

## **Lasioloma antillarum (Ascomycota: Pilocarpaceae), a new lichenized fungus from the Antilles, and the importance of posterior annotations of sequence data in public repositories**

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## *Lasioloma antillarum* (Ascomycota: Pilocarpaceae), a new lichenized fungus from the Antilles, and the importance of posterior annotations of sequence data in public repositories

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**Abstract:** We describe the new lichenized fungus *Lasioloma antillarum* Lücking, Högnabba & Sipman from the Netherlands Antilles. The new species is characterized by a corticolous growth habit, apothecia with shortly tomentose margins, and rather small (35–50 × 12–16 µm), muriform ascospores in numbers of 2(–4) per ascus. The material had originally been identified as *Calopadia phyllogena* (Müll. Arg.) Vězda, with associated sequence data, but in phylogenetic analyses consistently fell outside the latter genus. Its revised identification as a species of *Lasioloma* is consistent with its phylogenetic position and underlines the necessity of posterior annotations in public sequence repositories, in order to correct previous identifications.

**Key words:** Antilles, Ascomycota, inaccuracies in GenBank, integrative taxonomy, *Lasioloma*, Pilocarpaceae, sequence labelling

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## Introduction

*Pilocarpaceae* is a moderately sized family of predominantly tropical lichenized fungi often found on living leaves, but with several lineages growing on other substrata (Kalb & Vězda 1987; Lücking 2008; Sérusiaux & al. 2008; Brand & al. 2014; Sanders 2014; Gumboski 2015; Lücking & al. 2017; Guzow-Krzemińska & al. 2019). It consists of two major groups, one formed by the former family *Micareaceae* and one corresponding to the former family *Pilocarpaceae* s.str. (Andersen & Ekman 2005; Miadlikowska & al. 2014; Kraichak & al. 2018; Aptroot & al. in Hyde & al. 2019). The latter includes a number of genera characterized by peculiar conidiomata, so-called campylidia, which are more or less hood-shaped and adapted to dispersal of the conidia by

rain splash (Sérusiaux 1986, 1995; Lücking 2001, 2008; Sanders & al. 2016). These genera had formerly been assigned to the family *Ectolechiaceae* and the monogeneric family *Lasiolomataceae* (Hafellner 1984; Kalb & Vězda 1987; Lücking 1999).

*Calopadia* Vězda, *Lasioloma* R. Sant., *Sporopodium* Mont. and *Tapellaria* Müll. Arg. are the core genera in the campylidia-bearing lineages of *Pilocarpaceae* s.str. (Lücking 1999, 2008; Lücking & Sérusiaux 2001; Sérusiaux & al. 2008; Neuwirth & Stocker-Wörgötter 2017). *Calopadia*, *Lasioloma* and *Tapellaria* are similar to each other in thallus and ascoma morphology and share filiform conidia adapted to rain water dispersal. *Lasioloma* differs from the other two genera in the woolly prothallus, the pilose apothecial margins and the centrally branched conidia, whereas *Tapellaria* can be distinguished from *Calopadia* in the jet-

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black apothecia with purple hypothecium and anastomosing, net-like paraphyses (Lücking 1999, 2008).

Thus far, few phylogenetic studies exist for *Pilocarpaceae*, although the data available show an emerging picture of some genera being monophyletic and others para- or polyphyletic (Andersen & Ekman 2005; Miadlikowska & al. 2014; Kraichak & al. 2018; Aptroot & al. in Hyde & al. 2019; Wang & al. 2020). The genera *Calopadia* and *Lasioloma* have been resolved as closely related, whereas *Tapellaria* is phylogenetically more distant (Wang & al. 2020), in agreement with the different hamathecial anatomy of the latter. The most recent study resolved *Lasioloma* to be nested within a paraphyletic *Calopadia* (Wang & al. 2020), suggesting that the peculiar morphological features of *Lasioloma* evolved from a plesiomorphic residual corresponding to the morphology of *Calopadia* and the two genera should perhaps be merged. This topology had not been noticed before, as the complete set of taxa had not been analysed simultaneously in previous studies (Andersen & Ekman 2005; Miadlikowska & al. 2014; Aptroot & al. in Hyde & al. 2019).

Since this nested topology was caused by a single specimen identified as *Calopadia phyllogena* (Müll. Arg.) Vězda, collected in the Netherlands Antilles and first published in a broad-scale assessment of *Lecanoromycetes* as part of the AFTOL project (Miadlikowska & al. 2014), we set out to examine the taxonomic status of the underlying specimen, housed at B (*Sipman 54818*). We thereby envisioned three potential scenarios: (1) the specimen had been correctly identified, at least to genus level, making *Calopadia* indeed paraphyletic relative to *Lasioloma*; (2) the material consisted of a mixed collection, including genuine *C. phyllogena* but also thalli of *Lasioloma* that had accidentally been sequenced; (3) the material was misidentified and in reality represented a species of *Lasioloma*. The latter two options are not unlikely as mixed collections in these usually small lichens are common and some species and specimens of *Lasioloma* have reduced apothecial hairs, making them superficially similar to *Calopadia*. Some species of *Calopadia* have also been shown to produce a woolly prothallus (Lücking 1998, 2008).

Table 1. Voucher information and GenBank accession numbers for the specimens used in this study.

Species	Voucher	mtSSU	nuLSU	ITS	RPB1
<i>Calopadia foliicola</i>	Costa Rica, Lücking 16011 (BG)	AY567782	AY756318	AY756462	AY756381
<i>Calopadia</i> aff. <i>foliicola</i>	Thailand, KYW0035 (HMAS-L, RAMK)	MK957148	–	MK946953	–
<i>Calopadia</i> aff. <i>foliicola</i>	Thailand, KYW0036_31538 (HMAS-L, RAMK)	MK957157	–	MK946962	–
<i>Calopadia</i> aff. <i>foliicola</i>	Thailand, KYW0068 (HMAS-L, RAMK)	–	–	MK946951	–
<i>Calopadia</i> aff. <i>foliicola</i>	Thailand, KYW0251 (HMAS-L, RAMK)	MK957153	–	MK946958	–
<i>Calopadia lecanorella</i> (Nyl.) Kalb & Vězda	Costa Rica, Lücking 17252g (F)	EU601738	EU601751	–	–
<i>Calopadia perpallida</i> (Nyl.) Vězda	Costa Rica, Lücking 17098d (F)	EU601739	EU601752	–	–
<i>Calopadia phyllogena</i>	Netherlands Antilles, <i>Sipman 54818</i> (AFTOL-ID 4887) (B)	KJ766365	KJ766539	–	KJ766842
<i>Calopadia puiggarii</i> (Müll. Arg.) Vězda	China, 20180158 (HMAS-L, RAMK)	MK957167	–	MK946972	–
<i>Calopadia puiggarii</i>	China, HN20170381 (HMAS-L, RAMK)	MK957172	–	MK946975	–
<i>Calopadia puiggarii</i>	Thailand, KYW0036_31764 (HMAS-L, RAMK)	MK957156	–	MK946961	–
<i>Calopadia</i> sp. 1	China, HW-2018a (HKAS)	–	MF326268	–	–
<i>Calopadia subcoerulescens</i> (Zahlbr.) Vězda	Costa Rica, Lücking 16604 (F)	EU601740	–	–	–
<i>Calopadia</i> sp. 2	Thailand, KYW0365 (HMAS-L, RAMK)	MK957163	–	MK946968	–
<i>Lasioloma arachnoideum</i>	Costa Rica, Lücking 16005 (BG)	AY567783	–	AY756467	–
<i>Lasioloma</i> cf. <i>phycophorum</i>	Thailand, KYW0595 (HMAS-L, RAMK)	MK957155	–	MK946960	–
<i>Lasioloma</i> cf. <i>phycophorum</i>	Thailand, KYW0646 (HMAS-L, RAMK)	MK957158	–	MK946979	–
<i>Sporopodium antoninianum</i>	Costa Rica, Lücking 16002d (BG)	AY567785	–	AY756498	–

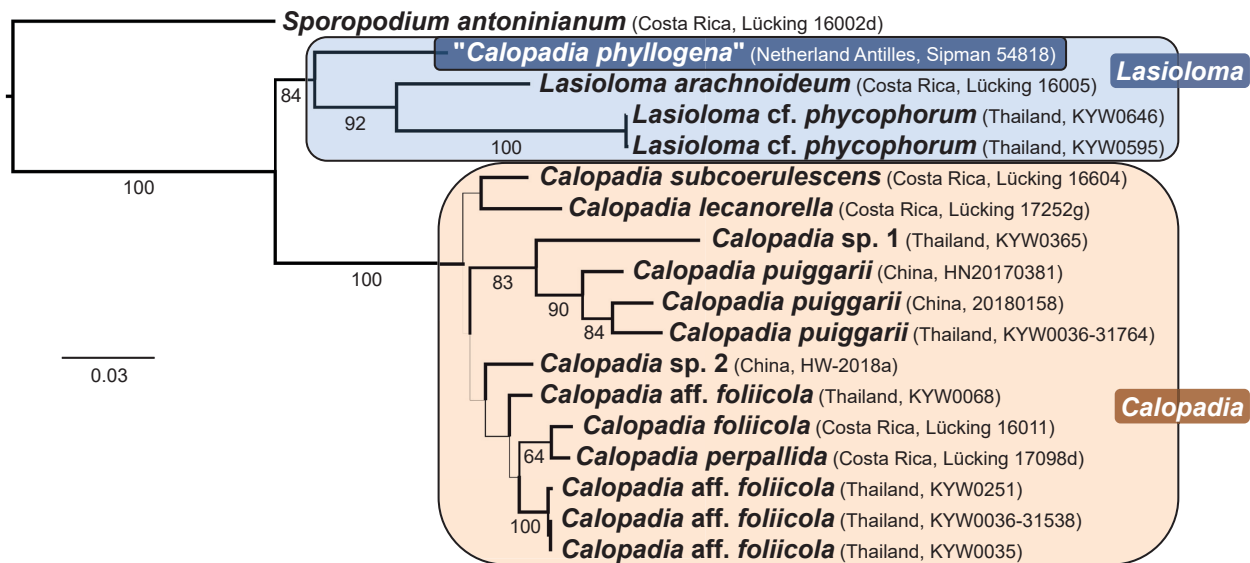


Fig. 1. Best-scoring maximum-likelihood tree of *Calopadia* and *Lasioloma* based on a 4-marker concatenated alignment (mtSSU, nuLSU, ITS, RPB1). The target specimen is supported sister to *Lasioloma arachnoideum*.

## Material and methods

To re-assess the phylogenetic placement of *Calopadia phyllogena* (Sipman 54818), we downloaded all available sequences from GenBank of the genera *Calopadia* and *Lasioloma*, with *Sporopodium antoninianum* Elix, Lumbsch & Lücking as outgroup, representing four markers (mtSSU, nuLSU, ITS, RPB1; Table 1). Separate alignments were prepared using MAFFT 7 (Katoh & Standley 2013) and potentially ambiguously aligned regions were assessed using the Guidance Web Server (Penn & al. 2010). Given that only few ambiguously aligned sites were detected and these did not affect backbone topology and support, all sites were maintained to achieve maximum terminal resolution. No supported conflict was detected between topologies from the individual markers and so the concatenated alignment was subjected to maximum likelihood tree search in RAxML 8 (Stamatakis 2014), under the universal GTR-Gamma model, with 1000 bootstrap pseudoreplicates.

The underlying specimen of *Calopadia phyllogena* (Sipman 54818) was re-examined morphologically and anatomically using a LEICA Zoom 2000 dissecting microscope and a ZEISS Axioskope compound microscope. Secondary chemistry was assessed following Orange & al. (2010).

## Results and Discussion

In our 4-marker phylogeny, the sequenced specimen originally identified as *Calopadia phyllogena* (Sipman 54818) was supported as sister to a clade including *Lasioloma arachnoideum* (Kremp.) R. Sant. from Costa Rica and two specimens originally identified as *L. arach-*

*noideum* from Thailand (Fig. 1; see below). The above clade was separate from a strongly supported clade on a long stem branch including all other sequenced species of *Calopadia* (Fig. 1). Revision of the underlying material identified as *C. phyllogena* revealed that it does not represent a species of *Calopadia*, as evident from the apothecial anatomy and the branched conidia, but indeed corresponds to the genus *Lasioloma*. We can therefore conclude that with current available data, *Calopadia* and *Lasioloma* are reciprocally monophyletic. Closer inspection of the material further revealed that the specimen in question represented an undescribed species in the genus *Lasioloma*, which is formally introduced below.

This case highlights the necessity of critically revising voucher material of sequences that exhibit unexpected phylogenetic relationships, and the need to properly identify underlying voucher material in sequence data. In the present case, with the information provided, we were able to readily trace the voucher specimen and assess its taxonomic status. In order to reflect the updated taxonomy, it is also necessary to update the corresponding sequence records, which can currently be done only by the original submitter.

The phylogeny also indicates further need for taxonomic revision of sequenced material. Thus, the Thai specimens originally identified as *Lasioloma arachnoideum* by Wang & al. (2020) formed a clade separate from the neotropical specimen (Fig. 1). The photograph in Wang & al. (2020: 383, fig. 4D) indicates that the sequenced material may represent *L. phycophorum* (Vain.) R. Sant., although the depicted specimen was not sequenced. Likewise, specimens identified as *Calopadia foliicola* (Fée) Vězda formed three separate clades (Fig. 1). Given that the species was described from the neotropics (Brazil; see Lücking 2008), the material

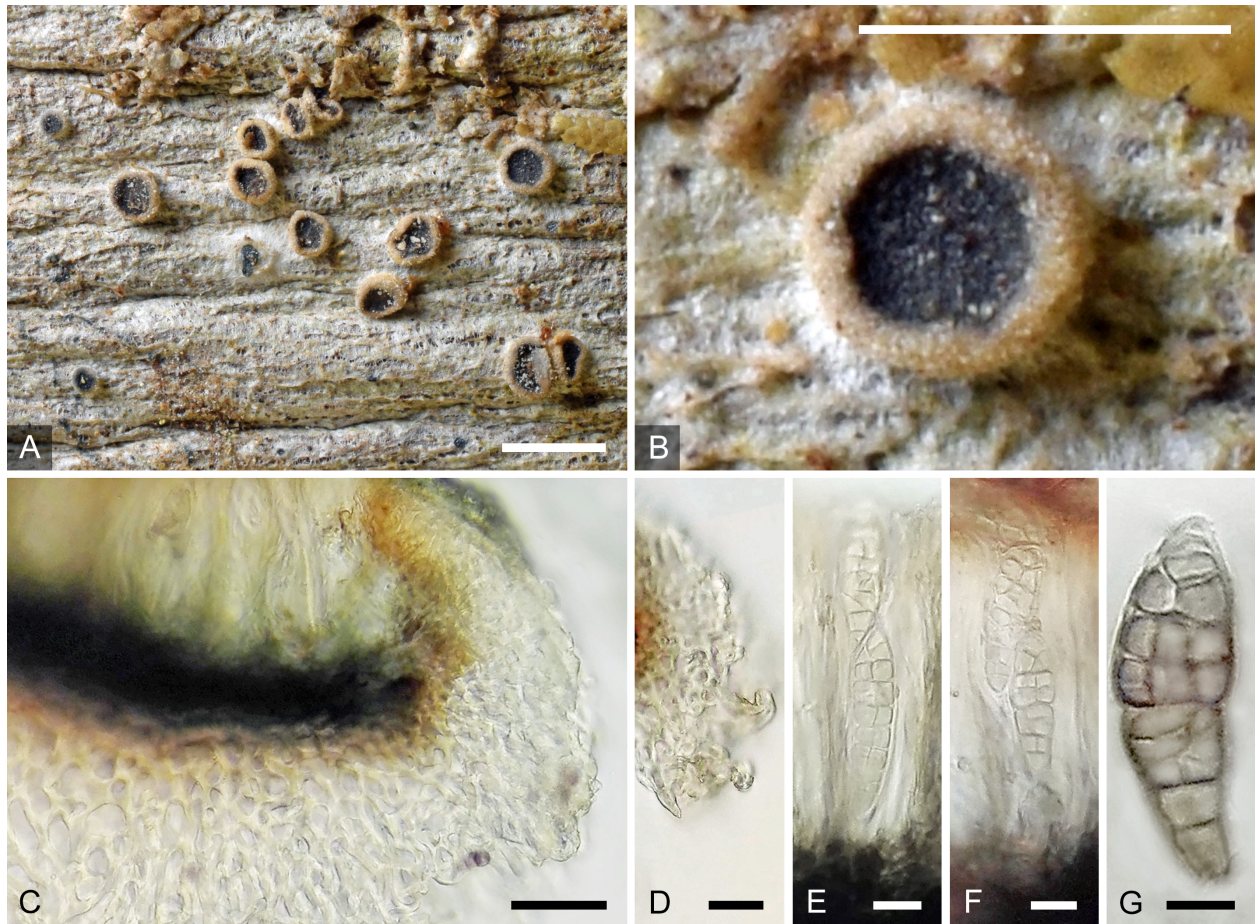


Fig. 2. Morphology and anatomy of *Lasioloma antillarum* (holotype). – A: thallus with apothecia and 1 campylidium (middle left); B: apothecium enlarged; C: section through apothecium showing hypothecium and excipulum; D: lateral excipulum enlarged showing irregular surface and protruding hairs; E, F: part of hymenium with asci and immature ascospores; G: mature ascospore. – Scale bars: A, B = 1 mm; C = 50  $\mu$ m; D = 20  $\mu$ m; E, F, G = 10  $\mu$ m.

from Thailand (Wang & al. 2020) needs to be revised. The photograph provided by Wang & al. (2020: 383, fig. 4B), corresponding to one of the three specimens (KYW0035) forming the terminal clade, fits *C. foliicola* except for the plane apothecial disc (distinctly convex in *C. foliicola*), so there appears to be some indication of more or less cryptic speciation in this genus, combined with geographic signal.

### Taxonomic treatment

*Lasioloma antillarum* Lücking, Högnabba & Sipman, **sp. nov.** – MycoBank MB 838956. – Fig. 2.

Holotype: Netherlands Antilles, Saba, Summit of Mt Scenery, along trail to western lookout, 17°38'05"N, 63°14'22"W, 825 m, disturbed mossy forest (hurricane damage), on bark, 8 Mar 2007, *H. J. M. Sipman 54818* (B 60 0143940).

*Diagnosis* — Differing from *Lasioloma spinosum* in the broader ascospores and the corticolous instead of foliicolous growth habit.

*Description* — Thallus corticolous, 1–3 cm in diam., 30–50  $\mu$ m thick, centrally continuous but toward margin with dispersed, irregular patches; surface smooth to uneven, white to pale grey. Photobiont chlorococcoid. Apothecia sessile, rounded, 0.5–0.8 mm in diam., 300–400  $\mu$ m high; disc plane to slightly concave, dark brownish grey, non-pruinose; margin thick, slightly prominent, cream-coloured, very shortly tomentose. Excipulum distinctly paraplectenchymatous, laterally 50–80  $\mu$ m broad, below hypothecium 200–300  $\mu$ m high, colourless, laterally with irregular surface and protruding, short hairs formed by single, unbranched hyphae. Hypothecium 30–60  $\mu$ m high, dark brown to brown-black. Apothecial base hyaline. Epitecium thin, 5–10  $\mu$ m high, yellowish brown. Hymenium 80–90  $\mu$ m high, colourless. Paraphyses branched and slightly anastomosing. Asci 80–90  $\times$  20–25  $\mu$ m. Ascospores 2(–4) per ascus, ellipsoid to fusiform, muriform, with 7–9 transverse and 1–3 longitudinal septa per segment, 35–50  $\times$  12–16  $\mu$ m, 2.7–3.5  $\times$  as long as broad, slightly constricted at middle septum, colourless. Campylidia sessile, 0.7–1 mm broad, 1–1.3 mm long (high); lobe well-developed, hood-shaped, dark aeruginous grey, non-pruinose; socle not apparent. Wall composed of 2 layers,

outer layer para- to prosoplectenchymatous with thick-walled cells, hyaline, 20–30 µm thick, inner layer para-plectenchymatous, dark aeruginous, 20–30 µm thick, layers bent around each other and separated by an additional inner, prosoplectenchymatous, yellowish, 15–25 µm thick layer to form a 5-layered wall 100–130 µm thick. Conidial cells lining inner wall surface. Conidia filiform, branched from centre and with 4 or 5 branches, each branch 3–5-septate, 30–40 × 1.5–2.5 µm, main branch slightly longer and thicker than others. Secondary chemistry: no substances detected by TLC.

*Etymology* — The epithet refers to the origin of the material in the Antilles.

*Remarks* — The new species is characterized by apothecia with rather short hairs and mostly 2-spored asci with small, muriform ascospores. Most species of *Lasioloma* have single-spored asci with large, muriform ascospores (Santesson 1952; Lücking & Sérusiaux 2001; Lücking 2008). Only three species have been described with smaller spores and 2–8-spored asci, namely *L. inexpectatum* R. Sant. & Lücking (Santesson & Lücking 1999), *L. pauciseptatum* Van den Boom (van den Boom & al. 2018) and *L. spinosum* Hafellner & Vězda (Vězda 1994). The first, described from Africa, differs in its smaller, 7-septate ascospores, whereas *L. pauciseptatum* from Suriname produces 4–8-spored asci and also distinctly smaller ascospores (see key below). Most similar in morphology and anatomy is *L. spinosum*, a foliicolous taxon from Indonesia. Besides the deviating distribution and substrate ecology, the latter has much narrower ascospores, 7–9 × as long as broad (see key below).

### Key to the known species of *Lasioloma*

In the following key, all validly described species in the genus are included. *Lasioloma heliotropicum* Bat. & M. P. Herrera is not a validly published name (no description, no reference to original material; Batista & Cavalcanti 1964) and its status could not be established (Lücking & al. 1998).

1. Apothecia as yet unknown; campylidia present; conidial branches with short terminal appendages; corticolous; neotropics (Nicaragua) . . . . . **2**  
     . . . . . *Lasioloma appendiculatum* Breuss  
 – Apothecia and usually also campylidia present; conidial branches lacking terminal appendages . . . . . **2**
2. Asci 2–8-spored; ascospores 20–55 × 3.5–16 µm . . . **3**  
 – Asci 1-spored; ascospores 60–115 × 15–35 µm . . . **6**
3. Ascospores 7-septate, 25–30 × 3.5–7 µm; foliicolous; African palaeotropics (Ivory Coast) . . . . .  
     . . . . . *Lasioloma inexpectatum* R. Sant. & Lücking  
 – Ascospores (sub-)muriform . . . . . **4**

4. Ascospores 20–27 × 8–12 µm, 2–2.5 × as long as broad, 4–8 per ascus; corticolous; neotropics (Suriname) . . . *Lasioloma pauciseptatum* Van den Boom  
 – Ascospores 35–55 × 5–16 µm, 3–9 × as long as broad, 2–4 per ascus . . . . . **5**
5. Ascospores 45–55 × 5–8 µm, 7–9 × as long as broad, submuriform, 2–4 per ascus; foliicolous; eastern palaeotropics (Indonesia) . . . . .  
     . . . . . *Lasioloma spinosum* Hafellner & Vězda  
 – Ascospores 35–50 × 12–16 µm, 2.7–3.5 × as long as broad, muriform, mostly 2 per ascus; corticolous; neotropics (Netherlands Antilles) . . . . .  
     . . . . . *Lasioloma antillarum* Lücking, Högnabba & Sipman
6. Thallus distinctly warty; medulla becoming yellow to reddish; corticolous; neotropics and African palaeotropics . . . . .  
     . . . . . *Lasioloma stephanellum* (Nyl.) Lücking & Sérus.  
 – Thallus smooth to uneven; medulla not pigmented; foliicolous . . . . . **7**
7. Cephalodia absent; thallus dispersed, smooth; woolly prothallus conspicuous; pantropical . . . . .  
     . . . . . *Lasioloma arachnoideum* (Kremp.) R. Sant.  
 – Vermicular cephalodia usually present; thallus continuous to marginally dispersed, smooth to uneven; woolly prothallus developed mostly marginally; eastern palaeotropics . . . . . **8**
8. Ascospores 90–115 µm long; thallus continuous, smooth . . . *Lasioloma phycophilum* (Vain.) R. Sant.  
 – Ascospores 60–90 µm long; thallus marginally dispersed, uneven . . . . . **9**
9. Apothecial disc dark greyish brown . . . . .  
     . . . . . *Lasioloma phycophorum* (Vain.) R. Sant.  
 – Apothecial disc light greyish brown . . . . .  
     . . . . . *Lasioloma trichophorum* (Vain.) R. Sant.

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### Supplemental content online

See <https://doi.org/10.3372/wi.51.51107>

Supplementary File S1. Concatenated alignment of four markers (in order: mtSSU, nuLSU, ITS, RPB1) for the taxa included in this study (in FASTA format).

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