



Review article

# Searching for clues for eighteen years: Deciphering the ecological determinants of *Cryptococcus gattii* on Vancouver Island, British Columbia

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## Abstract

*Cryptococcus gattii* emerged on Vancouver Island in 1999 for unknown reasons, causing human and animal fatalities and illness. The apparent emergence of this fungus in another temperate area, this time in the Pacific Northwest, suggests the fungus may have expanded its ecological niche. Yet studies that directly examine the potential roles of climatic and land use changes on *C. gattii* are still lacking. We aim to summarize the existing global literature on the ecology of *C. gattii*, with particular focus on the gap in knowledge surrounding the potential effects of climatic and land use changes. We systematically reviewed English peer-reviewed literature on the ecological determinants of *C. gattii*. We included studies published from January 1970 through June 2016 and identified 56 relevant studies for our review. We identified environmental isolations of *C. gattii* from 18 countries, spanning 72 separate regions across six continents. Fifty-three tree species were associated with *C. gattii*, spanning 10 climate classifications and 36 terrestrial ecoregions. No studies directly tested the potential effects of climatic changes (including climatic oscillations and global climate change) on *C. gattii*, while only one study directly assessed those of land use change. To improve model predictions of current and future distributions of *C. gattii*, more focus is needed on the potential effects of climatic and land use changes to help decrease the public health risk. The apparent emergence of *C. gattii* in British Columbia is also an opportunity to explore the factors behind emerging infectious diseases in Canada and elsewhere.

**Key words:** climate change, *Cryptococcus gattii*, human fungal pathogen, land use change, molecular type.

## Introduction

Over recent years, North America has witnessed the emergence or re-emergence of several traditionally tropical and subtropical diseases, such as the introduction of West Nile virus in New York in 1999<sup>1</sup> and the first autochthonous transmission of chikungunya virus in Florida in 2014.<sup>2</sup> It is also experiencing the spread of other diseases, such as Lyme disease in southern Ontario.<sup>3</sup> Accelerating climatic and land use changes are likely key factors driving these trends, though elucidating their specific roles from the web of other interacting drivers (e.g., global trade, migration, and travel) is daunting. Despite this complexity, the task of determining the ecological drivers of infectious organism emergence not only remains but is also becoming increasingly critical with accelerating climate change. Another complication is whether an emergence is a true emergence (i.e., the organism has never before been present in the environment it has been discovered in) or a new recognition (i.e., the organism existed in the environment previously and has only now been detected).

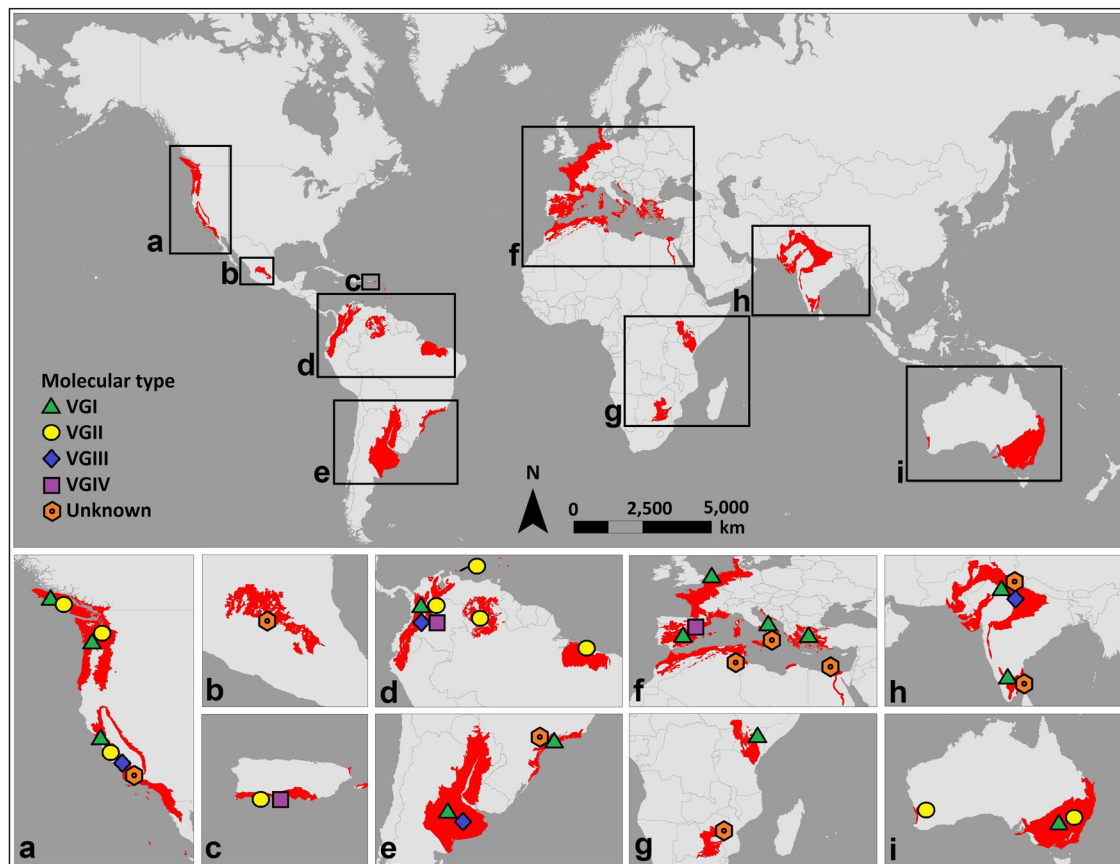
Cryptococcosis is a potentially fatal respiratory and neurological disease caused by inhalation of *Cryptococcus* fungal spores<sup>4</sup> and causes nearly 625,000 deaths globally each year.<sup>5</sup> Cryptococcosis caused by *C. gattii* was considered predominantly a tropical and subtropical disease until its discovery on Vancouver Island, British Columbia, in 1999,<sup>6</sup> despite clinical, veterinary, and environmental isolations in temperate zones prior to 1999.<sup>7–9</sup> Clinical isolation of *C. gattii* was rare in North America before the Vancouver Island outbreak, with one of the earliest known cases coming from a patient in West Virginia in 1924 (this isolate was only identified as *C. gattii* VGI decades later).<sup>10</sup> Retrospective analyses of clinical records showed that no clinical cases of *C. gattii* infections existed in Canada prior to 1999.<sup>6</sup> Whether environmental triggers helped *C. gattii* emerge for the first time or become detectable to humans after years or decades of existence on Vancouver Island in 1999 is unknown and needs to be explored. *C. gattii* is a facultative parasite; it can remain free-living on trees and in soil but will take advantage of the opportunity of infecting a mammal host, including humans.<sup>11</sup> Despite possible tropical and subtropical origins, now suggested to be in South America or Africa,<sup>12,13</sup> *C. gattii* has been associated with a variety of native Canadian trees, potentially facilitating its establishment and proliferation in the western Canadian temperate environment.

The *Cryptococcus* species complex is currently comprised of two species: *Cryptococcus neoformans* with serotypes A, D, and AD and *Cryptococcus gattii* with serotypes B and C.<sup>14</sup> Both species have two mating types: MAT $\alpha$  and MAT $\alpha$ , where the latter is more frequently

found in clinical and environmental isolates.<sup>15,16</sup> Molecular typing techniques of subgroups within the *C. gattii* species have evolved through the decades, with the application of many techniques including DNA<sup>17</sup> and polymerase chain reaction (PCR) fingerprinting,<sup>18</sup> random amplification of polymorphic DNA,<sup>19</sup> restriction fragment length polymorphism (RFLP),<sup>20</sup> amplified fragment length polymorphism (AFLP),<sup>21</sup> and multilocus sequence typing (MLST),<sup>22</sup> among many others. The multitude of molecular typing methods at the global scale created the need to standardize molecular nomenclature for the *C. neoformans* and *C. gattii* species.

A global standardized approach is crucial for comparison of results in different countries and across years, though this has not been without its challenges. Three of the molecular typing techniques produced the most comparable results when subtyping the *C. neoformans* and *C. gattii* species: PCR fingerprinting, AFLP, and MLST.<sup>16</sup> The first, coupled with RFLP of the *URA5* gene, differentiated *C. gattii* into four molecular types: VGI, VGII, VGIII, and VGIV.<sup>20</sup> The high discriminatory power of the AFLP typing method then led to the differentiation of AFLP4, AFLP5, AFLP6, AFLP7, and AFLP10 strains within the *C. gattii* species (where the last two were variations of VGIV),<sup>21,23</sup> in addition to two AFLP6 subtypes (AFLP6A and AFLP6B) in the Vancouver Island *C. gattii* outbreak.<sup>24</sup> MLST analyses further confirmed the VGI-VGIV molecular types from the PCR fingerprinting methods as well as the Vancouver Island outbreak subtypes VGIIa and VGIIb.<sup>25</sup> At present, the *C. gattii* complex is categorized into five molecular types (AFLP4/VGI, AFLP5/VGIII, AFLP6/VGII, AFLP7/VGIV, and AFLP10/VGIV)<sup>26</sup>, now argued to be five separate species called *C. gattii sensu stricto*, *C. bacilliformis*, *C. deuterogattii*, *C. tetragattii*, and *C. decagattii*, respectively.<sup>27</sup> Since the VGI-VGIV nomenclature was most prominent in our reviewed literature, with many studies published before the differentiation of VGIV into AFLP7 and AFLP10, we will hereafter use the VGI-VGIV nomenclature for this review.

The ongoing outbreak of *C. gattii* infections in the Pacific Northwest is attributed predominantly to VGIIa and to a lesser extent to VGIIb and VGI.<sup>24,28</sup> Another highly virulent subtype, VGIIc, is currently restricted to Oregon.<sup>29</sup> VGIII also appears to be expanding in the western United States and differs from the Vancouver Island outbreak (VIO) VGII strains by causing infections in immunocompromised individuals in Southern California and the southwestern United States.<sup>30</sup> Of all four molecular types, VGII has been reported as the most virulent and fertile in immune cell and whole animal experiments, demonstrating particularly high virulence in the Pacific Northwest.<sup>24,31</sup> Each molecular type also has a slightly different geographical distribution. VGI



**Figure 1.** Worldwide ecoregions from which *C. gattii* has been isolated from the environment. Ecoregions (in red) where environmental isolations of *Cryptococcus gattii* have been made worldwide, along with insets detailing (a) the Pacific Northwest, (b) Mexico, (c) Puerto Rico, (d) northern South America, (e) southeastern South America, (f) western Europe and the northern coast of Africa, (g) eastern Africa, (h) India, and (i) Australia, based on the terrestrial regions specified by Olson et al.<sup>46</sup> The molecular type(s) found in each ecoregion is/are superimposed. It is important to note that the ecoregions highlighted in red represent entire ecoregions within which *C. gattii* was isolated, and not necessarily the full extent of where *C. gattii* was found. Only areas that were specified in the review papers as environmental isolation areas of *C. gattii* were mapped. This excludes areas where only clinical and/or veterinary isolations have been made, as well as areas where only the country of environmental isolation was specified, and is likely an underrepresentation of the full extent of *C. gattii* in the global environment. This Figure is reproduced in color in the online version of *Medical Mycology*.

and VGII have widespread distributions worldwide, while VGIII and VGIV are more localised and less common.<sup>16,32</sup>

Vancouver Island has one of the highest annual incidences of *Cryptococcus gattii* infections among humans in the world.<sup>33</sup> From 1999 to 2015, 393 cases were reported in British Columbia (Source: BCCDC, 2016). As of 2004, *C. gattii* was discovered in the Pacific Northwestern United States, including Oregon and Washington, at least eight non-Pacific Northwest states, including California<sup>34</sup> and, most recently, in Nova Scotia, Canada.<sup>35</sup> Predictions of the full environmental extent of *C. gattii* are likely underestimates due to the lack of successful environmental isolations (see Fig. 1). For example, *C. gattii* VGIIa (identical to the Vancouver Island outbreak strain R265) was isolated from a Japanese patient in 2007 with no recent history of travel to disease-endemic areas.<sup>36</sup> To our knowledge, environmental isolations of *C. gattii* in Japan are still lacking but the lo-

cal acquisition of infection indicates more environmental sampling needs to be done. If Vancouver Island witnessed a new emergence of *C. gattii* in 1999, then its emergence in this temperate area and others in the Pacific Northwest suggests the fungus may have expanded its ecological niche. Yet, studies that directly examine the potential effects of climatic and land use changes on *C. gattii* are lacking. This gap in the literature exists despite the possibilities of importation, warming of ambient air, and deforestation playing roles in *C. gattii*'s Vancouver Island emergence.<sup>6,37</sup> Warmer temperatures can increase a plant's susceptibility to fungal colonization,<sup>38</sup> further supporting the need to study the potential effects of climatic changes. Aerosolization of *C. gattii* spores is aided by felling of colonized trees,<sup>37</sup> possibly explaining why *C. gattii* emerged on Vancouver Island in an area of expanding neighbourhoods and following construction of a new highway through forested areas in

the mid-1990s.<sup>6</sup> This further supports the need to study the possible effects of land use changes on *C. gattii* emergence and spread.

A previous review of the global distribution of *C. gattii*<sup>32</sup> found published work on clinical and veterinary isolations of the fungus nearly always outnumbered that for environmental isolations (see Springer and Chaturvedi<sup>32</sup>, Table 1). However, the movement of individuals who become infected and the lengthy incubation period of *C. gattii* currently estimated to last up to 3 years<sup>39</sup> complicates determining the actual location of exposure. There have also been tourists who contracted *C. gattii* after visiting Vancouver Island and were later diagnosed in their respective home countries.<sup>40–43</sup> For these reasons, this article focuses solely on environmental isolations, or isolations taken directly from an environmental source, of *C. gattii*. Through this review, we aim to summarize the existing global literature on the ecology of *Cryptococcus gattii*, with particular focus on the potential effects of climatic and land use changes.

## Methods

We performed systematic searches in three databases for peer-reviewed English journal literature on the ecology of *Cryptococcus gattii*: Google Scholar (<http://scholar.google.com>), ScienceDirect (<http://www.science-direct.com/>), and Web of Science (<http://thomsonreuters.com/thomson-reuters-web-of-science>). We included studies published from January 1970 up to and including June 2016. Each search varied based on the search engine input options but included the following three sets of inputs for topics: the first inputs for the species was “either/or” for “*Cryptococcus gattii*,” “*Cryptococcus neoformans* var. *gattii*,” or “*Cryptococcus bacillisporus*” (the last being synonymous with *C. gattii* in the late 1970s and early 1980s)<sup>44</sup>; the second inputs for the ecological or geographical study of *C. gattii* was “either/or” for “ecolog\*,” “geograph\*,” “distribution\*,” “ecological niche,” or “niche”; the third inputs for the effects of climate and land use changes was “either/or” for “climate,” “climate change,” “climat\* oscillation,” “land use,” “land use change,” or “environmental change\*.” The initial searches yielded a total of 402 papers. Studies were included if they successfully sampled for *C. gattii* in the environment. We therefore excluded any clinical studies where the fungus was isolated from a human or animal only. We also only included studies where ecological determinants of *C. gattii* were tested for. Based on these criteria, our search yielded 56 publications for review.

We identified climate characteristics where *C. gattii* was environmentally isolated using the Köppen-Geiger climate

classification scheme,<sup>45</sup> the most frequently used climate classification system. We used the most recent map based on observed climate characteristics for 1976–2000 (<http://koeppen-geiger.vu-wien.ac.at/shifts.htm>). We also identified the corresponding terrestrial ecoregions of the world where these samples were taken, based on the ecoregions classification map developed by Olson et al.<sup>46</sup> (Table S1) (<https://databasin.org/datasets/68635d7c77f1475f9b6c1d1dbe0a4c4c>). We overlaid each map over the mapped *C. gattii* sample regions in ArcMap v.10.1 (ESRI 2012, Redlands, CA, USA). Coordinates for the sample regions were taken based on the centroid of the city, town, or region where sampling occurred, unless exact coordinates were specified by the study.

## The geographical distribution of *C. gattii* worldwide

Retrospective analyses currently suggest that the earliest documented human case of *C. gattii* infection may have been from a patient in Europe in 1895.<sup>10,47</sup> However, *C. gattii* was first recognized as a variety of *C. neoformans* after it was isolated from a 7-year-old Congolese Bantu boy in 1970,<sup>48</sup> differentiated by its elongated, cigar-shaped form that was atypical of the round shape characteristic of *C. neoformans*.<sup>49</sup> Following the 1970 differentiation of *C. gattii* from *C. neoformans*, reports of *C. gattii* infections predominantly originated in tropical and subtropical regions, including parts of Africa, South America, Asia, and Australia. This led to the long-standing hypothesis that *C. gattii* is a tropical and subtropical pathogen,<sup>50,51</sup> despite isolations also being made in temperate areas. These included multiple *C. gattii* outbreaks in goats in Spain between 1990 and 1994,<sup>7</sup> as well as outbreaks in sheep and horses in southwestern Australia in the early 1990s.<sup>8</sup> The 1999 Vancouver Island outbreak of *C. gattii* renewed investigations into the ecological niche of the fungus, dismantling the hypothesis that the fungus was mainly restricted to tropical and subtropical regions.<sup>24</sup> Soon, clinical and/or veterinary isolations of *C. gattii* were made in temperate regions of the United States, Europe, and Asia<sup>31,52,53</sup> as well as Eastern Canada.<sup>35</sup> Human, veterinary, and environmental isolations of *C. gattii* are now widespread, found on every continent except Antarctica.

## Environmental isolations

Based on the present review of the peer-reviewed literature from 1970 to 2016, 56 publications identified isolations of *C. gattii* from the environment in 18 different countries, spanning 72 separate regions and six continents

**Table 1.** Overview of positive global environmental isolations of *Cryptococcus gattii*.

Country	Reference	Region	Molecular type	Mating type	Source	Collection year(s)	
Argentina	(55)	Buenos Aires City	...	...	Tree hollows	2001	
	(60)	Republica de Chile Park, de los Patricios Park, Centenario Park, and N. Avellaneda Park, Buenos Aires City	VGI	...	Tree hollows	2002	
	(59)	Resistencia, Chaco	VGHII	...	...	Tree hollows	2006–2007
		Rosario, Santa Fe		VGI	...	Tree hollows	2006–2007
		Parque España, La Paz, Entre Rios		VGI	...	Tree hollows	2006–2007
Australia	(77)	Barossa Valley (Barossa Reservoir and Nuriootpa), SA <sup>a</sup>	...	...	Bark, woody and leaf debris	1989	
		Balranald, NSW	...	...	Bark, woody debris	1989	
		Hay, NSW	...	...	Bark, woody debris	1989	
		Currumbin, Gold Coast, QLD	...	...	Bark and woody debris in tree hollows	1991	
	(9)	Mt. Annan, Greater Sydney, NSW	...	...	Plant debris	1991	
		Balranald, NSW	VGI	...	Bark, fruit, soil, woody debris	1989–1990	
		Tocumwal, NSW	VGI	...	Woody debris	1991	
		Mt. Annan, Greater Sydney, NSW	VGI	...	Woody debris	1991–1994	
		Currumbin, Gold Coast, QLD	VGI	...	Woody debris	1991–1993	
		Barossa Valley, SA	VGI	...	Air, bark, fruit, soil, woody and leaf debris	1989–1992	
Botswana	(79)	Busselton, WA	VGHII	...	Plant debris	1993	
		Balranald, NSW	VGI	$\alpha$ , a	Bark, soil, woody debris	1989–1996	
		Hay, NSW	VGI	$\alpha$	Soil, woody debris	1989–1990	
		Adelaide, SA	VGI	$\alpha$	Woody debris	1996	
		Gold Coast, QLD	VGI	$\alpha$	Woody debris	1996	
	(61)	Renmark, SA	VGI	$\alpha$ , a	Woody debris	1998	
		St. Ives, Sydney, NSW	VGI	$\alpha$	Tree detritus, woody debris	1997–1998	
		Port Macquarie, NSW	VGI	$\alpha$	Woody debris	1998	
		Pilliga, NSW	VGI	$\alpha$	Leaf debris	1998	
		Breza, NSW	VGI	$\alpha$	Leaf debris	1998	
	(100)	Coffs Harbour, NSW	...	$\alpha$	Branches, leaves, wood	1997–2000	
		Port Macquarie, NSW	...	$\alpha$	Branches, leaves, wood	1997–2000	
		Sydney, NSW	...	$\alpha$ , a	Branches, leaves, wood	1997–2000	
		Glenbrook, Blue Mountains National Park, Sydney	VGI	$\alpha$	Tree hollows	2000	
		Mt. Druitt, Sydney, NSW	VGI, VGHII	$\alpha$	Insect frass on bark of tree	<2003	
(62)	Francistown	...	...	Bark, tree hollows, soil	2012		

Table 1. (Continued).

Country	Reference	Region	Molecular type	Mating type	Source	Collection year(s)
Brazil	(101)	Rio de Janeiro	VGI	...	Bat guano	<1993
	(58)	Teresina	VGII	$\alpha$	Tree hollows	1993
	(57)	Teresina	VGII	$\alpha$	Tree hollows	1993–1997
	(82)	Ibirapuera Park, Sao Paulo	...	...	Bark, flowers, fruits, leaves, plant detritus, soil	1996–1997
	(56)	Ilha de Maracá	VGII	...	Tree hollows	1998
	(54)	Belém, Pará	VGII	...	Tree hollows	<2009
	(72)	Botafogo district, Rio de Janeiro	VGI	...	Tree hollows	2008–2010
	(99)	Santa Isabel do Rio Negro, Amazonas state	VGII	$\alpha, \alpha$	Dust from wooden houses	<2015
	(24)	Rathrevor Beach Provincial Park (Parksville), MacMillan Park (Cathedral Grove), Vancouver Island	VGII	$\alpha$	Air, bark, tree hollows, soil	2001–2002
Canada	(74)	Victoria, Duncan, Nanaimo, Parksville, Courtenay, Cameron Lake, and Campbell River, Vancouver Island	VGI, VGII	...	Air, bark, leaf debris, living trees, soil	2001–2003
	(37)	Vancouver Island	VGI, VGII	$\alpha$	Air, bark, footwear, leaves, soil, tree hollows, water, wheel wells of cars, woody debris	2003–2005
	(87)	Courtenay, Duncan, Nanaimo, Parksville, Port Alberni, Victoria, Vancouver Island	VGI, VGII	$\alpha$	Air, bark, tree hollows, leaves, soil, water, woody debris	2001–2006
Colombia	(31)	Mainland, British Columbia Gulf Islands	VGII VGI, VGII	... ...	Air Bark, soil, tree hollows, water, woody debris	2001–2006 2001–2005
	(75)	Mainland, British Columbia Vancouver Island	VGII VGII	... $\alpha$	Air Air, soil,	2001–2005 2001–2005
	(95)	Cúcuta	...	...	Air, bark, flowers, leaves, seeds, tree detritus	1997
	(71)	La Calera	...	<b>a</b>	Tree detritus	2003
	(85)	Bogota	...	...	Bark, soil, tree hollows	2003
		Bogota	VGII	<b>a</b>	Tree detritus, soil	2002–2003
		Cali	VGII	<b>a</b>	Tree detritus, soil	2002–2003
		Cúcuta	VGI, VGIII, VGIV	$\alpha$	Tree detritus, soil	2002–2003
		Cundinamarca	VGII	<b>a</b>	Tree detritus, soil	2002–2003
		Medellin	VGIV	$\alpha$	Tree detritus, soil	2002–2003
(93)	Bogota	VGII	<b>a</b>	Flowers and detritus	2007	
	Cúcuta	VGIII	<b>a</b>	Soil	2008–2009	
		Cúcuta	VGI	<b>a</b>		
		VGIII	$\alpha$			

Table 1. (Continued).

Country	Reference	Region	Molecular type	Mating type	Source	Collection year(s)
Egypt	(81)	Qatur and Tanta areas, Gharbia Governorate	...	...	Bark, flowers, living trees, soil, wood	1998
Greece	(73)	Athens Salamina Island	VGI	$\alpha$	Soil, tree hollows Tree hollows	2013
India	(92)	Ferozepur	...	...	Tree flowers	1995–1996
	(67)	Delhi/New Delhi (northwestern India)	...	...	Tree hollows	2000–2002
	(65)	Delhi (northwestern India)	...	...	Flowers	1999–2000
	(69)	Jabalpur City (Central India)	...	...	Tree hollows	2002–2004
	(53)	Delhi/New Delhi (northwestern India)	...	$\alpha$	Air, tree hollows	2000–2002
	(88)	Delhi, Union Territory; Trivuvannamalai, Tamil Nadu	VGI	$\alpha$	Soil, tree hollows	2004–2007
	(80)	Guindy National Park, Chennai, South India	...	...	Bark, debris of living trees	<2009
	(68)	Delhi (northwestern India)	VGI, VGIII	$\alpha$	Tree hollows	2002–2007
	(63)	Delhi (northwestern India)	VGI, VGIII	$\alpha$	Soil, tree hollows	2002–2007
Italy	(98)	Apulia	...	...	Debris of living trees, samples from animals, soil	1997
	(73)	Route Gallipoli–Collepasso Route Brindisi–Fasano	VGI	<b>a</b>	Tree hollows	2013
		Ragaina, Catania		<b>a</b>		
	(96)	Reggio Calabria (southern Italy)	VGI	$\alpha$	Debris of living trees and tree detritus	2009
Kenya	(66)	Nairobi	VGI	...	Bird nesting sites, tree hollows	2012–2013
Mexico	(90)	Mexico City	...	...	Debris of living trees, flowers, leaves, tree detritus	<1999
The Netherlands	(52)	Berg en Dal	VGI	<b>a</b>	Tree hollows	2011
Bonaire (Dutch Caribbean)	(70)	Lagun Goto Rincon village Hato village	VGII	$\alpha$	Tree hollows, woody debris	2013
Puerto Rico	(94)	Guanica Dry Forest, and western and southern regions	VGII, VGIV	$\alpha$	Soil, tree detritus, tree hollows	<2010

Table 1. (Continued).

Country	Reference	Region	Molecular type	Mating type	Source	Collection year(s)
Spain	(64)	Alicante	VGI	$\alpha$	Bark, tree hollows	<2012
		Barcelona	VGI	$\alpha$	Tree detritus, tree hollows	<2012
	(97)	El Perello, Tarragona	VGIV	$\alpha$	Woody debris	<2015
		Campello, Alicante	VGI	$\alpha$	Tree hollows	2014
	(73)	El Perello, Tarragona	VGIV	$\alpha$	Tree hollows	2013
	Mendivil, Navarra	VGI	$\alpha$	Bark	2014	
Tunisia	(84)	Sfax region (southern Tunisia)	...	$\alpha$	Bark, flowers, fruits, soil, woody debris	<2011
United States	(112)	Fort Point, San Francisco, California	...	...	...	1990
	(9)	San Francisco, California	VGII	...	Woody debris	<1996
		San Diego Zoo area, San Diego, California	VGIII, VGI	...	Woody debris	<1996
	(76)	California	...	...	Bark	<2000
	(31)	Northern Washington	VGII	...	Bark, fence post, soil, tree hollows, woody debris	2001–2005
	(87)	Lynden, Washington	VGII	...	Soil	2001–2006
(89)		Los Angeles, California	VGI	$\alpha$	Debris of living trees, soil	2011–2012
			VGIII	$\alpha, \alpha$	Debris of living trees, soil	2011–2012
Uruguay	(78)	Oregon	VGI, VGII	...	Bark, soil	2010–2011
	(102)	...	VGII	...	Wasp nest	<1993

Studies are organized by country, then by date of publication. Sections with “...” indicate that the information was not specified in the study. In studies where the years of collection were not specified, “<” is used to indicate the collection was made prior to the study’s publication year.

<sup>a</sup>NSW, New South Wales; QLD, Queensland; SA, South Australia; WA, Western Australia.



worldwide (Table 1). Isolations were most commonly made on or around various tree species, with positive samples most often taken by swabbing inside or sampling debris from hollows of living trees.<sup>24,37,54–73</sup> Other isolations were made through analyses of tree bark,<sup>9,24,31,37,62,64,71,73–84</sup> soil,<sup>9,24,31,37,62,63,71,73,74,78,79,82,85–89</sup> leaves,<sup>37,75,82,87,90,91</sup> flowers,<sup>65,81,84,90,92–94</sup> fruit,<sup>9,82,84,94</sup> plant detritus,<sup>64,75,82,85,90,93,95,96</sup> woody debris,<sup>9,31,37,77,79,80,83,84,87,89,90,96–98</sup> air,<sup>9,24,31,37,53,74,75,87</sup> and water.<sup>31,37,87</sup> *C. gattii* was also isolated from indoor dust in wooden houses,<sup>99</sup> wheel wells of cars,<sup>37</sup> insect and bat faeces,<sup>100,101</sup> animal enclosures,<sup>98</sup> bird nesting sites,<sup>66</sup> and wasp nests.<sup>102</sup> *C. gattii* has often been harder to isolate in the environment than *C. neoformans*, likely due to lower concentrations.<sup>84</sup> It is important to note that this review encompasses a variety of isolation methods from different environmental sources, making direct comparisons between studies more difficult.

Despite this collection of primary sources, the true global distribution of *C. gattii* is still likely underrepresented because environmental monitoring is often patchy.<sup>32</sup> Certain locations have, for instance, been excluded from this review due to clinical isolations but not environmental ones. For example, *C. gattii* has been clinically isolated in eight non-Pacific Northwest states since 2009, yet tests for *C. gattii* in the natural environment of these areas have not yet been attempted.<sup>34</sup>

### Variations in distributions of molecular types

The geographical distribution of *C. gattii* continues to evolve and patterns in the locations of the different molecular types are emerging. Molecular type VGI appears relatively widespread. Environmental isolations of VGI were made on six continents, excluding Antarctica (Fig. 1; Table 1). VGII is still primarily found in more tropical and subtropical areas,<sup>16</sup> though it appears to have adapted to the more temperate climates of areas such as southern Australia,<sup>9</sup> British Columbia, and parts of the Pacific Northwest.<sup>24</sup> Environmental isolations of molecular type VGIII are relatively less common, with positive samples in Argentina,<sup>59</sup> Colombia,<sup>85,86</sup> India,<sup>68</sup> and the United States<sup>9</sup> (Table 1). Molecular type VGIV shows the fewest positive environmental isolations worldwide, so far located in Columbia,<sup>85</sup> Puerto Rico,<sup>94</sup> and Spain.<sup>97</sup> This molecular type has yet to be environmentally isolated from North America. Mating type  $\alpha$  is more common both clinically and environmentally than mating type  $a$ ,<sup>24</sup> though reasons for this are still unknown. The ecological reasons for the distributions of each molecular type, including the possible ecological niche of each molecular type, are also still unknown.

### *C. gattii* in British Columbia

The emergence of *C. gattii* on Vancouver Island in 1999 contributed to dismantling the long-held hypothesis that the species was mainly restricted to tropical and subtropical niches.<sup>24</sup> The fungus infected immunocompetent individuals, including local residents, visiting tourists, as well as wild and domestic animals.<sup>24</sup> The search for the ecological niche of *C. gattii* on Vancouver Island began in 2001.<sup>74,103</sup> Animal, human, and environmental cases were found to be clustered along the eastern side of Vancouver Island in the rain shadow.<sup>104,105</sup> This area is defined by flora and soil unique to the Coastal Douglas Fir and Western Hemlock biogeoclimatic zone.<sup>105</sup> In this zone, summers are dry and average 17.6°C, while winters are mild, average 2.7°C<sup>24</sup> and rarely go below freezing.<sup>104</sup>

Vancouver Island not only gained worldwide scientific attention for the unexpected emergence of *C. gattii* in a new temperate zone but also for one of the highest reported incidences of *C. gattii* infections in the world.<sup>106</sup> Nearly all *C. gattii* isolates from the VIO (>97%) fall under the VGII molecular type,<sup>24</sup> with VGI also being isolated.<sup>31,37,87</sup> Every study of Canadian isolates included in this review also isolated VGII (Table 1). Whether *C. gattii* was recently introduced to the Canadian environment or existed undetected for years is still unclear. However, the match between a clinically isolated 1970s Seattle strain and the 1999 Vancouver Island outbreak VGII strain may suggest the latter.<sup>107</sup>

### The ecology of *C. gattii*

The ecology of *C. gattii* remained unknown until its discovered association with *Eucalyptus* trees in Australia in 1990.<sup>77,108</sup> This led to a plethora of investigations worldwide into *C. gattii* ecology, with focus primarily on plant debris, including particular emphasis on the hollows of tree trunks.<sup>9,56,59</sup> Despite the observation of expanding geographic distributions of *C. gattii* in the Pacific Northwest and the urgent need to better understand *C. gattii* ecology in order to forecast its expansion and possible reasons for its emergence in temperate areas, there is still minimal research on the biophysical determinants that strongly influence *C. gattii* dynamics.<sup>104,109</sup>

#### Abiotic factors

##### Temperature

Most studies that directly test the temperature thresholds of *C. gattii* appear to focus on the Vancouver Island outbreak VGII strains. The *C. gattii* strains in Vancouver have been found to be sensitive to temperatures below freezing.<sup>104,105</sup>

but showed high survival rates in seawater at 4°C.<sup>87</sup> The VGIIa and VGIIb subtypes within the VGII molecular type involved in the VIO also have the ability to grow at 37°C, the core human body temperature.<sup>110</sup> Their ability to infect a variety of mammals further suggests their tolerance exceeds 37°C, given that many mammals have body temperatures several degrees higher than those of humans.<sup>111</sup> In addition, they are also able to produce melanin to deal with stressors such as sunlight radiation and temperature.<sup>104</sup> Areas with greater amounts of solar radiation appear to promote *C. gattii* in air and trees.<sup>71,104</sup> This may explain how *C. gattii* has also been found in dry, hostile environments with constant sun exposure, with isolations of the fungus taken from succulent plants.<sup>94</sup>

Our use of the Köppen-Geiger climate classification map<sup>45</sup> revealed 28 studies with positive environmental sampling of *C. gattii* in other “warm temperate” climates as early as 1991 in California<sup>112</sup> (Table S1). Increased temperatures caused by climatic changes may also have facilitated the emergence of *C. gattii* into Vancouver’s temperate environment,<sup>107</sup> though this has not yet been explored. Likewise, climate change in other parts of the world may shrink *C. gattii*’s ecological niche in tropical and subtropical areas as temperatures gradually exceed tolerable ranges for the fungus.

### Water and air

Environmental isolations of *C. gattii* from water, including water bodies, precipitation, and moisture, were of the VGII strains from the VIO.<sup>31,37,87,113</sup> *C. gattii* was first isolated in freshwater and saltwater on Vancouver Island.<sup>87</sup> Saltwater, either filtered or unfiltered, better supported *C. gattii*, though the organism is not considered a true halophile.<sup>87</sup> The route of cryptococcal infection in sea animals is still unclear but could be due to runoff or air transport.<sup>24</sup> Yet, *C. gattii* also appears to grow in drier conditions, with clinical, veterinary, and environmental samples predominantly located in the rain shadow of Vancouver Island. Our use of the Köppen-Geiger classification scheme<sup>45</sup> revealed that the fungus can survive in a range of precipitation conditions, from “equatorial rainforest” climates to “arid” climates (Fig. 1, Table S1). Ecological niche modeling of this fungus in British Columbia forecasted that optimal ecological conditions fall within the Coastal Douglas-Fir and Coastal Western Hemlock regions.<sup>103</sup> These biogeoclimatic zones are defined by relatively drier conditions (650–1250 mm of annual precipitation) compared to British Columbia or Vancouver Island averages,<sup>114</sup> suggesting *C. gattii* does not require, or may even prefer, less moisture. This would support the hypothesis that *C. gattii* relies on aerolization of its spores for transfer to other lo-

cations or potential hosts; dry conditions would facilitate aerolization.

Aerosolization of spores may also facilitate the fungus’ transport to colonize other trees and soil. Though literature directly studying aerosolization of *C. gattii* is rare, concentrations of airborne cryptococci appear to show a seasonal pattern; concentrations peaked in August, the warmest and driest month in British Columbia.<sup>87</sup> In addition, cooler, wetter months, such as December, experienced a drop in concentrations of airborne cryptococci. This is likely due to the fungal propagules being washed out of the air during precipitation; no positive air samples of *C. gattii* were made during or shortly after rainy periods.<sup>87</sup> Relative humidity was also negatively correlated with airborne concentrations of *C. gattii*. Air sampled from inside tree hollows has also led to positive isolations of *C. gattii*,<sup>53</sup> but more analyses are needed to determine whether air is the predominant method of *C. gattii* transport to new locations.

### Soil

Trees were originally suggested to be the principal reservoir of *C. gattii* colonization, with particular emphasis on eucalypts.<sup>9,77,82,91</sup> However, first-attempt sampling of *C. gattii* on Vancouver Island revealed a greater proportion of positive isolations from soil samples than from swab samples of trees, stumps, shrubs, and cut logs.<sup>87</sup> In our review, 22 of the 56 studies isolated *C. gattii* from soil close to trees. Like many fungal species, concentrations of *C. gattii* are greatest in the top 15 cm of soil, usually due to temperature, humidity, and nutrient requirements.<sup>87</sup> This may also be due to their increased aerosolization when near the surface of the soil, as moderate winds may mobilize surface soil and increase *C. gattii* airborne concentrations.<sup>104</sup>

The soil in the Coastal Douglas Fir biogeoclimatic zone is acidic, with *C. gattii* and other acidophilic or acidotolerant fungi being isolated from soil samples with pH ranges of 4.3 to 7.5.<sup>87</sup> One sampling method of trees in the Coastal Douglas Fir region of Vancouver Island and mainland British Columbia measured both tree swabs as well as sampled soil associated with the root zone. Across trees where only the swab or soil sample was positive, positive soil samples were nearly twice as common (65%) as positive tree samples (35%).<sup>87</sup> Yet, whether a tree or its surrounding soil was first to be colonized remains difficult to determine.

## Biotic interactions

### Trees and eucalypt associations

We found 53 tree species associated with *C. gattii*, spanning 10 climate classifications<sup>45</sup> and 36 terrestrial ecoregions<sup>46</sup> (Table S1). Association with eucalyptus plants was originally considered the reason for *C. gattii* presence, including

in nonendemic areas.<sup>9,77</sup> However, Fortes et al.<sup>56</sup> noted that other areas where cryptococcosis is endemic, such as British Columbia, central Africa and Papua New Guinea, have shown negative sampling for the fungus from *Eucalyptus* species. *C. gattii* was soon found in wild tropical rainforests of Brazil without anthropic interference nor presence of eucalyptus tree species.<sup>56</sup> Over 60% of the papers (37/56) in this review isolated *C. gattii* from noneucalypt trees (Table S1). Other studies also noted no *Eucalyptus* species in areas where environmental isolates of *C. gattii* were positive,<sup>74</sup> or found that even with *Eucalyptus* species present, *C. gattii* was isolated on other trees endemic to the study region.<sup>61,73,87,88</sup> This suggested that the association between *C. gattii* and eucalypt species did not represent the total environmental niche of the fungal species, with more habitats open for colonization around the world.<sup>56</sup> *C. gattii* has also been found on trees in the middle of bustling urban centres,<sup>60,90</sup> as well as environments with nearly no anthropogenic disturbance nor introduced vegetation.<sup>56</sup> The natural degradation of trees has been hypothesized to be the precursor for the primary niche of *C. gattii*,<sup>57,86</sup> though this remains to be tested.

On Vancouver Island, *C. gattii* was isolated from a variety of noneucalypt species, including alder (*Alnus rubra*), western red cedar (*Thuja plicata*), Douglas fir (*Pseudotsuga menziesii*), Garry oak (*Quercus garryana*), and grand fir trees (*Abies grandis*) (all native to Canada).<sup>24</sup> *Eucalyptus* species are not native to Canada but have been introduced as ornamentals.<sup>74</sup> While some eucalypt species are able to withstand the USDA Zone 8 characteristics of the eastern side of Vancouver Island (where ‘zones’ are a horticultural guide to how well plants can tolerate cold temperatures), several of the eucalypt species from which *C. gattii* has been isolated, such as *Eucalyptus camaldulensis*, are intolerant to these conditions.<sup>74</sup>

### Potential vectors of *C. gattii*

*C. gattii* could be introduced to new locations through passive transport of the spores by bird and other animal migrations on their extremities or in their faeces.<sup>37,100,101</sup> The list of potential animal vectors is increasing. In addition to companion animals such as cats and dogs,<sup>31</sup> *C. gattii* infections have been identified in other species, such as marine mammals,<sup>115</sup> ferrets,<sup>116</sup> llamas,<sup>31</sup> horses,<sup>117</sup> goats,<sup>7</sup> koalas,<sup>118</sup> deer,<sup>35</sup> insects,<sup>100,102</sup> and birds.<sup>119</sup> In addition to their potential role as vectors, both companion and wild animals often act as important sentinels for human *C. gattii* infection.<sup>113,115,120</sup> The isolation of *C. gattii* from companion animals and wild animals with limited mobility is comparable to isolations taken directly from environmental sources (e.g., trees, soil) due to the animals’ limited range

and frequent to constant exposure to outdoor airborne organisms.<sup>120</sup> The relationship of insects with *C. gattii* is unclear, but insect frass and nests have been found in association with the fungus.<sup>100</sup> The fungus may have arrived after the frass or nest were produced, taking advantage of the digested organic material.<sup>100</sup> In addition, the presence of *C. gattii* in cactus lesions in the Guanica Dry Forest of Puerto Rico may be linked with birds and insects which create the lesions.<sup>94</sup> Isolations have also been made in droppings of caged birds, such as parrots.<sup>121</sup> *C. gattii* is not generally associated with bird excrement,<sup>74</sup> while *C. neoformans* is often isolated from the nests of birds, where there is an accumulation of faeces, limited exposure to sunlight and UV radiation,<sup>122,123</sup> and insufficient aeration.<sup>84</sup> The dispersal capacity of these various vectors is not yet clear, but anthropogenic dispersal of *C. gattii* through vehicles (e.g., car wheels) and footwear is possible.<sup>37</sup>

## Changing environments

While mechanical vectors may contribute to the expansion of *C. gattii* into Canada and other temperate zones, climatic and land use changes may play a greater role in creating hospitable environments where previous colonization may not have been possible. These changes may also improve environmental conditions to encourage the proliferation of already-existing *C. gattii* populations. Analysis of the emerging colonization of *C. gattii* in the Pacific Northwest and its associated clinical cases helps explore whether climatic and land use changes played a role in its emergence.<sup>74</sup>

### Climatic changes

The proposed tropical and subtropical origins of *C. gattii*<sup>12,13</sup> followed by an apparent emergence in a temperate Canadian climate in 1999 renewed global interest in the ecological niche of *C. gattii* and the possibility that climatic changes may have played a role. Climatic changes (including large-scale climatic oscillations, such as the El Niño Southern Oscillation, as well as global climate change) may have contributed to the establishment or proliferation of *C. gattii* in the Pacific Northwest. None of the reviewed studies directly tested the potential effects of climatic changes on *C. gattii* establishment or spread. In addition, only one study mentioned climate change as a possible factor in its emergence and facilitated spread of *C. gattii* in the Pacific Northwest.<sup>24</sup> To our knowledge, the effects of climatic changes on *C. gattii* distributions have not been directly studied in any region worldwide. However, association between *C. gattii* dynamics and seasonal or monthly regional weather patterns have been studied (e.g., Uejio et al.<sup>104</sup>). The relatively recent

environmental isolations of *C. gattii* in temperate regions such as Vancouver<sup>24</sup> and The Netherlands,<sup>52</sup> in addition to the clinical isolation of the fungus in humans with no recent travel history to disease-endemic areas (e.g., Japan),<sup>36</sup> suggest this organism is either expanding its geographical range into previously uncolonized areas or increasing in concentrations leading to enhanced environmental detection due to one or more environmental triggers, such as climatic or land use changes. Focus on climatic changes and their potential association with *C. gattii*, as well as their measured effects on other infectious organism distributions, has been urged.<sup>107,124</sup> One possibility is that *C. gattii* existed for several decades in the Pacific Northwest, only becoming detectable with increasingly favorable conditions, such as climatic changes. Another may be that introductions of *C. gattii* into the Pacific Northwest have occurred more than once, but warming conditions over the past decades, particularly the warming trend on the east coast of Vancouver Island,<sup>6</sup> eventually created an optimal environment for its establishment and spread. These hypotheses continue to be debated and need to be addressed with climatic change analyses for British Columbia, with particular focus on the initial area of emergence and any environmental changes it underwent before the first detection of *C. gattii* in 1999.

### Land use change

We found one study that directly tested the effects of land use change on *C. gattii* dispersal by analyzing levels of airborne *C. gattii* following forestry and municipal activities.<sup>37</sup> The possible effects of soil disturbance on *C. gattii* spread was explored in infected dogs and cats on Vancouver Island from 2001 to 2003 through a case-control study, finding that the animals living or active near commercial environmental disturbance areas had increased risk of *C. gattii* infection.<sup>125</sup> Companion animals serve as crucial sentinels of human risk to *C. gattii* infection, and wild animals may be considered even better environmental indicators due to their continuous exposure to the outdoors and, therefore, airborne infectious organisms.<sup>113,115,120</sup> Changes in land use have been speculated as one of the risk factors underlying increases in fungal spread and infection.<sup>126</sup> The spread of other fungal species has been found to be aggravated by soil disruption. For instance, the spread of the fungal *Fusarium* genus in Brazil has been encouraged by agricultural practices.<sup>126,127</sup> Similarly, the spread of *Coccidioides* species in and around California that cause coccidioidomycosis has also been correlated with soil disruption activities, such as agricultural, archaeological, and military practices.<sup>128</sup>

Aerosolization of *C. gattii* occurs through deforestation activities such as tree cutting, limb removal, and chipping.<sup>37</sup> Analyses of *C. gattii*-positive red alder and Douglas fir trees

before and after they were felled revealed increased airborne fungal concentrations for the red alder and increased concentrations for both trees after branch chipping.<sup>37</sup> Such activities increase aerosolization of the fungal spores, increasing risk of spread as well as exposure to nearby humans and other animals. Aerosolization would be further aggravated with wind. The effects of land use changes such as forestry need further exploration, particularly at larger spatial scales, to determine the spatial extent of land use effects on *C. gattii* dispersal.

### Future opportunities

*Cryptococcus gattii* is one of the primary aetiological agents of cryptococcosis and is of increasing global importance. Yet, 18 years after its emergence on Vancouver Island, the reasons behind its outbreak are still lacking. Specifically, did climatic changes and land use changes play a role in the 1999 VIO of *C. gattii* and its subsequent spread to the mainland of British Columbia in 2004? If so, what were their roles? Would the longer-term changes in climate have greater or lesser impacts than the more immediate effects of land use change?

In our review of environmental isolations of *C. gattii*, we found the fungus can survive in a variety of climates, including humid and arid conditions, as well as in association with at least 53 different tree species native to these sampled regions across six continents. Its ecological niche is therefore likely very flexible, suggesting the fungus may continue to expand its distribution. Analyses of ecological factors such as tree and soil associations with *C. gattii*, as well as the potential role of animals in increasing the extent and distribution of *C. gattii* colonization,<sup>91,100,102</sup> are needed. Analyses of the potential effects of climatic and land use changes on the geographical distribution of *C. gattii*, as well as possible interactions between changing environmental factors and ecological factors, are also needed to increase understanding of its ecological niche and what may drive its possible expansion into new areas. Which ecological factors (e.g., temperature, precipitation) changed prior to the 1999 *C. gattii* outbreak on Vancouver Island? What land use changes occurred on the area of *C. gattii* emergence? How did these factors change over time, and were these changes associated with the spread of *C. gattii* through the Pacific Northwest?

Spatiotemporal modeling of *C. gattii* is one method that may help answer these questions, and the 1999 VIO may serve as a unique opportunity to study its emergence and subsequent expansion. Improved modeling of *C. gattii* distributions will improve predictions of areas *C. gattii* will likely emerge in or expand to in the future. These methods may also shed light on the factors behind emerging

environmental infectious diseases in Canada and elsewhere. By increasing understanding in the ecology and dispersal mechanisms of *C. gattii*, as well as the potential effects of climatic and land use changes on its emergence and distribution, *C. gattii* could serve as a model for other infectious organisms around the world.

## Supplementary material

Supplementary data are available at [MMYCOL](https://www.mycology.com/online) online.

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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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