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# Diversity of the *Ganoderma* species in Uruguay

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**ABSTRACT**

*Ganoderma* is a cosmopolitan genus that includes a great diversity of species. Many of them have been historically described based only on morphological characteristics; however, due to their morphological plasticity, there is no complete understanding about their relationship and taxonomic status. Commonly applied names, particularly in the southern Neotropics, come from species of North Hemisphere distribution (e.g. *G. lucidum, G. resinaceum* and *G. applanatum*). The objective of the present work was to perform a survey of *Ganoderma* species thriving in Uruguay. We aimed to identify and characterize them through molecular, morphological and ecological analysis. The results confirm the presence of four reddish laccate species first registered for Uruguay (*G. dorsale, G. platense, G. martinicense* and *G. mexicanum*), and one non-laccate species (*G. australe* s.l.) composed of two clades. The species are morphologically differentiated mainly by its stipe, pilear surface, context, pores, basidiospores and cutis cells. Regarding the ecological data, the species present differences in substrate preferences. In addition, a taxonomic discussion regarding phylogenetic relationships and taxonomic status of Uruguayan *Ganoderma* species is presented.

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ITS; mycogeography; phylogeny; systematics; South American polypores

## Introduction

*Ganoderma* (P. Karst.) harbors at least 220 species, being the most diverse genus of Ganodermataceae [1–4]. However, due to their high diversity and phenotypic plasticity, the phylogenetic relationship and taxonomic status of many species remain unclear until now [5,6]. In the last 20 years, the use of molecular tools, mainly through the amplification and sequencing of Internal Transcribed Spacer (ITS), has been incorporated into systematic studies and into circumscription of *Ganoderma* species around the world. In this sense, through phylogenies based on molecular characters, the tendency in recent years has been to reinterpret the variations in characters of morphologically defined species, reinterpret the ecological relationships (relationship with hosts), determine their distribution and arrive at an understanding of the biogeographic processes that shape it [5,7–11].

*Ganoderma* species are morphologically characterized by the formation of sessile to stipitate basidiomata, with a glossy reddish laccate to opaque non-laccate cover, ellipsoid to ovoid double-walled basidiospores with truncated apex and endosporium with columnar ornamentations [12]. This cosmopolitan genus is comprised of parasitic and saprophytic species that decay the wood of plants from temperate and tropical areas around the world [6,13,14]. These fungi are described as white rot decayers that play a critical role in the dynamics of wood decomposition in tropical forests [15]. Moreover, they are the main cause of tree deterioration in public ornamental and commercial plantations [10,15–18].

In the last decades, some researchers have tried to elucidate the diversity of *Ganoderma* genus in the Neotropics, particularly Bazzalo and Wright [19], Gilbertson and Ryvarden [20], Gottlieb and Wright [21,22], Gottlieb et al. [7], Ryvarden [12,23], Torres-Torres and Dávalos [24], Torres-Torres et al. [25,26], and more recently Cabarroi-Hernández et al. [6] and Loyd et al. [11] for the northern limit of Neotropical distribution. Uruguay in particular harbors a great biodiversity due to its transitional condition and ecoregions diversity [27]. Some *Ganoderma* species have been historically recorded in Uruguay (Table 1), while Gazzano [28,29] and Martinez [30] have made more recent contributions to the diversity of the genus in Uruguay.

Some Uruguayan cited species as *G. applanatum* (Pers.) Pat. (= *G. lipsiense* (Batsch) G.F. Atk.), *G. sessile* Murrill, *G. lucidum* P. Karst. and *G. resinaceum* Boud were distributed out of the southern Neotropics [14,35,36], while others as *G. lorenzianum* (Kalchbr.)

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MS. Guillermo Morera. Main author of the work, he collected the specimens, analyzed the data and wrote the manuscript.Dra. Sandra Lupo Rizzo. Participated in field campaigns, molecular analysis and reviewed the manuscript.Dra. Sandra Alaniz Ferro. Participated in the revision and correction of the manuscript.Dr. Gerardo Robledo. Contributed in morphological and phylogenetic analyses, and reviewed the manuscript.

Supplemental data for this article can be accessed here.



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Pat., *G. nitens* (Fr.) Pat. lack of phylogenetic studies and their taxonomic status is unclear. Until now, there is no complete understanding on the systematic of the *Ganoderma* genus in Uruguay and its species diversity remains unknown.

The objective of the present work was to perform an updated survey of the *Ganoderma* species thriving in Uruguay and characterize them through molecular, morphological and ecological analysis. We also aimed to discuss the taxonomic status of the species of Uruguay and contrast with previous reports. We hypothesized that the *Ganoderma* specimens of Uruguay correspond to native species that have not been previously reported for the country. Those species are different from those registered to date (with names of species described from the Northern Hemisphere).

## Materials and methods

### *Fungal specimens, basidiomata description and host characterization*

Fresh *Ganoderma* basidiomata were collected from indigenous and urban ecosystems of Uruguay, during 2017 and 2018 field expeditions. Geographic location, host species and substrate condition (living tree, dead trunk, roots and stump) data were taken at the collection site following Urcelay and Robledo [37]. For fresh basidiomata, small pieces were aseptically taken from the context, placed into 2% malt extract agar (MEA) and incubated in darkness at 25°C. Pure cultures and basidiomata were deposited in MVHC. In addition, specimens from national herbaria (MVHC and MVM) were examined. Herbarium acronyms follow Thiers [38] (continuously updated, http://sweetgum.nybg.org/).

For morphological analyses and basidiomata identification, macroscopic and microscopic observations were made on basidiomata following the terminology and methodology according to authors [6,7,19,22–26]. Macroscopic features of basidiomata were analyzed and measured, particularly: pileus dimension, colour, texture, shape and appearance of surface, margin and stipe. The colour, presence of melanoid deposits and texture of context were also inspected and pores were described and measured (pores/mm). Microscopic features (basidiospores, chlamydospores in context, generative and somatic hyphae and cuticular cells) were analyzed with an optical microscope by mounting small sections of basidiomata with 5% KOH or Melzer's Reagent (to test for dextrinoid or amyloid reaction). In particular, for the analysis of the hyphal system, sections of basidiomata were treated for 24–48 h in 3% NaOH at 50–60°C [39]. Thirty basidiospores were measured from each specimen (length and width) and values were expressed as rank of mean values. Width was measured in the widest part of the spore and the length considered from the base to the truncated apex of the basidiospore. Then, Q ratio was calculated as the relation: length/width. Measures of cuticular cells were made from the middle part of the basidiomes.

Host relationships of each *Ganoderma* species in Uruguay were characterized by host range and native/exotic status. The preference of substrate condition was analyzed through the relative frequency of each *Ganoderma* species in each substrate condition (LT = stem of living tree, DT = dead trunks, S = stumps and R = soil, arising from roots of living or dead trees) following Urcelay and Robledo [37]. Then, substrate preference was determined by transforming this relative frequency into a percentage.

### *DNA extraction, PCR and sequencing*

DNA extractions from pure cultures were performed, using the CTAB protocol of Doyle and Doyle [40] with modifications [41]. The PCR reaction of the ITS region (including ITS1, 5.8s and ITS2) was performed. The following PCR primers were alternatively used: ITS1/ITS4 [42], ITS1-F/ITS4-B [43], and ITS4/ITS5 [42]. The PCR mixture was prepared in a 25 µl final volume, with 2 µl of genomic DNA solution (10 ng), 16 µl of mQ water, 0.25 µl of Taq polymerase (1 U), 0.5 µl of each primer (10 mM), 0.7 µl of 50 mM MgCl2, 2.5 µl of dntps (2.5 mM) and 2.5 µl (10X) of buffer. PCR reactions were performed in a MultiGene Optimax thermocycler (Labnet International Inc) with the cycling conditions as follows: 3 min at 94°C, followed by 35 cycles, each

**Table 1.** *Ganoderma* species recorded in Uruguay.

| Taxon | Author |
|---|---|
| *G. australe* (Fr.) Pat. | Felippone [6667] |
| *G. applanatum* (Pers.) Pat. (= *G. lipsiense* (Batsch) G.F. Atk.) | Felippone [66], Herter [31], Gazzano [28,29,32] Martínez [30] |
| *G. fornicatum* (Fr.) Pat. | Felippone [66] |
| *G. lorenzianum* (Kalchbr.) Pat. | Patouillard [65], Felippone [66] |
| *G. lucidum* (Curtis) P. Karst. | Spegazzini [33], Felippone [66], Wright & Blumenfeld [34], Gazzano [28,29,32], Martínez [30] |
| *G. nitens* (Fr.) Pat. | Patouillard [65], Felippone [66] |
| *G. resinaceum* Boud. | Felippone [66], Gazzano [32], Martínez [30] |
| *G. sessile* Murrill | Gazzano [29] |

**Table 2.** *Ganoderma* species, specimens, location, Gen Bank accession numbers for ITS sequences and reference source.

| Species/Voucher/Culture reference | Locality | ITS GenBank accession number | References |
|---|---|---|---|
| *Ganoderma adspersum* | | | |
| CBS351.74 | Belgium | X78742/X78763 | Moncalvo et al. [13] |
| GaTO00 | Italy | AM906057 | Guglielmo et al. [44] |
| *Ganoderma annulare* | | | |
| KCTC 16803 | Brazil | JQ520160 | GenBank |
| *Ganoderma applanatum* | | | |
| ATCC44053 | Japan | JQ520161 | GenBank |
| *Ganoderma australe* **s.l.** | | | |
| **MVHC 5620** | **Uruguay** | **MN191569** | **This work** |
| **MVHC 5564** | **Uruguay** | **MN191568** | **This work** |
| **MVHC 5582** | **Uruguay** | **MN191570** | **This work** |
| **MVHC 5659** | **Uruguay** | **MN191554** | **This work** |
| **MVHC 5660** | **Uruguay** | **MN191553** | **This work** |
| **MVHC 5668** | **Uruguay** | **MN191555** | **This work** |
| **MVHC 5601** | **Uruguay** | **MN191556** | **This work** |
| **MVHC 5605** | **Uruguay** | **MN191557** | **This work** |
| **MVHC 5661** | **Uruguay** | **MN191558** | **This work** |
| **MVHC 5646** | **Uruguay** | **MN191552** | **This work** |
| **MVHC 5640** | **Uruguay** | **MN191551** | **This work** |
| **MVHC 5641** | **Uruguay** | **MN191550** | **This work** |
| **MVHC 5680** | **Uruguay** | **MN191547** | **This work** |
| **MVHC 5647** | **Uruguay** | **MN191549** | **This work** |
| **MVHC 5587** | **Uruguay** | **MN191548** | **This work** |
| **MVHC 5568** | **Uruguay** | **MN191559** | **This work** |
| **MVHC 5645** | **Uruguay** | **MN191560** | **This work** |
| **MVHC 5697** | **Uruguay** | **MN191561** | **This work** |
| **MVHC 5717** | **Uruguay** | **MN191572** | **This work** |
| **MVHC 5711** | **Uruguay** | **MN191562** | **This work** |
| **MVHC 5722.2** | **Uruguay** | **MN191563** | **This work** |
| **MVHC 5724** | **Uruguay** | **MN191565** | **This work** |
| **MVHC 5725** | **Uruguay** | **MN191564** | **This work** |
| **MVHC 5746** | **Uruguay** | **MN191573** | **This work** |
| **MVHC 5688** | **Uruguay** | **MN191566** | **This work** |
| **MVHC 5708** | **Uruguay** | **MN191567** | **This work** |
| **MVHC 5579** | **Uruguay** | **MN191546** | **This work** |
| **MVHC 5732** | **Uruguay** | **MN191545** | **This work** |
| **NC 7177** | **Uruguay** | **MN191571** | **This work** |
| 0705 | Taiwan | X78750.1 | Moncalvo and Buchanan [5] |
| *Ganoderma concinnum* | | | |
| Robledo 3192 (FCOS) | Bolivia | MN077522 | Costa-Rezende et al. [53] |
| Robledo 3235 (FCOS) | Bolivia | MN077523 | Costa-Rezende et al. [53] |
| *Ganoderma dorsale* | | | |
| **MVHC 5588** | **Uruguay** | **MN191582** | **This work** |
| **MVHC 5701** | **Uruguay** | **MN191581** | **This work** |
| **MVHC 5653** | **Uruguay** | **MN191578** | **This work** |
| **MVHC 5648** | **Uruguay** | **MN191579** | **This work** |
| **MVHC 5654** | **Uruguay** | **MN191580** | **This work** |
| *Ganoderma ecuadoriense* | | | |
| ASL799 | Ecuador | KU128524 | Crous et al. [45] |
| PMC126 | Ecuador | KU128525 | Crous et al. [45] |
| Poly-2-4 | Ecuador | KU128526 | Crous et al. [45] |
| *Ganoderma gibbosum* | | | |
| XSD34 | China | EU273513 | GenBank |
| *Ganoderma lipsiense* | | | |
| BAFC2424 | Argentina | AF169977/8 | Gottlieb et al. [7] |
| *Ganoderma lobatum* | | | |
| BAFC2411 | Argentina | AF169989/90 | Gottlieb et al. [7] |
| CBS222.48 | USA | X78740/X78761 | Moncalvo et al. [13] |
| *Ganoderma lucidum* | | | |
| BAFC2419 | Argentina | AF170007/AF170008 | Gottlieb et al. [7] |
| BAFC2493 | Argentina | AF170009/A1470010 | Gottlieb et al. [7] |
| UMNUT1 | USA | MG654070 | Loyd et al. [11] |
| K 175217 | England | KJ143911 | Zhou et al. [46] |
| RYV 33217 T | Norway | Z37096/ Z37073 | Moncalvo et al. [13] |
| CBS 270.81 | France | Z37049/Z37099 | Moncalvo et al. [13] |
| Dai 2272 | Sweden | JQ781851 | Cao et al. [14] |
| Dai11593 | Finland | JQ781852 | Cao et al. [14] |
| BCRC 37043 | China | EU021460 | Wang et al. [9] |
| *Ganoderma martinicense* | | | |
| **MVHC 5635** | **Uruguay** | **MN191574** | **This work** |
| **MVHC 5583** | **Uruguay** | **MN191575** | **This work** |
| **MVHC 5637** | **Uruguay** | **MN191577** | **This work** |
| **MVHC 5684** | **Uruguay** | **MN191576** | **This work** |
| 246TX | USA | MG654185 | Loyd et al. [11] |
| Mart08_55 **T** | Martinique | KF963256 | GenBank |
| *Ganoderma mexicanum* | | | |
| **MVHC 5652** | **Uruguay** | **MN191583** | **This work** |

(*Continued*)

Table 2. (Continued).

| Species/Voucher/Culture reference | Locality | ITS GenBank accession number | References |
|---|---|---|---|
| XAL D.Jarvio 143 | Mexico | MK531823 | Cabarroi-Hernández et al. [6] |
| BAFC2580 | Brazil | AH008108 | Gottlieb et al. [7] |
| *Ganoderma multipileum* | | | |
| DAI 9447 | China | KJ143914 | Zhou et al. [46] |
| *Ganoderma multiplicatum* | | | |
| SPC9 | Brazil | KU569553 | Bolaños et al. [60] |
| CC8 | Brazil | KU569515 | Bolaños et al. [60] |
| URM 83346 | Brazil | JX310823 | Bolaños et al. [60] |
| *Ganoderma oerstedii* | | | |
| ATCC 52409 | Argentina | Z37058/Z37083 | Moncalvo et al. [13] |
| ATCC 52410 | Argentina | X78739/X78760 | Moncalvo et al. [13] |
| ATCC 52411 | Argentina | Z37059/Z37084 | Moncalvo et al. [13] |
| *Ganoderma parvulum* | | | |
| URM 83344 | Brazil | JX310819 | Correia de Lima Júnior et al. [57] |
| URM 2948 | Brazil | JX310821 | Correia de Lima Júnior et al. [57] |
| *Ganoderma platense* | | | |
| **MVHC 5586** | **Uruguay** | **MN191585** | **This work** |
| **MVHC 5565** | **Uruguay** | **MN191584** | **This work** |
| **MVHC 5721** | **Uruguay** | **MN191591** | **This work** |
| **MVHC 5732.2** | **Uruguay** | **MN191592** | **This work** |
| **MVHC 5686** | **Uruguay** | **MN191593** | **This work** |
| **MVHC 5692** | **Uruguay** | **MN191596** | **This work** |
| **MVHC 5687.2** | **Uruguay** | **MN191597** | **This work** |
| **MVHC 5687** | **Uruguay** | **MN191594** | **This work** |
| **MVHC 5690** | **Uruguay** | **MN191595** | **This work** |
| **NC 5332** | **Uruguay** | **MN191587** | **This work** |
| **NC 7187** | **Uruguay** | **MN191588** | **This work** |
| **NC 5104** | **Uruguay** | **MN191589** | **This work** |
| **MVHC 5694** | **Uruguay** | **MN191590** | **This work** |
| **MVHC 5691** | **Uruguay** | **MN191586** | **This work** |
| BAFC384 | Argentina | AH008109 | Gottlieb et al. [7] |
| *Ganoderma polychromum* | | | |
| 330OR | USA | MG654196 | Loyd et al. [11] |
| BJ280CA | USA | MG910492 | Loyd et al. [11] |
| *Ganoderma resinaceum* | | | |
| DP2 | Italy | AM906060 | Guglielmo et al. [44] |
| CIRM BRFM 753 | France | FJ805250 | GenBank |
| HMAS86599 | England | AY884177 | Wang et al. [9] |
| BR 4150 | France | KJ143915 | Zhou et al. [46] |
| GR-101 | India | GU451246 | Mohanty et al. [63] |
| CBS 22036 | The Netherlands | JQ520201 | Park et al. [60] |
| BCRC 36147 | The Netherlands | KJ143916 | Zhou et al. [46] |
| *Ganoderma sessile* | | | |
| 111TX | USA | MG654306 | Loyd et al. [11] |
| 103SC | USA | MG654304 | Loyd et al. [11] |
| 113FL | USA | MG654307 | Loyd et al. [11] |
| 228DC | USA | MG654319 | Loyd et al. [11] |
| 117TX | USA | MG654309 | Loyd et al. [11] |
| BAFC2373 | Argentina | AH008111 | Gottlieb et al. [7] |
| *Ganoderma* **sp.** | | | |
| MUCL27886 | India | AF255190 | Moncalvo and Buchanan [5] |
| LXT.8 | Vietnam | AF255188 | Moncalvo and Buchanan [5] |
| PKB96/330 | Japan | AF255105 | Moncalvo and Buchanan [5] |
| TAI-05 | Taiwan | AF255193/4 | Moncalvo and Buchanan [5] |
| CP331 | Papua New Guinea | AF255125 | Moncalvo and Buchanan [5] |
| JM97/31 | USA | AF255098 | Moncalvo and Buchanan [5] |
| JM98/132 | China | AF255115 | Moncalvo and Buchanan [5] |
| JM98/2 | South Africa | AF255149 | Moncalvo and Buchanan [5] |
| ME-GAN-24 | USA | AF255131/2 | Moncalvo and Buchanan [5] |
| RV-PR10 | Puerto Rico | AF255133 | Moncalvo et al. [13] |
| JMCR.132 | Costa Rica | AF255138 | Moncalvo and Buchanan [5] |
| MUCL40406 | Ecuador | AF255139 | Moncalvo and Buchanan [5] |
| MUCL40324 | French Guiana | AF255141 | Moncalvo and Buchanan [5] |
| NIAST824 | South Korea | AF255114 | Moncalvo and Buchanan [5] |
| HMAS60686 | China | AF255191/2 | Moncalvo and Buchanan [5] |
| BAFC2531 | Chile | AF255176 | Moncalvo and Buchanan [5] |
| BAFC2449 | Argentina | AF255187 | Moncalvo and Buchanan [5] |
| *Ganoderma subamboinense* **var.** *laevisporum* | | | |
| ATCC 52419 | Argentina | X78736 | Gottlieb et al. [7] |
| ATCC 52420 | Argentina | JQ520205 | Park et al. [60] |
| UMNFL 100 | USA | MG654373 | Loyd et al. [11] |
| *Ganoderma tornatum* | | | |
| BAFC2764 | Argentina | AF169993/4 | Gottlieb et al. [7] |
| BAFC2582 | Brazil | AF169985/6 | Gottlieb et al. [7] |
| BAFC1139 | Argentina | AF169979/80 | Gottlieb et al. [7] |
| *Ganoderma tuberculosum* | | | |
| BAFC2488 | Argentina | AH008114 | Gottlieb and Wright [22] |

(*Continued*)

Table 2. (Continued).

| Species/Voucher/Culture reference | Locality | ITS GenBank accession number | References |
|---|---|---|---|
| PLM684 | USA | MG654369 | Loyd et al. [11] |
| UMNFL160 | USA | MG654364 | Loyd et al. [11] |
| *Ganoderma zonatum* | | | |
| FL-02 | USA | KJ143921 | Zhou et al. [46] |
| 179NC | USA | MG654417 | Loyd et al. [11] |
| UMNFL105 | USA | MG654408 | Loyd et al. [11] |
| UMNSC4 | USA | MG654415 | Loyd et al. [11] |
| 123FL | USA | MG654416 | Loyd et al. [11] |
| BAFC2374 | Argentina | AH008110 | Gottlieb and Wright [22] |
| *Cristataspora coffeata* | | | |
| FLOR 50933 | Brazil | KU315204 | Costa-Rezende et al. [53] |
| *Foraminispora rugosa* | | | |
| HUEFS_DHCR560 | Brazil | MF409963 | Costa-Rezende et al. [53] |
| FLOR 52191 | Brazil | KU315200 | Costa-Rezende et al. [4] |

consisting of 60 s at 94°C, 45 s at 50°C, 60 s at 72°C, and a final extension step at 72°C for 5 min. PCR products were verified by electrophoresis in 1.0% agarose gels in TBE buffer, stained with EZ vision®One (Amresco®) and visualized under UV light transillumination. GeneRuler DNA Ladder Mix marker (Thermo) was used as molecular size marker. PCR products were purified and sequenced by Macrogen (Seoul, Korea). Sequences were submitted to GenBank (Table 2).

### Phylogenetic analyses

The sequences obtained were manually edited (visual inspection of sequences and chromatograms, resolution of conflicts and pair the extremes) with Bioedit V.7.0.5.3 [47] and incorporated into alignments with sequences of specimens from other parts of the world obtained from GenBank (Table 2). Multiple alignment was made using ProbCons 1.12 from the CIPRES Science Gateway [48]. Subsequently, the best evolutionary model for each region (ITS1, 5.8S and ITS2) was estimated using the Corrected Akaike Informational Criteria (AICc), implemented by the jModelTest2 v.1.6 software [49]. The phylogenetic analysis was conducted in two independent ways: Bayesian Inference (BI) and Maximum Likelihood (ML), performed with MrBayes 3.2.7 [50] and RAxML 8.2.12 [51], respectively, in CIPRES Science Gateway [52]. *Cristataspora coffeata* (Murrill) Robledo, Costa-Rezende and de Madrignac Bonzi (FLOR 50933) and *Foraminispora rugosa* (Berk.) Costa-Rezende, Drechsler-Santos and Robledo (FLOR 52191 and HUEFS DHCR560) were used as outgroup [4,53]. For the BI, two independent runs were performed, starting with random trees, with four independent and simultaneous chains, 10,000,000 MCMC generations, and maintaining 1 tree every 1000 generations. Burn in discarded values was indicated as 0.25. The estimated models for each partition were incorporated, as indicated below (see Results). The average standard deviation of split frequencies was limited to below 0.01. Convergence of the Markov chains to a stationary distribution was visually inspected using the Tracer v.1. 7. 1 program [54]. A GTRGAMMA nucleotide model and 1000 bootstrap iterations were indicated for the ML analysis. The rest of the parameter values were set by default. Since the topologies of trees obtained in each analysis were convergent, only the consensus BI tree is shown with values of Bayesian posterior probability (BPP) and ML bootstrap (ML) separated by cross bars (BPP/BS). A clade was considered strongly supported if it showed a 0.95 BPP and/or 80% BS [55].

## Results

### Fungal specimens, basidiomata description and host characterization

A total of 163 *Ganoderma* specimens from collections generated in this work ($n = 90$), MVHC ($n = 57$) and MVM ($n = 16$) were morphologically and ecologically analyzed (Tables 3 and 4). *Ganoderma australe* was the most commonly collected species, with 101 specimens. The 62 remaining specimens belong to four reddish laccate species: *G. mexicanum* ($n = 3$), *G. martinicense* ($n = 10$), *G. platense* ($n = 22$) and *G. dorsale* ($n = 27$).

Morphologically assigned species are presented in Figures 1 and 2. *Ganoderma* species of Uruguay were primarily differentiated by their pilear surface into two groups: reddish laccate and non-laccate (Table 3 and Figure 1).

The first group is composed of two species with great and robust basidiomes (*G. martinicense* and *G. platense*) and two species with smaller basidiomes (*G. dorsale* and *G. mexicanum*).

*Ganoderma martinicense* is characterized by a large, commonly substipitate basidiomata with a tuberculous concentric zonated pilear surface and conspicuous melanoid deposits in the homogeneous context. Microscopically, it is characterized by the presence of a distinct smooth basidiospores ornamentation, formed by free pillars and cutis cells with weak

**Table 3.** Morphological assessment of *Ganoderma* species found in Uruguay.

| | | | | Context | | Pores | | | Basidiospores | | Chlamydospores | | Cutis cells | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | Stipe | Surface | Margin | Color | Resinous/ melanoid deposits | Color | Shape | Pores/ mm | Size (μm) | Ornamentation | Description | Size (μm) | Shape | Melzer reaction | Size (μm) |
| ***G. australe* M1** | Rarely present, mostly sessile | Tubercular, rugose, concentric zonate, brownish to mate | Brownish, flat to slightly lobulated | Homogeneous, chocolate | Always present | Whitish to brownish | Circular to irregular | 4–6 | 8.3–10.3 × 5–6.6 | Thick, free to sub free pillars | In context, final or intercalary, globose to slightly ellipsoid, double-walled. | 10–13 × 9–11 | Branches of terminal skeletal hyphae embedded in melanoid substance | Null reaction | 30–70 × 3–5 |
| ***G. australe* M2** | Rarely present, mostly sessile | Concentric zonate, brownish to greyish | Brownish, flat to slightly lobulated | Homogeneous, chocolate | Always present | Whitish to brownish | Circular to irregular | 4–6 | 6.5–10.1 × 4.2–6.1 | Thick, free to sub free pillars | In context, very rare, slightly ellipsoid, double-walled. | 11 × 9 | Branches of terminal skeletal hyphae embedded in melanoid substance | Null reaction | 50–70 × 4–5 |
| ***G. dorsale*** | Always present, lateral, vertical | Rough, semi-concentric zonation, wine purplish to reddish | Reddish, flat to acute | Not fully homogeneous, first light brown becoming dark brown near the tubes | Always present | Whitish to brownish | Circular to irregular | 3–5 | 8–8.9 × 5.8–6.1 | Distinct, thick pillars, sometimes with anastomosed appearance | In context, rare, late or intercalary, double-walled. | 5–13 × 4–7.5 | Cylindrical to clavate, usually with apical protuberances (up to 2) | Strongly amyloid | 20–39 × 3–7.5 |
| ***G. martinicense*** | Substipitate to sessile | Tuberculous, rugose, concentric colored, first purplish, then reddish and yellowish toward the margin | Whitish, lobulated | Not fully homogeneous, alternate dark and light brown | Always present | Whitish to yellowish | Circular to irregular | 4–6 | 10–12.9 × 5.7–7.7 | Distinct, free pillars | In culture, terminal or intercalary, double walled, variably smooth, sometimes with appendages, yellowish.<br>In context, terminal or intercalary. | 8–12.5 × 8–11<br>9–12 × 5–8 | Spheroid pedunculated to clavate, occasionally with apical or basal branches | Weakly amyloid | 30–60 × 5–15 |

(*Continued*)

Table 3. (Continued).

| | | | | Context | | Pores | | | Basidiospores | | Chlamydospores | | Cutis cells | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxon | Stipe | Surface | Margin | Color | Resinous/ melanoid deposits | Color | Shape | Pores/ mm | Size (µm) | Ornamentation | Description | Size (µm) | Shape | Melzer reaction | Size (µm) |
| ***G. mexicanum*** | Present, lateral, horizontal | Rough, slightly semi-concentric zonation, shiny, bordered to reddish | Whitish, acute | Mostly light colored. Slightly heterogeneous, being darker near the tubes | Present, inconspicuous | Whitish to brownish | Circular to irregular | 4–6 | 7–9 × 5–6 | Inconspicuous, mostly free pillars | In context, frequent, final or intercalary, globose to slightly ellipsoid, double-walled. Dextrinoid. | 9–15.5 × 7–11 | Cylindrical to slightly clavate, generally without protuberances | Strongly amyloid | 30 × 4–6 |
| ***G. platense*** | Always sessile | Rough to variably striated, semi-orbicular zonation, shiny, wine purplish on board | Whitish, lobulated to acute | Not fully homogeneous, first light brown becoming dark brown near the tubes | Present, inconspicuous | Whitish to brownish | Angular | 3–4 | 7.5–9 × 4–6.2 | Inconspicuous, free pillars | In context, infrequent, terminal or intercalary, brownish. | 8.3–14.3 × 5.2–6.5 | Cylindrical to slightly clavate, sometimes with knots or apical constrictions | Strongly amyloid | 30–50 × 4–9 |

**Table 4.** Number of specimens, distribution and host characterization (species, native/exotic status and substrate preferences) of *Ganoderma* species collected and studied in this work.

| Taxon | Total specimens | Distribution (Departments) | Host characterization | | |
|---|---|---|---|---|---|
| | | | Native | Exotic | Substrate preferences |
| *G. australe* M1 | 13 | Lavalleja, Montevideo, Maldonado, Paysandú, Rivera, Rocha, Florida, Colonia, Flores | *Scutia buxifolia, Myrsine laetevirens, Alophyllus edullis, Lithraea* sp. | *Acacia melanoxylon, Gleditsia triachanthos, Tilia* sp., *Eucalyptus* sp., *Quercus* sp., *Salix* sp., *Grevillea* sp. | LT (45%), DT (33%) and S (22%) |
| *G. australe* M2 | 88 | Canelones, Lavalleja, Maldonado, Montevideo, Rivera, Rocha, Tacuarembó, Treinta y Tres, Florida, Cerro Largo, Río Negro, Artigas, Colonia, San José | *Alophillus edulis, Lithraea* sp., *Myrcianthes* sp., *Scutia buxifolia, Myrsine laetevirens* | *Acacia longifolia, Acacia melanoxylon, Acacia* sp., *Eucalyptus globulus, Robinia pseudoacacia, Prunus persica, Pinus pseudostrobus, Quercus suber, Salix* sp. | DT (46%), LT (39%), S (14%) and R (1%) |
| *G. dorsale* | 27 | Montevideo, Rocha, Treinta y Tres, Canelones, Rivera | *Alophyllus edulis, Lithraea* sp., *Ruprechtia salicifolia, Scutia buxifolia, Celtis* sp., *Sebastiana* sp. | *Quercus suber* | R (58%), LT (37%) and S (5%) |
| *G. martinicense* | 10 | Canelones, Montevideo, Paysandú, Rocha, Artigas | *Tipuana tipu* | *Acacia* sp. | R (87%) and S (13%) |
| *G. mexicanum* | 3 | Rocha, Paysandú | Unidentified wood | - | DT (100%) |
| *G. platense* | 22 | Canelones, Montevideo, Maldonado | - | *Acacia dealbata, Fraxinus* sp., *Platanus acerifolia, Salix* sp., *Quercus* sp., *Araucaria* sp. | LT (85%), DT (8%) S (7%) |

LT = stem of living tree, DT = dead trunks, S = stumps, R = soil arising from roots of living or dead trees.

reaction and spheroid-pedunculated shape. On the other hand, *G. platense* basidiomata are smaller, semi-orbicular zonate, homogeneous colored and always sessile. Its context is also different, presenting extremely inconspicuous melanoid lines and microscopically characterized by particular cutis elements with apical constrictions (Figure 2).

*Ganoderma mexicanum* produces stylized, flabelliform, laterally and horizontally stipitate basidiomata characterized by an almost light-colored context and basidiospores with fine ornamentation, whereas *G. dorsale* produces stylized, spatuliform, shell-shaped and almost laterally and vertically stipitate basidiomata with no homogeneous distinctive context. Its basidiospores present notorious rough ornamentation and almost cylindrical cutis cells with a strong amyloid reaction (Figure 2).

Specimens with non-laccate surfaces are morphologically very similar, discernible however by their pilear surface, which is distinctly tubercular in *G. australe* M1 and distinctly zonate in *Ganoderma australe* M2.

Herbaria specimens showed a morphology consistent with the species recorded in this study. In that sense, *G. lucidum* is a name previously used for specimens corresponding to the *G. dorsale, G. martinicense, G. platense* and *G. mexicanum* species. The name *G. resinaceum* was previously used to name specimens corresponding to *G. platense* and *G. martinicense*. The names *G. lipsiense, G. applanatum* and *G. marmoratum* were used to refer to specimens of *G. australe* in the broad sense (from now on sensu lato or s.l.). A morphological key for *Ganoderma* species found in Uruguay is presented below.

Host relationships of each *Ganoderma* species in Uruguay and presence in distinct departments are presented in Table 4. *Ganoderma martinicense* and *G. australe* were found alternatively in several departments growing on native or exotic trees. On the other hand, *G. platense* was only recorded in the southeast (Montevideo, Canelones and Maldonado departments), growing preferentially on exotic trees, *G. mexicanum* was only found on dead stems of native forest, and *G. dorsale*, almost exclusively on native trees. Regarding their substrate preferences, *G. mexicanum* and *G. australe* M2 were found preferentially on dead stem wood (100% and 46%, respectively); *G. martinicense* and *G. dorsale*, preferentially on roots (87% and 58%, respectively); and *G. australe* M1 and *G. platense*, preferentially on live stems (45% and 85%, respectively).

## *Phylogenetic analyses*

A total of 53 new sequences were generated from Uruguayan specimens. The dataset alignment resulted in 140 DNA sequences comprising 639 bp. The best evolutionary models for each partition were as follows: K80 + G (ITS1), JC (5.8 S), K80 + G (ITS 2). The partition scheme was K80 + G (ITS1) with -lnL = 1373.0169, and equal base frequencies as follows: A = 0.25, C = 0.25, G = 0.25, T = 0.25, JC (5.8 S) with -lnL = 300.8947 with equal base frequencies, and K80 + G (ITS 2) with 1498.7406 and equal base frequencies. The Bayesian Inference consensus tree is presented in Figure 3.

A total of 19 supported clades were recovered through a phylogenetic analyses (Figure 3). Sequences corresponding to Uruguayan specimens

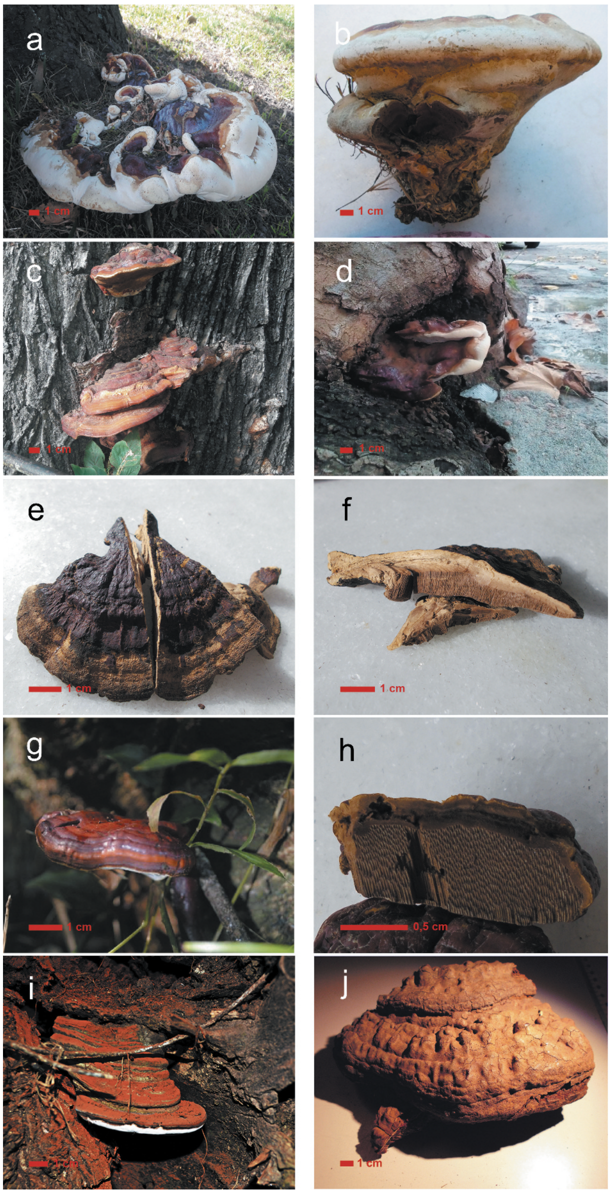


Figure 1. Basidiomata of *Ganoderma* species from Uruguay. *G. martinicense*: (a) (MVHC 5583) and (b) (MVHC 5635), *G. platense*: (c) (MVHC 5586) and (d) (MVHC 5687), *G. mexicanum* (MVHC 5652): (e) and (f), *G. dorsale*: (g) (MVHC 5648) and (h) (MVHC 5655), *G. australe* M2 (MVHC 5587): (i) *G. australe* M1 (MVHC 5582): (j).

were distributed in six terminal clades representing the following species (BPP/BS): *Ganoderma platense* Speg. (1/98), *Ganoderma dorsale* (Lloyd) Torrend (1/100), *G. martinicense* Welti & Courtec. (0.98/80), *G. mexicanum* Pat. (1/100) and *Ganoderma australe* s.l. composed of two terminal clades: one of them termed clade 1 (0.97/73), composed of specimens of *G. australe* M1 and the other one termed clade 2 (0.91/68) and composed of specimens of *G. australe* M2. The remaining clades represent next species: *G. multipileum* Hou. (0.96/97), *G. multiplicatum* (Mont.) Pat. (1/90), "*G. lucidum*" sensu authors [22] (0.76/94), *G. tuberculosum* Murrill (1/100), *G. concinnum* Ryvarden (1/100), *G. lucidum* (1/100), *G. resinaceum* (0.9/59), *G. polychromum* Murrill (1/96), *G. sessile* (0.99/82), *G. zonatum* Murrill (1/100), *G. ecuadoriense* W.A. Salazar, C.W. Barnes & Ordoñez (1/100) and two unnamed taxa of the *G. australe/applanatum* complex from the Neotropic and Asia (1/89) and the Northern Hemisphere (1/88).

Key to *Ganoderma* species from Uruguay

1. Reddish to wine-purplish pileus surface, glossy to opaque ........ 2

1´. Greyish brown pileus surface, matte, never reddish ........ *G. australe* s.l. **5**

2. Usually robust basidiomata, mostly sessile to occasionally substipitate ........ **3**

2´. Stylized basidiomata, slender, laterally to eccentrically stipitate ........ **4**

3. Pores 4–6/mm, basidiospores with thick endosporic pillars........ *G. martinicense*

3´. Pores 3–4/mm, basidiospores with thin and tiny endosporic pillars........ *G. platense*

4. Vertically stipitate basidiomata, brownish context and basidiospores with prominent endosporic pillars........ *G. dorsale*

4´. Horizontally stipitate basidiomata, cream colored context and basidiospores with tiny endosporic pillar........ *G. mexicanum*

5. Conspicuosly, tuberculous pileus surface, sometimes presenting concentric zones. . ........ *G. australe* M1

5´. Concentrically zonated to slightly tubercular pileus surface........ *G. australe* M2

## Discussion and integrative taxonomy

The taxonomic status of *Ganoderma* species in Uruguay was evaluated through ITS-based phylogenetic analyses in combination with morphological and ecological data. The topology recovered in our phylogenetic analyses is congruent with previous works [4–6]. Three main clades were recovered. One of them is composed of *Ganoderma* species with reddish laccate basidiomata, stipe and pilear surface, including traditional *G. lucidum* and *G. resinaceum* complexes (0.99/67). The second one is composed of almost-sessile *Ganoderma* species with non-laccate pilear surface, including *G. australe* and *G. applanatum* complexes (1/76). The third one is a small subclade of 2 reddish laccate species including *G. zonatum* and *G. ecuadoriense* (0.96/66). Studied Uruguayan

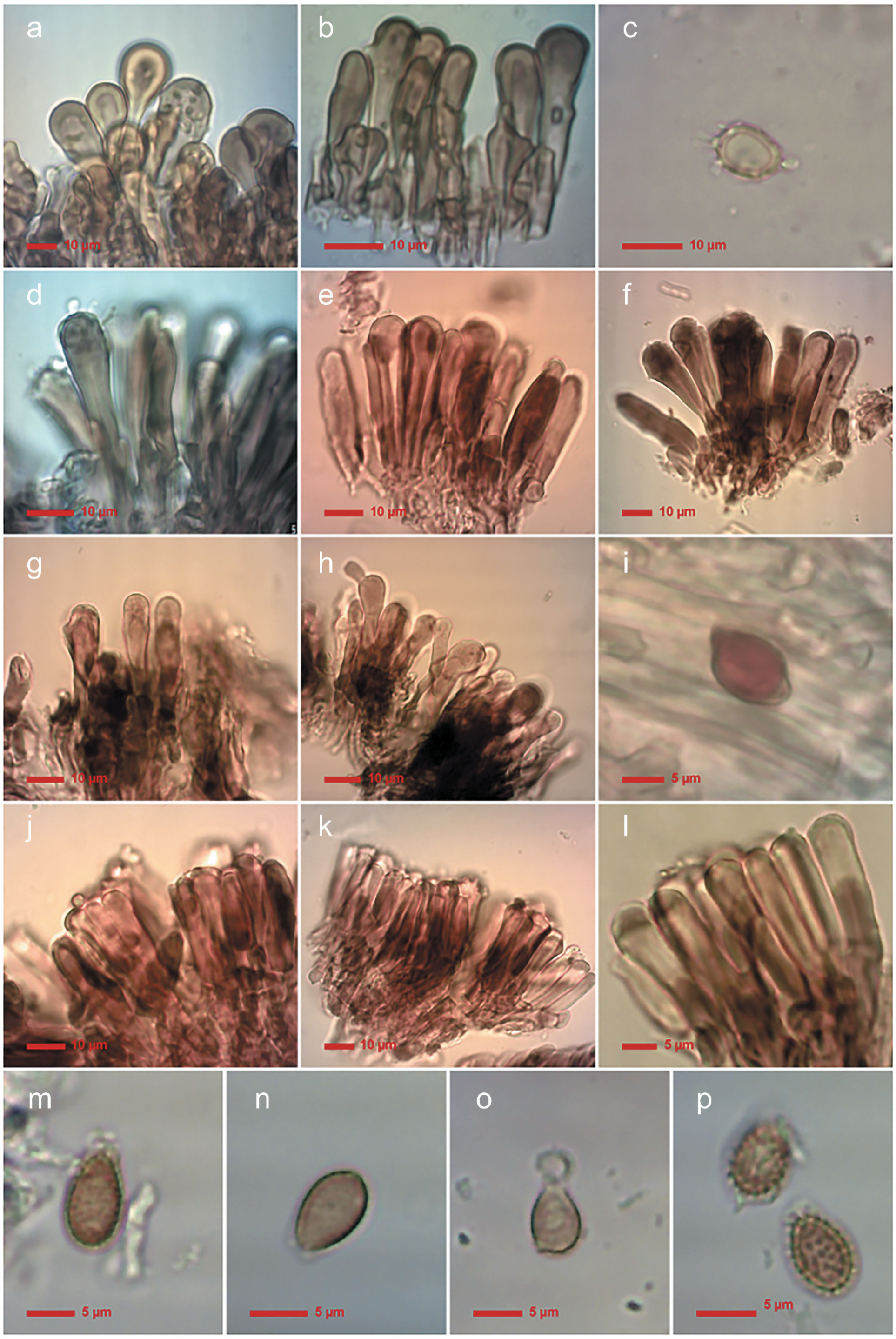


**Figure 2.** Microscopic features of laccate *Ganoderma* species from Uruguay. Pilear cells: *G. martinicense* (MVHC 5635): (a,b), *G. platense* (MVHC 5687): (d–f), *G. mexicanum* (MVHC 5652): (g–h), *G. dorsale* (MVHC 5655): (j–l). Chlamydospores from fresh *G. martinicense* (MVHC 5635) culture in MEA: (c) and dextrinoid characteristic chlamydospores from the context of *G. mexicanum* (MVHC 5652): (i). Basidiospores from *G. martinicense* (MVHC 5635): (m), *G. platense* (MVHC 5687): (n), *G. mexicanum* (MVHC 5652): (o) and *G. dorsale* (MVHC 5655): (p).

*Ganoderma* specimens are distributed in six clades representing species whose taxonomic resolutions are discussed below.

*Ganoderma martinicense* is characterized by developing short stipitate basidiomata of large dimensions (up to 30 cm upper view), with the pilear surface concentrically zonate, with melanoid incrustations in the context, pores 5–6/mm, basidiospores measuring 10–12 × 5–7 µm and non-amyloid pear-shaped to shortly cylindrical cutis cells. The species is distributed from SW North America through the Caribbean and the Neotropical Atlantic Rain Forests, [11,56], reaching Uruguay and NE Argentina. Ten of the studied specimens fit very well with that morphological description. Moreover, they are grouped with *G. martinicense* specimens, including the type in a strongly supported clade (1/88). Other names were used to label sequences of specimens that are conspecific with sequences of this

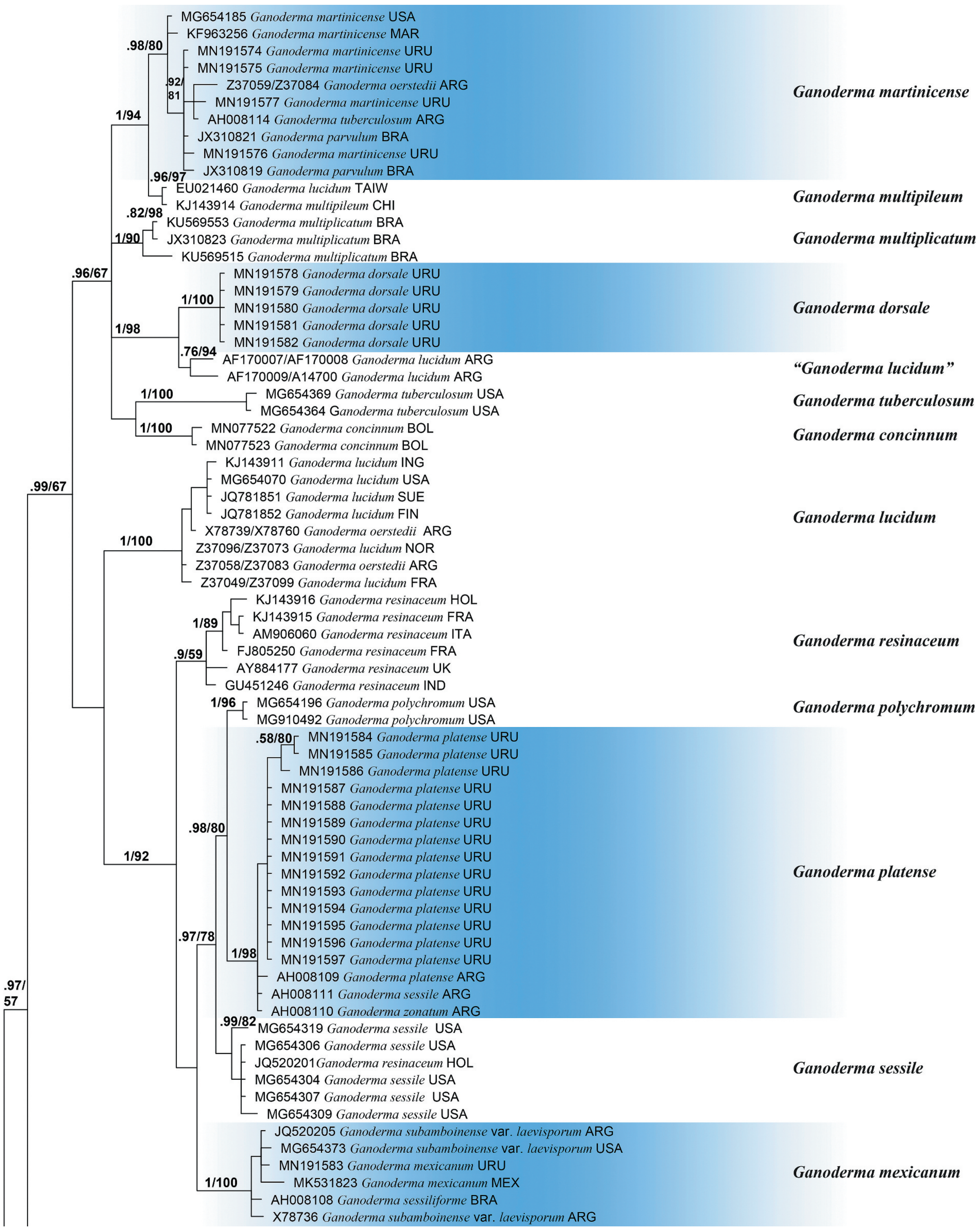

Figure 3. Consensus Bayesian Inference tree based on ITS sequence data used for positioning *Ganoderma* species found in Uruguay (highlighted in light blue). Values of Bayesian and Maximum likelihood are presented as PPB/BS. *Cristataspora coffeata* (FLOR 50933) and *Foraminispora rugosa* (FLOR 52191 and HUEFS DHCR560) were used as outgroup.

lineage (Figure 3), i.e. *G. tuberculosum* [7], *G. oerstedii* (Fr.) Murrill [13] *and G. parvulum* Murrill [57]. *Ganoderma tuberculosum* and *G. parvulum* form two different, distant and unrelated lineages [6,11]. *Ganoderma oerstedii* has been described as presenting a context lacking resinous lines, longer basidiospores with semi-rough columnar ornamentations: 12–15 × 8–10 μm [12] and 9–14 × 6–9 μm [19]. The pileipellis is formed by irregular, lobed and branched cells with up to seven short, wide protuberances [25]. Sequences of specimens from the type locality (San Juan de Puerto Rico) need to be included to determine

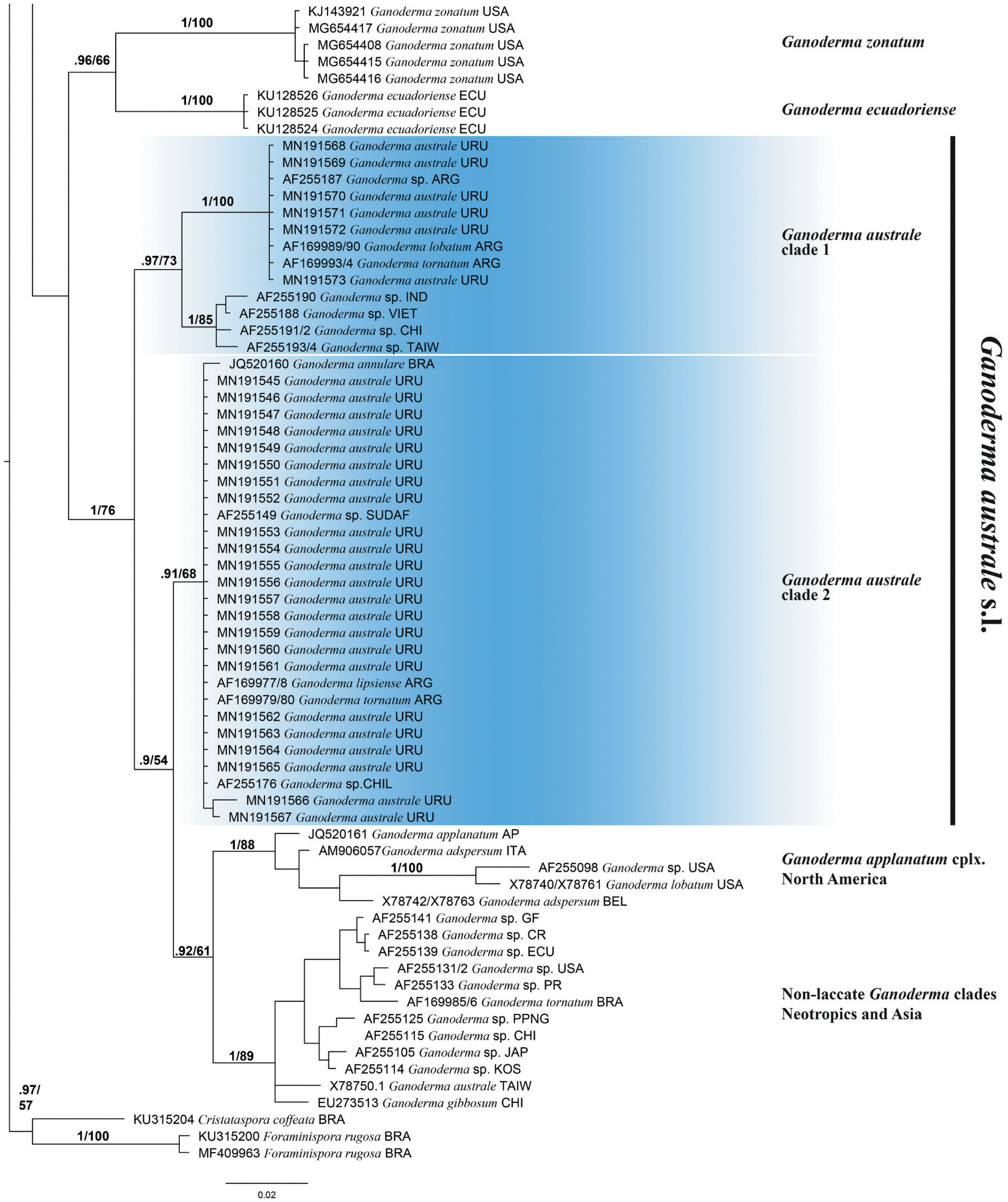


**Figure 3.** Continued.

the taxonomic status and the phylogenetic relationships of *G oerstedii*. The closest relative of *Ganoderma martinicense* is *G. multipileum* (1/94), an Asian species with small pores: 6–8/mm [9].

Five sequences of Uruguayan specimens are grouped together in a distinct, well-supported clade (1/100), unrelated to any available sequence. Specimens of this clade are characterized by a stylized basidiomata, laterally to centrally slender stipite, shiny reddish orange to violet colored radial and concentric zonate pilear surface, and melanoid bands in the context. Microscopically, the cutis is composed of clavate cells (Figure 2) and basidiospores with thick, often anastomosed endosporic ornamentations. The macro-morphological features suggest some Neotropical taxa including *Ganoderma elegantum* Ryvarden [12], *G. concinnum* [23] and *G. dorsale* [58]. *Ganoderma elegantum* is characterized by pores 6–7/mm, branched cutis cells (with up to six branches) and basidiospores with thin endosporic ornamentations [12]. *Ganoderma concinnum*, recorded in Colombia and Bolivia, is characterized by the formation of basidiomata with slender stipes of up to 20 cm long and it forms a different phylogenetic lineage [12,53]. *Ganoderma dorsale* (= *G. lucidum* var. *dorsale* in accordance with Torres-Torres et al. [25]) was described from Río Grande Do Sul in southern Brazil [59]. The macro and micro morphological characteristics of the specimens of this lineage agree with the description of the type specimen of *G. dorsale* [22,25] and fit well with the studied specimens: basidiomata shape and size, presence of melanoid lines in the context, basidiospores' shape and size, chemical reactions and shapes of pileipellis cells. Thus, considering morphology and distribution, *Ganoderma dorsale* is the most suitable name for the new clade of Uruguayan specimens. The closest relative is a phylogenetic clade named "*G. lucidum*" from NW Argentinean Yungas [7]. More sequences and morphological reassessments are necessary to evaluate the taxonomic status of the "*G. lucidum*" clade sensu Gottlieb et al. [7].

*Ganoderma multiplicatum*, also occurring in the Neotropics, is morphologically differentiated by its cutis cells with numerous protuberances [25,57,60,61]. The phylogenetic relationship between *G. multiplicatum, G. martinicense, G. multipileum, G. dorsale*, "*G. lucidum", G. tuberculosum* and *G. concinnum* remains unsolved.

*Ganoderma platense* is characterized by developing sessile basidiomata, usually imbricated, with a semi-orbicular zonate pilear surface context, usually with inconspicuous and discontinuous melanoid deposits, thin dissepiments and large pores (2–4/mm), pileipellis formed by cylindrical to slightly claviform elements, with apical constrictions and basidiospores measuring 9–13 × 5–8 µm, with thin, endosporic ornamentations [7,22,62]. The morphological characteristics of the studied specimens constituting this clade (Figures 1 and 2) agree with the description of *G. platense* and this name is hereinafter applied to the clade. Originally described from Buenos Aires (Argentina), it is currently known for growing on *Platanus acerifolia* trees of urban ecosystems of its type locality and on stumps of gallery forests of the Parana and Uruguay rivers [7,22,62]. Within the *G. platense* clade, two sequences correspond to specimens labelled as *G. sessile* and *G. zonatum* [7]; however, both species were phylogenetically circumscribed to distinct North American clades and are characterized by growing on hardwoods wood and monocots, respectively [11]. *Ganoderma platense* is grouped in the so called "resinaceum clade", together with *G. resinaceum* s.l. in Cabarroi-Hernández et al. [6], *G. polychromum* and *G. sessile* sensu Loyd et al. [11]. *Ganoderma resinaceum* encompasses North American, European and Asian populations, including more than one phylogenetic species. Nevertheless *G. resinaceum sensu auctores* from Eurasian descriptions present cutis cells with laterally diverticulate branches [22,25,63,64]. It contrasts with *G. platense*, which presents cylindrical to slightly clavate cells with apical constrictions. *Ganoderma polychromum* (Copel.) Murrill is distributed in North America, growing on hardwoods and characterized by a context without melanoid deposits, smaller pores (4–5 mm) and larger basidiospores measuring 10.8–13.2 × 6–7.5 µm [11].

*Ganoderma mexicanum* is known for its occurrence in Brazil, Colombia, Costa Rica, Cuba, French Guiana, Mexico, Nicaragua, South–eastern USA (Florida) [6], and now reported in Uruguay.

Morphologically, the studied specimen presents the diagnostic characters of light-colored context with variably abundant dextrinoid, smooth chlamydospores [6].

The sequences of non-laccate *Ganoderma* specimens collected in Uruguay were distributed in two clades: one of them with Southern Hemisphere specimens and the other one with specimens from South America and Asia [5]. Although the morphological analysis did not allow us to discriminate the specimens of each clade, the pilear surface generally appears tubercular and non-zonate in *G. australe* M1 and concentrically zonate *G. australe* M2. In addition, there seems to be ecological differences regarding host preferences: *G. australe* M1 specimens develop basidiomata on living hosts, whereas *G. australe* M2 on dead hosts. It was established that species of the *G. australe/ applanatum* complexes could have a recent origin (not earlier than 30 Ma), with a distribution pattern explained by a large-scale, episodic colonization model and subsequent distance isolation [5]. The recent origin, in addition to the remaining interfertility between specimens from both clades [5], may be the plausible explanation of the crypticity and lack of clear morphological differences for the specimens of the two clades of *G. australe*. Many names were proposed for sequence of specimens grouped in each clade: *G. tornatum* (Pers.) Bres., *G. lobatum* (Schwein.) G.F. Atk., *G. annulare* (Lloyd) Boedijn and *G. lipsiense* [7]. *Ganoderma annulare* and *G. tornatum* are largely considered synonyms of *G. australe*, and *Ganoderma lipsiense* was long considered a synonym of *G. applanatum* [21,25]. *Ganoderma applanatum* type locality is in the Northern Hemisphere and previous phylogenetic analyses suggested that it could be represented by a Northern Hemisphere clade [5,8]. Morphologically, *G. applanatum* lacks melanoid lines in the context [25], whereas all Uruguayan specimens present melanoid elements in the context. From the morphological, ecological and distribution data, M1 and M2 specimens should remain as *G. australe* s.l.

In this context, the previous records of *Ganoderma* in Uruguay (Table 1) should be questioned, and due to the absence of herbarium specimens, only speculations can be made in relation to published descriptions. Records of *G. lorenzianum* [65,66] have characteristics that resemble *G. mexicanum* due their morphological similarities regarding light colored context (picture in reference [67]), stipe and ovoid, smooth basidiospores (9–10 × 6–7 µm). *G. lorenzianum* is an earlier name than *G. mexicanum* but the description and picture offered in bibliography [67] and the low number of specimens of *G. mexicanum* analyzed in Uruguay is insufficient to assess the epitypification of these species. Focused studies on specimens corresponding to this species are urgently needed. In Uruguay, *Ganoderma orbiforme* (Fr.) Ryvarden (=*G. fornicatum* (Fr.) Pat.) was recorded by Felippone [66], but it currently forms a different phylogenetic clade [57] and no specimens or sequences corresponding to this species were recorded in the country. *Ganoderma nitens* was recorded by Patouillard [65] characterized by warty basidiospores, 10 × 7 µm, the type is currently lost [68], so this record could be attributed to *G. dorsale*, a single reddish laccate *Ganoderma* species of Uruguay with warty spores.

## Conclusions

An integrated comprehensive approach was carried out using morphological, molecular and ecological evidence to assess the diversity of *Ganoderma* in Uruguay. A total of five species (forming six supported clades) were found through the analysis of 163 basidiomata and 53 sequences. Among those species, *G. martinicense, G. mexicanum, G. platense* and *G. dorsale* were confirmed and identified for the first time in Uruguay. Particularly, *G. platense* and *G. dorsale* were first recovered in the phylogenetic analyses. On the other hand, non-laccate specimens were distributed in two clades and so far considered as *G. australe* s.l.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

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