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

Christian LECHAT (†) Abstract: Lasionectria saulensis sp. nov. is described and illustrated based on a collection on dead leaves of **Jacques FOURNIER** Astrocaryum sp. (Arecaceae) in French Guiana. This species is placed in Lasionectria based on its acremonium-like asexual morph, ascomata not changing colour in 3% KOH or lactic acid, and phylogenetic com-**Delphine CHADULI** parison of ITS and LSU sequences with known species of Lasionectria. Lasionectria saulensis is primarily **Anne FAVEL** characterised by non-stromatic, pale orange, subglobose, hairy ascomata with a conical apex, and striate ascospores. Based on comparison of morphological characteristics and molecular data with known Lasio-Ascomycete.org, 14 (3): 85-88 nectria species, L. saulensis is proposed as a new species. Mise en ligne le 11/07/2022 Keywords: Astrocaryum, ribosomal DNA, taxonomy. ڡ 10.25664/ART-0350 Résumé : Lasionectria saulensis sp. nov. est décrite et illustrée d'après une récolte effectuée sur feuille morte d'Astrocaryum sp. (Arecaceae) en Guyane française. Cette espèce est placée dans le genre Lasionectria d'après CC BY-NC-ND sa forme asexuée de type Acremonium, les ascomes ne changeant pas de couleur dans KOH à 3% ou dans l'acide lactique et la comparaison phylogénétique des séquences ITS et LSU avec les espèces connues de Lasionectria. Lasionectria saulensis se caractérise principalement par des ascomes dépourvus de stroma, orange pâle, subglobuleux, poilus, avec l'apex convexe et des ascospores striées. En se fondant sur la comparaison des caractères morphologiques et des données moléculaires avec les espèces connues, L. saulensis

> est proposée comme une espèce nouvelle. **Mots-clés :** ADN ribosomal, *Astrocaryum*, taxinomie.

Introduction

In the continuity of the inventorial survey of fungi in French Guiana (LECHAT *et al.*, 2022), an hypocrealean fungus was collected on a dead palm leaf of *Astrocaryum* sp. This fungus was morphologically characterised, cultured and phylogenetically analysed. ITS and LSU sequences were obtained from dry ascomata and compared with those of 24 species having an acremonium-like asexual morph, confirming its placement in *Lasionectria* (Sacc.) Cooke. Based on these results *Lasionectria saulensis* Lechat & J. Fourn. is proposed as a new species.

Materials 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, and the ascospore ornamentation was observed in unheated lactic cotton blue. The holotype is deposited in LIP herbarium (University of Lille, France). Cultures of living specimens were made on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 5 cm diam. incubated at 25°C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Oviedo, Spain): total DNA was extracted from dry specimens blending a portion of them 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 ddH₂O. PCR amplification was performed with the primers ITS1F and ITS4 (WHITE et al., 1990; GARDES & BRUNS, 1993) for ITS, while LROR and LR5 (VILGALYS & HESTER, 1990) were used to amplify the 28S nLSU region. PCR reactions were performed under a program consisting of a hot start at 95°C for 5 min, followed by 35 cycles at 94°C, 54°C and 72°C (45, 30 and 45 s respectively) and a final 72°C step for 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with primer ITS4. Chromatograms were checked searching for putative reading errors, and these were corrected. Phylogenetic analyses were performed online at www.phylogeny.limm.fr (DEREEPER *et al.*, 2008). Maximum likelihood phylogenetic analyses were performed with PhyML 3.0 aLRT (ZWICKL, 2006), using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric version of the approximate likelihood-ratio test, implemented in PhyML SH-aLRT (ANISIMOVA & GASCUEL, 2006).

Nomenclature follows MycoBank (Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands).

Taxonomy

Lasionectria saulensis Lechat & J. Fourn., sp. nov. Fig. 3 MycoBank: MB844375

Diagnosis: Differs from the most similar species *Lasionectria vulpina* by its longer and narrower ascospores, hyaline to pale yellow hairs instead of orange hairs, and its occurrence on dead palm leaves in French Guiana.

Holotype: FRENCH GUIANA, Saül, trail to Monts La Fumée, 3.63105° N, -53.205912° E, alt. 248 m, on dead leaves of *Astrocaryum* sp. (*Arecaceae*), 3 Apr. 2021, *leg*. C. Lechat, LIP CLLG21159, ex-holotype culture: no longer viable. GenBank sequences: ITS: ON206617 and LSU: ON206592.

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

Ascomata solitary or in groups of 2–3, superficial, scattered on substrate, non-stromatic, subglobose, (160–)180–200(–220) µm high, 160–180 µm diam. (Me = 190 × 170 µm, n = 10), becoming slightly cupulate when dry, easy to remove from substrate, not changing colour in 3% KOH or lactic acid. **Ascomatal apex** convex, concolourous, glabrous. **Ascomatal surface** composed of angular, thinwalled cells 8–14 × 6–12 µm with pale orange wall, bearing a crown of hairs around ostiolar region. **Hairs** flexuous 25–45(–50) × 3–5 µm wide, thick-walled, with wall 1.5–2 µm thick, septate, hyaline to pale yellow, arising from cells of upper ascomatal wall. **Ascomatal wall** 20–25 µm thick, composed of two regions: outer region 12–15 µm wide, of subglobose to elongated, thick-walled cells 4–7 × 2–3 µm, with pale orange walls 1.5–2.5 µm thick; inner region 8–10 µm wide,

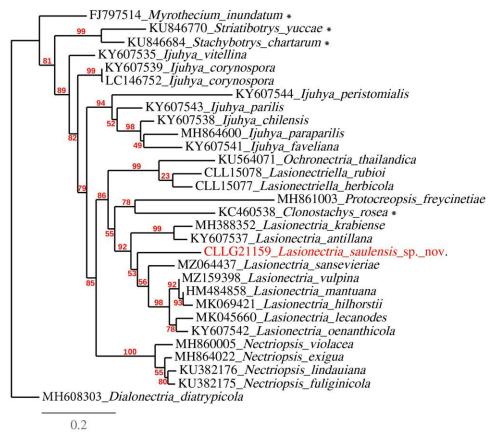


Fig. 1 – Maximum likelihood phylogeny (-InL = 2516.77551) of *Lasionectria saulensis* inferred by PhyML 3.0, model TS93 from a 650 bp matrix of ITS sequences, rooted with *Dialonectria diatrypicola* Lechat, J. Fourn. & Gardiennet (*Nectriaceae*). Species marked with * have a non-acremonium asexual morph.

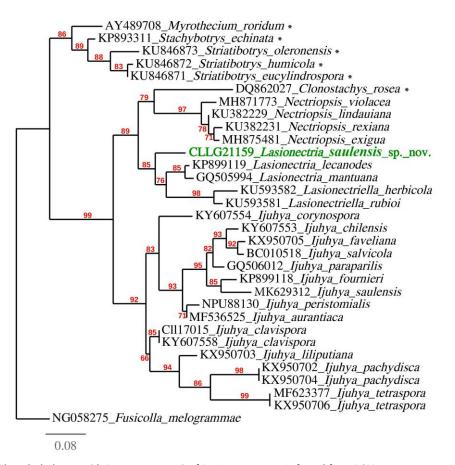


Fig. 2 – Maximum likelihood phylogeny (-InL = 2934.95332) of *Lasionectria* spp. inferred from LSU sequences, rooted with *Fusicolla melo-grammae* Lechat & Aplin (*Nectriaceae*). Species marked with * have a non-acremonium asexual morph.

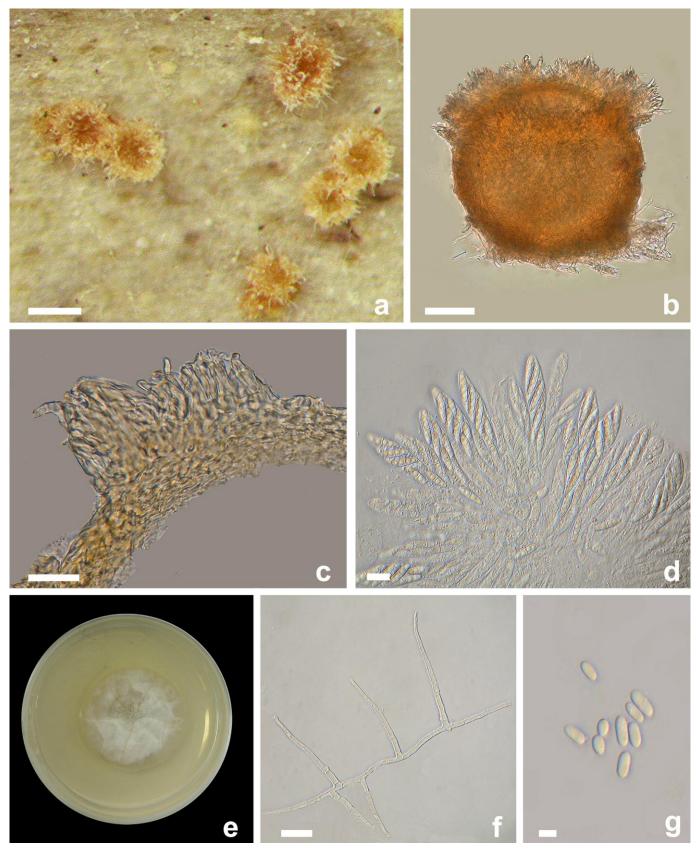


Fig. 3 – a-g: *Lasionectria saulensis* (Holotype CLLG21159); a: Dry ascomata on the substrate; b: Ascoma in water in side view; c: Section of ascomatal wall showing hairs arising from wall cells; d: Asci and ascospores in water; e: Culture at 2 weeks; f: Conidiophores in lactic acid; g: Conidia in lactic acid. Scale bars: a = 200 μ m; b = 50 μ m; c = 20 μ m; d, f = 10 μ m; g = 5 μ m.

of elongated, flattened thin-walled cells 7–12 × 1.5–2 µm, with hyaline walls 1–1.5 µm thick. **Asci** (55–)60–65(–70) × 8–9 µm (Me = 62.5 × 8.5 µm, n = 20), stipitate, clavate, apex rounded, with a refractive thickening, containing eight biseriate ascospores or biseriate above and uniseriate below. **Ascospores** (9.5–)10–12(–13) × 2.5–3 µm (Me = 11 × 2.8 µm, n = 30), narrowly ellipsoidal to fusiform, attenuated and rounded at ends, equally two-celled, with two drops in each cell, slightly constricted at septum, hyaline, striate.

Culture characteristics: After two weeks on PDA at 25°C, colony 25–30 mm diam., aerial mycelium white, without colouration in medium. Mycelium composed of septate, hyaline, smooth hyphae 2–2.5 µm diam. Conidiophores monomorphic, arising from aerial hyphae, macronematous, flexuous, hyaline 28–50 µm long, 2.5–3 µm diam. at base, faintly granulose, 1-septate, bearing terminal, subulate conidiogenous cells 30–38 µm long, with a non-flared collarette. Conidia ellipsoidal to subcylindrical, rounded at end, without abscission scar, aseptate, $5-8(-10) \times 2-2.5$ µm (Me = 6.5×2.2 µm, n = 30), hyaline, smooth. Unfortunately, the culture of this specimen is no longer viable.

Results and discussion

Within *Bionectriaceae*, *Lasionectria* is defined by hairy ascomata not changing colour in 3% KOH or lactic acid, ascomatal wall of two regions, hairs arising from cells of ascomatal wall, convex apex, striate ascospores and an acremonium-like asexual morph (LECHAT & FOURNIER, 2019; ROSSMAN *et al.*, 1999). *Lasionectria saulensis* fits well this definition, and our phylogenetic analyses of ITS and LSU sequences (Fig. 1–2) comparing *L. saulensis* with known bionectriaceous species having an acremonium-like asexual morph, unambiguously place the new species in *Lasionectria*. The conspicuous crown of hairs surrounding a glabrous apex may easily mislead to the closely related genus *ljuhya* Starbäck. In *ljuhya*, ascomatal wall differs in being only one-layered but, in critical cases like here, a formal generic placement requires molecular support.

Morphologically, *L. saulensis* is similar to *L. vulpina* (Cooke) Rossman & Samuels (Rossman *et al.*, 1999) but differs from it by having longer and narrower ascospores $(9.5-)10-12(-13) \times 2.5-3 \mu m$ vs. $(7-)8-11(-13) \times 3-4 \mu m$, white to pale yellow hairs vs. orange hairs, 89% similarity of ITS sequences, and its occurrence in tropical areas. Based on morphological features combined with the results of our phylogenetic analyses, *L. saulensis* is proposed as a new species.

<u>Note</u>: To our knowledge, *Lasionectria saulensis* is the first species of *Lasionectria* reported from French Guiana.

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Author's contributions

Christian Lechat was responsible for the conception of the study, cultures, morphological studies, phylogenetic analyses, design of figures and plates and writing a first draft. Jacques Fournier critically reviewed the first draft and proposed an improved version and took care of the registration at MycoBank. Delphine Chaduli and Anne Favel managed the culture collection in which the culture was to be deposited and took care of the registrations at GenBank. All authors except CL read and approved the final manuscript.

References

- ANISIMOVA M. & GASCUEL O. 2006. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Systematic Biology*, 55 (4): 539–552. doi: 10.1080/10635150600755453
- DEREEPER A., GUIGNON V., BLANC G., AUDIC S., BUFFET S., CHEVENET F., DUFA-YARD J.F., GUINDON S., LEFORT V., LESCOT M., CLAVERIE J.M. & GASCUEL O. 2008. — Phylogeny.fr: robust phylogenetic analysis for the nonspecialist. *Nucleic Acids Research*, 2008 Jul 1;36 (Web Server issue): W465–W469. doi: 10.1093/nar/qkn180
- GARDES M. & BRUNS T.D. 1993. ITS primers with enhanced specificity for Basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2 (2): 113–118. doi: 10.1111/j.1365 -294x.1993.tb00005.x
- LECHAT C. & FOURNIER J. 2019. Three new species of *ljuhya (Bionectriaceae, Hypocreales)* from metropolitan France, French Guiana and Spain, with notes on morphological characterization of *ljuhya* and allied genera. *Ascomycete.org*, 11 (2): 55–64. doi: 10.25664/art-0259
- LECHAT C., FOURNIER J. CHADULI D. & FAVEL A. 2022. *Hydropisphaera palmicola*, a new species from Saül (French Guiana). *Ascomycete.org*, 14 (2): 81–84. doi: 10.25664/art-0349
- Rossman A.Y., SAMUELS G.J., ROGERSON C.T. & LOWEN R. 1999. Genera of *Bionectriaceae*, *Hypocreaceae* and *Nectriaceae* (*Hypocreales*, Ascomycetes). *Studies in Mycology*, 42: 1–248.
- VILGALYS R. & HESTER M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172 (8): 4238–4246. doi: 10.1128/jb.172.8.4238-4246.1990
- WHITE T.J., BRUNS T., LEE S. & TAYLOR J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* INNIS M.A., GELFAND D.H., SNINSKY J.J. & WHITE T.J. (eds). *PCR Protocols:* a guide to methods and applications. Academic Press, New York, USA: 315–322. doi: 10.1016/b978-0-12-372180-8.50042-1
- ZWICKL D.J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. Dissertation. Austin, The University of Texas.



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