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A preliminary study of wood-decay fungi in forests of northwest Arkansas

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Abstract

The present study represented an effort to characterize the assemblage of wood-decay fungi associated with forest ecosystems in northwest Arkansas. Specimens of fungi were collected from two different study areas. These were Pea Ridge National Military Park and Devil's Den State Park. In addition, small pieces of coarse woody debris (usually dead branches) were collected in the two study areas, returned to the laboratory and placed in plastic incubation chambers to which water was added. Fruiting bodies appearing in these chambers over a period of several months were collected and handled in the same manner as specimens on decaying wood obtained in the field. All specimens were identified through sequencing of the internal transcribed spacer (ITS) ribosomal DNA region. A total of 111 taxa were recorded, the majority of which could be identified to species. Seventy-seven taxa were recorded as field collections, whereas 34 taxa were recorded from the incubation chambers. Surprisingly, the two sets of data did not share any examples in common.

Key words – Basidiomycota – coarse woody debris **–** ITS ribosomal DNA region **–** Ozarks

Introduction

In forest ecosystems, certain fungi (usually referred to as wood-decay fungi) play an essential role in the decomposition of the coarse woody debris derived from the trees and other woody plants (e.g., shrubs) present. The decomposition of this coarse woody debris is a critical process, since it is important in nutrient recycling, soil development and the carbon budget of these ecosystems. For example, some wood-decay fungi have the capability to degrade the lignin component of coarse woody debris (Blanchette 1991, Eriksson et al. 2012), which otherwise would accumulate over time. Other wood-decay fungi meet their energy requirements by absorbing molecules derived the breakdown of the cellulose component of coarse woody debris. The fruiting bodies of wood-decay fungi also can serve as a source of food, nutrients and a breeding place for various animals (Stephenson 2010).

Wood-decay fungi are common in the forests of Arkansas, but there has never been an effort to characterize the assemblage of species present. The objective of the present study was to obtain data on wood-decay fungi in two different areas in northwest Arkansas. These two areas were Pea Ridge National Military Park and Devil's Den State Park. Pea Ridge National Military Park (36°27'15" N, 94°02'05" W), which has a total area of approximately 1740 ha, was established in 1956 to protect the site of the Civil War Battle of Pea Ridge, which was fought on 7–8 March 1862.

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The park is located in northwest Arkansas near the Missouri border. Forested areas of the park are dominated by a mixture of several species of oak (*Quercus alba* L., *Q. velutina* Lam., *Q. stellata* Wangenh. and *Q. rubra* L.) and hickory (*Carya ovata* [Mill.] K. Koch, *C. texana* Buckley and *C. tomentosa* Sarg.). Devil's Den State Park (36°46'28" N, 94°14'30" W), consists of about 1011 ha and is located near the border between Arkansas and Oklahoma. The park, which was constructed by the Civilian Conservation Corps during the 1930's, protects the largest sandstone crevice area in the United States. The forests present are similar in composition to those found in Pea Ridge National Military Park, but contains an admixture of species such as red maple (*Acer rubrum* L. and *Acer saccharum* Marshall) generally characteristic of relatively more mesic site conditions Stephenson et al. 2007).

Materials & Methods

Sampling for wood-decay fungi

Each of the two study areas was visited on a number of occasions during the period of July to November 2017. Fruiting bodies of wood-decay fungi were located using an opportunistic search method as described by Cannon & Sutton (2004). When fruiting bodies were observed, they were photographed in the field and then removed from the substrate upon which they occurred with the aid of a knife or a small hatchet. Fruiting bodies were then placed in a plastic collecting box with multiple compartments or loosely wrapped in aluminum foil and returned the laboratory. In the laboratory, specimens were dried at a temperature between 42-55˚C on a food dehydrator, placed in plastic bags, and placed in the herbarium at the University of Arkansas (UARK) after being labeled with a unique collection number.

During a period extending from November 2017 to February 2018, the study areas were revisited and small pieces of coarse woody debris (usually dead branches) were collected. These were returned to the laboratory and placed in plastic incubation chambers (33 cm long by 17 cm wide and 11 cm deep) along with a small amount of water. These were maintained for approximately two months, and water was added whenever necessary to keep the sample material moist. It was anticipated that in the incubation chambers, at least some of the mycelia already present in the coarse woody debris would produce fruiting bodies. When this happened, the fruiting bodies were collected and preserved in the same manner as those collected directly in the field.

Samples and morphological observations

Morphological aspects of the specimens collected in the field or from incubation chambers were determined with the use of an AmScope stereomicroscope (Gilbertson & Ryvarden 1986, Sotome et al. 2013). Tentative identifications were based upon various morphological features such as the size, color and shape of the fruiting body and the presence or absence of such structures as a distinct cap or stipe. Identifications were made with the use of such sources of information as Gilbertson & Ryvarden (1986, 1987), Bessette et al. (1997), Barron (1999), Binion et al. (2008), Elliott & Stephenson (2018).

DNA extraction, PCR and sequencing

DNA was extracted from one or more representative specimens for each different taxon tentatively identified on the basis of morphological features of the fruiting body. This extraction was carried out using a Wizard® genomic purification kit (Promega Corporation, Madison, WI). Genomic DNA was amplified using the fungal-specific primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Toju et al. 2012). PCR amplifications were performed in a thermocycler programed for an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 sec, annealing at 50 °C for 45 sec, and extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The length of amplified products was confirmed by 1% agarose gel electrophoresis gel using 0.5 × TAE buffer, SYBR safe staining dye and 1 kb DNA ladder (New England Biolabs, Ipswich, MI). The amplicons

were sent for Sanger sequencing to GeneWiz (South Plainfield, NJ). The sequences obtained from the latter company were cleaned up and then identified by doing nucleotide blast searches against the NCBI database (www.ncbi,nlm,nih,gov).

Results

For identification of specimens sequences were blasted against the NCBI database with a blast option to identify the taxon involved. At 97% sequence similarity, sequences were considered as identified to species when there was a match with an existing sequence; at a lower % sequence identify, sequence were considered as identified only to genus. Although no single cutoff value has been universally established for species identification across the kingdom Fungi, 97% sequence similarity has been used in a number of other studies (Raja et al. 2017). A total of 111 taxa (Table 1) were recorded, the majority of which could be identified to species. Seventy-seven taxa were recorded as field collections, whereas 34 taxa were recorded from the incubation chambers. Surprisingly, the two sets of data did not share any examples in common.

The taxa identified belong to at least 37 different families, with representatives of the Polyporaceae, Mycenaceae, Marasmiaceae, Pluteaceae, Steccherinaceae, Stereaceae and Xylariaceae the most common. Twenty taxa belonged to just the Polyporaceae. *Mycena* was the genus represented by the most species (7). Although the focus of this study was directed towards wood-decay fungi, some of the taxa identified have a different ecological role and were simply associated with decomposing wood. For example, such is the case for *Cordyceps confragosa*, which is an entomopathogenic fungus.

Table 1 Taxa of wood-decay fungi recorded from northwest Arkansas. Note: %ID = percent sequence identity, F = field collection, I = incubation chamber collection and SGB = sequence in GenBank.

Taxon	Family	%ID	F	I	ANO
Antrodia serialis (Fr.) Donk	Fomitopsidaceae	**99**	**X**		KC585304.1
Auricularia americana Parmasto & I. Parmasto	Auriculaceae	**99**	**X**		JX065166.1
Bolbitius bisporus E.F. Malysheva	Bolbitiaceae	**99**	**X**		NR153611.1
Byssomerulius incarnates (Schwein.) Gilb.	Meruliaceae	**99**	**X**		MF773635.1
Cladosporium cladosporioides (Fresen.) G.A. de Vries	Davidiellaceae	**100**	**X**		MF476049.1
Clitopilus hobsonii (Berk.) P.D. Orton	Entolomataceae	**99**		**X**	FJ770395.1
Coprinellus radians (Fr.) Vilgalys, Hopple & Jacq.	Psathyrellaceae	**100**	**X**		KJ714004.1
Coprinellus sp. 1	Psathyrellaceae	**99**	**X**		KX611630.1
Cordyceps confragosa (Mains) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora	Clavicipitaceae	**99**		**X**	KT372853.1
Crucibulum laeve (Huds.) Kambly	Nidulariaceae	**98**	**X**		DQ071701.2
Cryptococcus yokohamensis Alshahni, Satoh & Makimura	Tremellaceae	**97**		**X**	HM222928.1
Daedaleopsis septentrionalis (P. Karst.) Niemela	Polyporaceae	**99**	**X**		HG973499.1
Daedaleopsis tricolor (Bull.) Bondartsev & Singer	Polyporaceae	**100**	**X**		KY235366.1
Eurotium rubrum Jos. König, Spieck. & W. Bremer	Trichocomaceae	**100**	**X**		EU001331.1
Exidia recisa (Ditmar) Fr.	Auriculariaceae	**99**	**X**		LC098751.1
Exidia sp. 1	Auriculariaceae	**99**	**X**		MF161299.1

Table 1 Continued.

Taxon	Family	%ID	F	I	ANO
Fuscoporia gilva (Schwein.) T. Wagner & M. Fisch.	Hymenochaetaceae	**100**	**X**		KU139196.1
Galiella rufa (Schwein.) Nannf. & Korf	Sarcosomataceae	**99**		**X**	AF485073.1
Ganoderma sp. 1	Ganodermataceae	**99**	**X**		AF255100.1
Gloeoporus dichrous (Fr.) Bres.	Meruliaceae	**100**	**X**		JQ673109.1
Gymnopus dryophilus (Bull.) Murrill	Omphalotaceae	**99**		**X**	DQ449974.1
Gymnopus earleae Murrill	Marasmiaceae	**99**		**X**	DQ449994.1
Gymnopus foliiphilus R.H. Petersen	Omphalotaceae	**100**	**X**		KY026721.1
Gymnopus luxurians (Peck) Murrill	Marasmiaceae	**99**		**X**	AY256709.1
Gymnopus subnudus (Ellis ex Peck) Halling	Marasmiaceae	**99**	**X**		KY026667.1
Hericium erinaceus (Bull.) Pers.	Hericiaceae	**99**	**X**		AY534583.1
Hohenbuehelia angustata (Berk.) Singer	Pleurotaceae	**100**	**X**		MG383816.1
Hydnochaete tabacina (Berk. & M.A. Curtis ex Fr.) Ryvarden	Hymenochaetaceae	**97**	**X**		JQ279562.1
Hymenochaete pinnatifida Burt	Hymenochaetaceae	**100**	**X**		KU975472.1
Hyphodermella rosae (Bres.) Nakasone	Phanerochaetaceae	**100**	**X**		KT962555
Hypoxylon crocopeplum Berk. & M.A. Curtis	Xylariaceae	**99**	**X**		KU683962.1
Infundibulicybe gibba (Pers.) Harmaja	Tricholomataceae	**99**	**X**		MG663274.1
Irpex lacteus (Fr.) Fr.	Steccherinaceae	**99**	**X**		KT272411.1
Lachnum virgineum (Batsch) P. Karst.	Hyaloscyphaceae	**99**		**X**	AB481268.1
Lenzites betulinus (L.) Fr.	Polyporaceae	**100**	**X**		KY313640.1
Lycoperdon pyriforme Schaeff.	Agaricaceae	**100**	**X**		MF161171.1
Marasmiellus juniperinus Murrill	Marasmiaceae	**99**		**X**	NR_119582.1
Marasmius pulcherripes Peck	Marasmiaceae	**98**		**X**	FJ917615.1
Marasmius sp. 1	Marasmiaceae	**95**		**X**	FJ917619.1
Microstoma floccosum (Sacc.) Raitv.	Sarcoscyphaceae	**99**	**X**		AF026309.1
Mycena aurantiomarginata (Fr.) Quél.	Mycenaceae	**99**		**X**	JF908479.1
Mycena haematopus (Pers.) P. Kumm.	Mycenaceae	**100**		**X**	MF686517.1
Mycena inclinata (Fr.) Quél.	Mycenaceae	**99**	**X**		DQ490645.1
Mycena leaiana (Berk.) Sacc.	Mycenaceae	**99**	**X**		JF908376.1
Mycena sp. 1	Mycenaceae	**95**	**X**		MG748570.1
Mycena sp. 2	Mycenaceae	**96**		**X**	FJ917615.1
Mycena sp. 3	Mycenaceae	**90**	**X**		DQ490645.1
Mycorrhaphium adustum (Schwein.) Maas Geest.	Steccherinaceae	**100**	**X**		JN710573.1
Nectria mariannaeae Samuels & Seifert	Nectriaceae	**99**		**X**	GU586835.1
Neofavolus alveolaris (DC.) Sotome & T. Hatt.	Polyporaceae	**99**	**X**		KP283508.1
Neofavolus sp. 1	Polyporaceae	**99**	**X**		KP283508.1
Neofavolus sp. 2	Polyporaceae	**99**	**X**		KP283508.1
Neofavolus sp. 3	Polyporaceae	**99**	**X**		KP283508.1
Nigroporus vinosus (Berk.) Murrill	Steccherinaceae	**100**	**X**		JX109857.1
Panellus stipticus (Bull.) P. Karst.	Mycenaceae	**99**	**X**		AB863032.1
Panus conchatus (Bull.) Fr.	Panaceae	**100**		**X**	MH016880.1
Panus lecomtei (Fr.) Corner	Polyporaceae	**99**		**X**	KP135329.1

Table 1 Continued.

Taxon	Family	%ID	F	I	ANO
Panus neostrigosus Drechsler-Santos & Wartchow	Polyporaceae	**99**		**X**	KU761235.1
Panus rudis Fr.	Polyporaceae	**99**	**X**		KU863048.1
Phaeomarasmius erinaceus (Peck) Singer	Inocybaceae	**99**	**X**		MG773816.1
Phanerochaete pseudosanguinea Floudas & Hibbett	Phanerochaetaceae	**100**	**X**		KP135097.1
Phlebiopsis flavidoalba (Cooke) Hjortstam	Phanerochaetaceae	**97**	**X**		KX065956.1
Pholiotina aeruginosa (Romagn.) M.M. Moser	Bolbitiaceae	**97**	**X**		KF515918.1
Pleurotus floridanus Singer	Bolbitiaceae	**100**		**X**	MG819742.1
Pleurotus ostreatus (Jacq. ex Fr.) P. Kumm.	Pleurotaceae	**99**		**X**	MH395969.1
Pleurotus sapidus Sacc.	Pleurotaceae	**100**	**X**		KY962449.1
Pluteus cervinus (Schaeff.) P. Kumm.	Pleurotaceae	**99**	**X**		KF306014.1
Pluteus chrysophlebius (Berk. & M.A. Curtis) Sacc.	Pleurotaceae	**99**	**X**		HM562125.1
Pluteus longistriatus (Peck) Peck	Pluteaceae	**99**	**X**		MH211936.1
Pluteus petasatus (Fr.) Gillet	Pluteaceae	**100**	**X**		KJ009707.1
Pluteus romellii (Britzelm.) Sacc.	Pluteaceae	**99**		**X**	KM983699.1
Pluteus sp. 1	Pleurotaceae	**95**	**X**		KY346856.1
Polyporus tuberaster (Jacq. ex Pers.) Fr.	Polyporaceae	**97**		**X**	KJ668474.1
Polyporus sp. 1	Polyporaceae	**100**	**X**		KU324794.1
Psathyrella sp. 1	Psathyrellaceae	**94**	**X**		KC992949.1
Pseudochaete tabacina (Sowerby) T. Wagner & M. Fisch.	Hymenochaetaceae	**98**	**X**		KJ140591.1
Resupinatus alboniger (Pat.) Singer	Pleurotaceae	**99**		**X**	KU355368.1
Resupinatus applicatus (Batsch) Gray	Tricholomataceae	**99**		**X**	KU355368.1
Rhizomarasmius pyrrhocephalus (Berk.) R.H. Petersen	Psathyrellaceae	**99**	**X**		DQ097369.1
Schizophyllum commune Fr.	Schizophyllaceae	**99**		**X**	EU853847.1
Schizopora ovispora (Corner) Hjortstam & Ryvarden	Schizoporaceae	**100**		**X**	KX857803.1
Scutellinia crinite (Bull.) Lambotte	Pyronemataceae	**99**		**X**	AY220797.1
Simocybe serrulata (Murrill) Singer	Inocybaceae	**99**	**X**		MF153085.1
Simplicillium lanosoniveum (J.F.H. Beyma) Zare & W. Gams	Cordycipitaceae	**100**		**X**	AB758126.1
Spongipellis pachyodon (Pers.) Kotl. & Pouzar	Hapalopilaceae	**100**	**X**		KP135302.1
Steccherinum bourdotii Saliba & A. David	Steccherinaceae	**99**	**X**		KY948818.1
Steccherinum laeticolor (Berk. & M.A. Curtis) Banker	Steccherinaceae	**99**	**X**		KY948823.1
*Steccherinum murashkinskyi (*Burt) Maas Geest.	Steccherinaceae	**99**	**X**		FJ798705.1
Stereum complicatum (Fr.) Fr.	Stereaceae	**99**	**X**		KU559368.1
Stereum ostrea (Blume & T. Nees) Fr.	Stereaceae	**100**	**X**		KU559366.1
Stereum sanguinolentum (Alb. & Schwein.) Fr.	Stereaceae	**99**		**X**	AY089730.1
Stereum sp. 1	Stereaceae	**100**	**X**		KJ832051.1

Table 1 Continued.

Taxon	Family	%ID	F	I	ANO
Stereum sp. 2	Stereaceae	**99**	**X**		KR135365.1
Stereum sp. 3	Stereaceae	**99**	**X**		KJ831876.1
Tetrapyrgos nigripes (Fr.) E. Horak	Marasmiaceae	**99**	**X**		DQ449942.1
Trametes conchifer (Schwein.) Pilát.	Polyporaceae	**100**	**X**		JN164988.1
Trametes versicolor (L.) Lloyd	Polyporaceae	**100**	**X**		MG554226.1
Trametes villosa (Sw.) Kreisel	Polyporaceae	**99**	**X**		JN164970.1
Trametopsis cervina (Schwein.) Tomšovský	Polyporaceae	**100**	**X**		MG663240.1
Trichaptum biforme (Fr.) Ryvarden	Polyporaceae (?)	**100**	**X**		MF773616.1
Trichoderma viride Pers.	Hypocreaceae	**99**		**X**	KM458804.1
Trichoderma sp. 1	Hypocreaceae	**99**		**X**	AB872440.1
Truncospora ohiensis Berk.	Polyporaceae	**98**		**X**	KT695324.1
Tyromyces galactinus (Berk.) Bondartsev	Polyporaceae	**100**	**X**		KY948829.1
Tyromyces kmetii (Bres.) Bondartsev & Singer	Polyporaceae	**99**	**X**		KF698747.1
Urnula craterium (Schwein.) Fr.	Sarcosomataceae	**99**	**X**		EU834222.1
Xeromphalina kauffmanii A.H. Sm.	Mycenaceae	**99**	**X**		MG663296.1
Xylaria cornu-damae (Schwein.) Berk.	Xylariaceae	**99**		**X**	AF163031.1
Xylaria hypoxylon (L.) Grev.	Xylariaceae	**100**	**X**		U47841
Xylaria sp. 1	Xylariaceae	**99**	**X**		KU683962.1
Xylaria sp. 2	Xylariaceae	**92**		**X**	JQ761642.1

Discussion

Although studies of the wood-decay fungi have been carried out in various localities throughout the world (e.g., Leiniger et al. 1997, Alemu 2013, Lyngdoh & Dkhar 2014, Bhutia 2016), the one described herein apparently represents the first such effort in the forests of northwest Arkansas. Indeed, we are not aware of any comparable studies anywhere in the Ozark physographic province, which encompasses norhern Arkansas and the southern half of Missouri while also extending westward into northern Oklahoma and southeast Kansas. Consequently, the prelimary results reported in this paper set the stage for future more comprehensive studies.

The total number of taxa reported (111) clearly indicates that the species richness of the assemblage of wood-decay fungi in the general study is quite high. Some of the taxa recorded, including such examples as *Mycena haematopus*, *Panellus stipticus*, *Pleurotus ostreatus*, *Schizophyllum commune*, and *Stereum complicatum* are common and widespread wood-decay fungi. Other taxa had sequences that did not match anything in the GenBank database. This would suggest that they are either rare (i.e., have been described but not yet sequenced) or are possibly new to science.

The most unexpected result of the present study is that the set of taxa recorded as field collections and the set of taxa ecorded from the incubation chambers did not share any examples in common. Field collections of fruiting bodies and the pieces of coarse woody debris placed in the incubation chambers were obtained at somewhat different times of the year, with the collecting periods overlapping only in November. Moreover, the pieces of coarse woody debris tended to be portions of branches, whereas the majority of fruiting bodies obtained as field collections typically occurred on logs. Although wood-decay fungi do differ with respect to some aspects of their biology (Worrall et al. 1997), it is difficult to see how this would account for such a profound difference, especially since the second author has observed such taxa as *Mycena haematopus*, recorded only in incubation chambers in the present study, commonly fruiting upon large logs. Clearly, the conditions that exist within an incubation chamber are not the same as those that exist in the field, and this might be a factor accounting for the observed differences. Nevertheless, it

seems exceedingly unlikely that these differences would persist as additional data from the two study areas become available.

The second author is not aware of any published study of wood-decay fungi in which incubation chambers have been used in efforts to document the taxa present at a particular locality. In the present study, the number of incubation chambers yielding fruiting bodies of fungi was quite high (56%), and since the chambers were monitored on a regular basis, the fruiting bodies could be collected when they were still in excellent condition. This certainly was a major advantage, both in terms of making identification easier and increasing the chances of being able to extract "good" DNA.

The fungal biota (all groups) of northwest Arkansas and adjacent areas of the Ozarks is not especially well-documented, since the appropriate surveys have never been carried out (Swartz 1933) However, based the limited body of data currently available (e.g., Discover Life [www.discoverlife.com] and GBIF [www.gbif.org]), there are no previous records from the region for a number of the taxa listed in Table 1. It is anticipated that additional more comprehensive studies will yield numerous additonal records.

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