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Neooccultibambusa jonesii, a novel taxon within Occultibambusaceae

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Abstract

A new species of *Neooccultibambusa*, *N. jonesii*, is introduced from *Ammophila arenaria* collected in Italy. The novel taxon is characterized by immersed to semi-immersed ascomata in fissures of bark, a two-layered peridium, hyaline to brown ascospores and in producing chlamydospores in culture; these characters are typical of *Neooccultibambusa*. Multigene phylogenies based on analysis of 28S, 18S and RPB2 sequence data, indicate that *N. jonesii* belongs in the family Occultibambusaceae and is closely related to *N. chiangraiensis*, but is phylogenetically distinct. *Neooccultibambusa jonesii* can be differentiated from *N. chiangraiensis* based on the number of septa, the larger and mature dark brown ascospores and in lacking a mucilaginous sheath. We provide a description and photoplates of the new species and compare it with related species, together with phylogenetic inferences based on combined sequence data.

Keywords – chlamydospores – Dothideomycetes – monocotyledons – RPB2–rDNA phylogeny – grass

Introduction

The family Occultibambusaceae was introduced by Dai et al. (2017) with *Occultibambusa* as the type genus. The family comprises four genera including *Occultibambusa*, *Seriascoma* and *Versicolorisporium* (Dai et al. 2017) and *Neooccultibambusa* (Doilom et al.

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2017). These taxa are saprobic on monocotyledons (Dai et al. 2017), such as bamboo, but also have been found on hardwoods, such as teak (*Tectona grandis*, Doilom et al. 2017).

The grass family (Poaceae, or Gramineae) is one of the most economically important plant families (Soreng et al. 2015). It provides human food in the form of cereals (for example, wheat, rice, barley, oats, millet, maize, sorghum) and sugar (sugar cane) (Raven & Johnson 1995, Sarwar et al. 2013). In addition, grasses feed cattle, provide the basis for most alcoholic drinks, as well as building materials (bamboo), thatch, and straw (Sarwar et al. 2013). A number of grasses yield essential oils (lemongrass) and raw materials for cosmetics (oats) (Paul & Julie 2003). The fungi on grasses are also thought be induce respiratory diseases (Purahong & Hyde 2011). Considering the importance of grasses, it is paramount that we understand the fungi that grow on grasses (Wong & Hyde 2001). Although there have been many minor studies on graminicolous fungi (Wong & Hyde 2001), it is only recently that their study has linked morphology and phylogeny (Dai et al. 2012, 2014a, b, c, Liu et al. 2011, 2012, 2014, 2015, Phookamsak et al. 2014, Wijayawardene et al. 2014).

Species of Occultibambusaceae are characterized by immersed, solitary to gregarious ascomata with black ostioles, broadly cylindrical to clavate, bitunicate asci, cellular pseudoparaphyses and broad-fusiform, hyaline to dark brown ascospores, with 1–3 septa. The asexual morphs generally are coelomycetous and morphologically diverse (Hatakeyama et al. 2008, Dai et al. 2017). In the present article, a novel *Neooccultibambusa* species differing from *N. chiangraiensis*, the generic type, is introduced based on phenotypic characteristics and supported with phylogenies generated from DNA sequence analyses.

Material and methods

Sample collection and specimen examination

Fresh materials from dead culms of *Ammophila arenaria* (Poaceae), collected in the Province of Ravenna, Italy in February 2016, were observed using a Motic SMZ 168 Series microscope. Hand sections of fruiting structures were mounted in water for microscopic studies and photomicrography. The fungus was examined with a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 450D digital camera connected to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for the Figures were processed with Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA). Isolations were carried from single ascospores, following a modified method of Chomnunti et al. (2014).

Voucher specimens are deposited in the herbarium of Mae Fah Luang University (Herb. MFLU) and the living cultures are deposited in culture collection of Mae Fah Luang University (MFLUCC), Thailand and Culture Collection of Kunming Institute of Botany (KUMCC) [the latter under Material Transfer Agreement (MTA)]. Faces of fungi and IF numbers are registered as in Jayasiri et al. (2015) and Index Fungorum (2016). New species establishment are based on recommendations as outlined by Jeewon and Hyde (2016).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from the growing mycelium after 14 days grown on MEA at 18ºC using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®) following the manufacturer's protocol (Hangzhou, P.R. China). DNA amplifications were performed by Polymerase Chain Reaction (PCR). The partial large subunit nuclear rDNA (LSU) was amplified with primer pairs LROR and LR5 (Vilgalys and Hester, 1990). The small subunit nuclear rDNA

(SSU) was amplified with primer pairs NS1 and NS4 (White et al. 1990). The RNA polymerase II second largest subunit (RPB2) gene was amplified with primers fRPB2 and fRPB2-7cR (Carbone and Kohn 1999, Liu et al. 1999, Sung et al. 2007).

The amplification procedure was carried in a 50 µl reaction volume containing 2 µl DNA, 25 µl PCR mix, 19 µl distilled water 2 µl of each primer. The PCR reactions for amplification of LSU, SSU and RPB2 were performed under standard conditions (White et al. 1990). Purification and sequencing of PCR products were carried at Shanghai Sangon Biological Engineering Technology and Services Co. (China). Newly generated sequences are deposited at NCBI GenBank under the accession numbers provided in Table 1.

Sequence alignment and phylogenetic analysis

LSU, SSU and RPB2 sequence data were verified before further analyses. All introns and exons were aligned individually. Ambiguously aligned regions with many leading or trailing gaps were excluded in alignments prior to tree building. Multiple sequence alignments were produced with MAFFT v. 6.864b (Katoh & Standley 2013) and further improved manually where necessary and datasets analysed under different optimality criteria as outlined by Jeewon et al (2002, 2003, 2013). The final phylogenetic tree used to infer the taxonomic placement of our new taxon was generated based on DNA sequence analyses of a concatenated dataset of LSU, SSU and RPB2.

Phylogenetic analyses were performed by using RAxML for maximum likelihood, PAUP v. 4.0b10 (Swofford 2002) for maximum parsimony and MrBayes v. 3.2 (Ronquist et al. 2011) for Bayesian analyses. Maximum-parsimony analysis was carried in order to obtain the most parsimonious tree. Trees were inferred using the heuristic search option with 1000 random sequence additions. Descriptive tree statistics for parsimony (Tree Length [TL], Consistency Index [CI], Retention Index [RI], Relative Consistency Index [RC] and Homoplasy Index [HI] were calculated for trees generated under different optimality criteria. Kishino-Hasegawa tests (KHT) (Kishino and Hasegawa 1989) were performed in order to determine whether trees were significantly different. Maximum parsimony bootstrap values equal or greater than 60 % are given near to each node (Fig. 1). A maximum likelihood analysis was performed at the CIPRES webportal (Miller et al. 2010) using RAxML-HPC2 on XSEDE (v 8.2.8) with default parameters and bootstrapping with 1000 replicates (Stamatakis 2006, Stamatakis et al. 2008). Maximum likelihood bootstrap values (MLBS) equal or greater than 60% are given at each node (Fig. 1).

An appropriate model of evolution was selected by using MrModeltest 2.2 (Nylander 2004) for each gene partition. Posterior probabilities (Rannala &Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2 (Ronquist et al. 2011). The Bayesian inference was conducted under different models for each partition of the matrix as evaluated by MrModeltest 2.2 (Nylander 2004). Six simultaneous Markov chains were run for 100000000 generations and every 1000th generation a tree was sampled. MCMC heated chain was set with a “temperature” value of 0.15. The distribution of log-likelihood scores was examined to determine stationary phase for each search and to decide if extra runs were required to attain convergence, using the program Tracer 1.5 (Rambaut & Drummond 2007). All sampled topologies beneath the asymptote (25%) were discarded as part of a burn-in procedure and the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree. Bayesian Posterior Probabilities (BPP) equal or greater than 0.90 is given near to each node (Fig. 1). Phylogenetic trees and data files were viewed FigTree v. 1.4 (Rambaut & Drummond 2008). Novel sequences generated in this study are deposited in

Table 1 Taxa used in the phylogenetic analysis and GenBank accession numbers (LSU, SSU and RPB2 sequence data) and species. The newly generated sequences are indicated in bold.

Species name	Family	Culture no.	Gene Bank accession no.		
			LSU	SSU	RPB2
Aigialus grandis	Aigialaceae	BCC 18419	GU479774	GU479738	GU479813
Aigialus mangrovis	Aigialaceae	BCC 33564	GU479777	GU479742	GU479816
Anteaglonium globosum	Anteagloniaceae	SMH 5283	GQ221911	NA	NA
Anteaglonium latirostrum	Anteagloniaceae	GKM 1119	GQ221874	NA	NA
Anteaglonium parvulum	Anteagloniaceae	SMH 5223	GQ221909	NA	NA
Anteaglonium abbreviatum	Anteagloniaceae	ANM 925a	GQ221877	NA	NA
Arthopyrenia salicis	Roussoellaceae	CBMAI 1330	JN903536	NA	NA
Arthopyrenia salicis	Roussoellaceae	CBS 368.94	AY538339	AY538333	GU371814
Bambusicola massarinia	Bambusicolaceae	MFLUCC 11-0389 ^{T}	JX442037	JX442041	KP761716
Bambusicola splendida	Bambusicolaceae	MFLUCC 11-0439 ^{T}	JX442038	JX442042	NA
Biatriospora marina	Biatriosporaceae	CY 1228	GQ925848	GQ925835	GU479823
Biatriospora sp.	Biatriosporaceae	E 11301D	LN626685	LN626675	LN626663
Biatriospora mackinnonii	Biatriosporaceae	CBS 110022	KF015609	GQ387553	KF015704
Biatriospora mackinnonii	Biatriosporaceae	CBS 674.75	KF015612	GQ387552	KF015703
Byssosphaeria salebrosa	Melanommataceae	SMH 2387	NA	NA	GU385162
Corynespora cassiicola	Corynesporaceae	CBS 100822	GU301808	GU296144	GU357772
Corynespora smithii	Corynesporaceae	CABI 5649b	GU323201	NA	GU371804
Decaisnella formosa	Lophiostomataceae	BCC 25617	GQ925847	GQ925834	GU479824
Delitschia chaetomioides	Delitschiaceae	SMH 3253.2	GU390656	NA	NA
Delitschia winteri	Delitschiaceae	AFTOL-ID 1599	DQ678077	DQ678026	DQ677975
Dendryphion europaeum	Torulaceae	CPC 22943	KJ869203	NA	NA
Dendryphion europaeum	Torulaceae	CPC 23231	KJ869202	NA	NA

Table 1 continued

Species name	Family	Culture no.	Gene Bank accession no.		
			LSU	SSU	RPB2
Elongatopedicellata lignicola	Roussoellaceae	MFLUCC 15-0642 T	NA	NA	NA
Fissuroma maculans	Aigialaceae	MFLUCC10-0886	NG042598	JN846734	NA
Halomassarina thalassiae	Trematosphaeriaceae	BCC 17054	GQ925849	GQ925842	NA
Halomassarina thalassiae	Trematosphaeriaceae	BCC 17055	GQ925850	GQ925843	NA
Halotthia posidoniae	Halotthiaceae	BBH 22481	GU479786	GU479752	NA
Helicascus nypae	Morosphaeriaceae	BCC36752	GU479789	GU479755	GU479827
Herpotrichia macrotricha	Melanommataceae	GKM 196N	GU385176	NA	NA
Hysterium angustatum	Hysteriaceae	CBS 236.34	NA	GU397359	FJ161117
Keissleriella cladophila	Lentitheciaceae	CBS 104.55	JX681090	GU296155	GU371735
Lentithecium fluviatile	Lentitheciaceae	CBS 122367	GU301825	GU296158	NA
Leptosphaeria biglobosa	Leptosphaeriaceae	CBS 303.51	GU301826	NA	NA
Leptosphaeria doliolum	Leptosphaeriaceae	CBS 505.75	GQ387576	GQ387515	KT389640
Longiostiolum tectonae		MFLUCC 12-0562	KU764700	KU712459	NA
Lophiostoma macrostomoides	Lophiostomataceae	GKM 1033	GU385190	NA	NA
Lophiotrema lignicola	Lophiotremataceae	CBS122364 T	GU301836	GU296166	FJ795462
Lophiotrema nucula	Lophiotremataceae	CBS 627.86	GU301837	GU296167	FJ795463
Massaria inquinans	Massariaceae	CBS122369	GU456322	GU456300	NA
Massarina velataspora	Morosphaeriaceae	BCC17059	GQ925852	GQ925841	NA
Mauritiana rhizophorae	Halotthiaceae	BCC 28866	GU371824	GU371832	NA
Melanommapulvis pyrius	Melanommataceae	CBS 124080	GU456323	GU456302	GU456350
Monotosporella tuberculata	Melanommataceae	CBS 256.84	GU301851	NA	NA
Montagnula opulenta	Didymosphaeriaceae	CBS 168.34	NG027581	NA	NA
Morosphaeria ramunculicola	Morosphaeriaceae	JK 5304B	GU479794	GU479760	GU479831

Table 1 continued

Species name	Family	Culture no.	Gene Bank accession no.		
			LSU	SSU	RPB2
Neooccultibambusa chiangraiensis	Occultibambusaceae	MFLUCC 12-0559 [T]	KU764699	KU712458	NA
Neooccultibambusa jonesii	**Occultibambusaceae**	**MFLUCC 16 0643**	**KY111437**	**KY111438**	**NA**
Neooccultibambusa sp.	Occultibambusaceae	MFLUCC 12-0564	NA	NA	NA
Neoroussoella bambusea	Roussoellaceae	MFLUCC 11-0124 [T]	KJ474839	NA	KJ474856
Occultibambusa bambusae	Occultibambusaceae	MFLUCC 11-0394	KU863113	NA	KU940171
Occultibambusa bambusae	Occultibambusaceae	MFLUCC 13-0855	KU863112	NA	KU940170
Occultibambusa fusispora	Occultibambusaceae	MFLUCC 11-0127	KU863114	NA	KU940172
Occultibambusa pustula	Occultibambusaceae	MFLUCC 11-0502	KU863115	NA	NA
Ophiosphaerella sasicola	Lentitheciaceae	KT 1706	AB524599	AB524458	AB539098
Paraconiothyrium minitans	Didymosphaeriaceae	CBS 122788	EU754173	EU754074	GU357807
Paradictyoarthrinium diffractum	Paradictyoarthriniaceae	MFLUCC 12-0557	KP744497	NA	NA
Paradictyoarthrinium diffractum	Paradictyoarthriniaceae	MFLUCC 13-0466	KP744498	KP753960	NA
Paradictyoarthrinium tectonicola	Paradictyoarthriniaceae	MFLUCC 12-0556	KP744499	NA	NA
Paradictyoarthrinium tectonicola	Paradictyoarthriniaceae	MFLUCC 13-0465[T]	KP744500	KP753961	NA
Pleomassaria siparia	Pleomassariaceae	AFTOL-ID 1600	DQ678078	DQ678027	DQ677976
Preussia funiculata	Sporormiaceae	CBS 659.74	GU301864	GU296187	GU371799
Preussia minima	Sporormiaceae	AFTOL-ID 1256	DQ678056	DQ678003	DQ677950
Prosthemium stellare	Pleomassariaceae	VM 20050611	AB553781	AB553650	NA
Pseudocoleodictyospora sukhothaiensis	Pseudocoleodictyosporaceae	MFLUCC 12-0554 [T]	KU764710	KU712471	KU712493
Pseudocoleodictyospora tectonae	Pseudocoleodictyosporaceae	MFLUCC 12-0385[T]	KU764704	KU712461	KU712491
Pseudocoleodictyospora tectonae	Pseudocoleodictyosporaceae	MFLUCC 12-0387	NA	KU712462	KU712492
Pseudocoleodictyospora thailandica	Pseudocoleodictyosporaceae	MFLUCC 12-0565 [T]	KU764701	KU712472	KU712494

Table 1 continued

Species name	Family	Culture no.	Gene Bank accession no.		
			LSU	SSU	RPB2
Quadricrura septentrionalis	Tetralosphaeriaceae	HHUF 28782	NG042327	AB524475	NA
Roussoella hysterioides	Roussoellaceae	HH 26988	AB524622	AB524481	AB539102
Roussoella nitidula	Roussoellaceae	MFLUCC 11-0182 [T]	KJ474843	NA	KJ474859
Roussoella nitidula	Roussoellaceae	MFLUCC 11-0634	KJ474842	NA	KJ474858
Roussoella pustulans	Roussoellaceae	KT 1709	AB524623	AB524482	AB539103
Seriascoma didymosporum	Occultibambusaceae	MFLUCC 11-0179	KU863116	NA	KU940173
Seriascoma didymosporum	Occultibambusaceae	MFLUCC 11-0194	KU863117	NA	KU940174
Sporidesmium australiense	Roussoellaceae	HKUCC 10833	DQ408554	NA	DQ435080
Subglobosporium tectonae	Pseudocoleodictyosporaceae	MFLUCC 12-0390	NA	KU712463	KU712495
Subglobosporium tectonae	Pseudocoleodictyosporaceae	MFLUCC 12-0393[T]	KU764703	KU712464	KU712485
Tetraplosphaeria sasicola	Tetralosphaeriaceae	KT 563	AB524631	AB524490	NA
Thyridaria rubronotata	Thyridariaceae	CBS 419.85	GU301875	NA	GU371728
Torula ficus	Torulaceae	CBS 595.96[T]	KF443385	KF443387	KF443395
Torula herbarum	Torulaceae	CBS 111855	KF443386	KF443391	KF443396
Torula herbarum	Torulaceae	CBS 379.58	CBS 379.58	KF443388	NA
Torula herbarum	Torulaceae	CPC 24114 [T]	KR873288	NA	NA
Torula hollandica	Torulaceae	CBS 220.69[T]	KF443384	KF443389	KF443393
Torula masonii	Torulaceae	CBS 246.57[T]	KR873290	NA	NA
Torula masonii	Torulaceae	CBS 245.57	KR873289	NA	NA
Trematosphaeria pertusa	Trematosphaeriaceae	CBS 122368	FJ201990	FJ201991	FJ795476
Versicolorisporium triseptatum	Occultibambusaceae	HHUF:28815	AB330081	AB524501	NA
Westerdykella ornata	Sporormiaceae	CBS 379.55	GU301880	GU296208	GU371803

[T] = Type strain.
NA = not available.

GenBank (Table 1) and the final matrices used for phylogenetic analyses were saved in TreeBASE (www.treebase.org; TB2:S20198).

Results

Phylogeny

Irrespective of the criteria used in the analyses of single or combined genes datasets, phylogenies generated were topologically congruent (results not shown). Fig. 1 represents the relationships of our new taxon. This dataset consisted of 2589 characters (LSU-863, SSU-951, RPB2-783) of which 1563 were constant, 179 variable parsimony uninformative characters and 847 parsimony informative characters (32%) while *Hysterium angustatum* CBS 236.34 was the outgroup taxon. The parsimony analysis of the data matrix resulted in three equally parsimonious trees and the best tree support values belong to the first tree (TL = 6000 steps, CI = 0.287, RI = 0.598, RC = 0.172 and HI = 0.713). The Bayesian analysis resulted in 2438 trees after 2438000 generations. The first 610 trees, representing the burn-in phase of the analyses were discarded, while the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree (split frequency was lower than 0.01). A best scoring RAxML tree resulted with the value of likelihood: -29664.999375. Phylogenetic trees obtained from maximum parsimony, maximum likelihood and Bayesian analysis yielded trees with similar overall topology at the species level and are in agreement with previous studies (Hyde et al. 2013, Dai et al. 2017, Doilom et al. 2017). The strain of *Neooccultibambusa jonesii* formed a sister clade to *Neooccultibambusa* sp. with high bootstrap value in Bayesian analysis (0.93BPP) and maximum parsimony (61 %), but lower value in maximum likelihood analysis. Therefore, a new species is introduced to accommodate this taxon within the genus *Neooccultibambusa*.

Taxonomy

Neooccultibambusa jonesii Jayasiri, Camporesi & K.D. Hyde, **sp. nov**

Index Fungorum number: IF552518; *Facesoffungi number*: FoF 02642, Fig. 2

Etymology: Named in honour of Professor E.B. Gareth Jones for his contributions to mycology.

Holotype: MFLU 16-0871

Saprobic on dead and aerial stems of *Ammophila arenaria* L. Sexual morph: *Ascomata* 104–155 µm high × 130–160 µm diameter ($\bar{x}$ = 128 × 151 µm, n = 10), scattered to gregarious, semi-immersed to densely erumpent, carbonaceous, dark brown, globose to subglobose. *Ostiole* with a small to large, flat, crest-like apex, with apex composed of pseudoparenchymatous cells. *Peridium* 16–22 µm thick near the ostiole, with inner layer composed of hyaline cells of *textura angularis*, outer layer dark brown, 5–6 layers. *Hamathecium* comprising of 1.3–2 µm diam., septate, hyaline, anastomosing and branched, cellular pseudoparaphyses, embedded in gelatinous matrix between and above the asci. *Asci* 47–76 × 8–10 µm diameter ($\bar{x}$ = 60 × 9 µm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical to clavate, with furcate pedicel, rounded at apex with a minute ocular chamber. *Ascospores* 15–20 × 2.5–4.5 µm ($\bar{x}$ = 17 × 4 µm, n = 30), uniseriate to biseriate, narrowly fusiform with acute ends, 1-septate, 2 septa in germinated spores (Fig 3a), constricted at the septa, with distinct oil drops in each cell, smooth-walled, hyaline, pale brown to dark brown. Asexual morph: *Colonies* circular, floccose, dark brown to black, with smooth, circular margin, 20 mm diameter in 3 weeks, *Mycelium* 2–3.5 µm wide, aerial to immersed, composed of pale brown to dark brown, septate, branched hyphae, slightly constricted at septa, walls with melanin deposits. *Conidiophores* macronematous, prostrate, flexuous, septate, branched, dark brown, semi- to immersed. *Conidiogenous cells* gangliar-type, terminal or intercalary. *Conidia* 10–14 (–16) × 8–14 (−17) ($\bar{x}$ = 13× 13 µm, n = 20), subglobose to globose, unicellular, initially subhyaline, becoming pale to dark brown at maturity, thick-walled, smooth, sometimes wall collapsing.

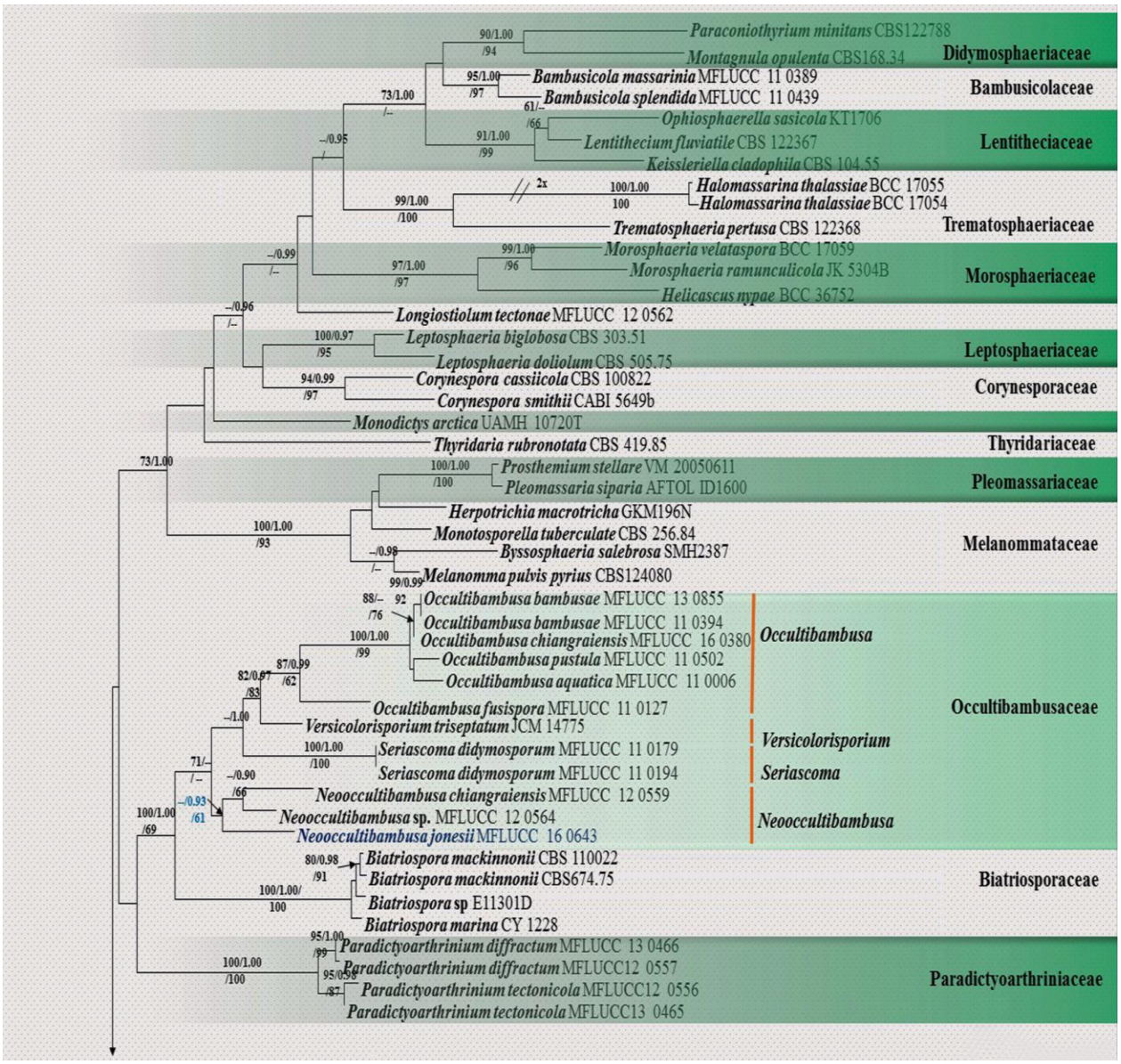

Fig. 1 Phylogram generated from maximum likelihood analysis (RAxML) based on combined LSU, SSU and RPB2 sequence data from species of Pleosporales. The tree is rooted to *Hysterium angustatum* CBS 236.34. Maximum Likelihood bootstrap values (MLBS) ≥60 %, Bayesian posterior probabilities (BPP) ≥0.90, and Maximum Parsimony (MP) ≥60 % are given at the nodes respectively. Some branches were shortened to fit the page – these are indicated by two diagonal lines with the number of times a branch was shortened indicated next to the lines. The new isolate is in blue.

Culture characteristics – Ascospores germinating on MEA within 24 hours. Germ tubes produced at both ends of ascospores and can see two septate stage of ascospore. Colonies on MEA reaching 20 mm diameter after 3 weeks, circular, convex or dome-shaped, cottony, aerial

in the center, immersed at the edge, edge entire, dense, with colonies becoming brownish grey after 3 weeks.

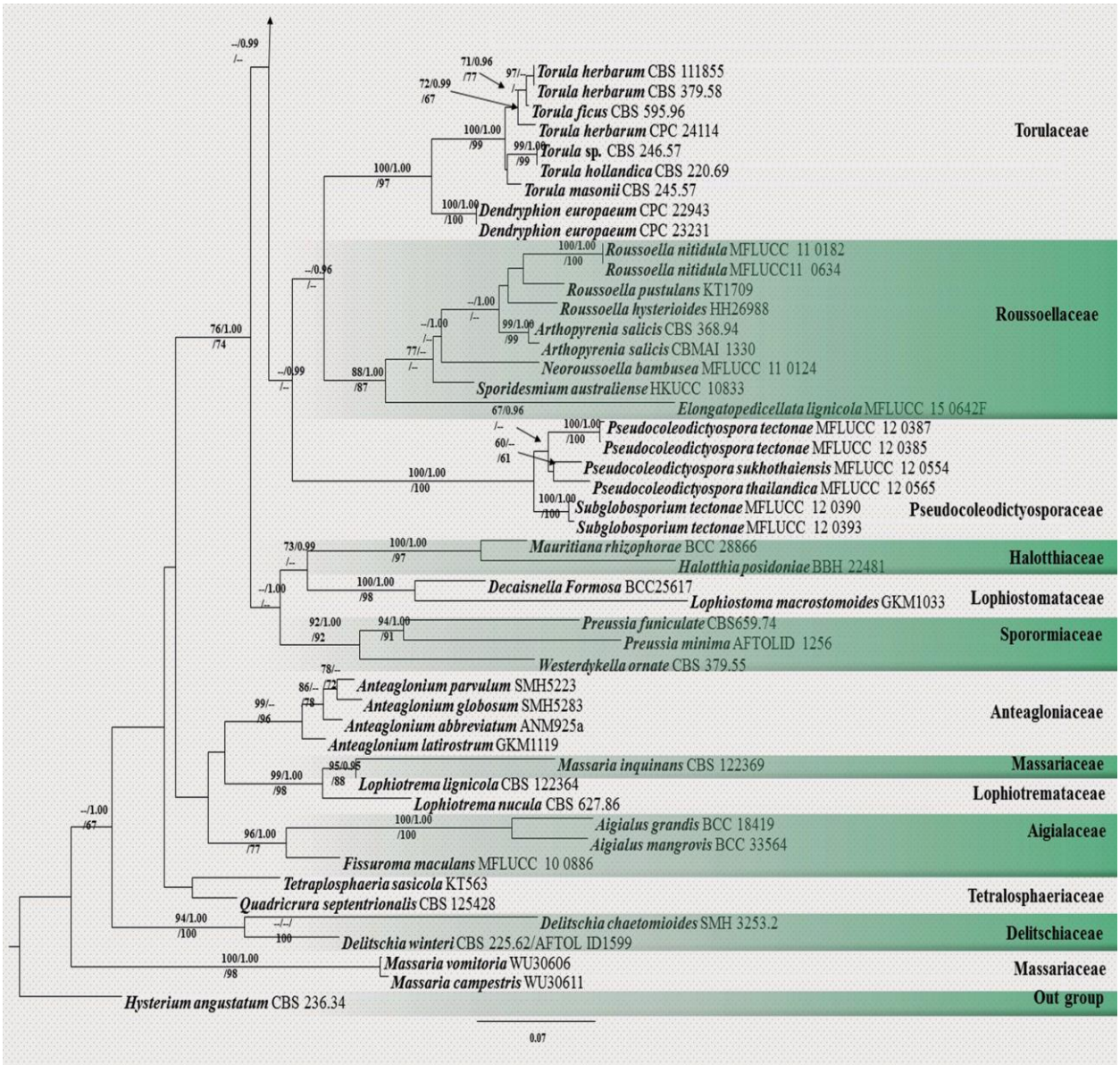

Fig 1 continued.

Material examined – ITALY, Province of Ravenna, Lido di Dante, dead and aerial stem of *Ammophila arenaria* (Poaceae), 9 February 2016, Erio Camporesi, IT 2813 (MFLU 16-0871, **holotype**); (**isotype** in KUN), ex-type living culture, MFLUCC 16 0643; KUMCC 16-0110.

Discussion

Neooccultibambusa, is represented by the generic type, *N. chiangraiensis*, and is similar to *Occultibambusa* in having dark brown, fusiform ascospores, surrounded by a gelatinous sheath. However, *Neooccultibambusa* produces cylindrical to subcylindrical asci and ascospores with 1–3 transverse septa and *Occultibambusa* produces broadly cylindrical to clavate asci and

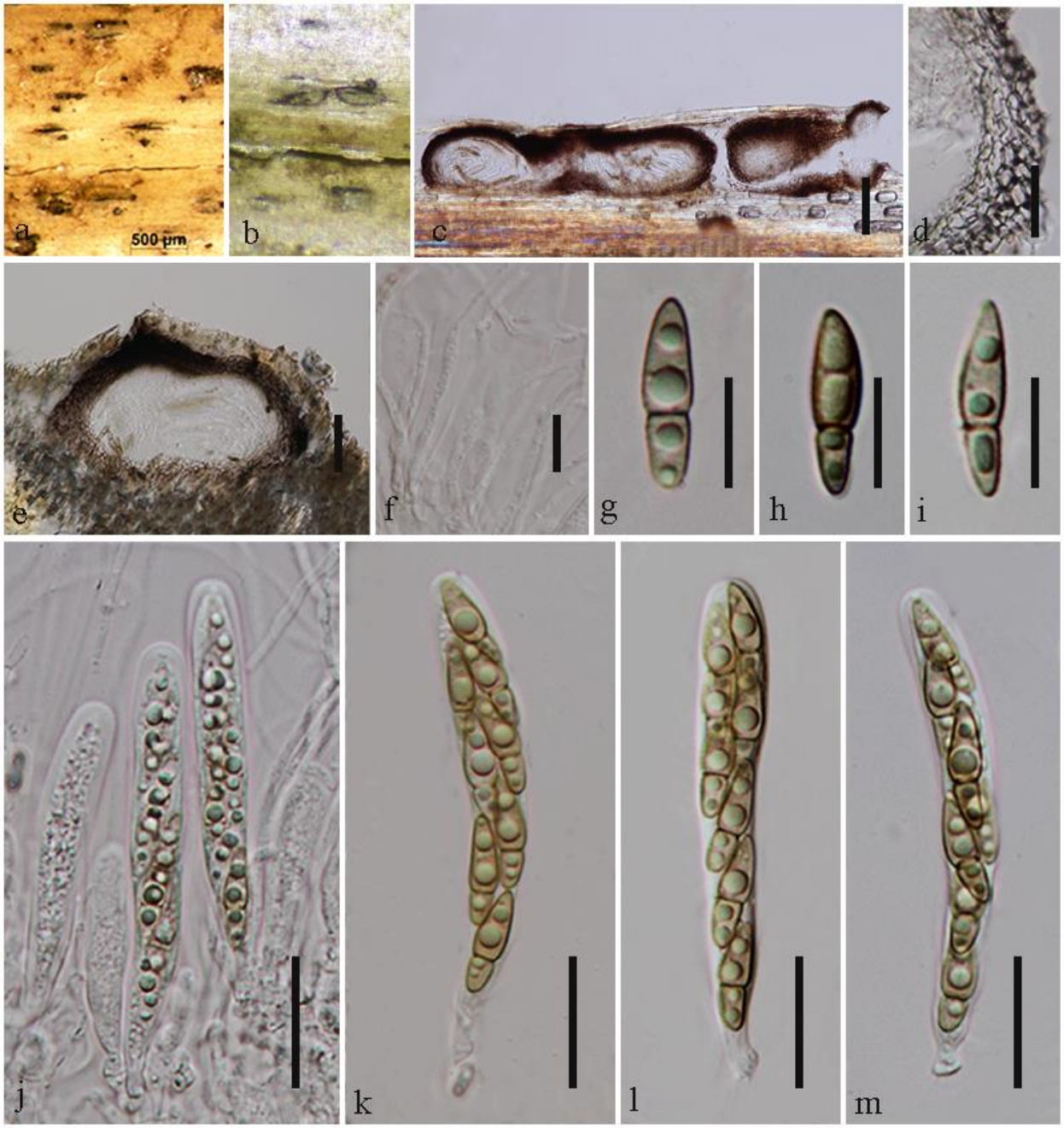

Fig. 2 – *Neooccultibambusa jonesii* (holotype). a, b Appearance of substrate with ascomata. c, e Sections of ascomata. d Peridium. f Pseudoparaphyses. g-i Ascospores. j-m Asci. Scale bars: c, e = 50 µm, d = 20 µm, f = 50 µm, g-i = 10 µm, j-m = 30 µm.

ascospores with 1 transverse septum. Phylogenetic analyses also separate *Neooccultibambusa* from *Occultibambusa* (Dai et al. 2017, Doilom et al. 2017). Currently *Neooccultibambusa* comprises two taxa, *N. chiangraiensis* (MFLUCC 12 0559) and an undescribed species, *Neooccultibambusa* sp. (MFLUCC 12 0564, Dai et al. 2017, Doilom et al. 2017). However, descriptions and illustrations of MFLUCC 12–0564 are not available and therefore *N. jonesii* is

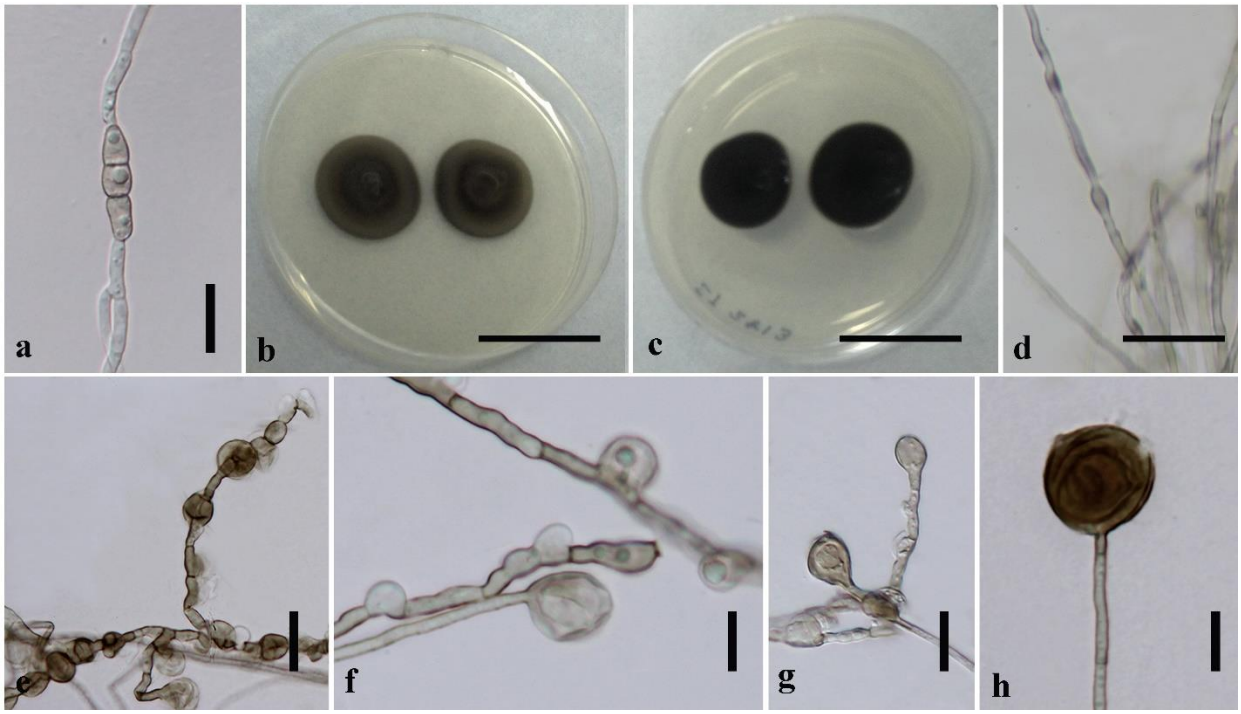

Fig. 3 – Morphology of *Neooccultibambusa jonesii* on MEA (from MFLUCC 16 0643). a Germinated ascospore in culture. b, c Colonies after 7 days from single spore isolation. d Mycelia with melanin deposits. e-g Conidia produced on hyphae. h Conidium. Scale bars: a, d-h = 10 μm, b, c = 4 cm.

compared with generic type, *N. chiangraiensis* (Doilom et al. 2017). *Neooccultibambusa jonesii* differs from *N. chiangraiensis* in having dark brown ascospores with prominent guttules, and smaller ascospores (15–20 × 2.5–4.5 μm). In addition, *Neooccultibambusa jonesii* can be segregated from *N. chiangraiensis* based on the number of septa (2, versus 1). The morphological differences are also supported by analysis of combined LSU, SSU and RPB2 sequence data. DNA sequence comparison between *N. chiangraiensis* and *N. jonesii* reveals some striking differences among nucleotides that further supports the establishment of the new taxon. In particular, there are 24 and 14 base pair differences in the LSU and SSU regions respectively between these two species.

Ammophila arenaria is a common grass species found in coastal sound dunes in many countries, with 241 associated fungal taxa (Treigienė 2011, Farr & Rossman 2016). In addition, a number of new species have been described from this host. For example, *Peziza fruticosa* and *Anthostomella ravennica* have recently been collected and described from Italy (Hyde et al. 2016). Many Dothideomycetes species in the genera *Didymosphaeria*, *Leptosphaeria*, *Mycosphaerella*, *Phaeosphaeria* and *Pleospora*, have been recorded from this host (Farr & Rossman 2016). Most of these records however, need confirming with molecular data.

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