# Reassessment of phylogenetic relationships of some lentinoid fungi with velutinate basidiomes based on partial 28S ribosomal RNA gene sequencing

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Lentinoid fungi characterised by villosity of pileus and/or stem mostly belong to the genera Lentinus and Panus. However, morphology-based taxonomy of some of these species has remained controversial and they used to be classified back and forth between the two genera. The aim of this study was to investigate the phylogenetic relationships of these velutinate lentinoid fungi based on partial sequences of nuclear large subunit (nucLSU) rDNA. Fourteen sequences generated from Cameroon specimens out of which eleven sequences of velutinate collections were included in the dataset of taxa belonging to the polyporoid clade; their phylogeny was obtained by Bayesian, maximum parsimony, and likelihood analyses. In all trees a clade comprising all *Lentinus* taxa was resolved, whereas taxa referred to as *Panus* spp. formed a strongly supported monophyletic group. Species with velutinate to tomentose basidiomes were distributed over both clades. Those with pilose-strigose basidiomes and corresponding to *Lentinus* subsect. Criniti grouped together in the Lentinus clade. Contrarily, species with velutinate to strigose basidiomes and thickwalled skeletocystidia, which are considered belonging to *Lentinus* sect. *Velutini*, nested in the Panus clade, but were nonmonophyletic. In this latter clade, the P. velutinus complex, including taxa with velutinate to hispid-strigose basidiomes and long-slender stems, was moderately supported. The results indicated that the combination of uninflated vegetative hyphae, unbranched skeletal hyphae, and a radiate hymenophoral trama type is phylogenetically supported and characterises those species in the Panus clade.

Keywords: lamellate Polyporales, Lentinus, Panus, hyphal pegs, nucLSU phylogeny, ribosomal RNA

Lentinoid fungi are substantially tropical, forming a seemingly variety of white-spored and lamellate basidiomata, but without any phylogenetic connection to Agaricales (Corner 1981, Pegler 1972, 1983). In spite of morphological and anatomical similarities, lentinoid fungi do not represent a natural group (Table 1). Ecologically, they are of importance for decomposition processes in forest ecosystems, being

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saprobes on dead hard wood with capacity to perform two types of wood decay: most of them cause white rots, but members of *Neolentinus* Redhead and Ginns and *Heliocybe* Redhead and Ginns cause brown-rots (Redhead and Ginns 1985; Thorn *et al.* 2000). Classification of lentinoid taxa based on molecular phylogenetic analyses revealed that they are phylogenetically diverse and strongly polyphyletic (Corner 1981, Pegler 1983, Singer 1986, Hibbett and Vilgalys 1993, Hibbett and Thorn 1994, Hibbett and Donoghue 1995, Thorn *et al.* 2000). They were placed in various clades with *Lentinellus* P. Karst. belonging to Russulales, *Heliocybe* and *Neolentinus* Closely related to Gloeophyllales, while *Faerberia* Pouzar, *Lentinus* Fr., and *Panus* Fr. were shown to belong to the Polyporales. Phylogenetic studies in support of ecological characteristics and also, to some extend, the phenotypic variation in lentinoid fungi, afforded thereby the improvement of generic classification.

Although comprehensive morphological investigations (Pegler 1972, 1975, 1983; Corner 1981) provided a better insight into the taxonomic structure of some genera, the situation remains blurred for some taxa in the genera Lentinus and Panus. Many synonyms in different combinations arose in Lentinus, Neolentinus, and Panus (Redhead and Ginns 1985, May and Wood 1995). In addition to Corner's and Pegler's contributions, other studies on lamellate fungi increased the taxonomic confusion around Lentinus and Panus (Kühner 1980, Singer 1986). All these morphological works resulted in two different approaches of classification. The Corner-Pegler classification (Corner 1981, Pegler 1983) privileged hyphal construction, whereas the Singer-Kühner treatment (Kühner 1980, Singer 1986) mostly emphasized the hymenophoral trama anatomy. Hibbett et al. (1993a, 1993b) found that the ontogeny of basidiomata and early morphology of hymenophore differ between Lentinus and Panus. These findings substantiated the view that lamellae in these genera result from convergent evolution. This lent further support to results from molecular analyses. Ontogenic features, however, can be barely applied in studying herbarium materials, and are thus of limited value for the taxonomy of Lentinus and Panus at species level. We have therefore used the Corner-Pegler approach for morphological examination of lentinoid collections included in this study.

We have initiated this study while studying collections of lentinoid fungi from Cameroon, with the result that some taxa with velutinate basidiomes assigned by Corner (1981) and Pegler (1983) to *Lentinus* belong to *Panus*. The main objective was to reassess the phylogenetic relationships and distribution of lentinoid fungi with velutinate basidiomes within the clades of *Lentinus* and *Panus*. To address this question, we sequenced a portion of nuclear large subunit rDNA of lentinoid collections from a three years survey in the Mbalmayo Forest Reserve (MFR), Cameroon, and retrieved previously published nucLSU sequences of selected members of the polyporoid clade from GenBank. DNA sequence data was analysed using Bayesian, maximum parsimony, and likelihood methods.

### **Materials and Methods**

Sampling and morphology

Specimens of lentinoid fungi used for sequencing in this study are listed in Table 2. Nomenclature follows that used by Corner (1981), Kühner (1980), Pegler (1983), and Singer (1986). Microscopic features were studied from free-hand thin sections mounted in 5 % KOH, and observed at  $1000 \times$  using an Olympus BX51TF microscope with bright field and phase contrast optics. Herbaria are abbreviated according to Holmgren et *al.* (1990).

Tab.	1.	_	Relevant	morphological	characters	differentiating	members	of	lentinoid
gene	ra.								

		White r	Brown rot			
Characters	Russulales	Po	olyporales	Gloeophyllales		
	Lentinellus	Lentinus	Panus	Faerberia	Heliocybe	Neolentinus
Pileus villose-hispid to squamulose	-	(+)	(+)	-	-	-
Pileus glabrous to scurfy	+	(+)	(+)	+	+	+
Cantharelloid form	-	-	-	+	-	-
Metuloid cystidia	-	(+)	(+)	-	-	-
Skeletoligative hyphae	-	+	-	-	-	-
Hyphal pegs	-	(+)	-	-	-	-
Clamp connections	+	+	+	+	-	+
Hymenophoral trama construction	descending	descending radiate	radiate	radiate	descending	descending

+ Character of almost all species, (+) character of many species, - character absent

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted as described in Douanla-Meli et al. (2005). Primers LR0R and LR5 (Vilgalys and Hester 1990) were used to amplify a portion of the nucLSU rDNA. PCR reactions were performed in 50 µL volume on a TGradient Thermocycler 96 (Biometra). The cycling parameters were an initial denaturation at 94 °C for 3 min, followed by 35 cycles consisting of 94 °C, 55 °C, and 72 °C for 30 s, 45 s, and 60 s, respectively, and a final extension step of 7 min at 72 °C. PCR products were purified with the QIAquick<sup>™</sup> PCR Purification Kit (QIAGEN) following the manufacturer's instructions. Amplification products were subsequently sequenced in both directions with PCR primers using the ABI PRISM<sup>™</sup> BigDye Terminator Cycle Se-

Species	Voucher	GenBank accession numbers
Lentinus squarrosulus Mont.	DMC 175	EU908177
L. squarrosulus Mont.	DMC 176	EU908176
L. squarrosulus Mont.	DMC 178	EU908178
Panus velutinus (Berk.) Pegler & R.W. Rayner (= L. velutinus Fr.)	DMC 683	EU908185
P. velutinus (Berk.) Pegler & R.W. Rayner (= L. velutinus Fr.)	DMC 694	EU908187
P. velutinus (Berk.) Pegler & R.W. Rayner (= L. velutinus Fr.)	DMC 695	EU908188
P. velutinus (Berk.) Pegler & R.W. Rayner (= L. velutinus Fr.)	DMC 734b	EU908186
P. velutinus (Berk.) Pegler & R.W. Rayner (= L. velutinus Fr.)	NAL318	GQ487335
P. velutinus var. glabrior Corner	DMC 174	EU908183
P. velutinus var. glabrior Corner	DMC 188	EU908184
P. similis (Berk. & Broome) T.W. May & A.E. Wood	DMC 189	EU908182
P. fasciatus (Berk.) Singer (= L. fasciatus Berk.)	DMC 184	EU908180
P. cf. fasciatus	DMC 696	EU908181
Panus sp.	DMC 182	EU908179

**Tab. 2.** – Specimens examined and sequenced by the authors in this study, vouchers, and GenBank accession numbers.

quencing Kit, Version 3.1 (Applied Biosystems), and analysed using an automated DNA sequencer ABI 3100. Contiguous nucleotide sequences were edited and assembled using SeqEd v1.0.3 (Applied Biosystems). GenBank accession numbers of new sequences are provided in Table 2. For additional sequences of lentinoid fungi and taxa of polyporoid clade from GenBank, accession numbers are given in the phylogenetic tree. *Geastrum saccatum* Fr., *Gomphus floccosus* (Schwein.) Singer, and *Sphaerobolus stellatus* Tode from the gomphoid-phalloid clade were selected as outgroup taxa.

#### Alignment and phylogenetic analyses

Sequences were aligned with ClustalX (Thompson *et al.*, 1997) and manually optimized in Se-Al 2.0a11 (Rambaut 2002). The nucLSU dataset was analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian MCMC inference (BI). MP analyses were implemented in the program PAUP\* 4.0b10 (Swofford 2002) and used characters defined as unordered, unweighted or reweighted according to the consistency index (CI) value. Gaps were treated as missing data. A heuristic search was performed with the following settings: 1000 random stepwise addition sequences, TBR branch swapping algorithm, MulTress and steepest descent in effect, retaining 100 trees after each replicate. Consistency indices (CI) and retention indices (RI) (Farris, 1989) were calculated to evaluate the amount of homoplasy in the dataset. For assessing character support, 1000 bootstrap replicates (Felsenstein 1985) were performed with stepwise random addition sequences, holding 10 trees at each step and retaining groups with frequency  $\ge 60$  %. Different substitution models were compared via the Akaike information criterion (AIC) with PAUP\* and MrModeltest 2.2 (Nylander 2004) to select the best-fit model of the dataset for BI and ML analyses. BI analysis was done in MrBayes 3.0b4 (Ronquist and Huelsenbeck 2003) using a metropolis-coupled Markov chain Monte Carlo (MC3) algorithm (Larget and Simon 1999). Six independent runs of 2 million generations were completed with six chains each. The Markov chains started from a random tree sampling every 100th generation. The log-likelihood scores of sample points were plotted against the generation time, and 25 % of trees sampled before reaching stationarity were discarded as burn-in. Bayesian posterior probabilities (BPP) and 50 % majority consensus tree were inferred from trees sampled after reaching likelihood convergence using the "sumt" command in MrBayes 3.0b4. ML analyses were conducted using the program RAxML v. 7.0.4 (Stamatakis et al. 2008). A tree was obtained by simultaneously running a fast bootstrap search of 100 pseudoreplicates followed by a search for the most likely tree. ML bootstrap values (MLBP)  $\geq 60$  % are given at the nodes (Fig. 1).

## Results

#### Phylogenetic analyses

Fourteen new sequences were generated in this study that varied from 909 to 958 bp. The nucLSU rDNA dataset included 81 sequences and the final alignments consisted of a total of 900 characters, 284 characters were variable, out of which 185 characters were parsimony informative. Unweighted parsimony analyses resulted in 390 900 equally most parsimonious trees (MPTs) on one island (length 1036 steps, CI = 0.375, RI = 0.714). All the MPTs were similar in topology and major groupings within the clades, except slight variation in the resolution of terminal nodes in *P. velutinus* clade. After reweighting the characters based on CI, intended to reduce the number of MPTs, weighted parsimony analyses resulted in the same number of 390 900 MPTs on one island (length 392.45 steps, CI = 0.575, RI = 0.753). The unweighted and weighted tree files compared in MacClade 4.05 (Maddison and Maddison 2001) revealed no consistent differences. Both unweighted and weighted analyses were concordant in the groupings and phylogenetic placement of Lentinus and Panus taxa, and have identical consensus trees (not shown). Weighted trees, however, were stronger supported by bootstrapping than unweighted trees.

Using MrModeltest 2.2 under AIC, the model estimated for BI and ML analyses was General Time Reversible model with gamma distributed substitution rates and assuming a portion of invariable sites (GTR+I+G). Parameters were estimated as followed: base frequencies

freqA = 0.2677, freqC = 0.1862, freqG = 0.2784, freqT = 0.2676; substitution rates R(a) [A-C] = 0.4340, R(b) [A-G] = 2.5630, R(c) [A-T] =1.1047, R(d) [C-G] = 0.4253, R(e) [C-T] = 6.9220, R(f) [G-T] = 1.0000;gamma shape parameter = 0.6380; proportion of invariable sites (pinvar) = 0.4809. The RAxML search yielded a most likely tree (not shown) with a log-likelihood -6454.208631. The matrix had 379 distinct alignment patterns, with 23 % of gaps or completely undetermined characters in the alignment. BI and ML trees were identical with main groups congruent to those present in the MP trees. In the Bayesian 50 % majority rule consensus tree presented in Fig. 1, the resolution of internal nodes varied in parts, but no conflict with consensus trees of MP analyses was observed. Overall topology of the trees corresponded closely to currently circumscribed polyporoid clades (Moncalvo et al. 2002, Binder et al. 2005). Regarding relationships in lentinoid fungi, the phylogenetic trees were also consistent with other molecular studies (Hibbett and Vilgalys 1993, Thorn et al. 2000) showing the separation of *Lentinus* and *Panus* in different clades within the polyporoid clade. The ingroup taxa were resolved into two large groups containing the Panus clade and the core Lentinus clade or Lentinus sensu stricto. The Panus clade (1.00 BPP, 85 % BSS, 85 % MLBP) was further divided into two, but poorly supported subclades. One subclade corresponded to P. velutinus complex that showed different clusters of P. fulvus, and P. similis sequences. Two sequences representing P. velutinus var. glabrior were closely related to P. similis but did not group together. Three species P. conchatus, P. strigellus and Panus sp. were resolved in a polytomy basal to this subclade. The second subclade included the moderately (0.95 BPP, 72 % BSS) supported group of *P. ciliatus* and *P.* fasciatus and the strongly supported (1.00 BPP, 92 % BSS) group of *P*. lecomtei and P. rudis. Sequences of Lentinus formed a polyphyletic group with other poroid taxa. Sequences of *Lentinus* taxa *L. bertieri*, L. crinitus, L. swartzii, which have pilose-strigose basidiomes with ciliate margins, grouped together with those of L. scleropus and L. sajor-caju.

#### Discussion

This study reports on the DNA phylogeny of lentinoid fungi with velutinate-strigose basidiomes belonging to *Lentinus* and *Panus*. Our sequence analysis like previous studies (Hibbett and Vilgalys 1993, Hibbett and Donoghue 1995, Hibbett *et al.* 1993, Moncalvo *et al.* 2000, 2002; Thorn *et al.* 2000) indicated that *Lentinus* and *Panus* constitute two different evolutionary lineages inside the polyporoid clade. In congruence to our study, *Panus* was closely related to the Meruliaceae taxa (Moncalvo *et al.* 2002, Binder *et al.* 2005) whereas *Lentinus* was used to be placed in a /polyporaceae clade (Moncalvo *et al.* 2002) or in a core polyporoid clade (Binder *et al.* 2005) and forms a polyphyletic



**Fig. 1.** – Bayesian rDNA phylogeny of velutinate to hispid-strigose lentinoid fungi based on nucLSU sequences. The tree shown is the 50 % majority-rule consensus tree from analysis with GTR + I + G, analysis searched for 2 million generations using 6 chains of MC<sup>3</sup> algorithm and sampling every 100<sup>th</sup> generation. Bayesian probabilities values (BPP) are indicated on the branches. Bootstrap values (BSS)  $\geq$  60 % from full heuristic bootstrap analyses performed using 1000 replicates and maximum likelihood bootstrap values (MLBP)  $\geq$  60 % calculated using RAxML are also indicated (see legend). Taxon labels in bold type indicate lentinoid taxa with velutinate to hispid-strigose basidiomes. Taxon name followed by exclamation point is dubious. The tree was rooted to *Geastrum saccatum, Gomphus floccosus*, and *Sphaerobolus stellatus*.

group (Hibbett and Vilgalys 1993). Given the many common morphological characters (e.g., radiate hymenophore, velutinate to hispidstrigose pileus and stem) characterising *Lentinus* and *Panus*, generic morphological classification of species in *Lentinus* sect. *Velutini* Pegler (Pegler 1983) has remained equivocal. The results of the present study help to solve the confusion occurring in morphology-anatomy-based taxonomy (Corner 1981, Pegler 1972, 1975, 1983). All the taxa having velutinate to hispid-strigose pileus and/or stem, uninflated generative hyphae, hymenophoral trama of radiate construction, and context consisting of unbranched skeletal hyphae, were included in the *Panus* clade. These characters accounted for the circumscription of *Lentinus* sect. *Velutini* (Pegler 1983).



Figs. 2–7. Morphology. 2, 3, Young and mature basidiomes of *Panus* cf. *fasciatus* (DMC 696). 4. Panus velutinus (DMC 694) with gelatinous hymenial layer. Figs. 5, 6, 7. Microscopic features. 5. *Panus* cf. *fasciatus* (DMC 696). 6. *Panus fasciatus* (DMC 184). 7. *Panus velutinus* (DMC 694). a. Section in hymenial layers. b. Cheilocystidia. c. Sclerocystidia. d. Skeletal hyphae. e. Tip of pileal or stem hair. f. Basidiospores. g. Basidia. Bars = 1 cm for 2–4, 5 µm for 5, and 10 µm for 6–7.

With recognition of *Panus* at generic rank (May and Wood 1995) in agreement with phylogenetic and ontogenic studies (Hibbett and Vilgalys 1993, Hibbett *et al.* 1993a), four species (*L. ciliatus* Lév., *L. hookerianus* Berk., *L. similis*, and *L. tephroleucus* Mont.) of the six in *Lentinus* sect. *Velutini* were synonymised with *Panus*. *Lentinus velutinus* first was combined into *P. velutinus* (Fries 1838). Pegler and Rayner (1969) proposed the new combination *P. fulvus* based on the synonym *L. fulvus* Berk. Later on, Corner (1981) described from Malaysia three further varieties, var. *fenestratus*, var. *glabrior*, and var. *nudicollum*, of *P. fulvus* based on pileal surface structure and gill spacing. Pegler (1983), however, rehabilitated *L. velutinus*, which is currently accepted (http://www.indexfungorum.org). According to the description given by Corner (1981) for P. fulvus and Pegler (1972, 1983) for L. velutinus, and our observations from the total of eight Cameroon collections, all these collections refer to the same species with skeletal hyphae unbranched or often with 1-3 short abortive processes, but never with binding processes (ligative hyphae) as typical for *Lentinus* species. This feature combined to the radiate hymenophoral trama (Hibbett et al., 1993a) is decisive for assigning this species to Panus, a placement largely supported by molecular phylogenetic analyses. The epithet 'velutinus' is retained as the oldest one and has priority against 'fulvus'. In the Panus clade, all trees resolved a P. velutinus group, the members of which are morphologically characterised by a slender stem, usually much longer (up to 25 cm long) than the pileus diameter, and arising from a pseudosclerotium. Sequences of *P. velutinus* were almost identical, except GQ487335 of the collection NAL318, which formed a long branch and presented 1 % base differences in pair sequences comparison with all other P. velutinus sequences. Morphological support to the phylogenetic resolution of this collection, however, is too weak and pertains only to the almost darker, chocolate brown colored, tortuous and deeply buried (of half-length) stem. Within the *P. velutinus* complex, all sequences included and representing P. similis did not group together. In all the analyses, the GenBank sequence AY615996 of the collection TENN58776 named P. fulvus rather grouped together with other three GenBank sequences of P. similis than with P. velutinus. Knowing that P. similis recently was named P. fulvus var. similis (Berk. and Broome) Corner; the collection TENN58776 possibly represents this variety. The voucher deposited in the Knoxville herbarium must be restudied to solve this problem. Morphologically, P. similis represents the most distinctive species in the *P. velutinus* complex, being easily recognised by a finely velutinate to glabrescent, radially sulcato-striate pileus with prominent striae and subdistant lamellulae.

Amongst collections of *P. velutinus*, DMC 694 (EU908187) matches well the gross morphology of *P. fulvus* var. *fulvus* (Berk.) Pegler and R.W. Rayner (Pegler and Rayner 1969, Corner 1981), but presents slight deviation. Its basidiomes have translucent pilei and were not found associated with pseudosclerotium as usual in *P. velutinus*. Moreover, the hymenial layers are firmly enclosed in resinous cement (Fig. 7a), a character previously neither recorded in *Lentinus* nor in *Panus*. Also, the basidiospores are  $6.5-7(-8) \times 3-3.5$  µm bigger and often retained embedded in this resinous matrix. NucLSU sequence of DMC 694 (EU908187) showed no pair base difference in comparison with other sequences in *P. velutinus*. Until other such specimens are collected for further investigations, we consider the above morphological character differences as potential geographical deviation of Cameroon material. Likewise, two other collections in the *P. velutinus* complex,

DMC 174 (EU908183) and DMC 188 (EU908184), macroscopically match the diagnostic features of *P. fulvus* var. glabrior. This variety was proposed by Corner (1981) from Malay Peninsula as a small P. fulvus var. fulvus, characterised by apparently glabrous pileal surface and more or less distant gills. Following the suggested name change of P. fulvus, this variety must be named P. velutinus var. glabrior and this change should be extended to the other two varieties var. *fenestratus*, and var. nudicollum. Macroscopically, P. velutinus var. glabrior further differs from *P. velutinus* in having a slightly shorter, more slender stem, often curved at base. According to our observations, P. velutinus var. glabrior preferentially grows on unburied, tiny twigs or bark of logs, and rather forms a small pseudosclerotium, sometimes also phoenicoid, thus growing on burnt woody material. Contrarily, P. velutinus forms a massive pseudosclerotium on the ground or bigger substrate often buried in the ground. In the phylogeny of nucLSU rDNA sequences, P. velutinus var. glabrior was well separated from P. velutinus. However, its two sequences failed to group together and the results revealed a close relationship between DMC 174 and P. similis (DMC 189). The obvious morphological similarity between P. similis and P. velutinus var. glabrior is only the radially striate to sulcatostriate pileus. A thorough comparative analysis of P. velutinus, P. velutinus var. glabrior, and P. similis was not possible with the taxon sampling of this study. Comprehensive morphological and molecular analyses including more East Asian specimens may help clarify the taxonomic situation and relationships in species of the P. velutinus complex.

Lentinus fasciatus, another species of Lentinus sect. Velutini, initially was transferred to Panus by Singer (962), a recombination confirmed by Pegler (1965) based on examination of type material. Although the name *L. fasciatus* has been later rehabilitated (Pegler 1983); our phylogeny indicated that this species belongs to Panus. Panus fas*ciatus* rather clustered with *P. ciliatus* and their separation from the *P*. *velutinus* group is congruent with basidiome morphology. Members of the *P. velutinus* complex usually have slender stems, up to two times longer than pileus diameter, whereas stems in P. ciliatus and P. fasciatus are only sometimes slender, but often short and stocky. Despite slight morphological differences, the collection DMC 696 (Panus cf. fasciatus, EU908181) grouped with P. fasciatus on a strongly supported branch. DMC 696 is, moreover, distinguished from all Panus with velutinate basidiomes in having smaller basidiomes of violet to reddish tints, hispid non sulcato-striate pileus, and a subcylindrical to fusoid stem, abrupt or attenuate towards the pileus (Fig. 2). Most Panus specimens have lilac-purple pigments at early stages, which are usually lost in mature basidiomes (Miller 1967, Corner 1981, Pegler 1983). In DMC 696, the lilac to reddish colour is persistent on mature basidiomes, and only fading in drying. Molecular relationship between DMC 696 and *L. fasciatus* is morphologically only corroborated by the strongly hispid basidiomes. *Lentinus fasciatus* has a short and stocky stem (Pegler 1983), and also differs in having bigger cylindrical basid-iospores (6–8.5 × 3–4  $\mu$ m). Among the hispid-strigose *Panus* species, DMC 696 phenetically also resembles *P. ciliatus* reported from South-east Asia and Australasia (Pegler 1983). *Panus ciliatus* differs in having a cinnamon brown to fuscous, asulcate but radially striate pileus, a densely ciliate and often lobed or incised margin, and a conspicuous fusoid pseudosclerotium. Microscopically, in addition to clavate-fusoid, constricted to nodulose thin-walled cheilocystidia present in both *P. ciliatus* and DMC 696, the latter also has longer, thick-walled subcylindrical cheilocystidia (Fig. 5b). Additional material is needed to address the taxonomic status of the species represented by DMC 696.

In spite of being merely based on quantitatively and geographically limited material of velutinate lentinoid fungi, our molecular phylogenetic analyses indicated that taxa with velutinate basidiomes are distributed in *Lentinus* as well as in *Panus*, and have provided evidence that all species previously placed in *Lentinus* sect. *Velutini* should properly be assigned to *Panus*. The combination of uninflated vegetative hyphae, unbranched skeletal hyphae, and radiate hymenophoral trama characterising all species in the *Panus* clade is thus phylogenetically supported. The *P. velutinus* complex includes morphologically similar, velutinate to hispid-strigose specimens with long and slender stems, but additional collections from wider geographical regions are needed to clarify the status of some species in this complex.

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