Clonostachys saulensis (*Bionectriaceae, Hypocreales*), a new species from French Guiana

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Abstract: *Clonostachys saulensis* sp. nov. is described and illustrated based on a collection on bark of dead liana in French Guiana. This species is placed in *Clonostachys* (= *Bionectria*) based on its clonostachys-like asexual morph, ascomata not changing colour in 3% KOH or lactic acid and phylogenetic comparison of ITS sequences with known species of *Clonostachys*. *Clonostachys saulensis* is primarily characterized by non-stromatic, smooth, pale brown, globose ascomata coated with a whitish powdery scurf from base up to half height and turning blackish upon drying. Based on comparison of morphological characteristics of sexual-asexual morphs and molecular data with known species, *C. saulensis* is proposed as a new species.
Keywords: Ascomycota, ribosomal DNA, taxonomy.

Résumé : *Clonostachys saulensis* sp. nov. est décrite et illustrée d'après une récolte effectuée sur écorce de liane morte en Guyane française. Cette espèce est placée dans le genre *Clonostachys* (= *Bionectria*) d'après sa forme asexuée de type clonostachys, les ascomes ne changeant pas de couleur dans KOH à 3% ou dans l'acide lactique et la comparaison phylogénétique des séquences ITS avec les espèces connues de *Clonostachys*. *Clonostachys saulensis* est principalement caractérisée par des ascomes globuleux, sans stroma, brun pâle, couverts d'une pellicule poudreuse blanchâtre de la base jusqu'à la moitié de la hauteur, devenant noirâtres en séchant. En se fondant sur la comparaison des caractères morphologiques et des données moléculaires avec les espèces connues, *C. saulensis* est proposée comme une nouvelle espèce.
Mots-clés : ADN ribosomal, Ascomycota, taxinomie.

Introduction

During an inventorial survey of fungi in Saül (French Guiana) in August 2018 (Lechat & Fournier, 2018; 2019), an intriguing hypocrealean fungus was collected on dead bark of the woody liana *Bauhinia* sp. (*Fabaceae*). Its scattered, superficial, non-stromatic subglobose ascomata were distinctive in being brown and coated in the lower half by a white powdery scurf and became more remarkable in turning blackish upon drying, making the contrast with the white scurf even more striking (Fig. 2 a and b). This fungus was morphologically characterized, cultured and an ITS sequence used in a phylogenetic analysis. In this paper we present our results leading to its placement in *Clonostachys* Corda (*Bionectriaceae*) and a description of the new species *C. saulensis*.

Material and methods

Dry specimens were rehydrated and examined using the method described by Rossman *et al.* (1999). Microscopic observations and measurements were made in water. The holotype specimen was deposited in LIP herbarium (University of Lille, France) and living cultures at CIRM-CF (Centre International des Resources Microbiennes, Marseille, France). Cultures of the living specimen were plated on PDA (Potato Dextrose Agar) with 5mg/l of streptomycin in Petri dishes 5 cm diam, incubated at 25° C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Santander, Spain) as follows: total DNA was extracted from dry specimens blending a portion using a micropestle in 600 µL CTAB buffer (CTAB 2%, NaCl 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65° C. A similar volume of chloroform:isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifuged for 10 min at 13,000 g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in 70% cold ethanol, centrifuged again for 2 min and dried. It was finally resuspended in 200 µL ddH_2O. PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) for ITS. Chromatograms were checked searching for putative reading errors, and these were corrected.

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (Tamura *et al.*, 2013). Nomenclature follows Mycobank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

Taxonomy

Clonostachys saulensis Lechat & J. Fourn., *sp. nov.* Fig. 2
Mycobank: MB830252

Diagnosis: Differs from all known species of *Clonostachys* in having pale brown ascomata becoming dark brown to nearly black when dry, coated with a white powdery scurf on the lower half.

Holotype: French Guiana, Saül, Gros Arbres trail, on dead bark of *Bauhinia* sp., 22 Aug. 2018, *leg*. C. Lechat, CLLG18023-A5 (LIP CLLG18023-A5), ex-holotype culture: BRFM 2782, ITS GenBank sequence: MK635054

Etymology: The specific epithet "*saulensis*" refers to Saül, the locality where this species was collected.

Ascomata solitary, superficial, scattered on substrate, non-stromatic, smooth in upper part, pale brown when fresh, becoming dark brown to nearly black when dry, subglobose, (380–)400–430 (–450) µm high, 380–420 µm diam (Me = 420 × 400 µm, n = 10), coated from base up to half height with a powdery, whitish, amorphous scurf, collapsing cupulate or laterally pinched when dry, difficult to remove from substrate, not changing colour in 3% KOH or lactic acid. Perithecial apex convex with a minute, concolorous, pointed papilla 50–70 µm diam, composed of thick-walled, ellipsoidal, elongated cells 7–10 µm long, 2–3 µm wide with wall 1 µm thick, pale yellowish brown. **Ascomatal wall** 45–55(–60) µm thick, composed of two regions: outer region 35–40 µm wide, of subglobose to ellipsoidal thick-walled cells 7–25 × 5–12 µm, with pale orange walls 1.5–2.5 µm thick; inner region 10–16 µm wide, of elongated, flattened thick-walled cells 7–15 × 4–6 µm, with hyaline walls 1.5–2.5 µm thick. **Ascomatal surface** composed of subglobose to ellipsoidal, subangular, thick-walled cells up to 25 µm in greatest dimension with wall 2–3 µm thick, partially covered by whitish, powdery scurf consisting of minute particles of ill-defined shape, not dissolving and not changing colour in 3% KOH. **Asci** (55–)70–80(–90) × (11–) 12–14(–18) µm (Me = 75 × 13 µm, n = 20), short stipitate, clavate, apex slightly flattened, with a refractive ring, containing eight biseriate ascospores or biseriate above and uniseriate below. **Ascospores** (13–)14–17(–18) × 4.5–5.5(–6) µm (Me = 15 × 5 µm, n = 30), narrowly ellipsoidal to fusiform with attenuated ends, equally two-celled, slightly constricted at septum, hyaline, spinulose.

Culture characteristics: After two weeks on PDA at 25° C, colony 30–40 mm diam, aerial mycelium white in centre, faintly zonate in median area with pale yellow to pale orange zonation lines, off-white at margin, without colouration in medium. Mycelium composed of septate, hyaline, smooth hyphae 2.5–3.5 µm diam. Conidiophores monomorphic, penicillate, arising from aerial hyphae, macronematous, flexuous, hyaline, stipe and lateral branches 15–30 long, 3–3.5(–4) µm diam, bearing subulate conidiogenous cells 12–20 µm long, 2.5–3 µm diam at base. Conidia hyaline, aseptate, oblong to subfusiform with rounded apex, attenuated towards base with or without a median, apiculate hilum, smooth-walled, 8.5–11(–12) long, 3.5–4 µm wide in the widest part.

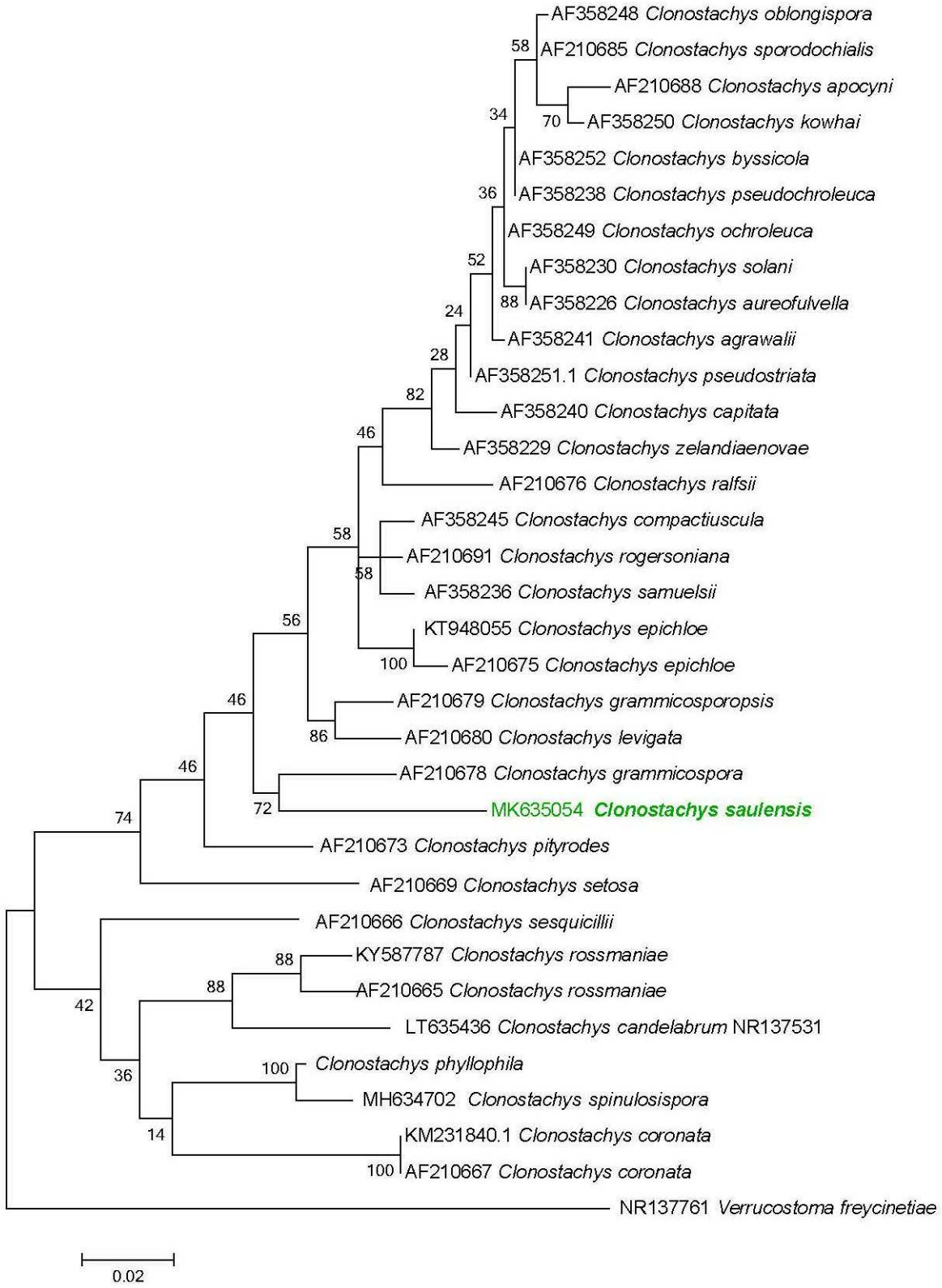

Fig. 1 – Maximum likelihood phylogeny (-lnL = 2516.77551) of *Clonostachys saulensis* inferred by PhyML 3.0, model TS93 from a 640 bp matrix of ITS sequences, rooted with *Verrucostoma freycinetiae*, which has acremonium-like asexual morph.

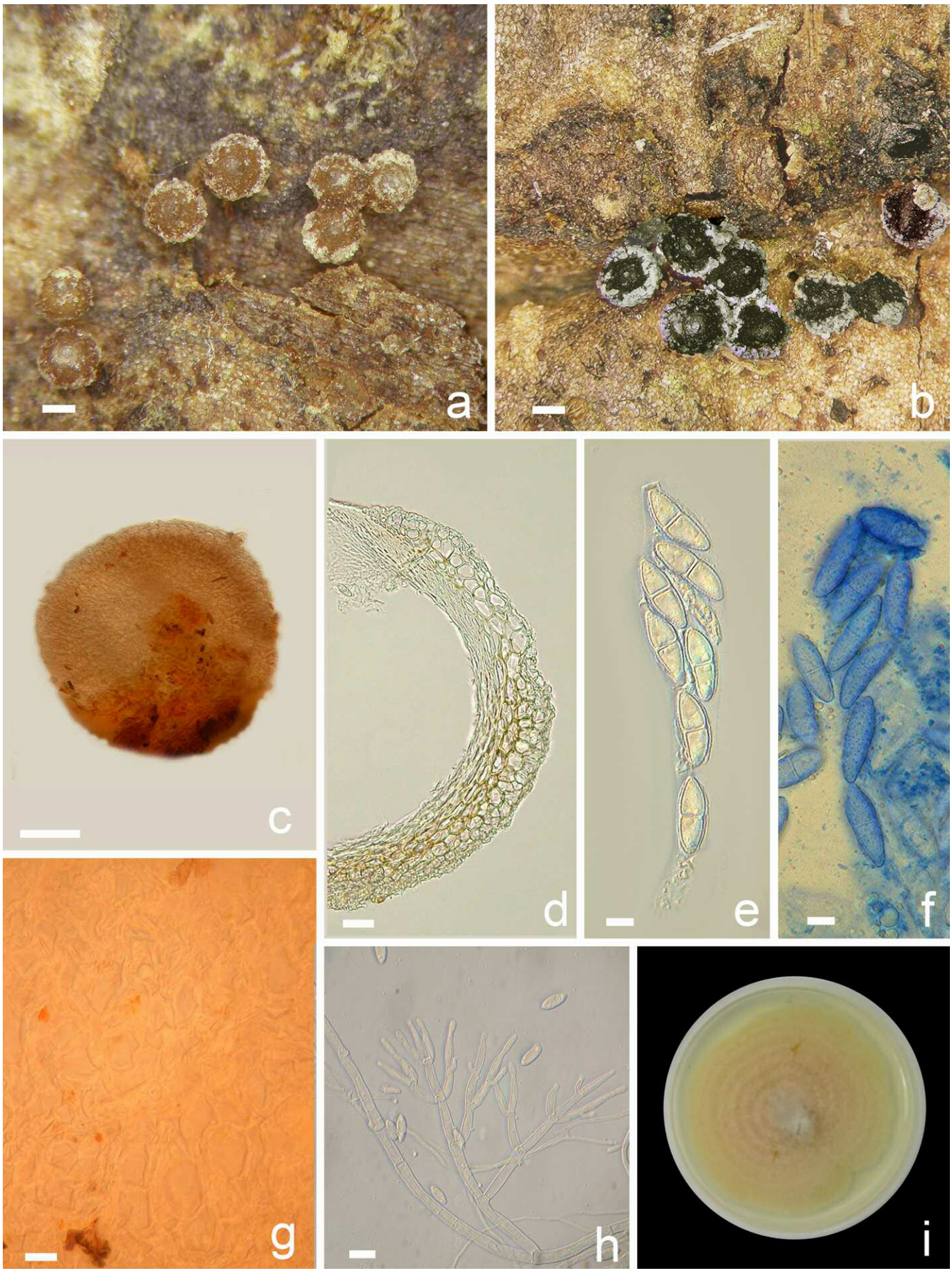

Fig. 2 – a-i: *Clonostachys saulensis* (Holotype LIL CLLG18023-A5); a: Fresh ascomata on the substrate; b: Dry ascomata appearing nearly black; c: Ascoma in water in side view; d: Vertical section of lateral ascomatal wall; e: Ascus and ascospores in water; f: Ascus and ascospores in lactic cotton blue showing a discrete spinulose ornamentation; g: Cells of ascomatal surface; h: Conidiophores and conidia in lactic acid; h: Culture at three weeks. Scale bars: a, b = 200 µm; c = 100 µm; d = 20 µm; e-h: = 5 µm.

Results and discussion

Clonostachys was reinstated by Rossman *et al.* (2013) replacing *Bionectria* Speg. to comply with the new rules of the International Code of Nomenclature (ICN) concerning pleomorphic fungi (Article 59.1, McNeill *et al.*, 2012). The family *Bionectriaceae* Samuels & Rossman is morphologically characterized by perithecial ascomata with light-coloured (white, pale tan, orange or brown) wall that does not change colour in 3% KOH or lactic acid (Rossman *et al.*, 1999; 2001). Within the *Bionectriaceae*, *Clonostachys* is defined by penicillate, frequently sporodochial asexual morphs and this segregation is supported by molecular evidence (Hirooka *et al.*, 2010; Rossman *et al.*, 2001; Schroers, 2001). There are sixty-five taxa reported in Index Fungorum (www.indexfungorum.org) including forty-two as *Bionectria*. Undoubtedly many unknown species have yet to be discovered, especially in tropical areas.

Based on the ascomata not changing colour in 3% KOH or lactic acid, clonostachys-like asexual morph obtained in culture and phylogenetic analysis of ITS sequences, the new species described above is unambiguously placed in *Clonostachys*.

The phylogenetic analysis carried out in the present study (Fig. 1), comparing *C. saulensis* with 29 species of *Clonostachys* places our fungus in a subclade along with *C. grammicospora* Schroers & Samuels with 95% similarity of their ITS sequences. Morphologically, *C. grammicospora* clearly differs from *C. saulensis* in having stromatic, orange and coarsely warted ascomata, smaller and striate ascospores as well as shorter conidia (Schroers, 2001).

Clonostachys saulensis is primarily characterized by non-stromatic, pale brown ascomata becoming dark brown to blackish when dry, a most unusual colour in *Clonostachys* which generally features pallid ascomata. Blackish ascomata in dry condition coated with white basal scurf, combined with the results of our phylogenetic analysis showing the unique position of this species in the *Clonostachys* clade justify the status of *C. saulensis* as a distinctive new species.

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