IDENTIFICATION OF *OUDEMANSIELLA CANARII* AND *O. CUBENSIS* (BASIDIOMYCOTA, PHYSALACRIACEAE) IN ARGENTINA USING MORPHOLOGICAL, CULTURE AND MOLECULAR ANALYSIS

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Abstract. Species of *Oudemansiella* are distributed worldwide mainly in tropical to south temperate zones. Morphological identification of the species is usually complex and may even be ambiguous, especially for *O. canarii* and *O. cubensis*, since they share many similar characters. They have been considered synonyms by several authors. There are no recent descriptions or detailed illustrations of *Oudemansiella* species from Argentina, and molecular data are practically null. In this work these species were described and illustrated making use of new collections, culture data, and nrITS phylogenetic analysis. It was not possible on the basis of the results to differentiate the species macroscopically, but these were differentiated at the microscopic level, mainly by their pileipellis structure. Phylogenetic analysis from nrITS sequences enabled the molecular identification of these two species, and the cultivation of basidiomes on lignocellulosic substrate allowed us to affirm that both species have a hemiangiocarpic development.

Keywords: *Agaricales*, *Basidiomycota*, ITS, phylogeny, taxonomy

The genus *Oudemansiella* Speg. (Basidiomycota, Physalacriaceae) was proposed based on *Agaricus platensis* Speg. (Spegazzini, 1880a) and combined in *Oudemansia* Speg. (Spegazzini, 1880b). Later, it was replaced by *Oudemansiella* (Spegazzini, 1881) because *Oudemansia* already existed in Malvaceae. In the diagnosis, Spegazzini (1880b, 1881) characterized this genus as not having a manifest veil and having a central stipe, a hemispheric, fleshy, and nonliquescent pileus, and membranose lamellae, first connate, then free. The diagnostic characters presented by Spegazzini (1881) to delimit *Oudemansiella* were so broad that most agaric fungi would fit the description (Petersen and Hughes, 2010). Because of this broad description, the concept of the genus *Oudemansiella* has changed over the years. Some authors (Moser, 1955; Singer, 1962a,b, 1964; Clémençon, 1979; Pegler and Young, 1986; Singer, 1986; Yang et al., 2009) considered *Oudemansiella* a broadly circumscribed genus, which included *Xerula* Maire and *Mucidula* Pat. species. On the other hand, other authors (Dörfelt, 1979; Boekhout and Bas, 1986; Redhead et al., 1987; Petersen and Halling, 1993; Corner, 1994, 1996; Petersen and Methven, 1994; Boekhout, 1999; Halling and Mueller, 1999; Contu, 2000; Mueller et al., 2001; Horak, 2005; Petersen and Hughes, 2005; Petersen and Nagasawa, 2006; Petersen and Baroni, 2007; Petersen, 2000, 2008a,b,c) considered *Oudemansiella*, *Xerula,* and *Mucidula* as separate genera. Yang et al. (2009) and Petersen and Hughes (2010) summarized the taxonomic history of the *Oudemansiella* and *Xerula* complex.

With the advancement of molecular studies, *Oudemansiella* was placed in the *Physalacriaceae*. Petersen and Hughes (2010) completed the previous morphological and molecular studies, proposed four new genera (*Hymenopellis* R.H. Petersen, *Paraxerula* R.H. Petersen, *Pointiculomyces* R.H. Petersen, and *Protoxerula* R.H. Petersen), and redefined other previously existing genera (*Dactylosporina* (Clémençon) Dörfelt, *Mucidula*, *Oudemansiella*, and *Xerula*). In these assessments the genus *Oudemansiella* has been circumscribed to species with pileus dry to generally viscid, usually with scattered floccules; lamellae white to off-white and subdistant; and stipe central, usually without veil remnants, or with rudimentary or fugacious veil. Microscopically, basidiospores are characterized by being large, globose to subglobose, and white in prints, their pleuro- and cheilocystidia are well developed, and the pileipellis is formed of a polycystoderm or an ixotrichodermium, constructed of hyphal chains with subspherical, keg-shaped, fusiform, filamentous, or rod-shaped cells (Petersen and Hughes, 2010). The number of *Oudemansiella* species sensu stricto has been limited by these revisionary studies and 36 are recognized (Index Fungorum, 2020), with worldwide distribution, mainly in tropical to south temperate zones (Yang et al., 2009).

Morphological identification of *Oudemansiella* species is usually complex and may be ambiguous, especially for *O. canarii* (Jungh.) Höhnel. and *O. cubensis* (Berk. and M.A. Curtis) R.H. Petersen, since they share many characteristics and have been considered synonyms by several authors

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(Horak, 1968; Pegler and Young, 1986; Singer, 1986; Wright and Albertó, 2002; Niveiro and Albertó, 2012). Many citations and descriptions of these two species should not be considered for identification purposes until further revisions of herbarium materials are carried out. Molecular data of *Oudemansiella* species are scarce and are often not accompanied by the corresponding morphological descriptions, which can lead to identification errors. In Argentina, *Oudemansiella* species sensu lato were cited by Spegazzini (1880b, 1881, 1899, 1926), Singer (1950), Singer and Digilio (1952), Singer (1964), Raithelhuber (1979, 1987, 1991, 1995, 2004), Wright and Albertó (2002), Wright and Wright (2005), and Wright et al. (2008), but there are no recent descriptions or detailed illustrations, and molecular data are practically null. The aim of this work is to describe and illustrate the two *Oudemansiella* species sensu stricto found in Argentina on the basis of new collections, culture data, and phylogenetic analysis formulated on molecular data.

Materials and Methods

Morphological Studies

The specimens were collected in northern and central Argentina. Strains were isolated in nature from wild specimens and conserved in glucose potato agar in the ICFC (INTECH Collection of Fungal Cultures, Laboratory of Mycology and Mushroom Cultivation, Chascomús, Argentina); reference in the WDCM database: 826. For the taxonomic identification, the specimens were analyzed macro- and microscopically following the criteria and terminology proposed by Alexopoulos and Mims (1985), Vellinga (1988), and Lodge et al. (2004). The color terminology followed Kornerup and Wanscher (1978). Microscopic observations were made from material mounted in a 5% KOH (v/w) with 1% floxin aqueous solution and Melzer's reagent to verify amyloid reaction (Wright and Albertó, 2002). The microscopic structures were measured directly through photographs taken with a Zeiss Discovery V20 SteREO camera using ZEN 2.6 (Blue Edition) software or with a BX 43 Olympus camera using Cell standard 3.0 software. For the basidiospores, "n=" indicates the number of basidiospores measured, "x=" the average value, "Q=" the Q-value (length/width), and "Qx=" the mean value of Q. The authors of scientific names agree with the *Index Fungorum* "Authors of Fungal Names" (Index Fungorum, 2020), and acronyms of herbaria follow the *Index Herbariorum* (Thiers, 2020). The collected material was dried, kept in the freezer for a week, and deposited as a reference in the CTES herbarium. One strain of each *Oudemansiella* species conserved in agar in the culture collection mentioned above was cultivated on wheat straw to obtain basidiomes in order to characterize the development. Spawn production, substrate preparation, spawning, incubation, and cultivation conditions followed the methodology proposed by Jaramillo Mejía and Albertó (2013) for cultivation of *Pleurotus ostreatus* (sterile technique), using plastic bags with substrate, and introducing the following modifications: 2-cm layer of casing soil formulated with 99% peat (Bertinat ™) and 1% $CaCO_3$ added after the incubation period. Mushrooms were grown in a room under controlled conditions with an air temperature of 24 C and relative humidity of 90%, and with periodic watering.

Molecular Genetic Analyses

Extracting DNA from basidiomes was performed in two ways: (1) by the Barcode of life Project following Ivanova and Grainger (2006) protocols, and (2) using EasyPure® Plant Genomic DNA Kit following the manufacturer's protocols. PCR amplification was performed following Ivanova et al. (2008). The nuclear ribosomal internal transcribed spacer region (ITS) was amplified using the basidiomycete-specific primer set: ITS1-F and ITS4-B (Gardes and Bruns, 1993). For samples processed through the Barcode of Life project, PCR products were sequenced in the Canadian Centre for DNA Barcoding (CCDB). For the rest of the samples, PCR products were sequenced at Macrogen, Korea.

Molecular Phylogeny

The dataset was compiled using our 15 sequences and 21 sequences selected from GenBank on the basis of BLAST results. *Hymenopellis radicata* (Relhan: Fr.) R.H. Petersen and *Xerula pudens* (Pers.) Singer were selected as outgroups (Table 1). Sequence editing and alignment were done in BioEdit (version 7.2.5). Sequences were aligned under the Clustal IW criteria. Phylogenetic reconstruction was inferred using maximum likelihood estimation (ML) and Bayesian inference (BI) separately. The best evolutionary model for ML was TrN+G; it was estimated using Bayesian information criterion (BIC) in jModelTest2 (version 1.6) (Guindon and Gascuel, 2003; Darriba et al., 2012). The evolutionary model used for BI was GTR+I+G. Maximum likelihood estimates were calculated using MEGA X (Kumar et al., 2018). The statistical support for the resulting phylogenies was assessed by bootstrapping with 1,000 replicates (Felsenstein, 1985). Bayesian inference was performed in MrBayes 3.2.7 (Ronquist et al., 2012), and it was carried out with two simultaneous runs of four Markov Monte Carlo chains. These were run for 10^6 generations, with sampling each 100 generations, and with a burnin of 1,001 generations; the final consensus was based on 17,959 trees used to estimate posterior probabilities. Only the topology of the ML tree is shown, indicating support values of Bayesian posterior probability/bootstrapping (BPP/BS) of each node. A node was considered to be strongly supported if it showed a BPP ≥ 0.95 and/or BS ≥ 90%, whereas moderate support was was indicated by a BPP ≥ 0.90 and/or BS ≥ 70%.

TABLE 1. List of sequences used in the phylogenetic analyses and their references.[1]

SPECIES	SPECIMEN VOUCHER	ORIGIN	GENBANK ACCESSION NUMBER	REFERENCE
Hymenopellis radicata	LE-BIN 1795	Russia	MK795851	Moiseenko et al. (unpubl.)
Hymenopellis radicata	TENN60080	USA	GQ913389	Petersen and Hughes (2010)
Oudemansiella australis	RV95/416	Australia	AF321473	Mueller et al. (2001)
Oudemansiella australis	RV95/297	Australia	AF321472	Mueller et al. (2001)
Oudemansiella canarii	JM98/221	China	AF321476	Mueller et al. (2001)
Oudemansiella canarii	TENN62802	USA	GQ892793	Petersen and Hughes (2010)
Oudemansiella canarii	FLAS-F-61207	USA	MH211812	Kaminsky et al. (unpubl.)
Oudemansiella canarii	**13v**	**Argentina**	**MT271880**	**This work**
Oudemansiella canarii	**14v**	**Argentina**	**MT272111**	**This work**
Oudemansiella canarii	**15v**	**Argentina**	**MT271881**	**This work**
Oudemansiella canarii	**22v**	**Argentina**	**MT271883**	**This work**
Oudemansiella canarii	**33v**	**Argentina**	**MT271882**	**This work**
Oudemansiella canarii	**36v**	**Argentina**	**MT272113**	**This work**
Oudemansiella canarii	**68v**	**Argentina**	**MT273085**	**This work**
Oudemansiella canarii	**3325**	**Argentina**	**MT272114**	**This work**
Oudemansiella canarii	**3326**	**Argentina**	**MT272115**	**This work**
Oudemansiella canarii	**3328**	**Argentina**	**MT272116**	**This work**
Oudemansiella canarii	**MA4**	**Argentina**	**MT272117**	**This work**
Oudemansiella canarii	**MA5**	**Argentina**	**MT272119**	**This work**
Oudemansiella canarii	**3060**	**Argentina**	**MT272112**	**This work**
Oudemansiella crassifolia	HKAS43500	China	AY665205	Zhang and Yang (unpubl.)
Oudemansiella crassifolia	S140148	China	MK886522	Hu et al. (unpubl.)
Oudemansiella cubensis	TENN51190	Costa Rica	GQ892794	Petersen and Hughes (2010)
Oudemansiella cubensis	TENN59771	Dominican Republic	GQ892791	Petersen and Hughes (2010)
Oudemansiella cubensis	TENN58954	Argentina	GQ892789	Petersen and Hughes (2010)

[1] Data in boldface correspond to the new sequences proposed in this work.

TABLE 1 CONT. List of sequences used in the phylogenetic analyses and their references.[1]

SPECIES	SPECIMEN VOUCHER	ORIGIN	GENBANK ACCESSION NUMBER	REFERENCE
Oudemansiella cubensis	TENN56534	Costa Rica	GQ892790	Petersen and Hughes (2010)
Oudemansiella cubensis	TENN49023	Puerto Rico	GQ892792	Petersen and Hughes (2010)
Oudemansiella cubensis	**MA2**	**Argentina**	**MT272118**	**This work**
Oudemansiella cubensis	**MA3**	**Argentina**	**MT271884**	**This work**
Oudemansiella cubensis (submitted as O. canarii)	RVPR100	Puerto Rico	AF321479	Mueller et al. (2001)
Oudemansiella cubensis (submitted as O. canarii)	RVPR33	Puerto Rico	AF321478	Mueller et al. (2001)
Oudemansiella cubensis (submitted as O. canarii)	RV96/35	Costa Rica	AF321477	Mueller et al. (2001)
Oudemansiella cubensis (submitted as O. canarii)	ECO-TA-HO 7876	Mexico	MF156259	Guillen-Navarro et al. (unpubl.)
Oudemansiella cubensis (submitted as O. canarii)	170854	Brazil	KJ620018	Vitola et al. (unpubl.)
Xerula pudens	178026	Sweden	AF321491	Mueller et al. (2001)
Xerula pudens	F. Popa1969	Germany	MF063189	Qin et al. (2018)

[1] Data in boldface correspond to the new sequences proposed in this work.

RESULTS

Taxonomy

Oudemansiella canarii (Jungh.) Höhnel, Siztgber. K. Akad. Wiss., Wien, math.- nat. Kl. 118: 276. 1909. TYPE: INDONESIA, Java. Mt. Halimun Nat. Park, loop trail from Cikiniki, ~1000 m elev., 8 Jan 1999, DE Desjadin, DED6886 (Neotype: SFSU, BO; Holotype: not present ex herb Junghuhn at L.). Fig. 1–2.

Basionym: *Agaricus canarii* Jungh. Praemissa in floram cryptogamicam Javae Insuae, Fasc. 1: 82. 1838.

Basidiomata gregarious in small cluster, xylophagous (Fig. 1A). *Pileus* up to 65 mm broad, hemispherical-convex when young, then plano-convex to plane (Fig. 1B). Surface markedly glutinous when young, embedded in translucent mucilage that disappears with maturity, hygrophanous dark brown (7F4-6) when young, clearing up when dehydrated, brownish orange (7C4-6), yellowish brown (5D-E4), finally yellow-white (4A2-3) on the disk to yellowish white (2A2) to white (2A1) at the margin at maturity. Glabrous or occasionally with scattered veil patches forming small, appressed, dark brown (7F4-6) to yellowish-brown (5D-E4) scales, in young specimens embedded in the glutinous surface. Margin smooth with tiny fragments of veil when young. *Context* thin, up to 2 mm, white (2A1) to ashen or pale (2A2, "yellowish-white"), odor farinaceus that accentuates and turns sweet when dry. *Lamellae* adnate with a decurrent tooth when young (Fig. 1C), then adnate to adnexed, ventricose to broadly ventricose, white (1A1) when young, and sometimes yellowish white (2A2) at maturity with a fimbriate margin under a lens, lamellulae of one or two orders. *Stipe* central (Fig. 1A,D), 15–80 × 4–9 mm, straight or curved, cylindrical, tapering to the apex, with a bulbose to subbulbose base, white (2A1) to pale (2A2), concolorous with the lamellae, solid, tough, fibrous, surface dry, longitudinally fibrillose, with small remnant fragments of veil toward the apex, which disappear at maturity. *Basidiome development* hemiangiocarpic (Fig. 1D). *Spore print* white (2A1) to pale (2A2) and extremely abundant (Fig. 1E). *Basidiospores* 16.79–19.96 × 17.69–21.99 μm, x = 18.11 × 19.13 μm, Qx = 1.00–1.16, Qx = 1.05, n = 25; globose to subglobose, smooth, nonamyloid, hyaline, thin walled, with multigranular contents and abundant guttules (Fig. 2A). *Basidia* 40–69 × 22–35 μm, clavate, 4-spored, hyaline, thick walled (Fig. 2B). *Pleurocystidia* 146–246 × 25.75—38.00 μm, lageniform, formed by a globose to subglobose base, and a narrower, cylindrical, long neck, thick walled at the base, thin walled toward the apex, homogeneous contents (Fig. 2C). *Cheilocystidia* 9–28 × 44–84 μm, clavate to broadly clavate, often pedicelate. *Hymenophoral trama* subregular, consisting of 4- to 35-μm-diam., thin-walled hyphae, nonamyloid. *Pileipellis* formed by two tissue types: (1) underneath, a more or less hymeniform layer, formed by spindle cells, cylindrical to

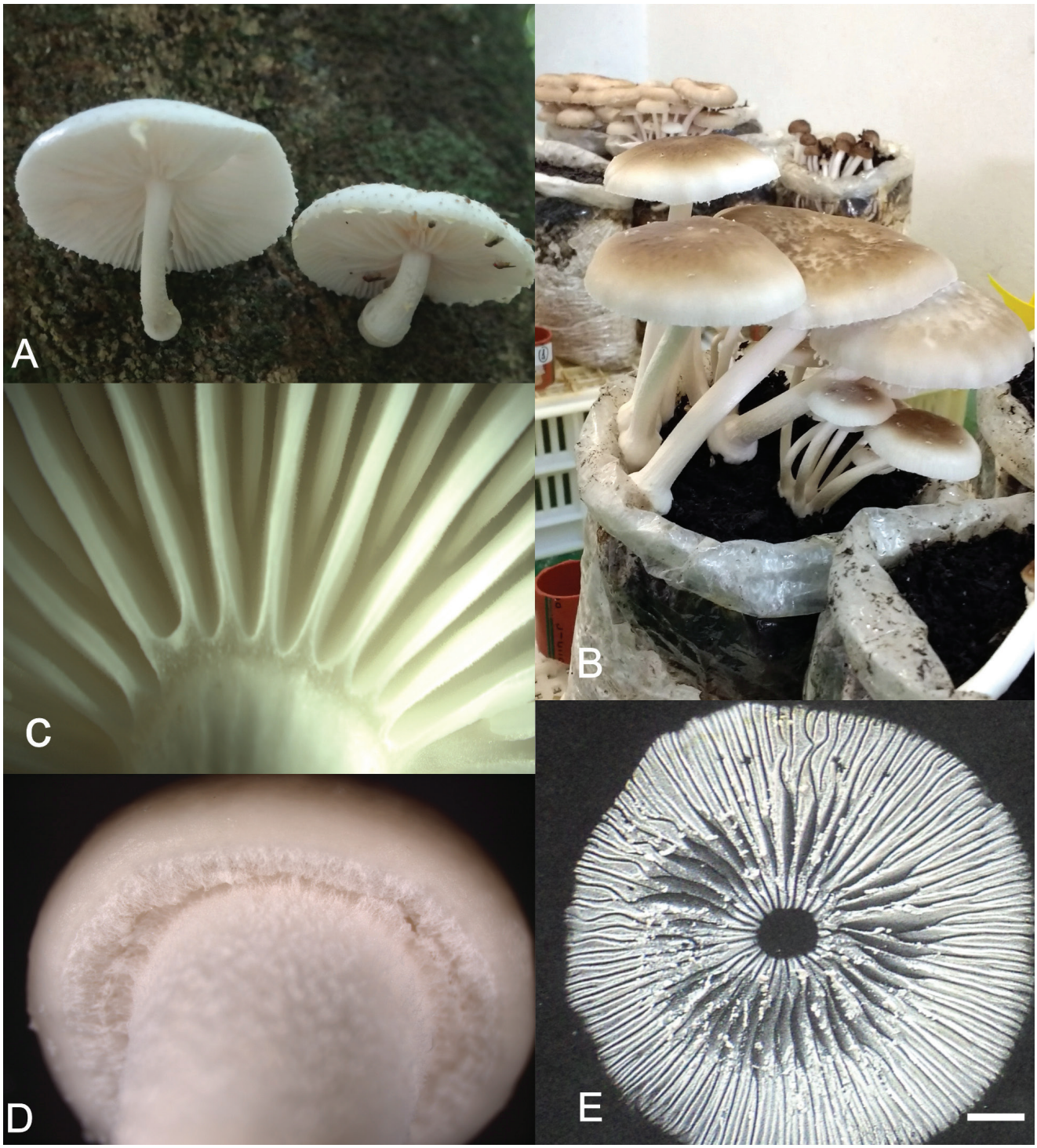

FIGURE 1. Basidiomes of *O. canarii*. **A**, general aspect; **B**, specimens obtained in culture; **C**, lamellae; **D**, partial veil; **E**, spore print. Bars = 1.3 cm (A); 2.6 cm (B); 0.15 cm (C); 0.3 cm (D); 0.92 cm (E).

thinly claviform, softly pigmented olive, 67–101 × 9.0–13.5 μm, thin walled and with homogeneous content; (2) on the surface, an ixotrichoderm formed by hyphae 4.7–6.75 μm in diam. and 90–202 μm in length, erect, occasionally branched and intermixed (Fig. 2D). Scales formed by erumpent chains cells 19.28–41.14 × 28.28–51.42 μm, broadly fusoid (basally) to subglobose or globose (apically) (Fig. 2E). *Stipitipellis* a cutis made up of smooth and parallel, 6.65- to 12.75-μm-diam. hyphae. *Caulocystidia* clavate, hyaline, thick walled (1.5 thick). *Clamp connections* present. Basidiomes obtained in culture showed a hemiangiocarpic development (Fig. 1D). Pileus 10–140 mm, convex when young and convex to plane when mature and without floccules. The other macro- and microscopic characteristics

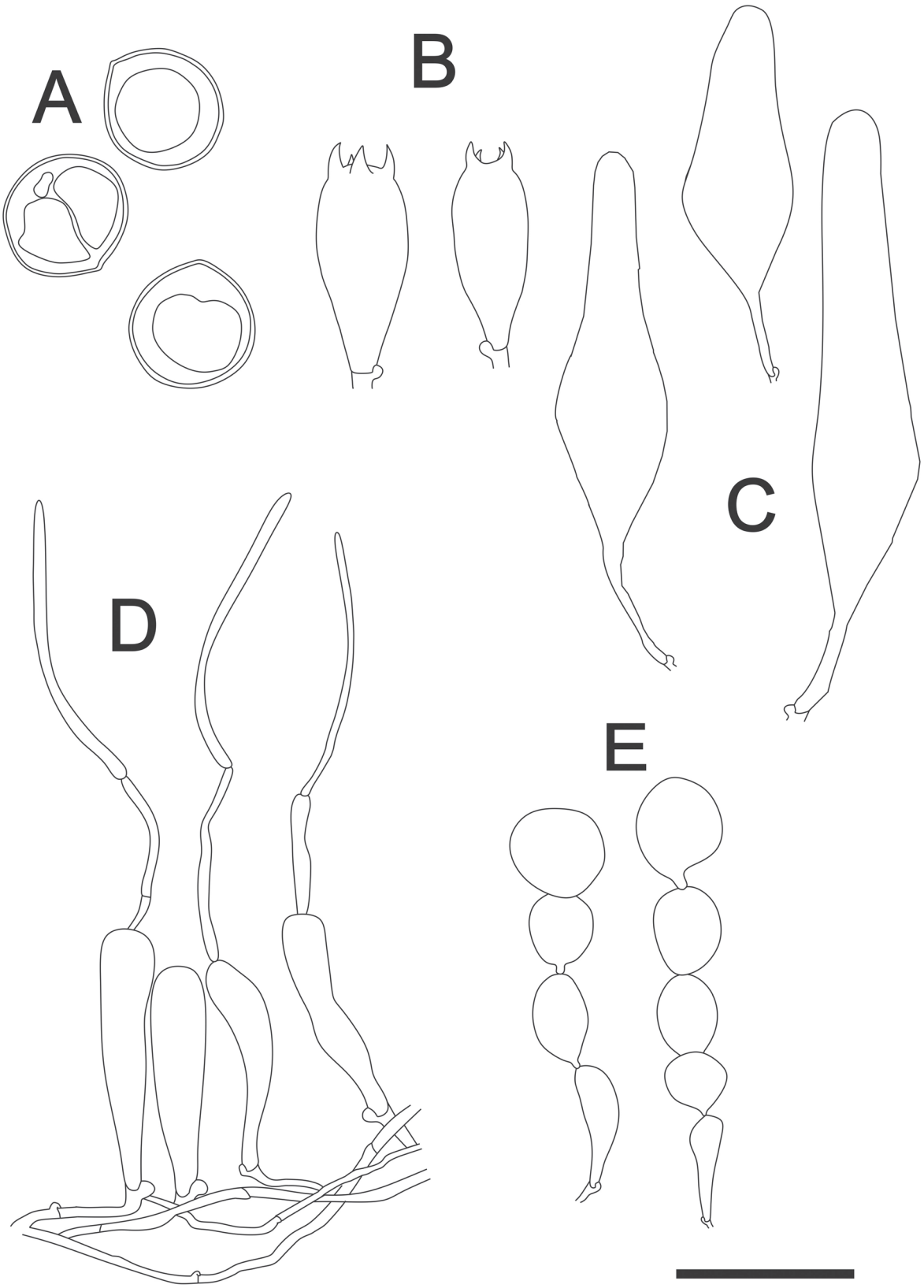

FIGURE 2. Microscopic characters of *O. canarii*. **A**, basidiospores; **B**, basidia; **C**, pleurocystidia; **D**, pileipellis elements; **E**, scale elements. Bars = 30 μm (A); 50 μm (B, E); 70μm (C, D).

agree with the descriptions of the basidiomata found in nature mentioned above.

Distribution: Southern Asia (Japan, Malaysia, Papua New Guinea, Solomon Islands) and tropical America (Argentina, Mexico, United States) (Petersen and Hughes, 2010). In Argentina, known in Buenos Aires, Corrientes, Misiones, and Tucumán Provinces.

Ecology: growing on live, decaying, or dead logs of gymnosperms and angiosperms. Solitary or forming small groups of 1–5 basidiomes.

Additional specimens examined: ARGENTINA, Buenos Aires, Reserva Ecológica Costanera Norte, 31°38'10"S, 60°40'31"W, 4/08/2016, J. Aliaga MA4 (CTES). Corrientes City, Province of Corrientes, 27°27'29"S, 58°49'17"W, 26/03/2017, N. Niveiro MA5 (CTES). Misiones, General Manuel Belgrano, Reserva Natural Estricta San Antonio, 26°03'00"S, 53°43'00"W, 23/03/2017, M. Alberti 22v, 33v, 36v (CTES). *Ib*. INTA, Campo Anexo General Manuel Belgrano, in *Araucaria angustifolia* forest, 26°38'29"S, 53°46'14"W, 23/03/2017, M. Alberti 43v (CTES). Guaraní, Reserva Privada Yasi Yateré, 27°13'26"S, 54°00'47"W, 809 m.s.n.m., 23/03/2017, M. Alberti 60v (CTES). Iguazú, Parque Nacional Iguazú, surroundings of CIES, 26°38'29"S, 53°46'14"W, 210 m.s.n.m., 22/03/2017, Ramírez 13v, 14v, 15v, 17v (CTES), *Ib*. Timbó station, N. Ramírez 16v (CTES). San Ignacio, Parque Provincial Teyú Cuaré, 27°33'43.3"S, 55°35'24.2"W, 18/10/2017, N. Niveiro MA3325, MA3326, MA3327, MA3328 (CTES). Santa Ana, Parque Provincial Cañadon de Profundidad, 27°33'23.1"S, 55°42'21.1"W, 16/10/2017, N. Niveiro MA3325 (CTES). San Pedro, Parque Provincial Moconá, Chachi trail, 27°08'35"S, 53°53'12"W, 25/03/2017, M. Alberti 68v (CTES). *Ib*. 26/03/2017, M. Alberti 93v (CTES). Reserva Privada Yagurundí, 26°41'40"S, 54°15'52"W, 23/03/2017, M. Alberti 57v (CTES).

Oudemansiella canarii is characterized by its white pileus with scattered brownish veil patches (but absent in basidiomes obtained from culture), surface markedly glutinose and darker (brownish) when young, and pileipellis formed by a superficial ixotricodermium composed by expanded cells mounted on a more or less hymeniform layer, formed by spindle cells, cylindrical to thinly claviform, olive pigmented. Pleurocystidia numerous and of large size (Petersen and Hughes, 2010). One of the most similar species that shares the pileipellis structure is *O. exannulata* (Cleland & Cheel) R.H. Petersen from eastern Australia. However, it differs from *O. canarii* by the absence of floccules in the pileus surface, and its larger pileus, up to 100 mm diam. (Petersen and Hughes, 2010). The macro- and microscopical characters of the Argentine specimens of *O. canarii* coincide with the description previously made by Corner (1994) and Petersen and Hughes (2010), although with minor differences. Petersen and Hughes (2010) describe larger basidiospores (up to 31 × 33 μm), with 1–4 sterigmated basidia and basidiomes usually with pinkish colorations. In this work, maximum measurements of basidiospores were 19.96 × 21.99 μm; the observed basidia were 4-sterigmated in all cases, and no pinkish colorations have been seen in any examined basidiomata.

Oudemansiella cubensis (Berk. & M. A. Curtis) R. H. Petersen, Nova Hedwigia, Beih. 137: 283. 2010. TYPE: CUBA. July 1857, C. Wright s.n. (FH). Fig. 3–4.

Basionym: *Agaricus (Amanita) cubensis* Berk. & M.A. Curtis. J. Linn. Soc. London 10: 282. 1869.

Synonyms: *Agaricus (Tricholoma) platensis* Speg. Anales Soc. Ci. Argent. 9(4): 161. 1880.

Oudemansia platensis (Speg.) Speg. Anales Soc. Ci. Argent. 10(6): 280. 1880. [*nom. gen. illeg*., Art. 53 ICNB].

Oudemansiella platensis (Speg.) Speg. Anales Soc. Ci. Argent. 12(1): 24. 1881.

Psalliota platensis (Speg.) Herter. Estudios Botanicos Region Uruguaya, III *Florula Uruguayensis Plantae Avasculares* (Montevideo): 43. 1933.

Basidiomata gregarious in small cluster, xylophagous (Fig. 3A). *Pileus* up to 80 mm broad, hemispherical-convex when young, then plano-convex to plane; glutinous when young, quickly turning viscid, and finally dry at maturity (in dry environmental conditions) (Fig. 3B, C); hygrophanous, brown (6E5-8) when young, becoming lighter brown when dehydrated (6D4), grayish brown (6D3), finally orange-white (6A2) in the disk to white (6A1) at the margin at maturity. Glabrous or occasionally with scattered veil patches forming appressed or recurved scales, concentrated in the center, more dispersed toward the margins, brown (7E4-6) to dark brown (7F4-6), varying considerably in size, forming from large patches (5–20 mm) to small and very abundant scales (1–3 mm) (Fig. 3D). Margin smooth with tiny fragments of veil when young. *Context* thin, up to 2 mm, white (2A1) to ashen or pale (2A2, "yellowish-white"), odor farinaceus, which accentuates and turns sweet when dry. *Lamellae* adnate when young, then adnexed, ventricose, subdistant to distant (Fig. 3E), white (1A1) when young, turning yellowish white (1A2) to pale yellow (1A3) at maturity, lamellulae of one or two orders. *Stipe* central, 17–80 × 4–8 mm, straight or curved, cylindrical, slightly tapering to the apex, with a subbulbose base, (Fig. 3A–F), white (2A1) to pale (2A2), concolorous with the lamellae, solid, tough, fibrous, surface dry, longitudinally fibrillose, with small remnant veil fragments toward the apex, which disappear at maturity. *Basidiome development* hemiangiocarpic (Fig. 3F). *Spore-print* white to pale and extremely abundant (Fig. 3G). *Basidiospores* 14.92–20.47 × 17.08–20.72 μm; x = 17.7 × 18.38 μm, Q = 1.00–1.20, Qx = 1.03, n = 25; globose to subglobose, multigranular contents and abundant guttules, nonamyloid, hyaline, smooth, thick walled (Fig. 4A). *Basidia* 45–70 × 23–36 μm, clavate, 4-spored, hyaline, thick walled (Fig. 4B). *Pleurocystidia* 140.0–190.3 × 25.38–39.24 μm, lageniform, formed by a globose to subglobose base, and a narrower, cylindrical, long neck, thick walled on the base, thin walled to the apex, homogeneous contents (Fig. 4C). *Cheilocystidia* of two types: (1) 13–28 × 39–83 μm, fusiform to broadly clavate thin walled; and (2) 12–40 × 61–150 μm, very broadly fusiform, thick walled. *Hymenophoral trama* subregular, consisting of 5- to 38-μm-diam., thin-walled hyphae, nonamyloid. *Pileipellis* formed by two tissue types: (1) underneath, a more or less hymeniform layer, formed by claviform cells,

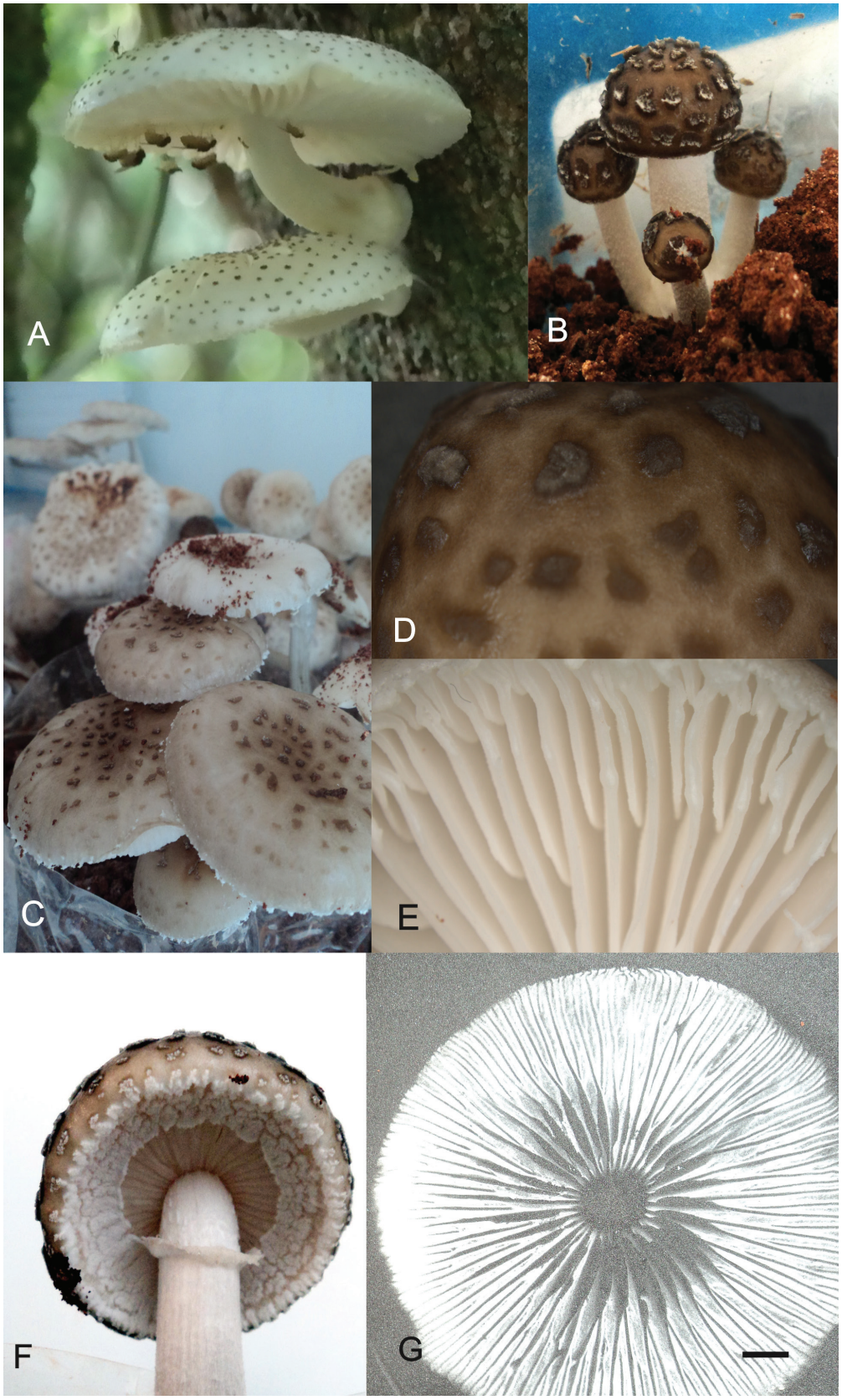

FIGURE 3. Basidiomes of *O. cubensis*. **A**, general aspect; **B**, primordia; **C**, specimens obtained in culture; **D**, detail of pileus scales; **E**, lamellae; **F**, partial veil; **G**, spore print. Bars = 0.75 cm (A); 1 cm (B, G); 1.75 cm (C); 0.3 cm (D, E); 0.43 cm (F).

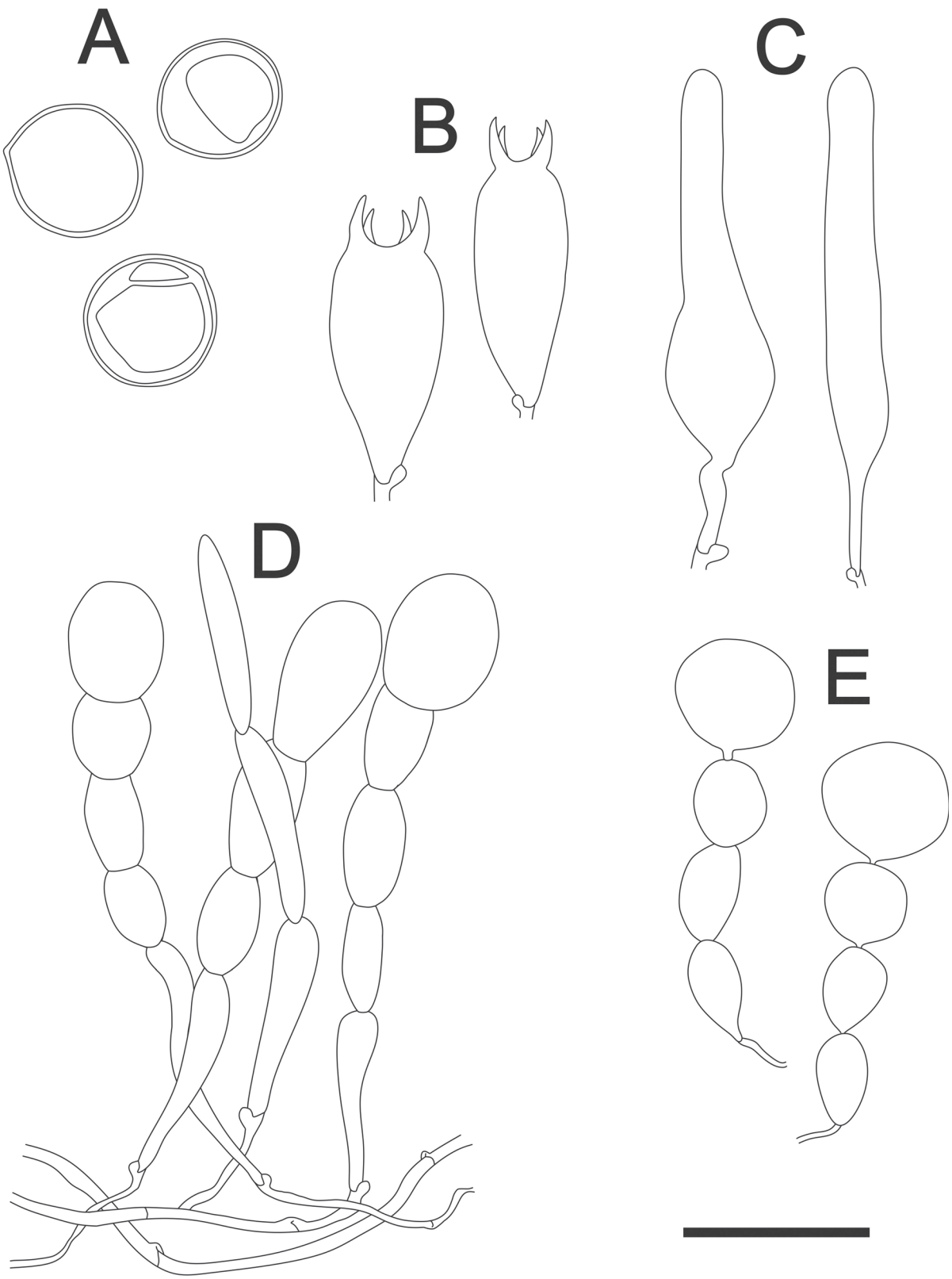

FIGURE 4. Microscopic characters of *O. cubensis*. **A**, basidiospores; **B**, basidia; **C**, pleurocystidia; **D**, pileipellis elements; **E**, scale elements. Bars = 25 μm (A, D); 50 μm (B); 70 μm (C); 80 μm (E).

20–80 × 8–10 μm, softly pigmented olive; and (2) the most superficial layer, formed by a polycystoderm, composed by chains of progressively inflated cells of 6–25 μm diam. (Fig. 4D). Scales formed by erumpent chains cells of 20–50 μm diam., broadly fusoid (basally) to subglobose or globose (apically) (Fig. 4E). *Stipitipellis* in a cutis made up of smooth and parallel, 6.68- to 14.63 μm diam. hyphae. *Caulocystidia* fusiform, hyaline, thick walled (1.4 thick). *Clamp connections* present. Basidiomes obtained by culture showed a hemiangiocarpic development (Fig. 2G). Pileus 15–99 mm diam., convex when young and convex to plane when mature with floccules present. The other macro- and microscopic characteristics coincide with the descriptions of the basidiomes found in nature mentioned above.

Distribution: Neotropical, from southern U.S.A. to northern Argentina: Argentina, Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, United States (Petersen and Hughes, 2010). In Argentina, known to Buenos Aires, Corrientes, and Misiones Provinces.

Ecology: growing on live, decaying, or dead logs of gymnosperms and angiosperms. Solitary or forming small groups of 1–4 basidiomes.

Additional specimens examined: ARGENTINA, Buenos Aires, Reserva Natural Municipal Santa Catalina, 34°46'46"S, 58°25'36"W, 27/07/2016, S. Ponce MA3 (CTES). Corrientes, San Cosme, Paso de la Patria, Estancia Las Lagunas, 27°22'15"S, 58°32''54"W, 21/04/2016, N. Niveiro 2930/MA2 (CTES).

Petersen and Hughes (2010) define six characters that separate *O. cubensis* from similar taxa: (1) smaller spores, (2) longer and slender basidia, (3) proximal swelling of pleurocystidia less than in other species, (4) immature pileus color dark brown rather than pallid gray, pallid yellow, or pinkish, (5) conspicuous cheilocystidia, and (6) pileipellis composed of chains of cells. However, spore size, basidia, cheilocystidia, and pleurocystidia are not completely conclusive characters because of overlapping measurements in many cases. The most suitable morphological character to differentiate this species is the white to pallid-brown pileus with scattered veil patches and a pileipellis composed of a superficial polycystoderm, formed by a chain of progressively inflated cells, and underneath a more or less hymeniform layer, formed by claviform cells softly pigmented olive. *Oudemansiella apalosarca* (Berk. & Broome) Höhn. from Sri Lanka and Oceania (as *O. australis* G. Stev. & G.M. Taylor) differs from *O. cubensis* in its pileus without floccules, dry surface, and larger spores (22–25 × 20–24 μm) (Petersen and Hughes, 2010). Descriptions of Argentine specimens of *O. cubensis* are in agreement with Petersen and Hughes (2010) and with descriptions made by Corner (1994) for *O. platensis*. In this work, basidiospores with a maximum size of 20.47 × 20.72 μm are reported, which are smaller than maximum sizes described by Petersen and Hughes (2010) (22.5 × 22 μm), and by Corner (1994) (15 × 25 μm). Also, these authors reported pleurocystidia greater than 250 μm long, whereas the maximum pleurocystidia length described in this work is 190.3 μm. Concerning basidia, Petersen and Hughes (2010) reported 2- to 4-sterigmated basidia, but in this work only 4-sterigmated basidia are described.

Phylogenetic Analysis

The original dataset consisted of 36 taxa and 682 positions. The resulting tree generated in BI agreed with the ML analysis. The species studied in this work were grouped in three well-defined clades: Clade *O. canarii* (1/91); Clade *O. cubensis* (1/84); and Clade *O. apalosarca* (1/90), in which *Oudemansiella* sequences named as *O. crassifolia* and *O. australis* from the Asian continent were grouped (both synonymized with *O. apalosarca* by Petersen and Hughes, 2010) (Fig. 5).

Discussion

Taking into consideration the works published by Petersen and Hughes (2010) and Niveiro and Albertó (2012), seven species of *Oudemansiella* were described for Argentina, some of which have not been revised yet. Species such as *O. aculeata* Raith. and *O. haasiana* Raith. have been poorly described and the type materials are presumably lost. After several field trips to collect mushrooms from the northern and central parts of the country, where we obtained various specimens belonging to the genus *Oudemansiella*, we found it was very difficult to determine them to specific level because of the great variation in the morphological characters that these collections presented. On the basis of morphological characters, we initially thought that the number of species present in the area was higher than we had previously assumed. Only the combined study of morphology and molecular characters allowed us to conclude that only two species were present in the area. Moreover, it was not possible to differentiate them at the macroscopic level, it being necessary to perform micromorphological studies, culture studies, and phylogenetic analyses to be able to identify them with certainty. At the microscopic level, they can be differentiated mainly by pileipellis structure, specifically its superficial layer: *O. canarii* has an ixotricodermium composed of expanded cells, whereas *O. cubensis* has a superficial polycystoderm, formed by a chain of progressively inflated cells. Some authors consider that it could be variable at different stages of development (Dörfelt, 1981; Corner, 1994; Yang et al., 2009).

Another morphological difference, noticed after repeated observations of basidiomes obtained in culture, is the consistency of the primordia, *O. canarii* primordia being more glutinous than in *O. cubensis*. However, this can vary greatly with environmental conditions, as well as the perception of the observer. In addition, when these species are cultivated under controlled conditions, they can be recognized by the presence of scales on the pileus in *O. cubensis*, which are absent in *O. canarii*. (Fig. 1C, 3C–E). Regarding basidiomes development, Corner (1994) pointed out that the development of species of the genus was gymnocarpic. The production of basidiomes in culture on substrate allowed us to observe the development of many specimens of both species. We can affirm that *O. canarii* and *O. cubensis* have a hemiangiocarpic development. According to Alexopoulos and Mims (1985), the hemiangiocarpic

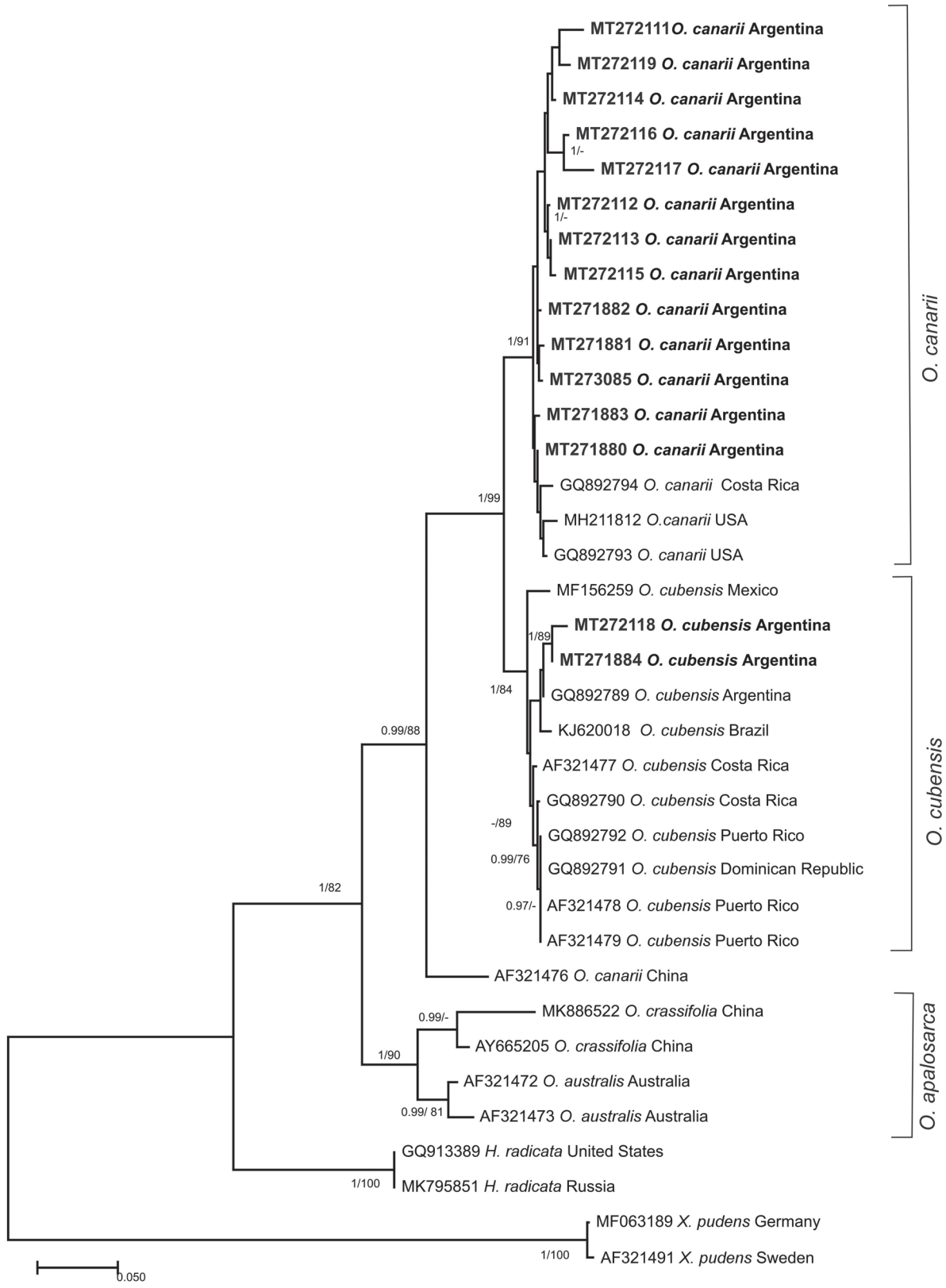

FIGURE 5. Molecular phylogeny inferred by Bayesian inference based on dataset of ITS sequences. Bayesian posterior probabilities above 0.9 (BPP ≥ 0.9) and bootstrap values above 70% (BS ≥ 70) are shown. Sequences obtained in this study are in bold.

development is characterized by the fact that during the first phases of basidiomal development, the hymenium is covered by the partial veil, which is a tissue that connects the margin of the pileus with the stipes. When the pileus expands, the partial veil tears and may or may not form a permanent ring. The phylogenetic analysis carried out from the ITS sequences allowed us to separate them into three well-supported clades (Fig. 5). Our sequences were grouped into two different clades: *O. canarii* and *O. cubensis*. These results agree with those obtained by Petersen and Hughes (2010), who also phylogenetically separated both species on the basis of ITS sequences. We assert that ITS sequences are optimal markers for phylogenetic molecular identification of these two species, which are at present the only ones found in Argentina belonging to genus *Oudemansiella* sensu stricto.

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