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ORIGINAL ARTICLE

Coniella lustricola, a new species from submerged detritus

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Abstract The draft genome, morphological description, and phylogenetic placement of *Coniella lustricola* sp. nov. (Schizoparmeaceae) are provided. The species was isolated from submerged detritus in a fen at Black Moshannon State Park, Pennsylvania, USA and differs from all other *Coniella* species by having ellipsoid to fusoid, inequilateral conidia that are rounded on one end and truncate or obtuse on the other end, with a length to width ratio of 2.8. The draft genome is 36.56 Mbp and consists of 870 contigs on 634 scaffolds (L50 = 0.14 Mb, N50 = 76 scaffolds), with 0.5% of the scaffold length in gaps. It contains 11,317 predicted gene models, including predicted genes for cellulose, hemicellulose, and xylan degradation, as well as predicted regions encoding for amylase, laccase, and tannase enzymes. Many members of the Schizoparmeaceae are plant pathogens of agricultural crops. This draft genome represents the first sequenced *Coniella* genome and will be a valuable tool for comparisons among pathogenic *Coniella* species.

Keywords Diaporthales · Schizoparmeaceae · Sordariomycetes · 1000 Fungal Genome Project

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Introduction

The family Schizoparmeaceae (Diaporthales) was erected by Rossman et al. (2007) and is known to contain several agriculturally important pathogenic species. Historically, this family included three genera, two producing only asexual morphs (*Coniella*, *Pilidiella*) and one producing sexual morphs (*Schizoparme*) (Rossman et al. 2007). This family has recently been reevaluated by Alvarez et al. (2016), who provided an in-depth historical analysis for each genus and revised the classification through a four-gene (ITS, LSU nrDNA, *rpb2*, *tef1*) phylogenetic analysis combined with morphological observations. They concluded that *Coniella* and *Pilidiella* were taxonomic synonyms, with the oldest anamorphic name, *Coniella*, having precedence over the name of the other asexual morph, *Pilidiella* (Alvarez et al. 2016).

The genus *Coniella* was erected by Von Höhnel (1918), typified by *C. pulchella*, and separated by Petrak and Sydow (1927) into *Euconiella* (dark conidia) and *Pseudoconiella* (pale conidia) (Alvarez et al. 2016). Petrak and Sydow (1927) erected the genus *Pilidiella* for species with hyaline to pale brown conidia. Historically, van der Aa (in Von Arx 1973) and Von Arx (1981) treated the two genera as distinct, Sutton (1980) and Nag Raj (1993) treated *Pilidiella* as a synonym of *Coniella*, and Castlebury et al. (2017) and Van Niekerk et al. (2004) suggested that the two genera are separated based on internal transcribed spacer (ITS) region and partial 28S nuclear ribosomal large subunit (LSU) sequence analyses. *Coniella*

was distinguished from *Pilidiella* by the former having brown conidia with a length to width ratio equal to or less than 1.5, while the latter having hyaline to pale brown conidia with a length to width ratio greater than 1.5 (Van Niekerk et al. 2004). Alvarez et al. (2016) determined that conidial color was not a good morphological characteristic based on molecular and morphological data, resulting in synonymy of *Pilidiella*, *Schizoparme*, and *Coniella*, with *Coniella* taking preference.

Many *Coniella* species are plant pathogens of economically valuable agricultural crops. *Coniella granati* (Sacc.) Petr. & Syd. infects *Punica granatum* L. (pomegranate), causing crown rot, fruit rot, and shoot blight in many countries, including China, Greece, Iran, Italy, Spain, Turkey, and in the state of California in the United States (Çeliker et al. 2012; Chen et al. 2014; Michailides et al. 2010; Mirabolfathy et al. 2012; Palou et al. 2010; Pollastro et al. 2016; Tziros and Tzavella-Klonari 2008). *Coniella diplodiella* (Speg.) Petr. & Syd. causes white rot of *Vitis vinifera* L. (wine grape), with up to 20–80% crop loss (Nag Raj 1981). This species is cosmopolitan, with optimal in vitro growth temperatures ranging from 23–27 °C (Šrobárová and Kakalíková 2007). In addition, *Coniella* species are known pathogens of *Fragaria* L. (strawberry), with *C. castaneicola* (Ellis & Everh.) B. Sutton causing fruit rot and *C. fragariae* (Oudem.) B. Sutton causing leaf diseases.

With the advent of whole genome sequencing, it is now possible to compare the genetic profile of similar species to determine genes that are important to different life strategies (Zhao et al. 2013). The goals of this paper are to: (1) introduce the draft genome for a new species of *Coniella*, (2) provide a brief review of the putative detritus degrading enzymes that can be predicted within the draft genome, and (3) describe and illustrate the new species of *Coniella* and elucidate its phylogenetic placement within the family Schizoparmeaceae. The genome of this fen-inhabiting species was sequenced to provide future insight into its enzymatic potential, ecological role, and value as a comparison to other *Coniella* species infecting economically valuable agricultural crops.

Materials and methods

Collection site

Four isolates with identical ITS rDNA regions were obtained from two separate collecting trips to Black Moshannon State Park in Pennsylvania (collection permit 2014-27). One isolate was obtained from a submerged detrital sample collected on August 11, 2014, while the other three isolates were obtained from three separate submerged detrital samples collected on November 24, 2014.

Collection and isolation methods

Submerged detritus was collected from areas where *Sphagnum* spp., *Sarracenia purpurea*, and *Drosera* spp. were present. Samples were obtained below the *Sphagnum* layer, approximately 15–20 cm below the water line. Detrital samples were immediately stored in sterile plastic bags on ice and processed within one week. Fungi were isolated by spreading 400 μL of a 1/100 serial dilution onto the surface of 90-mm tea agar plates (Mehrotra et al. 1982) supplemented with 0.2 g/L chloramphenicol (Fisher Scientific). Plates were wrapped with Parafilm (Fisher Scientific) and incubated at both 10 °C and 25 °C for two weeks. Individual colonies were transferred to separate potato dextrose agar (PDA) and malt extract agar (MEA) plates and incubated at 21 °C under 24 h darkness. Isolates were stored at 7 °C after visible colony growth occurred. Biomass for DNA extraction was obtained by growing isolate ILLS 80714 under shaken culture using a New Brunswick Scientific C24 incubator shaker set to 90 rpm. The isolate was grown in 50 mL of filtered (two layers of Whatman #1) potato dextrose broth at RT in 100-mL Erlenmeyer flasks.

Preservation methods

Dried culture specimens were deposited in the Illinois Natural History Survey (ILLS) fungarium. Living cultures were submitted to the Canadian Collection of Fungal Cultures.

Whole genome DNA extraction

DNA extraction of ILLS 80714 was completed by modifying the protocol of Healey et al. (2014). The modifications consisted of extending the chloroform:isoamyl alcohol protein extraction time from 5 min to 15 min, reducing the DNA precipitation time from 1 h to 15 min, increasing the centrifuge force from 5000 g to 14,000 g and time from 10 min to 15 min to pelletize the precipitated DNA, and adding two additional DNA wash cycles: 70% ethanol DNA wash followed by a final 95% ethanol DNA wash. All DNA extraction samples were stored at 4 °C until the quality checks were completed to prevent long strand DNA breakage by repeated thaw and unthaw cycles. The DNA samples were pooled and stored at − 20 °C after completion of the quality checks.

Genome sequencing methods

Using a Covaris LE220, 100 ng of DNA was sheared and 300-bp fragments were selected using SPRI beads (Beckman Coulter). The fragments were treated with end-repair, A-tailing, and ligation of Illumina compatible adapters (IDT, Inc.) using the KAPA-Illumina library creation kit (KAPA Biosystems). The prepared library was then quantified by

qPCR using the Kapa SYBR Fast Illumina Library Quantification Kit (Kapa Biosystems) and run on a Roche LightCycler 480 real-time PCR instrument. The quantified library was then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq paired-end cluster kit, v4, and Illumina's cBot instrument to generate a clustered flowcell for sequencing. Sequencing of the flowcell was performed on the Illumina HiSeq2500 sequencer using HiSeq TruSeq SBS sequencing kits, v4, following a 2x150 indexed run recipe.

Genome assembly methods

Genome sequencing was performed by the Joint Genome Institute as part of the 1000 Fungal Genome Project (http://1000.fungalgenomes.org). The *Coniella* genome was sequenced using Illumina (AXNNN HiSeq-1 TB Regular (DNA) 2x151 bp .3 kb). Each fastq file was QC filtered for artifact/process contamination and subsequently assembled with Velvet (Zerbino and Birney 2008). The resulting assembly was used to simulate a long mate-pair library with insert 3000 +/− 300 bp, which was then assembled with the original Illumina library with AllPathsLG release version R49403 (Gnerre et al. 2011). Mitochondria genome was assembled separately with AllPathsLG version R46652.

RNA extraction methods

Mycelium for RNA extraction was obtained by growing ILLS 80714 on multiple Petri plates of iron/lysine agar (Difco), water agar (1.5% agar) supplemented with autoclaved hemp seed, and PDA supplemented with 500 mg of 97% L-cysteine (Aldrich) at RT under 24 h darkness for 14 days. Fungal biomass was collected by scraping mycelium from the agar surface, flash frozen with liquid nitrogen, and ground in an autoclaved mortar with pestles (soaked in bleach solution for 1 h and wrapped in aluminum foil prior to autoclaving). During the grinding process, additional liquid nitrogen was added to keep the biomass frozen during the partitioning process. After weighing (~50 mg were transferred into 1.5-mL centrifuge tubes), tubes were immediately placed on ice to keep the fungal biomass frozen, and stored at − 80 °C until RNA extraction. RNA isolation was performed according to the RNeasy Plus Mini Handbook, Appendix E. The filtrate was then transferred to a clean 2-mL centrifuge tube for DNase digestion according to the RNeasy MinElute Cleanup Handbook, Appendix C. Small RNA (<200 nucleotides) was obtained and cleaned according to the RNeasy MinElute Cleanup Kit manual. The large RNA (>200 nucleotides) was isolated according to the RNeasy Plus Mini Kit and the resulting elution was cleaned following the RNeasy MinElute Cleanup Kit "cleanup and concentration" protocol. The final elution volume was adjusted to 20 μL. All RNA samples with good quantity were pooled and stored at − 20 °C.

Transcriptome sequencing methods

Transcriptome was sequenced using Stranded cDNA libraries that were generated using the Illumina TruSeq Stranded RNA LT Kit. mRNA was purified from 1 μg of total RNA using magnetic beads containing poly-T oligos. mRNA was fragmented and reversed transcribed using random hexamers and SSII (Invitrogen), followed by a second strand synthesis. The fragmented cDNA was treated with end-pair, A-tailing, adapter ligation, and eight cycles of PCR. The prepared library was then quantified by qPCR using the Kapa SYBR Fast Illumina Library Quantification Kit (Kapa Biosystems) and run on a Roche LightCycler 480 real-time PCR instrument. The quantified library was then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq paired-end cluster kit, v4, and Illumina's cBot instrument to generate a clustered flowcell for sequencing. Sequencing of the flowcell was performed on the Illumina HiSeq2500 sequencer using HiSeq TruSeq SBS sequencing kits, v4, following a 2x150 indexed run recipe.

Transcriptome assembly methods

Raw fastq file reads were filtered and trimmed using the JGI QC pipeline. Briefly, using BBDuk (https://sourceforge.net/projects/bbmap), raw reads were evaluated for artifact sequence by kmer matching (kmer = 25), allowing one mismatch and the detected artifact was trimmed from the 3′ end of the reads. RNA spike-in reads, PhiX reads, and reads containing any Ns were removed. Quality trimming was performed using the phred trimming method set at Q6. Finally, following trimming, reads under the length threshold were removed (minimum 50 bp). Filtered fastq files were used as input for de novo assembly of RNA contigs. Reads were assembled into consensus sequences using Trinity v2.1.1 (Grabherr et al. 2011). Trinity was run with default parameters except for the inclusion of –normalize_reads (an in-silico normalization routine) and –jaccard_clip (to minimize fusion of transcripts derived from gene dense genomes) options.

Genome annotation and identification of genes involved in plant detritus degradation and stress tolerance

The *Coniella* genome was annotated using the JGI annotation pipeline (Grigoriev et al. 2014). The genome was then searched for predicted proteins involved in plant detritus degradation using MycoCosm (Grigoriev et al. 2014) by searching for specific Enzyme Commission (EC) numbers. The resulting protein sequences were compared against the non-redundant protein sequences database in NCBI using

the protein to protein Basic Local Alignment Search Tool (BLASTp) (Altschul et al. 1990). A protein match with >80% query coverage and >60% ID were the criteria used to determine homology. The comparative analysis of *Coniella* genome expansions and contractions was performed by using MycoCosm (Grigoriev et al. 2014) to search gene family clustering runs across all available Sordariomycetes genomes.

Phylogenetic analyses

Phylogenetic placement of the new species within the current concept of *Coniella* was based on an analysis of sequences from four nuclear DNA regions as outlined by Alvarez et al. (2016) and Marin-Felix et al. (2017). The regions that were compared were: (1) entire ITS region, (2) partial LSU, (3) the second largest subunit of the RNA polymerase II gene (*rpb2*), and (4) partial translation elongation factor 1-alpha (*tef1*). Sequences for these gene regions were obtained from GenBank for previously published *Coniella* species, while gene sequences for the holotype and three paratypes of the new species were Sanger sequenced according to Promputtha and Miller (2010) (Table 1). Individual gene alignments were conducted in SeaView v4.5.3 (Galtier et al. 1996) using Muscle v3.7 (Edgar 2004). Ambiguous regions were removed from each gene alignment using Gblocks v0.91b (Castresana 2000), allowing for less strict flanking regions, gap positions within the final blocks, and smaller final blocks. Maximum likelihood (ML) analyses were conducted on each gene region using PhyML (Guindon and Gascuel 2003) under the GTR substitution model with four rate classes and optimized invariable sites based on the results from jModelTest 2.0 on XSEDE (2.1.6) (Posada 2008). The best nearest neighbor interchange and subtree pruning and regrafting tree improvement was implemented on the unrooted BioNJ starting tree and branch support was determined with 1000 nonparametric bootstrap replicates (Hustad et al. 2014). The resulting individual PhyML trees for each gene were examined for potential conflicts indicated by incongruent clades with ≥ 70% bootstrap support (Wiens et al. 2008). No significant conflicts were observed, so a final concatenated data set (3020 bp) of all gene regions was generated for Bayesian and ML analyses. Bayesian analysis was conducted using MrBayes 3.2.2 on XSEDE (3.2.6) and ML analysis was conducted with RAxML-HPC2 on XSEDE (8.2.9) through the CIPRES Science Gateway (Miller et al. 2010). ML analysis was conducted using the GTRGAMMA model for 1000 bootstrap replicates. Bayesian analysis was conducted using the GTR + I + G model with four rate classes and four independent chains and ran for 15 million generations with 0.25% burn-in. Nodes with ≥ 70% bootstrap support and ≥ 95% Bayesian posterior probability were considered significantly supported (Alfaro et al. 2003).

Morphological analyses

Light microscopic evaluation was conducted using an Olympus BX51 microscope with differential interference contrast (DIC) and equipped with an Olympus Q-Color 3 digital camera. Images were processed with Adobe Photoshop 7.0 (Adobe Systems Inc., Mountain View, California), and measurements were conducted using NIH Image 1.63. Observations and measurements of structures were made from freezing microtome sections or squash mounts of conidiomata mounted in distilled water. Conidial volume, measured as the average conidial length:width (l:w) ratio, is provided, as this character has been proven to be useful in distinguishing among *Coniella* taxa (Nag Raj 1993). A minimum of 20 measurements of each character were made, with the exception of the round basal cell (n = 10). India ink was used to detect the presence of mucilage. Morphological features were compared against previously described *Coniella* and *Pilidiella* species (Marin-Felix et al. 2017).

Data access

Genome assembly and annotation are available via JGI Fungal Genomics Portal MycoCosm (http://jgi.doe.gov/fungi; Grigoriev et al. 2014) and the whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession NSBW00000000. The version described in this paper is version NSBW01000000. The concatenated alignment file with ambiguous regions removed via Gblocks that was used in the phylogenetic analyses is available at TreeBASE: http://purl.org/phylo/treebase/phylows/study/TB2:S20699.

Results

Draft genome

The assembled genome size was 36.56 Mbp, with a sequencing read coverage depth of 139x. There were 870 contigs, comprising 634 scaffolds, of which 583 scaffolds were ≥ 2Kbp. The 76th largest scaffold captured approximately half of the assembled sequence (L50 = 0.14 Mbp). The 236 gaps represented 0.5% of the scaffold length. There were 11,317 gene models predicted, with an average gene length of 1718 bp, the average transcript was 1538 bp, the average exon was 599 bp, and the average intron was 117 bp. Sugar transporters and cytochrome p450 represent the largest gene families encoded in this genome. In addition, short chain dehydrogenase, carboxyl esterase, RAS, and amino acid permease families were prevalent.

Table 1 Sequences used in the phylogenetic analysis of *Coniella* species proposed by Alvarez et al. (2016) and Marin-Felix et al. (2017)

ID	Strain accession no.	GenBank accession no.				References
		ITS	LSU	*rpb2*	*tef1*	
Coniella						
africana	CBS 114133^{T} = CPC 405	AY339344	AY339293	KX833421	KX833600	Van Niekerk et al. (2004); Alvarez et al. (2016)
crousii		HQ264189	–	–	–	Rajeshkumar et al. (2011)
diplodiella	CBS 111858^{ET} = CPC 3708	AY339323	KX833335	KX833423	KX833603	Van Niekerk et al. (2004); Alvarez et al. (2016)
	CBS 112729 = CPC 3927	KX833520	KX833345	KX833433	KX833613	Alvarez et al. (2016)
diplodiopsis	CBS 116310 = CPC 3793	KX833532	KX833357	KX833443	KX833627	Alvarez et al. (2016)
	CBS 10923 = CPC 3933	AY339332	AY339287	KX833440	KX833624	Van Niekerk et al. (2004); Alvarez et al. (2016)
duckerae	VPRI 13689 = CBS 142045^{T}	KY924929	–	–	–	Marin-Felix et al. (2017)
erumpens	CBS 52378^{T}	KX833535	KX833361	KX833446	KX833630	Alvarez et al. (2016)
eucalyptigena	CBS 139893^{T}	KR476725	–	–	–	Crous et al. (2015a, b)
eucalyptorum	CBS 112640^{T} = CPC3904 = DFR 100185	AY339338	AY339290	KX833452	KX833637	Van Niekerk et al. (2004); Alvarez et al. (2016)
	CBS 114852	KX833556	KX833380	KX833464	KX833652	Alvarez et al. (2016)
fragariae	CBS 17249^{NT} = CPC 3930	AY339317	AY339282	KX833472	KX833663	Van Niekerk et al. (2004); Alvarez et al. (2016)
	CBS 45468	KX833571	KX833393	KX833477	KX833670	Alvarez et al. (2016)
fusiformis	CBS 141596^{T} = CPC 19722	KX833576	KX833397	KX833481	KX833674	Alvarez et al. (2016)
granati	CBS 132860	KX833577	KX833400	KX833484	KX833677	Alvarez et al. (2016)
hibisci	CBS 109757^{ET}	KX833589	–	–	KX833689	Marin-Felix et al. (2017)
javanica	CBS 45568^{T}	KX833583	KX833403	KX833489	KX833683	Alvarez et al. (2016)
koreana	CBS 14397	KX833584	AF408378	KX833490	KX833684	Alvarez et al. (2016)
lanneae	CBS 141597^{T} = CPC 22200	KX833585	KX833404	KX833491	KX833685	Alvarez et al. (2016)
limoniformis	CBS 111021^{T} = PPRI 3870 = CPC 3828 = ARC-MYC J 13102	KX833586	KX833405	KX833492	KX833686	Alvarez et al. (2016)
lustricola	DAOMC 251731^{T}	MF631778	MF631799	MF651900	MF651899	**Present study**
	DAOMC 251732	MF631779	MF631800	–	–	**Present study**
	DAOMC 251733	MF631780	MF631801	–	–	**Present study**
	DAOMC 251734	MF631781	MF631802	–	–	**Present study**
macrospora	CBS 52473^{T} = CPC 3935	KX833587	AY339292	KX833493	KX833687	Alvarez et al. (2016)
malaysiana	CBS 141598^{T} = CPC 16659	KX833588	KX833406	KX833494	KX833688	Alvarez et al. (2016)
nicotianae	CBS 87572^{T} = PD 72/793	KX833590	KX833407	KX833495	KX833690	Alvarez et al. (2016)
nigra	CBS 16560^{T} = IMI 181519 = IMI 181599 = CPC4198	AY339319	KX833408	KX833496	KX833691	Van Niekerk et al. (2004); Alvarez et al. (2016)
obovata	CBS 111025 = CPC4196 = IMI 261318	AY339313	KX833409	KX833497	KX833692	Van Niekerk et al. (2004); Alvarez et al. (2016)
paracastaneicola	CBS 141292^{T} = CPC 20146	KX833591	KX833410	KX833498	KX833693	Alvarez et al. (2016)
peruensis	CBS 110394^{T} = RMF 7401	KJ710463	KJ710441	KX833499	KX833695	Crous et al. (2015a, b); Alvarez et al. (2016)
pseudogranati	CBS 137980^{T}	KJ869132	–	–	–	Crous et al. (2014)
pseudostraminea	CBS 112624^{T} = IMI 233050	KX833593	KX833412	KX833500	KX833696	Alvarez et al. (2016)
quercicola	CBS 90469^{NT}	KX833595	KX833414	KX833502	KX833698	Alvarez et al. (2016)
solicola	CBS 76671^{T}	KX833597	KX833416	KX833505	KX833701	Alvarez et al. (2016)
straminea	CBS 14922 = CPC 3932	AY339348	AY339296	KX833506	KX833704	

Table 1 (continued)

ID	Strain accession no.	GenBank accession no.				References
		ITS	LSU	*rpb2*	*tef1*	
						Van Niekerk et al. (2004); Alvarez et al. (2016)
tibouchinae	CBS 131595^{T} = CPC 18512	JQ281774	KX833418	KX833507	JQ281778	Miranda et al. (2012); Alvarez et al. (2016)
wangiensis	CBS 132530^{T} = CPC 19397	JX069873	JX069857	KX833509	KX833705	Crous et al. (2012); Alvarez et al. (2016)
Melanconiella hyperopta	CBS 131696	JQ926281	JQ926281	KX833510	KX833706	Miranda et al. (2012); Alvarez et al. (2016)

Predicted genes related to detritus degradation and stress tolerance

The *Coniella* genome contains predicted genes for enzymes involved in the breakdown of cellulose, hemicellulose, pectin, and lignin (Table 2), all of which are useful for the degradation of plant detritus. Predicted cellulases include beta-glucosidases (EC 3.2.1.21), endoglucanases (3.2.1.4), and 1,4-beta-cellobiosidase (3.2.1.91). In the *Coniella* genome, we found several enzymes involved in the breakdown of hemicellulose, including 1,4-beta-xylosidase (3.2.1.37), alpha-N-arabinofuranosidase (3.2.1.55), and 1,4-alpha-galacturonidase (3.2.1.67). In addition, several pectinesterases (3.1.1.11) and polygalacturonases (3.2.1.15) for the breakdown of pectin were found. No protein models predicted proteins encoded for heme peroxidase (1.11.1.14), manganese-dependent peroxidase (1.11.1.13), or versatile peroxidases (1.11.1.16) that are known to be involved in lignin degradation. We found phenol oxidases (1.10.3.2) that are involved in lignin degradation as well as tannases (3.1.1.20), which are involved in the degradation of tannins. Related to stress tolerance, *Coniella* lacked a predicted gene for a low temperature viability protein involved in ribosome biogenesis 40S subunit production, possessed a predicted gene for cysteine-rich TM module stress response (CYSTM), had a loss of predicted genes in a stress-responsive A/B barrel domain gene family, and contained a predicted gene for aryl sulfotransferase.

Taxonomy

Coniella lustricola Raudabaugh, Iturr., & A.N. Mill., **sp. nov.** Fig. 1.

MycoBank MB 819905.

Etymology: Named after the type of habitat, a fen, in which the species was collected.

Diagnosis: *Coniella lustricola* differs from all other species in the genus by its fen habitat and having ellipsoid to fusoid conidia that are rounded on one end and truncate or obtuse on the other end, with inequilateral sides (7)8–11(11.5) × 3–4 μm, and a length/width ratio (l:w) of 2.8.

Description: Conidiomata superficial to semi-immersed on malt extract, oatmeal, and potato dextrose agar, solitary to gregarious, one to several ostioles per single conidiomata. Conidiomata pycnidial, unilocular, ostiolate, hyaline when young, obovate, 100–150 μm diam., gradually maturing to light brown and finally dark brown (~500 μm diam.); ostiole central to slightly eccentric, circular or oval, often situated in a conical or rostrate neck; trichomes hyaline and enclosing the ostiole when young, later dark-brown, formed near the ostiole, absent upon conidiomata maturation. Conidiomal wall 36–40 μm thick; in median section composed of two layers of roundish to polygonal to irregular cells of *textura angularis* (9.5)12.0–24.0 × 13.0– 21.5 μm; external layer 2–3 cells wide, pale-brown becoming dark-brown upon maturity; internal layer composed of two rows of hyaline to pale brown thin-walled cells; cells of inner wall giving rise to rounded, irregularly shaped cells, 4–7.5 × 3–6 μm diam. that give rise to a dense layer of conidiogenous cells. Conidiogenous cells discrete, subulate, hyaline, smooth-walled, with a flat base, tapering gradually towards the apex, or with visible periclinal thickenings, 10–17.5 × 2.0–4.0 μm, with a single terminal conidiogenous locus. Conidia unicellular, thin-walled, hyaline when young, walls turning brown at maturity, ellipsoid to fusoid, distal end rounded, proximal end truncate or obtuse, inequilaterally sided (7)8–11(11.5) × 3–4 μm, length/width ratio (l:w) 2.8, usually with two central guttules when mature versus several small guttules when young.

Holotype: United States, Pennsylvania, Centre County, near Philipsburg, Black Moshannon State Park, 40° 54.034′N, 78° 3.622′W, dried culture, isolated from submerged detritus, 11-Aug-2014, D.B. Raudabaugh and M. Woodley (ILLS 80714; ex type strain DAOMC 251731). Paratypes, same location, 24-Nov-2014, 40° 54.046′N, 78° 3.527′W (ILLS 80715; ex type strain DAOMC 251732); 40° 54.020′N, 78° 3.634′W (ILLS 80716; ex type strain DAOMC 251733); 40° 54.009′N, 78° 3.643′W (ILLS 80717; ex type strain DAOMC 251734).

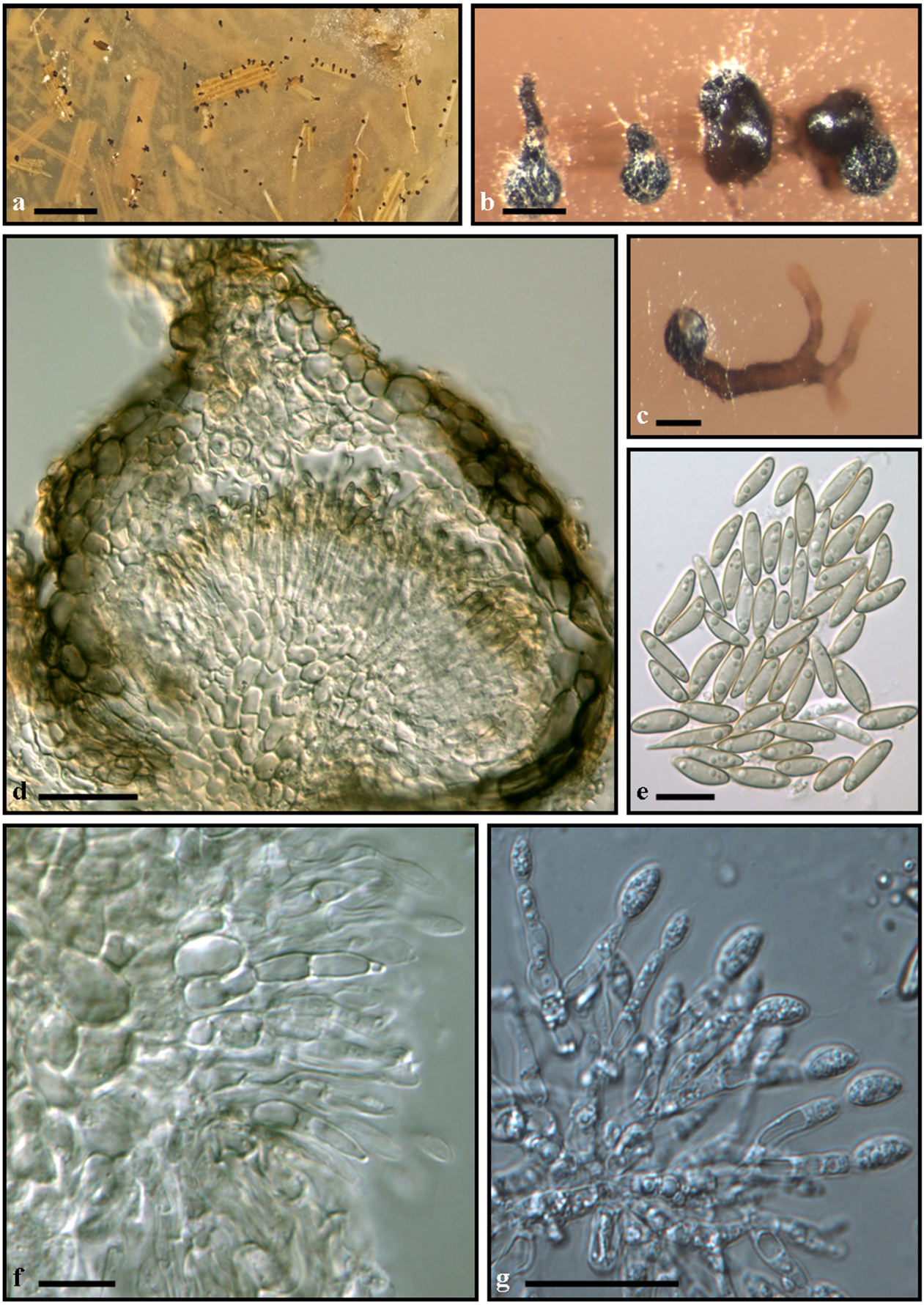
Table 2 Predicted enzymes within the *Coniella lustricola* genome involved in plant detritus degradation

Genome information			NCBI match			
Search query	Protein ID	Model name	Accession no.	GenBank description	% query	% ID
EC 3112-0	116432	CE116431_2445	KKY29585	[a]putative tannase subunit	100	61
EC 1103-2	47815	CE47814_2585	KUI73984	[b]laccase-2	99	68
EC 1111-7	15978	CE15977_125436	OCW42076	[c]catalase/peroxidase HPI	99	79
EC 1119-0	190009	CE190008_57424	KUI67018	[b]S-(hydroxymethyl)glutathione dehydrogenase	99	92
EC 3111-1	252360	CE252359_611	KUI60218	[d]putative pectinesterase A	97	67
EC 3211	156161	CE156160_920	KUI56593	[d]alpha-amylase A type-3	99	73
EC 3211-5	283041	CE283040_849	AAC24953	[e]endopolygalacturonase 4	99	79
EC 3212-0	362003	e_gw118201	KUI72634	[b]putative alpha/beta-glucosidase agdC	99	68
EC 3212-1	75718	CE75717_6008	KKY35357	[a]putative glycoside hydrolase family 3	99	72
EC 3213	451729	fgenesh1_pg34_#_43	KUI55350	[d]glucoamylase	98	77
EC 3213-1	484862	gm16835_g	KUI67627	[c]beta-galactosidase	95	76
EC 3213-7	367196	e_g-w1583671	KUI70894	[c]non-reducing end alpha-L-arabinofuranosidase BoGH43B	99	69
EC 3213-9	196407	CE196406_6093	KUI61960	[d]putative glucan endo-1,3-beta-glucosidase eglC	98	66
EC 3214	361344	e_gw114231	OCW34902	[c]endoglucanase 3 precursor	99	77
EC 3215-8	194392	CE194391_1155	KUI69627	[b]putative glucan 1,3-beta-glucosidase D	100	68
EC 3216-7	78695	CE78694_6744	KFX46954	[f]polygalacturonase	87	65
EC 3219-1	449139	fgenesh1_pg1_#_143	KPM43848	[g]putative exoglucanase type C	99	62

Species match: [a] *Diaporthe ampelina*, [b] *Valsa mali*, [c] *D. helianthi*, [d] *V. mali* var. *pyri*, [e] *Botrytis cinerea*, [f] *Talaromyces marneffei* PM1, [g] *Neonectria ditissima*

Phylogenetic and morphological placement

The ITS and LSU of ILLS 80714, ILLS 80715, ILLS 80716, and ILLS 80717 were identical. Phylogenetic analyses employing ML and Bayesian criteria of individual genes and the concatenated dataset suggested that *C. lustricola* and *C. erumpens* are sister taxa (Fig. 2). *Coniella lustricola* was distinguished from *Coniella* species lacking molecular information based on one or more of the following characters: conidial morphology and measurements, fungal host and habitat, and/or biogeography (Table 3).


a
b
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◀ **Fig. 1** Morphology of *Coniella lustricola*: **a** mature conidiomata on agar supplemented with *Miscanthus*, **b** mature conidiomata, **c** mature conidiomata with multiple ostioles, **d** cross-section of a mature conidiomata, **e** conidia, **f** irregular basal cell and conidiogenous cells, **f** conidiogenous cells with immature conidia. Scale bars: **a** = 2 cm; **b** = 400 μm; **c** = 300 μm; **d** = 50 μm; **e** = 10 μm; **f** = 10 μm; **g** = 20 μm

Discussion

This is the first report of a *Coniella* species isolated from submerged detritus within a fen habitat, constituting a completely different habitat for *Coniella* species, which commonly occur as plant pathogens, saprophytes, or as saprobes in soil. All species presently treated under the genus *Coniella* (Alvarez et al. 2016; Marin-Felix et al. 2017) were carefully compared morphologically and phylogenetically to *C. lustricola*. *Coniella lustricola* can be distinguished from its sister taxon, *C. erumpens* (Fig. 2), which is known only from rotten wood in Chile (Alvarez et al. 2016), by having a conidial l:w ratio of 2.8, as compared to 2.2 in *C. erumpens*. In addition, we distinguished *C. lustricola* from all remaining *Coniella* species (Table 3), providing further evidence that this species is distinct.

Coniella lustricola is the first *Coniella* species to have its genome sequenced. The *Coniella* genome is currently the smallest (36.56 Mbp) of the five additional Diaporthales genomes found in MycoCosm that range from 37.42 to 58.52 Mbp. At the class level, the *Coniella* genome size is close to the average Sordariomycetes genome size (42.46 Mbp, n = 108). *Coniella lustricola* appears to conform with most Ascomycota species in their ability to degrade cellulose and hemicellulose, but limited ability to degrade lignin (Dashtban et al. 2010). Our data corroborate this hypothesis on the basis of predicted gene models (Table 2). Because gene presence does not equate to gene function and fungi have a wide diversity and number of enzymes and vary in their ability to degrade plant cell walls (Zhao et al. 2013), further in vitro enzymatic assays, transcriptome experiments, and genomic

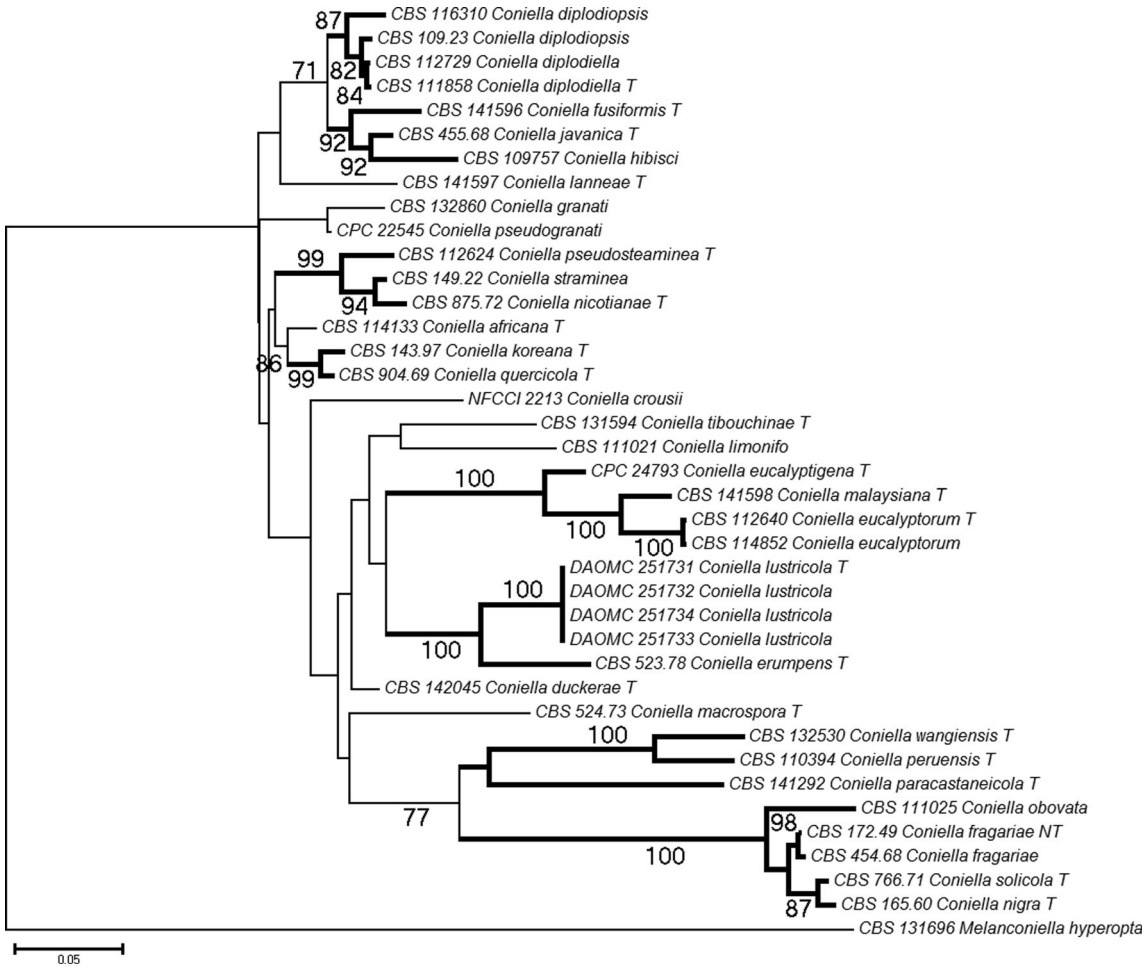

Fig. 2 Phylogram of the RAxML maximum likelihood analysis of *Coniella* species based on combined ITS, LSU, *rpb2*, and *tef1* nrDNA sequences. Bootstrap values ≥ 70% are shown above or below branches. Thickened branches indicate Bayesian posterior probabilities ≥ 95%. *Coniella lustricola* in shown in **bold**. The type species of *Coniella* is *C. fragariae*. T = sequences generated from type specimens

Table 3 Diagnostic features among *Coniella* and its synonym *Pilidiella* species lacking molecular data

Isolate	Conidia morphology	Host	Geography	Source
Coniella				
angustispora		*Psidium guajava*	Hawaii	Samuels et al. (1993)
australiensis	l:w = 1.4			Alvarez et al. (2016); Van Niekerk et al. (2004)
calamicola		*Daemonorops margaritae*	Hong Kong	Alvarez et al. (2016)
castaneicola	l = 15–29 μm, w = 2.5–3.5 μm			Sutton (1980)
citri	l = 8–19 μm, w = 3–4.5 μm	*Citrus medica*	India	Nag Raj (1993); Sharma and Agarwal (1977)
clypeata		decaying leaf	Japan	Alvarez et al. (2016)
costae	l = 19–28 μm; w = 7–7.5 μm			Dianese et al. (1993)
delicata	l = 7–9 μm, w = 2.5–3 μm	*Aerides crassifolia*	Thailand	Sutton (1980)
destruens	l = 12–13 μm, w = 4–5 μm, acutely rounded apices	*Eucalyptus grandis*	Hawaii	Van Niekerk et al. (2004)
eucalypticola	l = 19–29 μm, w = 2.5–3.5 μm	*Eucalyptus* sp.	India	Nag Raj (1976)
genistae		*Genista tinctoria*	Germany	Alvarez et al. (2016)
minima	globose to subglobose conidia l = 6.5–7.5 μm, w = 3.5–4.5 μm	*Eucalyptus camaldulensis*	Burma (Myanmar)	Alvarez et al. (2016); Sutton (1980)
musaiaensis	l = 15.5–22 μm, w = 4.5–5 μm	*Piliostigma thonningii*, *Acacia arabica*, and from soil	Sierra Leone, Pakistan, and India	Sutton (1980)
oryzae	ellipsoidal conidia	*Oryzae sativae*	Pakistan	Ahmad (1968)
petrakii	l = 10–15.5 μm, w = 4.5–7 μm	*Vitis vinifera*	India	Sutton (1980)
petrakioidea	l:w = 1.9			Van Niekerk et al. (2004)
populina		*Populus tremula*	Russia	Alvarez et al. (2016)
simba	strongly fusiform navicular	*Caesalpinia pulcherrima*	India	Sutton (1969)
stromatica	l = 12.4–19.7 μm, w = 8–10 μm, longitudinal germ slit	tree bark	Brazil	Alvarez et al. (2016)
terminaliae	globose to subglobose, l:w = 2.0			Miranda et al. (2012)
terminaliicola		*Terminalia superba*	Ecuador	Alvarez et al. (2016)
Pilidiella				
duvauicola		*Duvaue longifolia*	Argentina	Alvarez et al. (2016)
haraeana	l = 15–17.5 μm			Sydow and Sydow (1913)
jambolana	l = 19–22 μm, w = 3.5–4 μm	*Eugenia jambolana*	Pakistan	Ahmad (1967)
tamaricina		*Tamarix articulata*	Pakistan	Ahmad (1967)

l = length, w = width

comparisons are needed to determine the extent to which *C. lustricola* and its relatives can degrade plant detritus.

Interestingly, the *Coniella* genome has several unique features related to detritus degradation and stress tolerance. It possesses six pectate lyases that, so far, represent the fewest number found in Diaporthales genomes, followed by the eight pectate lyases in *Cryphonectria parasitica*, the causative agent of chestnut blight (Bramble 1936). *Coniella lustricola* possesses 13 tannases that represent the most numerous relative to other Diaporthales sequenced genomes. The next highest number of tannases in Diaporthales is 10, also found in *C. parasitica*. Lastly, the *Coniella* genome has three genes in peptidase family S64. This family is, so far, absent in other Diaporthales, and is known in *Saccharomyces cerevisiae* to process the membrane-bound transcription factor Stp1, inducing permease genes necessary for the uptake of amino acids (Abdel-Sater et al. 2004). *Coniella* lacked a predicted gene for a low temperature viability protein that is present in all other available Sordariomycetes genomes, and that is involved in ribosome biogenesis 40S subunit production (Loar et al. 2004). However, it possesses a predicted gene for cysteine-rich TM module stress response (CYSTM) that is known for stress tolerance across eukaryotes (Venancio and Aravind 2010), but that is lacking in all other Sordariomycetes. *Coniella lustricola* also has a loss of predicted genes in a stress-responsive A/B barrel domain gene family that is, so far, present in other Diaporthales species. The stress-responsive A/B barrel domain family has an unknown

function, but it is upregulated in *Populus balsamifera* during salt stress (Gu et al. 2004). *Coniella* possesses several predicted genes for aryl sulfotransferase. Aryl sulfotransferase enzymes have the potential to degrade toxic phenolic compounds that are a byproduct of biomass decomposition and phytoplankton extracellular metabolic products (Filipowicz et al. 2017; Osadchyy et al. 2016). Interestingly, some of these unique genetic differences (high tannase copy number, presence of peptidase family S64, and presence of aryl sulfotransferase genes) may indicate an adaptation to a high tannic acid, low nitrogen environment, and phenolic-rich detritus (Mettrop et al. 2014) typically found in fens.

According to the presented data, we propose *C. lustricola* as a new species in the genus *Coniella* based on molecular and morphological evidence. In addition, there is genomic evidence that this species can degrade hemicellulose, cellulose, pectin, and tannins. Lignin degradation is likely limited in most species in this genus. We concede that gene presence does preclude gene function; therefore, in vitro assays are needed to confirm predicted gene function. Presented genome data will contribute in understanding the enzymatic potential and ecological role of this fen-inhabiting species and allow its comparison with other *Coniella* species infecting agricultural crops.

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