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Leucoagaricus sabinae (Agaricaceae), a new species from the Dominican Republic

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Abstract: *Leucoagaricus sabinae* is proposed as a new species based on material collected in the Dominican Republic. This taxon is characterized macroscopically by the relatively small size, the dull gray-pink pileus, yellow gills immediately turning pink when touched and the ever-present white velar residues on the margin of the pileus. Microscopically the vaguely metachromatic to non-metachromatic spores, the scarce, cylindrical-flexuous cheilocystidia and the piles covering elements with parietal pigment are diagnostic. Based on molecular data (nrLSU, nrITS) this species belongs in the *Leucoagaricus/Leucocoprinus* clade of the Agaricaceae.

Key words: Lepiotaceae fungi, Agaricales, Caribbean, phylogeny.

Introduction: Among the lepiotaceous fungi in the Agaricaceae the *Leucoagaricus*/*Leucocoprinus* clade is particularly diverse in the tropics, with a lot of species awaiting discovery and formal description (Vellinga 2004a, b). The major mycofloristic studies of this group in the American tropical region date back to before the molecular era (Dennis 1952, 1961, 1970; Pegler 1983), and most of the molecular data currently available for this area come from the studies on ant-associated fungi and their free-living relatives (Vo et al. 2009) that do not focus on taxonomy.

Over the past ten years, one of the authors (C.A.) has collected and studied fungi in the Dominican Republic. More than 300 species of macrofungi have been recorded, and almost all voucher specimens are deposited in the herbarium of the Jardín Botánico Nacional Dr. Rafael Ma. Moscoso (Santo Domingo, Dominican Republic). Approximately 20% of the collections represent lepiotaceous fungi of different genera (*Chlorophyllum*, *Cystolepiota*, *Lepiota*, *Leucoagaricus*, *Leucocoprinus*) and are currently being studied and sequenced.

This contribution is focused on the description of a new species in the *Leucoagaricus*/*Leucocoprinus* clade characterized by small size, the dull gray-pink pileus, yellow gills immediately turning pink when touched, and the ever-present white velar residue on the margin of the pileus. The phylogenetic position of the species, based on molecular data, is also analyzed and discussed.

Materials and Methods: The basidiomata were photographed fresh, in habitat, using a digital camera Nikon coolpix 8400 and subsequently dried. Notes on fugacious characters (e.g. odor, taste) were made before drying. Collections were studied using standard procedures for morphological examination of lepiotaceous fungi (Candusso & Lanzoni 1990; Vellinga 2001). Microscopical preparations were mounted in 5% KOH, Congo Red, Melzer's

Reagent and Cresyl Blue. Microscopical observations were made with a Meiji optical microscope. Descriptive terms for morphological features follow Vellinga (1988, 2001). Color codes are from Munsell Soil Color Charts (Munsell Color 2009). The following abbreviations are used in the descriptions: avl for average length, avw for average width, Q for quotient of length and width and avQ for average quotient.

Protocols for DNA extraction, PCR and sequencing. Genomic DNA was isolated from 10 mg dried herbarium specimens with the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for ITS amplification (White et al. 1990, Gardes and Bruns 1993) and primers LR0R/LR6 (Vilgalys and Hester 1990, www.botany.duke.edu/fungi/mycolab) for LSU rDNA amplification. PCR was performed in a PE9700 thermal-cycler (Perkin-Elmer, Applied Biosystems) following the profiles given by Vizzini et al. (2011) for ITS and LSU. The PCR products were purified with the AMPure XP kit (Beckman) and sequenced by MACROGEN (Seoul, Republic of Korea). Sequence assembly and editing were performed with Geneious 5.3 (Drummond et al. 2010). The sequences are deposited in GenBank and accession numbers are given with the collection information. The newly generated nrLSU sequences were included in a general Agaricaceae dataset following the sampling of Vellinga et al. (2011). A very broad nrITS dataset with 323 sequences of *Leucoagaricus*/*Leucocoprinus* was also constructed to narrow down the closest relatives of our sequences (data not shown). Based on the results of these preliminary analyses and taking into account the results of BLAST searches for our sequences, a smaller nrITS dataset with representatives of the *Leucoagaricus* / *Leucocoprinus* clade was constructed and analyzed. For all datasets sequences were aligned using MAFFT version 7.110 (Katoh & Toh 2008) and alignments were manually corrected using

MacClade 4.08 (Maddison & Maddison 2002). A Maximum Likelihood analysis was run in the RAxML servers, version 7.2.8 (Stamatakis & et al. 2008), under a GTR model with one hundred rapid bootstrap replicates. Two species of *Macrolepiota* (*M. procera* and *M. rhodosperma*) were used as outgroup taxa for the ITS analysis and *Pseudobaespora pyrifer*a for the LSU analysis.

Results

Leucoagaricus sabiniae Angelini, Justo & Vizzini, sp. nov. (Figures 1–5)

Mycobank # 810631

Holotypus. Dominican Republic. Puerto Plata, Sosúa, on man-made litter in a deciduous forest, 30 November 2013, ANGE 306 (JBSD), nrITS KM983667, nrLSU KM983669.

Diagnosis. Characterized macroscopically by the relatively small size for the genus, the dull gray-pink pileus, yellow gills immediately turning pink when touched and the persistent white velar residues on the margin of the pileus. Microscopically the most distinctive characters are the vaguely metachromatic to non-metachromatic spores, the scarce, cylindrical-flexuous cheilocystidia and the pileus covering elements with incrusting, parietal pigment are diagnostic.

Etymology. The epithet is a dedication to Sabine Woght, for her logistic support of the field-work that has made possible the discovery of this new taxon.

Pileus 10–25 mm in diameter, convex when young, then flat without any hint of umbo; surface gray-pinkish, pinkish-brown if handled (Munsell 10R 6/1-6/4, 5/1-5/4), with a matte aspect, not hygrophanous, covered with whitish squamules and fibrils; margin not striate or only slightly in older specimens, with copious,

appendiculate, commonly triangular, white veil remnants. Lamellae free, rather sparse, ventricose, with numerous lamellulae, usually between 1 and 3 between lamella, white at first, then yellow, immediately turning pink when touched. Stipe 20–50 × 3–5 mm, cylindrical, a bit curved or flexuous in profile; surface white, turning pink-reddish when touched; in young specimens the surface smooth in the upper part and covered with veil floccules in the lower part; in older specimens the surface is almost completely smooth, sometimes with fugacious ring-like remnants; with thin, white rhizomorphs at base, that turn immediately pink when touched; fistulose. Context white to pink on pileus, and white to pink-reddish on stipe. Odor strong and unpleasant, like burnt rubber and/or fish. Taste similar to smell, bitter.

Spores 5.5–8 × 3.7–4.5 µm, avl × avw = 6.4 × 4.0, Q = 1.38–1.95, avQ = 1.62, ellipsoid to oblong, some phaseoliform, thick-walled, with evident and eccentric apiculus, without germ pore under an optical microscope, slightly dextrinoid, not or only vaguely metachromatic, acetic-ammonium reaction negative. Basidia tetra-sterigmate, clavate or cylindrical with a square base, 18–30 × 6.5–10 µm. Cheilocystidia 13.5–21 × 3–6 µm, cylindrical-capitulated, some with flexuous outline, infrequent and unevenly distributed along the lamella edge. Pleurocystidia absent. Pileus covering an intricate cutis, with septate, cylindrical, hyphae, some diverticulate; terminal elements cylindrical, up to 20 × 7.5 µm; hyphae with vacuolar grayish pigment and brown, incrusting parietal pigment, often appearing as a zebra-like pattern on the surface. Stipitipellis a cutis, with septate hyaline hyphae, 2–8.5 µm in diameter; at apex with some diverticulate, bifurcated hairs. Clamp-connections absent in all tissues.

Habit, habitat and distribution. Solitary or gregarious, on deciduous forest litter. So far, this species has been commonly found every year in the rainy autumn and winter period (November

to January) in the coastal area around Sosúa-Puerto Plata. For detailed botanical characteristics of the collection areas see Angelini & Losi (2013, 2014).

Additional collections examined.

Dominican Republic: Puerto Plata, Sosúa, on man-made litter in a deciduous forest, 21 November 2013, ANGE 305, nrITS KM983666, nrLSU KM983668; Puerto Plata, Sosúa, on man-made litter in a deciduous forest, 18 December 2013, ANGE 323; Puerto Plata, Sosúa, on man-made litter in a deciduous forest, 28 November 2014, ANGE 470.

Discussion: *Leucoagaricus sabinae* is characterized macroscopically by a unique combination of features: relatively small size, the dull gray-pink pileus, yellow gills immediately turning pink when touched and the white velar residues on the margin of the pileus. The new species shares the reddening of the gills and context after manipulation with species of *Leucoagaricus* section *Piloselli* Singer (Bon 1993, Vellinga 2010), but differs in having a context not greening in ammonia, scarce and cylindrical-flexuose cheilocystidia and the elements of the pileus covering elements with parietal, incrusting pigment.

A thorough literature search including monographic treatments and papers on lepiotoid fungi (e.g., Morgan 1906; Murrill 1914; Kauffman 1924; Beeli 1932, 1936; Dennis 1952, 1970; Smith 1954, 1966; Aberdeen 1962; Pegler 1968, 1972, 1975, 1977, 1983, 1986, 1987a, b, 1990; Pegler and Rayner 1969; Heinemann 1973, 1977, 1979, 1980; Wasser 1980; Natarajan and Manjula 1982; Enderle and Krieglsteiner 1989; Sundberg 1989; Candusso and Lanzoni 1990; Guzmán and Guzmán-Dávalos 1992; Bizio et al. 1993; Bon 1993; Akers 1997; Vellinga and Huijser 1999; Vellinga 2001, 2010a; Montoya and Bandala 2005; Wang and Yang 2005; Zelený 2006; Kosakyan et al. 2008; Kumar and Manimohan

2009; Albuquerque et al. 2010; Gierczyk et al. 2011; Liang and Yang 2011; Ferreira and Cortez 2012; Razaq et al. 2012; Kaur et al. 2013; Nawaz et al. 2013), highlighted the unique nature of this taxon: its features do not fit with the description of any published species.

The molecular analyses of the nrLSU (Fig. 6) and nrITS sequences (Fig. 7) also corroborated the uniqueness of *L. sabinae*. Molecularly the only available nrITS sequences that come close to *L. sabinae* are those generated from a Costa Rican collection labeled as *Lepiota flammeotincta* (U85331) and an undescribed *Leucoagaricus* species also from the Dominican Republic (*Leucoagaricus* sp. CA19; KM983716). Vellinga (2010) indicated that the sequence U85331 does not represent the true *Lepiota flammeotincta* Kauffman, but nothing is known about the actual characteristics of the collection from which the sequence was generated. The undescribed *Leucoagaricus* CA19, was also collected and studied by us, and differs from *L. sabinae*, in the yellow colors of the pileus and the clavate cheilocystidia. In more extensive nrITS analyses of the *Leucoagaricus/Leucocoprinus* clade (data not shown), the clade containing *L. sabinae*, “*Lepiota flammeotincta*” (U85331) and *Leucoagaricus* sp. CA19 (KM983716), consistently appears in a more inclusive group with species like *Leucocoprinus heinemannii* Migl., *Leucoagaricus dacrytus* Vellinga, *Leucoagaricus melanotrichus* (Malençon & Bertault) Trimbach and the species complex around *Lepiota atrodisca* Zeller.

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Fig. 1. *Leucoagaricus sabinae*: a), b), c) fresh specimens in habitat; d) in section. (Photos. C. Angelini)



Fig. 2. *Leucoagaricus sabinae*: a), b), c) fresh specimens in habitat. (Photos. C. Angelini)

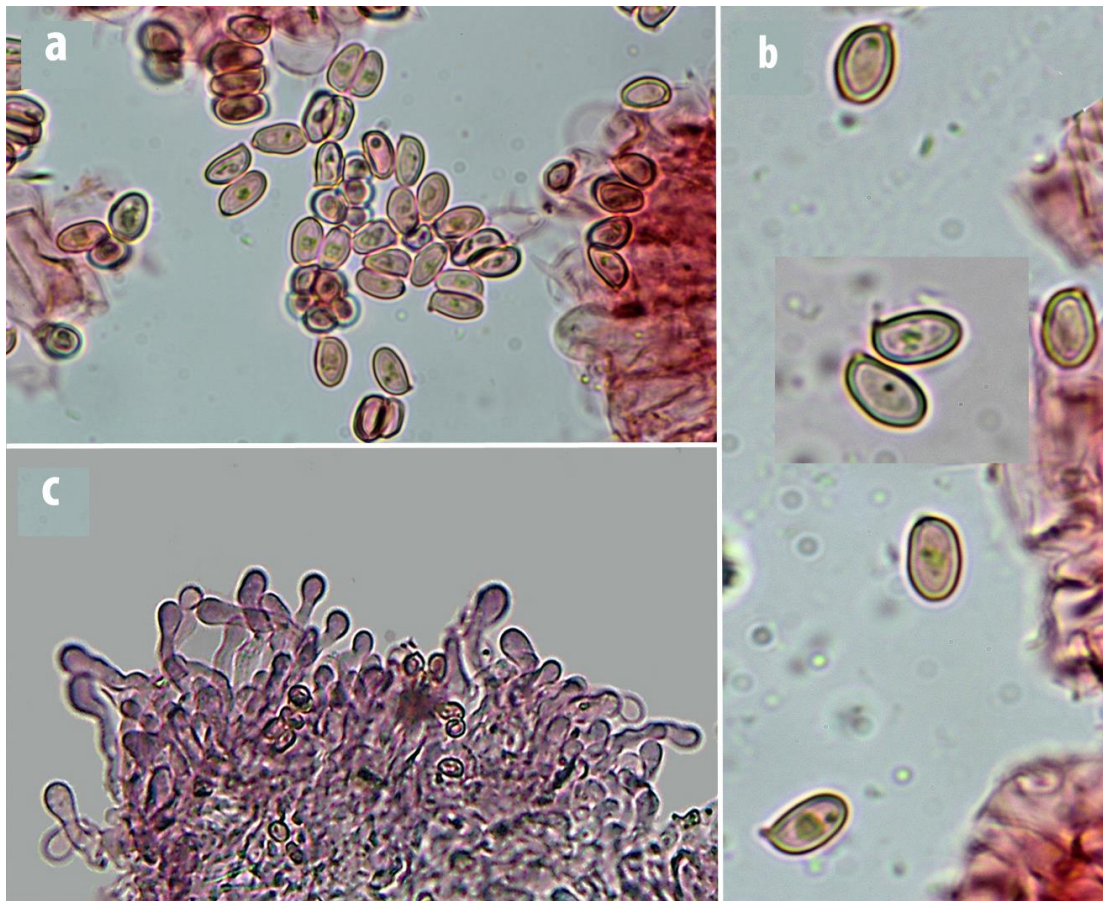


Fig. 3. *Leucoagaricus sabinae*: a), b), spores in congo red; c) cheilocystidia (Photos a, b, Claudio Angelini. Photo c, Alberto Bizzi)

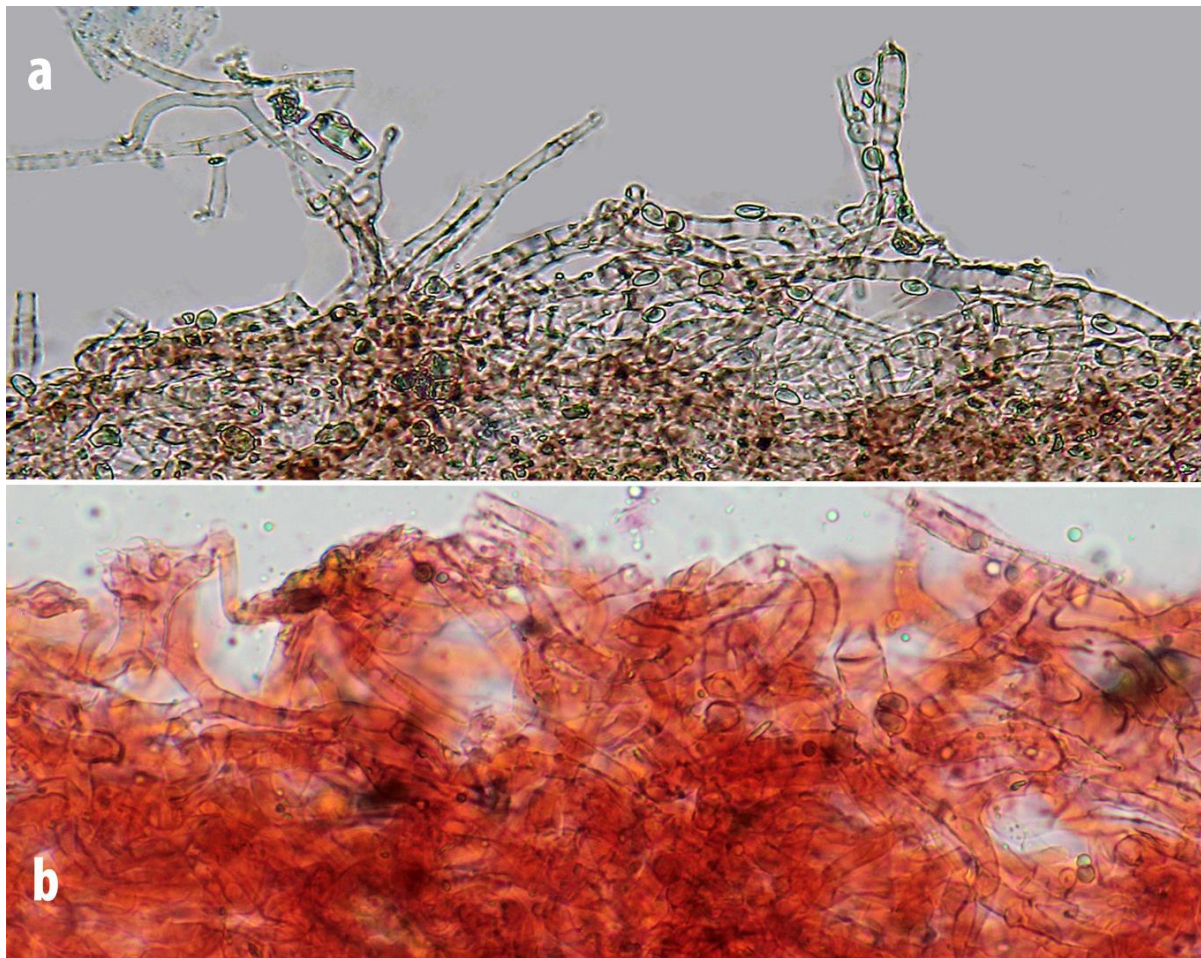


Fig. 4. *Leucoagaricus sabinae*: pileus covering a) in water; b) in congo red (Photos. Claudio Angelini)

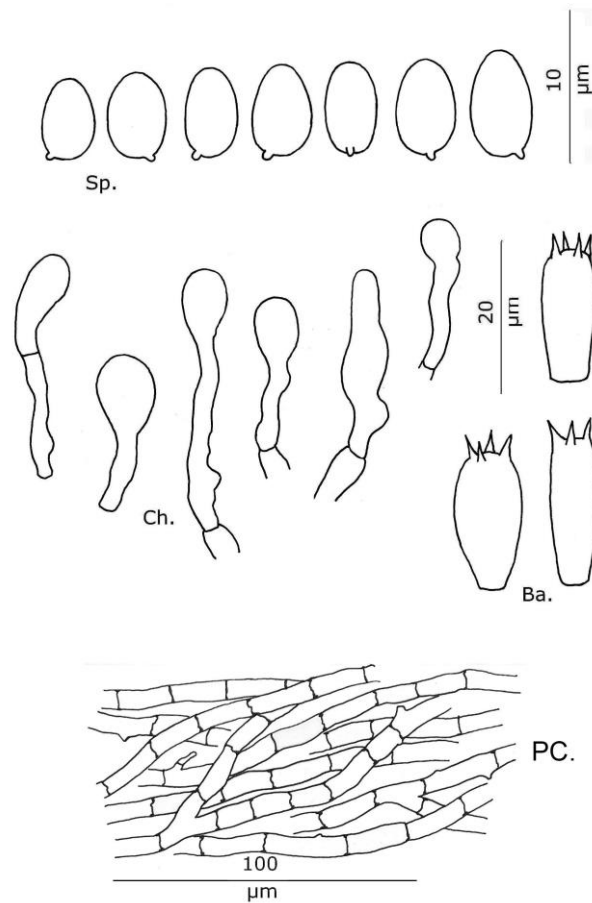


Fig. 5. Line drawings of microscopical characters Sp.) spores; Ch.) cheilocystidia; Ba.) basidia; PC) pileus covering elements. (A. Bizzi)

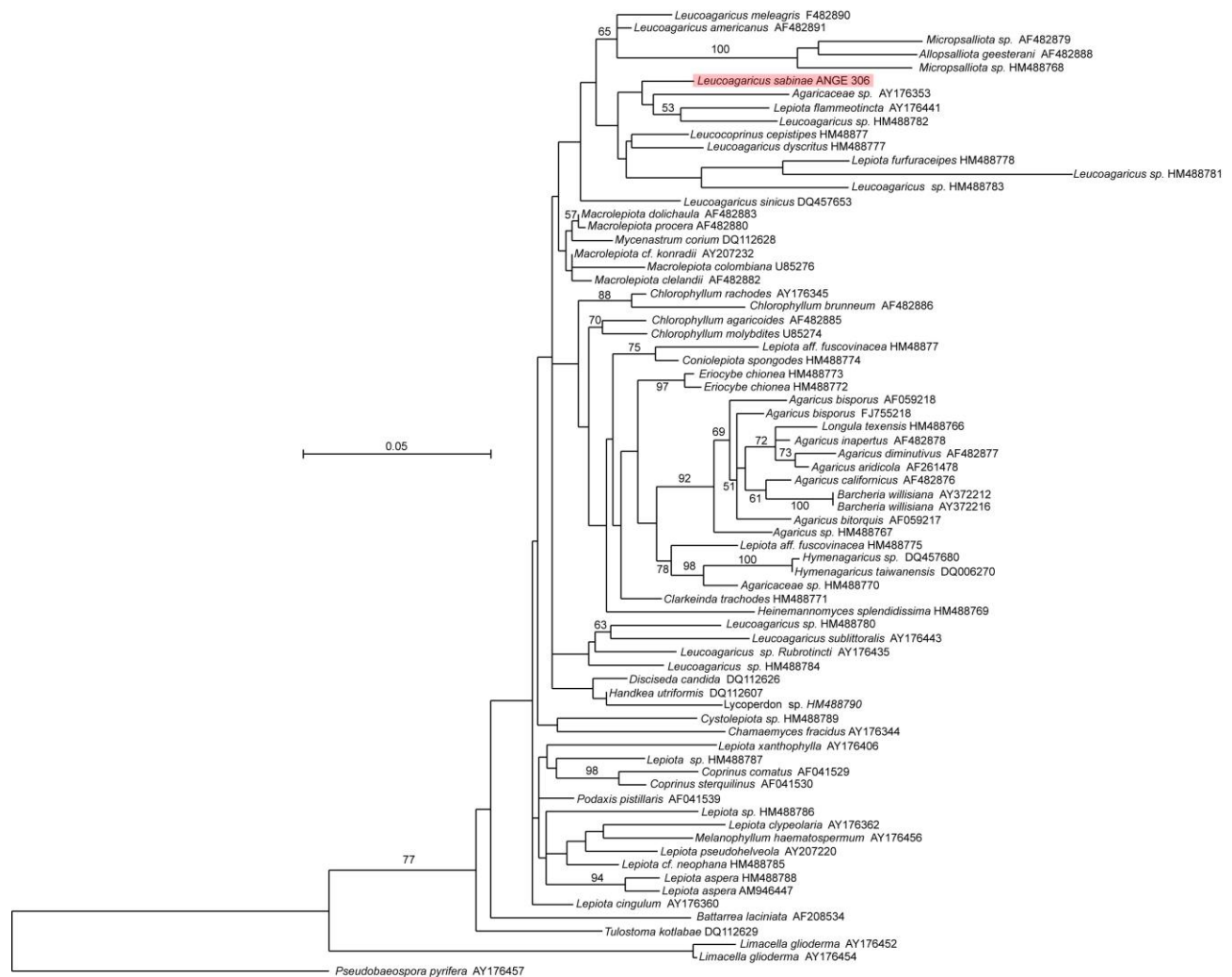


Fig. 6. Best tree from the maximum likelihood analyses of the nrLSU dataset. Only bootstrap values over 50 are shown.

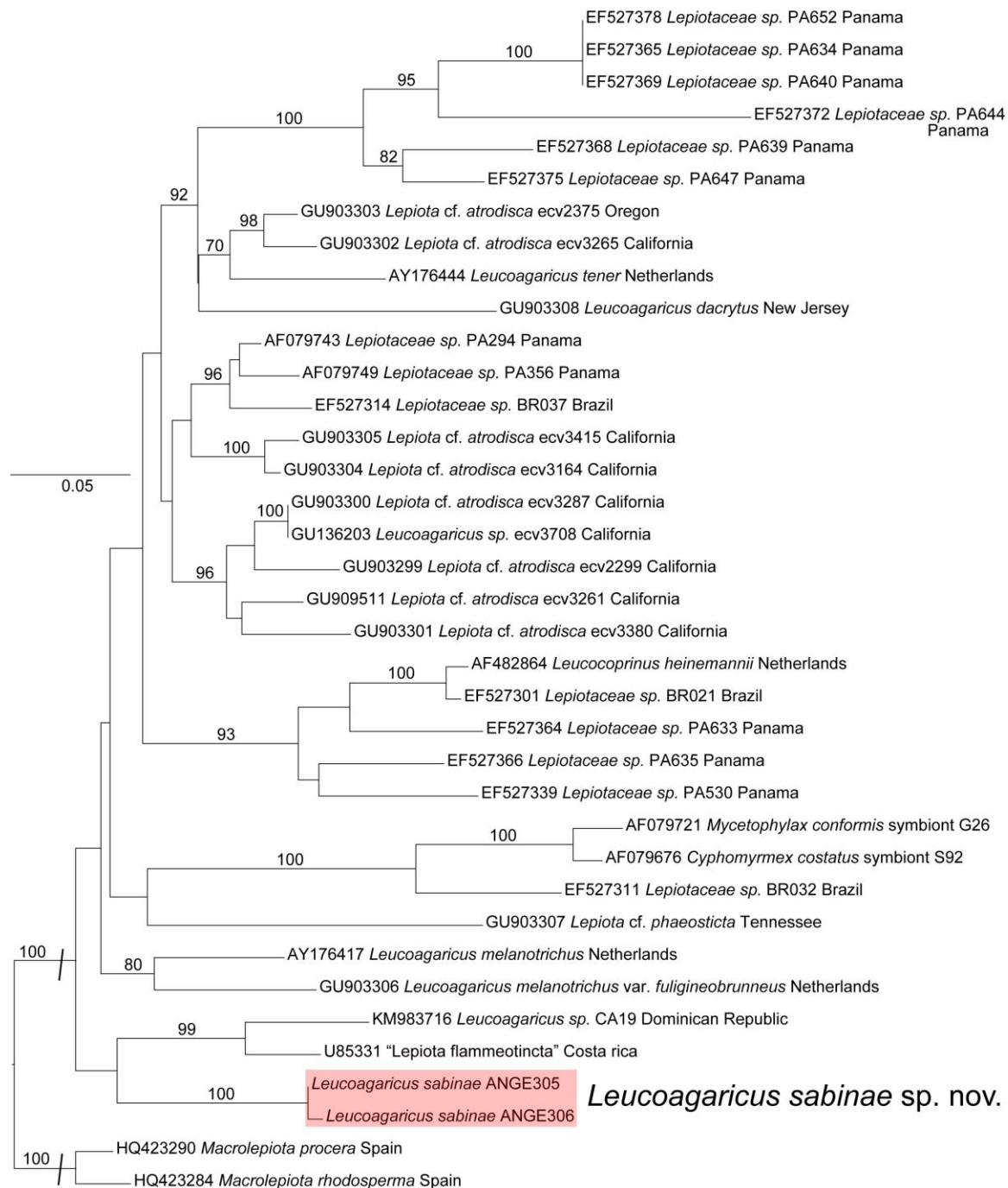


Fig. 7. Best tree from the maximum likelihood analyses of the nrITS dataset. Only bootstrap values over 70 are shown