Chrysonectria, a new genus in the *Nectriaceae* with the new species *C. finisterrensis* from France

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Abstract: *Chrysonectria finisterrensis* gen. and sp. nov. is described and illustrated, based on a collection from France on bark of *Quercus robur*. The acremonium-like asexual morph of this species was obtained in culture and sequenced. The genus is placed in the *Nectriaceae* based on ascomata changing colour in 3% KOH or lactic acid as defined by Rossman *et al.* (1999), acremonium-like asexual morph and phylogenetic analysis of its LSU sequence. Morphologically, *Chrysonectria* is primarily characterized by gregarious, corticolous, globose to subglobose, pale orange, non-stromatic ascomata with ascomatal surface covered by bright yellow hyphal elements arising from the subiculum, releasing orange yellow pigments in 3% KOH or yellow pigments in lactic acid.
Keywords: Acremonium-like, Ascomycota, *Hypocreales*, ribosomal DNA, taxonomy.

Résumé : *Chrysonectria finisterrensis* gen. et sp. nov. est décrite et illustrée d'après une récolte effectuée en France sur écorce de *Quercus robur*. La forme asexuée de cette espèce a été obtenue en culture et séquencée. Le genre est placé dans les *Nectriaceae* d'après les ascomes changeant de couleur dans KOH à 3% ou dans l'acide lactique, comme défini par Rossman *et al.* (1999), la forme asexuée de type acremonium et l'analyse phylogénétique de sa séquence LSU. *Chrysonectria* est principalement caractérisé par des ascomes globuleux à subglobuleux, orange pâle, non stromatiques, dont la surface est couverte d'hyphes jaune vif se développant à partir d'un subiculum et libérant un pigment jaune orangé dans la potasse à 3 %, jaune dans l'acide lactique.
Mots-clés : Acremonium, ADN ribosomal, Ascomycota, Hypocréales, taxinomie.

Introduction

In the continuation of our survey of hypocrealean fungi in Europe, a collection on bark of *Quercus robur* was intriguing because of the golden yellow colour of its unusual type of vestiture. It was assigned to the *Nectriaceae* Tul. & C. Tul. based on its pale orange ascomata changing colour in 3% KOH or lactic acid, which was confirmed by our phylogenetic analysis. This fungus was successfully cultured and yielded an acremonium-like asexual morph. Morphologically, it resembled some species of the bionectriaceous genus *Nectriopsis* Maire, but was precluded by the positive reaction of the ascomatal wall to KOH and the result of our analysis of its LSU sequence. We document herein the morphological and phylogenetic differences of this fungus with other genera of the *Nectriaceae*, leading to the recognition of the new genus *Chrysonectria*, accommodating the new species *Chrysonectria finisterrensis*. The affinities and differences of the new species with two strains of acremonium shown by the phylogenetic analysis are discussed.

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 specimen and paratypes were deposited in LIP herbarium (Lille) and living cultures in the CIRM Collection (Centre International des Resources Microbiennes, France). Cultures of the living specimen were made on PDA (Potato Dextrose Agar) with 5 mg/l of streptomycin in Petri dishes 9 cm diam., incubated at 25°C. DNA extraction, amplification, and sequencing were performed by ALVALAB (Santander, 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 65ºC. 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 LR0R and LR5 (Vilgalys & Hester, 1990) to amplify the 28S nLSU region. PCR reactions were performed under a program consisting of a hot start at 95 ºC for 5 min, followed by 35 cycles at 94 ºC, 54 ºC and 72 ºC (45, 30 and 45 s respectively) and a final 72 ºC step 10 min. Chromatograms were checked searching for putative reading errors, and these were corrected.

Analyses were performed online at www.phylogeny.lirmm.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 (CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands).

Taxonomy

Chrysonectria Lechat & J. Fourn., *gen. nov.* – MycoBank MB 826765

Diagnosis: Differs from other genera of the *Nectriaceae* having acremonium-like asexual morph by pale orange ascomata diffusing abundant, orange yellow pigment in 3% KOH, or bright yellow in lactic acid, overlain by golden yellow hyphal elements.

Asexual morph: acremonium-like.

Type species: *Chrysonectria finisterrensis* Lechat, J. Fourn. & Priou

Etymology: From Greek χρυσός = gold, for the colour of the hyphal elements covering ascomata.

Chrysonectria finisterrensis Lechat, J. Fourn. & Priou, *sp. nov.* – Figs. 1–3 – MycoBank MB 826766

Diagnosis: Ascomata superficial, non-stromatic, subglobose, not collapsing upon drying, pale orange, changing colour in 3% KOH and lactic acid, overlain by golden yellow hyphal elements; ascomatal wall 25–35 µm thick, of two regions; asci 8-spored, cylindrical to fusiform, with a refractive apical apparatus; ascospores 12–15 × 3–4 µm, subfusiform to narrowly clavate, two-celled, smooth. Asexual morph acremonium-like.

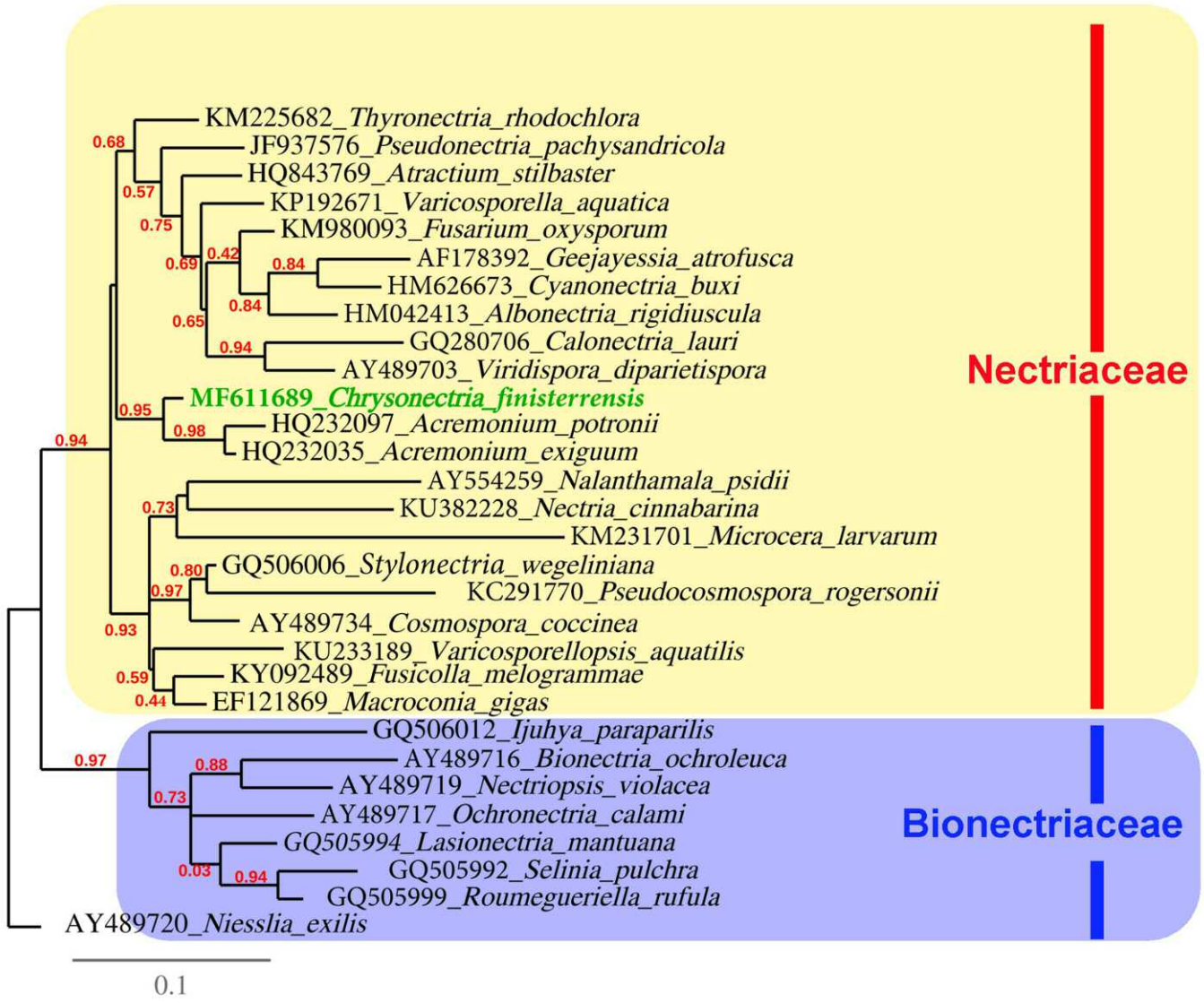

Fig. 1 – Maximum likelihood phylogeny (-lnL = 9036.31560) of *Chrysonectria finisterrensis* inferred by PhyML 3.0, model HKY85 from a 850 bp matrix of 28S rRNA sequences, rooted to *Niesslia exilis*, placing the new genus in the *Nectriaceae*. ML bootstrap support is given above or below the branches.

Etymology: The epithet "*finisterrensis*" refers to the French western department Finistère, where the new species was collected.

Holotype MBT383000: France, Finistère, Clohars-Carnoet, 47°48' 29.75" N 3°35'10.49 " W, alt. 61 m, on bark of *Quercus robur*, 26 Jan. 2017, *leg*. Y. Quelenn, communicated by J.-P. Priou, JPP17021 (LIP), ex-type culture deposited at CIRM (Centre International des Ressources Microbiennes, France) BRFM 2477. GenBank LSU: MF611689.

Ascomata gregarious, non-stromatic, globose to subglobose, not collapsing when dry, easily removed from substratum, partially immersed in a cottony, white to golden yellow subiculum, 180–220 µm high, 160–200 µm wide, pale orange but appearing bright yellow due to the golden yellow hyphal elements covering ascomatal wall, becoming dark orange when dry; upper third turning partially red in 3% KOH, releasing an abundant orange yellow pigment, but becoming yellow in lactic acid and releasing a bright yellow pigment. Ascomatal surface pale orange, obscured by golden yellow hyphal elements arising from subiculum. **Perithecial apex** brownish orange to brown around the pale yellow, translucent papilla. **Papilla** truncate, 30–40 µm diam., composed of subglobose to narrowly ellipsoidal, hyaline cells 4–10 × 2.5–3.5 µm, merging with periphyses. **Subiculum** composed of thin-walled hyphae proliferating upwards to cover ascomatal wall except ostiolar region, 2–3 µm diam with wall 0.5(–1) µm thick, septate, rounded at free ends, golden yellow, becoming dark orange in 3% KOH. **Ascomatal wall** excluding hyphal elements 25–35 µm thick, of two regions; outer region 15–25 µm thick, composed of thick-walled cells of undefined shape forming a *textura epidermoidea*, yellow to pale orange; inner region 8–10 µm thick composed of ellipsoidal, elongate, thin-walled, hyaline cells 5–10 × 3–5 µm. **Asci** evanescent, cylindrical to fusiform, containing eight irregularly biseriate ascospores, (50–)55–70(–75) × 8–9 µm, apically truncate with a refractive apical apparatus. **Ascospores** subfusiform to narrowly clavate, rounded at ends, equally 2-celled, usually with lower cell attenuated at base and narrower than upper cell, smooth, hyaline, (10–)12–15(–18) × 3–4(–4.5) µm, with 2–4 drops in each cell.

Cultural characteristics: Colony on PDA at 25°C, slow growing, reaching 10–15 mm diam after two weeks, grey to pale greenish brown, not producing pigmentation in medium, reverse pale brown. Conidiophores covering whole colony, yellow, usually simple or sometimes branched, arising from smooth hyphae 2–3 µm diam. Conidiogenous cells subulate, 20–28(–35) µm long with an unflared collarette, producing subglobose to widely ellipsoidal conidia, 1-

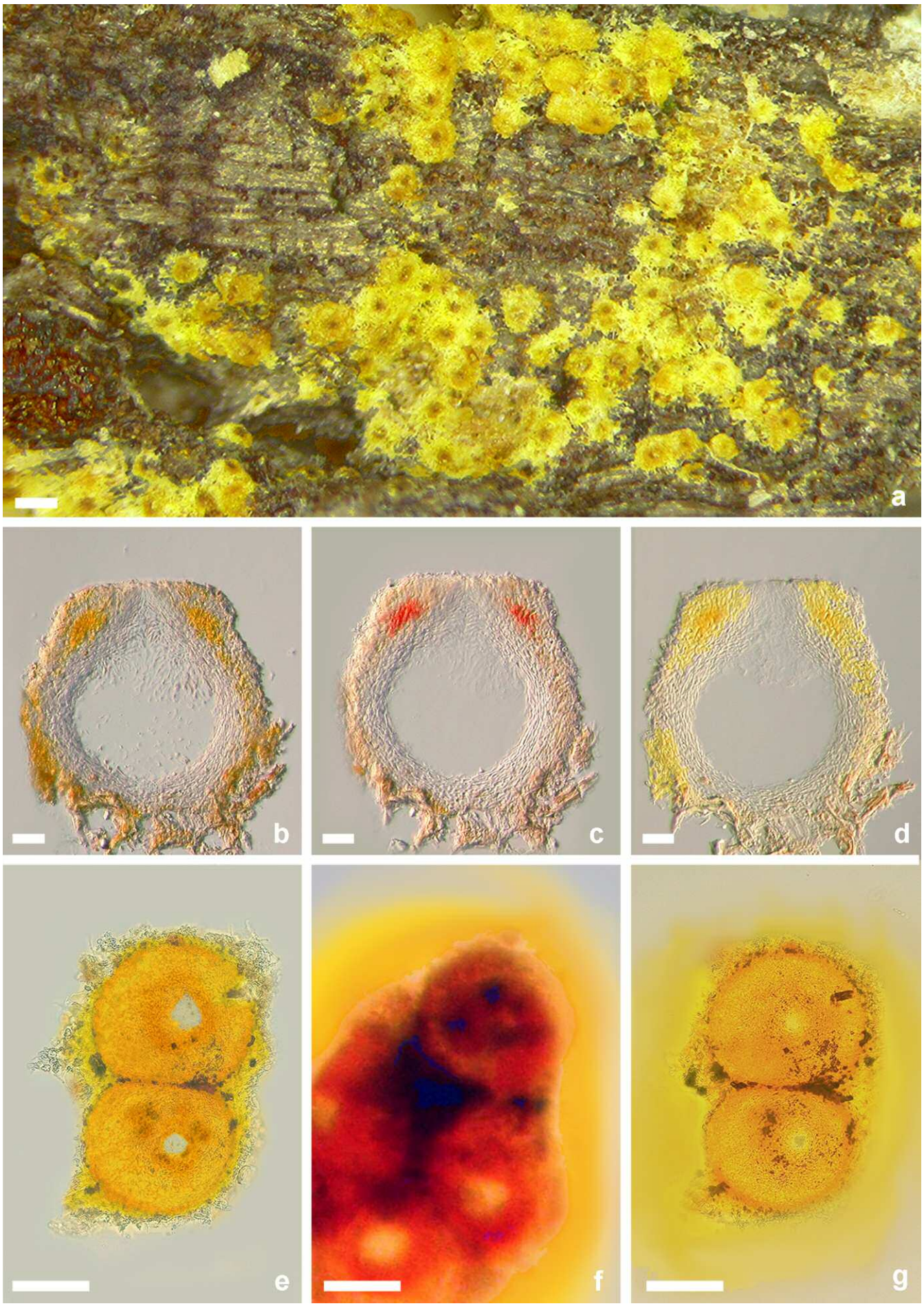

Fig. 2 – a-g: *Chrysonectria finisterrensis* (Holotype JPP17021); a: Ascomata on the substratum; b-d: Median vertical section of ascomata (b: In water, c: In 3% KOH, d: In lactic acid); e-g: Ascomatal apex in top view (e: In water, f: In 3% KOH, g: In lactic acid); Scale bars: a: 200 µm, b-d: 20 µm, e-g: 100 µm.

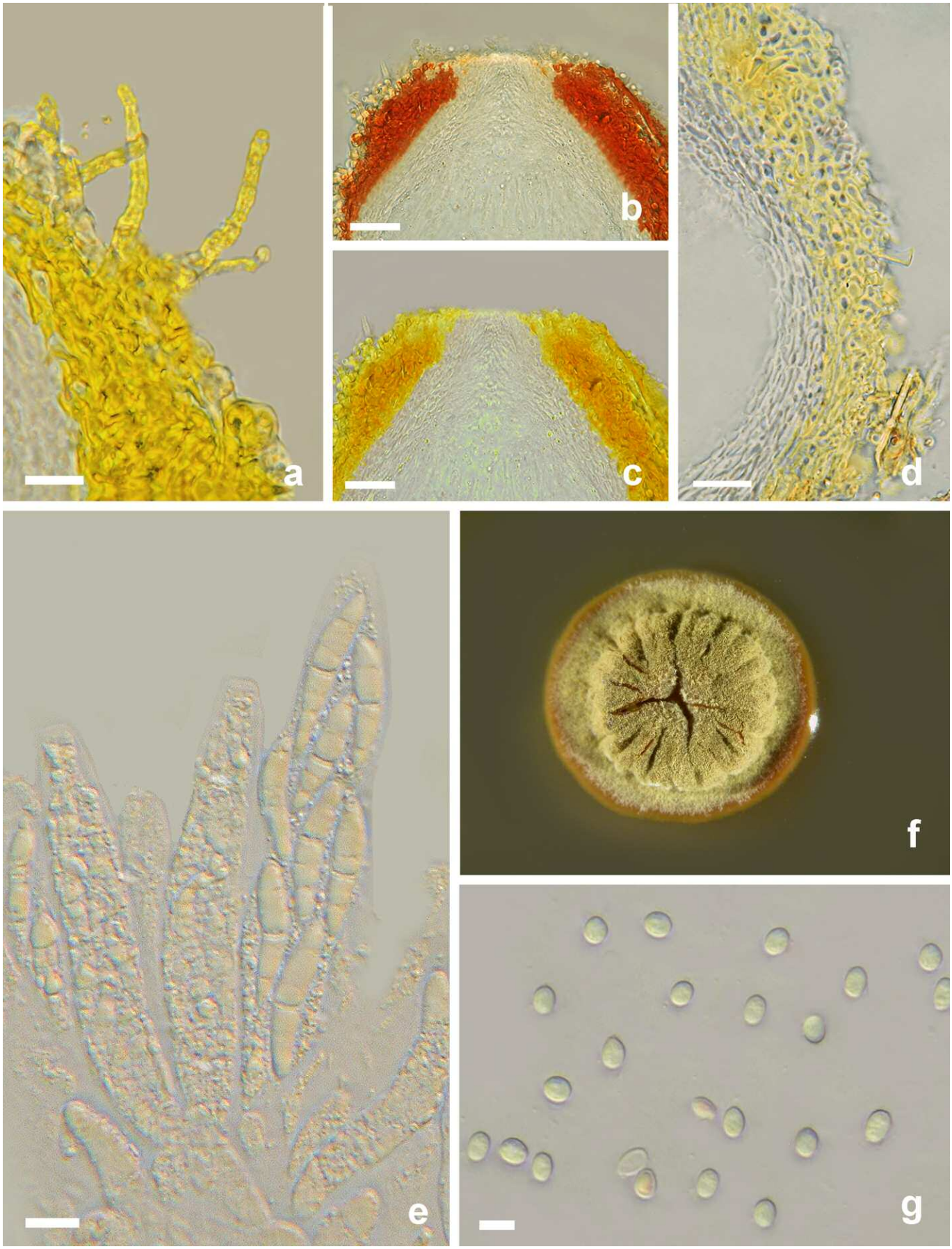

Fig. 3 – a-e: *Chrysonectria finisterrensis* (Holotype JPP17021); a: Ascomatal wall in vertical section showing the free ends of golden yellow, hyphal elements in lactic acid; b, c: Apex of perithecium, b: in 3% KOH, c: in lactic acid; d: Lateral ascomatal wall in vertical section in water; e: Asci and ascospores; f: Culture after two weeks; g: Conidia in water. Scale bars a, e: 10 µm, b-d: 20 µm, g: 5 µm.

celled, smooth, hyaline to pale yellowish en masse, 2.5–3.5(–4) × 2.5–3 µm without a visible abscission scar, symmetric or asymmetric.

Discussion

Hypocrealean fungi having soft-textured and brightly coloured ascomata changing colour in 3% KOH or lactic acid are considered to belong to the *Nectriaceae* as defined by Rossman *et al.* (1999), Hirooka *et al.* (2012) and Lombard *et al.* (2015). *Chrysonectria finisterrensis* matches these characteristics but the set of characters documented above does not match any known nectriaceous genus. Phylogenetic analysis, comparing it to 19 genera belonging to the *Nectriaceae* and 7 genera in the *Bionectriaceae* Samuels & Rossman, shows that the new genus is nested in an isolated clade within the *Nectriaceae*, clustering with *Acremonium exiguum* Gams (1975) and *A. potronii* Vuill. (1910), both species whose sexual morphs are unknown. This confirms the nectriaceous affinities of *Chrysonectria* and its distinctiveness within the known nectriaceous genera.

The asexual morph of *Chrysonectria finisterrensis* is primarily characterized by a grey to pale greenish brown colony, yellow conidiophores and subglobose to widely ellipsoidal conidia and compared with those of *A. exiguum* and *A. potronii*. *Acremonium exiguum*, known from Sri Lanka (Ceylan), clearly differs by forming a white to pinkish colony in culture, yielding smaller and more slender conidia 2–2.7(–3.5) × 1–1.5 µm (Gams, 1975) vs. 2.5–3.5(–4) × 2.5–3 µm. *Acremonium potronii*, isolated from the liquid of a human knee bursitis, was reported to have an optimal growth rate at 35–37 °C and to produce ovoid conidia 4–5 × 2–2.2 µm with a basal abscission scar, which are pink en masse. Morphological and ecological divergences of these species from *Chrysonectria* suggest that they are distinct, which is well supported by our phylogenetic analysis showing that *Chrysonectria* is placed within the same subclade but on a distant branch from both *Acremonium* species (Fig. 1).

Based on morphological characteristics, phylogenetic analysis and comparison with known genera in the *Nectriaceae*, *Chrysonectria* Lechat & J. Fourn. *gen. nov.* is proposed as a new genus with the type species *Chrysonectria finisterrensis* Lechat, J. Fourn. & Priou *sp. nov.*

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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., Dufayard J.F., Guindon S., Lefort V., Lescot M., Claverie J.M. & Gascuel O. 2008. — Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, 2008 Jul 1; 36 (Web Server issue): W465–9. doi: 10.1093/nar/gkn180

Gams W. 1975. — *Cephalosporium*-like Hyphomycetes: Some tropical species. *Transactions of the British Mycological Society*, 64 (3): 389–404. doi: 10.1016/S0007-1536(75)80138-0

Hirooka Y., Rossman A.Y., Samuels G.J., Lechat C. & Chaverri P. 2012. — A monograph of *Allantonectria*, *Nectria* and *Pleonectria* (*Nectriaceae*, *Hypocreales*, Ascomycota) and their pycnidial, sporodochial and synnematous anamorphs. *Studies in Mycology*, 71: 1–210. doi: 10.3114/sim0001

Lombard L., Merwe N.A. (van der), Groenewald J.Z. & Crous P.W. 2015. — Generic concepts in *Nectriaceae*. *Studies in Mycology*, 80: 189–245. doi: 10.1016/j.simyco.2014.12.002

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.

Vuillemin P. 1910. — Les conidiosporés. *Bulletin de la Société des Sciences de Nancy*, 11: 129–172.

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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