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Pseudogymnoascus destructans as the Agent of White-Nose Syndrome (WNS) in Bat Populations †

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Abstract: *Pseudogymnoascus destructans* is a psychrophilic fungus that causes white-nose syndrome (WNS), an emerging disease in North America. This fungus has caused unprecedented population declines. It has been described also in Europe and Asia, where has not caused significant mortality. The first evidence of WNS in North America is from a photograph of a hibernating bat taken during the winter of 2005-2006 in a cave near Albany, New York. *P. destructans* develops when body temperature decreases during winter hibernation. This fungus thrives in humid and cold conditions characteristic of caves. Infected bats can develop visible white fungal growth on the nose, ear, and wings, and awaken more frequently from torpor. It leads to physiologic changes that result in weight loss, dehydration, electrolyte imbalances, and the death of bats. The fungi can persist in the environments of underground bat hibernation sites and are believed to spread primarily by the natural movements of infected bats. Also, there is a strong possibility that it may also be transmitted by humans inadvertently carrying the fungus from cave to cave on their clothing and gear. WNS has a big impact on bat populations with high levels of mortality, particularly endangered species. Some populations will take many years to recover. The decline of bats also has an impact on the spread of diseases since many species of bat feed on insects' carriers of several pathogens.

Keywords: chiropter, bats, *Pseudogymnoascus destructans*, fungi, pathogen, white-nose syndrome

1. Introduction

Pseudogymnoascus destructans (formerly known as *Geomyces destructans*) is a psychrophilic fungus that is the etiologic agent of white-nose syndrome (WNS) in bats **[1]**. A fatal fungal disease that has devastated bat populations in North Hemisphere, particularly in North America **[2]**. The first report of *P. destructans* (WNS) was in North America in a photograph of a hibernating bat taken in the winter of 2005-2006 in a hibernaculum near Albany, New York (USA) **[3]**. In New York, the specie affected was little brown bats (*Myotis lucifugus)* were the first to be infected and had the higher mortality, resulting in population declines of 90-100% in caves **[4]**. Later this fungus with curved conidia was isolated from the skin of the nose and wing of *Myotis lucifugus* and *Myotis septentrionalis* **[2,5]**.

In 2013 after phylogenic analysis, these fungi were classified in the genus *Pseudogymnoascus* and the family *Pseudeurotiaceae* **[6]**. From there and in the next years it spread to other regions of North America and has killed millions of bats, threatening some species of extinction **[7]**. Although millions of bats have died in North America, mass mortality has not been observed among European bats infected by the fungus **[8]**. This fungi is not transmitted to humans.

2. Distribution and transmission

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P. destructans is present in 19 European countries that includes Belgium **[9]**, Croatia **[10]**, Czenia **[11]**, Estonia **[12]**, France **[13]**, Germany, Hungary **[14]**, Laktivia **[15]**, Luxembourg **[16]**, Netherlands, Poland **[12]**, Portugal **[17]**, Russia, Slovenia **[15]**, Slovakia **[18]**, Switzerland **[14]**, Ukraine **[12]**, United Kingdom **[19]**, Italy **[20]**. Also has been detected in China (Beijing, Jilin Liaoning, Shandong) **[21]**. In North America geographic distribution of *P. destructans* continues to increase is currently distributed in 38 states of the U.S.A. (Alabama, Arkansas, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Caroline, Tennese, Texas, Vermont, Virginia, Washington, West Virginia, Wisconsin) **[4,5,22]**and 5 Canadian provinces (New Brunswick, Nova Scotia, Ontario, Prince Edward Island, Quebec) **[4]** (Figure 1).

It is hypothesized that these fungi have been introduced to North America from Europe or Asia where are native **[16,21,23,24]**. The route of introduction is still unknown, but the subsequent spread throughout North America probably occurred due to the natural movement of bats **[25]**, contact with contaminated substrates and human clothing/equipment (particularly cave equipment) **[26]** and host/vector organisms (pathway vector) **[27]**.

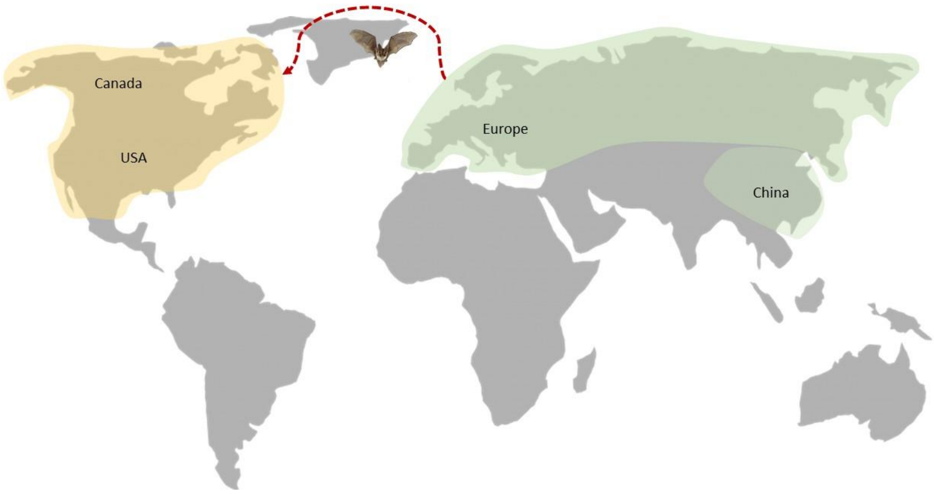

Figure 1. Distribution of *P. destructans.* (Yellow – Invasive, Green – Native).

3. Affected species

In North America, *P. destructans* has been described in eleven species of bats: Indiana bat *(Myotis sodalis*), gray bat (*Myotis grisescens*), little brown bat (*Myotis lucifugus*), the northern long-eared bat (*Myotis septentrionalis*), the big brown bat (*Eptesicus fuscus*), the tri-colored bat (*Perimyotis subflavus*), and the eastern small-footed bat (*Myotis leibii*), Virginia big-eared bat (*Corynorhinus townsendii virginianus*), the cave bat (*Myotis velifer*), the Silver-haired bat (*Lasionycteris noctivagans*), and the South-eastern bat (*Myotis austroriparius*) *[5,28]*.

In Europe has been observed in 13 bat species: Bechstein's bat (*Myotis bechsteinii),* Lesser mouse-eared bat (*Myotis blythii oxygnathus*), Brandt's bat (*Myotis brandtii*), pond bat (*Myotis dasycneme*), Daubenton's bat (*Myotis daubentonii*), Greater mouse-eared bat (*Myotis myotis*), whiskered bat (*Myotis mystacinus*), Geoffroy's bat (*Myotis emarginatus*), Northern bat (*Eptesicus nilssonii*), Lesser horseshoe bat (*Rhinolophus hipposideros*), Barbastell (*Barbastella barbastellus*), Brown long-eared bat (*Plecotus auritus*) and Natterer's bat (*Myotis nattereri*) *[15,18]*.

In Asia, the species where the fungi were observed were the Greater horseshoe bat (*Rhinolophus ferrumequinum),* least horseshoe bat *(Rhinolophus pusillus), l*arge-footed mouse-eared bat *(Myotis adversus),* eastern long-fingered bat *(Myotis macrodactylus),* Rickett's big-footed bat *(Myotis pilosus),* large myotis *(Myotis chinensis),* Ussuri tube-nosed bat *(Murina usseriensis),* greater tube-nosed bat *(Murina leucogaster),* and eastern water bat *(Myotis petax) [21].*

The species Rickett's big-footed bat *(Myotis pilosus)*, Indiana bat *(Myotis sodalis)*, grey bat *(Myotis grisescens),* little brown bat *(Myotis lucifugus)* according to the IUCN Red List of Threatened Species are considered threatened *[29]*.

4. Pathogen Characteristics and clinical presentation

It is psychrophilic fungi that grow at temperatures around 4 °C to 20 °C (the same temperatures that can be found in winter bat hibernacula) *[30]*. It belongs to the genus *Pseudogymnoascu*s, family *Pseudeurotiaceae* [5]. *P. destructans* can grow and sporulate (reproduce asexually via conidiation) on chitinaceous, keratinaceous, cellulosic, and lipid/protein-rich substrates (e.g. dead fish, mushrooms, fruit and insects) *[31]*. It can grow over a wide pH range (pH 5-11), high levels of sulfur compounds (cysteine, sulfite, sulfide), and in elevated levels of calcium in the environment *[31]*.

The transmission starts during autumn and has a high peak during the months of winter. During the summer the fungi vanish from the surviving animals and the prevalence during the summer months is almost null. However, the fungus may still be present in has the ability of persisted in the cave sediment even in the absence of bats *[32]* and in the skin of bats that utilize contaminated underground sites for daily torpor *[26]*.

4.1. Laboratory Diagnose

In the laboratory has a slow growth on cornmeal agar or Sabouraud dextrose agar incubated at 5 to 15°C for 16 days *[28]*. Colonies have a 1.0 mm diameter and are white marginally with grey to green powdery centres. The reverse side is uncoloured on cornmeal agar or brown in Sabouraud dextrose agar *[28,33]*. Microscopically has moderately thick-walled, curved conidia and erect, hyaline, smooth, narrow and thin-walled conidiophores *[28,34]*. Some virulence factors have also been identified in *P. destructans* that may contribute to skin invasion. Those include the over-production of riboflavin (vitamin B2) *[35]*, secretion of siderophores *[36]*, and excretion of a subtilisin-like serine protease that reduces collagen *[37]*. Also produces enzymes such naphthol-AS-B1-phosphohydrolase, β-glucosidase, leucine and valine arylamidase, esterase/esterase lipase/lipase, N-acetyl-β-glucosaminidase, acid and alkaline phosphatases, proteinases, and ureases *[38]*.

4.2. Clinical signs in bats

This fungi almost exclusively affects hibernating bats *[1,28]*. Gross clinical signals are fungal growth with 1 to 3 mm diameter, multifocal to coalescing white foci with a pinpoint black centre on the ears, nose, and wing membranes of hibernating bats (Fig. 2) *[5]*. In the wings is also possible to observe areas of depigmentation, splitting, and dryness of the patagia *[39]*. The animals infected also present behaviours alterations such as premature emergence from hibernation during the winter period *[40]*. Other systemic alterations that can be present are increasing evaporative water loss through the damaged skin, hypovolemia, hyperkalemia, acidosis, and hypotonic dehydration *[24,34]*.

In the *post-mortem*, the animals are emaciated and the fungi growing on the skin can be present or not. Histological exams of the skin can reveal cupping erosions and ulcers that are filled with densely packed, PAS- and GMS-positive fungal hyphae *[7]*. Animals that died during hibernation do not present an inflammatory response surrounding the invading hyphae, but in animals that emerged from hibernation is possible that they may exhibit a neutrophilic reaction as well oedema and necrosis *[7]*. Although mortality is very high some animals can survive and recover from the damage *[7,28]*.

Figure 2. Lesions in the skin of bat due to *P. destructans.* (Licence common use).

4.3. Diagnose and treatment

Methods to identify the fungus include fungal culture, histopathological examination, and PCR [4]. There is no practical treatment for colonies affected at the moment.

5. Social, environmental and Economic impacts

5.1. Social impacts

Some insectivorous bat species (e.g. *M. septentrionalis*) consume large quantities of mosquitoes each night [41]. Many of these mosquitos and other insects are carriers of many diseases that can affect humans. With the decline of bats due to *P. destructans* there is a reduction in the elimination of these vectors and a higher risk for transmission of vector-borne diseases [41].

5.2. Environmental impact

P. destructans affects all life stages of hibernating bats, and the mortality in hibernacula can be very high, resulting in significant and rapid decreases in bat abundance in the regions affected by this fungi [23]. In North American millions of bats have already died due to this fungi, leading to the decline of populations, with some common species (e.g. *Muotis lucifugus*) becoming almost inexistent in some regions [23,42]. Populations that have been affected by *P. destructans* have a slow recovery due to already low annual fecundity (one juvenile a year) and other causes of mortality such as wind-turbine collisions [43]. Some may never recover to the number before the infection. The alteration in bat populations can lead to alterations at the trophic level, damaged ecosystem services (e.e. pollination), negative impacts on agriculture, forestry, human and animal health and reduction of native biodiversity and threatened species [23].

5.3. Economic impacts

Insectivorous bats offer pest control services, by consuming insects that damage crops and forests, or vectors that carry diseases [44]. Bat species also are important pollinators and dispersers of seeds in tropical and subtropical regions [44]. These services save farmers millions of dollars every year. The mortality of bats due to these fungi is estimated to reduce these valuable and irreplaceable ecosystem services [45].

6. Conclusions

P. destructans has an enormous impact on the bat population, especially in North America, with so high levels of mortality that previously common bat species have become almost extinct.

There is limited understanding of both fungal pathogens and wildlife hosts, since fungal species, in general, are very poorly investigated compared to other taxa of pathogens. Antifungal medications existing are limited and those in use often have considerable side effects on the animals, and anti-fungal vaccines at still not available. There is also inadequate knowledge about the immunology, physiology, and ecology of *P. destructans* on the multiple species of bats infected. This has led to many challenges in the control and prevention of this disease [46].

Efforts to attend to this devastating disease have already been made, but in the future, more research and development of new drugs will be necessary to combat this disease.

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