Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes

Isabel Iturrieta González

UNIVERSITAT ROVIRA i VIRGILI

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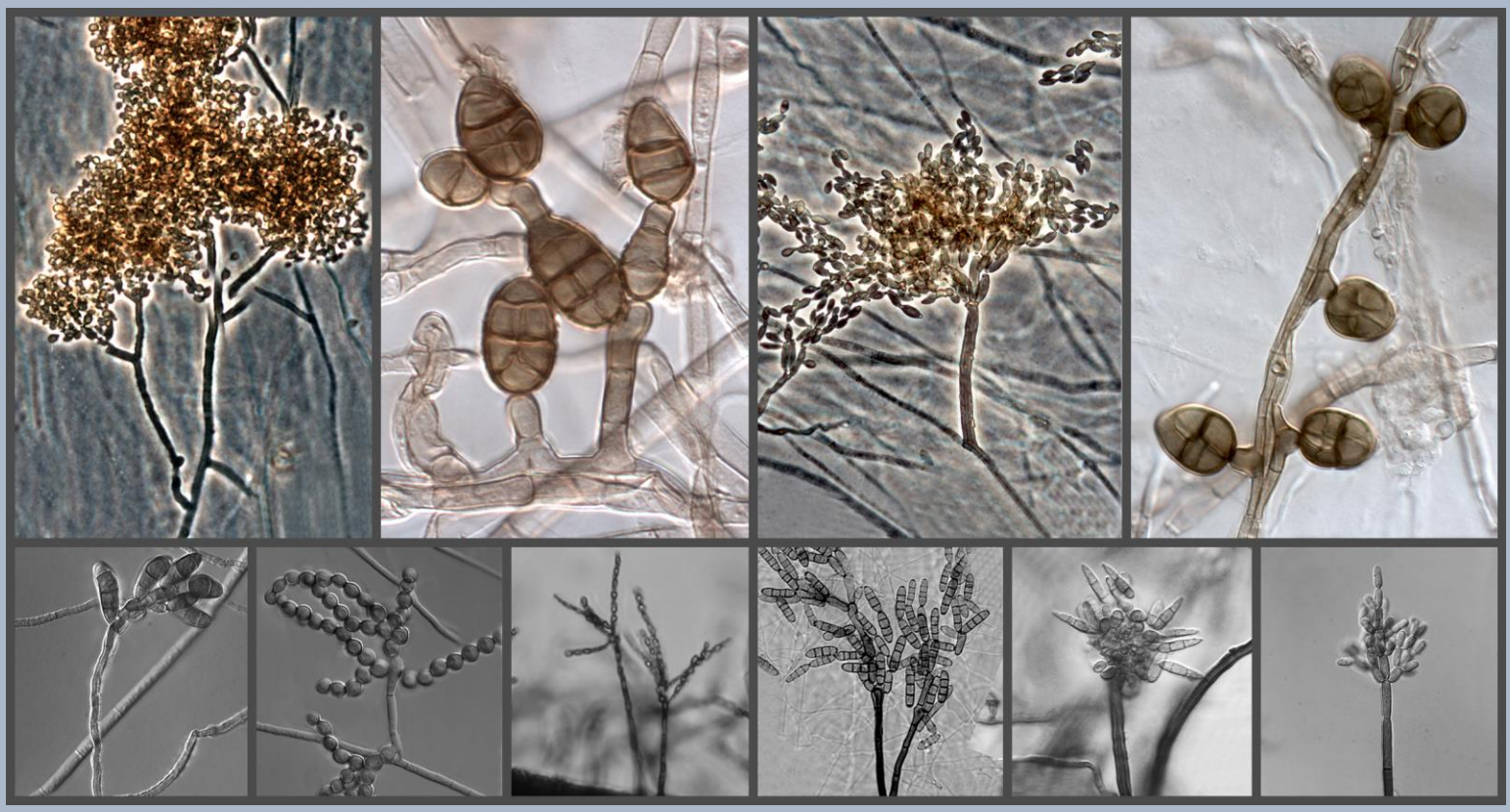
ISABEL ITURRIETA GONZÁLEZ



DOCTORAL THESIS
2020

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Doctoral Thesis

Directed by Drs. Josepa Gené Díaz, Dania García Sánchez and Josep Guarro Artigas

Departament de Ciències Mèdiques Bàsiques
Facultat de Medicina i Ciències de la Salut
Universitat Rovira i Virgili

Reus
2020

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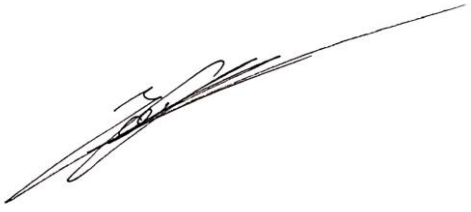
I STATE that the present study, entitled "**Identification and phylogeny of clinical and environmental isolates of *Alternaria*, *Cladosporium* and other genera of dematiaceous hyphomycetes**", presented by **Isabel Iturrieta González** for the award of the degree of Doctor, has been carried out under our supervision at the Department Ciències Mèdiques Bàsiques of this university, and it fulfils the requirements to obtain the International Doctorate Mention.

Reus, August 28th, 2020.

Doctoral Thesis Supervisors



Josepa Gené Díaz Dania García Sánchez Josep Guarro Artigas

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A mi abuelito Sergio

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ACKNOWLEDGEMENTS

En momentos como este son muchas las personas a las que me gustaría expresar mi gratitud. En primer lugar, quisiera agradecer a mis directores de tesis por haber sido excelentes guías durante todo el desarrollo de mi doctorado. Al Dr. Josep Guarro por haberme dado la oportunidad de realizar mi doctorado en esta unidad, formando parte de este grupo durante estos años y por siempre fomentar e impulsar el crecimiento, superación y desarrollo profesional no solo mío, sino de todos los doctorandos. A la Dra. Dania García por su apoyo, orientación, su buen humor, motivación y su buena disposición durante el transcurso de estos casi 4 años. Y quisiera agradecer de manera muy especial a mi directora de tesis la Dra. Josepa Gené, por su cercanía, por su inagotable dedicación y atención en cada uno de los trabajos, su prolijidad y profesionalismo, sus consejos, por todo el conocimiento transmitido y por haber sido un importante apoyo para mí, no solo en el ámbito académico sino también en el personal, le estaré siempre muy agradecida.

Al Dr. Patricio Godoy Martínez por su cercanía, su amistad, por todo el apoyo que siempre me ha dado y por ser el gran impulsor y gestor que hizo que comenzara este gran viaje que ha significado desarrollar mis estudios de postgrado en el área de la Micología fuera de Chile, le estaré eternamente agradecida.

Al Dr. Luis Zaror, por sus consejos, su cercanía, por ser quien desde mis primeras clases de Micología en la Universidad Austral de Chile despertó en mí el interés por comenzar a profundizar en esta área y por ser también el impulsor y gestor de haber realizado mi doctorado en esta unidad.

Al Dr. José Cano por su cercanía, motivación, por los consejos y por haber actuado en innumerables ocasiones como “el abogado del diablo” en las reuniones de taxonomía. Al Dr. Alberto Stchigel por su cercanía, consejos y tan interesantes conversaciones con las que siempre nos deleitaba. Y a todos los demás integrantes de la unidad con los que tuve la oportunidad de compartir, que forman y formaron parte de este grupo.

Al Dr. Pedro Crous por la oportunidad de desarrollar mi estancia en el centro de investigación que él dirije en Países Bajos, Westerdijk Fungal Biodiversity Institute. Y en especial al Dr. Sybren de Hoog por darme la

oportunidad de realizar mi estancia en su grupo de trabajo dentro del centro, participando en la actualización del “Atlas of Clinical Fungi”, junto a la Dra. Sarah Abdalla y el Dr. Abdullah M.S. Al-Hatmi. Les agradeceré siempre su simpatía, motivación, todos los conocimientos transmitidos y por tantos lindos momentos compartidos.

A Carme y Nuria, excelentes técnicos, por la gran labor que hacen cada día, por su profesionalismo, dedicación y mostrar siempre la mejor disposición en cada momento que necesité de su apoyo.

A mis compañeros de doctorado con los que tuve la oportunidad de compartir a lo largo de estos casi 4 años, Jõao, Adela, Patri, Nicomedes, Dámaris, Emmanuel, Miriam, Tere, Neto, Alba “new”, Dayana, Taysa y Youssef. Aunque con algunos de ellos fue un corto periodo, me llevo muy bonitos recuerdos.

A Edu, Andreu, Dani y Viry, lindas personas que el doctorado me permitió encontrar, les agradezco todo el apoyo, compañerismo, amistad y tantos momentos lindos que compartimos.

A tres personas muy especiales que me regaló este doctorado y que se transformaron en grandes amigas. Mis tres chicas bellas: mi querida Albi, Marce y Ana (Ani), por ser mi gran apoyo, por su amistad, eternas conversaciones, risas y una infinidad de momentos que vivimos juntas, en resumen, por estar ahí siempre, para los buenos y malos momentos. Sin duda este doctorado no habría sido lo mismo sin ustedes, las llevaré siempre en mi corazón.

A mis amigas y amigos de Chile que, a pesar de la distancia, siempre han estado presentes con palabras de apoyo y ánimo durante todos estos años.

Y finalmente a mi familia, que sin duda han sido mi mayor pilar, a mis padres, hermanos, abuelitos, tías, tíos, primos… gracias por todo su apoyo, por sus consejos, motivación, por estar siempre presentes y por sentirlos siempre a mi lado a pesar de la distancia.

Mi eterna gratitud a todos ustedes…

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LIST OF ABBREVIATIONS

act	Actin
AFG	Anidulafungin
Alt a1	Alternaria major allergen gene
AMB	Amphotericin B
ATCC	American Type Culture Collection
ATPase	Plasma membrane ATPase
BI	Bayesian Inference
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
bs	Bootstrap Support
Ca	Calcium
CBS	Westerdijk Fungal Biodiversity Institute
CFG	Caspofungin
CLSI	Clinical and Laboratory Standards Institute
comb. nov.	*Combinatio nova*, Latin expression meaning “new combination”
cm	Centimeter
cmdA	Partial calmodulin gene
CNM-CM	Mold Collection of the Spanish National Center for Microbiology, Madrid, Spain
CNS	Central Nervous System
Cu.	*Curvularia*

Cy.	*Cyphellophora*
diam.	Diameter
Dec.	December
DNA	Deoxyribonucleic Acid
e.g.	Latin expression exempli gratia, meaning “for example”
EMBL	European Molecular Biology Laboratory
ET	Ex-epitype
et al.	*Et alia*, Latin expression meaning "and others"
etc.	*Et cetera*, Latin expression meaning “and the rest”
Fig.	Figure
FMR	Facultad de Medicina de Reus
g	Gram
gapdh	Glyceraldehyde 3-phosphate dehydrogenase
GCPSR	Genealogical Concordance Phylogenetic Species Recognition
GTR	General Time Reversible
HIV	Human immunodeficiency virus
HKY	Hasegawa-Kishino-Yano
HMAS	Herbarium Mycologicum Academiae Sinicae
ICN	International Code of Nomenclature
i.e.	Latin expression id est, meaning "that is" or “namely”
l	Liter
ILD	Incongruence Length Difference

ITC	Itraconazole
ITS	Internal Transcribed Spacer
LSU	Large Subunit of the rRNA
MEA	Malt Extract Agar
MEC	Minimum effective concentration
MEGA	Molecular Evolutionary Genetics Analysis
MFG	Micafungin
MIC	Minimum inhibitory concentration
mm	Millimeter
ml	Milliliter
ML	Maximum-likelihood
N	Nitrogen
NCBI	National Center for Biotechnology Information
NGS	Next Generation Sequencing
Nov.	November
nrDNA	Nuclear ribosomal DNA
num.	Number
OA	Oat Agar
P	Phosphorus
PCA	Potato Carrot Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PNA	Pine Needle Agar
pp	Posterior probability

PSC	Posaconazole
rDNA	Ribosomal DNA
RNA	Ribonucleic acid
***rpb*1**	RNA polymerase II largest subunit
***rpb*2**	Second largest subunit of RNA polymerase II
SD	Standard deviation
SNA	Synthetic Nutrient-poor Agar
sp.	Species
sp. nov.	*Species nova*, Latin expression meaning “new species”
SSU	Small subunit ribosomal DNA
T	Ex-type
T93	Tamura Nei
TBF	Terbinafine
tef1	Translation elongation factor 1α
tub2	Partial β-tubulin gene
URV	Universitat Rovira i Virgili
UTHSC	University of Texas Health Science Center
UV	Ultraviolet
v.	Version
VRC	Voriconazole
wk	Week
WNS	White nose syndrome
µg	Microgram

µm	Micrometer
º C	Celsius degrees
&	And
%	Percentage

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1. INTRODUCTION

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
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1.1 Overview of the fungi

The kingdom Fungi comprises a large and heterogeneous group of heterotrophic eukaryotes that includes aquatic and terrestrial inhabitants with an important role as mutualists, decomposers, symbionts, free-living saprobes and pathogens associated with animals and plants. Traditionally, based on their morphological traits associated with reproduction, they were divided into four divisions (= phyla), i.e. *Ascomycota*, *Basidiomycota*, *Zygomycota* and *Chytridiomycota* (Whittaker 1969, Guarro et al. 1999). However, molecular and genomic analyses of these fungal groups revealed that the two latter were polyphyletic, giving rise to the proposal of other taxonomic high-level categories (Hibbet et al. 2007, McLaughlin et al. 2009, Spataphora et al. 2017). Therefore, currently the following nine phylogenetic well defined groups are recognized (Fig. 1): *Opisthosporidia*, *Chytridiomycota*, *Neocallimastigomycota*, *Blastocladiomycota*, *Zoopagomycota*, *Mucoromycota*, *Glomeromycota*, *Ascomycota* and *Basidiomycota* (Naranjo-Ortiz & Gabaldón 2019a).

Ascomycota corresponds to the largest phylum in the kingdom Fungi with about 64,000 known species, representing two-thirds of all the described fungal species (Naranjo-Ortiz & Gabaldón 2019a, Adl et al. 2019); however, environmental studies suggest the existence of a large amount of unknown taxa (Naranjo-Ortiz & Gabaldón 2019a, Adl et al. 2019). It contains a high diversity of organisms, including the “Archiascomycetes” (currently *Taphrinomycotina*) with unculturable fungi such as the human lung parasite *Pneumocystis*, the “Hemiascomycetes” (currently *Saccharomycotina*) that includes common components of the gut microbiota of insects and of the vertebrate mucosae such as *Candida* spp., and the “Euascomycetes” (currently *Pezizomycotina*), the most diverse group of ascomycetous fungi that includes common toxins, alkaloids and other secondary metabolite producers, as well as many important human pathogens causing conditions from unpleasant dermatological infections to life-threatening colonization of internal organs (Guarro et al. 1999, de Hoog et al. 2000, Heitman et al. 2017, Spataphora et al. 2017, Naranjo-Ortiz & Gabaldón 2019a,b). All genera included in this thesis belong in this latter group of ascomycetes.

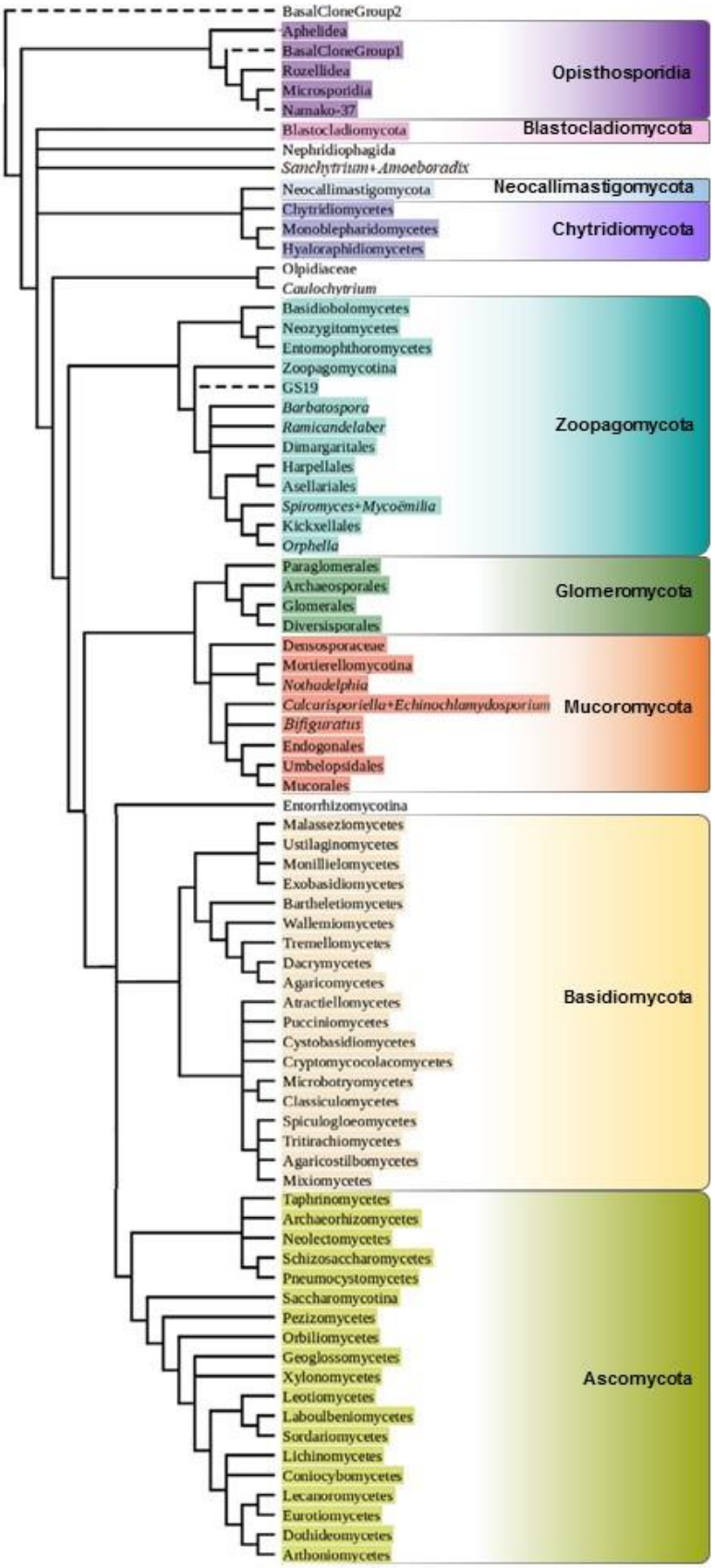

Figure 1. The fungal tree of life. The tree shows the currently described groups within the kingdom Fungi and colors correspond to the main clades representative of the phyla (Adapted and modified from Naranjo-Ortiz & Gabaldón 2019a).

INTRODUCTION

Although some fungi show unicellular organization (yeasts), most members of the *Pezizomycotina* are multicellular and are organized in tubular structures (filaments), called **hyphae**. Hyphae are **septate** and the septum is uni- or multi-porate and associated to a peroxisome-derived electrodense organelle called the **Woronin body**, which are absent in the two other main groups of ascomycetes, i.e. *Taphrinomycotina* and *Saccharomycotina* (Spataphora et al. 2017, Naranjo-Ortiz & Gabaldón 2019a). Filaments grow and branch giving rise to mycelial colonies, colloquially known as **molds**, though some members of this group have the ability to produce both forms (yeast/filaments) as response to shifts in temperature. These are the **dimorphic fungi** that produce yeast-like forms at temperature ranging from 35 to 37 ºC and the filamentous-like forms at 22–25 ºC (Gauthier 2017). Relevant human pathogens are dimorphic ascomycetes, such as *Histoplasma capsulatum, Blastomyces dermatitidis*, *Coccidioides immitis, Paracoccidioides brasiliensis*, *Sporothrix schenckii* or *Talaromyces marneffei* (Klein & Tebbets 2007).

The cell wall of the fungi has been broadly studied and characterized (Farkas 1979, Feofilova 2010, Gow et al. 2017, Kang et al. 2018). It is considered an essential and dynamic structure involved in morphogenesis, pathogenesis and viability of the fungal cell, so much so, that about one-fifth part of the yeast genome is involved in the biosynthesis of this structure (Gow et al. 2017). Structurally, the fungal wall is composed of β-(1.3) and β-(1.6) glucans forming an interchain with 3 to 4 % of chitin and other protein and polysaccharides whose composition varies between fungal species (Gow et al. 2017). Some fungi show in the inner wall complex polymerized phenolic compounds called melanin, which is a group of natural pigments, negatively charged, hydrophobic and with high molecular weight (White 1958). The level of melanin in the cell wall allows to discriminate ascomycetes and other fungi as **hyaline fungi** (absence of melanin) *vs* **dematiaceous or black fungi** (presence of melanin), showing vegetative and often reproductive structures with a fuscous color, ranging from olive or brown to black (Nosanchuk & Casadevall 2006, Latgé 2007).

The fungi, can propagate by sexual and asexual reproduction (Fig. 2), releasing to the environment a great number of spores that can resist extreme conditions and disperse over long distances. The sexual state, also known as **teleomorph,** generates spores by meiotic division of their nuclei, giving rise to ascospores. Conversely, the asexual state, or **anamorph,** produces dispersal propagules by mitotic division of their nuclei and commonly it generates a great number of asexual spores or conidia (Alexopoulos et al. 1996, Kirk et al. 2008). Some species can produce more than one asexual state, which are called **synanamorphs** (Seifert et al. 2011). When both states

of sporulation are present the whole fungi is named **holomorph**. In some cases, sexual and asexual morphs develop side-by-side, but in general they are produced at different times or on different substrates, when its life cycle includes the production of more than one independent form or spore stage, this organism is named **pleomorphic fungus** (Kirk et al. 2008). Most of the fungi included in *Pezizomycotina* are pleomorphic and only known from their asexual morphs, which are cited in older literature as “Deuteromycetes", “Hyphomycetes” or “Imperfect Fungi". However, nowadays these terms are no longer used in formal taxonomy (Seifert et al. 2011).

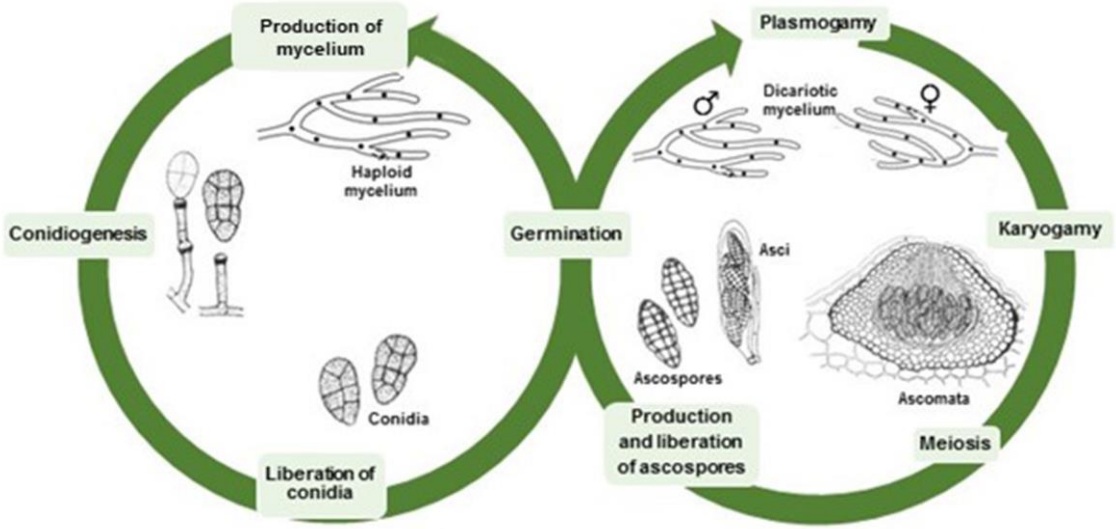

Figure 2. Asexual (anamorphic) and sexual (teleomorphic) cycle of a filamentous ascomycete (Adapted from Crous et al. 2009a, Watkinson 2015, Wallen & Perlin 2018).

The identification of anamorphic species has traditionally been based on conidia morphology and other morphological characters associated with fertile structures such as conidiogenous cells, conidiophores, and fruiting body, among others.

First, characters derived from **conidiophores** considering the degree of its complexity allows defining it in three different ways. If this structure is not morphologically distinguished from vegetative hyphae, it is named micronematous, those that are slightly differentiated from the hyphae are consider semimacronematous, and macronematous, those that are morphologically well distinguished from vegetative hyphae (Ellis 1971). Although this classification is obsolete, at the dawn of taxonomy, the hyphomycetes were divided by Saccardo (1886) into four families (*Mucedineae*, *Dematieae*, *Stilbeae* and *Tuberculariеae*) depending on whether the conidiophores were grouped or not grouped. Briefly, in *Mucedineae* and *Dematieae* they can be

solitary or at least separate along their length, in the *Stilbeae* long conidiophores are fused forming synnemata, whilst in the *Tuberculariеae* generally short conidiophores are aggregated on stromata usually forming tubercles or also denominated sporodochia (Hughes 1953). Another important characteristic of conidiophores is their ability to stop their growth or not after producing the first conidium, so we can differentiate between determinate or indeterminate conidiophores. The conidiophore can be branched or unbranched, in some genera these branched patterns of conidiophores are regularly defined (penicillate, verticillate, dichotomous, etc.) and are often used as a taxonomic character (Seifert et al. 2011).

In second place can mention the **conidiogenesis** mode, which refers to the basic processes involved in conidiogenesis, i.e. the ways in which walls are laid down (apical-, diffuse-, and ring-wall building), conidial initiation, conidial secession, conidial maturation, collarette production, and conidiogenous cell proliferation. However, previous attempts to use conidiogenesis as the basis for classification proved to be impracticable because of their inability to cope with taxa that failed to fit into the recognized taxonomic categories (Sutton 2014). The two principal types of conidium ontogeny are referred as **blastic** and **thalic** development**,** although up to 43 conidiogenic events that combine the characteristics of initiation, wall formation, conidia secession, proliferation of conidia cells, and number of conidia formed by conidiogenous cells have been recognized by Kirk et al. (2008).

Blastic conidia differentiate apically or laterally from a fertile hypha by the blowing out and de novo growth of part of the conidiogenous cell and are delimited from the parent by a basal septum. The **conidiogenous cell** can produce a unique conidiogenous locus and is denominated **monoblastic**, conversely if several conidiogenous loci are observed, the cell is denominated as **polyblastic** (Ellis 1971). Within the **blastic conidiogenesis** different types of ontogenies can be observed. In **tretic conidiogenesis** the conidia are originated by protrusion of the cell wall from the conidiogenous cell, generating channels or pores in the conidiogenous loci; if only one pore is produced it is denominated monotretic, or polytretic when two or more are observed (Kirk et al. 2008). In the **phialidic conidiogenesis**, the conidiogenous cell denominated phialide, produces conidia by extruding new cell walls through their open necks. The conidia may collect in droplets or adhere in chains where the youngest conidium is always closest to the fertile apex of the phialide (i.e., basipetal development) and the length of the phialide does not change during this process (Cole 1986). Phialides can be denominated monophialidic or polyphialidic depending whether they present one or more fertile opertures. Due to the apical wall rupture during

formation of the primary phialoconidium or at initiation of the second conidium, the remaining sleeve of wall material at the fertile apex is called collarette, and its size varies depending on the level at which the circumscissile rupture of the outer wall occurs (Hammill 1974, Cole 1986, Watkinson et al. 2015). The conidiogenous cells named annellide bearing also a basipetal succession of blastic conidia but the conidiogenous cell visibly elongates after delimitation of each conidium. The **annellidic conidiogenesis** gives rise to a series of ring like wall scars (annellations) at the conidiogenous cell apex, each scar representing the site where conidial secession and proliferation had occurred (Ellis 1971, Cole 1986). The production of conidia in chains can be denominated acropetal when the youngest is in the apex or basipetal if the new spores are formed at the base of the chain (Watkinson et al. 2015). The **thalic conidiogenesis** occurs by differentiation of a portion of a fertile hypha into a single, terminal or intercalary conidium (holothallic conidiogenesis), or by conversion and disarticulation of a hyphal segment into several conidia (arthric conidiogenesis) (Cole 1986, Watkinson et al. 2015). Through thalic proliferation fungi can also produce resistance structures like **chlamydospores,** formed by a complete cell of the hyphae producing thick, melanized, and often encapsulated cell walls (Watkinson et al. 2015).

Finally, the characters derived from the morphology of the conidia such as the presence and number of septa, the ornamentation and pigmentation of the walls, and the presence of appendages or mucilaginous layers have been widely used to delimit different taxonomic categories (Kirk et al. 2008).

1.3 Taxonomy and nomenclature

The fungi are classified in diverse taxonomic rank commonly denominated taxa that includes **Kingdom, Phylum, Class, Order, Family, Genus** and **Species,** although **subkingdom, subphylum, subclass** or **subfamily** can sometimes be used. Several filamentous asexual ascomycetes were traditionally classified into **Deuteromycetes**, which is, an artificial group not recognized as a real taxon, and whose species are characterized by only producing asexual structures (Guarro et al. 1999). The deuteromycetes were subdivided in **Hyphomycetes,** characterized by showing sterile mycelium or bearing conidia directly or on special branches of specialized hyphae (conidiophores), and **Coelomycetes,** characterized by producing numerous conidia in fruiting bodies (conidiomata), that can be spherical (pycnidia), with conidiogenous cells lining the inner cavity wall or are cup shaped (acervuli), in which case the conidiogenous cells form a palisade on the conidiomatal surface (Guarro et al. 1999).

INTRODUCTION

The species is the basic concept in taxonomy and one of the central terms in evolutionary biology and biologic diversity (Claridge et al. 1997). For a long time, in mycology the definition of species and superior taxa was based on the classical taxonomy, which was mainly focused on morphological characters. However, in recent years, the taxonomy has experienced important advances due to the introduction of other criteria that have resulted in the definition of different concepts of species i.e. morphological, biological, ecological and phylogenetic species concept (Giraud et al. 2008).

The **morphological concept of species** is based alone on morphological features of asexual and sexual fertile structures and the species are defined according to the degree of similarity of these structures (Guarro et al. 1999, Giraud et al. 2008). The **biological concept of species** relies on reproductive isolation and establishes that members of a species must be able to interbreed sexually; however, this concept cannot be applied to asexual fungi (Seifert et al. 1995). The **ecological concept of species** is based on differences produced by adaptation to a particular ecological niche (Giraud et al. 2008). With the incorporation of molecular tools the **concept phylogenetic of species** was erected, which is based on the genetic divergences using a genetic sequence (Giraud et al. 2008) and a species is defined as a monophyletic group or organisms with a common ancestor (Taylor et al. 2000, Cai et al. 2011). This concept is particularly appropriate in the study of fungi in which reproductive structures are not observed and comparison at morphological level result impossible.

The morphological concept of species was the most commonly used in mycology for a long time (Cai et al. 2011), it however shows some limitation derived from instability or variability of some characters under different conditions (Cai et al. 2011). For this reason, currently, the most widely used criteria is the **Genealogical Concordance Phylogenetic Species Recognition (GCPSR),** in which multi-gene sequencing and phylogenetic analysis allows the determining of the phylogenetic concordance of multiple unlinked genes to indicate a lack of genetic exchange and thus evolutionary independence; it is more finely discriminating than the other criteria being also better able to reveal **cryptic species**, i.e. species morphologically very similar or indistinguishable, but genetically divergent (Giraud et al. 2008, Cai et al. 2011).

The advent of molecular tools through the utilization of multi-locus phylogenetic analysis, together with morphologic characterization, has allowed to improve substantially the correct delineation of different taxa, triggering a significant

restructuring of the fungal taxonomy. In this sense, the International Fungal Barcoding Consortium performed in 2012, officially recommended the use of the internal transcribed spacer (ITS) region as a fungal DNA barcode for a primary identification (Schoch et al. 2012). The ITS was chosen as the best candidate for barcoding for several reasons, because it is easy to amplify, is a good marker for species-level identification due to its high information content, and because each fungal cell contains multiple copies of the nrRNA including also these regions. Therefore, ITS is generally used as DNA barcode for species identification (Xu 2016). The sequence of this region containing two variable zones (ITS1 and ITS2) separated by the conserved 5.8S nrRNA gen and externally flanked by 18S and 28S nrRNA genes allocated at the 5`-end of the ITS1 and at the 3` of the ITS2 respectively.

However, it has been seen that in several genera, ITS is not sufficiently informative to separate the species correctly. Therefore, percentage of identity to be considered as the cut-off for species delimitation remains controversial, because the interspecific variability of this region varies among different fungal taxonomic groups (Lücking et al. 2020). Then incorporation of sequences of more informative regions such as introns of different protein encoding genes, e.g. actin (*Act*), β-tubulin (*tub*2), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), translation elongation factor 1-alpha (*tef*1), among several others, have been introduced to the analyses for phylogenetic reconstruction (Guarro et al. 1999, Lücking et al. 2020).

Currently, the nomenclature of fungi is regulated by the International Code of Nomenclature for algae, fungi and plants (ICN) (Hawksworth et al. 2011, Norvell 2011). The denomination of the fungal species is performed using a Latin binomial, in which the first epithet corresponds to the genus and the second epithet denominates the species; only the first letter of the genus should be written with capital letter and both in italic. The name assigned for a species can be related with different factors, e.g. host species association, the locality (the country or region in which the fungus was collected) or personal names (as an honor to another mycologist) or other preferences of individuals (Guarro et al. 1999, Dayarathne et al. 2016)

Formerly, the fungus with a pleomorphic life cycle were denominated using two or more different scientific names, one for the sexual state and one or more for the asexual states; however, the nomenclature for species denomination has experienced sweeping modifications. With the establishment of the Article 59.1 of the ICN and the application of the principle “one fungus/one name”, the ancient dual nomenclature system has been abandoned and currently replaced with the use of a unique name for

each species (Hawksworth et al. 2011, Norvell 2011). Once it was accepted that it would be desirable for each fungus to have only one name, a second symposium entitled "One Fungus = Which Name?" was held in Amsterdam, to address the naming of pleomorphic fungi (Braun 2012). It was decided to maintain the oldest name, but with some exceptions (for example, if the latter name was the most widely used). Lists of the names accepted and rejected were proposed in order to minimize the impact of these changes mainly for medical mycology.

1.2 Fungal ecology

In general, fungi are the group with a highest number of species after insects (Wu et al. 2019), although it has been estimated that only 5–7 % of total species are known, of which only about 2.2–3.8 million are culturable under laboratory conditions. However, with the advent of large-scale environmental sequencing methods such figure has been increased and the number of species could reach up to 12 to 15 million (Seifert et al. 2011, Wu et al. 2019).

Fungi are ubiquitous organisms widely distributed in numerous habitats, the saprobed being the most common with the ability of obtaining nutrients from organic material in decomposition. They show a great plasticity due to their adaptation capacity, and have been recovered from many diverse substrates such as soil, wood, plant tissues, dung, insects, and even on other fungi. However, the quantities of nutrients, substratum physical features, water availability, humidity, and temperature among other biotic and abiotic conditions determine the success of colonization and maintenance of the species (Mueller et al. 2004). Although fungi can inhabit practically all environments, in this thesis we have focused on soil, plant litter, coprophilous inhabiting fungi and from clinical setting.

Soil constitutes an important reservoir for fungal propagules, which are considered the most abundant component of soil mycrobiota in terms of biomass ranging from microscopic single unicellular yeasts to filamentous macroscopic fungi (Anderson & Domsch 1978, Bridge & Spooner 2001). The majority of soil fungi are saprotrophs (soil-borne fungi); however, many of them can be parasites of plant roots (Crous et al. 2009a). Usually fungi are present as mycelium, spores (sexual or asexual), chlamydospores or sclerotial bodies, being the first state the most active in term of metabolic activity and the remaining are commonly present as dormant survival structures showing a limited activity related to soil nutrient cycle (Crous et al. 2009a).

These organisms are leading decomposers and their potential for reducing organic matter is more than 75 % greater than other soil microorganisms (Krishna & Mohan 2017). Due to their ability to produce a broad variety of extracellular enzymes, they are able to break down all kinds of organic matter, including the decomposition of complex, recalcitrant compounds from plants and animals, such as cellulose, hemicellulose, lignin and chitin (Boddy et al. 2007). Soil fungi also contribute to regulate the balance of carbon and nitrogen, promoting the redistribution of nutrients, maintenance of fertility and soil structure, but also participating as biological control against root pathogens and in the protection against drought (Wagg et al. 2014, El-Komy et al. 2015, Frąc et al. 2018). In addition, many fungal species possess the ability to act as an effective biosorbent of toxic metals such as cadmium, copper, mercury, lead, and zinc (Frąc et al. 2018).

The fungal population in soil varies within and across the biomes in relation to the diversity and litter composition, root density, and nutrient availability (Eldor 2015, Frąc et al. 2018). Pyrosequencing analysis of a large set of soil French and Italian samples from different ecosystems revealed the largest phyla in this substratum was the *Ascomycota* (Orgiazzi et al. 2013). Similar results were later obtained for a set of global soil samples by Tedersoo et al. (2015), who also demonstrated that the richness of this group peaked in tropical ecosystems, positively correlated to plant richness. Richness of tree species was also positively correlated with richness of soil fungi (Tedersoo et al. 2016). The ascomycetous fungal relative abundance and diversity can also be influenced by land use types. Although plant and soil properties have been demonstrated to be the main driving factors that explained soil fungal diversity, the impact of human disturbance and harvesting possess significant effects on fungal community (Lienhard et al. 2013, Yang et al. 2017). In this sense, the use of a metagenomic approach has proved that agricultural practices have an antagonistic effect on fungal soil diversity (Lienhard et al. 2013, Rascovan et al. 2013). Plant litter represents a major source of organic carbon in forest soils, and its decomposition is a crucial point in nutrient recycling. The term litter encompasses the layer of dead plant material present on soil surface or dead plant material that is detached from a living plant and constitute the main plant biomass-derived substrate for colonization of many saprophytic fungi (Vittal 1976). The decomposition of this substrate depends on several factors such as temperature, humidity, chemical composition of material and soil organisms and is considered a crucial pathway for nutrient to return to soil e.g. nitrogen, phosphorus, and calcium that are newly available for plants and microbial uptake (Krishna & Mohan 2017). Plant litter decomposition is a highly complex process

mediated by various fungal taxa, which possess a large arsenal of extracellular enzymes that play an important role in the cellulose, hemicellulose, pectin and lignin degradation (Prakash et al. 2015). A recent study performed by Prakash et al. (2015) in tropical dry forest showed that the most part of isolates were ascomycetous fungi that were able to produce lipases, cellulases, pectinases, pectate transeliminases, proteases, amylases and laccases enzymes in plate assays.

Biochemical decomposition of plant organic material is a sequential process which, at first, involves the loss of easily utilizable compounds (oligosaccharides, organic acids), followed by the degradation of the remaining highly recalcitrant compounds (lignin or suberin). These changes are related to microbial litter decomposers succession, which reveal the diversity of catabolic capabilities that are sequentially required to complete the process of litter decomposition (Frankland 1998, Osono & Takeda 2001). In consequence, fungal community is variable and dependent on chemical dynamic substrate changes, leading to the formation of different niches which potentially increase fungal diversity or to the creation of a more uniform environment with a potential decrease in diversity (Melillo et al. 1989, Dickie et al. 2012, Prakash et al. 2015).

Fungi involved in the decomposition of plant litter have long been studied mostly based on observational and culture-dependent methods (Koide et al. 2005, Osono 2005, Zhang et al. 2008, Osono et al. 2009), and have been divided into early, intermediate and late decomposers (Frankland 1998, Tang et al. 2005). Some studies demonstrated that the initial stages of litter decay is dominated by *Ascomycota* phylum, but its abundance decrease during the process of degradation as they are gradually replaced by fungi from the *Basidiomycota*, during the later stages of decomposition (Frankland 1998, Osono 2007, Voříšková & Baldrian 2013, Kirker et al. 2020). Although prolonged seasonal dry period and moisture limitation can prevent litter colonization by basidiomycetous fungi, then ascomycetes can prevail in intermediates and the late step of breakdown of vegetal organic materials (Prakash et al. 2015).

The application of next generation sequencing (NGS) technology to characterize leaf litter has enabled large level analysis and the resulting metagenomic capabilities has allowed researchers to analyze mixed microbial communities and dissecting complex and dynamic microbial communities that have been applied to forest soils, decaying wood, and standing trees. Furthermore, these methods have served to evaluate the contribution of phyllosphere inhabiting as well as foliar endophytes fungi in the decomposition leaf litter community (Voříšková & Baldrian

2013, Cordier et al 2012). Molecular and next-generation sequencing undoubtedly provide more complete estimates of fungal diversity of litter than traditional culture-based research, however a culture-based method is essential for investigating and exploting biologically active compounds of these valuable organisms.

Animal dung represents another rich ecological medium for fungal development, which holds high available carbon sources, such as hemicellulose, cellulose or lignin, likewise a high nitrogen content. Herbivorous dung also contains vitamins, growth factors, minerals, and a high water content with a pH around 6.5 that offer a propitious medium for fungal growth (Webster 1970, Lodha 1974). Coprophilous fungi play an important role in the ecosystems due to their ability to recycle nutrients in animal faeces (Hawksworth et al. 1983, Richardson 2001). In fact, they are the main consumers of faecal cellulose and lignin, producing biomass that is latter used by other microorganisms. Although fungi are reported from all types of dung, herbivore dung is considered a rich repository for the land mycobiota, whereas decomposition of carnivore and omnivorous dung, bacteria play the most important role (Bell 1983). Two distinct groups of fungi could be considered on dung, the strictly coprophilous which only grow on this substrate, and the fimicolous fungi that can also survive on other decaying organic matter (Soláns 1990, Doveri 2004, Sarrocco 2016). However, the coprophilous appellation is often used as a term in a wide sense. Dung decomposition by fungi occur through temporal and taxonomic succession that is ruled by a highly competitive pressure for space in order to produce their fruit bodies on the dung surface. Generally, *Mucoromycota* (zygomycetous fungi) are observed early in succession, followed by *Ascomycota*, and *Basidiomycota*, although some authors consider that the timing of appearance of these groups overlaps considerably (Lodha 1974). Since the beginning of the last century, when Saccardo (1902) listed more than 750 species from coprophilous origin and more than 150 genera from dung, few publications have been devoted to the coprophilous conidial fungi (Massee & Salmon 1902, Tubaki 1954, Bell 1983, Seifert et al. 1983, Subramanian 1983, Jeamjitt et al. 2006). Many authors consider that hyphomycetes are not true dung fungi, but they are mere pollutants arriving from air or soil, after the dung has been deposited. However, some asexual state of ascomycetous fungi being known only from dung shows that this is not a justified assumption (Seifert et al. 1983). The data available on coprophilous fungi are still very limited, dung mycobiota of only a few regions have been surveyed, and for many areas of the world, even the most elementary information is lacking. Taxonomic studies are of great importance in studying this large group of fungi and can contribute to a better understanding of the potential of the practical exploitation of

dung-inhabiting fungi, and also in terms of the discovery of new biological active compounds.

In the last decades, an increasing number of fungal species has been reported as pathogens for plants and animals, including human. Plant pathogens, constitute the cause of important economic losses in agriculture every year due to the damage produced in the pre- and post-harvest process of the crops affecting a broad range of plant species, e.g. rice, wheat, maize, potatoes, and soybean, which are considered the five most important crops globally (Almeida et al. 2019). The phytopathogenic species can be classified into three categories, **biotrophic** pathogens, those that can obtain nutrients directly from the living plant tissues and being mainly responsible for serious economic losses (Doehlemann et al. 2017), **necrotropic** those that produce necrosis of the infected zone of the plant or even kill tissue, however, they can also obtain nutrients from decaying leaves. The third group defined as **hemibiotropic** is composed of species with the ability to use both substrates (living and dead plant tissues), starting as biotrophs and then switch to become necrotrophs (Divon & Fluhr 2007).

The fungal infections in animals have generated devastating consequences. Mass deaths of bats in North America as a consequence of the white nose syndrome (WNS) caused by *Geomyces destructans* (Fisher et al. 2012); the chytridiomycosis caused by two species of the genus *Batrachochytrium*, that have contributed to the decline of at least 501 amphibian species (6.5 % of described amphibian species), 124 of them (25 %) being reduced by more than 90 %, and 90 species (18 %) being confirmed or presumed extinct in the wild (Scheele et al. 2019); or the fungal infection in honey bee brood caused by *Ascosphaera apis* (Jensen et al. 2013), are only some examples.

Human fungal infections constitute diverse clinical syndromes from which superficial infections, affecting skin and nail, are the most frequent together with mucosal infections of the oral and genital tracts, particularly vulvovaginitis that can affect 50 to 75 % of women in their childbearing years. Although with a considerably less incidence, the invasive fungal infection is one of the most feared by generate high rate of mortality bordering or exceeding 50 % (Brown et al. 2012). In the last few decades, the incidence of dematiaceous fungi has increased considerably, with more than 150 species and 70 different genera reported, affecting both, immunocompetent and immunocompromised patients with mortality rates that can reach up to 70 % in the latter (Revankar & Sutton 2010). These fungi can produce chromoblastomycosis,

eumycetoma and phaeohyphomycosis distinguished from each other by dependence of the histological findings. The chromoblastomycoses are more frequent in tropical regions and are characterized by the presence of sclerotic bodies in tissues of the patients. The eumycetoma correspond to a deep infection commonly observed in the lower extremities, distinguished by the presence of mycotic granules (Revankar 2007). And, finally, the phaeohyphomycosis is a term destined to englobe all other clinical syndromes caused by dematiaceous fungi, in which can only be observed septate pigmented, dichotomously branched hyphae and associated to superficial, respiratory, central nervous system or disseminated disease (Revankar 2007).

1.4 The genus *Alternaria*

1.4.1 Overview

Alternaria is a ubiquitous and complex genus with a great number of species, which are present in a wide range of substrates such as soil, air, water, plant debris, among others. They show a diverse ecological lifestyle that includes saprobic, pathogenic and entophytic species. Many *Alternaria* species are relevant phytopathogens that can affect a great variety of plants that include *Apiaceae*, *Asteraceae*, *Brassicaceae*, *Cyperaceae*, *Poaceae*, *Solanaceae* etc., causing significant economic losses to the agricultural and related sectors (Thomma 2003, Lawrence et al. 2016). Several species have been described as emerging human pathogens, mainly affecting immunocompromised patients, but also their spores are important airborne allergens causing asthmatic processes (de Hoog et al. 2011). In summary, few fungal taxa can match the global impact of *Alternaria* on native ecosystems and its affect on humans and human activities (Lawrence et al. 2013). For these reasons, correct identification of *Alternaria* species is of great value to researchers, medical mycologists and the public alike.

Alternaria species are commonly recognized by their darkly pigmented multi-celled conidia typically with transverse and longitudinal septa (dictyoconidia) and forming long and branched conidial chains. In the past, it was a representative genus of the hyphomycetes since many of its species produce exclusively the asexual morph. However, the discovery of sexual reproduction in some *Alternaria* species and phylogenetic studies placed the genus in *Pleosporaceae* (*Pleosporales, Dothideomycetes, Pezzizomycotyna, Ascomycota*). Currently, the genus includes more

than 300 accepted species (Lawrence et al. 2016), but its number is increasing when exploring new substrates or locations mainly by and using molecular tools.

1.4.2 Taxonomy and identification

The genus *Alternaria* was originally described by Nees von Esenbeck in 1816 with *A. tenuis* as the type specimen, but some years later the species was considered conspecific with *Torula alternata* and, in 1912, Keissler synonymized both with *A. alternata*, because this species was considered the type species of *Alternaria* (Nees von Esenbeck 1816, Keissler 1912). Not only has the uncertain position of the type of the genus complicated the classification of alternaria-like fungi since the inception of the genus, but also the taxonomy of *Alternaria* has been debated until recently. Before the advent of molecular technologies, the classification based on morphological traits of alternaria-like fungi involved successive revisions of *Alternaria* and similar genera by several authors (Woudenberg et al. 2013, 2017), but probably the most well known was that of Simmons whose extense work culminated in his "*Alternaria*. An identification manual" (Simmons 2007). Based on conidial morphology, he organized and delineated the 275 accepted species in species-groups, selecting one species as representative of each morphological group (Simmons 1992, 2007). However, more recently, diverse phylogenetic studies have demonstrated that several of these species-groups were monophyletic (Hong et al. 2005, Lawrence et al. 2012, 2013), and maintained as sections including the synonymy of several alternaria-like genera, such as *Allewia, Brachycladium, Chalastospora, Chmelia, Crivellia, Embellisia, Nimbya, Pseudoalternaria, Sinomyces, Teretispora, Ulocladium, Undiphilum* and *Ybotromyces* (Woudenberg et al. 2013). Thus, the genus is currently divided in 27 monophyletic sections (Lawrence et al. 2013, 2016, Woudenberg et al. 2013, 2014). Taxonomic traits and species composition of all *Alternaria* sections are summarized in Lawrence et al. (2016). Additional phylogenetic research has been carried out for some *Alternaria* section, such as *Porri*, *Alternaria*, *Pseudoalternaria* and *Infectoriae*, redefining and delineating new taxa for each of them, and highlighting the latter as one of the largest and most conflictive sections in *Alternaria* (Woudenberg et al. 2014, 2015, Poursafar et al. 2018).

1.4.2.1 Morphological identification

Morphologically, both the asexual and the sexual morphs of *Alternaria* have been well characterized, however the former morph is prevalent in most species (Fig. 3). This shows semi- to macronematous pigmented conidiophores, integrated conidiogenous cells, mono or polytretic condiogenesis and commonly showing

dictyoconidia, although phragmoconidia (conidia with only transverse septa) are also produced in several species (Woudenberg et al. 2013). Conidia can be solitary or arranged in branched or unbranched chains, ovoid, obovoid, cylindrical, narrowly ellipsoid or obclavate, and possess an apical beak or tapering apical cells, smooth- to verrucose-walled. Species with meristematic growth are also known (Simmons 2007, Lawrence et al. 2016). The teleomorph is less frequent and only observed in species of only seven sections; i.e., *Alternaria*, *Crivellia*, *Embellisioides*, *Eureka*, *Infectoriae*, *Nimbya* and *Panax* (Lawrence et al. 2016). It is characterized by dark brown ascomata, erumpent to almost superficial when mature, globose to ovoid, papillate, ostiolate and smooth or setose at maturity. Asci are bitunicate, fissitunicate, uni- or biseriate, cylindrical to clavate, containing (4–6–)8 ascospores, which show 3–7-transverse septa

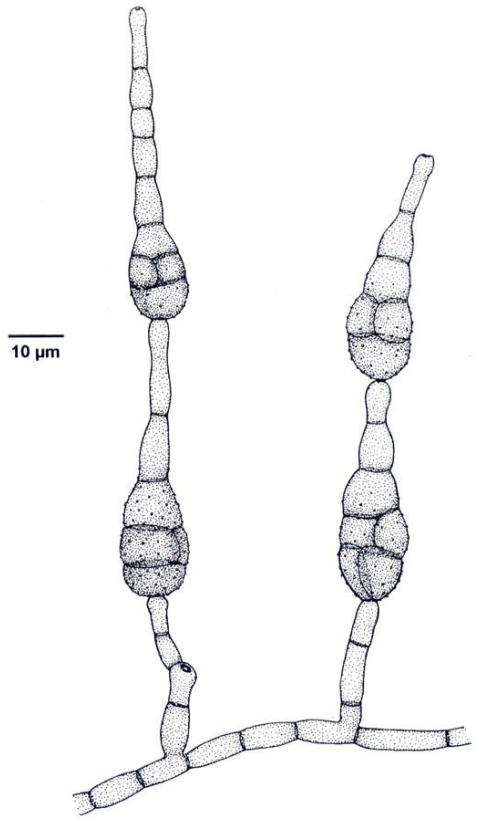

Figure 3. Conidiophores and conidia of *A. alternata* (Adapted from de Hoog et al. 2000).

and 1–2 series of longitudinal septa (Woudenberg et al. 2013, Lawrence et al. 2016). Colony and conidial morphology, together with the branching pattern of the conidial chains are useful to recognize species and sections (Simmons 2007, Lawrence et al. 2016). However, overlapping of morphological features among taxa, and the plasticity

of these fungi depending on culture conditions are handicaps for the correct identification of their species. Therefore, sequence analysis is now crucial for species recognition.

1.4.2.2 Molecular identification

Sequence analyses of the nuclear rDNA, including the intervening ITS regions, and different protein-encoding genes have been used to establish phylogenetic relationships among species-groups or sections of this genus (Woudenberg et al. 2013, Lawrence et al. 2014, Deng et al. 2018). However, Lawrence et al. (2013) provided a phylogenetic study based on the combination of five loci [(*gapdh*, *Alternaria* major allergen gene (*Alt a1*), *ATPase*, *act* and partial calmodulin gene (*cmdA*)] that supported the different sections of the genus, although with different degree of phylogenetic resolution. For instance, the ITS barcode is 100 % identical or nearly so among species of the same section (Woudenberg et al. 2015; Lawrence et al. 2016). Therefore, in addition to this marker, the combined analysis of other loci, such as *ATPase*, *gapdh*, second largest subunit of RNA polymerase II (*rpb2*) and *tef1*, have also been commonly used for species recognition (Woudenberg et al. 2013, Lawrence et al. 2016). Nevertheless, no standard combination has been proposed for resolving phylogenetic relationships in the different sections of this genus.

1.4.3 Clinical interest and antifungal susceptibility

The genus *Alternaria* includes several species able to cause opportunistic human infections. Although infections affecting immunocompetent patients have been reported, these fungi mainly affect immunocompromised patients and are currently considered an emerging fungal pathogen due to the increasing of cases in recent decades (Douglas et al. 2016). This phenomenon is probably associated to the notorious increase of the population with HIV infections, hematological affections such as leukemia or organ transplants (Pastor & Guarro 2008). Furthermore, Cushing's syndrome and the extensive use of intensive and aggressive medical practices such as surgery, the use of catheters, radiation, chemotherapy, and antibiotics treatments are some of the main risk factors associated with infections by these ubiquitous fungi (Guarro et al. 1999, Pastor & Guarro 2008).

The most frequent clinical manifestation due to *Alternaria* are cutaneous and subcutaneous infections, however, allergic sinusitis, bronchial asthma, pneumonitis and rhinitis are commonly reported (Shugar et al. 1981, Ogawa et al. 1997, Bush & Prochnau 2004, Stark et al. 2005, Pastor & Guarro 2008, Revankar & Sutton 2010,

Kpodzo et al. 2011, Levetin et al. 2016). In recent decades ocular mycosis, onychomycoses, (García-Martos et al. 2000, Konidaris et al. 2013, González-Vela et al. 2014), invasive infections including cerebral infection (Cardona et al. 2020), and, disseminated infection (Mirhendi et al. 2013) have also been described.

Alternaria alternata and *A. infectoria* have been the most common reported species, but in the majority of case reports the identification of these molds was based on morphological criteria and/or using sequence analysis of the ITS region. As mentioned before, identification of *Alternaria* isolates by morphology and ITS barcode allows to recognition of the section to which they belong, but not sufficiently adequate for an accurate identification at the species level (Pastor & Guarro 2008, Revankar & Sutton 2010, Lawrence et al. 2016).

The treatment of alternariosis depends on the level of infection and location, but commonly require surgical debridement in association with antifungal therapy (Pastor & Guarro 2008, Derber et al. 2010). However, the antifungal therapy and the optimal duration of treatment are not yet well defined. Nevertheless, in general, alternariosis shows a good response to conventional antifungal drugs, itraconazol being the most commonly used and with a successful outcome (Del Palacio et al. 1996, Pastor & Guarro 2008, Demirci et al. 2015), although therapeutic failures have also been reported (Gené et al. 1995, Gomes et al. 2011). Fluconazol does not show good activity against *Alternaria* infections (Pastor & Guarro 2008). Others antifungal drugs, such as amphotericin B (Lo Cascio et al. 2004) or terbinafine (Cardona et al. 2020) have also been used, however, voriconazole and posaconazol have shown to be a very effective therapeutic option, but more studies are required (Pastor & Guarro 2008, González-Vela et al. 2014).

1.5 The genus *Cladosporium*

1.5.1 Overview

Cladosporium is a dematiaceous genus, and the species of this genus are considered among the most common fungi in outdoor environments (Flannigan et al. 2002). Its species can be found in a wide range of substrates as soil, water, air, plant material, dung, food, or clinical specimens, but also in extreme ecological niches, including hypersaline environments (Zalar et al. 2007, Sandoval-Denis et al. 2015, 2016, Razafinarivo et al. 2016, Bensch et al. 2018, Temperini et al. 2018). They occupy diverse ecological modes, ranging from saprobes to endophytic. They can infect

animals, plants, even be parasites for other fungi (Heuchert et al. 2005, Sandoval-Denis et al. 2015, 2016). There are a limited number of plurivorous species, which do not appear to have any strong environmental preference such as *C. cladosporioides*, *C. herbarum* or *C. oxysporum* (Bensch et al. 2012). By contrast, some phytopathogenic species are often host-specific, causing typical leaf spots, discolorations, necrosis or shot hole symptoms on living or senescing leaves. The host plants are usually restricted to a single family, often only infecting few species of a single genus. Although rarely, some *Cladosporium* endophytic species show the ability to produce compounds with anti-bacterial, anti-cancer, anti-oxidative or immuno-suppressive properties (AlMatar & Makky 2016, Khan et al. 2016, Adorisio et al. 2019). In addition, a few *Cladosporium* species are efficient biological insecticides, particularly against insects that have developed resistance to chemical insecticides (Abdel-Baky & Abdel-Salam 2003). Therefore, the collection of *Cladosporium* isolates from different substrates and the discovery of new species represent a potential source of new compounds of pharmacological, agricultural or industrial interest.

Cladosporium is a typical genus that comprises of hyphomycetous species with very similar morphological features to many other asexual fungi. This and the occurrence of cladosporioid fungi on a wide range of substrates led to numerous species being assigned to *Cladosporium* since its inception (Link 1816), turning it quickly into one of the largest and most heterogeneous genera of hyphomycetous fungi. Prasil & de Hoog (1988) estimated around 540 species belonging to this genus and Dugan et al. (2004) published a checklist with 772 *Cladosporium* names. However, current sequence analyses and detailed observations of the morphological characters have demonstrated that *Cladosporium* is a monophyletic genus belonging to the *Cladosporiaceae* (*Cladosporiales, Dothideomycetes, Pezzizomycotyna*, *Ascomycota*), and encompassing 238 accepted species (Braun & Schubert 2007, Bensch et al. 2012, 2018, Crous et al. 2019b, Abdollahzadeh et al. 2020). However, this number could probably be expanded with the study of the diversity of cladosporium-like fungi from substrates poorly investigated.

1.5.2 Taxonomy and identification

The genus *Cladosporium* was introduced by Link in 1816 with four species, i.e. *C. herbarum*, *C. abietinum*, *C. atrum* and *C. aureum*, and leptotypified with *C. herbarum* in 1931 by Clements & Shear (Bensch et al. 2012). As mentioned before, the number of species in the genus increased rapidly, but with imprecise and brief descriptions in the literature for many taxa, making the genus problematic for the identification of its species (Bensch et al. 2012). Many authors followed a wide concept of the genus and

all hyphomycetous dematiaceous fungi with more or less differentiated conidiophores (macronematous) and producing amero- to phragmosporous conidia arranged in acropetal chains were assigned to *Cladosporium*. In addition, due to some *Cladosporium* species were associated with the teleomorphic genus *Mycosphaerella*, both genera were initially classified in the ascomycetous family *Mycosphaerellaceae*. Several revisions have been carried out along the history of *Cladosporium* (David 1997, Crous et al. 2001), but the first strong evidence on the great heterogeneity and polyphyly of *Cladosporium* was published by Braun et al. (2003). These authors also demonstrated that the sexual morphs of several *Cladosporium* species were phylogenetically distant from members of *Mycosphaerella* and represented a distinct teleomorphic genus proposed as *Davidiella*, which was established as the sexual genus for *Cladosporium* (Braun et al. 2003, Schoch et al. 2006). Further phylogenetic studies on *Mycosphaerella* and *Davidiella* highlighted that both genera were representatives of two distinct dothideomycetous families, namely *Mycosphaerellaceae* and *Cladosporiaceae*, respectively (Schoch et al. 2006, 2009). However, with the implementation of "one fungus/one name" criteria for pleomorphic fungi, the name of *Cladosporium* had priority over *Davidiella,* which currently is considered a synonym of the former (Bensch et al. 2012). It is of note is that recently the genus *Cladosporium* has been delineated as the unique member of the new order *Cladosporiales* (Abdollahzadeh et al. 2020).

The modern concept of *Cladosporium* was established by a polyphasic approach and many species originally placed in the genus were relocated, or were the basis for the proposal of new genera belonging to *Cladosporiaceae* or other families in *Capnidoales*, to other orders in *Dothideomycetes,* or even to other ascomycetous classes. For instance, some of the genera where old *Cladosporium* species have been reassessed in *Dothideomycetes* are: *Neocladosporium* (*Capnodiales*), *Rachicladosporium* (*Capnodiales*), *Penidiella* (*Capnodiales*), *Toxicocladosporium* (*Capnodiales*), *Verrucocladosporium* (*Capnodiales*), *Apenidiella* (*Mycosphaerellales*), *Ochrocladosporium* (*Pleosporales*), *Venturia* (*Venturiales*); in *Eurotiomycetes*: *Cladophialophora* and *Metulocladosporiella* (*Chaetothyriales*); or in *Leotiomycetes*: *Hormoconis* and *Rhizocladosporium* (*Helotiales*) (Braun et al. 2003, Crous et al. 2006, Seifert et al. 2007, Bensch et al. 2012, Bezerra et al. 2017). Some of those cladosporioid genera, commonly denominated cladosporium-like fungi, are treated in the next section since they have been identified in the present work (section 1.6).

The molecular studies also showed that the most frequently *Cladosporium* species isolated from outdoor and indoor environments, such as *C. herbarum*, *C. sphaerospermum* and *C. cladosporioides*, were in fact complexes of cryptic species,

genetically differentiated but with very similar morphological characteristics. Therefore, currently, for the identification of *Cladosporium* species or cladosporium-like fungi, in addition to morphological examination, sequence analyses of different gene markers is mandatory for their recognition (Bensch et al. 2010, 2012, 2015, 2018).

1.5.2.1 Morphological identification

The genus *Cladosporium* is morphologically characterized by the production of unbranched or branched conidiophores, micro- or macronematous, solitary or fasciculate; with integrated conidiogenous cells, terminal or subterminal, mono- or polyblastic, and showing a characteristic type of conidiogenous locus defined as a coronate structure (scar) which consists of a convex central dome surrounded by a raised periclinal rim and is present in the conidiogenous loci and also in the conidial hila (David 1997, Braun et al. 2003). From the conidiogenous cells are originated primary and secondary ramoconidia, but also intercalary conidia and terminal conidia, whose size gradually diminishes throughout the chain (Fig. 4). The conidia can be solitary (*in vivo*), or most commonly forming simple or branched acropetal chains, of variable shape (subglobose, ovoid, ellipsoid, fusiform, limoniform to subcylindrical or cylindrical), septation (aseptate or with transverse eusepta) and ornamentation (smooth, verruculose, verrucose or echinulate) (Bensch et al. 2012). The sexual morph of the genus*,* described only for few species (*C. allicinum*, *C. grevilleae*, *C. herbarum*, *C. macrocarpum C. silenes* and *C. variabile*), is characterized by globose and pseudothecial ascomata, showing 1(–3) periphysate ostiolar necks, with a peridium of 3–6 layers, black to red-brown, and of *textura angularis*; asci fasciculate, bitunicate, obovoid to broad ellipsoid or subcylindrical, straight to slightly curved, 8-spored; ascospores bi- or multiseriate, hyaline, obovoid to ellipsoid-fusiform, containing irregular luminar inclusions, mostly thick-walled, straight to slightly curved, frequently becoming brown and verruculose (Bensch et al. 2012, Marin-Felix et al. 2017a).

The morphological identification of the *Cladosporium* species is difficult because they show overlapping traits. However, diagnostic features to identify isolates of the *C. cladosporioides*, *C. herbarum* and *C. sphaerospermum* species complexes have been defined (Bensch et al. 2012, 2015, Marín-Felix et al. 2017a). The largest complex, *C. cladosporioides*, is characterized by the production of non-nodulose, mostly non-geniculate conidiophores and conidia with variable ornamentation, ranging from smooth, or nearly so, to irregularly verrucose–rugose or rough-walled, while that true verrucose conidia have not been described in this species complex (Bensch et al. 2015, Marin-Felix et al. 2017a). The species of the *Cladosporium herbarum* complex

can be distinguished by their ornamented conidia, ranging from minutely verruculose to verrucose, echinulate or spinulose, and most species show conidiophores with lateral swellings containing the conidiogenous cells (Bensch et al. 2015, Marin-Felix et al. 2017a). The species of *C. sphaerospermum* complex are characterized by non-nodulose neither geniculate, conidiophores poorly differentiated from the supported hyphae and by the production of numerous globose or subglobose, terminal and intercalary conidia with a variable ornamentation, ranging from smooth to minutely verruculose, verrucose or rugose (Zalar et al. 2007, Bensch et al. 2015, Marin-Felix et al. 2017a).

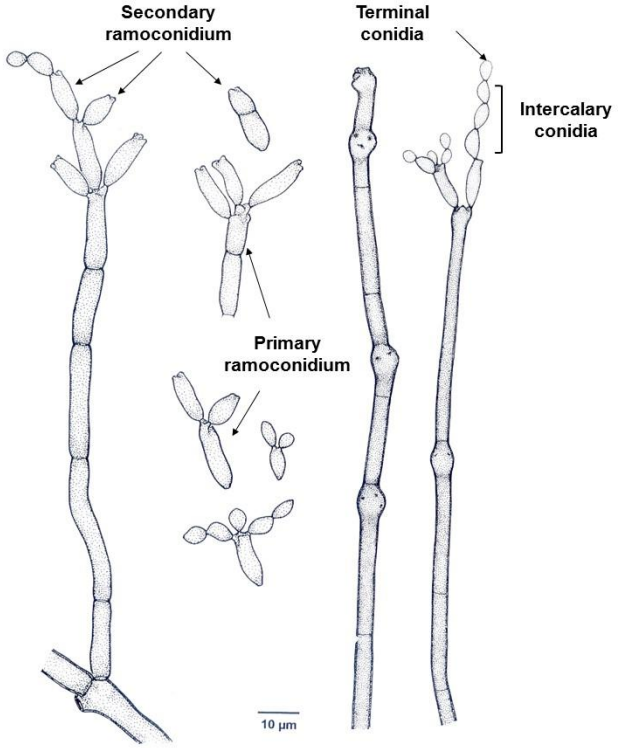

Figure 4. Conidiophore with primary and secondary ramoconidia, intercalary conidia, and small terminal conidia of *C. oxysporum* (Adapted from de Hoog et al. 2000).

1.5.2.2 Molecular identification

Although the standard ITS barcode demonstrated to be useful to distinguish most species belonging to the *C. sphaerospermum* species complex, it shows a limited resolution for species of the other mentioned complexes (Bensch et al. 2015). In fact, the different phylogenetic studies carried out on *Cladosporium* agree in that the ITS is a suitable locus for the identification of an isolate at the genus level, and to some extent even a specific complex, however additional loci are required for an accurate identification at the species level (Bensch et al. 2012, 2015). Other recommended

barcodes for that purpose are the actin (*act*) and the translation elongation factor 1-alpha (*tef*1) genes, being phylogenetic analyses with both loci able to discriminate all *Cladosporium* species currently accepted in the genus (Marin-Felix et al. 2017a, Bensch et al. 2018).

1.5.3 Clinical interest of *Cladosporium* species

Although in old literature some *Cladosporium* species were associated to severe human infections, such as chromoblastomycosis or systemic infections, the species able to cause such mycoses, such as *C. carrionii* and *C. bantianum*, among others, are currently moved to *Cladophialophora* (Queiroz-Telles et al. 2017). This genus belongs to the *Herpotrichiellaceae* of the order *Chaetothyriales* (de Hoog et al. 2000), which also includes several human opportunists (e.g., *Cyphellophora*, *Exophiala*, *Fonsecaea, Phialophora* or *Rhinocladiella*) (Crous et al. 2007b). *Cladosporium* is considered a rare human pathogen due to the saprophytic nature of its species, but a notable increase of case reports in immunocompetent and immunocompromised patients, as well as in animals, has been published in recent decades (Castro et al. 2013, Jayasinghe et al. 2017, Batra et al. 2019, Velázquez-Jiménez et al. 2019). Only isolates of the species *C. cladosporioides*, *C. herbarum*, *C. sphaeropermum*, *C. oxysporum* and *C. macrocarpum* have been described as agents of human infection (Gugnani et al. 2006, Maduri et al. 2015, Lalueza et al. 2011, Jayasinghe et al. 2017, Batra et al. 2019). Although most of these reports are doubtful cases (Sandoval-Denis et al. 2015, 2016), it is of note that a recent case of chromoblastomycosis attributed to *F. pedrosoi* (Torres et al. 2010), has been recently molecularly reidentified by the same authors as *C. langeronii* (Torres-Guerrero et al. 2018), an uncommon species of the complex *C. sphaerospermum*, which type strains was curiously also isolated from ulcero-nodular lesions in a Brazilian man (Bensch et al. 2012). There have been further *Cladosporium* species recovered from clinical specimens, but the lack of clinical evidence does not allow for them to be considered causal agents of disease (Sandoval-Denis et al. 2015, 2016). Considering that *Cladosporium* isolates of recent clinical reports were identified only with ITS analysis, the list of *Cladosporium* species can be expanded if an appropriated molecular approach is used for their identification. What is clear is that the diversity of *Cladosporium* species associated to human and animal infections still remains underestimated to date.

The reported most common route of infection by *Cladosporium* isolates is through traumatic inoculation or respiratory tract (Castro et al. 2013, Grava et al. 2016),

and the associated clinical syndromes are cutaneous and subcutaneous infection with formation of nodules or abscesses (Gugnani et al. 2006, Maduri et al. 2015), allergic reactions (Sellart-Altisent et al. 2007, Levetin et al. 2016), pulmonary infection (Castro et al. 2013), keratitis (Cheng et al. 2015) and, less frequently, invasive infection such as brain infection (Lalueza et al. 2011, Batra et al. 2019).

The treatment of infections by *Cladosporium* depends on the anatomic location and if they are cutaneous or subcutaneous, surgical excision is commonly required. The antifungal therapy has not yet been defined and there are a wide range of drugs used to treat *Cladosporium* infections (i.e. amphotericin B, 5-fluorocytosine, ketoconazole, miconazole, voriconazole, itraconazole, natamycin and potassium iodide). However, the drugs more effective for resolving such infections have been itraconazole and posaconazole (Castro et al. 2013, Batra et al. 2019), which have also resulted in being the most potent antifungals tested *in vitro* against *Cladosporium* species in Sandoval-Denis et al. (2015).

1.6 Other genera of dematiaceous hyphomycetes

In the present thesis, isolates belonging to other hyphomycetous dematiaceous genera that resulted of taxonomic interest were morphologically and molecularly studied (Fig. 5). Although some of them were isolated from human clinical specimens, such as a few *Curvularia* (*Cu.*) isolates, most of them were collected from soil or plant debris.

Some of these fungi belong to different ascomycetous taxa of uncertain taxonomic position at the family or order level such as *Matsushimaea* or *Pseudopenidiella*. Taxonomic position and a brief description of the genera identified in the present work are included below in alphabetical order.

1.6.1 *Apenidiella*

The genus *Apenidiella* was described by Quaedvlieg & Crous in 2014 to accommodate *Apenidiella strumelloidea* (formerly *Cladosporium strumelloideum*), isolated from leaf of *Carex* sp. collected from stagnant water in Russia (Quaedvlieg et al. 2014). It is a cladosporium-like genus classified in the *Teratosphaeriaceae* (*Capnodiales*), distinct to that of the genus *Cladosporium s.str.* (*Cladosporiaceae*) (Quaedvlieg et al. 2014). Morphologically, it is characterized by the production of both solitary macronematous and micronematous conidiophores, this latter reduced to conidiogenous cells. The

conidiogenous cells are terminal, integrated, bearing a unique set of ramoconidia with 1–3 terminal loci from which aseptate conidia are formed in branched chains. This genus is morphologically very similar to *Penidiella*, from which it can be differentiated by the absence of verticillate sets of ramoconidia and by multilocus sequence analysis of LSU and *rpb*2 regions (Quaedvlieg et al. 2014).

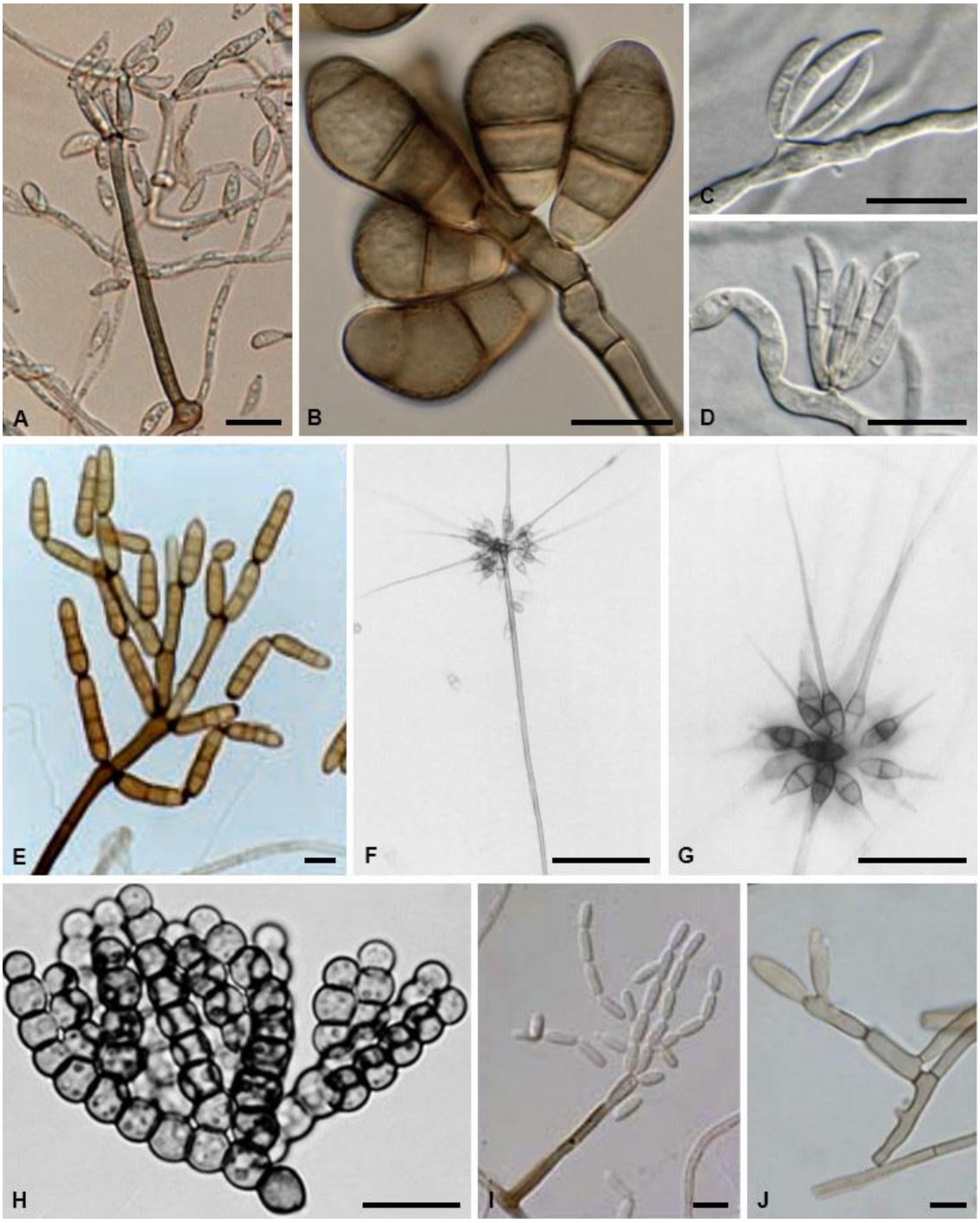

Figure 5. Other genera of dematiaceous hyphomycetes. **A**. *Apenidiella strumelloidea*

(ex-type CBS 114484). **B**. *Curvularia hominis* (ex-type CBS 136985). **C–D**. *Cyphellophora goniomatis* (ex-type CBS 146077). **E**. *Dendryphiella paravinosa* (ex-type CBS 141286). **F–G**. *Heliocephala zimbabweensis* (ex-type CBS 691.97). **H**. *Matsushimaea fasciculata* (dried type culture, MFC-2503). **I**. *Pseudopenidiella piceae* (ex-type CBS 131453). **J**. *Venturia fuliginosa* (ex-type HMAS 247007). Scale bars: F = 50 µm; G = 20 µm; the others = 10 µm. Picture A taken from Crous et al. (2007c); B from Marin-Felix et al. (2017a); C, D from Crous et al. (2019b); E from Crous et al. (2016); F, G from Decock et al. (1998); H from Matsushima (1975); I from Crous et al. (2012); J from Shen et al. (2016).

1.6.2 *Curvularia*

Curvularia is a monophyletic genus of the *Pleosporaceae* (*Pleosporales, Dothideomycetes, Pezizomycotina, Ascomycota*) described by Boedijn in 1933 and typified with *Cu. lunata*. The genus includes saprobes, endophytes and relevant pathogens for plants and animals, including humans. As phytopathogens mainly affect cereals and grasses and they are, subsequently, often a cause of significant economic losses in the agricultural sector (Kusai et al. 2016, Marin-Felix et al. 2017b, Mourão et al. 2017, Shirsath et al. 2018). Several species are emerging human opportunists, with *Cu. hawaiiensis*, *Cu. lunata* and *Cu. spicifera* being the most common species reported causing skin infections, sinusitis, pulmonary and ocular infections (mainly keratitis) in immunocompetent patients, and also systemic infections in immunocompromised patients such as cerebral phaeohyphomycoses (Carter & Boudreaux 2004, Razzaghi-Abyaneh et al. 2015, Bay et al. 2017, Beckett et al. 2017, Kiss et al. 2019).

The asexual morph of *Curvularia* is characterized by the production of euseptate conidia, usually curved due to the hypertrophy of one of the intermediate conidial cells. However, currently also included species with straight and distoseptate conidia (Marin-Felix et al. 2017a). The genus is currently well-delineated and its species identified by molecular approach using sequence analyses of the encoding gene markers ITS, *gapdh* and *tef*1 (Marin-Felix et al. 2017a). Based on this approach, several species of the pleosporalean genera *Bipolaris*, *Drechslera* and *Helminthosporium* have been transferred to *Curvularia* (Manamgoda et al. 2012, 2015, Tan et al. 2018).

1.6.3 *Cyphellophora*

The genus *Cyphellophora* (*Cy*.) is currently included in a monotypic family *Cyphellophoraceae* (*Chaetothyriales*, *Eurotiomycetes*, *Pezizomycotina*, *Ascomycota*)

(Réblová et al. 2013). It was introduced by de Vries in 1962 with *Cy. laciniata* as type species and successive molecular studies, based mainly on nrDNA sequence analyses, increased to nearly twenty the number of the species in the genus (Réblová et al. 2013, Madrid et al. 2016). *Cyphellophora* includes saprobes, endophytes as well as pathogenic species for plants but mainly for animals and humans (de Vries et al. 1986, Sutton et al. 1991, Decock et al. 2003). As human opportinists, *Cy. laciniata, Cy. pluriseptata*, and *Cy. suttonii* have been associated with skin and nail infections, and less frequently with pulmonary infections (de Vries et al. 1986, Sutton et al. 1991, Decock et al. 2003, Madrid et al. 2016).

Cyphellophora is morphologically characterized by the production of intercalary or terminal phialides with a prominent to indistinct collarette originating 1–3-septate and mostly curved conidia adhering in bundles (Decock et al. 2003, Feng et al. 2014, Attili-Angelis et al. 2014). The conidial morphology is considered the most relevant feature to distinguish this genus from their close relative *Phialophora*, which is comprised of species with aseptate and mostly globose to ellipsoid conidia (Madrid et al. 2016). Despite the recent studies based on DNA sequence data, the taxonomic structure of *Cyphellophora* is not well-defined since some species identified as *Phialophora*, still belonging to that genus, are members of the *Cyphellophora* lineage (Attili-Angelis et al. 2014). Barcodes used for the identification of its species are LSU, ITS and the partial β-tubulin gene (*tub2*) (Attili-Angelis et al. 2014).

1.6.4 *Dendryphiella*

Dendryphiella a genus of hyphomycetous dematiaceous fungi, proposed by Bubák & Ranojevič (Ranojevič 1914) and formerly typified with *D. interseminata*. However, it was considered a synonym of *D. vinosa* by Reisinger (1968), being therefore this later the type of the genus. Currently, it comprises 15 species usually associated with decaying leaves and wood, but with an uncertain phytopathogenic role (Crous et al. 2014, 2016, Liu et al. 2017, Hyde et al. 2018 Crous et al. 2019a, Ferreira & Barreto 2019).

Dendryphiella is characterized by the production of macronematous conidiophores with intercalary and terminal polytretic conidiogenous cells, usually swollen and with dark conidiogenous loci, originating conidia commonly septate, solitary or arranged in acropetal chains (Ellis 1971). Recent molecular studies, based on analyses of SSU and LSU rDNA, allocated *D. vinosa* in the pleosporalean family *Dictyosporiaceae* (Tanaka et al. 2015, Boonmee et al. 2016). It is of note that

sequence data of *Dendryphyella* species is very limited and, in addition to those of *D. vinosa*, only ITS and LSU sequences of *D. eucalyptorum*, *D. fasciculata*, *D. paravinosa*, *D. pitsanulokensis* and *D. stromaticola* are available for comparison (Crous et al. 2014, 2016, Liu et al. 2017, Hyde et al. 2018, Crous et al. 2019a).

1.6.5 *Heliocephala*

The genus *Heliocephala* was introduced by Rao in 1984 to accommodate *H. proliferans*, a fungus characterized by the production of macronematous conidiophores, bearing sets of monoblastic conidiogenous cells originating obclavate, sometimes rostrate, hooked phragmoconidia radially arranged in a compact head (Rao et al. 1984). Recently, it has been related to the *Microthyriaceae (Microthyriales, Dothideomycetes*) (Crous et al. 2018). *Heliocephala* comprises seven species, two previously classified in *Holubovaniella* (Castañeda-Ruiz 1985, Heredia-Abarca et al. 2011). Most of the species have been isolated from plant material, with the exception of *H. natarajanii*, which was described on basidiocarp of *Pisolithus tinctorius* (Rao et al. 1984, Castañeda-Ruiz 1985, Decock et al. 1998, Kumaresan & Srinivasan 2002, Heredia-Abarca et al. 2011, Mel´nik et al. 2013).

1.6.6 *Matsushimaea*

Matsushimaea was proposed by Subramanian in 1977 based on *Torula fasciculata*, a fungus described two years before by Matsushima from dead leaves of *Cinnamomum japonicum* in Tokyo, Japan (Matsushima 1975, Subramanian 1977). It could be defined as a cladosporium-like fungus by the production of branched chains of dark brown conidia growing directly on hyphae, but differentiated conidiophores are lacking (Subramanian 1977, Castañeda-Ruiz et al. 1996). Later, *M. fertilis* and *M. magna*, found on rotten and decaying leaf in Cuba and South Africa, respectively, were added to the genus (Castañeda-Ruiz et al. 1996, Matsushima 1996). No molecular data exist for any species of the genus, and its taxonomic position remains obscure.

1.6.7 *Pseudopenidiella*

The genus *Pseudopenidiella* was introduced by Crous & Koukol in 2012 to accommodate *P. piceae*, a cladosporium-like fungus isolated from needle litter of *Picea abies* in the Czech Republic (Crous et al. 2012). It was characterized by the production of dimorphic conidiophores (macro and microconidiophores), with terminal conidiogenous cells bearing 1–3 conidiogenous loci producing aseptate ramoconidia from which arise branched chains of aseptate conidia (Kirk 1983, Crous et al. 2012). It differs from the genus *Cladosporium* by the lack of thickened and darkly pigmented

coronate scars associated to the conidiogenous loci. Analyses of ITS and LSU sequences of the type species placed *Pseudopenidiella* far from other cladosporium-like genera, showing only weak phylogentic relationships with some *Heliocephala* and *Fusicladium* species. Therefore, it was considered *incertae sedis* in *Dothideomycetes* (Crous et al. 2012).

1.6.8 *Venturia*

The genus *Venturia* was proposed by Saccardo in 1882 and, due to the number of species described since then, it has become a large and complex genus with a rather uncertain taxonomic position of their species. Although in the Index Fungorum 279 species names are registered as *Venturia*, DNA data is only available for near to 25 species, including the type of the genus *V. inaequalis* (Marin-Felix et al. 2017a). Phylogeny of the small sub-unit ribosomal DNA (SSU), LSU, *tef1*, RNA polymerase II largest sub-unit (*rpb1*) and *rpb2* loci of some of these species place the genus in the family *Venturiaceae*, order *Venturiales,* rather than in *Pleosporales* as was previously stated (Zhang et al. 2011). The modern concept of the genus includes species with sexual and/or asexual morph (Marin-Felix et al. 2017a). The first is characterized by black pseudothecial ascomata, globose to subglobose, peridium of *textura angularis*, central ostiole and covered with setae; asci are bitunicate, oblong to obclavate; ascospores obliquely uniseriate to biseriate, ellipsoidal, uniseptate and slightly constricted at the septum. The asexual morph usually produces macronematous conidiophores, solitary or in small groups, but micronematous conidiophores reduced to conidiogenous cells are also present is some species; conidia can be solitary or forming acropetal chains, 0–3(–4)-septate. Due to the morphological pattern of the macronematous conidiophores, the genus is also considered a cladosporium-like fungi (Marin-Felix et al. 2017a). Previously, species with known sexual morph were included in *Venturia* and those with asexual morph were classified in *Fusicladium* (Crous et al. 2007b). However, due to several molecular studies and following the criteria of “one fungus/one name” for pleomorphic fungi, both genera were synonymized, giving *Venturia* priority. Subsequently several species of *Fusicladium* were transferred to *Venturia* (Rossman et al. 2015, 2016). Since LSU and ITS barcodes showed very low resolution to discriminate species in *Venturia*, it was recommended to combine ITS with *tef*1 and *tub*2 (Crous et al. 2007b, Zhang et al. 2011, Marin-Felix et al. 2017a).

Venturia includes relevant phytopathogenic fungi that affect a wide range of dicotyledonous plants and fruit crops worldwide, causing important economic loses (Belete & Boiraz 2017, González-Domínguez et al. 2017). Apple scab, also know as

black spot, is considered the most important disease in apple, with *V. inaequalis*, the type species, being the most common reported causal agent of this disease (Jha et al. 2009, Bowen et al. 2011, Berete & Boiraz 2017). Other relevant phytopathogens are:

V. pyrina affecting the European pear, *V. nashicola* the Asian pear, *V. carpophila* affecting the peach (*Prunus persica*) and *V. cerasi* the cherry (*Prunus cerasus*), to mention some of them (Park et al. 2000, Sokolova et al. 2014, Chen et al. 2017, González-Domínguez et al. 2017).

2. INTEREST AND OBJECTIVES

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

INTEREST AND OBJECTIVES

Fungal species are ubiquitous organisms widely distributed worldwide in different ecological systems such as saprobes, endophytes, symbionts or parasites. In recent decades, however, there has been an extended number of species associated with an increasing variety of fungal syndromes. In plants, they are responsible for important losses and damage to agricultural activities and food production (Godfray et al. 2016, Almeida et al. 2019). In animals, fungi have been documented with relevant impact in their biodiversity, being associated even with the extinction of some species (Fisher et al. 2012, Jensen et al. 2013, Scheele et al. 2019). As causal agents of human infections, the number of fungal opportunists has increased, especially with the rise of the immunocompromised population, mainly in patients with cancer and organ transplants. Nevertheless, fungi have many beneficial effects not only in the nature, but also as models of organisms for research on different biological aspects, or they are the basis in biotechnology to get biomolecules of medical, industrial or agricultural interest among several other applications (Abdel-Baky 2000, Abdel-Baky & Abdel-Salam 2003, Lou et al. 2013, AlMatar & Makky 2016, Irfan et al. 2018, Islam et al. 2019). However, despite its important role in the nature and different scientific areas, its biodiversity is still very unknown.

In recent decades, the use of sequence DNA analysis for fungal identification has revealed that their diversity is much higher than expected (Bensch et al. 2010, 2018, Lawrence et al. 2016, Tan et al. 2018). In fact, there is a significant number of species still undiscovered, not only present in unexplored ecological niches, but also associated with common substrates in our environment (soil, plant material, etc.) still poorly studied through molecular tools. It is well known that sequence analysis of nrDNA, including the ITS barcode, as well as of other genes, have allowed to described in recent years a great number of cryptic species in cosmopolitan genera such as *Aspergillus*, *Fusarium* or *Penicillium*, among them important human pathogens or producers of secondary metabolites with a wide range of applications in different fields. Therefore, delimiting and characterizing new species, as well as the correct identification of the fungi associated with a certain environment or substrate, will not only allow to know more precisely the fungal diversity and understand its role in that place, but it will also contribute to a better knowledge of the taxonomy of fungi through studying the phylogenetic relationships of, every time, an increasing number of known fungi. However, it is of note that, although the molecular approach has been extensively implemented in the fungal systematics, there are numerous genera of which the taxonomy still remains obscure.

Considering this context, we focused our study mainly on the cosmopolitan genera *Alternaria* and *Cladosporium*. Both genera include well-known species, among them important pathogens for plants, but also capable of causing serious infections in human and animals. However, species identification in both genera is realy difficult due to the high number of species they have and the overlapping of morphological features among taxa, what leads to DNA sequence analysis to be crucial for species recognition. In recent years, numerous cryptic species have recently been described based on polyphasic approach, including multilocus sequence analysis. Therefore, correct identification or delineation of their species to known the real species diversity, in general or associated to a particular substrate, or to clarify the taxonomic structure of a particular group, such as in the case of *Alternaria* section *Infectoriae*, is still needed in both genera.

Thus, the **general objective** of the present thesis was to study the diversity of *Alternaria*, *Cladosporium* and other morphologically similar or phylogenetic related genera from isolates of clinical and environmental origin, and to assess taxonomic position of those rare or undescribed species by molecular tools.

To carry out this objective, the following **specific objectives** were defined:

1. To obtain a great number of isolates belonging to the mentioned genera isolated from different substrates, including soil and plant debris collected in unexplored geographical locations, and especially isolates from substrates poorly studied by molecular tools, such as clinical specimens, submerged plant material or dung from herbivore animals.
2. To identify the isolates through morphological criteria and comparison of DNA barcodes recommended for the respective genera to get a confident identification and determine the true spectrum of species diversity in the different substrates studied.
3. To characterize by a polyphasic approach (i.e. DNA sequence data, morphology or physiology) the detected putative new species within the studied genera.
4. To determine, if necessary, the *in vitro* susceptibility testing for species from clinical origin.

3.- MATERIAL AND METHODS

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

3.1 Origin of the isolates

A total of 589 isolates were studied in the present thesis; 56 (9.5 %) from clinical origin, mostly recovered from human specimens (Table 1), and 533 (90.5 %) isolated from different environmental substrates (200 from soil, 204 from herbivore dung, and 129 from plant debris) (Table 2) (Fig. 6).

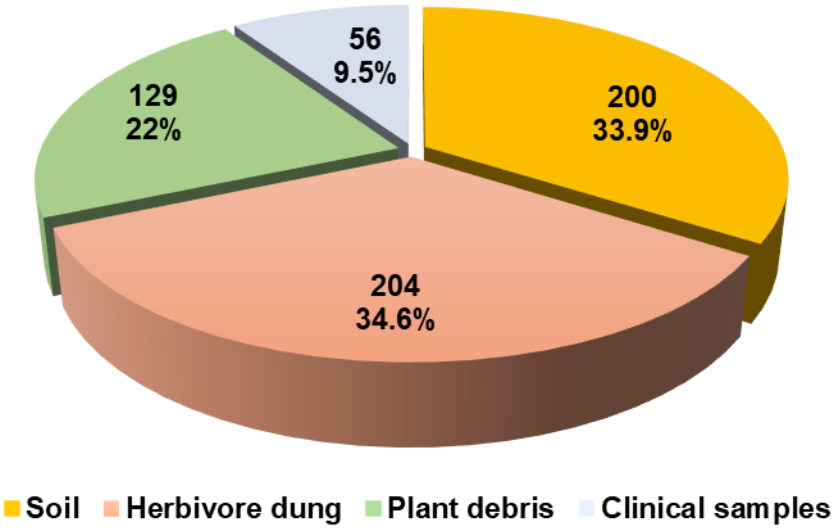

Figure 6. Distribution of the isolates studied

The clinical isolates were mainly received from culture collections of three different institutions: 16 (28.6 %) from the Fungus Testing Laboratory of the University of Texas Health Science Center – UTHSC (Texas, USA), 3 (5.4 %) from the South Reference Laboratory of Sant Joan University Hospital (Reus, Spain), and 37 (66 %) from the National Center for Microbiology of the Carlos III Health Institute (Madrid, Spain). The isolates were recovered from cutaneous (21), respiratory (12), subcutaneous (4), ocular (4), pericardial liquid (1) and bone biopsy (1) human specimens. Two of these isolates were from undocumented exudates, six have no documented origin and five from a clinical environment. These clinical isolates were received presumptively identified as *Alternaria* sp. (54) and *Curvularia* sp. (2).

Environmental isolates were obtained from samples collected in Spain (162 from soil, 204 from herbivore dung, and 108 from plant debris), Mexico (37 soil samples) and Vietnam (19 plant debris samples and 1 from soil), using the procedures described below.

Some types or reference strains from the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands) were used for comparison (Table 3).

3.2 Environmental samples processing and fungal isolation

3.2.1 Herbivore dung samples

Herbivore dung samples were processed using serial dilution technique and moist chambers methods (Crous et al. 2009a, Richardson 2001).

For serial dilution technique, approximately 1 g of dung sample was introduced into a tube with 9 ml of sterilized distilled water, containing chloramphenicol and dieldrin to inhibit the growth of bacteria and mites. The sample was homogenized thoroughly and serially diluted up to 10^{-2} or 10^{-3}. Finally, the pour plate method was used, which consisted of transferring 1 ml of each dilution to a sterile plastic Petri dish, and, subsequently, 20 ml of melted cooled medium poured into the plate and carefully homogenized. Two different media were chosen and prepared at the same time, i.e. PDA and PCA. Once the culture medium was solidified, the plates were incubated at room temperature and checked continuously for detectinc fungal presence for up to 1 month. A part of the dung samples was incubated in moist chambers. Several pieces of the sample were introduced into a sterile Petri dish (90 mm diameter) with a piece of sterile filter paper covering the bottom, which was humidified with drops of sterilized distilled water, containing chloramphenicol and dieldrin. Those chambers were incubated at room temperature and sterile water was added every 5 or 7 days to maintain wet conditions during the incubation. The samples were periodically examined for the fungal isolation for up to 3 months.

3.2.2 Soil samples

To process soil samples, we used the serial dilution and baiting technique according to Crous et al. (2009a) and Calduch et al. (2004), respectively.

The serial dilution technique was identical that for dung samples.

The baiting technique consisted in pouring a part of the soil sample into a sterile Petri dish (90 mm diameter) until covering at least half of the plate. Thereafter, a mixture of pieces of sterile filter paper and wood was distributed on the surface of the soil and moistened with sterilized distilled water with chloramphenicol and dieldrin using a sterile pipette and repeated every 5 or 7 days to maintain the humidity of the sample and promote the fungal growth. The plates were incubated at room temperature and checked periodically under stereomicroscope for up to 3 months.

3.2.3 Plant debris

Samples of plant debris, collected mainly in forest areas but also submerged plant material from fluvial origin, were incubated in moist chambers and prepared following the same procedure explained previously for dung samples. Sterile Petri dishes of 90 or 140 mm diameter were used depending on the size of the plant material. Submerged plant materials were washed with tap water for 1–2 minutes to reduce the presence of the sediment or mud with other organisms (protozoa, nematodes, insect and bacteria) that could impede proper fungal growth (Abdullah & Webster 1980, Castañeda-Ruiz et al. 2016).

The microfungi of interest grown on the samples were selected and isolated under a stereomicroscope (Leica Microsystems EZ4). With the help of a sterile dissection needle, conidia or small fragments of a single colony were transferred onto PDA plates to get pure cultures of cladosporium-like fungi, and on PCA plates to get cultures of alternaria-like fungi. For mixed cultures, isolates were repeatedly subcultured streaking technique until obtaining single colonies, which isolates were phenotypically and molecularly characterized.

3.3 Molecular analysis

3.3.1 DNA extraction and sequencing

Isolates growing on pure culture after 7 days of incubation at 25 ºC were used for the DNA extraction following a modified protocol described by Müller et al. (1998). The total DNA was used for the amplification of the selected genetic markers. All loci used in the present thesis and the respective primer pairs for their amplification are summarized in Table 4. PCR products were purified and stored at -20 ºC until sequencing, which was carried out at Macrogen Europe (Macrogen Inc. Madrid, Spain) with the same primer pairs used for the amplification of each loci. The sequences were assembled and edited in the software SeqMan v. 7.0.0 (DNAStar Lasergene, Madison, WI, USA) to get consensus ones, which were deposited in the GenBank database of the NCBI under GenBank/EMBL accession numbers.

3.3.2 Molecular identification and phylogenetic analysis

A preliminary molecular identification was performed using the recommended barcode for each genus, i.e. *tef*1 for *Cladosporium*, *rpb*2 for *Alternaria*, *gapdh* for *Curvularia* and ITS for the other genera (Schoch et al. 2012, Woudenberg et al. 2013, Bensch et al. 2015, Manamgoda et al. 2015). Consensus sequences of each isolate

were used for the BLAST research tool in Genbank and CBS databases. The isolates that showed a coverture and a percentage of identity higher than 98 % with sequences of type or reference strains of known species, their identification was considered with confidence. Those that showed percentage of identity lower than 98 %, were studied applying a phylogenetic multi-locus analyses, and phenotypic studies for their delineation at the species and/or genus level.

The phylogenetic analyses were performed first individually for each gene. Sequence alignments were accomplished in MEGA (Molecular Evolutionary Genetics Analysis) program version 6.0. (Tamura et al. 2013) through the ClustalW algorithm (Thompson et al. 1994) and refined with MUSCLE (Edgar 2004) or manually if necessary, in the same platform.

For the multi-locus sequence analyses, the phylogenetic congruence among loci, was evaluated by using the Incongruence Length Difference (ILD) test implemented in the Winclada program (Farris et al. 1994), and also by a visual comparison of the individual phylogenies. In the latter case, only when the position and monophyly of the terminal clades for each genetic marker was concordant, the concatenated study was performed.

Phylogenetic reconstructions were performed through the Maximum-likelihood (ML) and Bayesian Inference (BI) approaches. The best nucleotide substitution model for each locus and for the combined dataset was estimated in MEGA program and in jModelTest version 2.1.10 (Posada 2008) for ML and BI, respectively. The ML analysis was carried out with the same Mega program or with RAxML-HPC2 on XSEDE version 8.2.12 (Stamatakis et al. 2014) executed in the Cipres Science Gateway version 3.3 portal (Miller et al. 2010). Support of the internal branches was assessed by the Bootstrap method with 1,000 replications, where values ≥70 % were considered significant. The BI analysis was executed under MrBayes version 3.1.2 (Ronquist et al. 2012), two simultaneous runs being performed of 5,000,000 generations, four Markov chains and samples were stored every 1,000 generations. The 50 % majority-rule consensus tree and posterior probability values (pp) were calculated after discarding the first 25 % of the samples. A pp value of ≥ 0.95 was considered significant.

3.4 Phenotypic characterization

3.4.1 Macroscopic characterization

The phenotypic characterization of the selected isolates was carried out on the culture media recommended for the different genera. In summary, the culture media

and incubation conditions used for macroscopic characterization of the colonies were Potato Dextrose Agar (PDA; Pronadisa, Madrid Spain), Potato Carrot Agar (PCA; potato 20 g, carrot 20 g, agar 13 g, distilled water 1 l), Oatmeal Agar (OA; Oatmeal 30 g, agar 13 g, distilled water 1 l), Synthetic Nutrient-poor Agar (SNA; KH_2PO_4 1 g, KNO 1 g, $MgSO_4$×7H2O 0.5 g, KCl 0.5 g, Glucose 0.2 g, Sucrose 0.2 g, agar 14 g, distilled water 1 l) and Malt Extract Agar (MEA; Peptone 1 g, Glucose 20 g, Malt Extract 20 g, agar 15 g, distilled water 1 l), which were incubated at 25 ºC in darkness for 7, 14 or up to 30 days for those fungi with slower growth. After the incubation period, the colonies were measured, and their color of the obverse and reverse was described using the color notation from Kornerup & Wanscher (1978). To determine the minimum, optimum and maximum growth temperatures, the isolates were cultured on PDA and incubated at different temperatures ranging from 5 ºC up to 40 ºC for a period of 7 to 14 days.

3.4.2 Microscopic characterization

The microscopic characterization was performed using the culture media recommended for each genus or in the agar media on which the fungi showed a better sporulation. For instance, in the case of *Cladosporium* or similar fungi, microscopic features were evaluated with isolates growing on SNA after 7 days at 25 ºC, following Bensch et al. (2018). For *Alternaria* or alternaria-like isolates, they were examined on PCA after 7 days at 25 ºC, following Simmons (2007). In the case of *Curvularia* isolates, features were evaluated on PDA after 7 days at 25 ºC, according to Marin-Felix et al. (2017a). For other genera a combination of the following agar media were used. i.e. PDA, PCA, OA or Pine Needle Agar (PNA; agar-agar 15 g, distilled water 1 l and sterile pine needles placed in the surface).

For the microscopic observation and measurement of the structures, microscopic slides were prepared, with the help of a sterile needle and taking conidiophores growing between the colony margin and 2 cm inwards, using lactic acid or Shear´s solution as mounting liquid (Crous et al. 2009a). Wherever possible, at least 30 measurements (1000 magnification) of the microscopic structures were taken in order to facilitate the comparison with those closer species. In some genera, i.e. *Cladosporium* and *Curvularia*, the mean and standard deviation (SD) for the most of the structures were also reported.

3.5 In vitro antifungal susceptibility test

Since some of the *Alternaria* isolates studied here were found to be a causal agent of human cutaneous infection, the pattern of antifungal susceptibility was

determined as part of their characterization. The *in vitro* susceptibility testing was performed according to the protocol described in the document M38-A2 of the CLSI with some modification (CLSI 2008). The inoculum prepared from isolates with poor sporulation was made from hyphal fragments, and for isolates with abundant sporulation the inoculum was performed through conidial suspension using a pure culture on culture media that allow a good sporulation (PCA or OA after 7 to 14 days at 25 ºC). The conidia were collected using a sterile loop and transferred to a sterile tube containing sterile water with a drop of Tween 20 to facilitate the segregation of conidia. Eight antifungal drugs were tested, i.e. amphotericin B (AMB) (Sigma-Aldrich Quimica S.A., Madrid, Spain), itraconazole (ITC) (Jansen Pharmaceuticals, Beerse, Belgium), posaconazole (PSC) (Schering-Plough Research Institut, NJ, USA), voriconazole (VRC) (Pfizer S.A., Madrid, Spain), terbinafine (TBF) (Sigma Aldrich Química S.A., Madrid, Spain), anidulafungin (AFG) (Pfizer S.A., Madrid, Spain), caspofungin (CFG) (Merk & Co., Inc., Rahway, USA), and micafungin (MFG) (Astellas Pharma, Madrid, Spain). The microplates were prepared for each antifungal drug concentration ranging from 0.03 to 16 µg/ml, and the incubation was set to 35 ºC for 48 hours and read visually. The results were expressed on minimal inhibitory concentration (MIC) for AMB, azoles (ITC, PSC and VRC) and TBF and was defined as the lowest drug concentration that produced 100 % inhibition of visible fungal growth for AMB, ITC, PSC, and VRC and 80 % inhibition for TBF. For the echinocandins (AFG, CFG and MFG) the results were determined on the minimum effective concentration (MEC), which was defined as the lowest concentration of drug that generates morphological changes on the growth of the tested fungi (i.e. small, rounded, compact hyphal forms) as compared to the growth of the control (i.e. long hyphal cluster). All tests were performed in duplicate to assess reproducibility. The strain ATCC 22019 of *Candida parapsilosis* was used as quality control of the assays.

3.6 Registration and conservation of the isolates

Pure cultures of the isolates identified in the present thesis were deposited at the fungal collection of the Faculty of Medicine of the Rovira i Virgili University in Reus (FMR). Additionally, living cultures of rare and new species, as well as the respective holotypes, were also deposited at the Westerdijk (before CBS-KNAW) Fungal Biodiversity Institute (Utrecht, The Netherlands). Names for the new taxa were registered at the MycoBank database (Crous et al. 2004).

Three methods of conservation were adopted to ensure the survival of the isolates (i.e. sterile water, mineral oil and lyophilization).

3.6.1 Conservation in sterile water

Sporulating colonies of the isolates growing on PCA or OA were cut with a sterile scalpel, and five to six blocks of c.a. 0.5 cm were transferred to glass flasks, containing 2–3 ml of distilled water and sterile tap water, respectively, which were sealed and stored at room temperature in darkness (Castellani 1967).

3.6.2 Conservation with mineral oil

The isolates growing on different culture media were subcultured on agar slant glass tubes with screw caps. The agar media for storing were usually PDA, PCA and OA, depending on fungal species. The cultures were incubated at 25 ºC in darkness for 7 to 14 days or more, until their sporulation. Thereafter, sterile mineral oil was deposited on the colony to a level covering the totality of the agar slant of the tubes. The tubes were stored at room temperature in darkness (Buell & Weston 1947).

3.6.3 Lyophilization

Colony surface of the isolates in pure culture, growing and sporulating on PCA or OA, were scratched with a sterile scalpel, especially in the sporulated zone. The fungal mass was introduced into a sterile tube containing 3 ml of the solution Skim milk (Difco), and after homogenization, the suspension was distributed among three glass flasks provided with an airtight rubber stopper. The vials were introduced into the automatized lyophilizator VirTis Advantage 2.0 ES (SP Scientific, USA). To evaluate the viability and purity of the strain, one vial was used as quality control being cultivated on PDA. The two other vials were stored at room temperature in darkness.

Table 1. Clinical isolates included in the present thesis.

Species	Section	Original num.	FMR num.	Substrate of origin	Country
Alternaria arbusti	*Infectoriae*	DI18-125	17468	Nail	USA
		DI18-126	17472	Sinus	USA
A. anthropophila	*Infectoriae*	–	16235	Subcutaneous nodule	Spain
		CNM-CM2519	17278	Skin biopsy	Spain
		CNM-CM3823	17288	Subcutaneous nodule	Spain
		CNM-CM5813	17296	Pericardial liquid	Spain
A. atrobrunnea	*Infectoriae*	–	16868	Ulcerative skin lesion	Spain
A. guarroi	*Infectoriae*	–	16556	Ulcerative skin lesion	Spain
A. kordkuyana	*Pseudoalternaria*	DI18-42	17467	Knee Tissue	USA
A. malorum	*Chalastospora*	DI18-31	17455	Bronchial brush	USA
A. oregonensis	*Infectoriae*	DI17-117	16978	Right eye	USA
		CNM-CM3527	17286	Aqueous humor	Spain
		CNM-CM7809	17306	Environmental control	Spain
		CNM-CM8684	17309	Environmental control	Spain
A. rosae	*Pseudoalternaria*	DI18-34	17460	Leg	USA
Alternaria sp. 1	*Infectoriae*	CNM-CM5164	17294	Corneal exudate	Spain
		CNM-CM7518	17305	Bone biopsy	Spain
Alternaria sp. 2	*Infectoriae*	CNM-CM4287	17291	Skin	Spain

			CNM-CM6979	17300	Cutaneous exudate	Spain
	Alternaria sp. 3	*Infectoriae*	CNM-CM7186	17302	Cutaneous exudate	Spain
			CNM-CM8839	17308	Skin	Spain
	Alternaria sp. 4	*Infectoriae*	CNM-CM6629	17299	Cutaneous exudate	Spain
			CNM-CM7971	17307	Muscle biopsy	Spain
	Alternaria sp. 6	*Infectoriae*	CNM-CM1658	17276	Skin biopsy	Spain
			CNM-CM2925	17283	Unknown	Spain
			CNM-CM4692	17293	Skin	Spain
	Alternaria sp. 7	*Infectoriae*	CNM-CM4206	17289	Bronchial aspirate	Spain
			CNM-CM5572	17295	Skin biopsy	Spain
	Alternaria sp. 8	*Infectoriae*	CNM-CM6245	17297	Skin	Spain
			CNM-CM6980	17301	Cutaneous lesion	Spain
	Alternaria sp. 9	*Infectoriae*	DI17-130	16991	Tracheal aspirate	USA
			DI18-116	17470	Tissue, Leg right	USA
	Alternaria sp. 11	*Chalastospora*	DI17-120	16981	Right ethmoid contents	USA
	Alternaria sp.	*Infectoriae*	DI17-129	16990	Aspirate, right maxillary	USA
	Alternaria sp.	*Infectoriae*	DI18-35	17461	Eye	USA
	Alternaria sp.	*Infectoriae*	DI18-127	17465	Nasal Tissue	USA
	Alternaria sp.	*Infectoriae*	DI18-40	17466	Respiratory (tracheal suction)	USA

Alternaria sp.	*Infectoriae*	DI18-115	17469	Skin biopsy	USA
Alternaria sp.	*Infectoriae*	CNM-CM2349	17277	Unknown	Spain
Alternaria sp.	*Infectoriae*	CNM-CM2585	17279	Otic exudate	Spain
Alternaria sp.	*Infectoriae*	CNM-CM2757	17280	Unknown	Spain
Alternaria sp.	*Infectoriae*	CNM-CM2849	17281	Unknown	Spain
Alternaria sp.	*Infectoriae*	CNM-CM2852	17282	Unknown	Spain
Alternaria sp.	*Infectoriae*	CNM-CM3086	17284	Clinic environmental	Spain
Alternaria sp.	*Infectoriae*	CNM-CM3229	17285	Unknown	Spain
Alternaria sp.	*Infectoriae*	CNM-CM3659	17287	Sputum	Spain
Alternaria sp.	*Infectoriae*	CNM-CM4241	17290	Tracheal aspirate	Spain
Alternaria sp.	*Infectoriae*	CNM-CM4355	17292	Skin biopsy	Spain
Alternaria sp.	*Infectoriae*	CNM-CM6512	17298	Exudate	Spain
Alternaria sp.	*Infectoriae*	CNM-CM7291	17303	Cutaneous lesion	Spain
Alternaria sp.	*Infectoriae*	CNM-CM7292	17304	Cutaneous lesion	Spain
Alternaria sp.	*Infectoriae*	CNM-CM8941	17310	Environmental control	Spain
Alternaria sp.	*Infectoriae*	CNM-CM9012	17311	Environmental control	Spain
Alternaria sp.	*Infectoriae*	CNM-CM9201	17312	Abscess	Spain
Curvularia suttoniae	–	UTHSC 09-3575	10992	Leg wound	USA
	–	UTHSC 08-809	11690	Sphenoid sinus	USA

CNM-CM: Mold Collection of the Spanish National Center for Microbiology, Madrid, Spain; FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; UTHSC: University of Texas Health Science Center, San Antonio, USA.

Table 2. Isolates obtained from different environmental sources included in the present thesis.

Species	Section / Complex	FMR num.	Substrate of origin	Location	Country
Alternaria aconidiophora	*Infectoriae*	17111	Forest leaf litter	Vall de Boí, Alta Ribagorça	Spain
A. alternata	*Alternaria*	15721	Herbivore dung	Tejeda, The Canary Islands	Spain
		15722	Herbivore dung	Roque Nublo, The Canary Islands	Spain
		15723	Herbivore dung	Pico de Bandama, The Canary Islands	Spain
		15724	Herbivore dung	Road Puerto de las Nieves, The Canary Islands	Spain
		15725	Herbivore dung	North coast, The Canary Islands	Spain
		15726	Herbivore dung	Roque Nublo, The Canary Islands	Spain
		15799	Herbivore dung	Tejeda, The Canary Islands	Spain
		15886	Herbivore dung	Mogán, The Canary Islands	Spain
		15887	Soil	Villa Jiménez, Michoacán	Mexico
		15890	Herbivore dung	Roque Nublo, The Canary Islands	Spain
		15910	Garden soil	Salvador Vilaseca Institute, Reus	Spain
		15911	Garden soil	Villa Jiménez, Michoacán	Mexico

15918	Garden soil	Salvador Vilaseca Institute, Reus	Spain
15919	Garden soil	Salvador Vilaseca Institute, Reus	Spain
15920	Soil	Villa Jiménez, Michoacán	Mexico
15925	Soil	Villa Jiménez, Michoacán	Mexico
15928	Garden soil	Sitges, Barcelona	Spain
15978	Garden soil	Sitges, Barcelona	Spain
15929	Garden soil	Road Pont del Diable, Tarragona	Spain
15979	Soil	Villa Jiménez, Michoacán	Mexico
15980	Soil	Villa Jiménez, Michoacán	Mexico
15981	Soil	Villa Jiménez, Michoacán	Mexico
15982	Soil	Villa Jiménez, Michoacán	Mexico
15983	Soil	Villa Jiménez, Michoacán	Mexico
15985	Garden soil	Reus	Spain
15986	Garden soil	Reus	Spain
15987	Garden soil	Reus	Spain
15988	Garden soil	Reus	Spain
15989	Garden soil	Reus	Spain
16077	Soil	Villa Jiménez, Michoacán	Mexico

16078	Soil	Villa Jiménez, Michoacán	Mexico
16079	Garden soil	Reus	Spain
16086	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
16147	Plant debris	Tarragona	Spain
16148	Plant debris	Amphitheater park, Tarragona	Spain
16149	Plant debris	Amphitheater park, Tarragona	Spain
16151	Garden soil	Reus	Spain
16152	Garden soil	Reus	Spain
16296	Plant debris	Tarragona	Spain
16297	Plant debris	Tarraco Imperial square, Tarragona	Spain
16298	Garden soil	Amphitheater park, Tarragona	Spain
16300	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
16301	Garden soil	Mediterranean balcony, Tarragona	Spain
16302	Garden soil	Tarraco Imperial square, Tarragona	Spain
16303	Plant debris	Tarraco Imperial square, Tarragona	Spain

16350	Garden soil	Tarragona	Spain
16351	Garden soil	Mediterranean balcony, Tarragona	Spain
16352	Garden soil	Tarragona	Spain
16353	Plant debris	Amphitheater park, Tarragona	Spain
16355	Plant debris	Amphitheater park, Tarragona	Spain
16356	Garden soil	Amphitheater park, Tarragona	Spain
16357	Garden soil	Parc de la festa, Reus	Spain
16359	Garden soil	Tarragona	Spain
16360	Garden soil	Tarraco Imperial square, Tarragona	Spain
16361	Garden soil	Tarraco Imperial square, Tarragona	Spain
16362	Soil	Sierra del Montsant, Tarragona	Spain
16363	Garden soil	Parc de la festa, Reus	Spain
16364	Plant debris	Sierra del Montsant, Tarragona	Spain
16366	Garden soil	Parc Samà, Cambrils	Spain
16402	Garden soil	Parc Samà, Cambrils	Spain

16367	Garden soil	Parc de la festa, Reus	Spain
16368	Garden soil	Parc Samà, Cambrils	Spain
16369	Garden soil	Parc Samà, Cambrils	Spain
16370	Garden soil	Parc Samà, Cambrils	Spain
16403	Garden soil	Amphitheater park, Tarragona	Spain
16404	Garden soil	Parc de la festa, Reus	Spain
16405	Garden soil	Amphitheater park, Tarragona	Spain
16406	Garden soil	Parc de la festa, Reus	Spain
16407	Garden soil	Parc de la festa, Reus	Spain
16408	Garden soil	Parc de la festa, Reus	Spain
16409	Garden soil	Parc de la festa, Reus	Spain
16410	Garden soil	Parc Samà, Cambrils	Spain
16411	Garden soil	Parc Samà, Cambrils	Spain
16412	Garden soil	Parc Samà, Cambrils	Spain
16413	Plant debris	Amphitheater park, Tarragona	Spain
16414	Plant debris	Tarragona	Spain
16415	Plant debris	Tarragona	Spain
16416	Plant debris	Tarragona	Spain

16417	Plant debris	Tarragona	Spain
16419	Plant debris	Tarragona	Spain
16420	Garden soil	Parc Samà, Cambrils	Spain
16423	Plant debris	Alt Camp, Tarragona	Spain
16463	Herbivore dung	Alt Camp, Tarragona	Spain
16464	Soil	El Miracle beach, Tarragona	Spain
16467	Plant debris	Parc Samà, Cambrils	Spain
16468	Garden soil	Parc Samà, Cambrils	Spain
16469	Herbivore dung	Poblet, Tarragona	Spain
16472	Plant debris	Tarragona	Spain
16473	Garden soil	Parc Samà, Cambrils	Spain
16574	Herbivore dung	Montseny natural park, Barcelona	Spain
16551	Herbivore dung	Pontons, Barcelona	Spain
16552	Herbivore dung	Alt Camp, Tarragona	Spain
16575	Herbivore dung	Alt Camp, Tarragona	Spain
16577	Plant debris	Tarragona	Spain
16578	Herbivore dung	Pontons, Barcelona	Spain
16628	Soil	Els Ports natural park, Tarragona	Spain
16630	Soil	Montseny natural park,	Spain

		Barcelona	
16631	Soil	Montseny natural park, Barcelona	Spain
16647	Herbivore dung	Montseny natural park, Barcelona	Spain
16699	Herbivore dung	Els Ports natural park, Tarragona	Spain
16701	Herbivore dung	Els Ports natural park, Tarragona	Spain
16703	Herbivore dung	Els Ports natural park, Tarragona	Spain
17057	Herbivore dung	Arbolí, Tarragona	Spain
17097	Submerged plant debris	Road of Malafogassa, Barcelona	Spain
17107	Herbivore dung	Els Ports natural park, Tarragona	Spain
17113	Plant debris	Vall de Boí, Alta Ribagorça	Spain
17114	Plant debris	Vall de Boí, Alta Ribagorça	Spain
17115	Submerged plant debris	Cornudella de Montsant, Tarragona	Spain
17116	Submerged plant debris	Cornudella de Montsant, Tarragona	Spain
17269	Plant debris	Swamp of Siurana, Tarragona	Spain

		17270	Herbivore dung	Vall de Boí, Alta Ribagorça	Spain
		17362	Herbivore dung	Baells, Huesca	Spain
		17371	Herbivore dung	Els Ports natural park, Tarragona	Spain
		17662	Plant debris	Northest region	Vietnam
		17664	Plant debris	Northest region	Vietnam
A. altcampina	*Pseudoalternaria*	16476	Goat dung	Alt Camp, Tarragona	Spain
A. anthropophila	*Infectoriae*	17529	Herbivore dung	La Juncosa de Montmell, Tarragona	Spain
A. armoraciae	*Chalastospora*	17109	Herbivore dung	Vall de Boí, Alta Ribagorça	Spain
A. arborescens	*Alternaria*	15797	Herbivore dung	Peraleda del Zaucejo, Extremadura	Spain
		16085	Soil	Villa Jiménez, Michoacán	Mexico
		16155	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16354	Plant debris	Amphitheater park, Tarragona	Spain
		16358	Plant debris	Tarragona	Spain
		16511	Soil	Costa Montseny, Barcelona	Spain
		16471	Garden soil	Parc de la festa, Reus	Spain
		16474	Soil	Costa Montseny, Barcelona	Spain
		16475	Garden soil	Parc Samà, Cambrils	Spain

			16627	Herbivore dung	Montseny natural park, Barcelona	Spain
			16629	Soil	Montseny natural park, Barcelona	Spain
			16700	Herbivore dung	Els Ports natural park, Tarragona	Spain
			17096	Herbivore dung	Vall de Boí, Alta Ribagorça	Spain
			17363	Herbivore dung	Baells, Huesca	Spain
	A. atra	*Ulocladioides*	15761	Herbivore dung	Tejeda, The Canary Islands	Spain
			15762	Herbivore dung	Roque Nublo, The Canary Islands	Spain
			15763	Herbivore dung	Road north coast, The Canary Islands	Spain
			15764	Herbivore dung	Roque Nublo, The Canary Islands	Spain
			15766	Herbivore dung	Tejeda, The Canary Islands	Spain
			15924	Soil	Villa Jiménez, Michoacán	Mexico
			15990	Soil	Villa Jiménez, Michoacán	Mexico
			16076	Garden soil	Reus	Spain
			16083	Soil	Villa Jiménez, Michoacán	Mexico
			16109	Soil	Villa Jiménez, Michoacán	Mexico
			17063	Plant debris	Swamp of Siurana, Tarragona	Spain

			17709	Herbivore dung	Around the brugent river, Tarragona	Spain
			18179	Bird dung	Aitona, Lérida	Spain
	A. botrytis	*Ulocladium*	15600	Herbivore dung	Mallorca island	Spain
			15595	Herbivore dung	Mallorca island	Spain
			15758	Herbivore dung	Hontoria de la Cantera, Burgos	Spain
			15760	Herbivore dung	Sil canyon, Galicia	Spain
			15798	Herbivore dung	Peraleda del Zaucejo, Extremadura	Spain
			15884	Herbivore dung	Burgos	Spain
			15885	Herbivore dung	The Canary Islands	Spain
			15891	Herbivore dung	Roque Nublo, The Canary Islands	Spain
			15909	Garden soil	Salvador Vilaseca Institute, Reus	Spain
			15922	Herbivore dung	The Canary Islands	Spain
			16080	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
			16081	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
			16082	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain

		16084	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16108	Soil	Granja de Torrehermosa, Extremadura	Spain
		16146	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16150	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16153	Garden soil	Reus	Spain
		16304	Garden soil	Parc de la festa, Reus	Spain
		16401	Garden soil	Parc Samà, Cambrils	Spain
		16371	Garden soil	Tarragona	Spain
		16418	Herbivore dung	Poblet, Tarragona	Spain
		16480	Garden soil	Parc Samà, Cambrils	Spain
		17708	Herbivore dung	La Riba, Tarragona	Spain
A. cantlous	*Ulocladioides*	15888	Herbivore dung	Tejeda, The Canary Islands	Spain
		15889	Herbivore dung	The Canary Islands	Spain
		15921	Garden soil	Salvador Vilaseca Institute, Reus	Spain
		15923	Garden soil	Salvador Vilaseca Institute, Reus	Spain
		17058	Herbivore dung	Els Ports natural park, Tarragona	Spain

	A. chlamydosporifera	*Radicina*	17360	Rabbit dung	Baells, Huesca	Spain
	A. curvata	*Infectoriae*	16901	Goat dung	Els Ports natural park, Tarragona	Spain
	A. fimeti	*Infectoriae*	17110	Rodent dung	Arbolí, Tarragona	Spain
	A. gaisen	*Alternaria*	15800	Herbivore dung	Anaga mountain, The Canary Islands	Spain
	A. heterospora	*Ulocladioides*	15756	Herbivore dung	Road Tejeda, The Canary Islands	Spain
			15765	Herbivore dung	Mogán, The Canary Islands	Spain
			15927	Soil	Villa Jiménez, Michoacán	Mexico
			16365	Garden soil	Tarragona	Mexico
			16478	Herbivore dung	Alt Camp, Tarragona	Spain
	A. inflata	*Pseudoalternaria*	16477	Rabbit dung	Poblet, Tarragona	Spain
			16697	Rabbit dung	Poblet, Tarragona	Spain
	A. kordkuyana	*Pseudoalternaria*	17061	Submerged plant debris	Arbolí, Tarragona	Spain
			17062	Plant debris	Arbolí, Tarragona	Spain
			17372	Herbivore dung	Baells, Huesca	Spain
			17705	Herbivore dung	Capafons, Tarragona	Spain
	A. lawrencei	*Infectoriae*	17004	Goat dung	Els Ports natural park, Tarragona	Spain
	A. malorum	*Chalastospora*	17369	Rabbit dung	Sepulveda, Riaza	Spain

A. merytae	*Infectoriae*	16154	Herbivore dung	Peraleda del Zaucejo, Extremadura	Spain
A. montsantina	*Infectoriae*	17060	Plant debris	Swamp of Siurana, Tarragona	Spain
A. oregonensis	*Infectoriae*	16466	Herbivore dung	Alt Camp, Tarragona	Spain
A. pobletensis	*Chalastospora*	16448	Herbivore dung	Poblet, Tarragona	Spain
A. pseudoventricosa	*Infectoriae*	16900	Horse dung	Els Ports natural park, Tarragona	Spain
A. rosae	*Pseudoalternaria*	15720	Herbivore dung	Hontoria de la cantera, Burgos	Spain
		17376	Herbivore dung	Cerezo de Arriba, Riaza	Spain
		17377	Herbivore dung	Cerezo de Arriba, Riaza	Spain
		17378	Rabbit dung	Hermitage of the eternal Father, Riaza	Spain
A. slovaca	*Infectoriae*	16422	Herbivore dung	Alt Camp, Tarragona	Spain
Alternaria sp. 5	*Infectoriae*	17526	Herbivore dung	Pontons, Barcelona	Spain
	Infectoriae	17527	Herbivore dung	Pontons, Barcelona	Spain
Alternaria sp. 10	*Chalastospora*	17518	Herbivore dung	Pontons, Barcelona	Spain
Alternaria sp.	*Infectoriae*	16646	Plant debris	Els Ports natural park, Tarragona	Spain
Alternaria sp.	*Infectoriae*	17361	Herbivore dung	Els Ports natural park, Tarragona	Spain
Alternaria sp.	*Infectoriae*	17525	Herbivore dung	Pontons, Barcelona	Spain

Alternaria sp.	*Infectoriae*	17528	Herbivore dung	La Juncosa de Montmell, Tarragona	Spain
Alternaria sp.	*Infectoriae*	17589	Plant debris	Mallorca	Spain
Alternaria sp.	*Infectoriae*	17590	Plant debris	Mallorca	Spain
Alternaria sp.	*Infectoriae*	18175	Bird dung	Termens, Lérida	Spain
Apenidiella foetida	–	17266	Submerged plant debris	Arbolí, Tarragona	Spain
Cladophialophora boppii	–	4613	Rotten leaf	Havana city	Cuba
Cladosporium aggregatocicatricatum	*Herbarum*	15807	Herbivore dung	Anaga mountain, The Canary Islands	Spain
		16242	Plant debris	Tarragona	Spain
		17263	Submerged plant debris	Cornudella de Montsant, Tarragona	Spain
		17397	Herbivore dung	Baells, Huesca	Spain
		17400	Herbivore dung	Baells, Huesca	Spain
C. allicinum	*Herbarum*	16211	Plant debris	Amphitheater park, Tarragona	Spain
		16287	Garden soil	Parc Samà, Cambrils	Spain
		16387	Plant debris	Tarragona	Spain
		16878	Garden soil	Reus	Spain
		17070	Submerged plant debris	Arbolí, Tarragona	Spain

			17104	Submerged plant debris	Cornudella de Montsant, Tarragona	Spain
			17349	Herbivore dung	Baells, Huesca	Spain
			17353	Herbivore dung	Baells, Huesca	Spain
	C. angustiherbarum	*Herbarum*	17009	Plant debris	Vall de Boí, Alta Ribagorça	Spain
	C. anthropophilum	*Cladosporioides*	16087	Garden soil	Reus	Spain
			16535	Plant debris	Parc Samà, Cambrils	Spain
	C. aphidis	*Sphaerospermum*	16236	Plant debris	Tarragona	Spain
			16290	Garden soil	Tarragona	Spain
			16683	Plant debris	El Miracle beach, Tarragona	Spain
	C. asperulatum	*Cladosporioides*	16453	Herbivore dung	Alt Camp, Tarragona	Spain
			16454	Herbivore dung	Alt Camp, Tarragona	Spain
			17256	Herbivore dung	Els Ports natural park, Teruel	Spain
			17261	Soil	Road of Malafogassa, Barcelona	Spain
			17351	Herbivore dung	Baells, Huesca	Spain
			17355	Plant debris	Pallaresos, Tarragona	Spain
			17356	Plant debris	Pallaresos, Tarragona	Spain
			17358	Plant debris	Pallaresos, Tarragona	Spain
	C. australiense	*Cladosporioides*	15904	Soil	Villa Jiménez, Michoacán	Mexico
	C. caprifimosum	*Cladosporioides*	16532	Herbivore dung	Alt Camp, Tarragona	Spain

C. chubutense	*Cladosporioides*	17396	Rabbit dung	Castillejo de Mesleón, Riaza	Spain
		17398	Rabbit dung	Castillejo de Mesleón, Riaza	Spain
C. cladosporioides	*Cladosporioides*	15803	Herbivore dung	Pico de Bandama, The Canary Islands	Spain
		15805	Herbivore dung	Anaga mountain, The Canary Islands	Spain
		15808	Herbivore dung	Hontoria de la Cantera, Burgos	Spain
		15809	Herbivore dung	Peraleda del Zaucejo, Extremadura	Spain
		15893	Herbivore dung	Tejeda, The Canary Islands	Spain
		15900	Herbivore dung	Tejeda, The Canary Islands	Spain
		15902	Soil	Morelia, Michoacán	Mexico
		15903	Soil	Villa Jiménez, Michoacán	Mexico
		15905	Soil	Villa Jiménez, Michoacán	Mexico
		15912	Soil	Villa Jiménez, Michoacán	Mexico
		15934	Soil	Morelia, Michoacán	Mexico
		15935	Soil	Morelia, Michoacán	Mexico
		15975	Garden soil	Reus	Spain
		15976	Garden soil	Reus	Spain
		16091	Soil	Morelia, Michoacán	Mexico
		16099	Herbivore dung	Granja de Torrehermosa,	Spain

		Extremadura	
16157	Garden soil	Tarragona	Spain
16160	Garden soil	Mexico City	Mexico
16162	Herbivore dung	Tejeda, The Canary Islands	Spain
16193	Garden soil	Mediterranean balcony, Tarragona	Spain
16205	Plant debris	Amphitheater park, Tarragona	Spain
16213	Garden soil	Parc de la festa, Reus	Spain
16214	Garden soil	Parc de la festa, Reus	Spain
16224	Garden soil	Amphitheater park, Tarragona	Spain
16230	Garden soil	Mediterranean balcony, Tarragona	Spain
16237	Plant debris	Tarragona	Spain
16295	Garden soil	Parc Samà, Cambrils	Spain
16375	Garden soil	Amphitheater park, Tarragona	Spain
16377	Plant debris	Tarragona	Spain
16381	Garden soil	Parc Samà, Cambrils	Spain
16384	Garden soil	Parc Samà, Cambrils	Spain
16390	Garden soil	Parc Samà, Cambrils	Spain

16392	Garden soil	Mediterranean balcony, Tarragona	Spain
16393	Garden soil	Parc Samà, Cambrils	Spain
16394	Plant debris	Alt Camp, Tarragona	Spain
16455	Garden soil	Parc Samà, Cambrils	Spain
16534	Plant debris	Parc Samà, Cambrils	Spain
16536	Herbivore dung	Alt Camp, Tarragona	Spain
16586	Herbivore dung	Pontons, Barcelona	Spain
16587	Herbivore dung	Pontons, Barcelona	Spain
16654	Herbivore dung	Costa del Montseny, Barcelona	Spain
16685	Herbivore dung	Els Ports natural park, Tarragona	Spain
16686	Herbivore dung	Els Ports natural park, Tarragona	Spain
16687	Herbivore dung	Els Ports natural park, Tarragona	Spain
16688	Herbivore dung	Els Ports natural park, Tarragona	Spain
16689	Herbivore dung	Els Ports natural park, Tarragona	Spain
16690	Plant debris	La Garrocha, Gerona	Spain
16898	Herbivore dung	Els Ports natural park,	Spain

					Tarragona	
			17098	Plant debris	Swamp of Siurana, Tarragona	Spain
			17255	Herbivore dung	Els Ports natural park, Tarragona	Spain
	C. coprophilum	*Cladosporioides*	16101	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
			16164	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
	C. delicatulum	*Cladosporioides*	16103	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
			16156	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
			16204	Plant debris	Amphitheater park, Tarragona	Spain
			16233	Garden soil	Mediterranean balcony, Tarragona	Spain
			16531	Garden soil	Parc Samà, Cambrils	Spain
			16653	Herbivore dung	Poblet, Tarragona	Spain
			17352	Herbivore dung	Baells, Huesca	Spain
	C. dominicanum	*Sphaerospermum*	16207	Plant debris	Mediterranean balcony, Tarragona	Spain
			16510	Plant debris	Amphitheater park, Tarragona	Spain

C. europaeum	*Cladosporioides*	15812	Herbivore dung	Peraleda del Zaucejo, Extremadura	Spain
		16201	Garden soil	Parc de la festa, Reus	Spain
		16253	Garden soil	Amphitheater park, Tarragona	Spain
		16839	Garden soil	Amphitheater park, Tarragona	Spain
		17072	Bird dung	Cornudella del Montsant, Tarragona	Spain
C. floccosum	*Herbarum*	16451	Soil	Sierra del Montsant, Tarragona	Spain
		16622	Plant debris	Els Ports natural park, Tarragona	Spain
		16875	Garden soil	Reus	Spain
		16899	Herbivore dung	Els Ports natural park, Tarragona	Spain
		17008	Submerged plant debris	Road of Malafogassa, Barcelona	Spain
		17066	Plant debris	Arbolí, Tarragona	Spain
		17068	Plant debris	Arbolí, Tarragona	Spain
		17069	Plant debris	Arbolí, Tarragona	Spain
		17074	Herbivore dung	Cornudella de Montsant, Tarragona	Spain
		17075	Herbivore dung	Cornudella de Montsant,	Spain

					Tarragona	
			17099	Plant debris	Swamp of Siurana, Tarragona	Spain
			17260	Submerged plant debris	Cornudella de Montsant, Tarragona	Spain
			17348	Herbivore dung	Els Ports natural park, Teruel	Spain
			17375	Plant debris	Pallaresos, Tarragona	Spain
			17520	Herbivore dung	Pontons, Barcelona	Spain
			17521	Herbivore dung	Pontons, Barcelona	Spain
	C. fuscoviridum	*Cladosporioides*	16385	Garden soil	Parc Samà, Cambrils	Spain
	C. fusiforme	*Sphaerospermum*	16696	Herbivore dung	Els Ports natural park, Teruel	Spain
	C. halotolerans	*Sphaerospermum*	15930	Soil	Michoacán	Mexico
			15931	Garden soil	Salvador Vilaseca Institute, Reus	Spain
			15933	Garden soil	Salvador Vilaseca Institute, Reus	Spain
			15966	Garden soil	Tarragona	Spain
			15967	Garden soil	Campus URV, Tarragona	Spain
			15969	Garden soil	Tarragona	Spain
			15970	Garden soil	Tarragona	Spain
			16088	Soil	Villa Jiménez, Michoacán	Mexico
			16096	Soil	Pirineo aragonés	Spain

			16217	Soil	Amphitheater park, Tarragona	Spain
			16238	Garden soil	Parc de la festa, Reus	Spain
			16374	Garden soil	Parc de la festa, Reus	Spain
			16462	Soil	Northest region	Vietnam
	C. ipereniae	*Cladosporioides*	16590	Herbivore dung	Alt Camp, Tarragona	Spain
	C. lebrasiae	*Sphaerospermum*	16243	Plant debris	Tarragona	Spain
	C. lentulum	*Cladosporioides*	16288	Plant debris	Tarragona	Spain
			16389	Herbivore dung	Poblet, Tarragona	Spain
	C. licheniphilum	*Cladosporioides*	16619	Herbivore dung	Costa Montseny, Barcelona	Spain
	C. limoniforme	*Herbarum*	16090	Soil	Road Pont del Diable, Tarragona	Spain
			16163	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
			16221	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
			16229	Garden soil	Tarraco Imperial square, Tarragona	Spain
			16373	Garden soil	Parc de la festa, Reus	Spain
			15915	Soil	Villa Jiménez, Michoacán	Mexico
	C. macrocarpum	*Herbarum*	15917	Herbivore dung	Roque Nublo, The Canary Islands	Spain

16105	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
16159	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
16250	Soil	Sierra del Montsant, Tarragona	Spain
16251	Soil	Sierra del Montsant, Tarragona	Spain
16840	Herbivore dung	Poblet, Tarragona	Spain
16841	Herbivore dung	Els Ports natural park, Teruel	Spain
16844	Herbivore dung	Els Ports natural park, Teruel	Spain
17006	Herbivore dung	Els Ports natural park, Teruel	Spain
17071	Plant debris	Swamp of Siurana, Tarragona	Spain
17073	Bird dung	Arbolí, Tarragona	Spain
17102	Bird dung	Cornudella de Montsant, Tarragona	Spain
17103	Sheep dung	Ulldemolins, Tarragona	Spain
17259	Submerged plant debris	Cornudella del Montsant, Tarragona	Spain
17350	Herbivore dung	Els Ports natural park, Teruel	Spain
17370	Herbivore dung	Vall de Boí, Alta Ribagorça	Spain
17401	Rabbit dung	Riaza	Spain

		17710	Herbivore dung	Montblanc, Tarragona	Spain
C. michoacanense	*Sphaerospermum*	15914	Soil	Villa Jiménez, Michoacán	Mexico
		15932	Soil	Morelia, Michoacán	Mexico
C. parahalotolerans	*Sphaerospermum*	16624	Garden soil	Parc de la festa, Reus	Spain
C. perangustum	*Cladosporioides*	16095	Garden soil	Barcelona	Spain
		16194	Garden soil	Mediterranean balcony, Tarragona	Spain
		16206	Plant debris	Amphitheater park, Tarragona	Spain
		16210	Plant debris	Amphitheater park, Tarragona	Spain
		16240	Plant debris	Amphitheater park, Tarragona	Spain
		16286	Plant debris	Tarragona	Spain
		16388	Plant debris	Tarragona	Spain
		16589	Herbivore dung	Fageda costa Montseny, Barcelona	Spain
		16620	Plant debris	Amphitheater park, Tarragona	Spain
		16842	Herbivore dung	Costa del Montseny, Barcelona	Spain
		17588	Plant debris	Northest region	Vietnam
		17663	Plant debris	Northest region	Vietnam

C. phaenocomae	*Cladosporioides*	16588	Herbivore dung	Fageda, Costa del Montseny, Barcelona	Spain
		16846	Soil	Costa del Montseny, Barcelona	Spain
C. phyllophilum	*Cladosporioides*	16457	Herbivore dung	Poblet, Tarragona	Spain
		16459	Herbivore dung	Poblet, Tarragona	Spain
C. pseudocladosporioides	*Cladosporioides*	15894	Herbivore dung	Roque Nublo, The Canary Islands	Spain
		15901	Soil	Morelia, Michoacán	Mexico
		15972	Garden soil	Reus	Spain
		16093	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16094	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16100	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16102	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16208	Plant debris	Mediterranean balcony, Tarragona	Spain
		16222	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16254	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain

		16456	Herbivore dung	Alt Camp, Tarragona	Spain
		16585	Soil	Pontons, Barcelona	Spain
C. pseudotenellum	*Herbarum*	16231	Garden soil	Parc de la festa, Reus	Spain
C. ramotenellum	*Herbarum*	15811	Herbivore dung	Peraleda del Zaucejo, Extremadura	Spain
		15898	Herbivore dung	Peraleda del Zaucejo, Extremadura	Spain
		16089	Garden soil	Sitges, Barcelona	Spain
		16106	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16110	Soil	Castejón, Navarra	Spain
		16158	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
		16199	Plant debris	Tarragona	Spain
		16202	Garden soil	Tarraco Imperial square, Tarragona	Spain
		16212	Garden soil	Parc de la festa, Reus	Spain
		16218	Garden soil	Parc de la festa, Reus	Spain
		16219	Garden soil	Parc de la festa, Reus	Spain
		16228	Garden soil	Tarraco Imperial square, Tarragona	Spain
		16241	Garden soil	Tarraco Imperial square, Tarragona	Spain

		16244	Garden soil	Tarragona	Spain
		16248	Garden soil	Tarraco Imperial square, Tarragona	Spain
		16249	Plant debris	Tarragona	Spain
		16252	Plant debris	Tarragona	Spain
		16255	Plant debris	Tarragona	Spain
		16256	Garden soil	Tarragona	Spain
		16292	Garden soil	Parc Samà, Cambrils	Spain
		16537	Plant debris	Tarragona	Spain
		16652	Garden soil	Parc Samà, Cambrils	Spain
		16694	Plant debris	Els Ports natural park, Teruel	Spain
		16874	Garden soil	Reus	Spain
		17262	Plant debris	Arbolí, Tarragona	Spain
		17357	Herbivore dung	Els Ports natural park, Teruel	Spain
C. rugulovarians	*Cladosporioides*	15810	Herbivore dung	North coast, The Canary Islands	Spain
C. silenes	*Cladosporioides*	15895	Herbivore dung	Roque Nublo, The Canary Islands	Spain
		15896	Herbivore dung	Cruz de Tejeda, The Canary Islands	Spain
C. sinuosum	*Herbarum*	17005	Submerged plant debris	Road of Malafogassa, Barcelona	Spain

			17065	Plant debris	Arbolí, Tarragona	Spain
			17106	Submerged plant debris	Cornudella de Montsant, Tarragona	Spain
	C. sphaerospermum	*Sphaerospermum*	15892	Herbivore dung	Los Llanos de Aridane, The Canary Islands	Spain
			15913	Soil	Villa Jiménez, Michoacán	Mexico
			15965	Garden soil	Tarragona	Spain
			16097	Soil	Villa Jiménez, Michoacán	Mexico
			16195	Garden soil	Mediterranean balcony, Tarragona	Spain
			16196	Garden soil	Tarragona	Spain
			16198	Plant debris	Tarragona	Spain
			16215	Garden soil	Amphitheater park, Tarragona	Spain
			16216	Garden soil	Amphitheater park, Tarragona	Spain
			16220	Garden soil	Parc de la festa, Reus	Spain
			16225	Garden soil	Amphitheater park, Tarragona	Spain
			16246	Plant debris	Tarragona	Spain
			16289	Plant debris	Amphitheater park, Tarragona	Spain
			16291	Garden soil	Parc Samà, Cambrils	Spain

			16376	Plant debris	Tarragona	Spain
			16383	Garden soil	Parc Samà, Cambrils	Spain
			16450	Garden soil	Parc Samà, Cambrils	Spain
			16461	Garden soil	Parc Samà, Cambrils	Spain
			16530	Garden soil	Parc Samà, Cambrils	Spain
			16533	Garden soil	Parc Samà, Cambrils	Spain
			16581	Plant debris	El Miracle beach, Tarragona	Spain
			16651	Plant debris	Parc Samà, Cambrils	Spain
			16657	Garden soil	Parc Samà, Cambrils	Spain
			16684	Plant debris	Parc Samà, Cambrils	Spain
			16847	Herbivore dung	Vall Fosca	Spain
	C. submersum	*Herbarum*	17264	Submerged plant debris	Cornudella de Montsant, Tarragona	Spain
			17268	Submerged plant debris	Cornudella de Montsant, Tarragona	Spain
	C. subuliforme	*Cladosporioides*	15968	Garden soil	Campus URV, Tarragona	Spain
	C. tenellum	*Herbarum*	16107	Herbivore dung	Granja de Torrehermosa, Extremadura	Spain
			16232	Garden soil	Parc de la festa, Reus	Spain
			16460	Herbivore dung	Poblet, Tarragona	Spain
			16538	Herbivore dung	Alt Camp, Tarragona	Spain

			16623	Soil	El Miracle beach, Tarragona	Spain
			17359	Herbivore dung	Baells, Huesca	Spain
	C. tenuissimum	*Cladosporioides*	15916	Soil	Villa Jiménez, Michoacán	Mexico
			16391	Herbivore dung	Alt Camp, Tarragona	Spain
			17267	Plant debris	Arbolí, Tarragona	Spain
			17586	Plant debris	Northest region	Vietnam
			17713	Plant debris	Northest region	Vietnam
	C. velox	*Sphaerospermum*	15973	Garden soil	Reus	Spain
			15974	Garden soil	Reus	Spain
			16293	Garden soil	Parc Samà, Cambrils	Spain
	C. vicinum	*Cladosporioides*	15897	Herbivore dung	Pico de Bandama, The Canary Islands	Spain
			16200	Garden soil	Parc de la festa, Reus	Spain
			16203	Plant debris	Amphitheater park, Tarragona	Spain
			16209	Plant debris	Mediterranean balcony, Tarragona	Spain
			16227	Herbivore dung	The Canary Islands	Spain
			16650	Garden soil	Parc Samà, Cambrils	Spain
			16897	Herbivore dung	Els Ports natural park, Teruel	Spain
	C. westerdijkiae	*Cladosporioides*	16226	Garden soil	Tarragona	Spain

			16838	Herbivore dung	Alto Pirineo	Spain
	C. xylophilum	*Cladosporioides*	15801	Herbivore dung	Road Puerto de las Nieves, The Canary Islands	Spain
			15802	Herbivore dung	Tejeda, The Canary Islands	Spain
			15804	Herbivore dung	Caldera de Bandama, The Canary Islands	Spain
			15806	Herbivore dung	Anaga mountain, The Canary Islands	Spain
			16161	Herbivore dung	Road Puerto de las Nieves, The Canary Islands	Spain
			16223	Garden soil	Parc de la festa, Reus	Spain
			16379	Plant debris	Sierra del Montsant, Tarragona	Spain
			16382	Garden soil	Parc Samà, Cambrils	Spain
			16395	Garden soil	Parc Samà, Cambrils	Spain
			16452	Plant debris	Alt Camp, Tarragona	Spain
			16458	Herbivore dung	Poblet, Tarragona	Spain
			16655	Herbivore dung	Costa Montseny, Barcelona	Spain
			17007	Herbivore dung	Els Ports natural park, Teruel	Spain
			17712	Submerged plant debris	Mont-ral, Tarragona	Spain
	Cladosporium sp.	*Cladosporioides*	16656	Herbivore dung	Costa del Montseny, Barcelona	Spain

	Curvularia alcornii	–	17517	Plant debris	Northest region	Vietnam
	Cu. dactyloctenicola	–	17657	Plant debris	Northest region	Vietnam
		–	17658	Plant debris	Northest region	Vietnam
	Cu. eragrostidis	–	17661	Plant debris	Northest region	Vietnam
	Cu. geniculata	–	17515	Plant debris	Northest region	Vietnam
	Cu. hominis	–	17655	Plant debris	Northest region	Vietnam
		–	17660	Plant debris	Northest region	Vietnam
	Cu. inaequalis	–	18177	Bird dung	Aitona, Lérida	Spain
	Cu. oryzae	–	17516	Plant debris	Northest region	Vietnam
	Cu. paraverruculosa	–	17656	Soil	Villa Jiménez, Michoacán	Mexico
	Cu. vietnamensis	–	11956	Sorghum seed	Bogor, Java	Indonesia
			17659	Plant debris	Northest region	Vietnam
	Cyphellophora vietnamensis	–	17714	Unidentified dead leaves	Northest region	Vietnam
	Heliocephala variabilis	–	17592	Unidentified dead leaves	Northest region	Vietnam
	Matsushimaea monilioides	–	16505	Garden soil	Parc Samà, Cambrils	Spain
	Neodendryphiella mali	–	17003	Herbivore dung	Els Ports natural park, Teruel	Spain
		–	17524	Plant debris	Northest region	Vietnam
	N. michoacanensis	–	16098	Soil	Villa Jiménez, Michoacán	Mexico
	N. tarraconensis	–	16234	Garden soil	Amphitheater park,	Spain

				Tarragona	
Paradendryphiella arenaria	–	16400	Soil with sand	El Miracle beach, Tarragona	Spain
Pseudopenidiella gallaica	–	9234	Unidentified dead leaves	Natural Park of Monte Aloia, Galicia	Spain
P. vietnamensis	–	17593	Unidentified dead leaves	Northest region	Vietnam
Rachicladosporium cboliae	–	16294	Plant debris	Amphitheater park, Tarragona	Spain
Stemphylium vesicarium	–	16479	Soil	El Miracle beach, Tarragona	Spain
	–	16421	Soil	El Miracle beach, Tarragona	Spain
	–	17059	Herbivore dung	Arbolí, Tarragona	Spain
	–	17108	Plant debris	Arbolí, Tarragona	Spain
	–	16449	Plant debris	El Miracle beach, Tarragona	Spain
	–	17707	Herbivore dung	Montblanc, Tarragona	Spain
	–	17711	Herbivore dung	Mont-ral, Tarragona	Spain
	–	18173	Bird dung	Termens, Lérida	Spain
Venturia submersa	–	17405	Submerged plant debris	Hermitage of the eternal Father, Riaza	Spain

FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; URV: Universitat Rovira i Virgili.

Table 3. Type and reference strains received from the Westerdijk Fungal Biodiversity Institute (CBS).

Species	Received as	CBS num.	FMR num.	Substrate of origin	Country
Drechslera biseptata	*Dendryphiella vinosa*	117.14	16559	Soil	Scotland
Torula herbarum	*Dendryphiella infuscans*	381.81	16560	*Epilobium hirsutum* dead stem	The Netherlands
Dendryphiella paravinosa	*Dendryphiella vinosa*	118716	16562	*Geniostoma rupestre* var. *ligustrifolium* leaf lesions	New Zealand
Dendryphiella paravinosa	*Dendryphiella vinosa*	121797	9235	Plant debris	Spain
Neodendryphiella mali	*Diplococcium asperum*	139.95	16561	Apple leaf	Italy
Dendryphiella variabilis	*Dendryphiella vinosa*	584.96	16563	Dead leaf of Lauraceous tree	Cuba
Matsushimaea fasciculata	*Matsushimaea fasciculata*	167.97	16826	*Quercus ilex* leaf litter	Spain

FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain.

Table 4. Genetic markers and primers used in the different studies included in the present thesis.

Genera	Loci	Primers			References
		Sense	Name	Sequence (5'-3')	
Alternaria	***ATPase***	Forward	ATPDF1	ATCGTCTCCATGACCGAGTTCG	Lawrence et al. (2013)
		Reverse	ATPDR1	TCCGATGGAGTTCATGATAGCC	
	gapdh	Forward	gpd1	CAACGGCTTCGGTCGCATTG	Berbee et al. (1999)
		Reverse	gpd2	GCCAAGCAGTTGGTTGTGC	
	ITS	Forward	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
		Reverse	ITS4	TCCTCCGCTTATTGATATGC	
	rpb2	Forward	RPB2-5F2	GGGGWGAYCAGAAGAAGGC	Sung et al. (2007), Liu et al. (1999)
		Reverse	fRPB2-7cR	CCC ATR GCT TGY TTR CCC AT	
	***tef*1**	Forward	EF-728F	CATCGAGAAGTTCGAGAAGG	Carbone & Kohn (1999)
		Reverse	EF-986R	TACTTGAAGGAACCCTTACC	
Cladosporium	***act***	Forward	ACT-512F	ATGTGCAAGGCCGGTTTCGC	Carbone & Kohn (1999)
		Reverse	ACT-783R	TACGAGTCCTTCTGGCCCAT	
	ITS	Forward	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
		Reverse	ITS4	TCCTCCGCTTATTGATATGC	
	***tef*1**	Forward	EF-728F	CATCGAGAAGTTCGAGAAGG	Carbone & Kohn (1999)
		Reverse	EF-986R	TACTTGAAGGAACCCTTACC	
Curvularia	***gapdh***	Forward	gpd1	CAACGGCTTCGGTCGCATTG	Berbee et al. (1999)
		Reverse	gpd2	GCCAAGCAGTTGGTTGTGC	
	ITS	Forward	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
		Reverse	ITS4	TCCTCCGCTTATTGATATGC	
	***tef*1**	Forward	EF1-983	GCYCCYGGHCAYCGTGAYTTYAT	Schoch et al. (2009)
		Reverse	EF1-2218R	ATGACACCRACRGCRACRGTYTG	
Other dematiaceous genera	**ITS**	Forward	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
		Reverse	ITS4	TCCTCCGCTTATTGATATGC	
	LSU	Forward	NL1	GCATATCAATAAGCGGAGGAAAA	O'Donnell (1993)
		Reverse	NL4b	GGTCCGTGTTTCAAGACGG	

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

4. RESULTS

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

The fungi studied in the present thesis were preliminarily identified based on morphological features and later confirmed by sequence analysis of at least one informative phylogenetical marker.

As mentioned before, our study on the genus *Alternaria* was focused mainly on the species of section *Infectoriae*, since it is a poorly taxonomic characterized section that includes a few species described as causal agents of human infections. Of 54 clinical isolates presumptively identified, after molecular identification with the *rpb2* marker, 50 (92.6 %) were confirmed as members of that section, two (3.7 %) to section *Chalastospora* and other two (3.7 %) were included in section *Pseudoalternaria*, these two later sections being rarely reported from a clinical setting. All these isolates were also characterized by a multi-locus sequence analysis; however, only nine could be identified as known species of *Alternaria*; i.e., *A. arbusti* (2) and *A. oregonensis* (4) in section *Infectoriae*, *A. kordkuyana* (1) and *A. rosae* (1) in section *Pseudoalternaria*, and *A. malorum* (1) in section *Chalastospora*. Of the 45 isolates that did not match with any previously described *Alternaria* species, six were related to section *Infectoriae*, which were characterized and proposed as the new species *A. anthropophila*, *A. atrobrunnea* and *A. guarroi*. Of interest is that these three novel *Alternaria* were demonstrated to be causal agents of human cutaneous infection (see section 4.1.1 of the present thesis).

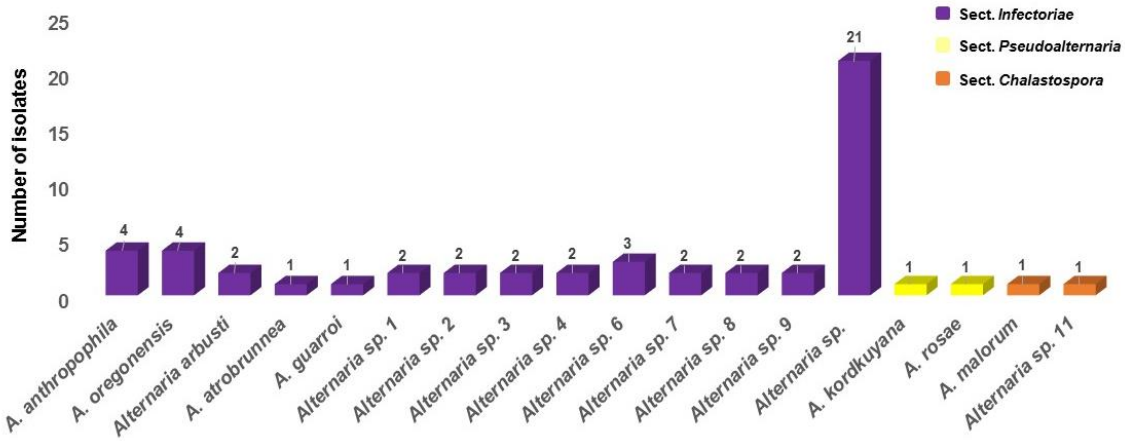

Figure 7. Diversity of *Alternaria* species identified from clinical sources

The other 39 unidentified isolates are still under study, although preliminary morphological and molecular results suggest that 18 of them are representatives of nine putative novel species (*Alternaria* sp. 1-4, 6-9 and 11), eight in section *Infectoriae*

and one in section *Chalastospora*. Details of those preliminary results are included in section 4.1.3. The species diversity of the clinical isolates of *Alternaria* is in Figure 7.

Among alternaria-like fungi isolated from environmental sources, 212 (96.4 %) were identified as belonging to *Alternaria* and eight to *Stemphylium vesicarium* (3.6 %) (Table 2). *Alternaria* isolates were distributed into seven sections, *Alternaria* (130 isolates, 61.3 %), *Ulocladium* (24 isolates, 11.3 %), *Ulocladioides* (23 isolates, 10.9 %) *Infectoriae* (19 isolates, 8.9 %), *Pseudoalternaria* (11 isolates, 5.2 %), *Chalastospora* (4 isolates, 1.9 %) and *Radicina* (1 isolate, 0.5 %) (Fig. 8).

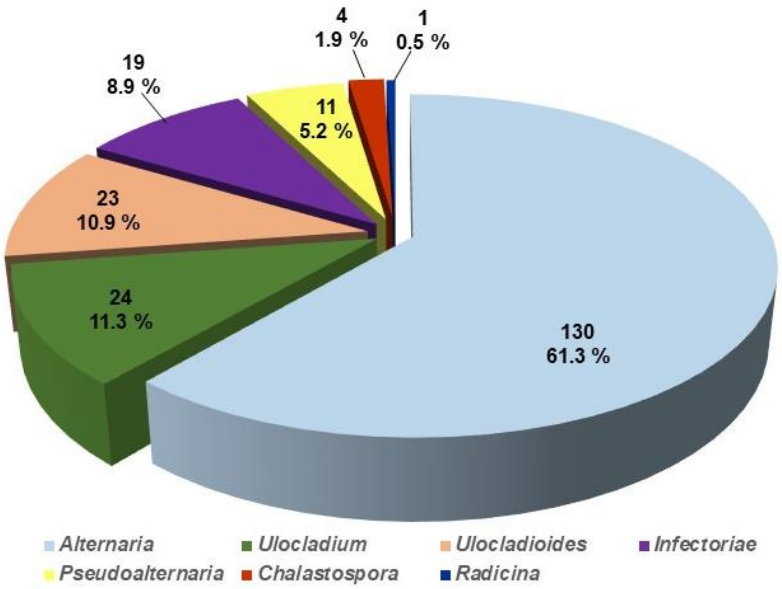

Figure 8. Distribution by section of the environmental isolates of *Alternaria*.

A total of 27 species of *Alternaria* were identified from environmental sources (Fig. 9), although two of them are not yet formally proposed (*Alternaria* sp. 5 and 10). The most frequent species was *A. alternata* with 115 isolates (54.2 %), followed by *A. botrytis* with 24 isolates (11.3 %) and *A. arborescens* with 14 isolates (6.6 %).

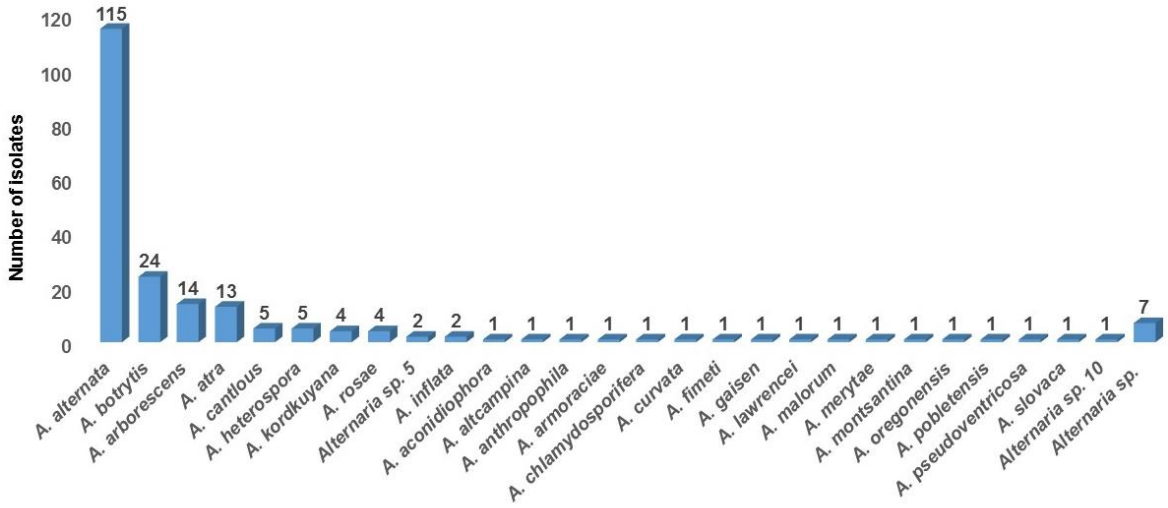

Figure 9. Diversity of *Alternaria* species identified from environmental sources.

The most frequently species identified in the section *Alternaria* was *A. alternata* with 115 isolates (88.5 %), followed by *A. arborescens* with 14 isolates (10.8 %) and *A. gaisen* with one isolate (0.7 %). In the section *Ulocladium* only *A. botrytis* was identified with 24 isolates (100 %). In the section *Ulocladioides* three species were identified, *A. atra* being the most prevalent with 13 isolates (56.6 %), followed by *A. cantlous* with five isolates (21.7 %), and *A. heterospora* with five isolates (21.7 %). In the section *Infectoriae* only three species were identified, *A. merytae*, *A. oregonensis* and *A. slovaca* with one isolate each, while 16 isolates (84.2 %) could not be identified. In the section *Pseudoalternaria* the most frequently identified species was *A. kordkuyana* with four isolates (36.4 %) and *A. rosae* with also four isolates (36.4 %), but three did not match with any known species in that section. Of the four isolates of the section *Chalastospora*, two were identified as *A. armoraciae* and *A. malorum*, respectively, and the other two could not be identified. The only isolate of the section *Radicina*, could not be identified.

The 22 above-mentioned unidentified *Alternaria* isolates were therefore characterized by multi-locus analyses. Results of the polyphasic approach allowed to delineate 11 of them as representatives of 10 new species, *A. aconidiophora*, *A. altcampina*, *A. chlamydosporifera*, *A. curvata*, *A. fimeti*, *A. inflata*, *A. lawrencei*, *A. montsantina*, *A. pobletensis*, *A. pseudoventricosa*, which have already been published in the article included in section 4.1.2. In addition, one isolate was identified as *A. anthropophila*, a new species published in section 4.1.1 of the present thesis, and the other 10 isolates of *Alternaria* not identified are morphological and molecularly characterized in section 4.1.3. The diversity of *Alternaria* species identified from environmental sources in the present thesis is illustrated in Figure 10.

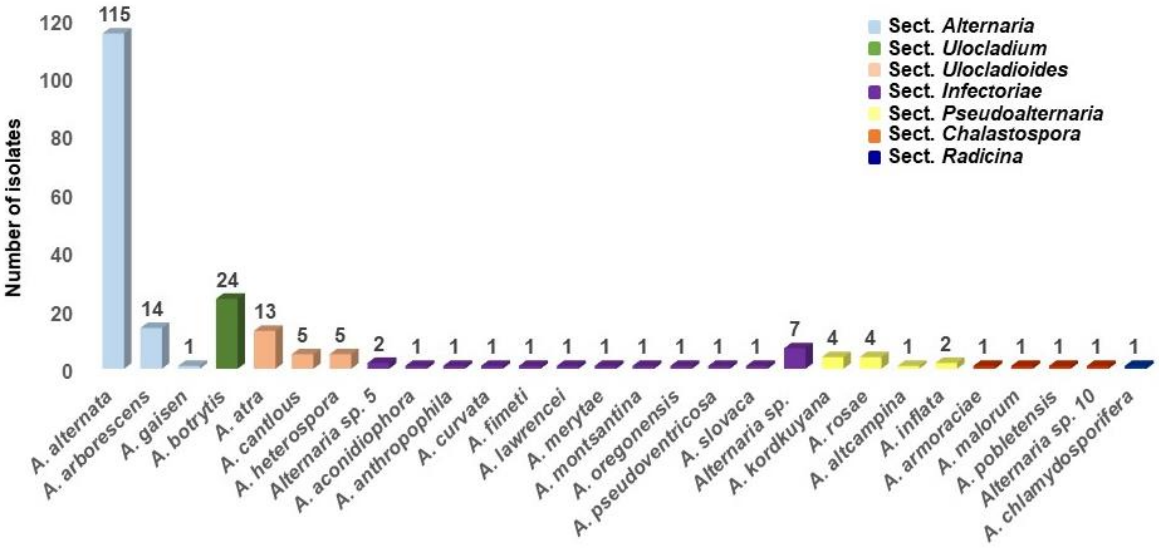

Figure 10. Diversity of *Alternaria* species identified from environmental sources according to the section.

Comparative results between the isolates of *Alternaria* collected on herbivore dung, soil and plant debris samples revealed that the first was the substrate with a higher diversity. From herbivore dung samples a total of 23 species were identified, *A. alternata* being the most prevalent species isolated, followed by *A. botrytis*, *A. atra* and *A. arborescens;* nine of them were described as new (*A. anthropophila, A. altcampina, A. chlamydosporifera, A. curvata, C. fimeti, A. inflata, A. lawrencei, A. pobletensis,* and *A. pseudoventricosa*). In both soil and plant debris samples, we identified only six species in each, *A. alternata* being the most frequent. The following species more isolated were: *A. botrytis*, *A. arborescens*, *A. atra*, *A. cantlous* and *A. heterospora* in soil samples, and *A. arborescens*, *A. kordkuyana*, *A. aconidiophora, A. atra* and *A. montsantina* in plant debris samples (Fig. 11).

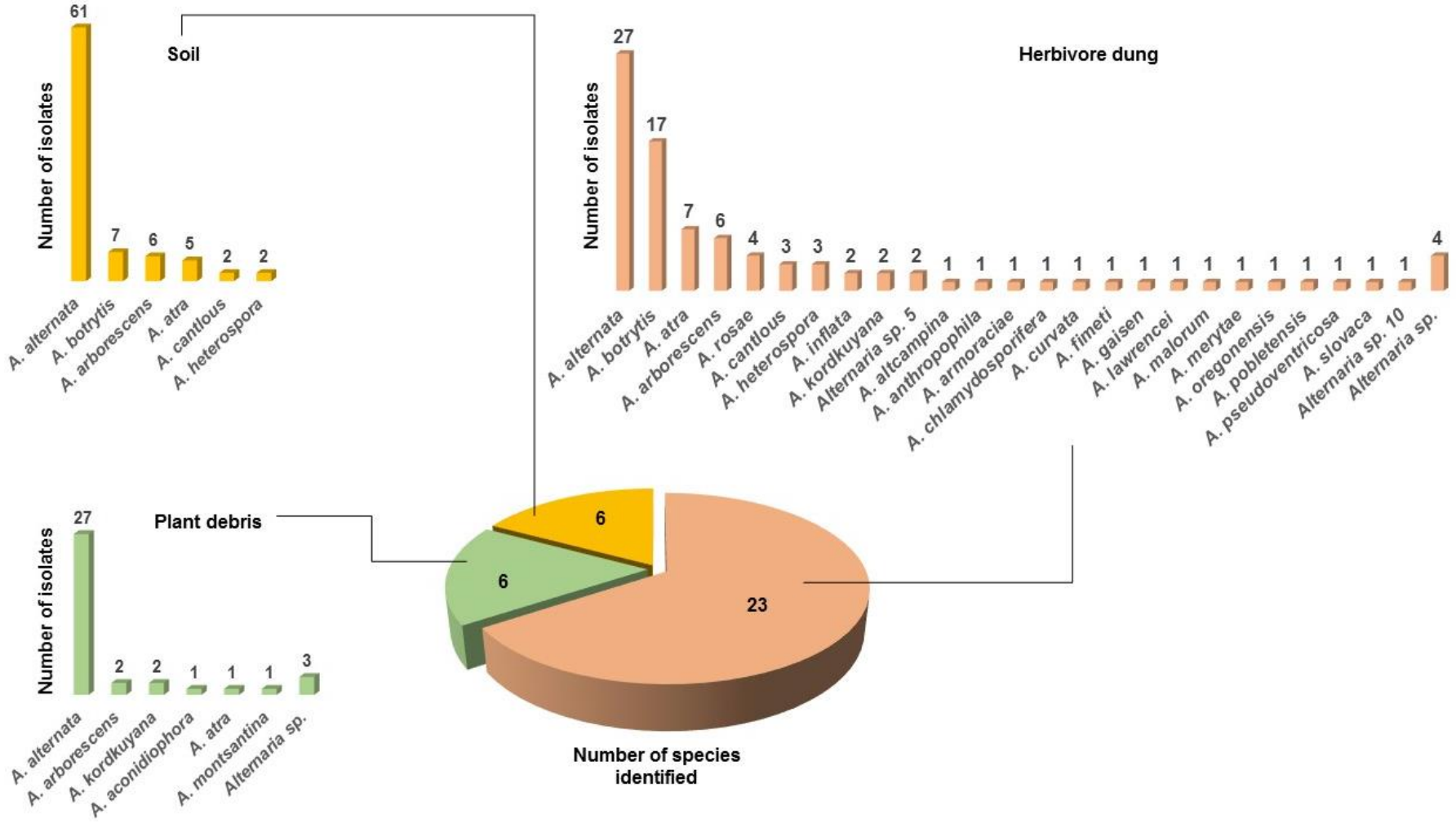

Figure 11. Diversity of *Alternaria* species identified according to the substrate of origin.

Of the total environmental cladosporium-like isolates identified, 287 belonged to the genus *Cladosporium* and 7 were identified as species of other cladosporium-like genera (i.e. *Apenidiella*, *Cladophialophora*, *Matsushimaea*, *Pseudopenidiella*, *Rachicladosporium*, *Venturia*) (Table 2). Five of these isolates identified as belonging to the cladosporium-like genera were proposed as new species, i.e. *Apenidiella foetida* (section 4.2.3), *Matsushimaea monilioides* (section 4.2.4), *Pseudopenidiella gallaica* and *P. vietnamensis* (sections 4.2.5 and 4.3.2 respectively) and *Venturia submersa* (section 4.2.6).

The *Cladosporium* s. str. Isolates were distributed among the three species complexes of the genus, i.e. 144 isolates (50.2 %) belonging to the *C. cladosporioides* complex, 92 isolates (32.1 %) to *C. herbarum* complex and 51 (17.7 %) to *C. sphaerospermum* complex (Fig. 12).

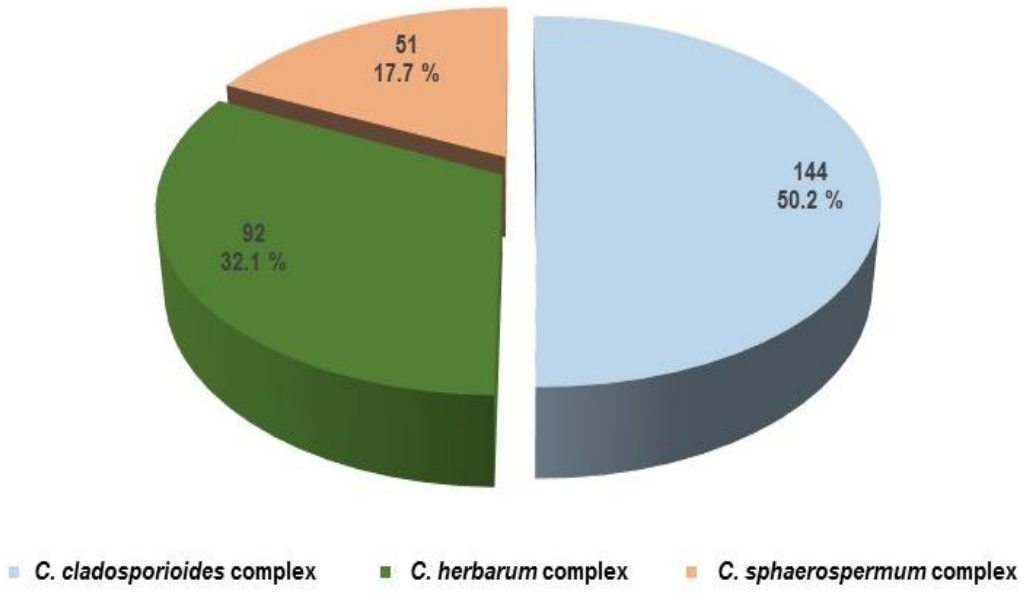

Figure 12. Distribution of the *Cladosporium* isolates in the species complex of the genus.

A total of 44 species of *Cladosporium* were identified from environmental sources. The most frequently isolated was *C. cladosporioides* with 50 isolates (17.4 %), followed by *C. ramotenellum* with 26 isolates (9.1 %), *C. sphaerospermum* with 25 isolates (8.7 %), *C. macrocarpum* with 18 isolates (6.3 %) and *C. floccosum* with 16 isolates (5.6 %) (Fig. 13).

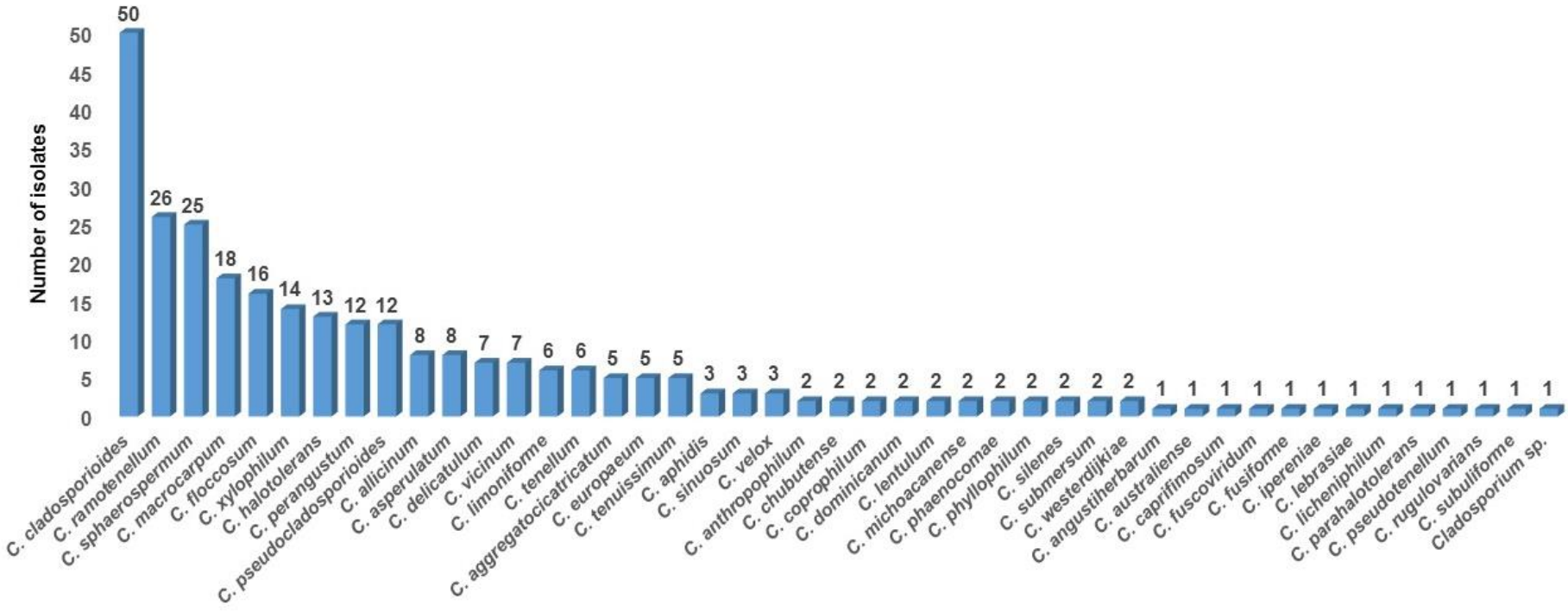

Figure 13. Diversity of *Cladosporium* species identified from environmental sources.

A total of 24 species of *Cladosporium* of the *C. cladosporioides* complex were identified, the most frequent was *C. cladosporioides* with 50 isolates (34.7 %), followed by *C. xylophilum* with 14 isolates (9.7 %), *C. perangustum* with 12 isolates (8.3 %), *C. pseudocladosporioides* with 12 isolates (8.3 %) and *C. asperulatum* with 8 isolates (5.5 %). In *C. herbarum* complex there were identified a total of 11 species, *C. ramotenellum* with 26 isolates (28.3 %) being the most frequently isolated, followed by *C. macrocarpum* with 18 isolates (19.6 %), *C. floccosum* with 16 isolates (17.4 %), *C. allicinum* with 8 isolates (8.7 %), *C. limoniforme* with 6 isolates (6.5 %) and *C. tenellum* with 6 isolates (6.5 %); and in *C. sphaerospermum* complex there were identified a total of 9 species, with *C. sphaerospermum* the most prevalent, with 25 isolates (49 %), followed by *C. halotolerans* with 13 isolates (25.5 %), *C. aphidis* with 3 isolates (5.9 %) and *C. velox* with 3 isolates (5.9 %). However, 10 isolates did not match with either of the accepted species in *Cladosporium* and they were selected for multi-locus sequence analysis for their final identification. The polyphasic approach of these interesting isolates allowed us to propose seven new species described in two publications (sections 4.2.1 and 4.2.2). The spectrum of species diversity in *Cladosporium* is represented in Figure 14.

Comparative results between the isolates of *Cladosporium* collected on herbivore dung, soil and plant debris samples revealed that the first two types of substrates showed a similar and higher diversity of species than the diversity found on plant material. From herbivore dung a total of 29 species were identified, those of *C. cladosporioides* with 19 isolates (17.9 %) being the most frequent, followed by *C. macrocarpum* with 14 isolates (13.2 %), *C. pseudocladosporioides* with 8 isolates (7.5 %), *C. xylophilum* with 8 isolates (7.5 %) and *C. floccosum* with 6 isolates (5.7 %). In soil samples were identified 28 species, with *C. cladosporioides* being the most prevalent with 24 isolates (21.8 %), followed by *C. sphaerospermum* with 16 isolates (14.5 %), *C. ramotenellum* with 14 isolates (12.7 %), *C. halotolerans* with 13 isolates (11.8 %) and *C. limoniforme* with 4 isolates (3.6 %). From plant debris there was identified a total of 22 taxa, with *C. floccosum* with 8 isolates (11.3 %) and *C. perangustum* with 8 isolates (11.3 %) being the two most frequent, followed by *C. cladosporioides* with 7 isolates (9.9 %), *C. ramotenellum* with 7 isolates (9.9 %) and *C. sphaerospermum* with 7 isolates (9.9 %) (Fig. 15).

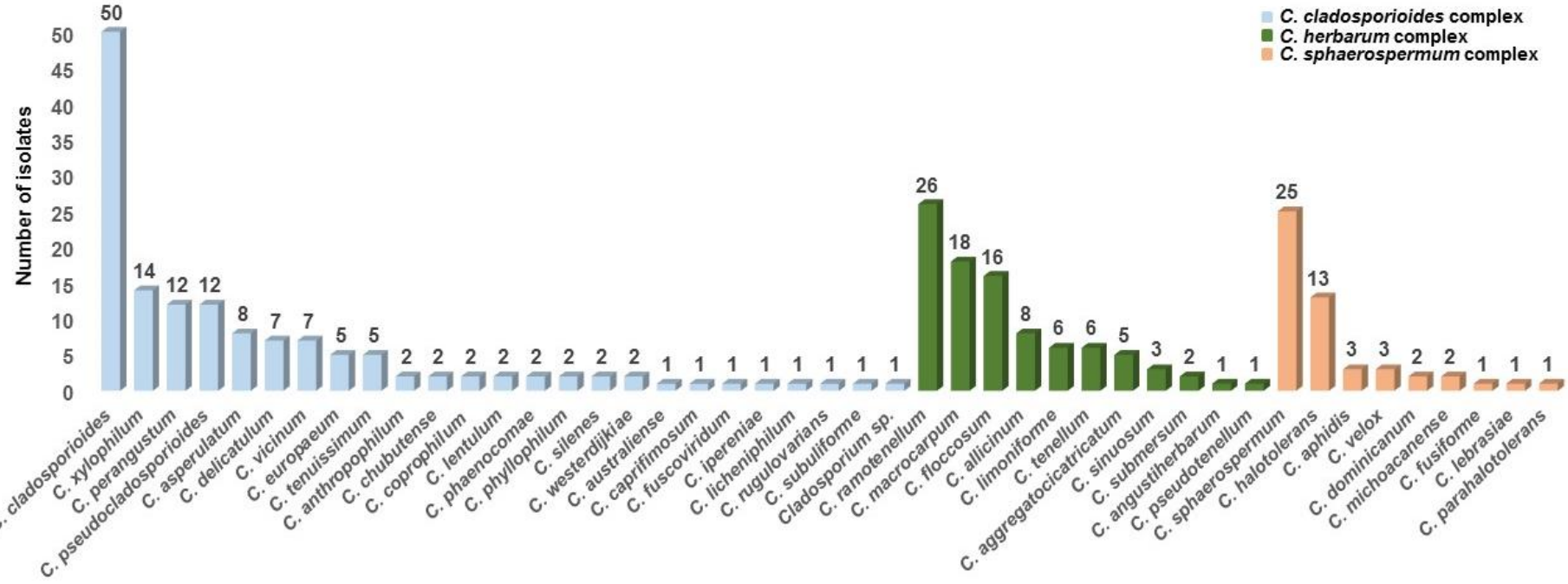

Figure 14. Diversity of *Cladosporium* species identified from environmental sources according to the species complex.

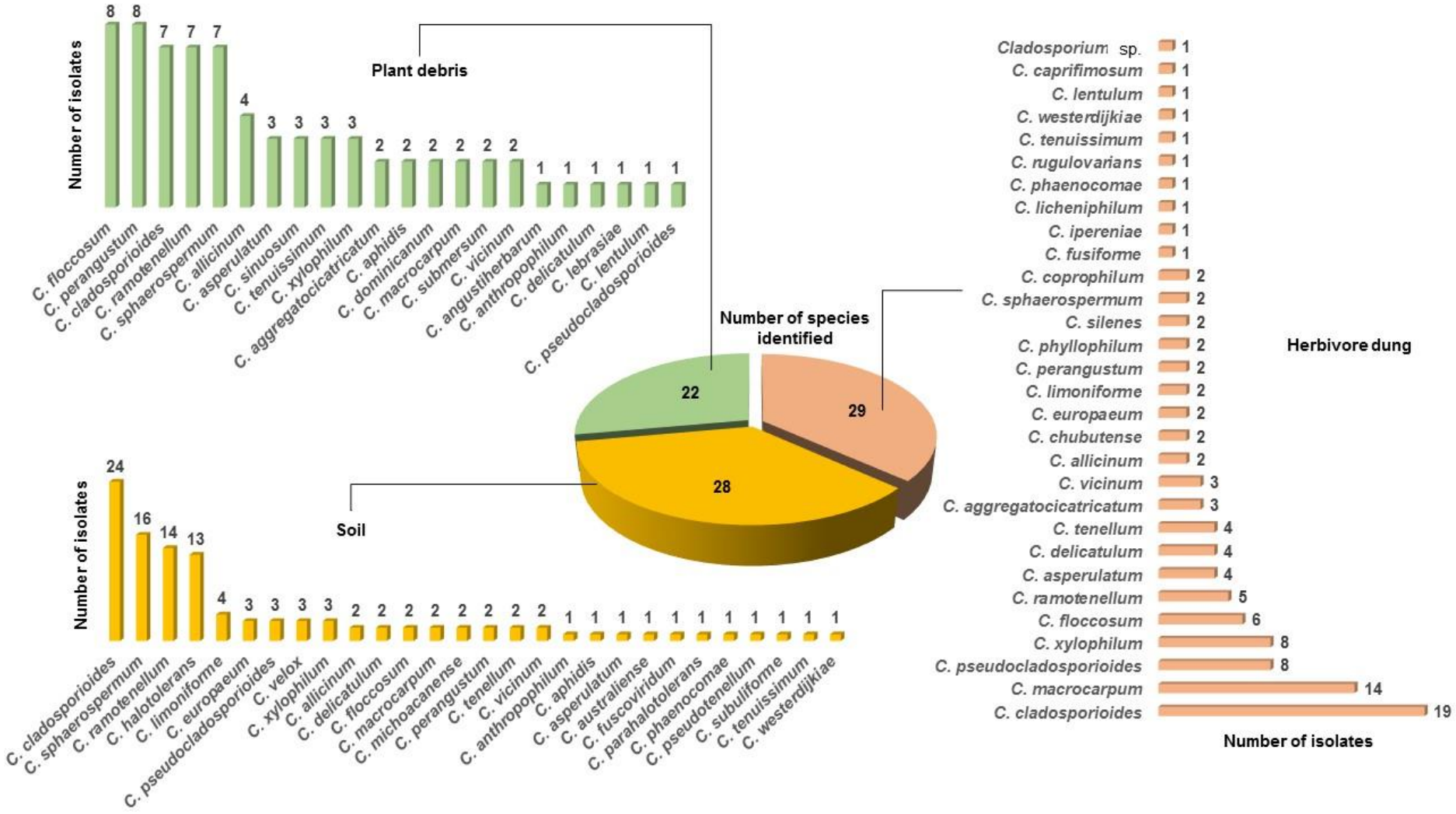

Figure 15. Diversity of *Cladosporium* species identified according to the substrate of origin.

Regarding isolates of other dematiaceous genera studied in the present thesis, there are included fungi belonging to *Curvularia*, *Dendryphiella, Paradendryphiella* and the new proposed genus *Neodendryphiella,* four pleosporalean genera that produce conidia from mono- or polytretic conidiogenous cells (poroconidia), and isolates delineated as new species of *Cyphellophora* and *Heliocephala*, two hyphomycetous genera that produce conidia from phialidic conidiogenous cells (phialoconidia). All these fungi are characterized and formaly proposed in the section 4.3 of the present thesis.

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

4.1 Results on the genus *Alternaria*

4.1.1 Polyphasic identification of three new species in *Alternaria* section *Infectoriae* causing human cutaneous infection.

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Published in: Mycoses 2019; 63(2): 212–224.

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Received: 23 July 2019 | Accepted: 9 October 2019
DOI: 10.1111/myc.13026

ORIGINAL ARTICLE

mycoses Diagnosis, Therapy and Prophylaxis of Fungal Diseases WILEY

Polyphasic identification of three new species in *Alternaria* section *Infectoriae* causing human cutaneous infection

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Summary

Background: Cutaneous phaeohyphomycosis is an emerging disease in immunocompromised patients, being *Alternaria* one of the most common genera reported as a causative agent. Species identification is not carried out mainly due to the complexity of the genus. Analysis of the ITS barcode has become standard for fungal identification, but in *Alternaria* it is only able to discriminate among species-groups or sections.

Methods: We present three cases of cutaneous infection caused by *Alternaria* isolates morphologically identified as belonging to section *Infectoriae*. They have been morphologically characterised and phylogenetically delineated with five molecular markers (ITS, *ATPase*, *gapdh*, *rpb2* and *tef1*).

Results: Mycotic infections have been diagnosed by repeated cultures and histopathological examination in two of the cases. The polyphasic approach has allowed to delineate three new species of *Alternaria* section *Infectoriae*, that is *A anthropophila*, *A atrobrunnea* and *A guarroi*. *ATPase* has been the only locus able to discriminate most of the species (29 out of 31) currently sequenced in this section, including *A infectoria* the commonest reported species causing alternariosis. Susceptibility test showed different antifungal patterns for the three species, although terbinafine was the most active in vitro drug against these fungi.

Conclusions: The *ATPase* gene is recommended as an alternative barcode locus to identify *Alternaria* clinical isolates in section *Infectoriae*. Our results reinforce the relevance of identification of *Alternaria* isolates at the species level and the necessity to carry out antifungal susceptibility testing to determine the most adequate drug for treatment.

KEYWORDS
Alternaria infectoria, alternariosis, antifungal susceptibility, immunocompromised patients, molecular identification, opportunistic infections, *Pleosporaceae*, taxonomy

1 | INTRODUCTION

Alternaria is a cosmopolitan dematiaceous genus, whose species are mostly saprophytic, but also endophytic and phytopathogenic, generating important damages in diverse agronomic products.[1,2] In the last decades, it also emerges as an important human opportunistic pathogenic mould especially affecting immunocompromised patients and commonly causing cutaneous and subcutaneous infections, but cases of rhinosinusitis, oculomycosis, onychomycosis and invasive disease have been also reported.[3-6] The increasing incidence of alternariosis in immunosuppressed population is mainly due to transplants (bone marrow or solid organ transplant), to patients with cancer treatments or to primary or acquired immunodeficiency.[3,4,7-9] In absence of any standard guidance, multiple therapeutic options have been used for these infections, including thermotherapy,[10] but itraconazole (ITC) has been the antifungal therapy most frequently used and, generally, with a satisfactory outcome.[3,4,11] However, posaconazole (PSC) is currently chosen as a better option since it usually has lower minimal inhibitory concentration (MIC) values, a better body tissues distribution and less drug interactions than ITC.[4,12]

Although the most commonly reported species are *A alternata*, *A infectoria*, *A tennuissima* and *A chartarum*, it is of note that species identification is not performed in practically half of the cases of alternariosis reported.[3,4] This is probably due to the great number of species described in the genus and to the difficulties in the interpretation of the morphological features of its isolates, which often lead to incorrect identification.[3] In particular, the morphological identification of *A infectoria* is problematic mainly due to the scarcity or lack of sporulation, producing white or nearly white colonies, mainly when grown on nutrient-rich media.[13,14] Therefore, alternative methods, such as DNA sequencing, are necessary for the correct identification of *Alternaria* species. In this sense, re-identification of *Alternaria* clinical isolates by sequence analysis highlights that *A infectoria*, rather than *A alternata*, is the most frequently identified species, at least as causative agent of cutaneous alternariosis.[4,15,16]

Several molecular taxonomic studies have contributed to stablish the modern concept of *Alternaria*. The genus is divided into 27 monophyletic subgeneric groups, called sections, and comprises about 280 accepted species.[17-21] Although the internal transcribed spacers (ITS) of the nuclear ribosomal DNA (rDNA) has been proposed as an universal barcode for fungal classification,[22] it shows a low discriminatory power for distinguishing closely related *Alternaria* species due to the limited numbers of informative sites.[2,17,19] In fact, *Alternaria* identification based on the ITS barcode is limited to species complex or section, being therefore required a multilocus sequence analysis for the species delimitation of these fungi.[2,17,21] Unlike other genera of human opportunists such as *Aspergillus* and *Penicillium*, standard methodology for molecular identification of *Alternaria* species has not been yet stablished, even combinations of different genes are used depending on the *Alternaria* section studied.[2,21,23] In the case of the section *Infectoriae*, the combination of ITS, RNA polymerase second largest subunit (*rpb2*), glyceraldehyde 3-phosphate dehydrogenase (*gapdh*), translation elongation factor 1-alpha (*tef1*) or plasma membrane ATPase (*ATPase*) have been used for species recognition.[17,20,21,24] Currently, this section comprises 36 species, of which only three have been described as opportunistic pathogenic species, that is *A infectoria*, *A caespitosa* and *A triticina*.[3,25]

Herein, we present three cases with skin and soft tissue infection due to *Alternaria* in immunocompromised patients, who were admitted in 2017 at the Sant Joan Universitary Hospital in Reus, Spain. The isolates from the three cases showed the typical morphological features of those species of *Alternaria* section *Infectoriae* (ie short primary conidiophores with one or several conidiogenous loci, and small conidia with few longitudinal septa forming branched chains). Based on the multilocus sequence analysis of five genes and on morphological data, the three isolates have been recognised as new species in the genus, which extend the list of *Alternaria* species able to cause human infection. The in vitro susceptibility profile against eight antifungal drugs is provided for the three fungi.

1.1 | Case reports

1.1.1 | Case 1

A 46-year-old male, with pulmonary transplantation 2 years earlier and under immunosuppressive therapy (tacrolimus 2.5 mg/12 h and mycophenolate mofetil 500 mg/12 h), presented to us with a painless nodule on his right lower extremity. A punch biopsy on the nodule was carried out for histopathological study and cultured following the recommendations of the Spanish Society of Infectious Diseases and Clinical Microbiology.[26] The haematoxylin and eosin (H&E) stain (Figure 1A) showed an important inflammatory granulomatous lesion with suppurative reaction. Grocott's methenamine silver (GMS) stain revealed abundant septate, hyphal elements in

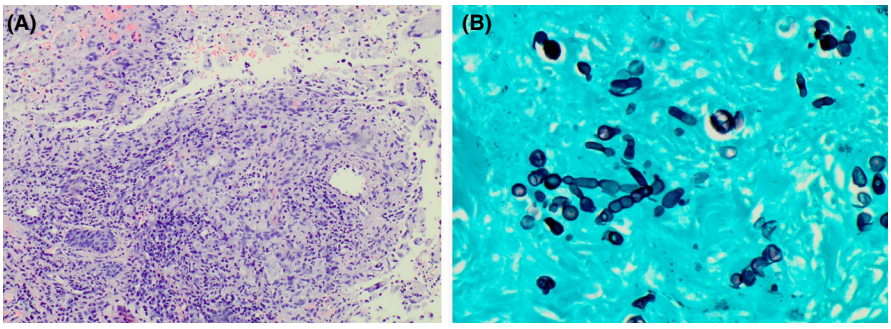

FIGURE 1 Histological section of the skin biopsy from case 1. Haematoxylin and eosin (H&E) staining showing an inflammatory granulomatous lesion (A); Grocott's methenamine silver (GMS) staining revealing septate hyphae (B). (original magnifications: H&E ×100, GMS ×400)

dermis and hypodermis (Figure 1B). After 72 h of incubation at 25 and 37°C on Sabouraud dextrose agar (SAB) supplemented with gentamicin and chloramphenicol (BioMérieux SA), tissue cultures formed numerous colonies of a melanised filamentous fungus. Cultures were negative for bacteria. On SAB, the sporulation was scarce, but morphological features of the few conidia produced allowed us to identify the fungus as *Alternaria* sp. The patient was treated with topical applications of voriconazole (VRC) for 20 days showing clinical improvement, with the subsequent exeresis of the nodule. At the 12-month follow-up visit, there was no relapse.

1.1.2 | Case 2

A 73-year-old male, with a clinical history of prostate adenocarcinoma in stage IV and receiving radiotherapy treatment, was admitted to the hospital due to a clinical condition compatible with sepsis. Several weeks earlier, he had successfully been treated with cloxacillin (1 g/6 h iv for 10 days) due to positive cultures for *Staphylococcus aureus* from an infected lesion on the right index that advanced to cellulitis. Three days after admission, the patient presented multiple skin ulcers on both lower extremities at the knees level. Skin biopsies from each knee were obtained and the histopathological analysis with H&E and GMS stains revealed in both biopsies abundant septate hyphae in dermis and hypodermis with a suppurative granulomatous inflammation similar to that observed in the first case (Figure 1). Cultures from the tissue biopsied on SAB at 25 and 37°C yielded colonies of a melanised filamentous fungus after 72 h of incubation, whose micromorphological features matched *Alternaria* sp. Bacteriological cultures were negative. The treatment was initiated with liposomal amphotericin B (LAMB) 2 mg/kg/24 h iv by 10 days, but it was replaced by VRC due to the side effects of the first drug. The VRC was administered intravenously 6 mg/kg/12 h at the first day followed by 4 mg/kg/12 h until complete 7 days. The patient evolved favourably with healing of the skin lesions and was discharged from the hospital with no apparent systemic signs of infection. He died 12 months after the diagnosis of alternariosis due to unrelated causes, with no recurrence of the fungal lesions.

1.1.3 | Case 3

A 71-year-old female was referred to the hospital due to a heart failure. She presented a multi-pathological clinical history, which consisted in arterial hypertension, diabetes mellitus, renal failure, with a chronic ischaemic heart disease history, severe and diffuse three-vessel disease and dilated myocardiopathy of ischaemic origin. On examination, the patient displayed numerous skin ulcerative lesions in her lower extremities. Exudate samples from different ulcers were cultured and colonies of a melanised filamentous fungus compatible with *Alternaria* were obtained on SAB after 72 h of incubation at 25 and 37°C. The ulcers improved with antibiotic treatment and nursing cures without specific treatment against *Alternaria*. One month later, the patient evolved with cardiogenic shock, multi-organ failure and *exitus*, but without a complete resolution of her cutaneous infection.

The three patients presented anaemia and thrombocytopenia at the time of diagnosis, only the first patient had acute renal failure and the last one had cholestasis. Clinical findings of the three patients at the admission are summarised in Table 1.

One fungal isolate from each case was send to the Mycology Unit of the University Rovira i Virgili (Spain) for species identification and antifungal susceptibility testing. The isolates were deposited as FMR 16235 (case 1), FMR 16556 (case 2) and FMR 16868 (case 3), and all of them were morphologically identified as *Alternaria* sp from section *Infectoriae*.

2 | MATERIALS AND METHODS

2.1 | DNA extraction and sequencing

We selected five phylogenetic markers used in previous molecular studies on *Alternaria* identification.[17,20,21] These were the complete ITS1-5.8SrDNA-ITS2 fragment, and fragments of the genes *rpb*2, *gapdh*, *tef*1 and *ATPase*. The DNA was extracted from strains cultured on potato dextrose agar (PDA; Pronadisa) after 7 days of incubation at 25°C in the dark, using the modified protocol of Müller et al.[27] The

TABLE 1 Clinical findings, treatment and outcome of the three reported cases

				Haematological conditions					
Case	Age/gender	Clinical history	Clinical presentation	Haemoglobin (g/dL)	Leucocytes (×10^3/uL)	Platelets (×10^3/uL)	Creatinine (mg/dL)	Primary therapy	Outcome
1	46/M	Pulmonary transplant (2 y prior), under immunosuppressive treatment	Solitary, nodule, right leg	12.7	6.27	120	1.46	VRC + surgery	Cure, no relapse
2	73/M	Prostate adenocarcinoma stage IV, under radiotherapy treatment	Multiple skin ulcers on knees	9.8	5.5	109	0.40	LAMB, replaced with VRC	Cure, no relapse (dead other reasons)
3	71/F	Hypertension, diabetes mellitus, renal failure, chronic ischaemic heart disease, polytreatment	Multiple ulcerative lesions in legs	10.8	5.8	83	0.74	Antibiotic, nursing cures	Improved (dead other reasons)

TABLE 2 *Alternaria* species included in the phylogenetic study and their respective origin and GenBank accession number

Species	Strains	Substrate	Locality	GenBank accession numbers[a]				
				ITS	*gapdh*	*ATPase*	*tef*1	*rpb2*
Alternaria abundans	CBS 534.83 (T)	*Fragaria* sp	New Zealand	JN383485	KC584154	JQ671802	KC584707	KC584448
A alternarina	CBS 119396 (T)	*Avena sativa*	USA/Wisconsin	JQ693648	JQ646289	JQ671817	**LR134367**	JQ905199
A anthropophila	FMR 16235 (T)	Human subcutaneous nodule	Spain/Catalonia	**LR537444**	**LR537034**	**LR537052**	**LR537046**	**LR537040**
	FMR 17278	Human skin biopsy	Spain/Aragón	**LR537030**	**LR537035**	**LR537054**	**LR537048**	**LR537042**
	FMR 17288	Human subcutaneous nodule	Spain/Galicia	**LR537032**	**LR537038**	**LR537055**	**LR537049**	**LR537043**
	FMR 17296	Pericardial liquid	Spain/Cantabria	**LR537445**	**LR537036**	**LR537053**	**LR537047**	**LR537041**
A arbusti	CBS 596.93 (T)	*Pyrus pyrifolia*	USA/California	JQ693644	JQ646365	JQ671940	FJ214902	**LR134184**
A armoraciae	CBS 118702 (T)	*Armoracia rusticana*	New Zealand	KC584182	KC584099	**LR134098**	KC584638	KC584379
A atrobrunnea	FMR 16868 (T)	Human ulcerative skin lesion	Spain/Catalonia	**LR537033**	**LR537039**	**LR537057**	**LR537051**	**LR537044**
A broccoli-italicae	CBS 118485 (T)	*Brassica oleracea* var *italica*	Australia	KM821536	KM821538	KY412557	**LR134262**	**LR134194**
A caespitosa	CBS 177.80 (T)	Human skin lesion	Spain	KC584250	KC584178	**LR134114**	KC584752	KC584492
A californica	CBS 119409 (T)	*Triticum aestivum*	USA/California	JQ693645	JQ646285	JQ671813	**LR134245**	**LR134181**
A cerasidanica	CBS 121923 (T)	Fruit of *Prunus avium*	Denmark/near Skaelskor	**LR135744**	**LR135747**	**LR135748**	**LR135745**	**LR135746**
A conjuncta	CBS 196.86 (T)	*Pastinaca sativa*	Switzerland	FJ266475	AY562401	JQ671824	KC584649	KC584390
A daucicaulis	CBS 119398 (T)	*Daucus carota*	Canada/Ontario	JQ693653	JQ646294	JQ671822	**LR134241**	**LR134177**
A ethzedia	CBS 197.86 (T)	*Brassica napus*	Switzerland	AY278833	AY278795	JQ671805	KC584657	KC584398
A frumenti	CBS 119401 (T)	Undetermined Poaceae	New Zealand	JQ693654	JQ646295	JQ671823	**LR134370**	**LR134172**
A graminicola	CBS 119400 (T)	Undetermined Poaceae	United Kingdom	JQ693650	JQ646291	JQ671819	**LR134249**	**LR134180**
A guarroi	FMR 16556 (T)	Human ulcerative skin lesion	Spain/Catalonia	**LR537031**	**LR537037**	**LR537056**	**LR537050**	**LR537045**
A hordeiaustralica	CBS 119402 (T)	*Hordeum vulgare*	South Australia	JQ693641	JQ646283	JQ671811	**LR134243**	**LR134179**
A hordeicola	CBS 121458 (T)	*Hordeum vulgare*	Southwest Norway	JQ693642	JQ646284	JQ671812	**LR134371**	**LR134175**
A humuli	CBS 119404 (T)	*Humulus lupulus*	France/Alsace	JQ693652	JQ646293	JQ671821	**LR134199**	**LR134174**
A incomplexa	CBS 121330 (T)	Canal mud	USA/Idaho	JQ693658	JQ646287	JQ671815	**LR134250**	**LR134185**
A infectoria	CBS 210.86 (T)	*Triticum aestivum*	United Kingdom	AF347034	AY278793	JQ671804	KC584662	KC584404
A intercepta	CBS 119406 (T)	*Viburnum* sp	USA/Chicago	JQ693656	JQ646297	JQ671826	FJ214927	**LR134170**
A merytae	CBS 119403 (T)	*Meryta sinclairii*	New Zealand	JQ693651	JQ646292	JQ671820	**LR134198**	**LR134119**

(Continues)

TABLE 2 (Continued)

Species	Strains	Substrate	Locality	GenBank accession numbers[a]				
				ITS	*gapdh*	*ATPase*	*tef1*	*rpb2*
A metachromatica	CBS 553.94 (T)	*Triticum aestivum*	South Australia	JQ693660	AY562404	JQ671809	FJ214931	JQ905189
A novae-zelandiae	CBS 119405 (T)	*Daucus carota*	New Zealand	JQ693655	JQ646296	JQ671825	**LR134197**	**LR134120**
A oregonensis	CBS 542.94 (T)	*Triticum aestivum*	USA/Oregon	FJ266478	FJ266491	JQ671827	KC584674	KC584416
A quercicola	CBS 141466 (T)	*Quercus brantii*	Iran/Fars province	KX228295	KX228362	**LR134115**	**LR134259**	**LR134188**
A roseogrisea	CBS 121921 (T)	*Helianthus annuus*	USA/North Dakota	**LR134102**	**LR134103**	**LR134104**	**LR134260**	**LR134192**
A slovaca	CBS 567.66 (T)	Human, lesion of ear	Czechoslovakia	KC584226	KC584150	**LR134368**	KC584702	KC584444
A triticimaculans	CBS 578.94 (T)	*Triticum aestivum*	Argentina/La Plata	JQ693657	JQ646280	JQ671806	FJ214930	**LR134183**
A triticina	CBS 763.84 (T)	*Triticum aestivum*	India/New Delhi	AY278834	JQ646281	JQ671808	FJ214942	**LR134186**
A ventricosa	CBS 121546 (T)	*Pyrus bretschneideri*	USA/Washington	JQ693649	JQ646290	JQ671818	KY352501	**LR134134**
A viburni	CBS 119407 (T)	*Viburnum* sp	USA/Chicago	JQ693647	JQ646288	JQ671816	**LR134200**	**LR134166**

Note: Newly generated sequences in this study and new species are indicated in bold.
Abbreviations: *ATPase*, plasma membrane ATPase; *gapdh*, glyceraldehyde 3-phosphate dehydrogenase; ITS, Internal Transcribed Spacers; *rpb2*, RNA polymerase second largest subunit; *tef1*, translation elongation factor 1-alpha.
[a]CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain. T: Ex-type strain.

amplification of the ITS region was carried out using the primer pairs ITS5/ITS4,[28] *rpb2* region using RPB2-5F2 and fRPB2-7cR,[29] *ATPase* using ATPDF1 and ATPDR1,[30] *gapdh* using gpd1 and gpd2,[31] and the partial gene *tef1* with the primers EF1-728F and EF1-986R.[32] The five regions were sequenced at Macrogen Europe (Macrogen Inc), using the same primer pairs and the consensus sequences were obtained in SeqMan software v. 7.0.0 (DNAStar Lasergene).

The novel sequences generated in the study were deposited in the NCBI´s GenBank nucleotide database (Table 2).

2.2 | Phylogenetic analysis

The phylogenetic analyses included sequences of 28 ex-type strains corresponding to most of the species that comprise the section *Infectoriae*, the three case isolates, and the ex-type strains of *A abundans* (CBS 534.83) and *A breviramosa* (CBS 121331) of the section *Chalastospora* as outgroup (Table 2). Additionally, sequences of tree unidentified clinical isolates of *Alternaria* from our collection that showed to be genetically similar to some of the case isolates were also included in the analyses (Table 2).

The alignment of each locus was performed in Mega software (Molecular Evolutionary Genetics Analysis) v.6.0.,[33] through Clustal W algorithm[34] and refined with MUSCLE[35] or manually if necessary. The combined analysis of the five phylogenetic markers was tested through incongruence length difference (ILD) implemented in the Winclada program.[36] Phylogenetic analysis was performed using the maximum-likelihood method (ML) under Mega software v.6.0. and Bayesian Inference (BI) approaches under MrBayes v. 3.2.6.[37] The best nucleotide substitution model determined using jModelTest[38] for the ML of the combined analysis of the five phylogenetical markers, was general time reversible with gamma distribution and invariant sites (G + I). For the BI phylogenetic analysis, the best nucleotide substitution model was chosen using jModelTest.[38] For the ITS region and *rpb2*, we used Kimura 2-parameter with invariant sites (K80 + I), for *gapdh* Kimura 2-parameter with gamma distribution (K80 + G), for *tef1* symmetrical model with gamma distribution (SYM + G) and for *ATPase* general time reversible with gamma distribution and invariant sites (GTR + G + I). The parameters settings used were two simultaneous runs of 5.000.000 generations, four Markov chains, sampled every 1000 generations. The 50% majority-rule consensus tree and posterior probability values (PP) were calculated after discarding the first 25% of the samples. For ML analysis, ML bootstrap values (BML) ≥70% were considered significant, and for BI, PP values of ≥0.95.

2.3 | Phenotypic study

Morphological characterisation of the isolates was carried out on potato carrot agar (PCA; potato 20 g, carrot 20 g, agar 13 g, distilled water 1 L), oatmeal agar (OA; oatmeal 30 g, agar 13 g, distilled water 1 L) and PDA after 7 days of incubation at 25°C in dark. Colour of the colonies in descriptions was based on Kornerup and Wanscher.[39] The measurements and description of the microscopic

structures were made after 14 days at 25°C in dark, from the specimen mounted in Shear's solution. The Zeiss Axio-Imager M1 light microscope (Zeiss) with a DeltaPix Infinity X digital camera was used to obtain the photomicrographs.

2.4 | Antifungal susceptibility

The in vitro antifungal susceptibility profile of the three clinical isolates was performed according to the CLSI M38-A2 method.[40] We evaluated antifungal activity of AMB (Sigma-Aldrich Quimica SA), VRC (Pfizer SA), PSC (Schering-Plough Research Institut), ITC (Jansen Pharmaceuticals), caspofungin (CFG) (Merk & Co., Inc), anidulafungin (AFG) (Pfizer SA), micafungin (MFG) (Astellas Pharma) and terbinafine (TBF) (Sigma-Aldrich Química SA). Microdilution plates were incubated at 35°C for 48 hours and read visually. The MIC was defined as the lowest drug concentration that produced 100% inhibition of visible fungal growth for AMB and azoles (ITC, PSC and VRC), and 80% for TBF. For the echinocandins (AFG, CFG and MFG), the minimum effective concentration (MEC) was determined microscopically as the lowest concentration of drug at where visible morphological changes in growth were observed (ie small, rounded, compact hyphal forms) compared to the growth in control. *Candida parapsilosis* ATCC 22019 was used as quality control strain for all tests that were performed in duplicate.

2.5 | Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as the research in this article related to micro-organisms.

3 | RESULTS

3.1 | Phylogeny

The topology of the phylogenetic trees for single partitions were congruent and according the ILD test ($P = 0.16$) could be combined. The final concatenated sequence alignment, with 36 strains and the five loci, comprised 3014 bp (ITS 526 bp, *rpb2* 573 bp, *ATPase* 1182 bp, *gapdh* 483 bp and *tef1* 250 bp) with 453 variable sites (ITS 26 bp, *rpb2* 118 bp, *ATPase* 153 bp, *gapdh* 94 bp and *tef1* 62 bp) of which 275 were phylogenetically informative (ITS 18 bp, *rpb2* 79 bp, *ATPase* 83 bp, *gapdh* 60 bp and *tef1* 35 bp). The topology of the trees inferred by the two phylogenetic methods (ML and BI) were basically the same, with minor differences in statistically supported groupings. The ML phylogenetic tree with the bootstrap and posterior probability values showed that the isolates of our patients were nested into the section *Infectoriae* (Figure 2). However, they appeared as lineages clearly distinct from the 28 ex-type strains of the species included in this study. Therefore, total of 31 lineages representing the different species in the section were recognised. Isolate FMR 16235 of case 1 was grouped with other isolates (FMR 17278, FMR 17288 and FMR 17296) of clinical origin, forming all a highly supported terminal clade. Isolates FMR 16556 and FMR 16868 of cases 2 and 3, respectively, grouped in a basal clade together with *A slovaca* and *A quercicola*. However, the phylogenetic relationship among them was uncertain because the internal branches showed low statistical support, and our isolates were located in independents branches, distant from the other accepted species. Based on the polyphasic approach, we propose and describe three new taxa in the section *Infectoriae* that were able to grow at 37°C and can be distinguished from related species by colony features and microscopic morphology of their conidia.

The individual phylogenetic analysis of each locus showed different abilities to discriminate among species in the section *Infectoriae*. ITS was able to differentiate less than 10% (3/31) of the species of the section included in the analysis, being only *A broccoli-italicae*, *A intercepta* and *A metachromatica* identified with this genetic marker. *Gapdh* was able to differentiate 45% (14/31) of the species. *Tef1* was able to separate 61% (19/31) of the species in the section. *Rpb2* resolved 68% (21/31) of the species, being unable to distinguish *A arbusti*, *A cerasidanica*, *A conjuncta*, *A ethzedia*, *A oregonensis*, *A roseogrisea*, *A triticimaculans*, *A triticina*, *A ventricosa* and *A viburni*. The highest percentage of species resolution, 94% (29/31), was achieved with the *ATPase* dataset. The only species that could not be distinguished were *A ethzedia* and *A triticimaculans*.

Results of the in vitro susceptibility testing were broadly variable among the three case isolates (Table 3). The drug that showed good activity for the isolates was TBF, with MICs ranging from 0.5 to 2 μg/mL. By contrast, AMB demonstrated poor activity for all them (2 to 8 μg/mL). The echinocandins (AFG, CFG and MFG) displayed good activity against FMR 16235 and FMR 16868, with values ranging between 0.03 and 1 μg/mL. Among these, MFG exhibited the lower MECs values (0.03 μg/mL for FMR 16868 and 0.125 μg/mL for FMR 16235). However, the three echinocandins showed poor activity against FMR 16556, with MECs ranging from 4 to 8 μg/mL. In general, the azoles showed low MICs values against FMR 16235 and FMR 16556 (between 0.125 and 2 μg/mL) with exception of ITC for FMR 16556 (MIC > 16 μg/mL). Conversely, no azole demonstrated activity against FMR 16868 (MICs from 8 to > 16 μg/mL).

3.2 | Taxonomy

Alternaria anthropophila Iturrieta-González, Gené, Alastruey & Dania García, sp nov.—MycoBank MB 829636, Figure 3

Etymology. Name referred to the source of the isolates, human clinical specimens.

Colonies on PDA reaching 71 mm diam. after 7 d at 25°C, white to greyish yellow (1A1/4B4), white at the periphery, flat, densely floccose, margin regular; reverse brown to greyish orange (6E4/5B4), white at the periphery. On PCA attaining 65 mm diam. after 7 d at 25°C, olive-brown (4D4), flat, slightly floccose, margin fimbriate; reverse olive-brown (4D4). On OA reaching 61 mm diam. after 7 d at 25°C, dull green (30E4), flat, slightly floccose, margin regular; reverse dull green (30E4).

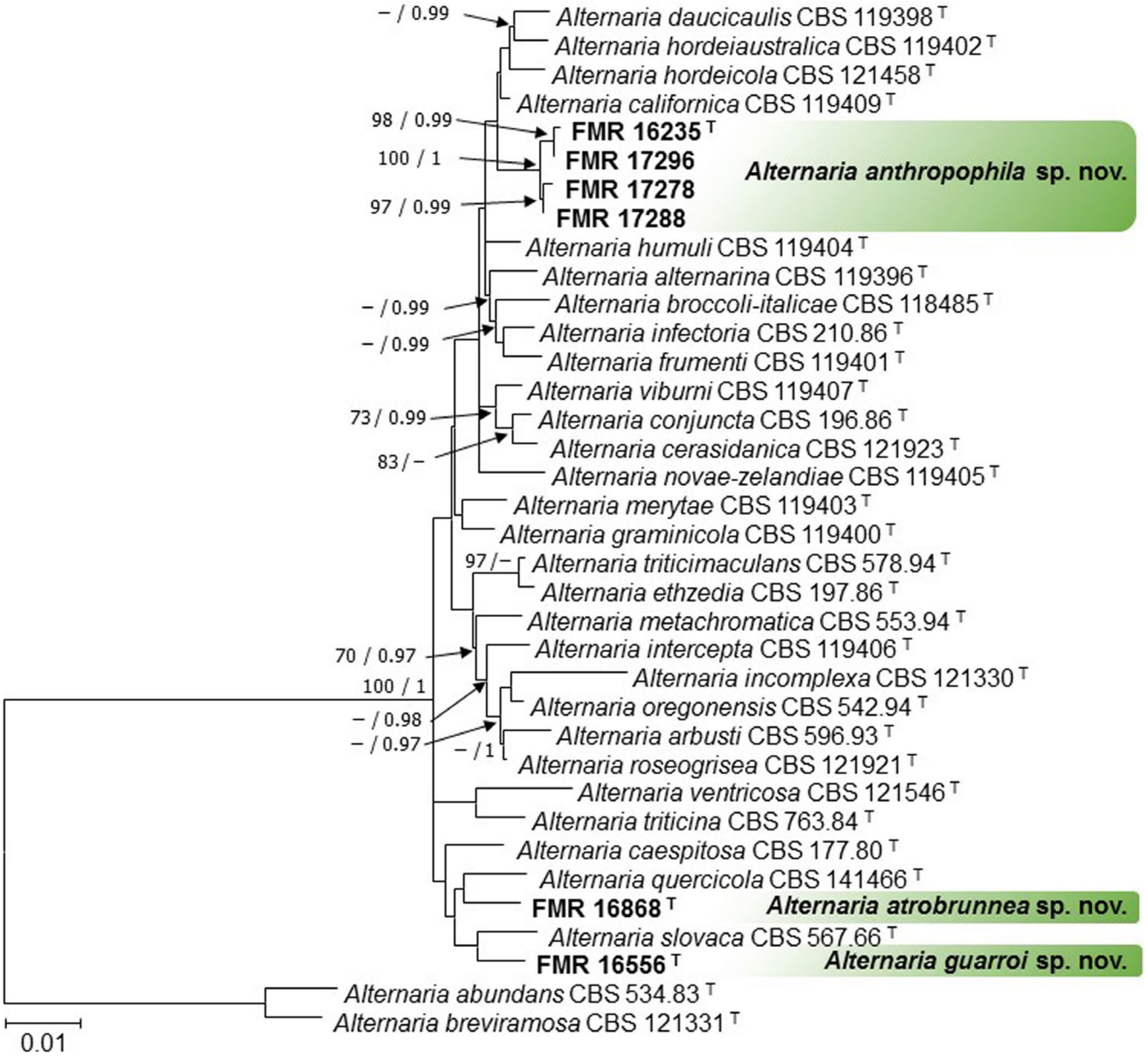

FIGURE 2 Maximum-likelihood (ML) tree constructed with ITS, *ATPase*, *gapdh*, *rpb2* and *tef1* sequences from 34 strains representatives of the section *Infectoriae*. The phylogenetic tree was rooted with *Alternaria abundans* and *A breviramosa*. Bootstrap support values for ML greater than 70% and Bayesian posterior probabilities greater than 0.95 are given near nodes. Names of species newly described here are indicated in bold. Branch lengths are proportional to distance. [T] Ex-type strain

Mycelium superficial and immersed, composed of septate, branched, 2-5 µm wide, smooth-walled to verruculose, hyaline to pale brown hyphae. Conidiophores solitary, arising directly from aerial hyphae, erect to slightly flexuous, occasionally geniculate at the apex, with up to 9 septa, unbranched or with up to two branches, 26-120 × 4-5(-7) µm, brown, smooth or verruculose, with 1-3 lateral or terminal conidiogenous loci. Conidia solitary or in chains of up to 9 conidia, commonly ellipsoidal, ovoid or obclavate, 11-63 × 6-11 µm, verruculose to verrucose, pale brown to brown, with some darkened middle transverse septa, (0-)2-4(-10) transverse septa, and 0-1 longitudinal or oblique septa per transverse segment; the primary conidia commonly produce secondary conidiophores that usually consist in a successive geniculate terminal extension, up to 150 µm long, bearing conidia solitary or in short chains. Sexual form not observed.

Cardinal temperatures for growth—Optimum 25°C, maximum 37°C, minimum 5°C.

Specimens examined. Spain, Aragón, human skin lesion, May 2003, *Ana Alastruey* (FMR 17278 = CNM 2519); Catalonia, Reus, human subcutaneous nodule, Feb. 2017, *Isabel Pujol* (holotype CBS H-23916, culture ex-type CBS 145587 = FMR 16235); Cantabria, human pericardial liquid, Jul. 2009, *Ana Alastruey* (FMR 17296 = CNM 5813); Galicia, human subcutaneous nodule, Dec. 2005, *Ana Alastruey* (FMR 17288 = CNM 3823).

TABLE 3 Results of in vitro antifungal susceptibility testing for the three case isolates of *Alternaria* section *Infectoriae*

Results (μg/mL) for	*A anthropophila* (Case 1-FMR 16235)		*A guarroi* (Case 2-FMR 16556)		*A atrobrunnea* (Case 3-FMR 16868)	
	MIC	MEC	MIC	MEC	MIC	MEC
AMB	2	—	2	—	8	—
ITC	0.125	—	>16	—	16	—
PSC	0.125	—	0.125	—	8	—
VRC	1	—	2	—	>16	—
AFG	—	0.5	—	8	—	0.06
CFG	—	0.5	—	4	—	1
MFG	—	0.125	—	4	—	0.03
TBF	0.5	—	1		2	—

Abbreviations: AFG, anidulafungin; AMB, amphotericin B; CFG, caspofungin; ITC, itraconazole; MFG, micafungin; PSC, posaconazole; TBF, terbinafine; VRC, voriconazole.

Note: *A anthropophila* is included in a poorly supported clade together with the plant-related species *A californica*, *A daucicaulis*, *A hordeicola* and *A hordeiaustralica*. *Alternaria californica* differs by producing unbranched and shorter conidiophores (30-100 μm) and conidia with up to 16 transverse septa.[41] The other three species (*A daucicaulis*, *A hordeicola* and *A hordeiaustralica*) are characterised by the production of smooth or slightly punctate conidia in culture and by the production of a *Lewia*-like sexual morph on natural substrates.[41,42]

Alternaria atrobrunnea Iturrieta-González, Pujol, Dania García & Gené, sp nov.—MycoBank MB 829637, Figure 4

Etymology. Name referred to the colour of the colony reverse on PDA.

Colonies on PDA reaching 75 mm diam. after 7 d at 25°C, grey to greyish yellow (4D1/4C8), flat, cottony, margin fimbriate; reverse yellowish brown to dark blond (5F4/5D4), yellowish grey at the periphery (4B2). On PCA attaining 83 mm diam. after 7 d at 25°C, olive to dark green (3E3/29F7), flat, floccose, margin regular; reverse dark green (29F3). On OA reaching 85 mm diam. after 7 d at 25°C, olive to dark green (3E3/29F4), flat, slightly floccose, margin regular; reverse dark green (29F8).

Mycelium superficial and immersed, composed of septate, branched, 2-6(-11) μm wide, subhyaline to pale brown, smooth to verruculose hyphae. Conidiophores solitary, arising directly from aerial hyphae, erect to slightly flexuous, with up to 8-septate, unbranched, 14-39 × 3-5 μm, brown, smooth or verruculose, with 1 terminal conidiogenous locus. Conidia solitary or in simple short chains with up to 5 conidia, ovoid or obclavate, 7-44 × 5-12 μm, verrucose to tuberculate, brown, with some darkened middle transverse septa, 3-8 transverse septa, and 0-1(-2) longitudinal or oblique septa per transverse segment; some primary conidia produce secondary conidiophores as lateral or terminal extensions from the conidial body, bearing conidia in short chains. Sexual form not observed.

Cardinal temperatures for growth—Optimum 25°C, maximum 37°C, minimum 5°C.

Specimen examined. Spain, Catalonia, Reus, exudate from an ulcerative skin lesion, Oct. 2017, *Isabel Pujol* (Holotype CBS H-23918, culture ex-type CBS 145589 = FMR 16868).

Note: *Alternaria atrobrunnea* is placed in a poorly supported basal clade of the phylogenetic tree (Figure 2) together with *A quercicola*, a species described from *Quercus brantii* (*Fagaceae*) in Iran.[43] However, *A quercicola* differs from our new species in the conidial ornamentation, being rough-walled towards the base and smooth-walled towards the apex, and in length, (25-)31-51(-57) μm long.[43]

Alternaria guarroi Iturrieta-González, Dania García, Pujol & Gené sp nov.—MycoBank MB 829638, Figure 5

Etymology. Name in honour of Josep Guarro for his extensive work on medical mycology.

Colonies on PDA reaching 80 mm diam. after 7 days at 25°C, white, flat, cottony, margin regular; reverse pale yellow (4A3). On PCA attaining 82 mm diam. after 7 days at 25°C, colourless, flat, with predominantly hyaline immersed mycelium, margin regular; reverse colourless. On OA reaching 83 mm diam. after 7 days at 25°C, golden grey (4C2), flat, slightly floccose, margin regular; reverse olive-brown (4E4).

Mycelium superficial and immersed, composed of septate, branched, 2-7 μm wide, smooth to verruculose, subhyaline to pale brown hyphae. Conidiophores solitary, erect to slightly flexuous, occasionally geniculate at the apex with up to 6–septate, mostly unbranched, 11-55 × 3-4 μm, brown, smooth or verruculose, with 1-3 lateral or terminal conidiogenous loci. Conidia solitary or in short unbranched chains of up to 5 conidia, ellipsoidal, obclavate or ovoid, 5-31 × 3-10(-12) μm, smooth to verruculose, brown, with some darkened middle transverse septa, 1-5(-9) transverse septa, and 0-1 longitudinal or oblique septa per transverse segment; these primary conidia produce secondary conidiophores that consist in a subapical extension from the conidial body, with a terminal conidiogenous locus bearing solitary or a short chain of conidia. Sexual form not observed.

Cardinal temperatures for growth—Optimum 25°C, maximum 37°C, minimum 5°C.

Specimen examined. Spain, Catalonia, Reus, biopsy from ulcerative skin lesion, Apr. 2017, *Isabel Pujol* (Holotype CBS H-23917, culture ex-type CBS 145588 = FMR 16556).

Note: *Alternaria guarroi* and *A slovaca* are in the same basal clade but with low statistical support. The latter species, which was also described from dermic human lesions in Bratislava (Czechoslovakia), differs from *A guarroi* in producing solely chlamydospores and sporadically blastospores,[17] which were originally described as brownish, aseptate, obovoid spores of 1-1.5 × 1 μm growing directly from mycelium cells and never germinating.[44]

4 | DISCUSSION

Phaeohyphomycosis is an increasingly recognised infection, in which *Alternaria* is one of the most commonly reported agents, mainly associated with both superficial and deep local infection in patients with impaired immunity, especially due to solid organ transplantation.[5,8,9,16,45-47] Pulmonary transplantation, prostrate

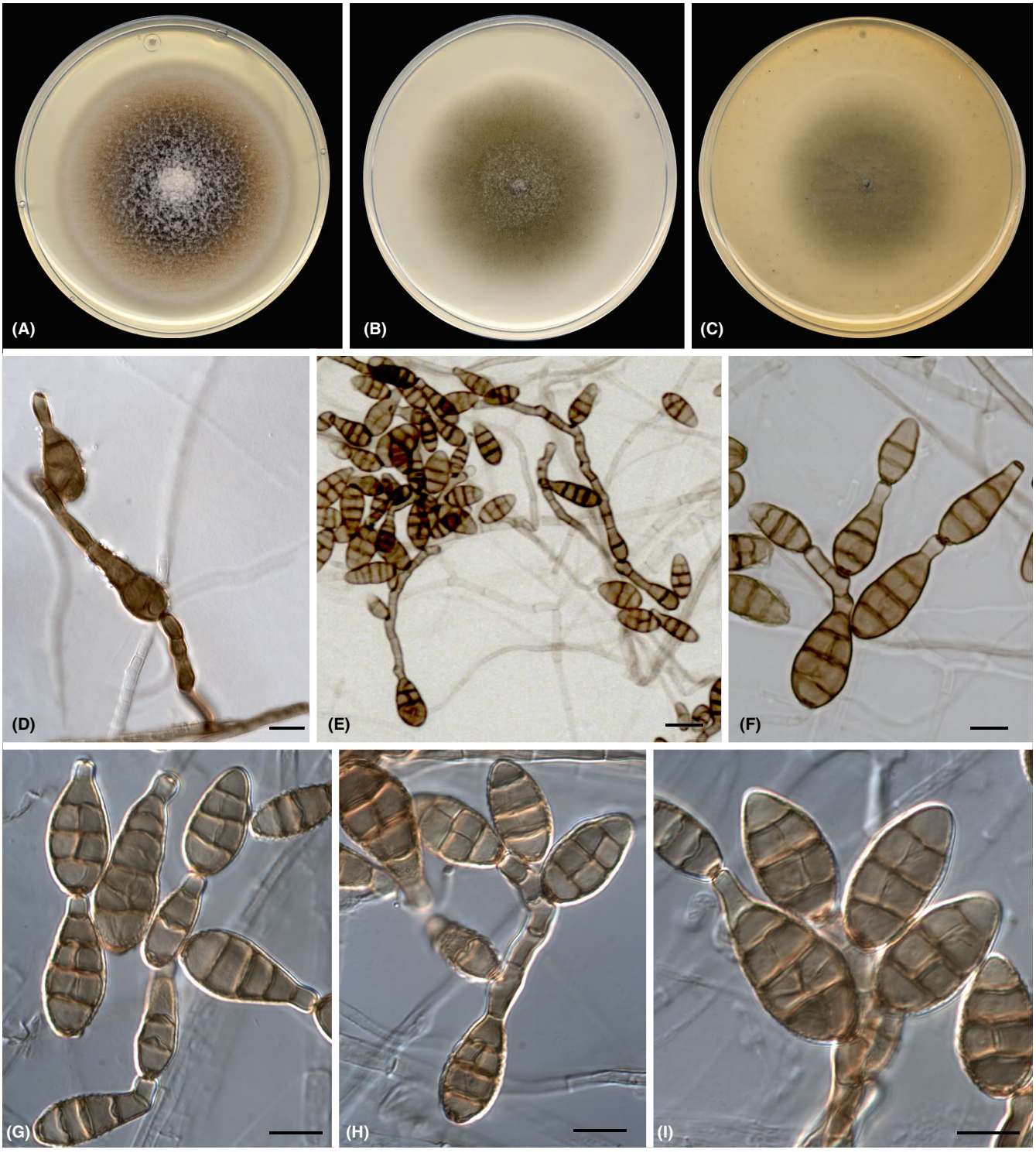

FIGURE 3 *Alternaria anthropophila* sp nov., FMR 16235. A-C. Colonies on (A) PDA, (B) PCA, (C) OA, at 25°C after 7d. D-G. Conidiophores and conidia at 25°C from PCA after 14 days (D) and from OA after 30 days (E-I). Scale bars E = 20 µm; D, F-I = 10 µm

adenocarcinoma and a multiple pathological clinical history were predisposing factors to induce an immunocompromised condition in our patients to suffer cutaneous infection by this ubiquitous fungal genus. Respective mycotic infections were diagnosed by repeated cultures, in addition to histopathological examination of the biopsy specimens with visible fungal elements in two of the cases.

Although several species have been associated as causal agent of alternariosis, *A infectoria* is the most frequent species identified in recent studies.[4,16,48,49] However, in most cases its identification is limited to morphological features of the fungus growing in culture and confirmed by the analysis of the ITS barcode. Even, in some cases, only ITS analysis is carried out directly from paraffin-embedded

FIGURE 4 *Alternaria atrobrunnea* sp nov., FMR 16868. A-C. Colonies on (A) PDA, (B) PCA, (C) OA, at 25°C after 7d. D-G. Conidiophores and conidia from PCA after 14 days. Scale bars D-G = 10 µm

tissue samples without culturing.[16,50] Nevertheless, as stated before, the so-called barcode for fungi has very limited resolution to distinguish closely related species in *Alternaria*, usually being only able to distinguish among *Alternaria* species-groups or sections.[17,19] Therefore, the incidence of *A infectoria* as causative agent of alternariosis is probably overestimated in the above-mentioned studies, since the analysis of ITS sequences only is able to confirm the relationship of the case isolate to *Alternaria* section *Infectoriae*. In the present study, sequence analysis of five loci (ITS, *ATPase*, *gapdh*, *tef*1 and *rpb*2) has allowed to distinguish most species currently accepted in section *Infectoriae* and to delineate three novel taxa in this group of *Alternaria*, that is *A anthropophila*, *A atrobrunnea* and *A guarroi* (Figure 2). Of note is that the former one has been phylogenetically strongly supported with sequences of other clinical isolates that could not be identified previously due to their limited sporulation in culture. However, the taxonomic structure of the section remains obscure, since the genetic markers used have not been able to resolve the phylogenetic relationships among many of the species included in the analysis. Anyway, we agree with Lawrence et al[19,30] in that the *ATPase* is a very suitable genetic markers for molecular identification of *Alternaria* species and this could be used in clinical laboratories for identifying these fungi. In fact, it has been able to discriminate practically all the species in *Alternaria* section *Infectoriae* included in this study (Figure S1). Identification at the species level in such section is relevant since, as the new species described here, it includes fungi able to growth at the body temperature and, therefore, with potential to cause animal and human infections. However, only the correct identification of *Alternaria* isolates can lead to determine the epidemiology of its species or to establish proper treatment to resolve infections caused by this complex group of fungi.

Given that no optimal treatment has been defined for *Alternaria* infections, several therapeutic options are used, depending on the status of the patient concerned and the extent of disease. The most commonly antifungal therapy includes ITC, VRC and PSC,[51] being

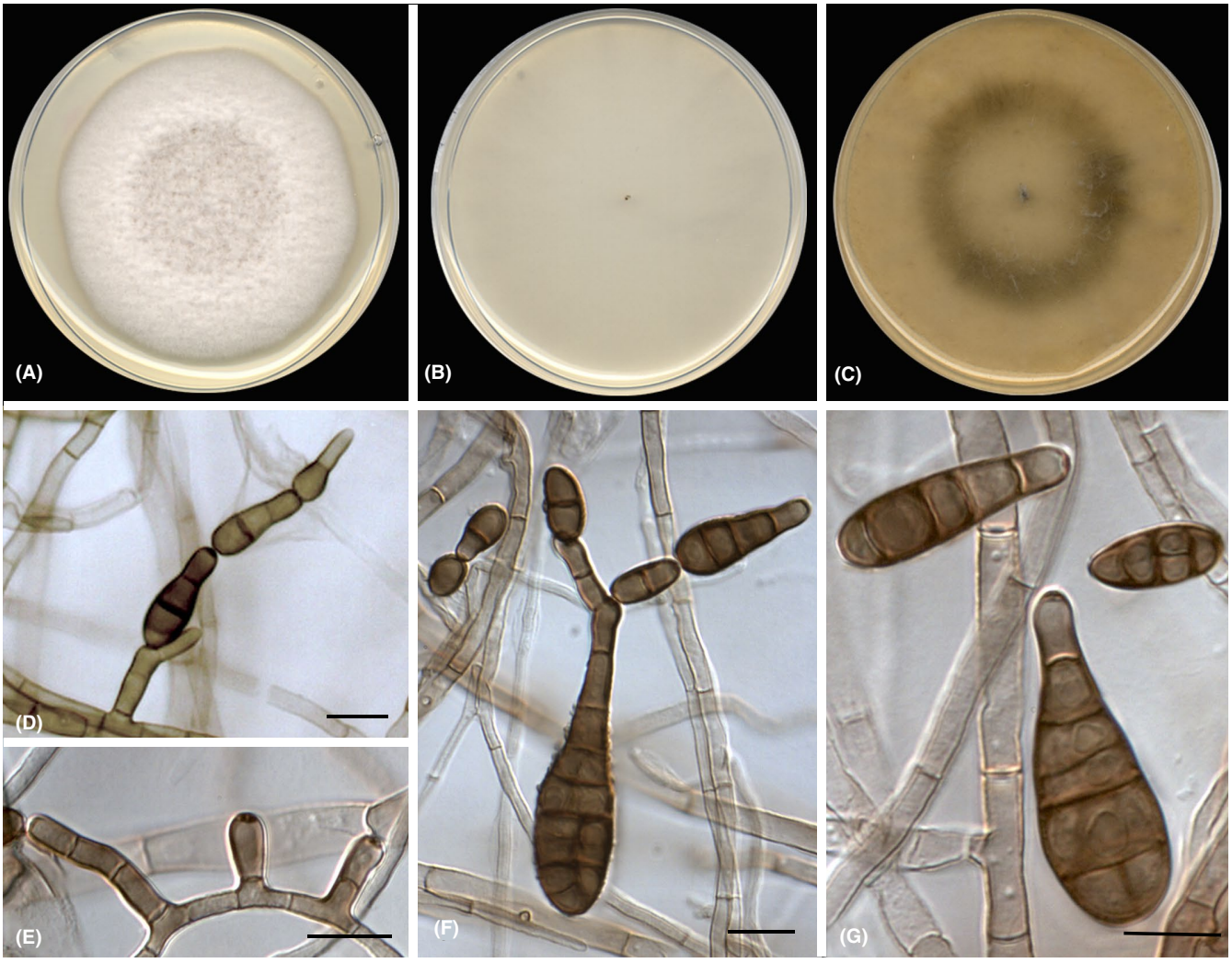

FIGURE 5 *Alternaria guarroi* sp nov., FMR 16556. A-C. Colonies on (A) PDA, (B) PCA, (C) OA, at 25°C after 7d. D-G. Conidiophores and conidia from PCA after 14 days. Scale bars D-G = 10 μm

the former widely used in cutaneous alternariosis and usually combined with surgical excision of involved tissue and reduction of the immunosuppression in cases of transplant recipients.[3-5,7,16,52] In our case, in the first patient the infection was resolved with surgical excision of the nodule and topical VRC; the second patient with multiple skin lesions was initially treated with iv LAMB but due to side effects this was switched to VRC, while multiple lesions in the third patient were improved with nursing cures without specific antifungal treatment but follow-up was not possible because she was exitus (Table 1). It of note however that, due to hepatic impairment as side effect reported for VRC[50,53] or the significant drug-drug interaction when using ITC,[11] currently PSC has shown to be a good option to treat cutaneous alternariosis.[4,50,54] This correlates with results of in vitro antifungal testing in several studies, which show that, among azoles, PSC is the most potent drug against *Alternaria* species.[7,55,56] In the present study, isolates of *A anthropophila* and *A guarroi* of the first two cases, respectively (Table 3), showed low MICs to this drug. Although it was not the azol of election for treatment of our patients but VRC, which showed moderate activity, they cured with the treatment. Conversely, no azole was active against *A atrobrunnea* isolated from the third case. This variable antifungal profile was also observed in the case of equinocandins, for which *A anthropophila* and *A atrobrunnea* exhibited low MEC values (0.03 to 1 μg/mL) respect to the high values (4 to 8 μg/mL) in *A guarroi* (Table 3). Different antifungal patterns have also been found in this class of drugs in other studies when susceptibility data are compared among *Alternaria* species.[55,56] Therefore, these results reinforce the relevance of identification of *Alternaria* isolates at the species level and the necessity to carry out antifungal susceptibility testing to determine the most successful drug for alternariosis treatment. However, only the analysis of susceptibility patterns of more isolates of well-delineated species will allow us to elucidate whether a correct identification can predict the best drug for treatment.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Isabel Iturrieta-González conceived the ideas, organised and analysed the data, and join in the writing. Dania García and Josepa Gené conceived the ideas, analysed the data and led the writing. Isabel Pujol, Simona Iftimorie, Vanesa Morente, Rosana Queralt and Frederic Ballester collected the clinical data; Marcela Guevara-Suarez supported the in vitro susceptibility testing, Ana Alastruey-Izquierdo supplied the strains and reviewed the text; Margarita Hernández-Restrepo supplied the sequences of type strains and reviewed the text.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Iturrieta-González I, Pujol I, Iftimie S, et al. Polyphasic identification of three new species in *Alternaria* section *Infectoriae* causing human cutaneous infection. *Mycoses*. 2020;63:212–224. https://doi.org/10.1111/myc.13026

Supplementary material

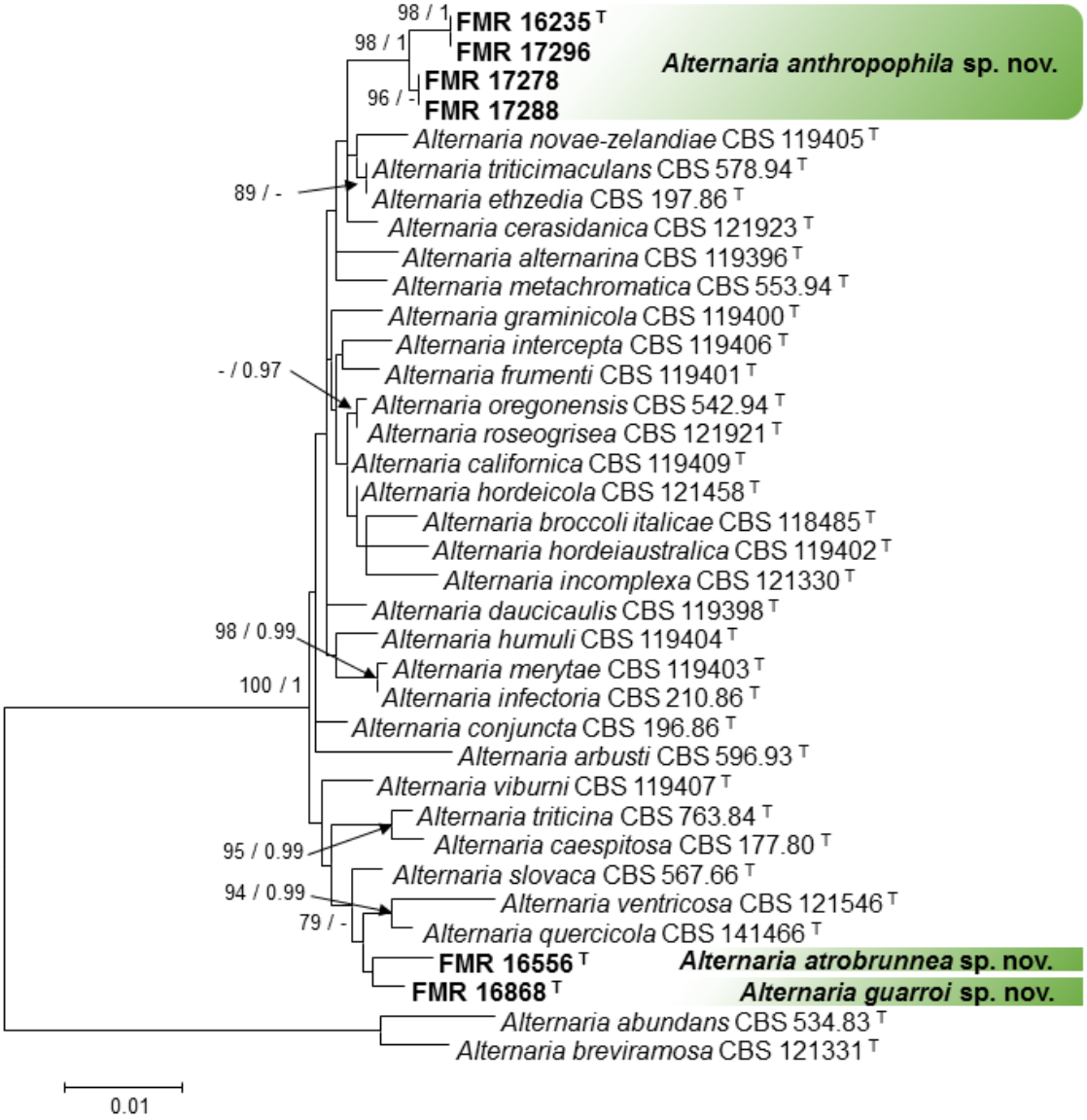

FIGURE S1. Maximum-likelihood (ML) tree constructed with *ATPase* sequences from 34 strains representatives of the section *Infectoriae*. The phylogenetic tree was rooted with *Alternaria abundans* and *A breviramosa*. Bootstrap support values for ML greater than 70% and Bayesian posterior probabilities greater than 0.95 are given near nodes. Names of species newly described here are indicated in bold. Branch lengths are proportional to distance. T Ex-type strain.

4.1.2 Genera of phytopathogenic fungi: GOPHY 3

Y. Marin-Felix, M. Hernández-Restrepo, I. Iturrieta-González, D. García, J. Gené, J.Z. Groenewald, L. Cai, Q. Chen, W. Quaedvlieg, R.K. Schumacher, P.W.J. Taylor, C. Ambers, G. Bonthond, J. Edwards, S.A. Krueger-Hadfield, J.J. Luangsa-ard, L. Morton, A. Moslemi, M. Sandoval-Denis, Y.P. Tan, R. Thangavel, N. Vaghefi, R. Cheewangkoon, and P.W. Crous

Published in: Studies in Mycology 2019; 94: 1–124.

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

available online at www.studiesinmycology.org

STUDIES IN MYCOLOGY 94: 1–124 (2019).

WESTERDIJK FUNGAL BIODIVERSITY INSTITUTE

Genera of phytopathogenic fungi: GOPHY 3

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Abstract: This paper represents the third contribution in the Genera of Phytopathogenic Fungi (GOPHY) series. The series provides morphological descriptions, information about the pathology, distribution, hosts and disease symptoms for the treated genera, as well as primary and secondary DNA barcodes for the currently accepted species included in these. This third paper in the GOPHY series treats 21 genera of phytopathogenic fungi and their relatives including: *Allophoma*, *Alternaria*, *Brunneosphaerella*, *Elsinoe*, *Exserohilum*, *Neosetophoma*, *Neostagonospora*, *Nothophoma*, *Parastagonospora*, *Phaeosphaeriopsis*, *Pleiocarpon*, *Pyrenophora*, *Ramichloridium*, *Seifertia*, *Seiridium*, *Septoriella*, *Setophoma*, *Stagonosporopsis*, *Stemphylium*, *Tubakia* and *Zasmidium*. This study includes three new genera, 42 new species, 23 new combinations, four new names, and three typifications of older names.

Key words: DNA barcodes, Fungal systematics, New taxa.

Taxonomic novelties: New genera: *Arezzomyces* Y. Marín & Crous, *Globoramichloridium* Y. Marín & Crous, *Wingfieldomyces* Y. Marín & Crous; **New species:** *Allophoma pterospermicola* Q. Chen & L. Cai, *Alternaria aconidiophora* Iturrieta-González, Dania García & Gené, *Alternaria altcampina* Iturrieta-González, Dania García & Gené, *Alternaria chlamydosporifera* Iturrieta-González, Dania García & Gené, *Alternaria curvata* Iturrieta-González, Dania García & Gené, *Alternaria fimeti* Iturrieta-González, Dania García & Gené, *Alternaria inflata* Iturrieta-González, Dania García & Gené, *Alternaria lawrencei* Iturrieta-González, Dania García & Gené, *Alternaria montsantina* Iturrieta-González, Dania García & Gené, *Alternaria pobletensis* Iturrieta-González, Dania García & Gené, *Alternaria pseudoventricosa* Iturrieta-González, Dania García & Gené, *Brunneosphaerella roupeliae* Crous, *Elsinoe picconiae* Crous, *Elsinoe veronicae* Crous, Thangavel & Y. Marín, *Neosetophoma aseptata* Crous, R.K. Schumach. & Y. Marín, *Neosetophoma phragmitis* Crous, R.K. Schumach. & Y. Marín, *Neosetophoma sambuci* Crous, R.K. Schumach. & Y. Marín, *Neostagonospora sorghi* Crous & Y. Marín, *Parastagonospora novozelandica* Crous, Thangavel & Y. Marín, *Parastagonospora phragmitis* Crous & Y. Marín, *Phaeosphaeriopsis aloes* Crous & Y. Marín, *Phaeosphaeriopsis aloicola* Crous & Y. Marín, *Phaeosphaeriopsis grevilleae* Crous & Y. Marín, *Phaeosphaeriopsis pseudoagavacearum* Crous & Y. Marín, *Pleiocarpon livistonae* Crous & Quaedvl., *Pyrenophora avenicola* Y. Marín & Crous, *Pyrenophora cynosuri* Y. Marín & Crous, *Pyrenophora novozelandica* Y. Marín & Crous, *Pyrenophora pseudoerythrospila* Y. Marín & Crous, *Pyrenophora sieglingiae* Y. Marín & Crous, *Pyrenophora variabilis* Hern.-Restr. & Y. Marín, *Septoriella germanica* Crous, R.K. Schumach. & Y. Marín, *Septoriella hibernica* Crous, Quaedvl. & Y. Marín, *Septoriella hollandica* Crous, Quaedvl. & Y. Marín, *Septoriella pseudophragmitis* Crous, Quaedvl. & Y. Marín, *Setophoma brachypodii* Crous, R.K. Schumach. & Y. Marín, *Setophoma pseudosacchari* Crous & Y. Marín, *Stemphylium rombundicum* Moslemi, Y.P. Tan & P.W.J. Taylor, *Stemphylium truncatulae* Moslemi, Y.P. Tan & P.W.J. Taylor, *Stemphylium waikerieanum* Moslemi, Jacq. Edwards & P.W.J Taylor, *Vagicola arundinis* Phukhams., Camporesi & K.D. Hyde, *Zasmidium thailandicum* Crous; **New combinations:** *Arezzomyces cytisi* (Wanas. *et al.*) Y. Marín & Crous, *Globoramichloridium indicum* (Subram.) Y. Marín & Crous, *Phaeosphaeria phoenicicola* (Crous & Thangavel) Y. Marín & Crous, *Pyrenophora poae* (Baudyš) Y. Marín & Crous, *Pyrenophora wirreganensis* (Wallwork *et al.*) Y. Marín & Crous, *Seiridium cupressi* (Nattrass *et al.*) Bonthond, Sandoval-Denis & Crous, *Seiridium pezizoides* (de Not.) Crous, *Septoriella agrostina* (Mapook *et al.*) Y. Marín & Crous, *Septoriella artemisiae* (Wanas. *et al.*) Y. Marín & Crous, *Septoriella arundinicola* (Wanas. *et al.*) Y. Marín & Crous, *Septoriella arundinis* (W.J. Li *et al.*) Y. Marín & Crous, *Septoriella bromi* (Wijayaw. *et al.*) Y. Marín & Crous, *Septoriella dactylidis* (Wanas. *et al.*) Y. Marín & Crous, *Septoriella elongata* (Wehm.) Y. Marín & Crous, *Septoriella forlicesenica* (Thambug. *et al.*) Y. Marín & Crous, *Septoriella garethjonesii* (Thambug. *et al.*) Y. Marín & Crous, *Septoriella italica* (Thambug. *et al.*) Y. Marín & Crous, *Septoriella muriformis* (Ariyaw. *et al.*) Y. Marín & Crous, *Septoriella rosae* (Mapook *et al.*) Y. Marín & Crous, *Septoriella subcylindrospora* (W.J. Li *et al.*) Y. Marín & Crous, *Septoriella vagans* (Niessl) Y. Marín & Crous, *Wingfieldomyces cyperi* (Crous & M.J. Wingf.) Y. Marín & Crous, *Zasmidium ducassei* (R.G. Shivas *et al.*) Y. Marín & Crous; **New names:** *Pyrenophora nisikadoi* Y. Marín & Crous, *Septoriella dactylidicola* Y. Marín & Crous, *Septoriella neoarundinis* Y. Marín & Crous, *Septoriella neodactylidis* Y. Marín & Crous; **Typification: epitypification:** *Ascochyta chrysanthemi* F. Stevens, *Pestalotia unicornis* Cooke & Ellis, *Rhynchosphaeria cupressi* Nattrass *et al.*

Available online 13 June 2019; https://doi.org/10.1016/j.simyco.2019.05.001.

Peer review under responsibility of Westerdijk Fungal Biodiversity Institute.

Studies in Mycology

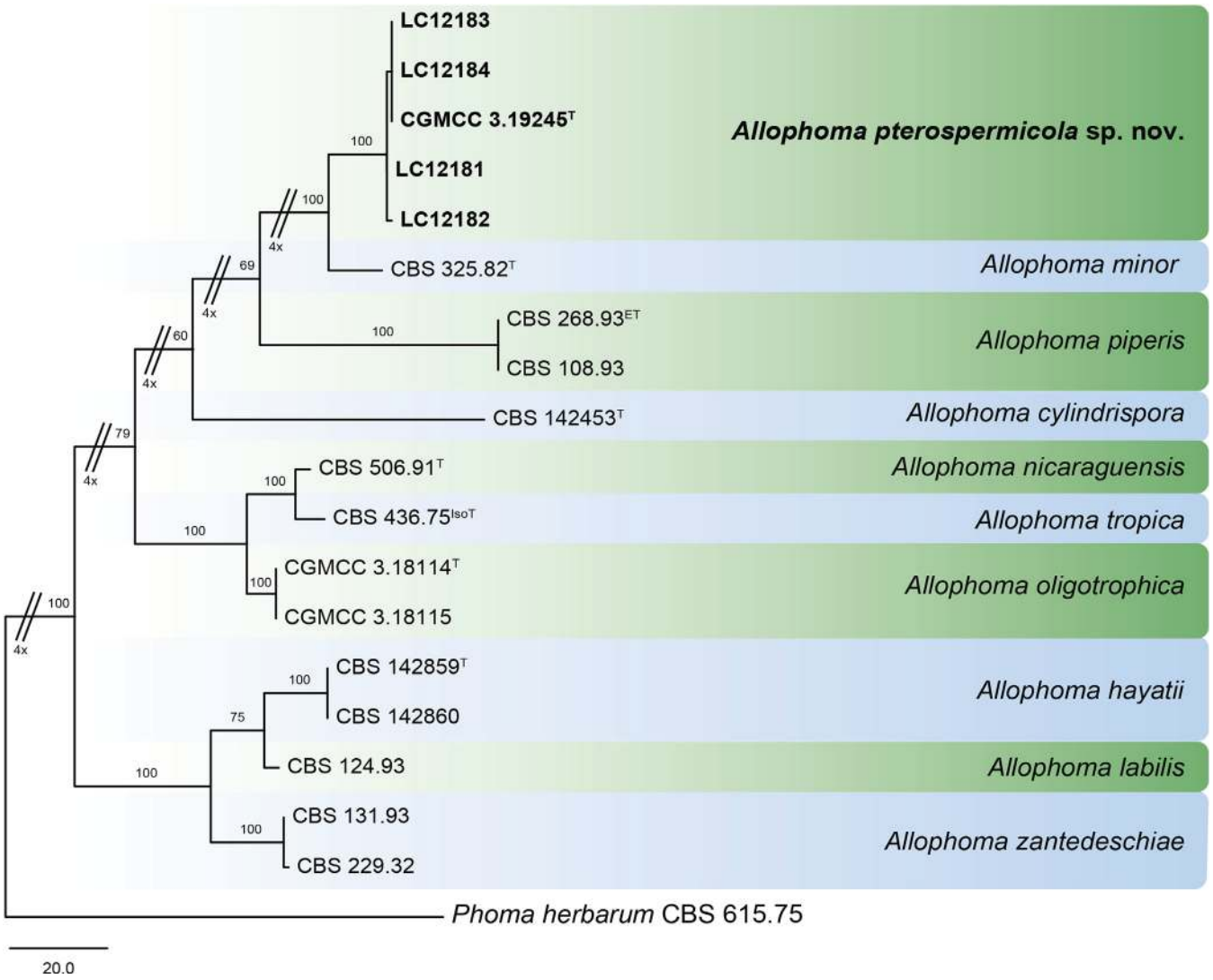

Fig. 2. Phylogenetic tree generated from a maximum parsimony analysis based on the combined LSU (860 bp), ITS (480 bp), *tub2* (333 bp) and *rpb2* (803 bp) sequences of all accepted species of *Allophoma*. The tree was rooted to *Phoma herbarum* CBS 615.75. Values above the branches represent parsimony bootstrap support values (> 50 %). Novel sequences and novel taxon are printed in **bold**. GenBank accession numbers are indicated in Table 1. ^{T}, ^{ET} and ^{IsoT} indicate ex-type, ex-epitype and ex-isotype strains, respectively. TreeBASE: S23493.

white near the margins. Application of NaOH results in a pale brownish olivaceous discolouration of the agar.

Typus: **China**, Guangxi, Nonggang National Nature Reserve, on diseased leaves of *Pterospermum xylocarpum* (*Sterculiaceae*), Jun. 2017, Z.Y. Ma (**holotype** HMAS 247983, culture ex-type CGMCC 3.19245 = LC 12185).

Additional materials examined: **China**, Guangxi, Nonggang National Nature Reserve, on diseased leaves of *Pterospermum xylocarpum* (*Sterculiaceae*), Jun. 2017, Z.Y. Ma, LC 12183; *ibid*., LC 12184; Guangxi, Jingxi, Gulongshan, on diseased leaves of *Maesa montana* (*Primulaceae*), Jun. 2017, Z.Y. Ma, LC 12181; *ibid*., LC 12182.

Notes: *Allophoma pterospermicola* represents the first report of a species in the family *Didymellaceae* on the two host genera *Pterospermum* (*Sterculiaceae*) and *Maesa* (*Primulaceae*). This species is closely related to *Al. minor*, but differs in producing longer conidiogenous cells [6–10 × 3–6 µm in *Al. pterospermicola vs.* 4–5.5(–6.2) × 3–4.5(–4.7) in *Al. minor*] and slightly narrower conidia [3–5.5 × 1.5–2 µm in *Al. pterospermicola vs.* (3–)3.5–4.5(–5) × 1.8–2.5(–3) µm in *Al. minor*]. In addition, *Al. pterospermicola* grows much slower on OA, MEA and PDA than *Al. minor*, and the latter species has only been recorded on *Syzygium aromaticum* (*Myrtaceae*) (Aveskamp *et al.* 2010).

Authors: Q. Chen & L. Cai

Alternaria Nees, Das System der Pilze und Schwämme: 72. 1816 (1816–1817). Fig. 4.
For synonyms see Woudenberg *et al.* (2013).

Classification: *Dothideomycetes*, *Pleosporomycetidae*, *Pleosporales*, *Pleosporaceae*.

Type species: *Alternaria alternata* (Fries) Keissler, basionym: *Torula alternata* Fr., Syst. Mycol. (Lundae) 3: 500. 1832 (nom. sanct.); additional synonyms listed in Woudenberg *et al.* (2015). Neotype designated by Simmons (1967): E.G.S. 11.050. Ex-epitype strain designated by de Hoog & Horré (2002): CBS 916.96 = E.G.S. 34.016.

DNA barcodes (genus): LSU, ITS.

DNA barcodes (species): ITS, *ATPase*, *gapdh*, *rpb2*, *tef1*. Table 2. Figs 5–7.

Ascomata small, solitary to clustered, erumpent to almost superficial at maturity, dark brown, globose to ovoid, apically

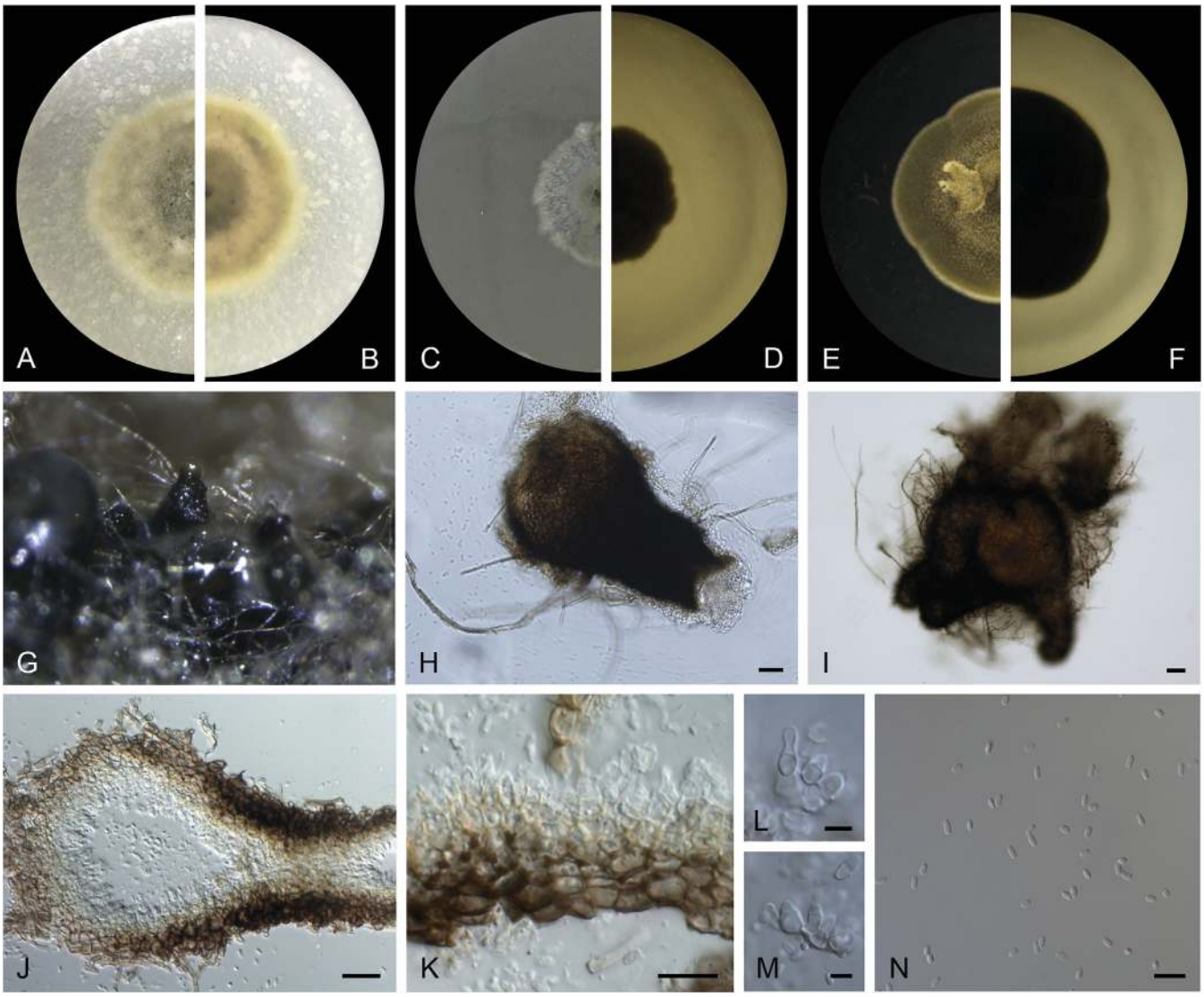

Fig. 3. *Allophoma pterospermicola* (ex-type CGMCC 3.19245). **A, B.** Colony on OA (front and reverse). **C, D.** Colony on MEA (front and reverse). **E, F.** Colony on PDA (front and reverse). **G.** Conidiomata sporulating on OA. **H, I.** Conidiomata. **J.** Section of conidioma. **K.** Section of conidiomatal wall. **L, M.** Conidiogenous cells. **N.** Conidia. Scale bars: H, J = 20 µm; I = 40 µm; K, N = 10 µm; L, M = 5 µm.

papillate, ostiolate, smooth or setose at maturity, with a thin ascomatal wall; *centrum* formed by a hamathecium with cellular pseudoparaphyses and asci in basal layer. *Asci* bitunicate, fissitunicate, uni- or biseriate, (4–6–)8-spored, cylindrical to cylindro-clavate, straight or somewhat curved, with a short furcate pedicel. *Ascospores* ellipsoid to fusoid, muriform, slightly constricted at septa, 3–7-transverse septa, 1–2 series of longitudinal septa through the two original central segments, end cells without septa, or with one longitudinal or oblique septum, or with a Y-shaped pair of septa, yellow-brown, smooth-walled, without guttules. *Conidiophores* macronematous or semi-macronematous, mononematous, simple or branched, pale brown or brown. *Conidiogenous cells* integrated, terminal becoming intercalary, mono- or polytretic and sympodial, cicatrised. *Conidia* solitary or in simple or branched chains, dry, ovoid, obovoid, cylindrical, narrowly ellipsoid or obclavate, beaked or non-beaked, pale or medium olivaceous brown to brown, smooth-walled or verrucose, with transverse and with or without oblique or longitudinal septa; *septa* can be thick, dark, an internal cell-like structure can be formed. Species with meristematic growth are known (adapted from Ellis 1976, Woudenberg *et al.* 2013, 2014, Grum-Grzhimaylo *et al.* 2016).

Culture characteristics: Colonies effuse, grey, olivaceous brown, dark blackish brown or black; mycelium immersed or partly superficial, composed of colourless, olivaceous brown or brown hyphae.

Optimal media and cultivation conditions: For morphological examinations the use of PCA and V-8 is recommended, incubated at moderate temperatures (*ca.* 22–25 ºC) under near-ultraviolet light (8 h light, 16 h dark), without humidity control, for 5–7 d or more if necessary (Simmons 2007). We also recommend microscopic examination of OA cultures due to the alterations observed on the conidial wall when grown on PCA.

Distribution: Worldwide.

Hosts: Mainly pathogens of a wide range of plant families, such as *Apiaceae*, *Asteraceae*, *Brassicaceae*, *Cyperaceae*, *Poaceae*, *Rosaceae*, *Rutaceae*, *Solanaceae*, among others (Thomma 2003, Lawrence *et al.* 2016). Some are implicated as human pathogens (de Hoog *et al.* 2011).

Disease symptoms: Most species are foliar pathogens, causing necrotic lesions as brown/black spots or “target spot” with the fungus residing in the central area, but also inducing leaf blight;

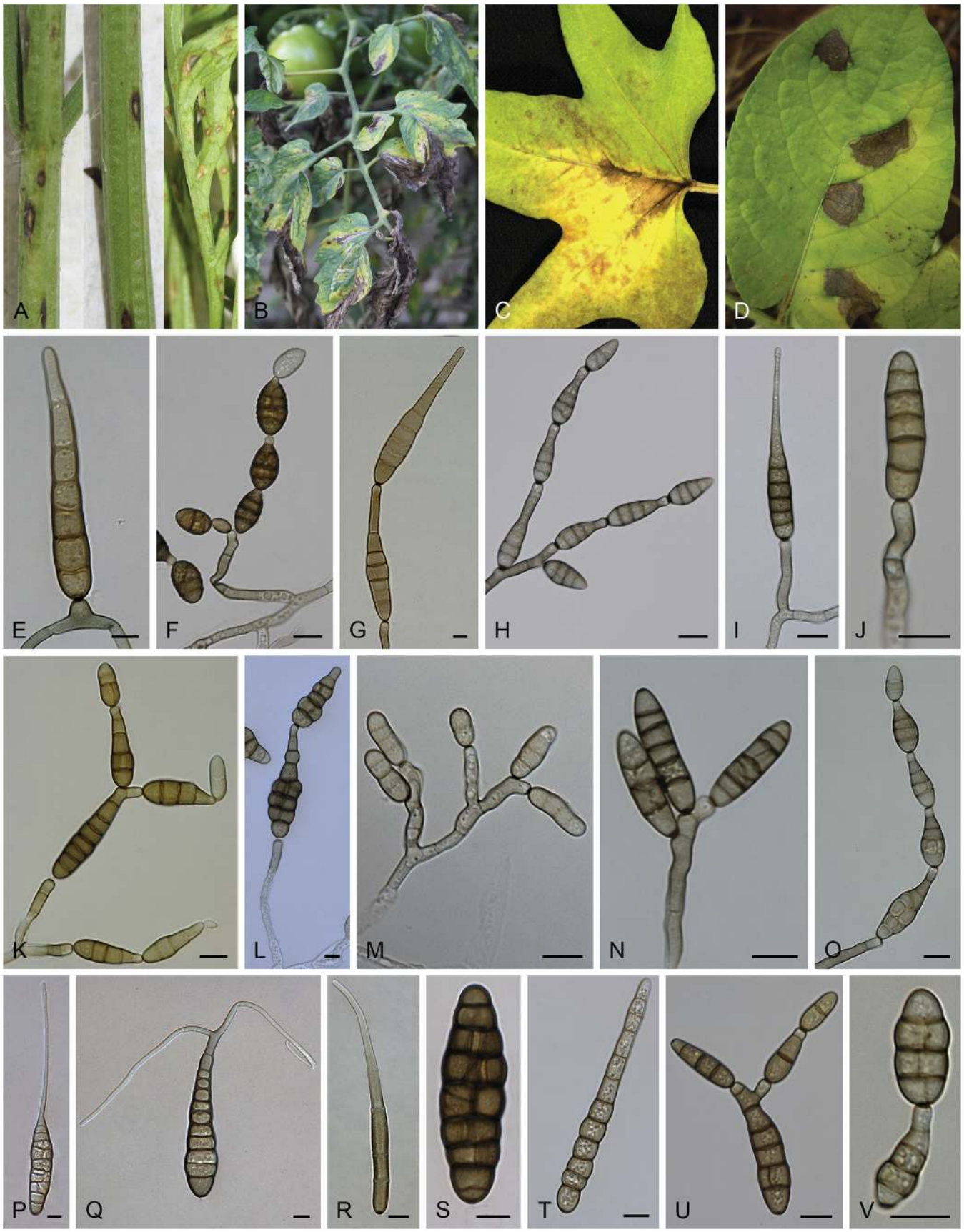

Fig. 4. *Alternaria* spp. **A–D.** Disease symptoms. **A.** *Alternaria dauci* on *Daucus carota*. **B.** *Alternaria linariae* on *Solanum lycopersicum*. **C.** *Alternaria neoipomoeae* on *Ipomoeae batatas* (Photo A.H. Thompson, ARC, South Africa). **D.** *Alternaria solani* on *Solanum tuberosum* (Photo J.E. van der Waals, University of Pretoria, South Africa). **E–V.** Asexual morph. **E–O.** Conidiophores. **E.** *Alternaria caricis*. **F.** *Alternaria chartarum*. **G.** *Alternaria cinerariae*. **H.** *Alternaria conjuncta*. **I.** *Alternaria elegans*. **J.** *Alternaria embellisia*. **K.** *Alternaria indefessa*. **L.** *Alternaria japonica*. **M.** *Alternaria penicillata*. **N.** *Alternaria proteae*. **O.** *Alternaria tenuissima*. **P–T.** Conidia. **P.** *Alternaria blumeae*. **Q.** *Alternaria calendulae*. **R.** *Alternaria perpunctulata*. **S.** *Alternaria carotiincultae*. **T.** *Alternaria triglochinicola*. **U, V.** Conidia producing secondary conidia. **U.** *Alternaria mimicola*. **V.** *Alternaria molesta*. Scale bars: 10 µm. Pictures A–D, P, Q taken from Woudenberg *et al.* (2014); E–O, R–V from Woudenberg *et al.* (2013).

Table 2. DNA barcodes of accepted *Alternaria* spp.

Species	Section	Isolates[1]	GenBank accession numbers[2]					References
			ITS	*gapdh*	*rpb2*	*tef1*	*ATPase*	
Alternaria abundans	*Chalastospora*	CBS 534.83^T	JN383485	KC584154	KC584448	KC584707	JQ671802	Woudenberg *et al.* (2013), Deng *et al.* (2018)
A. acalyphicola	*Porri*	CBS 541.94^T	KJ718097	KJ717952	KJ718271	KJ718446	–	Woudenberg *et al.* (2014)
A. aconidiophora	*Infectoriae*	FMR 17111^T	LR133931	LR133965	LR133967	LR133968	LR133969	Present study
A. agerati	*Porri*	CBS 117221	KJ718098	KJ717953	KJ718272	KJ718447	–	Woudenberg *et al.* (2014)
A. agripestis	*Porri*	CBS 577.94^T	KJ718099	JQ646356	KJ718273	KJ718448	–	Woudenberg *et al.* (2014)
A. allii	*Porri*	CBS 107.28^T	KJ718100	KJ717954	KJ718274	KJ718449	–	Woudenberg *et al.* (2014)
A. alstroemeriae	*Alternaria*	CBS 118809^T	KP124297	KP124154	KP124765	KP125072	–	Woudenberg *et al.* (2015)
A. altcampina	*Pseudoalternaria*	FMR 16476^T	LR133895	LR133900	–	–	LR133906	Present study
A. altemantherae	*Althernantherae*	CBS 124392	KC584179	KC584096	KC584374	KC584633	–	Woudenberg *et al.* (2013)
A. alternariacida	*Porri*	CBS 105.51^T	KJ718105	KJ717959	KJ718279	KJ718454	–	Woudenberg *et al.* (2014)
A. alternariae	*Ulocladium*	CBS 126989^T	AF229485	AY278815	KC584470	KC584730	–	Woudenberg *et al.* (2013)
A. alternarina	*Infectoriae*	CBS 119396^T	JQ693648	JQ646289	JQ905199	LR134367	JQ671817	Poursafar *et al.* (2018), Geng *et al.* (unpubl. data), present study
A. alternata	*Alternaria*	CBS 916.96^T	AF347031	AY278808	KC584375	KC584634	–	Woudenberg *et al.* (2013)
A. anagallidis	*Porri*	CBS 117128	KJ718106	JQ646338	KJ718280	EU130544	–	Woudenberg *et al.* (2014)
A. anigozanthi	*Eureka*	CBS 121920^T	KC584180	KC584097	KC584376	KC584635	–	Woudenberg *et al.* (2013)
A. anodae	*Porri*	PPRI 12376	KJ718110	KJ717963	KJ718284	KJ718458	–	Woudenberg *et al.* (2014)
A. aragakii	*Porri*	CBS 594.93^T	KJ718111	KJ717964	KJ718285	KJ718459	–	Woudenberg *et al.* (2014)
A. arborescens	*Alternaria*	CBS 102605^T	AF347033	AY278810	KC584377	KC584636	–	Woudenberg *et al.* (2013)
A. arbusti	*Infectoriae*	CBS 596.93^T	JQ693644	JQ646365	LR134184	–	JQ671940	Poursafar *et al.* (2018), present study
A. argyranthemi		CBS 116530^T	KC584181	KC584098	KC584378	KC584637	–	Woudenberg *et al.* (2013)
A. argyroxiphii	*Porri*	CBS 117222^T	KJ718112	JQ646350	KJ718286	KJ718460	–	Woudenberg *et al.* (2014)
A. armoraciae	*Chalastospora*	CBS 118702^T	KC584182	KC584099	KC584379	KC584638	LR134098	Woudenberg *et al.* (2013), present study
A. arrhenatheri	*Pseudoalternaria*	LEP 140372^T	JQ693677	JQ693635	–	–	JQ693603	Poursafar *et al.* (2018)
A. aspera	*Pseudoulocladium*	CBS 115269^T	KC584242	KC584166	KC584474	KC584734	–	Woudenberg *et al.* (2013)
A. atra	*Ulocladioides*	CBS 195.67^T	AF229486	KC584167	KC584475	KC584735	–	Woudenberg *et al.* (2013)
A. avenicola	*Panax*	CBS 121459^T	KC584183	KC584100	KC584380	KC584639	–	Woudenberg *et al.* (2013)
A. axiaeriisporifera	*Gypsophilae*	CBS 118715^T	KC584184	KC584101	KC584381	KC584640	–	Woudenberg *et al.* (2013)
A. azadirachtae	*Porri*	CBS 116444^T	KJ718115	KJ717967	KJ718289	KJ718463	–	Woudenberg *et al.* (2014)
A. bataticola	*Porri*	CBS 531.63^T	KJ718117	JQ646349	KJ718291	KJ718465	–	Woudenberg *et al.* (2014)
A. betae-kenyensis	*Alternaria*	CBS 118810^T	KP124419	KP124270	KP124888	KP125197	–	Woudenberg *et al.* (2015)

Table 2. (Continued).

Species	Section	Isolates[1]	GenBank accession numbers[2]					References
			ITS	*gapdh*	*rpb2*	*tef1*	*ATPase*	
A. blumeae	*Porri*	CBS 117364^T	KJ718126	AY562405	KJ718300	KJ718474	–	Woudenberg *et al.* (2014)
A. bornmuelleri	*Undifilum*	DAOM 231361^T	FJ357317	FJ357305	KC584491	KC584751	–	Woudenberg *et al.* (2013)
A. botryospora	*Embellisioides*	CBS 478.90^T	AY278844	AY278831	KC584461	KC584720	–	Woudenberg *et al.* (2013)
A. botrytis	*Ulocladium*	CBS 197.67^T	KC584243	KC584168	KC584476	KC584736	–	Woudenberg *et al.* (2013)
A. brassicae		CBS 116528	KC584185	KC584102	KC584382	KC584641	–	Woudenberg *et al.* (2013)
A. brassicaepekinensis	*Ulocladioides*	CBS 121493^T	KC584244	KC584170	KC584478	KC584738	–	Woudenberg *et al.* (2013)
A. brassicicola	*Brassicicola*	CBS 118699	JX499031	KC584103	KC584383	KC584642	–	Woudenberg *et al.* (2013)
A. brassicifolii	*Pseudoalternaria*	CNU 111118^T	JQ317188	KM821537	–	–	KY412558	Deng *et al.* (2018)
A. breviramosa	*Chalastospora*	CBS 121331^T	FJ839608	KC584148	KC584442	KC584700	LR134099	Woudenberg *et al.* (2013), present study
A. broccoli-italicae	*Infectoriae*	CBS 118485^T	KM821536	KM821538	LR134194	LR134262	KY412557	Deng *et al.* (2018), present study
A. burnsii	*Alternaria*	CBS 107.38^T	KP124420	JQ646305	KP124889	KP125198	–	Woudenberg *et al.* (2015)
A. caespitosa	*Infectoriae*	CBS 177.80^T	KC584250	KC584178	KC584492	KC584752	LR134114	Woudenberg *et al.* (2013), present study
A. calendulae	*Porri*	CBS 224.76^T	KJ718127	KJ717977	KJ718301	KJ718475	–	Woudenberg *et al.* (2014)
A. californica	*Infectoriae*	CBS 119409^T	JQ693645	JQ646285	LR134181	LR134245	JQ671813	Poursafar *et al.* (2018), present study
A. calycipyricola	*Panax*	CBS 121545^T	KC584186	KC584104	KC584384	KC584643	–	Woudenberg *et al.* (2013)
A. cantlous	*Ulocladioides*	CBS 123007^T	KC584245	KC584171	KC584479	KC584739	–	Woudenberg *et al.* (2013)
A. capsici-annui	*Ulocladium*	CBS 504.74	KC584187	KC584105	KC584385	KC584644	–	Woudenberg *et al.* (2013)
A. caricis	*Nimbya*	CBS 480.90^T	AY278839	AY278826	KC584467	KC584726	JQ671780	Woudenberg *et al.* (2013), Deng *et al.* (2018)
A. carotiincultae	*Radicina*	CBS 109381^T	KC584188	KC584106	KC584386	KC584645	–	Woudenberg *et al.* (2013)
A. carthami	*Porri*	CBS 635.80	KJ718131	KJ717981	KJ718305	KJ718479	–	Woudenberg *et al.* (2014)
A. carthamicola	*Porri*	CBS 117092^T	KJ718134	KJ717984	KJ718308	KJ718482	–	Woudenberg *et al.* (2014)
A. cassiae	*Porri*	CBS 478.81	KJ718135	KJ717985	KJ718309	KJ718483	–	Woudenberg *et al.* (2014)
A. catananches	*Porri*	CBS 137456^T	KJ718139	KJ717989	KJ718313	KJ718487	–	Woudenberg *et al.* (2014)
A. centaureae	*Porri*	CBS 116446^T	KJ718140	KJ717990	KJ718314	KJ718488	–	Woudenberg *et al.* (2014)
A. cerasidanica	*Infectoriae*	CBS 121923^T	LR135744	LR135747	LR135746	LR135745	LR135748	Present study
A. cesenica	*Infectoriae*	MFLUCC 13-0450^T	KP711383	–	–	KP711386	–	Liu *et al.* (2015)
A. cetera	*Chalastospora*	CBS 121340^T	JN383482	AY562398	KC584441	KC584699	LR134101	Woudenberg *et al.* (2013), Deng *et al.* (2018)
A. chartarum	*Pseudoulocladium*	CBS 200.67^T	AF229488	KC584172	KC584481	KC584741	–	Woudenberg *et al.* (2013)
A. cheiranthi	*Cheiranthus*	CBS 109384	AF229457	KC584107	KC584387	KC584646	–	Woudenberg *et al.* (2013)
A. chlamydospora	*Phragmosporae*	CBS 491.72^T	KC584189	KC584108	KC584388	KC584647	–	Woudenberg *et al.* (2013)

(*continued on next page*)

Table 2. (Continued).

Species	Section	Isolates[1]	GenBank accession numbers[2]					References
			ITS	*gapdh*	*rpb2*	*tef1*	*ATPase*	
A. chlamydosporigena	*Embellisia*	CBS 341.71	KC584231	KC584156	KC584451	KC584710	–	Woudenberg *et al.* (2013)
A. chlamydosporifera	*Radicina*	FMR 17360^T	LR133924	LR133927	LR133926	LR133929	–	Present study
A. cichorii	*Porri*	CBS 102.33^T	KJ718141	KJ717991	KJ718315	KJ718489	–	Woudenberg *et al.* (2014)
A. cinerariae	*Sonchi*	CBS 116495	KC584190	KC584109	KC584389	KC584648	–	Woudenberg *et al.* (2013)
A. cirsinoxia	*Porri*	CBS 113261^T	KJ718143	KJ717993	KJ718317	KJ718491	–	Woudenberg *et al.* (2014)
A. citrullicola	*Porri*	CBS 103.32^T	KJ718144	KJ717994	KJ718318	KJ718492	–	Woudenberg *et al.* (2014)
A. concatenata	*Pseudoulocladium*	CBS 120006^T	KC584246	AY762950	KC584480	KC584740	–	Woudenberg *et al.* (2013)
A. conidiophora	*Porri*	CBS 137457^T	KJ718145	KJ717995	-	KJ718493	–	Woudenberg *et al.* (2014)
A. conjuncta	*Infectoriae*	CBS 196.86^T	FJ266475	AY562401	KC584390	KC584649	JQ671824	Woudenberg *et al.* (2013), Poursafar *et al.* (2018)
A. conoidea	*Brassicicola*	CBS 132.89	AF348226	FJ348227	KC584452	KC584711	–	Woudenberg *et al.* (2013)
A. consortialis	*Ulocladioides*	CBS 104.31^T	KC584247	KC584173	KC584482	KC584742	–	Woudenberg *et al.* (2013)
A. crassa	*Porri*	CBS 110.38^T	KJ718147	KJ717997	KJ718320	KJ718495	–	Woudenberg *et al.* (2014)
A. cyamopsidis	*Porri*	CBS 364.67	KJ718156	KJ718003	KJ718329	KJ718504	–	Woudenberg *et al.* (2014)
A. cumini	*Eureka*	CBS 121329^T	KC584191	KC584110	KC584391	KC584650	–	Woudenberg *et al.* (2013)
A. cucumerina	*Porri*	CBS 116114^T	KJ718153	KJ718000	KJ718326	KJ718501	–	Woudenberg *et al.* (2014)
A. cucurbitae	*Ulocladioides*	CBS 483.81	FJ266483	AY562418	KC584483	KC584743	–	Woudenberg *et al.* (2013)
A. curvata	*Infectoriae*	FMR 16901^T	LR133898	LR133899	LR133901	LR133902	LR133905	Present study
A. dactylidicola	*Infectoriae*	MFLUCC 15-0466^T	KY703616	–	KY750720	–	–	Thambugala *et al.* (2017)
A. dauci	*Porri*	CBS 111.38^T	KJ718158	KJ718005	KJ718331	KJ718506	–	Woudenberg *et al.* (2014)
A. daucicaulis	*Infectoriae*	CBS 119398^T	JQ693653	JQ646294	LR134177	LR134241	JQ671822	Poursafar *et al.* (2018), present study
A. dennisii		CBS 476.90^T	JN383488	JN383469	KC584454	KC584713	–	Woudenberg *et al.* (2013)
A. deserticola	*Porri*	CBS 110799^T	KJ718249	KJ718077	KJ718424	KJ718595	–	Woudenberg *et al.* (2014)
A. dianthicola	*Dianthicola*	CBS 116491	KC584194	KC584113	KC584394	KC584653	–	Woudenberg *et al.* (2013)
A. dichondrae	*Porri*	CBS 200.74^T	KJ718167	KJ718012	KJ718340	KJ718515	–	Woudenberg *et al.* (2014)
A. didymospora	*Phragmosporae*	CBS 766.79	FJ357312	FJ357300	KC584455	KC584714	JQ671796	Woudenberg *et al.* (2013), Deng *et al.* (2018)
A. doliconidium	*Alternaria*	KUMCC 17-0263^T	MG828864	–	–	–	–	Wanasinghe *et al.* (2018)
A. echinaceae	*Porri*	CBS 116117^T	KJ718170	KJ718015	KJ718343	KJ718518	–	Woudenberg *et al.* (2014)
A. eichhorniae	*Alternaria*	CBS 489.92^T	KC146356	KP124276	KP124895	KP125204	–	Woudenberg *et al.* (2015)
A. elegans	*Dianthicola*	CBS 109159^T	KC584195	KC584114	KC584395	KC584654	–	Woudenberg *et al.* (2013)
A. ellipsoidea	*Gypsophilae*	CBS 119674^T	KC584196	KC584115	KC584396	KC584655	–	Woudenberg *et al.* (2013)
A. embellisia	*Embellisia*	CBS 339.71	KC584230	KC584155	KC584449	KC584708	–	Woudenberg *et al.* (2013)

Table 2. (Continued).

Species	Section	Isolates[1]	GenBank accession numbers[2]					References
			ITS	*gapdh*	*rpb2*	*tef1*	*ATPase*	
A. eryngii	*Panax*	CBS 121339	JQ693661	AY562416	KC584397	KC584656	–	Woudenberg *et al.* (2013)
A. ethzedia	*Infectoriae*	CBS 197.86^T	AY278833	AY278795	KC584398	KC584657	JQ671805	Woudenberg *et al.* (2013), Poursafar *et al.* (2018)
A. euphorbiicola	*Euphorbiicola*	CBS 119410	KJ718173	KJ718018	KJ718346	KJ718521	–	Woudenberg *et al.* (2014)
A. eureka	*Eureka*	CBS 193.86^T	JN383490	JN383471	KC584456	KC584715	–	Woudenberg *et al.* (2013)
A. fimeti	*Infectoriae*	FMR 17110^T	LR133920	LR133921	LR133923	LR133922	LR133925	Present study
A. forlicesenensis	*Infectoriae*	MFLUCC 13-0456^T	KY769657	–	–	–	–	Thambugala *et al.* (2017)
A. frumenti	*Infectoriae*	CBS 119401^T	JQ693654	JQ646295	LR134172	LR134370	JQ671823	Poursafar *et al.* (2018), present study
A. gaisen	*Alternaria*	CBS 632.93	KC584197	KC584116	KC584399	KC584658	–	Woudenberg *et al.* (2013)
A. geniostomatis	*Eureka*	CBS 118701^T	KC584198	KC584117	KC584400	KC584659	–	Woudenberg *et al.* (2013)
A. gossypina	*Alternaria*	CBS 104.32^T	KP124430	JQ646312	KP124900	KP125209	–	Woudenberg *et al.* (2015)
A. graminicola	*Infectoriae*	CBS 119400^T	JQ693650	JQ646291	LR134180	LR134249	JQ671819	Poursafar *et al.* (2018), present study
A. grandis	*Porri*	CBS 109158^T	KJ718239	JQ646341	KJ718414	EU130547	–	Woudenberg *et al.* (2014)
A. gypsophilae	*Gypsophilae*	CBS 107.41^T	KC584199	KC584118	KC584401	KC584660	–	Woudenberg *et al.* (2013)
A. hampshirensis	*Infectoriae*	MFLUCC 17-0783^T	MG828866	–	MG829247	–	–	Wanasinghe *et al.* (2018)
A. helianthiinficiens		CBS 208.86^T	JX101649	KC584120	KC584403	EU130548	–	Woudenberg *et al.* (2013)
A. heterospora	*Ulocladioides*	CBS 123376^T	KC584248	KC584176	KC584488	KC584748	–	Woudenberg *et al.* (2013)
A. hordeiaustralica	*Infectoriae*	CBS 119402^T	JQ693641	JQ646283	LR134179	LR134243	JQ671811	Poursafar *et al.* (2018), present study
A. hordeicola	*Infectoriae*	CBS 121458^T	JQ693642	JQ646284	LR134175	LR134371	JQ671812	Poursafar *et al.* (2018), present study
A. humuli	*Infectoriae*	CBS 119404^T	JQ693652	JQ646293	LR134174	LR134199	JQ671821	Poursafar *et al.* (2018), present study
A. hyacinthi	*Embellisioides*	CBS 416.71^T	KC584233	KC584158	KC584457	KC584716	–	Woudenberg *et al.* (2013)
A. incomplexa	*Infectoriae*	CBS 121330^T	JQ693658	JQ646287	LR134185	LR134250	JQ671815	Poursafar *et al.* (2018), present study
A. indefessa	*Cheiranthus*	CBS 536.83^T	KC584234	KC584159	KC584458	KC584717	–	Woudenberg *et al.* (2013)
A. infectoria	*Infectoriae*	CBS 210.86^T	AF347034	AY278793	KC584404	KC584662	JQ671804	Woudenberg *et al.* (2013), Poursafar *et al.* (2018)
A. inflata	*Pseudoalternaria*	FMR 16477 ^{T}	LR133930	LR133938	–	–	LR133966	Present study
A. intercepta	*Infectoriae*	CBS 119406^T	JQ693656	JQ646297	LR134170	–	JQ671826	Poursafar *et al.* (2018), present study
A. ipomoeae	*Porri*	CBS 219.79^T	KJ718175	KJ718020	KJ718348	KJ718523	–	Woudenberg *et al.* (2014)
A. iridiaustralis	*Alternaria*	CBS 118486^T	KP124435	KP124284	KP124905	KP125214	–	Woudenberg *et al.* (2015)
A. japonica	*Japonicae*	CBS 118390	KC584201	KC584121	KC584405	KC584663	–	Woudenberg *et al.* (2013)
A. jacinthicola	*Alternaria*	CBS 133751^T	KP124438	KP124287	KP124908	KP125217	–	Woudenberg *et al.* (2015)
A. jesenskae	*Porri*	CBS 133855^T	KJ718177	KJ718022	KJ718350	KJ718525	–	Woudenberg *et al.* (2014)

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Table 2. (Continued).

Species	Section	Isolates[1]	GenBank accession numbers[2]					References
			ITS	*gapdh*	*rpb2*	*tef1*	*ATPase*	
A. juxtiseptata	*Gypsophilae*	CBS 119673^T	KC584202	KC584122	KC584406	KC584664	–	Woudenberg *et al.* (2013)
A. kordkuyana	*Pseudoalternaria*	IRAN 16888F^T	MF033843	MF033826	–	–	MF033860	Poursafar *et al.* (2018)
		FMR 17061	LR133970	LR133998	–	–	LR134001	Present study
		FMR 17372	LR133995	LR133997	–	–	LR133999	Present study
A. kulundii	*Soda*	CBS 137525^T	KJ443262	KJ649618	KJ443176	KJ443219	–	Grum-Grzhimaylo *et al.* (2016)
A. lawrencei	*Infectoriae*	FMR 17004^T	LR133907	LR133908	LR133911	LR133912	LR133914	Present study
A. leptinellae	*Eureka*	CBS 477.90^T	KC584235	KC584160	KC584459	KC584718	–	Woudenberg *et al.* (2013)
A. leucanthemi	*Teretispora*	CBS 421.65^T	KC584240	KC584164	KC584472	KC584732	–	Woudenberg *et al.* (2013)
A. limaciformis	*Phragmosporae*	CBS 481.81^T	KC584203	KC584123	KC584407	KC584665	JQ671798	Woudenberg *et al.* (2013), Deng *et al.* (2018)
A. limicola	*Euphorbiicola*	CBS 483.90^T	KJ718178	JQ646329	KJ718351	KJ718526	–	Woudenberg *et al.* (2014)
A. linariae	*Porri*	CBS 105.41^T	KJ718180	KJ718024	KJ718353	KJ718528	–	Woudenberg *et al.* (2014)
A. longipes	*Alternaria*	CBS 540.94	AY278835	AY278811	KC584409	KC584667	–	Woudenberg *et al.* (2013)
A. lolii	*Embellisioides*	CBS 115266^T	JN383492	JN383473	KC584460	KC584719	–	Woudenberg *et al.* (2013)
A. macrospora	*Porri*	CBS 117228^T	KC584204	KC584124	KC584410	KC584668	–	Woudenberg *et al.* (2013)
A. malorum	*Chalastospora*	CBS 135.31	JQ693638	JQ646278	–	–	JQ671800	Poursafar *et al.* (2018)
		FMR 17369	LR134074	LR134077	–	–	LR134029	Present study
A. merytae	*Infectoriae*	CBS 119403^T	JQ693651	JQ646292	LR134119	LR134198	JQ671820	Poursafar *et al.* (2018), present study
A. metachromatica	*Infectoriae*	CBS 553.94^T	JQ693660	AY562404	JQ905189	FJ214931	JQ671809	Andersen *et al.* (2009), Poursafar *et al.* (2018), Geng *et al.* (unpubl. data)
A. mimicula	*Brassicicola*	CBS 118696^T	FJ266477	AY562415	KC584411	KC584669	–	Woudenberg *et al.* (2013)
A. molesta	*Phragmosporae*	CBS 548.81^T	KC584205	KC584125	KC584412	KC584670	–	Woudenberg *et al.* (2013)
A. montanica	*Porri*	CBS 121343^T	KJ718194	KJ718033	KJ718367	KJ718541	–	Woudenberg *et al.* (2014)
A. montsantina	*Infectoriae*	FMR 17060^T	LR133913	LR133915	LR133918	LR133919	LR133916	Present study
A. mouchaccae	*Phragmosporae*	CBS 119671^T	KC584206	AY562399	KC584413	KC584671	–	Woudenberg *et al.* (2013)
A. multiformis	*Ulocladioides*	CBS 102060^T	FJ266486	KC584174	KC584484	KC584744	–	Woudenberg *et al.* (2013)
A. multirostrata	*Porri*	CBS 712.68^T	KJ718195	JQ646362	KJ718368	EU130546	–	Woudenberg *et al.* (2014)
A. murispora	*Infectoriae*	MFLU 14-0758^T	NR_137964	–	–	–	–	Ariyawansa and Hyde (unpubl. data)
A. neoipomoeae	*Porri*	PPRI 11845^T	KJ718198	KJ718036	KJ718371	KJ718544	–	Woudenberg *et al.* (2014)
A. nepalensis	*Japonicae*	CBS 118700^T	KC584207	KC584126	KC584414	KC584672	–	Woudenberg *et al.* (2013)
A. nitrimali	*Porri*	CBS 109163^T	KJ718201	JQ646358	KJ718374	KJ718547	–	Woudenberg *et al.* (2014)
A. nobilis	*Gypsophilae*	CBS 116490	KC584208	KC584127	KC584415	KC584673	–	Woudenberg *et al.* (2013)

Table 2. (Continued).

Species	Section	Isolates[1]	GenBank accession numbers[2]					References
			ITS	*gapdh*	*rpb2*	*tef1*	*ATPase*	
A. novae-guineensis	*Porri*	CBS 116120^T	KJ718202	KJ718039	KJ718375	KJ718548	–	Woudenberg *et al.* (2014)
A. novae-zelandiae	*Infectoriae*	CBS 119405^T	JQ693655	JQ646296	LR134120	LR134197	JQ671825	Poursafar *et al.* (2018), present study
A. obclavata	*Chalastospora*	CBS 124120^T	KC584225	KC584149	KC584443	KC584701	LR134100	Woudenberg *et al.* (2013), present study
A. obovoidea	*Ulocladioides*	CBS 101229	FJ266487	FJ266498	KC584485	KC584745	–	Woudenberg *et al.* (2013)
A. obtecta	*Porri*	CBS 117367	KJ718204	KJ718041	KJ718377	KJ718550	–	Woudenberg *et al.* (2014)
A. oregonensis	*Infectoriae*	CBS 542.94^T	FJ266478	FJ266491	KC584416	KC584674	JQ671827	Woudenberg *et al.* (2013), Poursafar *et al.* (2018)
A. oudemansii	*Ulocladium*	CBS 114.07^T	FJ266488	KC584175	KC584486	KC584746	–	Woudenberg *et al.* (2013)
A. panax	*Panax*	CBS 482.81	KC584209	KC584128	KC584417	KC584675	–	Woudenberg *et al.* (2013)
A. papavericola	*Crivellia*	CBS 116606^T	FJ357310	FJ357298	KC584446	KC584705	–	Woudenberg *et al.* (2013)
A. paralinicola	*Porri*	CBS 116652^T	KJ718206	KJ718043	KJ718379	KJ718552	–	Woudenberg *et al.* (2014)
A. passiflorae	*Porri*	CBS 113.38	KJ718207	JQ646353	KJ718380	KJ718553	–	Woudenberg *et al.* (2014)
A. parvicaespitosa	*Pseudoaltemaria*	LEP 014858^T	MF033859	MF033842	–	–	KJ908217	Poursafar *et al.* (2018)
A. penicillata	*Crivellia*	CBS 116608^T	FJ357311	FJ357299	KC584440	KC584698	–	Woudenberg *et al.* (2013)
A. perpunctulata	*Althernantherae*	CBS 115267^T	KC584210	KC584129	KC584418	KC584676	–	Woudenberg *et al.* (2013)
A. petroselini	*Radicina*	CBS 112.41^T	KC584211	KC584130	KC584419	KC584677	–	Woudenberg *et al.* (2013)
A. petuchovskii	*Soda*	CBS 137517^T	KJ443254	KJ649616	KJ443170	KJ443211	–	Grum-Grzhimaylo *et al.* (2016)
A. peucedani		CNU 111485^T	KF728231	KF889361	–	–	–	Deng *et al.* (2014)
A. photistica	*Panax*	CBS 212.86^T	KC584212	KC584131	KC584420	KC584678	JQ671807	Woudenberg *et al.* (2013), Deng *et al.* (2018)
A. phragmospora	*Phragmosporae*	CBS 274.70^T	JN383493	JN383474	KC584462	KC584721	–	Woudenberg *et al.* (2013)
A. pipionipisi	*Porri*	CBS 116115^T	KJ718214	KJ718049	KJ718387	KJ718560	–	Woudenberg *et al.* (2014)
A. planifunda	*Embellisioides*	CBS 537.83^T	FJ357315	FJ357303	KC584463	KC584722	–	Woudenberg *et al.* (2013)
A. poaceicola	*Infectoriae*	MFLUCC 13-0346^T	KY026587	–	KY460971	–	–	Thambugala *et al.* (2017)
A. pobletensis	*Chalastospora*	FMR 16448^T	LR133896	LR133897	–	–	LR133903	Present study
A. porri	*Porri*	CBS 116699^T	KJ718218	KJ718053	KJ718391	KJ718564	–	Woudenberg *et al.* (2014)
A. proteae	*Embellisioides*	CBS 475.90^T	AY278842	KC584161	KC584464	KC584723	–	Woudenberg *et al.* (2013)
A. protenta	*Porri*	CBS 116696	KJ718221	JQ646335	KJ718394	KJ718567	–	Woudenberg *et al.* (2014)
A. pseudorostrata	*Porri*	CBS 119411^T	JN383483	AY562406	KC584422	KC584680	–	Woudenberg *et al.* (2013)
A. pseudoventricosa	*Infectoriae*	FMR 16900^T	LR133928	LR133935	LR133934	LR133936	LR133937	Present study
A. radicina	*Radicina*	CBS 245.67^T	KC584213	KC584133	KC584423	KC584681	–	Woudenberg *et al.* (2013)
A. ranunculi	*Porri*	CBS 116330^T	KJ718225	KJ718058	KJ718398	KJ718571	–	Woudenberg *et al.* (2014)

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Table 2. (Continued).

Species	Section	Isolates[1]	GenBank accession numbers[2]					References
			ITS	*gapdh*	*rpb2*	*tef1*	*ATPase*	
A. ricini	*Porri*	CBS 215.31^T	KJ718226	KJ718059	KJ718399	KJ718572	–	Woudenberg *et al.* (2014)
A. rosae	*Pseudoalternaria*	CBS 121341^T	JQ646279	JQ646279	–	–	JQ671803	Poursafar *et al.* (2018)
		FMR 15720	LR134076	LR134070	–	–	LR134004	Present study
		FMR 17376	LR134071	LR13403	–	–	LR134003	Present study
		FMR 17377	LR134073	LR134072	–	–	LR134028	Present study
A. roseogrisea	*Infectoriae*	CBS 121921^T	LR134102	LR134103	LR134192	LR134260	LR134104	Present study
A. rostellata	*Porri*	CBS 117366^T	KJ718229	JQ646332	KJ718402	KJ718575	–	Woudenberg *et al.* (2014)
A. saponariae	*Gypsophilae*	CBS 116492	KC584215	KC584135	KC584425	KC584683	–	Woudenberg *et al.* (2013)
A. scirpicola	*Nimbya*	CBS 481.90	KC584237	KC584163	KC584469	KC584728	JQ671781	Woudenberg *et al.* (2013), Deng *et al.* (2018)
A. scirpinfestans	*Nimbya*	EGS 49-185^T	JN383499	JN383480	–	JQ672404	JQ671783	Lawrence *et al.* (2012), Lawrence *et al.* (unpubl. data)
A. scirpivora	*Nimbya*	EGS 50-021^T	JN383500	JN383481	–	JQ672405	JQ671782	Lawrence *et al.* (2012), Lawrence *et al.* (unpubl. data)
A. scorzonerae	*Porri*	CBS 103.46	KJ718190	JQ646363	KJ718363	KJ718537	–	Woudenberg *et al.* (2014)
A. selini	*Radicina*	CBS 109382^T	AF229455	AY278800	KC584426	KC584684	–	Woudenberg *et al.* (2013)
A. sennae	*Porri*	CBS 477.81^T	KJ718230	JQ646344	KJ718403	EU130543	–	Woudenberg *et al.* (2014)
A. septospora	*Pseudoulocladium*	CBS 109.38	FJ266489	FJ266500	KC584487	KC584747	–	Woudenberg *et al.* (2013)
A. septorioides	*Brassicicola*	CBS 106.41^T	KC584216	KC584136	KC584427	KC584685	–	Woudenberg *et al.* (2013)
A. sesami	*Porri*	CBS 115264	JF780939	KJ718061	KJ718405	KJ718577	–	Woudenberg *et al.* (2014)
A. shukurtuzii	*Soda*	CBS 137520^T	KJ443257	KJ649620	KJ443172	KJ443214	–	Grum-Grzhimaylo *et al.* (2016)
A. sidae	*Porri*	CBS 117730^T	KJ718232	KJ718062	KJ718406	KJ718578	–	Woudenberg *et al.* (2014)
A. simsimi	*Dianthicola*	CBS 115265^T	JF780937	KC584137	KC584428	KC584686	–	Woudenberg *et al.* (2013)
A. silybi	*Porri*	CBS 134092^T	KJ718233	KJ718063	KJ718407	KJ718579	–	Woudenberg *et al.* (2014)
A. slovaca	*Infectoriae*	CBS 567.66^T	KC584226	KC584150	KC584444	KC584702	LR134368	Woudenberg *et al.* (2013), present study
A. smyrnii	*Radicina*	CBS 109380	AF229456	KC584138	KC584429	KC584687	–	Woudenberg *et al.* (2013)
A. solani	*Porri*	CBS 106.21	KJ718236	KJ718066	KJ718410	KJ718582	–	Woudenberg *et al.* (2014)
A. solani-nigri	*Porri*	CBS 113403	KJ718243	KJ718071	KJ718418	KJ718589	–	Woudenberg *et al.* (2014)
A. soliaridae		CBS 118387^T	KC584218	KC584140	KC584431	KC584689	–	Woudenberg *et al.* (2013)
A. solidaccana	*Brassicicola*	CBS 118698^T	KC584219	KC584141	KC584432	KC584690	–	Woudenberg *et al.* (2013)
A. sonchi	*Sonchi*	CBS 119675	KC584220	KC584142	KC584433	KC584691	–	Woudenberg *et al.* (2013)
A. steviae	*Porri*	CBS 117362^T	KJ718252	KJ718079	KJ718427	KJ718598	–	Woudenberg *et al.* (2014)
A. subcucurbitae	*Ulocladioides*	CBS 121491^T	KC584249	EU855803	KC584489	KC584749	–	Woudenberg *et al.* (2013)
A. tagetica	*Porri*	CBS 479.81	KC584221	KC584143	KC584434	KC584692	–	Woudenberg *et al.* (2013)

Table 2. (Continued).

Species	Section	Isolates[1]	GenBank accession numbers[2]					References
			ITS	*gapdh*	*rpb2*	*tef1*	*ATPase*	
A. tellustris	*Embellisia*	CBS 538.83^T	FJ357316	AY562419	KC584465	KC584724	JQ671794	Woudenberg *et al.* (2013), Deng *et al.* (2018)
A. terricola	*Ulocladioides*	CBS 202.67^T	FJ266490	KC584177	KC584490	KC584750	–	Woudenberg *et al.* (2013)
A. tillandsiae	*Porri*	CBS 116116^T	KJ718260	KJ718087	KJ718435	KJ718606	–	Woudenberg *et al.* (2014)
A. thalictrigena		CBS 121712^T	EU040211	KC584144	KC584436	KC584694	–	Woudenberg *et al.* (2013)
A. thunbergiae	*Porri*	CBS 116331^T	KJ718257	KJ718084	KJ718432	KJ718603	–	Woudenberg *et al.* (2014)
A. tomato	*Alternaria*	CBS 103.30	KP124445	KP124294	KP124915	KP125224	–	Woudenberg *et al.* (2015)
A. triglochinicola	*Eureka*	CBS 119676^T	KC584222	KC584145	KC584437	KC584695	–	Woudenberg *et al.* (2013)
A. triticimaculans	*Infectoriae*	CBS 578.94^T	JQ693657	JQ646280	LR134183	–	JQ671806	Poursafar *et al.* (2018), present study
A. triticina	*Infectoriae*	CBS 763.84^T	AY278834	JQ646281	LR134186	FJ214942	JQ671808	Andersen *et al.* (2009), Poursafar *et al.* (2018), Present study
A. tropica	*Porri*	CBS 631.93^T	KJ718261	KJ718088	KJ718436	KJ718607	–	Woudenberg *et al.* (2014)
A. tumida	*Embellisioides*	CBS 539.83^T	FJ266481	FJ266493	KC584466	KC584725	–	Woudenberg *et al.* (2013)
A. quercicola	*Infectoriae*	CBS 141466^T	KX228295	KX228362	LR134188	LR134259	LR134115	Crous *et al.* (2013), present study
A. vaccariae	*Gypsophilae*	CBS 116533	KC584223	KC584146	KC584438	KC584696	–	Woudenberg *et al.* (2013)
A. vaccariicola	*Gypsophilae*	CBS 118714^T	KC584224	KC584147	KC584439	KC584697	–	Woudenberg *et al.* (2013)
A. venezuelensis	*Porri*	CBS 116121^T	KJ718263	KJ718090	KJ718438	KJ718609	–	Woudenberg *et al.* (2014)
A. ventricosa	*Infectoriae*	CBS 121546^T	JQ693649	JQ646290	LR134134	KY352501	JQ671818	Poursafar *et al.* (2018), Fotedar *et al.* (unpubl. data), present study
A. viburni	*Infectoriae*	CBS 119407^T	JQ693647	JQ646288	LR134166	LR134200	JQ671816	Poursafar *et al.* (2018), present study
A. zinniae	*Porri*	CBS 117223	KJ718270	KJ718096	KJ718445	KJ718616	–	Woudenberg *et al.* (2014)

[1] CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CNU: Culture Collection Center of the Chungnam National University; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; EGS: Personal collection of Dr. E.G. Simmons; FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; IRAN: Fungal Culture Collections of the Iranian Research Institute of Plant Protection; KUMCC, Culture collection of Kunming Institute of Botany, Kunming, China; LEP: Mycological Herbarium of All-Russian Institute of Plant Protection, Saint Petersburg, Russia; MFLU and MFLUCC: Herbarium and culture collection of Mae Fah Luang University, Chiang Rai, Thailand, respectively; PPRI: ARC-Plant Protection Research Institute, Roodeplaat, South Africa. ^{T} indicates ex-type strains.

[2] ITS: internal transcribed spacers and intervening 5.8S nrDNA; *gapdh*: partial glyceraldehyde-3-phosphate dehydrogenase gene; *rpb2*: partial DNA-directed RNA polymerase II second largest subunit gene; *tef1*: partial translation elongation factor 1-alpha gene; *ATPase*: partial plasma membrane ATPase gene.

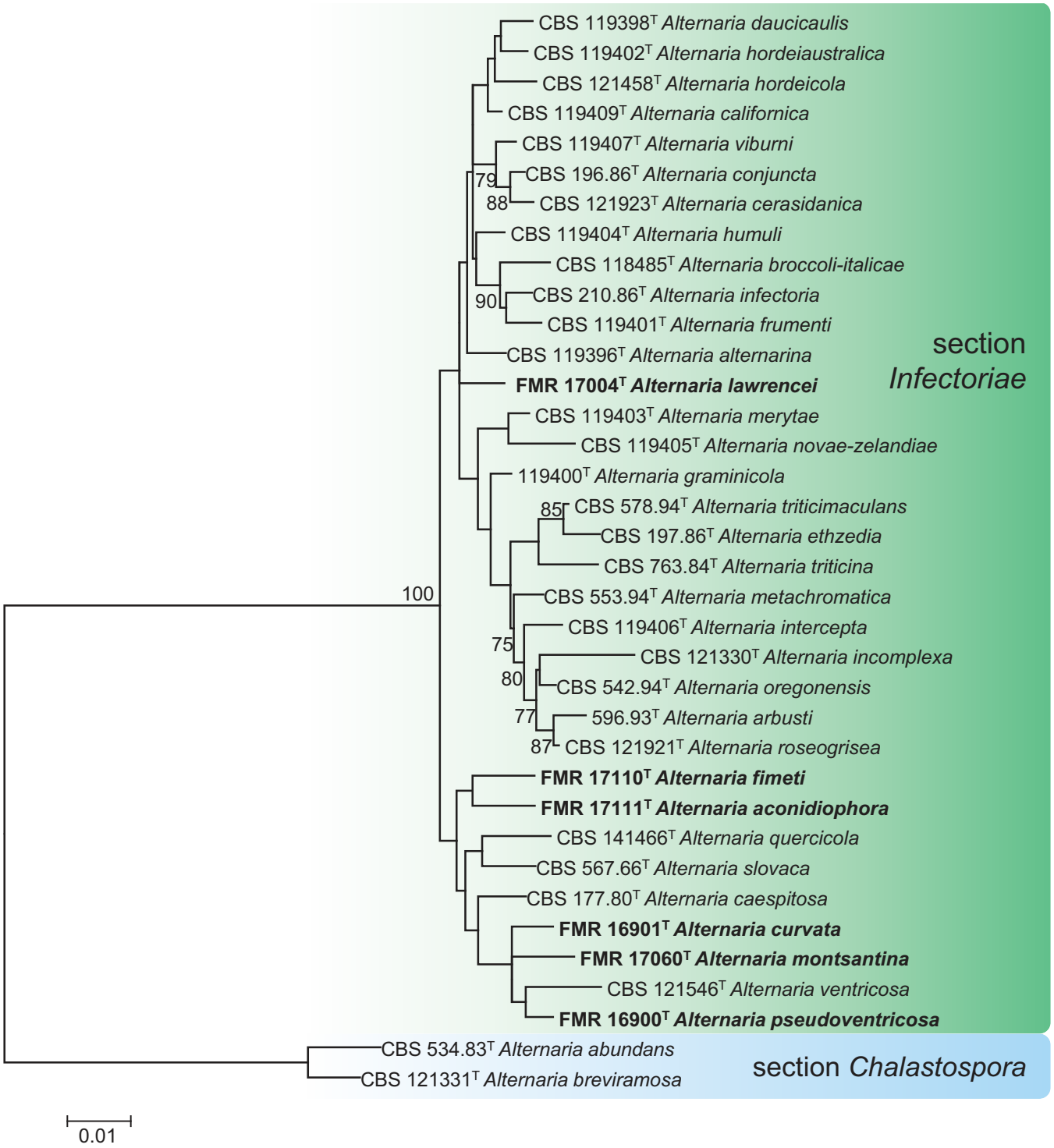

Fig. 5. Maximum Likelihood (ML) tree constructed with ITS (529 bp), *ATPase* (1180 bp), *gapdh* (489 bp), *rpb2* (573 bp) and *tef1* (239 bp) sequences of ex-type strains of the species in section *Infectoriae*. The phylogenetic tree was rooted to *Alternaria abundans* CBS 534.83 and *Alternaria breviramosa* CBS 121331 (section *Chalastospora*). Bootstrap support values above 70 % are shown at the nodes. GenBank accession numbers are indicated in Table 2. The novel species described in this study are indicated in **bold**. T indicates ex-type strain. TreeBASE: S23786.

seed-borne species may attack seedlings, resulting in damping-off, stem lesions or collar rot; sunken and dark lesions are present in roots, tubers, stems and fruits infections; some rots and decay are typical symptoms of post-harvest diseases (Laemmlen 2001, Thomma 2003, Lawrence *et al.* 2008). Phytotoxins are also produced during the invasion process as virulence factors which affect a wide spectrum of plant species. *Alternaria* toxins diffuse into host tissues resulting in a chlorotic or yellow halo around lesions, exacerbating the severity of the symptoms (Singh *et al.* 2015).

Notes: *Alternaria* is characterised mainly by its asexual morph with darkly pigmented multi-celled conidia, which are typically dictyosporous, some phragmosporous, and arranged single or in chains on the conidiophore. Some of these morphological features can also be observed in other closely related genera such as *Paradendryphiella* (Woudenberg *et al.* 2013) or *Stemphylium* (Woudenberg *et al.* 2017). However, *Paradendryphiella* mainly differs by its denticulate conidiogenous cells with prominent conidial scars aggregated at the apex of simple or branched conidiophores, and *Stemphylium* by showing

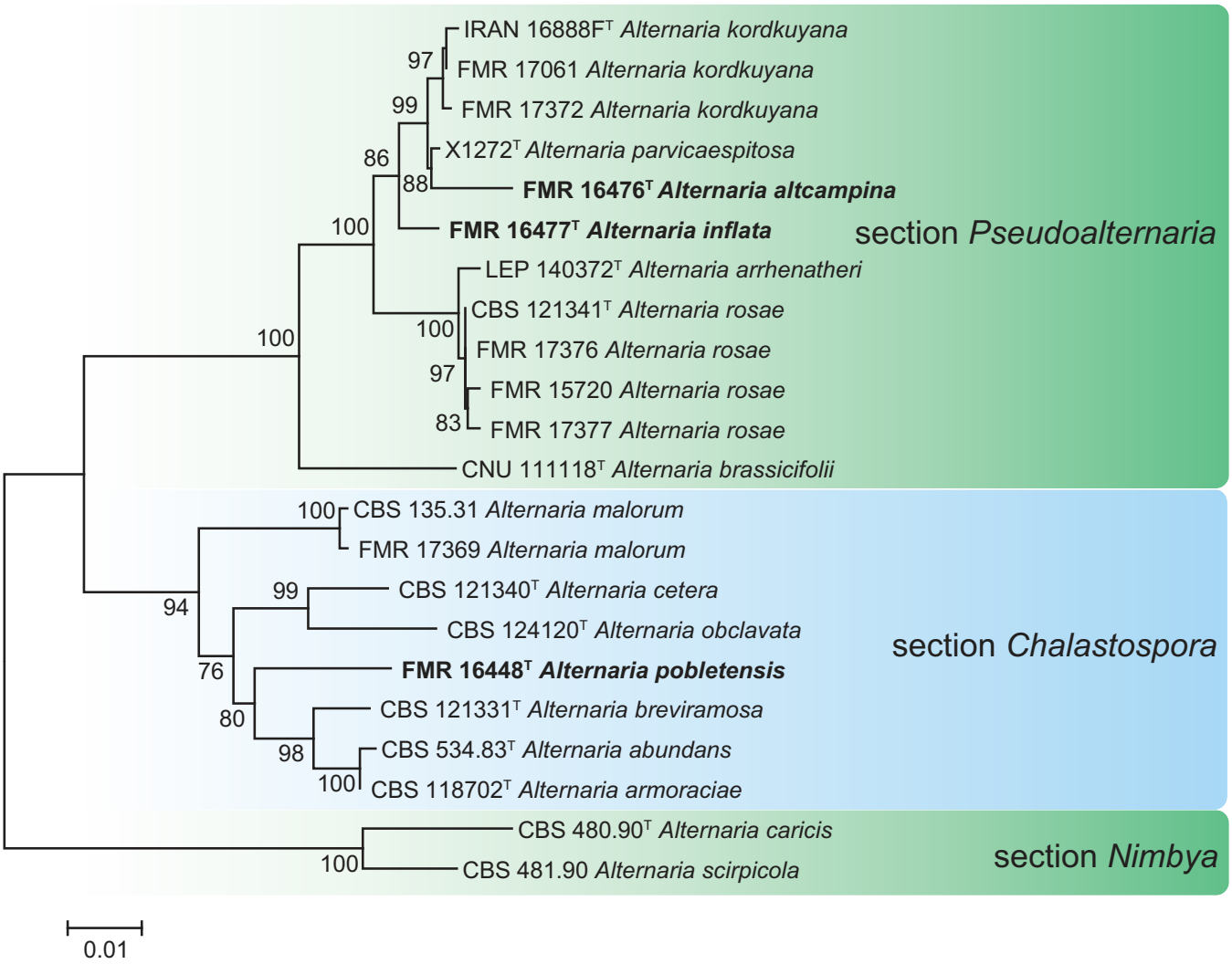

Fig. 6. Maximum Likelihood (ML) tree constructed with ITS (576 bp), *ATPase* (1198 bp) and *gapdh* (491 bp) sequences of ex-type strains of species in the sections *Pseudoalternaria* and *Chalastospora*. The phylogenetic tree was rooted to *Alternaria caricis* CBS 480.90 and *A. scirpicola* CBS 481.90 (section *Nimbya*). Bootstrap support values above 70 % are shown at the nodes. GenBank accession numbers are indicated in Table 2. The novel species described in this study are indicated in **bold**. ^{T} indicates ex-type strain. TreeBASE: S23787.

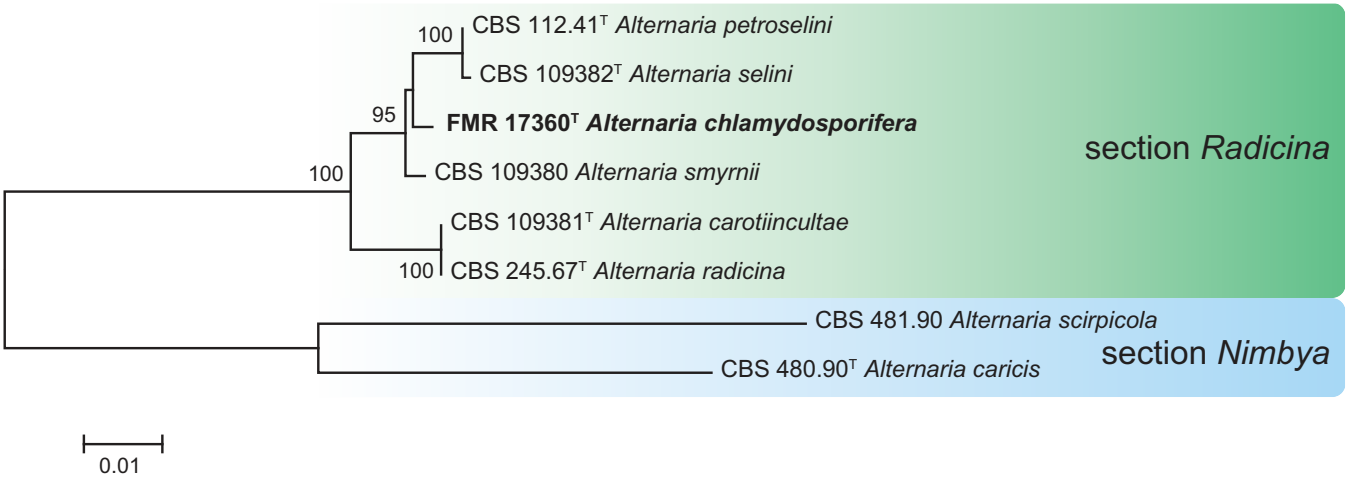

Fig. 7. Maximum Likelihood (ML) tree constructed with ITS (523 bp), *gapdh* (503 bp), *rpb2* (860 bp) and *tef1* (247 bp) sequences of ex-type strains of species in section *Radicina*. The phylogenetic tree was rooted to *Alternaria caricis* CBS 480.90 and *A. scirpicola* CBS 481.90 (section *Nimbya*). Bootstrap support values above 70 % are shown at the nodes. GenBank accession numbers are indicated in Table 2. The novel species described in this study are indicated in **bold**. ^{T} indicates ex-type strain. TreeBASE: S23788.

percurrent conidiophores with apically swollen conidiogenous cells.

Extensive morphological investigations of the genus *Alternaria* were carried out by Emory G. Simmons, which culminated with his monograph on *Alternaria* species identification (Simmons 2007). Based on the sporulation patterns and conidial morphology, he described several *Alternaria* species-groups which were typified by representative species (Simmons 1992). In recent years, based on molecular phylogenetic approaches using DNA sequence data, it has been shown that the main morphological groups identified by Simmons represent monophyletic species groups. Lawrence *et al.* (2013) provided the first strongly supported phylogenetic hypothesis among *Alternaria* lineages and elevated several of those monophyletic species groups to the taxonomic status of sections, each with a type species. Successive phylogenetic investigations added additional sections within the genus by synonymising genera such as *Allewia, Brachycladium, Chalastospora, Chmelia, Crivellia, Embellisia, Nimbya, Pseudoalternaria, Sinomyces, Teretispora, Ulocladium, Undiphilum* and *Ybotromyces* (Woudenberg *et al.* 2013, 2014, Lawrence *et al.* 2016). Therefore, the genus *Alternaria* currently comprises close to 280 species, most of them classified in 27 sections. Taxonomic traits and species composition of all *Alternaria* sections are summarised in Lawrence *et al.* (2016).

Considering, however, the overlap of morphological traits among *Alternaria* sections/species and that the culture conditions can greatly influence the morphology of these fungi, molecular identification is practically mandatory for the classification of *Alternaria* isolates. Although the ITS barcode is considered a good phylogenetic marker to define sections, it has limited discriminatory power to distinguish species, making multi-locus sequence analysis with several protein-coding loci essential for accurate species identification. While Woudenberg *et al.* (2013), in addition to the nrDNA regions, used the combination of *gapdh*, *rpb2* and *tef1* loci for redefining the genus, the combination of other phylogenetic markers has since been analysed to determine relationships and species delineation in studies on a particular section; *i.e.* ITS, *Alt a-1*, *endoPG, gapdh*, OPA10-2, *rpb2* and *tef1* for section *Alternaria* (Woudenberg *et al.* 2015); ITS, *ATPase*, *tef1* and *gapdh* for sections *Infectoriae* and *Pseudoalternaria* (Andersen *et al.* 2009, Deng *et al.* 2018, Poursafar *et al.* 2018); ITS, *Alt a-1*, *gapdh*, *rpb2* and *tef1* for section *Porri* (Woudenberg *et al.* 2014); and ITS, *Alt a-1* and *gapdh* for section *Sonchi* (Lawrence *et al.* 2012, Deng *et al.* 2014). Nevertheless, according to Lawrence *et al.* (2013) the plasma membrane *ATPase*, *cmdA*, and *Alt a-1* loci are the most informative markers for *Alternaria* species delimitation. However, considering that the latter locus unreliably amplifies some species within sect. *Infectoriae*, they suggested that the most suitable genetic markers for molecular identification at the species level are *ATPase* and *cmdA* genes (Lawrence *et al.* 2013, 2016). Unfortunately, the latter marker has not been used for the phylogeny of any of the above-mentioned sections.

Alternaria is a very successful pathogenic genus that causes disease on a great number of economically important plants, causing large economic losses due to the number of plant species affected and worldwide distributions of several *Alternaria* species (Meena *et al.* 2017). They are commonly described causing stem canker, leaf blight or leaf spot on a large variety of crops, including cereals, ornamentals, oil crops, vegetables such as broccoli, cauliflower, carrot, onion and potato, and fruits like apple, citrus, pear and strawberry, among others. Species in section *Alternaria*, such as *A. alternata*, *A. arborescens* or *A. tenuissima*, as well as others from sections *Alternantherae*, *Brassicicola*, *Crivellia*, *Gypsophilae*, *Nimbya*, *Radicina* or *Sonchi,* are frequently reported causing such diseases, but the largest group of phytopathogens in the genus is concentrated in section *Porri* (Lawrence *et al.* 2016, Meena *et al.* 2017). The most relevant plant pathogens in this latter section are *A. bataticola, A. porri, A. solani* and *A. tomatophila* (Woudenberg *et al.* 2014). *Alternaria* species also produce diverse phytotoxins, which affect their host plants at different stages of pathogenesis (Thomma 2003, Lawrence *et al.* 2008, Meena *et al.* 2017). Some of these phytotoxins have been evaluated by the European Food Safety Authority as potentially causing risks to human health (Meena *et al.* 2017).

In humans, *Alternaria* species are commonly associated with hypersensitivity pneumonitis, bronchial asthma, allergic sinusitis and rhinitis. To a lesser extent, they have been also described as causing paranasal sinusitis, ocular infections, onychomycosis, cutaneous and subcutaneous infections, granulomatous pulmonary disease, soft palate perforation and disseminated disease (Pastor & Guarro 2008, de Hoog *et al.* 2011).

In several surveys of microfungi from Spanish regions with different climates and biodiversity, samples of plant litter (leaves, bark and twigs) and dung of wild and farm herbivore animals (rabbits, rodents, goats, cattle and horses) were collected. From these samples, we found 16 interesting *Alternaria* isolates, belonging to sections *Infectoriae*, *Pseudoalternaria*, *Chalastospora* and *Radicina*. The multi-locus phylogenetic analysis based on five above-mentioned gene markers showed that 10 of them were undescribed species for the genus, and the others were identified as *A. kourtkuyana*, *A. rosae* and *A. malorum* (Figs 5–7). Most of these novel species have been isolated from herbivore dung, which appear to represent a reservoir of interesting *Alternaria* species which could represent potential plant pathogens.

References: Ellis 1976, Simmons 2007 (morphology); Laemmlen 2001, Thomma 2003, Lawrence *et al.* 2008, Meena *et al.* 2017 (plant infections); Pastor & Guarro 2008, de Hoog *et al.* 2011 (human infections); Woudenberg *et al.* 2013, 2014, 2015, Grum-Grzhimaylo *et al.* 2016, Lawrence *et al.* 2016, Poursafar *et al.* 2018 (morphology and phylogeny).

Alternaria aconidiophora Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829626. Fig. 8.

Etymology: Name refers to the lack of conidiophores from vegetative hyphae.

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 1–4 µm wide, septate, branched, hyaline to greyish, smooth-walled. *Conidiophores* absent. *Conidiogenous loci* inconspicuous on vegetative hyphae, scarce. *Conidia* commonly solitary at centre of the colony, globose, ovoid, near ellipsoid or obclavate, 12–31 × 7–12 µm, with some darkened middle transverse septa, 1–5 transverse, 0–1(–2) longitudinal or oblique septa per transverse segment, brown, smooth-walled. *Secondary conidiophores* present, may be formed apically from the conidial body as a short extension often geniculate, with one or two, terminal or subterminal conidiogenous loci. *Sexual morph* not observed.

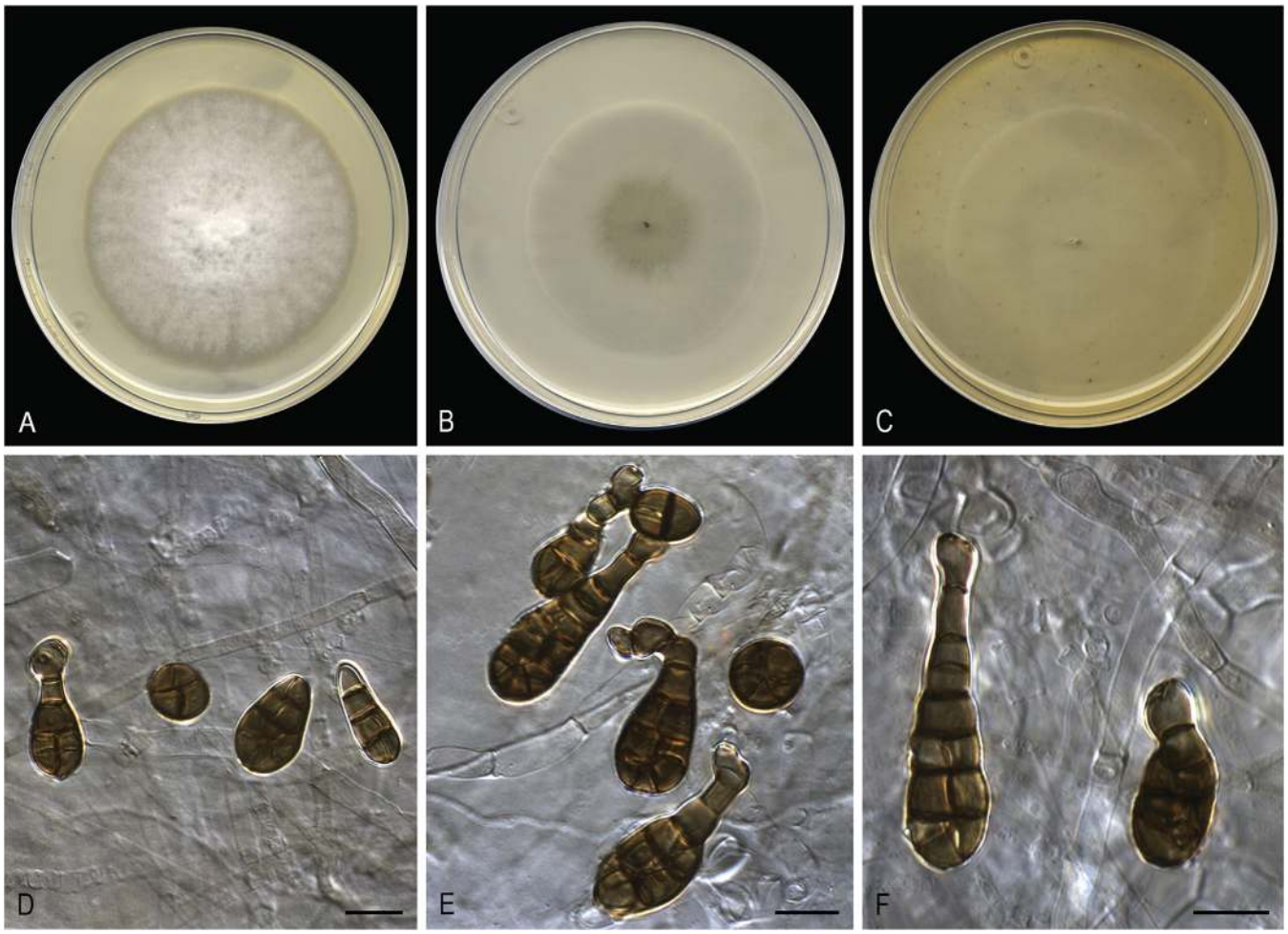

Fig. 8. *Alternaria aconidiophora* (ex-type FMR 17111). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–F.** Conidia. Scale bars = 10 µm.

Culture characteristics: Colonies on PDA reaching 64 mm diam after 1 wk at 25 °C, flat, cottony at centre, slightly radially folded towards the periphery, aerial mycelium abundant, margins regular; surface white (1A1); reverse yellowish white (4A2). On PCA attaining 54 mm diam, flat, aerial mycelium scarce, margins regular; surface greyish green to greenish grey (1D3/1B1); reverse greenish grey (1C2/1B1). On OA reaching 61 mm diam, flat, aerial mycelium scarce, margins regular; surface and reverse colourless.

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Typus: **Spain**, Catalonia, Alta Ribagorça, Vall de Boí, isolated from forest leaf litter, Dec. 2017, J. Gené (**holotype** CBS H-23891, culture ex-type CBS 145419 = FMR 17111).

Notes: *Alternaria aconidiophora* together with *A. fimeti*, both species introduced here from herbivore dung, are placed in an unsupported clade in *Alternaria* section *Infectoriae* (Fig. 5). Morphologically, the latter differs from *A. aconidiophora* in having conspicuous sporulation with well-differentiated conidiophores and verrucose conidia up to 44 µm long. The conidia of *A. aconidiophora* are smooth-walled and 12–31 µm long.

Alternaria altcampina Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829627. Fig. 9.

Etymology: Name refers to the region of Alt Camp (Catalonia), from where the fungus was collected.

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 2–4 µm wide, branched, pale yellowish brown to brown, septate, smooth-walled to verruculose. *Conidiophores* macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, unbranched, occasionally branched, up to 10-septate, 12–88 × 3–4 µm, brown becoming pale towards apex, smooth-walled, with 1 terminal and up to 3 subterminal conidiogenous loci. *Conidia* in branched chains, occasionally solitary, ovoid, obclaviform, ellipsoidal or somewhat cylindrical, 9–43 × 6–8 µm, with darkened middle transverse septa, (1–)2–3(–6) transverse, 0–1 longitudinal or oblique septa in up to 4 of the transverse segments, usually inconspicuous, pale yellowish to yellowish brown, verrucose. *Secondary conidiophores* commonly formed apically as a beak from conidial body, or as a lateral conidiogenous loci from body cells bearing conidia in short chains. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 73 mm diam after 1 wk at 25 °C, flat, densely floccose, aerial mycelium abundant, margins fimbriate; surface olive brown to blond (4D3/4C4), white at the periphery; reverse yellowish brown to orange-grey (5E4/5B2). On PCA attaining 66 mm diam, flat, granular, aerial mycelium scarce, margins regular; surface dark green (30F8); reverse dull green (30E4). On OA reaching 70 mm diam, flat, loosely floccose at centre, aerial mycelium scarce, margins regular; surface dark green (28F4); reverse dull green (29E3).

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

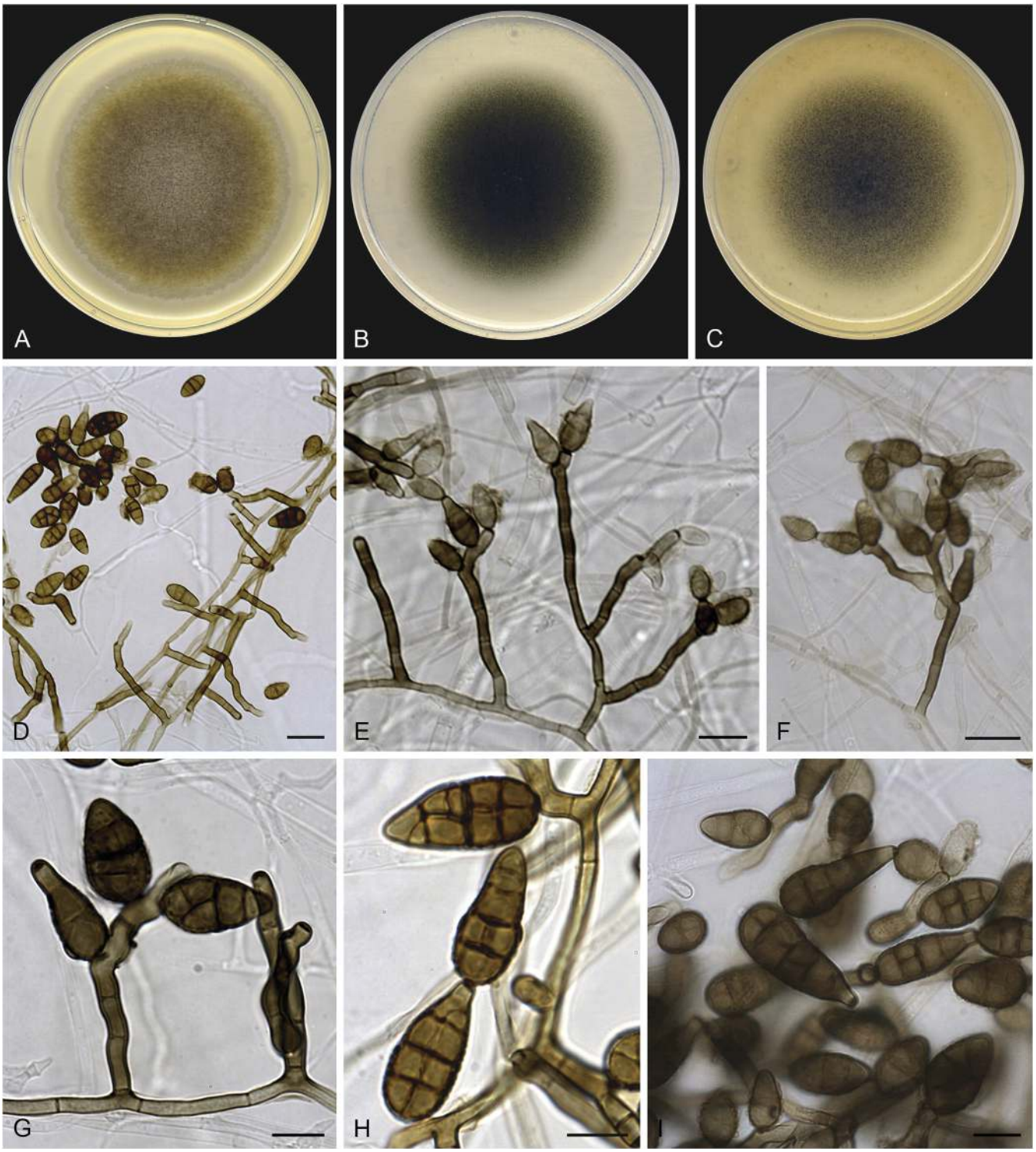

Fig. 9. *Alternaria altcampina* (ex-type FMR 16476). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–I.** Conidiophores and conidia. Scale bars: D–E = 20 µm; F–I = 10 µm.

Typus: **Spain**, Catalonia, Alt Camp, isolated from goat dung, Mar. 2017, I. Iturrieta-González, M. Guevara-Suarez & J. Guarro (**holotype** CBS H-23892, culture ex-type CBS 145420 = FMR 16476).

Notes: Based on the phylogeny of ITS, *ATPase* and *gapdh*, *A. altcampina* is classified in *Alternaria* section *Pseudoalternaria* (Fig. 6). It is closely related to the recently described species *A. parvicaespitosa,* which was isolated from harvested blueberry fruit (California, USA), and *A. kordkuyana*, isolated from symptomatic wheat heads of *Triticum aestivum* (Kordkuy, Iran). *Alternaria parvicaespitosa* differs in having smaller conidia (10–25 × 7–12 µm) with smooth to slightly punctulate outer walls (Gannibal & Lawrence 2016), and *A. kordkuyana* by its larger conidia [30–50(–60) × 7–11 µm] and shorter conidiophores (10–40 × 3–4 µm) (Poursafar *et al.* 2018).

Alternaria chlamydosporifera Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829628. Fig. 10.

Etymology: Name refers to the production of abundant chlamydospores in culture.

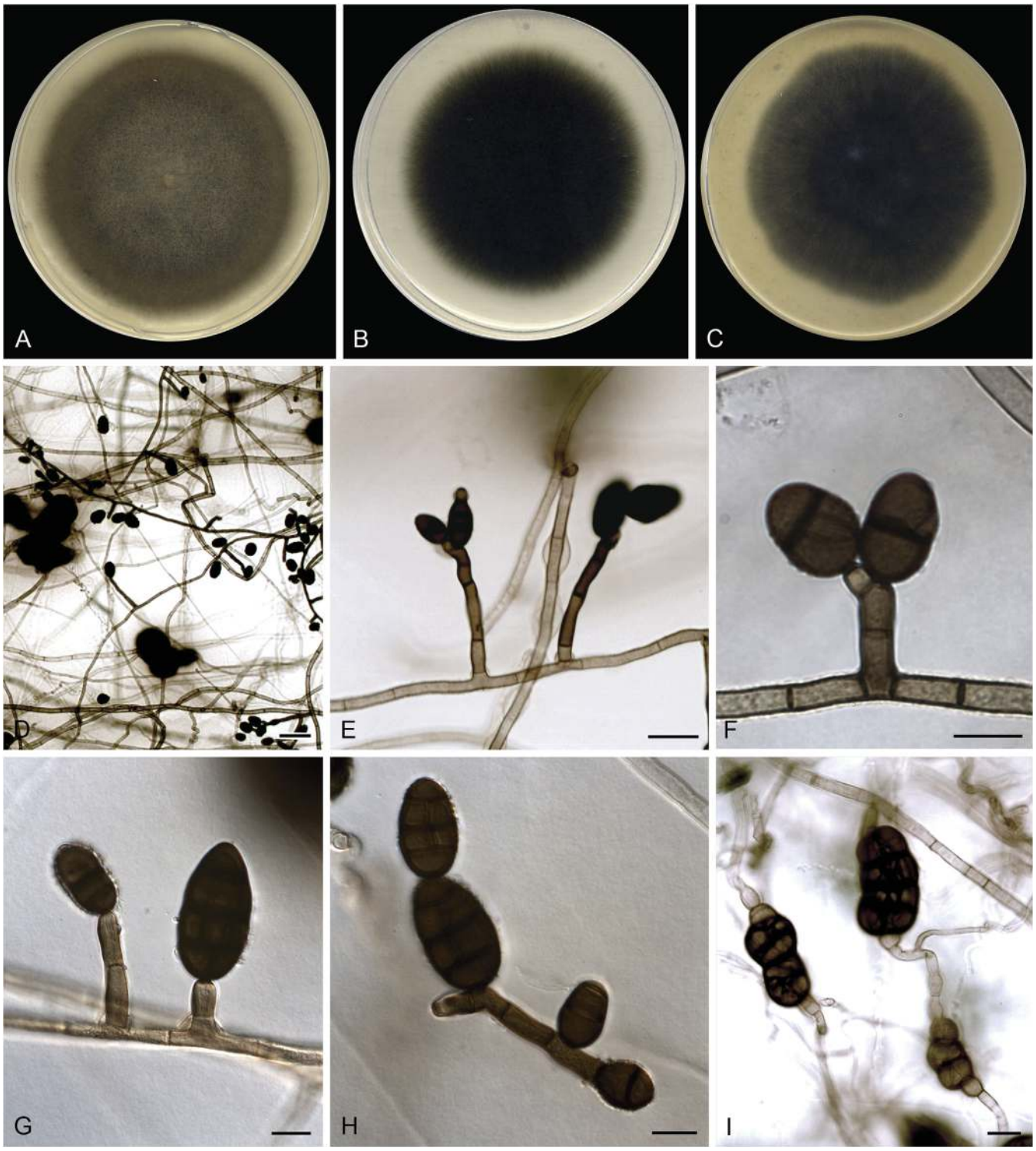

Fig. 10. *Alternaria chlamydosporifera* (ex-type FMR 17360). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–H.** Conidiophores and conidia. **I.** Chlamydospores. Scale bars: D = 50 µm; E, I = 20 µm; F–H = 10 µm.

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 3–6 µm wide, septate, branched, pale brown to brown, smooth-walled. *Conidiophores* macronematous, arising directly from aerial hyphae, erect to slightly flexuous, occasionally geniculate at apex, 1–4-septate, unbranched or scarcely branched, 14–140 × 3–5 µm, dark brown, verruculose, with 1–2 conidiogenous loci. *Conidia* mostly solitary, occasionally in short chains with up to two conidia, ellipsoidal or ovoid, occasionally subglobose, 12–41 × 7–20 µm, with darkened middle transverse septa, 1–3(–4) transverse, and 0–1(–2) longitudinal septa per transverse segments, brown to dark brown, verruculose. *Secondary conidiophores* can be formed apically from conidial body as a beak, geniculate, with 1–3 terminal or lateral conidiogenous loci, bearing solitary or short chains of conidia. *Chlamydospores* abundant, immersed, intercalary, irregular shape, rarely broadly ellipsoidal or clavate, muriform, sometimes showing central constriction, 60–91 × 32–57 µm, dark brown to black. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 82 mm diam after 1 wk at 25 °C, flat, densely floccose, aerial mycelium abundant, margins regular; surface greyish brown (5E3); reverse

black to greyish brown (5E3). On PCA attaining 68 mm diam, flat, with granular appearance by the presence of abundant chlamydospores, aerial mycelium scarce, margins regular; surface dark green (29F5); reverse dark green (30F8). On OA reaching 71 mm diam, flat, loosely floccose at centre, slightly granular towards the periphery, aerial mycelium scarce, margins slightly lobate; surface dark green (29F4); reverse dark green (29F4).

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Typus: **Spain**, Huesca, Baells, isolated from rabbit dung, Apr. 2018, G. Sisó & D. García (**holotype** CBS H-23893, culture ex-type CBS 145421 = FMR 17360).

Notes: *Alternaria chlamydosporifera* belongs to *Alternaria* section *Radicina* (Fig. 7). It is included in a well-supported clade (95 % BS) with *A. petroselini, A. selini* and *A. smyrnii*, which are pathogens of *Apiaceae* (Lawrence *et al.* 2016). *Alternaria petroselini* and *A. selini* can be easily differentiated by the lack of chlamydospores in culture and their larger (50–66 µm in *A. petroselini vs.* 48–65 µm in *A. selini*) and usually ellipsoidal conidia (Simmons 1995). Although *A. smyrnii*, the closest relative to *A. chlamydosporifera*, has been described as producing sclerotial knots in culture that are able to form fertile conidiophores, its conidia are considerably longer (67–96 µm) (Simmons 1995) than those of *A. chlamydosporifera* (12–41 µm long). In addition, we have never observed conidiophores associated with the chlamydospores of the latter species.

Alternaria curvata Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829628. Fig. 11.

Etymology: Name refers to the presence of curved conidia.

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 2–6 µm wide, septate, branched, hyaline to yellowish brown to brown, smooth-walled to verruculose. *Conidiophores* macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, usually unbranched, up to 14-septate, 23–80 × 3–5 µm, brown to dark brown, smooth-walled, with a terminal or occasionally a sub-terminal conidiogenous loci. *Conidia* forming branched chains, with up to 5 conidia in unbranched part, ovoid or nearly ellipsoidal, often slightly curved, 13–47(–70) × 4–16 µm, with darkened middle transverse septa, (0–)1–5(–7) transverse, and 0–2(–3) longitudinal or oblique septa per transverse segment, brown to dark brown, verrucose to tuberculate. *Secondary conidiophores* can be formed apically or laterally from the conidial body as a short extension bearing conidia in short chains. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 63 mm diam after 1 wk at 25 °C, flat, densely floccose, aerial mycelium abundant, margins regular; surface white to dull green (1A1/30D4); reverse dark green to olive yellow (30F8/2D6), white at the periphery. On PCA attaining 62 mm diam, flat, loosely floccose, aerial mycelium scarce, margins regular; surface olive (3F4); reverse dark green to grey (29F4/29B1). On OA reaching 61 mm diam, scarce aerial mycelium towards the periphery, margins regular; surface greyish green (30E5), with greyish mycelium tufts at centre; reverse dull green (29E4).

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Typus: **Spain**, Catalonia, Tarragona, Els Ports Natural Park, isolated from goat dung, Oct. 2017, G. Sisó & C. González-García (**holotype** CBS H-23894, culture ex-type CBS 145422 = FMR 16901).

Notes: *Alternaria curvata* was included in the section *Infectoriae*, forming an unsupported basal clade together with *A. montsantina* and *A. pseudoventricosa*, both introduced here, and *A. ventricosa* (Fig. 5). Morphologically, the former two species differ from *A. curvata* in lacking curved conidia. *Alternaria ventricosa* has asymmetrical conidia, due to the hyperplasia and hypertrophy of cells, especially on one side of the conidia (Roberts 2007). Other morphologically similar species are *A. fimeti* and *A. triticina*, which also have curved conidia. However, *A. triticina* is phylogenetically more distant and its conidia are strongly inequilateral and wider (up to 22 µm) (Simmons 2007) than those of *A. curvata* (4–16 µm wide). *Alternaria fimeti* can be differentiated from *A. curvata* by the production of longer conidiophores (22–182 µm in *A. fimeti vs.* 23–80 µm in *A. curvata*) and the absence or scarce development of secondary conidiophores.

Alternaria fimeti Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829630. Fig. 12.

Etymology: Name refers to the substrate where the species was isolated, herbivore dung.

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 2–5 µm wide, septate, branched, hyaline to subhyaline to pale yellowish, verruculose. *Conidiophores* semi- to macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, unbranched (can be slightly branched on OA), up to 9-septate, 22–182 × 1–5 µm, pale brown, smooth-walled, with 1 terminal conidiogenous locus. *Conidia* solitary or in short chains of up to six conidia, ovoid, obpyriform or obclavate, some slightly curved, 9–44 × 5–14(–23) µm, with darkened middle transverse septa, 0–5 transverse (up to 7 in OA), and 0–1(–2) longitudinal or oblique septa per segment, brown, verrucose. *Secondary conidiophores* only scarcely produced on OA as apical or lateral extension from conidial body, up to 25 µm long. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 66 mm diam after 1 wk at 25 °C, flat, densely floccose, aerial mycelium abundant, margins fimbriate; surface yellowish grey to yellowish white (3C2/3A2); reverse yellowish brown to light yellow (5E8/4A5). On PCA attaining 65 mm diam, flat, slightly floccose at centre, aerial mycelium scarce, margins regular; surface olive-brown (4F5); reverse olive-brown (4F8/4E4). On OA reaching 64 mm diam, flat, slightly floccose, scarce aerial mycelium, margins regular; surface dull green (30E5) with grey floccose area; reverse dull green (30E4).

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Typus: **Spain**, Catalonia, Priorat, Montsant Natural Park, Arbolí, isolated from small rodent dung, Feb. 2018, I. Iturrieta-González, E. Carvalho & J. Gené (**holotype** CBS H-23895, culture ex-type CBS 145423 = FMR 17110).

Note: *Alternaria fimeti* is placed in a clade of section *Infectoriae* together with *A. aconidiophora* (see notes of this latter species).

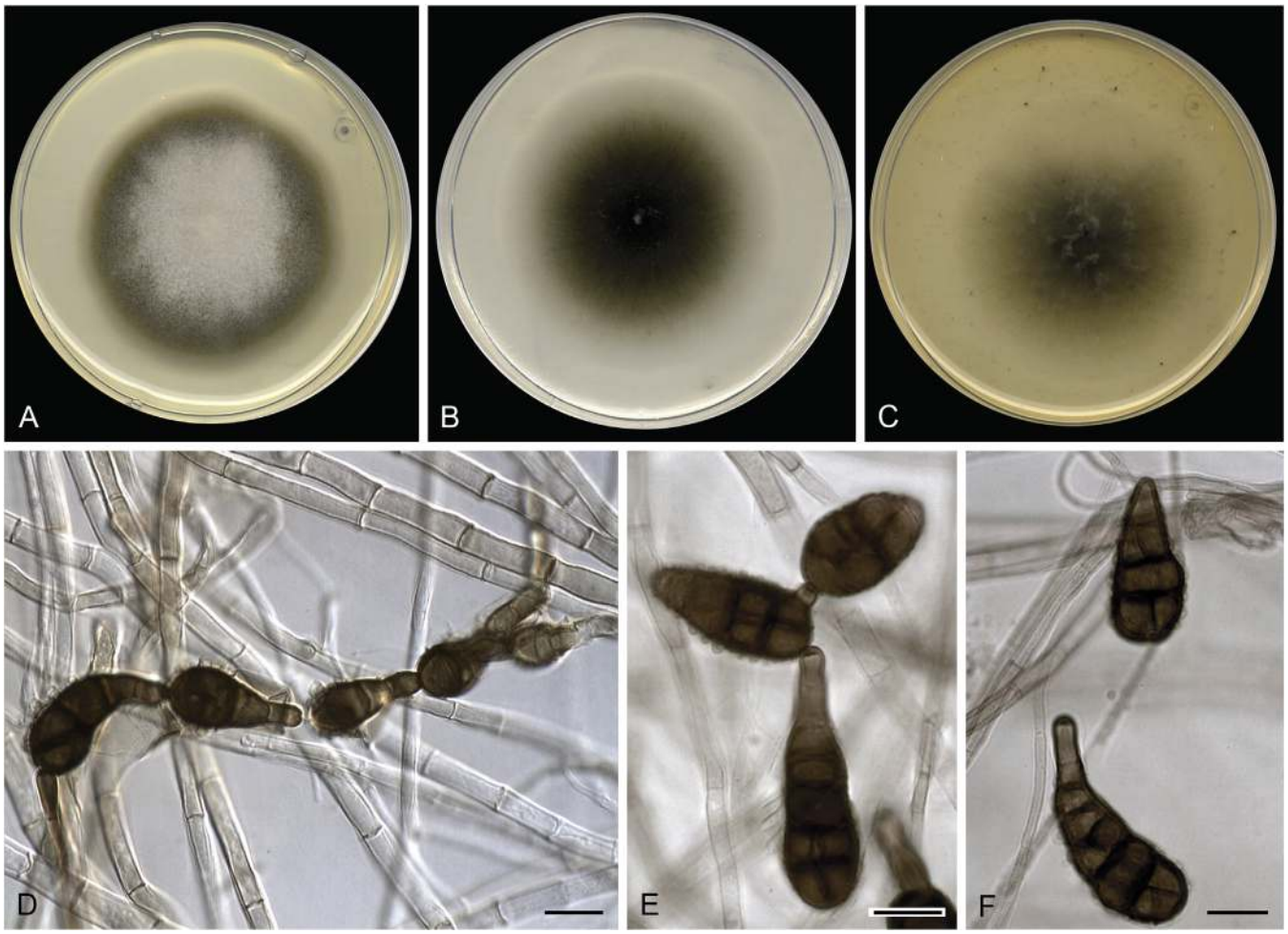

Fig. 11. *Alternaria curvata* (ex-type FMR 16901). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–F.** Conidiophore and conidia. Scale bars = 10 µm.

Alternaria inflata Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829631. Fig. 13.

Etymology: Name refers to the presence of swollen cells in the conidial body.

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* of 1–3 µm wide, septate, branched, hyaline to pale brown, smooth-walled to verruculose. *Conidiophores* arising laterally or terminally from aerial hyphae, erect to slightly flexuous, semi- to macronematous, up to 10-septate, commonly unbranched, 9–73(–105) × 2–5 µm, pale brown to brown, smooth-walled, with one terminal conidiogenous loci or up to three subterminal conidiogenous loci. *Conidia* solitary or in short chains with up to four conidia, broadly ellipsoidal or ovoid, 13–41 × 5–14 µm, often with some swollen cells protruding the conidium outline, some with darkened middle transverse septa, (1–)2–3(–5) transverse septa, and 0–2 longitudinal or oblique septa per transverse segment, brown, verruculose. *Secondary conidiophores* scarcely produced, as an apical extension up to 15 µm long, bearing conidia in short chains. *Chlamydospores* present, consisting of intercalary, thick-walled, brown swollen cells, up to 8 × 6 µm, arranged in chains or in clusters. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 62 mm diam after 1 wk at 25 °C, flat, aerial mycelium abundant, floccose, margins fimbriate; surface white (1A1); reverse greyish yellow to yellowish white (4C6/4A2). On PCA attaining 67 mm diam, flat, scarce aerial mycelium, margins regular; surface dull green to grey (30E4/30B1); reverse dark green to grey (30E4/30B1). On OA reaching 61 mm diam, flat, loosely floccose, margins regular; surface pale grey (1B1); reverse pale grey (1B1).

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Typus: **Spain**, Catalonia, Conca de Barberà, Poblet, isolated from rabbit dung, Mar. 2017, J. Guarro & I. Iturrieta-González (**holotype** CBS H-23896, culture ex-type CBS 145424 = FMR 16477).

Notes: Our phylogeny shows that *A. inflata* belongs to section *Pseudoalternaria* (Fig. 6). It clustered in a well-supported clade (86 % BS) with *A. altcampina*, *A. kordkuyana* and *A. parvicaespitosa,* but formed a single basal lineage representative of a distinct species. *Alternaria inflata* can be differentiated from all the species in the section by the production of chlamydospores and by the formation of broadly ellipsoidal conidia, usually with swollen cells protruding from the conidial body. In addition, *A. altcampina* also differs in the production of secondary conidiophores, *A. parvicaespitosa* in its shorter conidiophores (up to 70 µm) and conidia (10–25 µm) (Gannibal & Lawrence 2016), and *A. kordkuyana* in the production of longer conidial chains [up 5–8(–10) conidia] and conidia measuring 30–50(–60) × 7–11 µm with up to seven transverse septa (Poursafar *et al.* 2018).

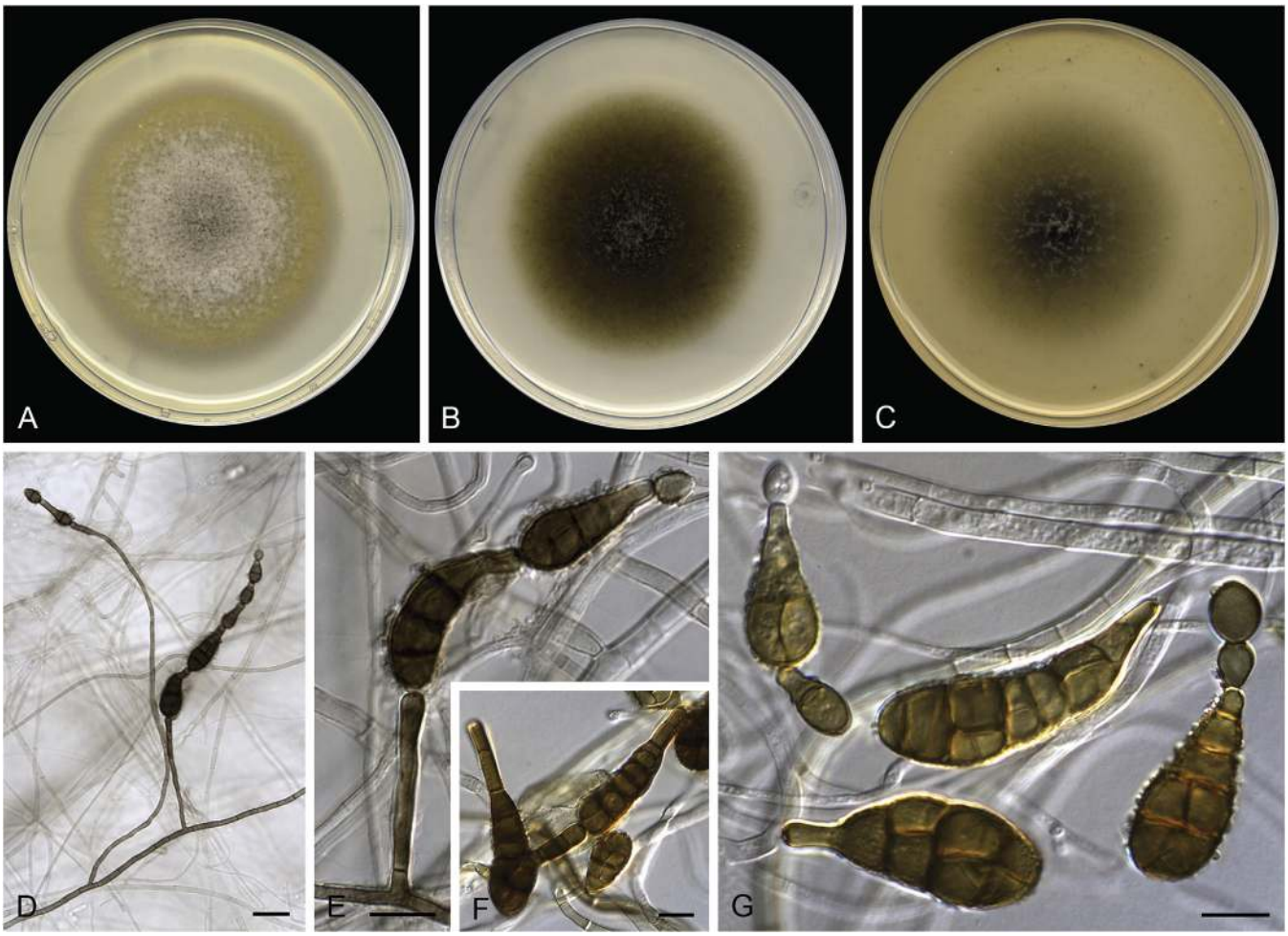

Fig. 12. *Alternaria fimeti* (ex-type FMR 17110). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–G.** Conidiophores and conidia. Scale bars: D = 20 µm; E–G = 10 µm.

With the additions of *A. altcampina* and *A. inflata* section *Pseudoalternaria* now comprises seven species. It is of note, however, that most of these taxa are only known from a single collection. In our survey of asexual fungi from herbivore dung, we identified several Spanish isolates belonging to other species in the section, namely *A. kordkuyana* and *A. rosae* (Fig. 6). Considering that most of the species in section *Pseudoalternaria* are mainly associated with herbaceous plants of the families *Brassicaceae, Ericaceae, Poacea* or *Rosaceae,* it is not surprising to find these fungi in faeces of herbivorous animals.

Alternaria lawrencei Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829632. Fig. 14.

Etymology: Name in honour of Daniel P. Lawrence for his contribution to the taxonomy of the genus *Alternaria.*

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 1–3 µm wide, septate, branched, pale brown, smooth-walled. *Conidiophores* macronematous, solitary, arising directly from aerial hyphae, erect to slightly flexuous, occasionally geniculate at apex, usually unbranched, up to 10-septate, 9–125 × 3–4(–5) µm, brown, smooth-walled, with 1–2 lateral or terminal conidiogenous loci; *micronematous conidiophores* also present, reduced to intercalary conidiogenous cells with a single conidiogenous locus on hyphae. *Conidia* solitary or in short chains, up to six conidia in the unbranched part, ovoid, obpyriform or obclavate, 6–71 × 7–15 µm, with darkened middle transverse septa, (1–)2–7(–9) transverse, and 0–2(–3) longitudinal or oblique septa, pale brown to brown, verrucose to tuberculate. *Secondary conidiophores* commonly formed apically on conidia as a geniculate extension with several conidiogenous loci, or as lateral extensions from cells of conidial body, up to 35 µm long, producing conidia solitary or in short chains. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 68 mm diam after 1 wk at 25 °C, low convex, cottony, aerial mycelium abundant, margins regular; surface white (1A1); reverse yellowish brown to greyish yellow (5E7/4B6). On PCA attaining 69 mm diam, low convex, slightly floccose, aerial mycelium relatively abundant at centre, margins regular; surface yellowish grey to olive (2D2/1E3); reverse dark green to olive (30F8/1E3). On OA reaching 63 mm diam, loosely floccose at centre, flat and scarce aerial mycelium towards the periphery, margins regular and diffuse; surface olive (2F4) to olive-grey (2B1); reverse olive to yellowish grey (2F8/2D2).

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Typus: **Spain**, Catalonia, Tarragona, Els Ports Natural Park, isolated from goat dung, Oct. 2017, G. Sisó & C. González-García (**holotype** CBS H-23897, culture ex-type CBS 145425 = FMR 17004).

Notes: Although *A. lawrencei* is clearly placed in section *Infectoriae*, the multi-locus analysis did not reveal any phylogenetic

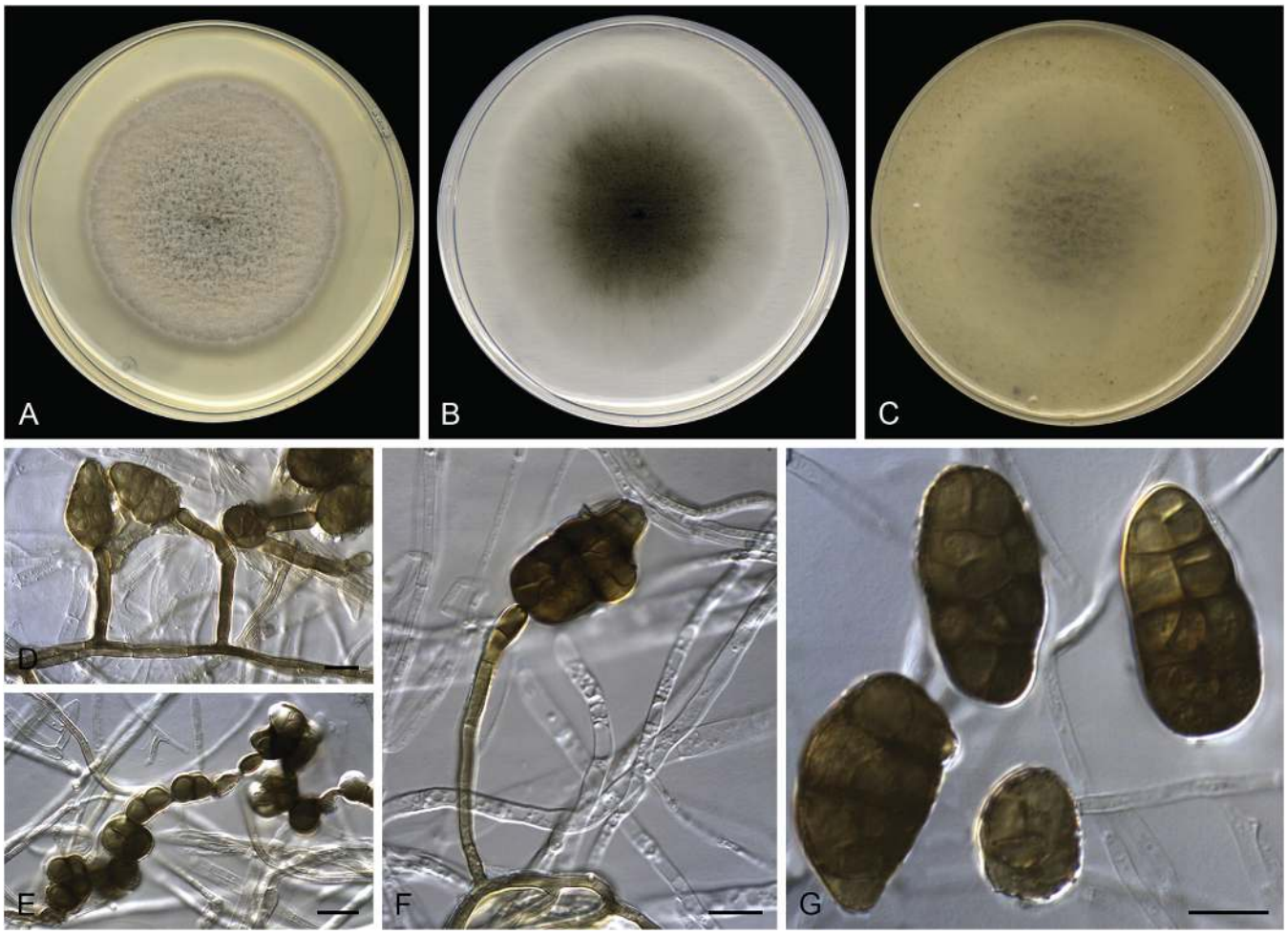

Fig. 13. *Alternaria inflata* (ex-type FMR 16477). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D, F–G.** Conidiophores and conidia. **E.** Chlamydospores. Scale bars = 10 µm.

relationship with any species included in the analysis (Fig. 4). It is of note, however, that eight species of the section (*i.e. A. cerasi, A. cesenica*, *A. dactylidicola*, *A. forlicesenensis*, *A. hampshirensis*, *A. litorea*, *A. murispora* and *A. poaceicola*) could not be included in our analysis due to their limited molecular data. Nevertheless, *A. cesenica*, *A. dactylidicola*, *A. forlicesenensis*, *A. hampshirensis*, *A. murispora* and *A. poaceicola* can be distinguished from *A. lawrencei* by the production of a sexual morph (Ariyawansa *et al.* 2015b, Liu *et al.* 2015, Thambugala *et al.* 2017, Wanasinghe *et al.* 2018), *A. cerasi* by its inequilateral conidia (Potebnia 1907, Simmons 2007), and *A. litorea* by the production of shorter primary conidiophores (40–50 µm long) and smooth-walled conidia that are 22–32 µm long (Simmons 2007).

Alternaria montsantina Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829633. Fig. 15.

Etymology: Name refers to the place, Montsant Natural Park (Catalonia), where the fungus was collected.

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 1–7 µm wide, septate, branched, usually forming hyphal coils, subhyaline to pale brown, smooth-walled to verruculose. *Conidiophores* macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, unbranched, up to 15-septate, 12–137 × 3–6 µm, often with geniculate apical portion containing intercalary and terminal conidiogenous loci, brown, smooth-walled to verruculose. *Conidia* solitary or in short chains with up to five conidia, subglobose, ovoid or obpyriform, 8–65 × 6–12 µm, with 1–3(–11) transverse septa, and 0–2 longitudinal or oblique septa, brown, verrucose to tuberculate. *Secondary conidiophores* commonly produced apically as a long, often geniculate extension, up to 105 µm long and 10-septate, bearing terminal conidial chains. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 76 mm diam after 1 wk at 25 °C, flat, densely floccose, aerial mycelium abundant, margins regular; surface pastel grey to greyish yellow (1C1/2C4) and with a white edge; reverse blond to white (4C4/1A1). On PCA attaining 70 mm diam, flat, loosely floccose at centre, aerial mycelium moderate, margins regular; surface olive brown to white (4D4/1A1); reverse olive to white (3D4/1A1). On OA reaching 75 mm diam, flat, cottony, margins regular; surface yellowish grey to olive (3D2/2F4) and white edge; reverse olive to white (2F4/1A1).

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Typus: **Spain**, Catalonia, Priorat, Montsant Natural Park, Swamp of Siurana, isolated from an unidentified twig, Feb. 2018, I. Iturrieta-González, E. Carvalho & J. Gené (**holotype** CBS H-23898, culture ex-type CBS 145426 = FMR 17060).

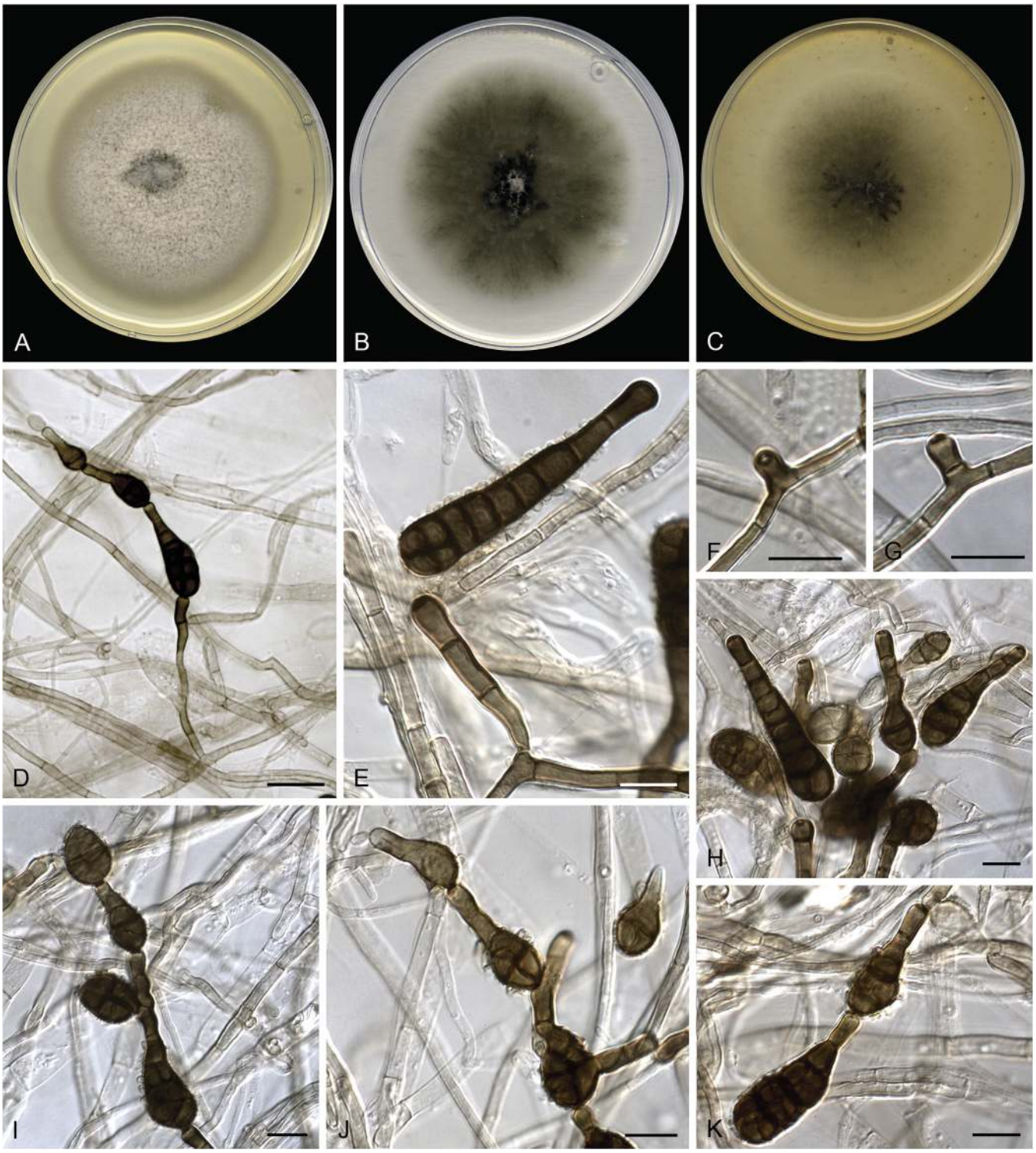

Fig. 14. *Alternaria lawrencei* (ex-type FMR 17004). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–K.** Conidiophores and conidia. Scale bars: D = 20 µm; E–K = 10 µm.

Notes: *Alternaria montsantina* is placed in a weakly supported basal clade of the section *Infectoriae,* together with *A. curvata*, *A. pseudoventricosa* and *A. ventricosa* (Fig. 5). Morphologically, this new species can be distinguished from *A. curvata* and *A. ventricosa* by the absence of curved or inequilateral inflated conidia. *Alternaria montsantina* differs from *A. pseudoventricosa* in the production of longer (12–137 µm) and often geniculate primary and secondary conidiophores, bearing solitary conidia or arranged in short chains (up to five conidia). Conidiophores in *A. pseudoventricosa* are 30–44 µm long, and the conidial chains include up to 19 conidia.

Alternaria pobletensis Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829634. Fig. 16.

Etymology: Name refers to the place, Poblet (Catalonia), from where the species was collected.

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 2–5 µm wide, branched, pale brown, septate, smooth-walled. *Conidiophores* semi- to macronematous, solitary, arising directly from aerial hyphae, erect to slightly flexuous, occasionally slightly geniculate at apex, unbranched or branched, up to 8-septate, 14–82 × 4–5(–6) µm, brown,

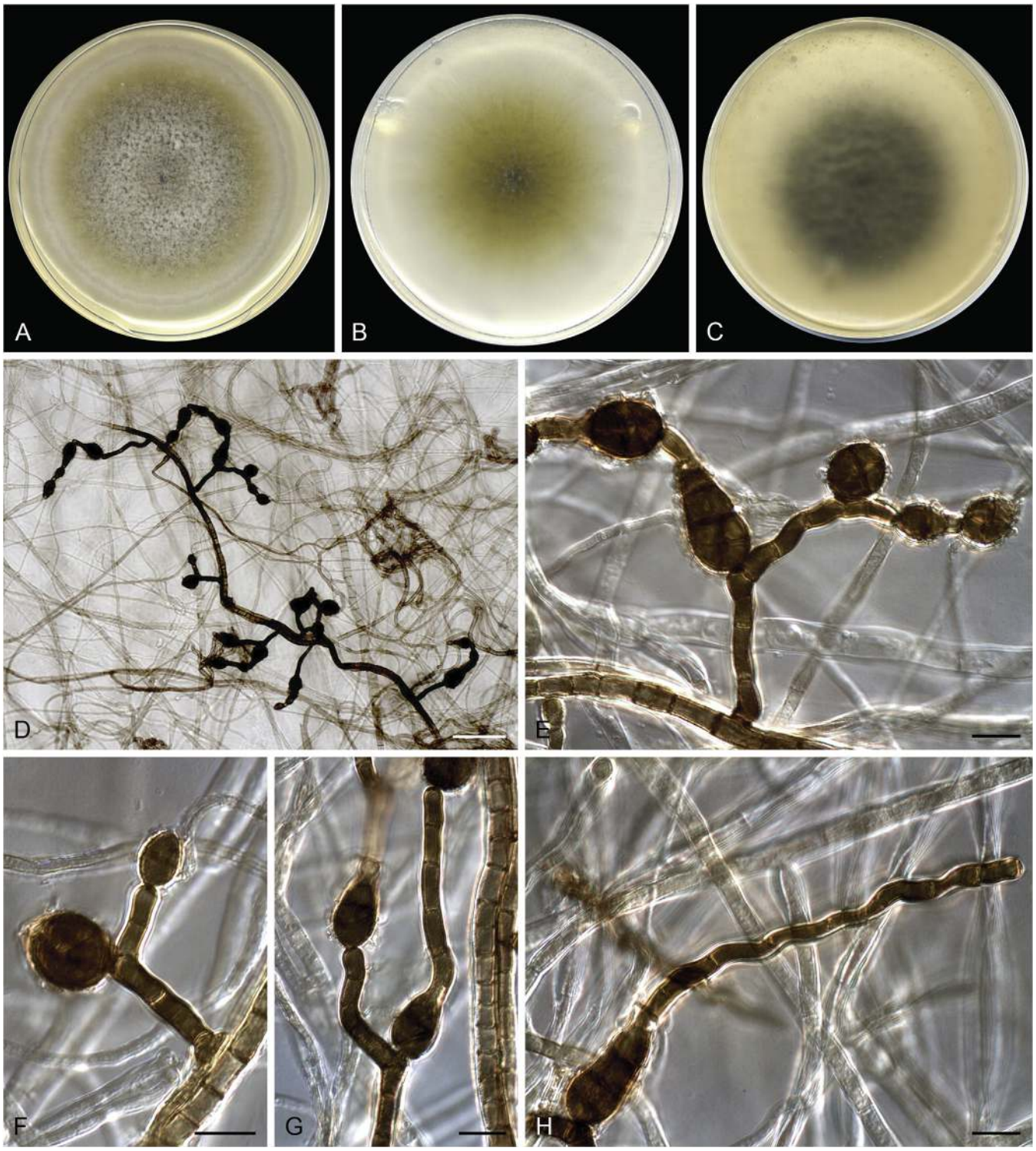

Fig. 15. *Alternaria montsantina* (ex-type FMR 17060). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–H.** Conidiophores and conidia. Scale bars: D = 50 µm; E–H = 10 µm.

smooth-walled, with 1–2 lateral or terminal conidiogenous loci. *Conidia* commonly in short, scarcely branched chains, with up to seven conidia, obpyriform or obclavate, some ellipsoidal or subcylindrical, 8–50 × 5–20 µm, (1–)3–7(–9) transverse septa, often middle septa darker, and 0–1(–2) longitudinal or oblique septa per transverse segment, pale brown to brown, smooth-walled or verruculose. *Secondary conidiophores* commonly produced apically as a short beak up to 11 µm long, or laterally from cells of conidial body, bearing conidia in short chains. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 46 mm diam after 1 wk at 25 °C, flat, floccose at the centre, velvety towards the periphery, aerial mycelium moderate, margins regular; surface olive (3F8), whitish at the periphery; reverse black to yellowish brown (5D5). On PCA attaining 58 mm diam, flat, velvety, margins regular; surface dark green to dull green (30F8/28D3); reverse dark green to dull green (30F8/28D3). On OA reaching 55 mm diam, flat, loosely floccose, margins regular; surface greyish green to dull green (29C3/29E4); reverse dark green to dull green (30F8/30E3).

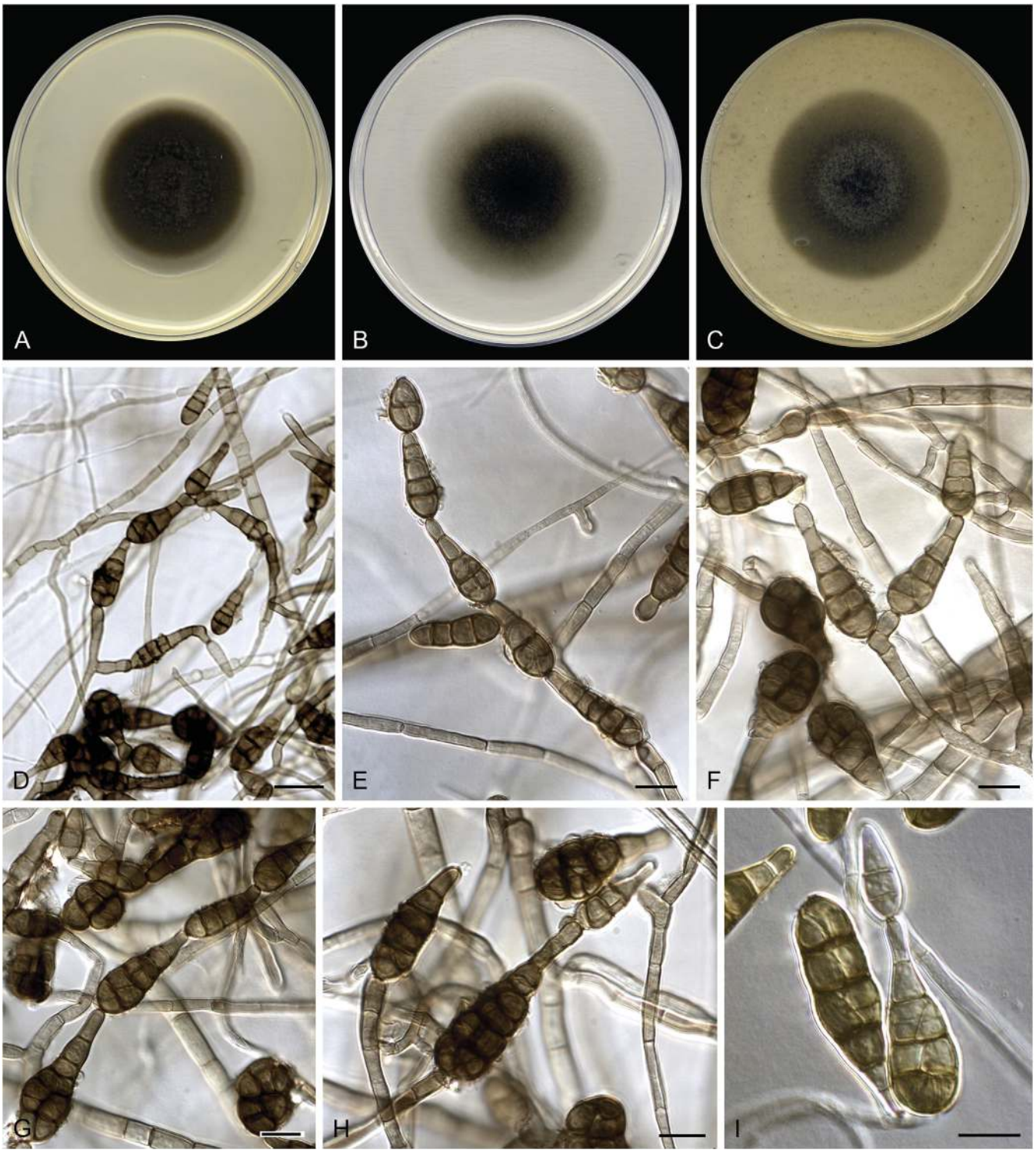

Fig. 16. *Alternaria pobletensis* (ex-type FMR 16448). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–I.** Conidiophores and conidia. Scale bars: D = 20 µm; E–I = 10 µm.

Cardinal temperature for growth: Optimum 25 °C, maximum 35 °C, minimum 5 °C.

Typus: **Spain**, Catalonia, Conca de Barberà, Poblet, isolated from unidentified herbivore dung, Mar. 2017, J. Guarro & I. Iturrieta-González (**holotype** CBS H-23899, culture ex-type CBS 145427 = FMR 16448).

Notes: *Alternaria pobletensis* clustered in section *Chalastospora* in a single branch clearly separated from the other six species that currently comprise the section (Fig. 7). Other species of section *Chalastospora* rarely produce conidia with longitudinal septa (Woudenberg *et al.* 2013); however, the conidia in *A. pobletensis* usually have two or more longitudinal or oblique septa. Its closest relative is *A. breviramosa*. This was originally described as *Chalastospora ellipsoidea*, found on *Triticum* (*Poaceae*) in Australia (Crous *et al.* 2009a), but later its name was changed to avoid confusion with *Alternaria ellipsoidea*, an already described species from section *Gypsophilae* (Woudenberg *et al.* 2013). Section *Gypsophilae* contains all *Alternaria* species that occur on *Caryophyllaceae* (Lawrence *et al.* 2016). *Alternaria breviramosa* differs from *A. pobletensis* by having shorter conidiophores (up to 25 µm), often reduced to

conidiogenous cells, and ellipsoidal, subcylindrical to fusoid conidia with only transverse septa (Crous *et al.* 2009a).

Alternaria pseudoventricosa Iturrieta-González, Dania García & Gené, ***sp. nov.*** MycoBank MB829635. Fig. 17.

Etymology: Name refers to the apparent phylogenetic relationship to *A. ventricosa.*

Asexual morph on PCA: *Mycelium* superficial and immersed. *Hyphae* 1–7 µm wide, septate, branched, hyaline to pale brown, smooth-walled. *Conidiophores* macronematous, arising laterally from aerial hyphae, erect to slightly flexuous, up to 4-septate, unbranched, 30–45 × 4–6 µm, brown, smooth-walled, with one terminal conidiogenous locus. *Conidia* commonly in unbranched chains, with up to 19 conidia, obpyriform or obclavate, 10–48(–66) × 5–14 µm, with darkened middle transverse septa, 1–7 transverse, 0–1 longitudinal or oblique septa, brown to dark brown, verrucose to tuberculate. *Secondary conidiophores* scarce, as a beak arising from the conidial body. *Sexual morph* not observed.

Culture characteristics: Colonies on PDA reaching 64 mm diam after 1 wk at 25 °C, flat, cottony at the centre, floccose towards the periphery, margins regular; surface white (1A1); reverse yellowish white (4A2). On PCA attaining 62 mm diam, flat towards the periphery, margins regular; surface dark green (29F4), with tuft of white aerial mycelium at centre; reverse dark green to grey (29F8/29B1). On OA reaching 67 mm diam, flat, loosely floccose, margins regular; surface dull green (29E4); reverse dull green (29E4).

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Typus: **Spain**, Catalonia, Tarragona, Els Ports Natural Park, isolated from horse dung, Oct. 2017, G. Sisó & C. González-García (**holotype** CBS H-23900, culture ex-type CBS 145428 = FMR 16900).

Notes: *Alternaria pseudoventricosa* and *A. ventricosa* clustered in an unsupported monophyletic basal clade in section *Infectoriae* (Fig. 5). They can be differentiated by their conidial morphology. Conidia in *A. ventricosa* are usually asymmetric, laterally swollen, and pale cinnamon brown (Roberts 2007). In contrast, those of *A. pseudoventricosa* are obpyriform or obclavate and brown to dark brown.

Authors: I. Iturrieta-González, D. García, M. Hernández-Restrepo & J. Gené

Brunneosphaerella Crous, Stud. Mycol. 64: 31. 2009. Fig. 18.

Classification: *Dothideomycetes*, *Dothideomycetidae*, *Capnodiales*, *Mycosphaerellaceae*.

Type species: *Brunneosphaerella protearum* (Syd. & P. Syd.) Crous, basionym: *Leptosphaeria protearum* Syd. & P. Syd. Epitype and ex-epitype strain designated by Crous *et al.* (2011): CBS H-20335, CBS 130597 = CPC 16338.

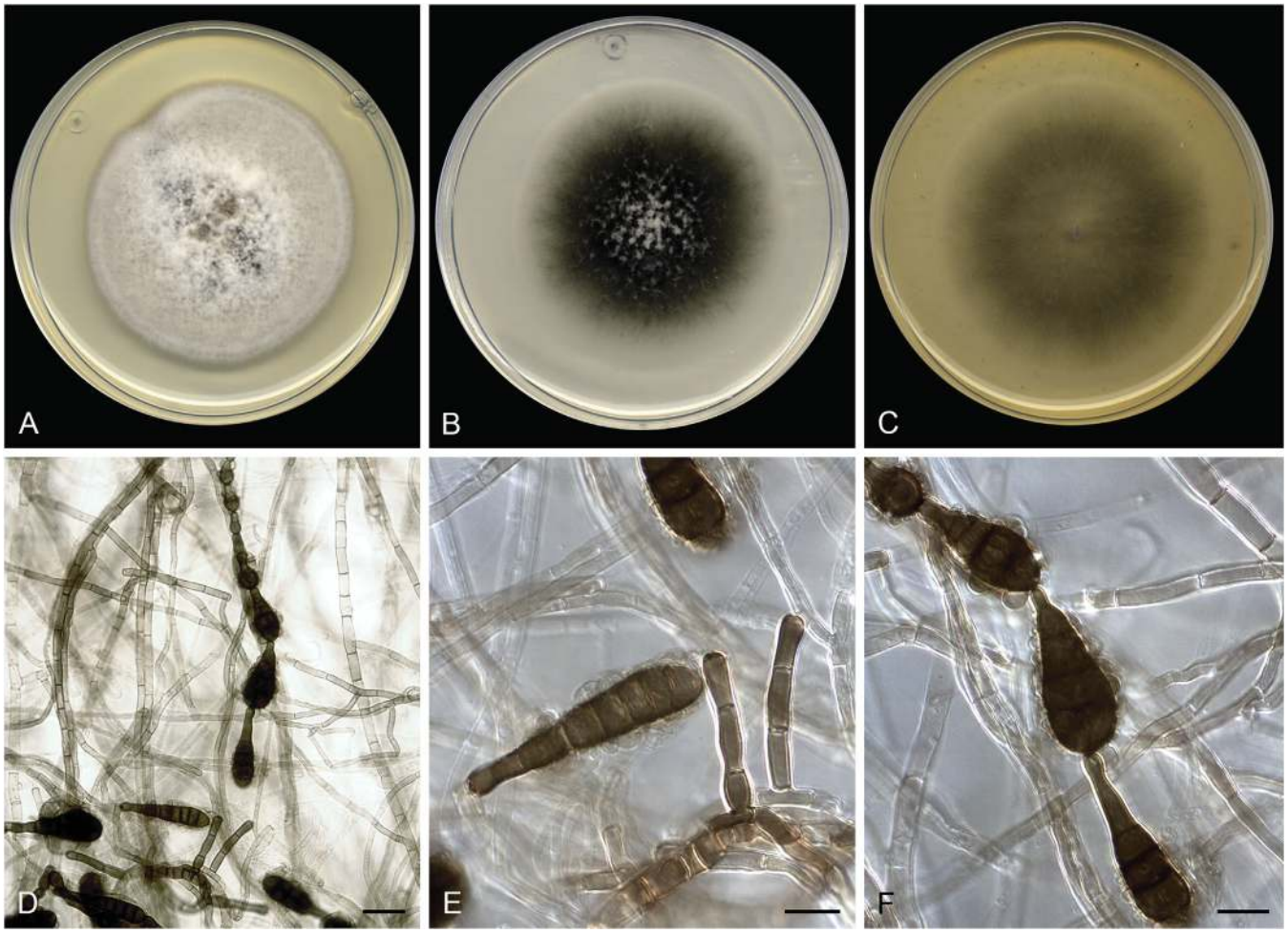

Fig. 17. *Alternaria pseudoventricosa* (ex-type FMR 16900). **A.** Colonies on PDA. **B.** Colonies on PCA. **C.** Colonies on OA. **D–F.** Conidiophores and conidia. Scale bars: D = 20 µm; E, F = 10 µm.

Notes: This species was initially introduced as *R. ducassei* to accommodate some isolates associated with a severe leaf speckle disease of Ducasse banana (*Musa acuminata* × *babisiana* cv. Pisang awak) in northern Queensland (Shivas *et al.* 2011). The authors noticed that this species was similar to *Zasmidium* in having pigmented conidiophores with integrated conidiogenous cells that sympodially proliferate near the apex, with slightly thickened and refractive scars and aseptate, subhyaline conidia also with slightly thickened and refractive hila. However, it was classified in *Ramichloridium* in preference to *Zasmidium* because, at the time, *Zasmidium* was a paraphyletic genus in the *Mycosphaerellaceae*. In our phylogenetic analysis based on the combined dataset, the ex-type strain of this species was located in the well-supported clade (100 % BS / 1 PP) representing *Zasmidium*, and therefore a new combination *Z. ducassei* is proposed. Moreover, additional isolates belonging to this species were obtained from the same host genus, but from different locale, Malaysia.

Zasmidium thailandicum Crous, ***sp. nov.*** MycoBank MB829647. Fig. 85.

Etymology: Named reflects the country from where it was collected, Thailand.

On SNA. *Conidiophores* solitary, arising from superficial hyphae, subcylindrical, pale brown, 1–3-septate, unbranched or branched below, 20–100 × (1.5–)2 µm. *Conidiogenous cells* subhyaline, smooth-walled, subcylindrical, apical and intercalary, apical part with well-defined rachis bearing minute (0.5 µm diam) slightly darkened scars, 10–30 × 1.5–2 µm. *Ramoconidia* fusoid to obclavate, hyaline, smooth-walled, aseptate, guttulate, 8–12(–17) × 2.5–3 µm. *Conidia* solitary, hyaline, smooth-walled, guttulate, aseptate, ellipsoid, apex obtuse, base protruding, truncate, 0.5–1 µm diam, (3–)4–4.5(–5) × (2–)2.5 µm.

Culture characteristics: Colonies erumpent, spreading, with moderate aerial mycelium and smooth, lobate margins, reaching 20 mm diam after 2 wk at 25 °C. On MEA, PDA and OA surface pale mouse grey, reverse mouse grey.

Typus: **Thailand**, Rachaburi province, Bangkok, on leaves of *Musa* sp. (*Musaceae*), 2010, P.W. Crous, HPC 2158 (**holotype** CBS H-23850, culture ex-type CBS 145027 = CPC 33960).

Notes: Zasmidium thailandicum is closely related to *Z. ducassei*. Moreover, both species have been reported from the same host genus, *Musa*, causing leaf spots on banana leaves. However, these species can be easily distinguished by the length of their conidia [5–10 µm in *Z. ducassei vs.* (3–)4–4.5(–5) µm in *Z. thailandicum*].

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ACKNOWLEDGEMENTS

Sincere thanks are due to the curators Tara Rintoul (DAOM) and Bevan Weir (ICMP and PDD). We also thank the MycoBank curator Konstanze Bench, and the technical staff at the Westerdijk Institute, Arien van Iperen (cultures and deposit of herbarium samples), Mieke Starink-Willemse (DNA isolation and sequencing) and Trix Merkx (deposit of isolates) for their invaluable assistance. Jacqueline Edwards thanks Robyn Brett, VPRI curatorial assistant, for maintaining and culturing the VPRI specimens and Tonya Wiechel for extracting DNA and performing PCR on the VPRI specimens. Sampling in Maryland was supported by start-up funds from the University of Alabama at Birmingham to S.A. Krueger-Hadfield. We are thankful to Dr. Paul Kirk for helpful advice regarding the treatment of the invalid name *Seiridium cupressi* (Guba) Boesew. The study of the genus *Alternaria* was supported by the Spanish Ministerio de Economía y Competitividad, Grant CGL2017-88094-P.

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Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

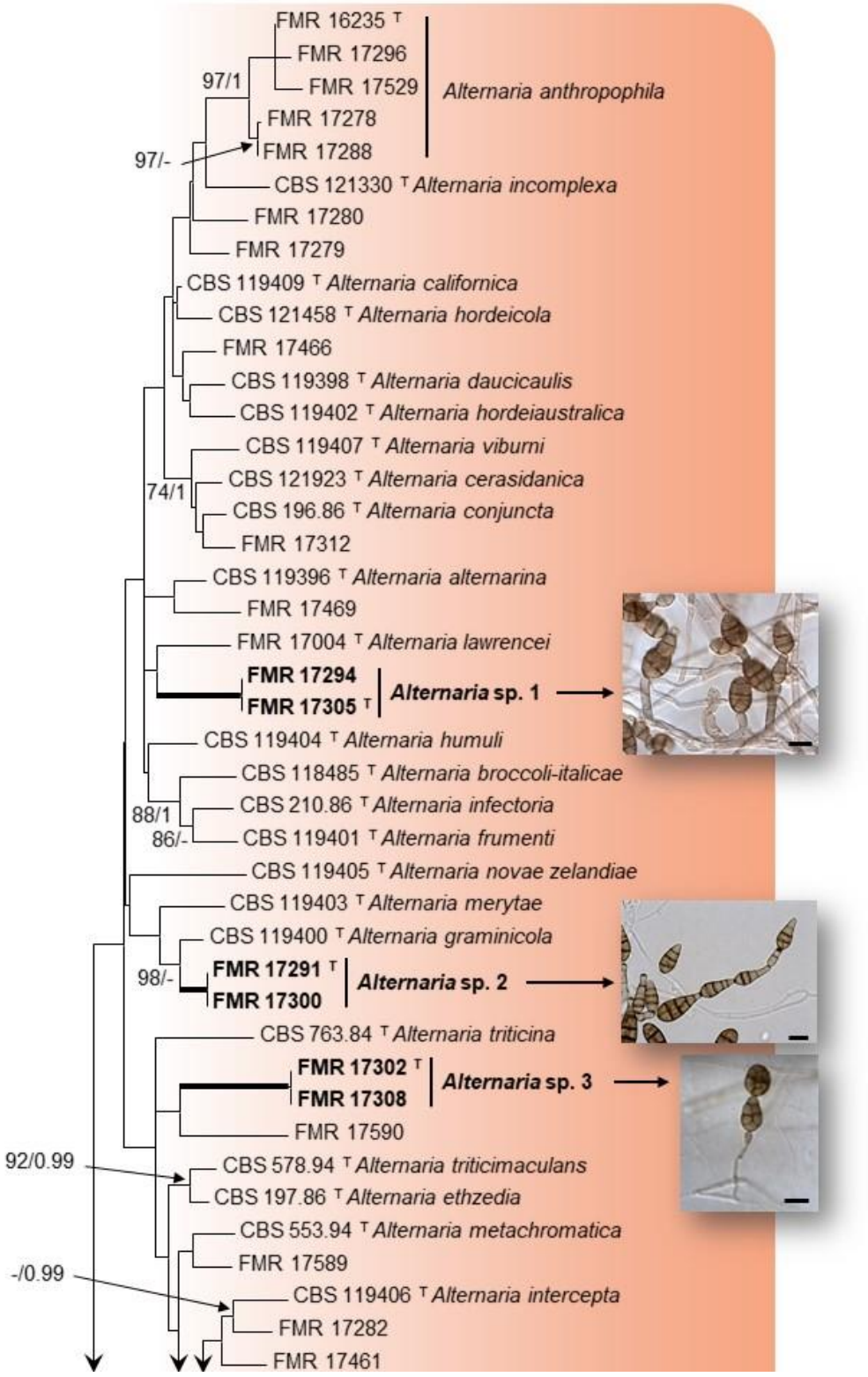
4.1.3 Preliminary results on molecular and phenotypic characterization of other *Alternaria* isolates from clinical and environmental sources collected in Spain and the USA

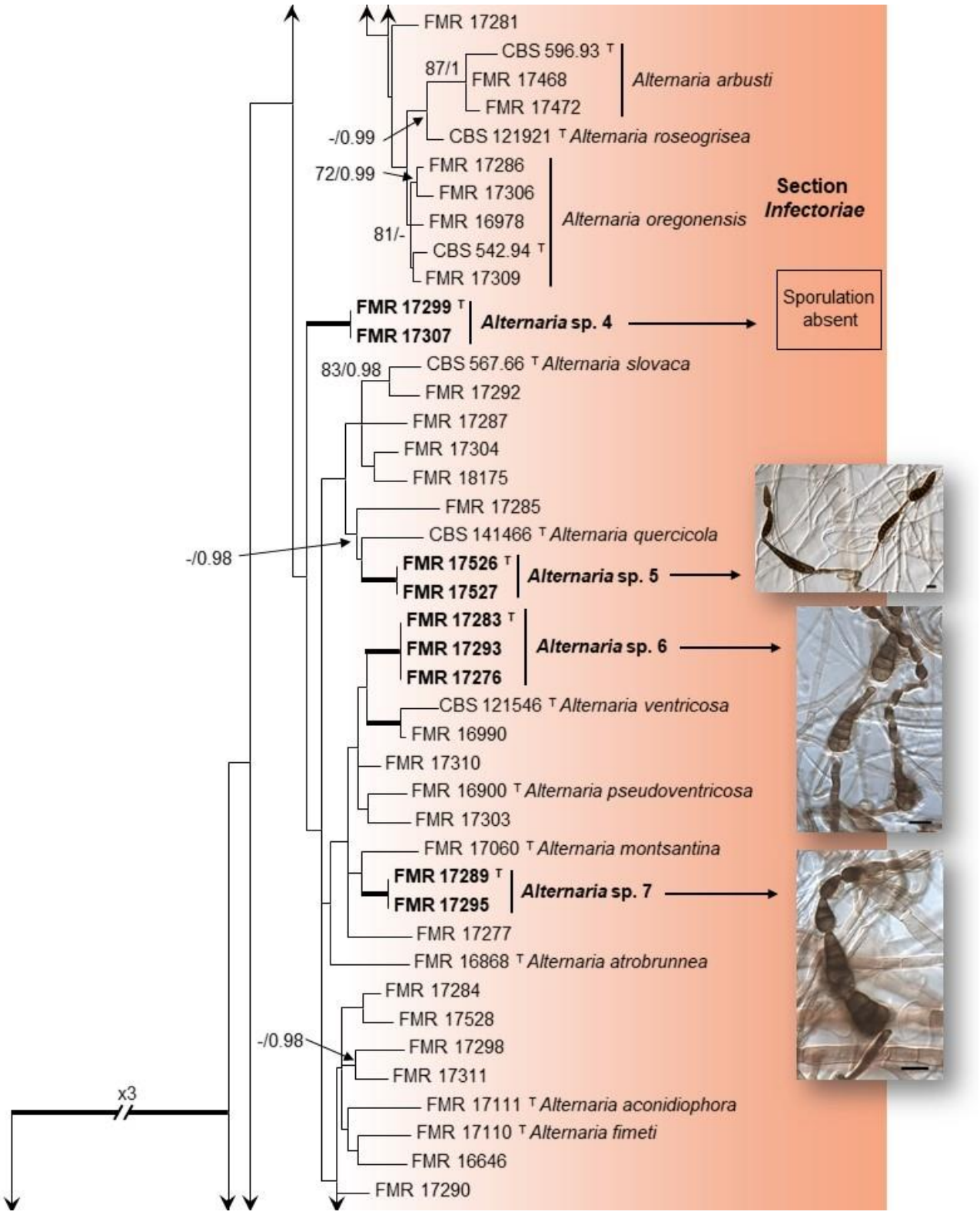
In preparation to be submited in: Persoonia

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

Herein are the preliminary results on the identification of 67 isolates morphologically interpreted as belonging to the *Alternaria* section *Infectoriae*, 51 from clinical origin (37 from the Carlos III Health Institute, Spain; 14 from the Fungus Testing Laboratory of the University of Texas, USA) and 16 from environmental origin. We isolated these latter from herbivore dung samples (12 isolates) and plant material (four isolates) collected mainly in Catalonia. However, sequence analysis of the *rpb*2 gene marker allowed us to confirm that 57 of these isolates belonged in the section mentioned above, six in the *Pseudoalternaria* section, and four in the *Chalastospora* section. As it is explained in previous sections (4.1.1 and 4.1.2) on *Alternaria* results, we carried out multi-locus sequence analyses combining different phylogenetic markers to resolve the phylogeny and taxonomy of unidentified isolates. That is ITS, *ATPase*, *gapdh*, *rpb*2 and *tef*1 for the phylogeny of the section *Infectoriae*, and the ITS, *ATPase* and *gapdh* loci were used for the sections *Chalastospora* and *Pseudoalternaria* (Lawrence et al. 2014, Deng et al. 2018, Poursafar et al. 2018).

Phylogenetic analysis of the combined dataset used for the section *Infectoriae* (Fig. 16) revealed that 10 isolates clustered in a single well-supported clade (ML ≥ 80 bs) with previously described *Alternaria* species, which are *A. anthropophila* (four isolates), *A. oregonensis* (four isolates) and *A. arbusti* (two isolates). The remaining 47 isolates did not match with well-established species and many of them could be considered as representatives of putative new species in the section, at least those with full-supported clades (100/1) that include more than one isolate (*Alternaria* sp. 1−9). The extreme genetic diversity in the section *Infectoriae* has previously been reported by Poursafar et al. (2018), however these authors preferred do not introduce any novel species since almost all of their strains were allocated in single unsupported branches. In our case, a considerable number of isolates (28 in total) is also represented by a single unsupported branch. Therefore, following the same criterion than the above-mentioned authors, those isolates will remain undescribed formally until to increase the number of specimens per clade to improve the robustness of the different phylogenetic species detected. Nevertheless, it is of note that several of those isolates have lost the ability to sporulate (26 in total), what hinders their proper morphological characterization. The nine novel species of the section *Infectoriae* we prepare for publication are characterized phenotypically below.


FMR 16235 T
FMR 17296
97/1
FMR 17529
Alternaria anthropophila
FMR 17278
FMR 17288
97/-
CBS 121330 T Alternaria incomplexa
FMR 17280
FMR 17279
CBS 119409 T Alternaria californica
CBS 121458 T Alternaria hordeicola
FMR 17466
CBS 119398 T Alternaria daucicaulis
CBS 119402 T Alternaria hordeiaustralica
CBS 119407 T Alternaria viburni
74/1
CBS 121923 T Alternaria cerasidanica
CBS 196.86 T Alternaria conjuncta
FMR 17312
CBS 119396 T Alternaria alternarina
FMR 17469
FMR 17004 T Alternaria lawrencei
FMR 17294
FMR 17305 T
Alternaria sp. 1
CBS 119404 T Alternaria humuli
CBS 118485 T Alternaria broccoli-italicae
88/1
CBS 210.86 T Alternaria infectoria
86/-
CBS 119401 T Alternaria frumenti
CBS 119405 T Alternaria novae zelandiae
CBS 119403 T Alternaria merytae
CBS 119400 T Alternaria graminicola
98/-
FMR 17291 T
FMR 17300
Alternaria sp. 2
CBS 763.84 T Alternaria triticina
FMR 17302 T
FMR 17308
Alternaria sp. 3
FMR 17590
92/0.99
CBS 578.94 T Alternaria triticimaculans
CBS 197.86 T Alternaria ethzedia
CBS 553.94 T Alternaria metachromatica
FMR 17589
-/0.99
CBS 119406 T Alternaria intercepta
FMR 17282
FMR 17461


FMR 17281
CBS 596.93 T
87/1
FMR 17468
FMR 17472
Alternaria arbusti
-/0.99
CBS 121921 T Alternaria roseogrisea
72/0.99
FMR 17286
FMR 17306
FMR 16978
81/-
CBS 542.94 T
FMR 17309
Alternaria oregonensis
Section Infectoriae
FMR 17299 T
FMR 17307
Alternaria sp. 4
Sporulation absent
83/0.98
CBS 567.66 T Alternaria slovaca
FMR 17292
FMR 17287
FMR 17304
FMR 18175
FMR 17285
CBS 141466 T Alternaria quercicola
-/0.98
FMR 17526 T
FMR 17527
Alternaria sp. 5
FMR 17283 T
FMR 17293
FMR 17276
Alternaria sp. 6
CBS 121546 T Alternaria ventricosa
FMR 16990
FMR 17310
FMR 16900 T Alternaria pseudoventricosa
FMR 17303
FMR 17060 T Alternaria montsantina
FMR 17289 T
FMR 17295
Alternaria sp. 7
FMR 17277
FMR 16868 T Alternaria atrobrunnea
FMR 17284
FMR 17528
-/0.98
FMR 17298
FMR 17311
x3
FMR 17111 T Alternaria aconidiophora
FMR 17110 T Alternaria fimeti
FMR 16646
FMR 17290

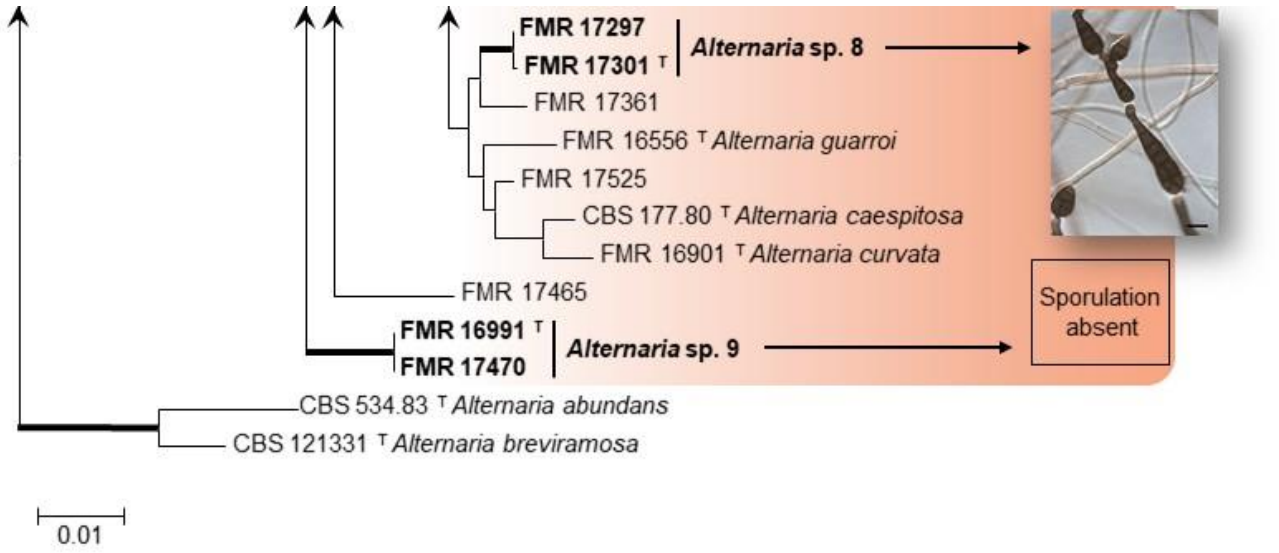


Figure 16. Maximum Likelihood (ML) tree constructed with ITS, *ATPase*, *gapdh*, *rpb2* and *tef1* sequences of ex-type strains of species in the section *Infectoriae*. The phylogenetic tree was rooted with *Alternaria abundans* CBS 534.83 and *A. breviramosa* CBS 121331 (section *Chalastospora*). The bootstrap values (bs) greater than 70 % and Bayesian posterior probabilities (pp) greater than 0.95 are given at the nodes (bs/pp). Bold branches indicate bs/pp of 100/1. The novel species proposed in this study are indicated in bold. T indicates ex-type strain. Scale bars = 10 µm.

Multi-locus sequence data of the section *Pseudoalternaria* (Fig. 17) allowed us to identify three isolates as *A. kordkuyana*, two of them being isolated from plant material and herbivore dung samples collected in Spain, and the third isolated from a knee tissue sample from the USA. Other three isolates were identified as *A. rosae*, two isolated from herbivore dung and the third one from a leg tissue sample received from the USA. Of the four isolates in the section *Chalastospora* (Fig. 17), two clustered with the clade representative of the well-established species *A. malorum*, one isolated from herbivore dung and the other from a human bronchial brush sample also from the USA. The other two isolates, obtained also from a Spanish dung sample and from an American human tracheal aspirate specimen, did not match with any of the currently accepted species of the section. Since they are allocated in two monophyletic highly-supported lineages (82/0.99 and 90/1, respectively), they are considered distinct taxa and prepared to be published soon. Both putative new species (*Alternaria* sp. 10−11) are characterized phenotypically below.

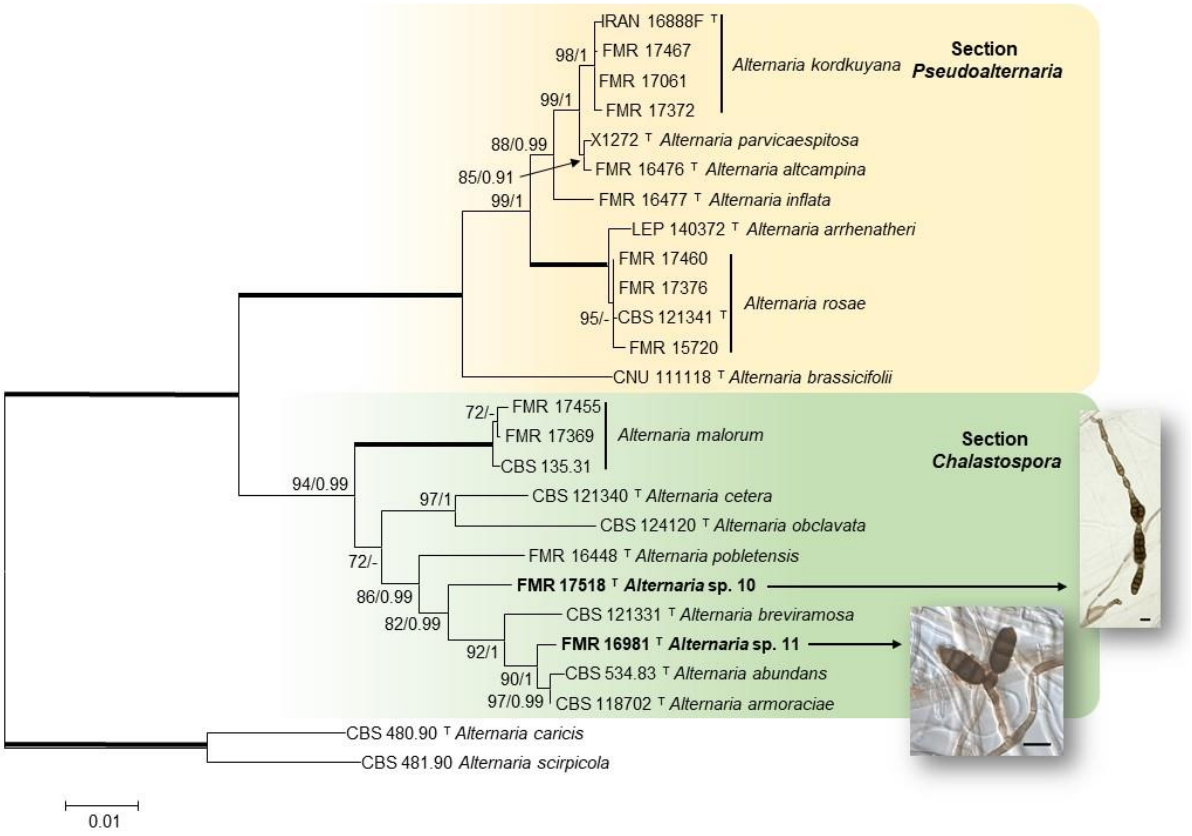


Figure 17. Maximum Likelihood (ML) tree constructed with ITS, *ATPase* and *gapdh* sequences of ex-type strains of species in the sections *Pseudoalternaria* and *Chalastospora*. The phylogenetic tree was rooted with *Alternaria caricis* CBS 480.90 and *A. scirpicola* CBS 481.90 (section *Nimbya*). The bootstrap values (bs) greater than 70 % and Bayesian posterior probabilities (pp) greater than 0.95 are given at the nodes (bs/pp). Bold branches indicate bs/pp of 100/1. The novel species proposed in this study are indicated in bold. T indicates ex-type strain. Scale bars = 10 µm.

Phenotypic characterization of the above-mentioned putative novel species of *Alternaria*

Alternaria sp. 1.

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 54−55 mm diam, with an irregular surface, orange white to white (6A2/1A1), cottony, aerial mycelium abundant, margins regular; reverse orange white (6A2/5A2). On PCA attaining 63−68 mm diam, olive brown to grey (2F4/4B1), flat, slightly floccose, aerial

mycelium scarce, margins regular; reverse yellowish brown to olive brown (5F8/4E3). On OA reaching 46−54 mm diam, flat, slightly floccose, scarce aerial mycelium, margins regular, surface and reverse olive brown (4D3) to colorless.

Microscopic characteristics on PCA at 25 ºC after 30 days: Mycelium superficial and immersed. Hyphae 1–4 µm wide, septate, branched, hyaline to pale brown, smooth-walled. Conidiophores macronematous, some reduced to conidiogenous cells on hyphae, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, usually unbranched, 6–59 × 3–4(–5) µm, pale brown, smooth-walled, with a terminal or occasionally a sub-terminal conidiogenous loci. Conidia solitary or forming branched chains, with up to 7 conidia in unbranched part, ovoid to ellipsoidal, obclavate, 10.5–28 × 5–11 µm, with darkened middle transverse septa, 1–5 transverse, and 0–1 longitudinal or oblique septa per transverse segment, brown, smooth. Secondary conidiophores, when present mainly on OA, formed apically with a single conidiogenous locus or as a short lateral extension on the conidial body bearing conidia. Alternarioid chlamydospores abundant, up to 350 µm long, with transverse and longitudinal septa, brown to dark brown, smooth-walled. Sexual morph not observed.

Cardinal temperature for growth: Optimum 20−25 °C, maximum 37 °C, minimum 5 °C.

Specimens examined: Spain, Andalucía, isolated from a human bone biopsy, *A. Alastruey-Izquierdo* (culture ex-type FMR 17305); Madrid, isolated from human corneal exudate, *A. Alastruey-Izquierdo* (FMR 17294).

***Alternaria* sp. 2.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 56−73 mm diam, white (1A1), cottony, aerial mycelium abundant, margins regular; reverse yellowish grey (4B2). On PCA attaining 56−65 mm diam, flat, slightly floccose, aerial mycelium scarce, margins regular; surface and reverse olive (2D3). On OA reaching 51−61 mm diam, flat, slightly floccose, scarce aerial mycelium, margins regular; surface and reverse yellowish grey (2C2).

Microscopic characteristics on SNA (supplemented with carnation pieces) at 25 ºC after 14 days: Mycelium superficial and immersed. Hyphae 1–5 µm wide, septate, branched, subhyaline to pale brown, smooth-walled. Conidiophores macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, branched and unbranched, 7–43 × 3–5 µm, sometimes reduced to conidiogenous cells, pale brown, smooth-walled, with a terminal or occasionally a sub-terminal conidiogenous loci. Conidia forming branched chains, with up to 6 conidia in unbranched part,

obclavate, ellipsoidal and ovoid, 7–43 × 4–16 µm, with darkened middle transverse septa, (0–)1–8 transverse, and 0–1 longitudinal or oblique septa per transverse segment, pale brown to brown, smooth. Secondary conidiophores abundant, apically or laterally from the conidial body as a short extension bearing conidia. Sexual morph not observed.

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Specimens examined: Spain, Andalucía, isolated from human skin sample, *A. Alastruey-Izquierdo* (culture ex-type FMR 17291); Catalonia, isolated from human cutaneous exudate, *A. Alastruey-Izquierdo* (FMR 17300).

***Alternaria* sp. 3.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 54−55 mm diam, slightly elevated, blond to white (4C4/1A1), cottony, aerial mycelium abundant, margins regular; reverse olive brown to orange white (4F8/5A2). On PCA attaining 56−66 mm diam, white (1A1), flat, slightly velvety towards the periphery, aerial mycelium scarce, margins regular; reverse white (1A1). On OA reaching 54−74 mm diam, flat, scarce aerial mycelium, margins regular; surface and reverse colorless.

Microscopic characteristics on PCA at 25 ºC after 30 days: Mycelium superficial and immersed. Hyphae 2–5 µm wide, septate, branched, pale brown, smooth-walled. Conidiophores semi-macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, sometimes reduced to conidiogenous cells, unbranched, 8–32.5(–171) × 3–6 µm, pale brown, smooth walled, with a terminal or occasionally a sub-terminal conidiogenous loci. Conidia solitary or forming short branched chains, with up to 4 conidia in unbranched part, subglobose to ovoid to ellipsoidal, 9–37 × 7–12 µm, with darkened middle transverse septa, 1–3(–5) transverse, and 0–1 longitudinal or oblique septa per transverse segment, brown, smooth-walled. Secondary conidiophores scarce, can be formed apically on the conidial body. Sexual morph not observed.

Cardinal temperature for growth: Optimum 20−25 °C, maximum 37 °C, minimum 5 °C.

Specimens examined: Spain, Andalucia, isolated from human cutaneous exudate, *A. Alastruey-Izquierdo* (Culture ex-type FMR 17302); Euskadi, isolated from human skin, A. Alastruey-Izquierdo (FMR 17308).

***Alternaria* sp. 4.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 53−68 mm diam, umbilicate, white (1A1), cottony, aerial mycelium abundant, margins regular; reverse orange white (5A2). On PCA attaining 47−70 mm diam, dark green (29F4) to white towards the periphery, flat, slightly velvety, aerial mycelium scarce, margins regular; reverse greenish grey (29F2) to white towards the periphery. On OA reaching 46−65 mm diam, flat, slightly velvety towards the periphery, scarce aerial mycelium, margins regular; surface and reverse brownish grey (5C2) to colorless towards the periphery.

Sporulation absent.

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Specimens examined: Spain, Aragón, isolated from human cutaneous exudate, *A. Alastruey-Izquierdo* (culture ex-type FMR 17299); Madrid, isolated from human muscle biopsy, *A. Alastruey-Izquierdo* (FMR 17307).

***Alternaria* sp. 5.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 59−60 mm diam, yellowish grey (2D2) to colorless, slightly convex, cottony, aerial mycelium abundant, margins regular; reverse dark green to dark blond (30F8/5D4), yellowish grey final edge (4B2). On PCA attaining 60−61 mm diam, flat, slightly velvety, aerial mycelium scarce, margins fimbriate; surface and reverse olive brown (4F4) to colorless towards the periphery. On OA reaching 59−60 mm diam, flat, slightly floccose, scarce aerial mycelium, margins regular; surface and reverse olive brown (4F4) to colorless towards the periphery.

Microscopic characteristics on PCA at 25 ºC after 7 days: Mycelium superficial and immersed. Hyphae 1–5 µm wide, septate, branched, subhyaline to yellowish brown, smooth-walled to verruculose. Conidiophores macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, unbranched, 10–59(–117) × 3–4(–5) µm, yellowish brown to brown, smooth-walled, with a terminal or occasionally a sub-terminal conidiogenous loci. Conidia solitary or forming short branched chains, with up to 9 conidia in unbranched part, ovoid, ellipsoidal, obclavate or subcylindrical, some of them slightly curved, 9–51 × 6–11.5 µm, with darkened middle transverse septa, 1–5(–10) transverse, and 0–1(–2) longitudinal or oblique septa per transverse segment, yellowish brown, smooth to tuberculate. Secondary conidiophores can be formed

apically or laterally from the conidial body as a short extension bearing conidia. Sexual morph not observed.

Cardinal temperature for growth: Optimum 25 °C, maximum 30 °C, minimum 15 °C.

Specimens examined: Spain, Barcelona province, Pontons (N 41.41397° E 1.52678°), isolated from herbivore dung, Jun. 2018, *J. Gené, J. Guarro & I. Iturrieta-González* (culture ex-type FMR 17526); Barcelona province, Pontons (N 41.41397° E 1.52680°), isolated from herbivore dung, Jun. 2018, *J. Gené, J. Guarro & I. Iturrieta-González* (FMR 17527).

***Alternaria* sp. 6.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 58−78 mm diam, greyish yellow (4B3), flat, cottony, aerial mycelium abundant, margins regular; reverse dark yellow to yellowish white (4C8/4A2). On PCA attaining 65−75 mm diam, greenish grey to olive grey (1B2/2D2), flat, floccose, aerial mycelium abundant, margins fimbriate; reverse white to olive grey (1A1/2D2). On OA reaching 57−70 mm diam, dark green (29F4), flat, scarce aerial mycelium, margins regular; reverse dark green (29F8).

Microscopic characteristics on PCA at 25 ºC after 7 days: Mycelium superficial and immersed. Hyphae 1–5 µm wide, septate, branched, subhyaline to pale brown, smooth-walled to verruculose. Conidiophores macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, usually unbranched, 15–127 × (3–)3.5–4(–5) µm, brown to dark brown, smooth-walled, with a terminal or occasionally a sub-terminal conidiogenous loci. Conidia solitary or forming branched chains, with up to 13 conidia in unbranched part, ovoid, ellipsoidal or obclavate, 8–55 × 4–12(–20) µm, with darkened middle transverse septa, (0–)1–7 transverse, and 0–1 longitudinal or oblique septa per transverse segment, brown to dark brown, smooth to verrucose. Secondary conidiophores can be formed apically or laterally from the conidial body as a short extension bearing conidia. Sexual morph not observed.

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Specimens examined: Spain, Madrid, isolated from unknown clinical sample, *A. Alastruey-Izquierdo* (culture ex-type FMR 17283); Euskadi, isolated from human skin biopsy, *A. Alastruey-Izquierdo* (FMR 17276); Castilla y León, isolated from human skin, *A. Alastruey-Izquierdo* (FMR 17293).

***Alternaria* sp. 7.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 77−80 mm diam, greyish yellow to pale grey (2B5/1B1), flat, velvety, aerial mycelium abundant, margins regular; reverse dark green (29F8) to black. On PCA attaining 78−80 mm diam, dark green (29F5), flat, floccose, abundant aerial mycelium, margins regular; reverse dark green (29F8). On OA reaching 76−78 mm diam, yellowish grey to olive (2D2/2F8), flat, abundant aerial mycelium, margins regular; reverse dark green (29F3).

Microscopic characteristics on PCA at 25 ºC after 7 days: Mycelium superficial and immersed. Hyphae 1–5 µm wide, septate, branched, subhyaline to pale brown, smooth-walled. Conidiophores macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, unbranched, 12.5–134 × 3–4.5(–5) µm, pale brown to brown, smooth-walled, with terminal conidiogenous loci. Conidia solitary or forming chains, with up to 6 conidia in unbranched part, ovoid or obclavate, sometimes curved, 8–51 × 4.5–14 µm, with darkened middle transverse septa, (0–)1–6(–7) transverse, and 0–1 longitudinal or oblique septa per transverse segment, brown to dark brown, smooth to verruculose. Sexual morph not observed.

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Specimens examined: Spain, Navarra, isolated from human bronchial aspirate, *A. Alastruey-Izquierdo* (culture ex-type FMR 17289); Madrid, isolated from human skin biopsy, *A. Alastruey-Izquierdo* (FMR 17295).

***Alternaria* sp. 8.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 73−74 mm diam, olive to greyish yellow (3E5/4B3), flat, velvety, aerial mycelium abundant, margins regular; reverse yellowish brown to brownish yellow (5F8/5C8). On PCA attaining 77−78 mm diam, olive to dark green (3E6/29F8), umbonate, densely floccose, abundant aerial mycelium, margins regular; reverse dark green (29F8). On OA reaching 63−64 mm diam, olive to dark green (3E6/29F8), flat, slightly floccose, slightly abundant aerial mycelium, margins regular; reverse dark green (29F8).

Microscopic characteristics on OA at 25 ºC after 14 days: Mycelium superficial and immersed. Hyphae 1–5 µm wide, septate, branched, pale brown to brown, smooth-walled. Conidiophores macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, usually unbranched, 19–156 × 3–5 µm, brown to dark brown, smooth-walled, with a terminal or occasionally a sub-terminal conidiogenous loci. Conidia forming branched chains, with up to 14 conidia in

unbranched part, ovoid, obclavate or subcylindrical, 11.5–52 × 6–11.5 µm, with darkened middle transverse septa, (1–)2–5(–7) transverse, and 0–1(–2) longitudinal or oblique septa per transverse segment, brown to dark brown, smooth to verruculose or verrucose. Secondary conidiophores can be formed apically or laterally from the conidial body as an extension bearing conidia. Sexual morph not observed.

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Specimens examined: Spain, Balearic islands, isolated from human cutaneous lesion, *A. Alastruey-Izquierdo* (culture ex-type FMR 17301); Castilla la Mancha, isolated from human skin, *A. Alastruey-Izquierdo* (FMR 17297).

***Alternaria* sp. 9.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 70−78 mm diam, pale grey to olive to olive brown (1B1/1E3/4D3) with a withe final edge, flat, slightly floccose, abundant aerial mycelium, margins regular; reverse yellowish brown to greyish yellow (5E4/4C5), white final edge. On PCA attaining 71−77 mm diam, olive (2F3) to colorless towards the periphery, flat, slightly granular, scarce aerial mycelium, margins regular; reverse dark green to olive (29F8/2F3). On OA reaching 72−73 mm diam, olive brown (4E3), flat, slightly floccose, scarce aerial mycelium, margins regular; reverse olive brown (4E4).

Sporulation absent.

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Specimen examined: USA, Colorado, isolated from human tracheal aspirate, *N. Wiederhold* (culture ex-type FMR 16991); Colorado, isolated from human tissue leg right, *N. Wiederhold* (FMR 17470).

***Alternaria* sp. 10.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 58−61 mm diam, blond to yellowish white (4C4/4A2), cottony, abundant aerial mycelium, margins regular; reverse yellowish brown (5F8/5D5), yellowish white final edge (4A2). On PCA attaining 49−50 mm diam, flat, slightly velvety, scarce aerial mycelium, margins regular; surface and reverse grey (1D4) to colorless towards the periphery. On OA reaching 55−56 mm diam, flat, slightly floccose, scarce aerial mycelium, margins regular; surface and reverse olive (4E3) to colorless.

Microscopic characteristics on OA at 25 ºC after 7 days: Mycelium superficial and immersed. Hyphae 1–4 µm wide, septate, branched, subhyaline to pale olivaceous, smooth-walled to verruculose. Conidiophores micronematous, semi-macronematous to macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, unbranched, 10.5–73 × 3–4.5 µm, pale olivaceous to yellowish brown, smooth-walled, with terminal conidiogenous loci. Conidia forming branched chains, with up to 15 conidia in unbranched part, ellipsoidal or obclavate, 10–40 × 4–14 µm, with darkened middle transverse septa, some of them with constricted septa, (1–)3–5(–7) transverse septa, and 0–1(–2) longitudinal or oblique septa per transverse segment, some of them show a muriform shape, 37–45 × 16–33 µm, yellowish brown to brown, smooth-walled. Secondary conidiophores can be formed apically as conidiogenous loci towards the conidial body. Sexual morph not observed.

Cardinal temperature for growth: Optimum 20 °C, maximum 30 °C, minimum 15 °C.

Specimen examined: Spain, Barcelona, Pontons (N 41.40590° E 1.50918°), isolated from herbivore dung, Jun. 2018, *J. Gené, J. Guarro & I. Iturrieta-González* (culture ex-type FMR 17518).

***Alternaria* sp. 11.**

Culture characteristics at 25 °C after 7 days: Colonies on PDA reaching 62−64 mm diam, yellowish grey (4B2), flat, densely floccose, abundant aerial mycelium, margins irregular; reverse olive brown (4F8/4D6). On PCA attaining 54−55 mm diam, olive (3E3) to white final edge, flat, floccose, abundant aerial mycelium, margins fimbriate; reverse olive (3F3), white final edge. On OA reaching 57−58 mm diam, yellowish brown (5F4), flat, slightly floccose, scarce aerial mycelium, margins regular; reverse brownish beige (6E3).

Microscopic characteristics on PCA at 25 ºC after 7 days: Mycelium superficial and immersed. Hyphae 1–4 µm wide, septate, branched, hyaline to pale brown, smooth-walled. Conidiophores macronematous, arising laterally or terminally from aerial hyphae, erect to slightly flexuous, branched and unbranched, 10–98 × 3–4.5(–5) µm, pale brown to brown, smooth-walled, with a terminal or occasionally a sub-terminal conidiogenous loci. Conidia solitary, cylindrical, ellipsoidal, ovoid, sometimes slightly curved, 10.5–25 × 6–11 µm, with darkened middle transverse septa, 1–3(–4) transverse, sometimes constricted at the septum, and 0–1 longitudinal or oblique septa per transverse segment, brown, tuberculate. Sexual morph not observed.

Cardinal temperature for growth: Optimum 25 °C, maximum 37 °C, minimum 5 °C.

Specimen examined: USA, Pensilvania, isolated from human right ethmoid contents, *N. Wiederhold* (culture ex-type FMR 16981).

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4.2 Results on the genus *Cladosporium* and cladosporium-like fungi

4.2.1 Six new *Cladosporium* species from environmental sources in Spain.

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Accepted for publication under major revisions: Journal of Fungi

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Article

Six New *Cladosporium* Species From Environmental Sources in Spain

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Received: 21 August 2020; Accepted: date; Published: date

Abstract: *Cladosporium* is a monophyletic genus in *Cladosporiaceae* (*Cladosporiales, Dothideomycetes*) which species are mainly found as saprobes and endophytes, but also includes fungi pathogenic for plants, animals and human. Species identification is currently based on three genetic markers; the internal transcribed spacer regions (ITS) of the rDNA, and partial fragments of actin (*ACT1)* and the translation elongation factor 1-α (*EEF1A*) genes. Using this phylogenetic approach and from morphological differences, we have recognized six new species originating from soil, herbivore dung or plant material collected at different Spanish locations. They are proposed as *Cladosporium caprifimosum*, *C. coprophilum*, *C. fuscoviridum* and *C. lentulum* belonging in the *C. cladosporioides* species complex, and *C. pseudotenellum* and *C. submersum* in the *C. herbarum* species complex. This study reveals that herbivore dung is an interesting substrate from which to explore the diversity of *Cladosporium* species.

Keywords: Cladosporiales; Cladosporiaceae; Hyphomycetes; Phylogeny; Taxonomy; Spain.

1. Introduction

Cladosporium is a ubiquitous genus in the family *Cladosporiaceae* of the recently proposed order *Cladosporiales* in the *Dothideomycetes* [1]. Their species are recovered from a wide range of substrates and have been reported to be among the most common fungi in both indoor and outdoor environments, including in extreme ecological niches [2–8]. Most *Cladosporium* species are saprophytic, but some have also been reported as endophytic, hyperparasites on other fungi and pathogenic on plants and animals, including humans [9–12]. Certain species show the ability to produce compounds of medical interest or are relevant as potencial biocontrol agents for plant disease [13-15].

Cladosporium is morphologically characterized mainly by its asexual form, which shows differentiated conidiophores producing acropetal chains of conidia from mono- or polyblastic conidiogenous cells. Both conidiogenous cells and conidia exhibit conidial scars with a unique coronate structure, which is composed of a central convex dome surrounded by a raised periclinal rim, usually thickened, refractive and darkly pigmented [16]. Based on these features and DNA phylogeny derived from the LSU nrRNA gene, the genus has been well-delineated and distinguished from other cladosporium-like genera such as *Hyalodendriella, Ochrocladosporium, Rachicladosporium, Rhizocladosporium, Toxicocladosporium, Verrucocladosporium* and the recently described genus *Neocladosporium* [17–18]. Phylogenetic relationships among species of *Cladosporium s. str.* have been studied extensively over the last decade by a multi-locus sequence analysis approach with sequences of the internal transcribed spacers (ITS) of the rDNA and of the two protein encoding genes, translation elongation factor 1-α (*EEF1A*) and actin (*ACT1*). The molecular approach combined with morphological features have allowed recognition of more than 230 species within the genus, which are

split into three species complexes, i.e. *Cladosporium cladosporioides*, *Cladosporium herbarum* and *Cladosporium sphaerospermum* complex [2–3,6,10,12,19–20].

While aiming to explore the diversity of microfungi from Spain, several interesting *Cladosporium* isolates have been recovered from different environmental samples. Using the above mentioned polyphasic approach, the taxonomy of those isolates has been resolved in six novel species for science; four classified into the *C. cladosporioides* species complex and two into the *C. herbarum* complex.

2. Materials and Methods

2.1. Samples and Isolates

Samples of soil, plant debris and herbivore dung were collected between 2016 and 2018 at various Spanish locations. Dilution plating methods were used for isolating fungi from soil and dung samples following the procedure described by Crous et al. [21] and a modified protocol described by Waksman [22], respectively. In addition, soil samples were also processed by baiting technique using small pieces of wood and filter paper as baits on the soil surface [23]. Samples of plant debris and also part of the herbivore dung were incubated in moist chambers following the procedures described by Castañeda-Ruiz et al. [24] and Richardson [25], respectively.

Among the cladosporium-like fungi found, we recovered eight isolates in pure culture which did not match with any of the currently accepted species within the genus *Cladosporium* (Table 1). The isolates were deposited in the culture collection of the Universitat Rovira i Virgili (FMR, Reus, Spain) and, once phylogenetically and morphologically characterized, living cultures of the novel species and dry cultures for holotypes were also deposited in the Westerdijk Fungal Biodiversity Institute (CBS; Utrecht, the Netherlands). Nomenclatural novelties and descriptions were deposited in MycoBank [26].

2.2. DNA Extraction, Amplification and Sequencing

Genomic DNA was extracted from cultures growing on potato dextrose agar (PDA; Pronadisa, Spain) after 7 days of incubation at 25 °C, following the modified protocol of Müller et al. [27]. Protocols listed previously in Sandoval-Denis et al. [10] were used for amplification and sequencing. The primer pairs used were ITS5/ITS4 [28] to amplify the ITS region including the 5.8S gene of the rDNA, EF-728F/EF-986R to amplify a partial fragment of the *EEF1A* gene, and ACT-512F/ACT-783R to amplify the *ACT1* gene [29]. PCR products were purified and stored at -20 °C until sequencing. The sequences were obtained using the same primers at Macrogen Europe (Macrogen Inc. Amsterdam, The Netherlands). Finally, the software SeqMan v. 7.0.0 (DNAStarLasergene, Madison, WI, USA) was used to assemble, edit and obtain the consensus sequences, which were then deposited in GenBank of NCBI (Table 1).

Table 1. Species, strains and GenBank accession number of the sequences included in this study.

Species	Strain no.[1]	Substrate[2]	GenBank nucleotide accession no. for[3]:		
			ITS	*ACT1*	*EEF1A*
Cercospora beticola	CBS 116456	*Beta vulgaris*	NR_121315	AY840458	AY840494
Cladosporium acalyphae	CBS 125982^T	*Acalypha australis*	HM147994	HM148481	HM148235
C. aerium	CBS 143356^T	Indoor air	MF472897	MF473747	MF473324
	DTO 323-G7	Indoor air	MF472899	MF473749	MF473326
C. aggregatocicatricatum	CBS 140493^T	Culture contaminant	KT600448	KT600645	KT600547
C. alboflavescens	CBS 140690^T	Animal BAL	LN834420	LN834604	LN834516
C. allii	CBS 101.81	Velvet spots on *Allium porrum*	JN906977	JN906996	JN906983
C. allicinum	CBS 121624^T	*Hordeum vulgare*	EF679350	EF679502	EF679425
	UTHSC DI-13-176	Human skin	LN834354	LN834538	LN834450
C. angulosum	CBS 140692^T	Human BAL	LN834425	LN834609	LN834521
C. angustiherbarum	CBS 140479^T	*Pinus ponderosa*	KT600378	KT600574	KT600475

C. angustisporum	CBS 125983^{T}	*Alloxylon wickhamii*	HM147995	HM148482	HM148236
C. angustiterminale	CBS 140480^{T}	*Banksia grandis*	KT600379	KT600575	KT600476
C. antarcticum	CBS 690.92^{T}	*Caloplaca regalis*	EF679334	EF679484	EF679405
C. anthropophilum	CBS 140685^{T}	Human BAL	LN834437	LN834621	LN834533
	CBS 674.82	Seed of *Gossypium* sp.	HM148014	HM148501	HM148255
C. arthropodii	CBS 124043^{ET}	*Arthropodium cirratum*	JN906979	JN906998	JN906985
C. asperulatum	CBS 126339	Leaf litter of *Eucalyptus* sp.	HM147997	HM148484	HM148238
	CBS 126340^{T}	*Protea susannae*	HM147998	HM148485	HM148239
C. australiense	CBS 125984^{T}	*Eucalyptus moluccana*	HM147999	HM148486	HM148240
C. austroafricanum	CBS 140481^{T}	Leaf litter	KT600381	KT600577	KT600478
C. basiinflatum	CBS 822.84^{T}	*Hordeum vulgare*	HM148000	HM148487	HM148241
C. caprifimosum	**FMR 16532^{T} (CBS 146918)**	**Goat dung**	**LR813198**	**LR813205**	**LR813210**
C. chalastosporoides	CBS 125985^{T}	Fruiting bodies of *Teratosphaeria proteae-arboreae*	HM148001	HM148488	HM148242
C. chasmanthicola	CBS 142612^{T}	Leaf spots of *Chasmanthe aethiopica*	KY646221	KY646224	KY646227
C. chubutense	CBS 124457^{T}	*Pinus ponderosa*	FJ936158	FJ936165	FJ936161
C. cladosporioides	CBS 112388^{T}	Air of indoor environment	HM148003	HM148490	HM148244
	CBS 113738	Grape bud	HM148004	HM148491	HM148245
C. colocasiae	CBS 386.64^{T}	*Colocasia esculenta*	HM148067	HM148555	HM148310
	CBS 119542	*Colocasia esculenta*	HM148066	HM148554	HM148309
C. colombiae	CBS 274.80B^{T}	*Cortaderia* sp.	FJ936159	FJ936166	FJ936163
C. coprophilum	**FMR 16101**	**Unidentified herbivore dung**	**LR813199**	**LR813204**	**LR813211**
	FMR 16164^{T} (CBS 144919)	**Unidentified herbivore dung**	**LR813201**	**LR813207**	**LR813213**
C. crousii	CBS 140686^{T}	Human BAL	LN834431	LN834615	LN834527
C. cucumerinum	CBS 171.52^{T}	*Cucumis sativus*	HM148072	HM148561	HM148316
	CBS 176.54	*Cucumis sativus*	HM148078	HM148567	HM148322
C. delicatulum	CBS 126344^{T}	Leaves of *Tilia cordata*	HM148081	HM148570	HM148325
	CBS 126342	Indoor air	HM148079	HM148568	HM148323
C. echinulatum	CBS 123191	*Dianthus barbatus*	JN906980	JN906999	JN906987
C. europaeum	CBS 116744	Leaves of *Acer pseudoplatanus*	HM148053	HM148540	HM148294
	CBS 134914^{T}	Indoor building material	HM148056	HM148543	HM148298
C. exasperatum	CBS 125986^{T}	*Eucalyptus tintinnans*	HM148090	HM148579	HM148334
C. exile	CBS 125987^{T}	Chasmothecia of *Phyllactinia guttata*	HM148091	HM148580	HM148335
C. fildesense	ChFC-554^{T}	Unidentified marine sponge	JX845290	MN233632	MN233633
C. flavovirens	CBS 140462^{T}	Human toenail	LN834440	LN834624	LN834536
C. flabelliforme	CBS 126345^{T}	*Melaleuca cajuputi*	HM148092	HM148581	HM148336
C. floccosum	CBS 140463^{T}	Humans ethmoid sinus	LN834416	LN834600	LN834512
C. funiculosum	CBS 122128	*Ficus carica*	HM148093	HM148582	HM148337
	CBS 122129^{T}	*Vigna umbellata*	HM148094	HM148583	HM148338
C. fuscoviridum	**FMR 16385^{T} (CBS 146920)**	**Garden soil**	**LR813200**	**LR813206**	**LR813212**
C. gamsianum	CBS 125989^{T}	*Strelitzia* sp.	HM148095	HM148584	HM148339
C. globisporum	CBS 812.96^{T}	Meat stamp	HM148096	HM148585	HM148340
C. grevilleae	CBS 114271^{T}	Leaves of *Grevillea* sp.	JF770450	JF770473	JF770472
C. herbaroides	CBS 121626^{T}	Hypersaline water	EF679357	EF679509	EF679432
C. herbarum	CBS 121621^{ET}	*Hordeum vulgare*	EF679363	EF679516	EF679440
C. hillianum	CPC 15458	Leaf of *Typha orientalis*	HM148098	HM148587	HM148342

	CBS 125988^{T}	Leaf of *Typha orientalis*	HM148097	HM148586	HM148341
C. inversicolor	CBS 401.80^{T}	Leaf of *Triticum aestivum*	HM148101	HM148590	HM148345
	CBS 143.65	Leaf of *Tilia* sp.	HM148100	HM148589	HM148344
C. ipereniae	CPC 16855	*Arctostaphylos pallida*	KT600395	KT600590	KT600492
	CBS 140483^{T}	*Puya* sp.	KT600394	KT600589	KT600491
C. iranicum	CBS 126346^{T}	Leaf of *Citrus sinensis*	HM148110	HM148599	HM148354
C. iridis	CBS 138.40^{ET}	Leaves of *Iris* sp.	EF679370	EF679523	EF679447
C. kenpeggii	CBS 142613^{T}	Leaves of *Passiflora edulis*	KY646222	KY646225	KY646228
C. lentulum	**FMR 16288^{T} (CBS 146921)**	**Unidentified leaf litter**	**LR813203**	**LR813209**	**LR813215**
	FMR 16389	**Unidentified herbivore dung**	**LR813202**	**LR813208**	**LR813214**
C. limoniforme	CBS 140484^{T}	*Musa acuminata*	KT600397	KT600592	KT600494
	CBS 113737	Grape berry	KT600396	KT600591	KT600493
C. licheniphilum	CBS 125990^{ET}	*Phaeophyscia orbicularis* and *Physcia* sp.	HM148111	HM148600	HM148355
C. longicatenatum	CBS 140485^{T}	Unknown plant	KT600403	KT600598	KT600500
C. longissimum	CBS 300.96 ^{T}	Soil along coral reef coast	DQ780352	EF101385	EU570259
C. lycoperdinum	CBS 126347	Galls of *Apiosporina morbosa*	HM148112	HM148601	HM148356
	CBS 574.78C	*Aureobasidium caulivorum*	HM148115	HM148604	HM148359
C. macrocarpum	CBS 121623^{NT}	*Spinacia oleracea*	EF679375	EF679529	EF679453
	UTHSC DI-13-191	Human face	LN834379	LN834563	LN834475
C. magnoliigena	MFLUCC 18–1559 ^{T}	Cone of *Magnolia grandiflora*	MK347813	-	MK340864
	MFLUCC 18–1557	Cone of *Magnolia grandiflora*	MK347811	-	MK340862
C. montecillanum	CBS 140486^{T}	Pine needles	KT600406	KT600602	KT600504
	CPC 15605	*Taraxacum* sp.	KT600407	KT600603	KT600505
C. myrtacearum	CBS 126349	*Eucalyptus placita*	HM148116	HM148605	HM148360
	CBS 126350^{T}	*Corymbia foelscheana*	HM148117	HM148606	HM148361
C. needhamense	CBS 143359^{T}	Indoor (office) air sample	MF473142	MF473991	MF473570
C. neerlandicum	CBS 143360^{T}	Swab sample	KP701887	KP702010	KP701764
C. neopsychrotolerans	CGMCC 3.18031^{T}	Soil of *Saussurea involucrata*	KX938383	KX938366	KX938400
	CGMCC 3.18032	Soil of *Saussurea involucrata*	KX938384	KX938367	KX938401
C. ossifragi	CBS 842.91^{ET}	Green leaf of *Narthecium ossifragum*	EF679381	EF679535	EF679459
C. oxysporum	CBS 125991	Soil, near the terracotta army	HM148118	HM148607	HM148362
	CBS 126351	Indoor air	HM148119	HM148608	HM148363
C. paracladosporioides	CBS 171.54^{T}	Unknown	HM148120	HM148609	HM148364
C. paralimoniforme	CGMCC 3.18103^{T}	Soil	KX938392	KX938375	KX938409
	CGMCC 3.18104	Soil	KX938393	KX938376	KX938410
C. parapenidielloides	CBS 140487^{T}	*Eucalyptus* sp.	KT600410	KT600606	KT600508
C. parasubtilissimum	CBS 143361^{T}	Indoor (bathroom) air	MF473170	MF474018	MF473593
	CPC 22396	Indoor (recreational vehicle) air	MF473171	MF474019	MF473594
C. perangustum	CBS 125996^{T}	*Cussonia* sp.	HM148121	HM148610	HM148365

C. phaenocomae	CBS 128769^{T}	*Phaenocoma prolifera*	JF499837	JF499881	JF499875
C. phlei	CBS 358.69^{ET}	*Phleum pratense*	JN906981	JN907000	JN906991
C. phyllactiniicola	CBS 126352^{T}	Chasmothecia of *Phyllactinia guttata*	HM148150	HM148639	HM148394
	CBS 126355	Chasmothecia of *Phyllactinia guttata*	HM148153	HM148642	HM148397
C. phyllophilum	CPC 13873	*Teratosphaeria proteae-arboreae* on *Protea arborea*	HM148155	HM148644	HM148399
	CBS 125992^{ET}	*Taphrina* sp. on *Prunus cerasus*	HM148154	HM148643	HM148398
C. pini-ponderosae	CBS 124456^{T}	*Pinus ponderosa*	FJ936160	FJ936167	FJ936164
C. prolongatum	CGMCC 3.18035	Soil of *Populus euphratica*	KX938395	KX938378	KX938412
	CGMCC 3.18036^{T}	Soil of *Populus euphratica*	KX938394	KX938377	KX938411
C. pseudiridis	CBS 116463^{T}	Leaf lesions on *Iris* sp.	EF679383	EF679537	EF679461
C. pseudotenellum	**FMR 16231^{T} (CBS 146922)**	**Garden soil**	**LR813145**	**LR813146**	**LR813196**
C. pseudochalastosporoides	CBS 140490^{T}	Pine needles	KT600415	KT600611	KT600513
C. pseudocladosporioides	CBS 125993^{T}	Outside air	HM148158	HM148647	HM148402
C. puyae	CBS 274.80A^{T}	*Puya goudotiana*	KT600418	KT600614	KT600516
C. ramotenellum	CBS 121628^{T}	Hypersaline water	EF679384	EF679538	EF679462
C. rectoides	CBS 125994^{T}	*Vitis flexuosa*	HM148193	HM148683	HM148438
	CBS 126357	*Plectranthus* sp.	HM148194	HM148684	HM148439
C. rhusicola	CBS 140492^{T}	*Rhus* sp.	KT600440	KT600637	KT600539
C. rugulovarians	CBS 140495^{T}	Leaf of unidentified *Poaceae*	KT600459	KT600656	KT600558
C. scabrellum	CBS 126358^{T}	*Ruscus hypoglossum*	HM148195	HM148685	HM148440
C. silenes	CBS 109082^{T}	*Silene maritima*	EF679354	EF679506	EF679429
C. sinense	CBS 143363^{T}	Indoor air	MF473252	MF474102	MF473675
C. sinuatum	CGMCC 3.18096^{T}	Alpine soil	KX938385	KX938368	KX938402
	CGMCC 3.18097	Alpine soil	KX938386	KX938369	KX938403
C. sinuosum	CBS 121629^{T}	*Fuchsia excorticata*	EF679386	EF679540	EF679464
	CBS 393.68	Air	KT600442	KT600639	KT600541
C. soldanellae	CBS 132186^{NT}	*Soldanella alpina*	JN906982	JN907001	JN906994
C. sphaerospermum	CBS 193.54	Human nails	DQ780343	EU570269	EU570261
C. spinulosum	CBS 119907^{T}	Hypersaline water	EF679388	EF679542	EF679466
C. subcinereum	CBS 140465^{T}	Human sputum	LN834433	LN834617	LN834529
C. subinflatum	CBS 121630^{T}	Hypersaline water	EF679389	EF679543	EF679467
	UTHSC DI-13-189	Human toenail	LN834391	LN834575	LN834487
C. submersum	**FMR 17264^{T} (CBS 146923)**	**Submerged plant material**	**LR813144**	**LR813195**	**LR813197**
C. subtilissimum	CBS 113754^{T}	Grape berry	EF679397	EF679551	EF679475
	CBS 113753	Bing cherry fruit	EF679396	EF679550	EF679474
C. subuliforme	CBS 126500^{T}	*Chamaedorea metallica*	HM148196	HM148686	HM148441
	CPC 15833	*Citrus* sp.	KT600453	KT600650	KT600552
C. tenellum	CBS 121634^{T}	Hypersaline water	EF679401	EF679555	EF679479
	CPC 11813	*Phyllactinia* sp. on leaves of *Corylus* sp.	EF679399	EF679553	EF679477
	CPC 12051	Hypersaline water	EF679400	EF679554	EF679478
	CPC 22290	Indoor air	MF473278	MF474128	MF473701
	CPC 22291	Indoor air	MF473279	MF474129	MF473702
	CPC 22410	Indoor air	MF473280	MF474130	MF473703
	DTO 127-D7	Air	KP701932	KP702054	KP701809

C. tenuissimum	CBS 125995^{ET}	*Lagerstroemia* sp.	HM148197	HM148687	HM148442
C. tianshanense	CGMCC 3.18033^{T}	Soil of *Saussurea involucrata*	KX938381	KX938364	KX938398
	CGMCC 3.18034	Soil of *Saussurea involucrata*	KX938382	KX938365	KX938399
C. tuberosum	CBS 140693^{T}	Human nasal biopsy	LN834417	LN834601	LN834513
	UTHSC DI-13-219	Human foot	LN834419	LN834603	LN834515
C. uwebraunianum	CBS 143365^{T}	Indoor (archive) air	MF473306	MF474156	MF473729
	DTO 072-C8	Indoor (archive) air	KP701873	KP701996	KP701750
C. variabile	CBS 121635^{ET}	*Spinacia oleracea*	EF679402	EF679556	EF679480
C. varians	CBS 126360	*Ulmus* sp.	HM148222	HM148713	HM148468
	CBS 126362^{T}	*Catalpa bungei*	HM148224	HM148715	HM148470
C. versiforme	CBS 140491^{T}	*Hordeum* sp.	KT600417	KT600613	KT600515
C. verruculosum	CGMCC 3.18099^{T}	Alpine soil	KX938388	KX938371	KX938405
	CGMCC 3.18100	Alpine soil	KX938389	KX938372	KX938406
C. verrucocladosporioides	CBS 126363^{T}	*Rhus chinensis*	HM148226	HM148717	HM148472
C. vicinum	CBS 143366^{T}	Indoor air	MF473311	MF474161	MF473734
	CBS 306.84	Urediniospores of *Puccinia allii*	HM148057	HM148544	HM148299
C. welwitschiicola	CBS 142614^{T}	Dead leaf of *Welwitschia mirabilis*	KY646223	KY646226	KY646229
C. westerdijkiae	CBS 113746^{T}	Bing cherry fruits	HM148061	HM148548	HM148303
	CPC 10150	*Fatoua villosa*	HM148062	HM148549	HM148304
C. wyomingense	CBS 143367^{T}	Indoor (living room) air	MF473315	MF474165	MF473738
C. xanthochromaticum	CBS 140691^{T}	Human BAL	LN834415	LN834599	LN834511
C. xylophilum	CBS 113749	Bing cherry fruits	HM148228	HM148719	HM148474
	CBS 125997^{T}	Dead wood of *Picea abies*	HM148230	HM148721	HM148476

[1] CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CGMCC: China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; ChFC: Chilean Fungal Collection, Chile; CPC: Culture collection of Pedro Crous, housed at CBS; DTO: Working collection of Jos Houbraken housed at CBS; FMR: Facultat de Medicina i Ciències de la Salut, Reus, Spain; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; UTHSC: Fungus Testing Laboratory at the University of Texas Health Science Center, San Antonio, TX, USA.^{T,} ^{ET,} ^{NT}, indicate ex-type, ex-epitype and ex-neotype strains, respectively. [2] BAL: bronchoalveolar lavage fluid. [3] ITS: Internal transcribed spacer regions of the rDNA and 5.8S region; *ACT1*: partial actin gene; *EEF1A*: partial translation elongation factor 1-alpha gene; Sequences newly generated in this study and novel species proposed are indicated in bold.

2.3. Sequence Alignment and Phylogenetic Analysis

The sequences obtained were compared with other fungal sequences deposited in the NCBI database through the BLASTn tool. Alignment of those sequences and the phylogenetic analysis for each locus were performed with the MEGA (Molecular Evolutionary Genetics Analysis) program v. 6.0. [30], using ClustalW algorithm [31] and refined with MUSCLE [32] or manually if necessary, on the same platform. Since the isolates under study were related to the *C. cladosporioides* and C. *herbarum* species complexes, we also carried out alignments including sequence data of ex-type and reference strains of all the species from both complexes retrieved from the GenBank and mainly published by Bensch et al. [6].

Phylogenetic reconstructions were made with the three phylogenetic markers (ITS, *ACT1* and *EEF1A*) recommended for an accurate identification at the species level [2,6,12] using Maximum Likelihood (ML), maximum parsimony (MP), and Bayesian Inference (BI) analyses, with the Mega software v. 6.0. for the former two [30] and with MrBayes v.3.2.6 for the latter one [33] . The combined

analysis of these three phylogenetic markers was tested visually and through Incongruence Length Difference (ILD) implemented in the Winclada programme [34].

Determined by Mega software v. 6.0., the best nucleotide substitution model for ML analysis of the *C. cladosporioides* complex was General Time Reversible with Gamma distribution and invariant sites (GTR+G+I), and for the *C. herbarum* complex the best was the Kimura 2-parameter with Gamma distribution and invariant sited (K2+G+I). Bootstrap support value ≥ 70 % was considered significant.

The MP analysis was performed using the heuristic search option with TBR (tree bisection and reconnection) branch swapping and 1,000 random sequence additions. Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RCI) were calculated. Bootstrap analysis was based on 1,000 replications [35]. Maximum parsimony bootstrap support value ≥ 70 % was considered significant.

Determined by jModelTest [36], the best nucleotide substitution models for the BI of the *C. cladosporioides* complex were Jukes Cantor with invariant sites (JC+I) for ITS, General Time Reversible with Gamma distribution and invariant sites (GTR+G+I) for *EEF1A* and Hasegawa-Kishino-Yano with Gamma distribution (HKY+G) for *ACT1;* and for the *C. herbarum* complex the best were the Kimura 2-parameter with Gamma distribution (K80+G) for ITS, Hasegawa-Kishino-Yano with Gamma distribution (HKY+G) for *EEF1A* and *ACT1.* The parameter settings used in these analyses were two simultaneous runs of 10,000,000 generations, and four Markov chains, sampled every 1,000 generations. The 50 % majority rule consensus tree and posterior probability values (PP) were calculated after discarding the first 25 % of the samples. A PP value of ≥ 0.95 was considered significant.

Final sequence alignments and trees generated in this study were registered in TreeBASE under the submission number 27016 (http://treebase.org).

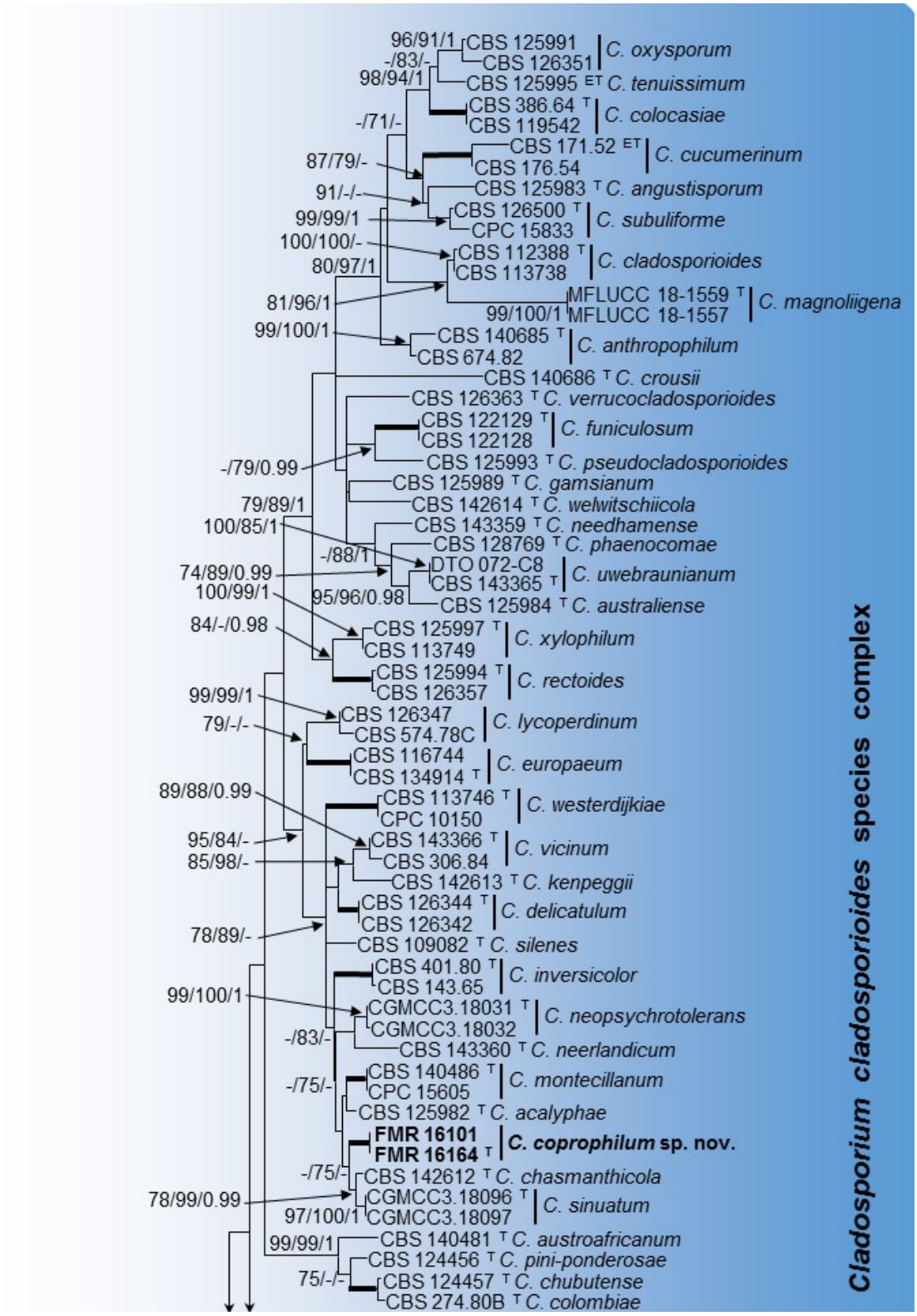
2.4. Phenotypic Studies

Microscopic features of the *Cladosporium* isolates were obtained from cultures growing on synthetic nutrient-poor agar (SNA; 1 g of KH_2PO_4, 1 g of KNO_3, 0.5 g of $MgSO_4$ x $7H_2O$, 0.5 g of KCl, 0.2 g of glucose, 0.2 g of sucrose, 14 g of bacteriological agar, 1 L of distilled water) after 7 to 14 days at 25 °C in the dark, mounted onto semi-permanent slides with Shear`s solution [6]. At least 30 measurements were taken to calculate length and width ranges of the conidia and ramoconidia, given as the mean ± standard deviation in the descriptions. Macroscopic characterization of the colonies was made on PDA (Pronadisa, Spain), oatmeal agar (OA; 30 g of oatmeal, 13 g of bacteriological agar, 1 L distilled water) and SNA after 14 days of incubation at 25 °C in darkness. Colour notation of the colonies in descriptions were from Kornerup & Wanscher [37]. In addition, cardinal temperatures for the fungal growth were determined on PDA cultures after 14 days at temperatures ranging from 5 to 40 °C at intervals of 5 °C.

3. Results

3.1. Phylogeny

Three individual phylogenies (ITS, *EEF1A* and *ACT1*), carried out for the *C. cladosporioides* and *C. herbarum* species complexes, were visually very similar and the ILD test showed that the three datasets loci were congruent in both complexes ($P = 0.16$) and could be combined. Phylogenies obtained by ML, MP and BI also showed a visual topological congruence. The combined alignment of the three mentioned loci datasets encompassed 101 sequences in the *C. cladosporioides* complex and 58 sequences in *C. herbarum* complex. The alignment for the former group comprised 1,062 bp (ITS 484 bp, *EEF1A* 315 bp and *ACT1* 263 bp), which included 447 bp variable sites (ITS 47 bp, *EEF1A* 262 bp and *ACT1* 138 bp) and 363 bp phylogenetically informative sites (ITS 25 bp, *EEF1A* 237 bp and *ACT1* 101 bp). Two species of the *C. sphaerospermum* complex, *C. sphaerospermum* CBS 193.54 and *C. longissimum* CBS 300.96, were included as outgroup in this first multi-locus phylogeny (Figure 1). For the maximum parsimony analysis the maximum of 1,000 equally most parsimonious trees were saved (Tree length = 1706; CI = 0,324; RI = 0.676; RCI = 0.219).


Cladosporium cladosporioides species complex
96/91/1
CBS 125991
-/83/-
CBS 126351
C. oxysporum
98/94/1
CBS 125995 ET C. tenuissimum
CBS 386.64 T
CBS 119542
C. colocasiae
-/71/-
CBS 171.52 ET
CBS 176.54
C. cucumerinum
87/79/-
91/-/-
CBS 125983 T C. angustisporum
99/99/1
CBS 126500 T
CPC 15833
C. subuliforme
100/100/-
CBS 112388 T
80/97/1
CBS 113738
C. cladosporioides
81/96/1
MFLUCC 18-1559 T
99/100/1
MFLUCC 18-1557
C. magnoliigena
99/100/1
CBS 140685 T
CBS 674.82
C. anthropophilum
CBS 140686 T C. crousii
CBS 126363 T C. verrucocladosporioides
CBS 122129 T
CBS 122128
C. funiculosum
-/79/0.99
CBS 125993 T C. pseudocladosporioides
CBS 125989 T C. gamsianum
79/89/1
CBS 142614 T C. welwitschiicola
100/85/1
CBS 143359 T C. needhamense
CBS 128769 T C. phaenocomae
-/88/1
74/89/0.99
DTO 072-C8
CBS 143365 T
C. uwebraunianum
100/99/1
95/96/0.98
CBS 125984 T C. australiense
84/-/0.98
CBS 125997 T
CBS 113749
C. xylophilum
CBS 125994 T
CBS 126357
C. rectoides
99/99/1
CBS 126347
79/-/-
CBS 574.78C
C. lycoperdinum
CBS 116744
CBS 134914 T
C. europaeum
89/88/0.99
CBS 113746 T
CPC 10150
C. westerdijkiae
95/84/-
CBS 143366 T
85/98/-
CBS 306.84
C. vicinum
CBS 142613 T C. kenpeggii
CBS 126344 T
CBS 126342
C. delicatulum
78/89/-
CBS 109082 T C. silenes
CBS 401.80 T
CBS 143.65
C. inversicolor
99/100/1
CGMCC3.18031 T
CGMCC3.18032
C. neopsychrotolerans
-/83/-
CBS 143360 T C. neerlandicum
CBS 140486 T
-/75/-
CPC 15605
C. montecillanum
CBS 125982 T C. acalyphae
FMR 16101
FMR 16164 T
C. coprophilum sp. nov.
-/75/-
CBS 142612 T C. chasmanthicola
78/99/0.99
CGMCC3.18096 T
97/100/1
CGMCC3.18097
C. sinuatum
99/99/1
CBS 140481 T C. austroafricanum
CBS 124456 T C. pini-ponderosae
75/-/-
CBS 124457 T C. chubutense
CBS 274.80B T C. colombiae

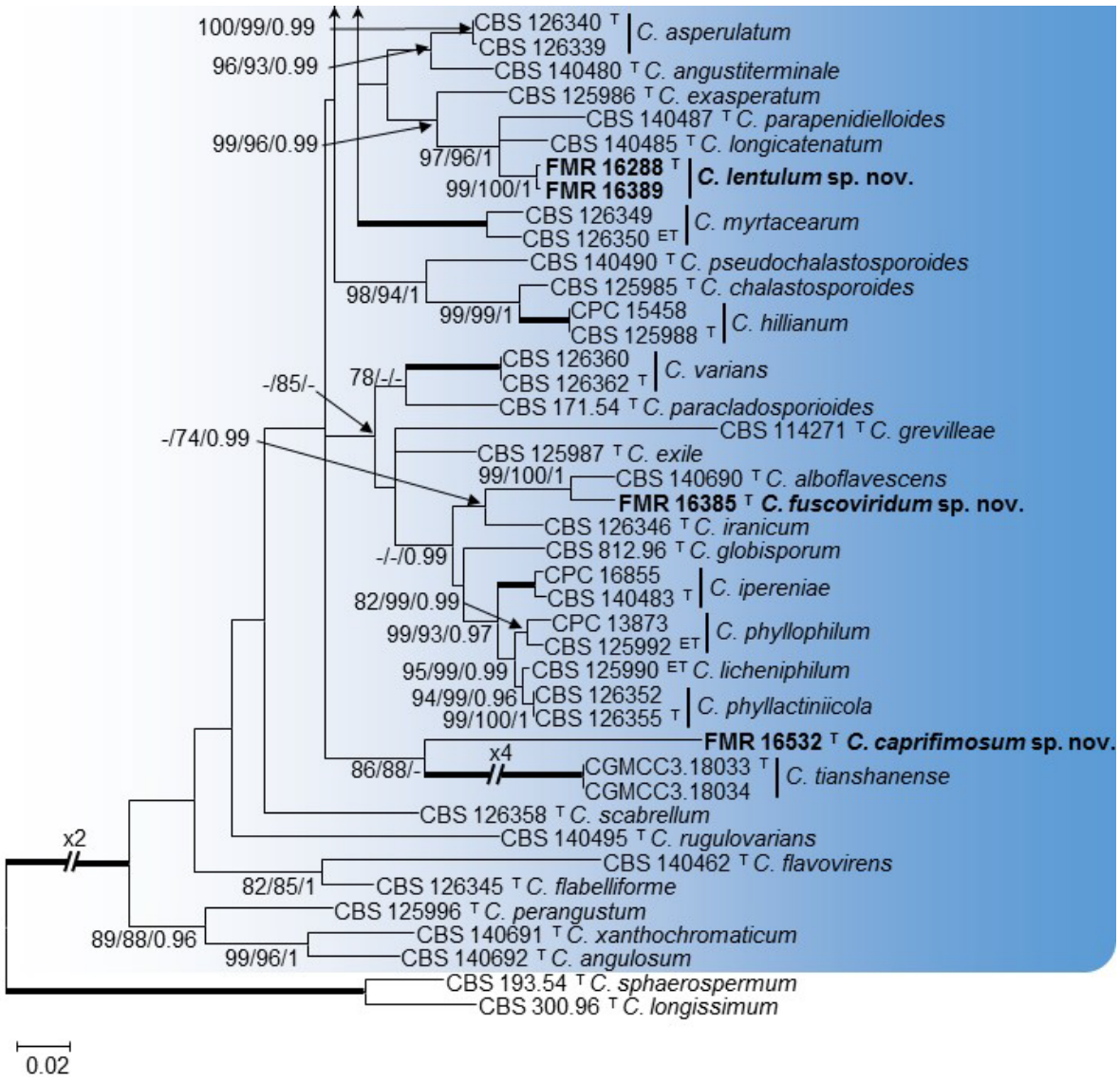


Figure 1. Maximum likelihood (ML) tree obtained from the combined analysis of ITS, *EEF1A* and *ACT1* sequences of 101 strains from the *C. cladosporioides* complex. The tree is rooted with *C. sphaerospermum* CBS 193.54 and *C. longissimum* CBS 300.96. Numbers on the branches represent ML bootstrap support values (MLBS) ≥70 %, followed by Maximum Parsimony bootstrap support values (PBS) ≥70 % and Bayesian posterior probabilities (PP) ≥0.95, lower values are indicate as "-". Bold branches indicate MLBS/PBS/PP of 100/100/1. Names of species newly described are indicated in bold. Branch lengths are proportional to distance. T Ex-type strain. ET Ex-epitype strain.

For the *C. herbarum* species complex, the alignment comprised 1,057 bp (ITS 503 bp, *EEF1A* 309 bp and *ACT1* 245 bp) with 407 bp variable sites (ITS 101 bp, *EEF1A* 186 bp and *ACT1* 120 bp) and 240 bp phylogenetically informative sites (ITS 27 bp, *EEF1A* 123 bp and *ACT1* 90 bp), using *Cercospora beticola* (CBS 116456) as outgroup (Figure 2). For the maximum parsimony analysis the maximum of 1,000 equally most parsimonious trees were saved (Tree length = 898; CI = 0,537; RI = 0,661; RCI = 0,355).

The eight unidentified isolates did not match any known lineage of *Cladosporium* species, six were related to the *C. cladosporioides* species complex and two to the *C. herbarum* complex, and together they represented six new phylogenetic species in the genus.

In the combined phylogeny of the *C. cladosporioides* complex, 71 species were delineated (Figure 1). The isolates FMR 16101 and FMR 16164 formed a strongly-supported terminal clade representative for a unique taxon, but with an uncertain phylogenetic position due to the lack of statistical support for the nearest lineages of ex-type strains of known species. A second undescribed monophyletic

terminal clade included FMR 16288 and FMR 16389, which grouped with the lineages of *C. exasperatum*, C. *parapenidielloides* and *C. longicatenatum* in a clade with highly supported values (99 MLBS / 96 PBS / 0.99 PP). FMR 16532 and FMR 16385 formed two monophyletic branches; the former being related (86 MLBS / 88 PBS / - PP) but very distant from the clade of *C. tianshanense*, while FMR 16385 was closely related to the ex-type strain of *C. alboflavescens* (99 MLBS / 100 PBS / 1 PP). The percentages of identity between these latter two fungi (97.79 % for *ACT1* and 96.75 % for *EEF1A*) together with morphological differences observed allow us to consider them distinct taxa.

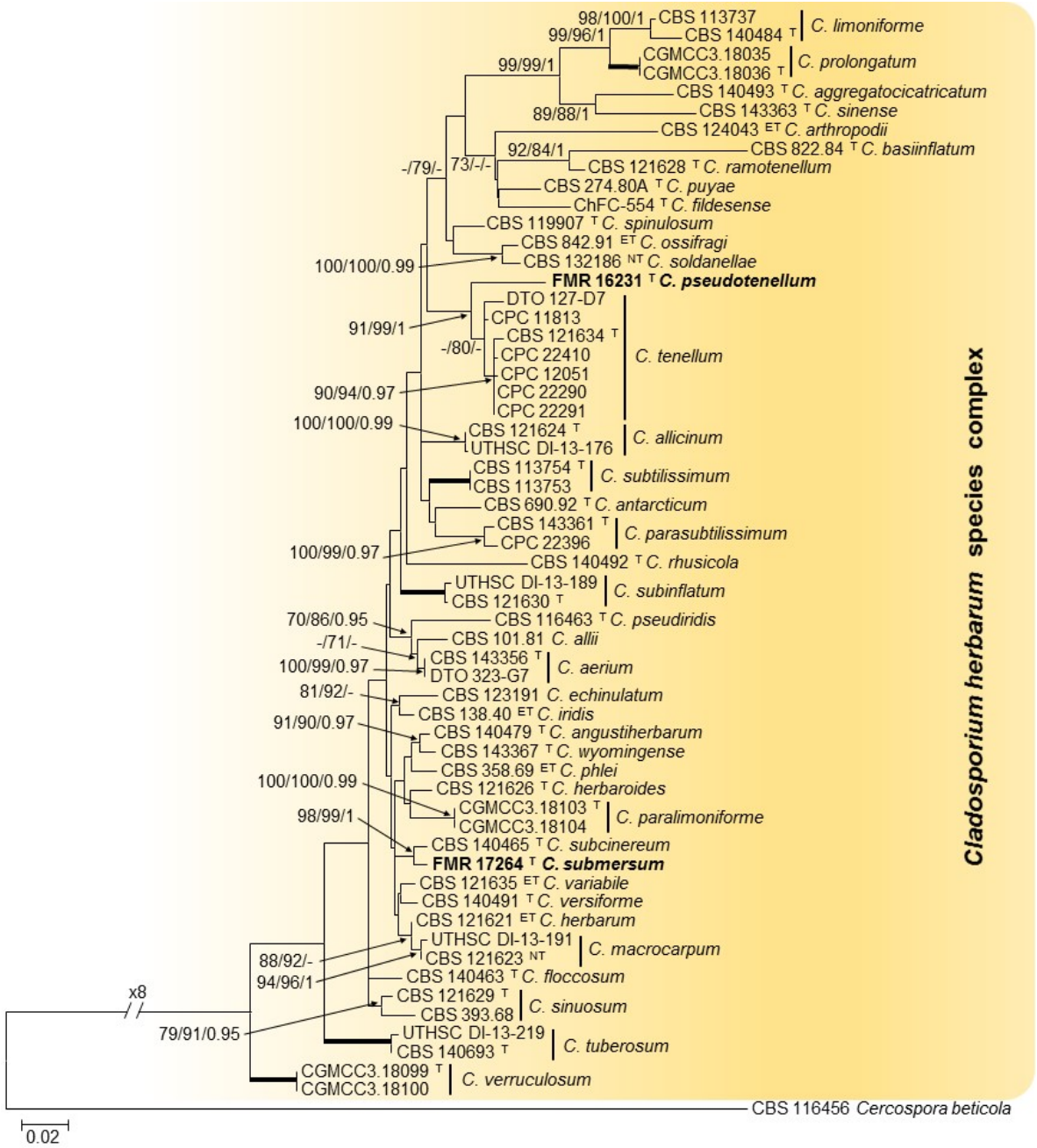


Figure 2. Maximum likelihood (ML) tree obtained from the combined analysis of ITS, *EEF1A* and *ACT* sequences of 58 strains from *C. herbarum* complex. The tree is rooted with *Cercospora beticola* CBS 116456. Numbers on the branches represent ML bootstrap support values (MLBS) ≥70 %, followed by Maximum Parsimony bootstrap support values (PBS) ≥70 % and Bayesian posterior probabilities (PP) above 0.95, lower values are indicate as "-". Bold branches indicate MLBS/PBS/PP of 100/100/1. Names of species newly described are indicated in bold. Branch lengths are proportional to distance. T Ex-type strain. ET ex-epitype strain. NT ex-neotype strain.

In the *C. herbarum* complex, 40 species were phylogenetically well-delimited, including two novel lineages each represented by FMR 16231 and FMR 17264 (Figure 2). Both were genetically and morphologically differentiated from their closest relatives, *C. tenellum* and *C. subcinereum*, respectively. The percentages of identity observed between the isolate FMR 16231 and the type species of *C. tenellum* (CBS 121634) were 97.78 %, 83.76 % and 100 % for *ACT1*, *EEF1A* and ITS, respectively, and between FMR 17264 and the type species of *C. subcinereum* (CBS 140465) were 98.57 %, 95.98 % and 100 % for *ACT1*, *EEF1A* and ITS, respectively.

The six novel phylogenetic species are described and illustrated in the taxonomy section below.

3.1. Taxonomy

Cladosporium caprifimosum Iturrieta-González, Dania García, Gené, sp. nov.—MycoBank MB 836074 (Figure 3).

Etymology: The name refers to goat dung, the substrate where the species was isolated.

Mycelium superficial and immersed, composed of septate, branched, subhyaline, smooth to verruculose hyphae, 1–2 μm wide. *Conidiophores* dimorphic, micronematous or macronematous, arising from lateral or terminal hyphae, erect to slightly flexuous, non-nodulose, septate, branched or unbranched, 8–137 μm long, 2–4 μm wide, pale brown, slightly verrucose. *Conidiogenous cells* integrated, terminal, cylindrical, sometimes geniculate at the apex, 22–44 × 3–4 μm, bearing up to four

Conidiogenous loci, darkened and refractive. *Ramoconidia* aseptate, almost cylindrical, 10–24 × 2–4 μm [av. (± SD) 15.8 (± 3.4) × 3.1 (± 0.45)], olive to pale brown, smooth. *Conidia* forming branched chains, with up to five conidia in the terminal unbranched part, aseptate, olive to pale brown, smooth; *small terminal conidia* ellipsoidal to obovoid, 3–7 × 2–3.5 μm [av. (± SD) 5.7 (± 0.83) × 2.4 (± 0.43)]; *intercalary conidia* ellipsoidal to somewhat fusiform, 6–11.5 × 2–3 μm [av. (± SD) 7.8 (± 1.06) × 2.6 (± 0.39)]; *secondary ramoconidia* ellipsoidal to almost cylindrical, 9–14 × 2.5–3.5 μm [av. (± SD) 11.3 (± 1.6) × 2.9 (± 0.26)].

Culture characteristics after 14 days at 25 °C: Colonies on OA reaching 24–25 mm diam. dark green (30F8), flat, slightly dusty, aerial mycelium scarce, margin regular; reverse dark green (30F8) to black. On PDA attaining 34–35 mm diam. olive (3E6/3F4), slightly umbonate, radially folded, velvety, aerial mycelium scarce, margin slightly lobate; reverse dark green (30F8) to olive (3E4). On SNA reaching 25–26 mm diam. olive (3E8), flat, dusty, aerial mycelium scarce, margin regular; reverse dark green (30F8) to black.

Cardinal temperature for growth: optimum 20 °C, maximum 30 °C, minimum 5 °C.

Specimen examined: Spain, Catalonia, Tarragona province, La Fatarella, from goat dung, Mar. 2017, *I. Iturrieta-González, M. Guevara-Suarez & J. Guarro* (Holotype CBS H-24469; cultures ex-type FMR 16532, CBS 146918).

Notes: The only species related with *C. caprifimosum* is *C. tianshanense* (Figure 1), a fungus isolated from alpine tundra soil in China [38]. However, they show a considerable genetic distance (14.7 % considering the combined analysis of ITS, *ACT1* ant *EEF1A*). In addition, *C. tianshanense* clearly differs from the new species by its psychrotolerant behaviour, with an optimal temperature for growth at 15 °C and no growth at 25 °C, and differs morphologically, by having shorter conidiogenous cells (6–21 μm; 22–44 μm in *C. caprifimosum*) and shorter (5–15; up to 24 μm in *C. caprifimosum*), 0–1-septate and asperulate to verruculose ramoconidia [38].

Cladosporium coprophilum Iturrieta-González, Dania García, Gené, sp. nov.—MycoBank MB 836075 (Figure 4).

Etymology: Name refers to the substrate where the species was isolated, unidentified herbivore dung.

Mycelium superficial and immersed, composed of septate, branched, pale brown, smooth hyphae, 3–5 μm wide. *Conidiophores* macronematous, arising laterally or terminally from hyphae, erect to slightly flexuous, non-nodulose, septate, unbranched, up to 124 μm long, 3–4 μm wide, pale brown, smooth. *Conidiogenous cells* integrated, terminal, rarely intercalary, cylindrical, (7–)14–33 × (2–)3–4 μm, bearing up to 3 conidiogenous loci, slightly darkened and refractive. *Ramoconidia* 0(–1)-septate, subcylindrical to cylindrical, 9–19 μm long × 3–5 μm [av. (± SD) 12.3 (± 2.8) × 3.9 (± 0.54)], pale brown,

smooth. *Conidia* forming branched chains, with up to five conidia in the terminal unbranched part, aseptate, pale brown, smooth to verruculose; *small terminal conidia* ellipsoidal to slightly obovoid, 4.5–7 × 2.5–4 µm [av. (± SD) 6 (± 0.64) × 3.1 (± 0.31)]; *intercalary conidia* ellipsoidal, 6–10.5 × 2.5–4 µm [av. (± SD) 7.7 (± 1.32) × 3.3 (± 0.37)]; *secondary ramoconidia* subcylindrical to cylindrical, 7–12.5 µm long × 3–5 µm [av. (± SD) 9.6 (± 1.7) × 4.2 (± 0.51)].

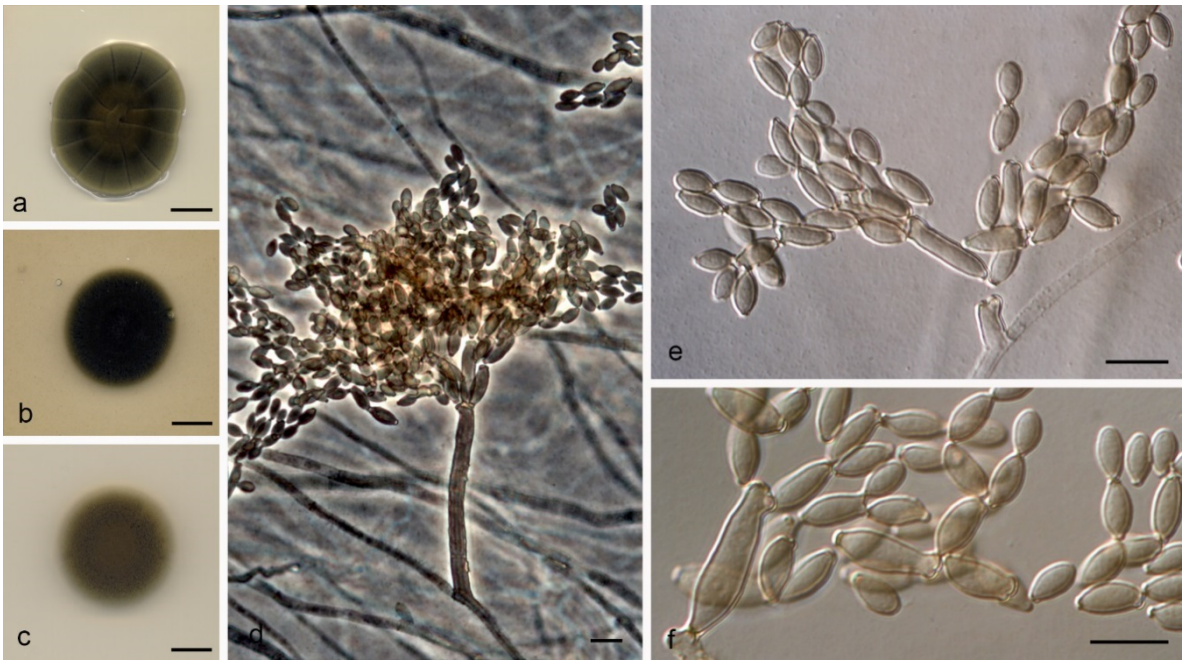


Figure 3. *Cladosporium caprifimosum* (ex-type FMR 16532). (**a–c**) Colonies on PDA, OA and SNA after 14 days at 25 °C. (**d–f**) Conidiophores and conidia. Scale bars (**a–c**) = 10 mm; (**d–f**) = 10 µm.

Culture characteristics after 14 days at 25 °C: Colonies on OA reaching 21–22 mm diam. olive (2F6) to black, dark green margin (30F4), flat, slightly dusty at the center, aerial mycelium scarce, margin regular; reverse dark green (30F8) to black. On PDA attaining 36–37 mm diam. olive (2F6/2E3), greenish grey margin, slightly depressed and irregularly folded at the centre, velvety, aerial mycelium scarce, margin regular; reverse dark green (30F8/27F3). On SNA reaching 27–28 mm diam. olive (3F6/2F8), flat, slightly dusty, aerial mycelium scarce, margin regular; reverse dark green (30F8) to black.

Cardinal temperature for growth: optimum 20 °C, maximum 25 °C, minimum 5 °C.

Specimens examined: Spain, Extremadura, Badajoz province, Granja de Torrehermosa, unidentified herbivore dung, Jan. 2017, *J. Cano* (Holotype CBS H-24470; cultures ex-type FMR 16164, CBS 144919); Badajoz province, Granja de Torrehermosa, unidentified herbivore dung, Mar. 2017, *J. Cano* (FMR 16101).

Notes: Based on the multi-locus analysis (Figure 1), *C. coprophilum* is allocated to a terminal low-supported clade together with *C. chasmanthicola* and *C. sinuatum*, species recently described from leaf spots of *Chasmanthe aethiopica* in South Africa [12] and Alpine soil in China [38], respectively. The new species is distinguished from *C. chasmanthicola* by the production of smooth hyphae (smooth to distinctly verrucose or irregularly rough-walled in C. *chasmanthicola)*, longer conidiogenous cells (up to 33 vs up to 24 µm), shorter ramoconidia (9–19 vs 15–33 µm) with fewer septa [(0(–1) vs 0–1(–3)-septate], and longer terminal conidia (4.5–7 vs 2.5–4.5 µm) [12]. *Cladosporium coprophilum* differs from *C. sinuatum* by the production of aseptate intercalary conidia (0–1-septate in *C. sinuatum*). In addition, *C. sinuatum* is characterized by distinctive geniculate-sinuous conidiophores and rather faster growth on OA (40–45 mm vs 21–22 mm in *C. coprophilum* after 14 d at 25 °C) [38].

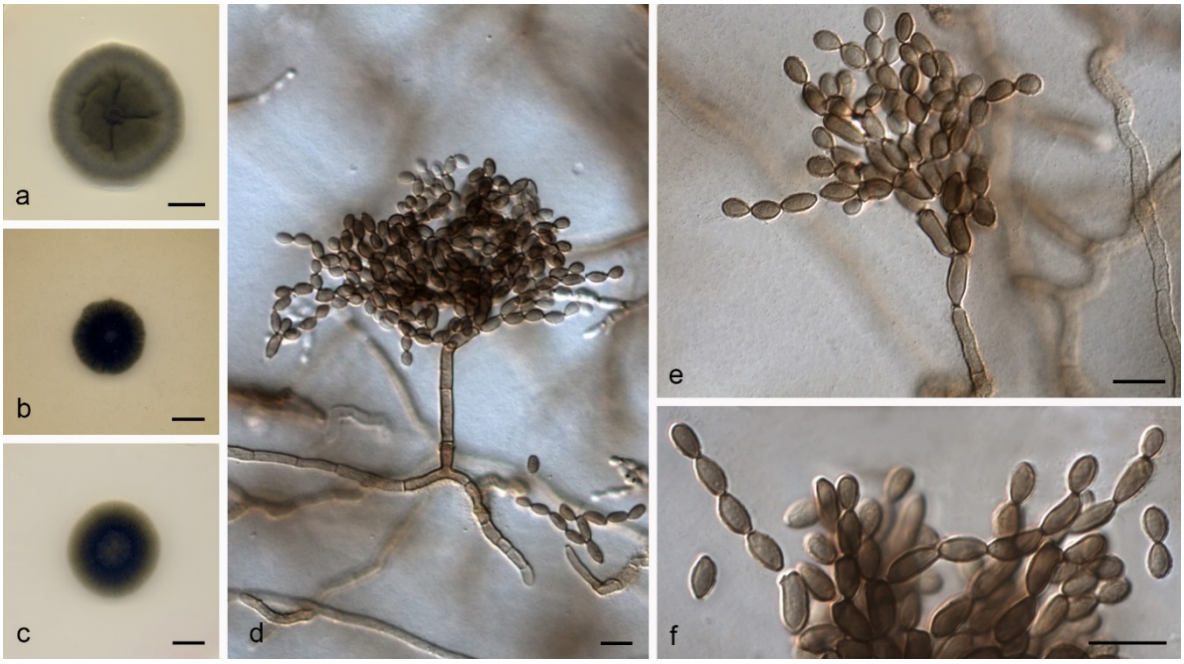


Figure 4. *Cladosporium coprophilum* (ex-type FMR 16164). (**a–c**) Colonies on PDA, OA and SNA after 14 days at 25 °C. (**d–f**) Conidiophores and conidia. Scale bars (**a–c**) = 10 mm; (**d–f**) = 10 μm.

Cladosporium fuscoviridum Iturrieta-González, Dania García, Gené, sp. nov.—MycoBank MB 836076 (Figure 5).

Etymology: Name refers to the dark green reverse of the colonies of the species growing in all agar media tested.

Mycelium superficial and immersed, composed of septate, branched, subhyaline to pale brown, smooth to verruculose hyphae, 1–3 μm wide. *Conidiophores* semi-macronematous to macronematous, arising laterally and terminally from hyphae, sometimes reduced to conidiogenous cells, septate, erect to slightly flexuous, branched or unbranched, sometimes geniculate at the apex, up to 56 μm long, 3–4 μm wide, pale brown, smooth to verruculose. *Conidiogenous cells* terminal and subterminal, cylindrical to slightly clavate, 8–27 × 3–4 μm, bearing up to 4 conidiogenous loci, darkened and refractive. *Ramoconidia* 0–1(–3)-septate, subcylindrical to ellipsoidal, 7.5–22 × 2.5–4 μm [av. (± SD) 12.8 (± 3.9) × 3 (± 0.43)], pale brown, smooth to verruculose. *Conidia* in branched chains with up to 4 conidia in the terminal unbranched part, pale brown, smooth to verruculose, with protuberant, slightly darkened and refractive hila; *small terminal conidia* aseptate, globose, subglobose to obovoid, 3–6 × 2–3.5 μm [av. (± SD) 4.5 (± 0.66) × 3 (± 0.39)]; *intercalary conidia* aseptate, ellipsoidal to somewhat limoniform, 4.5–7 × 2.5–4 μm [av. (± SD) 5.7 (± 0.70) × 3.2 (± 0.36)]; *secondary ramoconidia* 0(–1)-septate, subcylindrical to ellipsoidal 6–11.5 × 2.5–4 μm [av. (± SD) 8.8 (± 1.64) × 3.1 (± 0.40)].

Culture characteristics after 14 days at 25 °C: Colonies on OA reaching 31–32 mm diam. olive (3F8) to dark green (30F5), olive final edge (2F8), flat, velvety, aerial mycelium scarce, margin regular; reverse dark green (30F5) to black. On PDA attaining 44–46 mm diam. grey to olive to olive yellow (3D1/2E5/2C6), white at the final edge, flat, velvety, aerial mycelium scarce, margin regular; reverse dark green (30F8) to black, with a whitish final edge. On SNA reaching 34–35 mm diam. olive (3F8), flat, velvety, aerial mycelium scarce, margin regular; reverse dark green (30F8), olive final edge (3F3).

Cardinal temperature for growth: optimum 25 °C, maximum 30 °C, minimum 5 °C.

Specimen examined: Spain, Catalonia, Tarragona province, Cambrils, Samà Park, garden soil, Feb. 2017, *I. Iturrieta-González* & *J. Gené* (Holotype CBS H-24471; cultures ex-type FMR 16385, CBS 146920).

Notes: *Cladosporium fuscoviridum* is closely related to *C. alboflavescens* (Figure 1), a monotypic species described from an animal respiratory specimen collected in California [10]. The species can be distinguished by their colony and microscopic features; i.e. *C. fuscoviridum* has darker colonies and faster growth rates at 25 °C after 2 wk on the three media tested (OA, 31–32 vs 20–23 mm; PDA, 44–46

vs 34–36 mm; SNA, 34–35 vs 20–25 mm), shorter conidiophores (up to 56 µm vs up to 130 µm long in *C. alboflavescens*), and 0–3-septate (aseptate in *C. alboflavescens*) shorter (7.5–22 vs 11–36 µm) ramoconidia. *Cladosporium iranicum* is related with *C. fuscoviridum* and *C. alboflavescens*, but can be easily distinguished from them by its larger conidiophores (40–180(–135) µm), with chains of up to 10 conidia in the terminal unbranched part, and a faster growth rate on PDA (56–60 mm after 14 d at 25 °C) [2].

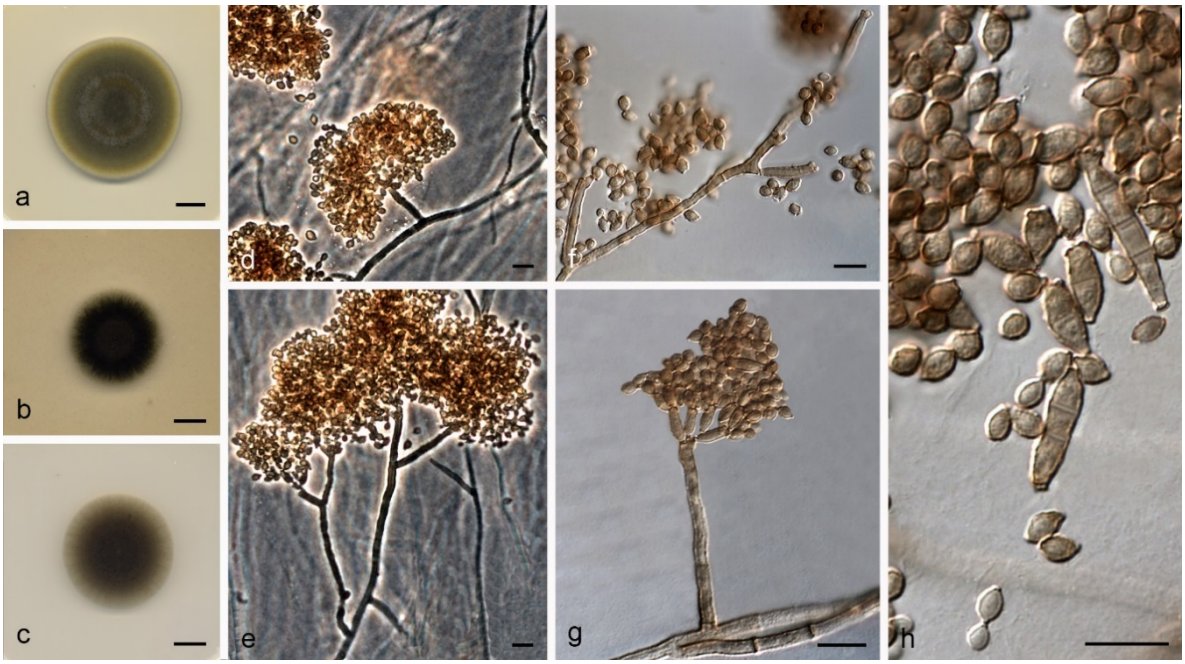


Figure 5. *Cladosporium fuscoviridum* (ex-type FMR 16385). (**a–c**) Colonies on PDA, OA and SNA after 14 days at 25 °C. (**d–h**) Conidiophores and conidia. Scale bars (**a–c**) = 10 mm; (**d–f**) = 10 µm.

Cladosporium lentulum Iturrieta-González, Dania García, Gené, sp. nov.—MycoBank MB 836077 (Figure 6).

Etymology: Name refers to its slower growth with respect to the phylogenetically related species.

Mycelium superficial and immersed, composed of septate, branched, subhyaline to yellowish brown, smooth to verruculose hyphae, 1–4 µm wide. *Conidiophores* macronematous, arising laterally and terminally from hyphae, septate, erect to slightly flexuous, unbranched, sometimes geniculate at the apex, occasionally branched, up to 406 µm long, 3–4 µm wide, pale brown to brown, smooth to verrucose. *Conidiogenous cells* integrated, terminal and subterminal, cylindrical to subcylindrical, 11–27 × 2–4(–5) µm, bearing up to 5 conidiogenous loci, darkened and refractive. *Ramoconidia* 0(–2)-septate, subcylindrical to cylindrical, 10.5–23 × 2.5–4.5 µm [av. (± SD) 14.2 (± 2.61) × 3.2 (± 0.52)]; pale brown, smooth to verruculose. *Conidia* forming branched chains with up to 5 conidia in the unbranched part of the chain, pale brown, smooth to slightly verruculose, with protuberant, slightly darkened and refractive hila; *small terminal conidia* aseptate obovoidal to ellipsoidal, 4.5–7.5 × 1.5–2.5 µm [av. (± SD) 5.8 (± 0.81)) × 2.7 (± 0.29)]; *intercalary conidia* 0(–1)-septate, ellipsoidal to subcylindrical, 6–10.5 × 2–3 µm [av. (± SD) 8.4 (± 1.31) × 2.3 (± 0.34)]; *secondary ramoconidia* 0(–1)-septate, ellipsoidal to subcylindrical, slightly constricted at septum when present, 7.5–14.5 × 2–3 µm [av. (± SD) 10.5 (± 2.05) × 2.5 (± 0.30)].

Culture characteristics after 14 days at 25 °C: Colonies on OA reaching 19–20 mm diam. olive (3F8), flat, velvety, aerial mycelium scarce, margin regular; reverse dark green (30F8) to black. On PDA attaining 28–36 mm diam. dark green (27F8), with a whitish final edge, slightly umbonate, radially folded, velvety, aerial mycelium scarce, margin slightly lobulate; reverse olive brown (4E4), whitish at the edge. On SNA reaching 22–23 mm diam. olive (3F5), flat, slightly dusty, aerial mycelium scarce, margin fimbriate; reverse dark green (30F8) to black.

Cardinal temperature for growth: optimum 20 °C, maximum 30 °C, minimum 5 °C.

Specimens examined: Spain, Catalonia, Tarragona province, Tarragona, unidentified leaf litter, Feb. 2017, *I. Iturrieta-González* (Holotype CBS H-24472; cultures ex-type FMR 16288, CBS 146921); Tarragona province, Poblet, unidentified herbivore dung, Mar. 2017, *I. Iturrieta-González*, *M. Guevara-Suarez* & *J. Guarro* (FMR 16389).

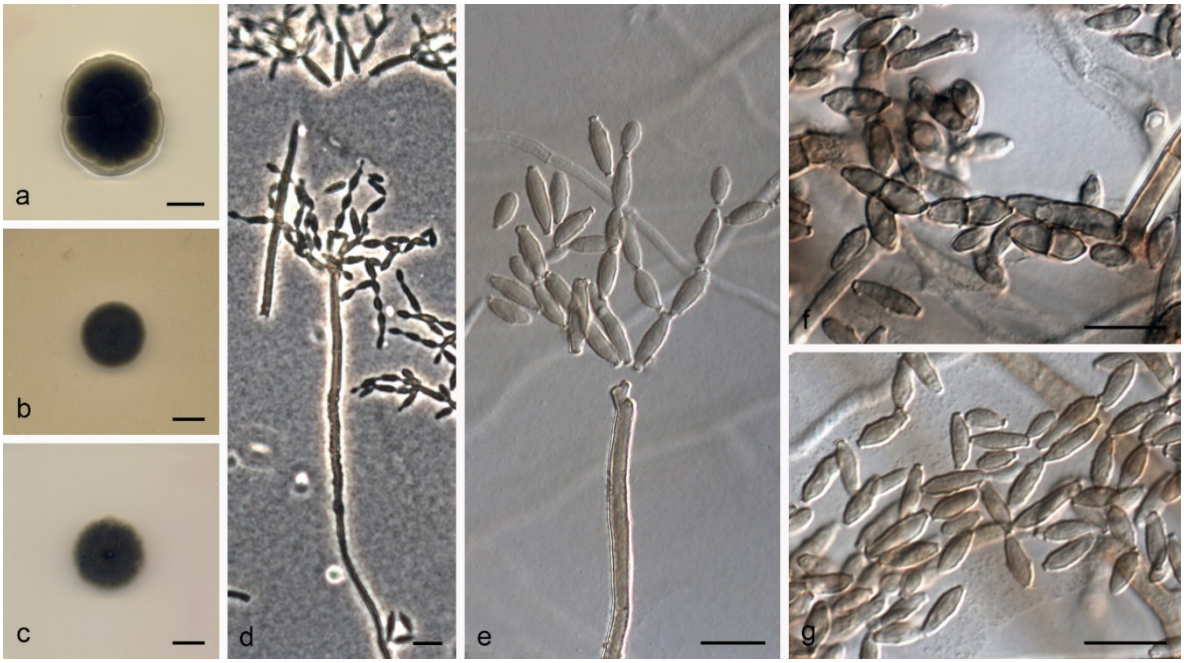


Figure 6. *Cladosporium lentulum* (ex-type FMR 16288). (**a–c**) Colonies on PDA, OA and SNA after 14 days at 25 °C. (**d–g**) Conidiophores and conidia. Scale bars (**a–c**) = 10 mm; (**d–f**) = 10 μm.

Notes: Our phylogeny (Figure 1) shows *C. lentulum* included in a well-supported terminal clade together with the ex-type strains of *C. exasperatum*, *C. parapenidielloides* and *C. longicatenatum*, three species all described from plant material collected in Australia [2,20]. However, it shows a sufficient genetic distance to be considered a distinct species from the closest, *C. parapenidielloides* and *C. longicatenatum* (3.9 % and 2 % respectively, considering the combined analysis with the three markers). Phenotypically, *C. lentulum* can be distinguished from its counterparts mainly by its slower growth, especially on OA at 25 °C after 14 d (19–20 mm vs 39–54 mm for *C. exasperatum*, 42–55 mm for *C. parapenidielloides* and 43–54 mm for *C. longicatenatum*). In addition, our new species shows shorter ramoconidia (10.5–23 μm) than *C. exasperatum* and *C. longicatenatum* (19–40 μm and 22–42 μm, respectively); ramoconidia in *C. parapenidielloides* are absent; the conidia in *C. lentulum* are smooth or nearly so, while those of *C. exasperatum* and *C. longicatenatum* possess a unique verruculose-rugose conidial surface ornamentation, especially prominent in the former; and conidiophores in *C. parapenidielloides* are much shorter (up to 67 μm) than those observed in *C. lentulum* (up to 406 μm) [2,20].

Cladosporium pseudotenellum Iturrieta-González, Dania García, Gené, sp. nov.—MycoBank MB 836078 (Figure 7).

Etymology: The name refers to "*C. tenellum*", the closest phylogenetic species.

Mycelium superficial and immersed, composed of septate, branched, subhyaline to pale brown, smooth-walled, occasionally tuberculate and with abundant swellings, hyphae, 2–3(–4.5) μm wide. *Conidiophores* macronematous, arising laterally or terminally from hyphae, erect to slightly flexuous, non-nodulose, occasionally geniculate at the apex, septate, unbranched, occasionally branched, up to 146 μm long, 2.5–3 μm wide, pale brown, smooth to slightly verruculose. *Conidiogenous cells* integrated, terminal or intercalary, cylindrical, sometimes geniculate, 15–32 × 2.5–3 μm, with up to five conidiogenous loci, thickened, darkened and refractive, often crowded at the apex. *Ramoconidia* rarely formed, 0(–1)-septate, ellipsoidal to subcylindrical, 9–14.5 × 4–5.5 μm [av. (± SD) 11.6 (± 1.60) × 4.6 (± 0.44)], pale brown, verruculose. *Conidia* forming branched chains, with up to four conidia in the

terminal unbranched part, aseptate, pale brown, verruculose to verrucose; *small terminal conidia* subglobose to obovoid, 4–7 × 3–5 µm [av. (± SD) 5.8 (± 0.77) × 3.9 (± 0.60)]; *intercalary conidia* ellipsoidal to limoniform, 6–8.5 × 3–5 µm [av. (± SD) 7.4 (± 0.73) × 3.8 (± 0.50)]; *secondary ramoconidia* 0(–2)-septate, ellipsoidal to subcylindrical, 7–12.5 × 4–5 µm [av. (± SD) 9.6 (± 1.76) × 4.4 (± 0.33)] with 1–3 distal hila.

Culture characteristics after 14 days at 25 °C: Colonies on OA reaching 21–22 mm diam. olive (2F8/2F4), flat, velvety, aerial mycelium scarce, margin fimbriate; reverse dark green (30F8) to black. On PDA attaining 29–30 mm diam. olive grey (3E2/3F2), paler at the periphery, radially folded, velvety, aerial mycelium scarce, margin slightly lobate; reverse dark green (30F8) to black. On SNA reaching 21–22 mm diam. olive (2F8), flat, slightly dusty, aerial mycelium scarce, margin fimbriate; reverse dark green (30F8) to black.

Cardinal temperature for growth: optimum 20 °C, maximum 30 °C, minimum 5 °C.

Specimen examined: Spain, Catalonia, Tarragona province, Reus, garden soil, Feb. 2017, *I. Iturrieta-González* (Holotype CBS H-24473; cultures ex-type FMR 16231, CBS 146922).

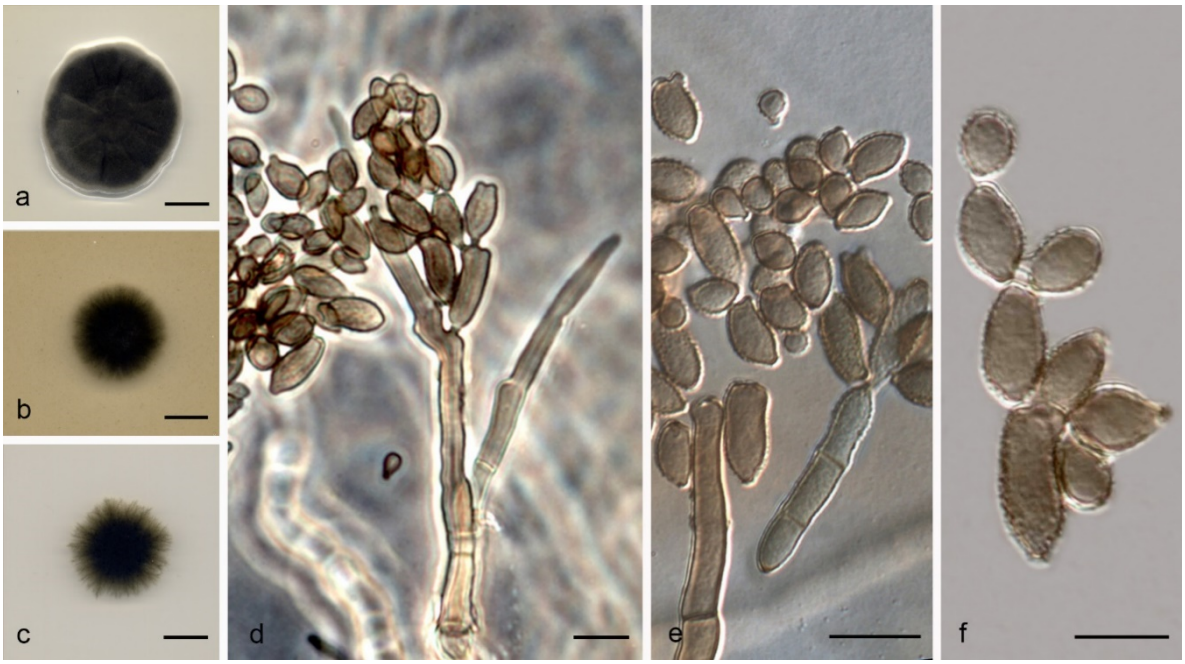


Figure 7. *Cladosporium pseudotenellum* (ex-type FMR 16231). (**a–c**) Colonies on PDA, OA and SNA after 14 days at 25 °C. (**d–f**) Conidiophores and conidia. Scale bars (**a–c**) = 10 mm; (**d–f**) = 10 µm.

Notes: Based on the phylogeny of the *C. herbarum* complex (Figure 2), *C. pseudotenellum* is closely related with *C. tenellum*, a species originally described from hypersaline water in Israel, later found on *Phyllactinia* sp. (Erysiphaceae), and in indoor air samples collected in USA [3,6,19]. Our species differs from *C. tenellum* by the absence of micronematous conidiophores and by having shorter macronematous conidiophores (up to 146 µm vs up to 200 µm), shorter conidiogenous cells (15–32 µm vs 6–40 µm), with few conidiogenous loci (up to five vs up to 10 or more in *C. tenellum)*, and shorter ramoconidia (9–14.5 vs up to 32 µm). In addition, the conidia in *C. pseudotenellum* are aseptate, while those of *C. tenellum* are 0–1(–3)-septate [3,19].

Cladosporium submersum Iturrieta-González, Dania García, Gené, sp. nov.—MycoBank MB 836079 (Figure 8).

Etymology: Name refers to the aquatic habitat where the substrate (submerged plant material) of the fungus was collected.

Mycelium superficial and immersed, composed of septate, branched, subhyaline to pale brown, smooth-walled to verruculose hyphae, 1–3 µm wide. *Conidiophores* dimorphic, micronematous or macronematous, arising laterally and terminally from hyphae, erect to slightly flexuous, nodulose, geniculate at the apex, septate, unbranched, occasionally branched with small prolongations just below the septum, up to 77 µm long, 3–5 µm wide, pale brown to brown, smooth to verruculose.

Conidiogenous cells integrated, terminal and intercalary, geniculate, nodulose, 11–28 × 3–6 µm, bearing up to five conidiogenous loci, darkened and refractive. *Ramoconidia* rarely formed, 0(–1)-septate, sometimes constricted at the septum when present, cylindrical to subcylindrical, 10.5–24 × 4.5–7 µm [av. (± SD) 16 (± 3.6) × 6.1 (± 1.03)], pale brown, verruculose to verrucose. *Conidia* forming short branched chains, pale brown, verrucose, occasionally verruculose, with protuberant and slightly darkened hila; *small terminal conidia* aseptate, ovoid to ellipsoidal, 6–12.5 × 3.5–7 µm [av. (± SD) 7.8 (± 1.63) × 4.8 (± 0.79)]; *intercalary conidia* and *secondary ramoconidia* 0–1-septate, ellipsoidal or subcylindrical, 7.5–16 × 4.5–8 µm [av. (± SD) 11 (± 2.18) × 5.7 (± 0.99)].

Culture characteristics after 14 days at 25 °C: Colonies on OA reaching 22–23 mm diam. brownish grey to olive brown (4E2/4E4), umbonate, velvety, aerial mycelium scarce, margin slightly irregular and fimbriate; reverse dark green to olive brown (6F8/4E3). On PDA attaining 26–28 mm diam. olive (3F3/1F5), slightly umbonate, radially folded, velvety, aerial mycelium scarce, margin irregularly undulate; reverse dark green (30F9) to black with brownish red (9C6) areas observed between 15 and 20 °C and a white edge. On SNA reaching 21–22 mm diam. olive (3E3), slightly umbonate, loosely cottony, margin fimbriate; reverse dark olive brown to golden grey (3E3/4C2).

Cardinal temperature for growth: optimum 20 °C, maximum 35 °C, minimum 5 °C.

Specimen examined: Spain, Catalonia, Tarragona province, Cornudella del Montsant, Siurana's swamp, submerged plant material, Feb. 2018, *I. Iturrieta-González, E. Carvalho & J. Gené* (Holotype CBS H-24474; cultures ex-type FMR 17264, CBS 146923).

Notes: *Cladosporium submersum* is related to *C. subcinereum*, and morphologically differentiated by having shorter conidiophores (up to 77 µm vs up to 140 µm), shorter conidiogenous cells (11–28 vs 16–38 µm), shorter ramoconidia (10.5–24 vs 19–59 µm), and longer terminal conidia (6–12.5 vs 5–7 µm), which are ovoid to ellipsoidal in our species and globose to subglobose in *C. subcinereum* [10]. In addition, *C. submersum* exhibited a colony reverse on PDA with brownish red areas, a feature that is absent in *C. subcinereum*.

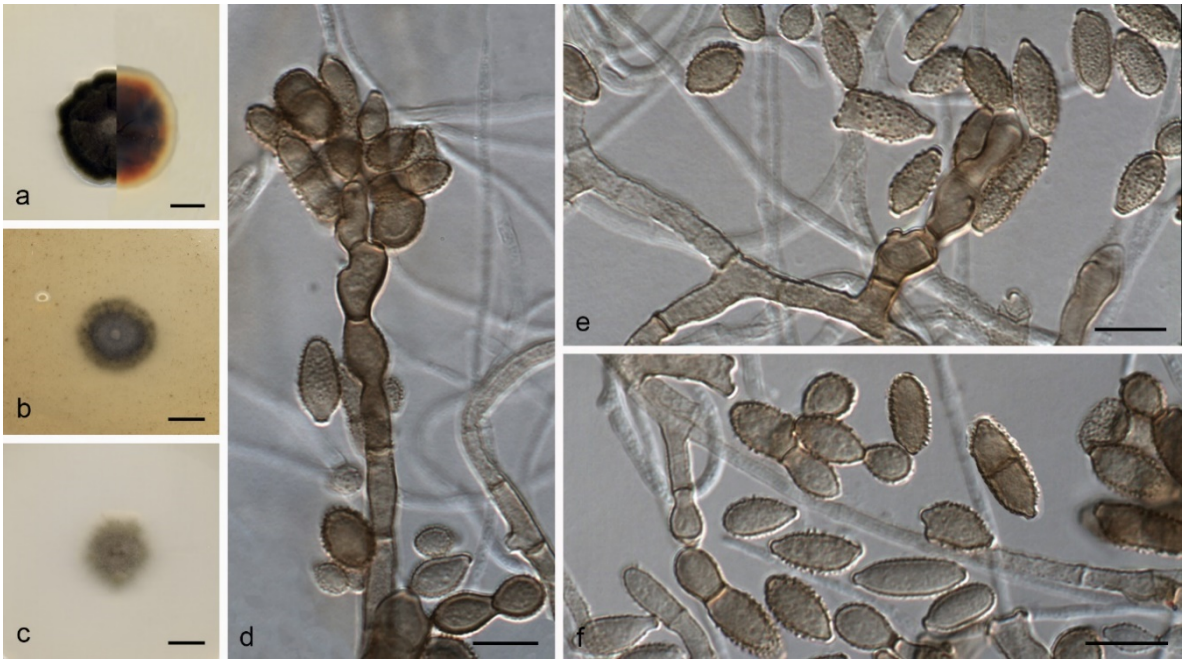


Figure 8. *Cladosporium submersum* (ex-type FMR 16264). (**a–c**) Colonies on PDA (front at 25 °C and reverse at 20 °C), OA and SNA at 25 °C after 14 days. (**d–f**) Conidiophores and conidia. Scale bars (**a–c**) = 10 mm; (**d–f**) = 10 µm.

4. Discussion

Cladosporium is a well-delineated genus, the taxonomic structure and phylogenetic relationships of its species have been investigated in several studies over the last decade, so far giving rise to a genus

of more than two hundred well-stablished species [2–3,6,10,12,21,39–41]. However, this species number will continue to expand through the study of soil, which is a proven pool of fungal species that remain undescribed, and other substrates poorly investigated by molecular tools for fungal diversity [42–43]. In this context, in the survey of microfungi from various Spanish locations from which samples of soil, dung from different herbivorous animals and plant debris were collected, a set of *Cladosporium* isolates were obtained in pure culture. Using the molecular approach for *Cladosporium* identification [3,12], nearly 40 species were confidently identified as belonging to known species (data not shown), a part of the eight isolates that are described here as *C. caprifimosum, C. coprophilum*, *C. fuscoviridum, C. lentulum*, *C. pseudotenellum* and *C. submersum*. Of note is that almost all the specimens in the present study (7/8) were isolated directly from the natural substratum incubated in moist chambers or from baiting technique plates. Although *Cladosporium* isolates are commonly detected by plating methods, the slow growth rate or the low spore concentration of some cladosporium-like fungi compared to other fungi present in a given substrate is probably a handicap to detection and/or isolation of interesting *Cladosporium* species. Therefore, as recommended by Crous [44] for similar fungi, techniques based on fungal isolation directly from the natural substratum should be considered of choice for future studies of *Cladosporium* species diversity.

To our knowledge, *Cladosporium* species as dung inhabiting fungi have been reported in a very few studies, *C. cladosporioides* and *C. herbarum* being the most reported species [45–51]. However, in all those studies, fungal identification was based exclusively on morphological features. Only *C. herbarum* has been reported recently from crown droppings and identified molecularly, but using only the ITS barcode [52]. In our case, the three new species isolated on herbivore dung (i.e. *C. caprifimosum, C. coprophilum*, and *C. lentulum)* showed the typical morphological features attributed to the *C. cladosporioides* species complex. However, their identification would have been difficult with morphological features alone, even with the analysis of their ITS sequences, since they were almost identical as reported in previous studies for many other *Cladosporium* species [2,3,12]. Therefore, only sequence analysis with *ACT1* and *EEF1A* will allow us to know the real spectrum of *Cladosporium* diversity from this understudied substrate by molecular tools.

Although no temperature studies have been systematically applied to characterize most *Cladosporium* species [3,6,20], we agree with Ma et al. [38] that cardinal temperatures for growth can help to differentiate certain species in their respective complexes. While species in the *C. sphaerospermum* complex show a maximum temperature for growth of no more than 30–32 °C, *C. halotolerans* was able to grow at 35 °C [4]. Similarly, although most species of the *C. cladosporioides* complex do not tolerate high temperatures, *C. angulosum*, *C. angustisporum, C. anthropophilum*, *C. flavovirens, C. funiculosum, C. pseudocladosporioides*, *C. subuliforme* and *C. tenuissimum* were able to grow at 35 °C [4,10]. To date, no member of the *C. herbarum* complex was found to be able to grow above 30 °C, however one of the novel species of the complex described here, *C. submersum*, had a maximum growth at 35 °C. On the contrary, the recently described species *C. neopsychrotolerans* and *C. tianshanense* from the complex *C. cladosporioides* and *C. psychrotolerans* from the complex *C. sphaerospermum* showed a psychrophilic behavior [38–39], demonstrating in part the ability of *Cladosporium* species to adapt to different environmental conditions.

Acknowledgments: This study was supported in part by the Spanish Ministerio de Economía y Competitividad, grant CGL2017-88094-P.

Author Contributions: I. I.-G. and J.G. conceived the ideas, organised and analysed the data, joined and led the writing, and collected part of the samples; D.G. organised and analysed the data, and led the writing.

Competing of interest: The authors declare no conflict of interest.

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Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

4.2.2 *Cladosporium michoacanense* sp. nov.

Isabel Iturrieta-González, Josepa Gené & Dania García.

Published in: Persoonia (Fungal Planet description sheets) 2018; 40: 266– 267.

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

Fungal Planet 722 – 13 July 2018

Cladosporium michoacanense Iturrieta-González, Gené & Dania García, *sp. nov.*

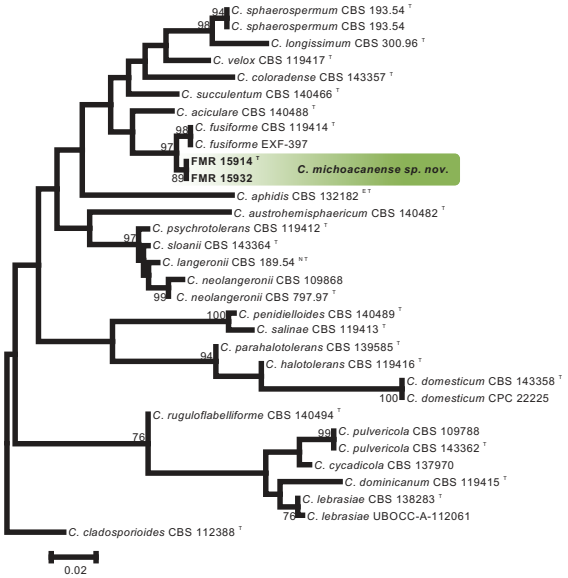
Etymology. Name refers to Michoacán, the geographical area where the fungus was collected.

Classification — *Cladosporiaceae*, *Capnodiales*, *Dothideomycetes*.

Colonies sporulating on synthetic nutrient-poor agar. *Mycelium* consisting of branched, septate, smooth, brown, 2–3 µm wide hyphae. *Conidiophores* macronematous, erect to slightly flexuous, 1–16-septate, branched or unbranched, pale to medium olivaceous brown, smooth, verruculose to tuberculate 24–552 × 3–3.5 µm. *Conidiogenous cells* terminal, cylindrical, 14–20 × 2–3 µm, bearing 2–4 subdenticulate loci, 1 µm wide, thickened, darkened and refractive. *Primary ramoconidia* 0–1-septate, pale brown, smooth to somewhat tuberculate, cylindrical to subcylindrical, 11–31 × 2–3 µm, with up to three distal hila; hilum thickened, darkened and refractive. *Secondary ramoconidia* aseptate, pale brown, smooth, cylindrical to subcylindrical, 10–15 × 2–3 µm, with up to 4 distal hila. *Conidia* in branched chains, with up to 4 conidia in the terminal unbranched part, aseptate, pale brown, smooth, with protuberant and darkened hila; intercalary conidia, ellipsoidal and obovoid, 5–12.5 × 2–3.5 µm; small terminal conidia subglobose, obovoid, pyriform, ellipsoidal, occasionally fusiform, 2.5–6.5 × 1.5–2 µm.

Culture characteristics — (at 25 °C in 2 wk): Colonies on PDA up to 34 mm diam, slightly dusty to velvety, radially folded, olive to dull green (3F3/28E4) (Kornerup & Wanscher 1978), aerial mycelium scarce, margin regular; reverse dark green (28F8); exudate scarce, consisting of small colourless droplets on the colony surface. On OA, up to 23 mm diam, slightly dusty, flat, olive to dark green (2F4/29F8), aerial mycelium scarce, margin irregular; reverse dark green (29F8) to black. On SNA, up to 22 mm diam, slightly dusty, flat, olive (3F4-8), aerial mycelium scarce, margin regular; reverse olive (2F4).

Cardinal temperature for growth — Optimum 20 °C, maximum 30 °C, minimum 5 °C.



Colour illustrations. Villa Jiménez, Michoacán (Imagen Credit Marco A. Ambris), Mexico; colony sporulating on PDA after 2 wk at 25 °C; conidiophores and conidia on SNA after 7 d at 25 °C. Scale bars = 10 µm.

Typus. Mexico, Michoacán, Villa Jiménez, from soil, Sept. 2016, leg. *E. Rodriguez-Andrade* (holotype CBS H-23245, cultures ex-type FMR 15914 = CBS 143588, ITS, LSU, *actA* and *tef1* sequences GenBank LT907958, LT934506.1, LT907961 and LT907945, MycoBank MB823063).

Additional material examined. Mexico, Michoacán, Morelia, from soil, Sept. 2016, leg. *E. Rodriguez-Andrade*, FMR 15932, ITS, *actA* and *tef1* sequences GenBank LT907944, LT907960 and LT907959.

Notes — *Cladosporium michoacanense* belongs to the *C. sphaerospermum* complex (Bensch et al. 2018). Based on the combined analysis of ITS, *actA* and *tef1* markers, its closest relative is *C. fusiforme*. However, the lineage formed by the two isolates of *C. michoacanense* received a high statistical support and showed a phylogenetic distance of 1 % with respect to the lineage of the ex-type strain of *C. fusiforme* (CBS 119414). *Cladosporium fusiforme* differs from our novel species in several morphological aspects, such as in having shorter conidiophores (up to 200 µm long), larger primary (15–40 µm long) and secondary ramoconidia ((7–)8–24(–31) µm long), and terminal conidia commonly being fusiform (Zalar et al. 2007). *Cladosporium michoacanense* exhibits small conidia of varied shape (subglobose, ellipsoidal, obovoid, pyriform), but rarely fusiform.

Based on a megablast search of NCBIs GenBank nucleotide database using LSU sequences, the closest species were *C. sphaerospermum* (GenBank DQ780351.2; Identities = 840/844 (99 %), Gaps = 1/844 (0 %)), *C. longissimum* (GenBank DQ780352.2; Identities = 838/844 (99 %), Gaps = 1/844 (0 %)) and *C. langeronii* (GenBank DQ780380.2; Identities = 836/844 (99 %), Gaps = 1/844 (0 %)). The closest hits using ITS sequences were *C. cladosporioides* (GenBank JF911745.1; Identities 499/500 (99 %), Gaps = 0/500 (0 %)), *C. succulentum* (GenBank LN834434.1; Identities = 501/511 (98 %), Gaps = 5/511 (0 %)) and *C. crousii* (GenBank NR_148192.1; Identities = 500/511 (98 %), Gaps = 3/511 (0 %)). The closest hits using the *actA* sequences were *C. fusiforme* (GenBank KJ596640.1; Identities = 205/216 (95 %), Gaps = 4/216 (1 %)), *C. aciculare* (GenBank KT600607.1; Identities = 214/232 (92 %), Gaps = 0/232 (0 %)) and *C. velox* (GenBank KT600654.1; Identities = 202/225 (90 %), Gaps = 2/225 (0 %)). The closest hits with *tef1* sequences were *C. fusiforme* (GenBank KJ596595.1; Identities = 236/252 (94 %), Gaps = 3/252 (1 %)), *C. aciculare* (GenBank KT600509.1; Identities = 236/263 (90 %), Gaps = 1/262 (0 %)) and *C. velox* (GenBank KT600556.1; Identities = 216/258 (84 %), Gaps = 4/258 (1 %)).

Maximum likelihood tree obtained from the combined analysis of ITS, *actA* and *tef1* sequences of the *C. sphaerospermum* species complex (Bensch et al. 2018). Bootstrap support values above 70 % are indicated on the nodes. The alignment included 977 bp and was performed with ClustalW. The Kimura 2-parameter with Gamma distribution (G) was used as the best nucleotide substitution model. Both the alignment and tree were constructed with MEGA v. 6.0 (Tamura et al. 2013). The new species proposed herein is in the green box and ex-type, ex-epitype and ex-neotype strains are indicated with T, ET and NT, respectively.

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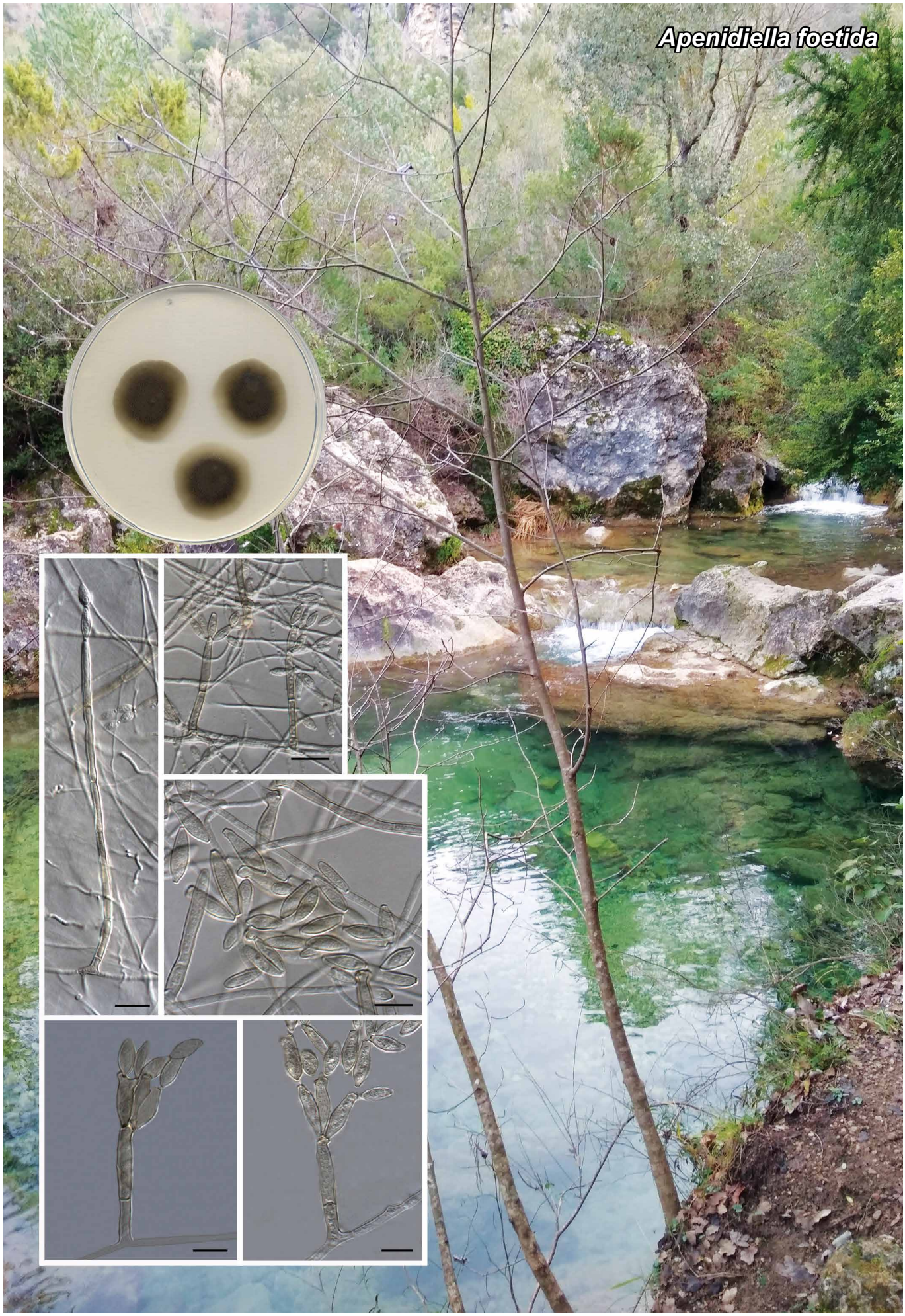
4.2.3 *Apenidiella foetida* sp. nov.

Isabel Iturrieta-González, Josepa Gené & Dania García.

Published in: Persoonia (Fungal Planet description sheets) 2019; 42: 376–377.

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González


Apenidiella foetida

Fungal Planet 904 – 19 July 2019

Apenidiella foetida Iturrieta-González, Gené, Dania García, *sp. nov.*

Etymology. Name refers to the unpleasant odour produced in older cultures.

Classification — *Teratosphaeriaceae*, *Capnodiales*, *Dothideomycetes*.

Mycelium consisting of branched, septate, subhyaline to pale olivaceous, smooth-walled, 1–2 µm diam hyphae. *Conidiophores* mononematous, macronematous, unbranched, erect, subcylindrical, up to 6-septate, pale olivaceous, smooth-walled, up to 130 µm long, 3–5 µm wide. *Conidiogenous cells* terminal, integrated, mono- or polyblastic, with up to 5 conidiogenous loci thickened and darkened, commonly giving rise to a set of ramoconidia at the same level, ramoconidia at different levels also present, pale olivaceous, smooth-walled, 18–27 × 3–4 µm. *Ramoconidia* aseptate, with up to 2–3(–4) terminal conidiogenous loci thickened and darkened, pale olivaceous, smooth-walled, some slightly verruculose, 12–21 × 4–5 µm, forming conidia in acropetal chains. *Conidia* aseptate, fusiform, limoniform or lanceolate, pale olivaceous, smooth-walled, some slightly verruculose, 7–21 × 3–5 µm. *Sexual morph* not observed.

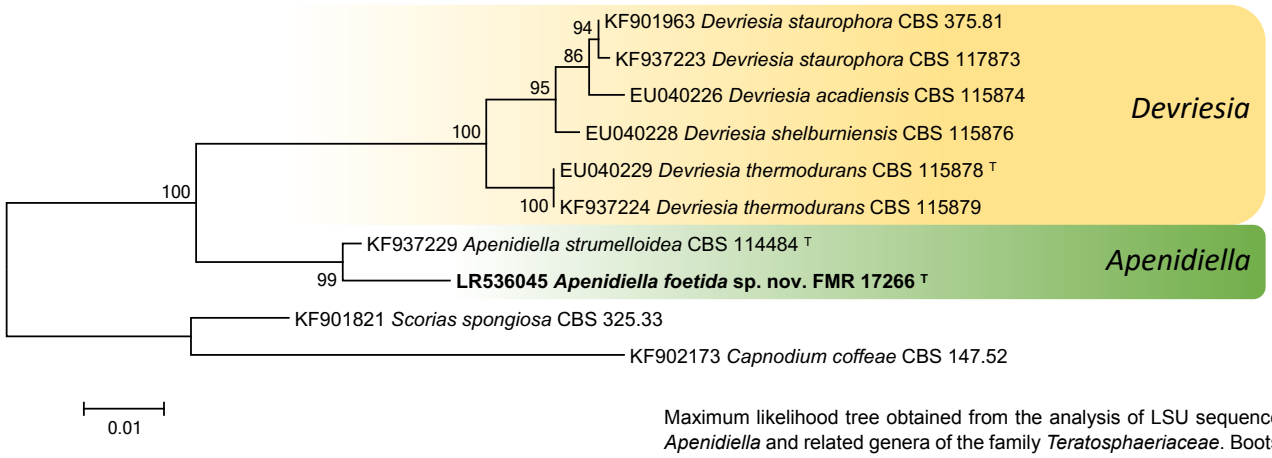
Culture characteristics — Colonies on PDA reaching 28–33 mm diam after 30 d at 25 °C, olive brown (4F3) (Kornerup & Wanscher 1978), velvety, radially folded, aerial mycelium scarce, regular margin; reverse dark green (30F8) to black. On PCA reaching 27 mm after 30 d at 25 °C, olive (3F3/3E3), slightly granular, flat, aerial mycelium scarce, regular margin; reverse yellowish brown to greyish brown (5F8/5E3). On OA reaching 20–23 mm diam after 30 d at 25 °C, olive (3F3), slightly granular, flat, aerial mycelium scarce; reverse yellowish brown (5F8/5F4). An unpleasant smell was appreciated in old cultures of PCA and OA.

Cardinal temperature for growth — Optimum 25 °C, maximum 28 °C, minimum 5 °C.

Typus. Spain, Catalonia, Baix Camp, Arbolí River, on submerged plant debris, Feb. 2018, *I. Iturrieta-González*, *E. Carvalho & J. Gené* (holotype CBS H-23919, culture ex-type CBS 145590 = FMR 17266; ITS and LSU sequences GenBank LR536044 and LR536045, MycoBank MB830227).

Notes — *Apenidiella* is a monotypic genus recently introduced in the family *Teratosphaeriaceae* to accommodate *A. strumelloidea* (previously *Cladosporium strumelloideum*), a fungus isolated from a leaf of *Carex* sp. collected in stagnant water from the Sutka River in Russia (Crous et al. 2007, Quaedvlieg et al. 2014). Interestingly, the novel species was recovered from a similar habitat than the type species of the genus. *Apenidiella strumelloidea* differs from *A. foetida* in having shorter conidiophores (up to 80 µm long) and conidiogenous cells (8–12 µm) and its conidia frequently show one side flat and the other convex, even slightly curved conidia are also present (Crous et al. 2007). In addition, in *A. strumelloidea* macro- and microconidiophores were described, while in our species only macroconidiophores were observed.

Based on a megablast search of NCBIs GenBank nucleotide database, the **LSU** sequence of *A. foetida* showed a similarity of 98.82 % (839/849) with that of *A. strumelloidea* (CBS 114484, GenBank KF937229), while the similarity between **ITS** sequences (GenBank LR536044 vs GenBank EU019277) was 93.67 % (459/490).



Maximum likelihood tree obtained from the analysis of LSU sequences of *Apenidiella* and related genera of the family *Teratosphaeriaceae*. Bootstrap support values above 70 % are indicated on the nodes. The alignment included 751 bp and was performed with ClustalW. Kimura 2 parameters with Gamma distribution (K2+G) was used as the best nucleotide substitution model. Both the alignment and tree were constructed with MEGA v. 6 software (Tamura et al. 2013). The new species proposed in this study is indicated in **bold**. A superscript T denotes ex-type cultures.

Colour illustrations. Arbolí, Catalonia, Spain. Colony sporulating on PCA after 30 d at 25 °C, and conidiophores and conidia after 14 d at 25 °C. Scale bars = 10 µm.

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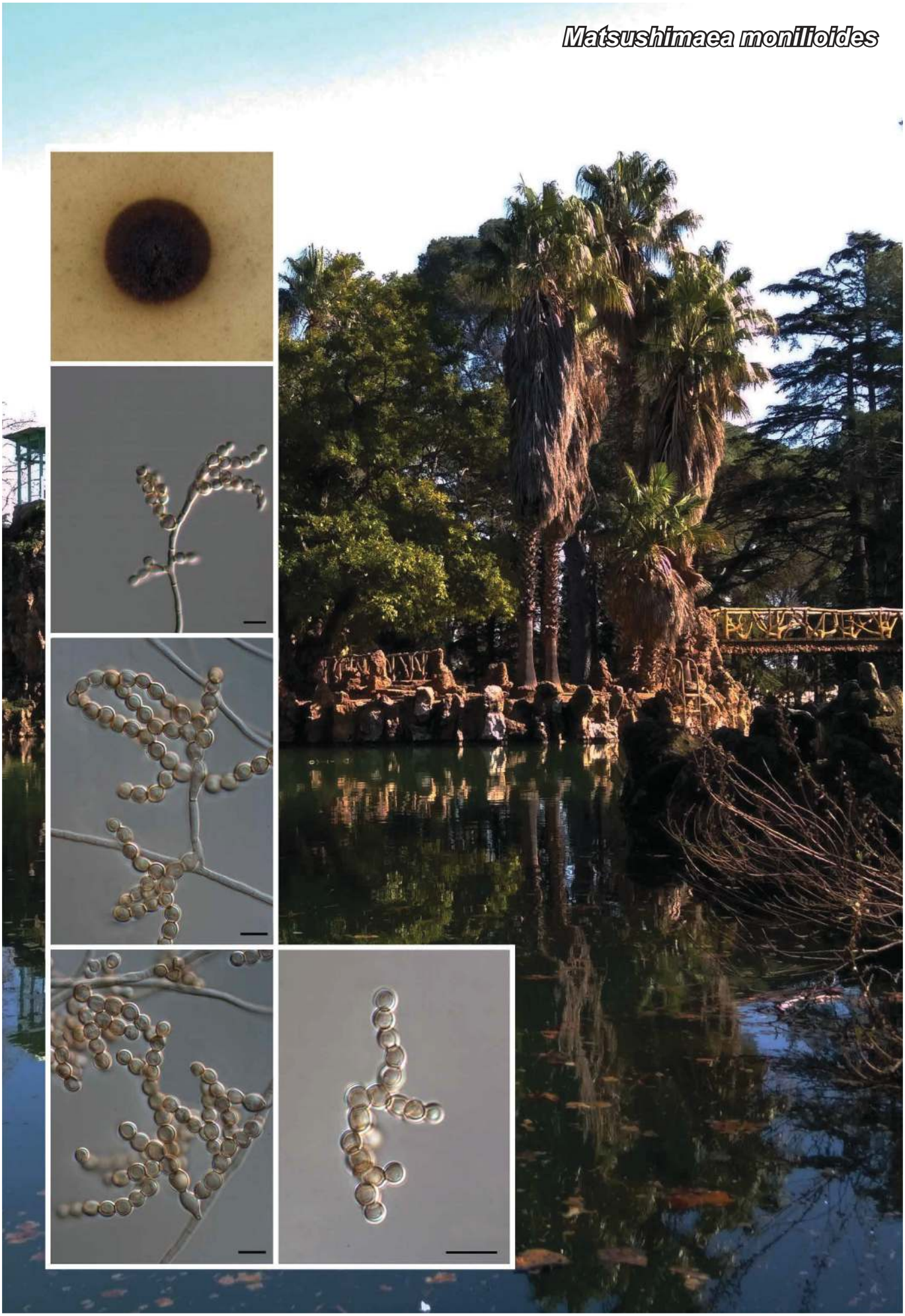
4.2.4 *Matsushimaea monilioides* sp. nov.

Isabel Iturrieta-González, Dania García & Josepa Gené.

Published in: Persoonia (Fungal Planet description sheets) 2018; 40: 304–305.

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González



Fungal Planet 741 – 13 July 2018

Matsushimaea monilioides Iturrieta-González, Dania García & Gené*, sp. nov.*

Etymology. Name refers to the moniliform filaments in conidia.

Classification — *Sympoventuriaceae*, *Venturiales*, *Dothideomycetes*.

Mycelium consisting of branched, septate, olive, smooth-walled, 1–2 µm diam hyphae, frequently forming hyphal coils, occasionally with irregular swellings not constricted at the septa. *Conidiophores* micronematous, often reduced to conidiogenous cells with conidia arising directly on hyphae. *Conidiogenous cells* integrated, mono- or polyblastic, intercalary or terminal, elongated, 7–14.5 × 2–4 µm, pale brown, smooth-walled. *Conidia* solitary, sessile or on short protrusions, irregularly shaped, composed of a basal cell from which arise acropetal chains of cells, giving place to moniliform, septate, often branched filaments, up to 46 µm long and 2–4.5 µm wide, remaining attached at maturity; cells globose, subglobose, ellipsoidal to somewhat pyriform, 2.5–5.5 × 2–4.5 µm, brown, smooth-walled. *Sexual morph* not observed.

Culture characteristics — Colonies on PDA reaching up to 13 mm diam after 14 d at 25 °C, yellowish brown, velvety, flat, aerial mycelium scarce, margin entire; reverse dark brown. On OA up to 14 mm diam after 14 d at 25 °C, dark brown, dusty, flat; reverse dark brown. No growth at 37 °C.

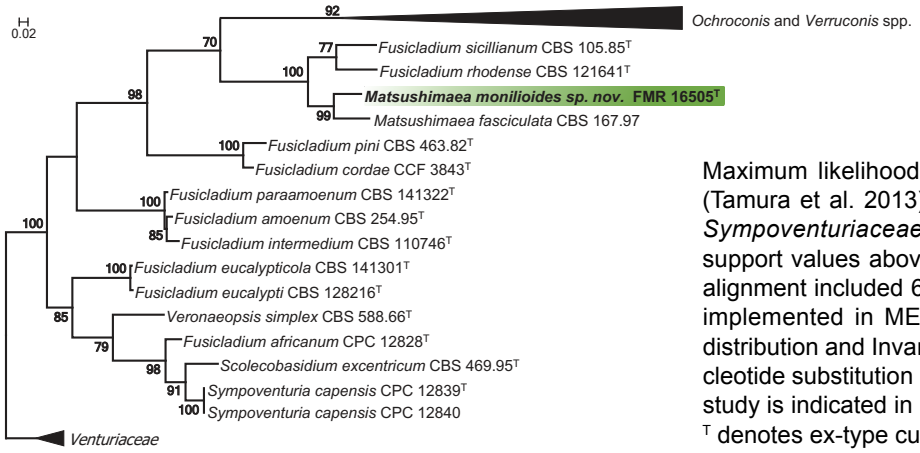
Typus. Spain, Catalonia, Tarragona, Parc Samà, garden soil, Feb. 2017, *J. Gené & I. Iturrieta-González* (holotype CBS H-23392; cultures ex-type FMR 16505 = CBS 143867, ITS and LSU sequences GenBank LT883468 and LT883469, MycoBank MB823930).

Notes — The genus *Matsushimaea* was erected by Subramanian (1977) to accommodate *Torula fasciculata*, a fungus described by Matsushima (1975) and characterised by the production of sessile branched conidia arising directly from vegetative hyphae. In addition to the type, *M. fasciculata*, the genus currently includes two other species, *M. fertilis* (Castañeda-Ruiz et al. 1996) and *M. magna* (Matsushima 1996). The three species were found on leaf litter from Japan, Cuba and South Africa, respectively. Considering the lack of molecular data for *Matsushimaea* and that only for *M. fertilis* ex-type cultures were available for comparison, we selected a reference strain of *M. fasciculata* (CBS 167.97), which morphological features fit with those of the protologue of the species, in order to elucidate the phylogenetic position of the genus among ascomycetes and determine its relationships with our fungus. A phylogenetic analysis with the rDNA operon (ITS and LSU) placed the CBS strain of *M. fasciculate* in the family *Sympoventuriaceae* and it was closely related to our strain. However, both strains showed genetic differences (99 % similar with LSU, 86 % with ITS) enough to be considered distinct species.

Matsushimaea monilioides morphologically resembled *M. fertilis*. However, a megablast search with ITS and LSU sequences of the ex-type strain (INFAT C93/204 = IMI 358617) of this latter species showed it was related to the genus *Cladophialophora* (*Herpotrichiellaceae*, *Chaetothyriales*), being highly similar to the sequences of the ex-type of *C. boppii* (CBS 126.86; LSU 100 % similar with GenBank FJ358233 and ITS 98 % similar with GenBank NR_131297). Therefore, *M. fertilis* was excluded in the present phylogenetic analysis.

Based on a megablast search of NCBIs GenBank nucleotide database, the closest hits using LSU sequence of *M. monilioides* with other sympoventuriaceous species were *Fusicladium sicillianum* (CBS 105.85; GenBank FN398150.1) with a similarity of 95 % (531/557) and *Fusicladium rhodense* (CBS 121641; GenBank EU035440.1) also 95 % (812/855) similar. The closest hits using the ITS sequence were *F. rhodense* (CBS 121641; GenBank EU035440.1) and *F. sicillianum* (CBS 105.85; GenBank FN549914.1) with a similarity of 86 % (402/470) and 85 % (390/459), respectively.

Matsushimaea fasciculata and *M. magna* morphologically differ from our fungus in conidial morphology; while the conidia of the former are more regularly shaped, obconical to cupulate and measure 30–45 µm long (Matsushima 1975), those of *M. magna* are larger, up to 100 µm long (Matsushima 1996).



Maximum likelihood tree inferred with MEGA v. 6 software (Tamura et al. 2013) from the analysis of ITS sequences of *Sympoventuriaceae* and *Venturiaceae* families. Bootstrap support values above 70 % are indicated on the nodes. The alignment included 665 bp and was performed with ClustalW implemented in MEGA. Tamura 3-parameter with Gamma distribution and Invariant sites (G+I) was used as the best nucleotide substitution model. The new species proposed in this study is indicated in **bold** face in the green box. A superscript T denotes ex-type cultures.

Colour illustrations. Parc Samà, Tarragona, Spain; colony sporulating on OA and conidia after 14 d at 25 °C. Scale bars = 10 µm.

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4.2.5 *Pseudopenidiella gallaica* sp. nov.

Isabel Iturrieta-González, Dania García & Josepa Gené.

Published in: Persoonia (Fungal Planet description sheets) 2018; 41: 396–397.

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González


Pseudopenidiella gallaica

Fungal Planet 859 – 13 December 2018

Pseudopenidiella gallaica Iturrieta-González, Dania García, Gené, *sp. nov.*

Etymology. Name refers to the Spanish region where the species was collected.

Classification — *Microthyriaceae*, *Microthyriales*, *Dothideomycetes*.

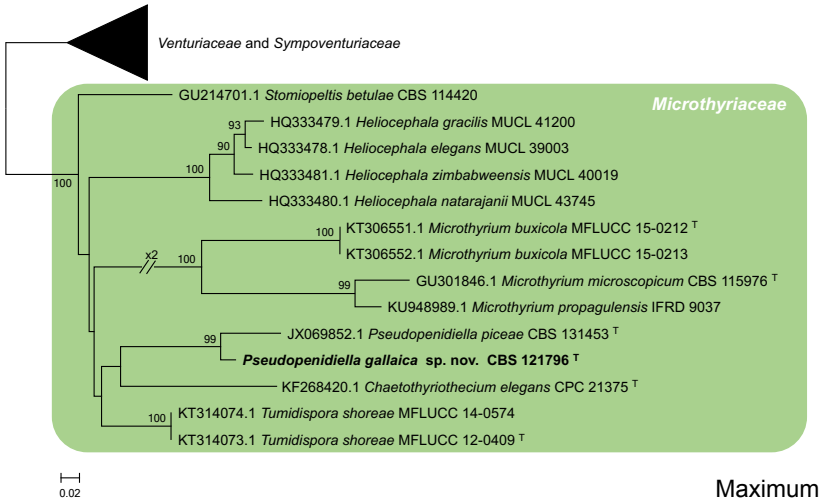
Mycelium consisting of branched, septate, pale brown, smooth-walled to verruculose hyphae of 1–1.5 µm diam. *Conidiophores* mononematous, dimorphic: microconidiophores reduced to conidiogenous cells on hyphae, 13–19 µm high, apex truncate 2 µm wide, pale brown; macroconidiophores unbranched, erect, subcylindrical, with up to 3-septate, pale brown to brown, often verruculose towards the apex, smooth- and thick-walled towards an often swollen base, up to 55 µm long (up to 120 µm long on the natural substratum), 2–3 µm wide. *Conidiogenous cells* terminal or subterminal, mono- or polyblastic, with up to 4 inconspicuous conidiogenous loci, verruculose, pale brown, 11–21.5 × 1.5–3 µm. *Ramoconidia* subcylindrical, aseptate, pale brown, smooth to verruculose, 7.5–11 × 2–3 µm, forming conidia in acropetal branched chains. *Conidia* cylindrical to ellipsoidal, aseptate, pale brown, smooth-walled to verruculose, 6–12 × 1–3 µm. *Sexual morph* not observed.

Culture characteristics — Colonies on PDA reaching 8–9 mm diam after 30 d at 25 °C, golden grey to black, velvety, erumpent, aerial mycelium scarce,feathery margin; reverse dark brown to black. On OA reaching 5–6 mm diam after 30 d at 25 °C, olive brown to black, slightly dusty, flat, aerial mycelium scarce; reverse dark brown to black.

Typus. Spain, Galicia, Pontevedra, Natural Park of Monte Aloia, on unidentified dead leaves, Feb. 2006, *J. Mena & C. Silvera* (holotype FMR H-9234, cultures ex-type CBS 121796 = FMR 9234; ITS and LSU sequences GenBank LT984842 and LT984843, MycoBank MB828082).

Notes — *Pseudopenidiella* was introduced to accommodate *P. piceae* (Crous et al. 2012b), a hyphomycetous fungus morphologically similar to *Cladosporium*, but phylogenetically distant to the family *Cladosporiaceae* (*Capnodiales*, *Dothideomycetes*). The genus was characterised by the formation of dimorphic conidiophores with terminal aseptate ramoconidia producing branched conidial chains, and by the absence of coronate-type scars on conidia or conidiogenous cells. In addition to the type, *P. pini* (formerly *Polyscytalum pini*; Kirk 1983) is currently included in *Pseudopenidiella* (Kirk 2014). However, the phylogeny of this latter species is obscure since only herbarium material (holotype IMI 242163) is available for comparison. *Pseudopenidiella pini* is characterised by the production of short and broad denticulate conidiogenous cells, a feature not described in *Pseudopenidiella*. *Pseudopenidiella gallaica* differs from *P. piceae* in its shorter conidiophores (up to 55 µm long in culture – up to 120 µm on the natural substratum – vs 150 µm long in *P. piceae*) and slightly longer conidia (up to 12 µm in *P. gallaica* vs up to 10 µm in *P. piceae*).

Based on a megablast search of NCBIs GenBank nucleotide, LSU sequence of *P. gallaica* showed a similarity of 95 % (742/785) with that of *P. piceae* (CBS 131453, GenBank NG_042681); while ITS sequence did not reveal any close hits. Our phylogenetic reconstruction shows that *Pseudopenidiella* is related to the members of the family *Microthyriaceae* (Abarca et al. 2011, Singtripop et al. 2016).



Maximum likelihood tree obtained from the analysis of LSU sequences of *Pseudopenidiella* and related genera of the family *Microthyriaceae*. Bootstrap support values above 70 % are indicated on the nodes. The alignment included 555 bp and was performed with ClustalW. Tamura Nei with Gamma distribution (G) was used as the best nucleotide substitution model. Both the alignment and tree were constructed with MEGA v. 6 software (Tamura et al. 2013). The new species proposed in this study is indicated in **bold** face. A superscript T denotes ex-type cultures.

Colour illustrations. Natural Park of Monte Aloia, Pontevedra, Galicia, Spain; colony sporulating on PDA after 30 d at 25 °C and conidia after 10 d at 25 °C. Scale bars = 10 mm (colony) and = 10 µm (microscopic structures).

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4.2.6 *Venturia submersa* sp. nov.

Isabel Iturrieta-González, Josepa Gené & Dania García.

Published in: Persoonia (Fungal Planet description sheets) 2019; 43: 418–419.

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

Fungal Planet 1040 – 18 December 2019

Venturia submersa Iturrieta-González, Gené, Dania García, *sp. nov.*

Etymology. Referring to the fungus growing on submerged plant debris.

Classification — *Venturiaceae*, *Venturiales*, *Dothideomycetes.*

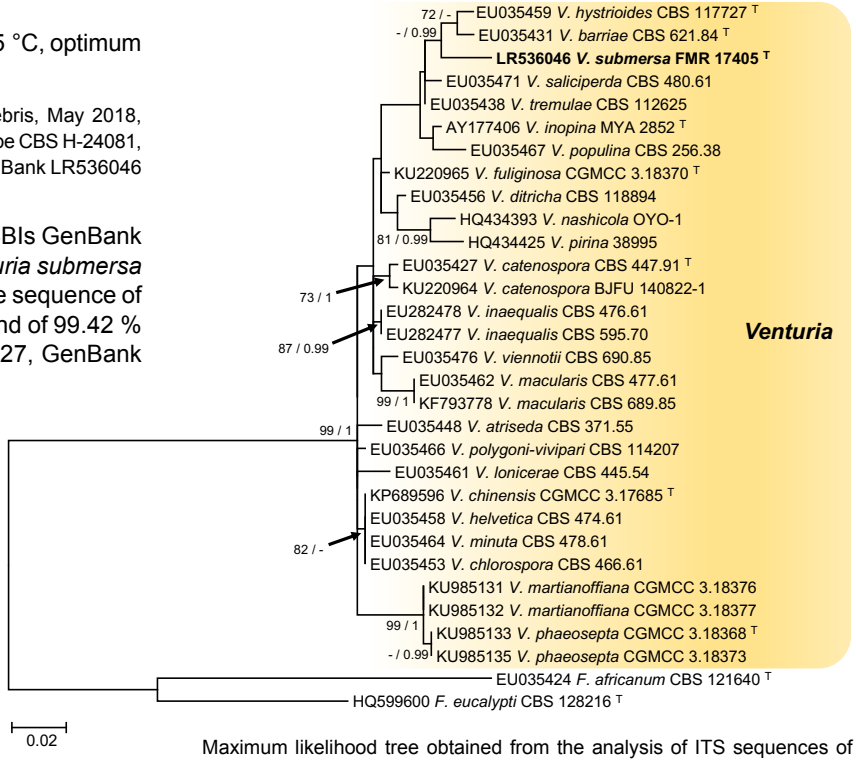
Mycelium consisting of branched, septate, subhyaline to pale olivaceous, smooth-walled 2–5 µm diam hyphae. *Conidiophores* mononematous, growing laterally on hyphae, micronematous, reduced to a conidiogenous cell, or macronematous, erect, unbranched, more rarely branched, subcylindrical, pale olivaceous, smooth-walled, up to 30 µm long. *Conidiogenous cells* terminal, polyblastic, with up to three denticle-like conidiogenous loci, smooth-walled, pale olivaceous, 11–24 × 2–4 µm, forming conidia in simple or branched acropetal chains. *Ramoconidia* 0(–1)-septate, cylindrical, with truncate base, up to three terminal or subterminal conidiogenous loci, smooth-walled, pale olivaceous, 13–24 × 2–4 µm. *Conidia* fusiform, ellipsoidal or cylindrical, 0(–2)-septate, pale olivaceous, smooth-walled, 7–15 × 3–4(–5) µm. *Sexual morph* not observed.

Culture characteristics at 25 °C in 2 wk — Colonies on potato dextrose agar (PDA) reaching 9 mm diam, grey (4F1), velvety, umbonate, aerial mycelium scarce, regular margin; reverse black. On potato carrot agar (PCA) reaching 8–10 mm, brownish grey (4F2), velvety, flat, aerial mycelium scarce, regular margin; reverse black. On oatmeal agar (OA) reaching 8–10 mm diam, grey (4F1), velvety, flat, aerial mycelium scarce, regular margin; reverse dark brown (6F8).

Cardinal temperature for growth — Minimum 5 °C, optimum 20 °C, maximum 25 °C.

Typus. Spain, Segovia, Riaza, on submerged plant debris, May 2018, *I. Iturrieta-González, V. Magaña-Dueñas & D. García* (holotype CBS H-24081, cultures ex-type FMR 17405; ITS and LSU sequences GenBank LR536046 and LR536048, MycoBank MB831789).

Notes — Based on a megablast search of NCBIs GenBank nucleotide database, the LSU sequence of *Venturia submersa* showed a similarity of 99.77 % (857/859) with the sequence of *V. barriae* (CBS 621.84, GenBank EU035431) and of 99.42 % (854/859) with that of *V. hystrioides* (CBS 117727, GenBank EU035459); while the ITS sequence was 96.44 % (488/506) similar with that of the latter species (CBS 117727, GenBank EU035459) and 95.46 % (484/507) with *V. barriae* (CBS 621.84, GenBank EU035431). The phylogenetic reconstruction using ITS barcodes of different accepted *Venturia* species, including the type *V. inaequalis*, showed that the new species was located in an unsupported clade together with *V. barriae*, *V. hystrioides, V. inopina*, *V. populina*, *V. tremulae* and *V. saliciperda*, being closely related with the former two species. *Venturia barriae*, formerly *Fusicladium fagi*, and *V. hystrioides,* formerly *Capronia hystrioides,* were described from decaying leaves of *Fagus sylvatica* and from scar of cherry fruit, respectively (Dugan et al. 1995, Crous et al. 2007c, Rossman et al. 2015). Morphologically, our new species differs from *V. barriae* in having longer conidiophores (up to 30 µm long vs up to 15 µm long in *V. barriae*), commonly aseptate and shorter conidia (7–15 µm vs up to 40 µm in *V. barriae*), and slower growth on PDA after 4 wk in darkness (23 mm in *V. submersa* vs 50 mm at 25 °C in *V. barriae*). *Venturia hystrioides* differs from *V. submersa* in the absence of macronematous conidiophores, larger ramoconidia (up to 30 µm long) with more septa (0–3), and by its more rapid growth on PDA and OA (reaching 40 mm after 2 wk at 25 °C in dark) (Crous et al. 2007c).



Maximum likelihood tree obtained from the analysis of ITS sequences of the genus *Venturia*. The alignment included 502 bp and was performed with ClustalW. Both the alignment and tree for ML were constructed with MEGA v. 6 software (Tamura et al. 2013) and Bayesian Inference (BI) approaches under MrBayes v. 3.2.6 (Ronquist et al. 2012). Kimura 2-parameters with Gamma distribution (K2+G) was used as the best nucleotide substitution model for ML and Hasegawa-Kishino-Yano with Gamma distribution (HKY+G) for BI. Bootstrap support values for ML greater than 70 % and Bayesian posterior probabilities greater than 0.95 are given near nodes. The new species proposed in this study is indicated in **bold** face. A superscript T denotes ex-type cultures.

Colour illustrations. Riaza, Segovia, Spain. Colony sporulating on PCA after 2 wk at 25 °C; conidiophores and conidia after 10 d. Scale bars 10 mm (colony), 10 µm (microscopic structures).

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4.3. Results on other hyphomycetous dematiaceous fungi

4.3.1 Three new *Curvularia* species from clinical and environmental sources.

Isabel Iturrieta-González, Josepa Gené, Nathan Wiederhold, Dania García.

Published in: Mycokeys 2020; 68: 1–21.

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

MycoKeys 68: 1–25 (2020)
doi: 10.3897/mycokeys.68.51667
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Three new *Curvularia* species from clinical and environmental sources

Isabel Iturrieta-González[1], Josepa Gené[1], Nathan Wiederhold[2], Dania García[1]

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Academic editor: N. Wijayawardene | Received 3 March 2020 | Accepted 18 April 2020 | Published 17 June 2020

Citation: Iturrieta-González I, Gené J, Wiederhold N, García D (2020) Three new *Curvularia* species from clinical and environmental sources. MycoKeys 68: 1–21. https://doi.org/10.3897/mycokeys.68.51667

Abstract

Curvularia is a Pleosporalean monophyletic genus with a great diversity of species, including relevant phytopathogenic, animal and human pathogenic fungi. However, their microscopic identification is difficult due to overlapping morphological features amongst species. In recent years, multi-locus sequence analysis using the ITS region of the rDNA and fragments of the genes *gapdh* and *tef*1 revealed numerous cryptic species, especially in isolates that commonly produced 3-septate conidia. Therefore, based on sequence analysis of the above-mentioned DNA barcodes recommended for species delineation in *Curvularia*, we propose three novel species, *C. paraverruculosa*, *C. suttoniae* and *C. vietnamensis*, isolated from soil, human clinical specimens and plant material, respectively, collected in different countries. These new species are morphologically characterised and illustrated in the present study. *Curvularia paraverruculosa* differs from its counterparts, *C. americana* and *C. verruculosa*, mainly by its narrower conidia. *Curvularia suttoniae* and *C. vietnamensis* are closely related to *C. petersonii*, but the former two have larger conidia.

Keywords

Ascomycetes, Dematiaceous hyphomycetes, phylogeny, Pleosporaceae, taxonomy

Introduction

The genus *Curvularia* Boedijn (1933), typified by *C. lunata* (Wakker) Boedijn, belongs in Pleosporaceae, Pleosporales (Wijayawardene et al. 2018). Members of *Curvularia* show different life modes, i.e. saprophytic, endophytic and also pathogenic on plants and animals (Marin-Felix et al. 2017a). Phytopathogenic species can affect wild grasses

and staple crops, such as rice, maize, wheat or sorghum and give rise to serious losses in agricultural production (Gautam et al. 2013, Manamgoda et al. 2015, Marin-Felix et al. 2017a, Tan et al. 2018). The endophytic species have garnered interest in recent years for their use in the production of bio-based products that are beneficial to living organisms and the environment (Bengyella et al. 2019). Since the first report of *Curvularia* as a human pathogen in a patient with mycetoma (Baylet et al. 1959), other clinical presentations have been reported, such as superficial and deep infections that mainly affect the respiratory tract but can even cause cerebral phaeohyphomycosis with an extremely poor prognosis (de Hoog et al. 2000).

The genus is morphologically distinguished mainly by its asexual morph, which shows sympodial conidiophores with mono- to polytretic conidiogenous cells and transversally septate conidia. Typically, the conidia in *Curvularia* are curved due to the hypertrophy of one of the intermediate cells and they are euseptate (Ellis 1971), although other authors opine that the conidia in *Curvularia* are distoseptate (Sivanesan 1987, Seifert et al. 2011, Madrid et al. 2014). The species of *Bipolaris* and *Exserohilum* have typically straight and distoseptate conidia; however, some of them have been transferred to *Curvularia*, based on their DNA sequence analyses (Manamgoda et al. 2012, Hernández-Restrepo et al. 2018, Tan et al. 2018). Furthermore, due to the overlapping of morphological characters amongst certain species of *Curvularia*, such as conidial size, shape and septation, an accurate identification at the species level is difficult without a DNA sequence analysis (da Cunha et al. 2013, Madrid et al. 2014, Manamgoda et al. 2015). Several cryptic species have been described recently using only multi-locus sequence analyses of the recommended DNA barcodes for species delimitation, i.e. the internal transcribed spacer (ITS) region of the rDNA and the protein-coding loci glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and translation elongation factor 1-a (*tef*1) (Marin-Felix et al. 2017a, Tan et al. 2018). Nearly 130 species have so far been accepted in *Curvularia*, including the species classified previously in the teleomorphic genera *Cochliobolus* and *Pseudocochliobolus* after applying the current criteria for fungal nomenclature (Manamgoda et al. 2012, 2015, Madrid et al. 2014, Hyde et al. 2017, Marin-Felix et al. 2017a, 2017b, Dehdari et al. 2018, Heidari et al. 2018, Hernández-Restrepo et al. 2018, Liang et al. 2018, Mehrabi-Koushki et al. 2018, Tan et al. 2018, Tibpromma et al. 2018, Kiss et al. 2019, Raza et al. 2019, Zhang et al. 2020).

Based on a polyphasic approach, combining morphological and phylogenetic analyses, three novel *Curvularia* species are proposed here, isolated from human clinical specimens in the USA, soil in Mexico and seed and plant debris in Vietnam and Indonesia, respectively.

Material and methods

Origin of isolates

Five unidentified *Curvularia* isolates, maintained in the fungal collection of the Medical School of the Rovira i Virgili University (FMR; Reus, Spain), were included in the

study. Two of these (FMR 10992, FMR 11690) were isolated from human specimens in the USA by Deana A. Sutton of the Fungus Testing Laboratory at the University of Texas Health Sciences Center (UTHSC; San Antonio, USA) and the other three (FMR 11956, FMR 17656, FMR 17659) were isolated from environmental samples; the first from sorghum seeds collected in Indonesia, the second from soil collected in the Mexican region of Michoacán and the third from unidentified plant material collected in the north-east of Vietnam.

DNA extraction, PCR, sequencing and phylogenetic analysis

The fungal DNA was extracted from colonies growing on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) for 7 to 10 days at 25 °C in darkness and following the protocol of Müller et al. (1998). The ITS barcode, including the 5.8S gene and the genes *gapdh* and *tef*1 were analysed following Marin-Felix et al. (2017a). Amplification was carried out using the primer pairs ITS5/ITS4 for the ITS region (White et al. 1990), gpd1/gpd2 for *gapdh* (Berbee et al. 1999) and EF983/2218R for *tef*1 (Schoch et al. 2009). The PCR products were purified and stored at -20 °C until sequencing. The same pairs of primers used for the amplification were also used to obtain the DNA sequences, which were processed at Macrogen Europe (Macrogen Inc., Madrid, Spain). The sequences of each isolate were edited using SeqMan v. 7.0.0 (DNAStar Lasergene, Madison, WI, USA) to obtain the consensus sequences.

We made a preliminary comparison of *gapdh* sequences generated from our isolates with those of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLASTn) for their molecular identification. To establish the phylogenetic position of unidentified isolates with respect to the most accepted species in *Curvularia*, we carried out individual (data not shown) and combined alignments of the three loci complemented by all available sequences of the ex-type and reference strains of *Curvularia* species retrieved from NCBI (Table 1). Based on this first phylogeny of the genus, a more restricted multi-locus analysis was carried out, including only those *Curvularia* species most related to the isolates under study. The alignments were made in the MEGA (Molecular Evolutionary Genetics Analysis) software v.6.0. (Tamura et al. 2013), using ClustalW algorithm (Thompson et al. 1994), refined with MUSCLE (Edgar 2004) in the same platform and manually adjusted as necessary. Phylogenetic reconstructions were made using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches under RAxML-HPC2 on XSEDE v.8.2.12 (Stamatakis et al. 2014) in CIPRES Science gateway portal (Miller et al. 2010) and MrBayes v. 3.2.6 (Ronquist et al. 2012), respectively.

For the ML analysis, the best nucleotide substitution model for the combined analysis of ITS, *gapdh* and *tef*1, determined using the MEGA programme, was Kimura 2-parameters with Gamma distribution (K2+G); the combined analysis of these three phylogenetic markers was tested through Incongruence Length Difference (ILD) implemented in the Winclada programme (Farris et al. 1994). ML bootstrap values (bs) ≥ 70% were considered significant.

Table 1. Species included in this study, their substrate, origin and GenBank accession numbers.

Species	Strain no[1]	Substrate	Country	Genbank accession no.[2]		
				ITS	*gapdh*	*tef1*
Bipolaris maydis	CBS 136.29 T	*Zea mays*	USA	AF071325	KM034846	KM093794
B. saccharicola	CBS 155.26 T	Unknown	Unknown	KY905674	KY905686	KY905694
Curvularia aeria	CBS 294.61 T	Air	Brazil	HF934910	HG779148	–
C. affinis	CBS 154.34 T	Unknown	Indonesia	KJ909780	KM230401	KM196566
C. ahvazensis	CBS 144673 T	*Zinnia elegans*	Iran	KX139029	MG428693	MG428686
C. akaii	CBS 317.86	*Themada triandra* subsp. *japonica*	Japan	KJ909782	KM230402	KM196569
C. akaiiensis	BRIP 16080 T	Unknown	India	KJ415539	KJ415407	KJ415453
C. alcornii	MFLUCC 10-0703 T	*Z. mays*	Thailand	JX256420	JX276433	JX266589
C. americana	UTHSC 08-3414 T	Human ankle	USA	HE861833	HF565488	–
	UTHSC 07-2649	Human toe tissue	USA	HE861834	HF565486	–
	UTHSC 08-84	Human nasal sinus	USA	HG779015	HG779115	–
	UTHSC 08-278	Human peritoneal dialysis fluid	USA	HE861832	HF565487	–
	UTHSC 08-2697	Human leg	USA	HG779016	HG779117	–
C. annelliconidiophori	CGMCC3.19352 T	Roots of *Saccharum officinarum*	China	MN215641	MN264077	MN263935
C. asiatica	MFLUCC 10-0711 T	*Panicum* sp.	Thailand	JX256424	JX276436	JX266593
C. australiensis	BRIP 12044 T	*Oryza sativa*	Australia	KJ415540	KJ415406	KJ415452
	CBS 172.57	*O. sativa* seeds	Vietnam	JN601026	JN601036	JN601003
C. australis	BRIP 12521 T	*Sporobolus caroli*	Australia	KJ415541	KJ415405	KJ415451
C. bannonii	BRIP 16732 T	*Jacquemontia tamnifolia*	USA	KJ415542	KJ415404	KJ415450
C. beasleyi	BRIP 10972 T	*Chloris gayana*	Australia	MH414892	MH433638	MH433654
	BRIP 15854	*Leersia hexandra*	Australia	MH414893	MH433639	MH433655
C. beerburrumensis	BRIP 12942 T	*Eragrostis bahiensis*	Australia	MH414895	MH433634	MH433657
C. boeremae	IMI 164633 T	*Portulaca oleracea*	India	MH414911	MH433641	–
C. borreriae	CBS 859.73	Volcanic ash soil	Chile	HE861848	HF565455	–
C. bothriochloae	BRIP 12522 T	*Bothriochloa bladhii*	Australia	KJ415543	KJ415403	KJ415449
C. brachyspora	CBS 186.50	Soil	Indonesia	KJ922372	KM061784	KM230405
C. buchloes	CBS 246.49 T	*Buchloë dactyloides*	USA	KJ909765	KM061789	KM196588
C. carica-papayae	CBS 135941 T	*Carica papaya*	India	HG778984	HG779146	–
C. chiangmaiensis	CPC 28829 T	*Z. mays*	Thailand	MF490814	MF490836	MF490857
C. chlamydospora	UTHSC 07-2764 T	Human toe nail	USA	HG779021	HG779151	–
C. chonburiensis	MFLUCC 16-0375 T	Dead leaf of *Pandanus* sp.	Thailand	MH275055	MH412747	–
C. clavata	BRIP 61680	*Oryza* sp.	Australia	KU552205	KU552167	KU552159
C. cymbopogonis	CBS 419.78	*Yucca* leaf spot	Netherlands	HG778985	HG779129	–
C. coatesiae	BRIP 24261 T	*Litchi chinensis*	Australia	MH414897	MH433636	MH433659
C. coicis	CBS 192.29 T	*Coix lacryma-jobi*	Japan	AF081447	AF081410	JN601006
C. coimbatorensis	SZMC 22225 T	Human corneal scraping	India	MN628310	MN628306	MN628302
C. colbranii	BRIP 13066 T	*Crinum zeylanicum*	Australia	MH414898	MH433642	MH433660
C. comoriensis	CBS 110673	Unknown	Unknown	LT631357	LT715841	–

Species	Strain no[1]	Substrate	Country	Genbank accession no.[2]		
				ITS	*gapdh*	*tef1*
C. crassiseptum	CBS 503.90 T	Plant material	Nigeria	LT631310	LT715882	–
C. crustacea	BRIP 13524 T	*Sporobolus* sp.	Indonesia	KJ415544	KJ415402	KJ415448
C. dactyloctenicola	CPC 28810 T	*Dactyloctenium aegyptium*	Thailand	MF490815	MF490837	MF490858
C. dactyloctenii	BRIP 12846 T	*Dactyloctenium radulans*	Australia	KJ415545	KJ415401	KJ415447
C. deightonii	CBS 537.70	*Sorghum vulgare*	Denmark	LT631356	LT715839	–
C. determinata	CGMCC3.19340 T	Leaves of *S. officinarum*	China	MN215653	MN264088	MN263947
C. elliptiformis	CGMCC3.19351 T	Roots of *S. officinarum*	China	MN215656	MN264091	MN263950
C. ellisii	CBS 193.62 T	Air	Pakistan	JN192375	JN600963	JN601007
C. eragrosticola	BRIP 12538 T	*Eragrostis pilosa*	Australia	MH414899	MH433643	MH433661
C. eragrostidis	CBS 189.48	*Sorghum* seed	Indonesia	HG778986	HG779154	–
C. falsilunata	CGMCC3.19329 T	Roots of *S. officinarum*	China	MN215660	MN264093	MN263954
C. flexuosa	CGMCC3.19447 T	Roots of *S. officinarum*	China	MN215663	MN264096	MN263957
C. gladioli	CBS 210.79	Gladiolus leaf	Romania	HG778987	HG779123	–
C. geniculata	CBS 187.50	*Andropogon sorghum* seed	Indonesia	KJ909781	KM083609	KM230410
C. graminicola	BRIP 23186 T	*Aristida ingrata*	Australia	JN192376	JN600964	JN601008
C. guangxiensis	CGMCC3.19330 T	Roots of *S. officinarum*	China	MN215667	MN264100	MN263961
C. gudauskasii	DAOM 165085	Unknown	Unknown	AF071338	AF081393	–
C. harveyi	BRIP 57412 T	*Triticum aestivum*	Australia	KJ415546	KJ415400	KJ415446
C. hawaiiensis	BRIP 11987 T	*O. sativa*	USA	KJ415547	KJ415399	KJ415445
C. heteropogonicola	BRIP 14579 T	*Heteropogon contortus*	India	KJ415548	KJ415398	KJ415444
C. heteropogonis	CBS 284.91 T	*H. contortus*	Australia	KJ415549	JN600969	JN601013
C. hominis	CBS 136985 T	Human cornea	USA	HG779011	HG779106	–
C. homomorpha	CBS 156.60 T	Air	USA	JN192380	JN600970	JN601014
C. inaequalis	CBS 102.42 T	Soil	France	KJ922375	KM061787	KM196574
C. intermedia	CBS 334.64	*Avena versicolor*	USA	HG778991	HG779155	–
C. ischaemi	CBS 630.82 T	*Ischaemum indicum*	Solomon Islands	MH861533	JX276440	–
C. kenpeggii	BRIP 14530 T	*Triticum aestivum*	Australia	MH414900	MH433644	MH433662
C. kusanoi	CBS 137.29	*Eragrostis major*	Japan	JN192381	LT715862	JN601016
C. lamingtonensis	BRIP 12259 T	*Microlaena stipoides*	Australia	MH414901	MH433645	MH433663
C. lunata	CBS 730.96 T	Human lung biopsy	USA	JX256429	JX276441	JX266596
C. malina	CBS 131274 T	*Zoysia matrella*	USA	JF812154	KP153179	KR493095
C. manamgodae	CGMCC3.19446 T	Roots of *S. officinarum*	China	MN215677	MN264110	MN263971
	LC13495	Roots of *S. officinarum*	China	MN215678	MN264111	MN263972
C. mebaldsii	BRIP 12900 T	*Cynodon transvaalensis*	Australia	MH414902	MH433646	MH433664
	BRIP 13983	*Cynodon dactylon* x *C. transvaalensis*	Australia	MH414903	MH433647	MH433665
C. micropus	CBS 127235 ET	*Paspalum notatum*	Georgia	HE792934	LT715859	–

Species	Strain no[1]	Substrate	Country	Genbank accession no.[2]		
				ITS	*gapdh*	*tef1*
C. microspora	GUCC 6272 T	*Hippeastrum striatum*	China	MF139088	MF139106	MF139115
C. miyakei	CBS 197.29 T	*Eragrostis pilosa*	Japan	KJ909770	KM083611	KM196568
C. mosaddeghii	IRAN 3131C T	*Syzygium cumini*	Iran	MG846737	MH392155	MH392152
C. muehlenbeckiae	CBS 144.63 T	*Sorghum* sp.	USA	MH858242	HG779108	KM196578
C. neergaardii	BRIP 12919 T	*O. sativa*	Ghana	KJ415550	KJ415397	KJ415443
	CBS 276.91	Unknown	Australia	LT631362	LT715848	–
C. neoindica	IMI 129790 T	*Brassica nigra*	India	MH414910	MH433649	MH433667
C. nicotiae	BRIP 11983 T	Soil	Algeria	KJ415551	KJ415396	KJ415442
C. nodosa	CPC 28800 T	*Digitaria ciliaris*	Thailand	MF490816	MF490838	MF490859
	CPC 28801	*Brachiaria reptans*	Thailand	MF490817	MF490839	MF490860
C. nodulosa	CBS 160.58	*Eleusine indica*	Unknown	JN601033	JN600975	JN601019
C. oryzae	CBS 169.53 T	*O. sativa*	Vietnam	KP400650	KP645344	KM196590
C. ovariicola	CBS 470.90 T	*Eragrostis interrupta*	Australia	JN192384	JN600976	JN601020
C. pallescens	CBS 156.35 T	Air	Indonesia	KJ922380	KM083606	KM196570
C. palmicola	MFLUCC 14-0404 T	Dead branches of *Acoelorrhaphe wrightii*	Thailand	MF621582	–	–
C. pandanicola	MFLUCC 15-0746 T	Dead leaf of *Pandanus* sp.	Thailand	MH275056	MH412748	MH412763
C. papendorfii	CBS 308.67 T	*Acacia karroo*	South Africa	KJ909774	KM083617	KM196594
C. paraverruculosa	**FMR 17656 T**	**Soil**	**Mexico**	**LR736641**	**LR736646**	**LR736649**
C. petersonii	BRIP 14642 T	*D. aegyptium*	Australia	MH414905	MH433650	MH433668
C. perotidis	CBS 350.90 T	*Perotis rara*	Australia	JN192385	KJ415394	KM230407
C. phaeospara	CGMCC3.19448 T	Roots of *S. officinarum*	China	MN215686	MN264118	MN263980
C. pisi	CBS 190.48 T	*Pisum sativum*	Canada	KY905678	KY905690	KY905697
C. plantarum	CGMCC3.19342 T	Roots of *S. officinarum*	China	MN215688	MN264120	MN263982
C. platzii	BRIP 27703b T	*Cenchrus clandestinum*	Australia	MH414906	MH433651	MH433669
C. polytrata	CGMCC3.19338 T	Roots of *S. officinarum*	China	MN215691	MN264123	MN263984
C. portulacae	BRIP 14541 T	*Portulaca oleracea*	USA	KJ415553	KJ415393	KJ415440
C. prasadii	CBS 143.64 T	*Jasminum sambac*	India	KJ922373	KM061785	KM230408
C. protuberans	CGMCC3.19360 T	Leaves of *S. officinarum*	China	MN215693	MN264125	MN263986
C. protuberata	CBS 376.65 T	*Deschampsia flexuosa*	UK	KJ922376	KM083605	KM196576
C. pseudobrachyspora	CPC 28808 T	*Eleusine indica*	Thailand	MF490819	MF490841	MF490862
C. pseudolunata	UTHSC 09-2092 T	Human nasal sinus	USA	HE861842	HF565459	–
C. pseudorobusta	UTHSC 08-3458	Human nasal sinus	USA	HE861838	HF565476	–
C. radici-foliigena	CGMCC3.19328 T	Roots of *S. officinarum*	China	MN215695	MN264127	MN263988
	LC11956	Roots of *S. officinarum*	China	MN215698	MN264130	MN263991
C. radicicola	CGMCC3.19327 T	Roots of *S. officinarum*	China	MN215699	MN264131	MN263992
	LC11953	Roots of *S. officinarum*	China	MN215700	MN264132	MN263993
C. ravenelii	BRIP 13165 T	*Sporobolus fertilis*	Australia	JN192386	JN600978	JN601024
C. reesii	BRIP 4358 T	Air	Australia	MH414907	MH433637	MH433670

Species	Strain no[1]	Substrate	Country	Genbank accession no.[2]		
				ITS	*gapdh*	*tef1*
C. richardiae	BRIP 4371 T	*Richardia brasiliensis*	Australia	KJ415555	KJ415391	KJ415438
C. robusta	CBS 624.68 T	*Dichanthium annulatum*	USA	KJ909783	KM083613	KM196577
C. rouhanii	CBS 144674 T	*Syngonium vellozianum*	Iran	KX139030	MG428694	MG428687
C. ryleyi	BRIP 12554 T	*Sporobolus creber*	Australia	KJ415556	KJ415390	KJ415437
C. saccharicola	CGMCC3.19344 T	Roots of *S. officinarum*	China	MN215701	MN264133	MN263994
C. sacchari-officinarum	CGMCC3.19331 T	Leaves of *S. officinarum*	China	MN215705	MN264137	MN263998
C. senegalensis	CBS 149.71	Unknown	Nigeria	HG779001	HG779128	–
C. shahidchamranensis	IRAN 3133C T	Crude oil contaminated soil	Iran	MH550084	MH550083	–
C. soli	CBS 222.96 T	Soil	Papua New Guinea	KY905679	KY905691	KY905698
C. sorghina	BRIP 15900 T	*Sorghum bicolor*	Australia	KJ415558	KJ415388	KJ415435
C. spicifera	CBS 198.31	*Capsicum anuum*	Cyprus	HF934916	HG779136	–
	CBS 274.52	Soil	Spain	JN192387	JN600979.	JN601023
C. sporobolicola	BRIP 23040b T	*Sporobolus australasicus*	Australia	MH414908	MH433652	MH433671
C. subpapendorfii	CBS 656.74 T	Soil	Egypt	KJ909777	KM061791	KM196585
C. suttoniae	**FMR 10992 T**	**Human leg wound**	**USA**	HE861828	HF565479	**LR736651**
	FMR 11690	**Human sphenoid sinus**	**USA**	HE861826	HF565477	**LR736650**
C. tamilnaduensis	SZMC 22226 T	Human corneal scraping	India	MN628311	MN628307	MN628303
	SZMC 26758	Human corneal scraping	India	MN628308	MN628304	MN628300
	SZMC 26759	Human corneal scraping	India	MN628309	MN628305	MN628301
C. thailandicum	MFLUCC 15-0747 T	Decaying leaves of *Pandanus* sp.	Thailand	MH275057	MH412749	MH412764
C. trifolii	CBS 173.55	*Trifolium repens*	USA	HG779023	HG779124	–
C. tripogonis	BRIP 12375 T	*Tripogon loliiformis*	Australia	JN192388	JN600980	JN601025
C. tropicalis	BRIP 14834 T	*Coffea arabica*	India	KJ415559	KJ415387	KJ415434
C. tsudae	ATCC 44764 T	*Chloris gayana*	Japan	KC424596	KC747745	KC503940
	BRIP 10967	Leaf tip blight of *C. gayana*	Australia	KC424604	KC747754	KC503949
C. tuberculata	CBS 146.63 T	*Z. mays*	India	JX256433	JX276445	JX266599
C. umbiliciformis	CGMCC3.19346 T	Roots of *S. officinarum*	China	MN215711	MN264142	MN264004
C. uncinata	CBS 221.52 T	*O. sativa*	Vietnam	HG779024	HG779134	–
C. variabilis	CPC 28815 T	*Chloris barbata*	Thailand	MF490822	MF490844	MF490865
	CPC 28816	*Imperata cylindrica*	Thailand	MF490823	MF490845	MF490866
C. verruciformis	CBS 537.75	*Lobibyx* sp. feather	New Zealand	HG779026	HG779133	–
C. verruculosa	CBS 149.63	*Elaeis guineensis*	Nigeria	HF934909	HG779110	–
	CBS 150.63	*Punica granatum* leaf	India	KP400652	KP645346	KP735695
	CPC 28792	*C. dactylon*	Thailand	MF490825	MF490847	MF490868
	CPC 28809	*E. indica*	Thailand	MF490824	MF490846	MF490867

Species	Strain no[1]	Substrate	Country	Genbank accession no.[2]		
				ITS	*gapdh*	*tef*1
C. vietnamensis	**FMR 17659 T**	**Unidentified dead leaves**	**Vietnam**	**LR736642**	**LR736644**	**LR736647**
	FMR 11956	***Sorghum* seed**	**Indonesia**	**LR736652**	**LR736643**	**LR736648**
C. warraberensis	BRIP 14817 T	*D. aegyptium*	Australia	MH414909	MH433653	MH433672
C. xishuangbannaensis	KUMCC 17-0185 T	Decaying leaves of *Pandanus amaryllifollus*	China	MH275058	MH412750	MH412765

[1] ATCC: American Type Culture Collection, Virginia, USA; BRIP: Queensland Plant Pathology Herbarium, Brisbane, Australia; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; CGMCC: China General Microbiological Culture Collection Center, China; CPC: Culture collection of Pedro Crous, housed at Westerdijk Fungal Biodiversity Institute; DAOM: Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; GUCC: Department of Plant Pathology, Agriculture College, Guizhou University, P.R. China; IMI: International Mycological Institute, Kew, UK; IRAN: Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Iran; KUMCC: Culture Collection of Kunming Institute of Botany, Kunming, China; LC: Personal culture collection held in the laboratory of Prof. Lei Cai, China; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Ria, Thailand; MUCL: Mycothe`que de l'Universite´ Catholique de Louvain, Louvain-la-Neuve, Belgium; SZMC: Szeged Microbiological Collection at the Department of Microbiology, Faculty if Science and Informatics, University of Szeged, Hungary; UTHSC: Fungus Testing Laboratory, Department of Pathology at the University of Texas Health Science Center, San Antonio, Texas, USA. T and ET indicate ex-type and ex-epitype strain.
[2]Sequences newly generated in this study and novel species proposed are indicated in bold.

For the BI phylogenetic analysis, the best nucleotide substitution model was determined using jModelTest (Posada 2008). For the ITS region, we used Kimura 2-parameter with Invariant sites (K80+I), for *gapdh* General Time Reversible with gamma distribution (GTR+G) and for *tef*1 General Time Reversible with invariant sites (GTR+I). The parameter settings used were two simultaneous runs of 5M generations, four Markov chains, sampled every 1000 generations. The 50% majority-rule consensus tree and posterior probability values were calculated after discarding the first 25% of the samples. A posterior probability (pp) value of ≥ 0.95 was considered significant.

Sequence data generated in the present study were deposited in GenBank (Table 1) and the alignments in TreeBASE (http://treebase.org).

Phenotypic study

Macroscopic characterisation of the colonies was made on PDA, oatmeal agar (OA; oatmeal 30 g, agar 13 g, distilled water 1 litre) and potato carrot agar (PCA; potato 20 g, carrot 20 g, agar 13 g, distilled water 1 litre), after 7 days at 25 °C in darkness. Colours of the colonies in descriptions were based on Kornerup & Wanscher (1978). Cardinal temperatures for growth were obtained on PDA after 7 days in darkness.

Microscopic features were studied from the specimens mounted in Shear's solution growing on the same media (Madrid et al. 2014). At least 30 measurements were taken for the calculation of conidial and conidiophores length and width ranges, which are also reported as the mean plus or minus standard deviation in the descriptions.

Photomicrographs were taken using a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity X digital camera.

Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004). Ex-type cultures and holotypes, which were dried cultures, were deposited at the Westerdijk Fungal Biodiversity Institute from Utrecht (CBS, The Netherlands).

Results

BLASTn results with *gapdh* sequences showed that the isolate FMR 17656 was ≤ 97.6%, similar to *C. verruculosa* CPC 28792; FMR 11956 and FMR 17659 showed a similarity of 93.31% and 93.6%, respectively, with *C. spicifera* CBS 198.31; and isolates FMR 10992 and FMR 11690 both exhibited a similarity of 94.7% with the ex-type strain of *C. petersonii* (BRIP 14642). Sequence similarity with this marker between FMR 11956/17659 and FMR 10992/11690 was 97%. These values suggested that the unidentified isolates represented putative new species for the genus, which were then confirmed by multi-locus sequence analysis of ITS, *gapdh* and *tef*1 barcodes. The combined analysis included 128 sequences representing 126 taxa in the genus *Curvularia* and these were rooted with *Bipolaris maydis* (CBS 136.29) and *B. saccharicola* (CBS 155.26) (Suppl. material 1: Fig. S1). The alignment comprised a total of 1928 bp (ITS 432, *gapdh* 573 bp and *tef*1 923 bp), including 546 variable sites (ITS 119 bp, *gapdh* 253 bp and *tef*1 174 bp) and 445 phylogenetically informative (ITS 83 bp, *gapdh* 233 bp and *tef*1 129 bp). The unidentified isolates were allocated to three single lineages in the same clade (74/0.99) together with sequences of the ex-type strains of *C. americana* (UTHSC 08-3414), *C. petersonii* (BRIP 14642) and *C. verruculosa* (CBS 150.63), but with enough distance to be considered distinct species. The two clinical isolates (FMR 10992 and FMR 11690) formed a fully-supported clade closely related to isolates FMR 11956 and FMR 17659, which were collected in Indonesia and Vietnam, respectively and to *C. petersonii*. The fifth isolate (FMR 17656) was related to *C. verruculosa* and *C. americana*, but formed an independent and distant branch from the previously-mentioned species.

In order to evaluate possible intra- and inter-specific variability within the species and to confirm the novelty of these fungi, we performed a multi-locus analysis, including only those sequences of the species that were more related to the unidentified *Curvularia* isolates (Fig. 1). The alignment comprised a total of 1894 bp (ITS 409, *gapdh* 562 bp and *tef*1 923 bp), with 298 variable sites (ITS 66 bp, *gapdh* 135 bp and *tef*1 97 bp) and 240 being phylogenetically informative (ITS 51 bp, *gapdh* 117 bp and *tef*1 72 bp). The phylogenetic analyses show that these isolates indeed represent three new species, which are described and illustrated in the Taxonomy section. The species can be morphologically differentiated mainly by features of their conidia (Table 2).

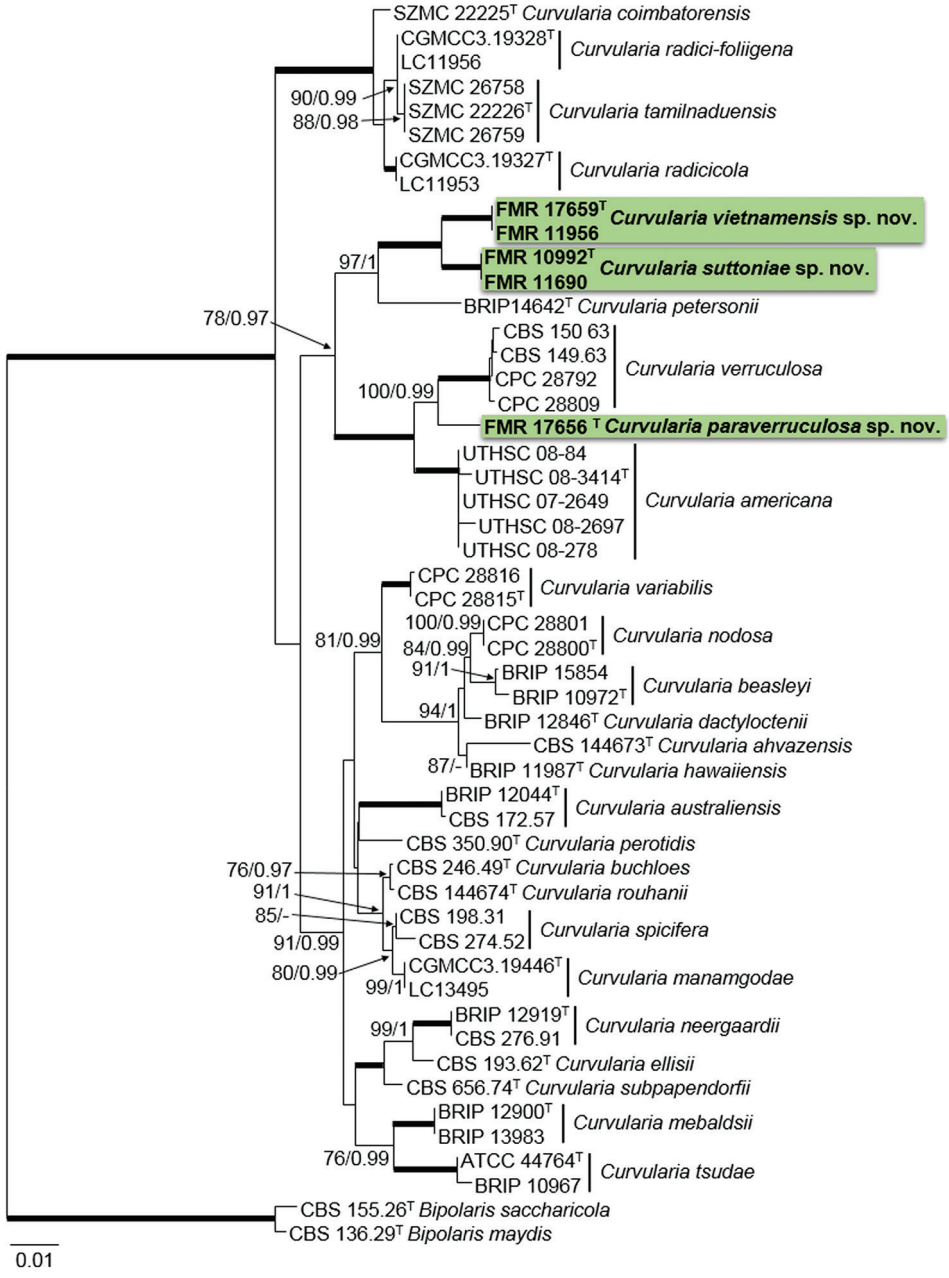

Figure 1. Phylogenetic tree of the *Curvularia* species most related to the new taxa based on Maximum Likelihood analysis obtained by RAxML, using the combined analysis of ITS, *gapdh* and *tef*1 and rooted with *Bipolaris maydis* CBS 136.29 and *Bipolaris saccharicola* CBS 155.26. Bootstrap values (bs) greater than 70% and Bayesian posterior probabilities (pp) greater than 0.95 are given at the nodes (bs/pp). Bold branches indicate bs/pp of 100/1. The novel species are highlighted in bold. Ex-type isolates are marked with a superscript T.

Table 2. Conidial features of the novel *Curvularia* species proposed here and of their closest relatives.

Species	Size (µm)	Septum no.	Ornamentation	References
C. americana	13–28 × 7–15	3–4	Smooth upper cells, verruculose basal cell	Madrid et al. (2014)
C. palmicola	23.9–34.7 × 9.3–15.7	3	Smooth	Hyde et al. (2017)
C. paraverruculosa	11–37 × 8–12	3(–4)	Verruculose to verrucose	Present study
C. petersonii	(15–)17–19(–21) × (5–)5.5–6(–7)	3	Smooth	Tan et al. (2018)
C. suttoniae	8–22 × 5–9	(2–)3	Smooth upper cells, verruculose basal cell	Present study
C. verruculosa	20–40 × 12–17	3	Rough to verruculose	Sivanesan (1987)
C. vietnamensis	15–28 × 5–12	(1–)3(–4)	Smooth	Present study

Taxonomy

Curvularia paraverruculosa **Iturrieta-González, Gené & Dania García, sp. nov.**
MycoBank No: 833024
Fig. 2

Etymology. Name refers to the phylogenetic closeness to *Curvularia verruculosa*.

Type. Mexico, Michoacán, Villa Jiménez, from soil, Sept 2016, *E. Rosas de Paz.* (holotype CBS H-24293, culture ex-type FMR 17656, CBS 146220).

Description (PDA at 25 °C). *Mycelium* composed of branched, septate, subhyaline to pale brown, thin- and smooth-walled hyphae, 2–4 µm wide. *Conidiophores* semi- to macronematous, mononematous, septate, straight or flexuous, geniculate at upper part, unbranched or slightly branched, smooth-walled, yellowish-brown to brown, 19–85(–145) × 3–6 µm (av. (±SD) 49.6 (±43.8) × 4.6 (±0.69)). *Conidiogenous cells* terminal or intercalary, polytretic, proliferating sympodially, yellowish-brown, with darkened scars, subcylindrical, 4–6 µm wide. *Conidia* 3(–4)-septate, mostly curved at the third cell from base which is usually larger than the others, sometimes apically bifurcate, verruculose to verrucose, apical and basal cells subhyaline to pale brown, middle cells brown, 11–37 × 8–12 µm (av. (±SD) 24 (±18.38) × 9.58 (±1.66)); hila slightly protuberant, thickened and darkened. Sexual morph not observed.

Culture characteristics (7 d at 25 °C). *Colonies* on PDA reaching 45 mm diam., dark green (30F8), final edge whitish, velvety, flat, margin regular and fimbriate; reverse dark green (30F8). On PCA and OA, reaching 58–60 mm diam., dark green (30F8), final edge whitish, slightly floccose, flat, margin regular and fimbriate; reverse dark green (30F8). Sporulation was abundant on the three media.

Cardinal temperature for growth. Optimum 30 °C, maximum 37 °C, minimum 15 °C.

Distribution. Mexico.

Notes. *Curvularia paraverruculosa* is allocated phylogenetically to a strongly-supported clade (100/1) with *C. verruculosa* and *C. americana* (Fig. 1). All three species commonly have 3-septate conidia, but these can be distinguished by their size and

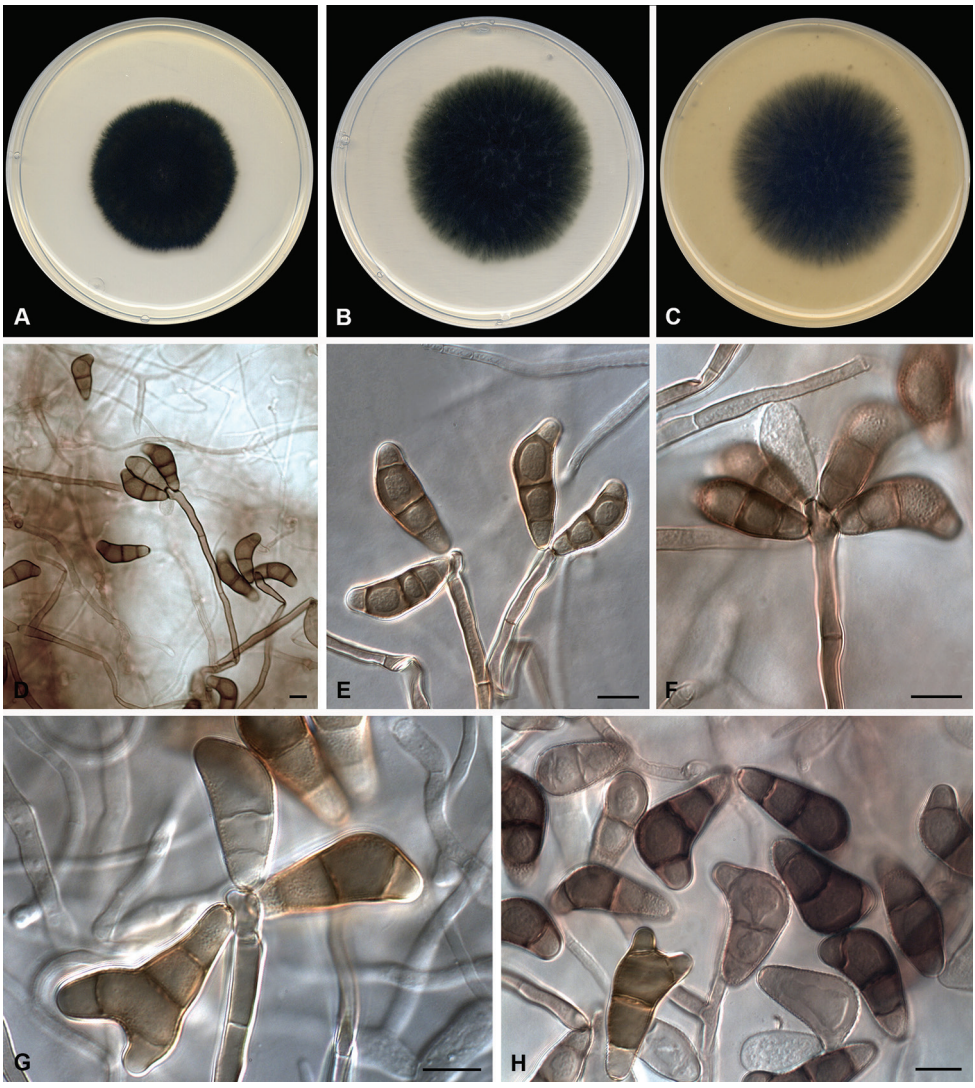

Figure 2. *Curvularia paraverruculosa* sp. nov. (ex-type FMR 17656). **A–C** Colonies on PDA, PCA and OA, respectively, at 25 °C after 7 d **D–H** conidiophores and conidia. Scale bars: 10 µm.

ornamentation. Although conidia in *C. verruculosa*, the closest phylogenetic species and *C. paraverruculosa* are entirely verruculose, they are larger in the former (20–40 × 12–17 µm) (Sivanesan 1987). Furthermore, *C. paraverruculosa* also produces apically bifurcate conidia (Fig. 2), which have not been described in *C. verruculosa*. The conidia of *C. americana* are smaller (13–28 × 7–15 µm) and smooth-walled with a slightly verruculose basal cell (Madrid et al. 2014). In addition, microconidiation, described in *C. americana*, has not been observed in *C. paraverruculosa*.

Curvularia suttoniae **Iturrieta-González, Wiederhold, Gené & Dania García, sp. nov.**
MycoBank No: 833025
Fig. 3

Etymology. Named in honour of the American mycologist Deanna A. Sutton for her contribution to the body knowledge of microfungi.

Type. USA, Texas, from a human leg wound, 2009, *D.A. Sutton* (holotype CBS H-24294, culture ex-type UTHSC 09-3575, CBS 146221, FMR 10992).

Description (PDA at 25 °C). *Mycelium* consisting of branched, septate, pale brown, smooth-walled to verruculose hyphae, 1–4 µm wide. *Conidiophores* mononematous, semi- to macronematous, erect to slightly flexuous, geniculate at the apex, unbranched or branched, smooth-walled to verruculose, pale brown, 43–103 × 3–5 µm (av. (±SD) 80 (±32.35) × 3.7 (±0.67)). *Conidiogenous cells* terminal, subterminal or intercalary, polytretic, proliferating sympodially, pale brown, darkened scars, subcylindrical to slightly swollen, 3–5 µm wide. *Conidia* (2–)3-septate, straight or curved, with the third cell often larger than the rest, apical and middle cells smooth-walled, basal cell verruculose, pale brown to brown, apical and basal cells paler than the middle cells, 8–22 × 5–9 µm (av. (±SD) 15 (±9.89) × 6.88 (±1.18)); hila protuberant, thickened and darkened. Sexual morph not observed.

Culture characteristics (7 d at 25 °C). Colonies on PDA reaching 66–68 mm diam., yellowish-grey (4B2), velvety, flat, margin slightly irregular and fimbriate; reverse black to brownish-orange (5C4); soluble pigment brown (6E6) present in cultures between 30–37 °C. On PCA, reaching 67 mm diam., olive grey (3D2), slightly floccose at the centre, flat, margin regular and whitish; reverse olive grey (3D2), whitish towards periphery. On OA, reaching 64 mm diam., olive grey (3F2), slightly floccose at the centre, flat, margin regular and whitish; reverse olive grey (3F2). Scarce sporulation on the three media.

Cardinal temperature for growth. Optimum 25–30 °C, maximum 37 °C, minimum 5 °C.

Distribution. USA.

Additional specimen examined. USA, South Carolina, from human sphenoid sinus, 2008, *D.A. Sutton* (UTHSC 08-809, FMR 11690).

Notes. *Curvularia suttoniae* is included in a well-supported clade with *C. petersonii* and *C. vietnamensis*, the latter also described here. Although the three species are clearly differentiated phylogenetically (Fig. 1), they can be distinguished only by subtle morphological features. While the conidia of *C. petersonii* and *C. vietnamensis* are entirely smooth, those of *C. suttoniae* show verruculose basal cells. Furthermore, the conidia in *C. petersonii* are narrower (5–7 µm wide) (Tan et al. 2018) and, in *C. vietnamensis*, they are larger (15–28 × 5–12 µm) than those of *C. suttoniae* (8–22 × 5–9 µm). In addition to these morphological features, *gapdh* sequences easily distinguish the two latter species.

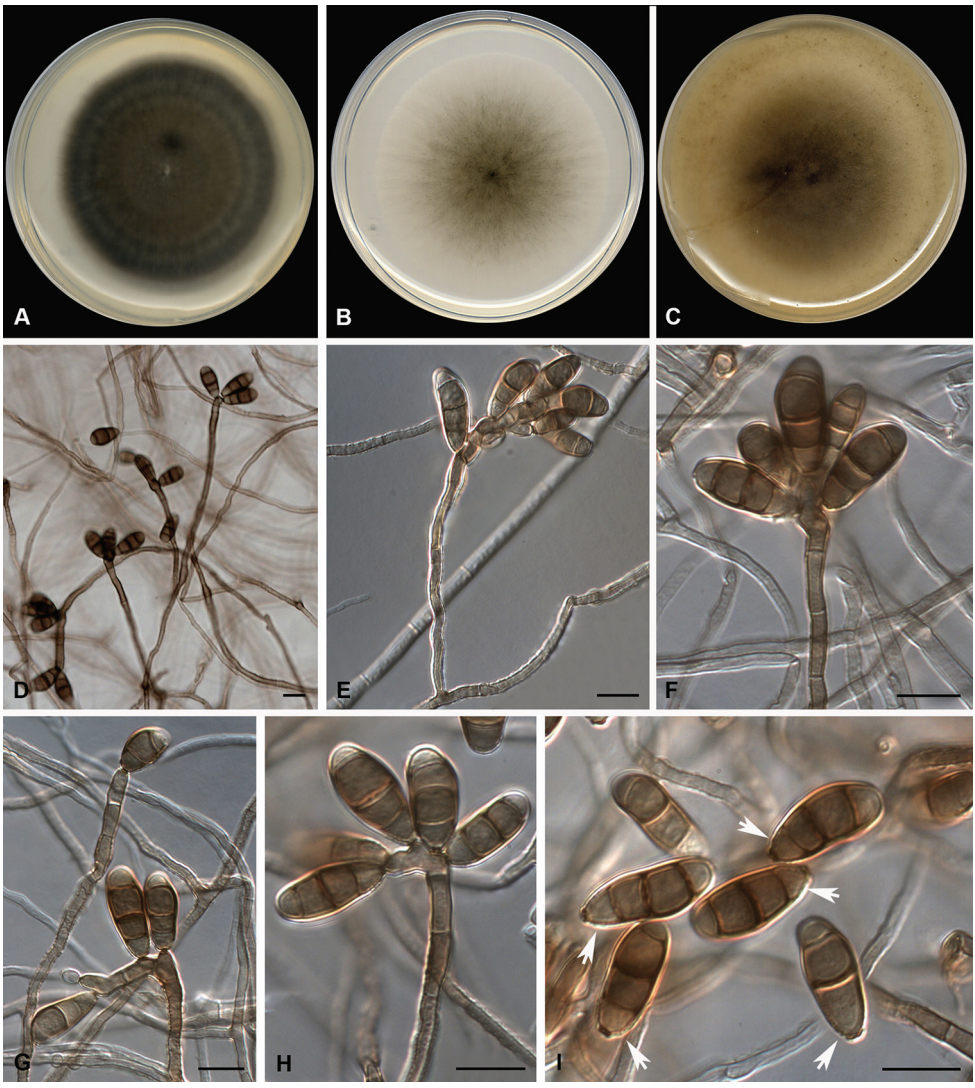

Figure 3. *Curvularia suttoniae* sp. nov. (ex-type FMR 10992). **A–C** Colonies on PDA, PCA and OA, respectively, at 25 °C after 7 d **D–I** conidiophores and conidia with verruculose basal cells (arrows). Scale bars: 10 µm.

Curvularia vietnamensis **Iturrieta-González, Gené & Dania García, sp. nov.**
MycoBank No: 833027
Fig. 4

Etymology. Name refers to the country where the species was collected.

Type. Vietnam, north-east region, on an unidentified dead leaf, Aug 2011, *J. Guarro* (holotype CBS H-24295, culture ex-type CBS 146222, FMR 17659).

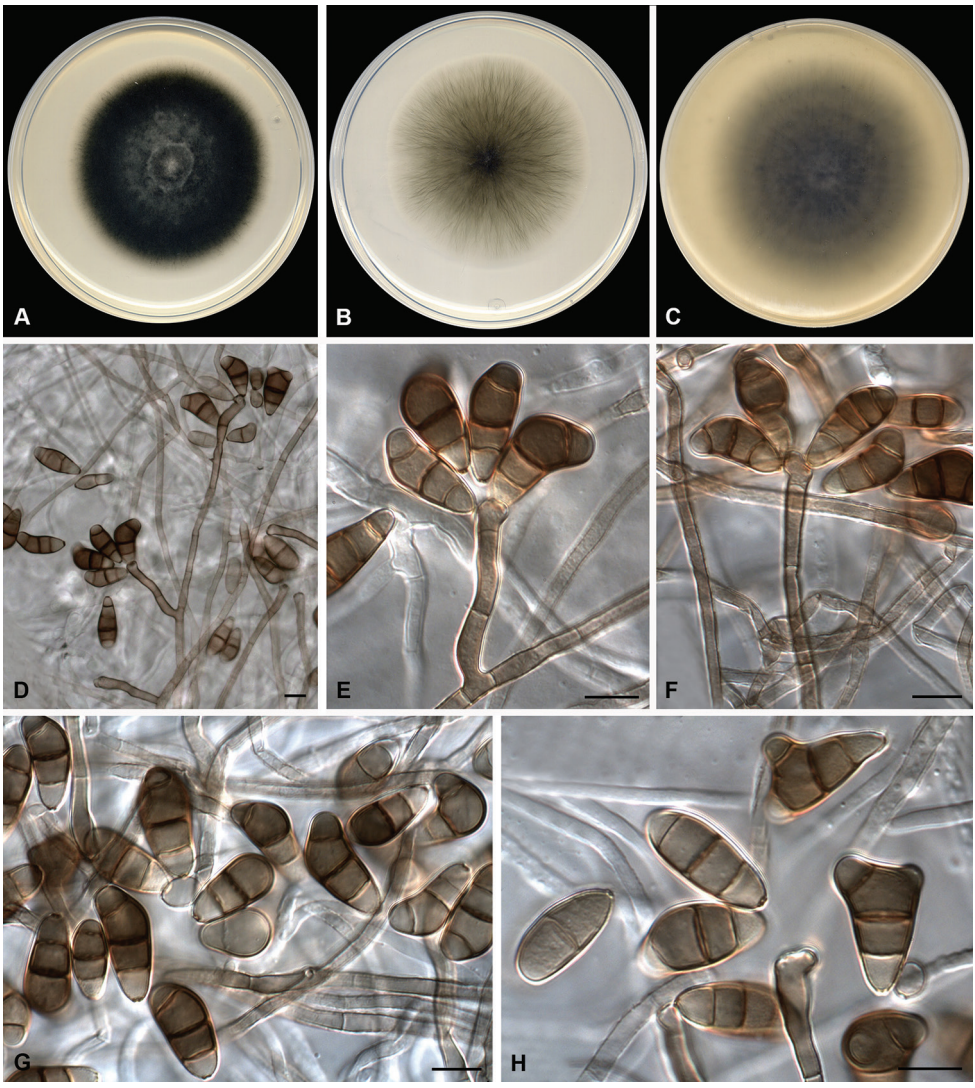

Figure 4. *Curvularia vietnamensis* sp. nov. (ex-type FMR 17659). **A–C** Colonies on PDA, PCA and OA, respectively, at 25 °C after 7 d **D–H** conidiophores and conidia. Scale bars: 10 µm.

Description (PDA at 25 °C). *Mycelium* composed of branched, septate, subhyaline to pale brown, thin and smooth-walled to verruculose hyphae, 2–4 µm wide. *Conidiophores* macronematous, mononematous, septate, straight or flexuous, sometimes slightly geniculate at upper part, unbranched to slightly branched, smooth to verruculose, pale brown to brown, 11–136(–194) × 3–6 µm (av. (±SD) 92.2 (±72.86) × 4.21 (±0.85)). *Conidiogenous cells* terminal or intercalary, mono- or polytretic, proliferating sympodially, pale brown, with darkened scars, subcylindrical to swollen, 3–7 µm wide.

Conidia (1–)3(–4)-septate, curved, with the third cell from base unequally enlarged, some apically bifurcate, smooth-walled, apical and basal cells pale brown, middle cells brown, 15–28 × 5–12 µm (av. (±SD) 21.38 (± 3.44) × 9.34 (±1.83)); hila slightly protuberant, thickened and darkened. Sexual morph not observed.

Culture characteristics (7 d at 25 °C). *Colonies* on PDA reaching 62 mm diam., greenish-grey to dark green (28C2/29F8), final edge white, umbonate, densely floccose, margin regular; reverse grey (29F1), final edge pale grey (1B2). On PCA, reaching 58 mm diam., olive grey to grey (3F2/3B1), slightly floccose at the centre, margin regular, final edge whitish; reverse olive grey to grey (3F2/3B1). On OA, reaching 74 mm diam., olive (2F3) slightly floccose at the centre, margin regular, flat; reverse olive to greenish-grey (2F3/1C2). Sporulation abundant mainly on PCA and OA.

Cardinal temperature for growth. Optimum 30 °C, maximum 37 °C, minimum 15 °C.

Distribution. Indonesia and Vietnam.

Additional specimen examined. Indonesia, from Sorghum seed, 1948, *J. van der Vecht* (CBS 188.48 = FMR 11956).

Notes. See *C. suttoniae* described above.

Discussion

As in other Pleosporalean genera, *Curvularia* is currently a well-delineated genus on the basis of molecular data (Manamgoda et al. 2015, Marin-Felix et al. 2017a). However, morphological features and analyses of the ITS barcode are insufficient to accurately identify *Curvularia* species. Thus, the multi-locus sequence analysis of different gene markers (i.e. LSU, ITS, *gapdh*, *rpb*2 and *tef*1) has been used to study the species diversity in *Curvularia* and phylogentic relationships with other similar genera (Hernández-Restrepo et al. 2018, Manamgoda et al. 2012, 2015, Madrid et al. 2014, Marin-Felix et al. 2017a, 2017b, Tan et al. 2018). Marin-Felix et al. (2017a) regarded ITS, *gapdh* and *tef*1 as the DNA barcodes for species delineation in the genus. During the last three years, numerous new *Curvularia* species have been introduced (Hyde et al. 2017, Marin-Felix et al. 2017a, 2017b, Dehdari et al. 2018, Heidari et al. 2018, Liang et al. 2018, Mehrabi-Koushki et al. 2018, Tan et al. 2018, Tibpromma et al. 2018, Kiss et al. 2019, Raza et al. 2019, Zhang et al. 2020). Novel species are found, not only on fresh material collected in various geographical regions, but also in re-evaluation of *Curvularia* isolates deposited in fungal collections and earlier identified by morphological features or ITS sequence analysis.

The five isolates, studied here, showed morphological similarity with *C. americana* or *C. lunata* (Sivanesan 1987, Madrid et al. 2014), but they also showed subtle variations that did not match with these species. Multi-locus analysis of the recommended barcodes facilitated the delineation of the novel species *C. paraverruculosa*, *C. suttoniae* and *C. vietnamensis*, which were closely related to the known species *C. americana*, *C. petersonii* and *C. verruculosa* (Fig 1).

As in the case of *C. suttoniae*, other related species, such as *C. americana* and *C. verruculosa*, have also been associated with clinical specimens previously (da Cunha et al. 2013, Madrid et al. 2014). However, the role of all these fungi in human diseases has never been proven. Contrary to that, the recently described species *C. coimbatorensis* and *C. tamilnaduensis* were shown to be causal agents of fungal keratitis in India (Kiss et al. 2019). These two latter species, as with *C. suttoniae* and *C. vietnamensis* in our case, could only be molecularly differentiated by *gapdh* and *tef*1 loci; ITS sequence similarity between *C. coimbatorensis* and *C. tamilnaduensis* was 99% (Kiss et al. 2019) and between *C. suttoniae* and *C. vietnamensis*, it was 100%. Therefore, considering clinical laboratories commonly use ITS barcode for fungal diagnosis, not only will the diversity of *Curvularia* species remain obscure in the clinical setting, but also, subsequently, the epidemiology of its species associated with human or animal diseases. Our results suggest that *gapdh* and *tef*1 loci could be good alternatives as barcodes for *Curvularia* identification, since both have a high discriminatory power amongst species. However, *gapdh* would be the recommended locus because there are more sequences available for different species in the genus.

The ITS analysis revealed that *C. palmicola*, only known for its type specimen found on dead branches of *Acoelorrhaphe wrightii* in Thailand (Hyde et al. 2017), is also closely related to the novel species described here. However, this fungus was not included in our concatenate analysis since sequences of *gapdh* and *tef*1 were not available for comparison. Nevertheless, *C. palmicola* can be distinguished morphologically from our species mainly by having conidia with constricted wall at the septum level. Furthermore, *C. palmicola* has longer conidia (23.9–34.7 µm) than *C. suttoniae* (8–22 µm) and C. *vietnamensis* (15–28 µm) and it differs from *C. paraverruculosa* by its smooth-walled conidia.

Despite the fact that DNA sequence analysis is currently mandatory for *Curvularia* identification, two species were recently characterised exclusively, based on morphological data and host association, i.e. *C. tremae* on living leaves of *Trema orientalis* (Haldar 2017) and *C martyniicola* on *Martynia annua* (Kumar and Singh 2018), both from India. *Curvularia tremae* produces up to 4-septate and larger conidia (average length 152.21 µm and 67.75 µm wide at the broadest part) than those described here. Despite the conidia being mostly 3-sepetate, as in our species, *C. martyniicola* differs by having longer conidiophores (95–200 µm) than those of *C. paraverruculosa* (19–85(–145) µm) and *C. suttoniae* (43–103 µm) and by larger conidia (25–45 × 10–15 µm) than those observed in *C. vietnamensis* (15–28 × 5–12 µm).

Acknowledgements

The authors are grateful to Sándor Kocsubé of the University of Szeged (Hungary) for sending the sequences of *C. tamilnaduensis* and *C. coimbatorensis* used in this study. The study was supported by the Spanish Ministerio de Economía y Competitividad, grant CGL2017-88094-P.

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Supplementary material 1

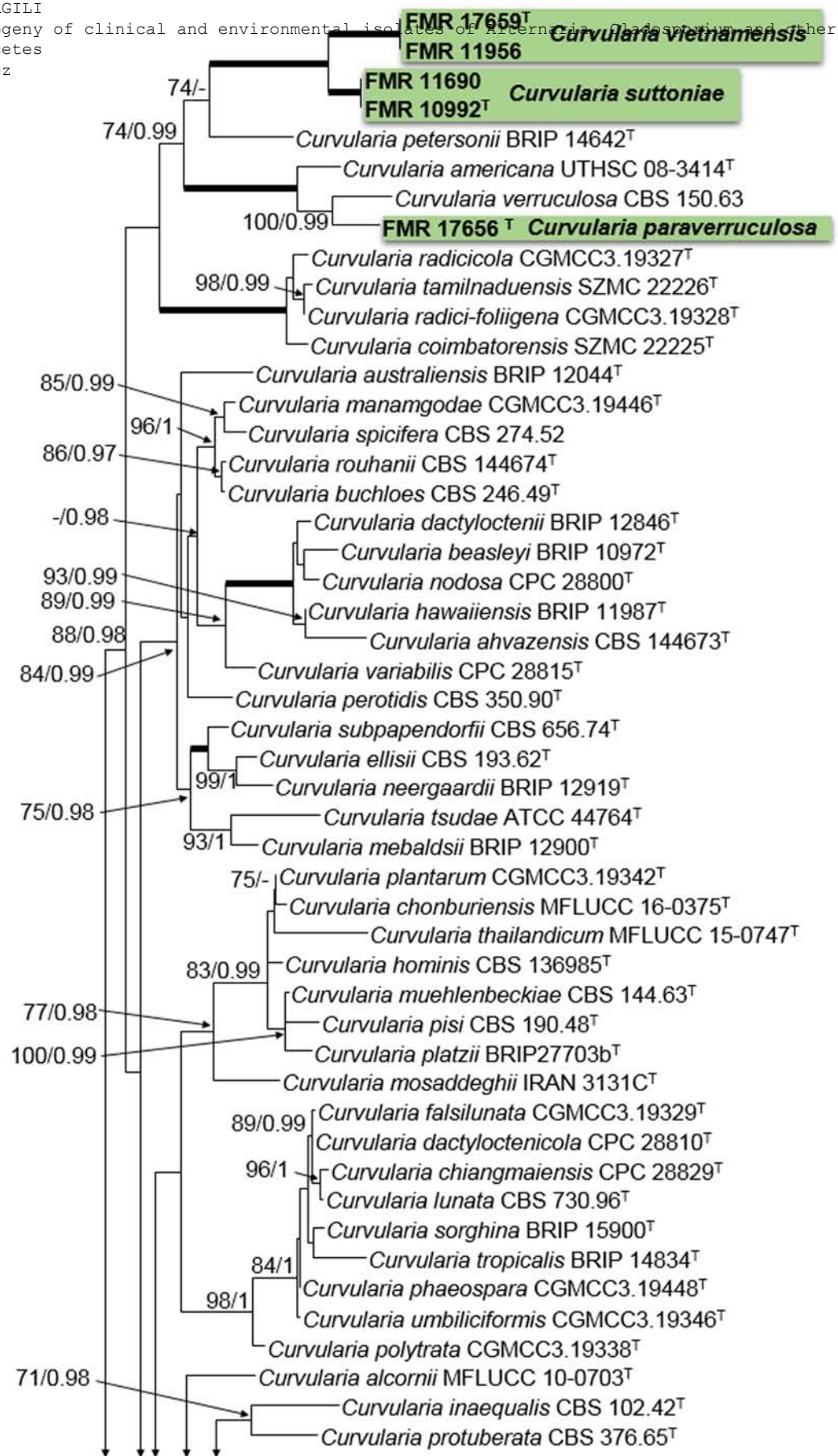
Figure S1. Phylogenetic tree of the genus *Curvularia* based on Maximum Likelihood analysis obtained by RAxML, using the combined analysis of ITS, *gapdh* and *tef*1 and rooted with *Bipolaris maydis* CBS 136.29 and *Bipolaris saccharicola* CBS 155.26

Authors: Isabel Iturrieta-González, Josepa Gené, Nathan Wiederhold, Dania García

Data type: phylogenetic tree

Explanation note: Bootstrap values (bs) greater than 70% and Bayesian posterior probabilities (pp) greater than 0.95 are given at the nodes (bs/pp). Bold branches indicate bs/pp of 100/1. The novel species are highlighted in bold. Ex-type and ex-epitype strain are marked with a superscript T and ET, respectively.

Link: https://doi.org/10.3897/mycokeys.68.51667.suppl1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FMR 10992T
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74/0.99
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Curvularia americana UTHSC 08-3414T
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100/0.99
FMR 17656 T
Curvularia paraverruculosa
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Curvularia radici-foliigena CGMCC3.19328T
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Curvularia beasleyi BRIP 10972T
93/0.99
Curvularia nodosa CPC 28800T
89/0.99
Curvularia hawaiiensis BRIP 11987T
88/0.98
Curvularia ahvazensis CBS 144673T
84/0.99
Curvularia variabilis CPC 28815T
Curvularia perotidis CBS 350.90T
Curvularia subpapendorfii CBS 656.74T
Curvularia ellisii CBS 193.62T
99/1
Curvularia neergaardii BRIP 12919T
75/0.98
Curvularia tsudae ATCC 44764T
93/1
Curvularia mebaldsii BRIP 12900T
75/-
Curvularia plantarum CGMCC3.19342T
Curvularia chonburiensis MFLUCC 16-0375T
Curvularia thailandicum MFLUCC 15-0747T
83/0.99
Curvularia hominis CBS 136985T
Curvularia muehlenbeckiae CBS 144.63T
77/0.98
Curvularia pisi CBS 190.48T
100/0.99
Curvularia platzii BRIP27703bT
Curvularia mosaddeghii IRAN 3131CT
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96/1
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Curvularia lunata CBS 730.96T
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84/1
Curvularia tropicalis BRIP 14834T
98/1
Curvularia phaeospara CGMCC3.19448T
Curvularia umbiliciformis CGMCC3.19346T
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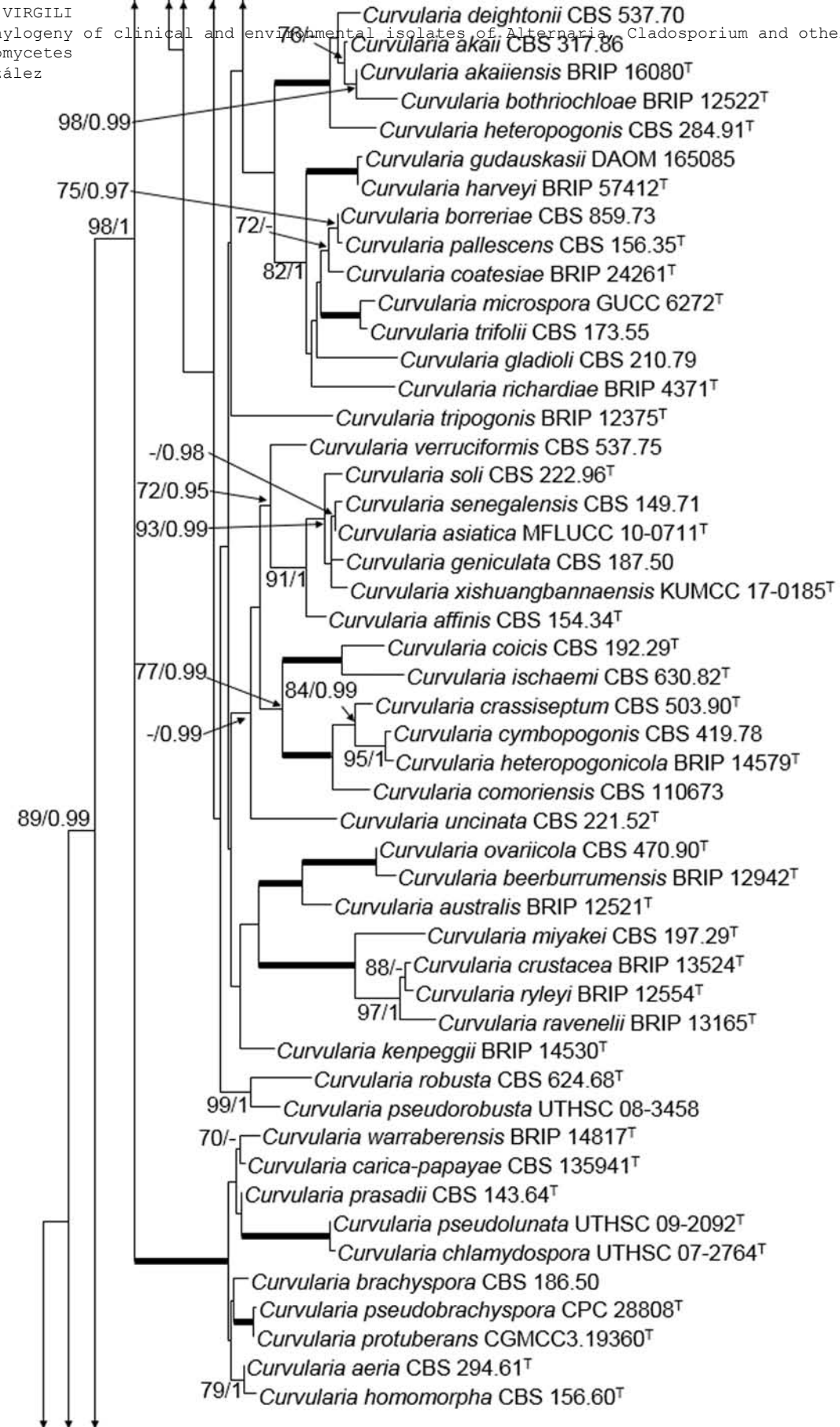
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Curvularia borreriae CBS 859.73
Curvularia pallescens CBS 156.35T
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Curvularia richardiae BRIP 4371T
Curvularia tripogonis BRIP 12375T
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Curvularia affinis CBS 154.34T
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Curvularia ischaemi CBS 630.82T
Curvularia crassiseptum CBS 503.90T
Curvularia cymbopogonis CBS 419.78
Curvularia heteropogonicola BRIP 14579T
Curvularia comoriensis CBS 110673
Curvularia uncinata CBS 221.52T
Curvularia ovariicola CBS 470.90T
Curvularia beerburrumensis BRIP 12942T
Curvularia australis BRIP 12521T
Curvularia miyakei CBS 197.29T
Curvularia crustacea BRIP 13524T
Curvularia ryleyi BRIP 12554T
Curvularia ravenelii BRIP 13165T
Curvularia kenpeggii BRIP 14530T
Curvularia robusta CBS 624.68T
Curvularia pseudorobusta UTHSC 08-3458
Curvularia warraberensis BRIP 14817T
Curvularia carica-papayae CBS 135941T
Curvularia prasadii CBS 143.64T
Curvularia pseudolunata UTHSC 09-2092T
Curvularia chlamydospora UTHSC 07-2764T
Curvularia brachyspora CBS 186.50
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Curvularia protuberans CGMCC3.19360T
Curvularia aeria CBS 294.61T
Curvularia homomorpha CBS 156.60T
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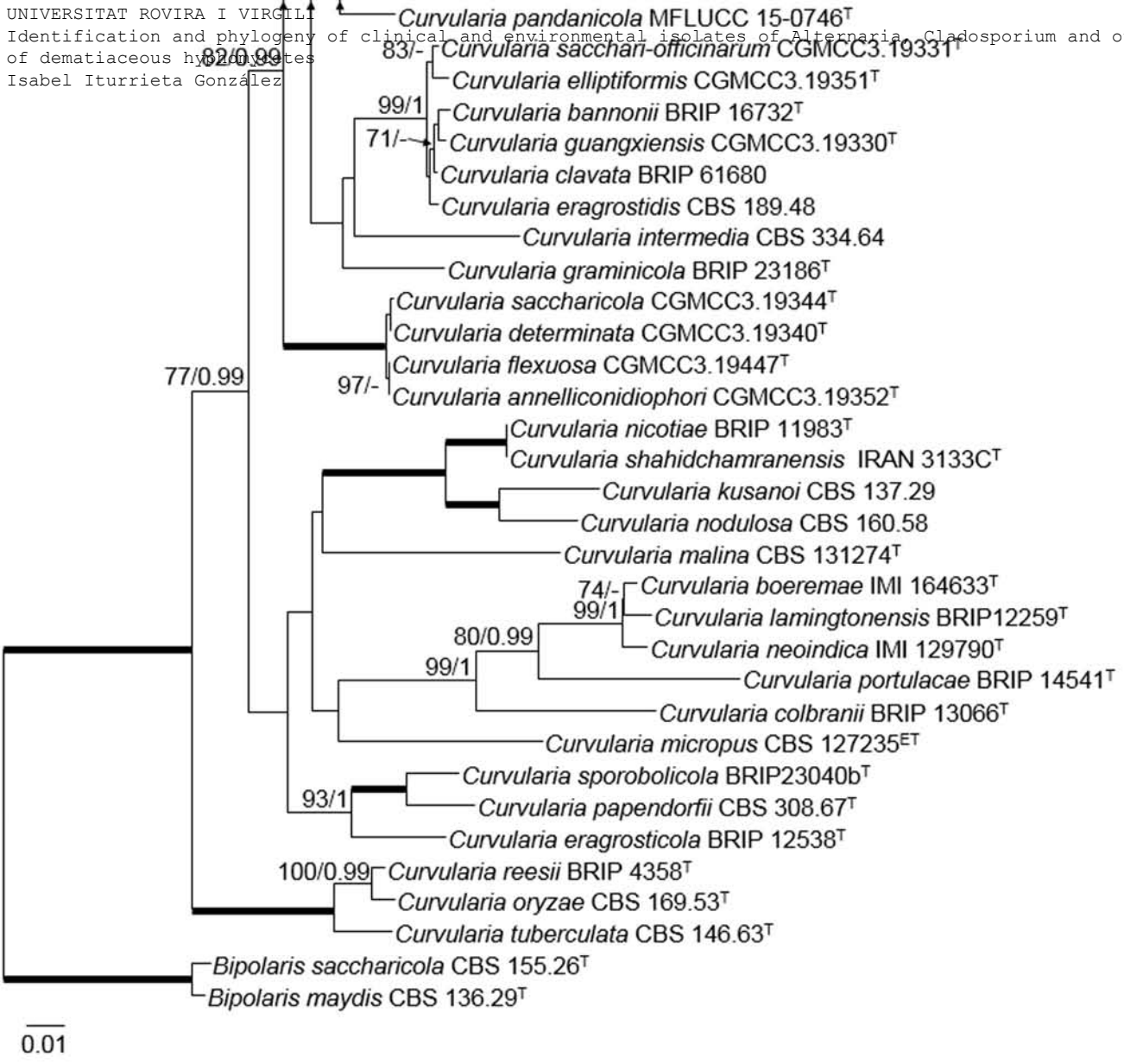

4.3.2 *Heliocephala variabilis* and *Pseudopenidiella vietnamensis:* Two New Hyphomycetous Species in the *Microthyriaceae (Dothideomycetes)* from Vietnam

Isabel Iturrieta-González, Dania García, Josep Guarro, Josepa Gené

Published in: Microorganisms 2020, 8(4): 478; doi:10.3390/microorganisms8040478

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

microorganisms

MDPI

Article

Heliocephala variabilis and *Pseudopenidiella vietnamensis*: Two New Hyphomycetous Species in the Microthyriaceae (Dothideomycetes) from Vietnam

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Received: 29 February 2020; Accepted: 26 March 2020; Published: 27 March 2020

Abstract: In a survey of microfungi from plant debris collected in Vietnam, two new hyphomycetous species were found, which belong to the genera *Heliocephala* and *Pseudopenidiella* and the family *Microthyriaceae* (*Microthyriales*, *Dothideomycetes*). Maximum Likelihood and Bayesian Inference sequence analyses of the internal transcribed spacers (ITS) and large subunit (LSU) of the ribosomal DNA barcodes allowed assessing the phylogenetic relationships of the new species with other species of the respective genera. *Heliocephala variabilis* sp. nov. was closely related to *Heliocephala elegans*, *Heliocephala gracilis*, and *Heliocephala zimbabweensis*, from which it was morphologically distinguished by its smaller conidiophores and non-rostrate conidia of up to four septa on the natural substratum. *Pseudopenidiella vietnamensis* sp. nov. was related to *Pseudopenidiella piceae* and *Pseudopenidiella podocarpi* and differed from the former principally by its lack of microconidiophores and from *P. podocarpi* by having larger macroconidiophores and smooth conidia. Key morphological features to distinguish the accepted species in *Heliocephala* and *Pseudopenidiella* are also provided. In addition, *Pseudopenidiella pini* was excluded from the genus on the basis of its morphological features.

Keywords: hyphomycetes; dematiaceous fungi; phylogeny; taxonomy; Vietnam

1. Introduction

Vietnam is one of the twenty most bio-diverse countries in the world [1–3]. Its very diverse ecological niches, climatic conditions, and high level of plant endemism would suggest that the country also has great fungal diversity, although this has been not extensively studied so far [4–6]. During a survey of asexual microfungi from plant debris carried out in the Northeast of Vietnam, two interesting specimens, belonging to the genera *Heliocephala* and *Pseudopenidiella* (*Microthyriaceae*, *Microthyriales*, *Dothideomycetes*) [7] were isolated and deposited in the culture collection at the Medicine Faculty of the Universitat Rovira i Virgili (Reus, Spain) for subsequent studies.

The genus *Heliocephala* was introduced by Rao et al. [8] based on *Heliocephala proliferans*, a hyphomycetous fungus characterized by the production of solitary macronematous conidiophores, bearing a terminal compact clusters of monoblastic conidiogenous cells that can arise more or less radially from short branches (metula-like) and produce obclavate, rostrate, or hooked conidia. Based on DNA data and morphological features, the genus was emended by Heredia-Abarca et al. [9] to include the species of *Holubovaniella* [10], the latter subsequently being considered a synonym of *Heliocephala*. Currently, *Heliocephala* comprises seven species, most of them isolated from plant material, with the exception of *Heliocephala natarajanii*, which was found along with a basidiocarp of *Pisolithus tinctorius* [11].

Crous et al. [12] introduced the genus *Pseudopenidiella* to accommodate *Pseudopenidiella piceae* recovered from the needle litter of *Picea abies* in the Czech Republic. Morphologically, the genus is

characterized by the presence of micro- and macroconidiophores, with aseptate conidia and ramoconidia arranged in branched acropetal chains. It shows a conidiogenous apparatus similar to those of the genera *Cladosporium*, *Digitopodium*, and *Penidiella*, but mainly differs from them by the lack of darkened and coronate-type scars in both conidia and conidiogenous cells and by the absence of rhizoids associated with the conidiophores. Phylogenetically, three species have been recognized in the genus, *Pseudopenidiella podocarpi* being the most recently described on leaves of *Podocarpus latifolus*, collected in South Africa [13].

Based on a polyphasic approach, we propose two novel hyphomycetous fungi from Vietnam, *Heliocephala variabilis* and *Pseudopenidiella vietnamensis*, which are described and illustrated below.

2. Material and Methods

2.1. Samples and Isolates

Samples of plant debris were collected in the Northeast region of Vietnam in 2011 and treated according to Hernández-Restrepo et al. [14]. They were placed in moist chambers and incubated at room temperature (22 °C) in darkness, being examined periodically under a stereomicroscope over a 3-month period. The fungi were isolated on potato dextrose agar (PDA; Pronadisa, Madrid, Spain), and the interesting specimens were preserved at room temperature on PDA slant cultures covered with mineral oil.

2.2. DNA Extraction, PCR, Sequencing and Phylogenetic Analyses

Isolates were grown on PDA for 14 days at 25 °C in darkness, and DNA was extracted from mycelium through the modified protocol of Müller et al. [15]. For identification purposes, we performed the PCR using the primer pairs ITS5/ITS4 and NL1/NL4b to amplify the region spanning the internal transcribed spacers (ITS) 1 and 2, including the 5.8S gene, and the D1/D2 domain of the large subunit (LSU) of the nuclear ribosomal (nr)DNA, respectively, following the protocol of Cano et al. [16]. The PCR products were purified and stored at −20 °C until sequencing at Macrogen (Madrid, Spain).

ITS and LSU sequences of the unidentified isolates were compared with those of other fungi deposited at the National Center for Biotechnology Information (NCBI) by the Basic Local Alignment Search Tool (BLAST). To assess the taxonomic position and phylogenetic relationships of our fungi, we carried out single analyses of the ITS and LSU sequences obtained here and those available of *Heliocephala* and *Pseudopenidiella* species and other members of the family *Microthyriaceae*. Since a similar topology of the phylogenetic trees obtained from the previous analyses was observed, a combined analysis of the two markers was performed to obtain a more robust phylogeny of the fungi studied. All the sequences, which were mainly taken from previous studies [7,9,12,13,17–20], were retrieved from GenBank, including those of *Venturia catenospora* and *Venturia inaequalis* used as outgroup (Table 1). The alignments were made in the MEGA (Molecular Evolutionary Genetics Analysis) software v.6.0. [21], using the ClustalW algorithm [22] and refined with MUSCLE [23] or manually if necessary, on the same platform. Phylogenetic reconstructions were made using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches using the MEGA software v. 6.0. [21] and MrBayes v. 3.2.6 [24], respectively.

For the ML analysis of the ITS and LSU regions, the best nucleotide substitution model determined by the same program was the Tamura–Nei model with Gamma distribution and invariant sites (T93+G+I); ML bootstrap values (BML) ≥ 70 % were considered significant. For the BI analysis, the best nucleotide substitution model was determined using jModelTest [25]. For the ITS region, we used the Kimura 2-parameter with Gamma distribution (K80+G) and for the LSU region, we used General Time Reversible with Gamma distribution and invariant sites (GTR+G+I). The parameters used were two simultaneous runs of 5,000,000 generations, four Markov chains, sampled every 1000 generations. The 50% majority-rule consensus tree and posterior probability values (PP) were calculated after discarding the first 25% of the samples. A PP value ≥ 0.95 was considered significant.

Table 1. Species included in this study, their substrate, origin, and GenBank accession numbers.

Species	Strain Number [1]	Substrate	Country	Genbank Accession No. [2]	
				ITS	LSU
Chaetothyriothecium elegans	CPC 21375 T	Leaves of *Castanopsis* sp.	Thailand	-	KF268420
Heliocephala elegans	MUCL 39003	Fallen leaf of *Andira inermis*	Cuba	HQ333478	HQ333478
H. gracilis	CBS 369.86 IT	Fallen leaf of *Matayba oppositifolia*	Cuba	HQ333479	HQ333479
H. natarajanii	MUCL 43745 T	Basideocarp of *Pisolithus tinctorius*	India	HQ333480	HQ333480
H. zimbabweensis	CBS 691.97 T	Unidentified leaf litter	Zimbabwe	HQ333481	HQ333481
H. variabilis sp. nov.	FMR 17592 T	Unidentified dead leaves	Vietnam	**LR536989**	**LR588212**
Microthyrium buxicola	MFLUCC 15-0212 T	Leaves of *Buxus* sp.	Italy	-	KT306551
	MFLUCC 15-0213	Leaves of *Buxus* sp.	Italy	-	KT306552
M. microscopicum	CBS 115976 T	-	The Netherlands	-	GU301846
M. propagulensis	IFRD 9037 T	Fallen leaves of *Castanopsis histrix*	China	-	KU948989
Pseudopenidiella piceae	CBS 131453 T	Needle litter of *Picea abies*	Czech Republic	JX069868	JX069852
P. gallaica	CBS 121796 T	Unidentified dead leaves	Spain	LT984842	LT984843
P. podocarpi	CBS 146067 T	Leaves of *Podocarpus latifolius*	South Africa	MN562140	MN567647
	CPC 37094	Leaves of *P. latifolius*	South Africa	MN562141	MN567648
P. vietnamensis sp. nov.	FMR 17593 T	Unidentified dead leaves	Vietnam	**LR536990**	**LR536991**
Stomiopeltis betulae	CBS 114420	*Betula* sp.	Sweden	GU214701	GU214701
Tumidispora shoreae	MFLUCC 12-0409 T	Dead leaves of *Shorea* sp.	Thailand	-	KT314073
	MFLUCC 14-0574	Dead leaves of *Shorea* sp.	Thailand	-	KT314074
Venturia catenospora	CBS 447.91 T	Leaf spot of *Salix triandra*	Germany	EU035427	MH873940
V. inaequalis	CBS 476.61	Fruit of *Malus sylvestris*	Belgium	EU282478	GU456336

[1] CBS: culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: culture collection of Pedro Crous housed at the CBS; IFRD: International Fungal Research and Development Centre Research Institute of Resource Insects, Kunming; FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Ria, Thailand; MUCL: Mycothèque de L'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; T and IT: ex-type and ex-isotype strain, respectively. [2] Sequences newly generated in this study are indicated in bold.

Sequences of the fungi studied here were deposited in GenBank (Table 1), and the alignment used was deposited in TreeBASE (submission number S25760).

2.3. Phenotypic Study

Microscopic characterization of the isolates was carried out on potato carrot agar (PCA; potato 20 g, carrot 20 g, agar 13 g, distilled water 1 L) and oatmeal agar (OA; Oatmeal 30 g, agar 13 g, distilled water 1 L) after 30 days of incubation to get sporulation. Measurements and descriptions of the structures were taken from the specimens mounted in Shear's solution or lactic acid (100% *v*/*v*). Photomicrographs were obtained using a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity X digital camera.

Macroscopic characterization of the colonies was done on PDA, OA, and PCA after 30 days of incubation at 25 °C in darkness. Cardinal temperatures for growth were obtained on PDA incubated at 5, 15, 20, 25, 30, 35, 37, and 40 °C after 14 days in darkness. The colony colors in descriptions are based on Kornerup and Wanscher [26].

Nomenclatural novelties and descriptions were deposited in MycoBank [27]. Ex-type cultures and holotypes, which were dried cultures, were deposited at the Westerdijk Fungal Biodiversity Institute (CBS, Utrecht, The Netherlands).

3. Results

BLAST analyses with LSU and ITS sequences confirmed the morphological identification of FMR 17592 and FMR 17593 at the genus level but revealed a relatively low percentage of identity with respect to other species, suggesting they were novel species of *Heliocephala* and *Pseudopenidiella*, respectively. The similarity of LSU sequences between FMR 17592 and other *Heliocephala* species (i.e., *Heliocephala gracilis*, *Heliocephala zimbabweensis*, *Heliocephala elegans*, and *H. natarajanii*) ranged from 94.04% to 96.73%. In the case of FMR 17593, similarity was 97.68% to *P. podocarpi*, 96.24% to *P. piceae*, and 95.80% to *Pseudopenidiella gallaica*. ITS sequences showed lower percentages of identity, with a maximum of 95.10% between FMR 17592 and the *Heliocephala* species mentioned above and of ≤89.25% between FMR 17593 and the species of *Pseudopenidiella* analyzed. A combined analysis of the two loci (ITS and LSU) revealed the status of these fungi with respect to the other species of *Heliocephala* and *Pseudopenidiella* and allied genera of the family *Microthyriaceae* (Figure 1). The total alignment included 20 sequences and comprised 1501 bp, from which 604 bp were variable, 864 bp conserved, and 460 bp phylogenetically informative. FMR 17592 and FMR 17593 were included in the full-supported clades representatives of the genera above mentioned and were genetically and morphologically differentiated from their closest phylogenetic relatives.

Key morphological features that distinguish the accepted species of *Heliocephala* and *Pseudopenidiella*, including the new taxa described below, are summarized in Tables 2 and 3.

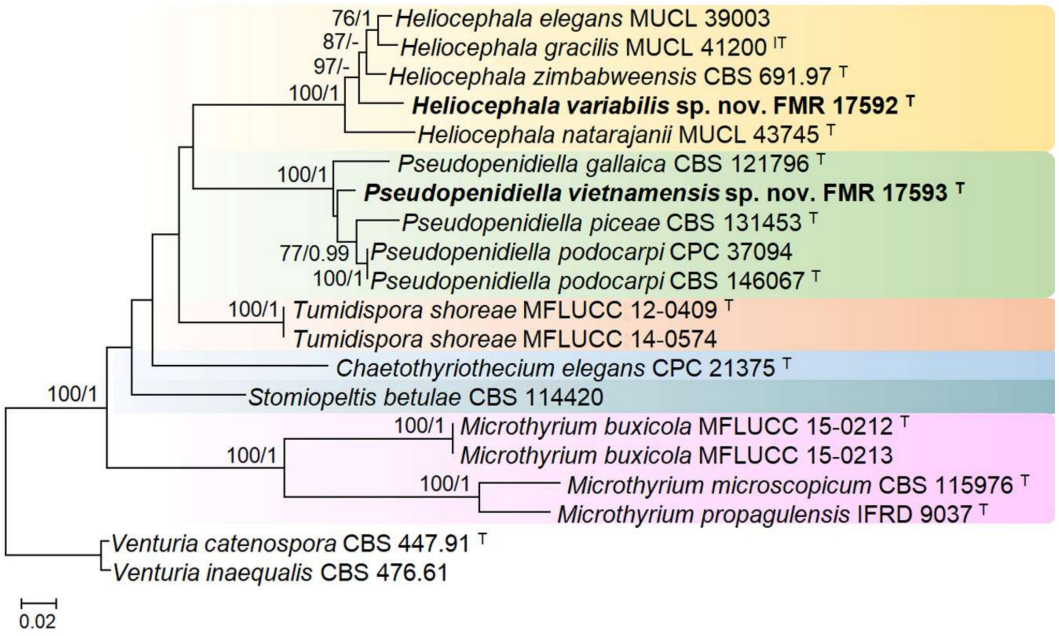

Figure 1. Maximum Likelihood (ML) tree constructed with the internal transcribed spacers (ITS) and large subunit (LSU) sequences of 18 strains representative of the family *Microthyriaceae* (*Microthyriales*). The phylogenetic tree was rooted with *V. catenospora* and *V. inaequalis* (*Venturiaceae*, *Venturiales*). Bootstrap support values for ML greater than 70% and Bayesian posterior probabilities greater than 0.95 are given near nodes. The names of the newly described species are in bold. Branch lengths are proportional to distance; ^{T} Ex-type strain; ^{IT} Ex-isotype strain.

Table 2. Key morphological features distinguishing the accepted *Heliocephala* species.

Species	Conidiophore Size *	Conidia				References
		Size *	Septum No.	Ornamentation	Rostrum	
H. elegans	250–700 × 7–11	8–25 × 3–4	1–3	Smooth	Present, straight	[10]
H. gracilis	80–350 × 7–10	4–12.5 × 2–5	0–1	Smooth	Absent	[10]
H. natarajanii	up to 109 × 1.5–3.5	(8.5–)17–34(–103) × (1.5–) 2.5–4.5(–6.5)	2(–3)	Basal cell verruculose	Present, straight, curved or uncinate	[11]
H. proliferans	up to 210 × 3.5–4	(10–)15–50(–200) × 3–4	2	Basal cell verruculose	Present, straight or curved	[8]
H. triseptata	21–40 × 7–19	15–27 × 3.5–4.5	3	Smooth	Present, straight	[9]
H. variabilis	up to 153 × 4–6	4–26 × 3–6	(0–)1–3(–4)	Smooth to verruculose	Absent	Present study
H. vietnamensis	210–340 × 6–8	14–17 × 2.8–3.8	3	Smooth	Absent	[28]
H. zimbabweensis	180–240 × 3–4	23–125 × 3.5–5.3	2	Smooth	Present, straight	[29]

* in µm.

Table 3. Key morphological features distinguishing the accepted *Pseudopenidiella* species.

Species	Macroconidiophore Size *	Microconidiophore	Ramoconidia Size *	Conidia		References
				Size *	Ornamentation	
P. gallaica	up to 120 × 2–3	Present	7.5–11 × 2–3	6–12 × 1–3	Smooth to verruculose	[7]
P. piceae	up to 150 x 3–4	Present	8–12 × 2–3	(6–)7–9(–10) × (2.5–)3	Finely verruculose	[12]
P. podocarpi	10–110 × 3–4	Absent	(9–)12–13 × (2.5–)3–3.5	(9–)11–12(–15) × 2.5(–3)	Verruculose	[13]
P. vietnamensis	up to 236 × 3–5	Absent	7–13 × 3–4	5–10 × 2–3	Smooth	Present study

* in µm.

Taxonomy

Heliocephala variabilis Iturrieta-González, Gené, Dania García, sp. nov.—MycoBank MB 833179 (Figure 2).

Figure 2. *H. variabilis* (ex-type FMR 17592). (**A**–**C**). Colonies on potato dextrose agar (PDA), potato carrot agar (PCA), and oatmeal agar (OA), respectively, after 30 days at 25 °C. (**D**–**J**). Conidiophores and conidia. Scale bars: (**D**,**E**) = 20 µm; (**F**–**J**) = 10 µm.

Etymology: Name refers to the variation in the conidial morphology.

Mycelium consisting of branched, septate, subhyaline to pale brown, smooth to verrucose hyphae 1–2 µm wide. Conidiophores macronematous, rarely semi-macronematous, mononematous, erect, subcylindrical, with up to seven septa, brown, pale brown towards the apex, smooth-walled, up to 153 µm long (up to 148 µm long on the natural substratum), 4–6 µm wide, commonly 2–3 closely packed primary branched, from which 1–2 secondary metula-like branches are commonly present. Conidiogenous cells terminal, monoblastic, discrete, ampuliform, smooth-walled, pale brown, 3–13 × 2–3 µm. Conidia solitary, broadly ellipsoidal, subcylindrical or obclavate, smooth-walled to slightly verruculose, pale brown, (0–)1–3-septate (up to 4-septate on the natural substratum): 0–1-septate, 4–11 × 3–6 µm; 2–3-septate 15–26 × 3–5 µm; 4-septate 24–26 × 3.8–4.3 µm. Sexual morph not observed.

Culture characteristics after 30 days at 25 °C—Colonies on PDA reaching 17–18 mm of diameter, olive color (3F7) with some areas olive-brown (4E4), velvety, convex, aerial mycelium scarce, margin slightly irregular; reverse dark brown (7F8) to black. On PCA, reaching 18 mm of diameter, yellowish

brown (5E4), black at the periphery, velvety, flat, aerial mycelium scarce, margin slightly irregular; reverse dark brown (7F8) to black. On OA, reaching 23 mm of diameter, yellowish brown (5E4), black at the periphery, umbonate, slightly floccose at the center, velvety towards the periphery, margin entire and slightly fimbriate; reverse dark brown (7F8) to black.

Cardinal temperatures for growth: Minimum 15 °C, optimum 25 °C, maximum 30 °C.

Specimen examined: Vietnam, north-east region, on unidentified dead leaf, Aug. 2011, Josep Guarro (holotype CBS H-24291, culture ex-type CBS 146334 = FMR 17592).

Diagnosis: *H. variabilis* differs from four closely related species, i.e., *H. elegans*, *H. gracilis*, *H. zimbabweensis*, and *H. natarajanii*, by the size of its macronematous conidiophores and the septation of conidia (Table 2).

Pseudopenidiella vietnamensis Iturrieta-González, Dania García, Gené, sp. nov.—MycoBank MB 833180 (Figure 3).

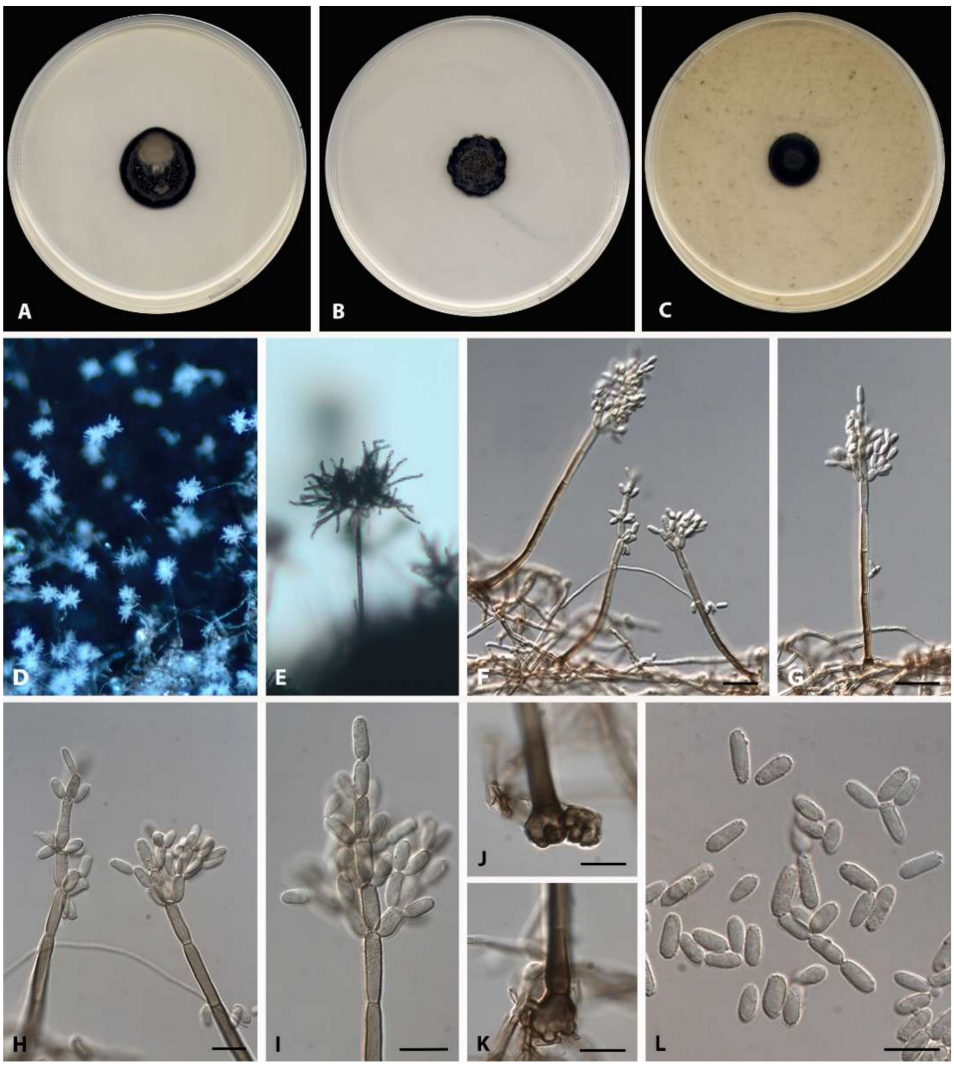

Figure 3. *P. vietnamensis* (ex-type FMR 17593). (**A**–**C**) Colonies on PDA, PCA, and OA, respectively, after 30 days at 25 °C. (**D**–**I**,**L**) Conidiophores and conidia. (**D**,**E**) Conidiophores under stereomicroscope. (**J**,**K**) Detail of conidiophore basal cells. Scale bars: (**F**,**G**) = 20 μm; (**H**–**L**) = 10 μm.

Etymology: Name refers to Vietnam, the country where the fungus was collected.

Mycelium consisting of branched, septate, pale brown, smooth-walled hyphae 1–3 μm wide. Conidiophores macronematous, mononematous, unbranched, erect to slightly flexuous, subcylindrical, with up to 10-septate, pale brown to brown, smooth-walled, verruculose towards the apex, swollen

and often lobate basal cell, up to 236 µm long, 3–5 µm wide; microconidiophores not observed. Conidiogenous cells terminal, polyblastic, with up to three inconspicuous conidiogenous loci, verruculose, pale brown, 12–18 × 2–4 µm. Ramoconidia cylindrical, aseptate, pale brown, verruculose, 7–13 × 3–4 µm, forming conidia in acropetal branched chains. Conidia cylindrical, aseptate, pale brown, smooth-walled to verruculose, 5–10 × 2–3 µm. Sexual morph not observed.

Culture characteristics after 30 days at 25 °C: Colonies on PDA reaching 23 mm of diameter, grey (7F1) with some regions greyish brown (5D3), black at the edge, velvety, slightly convex, aerial mycelium scarce, margin entire; reverse dark brown (7F8) to black. On PCA, reaching 17 mm of diameter, brownish grey (5F2), velvety, slightly convex, aerial mycelium scarce, margin undulate; reverse dark brown (7F8) to black. On OA, reaching 15 mm of diameter, brownish grey (5F2), black at the edge, finely granular, flat, aerial mycelium scarce, margin entire; reverse dark brown (7F6) to black.

Cardinal temperatures for growth: Minimum 15 °C, optimum 25 °C, maximum 30 °C.

Specimen examined: Vietnam, north-east region, on unidentified dead leaf, Aug. 2011, Josep Guarro (holotype CBS H-24292, culture ex-type CBS 146219 = FMR 17593).

Diagnosis: *P. vietnamensis* differs from *P. piceae* and *P. gallaica* in the lack of microconidiophores, and from *P. podocarpi* in the size of their macronematous conidiophores (Table 3).

4. Discussion

Sequence analysis of the ITS and LSU barcodes were enough to resolve the taxonomy of the fungi under study and attribute the species to the monophyletic genera *Heliocephala* and *Pseudopenidiella*. However, phylogenetic relationships to other genera in the family *Microthyriaceae* remained obscure with the present taxon sampling, due to the lack of statistical support in the main clades obtained in the analysis (Figure 1). Despite DNA data not being available for all species of the mentioned genera, the novel species, *H. variabilis* and *P. vietnamensis,* showed morphological traits that clearly allowed their distinction from the other species of the respective genera (Tables 2 and 3).

Heliocephala variabilis was phylogenetically close to *H. elegans, H. gracilis*, and *H. zimbabweensis.* The first two species, which were previously included in the genus *Holubovaniella* [10], could be differentiated from *H. variabilis* by having much more robust conidiophores (up to 700 × 11 µm in *H. elegans*; up to 350 × 10 µm in *H. gracilis*) that usually proliferate, showing several clusters of short branches and intercalary conidiogenous cells. In addition, on the natural substratum, our species showed conidia with up to four septa, while those of *H. elegans* and *H. gracilis* are 1–3- and 0–1-septate, respectively [10]. *Heliocephala zimbabweensis* resembles *H. proliferans*, and both differ from *H. variabilis* by having longer conidiophores (up to 210 µm in *H. proliferans*; up to 240 µm in *H. zimbabweensis*) and two-septate conidia with a very long and filiform rostrum, subsequently showing much longer conidia (10–200 µm in *H. proliferans*; 23–125 µm in *H. zimbabweensis*) than the species proposed here. Another feature exclusive to *H. proliferans* and *H. zimbabweensis* is the presence of a secondary cluster of conidiogenous cells at the apex of the conidial rostrum [8,29]. The great morphological similarity of these two species suggest they could be conspecific, but the lack of DNA data from the type is a handicap to elucidating the taxonomy of these fungi. Other two *Heliocephala* species with no molecular data are *Heliocephala triseptata* and *Heliocephala vietnamensis* [9,28], but the protologue of both taxa only describes conidia with three septa, and the conidiophores of the former are the smallest ones in the genus (21–40 µm long), while those of *H. vietnamensis* are longer (up to 340 µm) than those observed in *H. variabilis* (up to 153 µm long).

Although the three species of *Pseudopenidiella* are phylogenetically well differentiated, morphologically they are very similar, and even their conidiogenous apparatus resembles that of other cladosporium-like fungi that belong to the order *Capnodiales,* such as *Penidiella* or *Apenidiella* and other related genera [30,31]. *Pseudopenidiella* species can be only distinguished by subtle differences in their macroconidiophores and by the presence or absence of microconidiophores, as summarized in Table 3. It is worth mentioning that, based exclusively on morphological data, a fourth species named *Pseudopenidiella pini* was introduced in the genus by Kirk [32]. This was based on *Polyscytalum*

pini, which was described from several specimens collected on decaying needles of *Pinus sylvestry*, mainly in the United Kingdom. However, none of these specimens is currently available for molecular comparison. Some of the features described and illustrated in its protologue, such as the presence of denticulate conidiogenous cells and one-septate (ramo-) conidia [33], do not fit with the generic concept of *Pseudopenidiella* [12]; therefore, we prefer to exclude *P. pini* from the genus until further studies with additional new collections of the species can confirm its position.

Author Contributions: I.I.-G. conceived the ideas, organized and analyzed the data, and joined in the writing; D.G. and J.G. (Josepa Gené) conceived the ideas, analyzed the data, and led the writing; J.G. (Josep Guarro) collected the samples and led the writing. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Spanish Ministerio de Economía y Competitividad, grant CGL2017-88094-P.

Conflicts of Interest: The authors declare no conflict of interest.

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Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

4.3.3 *Neodendryphiella*, a novel genus of the *Dictyosporiaceae* (*Pleosporales*)

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Published in: MycoKeys 2018; 37: 19–38

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MycoKeys 37: 19–38 (2018)
doi: 10.3897/mycokeys.37.27275
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Neodendryphiella, a novel genus of the Dictyosporiaceae (Pleosporales)

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Academic editor: *M. Haase* | Received 12 April 2018 | Accepted 13 July 2018 | Published 26 July 2018

Citation: Iturrieta-González I, Gené J, Guarro J, Castañeda-Ruiz RF, García D (2018) *Neodendryphiella*, a novel genus of the Dictyosporiaceae (Pleosporales). MycoKeys 37: 19–38. https://doi.org/10.3897/mycokeys.37.27275

Abstract

In a survey of soil and herbivore dung microfungi in Mexico and Spain, several dendryphiella-like species were found. Phylogenetic analyses based on ITS and LSU sequences showed that these fungi belonged to the family Dictyosporiaceae (Pleosporales) and represent an undescribed monophyletic lineage distant from *Dendryphiella*. Therefore, the genus *Neodendryphiella* is proposed to accommodate three new species, *N. mali*, *N. michoacanensis* and *N. tarraconensis*. The novel genus shares morphological features with *Dendryphiella* such as differentiated conidiophores and polytretic integrated conidiogenous cells, that produce acropetal branched chains of conidia. *Neodendryphiella* differs in the absence of nodulose conidiophores bearing conidiogenous cells with pores surrounded by a thickened and darkened wall, typical features in the conidiogenous apparatus of *Dendryphiella*. In addition, the phylogenetic and morphological analysis of several reference strains of different *Dendryphiella* species, available for comparison, support the proposal of *D. variabilis* **sp. nov.**, which mainly differs from the other species of the genus by having conidia up to 7 septa and highlight that *D. vinosa* and *D. infuscans* are obscure species that require further taxonomic review.

Keywords

Dendryphiella, Ascomycota, Phylogeny, Taxonomy

Introduction

In an ongoing survey of asexual microfungi from soil and herbivore dung, several interesting specimens morphologically consistent with *Dendryphiella* were found from samples collected in Mexico and Spain. *Dendryphiella* is a dematiaceous hyphomycete proposed by Bubák and Ranojevič (Ranojevič 1914) and typified with *D. interseminata*, which is currently considered a synonym of *D. vinosa* (Reisinger 1968). *Dendryphiella vinosa* is a saprobic fungus commonly found on plant debris, especially on the decaying herbaceous stems of several plants (Ellis 1971, Mercado Sierra et al. 1997). The genus is characterised by pigmented conidiophores, with terminal or intercalary polytretic conidiogenous cells, with dark scarring on the nodose swellings, producing acropleurogenous, solitary or catenate conidia, which are commonly multi-septate and cylindrical with rounded ends (Ellis 1971). Although Index Fungorum and MycoBank list 17 taxa in *Dendryphiella*, a recent review of literature reported only 12 species are accepted, including the newly proposed *D. fasciculata* (Liu et al. 2017). *Dendryphiella pitsanulokensis* is the latter species added to the genus (Hyde et al. 2018). Previous phylogenetic studies, conducted mainly from sequence data of the 18S nrDNA (SSU), 28S nrDNA (LSU) and the internal transcribed spacer (ITS) nrDNA regions, showed that the marine species *D. arenariae* and *D. salina* were phylogenetically distant from the type *D. vinosa* and related to the Pleosporaceae (Gareth Jones et al. 2008, Suetrong et al. 2009). Both species were therefore moved to the genus *Paradendryphiella* (Woudenberg et al. 2013) and, more recently, *D. vinosa* was included in the family Dictyosporiaceae (Tanaka et al. 2015, Boonmee et al. 2016). However, DNA sequence data for *Dendryphiella* species is very limited to create a robust taxonomy for the genus. Only LSU and/or ITS sequences of *D. eucalyptorum*, *D. fasciculata*, *D. paravinosa*, *D. pitsanulokensis* and *D. vinosa* are available (Crous et al. 2014, 2016, Liu et al. 2017, Hyde et al. 2018). In addition, with the exception of the first four mentioned, there is no ex-type culture of other species of this genus and only reference strains of *D. vinosa* and *D. infuscans* are available in public collections for comparison.

Despite the similarity of our soil isolates to *Dendryphiella*, a preliminary study revealed that they showed a low sequence relationship with members of this genus. On the other hand, they were closely related to the strain CBS 139.95 of *Diplococcium (Di.) asperum*, which was proven to be related to the Dictyosporiaceae (Shenoy et al. 2010, Boonmee et al. 2016). It is well known that the genus *Diplococcium* is highly polyphyletic, with species distributed across different classes of the Ascomycota, with its type species, *Di. spicatum,* being related to the Helotiales in Leotiomycetes (Shenoy et al. 2010, Hernández-Restrepo et al. 2017).

The aim of the present study was to resolve the taxonomy of these dendryphiella-like fungi which, based on analysis of the ITS and LSU loci, might represent a new genus in Dictyosporiaceae.

Material and methods

Sampling and fungal strains studied

Soil and dung samples collected in different geographical regions (Mexico and Spain) were studied using the wood baiting technique, moist chambers and dilution plating method according to Calduch et al. (2004). Using the first two techniques, we found three interesting dendryphiella-like fungi, which were isolated on Potato Dextrose Agar (PDA; Pronadisa, Madrid Spain) and incubated at room temperature in the dark. Additionally, six strains from the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (CBS), which corresponded to *D. vinosa* (CBS 117.14, CBS 118716, CBS 121797 and CBS 584.96), *D. infuscans* (CBS 381.81) and *Di. asperum* (CBS 139.95) were included in the study for morphological and sequence comparison (Table 1).

DNA extraction, sequencing and phylogenetic analysis

The isolates were cultured on PDA for 7 days at 25 °C in darkness. The DNA was extracted through the modified protocol of Werner et al. (1998). The primer pairs ITS5/ITS4 and NL1/NL4b were used to amplify ITS regions, including the 5.8S gene and the D1/D2 domain of the LSU of the nrDNA, respectively, following Cano et al. (2004). PCR products were purified and stored at -20 °C until sequencing. The same pairs of primers were used to obtain the sequences at Macrogen Europe (Macrogen Inc. Amsterdam, The Netherlands). Finally, the sequences were assembled and edited using SeqMan v. 7.0.0 (DNAStar Lasergene, Madison, WI, USA) to obtain the consensus sequences.

The sequences generated in the present study were compared with those of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). Alignments for each locus were made with the MEGA (Molecular Evolutionary Genetics Analysis) software v. 6.0. (Tamura et al. 2013), using the ClustalW algorithm (Thompson et al. 1994) and refined with MUSCLE (Edgar 2004) or manually, if necessary, on the same platform. The alignment included our sequences complemented with available sequences of NCBI and NITE Biological Resource Center (NBRC) of species that conformed the different genera of the family Dictyosporiaceae (Table 1). This determined the phylogenetic position of the dendryphiella-like isolates in this group of fungi. Phylogenetic reconstructions with ITS and LSU sequences were made using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches under the MEGA software v. 6.0. (Tamura et al. 2013) and MrBayes v. 3.2.6 (Ronquist et al. 2012), respectively.

For the ML phylogenetic analysis of the LSU region, the best nucleotide substitution model determined by the same programme was the Kimura 2-parameter

Table 1. Species included in this study, their origin and GenBank accession numbers.

Species	Original identification	Strain number[1]	Country	Genbank accession no.[2]	
				ITS	LSU
Aquaticheirospora lignicola		RK-2006a (T)	Thailand	AY864770	AY736378
Cheirosporium triseriale		HMAS 180703 (T)	China	EU413953	EU413954
Drechslera biseptata	***Dendryphiella vinosa***	**CBS 117.14**	**Scotland**	**LT963770**	**LT963509**
Dendryphiella eucalyptorum		CBS 137987 (T)	Spain	KJ869139	KJ869196
Dendryphiella fasciculata		MFLUCC 17-1074 (T)	Thailand	MF399213	MF399214
Dendryphiella paravinosa	***Dendryphiella vinosa***	**CBS 118716**	**New Zealand**	**LT963357**	**LT963359**
Dendryphiella paravinosa	***Dendryphiella vinosa***	**CBS 121797**	**Spain**	**LT963354**	**LT963355**
Dendryphiella paravinosa		CBS 141286 (T)	Italy	KX228257	KX228309
Dendryphiella variabilis	***Dendryphiella vinosa***	**CBS 584.96 (T)**	**Cuba**	**LT963453**	**LT963454**
Dendryphiella vinosa		NBRC 32669	Japan	DQ307316	03266901[3]
Dendryphiella vinosa		–	–	–	EU848590
Dictyocheirospora bannica		KH 332 (T)	Japan	LC014543	AB807513
Dictyocheirospora pseudomusae		KH 412	Japan	LC014549	AB807516
Dictyocheirospora rotunda		MFLUCC 14-0293b (T)	Thailand	KU179099	KU179100
Dictyosporium bulbosum		yone 221	Japan	LC014544	AB807511
Dictyosporium elegans		NBRC 32502 (T)	Japan	DQ018087	DQ018100
Dictyosporium strelitziae		CBS 123359 (T)	South Africa	FJ839618	FJ839653
Digitodesmium bambusicola		CBS 110279 (T)	Philippines	DQ018091	DQ018103
Gregarithecium curvisporum		KT 922 (T)	Japan	AB809644	AB807547
Jalapriya inflata		NTOU 3855	UK	JQ267362	JQ267363
Jalapriya pulchra		MFLUCC 15-0348 (T)	China	KU179108	KU179109
Jalapriya toruloides		CBS 209.65	–	DQ018093	DQ018104
Neodendryphiella mali	***Diplococcium asperum***	**CBS 139.95 (T)**	**Italy**	**LT906655**	**LT906657**
Neodendryphiella mali	***Dendryphiella* sp.**	**FMR 17003**	**Spain**	**LT993734**	**LT993735**
Neodendryphiella michoacanensis	***Dendryphiella* sp.**	**FMR 16098 (T)**	**Mexico**	**LT906660**	**LT906658**
Neodendryphiella tarraconensis	***Dendryphiella* sp.**	**FMR 16234 (T)**	**Spain**	**LT906659**	**LT906656**
Paradendryphiella arenaria		CBS 181.58 (T)	France	KF156010	KC793338
Paradendryphiella salina		CBS 142.60	United Kingdom	DQ411540	KC793339
Pseudocoleophoma calamagrostidis		KT 3284 (T)	Japan	LC014592	LC014609
Pseudocoleophoma polygonicola		KT 731 (T)	Japan	AB809634	AB807546
Pseudodictyosporium elegans		CBS 688.93 (T)	Taiwan	DQ018099	DQ018106
Pseudodictyosporium wauense		NBRC 30078	Japan	DQ018098	DQ018105
Torula herbarum	***Dendryphiella infuscans***	**CBS 381.81**	**Netherlands**	**LT963446**	**LT963455**

[1]CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; HMAS: The Mycological Herbarium of the Chinese Academy of Science; KH: K. Hirayama; KT: K. Tanaka; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NBRC: NITE Biological Resource Centre, Japan; NTOU: Institute of Marine Biology, National Taiwan Ocean University; RK: R. Kodsueb; yone: H. Yonezawa. (T): ex-type strain.

[2]Sequences newly generated in this study are indicated in bold.

[3]Number of sequence of the NBRC database.

with Gamma distribution and, for the ITS region, it was the General Time Reversible model with Gamma distribution. The combined analysis of these two phylogenetic markers was tested through Incongruence Length Difference (ILD) implemented in the Winclada programme (Farris et al. 1994). For the combined analysis of LSU and ITS sequences, the best nucleotide substitution model was the General Time Reversible with Gamma distribution and Invariant sites (G+I). ML bootstrap values (BML) ≥70% were considered significant.

For the BI phylogenetic analysis, the best nucleotide substitution model was determined using jModelTest (Posada 2008). For the LSU region, we used the Kimura 2-parameter with Gamma distribution (K80+G) and, for the ITS symmetrical model, we used Gamma distribution (SYM+G). The parameter settings used were two simultaneous runs of 5M generations, four Markov chains, sampled every 1000 generations. The 50% majority-rule consensus tree and posterior probability values (PP) were calculated after discarding the first 25% of the samples. A PP value of ≥0.95 was considered significant.

The DNA sequences and alignments generated in this study were deposited in GenBank (Table 1) and in TreeBASE (http://treebase.org), respectively.

Phenotypic study

The microscopic characterisation of the fungi studied was carried out according to Marin-Felix et al. (2017), using autoclaved pine twig arranged on the surface of water agar (PNA) after 7 days at 25 °C in darkness. Measurements and descriptions of the structures were taken from the specimens mounted in Shear's solution. Photomicrographs were obtained using a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity × digital camera.

Macroscopic characterisation of the colonies was made on PDA, Oatmeal Agar (OA; Oatmeal 30 g, agar 13 g, distilled water 1 l), Potato Carrot Agar (PCA; potato 20 g, carrot 20 g, agar 13 g, distilled water 1 l), SNA (KH_2PO_4 1 g, KNO 1 g, $MgSO_4 \times 7H_2O$ 0.5 g, KCl 0.5 g, Glucose 0.2 g, Sucrose 0.2 g, agar 14 g, distilled water 1 l) and Malt Extract Agar (MEA; Peptone 1 g, Glucose 20 g, Malt Extract 20 g, agar 15 g, distilled water 1 l) after 14 days at 25 °C in darkness. Colony colours in descriptions were matched with Kornerup and Wanscher (1978). Cardinal temperatures for growth were obtained on PDA after 14 days in darkness.

Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004). Ex-type cultures and holotypes, which consisted of dried cultures, were deposited at the CBS. Additionally, living cultures of the new species were also preserved in the Faculty of Medicine in Reus (FMR, Spain).

Results

The BLAST query revealed that LSU sequences of our dendryphiella-like isolates (FMR 16098, FMR 16234 and FMR 17003) showed a high percentage of identity (99%) with that of the isolate CBS 139.95 of *Di. asperum* and all of them were related to the Dictyosporiaceae. However, they showed a sequence identity of between 96-97% with LSU sequences of *Dictyosporium* species and other members of this family, including several species of *Dendryphiella* deposited in the GenBank. The ITS sequences did not match significantly any of those deposited in the NCBI database.

We carried out individual and combined analyses with the LSU and ITS loci to assess relationships with members of the Dictyosporiaceae, including reference strains of *D. vinosa* and *D. infuscans* sequenced in the present study. Single phylogenies of LSU and ITS loci encompassed 31 and 30 sequences, respectively, representing 12 genera and including *Paradendryphiella arenaria* and *P. salina* (Pleosporaceae) as outgroup (Figs. S1 and S2 in the supplementary material). LSU analysis comprised 630 bp from which 111 bp were variable and 84 bp phylogenetically informative. The ITS comprised 496 bp, 266 bp being variable and 206 bp being phylogenetically informative. The topology of trees for single loci were very similar and the ILD test showed that the LSU and ITS datasets loci were congruent (P = 0.16) and could be combined. The final combined analysis encompassed 30 sequences and comprised 1126 bp (ITS 496 bp, LSU 630 bp). The ML tree showed that FMR 16098, FMR 16234, FMR 17003 and CBS 139.95 clustered together in a well-supported undescribed monophyletic lineage representing a new genus in the family (Fig. 1). The LSU and ITS sequence comparison of the four isolates revealed them as different taxa. The low identity values together with the morphological differences found amongst them allow us to propose three new species in this new genus, which are described below.

Regarding the five *Dendryphiella* strains included in this study, only three (CBS 118716, CBS 121797 and CBS 854.96) nested in the well-supported clade of *Dendryphiella* and none of them matched sequences representative of the type species of the genus *D. vinosa* (DQ 307316.1, EU848590.1 and NBRC-03266901) and used previously by other authors to establish the relationship of *D. vinosa* with the Dictyosporiaceae (Gareth Jones et al. 2008, Crous et al. 2014, 2016, Tanaka et al. 2015, Boonmee et al. 2016, Liu et al. 2017). The strains CBS 118716 and CBS 121797 matched the ex-type strain of *D. paravinosa* (CBS 141286); while CBS 584.96 nested in a terminal subclade with *D. fasciculata* and *D. paravinosa*, but it was placed in a single branch representative of a distinct taxa (Fig. 1). Its genetic difference and the production of conidia with up to 7 septa, a distinct morphological feature with respect to the accepted species of *Dendryphiella* (Liu et al. 2017, Hyde et al. 2018), justify the proposal of a new species in this genus. The other two isolates that had been received as *Dendryphiella* did not belong to this genus. The oldest reference strain of *D. vinosa* (CBS 117.14) corresponded to *Drechslera biseptata* and the strain previously identified as *D. infuscans* (CBS 381.81) matched *Torula herbarum*. The molecular identification of all the isolates included in this study is provided in Table 1.

Taxonomy

Neodendryphiella **Iturrieta-González, Dania García & Gené, gen. nov.**
MycoBank: MB824664

Etymology. The name refers to the morphological similarity with *Dendryphiella*.

Type species. *Neodendryphiella tarraconensis* Iturrieta-González, Gené & Dania García.

Description. *Conidiophores* semi-macronematous to macronematous, mononematous, erect or slightly flexuous, unbranched or branched towards the apical region, septate, subhyaline to brown, smooth to verrucose, cylindrical, some slightly swollen in the conidiogenous loci. *Conidiogenous cells* integrated, terminal or intercalary, polytretic, cylindrical or clavate, forming conidia in acropetal branched chains. *Ramoconidia* aseptate or septate, pale brown, smooth to verruculose, mostly cylindrical or subcylindrical, rounded apex and truncate base, with several pores and conidial scars often thickened and darkened. *Conidia* blastocatenate, aseptate or septate, pale brown, verruculose to verrucose, ellipsoidal, doliiform, clavate or subcylindrical, with scars thickened and darkened. *Sexual morph* not observed.

Distribution. Italy, Mexico and Spain.

Neodendryphiella mali **Iturrieta-González, Gené & Dania García, sp. nov.**
MycoBank: MB824665
Fig. 2

Etymology. Name refers to the substrate, *Malus domestica,* where the type strain of the species was collected.

Type. Italy, Dipt. Prot. Valor. Agroalimentare, from leaf of *Malus domestica*, Feb. 1995, A. Cesari (holotype CBS H-23477, culture ex-type CBS 139.95).

Description. *Mycelium* superficial and immersed, composed of septate, branched, smooth to verruculose, hyaline to pale brown hyphae of 1–3 µm wide. *Conidiophores* semi-macronematous to macronematous, mononematous, erect or slightly flexuous, branched or unbranched, up to 11-septate, cylindrical, up to 385 µm long, 3–4 µm wide, brown, usually darker toward the base, smooth to verrucose. *Conidiogenous cells* terminal and intercalary, mostly cylindrical, 8–38 × 3–4(–5) µm, with 1–4 pores. *Ramoconidia* 0–1-septate, with up to 3 terminal and lateral pores, pale brown, smooth to verruculose, mostly cylindrical, (11–)15–17(–21) × 3–4 µm. *Conidia* catenate, with up to 10 conidia in the terminal unbranched part, (0–)1-septate, usually not constricted at the septum, pale brown, verruculose to verrucose, ellipsoidal, doliiform or subcylindrical with more or less rounded ends, 4–15 × 3–5 µm.

Culture characteristics (14 d at 25 °C). Colonies on PDA reaching 22 mm diam., convex, slightly convoluted at the centre, pastel grey to white (1C1/1A1), aerial mycelium scarce, with slightly fimbriate margin; reverse olive brown to yellowish-brown

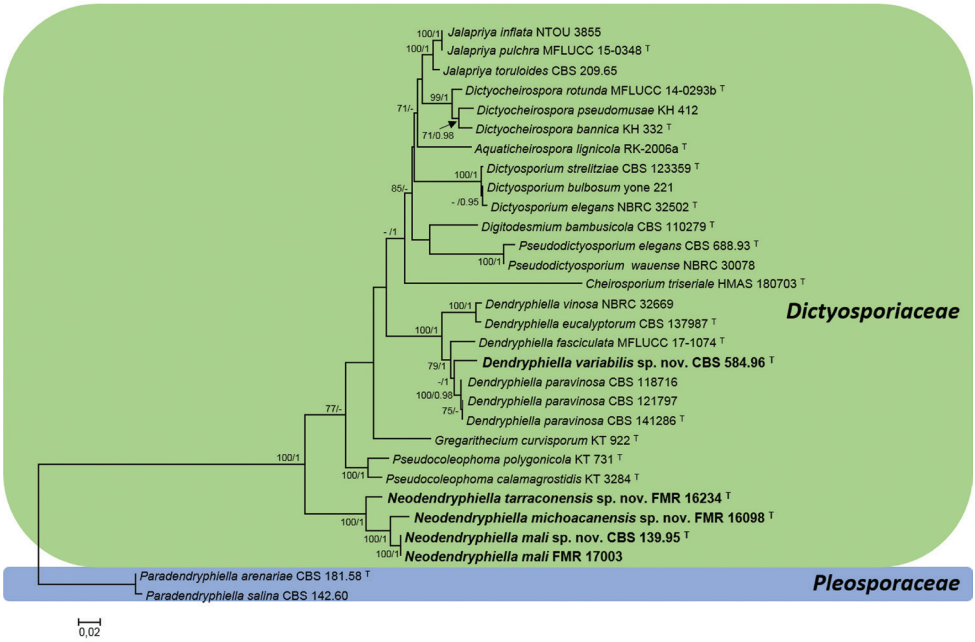

Figure 1. Maximum Likelihood (ML) tree constructed with the ITS and LSU sequences of 30 strains representatives of different taxa in the families Dictyosporiaceae and Pleosporaceae. The phylogenetic tree was rooted with *Paradendryphiella arenaria* and *P. salina*. Bootstrap support values for ML greater than 70% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. Names of species newly described here are indicated in bold. Branch lengths are proportional to distance. [T] Ex-type strain.

(4D3/3A2). On PCA attaining 23 mm diam., flat, olive brown to greyish-beige (4F8/4C2), aerial mycelium scarce, slightly fimbriate margin; reverse greyish-beige to brownish-grey (4C2/4D2). On OA reaching 40 mm diam., flat, granular, yellowish-brown to reddish-yellow (5E8/4B7), aerial mycelium scarce, with a regular margin; reverse olive brown to yellowish-brown (4D8/4B7). On SNA attaining 24 mm diam., flat, slightly granular, olive brown to grey (4F8/4B1), aerial mycelium scarce, with fimbriate margin; reverse yellowish-brown (5F7/5E4). On MEA reaching 11–15 mm diam., umbonate, slightly cerebriform towards the periphery, velvety, olive grey (3E2), with irregular margin; reverse olive grey (3E2).

Cardinal temperature for growth. Optimum 25 °C, maximum 30 °C, minimum 10 °C.

Distribution. Italy and Spain.

Additional isolates examined. Spain, Els Ports de Beseit Natural Park, Teruel, from herbivore dung, Oct. 2017, Dania García (FMR 17003)

Notes. Although LSU sequences of *N. mali* (CBS 139.95 and FMR 17003) were very similar to those of *N. michoacanensis* (FMR 16098) and *N. tarraconensis* (FMR 16234), ITS regions showed a similarity of 96.2% (identities = 441/458, gaps 2/458 (0 %)) with respect to *N. michoacanensis* and of 92.3% (identities = 423/458, gaps 1/458

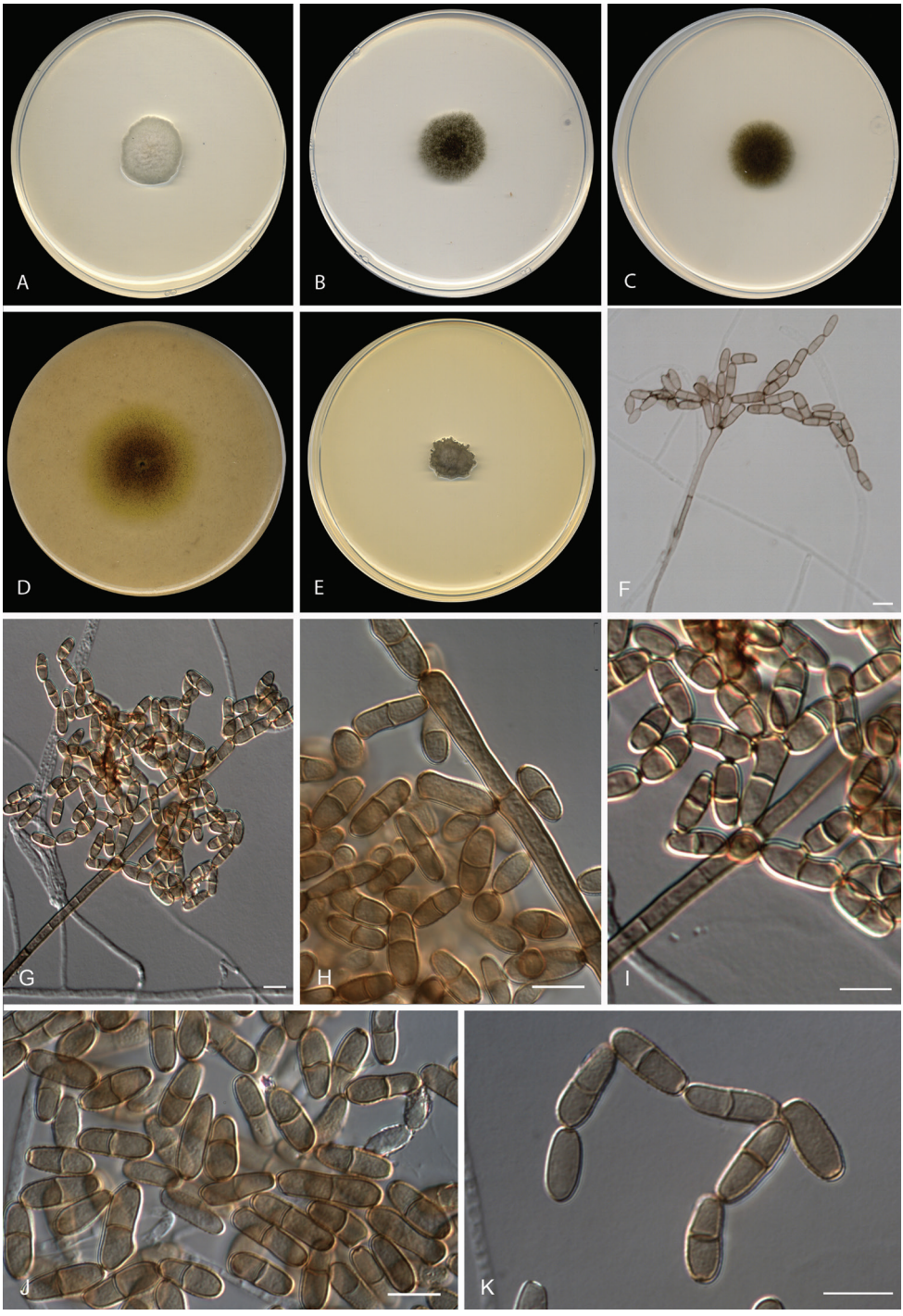

Figure 2. *Neodendryphiella mali* sp. nov. (ex-type CBS 139.95). **A–E** Colonies on **A** PDA **B** PCA **C** SNA **D** OA **E** MEA at 25 °C after 14 d **F–K** Conidiophores and conidia. Scale bars: 10 µm (**F–K**).

(0%)) with respect to *N. tarraconensis*. ITS sequences of the two latter species described below were 92.1% similar (identities = 422/458, gaps 0/458 (0%)).

Neodendryphilla mali is morphologically very similar to *N. michoacanensis* since both have conidia and ramoconidia 0–1-septate; however, *N. michoacanensis* has shorter conidiophores (up to 280 µm long) and terminal conidial branches with fewer conidia (up to 4 per branch), which measure 5–16(–18) × 3–6 µm. In addition, 2-septate conidia can also be present in *N. michoacanensis* and this species tends to grow faster than *N. mali* on PDA (34 mm vs 22 mm diam. after 14 d, respectively) and PCA (42 mm vs 23 mm diam. after 14 d, respectively). *Neodendryphiella mali* also resembles *D. infuscans,* but the latter exhibits longer conidiophores, up to 500 µm and smooth to minutely verruculose conidia with up to 2 septa (Ellis 1971). However, the protologue of *D. infuscans* (as *Cladosporium infuscans*; Thümen 1879), which was based on a specimen collected in Aiken (USA), describes conidia 0–1-septate, smooth-walled and up to 10 µm long. No living culture of the type specimen was preserved for further comparison.

As mentioned before, the strain CBS 139.95 was identified as *Di. asperum* and found by other authors to be related with dictyosporium-like fungi (Shenoy et al. 2010, Tanaka et al. 2015). However, the protologue of *Di. asperum* was characterised by single or fasciculate conidiophores, which were up to 250 µm long, bearing terminal or subterminal, short and unbranched chains of conidia with only 1 septum (Pirozynski 1972), morphological features that do not fit with those observed in the abovementioned strain. We therefore concluded that it was a misidentified strain and clearly represents a different species. At any rate, it is of note that the taxonomy of *Di. asperum* is controversial because of the different interpretation of the morphological features of Pirozynski's specimen (DAOM 133941c isotype). Holubová-Jechová (1982) described conidiogenous cells showing inconspicuous denticles or conidiogenous scars instead of the typical pores in conidiogenous cells of *Diplococcium* and suggested excluding this species from the genus. On the other hand, Goh and Hyde (1998) re-examined the isotype of *Di. asperum* and observed the typical pores of tretic conidiogenesis, considering it an acceptable species for *Diplococcium*. However, since only herbarium material is preserved for comparison (Pirozynski 1972), its phylogeny remains uncertain.

Neodendryphiella michoacanensis **Iturrieta-González, Dania García & Gené, sp. nov.**
MycoBank: MB824666
Fig. 3

Etymology. Name refers to Michoacán, the geographical area where the fungus was collected.

Type. Mexico, Michoacán, Villa Jiménez, from soil, Sept. 2016, E. Rodriguez-Andrade (holotype CBS H-23478; culture ex-type CBS 144323 = FMR 16098).

Description. *Mycelium* superficial and immersed, composed of septate, branched, smooth to verruculose and hyaline to pale brown hyphae of 1–3 µm wide. *Conidi-*

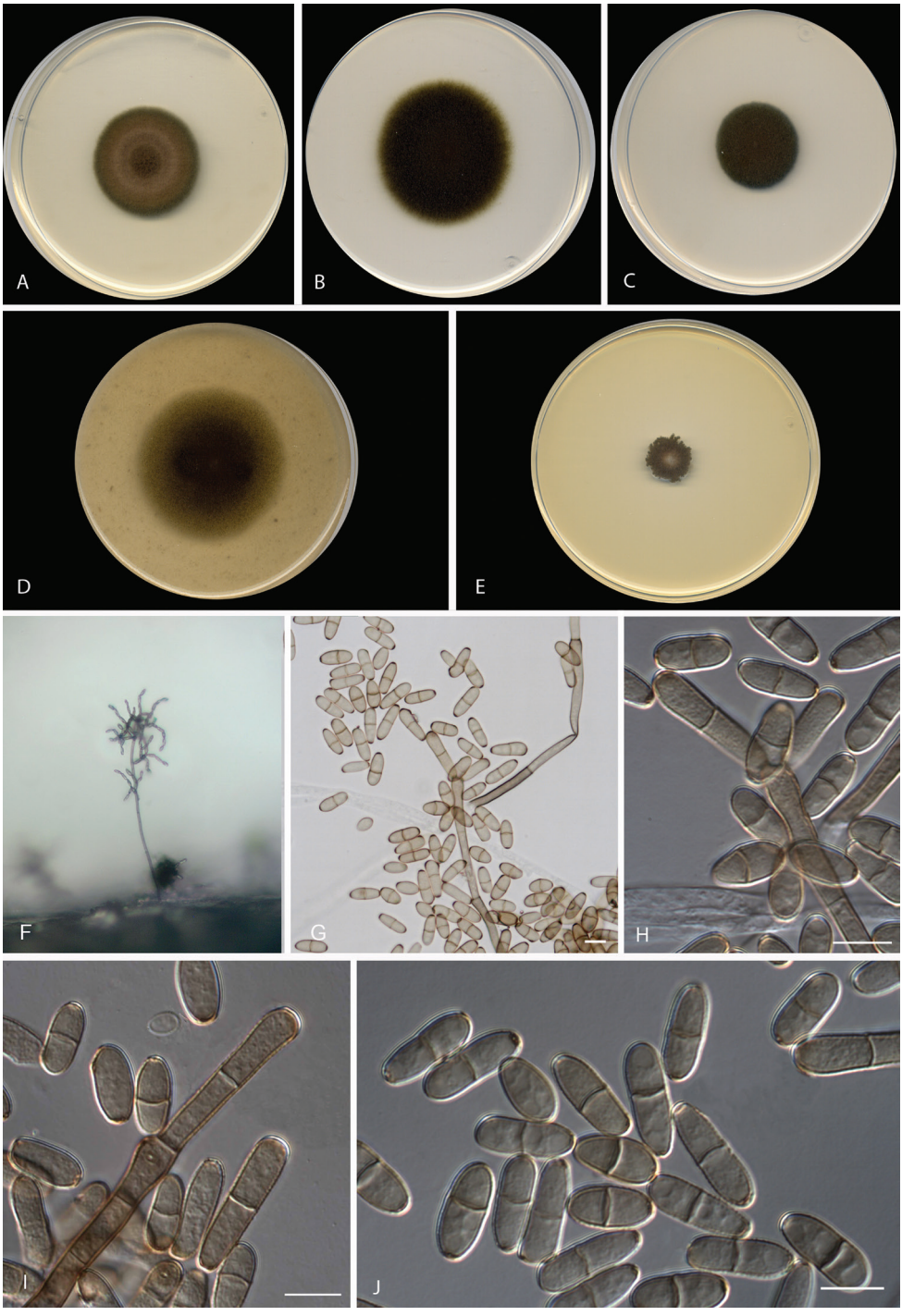

Figure 3. *Neodendryphiella michoacanensis* sp. nov. (ex-type FMR 16098). **A–E** Colonies on **A** PDA **B** PCA **C** SNA **D** OA **E** MEA at 25 °C after 14 d **F–J** Conidiophores and conidia. Scale bars: 10 µm (**G–J**).

ophores semi-macronematous to macronematous, mononematous, erect or slightly flexuous, slightly branched, 1–13 septate, cylindrical or slightly swollen in the conidiogenous loci, 44–280 × 2–4 µm, brown, usually darker toward the base, smooth or verruculose, verrucose at the base. *Conidiogenous cells* terminal and intercalary, cylindrical or clavate, 11–62 × 3–5 µm, with up to 3 pores. *Ramoconidia* (0–)1-septate, with up to 4 terminal or subterminal pores, pale brown, smooth to verruculose, cylindrical, subcylindrical, to slightly clavate, with more or less rounded apex and truncate base, 12–23 × 3–4(–5) µm. *Conidia* catenate, with up to 4 conidia in the terminal unbranched part, (0–)1(–2)-septate, some slightly constricted at the septum, pale brown, verruculose to verrucose, ellipsoidal or subcylindrical, 5–16(–18) × 3–6 µm.

Culture characteristics (14 d at 25 °C). Colonies on PDA reaching 34 mm diam., slightly umbonate, velvety, olive brown (4F6/4E8), with slightly fimbriate margin; reverse dark green (30F8) to black. On PCA attaining 42 mm diam., flat, granular, olive brown (4F8), aerial mycelium scarce, fimbriate margin; reverse dark green to olive brown (30F8/4F8). On OA reaching 48 mm diam., flat, granular, yellowish-brown to olive (5F4/3D4), aerial mycelium scarce, with a regular margin; reverse brownish-grey to greyish-yellow (4D2/3B6). On SNA attaining 22 mm diam., flat, slightly granular, olive brown (4F8), aerial mycelium scarce, with slightly fimbriate margin; reverse dark green (30F8) to black. On MEA reaching 13–15 mm diam., slightly umbonate, flat towards the periphery, velvety, yellowish-grey to olive (3C2/3F8), with white irregular margin; reverse olive grey to dark green (3E2/30F8).

Cardinal temperature for growth. Optimum 25 °C, maximum 30 °C, minimum 10 °C.

Distribution. México.

Notes. *Neodendryphiella michoacanensis* morphologically resembles *N. mali*, in its conidiogenous apparatus with 0–1-septate ramoconidia, but the latter differs by having longer conidiophores (up to 385 µm), terminal conidial chains with up to 10 conidia and its conidia are 0–1-septate and smaller (4–15 × 3–5 µm). *Neodendryphiella michoacanensis* also resembles *D. uniseptata* in their conidial morphology, but ramoconidia of the latter species are often aseptate and can be up to 30 µm long (Matsushima 1971). *Dendryphiella uniseptata* is only known from the type material, which was collected in Honiara (Japan) and no ex-type culture was preserved. This species was considered a synonym of *D. infuscans* by Matsushima (1975) but not accepted by Liu et al. (2017).

Neodendryphiella tarraconensis **Iturrieta-González, Gené & Dania García, sp. nov.**
MycoBank: MB824667
Fig. 4

Etymology. Name refers to Tarragona, the geographical area where the fungus was collected.

Type. Spain, Tarragona, from garden soil, Feb. 2017, I. Iturrieta-González (holotype CBS H-23479, culture ex-type CBS 144324 = FMR 16234).

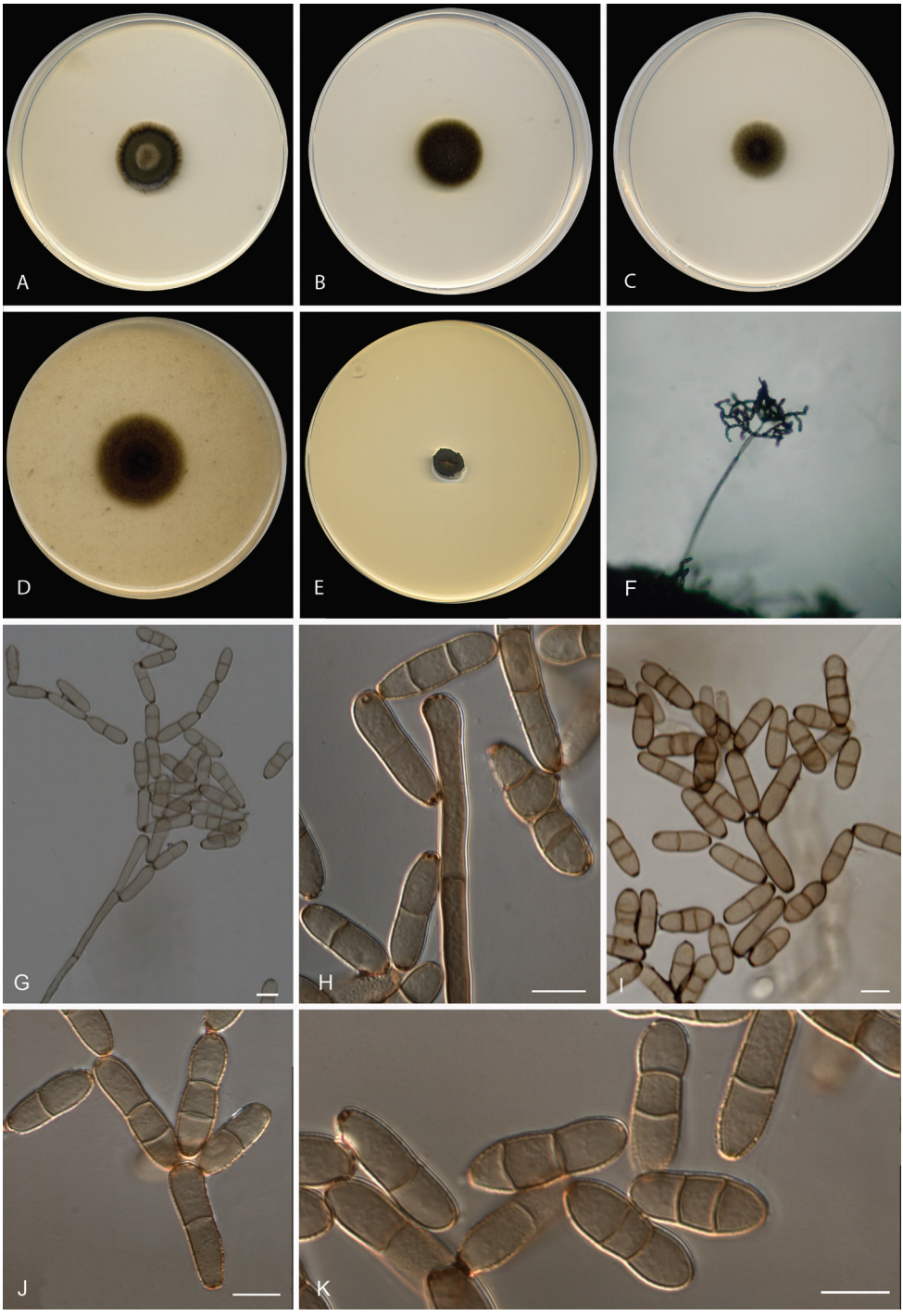

Figure 4. *Neodendryphiella tarraconensis* sp. nov. (ex-type FMR 16234). **A–E** Colonies on **A** PDA **B** PCA **C** SNA **D** OA **E** MEA at 25 °C after 14 d **F–K** Conidiophores and conidia. Scale bars:10 µm (**G–K**).

Description. *Mycelium* superficial and immersed abundant, composed of septate, branched, smooth to verruculose, hyaline to pale brown hyphae, 1–2 µm wide. *Conidiophores* macronematous, mononematous, erect or slightly flexuous, branched or unbranched, up to 6-septate, cylindrical, 19–185 × 2–5 µm, brown, smooth, darker and finely verrucolose towards the base. *Conidiogenous cells* terminal and intercalary, subcylindrical to clavate, 9–35 × (2–)3–4(–5) µm, with up to 2 pores. *Ramoconidia* (0–)1–2(–3)-septate, usually slightly constricted at the septa, with up to 3 terminal and subterminal pores, pale brown, smooth to verruculose, mostly cylindrical, with rounded apex and truncate base, 12–21(–23) × 4–5 µm. *Conidia* catenate, with up to 7 conidia in the terminal unbranched part, (0–)1–2-septate, pale brown, verruculose, ellipsoidal or subcylindrical with more or less rounded ends, 6–21 × 3–6(–7) µm; when 1-septate, the septum is often submedial and slightly constricted, when 2-septate, usually constricted at only one septum.

Culture characteristics (14 d at 25 °C). Colonies on PDA reaching 23 mm diam., umbonate, velvety, greyish-brown to olive brown (5E3/4F8), with slightly fimbriate margin; reverse dark green (30F8) to black. On PCA attaining 24 mm diam., flat, velvety, olive brown (4F8), slightly fimbriate margin, reverse dark green to olive brown (28F5/3B2) with a pale yellow (4A3) diffusible pigment. On OA reaching 30 mm diam., flat, slightly granular, yellowish-brown to olive brown (5F8/4F4), aerial mycelium scarce, with regular margin; reverse yellowish-brown to olive brown (5F8/4F4). On SNA attaining 21 mm diam., flat, slightly granular, yellowish-brown to olive (5F4/3F5), aerial mycelium scarce, with fimbriate margin; reverse yellowish-brown to olive (5F4/3F5). On MEA reaching 8-10 mm diam., slightly elevated but depressed at the centre, radially folded, velvety, olive (2F8), with irregular margin; reverse olive (2F4).

Cardinal temperature for growth. Optimum 25 °C, maximum 30 °C, minimum 10 °C.

Distribution. Spain.

Notes. In addition to the genetic differences mentioned above, *N. tarraconensis* differs from the other two species in the genus by the presence of ramoconidia with up to 3 septa and conidia from terminal branches with mostly 1–2-septate. It is noteworthy that 1-septate conidia usually show a slightly longer basal cell since the septum is submedial and, when 2-septate, often only one of the septa is constricted, features not described in any species of *Dendryphiella* and *Neodendryphiella*.

Dendryphiella variabilis **Iturrieta-González, Dania García & Gené, sp. nov.**
MycoBank: MB824668
Fig. 5

Etymology. Name refers to the variable number of septa in the conidia.

Type. Cuba, from a dead leaf of a Lauraceous tree, 1996, R.F. Castañeda (holotype CBS H-23476; ex-type cultures CBS 584.96 = INIFAT C95/105-4 = MUCL 39840 = FMR 16563).

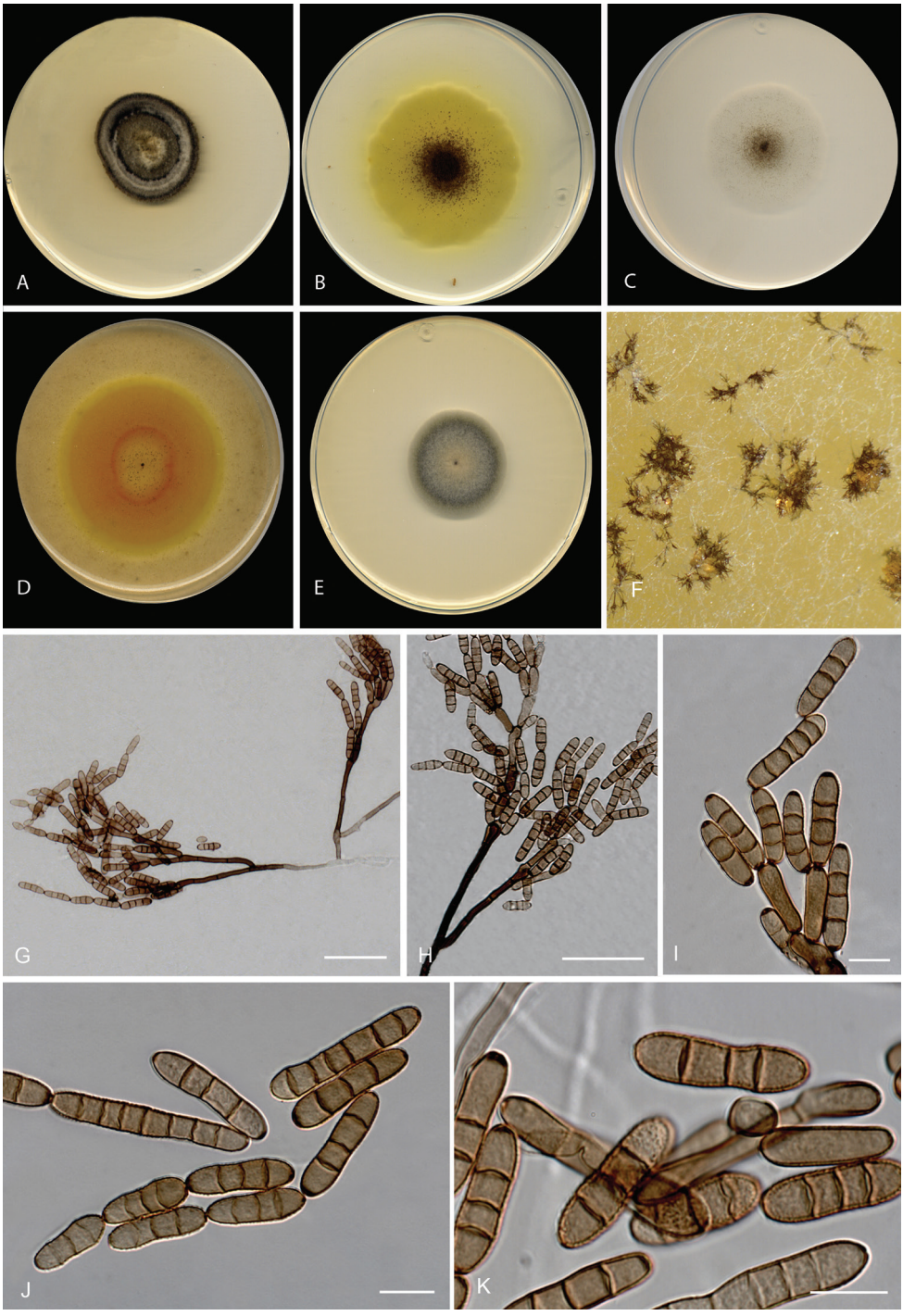

Figure 5. *Dendryphiella variabilis* sp. nov. (ex-type CBS 584.96). **A–E** Colonies on **A** PDA **B** PCA **C** SNA **D** OA **E** MEA at 25 °C after 14 d **F** Exudates and conidiophores produced on OA **G–K** Conidiophores and conidia. Scale bars: 50 µm (**G–H**), 10 µm (**I–K**).

Description. *Mycelium* superficial and immersed, composed of septate, branched, smooth to verruculose hyaline to pale brown hyphae, 1–3 µm wide. *Conidiophores* macronematous, mononematous, often arranged in loose fascicules, erect or slightly flexuous, branched, 1–8-septate, nodulose toward the apex, up to 143 µm long, 2–6 µm wide, brown, smooth to verruculose. *Conidiogenous cells* terminal and intercalary, sympodially extended towards the apex, with 1–5 pores surrounded by a thickened and darkened wall, clavate, 7–37 × 3–6(–7) µm. *Ramoconidia* (0–)2–3-septate, cylindrical to subcylindrical, with rounded ends, 16–27 × 5–6 µm, usually with 2 apical pores, conidial scars thickened and darkened. *Conidia* in short branched chains, with up to 5 conidia in the terminal unbranched part, (0–)3(–7)-septate, some constricted at the medial septum, pale brown, verruculose to verrucose, cylindrical or subcylindrical, with rounded ends, 6–44 × 4–6 µm, conidial scars often thickened and darkened. *Sexual morph* not observed.

Culture characteristics (14 d at 25 °C). Colonies on PDA reaching 30–33 mm diam., slightly umbonate, flat towards the periphery, velvety, irregularly coloured yellowish-grey to olive brown (4B2/4D3) and brownish-grey to yellowish-brown (5F2/5F4), with irregular margin; reverse yellowish-brown (5F8) to black. On PCA attaining 48 mm diam., flat, granular to velvety, yellowish-brown (5F8), aerial mycelium scarce, undulate margin; reverse olive to greyish-yellow (3F4/3B4), with a pale yellow diffusible pigment. On OA reaching 58 mm diam., flat, slightly granular, blond to reddish-yellow (5C4/4A7), light yellow (4A4) at the periphery, aerial mycelium scarce, with a regular margin, with scarce pale brown exudate; reverse same colouration with the colony surface. On SNA attaining 40 mm diam., flat, slightly granular to velvety, yellowish-brown to grey (5F7/4B1), with fimbriate margin; reverse brownish-grey to white (5D2/1A1). On MEA reaching 32 mm diam., flat, cottony, yellowish-grey to olive (4B2/3F4), yellowish-grey (3B2) at the periphery, with regular margin; reverse dark green to white (30F8/1A1).

Cardinal temperature for growth. Optimum 25 °C, maximum 30 °C, minimum 15 °C.

Distribution. Cuba.

Notes. *Dendryphiella variabilis* differs from *D. paravinosa* mainly by having longer conidia (up to 44 µm), which can have up to 7 septa. The conidia of *D. paravinosa* are up to 3-septate and measure (10–)24–27(–33) × (6–)7(–7.5) µm (Crous et al. 2016). The only species of the genus reported with conidia up to 5-septate are *D. eucalyptorum* and *D. vinosa*, but they are smaller, measuring (19–)20–23(–25) × 5(–7) µm in the former (Crous et al. 2014) and 13–39 × 4–8 µm in the latter (Ellis 1971). The other closely related species to *D. variabilis* is *D. fasciculata* (Fig. 1), but it mainly differs by the presence of fasciculate conidiophores and 3-septate conidia (Liu et al. 2017).

Discussion

The present study proposes the genus *Neodendryphiella* based on the analysis of the ITS and LSU sequences, which represented an undescribed monophyletic lineage related but phylogenetically distant from the morphologically similar genus *Dendryphiella*.

Both genera belong to the Dictyosporiaceae (Dothideomycetes) and share similar conidiophore morphology with polytretic conidiogenous cells forming usually septate conidia arranged in acropetal branched chains. *Dendryphiella* can be differentiated by the presence of nodulose conidiophores and conidiogeneous cells with pores surrounded by a thickened and darkened wall, which are absent in *Neodrendryphiella*. Other genera of the Dothideomycetes, although accommodated in different orders or families with a similar conidiogenous apparatus are *Dendryphion* (Toluraceae, Pleosporales) (Crous et al. 2014, Crous et al. 2015), *Dendryphiopsis* (Kirschsteiniotheliaceae, Kirschsteiniotheliales) (Su et al. 2016, Hernández-Restrepo et al. 2017) and *Paradendryphiella* (Pleosporaceae, Pleosporales) (Woudenberg et al. 2013). However, the genus *Diplococcium* in Leotiomycetes also shows similar asexual propagules (Shenoy et al. 2010, Hernández-Restrepo et al. 2017), which complicates the classification of these fungi based exclusively on morphological features.

Our phylogenetic study not only allowed us to distinguish very similar isolates in three distinct species, *N. mali, N. michoacanensis* and *N. tarraconensis*, but also helped us to correctly identify some strains that had previously been attributed to *Dendryphiella* (Table 1). In addition, it is of note that, considering the species accepted in *Dendryphiella* (Liu et al. 2017, Hyde et al. 2018), this genus seems to be morphologically heterogeneous and probably polyphyletic. It includes species with apparently polyblastic denticulate conidiogenous cells, such as *D. eucalypti* (Matsushima, 1983) or *D. uniseptata* (Matsushima, 1971), rather than polytretic conidiogenous cells typical of *Dendryphiella* (Rao and Narania 1974, Crous et al. 2014, 2016) or species that produce solitary conidia, such as *D. cruzalmensis* (Batista, 1946) or *D. lycopersicifolia* (Batista & Peres, 1961). In this scenario, therefore, *Dendryphiella* requires a further taxonomic re-evaluation. However, taking into account that only herbarium material is available for the type *D. vinosa* (preserved in the Kew herbarium, as *Helminthosporium vinosum*) there is a need to re-collect this species from the type locality (Cuba) for epitypification and giving nomenclature stability to the genus.

Acknowledgements

This study was supported by the Spanish Ministerio de Economía y Competitividad, Grant CGL2017-88094-P.

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Supplementary material 1

***Neodendryphiella* gen. nov. Tree LSU**

Authors: Isabel Iturrieta-González, Josepa Gené, Josep Guarro, Rafael F. Castañeda-Ruiz, Dania García

Link: https://doi.org/10.3897/mycokeys.37.27275.suppl1

Supplementary material 2

***Neodendryphyella* gen. nov. Tree ITS**

Authors: Isabel Iturrieta-González, Josepa Gené, Josep Guarro, Rafael F. Castañeda-Ruiz, Dania García

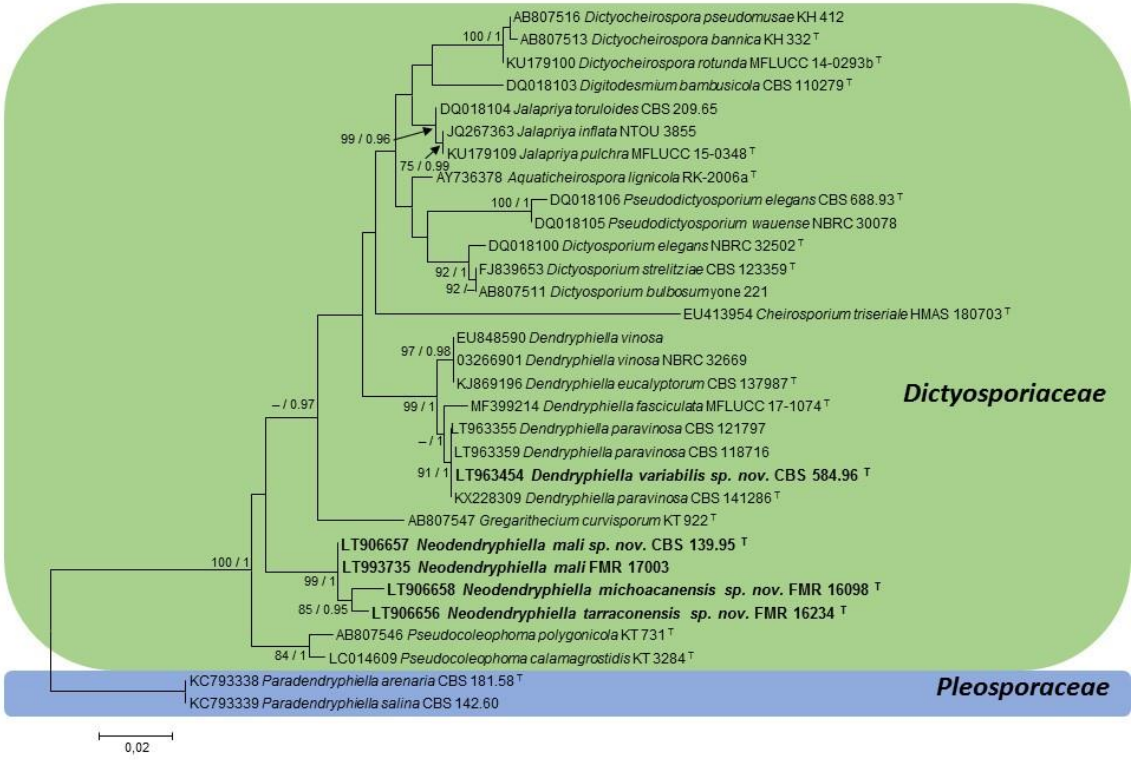
Link: https://doi.org/10.3897/mycokeys.37.27275.suppl2

Supplementary material 1

***Neodendryphiella* gen. nov. Tree LSU**

Authors: Isabel Iturrieta-González, Josepa Gené, Josep Guarro, Rafael F. Castañeda-Ruiz, Dania García

Link: https://doi.org/10.3897/mycokeys.37.27275.suppl1



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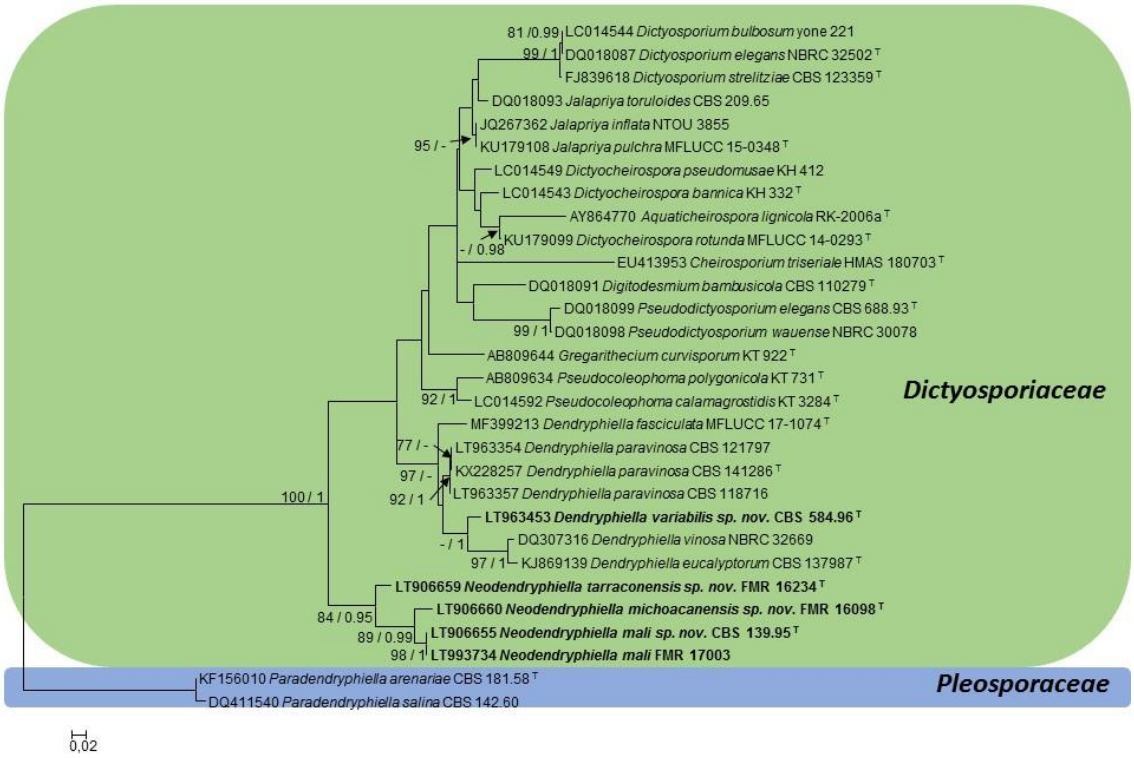
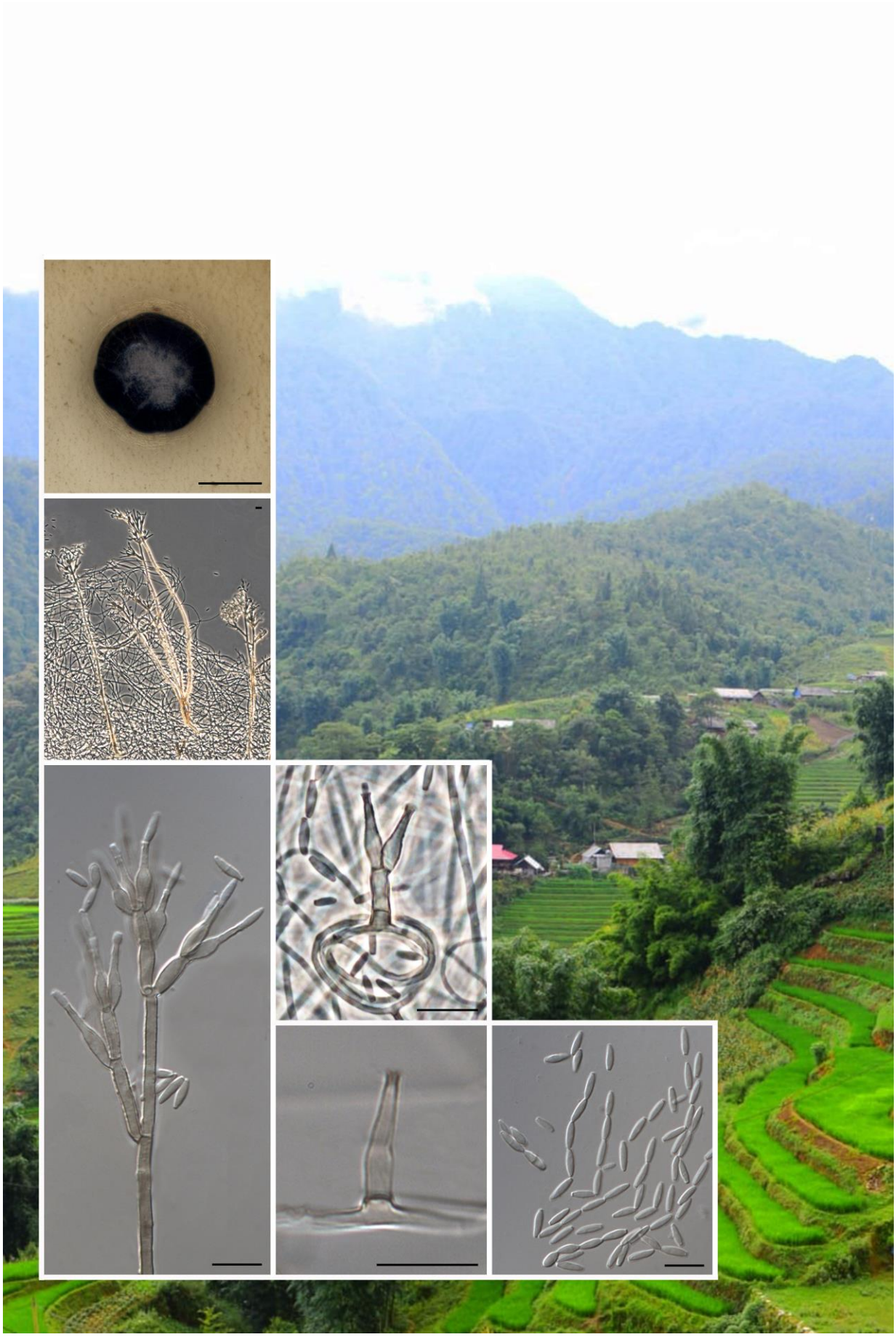


4.3.4 *Cyphellophora vietnamensis* sp. nov.

Isabel Iturrieta-González, Dania García, Guarro & Gené.

Submited for publication in: Persoonia (Fungal Planet description sheets)

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González



Cyphellophora vietnamensis Iturrieta-González, Dania García, Guarro & Gené, sp. nov.

Etymology. Name referred to the geographical region where the fungus was collected.

Classification – *Cyphellophoraceae, Chaetothyriales, Eurotiomycetes.*

Mycelium consisting of branched, septate, subhyaline to pale olivaceous, smooth-walled hyphae, 1–1.5 µm diam. *Conidiophores* commonly macronematous, mononematous or in groups of 2–4, growing laterally or terminally on hyphae, erect, more or less penicillate branched, up to 250 µm long, with stipe pale brown to brown, smooth- and thick-walled; branches bearing terminally groups of 2–3 phialides, pale brown, asperulate to verruculose; micronematous conidiophores also present, consisting in phialides growing directly or on short supporting cells from vegetative hyphae. *Phialides* langeniform, 12–20 × 2–3.5 µm at the broad part, tapering to a long cylindrical neck with a conspicuous collarete slightly darker than the rest of the phialide, pale olivaceous, smooth-walled. *Conidia* in long unbranched chains (up to 90 conidia), 0(–1)-septate, ellipsoidal to somewhat fusiform, with truncate ends, obovoid when terminally, pale olivaceous, smooth-walled, 4–7 × 1–2 µm. *Chlamydospores* absent. *Sexual morph* not observed.

Culture characteristics – Colonies on PDA reaching 18–19 mm diam after 2 wk at 25 ºC, brownish grey to grey (4D2/4B1), final edge olive (2F8), velvety, radially folded, aerial mycelium scarce, irregular margin; reverse olive (2F8). On PCA reaching 18–20 mm after 2 wk at 25 ºC, olive grey to olive (3D2/3F8), velvety, flat, aerial mycelium scarce, regular margin; reverse olive (2F8). On OA reaching 18–19 mm diam after 2wk, pale grey to olive (1B1/2F8), velvety at the centre, flat, aerial mycelium scarce, irregular margin; produce a metallic brightness on the border of the colony; reverse olive (2F8). Urease positive; laccase production negative.

Cardinal temperatures for growth – Minimum 15 ºC, optimum 25 ºC, maximum 30 ºC.

Typus. VIETNAM, Northeast region, on unidentified dead leaf, Aug. 2011, *J. Guarro* (holotype H-24475, cultures ex-type FMR 17714, CBS 146924; ITS, LSU and *tub*2 sequences GenBank LR814107, LR814108 and LR814116, MycoBank MB 836045).

Notes – Based on a megablast search of NCBIs GenBank nucleotide, LSU sequence of *C. vietnamensis* showed a similarity of 98.22 % (829/844) with the sequence of *C. oxyspora* (CBS 698.73, GenBank NG_067405) and 97.75 % (825/844) with that of *C. suttonii* (CBS 125441, GenBank MH874978); while ITS sequence was 96.71 %

(558/577) similar with that of *Phialophora capiguarae* (ex-type strain CBS 132767, GenBank KF928464) and a 88.61 % (537/606) respect to *C. oxyspora* (IFM 51368, GenBank AB190870). Phylogenetic reconstruction with ITS, LSU and *tub2* loci (Attili-Angelis et al. 2014) of the accepted species of *Cyphellophora* and *Phialophora*, including the type species of the respective genera (i.e. *C. laciniata* CBS 190.61 and *P. verrucosa* CBS 140326), showed that the new species is allocated in a strongly supported clade with *C. oxyspora* and *P. capiguarae,* but being closely related to the latter species*.* Our phylogeny supports that *P. capiguarae* as well as *P. attae*, both described by Attili-Angelis et al. (2014), belongs to the *Cyphellophora* clade. Therefore, respective new combinations are proposed below.

Morphologically, *C. vietnamensis* differs from *P. capiguarae* mainly by having unbranched chains of conidia, which are smaller (4–7 × 1–2 µm vs 6.5–9 × 1.9–2.5 µm in *P. capiguarae*) and commonly aseptate, absence of chlamydospores, and a moderately faster growth (PDA, 18–19 mm vs 13–14 mm in *P. capiguarae*; OA, 18–19 mm vs 14–15 mm in *P. capiguarae*) after 2 wk at 25 ºC. *Cyphellophora vietnamensis* clearly differs from *C. oxyspora* (Gams & Holubová-Jechová 1976, Réblová et al. 2013) by its long penicillate conidiophores.

Cyphellophora attae (Attili-Angelis, Duarte, Stielow & de Hoog) Iturrieta-González, Gené, Dania García, comb. nov.

Basionym: Phialophora attae Attili-Angelis, Duarte, Stielow & de Hoog, Fungal diversity 65: 68 (2014).

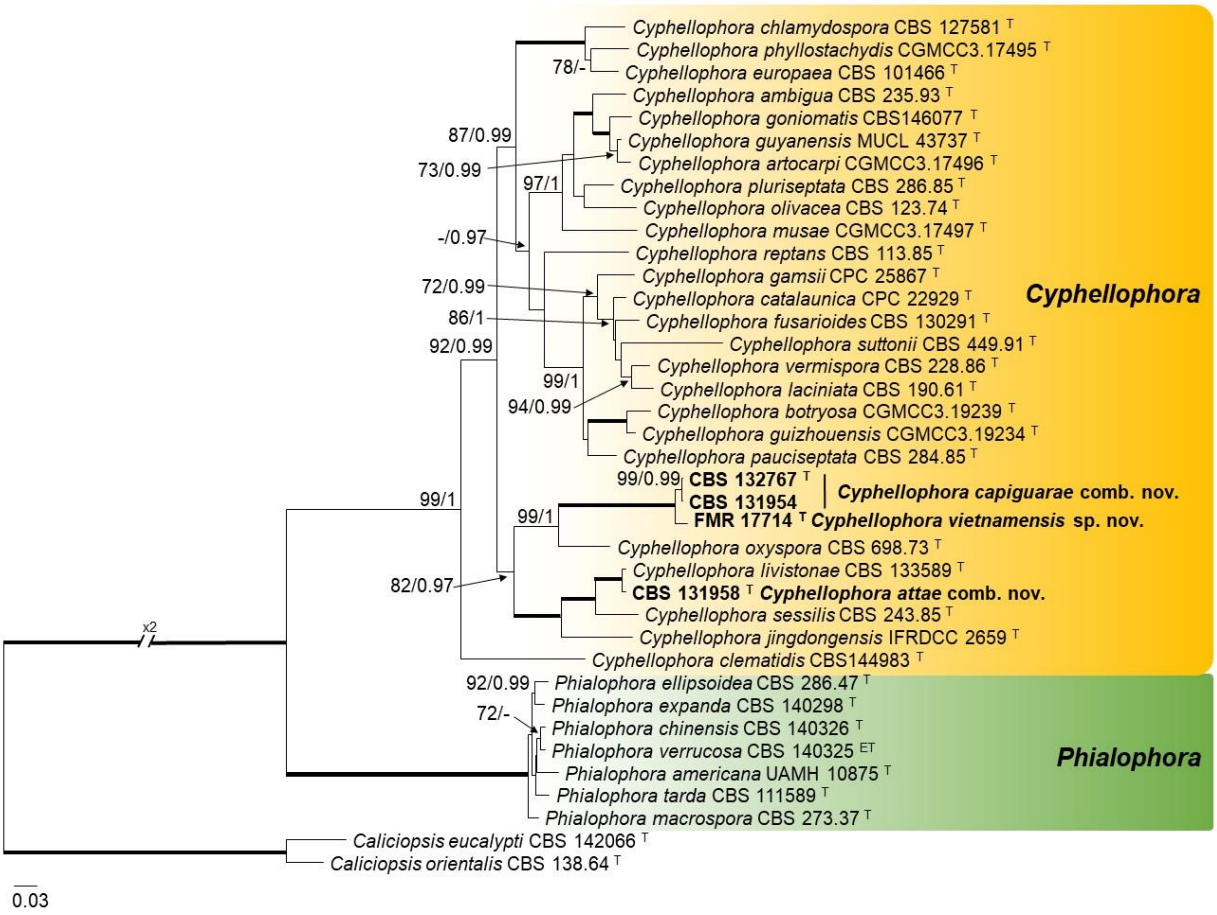
Typus. BRAZIL, Fazenda Santana, Botucatu, São Paulo, from the cuticle of *Atta capiguara* gynes, Nov. 2008, *A.P.M. Duarte*, *F.L.A. Guedes* and *D. Attili-Angelis* (holotype and cultures ex-type CBS 131958; ITS, LSU and *tub2* sequences GenBank KF928463, KF928527 and KF928591; MycoBank MB 836046).

Notes – *Cyphellophora attae* is closely related to *C. livistonae* (Crous et al 2012, Madrid et al 2016) and *C. sessilis* (de Hoog et al. 1999, Réblová et al. 2013), both species formerly classified in *Phialophora*. Morphologically, *C. attae* can be differentiated from *C. livistonae* by the production of shorter [1.6–4.2 vs (4–)7–8(–10) µm] and aseptate conidia, and by the absence of chlamydospores, Chlamydospores in *C. livistonae* are intercalarly, 0–1-septate, measuring 8–10 × 3–5 µm (Crous et al. 2012). *Cyphellophora sessilis* differs by its shorter (up 3 µm; up to 4.2 in *C. attae*) and obovoidal conidia (broadly ellipsoidal in *C. attae*).

Cyphellophora capiguarae (Attili-Angelis, Duarte, Pagnocca & de Vries) Iturrieta-González, Gené, Dania García, comb. nov.

Basionym: Phialophora capiguarae Attili-Angelis, Duarte, Pagnocca & de Vries, Fungal diversity 65: 70 (2014).

Typus. BRAZIL, Fazenda Santana, Botucatu, São Paulo, from cuticle of *Atta capiguara* gynes, Dec. 2009, *F.C. Pagnocca, N.S. Nagamoto, A.P.M. Duarte* and *D. Attili-Angelis* (holotype and cultures ex-type CBS 132767; ITS, LSU and *tub*2 sequences GenBank KF928464, KF928528 and KF928592; MycoBank MB 836047).



Maximum likelihood tree obtained from the combined analysis of ITS, LSU and *tub*2 sequences of the genus *Cyphellophora* and representative species of the genus *Phialophora*. The alignment included 1965 bp performed with ClustalW. The ML was constructed under RAxML-HPC2 on XSEDE v.8.2.12 (Stamatakis et al. 2014) in Cipres Science gateway portal (Miller et al. 2010) and Bayesian Inference (BI) approaches under MrBayes v. 3.2.6 (Ronquist et al., 2012). Tamura Nei with gamma distribution (T93+G) was used as the best nucleotide substitution model for ML and for BI were used General Time Reversible with gamma distribution (GTR+G) for ITS, General Time Reversible with gamma distribution and invariant sites (GTR+G+I) for LSU and

Hasegawa-Kishino-Yano with gamma distribution and invariant sites (HKY+G+I) for *tub*2. Bootstrap support values for ML greater than 70 % and Bayesian posterior probabilities greater than 0.95 are given near nodes. Bold branches indicate bs/pp of 100/1. The new species and new combinations proposed are indicated in **bold** face. A superscript T or ET denotes ex-type or ex-epitype cultures.

Colour illustrations. Vietnam, Northeast region, colony sporulating on OA after 2 wk at 25 ºC; conidiophores, phialides and conidia after 18 d. Scale bars of colony = 10 mm and microscopic structures = 10 µm.

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Acknowledgements

This study was partially supported by the Spanish Ministerio de Economía, Industria y Competitividad (grant CGL2017-88094-P).

5. SUMMARIZING DISCUSSION

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

The testing of metagenomics studies on fungal biodiversity has been increasing especially in the last two decades (Cuadros-Orellana et al. 2013, Nilsson et al. 2019). All these studies confirm the hypothesis that fungal diversity is much greater than the number of currently known species (near to 100 thousand) (Hawksworth 2001, Blackwell 2011). The most recent estimation of fungal species diversity on Earth is near to 3.8 million species (Hawksworth & Lücking 2017) and, surprisingly, merely <1 % of those species have been cultivated with established protocols, which renders large taxonomic groups undescribed and virtually unknown to science (Tedersoo et al. 2017). This incredible number of undiscovered species not only inhabits unexplored ecological habitats, but also within substrates from our environment which fungal biodiversity has been understudied by molecular tools (Luo et al. 2014, Hawksworth & Lücking 2017, Hyde et al. 2018, Naranjo-Ortiz & Gabaldón 2019a). To identify well-established species from new habitats or substrates or to characterize and obtain cultures of rare or novel fungi is not only relevant for a better understanding of the role of the species in their habitats, but also to finding microorganisms which are producers of interesting molecules with a wide range of applications.

Our study demonstrated that despite the important investigation of cosmopolitan and well-delineated genera, such as *Alternaria* and *Cladosporium*, there are still interesting isolates that deserve attention. The study of the fungal diversity of some interesting substrates such as clinical sources or herbivore dung, which biodiversity is still understudied by molecular tools, has allowed the characterization and description of a considerable number of new species. This research contributes, therefore, to improve the knowledge of fungal diversity, in general, but also to determine the spectrum of fungi associated to such substrates for a better understand of their role from an ecological or epidemiological point of view. Many of these fungi are currently available in public cultures collections to the scientific community for further studies.

5.1 Comments on the genus *Alternaria* and related fungi

As mentioned in the introduction, the taxonomy of the genus *Alternaria* has been investigated in successive phylogenetic studies, being currenly a well-delineated genus and taxonomically structured in 27 sections (Lawrence et al. 2013, 2016, Woudenberg et al. 2013, 2014, 2015). However, the section *Infectoriae* is recognized as one of the largest and most complicated one (Poursafar et al. 2018). Isolates of this section commonly produce small spores, with a rather variable morphology, and tend to show poor sporulation when they grow *in vitro*, hindering their morphological identification (de Hoog & Horré 2002, Pastor & Guarro 2008). For instance, erroneous

identifications of *A. infectoria* by *A. alternata* commonly occurs in the diagnosis of human infections (de Hoog et al. 2011), and in many cases, these small-spored *Alternaria* from clinical sources are only identified at the genus level or section if only ITS analysis is used for their identification. Therefore, to advance in the taxonomy of this section through defining proper molecular markers for delineating species is relevant for the correct identification of causal agents of human infections and also to help to understand their role in the environment. In previous molecular studies of the section *Infectoriae*, only three genetic markers (ITS, *gapdh* and *ATPase*) were used for its phylogenetic investigation (Lawrence et al. 2014, Deng et al. 2018, Poursafar et al. 2018). Therefore, for a more robust phylogeny two additional markers (*rpb*2 and *tef*1) that proved to be good loci for the phylogeny of the section (Woudenberg et al. 2013) were used for our multi-locus approach. Examination of those small-spored *Alternaria* from clinical and environmental samples, morphologically identified as belonging mostly in section *Infectoriae*, and the multi-locus sequence analysis of the five loci allow the discrimination of not only all the known species described so far in the section, but also to propose nine new species. Three of these new species, *A. anthropophila*, *A. atrobrunnea* and *A. guarroi*, caused cutaneous infections, while the others, *A. aconidiophora, A. curvata, A. fimeti, A. lawrencei, A. montsantina* and *A. pseudoventricosa* were isolated from environmental substrates, mainly herbivore dung. A common feature of all those species is their ability to grow at 37 ºC, which is essential to cause infections in mammals (Casadevall 2007). The new species and human pathogen described as *A. anthropophila* was also found on an herbivore dung sample collected in Catalonia, the same geographical origin as the type strain of the species. In addition, it is of note that *A. anthropophila* seems to be a species of wide distribution in our country, since other clinical isolates studied were from Aragon, Cantabria and Galicia. Other species of the section *Infectoriae* identified in the present study are *A. arbusti* isolated from two clinical samples in the USA; *A. merytae* and *A. slovaca,* both isolated from unidentified herbivore dung collected in Extremadura and Catalonia, respectively; and *A. oregonensis* found on different substrates collected in Spain and USA. Interestingly, these three fungi can be considered rare species since they are practically known only through their type specimens; *A. merytae* was described on *Merita sinclari* from New Zealand (Simmons 2002), *A. oregonensis* on a leaf spot in *Triticum aestivum* from the USA, and *A. slovaca* from a human biopsy from Slovakia (Woudenberg et al. 2013). The most surprising fact in our study on the section *Infectoriae* was that no isolate was identified as the well-known medical species *A. infectoria* (de Hoog et al. 2000), indicating that this is probably an overestimated fungi, at leats as human opportunistic pathogen.

Despite *Alternaria* species from section *Infectoriae* being well-delineated through the multi-locus sequence analysis mentioned above, and that we confirmed the *ATPase* locus as a suitable marker for molecular identification of species in the section (see sections 4.1.1 and 4.1.2), as well as for other species in the genus (Lawrence et al. 2013, 2016), there are still more than 40 isolates (39 from clinical and 10 from environment sources) which could not be assigned to any well-stablished *Alternaria* species (Figs 7, 9−11). Although some of them are in process of being described formaly, for the time being some others will remain undescribed due to still showing an unclear phylogeny or because they have lost the ability to sporulate, which impedes their morphological charaterization. Scarcity or lack of sporulation and production of white colonies are well-known features in members of *Infectoriae* section when grown *in vitro* (Andersen & Thrane 1996, de Hoog & Horré 2002, Pastor & Guarro 2008) and, despite the attemps to stimulate conidiation, even following protocols described for that purpose (Nishikawa & Nakashima 2020), numerous isolates of species of this section remain sterile.

The absence of sporulation is a handicap not only for a proper species characterization, but also to investigate the *in vitro* antifungal susceptibility patterns of clinical isolates, since this hinders the standardization of the inoculum and the results obtained from the tests are unreliable. This was the problem we found when we tried to determine the antifungal susceptibility of the clinical isolates of the *Infectoriae* section. Therefore, we limited our study to test only the new human pathogens *A. anthropophila, A. atrobrunnea* and *A. guarroi* described here. Although antifungal therapy for *Alternaria* infections is not yet defined, ITC, VRC and PSC are the most common drugs used to resolve those infections (Chowdhary et al. 2014). However, due to VRC having hepatic toxicity and ITC showing drug interactions (Park et al. 2012, Mihăilă 2015, Doß et al. 2017), currently PSC seems to be the best option for alternariosis treatment (Bajwa et al. 2017). Our results confirm that PSC showed low MICs for *A. anthropophila* and *A. guarroi*, however no azol tested exhibited good activity on *A. atrobrunnea*. With respect to equinocandins, while *A. anthropophila* and *A. atrobrunnea* showed low MECs (0.03 to 1 µg/ml), these were considerably high (4 to 8 µg/ml) in *A. guarroi.* These results reinforce the importance of the correct identification of the fungi at the species level, but also that antifungal susceptibility deserves further investigation in *A. infectoria* species complex to dilucidate such differences.

Other small-spored *Alternaria* isolates from clinical and environmental sources identified in the present thesis belong to the sections *Alternaria*, *Chalastospora*,

Ulocladium, *Ulocladioides*, *Pseudoalternaria* and *Radicina*. The former section includes more than half of the total studied isolates (130, 61.3 %), *A. alternata* (115 isolates, 54.2 %) being the most frequent species identified, as expected (Simmons 2007). Other species identified in this section were *A. arborescens* and *A. gaisen*, but in a considerable smaller proportion (14, 6.6 % and 1, 0.5 %) than *A. alternata*. It is of note that *A. arborescens* is, in fact, recognised by Woudenberg et al. (2015) as a complex of species still unresolved and its isolates are recovered from many environmental sources as in our case (soil, plant debris and herbivore dung), while *A. gaisen* is a well-stablished species described as phytopatogen on pear (Woudenberg et al. 2013, 2015). We found this latter species from an herbivore dung sample collected in The Canary Islands.

In addition to *A. pobletensis*, the new species from dung described in section *Chalastospora*, other species identified in the section are *A. armoraciae* and *A. malorum.* While the former is only known from the type material (Simmons 2007), the latter, originally identified as *Cladosporium malorum*, is a prevalent phytopatogen with a wide range of hosts (Goetz & Dugan 2006). Therefore, it is not rare to find it associated to herbivore dung, as in our case, but also we identified this species from a human clinical specimen without proved pathogenicity. However, it is of note that *A. malorum* has been reported as causal agent of a human disseminated infection (Mirhendi et al. 2013).

For sections *Ulocladium* and *Ulocladioides*, the most frequent identified have been *A. botrytis* and *A. atra*, respectively, two cosmopolitan and widespread species commonly found on soil and plant debris (Ellis 1976, Domsch et al. 2007). Both species have been abundantly isolated from all environmental samples we collected. *Alternaria cantlous* and *A. heterospora*, of the section *Ulocladioides*, have also been identified exclusively from environmental samples, although in a smaller proportion than the previous species (Table 2). Little is known on the ecology or distribution of both fungi since they are only registered from the type location in China (Wang et al. 2009, 2010); we found both species in samples collected in different Spanish locations, including The Canary Islands. Therefore, they seem to be of wide distribution. Finaly, in addition to the new species *A. chlamydosporifera* described for *Radicina* section, and *A. altcampina* and *A. inflata* for *Pseudoalternaria* section, described all from herbivore dung collected in our country, two other species of this latter section, *A. kordkuyana* and *A. rosae*, have been found in clinical specimens from the USA but also isolated from dung and plant debries collected in Spain. According to the literature reviewed, these can be considered rare species since both are monotypic and only known from

their type strains collected in New Zealand (Simmons 2007) and Iran (Poursafar et al. 2018), respectively. Species of all these sections have been molecularly identified with confidence by multi-locus sequences analysis of ITS, *gapdh* and *ATPase* gene markers as used in other studies for these group of fungi (Lawrence et al. 2014, Deng et al. 2018, Poursafar et al. 2018).

To our knowledge, studies on *Alternaria* diversity from herbivore dung, of which identification has been carried out by sequence analyses are practically lacking. We can only mention the study of Torbati et al. (2016) on diversity of coprophilous ascomycetes from bird droppings collected in Iran, in which based on molecular data from ITS-rDNA region only *A. alternata* was identified, among other ascomycetes. Considering other studies on coprophilous fungi, the identification of the species has been done exclusively on morphological traits. And, with respect to *Alternaria,* only *A. alternata*, *A. atra*, *A. botrytis* and *A. chartarum* have been reported from animal faeces (Seifert et al. 1983, Jeamjitt et al. 2006, Masunga et al. 2006, Piontelli et al. 2006, Basumatary & McDonald 2017, Mohammed et al. 2017). Therefore, this is the first attempt to assess *Alternaria* diversity using a proper molecular approach for its species identification. According to our results, this substrate shows not only a high diversity in *Alternaria* species, which it is not surprising since these fungi are commonly associtated with plant material, but also it represents an excellent reservoir of many interesting and new species for science for *Alternaria* and other fungi, as we can see latter. In fact, we arrived to the same conclusion in respect to the diversity of penicillium-like fungi isolated from herbivore dung collected exclusively in our country (Guevara et al. 2020). Regarding the 92 *Alternaria* isolates we obtained in pure cultures from dung of various herbivore animals, a total of 23 species has been identified with condifence (Fig. 11). Despite the most commonly recovered are cosmopolitan species, such as *A. alternata* (27, 29.3 %), *A. botrytis* (17, 18.5 %), *A. atra* (7, 7.6 %) and *A. arborescens* (6, 6.5 %), by order of frequency, other uncommon or monotypic species have also been found, as mentioned above, such as *A. armoraciae*, *A. cantlous*, *A. heterospora*, *A. gaisen*, *A. kordkuyana*, *A. rosae*, *A. merytae*, *A. oregonensis* or *A. slovaca.* However, what is surprising is the proposal of eight novel species (*A. altcampina, A. chlamydosporifera, C. curvata, C. fimeti, C. inflata, A. lawrencei, A. pobletensis,* and *A. pseudoventricosa*), and other two in preparation (*Alternaria* sp. 5 and *Alternaria* sp. 10), isolated exclusively from herbivore dung. Nevertheless, ecological role of all these interesting *Alternaria* species on that substrate remains unclear and would merit further investigation.

Unlike herbivore dung samples, we found a much lower *Alternaria* species diversity in soil and plant debris, six species in each type of samples (Fig. 11), again *A. alternata* being the most common species identified from both substrates. Of mention is the proposal of *A. aconidiophora* and *A. montsantina* as new species isolated exclusively from plant material, in addition to other three isolates currently studied since their sequence analysis shows they are undescribed species for science (FMR 16646, FMR 17589 and FMR 17590).

Among related genera to *Alternaria,* we only identify isolates belonging to the genus *Stemphylium*, all being identified as *S. vesicarium.* This species has been isolated from the three types of environmental samples we studied; that means it is a ubiquitous fungus in our environment. Reports on *Stemphylium* species from dung are limited to *S. botryosum* and *S. asperulum,* which were described from human faeces (Seifert et al. 1983). *Stemphylium vesicarium* is mainly described as phytopathogen on a wide range of hosts (Köhl et al. 2009, Misawa & Yasuoka 2012, Woudenberg et al. 2017, Gazzetti et al. 2019).

5.2 Comments on the genus *Cladosporium* and cladosporium-like fungi

During the last decade, *Cladosporium* has been extensively reviewed based on phylogenetic analyses of several loci, currently being a well-delineated genus with more than two hundred species (Zalar et al. 2007, Crous et al. 2007a, 2019b, Schubert et al. 2007, Bensch et al. 2010, 2012, 2015, 2018, Sandoval-Denis et al. 2016, Bezerra et al. 2017, Marin-Felix et al. 2017a, Jayasiri et al. 2019). Species previously classified in the genus, which resulted to be phylogentically unrelated to *Cladosporium* s. str., were the basis of the proposal of several new genera such as *Apenidiella* (Quaedvlieg et al. 2014). This and other cladosporium-like genera are treated below since interesting or new species for science have been found in the present thesis.

Based on sequence analyses of the recommended barcodes (ITS, *act* and *tef*1) for delimiting species in *Cladosporium* s. str. (Marin-Felix et al. 2017a), we identified 287 isolates from environmental samples (soil, herbivore dung and plant debris) in 37 known species of *Cladosporium*. However, ten isolates did not match with any well-stablished *Cladosporium* species, resulting in seven new phylogenetic species for the genus, proposed as *C. caprifimosum*, *C. coprophilum*, *C. fuscoviridum,* C. *michoacanense, C. lentulum, C. pseudotenellum* and *C. submersum,* and one, represented by FMR 16656, that is still under study (Fig. 13). These species were distributed in the three *Cladosporium* species complex, the *C. cladosporioides* complex being the most prevalent and rich in species, as it has been reported in previous

studies (Bensch et al. 2010, 2012, 2015, 2018, Sandoval-Denis et al. 2016, Marin-Felix et al. 2017a). In such complex, the most commonly identified species have been *C. cladosporioides* followed by *C. xylophilum*, *C. perangustum*, *C. pseudocladosporioides*, *C. asperulatum*, *C. delicatum*, *C. vicinum*, *C. europaeum* or *C. tenuissimum*. All these species were isolated from the different type of substrates we studied and collected from several locations, which means they are ubiquitous in our environment, even despite the recent description of some of them, such as *C. vicinum*. This species was described by Bensch et al. (2018) from indoor environment samples collected in the USA and New Zealand, but also on plants from this latter country or associated to other fungi collected in the UK and South Africa; now also from our country (The Canary Islands, Catalonia and Teruel). *Cladosporium anthropophilum*, which was reported for the first time in our country and was originally described almost exclusively from human clinical samples in the USA (Sandoval-Denis et al. 2016), can be now considered a wide spread species over the world (Bensch et al. 2018). Other species identified in the complex which deserve attention can be, for instance: *C. australiense* and *C. ipereniae*, two uncommon species from which few specimens are known (Bensch et al. 2010, 2015, Segers et al. 2015); or the monotypic species *C. chubutense,* only known from the type collection in Argentina (Schubert et al. 2009), *C. phaenocomae* from South Africa (Crous & Groenewald 2011), *C. rugulovarians* from Brazil (Bensch et al. 2015), *C. silenes* from the UK (Crous et al. 2011) and *C. subuliforme* from Thailand (Bensch et al. 2010).

Among the species identified in the *C. herbarum* complex, the most frequently isolated have been *C. ramotenellum*, followed by *C. macrocarpum*, *C. floccosum*, *C. allicinum*, *C. limoniforme*, *C. tenellum* and *C. aggregatocicatricatum*. Recent molecular studies show that *C. floccosum* and *C. ramotenellum* are common saprobic species, occurring on various substrates with a wide geographical distribution (Bensch et al. 2015, 2018, Sandoval-Denis et al. 2016) and now also confirmed in Spain. Other interesting species identified in the complex is *C. angustiherbarum*, only reported previously from Australia and the USA (Bensch et al. 2018). However, surprisingly, *C. herbarum*, the most commonly reported species in the complex has not been identified in our study. To the contrary, most identified species in the *C. sphaerospermum* complex have been *C. sphaerospermum* as expected, followed by *C. halotolerans*. While the former has been isolated from all type of studied samples, *C. halotolerans* has been found exclusively from soil samples. Among the other species identified in a smaller proportion and related to this group of cladosporia, we can highlight *C. aphidis*, a species usually associated to aphidis or plant material infested by these insects

(Bensch et al. 2012), *C. dominicanum* known from different substrates (Bensch et al. 2015, 2018) and, in our case, found exclusively on plant debris, or *C. parahalotolerans* and *C. velox* mainly isolated from indoor environment samples (Bensch et al. 2018), both found in garden soil samples. However, the most interesting is the isolate identified as *C. lebrasiae,* a monotypic species which protologue was described from milk bread in France (Razafinarivo et al. 2016) and we found it on plant debris collected in Tarragona.

As in the case of the genus *Alternaria*, this represents the first study on *Cladosporium* diversity from herbivore dung, which species have been identified by sequence analyses of the apropriate barcodes. For this reason, the biodiversity found in respect to these fungi has been much higher than that described before. To our knowledge, only *C. cladosporioides*, *C.cucumerinum* and *C. herbarum* were reported in previous studies on coprophilous ascomycetes, and these were usually identified morphologically or by ITS analysis (Seifert et al. 1983, Jeamjitt et al. 2006, Masunga et al. 2006, Piontelli et al. 2006, Thilagam et al. 2015, Torbati et al. 2016). In the present thesis, we identified 29 species, including three new for science (*C. caprifimosum, C. coprophilum* and *C. lentulum*), all found in different Spanish locations. The most prevalent species identified on this substrate was *C. cladosporioides* (19, 17.9 %), followed by *C. macrocarpum* (14, 13.2 %), *C. pseudocladosporioides* (8, 7.5 %), *C. xylophilum* (8, 7.5 %) and *C. floccosum* (6, 5.7 %). On the contrary, the species only found on herbivore dung were *C. chubutense*, *C. fusiforme*, *C. ipereniae*, *C. licheniphilum*, *C. phyllophilum*, *C. rugulovarians*, *C. silenes*, all of them of the *C. cladosporioides* complex, except C. *fusiforme* of the *C. sphaerospermum* complex. However, the role of these fungi on dung remains obscure until further investigations. In addition, a preliminary comparative analysis of *tef*1 sequence of an isolate (FMR 16656), found from unidentified herbivore dung collected in the Montseny Natural Park, did not match with any described species in the genus, showing a low percentage of identity (89.95 %) with two species of the *C. cladosporioides* complex, i.e. *C. anthropophilum* and *C. pseudocladosporioides*. This putative new species will not be formally proposed until completing the morphological and DNA data for its characterization.

A similar proportion of species has been found from soil as well as from herbivore dung, namely 28 species including the new ones, *C. fuscoviridum* and *C. pseudotenellum* from Spain and *C. michoacanense* from Mexico. Again, *C. cladosporioides* has been the most prevalent (24, 21.8 %), followed by *C. sphaerospermum* (16, 14.5 %), *C. ramotenellum* (14, 12.7 %) and *C. halotolerans* (13,

11.8 %). As mention above, it is of note that this latter species has been exclusively isolated from the soil samples studied, as well as *C. australiense*, *C. subuliforme*, *C. parahalotolerans* and *C. velox*. Similarly, we identified 22 species from plant debris, including *C. submersum* and *C. lentulum* described as new for science. In this substrate however, the most prevalent species have been *C. floccosum* (8, 11.3 %) and *C. perangustum* (8, 11.3 %), followed by *C. cladosporioides* (7, 9.9 %), *C. ramotenellum* (7, 9.9 %) and *C. sphaerospermum* (7, 9.9 %). In addition to *C. submersum*, other species isolated exclusively from this substrate have been *C. dominicatum*, found previously in other subtrates from different countries (Bensch et al. 2018); and *C. angustiherbarum* and *C. sinuosum*, two monotypic species the protologues of which were described on *Pinus ponderosa* in the USA (Bensch et al. 2015) and on *Fuchsia excorticate* in New Zealand (Schubert et al. 2007), respectively.

Mainly from plant debris we also identified other dematiaceous hyphomycetes morphologically similar to the genus *Cladosporium.* However, all of them have a significant morphological difference in their conidiogenous apparatus in respect to members of the *Cladosporium* s. str., that is the lack of a coronate structure associated to the conidiogenous loci and conidial hila (Braun et al. 2003, Bensch et al. 2012). In addition to their morphological features, the taxonomy of these fungi have been resolved by sequence analyses of the rDNA operon (ITS and LSU). With this polyphasic approach, we characterized and proposed the following new cladosporiod species *Apenidiella foetida*, *Matsushimaea monilioides*, *Pseudopenidiella gallaica* and *P. vietnamensis,* and *Venturia submersa.* In addition, we identified the interesting fungus *Rachicladosporium cboliae*, a monotypic species originally described from twig litter collected in the USA (Crous et al. 2009b).

Apenidiella foetida was isolated from submerged plant material collected in a stream from the Arbolí Mountains (Catalonia). It was phylogenetically related to the type species of the genus, *Ap. strumelloidea*, a fungus isolated from a similar habitat to our fungus but from Russia (Crous et al. 2007c, Quaedvlieg et al. 2014). Currently, the genus includes other species, *Ap. antarctica,* which has been isolated from permafrost in Antarctica (Crous et al. 2019b). The locations where the *Apenidiella* species have been found clearly suggests an adaptation of these fungi to aquatic habitats with cold temperatures. Although our species was found in a temperate region, it shows a psychrophilic tendence since *in vitro* exhibited the ability to grow at 5 ºC.

A morphologically similar genus, but unrelated phylogenetically to *Apenidiella* (*Teratosphaeriaceae*, *Capnodiales*), is *Pseudopenidiella*. At the beginning of the thesis,

this genus was taxonomically defined as *incertae sedis* in *Dothydeomycetes*. However, phylogenetic analysis with the above mentioned rDNA loci of *P. piceae*, the type species of the genus (Crous et al. 2012), and our two new additions allowed us to resolve the taxonomy of *Pseudopenidiella* in the family *Microthyriaceae* of the order *Microthyriales*. Collection of data from *P. piceae, P. gallaica*, *P. vietnamensis*, and from the last new addition in the genus, *P. podocarpi* (Crous et al. 2019b), suggests that *Pseudopenidiella* is a saprobic genus occurring on plant material with a wider geographic distribution, since its species have been found in Czech Republic, Spain, Vietnam and South Africa, respectively.

Other hyphomycetous genus with a cladosporiod resemblance, and also with an uncertain taxonomic position among ascomycetes at the beginning of the thesis, was *Matsushimaea.* In that case, the problem was the absence of sequence data of the type species of the genus, *M. fasciculata*, and also from *M. fertilis* and *M. magna,* the other species described to date in *Matsushimaea* (Subramanian 1977, Castañeda-Ruiz et al. 1996, Matsushima 1996). Since the fungus under study, FMR 16505, showed intermediate morphological features between *M. fertilis*, which ex-type strain was kept in our culture collection, and *M. fasciculata*, which a reference strain was available in the CBS-KNAW fungal collection, a comparative sequence analysis was carried with those fungi. *Matsushimaea magna* could not be included in the study because there were no strains of this species available in any public repository of fungal cultures. While sequence analyses of LSU and ITS barcodes revealed that our isolate and that of *M. fasciculata* were phylogenetically related, allocating both fungi into the family *Sympoventuriaceae* (*Venturiales*), the sequences of *M. fertilis* ex-type strain resulted practically identical to those of the species *Cladophialophora boppii* (*Herpotrichiellaceae, Chaetothyriales*). Therefore, in addition to describing *M. monilioides* as a new species in the genus, this study allowed us to resolve the taxonomic position of *Matsushimaea*, and to consider *M. fertilis* and *Cl*. *boppii* as conspecific.

The last cladosporium-like isolate characterized in the thesis was also a member of the order *Venturiales*, as in the case of *Matsushimaea,* but belonged to the family *Venturiaceae*. However, its morphological traits, i.e. acroperal conidial chains born on polyblastic conidiogenous cells with denticulate or subdenticulate and truncate conidiogenous loci, matched better with features of the asexual morphs of members of the genus *Venturia* (Crous et al. 2007b). Sequence comparison of the ITS region with other species of the genus showed a low percentage of identity (≤ 96 %), confirming the phylogenetic novelty of the isolate proposed as *V. submersa*. Species of the genus

Venturia are defined as parasitic on dichotiledoneous plants (Zhang et al. 2011). Although this novel species was isolated from unidentified plant debris collected in a stream from Riaza (Segovia), its ecological role or pathogenic behaviour will remain uncertain until further studies with new specimens.

It is interesting to note that all the new cladosporium-like species were isolated directly from the sample (herbivore dung, soil and plant material) incubated in moist chambers. Soil and herbivore dung samples were also prepared by dilution plating technique, however, probably due to the slow growth and reduced colony dimensions of these fungi on agar cultures, it was problematic its detection. Therefore, according to our experience, techniques based on fungal isolation directly from the natural substratum should be considered of choice for futures studies on the diversity of cladosporium-like fungi.

5.3 Comments on other hyphomycetous genera identified in the thesis

Since we investigate substrates from which fungal diversity is poorly known, we found other interesting dematiaceous hyphomycetes of which the morphology was really confused. We must recognize that thanks to their sequence data, they could be allocated in genera the morphology of which did not fit properly with the specimen found, as in the case of the novel *Cyphellophora* species proposed here (section 4.3.4). On the other hand, others represented, in fact, cryptic fungi not only at the species level, as the new *Curvularia* or *Heliocephala* species proposed (section 4.3.1 and 4.3.2 respectively), but also at the genus level. In this sense, the characterization of dendryphiella-like isolates allowed us to propose the novel genus with the name of *Neodendryphiella* (section 4.3.3).

We introduce the genus *Neodendryphiella* on the bases of three new species, *N. mali*, *N. michoacanensis* and *N. tarraconensis*, isolated from various substrates (plant material, herbivore dung and soil) and collected in different countries (Italy, Mexico and Spain). Despite the resemblance of the unidentified isolate to those of the genus *Dendryphiella*, the phylogeny obtained with ITS and LSU sequences revealed that species of *Dendryphiella* and those dendryphiella-like isolates formed two separated lineages, being representatives of distinct genera into the pleosporalean family *Dictyosporiaceae*. Despite *Dendryphiella* species being considered common fungi inhabiting plant material (Ellis 1971, 1976), DNA data of its species is very scarce. So, we requested at the CBS collection (Utrecht, The Netherlands) several reference strains of *D. vinosa*, the type species of the genus, and *D. infuscans*, in order to get a more robust phylogeny of this group of fungi. It is important to mention that ex-

type strains of the respective species are not available in any public repository. However, to our surprise, many of these strains were misidentified. For instance, CBS 381.81 received as *D. infuscans* was re-identified as *Torula herbarum,* or the four strains received as *D. vinosa* were re-identified as *Drechslera biseptata* (CBS 117.14), *Dendryphiella paravinosa* (CBS 118716 and CBS 121797) and, based on our phylogenetic analysis, we found the last one (CBS 584.96) as representative of a new species in the genus, which was proposed as *D. variabilis*. These findings highlight the relevance of corroborating the identification of strains despite proceeding from reference centers, and the necessity of updating in those institutions the strain identification by sequence analysis, mainly of those strains deposited before the molecular era for fungal identification.

With respect to the genus *Curvularia,* we examined 14 isolates, two of them were from human clinical samples (leg wound and sphenoid sinus), originating from the USA, and the remaining isolates were from environmental substrates, nine from plant debris collected in Vietnam, one from a Mexican soil sample, one from herbivore dung collected in Spain, and another one from plant debris collected in Indonesia. Some of these isolates were identified as *Cu. geniculata*, *Cu. oryzae*, *Cu. alcornii*, *Cu. hominis*, *Cu. dactyloctenicola*, *Cu. eragrostidis* and *Cu. inaequalis* (Table 2) and confirmed molecularly by *gapdh* sequence comparison in the NCBI database through the BLAST tool. Nevertheless, six isolates showed a low percentage of identity (< 97 %) in respect to those sequences of *Curvularia* species deposited in that database, being therefore considered putative new species for the genus and confirmed by multi-locus analysis with the recommended barcodes (Marin-Felix et al. 2017a). After their phylogenetic delineation, we could establish morphological differences that allowed us to distinguish them microscopically from other *Curvularia* species usually producing 3-septate conidia. The species proposed from environmental samples were *Cu. paraverruculosa* and *Cu. vietnamensis*, and from the clinical specimens was *Cu. suttoniae*. Since no clinical data of the patients nor histopathological diagnosis for the latter species were available, its pathogenic role in human disease remains to be elucidated. It is important to highlight that only the correct identification of the fungi at the species level will allow us to delve into the knowledge of the biology of a species in particular. According to our data, this confidable species identification in *Curvularia* would be insured with sequence analysis of the *gapdh* gene marker, as the *ATPase* is for *Alternaria* or *tef*1 for *Cladosporium* species.

6. CONCLUSIONS

Identification and phylogeny of clinical and environmental isolates of Alternaria, Cladosporium and other genera of dematiaceous hyphomycetes
Isabel Iturrieta González

CONCLUSIONS

From the study of clinical and environmental isolates of the genus *Alternaria* we conclude that:

1. The molecular approach combining the sequences of the loci ITS, *ATPase*, *gapdh, rpb*2 and *tef*1 showed good results to delineate *Alternaria* species in section *Infectoriae*, while the combination of three or four of these loci has been enough to establish phylogenetic relationships and distinguish species in other *Alternaria* sections treated in the present study, such as *Chalastospora*, *Pseudoalternaria* and *Radicina*.

2. While analysis of the ITS barcode helps to identify *Alternaria* isolates at the section level, sequence analyses of *rpb*2 or *ATPase*, this latter especially for the section *Infectoriae*, are useful loci to identify isolates at the species level or detect undescribed lineages for the genus.

3. The polyphasic approach on the set of *Alternaria* isolates allowed to propose 13 new species: *A. aconidiophora, A. anthropophila*, *A. atrobrunnea, A. curvata, A. fimeti, A. guarroi*, *A. lawrencei, A. montsantina* and *A. pseudoventricosa* for the section *Infectoriae*; *A. alcampina* and *A. inflata* for the section *Pseudoalternaria*; *A. pobletensis* for the section *Chalastospora*; and *A. chlamydosporifera* for the section *Radicina*. Furthermore, 11 putative new species are being characterized to be formally proposed, most of them isolated from clinical specimens.

4. The new species *A. anthropophila, A. atrobrunnea* and *A. guarroi* have been demonstrated to cause cutaneous infections. Since their *in vitro* antifungal susceptibility profile seems to be species-dependent, the correct identification of *Alternaria* isolates at the species level could be relevant for the proper treatment of alternariosis.

5. From the set of *Alternaria* clinical isolates *A. rosae* and *A. kordkuyana* of the section *Pseudoalternaria* have been isolated for the first time from clinical specimens, but with unknown pathogenic role.

6. Since no clinical isolates of *A. infectoria* have been isolated in this study, we consider this species overestimated as human opportunis in the medical literature.

7. In the set of *Alternaria* isolates from environmental sources 27 species have been identified and distributed in seven sections: *Alternaria*, *Chalastospora*,

Infectoriae, *Ulocladium*, *Ulocladioides*, *Pseudoalternaria* and *Radicina*, being *A. alternata* of the former section the most prevalent species identified.

8. The herbivore dung, unlike soil and plant debris samples, has shown to be an excellent substrate to find *Alternaria* isolates of taxonomic interest. From the 23 species identified on the former substrate, eight were new for science (*A. altcampina*, *A. chlamydosporifera, A. curvata, A. fimeti, A. inflata, A. lawrencei, A. pobletensis* and *A. pseudoventricosa*) and other nine (*A. armoraciae*, *A. cantlous*, *A. heterospora*, *A. gaisen*, *A. kordkuyana*, *A. rosae*, *A. merytae*, *A. oregonensis* or *A. slovaca*) can be considered rare species since, to date, there are few specimens registered or they are only known from the type specimen.

From the study of environmental isolates of the genus *Cladosporium* and cladosporium-like fungi, we conclude that:

9. The *tef*1 is a suitable gene marker to identify and detect interesting *Cladosporium* species, and the combination of ITS, *act* and *tef*1 is necessary to characterize novel species in the genus.

10. The set of isolates of *Cladosporium* s. str. were identified in 37 known species, in addition to eight, which were found to be new species for science; seven described and proposed as *C. caprifimosum*, *C. coprophilum*, *C. fuscoviridum, C. lentulum, C. michoacanense, C. pseudotenellum* and *C. submersum,* and an eighth that are still under phenotypic characterization.

11. Among the known species identified *C. angustiherbarum, C. australiense, C. chubutense*, *C. lebrasiae, C. ipereniae*, *C. phaenocomae*, *C. rugulovarians*, *C. silenes* and *C. subuliforme* can be considered rare species since, to date, there are few specimens registered or they are monotypic.

12. All the species identified have been distributed among three species complexes, the *C. cladosporioides* complex being the most rich in species and *C. cladosporioides* the most prevalent species among the set of cladosporia studied, followed in order of frequence by *C. ramotenellum, C. sphaerospermum*, *C. macrocarpum*, *C. floccosum, C. xylophilum, C. halotolerans, C. perangustum* and *C. pseudocladosporioides*.

13. Herbivore dung and soil samples have shown slightly higher species diversity than plant debris samples, but since the spectrum of species found in each type is different, they can be considered interesting substrates to continue exploring the biodiversity of the genus *Cladosporium*.

14. Five cladosporium-like fungi, mostly from plant debris, have been described as new species and resolved their taxonomic position on the bases of LSU and ITS phylogeny. They are: *Apenidiella foetida* placed into the family *Teratosphaeriaceae (Capnodiales*), *Matsushimaea monilioides* in the family *Sympoventuriaceae* and *Venturia submersa* in the family *Venturiaceae*, both genera allocated in the order *Venturiales*, and *Pseudopenidiella gallaica* and *P. vietnamensis* placed into the family *Microthyriaceae* (*Microthyriales).*

From the study of other dematiaceous genera included in the present thesis we concluded that:

15. Phylogenetic analysis of the ITS and LSU regions has allowed us to propose the new genus *Neodendryphiella* in the family *Dictyosporiaceae* (*Pleosporales*), with the three new species, *N. tarraconensis*, *N. michoacanensis* and *N. mali*, and the closely related new species *Dendryphiella variabilis*; as well as to describe the new species *Heliocephala variabilis* allocated in the family *Microthyriaceae*.

16. Multi-locus sequence analysis of ITS, *gapdh* and *tef*1 of the interesting isolates belonging to the genus *Curvularia* has allowed us to propose three new species *Cu. paraverruculosa* and *Cu. vietnamensis* isolated from soil and plant debris respectively, and *Cu. suttoniae* isolated from human clinical specimens.

17. Multi-locus sequence analysis of ITS, LSU and *tub*2 loci has allowed us to describe the novel species *Cyphellophora vietnamensis* and to propose two new combinations in the genus *Cy. attae* and *Cy. capiguarae,* based on two species previously classified in the genus *Phialophora*.

18. Since many of the interesting fungi investigated in the present study have been isolated directly from the natural substratum (herbivore dung, soil and plant material) incubated in moist chambers or from baiting technique plates, these should be procedures of choice for futures studies of dematiaceus hyphomycetous diversity.

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7. REFERENCES

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