Journal of Arid Environments 219 (2023) 105089

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Characterizing fungal communities on Mojave desert tortoises (*Gopherus agassizii*) in Arizona to uncover potential pathogens

Daniel R. Kollath [a,*], Greer A. Dolby [b,c], Timothy H. Webster [b,d], Bridget M. Barker [a]

[a] The Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, Arizona, USA
[b] School of Life Sciences, Arizona State University, Tempe, Arizona, USA
[c] Department of Biology, University of Alabama at Birmingham, Birmingham, Alabama, USA
[d] Department of Anthropology, University of Utah, Salt Lake City, Utah, USA

ARTICLE INFO

Keywords:
Conservation
Disease ecology
Fungal pathogens
Mycobiome
Tortoise
Onygenales

ABSTRACT

The Mojave Desert tortoise (*Gopherus agassizii*) is a threatened reptile endemic to the southwestern United States. There are many factors contributing to its population decline such as human-driven habitat destruction and infectious diseases, which might include emerging fungal pathogens. We analyzed the fungal communities of two tortoises from northwest Arizona and compared the host mycobiome to the burrow mycobiome. Our main goal was to characterize the fungal community living on the Mojave Desert tortoise and to determine whether there are differences in community composition and potential pathogen abundance between the soil from burrows and tortoises' mycobiome. Dissimilarities in communities would indicate that there are fungi adapted to the animal niche that are rarely present in soil and vice versa. Metagenomic analysis of ITS2 amplicons revealed significant differences in fungal communities between the substrate and samples taken from the animals. The tortoise samples show higher alpha diversity than the soil and are distinct from soil samples based on beta diversity analysis. We observed significantly greater relative abundance of potential fungal pathogens from the order Onygenales in the tortoise samples than the soil. These mycobiome data suggest that there is a unique fungal community occupying the desert tortoise host niche and this community contains a higher relative abundance of potential fungal pathogens. We argue that these fungi may pose a risk for increased morbidity and mortality among desert tortoises, which can in turn drive population decline.

1. Introduction

Tortoises and turtles have the highest risk for extinction of any higher vertebrate group, with greater than 300 species threatened (Stanford et al., 2020). Habitat loss, life history, climate change, and illegal poaching by humans are factors contributing to these extinction threats (Allison et al., 2018). Additionally, infectious diseases threaten tortoise populations, with *Mycoplasma*, *herpesviruses* and *ranaviruses* linked to major die-offs (Jacobson et al., 2014; Johnson et al., 2008; Sim et al., 2015; Tompkins et al., 2015). However, recent emergence of fungal infections in amphibians and reptiles (*e.g.*, chytridiomycosis and ophidiomycosis) suggest fungi should be investigated as potential threats to tortoise populations (Tompkins et al., 2015; Berger et al., 1998; Lorch et al., 2016).

The Mojave Desert tortoise (*Gopherus agassizii)* is listed as threatened under the federal Endangered Species Act and occurs predominantly in areas north and west of the Colorado River in the Mojave Desert (Allison et al., 2018). These long-lived reptiles spend most of their life sequestered in underground burrows or dens that potentially make them vulnerable to soil-borne pathogens, such as fungi in the order Onygenales (Allison et al., 2018; Nagy et al., 1986; Wilson et al., 1999). Many fungi within the Onygenales order are tightly associated with animals and may cause significant disease in otherwise healthy mammals and reptiles, such as Coccidioides spp, many dermatophytes, and Ophidiomyces (Lorch et al., 2016; Coleine et al., 2022; Woodburn et al., 2021). Animal burrows are environmental reservoirs for pathogens including bacterial and fungal species that can infect animals (Bechtel et al., 2021; Kollath, 2019; Kaufmann et al., 2021). Desert tortoises often share burrows with conspecifics, making multiple animals susceptible to infection if the burrows contain pathogens (Harless et al., 2009). Environmental fluctuations due to climate change may also play a role in tortoise susceptibility to fungal disease. It is predicted that a warming

* Corresponding author. The Pathogen and Microbiome Institute, Northern Arizona University, PO Box 4073, Flagstaff, AZ 86011, USA.
E-mail address: drk87@nau.edu (D.R. Kollath).

https://doi.org/10.1016/j.jaridenv.2023.105089
Received 19 October 2022; Received in revised form 3 August 2023; Accepted 25 October 2023

climate will lead microorganisms, particularly fungi, to become more heat-adapted and increase in virulence, making native microbes that tortoises encounter potentially pathogenic (Casadevall, 2022; Nnadi et al., 2021). Indeed, there is evidence of wild Mojave Desert tortoises being infected by fungal pathogens. In one report, a tortoise was diagnosed with fungal pneumonia and nine others had either cutaneous dyskeratosis of the skin or shell necrosis, several of which exhibited fungal hyphae (Homer et al., 1998). This short communication highlights the need for further investigation of pathogen potential within fungal communities. In this study, we examined the fungal community of the exterior (shell, skin, and cloaca) of two animals and compared these fungal communities with soil fungal communities from associated tortoise burrows.

In summary, we 1) examined relative quantities of Onygenales species between samples (tissues, animals, soils) 2) examined differences in overall fungal communities between samples and 3) examined differences in diversity of the overall communities among samples. This approach showed that certain groups live primarily on the tortoise, and contain potential pathogens. Based on this small sample, we hypothesize that a greater relative abundance of keratinophilic fungi, such as those from the genus *Microsporum* and *Chrysosporium*, are present on tortoises compared to the soil substrate. These types of fungi have been shown to cause disease in reptiles, including turtles (Woodburn et al., 2021; Mitchell et al., 2013), which makes it important to fully test these hypotheses.

2. Methods

2.1. Sample collection

The two wild tortoises (Tortoise 1 sample ID GopTBB1 and Tortoise 2 sample ID GopTBB2) were located near Lake Mead, Arizona, east of the Colorado River that creates the border of Arizona and Nevada. The animals appeared to be healthy with no obvious sign of disease. Swabs were taken from the entirety of the top of the shell, on the entirety of the skin on the underside and on the underside of all four feet of the tortoises as well as the exterior of the cloaca, resulting in six total swabs from each tortoise. Sterile foam-tipped specimen collections swabs (Puritan Medical Products, Pittsfield, ME, USA) were used. Swabs were swiped across the surface of the various body parts a total of 25 times to ensure the greatest number of microbes were gathered. Each of the two animals received the same swabbing regimen. After sample collection the animals were temporarily held in a water bath where they were able to drink water before being released. These tortoises were genotyped as *G. agassizii* (Dolby et al., 2021; AZGFD Heritage Project: #I17001) but are not protected under the species listing decision (USFWS, 1994). All handling followed IACUC protocol 16–1508R and Lake Mead National Recreation Area Scientific Research and Collecting Permit #1024-0236. Tortoises were released immediately following sample collection recovery. Swabs were submerged in RNA*later* (ThermoFisher Scientific) solution and stored at 4° Celsius for up to a week until downstream processing was completed.

Soils were collected from inside of tortoise burrows using a garden trowel that was sterilized with seventy percent ethanol between each sample. Five total burrows were sampled, two samples per burrow. Burrows were chosen based on size and shape that were consistent with tortoise burrows in this region. The DNA was pooled from the two replicates for sequencing. Each burrow was analyzed separately. Approximately 500 mg were collected and stored in sterile specimen containers and kept in the dark at room temperature until DNA was extracted. One burrow housed tortoise GopTBB2 and the other four were suspected tortoise burrows within in the same area that the two animals were found.

2.2. DNA extraction and ITS sequencing

Total genomic DNA from swabs and soils was extracted using the Qiagen DNeasy PowerSoil Pro kit (Qiagen, Valencia, CA) following the manufacturer's protocol. A step of 5-min homogenization at 6 m/s was included to ensure fungal cells were completely lysed. DNA was quantified by PicoGreen (Life Technologies, Inc, Carlsbad, CA) fluorescence, and normalized to a final concentration of approximately 10 ng/μL.

2.3. ITS2 amplification and amplicon sequencing

To determine the composition of the fungal community, each DNA sample underwent Illumina-based amplicon sequencing with fungal-specific ribosomal DNA (rDNA) primers targeting the ITS2 region (Alvarado et al., 2018; Taylor et al., 2016). Briefly, two amplification steps were used to generate amplicons for sequencing. The first amplification round utilized the locus-specific PCR primers with a universal 5′ tail sequence 5.8S-Fun (5′-AACTTTYRRCAAYGGATCWCT-3′) and ITS-4-Fun (5′-AGCCTCCGCTTATTGATATGCTTAART-3′). PCR was carried out in 25 μL reactions containing 1X Phusion Green Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA), an additional 1.5 mM $MgCl_2$ (3.0 mM total), 200 nM each primer, and 2 μL each normalized DNA sample or 5 μL each dilute DNA sample (see above). Cycling conditions were 95 °C for 2 min, then 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 60 °C for 4 min. PCR products were visualized on 1% agarose gel, purified by bead-prep, and diluted tenfold. Indexing was done during the second PCR to tag each sample using 12-bp Golay barcodes annealed to the ITS-4 Fun primer. Indexing sequences were added in second round amplification using primers matching the universal tails at the 3′-end (5′-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNN-CCTATGTGGAGAGCCAGTAA-3′, 5′-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTCAACGCTCA-CTACTGCGA-3′) (Alvarado et al., 2018). Reaction conditions were the same as first round amplification, but contained 100 nM each primer, 5 μL purified, diluted template, and cycling was carried out for only 15 cycles. The resulting pool was sequenced on a MiSeq Desktop Sequencer (Illumina, Inc., San Diego, CA) in 2 × 300 bp mode. Custom sequencing primers were used which match the universal tail sequences from first round amplification (read 1: 5′-CCTATGTGGAGAGCCAGTAAGCGATGCTATGGT-3′; read 2: 5′- GTCAACGCTCACTACTGCGATTACCCAAGTCAG-3′; index 1: 5′-CTGACTTGGGTAATCGCAGTAGTGAGCGTTGAC-3′) (Alvarado et al., 2018). Data processing was done using pipe craft, a bioinformatics pipeline designed for fungal amplicon sequences (Anslan et al., 2017). Pre-processing steps with associated bioinformatics program were as followed; assembling of paired reads using vsearch, quality filtering and trimming of sequences using Dada2, sequences shorter than 160 base pairs were discarded and the maximum number of expected errors (E-Max) was set to 0.25, demultiplexing using cutadapt, chimeric filtering using vsearch gene extraction using ITSx and clustering using CDHIT (Anslan et al., 2017). Finally, taxonomy was assigned using BLAST + against the UNITE database. The OTU table was used to explore differences in alpha diversity using Shannon's Index, beta diversity (differential abundance of individual taxa using Bray-Curtis), and comparisons of taxa relative abundances using the "tidyMicro" R-package (Carpenter et al., 2021). A sequence identity threshold of 97% was used to for OTU identification, rare taxa were filtered out of our OTU table for analysis and a rarefaction analysis, to check if the sampling depth was sufficient by examining if the curves reached saturation, was done on our data using the Rarefy R package (Thouverai et al., 2020). A relative abundance of 0.20% was used to filter out rare taxa. The total fungal community was included in analyses.

3. Statistical analyses

Shannon's diversity and OTU richness were separately compared among tissue swabs and animals with Kruskal–Wallis tests. Post-hoc Dunn's pairwise test was also performed with P-values adjusted with Benjamini Hochberg correction. To examine beta-diversity among tissue swabs and animals we visualized the communities using principal coordinate analysis using Bray-Curtis dissimilarities. We further compared communities using permutational analysis of variance (PERMANOVA) with 999 permutations.

4. Results

4.1. Mycobiome sequencing

A total of 160,148 ITS amplicon sequences were retained after the removal of low-quality reads. The sequences from 17 samples had a median sequencing depth of 10,038, ranging from 7532 to 13,464 reads per sample.

4.2. Richness of Onygenales

Rarefied data was used to compare Richness of Onygenales. We did not find a significant difference in Onygenales OTU richness among the parts of the tortoise and the soil substrate using Kruskal-Wallis non parametric tests (p-value = 0.8, Fig. 1a). Interestingly, when comparing the richness of Onygenales from each sampled tortoise there was a difference (p-value = 0.01). Dunn's Post-hoc pairwise test show Tortoise One had significantly lower Onygenalean richness than the soil (p = 0.017) and Tortoise Two had significantly greater Onygenalean richness compared to the soil (p = 0.03, Fig. 1b). The most common families represented in the order are Onygenaceae, Gymnoascaceae, and Ajellomycetaceae, as well as OTUs that are unidentified at the family level. It appears that there is an influence of the individual animal on the relative amount of potential pathogenic fungi living on the exterior of the animal.

4.3. Beta diversity comparison of the whole fungal community

The PERMANOVA based on Bray-Curtis dissimilarities found that there were no differences in fungal communities between body areas (Df = 3, F = 1.22, pseudo p-value = 0.29). However, upon examination of the principal coordinate analysis (PCoA), the soil samples are clustering together away from the samples taken from tortoises and the two fungal communities from the different tortoise's cluster separately (Fig. 2a). This is reflected in a second PERMANOVA, which identified a statistical difference in the fungal communities among individual tortoises and the soil samples (Fig. 2b; Df = 2, F = 2.93, pseudo p-value = 0.024) (Fig. 2b).

4.4. Alpha diversity comparison if the whole fungal community

Good's coverage and the subsequent Alpha-diversity measurements were calculated through rarefied bootstrap analysis on the OTU level. Rare taxa were removed from the analysis with a Good's coverage below 80% were removed from this analysis. Alpha fungal diversity is based on the Shannon Index. Significance was tested via a Kruskal-Wallis non-parametric test and then with Dunn's Post-hoc pairwise test. There is a significant difference between parts of the tortoise ($p = 0.03$) and pairwise analysis shows differences among different areas of the body and the soil substrate (indicated by asterisks; cloaca $p < 0.001$, shell $p < 0.001$, and skin $p < 0.001$, Fig. 3a). There are also significant differences between the fungal communities of the sampled animals as a whole ($p = 0.01$) and pairwise analysis shows differences among the animals and soil (indicated by asterisks; animal one $p < 0.001$, animal two $p < 0.001$, Fig. 3b). The communities of the animals are also different from each other ($p=<0.001$). It appears that the order *Pleosporales* is overly abundant relative to other fungal taxa in the soil samples compared to the samples taken from the tortoises.

4.5. Relative abundances of different fungal orders

The most abundant fungal orders on the cloaca are Eurotiales, Sordariales, and Onygenales. The most abundant taxa on the shell are Pleosporales, Dothideales, and Eurotiales. The most abundant taxa on the skin are Pleosporales, Eurotiales, and Chaetothyriales (Fig. 4a). The most abundant taxa in the soil are Pleosporales, and unidentified fungi (Fig. 4). The most abundant taxa on tortoise GopTBB1 are as a whole are Pleosporales, Dothideales, and Chaetothyriales. The most abundant taxa on tortoise GopTBB2 are Eurotiales, Pleosporales, and Onygenales (Fig. 4b).

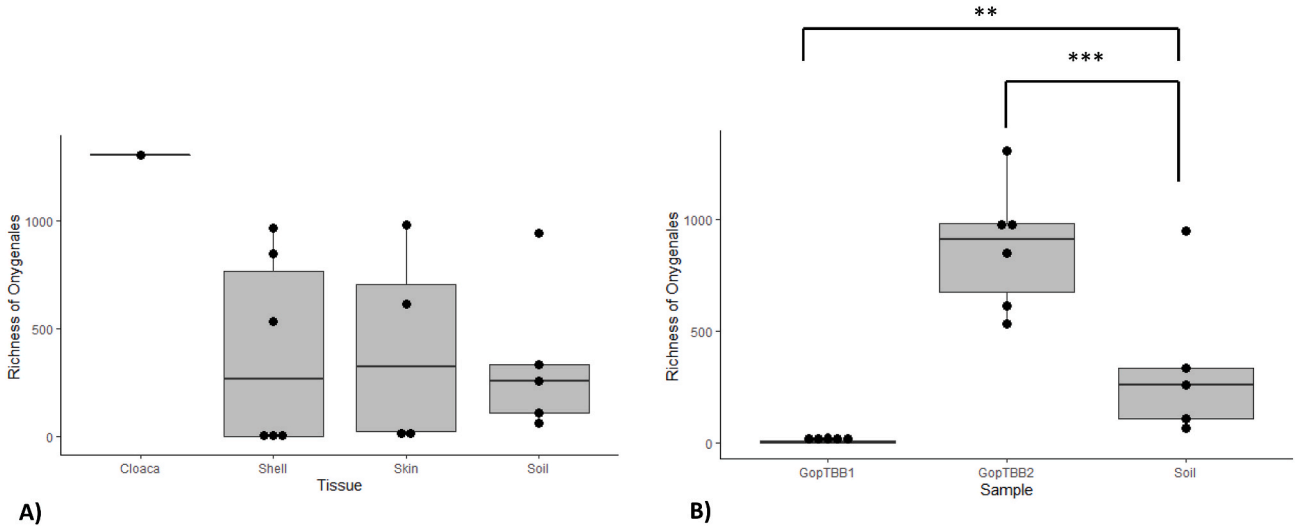

Fig. 1. Richness of Onygenales among the tortoise fungal community and the soil substrate: A) different parts of the tortoise compared to the soil and B) the two sampled animals compared to the soil. Asterisks indicate that both tortoises are different from the soil substrate GopTBB1 lower and GopTBB2 higher. Tortoises GopTBB1 and GopTBB2 also differ from each other.

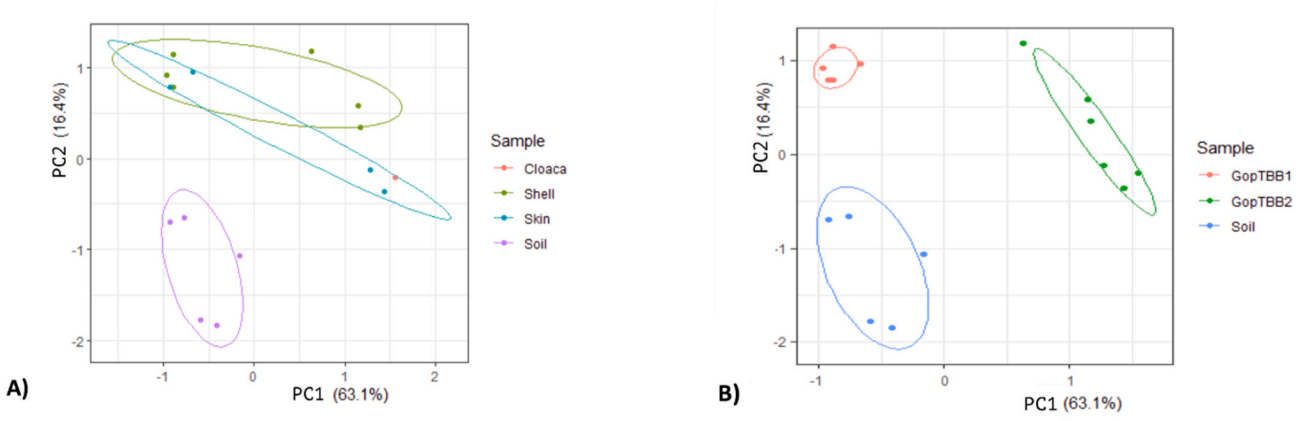

Fig. 2. Beta-Diversity of the tortoise fungal communities and soil substrate: Principal coordinate analysis (PCoA) of **A**) body areas to the soil and B) the two sampled animals to the soil using Beta diversity based on Bray–Curtis dissimilarities matrix following normalization to proportions. Ellipses show 95% confidence intervals (95% CIs) of each sample. Each point represents the fungal community of an animal, soil, or part of the animal.

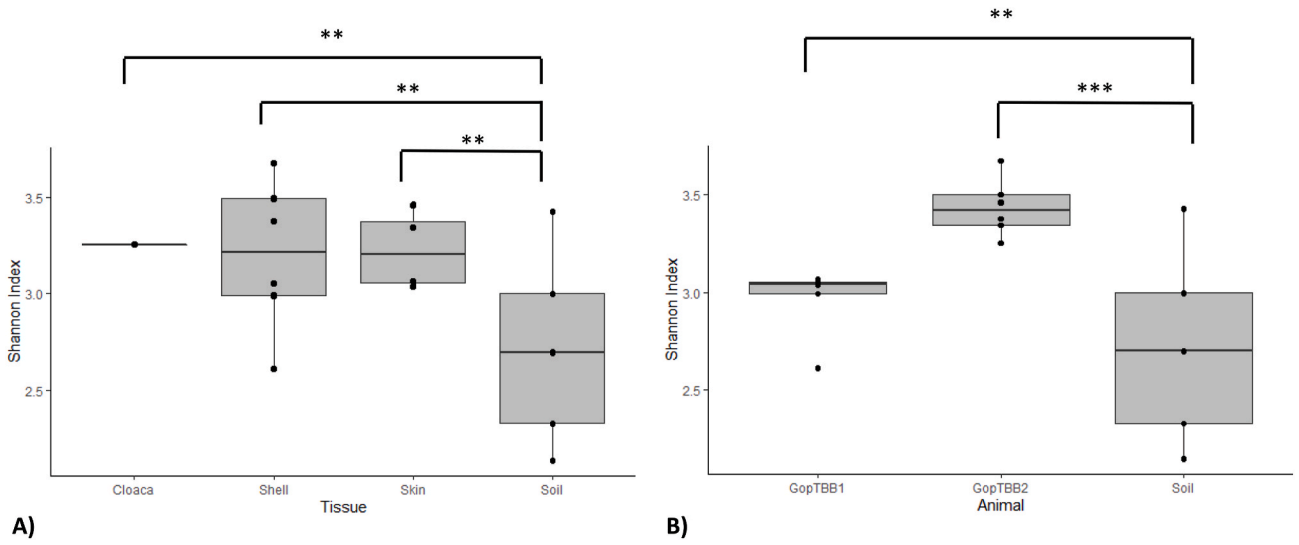

Fig. 3. Comparisons of fungal alpha diversity based on Shannon's index in the tortoise fungal community and the soil substrate: A) parts of the tortoise and the soil and B) the two sampled animals and the soil. Asterisks indicate statistical significance.

5. Discussion

In this study, we aimed to understand the exterior mycobiome of the threatened Mojave Desert tortoise to determine whether this species is at risk of being colonized by potential pathogens that could cause dermatomycoses. We hypothesized that a greater relative abundance of these potential pathogens would be associated with Mojave Desert Tortoises and based on this data this was supported. The sample size is too small ($n = 2$) to come to definitive conclusions; however, we hope this study sheds light on infectious disease investigations for desert tortoise populations. Our analysis of the exterior fungal communities of two tortoises showed distinct differences between the tortoises and surrounding soil that they inhabit, suggesting a fungal community was adapted to survive on the exterior of each specific reptile. Some of the members of the mycobiome could be pathogens, especially the high relative abundance of members of the order Onygenales. Notably, we discovered that one of the tortoises had higher species richness of the order Onygenales than the other tortoise as well as higher than the surrounding soil, although showing no signs of disease. Interestingly, the two animals' fungal communities differed significantly. This suggests increasing sample size would reveal additional host specific fungi and the discovery of additional diversity.

We focused on the potentially pathogenic Onygenales fungal taxa present on the high keratin environment of the tortoise exterior by comparing fungal communities living on the desert tortoise to those in the surrounding soil. Initially, we did not observe a significant difference in OTU richness between the soil and the tortoise samples. However, in examining tortoises and body regions separately, we did observe differences in the whole fungal communities of Onygenales richness, alpha diversity, and beta diversity consistent with a difference in fungal communities between the tortoises and the environment and a higher relative abundance of potential pathogens on tortoises (Fig. 4).

Specifically, Tortoise Two exhibited a higher number of potential pathogenic fungi in the order Onygenales. This result was concerning to us, as the order Onygenales contains a high density of fungal pathogens and keratinophilic fungi that could cause disease under the appropriate conditions. Most notably, we detected a species from the genus *Chrysoporium*—this genus can cause severe and fatal dermatomycoses in several different reptile species (Bertelsen et al., 2005).

In summary, we found that the Mojave Desert tortoise had fungal communities distinct from the soil in the surrounding environment. The tortoise communities had a high relative abundance of species in the order Onygenales that could have the potential to cause morbidity and mortality in tortoise populations. With the warming climate there is a

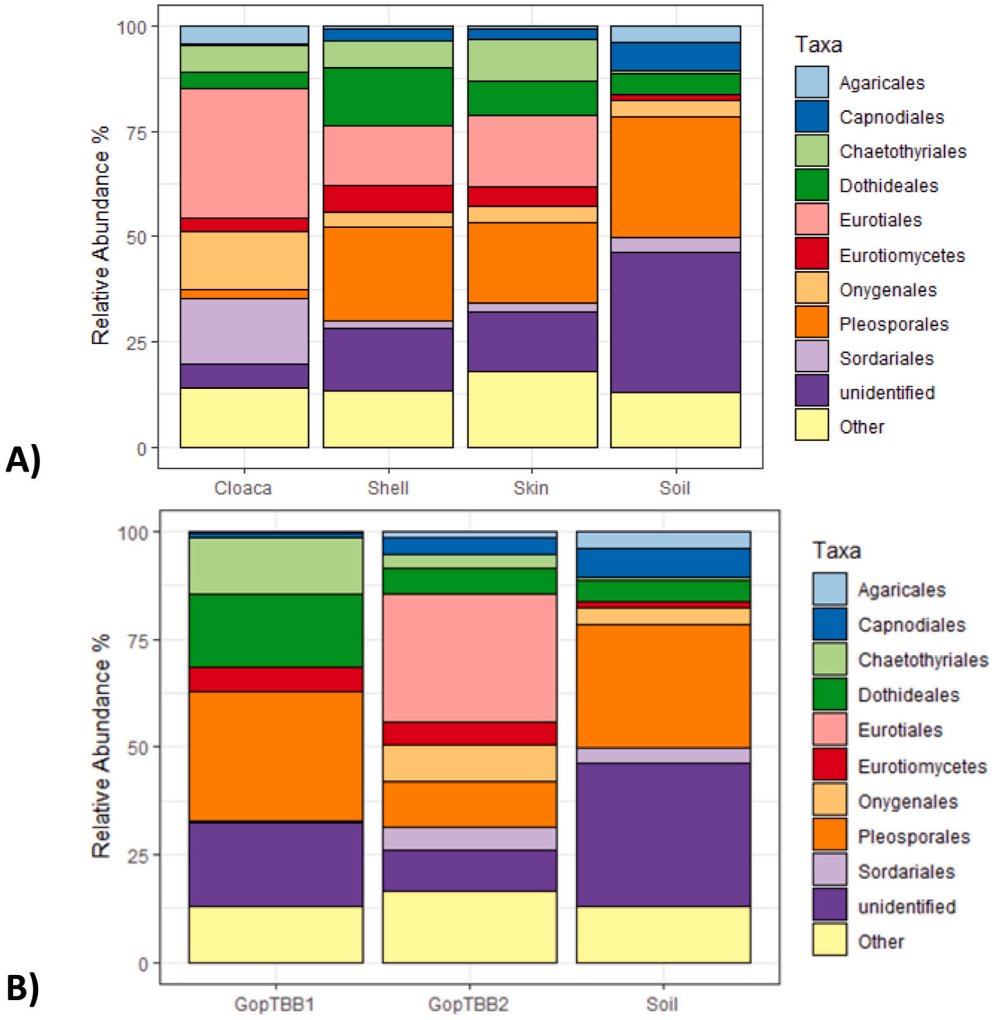

Fig. 4. Relative abundances of fungal orders found on the desert tortoises and soil substrate. The top ten most abundant fungal orders are shown in the figure legend. A) is comparing the fungal profiles of different swab sites compared to the soil substrate and B) is comparing fungal profiles between the two animals and the soil substrate.

higher risk of mycoses because of range expansion, thermotolerance, and immunosuppression of hosts, (Nnadi et al., 2021) and fungi already present in this tortoise population could present an increased risk of disease in the future. It is thus not only important to identify the known agents of reptilian disease, such as *Mycoplasma* and *Ranavirus*, but also to recognize these emerging fungi as potential threats to biodiversity in conservation efforts for this and other species.

6. Study limitations

We acknowledge that study is limited by use of two animals and is observational at a single time point. Increasing the sample size will improve assessment of total diversity of fungi living on these tortoises and uncover rare species that we likely missed due to small sample size.

CRediT authorship contribution statement

Daniel R. Kollath: Conceptualization, Methodology, Writing – original draft, Investigation, Visualization. **Greer A. Dolby:** Methodology, Investigation, Funding acquisition, Writing – review & editing. **Timothy H. Webster:** Methodology, Investigation, Funding acquisition, Writing – review & editing. **Bridget M. Barker:** Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

No conflict of interest declared.

Data availability

Sequence data have been submitted to a public repository.

Acknowledgments

We would like to thank Dale DeNardo, Kenro Kusumi, Melissa Wilson, Cristina Jones, and Paul Frank for their help in obtaining samples, permits, and funding for field work to collect these samples. We would also like to thank the Genetics core (formerly EnGGen) at Northern Arizona University for performing the next generation sequencing. This work was funded by AZGF Heritage Grant #I17001.

References

Allison, L.J., McLuckie, A.M., 2018. Population trends in Mojave desert tortoises (Gopherus agassizii). Herpetol. Conserv. Biol. 13 (2), 433–452.
Alvarado, P., et al., 2018. Detection of Coccidioides posadasii from xerophytic environments in Venezuela reveals risk of naturally acquired coccidioidomycosis infections. Emerg. Microb. Infect. 7 (1), 1–13.

Anslan, S., et al., 2017. PipeCraft: flexible open-source toolkit for bioinformatics analysis of custom high-throughput amplicon sequencing data. Molecular ecology resources 17 (6), e234–e240.
Bechtel, M.J., et al., 2021. Borreliosis transmission from ticks associated with desert tortoise burrows: examples of tick-borne relapsing fever in the Mojave Desert. Vector Borne Zoonotic Dis. 21 (8), 635–637.
Berger, L., et al., 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. Proc. Natl. Acad. Sci. USA 95 (15), 9031–9036.
Bertelsen, M.F., et al., 2005. Fatal cutaneous mycosis in tentacled snakes (Erpeton tentaculatum) caused by the Chrysosporium anamorph of Nannizziopsis vriesii. J. Zoo Wildl. Med. 36 (1), 82–87.
Carpenter, C.M., et al., 2021. tidyMicro: a pipeline for microbiome data analysis and visualization using the tidyverse in R. BMC Bioinf. 22 (1), 1–13.
Casadevall, A., 2022. Immunity to invasive fungal diseases. Annu. Rev. Immunol. 40, 121–141.
Coleine, C., et al., 2022. Humidity and low pH boost occurrence of Onygenales fungi in soil at global scale. Soil Biol. Biochem. 167, 108617.
Harless, M.L., et al., 2009. Home range, spatial overlap, and burrow use of the desert tortoise in the West Mojave Desert. Copeia 2009 (2), 378–389.
Homer, B.L., et al., 1998. Pathology of diseases in wild desert tortoises from California. J. Wildl. Dis. 34 (3), 508–523.
Jacobson, E.R., et al., 2014. Mycoplasmosis and upper respiratory tract disease of tortoises: a review and update. Vet. J. 201 (3), 257–264.
Johnson, A.J., et al., 2008. Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. J. Wildl. Dis. 44 (4), 851–863.
Kaufmann, C., Cassin-Sackett, L., 2021. Fine-Scale spatial structure of soil microbial communities in burrows of a keystone rodent following mass mortality. Frontiers in Ecology and Evolution 9, 758348.
Kollath, D.R., et al., 2019. Investigating the role of animal burrows on the ecology and distribution of Coccidioides spp. in Arizona soils. Mycopathologia 185, 145–159.
Lorch, J.M., et al., 2016. Snake fungal disease: an emerging threat to wild snakes. Phil. Trans. Biol. Sci. 371 (1709), 20150457.
Mitchell, M.A., Walden, M.R., 2013. Chrysosporium anamorph Nannizziopsis vriesii: an emerging fungal pathogen of captive and wild reptiles. Veterinary Clinics: Exotic Animal Practice 16 (3), 659–668.
Nagy, K.A., Medica, P.A., 1986. Physiological ecology of desert tortoises in southern Nevada. Herpetologica 73–92.
Nnadi, N.E., Carter, D.A., 2021. Climate change and the emergence of fungal pathogens. PLoS Pathog. 17 (4), e1009503.
Sim, R.R., et al., 2015. Identification of a novel herpesvirus in captive Eastern box turtles (Terrapene carolina carolina). Vet. Microbiol. 175 (2–4), 218–223.
Stanford, C.B., et al., 2020. Turtles and tortoises are in trouble. Curr. Biol. 30 (12), R721–R735.
Taylor, D.L., et al., 2016. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. Appl. Environ. Microbiol. 82 (24), 7217–7226.
Thouverai, E., et al., 2020. Rarefy: Rarefaction Method.
Tompkins, D.M., et al., 2015. Emerging infectious diseases of wildlife: a critical perspective. Trends Parasitol. 31 (4), 149–159.
Wilson, D.S., et al., 1999. Winter activity of juvenile desert tortoises (Gopherus agassizii) in the Mojave Desert. J. Herpetol. 33 (3), 496–501.
Woodburn, D.B., et al., 2021. Shell lesions associated with Emydomyces testavorans infection in freshwater aquatic turtles. Veterinary pathology 58 (3), 578–586.