Lasionectriopsis, a new genus in the *Bionectriaceae*, with the new species *L. verrucospora*

Christian Lechat
Pierre-Arthur Moreau
Hans Bender

Abstract — *Lasionectriopsis verrucospora* gen. and sp. nov. is described and illustrated based on a collection in Germany. The asexual morph of this fungus was obtained in culture and 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 subglobose ascomata, semi-immersed in a subiculum, whitish to pale orange and verruculose ascospores. Two species so far only known by their asexual morph (*Acremonium pteridii* and *A. spinosum*) are recombined in *Lasionectriopsis* based on molecular data.

Key words — acremonium-like, Ascomycota, *Hypocreales,* ribosomal DNA, taxonomy.

Résumé — *Lasionectriopsis verrucospora* gen. et sp. nov. est déctrit et illustré d'après une récolte effectuée en Allemagne. La forme asexuée de ce champignon a été obtenue en culture et la cultures 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 sub-globuleux, semi-immergés dans un subiculum, blanchâtres 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 —acremonium-morphe, ADN ribosomal, Ascomycota, *Hypocreales,* taxonomy.

Introduction:

In the continuation of the survey of hypocrealean fungi, an intriguing fungus was collected on dead wood of *Fagus sylvatica* L. (*Fagaceae*), which did not match any known genus. The ascomata not changing colour in 3% KOH or lactic acid, almost completely covered by hyphal elements recall some members of *Lasionectria* (Sacc.) Cooke, but 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-region and striate ascospores. Molecular analysis of LSU sequences shows that our fungus belongs to the *Bionectriaceae*, which is in agreement with the morphology of its sexual and asexual morphs. Morphological comparison with known genera belonging to the *Bionectriaceae* reported in literature (HIROOKA *et al*., 2010; LECHAT & FOURNIER, 2016a; 2016b; LECHAT, FOURNIER & MOREAU, 2016; LECHAT *et al.* 2017; LECHAT & FOURNIER, 2017; LECHAT & FOURNIER, 2018; 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 verrucospora.*

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 CBS Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Cultures of the living specimen were made on PDA (Potato Dextrose Agar) with 5mg/l of streptomycin in Petri dishes 9 cm diam., incubated at 25°C. DNA extraction, amplification, and sequencing were performed by ALVALAB (E-39012 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 ddH2O. 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). New taxa are registered in MycoBank (CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands).

Taxinomic novelties:

Lasionectriopsis gen. nov. Lechat & P.-A. Moreau

MycoBank MB XXXXXX

Lasionectriopsis verrucospora Lechat, P.-A. Moreau & H. Bender sp. nov.

MycoBank MB XXXXXX

Lasionectriopsis pteridii (Gams) Lechat & P.-A. Moreau comb. nov.

MycoBank MB XXXXXX

Basionym: *Acremonium pteridii* W. Gams & J.C. Frankland in Gams, Cephalosporium-artige Schimmelpilze: 81, 1971; MycoBank MB 308186

Taxonomy

Lasionectriopsis Lechat & P.-A. Moreau ***gen. nov.***

MycoBank MB XXXXXX

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

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

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

Lasionectriopsis verrucospora Lechat, P.-A. Moreau & H. Bender **sp. nov. Fig. 2**

MycoBank MB XXXXXX,

Diagnosis: Differs from all known genera belonging to the *Bionectriaceae* having acremonium-like asexual morph by its ascomata semi-immersed in a white subiculum, non-stromatic, subglobose, pale yellow to pale orange, not changing colour in 3% KOH or lactic acid; Ascospores 8.5–9.5 × 2.5–3 µm, 1-septate, hyaline, verruculose.

Etymology: The epithet "*verrucospora*" refers to verruculose ascospores.

Holotype: GERMANY: Munchengladbach, on dead wood of *Fagus sylvatica*, 02 Sept. 2017, *leg*. H. Bender, CLL 17022 (LIP), ex-type culture CBS 143538**,** GenBank LSU sequence: XXXXXX

ASCOMATA gregarious, non-stromatic, globose, 180–220µm 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 containing ostiolar opening 30–40 µm diam. conical, slightly darker than the venter, composed of subglobose to narrowly clavate cells with wall pale orange, merging with the periphyses. ASCOMATAL WALL 25–35 µm thick, of a single region composed of subglobose to globose or ellipsoidal, thick walled 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 the upper part, uniseriate in the lower, apex rounded 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), ellipsoidal to widely fusiform, 1-septate, slightly constricted or not at the septum, verruculose, hyaline, pale yellow *en masse*.

Cultural characteristics:

After two weeks at 25°C on Difco PDA containing 5 mg/L streptomycin, colony 6–7 cm diam., withish to cream, not diffusing coloration 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 a not flared 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.

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

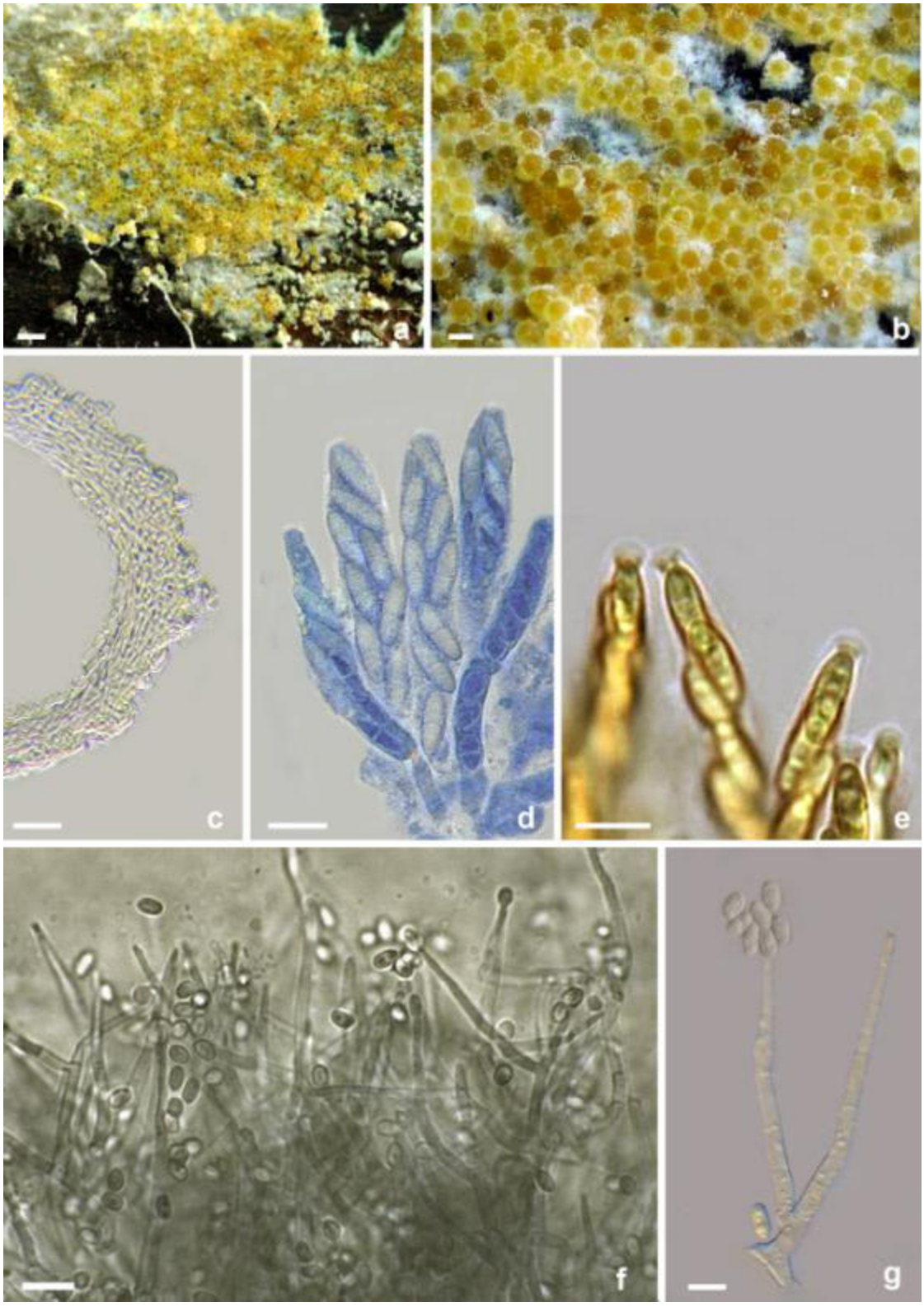

Fig. 2: a-g: *Lasionectriopsis verrucospora* (Holotype); a, b: Ascomata in natural environment; c: Lateral ascomatal wall in vertical section in water; d: Asci and ascospores in

lactic cotton blue; e: Asci with a ring in Melzer; 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;g: 1 cm; f: 10 µm; g = 5 µm.

Results and discussion

Lasionectriopsis verrucospora is characterized b*y* nonstromatic, subglobose, 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* (Sacc.) Cooke (1884), which likewise have acremonium-like asexual morph, but these species primarily differ from *Lasionectriopsis* in having ascomatal wall composed of two regions and striate ascospores.

Members of 14 genera in the *Bionectriaceae* whose 12 having acremonium-like asexual morph where included in our phylogenetic analysis (Fig. 1), as well as three species of *Bionectria* Speg. which have clonostachys-like asexual morph 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 *Acremonium spinosum* (Negroni) W. Gams, whose sexual morphs are unknown (GAMS 1971). This clade was already identified by SUMMERBELL *et al.* (2011) as the "pteridii-clade", nested in the "*Gliomastix/Bionectria*-clade" (corresponding to the family *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 and we think that it is conspecific to *A. pteridii*. Phylogenetically, *L. verrucospora* differs from *A. pteridii* by 7 substitutions and 1 insertion/deletion (98.8 % of similarity on a 774-position-long alignment); considering such differences on a relatively conserved marker, we do not retain the hypothesis of a conspecificity between *L. verrucospora* and any of the taxa cited above.

Finally, based on morphological features and molecular data we propose the recombination of *A. pteridii* in *Lasionectriopsis*, as *Lasionectriopsis pteridii* (Gams) Lechat & P.-A. Moreau *comb. nov*.

Acknowledgments:

We gratefully acknowledge Dr Amy Rossman, Oregon State University, Corvallis, U.S.A., for her advice and scientific help and for her presubmission review.

Literature cited:

ANISIMOVA M. & GASCUEL O. 2006. — Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Systematic Biology*, 55: 539–552.

COOKE M.C. 1884. *Synopsis Pyrenomycetum*. *Grevillea*, 12(64):102–113.

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 Res. 2008 Jul 1;36 (Web Server issue): W465–9.

GAMS W. 1971 — Cephalosporium-artige Schimmelpilze (Hyphomycetes) *Gustav Fischer Jena.*: 1-262

HIROOKA, Y., KOBAYASHI, T., ONO, T., ROSSMAN, A.Y. & CHAVERRI, P. 2010. — *Verrucostoma*, a new genus in the *Bionectriaceae* from the Bonin Islands, *Japan. Mycologia*, 102(2): 418–429.

LECHAT C., FOURNIER J. & COURTECUISSE R. 2015. *Verrucostoma martinicense* Lechat, J. Fourn. & Courtec. Fungal Planet 366. *Persoonia*, 34: 254–255.

LECHAT C. & FOURNIER J. 2016a. — *Lasionectriella*, a new genus in the *Bionectriaceae*, with two new species from France and Spain, *L. herbicola* and *L. rubioi*. *Ascomycete.org*, 8(2): 59–65.

LECHAT C., FOURNIER J. & MOREAU P.-A. 2016b. — *Xanthonectria*, a new genus for the nectrioid fungus *Nectria pseudopeziza*. *Ascomycete.org*, 8(4): 172–178.

LECHAT C. & FOURNIER J. 2017. — Four new species of *Ijuhya* (*Bionectriaceae*) from Belgium, metropolitan France and French Guiana. *Ascomycete.org*, 9 (1) : 11-18.

LECHAT C. & FOURNIER J. 2018. — *Flammocladiella decora,* a new combination to accommodate the hypocrealean fungus *Nectria decora. Ascomycete.org,* 10(1) : 48–54.

LECHAT C., FOURNIER J., VEGA M. & PRIOU J.-P. 2018. — *Geonectria,* a new genus in the *Bionectriaceae* from France. *Ascomycete.org*, 10 (2) : 91–95

LECHAT C., HAIRAUD M. & RUBIO E. 2017. — A new species of *Striatibotrys* from Asturias (Spain), *S. asturiensis. Ascomycete.org, 9 (5) : 153-155.*

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.

SCHROERS H-J. 2001. — A monograph of *Bionectria* (Ascomycota, *Hypocreales, Bionectriaceae*) and its *Clonostachys* anamorphs. *Studies in Mycology*, 46:1–214.

SUMMERBELL R.C., GUEIDAN C., SCHROERS H-J., DE HOOG G.S., STARINK M., AROCHA ROSETE Y., GUARRO J. & SCOTT A. 2011 *Acremonium* phylogenetic overview and revision of *Gliomastix, Sarocladium*, and *Trichothecium. Studies in Mycology,* 68: 139-162

VILGALYS R. & HESTER M. 1990. — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172: 4238–4246.

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.