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## ***Steccherinum filiferum* sp. nov. from the neotropics, and a new combination for *Odontia laxa***

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**ABSTRACT**—The new species *Steccherinum filiferum*, from Puerto Rico and Amazonian rainforests of Ecuador, is described. It is characterized by effused basidiomata with hyphal cords, a monomitic hyphal system, simple-septate hyphae, and minute hymenophoral aculei with encrusted, septate, *Candelabrochaete*-like cystidia. Because *Odontia laxa* [= *Odontium laxum*] is morphologically and phylogenetically similar to *S. filiferum*, the new combination *Steccherinum laxum* is proposed, after studying its type. Scanning electron microscopy studies show that *S. filiferum* and *S. laxum* can be differentiated by crystal size and arrangement on the cystidia. A key to the *Steccherinum* species with simple-septate generative hyphae is provided.

**KEY WORDS**—Caribbean, palm leaves, *Polyporales*, septocystidia, *Steccherinaceae*

## Introduction

*Steccherinum* Gray (*Steccherinaceae*, *Polyporales*) is a large genus with worldwide distribution. Ten species were listed for temperate northern hemisphere (Jülich & Stalpers 1980) and 27 species for tropics, subtropics, and the southern hemisphere (Hjortstam & Ryvarden 2007). Seventy-two species are accepted in this genus according to MycoBank (Robert & al. 2022). The species of the genus are saprobes on decaying wood, bark, and soft plant debris, and an ability for white rot is indicated (Ginns & Lefebvre 1993).

The morphological concept of this genus changed significantly over the last 50 years. According to an earlier concept (Maas Geesteranus 1974), *Steccherinum* species are characterized by spinose hymenophore, dimitic hyphal system, clamped generative hyphae, thick-walled tramal cystidia with crystalline incrustations, and variously shaped basidiomata from effused, effused-reflexed, to flabelliform and short-stipitate. In subsequent taxonomic works, three species with simple-septate generative hyphae (Svrček 1973, Ryvarden 1978, Hjortstam 1984, Liu & Dai 2021, Wu & al. 2021) and several species with poroid hymenophore (Knudsen & Hansen 1996, Miettinen & Ryvarden 2016) were included in this genus. As a consequence, the morphological distinctions among the genera *Irpex* Fr., *Steccherinum*, and *Junghuhnia* Corda were blurred. The polyphyletic nature of the two latter genera was confirmed by a multigene phylogenetic reconstruction (Miettinen & al. 2012). A further expansion of *Steccherinum* morphological concept resulted after studies by Liu & Dai (2021) who described *S. fragile* Z.B. Liu & Y.C. Dai, a species possessing a monomitic hyphal system, simple septa, poroid hymenophore, and lack of cystidia.

In this paper, a new species of *Steccherinum* is described from the neotropics, and *Odontia laxa* is transferred to *Steccherinum*.

## Materials & methods

Specimens of the new *Steccherinum* were obtained during collection trips to Puerto Rico (K.H. Larsson, in 1996) and to South and Central America (E. Yurchenko, in 2019). In Ecuador, specimens were collected on dead wood along a route and in a sample plot 20×20 m in forests less influenced by human activities. Specimens examined are deposited in the Herbariums of Białystok University of Technology, Hajnówka, Poland (BLS) and University of Gothenburg, Göteborg, Sweden (GB).

Basidiome descriptions are based on dry specimens. Micromorphology was studied on basidiome sections mounted in 3% aqueous KOH solution. Crystalline incrustations and spores were examined in Melzer's reagent, and

cyanophily of spores was observed in 0.02% cotton blue in 50% aqueous lactic acid. A Nikon Eclipse Ni-U light microscope (Nikon Corp., Japan) was used for measurements, mostly under  $\times 1000$  magnification using the NIS-Elements Br imaging software (Nikon Corp.). Mean spore length (L) and width (W) were calculated as arithmetic averages for 30 randomly selected spores. Spore quotient, Q, is the length/width ratio for individual spores.

Scanning electron (SEM) images were obtained using Phenom G2 pro desktop microscope (Labmate, UK). Pieces of basidiomata, 3–4 mm in extent, were removed from dried specimens, attached by double-sided adhesive film to metallic stands, specific to this model of microscope, and coated with 1.4 nm layer of gold in a Leica EN ACE200 vacuum coater (Leica Microsystems, Germany).

DNA extraction was done with E.Z.N.A.<sup>®</sup> Fungal DNA Mini Kit (Omega Bio-Tek, VWR, Radnor, Pennsylvania, USA). Primers ITS1, ITS2, ITS3 (White & al. 1990) and ALR0 (Collopy & al. 2001) were used for DNA amplification of ribosomal internal transcribed spacer (ITS) region that included the ITS 1, ITS 2, and 5.8S gene. PCR products were purified with a DNA Clean & Concentrator<sup>®</sup>-5 kit (Zymo Research, Irvine, California, USA), then sequenced by LGC Genomics GmbH (Berlin, Germany). The ITS sequence was edited and assembled with MEGA X (Kumar & al. 2018) and the five quality check guidelines were applied (Nilsson & al. 2012). Sequence was deposited in NCBI GenBank (<https://www.ncbi.nlm.nih.gov/genbank>).

## Results

The new ITS sequence OP279612 from a specimen from Ecuador (BLS M-5230) is 99.1% similar to JN710524 (*Candelabrochaete* sp., Miettinen & al. 2012), obtained from a specimen collected in Puerto Rico (KHL 9495, GB-0087369). We believe that the 0.9% difference in ITS sequences (with three mismatch positions and three positions with ambiguity symbols) indicate that they are the same species. The ITS sequence of *Odontium laxum* JN710577, based on KHL 12268, had the next highest similarity (89.75%) with our new sequence in a BLAST search.

The phylogenetic analysis of Miettinen & al. (2012, Fig. 4) placed the new species as a sister taxon to *Odontium laxum* KHL 12268 within the clade of *Steccherinum* s.str. To ascertain the identity of *O. laxum* KHL 12268, we compared it with an isotype specimen at GB. Both specimens have effused, minutely odontoid basidiomata (FIG. 1) with similar hyphae, hymenial elements, basidiospores, and crystalline incrustations on the cystidia (FIG. 2).

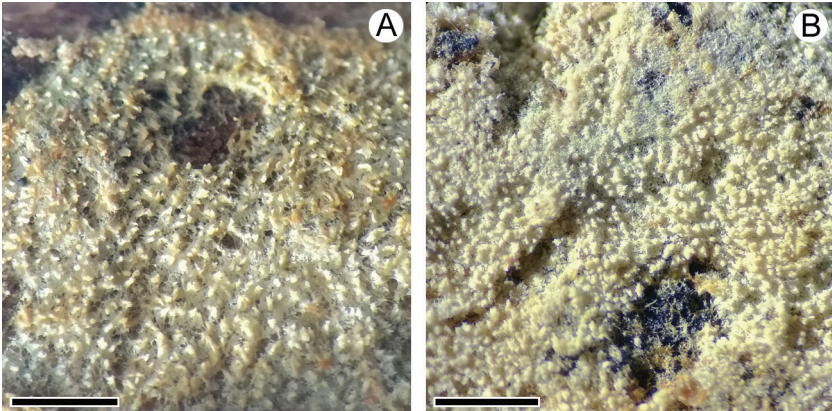


FIG. 1. *Steccherinum laxum*, macromorphology: A. GB KHL 12268; B. isotype of *Odontia laxa* (GB 19282). Scale bars = 1 mm.

Both specimens possess similar hyphal cords also. The basidiospores were similar in size and shape:  $L = 2.8 \mu\text{m}$ ,  $W = 1.8 \mu\text{m}$  in KHL 12268;  $L = 2.9 \mu\text{m}$ ,  $W = 1.9 \mu\text{m}$  in the isotype. Specimen KHL 12268 has a denser hyphal composition of aculei and 5-7 aculei/mm, compared to 6-8 aculei/mm in the isotype. Also, encrusted cystidia are barely visible or absent in some aculei in KHL 12268, whereas they are numerous and more distinctly encrusted in the isotype, resulting in a setose appearance of the aculei. From these observations, we conclude that KHL 12268 is conspecific with the isotype of *O. laxum*. Based on these phylogenetic and morphologic results, we propose the new combination *Steccherinum laxum* below.

The morphology of the new species was compared with the descriptions of all species of *Steccherinum*, *Candelabrochaete* Boidin, and *Odontidium* Parmasto, listed in Index Fungorum (<http://www.indexfungorum.org/names/names.asp>) with enough information for an adequate comparison. Because no published taxon was like our new species in micro- and macromorphology, and based on ITS sequence data, we propose a new species, described below.

The new taxon, *Steccherinum filiferum*, is morphologically similar or even identical with *S. laxum* in its hyphal, basidial, and spore morphology. However, we observed differences in crystalline incrustations on the cystidia in KOH solution and Melzer's reagent. SEM study revealed significant differences in the size and attachment of the crystals. In *S. laxum*, the crystals are primarily large, plate-like, and broadly attached (FIG. 3A), giving the cystidia a rough, thick-walled appearance at  $\times 1000$  magnification (FIG. 2D). In comparison, the

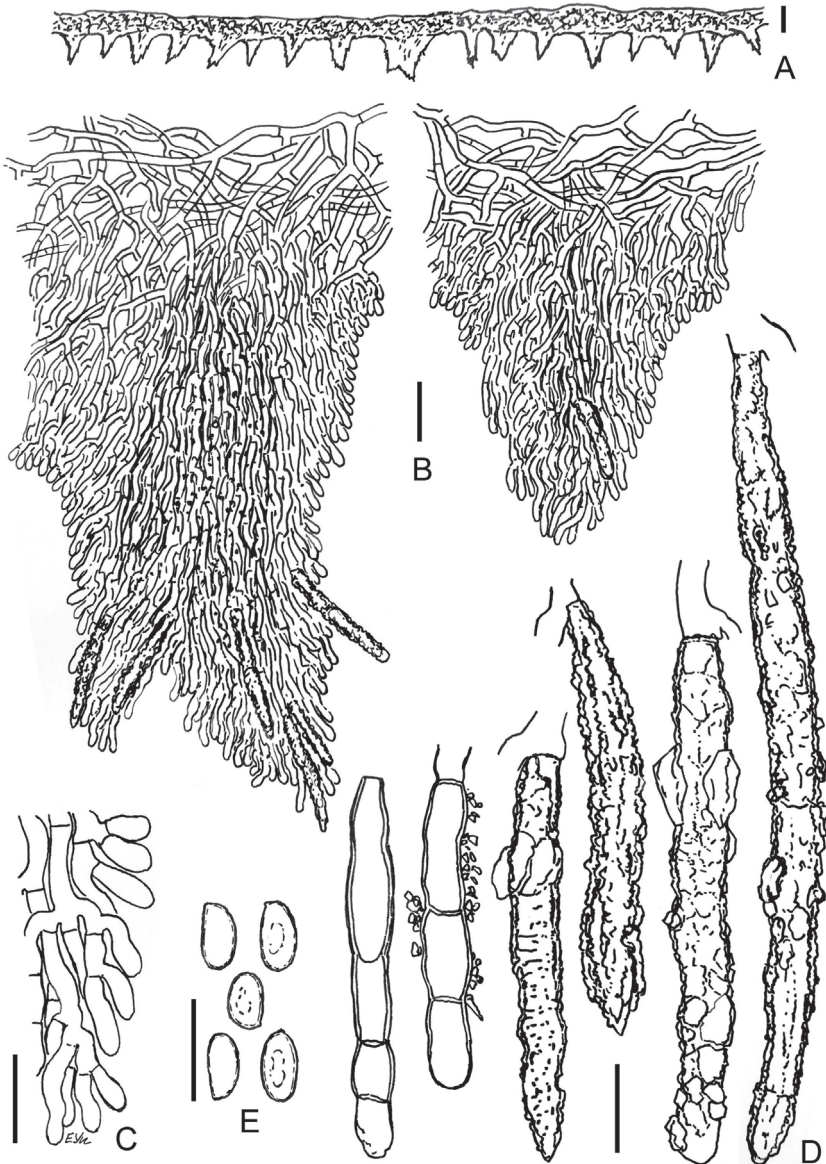


FIG. 2. *Steccherinum laxum* (GB KHL 12268): A, B. vertical sections through basidioma; C. portion of aculeal apex; D. smooth and variously encrusted cystidia; E. basidiospores. Scale bars: A = 100  $\mu$ m; B = 20  $\mu$ m; C, D = 10  $\mu$ m; E = 5  $\mu$ m.



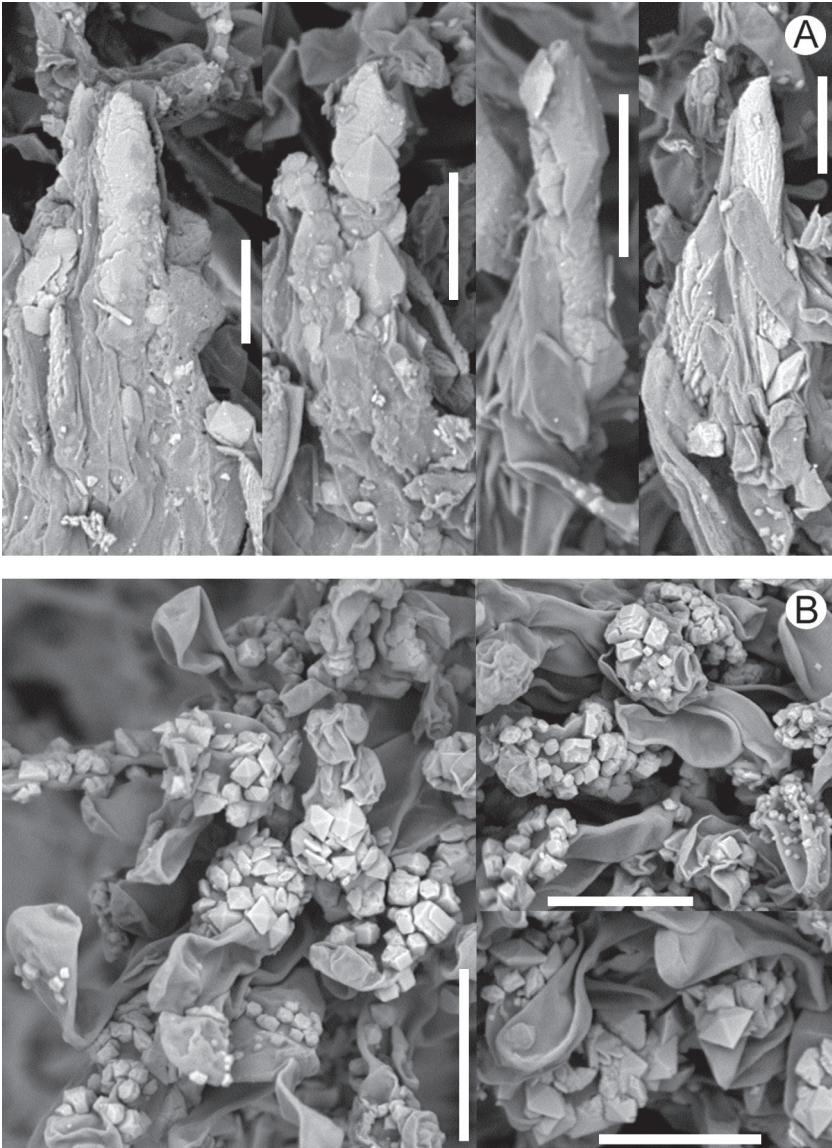


FIG. 3. SEM images of crystalline deposits on cystidia: A. *Steccherinum laxum* (isotype, GB 19282); B. *Steccherinum filiferum* (holotype, GB-0087369 [KHL 9495]). Scale bars = 10  $\mu$ m.

crystals in the new *Steccherinum* are smaller, distinct, aggregated, and appear loosely attached (FIG. 3B).

## Taxonomy

*Steccherinum filiferum* Yurchenko & K.H. Larss., **sp. nov.**

FIGS 3B, 4-6

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Differs from *S. laxum* by its cystidia encrusted with loosely attached aggregates of isodiametric crystals, and lack of numerous smooth hyphoid elements in upper part or aculei.

TYPE: USA, Puerto Rico, Isabela municipality, Bosque Estatal de Guajataca (Guajataca Commonwealth Forest), Montañas Aymamón, 18.4242N 66.9678W, 230 m a.s.l., moist subtropical forest, on decaying corticated branch of angiosperm tree on the ground, leg. K.-H. Larsson, 26.VI.1996, KHL9495 (**Holotype**, GB-0087369; GenBank JN710524, JN710613, JN710662, JN710713).

ETYMOLOGY: from the Latin *filiferum* = carrying threads, due to the presence of hyphal cords.

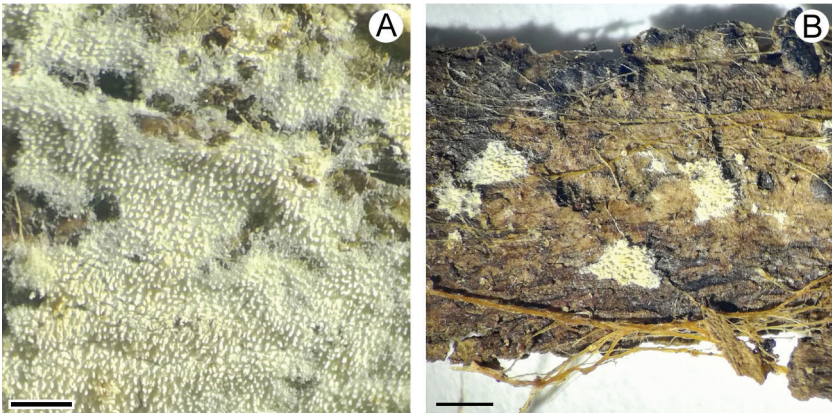


FIG. 4. *Steccherinum filiferum*, macromorphology: A. holotype, GB-0087369 (KHL 9495); B. BLS-M 5228, with basidiomata and hyphal cords. Scale bars = 1 mm.

**BASIDIOMA** effused, from 0.5-3 mm to 5 cm and more in extent, cream or pale ochraceous, soft-membranous and very fragile when young, membranous in older state, minutely odontoid, between aculei 25-100  $\mu$ m thick, discontinuous to continuous or minutely cracked. **ACULEI** subcylindrical, blunt, 50-150  $\mu$ m high, 30-80  $\mu$ m in diam (5-8/mm). **MARGIN** abrupt or arachnoid and then concolorous with hymenial surface, 0.3-0.5 mm wide.

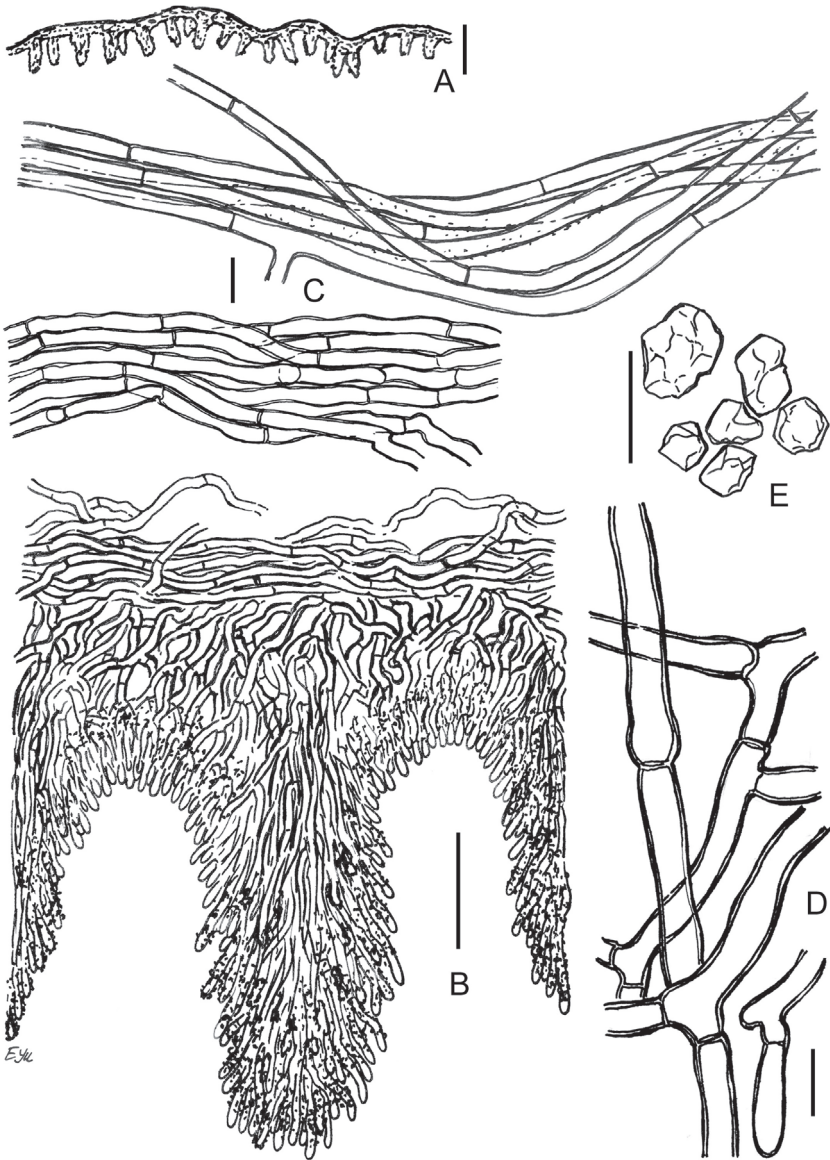


FIG. 5. *Steccherinum filiferum* (holotype, GB-0087369 [KHL 9495]): A, B. vertical sections through basidioma; C. hyphal cords; D. subicular hyphae; E. crystals from subiculum. Scale bars: A = 200  $\mu$ m; B = 50  $\mu$ m; C-E = 10  $\mu$ m.



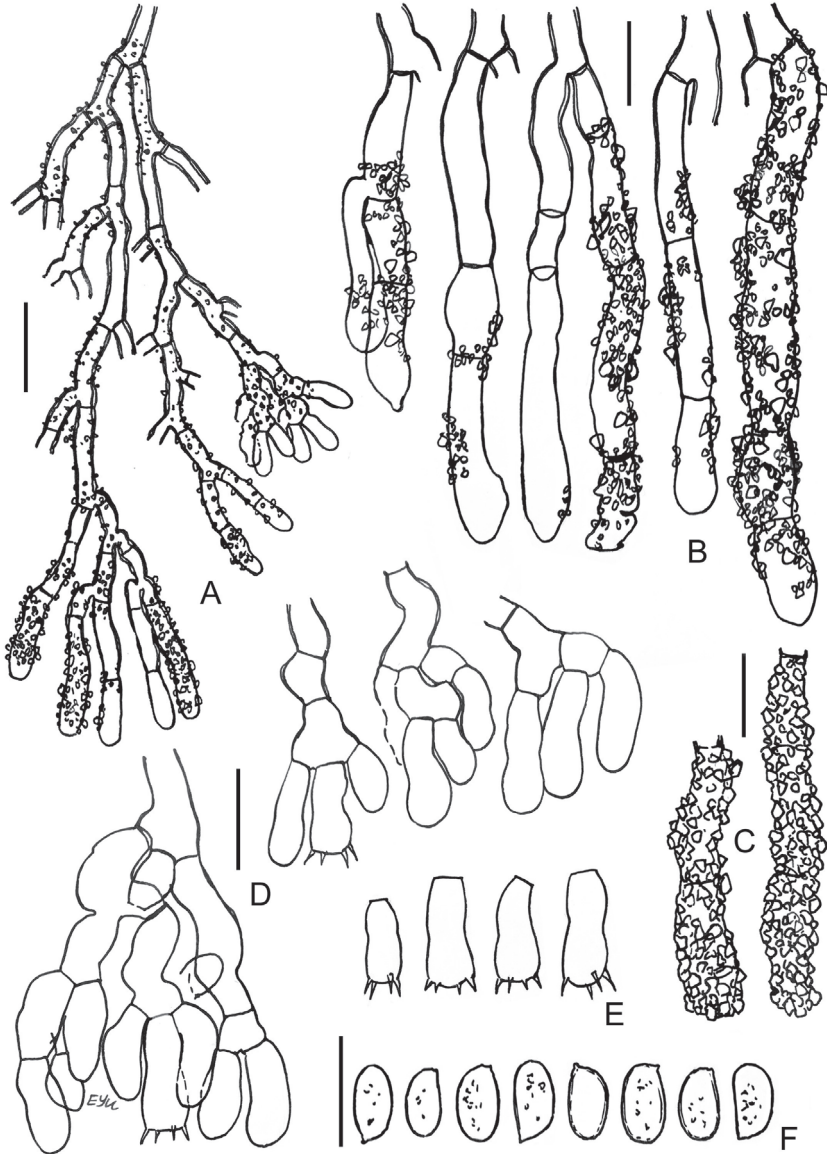


FIG. 6. *Steccherinum filiferum* (holotype, GB-0087369 [KHL 9495]): A. a portion of hyphae from aculeal trama ending by cystidia and basidioles; B. cystidia in KOH; C. cystidia in Melzer's reagent; D. portions of hymenium and subhymenial hyphae; E. basidia; F. basidiospores. Scale bars: A = 20  $\mu$ m; B-E = 10  $\mu$ m; F = 5  $\mu$ m.

**HYPHAL SYSTEM** monomitic, hyphae simple-septate, colorless to pale yellow, smooth or loosely encrusted. **HYPHAL CORDS** present between fruitbody patches and in subiculum, yellowish or ochraceous, 15–100(–150)  $\mu\text{m}$  diam; consisting of 2–40 hyphae, individual hyphae 3.3–8.5  $\mu\text{m}$  wide. **SUBICULAR HYPHAE** (3–)3.5–6  $\mu\text{m}$  diam, with some segments inflated to 8.5  $\mu\text{m}$ , often branched at right angles or nearly so, thin- to thick-walled, walls up to 1.5  $\mu\text{m}$  thick and appearing layered. **SUBHYMENIAL HYPHAE** 2.5–5  $\mu\text{m}$  diam, thin-walled, short-celled.

**CYSTIDIA** (projecting hyphae) in aculei with 0–2 septa, in upper part cylindrical or slightly clavate, rarely submucronate, 4–6  $\mu\text{m}$  wide, richly encrusted by isodiametric bipyramidal crystals 1–3  $\mu\text{m}$  in size in Melzer's reagent, loosely encrusted in KOH, rarely naked, colorless or pale yellowish.

**BASIDIOLES** ovoid, short clavate or short cylindrical, 6–13.5  $\times$  (3.2–)4–5  $\mu\text{m}$ , colorless. **BASIDIA** short clavate or subcylindrical, 10–13  $\times$  4.5–4.7  $\mu\text{m}$ , thin-walled, colorless, smooth or barely encrusted; sterigmata four, minute, about 1.5  $\times$  0.3  $\mu\text{m}$ . **BASIDIOSPORES** narrowly elliptic, oblong or short cylindrical, 3–3.5(–4)  $\times$  1.7–2.3  $\mu\text{m}$  ( $L = 3.2 \mu\text{m}$ ,  $W = 1.9 \mu\text{m}$  in holotype),  $Q = 1.7$ –2, thin-walled (appearing slightly thick-walled at  $\times 1000$  magnification), sometimes guttulate, inamyloid, acyanophilous; apiculus small or indistinct.

**ECOLOGY & DISTRIBUTION**—So far known from Puerto Rico and Ecuador; inhabits dead branches and remains of palm leaves in evergreen moist subtropical forest and tropical rainforest.

**ADDITIONAL SPECIMENS EXAMINED**—**ECUADOR, ORELLANA PROVINCE, between Puerto Francisco de Orellana and El Dorado**, right bank of the Napo River, 0.4851S, 76.9495W, 260 m a.s.l., rainforest, on fallen woody stem, leg. E. Yurchenko, 20.VII.2019, EYu190720-59 (BLS M-5227); **vicinity of Flor de Oriente**, 0.5042S 76.9819W, 295 m a.s.l., rainforest, on dead rachis of a palm leaf, leg. E. Yurchenko, 21.VII.2019, EYu190721-39 (BLS M-5230; GenBank OP279612); on fallen twig, leg. E. Yurchenko, 21.VII.2019, EYu190721-42 (BLS M-5228); on decaying fallen palm leaf, leg. E. Yurchenko, 21.VII.2019, EYu190721-43a (BLS M-5229).

**COMMENTS**—Most basidiomata in specimens from Ecuador are small, 0.5–3 mm in extent, exceptionally up to 15 mm. The substratum under and around the basidiomata is covered by hyphal cords, especially numerous and notable in BLS-M 5228 (FIG. 4B). Three of four Ecuadorian specimens (BLS M-5228, 4229, 5230) were found within one 400 m<sup>2</sup> sample plot.

***Steccherinum laxum* (L.W. Mill.) K.H. Larss. & Yurchenko, comb. nov.**

MB 845419

≡ *Odontia laxa* L.W. Mill., *Mycologia* 26(1): 18 (1934).

≡ *Odonticium laxum* (L.W. Mill.) Ryvarde, *Norweg. J. Bot.* 25: 296 (1978).

SPECIMENS EXAMINED—*Steccherinum laxum*: **USA, ?OHIO**, 'on elm', leg. Morgan, 30.VII.1893 (GB 19282, **isotype** of *Odontia laxa*); **TENNESSEE, Cosby**, Great Smoky Mountains National Park, along Snake Den Ridge Trail, mixed hardwood-*Thuja* forest, on decaying unidentified wood, leg. K.H. Larsson, 14.VII.2004, KHL 12268 (GB, as *Odonticium laxum*; GenBank JN710577, JN710642, JN710694, JN710729).

**A key to *Steccherinum* sensu lato species with simple-septate hyphae**

1. Hyphal system monomitic, pseudocystidia absent ..... 2
1. Hyphal system dimitic, pseudocystidia present ..... 5
2. Basidioma stipitate; cystidial elements absent; hyphae more or less constricted at septa ..... ***S. minutissimum***  
Maas Geesteranus (1974) noted that this species is not a true *Steccherinum* but did not give an opinion about its generic position.
2. Basidioma effused; cystidial elements present or absent; hyphae rarely slightly constricted at septa ..... 3
3. Hymenophore poroid; cystidial elements absent ..... ***S. fragile***
3. Hymenophore minutely odontoid; cystidial elements present ..... 4
4. Aculei in the upper part bearing cylindrical, one-celled or septate cystidia, more or less encrusted by small, loosely attached crystals, partly dissolving or detaching in KOH ..... ***S. filiferum***
4. Aculei in upper part bearing numerous, smooth hyphoid elements, and scarce, one-celled or septate cystidia, more or less encrusted by large, plate-like, broadly attached crystals, stable in KOH ..... ***S. laxum***
5. Basidioma effused-reflexed, spores  $3-5.3 \times 2.5-3.5 \mu\text{m}$  ..... ***S. rubigimaculatum***
5. Basidioma effused; spores  $2.5-4 \times 1.3-2.5 \mu\text{m}$  ..... 6
6. Basidioma easily detachable from the substratum; aculei up to 0.5 mm long, with conspicuous short-celled terminal hyphae ..... ***S. subcrinale***
6. Basidioma closely adnate; aculei 0.1-0.3 mm long, without short-celled terminal hyphae ..... 7
7. Basidioma coriaceous, tough; spores oblong-ellipsoid or short cylindrical,  $1.3-1.5 \mu\text{m}$  wide ..... ***S. aridum***
7. Basidioma soft; spores ellipsoid or broadly ellipsoid,  $2-2.2 \mu\text{m}$  wide ..... ***S. cremeoalbum***

## Discussion

The first collection of *Steccherinum filiferum* (the holotype collection, KHL 9495) was tentatively identified as a *Candelabrochaete* s.lat., due to its monomitic simple-septate hyphal system and the septate, cystidium-like hyphae at the apex of the aculei. *Candelabrochaete* is a paraphyletic genus (Justo & al. 2017, Li & al. 2022) typified by *C. africana* Boidin, described from Central African Republic. Available sequence of *C. africana* (from Puerto Rico and may not be conspecific with African collections) places it in an isolated position within the *Polyporales*, far removed from the *Steccherinum* clade (Miettinen & al. 2012, Justo & al. 2017). The cystidia in *S. filiferum* may be interpreted as hyphal ends originating from the aculeal trama and not morphologically homologous to septocystidia in *C. africana*. We considered placing the new species in *Odonticium* also, because of its monomitic hyphal system and simple septa. However, this genus is typified by *O. romellii* (S. Lundell) Parmasto, a distinct species with a dense trama and allantoid spores, that belongs in the *Hymenochaetales* (Larsson & al. 2006, Liu & al. 2019).

In their study of the *Steccherinaceae*, Miettinen & al. (2012) showed that *Steccherinum* s.lat. is polyphyletic and discussed the difficulty of defining a monophyletic *Steccherinum* s.str. Both *S. filiferum* and *S. laxum* have morphological features that are atypical for *Steccherinum*, a monomitic hyphal system and loose, open texture of subiculum. They form a strongly supported lineage within the *Steccherinum*-clade (Figure 4 in Miettinen & al. 2012). Although it may be defensible to create a new genus to accommodate these morphologically deviant taxa, following this line of reasoning would necessitate the creation of genera for *Steccherinum tenue* Burds. & Nakasone, *Steccherinum collabens* (Fr.) Vesterh., *Steccherinum nitidum* complex, and other strongly supported clades with typical *Steccherinum* characters. We believe, however, that the best solution is to include the *S. laxum* clade in *Steccherinum* s.lat. rather than divide the genus into many, smaller and morphologically unrecognizable genera.

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