Widespread Bat White-Nose Syndrome Fungus, Northeastern China

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To the Editor: Emerging infectious diseases have caused catastrophic declines in wildlife populations, and the introductions of many pathogen have been linked to increases in global trade and travel (*1*). Mapping the distribution of pathogens is necessary to identify species and populations at risk and identify sources of pathogen spillover and introduction. Once pathogen distributions are known, management actions can be taken to reduce the risk for future global spread (*2*).

Bats with symptoms of white-nose syndrome (WNS) were first detected in the United States in 2006, and the disease has subsequently caused precipitous declines in temperate bat populations across eastern North America (*3*,*4*). *Pseudogymnoascus destructans*, the causative agent of WNS, is a cold-growing fungus that infects bats' skin during hibernation, leading to more frequent arousals from torpor and death (*3*). *P. destructans* is widespread throughout Europe (*5*), but, to our knowledge, its presence in Asia has not been documented.

We sampled bats and hibernacula surfaces (cave walls and ceilings) across northeastern China during 2 visits (June–July 2014 and March 2015) using a previously described swab-sampling technique (*6*). Bats were captured inside caves and at their entrances. DNA was extracted from samples by using a modified QIAGEN DNeasy blood and tissue kit (QIAGEN, Valencia, CA, USA) and tested in duplicate for the presence of *P. destructans* with a quantitative real-time PCR (qPCR) (*6*,*7*).

In the summer of 2014 and winter of 2015, we collected 385 samples from hibernacula surfaces at 12 sites in 3 provinces and 1 municipality (Figure, panel A) and 215 samples from 9 species of bats at 10 sites (summer: *Rhinolophus ferrumequinum, Rhinolophus pusillus, Myotis adversus, Myotis macrodactylus, Myotis pilosus, Myotis chinensis, Murina usseriensis*; winter: *R. ferrumequinum, Murina leucogaster, Myotis petax*). During the summer, *P. destructans* was widely distributed across the study region with positive samples (determined on the basis of qPCR results) obtained from cave surfaces at 9 of 12 sites and from bats at 2 of the 9 sites where bats were sampled (Figure, panel A).

Prevalence of *P. destructans* was low during summer in the environment (mean prevalence across sites 0.06 ± 0.03) and in bats. Bats of 3 species tested positive for *P. destructans* in the summer: *M. macrodactylus* (1/10), *M. chinensis* (1/1), and *M. ussuriensis* (1/1). *P. destructans* was not detected in bats of 4 other species, of which >20 individual animals of each species were sampled (*R. ferrumequinum*, *R. pusillus*, *M. pilosus*, and *M. adversus*). The low prevalence of *P. destructans* in bats and on hibernacula surfaces in China during the summer was similar to comparable results from studies in North America (*6*).

In winter, prevalence at the 2 sites we revisited was much higher; 75% of 85 samples from 3 species tested positive, including samples from 16/17 *M. petax* bats. We also detected *P. destructans* in bats from 2 additional species (*R. ferrumequinum* [11/19 bats] and *M. leucogaster* [11/16 bats]).

In addition, during March 2015, we observed visual evidence of *P. destructans* in bats (*M. petax*; Figure, panel C) and obtained 2 fungal cultures from swab specimens taken from these bats. To isolate *P. destructans* from these samples, we plated swab specimens from visibly infected bats on Sabouraud dextrose agar at 10°C. We identified potential *P. destructans* isolates on the basis of morphologic characteristics. DNA was then extracted from 2 suspected fungal cultures and tested for *P. destructans* by qPCR, as previously described.

To further confirm the presence of *P. destructans*, we prepared the fungal isolates for Sanger sequencing (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-1314-Techapp1.pdf). The 600-nt amplification products from these 2 isolates were sequenced and found to be 100% identical to the *P. destructans* rRNA gene region targeted for amplification. In addition, using BLAST (http://www.ncbi.nlm.nih.gov/blast.cgi), we found that sequences were a 100% match with isolates from Europe (GenBank accession no. GQ489024) and North America (GenBank accession no. EU884924). This result confirms that the same species of fungus occurs on all 3 continents. We also obtained wing biopsy punches from these bats and found lesions characteristic of WNS by histopathologic examination (Figure, panel B; online Technical Appendix).

The occurrence of *P. destructans* at most sites sampled indicates that this pathogen is widespread in eastern

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Asia (Figure, panel A). The presence of *P. destructans* in bats from 6 species in China and on bats in 13 species in Europe (*8*) confirms the generalist nature of this fungus and suggests that it may occur throughout Eurasia (Figure, panel D).

Decontamination and restrictions on the use of equipment that has been used in caves in Asia would help reduce the probability of introducing *P. destructans* to uninfected bat populations (e.g., western North America, New Zealand, southern Australia, and temperate areas of South

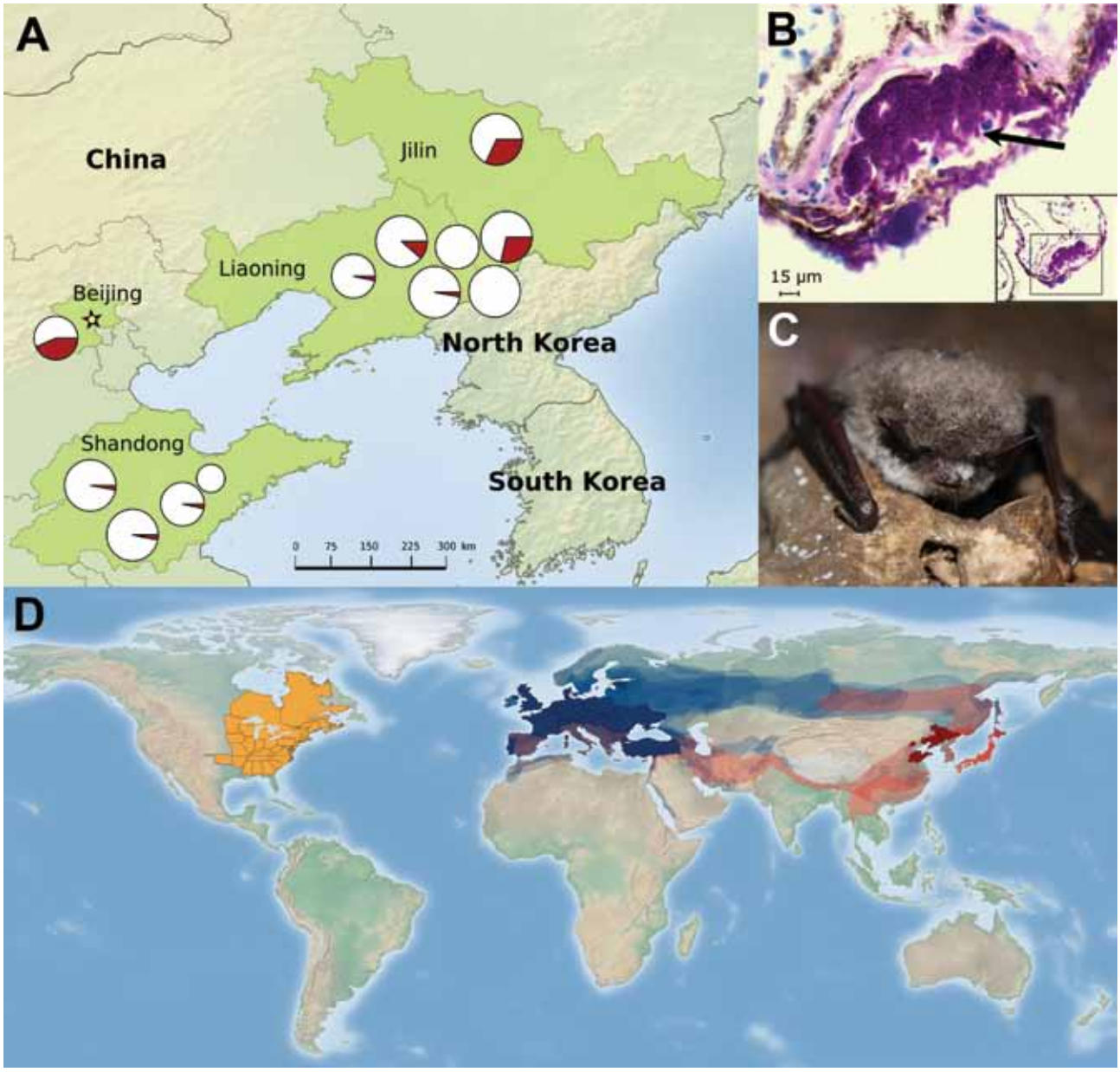

Figure. A) Distribution of *Pseudogymnoascus destructans* in cave environments during summer at 9 sites in northeastern China. Pie charts show the prevalence of *P. destructans,* and the size of pie graphs indicates the number of samples taken at each site (range 10–35). B) Histologic wing cross-section from *Myotis petax* bat collected in March 2015 with cup-like lesion (arrow) diagnostic of white-nose syndrome (periodic acid–Schiff staining). C) *M. petax* bat found in a cave in Jilin, China, showing visible signs of white-nose syndrome, March 2015. D) Documented global distribution of *P. destructans*. Areas in solid black represent the provinces and countries in China and Europe, respectively, where *P. destructans* was detected in this study and from previous research (*5*). Semitransparent regions show the species ranges (range data taken from http://www.iucnredlist.org/) for the bat species detected with *P. destructans* in Asia (n = 6) and Europe (n = 13) (*8*) and possible distribution of *P. destructans*. The solid black region in North America shows the extent of *P. destructans* spread as of May 15, 2015 (https://www.whitenosesyndrome.org/resources/map). A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/22/1/15-1314-F1.htm).

America). These measures would also reduce the risk of introducing new strains of *P. destructans* to regions where bats are already infected (e.g., eastern North America and Europe). These measures are necessary to prevent the devastating effects this pathogen has had on bats in North America and would help maintain the ecosystem services that bats provide (*9,10*).

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References

1. Daszak P, Cunningham AA, Hyatt AD. Emerging infectious diseases of wildlife—threats to biodiversity and human health. Science. 2000;287:443–9. http://dx.doi.org/10.1126/science.287.5452.443
2. St John RK, King A, de Jong D, Bodie-Collins M, Squires SG, Tam TW. Border screening for SARS. Emerg Infect Dis. 2005;11:6–10. http://dx.doi.org/10.3201/eid1101.040835
3. Warnecke L, Turner JM, Bollinger TK, Lorch JM, Misra V, Cryan PM, et al. Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. Proc Natl Acad Sci. 2012;109:6999–7003. http://www.pnas.org/content/109/18/6999 [cited 2015 Nov 30].
4. Langwig KE, Hoyt JR, Parise KL, Kath J, Kirk D, Frick WF, et al. Disease dynamics of white-nose syndrome invasion, midwestern United States, 2012–2014. Emerg Infect Dis. 2015;21:1023–6. http://dx.doi.org/10.3201/eid2106.150123
5. Puechmaille SJ, Wibbelt G, Korn V, Fuller H, Forget F, Muhldorfer K, et al. Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. PLoS ONE. 2011;6;e19167. http://dx.doi.org/10.1371/journal.pone.0019167
6. Langwig KE, Frick WF, Reynolds R, Parise KL, Drees KP, Hoyt JR, et al. Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. Proc Biol Sci. 2015;282:20142335. http://dx.doi.org/10.1098/rspb.2014.2335
7. Muller LK, Lorch JM, Lindner DL, O'Connor M, Gargas A, Blehert DS. Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of *Geomyces destructans*. Mycologia. 2013;105:253–9. http://dx.doi.org/10.3852/12-242
8. Zukal J, Bandouchova H, Bartonicka T, Berkova H, Brack V, Brichta J, et al. White-nose syndrome fungus: a generalist pathogen of hibernating bats. PLoS ONE. 2014;9:e97224. http://dx.doi.org/10.1371/journal.pone.0097224
9. Maine JJ, Boyles JG. Bats initiate vital agroecological interactions in corn. Proc Natl Acad Sci U S A. 2015 Sep 14. pii: 201505413. [Epub ahead of print].
10. Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH, Kilpatrick AM, et al. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. Ecology Letters. 2012;15:1050-7. http://dx.doi.org/10.1111/j.1461-0248.2012.01829.x

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New Clinical Strain of *Neisseria gonorrhoeae* with Decreased Susceptibility to Ceftriaxone, Japan

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To the Editor: In 2009, 2010, and 2013, *Neisseria gonorrhoeae* strains H041 (ceftriaxone MIC of 2 mg/L), F89 (ceftriaxone MIC of 1 mg/L), and A8806 (ceftriaxone MIC of 0.5 mg/L) were isolated from samples from patients in Japan (*1*), France (*2*) and Australia (*3*), respectively. In Japan, no other clinical *N. gonorrhoeae* strains with decreased susceptibility to ceftriaxone were reported until 2014, when clinical strain GU140106 (ceftriaxone MIC of 0.5 mg/L) was isolated from a man in in Nagoya, Japan. We report details of this case and sequencing results of the *penA* gene for the strain. The study was approved by the Institutional Review Board of the Graduate School of Medicine, Gifu University, Japan.

N. gonorrhoeae strain GU140106 was isolated from a urethral swab sample from a man with acute urethritis. The man had received fellatio, without condom use, from a female sex worker in Nagoya in December 2013. He visited our clinic in January 2014 for urethral discharge. Culture of a urethral swab sample was positive for *N. gonorrhoeae*. We used the Cobas 4800 CT/NG Test (Roche Molecular Systems Inc., Pleasanton, CA, USA) to test a first-voided urine sample; results were positive for *N. gonorrhoeae* but negative for *Chlamydia trachomatis*. The infection was treated with a single-dose regimen of ceftriaxone (1 g) administered by intravenous drip infusion. Two weeks later,

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Technical Appendix

DNA Amplification and Sequencing

We used conventional PCR with *Pseudogymnoascus*-specific primers developed by Lorch et al. (*1*) [nu-SSI(1506)-184–9-Gd (5′-GGGGACGTCCTAAAGCCT-3′) nu-5.8S-144–3-Gd (5′-TTGTAATGACGCTCGGAC-3′)] for amplifying a fragment of the *P. destructans* ribosomal RNA gene between the small subunit (SSU; 18S) and the internal transcribed spacer region. PCR was conducted by using *Taq* DNA polymerase (Life Technologies, Grand Island, NY, USA), per the manufacturer's instructions. Reactions included 2 μL of extracted DNA (1 ng/μL) in a volume of 20 μL, and the PCR cycling conditions consisted of an initial denaturation at 95°C for 10 min, followed by 40 cycles of 94°C for 1 min, 60°C for 30 s, and 72°C for 1 min, and a final extension of 72°C for 10 min. Five microliters of PCR product were loaded onto a 2% agarose gel and run at 100 V for 45 min. The generation of a 600-nucleotide fragment confirmed the presence of *P. destructans* DNA in the isolates. PCR products were cleaned by using ExoSAP-IT (Affymetrix, Cleveland, OH, USA), following the manufacturer's instructions, and then sequenced by using the PCR primers with BigDye Terminator v3.1 chemistry (Applied Biosystems, Foster City, CA, USA). Reaction products were analyzed on an Applied Biosystems 3130xl automated genetic analyzer (Life Technologies). Sequencing reaction results for complementary strands were assembled and edited in Sequencher 5.3 (Gene Codes, Ann Arbor, MI, USA). These sequences were aligned to the *P. destructans* designated type isolate 20631–21 (GenBank accession no. FJ231098) in BioEdit v7.1.3.0 (*2*) and found to be identical to the aligned region.

Histology Sample Collection

Two bats that exhibited visible signs of fungal growth were hand captured alive in the field and brought to back to the College of Animal Science and Technology in Changchun, China. An ultraviolet flashlight (395 nm; LEDwholesalers, Hayward, CA, USA) was used to target areas of the wing with potential fungal lesions and a small 3-mm biopsy punch (Miltex, Plainsboro, NJ, USA) specimen was collected (*3*). The wing biopsy specimens were immediately placed in 10% neutral buffered formalin. Wing membranes were rolled to maximize surface area being examined. Protocols described in Meteyer et al. (*4*) were followed for staining, fixing, and sectioning of wing tissue. Stained wing tissues were examined for characteristic lesions of white-nose syndrome at 60× magnification power. Bats were allowed to recover for several days in an isolated flight cage and then returned to the cave where they were captured.

References

1. Lorch JM, Gargas A, Meteyer CU, Berlowski-Zier BM, Green DE, Shearn-Bochsler V, et al. Rapid polymerase chain reaction diagnosis of white-nose syndrome in bats. J Vet Diagn Invest. 2010;22:224–30. PubMed http://dx.doi.org/10.1177/104063871002200208

2. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series; 1999. p. 95–8.

3. Turner GG, Meteyer CU, Barton H, Gumbs JF, Reeder DM, Overton B, et al. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. J Wildl Dis. 2014;50:566–73. PubMed http://dx.doi.org/10.7589/2014-03-058

4. Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green DE, Shearn-Bochsler V, et al. Histopathologic criteria to confirm white-nose syndrome in bats. J Vet Diagn Invest. 2009;21:411–4. PubMed http://dx.doi.org/10.1177/104063870902100401