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TAXONOMIC DESCRIPTION

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Gongronella eborensis sp. nov., from vineyard soil of Alentejo (Portugal)

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Abstract

This study describes a novel fungal species belonging to the genus *Gongronella*. During a previous work focusing on metalaxyl degradation by *Mucorales* strains, two isolates from vineyard soil samples collected in the Alentejo region, south Portugal, were identified as a putative novel species based on combined molecular and MALDI-TOF MS data. This new species is described here using a polyphasic approach that combines morphology, internal transcribed spacer of ribosomal DNA (ITS) and 28S ribosomal DNA (LSU) sequence data analysis and proteomic profiling by MALDI-TOF MS. Phenotypic and molecular data enabled this novel species to be clearly distinguished from other *Gongronella* species with results of combined ITS+LSU analysis showing that the *Gongronella* species is related to *Gongronella butleri* and *Gongronella brasiliensis*. Therefore, from the results of morphological and molecular analyses, isolates MUM 10.262 and MUM 10.263 seem to represent a new *Gongronella* species and the name *Gongronella eborensis* sp. nov. is proposed, with the ex-type strain MUM 10.262 (=CCMI 1100=CBS 128763).

INTRODUCTION

Within the Filo Mucoromycota of the Mucoromyceta subkingdom [1], the order *Mucorales* comprises predominantly terrestrial, ubiquitous and fast-growing saprotrophic fungal species. Some strains have been described as beneficial to plant growth [2], others display characteristics that allow their use in food fermentation [3, 4] and some are responsible for spoilage of foodstuff [5]. Like other filamentous fungi, *Mucorales* have an important role in nature as decomposers, including the potential to degrade xenobiotic or recalcitrant compounds, solubilize non-soluble minerals and potential for enzyme production [6–9], which makes them important candidates for biotechnological applications such as bioremediation processes or production of industrially relevant enzymes. Finally, within *Mucorales*, several species are described as etiological agents of human opportunistic infections (mucormycosis) showing an alarming increase of report cases in recent years [10].

Gongronella has a worldwide distribution with moderate frequencies in subtropical regions and warm climates [11, 12]. The Alentejo region in Portugal has a Mediterranean climate characterized by hot and dry summers. Species belonging to this genus have been isolated from arable soils, marshes, wasteland, anthills, garden soil and palm plantations [13–19]. Taxonomy is mainly based on morphological characters: coenocytic hyphae and asexual reproduction based on specialized structures (sporangia) with constriction between apophysis and sporangium, and most species form zygospores as result of sexual reproduction. Nevertheless, the highly similar morphological characteristics hinder classification and identification.

More recently, with the application of DNA-based approaches, several coordinated efforts to improve species identification have taken place. Examples include the Fungal Barcoding Consortium [20] in proposing a universal DNA barcode

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Keywords: *Cunninghamellaceae*; ITS; LSU; *Mucorales*; phylogeny; taxonomy.
Abbreviations: ATCC, American Type Culture Collection; CBS-KNAW, Westerdijk Fungal Biodiversity Institute; CCMI, Culture Collection of Industrial Microorganisms; CGMCC, China General Microbiological Culture Collection Center; DSM, DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH; EML, Environmental Microbiology Laboratory Fungarium; IMI, CABI culture collection (IMI-International Mycological Institute); LSHB, London School of Hygiene and Tropical Medicine; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MUM, Micoteca da Universidade do Minho; MUM-H, Micoteca da Universidade do Minho Herbário; NCBI, National Center for Biotechnology Information; NRRL, Agricultural Research Service Culture Collection; QM, Quartermaster Research and Development Center; UNESCO, United Nations Educational, Scientific and Cultural Organization; URM, University Recife Mycology (Federal University of Pernambuco-UFPE).
The GenBank accession numbers of strain MUM 10.262^T (CBS 128763, CCMI 1100) are ITS region sequence KT809408; LSU region sequence MN947301. The GenBank accession numbers of strain MUM 10.263 (CCMI 1101) are ITS region sequence GU244500; LSU region sequence MN947302.
One supplementary file is available with the online version of this article.

Table 1. Strains used for phylogenetic analysis of the two isolates of a new *Gongronella* species isolated from vineyard soil samples in Alentejo, Portugal

GenBank accession numbers of the ITS and LSU sequences used to reconstruct the phylogenetic trees Fig. 1. T, type strain; NT, neotype strain; sequences in bold were generated in the present study.

Species	Culture collection number	GenBank accession numbers	
		ITS	LSU
***Gongronella eborensis* sp. nov.**	**MUM 10.262^{T}=CCMI 1100^{T}=CBS 128763^{T}**	KT809408	**MN947301**
	MUM 10.263=CCMI 1101	GU244500	**MN947302**
Gongronella brasiliensis	URM 7487^{T}	NR_155148	KY114932
	URM 7488	KY114931	KY114933
Gongronella butleri	CBS 216.58^{T}=IMI 071628^{T}=LSHB BB321^{T}=QM 7847^{T}	MH857761	MH869292
	CBS 415.67	MH859014	MH870714
Gongronella guangdongensis	CGMCC 2.15212^{T}	NR_158464	**MN947303**
	CGMCC 2.15213	KC462740	**MN947304**
Gongronella koreana	EML-TS2Bp^{T}	KP636529	KP636530
	EML-TS2Bp-2	KP835545	KP835542
Gongronella lacrispora	ATCC 24412^{T}=MUM 10.258^{T}=CBS 244.62^{T}=DSM 1169^{T}=NRRL 2643^{T}	GU244498	JN206609
Gongronella orasabula	EML-QF 12-1^{T}	NR_148087	KT936263
	EML-QF 12–2	KT936270	KT936264
Gongronella sichuanensis	CGMCC 3.19651^{T}	MK813373	MK813855
	CGMCC 3.19652	MK813374	MK813856
	CGMCC 3.19653	MK813375	MK813857
Gongronella zunyiensis	CGMCC 3.19899^{T}	MN453856	MN453853
	CGMCC 3.19900	MN453857	MN453854
	CGMCC 3.19901	MN453858	MN453855
Circinella lacrymispora	CBS 101757^{T}	NR_103650	NG_066157
Cunninghamella echinulata	CBS 156.28^{NT}	JN205895	MH877699

marker for fungi, genetic diversity inventories of major taxonomic groups such as the one published by Walther *et al.* [21] on *Mucorales* DNA barcoding and the recent Westerdijk Fungal Biodiversity Institute barcoding project [22]. These tools have been used to assist species delimitation and have led to an increase of the number of newly described taxa. In fact, six out of the eight currently recognized *Gongronella* species have been described in the last 5 years [14–19].

As part of a study that investigated the ability to degrade metalaxyl by different *Mucorales* species isolated from vineyard soil collected in Alentejo (South Portugal), two *Gongronella* isolates could not be assigned to previously described species [23, 24]. Here we report the polyphasic approach that was applied to describe a novel *Gongronella* species, *Gongronella eborensis* sp. nov. (*Cunninghamellaceae*, *Mucorales*).

METHODS

Fungal isolates from vineyard soil samples

Soil samples were obtained from two adjacent vineyards fields in the Alentejo region (Évora, South Portugal) that were subjected, during 16 (site A, 38° 29′ 39.0″ N 7° 34′ 37.3″ W) and 8 (site B, 38° 29′ 41.5″ N 7° 34′ 41.6″ W) years, respectively, to annual applications of a commercial fungicide containing metalaxyl as described in Martins *et al.* [23]. Two isolates, mycelium-3A from site A and mycelium-3B from site B, were isolated following the isolation and identification of soil microbial populations protocol previously described in [23]. Both isolates are deposited in Micoteca da Universidade do Minho (MUM) culture collection, Braga, Portugal, with the accession numbers MUM 10.262 (mycelium-3A) and MUM 10.263 (mycelium-3B). The ex-type strain (MUM 10.262) is also deposited at CCMI (Culture Collection of Industrial Microorganisms, Lisbon, Portugal) with accession number CCMI 1100 and at CBS-KNAW (Westerdijk Fungal Biodiversity Institute) with accession number CBS 128763.

Morphological characterization

For morphological analysis, fungal strains were inoculated in 60 mm potato dextrose agar (PDA) plates and grown at 25 °C for 5 days in the dark. Digital images of the colonies were obtained using a Canon EOS 5D Mark II camera as described elsewhere [25]. Colours and codes presented in the description are those from the Methuen Handbook of Colour [26]. Wet mounts were prepared by staining the fungal samples with lactophenol cotton blue (Merck) and the slides were examined under light optical microscope using a Leica DM5000B apparatus to describe and measure micro-morphological characteristics. Samples were prepared for scanning electron microscopy according to [27] and were added to aluminium pin stubs with electrically conductive carbon adhesive tape (PELCO Tabs) on a Phenom Standard Sample Holder (SH) at 5 or 10 Kv and a spot size of 3.3. The samples were 10 Angstrom Au coated. Characterization was performed using a desktop scanning electron microscope (Phenom ProX). Results were acquired using the ProSuite software version 3.0.

Fungal molecular identification

Internal transcribed spacer of ribosomal DNA (ITS) sequence data was previously obtained for the two *Gongronella* isolates MUM 10.262 and MUM 10.263 [24]. Partial 28S ribosomal DNA (LSU) sequence data for the two *Gongronella* isolates and for *G. guangdongensis* CGMCC 2.15212 and CGMCC 2.15213 strains were obtained as follows. Amplification was

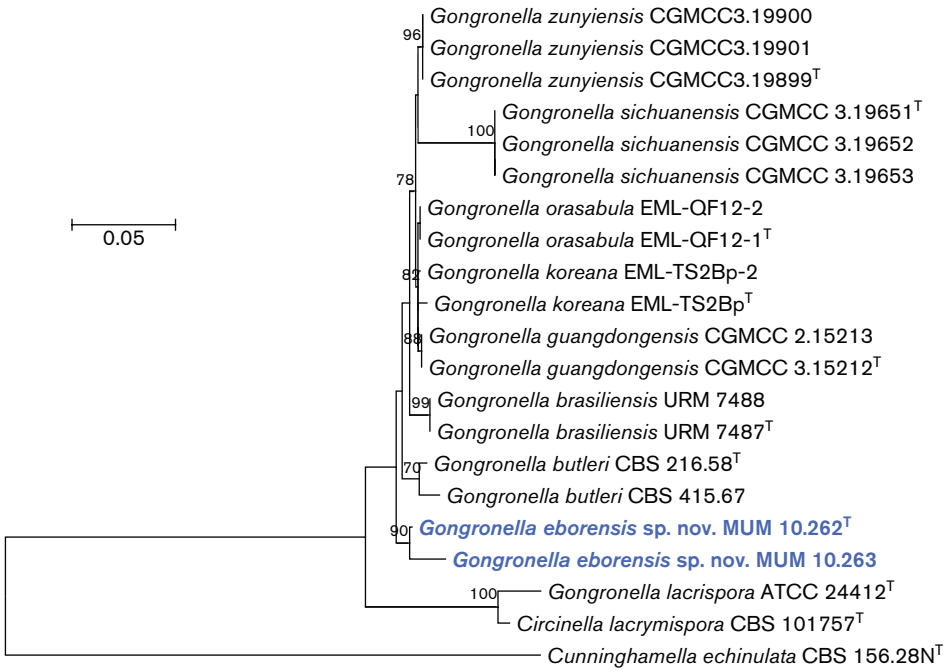

Fig. 1. Combined phylogeny for ITS and LSU sequence data of the two *Gongronella eborensis* sp. nov. isolates (MUM 10.262^T and MUM 10.263) with other *Gongronella* species detailed in Table 1. *Cunninghamella echinulata* CBS 156.28 was used as an outgroup. Selected model: T92+G. The percentage of trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches. Bold branches are supported with ≥0.95 Bayesian posterior probability. The tree is drawn to scale with branch lengths measured in the number of substitutions per site. All positions with less than 95% site coverage were eliminated. There were a total of 1091 positions in the final dataset. T, ex-type strain; NT, neotype strain.

performed using 0.2 µm LR0R/LR5 primers [28], 1×VWR Taq DNA Polymerase Master Mix with 1 mM $MgCl_2$ (VWR) and approximately 50 ng template DNA in a 50 µl reaction volume. The used PCR (Biorad) cycling protocol was: 95 °C for 5 min; 35 cycles of 95 °C for 1 min, 56 °C for 45 s, 72 °C for 90 s; 72 °C for 10 min. Amplification success was verified in 1% agarose gel and obtained amplicons were purified using the NZYGelpure kit (NZYtech) and sent for Sanger sequencing to Stab Vida Lda (Madan Parque). Phylogenetic analysis was performed through analysis of ITS and LSU data of the *Gongronella* isolates MUM 10.262 and 10.263 against different *Gongronella* species sequences retrieved from the NCBI database (Table 1). Alignment was performed using MUSCLE [29] followed by visual inspection and, when necessary, manual correction using MEGA7.0 [30]. Poorly aligned positions and divergent regions were eliminated using the Gblocks tool [31]. Data sets were concatenated using the online tool *FaBox* [32] in order to perform a multigene phylogeny reconstruction. The most suitable substitution model was determined based on the lowest Bayesian information criterion. Maximum-likelihood (ML) trees based on Tamura three-parameter [33] or Kimura two-parameter [34] substitution models and 1000 bootstrap replications [35] were calculated using MEGA7.0. All positions with less than 95% site coverage data were eliminated. Estimates of evolutionary divergence between sequences were calculated in MEGA7.0 using the same model. Bayesian posterior probabilities (pp) of branches were computed in MrBayes 3.2 [36] using settings for the best-fit model selected by the Akaike information criterion in MrModeltest 2.4 [37] (HKY+G for ITS analysis, GTR+G for LSU and the multigene analyses).

RESULTS AND DISCUSSION

Molecular characterization

To predict the phylogenetic placement of MUM 10.262 and 10.263 within the genus *Gongronella*, individual and concatenated sequences of ITS and LSU were aligned against those of the available species (Fig. 1, File S1, available in the online version of this article).

In 2012, ITS was proposed as the universal DNA barcode for fungal species [20] and some studies have shown its discriminative power in *Mucorales* [38–40]. Nevertheless, a biodiversity study of *Mucorales* has shown that, for some groups, this region can be variable to a degree where alignment is impaired and sister species are not confidently grouped, with LSU being an adequate alternative marker [21].

The use of rDNA regions for fungal identification has several advantages when compared with the use of functional genes [41]. As these regions are under different rates of evolution, varying levels of genetic variation can be observed. ITS, due to the presence of sequences that are not necessary for ribosome

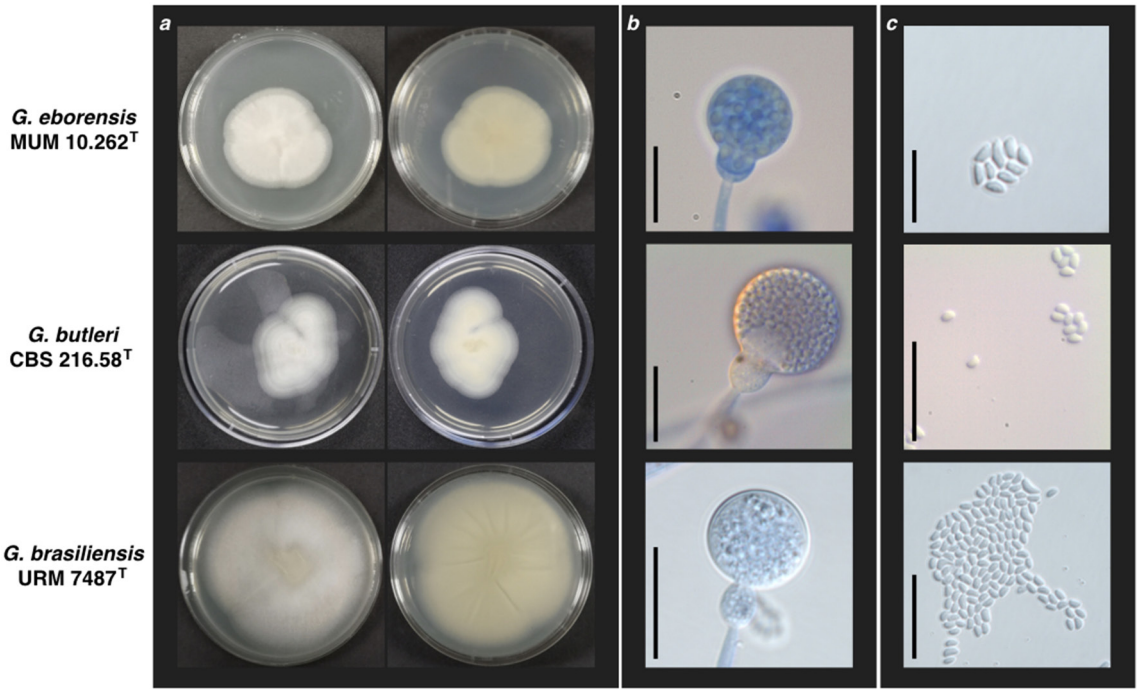

Fig. 2. Morphological comparison of *Gongronela eborensis* sp. nov. MUM 10.262 with closely related *G. butleri* CBS 216.58 and *G. brasiliensis* URM 7487. (a) Colonies on PDA at 25 °C, 5 days: obverse on the left and reverse on the right. (b) Sporangia under light microscope (Bars, 20 µm). (c) Sporangiospores under light microscope (bars, 20 µm).

function, experience lower evolutionary pressure, leading to higher sequence variability that enables the discrimination at species level [42, 43]. In fact, it is estimated that ITS barcoding is effective for species discrimination across more than 70% of the fungi tested [20]. On the other hand, LSU evolves more slowly and is more informative for intermediate taxonomic levels [42]. Nevertheless, it has been shown to be a valuable marker by itself or when combined with ITS [20, 41, 44]. When looking to the single-region trees in File S1, the differences in discrimination power between ITS and LSU are noteworthy. While ITS resolves all species in monophyletic clades supported by more than 0.95 pp (except *G. koreana*), LSU is not able to differentiate the majority of species, creating a group (even though it is not statistically supported) that includes all five species originally described from Asian countries. In both trees, the *Gongronella* isolates (MUM 10.262 and 10.263) are grouped in a well-defined and supported monophyletic branch (bootstrap support of 87% and >0.95 pp).

As reviewed by Walther *et al.*, *Mucorales* genomes are highly dynamic and the number of studies that use at least two unlinked markers is low [45]. For that reason, although it is not an optimal situation, molecular based taxonomy in *Mucorales* usually only considers a single locus, normally ITS alone or combined with LSU [45]. In fact, we were confronted by the lack of reference sequences for other loci from *Gongronela* species, a situation that has been previously reported [18, 45]. Nevertheless, we believe that the combination of both ITS and LSU rDNA regions can improve the accuracy of the phylogenetic placement by increasing the number of analysed bases. As suggested by Porras-Alfaro *et al.*, longer sequences provide a higher discriminatory power than shorter sequences, for either the ITS or LSU regions, regardless of the classification method [41].

The combined ITS+LSU phylogenetic analysis presented here comprised 21 nucleotide sequences and a total of 1091 positions in the final data set. In the obtained phylogenetic tree (Fig. 1), with the exception of *G. koreana*, isolates for each *Gongronella* species were monophyletically grouped with all being well supported by either the bootstrap values (>70%) or the Bayeasian posterior probabilities (>0.95). The two new *Gongronella* strains clustered in a group with 90% of bootstrap support and 1.00 posterior probability, being more closely related to *G. butleri* and *G. brasiliensis*.

The use of MALDI-TOF MS has recently been shown to be a reliable and rapid tool to identify *Mucorales* at species level [46]. Previously reported proteomic data of the two *Gongronella* isolates MUM 10.262 and 10.263 [24] defined them as a putative new species despite differences between the spectra of both strains were observed, which is now supported by the combined phylogenetic analysis of ITS and LSU regions. Also worth noticing is that, despite no differences were observed between the two LSU sequences, the number of base substitutions per site between ITS sequences of *Gongronella* isolates MUM 10.262 and 10.263 was of 3.62%, which is on

Table 2. Comparison of micro-morphological characters of *Gongronella eborensis* MUM 10.262^T with closely related taxa

	G. eborensis	*G. brasiliensis**	*G. butleri*†
Sporangiophores	Erect, 46–94×1.5–3.0 µm, irregularly branched in monopodial fashion, smooth-walled, hyaline, one septum below the apophysis	Erect, straight or slightly recumbent, 26.5–320×2.5–5 µm, smooth-walled, hyaline, one or two septa below the apophysis	Erect, 40–156(–340)×2–6(–7) µm, smooth to very faintly roughened, hyaline, branching irregularly or simply, one septum below the apophysis
Sporangia	Globose to subglobose, with many spores, wall thin and smooth, 7.5–16×7.0–13.0 µm, apophysis globose to subglobose 3.5–6.5×3.0–7.0 µm, with columellae hemispherical to subglobose and collarette	First yellowish then becoming light brownish, globose, subglobose, 9.5–30 µm diam., smooth-walled, some leaving collars, apophysis, smooth-walled, variable in shape, globose, (3–)4–5(–6) µm diam., vase-shaped, (3–)4×12 (–14.5) µm and ellipsoidal, 5–10(–12)×3–7(–8.5) µm, columellae globose, subglobose, (3–)4–8(–9) µm or conical-cylindrical, 1.5–2.5×2–3 µm, some with an evident collar	Globose, white to yellow-tinted or faintly greyish olive in reflected light, many spores, wall thin and smooth, 7–32 µm in diam, apophysis 3.3–13(–32) µm in diam and 4.5–11 µm in length, hemispherical to urn-shaped, smooth, columellae hemispherical or dome-shaped, and with typically distinct collars
Sporangiospores	Reniform to fusiform-elliptical, hyaline, smooth-walled, 2.6–3.8×1.2–1.6 µm in diameter	Variable in shape, hyaline, smooth-walled, some containing oil droplets, reniform, 1.5–4×1.5–2.5 µm, ellipsoid to fusiform, 2–6.5×1.5–3 µm, ellipsoid with a flattened end, some almost falciform, 2.5–7.5×1.5–4 µm	Oval to flattened on one side to almost reniform, hyaline, thin-walled, smooth, 2.2–4.5×1.6–2.5 µm, typically 3.5 µm long
Chlamydospores	Not observed	Present in the aerial mycelium, globose, subglobose and doliiform	Present in substrate mycelium, globose to irregularly ovoid
Zygospores	Not observed	Not observed	18–36 µm in diam, regularly globose, roughened with short blunt spines about 2 µm long, brown to dark brown

*Data derived from Tribpromma *et al.* in [17].
†Data derived from Hesseltine and Ellis in [48].

the lower-end range of the ITS intraspecific variability values observed by Walther *et al.* [21]. In fact, the authors suggested that this variability could be relatively high in *Mucorales* (maximum observed of 13.3% in *Cunninghamella echinulata*) even in well-defined and sampled taxonomic groups (>5% dissimilarity in *Lichtheimia*) [21]. Unfortunately, no values for the genus *Gongronella* were presented in that study.

The rate at which new *Gongronella* species are being described (six species in the last 5 years plus the present study) reinforces the ideas of Hoffmann *et al.* [47] and Tibpromma *et al.* in [17] that state that more *Gongronella* isolates need to be studied and that the extensive use of molecular tools is of great aid to species delimitation, especially in this taxon.

It is also interesting to see that, when analysing individual and combined ITS and LSU sequences (Fig. 1, File S1), the ex-type strain of *Circinella lacrymispora* clusters with the ex-type strain of *G. lacrispora* in a branch supported by 100% of bootstrap replicates and 1.00 pp. This is in accordance with and further supports the conclusion of Walther *et al.* [21] that conspecificity between *C. lacrymispora* and *G. lacrispora* should be considered and the taxonomy of *C. lacrymispora* revised.

Macro- and micro-morphology analyses

Traditionally, *Mucorales* taxonomy is based on strain morphological characterisation including colour and texture of the colonies as well as their vegetative and reproductive structures. The new *Gongronella* species described here is characterized by white colonies and slow growth rates. Colonies reach approximately 30 mm in diameter after 5 days growth at 25 °C on PDA plates, taking up to 15 days to cover the full plate. Micro-morphologically, this species is characterized by branched and erect sporangiophores falling down in the well-disseminated concept of 'pin moulds' for these taxa, sporangia globose with constriction between apophysis and sporangium (this being a key characteristic of the genus *Gongronella*). Comparison of its macro-morphology with that of related species (Fig. 2) is in accordance with the phylogenetic analysis results. *Gongronella* sp. MUM 10.262 and 10.263 colonies resemble those of *G. butleri* type strain in their size and texture but there are slight differences in the reverse colony colour, with *G. butleri* colonies being closer to white than the yellowish white/pale colonies of *Gongronella* species. When the same comparison is done with the *G. brasiliensis* type strain, major differences in colony texture and growth rates are observed. Micro-morphology observation supports the differentiation of *Gongronella* species (Table 2, Fig. 3). In comparison with *G. butleri* and *G. brasiliensis*, sporangia are smaller and the apophysis is not only smaller but also differently shaped. Sporangiospores are more similar to *G. butleri* (reniform to fusiform-elliptical in *Gongronella* species) and contrast with the more variable ones of *G. brasiliensis*, however they are smaller in both cases.

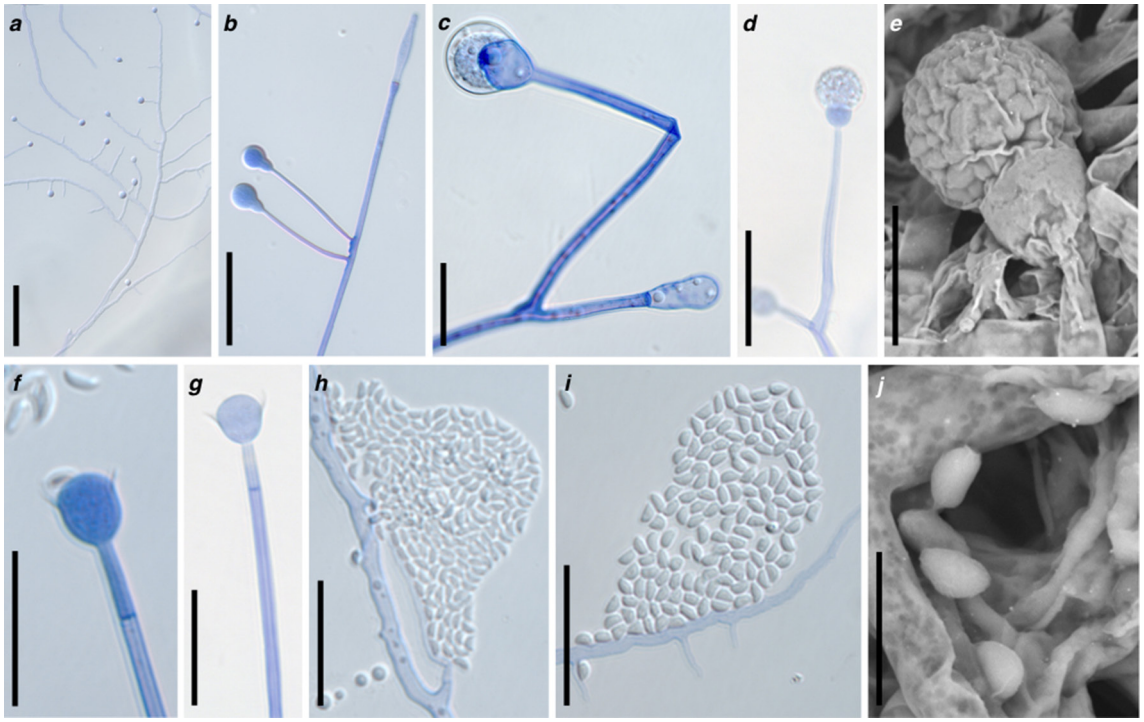

Fig. 3. *Gongronella eborensis* sp. nov. micro-morphological characters under light microscopy and as observed using scanning electron microscopy. (a) MUM 10.262 branching pattern with young sporangiophores; (b) MUM 10.262 sporangiophores with apophysis; (c) MUM 10.262 mature sporangiophore and columellae; (d) MUM 10.263 sporangiophore with apophysis; (e) MUM 10.262 mature sporangiophore; (f) MUM 10.262 columellae with collarette and septum; (g) MUM 10.263 columellae with collarette and septum; (h) MUM 10.262 sporangiospores; (i) MUM 10.263 sporangiospores; (j) MUM 10.262 sporangiospores. Bars: (a,b) 50 µm; (c,d,f,g,h,i) 20 µm; (e) 8 µm; (j) 5 µm.

Incidence and ecology

Gongronella strains MUM 10.262 and 10.263 were isolated near Montoito village (Évora, Portugal) from soil samples of vineyards fields submitted to repeated annual metaxyl treatments (16 and 8 years, respectively). Sampling sites can be defined as predominantly Mediterranean luvisols and more details regarding the physicochemical properties can be found in [23]. Recorded values from 1981 to 2010 show that the average lower temperature in the coldest month is around 6 °C and that the average maximum is around 31 °C in the hottest, with temperatures reaching values as high as 46 °C in certain years. Average monthly precipitation values vary from 4.1 mm to 95.1 mm (air temperature and precipitation data obtained from www.ipma.pt/pt/oclima/normais.clima/1981-2010/007/, accessed on 17/01/2020).

Until now, *Gongronella* strains MUM 10.262 and 10.263 have only been found in soils treated with repeated applications of a commercial fungicide (metalaxyl). MUM 10.262 has been previously submitted to metalaxyl sensitivity, tolerance and dissipation studies [23, 24] that have shown that this strain has high tolerance (up to 1 gl^{-1}) and ability to degrade and dissipate metalaxyl. Martins *et al.* [24] suggested that there could be secondary metabolism alterations to produce the necessary enzymes to degrade this fungicide. Since both *Gongronella* strains MUM 10.262 and 10.263 have been exposed to metalaxyl for different periods of time, one can assume that the differences found between the MALDI-TOF MS spectra [24] are a consequence of different stages of metalaxyl adaptation. The existence of proteomic variation is also a reflection of genomic variability at the intraspecific level, as exemplified by the observed variability detected in the ITS region.

These current results suggest that MUM 10.262 has great potential and it should be further explored for applications of soil bioremediation and for metalaxyl-degradation.

DESCRIPTION OF *GONGRONELLA EBORENSIS* M.R. MARTINS, C. SANTOS, C. SOARES, C, SANTOS & N. LIMA SP. NOV.

Gongronela eborensis (e.bo.ren´sis. N.L. fem. adj. *eborensis* pertaining to Ebora, which was an old Romanic municipality named by the emperor Julius Caesar. Currently, the Portuguse city name is Évora. The city has been classified as a World Heritage site since 1986 by UNESCO and is the capital of the Alentejo region where this species was collected) Figs 2 and 3.

Typus. Portugal, region of Alentejo, district of Évora, vineyard soil from Montoito village, isolated by Maria Rosário Martins

and Pablo Pereira as mycelium-3A in October 1999 (holotype MUM-H 10.262, culture ex-type MUM 10.262).

Diagnosis. Slow growth on PDA medium, branched and erect sporangiophores, sporangia globose to subglobose with columellae present and sporangiospores reniform to fusiform-elliptical, hyaline and smooth. Chlamydospores and zygospores not observed.

Description. Colony diameter on PDA, 5 days at 25 °C: 28–32 mm.

Colony characteristics. Colonies raised, margins low, wide and entire; texture lanose; white (A1) colony obverse; yellowish white to pale (2A2) colony reverse; rhizoids absent.

Micro-morphology. Sporangiophores erect, branched 46–94×1.5–3.0 µm long, hyaline, smooth, always with a septum under the apophysis, branching irregularly or simply. Sporangia 7.5–16.0×7.0–13.0 µm, globose to subglobose with columellae (11.5–5.5×8.2–3.2 µm), hemispherical to subglobose, hyaline and many spores, sporangial wall thin and smooth, apophysis globose to subglobose 3.5–6.5×3.0–7.0 µm diameter, hyaline. Sporangiospores reniform to fusiform-elliptical, 2.6–3.8×1.2–1.6 µm, hyaline and smooth. Rhizoides, chlamydospores and zygospores not observed.

ITS barcode. KT809408 (alternative markers: LSU=MN947301).

Other isolate examined. MUM 10.263 isolated by Maria Rosário Martins and Pablo Pereira as mycellium-3B in October 1999 from vineyard soil, Montoito village, district of Évora, region of Alentejo, Portugal. ITS barcode: GU244500 (alternative markers: LSU=MN947302).

Mycobank number: MB834083

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Author contributions
M.R.M. isolated and performed the first strain characterizations; Ce.S. and Cl.S. performed additional phenotyping characterizations; M.R.M. and Ca.S. performed molecular analysis; M.R.M. and N.L. were responsible for obtaining funds for the wet work; M.R.M., Ca.S. and N.L. drafted the original version of the manuscript. All authors equally reviewed the final manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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