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UNIVERSIDAD DE GUADALAJARA



***Pluteus horakianus*, una especie nueva de
México, con base en datos morfológicos y
moleculares**

**TRABAJO DE TITULACIÓN EN LA MODALIDAD DE
ARTÍCULO CIENTÍFICO**

**QUE PARA OBTENER EL TÍTULO DE:
LICENCIADO EN BIOLOGÍA**

**PRESENTA
ADRIÁN ANTONIO GALVÁN CORONA**

Las Agujas, Zapopan, Jal., noviembre 09 de 2009

Dra. Georgina Adriana Quiroz Rocha
 Presidente del Comité de Titulación
 Licenciatura en Biología
 Centro Universitario de Ciencias Biológicas y Agropecuarias
 Presente

Por este conducto me permito poner a su consideración mi anteproyecto de titulación modalidad Artículo científico publicado, titulado: "Pluteus horakianus, a new species from Mexico, based on morphological and molecular data", el cual se anexa para que sea turnado al Comité de Titulación de esta carrera para su revisión y, en su caso, aprobación.

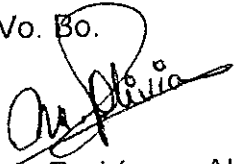
Así mismo, me permito proponer como director/a de titulación a: Dra. Olivia Rodríguez Alcántar y como asesores a: Dra. Laura Guzmán Dávalos y Dra. Alma Rosa Villalobos Arámbula.

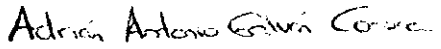
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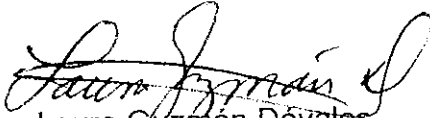
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Lugar y fecha
 Las Agujas, Zapopan, Jal., 28 de julio del 2009



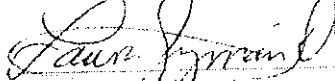
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Universidad de Guadalajara
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Coordinación de carrera de Licenciado en Biología

C. Adrián Antonio Galván Corona

PRESENTE

Manifestamos a usted que con esta fecha ha sido aprobado su tema de titulación en la modalidad de: **INVESTIGACIÓN Y ESTUDIOS DE POSGRADO** opción **SEMINARIO DE INVESTIGACIÓN** con el título: "*Pluteus horakianus*, a new species from Mexico, based on morphological and molecular data)" para obtener la Licenciatura en Biología.

Al mismo tiempo le informamos que ha sido aceptado como Directora de dicho trabajo a la Dra. Olivia Rodríguez Alcántar y como asesora a la Dra. Laura Guzmán Dávalos y Dra. Alma Rosa Villalobos Arámbula.

Sin más por el momento, reciba un cordial saludo.

ATENTAMENTE
"PIENSA Y TRABAJA",
"2009, Año del Bicentenario de Charles Darwin"
Las Agujas, Zapopan, Jal., 7 de octubre de 2009.



DRA. GEORGINA ADRIANA QUIROZ ROCHA
PRESIDENTE DEL COMITÉ DE TITULACIÓN

BIOL. MARGARITO MORA NÚÑEZ
SECRETARIO DEL COMITÉ DE TITULACIÓN

Este trabajo se realizó en el Laboratorio de Micología del Departamento de Botánica y Zoología de la Universidad de Guadalajara, bajo la dirección de la Dra. Olivia Rodríguez Alcántar. Así mismo se contó con la asesoría de la Dra. Laura Guzmán Dávalos y la Dra. Alma Rosa Villalobos Arámbula.

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Indudablemente a mis padres que pese a cualquier adversidad, se las arreglaron para que pudiera continuar y fueron el máximo apoyo para cumplir mi anhelo de convertirme en Biólogo.

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Y a Olivia Rodríguez, que me contagió su ánimo y brindó todo su apoyo para concluir esta etapa de formación.

¡A ustedes, gracias por todo!

***Pluteus horakianus*, a new species from Mexico, based on morphological and molecular data**

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Rodríguez, O., Galván-Corona, A., Villalobos-Arámbula, A.R., Vargas, G., Guzmán-Dávalos, L. (2008). *Pluteus horakianus*, a new species from Mexico, based on morphological and molecular data. *Sydowia XX (X)*: pp-pp.

Pluteus horakianus is described here from *Quercus*, *Pinus-Quercus* and subtropical-montane cloud forests in Western Mexico. The species is morphologically similar to *P. aurantiorugosus* but is distinguished by its red pileus, the marginate lamellae with scarlet to orange-reddish edges, the red tinges on the stipe and the small and fragile basidiomata. The phylogeny inferred from ITS rDNA sequences revealed that *P. horakianus* represents a distinctive species within the well supported *Celluloderma* clade distantly related to the morphologically similar *P. aurantiorugosus*. A morphological description of the new species is supplemented by a photograph of a fresh basidioma and illustrations of the micromorphological features.

Keywords: Agaricales, Basidiomycota, ITS rDNA sequences, phylogeny, *Pluteaceae*.

The genus *Pluteus* Fr. refers to saprobic, lignicolous to rarely terricolous agaric fungi characterized by free lamellae, sordid pink to brownish pink spore print, the absence of a ring and volva on the stipe and mainly by the bilateral inverse lamella trama (Singer 1986). The traditional taxonomy of *Pluteus* is based on microscopic features, in particular on the structure of the pileipellis and the presence and/or absence of both cheilocystidia and pleurocystidia (Kühner and Romagnesi 1953, Singer 1956, 1958; Horak 1964, Horak and Heinemann 1978, Orton 1986, Vellinga 1990, Banerjee and Sundberg 1993, 1995). According to Minnis *et al.* (2006), *Chamaeota*, a genus morphologically similar to *Pluteus* but with partial veil, is probably obsolete because they found *C. mamillata* (Longyear) Murrill nested in the *Pluteus* clade using LSU rDNA sequences. Perhaps the description of the genus *Pluteus* should be modified to include the presence of partial veil.

Approximately 33 taxa of *Pluteus* are reported from Mexico; however, based on our field observations, several more taxa are expected to be found (Rodríguez and Guzmán-Dávalos 1997, 1999, 2001, 2007). In this study, a re-examination of collections previously identified as *P. aurantiorugosus* (Trog) Sacc. revealed a distinct taxon. Thus, the new species, *P. horakianus*, is proposed. Due to the orange to scarlet pileus, macromorphologically, these two species of *Pluteus* are quite similar; however, *P. horakianus* is readily separated from *P. aurantiorugosus* by its reddish lamellar edges and the red tinges on the stipe. ITS rDNA-based analyses confirm the status of *P. horakianus* as a distinct taxon.

Materials and methods

Morphological study

Micromorphological observations were obtained from hand-made sections of basidiomata or gill fragments mounted in 3% KOH. Basidiospores were measured in 3 % KOH using a calibrated optical micrometer in a Zeiss K-7 optical microscope (oil immersion objective 100x). Measurements are given as follow: (minimum) range (maximum), mean values (\bar{x}), Q = length/width ratio [N = number of samples, n = sample size]. The length of basidia includes the sterigmata. The terms for the descriptions follows those of Vellinga (1998) and occasionally those of Largent (1986). Colour designations within parentheses are from Kornerup and Wanscher (1989). Illustrations were made with the aid of a drawing tube. The acronyms of herbaria and the citation of the authors follow Holmgren *et al.* (1990) and Kirk and Ansell (1992), respectively. The studied collections are conserved in the following herbaria: IBUG (Institute of Botany, University of Guadalajara, Zapopan, Mexico) and ZT (Eidgenössische Technische Hochschule Zurich, Switzerland).

DNA extraction, PCR amplification, sequencing and sequence assembly

Genomic DNA was isolated from small pieces (ca. 4 mg) of dried herbarium material (including pileipellis, context and lamellae) by using the protocol of salt-extraction with 1% PVP (Aljabani and Martínez 1997) with several modifications. Pelleted DNA was re-suspended in 30 μ l, 50 μ l or 80 μ l of EDTA and stored at 4 °C until used. The genomic sequence of the internal transcribed spacer (ITS) containing the ITS1, 5.8S and ITS2 regions was amplified by PCR using the pair primers ITS1F-ITS4 (Vilgalys and Hester 1990). Each

Tab. 1. - *Pluteus* and outgroup specimens used in this study.

DNA num.	Species	Section or family for outgroups	Collector, number and herbarium	Locality	Date of collection	GenBank No.
113	<i>P. albospitatus</i> var. <i>poliobasis</i> Singer	Hispiderma	O. Rodríguez 2951 (IBUG)	Mexico	Jul 19, 2006	FJ375244
160	<i>P. aurantiorugosus</i> (Trog) Sacc.	Celluloderma	J.C. Zamora 15720 (AH)	Spain	Nov 5, 2001	FJ375248
216	<i>P. aurantiorugosus</i>	Celluloderma	L. Guzmán-Dávalos 6581 (IBUG)	Mexico	Jul 20, 1997	FJ375249
6	<i>P. cervinus</i> (Schaeff.) P. Kumm.	Pluteus	L. Guzmán-Dávalos 3513 (IBUG)	Mexico	Sep 5, 1986	FJ375241
242	<i>P. diverticulatus</i> Corriol	Celluloderma	0092579 (Holotype, PC)	France	Sep 1, 1950	FJ375247
58	<i>P. horakianus</i> O. Rodr. - Alcántar	Celluloderma	L. Guzmán-Dávalos 7488 (IBUG)	Mexico	Aug 31, 1998	FJ375250
60	<i>P. horakianus</i> O. Rodr. - Alcántar	Celluloderma	L. Guzmán-Dávalos 7271 (Holotype, IBUG)	Mexico	Jul 24, 1998	FJ375251
114	<i>P. nigrolineatus</i> Murrill	Hispiderma	O. Rodríguez 1548 (IBUG)	Mexico	Aug 14, 1996	FJ375245
222	<i>P. pellitus</i> (Pers.) P. Kumm.	Pluteus	J. García 9934	Mexico	Sep 1, 1996	FJ375243
100	<i>P. petasatus</i> (Fr.) Gillet	Pluteus	O. Rodríguez 2587 (IBUG)	Mexico	Jul 17, 2004	FJ375242
119	<i>P. romelli</i> (Britzelm.) Lapl.	Celluloderma	O. Rodríguez 1565 (IBUG)	Mexico	Aug 31, 1996	FJ375246
85	<i>P. thomsonii</i> (Berk. & Broome) Dennis	Celluloderma	95091602	France		FJ375252
155	<i>P. thomsonii</i>	Celluloderma	F. Pardo s.n. (AH)	Spain	Nov 2, 2001	FJ375253
GB ^a	<i>Chamaeota sinica</i> J.Z. Ying	Pluteaceae				DQ182505
GB ^b	<i>Entoloma blotamii</i> (Berk.) Sacc.	Entolomataceae				EF330938
GB ^c	<i>Entoloma nitidum</i> Quéf.	Entolomataceae				AY228340
GB ^d	<i>Entoloma sericeum</i> (Bull.) Quéf.	Entolomataceae				AF357020
GB ^e	<i>Limacella glioderma</i> (Fr.) Maire	Amanitaceae				AY176451
176	<i>Volarrella gloiocephala</i> (DC.) Boekhout & Enderle	Pluteaceae	L. Guzmán-Dávalos 8444	USA	Sep. 13, 2000	FJ375254

^a Sequence downloaded from GenBank, Matheny and Hibbett (2005). ^b Sequence downloaded from GenBank, Denis *et al.* (2007).

^c Sequence downloaded from GenBank, Acorn *et al.* (2003). ^d Hofstetter *et al.* (2002). ^e Vellinga (2004).

25 μ l PCR reaction volume contained 16.9 μ l of sterile double-distilled water, 2.4 μ l of 10X reaction buffer (100 μ M Tris, 500 μ M KCl), 1.2 μ l MgCl₂ (Applied Biosystems), 1.2 μ l of 5 mM dNTPs, 0.1 μ l of Taq DNA polymerase 5U/ μ l (Applied Biosystems), 0.5 μ l of each 10 μ M primer, 1.2 μ l of BSA (bovine serum albumin; New England Bio Labs), and 1 μ l of DNA template. PCR amplifications were performed in a MJ Research PTC 200 thermocycler. The DNA was denatured at 95 °C for 3 min. Thirty five cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 45 s, and extension at 72 °C for 2 min were followed by 15 cycles of 95 °C for 1 min, 50 °C for 45 s and 72 °C for 2 min increasing 5 s each cycle with an extension step of 72 °C for 10 min and final incubation at 4 °C. Amplification products were size-fractionated on 1.5% agarose gels (NuSieve, FMC Bioproducts). The gels were stained with ethidium bromide and photographed under UV light. The PCR product was purified with GFXtm purification kit (Amersham Biosciences) and was subsequently sequenced by capillary electrophoresis on an ABI-Prism 310 Genetic Analyzer (Applied Biosystems). Resulting chromatograms were visually checked, manually corrected when necessary using Chromas 1.45 (McCarthy 1996–1998) and edited, assembled and aligned with MacClade 4.0 (Maddison and Maddison 2000) and subsequently increased by eye.

Phylogenetic analyses

Thirteen new *Pluteus* sequences and one new *Volvariella* sequence were generated and deposited in GenBank under the accession numbers (FJ375241–FJ375254). Among them, two sequences of *P. aurantiorugosus* originating from Mexico and Spain, respectively, were included in the evaluation of the new Mexican taxon. Furthermore, five sequences were obtained from GenBank (Tab. 1). The trees were rooted with species of *Volvariella*, *Limacella*, *Chamaeota* and *Entoloma*. Phylogenetic trees were inferred with PAUP* 4.0b10 (Altevec) (Swofford 2000). Branch-and-bound searches were performed using the criterion of maximum parsimony with furthest addition sequence, branches collapsed if maximum branch length is zero, only minimal trees were kept, and MulTrees option in effect. Gaps were treated as missing characters. Starting trees were obtained via stepwise addition. Relative branch support was estimated with 1000 bootstrap replications (Felsenstein 1985) with the same parameters mentioned previously. The parsimony tree scores, including tree length and consistency, retention, rescaled consistency and homoplasy indices (CI, RI, RC and HI) excluding uninformative characters, were calculated.

Taxonomy

Pluteus horakianus O. Rodr.-Alcántar, sp. nov. (Figs. 1, 3–7, 8–12)
Mycobank MB511993

Pileus 10–25 mm, primo umbonatus vel campanulatus, dein planoconvexus, rugosus ad discum, translucide striatus marginem versus, ruber, rubroaurantiacus vel brunneoruber, siccus. Lamellae liberae, primo aurantiacae dein aurantiacorubrae, ad aciem persistenter rubrae vel aurantiacorubrae. Stipes 15–40 × 1–3 mm, cylindricus, aequalis, curvatus, aurantioruber vel aurantiacus, fibrillosus, siccus. Caro albidula vel pallide lutea. Odor saporque nulli. Basidiosporae 5–7.5 (8) × 4.5–6 (6.5) μm, subglobose vel late ellipsoideae, leves. Basidia 22–32 × 6.5–9.5 μm, clavata, 4-sporigera, defibulata. Cheilocystidia (25) 30–55 (60) × (8) 10–22 μm, clavata, pigmento impleta. Pleurocystidia 40–80 × 12–23 μm, polymorphica, lageniformia, subfusiformia vel clavata, hyalina. Caulocystidia nulla. Pileipellis e cellulis vesiculososis vel clavatis hymenodermium formantibus, 20–70 (75) × (9) 12–30 (40) μm, pigmento pallide luteobrunneo impletis. Fibulae nullae. Habitatio ad lignum putridum in silvis mixtis (Pinus, Quercus). Mexico. Jalisco, San Sebastián del Oeste, 1900 m, 24 August 1998, L. Guzmán-Dávalos 7271 (holotypus, IBUG).

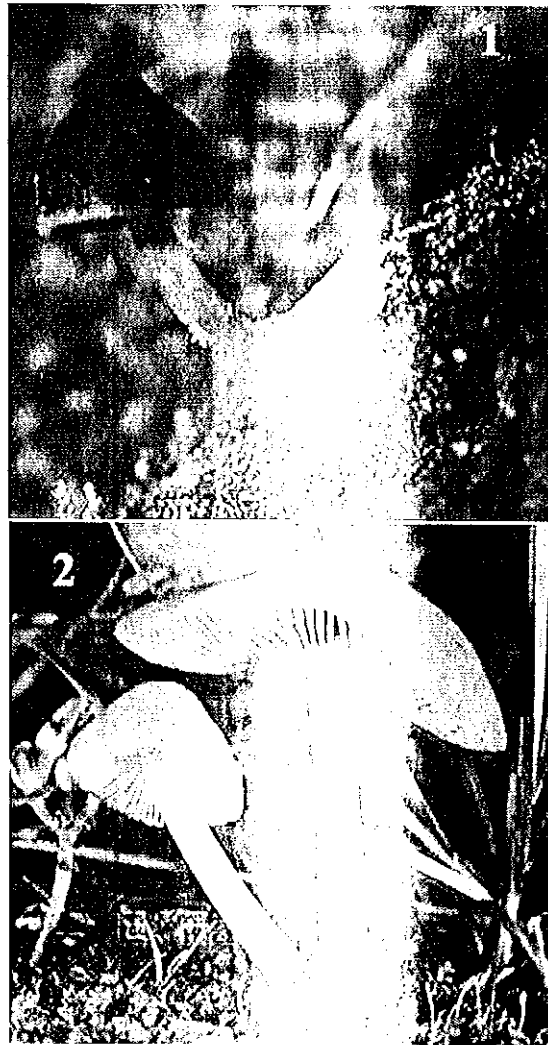
Pileus 10–25 mm, at first subumbonate, umbonate to campanulate, becoming convex to plano-convex in mature specimens, rugose at disk but smooth towards incurved, translucently striate margin; surface velvety, dry, at first dark red (11C8) to dark reddish-brown (8F7–8, 9F8) becoming orange-red (8A8), reddish-orange (7B7), brownish-red (8D8), oxide-red (8E8) to reddish-brown (9E8) in age. – Lamellae free, crowded, rather broad, ventricose, at first orange (5A7), brownish-orange (6B7), turning to pinkish, reddish-orange (7B7) to pale orange (5A3) in age, lamellar edges distinctly and persistently red, scarlet, red-orange (8A8) to reddish-orange (7B7). – Stipe 15–40 × 1–3 mm, central, equal, slightly curved, glabrous to innately silky-fibrillose, longitudinally striate, fragile, solid to hollow, upper half at first scarlet (9A8), turning dark orange (5A8) to orange (6A7, 6B8) in aged specimens, lower half towards base scarlet (9A8) to orange-red (8A8), with cottony, whitish, basal mycelium. – Context 1–2 mm thick in pileus, fleshy-fibrous, whitish to yellowish-white (4A2), unchanging upon exposure. – Smell and taste not distinctive.

Basidiospores 5–7.5 (8) × 4.5–6 (6.5) μm, \bar{x} = 6.5 × 5.3 μm, Q = 1.07–1.3 (1.5) [N = 7, n = 24], subglobose to broadly ellipsoid then ellipsoid, smooth, wall slightly thickened to thin, hyaline. – Basidia 22–32 × 6.5–9.5 μm, clavate, 4-spored, hyaline with refringent content. – Cheilocystidia (25) 30–55 (60) × (8) 10–22 μm, clavate, occasionally utriform, thin-walled, hyaline. – Pleurocystidia 40–80 × 12–23 μm, polymorphic, ranging in shape from lageniform with short or elongate neck, utriform, subfusiform or clavate, thin-walled, hyaline. – Caulocystidia absent. – Pileipellis a hymeniderm composed of sphaeropedunculate, vesiculose or clavate cells, mea-

suring 20–70 (75) × (9) 12–30 (40) μm, apex rarely subacute or mucronate, thin-walled, filled with yellowish-brown dissolved cytoplasmatic pigment. – Oleiferous hyphae absent. – Lamella trama bilateral inverse. – Clamp connections absent.

Etymology. – Named in honour to Dr. Egon Horak for his contributions to the genus *Pluteus*.

Habitat. – Lignicolous, gregarious, on rotten wood or woody debris among moss, both in disturbed *Quercus* forest, *Pinus-Quercus* forest and subtropical-montane cloud forest, 1300–2240 m.



Figs. 1–2: *Pluteus horakianus*: 1. Basidiomata (S. Guzmán-Dávalos 7271 holotype, photo. E. Fanti). *Pluteus aurantiorugosus*: 2. Basidiomata (O. Rodríguez 3002, photo. E. Fanti).

Materials examined. – MEXICO, COLIMA, Municipality of Minatitlán, El Terrero, 1940 m, 22 Jun 1995, *leg. et det.* O. Rodríguez 1090 (IBUG). JALISCO, Municipality of San Sebastián del Oeste, road to La Mina, El Real Alto, 2070 m, 29 Aug 1994, *leg. et det.* O. Rodríguez 1044 (IBUG); Cañada de Milpillas, road Milpillas - La Virgencita, 1300–1400 m, 13 Aug 1998, *leg.* L. Guzmán-Dávalos 7319, *det.* O. Rodríguez (IBUG). NAYARIT, Municipality of Tepic, Reserva Ecológica del Cerro de San Juan, La Capilla, 2020–2240 m, 31 Jul 1991, *leg.* J.L. Villarruel-Ordaz 91, *det.* O. Rodríguez (FCME); 26 Sep 1991, *leg.* Gutiérrez-Ruíz 212, *det.* O. Rodríguez (FCME); km 6.3 road to El Cuarenteño, from km 4 Tepic-Miramar highway, 1430 m, 31 Aug 1998, *leg.* L. Guzmán-Dávalos 7488, *det.* O. Rodríguez (IBUG).

Holotypus. – JALISCO, Municipality of San Sebastián del Oeste, approximately 10 km from San Sebastián del Oeste to La Bufa, 1900 m, 24 Jul 1998, *leg.* L. Guzmán-Dávalos 7271, *det.* O. Rodríguez (IBUG, holotype; ZT, isotype).

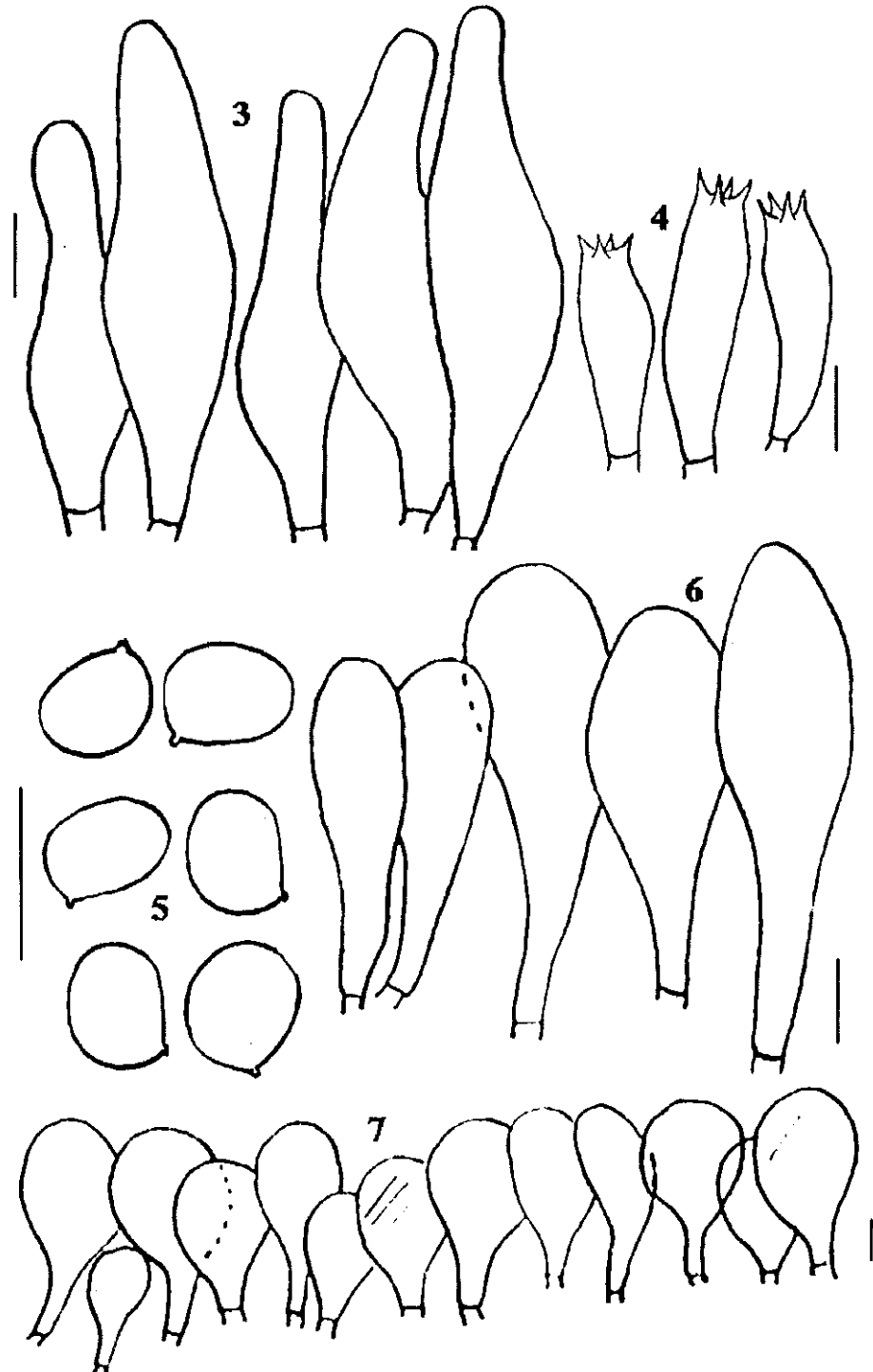
Results of phylogenetic analyses

The initial alignment of the 19 ITS sequences consisted of 773 nucleotide positions. Both ends of the sequences and ambiguous regions were excluded from the analyses, leaving 311 characters. Of these, 49 were parsimony informative. Equally weighted parsimony analysis produced five trees with a tree length of 159 steps; CI = 0.572, HI = 0.427, RI = 0.763 and RC = 0.523. The phylogram shown (Fig. 13) has the same topology as the strict-consensus tree. *Pluteus* is monophyletic with a bootstrap support of 83%. Three clades were found, each representing the taxonomically accepted sections of the genus. Macromorphologically, *P. horakianus* and *P. aurantiorugosus* are species belonging to same well supported clade that represent the section *Celluloderma* Fayod (Singer 1986), but they actually belong to distinct subclades (Fig. 13).

Discussion

Macromorphologically, *Pluteus horakianus* is distinguished by small, fragile and colourful basidiomata with red pileus; red, scarlet, red-orange to reddish-orange lamellar edges and red tinges on the stipe. Micromorphologically, it is characterized by mainly lageniform pleurocystidia and clavate cheilocystidia. However, the shape of both, the cheilocystidia and pleurocystidia, can vary considerably, e.g. the collection Guzmán-Dávalos 7488 has slender clavate cheilocystidia, and the coll. Guzmán-Dávalos 7271 (holotype), showed slender subfusiform pleurocystidia (Figs. 8–13).

Morphotaxonomically, the most similar species is *P. aurantiorugosus* (Fig. 2), which is reported from Europe, North America and Northern Africa (e.g. Lange 1936, Kühner and Romagnesi 1953, Singer 1956, 1958; Horak, 1964, Homola 1972, Dermek 1987, Vellinga 1990, Citérin and Eyssartier 1998, Consiglio 2000). *Pluteus aur-*



Figs. 3-7: *Pluteus horakianus*, 3: pleurocystidia, 4: basidia, 5: basidiospores, 6: cheilocystidia, 7: pileipellis (Gutiérrez-Ruiz 212, FCME) (5: scale = 20 μm , 3-4, 6-7 = 10 μm).

antiorugosus is described to possess scarlet, orange to red-orange basidiomes, but the lamellar edges are not marginate as in *P. horakianus* and the basidiospores are smaller ($5.7\text{--}6.7 \times 4.3\text{--}5 \mu\text{m}$, according to Consiglio 2000). Several Mexican collections (Rodríguez 1090, J.L.Villarruel-Ordaz 91 and Gutiérrez-Ruiz 212) were previously misidentified as *P. aurantiorugosus* (Rodríguez and Guzmán-Dávalos 2000). Subsequent re-examination of this material revealed that these collections represented an undescribed species, which is formally described here as a new taxon. It is also important to mention that in dried specimens of *P. horakianus* several specific macromorphological characters (e.g. the yellowish-orange-tinges on the stipe and the reddish-brown pileus) can be readily recognized.

There are other superficially similar species with orange to reddish coloration on the stipe. *Pluteus aurantiopustulatus* E. Horak from has a different type of pileipellis and for this reason it is included in sect. *Hispidoderma* Fayod (Horak 1977). *Pluteus aurantipes* Minnis, Sundberg & Nelsen, also in sect. *Hispidoderma*, has a pileipellis consisting of a trichoderm and lacks pleurocystidia (Minnis *et al.* 2006). *Pluteus flammipes* E. Horak, *P. laetifrons* (Berk. & M.A. Curtis) Sacc. and *P. laetus* Singer also are reported to have basidiomes with predominantly orange to reddish colours, but differ micromorphologically in the form and size of the cystidia, or as in *P. laetus*, by having a pileipellis typical for subsect. *Mixtini* Singer (Singer 1958; Horak 1964).

Moncalvo *et al.* (2002) have shown that the genus *Pluteus* is monophyletic. In the present contribution, the analyses also support monophyly of *Pluteus* with 83% of bootstrap support (BS). Additionally, the three widely accepted sections in the genus form well supported distinct clades (Fig. 13): Sect. *Celluloderma* (91% BS), Sect. *Hispidoderma* (93% BS), and Sect. *Pluteus* (96% BS). Micro-morphologically, the species of the Sect. *Celluloderma* are characterized by having basidiomata with thin-walled pleurocystidia and a pileipellis made of a hymeniderm of sphaeropedunculate cells. The taxa of Sect. *Hispidoderma* are distinguished by having thin-walled pleurocystidia and a palisadic pileipellis structure with distinctive pileocystidia. Finally, the species of Sect. *Pluteus* are characterized by having species with conspicuous metuloid pleurocystidia and a pileipellis composed of filamentous hyphae that form either a cutis or a trichoderm (Fayod 1889, Lange 1917, 1936; Kühner and Romagnesi 1953, Singer 1956, 1958, 1986; Horak 1964, Orton 1986, Vellinga 1990, Banerjee and Sundberg 1995).

Based upon analyses of ITS rDNA data, the new Mexican species, *Pluteus horakianus*, represents a distinctive taxon belonging to Sect. *Celluloderma*. Molecular data referring to European and Mexican specimens of *P. aurantiorugosus* were compared with those of *P.*

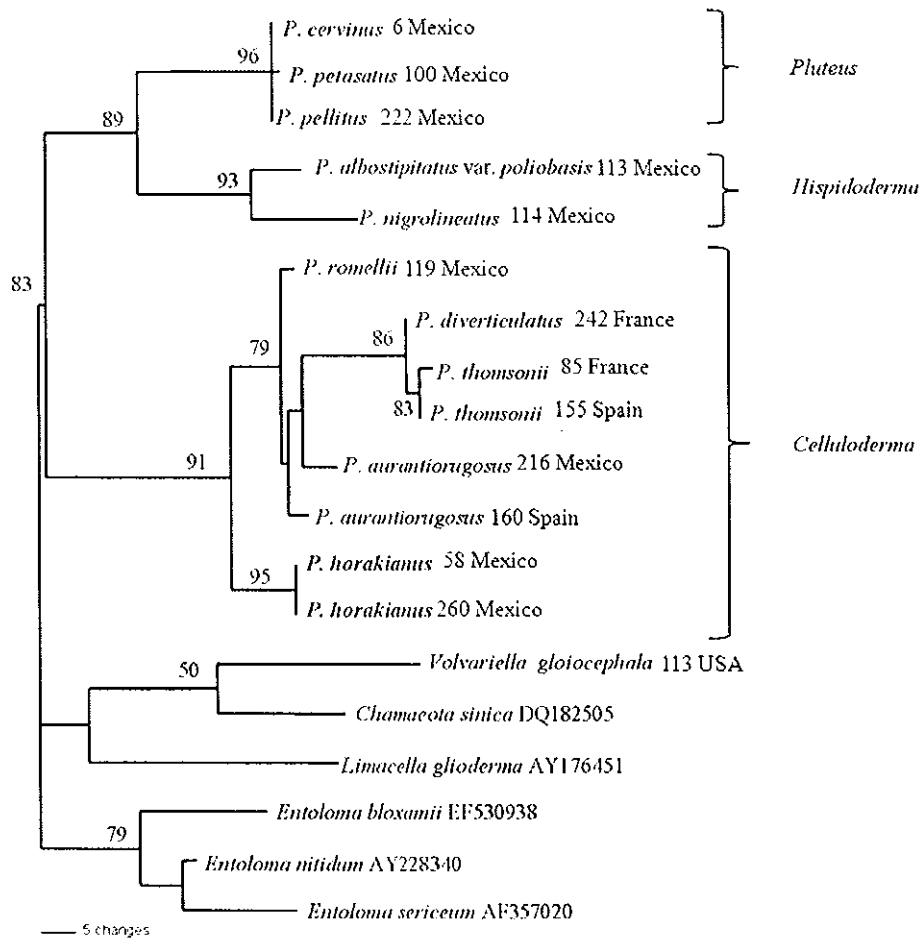


Fig. 13. One of the three phylograms resulting from a branch-and-bound search, of 10 species (13 samples) of *Pluteus*, and six outgroups, based on ITS rDNA sequence data. Tree length = 159 steps, parsimony informative characters = 49, CI excluding uninformative characters = 0.572, RI = 0.763, RC = 0.523. Bootstrap values > 50% obtained from a branch-and-bound search with 1000 replicates are given above each branch.

horakianus. As illustrated in the phylogram the two species belong to *Celluloderma* clade, but as two distinctive clusters (Fig. 13). Within the *Celluloderma* clade, *P. horakianus* was in a basal position (91% BS). Other species included in this clade are *P. diverticulatus* Corriol and the related *P. thomsonii* (Berk. & Broome) Dennis (86% BS). Both taxa belong to Subsect. *Mixtini* and have the *Mixtini*-type pileipellis. *Pluteus romellii* (Britzelm.) Lapl., characterized by *Cellulodermini*-type pileipellis belongs to the Subsect. *Cellulodermini*. It is interesting to note that the two collections of *P. aurantiorugosus* are not grouped together. This may indicate that the specimen from Mexico could represent a different species distinct from the Eur-

ocean specimen. The latter specimen has some morphological differences such as larger pilleipellis elements while the Mexican specimen has smaller elements. It is necessary to study more material to see if there is a distinct species in Mexico that is separate from *P. aurantiorugosus* described from Europe.

The Sect. *Pluteus* clade encompasses *P. cervinus* (Schaeff.) P. Kumm. and the macroscopically similar *P. pellitus* (Pers.) P. Kumm. and *P. petasatus* (Fr.) Gillet. Representatives of Sect. *Hispidoderma* including both *P. albostipitatus* var. *poliobasis* Singer and *P. nigrolineatus* Murrill have been analysed, and they are found to belong to the same clade.

The molecular data presented in Fig. 13 show phylogenetic affinities between Sect. *Pluteus* and Sect. *Hispidoderma* (89% BS). Our results, however, do not correspond to those published by Moncalvo *et al.* (2002) where Sect. *Celluloderma* and Sect. *Hispidoderma* are in the same clade. Further research is needed to clarify the phylogenetic relationships between the different sections of *Pluteus*.

In conclusion, the examined collections of the new Mexican *Pluteus horakianus* yield enough morphological and molecular differences that it can be considered as a distinct taxon. Currently, this species is known from several localities in Western Mexico (Colima, Jalisco and Nayarit States) where it is found on rotting wood and debris in temperate *Quercus*, *Pinus-Quercus* and mixed cloud forests.

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