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## Genomic Epidemiology of Large Blastomycosis Outbreak, Ontario, Canada, 2021

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Using phylogenomic analysis, we provide genomic epidemiology analysis of a large blastomycosis outbreak in Ontario, Canada, caused by *Blastomyces gilchristii*. The outbreak occurred in a locale where blastomycosis is rarely diagnosed, signaling a possible shift in geographically associated incidence patterns. Results elucidated fungal population genetic structure, enhancing understanding of the outbreak.

North American blastomycosis is an infection most commonly caused by environmental dimorphic fungi *Blastomyces dermatitidis* and *B. gilchristii*. Infections range from asymptomatic to severe, typically presenting as respiratory illness, with possible systemic dissemination (1,2). The geographic range of *B. dermatitidis* and *B. gilchristii* fungi spans the eastern half of North America, including Ontario, Canada (2,3). Although overall incidence rates are low, isolated cases of blastomycosis are diagnosed regularly among populations in endemic areas (2,3), and clusters and outbreaks occur due to common environmental exposures (1,4–7).

We describe a large genomic epidemiology investigation of a blastomycosis outbreak in Constance Lake First Nation, a small community (population <2,000) in northeastern Ontario, Canada, in a locale where blastomycosis has rarely been encountered (2). We studied samples from 181 patients that were received by the Public Health Ontario Laboratory during November 2021–May 2022. By August 2022, we identified *B. gilchristii* fungus, by using multilocus sequence typing (8), in cultures from 40 persons linked to the outbreak (37 community residents and 3 persons [deemed travel A, B, and C] who visited the community during the possible exposure window). We observed that most positive cultures (35/40) were derived from specimens collected during a 7-week period—mid-November 2021 through December

2021—and most (39/40) were obtained from respiratory specimens (Table). Patients spanned all age groups; 55% were male and 45% female (Table).

We performed whole genome short-read sequencing (Illumina, <https://www.illumina.com>) on outbreak isolates and 21 other randomly selected *B. gilchristii* isolates (Appendix, <https://wwwnc.cdc.gov/EID/article/30/7/23-1594-App1.pdf>) cultured from specimens of patients from northern and eastern Ontario in July 2019–July 2022 (BioProject no. PRJNA890593). This collection of sequenced isolates contributes substantially to the number of *B. gilchristii* genomes available to advance research on this important pathogen. We conducted single-nucleotide variant (SNV) analysis to determine genetic diversity and relatedness between the 61 Ontario isolates plus 4 isolates from patients in Minnesota, USA (BioProject no. PRJNA786864), by using MycoSNP v0.22 (<https://github.com/CDCgov/mycosnp-nf>) with reference genome *B. gilchristii* SLH14081 (GenBank accession no. GCA\_000003855.2) and conducted phylogenomic analysis (neighbor-joining method) using MEGA11 (<https://www.megasoftware.net>) (1).

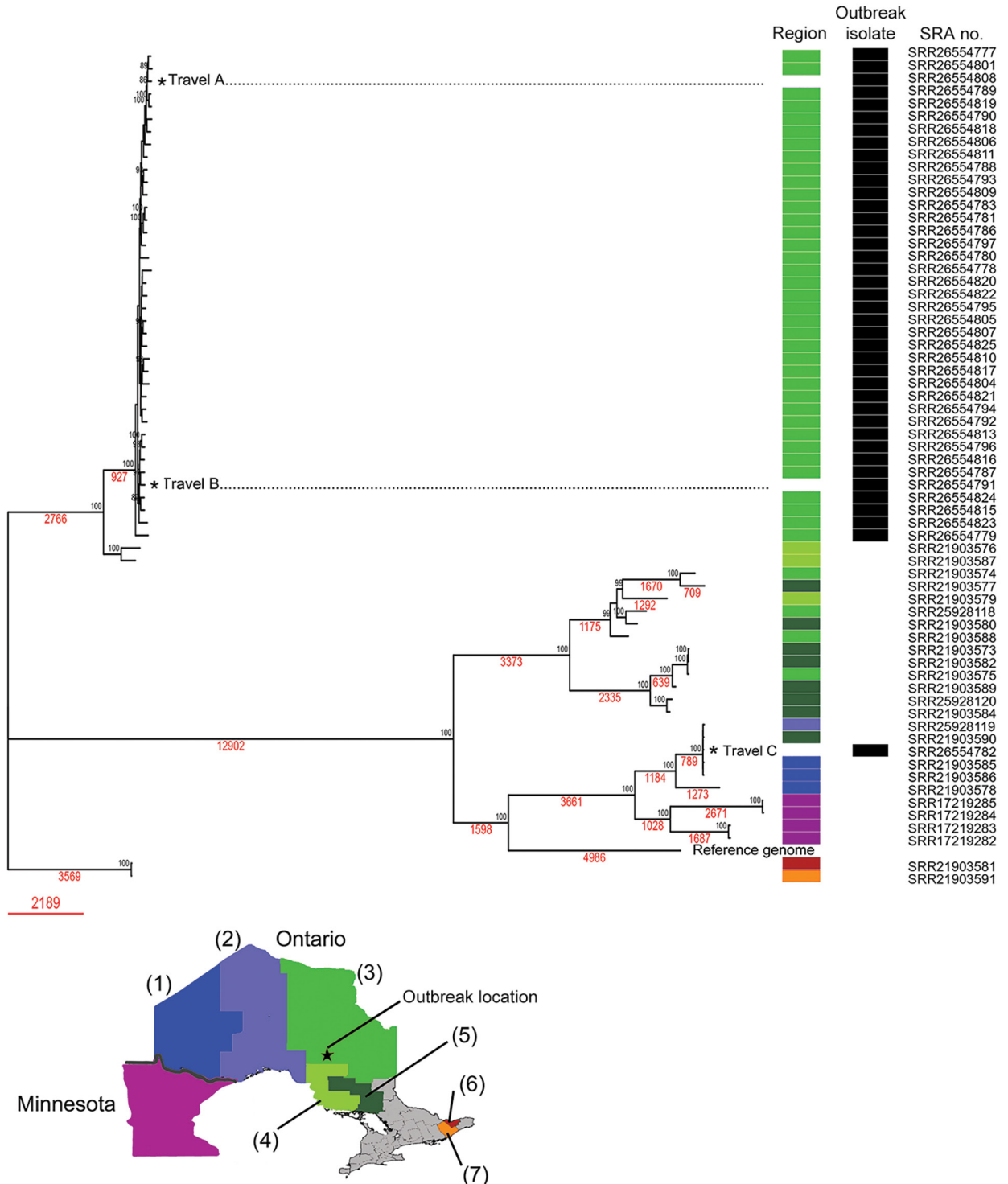
Phylogenomic analysis of SNVs suggested that 39 of the 40 outbreak isolates were genetically highly similar, including isolates from 2 nonresidents who had traveled to the impacted community (travel A and B) (Figure). By contrast, the isolate from travel C was genetically dissimilar from the other outbreak isolates and likely represents a sporadic case acquired elsewhere (Figure). High genetic similarity between outbreak isolates and the brief 7-week timeframe for symptom onset for 90% of cases suggests a shared discrete exposure window. The overall analysis depicts multiple genetically distinct populations of *B. gilchristii* fungi separated by thousands of SNVs, correlated with different geographic regions.

To validate the number of SNV differences between isolates, we separately investigated 9 pairs of biologic replicates (isolates from separate specimens from the same person) and 3 technical replicates from 2 other randomly selected isolates (Appendix). We found that 8 of 9 biologic replicates and all technical replicates averaged 12 (range 5–20) SNVs between corresponding isolates. In contrast, the number of SNVs between 39 outbreak isolates (excluding travel C) averaged 380 (range 96–778); 1 pair of biologic replicates differed by 192 SNVs. By comparison, 5 other clusters of isolates, including 2 clusters from Minnesota patients, had <100 SNVs between isolates. Taken together with the timing of the outbreak, the SNV differences between isolates suggests a shared exposure to a genetically similar yet heterogeneous fungal population, rather than a genetically identical point source. We theorize that the pair of biologic replicates differing by 192 SNVs might represent infection by 2 different strains of the fungal population. Of note, the outbreak locale possesses several environmental niches considered suitable reservoirs for *Blastomyces* spp. fungi, namely waterway-adjacent and forested areas, with coniferous trees supplying acidic soil rich in decaying organic material (7).

During the past 40 years, reports have documented several outbreaks and case clusters of North American blastomycosis (1,4–7). Most ascribe exposure to a discrete time and environmental locale, although that presumption is challenging to confirm because of the variable incubation time (1–6 months) (6) and patient travel. Recently, genomic epidemiologic information gleaned from whole-genome bioinformatics analysis has enhanced the description of outbreaks and case clusters of fungal pathogens (9,10), including *B. gilchristii* (1), enabling a more fulsome understanding of disease acquisition. In this study, we have interpreted the genomic epidemiology of a large outbreak of *B. gilchristii* within the context of several contemporaneous, genetically unrelated isolates to describe pathogen population genetic structure. We consider such genomic epidemiologic analyses useful in identifying cases caused by genetically related strains and, when combined with other epidemiologic factors including patient travel, pinpointing the timing and location of exposure. Epidemiologic investigation combined with pathogen phylogenomic analyses enables improved understanding of blastomycosis outbreaks and disease dynamics. This detailed information might help elucidate ecologic variables (possibly altered by climate change or modified land-use patterns) that influence disease acquisition (3). We believe that increased awareness of pathogen range and risk can aid prompt future case diagnoses.

**Table.** Clinical characteristics of culture-confirmed outbreak cases from a large blastomycosis outbreak, Ontario, Canada, 2021

Characteristic	Culture-confirmed cases, no.
Patient age, y	
0–19	13
20–34	7
35–49	13
50–69	7
Patient sex	
M	22
F	18
Date collected	
2021 Nov	23
2021 Dec	12
2022 Jan–Aug	5
Specimen type	
Sputum	37
Other	3



**Figure.** Phylogenomic analysis of whole-genome single-nucleotide variants by neighbor-joining method of *Blastomyces gilchristii* isolates from a large blastomycosis outbreak, Ontario, Canada, 2021. Percentage of trees of 500 bootstrap replications in which the associated taxa clustered together is shown next to the nodes. The tree is drawn to scale, with branch lengths measured in number of substitutions per site (red text). There were 45,321 positions in the final dataset. Outbreak isolates are designated with black bars. Colors indicate geographic region in which the patient resided is as shown on map, including cases from Minnesota, USA; numbers indicate regions: (1) Northwest, (2) Thunder Bay District, (3) Porcupine, (4) Algoma, (5) Sudbury, (6) Ottawa, (7) Leeds/Grenville/Lanark. The geographic regions of residence for the travel cases were not available. SRA, National Center for Biotechnology Information Sequence Read Archive.

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We respectfully acknowledge the devastating impact this outbreak has had on the Constance Lake First Nation community and beyond. We are grateful for the permission granted by the community to share these findings to enhance our collective understanding of blastomycosis to help mitigate illness. We also acknowledge the support from Public Health Ontario staff, Indigenous Services Canada, and Porcupine Health Unit, especially Jo Ann Majerovich and Lianne Catton.

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## Serosurvey of Chikungunya Virus in Old World Fruit Bats, Senegal, 2020–2022

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We conducted a cross-sectional serosurvey for chikungunya virus (CHIKV) exposure in fruit bats in Senegal during 2020–2023. We found that 13.3% (89/671) of bats had CHIKV IgG; highest prevalence was in *Eidolon helvum* (18.3%, 15/82) and *Epomophorus gambianus* (13.7%, 63/461) bats. Our results suggest these bats are naturally exposed to CHIKV.

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that has caused >10 million cases in >125 countries and territories in the past 2 decades. Chikungunya disease is characterized by acute and chronic signs and symptoms in humans and can sometimes lead to neurologic complications and fatal outcomes (1). CHIKV is transmitted among humans mainly by *Aedes aegypti* and *Ae. albopictus* mosquitoes

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# Genomic Epidemiology of Large Blastomycosis Outbreak, Ontario, Canada, 2021

## Appendix

**Appendix Table.** Description of study isolates and BioProject PRJNA890593 sequence read accession (SRA) numbers from an analysis of genomic epidemiology of large blastomycosis outbreak, Ontario, Canada, 2021\*

Sample no. (Travel case)	SRA no.	Outbreak isolate	Duplicate†	Collection date	Specimen type	Geographic region (Ontario Public Health unit)
21X008	SRR26554818	yes	na	2021-Nov	sputum	Porcupine
21X012	SRR26554788	yes	na	2021-Dec	sputum	Porcupine
21X030	SRR26554824	yes	na	2021-Nov	sputum	Porcupine
21X039 (Travel A)	SRR26554808	yes	na	2021-Nov	sputum	not available
21X054	SRR26554813	yes	na	2021-Nov	sputum	Porcupine
21X057	SRR26554792	yes	na	2021-Nov	sputum	Porcupine
21X063	SRR26554817	yes	na	2021-Nov	sputum	Porcupine
21X118	SRR26554816	yes	na	2021-Nov	sputum	Porcupine
21X121	SRR26554815	yes	na	2021-Nov	sputum	Porcupine
21X123	SRR26554811	yes	na	2021-Nov	sputum	Porcupine
21X315	SRR26554786	yes	na	2021-Dec	sputum	Porcupine
21X460	SRR26554821	yes	na	2021-Dec	respiratory specimen	Porcupine
21X587	SRR26554806	yes	na	2021-Dec	respiratory specimen	Porcupine
21X590	SRR26554787	yes	na	2021-Dec	sputum	Porcupine
21X701 (Travel B)	SRR26554791	yes	na	2021-Dec	sputum	not available
21X758	SRR26554805	yes	na	2021-Dec	sputum	Porcupine
21X848	SRR26554807	yes	na	2021-Dec	sputum	Porcupine
21X866	SRR26554796	yes	na	2021-Nov	sputum	Porcupine
21X867	SRR26554781	yes	na	2021-Nov	sputum	Porcupine
21X869	SRR26554801	yes	na	2021-Nov	sputum	Porcupine
21X871	SRR26554795	yes	na	2021-Nov	sputum	Porcupine
21X883	SRR26554825	yes	na	2021-Nov	sputum	Porcupine
21X914	SRR26554793	yes	na	2021-Nov	sputum	Porcupine
21X923	SRR26554823	yes	na	2021-Nov	sputum	Porcupine
21X930	SRR26554820	yes	na	2021-Nov	sputum	Porcupine
22X011	SRR26554780	yes	na	2022	other	Porcupine
22X115 (Travel C)	SRR26554782	yes	na	2022	sputum	not available
22X253	SRR26554779	yes	na	2022	sputum	Porcupine
22X720	SRR26554778	yes	na	2022	sputum	Porcupine
21X565	SRR26554775	yes	Patient A <sup>1</sup>	2021-Nov	respiratory specimen	Porcupine
21X870	SRR26554797	yes	Patient A	2021-Nov	sputum	Porcupine
21X127	SRR26554809	yes	Patient B	2021-Nov	sputum	Porcupine
21X264	SRR26554776	yes	Patient B	2021-Dec	sputum	Porcupine
21X955	SRR26554798	yes	Patient C	2021-Dec	sputum	Porcupine
21X998	SRR26554819	yes	Patient C	2021-Nov	sputum	Porcupine
21X288	SRR26554794	yes	Patient D	2021-Dec	sputum	Porcupine
21X776	SRR26554803	yes	Patient D	2021-Dec	sputum	Porcupine
21X7289	SRR26554774	yes	Patient E	2021-Dec	sputum	Porcupine
21X933	SRR26554822	yes	Patient E	2021-Nov	sputum	Porcupine
21X258	SRR26554804	yes	Patient F	2021-Dec	sputum	Porcupine
21X760	SRR26554773	yes	Patient F	2021-Dec	sputum	Porcupine
21X917	SRR26554799	yes	Patient G	2021-Dec	sputum	Porcupine

Sample no. (Travel case)	SRA no.	Outbreak isolate	Duplicate†	Collection date	Specimen type	Geographic region (Ontario Public Health unit)
21X942	SRR26554789	yes	Patient G	2021-Dec	sputum	Porcupine
21X290	SRR26554790	yes	Patient H	2021-Dec	sputum	Porcupine
21X849	SRR26554800	yes	Patient H	2021-Dec	sputum	Porcupine
21X840	SRR26554802	yes	Patient I	2022	sputum	Porcupine
22X096	SRR26554777	yes	Patient I	2022	sputum	Porcupine
21X125	SRR26554810	yes	TechRep1	2021-Nov	sputum	Porcupine
21X125B	SRR26554814	yes	TechRep1	2021-Nov	sputum	Porcupine
21X125C	SRR26554812	yes	TechRep1	2021-Nov	sputum	Porcupine
21X350	SRR26554783	yes	TechRep2	2021-Dec	sputum	Porcupine
21X350B	SRR26554785	yes	TechRep2	2021-Dec	sputum	Porcupine
21X350C	SRR26554784	yes	TechRep2	2021-Dec	sputum	Porcupine
19X156	SRR21903574	no	na	2019-Mar	sputum	Porcupine
19X159	SRR21903581	no	na	2019-Jul	BAL	Ottawa
19X280	SRR21903589	no	na	2019-Jul	sputum	Sudbury
19X301	SRR21903590	no	na	2019-Nov	sputum	Sudbury
19X542	SRR21903588	no	na	2019-Jul	bronchial washing fluid	Porcupine
19X611	SRR21903591	no	na	2019-Jul	BAL	Leeds, Grenville, Lanark
20X036	SRR21903586	no	na	2020-Nov	sputum	Northwest
20X504	SRR21903587	no	na	2020-Aug	sputum	Algoma
20X548	SRR21903584	no	na	2020-Nov	sputum	Sudbury
20X814	SRR21903582	no	na	2020-Aug	BAL	Sudbury
20X822	SRR21903585	no	na	2020-Sep	sputum	Northwest
21X285	SRR21903577	no	na	2021-Oct	bronchial washing fluid	Sudbury
21X289	SRR21903573	no	na	2021-Aug	ankle aspirate	Sudbury
21X331	SRR21903580	no	na	2021-Oct	sputum	Sudbury
21X378	SRR21903576	no	na	2021-Nov	BAL	Algoma
21X597	SRR21903578	no	na	2021-Jul	sputum	Northwest
21X805	SRR21903575	no	na	2021-Jan	sputum	Porcupine
21X982	SRR21903579	no	na	2021-Jan	sputum	Algoma
22X325	SRR25928120	no	na	2022-Jul	BAL	Sudbury
22X343	SRR25928119	no	na	2022-Apr	bronchial washing fluid	Thunder Bay District
22X864	SRR25928118	no	na	2022-Apr	BAL	Porcupine

\*Hosts in all instances were homo sapiens. BAL, bronchoalveolar lavage fluid; na, not applicable.

†Patient identifiers were randomly assigned