Two new species of *Chaetopsina* (*Nectriaceae*) from Saül (French Guiana)

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Abstract: Two new species of *Chaetopsina* are described and illustrated from specimens collected in French Guiana. Based on morphological divergences of both sexual and asexual morphs from known *Chaetopsina* species as well as phylogenetic analysis of ITS and LSU sequences, *C. guyanensis* and *C. saulensis* are proposed as new species. In addition, two new combinations in *Chaetopsina* are proposed.
Keywords: Ascomycota, *Astrocaryum*, *Bauhinia*, *Chaetopsinectria*, *Hypocreales*, *Mariannaea*, new combinations, ribosomal DNA, taxonomy.

Résumé : deux nouvelles espèces de *Chaetopsina* sont décrites et illustrées à partir de matériel récolté en Guyane française. En se fondant sur les différences morphologiques des stades sexués et asexués avec les espèces de *Chaetopsina* connues, ainsi que sur l'analyse phylogénétique des séquences ITS et LSU, *C. guyanensis* et *C. saulensis* sont proposées comme espèces nouvelles. En outre, deux nouvelles combinaisons sont proposées et trois espèces sont réintégrées dans le genre *Chaetopsina*.
Mots-clés : ADN ribosomal, Ascomycota, *Astrocaryum*, *Bauhinia*, *Chaetopsinectria*, Hypocréales, *Mariannaea*, nouvelles combinaisons, taxinomie.

Introduction

A field trip carried out over one week in August 2018 in Saül (French Guiana) led to the discovery of several new species of *Hypocreales*, three of which have been described (Lechat & Fournier, 2019a; 2019b; Lechat *et al.*, 2019). Two additional collections, one on a dead corticated liana and one on dead leaves of a palm, featured scattered, superficial, red perithecial ascomata associated with brown upright conidiophores of an asexual morph assignable to *Chaetopsina* Rambelli based on the type species, *C. fulva* Rambelli. Both collections were studied morphologically, then successfully cultured and sequenced. We present here the morphological, cultural and molecular data supporting the placement of both collections in *Chaetopsina* as the new species *C. guyanensis* and *C. saulensis*.

We follow Rossman *et al.* (2016) who recommended *Chaetopsina* as the correct name for the nectriaceous fungi associated with a chaetopsina-like asexual morph formerly placed in *Nectria* (Fr.) Fr., *Cosmospora* Rabenh or *Chaetopsinectria* J. Luo & W.Y. Zhuang. Accordingly, we propose two new combinations to accommodate the basionyms *Nectria chaetopsinae* Samuels and *N. macrochaetopsinae* Samuels and recognize three taxa formerly placed in *Chaetopsinectria* in the genus *Chaetopsina*.

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. The holotype specimens were deposited in LIP herbarium (University of Lille) and living cultures at CIRM-CF (Centre International des Resources Microbiennes, Marseille, France). Cultures of the living specimens were plated on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 5 cm diam, incubated at 25°C. After growth of cultures for 7–10 days, genomic DNA was extracted from a portion of fresh mycelium using the Nucleospin plan II kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. For the cell lysis step, the mycelium was fragmented using FastPrep-24™ 5G Benchtop Homogenizer in a lysing Matrix A tube containing the lysis buffer PL1 and RNAse. The sample obtained was purified following the Nucleospin plant II protocol (steps 3 to 7). ITS5 and ITS4 primers (White et al., 1990) were used for PCR amplification and sequencing reaction. The ITS1-5.8S rRNA gene-ITS2 was amplified from 1 µl genomic DNA in 50 µl PCR using CloneAmp Hifi PCR Premix (Takara). An automated thermal cycler (Mastercycler, Eppendorf, Germany) was used for amplification reactions. 35 cycles of 10 s denaturation at 98°C was followed by 5 s of annealing at 55°C and 5 s of elongation at 72°C. The PCR products were checked on FlashGel™ DNA System (Lonza, Switzerland), and sequenced by GENEWIZ (Leipzig, Germany). Chromatograms were checked searching for putative reading errors, and these were manually corrected. All the nucleotide sequences were deposited in GenBank. Sequences generated in this study and those obtained from GenBank were aligned under Clustal W (Thomson *et al.*, 1994). The evolutionary history conducted using MEGA version 6 (Tamura *et al.*, 2013) was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993).

Taxonomy

Chaetopsina guyanensis Lechat & J. Fourn., *sp. nov.* Fig. 3
Mycobank: MB 831254

Diagnosis: Differs from all known *Chaetopsina* species in having faintly spinulose ascospores and fusiform conidia in nature.

Holotype: French Guiana, Saül, Gros Arbres trail, on bark of dead *Bauhinia* sp. (*Fabaceae*), 24 Aug. 2018, *leg.* C. Lechat CLLG18038-B (LIP CLLG18038-B), ex-type culture: BRFM 2848, ITS GenBank sequence: MN017105.

Etymology: The epithet *guyanensis* refers to French Guiana where this species was collected.

Ascomata scattered or clustered in groups of 5–10, superficial, non-stromatic, seated on lenticels of bark, subglobose to obpyriform, with an acutely conical apex, 260–300 µm high, 180–280 µm diam (Me = 280 × 240 µm, n = 15), occasionally laterally collapsing when dry, orange when immature, becoming brownish orange to reddish brown when mature with paler papilla, shining, turning dark red in 3% KOH, yellow in lactic acid. Ascomatal surface composed of cells of undefined shape, forming a *textura epidermoidea*. Ascomatal wall in vertical section 15–20 µm thick, of a single region composed of thick-walled globose to subglobose thick-walled cells 3–10 × 3–8 µm with orange wall 1.5–3 µm thick, forming a *textura epidermoidea*, becoming ellipsoidal to slightly elongated towards interior. Apex of a palisade of vertically arranged, cylindrical to narrowly clavate cells 6–10 × 2–3 µm. **Asci** evanescent, unitunicate, clavate, short stipitate, rounded at apex, without ring, 60–75 × 7–8 µm, 8-spored, ascospores apically biseriate, uniseriate below. Moniliform **paraphyses** inserted between asci, up to 12 µm wide toward base. **Ascospores** (9.5–)10–12.5(–13) × 3.5–4.2 µm (Me = 11.5 × 3.8 µm, n = 40), oblong-ellipsoid with obtuse ends, straight to slightly curved, equally two-celled, slightly constricted at septum, hyaline, faintly spinulose, with ornamentation only visible in lactic cotton blue.

Asexual morph in natural environment: Conidiophores sparse, difficult to spot under the dissecting microscope, pale brown to dark

reddish brown, gradually tapering and becoming paler to hyaline at apex, becoming yellow in lactic acid, erect, septate, 360 × 8–10 µm, up to 15 µm diam at base, thick-walled with wall 2 µm thick, branched at tip with thin-walled branches bearing long lageniform, polyblastic conidiogenous cells 20–26 × 5–8 µm, producing fusiform, slightly curved, hyaline, aseptate, smooth conidia 9–10.5(–11.5) × 2.5–3 µm.

Cultural characteristics: Colony slow growing, 2–2.5 cm diam after three weeks at 25°C, white to pale yellow in centre, white at margin with brownish orange strands penetrating medium, diffusing yellow; conidiophores arising from aerial mycelium, composed of hyaline, septate, smooth hyphae 2.5–3 µm diam. Conidiophores smooth, hyaline, simple or branched bearing subcylindrical to subulate, polyblastic conidiogenous cells 30–50 × 3–4 µm with a flared collarette, producing narrowly ellipsoidal conidia with rounded apex, hyaline, acute at base, aseptate, 6–9(–11) × 3–3.5(–4) µm.

Known distribution: French Guiana.

Notes: *Chaetopsina guyanensis* differs from all known species in the genus by having spinulose ascospores. Its asexual morph in culture differs from that observed in nature in having different conidia in size and shape, as previously noted by Samuels (1985) regarding *N. chaetopsinae*, *N. chaetopsinae-polyblastiae* and *N. chaetopsinae-catenulatae*.

Chaetopsina saulensis Lechat & J. Fourn., *sp. nov.* Fig. 4
Mycobank MB 831255

Diagnosis: Similar to *C. macrochaetopsinae* from which it differs in having striate and larger ascospores.

Holotype: French Guiana, Saül, Roche Bateau trail, on dead palm leaf of *Astrocaryum vulgare* Mart. (*Arecaceae*), 23 Aug. 2018, *leg.* C. Lechat CLLG18029 (LIP), ex-type culture: BRFM 2845. ITS and LSU GenBank sequences: MN017104 and MN017106.

Etymology: The epithet *saulensis* refers to Saül (French Guiana) where this species was collected.

Ascomata solitary, superficial, scattered, non-stromatic, seated among conidiophores of a chaetopsina-like asexual morph, subglobose to obpyriform with an acutely conical apex, 200–250 µm high, 180–240 µm diam (Me = 230 × 220 µm, n = 20), not collapsing when dry, orange when immature, becoming dark orange to red when mature, shining, turning dark red in 3% KOH, yellow in lactic acid. Ascomatal surface composed of cells of undefined shape, forming a *textura epidermoidea*, translucent, showing asci and ascospores

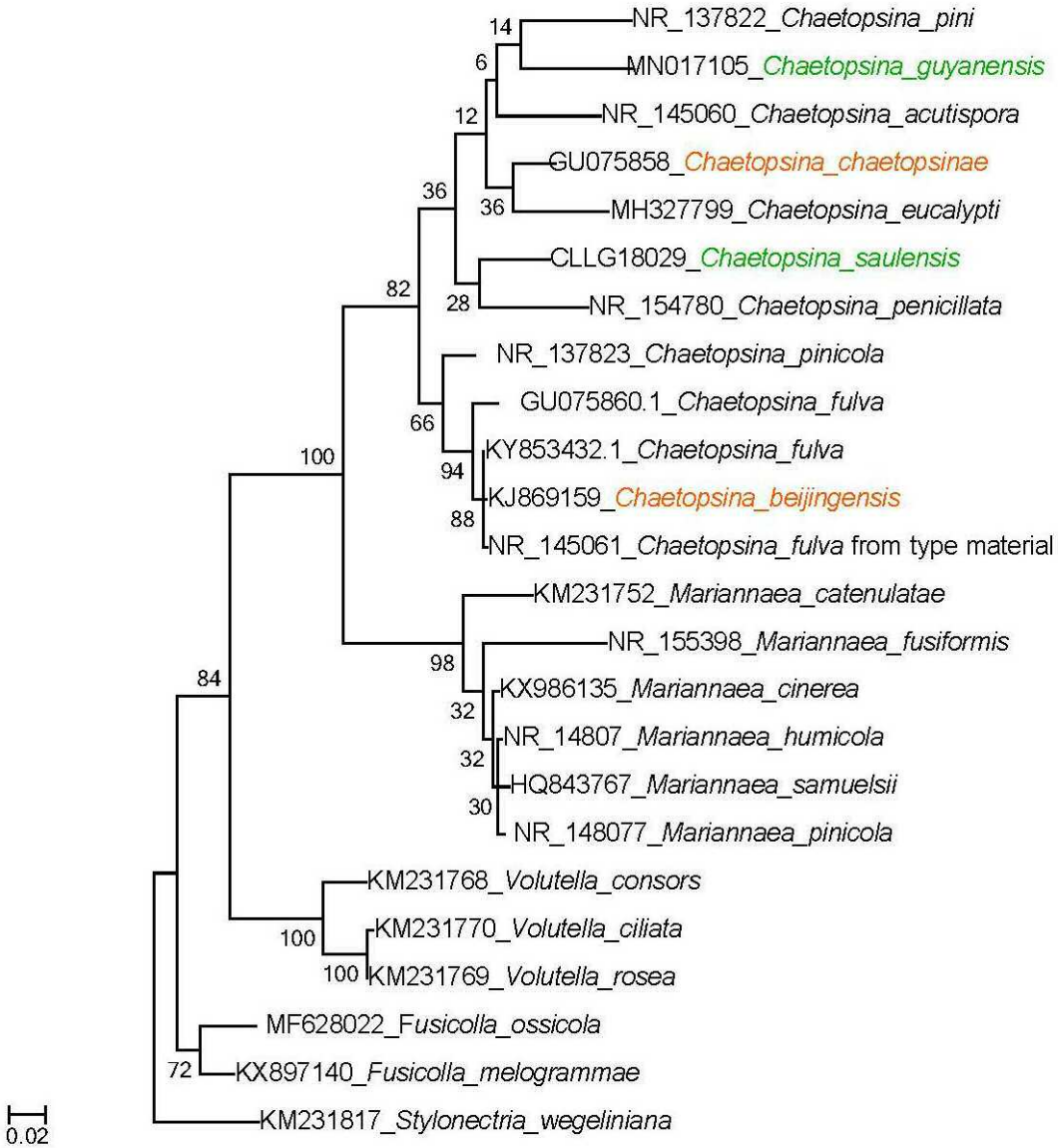

Fig. 1 – Maximum likelihood phylogeny (-lnL = 2389.5567) of *Chaetopsina* spp. inferred by Tamura-Nei model from a 640 bp matrix of ITS sequences, rooted with *Stylonectria wegeliniana*.

through ascomatal wall. Ascomatal wall in vertical section 10–15 µm thick, of a single region composed of thick–walled globose, subglobose to ellipsoidal cells 3–10 × 3–8 µm with orange wall 1.5 µm thick. Apex of a palisade of vertically arranged, cylindrical to narrowly clavate cells 10–15 × 1.5–2 µm diam merging with periphyses. **Asci** evanescent, unitunicate, clavate, short-stipitate, attenuated at rounded apex, without ring, 60–70 × 16–20 µm, 4–6–8-spored, ascospores multiseriate. Moniliform **paraphyses** inserted between asci up to 20 µm wide toward base. **Ascospores** narrowly fusiform, slightly curved (35–)40–58(–62) × 8–9(–10) µm (Me 49 × 7.5 µm, n = 50), equally two-celled, not constricted at septum, hyaline, spirally striate with cyanophilous ends.

Asexual morph on natural environment: Conidiophores abundant, pale brown to brownish orange, becoming yellow in lactic acid, erect, bulbous at base, setiform, unbranched, septate, up to 320 µm long, 8–9 µm diam, thick-walled with wall 2 µm thick, bearing at tip or toward base lageniform, monoblastic conidiogenous cells 5–6 × 3.5–4 µm, producing cylindrical, straight, hyaline, aseptate, smooth conidia 9–15 × 1.5 µm, attenuated at base with an abscission scar.

Cultural characteristics: Colony slow growing, 2–3 cm diam after two weeks at 25°C, white in centre and at margin, pale carmine red in middle area; aerial mycelium floccose, composed of hyaline to orange, septate, hyphal elements bearing smooth, hyaline, aseptate conidiophores 35–55 × 4–5 m with a single terminal conidiogenous cell 20–25 × 4–4.5 µm with a flared collarette, producing cylindrical, straight, hyaline, aseptate conidia 27–40 × 4–4.5 µm, attenuated at base with a small abscission scar. Culture of this specimen is no longer viable.

Known distribution: French Guiana.

Notes: *Chaetopsina saulensis* has the longest ascospores in the genus, which are striate (35–)40–58(–62) × 8–9(–10) µm, while those of the similar species *C. macrochaetopsinae* are smooth-walled and slightly smaller 36–41.5(–43.5) × 6–7.2(–8) µm. As noted in *C. guya-*

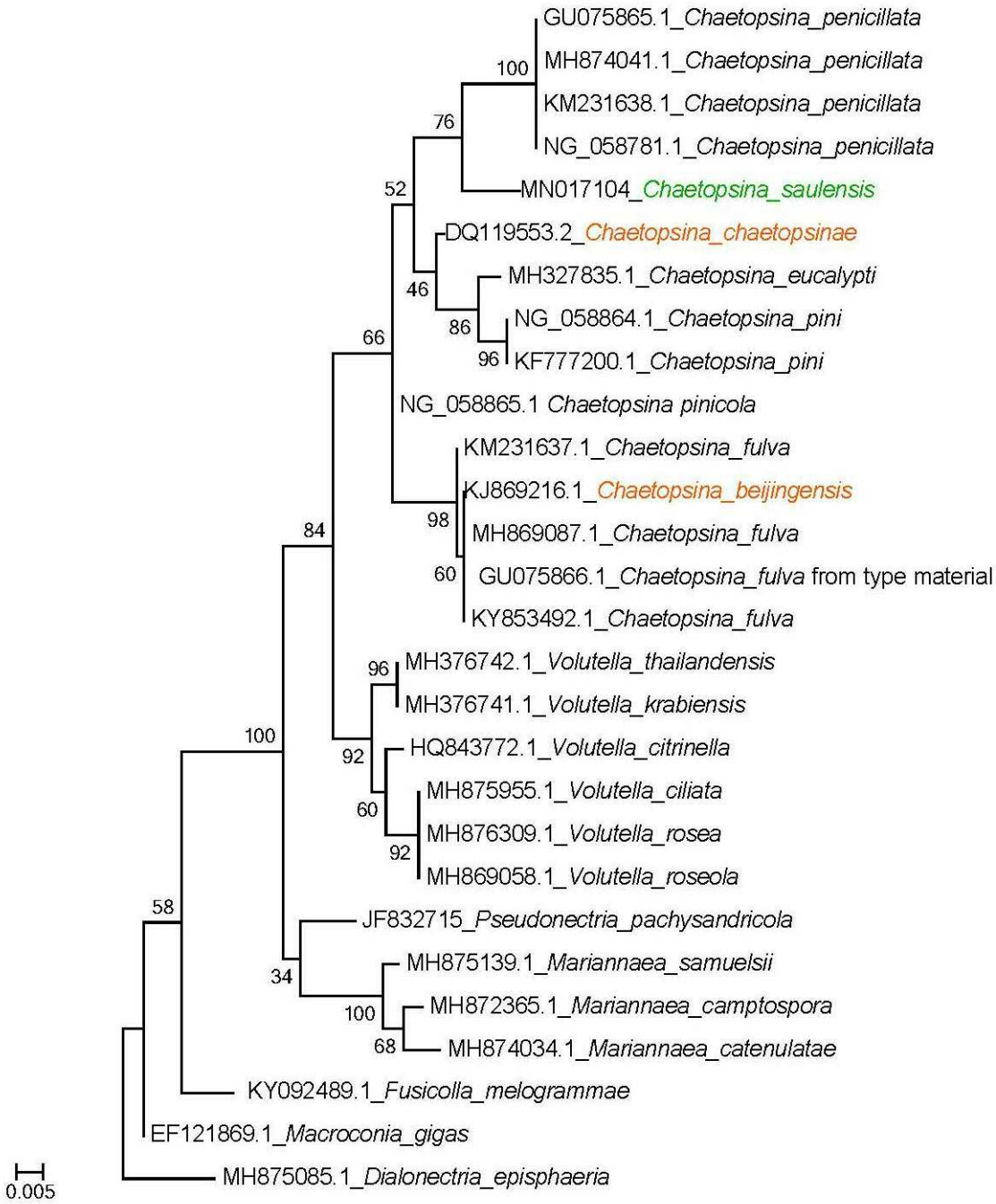

Fig. 2 – Maximum likelihood phylogeny (-lnL = 1931.79365) of *Chaetopsina* spp. inferred by Tamura-Nei model from a 810 bp matrix of LSU sequences, rooted with *Dialonectria episphaeria*.

nensis and other known species, this fungus has longer conidia in culture than in nature.

Discussion

Chaetopsina was originally regarded as a hyphomycetous genus described by Rambelli (1956), characterized by upright, pigmented, thick-walled conidiophores with apical or lateral hyaline branches bearing monoblastic phialides, without a known sexual morph. *Chaetopsina* occurs on leaves, bark, dead palm leaves or ascomycetous stromata, and all known species are reported in tropical and neotropical areas, except *C. fulva* which is cosmopolitan.

Samuels (1985) established the connection between some *Chaetopsina* asexual morphs with the sexual morphs of four new hypocrealean species assigned to the *Nectria episphaeria* group in the broad sense, based on similar peridial anatomy. The sexual morphs associated with *Chaetopsina* asexual morphs were thereafter placed in *Cosmospora* (Rossman *et al.*, 1999; Nirenberg & Samuels, 2000; Zhuang & Zhang, 2002; Hosoya & Tubaki, 2004; Nong & Zhuang, 2005; Hirooka *et al.*, 2008; Rossman *et al.*, 2008). Based on molecular data, a new genus *Chaetopsinectria* was proposed by Luo & Zhuang (2010) to accommodate them. As recommended by Rossman *et al.* (2016), *Chaetopsina* was selected as the correct name for this genus replacing the now synonymous generic name for the sexual morph.

After the description by Rambelli (1956), a number of hyphomycetous species were added to *Chaetopsina* as discussed in detail by Samuels (1985) and compared with closely related genera. He retained the red-brown conidiophores turning yellow in lactic acid as the unique morphological feature characterizing *Chaetopsina*. A comprehensive overview of the taxonomic and nomenclatural concept of *Chaetopsina* can be found in Seifert *et al.* (2011).

Based on morphological characteristics of the sexual and asexual morphs including the ascomatal wall anatomy, the two species described above match well the concept of *Chaetopsina*. Our phylogenetic analysis (Figs. 1–2) shows that their ITS and LSU sequences cluster with those of eight known species of *Chaetopsina*, including the type species *C. fulva*, which unambiguously supports their placement in this genus.

Morphologically, *C. guyanensis* resembles *C. chaetopsinae-polyblastiae* Samuels in having nearly the same size and shape of ascospores, a similar morphology in culture and conidia in nature differently shaped from those in culture, as well as its occurrence in South America. It differs from *C. chaetopsinae-polyblastiae* (Samuels, 1985) by significantly larger ascomata 260–300 × 180–240 µm vs 145–160 × 115–145 µm, ornamented ascospores and fusiform curved conidia in nature, the two latter features being unique within the genus. In our phylogenetic tree (Fig. 1), *C. guyanensis* is nested on a sister branch to *C. eucalypti* Crous, a species only known from Australia on *Eucalyptus* leaf litter (Crous *et al.*, 2018), and *C. pini* Crous & Cheew., only known from Thailand on needle litter of *Pinus caribaea* (Crous *et al.*, 2013). Only known as asexual morph, both species differ from *C. guyanensis* in having larger conidia and less than 88% similarity of ITS sequences.

Chaetopsina saulensis differs from all known species in having long fusiform ascospores. Only *C. macrochaetopsinae* (≡ *N. macrochaetopsinae*) is reported to have a similar ascospore morphology (Samuels *et al.*, 1990), but *C. saulensis* differs in having spirally striate ascospores, (35–)40–58(–62) × 8–9(–10) µm, with cyanophilous tips, while *N. macrochaetopsinae* has smooth-walled, smaller ascospores, 36–41.5(–43.5) × 6–7.2(–8) µm, without cyanophilous tips. *Chaetopsina saulensis* appears phylogenetically related to *C. penicillata* Samuels (Figs. 1–2), which differs from our fungus in having smaller ascospores and much larger conidia (Samuels, 1985) with only 91% and 97% similarity in ITS and LSU sequences respectively. No ITS or LSU sequences of *C. macrochaetopsinae* are available for comparison.

To comply with the recommendations made by Rossman *et al.* (2016) to stabilize the nomenclature of nectriaceous fungi with chaetopsina-like asexual morphs, we propose the new combinations *Chaetopsina chaetopsinae* (Samuels) Lechat & J. Fourn. comb. nov. to accommodate *Nectria chaetopsinae* Samuels, as well as *Chaetopsina macrochaetopsinae* (Samuels) Lechat & J. Fourn. comb. nov. to accommodate *Nectria macrochaetopsinae* Samuels.

Based on a MEGABLAST search of NCBIs GenBank nucleotide database, the closest hits using the ITS or LSU sequences to *C. fulva* are *C. beijingensis* Crous & Y. Zhang ter and *C. pinicola* Crous & Cheew., which are placed in a subclade distant from *N. chaetopsinae* Samuels as showed in our ML trees (Figs. 1–2). Accordingly, we recognize *N. chaetopsinae* as a distinct species *Chaetopsina chaetopsinae* (Samuels) Lechat & J. Fourn. to accommodate *Nectria chaetopsinae*. *Chaetopsina fulva* is only known as an asexual morph, although it was regarded as synonymous with *Nectria chaetopsinae* by Rossman *et al.* (2016). However, *C. fulva* has conidia 8–12 × 1.5 µm (Kirk & Sutton, 1985) while those of *Nectria chaetopsinae* are (5–)5.6–7.2(–8.5) × 1–1.3(–1.7) µm (Samuels, 1985), which suggests that these names are not synonyms. This conclusion is confirmed by our molecular data showing they are not phylogenetically closely related (Figs. 1–2).

Morphologically, *C. beijingensis* is similar to *C. fulva* and considered herein a synonym, that is well supported by our phylogenetic analyses of ITS and LSU sequences showing 99.6% and 100 % similarity respectively.

Based on their morphology, *C. penicillata* Samuels (syn. *Nectria chaetopsinae-penicillatae*) and *C. polyblastia* Samuels (syn. *Nectria chaetopsinae-polyblastiae*) are recognized in *Chaetopsina*.

Based on a multigene phylogenetic analysis, Lombard *et al.* (2015) recombined *Nectria chaetopsinae-catenulatae* (Samuels), ex-type culture CBS 491.92, as *Mariannaea catenulatae* (Samuels) L. Lombard & Crous. The genus *Mariannaea* is phylogenetically clearly distinct from *Chaetopsina* and morphologically different in featuring globose ascomata not changing colour in KOH and a flat apex, as well as conidia produced in imbricate chains according to Samuels & Seifert (1991) and Gräfenhan *et al.* (2011). In this context, we think that the culture CBS 491.92 is misidentified and is recognized as a species of *Mariannaea*.

Taxonomic novelties

Chaetopsina guyanensis Lechat & J. Fourn., *sp. nov.* – Mycobank: MB 831254

Chaetopsina saulensis Lechat & J. Fourn., *sp. nov.* – Mycobank: MB 831255

Chaetopsina chaetopsinae (Samuels) Lechat & J. Fourn., *comb. nov.* – Mycobank: MB 831332

Basionym: *Nectria chaetopsinae* Samuels, *Mycotaxon*, 22 (1): 18 (1985).

≡ *Cosmospora chaetopsinae* (Samuels) Rossman & Samuels (1999).

≡ *Chaetopsinectria chaetopsinae* (Samuels) J. Luo & W.Y. Zhuang (2010).

Chaetopsina macrochaetopsinae (Samuels) Lechat & J. Fourn., *comb. nov.* – Mycobank: MB 831333

Basionym: *Nectria macrochaetopsinae* Samuels, *Memoirs of the New York Botanical Garden*, 59: 40 (1990).

≡ *Cosmospora macrochaetopsinae* (Samuels) Rossman & Samuels (1999).

Additional species:

Chaetopsina penicillata Samuels (1985).

≡ *Nectria chaetopsinae-penicillatae* Samuels (1985).

≡ *Cosmospora chaetopsinae-penicillatae* (Samuels) Rossman & Samuels (1999).

≡ *Chaetopsinectria chaetopsinae-penicillatae* (Samuels) J. Luo & W.Y. Zhuang (2010).

Chaetopsina polyblastia Samuels (1985).

≡ *Nectria chaetopsinae-polyblastiae* Samuels (1985).

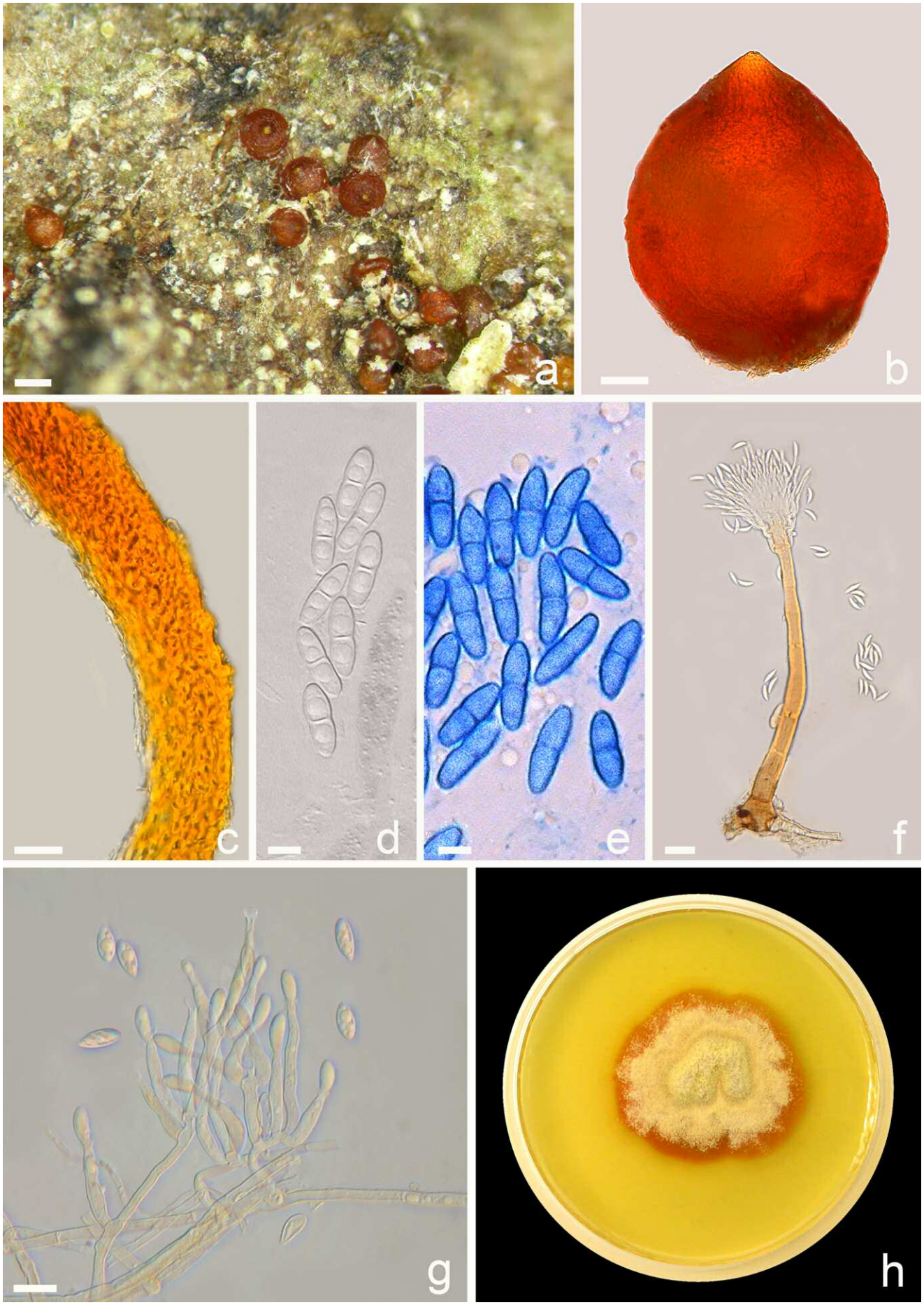

Fig. 3 – a–h: *Chaetopsina guyanensis* (Holotype CLLG18038-B); a: Ascomata in natural environment; b: Close-up of ascoma in side view in water; c: Lateral ascomatal wall in vertical section in water; d: Ascus and ascospores in water; e: Ascospores in lactic cotton blue not heated; f: Conidiophore and conidia from nature; g: Conidiophores and conidia from culture; h: Culture after three weeks. Scale bars: a = 200 µm; b = 50 µm; c, g = 10 µm; d, e = 5 µm; f = 20 µm.

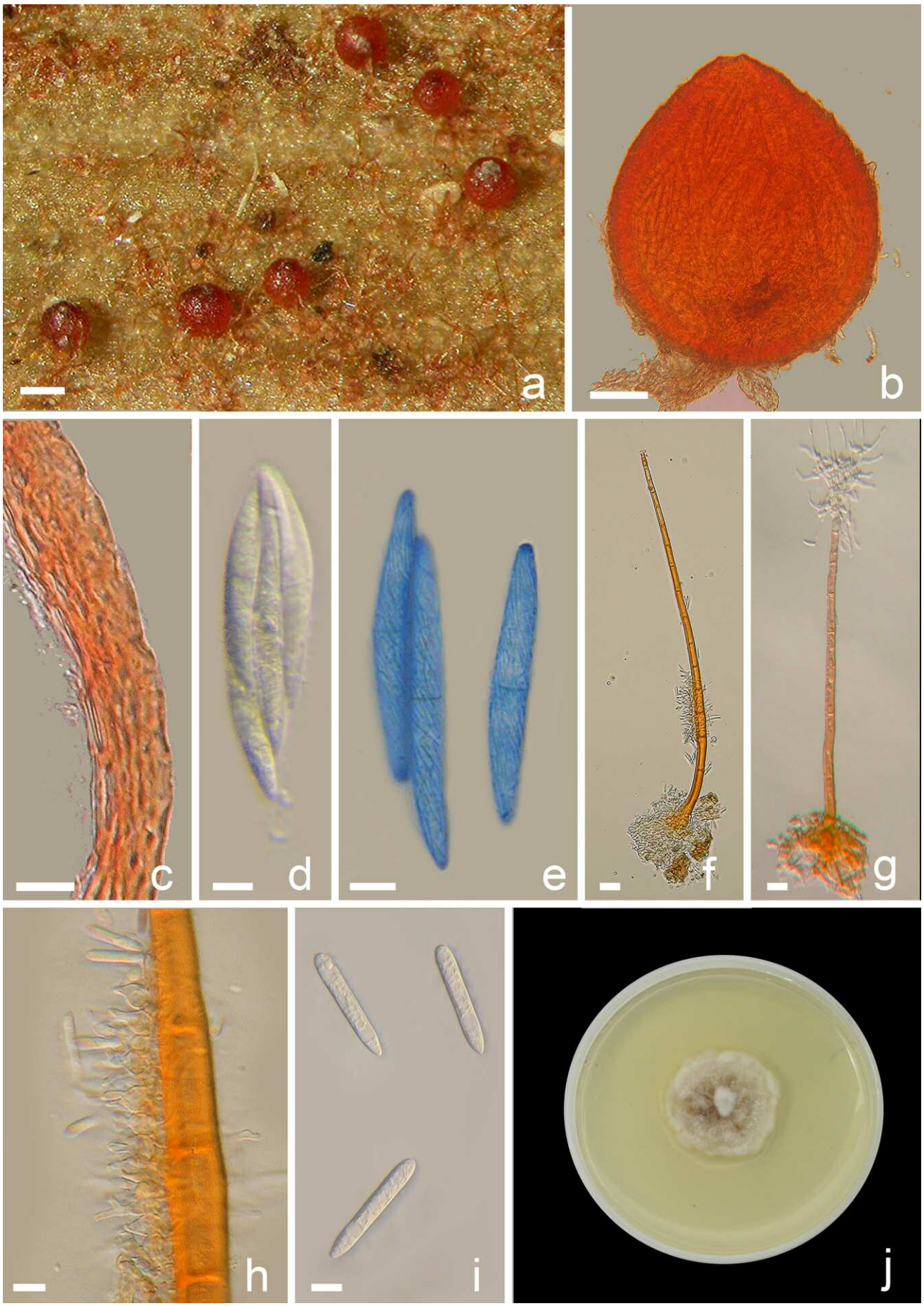

Fig. 4 – a–h: *Chaetopsina saulensis* (Holotype CLLG18029); a: Ascomata in natural environment; b: Close-up of ascoma in side view in water; c: Lateral ascomatal wall in vertical section in water; d: Ascus and ascospores in water; e: Ascospores in cotton blue, showing striate ornamentation and cyanophilous tips; f: Conidiophore with lateral conidiogenous cells toward base; g: Conidiophore with conidiogenous cells at tip; h: Close-up of conidiogenous cells from nature; i: Conidia from culture; j: Culture after two weeks. Scale bars: a = 200 µm; b = 50 µm; c–g, i = 10 µm; h = 5 µm.

≡ *Cosmospora chaetopsinae-polyblastiae* (Samuels) Rossman & Samuels (1999).

≡ *Chaetopsinectria chaetopsinae-polyblastiae* (Samuels) J. Luo & W.Y. Zhuang (2010).

Chaetopsina catenulata Samuels (1985).

≡ *Nectria chaetopsinae-catenulatae* Samuels (1985).

≡ *Cosmospora chaetopsinae-catenulatae* (Samuels) Rossman & Samuels (1999).

≡ *Chaetopsinectria chaetopsinae-catenulatae* (Samuels) J. Luo & W.Y. Zhuang (2010).

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