For this, the specimens were reviewed in detail in order to

➤ Ref. bibliográfica: Chamorro-Martínez, H. A.; Raymundo, T.; Martínez-González, C. R.; Aguirre Acosta, E.; Valenzuela, R. 2022. Two new stipitate species of *Phylloporia* (Basidiomycota, Hymenochaetaceae) from Chamela Biology Station, U.N.A.M. in Jalisco, Mexico. *Lilloa 59* (Suplemento): 359-375. **doi**: https://doi.org/10.30550/j.lil/2022.59.S/2022.09.28
➤ Recibido: 19 de julio 2022 – Aceptado: 28 de septiembre 2022 – Publicado en línea: 18 de octubre 2022.
➤ URL de la revista: http://lilloa.lillo.org.ar

describe their macroscopic and microscopic characteristics, DNA extraction was performed and the 28S rDNA (LSU) was amplified by PCR. Phylogenetic analyzes were performed using Bayesian Inference and Maximum Likelihood methods. It is corroborated that *Phylloporia rajchenbergii* and *P. ryvardenii* differ from the species described in the genus due to the color of the pileus, the size of the pores, the type of context (homogeneous in *P. ryvardenii*) and the size of the basidiospores; in addition, both species form different clades in the phylogenetic analyzes carried out.

Keywords — Hymenochaetales; new species; phylogeny; taxonomy.

RESUMEN

Las especies clasificadas en *Phylloporia* se caracterizan por poseer basidiomas anuales a perennes, con hábitos resupinados, pileado-sésiles a pileado-estipitados y contexto doble. Ellas presentan sistema hifal monomítico hasta dimítico, las basidiosporas son globosas a cilíndricas, de pared engrosada, amarillentas a marrón pálidas en KOH. El género incluye 72 especies descritas a nivel mundial, y en México solo se han registrado ocho taxones, cinco de los cuales han sido caracterizados molecularmente. El objetivo de este trabajo consiste en describir e ilustrar dos nuevas especies estipitadas de *Phylloporia* de la Estación de Biología Chamela ubicada en el municipio de La Huerta en el estado de Jalisco, México: *P. rajchenbergii* y *P. ryvardenii.* Para ello, se revisaron detalladamente los ejemplares con el fin de describir sus características macroscópicas y microscópicas, se realizó extracción de ADN y se amplificó por PCR el 28S ADNr (LSU). Los análisis filogenéticos se realizaron utilizando los métodos de Inferencia Bayesiana y Máxima Verosimilitud. Se corrobora que *P. rajchenbergii* y *P. ryvardenii* se diferencian de las especies descritas en el género por el color del píleo, el tamaño de los poros, el tipo de contexto (homogéneo en *P. ryvardenii*) y el tamaño de las basidiosporas; además, ambas especies forman clados distintos en los análisis filogenéticos llevados a cabo.

Palabas clave — Filogenia; Hymenochaetales; especies nuevas; taxonomía.

INTRODUCTION

Phylloporia species are characterized by annual or perennial, resupinate or pileate, sessile or stipitate basidiomata, duplex context with a black line separating an upper tomentum, persistent and of variable thickness. Microscopically by a monomitic to rarely dimitic hyphal system, absence of setal elements and small (< 6 μm long), subglobose to cylindrical, yellow, thick-walled, non-dextrinoid and frequently collapsed basidiospores. Nutritional strategies of *Phylloporia* species are not yet fully understood, it is known they cause white rot on different parts of living angiosperms, but relationships and effects on their host physiology have been rarely studied (Esquivel & Carranza, 1996; Yombiyeni *et al*., 2015). However, *Phylloporia* species have been cited as phytopathogens with high host-specialization (Valenzuela *et al*., 2011;

Zhou & Dai, 2012; Decock *et al*., 2015), although several species can be found to be saprophytic in dead aerial wood (Douanla-Meli *et al.*, 2007), or terrestrial, connected to living/dead wood (Wagner & Ryvarden, 2002; Ipulet & Ryvarden, 2005; Yombiyeni *et al*., 2015). Additionally, Yombiyeni *et al*. (2015) refer to the association between habit and the plant organ with the basidiomata in *Phylloporia* as the «morpho-ecologycal type», and stipitate basidiomata emerging from soil or associated to root had been named «*P. spathulata*» or «stipitate on root» morpho-ecological type (Ferreira-Lopes *et al.*, 2016). Of the 72 described species of *Phylloporia* (Index Fungorum, 2022), eight have *P. spathulata* morpho-ecological type and tropical distribution (Dai, 2010; Yombiyeni *et al*., 2015; Ferreira-Lopes *et al.*, 2016; Wu *et al*., 2019). So far, five taxa with stipitate basidiomata have been recorded in the neotropics: *P. elegans* Ferreira-Lopes, Robledo & Drechsler-Santos, *P. nodostipitata* Ferreira-Lopes & Drechsler-Santos, *P. solicola* Oliveira-Filho & Gibertoni, *P. verae-crucis* (Berkeley ex Saccardo) Ryvarden, and *P. spathulata* (Wagner & Ryvarden, 2002; Ryvarden, 2004; Valenzuela *et al.*, 2011; Ferreira-Lopes *et al.*, 2016; Wu *et al*., 2019).

In Mexico, it has been cited eight *Phylloporia* species: *P. bibulosa* (Lloyd) Ryvarden, *P. chrysites* (Berk.) Ryvarden, *P. fruticum* (Berk & M. A. Curtis) Ryvarden, *P. pectinata* (Klotzsch) Ryvarden, *P. pulla* (Mont. & Berk.) Decock & Yombiy, *P. rzedowskyi* R. Valenz. & Decock, *P. spathulata* (Hook.) Ryvarden, *P. ulloae*, and *P. verae-crucis* (Guzmán, 1983; Anell & Guzmán, 1987, 1988; Valenzuela & Chacón, 1991; Ryvarden & Guzmán, 1993; Valenzuela *et al*., 2002; Romero *et al*., 2010; Valenzuela *et al*., 2011; Álvarez *et al*., 2016), of which *P. spathulata* and *P. verae-crucis* have pileate-stipitate terrestrial basidiomata.

During the review of the specimens deposited in the fungal collection of the Escuela Nacional de Ciencias Biológicas of the Instituto Politécnico Nacional (ENCB) and in the collection of fungi of Instituto de Biología of the Universidad Nacional Autónoma de México (MEXU) as result from mycological expeditions carried out about a decade ago, a great diversity of *Phylloporia* was observed, and several stipitate specimens previously tagged as *P*. aff. *spathulata* were described with a distinctive character set of the above taxa. Therefore, the objective of this work is to describe two new stipitate *Phylloporia* species from the tropical dry forest for the Chamela Biology Station, based on morphological, ecological, and molecular data.

MATERIAL AND METHODS

Field work

Specimens were collected between 2008 and 2011 in the Chamela Biology Station, U.N.A.M. in the municipality of La Huerta, Jalisco State, Mexico (Fig. 1) (19°27'2.1"N, 105°01'33"W, 250 m asl, coordinates and elevation were obtained with a Garmin Etrex 10 GPS, Kansas City, USA). The Chamela-Cuixmala Biosphere Reserve belongs to the Pacific Lowlands Province (Morrone *et al.*, 2022), and comprises vegetation in advanced stage of succession classified as tropical dry forest (Bullock, 1986) with *Caesalpinia eriostachys* Benth. and *Amphypterigium adstringens* (Schltdl.) Standl. as dominant species according to Ceballos *et al.* (1999). The specimens are

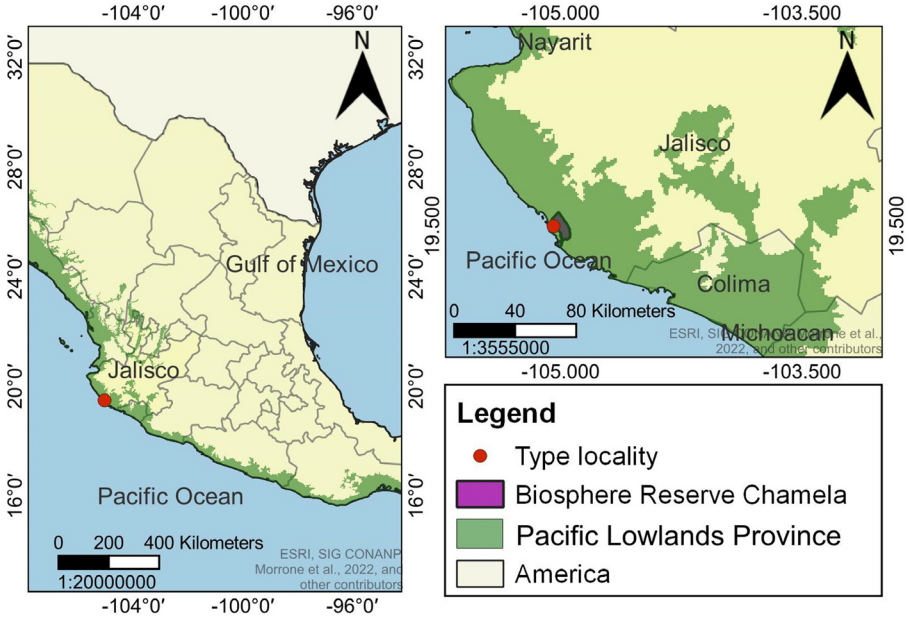

Fig. 1. Map of the Type locality of *Phylloporia rajchenbergii* and *P. ryvardenii* in the Chamela Biological Station, Chamela Biosphere Reserve, Jalisco, Mexico.

Fig. 1. Mapa de la localidad tipo de *Phylloporia rajchenbergii* y *P. ryvardenii* en la Estación Biológica Chamela, Reserva de la Biosfera Chamela, Jalisco, México.

deposited in the fungal collection in the ENCB and MEXU Herbaria (acronyms follows Thiers, 2022 [continuously updated]).

Morphological analyses

Morphological examinations were conducted using protocols outlined by Ryvarden (1991) and Valenzuela (2011). Basidiomata color were coded following Methuen Handbook of Colour (Kornerup & Wanscher, 1978), indicated in parentheses in the description. Shape and number of pores per mm were observed with a stereomicroscope (LEICA, S9E, Germany). For the microscopic study and structures measurements, temporary preparations were made in 70% alcohol and 5% potassium hydroxide (KOH) to elaborate description and measurements of hyphal structure, basidiospores and other microscopic structures with a micrometric scale in an optical microscope (MO; Primo Star, Carl Zeiss, Germany). Measurements of microscopic structures followed sampling of 30 measures per structure per specimen, with 5% of the measurements excluded from each end and given in parentheses.

DNA extraction, amplification, and sequencing

The DNA was obtained from herbarium specimens. The CTAB protocol of Martínez-González *et al*. (2017) was used to extract genomic DNA. The DNA was quantified with a Nanodrop 2000c (Thermo Scientific™, Wilmington, USA). The ribosomal

large subunit (LSU) region was amplified with the primer LR0R (Cubeta *et al*., 1991) and LR5 (Vilgalys & Hester, 1990). The PCR reaction contained the following: enzyme buffer 1x, Taq DNA polymerase, 0.8 deoxynucleoside triphosphates (0.2 mM each), 100 ng DNA, 20 pmol of each primer and 2 units of GoTaq DNA (Promega, USA), with a final volume of 15 μL. The amplification was run as follows: denaturalization at 94 °C for 4 min., 35 cycles of denaturalization at 94 °C for 45 sec, annealing at 50 °C for 1 min and final elongation at 72 °C for 5 min. All the PCR reactions were undertaken in a Peltier Thermal Cycler PTC-200 (BIORAD, México). The PCR products were verified by agarose gel electrophoresis. The gels were run at 1 h at 95 V cm{ ³ in 1.5% agarose and 1× TAE buffer (Tris Acetate-EDTA). The gel was stained with GelRed (Biotium, USA) and the bands were visualized in an Infinity 3000 transilluminator (Vilber Lourmat, Germany). The amplified products were purified with the ExoSAP Purification kit (Affymetrix, USA), following the manufacturer's instructions. They were quantified and prepared for the sequence reaction using a BigDye Terminator v. 3.1 (Applied Biosystems, USA). These products were sequenced in both directions with an Applied Biosystem model 3730XL (Applied BioSystems, USA).

Sequence assembly

The sequences of both strands of each of the genes were analyzed, edited and assembled using the BioEdit version 7.0.5 (Hall, 1999) to generate a consensus sequence. These consensus sequences were compared with those deposited in the GenBank of the National Center for Biotechnology Information (NCBI), using the tool BLASTN 2.2.19 (Zhang *et al*., 2000).

Phylogenetic analysis

In order to study phylogenetic relationships, our newly produced sequences of four individuals of *Phylloporia* were added to reference sequences of LSU deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/genbank/). The LSU region was aligned using the online version of MAFFT v7 (Katoh *et al*., 2002, 2017; Katoh & Standley, 2013). Alignments were reviewed in PhyDE V. 10.0 (Müller *et al*., 2005), followed by minor manual adjustments to ensure character homology between taxa. The matrix was composed of 88 specimens representing 49 species (905 characters). Phylogenetic inferences were estimated with maximum likelihood in Rax-ML v. 8.2.10 (Stamatakis, 2014) with a GTR + G model of nucleotide substitution. To assess branch support, 1000 nonparametric rapid bootstrap pseudoreplicates were run with the GTRGAMMA model. For Bayesian posterior probability, the best evolutionary model for alignment was sought using PartitionFinder v.2 (Lanfear *et al*., 2014, 2017; Frandsen *et al*., 2015). Phylogenetic analyses were performed using MrBayes v. 3.2.6 x64 (Huelsenbeck & Ronquist, 2001). The information block for the matrix included two simultaneous runs, four Montecarlo chains, temperature set to 0.2 and sampling 10 million generations (standard deviation d<0.1) with trees

sampled every 1000 generations. The first 25% of samples were discarded as burn-in, and stationarity was checked in Tracer v. 1.6 (Rambaut *et al*., 2014). Trees were visualized and optimized in FigTree v. 1.4.4 (Rambaut *et al.*, 2014), and they were edited in Adobe Illustrator vCS4 (Adobe Systems, Inc., San Jose, CA).

RESULTS

Phylogenetic analysis

The two simultaneous Bayesian runs continued until the convergence parameters were met, and the standard deviation fell below 0.001 after 10 million generations. No significant changes in tree topology trace or cumulative split frequencies of selected nodes were observed after about 2.5 million generations, so the first 2,500,000 samples trees (25%) were discarded as burn-in. Both the Bayesian analyses and Maximum Likelihood (Fig. 2) recovered *P. rajchenbergii* and *P. ryvardenii* supporting the two undescribed lineages distinctive from related species of *Phylloporia*.

Taxonomy

Phylloporia rajchenbergii Chamorro-Martínez, Raymundo, Martínez-González & R. Valenz. *sp. nov*. Fig. 3, 5A.
Mycobank: MB844872.

Type.— MEXICO. MEXICO, Jalisco, municipality La Huerta, Reserva de la Biosfera Chamela-Cuixmala, km 50 Barra de Navidad-Puerto Vallarta highway, Chamela Biology Station, U.N.A.M., Eje Central, 19º27'2.1"N 105º01'33"O, 250 m asl, 27-IX-2010, *R. Valenzuela 13977* (Holotype: ENCB, Isotype: MEXU; LSU OP482250).

Etymology.— The species is named in honor to Dr. Mario Rajchenberg by its contributions to the taxonomy of polypores in the world.

Diagnosis.— *Phylloporia rajchenbergii* differs from *P. spathulata* by its reddish-brown, zonate pileus, its smaller pores, polygonal to elongate, and its wider, ellipsoid basidiospores, lacks cistidioles and grows in soil in tropical dry forest.

Basidiomata annual, gregarious, laterally stipitate, 10–55 mm total length, spathulate. Pileus spathulate to reniform in outline, applanate to convex in section, 5–28 mm diameter, 4–5 mm thick, the surface covered with a light tomentum, shine in the center, opaque towards margin, orange (6B7) in young specimens, light brown (6D7) to reddish brown (8D7), concentrically zonate, sulcate, with zones dark brown (8F6), paler to the margin, light yellow (4A5) to brown (6E6), dark brown (7F8) when old or bruised, brownish grey (6F8) when dry. Margin sterile, involute to acute, white, pale yellow (4A3) to yellowish brown (5D6). Hymenophore poroid, orange yellow (4B7), ochre (5C7), golden brown (5D7) to yellowish brown (5D6,5E8) pores decurrent that extends to one-third upper part of the stipe; 14–16 per mm, polygonal to elongate, irregular in some parts, dissepiment slightly fimbriate; tube layer up

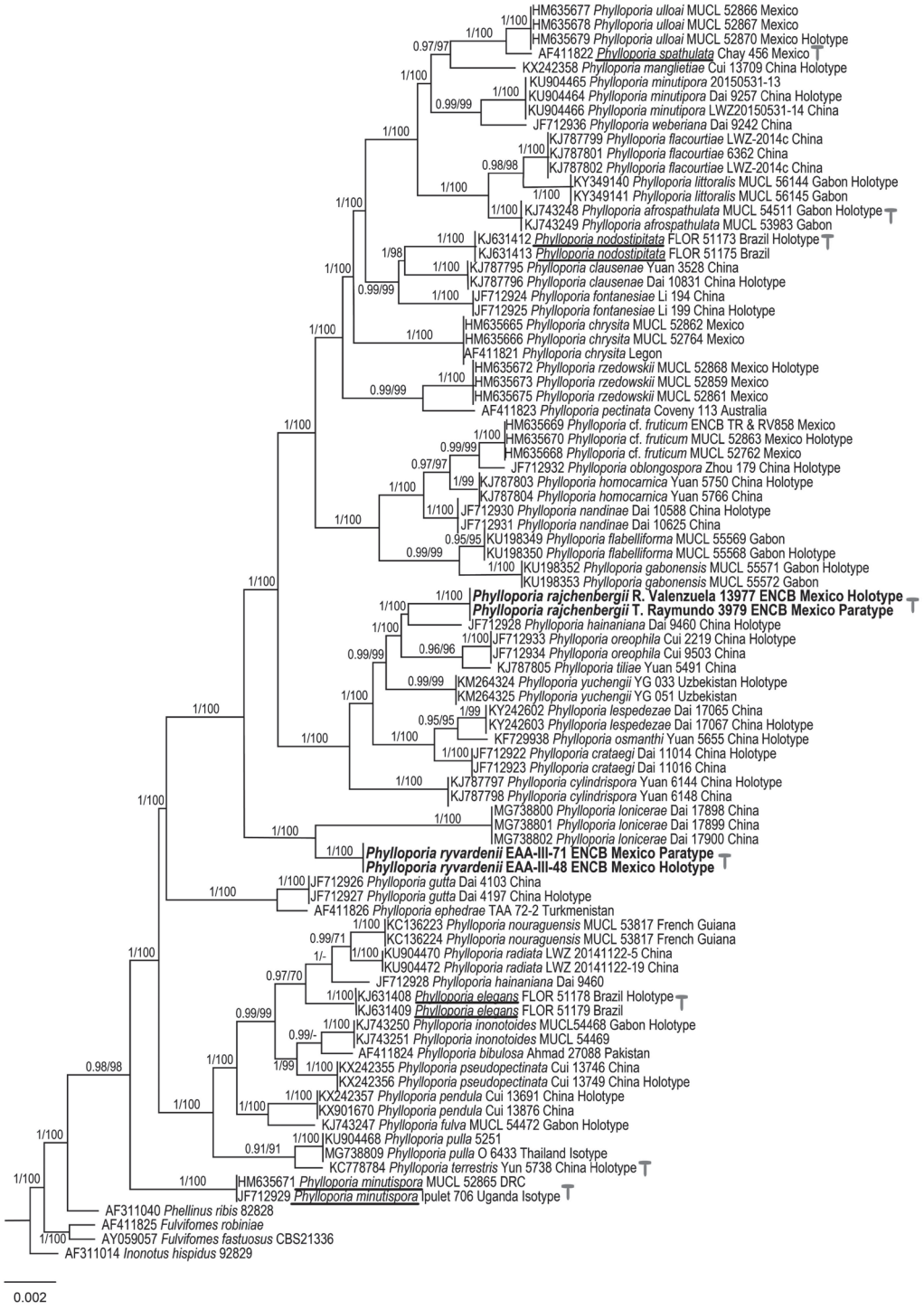

Fig. 2. Bayesian inference (BI) tree from the LSU sequence data from 88 specimens. The values above the branches represent Bayesian posterior probabilities (PP, left) and bootstrap values (BS, right). The scale bar represents the expected number of nucleotide substitutions per site. Names in bold represent samples sequenced for this study. Underlined names represent stipitate taxa with neotropical distribution and the symbol represents stipitate habit.

Fig. 2. Árbol de inferencia bayesiana (BI) con base en secuencias LSU de 88 especímenes. Los valores por encima de las ramas representan probabilidades posteriores bayesianas (PP, izquierda) y bootstrap (BS, derecha). La barra representa el número esperado de sustituciones de nucleótidos por sitio. Los nombres en negrita representan muestras secuenciadas para este estudio. Los nombres subrayados representados taxones estipitados con distribución neotropical y el símbolo representa hábito estipitado.

Fig. 3. *Phylloporia rajchenbergii* R. Valenzuela 13977 (Holotype). A) Basidiomata. B) Duplex context with a black line separating an upper tomentum and a lower compact context. C). Pores and margin. D) Basidiospores. Photos by Hannya Chamorro. A, scale = 10 mm; B, scale = 2 mm; C, scale = 1 mm; D, scale = 5 μm.

Fig. 3. *Phylloporia rajchenbergii* R. Valenzuela 13977 (Holotipo). A) Basidiomas. B) Contexto dúplex con línea negra separando un tomento superior y un contexto compacto inferior. C) Poros y margen. D) Basidiosporas. Fotos por Hannya Chamorro. A, escala = 10 mm; B, escala = 2 mm; C, escala = 1 mm; D, escala = 5 μm.

to 1 mm deep, yellowish brown (5E4). Stipe lateral, cylindrical to irregular, erect, tomentose, 10–36 mm length, 2–3 mm diameter, ochre (5C7), yellowish brown (5E8) to dark brown (8F6), thickened to the base, irregular, 5–14 mm length, tomentose to spongy, tomentum of context from the stipe the same color and texture from tomentum of context from the pileus. Context duplex, up to 2 mm total thick, azonate, with a black line separating an upper loose tomentum and a lower denser context and it extends to the stipe, upper tomentum fibrous, up to 0.8 mm thick, yellowish brown (5E8) to reddish brown (8E8); lower context compact and dense, up to 1.5 mm thick at the base, very thin (<1 mm) to the margin, yellowish brown (5D5).

Hyphal system monomitic in all parts; hyphae simple septate, thin- to thick-walled, light yellow to golden in KOH; in the tomentum interwoven, unbranched, thin- to thick-walled, 3.2–4.0 μm wide; in the lower context subparallel to interwoven, unbranched, thick-walled, 2.4–5.6 μm; in the hymenophoral trama sub-parallel to interwoven, unbranched, thin-walled to thick-walled, 4–4.8 μm wide. Sterile elements not seen. Basidia 5–5.5 × 4.5–4.8 μm, clavate, hyaline in KOH, 4-spored, sterigmata 1–1.6 μm long. Basidiospores ellipsoid, pale yellow to golden yellow in KOH, slightly thick-walled, smooth, inamyloid, (2.4–)3.2–3.6(–4) × 2.4–3.2(–3.4) μm (ave = 3.3 × 2.6 μm, aveQ = 1.28).

Substrates.— Known so far growing from soil on roots from unidentified angiosperms in tropical dry forest.

Distribution.— This species is known from Chamela-Cuixmala Biosphere Reserve and belongs to the Pacific Lowlands Province in the Neotropical region.

Additional studied material.— MEXICO, Jalisco, municipality La Huerta, Reserva de la Biosfera Chamela-Cuixmala, km 50 Barra de Navidad-Puerto Vallarta highway, Chamela Biology Station, Eje Central, 19º27'2.1"N 105º01'33"O, 250 m asl, 27-IX-2010, *R. Valenzuela 13954, 13989* (ENCB), *T. Raymundo 3524* (ENCB), 17-IX-2011, *P. Garma 616* (ENCB), *T. Raymundo 3979* (ENCB; LSU OP482251), *R. Valenzuela 14472* (ENCB).

Observations.— This species is characterized by pileate-stipitate basidiomata, up to 55 mm long, growing from soil probably associated with roots, by having reddish-brown pileus, zonate, pores 14–16 per mm, polygonal to elongate, and ellipsoid basidiospores, 3.2–3.6 × 2.4–3.2 μm. The two new species described here are morphologically similar, and belong to the *P. spathulata* complex, both species share similar size of basidiospores, but they differ in the color of the pileus surface, *P. rajchenbergii* has a duplex context with black line while *P. ryvardenii* has homogeneous context without black line, except in the stipe. The sister clade of *P. rajchenbergii* is *P. hainaniana* Y.C. Dai & B.K. Cui and it is separated by its sessile basidiomata, pores 4-6 per mm, and larger basidiospores (4.6–5.6 × 3.0–3.6 μm) (Cui *et al*., 2010). *Phylloporia spathulata* has laterally stipitate basidiomata, however it differs by having azonate pileus, 7–9 pores per mm (vs 14–16 pores) and basidiospores that are elongated to cylindrical (3–4 × 2–2.5 μm vs. 3.2–3.6 × 2.4–3.2 μm).

Phylloporia ryvardenii Chamorro-Martínez, Raymundo, Aguirre-Acosta & R. Valenz. *sp. nov*. Fig. 4, 5B.
Mycobank: MB844873.

Type.— MEXICO, Jalisco, municipality La Huerta, Chamela-Cuixmala Biosphere Reserve, km 50 Barra de Navidad-Puerto Vallarta highway, Chamela Biology Station, U.N.A.M., Road Tejones, 19º27'2.1"N 105º01'33"O, 250 m asl, 04-IX-2008, *E. Aguirre-Acosta et al. III-48* (Holotype: MEXU, Isotype: ENCB; LSU OP482252).

Etymology.— The species is named in honor to Dr. Leif Ryvarden by its contributions to the taxonomy of polypores in the world.

Diagnosis.— *Phylloporia ryvardenii* differs from *P. minutispora* by its neotropical distribution, its yellowish-brown pileus, zonate, its smaller pores, polygonal to irregular, its homogeneous context without black line, and its larger and ellipsoid basidiospores. *Phylloporia ryvardenii* lacks cistidioles and grows in soil in tropical dry forest.

Basidiomata annual, gregarious, laterally stipitate, 22–53 mm total length. Pileus spathulate, sometimes multiple pilei fused with independent stipes, 7–18 mm diameter, 4–5 mm thick, the surface covered with a light tomentum, finely velutinate, shiny, yellowish brown (5E8) to golden brown (5D7), zonate, yellowish ochre (5C7); marginal areas paler, greyish orange (5B5); margin involute to acute, sterile, greyish yellow (4B5) then golden (4C6). Hymenophore poroid, yellowish brown (5E4), pores slightly decurrent that extends in some specimen to one-fifth to one-fourth upper part of the stipe; 10–12 per mm, polygonal to irregular, dissepiment slightly thickened, entire; tube layer up to 1 mm deep, yellowish brown (5E4). Stipe lateral, cylindrical to flattened, slender, tomentose, 15–18 mm total length, 1–3 mm diameter, concolorous with the pileus, thickened base, bulbose to irregular, 5–7 mm length, tomentum, and context from the stipe the same color and texture from tomentum, and context from the pileus. Context homogeneous, azonate, black line lacks in the pileus context but present in stipe context, up to 0.5 mm thick, fibrous to corky, light yellowish brown (5E8).

Hyphal system monomitic in all parts, hyphae simple septate, thin- to thick-walled, light yellow to golden in KOH; in the context, subparallel to interwoven, unbranched or with few branches, thick-walled, 4.8–5.6 μm wide; in the hymenophoral trama parallel, subparallel to interwoven, unbranched or with few branches, slightly thick-walled, 3.2–4.0 μm wide. Cistidioles or sterile elements not seen. Basidioles 4.8–5.6 × 3.2–4.0 μm, hyaline, subglobose, thin-walled. Basidia 6–6.5 × 4–4.8 μm, clavate, thin-walled, hyaline in KOH, 4-sterigmate, 1.0–1.6 μm long. Basidiospores ellipsoid, very pale yellow to light golden in KOH, slightly thick-walled, smooth, inamyloid, (3.6–)3.8–4(–4.4) × 3–3.2(–3.4) μm (ave = 3.9 × 3.1 μm, aveQ = 1.25).

Substrates.— known so far growing from soil or roots from non-determined angiosperms in tropical dry forest.

Distribution.— This species is known from Chamela-Cuixmala Biosphere Reserve and belongs to the Pacific Lowlands Province in Neotropical region.

Fig. 4. *Phylloporia ryvardenii* E. Aguirre-Acosta *et al.* III-48 (Holotype). A) Basidiomata. B) Homogeneous context. C) Pores and margin. D) Basidiospores. Photos by Hannya Chamorro-Martínez. A, scale = 10 mm; B, scale = 5 mm; C, scale = 1 mm; D, scale = 5 μm.

Fig. 4. *Phylloporia ryvardenii* E. Aguirre-Acosta et al. III-48 (Holotipo). A) Basidiomas. B) Contexto homogéneo. C) Poros y margen. D) Basidiosporas. Fotos por Hannya Chamorro-Martínez. A, escala = 10 mm; B, escala = 5 mm; C, escala = 1 mm; D, escala = 5 μm.

Additional studied material.— MEXICO, Jalisco, municipality La Huerta, Chamela-Cuixmala Biosphere Reserve, km 50 Barra de Navidad-Puerto Vallarta highway, Chamela Biology Station, U.N.A.M., Road Tejones, 19º27'2.1"N 105º01'33"O, 250 m asl, 04-IX-2008, *E. Aguirre-Acosta et al. III-71* (MEXU, ENCB, LSU OP482253).

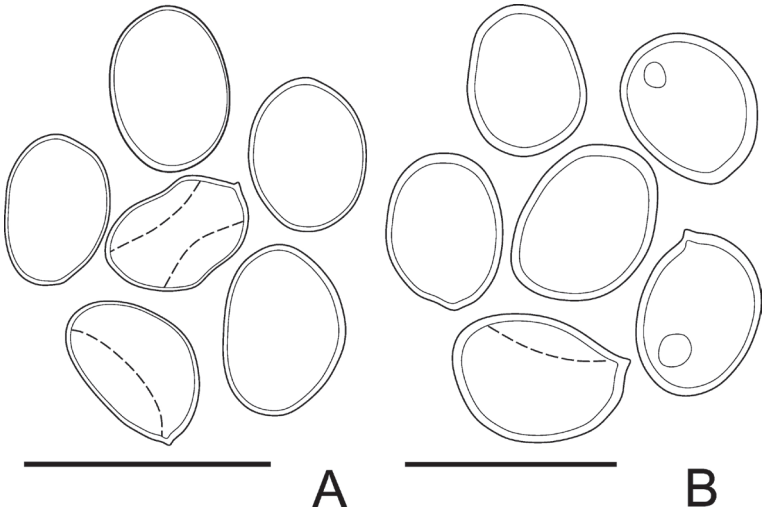

Fig. 5. Basidiospores of holotypes of A) *Phylloporia rajchengergii* and B) *P. ryvardenii*, with dotted lines representing flattened sides. Drawings by Hannya Chamorro-Martínez. A, scale = 5 μm; B, scale = 5 μm.

Fig. 5. Basidiosporas de los tipos de A) *Phylloporia rajchengergii* y B) *P. ryvardenii*, con líneas punteadas que representan lados aplanados. Dibujos de Hannya Chamorro-Martínez. A, escala = 5 μm; B, escala = 5 μm.

Observations.— This species is characterized by pileate-stipitate basidiomata, up to 53 mm long, growing from soil probably associated with roots, by having yellowish-golden brown pileus, zonate, pores 10–12 per mm, polygonal to irregular, homogeneous context and ellipsoid basidiospores, 3.8–4 × 3–3.2 μm. *Phylloporia minutispora* Ipulet & Ryvarden has pileate-stipitate basidiomata growing on soil, and homogeneous context; however, it differs by having Paleotropical distribution (Zhou & Dai, 2012) larger pores (7–9 per mm vs. 10–12 pores per mm), and basidiospores broadly ellipsoid to subglobose (2–3 × 2.5 μm vs 3.8–4 × 3–3.2 μm). The sister clade of *P. ryvardenii* is *P. lonicerae* W.M. Qin, Xue W. Wang, T. Sawahata & L.W. Zhou that it is separated by its sessile basidiomata, pores 6-8 per mm, and grow on *Lonicera japonica* Thunb. (Caprifoliaceaea) (Qin *et al*., 2018).

DISCUSSION

The determination of the poliporoid fungi (especially for the order Hymenochaetales) has been proposed not only taking into consideration morphological and molecular characters but also ecological characters such as the type of vegetation, distribution, and host specificity (Valenzuela *et al*., 2011 and Amalfi *et al*., 2012). Most *Phylloporia* species grow parasitically on living hardwoods, and speciation in the genus seems to be driven by the process of colonizing and adapting to new hosts (Wu *et al*., 2019; Zhou *et al*., 2022). The similar morphology at the interspecific level makes the genus taxonomically difficult, in any case, the integrative taxonomy with the use of molecular characters, host specificity and geographical distribution have contributed to separate cryptic species, in *P. bibulosa*, *P. chrysites*, *P. pectinata*, and *P. spathulata*, among other species. The topology of the tree corroborates the previous topologies

based on the LSU sequences, that show *Phylloporia* as a monophyletic group (Fig. 2); however, they also show a considerable number of subclades in which taxa with different morpho-ecological types, biogeographical distribution or even hyphal structure appear phylogenetically related (Wu *et al*., 2019; Zhou *et al.*, 2022).

The number of stipitate species of *Phylloporia* increases to ten, with mayor richness in neotropical regions, so far seven taxa have been cited from the neotropics: *P. elegans*, *P. nodostipitata*, *P. rajchenbergii*, *P. ryvardenii*, *P. solicola*, *P. spathulata* and *P. verae-crucis*. All neotropical «stipitate on roots» species are morphologically similar, share basidiospores shape and size, presumably grow associated with roots (of non-determined angiosperms) plus their known geographic distribution are very restricted, except for *P. spathulata s. s.*; but are phylogenetically distant from each other as mentioned in other reviews (Ferreira-Lopes *et al.,* 2016; Wu *et al*., 2020), these characters could be the result of host speciation and adaptative response to neotropical microecosystems, in any case, specialized studies are necessary to test this hypothesis.

While *P. rajchenbergii* is related to *P. hainaniana*, which have an imbricate basidiomata and was described from southern China growing on living angiosperm tree, *P. ryvardenii* is related to *P. lonicerae* (Fig. 2), a sessile species described from Japan, growing on living vine of *Lonicera japonica* (Quin *et al*., 2018); phylogenetic closeness between sessile and stipitate taxa from different biogeographic realms had already been mentioned previously (Ferreira-Lopes *et al.,* 2016; Wu *et al*., 2019), but it is necessary further ecological, evolutive and biogeographical studies to elucidate the reason for these results. It has been proposed that phylogenetic inference based on multiple genetic markers would offer a solution, but most *Phylloporia* described taxa are represented by a limited number of sequences and only some species have type sequences available with different genetic markers in GenBank (Zhou *et al*., 2022), therefore, it is expected to continue working on the determination of *P. aff. spathulata* collections while the available sequences and ecological datasets increase.

CONCLUSIONS

Apart from the morphological similarities between the stipitate species of the Neotropical region, it is possible to affirm that they comprise separate phylogenetic lineages. Currently, species of *Phylloporia* with a «stipitate on roots» morpho-ecological type in the neotropical region includes seven species: *P. elegans*, *P. nodostipitata*, *P. rajchenbergii*, *P. ryvardenii*, *P. solicola*, *P. spathulata* and *P. verae-crucis*, showing the highest species richness with this morpho-ecological type. However, its ecological and evolutionary relationships have not been described with certainty, so it is likely this number will increase with the study of American collections with stipitate basidiomata that has been cited or tagged as *P. spathulata.*

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support received from the CONACyT (Mexico) through the project 252934, and IPN for supporting research through in the projects SIP20220030 and SIP-20221348. The authors gratefully acknowledge to the authorities of Chamela Biology Station, U.N.A.M. for the facilities granted to carry out this study.

BIBLIOGRAPHY

Álvarez, V. I., Raymundo, T. & Valenzuela, R. (2016). Los hongos poliporoides de la Huasteca Potosina, San Luis Potosí, México. *Polibotánica 41*: 31-48. https://doi.org/10.18387/polibotanica.41.2

Amalfi, M., Raymundo, T., Valenzuela, R. & Decock, C. (2012). *Fomitiporia cupressicola* sp. nov., a parasite on *Cupressus arizonica*, and additional unnamed clades in the southern USA and northern Mexico, determined by multilocus phylogenetic analyses. *Mycologia 104* (4): 880-893. https://doi.org/10.3852/11-196

Anell, J. C. & Guzmán, G. (1987). Especies de poliporáceos citadas del Estado de Veracruz. *Scientia Fungorum 3*: 137-148. https://doi.org/10.33885/sf.1987.3.693

Anell, J. C. & Guzmán, G. (1988). Nuevos registros de los hongos del grupo de los poliporáceos del estado de Veracruz. *Scientia Fungorum 4*: 25-42. https://doi.org/10.33885/sf.1988.3.708

Bullock, S. H. (1986). Climate of Chamela, Jalisco, and trends in the south coastal region of Mexico. *Archives for Meteorology, Geophysics, and Bioclimatology, Series B, 36* (3-4): 197-316.

Ceballos, G., Székely, A., García, A., Rodríguez, P. & Noguera, F. (1999). *Programa de manejo de la Reserva de la Biosfera Chamela-Cuixmala*. Instituto Nacional de Ecología, Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAP). Ciudad de México, México.

Cubeta, M. A., Echandi, E., Abernethy, T., & Vilgalys, R. (1991). Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of an amplified ribosomal RNA gene. Phytopathology, 81(11), 1395-1400.

Cui, B. K., Yuan H. S. & Dai, Y.C. (2010). Two new species of *Phylloporia* (Basidio mycota,Hymenochaetaceae) from China. *Mycotaxon 113*: 171-178. https://doi.org/10.5248/113.171

Dai, Y. C. (2010). Hymenochaetaceae (Basidiomycota) in China. *Fungal diversity 45* (1): 131-343. https://doi.org/10.1007/s13225-010-0066-9

Decock, C., Yombiyeni, P. & Memiaghe, H. (2015). Hymenochaetaceae from the Guineo-Congolian rainforest: *Phylloporia flabelliforma* sp. nov. and *Phylloporia gabonensis* sp. nov., two undescribed species from Gabon. *Cryptogamie Mycologie 36* (4): 449-467. https://doi.org/10.7872/crym/v36.iss4.2015.449

Douanla-Meli, C., Ryvarden, L. & Langer, E. (2007). Studies of tropical African pore Fungi (Basidiomycota, Aphyllophorales): three new species from Cameroon. *Nova Hedwigia 84*: 409-420. https://doi.org/10.1127/0029-5035/2007/0084-0409

Esquivel, R. E. & Carranza, J. (1996). Patogenicidad de *Phylloporia chrysita* (Aphyllophorales: Hymenochaetaceae) sobre *Erythrochiton gymnanthus* (Rutaceae). *Revista de Biología tropical 44* (S4): 137-145.

Ferreira-Lopes, V., Robledo, G. L., Reck, M. A., Neto, A. G. & Drechsler-Santos, E. R. (2016). *Phylloporia spathulata sensu stricto* and two new South American stipitate species of *Phylloporia* (Hymenochaetaceae). *Phytotaxa 257* (2): 133-148. https://doi.org/10.11646/phytotaxa.257.2.3

Frandsen, P. B., Calcott, B., Mayer, C. & Lanfear, R. (2015). Automatic selection of partitioning schemes for phylogenetic analyses using iterative *k*-means clustering of site rates. *BMC Evolutionary Biology 15*: 1-17. https://doi.org/10.1186/s12862-015-0283-7

Guzmán, G. (1983). Los hongos de la península de Yucatán II. Nuevas exploraciones y adiciones micológicas. *Biotica 8*: 71-87.

Hall, T. A. (1999). "BioEdit: a user-friendly biological sequence alignment editor and análisis program for Windows 95/98/NT". *Nucleic Acids Symposium Series 41*: 95-98.

Huelsenbeck, J. P. & Ronquist, F. (2001). MrBayes: Bayesian inference of phylogeny. *Bioinformatics 17* (8): 754-755. https://doi.org/10.1093/bioinformatics/17.8.754

Index Fungorum. (2022). An international project to index all formal names in the Fungi Kingdom. Recuperated from: http://www.indexfungorum.org/names/Names.asp. Accessed 08 July 2022.

Ipulet, P. & Ryvarden, L. (2005). New and interesting polypores from Uganda. *Synopsis Fungorum* 20: 87-99.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research 30*(14): 3059-3066.

Katoh, K. & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution 30* (4): 772-780. https://doi.org/10.1093/molbev/mst010

Katoh, K., Rozewicki, J. & Yamada, K. D. (2017). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics 20* (4): 1160-1166. https://doi.org/10.1093/bib/bbx108

Kornerup, A. J. & Wanscher, H. (1978). *Methuen Handbook of Colour.* (3° Ed). Eyre Methuen, London.

Lanfear, R., Calcott, B., Kainer, D., Mayer, C. & Stamatakis, A. (2014). Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology 14:* 1-14. https://doi.org/10.1186/1471-2148-14-82

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T. & Calcott, B. (2017). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution 34* (3): 772-773. https://doi.org/10.1093/molbev/msw260

Martínez-González, C. R., Ramírez-Mendoza, R., Jiménez-Ramírez, J., Gallegos-Vázquez, C. & Luna-Vega, I. (2017). Improved method for genomic DNA extraction for *Opuntia* Mill. (Cactaceae). *Plant Methods 13*: 1-10.

Morrone, J. J., Escalante, T., Rodríguez-Tapia, G., Carmona, A., Arana, M. & Mercado-Gómez, J. D. (2022). Biogeographic regionalization of the Neotropical region: new map and shapefile. *Anais da Academia Brasileira de Ciências 94* (1): 20211167. https://doi.org/10.1590/0001-3765202220211167

Müller, K., Quandt, D., Müller, J. & Neinhuis, C. (2005). *PhyDE®-Phylogenetic data editor. Program distributed by the authors, versión 10.0.* Available from: https://www.phyde.de (accessed 16 April 2022).

Qin, W. M., Wang, X. W., Sawahata, T., Zhou, L. W. (2018). *Phylloporia lonicerae* (Hymenochaetales, Basidiomycota), a new species on *Lonicera japonica* from Japan and an identification key to worldwide species of *Phylloporia*. *MycoKeys 30*: 17-30. https://doi.org/10.3897/mycokeys.30.23235

Rambaut, A., Suchard, M. A., Xie, D. & Drummond, A. J. (2014). *Tracer v1.6*. Available from: http://beast.bio.ed.ac.uk/Tracer (accessed 16 April 2022).

Romero-Bautista, L., Pulido-Flores, G. & Valenzuela, R. (2010). Estudio micoflorístico de los hongos poliporoides del estado de Hidalgo, México. *Polibotanica 29*: 1-28.

Ryvarden, L. (1991). *Genera of Polypores, Nomenclature and Taxonomy.* Synopsis Fungorum 5. Fungiflora, Oslo, Noruega.

Ryvarden, L. (2004). *Neotropical polypores I. Introduction, Ganodermataceae and Hymenochaetaceae*. Sinopsis Fungorum 19. Fungiflora, Oslo.

Ryvarden, L. & Guzmán, G. (1993). New and interesting polypores from Mexico. *Mycotaxon 47*: 1-23.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics 30*: 1312-1313. https://doi.org/10.1093/bioinformatics/btu033

Thiers, B. (2022) *Index Herbariorum*: a global directory of public herbaria and associated staff. New York Garden's Virtual Herbarium. In: New York Garden's Virtual Herbarium. Recuperated from http://sweetgum.nybg.org/ih/. Accessed 08 July 2022.

Valenzuela, R. (2011). *Revisión de las especies con himenóforo poroide de la familia Hymenochaetaceae (Aphyllophorales, Hymenomycetes) en México*. (Tesis doctoral). Universidad Nacional Autónoma de México. Ciudad de México, México.

Valenzuela, R. & Chacón-Jiménez, S. (1991). Los Poliporáceos de México, III. Algunas especies de la Reserva de la Biósfera El Cielo, Tamaulipas. *Scientia Fungorum 7*: 39-69. https://doi.org/10.33885/sf.1991.3.773

Valenzuela, R., De la Huerta, C. P. & Fernández-Nava, R. (2002). Los Poliporáceos de México V. Algunas especies del norte del estado de Querétaro. *Polibotánica 9*: 85-122.

Valenzuela, R., Raymundo, T., Cifuentes, J., Castillo, G., Amalfi, M. & Decock, C. (2011). Two undescribed species of *Phylloporia* from Mexico based on morphological and phylogenetic evidence. *Mycological Progress 10* (3): 341-349. https://doi.org/10.1007/s11557-010-0707-0

Vilgalys, R. & Hester, M. (1990). Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology 72* (8): 4238-4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wagner, T. & Ryvarden, L. (2002). Phylogeny and taxonomy of the genus *Phylloporia* (Hymenochaetales). *Mycological Progress 1* (1): 105-116. https://doi.org/10.1007/s11557-006-0009-8

Wu, F., Ren, G., Wang, L., Oliveira-Filho, J. & Gibertoni, T. (2019). An updated phylogeny and diversity of *Phylloporia* (Hymenochaetales): eight new species and keys to species of the genus. *Mycological Progress 18*: 615-639. https://doi.org/10.1007/s11557-019-01476-4

Yombiyeni, P., Balezi, A., Amalfi, M. & Decock, C. (2015). Hymenochaetaceae from the Guineo-Congolian rainforest: three new species of *Phylloporia* based on morphological, DNA sequences and ecological data. *Mycologia 107* (5): 996- 1011. https://doi.org/10.7872/crym/v36.iss4.2015.449

Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology 7*: 203-214.

Zhou, L. W. & Dai, Y. C. (2012). Phylogeny and taxonomy of *Phylloporia* (Hymenochaetales): new species and a worldwide key to the genus. *Mycologia 104* (1): 211-222. https://doi.org/0.3852/11-093

Zhou, M., Wu, F., Dai, Y.-C. & Vlasák, J. (2022). Two new species of *Phylloporia* (Hymenochaetales) from the Neotropics. *MycoKeys 90*: 71-83. https://doi.org/10.3897/mycokeys.90.84767