Obba and Sebipora, new polypore genera related to Cinereomyces and Gelatoporia (Polyporales, Basidiomycota)

# **Otto Miettinen & Mario Rajchenberg**

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ORIGINAL ARTICLE

## *Obba* and *Sebipora*, new polypore genera related to *Cinereomyces* and *Gelatoporia* (Polyporales, Basidiomycota)

Otto Miettinen · Mario Rajchenberg

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Abstract Cinereomyces clade is a newly proposed monophyletic group of polypores containing currently four genera and five species, including two promising biopulping fungi, Ceriporiopsis rivulosa and C. subvermispora. The Cinereomyces clade is well-delimited in nrDNA-based phylogenetic analysis, but its position in Polyporales remains unclear. Its closest relative may be found in the core polyporoid clade. Only a few morphological characters are common for all the species in the clade, e.g. CB- and CRB+ hyphae, white fruiting bodies, presence of oil, and middlesized spores. Culturally, the species are unified by producing simple-septate generative hyppae in the margin, which produce simple-clamped hyphae backwards. The genus Gelatoporia is the correct place for Ceriporiopsis subvermispora. Two new genera are described in the group: Obba to incorporate C. rivulosa and a new austral species, Obba valdiviana, known from southern Argentina and recorded here also from Tasmania, and Sebipora to accommodate a new species from tropical Asia, S. aquosa. ITS sequences imply that Eurasian Gelatoporia subvermispora may belong to a different species from the North American one. G. subvermispora is recorded as new to Indonesia.

**Keywords** Ceriporiopsis rivulosa · Ceriporiopsis subvermispora · Diplomitoporus lindbladii · Taxonomy · Biopulping

O. Miettinen (⊠) Botanical Museum, University of Helsinki, Helsinki, PO Box 7, 00014, Finland e-mail: otto.miettinen@helsinki.fi

M. RajchenbergCentro de Investigación y Estensión Forestal Andino Patagónico (CIEFAP),C. C. 14, 9200 Esquel,Chubut, Argentina

#### Introduction

The generic position of the polypore *Ceriporiopsis rivulosa* (Gilbertson and Ryvarden 1986) has long been uncertain. Besides *Ceriporiopsis*, it has been placed in *Physisporinus* (Ryvarden 1984) and *Rigidoporus* (David 1971). Morphologically, it does not fit nicely in any of the abovementioned genera in the strict sense. On the other hand, cultural and enzymatic characters have placed it in the vicinity of *Gelatoporia subvermispora* (*=Ceriporiopsis subvermispora*), another effused polypore species of uncertain generic affinity (David 1971; Hakala et al. 2005; Nakasone 1981; Rajchenberg 1995).

Interestingly, both species have shown promise for use in biopulping, a process where wood chips are treated with white-rot fungi prior to mechanical pulping in the paper industry (Akhtar et al. 2000; Fackler et al. 2007; Maijala et al. 2008). These fungi decompose lignin selectively when wood chips are pre-treated with them, reducing the need of energy in pulping and even improving paper quality. Consequently, their taxonomic positions are of interest to a wider audience than just taxonomists.

When Niemelä (1985) described the genus *Gelatoporia* based on morphological characters, he included two species in the genus: *G. subvermispora* and *G. pannocincta*. However, cultural characters and sexual behaviour do not support close affinity of these two species, and rather indicate affinity of *G. pannocincta* with *Gloeoporus dichrous* (David 1972).

DNA-sequence-based phylogenetic studies have confirmed the conclusions of cultural studies in this case: *Ceriporiopsis rivulosa* and *Gelatoporia subvermispora* are indeed closely related (Tomšovský et al. 2010). Also, *Gelatoporia pannocincta* and *Gloeoporus dichrous* are closely related to each other and belong to the *Byssomerulius*  family, phlebioid clade of Polyporales, but are not related to *Ceriporiopsis rivulosa* (Binder et al. 2005; Larsson 2007; Tomšovský et al. 2010; Wu et al. 2010; Miettinen & Larsson 2010).

Phylogenetic studies have further shown that *Cinereo-myces lindbladii* (=*Diplomitoporus lindbladii*) is also related to *Gelatoporia subvermispora* and *Ceriporiopsis rivulosa*. During our studies in the southern hemisphere, we have encountered two further taxa in this species group. Together, they form a well-supported monophyletic group, called *Cinereomyces* clade hereafter, but the position of this group inside Polyporales is uncertain. In their larger study, Binder et al. (2005) placed *Cinereomyces lindbladii* inside the core polyporoid clade, whereas in a more restricted taxon sampling, Miettinen and Larsson (2010) left it outside.

In this article, we study the generic arrangements inside the *Cinereomyces* clade using nrDNA sequences and morphological methods, and the phylogenetic position of the *Cinereomyces* clade in Polyporales.

#### Materials and methods

We chose reference taxa for our phylogenetic analysis based on Binder et al. (2005), Dai et al. (2006), Matheny et al. (2007), Larsson (2007), Larsson et al. (2004), Lindner and Banik (2008), Robledo et al. (2009), Sotome et al. (2008), and through searches in GenBank. A total of 25 new nrDNA sequences were produced for this study (Table 1), while reference sequences of 79 specimens were obtained from GenBank (Tables 1 and 2). In addition to herbarium specimens, strains from University of Helsinki affiliated Fungal Biotechnology Culture Collection (FBCC) were utilised. Reference sequences were sampled from Polyporales with the aim of providing representatives from all major clades within the order (sensu Binder et al. 2005; Larsson et al. 2004; Larsson 2007). Certain divergent groups of brown-rot fungi, which often cause problems in nrDNA 28S-based phylogenetic analysis (Fibroporia, Laetiporus, Pycnoporellus, Sparassis, Taiwanofungus, Wolfiporia), were included in the analysis in the initial stages, but excluded later on. Their inclusion in the analysis did not affect the core results of this study, nor are they closely related to the Cinereomyces clade.

Specimens used for this study are listed below. DNA of specimens marked with an asterisk (\*) has been utilised in this study. Herbarium abbreviations follow Index Herbarorium standard, except for CIEFAP, which stands for the fungal herbarium of Centro de Investigación y Estensión Forestal Andino Patagónico, Argentina.

#### Specimens studied

*Atraporiella neotropica* Ryvarden. **Belize**. Cayo, 20. XI.2001 Ryvarden 44447 (O\*, holotype).

*Ceriporiopsis gilvescens* (Bres.) Domański. **Czech Republic**. Central Bohemia, Sázava 21.IX.1991 Niemelä 5516 (H\*). **Poland**. Małopolska, Kraków county, 1.IV.2009 Niemelä 8550 (H).

*Cinereomyces lindbladii* (Berk.) Jülich. **Finland**. Uusimaa, Inkoo, 24.V.2003 Kotiranta 19911 (H\*). Pohjois-Karjala, Ilomantsi, 28.8.1996 Oivanen s.n. (FBCC 117\*).

*Dichomitus squalens* (P. Karst.) D.A. Reid. **Russia**. Ryazan, Spassky, 16.VIII.2006 Zmitrovich s.n. (H\*).

Gelatoporia subvermispora (Pilát) Niemelä. China. Heilongjiang, Yichun, on Picea, 7.VII.1999 Y.C. Dai 3120 (H\*). Indonesia. Jambi, Kerinci, 900–1,100 m asl, mountain rainforest, on Pinus merkusii, 29.VII.2004 Miettinen 9079 (ANDA, H\*). Finland. Kittilän Lappi, Kittilä, on Picea, 24.VIII.2000 Kinnunen 1052 (H\*). Poland. Podlesie, Hajnówka, Białowieża, on Carpinus, 19.V.1996 Niemelä 5978 (H\*). Russia. Nizhny Novgorod, Sharanga, on Populus, Spirin 2156 (H\*). Perm, Kvarkush, on Picea, 6.VIII.2005 Kotiranta 20823 (H\*).

*Obba rivulosa* (Berk. & M.A. Curtis) Miettinen & Rajchenb. **Argentina**. Salta, Baritú National Park, 1190 m asl, 22°26'10.8"S, 64°43'40.2"W, 9.V.2007 G. Robledo 1473 (CORD). **Cuba** (see type). **Finland**. Uusimaa, Helsinki, 18. IX.1998 Kotiranta 16702 (H\*). Etelä-Häme, Lammi, 14. X.1998 T. Hakala s.n. (strain FBCC 938\* alias T241i), 14.-16.X.2003 Miettinen 8054 (H\*). Etelä-Häme, Tammela, 21. X.2003 Penttilä 15077 (H\*). Pohjois-Karjala, Ilomantsi, 7. IX.2003 Penttilä 14441 (H6002108\*). Pohjois-Karjala, Lieksa, 3.-4.IX.2003 Penttilä 14135 & Oinonen PTO8 (H\*). **USA**. Oregon, Lincoln county, 3.X.1973 Larsen s.n. (H, ex FP133696).

Obba valdiviana (Rajchenb.) Miettinen & Rajchenb. Argentina. Chubut, Los Alerces National Park, Lake Menéndez, Alerzal, 15.III.1993 Rajchenberg 10736 (BAFC 33238 holotype, CIEFAP isotype, strain Rajchenberg 159\*), Ibid., 3.V.2010 Rajchenberg 12292 (CIEFAP). Río Negro, Nahuel Huapi National Park, Puerto Blest, pathway to Los Cántaros, 9.X.1995 Rajchenberg 11043 (CIEFAP\*). Chile. Osorno, Puyehue National Park, Las Termas, 450 m asl, 15.IV.1988 Rajchenberg 4157 (BAFC 31280). Los Ríos, Futrono commune, ca. Lake Maihue, forest of Aetoxicon punctatum, Laureliopsis philippiana, Nothofagus obliqua, Eucryphia cordifolia, 3.II.2010 P. Sandoval G53 (SGO). Australia. Tasmania, Dalco Creek, 43°23'S, 147°01'E, on Eucalyptus, 15. III.2009 G. Gates FF503 (HO 557493\*). Tasmania, Liffey Falls, 41°42"S, 146°46'E, on Eucalyptus, 7. III.2009 G. Gates FF484 (HO 557492, strain CIEFAP 336\*).

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#### Table 1 List of sequenced specimens in Cinereomyces clade used in this study, and other newly sequenced specimens

Taxon	Herbarium specimen	Culture	Origin	ITS/5.8S	LSU
Atraporiella neotropica	*Holotype		Belize	HQ659221	HQ659221
Ceriporiopsis gilvescens	*Niemelä 5516		Czech Rep.	HQ659222	HQ659222
Cinereomyces lindbladii FI	Kotiranta 19911		Finland	FN907909	FN907909
C. lindbladii FI 2		*FBCC 117	Finland	HQ659223	
C. lindbladiiKEW		KEW 212			AF518615
C. lindbladii NO	KH Larsson 12078		Norway	FN907906	FN907906
Dichomitus squalens	*Zmitrovich s.n.		Russia	HQ659224	HQ659224
Gelatoporia subvermispora CN	*Dai 3120		China	HQ659226	
G. subvermispora CZ	BRNU 592909		Czech Rep.	FJ496694	FJ496706
G. subvermispora FI	*Kinnunen 1052		Finland	HQ659225	
G. subvermispora ID	*Miettinen 9079		Indonesia	HQ659229	
G. subvermispora PL	*Niemelä 5978		Poland	HQ659227	
G. subvermispora RU	Kotiranta 20823		Russia	FN907911	FN907911
G. subvermispora RU 2	*Spirin 2156		Russia	HQ659228	
G. subvermispora US		KCTC 6891 (=CBS 347.63)	USA, CO	FJ496695	FJ496707
G. subvermispora US 2		CBS 347.63 (=KTCT 6891)	USA, CO	FJ349621	
G. subvermispora US 3		ATCC 90467 (=FP 90031)	USA, MD	FJ545252	
G. subvermispora US 4		FP 90031 (=ATCC 90467)	USA, MD	FJ713106	
G. subvermispora WS1		WS1	AY089736		
Obba rivulosa CA		KCTC 6892 (=CBS 434.48)	Canada	FJ496693	FJ496710
O. rivulosa CBS 433.48		CBS 433.48		FJ496692	
O. rivulosa FI	*Miettinen 8054		Finland	HQ659231	HQ659231
O. rivulosa FI 2		*FBCC 938	Finland	HQ659233	
O. rivulosa FI 3	*Kotiranta 16702		Finland	HQ659232	
O. rivulosa FI 4	*Penttilä 14135		Finland	HQ659234	
O. rivulosa FI 5	Penttilä 14441		Finland	FJ496691	
O. rivulosa FI 6	*Penttilä 15077		Finland	HQ659230	
O. rivulosa US		JLL 10602	USA, CA	AY219363	
Obba valdiviana AR 1	isotype	*CIEFAP 159	Argentina	HQ659237	
O. valdiviana AR 2	*Rajchenberg 11043		Argentina	HQ659238	
O. valdiviana AU 1	*Gates FF503		Australia	HQ659235	HQ659235
O. valdiviana AU 2		*CIEFAP 336	Australia	HQ659236	HQ659236
Piloporia sajanensis	*Manninen 2733a		Finland	HQ659239	HQ659239
Sebipora aquosa	*Miettinen 8680.1		Indonesia	HQ659240	HQ659240
S. aquosa 2	*holotype		Indonesia	HQ659242	
S. aquosa 3	*Miettinen 9265.1		Indonesia	HQ659243	
S. aquosa 4	*Miettinen 12032		Indonesia	HQ659241	
Tyromyces chioneus	*Miettinen 7487		Finland	HQ659244	HQ659244

Specimens marked with an asterisk (\*) have been sequenced for this study. Two-letter codes after species names denote country of origin (ISO 3166)

*Piloporia sajanensis* (Parmasto) Niemelä. **Finland**. Inarin Lappi, Inari, 15.VI.2006 Manninen 2733a (H\*).

*Tyromyces chioneus* (Fr.) P. Karst. Finland. Etelä-Häme, Lammi, 3.IX.2003 Miettinen 7487 (H\*).

Sebipora aquosa Miettinen. Indonesia. Riau, Rokan Hilir (see holotype\*). Riau, Pekanbaru, open yard on a burned log, 1.XII.2007 Miettinen 12032 (ANDA, H\*).

Riau, Indragiri Hulu, Bukit Aluran Babi, 0°50.402'S, 102°14.006'E, secondary half-open rainforest, on a log (60 cm in diameter, decay stage 3/5), 28.VI.2004 Miettinen 8680.1 (ANDA, H\*). Papua, Teluk Wondama, Werianggi N, 2°27.168'S, 134°9,100'E, small opening in a primary forest, on a log (25 cm in diameter, decay stage 4/5), 6. IX.2004 Miettinen 9265.1 (MAN, H\*).

Table 2 (continued)

 Table 2
 List of taxa and nrDNA sequences retrieved from GenBank with accession numbers

Taxon	ITS/5.8S	LSU
Abundisporus sclerosetosus	FJ411101	FJ393868
Amaurodon viridis	AM490942	AM490942
Amylocystis lapponica	EU118603	EU118603
Antrodia albida	DQ491414	AY515348
Antrodiella pallescens	FN907921	FN907921
Bjerkandera adusta	DQ060097	AF287848
Boletopsis leucomelaena	DQ484064	DQ154112
Byssomerulius corium	AY463389	AY586640
Ceraceomyces violascens	EU118611	EU118612
Ceriporia viridans	AF347109	AF347109
Ceriporiopsis aneirina	EU340895	EU368503
Cerrena unicolor	FN907915	FN907915
Coriolopsis caperata	AB158316	AB158316
Coriolopsis gallica	AY684172	AY855913
Cryptoporus volvatus		AY858356
Dacryobolus karstenii	EU118624	EU118624
Dentocorticium sulphurellum		AF261539
Diplomitoporus crustulinus	FN907907	FN907907
Diplomitoporus overholtsii		AY333813
Donkioporia expansa	FJ411104	FJ393872
Earliella scabrosa		AY333812
Epithele typhae	DQ486701	DQ457665
Fomes fomentarius		AF311047
Ganoderma adspersum	AM269771	AM269829
Gloeoporus dichrous	EU118627	EU118627
Gloeoporus pannocinctus	AF141612	AF141612
Grammothele fuligo		AJ406506
Grifola frondosa	AY218415	AY218413
Grifola sordulenta	AY854085	AY645050
Hvphoderma definitum	DQ677493	DO677493
Hvphoderma setigerum	FN907905	FN907905
Hvphoderma transiens	DO677504	DO677504
Hvphodermella corrugata	EU118630	EU118630
Hypochnicium polonense	EU118635	EU118635
Ischnoderma benzoinum		AJ406543
Lentinus tigrinus	AY218419	AF518627
Lenzites betulinus	AY463436	AY586683
Lopharia cinerascens	AY463440	AY 586687
Megasporoporia cavernulosa		AY351933
Merulionsis taxicola	EU118648	EU118648
Merulius tremellosus	AF141632	AF141632
Microporellus sp	FI411106	FI393874
Microporus xanthopus	1911100	AY333799
Phanerochaete sordida	EU118653	EU118653
Perenninoria fraxinea	EU110000	EU110000
Perenniporta medulla-panis	FJ411087	FI393875
Perenninoria martius	FI411097	F1393859
Perenniporia ochroleuca	FJ411097	FJ393864
I I I I I I I I I I I I I I I I I I I		22220001

Taxon	ITS/5.8S	LSU
Perenniporia subacida	FJ411103	FJ393880
Perenniporiella chaquenia	FJ411085	FJ393857
Polyporus alveolaris	AB070828	AJ487937
Polyporus brumalis	AF347108	AF347108
Polyporus dictyopus	AF516561	AJ487945
Polyporus tuberaster	AF516596	AJ488116
Postia guttulata	EU118650	EU118650
Pycnoporus cinnabarinus	AY463457	AY586703
Pyrofomes demidoffii	FJ411105	FJ393873
Scopuloides hydnoides	EU118665	EU118665
Skeletocutis amorpha	FN907913	FN907913
Skeletocutis chrysella	FN907916	FN907916
Skeletocutis jelicii	FJ496690	FJ496727
Steccherinum ochraceum	EU118669	EU118670
Trametes elegans	FJ372692	FJ372714
Trametes hirsuta	AB158313	AB158313
Trametes ochracea	AY684177	AY855908
Vuilleminia comedens	AY463482	AY586725

#### DNA and phylogenetic methods

DNA was extracted from herbarium specimens or cultures. DNeasy plant mini kit (Oiagen, Hilden) was used for DNA extraction, following the manufacturer's recommendations. For part of the specimens, standard CTAB-chloroform extraction was used. PCR reactions were carried out using Ready-To-Go PCR beads (Amersham Pharmacia Biotech, Uppsala) or Phusion High-Fidelity DNA Polymerase (Finnzymes). Primers used to amplify ITS region were ITS1F, ITS1, ITS4, ITS4B and LR21 (depending on the specimen), and for partial LSU region LR0R and LR7 (Gardes and Bruns 1993; Hopple and Vilgalys 1999; White et al. 1990). Amplified products were purified using Qiaquick spin columns (Qiagen) or Geneclean Turbo kit (Qbiogene). Primers used for ITS sequencing were ITS1, ITS4 and LR22, and for LSU CTB6, LR5, LR3R, and LR7 (White et al. 1990, Hopple and Vilgalys 1999, http://www. aftol.org, http://plantbio.berkeley.edu/bruns). Sequencing was done by Macrogen (Korea). Sequences were edited and assembled using ChromasPro 1.42 (Technelysium).

The gene regions in the phylogenetic analysis were nrDNA ITS and partial 28S. Limits of ITS1, 5.8S, and ITS2 were determined with ITS extractor (Nilsson et al. 2010). Sequences were aligned using MAFFT 6.708 with strategy G-INS-i (Katoh et al. 2005; Katoh and Toh 2008) and adjusted manually using PhyDE 0.995 (Müller et al. 2008). Out of 78 LSU-sequenced specimens, 5 lacked 5.8S, and another 13 had significantly (>100 bp) shorter

LSU than others. The part of ITS-LSU region used in tree building comprised of about 970 bp of LSU from the start, ca. 80 bp of ITS1 and ca. 70 bp of ITS2. After removing unalignable regions, 1,277 characters remained, of which 436 were variable and 325 parsimony informative. In ITS analysis, the aligned dataset of 30 sequences contained 574 characters, of which 103 were variable and 93 parsimony informative. Genetic distances between ITS sequences were calculated from the manual alignment.

Parsimony analyses were executed in PAUP 4.0b10 (Sinauer Associates, David Swofford) under Windows XP. All transformations were considered unordered and equally weighted. Gaps were treated as missing data. Branches were collapsed if zero length was possible (pset collapse= minbrlen). Heuristic searches used a thousand random taxon addition replicates and tree bisection-reconnection (TBR) branch swapping with other options using the program's default settings. Topology was constrained so that Amaurodon viridis (outgroup), Boletopsis leucomelaena and Vuilleminia comedens were forced together. Relative robustness of clades was estimated through BS analysis using PAUP. Settings used were 1,000 BS replicates with 10 random addition sequences per replicate, TBR branch swapping, and otherwise default settings. For a Bayesian inference of phylogeny, a Bayesian inference run was done with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using eight chains and three runs in parallel, partition to 5.8S and 28S, GTR+I+G substitution model in both partitions, and temp=0.092. Topology was constrained as in parsimony analysis. The analysis was run for 30 million generations, sampling every 3,000 generations. Burnin was set to 2,500 trees. Otherwise default settings were used. ITS analysis used similar settings but without constraints and partitions, was run for 2 million generations sampling every 1,000 generations, and burn-in set to 500 trees. Both analysis were done running eight processors in parallel in CSC-IT Center for Science (http://csc.fi).

#### Morphology

During microscopic studies, the mountant media used were Cotton Blue (CB), Melzer's reagent (IKI), 5% KOH, and Cresyl Blue (CRB, Merck 1280). Measurements and drawings were made in CB. Entry CB+ means cyanophily, CB- acyanophily, and IKI- means neither amyloid nor dextrinoid reaction. Cresyl Blue reaction terms follow Tortić (1976): strong lilac reaction of hyphae, i.e. metachromatic reaction, is marked CRB+; blue reaction (orthochromatic) CRB-. For further references on the use of CB in Aphyllophorales, the reader is referred to Kotlaba and Pouzar (1964), Jülich (1974), and Niemelä (2005).

Measurements were done using  $\times 1,000$  magnification and phase contrast illumination (see Miettinen et al. 2006 for further detail). Pores were measured by subjectively choosing as straight line of pores as possible and measuring how many fit per mm. Sketches were vectorised in CorelDRAW X3. Terms on nuclear behaviour follow Boidin (1971).

#### Results

In Bayesian analysis of both the ITS and ITS+LSU datasets, the separate runs converged well (average SD of split frequencies constantly <0.01). In the case of the ITS dataset, parsimony analysis produced identical trees with Bayesian analysis with similar bootstrap support values. Also, the strict consensus parsimony tree of the ITS+LSU dataset supported all the results discussed below, though partly with low bootstrap support, differing from the Bayesian results only in some deeper nodes irrelevant to this paper. Only the results of Bayesian analysis are shown here.

The *Cinereomyces* clade is very well supported both in Bayesian and parsimony analysis, sitting on a long branch in the phylogram (Fig. 1). However, the position of the *Cinereomyces* clade within Polyporales remains unclear: it does not group either with *Tyromyces* clade (as suggested by Miettinen and Larsson 2010), nor with core polyporoid clade (Binder et al. 2005). This is consistent with results of Tomšovský et al. (2010). Though results are far from conclusive, a closer relationship with the core polyporoid clade seems more likely.

Within Cinereomyces clade, nrDNA analysis (Fig. 1) as well as ITS-based analysis (Fig. 2), show four separate, well-delimited groups (7-12% ITS difference between the groups, 0-4% within). This leaves open two kinds of taxonomic solutions inside the clade: whether to lump everything in one genus (Cinereomyces) or place the species in four different genera. Lumping would result in a morphologically very heterogenous genus, since Cinereomyces lindbladii is dimitic and the rest monomitic, since spore and basidium shapes show large variation (Figs. 3 and 4), and as sexual characters between the genera vary considerably (Table 3). Such a genus would be in practice unusable since it cannot be properly characterised. Thus, we describe below two new genera: Obba to accommodate Ceriporiopsis rivulosa and Ceriporiopsis rivulosa var. valdiviana, and Sebipora for an Indonesian, undescribed species. This solution leaves Cinereomyces and Gelatoporia as monotypic genera for the time being.

ITS sequences of *Gelatoporia subvermispora* indicate that North American (n=3) and Eurasian material (n=7) might not belong to the same species. ITS sequence differences between continents are small, occurring in six nucleotide bases only (1.1%). However, within-continent



◄ Fig. 1 Consensus phylogram of the 22,503 trees retained in the Bayesian analysis of nrDNA ITS and LSU. *Numbers* represent Bayesian posterior probabilities. Branch lengths reflect expected changes per site as indicated by the *scale*. Clade names follow Binder et al. (2005), family names Larsson (2007), and order names Hibbett et al. (2007)

sequences are homogenous in those sites, but consistently different between the two continents. At the very least, it appears that there is a geographic structure within the species, which should be taken into account when strains of *G. subvermispora* are utilised in biotechnological studies. So far, the strains used in biopulping studies derive from North America (e.g. FP-90031, CZ-3, ATCC 90467; Akhtar et al. 1992; Amirta et al. 2006; Atik et al. 2006; Galkin et al. 1998; Harreither et al. 2007). *Gelatoporia subvermispora* has been described from North America, so it is the Eurasian species that would require a new name in case there are two species involved instead of one.

Fig. 2 *Cinereomyces* clade consensus phylogram of the 4,503 trees retained in the Bayesian analysis of nrDNA ITS region. *Numbers* represent Bayesian posterior probabilities. Branch lengths reflect expected changes per site as indicated by the *scale* 

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Similarly in the available sequences of *Obba rivulosa*, the North American material (n=3) differs consistently from Finnish material (n=6) in four bases. The sample and difference are too small for taxonomic conclusions, but this is an indication that North American and North European population may have diverged.

#### Discussion

#### Position of Cinereomyces clade

In their wide sampling of homobasidiomycetes, Binder et al. (2005) used a considerably larger dataset of nuclear rDNA than here, and also utilised mitochondrial rDNA. In that analysis, *Dentocorticium sulphurellum* formed a monophyletic group with the core polyporoid clade, with





Fig. 3 Obba valdiviana, isotype: a hymenial cells and tramal crystals, b spores. Obba rivulosa, Miettinen 8054: c spores. Cinereomyces lindbladii, Kotiranta 19911: d spores. Gelatoporia subvermispora, Kotiranta 20823: e spores, f encrusted hyphal ends in lower trama

a 95% boostrap support. Their full dataset analysis, where most taxa were represented by sequence data comparable with this study, further grouped *Cinereomyces lindbladii* with *D. sulphurellum* and the core polyporoid clade, albeit with no bootstrap support. The placement of *D. sulphurellum* inside the core polyporoid clade also receives support from Wang et al. (2004), utilising nrDNA and rpb2, and Tomšovský et al. (2010), using nrDNA LSU and mt-rDNA SSU. Wang et al. did not include members of *Cinereomyces* clade in their analysis, but Tomšovský et al. (2010) did, and as in our analysis, *Cinereomyces* clade formed a monophyletic group neither with the core polyporoid clade nor with *D. sulphurellum*.

In all these analyses, *Cinereomyces* clade nests within the same part of the tree with the core polyporoid clade and *Tyromyces* clade, even though their mutual positions are unresolved. It appears that nrDNA ITS and LSU alone are insufficient to solve deep nodes properly in Polyporales. A multi-gene study with wide taxon sampling of Polyporales would be needed to resolve those deeper nodes. For the time being we consider *Cinereomyces* clade as a separate entity from the core polyporoid clade Polyporales.

#### Characters of Cinereomyces clade

Species in the *Cinereomyces* clade share a number of characters: resupinate pale-coloured and poroid fruiting bodies, encrustation in trama or tube mouths, medium-sized spores, hyaline or shiny oily substance in trama, CRB+ but CB– hyphae, and cultural characters. In addition, all species except *Sebipora aquosa* have hyphal walls swelling in KOH, all species except *Cinereomyces lindbladii* have partly agglutinated hyphae, and all species have one or two prominent oil drops inside spores except *Gelatoporia subvermispora*. These morphological characters are shared by many polypores, with the possible exception of cultural characters, and fail to differentiate *Cinereomyces* clade as a group apart from other polypore genera. Table 3 compares the main characters of the genera in this group.

Ecologically, the species in this clade are white-rot causing saprotrophs growing in coarse woody debris. Notably, all the species with the exception of *Obba valdiviana* have frequently been found on burned wood, though none are restricted to it.

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#### Mycol Progress (2012) 11:131–147



Fig. 4 Sebipora aquosa. Spores from a holotype, b Miettinen 8680.1 and c Miettinen 9265.1. Holotype, d context, e trama, f tramal encrustation and g hymenial cells

Cultures of *Cinereomyces lindbladii*, *Gelatoporia subvermispora*, *Obba rivulosa* and *O. valdiviana* pertain to group 54 of Nobles' (1958) classification. Group 54 is characterised by taxa that produce in culture broad, simpleseptate marginal hyphae, which give rise to narrower, simple-clamped hyphae that dominate backwards and throughout the mature mycelium. Cultures also give positive extracellular oxidases reactions, grow rapidly (i.e. dishes covered in 1-2 weeks), their simple-septate hyphae become thickened and appear gelatinous, and the mycelium

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	Cinereomyces	Gelatoporia	Obba	Sebipora
Mating system	Tetrapolar	Bipolar	Bipolar (and tetrapolar?)	NA
Nuclear behaviour	Astatocoenocytic	NA	Astatocoenocytic	NA
Annual/perennial	Short-lived perennial	Annual	Annual to biennial	Annual
Hyphal system	Dimitic	Monomitic	Monomitic	Monomitic
Spore shape	Broad allantoid	Allantoid	Subglobose - broadly ellipsoid	Cylindrical
Spore wall	Thin	Thin	Slightly thick-walled	Thin
Cystidioles	Poorly differentiated	Poorly differentiated	Characteristic	Absent
Tramal crystals	Irregular, if present mostly large plates	Fine encrustation, large plates	Rosettes, plates	Fine encrustation, large plates
KOH reaction	Skeletal hyphae swell and slowly dissolve	Hyphal walls swell inwards and gelatinise	Hyphal walls swell inwards and gelatinise	KOH- though walls slightly thicker than in CB, IKI
Distribution	Temperate-boreal, northern hemisphere	Temperate-boreal, northern hemisphere	Temperate-subtropical, northern and southern hemisphere	Tropical Asia

Tab	le 3	C	Comparison	of	genera	in	Cinereomyces	clade
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NA Not available

forms chlamydospores and/or conidia. Nobles' group 54 also included other corticioid and poroid species from the unrelated genera *Aurantiporus, Byssomerulius, Hapalopilus, Ischnoderma*, and *Phlebia*. All taxa that Nobles studied had a bipolar mating system and astatocoenocytic nuclear behaviour with the exception of *Hapalopilus salmonicolor*, which is tetrapolar and heterocytic.

Nakasone (1981) pointed out that cultures of *Obba rivulosa*, *Cinereomyces lindbladii* and *Gelatoporia sub-vermispora* are nearly indistinguishable. For instance, they all produce unique, slender "binding hyphae" in the aerial mat. The species differ in their optimum growth temperature, growth at 40°C, and ability to grow on cabbage extract agar.

Considerable interest has been shown on the biopulping abilities of *Gelatoporia subvermispora* and *Obba rivulosa*. We suggest that other species in the clade might show potential for that purpose, since besides being closely related they also share very similar cultural characters and are aggressive white-rotters in the nature.

#### TAXONOMY

#### Cinereomyces Jülich

Bibliotheca Mycologica 85: 400, 1981

Type species: Cinereomyces lindbladii (Berk.) Jülich

The type species has formerly been included in *Diplomitoporus*. Spirin (2005) included *Skeletocutis lenis* and *S. vulgaris* in *Cinereomyces*, but Miettinen and Larsson (2010) showed them to belong to a new genus in Hymenochaetales, *Sidera*, and concluded that *Ciner*-

*eomyces* should be considered monotypic, i.e. containing only *C. lindbladii*. They also provided a comparison of *Cinereomyces* to *Diplomitoporus*, accompanied with drawings of skeletal hyphae and spores. For cultural characters, Nobles (1958, 1965), Stalpers (1978), Nakasone (1981), and David (1982) provide further detail.

*Cinereomyces* has acyanophilous (CB–) and metachromatic (strongly lilac, CRB+) hyphae. In *Diplomitoporus flavescens*, the type species of *Diplomitoporus*, hyphae are orthochromatic (dark blue, CRB–) and cyanophilous (CB+). *Sidera lenis*, in turn, has less strongly but clearly lilac hyphae in CRB; the hyphae are also acyanophilous.

#### Gelatoporia Niemelä

Karstenia 25: 22, 1985

Type species: Gelatoporia subvermispora (Pilát) Niemelä

Niemelä (1985) gives a detailed description of the type species accompanied with a drawing. For cultural characters, see Nobles (1958, 1965), Nakasone (1981), and Stalpers (1978). We consider this genus currently monotypic. *Gloeoporus pannocinctus* (Romell) J. Erikss., which had been included in *Gelatoporia*, differs from the type species *G. subvermispora* by presenting tetrapolar sexuality and heterocytic nuclear behaviour (David 1972) as opposed to bipolar sexuality (nuclear behaviour unknown). Phylogenetic analysis place it close to *Gloeoporus dichrous* (Fr.) Bres. in the *Byssomerulius* family, phlebioid clade (Binder et al. 2005; Miettinen and Larsson 2010; Tomšovský et al. 2010; Wu et al. 2010). Morphologically, *Gloeoporus* species differ from *Gelatoporia* in their layered fruiting bodies with partly fluffy subiculum contrasting to dense trama, and a cyanophilous reaction of hyphae.

*Gelatoporia subvermispora* seems to have an exceptionally wide distribution, and the current concept as previously discussed may actually contain two species. The Indonesian record, the first from that country, comes from a log of introduced *Pinus merkusii* in a forest plantation about 1 km above sea level.

Obba Miettinen & Rajchenb. gen. nov.

#### MB 519509

*Type species Obba valdiviana* (Rajchenb.) Miettinen & Rajchenb.

*Etymology Obba* (Lat.), a type of household container for liquids used in Rome; refers to the broad bottle-shaped cystidioles characteristic of the genus.

*Description* Basidioma lignicola, resupinatum, pallidum, hymenophorum poroideum. Systema hypharum monomiticum, basidia magna (plus quam  $15 \times 6 \mu m$ ). Cystidiola frequentia, mammiformia vel subulata. Sporae globosae vel subglobosae, gutta olei magna. Putrificatio ligni alba.

**Basidiocarp** resupinate, annual to biennial, white when fresh, cream to ochraceous when dry, a few millimetres up to 13 cm long, up to 3 mm thick. Consistency soft when fresh, drying hard to brittle. Subiculum thin, cartilaginous line when dry.

**Hyphal system** monomitic, clamps present, CB–, IKI–, CRB+. Hyphae mostly thin-walled to slightly thick-walled, mostly 2–3  $\mu$ m in diameter. Coarse crystal rosettes in trama and tube mouths, (2)4–7(9)  $\mu$ m in diameter, also rhomboidal plates. Subicular hyphae often agglutinated. Tramal hyphae interwoven to subparallel, tissue rather dense, upper trama may be agglutinated. In KOH part of hyphal walls swell inwards and gelatinise, resulting in a capillary, distinct lumen. Free oil droplets present in microscopic slides.

#### Cystidia none.

**Hymenium**. Cells large (about  $15-20 \times 6-9 \mu m$ ), with oily contents. Basidia mostly clavate, with 4 sterigmata. Cystidioles subulate to mammiform, common.

**Basidiospores** subglobose to globose, CB(+), plasma stained in CB, with a large hyaline oil droplet, thin- to slightly thick-walled, smooth.

*Distribution* Eurasia, Australia (Tasmania), the Americas, from subtropics to boreal zone.

*Ecology* Associated with white-rot on trunks of gymnosperms and angiosperms.

*Sexuality* Bipolar, possibly also tetrapolar (David 1971; see *O. rivulosa*).

Nuclear behaviour Astatocoenocytic.

*Remarks* Morphologically, the species of *Obba* are similar to *Physisporinus* species with subglobose spores, large hymenial cells and monomitic hyphal structure. For instance, Ryvarden and Gilbertson (1994) and Niemelä (2005) have placed *O. rivulosa* there. Species of *Physisporinus* differ from *Obba* species in having simple septa and showing quite different cultural characters, such as the formation of plectenchymatous areas and/or cuticular cells (Stalpers 1978). Most *Physisporinus* species seem to belong to the *Meripilus* clade and are not related to the *Cinereomyces* clade (Fig. 1).

*Obba* shares a monomitic, clamped hyphal structure with several other polypore genera including *Atraporiella*, *Ceriporiopsis, Erastia, Hapalopilus* and *Raduliporus*. Type species of these genera never show quite the same combination of characters as species of *Obba*: white, thin, resupinate fruiting bodies, large hymenial cells, abundant cystidioles and subglobose, (very) slightly thick-walled spores. DNA data, when available, shows that those genera are not related to the *Cinereomyces* clade (Fig. 1). Microscopically somewhat similar *Aurantiporus* and *Hapalopilus* species are characterised by strongly pigmented and fleshy fruiting bodies, which are pileate in the case of the type species. Species of *Spongipellis* have pileate, large and fleshy fruiting bodies, and clearly more thick-walled and cyanophilous large spores.

Differences and similarities between genera in *Cinereomyces* clade are summarised in Table 3. *Gelatoporia* and *Obba* share a monomitic hyphal structure, which is often partly agglutinated and partly embedded in a gelatinous matrix; rather fine encrustation in trama and particularly on hyphal ends; CB– but CRB+hyphae, similar KOH reaction, and somewhat similar-looking fruiting bodies. Both have a bipolar mating system and similar cultural features (i.e. formation of simple septate hyphae in the growing margin; David 1971; Nakasone 1981; Rajchenberg 1995).

Could *Ceriporiopsis rivulosa* be included in *Gelatoporia* instead of creating a new genus for it? Analysis based on nrDNA does not support that conclusion (Fig. 2). Certain key characters also argue against such an arrangement: narrow allantoid, thin-walled spores (*Gelatoporia*) versus subglobose, slightly thick-walled spores (*Obba*), and size and shape of basidia and cystidioles, which are small in *Gelatoporia* and large in *Obba*. *Gelatoporia subvermispora* possesses slightly but clearly thick-walled hyphae whereas *Obba* species mostly thin-walled. Based on spore characters, Niemelä (1985) refrained from combining *O. rivulosa* in *Gelatoporia*.

*Obba rivulosa* (Berk. & M.A. Curtis) Miettinen & Rajchenb. comb. nov.

MB 519510 Fig. 3c

*Basionym Polyporus rivulosus* Berk. & M.A. Curtis, J Linn Soc Bot 10:318, 1869. Type: Cuba, Wright 154 (lectotype in S! selected here, no F5556, syntypes at least in K, NY!).

Description Basidiocarp annual, resupinate, white when fresh, drying cream to light ochraceous, occasionally with brown stains (type specimen), usually forming small elongated patches a few millimeters to centimeters in width, up to  $13 \times 5$  cm, up to 2 mm thick, according to Lowe (1966) up to 3 mm thick. Consistency soft when fresh, fragile when dry but thick subiculum resinous hard when dry; old fruiting bodies often detaching easily from substrate, young ones firmly attached. Pores rather regular, thin-walled, (rounded) angular, mouths smooth, white when fresh and dry, tubes cream to ochraceous to brownish resinous when dry, American material 3-5/mm (Gilbertson and Ryvarden 1986; Lowe 1966), Finnish material 5-7/mm, tubes 0.4-2.5 mm long. Subiculum a thin line, watery white when fresh, often turns brownish resinous upon drying, 0.1-0.3 mm thick, according to Gilbertson and Ryvarden (1986) up to 1 mm thick. Margin usually distinct, thinning out, without a sterile margin or with a thin (<1 mm) sterile margin, which is watery when fresh.

**Hyphal system** monomitic, CB–, IKI–, CBR+, hyphal walls swell inwards in KOH. Hyphae thin- to slightly thick-walled, rather similar throughout the fruiting body, (1.5) 2.2–3.0(4.2)  $\mu$ m in diameter in subiculum, (1.3)2.2–2.9 (3.4)  $\mu$ m in diameter, in KOH up to 4  $\mu$ m in trama, clamps present, simple septa rare. Crystals always present, common in trama and tube mouths as rather small rhomboidal plates and irregular rosettes. Subiculum usually at least partly agglutinated, hyphae horizontally arranged and parallel to subparallel, sinuous, thin- to slightly thick-walled. Tramal hyphae interwoven to subparallel, in lower trama thin-walled, in upper trama thin- to slightly thick-walled, often partly agglutinated. Subhymenium dense. Free-floating oil drops usually common in microscopic slides.

Cystidia none.

**Hymenium** cells commonly with oily contents, detach easily in KOH. Basidia broad clavate to cylindrical to slightly constricted,  $16-27 \times 6-9.5 \mu m$ , with 4 sterigmata up to 4  $\mu m$  long, with basal clamp. Cystidioles fusiform to mammiform, numerous,  $12-27 \times 4.8-8.8 \mu m$ .

**Basidiospores** subglobose to broad ellipsoid, hyaline, CB(+), IKI-, thin- to slightly thick-walled, smooth, (4.4)  $4.6-5.2(5.5) \times (3.6)3.7-4.3(4.7)$  µm. L=4.89 µm, W=4.00 µm, Q'=1.1-1.3(1.4), Q=1.22, n=120/4. *Distribution* North America, Caribbean, and Eurasia, from subtropical to boreal vegetation zone. The species is evidently more common in North America than in Europe. Robledo (2009) reported it from the subtropical Yungas forest that lies in the eastern slope of the Andes (NW Argentina).

*Ecology* Associated with white-rot on fairly decayed conifer trunks: *Abies, Pinus, Pseudotsuga, Sequoia, Thuja* and *Tsuga*, and, rarely, angiosperms such as *Alnus, Castanea, Populus* and *Quercus*. Host of the lectotype is angiosperm (studied by Tuuli Timonen and Pirkko Harju, H). A relatively high proportion of the collections of this species have been made on charred wood, both in America and Europe (Bernicchia 2005; Kotiranta 1985; Niemelä 2005). In Finland, this otherwise rare species is sometimes common on old forest fire areas (Penttilä and Kotiranta 1996). However, the species is certainly not restricted to charred wood or burned forest. Gilbertson and Ryvarden (1986) report it from living conifers as well.

*Cultural characters* See David (1971), Nakasone (1981), Nobles (1965), and Stalpers (1978). The species is characterised by its ability to grow on cabbage extract agar as opposed to *Cinereomyces lindbladii* and *Gelatoporia subvermispora* (Nakasone 1981).

*Sexuality* Repeatedly recorded as bipolar for North American specimens (Nobles et al. 1957; Nakasone 1981) but tetrapolar for two European materials (David 1971).

Nuclear behaviour Astatocoenocytic (David 1971).

*Remarks* Along with our own data, we have utilised Bernicchia (2005), Dai (1998), Gilbertson and Ryvarden (1986), Kotiranta (1985), Lowe (1966), Melo and Tellería (1992), Niemelä (2005), and Ryvarden and Gilbertson (1994) in writing the macroscopic, distribution and ecology descriptions. Kotiranta (1985) provides a microscopic drawing of *O. rivulosa*.

David (1971) reports that her European cultures differ in their sexuality from North American material (tetrapolar vs bipolar). She also reports considerably larger pore size (2–3/mm) than in Finnish (5–7/mm) or American material (3–5/mm). Difference in sexuality may indicate different species as has been shown for, e.g., several *Antrodia* taxa that, despite being morphologically very similar, represent different biological, non-compatible taxa (David and Déquatre 1984, 1985). On the other hand, David (1971) recorded formation of dikaryons between North American cultures and monosporic European cultures.

ITS and pore size differences indicate that *O. rivulosa* may be a species complex even after the separation of *O. valdiviana* from it. It should be noted that the type specimen

Species/specimen	Length	L	Width	W	Q'	Q	п
Obba rivulosa	(4.4)4.6-5.2(-5.5)	4.89	(3.6)3.7-4.3(-4.7)	4.00	1.1-1.3(-1.4)	1.22	120/4
Lectotype	(4.5)4.6-5.1(-5.2)	4.86	(3.7)3.8-4.1(-4.4)	3.94	1.1-1.3(-1.4)	1.23	30
Larsen 3.X.1973	4.5-5.2(-5.5)	4.84	(3.6)3.7-4.4(-4.5)	3.97	1.1-1.3(-1.4)	1.22	30
Miettinen 8054	4.6-5.2	4.89	(3.7)3.8-4.6(-4.7)	4.11	1.1–1.3	1.19	30
Penttilä 15077	(4.4)4.5-5.5	4.96	3.7-4.3	3.99	1.1–1.4	1.24	30
Obba valdiviana	(4.5)4.6-5.3(-5.6)	4.99	4.2-5.0(-5.2)	4.63	1.0-1.2	1.08	90/3
Isotype	(4.6)4.7-5.2(-5.5)	5.00	(4.3)4.4-5.1(-5.2)	4.72	1.0-1.1	1.06	30
Gates FF503	4.5-5.4	4.79	4.2-5.0(-5.1)	4.48	1.0-1.1	1.07	32
Rajchenberg 4157	4.6-5.2	4.94	(4.2)4.3-5.0	4.66	1.0-1.1	1.06	30
Rajchenberg 11403	(4.5)4.6-5.5(-5.6)	5.02	4.2-4.9(-5.2)	4.52	1.0-1.2	1.11	30
Sebipora aquosa	(5.2)5.7-7.6(-8.0)	6.44	(2.0)2.1-2.7(-2.8)	2.39	(2.2)2.3-3.3(-3.6)	2.70	96/3
Holotype	5.6-7.2(-8.0)	6.29	(2.2)2.3-2.8	2.50	2.2-2.9(-3.0)	2.52	32
Miettinen 9265.1	(5.2)5.8-7.6(-8.0)	6.68	2.0-2.6(-2.7)	2.29	(2.2)2.5-3.6	2.91	32
Miettinen 8680.1	(5.6)5.7–7.6	6.34	2.2–2.8	2.37	2.4-3.0	2.67	32

**Table 4** Spore measurements of Obba rivulosa, O. valdiviana and Sebipora aquosa. Composite statistics of O. valdiviana exclude Tasmanianspecimen, Gates FF503

was collected in subtropical Cuba, whereas the current species concept is based mainly on temperate North American collections. Morphologically, the holotype is fairly similar to northern specimens, and spore size is nearly identical nearly identical (Table 4). Fresh collections from subtropical North America or Caribbean would be needed to clarify species range and limits of the type species.

*Obba valdiviana* (Rajchenb.) Miettinen & Rajchenb. stat. et comb. nov.

#### MB 519511 Fig. 3a-b

*Basionym Ceriporiopsis rivulosa* (Berk. & M.A. Curtis) Gilb. & Ryvarden var. *valdiviana* Rajchenb., Bol Soc Argent Bot 30:153, 1995. Holotype: Argentina, Chubut, Futaleufú, Los Alerces National Park, 15.III.1993 Rajchenberg 10736 (BAFC 33238, isotype CIEFAP!).

Description **Basidiocarp** annual to biennial, resupinate, snow-white, initially forming small, circular to elongated bodies up to  $13 \times 9$  cm, but fusing and often covering large surfaces. Consistency hygrophanous and fleshy when fresh, hard and waxy to bony and detaching from substrate upon drying, yet easy to cut with a razor blade. Pore mouths felty and white, often resinous in cross section, 6–8/mm, tubes 0.5-5 mm long, up to 11 mm in Tasmanian material. Subiculum white when fresh and brown cartilaginous line when dry, 0.1-0.3(0.5) mm. Sterile margin distinct or not, up to 1 mm wide, felty, slightly separated from substrate but always well attched to it. **Hyphal system** monomitic, CB–, IKI–, CRB+, hyphal walls swell inwards in KOH. Hyphae thin- to slightly thick-walled, (1.5)2.0-3.7(4.2) µm in diameter in subiculum, (1.5)2.0-2.7(3.7) µm in trama, clamps present. Irregular crystal rosettes common in trama and tube mouths, typically 5–7 µm in diameter, in upper trama also some rhomboidal plate-spresent, up to 20 µm long. Subiculum partly to fully agglutinated, hyphae mostly horizontally arranged and subparallel, in agglutinated parts sinuous and difficult to tell and break apart, partly covered with slime: tissue rather dense, hyphae intervowen to subparallel, thin-walled at least in lower trama. Subhymenium tightly arranged, hyphae intervoven. Small oil droplets floating around in microscopic slides.

#### Cystidia none.

**Hymenium**. Cells commonly with oil droplets. Basidia broad clavate to rarely constricted cylindrical,  $16.5-22 \times 6.5-9$  µm, with 4 sterigmata. Cystidioles fusiform to mammiform, common,  $13-20 \times 6-8.2$  µm.

**Basidiospores** globose to subglobose, hyaline, CB(+), IKI–, with thin to slightly thickened walls, smooth, (4.5)  $4.6-5.3(5.6)\times4.2-5.0(5.2)$  µm, L=4.99 µm, W=4.63 µm, Q'=1.0–1.2, Q=1.08, *n*=90/3 based on the Argentinian material, with a prominent hyaline, oily-like guttule in the othewise cyanophilous cytoplasm.

*Distribution* Temperate southern Argentina and Chile. Two recent finds have been made in Tasmania.

*Ecology* Associated with white pocket rot on strongly decayed trunks of *Nothofagus dombeyi* and undetermined broad-leaved tree species. In Tasmania, it has been collected from *Eucalyptus* sp. logs over 90 cm in diameter.



**Fig. 5** Size of individual spores of *O. rivulosa* ( $\circ$ ) and *Obba valdiviana* (+; black Argentinian, blue/grey Tasmanian). The points have been jittered, i.e., moved randomly within 0.05  $\mu$ m around the original value along both axes

*Cultural features* See Rajchenberg (1995) for a complete description. In brief, the mycelium is characterised by a rapid growth, with generative hyphae being simple-septate in the margin but becoming clamped backwards. Microscopically, they form thick-walled generative hyphae, narrow, capilliform, much branched hyphae forming a net-like cluster and chlamydospores, described as "binding hyphae" by Nakasone (1981).

Sexuality Unknown.

#### Nuclear behaviour Astatocoenocytic.

*Remarks Obba rivulosa* differs from *O. valdiviana* by larger pores and narrower, broadly ellipsoid basidiospores (Figs. 3 and 5, Table 4). Known distributions of the species do not overlap.

Preliminary di×mono compatibility tests (Hallenberg 1984) performed between the polyspermic culture obtained from *O. valdiviana* holotype (CIEFAP culture collection

159) and 4 monosporous cultures of *O. rivulosa* from USA (FP 133035) gave negative results (Rajchenberg 1995). Nakasone (1981) described the strain derived from FP 133035 (USA, Oregon, Mary's Peak, on *Tsuga*, 8. XI.1971 MJ Larsen) in her culture study of *O. rivulosa*, and we have studied fruiting bodies collected from the same area for this study.

The Tasmanian material differs from the Argentinian material genetically (ITS distance 1.3%). No clear morphological character separate the two (Fig. 5). Further sampling and closer study are needed to determine whether the two are separate species.

Sebipora Miettinen gen. nov.

MB 519512

Type species: Sebipora aquosa Miettinen sp. nov.

MB 519513

Figs. 4 and 6

*Holotype* Indonesia, Sumatra, Riau, Rokan Hilir, Desa Sungai Maju, 2°0.790'N, 100°40.546'E, under 40 m above sea level, slash and burn site in a peat swamp forest area, on a partially buried piece of wood (15 cm in diameter, decay stage 2/5), 8.VII.2004 Miettinen 8868 (ANDA, isotype H).

*Etymology Sebum* (Lat.), refers to the tallow look of pore surface of the type species, *aquosus* (Lat.), watery, refers to fresh fruiting bodies of the species.

*Description* Basidioma lignicola, resupinatum vel pileatum, dilute ochraceum vel pallidum, hymenophorum poroideum, porae 4–6 per mm. Systema hypharum monomiticum,



Fig. 6 Fresh fruiting body of Sebipora aquosa, Miettinen 12032

hyphae tenuitunicatae vel leviter crassitunicatae, CB–, KOH–, IKI -, CRB+, 2.6–4.6  $\mu$ m in diametro. Trama inferiore crusta arenoidea vel fasciculis spinosis crystallorum parvis tectum. Sporae cylindricae, leviter curvata, 1–2 guttis olei magnis, 5.7–7.6×2.1–2.7  $\mu$ mVB.

**Basidiocarp** resupinate to pileate, annual, pure white when fresh, upper surface hairless, azonate, in dry specimens shiny cream with greyish, yellowish and brownish hues, pore surface turning greyish, sometimes with yellow or brown hues,  $2-10 \times 1-5$  cm wide, caps projecting up to 3 cm, 1-9 mm thick, always broadly attached. Consistency rubber-like but easy to tear apart when fresh, often watery, hard when dry. Pores rather regular, rounded angular, rather thin-walled, tubes white to cream in cross section when dry, 4-6/mm, 1-4 mm long. Subiculum white, homogenous, subiculum of the resupinate part 0.1-0.4 mm, cap context 1-6 mm thick. Cap margin sharp, resupinate margin thinning out but well delineated. Mycelial cords absent.

Hyphal system monomitic, CB-, IKI-, KOH-, CRB+. Hyphae rather homogenous throughout the fruiting body, (2.0)2.7-3.9(4.8) µm in diameter in subiculum, (2.2)2.6-3.7(5.0) µm in trama, clamps always present. Crystals present as sandy encrustation in tube mouths and trama or small thorny crystal clusters attached to tramal hyphae, scanty to abundant; sometimes also large rhomboidal plates and irregular crystal clusters in subiculum and upper trama. Subicular hyphae interwoven, slightly thick-walled to thickwalled, lumen very distinct and usually at least half of the total width, walls 0.3-1.7 µm thick. Contextual hyphae subparallel, horizontal, slightly thick-walled to thickwalled, relatively loose, (2.7)3.2-4.6(5.8) µm in diameter. Upper surface consists of partly agglutinated layer of interwoven hyphae. Tramal hyphae subparallel, slightly thick-walled, tissue dense, partly agglutinated. Subhymenial hyphae thin-to slightly thick-walled. Free-floating, hyaline oil in microscopic slides present, scanty to abundant.

Cystidia absent.

**Hymenium**. Basidia clavate,  $11-20.5 \times 4.8-6.5 \mu m$ , with 4 sterigmata, 2.2–3  $\mu m$  long. Cystidioles absent.

**Basidiospores** cylindrical, often slightly curved, thinwalled, smooth,  $(5.2)5.7-7.6(8.0) \times (2.0)2.1-2.7(2.8)$  µm, L=6.44 µm, W=2.39 µm, Q'=(2.2)2.3-3.3(3.6), Q=2.7, n=96/3, with 1–2 oil drops, apiculus inconspicuous.

*Distribution* Low altitudes in Sumatra and New Guinea. Probably not rare.

*Ecology* Found in half-open secondary rainforest as well as open yard on a burned tree trunk. Subtrate was in all cases a fallen angiosperm tree trunk over 15 cm in diameter.

*Remarks* Table 3 compares *Sebipora* with the other genera in *Cinereomyces* clade.

In the traditional genus system of polypores, pileate specimens of *Sebipora aquosa* would have been placed in *Tyromyces*, a dumping place for monomitic white-rot species with thin-walled spores. All legitimate names of *Ceriporiopsis* and *Tyromyces* registered in MycoBank as of August 1, 2010 were checked against *S. aquosa*, as well as all species described by E.J.H. Corner, following the interpretion of Hattori (2001a, b, 2002, 2003a, b) when available. No match was found.

In the key of monomitic *Tyromyces* species in Malesia by Corner (1992), *T. pinguis* comes closest. According to the protologue, the species has a "fatty vitrous appearance" and similar-sized spores. Hattori (2003a) studied the type of the species, which is in poor condition, and referred it to *Ceriporia* as it has simple septa. *Tyromyces levis* Corner from the Solomon Islands has rather similar spores and is half-resupinate, but its spores are larger, caps have a tomentose surface, and basidia are 2-sterigmate (Hattori 2003a).

The genus *Tyromyces* sensu stricto, typified by *T. chioneus*, is somewhat similar microscopically: occasional encrustation on hyphae that are CB– and CRB+, cylindrical spores, and oily substance. However, fruiting bodies of *T. chioneus* turn clearly dimitic when aging, and even when young its subicular hyphae have characteristic thick-walled, finger-like projections also present for instance in related *T. canadensis* (Niemelä 1985), but which are lacking in *Sebipora*. Phylogenetically *Tyromyces* s. str. is not closely related to the *Cinereomyces* clade (Fig. 1).

Other monomitic white-rot genera with resupinate fruiting bodies include *Atraporiella*, *Ceriporiopsis*, *Raduliporus*, *Pouzaroporia* and *Sidera*. In the strict sense, none of them are closely related to the *Cinereomyces* clade (Fig. 1; Miettinen and Larsson 2010; Tomšovský et al. 2010). All the above mentioned genera in the strict sense include only the type species or a few monomitic species, so comparison of the genera is essentially a comparison of the type species. Considering spore shape and hyphal structure, morphologically the closest generic type is *Ceriporiopsis gilvescens* with a monomitic, dense hyphal structure with oily substance and cylindrical spores. Its spores are curved, smaller and aguttulate, no encrustation is present, and its fruiting bodies are strictly effused and bright coloured.

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