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Temperate Climate Niche for *Cryptococcus gattii* in Northern Europe

To the Editor: *Cryptococcus gattii* was considered to be geographically restricted to countries with tropical and subtropical climates until 1999, when an outbreak of cryptococcosis in humans and animals occurred in the temperate climate of Vancouver Island, British Columbia, Canada (1). Montagna et al. reported the first environmental *C. gattii* in Europe from the Mediterranean region of Italy; these authors isolated it from 11 (4.3%) of 255 samples of plant detritus of *Eucalyptus camaldulensis* trees collected from the residential locality of an autochthonous case of cryptococcal meningitis caused by *C. gattii* in Apulia (2). These observations were recently substantiated by the isolation of *C. gattii* from plant debris of trees belonging to *Ceratonia siliqua* (carob), *Pinus halepensis* (stone pine), and *E. camaldulensis* in Spain (3). We report environmental isolation of the primary pathogenic fungus *C. gattii* from a forest in Berg en Dal, the Netherlands, which extends its geographic distribution to the temperate climate of northern Europe.

We investigated 112 decayed wood samples collected from inside trunk hollows of 52 living trees belonging to 5 families during April–May 2011 in Nijmegen, the Netherlands. The trees sampled were chestnut (*Castanea sativa*, n = 24), Douglas fir (*Pseudotsuga menziesii*, n = 17), oak (*Quercus macranthera*, n = 6), walnut (*Juglans regia*, n = 3), and mulberry (*Morus alba*, n = 2). The main criterion in selecting a tree for sampling was advanced age and presence of large trunk hollows variably sheltered from sunlight. The sampled sites had no bird nests and were apparently free from avian excreta. The decayed wood samples were collected with an in-house swabbing technique by using simplified Staib niger seed agar as described (4). The plates were incubated at 30°C and periodically observed up to 7 days for isolation of *C. gattii* and *C. neoformans*. Suspected colonies of *Cryptococcus* spp. were purified by dilution plating and identified by their morphologic and biochemical profiles, including development of blue color on L-canavanine-glycine bromothymol blue medium.

Identity of the isolates was confirmed by sequencing the internal transcribed spacer and D1/D2 regions, and they were genotyped by using amplified fragment-length polymorphism (AFLP) fingerprinting and multilocus sequence typing (MLST). The MLST loci *CAP10*, *CAP59*, *GPD1*, *IGS*, *LAC1*, *MPD1*, *PLB1*, *SOD1*, *TEF1a*, and *URA5* of the environmental *C. gattii* isolates were amplified and sequenced, and data were compared with MLST data from a large *C. gattii* population study (5) and with a recently published set of clinical, animal, and environmental *C. gattii* isolates from Mediterranean Europe and the Netherlands (Figure) (3,6,7). In addition, the mating type was determined with PCR by using mating type-specific primers for the *STE12a* and α alleles (8).

Four strains of *C. neoformans* species complex were isolated from the 112 decayed wood samples examined from 52 trees. One strain that originated from an oak tree (*Q. macranthera*), was identified as *C. neoformans* var. *grubii*. The remaining 3 strains, all originating from different hollows in a Douglas fir tree, were identified as *C. gattii* genotype AFLP4/VGI and mating type α . The strains were deposited at the CBS-KNAW (Centraalbureau voor Schimmelcultures/Royal Netherlands Academy of Arts and Sciences) Fungal Biodiversity Centre (accession nos.

CBS12349, CBS12355, and 12356), and the sequences were deposited in GenBank (accession nos. JN982044–JN982073).

MLST analysis showed that the *C. gattii* isolates in our study are more closely related to the clinical isolate from the Netherlands (6) and to the clinical and environmental *C. gattii* isolates (AFLP4/VGI) reported from the Netherlands and other countries in Europe than to isolates from outside Europe (3,7,8). The autochthonous *C. gattii* AFLP4/VGI isolate, CBS2502 (earlier identified as *C. neoformans*) isolate from the Netherlands was

recovered postmortem in 1957 from the lungs of a pregnant woman with cryptococcosis (6). This patient came from a low socioeconomic strata, was unlikely to have traveled outside the Netherlands, and probably acquired the infection indigenously from an environmental source (6).

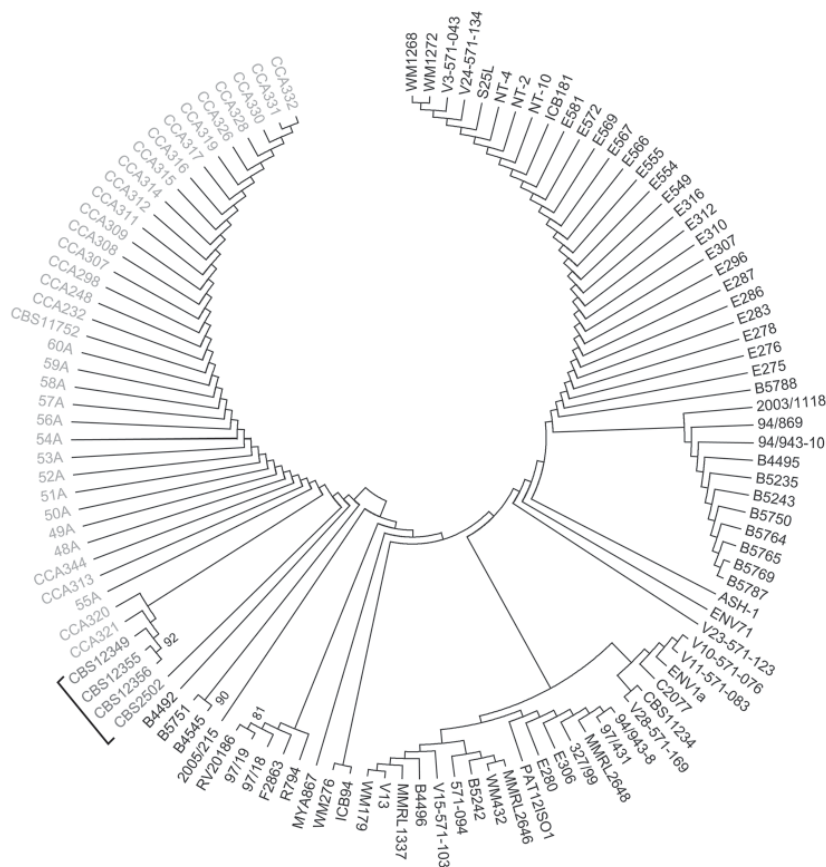
Furthermore, genotype AFLP4/VGI appears to be the genotype of *C. gattii* prevalent in Europe (3,7,8). Outside Europe, *C. gattii* has been reported from Douglas fir trees in Vancouver Island, British Columbia, Canada; however, those isolates represented another molecular type, i.e., AFLP6/VGII (9). Genotype AFLP4/VGI *C. gattii* isolates have been implicated in human infections in that region, but to our knowledge, no environmental isolates have been found until now.

Our detection of *C. gattii* in the environment and its previous isolation from a clinical case in the Netherlands suggests that this pathogen is endemic to the temperate climate of northern Europe. This suggestion agrees with the concept emerging from a decade of investigations in Canada and the Pacific Northwest that the geographic distribution of *C. gattii* extends to the temperate region, albeit with another AFLP genotype (1,9,10). Further environmental studies are likely to show a wider spectrum of host trees and higher environmental prevalence of *C. gattii* in this continent than what appears in the literature.

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Figure. Unrooted bootstrap maximum-likelihood phylogenetic multilocus sequence typing analysis of *Cryptococcus gattii* genotype AFLP4/VGI isolates based on 7 unlinked nuclear loci (5). Bracket indicates the 3 *C. gattii* isolates from the Netherlands cultured from Douglas fir (*Pseudotsuga menziesii*) (CBS12349, CBS12355, CBS12356) and 1 clinical isolate from 1957 from the Netherlands (CBS2502) (6). Isolates in gray to the left of the bracket are the previously observed European Mediterranean cluster, with clinical, animal and environmental isolates (3). All other *C. gattii* AFLP4/VGI isolates originate mainly from Australia, Africa, and South America, as described (5). The isolates from the Netherlands are closely related to isolates that originated from the Mediterranean region. Numbers next to branches show bootstrap support (≥ 80). A color version of this figure is available online (wwwnc.cdc.gov/EID/article/18/1/11-1190-F1.htm).



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Pulmonary Infection Caused by *Mycobacterium conceptionense*

To the Editor: *Mycobacterium conceptionense* was first identified in 2006 from a patient with posttraumatic osteitis (1). Since then, 3 more isolates have been recovered from a subcutaneous abscess (2), a wound after breast surgery (3), and an abscess after a fat injection (4). During November 2009 through April 2010, *M. conceptionense* was isolated from sputum from 4 patients in 2 tertiary hospitals in South Korea.

Patient 1, a 69-year-old woman, was admitted to Seoul National University Bundang Hospital in 2005 with fever and pleuritic chest pain. She had a long history of recurrent fever and cough. Computed tomography (CT) showed multifocal nodular lung lesions with lymphadenopathy. After 7 days of treatment with cefuroxime and azithromycin, the patient's fever subsided and radiographic lesions disappeared. She was discharged

with negative culture results. After discharge, she had recurrent episodes of fever, and CT showed waxing and waning pulmonary lesions. Nontuberculous mycobacteria (NTM) species were isolated from some sputum cultures: *M. smegmatis* in 2006; *M. avium* in 2007; and *M. intracellulare* in 2008 and 2009. In February and April 2010, her respiratory symptoms and chest CT findings indicated more severe disease, and *M. conceptionense* grew in sputum cultures. After treatment with clarithromycin, rifampin, and ethambutol for 2 months, the patient's symptoms improved and sputum culture results were negative.

Patient 2, a 70-year-old man with Parkinson disease, was referred to Seoul National University Bundang Hospital in November 2009 for a small nodular lung lesion detected by CT during a medical checkup. He exhibited no pulmonary symptoms. Routine laboratory test results were within normal limits. *M. conceptionense* was isolated from sputum. Clarithromycin was prescribed for 10 days, and the patient remains asymptomatic.

Patient 3, a 70-year-old man with tongue cancer, was admitted to Seoul National University Hospital in March 2010 with exacerbated dyspnea. In November 2009, CT had indicated new nodular lung lesions and chemotherapy had been started. Chest CT in 2010 showed increased size and extent of nodular infiltration, which suggested pulmonary infection rather than cancer metastasis. From 2 sputum samples, 2 isolates of *M. conceptionense* were identified. In addition, *Streptococcus pneumoniae* grew in blood and sputum cultures. Despite treatment with broad-spectrum antimicrobial drugs, the patient died of respiratory failure.

Patient 4, a 53-year-old man, sought care at Seoul National University Hospital in 2008 for chest discomfort. Other than having diabetes mellitus, he had been healthy. Chest