Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/JWD-D-21-00160: Caitlin J. Campbell, David M. Nelson, J. Edward Gates, H. Lisle Gibbs, Elizabeth R. Stevenson, Becky Johnson, Juliet Nagel, Regina Trott, Jamin G. Wieringa, and Hannah B. Vander Zanden. White-nose Syndrome Pathogen *Pseudogymnoascus destructans* Detected on Migratory Tree-roosting Bats.

Supplementary detailed swabbing methods and results

Skin swabs from the muzzle and forearm were collected from wild-caught live bats (in Maryland and Delaware as part of ongoing *Pd* monitoring under appropriate permits, e.g., Nagel and Gates [2017]) according to USGS monitoring and decontamination protocols. Traditional face-and-forearm swabbing of live bats aims to detect *Pd* on the body parts with highest *Pd* loads in WNS-susceptible species (National Wildlife Health Center 2020). As WNS-resistant species may encounter and retain *Pd* on parts of the body more likely to encounter cave substrate, we also swabbed the furred region of the plagiopatagium and the foot of whole carcasses when possible (Figure 1). For both methods, we rolled a purified water moistened sterile swab three times along the regions of targeted tissue. Extraction of DNA from swabs was conducted according to a protocol modified from Verant et al. (2016): samples were first treated with a sorbitol buffer containing 1M sorbitol, 100mM EDTA, 14mM 2-mercaptoethanol and 200 units of lyticase. We added 600µL of buffer to a swab, contained in a 1.5ml tube, and gently mixed before incubating at 30°C for 30 minutes. The swab was then removed and the sample centrifuged at 300xg for 10 minutes. The supernatants were pipetted off and the protocol for the QIAamp microkit using carrier RNA was followed subsequently.

Samples were tested for *Pd* DNA using a qPCR assay (Verant et al. 2016). PCR reactions consisted of the following components and final concentrations: 1x Express qPCR Supermix Universal, 400nM of forward and reverse primers, 250 nM of probe, 500 nM ROX reference dye, 5 µL of sample, and brought up to 20 µL with sterile water. A positive control of genomic DNA from *Pd* obtained from American Type Culture Collection (ATCC, MYA-4855D) and negative (sterile water added as template) controls were run alongside samples. All samples and controls were run as duplicates on each plate. PCR parameters, including 50°C for 2 minutes, 95°C for 20 seconds, and 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds, were carried out with an Applied Biosystems StepOnePlus Real-Time PCR system. The Cq was automatically generated for each plate and the results of each assay plotted and inspected for suitability. Laboratory analyses were conducted at the University of Maryland Center for Environmental Science Appalachian Laboratory.

One fur swab sample from an eastern red bat (not listed in Table 3) had a C_T value of 8.2, well below that of the positive control and of C_T values found in prior studies of *Pd* on bats (Verant et al. 2016), and a flat amplification curve, both of which suggests spurious amplification; therefore this sample was not considered to be *Pd* positive.

Code to reproduce summary tables and figures are available at: https://github.com/cjcampbell/Pd_DetectionMigratoryBats DOI: 10.5281/zenodo.5807799

LITERATURE CITED

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Verant ML, Bohuski EA, Lorch JM, Blehert DS. 2016. Optimized methods for total nucleic acid extraction and quantification of the bat white-nose syndrome fungus, *Pseudogymnoascus destructans*, from swab and environmental samples. *J Vet Diagn Invest* 28:110–118.