Research article

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Humaria setimarginata (Pyronemataceae, Ascomycota), a new species from Mexico

Humaria setimarginata (Pyronemataceae, Ascomycota), una nueva especie de México

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Abstract:

Background and Aims: *Humaria* (Pyronemataceae, Ascomycota) is an ectomycorrhizal genus of fungi, mainly distributed in temperate forest. It is characterized by cupuliform to discoid apothecia that are covered with abundant hairs throughout the exterior of the brown ascomata, and by ellipsoid, hyaline, warty ascospores. There are 66 accepted species of which only one has been recorded in Mexico. The present study aims to describe a new species of *Humaria* with morphological, ecological and molecular data, found in *Quercus* forests from Tamaulipas, Mexico.

Methods: The specimens were collected in 2019 in the Victoria municipality, Tamaulipas, Mexico. Description and morphological studies were performed according to traditional mycological techniques. Studied material was deposited in the José Castillo Tovar Mycological Herbarium of the Instituto Tecnológico de Ciudad Victoria (ITCV) and the Escuela Nacional de Ciencias Biológicas Herbarium (ENCB) of the Instituto Politécnico Nacional. Phylogenetic analyses were performed using ITS and LSU sequences of the nuclear rDNA.

Key results: *Humaria setimarginata* sp. nov. is described and illustrated. This species is well differentiated by its morphological, molecular and ecological characteristics. It forms typically cupuliform apothecia with straight margin, abundant dark brown colored short hairs at the margin, and a greyish-white hymenium. It presents a dextrinoid reaction in the ectal excipulum in contact with Melzer´s reagent. It grows around *Quercus rysophylla* and *Q. polymorpha* in oak forests.

Conclusions: *Humaria setimarginata* is the second species of this genus reported in Mexico, after *Humaria hemisphaerica*. There are several collections under the name *Humaria* sp. that need a revision, to expand the diversity knowledge of this genus in this country. It is possible that several species exist, considering that this genus is ectomycorrhizal and the diversity of its potential hosts is high.

Key words: ectomycorrhizal fungi, northeast of Mexico, Pezizales, *Quercus* spp.

Resumen:

Antecedentes y Objetivos: *Humaria* (Pyronemataceae, Ascomycota) es un género de hongos ectomicorrízicos, distribuidos generalmente en bosques templados. Se caracteriza por apotecios cupuliformes a discoides que están cubiertos con abundantes pelos en todo el exterior de los ascomas marrones, y por ascosporas elipsoides, hialinas y verrucosas. Son 66 especies aceptadas de las cuales solo una ha sido registrada en México. El presente estudio tiene como objetivo describir una nueva especie de *Humaria,* con datos morfológicos, ecológicos y moleculares, encontrada en el bosque de *Quercus* de Tamaulipas, México.

Métodos: Los ejemplares fueron recolectados en 2019 en el municipio Victoria, Tamaulipas, México. La descripción y los estudios morfológicos se realizaron según las técnicas micológicas tradicionales. El material estudiado fue depositado en el Herbario Micológico José Castillo Tovar del Instituto Tecnológico de Ciudad Victoria (ITCV) y en el Herbario de la Escuela Nacional de Ciencias Biológicas (ENCB) del Instituto Politécnico Nacional. Los análisis filogenéticos se realizaron utilizando secuencias ITS y LSU del ADNr nuclear.

Resultados clave: *Humaria setimarginata* sp. nov. se describe e ilustra. Esta especie se diferencia bien por características morfológicas, moleculares y ecológicas. Forma un apotecio típicamente cupuliforme, con margen recto, abundantes pelos cortos color marrón oscuro en el margen y un himenio blanco grisáceo. Presenta una reacción dextrinoide en el excípulo ectal en contacto con el reactivo Melzer. Crece alrededor de *Quercus rysophylla* y *Q. polymorpha* en bosques de encino.

Conclusiones: *Humaria setimarginata* es la segunda especie de este género que se reporta para México, después de *Humaria hemisphaerica*. Hay varias colecciones bajo el nombre de *Humaria* sp. que necesitan una revisión, para ampliar el conocimiento de la diversidad de este género en el país. Es posible que existan varias especies, considerando que este género es ectomicorrícico y la diversidad de sus hospedantes potenciales es alta.

Palabras clave: hongos ectomicorrícicos, noreste de México, Pezizales, *Quercus* spp.

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Introduction

The name *Humaria* (Fr.) Boud. was first used by Fries (1822) in the rank of tribe of his broad genus *Peziza* Dill. ex Fr. Cooke (1879), following Fries´ definition, used the rank of subgenus; then Boudier (1885) erected it at the rank of genus. Clements and Shear (1931) selected *Humaria leucoloma* (Hedw.) Boud. as lectotype of this genus. With such a typification *Humaria leucoloma* becomes an obligate synonym of *Octospora* Hedw. Fuckel (1870) used the name *Humaria* with his own definition, encompassing several species of the series *Lachnea* defined by Fries (1822) or the tribe *Lachnea* (Fr.) Boud. Its type has been designated by Denison (1959) with *Humaria hemisphaerica* (F.H. Wigg.) Fuckel (*Peziza hemisphaerica* F.H. Wigg.) and must be followed. *Humaria* became the type of family Humariaceae proposed by Velenovský (1934), then accepted by subsequent authors such Le Gal (1947), Dennis (1960), Moser (1963), Berthet (1964), Rifai (1968), until Eckblad (1968) emended the family Pyronemataceae to widen its definition, including Humariaceae. This has been followed by Korf (1972) and subsequent authors. The modern classification based on molecular phylogeny confirmed the position of *Humaria* inside the family Pyronemataceae inside its own linage (Perry et al., 2007; Hansen et al., 2013; Van Vooren et al., 2021). Fuckel (1870) characterized this genus as having terrestrial ascomata, cupuliform apothecia when young, discoid when mature, gregarious, sessile, with tomentose hairs; asci cylindrical, elongated, containing 8 spores; ascospores oval to oblong-oval, containing 1-3 guttules, hyaline; filiform paraphyses. Among the 512 names listed in the Index Fungorum (2022) database under *Humaria* as genus, many of them are now combined in other genera, but 66 names are “accepted” in this repository, although many of them are old names, hard to interpret in a modern sense. *Humaria* represents an ectomycorrhizal genus of fungi, associated with different deciduous trees like *Quercus* spp., *Fagus sylvatica* L., *Tilia cordata* Mill. (Tedersoo et al., 2006; Erős-Honti et al., 2008), *Carya* spp. (Rudawska et al., 2018), as well as conifers like *Pinus* spp. (Tedersoo et al., 2006). On the American continent, only two species have been described: *H. cazaresii* (M.E. Sm. & Trappe) M.E. Sm., Healy & P. Alvarado and *H. hemisphaerica*. In Mexico, *H. hemisphaerica* has been cited from Durango, Guerrero, Hidalgo, Jalisco, Mexico City, Mexico State, Michoacán, Morelos, Oaxaca, Sonora and Tamaulipas, frequently in *Pinus-Quercus* and *Quercus* forests, montane cloud forests, and in coniferous forests (Chacón and Guzmán, 1983; Frutis and Guzmán, 1983; Bautista et al., 1986; Díaz-Barriga et al., 1988; Heredia, 1989; Pompa-González and Cifuentes, 1991; Esqueda et al., 1992; García Jiménez and Guevara Guerrero, 2005; García Jiménez and Valenzuela, 2005; Villarruel-Ordaz and Cifuentes, 2007; Raymundo et al., 2012, 2013; Gándara et al., 2014; García et al., 2014; Rodríguez-Alcántar et al., 2018, 2019). However, only Bautista et al. (1986) and Ortega López (2015) have given a detailed description. The present study aims to describe a new *Humaria* species in oak forest from Tamaulipas, Mexico based on morphological, ecological and molecular data.

Materials and Methods

Study material

Specimens were collected in 2019 in the Victoria municipality, Tamaulipas, located in the Sierra Madre Oriental (Fig. 1). The specimens were deposited in the José Castillo Tovar Mycological Herbarium of the Instituto Tecnológico de Ciudad Victoria (ITCV) and the Escuela Nacional de Ciencias Biológicas Herbarium (ENCB) of the Instituto Politécnico Nacional.

Morphological analyses

The collected material was examined following the traditional techniques proposed by Cifuentes et al. (1986). The specimens were described when fresh, macroscopic characters, like size, shape, color, and possible hosts were recorded. The terminology used herein refers to the Nuevo Diccionario Ilustrado de Micología (Ulloa and Hanlin, 2006). Colors are indicated according to the color table of Kornerup and Wanscher (1978). Longitudinal sections of dried apothecia were rehydrated with 70% alcohol, observed in 5% KOH, and in water. Ornamentation of ascospores, and other structures were observed using Melzer´s staining reagent, as well as cotton blue in lactophenol.

The microscopic structures were observed using an optical microscope (OM) (Axiostar Plus, Zeiss, Jena, Germa-

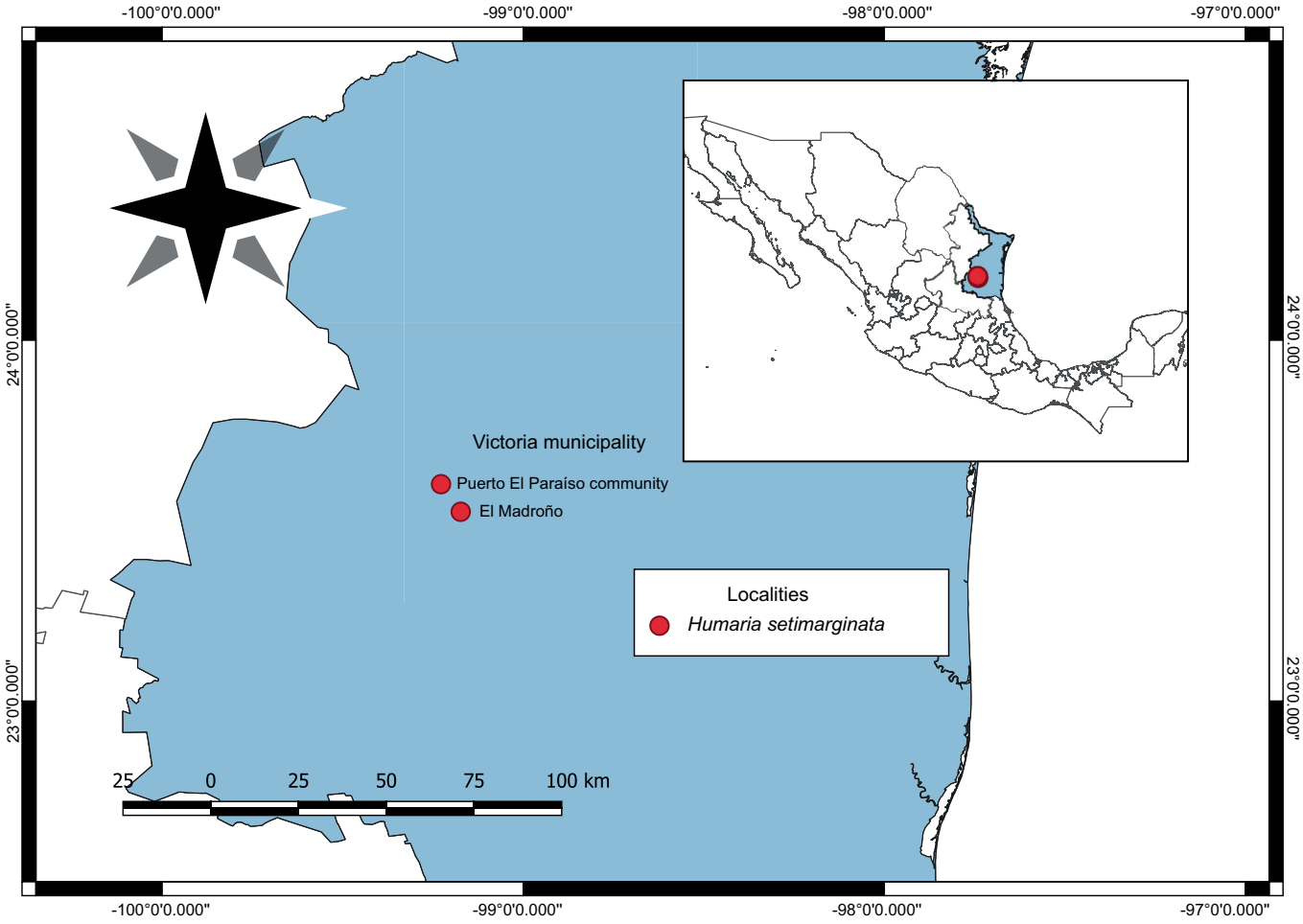

Figure 1: Localities of *Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez, in Tamaulipas, Mexico.

ny). Photographs were taken with a Rebel T-1i camera and a 100 mm macro lens (Canon, Tokyo, Japan). Scanning electron microscopy (SEM; SU1510, Hitachi High Technologies, Tokyo, Japan) was used to observe the ornamentation of the ascospores in detail. The works of Dennis (1981), Fuckel (1870) and Beug et al. (2014) were used to differentiate the species.

DNA extraction, amplification and sequencing

Total DNA was extracted from dried herbarium specimens using a modified version of the protocol of Martínez-González et al. (2017) protocol. PCR amplification, based on Mullis and Faloona (1987), included 35 cycles with an annealing temperature of 54 °C, and was carried out with the ITS5 and ITS4 primers (White et al., 1990; Gardes and Bruns, 1993) for the ITS nrDNA region, and LR0R and LR5 primers (Vilgalys and Hester, 1990; Cubeta et al., 1991) for the 28S nrDNA region (LSU).

PCR products were verified by agarose gel electrophoresis. The gels were run for 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), at the Instituto de Biología of the Universidad Nacional Autónoma de México (UNAM). The obtained sequences were compared with the original chromatograms to detect and correct possible reading errors.

Phylogenetic analysis

To explore the phylogenetic relationship of the new species, an alignment was made based on the taxonomic sampling employed by Erős-Honti et al. (2008), Healy et al. (2022) and sequences deposited in the Gen Bank NCBI database (GenBank, 2022).

Each gene region was independently aligned using the online version of MAFFT v. 7 (Katoh et al., 2002, 2017; Katoh and 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 matrices consisted of 31 taxa for ITS (700 characters) and 18 taxa for LSU (962 characters) (Table 1). The aligned matrices were concatenated into a single matrix (34 taxa, 1662 characters). Phylogenetic inferences were estimated with Maximum Likelihood in RAxML v. 8.2.10 (Stamatakis, 2014) with a GTR + G model of nucleotide substitution. To assess branch support, 1000 bootstrap replicates were run with the GTRGAMMA model. For Bayesian posterior probability, the best evolutionary model for alignment was sought using PartitionFinder v. 2.0 (Lanfear et al., 2014; 2016; Frandsen et al., 2015).

Phylogenetic analyses were also performed using MrBayes v. 3.2.6 ×64 (Huelsenbeck and 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 ≤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 (Rambaut et al., 2014). The two simultaneous Bayesian runs continued until the convergence parameters were met, and the standard deviation fell below 0.0001 after 10 million generations.

No significant changes in tree topology trace or cumulative split frequencies of selected nodes were observed after about 0.37 million generations, so the first 2,500,000 sampled trees (25%) were discarded as burn-in. Trees were visualized and optimized in FigTree v. 1.4.4 (Rambaut et al., 2014).

Results

Molecular analyses

We successfully amplified and sequenced the ITS and LSU region from the holotype of our *Humaria* collection. Both the Bayesian and Maximum Likelihood analyses (Fig. 2) recovered *Humaria setimarginata,* supporting the existence of a new taxon distinctive from related species of *Humaria* (1 Bayesian Posterior Probability (PP) and 100% bootstrap values (BS) for Maximum Likelihood). Thus, *Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez is proposed as a new species for science (see Taxonomy).

Taxonomy

Ascomycota

Pezizomycetes

Pezizales

Pyronemataceae

Humaria setimarginata Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez, sp. nov. Figs. 3, 4, 5, 6, 7. MycoBank no. MB 841440.

TYPE: MEXICO. Tamaulipas, Victoria municipality, El Madroño, 1443 m a.s.l., 23°36'16.32''N, 99°13'45.2''W, 11.XI.2019, *M. Sánchez 1889* (holotype: ITCV!, isotype: ENCB!).

Humaria setimarginata is characterized by the size, 60-353 × 13-20 µm, and position of marginal hairs, by a dextrinoid reaction in the ectal excipulum with Melzer´s reagent, and ascospores 19-25 × 10-15 µm, ellipsoid to oblong-ellipsoid.

Apothecial ascomata solitary or gregarious, 10-15 mm diameter, sessile, deeply cupulate, with a greyish white (1B1) hymenium when fresh, light orange (5A4)

Table 1: GenBank (2022) accession numbers corresponding to the sequences used in the phylogenetic analyses. The accession numbers of the new species are in bold.

Species name	Voucher Number	GenBank Accession	
		ITS	LSU
Genea gardneri Gilkey	SOC 690	AY830857	-----
Genea gardneri Gilkey	src831	DQ206850	-----
Genea gardneri Gilkey	src867	DQ206851	-----
Genea harknessii Gilkey	Trappe 13313	DQ220334	-----
Genea harknessii Gilkey	Trappe 11775	DQ220335	-----
Genea verrucosa Vittad.	AH44208	KJ938935	-----
Genea verrucosa Vittad.	BP104856	KJ938936	-----
Humaria cazaresii (M.E. Sm. & Trappe) M.E. Sm., Healy & P. Alvarado	Trappe18044	DQ206863	----
Humaria hemisphaerica (F.H. Wigg.) Fuckel	Andy 10/15/03	-----	AY789389
Humaria hemisphaerica (F.H. Wigg.) Fuckel	BAP 320 (FH)	-----	DQ220352
Humaria hemisphaerica (F.H. Wigg.) Fuckel	HKAS 82077	MG871304	MG871339
Humaria hemisphaerica (F.H. Wigg.) Fuckel	FHKH03100	DQ200832	DQ220353
Humaria hemisphaerica (F.H. Wigg.) Fuckel	JMP0104	EU819470	-----
Humaria hemisphaerica (F.H. Wigg.) Fuckel	K (M) 187356	MZ159485	-----
Humaria hemisphaerica (F.H. Wigg.) Fuckel	GO-2009-385	KC152113	-----
***Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez**	**Type ITCV**	**OP521892**	**OP529804**
Humaria sp.	FLAS-F-61555	MG019762	MG019797
Humaria sp.	FLAS-F-61554	MG019761	MG019796
Humaria sp.	LY NV2012.10.16	MG019765	MG019800
Humaria sp.	LY NV2014.08.35	MG019768	-----
Humaria sp.	LY NV2014.07.19	MG019767	-----
Humaria sp.	LY NV2012.10.16	MG019765	MG019800
Humaria sp.	ISC 443646	MG019764	MG019799
Humaria sp.	ISC 437685	MG019763	MG019798
Humaria sp.	FLAS F-61548	MG019759	MG019794
Humaria sp.	FLAS F-61555	MG019762	MG019797
Humaria sp.	FLAS F-61554	MG019761	MG019796
Humaria sp.	FLAS F-61552	MG019760	MG019795
Humaria sp.	FH 823753	MG019757	MG019792
Humaria sp.	FH 00304592	MT505219	MT505178
Humaria sp.	FLAS F-66262	MN653025	MZ018863
Humaria sp.	GEN 4	OP177902	-----
Humaria sp.	FLAS-F-68407	OM672766	-----

when dry, outer surface dark brown (6F8), covered with scattered short hairs, margin entire, with dense dark brown hairs; marginal hairs, 60-353 × 13-20 µm, wall 2-5 µm thick, with 1-14 septa, brown, superficial, having a single napiform base, with slightly rounded apex; excipular hairs similar, scattered, with a sharp apex; ectal excipulum 138-225 µm thick, of *textura angularis*, composed of angular to subglobose cells, 15-55 × 10-30 µm, walls of cells brown or hyaline, tissues turning greyish ruby (12C7) to reddish in Melzer´s reagent (dextrinoid

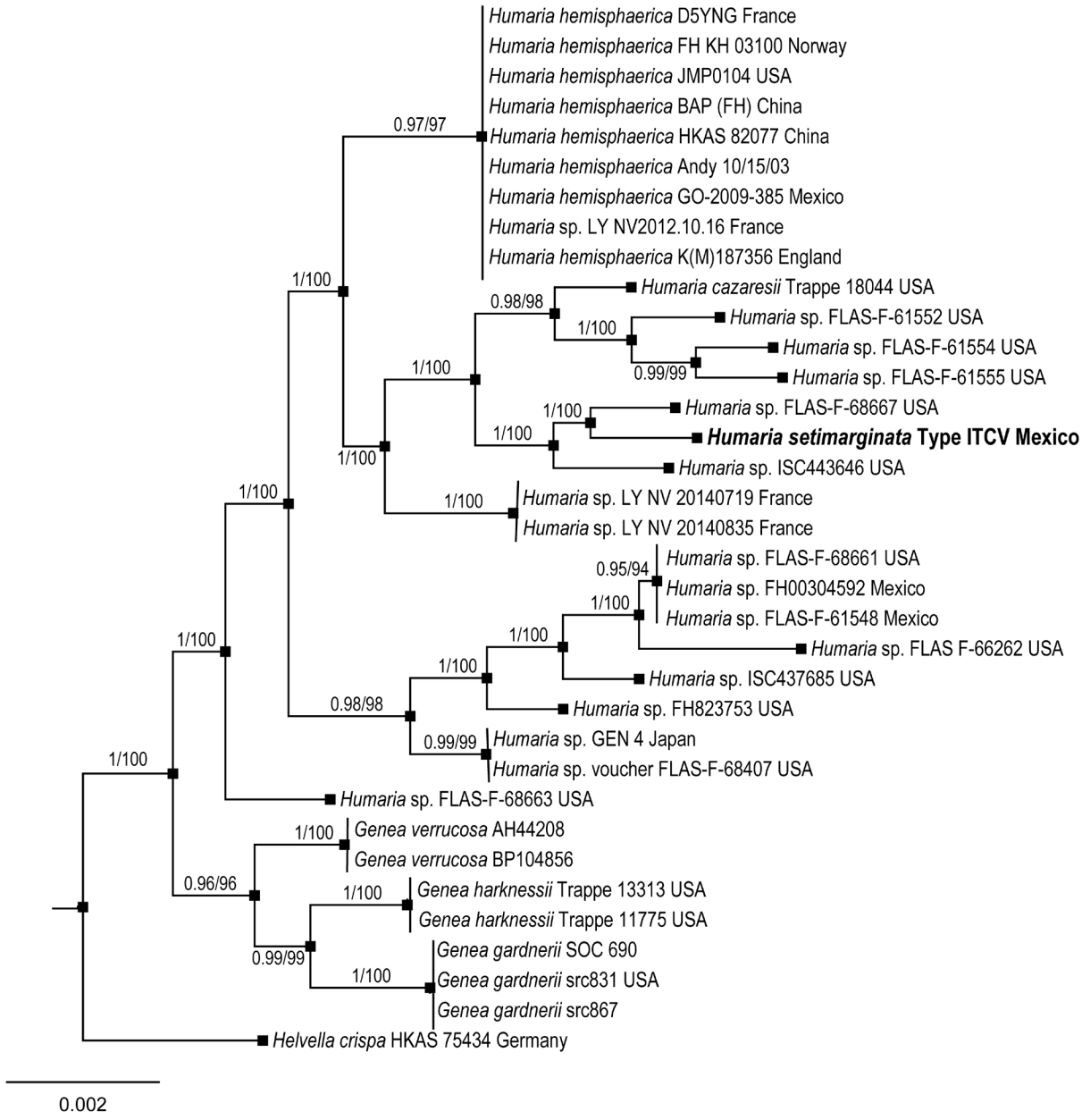

Figure 2: Phylogram of Bayesian inference (BI) tree from the ITS and LSU sequence data of 34 specimens. The values above branches represent Bayesian posterior probabilities (PP) and bootstrap values (BS) for Maximum Likelihood, respectively. The scale bar represents the expected number of nucleotide substitutions per site. Sequences obtained from this study are in bold.

reaction); medullary excipulum 80-130 µm thick, of *textura intricata*, made of hyphae 4-8 µm diameter, hyaline, without reaction in Melzer´s reagent; paraphyses hyaline, septate, filiform, 5-7 µm diameter at the apex, widened, some widened or having a bulge in the top cell; asci 217-237 × 12-15 µm, cylindrical, with croziers, hyaline, containing 8 uniseriate spores, inamyloid; ascospores 19-25 × 10-15 µm (X=21.7 × 12.5 µm, n=60), Q=1.5-2, Qm=1.7, ellipsoid to oblong-ellipsoid, hyaline, ornamented with cyanophilous warts, 1 µm high (side view), disappearing with 5% KOH.

Habitat: on soil, under *Quercus rysophylla* Weath. and *Q. polymorpha* Schltdl. & Cham.

Distribution: only known from the type locality.

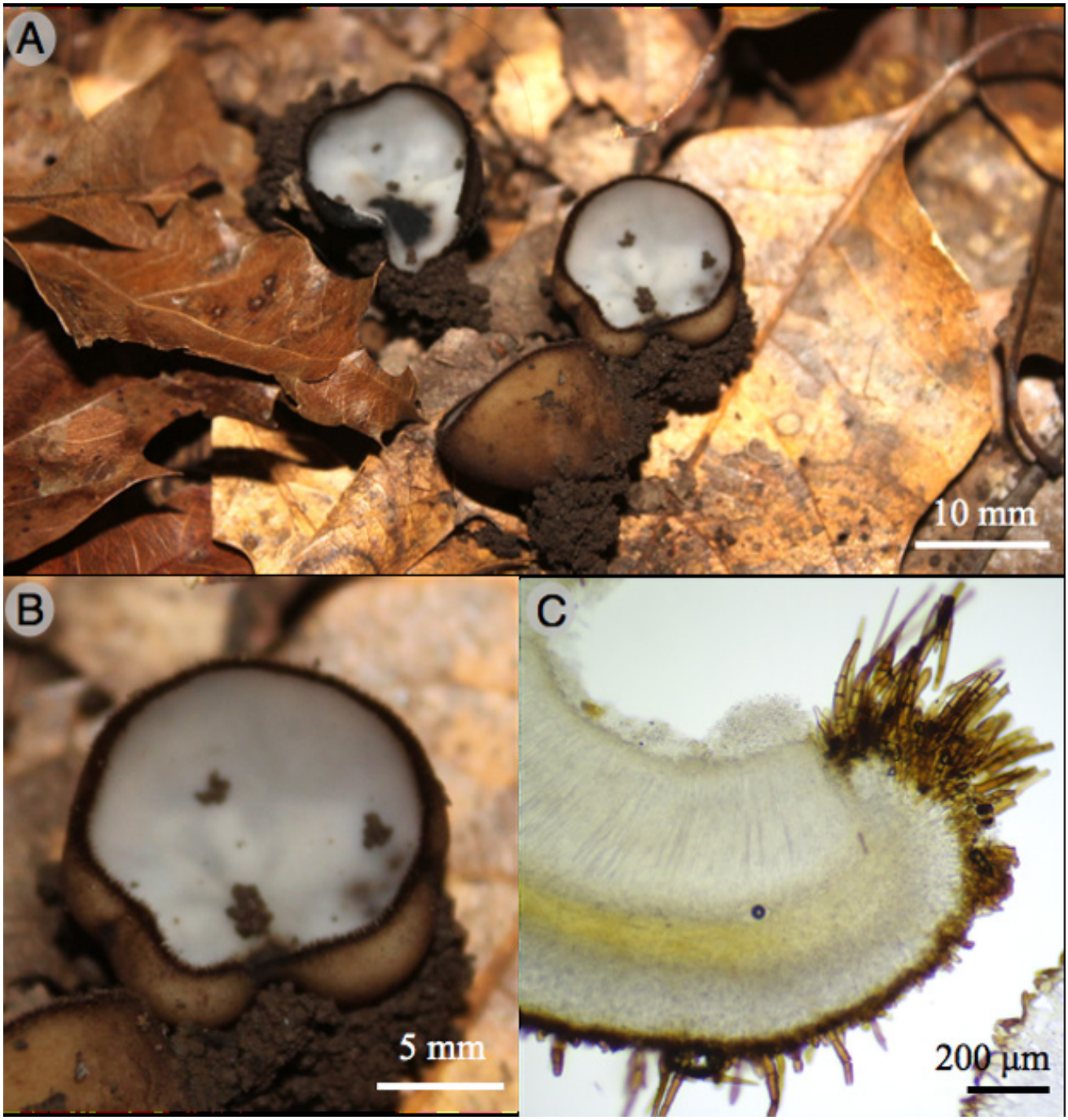

Figure 3: *Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez. A., B. apothecia; C. longitudinal section of the apothecium.

Etymology: from Latin *seta*, meaning "hair", and *margo*, meaning "edge, margin", referring to the abundant hairs in the margin of the apothecium.

Additional material examined: MEXICO. Tamaulipas, Victoria municipality, Puerto El Paraíso community, 1650 m a.s.l., 23°31'38.99''N, 99°12'20.04''W, 01.XI.2019, *M. Sánchez 1847* (ITCV), *1852* (ITCV).

Notes: *Humaria setimarginata* is characterized by its abundant, short marginal hairs, contrary to *H. hemisphaerica* which has longer hairs (sometimes reaching more than 1000 µm, see Table 2), mostly concentrated on the edge of the margin. This species also differs from *H. hemisphaerica* by the dextrinoid reaction of the ectal excipulum with Melzer's reagent and by slightly smaller ascospores (24 × 14 µm). It also differs from *H. cazaresii*, which is a hypogeous species

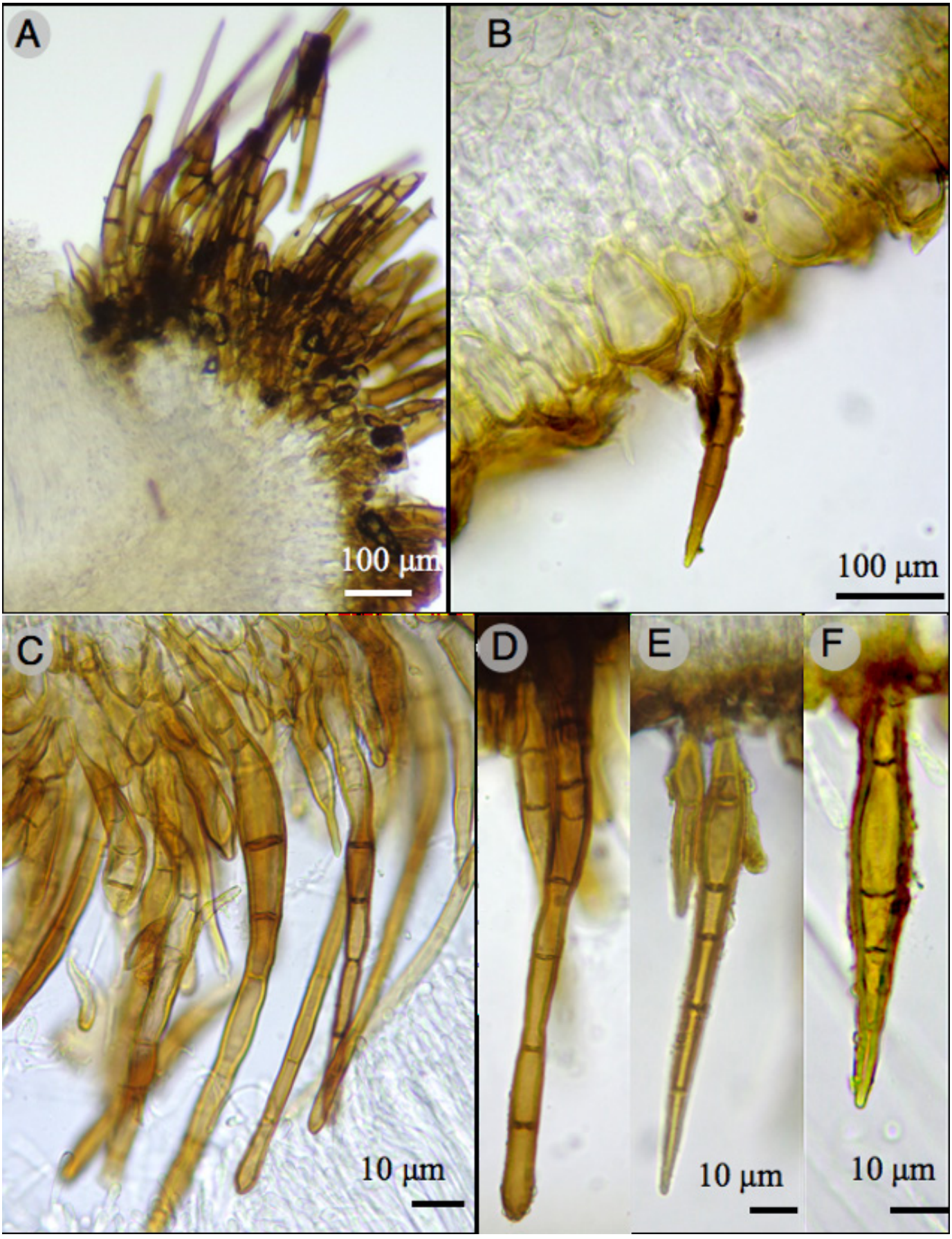

Figure 4: *Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez. A-F. hairs of the apothecium.

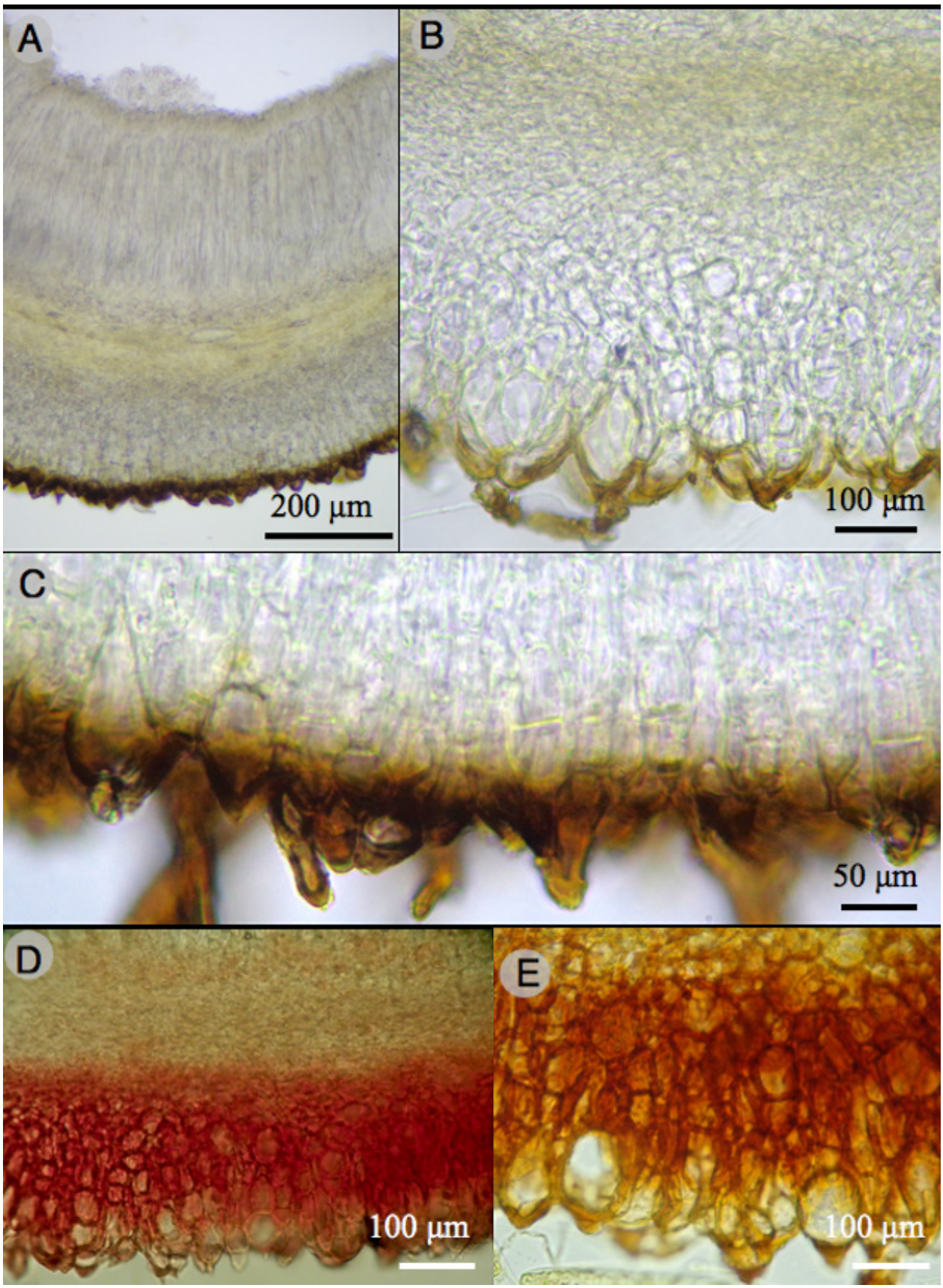

Figure 5: *Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez. A. longitudinal section of the apothecium; B., C. ectal excipulum; D., E. dextrinoid reaction in Melzer´s reagent of the ectal excipulum.

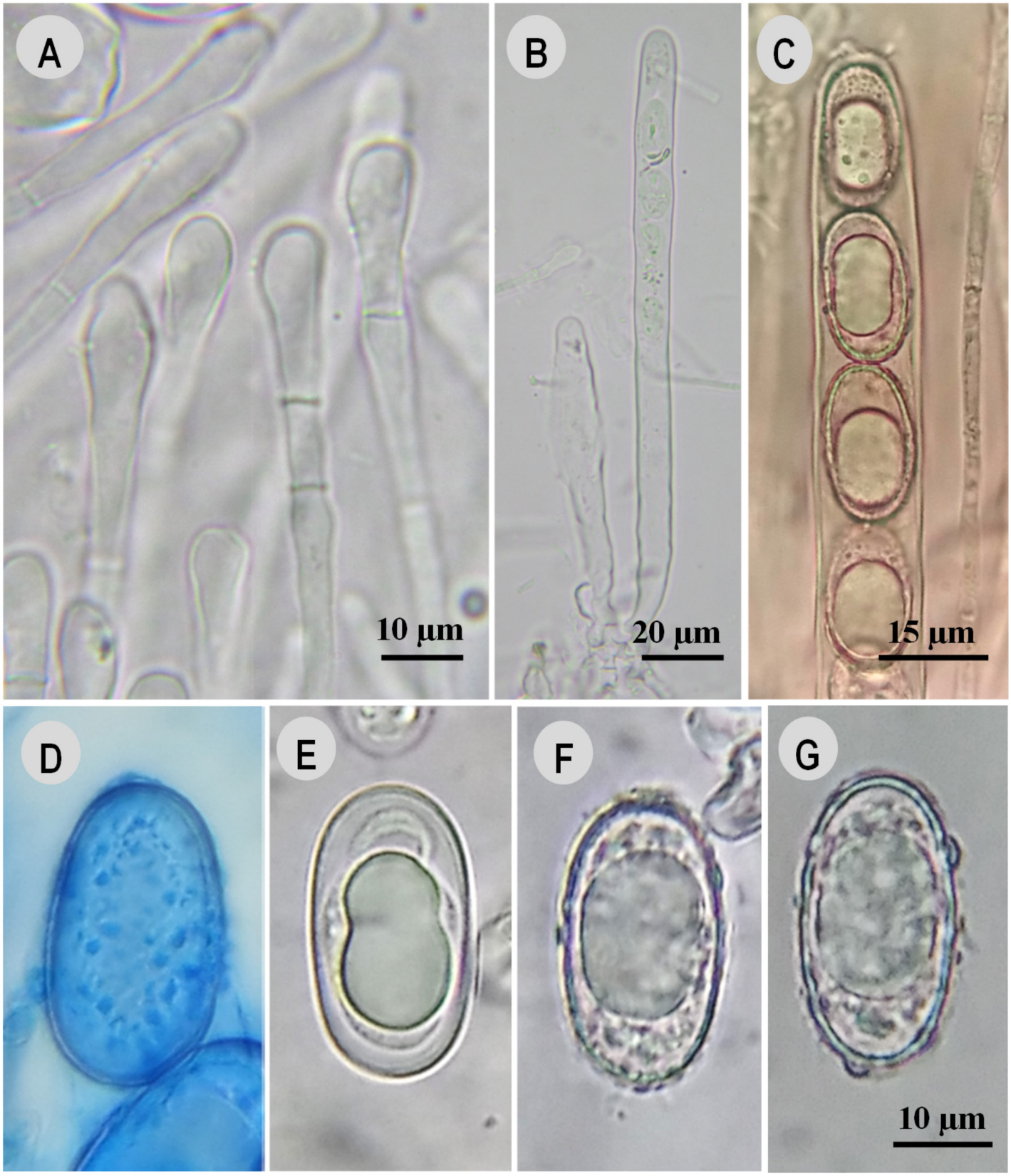

Figure 6: *Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez. A. paraphyses; B. asci; C. asci and ascospores; D. ascospore in cotton blue; E. ascospore in 5% KOH, ornamentation having dissolved; F., G. ascospores in water.

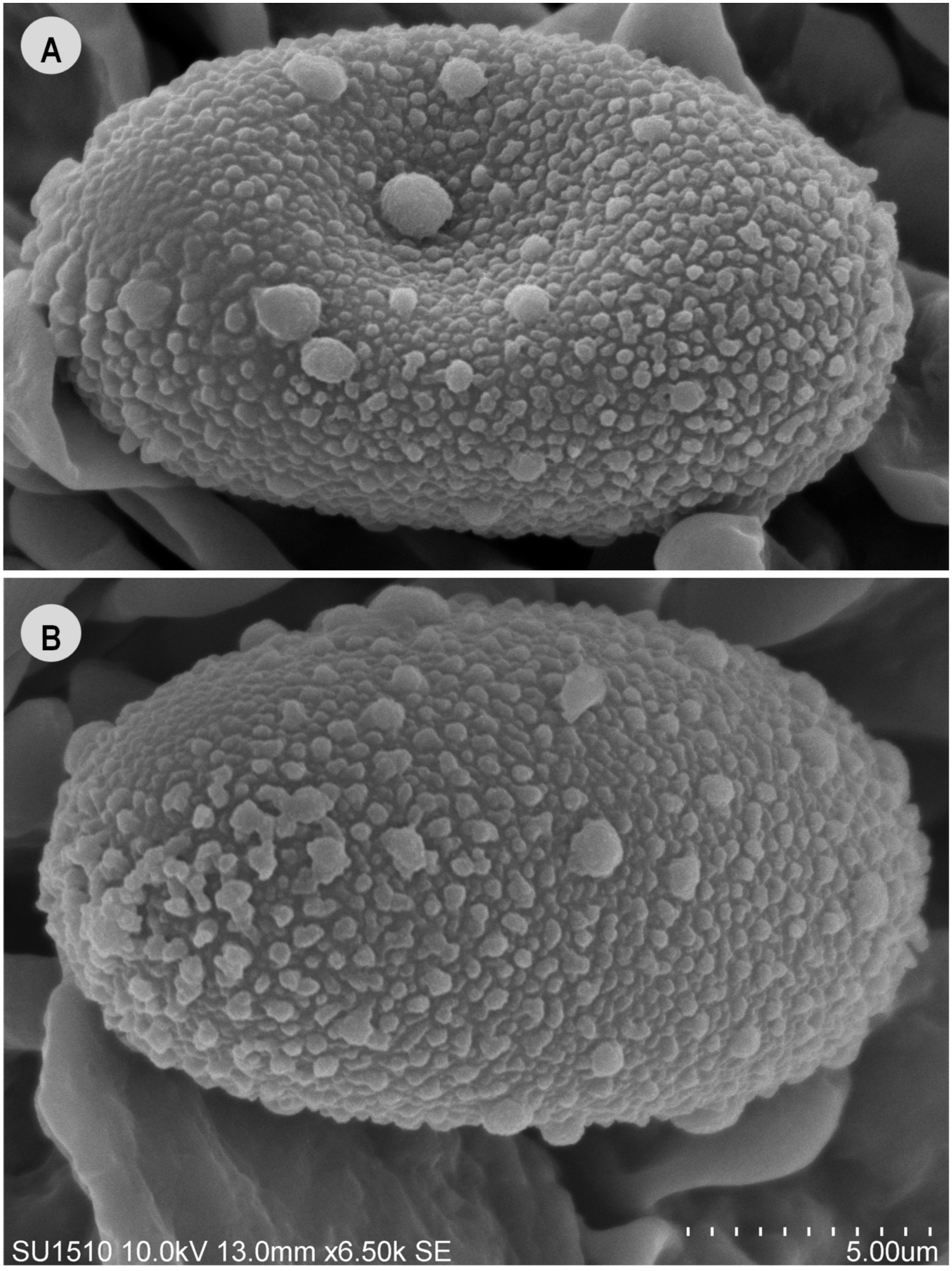

Figure 7: *Humaria setimarginata* Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez. A., B. ascospores with SEM.

Table 2: Comparison of species of *Humaria* (Fr.) Boud, in the American continent with species of the *Humaria hemisphaerica* (F.H. Wigg.) Fuckel complex.

Species	Distribution	Apothecia	Hymenium	Marginal hairs	Asci	Ascospores	Paraphyses	Reference
H. cazaresii (M.E. Sm. & Trappe) M.E. Sm., Healy & P. Alvarado	USA	10 mm diameter, hypogeous	No data	Without hairs	170-220 × 10-13 µm	18-21 × (12) 14-16 µm	2.5- 5 µm	Smith et al., 2006
H. hemisphaerica (F.H. Wigg.) Fuckel	England	30 mm diameter	White or whitish	500-1000 × 20 µm	350 × 20 µm	20-24 × 10-12 µm	7-8 µm	Dennis, 1981
H. hemisphaerica (F.H. Wigg.) Fuckel	Europe and USA	20-30 mm diameter,	White or whitish	400-500 × 15-20 µm	325 × 15-18 µm	25-27 × 12-15 µm	7-8 µm	Seaver, 1928
H. hemisphaerica (F.H. Wigg.) Fuckel	France	6-30 mm diameter	Dull white to pale grey	120-1300 ×17-28 µm	205-270 × 16-25 µm	20-24 × 11.5-14 µm	5-9 µm	Van Vooren, 2014
H. hemisphaerica (F.H. Wigg.) Fuckel	Germany	No data	White	No data	168 × 16 µm	24 × 14 µm	No data	Fuckel, 1870
Humaria hemisphaerica (F.H. Wigg.) Fuckel	Mexico	10-30 mm diameter	Whitish	770-850 × 20-22 µm	250-300 × 13.2-16.5 µm	19.8-20.9 × 11-12.1 µm	5.5-7.7 µm	Bautista et al., 1986
Humaria hemisphaerica (F.H. Wigg.) Fuckel	Mexico	15-30 mm diameter	Whitish	400-830 × 13-19 µm	190-230 × 10-13 µm	13-19 × 6-8 µm	3-4 µm	Ortega-López, 2015
H. hemisphaerica (F.H. Wigg.) Fuckel	Turkey	10-30 mm diameter, 5-15 mm high	Whitish or greyish	No data	No data	22-27 × 12-15 µm	No data	Sesli, 1998
H. hemisphaerica (F.H. Wigg.) Fuckel	USA	10-30 mm diameter	Whitish to pale grey	No data	230-270 (350) × 19-23 µm	22-27 × 10-13 µm	No data	Beug et al., 2014
H. setimarginata Sánchez-Flores, Raymundo, Van Vooren & García-Jiménez	Mexico	10-15 mm diameter	greyish white	60-353 × 13-20 µm	217-237 × 12-14 µm	19-25 × 10-15 µm	5-7 µm	This study

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which has smaller ascospores 18-21 × (12)14-16 µm (Smith et al., 2006). Finally, *H. setimarginata* is considered to be associated with *Q. rysophylla* and *Q. polymorpha*.

Discussion

Humaria setimarginata sp. nov. only known from the type specimen in *Quercus* forests in Mexico, is proposed based on the combination of morphological, ecological, and molecular characters. One of the main characters that differentiates this species is the dextrinoid reaction of the ectal excipulum in Melzer's reagent, a feature that has not been seen in other specimens or reported in current descriptions of *H. hemisphaerica* or any other species of *Humaria*.

In addition, *H. hemisphaerica* differs from *H. setimarginata* in the size of ascospores and the size of the marginal hairs (see Table 2). One of the problems in differentiating *H. hemisphaerica* is the wide range of macro and microscopic characters that have been provided in the literature. Dennis (1981) described the latter with slightly narrower ascospores 20-24 × 10-12 µm, wider asci 350 × 20 µm and wider paraphyses 7-8 µm. Fuckel (1870) gives ascospores measuring 24 × 14 µm. Sesli (1998) described larger ascospores, 22-27 × 12-15 µm. Van Vooren (2014) indicated 20-24(-25) × (11-)11.5-14 µm for ascospore dimensions. In Mexico, Bautista et al. (1986) described this species as having longer hairs, 770-850 × 20-22 µm, and smaller and narrower ascospores 19-21 × 11-12 µm. Ortega López (2015), in his master's thesis, described it with larger and narrower hairs, 400-830 × 10-13 µm, and with smaller and narrower ascospores, 13-19 × 6-8 µm.

All these variations in spore size and hair dimensions suggest the existence of several potential distinct species named under *H. hemisphaerica*. Our phylogeny (Fig. 2) shows that such a diversity exists in the genus *Humaria*. A detailed revision of the *H. hemisphaerica* species complex is required, including type study, but this is beyond the aim of our article.

Reports of *Humaria* species in Mexico are scarce, as are their descriptions; this is probably due to the morphological similarity of the collections made in the country. However, the macro and microscopic differences could cause them to be classified as different taxa. Considering that *Humaria* is an ectomychorrizal genus (Tedersoo et al., 2006; Erős-Honti et al., 2008) and the country has a high diversity of ecosystems, being the home of 161 species of *Quercus* (Valencia-A, 2004) and 50 of *Pinus* (Gernandt and Pérez-de la Rosa, 2014; Pérez-de la Rosa and Gernandt, 2017), we believe that a comprehensive review of the genus is needed. It is imperative that morphological and phylogenetic studies of other collections identified as *Humaria hemisphaerica* be carried out, as well as their ecology.

Author contributions

MSF, TR and JGJ conceived and designed the study. MSF collected the species. MSF, TR, NVV and JGJ contributed to the acquisition of important data for the study. CRMG extracted the DNA and realized amplification and phylogenetic analysis. The photos were taken by MSF. All authors contributed to the discussion, review, and approval of the final manuscript.

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