

Neocosmospora lechatii (Nectriaceae) a new species from French Guiana

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Ascomycete.org, 14 (4-5) : 165–171

Mise en ligne le 03/12/2022

doi 10.25664/ART-0360



Abstract: A description of *Neocosmospora lechatii* sp. nov. is presented, based on two collections on dead wood of unknown origin collected in French Guiana. The specimens were obtained in culture and six loci were amplified, sequenced, and compared with the currently known phylogenetic breadth of *Neocosmospora*. The new species is described, illustrated, and morphological comparisons are made to its closest relatives.

Keywords: Ascomycota, fusarioid, Hypocreales, new species, taxonomy.

Résumé : une description de *Neocosmospora lechatii* sp. nov. est présentée, à partir de deux récoltes sur bois mort d'origine inconnue, effectuées en Guyane française. Les spécimens ont été obtenus en culture et six loci ont été amplifiés, séquencés et comparés à l'étendue phylogénétique connue des *Neocosmospora*. La nouvelle espèce est décrite, illustrée et des comparaisons morphologiques sont effectuées avec les plus proches parents.

Mots-clés : Ascomycota, fusarioïde, Hypocreales, nouvelle espèce, taxinomie.

Introduction

The genus *Neocosmospora* E.F. Sm. (Nectriaceae), which is allied to *Fusarium* Link (LOMBARD *et al.*, 2015; CROUS *et al.*, 2021) is the second largest hypocrealean genus of fusarioid fungi, presently encompassing more than 100 accepted species (www.fusarium.org; CROUS *et al.*, 2021). *Neocosmospora* spp. colonise a vast diversity of substrates and geographical ranges. They are associated with many different ecological niches, including devastating plant pathogens and opportunistic human pathogenic species, also including endophytes, saprophytes, and mycotoxin producers (SANDOVAL-DENIS *et al.*, 2019).

Species of *Neocosmospora* are characterised by conspicuous dark orange to red perithecia, with two-layered, often coarsely warted perithecial walls that change colour in both 3% KOH and lactic acid; and ornamented, commonly striated, (0–)1-septate, yellow-brown ascospores (ROSSMAN *et al.*, 1999; SANDOVAL-DENIS *et al.*, 2019). The asexual morphs of *Neocosmospora* are commonly encountered in nature, often being characterised by robust fusarioid conidia, formed either on sporodochia or on tall, solitary aerial conidiophores, the latter typically showing long, tapered phialidic conidiogenous cells.

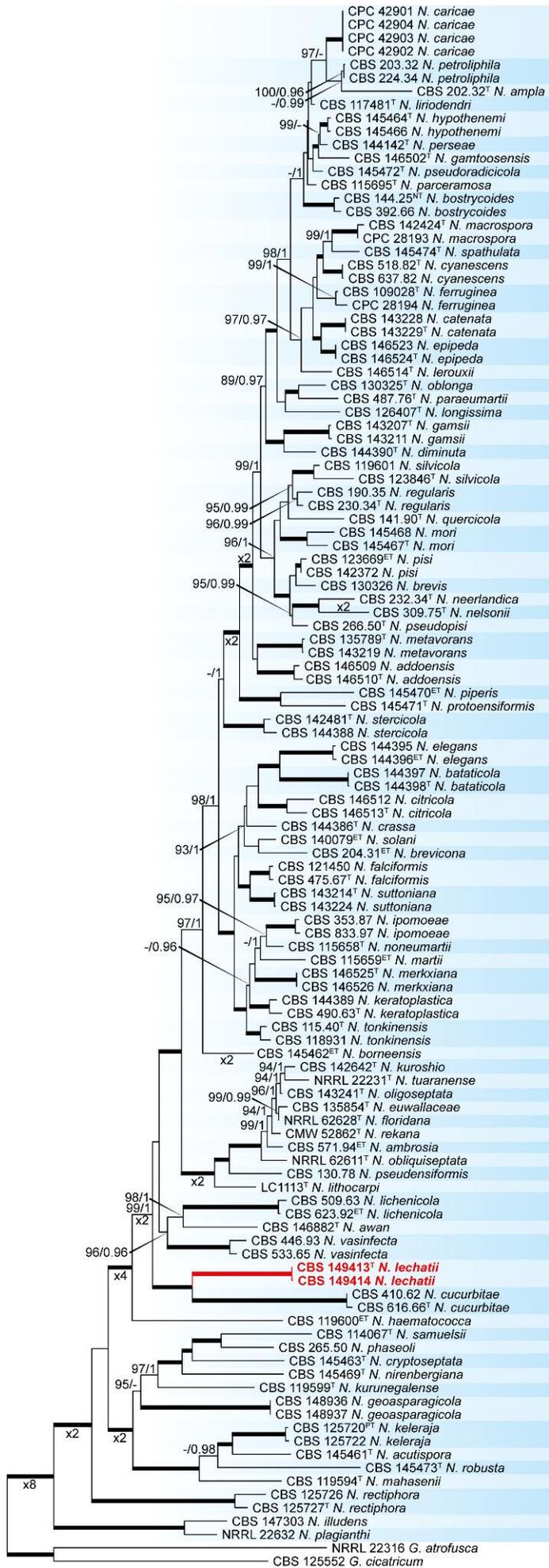
During a survey of microfungi in French Guiana an interesting fusarioid fungus was found that morphologically resembled *Neocosmospora*, producing typical red, warted perithecia, and large 1-septate, coarsely striate ascospores. The fungus was isolated and preliminarily identified as a *Neocosmospora* sp. Based on its morphological characters, and molecular phylogenetic analyses, the specimens are determined to represent a novel species, described here as *Neocosmospora lechatii* in honour of its collector.

Materials and methods

The specimens were examined and single-spore cultures established using methods described elsewhere (LECHAT & FOURNIER, 2015). Living cultures were sent to the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands) for confirmation of its identity and permanent storage. Fungal cultures were maintained in Oatmeal Agar (OA, recipe in CROUS *et al.*, 2019) plates and slants. Fungal growth rates and pigment production were studied on Potato Dextrose Agar (PDA, recipe in CROUS *et al.*, 2019), additional colony features were recorded on OA and Synthetic Nutrient-poor Agar (SNA; NIRENBERG, 1976). Colour notations follow those of RAYNER (1970). Micro-morphological features were studied as outlined in CROUS *et al.* (2021) from 7–10-d-old cultures grown on SNA and Carnation

Leaf Agar (CLA; FISHER *et al.*, 1982), incubated at 24 °C, under a 12 h near-UV light/dark cycle. Unless otherwise defined, measurements and photomicrographs were recorded using sterile water as mounting medium with a Nikon Eclipse 80i compound microscope with Differential Interference Contrast (DIC) optics and a Nikon AZ100 dissecting microscope, both equipped with a Nikon DS-Ri2 high-definition colour digital camera and the Nikon software NIS-elements D v. 5.11.02. Additional images of the fungus growing on its natural substrate were obtained from the records of the late C. Lechat and incorporated on the morphological analyses and photo plates.

For DNA isolation isolates were grown for 7 d on Malt Extract Agar (MEA, recipe in CROUS *et al.*, 2019), incubated as described above. Mycelium was scrapped from the surface of the colony using a sterile scalpel and processed using the Wizard® Genomic DNA purification Kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's instructions. Fragments of six DNA loci i.e., partial calmodulin gene (*cal*), the internal transcribed spacer regions with intervening 5.8S nrRNA gene (ITS), a fragment of the 28S large subunit of the nrDNA (LSU), partial DNA-directed RNA polymerase II gene largest subunit (*rpb1*), partial DNA-directed RNA polymerase II gene second largest subunit (*rpb2*) and partial translation elongation factor 1-alpha gene (*tef1*) were amplified using conditions and primer pairs as indicated elsewhere (CROUS *et al.*, 2021; GUARNACCIA *et al.*, 2021). Bidirectional sequences were generated using the same primer pairs as for PCR amplification on an Applied Biosystems 3730xl DNA analyser (Life Technologies, Carlsbad, California, USA). Sequences were assembled using Geneious Prime v. 2022.0.1 (Biomatters Ltd., Auckland, New Zealand). Gene sequence datasets of all six loci included in this study, representing the known species diversity of *Neocosmospora*, were downloaded from GenBank, and single-gene alignments were built using MAFFT v. 7.450 (KATO H *et al.*, 2013) as implemented in Geneious Prime, and manually checked using MEGA v. 6 (TAMURA *et al.*, 2007). Single-gene phylogenies were carried out using Maximum Likelihood (ML) in IQ-TREE v. 2.1.2 (MINH *et al.*, 2020) and Bayesian Inference (BI) using MrBayes v. 3.2.7a (RONQUIST & HUELSENBECK, 2003) as indicated in CROUS *et al.* (2021). The phylogenetic results were visually compared for topological conflicts, and a 6-gene multi-locus phylogenetic analysis was carried out as specified above. Alignments, phylogenetic trees and accession data of strains included in the analyses were deposited in Figshare.com (project number 149332, doi: 10.6084/m9.figshare.21195601.v1 and 10.6084/m9.figshare.21176314.v1). The taxonomic novelty was deposited in MycoBank, and DNA sequence data generated in this study in GenBank.



IQ-TREE phylogeny
 6 genes
 5344 sites
cal = 586
 ITS = 481
 LSU = 482
rpb1 = 1491
rpb2 = 1613
tef1 = 691
 1449 informative sites
 Best ML tree = -43633.577
 0.05

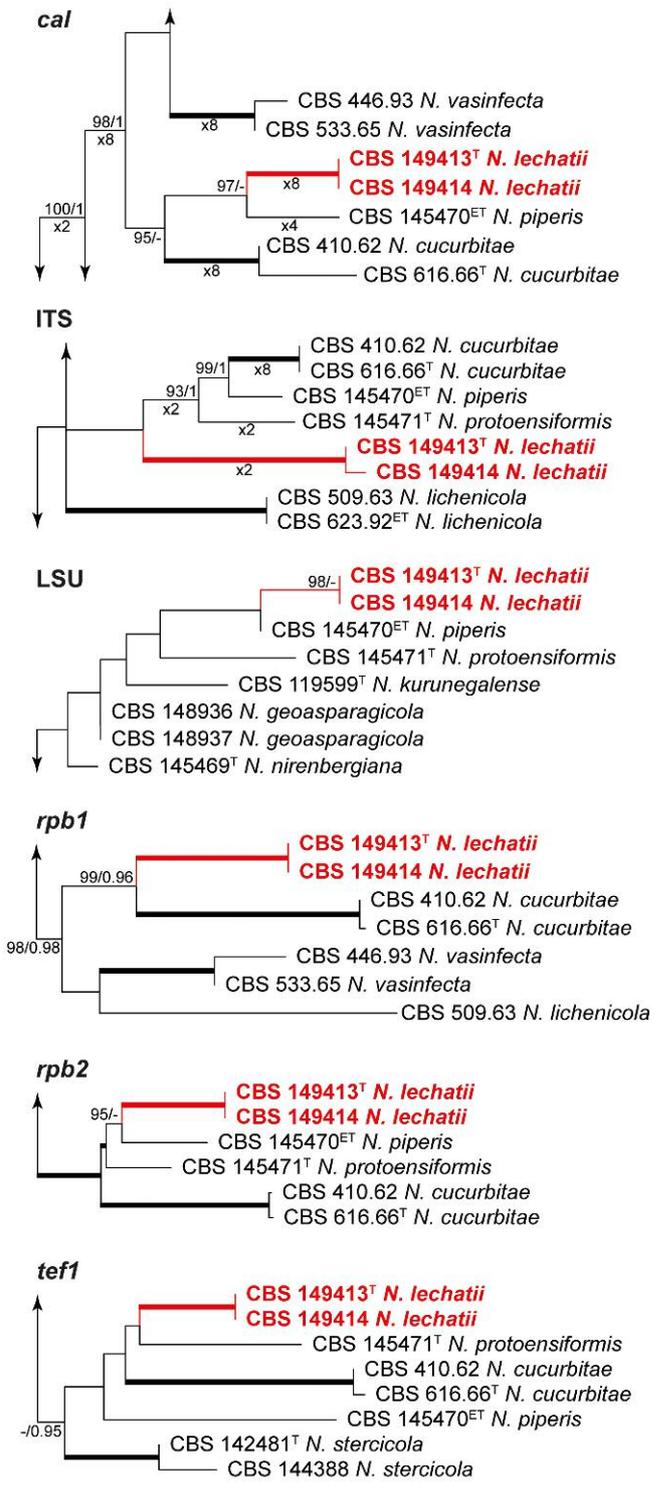


Fig. 1 (previous page) – Left: IQ-TREE phylogram obtained from the combined *cal*, ITS, LSU, *rpb1*, *rpb2* and *tef1* partial sequences of representative *Neocosmospora* spp.; species clades are indicated with colour boxes. Alignment basic statistics and tree scale are indicated inside the box. Right: partial view of relevant clades obtained by the single locus phylogenies (full trees available at Figshare.com under project number 149332 (doi: 10.6084/m9.figshare.21195601.v1 and 10.6084/m9.figshare.21176314.v1). Numbers at the nodes correspond to IQ-TREE bootstrap (ML-BS) values $\geq 95\%$ followed by Bayesian posterior probabilities (BI-PP) ≥ 0.95 . Bold branches indicate full support (ML-BS = 100, BI-PP = 1). The trees are rooted to *Geejayessia atrofusca* NRRL 22316 and *G. cicatricum* CBS 125552. The scale bar indicates the expected number of nucleotide substitutions per site. The new species proposed here is indicated in red font. Ex-epitype, ex-neotype, ex-paratype and, and ex-type strains are indicated with superscript ET, NT, PT and T, respectively.

Results

The phylogenetic analysis included 5344 sites from six loci (*cal* 586, ITS 481, LSU 482, *rpb1* 1491, *rpb2* 1613, and *tef1* 691) of which 3478 were invariable (*cal* 350, ITS 312, LSU 419, *rpb1* 980, *rpb2* 1046, and *tef1* 371), and 1449 were informative (*cal* 180, ITS 128, LSU 37, *rpb1* 402, *rpb2* 459, and *tef1* 243). Evolutionary models for each data partition as calculated for ML were TNe+I+G4 for *cal* and LSU, TIM2e+R3 for ITS, TIM3e+I+G4 for *rpb1* and *rpb2*, and SYM+R4 for *tef1*; while GTR+I+G was selected for all loci under BI. The ML best tree (log-likelihood -43633.557) was found after 176 iterations. The BI lasted for 3.27 M generations and recovered 6542 trees from which 4908 were included in the final analyses. The phylogeny included sequences from 117 strains, representing 79 known *Neocosmospora* spp., two novel isolates (CBS 149413 and CBS 149414) obtained in culture from specimens collected in French Guiana, and two outgroup taxa (*Geejayessia atrofusca* NRRL 22316 and *G. cicatricum* CBS 125552). The results from ML and BI were identical, hence only the ML topology is shown in Fig. 1, with both ML bootstrap (ML-BS) and BI posterior probability (BI-PP) values added at the nodes.

The combined phylogeny resolved 80 species-level clades in *Neocosmospora* (known species published with two or less loci, and phylogenetic species yet not formally described were excluded from the analyses). Isolates CBS 149413 and CBS 149414 were resolved as a well-delimited, fully supported (ML-BS = 100, BI-PP = 1) clade, related but phylogenetically distinct to *Neocosmospora cucurbitae* (p-distances: 0.05 for the combined dataset, *rpb2*, and *tef1*; ITS 0.09, *cal* 0.07, *rpb1* 0.04, and LSU 0.03). Single-gene phylogenies confirmed the previous results; strains CBS 149413 and CBS 149414 were resolved as a distinct, partially (LSU) to fully supported (*cal*, ITS, *rpb1*, *rpb2* and *tef1*) clade with every locus tested. This novel clade is therefore described below as the novel species, *Neocosmospora lechatii*.

Taxonomy

Neocosmospora lechatii Sand.-Den. & Crous, *sp. nov.* Figs. 2–4. MycoBank: MB 845723

Diagnosis: Differs from all known *Neocosmospora* spp. by forming two types of sporodochia; and from *N. acutispora* and *N. samuelisii* by the presence of microconidia and a sexual morph. Differs from *N. cucurbitae* and *N. mori* by its homothallic mating behaviour, shorter, falcate aerial macroconidia, and its wider, robust, predominantly 7-septate sporodochial macroconidia.

Holotype: FRENCH GUIANA, Saül, Gros Arbres trail, on unidentified dead wood, 26 March 2021, *leg.* C. Lechat (holotype designated here CBS H-25085), ex-type culture CBS 149413 = CLLG21062 = CPC 42648. GenBank accession numbers: *cal* = OP481878, ITS = OP497971, LSU = OP497975, *rpb1* = OP481880, *rpb2* = OP481882, and *tef1* = OP481884.

Etymology: In honour of the late French mycologist Christian Lechat (17 March 1952 – 7 Jan. 2022), who made a significant contribution to the systematics of hypocrealean fungi.

Perithecia superficial, non-stromatic, solitary or in small groups, dark orange to red, globose to broadly subglobose, 363–644 μm diam., warted, dark red in 3% KOH, yellow in 100% lactic acid.

Perithecial wall of two regions: outer region pigmented, composed of thick-walled subglobose to angular cells; inner region hyaline, composed of flattened cells, compacted, and oriented parallel to surface of perithecial wall; apex composed of vertically elongated, thin-walled cells. **Asci** cylindrical to clavate, (104–)116–146(–153) \times 11–12.5(–13.5) μm (av. 131 \times 12.2 μm), apex simple, 8-spored, ascospores uniseriate. **Ascospores** ellipsoid, (13–)14–17(–20) \times (6–)7.5–10 μm (av. 15.6 \times 8.3 μm), 1-septate, hyaline, becoming golden-brown at maturity, coarsely striate. **Conidiophores** abundantly formed on aerial mycelium or in sporodochia. Aerial conidiophores erect or prostrate, borne on the agar substrate and aerial mycelium, 42–165 μm tall, often simple, or branched laterally and sympodially, bearing terminal single phialides; aerial conidiogenous cells monophialidic, subulate to subcylindrical, smooth- and thin-walled, 18.5–71 \times 2.5–5 μm , with short, flared apical collarettes, and often conspicuous periclinal thickening. **Aerial conidia** of two types: i) microconidia ovoid to ellipsoid, smooth- and thin-walled, often with a narrow, flattened base, 0(–1)-septate, 6–13.5(–25.5) \times (3–)4–6 μm (av. 10 \times 4.2 μm); ii) macroconidia falcate to fusoid, gently dorsiventrally curved or almost straight, robust, apical cell blunt, basal cell obtuse, poorly- to well-developed foot-shaped, 2–4(–5)-septate, predominantly 3-septate, 2-septate conidia: (24.5–)27.5–39 \times (4.5–)5–6 μm (av. 32.7 \times 5.6 μm); 3-septate conidia: (26–)27.5–38(–40) \times (4.5–)5.5–6 μm (av. 32.9 \times 5.5 μm); 4-septate conidia: (42.5–)43–48.5(–50.5) \times 4.5–6 μm (av. 46.7 \times 5.4 μm); 5-septate conidia: 50.1 \times 5.9 μm (only one element observed); overall: (24–)28.5–45(–50) \times 4.5–6 μm (av. 36.5 \times 5.5 μm), borne at the tip of monophialides and accumulating on false-heads. **Sporodochia** produced abundantly on the surface of carnation leaves and less commonly on the agar surface, of two types: i) microsporodochia pale luteous to pale green. Microsporodochial conidiophores 32–77 μm tall, laterally, and verticillately branched bearing terminal phialides; microsporodochial conidiogenous cells monophialidic, subulate to subcylindrical, (11–)17–27.5(–33) \times 2.5–4 μm , smooth and thin-walled, with conspicuous periclinal thickening and a short apical collarette. Microsporodochial conidia morphologically indistinguishable from aerial microconidia. ii) macrosporodochia pale luteous to amber. Macrosporodochial conidiophores 30–54 μm tall, laterally, verticillately, and irregularly branched bearing terminal single or paired phialides; macrosporodochial conidiogenous cells monophialidic, doliiform to subcylindrical, (11.5–)14–20(–24) \times 3.5–5.5 μm , smooth and thin-walled, often proliferating percurrently, periclinal thickening absent or conspicuous, short, flared collarette often visible. Macrosporodochial conidia falcate, gently dorsiventrally curved, robust, apical cell blunt to slightly papillate and curved, basal cell well-developed, foot-shaped, 5–9-septate, predominantly 7-septate, hyaline, smooth- and thick-walled; 5-septate conidia: (46.5–)55–73.5 \times 5.5–6.5 μm (av. 64.1 \times 5.9 μm); 6-septate conidia: (65–)67.5–76(–81.5) \times 5.5–7 μm (av. 71.7 \times 6.2 μm); 7-septate conidia: (68.5–)73–82.5(–88) \times 6–7 μm (av. 77.6 \times 6.3 μm); 8-septate conidia: (70–)72–84.5(–92) \times 6–7 μm (av. 78 \times 6.3 μm); 9-septate conidia: (83–)84–91(–95) \times 6–7 μm (av. 87.5 \times 6.6 μm); overall: (46.5–)69–84(–95) \times 5.5–7 μm (av. 76.6 \times 6.3 μm). **Chlamydospores** subspherical to spherical, smooth- and thin-walled, 5–10.5 μm diam, hyaline, formed intercalary or terminally on hyphae and conidia, single or in groups.

Cultural characteristics: Colonies at 25 °C for 7 days covering an entire 9-cm-diam. Petri dish on all media tested: On PDA, luteous to

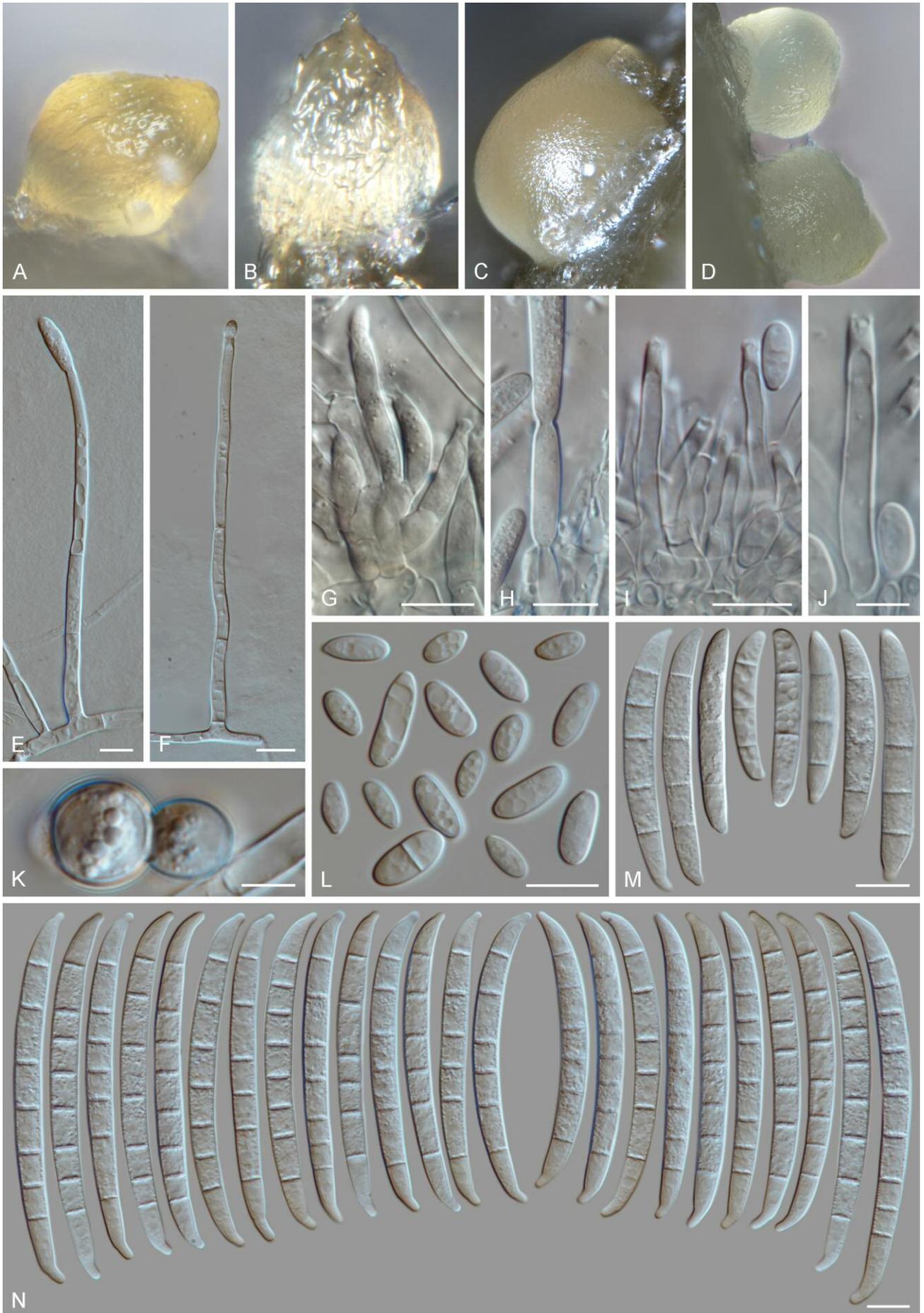


Fig. 2 – A–N: *Neocosmospora lechatii* sp. nov. (CBS 149413 ex-type), asexual morphology *in vitro*. A–D: sporodochia produced on the surface of carnation leaves; A, B: macrosporodochia; C, D: microsporodochia; E, F: aerial conidiophores and conidiogenous cells; G, H: macrosporodochial conidiophores and conidiogenous cells; I, J: microsporodochial conidiophores and conidiogenous cells; K: chlamyospores; L: microconidia; M: aerial macroconidia; N: sporodochial macroconidia. Scale bars: J, K = 5 μ m, all others = 10 μ m.

umber with white concentric patches of aerial mycelium, umber at centre, flat, felty to cottony, margin entire to slightly filamentous; reverse umber, ochreous to umber at centre, without diffusible pigments. On OA, luteous to umber with white concentric patches of aerial mycelium, umber at centre, flat, felty to cottony, margin entire to slightly filamentous; reverse umber, ochreous to umber at centre, without diffusible pigments. On SNA, white to pale buff with white concentric rings of short aerial mycelium, flat, felty to cottony; reverse white, without diffusible pigments.

Additional specimen examined: FRENCH GUIANA, Saül, Gros Arbres trail, on unidentified dead wood, 27 March 2021, leg. C. Lechat, CBS H-25086, cultures CBS 149414 = CLLG21075 = CPC 42649. GenBank accession numbers: *cal* = OP481879, ITS = OP497970, LSU = OP497974, *rpb1* = OP481881, *rpb2* = OP481883, and *tef1* = OP481885.

Known distribution: French Guiana.

Discussion: The novel species *Neocosmospora lechatii* was originally assigned to this genus by its collector (C. Lechat) based on its morphological features, characterised by the production of abundant, typically warted, bright red perithecia, coarsely striated 1-septate ascospores and a fusarioid asexual morph producing micro- and macroconidia on long aerial conidiophores. These initial observations were here confirmed, and proved these specimens to belong to an undescribed, novel South American species of *Neocosmospora*.

Morphological variation of asexual characters is known to occur in *Neocosmospora*, e.g., the asymmetrically clavate macroconidia shown by insect symbiont species of the Ambrosia clade of *Neocosmospora* (FREEMAN *et al.*, 2013), the cylindrical conidia of *N. tonkinensis* (BUGNICOURT, 1939; SANDOVAL-DENIS *et al.*, 2019); and the reduced, acre-

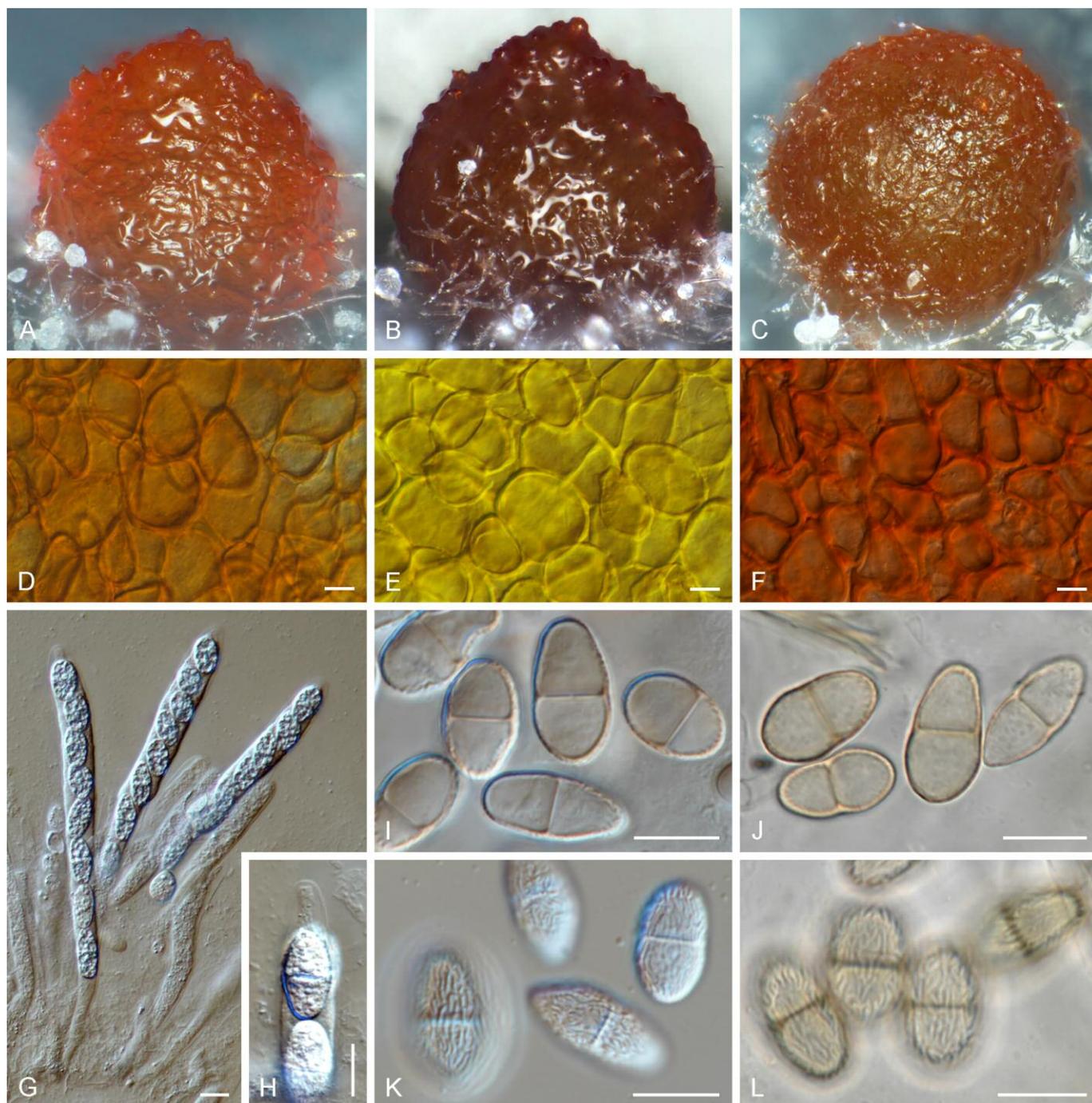


Fig. 3 – A–L: *Neocosmospora lechatii* sp. nov. (CBS 149413), sexual morphology *in vitro*. A–C: perithecia; D–F: detail of perithecial wall (D in water, E in lactic acid, and F in 3% KOH); G, H: asci; I–L: ascospores. Scale bars = 10 µm.

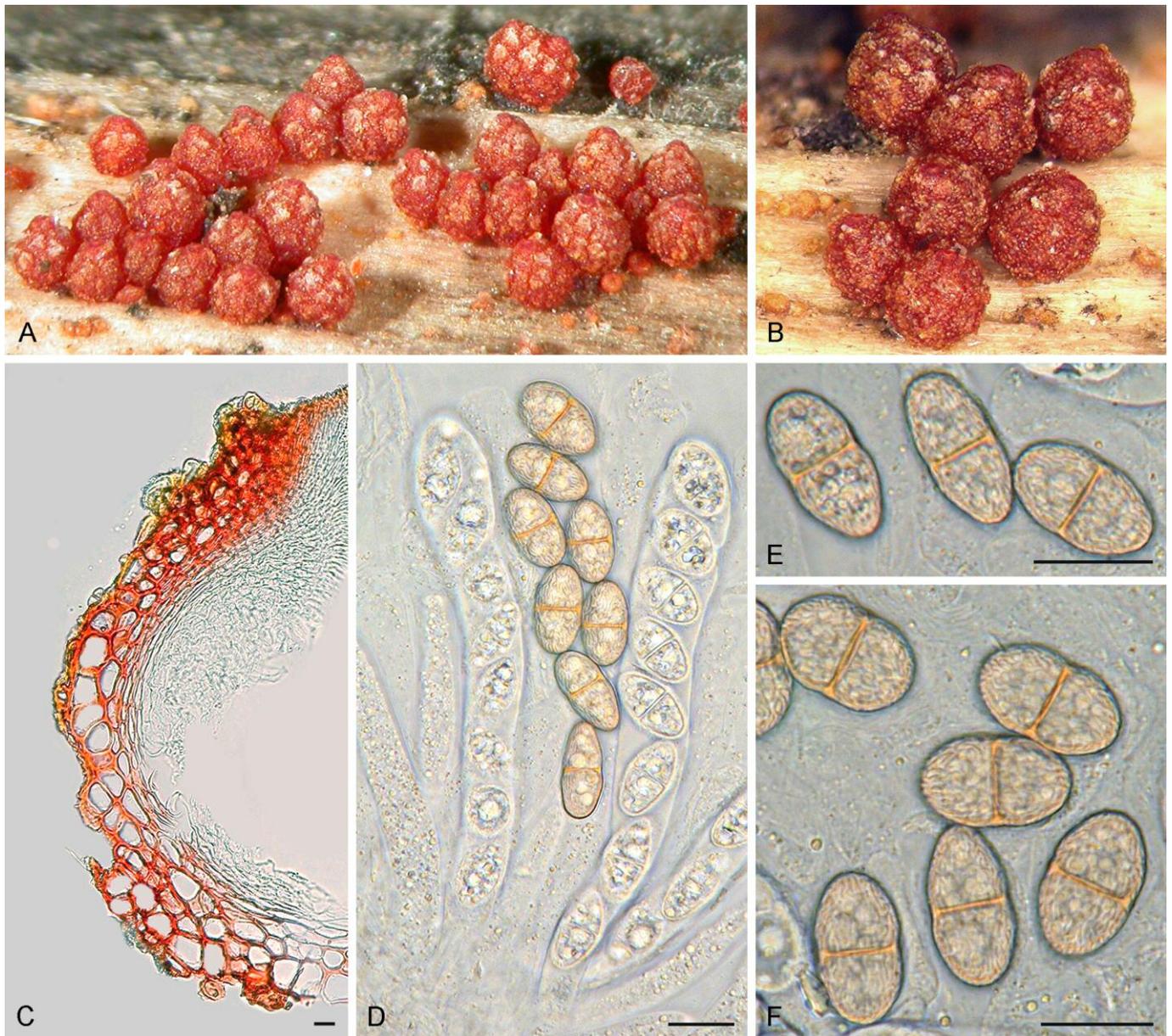


Fig. 4 – A–F: *Neocosmospora lechatii* sp. nov. (CLLG21075), sexual morphology on natural host. A, B: perithecia; C: detail of perithecial wall; D: asci; E, F: ascospores. Scale bars C = 20 µm, all others = 10 µm. All images in this figure by Christian Lechat.

monium-like conidial structures often found in *N. falciformis*, and common in *N. vasinfecta* (SMITH, 1899; SUMMERBELL & SCHROERS, 2002; SANDOVAL-DENIS *et al.*, 2019). Similarly, species of *Neocosmospora* have been described forming a combination of sporodochial macroconidia mixed with reduced sporodochial conidial forms in the same sporodochium (AOKI *et al.*, 2005, 2012). *Neocosmospora lechatii* is unique from all currently known species of *Neocosmospora* by the presence of two distinct sporodochial types, here termed micro- and macrosporodochia. Typical falcate, multiseptate macroconidia were observed from the latter; microsporodochia on the other hand, although it retains the basic sporodochial organization known in fusarioid fungi, was seen to produce only microconidia from short subulate phialides, more reminiscent of the conidiogenous cells typically observed in tall aerial conidiophores.

Macroconidial dimensions of the novel species are in the size and septation range reported for *Neocosmospora cucurbitae*, its closest phylogenetic and morphological relative, being also similar to those of *N. acutispora*, *N. mori* and *N. samuelsii* (SANDOVAL-DENIS *et al.*, 2019). However, compared with the phylogeny, all the above-mentioned species are clearly separated genealogically. Additionally, every locus commonly used for identification of fusarioid fungi (*cal*, ITS, LSU, *rpb1*, *rpb2*, and *tef1*) showed enough resolution to identify *N. lechatii*, even by using rDNA sequences alone. Morphologically,

N. lechatii can be distinguished from *N. acutispora* and *N. samuelsii* by the absence of microconidia, and larger, more robust aerial macroconidia in the latter two species. *Neocosmospora lechatii* and *N. mori* have similar sized sporodochial conidia; however, those of *N. lechatii* are predominantly 7-septate (vs. 5-septate in *N. mori*) and besides being more commonly septate, macroconidia of *N. lechatii* have a more pronounced dorsal curvature and are overall wider and more robust than those of *N. mori*. Although morphologically very similar to *N. cucurbitae*, apart from the presence of two distinct sporodochial types, a number of other features differentiate *N. lechatii* from the former taxon, including the shorter and less septate aerial microconidia (0(–1)-septate and up to 25.5 µm long in *N. lechatii* vs. up to 3-septate and 42 µm long in *N. cucurbitae*). Furthermore, its short falcate aerial macroconidia (vs. often cylindrical in *N. cucurbitae*) and the predominantly 7-septate, wider, robust, sporodochial macroconidia with well-developed foot-cells (vs. predominantly 5-septate in *N. cucurbitae*) also distinguish these taxa.

Both *N. cucurbitae* and *N. mori* have been recorded producing sexual morphs. The first under the names *Fusarium* (*Hypomyces*) *solani* f. sp. *cucurbitae* race 1, Mating Population (MP) I, or *Nectria haematococca* MPI (MATUO & SNYDER, 1973; MEHL & EPSTEIN, 2007), and the second taxon also reported as *Fusarium* (*Hypomyces*) *solani* f. sp. *mori* MP III, or *Nectria haematococca* MP III (MATUO & SNYDER, 1973;

O'DONNELL, 2000). However, sexual morphs of these two species could not be obtained in culture for further comparisons with *N. lechatii*. Nevertheless, significant differences in mating behaviour are already evident between these taxa. While both *N. cucurbitae* and *N. mori* are heterothallic species, which prevented obtaining the sexual morphs for comparison here (MATUO & SNYDER, 1973), *N. lechatii* is clearly homothallic, producing abundant perithecia with mature ascospores based on single spore cultures.

Acknowledgements

Christian Lechat was an amateur mycologist from France who has made a significant contribution to the field of hypocrealean fungal taxonomy and microfungi in general. He not only collected many new and interesting species of microfungi from the French West Indies and Europe, but also established the internet site known as "AscoFrance", which brought many amateur and specialist mycologists together, and in so doing helped mycologists find many evasive fungal taxa required for their research. To this modest, but highly dedicated mycologist, we pay homage.

We thank Jacques Fournier for providing original morphological and collection data from the personal records of C. Lechat.

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