

The sequestrate genus *Rosbeeva* T. Lebel & Orihara *gen. nov.* (Boletaceae) from Australasia and Japan: new species and new combinations

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Abstract The sequestrate genus *Chamonixia* has been shown to have affinities to the Boletales, in particular the genus *Leccinum*. Australasian and Japanese species of *Chamonixia* were examined using morphological and molecular (ITS and nLSU rDNA) data and found to also have affinities with *Leccinum* and *Leccinellum*, however they form a distinct clade separate from the European type species *C. caespitosa* Rolland and North American species. A new genus, *Rosbeeva* T. Lebel & Orihara *gen. nov.*, is proposed for the Australasian, Japanese and Chinese taxa. The species *R. mucosa* (Petri) T. Lebel *comb. nov.* is restricted in distribution to Singapore and Borneo,

and *R. pachyderma* (Zeller & C.W. Dodge) T. Lebel *comb. nov.* to New Zealand, with Australian collections considered to belong to a revised *R. vittatispora* (G.W. Beaton, Pegler & T.W.K. Young) T. Lebel *comb. nov.* or a new species *R. westraliensis* T. Lebel *sp. nov.* The Chinese species *R. bispora* (B.C. Zhang & Y.N. Yu) T. Lebel & Orihara *comb. nov.* is transferred to the new genus based upon morphological data. Two new species from Japan, *Rosbeeva eucyanea* Orihara and *R. griseovelutina* Orihara, are also described and illustrated. A key to all species of *Rosbeeva* is provided. Due to the highly modified gastroid sporocarp forms of both *Chamonixia* and *Rosbeeva*, many macroscopic characters of use in agaricoid taxonomy are difficult to interpret. However, color change and texture of sporocarps are of some use to distinguish genera and species. Microscopic characters such as spore shape, dimensions, and ornamentation, and pileipellis and hymenophoral trama structure, are essential for determining genera and species.

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Introduction

The genus *Chamonixia* was described from France for a single species, *Chamonixia caespitosa* Rolland (Rolland 1899). The genus was characterised by the white, fibrillose, indehiscent pileus which stained cerulean blue on handling or exposure to air, a loculate hymenophore of small round to oval chambers, initially pale becoming brown, stipe almost lacking, basidia bisporic, the spores elliptic, brown with 8–10 irregular ridges along their

length. Zeller and Dodge (1934) described several new species, emphasising the conspicuously striate or longitudinally ribbed spores rather than the colour change of the sporocarp, and transferred all taxa to the genus *Gautieria*. Smith and Singer (1959) retained *Chamonixia* as a distinct genus with sporocarps that generally stained blue, and revised the North American species. Corner and Hawker (1953) added two new species from south-east Asia. However, it was Beaton et al. (1985) who expanded the genus circumscription to include Australasian taxa with white to ochraceous sporocarps, which may stain blue or greenish on handling, basidia 2- or 4- spored, and spores with 4–6 longitudinal ridges.

Based on spore characters, Rolland (1899) and Pegler and Young (1989) considered *Chamonixia* to be related to *Gautieria* in the Cortinariaceae and Gautieriaceae respectively. However, the similarity in spore morphology was found to be superficial, and the 2 genera were suggested to have affinities to different orders, the Gomphales for *Gautieria* and Boletales for *Chamonixia* (Beaton et al. 1985; Binder and Bresinsky 2002). The presence of the bolete fungal parasite *Sepdonium* on sporocarps provided further support for placement of *Chamonixia* in the Boletales (Sahr et al. 1999). The sequestrate genus *Rhodactina* also has longitudinally ridged/winged spores, however it is quite distinct morphologically, lacking the blue staining of the sporocarp and the spores are deep purple. Analyses of DNA data has confirmed that *Gautieria* is related to the Ramariaceae (Giachini et al. 2010; Humpert et al. 2001), *Austrogautieria* to the Hysterangiales (Hosaka et al. 2006), and *Rhodactina* and *Chamonixia* to the Boletaceae (Binder and Bresinsky 2002; Kretzer and Bruns 1999; Yang et al. 2006).

Currently nine species of *Chamonixia* are known worldwide. The type species (*C. caespitosa*), from Europe, North America, and China (may not be this taxon), three from the USA (*C. ambigua*, *C. caudata* and *C. brevicolumna*), one from New Zealand (*C. pachydermis*), one endemic to Australia (*C. vittatispora*), two from Australia and Singapore/Malaysia (*C. mucosa* and *C. octorugosa*), and one from China (*C. bisporea*). However, preliminary morphological examination of Australian, New Zealand and Japanese collections suggested that they might differ from the original concept of *Chamonixia*. Analysis of DNA regions ITS 1+2, 5.8S and nLSU were undertaken to confirm relationships of the Australasian and Japanese taxa. Numerous collections were examined morphologically to determine species characters and boundaries. We present a circumscription of a new genus *Rosbeeva* T. Lebel & Orihara, descriptions and illustrations of previously described and three new species, and a key to all species of *Rosbeeva*.

Materials & methods

Molecular analyses

Taxon sampling

The regions of nuclear ribosomal DNA data used for examination of species included the ITS1–5.8S–ITS2 and the 5' end of the nuclear large subunit (nLSU). Sequences were derived from fresh or herbarium material or were retrieved from GenBank. Three alignment datasets were created for analyses: i) ITS1–5.8S–ITS2, ii) nLSU, and iii) ITS1–5.8S+nLSU. The ITS1–5.8S–ITS2 alignment included 55 sequences, representing 28 species, covering a range of species within *Xerocomus Boletus*, *Leccinum*, *Leccinellum*, *Chamonixia*, *Rosbeeva*, and *Octaviania*. *Xerocomus ferrugineus* was selected as outgroup for this analysis. The final nLSU alignment included 66 sequences, representing 36 species. The final ITS1–5.8S+nLSU alignment included 58 sequences, representing 31 species. Sequences of *Pulveroboletus* and *Retiboletus spp* which are close but not sister to the ingroup, and still easily alignable, were selected as outgroup. Fifty two novel sequences of Australian and Japanese taxa were included in the various alignments and analyses (Table 1 lists all taxa with corresponding herbarium and GenBank accession numbers). Unfortunately no material of *Rosbeeva bisporea* from China, or *R. mucosa* and *Chamonixia octorugosa* from South-East Asia was available for sequencing.

Nucleic acid preparation, amplification and sequencing

Genomic DNA of the Australian specimens was isolated with the QIAGEN DNeasy® Plant Mini Kit, following the manufacturer's protocol. The targeted regions were amplified from purified DNA using standard fungal primer pairs: ITS1/ITS4B and ITS5/ITS4 (Gardes and Bruns 1993; White et al. 1990); or 5.8SR/LR7 and LROR/LR5 (www.biology.duke.edu/fungi/mycolab/primers.htm). Reactions were conducted in a volume of 25 µl and contained 1.25 U Qiagen HotStar Taq DNA Polymerase, 10 pmol of each primer, 1.5 mM MgCl₂ and 0.25 mM each dNTP. Amplifications were performed in an Eppendorf Mastercycler Gradient Thermal Cycler. Cycling conditions consisted of a 10-min activation at 95°C, followed by 30 cycles of 30 s at 94°C, 30 s at 54°C (nLSU) or 58°C (ITS) and 1 min at 72°C. These cycles were followed by 5 min of final extension at 72°C, after which the product was held at 4°C. Products of amplification were purified using the Concert Rapid PCR Purification System (Life Technologies).

DNA of the Japanese specimens was isolated with the Indicating FTA Cards (Whatman International Ltd, Maid-

Table 1 Taxa sampled for DNA analyses, including DNA region, Genbank numbers, geographic region. Names in bold are new species or novel sequences

Taxon	Collection or strain	Geographic location	Genbank ITS	Genbank LSU
<i>Austroboletus gracilis</i>	M112/96	USA		DQ534624
<i>Austroboletus niveus</i>	M312			DQ534622
<i>Austroboletus novaehelandiae</i>	M50	NZ		DQ534623
<i>Austroboletus mucosus</i>	TH6300			AY612798
<i>Boletus erythropus</i>	MA-Fungi 47702		AJ419188	
<i>Chamonixia caespitosa</i>	strain 92/83	Bavaria	DQ534565	
<i>Chamonixia caespitosa</i>	Trappe 12656	USA	EU846310	
<i>Chamonixia caespitosa</i>	OSC 118291	USA	EU834197	
<i>Chamonixia caespitosa</i>	p693i	USA	EU669385	EU669427
<i>Chamonixia caespitosa</i>	p071i	USA	EU669208	EU669260
<i>Chamonixia caespitosa</i>	Trappe 12768	USA	EU852804	
<i>Chamonixia caespitosa</i>				AF336245
<i>Leccinellum albellum</i>				AY612811
<i>Leccinellum carpini</i>		Netherlands	AF454588	AF454588AF139691
<i>Leccinellum corsicum</i>				AF139693
<i>Leccinellum crocipodium</i>				AF139694
<i>Leccinellum crocipodium</i>		Netherlands	AF454590	AF454590
<i>Leccinellum lepidum</i>				AF139698
<i>Leccinellum lepidum</i>	SU70		DQ131631	
<i>Leccinum aeruginum</i>	8909241AE			DQ534618
<i>Leccinum atrostitatum</i>	REHalling 3081 NYBG	USA	AY538833	
<i>Leccinum aurantiacum</i>		Belgium	AF454569	AF454569
<i>Leccinum holopus</i>		Sweden	AF454563	AF139697
<i>Leccinum manzanitae</i>	TDB-969			DQ534613
<i>Leccinum melaneum</i>	Lm1	Germany		DQ534616
<i>Leccinum palustre</i>		Sweden	AF454587	
<i>Leccinum piceinum</i>		Austria		AF454578
<i>Leccinum quercinum</i>		Netherl		AF454591
<i>Leccinum rigidipes</i>		Netherl	AF454584	AF454584
<i>Leccinum roseotinctum</i>		Swed	AF454575	
<i>Leccinum rugosiceps</i>				AY612813
<i>Leccinum scabrum</i>		Sweden	AF454583	AY612814
<i>Leccinum scabrum</i>	860916/3GL			AF139705
<i>Leccinum schistophilum</i>	921024/1 GL			DQ534615
<i>Leccinum snellii</i>	REHalling 4472		AY538845	
<i>Leccinum subglabripes</i>				AF139688
<i>Leccinum talamancae</i>	REHalling 8001	Costa Rica	AY544779	
<i>Leccinum variicolor</i>		Sweden	AF454570	AF139706
<i>Octaviania asterosperma</i>	K(M)102511		EU784378	
<i>Octaviania asterosperma</i>	K(M)81259		EU784379	
<i>Octaviania asterosperma</i>				DQ534619
<i>Octaviania</i> sp.	KPM-NC-0015344	Japan	EU414289	EU414290
<i>Octaviania</i> sp.	KPM-NC-0015344	Japan	EU414291	EU414292
<i>Octaviania tasmanica</i>	PDD89058		GU222293	
<i>Octaviania tasmanica</i>	MEL2128500			HQ647146
<i>Octaviania tasmanica</i>	MEL2079332		HQ647145	
<i>Octaviania tasmanica</i>	TL2329		HQ647144	HQ647147
<i>Pulveroboletus curtisii</i>				AY612820

Table 1 (continued)

Taxon	Collection or strain	Geographic location	Genbank ITS	Genbank LSU
<i>Retiboletus flavoniger</i>	RH7189			AF456829
<i>Retiboletus ornatipes</i>				AF456817
<i>Retiboletus griseus</i>				AF456834
<i>Retiboletus retipes</i>				AF456831
<i>Retiboletus retipes</i>				AF456811
<i>Rosbeeva eucyanea</i>	TUMH-40253 (<i>Orihara 980</i>)	Japan	HQ693875	HQ693880
<i>Rosbeeva eucyanea</i>	TNS-F-36986 (<i>Orihara 782</i>)	Japan	HQ693874	HQ693879
<i>Rosbeeva griseovelutina</i>	TNS-F-36990 (<i>Orihara 982</i>)	Japan	HQ693876	HQ693881
<i>Rosbeeva griseovelutina</i>	TUMH-40266 (<i>Orihara 946</i>)	Japan	HQ693878	HQ693883
<i>Rosbeeva griseovelutina</i>	TNS-F-36992 (<i>Orihara 1021</i>)	Japan	HQ693877	HQ693882
<i>Rosbeeva pachyderma</i>	PDD89084	NZ	GU222301	
<i>Rosbeeva pachyderma</i>	M42	NZ		DQ534620
<i>Rosbeeva pachyderma</i>	MEL2079350	NZ	HQ647138	HQ647157
<i>Rosbeeva vittatispora</i>	JMT15656	ACT	HQ647125	
<i>Rosbeeva vittatispora</i>	KV503 MTL1116117	NSW	HQ647126	
<i>Rosbeeva vittatispora</i>	KV666 MTL1176177	NSW	HQ647128	
<i>Rosbeeva vittatispora</i>	KV693 MTL1184185	NSW	HQ647129	
<i>Rosbeeva vittatispora</i>	MEL2079331	Tas	HQ647134	HQ647165
<i>Rosbeeva vittatispora</i>	MEL2321928	Tas	HQ647141	HQ647158
<i>Rosbeeva vittatispora</i>	MEL2293673	Vic	HQ647136	HQ647163
<i>Rosbeeva vittatispora</i>	REH8674 MEL2264998	Tas	HQ647142	HQ647159
<i>Rosbeeva vittatispora</i>	MEL2078292	Tas	HQ647139	HQ647155
<i>Rosbeeva vittatispora</i>	MEL2329434 TL2115	Vic.	HQ647137	HQ647154
<i>Rosbeeva vittatispora</i>	TL2348	Tas	HQ647135	HQ647149
<i>Rosbeeva vittatispora</i>	TL2701	Vic	HQ647132	HQ647148
<i>Rosbeeva vittatispora</i>	TL2338	Tas	HQ647133	HQ647150
<i>Rosbeeva vittatispora</i>	TL2342	Tas	mtl2015	HQ647151
<i>Rosbeeva vittatispora</i>	JMT15537			HQ647152
<i>Rosbeeva vittatispora</i>	REH8679 MEL2265003	Tas		HQ651912
<i>Rosbeeva vittatispora</i>	REH8852 MEL2297066	Vic.		HQ651913
<i>Rosbeeva vittatispora variant a</i>	KV584 MTL1140141	NSW	HQ647127	
<i>Rosbeeva vittatispora variant a</i>	MEL2128491	NSW	HQ647143	HQ647156
<i>Rosbeeva westraliensis</i>	MEL2231712	WA	HQ647140	HQ647162
<i>Rosbeeva westraliensis</i>	MEL2322708	WA		HQ647160
<i>Rosbeeva westraliensis</i>	MEL2219011	WA	HQ647130	HQ647161
<i>Rosbeeva westraliensis</i>	JMT14692	WA	HQ647131	HQ647153
<i>Rosbeeva westraliensis</i>	MEL2233755	WA		HQ647164
<i>Xerocomus chrysenteron</i>			DQ822793	
<i>Xerocomus badius</i>			AJ889926	
<i>Xerocomus ferrugineus</i>	gs1236		DQ066404	
<i>Xerocomus pruinatus</i>	M28		EU350582	
<i>Xerocomus zelleri</i>			DQ822794	

stone, UK) according to the manufacturer's instructions. The ITS and nLSU regions of the rDNA were amplified by PCR using Ampdirect Plus (Shimadzu Corporation, Kyoto, Japan) without purification using the same primers as the

Australian specimens. For each amplification, the PCR reaction mixture contained a 2.0 mm small disc of the FTA Card, 8–10 µl of 2× Ampdirect Plus, 0.5 units of Nova *Taq* (Shimadzu Corporation, Kyoto, Japan), and 0.35 µM of

each primer, adding distilled water to 20 μ l. The reaction was performed on a Astec Program Temp Control System PC-812 (Astec, Fukuoka, Japan) as follows: initial incubation at 95°C for 10 min; in ITS amplification, a subsequent step of 10–12 cycles at 94°C for 30 s, 60°C for 60 s (decreasing 1°C per cycle in the last 5 cycles), and 72°C for 60 s (extending 1 s per cycle in the last 5 cycles), followed by 25 cycles at 94°C for 30 s, 55°C for 60 s, and 72°C for 66 s (extending 1 s per cycle; thus the final elongation time is 90 s), whereas in nLSU amplification, 35 cycles at 94°C for 30 s, 50°C for 60 s, and 72°C for 60 s (extending 1 s per cycle in the last 30 cycles), followed by 5 cycles at 94°C for 30 s, 50°C for 60 s, and 72°C for 90 s; and a final elongation (polymerization) step at 72°C for 7 min. All amplified PCR products were purified with ExoSAP-IT (USB Corporation, Cleveland, Ohio, USA) according to the manufacturer's instructions.

Purified DNA was directly sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Kit with primers for the ITS region ITS1, ITS1F, ITS5, and ITS4 and for the nLSU, LROR, LR5, and LR3R. Sequencing from the Australian and Japanese materials was carried out by means of an ABI model Automated 377 DNA Sequencer and an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA), respectively.

Assembly and manual editing of sequences for each region were performed with Sequencher 4.3 (GeneCodes). Sequences were then transferred to BioEdit v7.0.9 (Hall 2007) for alignment. Alignments were automated with ClustalX v2.0 (Thompson et al. 1997) and the alignment then manually edited. Previously unpublished sequence data are deposited in GenBank (Table 1).

Phylogenetic analysis

All transformations were weighted equally. Gaps in the alignment were treated as missing data. Both the ITS and LSU datasets contained few ambiguous regions, and thus, all indels were included in the analyses. All parsimony trees were rooted by the outgroup method (Maddison et al. 1984). Maximum parsimony analyses were performed using PAUP* 4.0b10 (Swofford 2002). Heuristic searches of the dataset were conducted with 1000 replicates of random addition sequence, tree bisection-connection (TBR) branch swapping and MULTREES on. Nodal support was tested by bootstrapping of 200 replicates with the heuristic search option (TBR and MULTREES off), including groups compatible with 50% majority rule consensus, with 10 random addition sequences.

Bayesian analyses were conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The general time reversible model under the assumption of a discrete

gamma-shaped rate variation with a proportion of invariable sites (GTR+I+G) was estimated as the best-fit likelihood model for both nLSU and ITS datasets with MrModeltest 2.2 (Nylander 2004) and PAUP* 4.0b10. Posterior probabilities (PP) were approximated by the Metropolis-coupled Markov chain Monte Carlo method. Two parallel runs were conducted with one cold and seven heated chains each for 5 000 000 generations, starting with a random tree. The seven chains were heated at 0.15 for both datasets. Trees were saved to a file every 100th generation. We judged that the two runs reached convergence when the average standard deviation of split frequencies continuously dropped below 0.01. Trees obtained before reaching convergence were discarded with the burn-in command, and the remaining trees were used to calculate a 50% majority consensus topology and to determine PP for individual branches.

Morphology

Macroscopic characters were described directly from fresh material and collectors' notes associated with herbarium material. Colours are described in general terms only. Fresh material was dried in a food dehydrator at 35°C for 12 h. Habitat, associated plant communities, and fruiting season are based on field notes.

Hand-cut sections of fresh and dried material were mounted in 5% aqueous solution of KOH, then stained with Congo Red. To determine the amyloid reaction, dried material was stained with Melzer's reagent. Measurements were made at $\times 400$ or $\times 1000$ with a calibrated ocular micrometer. Spore dimensions are given as length range \times width range, mean length \times width ($n=15$ unless specified). The length: width ratio (Q) is presented as the range of Q values. Measurements do include the apiculus but not the ornamentation. Basidia and cystidia dimensions are given as length range \times width range ($n=10$). Material for scanning electron microscopy (SEM) was sputter-coated with gold and photomicrographs taken using a JEOL JSM-5600 or a HITACHI S-800 machine. Illustrations and descriptions are in terms of structures visible by light microscopy and are based on type material (except where noted), though scanning electron microscope photographs were referred to for interpretation of spore ornamentation patterns.

Specimens examined in this study were deposited in the National Herbarium of Victoria (MEL), Western Australian Herbarium (PERTH), National Museum of Nature and Science, Tsukuba, Ibaraki, Japan (TNS), the Tottori University Mycological Herbarium, Tottori, Japan (TUMH), and the Natural History Museum and Institute, Chiba, Japan (CBM).

Results

Both parsimony and Bayesian analyses gave similar results for all datasets, however only the ITS and LSU Bayesian results are presented here (Figs. 1, 2). The two MCMC runs reached convergence after ca. 900 K and ca. 1.26 M generations in the ITS and LSU datasets, respectively. Trees generated before the convergence were discarded and the remaining 82002 and 74802 trees in each dataset were used for PP calculation. In the ITS Bayesian analysis, likelihoods of best state for “cold” chain of the two runs were −5048.54 and −5057.6 respectively. In the LSU Bayesian analysis, the maximum likelihoods of the two runs were −4831.61 and −4848.76. The ITS parsimony analysis resulted in 79053 trees of 3242 steps, CI=0.878, RI=0.751 and the nLSU parsimony analysis resulted in 64001 trees of 2069 steps, CI=0.773, RI=0.687. The separation of Australasian and Japanese taxa into a new genus, *Rosbeeva*, distinct from the European and North American species of *Chamonixia* is strongly supported. Analyses also show strong support for a distinct lineage including the genera *Leccinum*/ *Leccinellum*/ *Octaviania*/ *Chamonixia*/ *Rosbeeva*. Although, relationships between genera were not well-resolved in either ITS or nLSU analyses, *Leccinellum* (ex. *Leccinum* section *Luteoscabra*) is the probable sister clade to *Rosbeeva*, although only *Leccinellum lepidum* or *L. albellum* were consistently supported as a sister taxon.

Within the *Chamonixia* clade there is low support for a distinct European taxon, *C. caespitosa*, and two North American taxa. Within *Rosbeeva* the two new Japanese species, *R. eucyanea* and *R. griseovelutina*, were well differentiated from the Australasian taxa in the ITS analyses and both showed distinct monophyly in the ITS and nLSU phylogenies. However, there is little support for distinct species clades within the Australasian taxa. In analyses of ITS, two taxa, *Rosbeeva westraliensis* (pp 0.97) and *R. vittatispora* ‘variant a’ (pp. 0.95) appear distinct from the widespread Australian species *R. vittatispora* and the New Zealand *R. pachyderma*. As no distinctive morphological characters could be found, *R. vittatispora* ‘variant a’ is described, for the moment, as a variant form within *R. vittatispora*. However, *R. pachyderma* is morphologically distinct from *R. vittatispora*, in the size, lack of cyanescence, texture and smell of the sporocarp, and spore size and ornamentation. In analyses of nLSU one sequence of *R. pachyderma* is excluded and basal to the *Rosbeeva* clade, and a second cannot be distinguished from other Australasian taxa; however both ITS sequences are within *Rosbeeva* with other Australasian taxa. Extraction and sequencing of DNA was attempted unsuccessfully from several other *R. pachyderma* collections to try and elucidate the position of this taxon.

Morphologically the Australian, New Zealand and Japanese taxa differ from the type species *C. caespitosa* in the generally firmer sporocarp texture, inconsistent blue staining on handling or exposure (being absent, blueish, blueish-green, or even with some rufous-pink tints), partially gelatinised pellis and hymenophore, and spores ornamented with 3–5 instead of 6–10 ridges (Fig. 3). Many of the morphological characters used to differentiate the Australasian species are continuously variable from one collection to another and dependant upon maturity of the sporocarp. Colour changes are also variable within a collection, with cyanescence absent, present as small patches, or extending over much of the sporocarp. Differentiation between green and blue tones is also problematic, and may change over time after exposure or bruising. Hymenophore colour also changes with maturity of the spores, initially white then pale tan with a pinkish or slightly orange tint, becoming rusty brown or dark brown eventually. Within a sporocarp, spore size and ornamentation height can also vary considerably.

Taxonomy

Rosbeeva T. Lebel & Orihara gen. nov.—MycoBank 519395

*Basidiomata hypogaea vel emergentia, usque ad 50 mm diam., subglobosa ellipsoidea, pyriforma valde lobata irregulater, albida vel pallide cinerea initio sericea dein sordide cinerea plerumque cyanescentia ubi exposita, laevigata vel tomentosa. Hymenophora initio albida dein pallide cinnamomeae ad maturitatem atrobrunnea, loculata, cellulis minutis. Stipes absens vel a pede basali reductus, albidus vel cremeus, aliqua species lilacescentes vel cyanescentes. Sporae 9–22 (–32) × 3–8 μm, ellipsoideae vel fusioideae, subhyalinae vel luteobrunneae, laeves, 3–5 cristis longitudinalibus 0.25–3 μm altis ornatos; aspectu polari angulatae vel stellatae. Hyphae gelatinosae vel subgelatinosae. Pileipellis cutis vel trichodermis infirmis. **Type:** *Rosbeeva pachyderma* (Zeller & C.W. Dodge) T. Lebel comb. nov.*

Etymology: *Ros*=*Ross*; *beeva*=*Beever*. In honour of New Zealand pathologist, mycologist and truffle expert Ross Beever (1946–2010). A quiet, unassuming gentleman and rigorous scientist, with open and honest views on the world and mycology, and a mentor to many younger scientists.

Basidiocarps hypogaeal to emergent, solitary or in groups, 10–50 mm diam., subglobose, ellipsoid, pyriform or irregularly lobed; white to pale grey with a silky lustre initially, becoming dingy grey to dark grey overall and often cyanescent on bruising or exposure to air, smooth, finely tomentose or glabrous, indehiscent. *Hymenophore*



Fig. 1 Bayesian 50% majority rule consensus topology based on nuclear LSU rDNA dataset of 66 sequences. Bayesian posterior probabilities (pp) are indicated above or below branches at nodes

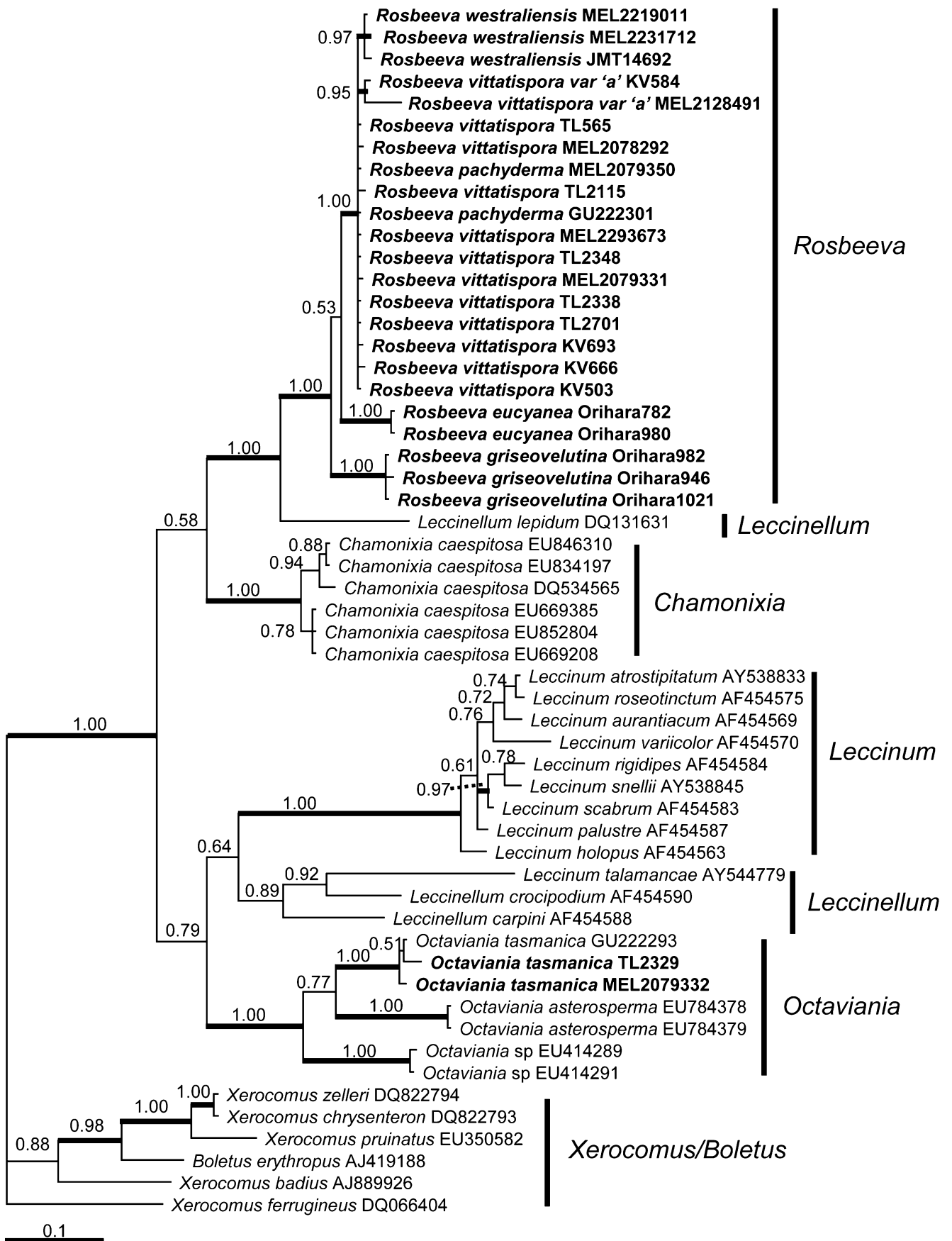


Fig. 2 Bayesian 50% majority rule consensus topology based on nuclear ITS1–5.8S–ITS2 rDNA dataset of 55 sequences. Bayesian posterior probabilities (pp) are indicated above or below branches at nodes

initially white becoming pale pinkish to cinnamon brown, then maturing to dark brown or blackish brown, firm, loculate, of minute irregular chambers, and thin tramal plates. *Stipe-columella* reduced to a small inserted basal pad or absent; cartilagenous, white to cream, in some species staining lilac-pink or blue. *Basidiospores* 9–22 (–32) × 3–8 μm, symmetric, ellipsoid to fusoid, subhyaline when immature, becoming golden brown to fuscous at maturity, with slightly thickened, smooth wall forming 3–5 longitudinal, meridian ridges sometimes forked, 0.25–3 μm high, with plane or strongly concave facets; in polar view appearing barely angular to star-shaped. *Basidia* short clavate to cylindrical,

with slender sterigmata. *Hymenium* developed when immature but collapsed at maturity, hyaline, of regular hyphae 2–5 μm diam., gelatinised to some degree; *basidioles* cylindrical or clavate to short clavate or obovoid; *hymenial cystidia* present or absent. *Subhymenium* generally not developed. *Pileipellis* a repent cutis or poorly developed trichodermium of hyaline hyphae, thin-walled, gelatinised or not. *Clamp connections* absent in all tissues.

Rosbeeva differs from *Chamonixia* in the texture of the sporocarp, firmer and less fleshy in *Rosbeeva*, and the overall spore shape and number of ridges, ellipsoid to fusiform with 3–5 ridges rather than subglobose to broadly ellipsoid with 6–10 ridges. *Chamonixia* species also tend to have a more fully developed trichodermial pileipellis rather than a cutis.

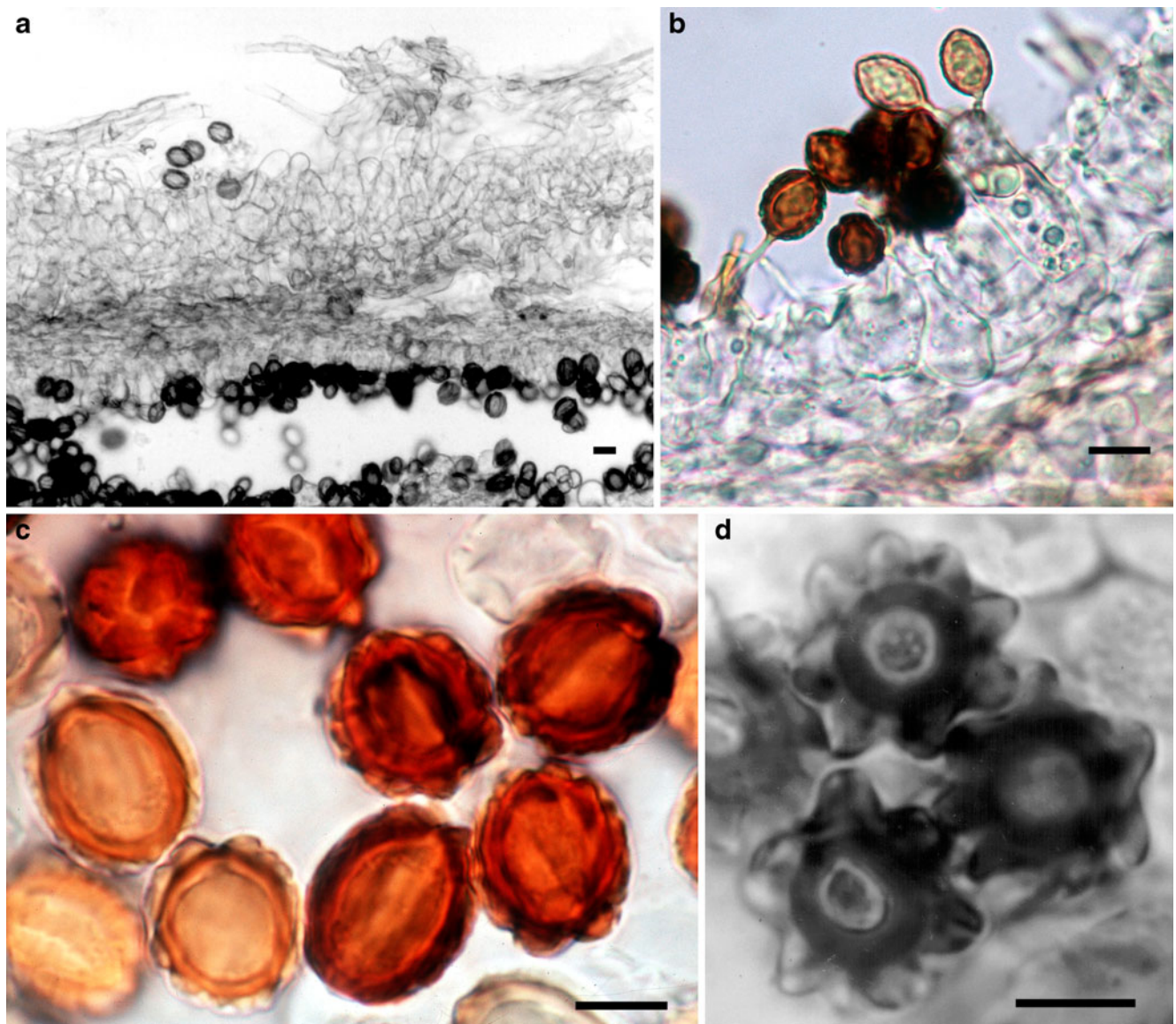


Fig. 3 *Chamonixia caespitosa* (Lange 3268) **a** trichodermial pileipellis; **b** subhymenium, basidia and spores; **c** longitudinal view of spores; **d** polar view of spores

Key to species of *Rosbeeva*

1. Sporocarp not cyanescent on bruising, with no grey tints *R. pachyderma*
- 1: Sporocarp cyanescent overall or in patches, greenish blue or indigo, may have grey tints 2
2. Spores mostly 9–15×3–6 μm, ridges 0.25–2 μm high 3
- 2: Spores mostly larger than 15×8 μm, ridges 1–2.5 μm high 4
3. Spores fusiform 12–14 (–16.5)×3–4.5 μm; sporocarps bruising deep blue overall; known only from Western Australia *R. westraliensis* sp. nov.
- 3: Spores ellipsoid to fusiform 9–12×4–5.5 μm; sporocarp bruising variable and patchy, absent, greenish-blue to deep blue; widespread in eastern Australia *R. vittatispora*
4. Sporocarp velvety, weakly and gradually becoming blue-green to griseous in patches; hyphae of pileipellis partly inflated and becoming a trichoderm; Spores fusiform to fusoid, $Q=2-3.3$ *R. griseovelutina* sp. nov.
- 4: Sporocarp smooth to felty, distinctly cyanescent; pileipellis repent or a cutis; Spores ellipsoid to fusoid, Q values mostly less than 2.....5
5. Basidia constantly 2-spored; peridial hyphae 2–3 μm broad; China.....*R. bispora*
- 5: Basidia 2-, 3-and 4-spored; peridial hyphae exceeding 5 μm broad; Southeast Asia or Japan.....6
6. Odour of dill; Spores (13–) 15–17 μm (mean 15.4 μm) in length, Singapore, Malaysia.....*R. mucosa*
- 6: Odour sweetish; Spores 14–20.5 μm (mean 17.5 μm) in length; Japan *R. eucyanea* sp. nov.

Rosbeeva bispora (B.C.Zhang & Y.N.Yu) T.Lebel & Orihara, *comb.nov.*

Mycobank 519399

≡ *Chamonixia bispora* B.C.Zhang & Y.N.Yu, *Mycotaxon* 35(2): 278, Figs. 1–4 (1989). T: CHINA. Guangdong Prov., Dinghu Mountain Natural Reserve, near Qing Yun Temple. Wang You-zao, Zhen Wan-ling, Li Jian-qing, 13.x. 1982 (GDIMH 5688).

Distribution and habitat: Epigeous, solitary on the ground in broad-leafed woods. October.

Notes—As the focus of this paper is on the Australasian and Japanese species, material of *Rosbeeva bispora* from China was not examined. However, the original description and illustrations are clear and concise (Zhang and Yu 1989). *Rosbeeva bispora* is somewhat atypical of the genus in that the sporocarps are epigeal rather than hypogaeal. This species may be differentiated by the combination of white to greyish brown fresh sporocarps, 2-spored basidia, and large spores (15–21×10–12 μm). The cyanescent reaction of the pileus and hymenophore on bruising, ellipsoid to sub-fusoid spores with 3–4 longitudinal ridges, and pileipellis a repent cutis are all characters supporting placement in *Rosbeeva* rather than *Chamonixia*.

Rosbeeva eucyanea Orihara, *sp. nov.*

Mycobank 519400—Figs. 4a, 5a, 6a–c.

Basidiomata solitaria vel aggregata, hypogaea vel subepigaea, usque ad 26 mm diam., subglobosa vel depressoglobosa, stipite brevi, reducto, superficie alba vel eburnea, valde cyanescenti. Peridium molle, pannosum, ex hyphis repentibus, subparallelis usque intertextis, dilutitunicatis, 2–19 μm latis compositum. Hymenophora fusca usque atrobrunnea, loculis minutis, irregularibus. Columella nulla. Cystidium hymenii nullum. Basidia doliiformia vel brevi-

clavata vel cylindrica ut plurimum 4-spora. Basidiosporae 14.2–20.5 (–23.4)×7.7–10.9 μm, Q=1.6–2.2, symmetricae, ellipsoideae vel fusoidae, hyalinae usque cinnamomeae, cum 3–5 costis usque ad 2.5 μm alta; aspectu polari angulatae vel stellatae. Odor suaves ad maturitatem. T: JAPAN. Tottori Pref., Tottori-shi, Ouchidani, Under *C. sieboldii*, M. Ohmae, Orihara980, 8.vii.2009 (TNS-F-36986) (holotype; designated here)

Etymology. The epithet (*eu*=true; *cyanea*=ultramarine blue) refers to its rapid, strong colour change to deep blue, which is markedly vivid even among the members of the genus.

Sporocarps solitary to gregarious, hypogeous to subepigeous, up to 26 mm in diam, subglobose to depressed-globose, rubbery, with reduced stipe at the base; *surface* felty, white to whitish grey, sometimes with pinkish tints, rapidly turning indigo-blue to ultramarine blue where touched or exposed to air and finally becoming blackish blue. *Pileus* persistent, soft, felty, context white, more developed at the bottom than the top. *Hymenophore* whitish when immature becoming chocolate-brown at maturity, rapidly turning indigo to ultramarine blue or pinkish when cut, of minute, subspherical locules. *Sterile base* present, forming reduced, white to slightly translucent stipe, showing the same colour change as the hymenophore, white mycelial tufts developed at the bottom. *Columella* absent. *Odour* sweetish at maturity.

Basidiospores 14.2–20.5 (–23.4)×7.7–10.9 μm (mean 17.5×9.4 μm; $n=50$), $Q=1.6-2.2$ (mean 1.9), ellipsoid to fusoid, colourless at first then becoming dark reddish brown at maturity, with 3–5 (mostly 4) longitudinal ridges up to 2.5 μm high in water connecting one another, walls 0.6–1.4 μm thick, with hilar appendix 1.6–3.2 μm long. *Basidia*



Fig. 4 Sporocarp diversity of Australasian and Japanese species. **a** *Rosbeeva eucyanea* (TNS-F-40253); **b** *Rosbeeva griseovelutina* (TNS-F-36989, holotype); **c** *Rosbeeva pachyderma* (Lebel 197); **d** *Rosbeeva*

vittatispora (TO-AUS-46); **e** *Rosbeeva vittatispora* variant 'a' (Vernes 584); **f** *Rosbeeva westraliensis* (MEL2233755, holotype)

(11.6–) 18.8–24.4×(6.4–) 10–13.7 μm, doliiform to short clavate to cylindrical, colourless to yellow, 4- or more rarely

2- or 3-spored. *Hymenial cystidia* absent. *Hymenium* developed when immature but collapsed at maturity,

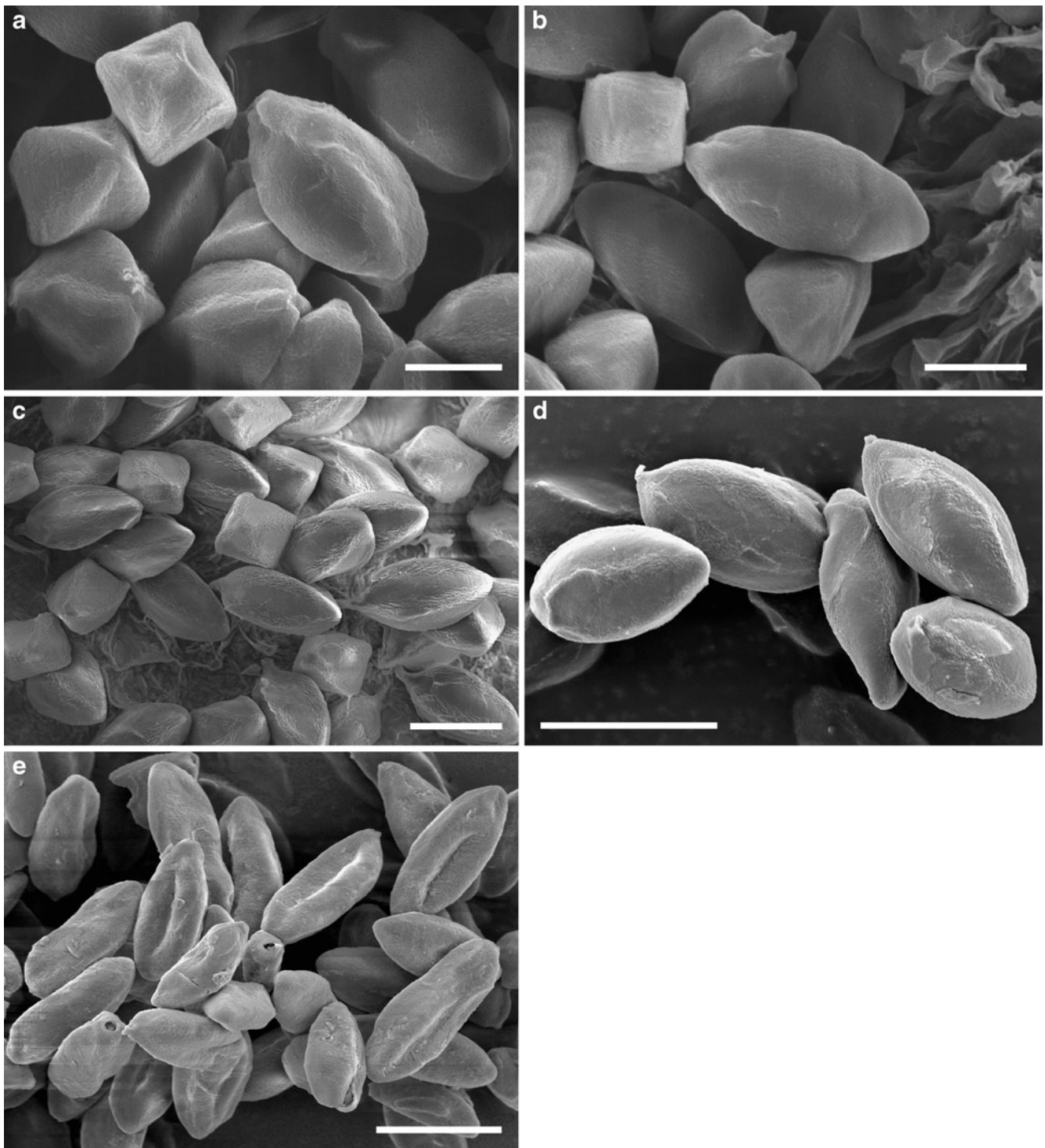


Fig. 5 Scanning electron microscope images of spores. **a** *Rosbeeva eucyanea* (TNS-F-36986, holotype); **b** *Rosbeeva griseovelutina* (TNS-F-36989, holotype); **c** *Rosbeeva pachyderma* (Lebel 197); **d** *Rosbeeva*

vittatispora (Beaton #77, isotype MELU); **e** *Rosbeeva westraliensis* (MEL2233755, holotype)

colourless, hyaline; *basidioles* clavate to short clavate or doliiform up to 23 μm high and 17 μm broad. *Subhymenium* not developed. *Hymenophoral trama* subgelatinous, of colourless, loosely interwoven to parallel, occasionally

branched, non-inflated, thin-walled ($< 0.5 \mu\text{m}$ thick), straight or more rarely sinuate, filamentous hyphae 2–9 μm broad partly stained blue or ochraceous. *Context of sterile base* brownish in dried specimen, of densely

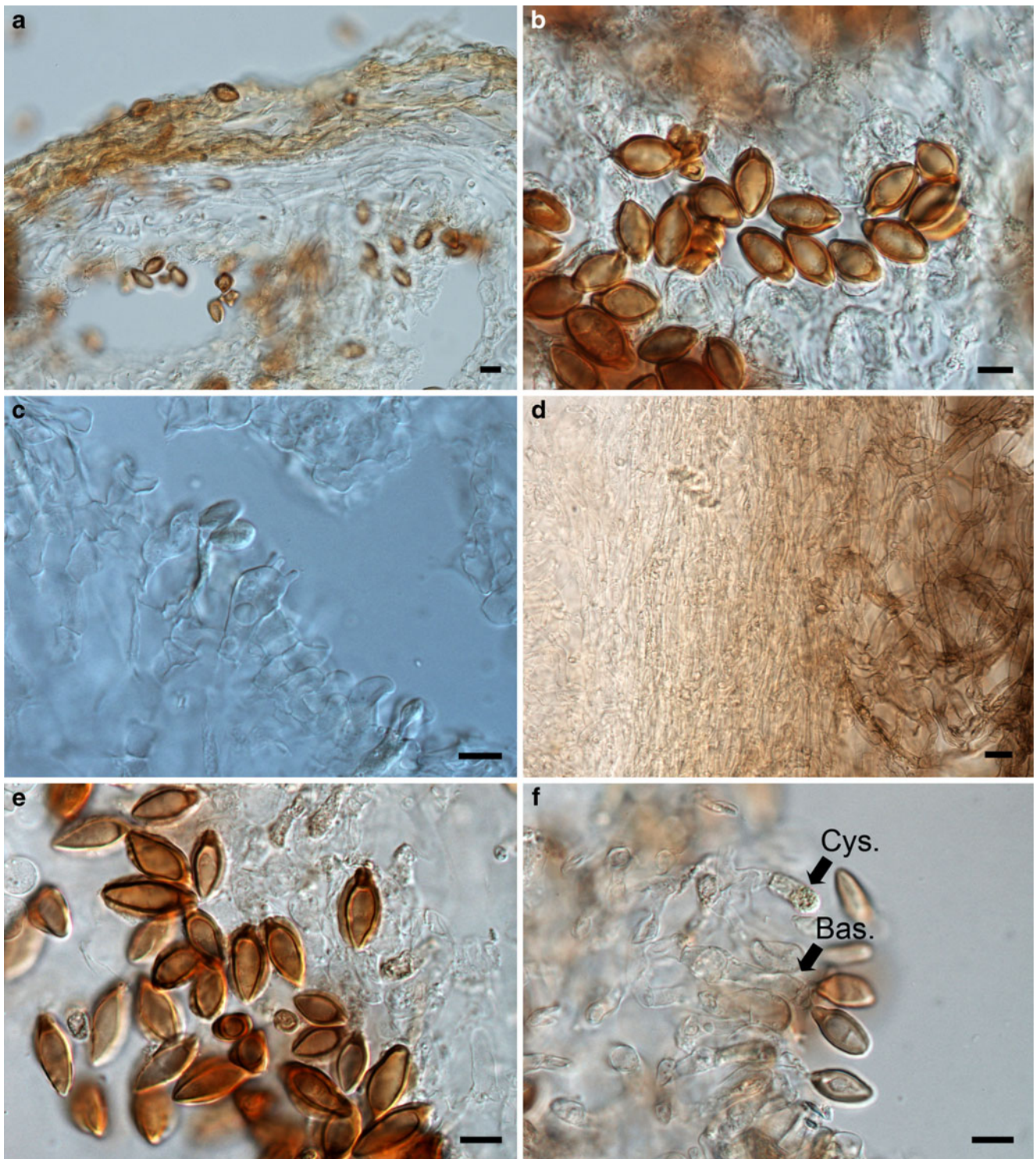


Fig. 6 *Rosbeeva eucyanea* (TNS-F-36986) a–c. **a** pileipellis; **b** spores; **c** hymenium and basidia; *Rosbeeva griseovelutina* (TNS-F-36989) d–f. **d** pileipellis; **e** spores; **f** 2-spored basidium ('Bas') and hymenial cystidium ('Cys')

interwoven, septate, non-inflated, thin-walled ($0.8\ \mu\text{m}$ broad) filamentous hyphae $2\text{--}12\ \mu\text{m}$ broad. *Pileipellis* up to $600\ \mu\text{m}$ thick in dried specimen, white to ochraceous-brown, partly stained blue where injured when fresh, of repent, occasionally inflated, septate, thin-walled ($> 0.8\ \mu\text{m}$

thick) filamentous hyphae $2\text{--}19\ \mu\text{m}$ broad readily crushed by pressure and loosely interwoven or subparallel at surface.

Habitat, distribution and season—Under *Castanopsis sieboldii* (Makino) Hatus. and *C. cuspidata* (Thunberg ex

Murray) Schottky; JAPAN (Southwestern Honshu and Kyusyu); Summer (June–September).

Specimens examined—JAPAN. Tottori Pref., Tottori-shi, Ouchidani, under *C. sieboldii*, M. Ohmae Orihara980, 8.vii.2009 (holotype TNS-F-36986; isotype TUMH49251); the same locality, M. Ohmae Orihara1032, 16.viii.2009 (TNS-F-36987); Aichi Pref., Okazaki-shi, Kitayama, S. Honda Orihara1312, 21.viii.2009 (TUMH40254); Mie Pref., Kameyama-shi, Seki-cho, near Shoho-ji Temple, under *C. cuspidata*, M. Taniguchi & T. Orihara Orihara782, 2.vi.2008 (TUMH40253); Hiroshima Pref., Hiroshima-shi, Higashi-ku, Fukuda, Toshikazu Imoto & Saya Imoto, 13.vii.2002 (CBM-FB-31453); Saga Pref., Nishi-matsuura-gun, Arita-cho, near the Arita Dam, under *C. sieboldii* and evergreen *Quercus* sp., M. Sadamatsu Orihara1247, 5.ix.2010 (TNS-F-36988).

Notes—Three of the Asian *Rosbeeva* species, *R. bispora*, *R. mucosa* and *R. eucyanea* share strong morphological similarities (Corner and Hawker 1953, Zhang and Yu 1989): they all have white to greyish sporocarps with bluish discoloration and ellipsoid to short fusoid basidiospores with mostly 4 longitudinal ridges. *Rosbeeva bispora*, however, can be distinguished from *R. eucyanea* by its 2- rather than 4-spored basidia and hyphae of the peridiopellis that are non-inflated and narrower (2–3 μm broad) than that of the latter species; *Rosbeeva mucosa* and the Australasian *Rosbeeva* spp. tend not to cyanesce to the same degree or as deeply blue as *R. eucyanea*, and they all have smaller basidiospores.

***Rosbeeva griseovelutina* Orihara, sp. nov**

Mycobank 519401.—Figs. 4b, 5b, 6d–f

*Basidiomata solitaria vel aggregata, hypogaea vel subepigaea, usque ad 25 mm diam., subglobosa vel depresso-globosa, stipite brevi, reducto, superficie velveta, albida vel schistacea vel grisea, tactu paulatim cyanescenti vel nigrescenti. Peridium molle; stratum externum ex hyphis repentibus vel trichodermialibus, plus minusve perpendicularibus, in parte inflatis, 3.5–45 μm latis compositum; stratum internum ex hyphis subparallelis, non inflatis, quam hyphis strati externi angustioribus. Hymenophora fusca, loculis minutis, irregularibus. Columella saepe preasens, diaphana, radiata. Cystidia hymeniorum 16.8–21–26 \times 4.6–6.7–8.4 μm , abundantia, cylindrata vel cylindro-clavata. Basidia breviclavata vel cylindrica ut plurimum 2-spora. Basidiosporae 14.4–31.9 \times 6.7–10.4 μm , $Q=2-3.3$, fusioideae vel fusiformes, hyalinae usque cinnamomeae, cum 3 vel 4 costis usuque ad 2.5 μm alta. Odor suaves vel 'rubbery' ad maturitatem. T: JAPAN. Hyogo Pref., Kobe-shi, Shiogahara, under *C. sieboldii* and *Q. glauca*, T. Orihara 1195, 18.vii.2010 (TNS-F-36989) (holotype designated here).*

Etymology. Latin, *griseo-* (= griseous), and *velutina* (= velvety) refer to the grey velvety character of the peridium.

Sporocarps solitary to gregarious, hypogeous to subepigeous, up to 25 mm in diam, subglobose to depressed-globose, rubbery, with more or less reduced stipe at the base; *surface* velvety, white when immature becoming whitish grey to dark grey or griseous often covered with patchy, repent, darker-coloured, cottony mycelial tufts at maturity, gradually and weakly turning blackish grey or blue-green where touched or bruised. *Pileus* persistent, thin, soft. *Hymenophore* whitish at first becoming chocolate-brown at maturity, occasionally turning blue-green especially around larvae tunnels when cut, of minute, irregular-shaped locules. *Sterile base* pulvinate or becoming radially branched columella, translucent, forming more or less reduced, whitish stipe at the base of sporocarps, occasionally turning the same colour as hymenophore when cut, with whitish mycelial tufts radiating from the bottom. *Odour* sweetish to rubbery, becoming stronger at maturity.

Basidiospores 14.4–31.9 \times 6.7–10.4 μm (mean 22.2 \times 8.7 μm ; $n=50$), $Q=2-3.3$ (mean 2.6), fusoid to fusiform, colourless at first then becoming dark reddish brown at maturity, with 3 or more rarely 4 smooth, longitudinal ridges up to 2.5 μm high in water connecting one another, walls 0.5–1.7 μm thick, with developed hilar appendix 1.8–6.7 μm long. *Basidia* 15.9–25.7 \times (5.9–) 6.8–11 μm , short clavate to cylindrical, colourless, 2- or rarely 3-spored. *Hymenial cystidia* 16.8–21–26 \times 4.6–6.7–8.4 μm ($n=15$), abundant, cylindrical to cylindro-clavate, granulate, colourless to yellowish in 3% KOH. *Hymenium* developed when immature but collapsed at maturity, hyaline; *basidioles* cylindrical or clavate to short clavate. *Subhymenium* not developed. *Trama* subgelatinous, of colourless, parallel to subparallel, sparse, partly branched, non-inflated, thin-walled (< 0.5 μm thick), straight filamentous hyphae 2.5–9 μm broad. *Context of stipe* subgelatinous, of densely interwoven, translucent, non-inflated filamentous hyphae 2–13 μm broad, walls up to 1.2 μm thick. *Pileipellis* thin, up to 350 μm thick; *outermost hyphae* repent or trichoderm, colourless at first turning blue-green or black where injured, running more or less vertically, partly swollen and branched, finely septate, thin-walled (> 0.8 μm thick), filamentous to subspherical, 3.5–45 μm broad, grading to inner context; *hyphae of inner context* parallel to surface, more or less narrower and less inflated than the outer, running in various directions in most part, grading to inner trama. *Clamp connections* absent in all tissues.

Habitat, distribution and season—In/on sandy soil under *Castanopsis sieboldii* (Makino) Hatus., *C. sieboldii* var. *lutchuensis* (Koidz) H. Ohba, *Quercus serrata* Murray, *Q. glauca* Thunberg or *Fagus crenata* Blume; JAPAN (Honsyu and the Ryukyu Archipelago [Amami-oshima Isl.]); Summer to late Autumn (July–November).

Specimens examined—JAPAN. Hyogo Pref., Kobe-shi, Shiogahara, T. Orihara1195, 18.vii.2010 (holotype TNS-F-

36989; isotype TUMH40257); the same locality, *M. Ohmae Orihara982*, 18.vii.2009 (TNS-F-36990, TUMH40256); Okayama Pref., Kagamino-cho, under *Fagus crenata*, *T. Orihara1261*, 25.ix.2010 (TNS-F-36991, TUMH40264); Tottori Pref., Tottori-shi, Ouchidani, *T. Orihara1001*, 25.vii.2009 (TUMH40259); the same locality, *T. Orihara1021*, 8.viii.2009 (TNS-F-36992, TUMH40260); the same locality, *M. Ohmae Orihara1031*, 16.viii.2009 (TUMH40261); the same locality, *T. Orihara1187*, 11.vii.2010 (TUMH40262); the same locality, *M. Ohmae Orihara1266*, 20.ix.2010 (TNS-F-12693, TUMH40263); the same locality, *E. Nagasawa92-334*, 25.x.1992 (TUMH40267); Kyoto Pref., Kyoto-shi, Sakyo-ku, Mt. Hiei, Kirara-zaka, *T. Orihara328*, 18.viii.2005 (TUMH40258); Kagoshima Pref., Amami-oshima Isl., Kinsakubaru Natural Forest, *M. Ohkubo Orihara764*, 18.xi.2007 (TUMH40265); Amami-oshima Isl., Yamato-son, on southeastern foot of Mt. Yuwan, *T. Orihara946*, 29.xi.2008 (TUMH40266).

Notes—*Rosbeeva griseovelutina* may be readily recognized by the unique velvety pileus with minute blackish patches of fibrous mycelial tufts. The pileus is also distinctive in that it becomes grey to griseous at maturity. Microscopically, large, fusoid to fusiform basidiospores, abundant hymenial cystidia, and trichodermial hyphae of the pileipellis place the species in a morphologically unique position.

Ecologically, most of the specimens of *R. griseovelutina* examined were collected in well drained, sandy soil in mid-summer, suggesting its strong habitat preference. On the other hand, it does not have strong preference for types of vegetation compared to *R. eucyanea*, which almost specifically occurs under *Castanopsis* species. This unique ecological requirement could also explain its rather sparse records in Japanese mycoflora although further accumulation of records of the species would be required to confirm this.

Rosbeeva mucosa (Petri) T.Lebel, *comb.nov.*

Mycobank 519397—Fig. 7

≡ *Hymenogaster mucosus* Petri in *Malpighia* 14:130, pl 2/11, 13–16 (1900). **T: BORNEO.** Sarawak, *O. Beccari 1867* (NY)

≡ *Gautieria mucosa* (Petri) Dodge & Zeller in *Ann. Mo. Bot. Gard.* 21:703, pl 18/20 (1934)

≡ *Chamonixia mucosa* (Petri) Corner & Hawker in *Trans. Brit. Mycol. Soc.* 76: 131 Fig. 2a (1953)

Basidiomata subhypogaeal to emergent, up to 30 mm diam, irregularly globose to subglobose, depressed at apex. **Pileus** minutely silky, subtomentose to felty, dry, smooth, white discolouring blue-green when bruised. **Hymenophore** white then purplish-fuscous, firm, becoming subgelatinous and rubbery, eventually deliquescing, loculate to labyrinthine, chambers minute. **Stipe-columella** absent or present as small basal pad, 3.5×2 mm, with few short strands

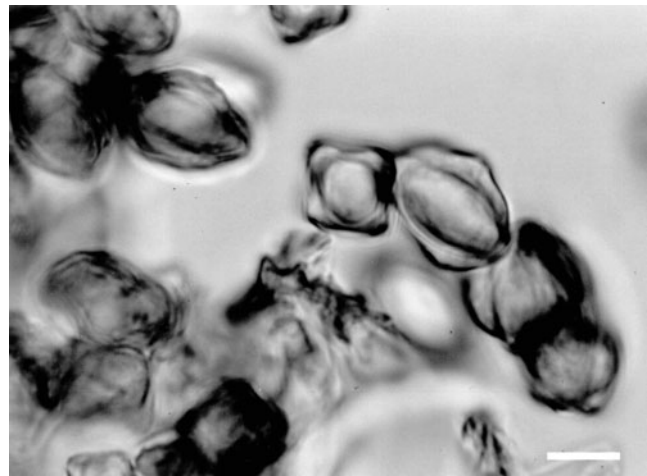


Fig. 7 *Rosbeeva mucosa* spores (Beccari 1867, holotype)

radiating, white, rapidly turning pink then blue-green or indigo when cut or exposed to air. **Odour** strong of dill.

Basidiospores (13–) 15–17×(7–) 8–9 μm, (mean 15.4×8.1), $Q=1.76–2.05$, ellipsoidal to fusoid, initially hyaline becoming brown, with mostly 4– rarely 5 longitudinal ridges, 1–2.5 μm high, which are rounded or with a wavy edge, generally quadrangular in polar view. **Basidia** 14–22×8–13 μm, hyaline, clavate or pyriform, mostly 2– rarely 4-spored; **basidioles** broadly clavate or obovoid, up to 19 μm high and 15 μm broad. **Hymenial cystidia** absent. **Subhymenium** not developed. **Hymenophoral trama** variable in width, 150–320 μm, gelatinous, of loosely interwoven to parallel, hyaline non-inflated, thin-walled (< 0.5 μm thick) filamentous hyphae 2–7 μm diam. **Pileipellis** well developed, up to 580 μm thick, a repent cutis of interwoven, irregularly branched, hyaline hyphae 6–7 μm diam, with some scattered inflated elements 9–12 μm, golden yellow in 3% KOH, overlying a broad context of more loosely interwoven, gelatinised hyaline hyphae 4–7 μm diam.

Specimens examined: BORNEO: Sarawak, *O. Beccari 1867* (prepared slide NY); SINGAPORE: Reservoir Jungle, vii.1929, *Corner 736* (prepared slide NY; fragment SING 0033753).

Habitat, distribution and season—Among leaf litter, subhypogaeal to emergent in jungle. Singapore and Borneo.

Notes—Unfortunately neither fresh material nor more recent collections of *Rosbeeva mucosa* from south east-Asia were available for examination, and type material was in such poor condition that DNA was not extracted. The description provided here is taken from Petri (1900), Corner and Hawker (1953), and examination of prepared slide material from NY of the type from Borneo and Corner 736 from Singapore (NY slide and fragment SING). The holotype of *R. mucosa* from Borneo and the Corner collection from Singapore match well, but differ markedly

from the Beaton et al. (1985) description and Australian collections. Beaton et al. (1985) apparently did not examine the holotype of *R. mucosa*. The type collection has a white then purplish-fuscous rubbery hymenophore; basal pad which rapidly discolours lilac pink, then blue-green or indigo when cut or exposed to air; spores 15–17×8–10 µm; and a strong odour of dill. The two Australian collections mentioned in Beaton et al. (1985) have a white then brownish, firm hymenophore; basal pad discoloration absent or if present faintly bluish, never lilac; spores 10–13×4–6 µm; and a mild odour or strongly of petroleum.

The many morphological differences suggest that the Asian and Australian material are two different taxa, the stellate polar view of the spore ornamentation being the only character in common. *Rosbeeva mucosa* is here restricted to the Borneo and Singapore collections, with Australian material determined as part of a broadly defined *R. vittatispora* (see description for further discussion). Although no associated plant information is available, it is probable that Dipterocarpaceae or Fagaceae are the mycorrhizal hosts.

Rosbeeva pachyderma (Zeller & C.W. Dodge) T. Lebel, *comb. nov.*

Mycobank 519396—Figs. 4c, 5c, 8

≡ *Hymenogaster pachydermis* Zeller & C.W. Dodge, *Annals of the Missouri Botanical Garden* 21: 637 (1934). T: NEW ZEALAND. South Island, Nelson, Mt Dun, *JC Neill, Cunningham 1096*, 27.v.1923 (NY)

≡ *Chamonixia pachydermis* (Zeller & C.W. Dodge) G.W. Beaton, Pegler & T.W.K. Young, *Kew Bulletin* 40(3): 584 (1985)

= *Gautieria novae-zelandiae* G. Cunn. [as ‘novaezelandiae’] [nom. inval., Art. 36.1], *Transactions and Proceedings of the Royal Society of New Zealand* 67(4): 409 (1938). T: PDD8331

= *Hysterangium sclerodermum* sensu G. Cunn., *The Gasteromycetes of Australia and New Zealand* 67 (1944) (misapplication of *Hysterangium sclerodermum* (Cooke) G. Cunn. (1934))

Basidiomata hypogaeal to often emergent, solitary or caespitose, (12–) 20–50 mm diameter, tuberiform, pyriform or subglobose, smooth to roughened with exposure, sometimes cracking, from off-white to yellow ochre to brilliant iodine greenish-blue in large patches, not cyanescent when bruised, drying pallid ferruginous. *Pileus* well developed, 1–2 mm thick, white or cream coloured in section, not cyanescing. *Hymenophore* initially off-white becoming rusty dark brown to chocolate brown at maturity, firm to rubbery, loculate, chambers fine, 2–3 to 1 mm, gel-filled. *Stipe-columella* reduced to a few narrow branches arising from a small, 2–6 mm wide×2–3 mm high, inserted sterile basal pad, white to translucent, cartilaginous, more

conspicuous in immature sporocarps. *Odour* strongly earthy and slightly of bleach.

Basidiospores (10.5–) 11–14 (–15)×(6–) 8–10 µm, (mean 13.2×8.3 µm), *Q*=1.38–1.61; ellipsoid to short fusoid, golden brown to brown in KOH, with a thickened wall, apex bluntly pointed, with 4–5 longitudinal ridges, 1.5–3 µm tall, which are rounded or with a wavy edge, and spiraling slightly on some spores. *Basidia* 14–21×6–13 µm, cylindrical to clavate, 4-spored; basidioles 10–15×5–11 µm, clavate to broadly clavate. *Hymenial cystidia* absent. *Subhymenium* poorly developed. *Hymenophoral trama* broad, 150–325 µm thick, of gelatinized, interwoven hyaline hyphae, 3–9 µm diam. *Peridiopellis* a narrow 50–75 µm thick, repent epicutis, of subparallel or partially interwoven hyaline, subgelatinised thin-walled hyphae, 3–9 µm diam., which become yellow in 3% KOH, with scattered slightly inflated hyphal elements, 5–13 µm diam.; overlying a broad context, up to 1200 µm thick, of interwoven gelatinised hyaline hyphae 3–6 µm diam.

Specimens examined: NEW ZEALAND: **North Island**: Wellington, Tongariro National Park, Chateau Tongariro, *J. Carter*, iv.1931 (type of *G. novaezelandiae*, K; PDD8331); Wellington, York Bay, *EH. Etkinson 1100*, 4.ii.1923; Wellington, Mangatorutoro Stream, *JM. Dingley 6165*, iii.1948 (K78510); Wellington, Tararua Range, Mt Reeves, *TC Birch* iii.1931 (PDD8334); Kaimanawa Forest Park, South of Taupo, Te Iringa hut track, off Clements rd, *R. Vilgalys 95/646*, 17.vi.1995 (MEL2246945); Bay of Plenty, Urewera National Park, Gisborne Huiairau Range, *T. Lebel & M. Castellano JMT15523*, 25.v.1995 (MEL; PDD); Bay of Plenty, Urewera National Park, Huiairau Range, *R. Beever, F. Camacho & M. Castellano JMT15528*, 25.v.1995 (MEL; PDD); Urewera National Park Lake Waikareiti trail shelter, *JM. Trappe 15537, T. Lebel & F. Camacho*, 26.v.1995 (MEL; PDD); Taupo, Tongariro National Park, Mangahua Campsite, *JM. Trappe 15575 & M. Castellano*, 28.v.1995 (MEL; PDD); Taupo, Tongariro National Park, Whakapapui Track, *JM. Trappe 15572 & F. Camacho*, 28.v.1995 (MEL; PDD); Taupo, Turangi, Kiko Rd end, Kiko Track, *F. Camacho & T. Lebel JMT15589*, 28.v.1995 (MEL; PDD); Murchison, Tapawere Rd, *D. Russell JMT12625*, 25.ix.1992 (MEL); Murchison, Skyline Track, *D. Russell JMT12588*, 2. ix.1992 (PDD); **South Island**: Fiordland National Park, Te Anau Valley, Greebe Saddle, *T. Lebel 197*, 9.v.2000 (MEL2079341); Fiordland National Park, Te Anau to Milford Sound Rd, The Chasm trail, approx. 50 m from trailhead, *T. Lebel 193*, 7.v.2000 (MEL2079350); Catlins Forest Park, Catlins River, *TW. May 1678*, 10.v.1995 (MEL2297174).

Habitat, distribution and season—Hypogaeal or more often emergent, in beech (*Nothofagus fusca*, *N. solandrii* and *N. menziesii*) forest. New Zealand. February–October.

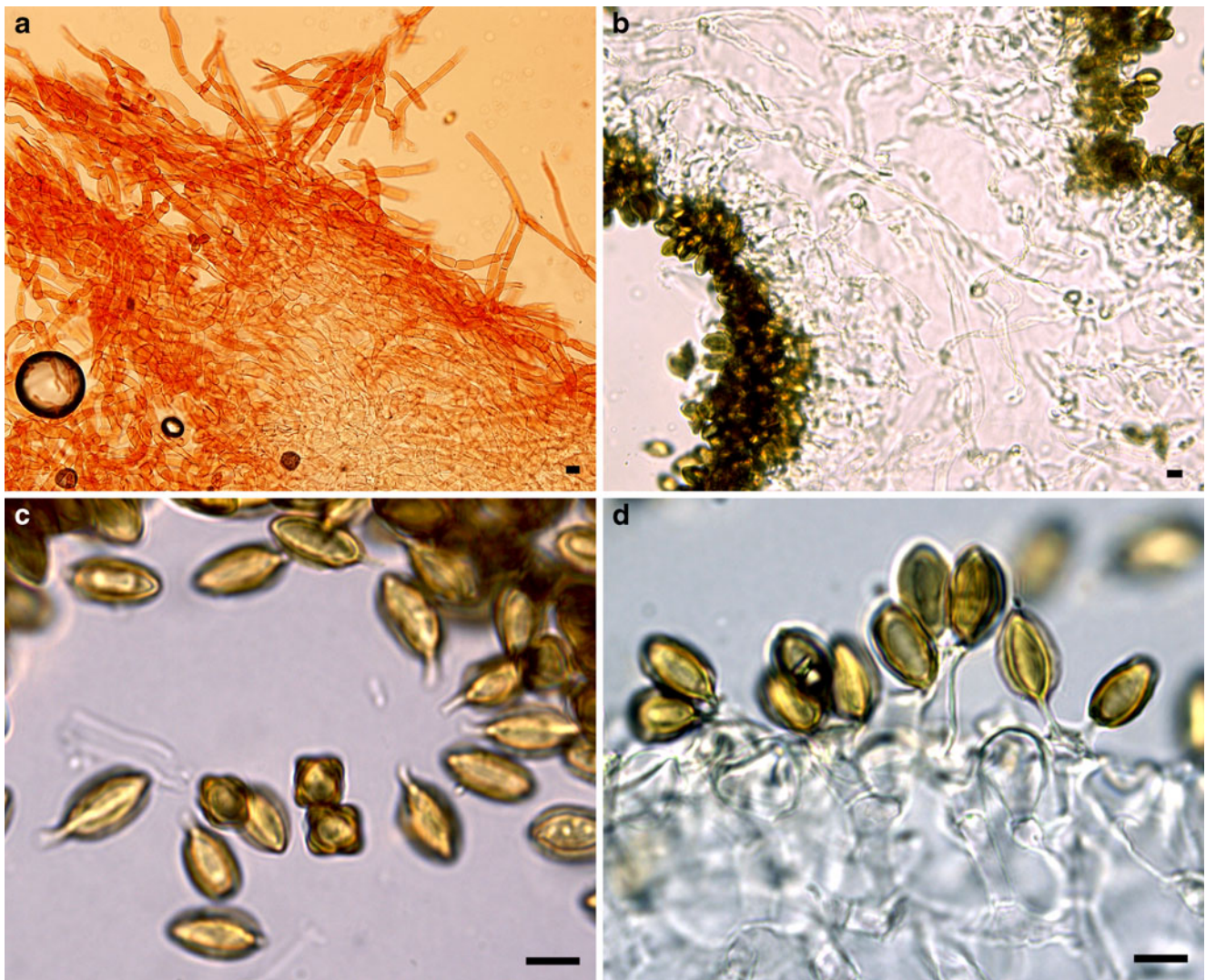


Fig. 8 *Rosbeeva pachyderma* (Lebel 197): **a** cutis pileipellis; **b** hymenophoral trama; **c** spores; **d** subhymenium, basidia and spores

Notes—*Rosbeeva pachyderma* is restricted in distribution to New Zealand, and differs from other Australasian species in the non-cyanescent, greenish-blue pileus, generally larger emergent sporocarps, more deeply coloured spores, and the much broader pileus and hymenophoral trama. In our examination of type material and recent collections, the structure of the pileus was found to be consistently hyphal with some scattered inflated elements, but never pseudoparenchymatous as described in the protologue of *Hymenogaster pachydermis* (Zeller and Dodge 1934). Analyses of DNA did not provide any support for differentiation of *R. pachyderma* from Australasian or Japanese taxa.

The type of *Hymenogaster pachydermis* (NY) from Mt Dun, and one of the collections cited by Cunningham (1979) from Mt Reeves (PDD8334) are immature specimens of the species, the spores are shorter and the ornamentation not as high or robust, and paler in colour.

The Australian collection placed under this name by Beaton et al. (1985) is a specimen of *R. vittatispora*. The Zeller 2260 collection from the USA listed in Beaton et al. (1985) is a *Chamonixia* sp., with broadly ellipsoid spores that have 8 distinct ridges.

All material of *Rosbeeva pachyderma* examined occurred in association with the three main species of *Nothofagus* found in New Zealand. *Nothofagus* species occur on a range of soil types, and altitudinal zones, from the coast to sub-alpine areas; as such *R. pachyderma* appears to have a broad ecological requirement.

Rosbeeva vittatispora (G.W.Beaton, Pegler & T.W.K. Young) T.Lebel, *comb. nov.*

Mycobank 519398—Figs. 4d–e, 5d, 9a–b

≡ *Chamonixia vittatispora* G.W.Beaton, Pegler & T.W.K. Young *Kew Bulletin* 40 (3): 586 (1985). **T: AUSTRALIA.** Victoria, Eildon, bush at end of 8th st, *G.Beaton* #77, 10. vii.1974 (type K; isotype MELU).

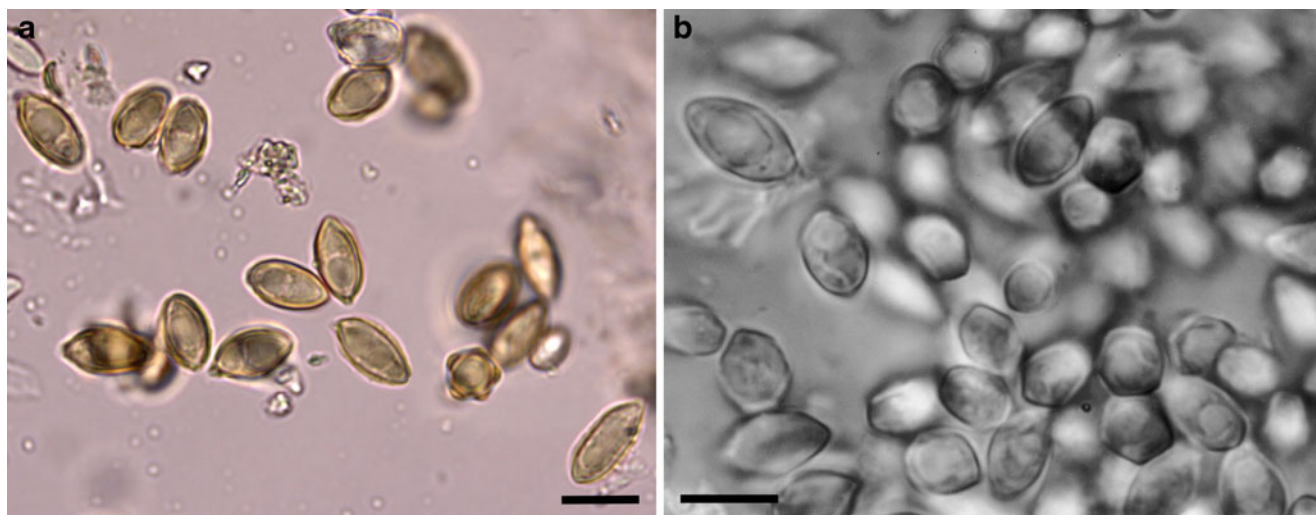


Fig. 9 *Rosbeeva vittatispora* variation in spore shape and ornamentation. **a** (Beaton #77 isotype MELU); **b** (Lebel 2348)

Basidiomata hypogaeal, 10–30 mm diam, irregularly globose, subglobose, or pyriform. *Pileus* thin, dry, smooth, white to pale greyish to buff with a silky lustre, barely staining greenish-yellow to greenish-blue or indigo in patches or eventually greenish-blue overall. *Hymenophore* white then pale tan, maturing to rusty brown or dark brown, firm becoming subgelatinous and rubbery, loculate to labyrinthine, chambers minute, becoming slightly greenish around insect damage. *Stipe-columella* absent or present as a small, inserted basal pad, 1–3.0×1–2.0 mm, white to translucent, sometimes turning blue-green when cut or exposed to air; sometimes a single white rhizomorph at base. *Odour* becoming stronger as sporocarp matures, of natural gas or kerosene with hints of fermenting fruit, unpleasant.

Basidiospores 9–12 (–13)×4.0–5.5 (–6.0) μm , (mean 11.9×5.1), $Q=1.65\text{--}2.2$, short ellipsoid to fusiform, initially hyaline becoming brown, with a slightly thickened wall, with mostly 4- rarely 5 longitudinal ridges, 0.25–2.0 μm high, in polar view appearing barely angular when immature, becoming distinctly quadrangular. *Basidia* 17–21×5–11 μm , hyaline, cylindrical to clavate, 2- or 4-spored; *basidioles* clavate to broadly clavate, 10–16×5–10 μm . *Hymenial cystidia* absent. *Subhymenium* poorly developed. *Hymenophoral trama* narrow, 60–140 μm thick, of loosely interwoven to parallel, hyaline non-inflated, thin-walled, subgelatinised hyphae 2–6 μm diam. *Pileipellis* thin, 20–75 μm thick, mostly a repent cutis of interwoven, irregularly branched, gelatinised hyaline to pale brown hyphae 4–8 μm diam, golden yellow in 3% KOH, with scattered, irregular patches of a weak trichodermium of partly swollen and branched, finely septate, subspherical elements 5–9 μm diam, becoming narrower and more filamentous towards the hymenophore.

Specimens examined: VIC: Eildon, bush at end of 8th st, *G.Beaton* #77, 10.vii.1974 (type K78513; isotype MELU); Great Otway Range National Park, Phillips Track, Triplet Falls, *G.Beaton* #57, 22.ix.1982 (K78578); Great Otway Range National Park, Wait-a-while rd from Youngs Creek to Lavers Hill, \pm 1 km from jct with Lavers Hill rd, *T.Lebel* 2701, 29.vi.2010 (MEL); near Marysville, Cumberland Reserve *Beaton* 12.i.83 (K78509); Eildon, Snobs Creek rd, old campsite, *K&G Beaton*, 3.iv.1982 (MELU); Lower Glenelg National Park, south of Heath Rd-Mt Deception junction, *K&G Beaton*, 12.ix.1982 (MELU); Warburton, Acheron Gap, *K&G Beaton*, 12.i.1983 (MELU); Acheron Way, Lake Mountain turn-off, *K&G Beaton*, 10.iv.1983 (MELU); Rubicon, 21 km along Blue Range road, *K&G Beaton*, 27.iv.1983 (MELU; MEL); Bullfight rd off Snobs Creek rd, *K&G Beaton*, 20.v.1984 (MELU); Alexandra, Crystal Creek *GA.Crichton* A3, 24.v.1963 (MEL2057768); Eildon, Dry Creek Rd, *GA.Crichton* L313, 6.vi.1969 (MEL2059185); Old Healesville Rd, Dom Saddle, *GA.Crichton* L119, 8.ix.1968 (MEL2058680); Great Otway Range National Park, Great Ocean Hwy, 30.6 km N of Erskine River, *M.Castellano* & *T.Lebel* JMT14208, 11.vii.1993 (MEL2124023); Eildon State Park, Eildon–Jamieson Rd, 9.6 km from Jamieson turnoff, *M.Castellano* & *T.Lebel* JMT14180, 10.vii.1993 (MEL2124027); Brisbane Ranges, rd to Steiglitz, *T.Lebel* 235, 4.ix.2000 (MEL2063454); Western Hwy between Horsham and Ararat, *T.Lebel* 228, 30.vii.2000 (MEL2063438); Langhi Ghiran State Forest, picnic area, *T.Lebel* 2115, 30.v.2004 (MEL2329434); Grampians National Park, Redman Rd, 1st track, *W.Colgan* III JMT 14351, 13.vii.1993 (MEL); Grampians National Park, rd to Zumstein Falls, 1 km from Halls Gap, *JM.Trappe* 14339 & *M.Castellano*, 13.vii.1993 (MEL);

TAS: Maydena to Strathgordon rd, 20 km W of #5 track, *T.Lebel* 179, 9.iv.2000 (MEL2079331); Tarkine Wilderness region, Waratah to Savage River Rd, 4 km from jct main rd, *T.Lebel* 2250, 22.iv.2006 (MEL); Tarkine Wilderness region, rd from Savage River mine to Corinna, *K.Syme RE. Halling* 8679 4.v.2005 (MEL2265003); Mt Field National Park, start of Lyrebird track, *T.Lebel* 163 & *K.Symes*, 7. iv.2000 (MEL2078292); Mt Field National Park, 45 m up Lyrebird track, *T.Lebel* 165, 7.iv.2000 (MEL2078294); Mt Field National Park, 25 m up Lyrebird track, *T.Lebel* 166, 7. iv.2000 (MEL2078295); Blue Tier National Park, Mt Michael track, Poimena, *T.Lebel* 2348, 18.v.2010; Cradle Mountain-Lake St Clair National Park, Lake St Clair, Watersmeet track, *T.Lebel* 2338, 16.v.2010 (MEL); Cradle Mountain-Lake St Clair National Park, Lake St Clair, Watersmeet track *T.Lebel* 2342, 16.v.2010 (MEL);

NSW: Noojee State Forest, *T.Lebel* 1091, 25.ii.2002; June Caves, *T.Lebel* 167, 8.iv.2000 (MEL2078320); Mt Kaputar National Park, plot MD GW1, *K.Vernes* 503, *M.Danks and T.Lebel*, 16.vii.2007 (MEL); Coolangubra National Park, Waratah Rd, 3.3 km NE junction Coolangubra Forest Way, *AW.Claridge, W.Colgan III, & A.Jumponnen JMT18749*, 3.vi.1996 (MEL266708); Tantara National Park, Unnamed track, 0.3 km NE junction of Tantara mountain rd, *AW.Claridge & W.Colgan III JMT18792*, 3.v.1996 (MEL266597); Yambulla State Forest, Falkner Rd, 0.7 km E junction of Kallack Rd, *B.Gunn & AW.Claridge* 1086, 9.vii.1996 (MEL266729); Bondi Gulf Nature reserve, Bondi Gulf Rd, 7.1 km E Cann Valley Highway, *A.Jumponnen & JM Trappe* 18933, 5. vi.1996 (MEL266605); New England National Park, plot MD NS4, *K.Vernes* 666, *M.Danks and T.Lebel*, 20.vii.2007; New England National Park, plot MD WS5 *K.Vernes* 675, *M.Danks and T.Lebel*, 20.vii.2007; New England National Park, plot MD GW6, *K.Vernes* 693, *M.Danks and T.Lebel*, 20.vii.2007; Monga State Forest, Milo Rd 3.3 km N of junction with Saddleback Rd, *JM.Trappe* 15800 & *T.Lebel*, 3.vi.1995 (MEL);

ACT: Namadgi National Park, Brindabella Ranges, Bulls Head, *JM.Trappe* 15656 & *F.Camacho*, 1.vi.1995 (MEL);

QLD: Herberton, *J.Garbarye* NQ31, v.1986 (AQ642648); north slope above Herberton, *J.Garbarye* NQ27, v.1986 (AQ642651).

Habitat, distribution and season—hypogaeal in mixed coastal, mallee or tall wet *Eucalyptus*, *Acacia* and *Leptospermum* spp. forest and woodland in Victoria, New South Wales and QLD, and in cool temperate rainforest of *Nothofagus* or *Eucalyptus* spp. in Victoria and Tasmania. April–Sept.

‘variant a’ Fig. 4e

Sporocarps 8–28 (–32) × 8–26 (–28) mm, globose to subglobose, overall white initially bruising deep indigo in

patches on exposure or handling, with scattered fibrils on surface staining indigo on exposure, firm textured. *Hymenophore* becoming brown with maturity. *Stipe* 1–2 × 2 mm, present as small basal pad, translucent cartilagenous with some blue staining. *Odour* strong kerosene in mature specimens. *Basidiospores* 9–11.5 × 4.0–5.5 μm. *Basidia* 4-spored.

Specimens: **NSW:** Mt Kaputar National Park, plot MD GW3, *K.Vernes* 584, *M.Danks and T.Lebel*, 17.vii.2007 (MEL); Namadgi State Forest, off Wog Way, 1.1 km from junction with Imlay rd to south of Wog Way *T.Lebel* 482, 30.v.2001 (MEL2128491); **TAS:** Near Waratah-Corinna rd, 7 km south-west of Waratah, 4 km down Savage River rd, *T.Lebel* FT250, 22.iv.2006 (MEL2321928); Cradle Mountain National Park, Dove Lake Track, *R.E.Halling* 8674, 1. v.2005 (MEL2264998)

Habitat, distribution and season—in New South Wales, hypogaeal in mixed *Eucalyptus*, *Acacia* and *Leptospermum* spp. woodland, and in Tasmania, hypogaeal in cool temperate rainforest of *Nothofagus*, *Leptospermum*, *Eucalyptus*, and *Arthrotaxis cupressoides*. May–July.

Notes—The species boundaries of *R. vittatispora* are broadened to include many Australian collections originally listed under the names *R. mucosa* and *R. pachyderma*. This is partly due to the lack of supporting morphological characters and also to the low variation in the ITS regions, making differentiation of more than one taxon problematic. Examination of many herbarium collections, showed that characters such as spore size and ornamentation height, and presence of cyanescing are continuously variable within and between collections. As the sporocarps mature, the intensity of the cyanescing reaction can increase or decrease, the hymenophore changes from white to pale tan then rusty brown to dark brown and the spore ornamentation becomes more obviously ridged and higher, as the spores mature. Approximately half of the collections examined contain only immature material (i.e. hymenophore white or barely tinted), and are lacking details such as colour changes of fresh material. This includes the type collection of *R. vittatispora*, which contains only immature specimens, with white to pale tan hymenophore and spores with very low ornamentation.

Australian collections listed in Beaton et al. (1985) under the name ‘*Chamonixia mucosa*’, on the basis of spores with more robust spore ornamentation and darker hymenophore, are considered to be mature specimens of *Rosbeeva vittatispora*. No other morphological characters were found to be consistent or distinctive, nor was there any support in molecular analyses for separation of a distinct taxon for collections with these characters.

In analyses of ITS, posterior probability support (0.95; Fig. 1) was found for a group of sequences which we

distinguish as a variant form of *R. vittatispora*, but do not name formally, ‘variant a’. We could find no morphological characters at this time that were unique to this group of collections, and that did not fall within the range of *R. vittatispora*. A short description of ‘variant a’, with selected specimens, is provided. *Rosbeeva vittatispora* as currently understood, is a widespread species in south-eastern Australia, and under collected in Western Australia, Queensland and South Australia. It is possible with further analyses of different regions of DNA (i.e. *rpb2* or *atp6*) that greater delimitation of species may be supported. This species can be distinguished from *R. westraliensis* T.Lebel sp. nov. by the shorter, fatter spores, odour of natural gas or kerosene rather than acrid, chemical, and tendency to greenish-blue rather than deep blue tints in cyanescence of the sporocarp on bruising. Further collections from Queensland, South Australia, New Caledonia and South-east Asia are needed to clarify species boundaries of *R. vittatispora* and *R. mucosa*.

Rosbeeva westraliensis T.Lebel, sp. nov.

MycoBank 519402—Figs. 4f, 5e, 10

Basidiomata aggregata, hypogaea, usque ad 32 mm diam., globosa vel irregulater elongata, albida vel pallide cinnerea initio sericea cyanescentia vel indica post contusa. Hymenophora initio albida dein pallide brunnea, ad maturitatem atrobrunnea, cyanescentia post contusa, loculata, cellulis minutis. Stipes absens vel a pede basali reductus, albidus vel cremeus. Basidiosporae 12.0–14.5 (–16.5) × 3.0–4.5 (–5.0) μm, fusoidae, hyalinae vel lueto-brunneae, ad maturitatem brunneae, laeves, 4–5 cristis longitudinalibus 1–2 μm altis ornatis; aspectu polari angulatae vel stellatae. Hyphae gelatinosae vel subgelatinosae. Pileipellis cutis. T: AUSTRALIA. Western Australia, 20 km W of Ravensthorpe, Phillips rd crossing, South

Coast Hwy, *K.Syme 1130/00 & M.Hart*, 20.viii.2000 (holotype MEL2233755 designated here; isotype PERTH07981880).

Etymology. The epithet *westraliensis* refers to the distribution of the species, currently known only from Western Australia.

Sporocarps hypogaeal, gregarious, up to 32 mm diam, globose to irregularly elongate, dry, firm, minutely felty when young, white with silky, slightly reflective lustre, white becoming pale greyish then bruising blue to deep indigo, in small patches, with handling becoming pale dirty greyish blue overall with deeper indigo blue patches; with some fine white bruising blue rhizomorphs scattered loosely over the surface. *Pileus* thin, white in cross section, not changing colour. *Hymenophore* firm, white initially becoming unevenly brown then eventually dark brown, with some blue staining around insect damage, loculate, chambers minute. *Stipe columella* absent or as small sterile basal inserted pad 1–2 × 1 mm, white to translucent, sometimes with thin strands radiating. *Basal mycelium* white. *Odour* strong, slightly acrid chemical.

Basidiospores 12.0–14.5 (–16.5) × 3.0–4.5 (–5.0) μm, (mean 14.2 × 3.9 μm), *Q* = 3.0–4.14, fusoid, initially hyaline to pale yellow, becoming brown at maturity, immature spores faintly dextrinoid in Melzers reagent; ornamentation 1.0–2.0 μm high, of 4–5 irregular longitudinal ridges, wavy and occasionally interrupted; in polar view appearing barely quadrangular or pentagonal. *Basidia* 24–37 × 7–12 μm, hyaline, clavate to broadly clavate, 2-, 3- or 4-spored; basidioles 10–14 × 9–13 μm, broadly clavate. *Hymenial cystidia* absent. *Subhymenium* not well developed. *Hymenophoral trama* narrow, 45–130 μm thick, of loosely interwoven, hyaline non-inflated, thin-walled, gelatinised hyphae 2–6 μm diam. *Pileipellis* a repent cutis, 80–200 μm

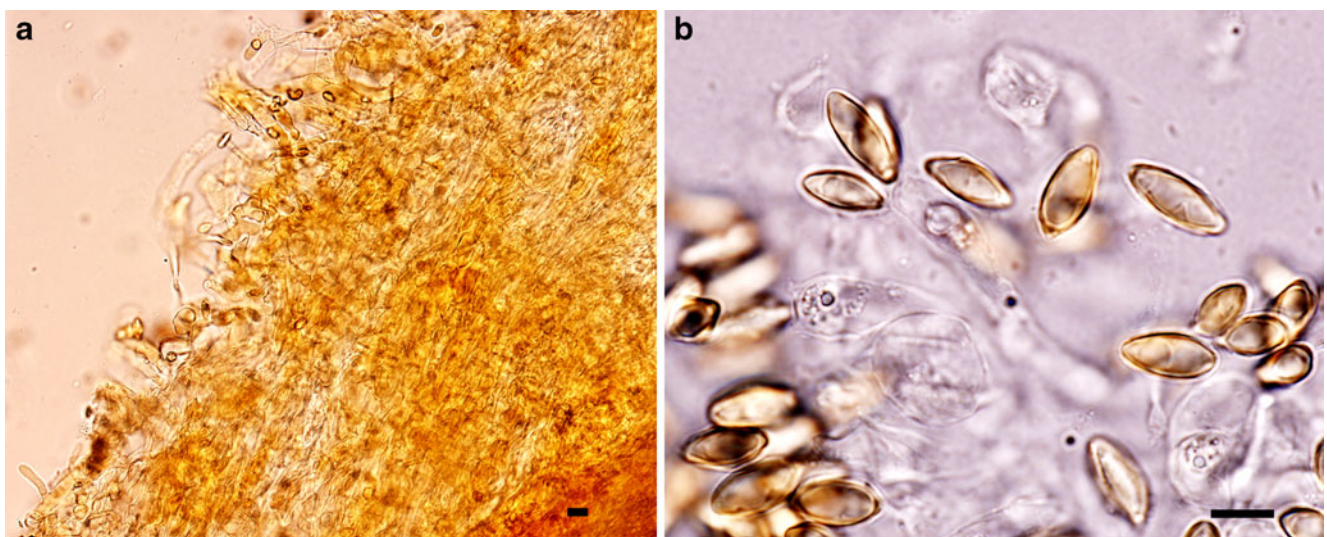


Fig. 10 *Rosbeeva westraliensis* (MEL2233755, holotype): **a** cutis pileipellis; **b** basidia and spores

thick, of subparallel to interwoven hyaline, subgelatinised thin-walled hyphae, 4–8 μm diam., which become yellow in 3% KOH.

Specimens examined—Western Australia: Dryandra State Forest, Tomingley Rd, one-way section, *K. Syme 1105/00*, 13.vii.2000 (MEL2219011); Dandalup Rd to Dwellingup, *JM.Trappe 14719 & M.Castellano*, 19.vii.1993 (MEL); Nanga Rd, *JM.Trappe 14692 & M.Castellano*, 19.vii.1993 (MEL); Mundaring, Ashendon Rd, *M.Castellano & W.Colgan III JMT14626*, 17.vii.1993 (MEL); Dryandra State Forest, Tomingley Rd, *K Syme 1106/00*, 13.viii.2000 (MEL2219022); Denmark, old drive-way through South Coast Hwy, loc. 406, *K.Syme 1256/03*, 20.vi.2003 (MEL2231712A, PERTH07982224); Sir James Mitchell National Park, *N.Malajczuk JMT6913*, 10.ix.1982 (MEL2124034); Jarrahdale, Langford Park, off Jarrahdale rd, *T.Lebel & M. Castellano JMT 14655*, 19.vii.1993 (MEL2322708); Dryandra State Forest, Williams to York rd, 4.6 km from Wandering rd junct, *M. Castellano JMT14959*, 23.vii.1993 (MEL2322707).

Habitat, distribution and season—*Allocausarina* spp. and open *Eucalyptus* woodland with low mixed shrub understory, sandy soils. Western Australia.

Notes—*Rosbeeva westraliensis* can be distinguished by the sporocarps that are initially white becoming pale grey then bruising blue to deep indigo with fine white bruising blue rhizomorphs scattered loosely over the surface. Microscopically the narrower and slightly longer, fusoid spores distinguish this species from the closely related *R. vittatispora* and ‘variant a’. It is possible that this species also occurs in South Australia, however the few existing herbarium collections either were determined to not belong to the genus or were immature and lacking field data, thus not able to be placed with any consistency within a particular species.

Excluded taxa

Austrogautieria octorugosa (Corner & Hawker) T.Lebel, *comb. nov.*

Mycobank 519403

≡ *Chamonixia octorugosa* Corner & Hawker, *Trans. Br. Mycol. Soc.* 36(2): 133 (1953)

The type collection *EJH Corner 748* (K78517) in Kew, which has been stored in alcohol, was examined. The colour change of the sporocarp to fuscous purple rather than blueish green or indigo, and large spores with 7–9 parallel, interconnected ridges 1–2 μm high, which converge at the apex, and have subacute margins are typical of *Austrogautieria* species rather than *Chamonixia* or *Rosbeeva*. The gelatinised hyphae of pileus and hymenophore, and well developed trichodermial pileipellis are also characters more typical of *Austrogautieria*. An annotation slip present in the type collection packet reads: ‘*annotated MA Castellano 24 October 1988*= *Austrogautieria octor-*

ugosa’. However, the nomenclatural change was never published.

Unfortunately no recent material was available for further study or DNA analyses.

Discussion

The sequestrate form has arisen multiple times within the Boletaceae and, in particular, three times with affinities to *Leccinum* and *Leccinellum*. Two of the genera have elongate ridged spores, *Chamonixia* (Binder and Bresinsky 2002) and *Rosbeeva*, and *Octaviana* has globose spinose spores (Orihara et al. 2010). Members of the sequestrate genus *Rhodactina* also have spores with elongated ridges; and a sequence of *R. incarnata* has recently been shown to have affinities to the Boletaceae, though not necessarily within the *Leccinum/Leccinellum/Chamonixia/Rosbeeva/Octaviana* lineage (Yang et al. 2006). Except for *Rhodactina*, all the sequestrate genera have a cyanescing reaction of the pileus to some degree. Both *Leccinum* and *Leccinellum* have smooth, subfusiform spores, scabers on the stipe (light or dark), and may or may not have a blueing or reddening reaction of the stipe or pileus context.

The lack of support for genus level relationships in the *Leccinum/Leccinellum/Chamonixia/Rosbeeva/Octaviana* clade precludes determining whether the ancestral state is the epigeal boletoid sporocarp form. This seems the most likely scenario as with few exceptions, in lineages with gastroid and/or secotioid sporocarp forms, the sequestrate taxa are in the terminal branches of clades (Hosaka et al. 2006; Justo et al. 2010; Lebel and Tonkin 2008; Peintner et al. 2002). Other studies of boletaceous fungi have also had similar lack of resolution of genus level relationships (Binder and Hibbett 2006; Desjardin et al. 2008; Halling et al. 2007). One possible classification would be to collapse the five genera into a single large genus *Leccinum*. However, there are good morphological and molecular supporting characters to continue distinguishing these taxa as distinct genera.

The novel genus *Rosbeeva* is supported both morphologically and phylogenetically as distinct from *Chamonixia*, *Octaviana* (Fig. 1) and *Rhodactina* (Yang et al. 2006). Although molecular data were unavailable for *Rosbeeva bispora* and *R. mucosa*, morphological characters support placement of these taxa within the new genus. *Chamonixia octorugosa* is, based on spore ornamentation a member of the genus *Austrogautieria*, not *Rosbeeva*. Further collecting within China, Japan, South-East Asia, New Caledonia, and Australia (South Australia and Queensland in particular), would be helpful to define relationships between taxa and may bring to light further novel taxa. Areas such as South-East Asia (including Papua New Guinea), and New

Caledonia, are an intriguing location as the distributions of northern and southern ectomycorrhizal plant associates intersects here. We know the genus occurs in Papua New Guinea as the spores can be found in the diet of mycophagous mammals (Vernes and Lebel 2011).

Leccinum and *Leccinellum* are most diverse in North America and Europe, with most species host-specific to members of the Betulaceae, Pinaceae and Fagaceae (den Bakker and Noordeloos 2005). In Australia, only the species, *Leccinum australiense* Bougher & Thiers is known in association with *Eucalyptus*, and from Malaysia *L. intusrubens* (Corner) Hongo in association with *Castanopsis* (Bougher and Thiers 1991; Corner 1972; Hongo 1983). In Japan and China, over ten species of *Leccinum* and *Leccinellum* are known, some being common in temperate, deciduous forests. Extensive collecting of boletaceous fungi in Australasia in the last few years has failed to uncover any further *Leccinum* or *Leccinellum* species (pers.comm. R Halling and R. Robinson). Although not common, both *Rosbeeva* and *Octaviania* are widespread within Australasia and Japan, in varied habitats (subtropical to cool temperate rainforest and dry sclerophyll woodlands) and associated with a wide range of hosts (*Eucalyptus*, *Nothofagus*, *Castanopsis* and *Fagus*). The broader distribution of the two genera is intriguing: Australia, New Zealand, Singapore, Borneo and Papua New Guinea (unconfirmed for *Octaviania*), Japan and China. The genus *Chamonixia* is widespread but not common in Europe, North America, Russia and China (Mleczko et al. 2009). The lack of corresponding macro- or micro-morphological characters to link the sequestrate to the boletoid genera, and lack of support at the genus level in analyses of DNA sequence data suggest that there are some taxa missing from the analyses of the *Leccinum/Leccinellum/Chamonixia/Rosbeeva/Octaviania* lineage. As BLAST searches of sequence data found no ‘hidden’ matches, and no unique herbarium material was uncovered, we presume that these missing taxa are either no longer extant or perhaps, still lie undiscovered.

At the species level, the Japanese taxa are distinct from the Australasian, with the Chinese *R. bispora* morphologically at least more similar to the Japanese taxa. However, species level differentiation within Australasian taxa is difficult, due to the low level of variation in ITS sequences and continuity of variation in morphological characters. *Rosbeeva westraliensis* collections consistently had more fusoid, longer spores, and a restricted plant association with *Allocasuarina* spp. This is one of the few Australasian species which is supported in Bayesian analyses of ITS sequences (Fig. 1). Another possible taxon, ‘variant a’ within *R. vittatispora*, is not formally named as we could not find any supporting morphological characters unique to this group of specimens.

While the two Japanese species, *R. eucyanea* and *R. griseovelutina*, are both monophyletic and sister species, their relationship to the Australasian *Rosbeeva* spp. is not resolved in the ITS phylogeny. In the nLSU phylogeny, the two species fell into the terminal clade with *R. vittatispora* and *R. westraliensis* (Fig. 1). At this point we cannot conclude chronological order of species differentiation within the *Rosbeeva* clade. However, relatively long branches of *R. eucyanea* and *R. griseovelutina* lineages in the phylograms suggest high potential phylogenetic diversity of *Rosbeeva* in Japan and the surrounding area (Faith 1992; Faith and Baker 2006).

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