Lasionectriopsis, a new genus in the *Bionectriaceae*, based on the new species *L. germanica*

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Abstract: *Lasionectriopsis germanica* gen. and sp. nov. is described and illustrated based on a collection from Germany. The asexual morph of this fungus was obtained in culture and the culture was sequenced. The genus is placed in the *Bionectriaceae* based on ascomata not changing colour in 3% KOH or lactic acid, and phylogenetic comparison of LSU sequences with species in 14 genera of the *Bionectriaceae*. *Lasionectriopsis* is primarily characterized by whitish to pale orange, globose ascomata, semi-immersed in a subiculum, and verruculose ascospores. Based on molecular data, two species known only as asexual morphs, *Acremonium pteridii* and *A. spinosum*, are recombined in *Lasionectriopsis*.
Keywords: acremonium-like, Ascomycota, *Hypocreales*, ribosomal DNA, taxonomy.

Résumé : *Lasionectriopsis germanica* gen. et sp. nov. est décrit et illustré d'après une récolte effectuée en Allemagne. La forme asexuée de ce champignon a été obtenue en culture et cette dernière a été séquencée. Le genre est placé dans les *Bionectriaceae* d'après les ascomes ne changeant pas de couleur dans KOH à 3% ou dans l'acide lactique et la comparaison des séquences LSU avec des espèces représentant 14 genres de *Bionectriaceae*. *Lasionectriopsis* est caractérisé par des ascomes globuleux, semi-immergés dans un subiculum, blanchâtre à orange pâle et des ascospores verruculeuses. Deux espèces connues jusqu'à présent par leur seul stade asexué, *Acremonium pteridii* et *A. spinosum*, sont recombinées en *Lasionectriopsis* sur la base de données moléculaires.
Mots-clés : ADN ribosomal, Ascomycota, *Hypocreales*, taxinomie, type-acremonium.

Introduction

In the continuation of the survey of hypocrealean fungi, an intriguing fungus was collected on decorticated, dead wood of *Fagus sylvatica* L. (*Fagaceae*), which did not match any known genus. The ascomata do not changing colour in 3% KOH or lactic acid, and are almost completely covered by hyphal elements recalling some members of *Lasionectria* (Sacc.) Cooke (Cooke, 1884). However, the specimen described herein primarily differs from them in having ascomatal wall of a single region and verruculose ascospores, while all known species of *Lasionectria* display ascomatal wall composed of two-regions and striate ascospores. Molecular analysis of LSU sequences shows that our fungus belongs to the *Bionectriaceae*, which agrees with the morphology of its sexual and asexual morphs. Morphological comparison with known genera belonging to the *Bionectriaceae* (Hirooka *et al*., 2010; Lechat & Fournier, 2016a; 2016b, 2017, 2018; Lechat *et al*., 2016, 2017; Rossman *et al*., 1999; Schroers, 2001), as well as phylogenetic analysis of LSU sequences suggest that this fungus represents a previously undescribed genus in the *Bionectriaceae*. Accordingly, the new genus *Lasionectriopsis* is proposed to accommodate the new species *Lasionectriopsis germanica*.

Materials and methods

Dry specimens were rehydrated and examined using the method in Rossman *et al*. (1999). Microscopic observations and measurements were made in water. The holotype specimen was deposited in the LIP herbarium (University of Lille, France) and living cultures in the CBS Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Cultures of the living specimen were plated 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) as follow: Total DNA was extracted from dry specimens blending a portion 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 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°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 10 min. Chromatograms were checked 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). Phylogenetic tree was rooted with *Stephanonectria keitii* Schroers & Samuels whose asexual morph is myrothecium-like (Schroers & Samuels, 1999). New taxa are registered in MycoBank (CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands).

Taxonomy

Lasionectriopsis Lechat & P.-A. Moreau, *gen. nov*. – MycoBank MB 828975

Diagnosis: Distinguished from other bionectriaceous genera having acremonium-like asexual morph by the combination of ascomata semi-immersed in a subiculum, ascomatal wall of a single region and verruculose ascospores.

Type species: *Lasionectriopsis germanica* Lechat, P.-A. Moreau & H. Bender

Etymology: *Lasionectriopsis* refers to the morphological similarity with *Lasionectria* (Sacc.) Cooke

Lasionectriopsis germanica Lechat, P.-A. Moreau & H. Bender, *sp. nov.* – MycoBank MB 828976 – Fig. 2

Holotype: Germany, Viersen (MTB 4704/3), on decorticated, dead wood of *Fagus sylvatica*, 2 Sept. 2017, *leg*. H. Bender, CLL 17022 (LIP), ex-type culture CBS 143538, GenBank LSU sequence: MK276528.

Etymology: The epithet "*germanica*" refers to Germany where this species was collected.

Ascomata gregarious, non-stromatic, globose, 180–220 µm diam., whitish, pale yellow to pale orange becoming brownish orange and cupulate or laterally pinched when dry, semi-immersed in a whitish subiculum composed of hyaline, septate hyphae 2–

2.5 µm diam. proliferating to cover ascomatal wall except ostiolar region. Perithecial apex with ostiolar opening 30–40 µm diam., conical, slightly darker than venter, composed of subglobose to narrowly clavate cells with pale orange wall, merging with periphyses. **Ascomatal wall** 25–35 µm thick, of a single region composed of subglobose to globose or ellipsoidal, cells 2.5–6 × 2–2.5 µm, with pale yellow walls 1–1.5 µm thick. **Asci** unitunicate, clavate, short stipitate (40–)45–52(–55) × 7–8.5 µm (Me = 50 × 7 µm, n = 20), with 8 ascospores biseriate or irregularly disposed in upper part, uniseriate in lower part, apex with a ring. **Paraphyses** evanescent between asci, filamentous to narrowly moniliform up to 5 µm diam. at base. **Ascospores** (8–) 8.5–9.5(–10) × 2.5–3(–3.5) µm (Me = 9 × 2.6 µm, n = 50), narrowly ellipsoidal to fusiform, 1-septate, slightly constricted or not at septum, verruculose, hyaline, pale yellow en masse.

Cultural characteristics: After two weeks, colony 6–7 cm diam., whitish to cream, colour not diffusing into medium, aerial mycelium white, producing an abundant acremonium-like asexual morph at margin; conidiophores simple or branched, 2.5–3 µm diam., flexuous, smooth, arising from smooth, septate hyphae 2–2.5 µm diam., with a simple conidiogenous cell 20–30 µm long, 2–2.5 µm diam. at base, septate, subulate with an unflared collarette, producing ellipsoidal to subcylindrical, hyaline, smooth, non-septate conidia (3.5–)4.5–5(–5.5) × 2.5–3 µm with rounded apex, attenuated at base with a basal abscission scar, grouped at tip of phialides to form a mucous head. Chlamydospores not observed. Conidiophores and conidia identical to those observed in natural environment.

Results and discussion

Lasionectriopsis germanica is characterized by nonstromatic, globose, whitish or pale yellow to pale orange ascomata not changing colour in 3% KOH or lactic acid, semi-immersed in a white subiculum, ascomatal surface almost completely covered by hyphal elements arising from the subiculum, ascomatal wall of a single region, verruculose ascospores and an acremonium-like asexual morph. Based on these characters, this fungus belongs to the *Bionectriaceae* as defined by Rossman *et al.* (1999) and Schroers (2001). At first glance, morphological characteristics of this fungus recall some species of *Lasionectria*, which likewise have acremonium-like asexual morph, but these species primarily differ from *Lasionectriopsis* in having an ascomatal wall composed of two regions and striate ascospores.

Members of 14 genera in the *Bionectriaceae* of which 12 having acremonium-like asexual morphs were included in our phylogenetic analysis (Fig. 1), as well as three species of *Clonostachys* Corda (= *Bionectria* Speg.) which have clonostachys-like asexual morphs as defined by Schroers (2001). *Stephanonectria* Schroers & Samuels whose asexual morph is myrothecium-like (Schroers & Samuels, 1999) was included as outgroup.

Our phylogenetic tree (Fig. 1) shows that *Lasionectriopsis* is nested in a well-supported subclade within the *Bionectriaceae*, including sequences from type collections of *Acremonium pteridii* Gams and *A. spinosum* (Negroni) W. Gams, whose sexual morphs are unknown (Gams, 1971). This clade was identified by Summerbell *et al.* (2011) as

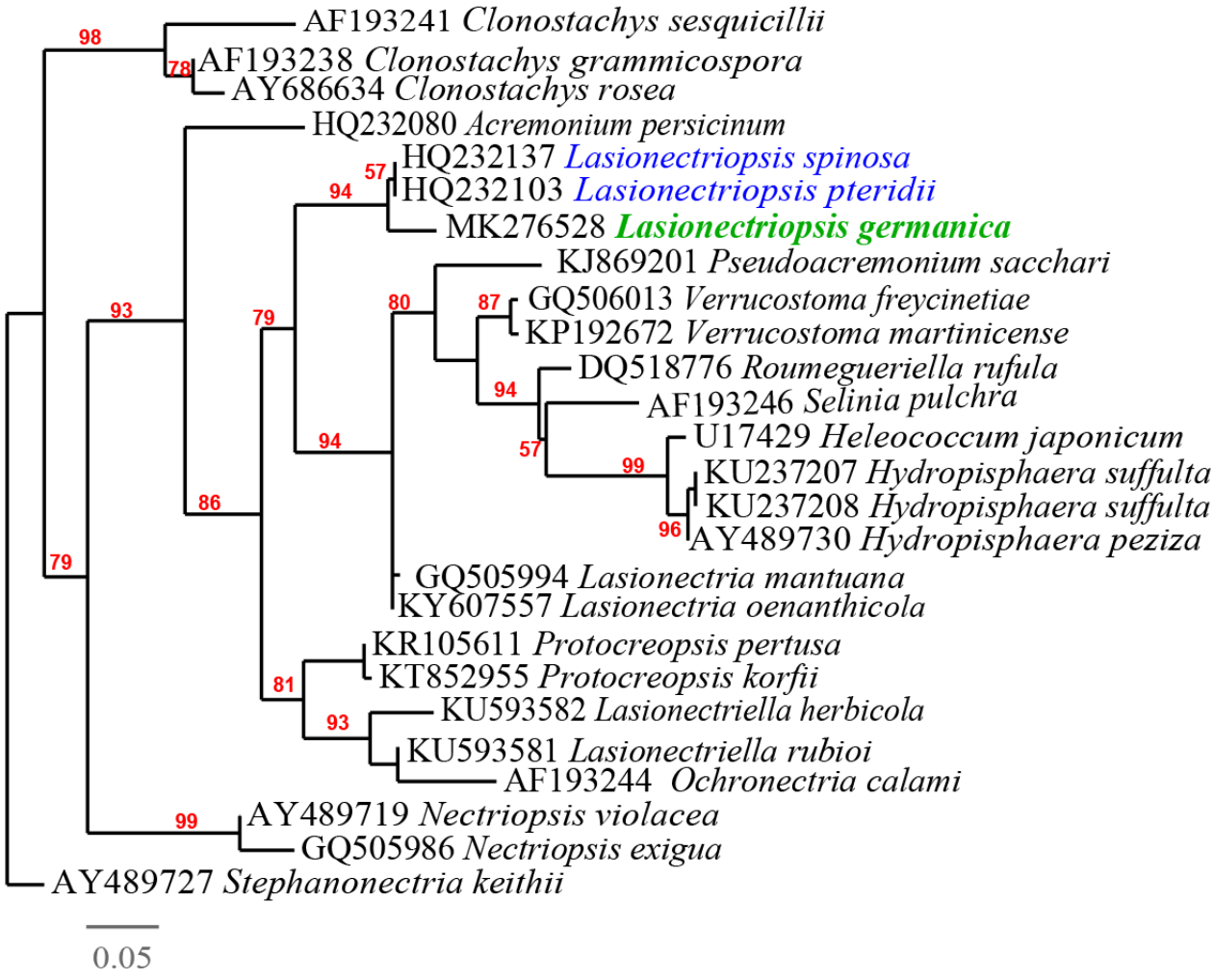

Fig. 1 – Maximum likelihood phylogeny (-lnL = 2363.56339) of *Lasionectriopsis germanica* inferred by PhyML 3.0, model HKY85 from a 932 bp matrix of 28S rRNA sequence, rooted with *Stephanonectria keithii*.

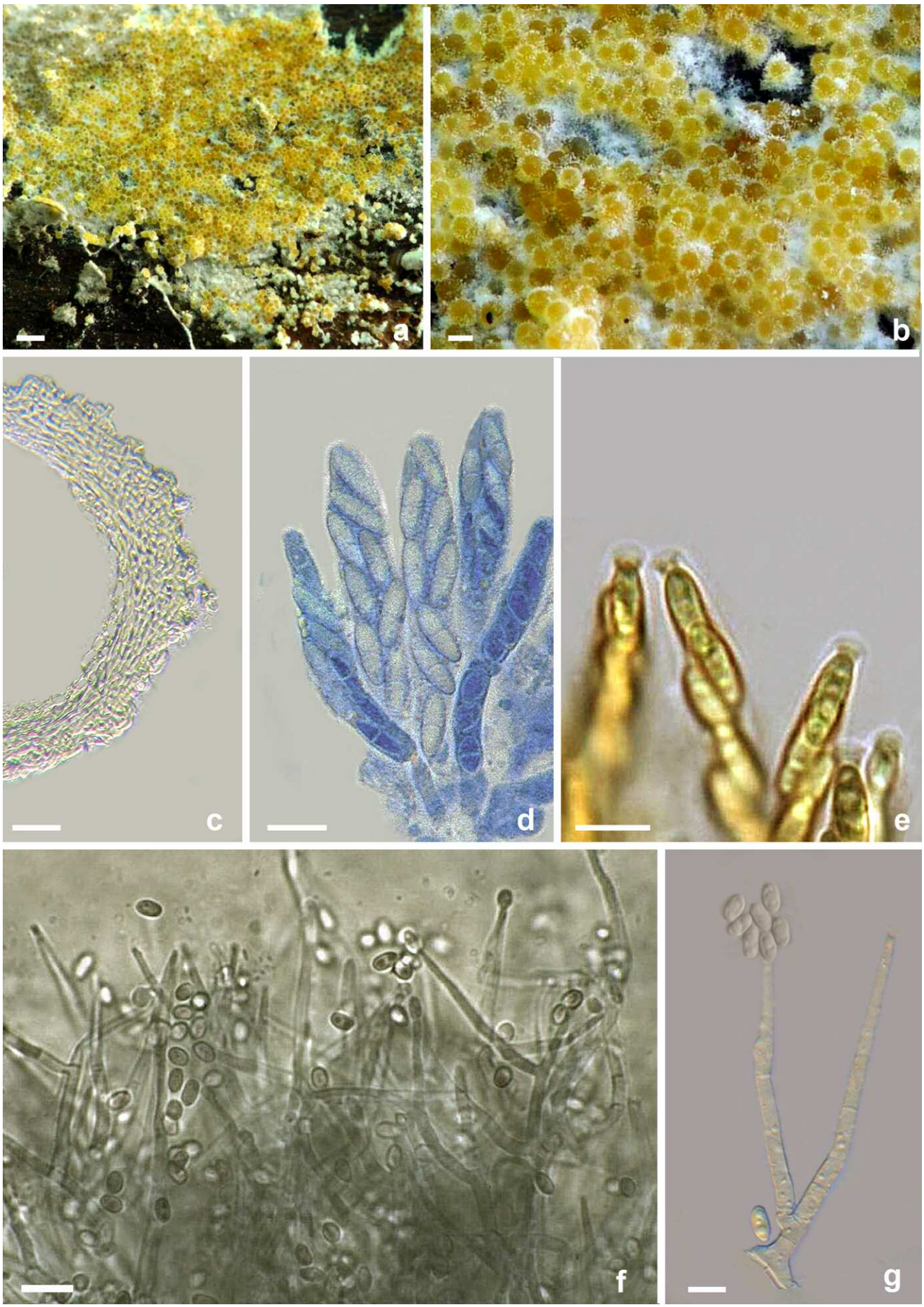

Fig. 2 – a-g: *Lasionectriopsis germanica* (Holotype); a, b: Ascomata in natural environment; c: Lateral ascomatal wall in vertical section in water; d: Asci and ascospores in lactic cotton blue; e: Asci with a ring in Melzer's; f: Conidiophores and conidia covering ascomatal wall; g: Conidiophores and conidia from culture in water. Scale bars: a = 1 cm; b = 200 µm; c = 20 µm; d = 10 µm; e = 5 µm; f = 10 µm; g = 5 µm.

the "pteridii-clade", nested in the "Gliomastix/Bionectria-clade" (corresponding to the *Bionectriaceae*). With the discovery of this sexual morph, at present the only one reported in this clade, we should be able to interpret the "pteridii-clade" morphologically but the sequences of *A. pteridii* and *A. spinosum* available in GenBank are 100% identical, that suggests that the sequence from *A. spinosum* was misidentified. Phylogenetically, *L. germanica* differs from *A. pteridii* by 7 substitutions and 1 insertion/deletion (98.8 % of similarity on a 774-position-long alignment). Considering such differences in a relatively conserved marker, we reject the hypothesis of conspecificity between *L. germanica* and any of the taxa cited above.

Finally, based on morphological features and molecular data, the combinations of *A. pteridii* and *A. spinosum* in *Lasionectriopsis*, as *Lasionectriopsis pteridii* (Gams) Lechat & P.-A. Moreau and *L. spinosa* Lechat & P.-A. Moreau are proposed below.

Taxonomic novelties

Lasionectriopsis pteridii (W. Gams) Lechat & P.-A. Moreau, *comb. nov.* – MycoBank MB 828977

Basionym: *Acremonium pteridii* W. Gams & J.C. Frankland, in Gams, *Cephalosporium*-artige Schimmelpilze: 81 (1971).

Lasionectriopsis spinosa (W. Gams) Lechat & P.-A. Moreau, *comb. nov.* – MycoBank MB 828978

Basionym: *Cephalosporium spinosum* Negroni, *Compt. Rend. Soc. Biol., Paris*: 480 (1933).

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