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Rare *Pleurotus* species with veiled basidiomata from the Neotropics: neotypification of *Pleurotus magnificus* and epityfication of *Pleurotus rickii*

FELIPE BITTENCOURT[1,4,*], MARIANA DE PAULA DREWINSKI[2,3,5], NELSON MENOLLI JR.[2,3,6] & ELISANDRO RICARDO DRECHSLER-SANTOS[1,7]

[1] *Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas, Departamento de Botânica, Universidade Federal de Santa Catarina, Campus Universitário Trindade, CEP: 88040-900, Florianópolis, Santa Catarina, Brazil.*

[2] *Núcleo de Pós-graduação Stricto Sensu, Pós-graduação em Biodiversidade Vegetal e Meio Ambiente, Instituto de Pesquisas Ambientais, Av. Miguel Stefano 3687, Água Funda, 04301-012, São Paulo, São Paulo, Brazil.*

[3] *IFungiLab, Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP), Câmpus São Paulo (SPO), Departamento de Ciências da Natureza e Matemática (DCM), Subárea de Biologia (SAB), Rua Pedro Vicente 625, 01109-010, São Paulo, São Paulo, Brazil.*

[4] *94bittencourt@gmail.com; https://orcid.org/0000-0003-4285-6213*

[5] *maridrewinski@gmail.com; https://orcid.org/0000-0002-7299-8477*

[6] *menollijr@yahoo.com.br; https://orcid.org/0000-0002-1841-8179*

[7] *drechslersantos@yahoo.com.br; https://orcid.org/0000-0002-3702-8715*

* *Corresponding author*

Abstract

Knowledge of *Pleurotus* in Brazil dates back to the 19th century, when European mycologists studied and described numerous Neotropical taxa. Some of these long-described species remain poorly known, mostly due to the lack of recent records, detailed morphological descriptions, and molecular data. During field surveys in Southeast and Southern Brazil, specimens of two rare *Pleurotus* species with veiled basidiomata, i.e. *P. magnificus* and *P. rickii*, were collected. These species are recorded for the first time in the state of Santa Catarina, Brazil, and *P. magnificus* for the first time in the states of Minas Gerais, Paraná and São Paulo. Updated and detailed morphological descriptions are provided. DNA sequences of the ITS and nLSU molecular markers were obtained for both species, and phylogenetic analyses were conducted to explore their relationships within *Pleurotus*. A neotype is designated for *P. magnificus*, and a lectotype and epitype are designated for *P. rickii*.

Key words: Atlantic Forest, lectotypification, phylogeny, taxonomy, threatened species.

Introduction

The history of mycology in Brazil is deeply intertwined with its colonization. After a few contributions during the 18th century, mainly by Portuguese naturalists, Brazilian ports opened to other European countries in the early 19th century (Fidalgo 1985). Throughout the 19th and early 20th centuries, Brazilian fungi were predominantly studied by European mycologists who either visited Brazil (e.g. Rolf Singer), settled here (e.g. Alfred Möller, Johannes Rick), or received samples from colleagues in Brazil (e.g. Giacomo Bresadola). These centuries were marked by the description of numerous species, a period when morphological comparison was challenging since many obscure publications were not widely available and many descriptions lacked morphological details.

Pleurotus (Fries 1821: 178) P. Kummer (1871: 24) is a well-known genus of agarics of significant economic importance due to their edibility and medicinal use (Li *et al.* 2021). Guzmán (2000) recognized 24 species of *Pleurotus*, but this number has increased as many new species have been described in recent decades. Although extensively studied, several taxonomic complexes are known within the genus [e.g. *P. djamor* (Rumphius ex Fries 1821: 185) Boedijn (1959: 292), *P. eryngii* (DeCandolle 1815: 47) Quélet (1872: 112) and *P. ostreatus* (Jacquin 1774: 3) P. Kummer (1871: 104) (Vilgalys *et al.* 1993, Liou 2000, Zervakis *et al.* 2014, 2019)], and some species are poorly understood, with knowledge based primarily on outdated publications and a few, sometimes lost, collections [e.g. *P. aggregatus* Bresadola (1896: 276), which lacks preserved material and DNA sequences (Menolli *et al.* 2014)].

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Recently, some taxonomic studies have focused on 'rediscovering' previously described species (Gargano *et al.* 2011, Liu *et al.* 2016), highlighting the importance of revisiting historical works.

Valuable contributions to *Pleurotus* diversity in Brazil were made mainly by Rolf Singer (1951, 1952, 1954, 1957, 1973, 1989), one of the most important mycologists on agarics of the 20th century. Father Johannes Rick, regarded as the father of Brazilian mycology (Fidalgo 1962), also made substantial contributions in the early decades of the 20th century, with his extensive knowledge of fungi from Southern Brazil (Rick 1906, 1907, 1930, 1937, 1938, 1961). Additional notable contributions on the knowledge of *Pleurotus* in Southern Brazil were made by Pereira (1988) and Putzke (2002). Pereira (1988) revisited the diversity of *Pleurotus* in Rio Grande do Sul, primarily based on Rick's contributions and collections, but he was unable to study some seemingly rare species such as *P. anastomosans* Rick (1930: 116) and *P. magnificus* Rick (1906: 22) due to the lack of preserved material.

More recently, Menolli *et al.* (2014) conducted a comprehensive study on the diversity of *Pleurotus* in Brazil, which included DNA sequencing and phylogenetic analysis of five species found in the country. This study included a poorly known species described after Rick's collections in Southern Brazil, which had never been sequenced before: *Pleurotus rickii* Bres. (1920: 27). Additionally, the authors listed 62 *Pleurotus* names reported for Brazil, many of which are treated as synonyms, *nomina dubia*, species from other genera, or lack preserved material or recent collections, many from past centuries. Given the high number of uncertain names of *Pleurotus* reported for Brazil, it is necessary to integrate this historical knowledge into contemporary taxonomic research of Brazilian funga.

During field surveys in the Atlantic Forest of South and Southeast Brazil, specimens of *Pleurotus* were collected and identified as *P. magnificus* and *P. rickii*. Our aim was to review the taxonomic status of both species thorough bibliographic research, provide updated morphological descriptions, and investigate their phylogenetic relationships within the genus *Pleurotus* using new DNA sequences.

Material & Methods

Sampling

The specimens were collected in the South and Southeast regions of Brazil, within the *Araucaria* Forest, Atlantic, and Parana Forest provinces according to the regionalization proposed by Morrone (2014). A map illustrating the distribution of the species (Fig. 1) was created using QGIS 2.28.3 'Firenze' (QGIS Development Team 2023), utilizing shapefiles provided by Morrone *et al.* (2022), IBGE (https://ibge.gov.br/), and Efrain Maps (http://www.efrainmaps.es). The specimens were deposited in FIFUNGI, FLOR, FURB, and SP Herbaria [acronyms follow Thiers (2014 [continuously updated], http://sweetgum.nybg.org/science/ih/)].

Morphological analysis

Macroscopic descriptions were based on fresh and dried basidiomata, with terminology following Senthilarasu & Kumaresan (2018) and colors according to Kornerup & Wanscher (1978). Basidiospore shapes were described following Bas (1969) classification. For microscopic studies, free-hand sections of dried basidiomata were prepared using 5% KOH plus 1% phloxine or 1% Congo red as the mounting medium (Largent *et al.* 1977, Ryvarden 1991). Measurements were made using ImageJ software (Abràmoff *et al.* 2004). The size of microscopic elements is given as values (or an interval) followed by a 5% variation in parentheses, when pertinent. The notation [a/b/c] at the beginning of a set of basidiospores data is to be read as "(a) basidiospores were measured from (b) basidiomata taken from (c) collections". The following abbreviations were used: ave. = average, diam. = diameter, Q = length/width ratio, Qm = mean length/width ratio from basidiospores measurements.

DNA extraction, PCR amplification and Sequencing

Dried basidiomata fragments were used for the DNA extraction with CTAB method according to Góes-Neto *et al.* (2005). The primer pairs ITS8-F/ITS6-R (Dentinger *et al.* 2010) and LR0R/LR7 (Bunyard *et al.* 1994) were used to amplify the ITS (ITS1-5.8S-ITS2) and nLSU (28S) markers, respectively. All PCR products were purified with PEG 20% (Polyethylene glycol 8,000 plus NaCl 2.5M), and sequencing reactions of PCR products were prepared with addition of a mix composed by 1 µl BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), 1 µl 5× Buffer, 1 µl primer (10 pmol/l), 5 µl H_2O q.s.p., and 2 µl of the purified PCR product. The same primers used to amplify the markers were used for bidirectional sequencing. Sequencing was performed by Myleus Biotecnologia, Minas Gerais, Brazil.

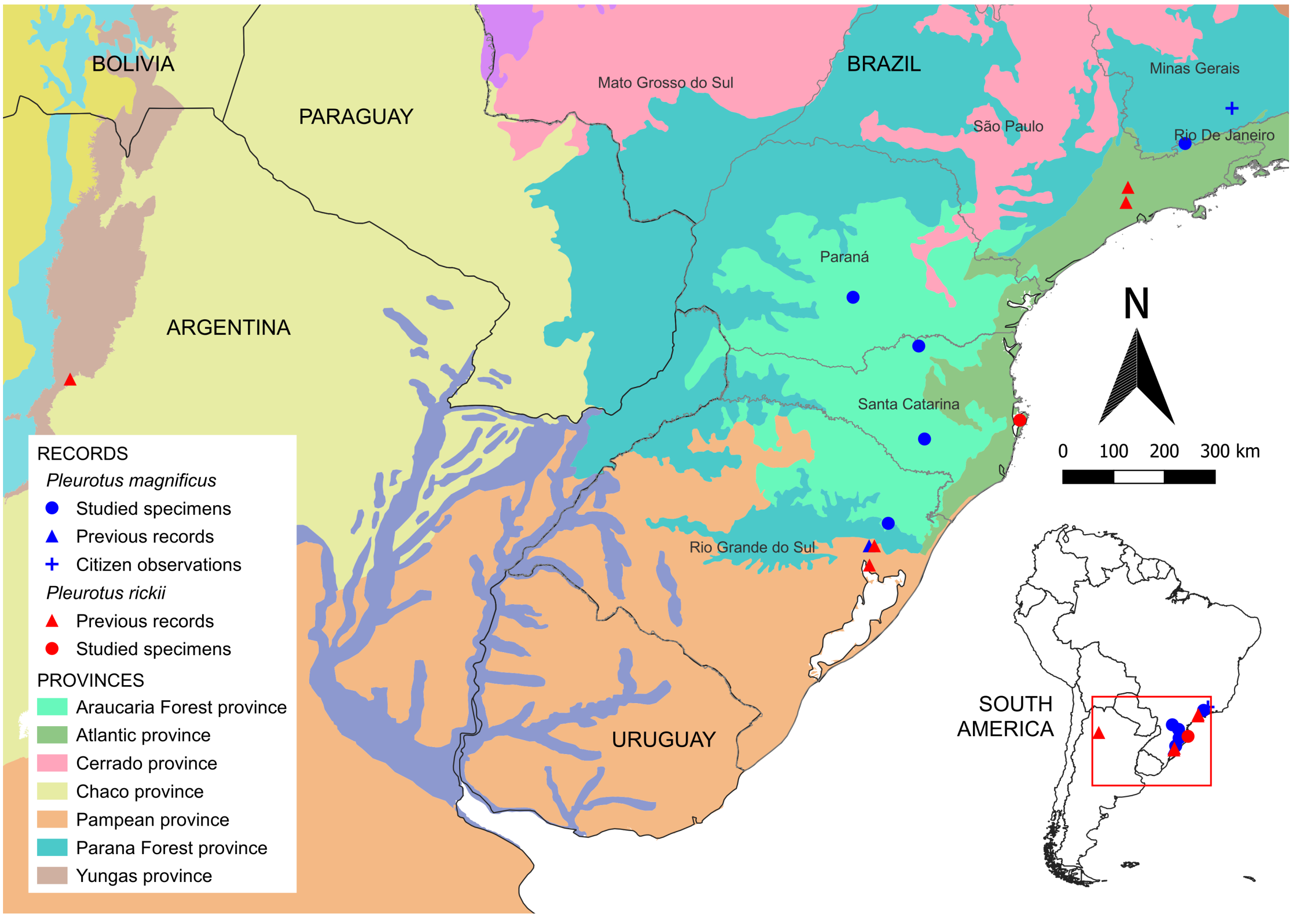

FIGURE 1. Distribution map of *Pleurotus magnificus* (blue points) and *P. rickii* (red points) in South America. Colored areas are delimitations of biogeographic provinces according to Morrone (2014).

Phylogenetic molecular analyses

For the phylogenetic analyses, a matrix of ITS and nLSU sequences was built by including 64 ITS and 42 nLSU sequences deriving from 69 *Pleurotus* specimens. All sequences are available in GenBank® (https://www.ncbi.nlm.nih.gov/) and were recovered mainly from Menolli *et al.* (2014) and Zervakis *et al.* (2019), besides other neotropical *Pleurotus* species, and morphologically-related taxa. Additionally, two specimens of *Hohenbuehelia* were designated as outgroups in both datasets based on previous studies on Agaricales (Matheny *et al.* 2006). All materials and sequences used in the molecular analyses are listed in Table 1.

Generated sequences were assembled and manually corrected with Geneious v. 6.1.8 (Kearse *et al.* 2012), then automatically aligned with MAFFT v.7 (Katoh & Standley 2013) with the remaining dataset under G-INS-i strategy. The alignment was manually adjusted in MEGA7 (Kumar *et al.* 2016) when necessary.

We used ModelFinder (Kalyaanamoorthy *et al.* 2017) implemented in the IQ-TREE software (Nguyen *et al.* 2015) to estimate the best-fit partitioning strategy and the best-fit model of nucleotide evolution for the dataset using four partitions (ITS1; 5.8S; ITS2; 28S). Models were restricted to those implemented in MrBayes 3.2 (Ronquist *et al.* 2012). Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses were applied to the dataset using the partition scheme and evolutionary models defined by ModelFinder.

ML searches were conducted with IQ-TREE software (Nguyen *et al.* 2015). The analysis initially involved 100 ML searches, each one starting from one randomized stepwise addition parsimony tree. Branch supports were calculated using the UFBoot (ultrafast bootstrap approximation) (Hoang *et al.* 2018) implemented in IQ-TREE with 1000 replications. BI was performed following Costa-Rezende *et al.* (2020) with MrBayes 3.1.2 software, performed in the CIPRES science gateway (Miller *et al.* 2011, http://www.phylo.org/). Chain convergence was determined using Tracer 1.7.2 (http://tree.bio.ed.ac.uk/software/tracer/). A node was considered strongly supported if it showed BPP $\geq$ 0.95 and/or BS $\geq$ 95% (Hyde *et al.* 2013, Minh *et al.* 2020) (EES $\geq$ 200).

TABLE 1. List of vouchers with the respective country of origin and accession number from GenBank® (https://www.ncbi.nlm.nih.gov/genbank/) of ITS and nLSU sequences used in the molecular phylogenetic analysis.

Taxon	Country	Voucher	Reference	ITS	nLSU
Hohenbuehelia atrocoerulea	Hungary	AMB:18080	Constiglio & Setti (2017)	KU355304	KU355389
Hohenbuehelia carlothornii	Costa Rica	03-RGTSN-519	Koziak *et al.* (2007)	EF409757	EF409757
Pleurotus abalonus	China	CBS 803.91	Zervakis *et al.* (2014)	AY315806	-
Pleurotus abalonus	India	CBS 615.80	Zervakis *et al.* (2014)	AY315810	-
Pleurotus abalonus	South Korea	P25	Unpublished	KY962441	KY963025
Pleurotus albidus	Argentina	BAFC 50.261	Unpublished	AF345659	-
Pleurotus albidus	Brazil	SP445812	Menolli *et al.* (2014)	KF280334	-
Pleurotus albidus	Brazil	SP445785	Menolli *et al.* (2014)	KF280335	-
Pleurotus australis	Australia	VT1953	Zervakis *et al.* (2014)	AY315758	-
Pleurotus australis	New Zealand	PDD59215	Zervakis *et al.* (2014)	AY315761	-
Pleurotus australis	unknown	RV95/568	Zervakis *et al.* (2014)	AY315762	AF261432
Pleurotus calyptratus	Czech Republic	D1839	Unpublished	-	AF135177
Pleurotus calyptratus	Slovakia	CBS 325.85	Unpublished	EU424283	EU365640
Pleurotus calyptratus	unknown	HMAS63355	Li (2005)	AY562495	AY562496
Pleurotus citrinopileatus	China	HMAS63344	Li & Yao (2005)	AY696301	-
Pleurotus citrinopileatus	China	HKAS85956	Liu *et al.* (2015)	KP867919	KP867910
Pleurotus citrinopileatus	Malaysia	FSCC1 (PCY1)	Avin *et al.* (2012)	JN234853	-
Pleurotus citrinopileatus	South Korea	P68	Unpublished	KY962484	KY963068
Pleurotus cornucopiae	England	D383	Vilgalys & Sun (1994)	U04079,U04118	U04146
Pleurotus cornucopiae	Germany	D1166	Vilgalys & Sun (1994)	U04059,U04098	U04135
Pleurotus cornucopiae	South Korea	P59	Unpublished	KY962485	KY963069
Pleurotus cystidiosus	United States	D420	Zervakis *et al.* (2014)	AY315767	-
Pleurotus cystidiosus	United States	D419	Zervakis *et al.* (2014)	AY315774	-
Pleurotus djamor	Brazil	SP392850	Menolli *et al.* (2014)	-	EU165505
Pleurotus djamor	China	d8	Li & Yao (2004)	-	AY524785
Pleurotus djamor	Papua New Guinea	CBS 100134	Unpublished	EU424287	EU365644
Pleurotus djamor	unknown	RV95/920	Moncalvo *et al.* (2000)	-	AF042575
Pleurotus djamor	unknown	CBS 665.85	Unpublished	EU424288	EU365645
Pleurotus dryinus	Germany	CBS 481.72	Vu *et al.* (2019)	EU424292	MH872241
Pleurotus dryinus	Italy	AMB:18868	Vizzini *et al.* (2024)	OR863471	OR863538
Pleurotus dryinus	Netherlands	CBS 278.90	Unpublished	AY265824	EU365647
Pleurotus dryinus	Netherlands	CBS 804.85	Unpublished	EU424294	EU365651
Pleurotus dryinus	United States	ECS-1108	Huerta *et al.* (2010)	GU722278	-
Pleurotus euosmus	South Korea	P100	Unpublished	MG282440	MG282499
Pleurotus euosmus	South Korea	P147	Unpublished	MG282486	MG282546
Pleurotus euosmus	United Kingdom	CBS 307.29	Unpublished	AY368659	EU365654
Pleurotus ferulaginis	Italy	PN10	Zervakis *et al.* (2014)	KF743829	-
Pleurotus ferulaginis	Slovenia	HIK132	Zervakis *et al.* (2014)	HM998831	-
Pleurotus fuscosquamulosus	Brazil	CCIBt 2404	Menolli *et al.* (2014)	KF280331	-
Pleurotus fuscosquamulosus	Italy	Baglivo s.n.	Vizzini *et al.* (2024)	OR863472	-
Pleurotus giganteus	Australia	MEL:2382605	Unpublished	KP012913	-
Pleurotus giganteus	China	LS	Unpublished	HM245780	-
Pleurotus giganteus	Thailand	CMU54-1	Kumla *et al.* (2013)	JQ724360	JQ724361
Pleurotus levis	Mexico	IE-771	Unpublished	KC894735	-
Pleurotus levis	South Korea	P136	Unpublished	MG282475	MG282535

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TABLE 1. (Continued)

Taxon	Country	Voucher	Reference	ITS	nLSU
Pleurotus levis	United States	DPL6135	Petersen *et al.* (2015)	KP026244	-
Pleurotus magnificus	Brazil	FIFUNGI 393	This study	PP836276	-
Pleurotus magnificus	Brazil	FLOR 79344	This study	PP836277	PP836273
Pleurotus magnificus	Brazil	FURB 44086	This study	PP836278	PP836274
Pleurotus opuntiae	Italy	SAF 250	Zervakis *et al.* (2019)	MH620770	MK182779
Pleurotus opuntiae	Italy	SAF 251	Zervakis *et al.* (2019)	MH620771	MK182780
Pleurotus opuntiae	Italy	SAF 252	Zervakis *et al.* (2019)	MH620772	MK182781
Pleurotus ostreatus	Austria	AFTOL-ID 564	Unpublished	AY854077	AY645052
Pleurotus ostreatus	France	CBS 291.47	Unpublished	EU424309	-
Pleurotus placentodes	China	HKAS51745	Liu *et al.* (2015)	KR827693	KR827695
Pleurotus placentodes	China	HKAS57781	Liu *et al.* (2016)	KR827694	KR827696
Pleurotus pulmonarius	Brazil	CCIBt 2963	Menolli *et al.* (2014)	KF280340	-
Pleurotus pulmonarius	Netherlands	CBS 507.85	Vu *et al.* (2019)	-	MH873590
Pleurotus pulmonarius	New Zealand	ICMP 18163	Unpublished	MH395973	MH395998
Pleurotus pulmonarius	United States	MO 324286	Unpublished	MK049947	MK049945
Pleurotus purpureoolivaceus	unknown	RHP3588.8	Moncalvo *et al.* (2000)	-	AF042576
Pleurotus rattenburyi	Australia	CBS 175.94	Unpublished	EU424315	-
Pleurotus rattenburyi	South Korea	P112	Unpublished	MG282451	MG282511
Pleurotus rickii	Brazil	SP445788	Menolli *et al.* (2014)	KF280341	-
Pleurotus rickii	Brazil	SP445787	Menolli *et al.* (2014)	KF280342	-
Pleurotus rickii	Brazil	FLOR 79342	This study	PP836279	PP836275
Pleurotus smithii	Mexico	CBS 680.82	Unpublished	EU424317	-
Pleurotus smithii	Mexico	P105	Unpublished	MG282445	MG282504
Pleurotus tuberregium	Indonesia	NedaS467	Isikhuemhen *et al.* (2000)	AF109975	-
Pleurotus tuberregium	unknown	ACCC50657	Unpublished	EU424319	EU365676

Results

Molecular phylogeny

For this study we generated four new sequences of ITS and three of nLSU markers. The final alignment resulted in 1775 characters, of which 616 were variable positions and 486 were parsimony-informative positions. The overall topologies obtained from ML and BI analyses of both datasets were highly congruent for well-supported clades, and only the best-scoring ML tree is displayed (Fig. 2). The retrieved *Pleurotus* clade was recovered with maximum support values (BS and PP), which includes five sequences obtained from *P. magnificus* (three ITS, two nLSU) and two from *P. rickii* (one ITS and nLSU each).

Newly provided sequences of *P. rickii* from Southern Brazil clustered in a clade with maximum support (100/1) with two other sequences of *P. rickii* previously reported from Southeastern Brazil (Menolli *et al.* 2014). *Pleurotus rickii* is sister to a clade containing sequences of *Pleurotus citrinopileatus* Singer (1943: 149), *Pleurotus cornucopiae* (Paulet 1808–1835: pl. 28) Quélet (1885: 278), and *Pleurotus euosmus* (Berkeley in Hussey 1847: pl. 75) Saccardo (1887: 358) (98/1), forming a clade with full support (100/1). Sequences of *P. magnificus* clustered with maximum support (100/1), but the analysis was unable to resolve its relationships with other *Pleurotus* species except for placement within the terminal clade of species subtended by *P. dryinus* (Persoon 1800: 96) P. Kummer (1871: 104) (-/0.99).

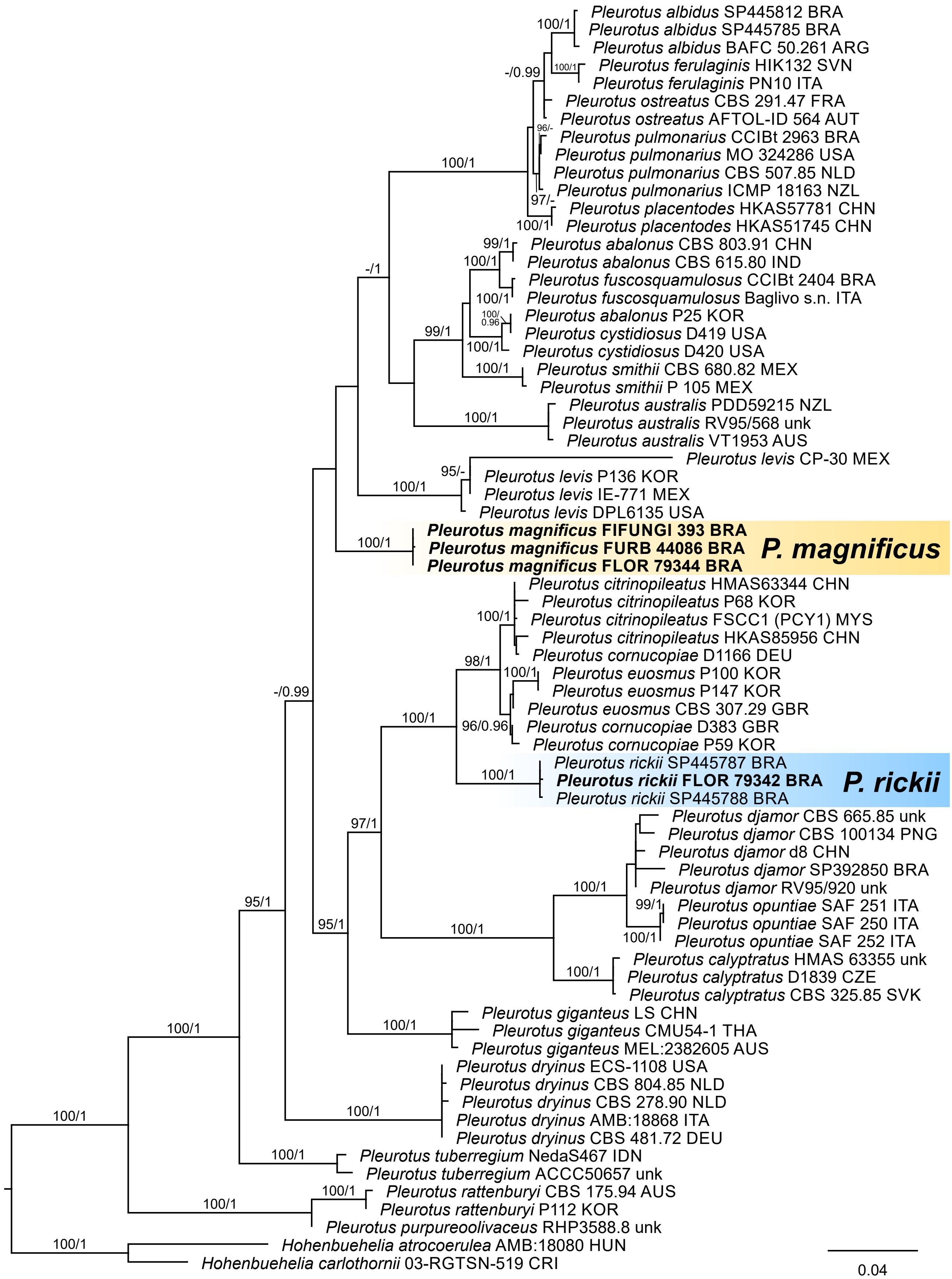

FIGURE 2. Best tree of Maximum Likelihood analysis of *Pleurotus* based on ITS and nLSU molecular markers. Branch support values are shown as BS/BPP above or below branches when ≥95 for bootstrap and ≥ 0.95 for Bayesian posterior probabilities. Two specimens of *Hohenbuehelia* were selected as outgroups. Specimens sequenced in this study are highlighted in bold. Location is present at the end of each terminal as country code (ISO 3166-1), unk= unknown location.

Taxonomy

Pleurotus magnificus Rick, Brotéria, Rev. scienc. nat. Colleg. S. Fiel 5: 22 (1906). Figs 3, 4

Neotype (designated here, MBT10022153):—BRAZIL. Santa Catarina: Três Barras, Floresta Nacional de Três Barras, 824 m a.s.l., 26°12'59''S, 50°18'07''W, yellow basidioma on fallen trunk with ca. 35 cm diameter, 2 June 2014, F. Bittencourt 134 (FURB 44086!).

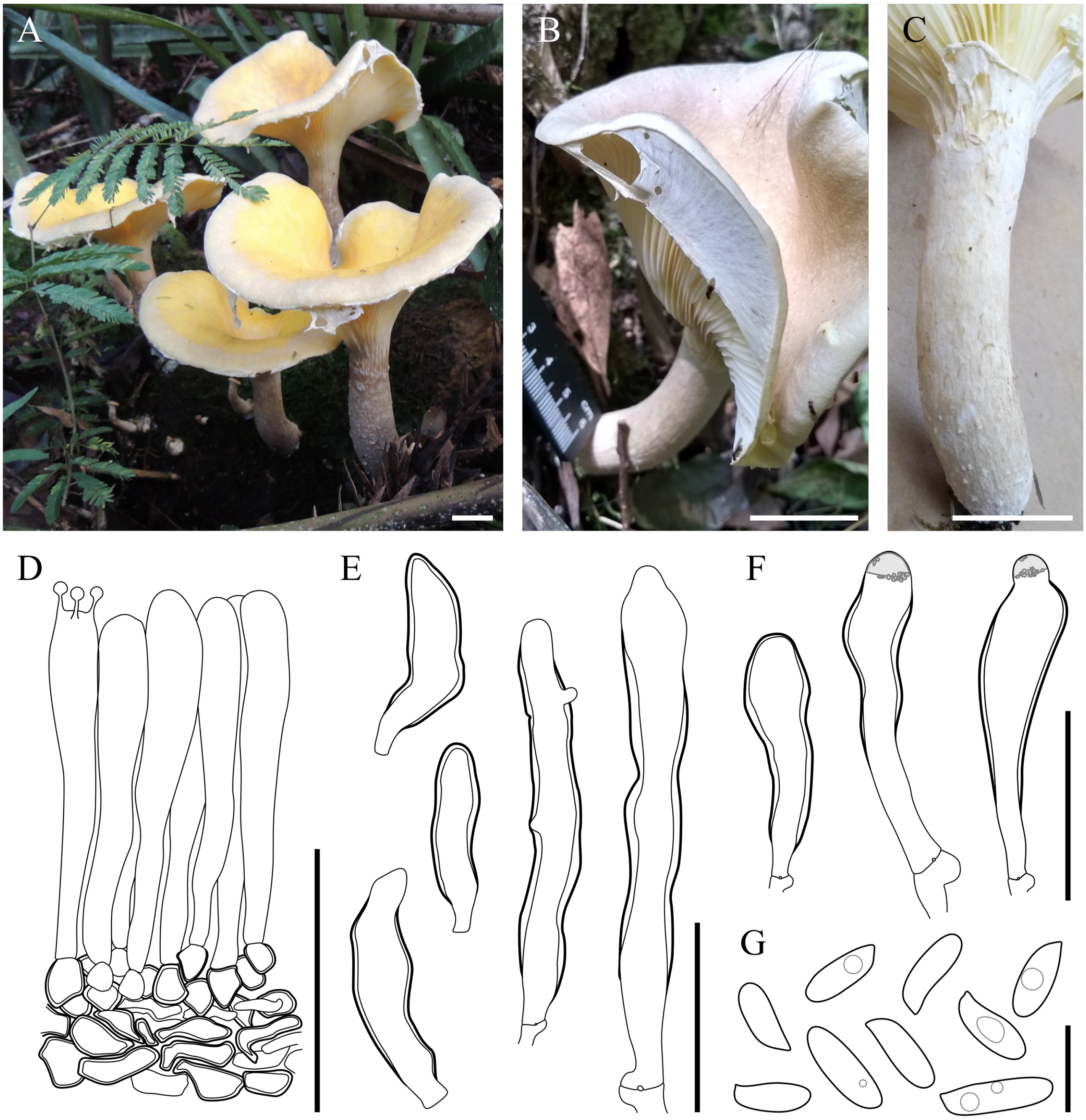

FIGURE 3. Morphological features of *Pleurotus magnificus*. A. Habit (FURB 44086, neotype); B. Detail of the partial veil remnants (SP 512761); C. Detail of the stipe and annulus (SP 512761); D. Hymenium and part of subhymenium (FURB 44086); E. Cheilocystidia (FURB 44086); F. Pleurocystidia (FURB 44086); G. Basidiospores (FURB 44086). Scale bars: A, B, C: 2 cm; D, E, F = 20 µm; G = 10 µm. Photos by F. Bittencourt (A) and M.P. Drewinski (B, C), illustrations by F. Bittencourt.

FIGURE 4. Known extant material of *Pleurotus magnificus* from 20th century. A. Rick 55 (FH 00543855); B. Plate from Theissen (1912); C. Rick 51 (Lloyd 740091). Photos provided by the Farlow Herbarium of Harvard University (A) and the U.S. National Fungus Collections (BPI) (C).

Specimens examined:—BRAZIL. Paraná: Guarapuava, Parque Municipal das Araucárias, 23 February 2018, M.P. Drewinski MPD186 (SP 512760); Jordão, Casa dos Drewinski, 12 February 2020, M.P. Drewinski MPD635 (SP 512761). Santa Catarina: Painel, Pedras Brancas, next to Rio Caveiras, 27°51'54"S, 50°11'59"W, 8 May 2018, F. Bittencourt 1078 (FLOR 79344); Três Barras, Floresta Nacional de Três Barras, 824 m a.s.l., 26°12'59"S, 50°18'07"W, yellow basidioma on fallen trunk with ca. 35 cm diameter, 2 June 2014, F. Bittencourt 134 (FURB 44086). São Paulo: São Bento do Sapucaí, Restaurante Entrevillas, 20 April 2022, Marcelo Sulzbacher leg. N. Menolli Jr. NMJ443 (FIFUNGI 393). Rio Grande do Sul: Canela, on dead wood, 21 April 2018, J.M. Timm 46/18 (FLOR 79343); São Leopoldo, in pino, 1908, J. Rick (FH 00543855) [photograph]; unknown location, J. Rick s/n, 27584 (BPI 740091 [photograph!]).

Description:—*Basidiomata* gregarious, scattered to caespitose (Fig. 3A). *Pileus* narrowly to broadly-shallowly depressed, 62–200 mm diam.; surface glabrous, smooth, wrinkled when dry, light yellow (1A4), yellowish white (4A2), yellowish grey (2B2, 2C2, 3C2), greyish yellow (4B4) when fresh, dull yellow (3B4), greyish-orange (5B5), brownish orange (5C6), orange or deep orange (6A7-8) when dry; margin often brighter, incurved when young, involute at maturity, incurved when dry; context up to 5 mm near the pileus center, yellowish white (2A2, 4A2) to yellowish gray (2B2, 4B2). *Lamellae* deeply decurrent, close, narrow, anastomosing near the stipe, pale yellow (3A3), orange gray (5B2) to grayish orange (5B3) when fresh, light brown (6D8), greyish orange (5B6) to brownish orange (6C8, 5C6) when dry; lamellar edges entire and usually darker in dried specimens; lamellulae present, 1–4 tiers. *Partial veil* present in young basidiomata, fugacious (Fig. 3B-C), but remaining for a time as appendiculate fragments on the pileus margin (Fig. 3A), single, yellowish white (4A2), annulus not seen. *Stipe* central to subeccentric, fleshy fibrous, solid, cylindrical to slightly attenuated upward, yellowish grey (4B2), greyish yellow (4B4), to brownish grey (5D2) when fresh, greyish orange (5B4-5) to brownish orange (5C5-6) when dry, 68–84 mm long, 9–23 mm thick, glabrous or with small fragile white squamules (Fig. 3C), fading in dry specimens.

Basidiospores [60/3/3] (8.4–)8.9–12.8(–13.8) × (3.2–)3.4–4.4(–4.6) µm (avg. = 10.7 × 3.9 µm), Q = 2.3–3.5, Qm = 2.8, IKI-, cylindrical to bacilliform, sometimes slightly wider at the anterior portion in side view, smooth, thin-walled, often bearing one or two guttules (Fig. 3G). *Basidia* (28–)30–46(–49) × (5.5–)6.0–8.0(–9.0) µm, cylindrical-clavate, hyaline, thin-walled or rarely thick-walled at base in some specimens, 4-sterigmate, with a basal clamp. *Pleurocystidia* (23–)28–38(–43) × 6.0–7.0(–7.5) µm, narrowly lageniform to narrowly obpyriform, seldom contracted in the median portion or close to the apex, apex obtuse, sometimes subcapitate, often apically mucronate to rostrate, thin- to thick-walled in the basal portion or toward to the apex, often bearing numerous small guttules or one wider in the apex, scattered or sometimes absent (Fig. 3F). *Cheilocystidia* 19–53(–62) × (3.5–)4.0–8.0(–10.0) µm, narrowly clavate, narrowly obpyriform, narrowly obutriform, obutriform or cylindrical, tapering towards the base, apex often flexuose, obtuse to almost acute, sometimes subcapitate, rarely mucronate to rostrate, thin- to more frequently thick-walled, rarely with small lateral tips, abundant (Fig. 3E). *Hyphal system* dimitic with clamp connections. *Lamellar trama* irregular, generative hyphae hyaline, frequently branched, sometimes very inflated, thin- to slightly thick-walled (up to 1 mm thick), (2.5–)3.0–9.0(–15) µm diam.; skeletal hyphae aseptate, rarely branched, hyaline, thick-walled (up to 2.5 mm thick), often solid, (2.5–)3.0–7.0(–8.0) µm. *Subhymenium* well developed, 16.0–30 µm wide, with pseudoparenchymatous, isodiametric, hyaline cells, (2.5–)3.0–12.5(–20) µm diam. *Lamellar edges* sterile, with cheilocystidia and slender hyphae 2–4 µm wide projecting up to 130 µm. *Pileipellis* a cutis, 11–60 µm wide, composed of generative hyphae repent to radially arranged, yellowish to brownish, thin- to slightly thick-walled (up to 0.5 µm thick), 2.0–6.0(–6.5) µm diam.; skeletal hyphae rare, similar to tramal skeletal hyphae. *Pileus hyphal system* composed of irregularly arranged thin- to slightly thick-walled (up to 1.0 µm thick), colorless and hyaline, generative hyphae (3.0–)3.5–13.5(–14.0) µm diam.; skeletal hyphae aseptate, rarely and rarely branched, hyaline, thick-walled (up to 2.5 mm thick), (3.0–)3.5–7.0 µm diam. *Stipitipellis* similar to pileipellis, composed of generative hyphae (5.0–)6.9–11.0 µm diam. *Stipe hyphal system* composed of irregularly arranged thin- to slightly thick-walled (up to 1.0 µm thick), colorless and hyaline, generative hyphae, (3.3–)3.6–7.5(–7.7) µm diam.; skeletal hyphae aseptate, rarely and rarely branched, hyaline, thick-walled (up to 2.5 mm thick), (2.8–)3–5.2(–5.7) µm diam.

Ecology and distribution:—On dead trunks of gymnosperms (FH 00543855, "in pino") and angiosperms [i.e. *Psidium* (Rick 1906)]. Associated with the *Araucaria* Forest province and high-elevation sites in lower latitudes. Currently known from Southern and Southeastern Brazil, in the states of Rio Grande do Sul (Rick 1906), Minas Gerais, Paraná, Santa Catarina, and São Paulo (*Araucaria* Forest, Parana Forest and Pampean provinces, this work) (Fig. 1).

Pleurotus rickii Bres., Annales Mycologici 18(1-3): 27. (1920). Figs 5, 6

Lectotype (designated here, MBT10022150):—BRAZIL. Rio Grande do Sul: São Leopoldo, *ad ligna*, J. Rick 135 (S-F180929 [photograph!]) (Fig. 6A-B).

Epitype (designated here, MBT10022152):—BRAZIL. Santa Catarina: Florianópolis, Unidade de Conservação Ambiental Desterro (UCAD), 27°31'52"S, 48°30'45"W, on dead fallen trunk, 8 September 2017, F. Bittencourt, G.F.A. Flores, G. Scheibler & T. Kossmann 916 (FLOR 79342!).

Specimens examined:—BRAZIL. Santa Catarina: Florianópolis, Unidade de Conservação Ambiental Desterro (UCAD), 27°31'52"S, 48°30'45"W, on dead fallen trunk, 8 September 2017, F. Bittencourt, G.F.A. Flores, G. Scheibler & T. Kossmann 916 (FLOR 79342). Rio Grande do Sul: São Leopoldo, in wood, trunk, subcespitose (*in ligna, ad truncos, subcaespitosus*), 1905, J. Rick 363 (S-F180930) (original syntype) [photograph, Fig. 6C–D].

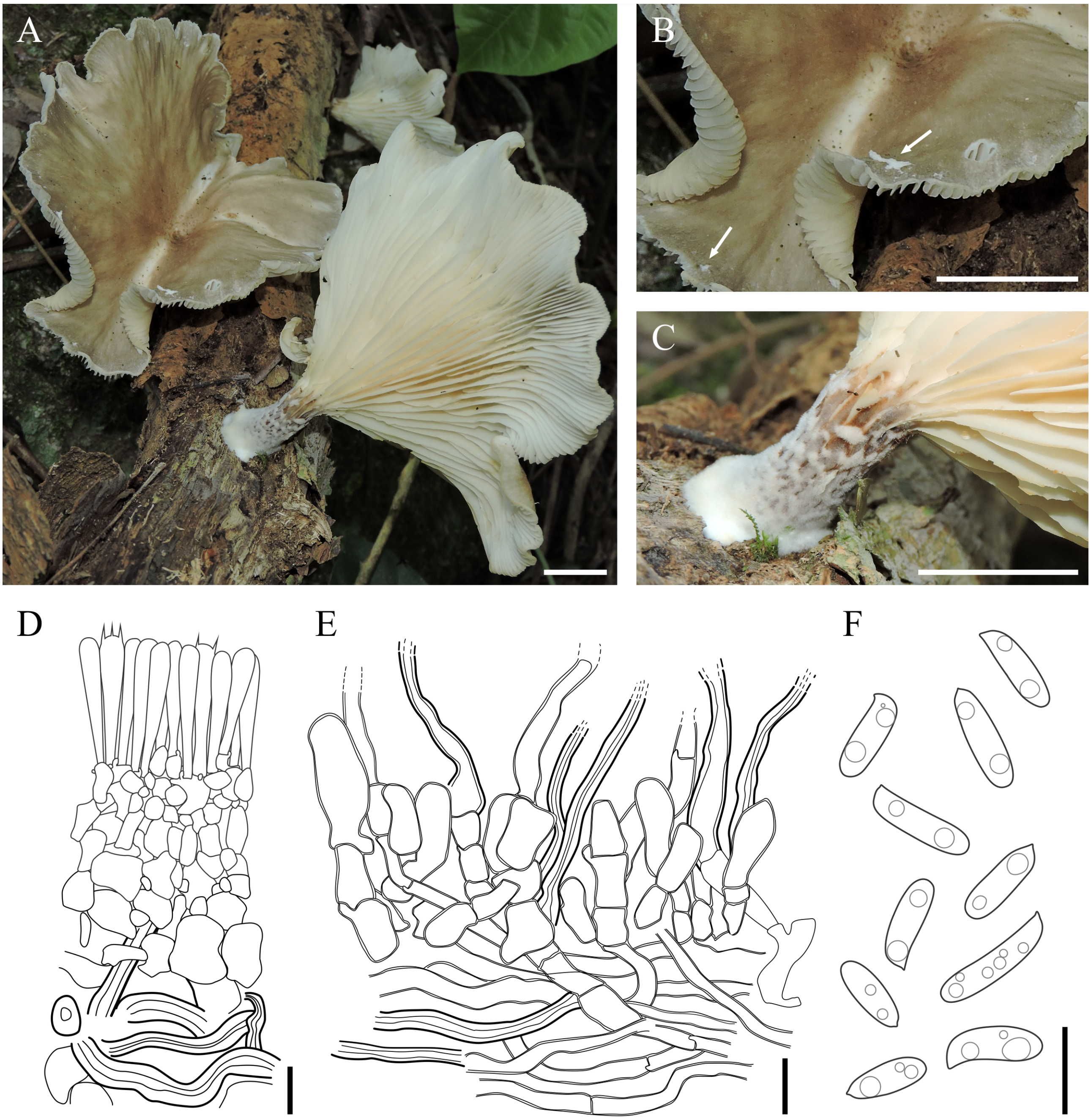

FIGURE 5. Morphological features of *Pleurotus rickii* (FLOR 79342, epitype). A. Habit; B. Pileus surface, showing the minute squamules and partial veil remnants (white arrows); C. Detail of the basal mycelium at the stipe; D. Hymenium, subhymenium and part of trama; E. Pileipellis; F. Basidiospores. Scale bars: A, B, C: 2 cm; D, E, F = 10 µm. All photos and illustrations by F. Bittencourt.

FIGURE 6. *Pleurotus rickii* syntypes at S herbarium. A: S-F180929 (Rick 135, designated here as lectotype); B: Rick's original notes of S-F180929; C: S-F180930 (Rick 263); D: Rick's original notes of S-F180930. Photos provided by the © Swedish Museum of Natural History, Stockholm; image reproduced with permission.

Description:—*Basidiomata* gregarious to scattered (Fig. 5A), rarely solitary. *Pileus* narrowly to broadly-shallowly depressed, 43–129 mm diam.; surface greyish-brown (2C3) when fresh, dark grayish-yellow (5D5) when dry, on young specimens with scattered minute squamules more abundant towards the center, in mature specimens with only a few remaining in the center, wrinkled when dry; margin brighter, incurved when young, involute at maturity; context up to 8 mm wide near the pileus center, white (1A1) to yellow when dry. *Lamellae* deeply decurrent, close, narrow, white to pale yellow (5A1) when fresh, brownish-yellow (5C7) in dried specimens; lamellar edges entire and concolorous; lamellulae present, 1–3 tiers. *Partial veil* present in young basidiomata, fugacious, single, pale yellow (5A1), leaving remnants on the pileus surface (Fig. 5B), annulus not seen. *Stipe* central to subeccentric, fleshy fibrous, solid, cylindrical to slightly attenuated upward, pale yellowish brown (2E4, 6E5), 16–27 mm long, 4–12 mm thick, with dark scales along it more easily seen on dried specimens, whitish mycelium present in the base (Fig. 5C).

Basidiospores [20/1/1] (8.3–)8.7–13.2(–14.1) × (2.8–)3.1–4.6 µm (avg. = 10.5 × 3.9 µm), Q = 2.1–3.5, Qm = 2.7, IKI- (n=20), cylindrical to bacilliform, smooth, thin-walled, often bearing one or two guttules (Fig. 5F). *Basidia* (26–)27–40(–44) × 5.5–7.0 µm, cylindrical-clavate, hyaline, thin-walled, 4-sterigmate, with a basal clamp. *Pleurocystidia* and *cheilocystidia* absent. *Hyphal system* dimitic with clamp connections. *Lamellar trama* irregular, generative hyphae hyaline, frequently branched, sometimes very inflated, thin- to slightly thick-walled (up to 1 µm thick), 3.0–11.0(–15.0) µm diam., skeletal hyphae aseptate, rarely branched, hyaline, thick-walled (up to 1.0 µm thick). *Subhymenium* well developed, 24–46 µm wide, with pseudoparenchymatous, isodiametric, hyaline cells, (2.5–)3.0–7.0(–7.8) µm wide (Fig. 5D). *Pileipellis* a cutis, 15–90 µm wide, composed of generative hyphae repent to radially arranged, yellowish to brownish, thin- to slightly thick-walled (up to 0.5 µm thick), (1.9–)3.0–5.5(–5.8) µm diam., sometimes with individual cylindrical hyphae or hyphal bundles projecting up to 30 µm upwards; skeletal hyphae rare, 3.2–4.8 µm diam. (Fig. 5E). *Pileus hyphal system* composed of irregularly arranged thin- to slightly thick-walled (up to 1.0 µm thick), colorless and hyaline, generative hyphae (2.1–)2.3–12.8(–13.5) µm diam.; skeletal hyphae aseptate, rarely branched, hyaline, thick-walled (up to 2.0 µm thick), (2.6–)2.8–5.4 µm diam. *Stipitipellis* similar to pileipellis, composed of generative hyphae 2.5–5.0(–6.0) µm diam., skeletal hyphae (1.5–)2.0–4.0(–5.0) µm diam. *Stipe hyphal system* composed of irregularly arranged thin- to slightly thick-walled (up to 1.0 µm thick), colorless and hyaline, generative hyphae, 2–8 µm diam.; skeletal hyphae aseptate, rarely branched, hyaline, thick-walled (up to 1.0 µm), 2.5–6.0 µm diam.

Ecology and distribution:—On dead trunks of unidentified angiosperms in tropical and subtropical forests. Recorded for Argentina (Yungas province, Lechner *et al.* 2004) and Brazil, where it is known for the states of Rio Grande do Sul (Rick 1907, 1937, 1961, Bresadola 1920), Santa Catarina (present work), and São Paulo (Menolli *et al.* 2014), in the Southern and Southeastern regions of Brazil (Atlantic Forest and Pampean provinces) (Fig. 1).

Discussion

It has been over a century since the publications of *P. rickii* and *P. magnificus*. Still, the valuable information presented in this paper continues to expand the knowledge about these two rare veiled *Pleurotus* species. The known distribution of *P. magnificus* and *P. rickii* is extended, presenting the first record of both species from the state of Santa Catarina as well as the first records of *P. magnificus* from the states of Minas Gerais, Paraná and São Paulo.

Pleurotus magnificus is a poorly known species described by Rick (1906) based on collections from Southern Brazil. The yellowish color of the basidiomata when fresh, the glabrous pileus, and the deeply decurrent lamellae are considered as key characters to recognize *P. magnificus* in the field, and to distinguishing it from *P. rickii*, which also has centrally stipitate basidiomata and conspicuous partial veil. The largest studied specimen's pileus reaches up to 100 mm broad, but the species' pileus can reach up to 200 mm (Rick 1906). *Pleurotus levis* (Berkeley & M.A. Curtis 1853: 427) Singer (1951: 271) is morphologically related, sharing the large and yellowish basidiomata, and presence of a partial veil, but differs from *P. magnificus* due to the paler yellow basidiomata, initially tomentose pileus center, villose to strigose stipe base, broader basidiospores (4–5.7 µm wide), and absence of cystidia (Pegler 1983). *Pleurotus levis* is known to occur only in the Northern Hemisphere, including Honduras, Mexico, and the southeastern USA (Pegler 1983). According to Rick (1906) and Singer (1954), *P. magnificus* is also morphologically similar to *Pleurotus corticatus* (Fries 1815: 92) P. Kummer (1871: 104) (=*P. dryinus*), sharing the centrally stipitate and large basidiomata with the presence of a partial veil (sometimes interpreted as an evanescent ring). However, our phylogenetic findings shows that *P. magnificus* is not closely related to *P. dryinus* or *P. rickii*.

After the original description of *P. magnificus*, this is the first published record of the species. One specimen collected by Camille Torrend and labeled as *P. magnificus* is deposited at the URM Herbarium (http://www.splink.org.br/). However, communication with the herbarium team revealed that the box containing the specimen is empty, making it impossible to confirm the record. Torrend primarily studied myxomycetes and polypores from northeastern Brazil, and to our knowledge he hasn't published any paper that cited *P. magnificus*.

Thanks to citizen science efforts and the species' distinctive appearance in the field, its presence has been documented in additional locations in recent years (Fig. 1). The current distribution indicates an association with the *Araucaria* Forest, known for its cold winters, as well as high-elevation areas (above 1,000 m a.s.l.) in lower latitudes, including the 'Serra da Mantiqueira' mountain range in the states of Minas Gerais and São Paulo (Fig. 1). While there is no clear evidence that *P. magnificus* is associated with a specific host species, it seems possible that its distribution overlaps with that of the Brazilian pine, *Araucaria angustifolia* (Bertol.) Kuntze (see Tagliari *et al.* 2021). However, given the scarcity of distribution data for *P. magnificus*, this connection requires further investigation. *Pleurotus magnificus* has been assessed under IUCN Red List criteria as Endangered - EN (Menolli *et al.* 2024) due to its restricted distribution.

In the original description, Rick (1906) did not cite any specimens that served as original material to describe *P.*

magnificus, nor did he provide any photograph or illustration. In our searches through online databases of herbaria where J. Rick deposited his collections [PACA, BPI, FH, IACM, S, R, and SFPA (today incorporated in BLA), see Fidalgo 1962], no specimen that could correspond to the type of *P. magnificus* was found. PACA Herbarium was visited personally by FB but there were no specimens that could serve as original material for *P. magnificus*, as previously noted by Pereira (1988). In the FH Herbarium there is a specimen collected by J. Rick and labeled as *P. magnificus* (FH 00543855), collected two years after the species description (Fig. 4A). Another specimen collected by J. Rick and seemingly examined by C.G. Lloyd, according to the label, is currently held in BPI Herbarium (BPI 740091) (Fig. 4C). In this collection, no indication of type specimen and collection date are presented, and the sample is accompanied with a small note written by Rick with "*mostly mesopodial, with velum*". This information is not in accordance with the protologue, which states "*stipite excentrico*" and makes no references to a veil, only a ring ("*annulo fibroso, evanescente*") (Rick 1906). Thus, it is very unlikely that it represents the specimen used to describe the species.

Theissen (1912) published a comprehensive review of the 'hymenomycetes' from the Brazilian state of Rio Grande do Sul, mainly based on Rick's previous works and unpublished records. This work presented for the first time original photographs of some species identified by J. Rick himself, among them a fresh basidiomata of *P. magnificus* (Fig. 4B). It is unclear whether this photograph corresponds to the specimen utilized in the protologue, as it lacks any information beyond the species name and author. Consequently, it is not possible to confirm its authenticity as original material for *P. magnificus*, i.e., available prior to the taxon's publication. No other material of *P. magnificus* that could be the type specimen is known to exist.

In accordance with Article 9.8 of the Code (Turland *et al.* 2018), we designate the specimen FURB 44086 (Fig. 3A) as neotype. We believe that this specimen precisely represents the original concept of the species as established by Rick (1906). The neotype was collected near the type locality (Southern Brazil) and provides essential data for reliable and modern taxonomic studies, including an updated description, field photographs, and DNA sequences.

Pleurotus rickii is also a rare species, originally described from collections by Rick (Bresadola 1920) and has been sampled only a few times since its description. It is distributed in the Atlantic Forest and Pampean provinces of the South and Southeastern Brazil coastal regions, and the Yungas rainforest of Northern Argentina (Fig. 1), possibly also present in other tropical forests of South America. The species has been assessed under IUCN Red List criteria as Near Threatened - NT (Bittencourt *et al.* 2021) due to its estimated small population (5,000 to 10,000 mature individuals) and continued decline of its habitat.

Morphologically, *P. rickii* is recognized in the field by its grayish to pale brown pileus surface with remnants of the partial veil present on the margin, and stipe with white basal mycelium and dark strands and spots of agglutinated hyphae. Although Pereira (1988) described cheilocystidia as rare to absent in mature basidiomata, none were found in the examined specimens. The description of *P. rickii* after Argentinean specimens (Lechner *et al.* 2004) shows some important morphological variation, such as smaller pilei and basidia, which could be attributed to the different habitat.

Pleurotus dryinus is similar to *P. rickii* but lacks the white basal mycelium and dark scales on the stipe (Zervakis & Balis 1996, Venturella *et al.* 2015), and is capable of forming anamorphic structures (Shnyreva & Shnyreva 2015). The species is frequently regarded as limited to the Northern Hemisphere (Zervakis & Balis 1996, Petersen & Krisai-Greilhube 1999, Venturella *et al.* 2015). Although Pereira (1988) recorded *P. dryinus* in Brazil, its occurrence in South America needs further investigation, requiring detailed morphological and phylogenetic comparison with specimens from Europe and North America.

Phylogenetically, *Pleurotus rickii* is sister to a clade containing specimens identified as *P. cornucopiae*, *P. euosmus*, and *P. citrinopileatus* (Fig. 2), from which it can be easily distinguished by the presence of a partial veil (Petersen & Krisai-Greilhuber 1999, Zervakis *et al.* 2019).

Prior to the description of *P. rickii*, this taxon had been studied by Rick (1907) under the name of *Armillaria procera* Spegazzini (1889: 385). In this paper, Rick provided a brief description of the specimens along with a single photograph (Rick 1907). Bresadola (1920) later described *P. rickii*, directly referencing Rick's description and illustration: "Armillaria procera *Rick: Agar. et Polyp. Bras. in Broteria Band II, p. 71, tab. VIII, f. 3 non Speg*." He cited two collections, Rick 135 and Rick 363, effectively designating them as the syntypes. These specimens are currently held at the S herbarium (Swedish Museum of Natural History, http://herbarium.nrm.se/) as S-F180929 and S-F180930, respectively. Based on morphological studies, *P. rickii* was later infrequently recorded in Brazil (Saccardo & Trotter 1925, Singer & Digilio 1951, Singer 1954, Pereira 1988, Raithelhuber 1991) and Argentina (Lechner *et al.* 2004). A study focused on phylogenetic analysis of *Pleurotus* species in Brazil provided the first ITS sequences of *P. rickii*, which were obtained from specimens collected in Southeast Brazil (São Paulo) and confirmed its position within *Pleurotus* (Menolli *et al.* 2014).

We had access to photographs of *P. rickii* syntypes held at the S Herbarium, which are in rather poor condition for further taxonomic studies (Fig. 6), especially S-F180930, which is crumbled into many pieces (Fig. 6C). In accordance with articles 9.3, 9.11, and 9.12 of the International Code of Nomenclature for algae, fungi, and plants (Turland *et al.* 2018), we designate the collection S-F180929 as a lectotype, which is the best preserved specimen among the syntypes. Additionally, we designate the specimen FLOR 79342 as an epitype in accordance with article 9.9 of the Code (Turland *et al.* 2018), since valuable morphological characteristics are no longer discernible and DNA cannot be obtained from the lectotype, which are essential for reliable identification and interpretation of the taxon.

It is worth mentioning that, like other species of *Pleurotus*, *P. magnificus* and *P. rickii* have a great economic potential as edible mushrooms (Li *et al.* 2021). According to Singer & Digilio (1951), *P. rickii* is very palatable. *Pleurotus magnificus* was recently harvested in the states of Minas Gerais, where it has been called 'giant forest oyster mushroom' and 'carapicu-amarelo' (Thiago van den H. Comenale, pers. comm.), and Rio Grande do Sul, and it has been consumed fried and roasted (Jeferson Müller Timm, Marcelo Sulzbacher, Thiago van den H. Comenale, pers. comm.) (Fig. 7).

Besides their potential as edible species, numerous bioactive compounds have been reported in other *Pleurotus* species such as *Pleurotus albidus* (Berkeley 1843: 633) Pegler (1983: 219) (Castro-Alves *et al.* 2017, Castro-Alves & Nascimento 2018), *Pleurotus djamor* (Carbonero *et al.* 2012, Maity *et al.* 2021), *Pleurotus eryngii* (Calabretti *et al.* 2021), and *Pleurotus ostreatus* (González-Palma *et al.* 2016, Khan *et al.* 2017, Koutrotsios *et al.* 2017). Most of these compounds are β-glucans, which have been linked to immunological activities (Carbonero *et al.* 2012, Castro-Alves *et al.* 2017, Castro-Alves & Nascimento 2018), anticarcinogenic effects (Maiti *et al.* 2011), antioxidant properties (Khan *et al.* 2017), antitumor effects (Venturella *et al.* 2015), and other beneficial health effects (Guzmán 2000, Patel *et al.* 2012). *Pleurotus* species are also known for their ability to kill nematodes (Lee *et al.* 2023), positioning them as potential agents for biological control in agriculture, as nematodes affects a wide range of crops. Hence, *P. rickii* and *P. magnificus* present significant economic potential that remains to be explored, and we believe the information provided in this paper will greatly support future studies on *Pleurotus* species to better understand their diversity, distribution, biology, and applications. In addition, concerning trend of habitat loss and deterioration for endangered species reinforces the urgent need for continued research and conservation efforts to protect poorly-known and overlooked species.

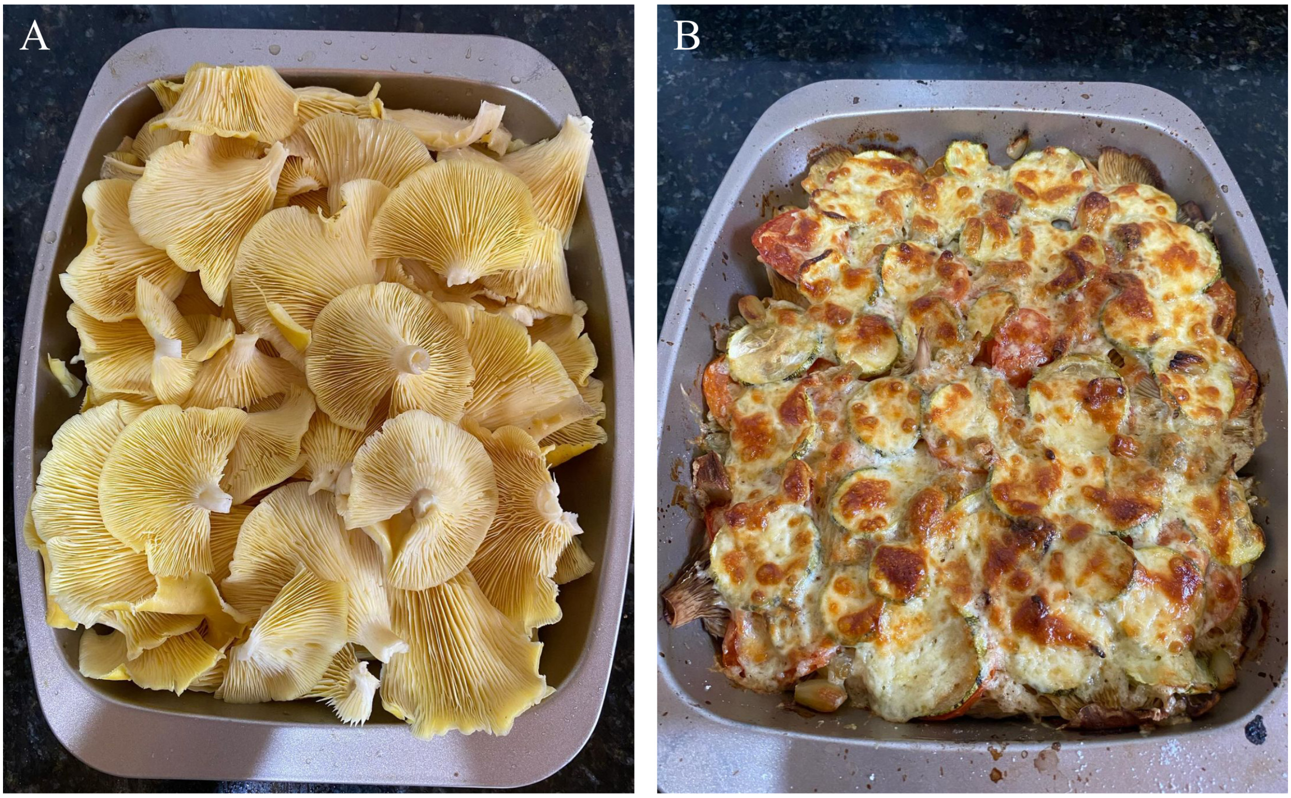

FIGURE 7. Using *P. magnificus* for preparing dishes. **A.** Fresh edible basidiomata; **B.** Finished dish. Photos by Thiago van den H. Comenale.

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