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RESEARCH ARTICLE

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Malassezia vespertilionis sp. nov.: a new cold-tolerant species of yeast isolated from bats

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Key words

Chiroptera
evolution
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Malassezia
Myotis
new species
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Abstract *Malassezia* is a genus of medically-important, lipid-dependent yeasts that live on the skin of warm-blooded animals. The 17 described species have been documented primarily on humans and domestic animals, but few studies have examined *Malassezia* species associated with more diverse host groups such as wildlife. While investigating the skin mycobiota of healthy bats, we isolated a *Malassezia* sp. that exhibited only up to 92 % identity with other known species in the genus for the portion of the DNA sequence of the internal transcribed spacer region that could be confidently aligned. The *Malassezia* sp. was cultured from the skin of nine species of bats in the subfamily *Myotinae*; isolates originated from bats sampled in both the eastern and western United States. Physiological features and molecular characterisation at seven additional loci (D1/D2 region of 26S rDNA, 18S rDNA, chitin synthase, second largest subunit of RNA polymerase II, β-tubulin, translation elongation factor EF-1α, and minichromosome maintenance complex component 7) indicated that all of the bat *Malassezia* isolates likely represented a single species distinct from other named taxa. Of particular note was the ability of the *Malassezia* sp. to grow over a broad range of temperatures (7–40 °C), with optimal growth occurring at 24 °C. These thermal growth ranges, unique among the described *Malassezia*, may be an adaptation by the fungus to survive on bats during both the host's hibernation and active seasons. The combination of genetic and physiological differences provided compelling evidence that this lipid-dependent yeast represents a novel species described herein as *Malassezia vespertilionis* sp. nov. Whole genome sequencing placed the new species as a basal member of the clade containing the species *M. furfur*, *M. japonica*, *M. obtusa*, and *M. yamatoensis*. The genetic and physiological uniqueness of *Malassezia vespertilionis* among its closest relatives may make it important in future research to better understand the evolution, life history, and pathogenicity of the *Malassezia* yeasts.

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INTRODUCTION

Members of the genus *Malassezia* are lipid-dependent fungi specialised to live on the skin of humans and other euthermic animals. *Malassezia* is the sole genus in the class *Malasseziomycetes*, and the 17 described species appear to be part of the natural skin mycobiome of animals (Wang et al. 2014). At least five species have been regularly associated with dermatitis or other types of skin disorders in humans (reviewed by Gaitanis et al. 2012), and *M. pachydermatis* is described as the cause of otitis externa in domestic dogs (Gustafson 1955, Bond et al. 2004). However, these potentially pathogenic species of *Malassezia* are also found on areas of normal skin of afflicted patients and on asymptomatic individuals, making it unclear what role the fungi play in skin disease (reviewed by Gaitanis et al. 2012). *Malassezia furfur*, *M. pachydermatis*, and *M. sympodialis* have also occasionally been implicated as causes of sepsis in infants and immunocompromised patients (reviewed by Gaitanis et al. 2012, Aguirre et al. 2015, Patron 2016), and it has even been hypothesised that *Malassezia* could play a role in promoting certain forms of skin cancer (Gaitanis et al. 2011).

In addition to their medical ambiguity, relatively little is known about the diversity and ecology of *Malassezia*. Members of the genus were traditionally identified on the basis of phenotypic traits, and prior to 1996 there were only three recognised species of *Malassezia*. More recent application of molecular techniques to assist with species characterisation has facilitated the ability to distinguish cryptic species and has increased the known diversity of the genus (e.g., Sugita et al. 2002, Hirai et al. 2004, Cabañes et al. 2007, 2016, Honnavar et al. 2016). Sugita et al. (2010) present a list of the animal hosts from which various species of *Malassezia* have been recovered. However, many reports used to generate that list lacked molecular data to support the identification of the *Malassezia* species that were isolated, and potentially novel taxa may have been overlooked. Indeed, diversity of the genus is likely much higher than currently documented (Amend 2014, Cabañes 2014). Ten of the 17 *Malassezia* species are most closely associated with humans, and were discovered through culture-based surveys of diseased skin; the remaining seven species (*M. brasiliensis*, *M. caprae*, *M. cuniculi*, *M. equina*, *M. nana*, *M. pachydermatis*, and *M. psittaci*) have been isolated primarily from animals (reviewed by Sugita et al. 2010, Cabañes et al. 2016), with *M. pachydermatis* reported as a zoonotic pathogen (Chang et al. 1998). Some of the zoophilic members of the group appear to have a broad host range, while others are more host-specific (reviewed by Guého-Kellerman et al. 2010, Sugita et al. 2010). Little work has been done with broader taxonomic

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host groups, and the relatively small number of described species of *Malassezia* is likely the result of sampling bias, which is skewed toward humans and domestic animals. Given the host specificity of some species of *Malassezia*, many more taxa may be discovered when a broader range of host species (especially wildlife) are sampled. Such undiscovered species of *Malassezia* could be important in further elucidating the taxonomy, evolution, ecology, and pathogenicity of this group of medically important fungi.

While investigating the mycobiota on the skin of bats, we detected a putative *Malassezia* sp. that was genetically distinct from other known members of the genus. Here we describe the isolation, occurrence, and characterisation of this novel species.

MATERIALS AND METHODS

Isolation of Malassezia from bats

Samples were collected in 2014, 2016, and 2017 under the U.S. Geological Survey - National Wildlife Health Center (NWHC) Animal Care and Use Committee Protocols #EP140212 and #EP081124-A2, with all necessary permits and permissions for the sites and species sampled. Hibernating bats (Fig. 1) were captured by hand and active bats were captured in mist nets. Gloves were changed between animals to prevent cross-contamination. The animals were sampled non-lethally using sterile Pur-Wraps® polyester-tipped swabs (Puritan Medical Products Company LLC, Guilford, Maine, USA) pre-moistened with 150 µL of sterile nuclease-free water. Swabs were gently rolled back-and-forth three times across the skin of the forearm and wing membrane between the elbow and wrist joints. Samples were then placed in sterile microcentrifuge tubes, stored chilled for up to 48 h, and shipped on ice to the NWHC. A total of 264 samples were obtained from thirteen sites in seven states (one site in Alabama, USA; one site in California, USA; one site in Kentucky, USA; one site in Missouri, USA; one site in Pennsylvania, USA; two sites in New York, USA; and six sites in Wisconsin, USA), representing ten bat species (*Lasionycteris noctivagans*, *Myotis californicus*, *Myotis grisescens*, *Myotis leibii*, *Myotis lucifugus*, *Myotis septentrionalis*, *Myotis sodalis*, *Myotis thysanodes*, *Myotis yumanensis*, and *Perimyotis subflavus*). A list of all individual bats sampled for the project is provided in Table 1.

Upon arrival at the laboratory, swabs were streaked onto Leeming and Notman agar (LNA; 10 g bacteriological peptone, 0.1 g yeast extract, 5 g glucose, 8 g desiccated ox bile, 1 mL glycerol, 0.5 g glycerol monostearate, 0.5 g Tween 60, 10 mL whole fat cow's milk, 0.5 g chloramphenicol, 0.5 g cycloheximide, 15 g agarose per litre, pH 6.0; modified slightly from Leeming & Notman (1987)) and incubated at 7 °C. Plates were checked weekly for a total of 12 wk, and any colonies resembling *Malassezia* were transferred to fresh LNA. Isolates were identified by sequencing the ITS as described by Lorch et al. (2015).

Whole genome sequence analysis

Isolate CBS 15041 (NWHC 44797-103; UAMH 11924) was selected for whole genome sequencing to further resolve the taxonomy of the bat-associated *Malassezia*. Nucleic acid was obtained using a phenol-chloroform extraction. Library preparation and next-generation sequencing was performed by the University of Wisconsin Biotechnology Center DNA Sequencing Facility using the genomic Nextera XT DNA Library Prep Kit (Illumina Inc., San Diego, CA) and the Illumina MiSeq Next Generation Sequencer platform. Sequence data was processed and assembled using JAAWS (https://github.com/nextgenusfs/jaaws). Briefly, the paired-end 250-bp MiSeq sequence reads (2×250) were processed with trimmomatic v. 0.36 (Bolger et al. 2014) to remove adapter sequences and phiX spike-in was removed using bowtie2 v. 2.3.2 (Langmead & Salzberg 2012) alignment to the phiX genome (NC_001422). The data were then assembled into scaffolds with Spades v. 3.9.0 (Bankevich et al. 2012). The subsequent assembly was cleaned using Blobtools v. 0.9.19 (Laetsch & Blaxter 2017) and filtered for unexpected coverage, mitochondrial DNA, contamination, and scaffolds less than 1 kb in length. Finally, the cleaned assembly was error corrected using five iterations of Pilon v. 1.22 (Walker et al. 2014). The genome of the bat-associated *Malassezia* was annotated with funannotate v. 0.7.0 while the 28 genomes of *Malassezia* species previously sequenced (Wu et al. 2015; Table 2) were annotated using funannotate v. 0.5.3 (https://github.com/nextgenusfs/funannotate). Conserved orthologues were identified using BUSCO2 (Simão et al. 2015) basidiomycete database using the busco wrapper script in Phyloma (https://github.com/nextgenusfs/phyloma). The concatenated protein sequences of 254 conserved BUSCO2 orthologues were aligned using MAFFT v. 7.305b (Katoh & Standley 2013)

Fig. 1 Hibernating bats, such as these *Myotis* sp., were sampled for this study by swabbing wing skin.

Table 1 List of individual bats sampled for this study.

Individual identifier	Location	Host species	Sampling date	*Malassezia vespertilionis* isolated
24716-001	Wisconsin, USA (site #1)	*Myotis septentrionalis*	03 March 2014	no
24716-002	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-003	Wisconsin, USA (site #1)	*Myotis septentrionalis*	03 March 2014	no
24716-004	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-005	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-006	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-007	Wisconsin, USA (site #1)	*Myotis septentrionalis*	03 March 2014	yes
24716-008	Wisconsin, USA (site #1)	*Myotis septentrionalis*	03 March 2014	yes
24716-009	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-010	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-011	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-012	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-013	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-014	Wisconsin, USA (site #1)	*Myotis septentrionalis*	03 March 2014	yes
24716-015	Wisconsin, USA (site #1)	*Myotis lucifugus*	03 March 2014	no
24716-016	Wisconsin, USA (site #1)	*Perimyotis subflavus*	03 March 2014	no
24716-017	Wisconsin, USA (site #1)	*Myotis septentrionalis*	03 March 2014	no
24716-018	Wisconsin, USA (site #1)	*Myotis septentrionalis*	03 March 2014	no
24716-019	Wisconsin, USA (site #1)	*Myotis septentrionalis*	03 March 2014	no
24716-020	Wisconsin, USA (site #1)	*Myotis* sp.*	03 March 2014	no
24738-002	Wisconsin, USA (site #2)	*Myotis lucifugus*	10 March 2014	yes
24738-005	Wisconsin, USA (site #2)	*Myotis lucifugus*	10 March 2014	no
24738-006	Wisconsin, USA (site #2)	*Myotis lucifugus*	10 March 2014	no
24738-008	Wisconsin, USA (site #2)	*Myotis lucifugus*	10 March 2014	no
24738-011	Wisconsin, USA (site #2)	*Myotis lucifugus*	10 March 2014	no
24738-013	Wisconsin, USA (site #2)	*Myotis lucifugus*	10 March 2014	yes
24738-022	Wisconsin, USA (site #2)	*Myotis lucifugus*	10 March 2014	no
24738-025	Wisconsin, USA (site #2)	*Myotis* sp.*	10 March 2014	yes
44767-001	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-002	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-003	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-004	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-005	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-006	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-007	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-008	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-009	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-010	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-011	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-012	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-013	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-014	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-015	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-016	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-017	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-018	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-019	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-020	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-021	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-022	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-023	Kentucky, USA	*Myotis grisescens*	04 March 2014	no
44767-024	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-025	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-026	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-027	Kentucky, USA	*Myotis sodalis*	04 March 2014	no
44767-028	Kentucky, USA	*Myotis sodalis*	04 March 2014	no
44767-029	Kentucky, USA	*Myotis lucifugus*	04 March 2014	no
44767-030	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-031	Kentucky, USA	*Myotis grisescens*	04 March 2014	yes
44767-032	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-033	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-034	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-035	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-036	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-037	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-038	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-039	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-040	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-041	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-042	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-043	Kentucky, USA	*Myotis sodalis*	04 March 2014	no
44767-044	Kentucky, USA	*Myotis sodalis*	04 March 2014	yes
44767-045	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-046	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-047	Kentucky, USA	*Perimyotis subflavus*	04 March 2014	no
44767-048	Kentucky, USA	*Myotis sodalis*	04 March 2014	no
44768-001	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-002	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-003	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-004	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-005	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-006	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-007	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-008	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-009	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-010	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-011	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-012	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	yes
44768-013	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no

Table 1 (cont.)

Individual identifier	Location	Host species	Sampling date	*Malassezia vespertilionis* isolated
44768-014	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-015	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-016	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-017	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-018	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-019	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-020	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	yes
44768-021	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	yes
44768-022	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-023	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-024	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-025	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	yes
44768-026	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-027	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-028	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-029	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-030	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44768-031	New York, USA (site #1)	*Myotis lucifugus*	19 March 2014	no
44769-001	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-002	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	no
44769-003	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	no
44769-004	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-005	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	no
44769-006	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-007	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	no
44769-008	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-009	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	no
44769-010	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-011	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	no
44769-012	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	no
44769-013	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-014	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-015	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	no
44769-016	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-017	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-018	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-019	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-020	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-021	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-022	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-023	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-024	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44769-025	Wisconsin, USA (site #3)	*Myotis lucifugus*	28 March 2014	yes
44797-032	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-033	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-034	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-035	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	yes
44797-036	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-037	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-038	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-039	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-040	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-041	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-042	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-043	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-044	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-045	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-046	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-047	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-048	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-049	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-050	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-051	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-052	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-053	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-054	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-055	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-056	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-057	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-058	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-059	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-060	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-061	New York, USA (site #2)	*Myotis lucifugus*	15 January 2015	no
44797-062	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-063	Missouri, USA	*Perimyotis subflavus*	24 February 2015	no
44797-064	Missouri, USA	*Myotis sodalis*	24 February 2015	yes
44797-065	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-066	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-067	Missouri, USA	*Perimyotis subflavus*	24 February 2015	no
44797-068	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-069	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-070	Missouri, USA	*Perimyotis subflavus*	24 February 2015	no
44797-071	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-072	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-073	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-074	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-075	Missouri, USA	*Perimyotis subflavus*	24 February 2015	no
44797-076	Missouri, USA	*Myotis sodalis*	24 February 2015	no
44797-077	Missouri, USA	*Perimyotis subflavus*	24 February 2015	no

Table 1 (cont.)

Individual identifier	Location	Host species	Sampling date	*Malassezia vespertilionis* isolated
44797-078	Missouri, USA	*Perimyotis subflavus*	24 February 2015	no
44797-100	Wisconin, USA (site #4)	*Myotis septentrionalis*	27 January 2015	yes
44797-101	Wisconin, USA (site #4)	*Myotis septentrionalis*	27 January 2015	no
44797-102	Wisconin, USA (site #4)	*Myotis septentrionalis*	27 January 2015	no
44797-103	Wisconsin, USA (site #1)	*Myotis septentrionalis*	28 January 2015	yes
44797-104	Wisconsin, USA (site #1)	*Myotis septentrionalis*	28 January 2015	yes
44797-105	Wisconsin, USA (site #1)	*Myotis septentrionalis*	28 January 2015	no
44797-106	Wisconsin, USA (site #1)	*Myotis septentrionalis*	28 January 2015	no
44797-107	Wisconsin, USA (site #1)	*Myotis septentrionalis*	28 January 2015	no
44797-108	Wisconsin, USA (site #1)	*Myotis septentrionalis*	28 January 2015	no
44797-109	Wisconsin, USA (site #1)	*Myotis septentrionalis*	28 January 2015	no
44797-110	Wisconsin, USA (site #1)	*Myotis septentrionalis*	28 January 2015	no
44797-111	Wisconsin, USA (site #5)	*Myotis septentrionalis*	29 January 2015	no
44797-112	Wisconsin, USA (site #5)	*Myotis septentrionalis*	29 January 2015	no
44797-113	Wisconsin, USA (site #5)	*Myotis septentrionalis*	29 January 2015	no
44797-114	Wisconsin, USA (site #5)	*Myotis septentrionalis*	29 January 2015	no
44797-115	Wisconsin, USA (site #5)	*Myotis septentrionalis*	29 January 2015	yes
44797-116	Wisconsin, USA (site #5)	*Myotis septentrionalis*	29 January 2015	no
44797-123	Wisconsin, USA (site #6)	*Myotis septentrionalis*	02 March 2015	no
44797-124	Wisconsin, USA (site #6)	*Myotis septentrionalis*	02 March 2015	yes
44797-125	Wisconsin, USA (site #6)	*Myotis septentrionalis*	02 March 2015	no
44797-126	Wisconsin, USA (site #6)	*Myotis septentrionalis*	02 March 2015	no
44797-127	Wisconsin, USA (site #6)	*Myotis septentrionalis*	02 March 2015	yes
44797-128	Wisconsin, USA (site #6)	*Myotis septentrionalis*	02 March 2015	no
44797-129	Wisconsin, USA (site #6)	*Perimyotis subflavus*	02 March 2015	no
44797-130	Wisconsin, USA (site #6)	*Myotis septentrionalis*	02 March 2015	no
44797-131	Wisconsin, USA (site #6)	*Myotis septentrionalis*	02 March 2015	yes
44797-132	Alabama, USA	*Perimyotis subflavus*	11 February 2015	no
44797-133	Alabama, USA	*Myotis grisescens*	11 February 2015	no
44797-134	Alabama, USA	*Perimyotis subflavus*	11 February 2015	no
44797-135	Alabama, USA	*Myotis sodalis*	11 February 2015	yes
44797-136	Alabama, USA	*Myotis grisescens*	11 February 2015	no
44797-137	Alabama, USA	*Myotis grisescens*	11 February 2015	yes
44797-138	Alabama, USA	*Myotis grisescens*	11 February 2015	yes
44797-139	Alabama, USA	*Myotis grisescens*	11 February 2015	yes
44797-140	Alabama, USA	*Perimyotis subflavus*	11 February 2015	no
44797-141	Alabama, USA	*Myotis grisescens*	11 February 2015	yes
44797-142	Alabama, USA	*Myotis grisescens*	11 February 2015	no
44797-143	Alabama, USA	*Myotis grisescens*	11 February 2015	yes
44797-144	Alabama, USA	*Myotis grisescens*	11 February 2015	no
44797-145	Alabama, USA	*Perimyotis subflavus*	11 February 2015	no
44797-146	Alabama, USA	*Myotis grisescens*	11 February 2015	no
44797-147	Alabama, USA	*Myotis grisescens*	11 February 2015	no
44797-148	Alabama, USA	*Myotis grisescens*	11 February 2015	yes
44797-149	Alabama, USA	*Perimyotis subflavus*	11 February 2015	no
44797-150	Alabama, USA	*Myotis grisescens*	11 February 2015	yes
44797-151	Alabama, USA	*Myotis grisescens*	11 February 2015	no
44797-152	Alabama, USA	*Myotis grisescens*	11 February 2015	yes
44797-153	Alabama, USA	*Myotis sodalis*	11 February 2015	yes
44797-154	Alabama, USA	*Myotis grisescens*	11 February 2015	no
44797-155	Alabama, USA	*Myotis grisescens*	11 February 2015	no
45701-660	California, USA	*Myotis yumanensis*	07 May 2016	no
45701-661	California, USA	*Myotis* sp.**	02 May 2016	no
45701-663	California, USA	*Myotis yumanensis*	18 April 2016	no
45701-664	California, USA	*Myotis yumanensis*	02 May 2016	yes
45701-665	California, USA	*Myotis californicus*	07 May 2016	yes
45701-666	California, USA	*Myotis californicus*	02 May 2016	no
45701-668	California, USA	*Myotis yumanensis*	09 May 2016	yes
45701-669	California, USA	*Myotis yumanensis*	18 April 2016	no
45701-671	California, USA	*Myotis yumanensis*	09 May 2016	no
45701-672	California, USA	*Myotis californicus*	02 May 2016	no
45701-674	California, USA	*Myotis californicus*	26 April 2016	no
45701-675	California, USA	*Myotis yumanensis*	07 May 2016	no
45701-676	California, USA	*Myotis* sp.**	02 May 2016	yes
45701-677	California, USA	*Myotis yumanensis*	18 April 2016	yes
45701-678	California, USA	*Myotis californicus*	18 April 2016	yes
45701-680	California, USA	*Myotis* sp.**	18 April 2016	no
45701-681	California, USA	*Myotis* sp.**	09 May 2016	no
45701-682	California, USA	*Myotis californicus*	19 April 2016	yes
45701-683	California, USA	*Myotis yumanensis*	20 April 2016	no
45701-684	California, USA	*Myotis californicus*	09 May 2016	no
45701-685	California, USA	*Myotis yumanensis*	20 April 2016	no
45701-686	California, USA	*Myotis thysanodes*	26 April 2016	yes
45701-687	California, USA	*Myotis yumanensis*	20 April 2016	no
45701-688	California, USA	*Myotis yumanensis*	20 April 2016	yes
45701-689	California, USA	*Myotis* sp.**	02 May 2016	no
45701-691	California, USA	*Myotis californicus*	16 May 2016	yes
45701-696	California, USA	*Myotis californicus*	16 May 2016	no
45701-699	California, USA	*Lasionycteris noctivagans*	16 May 2016	no
45701-714	California, USA	*Lasionycteris noctivagans*	16 May 2016	yes
45701-719	California, USA	*Myotis californicus*	16 May 2016	yes
45704-161	Pennsylvania, USA	*Myotis leibii*	26 February 2016	yes
46375-001	California, USA	*Myotis californicus*	08 May 2017	yes
46375-002	California, USA	*Myotis californicus*	08 May 2017	yes
46375-003	California, USA	*Lasionycteris noctivagans*	08 May 2017	yes
46375-004	California, USA	*Lasionycteris noctivagans*	08 May 2017	no

* either *Myotis lucifugus* or *Myotis septentrionalis*.
** either *Myotis lucifugus* or *Myotis yumanensis*.

Table 2 Fungal genomes and summary statistics used for whole genome phylogenic analysis.

Species	Strain identifier	GenBank accession number	Locus_tag	Assembly size (Mb)	Number of scaffolds	Scaffold N50 (Kb)	Percent GC	Protein coding gene models
Malassezia caprae	CBS 10434*	GCA_001264625.1	MCA1	7.58	229	110	59.78%	3.553
M. cuniculi	CBS 11721*	GCA_001264635.1	MCU1	7.459	76	522	58.99%	3.167
M. dermatis	CBS 9169*	GCA_001264665.1	MDM1	7.54	111	189	59.10%	3.538
	JCM 11348	GCA_001600775.1	MDM2	7.551	18	1.325	58.98%	3.544
M. equina	CBS 9969*	GCA_001264685.1	MEQ1	7.658	117	372	58.00%	3.232
M. furfur	JPLK 23	GCA_001265065.1	MFU6	7.79	2092	14	64.18%	3.087
	CBS 1878*	GCA_001265055.1	MFU1	13.865	3460	14	63.91%	5.418
	CBS 4172	GCA_001264895.1	MFU2	14.347	3453	15	64.23%	5.672
	CBS 7019*	GCA_001264875.1	MFU3	13.707	3262	15	63.70%	5.565
	CBS 7710	GCA_001264865.1	MFU4	15.232	4053	14	63.89%	5.835
	CBS 7982	GCA_001265045.1	MFU5	7.877	1694	20	64.06%	3.158
M. globosa	CBS 7966*	GCA_001264805.1	MGL2	8.94	113	724	52.02%	4.245
	CBS 7874	GCA_001264815.1	MGL1	8.938	138	398	51.87%	4.191
	CBS 7990	GCA_001264795.1	MGL3	8.884	108	414	52.07%	3.703
M. japonica	CBS 9431*	GCA_001264785.1	MJA1	8.341	295	66	62.38%	4.215
	JCM 11963	GCA_001600795.1	MJA2	8.364	16	814	62.33%	4.122
M. nana	CBS 9557*	GCA_001265015.1	MNA1	7.607	95	492	57.93%	3.785
	JCM 12085	GCA_001600835.1	MNA2	7.579	13	1.323	57.96%	3.734
M. obtusa	CBS 7876*	GCA_001264985.1	MOB1	7.842	1709	22	62.15%	2.893
M. pachydermatis	CBS 1879*	GCA_001264975.1	MPA1	8.158	61	957	55.08%	4.134
M. restricta	CBS 7877*	GCA_001264765.1	MRE1	7.249	90	402	55.83%	3.556
	CBS 8742	GCA_001264725.1	MRE2	7.26	69	666	55.79%	3.569
M. slooffiae	CBS 7956*	GCA_001264965.1	MSL1	8.425	1641	15	65.82%	3.422
M. sympodialis	ATCC 42132	GCA_001264925.1	MSY1	7.546	824	54	58.77%	3.055
	ATCC 44340	GCA_001264715.1	MSY2	7.562	769	59	58.88%	3.080
	ATCC 96806	GCA_001264705.1	MSY3	7.526	1030	44	58.80%	3.946
M. vespertilionis	CBS 15041*	GCA_002818225.1	MVES	7.581	14	844	56.62%	3.791
M. yamatoensis	CBS 9725*	GCA_001264885.1	MYA1	8.106	49	1.447	49.62%	3.971

* type or neotype strain.

and trimmed using trimAl v. 1.4.rev15 (Capella-Gutiérrez et al. 2009). A maximum-likelihood phylogeny was estimated using RAxML v. 8.2.10 (Stamatakis 2014) (PROTGAMMALG, 100 bootstrap replicates). As a secondary method, a Bayesian phylogeny was estimated using MrBayes v. 3.2.6 (Ronquist & Huelsenbeck 2003) through the CIPRES Science Gateway (Miller et al. 2010). For the Bayesian analysis, an LG model with gamma-distributed rate variation across sites was used. To generate the 50 % majority rule consensus tree, two runs, each with 1 000 000 generations and four chains, were performed. The chains were sampled every 250 generations with the first 25 % of sampled values discarded as burn-in.

Multilocus sequence analysis

To determine whether the isolates of *Malassezia* from bats represented a single species, seven loci (in addition to ITS) from 12 isolates (Table 3) were also examined: the D1/D2 region of 26S rDNA (hereafter referred to as D1/D2), the 18S rDNA, chitin synthase *CHS2*, second largest subunit of RNA polymerase II (*RPB2*), β-tubulin (β-tub), translation elongation factor EF-1α (*TEF1*), and minichromosome maintenance complex component 7 (*MCM7*). DNA was extracted using the methods of Lorch et al. (2015). The D1/D2 region was amplified using primers NL4 (5'-GGT CCG TGT TTC AAG ACG G-3') and NL1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG-3') (O'Donnell 1993); cycling conditions: 94 °C for 5 min; 30 cycles of 94 °C for 45 s, 51 °C for 1 min, and 72 °C for 3 min; and a final extension of 72 °C for 10 min. The 18S rDNA was amplified with forward (5'-ATC TGG TTG ATC CTG CCA GT-3') and reverse (5'-TCC TCC GCT TAT TGA TAT GC-3') primers described by Sugita & Nakase (1999); cycling conditions: 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 2 min and 30 s; and a final extension of 72 °C for 10 min. A portion of *CHS2* was amplified with primers ChiSyn2f (5'-CTG AAG CTT CAN ATG TAY AAY GAR GAY-3') and ChiSyn2r (5'-GTT CTC GAG YTT RTA YTC RAA RTT YTG-3') (Bowen et al. 1992, Cabañes et al. 2007); cycling conditions: 94 °C for 5 min; 45 cycles of 94 °C for 1 min, 50 °C for 2 min, and 72 °C for 3 min; and a final extension of 72 °C for 6 min. A fragment of *RPB2* was amplified with primers fRPB2-5F (5'-GAY GAY MGW GAT CAY TTY GG-3') and fRPB2-7cR (5'-CCC ATR GCT TGY TTR CCC AT-3') (Liu et al. 1999); cycling conditions: 94 °C for 4 min; 40 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1 min; and a final extension of 72 °C for 8 min. A portion of β-tub was amplified with primers F-βtub (5'-CAR GCY GGT CAR TGY GGT AAC CA-3') and F- βtub4r (5'-GCC TCA GTR AAY TCC ATY TCR TCC AT-3') (Einax & Voigt 2003); cycling conditions: 95 °C for 5 min; 30 cycles of 95 °C for 30 s, 50 °C for 1 min, and 72 °C for 1 min; and a final extension of 72 °C for 10 min. A portion of *TEF1* was amplified with primers EF1-983F (5'-GCY CCY GGH CAY CGT GAY TTY AT-3') and EF1-2218R (5'-ATG ACA CCR ACR GCR ACR GTY TG-3') (Rehner & Buckley 2005); cycling conditions: 94 °C for 2 min; 47 cycles of 94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min and 40 s; and a final extension of 72 °C for 10 min. A portion of *MCM7* was amplified with primers MCM7-709 (5'-ACN MGN GTN TCV GAY GTH AAR CC-3') and MCM7-1348 (5'-GAY TTD GCN ACN CCN GGR TCW CCC AT-3') (modified slightly from Schmitt et al. 2009); cycling conditions as described for *TEF1*. Reactions were carried out using GoTaq® Flexi DNA polymerase (Promega Corporation, Madison, WI) according to the manufacturer's instructions (with final concentrations of 1.5 mM $MgCl_2$ solution and 1 µM of each primer) except that twice the recommended amount of Taq polymerase (2.5 U) and 1–3 µL of template were added per 50 µL reaction. When necessary, PCR products were gel-purified prior to sequencing using the QIAquick Gel Extraction Kit (Qiagen Inc., Valencia, CA). All PCR products were sequenced in both directions using the same primers described for the initial amplifications. Additional internal sequencing primers were used for some loci: 18S rDNA, forward primers (5'-GCT ACC ACA TCC AAG GAA GG-3', 5'-CTG CGA AAG CAT TTG CCA AGG-3', 5'-TCT GGG CCG CAC GCG CGC TAC ACT G-3')

Table 3 List of GenBank accession numbers for individual locus *Malassezia* sequence data generated for this project. Existing sequence data used for the multilocus phylogenetic analysis are listed at the end of the table. GenBank accession numbers beginning with 'L' represent the whole genome contig from which sequence data for a given locus was obtained.

			GenBank accession numbers							
Isolate identifier	Other identifier	Species	ITS	D1/D2	18S rDNA	β-tub	*TEF1*	*RPB2*	*MCM7*	*CHS2*
NWHC 24716-007		*M. vespertilionis*	MF669399	NA	NA	NA	NA	NA	NA	NA
NWHC 24716-008		*M. vespertilionis*	MF669400	NA	NA	NA	NA	NA	NA	NA
NWHC 24716-014	CBS 15042	*M. vespertilionis*	MF669401	MF669387	MF669375	MF669327	MF669315	MF669351	MF669363	MF669339
NWHC 24738-002		*M. vespertilionis*	MF669402	NA	NA	NA	NA	NA	NA	NA
NWHC 24738-013		*M. vespertilionis*	MF669403	NA	NA	NA	NA	NA	NA	NA
NWHC 24738-025		*M. vespertilionis*	MF669404	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-031		*M. vespertilionis*	MF669405	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-033		*M. vespertilionis*	MF669406	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-034	CBS 15043	*M. vespertilionis*	MF669407	MF669388	MF669376	MF669328	MF669316	MF669352	MF669364	MF669340
NWHC 44767-035		*M. vespertilionis*	MF669408	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-036		*M. vespertilionis*	MF669409	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-037	UAMH 11925	*M. vespertilionis*	MF669410	MF669389	MF669377	MF669329	MF669317	MF669353	MF669365	MF669341
NWHC 44767-038		*M. vespertilionis*	MF669411	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-039		*M. vespertilionis*	MF669412	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-040		*M. vespertilionis*	MF669413	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-041		*M. vespertilionis*	MF669414	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-042		*M. vespertilionis*	MF669415	NA	NA	NA	NA	NA	NA	NA
NWHC 44767-044		*M. vespertilionis*	MF669416	NA	NA	NA	NA	NA	NA	NA
NWHC 44768-012		*M. vespertilionis*	MF669417	NA	NA	NA	NA	NA	NA	NA
NWHC 44768-020		*M. vespertilionis*	MF669418	NA	NA	NA	NA	NA	NA	NA
NWHC 44768-021		*M. vespertilionis*	MF669419	NA	NA	NA	NA	NA	NA	NA
NWHC 44768-025	CBS 15044	*M. vespertilionis*	MF669420	MF669390	MF669378	MF669330	MF669318	MF669354	MF669366	MF669342
NWHC 44769-001		*M. vespertilionis*	MF669421	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-004		*M. vespertilionis*	MF669422	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-006		*M. vespertilionis*	MF669423	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-008		*M. vespertilionis*	MF669424	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-010		*M. vespertilionis*	MF669425	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-013		*M. vespertilionis*	MF669426	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-014		*M. vespertilionis*	MF669427	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-016		*M. vespertilionis*	MF669428	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-017		*M. vespertilionis*	MF669429	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-018		*M. vespertilionis*	MF669430	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-019		*M. vespertilionis*	MF669431	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-020		*M. vespertilionis*	MF669432	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-021		*M. vespertilionis*	MF669433	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-022		*M. vespertilionis*	MF669434	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-023		*M. vespertilionis*	MF669435	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-024		*M. vespertilionis*	MF669436	NA	NA	NA	NA	NA	NA	NA
NWHC 44769-025		*M. vespertilionis*	MF669437	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-035	CBS 15045	*M. vespertilionis*	MF669438	MF669391	MF669379	MF669331	MF669319	MF669355	MF669367	MF669343
NWHC 44797-064		*M. vespertilionis*	MF669439	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-100		*M. vespertilionis*	MF669440	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-103*	CBS 15041*, UAMH 11924*	*M. vespertilionis*	MF669441	MF669392	MF669380	MF669332	MF669320	MF669356	MF669368	MF669344
NWHC 44797-104		*M. vespertilionis*	MF669442	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-115		*M. vespertilionis*	MF669443	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-124		*M. vespertilionis*	MF669444	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-127		*M. vespertilionis*	MF669445	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-131		*M. vespertilionis*	MF669446	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-135		*M. vespertilionis*	MF669447	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-137	CBS 15046	*M. vespertilionis*	MF669448	MF669393	MF669381	MF669333	MF669321	MF669357	MF669369	MF669345
NWHC 44797-138		*M. vespertilionis*	MF669449	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-139		*M. vespertilionis*	MF669450	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-141		*M. vespertilionis*	MF669451	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-143		*M. vespertilionis*	MF669452	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-148		*M. vespertilionis*	MF669453	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-150		*M. vespertilionis*	MF669454	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-152		*M. vespertilionis*	MF669455	NA	NA	NA	NA	NA	NA	NA
NWHC 44797-153		*M. vespertilionis*	MF669456	NA	NA	NA	NA	NA	NA	NA

Table 3 (cont.)

Isolate identifier	Other identifier	Species	GenBank accession numbers							
			ITS	D1/D2	18S rDNA	β-tub	*TEF1*	*RPB2*	*MCM7*	*CHS2*
NWHC 45701-664		*M. vespertilionis*	MF669457	NA	NA	NA	NA	NA	NA	NA
NWHC 45701-665	CBS 15048	*M. vespertilionis*	MF669458	MF669394	MF669382	MF669334	MF669322	MF669358	MF669370	MF669346
NWHC 45701-668		*M. vespertilionis*	MF669459	NA	NA	NA	NA	NA	NA	NA
NWHC 45701-676		*M. vespertilionis*	MF669460	NA	NA	NA	NA	NA	NA	NA
NWHC 45701-677		*M. vespertilionis*	MF669461	NA	NA	NA	NA	NA	NA	NA
NWHC 45701-678		*M. vespertilionis*	MF669462	NA	NA	NA	NA	NA	NA	NA
NWHC 45701-682	CBS 15051	*M. vespertilionis*	MF669463	MF669395	MF669383	MF669335	MF669323	MF669359	MF669371	MF669347
NWHC 45701-686	CBS 15049	*M. vespertilionis*	MF669464	MF669396	MF669384	MF669336	MF669324	MF669360	MF669372	MF669348
NWHC 45701-688		*M. vespertilionis*	MF669465	NA	NA	NA	NA	NA	NA	NA
NWHC 45701-691	CBS 15050	*M. vespertilionis*	MF669466	MF669397	MF669385	MF669337	MF669325	MF669361	MF669373	MF669349
NWHC 45701-714		*M. vespertilionis*	MF669467	NA	NA	NA	NA	NA	NA	NA
NWHC 45701-719		*M. vespertilionis*	MF669468	NA	NA	NA	NA	NA	NA	NA
NWHC 45704-161		*M. vespertilionis*	MF669469	NA	NA	NA	NA	NA	NA	NA
NWHC 46375-001		*M. vespertilionis*	MF669470	NA	NA	NA	NA	NA	NA	NA
NWHC 46375-002		*M. vespertilionis*	MF669471	NA	NA	NA	NA	NA	NA	NA
NWHC 46375-003	CBS 15047	*M. vespertilionis*	MF669472	MF669398	MF669386	MF669338	MF669326	MF669362	MF669374	MF669350
M9927*	CBS 9169*	*M. dermatis*	AB070356	AB070361	KF706452.1	LFFX01000095.1	LFFX01000013.1	LFFX01000105.1	LFFX01000023.1	LFFX01000059.1
CBS 1878*		*M. furfur*	AY743634	AY743602	EU192363.1	LFGI01000839.1	LFGI01000568.1	LFGI01001900.1	LFGI01002103.1	LFGI01003290.1
CBS 7019*		*M. furfur*	AY743635	AY743603	NA	LFGG01001873.1	LFGG01000118.1	LFGG01002621.1	LFGG01003117.1	LFGG01000889.1
NCPF 3349		*M. furfur*	NA	NA	AY083223.1	NA	NA	NA	NA	NA
CBS 9431*		*M. japonica*	EF140669	EF140672	KF706458.1	LFDB01000216.1	LFDB01000004.1	LFDB01000183.1	LFDB01000119.1	LFDB01000111.1
CBS 7876*		*M. obtusa*	AY387137	AY743629	NA	LFGC01001328.1	LFGC01000155.1	LFGC01000795.1	LFGC01001067.1	LFGC01000917.1
CBS 7968		*M. obtusa*	NA	NA	EU192365.1	NA	NA	NA	NA	NA
CBS 1879*		*M. pachydermatis*	AY387139	AY743605	LFGB01000057.1	LFGB01000029.1	LFGB01000018.1	LFGB01000009.1	LFGB01000036.1	LFGB01000046.1

* type or neotype strain. NA = sequence data not available or not used for analyses.

and reverse primers (5'-TGG AAT TAC CGC GGC TGC TGG CAC C-3', 5'-TCC TTG GCA AAT GCT TTC GCA G-3', 5'-CCG TCA ATT CCT TTA AGT TTC AGC C-3', 5'-AAG GTC TCG TTC GTT ATC G-3', 5'-GAC GGG CGG TGT GTA CAA AGG GCA G-3') (Sugita & Nakase 1999); *RPB2*, RPB2-6f (5'-TGG GGH ATG GTV TGY CCB GC-3') and RPB2-6r (5'-GCV GGR CAB ACC ATD CCC CA-3') (modified slightly from Liu et al. 1999); and *TEF1*, EF1-1577F (5'-CAG GAY GTN TAC AAG ATY GGT GG-3') and EF1-1567R (5'-ACH GTR CCR ATA CCA CCR ATC TT-3') (Rehner & Buckley 2005).

A phylogenetic analysis was conducted using newly generated sequences from the bat-associated isolates of *Malassezia* and from existing sequence data in GenBank for type cultures of a subset of the recognised species of *Malassezia* for which sufficient sequence data were available (Table 3). Members of the genus residing in the same core clade as the type isolate (as determined by the whole-genome analysis described above) were included in this analysis as were representatives from the other two core clades described by Wu et al. (2015). Sequence data for protein-coding genes were obtained from whole genome sequences deposited in GenBank by Wu et al. (2015), while that for multicopy genes originated from various sources (see Table 3). For *M. obtusa* and one isolate of *M. furfur*, 18S sequence data were not available for the type isolates; instead, sequence data from non ex-type strains were substituted for that locus.

Nucleotide sequences were aligned independently for each locus using MUSCLE in MEGA v. 6 (Tamura et al. 2013), and all gaps were deleted. MEGA 6 was also used to determine the best substitution model for each locus. A multigene phylogenetic analysis was then conducted by concatenating the final alignments of all eight loci. A Bayesian analysis was run as described above, except that 5 000 000 generations were used for each run and the sampling frequency was set to 1 000. Data was partitioned by locus and (for coding genes) by nucleotide codon position. A Kimura 2-parameter model with gamma distribution was applied to the non-coding loci (ITS, 18S rDNA, and D1/D2); a Kimura 2-parameter model with gamma distribution and invariant sites was used for *CHS2*; a general time-reversible model with gamma distribution was used for β-tub, *TEF1*, and *RPB2*; and an HKY model with gamma distribution and invariant sites was applied to *MCM7*.

Physiological and morphological characterisation of isolates

Seven isolates of the bat-associated *Malassezia* sp. that were analysed genetically were also characterised physiologically and morphologically using criteria commonly employed to distinguish species in the genus (Guého-Kellerman et al. 2010). To determine the influence of temperature on growth, all isolates were incubated at various temperatures as described by Guého et al. (1996). Due to the fastidious nature of the isolates, growth temperature experiments were conducted on LNA instead of modified Dixon's agar (mDA; Guillot et al. 1996). Growth was assessed at the following temperatures: 7, 24, 30, 37, and 40 °C. Inclusion of growth characteristics at 7 °C and 24 °C are not standard for *Malassezia*, but were performed because the fungus was isolated from hibernating bats, which maintain low body temperatures. Plates were inoculated by transferring cells with an inoculating loop and streaking for isolated colonies. The diameter of isolated colonies was measured and colony morphology descriptions were recorded every 10 d for a total of 50 d. Cell morphology was assessed from wet mounts with lactophenol cotton blue stain conducted on 10-d-old cultures that were grown on LNA at 24 °C.

The ability of the bat-associated *Malassezia* isolates to grow on mDA and without lipid supplementation on Sabouraud dextrose

agar (SDA) was tested by inoculating these media as described above. Utilisation of different types of Tween (i.e., 0.1 % Tween 80, 0.5 % Tween 40, 0.5 % Tween 60, and 10 % Tween 20) was tested according to the methodologies of Guého et al. (1996). The seven isolates were also characterised using the Tween Diffusion and Cremophor EL Assimilation Tests (Guillot et al. 1996). For all of these experiments, cultures were incubated at 32 °C and checked every seven days for a total of 50 d. Tests were also performed at 24 °C to ensure that lack of positive results was not due to incubation at temperatures outside the optimal growth range for the *Malassezia* sp.

Additional physiological tests included catalase reaction and β-glucosidase activity. The catalase reaction was performed by harvesting a loop full of cells from a 7-d-old culture grown on LNA at 24 °C, smearing the cells onto a glass slide, and adding one drop of 3 % hydrogen peroxide (Guého et al. 1996). The ability to hydrolyse esculin (i.e., β-glucosidase activity) was tested following the methods of Mayser et al. (1997) except that 7-d-old cultures grown on LNA were used to inoculate the medium due to the slower growth rate of the bat-associated isolates relative to other species of *Malassezia*. The esculin agar tubes were incubated at 32 °C and checked daily for 14 d, and thereafter they were checked every seven days for an additional 30 d. The test was considered positive if a black precipitate was produced. Mating experiments were not performed.

RESULTS

Isolation of Malassezia from bats

Fungal colonies that resembled *Malassezia* were visible on LNA medium after 40–50 d of incubation at 7 °C. These putative *Malassezia* colonies were observed growing in culture from samples collected from 75 of the 264 (28 %) bats examined. These included nine host species from seven U.S. states (Table 1; Fig. 2). The ITS sequences of 74 isolates shared 99.7–100 % sequence identity with one another across the approximately 760-bp fragment that was analysed. The remaining isolate had an ITS sequence divergent from the other 74 isolates and was not included in further analyses. When searched against the GenBank database, ITS sequences of the 74 bat-associated isolates most closely matched *M. japonica* (92 % sequence identity) within the portion of the sequences corresponding to the 5.8S rDNA; however, ITS1 and ITS2 were highly divergent from sequences that resided within GenBank. The ITS sequence data are available in GenBank (MF669451–MF669472).

Some isolates of the *Malassezia* sp. lost vigour and eventually failed to grow after several passes on LNA. Transfer of these isolates to mDA did not cause reinvigoration but rather seemed to facilitate further decline of the cultures. The reason for this was not determined. Despite losing some isolates, the majority grew well in the laboratory. Representative isolates were deposited in the Westerdijk Fungal Biodiversity Institute and the UAMH Centre for Global Microfungal Biodiversity culture collections (Table 3).

Whole genome sequence analysis

The Whole Genome Shotgun project was deposited at DDBJ/ENA/GenBank under the WGS Project PECA00000000 (accession GCA_002818225.1). The version described in this paper is version PECA01000000. Raw sequencing data is available from the NCBI SRA via the accession SRP121079. The genome of the *Malassezia* isolated from bats was 7.581 Mb, contained in 14 scaffolds, which is consistent with other *Malassezia* species (Boekhout et al. 1998, Wu et al. 2015). The annotated genome is estimated to contain 3 791 protein coding gene models (Table 2). Twenty-seven genomes of *Malassezia* species were obtained from NCBI GenBank, however most of these data contained only assemblies without annotated gene models. Thus, all genome assemblies were re-annotated using funannotate in order to generate comparable annotations between species. The number of predicted genes was similar to previously published reports (Table 2) (Wu et al. 2015).

Fig. 2 Map of the United States, showing the locations of sampling sites from which bats yielded isolates of *Malassezia vespertilionis* sp. nov. States from which isolates were obtained are labelled (AL = Alabama; CA = California; KY = Kentucky; MO = Missouri; NY = New York; PA = Pennsylvania; WI = Wisconsin).

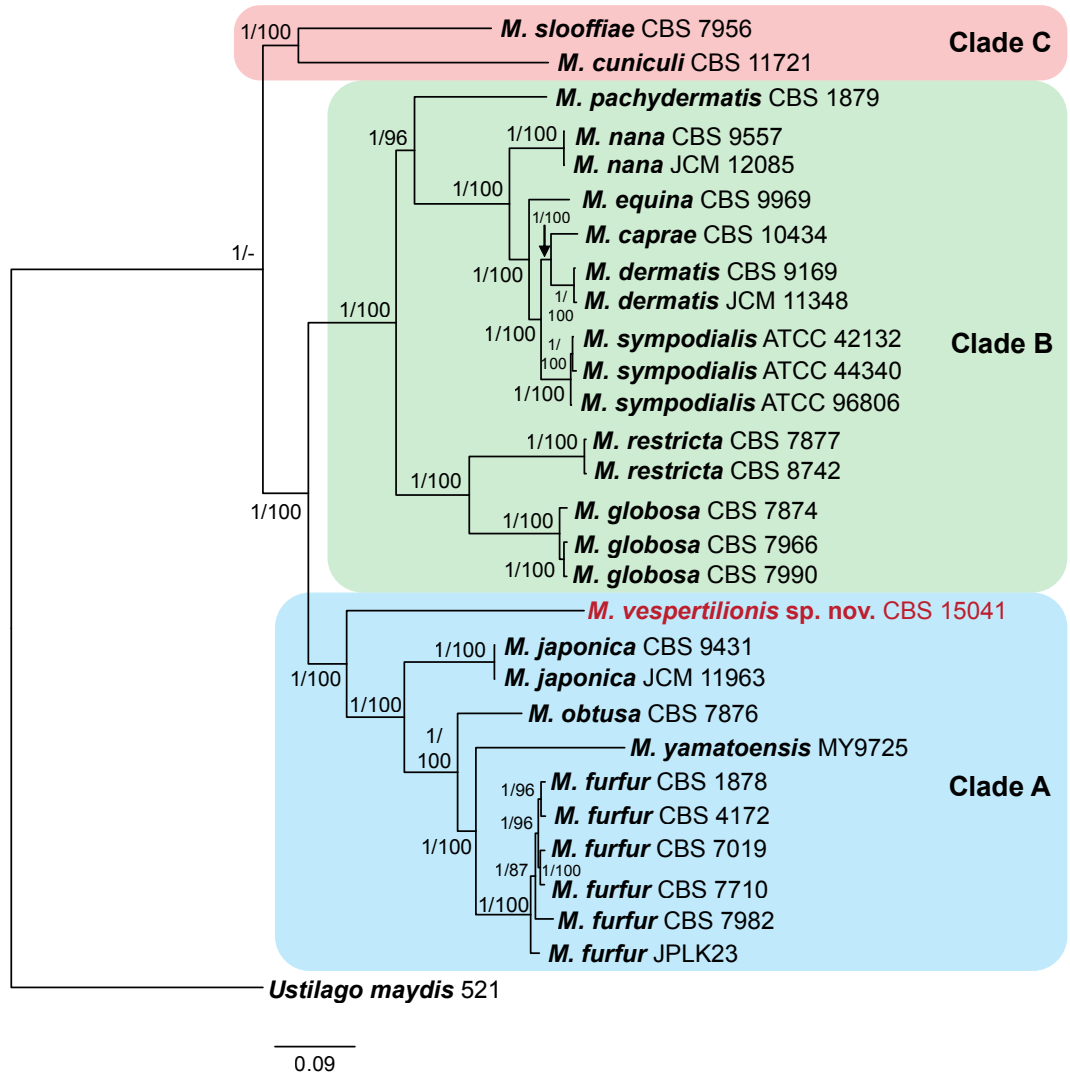

Fig. 3 Phylogenetic tree of the genus *Malassezia* based on concatenated amino acid sequences of 254 conserved orthologues. The tree from the Bayesian analysis is shown, but the tree generated from the maximum likelihood analysis had an identical topology. Posterior probabilities (Bayesian)/bootstrap values (maximum likelihood), respectively, are shown at the nodes. *Ustilago maydis* was used to root the tree. Clades A, B, C as described by Wu et al. (2015) are illustrated. Based on the analyses, *M. vespertilionis* sp. nov. is a basal member of clade A.

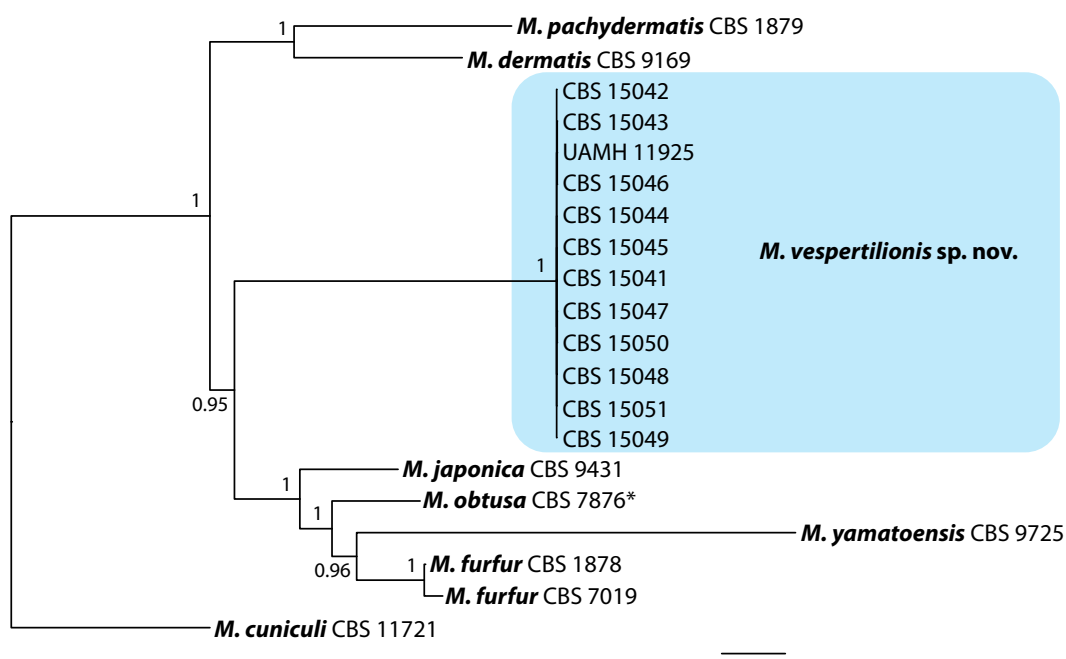

Fig. 4 Phylogenetic tree resulting from a Bayesian analysis of concatenated nucleotide sequences from eight loci (ITS, 18S rDNA, D1/D2 region, and portions of the β-tub, *TEF1*, *MCM7*, *RPB2*, *CHS2* genes) of 12 *Malassezia* isolates from bats, all *Malassezia* species from clade A for which sufficient genetic data was available, and representative members from clades B and C (Wu et al. 2015). Posterior probabilities are presented at each node. All examined isolates from bats formed a well-supported clade, suggesting that they represent a single taxon referred to herein as *M. vespertilionis* sp. nov.

Table 4 Physiological characteristics of the various species of *Malassezia*. Seven isolates of *M. vespertilionis* were characterized and the characteristics are summarized ('Overall') for the species based on those results. Information from other species of *Malassezia* were taken from previous summaries and original data by Cabañes et al. 2011, 2016, and Honnavar et al. 2016. Results are displayed as pos (positive reaction/test), neg (negative reaction/test), weak (weak positive reaction/test), variable (different isolates produce variable results), and ? (no information available for this specific test). When multiple results are listed for a given test/reaction, the one listed first is the most common result obtained from the isolates examined; results in parentheses are observed only rarely.

				Utilization					Tween Diffusion				Activity		Growth	
Species	Cell morphology	Growth mDA	Lipid dependency	Tween 20	Tween 40	Tween 60	Tween 80	Cremophor EL	Tween 20	Tween 40	Tween 60	Tween 80	Catalase	β-gluco-sidase	37 °C	40 °C
M. arunalokei	ovoidal, globose	pos	pos	neg	neg	neg	variable	neg	?	?	?	?	neg	neg	pos	neg
M. brasiliensis	ovoidal, ellipsoidal	pos	pos	pos	pos	pos	pos	pos	?	?	?	?	pos	neg	pos	pos
M. caprae	globose, ellipsoidal	pos	pos	neg	pos	pos	pos, (neg)	neg	neg	pos	pos	pos, (neg)	pos	pos, (neg)	neg, (weak)	neg
M. cuniculi	globose	neg, weak	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	pos	pos	pos	pos
M. dermatis	ellipsoidal, globose	pos	pos	pos	pos	pos	pos	pos, weak	pos	pos	pos	pos	pos	?	pos	pos
M. equina	ellipsoidal	pos	pos	weak	pos	pos	pos	neg	weak	pos	pos	pos	pos	neg	weak	neg
M. furfur	globose, ellipsoidal, cylindrical	pos	pos	pos	pos	pos	pos	pos	pos, (neg)	pos, (neg)	pos, (neg)	pos, (neg)	pos, (neg)	neg, (weak)	pos	pos
M. globosa	globose	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	pos	neg	neg, (weak)	neg
M. japonica	globose, ellipsoidal	pos	pos	neg	weak	pos	neg	?	neg	weak	pos	neg	pos	?	pos	neg
M. nana	ellipsoidal	pos	pos	variable	pos	pos	weak	neg	variable	pos	pos	weak	pos	neg	pos	variable
M. obtusa	ellipsoidal, cylindrical	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	pos	pos	neg, (weak)	neg
M. pachydermatis	ellipsoidal	pos	neg, weak	pos	pos	pos	pos	pos	pos	pos	pos	pos	pos, (weak)	pos, (neg)	pos	pos
M. psittaci	globose, ovoidal	pos	pos	pos	pos	pos	pos	pos	?	?	?	?	pos	neg	neg	neg
M. restricta	globose, ellipsoidal	pos	pos	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	variable	neg
M. slooffiae	ellipsoidal, cylindrical	pos	pos	pos, weak	pos	pos	neg, weak	neg	pos, weak, (neg)	pos	pos	neg, (weak)	pos	neg	pos	pos
M. sympodialis	ellipsoidal	pos	pos	neg, weak	pos	pos	pos	neg, weak	neg, weak	pos	pos	pos	pos	pos	pos	pos
M. yamatoensis	ellipsoidal	pos	pos	pos	pos	pos	pos	?	pos	pos	pos	pos	pos	?	pos	neg
M. vespertilionis (Overall)	ellipsoidal/ovoid; rarely globose	pos	pos	weak*	pos	pos	weak*	neg	neg, (weak)	weak, pos	pos	variable	neg	neg	weak	weak
M. vespertilionis isolate CBS 15041	ellipsoidal/ovoid; rarely globose	pos	pos	weak	pos	pos	weak	neg	neg	weak	pos	weak	neg	neg	weak	weak
M. vespertilionis isolate CBS 15042	ellipsoidal/ovoid; rarely globose	pos	pos	weak	pos	pos	weak	neg	neg	weak	pos	neg	neg	neg	weak	weak
M. vespertilionis isolate CBS 15043	ellipsoidal/ovoid; rarely globose	pos	pos	weak	pos	pos	weak	neg	weak	pos	pos	pos	neg	neg	weak	weak
M. vespertilionis isolate CBS 15044	ellipsoidal/ovoid; rarely globose	pos	pos	weak	pos	pos	weak	neg	neg	pos	pos	weak	neg	neg	weak	weak
M. vespertilionis isolate CBS 15045	ellipsoidal/ovoid; rarely globose	pos	pos	weak	pos	pos	weak	neg	neg	weak	pos	weak	neg	neg	weak	weak
M. vespertilionis isolate CBS 15046	ellipsoidal/ovoid; rarely globose	pos	pos	weak	pos	pos	weak	neg	neg	weak	pos	weak	neg	neg	weak	weak
M. vespertilionis isolate UAMH 11925	ellipsoidal/ovoid; rarely globose	pos	pos	weak	pos	pos	weak	neg	neg	weak	pos	weak	neg	neg	weak	weak

* generally grew well when test was performed at 24 °C

Using the annotated genomes and *Ustilago maydis* as an outgroup, we identified core conserved gene models in each genome using BUSCO v. 2 orthologous groups and generated a concatenated alignment of 254 gene models found in all 29 genomes studied. Using both maximum likelihood and Bayesian methods, the bat-associated *Malassezia* was placed as the most basal member of clade A (Fig. 3).

Multilocus sequence analysis

To demonstrate that all of the *Malassezia* isolates from bats represented a single taxon, multilocus sequencing analysis was performed on 12 isolates (including the type isolate). The isolates for the analysis were selected to represent a broad range of host species, geographic locations, and strains with slight sequence variations in the ITS region. The portions sequenced of D1/D2 region, β-tub, *TEF1*, and *RPB2* were 100 % identical among the 12 isolates, and the ITS region, 18S rDNA, *MCM7*, and *CHS2* shared at least 99.7, 99.9, 99.8, and 99.8 % sequence identity, respectively, between the isolates examined. The sequences generated for the multilocus analysis are available in GenBank (D1/D2: MF669394–MF669398; 18S rDNA: MF669382–MF669386; β-tub: MF669334–MF669338; *TEF1*: MF669322–MF669326; *RPB2*: MF669358–MF669362; *MCM7*: MF669370–MF669374; *CHS2*: MF669346–MF669350).

The final alignments used for the phylogenetic analysis consisted of the following numbers of characters: ITS region, 402 characters; 18S rDNA, 1065 characters; D1/D2 region, 541 characters; β-tub, 1041 characters; *TEF1*, 987 characters; *MCM7*, 600 characters; *RPB2*, 1086 characters; and *CHS2*, 534 characters. The small number of characters included in the ITS region alignment was due to high divergence between the different *Malassezia* species within the ITS1 and ITS2 regions, resulting in their subsequent removal from the alignment. Thus, the final 'ITS' alignment consisted almost entirely of sequence data representing the 5.8S rDNA which is more highly conserved among species of *Malassezia*. The Bayesian analysis based on the eight concatenated sequences produced a tree that showed the same relationships among the subset of species included as the phylogenetic analysis based on whole genome sequencing. The 12 *Malassezia* isolates from bats all grouped together into a well-supported clade that likely represented a single species (Fig. 4).

Physiological and morphological characterisation of isolates

The *Malassezia* sp. isolated from bats grew at all temperatures tested (7, 24, 30, 37, and 40 °C), with best growth (i.e., largest colony diameters) occurring at 24 °C. At higher temperatures, growth was slower. This was problematic since standard physiological tests used to characterise species of *Malassezia* are typically conducted at 32 °C for incubation periods that were too brief to allow for sufficient growth of this novel taxon from bats (Guého et al. 1996). Thus, we conducted most tests at both 24 °C and 32 °C and allowed cultures to incubate for up to 50 d. Test results at the two temperatures were generally equivalent, although positive test results often required longer incubation times and produced weaker results at 32 °C compared to 24 °C. Detailed descriptions of growth at different temperatures, cell morphology, and colony morphology are presented in the species description and in Table 4. Colony and cell morphology are also shown in Fig. 5.

The bat-associated *Malassezia* grew on mDA, but some isolates began to lose vigour (even more so than previously described on LNA) after several transfers on the medium. All isolates were lipid-dependent, failing to grow on SDA. Growth occurred on a variety of tween lipid sources, but not in the presence of Cremophor EL. The isolates were catalase and β-glucosidase negative. More detailed information is provided in the species description and in Table 4.

SPECIES DESCRIPTION

Malassezia vespertilionis J.M. Lorch & Vanderwolf, *sp. nov.* — MycoBank MB822382; Fig. 5

Etymology. The species epithet refers to the host from which the fungus was isolated (n. vespertilio, Latin for bat; gen. n. vespertilionis, of a bat).

Holotype. USA, Wisconsin, swab of wing skin of hibernating *Myotis septentrionalis*, 28 Jan. 2014, *J.P. White* (U.S. National Fungus Collections BPI 910536; culture ex-type CBS 15041 = UAMH 11924).

Colonies are approximately 0.5–1.0 mm diam after 10 d of growth at 24 °C on LNA; 2–5 mm diam after 40 d. At 10 d, colonies are cream-coloured, flat to slightly convex, somewhat glossy, have entire margins, and have a crumbly or waxy consistency. At 40 d, colonies have irregular margins, are slightly raised, and have a prominent papilla near the centre (Fig. 5). Cells are ellipsoid or ovoid to (rarely) globose, ranging in size from 2–3 × 2–4 µm (typically 2 × 3 µm) (Fig. 5). Buds are formed monopolarly, usually on a narrow base. Growth occurs (sometimes poorly) on mDA; no growth observed on SDA. Isolates are catalase and β-glucosidase negative. Variable in lipid utilisation: no growth observed for Cremophor EL, weak or no growth for Tween 20, usually weak growth for Tween 80, weak to good growth for Tween 40, and good growth for Tween 60. Growth occurs across a range of temperatures on LNA, but is slower at temperatures above and below 24 °C; specifically, individual pinpoint colonies are visible by day 30 at 7 °C and 30 °C, and on day 40 at 37 °C. Growth is evident at 40 d for

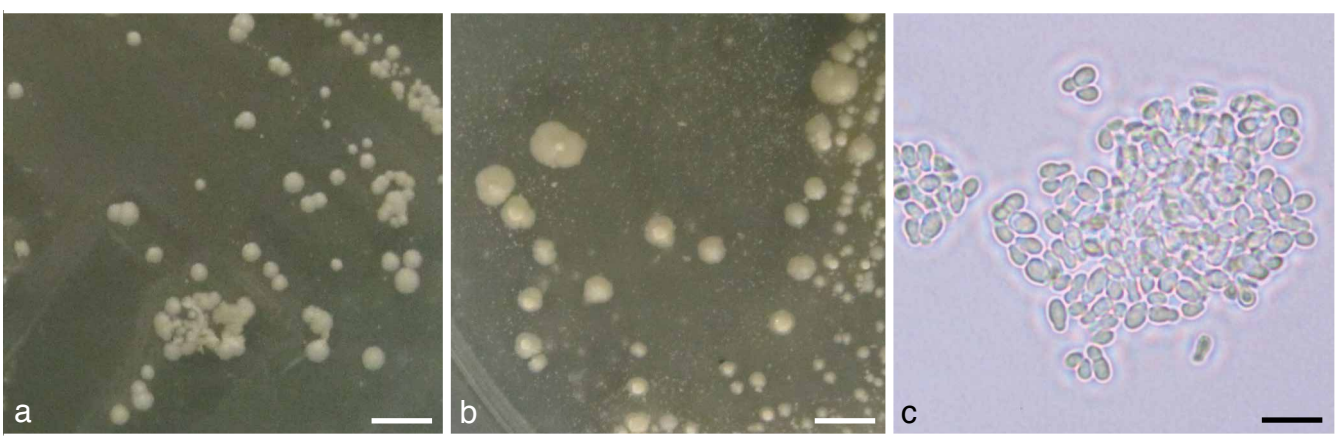

Fig. 5 Colony and cell morphology of *M. vespertilionis* sp. nov. grown on Leeming and Notman Agar at 24 °C. a. Colony size and morphology after 10 d of growth; b. colony size and morphology after 40 d of growth; c. cell morphology of 10-d-old culture. — Scale bars: a, b = 4 mm; c = 5 µm.

cultures grown at 40 °C on areas of the medium where cells are placed at high densities; however, individual colonies are not grossly discernible. A sexual state was not observed; however, mating experiments were not explicitly performed.

Additional specimens examined for physiological characteristics and multilocus DNA sequencing. USA, Wisconsin, swab of wing skin of a hibernating *Myotis septentrionalis*, 3 Mar. 2014, *J.P. White*, CBS 15042; Kentucky, swab of wing skin of hibernating *Myotis sodalis*, 4 Mar. 2014, *M.L. Verant*, CBS 15043; Kentucky, swab of wing skin of hibernating *Myotis sodalis*, 4 Mar. 2014, *M.L. Verant*, UAMH 11925; New York, swab of wing skin of hibernating *Myotis lucifugus*, 19 Mar. 2014, *M.L. Verant*, CBS 15044; New York, swab of wing skin of hibernating *Myotis lucifugus*, 15 Jan. 2015, *M.L. Verant*, CBS 15045; Alabama, swab of wing skin of hibernating *Myotis grisescens*, 11 Feb. 2015, *N. Sharp*, CBS 15046.

Additional isolates for which multilocus DNA sequencing was conducted. USA, California, swab of wing skin of *Lasionycteris noctivagans*, 8 May 2017, *T.J. Weller*, CBS 15047; California, swab of wing skin of *Myotis californicus*, 7 May 2016, *T.J. Weller*, CBS 15048; California, swab of wing skin of *Myotis thysanodes*, 26 Apr. 2016, *T.J. Weller*, CBS 15049; California, swab of wing skin of *Myotis californicus*, 16 May 2016, *T.J. Weller*, CBS 15050; California, swab of wing skin of *Myotis californicus*, 19 Apr. 2016, *T.J. Weller*, CBS 15051.

DISCUSSION

The diversity of species within the genus *Malassezia* has been expanded in recent years due to increased sampling and use of genetic and molecular tools for distinguishing taxa. In the current study, we report on a species of *Malassezia* that is relatively common on skin of North American bats (i.e., cultured from 28 % of 264 individuals sampled). Isolates of this *Malassezia* sp. from bats were physiologically and genetically similar to one another. Specifically, sequences of four loci (D1/D2 region, β-tub, *TEF1*, and *RPB2*) were identical between isolates. Minimal variation in the ITS region (99.7 % sequence identity), 18S rDNA (99.9 % sequence identity), and portions of the *MCM7* (99.8 % sequence identity) and *CHS2* (99.8 % sequence identity) genes were within the intraspecific variation documented in other species of *Malassezia* (e.g., Makimura et al. 2000, Hirai et al. 2004, Cabañes et al. 2007). As a group, the bat-associated isolates were highly similar to one another, and they were sufficiently distinct from all other known taxa. Thus, we propose that these bat-associated isolates represent the novel species *M. vespertilionis*.

All isolates of *M. vespertilionis* subjected to physiological tests were catalase negative, a trait shared only with *M. arunalokei*, *M. restricta*, and some strains of *M. furfur* and *M. pachydermatis* (Guého et al. 1996, Guillot et al. 1998, reviewed by Batra et al. 2005, Honnavar et al. 2016). In contrast to *M. arunalokei* and *M. restricta*, *M. vespertilionis* is capable of growth (albeit slow) at 40 °C and can utilise multiple types of Tween. It can be distinguished from catalase negative strains of *M. pachydermatis* based on ability of the latter to grow on SDA without the supplementation of lipids. Differentiation of *M. vespertilionis* from *M. furfur* based on physiological tests alone may be problematic due to reported variation in *M. furfur* (Batra et al. 2005). We examined a number of isolates of *M. vespertilionis* and found some variability in results of the physiological tests for this species as well. Interpretation of physiological tests can be challenging (Gupta et al. 2004), and the inability of *M. vespertilionis* to grow sufficiently to produce positive results under the standard incubation procedures set forth by Guého et al. (1996) further complicates the use of these tests for identification. Thus, although physiological tests can often separate *M. vespertilionis* from other described taxa and may be helpful for some applications, we encourage the use of DNA sequencing (e.g., ITS region) to confirm identification.

To date, *Malassezia* species have been isolated primarily from euthermic animals that maintain constant core body temperature near 37 °C. This is consistent with the statement made by Guého-Kellerman et al. (2010) that members of the genus do not endure temperatures below 28 °C. Most of the bat species from which *M. vespertilionis* was isolated hibernate for up to seven months of the year at which time their body temperature is close to that of the surrounding environment. Specifically, *Myotis lucifugus*, *Myotis sodalis*, and *Myotis septentrionalis* were reported to prefer winter hibernacula with average air temperatures around 7.2, 8.5, and 9.1 °C, respectively (Brack 2007). In the active season, a bat's body temperature may exceed 40 °C; however, bats often use bouts of torpor even during the active season, such that their body temperatures frequently fluctuate, sometimes approaching ambient temperature (Hock 1951, Studier 1981, Willis & Cooper 2009). Thus, the skin temperature of bats is highly variable, and this may explain the wide range of temperatures under which *M. vespertilionis* is capable of growth (from at least 7 °C to 40 °C). The ability to grow at such cool temperatures is noteworthy and may be unique to *M. vespertilionis* among the *Malassezia*. However, the lower growth limits of most *Malassezia* species have not been expressly described in literature, making comparisons difficult. The detection of DNA of uncultured malassezia-like organisms on corals and in terrestrial and marine environments suggests that other undescribed species of *Malassezia* may be capable of growth at ambient temperatures or reside on poikilothermic hosts (reviewed by Amend 2014).

Previous phylogenetic studies of *Malassezia* have demonstrated disparities in the relationships between species when different loci were analysed (Cabañes et al. 2007, Castellá et al. 2014). This genealogical discordance has made deciphering taxonomic relationship among all members of the genus difficult. Wu et al. (2015) was able to better resolve *Malassezia* phylogeny through whole genome sequencing and concatenation of 164 genes. This phylogeny resulted in three main clades: clade A consists of *M. furfur*, *M. obtusa*, *M. yamatoensis*, and *M. japonica*; clade B contains *M. sympodialis*, *M. dermatis*, *M. caprae*, *M. equina*, *M. nana*, *M. pachydermatis*, *M. globosa*, and *M. restricta*; and clade C is comprised of *M. cuniculi* and *M. slooffiae*. Using the sequence data generated by Wu et al. (2015) and the whole genome sequence of *M. vespertilionis* produced in this study, we conducted a phylogenetic analysis using amino acid sequences of 254 core genes. The resulting phylogenetic tree was identical to that of Wu et al. (2015) except that our analysis suggested that *M. obtusa* is basal to the subclade consisting of *M. furfur* and *M. yamatoensis* (Fig. 3). The *Malassezia* tree of Wu et al. (2015) had placed *M. yamatoensis* basal to *M. furfur* and *M. obtusa*; however, in that study the node was less well-supported compared to other species-level relationships. The slight change in tree topology and greater support for relationships within clade A in our tree may be due to the inclusion of more genes and *M. vespertilionis* in the analysis. These findings suggest that while sequencing loci traditionally used to differentiate species of *Malassezia* (e.g., ITS, D1/D2, β-tub, and *CHS2* (Cabañes et al. 2007, 2011, 2016, Castellá et al. 2014)) can be useful in identifying novel species, whole genome sequencing may be necessary to generate enough genetic data to fully resolve the relationship of those novel species with existing taxa.

Our phylogenetic analyses indicate that *M. vespertilionis* is the most basal member of clade A. *Malassezia arunalokei*, *M. brasiliensis*, and *M. psittaci* were not included in the analyses because genetic data is available for only three loci each for these newly-described species (Cabañes et al. 2016, Honnavar et al. 2016). Based on existing sequence data, *M. arunalokei*, *M. brasiliensis*, and *M. psittaci* share only about 75–87 %, 90–91 %, and 78–84 % DNA sequence identity, respectively, with *M. vespertilionis* in the ITS region, D1/D2 region, and

portion of the β-tubulin gene. Furthermore, previous analyses indicate that *M. arunalokei* is a member of clade B (Honnavar et al. 2016). *Malassezia brasiliensis* and *M. psittaci* are sister taxa to *M. furfur* and *M. yamatoensis*, respectively, which are both divergent from *M. vespertilionis* (Cabañes et al. 2016).

The genus *Malassezia* may be more diverse than currently documented due to the difficulty of transporting and culturing many fragile and fastidious members of the genus, the historic use of morphological and physiological characteristics as the sole criteria to identify species (which can fail to distinguish cryptic species), and a lack of sampling of diverse taxonomic host groups (Amend 2014, Cabañes 2014). In the only other published study in which bats were specifically surveyed for *Malassezia*, Gandra et al. (2008) reportedly cultured *M. furfur*, *M. globosa*, *M. pachydermatis*, and *M. sympodialis* (based on physiological and morphological characteristics) from Pallas' mastiff bats (*Molossus molossus*) in Brazil. No DNA sequence data were generated and the isolates were apparently not deposited in a public culture collection, making it difficult to ascertain their true species assignments. However, because all isolates from Gandra et al. (2008) were either catalase positive or lipid-independent (representing significant physiological deviations from *M. vespertilionis*) it may be that *M. vespertilionis* has not previously been isolated due to a lack of sampling effort of temperate bat species. Indeed, few studies have examined the fungal communities associated with bats, and those that have did not utilise fungal growth media suitable for the isolation of lipid-dependent species of *Malassezia* (Grose & Marinkelle 1966, Larcher et al. 2003, Voyron et al. 2011, Johnson et al. 2013, Vanderwolf et al. 2013). Njus (2014) detected *Malassezia* spp. on the skin of bats by conducting fungal community analyses with next generation sequencing. Based on reported sequence identities with other species of *Malassezia*, at least some of the *Malassezia* detected by Njus (2014) may represent *M. vespertilionis*. However, DNA sequence data from that project were not available in GenBank at the time of our study to confirm this.

Malassezia yeasts are best known for their association with certain skin ailments. For example, *M. pachydermatis* has been implicated as the cause of dermatitis in (among other species) rhinoceroses (Bauwens et al. 1996), dogs (Gustafson 1955, Bond et al. 2004), and sea lions (Guillot et al. 1998, Nakagaki et al. 2000). More often, the role of *Malassezia* in skin diseases of animals is unknown, and targeting skin lesions for culture and PCR analyses likely masks the frequency with which these fungi act as commensals. For example, Neves et al. (2017) isolated *Malassezia* from a relatively high percentage (32.8 %) of free-ranging golden-headed lion tamarins (*Leontopithecus chrysomelas*) in Brazil; none of the animals had skin lesions. Similarly, none of the 74 bats from which isolates of *M. vespertilionis* were recovered in this study showed visible signs of dermatitis at the time they were sampled. Thus, there is no current evidence that *M. vespertilionis* acts as a pathogen; instead, the yeast is likely a normal component of the skin mycobiome of bats.

In this study, *M. vespertilionis* was isolated from nine species of bats in seven U.S. states. With the exception of *Lasionycteris noctivagans*, all of these bats were species of *Myotis*, a diverse and widely distributed genus in North America. This, coupled with the detection of the fungus in both the eastern and western United States (Fig. 2), may indicate that the yeast is more widespread than our survey indicates. *Myotis* also has representatives throughout much of South America, Eurasia, Africa, and Australia, and additional sampling is necessary to determine whether *M. vespertilionis* may have a more global distribution. If this is shown to be the case, *M. vespertilionis* could prove to be an important species in which to study *Malassezia* genetics and evolution. All other known species of *Malassezia* occur on humans and domestic animals, which have been transported across the world. This, combined with the possibility of interspecific recombination (Cabañes et al. 2007, Castellá et al. 2014) when different species come into contact with one another, makes it difficult to understand historic patterns of geographic distribution, variation, and genetic exchange between strains. Bat species, however, have not been subjected to such extensive and recent human-assisted global movements, nor do they frequently come into close contact with other animal hosts that might facilitate genetic transfer between different species of *Malassezia*. Thus, if *M. vespertilionis* is found on other continents, genetic distinctions between strains may still be intact and provide important opportunities for future research into the evolution of these fungi and the role that humans have played in shaping the genetic structure and pathogenicity of *Malassezia* species.

Data accessibility

All relevant metadata related to this manuscript can be found in the tables of this manuscript, in GenBank, or in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S21466).

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REFERENCES

Aguirre C, Euliarte C, Finquelievich J, et al. 2015. Fungemia and interstitial lung compromise caused by Malassezia sympodialis in a pediatric patient. Revista Iberoamericana de Micología 32 (2): 118–121.

Amend A. 2014. From dandruff to deep-sea vents: Malassezia-like fungi are ecologically hyper-diverse. PLoS Pathogens 10 (8): e1004277.

Bankevich A, Nurk S, Antipov D, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19: 455–477.

Batra R, Boekhout T, Guého E, et al. 2005. Malassezia Baillon, emerging clinical yeasts. FEMS Yeast Research 5: 1101–1113.

Bauwens L, De Vroey C, De Meurichy W. 1996. A case of exfoliative dermatitis in a captive Southern white rhinoceros (Ceratotherium simum simum). Journal of Zoo and Wildlife Medicine 27: 271–274.

Boekhout T, Kamp M, Guého E. 1998. Molecular typing of Malassezia species with PFGE and RAPD. Medical Mycology 36: 365–372.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114–2120.

Bond R, Patterson-Kane JC, Lloyd DH. 2004. Clinical, histopathological and immunological effects of exposure of canine skin to Malassezia pachydermatis. Medical Mycology 42: 165–175.

Bowen AR, Chen-Wu JL, Momany M, et al. 1992. Classification of fungal chitin synthases. Proceedings of the National Academy of Sciences, USA 89: 519–523.

Brack Jr V. 2007. Temperature and locations used by hibernating bats, including Myotis sodalis (Indiana bat), in a limestone mine: implications for conservation and management. Environmental Management 40: 739–746.

Cabañes FJ. 2014. Malassezia yeasts: how many species infect humans and animals? PLoS Pathogens 10: e1003892.

Cabañes FJ, Coutinho SD, Puig L, et al. 2016. New lipid-dependent Malassezia species from parrots. Revista Iberoamericana de Micologia 33: 92–99.

Cabañes FJ, Theelen B, Castellá G, et al. 2007. Two new lipid-dependent Malassezia species from domestic animals. FEMS Yeast Research 7: 1064–1076.

Cabañes FJ, Vega, S, Castellá G. 2011. Malassezia cuniculi sp. nov., a novel yeast species isolated from rabbit skin. Medical Mycology 49: 40–48.

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25: 1972–1973.

Castellá G, Coutinho SD, Cabañes FJ. 2014. Phylogenetic relationships of Malassezia species based on multilocus sequence analysis. Medical Mycology 52: 99–105.

Chang HJ, Miller HL, Watkins N, et al. 1998. An epidemic of Malassezia pachydermatis in an intensive care nursery associated with colonisation of health care workers' pet dogs. New England Journal of Medicine 338: 706–711.

Einax E, Voigt K. 2003. Oligonucleotide primers for the universal amplification of β-tubulin genes facilitates phylogenetic analyses in the regnum Fungi. Organisms Diversity and Evolution 3: 185–194.

Gaitanis G, Magiatis P, Hantschke M, et al. 2012. The Malassezia genus in skin and systemic diseases. Clinical Microbiology Reviews 25: 106–141.

Gaitanis G, Velegraki A, Magiatis P, et al. 2011. Could Malassezia yeasts be implicated in skin carcinogenesis through the production of aryl-hydrocarbon receptor ligands? Medical Hypotheses 77: 47–51.

Gandra RF, Gambale W, De Cássia Garcia Simão R, et al. 2008. Malassezia spp. in acoustic meatus of bats (Molossus molossus) of the Amazon Region, Brazil. Mycopathologia 165: 21–26.

Grose E, Marinkelle CJ. 1966. Species of Sporotricum, Trichophyton and Microsporum from Columbian bats. Tropical and Geographical Medicine 18: 260–263.

Guého E, Midgley G, Guillot J. 1996. The genus Malassezia with description of four new species. Antonie van Leeuwenhoek 69: 337–355.

Guého-Kellerman E, Boekhout T, Begerow D. 2010. Biodiversity, phylogeny and ultrastructure. In: Boekhout T, Guého-Kellerman E, Mayser P, et al. (eds), Malassezia and the skin: science and clinical practice: 17–63. Springer, Germany.

Guillot J, Guého E, Lesourd M, et al. 1996. Identification of Malassezia species: A practical approach. Journal de Mycologie Médicale 6: 103–110.

Guillot J, Petit F, Degorce-Rubiales F, et al. 1998. Dermatitis caused by Malassezia pachydermatis in a California sea lion (Zalophus californianus). Veterinary Record 142: 311–312.

Gupta AK, Boekhout T, Theelen B, et al. 2004. Identification and typing of Malassezia species by amplified fragment length polymorphism and sequence analyses of the internal transcribed spacer and large-subunit regions of ribosomal DNA. Journal of Clinical Microbiology 42: 4253–4260.

Gustafson BA. 1955. Otitis externa in the dog: a bacteriological and experimental study. PhD thesis, Royal Veterinary College, Sweden.

Hirai A, Kano R, Makimura K, et al. 2004. Malassezia nana sp. nov., a novel lipid-dependent yeast species isolated from animals. International Journal of Systematic and Evolutionary Microbiology 54: 623–627.

Hock RJ. 1951. The metabolic rates and body temperatures of bats. The Biological Bulletin 101: 289–299.

Honnavar P, Prasad GS, Ghosh A, et al. 2016. Malassezia arunalokei sp. nov., a novel yeast species isolated from seborrheic dermatitis patients and healthy individuals from India. Journal of Clinical Microbiology 54: 1826–1834. doi: https://doi.org/10.1128/JCM.00683-16.

Johnson LJAN, Miller AN, McCleery RA, et al. 2013. Psychrophilic and psychrotolerant fungi on bats and the presence of Geomyces spp. on bat wings prior to the arrival of white-nose syndrome. Applied and Environmental Microbiology 79: 5465–5471.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780.

Laetsch DR, Blaxter ML. 2017. BlobTools: Interrogation of genome assemblies [version 1; referees: awaiting peer review]. F1000Research 6: 1287.

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9: 357–359.

Larcher G, Bouchara JP, Pailley P, et al. 2003. Fungal biota associated with bats in western France. Journal de Mycologie Médicale 13: 29–34.

Leeming JP, Notman FH. 1987. Improved methods for isolation and enumeration of Malassezia furfur from human skin. Journal of Clinical Microbiology 25: 2017–2019.

Liu YL, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16: 1799–1808.

Lorch JM, Minnis AM, Meteyer CU, et al. 2015. The fungus Trichophyton redellii sp. nov. causes skin infections that resemble white-nose syndrome of hibernating bats. Journal of Wildlife Diseases 51: 36–47.

Makimura K, Tamura Y, Kudo M, et al. 2000. Species identification and strain typing of Malassezia species stock strains and clinical isolates based on the DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. Journal of Medical Microbiology 49: 29–35.

Mayser P, Haze P, Papavassilis C, et al. 1997. Differentiation of Malassezia species: selectivity of Cremophor EL, castor oil and ricinoleic acid for M. furfur. British Journal of Dermatology 137: 208–213.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010: 1–8. New Orleans, Louisiana.

Nakagaki K, Hata K, Iwata E, et al. 2000. Malassezia pachydermatis isolated from a South American sea lion (Otaria byronia) with dermatitis. The Journal of Veterinary Medical Science 62: 901–903.

Neves JJ, Francelino M, Silva FG, et al. 2017. Survey of Malassezia sp. and dermatophytes in the cutaneous microbiome of free-ranging golden-headed lion tamarins (Leontopithecus chrysomelas – Kuhl, 1820). Journal of Medical Primatology 46: 65–69.

Njus KA. 2014. Molecular techniques for the identification of commensal fungal populations on cave roosting bats. Master's thesis. University of Akron, USA. http://rave.ohiolink.edu/etdc/view?acc_num=akron1403716687.

O'Donnell K. 1993. Fusarium and its near relatives. In: Reynolds DR, Taylor JW (eds), Fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics: 225–233. CAB International, UK.

Patron RL. 2016. A 34-year-old man with cough, lung nodules, fever, and eosinophilia. Clinical Infectious Diseases 63 (11): 1525–1526.

Rehner SA, Buckley E. 2005. A Beauveria phylogeny inferred from nuclear ITS and EF1-alpha sequences: evidence for cryptic diversification and links to Cordyceps teleomorphs. Mycologia 97: 84–98.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Schmitt L, Crespo A, Divakar PK, et al. 2009. New primers for promising single-copy genes in fungal phylogenetics and systematics. Persoonia 23: 35–40.

Simão FA, Waterhouse RM, Ioannidis P, et al. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31: 3210–3212.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313.

Studier EH. 1981. Energetic advantages of slight drops in body temperature in little brown bats, Myotis lucifugus. Comparative Biochemistry and Physiology Part A: Physiology 70: 537–540.

Sugita T, Boekhout T, Velegraki A, et al. 2010. Epidemiology of Malassezia-related skin diseases. In: Boekhout T, Guého-Kellerman E, Mayser P, et al (eds), Malassezia and the skin: science and clinical practice: 65–119. Springer, Germany.

Sugita T, Nakase T. 1999. Non-universal usage of the leucine CUG codon and the molecular phylogeny of the genus Candida. Systematic and Applied Microbiology 22: 79–86.

Sugita T, Takashima M, Shinoda T, et al. 2002. New yeast species, Malassezia dermatis, isolated from patients with atopic dermatitis. Journal of Clinical Microbiology 40: 1363–1367.

Tamura K, Stecher G, Peterson D, et al. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Vanderwolf KJ, McAlpine DF, Malloch D, et al. 2013. Ectomycota associated with hibernating bats in eastern Canadian caves prior to the emergence of white-nose syndrome. Northeastern Naturalist 20: 115–130.

Voyron S, Lazzari A, Riccucci M, et al. 2011. First mycological investigation on Italian bats. Hystrix 22: 189–197.

Walker BJ, Abeel T, Shea T, et al. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9: e112963.

Wang Q-M, Theelen B, Groenewald M, et al. 2014. Moniliellomycetes and Malasseziomycetes, two new classes in the Ustilaginomycotina. Persoonia 33: 41–47.

Willis CKR, Cooper CE. 2009. Techniques for studying thermoregulation and thermal biology in bats. In: Kunz TH, Parsons S (eds), Ecological and behavioral methods for the study of bats: 646–658. The John Hopkins University Press, USA.

Wu G, Zhao H, Li C, et al. 2015. Genus-wide comparative genomics of Malassezia delineates its phylogeny, physiology, and niche adaptation on human skin. PLoS Genetics 11: e1005614.