Pan-European Distribution of White-Nose Syndrome Fungus (*Geomyces destructans*) not Associated with Mass Mortality

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Abstract

Currently being written.

Introduction

White nose-syndrome (WNS) is a devastating disease causing mass mortalities in hibernating bats in North-America. In May 2009, it was estimated that over one million bats had died from the disease which was first documented in February 2006 at Howe Cave, West of Albany, New York [1]. A visually conspicuous white fungus grows on the face, ears, or wings of stricken bats with hyphae penetrating deep into the connective tissue of glabrous skin and snout [2] and causing severe damage [3]. The fungus associated with WNS is a newly described, psychrophilic (cold-loving) species (*Geomyces destructans*) [4], closely related to other psychrophilic species of *Geomyces* [5,6]. Although it is not yet proven whether *G. destructans* is the causative agent of the disease or if it needs to be regarded as a secondary infection, the fungus is always found on bats at sites with hibernating bats mass mortalities [7]. To date, bacteriologic, virologic, parasitologic and pathologic evaluations as well as toxic contaminants exposure studies did not identify any other agents/cause of death, which reinforces the idea that *G. destructans* is the causative agent [2,7,8,9].

G. destructans has been extensively found in different species of bats in North-America, from the states of Ontario and Quebec in Canada down to North Carolina and Oklahoma in the USA. Three recent studies investigating samples collected in 2008-2010 have shown that *G. destructans* was also present in six European countries (France, Germany, Switzerland, Czech Republic, Slovakia & Hungary) [6,10,11]. Nevertheless, the geographic coverage of these studies was limited to one or a few countries, while the extent of the distribution of *G. destructans* in Europe remains poorly known. In this paper, we combine previously published data

on the distribution of *G. destructans* in Europe [6,10,11] with new data from twelve countries covering 2,400 km from West to East (France to Turkey) and 1,900 km from North to South (Estonia to Turkey) and demonstrate the widespread presence of the fungus in Europe without associated mass mortality.

Results

Published data on *Geomyces destructans* in European bats, 2008-2010

Although photographs of bats with fungal growth similar to *G. destructans* were published in Germany in the 1980's [12], and also taken in the 1990's in the Czech Republic [10], there has been no confirmed record of *G. destructans* in Europe prior to 2008. In 2010, *G. destructans* has been confirmed *via* morphological and genetic analyses from samples collected during the winters 2007/2008, 2008/2009 and 2009/2010 in six European countries [6,10,11]. In France, Hungary, Switzerland and Slovakia, the fungus has been confirmed from 1-2 location(s) while it has been confirmed at 8 sites in Germany and 23 sites in the Czech Republic [6,10,11]. All *G. destructans* have been isolated from hairs, swabs or touch imprints from bats [6,10,11]. In Europe, eight *Myotis* species have been identified to be colonized with *G. destructans*: *M. myotis*, *M. blythii* (referred to as *M. oxygnathus* in [11]), *M. mystacinus*, *M. daubentoni*, *M. dasycneme*, *M. nattereri*, *M. bechsteinii* and *M. brandtii*. Species from other families were present in the caves with infected individuals (e.g. Miniopteridae: *Miniopterus schreibersii*; Rhinolophidae: *Rhinolophus hipposideros* and *R. ferrumequinum*), but no *G. destructans* has been confirmed from these species. Previous extensive surveys of cave fungi in Europe [i.e.

13,14,15] or fungi associated with insects hibernating in underground sites [16] never reported *G. destructans* in their inventory, although some other species of *Geomyces* were recovered [13,14,15].

New data on *G. destructans* in Europe 2005-2010

During winter hibernation counts, a total of 66 bats from 48 sites in twelve European countries were reported to have visible white fungal growth (Table 1, Fig. 1). This represents the first records from eight countries (Austria, Belgium, Denmark, Estonia, The Netherlands, Poland, Romania, Turkey and Ukraine). Sixty six bats were alive while two of them were found dead in the hibernacula. These 66 bats belonged to eight different *Myotis* species, *M. myotis* (42), *M. mystacinus* (9), *M. dasycneme* (6), *M. daubentoni* (3), *M. myotis/blythii* (2), *M. blythii* (1), *M. nattereri* (1), *M. escalerai*/sp. *A* (1) and *M. brandtii* (1). Of these, molecular and morphological identification of the colonizing fungus were carried out on 22 cases, while only photographic evidence was obtained for a further 29 cases (Table 1 & Fig. 2). The remaining 15 cases were based on reports of visual observations of a white fungal growth on bat snouts and/or ears, which was very similar to pictures presented in Fig. 2.

The temporal range of reported cases of live bats with white-fungus was not evenly distributed throughout the winter/spring, with about 2/3rd of the cases reported in March (42/66; Fig. 3). The number of reported cases nearly tripled between February (16 cases) and March (42 cases). The earliest case was reported on January 17th from Belgium and the two latest cases were observed on May 23rd in Estonia and June 25th in France (Table 1 & Fig. 3A, K).

Geomyces destructans identification

Out of a total of 66 bats with fungal growth, 21 were sampled, 16 with touch imprints and 5 with cotton swabs. The 21 bats sampled (19 alive and 2 dead) belonged to the *Myotis* species from which *G. destructans* was previously isolated (see list above). In some cases, we were not able to discriminate between *M. myotis* and *M. blythii* (referred to as *M. myotis/blythii*) as well as between the newly recognized *M. escalerai* [17,18] and *Myotis* sp. *A* [19], a yet undescribed cryptic species from the *M. nattereri* species complex [17,20]. Additionally, swab samples were collected from the tunnel wall of an Estonian hibernaculum. On the 23rd of May 2010, a *M. brandtii* was observed in this hibernaculum with white fungal growth on its snout (Fig. 1A) but no sample was collected at the time. When the site was revisited for sample collection on the 1st of June 2010, the bat had left the site so samples were collected by swabbing the walls of the tunnel where the bat was seen 9 days before. Four cotton swabs were used to sample different areas a few centimetres around the location where the bat was observed. The four swabs were then streaked onto four Sabouraud's agar plates each and monitored regularly to physically remove any fungal growth that was not similar to *G. destructans*. Although the amount of fungi varied per swab sample, *G. destructans* was recovered from all four swabs, which from now on are considered as one sample, bringing the total of samples analysed to 22. No mass mortality was reported at any of the sites investigated.

Out of 22 samples investigated in the laboratory, 14 of the 16 touch imprint samples presented characteristic conidia when observed under a microscope and two of them were doubtful; none of the cotton swabs were inspected under a microscope prior to culture. Cultures from 17 of these samples were successful (no cultures were done for 2 samples). The two dead bats investigated did not reveal the presence of *G.*

145 *destructans* but other fungal species such as *Mucor* sp. and *Cladosporium* sp. (data
146 not shown).
147 DNA was isolated from the 17 cultures of which 15 showed morphological
148 similarity with *G. destructans* (e.g. curved conidia) and from five touch imprints
149 without culture available (n=2) or with unsuccessful culture attempts (n=3).
150 Amplification and sequencing of the internal transcribed spacer (ITS) region (ITS1,
151 5.8S, and ITS2) was preferred over the small subunit (SSU) rDNA as it was shown to
152 be more informative and was comparable to both, European [6,11] and North
153 American *G. destructans* [7,21]. All sequences obtained (Accession Numbers:
154 xxxxxx-xxxxxxx) were identical and showed 100% similarity with previously
155 published *G. destructans* ITS sequences available on GenBank (retrieved on October
156 13th) [6,7,11,21].
157

Discussion

G. destructans has been first identified in Europe in 2008-2009 [6,11] but increasing photographic evidence suggest that the species was present in Europe well before this date [this study; 10,12]. A certain number of studies investigated fungi species present in European caves, including bat guano [13,14,22,23] and although most of them report *Geomyces* species, no *Geomyces* species with curved conidia (so far typical of *G. destructans*) have ever been reported. In the Czech Republic, Kubátová & Dvořák [16] investigated fungi associated with insects hibernating in underground sites but did not find *Geomyces* species. To our knowledge, only one study in Europe has investigated fungi present in bat's skin and hair samples where, based on our current knowledge, *G. destructans* is most likely to be found. During the winter 1999/2001, Larcher *et al.* [24] collected 25 samples of hair and skin swabs from six species, including three *Myotis myotis*, but did not find any *Geomyces* species. It is important to note that most fungal cultures have been carried out at temperatures above 24-25°C, temperatures at which *G. destructans* does not grow [4,21]. A better representation of temperate caves fungal diversity might be obtained by also culturing fungi at temperatures in the range of 10-15°C, which is more representative of temperatures encountered in European caves. Finally, to confirm the presence of *G. destructans* in Europe prior to 2009, historical collections of bat specimens (or eventually cave soil samples), especially specimens collected during the hibernation period, should be screened for the fungus. Retrospective investigations have provided valuable information on the historic distribution of *Batrachochytrium dendrobatidis* [25], the etiologic agent of chytridiomycosis which is now decimating amphibian populations on all continents except Antarctica [26].

Combining previously published data from France, Germany, Switzerland, Hungary, The Czech Republic and Slovakia [6,10,11], additional data collected from France, Germany and Hungary (this study), and new data from Belgium, The Netherlands, Poland and Estonia (this study), we demonstrate here that *G. destructans* is widespread in Europe. We consider the photographic evidence of bats with white fungus matching the characteristic growth pattern (e.g. Fig. 1; pictures from Ukraine and Turkey) to highly likely represent *G. destructans*, because so far all tested live European bats with such white fungal growth on their nose, similar to Fig. 1, have been confirmed to carry *G. destructans*. These findings further support the fact that *G. destructans* is widespread across Europe. As depicted in Fig. 1, most *G. destructans* cases (confirmed and suspected) are found from North-eastern France through Belgium, The Netherlands, Germany and the Czech Republic. However, it is not known whether this pattern reflects a true higher occurrence and/or prevalence of the fungus in these regions or if it is at least partly due to sampling bias whereby the fungus is more likely to be found in regions with a higher number of underground sites visited every winter or in regions were the fungus is specifically searched for. It is most likely hat this large scale pattern is likely due to a sampling bias as for example, the largest number of sites reported with *G. destructans* in the Czech Republic (76 localities with suspect or confirmed *G. destructans*) is also associated with the largest number of sites where the species was looked for (over 800) [10]. Similarly, it is unknown whether *G. destructans* is absent or rare in the Mediterranean region where it has not been found yet despite specific searches (i.e. Italy,[27]).

The number of observations of bats with white fungal growth per week highlights an increasing prevalence as winter passes and a sudden drop when bats emerge from hibernation in spring. This suggests that either bats acquire *G.*

destructans late during the hibernation period or that the fungus is carried by the bat at the onset of hibernation but needs time to develop as a visible white fungal growth. At present, virtually nothing is known about how exactly *G. destructans* is transmitted to the bats, either *via* the environment or *via* bat to bat contacts. Although under laboratory conditions, *G. destructans*' growth rate >10 mm per month is commonly reported for a wide range of media and temperatures in the range of 4-10 °C [4,6,10,11,21] , which are temperatures commonly selected by bats for hibernation [28], the fungus' growth rate might substantially differ under natural conditions. Other climatic factors, particularly humidity, might also influence *G. destructans* growth rate. The importance of relative humidity has been suggested by various researchers based on field observations; nevertheless, its influence on *G. destructans* growth rate has never been assessed. Differences in temperature and/or humidity might help explaining the regional differences in prevalence observed between submountainous humid to mesic regions (high prevalence) *versus* mountainous and limestone regions (low prevalence) observed in the Czech Republic [10]. Furthermore, during hibernation, bats rouse every two weeks in average [29,30] and groom the fungus off [10], which will considerably reduce the chance of the fungus to become apparent. Although it is not possible to clearly identify the mechanism responsible for the sudden increase in *G. destructans* prevalence in late February and March, the data suggest that shorter winter periods should be associated with lower *G. destructans* prevalence. This prediction seems to hold as in the Mediterranean region, associated with shorter hibernation periods, no bats with visually conspicuous fungal growth has been reported yet, although, winter cave surveys were carried out around the Mediterranean Sea. The case reported from Southern France (June 25th 2010, Fig. 3K) was found in the Pyrenees' mountains at *ca.* 1700 m a.s.l. and hence, is not considered

as belonging to the Mediterranean climatic region. More surveys are however necessary to uncover whether the length of the hibernation period and/or climatic conditions have a direct or indirect effect on *G. destructans* growth rate and prevalence on bats.

It is crucial that the change in prevalence over the hibernation period is considered when comparing prevalence across sites and/or years. Our results show that bats with fungal growth are first seen in January, then the prevalence slowly increases in February and peaks in March, while in April, when bats emerge from hibernation, the prevalence drops again. Our results are in agreement with recent results from the Czech Republic where on the winter 2009/2010, the number of sites with bats with white fungal growth remarkably increased from 4.1% in January/February (33/800 sites; regular bat monitoring) to 77.5% in late February/March (76/98 sites; additional inspections) [10]. This study reported that this increase in *G. destructans* prevalence was "*suggestive of an epizootic spread of the fungus*" [10]; we propose an alternative explanation whereby the increase in prevalence of *G. destructans* in late winter (March) has regularly (yearly) happened in Europe but has been unnoticed in the past as nearly all hibernation counts were carried out between December and mid-February when *G. destructans* prevalence is low, but not in March [31] when the prevalence of *G. destructans* is the highest (Fig. 3). By increasing the sample size, some cases might be reported earlier in the hibernation season or later through the summer, but we expect that the general pattern observed will not change. Prevalence here does not directly refer to the prevalence of *G. destructans* on bats but rather to the prevalence of visible signs of *G. destructans* growing on them. Our ability to detect of *G. destructans* growth on bats can substantially differ with proximity to the bats (i.e. low ceiling *vs.* high ceiling), the

location of the bat (ceiling *vs.* crevices), etc. Despite these difficulties in assessing the prevalence, in agreement with other studies [6,10,11], our data demonstrate that the species most commonly encountered with *G. destructans* growth is the largest European *Myotis* species, *Myotis myotis*.

We report here two individual bats with white fungal growth around their nose (one confirmed as *G. destructans*) from May and June, both individuals being in torpor in cold underground sites. This represents the first mention of individuals with *G. destructans* colonisation outside of the hibernation period and raises questions about the role of these individuals in the persistence of the fungus in the bat population. During the summer period, while females aggregate in colonies to raise their young, it remains largely unknown where males are roosting [e.g. 32]. Generally, some males can be present in maternity colonies but they represent only a minority if we assume a female to male ratio of 1/1. Furthermore, during the swarming season in late summer/autumn, large numbers of individuals aggregate in caves, mines or tunnels and come in close contact with each other (chasing, mating, etc.) [32,33,34,35,36,37], which could represent a place and time where *G. destructans* is transmitted between individuals. We also report the isolation of *G. destructans* from the environment surrounding hibernating bats. The presence of viable spores of *G. destructans* on the surfaces of the hibernation sites has large implications for the understanding of the transmission mechanisms. It seems likely that cave walls could serve as a passive vector and/or reservoir for *G. destructans* spores. It is not yet known how long these spores can remain viable for but as most spores are viable for an extended period of time, bats entering these sites in autumn (for swarming and/or hibernation) could become contaminated with *G. destructans* spores left from bats of the previous winter. In North-American, Lindner *et al.* [38] successfully amplified

ITS sequences identical to *G. destructans* DNA from soil samples collected during the winter 2008-2009 at three bat hibernacula and stressed the importance of the environment as a reservoir and its possible importance on *G. destructans* dynamics and therefore WNS. Our results confirm the view of Lindner *et al.* [38] and further suggest that more work is needed to understand the exact role of walls (reservoir, passive vector, etc.) as they are in physical contact with bats.

The wide distribution of *G. destructans* in Europe and the absence of associated mortality supports the hypothesis that *G. destructans* has co-evolved with European bats and only recently arrived in North America where it is causing unprecedented mass mortalities [6,7,10,11]. Nevertheless, it is not yet known whether the fungus alone is causing the mortality or whether it needs to be associated with other pathogens such as viruses to cause mass mortality. Recent work on the Colony Collapse Disorder affecting bees in North America, Europe and Asia has shown that the presence of the microsporidian *Nosema* alone cannot explain the disorder [39] but the association between *Nosema* and an invertebrate iridescent virus is always found in colonies suffering from the disorder [40]. Further studies are needed to investigate pathogens found in healthy bats and bats dying from WNS in North America [6].

Phylogeographic studies on European bat species have shown that in the last 100,000 years, some species colonized Europe from Western Asia [41], including *Myotis blythii* [42,43] which has been found with *G. destructans* [11]. We can therefore speculate that *G. destructans*' distribution is probably not limited to Europe and possibly extends eastwards into Russia, Western and Central Asia. Further surveys are necessary to clarify the global distribution of *G. destructans*.

Conclusions

We have shown here that *G. destructans*, the most likely causative agent of WNS in North America, is widespread in Europe, but not associated with mass mortality. The prevalence of visible fungal growth on bats increases in February/March before sharply decreasing when bats emerge from hibernation. We also isolated viable *G. destructans* from the walls of an underground site suggesting that hibernacula walls could be a passive vector and or reservoir for *G. destructans* and therefore, might play an important role in the transmission process. Further research is needed to clarify the global prevalence of *G. destructans* and identify variables (e.g. temperature, humidity, hibernation length, etc.) explaining regional differences. Finally, further research needs to be carried out in different parts of the globe, especially temperate region of the Northern and Southern hemispheres, to precisely determine *G. destructans* distribution.

Materials and Methods

Samples collection

During ongoing population censuses carried out in different countries across Europe, information on bats with visible white fungal growth on snouts and/or ears was recorded. Whenever possible, sterile dry cotton swabs [6] or adhesive tape touch imprints [11] were used to collect the fungal material from the bats. In Estonia, samples were collected from the wall of the tunnel where a bat with characteristic white fungus was observed nine days prior to the sampling. Where no sample collection was possible, a photograph was taken of the bat (photographic record). In

cases where neither sample collection nor photographic evidence was obtained, the record was classified as visual observation. Live hibernating bats with powdery, white fungal growth on their noses were considered as *G. destructans* suspects but not WNS suspects as apart from the presence of the fungus associated with WNS in North-America, there is presently no data supporting the occurrence of WNS in Europe and the fungus has not (yet) been identified on dead bats [11,44]. Although, given that *G. destructans* prevalence can reach high levels in some species (i.e. *Myotis myotis*) in late winter (especially in March), it can be expected, that by chance some bats dying from causes unrelated to the presence of *G. destructans* will also be carrying the fungus. But unless the criteria for the diagnosis of WNS are met (confirmation by histo-pathology and PCR) [2] dead bats with fungal growth in Europe cannot be considered as WNS suspects. Instead, various species of fungi have been identified on dead bats [11,27], many of them potentially being just post-mortem colonisations.

Fungal cultures

In the laboratory, samples were treated as in [6] for swabs and following [11] for touch imprints. Briefly, swabs were streak-plated onto plates of Sabouraud's agar, supplemented with 0.1% mycological peptone. For touch imprints, small areas with fungal conidia characteristic of *G. destructans* were identified by light microscopy and the tape was disinfected and excised before being transferred for culture to Sabouraud's agar. The plates were sealed with parafilm and incubated inverted in the dark at 10°C. A fungal growth developed within 14 days, from which single spore cultures were established.

Molecular identification

Each culture was sequenced for one molecular marker, the rRNA gene internal transcribed spacer (ITS) region (ITS1, 5.8S, and ITS2) to further confirm species identity. The DNA extraction, PCR amplification and DNA sequencing followed protocols described in Puechmaille et al. [6]. Briefly, DNA was extracted using the Qiagen Blood and Tissue kit following the manufacturer's instructions with slight modifications (after step 3, we added an incubation time of 10 minutes at 70°C). PCR reactions were carried out in 25 µL containing 1 µL of DNA extract (at 10-75 ng/µL), 1.5 mmol/L $MgCl_2$, 0.4 µmol/L each primer (Forward: ITS4, 5'-TCCTCCGCTTATTGATATGC-3'; Reverse: ITS5, 5'-GGAAGTAAAAGTCGTAACAAGG -3'; [45]), 0.2 mmol/L dNTP, 1x PCR buffer and 1 U Platinum Taq DNA Polymerase High Fidelity (Invitrogen). PCR cycling conditions were; initial step 15' at 95°C, then 10 cycles of 30" at 95°C, 1'45" at 60°C (reduce of 2°C every 2 cycles), 1' at 72°C, following by 30 cycles of 30" at 95°C, 1'45" at 50°C and 1' at 72°C. PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea) in both directions using the PCR primers. Complementary sequences were assembled and edited for accuracy using CodonCode Aligner 3.0.3 (www.codoncode.com/aligner/download.htm).

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499 Tables

500 Table 1. Confirmed and suspected *Geomyces destructans* records from Europe.

501

	Country	Lat	Lon	Date	Species	Culture	GenBank No.
Samples	France	47.7	-2.1	04/03/2010	*Myotis myotis*	Yes	
	France*	49.9	4.1	04/03/2010	*Myotis mystacinus*	Yes	
502	France*	50.6	2.5	22/02/2010	*Myotis nattereri*	Yes	
	Belgium	49.8	5.3	03/04/2010	*Myotis myotis*	Yes	
	Belgium	50.8	5.6	18/03/2010	*Myotis mystacinus*	no	
	Belgium	50.8	5.6	18/03/2010	*Myotis mystacinus*	yes	
	Netherlands	52.0	5.8	09/03/2010	*Myotis daubentonii*	no	
	Netherlands	52.1	4.3	02/2010	*Myotis dascycneme*	no	
	Germany	49.7	7.4	10/03/2010	*Myotis myotis*	yes	
	Germany	49.8	9.6	22/03/2010	*Myotis myotis*	yes	
	Germany	50.7	13.7	20/03/2010	*Myotis myotis*	yes	
	Germany	50.9	7.5	18/04/2009	*Myotis myotis*	no	
	Germany	51.2	8.1	21/03/2010	*Myotis mystacinus*	yes	
	Germany	51.2	8.1	21/03/2010	*Myotis mystacinus*	yes	
	Germany	51.2	8.1	07/03/2010	*Myotis myotis*	yes	
	Germany	51.2	8.1	07/03/2010	*Myotis myotis*	yes	
	Germany	52.3	9.5	23/03/2010	*Myotis myotis*	yes	
	Germany	52.3	9.4	23/03/2010	*Myotis mystacinus*	yes	
	Hungary	47.1	17.6	24/03/2010	*Myotis myotis*	no	
	Hungary	47.1	17.6	24/03/2010	*Myotis myotis*	yes	
	Poland	50.8	16.7	07/03/2010	*Myotis myotis*	Yes	
	Estonia[#]	59.3	24.6	01/06/2010	*Myotis brandtii*	Yes	
Photographs	France	44.8	1.6	25/04/2008	*Myotis myotis*	-	
	France	42.6	2.2	26/06/2010	*Myotis escalerai/sp. A*	-	
	France	47.7	-2.1	04/03/2010	*Myotis myotis*	-	
	France	45.0	2.0	13/02/2010	*Myotis myotis*	-	
	France	45.0	2.0	13/02/2010	*Myotis myotis*	-	
	France	47.3	6.2	04/03/2010	*Myotis myotis*	-	
	France	47.3	6.2	04/03/2010	*Myotis myotis*	-	
	France	47.3	6.2	04/03/2010	*Myotis myotis*	-	
	France	50.4	3.5	01/03/2008	*Myotis mystacinus*	-	
	France	47.2	1.4	24/02/2010	*Myotis myotis*	-	
	France	47.2	1.4	24/02/2010	*Myotis myotis*	-	
	Belgium	50.8	5.6	09/02/2008	*Myotis dasycneme*	-	
	Belgium	50.8	5.6	20/03/2008	*Myotis daubentonii*	-	
	Belgium	50.8	5.6	17/01/2010	*Myotis dasycneme*	-	
	Belgium	50.3	5.9	07/03/2010	*Myotis myotis*	-	
	Belgium	50.8	5.7	13/03/2010	*Myotis dasycneme*	-	
	Netherlands	52.0	5.7	04/03/2010	*Myotis mystacinus*	-	
	Danmark	56.4	9.1	14/03/2010	*Myotis myotis*	-	
	Germany	51.8	10.8	02/02/2008	*Myotis myotis*	-	
	Germany	51.6	10.5	07/02/2010	*Myotis myotis*	-	
	Germany	51.7	10.3	20/03/2010	*Myotis myotis*	-	
	Germany	52.3	9.5	23/03/2010	*Myotis mystacinus*	-	
	Germany	52.3	9.5	23/03/2010	*Myotis dascyneme*	-	
	Austria	46.8	16.0	07/02/2007	*Myotis myotis*		
	Poland	50.8	16.7	07/03/2010	*Myotis myotis*	-	
	Romania	46.8	22.6	29/03/2008	*Myotis blythii*	-	
	Romania	45.4	25.2	14/03/2009	*Myotis myotis/blythii*	-	
	Turkey	41.9	27.9	22/03/2009	*Myotis myotis/blythii*	-	
	Ukraine	48.8	26.6	13/02/2010	*Myotis myotis*	-	
Visual observations	France	49.1	6.6	06/04/2009	*Myotis myotis*	-	
	France	48.5	6.9	28/02/2009	*Myotis myotis*	-	
	France	48.3	7.1	29/03/2009	*Myotis myotis*	-	
	France	48.3	5.7	16/03/2008	*Myotis myotis*	-	
	France	47.9	6.8	03/03/2010	*Myotis myotis*	-	
	France	47.9	6.8	03/03/2010	*Myotis myotis*	-	
	France	49.5	5.2	04/03/2010	*Myotis myotis*	-	
	France	48.9	0.3	06/02/2010	*Myotis myotis*	-	
	France	47.2	5.7	20/02/2010	*Myotis myotis*	-	
	France	47.2	5.7	20/02/2010	*Myotis myotis*	-	
	France	47.2	5.7	20/02/2010	*Myotis myotis*	-	
	Germany	50.9	13.3	23/03/2010	*Myotis daubentonii*	-	
	Germany	49.9	7.4	14/03/2010	*Myotis myotis*	-	
	Netherlands	52.1	4.3	10/03/2005	*Myotis dascycneme*	-	
	Romania	47.0	22.4	08/04/2008	*Myotis myotis*	-	

503 * Dead bat

504 # Environmental sample (see text for further explanations)

505

Figure Legends

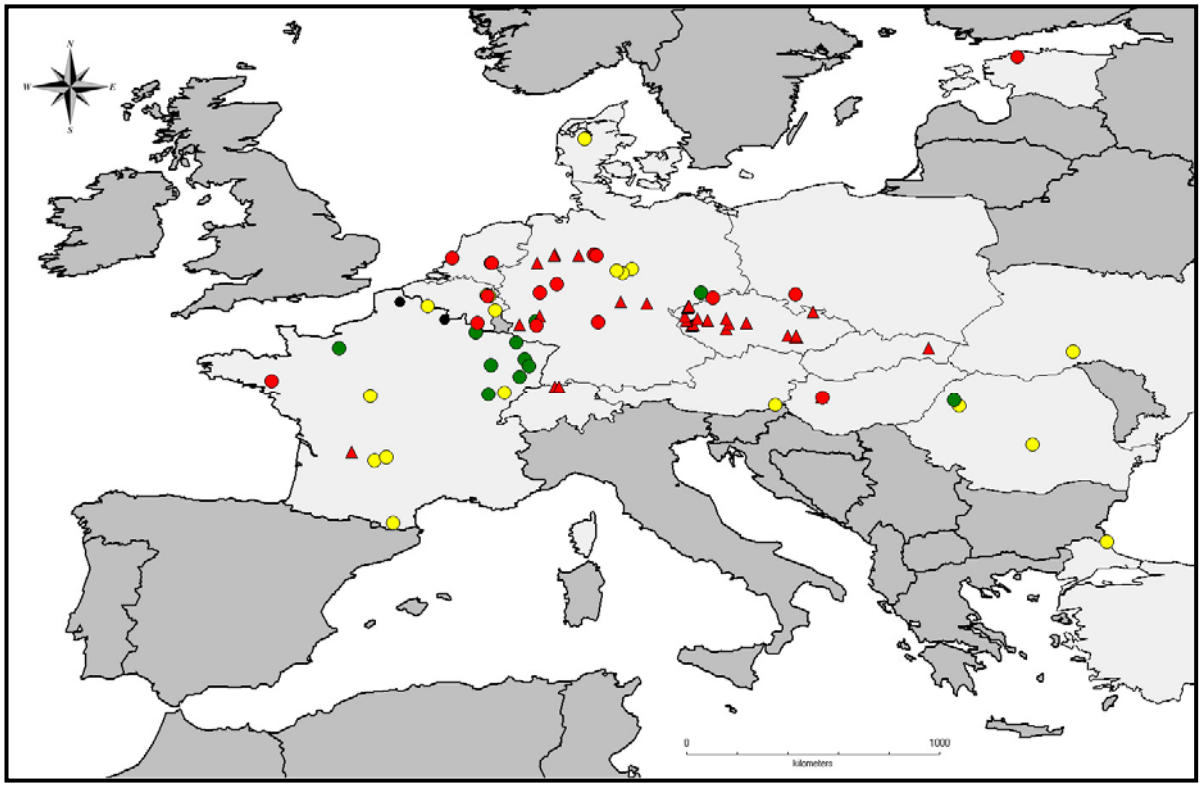

Fig. 1. Distribution of confirmed and suspected records of *G. destructans* in Europe. Data is presented for confirmed *G. destructans* records in red (circles, this study; triangles, published records), photographic evidence in yellow, visual reports in green. Dead bats without *G. destructans* are depicted as black circles.

Fig. 2. Photographic evidence showing bats with confirmed *G. destructans* growth from (A) Estonia (May 23rd 2010, © L. Lutsar), (B) Poland (March 7th 2010, © A. Wojtaszewski), (C) Belgium (March 18th 2010, © B. Mulkens), (D) France (March 4th 2010, © Y. Le Bris), (E) Netherlands (March 9th 2010, © T. Bosch) or bats with white-fungal growth suspected as *G. destructans* from (F) Austria (February 2nd 2007, © O. Gebhardt), (G) Germany (March 23rd 2010, © K. Passior), (H) Belgium (March

551 7th 2010, © F. Forget), (I) France (February 13th 2010, © J. Vittier), (J) Ukraine

552 (February 13th 2010, © A.-T. Bashta), (K) France (June 25th 2010, © F. Blanc), (L)

553 Turkey (March 22nd 2009, © M. Doker), and (M) Romania (March 29th 2008, © B.

554 Szilárd).

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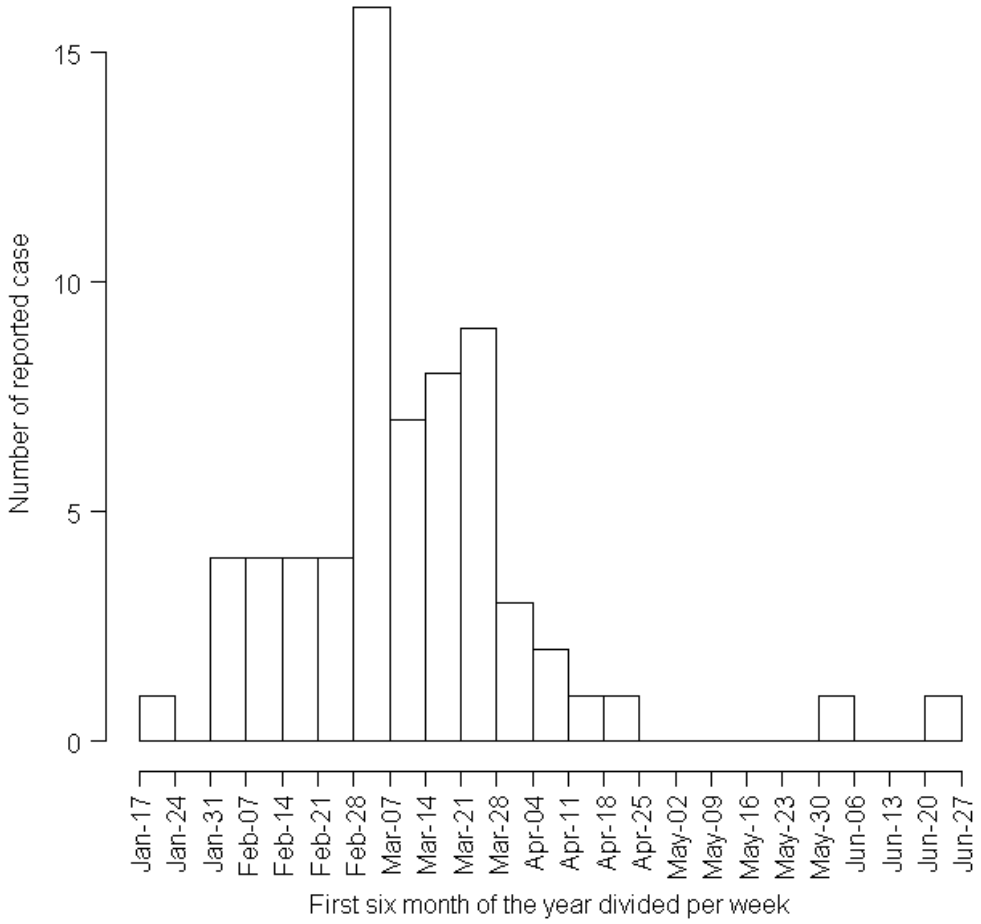

Fig. 3.Graph showing the number of live bats reported with white fungal growth (n=64) per week, starting on the first observation on January 17th and ending in June 25th after the last observation.