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Three independent evolutionary events of sequestrate *Lactifluus* species in Australasia

T. Lebel^{1,2,3*}, J.A. Cooper³, M.A. Castellano⁴, J. Nuytinck⁵

¹Botanic Gardens and State Herbarium of South Australia, Hackney Rd, Adelaide, SA 5000, Australia

²Royal Botanic Gardens Victoria, Birdwood Avenue, South Yarra, Victoria, 3141 Australia

³Mannaki Whenua - Landcare Research, P.O. Box 69040, Lincoln 7640, New Zealand

⁴USDA Forest Service, Northern Research Station, 3200 Jefferson Way, Corvallis, Oregon, USA

⁵Naturalis Biodiversity Center, Darwinweg 2, 2333 CR Leiden, The Netherlands

*Corresponding author: Teresa.Lebel@sa.gov.au

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Abstract: Three Australian species with sequestrate basidiome forms are recorded for the first time in the genus *Lactifluus* based on nuclear ITS-LSU and morphological data. These species represent three rare independent evolutionary events resulting in sequestrate basidiomes arising from agaricoid species in three different sections in two subgenera. All three species have highly reduced basidiome forms, and no species with intermediate forms have been found. *Lactifluus dendriticus* is unique in the genus in having highly branched, dendritic terminal elements in the pileipellis. We provide full descriptions of two species: *Zelleromyces dendriticus* (= *Lactifluus dendriticus* comb. nov.) in *Lactifluus* subg. *Lactifluus* sect. *Gerardii*, and *Lactifluus geoprofluens* sp. nov. in *Lf.* subg. *Lactifluus* sect. *Lactifluus*. A reduced description is provided for the third, *Lactifluus* sp. prov. KV181 in *Lf.* subg. *Pseudogymnocarpi* sect. *Pseudogymnocarpi*, as it is currently known from a single sequence.

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INTRODUCTION

Distinguishing species of *Lactarius* and *Lactifluus* is difficult enough, but the loss or reduction of macroscopic features in sequestrate species makes them equally problematic to distinguish between genera. In *Lactarius*, sequestrate basidiome forms are known from three main subgenera: subg. *Plinthogalus*, subg. *Russularia*, and subg. *Lactarius* (Dring & Pegler 1978, Beaton *et al.* 1984, Desjardin 2003, Nuytinck *et al.* 2003, Eberhardt & Verbeken 2004, Verbeken & Walley 2010, Wang *et al.* 2012, Verbeken *et al.* 2014a, b, Sang *et al.* 2016, Beenken *et al.* 2016, De Crop *et al.* 2017, Vidal *et al.* 2019). All of the Australasian lactarioid sequestrate forms were historically described in the genera *Zelleromyces*, *Arcangeliella* or *Gastrolactarius*, based on morphology, following Northern Hemisphere circumscriptions of genera. Apart from a regional treatment of species of *Zelleromyces* by Beaton *et al.* (1984) for Victoria, in which the descriptions and illustrations to six species were provided, Australian species have been little studied. Bougher & Syme (1998) provided a further description of *Z. daucus* from Western Australia, Grgurinovic (1997) short descriptions and a key to three South Australian species, and Trappe & Claridge (2003, 2008) descriptions of two more species. However, the diversification of sequestrate basidiome forms in *Lactarius* in Australasia is known to be high, with eight species currently described and a further 20–25 species remaining to be described (Beaton *et al.* 1984, Bougher & Lebel 2001, Lebel 2002b, Trappe & Claridge 2003, 2008, Lebel *et al.*

unpub. data). However, no phylogenetic analyses of subgenus or sectional placement has as yet been completed for these taxa or the related epigeal taxa, and species circumscription remains based on morphology.

While conducting research on Australasian sequestrate lactarioid taxa and the *Lactifluus clarkeae* species complex, DNA sequencing revealed species with affinities to *Lactifluus* rather than *Lactarius*. This unexpected finding led us to study their morphological characters in detail, test species delimitation and further examine the phylogenetic relationships between agaricoid and sequestrate *Lactifluus* species, to determine whether sequestrate forms in *Lactifluus* are the result of independent evolutionary events, similar to the pattern seen in *Lactarius* (Verbeken *et al.* 2014a, Sheedy *et al.* 2016). Here we provide descriptions where possible and confirm placement of the taxa in different subgenera within *Lactifluus*.

MATERIALS AND METHODS

Morphology

Several hundred collections from Australasian herbaria and fungaria (MEL, PERTH, BRI, AD, HO, CANB, PDD) and material held at Oregon State Herbarium (OSC) were examined from diverse geographic regions and habitat types across Australia. Detailed coordinate and elevation data for collections mentioned here are available online through the Atlas of Living Australia

(<https://www.ala.org.au/>). Collections matching morphological characters of the known *Lactifluus* related taxa, or with typical *Lactifluus* pileipellis structure and cystidia form (approximately 35 collections), were examined in greater detail and sampled for DNA sequencing. Macroscopic characters are described and measured from fresh material, field notes of other collectors, or dried herbarium collections. Measurements taken using dried fungarium material are listed as such and are estimated to be approximately 30 % smaller than measurements taken from fresh specimens. This estimate is based on fresh and dry weights of 30 recent collections of lactarioid sequestrate basidiomes. Colours are described in general terms from field observations in daylight conditions. Habitat, associated plant communities, sporulating season, presence and nature of latex, fresh odour, and taste are based on field notes.

Microscopic characters are described from examination of dried fungarium material. Hand-cut sections were rehydrated in 5 % KOH solution then mounted in congo red to observe the hymenium, trama, and pileipellis. Spore size, shape, ornamentation and amyloidity were observed in lamellae tissue mounted in Melzer's reagent. Measurements of microscopic characters were taken on an Olympus BX-52 microscope at $\times 400$ or $\times 1\,000$ using an Olympus DP-73 camera attachment and measurement tools in cellSens standard (v. 1.16). Microscopic measurements are given as a raw range of length $\times$ width with mean $\pm$ standard deviation (SD) of n measurements in parentheses. The length/width quotient (Q) of individual spores is presented as the raw range of Q values with mean $\pm$ standard deviation (SD) of n measurements in parentheses. Basidia, basidioles, and cystidia measurements are given as length (not including sterigmata) $\times$ width at widest point, and width at base or apex. Pseudocystidia, laticiferous hyphae, and hyaline hyphae measurements are given as a raw range of diameters. In highly reduced sequestrate basidiomes there is some difficulty in determining position of cystidial elements, thus in descriptions provided here they are referred to as hymenial cystidia.

Scanning electron microscopy (SEM) of gold-sputtered basidiospores mounted on carbon tape was performed using a Thermo Fisher Scientific XL30 FEG microscope (Waltham, USA) at the University of Melbourne Biosciences Microscopy Unit.

All photographs are based on the type collections unless otherwise stated. Names of fungaria are abbreviated according to Thiers (2011) (<http://sweetgum.nybg.org/ih/> — continuously updated). We use the abbreviation 'Lf.' to distinguish *Lactifluus* from *Lactarius* ('L.') when discussing species in these genera.

Molecular studies

Protocols for DNA extraction (Qiagen Plant DNeasy kit, Germantown, USA or the EZNA forensic kit, Omega Bio-tek, Norcross USA, for samples older than 1995), PCR, and sequencing followed those in Lebel & Syme (2012) and Lebel *et al.* (2015) and the references therein. Assembly, manual editing, and preliminary alignment of sequences were performed within Geneious v. 9.1.7 (Biomatters Ltd). Individual alignments for the ITS and LSU were then manually trimmed in BioEdit v. 7.1.3 (Hall 2011) and some final manual editing done in Geneious v. 9.1.7. The concatenated alignment and phylogenetic trees are available from the Landcare Research datastore <https://doi.org/10.7931/n4fc-4z93>.

Sequences of the ITS and LSU from similar species (based on blast searches), and representative taxa to cover subgenera

and sections within *Lactifluus* were retrieved from GenBank and UNITE (Köljalg *et al.* 2013), to generate a concatenated alignment. This was done with the on-line version of MAFFT v. 7 (Katoh *et al.* 2019). Three species of *Multifurca* were utilised as outgroup. Novel sequences representing collections from Australasia and other regions generated for this study are listed in Table 1 with relevant GenBank accession numbers, and all sequences utilised in analyses. Sequences labelled as provisional taxa "Lf. sp. 1–13" are numbered according to a broader analysis of Australasian species of *Lactifluus* which will be published elsewhere; we have retained the numbering convention for consistency.

Phylogenetic analyses of the concatenated ITS+LSU alignment was performed with Maximum Likelihood (ML) in RAxML v. 8.2.12 (Stamatakis *et al.* 2014) using the CIPRES Science Gateway v. 3.3 (Miller *et al.* 2010). The final alignment comprised 211 specimens (195 ITS and 146 LSU sequences), consisting of 2 087 bp including gaps. Gaps in alignments were treated as missing data. The tree was visualised in FigTree v. 1.4.2 (Rambaut 2009).

RESULTS

Sequestrate forms arose independently in at least three sections in two subgenera of *Lactifluus*: subg. *Lactifluus* sect. *Gerardii* (Lf. *dendriticus* comb. nov.) and sect. *Lactifluus* (Lf. *geoprofluens* sp. nov.), and subg. *Pseudogymnocarpi* sect. *Pseudogymnocarpi* (Lf. sp. prov. KV181). *Lactifluus dendriticus* comb. nov. appears to be a wide-spread species from eastern Australia, ectomycorrhizal with *Eucalyptus* and *Acacia*. It is in a strongly supported clade, sister to Lf. *wirrabara*, three provisional Australian species with agaricoid basidiomes (Lf. sp. prov. 10–12), an environmental sequence with unknown basidiome form Lf. sp. prov. 13 from Australia, Lf. *limbatus* from Malaysia, Lf. *sinensis* from China, Lf. *coniculus* from Sri Lanka, and Lf. *midnapurensis* from India in section *Gerardii* (Fig. 1). All of these Asian taxa are associated with lowland tropical rainforest dominated by Dipterocarp taxa (Stubbe *et al.* 2012, Song *et al.* 2017, Phookamsak *et al.* 2019).

Lactifluus geoprofluens sp. nov. is in a weakly supported clade with two agaricoid species Lf. *jetiae* nom. prov. (Fig. 3C) and Lf. *rugulostipitatus* nom. prov. (in press; these provisional species will be published elsewhere), a third provisional species known only from environmental sequences (Lf. sp. prov. 8), several undescribed taxa with agaricoid basidiomes from Thailand and Japan, and Lf. *distantifolius* and Lf. *Longipilus* (Fig. 2). More data are required to reliably recognise sister relationships as branch support values are low in this mixed clade of mostly undescribed taxa.

A single sequence of the sequestrate *Lactifluus* sp. prov. KV181 is in a clade with a mix of species from diverse geographic locations, Lf. *pseudoluteopus* from Thailand, Lf. *hygrophoroides* from North America, Lf. *rugatus* from Southern Europe, and Lf. *holophyllus* and Lf. *luteolamellatus* from South Korea in sect. *Pseudogymnocarpi*. While we did take a small sample for DNA in the field, unfortunately, the single collection of Lf. sp. prov. KV181 was destroyed by mould contamination after drying and discarded, and no further collections of this sequestrate taxon were found while looking through abundant material of sequestrate lactarioid taxa in herbaria. Thus while a full description cannot be provided at this time, we do provide a photo of basidiomes, and a partial description, in the hope that further material will be found.

Table 1. Specimens used in the phylogenetic analysis, including infrageneric taxon, species (as originally identified in the field or as labelled in GenBank), herbarium numbers and typification, country of origin, and ITS/LSU GenBank accession numbers. New sequences generated for this study are indicated in bold. Abbreviations used: AU - Australia, NZ - New Zealand, NCal - New Caledonia, WA - Western Australia, VIC - Victoria, TAS - Tasmania, SA - South Australia, NT - Northern Territory, NSW - New South Wales, QLD - Queensland, Frisland - Fraser Island.

(Infrageneric) taxon	Species	Herbarium or collection number	Country	GenBank accession numbers	
				ITS	LSU
<i>Multifurca</i>	<i>Multifurca ochricompacta</i>	BB02107	n/a	DQ421984	DQ421984
	<i>Multifurca</i> sp.	MEL238568	AU	MW134734	MW128106
	<i>Multifurca stenophylla</i>	CWD584	AU	JX266628	JX266633
	<i>Multifurca zonaria</i>	FH12-009	Thailand	KR364083	KR364212
subg. <i>Gymnocarpi</i> sect. <i>Gymnocarpi</i>	<i>Lactifluus foetens</i>	ADK3688 BR	Benin	KR364022	KR364149
	<i>Lactifluus gymnocarpus</i>	EDC12-047 GENT	Cameroon	KR364065	KR364194
	<i>Lactifluus tanzanicus</i>	TS1277	Tanzania	KR364037	n/a
subg. <i>Gymnocarpi</i> sect. <i>Luteoli</i>	<i>Lactifluus brunneoviolascens</i>	AV13-038 GENT	Italy	KR364123	KR364246
	<i>Lactifluus caliendrifer</i>	KW378 GENT Holotype	Thailand	MK517655	n/a
	<i>Lactifluus luteolus</i>	AV05-253 GENT	USA	KR364016	KR364142
	<i>Lactifluus russuliporus</i>	REH9398 NY Holotype	AU	KR364097	KR364229
subg. <i>Gymnocarpi</i> sect. <i>Nebulosi</i>	<i>Lactifluus chiapanensis</i>	V.M.Bandala 4374A GENT	Mexico	GU258297	GU265580
	<i>Lactifluus guadeloupensis</i>	RC_Guad11-023 LIP Holotype	Guadeloupe	KP691412	KP691421
subg. <i>Gymnocarpi</i> sect. <i>Panuoidei</i>	<i>Lactifluus panuoides</i>	G128	Guyana	KJ786647	KJ786551
subg. <i>Gymnocarpi</i> sect. <i>Phlebonemi</i>	<i>Lactifluus</i> aff. <i>phlebonemus</i>	EDC12-023 GENT	Cameroon	KR364062	KR364191
subg. <i>Gymnocarpi</i> sect. <i>Tomentosi</i>	<i>Lactifluus albens</i>	MEL2231695 Type	AU_WA	MW134740	MW128112
	<i>Lactifluus aurantioruber</i>	MEL2257827	AU_TAS	MW134745	MW128115
	<i>Lactifluus clarkeae</i>	MEL2101947 Epitype	AU_SA	MW134754	MW128120
	<i>Lactifluus clarkeae</i>	PDD102596	NZ	MW134770	MW128130
	<i>Lactifluus flocktonae</i>	MEL2238290 Epitype	AU_VIC	JX266621	JX266637
	<i>Lactifluus psammophilus</i>	MEL2238407 Type	AU_VIC	MW134791	MW128144
	<i>Lactifluus pseudoflocktonae</i>	MEL2238269 Holotype	AU_VIC	MW134801	MW128151
	<i>Lactifluus</i> sp. 1	environmental sample CMMY30M1	New Caledonia	KY774240	n/a
	<i>Lactifluus</i> sp. 5	PGK13-130 Nothofagus	New Caledonia	KP691436	KR605507
	<i>Lactifluus</i> sp. 6	environmental sample KT-26 Tristaniopsis	New Caledonia	LC271308	n/a
subg. <i>Lactariopsis</i> sect. <i>Albati</i>	<i>Lactifluus vellereus</i>	UE20.09.2004-22 UPS	n/a	DQ422034	DQ422034
subg. <i>Lactariopsis</i> sect. <i>Edules</i>	<i>Lactifluus edulis</i>	FN05-628 GENT	Malawi	KR364020	KR364147
subg. <i>Lactariopsis</i> sect. <i>Lactariopsis</i>	<i>Lactifluus annulatoangustifolius</i>	BB00-1518 PC	Madagascar	AY606981	KR364253
	<i>Lactifluus velutissimus</i>	JD886	Congo	KR364075	KR364204
subg. <i>Lactariopsis</i> sect. <i>Neotropicus</i>	<i>Lactifluus venezuelanus</i>	RC_Gaud11-017 LIP	Guadeloupe	KP691411	KP691420
subg. <i>Lactifluus</i> sect. <i>Allardii</i>	<i>Lactifluus allardii</i>	AV05-286 GENT	USA	KF220015	KF220124
subg. <i>Lactifluus</i> sect. <i>Ambicystidiati</i>	<i>Lactifluus ambicystidiatus</i>	KUN_F88179	China	KR908670	KR908672
subg. <i>Lactifluus</i> sect. <i>Gerardii</i>	<i>Lactifluus auriculiformis</i>	AV12-050 GENT Holotype	Thailand	KR364086	KR364216

Table 1. (Continued).

(Infrageneric) taxon	Species	Herbarium or collection number	Country	GenBank accession numbers	
				ITS	LSU
subg. <i>Lactifluus</i> sect. <i>Gerardii</i>	<i>Lactifluus bhandaryi</i>	TENN 051830 Holotype	Nepal	KR364111	n/a
	<i>Lactifluus conchatulus</i>	LTH457 GENT Isotype	Thailand	GU258296	GU265659
	<i>Lactifluus coniculus</i>	DS07-496 GENT Holotype	Sri Lanka	GU258236	GU265594
	<i>Lactifluus coniculus</i>	DS07-497 GENT	Sri Lanka	GU258237	GU265595
	<i>Lactifluus dendriticus</i> comb. nov.	MEL2063377	AU_VIC	MW471118	MW471115
	<i>Lactifluus dendriticus</i> comb. nov.	MEL2292167_CANB748620	AU_VIC	MW471119	MW471116
	<i>Lactifluus dendriticus</i> comb. nov.	MEL2326248	AU_VIC	MW471120	n/a
	<i>Lactifluus dendriticus</i> comb. nov.	MEL2126553	AU_VIC	MW471121	n/a
	<i>Lactifluus dendriticus</i> comb. nov.	MEL2061947	AU_NSW	MW471122	n/a
	<i>Lactifluus fuscomarginatus</i>	GO2010-144	Mexico	KC152157	n/a
	<i>Lactifluus genevieveae</i>	G.Gates_D.Ratkowsky 17-2-2005	AU	GU258294	GU265657
	<i>Lactifluus gerardiellus</i>	KW386 GENT Holotype	Thailand	KX889845	KX889844
	<i>Lactifluus gerardii</i>	AV05-375 GENT	USA	GU258254	GU265616
	<i>Lactifluus gerardii</i>	Desjardin3630	USA	GU258220	n/a
	<i>Lactifluus igniculus</i>	LE262983 Type	Vietnam	JX442759	n/a
	<i>Lactifluus indicus</i>	CAL 1282 Holotype	India	KU145119	KU145121
	<i>Lactifluus leae</i>	AV-RW04-90 GENT	Thailand	GU258244	
	<i>Lactifluus leae</i>	FH12-13 GENT	Thailand	KF432957	
	<i>Lactifluus leonardii</i>	P.Leonard 35607	AU	GU258295	GU265658
	<i>Lactifluus leonardii</i>	G.Gates 29-1-2002	AU	GU258304	GU265664
	<i>Lactifluus limbatus</i>	DS06-230 GENT	Malaysia	GU258222	GU265578
	<i>Lactifluus limbatus</i>	DS06-247 GENT	Malaysia	GU258223	GU265579
	<i>Lactifluus midnapurensis</i>	CAL 1516 Holotype	India	KY785175	KY785177
	<i>Lactifluus ochrogalactus</i>	E.Nagasawa 80-102 TMI Type	Japan	GU258280	
	<i>Lactifluus parvigerardii</i>	KUN_F61367 Holotype	China	JF975641	JF975642
	<i>Lactifluus petersenii</i>	AV05-267 GENT	USA	GU258282	GU265643
	<i>Lactifluus pulchrellus</i>	KW304_FH12-037 GENT Holotype	Thailand	KR364092	KR364223
	<i>Lactifluus raspei</i>	EDC14-517 Holotype	Thailand	KX889849	n/a
	<i>Lactifluus reticulatovenosus</i>	Horak 6472 GENT Holotype	Indonesia	GU258286	GU265649
	<i>Lactifluus robustus</i>	K16053113	China	KY353803	KY353806
	<i>Lactifluus robustus</i>	K15052822	China	KY353802	KY353805
	<i>Lactifluus sepiaceus</i>	MEL2300727	AU	GU258293	GU265656
	<i>Lactifluus sepiaceus</i>	MEL1054958	AU_VIC	MW134808	n/a
	<i>Lactifluus sepiaceus</i>	P.Leonard 40509	NZ	GU258287	GU265650
	<i>Lactifluus sinensis</i>	K15060710 Holotype	China	KT900208	n/a
	<i>Lactifluus sinensis</i>	K15070203	China	KT900209	n/a
	<i>Lactifluus sinensis</i>	environmental sample HIB12	China	JX457047	n/a
	<i>Lactifluus</i> sp.	DS06-003 GENT	Malaysia	GU258231	GU265588
	<i>Lactifluus</i> sp.	AV05-283 GENT	USA	GU258259	n/a
	<i>Lactifluus</i> sp.	DPLewis6983	USA	GU258272	n/a
	<i>Lactifluus</i> sp.	Desjardin3564	n/a	GU258273	n/a
	<i>Lactifluus</i> sp. 10	P.Leonard 10409	AU	JF731001	JF731003
	<i>Lactifluus</i> sp. 10	MEL2305122	AU_QLD	MW134809	n/a
	<i>Lactifluus</i> sp. 10	MEL2332066	AU_QLD	MW134810	n/a
	<i>Lactifluus</i> sp. 11	PL26078	AU	n/a	MW128157

Table 1. (Continued).

(Infrageneric) taxon	Species	Herbarium or collection number	Country	GenBank accession numbers	
				ITS	LSU
subg. <i>Lactifluus</i> sect. <i>Lactifluus</i>	<i>Lactifluus</i> sp. 12	R.E.Halling 6800	AU	JF731000	JF731002
	<i>Lactifluus</i> sp. 13	environmental sample RFLP61	AU	DQ388868	n/a
	<i>Lactifluus</i> sp. 13	environmental sample Toosoi16	AU	KC222796	n/a
	<i>Lactifluus</i> sp. 13	environmental sample Toosoi56	AU	KC222836	n/a
	<i>Lactifluus subgerardii</i>	AV05-389 GENT	USA	GU258271	n/a
	<i>Lactifluus wirrabara</i>	G.Gates_D.Ratkowsky 12-07-2003	AU	GU258306	GU265666
	<i>Lactifluus wirrabara</i>	G.Gates_D.Ratkowsky 17-01-2002	AU	GU258305	GU265665
	<i>Lactifluus wirrabara</i>	G.Gates_D.Ratkowsky 24-01-2004	AU	GU258307	n/a
	<i>Lactifluus wirrabara</i>	JET943 MEL	AU	GU258291	n/a
	<i>Lactifluus acicularis</i>	DS07-456 GENT	Thailand	HQ318224	HQ318125
	<i>Lactifluus acicularis</i>	KVP08-033 GENT	Thailand	HQ318242	HQ318150
	<i>Lactifluus corrugis</i>	JN2004-015 GENT	USA	JQ753820	JQ348262
	<i>Lactifluus corrugis</i>	AV05-291 GENT	USA	JQ753823	JQ348266
	<i>Lactifluus crocatus</i>	LTH268 GENT	Thailand	HQ318266	HQ318181
	<i>Lactifluus crocatus</i>	LTH202 GENT	Thailand	HQ318248	HQ318157
	<i>Lactifluus dissitus</i>	AV-KD-KVP09-082 GENT	India	n/a	JN389035
	<i>Lactifluus distantifolius</i>	DS07-461 GENT Isotype	Thailand	HQ318223	HQ318124
	<i>Lactifluus distantifolius</i>	LTH288 GENT	Thailand	HQ318274	HQ318193
	<i>Lactifluus geoprofluens</i> sp. nov.	MEL2145804 Holotype	AU_VIC	MW471123	MW471117
	<i>Lactifluus jetiae</i>	MEL2238281 Holotype	AU_VIC	MW134811	MW128158
	<i>Lactifluus jetiae</i>	MEL2341759	AU_VIC	MW134813	n/a
	<i>Lactifluus leptomerus</i>	AV-KD-KVP09-130 GENT	India	JN388971	JN389022
	<i>Lactifluus leptomerus</i>	AV-KD-KVP09-131 GENT Holotype	India	JN388972	JN389023
	<i>Lactifluus longipilus</i>	LTH273 GENT	Thailand	HQ318276	HQ318195
	<i>Lactifluus longipilus</i>	LTH168 GENT	Thailand	HQ318235	HQ318143
	<i>Lactifluus maenamensis</i>	KD 16-008	India	MF928075	n/a
	<i>Lactifluus mexicanus</i>	Montoya5276 Holotype	Mexico	MK211181	MK211190
	<i>Lactifluus oedematopus</i>	AV07-079 GENT	Belgium	JQ753835	JQ348270
	<i>Lactifluus oedematopus</i>	RW1228 GENT	France	HQ318216	HQ318116
	<i>Lactifluus oedematopus</i>	KVP12-001 GENT Neotype	Germany	KR364100	KR364232
	<i>Lactifluus pagodicystidiatus</i>	MEL2121979	AU_VIC	MW134815	MW128161
	<i>Lactifluus pagodicystidiatus</i>	MEL2150777 Holotype	AU_VIC	MW134816	MW128162
	<i>Lactifluus pallidilamellatus</i>	Leticia Montoya 4716	Mexico	JQ753824	JQ348268
	<i>Lactifluus pinguis</i>	LTH117 GENT Holotype	Thailand	HQ318211	HQ318111
	<i>Lactifluus pinguis</i>	LTH169 GENT	Thailand	HQ318221	HQ318121
	<i>Lactifluus rugulostipitatus</i>	MEL2329677 Holotype	AU_NT	MW134817	MW128163
	<i>Lactifluus rugulostipitatus</i>	MEL2329673	AU_NT	MW134819	n/a
	<i>Lactifluus</i> sp.	KIINA158 GENT	China	HQ318225	HQ318126
	<i>Lactifluus</i> sp.	AV-KD-KVP09-134 GENT	India	JN388978	JN389026
	<i>Lactifluus</i> sp.	AV-KD-KVP09-128	India	n/a	JN389020
	<i>Lactifluus</i> sp.	AV-KD-KVP09-137	India	n/a	JN389027
	<i>Lactifluus</i> sp.	AV-KD-KVP09-129	India	n/a	JN389021
	<i>Lactifluus</i> sp.	OSA-My-3993	Japan	n/a	AB238645
	<i>Lactifluus</i> sp.	OSA-My-3998	Japan	n/a	AB238650
	<i>Lactifluus</i> sp.	OSA-My-4003	Japan	n/a	AB238655

Table 1. (Continued).

(Infrageneric) taxon	Species	Herbarium or collection number	Country	GenBank accession numbers	
				ITS	LSU
	<i>Lactifluus</i> sp.	OSA-My-3994	Japan	n/a	AB238646
	<i>Lactifluus</i> sp.	OSA-My-4014	Japan	n/a	AB238666
	<i>Lactifluus</i> sp.	OSA-My-4015	Japan	n/a	AB238667
	<i>Lactifluus</i> sp.	OSA-My-3999	Japan	n/a	AB238651
	<i>Lactifluus</i> sp.	LTH313 GENT	Thailand	HQ318272	HQ318190
	<i>Lactifluus</i> sp.	LTH133 GENT	Thailand	HQ318212	HQ318112
	<i>Lactifluus</i> sp.	KVP08-006 GENT	Thailand	HQ318229	HQ318136
	<i>Lactifluus</i> sp.	LTH231 GENT	Thailand	HQ318278	HQ318197
	<i>Lactifluus</i> sp.	LTH123 GENT	Thailand	HQ318222	HQ318122
	<i>Lactifluus</i> sp.	LTH294 GENT	Thailand	HQ318273	HQ318191
	<i>Lactifluus</i> sp.	KVP08-021 GENT	Thailand	HQ318233	HQ318140
	<i>Lactifluus</i> sp.	LTH170 GENT	Thailand	HQ318252	HQ318165
	<i>Lactifluus</i> sp.	LTH264 GENT	Thailand	HQ318264	HQ318179
	<i>Lactifluus</i> sp.	KVP08-008 GENT	Thailand	HQ318231	HQ318138
	<i>Lactifluus</i> sp.	LTH249 GENT	Thailand	n/a	HQ318176
	<i>Lactifluus</i> sp.	LTH284 GENT	Thailand	HQ318253	HQ318166
	<i>Lactifluus</i> sp.	KVP08-026 GENT	Thailand	HQ318238	HQ318146
	<i>Lactifluus</i> sp.	DED7577	USA	n/a	HQ318188
	<i>Lactifluus</i> sp.	AV05-394 GENT	USA	GU258300	GU265660
	<i>Lactifluus</i> sp.	EIU-ASM10990	USA	JQ358921	JN940236
	<i>Lactifluus</i> sp.	AV04-209 GENT	USA	JN388977	JN388998
	<i>Lactifluus</i> sp.	EIU-ASM11130	USA	JQ358938	JN940223
	<i>Lactifluus</i> sp.	SAM310809-02 TENN	USA	MF773609	n/a
	<i>Lactifluus</i> sp.	MycoMap10398	USA	MH975019	n/a
	<i>Lactifluus</i> sp.	AV05-337 GENT	USA	JQ753821	n/a
	<i>Lactifluus</i> sp.	AV04-167 GENT	n/a	JQ753827	JQ348273
	<i>Lactifluus</i> sp. 8	environmental sample RFLP38	AU_QLD	DQ388845	n/a
	<i>Lactifluus</i> sp. 8	environmental sample RFLP39	AU_QLD	DQ388846	n/a
	<i>Lactifluus</i> sp. 8	environmental sample RFLP5	AU_QLD	DQ388812	n/a
	<i>Lactifluus</i> sp. 9	REH9320 NY	AU	KR364096	KR364228
	<i>Lactifluus</i> sp. 9	environmental sample Toosoil58	AU_QLD	KC222838	n/a
	<i>Lactifluus subvolemus</i>	KVP08-048 GENT	Slovenia	JQ753927	JQ348379
	<i>Lactifluus subvolemus</i>	LAS75_092-A	Sweden	n/a	JQ348348
	<i>Lactifluus versiformis</i>	AV-KD-KVP09-047 GENT	India	JN388964	JN389032
	<i>Lactifluus versiformis</i>	AV-KD-KVP09-014 GENT Holotype	India	JN388963	JN389029
	<i>Lactifluus vitellinus</i>	LTH348 GENT	Thailand	HQ318251	HQ318164
	<i>Lactifluus vitellinus</i>	KVP08-024 GENT Holotype	Thailand	HQ318236	HQ318144
	<i>Lactifluus volemus</i>	UE09.08.2004-5 UPS	n/a	DQ422008	DQ422008
subg. <i>Lactifluus</i> sect. <i>Piperati</i>	<i>Lactifluus albopicroi</i>	MDB_F12_18	AU_NT	MN598888	MN598864
	<i>Lactifluus albopicros</i>	MEL2297391 Type	AU_VIC	MN598874	MN598855
	<i>Lactifluus austropiperatus</i>	PERTH07550324 Type	AU_QLD	MN614115	MN614111
	<i>Lactifluus austropiperatus</i>	MEL2150778	AU_VIC	MN614116	MN614112
	<i>Lactifluus dwaliensis</i>	LTH67 GENT	Thailand	KF220108	KF220203
	<i>Lactifluus glaucescens</i>	M.Lecomte_2002-20-9-3	France	KF220031	KF220134
	<i>Lactifluus leucophaeus</i>	AV97-382 GENT	Papua New Guinea	GU258299	GU265640

Table 1. (Continued).

(Infrageneric) taxon	Species	Herbarium or collection number	Country	GenBank accession numbers	
				ITS	LSU
subg. <i>Lactifluus</i> sect. <i>Tenuicystidiati</i>	<i>Lactifluus piperatus</i>	M.Lecomte_2001-8-19-23	France	KF220120	KF220212
	<i>Lactifluus subpruinus</i>	KUN_F76034	China	KC154110	KC154136
	<i>Lactifluus tropicosinicus</i>	KUN_F59626	China	KC154120	KC154146
subg. <i>Pseudogymnocarpi</i>	<i>Lactifluus armeniacus</i>	EDC14-501 GENT Holotype	Thailand	KR364127	n/a
	<i>Lactifluus</i> sp.	TENN065929	USA	KR364102	KR364233
	<i>Lactifluus</i> sp.	JN2011-012 GENT	Vietnam	KR364045	KR364171
	<i>Lactifluus</i> sp. 7	AQ797939	AU_QLD	n/a	MW128164
	<i>Lactifluus</i> sp. 7	FG2018031	AU_QLD	MW134820	MW128165
	<i>Lactifluus</i> sp. 7	AQ794627	AU_QLD	MW134821	MW128166
subg. <i>Pseudogymnocarpi</i> sect. <i>Pseudogymnocarpi</i>	<i>Lactifluus volemoides</i>	TS0705 Holotype	Tanzania	KR364038	KR364165
	<i>Lactifluus</i> cf. <i>pseudogymnocarpus</i>	AV05-085 GENT	Malawi	KR364012	n/a
	<i>Lactifluus</i> cf. <i>pumilus</i>	EDC12-066 GENT	Cameroon	KR364067	n/a
	<i>Lactifluus flavellus</i>	MD393 Holotype	Togo	LK392594	n/a
	<i>Lactifluus gymnocarpoides</i>	JD885	DR Congo	KR364074	n/a
	<i>Lactifluus holophyllus</i>	ASIS19960	South Korea	MF611684	MF611659
	<i>Lactifluus holophyllus</i>	ASIS22632	South Korea	MF611685	n/a
	<i>Lactifluus holophyllus</i>	SFC20150812-63 Holotype	South Korea	MF611683	n/a
	<i>Lactifluus hygrophoroides</i>	AV05-251 GENT	USA	HQ318285	HQ318208
	<i>Lactifluus hygrophoroides</i>	EIU-ASM10004 clone c4	USA	JQ358911	n/a
	<i>Lactifluus longisporus</i>	AV94-557 GENT	Burundi	KR364118	KR364244
	<i>Lactifluus longisporus</i>	AV11-025 GENT	Tanzania	KR364054	n/a
	<i>Lactifluus luteolamellatus</i>	MHHNU8297	China	MK167429	n/a
	<i>Lactifluus luteolamellatus</i>	SFC20150818-39	South Korea	MF611680	n/a
	<i>Lactifluus luteopus</i>	AV94-463 GENT Type	Burundi	KR364119	n/a
	<i>Lactifluus luteopus</i>	EDC11-087 GENT	Tanzania	KR364049	KR364176
	<i>Lactifluus medusae</i>	EDC12-152 GENT	Cameroon	KR364069	n/a
	<i>Lactifluus pseudohygrophoroides</i>	SFC20140821-45 Holotype	South Korea	MF611682	MF611657
	<i>Lactifluus pseudohygrophoroides</i>	SFC20150813-71	South Korea	MF611681	n/a
	<i>Lactifluus pseudoluteopus</i>	LTH155 GENT	Thailand	HQ318286	HQ318210
	<i>Lactifluus pseudoluteopus</i>	FH12-026 GENT	Thailand	KR364084	n/a
	<i>Lactifluus pseudoluteopus</i>	environmental sample CD15	Thailand	FJ644702	n/a
	<i>Lactifluus rugatus</i>	EP 1212_7 LGAM-AUA	Greece	KR364104	KR364235
	<i>Lactifluus rugatus</i>	PA2010R	Greece	MH125243	n/a
	<i>Lactifluus rugatus</i>	4_01_2015	Italy	KU885436	n/a
	<i>Lactifluus</i> sp.	environmental sample L7524_Russ MAD37	Madagascar	FR731264	n/a
	<i>Lactifluus</i> sp.	environmental sample T071b	Thailand	JN969388	n/a
	<i>Lactifluus</i> sp.	MycoMap6251	USA	MK560130	n/a
	<i>Lactifluus</i> sp.	MycoMap6284	USA	MK560131	n/a
	<i>Lactifluus</i> sp.	FLAS-F-61011	USA	MH016945	n/a
	<i>Lactifluus</i> sp.	KUNF58696	n/a	KC154100	n/a
	<i>Lactifluus</i> sp.	KV181	AU_NSW	MW471124	n/a
	<i>Lactifluus sudanicus</i>	AV11-174 MD105	Togo	HG426469	KR364186
	<i>Lactifluus sudanicus</i>	MD148	Togo	HG426476	n/a

Fig. 1. Maximum Likelihood tree based on ITS and LSU sequences for subgenus *Lactifluus* sections *Gerardii*, *Piperati*, *Ambicystidiati*, and *Allardi*; subgenus *Gymnocarpi* sections *Tomentosi*, *Phlebonemi*, *Panuioidei*, *Nebulosi*, *Luteoli*, *Gymnocarpi*, and *Lf. vellereus* from *Lf. sect. Albati*, subg. *Lactariopsis*, with outgroup *Multifurca*. Bold lines indicate ML support $\geq 90\%$. Bold text sequences of sequestrate taxa generated for this study. Red text: Australian specimens or sequences, blue text: New Zealand specimens, green text: New Caledonia specimens or sequences.

Fig. 2. Maximum Likelihood tree based on ITS and LSU sequences for subgenus *Lactifluus* sections *Lactifluus* and *Tenuicystidiati*; and subgenus *Pseudogymnocarpi* section *Pseudogymnocarpi* and an unnamed clade, and exemplars from sections *Lactariopsis*, *Neotropicus* and *Edules*. Bold lines ML ≥ 90 %. Bold text sequences generated for this study. Red text: Australian specimens or sequences.

Taxonomy

Section *Gerardii*

Lactifluus dendriticus (T. Lebel) T. Lebel, J. Cooper & Nuytinck, *comb. nov.* MycoBank MB 838127. Figs 3B, 4A–F.

Basionym: *Zelleromyces dendriticus* T. Lebel, *Australasian Mycologist* **21**: 4. 2002. figs 1–4. MB 373383.

Etymology: The specific epithet, “*dendriticus*” (L), refers to the dendritic terminal element of the pileipellis.

Diagnosis: This species can be distinguished by the pale sequestrate basidiomes, unique dendritic terminal elements forming a short turf in the pileipellis, and globose spores.

Typus: **Australia**, Victoria, Errinundra National Park, The Gap Scenic Reserve, Gunmark Road, 1.3 km southeast of Survey

Fig. 3. Basidiomata of new sequestrate species and representative agaricoid species. **A.** *Lactifluus wirrabara* (JET 943; photo J.E. Tonkin). **B.** *Lf. dendriticus* (photo T. Lebel). **C.** *Lf. jetiae* (photo J.E. Tonkin). **D.** *Lf. geoprofluens* (photo L. Vaughan). **E.** *Lf. rugatus* (photo U. Pero). **F.** *Lf. sp. prov.* KV181 (photo T. Lebel). Scale bars = 10 mm

Fig. 4. *Lactifluus dendriticus*. **A.** Cross-section of pileipellis with dendritic terminal elements. **B.** Scalp section showing dendritic terminal elements. **C.** Dendritic terminal elements. **D.** Basidiospores. **E.** Hymenial trama with pseudocystidia. **F.** Scanning Electron Microscopy photo micrograph of basidiospores. Scale bars: A–D, F = 10 µm, E = 50 µm.

Road, Claridge site 124, 28 May 1996, A. Jumpponen & J. Trappe 18478 (holotype MEL 2063482, isotype OSC 80519).

Basidiomes hypogaeal, sequestrate, 5–25 × 5–20 mm, globose to subglobose, slightly irregular or furrowed near point of attachment; locules exposed at point of attachment in some basidiomata as pileus is incomplete, surface dry, smooth to minutely fibrillose or verrucose, white with a faint yellowish tinge and often with small darker yellow-brown patches, pileipellis thin, off-white in cross-section. *Hymenophore* loculate, locules large 0.5–2 mm diam, somewhat irregular, white when young, becoming pale yellow to pale brown-yellow with maturity, pale tan when dried. *Stipe-columella* absent. *Latex* scant to abundant, white, unchanging. *Taste* mild. *Odour* mildly sweet, pleasant. *Basidiospores* 9.0–10.8 × 8.5–10.6 µm ($\bar{x}$ = 9.8 ± 0.53 × 9.69 ± 0.64, n = 50), globose [Q=1.0–1.05 ($\bar{x}$ = 1.01 ± 0.02, n = 50)], walls weakly amyloid, ornamentation a fine, complete to almost complete regular mesh reticulum ± 0.5–0.7 µm high, with few scattered, isolated warts also present; hilar appendix small, central, plage absent. *Basidia* 33–65 × 7–13 µm, elongated clavate to cylindrical or rarely narrowly ventricose, hyaline, thin-walled, with 2 or 4 robust, slightly curved sterigmata 4–10 × 2–3.5 µm. *Hymenophoral trama* 14–22 µm wide, a narrow central strand of crowded, hyaline hyphae 2–4 µm diam, with slightly thickened walls intermixed with scattered sinuous laticiferous hyphae 3–7.5 µm diam, sphaerocytes absent; subhymenium 15–35 µm wide, with two or three tiers of ± isodiametric cells 8–15 µm diam. *Pseudocystidia* absent or when present, 5–11 µm diam, thin-walled, cylindrical to narrowly clavate, scattered, with oily refractive contents; arising in trama from laticiferous hyphae extending up through the subhymenium. *Pileipellis* 18–45 µm wide, composed of a very narrow cutis with an almost complete turf of thick-walled (throughout length) dendritic terminal elements and scattered rare laticiferous hyphae when young, this turf becoming patchy as basidiomata expands; no gelatinous matter or very little in some basidiomes apparent amongst terminal elements dendritic terminal elements less branched and intricate when young, variously and often elaborately branched when older, coralloid to irregularly repeatedly branched with coralloid tips, 18–32 µm high, 4–25 µm wide, 3–6 µm at base, walls thick. *Pileus trama* 32–60 µm wide, of tightly interwoven, subgelatinous, hyaline hyphae 2–4 µm diam, more crowded towards the surface than within, with scattered to common, sinuous laticiferous hyphae 3–8 µm diam, refractive in KOH; sphaerocytes absent.

Ecology and Distribution: Sporulating in April–July in small to large groups, at mid to higher elevations in the mountains of south eastern Australia in Victoria, Tasmania, and New South Wales. Hypogaeal in mixed forests of *Eucalyptus fastigata*, *E. cypellocarpa*, *E. radiata*, *E. dalrympleana*, *E. globoidea*, *E. obliqua*, *E. pauciflora*, *E. stellulata*, *E. regnans*, *Acacia dealbata*, *A. melanoxylon*, *A. aculeatissima*, *A. cognata*, or *A. mearnsii*.

Additional specimens examined: **Australia**, Victoria, Kinglake National Park, 120 m down Mountain Creek Track, 8 Jul. 1993, M.A. Castellano OSC 80520 (MEL 2063479); East Gippsland, Errinundra National Park, The Gap Scenic Reserve, Gunmark Road, 1.3 km SE of Survey Road, Claridge Site 124, 30 May 2003, A.W. Claridge, W. Colgan III, A. Jumpponen, I. Kratzer AWC5079 (CANB 748631); *ibid.*, Gap Road, 50 m E of Bonang River Bridge, Claridge Site 117, 9 May 2003, A.W. Claridge AWC4731 (CANB 65451); *ibid.* Claridge Site 117, 27 May 1999, J.M. Trappe AWC2589 (MEL 2105044); *ibid.*, 27

May 1999, J.M. Trappe AWC2588 (MEL 2105043); *ibid.*, Gap Road, 3.9 km E of Bonang Highway, Claridge site 118, 28 May 1996, J.M. Trappe 18434 (MEL 2063477); *ibid.*, Gap Road, 2.1 km west of track to Result Creek Falls, Claridge Site 138, 11 Jun. 1996, J.M. Trappe 19022 (MEL 2063472); *ibid.*, Gap Road, 5.2 km E of Junction with Bonang Highway, Claridge Site 119, 9 May 2003, J.M. Trappe & A.W. Claridge AWC4741 (DAR 76661, CANB 748620, MEL 2292167) (also in K, NY, BPI, FH); *ibid.*, Claridge Site 119, 26 May 2001, T. Lebel AWC3994 (CANB 736252); *ibid.*, Gap Road, 2.1 km west of track to Result Creek Falls, Claridge Site 138, 11 Jun. 1996, A.M. Jumpponen JMT19021 (MEL 2063473); Rich Forest Management Block, Jack Road, 0.7 km N of Winter Road, Claridge Retrospective Study Site R19, 27 Apr. 1996, A.W. Claridge AWC352 (CANB 669623); Lind National Park, Euchre Valley Road, 0.2 km W of Junction with Lind Park Road, Claridge Site 74, 29 May 1999, A.W. Claridge AWC2746B (MEL 210507); Murrungowar Forest Management Block, Princes Highway, 0.4 km E of Junction with Bendoc Ridge Road, Claridge Site 64, 26 May 1999, T. Lebel AWC2556 (MEL 2105041); 2.4 km N of Baw Baw National Park, 16 Jun. 1994, J.M. Trappe H6792 (PERTH 7593031); 20 km west of Mansfield near Mt Buller, 9 Jul. 1993, T. Lebel JMT14063 (MEL 2063478); Alpine National Park, Black Mountain Road, Rams Horn Track. Claridge Site No. 78, 11 Nov. 2008, A.W. Claridge JMT18089 (MEL 2326248); Noojee State Park, ca. 1 km from Tooronga Road, along Link Road in small carpark area, 25 Feb. 2002, S.H. Lewis 791 (MEL 2061947); North east of Marysville on Lady Talbot Drive, wishing Well Track, 19 Apr. 1999, T. Lebel TL5 (MEL 2063377); New South Wales, Bombala, Bondi Gulf Nature Reserve, Bondi Gulf Road, 4 km E of Cann Valley Highway, Claridge Site 53, 15 Jun. 1999, J.M. Trappe AWC2972 (CANB 748185); Tasmania, Break O'Day, Elephant Pass, 16 kms southeast of St Marys, 2 May 1990, N. Malajczuk MEL 2063481; Elephant Pass, 2 May 1990, J.M. Trappe H1373 (PERTH 7673167).

Notes: A revised description of *Lactifluus dendriticus* is provided here to enable better comparisons to other taxa, and to incorporate more data from fresh collections. Detailed illustrations of spores, pileipellis and hymenophore structure and constituent elements were provided as part of the original description (Lebel 2002). *Lactifluus dendriticus* is widespread in eucalypt forests occurring in regions with relatively higher rainfall. Its basidiomata do not always produce a latex when cut or bruised. Microscopically, *Lactifluus dendriticus* is unique, with distinct highly branched pileal terminal elements (Fig. 4A–C) that have not been observed in any other species of sequestrate or currently known agaricoid *Russulales* taxa.

Taxa in the larger clade, *Lactifluus wirrabara*, *Lf. limbatus*, *Lf. coniculus*, *Lf. sinensis*, and *Lf. midnapurensis* all have brownish agaricoid basidiocarps (Fig. 3A), and spores with a low almost complete to complete reticulum; however these species have smaller, subglobose to broadly ellipsoid spores, and very different pileicystidia or pileal terminal elements.

Section *Lactifluus*

Lactifluus geoprofluens T. Lebel, Castellano, Claridge & Trappe, *sp. nov.* MycoBank MB 838126. Figs 3D, 5A–F.

Etymology: The species epithet, “*profluens*”, from Latin, meaning “freely flowing” referring to the abundantly flowing latex in cut basidiomes of this species, and “*geo*” in reference to the hypogaeal basidiomes.

Diagnosis: Differs from other currently known lactarioid sequestrate taxa by the faint fishy odour, abundant white latex, globose to occasionally barely subglobose basidiospores, up to

10.5 × 10.0 µm, ornamented with a robust amyloid reticulum, and presence of dendritic pileal terminal elements

Typus: **Australia**, Victoria, East Gippsland, Rich Forest Management Block, near Jack Road 0.7 km N of junction with Winter Road, Claridge site R19, 29 Feb. 1996, A.W. Claridge AWC201 (holotype MEL 2145804).

Basidiomes hypogaeal, sequestrate, 5–15 mm broad, globose to subglobose or irregular, surface dry, smooth to rugulose and irregularly folded radially particularly around the point of attachment to substrate, minutely pruinose and velutinous, pale orange-ochre to almost reddish-brown in folds; pileus in cross-section thin (≤ 0.5 mm), pale orange becoming orange-brown in dried specimens. *Hymenophore* loculate, locules

Fig. 5. *Lactifluus geoprofluens* **A.** Lampropallisade pileipellis terminal elements (te), subpellis (sp) and context (c). **B.** Pileipellis terminal elements (te) and subpellis (sp). **C.** Hymenial trama. **D–E.** Basidiospores. **F.** Scanning Electron Microscopy photo micrograph of basidiospores. Scale bars: A, C = 50 µm; B = 20 µm; D–F = 10 µm.

0.5–2 mm, labyrinthine and vaguely radial from columella attachment, pale orange-ochre to reddish orange-brown. *Stipe-columella* present or absent, much reduced if present and variable, 0.5–3 mm long $\times$ 0.5–2 mm wide, irregularly cylindrical, slightly tapering towards the base, orange-ochre to reddish-brown in patches, minutely pruinose and finely rugulose; context golden to reddish-brown. *Latex* white, unchanging, abundant along the pileipellis edges and the interior of the hymenophore. *Taste* mild. *Odour* not obvious or slightly fishy when fresh. *Basidiospores* $8.0\text{--}10.5 \times 8.0\text{--}10.0 \mu\text{m}$ ($\bar{x} = 9.85 \pm 0.43 \times 9.79 \pm 0.35$, $n = 40$), globose to occasionally barely subglobose [$Q = 1.00\text{--}1.05$ ($\bar{x} = 1.02 \pm 0.02$, $n = 40$)], walls amyloid between ridges and plage distally to completely amyloid, ornamentation reticulate with ridge apices to $1 \mu\text{m}$ tall. *Basidia* $31\text{--}52 \times 8\text{--}14 \mu\text{m}$ ($\bar{x} = 44.18 \pm 5.33 \times 11.25 \pm 1.08$, $n = 19$), 3–6 μm wide at base ($\bar{x} = 3.87 \pm 0.54$, $n = 16$), clavate, mostly 4-spored but occasionally 3-spored; sterigmata $3\text{--}10 \times 1\text{--}2.5 \mu\text{m}$, ($\bar{x} = 7.18 \pm 2.20 \times 2.00 \pm 0.51$, $n = 12$); basidioles $30\text{--}51 \times 6\text{--}11 \mu\text{m}$ ($\bar{x} = 39.89 \pm 4.46 \times 9.14 \pm 1.41$, $n = 14$), 2–5 μm wide at base ($\bar{x} = 3.36 \pm 0.74$, $n = 14$), cylindrical to barely clavate. *Hymenophoral trama* comprised of interwoven and parallel tightly packed hyphae 2–3 μm diam, interspersed with sinuous laticiferous hyphae 5–7 μm diam ($\bar{x} = 5.64 \pm 0.31$, $n = 8$) and occasional sphaerocytes $30\text{--}45 \times 17\text{--}38 \mu\text{m}$; *subhymenium* composed of chains of 3–4 inflated cells $8\text{--}21 \times 6\text{--}17 \mu\text{m}$ ($\bar{x} = 11.30 \pm 6.70 \times 10.40 \pm 5.72$, $n = 18$), laticiferous hyphae present and occasionally extending into pseudocystidia 3–8 μm diam., thin-walled, cylindrical to slightly tortuous, with oily refractive contents. *Hymenial cystidia* scarce, when present not well differentiated, $31\text{--}52 \times 7\text{--}11 \mu\text{m}$ ($\bar{x} = 46.34 \pm 4.78 \times 9.56 \pm 1.16$, $n = 12$), subcylindrical tapering to apex, apex mucronate or obtuse, rarely emergent above hymenium, hyaline, thin-walled, contents scattered when present. *Pileipellis* a lamprolalisade; subpellis composed of 3–6 layers of thin-walled polygonal cells $8\text{--}19 \times 7\text{--}17 \mu\text{m}$ ($\bar{x} = 15.02 \pm 3.28 \times 13.20 \pm 2.06$, $n = 12$) μm ; lamprocystidia $18\text{--}27 \times 2\text{--}5 \mu\text{m}$ ($\bar{x} = 24.32 \pm 4.12 \times 3.73 \pm 1.02$, $n = 15$), elongate cylindrical and slightly sinuate, tapering toward apex, apex mucronate or capitate or obtuse, thick-walled, basal width 2–5 μm ($\bar{x} = 3.73 \pm 1.02$, $n = 15$), and apical width 1–3 μm ($\bar{x} = 1.59 \pm 0.35$, $n = 15$); *pileus trama* similar to hymenophoral trama, heteromerous, hyphae tightly compressed and interwoven, sphaerocytes not abundant.

Ecology and distribution: Sporulating in February–June in cool temperate forests in NE Victoria. Hypogaeal in mixed forests of *Eucalyptus maculata*, *E. muellerana*, *E. paniculata*, *E. obliqua*, *E. cypellocarpa*, *E. delegatensis*, *Angophora floribunda*, *E. longifolia*, *E. muellerana*, *E. sieberi*, *E. baxteri*, *E. botryoides*, with an *Acacia cognata*, *A. verticillata*, *A. melanoxylon*, *A. mucronata*, *A. longifolia*, *A. mearnsii*, *A. implexa* understorey.

Additional specimens examined: **Australia**, Victoria, East Gippsland, Mimosa Rocks National Park, Tanja-Tathra Road, 0.5 km N of junction with track Leading to ‘Gillards’, Claridge Site 40, 7 Jun. 2003, J.M. Trappe, A.W. Claridge, A. Jumpponen AWC5539 (OSC 148637); *ibid.*, Murrangowar Forest Management Block, Bendoc Ridge Road, 0.4 km from Princes Hwy, Claridge Site 66, 2 Jun. 2003, M. Vavrek AWC5306 (OSC 148635); *ibid.*, Cape Conran-Sydenham Inlet Coastal Park, Swampy Creek trail, Crossing of Swampy Creek, Claridge site 128, 31 May 2003, A. Jumpponen AWC5204 (OSC 148634); *ibid.*, East Gippsland, Rich Forest Management Block, junction of Mills Road and Jack Rich Divide Track,

Claridge Retrospective study site R8, 18 Jul. 1996, B. Gunn JMT19613 and AWC452 (OSC 159173); *ibid.*, Nunniong Forest Management Block, off Nunniong Road, Claridge site 106 (relocated 1999), 18 May 2001, T. Lebel AWC3614 (OSC 148633); Ben Boyd National Park, Bittangabee Picnic Area, Bittangabee Creek, Crossing of Walking Track leading N from Picnic Area, Claridge Site 52, 4 Jun. 2003, A. Jumpponen AWC5415 (OSC 148636); Lind National Park, Euchre Valley Road, 0.2 km W of Junction with Lind Park Road, Claridge site 13 (1999 relocation), 29 May 2001, W. Colgan III AWC4223 (OSC 148632).

Notes: This hypogaeous, sequestrate fungus is known from cool-temperate mixed species *Eucalyptus* forest in East Gippsland, Victoria. It differs from the other two sequestrate taxa in often having a distinct columella, radial-locular hymenophore and abundant white latex in all tissues. *Lactifluus geoprofluens* shares with other species in *Lactifluus* section *Lactifluus* the fishy odour, velutinous orange-brown pileus and a lamprolalisade pileipellis structure. The basidiospores in *Lf. geoprofluens* are large (up to $10.5 \times 10 \mu\text{m}$) and globose ($Q = 1\text{--}1.05$), unique among the Australian section *Lactifluus* species.

Section *Pseudogymnocarpi*

Lactifluus sp. prov. KV181 Fig. 3F.

Basidiomes sequestrate, up to 19 mm broad, globose to subglobose, surface dry, smooth to minutely pruinose and velutinous, pale creamy yellow-tan with some minor slightly darker bruising; pileipellis in section thin ($\leq 0.5 \text{ mm}$), pale tan. *Hymenophore* loculate, empty locules 0.5–2 mm, labyrinthine, orange-ochre. *Stipe* absent; *columella* present, percurrent central strand, 0.5–2 mm wide, white to pale cream. *Latex* white turning slightly yellowish, abundant, particularly along the pileipellis edges and the interior of the hymenophore near columella. *Taste* mild. *Odour* faint, fungal.

Distribution and habitat: Sporulating in June in cool temperate open woodland in the eastern tablelands of New South Wales. Hypogaeal in mixed *Eucalyptus* forest.

Sequence data: **Australia**, New South Wales, Gibraltar Range National Park, Mulligans Dr., 16 Jun. 2006, K. Vernes, T. Lebel & A. O'Malley KV181.

Notes: We provide the image of the basidiome and general notes in the hope that further material will be found. Our analyses show that this taxon is in a strongly supported section *Pseudogymnocarpi*, however support values within the clade are low (Fig. 2). The majority of agaricoid species currently known within this clade (e.g. *Lf. rugatus*, *Lf. pseudoluteopus*, *Lf. hygrophoroides*) have pale orange to orange-red pilei and stipe, pale coloured lamellae, abundant white latex (Fig. 3E), and an association with *Fagaceae* or *Pinaceae*. The lack of microscopic detail, and additional data for Australasian agaricoid taxa or environmental sequences, precludes any statements regarding how well this sequestrate taxon fits in this clade generally.

DISCUSSION

Both agaricoid and pleurotoid basidiome forms have been found in *Lactifluus*. In this predominantly agaricoid genus, the

pleurotoid habit has at least seven different origins in three subgenera: subg. *Lactifluus* section *Gerardii*, subg. *Lactariopsis* and subg. *Gymnocarpi* (De Crop *et al.* 2017). No pleurotoid forms are thus far known in *Lactarius*, but they have been found in *Russula* (Buyck & Horak 1999, Henkel *et al.* 2000). Before the present study, sequestrate forms were not known from *Lactifluus*, but have evolved many times over in *Lactarius*; the evolution of a sequestrate basidiome form thus appears to be a rare event in *Lactifluus*.

All three sequestrate *Lactifluus* species have a phylogenetic affinity with Southeast Asian taxa. Some, like *Lf. geoprofluens* and *Lf. dendriticus*, probably evolved in Australia from local ancestors (that have a SE Asian descent - or other way around; cannot be determined at this point). However, the isolated position of *Lf. sp. prov.* KV181 is intriguing, as apart from Asian relatives, a European and a North American species are also closely related. This could be due to under sampling of the Australian and Malesian taxa in particular. However, placement within section *Pseudogymnocarpi* is well supported (BS 98 %).

While the number of species in *Lactarius* with sequestrate basidiome forms is much greater (in the order of 25–30 undescribed and nine named species known for Australasia; and a further 22 spp. worldwide) than in *Lactifluus*, all sequestrate taxa also appear to have evolved as independent and isolated incidents within the genus (Trappe *et al.* 2002, Desjardin 2003, Verbeken *et al.* 2014a, Sheedy *et al.* 2016). None of the sequestrate taxa have evolved into a clade containing a large diversity of species with similar basidiome forms, all indicative of relatively recent origin of this basidiome form in *Lactarius* (Verbeken *et al.* 2014a) and *Lactifluus*. The recent description of several sequestrate *Lactarius* from tropical forests (Verbeken *et al.* 2014a, b, Buyck *et al.* 2017), and discovery of hidden diversity of Australasian *Lactifluus* species lend support to the theory that sequestrate basidiome forms are essentially an excellent way to maintain a fairly constant relative humidity in the enclosed hymenophore, allowing full development of spores to ensue, regardless of arid, seasonally dry or tropical, seasonally waterlogged conditions (Beever & Lebel 2014).

Unsurprisingly, given the trouble with morphological differentiation of *Lactifluus* and *Lactarius* agaricoid species, it is also difficult to distinguish taxa with sequestrate basidiomes. As a broad generalisation, in Australia, the majority of sequestrate *Lactarius* have yellow to orange to red tinged basidiomes, whereas two of the three *Lactifluus* taxa described in this study have white to pale cream basidiomes. Pale basidiome colouring is more common in Australasian sequestrate russuloid taxa (Beaton *et al.* 1984, Lebel & Trappe 2000, Lebel & Castellano 2002, Lebel 2002a, b, 2003a, b, Lebel & Tonkin 2007). General trends in microscopic differentiation between *Lactarius* and *Lactifluus* were formulated by Verbeken & Nuytinck (2013) and can be summarised as: (i) thick-walled elements in the pileipellis and stipitipellis, as well as lamprocystidia, are generally present in *Lactifluus* and very rarely observed in *Lactarius*, and (ii) a hymenophoral trama composed of sphaerocytes (as in *Russula*) is common in *Lactifluus* but is rarely observed in *Lactarius*. Both trends don't seem to hold up for the sequestrate *Lactifluus* species. The loss of thick-walled elements in pileus and hymenium in sequestrate forms could be due to increased folding and localisation of the hymenophoral trama in combination with loss of ballistospory (*i.e.* no longer require spacing to allow for spore drop). As the basidiomes are submersed in the soil, dispersal of spores by vectors such as marsupial mammals or insects, is

highly likely; by having thin walls the ripe basidiomes would be easier to break open or eat. The highly branched, angular pileal dendritic terminal elements of *Lf. dendriticus* are unique. The presence of distinctively shaped pileicystidia or terminal elements is more typical of species of *Russula* but rarely seen in milkcaps (Verbeken 1996a, Verbeken & Walley 2010). Initially thought to be a contaminant, perhaps a mycenoid mycoparasite or virus, but these terminal elements are found consistently in multiple basidiomes from geographically distant collections, and no DNA sequences for mycenoid taxa were ever recovered as contaminants.

In *Lactifluus* section *Gerardii* the majority of species have a brown stipe and pileus contrasting with white mostly distant lamellae, reticulate spore ornamentation < 2 µm high, a palisade pileipellis structure, and are generally lacking in macrocystidia (Stubbe *et al.* 2010, De Crop *et al.* 2017, 2018). The basidiomes can also be small, white and pleurotoid, with thick-walled terminal elements in the pileipellis (Stubbe *et al.* 2012, Latha *et al.* 2016, De Crop *et al.* 2018). Several of the agaricoid and pleurotoid taxa have latex that changes colour on exposure, however this is not the case for *Lf. dendriticus*. While the pale sequestrate basidiomes of *Lf. dendriticus* can be easily mistaken for sequestrate russuloid taxa as latex production is variable, the more open form of locules in the hymenophoral tissue tend to suggest a lactarioid taxon. However, it is the unique dendritic pileal terminal elements of *Lf. dendriticus* that are intriguing. How does a cystidium evolve from a simple filamentous, perhaps irregularly capitate form, to a highly branched, sharp angled form?

Lactifluus geoprofluens is readily distinguished from Australian and international species in *Lf.* section *Lactifluus* by lacking lamprocystidia, having large globose basidiospores and a sequestrate basidiome form. It is the first known sequestrate member of *Lf.* section *Lactifluus* (De Crop *et al.* 2017). Unfortunately, we do not have an image of the fresh basidiomes of *Lf. geoprofluens*, however the field notes all state pale orange-ochre to reddish brown in colour, with slightly paler hymenophoral tissue.

We currently lack data to describe *Lf. sp. prov.* KV181 as a new species, but we do provide a picture and a description of field characteristics. It is the first time a non-agaricoid representative of *Lf.* subg. *Pseudogymnocarpi* is documented, and the first Australasian representative in section *Pseudogymnocarpi*. We look forward to obtaining new material for further examination.

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