Chaetopsina pnagiana (*Nectriaceae, Hypocreales*), a new holomorphic species from Saül (French Guiana)

Christian LECHAT
Jacques FOURNIER

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Abstract: A new species of *Chaetopsina* is described and illustrated from a collection in French Guiana. Based on morphological divergences of both sexual and asexual morphs from known *Chaetopsina* species as well as phylogenetic analyses of ITS and LSU sequences, *C. pnagiana* is proposed as new species. A dichotomous key to holomorphic species of *Chaetopsina* is provided.
Keywords: Ascomycota, French Guiana, ribosomal DNA, taxonomy.

Résumé : Une nouvelle espèce de *Chaetopsina* est décrite et illustrée à partir de matériel récolté en Guyane française. En se fondant sur les différences morphologiques des stades sexué et asexué avec les espèces de *Chaetopsina* connues, ainsi que sur les analyses phylogénétiques des séquences ITS et LSU, *C. pnagiana* est proposée comme espèce nouvelle. Une clé dichotomique des espèces holomorphiques de *Chaetopsina* est proposée.
Mots-clés : ADN ribosomal, Ascomycota, Guyane française, taxinomie.

Introduction

An inventorial survey of fungi of Saül (French Guiana), initiated in 2018 by the Parc National Amazonien de Guyane (PNAG), lead to the discovery of several new species of *Chaetopsina* Rambelli (Lechat & Fournier, 2019c), *Clonostachys* Corda (Lechat & Fournier, 2018; Lechat *et al*., 2019), *Ijuhya* Starbäck (Lechat & Fournier, 2019b) and *Pleiocarpon* L. Lombard & D. Aiello (Lechat & Fournier, 2019a). In continuation of this survey, a field trip took place over one week in June 2019 during which additional undescribed species were discovered. We present and document in the following a new species of *Chaetopsina* collected on bark of an unidentified liana. Based on red, superficial, non-stromatic perithecial ascomata changing colour in 3% KOH or lactic acid, associated with brown upright conidiophores of its asexual morph becoming yellow in lactic acid, our fungus unambiguously belongs to *Chaetopsina* as delimited by Samuels (1985). Based on morphological characteristics and phylogenetic analyses of ITS and LSU sequences, this fungus is shown to significantly deviate from known species in *Chaetopsina* (Samuels, 1985; Lechat & Fournier, 2019c). Thus, we introduce the new species *Chaetopsina pnagiana* to accommodate this collection.

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 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). LR0R and LR5 (Vilgalys & Hester, 1990) were used to amplify the 28S nLSU region. Chromatograms were checked searching for putative reading errors, and these were manually corrected. The nucleotide sequence was deposited in GenBank. Sequence 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).

The epithet "*pnagiana*" was formed following a nomenclatural proposal regarding the epithets derived from acronyms (Dorr, 2009).

Taxonomy

Chaetopsina pnagiana Lechat & J. Fourn., *sp. nov.* – Fig. 3 – MB 833854.

Diagnosis: Differs from all known *Chaetopsina* species having smooth-walled ascospores with obtuse ends by conidia of asexual morph in nature up to 32 µm long.

Holotype: French Guiana, Saül, trail to Monts La Fumée, on lenticels of bark of an unidentified dead liana, 26 Jun. 2019, *leg*. C. Lechat CLLG19034 (LIP), ex-type culture: BRFM3055, ITS and LSU GenBank sequences: MN886609 and MN886607.

Etymology: The epithet *pnagiana* refers to PNAG (Parc National Amazonien de Guyane) where this species was collected.

Ascomata scattered or clustered in groups of 3–12, superficial, non-stromatic, seated on lenticels of bark, among conidiophores of its asexual morph, obpyriform, with an acutely conical apex, 180–220 µm high, 150–180 µm diam (Me = 200 × 165 µm, n = 15), not collapsing when dry, orange when immature, becoming bright red when mature with paler papilla, shining, turning purple in 3% KOH, yellow in lactic acid. **Ascomatal surface** composed of cells of undefined shape, forming *textura epidermoidea*. **Ascomatal wall** 15–20 µm thick in vertical section, of a single region composed of thick-walled, globose to subglobose cells, 3.5–12 × 3–5 µm, with orange wall, 1.5–3 µm thick, becoming paler and slightly elongated towards interior. Apex of a palisade of vertically arranged, cylindrical to narrowly clavate cells, 9–18 × 2–2.5 µm. **Asci** unitunicate, clavate, short-stipitate, with a flat apex, without ring, 60–70 × 10–12 µm, containing eight biseriate ascospores. **Ascospores** (14–)15–19(–20) × 4–4.7(–5.5) µm (Me = 17 × 4.5 µm, n = 30), fusiform with obtuse

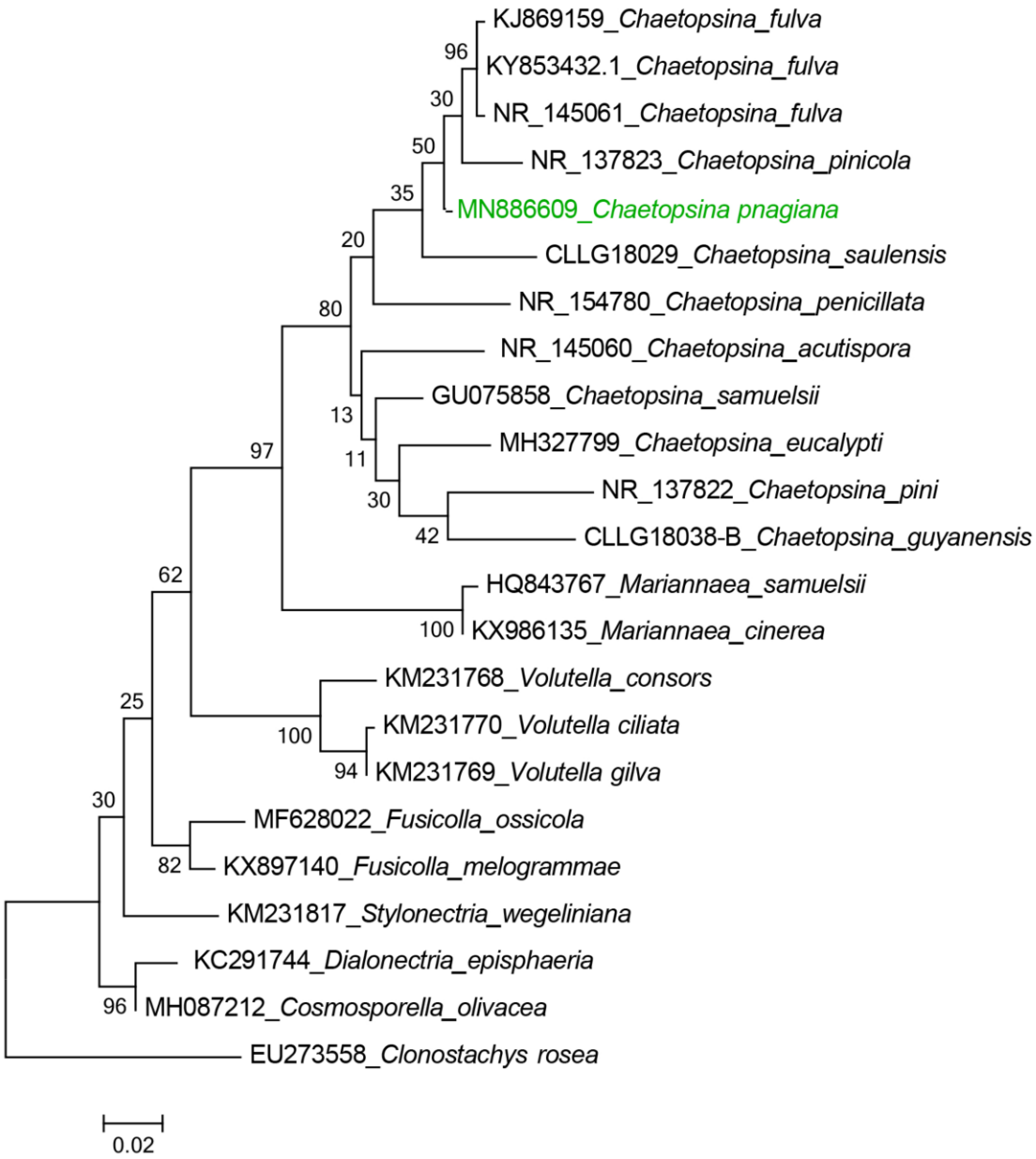

Fig. 1 – Maximum likelihood phylogeny (-lnL = 1964.40) of *Chaetopsina* spp. inferred by Tamura-Nei model from a 620 bp matrix of ITS sequences, rooted with *Clonostachys rosea*.

ends, slightly curved, equally two-celled, slightly constricted at septum, hyaline, with 1–3 pale yellow drops in each cell, smooth-walled.

Asexual morph in natural environment: Conidiophores scattered, pale brown to dark reddish brown, becoming yellow in lactic acid, erect, septate, 100–140 × 8–10 µm, up to 15 µm diam. at bulbous base, thick-walled with wall 1.5–2 µm thick, branched at tip with thin-walled branches bearing lageniform, polyblastic, hyaline conidiogenous cells, 12–18 × 3–5 µm, producing fusiform, slightly curved, hyaline, aseptate, smooth conidia (25–)28–32(–34) × 7–9 µm.

Cultural characteristics: Colony slow-growing, 2–2.5 cm diam after two weeks at 25°C, entirely white, diffusing a carmine coloration in medium around margin; aerial mycelium floccose, composed of smooth, hyaline to brownish orange, septate, hyphal elements, 2–2.5 µm diam, not sporulating after four weeks.

Results and discussion

Chaetopsina was introduced by Rambelli (1956) with *C. fulva* Rambelli as type species. Today 23 species are recognized in the genus, but only eight are currently known as sexual morph, which were included in the key below. The generic concept of *Chaetopsina* was recently discussed and documented by Lechat & Fournier (2019c), and the new species described above matches well this concept. At first glance, the sexual morph of *Chaetopsina pnagiana* is morphologically similar to all known species of the genus in having reddish brown to bright red, non-stromatic ascomata with an acute apex, turning purple in 3% KOH or yellow in lactic acid, with cells of ascomatal surface of undefined shape, forming *textura epidermoidea*, and growing associated with upright conidiophores of its chaetopsina-like asexual morph. *Chaetopsina* species can be segregated by their morphological characteristics, such as ascomatal dimensions and wall anatomy, size, shape and ornamentation of ascospores and conidia of asexual morph. The new species primarily differs from all known *Chaetopsina* species by its asexual morph in nature having the longest conidia in the genus, up to 32 µm long, when, in known *Chaetopsina* species, conidia do not exceed 16 µm long in nature, except *C. penicillata* Samuels, whose conidia measure up to 22 µm long. However, *C. penicillata* differs from our fungus in having striate ascospores, up to 41 µm long. Our phylogenetic anal-

Dichotomous key to the species of *Chaetopsina* based on sexual morph

1. Ascospores finely spinulose, 10–12.5 × 3.5–4.2 µm; French Guiana ..... ***C. guyanensis***
1. Ascospores smooth or striate **2**
2. Ascospores smooth **3**
2. Ascospores striate **7**
3. Ascospores 7.8–9.3(–10) × 2–2.7(–3) µm; New Zealand ..... ***C. chaetopsinae***
3. Ascospores over 10 µm long **4**
4. Ascospores less than 17 µm long **5**
4. Ascospores over 17 µm long **6**
5. Ascomata 250–280 µm high × 145–280 µm wide with broadly obtuse apex; South America ..... ***C. catenulata***
5. Ascomata 145–160 µm high × 115–145 µm wide with acute apex; French West Indies, South America ..... ***C. polyblastia***
6. Ascospores (14–)15–19(–20) × 4–4.7(–5.5) µm; French Guiana ..... ***C. pnagiana***
6. Ascospores 24–42 × 6–10 µm; Indonesia ..... ***C. macrochaetopsinae***
7. Ascospores 36–41 × 5.8–9.8(–11) µm; Ecuador, French West Indies, Jamaica, New Zealand ..... ***C. penicillata***
7. Ascospores 40–58(–62) × 8–9(–10) µm; French Guiana ..... ***C. saulensis***

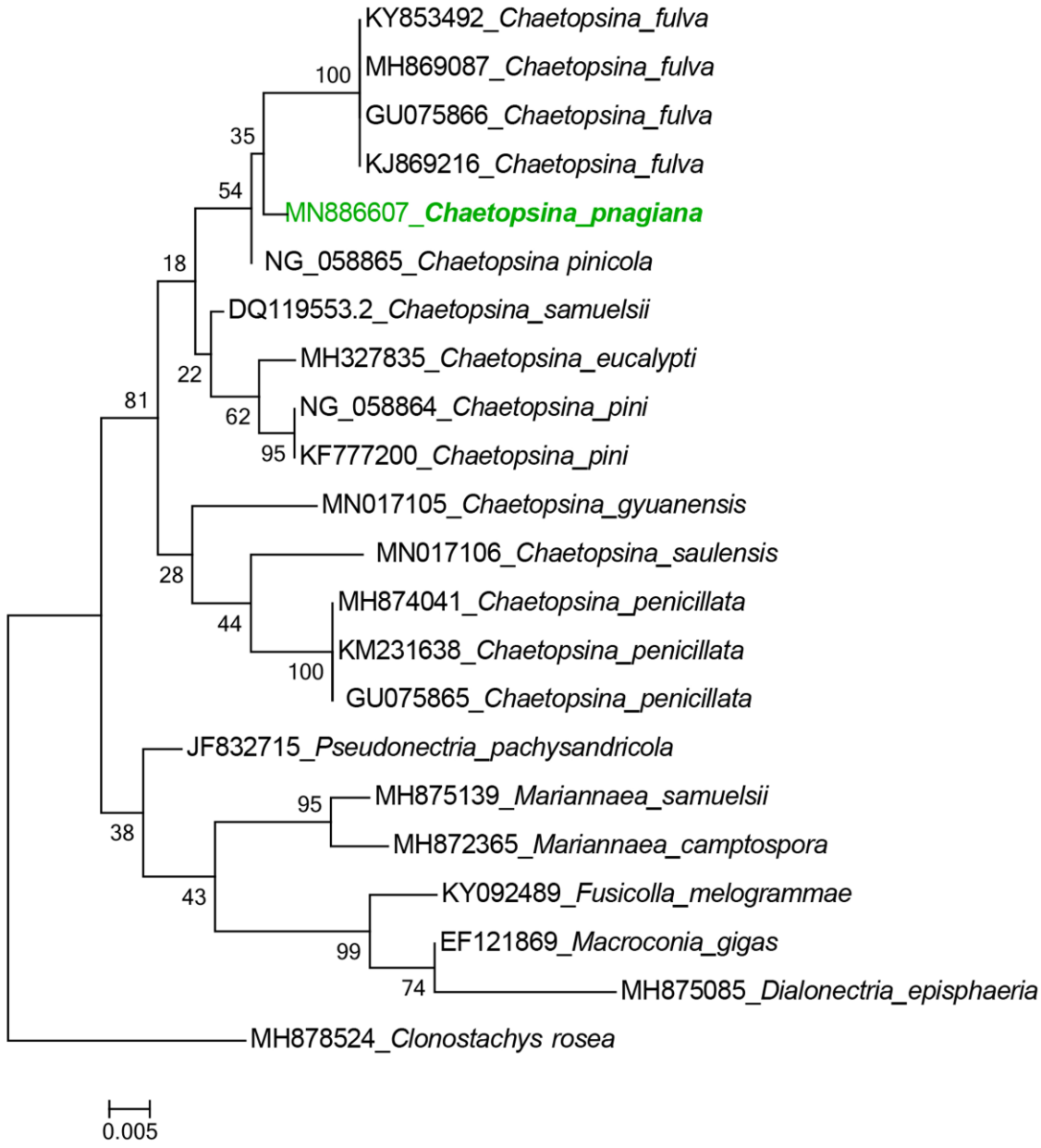

Fig. 2 – Maximum likelihood phylogeny (-lnL = 2023.84) of *Chaetopsina* spp. inferred by Tamura-Nei model from a 860 bp matrix of LSU sequences, rooted with *Clonostachys rosea*.

yses (Figs. 1–2) show that ITS and LSU sequences unambiguously place our fungus in *Chaetopsina*, on an isolated branch, close to *C. pinicola* Crous & Cheew. However, this species is only known in the asexual morph occurring on *Pinus* needles in Thailand (Crous *et al.*, 2013), differing primarily in having smaller, subcylindrical conidia (11–)13–15(–17) × 2(–2.5) µm vs (25–)28–32(–34) × 7–9 µm in *C. pnagiana*, both species having only 96.3% and 98.4% similarity of their ITS and LSU sequences respectively. Introduced after *C. guyanensis* Lechat & J. Fourn. and *C. saulensis* Lechat & J. Fourn. (Lechat & Fournier, 2019c), *C. pnagiana* is the eighth known holomorphic

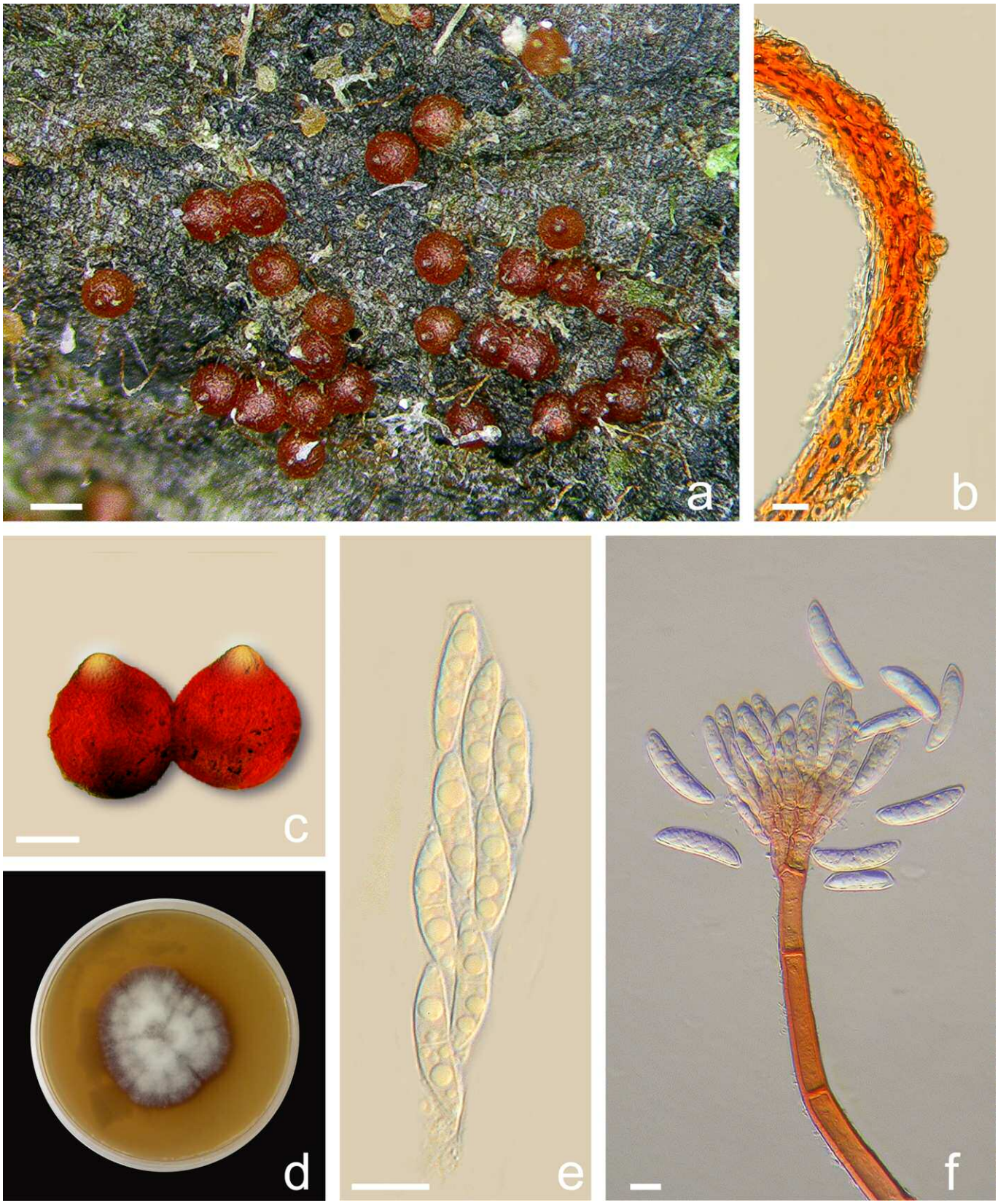

Fig. 3 – a-f: *Chaetopsina pnagiana* CLLG19028 (Holotype); a: Ascomata in natural environment; b: Lateral ascomatal wall in vertical section in water; c: Close-up of ascomata in water; d: Culture at three weeks; e: Ascus and ascospores in water; f: Conidiophores and conidia from nature in water. Scale bars: a = 200 µm; b, e, f = 10 µm; c = 100 µm.

species of this genus and the third new species discovered in a short time in Saül, suggesting that there are possibly still numerous unknown species to be discovered.

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1: C. Lechat – 64 route de Chizé, 79360 Villiers-en-Bois, France – lechat@ascofrance.fr

2: J. Fournier – Las Muros, 09420 Rimont, France – jfournzeroneuf@gmail.com