

Morphological and phylogenetic studies of two new neotropical species of *Loweomyces* (Polyporales, Basidiomycota)

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Abstract The genus *Loweomyces* is characterized by resupinate to pileate, sessile to stipitate basidiomes, monomitic to dimitic hyphal system with presence of pseudoskeletal hyphae, thin-walled hymenial cystidia, and usually cyanophilous spore and hyphal walls. The genus has not been widely accepted and some authors placed *Loweomyces* species in *Abortiporus*, *Tyromyces* or *Antrodiella*. During a survey of polypores in southern Brazil, two unidentified species with similar features to *Loweomyces* spp. were found. Morphological and molecular studies were carried out in order to understand their morphological and evolutionary relationships and to confirm their phylogenetic placement and taxonomic identity. DNA sequences of the internal transcribed spacer and large subunit of ribosomal RNA gene were used for phylogenetic studies. Both studied species have similar morphological characteristics to other *Loweomyces* spp. and group close to *L. fractipes* phylogenetically; therefore, *Loweomyces spissus* and *L. tomentosus* are proposed as new species. Full descriptions, illustrations of microscopic features, and an identification key to the accepted species in the genus are presented.

Keywords Taxonomy · Phylogeny · Wood-decaying fungi · Diversity

Introduction

Loweomyces, described by Kotlaba and Pouzar (1976) as a subgenus of *Spongipellis* Pat. and later raised by Jülich (1982) to genus, is characterized by species with resupinate to stipitate basidiomes and a monomitic to dimitic hyphal system with presence of pseudoskeletal hyphae. It also presents thin-walled hymenial cystidioid elements (often rare and difficult to observe), and cyanophilous spore and hyphal walls. There are four names associated with *Loweomyces* in MycoBank databases, i.e. *L. fractipes* (Berk. & M.A. Curtis) Jülich (1982), *L. sibiricus* (Penzina & Ryvardeen) Spirin (Zmitrovich et al. 2006), *L. subgiganteus* (Berk. & M.A. Curtis) Spirin (Zmitrovich et al. 2006), and *L. wynneae* (Berk. & Broome) Jülich (1982). However, only *L. fractipes* and *L. wynneae* have had their taxonomic positions confirmed by molecular data, being placed in the residual polyporoid clade (Miettinen et al. 2012; Binder et al. 2013).

Loweomyces fractipes, the generic type, is characterized by white-ochraceous, soft, pileate, dimidiate to fan-shaped (sometimes almost effused-reflexed), usually centrally-laterally stipitate basidiomes, monomitic hyphal system with presence of thick-walled hyphae in the stipe and context and broadly ellipsoid to subglobose, thin-walled, 4.5–6 × 4–5 μm basidiospores (Ryvardeen and Gilbertson 1993). *Loweomyces wynneae* differs in thinner, sessile to laterally substipitate, ochraceous, saffron yellow or pale buff basidiomata, irregular in shape (usually spatulate-flabelliform), with thin ochraceous rhizomorphs in the margin, smaller, ovoid to subglobose, thin-walled, 3–4 (4.5) × 2.3–3 μm basidiospores, and an abundance of thick-walled hyphae, but occasional

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clamps can be observed in thick-walled hyphae, in transitions between generative and skeletal hyphae (Bernicchia 2005; Ryvarden and Gilbertson 1993).

Both species were also treated in the genus *Abortiporus* Murrill (Rauschert 1988; Gilbertson and Ryvarden 1986). However, *A. biennis*, its type species, has a distinctly monomitic hyphal system and gloecystidia. *Tyromyces* P. Karst. has similar morphological characteristics to *Loweomyces*, but differs in the absence of cystidia, in having non-cyanophilous hyphae and spores, and in being characterized by distinctly monomitic species, with the exception of *T. chioneus* (Fr.) P. Karst., which has skeletal hyphae. *Spongipellis* Pat. is another genus characterized by a cyanophilous reaction of spores, but its type species, *S. spumeus* (Sowerby) Pat., has a monomitic hyphal system without distinctly thick-walled hyphae, larger basidia and thick-walled basidiospores. *Loweomyces wynneae* was also placed in *Antrodiella* Ryvarden & I. Johans. (Spirin 2001), which differs in having a dimitic hyphal system with thin-walled generative hyphae.

Molecular data revealed that *L. wynneae* and *L. fractipes* are unrelated to *Abortiporus*, *Antrodiella*, *Spongipellis* or *Tyromyces* (Miettinen et al. 2012). The other two *Loweomyces* spp., *L. sibiricus* and *L. subgiganteus*, have not been subject to a molecular study until now. Both species were formerly placed in *Tyromyces* (Penzina and Ryvarden 1998; Ryvarden 1984) and combined in *Loweomyces* by Zmitrovich et al. (2006) due to subglobose cyanophilous spores, cyanophilous hyphae and presence of leptocystidia (hydropleurous gloecystidia). However, leptocystidia do not seem to be typical structures of the generic type *L. fractipes*; therefore, a revision of the taxonomic position of *L. sibiricus* and *L. subgiganteus* is needed.

In a recent survey of polypores from southern Brazil, two unidentified species were collected and studied. Morphological and molecular data indicate their relationship with *L. fractipes* and *L. wynneae*; thus, two new species in the genus are proposed. Full descriptions, images, illustrations and an identification key are presented.

Material and methods

Specimens were collected in southern Brazil in areas of Araucaria Forest in Paraná and Rio Grande do Sul States. The Araucaria Forest is characterized by subtropical vegetation with dominated by the coniferous tree *Araucaria angustifolia* (Bertol.) Kuntze. Additional specimens of the *Loweomyces* group from O herbarium (herbarium abbreviations follow Thiers 2016), including a Brazilian specimen previously sequenced by Miettinen et al. (2012, as *Antrodiella* sp.), were studied for morphological comparison. For micromorphology studies, hand-cut sections of the

basidiomes were prepared on microscope slides with 3 % KOH solution. To observe hyphae separately and interpret the hyphal system, small pieces of the basidiomes were kept in 3 % NaOH solution under 60 °C for about 12 h. The samples were then used to prepare microscope slides. Aqueous phloxine solution (1 %) was used for staining. All microscopic structures observed were measured with the aid of an eyepiece micrometer. Whenever possible, a minimum of twenty measurements were taken from each structure present. Drawings of the microstructures were made with the aid of a microscope drawing tube (1,000× magnification).

For DNA extraction, DNeasy Plant Mini Kit (QIAGEN) and magnetic-bead technology of MagNA Pure compact system (Roche) were used. When needed, DNA was purified using DNA Clean & Concentration Kit (Zymo Research). DNA amplifications of the internal transcribed spacer (ITS) and large subunit (LSU) regions of ribosomal RNA gene were performed using ITS5/ITS4-Basidio (Nicolcheva and Bärlocher 2004) primer combination for ITS and LR0R/LR6 (Moncalvo et al. 2000) for LSU. The PCR regimes followed Tomšovský et al. (2010a). In samples where PCR did not yield satisfactory results, a nested PCR was performed following Tomšovský et al. (2010b). Amplified products were sent for custom purification and sequencing to MacroGen Ltd. (Korea).

The sequences obtained were initially edited in BioEdit (Hall 1999) and adjusted manually when needed. Reference sequences were chosen based on studies of Miettinen et al. (2012), Binder et al. (2013), Westphalen et al. (2016), and through BLAST searches in the NCBI database (National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov>). Sequences selected for this study are summarized in Table 1. The dataset of sequences was aligned using the MAFFT online server (<http://mafft.cbrc.jp/alignment/server/>) and used for the construction of phylogenetic trees applying Bayesian inference in the software MrBayes 3.2.2 (Ronquist et al. 2012) and Maximum Likelihood in RAxML-HPC v. 8 (Stamatakis 2014). The evolutionary models were inferred with the jModelTest2.c1.4 (Darriba et al. 2012) using AIC criterion. The best fit model selected was GTR+I+G. The Bayesian analyses were run for 10 million generations, sampling every 1,000 generations. Burn-in was set to 10 % of the trees. For Maximum Likelihood, a GTRCAT model of evolution was used. Number of bootstrap replicates was halted automatically (autoMRE) and 108 replicates were used. All analyses were conducted in CIPRES Science Gateway (Miller et al. 2010).

Results

Our phylogenetic analysis indicates that *Loweomyces* is well supported in the residual polyporoid clade, related to the

Table 1 List of sequences used in this study. *Sequences obtained in this study. — Sequences found on GenBank without a published reference

Specimen	GenBank No.		References
	ITS	LSU	
<i>Abortiporus biennis</i>	KP135300	KP135300	Floudas and Hibbett 2015
<i>Antella americana</i>	JN710509	JN710509	Miettinen et al. 2012
<i>Antrodia heteromorpha</i>	KC543145	KC543145	Spirin et al. 2013
<i>Antrodiella faginea</i>	JN710514	JN710514	Miettinen et al. 2012
<i>Antrodiella semisupina</i>	JN710521	JN710521	Miettinen et al. 2012
<i>Antrodiella romellii</i>	JN710520	JN710520	Miettinen et al. 2012
<i>Antrodiella palescens</i>	JN710518	JN710518	Miettinen et al. 2012
<i>Antrodiella</i> sp.	JN710522	JN710522	Miettinen et al. 2012
<i>Aurantiporus alborubescens</i>	JQ821319	JQ821318	Dvořák et al. 2014
<i>Aurantiporus fissilis</i>	HQ728292	HQ729002	Tomšovský 2012
<i>Bjerkandera adusta</i>	KT305935	KT305935	Westphalen et al. 2015
<i>Bjerkandera fumosa</i>	KT305937	KT305937	Westphalen et al. 2015
<i>Ceriporiopsis gilvescens</i>	HQ659222	HQ659222	Miettinen and Rajchenberg 2012
<i>Cymatoderma dendriticum</i>	JN649339	JN649339	Sjökvist et al. 2012
<i>Flaviporus brownii</i>	JN710537	JN710537	Miettinen et al. 2012
<i>Flaviporus brownii</i>	JN710538	JN710538	Miettinen et al. 2012
<i>Flaviporus liebmanii</i>	JN710540	JN710540	Miettinen et al. 2012
<i>Flaviporus liebmanii</i>	JN710542	JN710542	Miettinen et al. 2012
<i>Irpex lacteus</i>	JX109852	JX109852	Binder et al. 2013
<i>Junghuhnia atumnale</i>	JN710549	JN710549	Miettinen et al. 2012
<i>Junghuhnia crustacea</i>	JN710554	JN710554	Miettinen et al. 2012
<i>Junghuhnia nitida</i>	JN710560	JN710560	Miettinen et al. 2012
<i>Laetiporus sulphureus</i>	EU840565	EU884420	Tomšovský and Jankovský 2008
<i>Loweomyces fractipes</i>	KP859304	KP859309	—
<i>Loweomyces fractipes</i>	JN710568	JN710568	Miettinen et al. 2012
<i>Loweomyces fractipes</i>	JN710569	JN710569	Miettinen et al. 2012
<i>Loweomyces fractipes</i>	JN710570	JN710570	Miettinen et al. 2012
<i>Loweomyces fractipes</i> (MT 13/2012)*	KX378866	KX378866	
<i>Loweomyces spissus</i> (MCW 468/13)*	KX378867	KX378867	
<i>Loweomyces spissus</i> (MCW 471/13)*	KX378868	KX378868	
<i>Loweomyces spissus</i> (MCW488/14)*	KX378869	KX378869	
<i>Loweomyces tomentosus</i> (MCW 366/12)*	KX378870	KX378870	
<i>Loweomyces tomentosus</i> (MCW 382/12)*	KX378871	KX378871	
<i>Loweomyces wynneae</i> (DD 846/08)*	KX378865	KX378865	
<i>Loweomyces wynneae</i>	JN710604	JN710604	Miettinen et al. 2012
<i>Loweomyces wynneae</i>	JN710605	JN710605	Miettinen et al. 2012
<i>Phanerochaete chrysosporium</i>	HQ188436	GQ470643	James et al. 2011
<i>Phanerochaete sordida</i>	EU118653	EU118653	Larsson 2007
<i>Phlebia radiata</i>	AY854087	AF287885	Floudas and Hibbett 2015
<i>Podoscypha multizonata</i>	JN649360	JN649360	Sjökvist et al. 2012
<i>Skeletocutis nivea</i>	JX109858	JX109858	Binder et al. 2013
<i>Spongipellis spumeus</i>	HQ728285	HQ729017	Tomšovský 2012
<i>Spongipellis spumeus</i>	HQ728287	HQ729021	Tomšovský 2012
<i>Steccherinum bourdotii</i>	JN710584	JN710584	Miettinen et al. 2012
<i>Steccherinum ochraceum</i>	JN710590	JN710590	Miettinen et al. 2012
<i>Steccherinum tenuispinum</i>	KM411452	KM411452	—

Table 1 (continued)

Specimen	GenBank No.		References
	ITS	LSU	
<i>Trametopsis cervina</i>	AY684175	AY855907	Tomšovský et al. 2006
<i>Trulla dentipora</i>	JN710512	JN710512	Miettinen et al. 2012
<i>Trulla duracina</i>	JN710513	JN710513	Miettinen et al. 2012
<i>Tyromyces polyporoides</i>	JN710602	JN710602	Miettinen et al. 2012
<i>Tyromyces chioneus</i>	HQ659244	HQ659244	Miettinen and Rajchenberg 2012

genera *Antrodiella*, *Antella* Miettinen, *Flaviporus* Murrill, *Junghuhnia* Corda, *Steccherinum* Gray, and *Trulla* Miettinen & Ryvarden, but separated from them. The morphological and molecular data obtained support the description of two new species in *Loweomyces* (Fig. 1), *Loweomyces tomentosus* and *Loweomyces spissus*, spp. nov. The hyphal system in both

species is difficult to interpret as either monomitic or dimitic as it has many branched to unbranched thick-walled hyphae with sparse clamps. Morphological analysis of other *Loweomyces* spp. show that *L. fractipes* is distinguished by a more distinctly monomitic hyphal system in the pileus, while the new species have a dimitic (or seemingly dimitic)

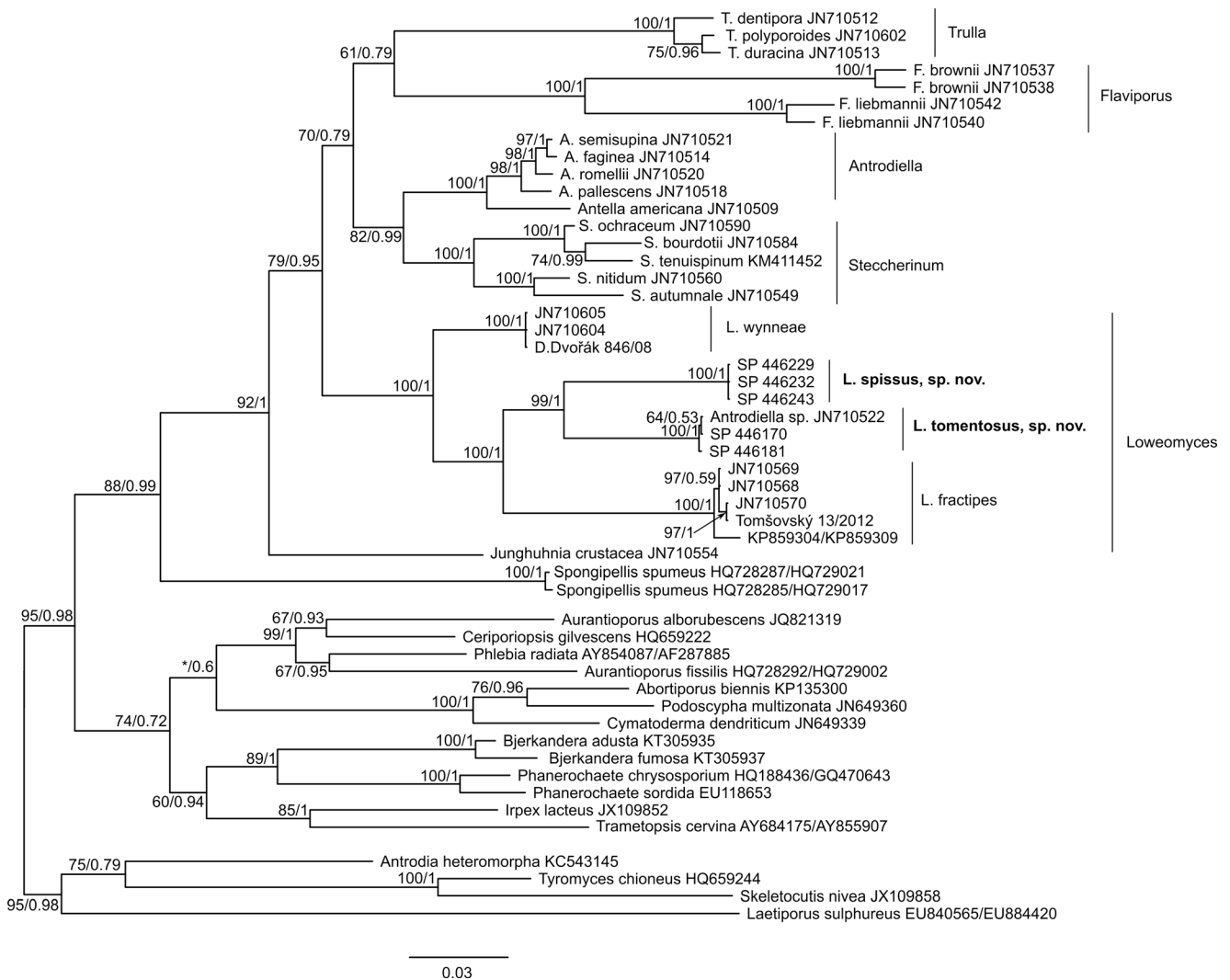


Fig. 1 Phylogenetic tree of ITS-LSU region conducted by Bayesian analysis (for legends to numbers, see Table 1). Numbers at branches indicate maximum likelihood bootstrap proportion and Bayesian

posterior probability values. The asterisk (*) marks different topology in both analyses. The bar indicates number of expected substitutions per position

structure, more similar to *L. wynneae* (see Discussion for more comments). Morphological and molecular analysis also showed that the specimen collected in Paraná State previously identified as *Antrodiella* sp. and sequenced by Miettinen et al. (2012) belongs in *L. tomentosus*.

Taxonomy

Loweomyces spissus Westphalen, Tomšovský & Rajchenb., *sp. nov.* (Figs. 2a and 3a–g)

MB817929

Holotype: BRAZIL. Paraná: Piraquara, Morro do Canal, 04. IX.2013, M.C. Westphalen 466/13 (SP 446227).

Etymology: *spissus* – refers to the hard consistency of the basidiomes when comparing to other species of the genus.

Basidiomes annual, pileate, sessile, dimidiate, imbricate, sometimes laterally fused, tough and somewhat watery when fresh, becoming dense and sub-woody when dried, up to 6.5 cm wide and 3 cm long. Pileus surface with faintly sulcate zones, smooth or sometimes with irregular and variable tuberculate growths, with a thin hirsute to velutinous layer in younger specimens, glabrous near the margins, cream-coloured when fresh, becoming slightly paler or unchanging upon drying; margin acute to somewhat lobed, concolorous with the pileus surface, up to 1 mm wide. Context dense, fibrous, homogenous or sometimes with a very thin inconspicuous tomentum formed by the pilear covering hyphae, up to 1.5 mm thick. Pore surface white to cream, pores small, regular, round

to angular, 7–9 per mm, dissepiments entire; tubes dense, concolorous with the pore surface, up to 4 mm deep.

Hyphal system dimitic. Generative hyphae clamped, thin- to thick-walled, hyaline, unbranched to branched, straight to sinuous, 2–5 µm wide; skeletal hyphae thick walled, often almost solid, hyaline, tortuous to straight, unbranched or with few branches, 3–6 µm wide; hyphae densely arranged and with transitional forms between skeletal and generative hyphae. Thin-walled cystidioles present, sometimes difficult to observe, ventricose, lageniform or cylindrical. Basidia clavate, 4-sterigmate, 10–12 × 4–5.5 µm. Basidiospores widely-ellipsoid to subglobose, hyaline, thin-walled, IKI-, 2.5–3.5 × 2–3 µm.

Habitat: Growing on logs of unidentified angiosperms in areas of *Araucaria* Forest (approximately 1,000 m a.s.l.) in south Brazil.

Comments: *Loweomyces spissus* is characterized by cream basidiomes that become tough and dense when dried. Its consistency, lack of a distinct tomentum, and the small pores distinguish it from other species of the genus. Microscopically, it has thick-walled hyphae that we interpret as true skeletal hyphae. However, transitional forms between generative and skeletal hyphae can be observed (Fig. 3f). The hyphae are densely arranged; nevertheless, our observations after NaOH treatment indicate (a) the presence of very long segments with no clamps, and (b) the septa in thick-walled hyphae seem to be rarer than in *L. tomentosus*. Therefore, we decided to treat this taxon as dimitic. In *L. tomentosus* clamps are more easily observed in the tomentum where hyphae are loosely arranged, so it is possible that the lack of this layer in *L. spissus* and the dense arrangement of hyphae make the observation of clamps difficult.

Examined specimens: BRAZIL. Paraná: Piraquara, Morro do Canal, 04.IX.2013, M.C. Westphalen 468/13 (SP 446229) and M.C. Westphalen 471/13 (446232); Ibid. 26.I.2014, M.C. Westphalen 488/14 (SP 446243)

Loweomyces tomentosus Westphalen, Tomšovský & Rajchenb. *sp. nov.* (Figs. 2b and 3h–m)

MB817930

Holotype: BRAZIL. Rio Grande do Sul: São Francisco de Paula, FLONA, 16.IV.2012, M.C. Westphalen 366/12 (SP 446170).

Etymology: *tomentosus* – refers to the conspicuous tomentum layer present in the species.

Basidiomes annual to biannual, pileate, sessile, dimidiate, semicircular, imbricate, very soft when fresh, corky and very light-weight when dried, up to 8 cm wide and 4.5 cm long. Pileus surface with faintly sulcate zones, soft and watery in fresh specimens, smooth to somewhat velutinate due to the presence of a spongy tomentum formed of matted hyphae, pale yellow when fresh, becoming paler cream when dried; margin acute, white to cream, up to 4 mm wide. Context thick with two distinct layers, upper layer soft and spongy, forming



Fig. 2 *Loweomyces spissus* (a) and *Loweomyces tomentosus* (b). Scale bar = 2 cm

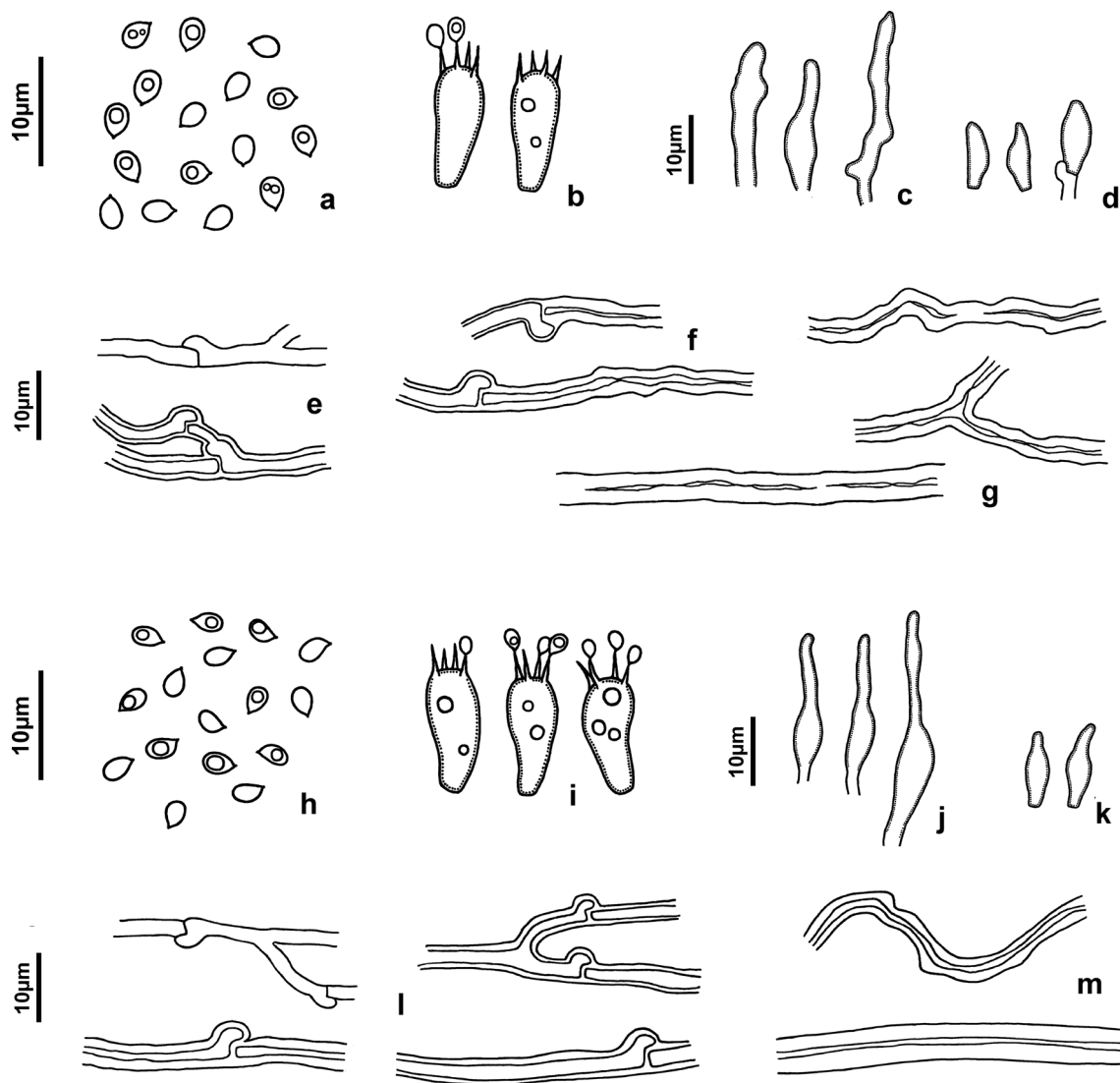


Fig. 3 *Loweomyces spissus* (a–g) and *L. tomentosus* (h–m). **a.** Basidiospores. **b.** Basidia. **c.** Cystidioid elements. **d.** Cystidioles. **e.** Generative hyphae. **f.** Transitory hyphae. **g.** Skeletal hyphae. **h.**

Basidiospores. **i.** Basidia. **j.** Cystidioid elements. **k.** Cystidioles. **l.** Generative hyphae. **m.** Nonseparate skeletal-like hyphae. Illustrations by M.C. Westphalen

a loose tomentum, up to 1.5 mm thick in dried specimens, when fresh at least 3 mm thick, lower layer very thin, forming a dense region above the tubes, up to 0.5 mm thick when fresh. Pore surface white to cream, pores regular, mostly angular, 4–6 per mm, dissepiments entire; tubes somewhat fibrous, concolorous with the pore surface, up to 5 mm deep.

Hyphal system mono-dimitic. Generative hyphae clamped, thin to thick walled, hyaline, smooth, straight to sinuous, branched, more rarely unbranched; thick-walled hyphae with long segments lacking septa which may be interpreted as skeletal hyphae, but sparse clamps can also be observed on some thick-walled hyphae; clamped hyphae 2.5–5 µm wide, easily observed in the tomentum, aseptate hyphae 3.5–6.5 µm wide; hyphae loosely arranged in the tomentum layer and more densely arranged above the tubes and in the trama. Thin-walled cystidioid elements present, ventricose, fusoid to

lageniform and elongated, sometimes difficult to observe. Basidia clavate, 4-sterigmate, 9–12 × 4–5 µm. Basidiospores ellipsoid, hyaline, thin-walled, IKI-, 3–3.5 × 2–2.5 µm.

Habitat: Growing on stumps of unidentified angiosperms.

Comments: *Loweomyces tomentosus* is characterized by soft and somewhat watery basidiomes when fresh, with a pale yellow pilear surface and white to cream, large pores. Microscopically, it has very small ellipsoid basidiospores and thick-walled hyphae with infrequent clamps. The clamped hyphae seem more abundant in the tomentum, but it is possible that they are just more easily observed due to the loose arrangement of hyphae in that layer. The hyphal system of this species is complex to define due to the presence of many transitory hyphae between generative and skeletal forms. Even though clamps can be observed in thick-walled hyphae,

some wider, 5–6 μm wide, very thick-walled hyphae present in the basidiomes easily break when compressed, making it difficult to verify the presence of clamps. Following the classification by Zmitrovich et al. (2006), it can be defined as sub-dimitic, which is applied when many thick-walled hyphae are rarely septate. The presence of more skeletal-like hyphae distinguishes *L. tomentosus* from *L. fractipes*, the latter having pileus tissue distinctly monomitic to sub-monomitic, i.e., with presence of slightly thick-walled clamped supporting hyphae (Zmitrovich et al. 2006). In addition, *L. fractipes* has larger spores (4.5–6 \times 4–5 μm). *Loweomyces wynneae* differs in its spatulate to flabelliform basidiomes with uneven, wavy margins and a radially strigose ochraceous, saffron yellow to pale buff pileus surface and thin, somewhat lacerate, dissepiments.

Examined specimens: BRAZIL. Rio Grande do Sul: São Francisco de Paula, FLONA, 30.IV.2012, M.C. Westphalen 382/12 (SP 446181); Viamão, Parque Saint Hilaire, 25.V.2009, M.A. Reck 091/09 (ICN).

Examined specimens of other *Loweomyces* spp.: *L. fractipes*: SLOVAKIA. Bratislava, Svätý Jur, Šúr national nature reserve, dead wood of *Alnus glutinosa*, 18.VI.2012, M. Tomšovský 11/2012, 12/2012, 13/2012 and 14/2012 (BRNL). *L. wynneae*: CZECH REPUBLIC. Brno, Křtiny, U Výпустku nature reserve, 370 m a.s.l., on the ground and rotted stumps, 31.X.2008, leg. et det. D. Dvořák, rev. P. Vampola; D. Dvořák 846/08 (BRNU). SLOVAKIA. Trenčín, Vršatské Podhradie, valley under Chmelová hill (ca 350 m NE of the top), ca 740–790 m a.s.l., primeval beech forest, on fallen bark of *Fagus sylvatica*, 1.IX.2005, leg. D. Dvořák, det. P. Vampola; D. Dvořák 254/05 (BRNU).

Identification key to accepted species of *Loweomyces*

1 Basidiomes dense, tough when fresh becoming sub-woody when dried, pores 7–9/mm, context homogenous or with a very thin inconspicuous tomentum layer.
L. spissus

1* Basidiomes soft when fresh, light-weight and somewhat brittle when dried, pores 3–6/mm, context duplex, with a soft tomentum layer and a denser layer above the tubes.

2 Pileus surface white to cream when fresh, hyphal system strictly monomitic, thick-walled generative hyphae seen on the context and stipe, spores 4.5–6 \times 4–5 μm .
L. fractipes

2* Pileus surface pale yellow to ochraceous when fresh, hyphal system mostly dimitic, thick-walled hyphae throughout the basidiomes with variable presence of clamps, spores 3–3.5 \times 2–2.5 μm .

3 Basidiomes semicircular, margins entire, pileus surface pale yellow, becoming cream when dried. *L. tomentosus*

3* Basidiomes spatulate to flabelliform, margins uneven, pileus surface ochraceous or darker (saffron yellow, pale buff), margin paler. *L. wynneae*

Discussion

Morphological and molecular data obtained in this study corroborate the need to describe two new species in *Loweomyces*. These are the first species of the genus described from the Neotropics; *L. fractipes* is known from North America (Berkeley 1872) and *L. wynneae* from Europe (Berkeley and Broome 1859). Sequences labeled as *L. fractipes* from Brazil available in GenBank (KP859304 and KP859309) were added to our dataset. Even though there are differences in ITS-LSU sequences (16 different nucleotides between Brazilian and other sequences from North America, which corresponds to approximately 1 %), some molecular diversity may be expected at species level when specimens from different regions are compared. The possibility that the Brazilian specimen might be an undescribed, tropical sister species of the temperate *L. fractipes* was considered. Unfortunately, the voucher specimen of the Brazilian *L. fractipes* (RP 197) could not be located for morphological comparison. Therefore, more collections and molecular data are needed to verify the presence of *L. fractipes* in the Neotropics.

The hyphal system in *Loweomyces* seems to be variable and difficult to interpret, with species from distinctly monomitic to truly dimitic, and often with thick-walled hyphae with variable presence of clamp connections. *Loweomyces tomentosus* has more easily observed clamps in the thick-walled hyphae (especially in the tomentum), but long aseptate thick-walled hyphal sections can also be observed. *Loweomyces spissus* has a more distinctly dimitic hyphal system with thickened hyphae with very long segments without clamps that we interpret as true skeletal hyphae. Nevertheless, clamp connections can also be seen in some thick-walled hyphae, mostly forming a transition between generative and skeletal hyphae, similar to what can be observed in *L. wynneae*. *Loweomyces fractipes* is the only species that can be more clearly interpreted as monomitic, presenting abundant clamps and thin to slightly thick-walled hyphae in the pileus and thick-walled supporting skeletal-like hyphae in the stipe. Ryvarden and Gilbertson (1993) commented on the presence of skeletal-like hyphae with sparse clamps on the stipe of *L. fractipes* specimens, a feature not observed in the specimens studied. In addition, *L. fractipes* basidiospores and hyphae are described as cyanophilous but the reaction was negative in the newly described Brazilian taxa. However, we also did not observe marked cyanophily in the herbarium specimens of *L. fractipes*, but the spores of

L. wynneae had a weak to moderate cyanophilous reaction. Therefore, the intensity of the reaction is variable in the genus.

The variability observed in the hyphal system and in cyanophily may correlate to the rather long branches in our phylogenetic tree, which indicate substantial molecular differentiation. Thus, in future the genus might be split into smaller, more homogeneous genera. Although both, *L. spissus* and *L. tomentosus* have a different hyphal structure as compared to *L. fractipes*, we prefer to keep them under *Loweomyces*. Micromorphologically, *L. tomentosus* and *L. spissus* are more similar to *L. wynneae*, while phylogenetically they are closer to *L. fractipes*. Therefore, the relations of the taxa are not clear and there is not enough evidence to separate them in different genera.

Cultures (monosporic and polysporic) of the two new Neotropical species were obtained from spore prints and both had a similar growth behaviour. After germination of spores, cultures were kept at 25 °C and mycelium growth continued slowly, forming a thick crust where pores started to develop. However, after a short period, the growth stopped and attempts to transfer the mycelium to different media (malt extract agar, potato dextrose agar, and water agar) did not result in a growing culture; therefore, further studies were impossible. This may be an interesting biological feature of the genus and a way to distinguish it from *Tyromyces* and *Antrodiella* species. However, to ensure if this is a relevant character and that it applies to all species in *Loweomyces*, further cultural studies are needed. Studies of the mating system and nuclear behaviour would be valuable to better understand polypore taxonomy and to assist discrimination of morphologically similar genera (Rajchenberg 2011).

In addition to the new species described here, our molecular data (unpubl.) indicate the occurrence of another possibly undescribed Brazilian species in *Loweomyces*. Because of incomplete sampling this species was not included in this study, but the occurrence of several new species may indicate that the genus has a diversity hotspot in the Neotropics that has been overlooked in these regions until now. The temperate specimens of *L. fractipes* and *L. wynneae* were usually recorded on small pieces of wood or on ground with wood litter in humid places, which indicates some affinity to humid biomes. The high morphological similarity of the newly described species with *Tyromyces* and especially *Antrodiella* spp. may also cause confusion and misidentifications. Detailed analysis of the hyphal system in polypores is extremely important. The traditional discrimination of monomitic, dimitic, or trimitic hyphal systems does not always correspond to hyphal structure of some genera and many intermediary forms may be observed (Zmitrovich et al. 2006). Another example of hyphal variability in polypores is the genus *Trametopsis* Tomšovský, which has a dimitic hyphal system with a trimitic aspect (Tomšovský 2008).

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