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Not every edible orange milkcap is *Lactarius deliciosus*: first record of *Lactarius quieticolor* (sect. *Deliciosi*) from Brazil

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Summary

The natural distribution of species of *Lactarius* sect. *Deliciosi* is mainly in the northern hemisphere, where they grow in ectomycorrhizal symbiosis mainly with conifers. Several species in this section are regionally well known and appreciated due to their culinary use. In South America, there is limited knowledge of their presence and harvesting, while their culinary value remains underexploited. Recently, field campaigns in pine plantations in southern Brazil revealed wide presence of *Lactarius* sect. *Deliciosi* species. Morphological and molecular identification approaches confirmed that all Brazilian collections correspond to one European species, *Lactarius quieticolor.* Fruiting bodies occurred in plantations of *Pinus taeda* and/or *P. elliottii*, on acidic soils, and under humid climate with mild to hot summers. A review of edibility and organoleptic properties confirmed both *L. quieticolor* and its commonly misapplied name, *L. deliciosus,* in South America as edible and of high quality. Several other *L.* sect. *Deliciosi* species are less appreciated or with unknown palatability. Due to low potential for long-distance dispersal, an ectomycorrhizal partner switch from European to North American pine species is proposed, which may have happened in South America for both allochthonous symbiotic partners. There is still a possibility that other combinations were established, including combinations with less valuable species from *L.* sect. *Deliciosi*.

Key words: ectomycorrhizal fungi, edible mushroom, Russulaceae, Pinaceae, pine plantation

Introduction

Among macrofungi (mushrooms), both saprotrophic fungi such as oyster mushrooms, shiitake, button mushroom, etc., and symbiotic/mycorrhizal fungi such as boletes, truffles, milk caps and chanterelles, have been traditionally harvested and consumed in large quantities in many countries in Europe, Africa and Asia. The tradition of eating wild mushrooms, including milk caps, is also strong in several North and Central American countries, like the U.S.A., Mexico and Guatemala (ROWE 1997, PÉREZ-MORENO et al., 2008). On the other hand, in South America, the tradition of cultivating fungi or collecting them in the wild for consumption has remained limited (BOA, 2004). Only limited knowledge exists on the use of native edible fungi in South America (HENKEL et al., 2004; DIAS, 2010; SANUMA et al., 2016; GAMBOA-TRUJILLO et al., 2014, 2018) while the presence and potential culinary use of introduced species remains underexploited (VALENZUELA, 2003; NIVEIRO et al., 2009; SULZBACHER et al., 2013, 2019; TOLEDO et al., 2016).

Two of the most frequently planted introduced ectomycorrhizal species of forest trees in South America are eucalypts (*Eucalyptus* spp.), native to Australasia, and pines (*Pinus* spp.), originating from North America and Eurasia (FAO, 2001). The first pine seedlings were brought to Brazil by European immigrants as ornamentals and for timber production, at the end of 19th century, with the introduction of *Pinus canariensis* C. Sm. from the Canary Islands to the state of Rio Grande do Sul (SHIMIZU, 2008). However, the introduction of pine in Brazil was boosted by resin production, timber and cellulose industry (SHIMIZU and AMARAL, 1987; SCHÜHLI et al., 2016). *Pinus sylvestris* L., *P. pinaster* Aiton, P. Roxburgh Sarg., *Pinus nigra* J.F. Arnold, *P. halepensis* Miller and *P. cembra* L. from Eurasia and other 16 pine species from North America were introduced in São Paulo State (LÖFGREN, 1906), serving as the basis for the beginning of the paper industry. *Pinus taeda* L. and *P. elliottii* Engelm. are the two most widely planted North American species in subtropical South America, due to their ability for adaptation to the climate and acidic soils, rich in organic matter (DOBNER Jr. et al., 2019). While eucalypts host few edible ectomycorrhizal fungi, of which the palatability is most often unknown, pines in their native range of distribution are known to host several edible and highly priced species of ectomycorrhizal genera, such as *Boletus* L. (HALL et al., 1998), *Suillus* Gray (DEWI et al., 2016), *Hydnum* L. (FENG et al., 2016), *Rhizopogon* Fr. (SULZBACHER et al., 2016b) and *Lactarius* Pers. (WANG et al., 2019).

Studies of ectomycorrhizal fungal communities associated with North American pines in Brazil revealed more than 30 species of Agaricomycetes distributed in 14 genera (SULZBACHER et al., 2013). The genus *Lactarius* is represented by four agaricoid species, namely *L. argillaceifolius* Hesler & A.H. Sm., *L. deliciosus* (L.) Gray, *L. fragilis* (Burl.) Hesler & A.H. Sm. and *L. rufus* (Scop.) Fr. (SÁ et al., 2013; SULZBACHER et al., 2013), and one recently described sequestrate species, *L. taedae* Silva-Filho, Sulzbacher & Wartchow (SILVA-FILHO et al., 2018).

Globally, *Lactarius* sect. *Deliciosi* (Fr.) Redeuilh, Verbeken & Walleyn comprises roughly 40 species (Tab. 1) that are easily recognizable in the field by their conspicuous orange, pink, vinaceous red, brownish grayish, lilac or blue basidiomata (NUYTINCK, 2004, 2007), which produce orange, red or blue latex, several of them changing to greenish when bruised or touched (NUYTINCK and VERBEKEN, 2005). The natural distribution of this monophyletic group is in the northern hemisphere (NUYTINCK et al., 2007), associating with *Pinus* L., *Picea* Link, *Abies* D. Don., *Tsuga* Carrière, *Alnus* Mill., *Quercus* L., *Larix* Mill., *Lithocarpus* Blume & *Arctostaphylos* Adans. (HESLER and SMITH, 1979). Occurrence of *Lactarius* sect. *Deliciosi* species in the southern hemisphere is related with an unintentional co-introduction with their ectomycorrhizal host trees (DICKIE et al., 2010; SULZBACHER et al., 2018), used in reforestation or ornamental programs in South America, Oceania and some African countries (GARRIDO, 1986; SHIMIZU and AMARAL, 1987; DUNSTAN et al., 1998; SULZBACHER et al., 2013; CHÁVEZ et al., 2015).

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Tab. 1: List of edibility and quality assessment based on literature and personal experiences published on the internet, host tree genera, and area of known distribution of *Lactarius* sect. *Deliciosi* species.

Species	**edibility and literature-based unscientific quality assessment**	**Host genera**	**Area of natural distribution**	**Reference**
Lactarius abieticola X.H.Wang	edible	*Abies*	Asia	Wang (2016)
Lactarius aestivus Nuytinck & Ammirati	edible	*Abies*, Tsuga	North America	Nuytinck and Ammirati (2014)
Lactarius akahatsu Nobuj. Tanaka.	edible (consumed in Thailand)	*Pinus*	Asia	Nuytinck et al. (2006b), Le et al. (2007)
Lactarius aurantiosordidus Nuytinck & S.L. Miller	edible	*Picea*	North America	Siegel and Schwarz (2016)
Lactarius barrowsii Hesler & A.H. Sm.	edible and good	*Pinus*	North America	States (2016)
Lactarius chelidonium var. *chelidonium* (A.H. Sm.) Hesler & A.H. Sm.	edible	*Pinus*	North America	http://www.fungikingdom.net
Lactarius cyanopus Basso	edible	*Pinus*	Europe	Nuytinck and Verbeken (2005)
Lactarius deliciosus (L.) Gray	edible excellent, good if cooked	*Pinus*	Europe and Asia	Nuytinck et al. (2006a) https://www.mykoweb.com
Lactarius deliciosus var. *areolatus* A.H. Sm.	unknown	*Picea*, *Pinus*	North America	http://www.mushroomexpert.com
Lactarius deliciosus var. *deterrimus* (Gröger) Hesler & A.H. Sm.	edible, contains mutagenic compounds	*Pinus*	North America	Anke et al. (1989), http://www.mushroomexpert.com
Lactarius deterrimus Gröger	edible, slightly bitter	*Picea*	Europe	https://sites.google.com/site/scottishfungi/species-profiles/lactarius-deterrimusfalse-saffron-milkcap
Lactarius fennoscandicus Verbeken & Vesterh.	edible, slightly bitter, not highly valued	*Picea*	Europe	rogersmushrooms.com
Lactarius guangdongensis X.H. Wang, Y. Han & C.Z. Liang	unknown	*Pinus*	Asia	Han et al. (2019)
Lactarius hatsudake Nobuj. Tanaka	edible (consumed in Japan, China, Korea and Russia)	*Pinus*	Asia	Nuytinck et al. (2006b)
Lactarius hengduanensis X.H. Wang	edible	*Picea*	Asia	Wang (2016)
Lactarius horakii Nuytinck & Verbeken	unknown	*Pinus*	Asia	Nuytinck et al. (2006b), Le et al. (2007)
Lactarius indigo var. *diminutivus* Hesler & A. H. Sm.	edibility not known	*Pinus*	North America	Nuytinck et al. (2006a), Bessette and Bessette (2019)
Lactarius indigo (Schwein.) Fr.	edible, average to delicious	*Pinus*, *Quercus*	North and Central America	http://www.mushroomexpert.com, Nuytinck et al. (2007), Bessette and Bessette (2019)
Lactarius laeticolor (S. Imai) Imazeki ex Hongo	edible	*Abies*	Asia	Nuytinck et al. (2006b)
Lactarius miniatosporus Montoya & Bandala	unknown	*Pinus*	Central America	Montoya and Bandala (2004)
Lactarius paradoxus Beardslee & Burl.	edible, good and tasty	*Pinus*, *Quercus*	North and Central America	Metzler and Metzler (2010) https://morelmushroomhunting.com
Lactarius porninsis Rolland	edible cooked, not edible raw	*Larix*	Europe, Asia	Nuytinck and Verbeken (2005), https://www.funghiitaliani.it
Lactarius pseudodeliciosus Beardslee & Burl.	edible	*Pinus*, *Quercus*	North America	Nuytinck et al. (2006a)
Lactarius pseudohatsudake X.H. Wang	edible	*Picea*	Asia	Wang (2016)
Lactarius quieticolor Romagn.	edible, good quality and excellent flavor	*Pinus*	Europe	Nuytinck and Verbeken (2005) Sulzbacher et al. (2018) http://www.fichasmicologicas.com
Lactarius rubrilacteus Hesler & A.H. Sm.	edible	*Pinus*, *Pseudotsuga*, *Arctostaphylos*	North America	Nuytinck et al. (2006), https://www.mykoweb.com
Lactarius rubriviridis Desjardin, H.M. Saylor & Thiers	unknown but probably edible	*Pinus*, *Abies*	North America	Desjardin et al. (2015)
Lactarius salmoneus Peck	edible, not highly valued	*Pinus*	North America	Nuytinck (2004), Fischer and Bessette (2010)

Lactarius salmonicolor R. Heim & Leclair	edible, mildly acrid to bitter	*Abies*	Europe	Nuytinck and Verbeken (2005)
Lactarius sanguifluus (Paulet) Fr.	edible, highly prized	*Pinus*	Europe	Nuytinck and Verbeken (2005)
Lactarius semisanguifluus R.Heim & Leclair	edible, less tasty	*Pinus*	Europe	http://www.gobe.si
Lactarius splendens Hesler & A.H. Sm.	unknown but probably edible	*Larix*	North America	Nuytinck et al. (2017)
Lactarius subindigo Verbeken & E. Horak	unknown	*Castanopsis*, *Lithocarpus*, *Pinus*, *Quercus*	Asia, Australasia	Nuytinck et al. (2006b), Nuytinck et al. (2007)
Lactarius subpurpureus Peck	unknown	*Tsuga*	North America	Nuytinck et al. (2006a)
Lactarius thakalorum Bills & Cotter	unknown	*Pinus*	Asia (Nepal)	Nuytinck et al. (2006b)
Lactarius thyinos A.H. Sm.	edible	unknown	North America	Nuytinck et al. (2006a)
Lactarius vinosus (Quél.) Bataille	edible	*Pinus*	Europe - Mediterranean	Nuytinck and Verbeken (2005)
Lactarius vividus X.H. Wang, Nuytinck & Verbeken	edible	*Pinus*	Asia	Wang et al. (2015)

Pine plantations in Brazil and other parts of South America cover almost 4.7 M ha (data for 2000 by the Food and Agriculture Organization; FAO, 2001), all with a high potential for production of one or more edible *Lactarius* sect. *Deliciosi* species. For this reason, we aimed to collect and determine commercialized *Lactarius* sect. *Deliciosi* collections entering the human food chain to pin down their precise identity, and thus to ensure their authenticity, safe trading and consumption. We focused on fungal collections that originated from introduced pine forests and timber plantations in southern Brazil, for their identification, positioning for biogeographical origin, and finally reconsidering the potential for their culinary use.

Materials and methods

Collecting area

Fresh specimens for identification were collected during fungus mapping forays in the years 2017 and 2018 (Sulzbacher et al., 2018) in pine forests and forest plantations in municipalities of southern Brazil, in Rio Grande do Sul state (Itaara, coord. 29°36'35'' S 53°45'53'' W; São Francisco de Paula, coord. 29°26'53'' S 50°35'01'' W; Santa Maria, coord. 29°38'41'' S 53°56'3'' W and 29°41'59.9'' S 53°42'40.5'' W; São Gabriel, coord. 30°25'57,8'' S 54°22'05,3'' W) and in Santa Catarina state, Urupema, (coord. 27°57'10'' S 49°52'23'' W). Quantitative data about harvesting in fifteen sites were provided by Terroir Sul (Santa Maria, RS, Brazil), a company that commercializes fresh and dry *L*. sect. *Deliciosi* fruiting bodies locally.

Morphological analysis

Macromorphological characteristics were described from the fresh material of six sites. Colour names and codes followed Kornerup and Wanscher (1978). Micromorphological characters were studied on dried material. Spores were mounted using Melzer's reagent, and then visualized and measured in lateral view, and ornamentation was excluded from measurements. Other structures were measured and visualized in 5% KOH and Congo red. For statistics, 60 basidiospores of three basidiomata of each collection were measured. In the basidiospore description, L(W) = length (width) average from a single basidioma, **Q** is the quotient between the length and width, and **Qm** is the median value of Q. For scanning electron microscopy, the dried material of one basidioma was directly stuck to an adhesive layer on a small clean coverslip, then coated with gold and observed with a scanning electron microscope JEOL JSM-6360LV (JEOL U.S.A. Inc., Peabody, MA, U.S.A.). Specimens are deposited in the JPB and in SMDB herbaria (Thiers, 2020, continuously updated).

Molecular methods

Total fungal DNA was extracted using the Qiagen DNeasy Plant MiniKit, according to the manufacturer's instructions. Extracted DNA was re-suspended in pre-warmed sterile milli-Q water to the approximate final concentration of 100 ng $\mu l^{-1}$and kept at -80 °C. The DNA extracts are kept at the Slovenian Forest Gene Bank (Slovenian Forestry Institute DNA library) under accession numbers E18/15-14, 15, and 16. The complete nuc-ITS-rDNA spacer (ITS) was amplified using primer pairs ITS1/ITS4 (White et al., 1990). PCR reactions were performed as follows: 1.0 µl DNA; 2.5 µl PCR buffer 10×; 3.0 µl dNTPs (1.5 mM); 2.0 µl $MgCl_2$ (20 mM); 3.0 µl of each primer (25 pmol); 0.5 U Taq polymerase (5 U μl^{-1}); and 10.5 µl of ultrapure water. PCR conditions followed Sulzbacher et al. (2016a). Amplifications were done in a GeneAmp$^{®}$ PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, U.S.A.). Prior to sequencing, PCR products were purified from agarose gel using the Wizard SV Genomic DNA Purification System (Promega Corporation, Madison, WI, U.S.A.). Both DNA strands were sequenced separately at Macrogen Europe B.V. (Amsterdam, The Netherlands) with the same primers as used for amplification. Sequencher 5.4.6 (Gene Codes Corporations, Ann Arbor, MI, U.S.A.) was used to assemble the consensus sequence from the two strands of each isolate.

Phylogenetic analysis

For this study, we assembled a dataset of all newly generated sequences from Brazil, available nrITS sequences of the *Lactarius* sect. *Deliciosi* retrieved from the GenBank on October 20, 2019, and additional specimens from subgenera *Lactarius* Pers. and *Russularia* (Fr. ex Burl.) Kauffman (Tab. 2). *Lactarius acris* (Bolton) Gray and *Lactarius pterosporus* Romagn. were used as an outgroup. The dataset was aligned using MAFFT v.7 (Katoh and Standley, 2013), under the E-INS-i criteria. Seaview v.4 (Gouy et al., 2010) was used for visualization and manipulation with the alignment, and the Gblocks v0.91b was used to eliminate poorly aligned positions in the alignment, with settings allowing gaps within selected blocks, smaller blocks and bigger segments with contiguous non-conserved positions (Castresana, 2000). JModelTest 2v.1.6 was used to select the best nucleotide substitution model using the Akaike Information Criterion (AIC) (Darriba et al., 2012). RAxML v8.2.X (Stamatakis, 2006) and MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) were used for Maximum likelihood estimation and Bayesian phylogeny inferences, respectively. The jModelTest 2v.1.6, RAxML v8.2.X and MrBayes 3.1.2 were used in the CIPRES Science Gateway 3.1

Tab. 2: Specimens, voucher collection, origin and GenBank accession numbers of nrITS sequences used in the molecular analyses

Specimen	ITS Accession n°	Voucher collection	Origin	Reference
Lactarius acris	DQ421988	EU014	Germany	Unpublished
L. aestivus	KJ769667	JFA 13112	U.S.A.	Nuytinck and Ammirati (2014)
L. aestivus	KJ769670	JN 2008-032	U.S.A.	Nuytinck and Ammirati (2014)
L. akahatsu	EF685045	22601	Japan	Nuytinck et al. (2007)
L. akahatsu	EF685097	AV 04-141	Thailand	Nuytinck et al. (2007)
L. aurantiosordidus	EF685096	SLM 216-03	U.S.A.	Nuytinck et al. (2007)
L. chelidonium	EF685049	SLM 9649	U.S.A.	Nuytinck et al. (2007)
L. controversus	KF241544	AV00-117	Italy	Verbeken et al. (2014)
L. decipiens	KF432973	AV2000-137	Italy	Wisitrassameewong et al. (2014)
L. deliciosus	DQ922489	AV 2000-104	Italy	Nuytinck and Verbeken (2007)
L. deliciosus	DQ922485	JN 2001-005	Sweden	Nuytinck et al. (2007)
L. deliciosus	DQ922486	SLM 9878	U.S.A.	Nuytinck et al. (2007)
L. deliciosus var. *areolatus*	EF685055	B. Kropp (RMS)	U.S.A.	Nuytinck and Verbeken (2007)
L. deterrimus	DQ922515	JN 2001-053	Slovakia	Nuytinck and Verbeken (2007)
L. deterrimus	DQ922519	JN 2001-099	Italy	Nuytinck and Verbeken (2007)
L. eucalypti	EU019923	MEL2238273	Australia	Lebel and Tonkin (2007)
L. fenoscandicus	DQ922510	AV 97-530	Sweden	Nuytinck and Verbeken (2007)
L. fenoscandicus	DQ922500	JV 95-330	Sweden	Nuytinck and Verbeken (2007)
L. hatsudake	EF685063	hat38541	China	Nuytinck et al. (2007)
L. horakki	EF685069	J. 8336	Indonesia	Nuytinck et al. (2007)
L. horakki	EF685070	J. 9983	Indonesia	Nuytinck et al. (2007)
L. indigo	EF685067	SLM 957	U.S.A.	Nuytinck et al. (2007)
L. imperceptus	EU819485	JMP0044	U.S.A.	Palmer et al. (2008)
L. inconspicuus	KF433001	KW100	Thailand	Wisitrassameewong et al. (2015)
L. indigo	EF685065	BK 29-10-2000-1	Belize	Nuytinck et al. (2007)
L. indigo var. *diminutivus*	EF685066	MCA 81	U.S.A.	Nuytinck et al. (2007)
L. laeticolors	EF685077	lae23149	Japan	Nuytinck et al. (2007)
L. laeticolors	EF685091	OKM 21714	South Korea	Nuytinck et al. (2007)
L. miniatosporus	EF685078	3830	Mexico	Nuytinck et al. (2007)
L. paradoxus	EF685081	DM 1-02-2002	U.S.A.	Nuytinck et al. (2007)
L. pornisis	DQ922547	JN 2001-082	Slovakia	Nuytinck and Verbeken (2007)
L. pornisis	DQ922546	JN 2002-019	Italy	Nuytinck and Verbeken (2007)
L. pseudodeliciosus	EF685082	DM 02-2002	U.S.A.	Nuytinck et al. (2007)
L. pterosporus	KF432963	JN2012-037	Germany	Wisitrassameewong et al. (2014)
L. quieticolor	MT007126	M.A. Sulzbacher 520	Brazil	This study
L. quieticolor	MT007127	M.A. Sulzbacher 518	Brazil	This study
L. quieticolor	MT007128	M.A. Sulzbacher 517	Brazil	This study
L. quieticolor	DQ922498	JN 2001-004	Sweden	Nuytinck and Verbeken (2007)
L. quieticolor	DQ922492	JN 2000-011	Belgium	Nuytinck and Verbeken (2007)
L. quieticolor	KX610696	IK-00481	Poland	Kalucka et al. (2016)
L. quieticolor	AF140269	ue141	Germany	Eberhardt et al. (2000)
L. quieticolor	KJ769675	JN 2001-130	France	Nuytinck and Ammirati (2014)
L. quieticolor	KJ769676	RW&AV 3193	Czech Republic	Nuytinck and Ammirati (2014)
L. quieticolor	EF565902	S/N	South Africa	Unpublished
L. quieticolor	EF685092	SLM19203	Sweden	Nuytinck et al. (2007)
L. quieticolor	MT335831	CONC–F 0810	Chile	Chávez et al. (2015)
L. quieticolor	MT335832	CONC–F 0811	Chile	Chávez et al. (2015)
L. quieticolor	MT335833	CONC–F 0812	Chile	Chávez et al. (2015)
L. rubrilacteus	EF685083	AEF 1055	U.S.A.	Nuytinck et al. (2007)
L. rubrilacteus	EF685084	SLM 19-04	U.S.A.	Nuytinck et al. (2007)
L. rubriviridis	EF685088	DED 7312	U.S.A.	Nuytinck et al. (2007)
L. salmoneus	EF685090	BK 11-08-2000-1	U.S.A.	Nuytinck et al. (2007)
L. salmonicolor	DQ922548	JN 2001-087	Belgium	Nuytinck and Verbeken (2007)
L. salmonicolor	DQ922549	JN 2001-123	France	Nuytinck and Verbeken (2007)
L. salmonicolor	DQ922552	JN 2002-027	Italy	Nuytinck and Verbeken (2007)
L. sanguifluus	DQ922539	JN 2000-008	Belgium	Nuytinck and Verbeken (2007)
L. sanguifluus	AY332546	JN 2001-050	Slovakia	Nuytinck and Verbeken (2003)
L. semisanguifluus	AY332553	AV 96-1032	Belgium	Nuytinck and Verbeken (2003)
L. semisanguifluus	AY332555	CL96-08	Sweden	Nuytinck and Verbeken (2003)
L. semisanguifluus	AY332556	JN 2001-093	Italy	Nuytinck and Verbeken (2003)
L. sublacarioides	KF432999	KW011	Thailand	Wisitrassameewong et al. (2014)
L. subindigo	EF685073	MC 05-300	Nepal	Nuytinck et al. (2007)
L. subindigo	EF685068	Kamal 259-99	India	Nuytinck et al. (2007)
L. subpurpureus	EF685100	MH KIINA 114	U.S.A.	Nuytinck et al. (2007)
L. subpurpureus	EF685099	JHR 574	U.S.A.	Nuytinck et al. (2007)
L. stephensii	AY331012	RW2930	Belgium	Nuytinck and Verbeken (2003)
L. taedae	MG873443	M.A. Sulzbacher 510	Brazil	Silva-Filho et al. (2019)
L. thyinos	EF685102	SLM 9648	U.S.A.	Nuytinck et al. (2007)
L. vinosus	DQ922542	AP & GH 230	Turkey	Nuytinck and Verbeken (2007)
L. vinosus	AY332550	JN 2001-11	Spain	Nuytinck and Verbeken (2003)

(Miller et al., 2010). A node was considered significantly supported if it received a BS ≥ 60% and/or BPP ≥ 0.95. The final alignment and the resultant topologies were deposited in TreeBASE, study ID: 25990.

Results

Occurrence and taxonomy

Lactarius sect. *Deliciosi* specimens were collected frequently and appear to be widely distributed in pine plantations and pine forests in southern Brazil. In the sampling years 2017 and 2018, and also in subsequent years, a minimum of 80 kilograms of fresh *Lactarius* sect. *Deliciosi* fruiting bodies were collected annually for commercial purposes, solely by the Terroir Sul company (Santa Maria, RS, Brazil), in the area ranging from the central region of Rio Grande do Sul State (12 sites) to the state of Santa Catarina (3 sites). These mushrooms were regularly traded under the name *Lactarius deliciosus* (Fig. 1). A detailed morphological and molecular study of collections, considered for the present analysis, positioned all analysed basidiomata in *Lactarius quieticolor* Romagn.

Lactarius quieticolor Romagn., Revue de Mycologie 23(3), 280 (1958)
= *Lactarius deliciosus* var. *quieticolor* (Romagn.) J. Blum, Les Lactaires. Etudes Mycologiques: 203 (1976)

Pileus (20-)65-91(-110) mm diam., convex to plane-convex, slightly depressed, becoming infundibuliform in mature basidiomata, pale orange (5A3, 6A4), orange (6A6), brownish orange (6C4, 6C6), grey (6B1); margin at first incurved, becoming straight; surface smooth, to finely fibrillose, concentrically zoned, viscid in young basidiomata, becoming dry at maturity; context firm, up to 9 mm thick near to insertion of stipe, 2-4 mm thick near to margin, white (1A1), light orange (5A4) to greyish orange (5B5), becoming olive (2F7) when bruised (Fig. 2). Lamellae subdecurrent to decurrent, densely arranged, 4-6 mm broad, sometimes forked towards the cap margin, greyish orange (6B4); margin smooth, conspicuously paler, pale orange (5A2); lamelullae of different sizes (Fig. 2). Stipe (21-)45-52 × (6-)13-18 mm, central, cylindrical, sometimes tapering downwards, curved at lower median portion, pale orange (5A3, 6A4), orange (6A6), brownish orange (6C4, 6C6), grey (6B1); surface smooth, slightly scrobiculate, sometimes spotted, dry; turning hollow with cavity 2-3 mm diam., context white (1A1) or very pale brown (10YR8/3) (Fig. 2). Latex not abundant, reddish orange, unchanged. Spore print not performed. Smell and taste not tested.

Fig. 2: *Lactarius quieticolor* collection M.A. Sulzbacher 520. Basidiomata in their natural habitat in pine plantation near the campus of the Federal University of Santa Maria, Santa Maria, Brazil, under *Pinus taeda*. Scale Bar = 10 mm. Photos: Marcelo A. Sulzbacher

Basidiospores 7-9(-9.5) × (5-)6-7(-8) µm; L= 7.4 µm, W= 6.3 µm, Q= 1.07-1.40, Qm= 1.19; subglobose to ellipsoid, predominantly subellipsoid, thin-walled, hyaline, ornamentation amyloid, up to 0.8 µm high, composed of a broad ridges and isolated warts, forming a coarse reticulum (Fig. 4); plage slightly amyloid; hilar appendix up to 3 µm long, inamyloid (Figs. 3A, 4). Basidia 53-63 × 9-13 µm, cylindrical to subclavate, predominantly 4-spored, more rarely 2-spored, often containing oil-droplets and needle-shaped content; sterigmata up to 5 µm long. (Fig. 3B). Pleuromacrocystidia scarce to frequent, 38-52 × 4-8 µm, subfusiform, with an acute apex, sometimes moniliform, thin-walled, with needle-shaped content; slightly emergent (Fig. 3B). Pleuropseudocystidia frequent, 3-6 µm diam., filiform to cylindrical,

Fig. 1: *Lactarius* sect. *Deliciosi* traded as '*L. deliciosus*' at local markets in South America: **A.** Dried and packed fungi obtained from a market; **B**. Freshly collected fungi ready for consumption and included in this study by courtesy of the trading company. Photos: Thaís Becker Ventura (A) and Marcelo A. Sulzbacher (B)

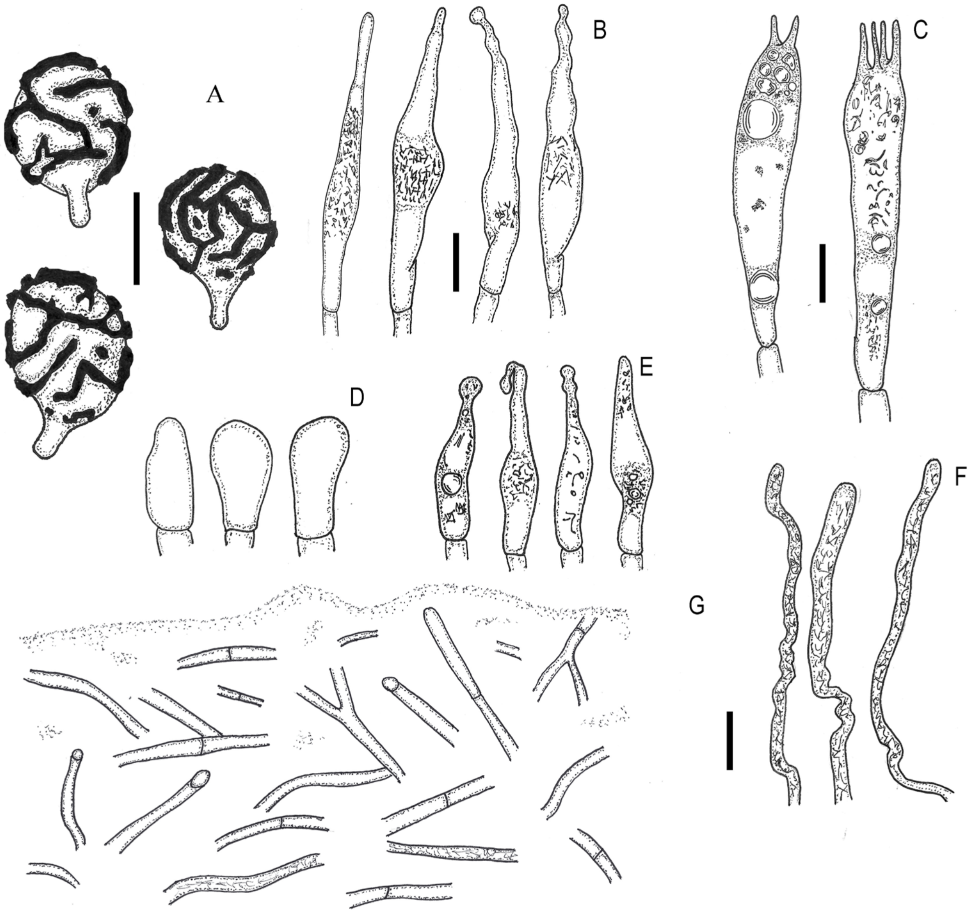

Fig. 3: Microscopic drawings of *Lactarius quieticolor* collection M.A. Sulzbacher 520. **A**. Basidiospores; **B**. Pleuromacrocystidia; **C**. Bi- and tetra-spored basidia; **D**. Cheiloleptocystidia; **E**. Cheilomacrocystidia; **F**. Pleuropseudocystidia; **G**. Pileipellis. Scale bar = 10 µm. Drawing: Alexandre G.S. Silva-Filho.

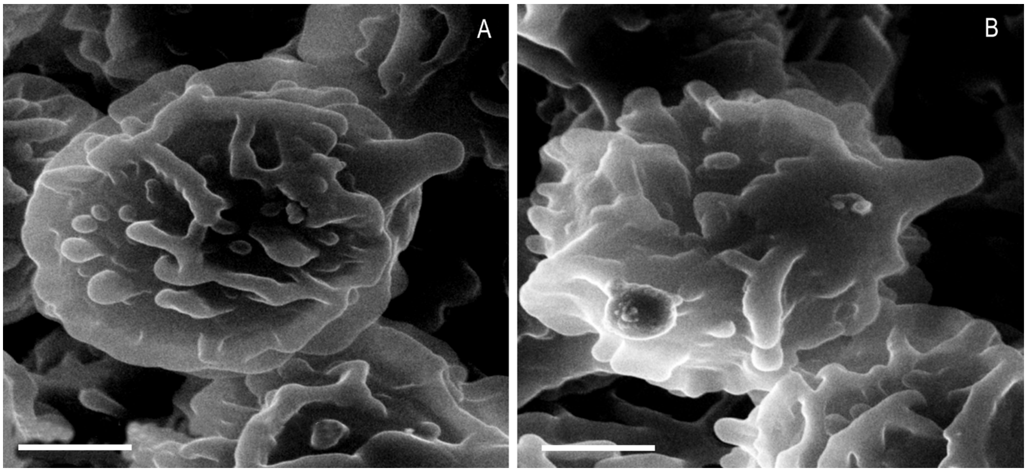

Fig. 4: Scanning electron microscopy photograph of gold-coated basidiospores of *Lactarius quieticolor,* collection M.A. Sulzbacher 520. Scale bar = 2 µm. Micrography: Alexandre G.S. Silva-Filho

flexuous in the lower portion, not projecting above the hymenium (Fig. 3F). Lamellar edge sterile, composed of scarce cheilomacrocystidia and frequent cheiloleptocystidia: cheilomacrocystidia 18-30 × 4-7 µm, subfusiform to fusiform, with moniliform apex, thin-walled, with needle-shaped content, slightly emergent (Fig. 3E); cheiloleptocystidia 14-20 × 6-9 µm, subclavate to clavate, thin-walled, hyaline (Fig. 3G). Subhymenium composed of cylindric to globose hyphae. Hymenophoral trama irregular composed of cylindrical hyphae 3-5 µm diam., smooth, thin-walled, hyaline and abundant laticifers 4-11 µm diam. Pileipellis an ixocutis, 80-180 µm thick, composed of interwoven hyphae, 2-6 µm diam., sometimes forked, smooth, thin-walled, hyaline, rarely oleiferic or laticiferic. Pileal trama composed of cylindrical hyphae 2-6 µm diam., smooth, thin-walled, hyaline and frequently mixed with laticifers 5-10 µm diam. (Fig. 3G). Stipitipellis a cutis composed by interwoven hyphae 3-5 µm diam., smooth, thin-walled, hyaline. Clamp-connections absent in all tissues examined.

Material examined: BRAZIL, RIO GRANDE DO SUL: Santa Maria, Boca do Monte, Estação Experimental de Silvicultura – FEPAGRO, under *Pinus elliottii* and *P. taeda*, 29°38'41''S and 53°56'3''W, 08.VI.2017, *leg.* M.A. Sulzbacher 520 (JPB); *ibid*, 12.VII.2018, *leg.* M.A. Sulzbacher 518 (JPB); Campus da Universidade Federal de Santa Maria, under *Pinus taeda*, 29°41'59.9''S 53°42'40.5''W, 28.VI.2018, M.A. Sulzbacher 517 (JPB); São Gabriel, under *Pinus* sp., 30°25'57,8''S and 54°22'05,3''W, 06.VII.2012, (SMDB 17523); Santa Maria, Boca do Monte, Estação Experimental de Silvicultura – FEPAGRO, under *Pinus* sp., 29°38'41''S53°56'3''W, 18.VII.2013, (SMDB 17524).

Habitat and distribution: Brazilian samples were collected in all surveyed plantations in all studied sites in Santa Catarina and Rio Grande do Sul (Brazil). Basidiomata were found in pine plantations of *Pinus taeda* and/or *P. elliottii*, solitary, on soil, partially or completely under thick needle duff. Prevailing soil types were oxisols, ultisols, mollisols or alfisols, usually ploughed prior to plantation setup, with pH ranging from 4.5 to 6.5. Climate at sites is always humid with mild to hot summers (Cfa-Cfb) and precipitation ranging from 1300-2200 mm annually.

Molecular phylogenetic results

In nrITS dataset, the alignment resulted in 856 characters (including introduced gaps). After a gblocks elimination of smaller blocks and bigger segments with contiguous non-conserved positions, the final dataset resulted in 678 informative characters. Both RAxML and Bayesian analysis yielded similar tree topologies, and the one inferred from Bayesian analyses is shown (Fig. 5). The *Lactarius* sect. *Deliciosi* clade was well supported (0.99 BPM) in Bayesian inference; on the other hand, *L. quieticolor* was supported (67% BS) only by Maximum likelihood estimation. All three samples from three different plantations in Brazilian forests grouped in the clade of *L. quieticolor*, confirming their morphological attribution. Collections from South America clustered in the same clade with European collections without any significant geographical distinction, indicating that *Lactarius quieticolor* collected in South America originates from a European ITS haplotype pool.

Discussion

There have been several past records on the occurrence of *Lactarius* sect. *Deliciosi* basidiomata in South America (e.g. Mikola, 1969). In Brazil, the country with the largest areas planted with pines (FAO, 2001), we found *L. deliciosus* reported in several fungal inventories (Guerrero and Homrich, 1999; Giachini et al., 2000; Sobestiansky, 2005; de Meijer, 2006) and reported as an edible mushroom from pine plantations in Brazil. The first identification of *L. quieticolor* in South America from several *P. radiata* plantations in the southern Mediterranean part of Chile (Chávez et al., 2015) had already put the validity of previous identifications of *L.* "*deliciosus*" in the region in doubt. This is now supported and extended by our results.

Collections gathered from different sites and in different years in Santa Catarina and Rio Grande do Sul states (Brazil) constantly matched the morphological and phylogenetic species concept of *L. quieticolor* (Romagnesi, 1958). There were only few morphological deviations from the original description, such as unchanging reddish orange latex, whereas European collections exude orange latex turning red with time (Reil, 1992; Nuythinck and Verbeken, 2005), and occasional bi-spored basidia, which have not been reported for *L. quieticolor* in the literature so far, and therefore could potentially mislead identification based solely on (macro)morphological characters.

Ecological factors play a crucial role in the specificity of fungal partners in ectomycorrhizal symbiosis. All species of *Lactarius* sect. *Deliciosi* are ectomycorrhizal, which makes them dependent on a specific host plant and habitat conditions (van der Linde et al., 2018). Indirectly, climate conditions can affect ectomycorrhizal fungi through their host-plant productivity in native and introduced vegetation (Grupe et al., 2018), while (un)favourable soil conditions can directly affect survival of the fungal partners in ectomycorrhiza. An outstanding example of soil conditions shaping the distribution and survival of mycorrhizal fungi is the case of true truffles (*Tuber* spp.), for which a high pH and a high level of carbonate in soil is a prerequisite for establishment and fruiting. At the same time, it is also a competitive advantage (García-Montero et al., 2008), and is therefore a prerequisite for truffle cultivation (Fischer et al., 2017). Soil pH is likely to affect the presence and distribution of *Lactarius* sect. *Deliciosi* in South America (Chávez et al., 2015). *Lactarius quieticolor* grows in acidic soil, while *L. deliciosus* prefers more or less neutral calcareous soils (Guerin-Laguette et al., 2000; Nuyntink and Verbeken, 2005). The absence of pine plantations on neutral or calcareous soils in Brazil and the successful growth of pines in acidic soil regions, with pH ranging from <4.5 to 6.5 (Batjes, 2012), suggests that the establishment of *L. deliciosus* is likely limited by this environmental factor, but these conditions benefit *L. quieticolor* in vast areas of northern, central and eastern South America.

Adequate identification of many non-timber forest products, including fungi, is a key quality indicator that determines the price that the buyers will pay for a particular commercialized mushroom (Lowore and Boa, 2001). In *L.* sect. *Deliciosi,* identification to species level used to be less significant as until recently all known species were regarded as edible (Nuytinck et al., 2007). However, during the last decade several new species have been described, such as *L. vividus* Wang, Nuytinck & Verbeken (Wang et al., 2015), *L. hengduanensis* X.H. Wang (Wang, 2016), *L. guangdongensis* X.H. Wang, Y. Han & C.Z. Liang (Han et al., 2019) and several other Asian and North American species (see Tab. 1 for details and references) for which edibility still needs to be confirmed. Some of these newly described species are difficult to separate from known species, due to close taxonomic affinity between them (Nuytinck et al., 2007), and thus a misidentification and consequential selling of a "wrong", less prized, or potentially non-edible species under a more favoured name is likely. Fresh *L. deliciosus* is the most popular and highly priced species in *L.* sect. *Deliciosi*, with average annual prices in the years 2002 and 2008 fluctuating between 6.08€ kg^{-1} and 16.22€ kg^{-1} on the Barcelona market (Voces et al., 2012), while some other sellers requested up to 22€ kg^{-1} for fresh *L. deliciosus* (www.selbyshrooms.com.au), based on consumer value. Prices for raw wholesale fungi are lower and range around 4€ kg^{-1} without packaging to around 6€ kg^{-1} for packed fungi (Da Re et al., 2015). So far, there is almost no fresh *L. "deliciosus"* sold in Brazil, nor were we able to find available prices for fresh *L. quieticolor.* Since different authors (Tab. 1) recognized both *L. deliciosus* and *L. quieticolor* as "excellent, good if cooked", and "edible, good quality and excellent flavour", respectively, the confusion from the culinary point of view would not significantly affect consumers. The confusion at this point may only be regarded as fraud as long as only these two species are in question.

The dispersal of different species is not only due to recent trading but also due to past events. The phylogeographic study of *L. quieticolor* and closely related species proved that continental borders and host species borders are not a migration limit for several ectomycorrhizal species; thus, the transfer of species with unknown edibility may only be a question of time. There are several studies on the distribution of alien and potentially invasive fungal species at regional or global level (Dickie et al., 2010, 2016; Fuentes et al., 2020). Besides trans-continental migration, the case of *L. quieticolor* is also an intriguing example of a host switch. Originally, *L. quieticolor* was

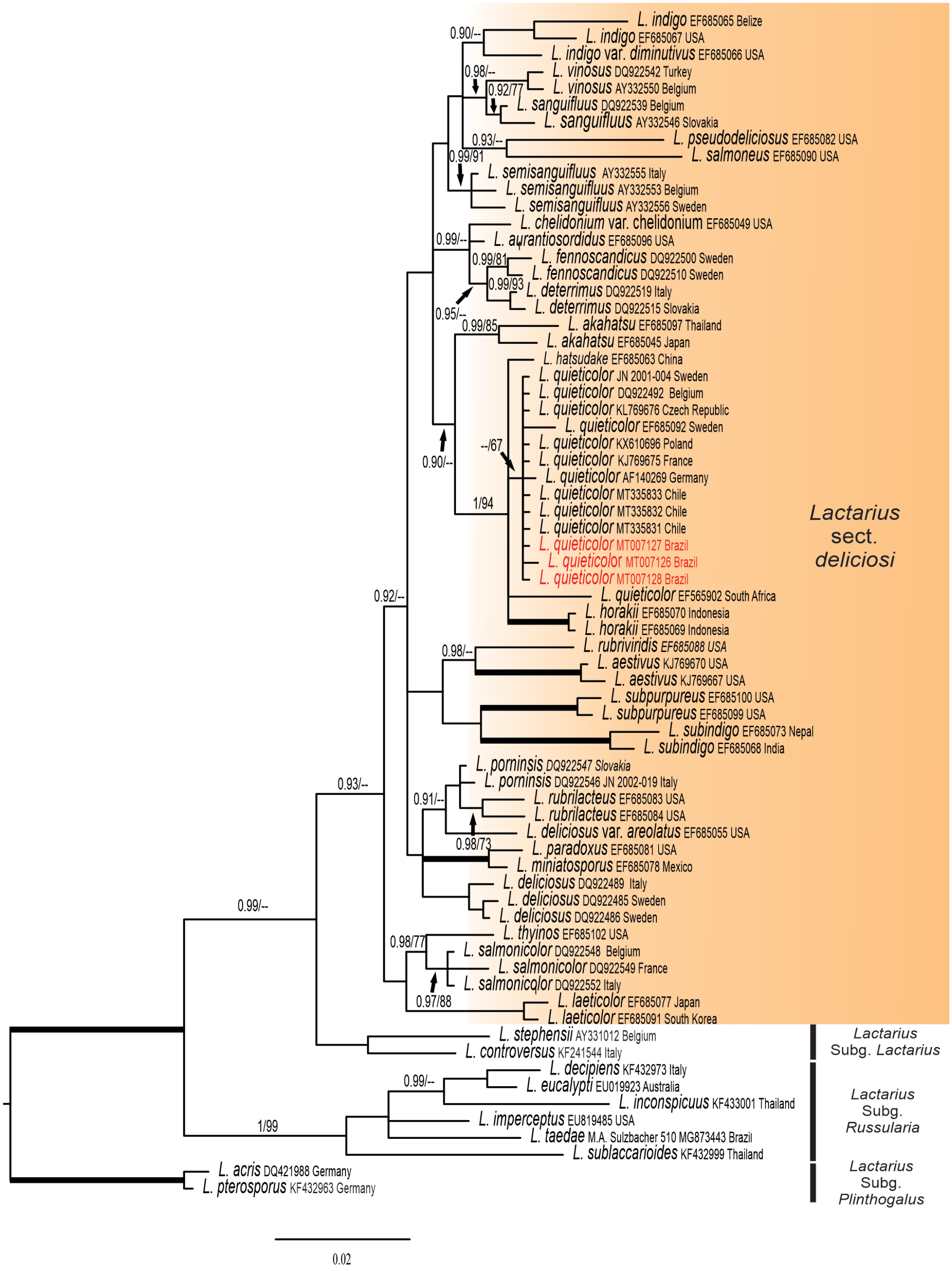

Fig. 5: A combined phylogeny of Bayesian analysis and Maximum likelihood of representative collections of *Lactarius* sect. *Deliciosi* based on the complete nuc-ITS-rDNA spacer (ITS). The specimens of the Brazilian *Lactarius quieticolor* are noted in red. Thicker lines represent branches with maximum values of bootstrap values and posterior probabilities (100% BS / 1.0 BPM). Bayesian posterior probabilities and bootstrap values are indicated if they exceed 0.95, and 60% respectively. The bar scale represents the expected number of nucleotide changes per site.

described growing under *P. sylvestris* (ROMAGNESI, 1958), a widely distributed Eurasian tree native to montane, continental, alpine and boreal climates (BURNS and HONKALA, 1990; MÁTYÁS et al., 2004). Some other collections were reported growing under *P. pinaster* (NUYNTINK and VERBEKEN, 2005), indigenous to North Africa and South Europe. Specimens reported from Chile grow associated with *P. radiata* (CHÁVEZ et al., 2015), the most planted species in that country (FAO, 2001), but native to the Pacific southwest of the U.S.A. and Mexico (BURNS and HONKALA, 1990). In this study, basidiomata of *L. quieticolor* were collected in *P. taeda* plantations, originating from eastern and south-eastern U.S.A. (BURNS and HONKALA, 1990) and planted in humid climates with mild to hot summer. Collections of *L. quieticolor* were either con-haplotypic with collections from various parts of Europe or showed a minor deviation from the dominant haplotype, but without supported geographic pattern and still within the cluster of *L. quieticolor*. Thus, it is plausible that the mycobiont has been transported and introduced from Europe attached to seeds or seedlings of *Pinus sylvestris* and/or *P. pinaster*. Other evidence that sustains this hypothesis is the unviable long-range (intercontinental) dispersion of thin-walled and unmelanized basidiospores of *Lactarius* (Chávez et al., 2015). This also support the proposal that collections from Chile and Brazil belong to this species and share its characteristics without any noticeable evolution or spatially differentiating changes in the analysed DNA marker.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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