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First confirmed records of *Geomyces destructans* (Blehert & Gargas 2009) in Austria

by

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Synopsis: Many studies have been conducted for a better understanding of the dramatic decline of millions of bats in North America for which the fungus, *Geomyces destructans*, is responsible. However, European bats seem to cope with it and individuals convalesce from this psychrophilic pathogen.

Our study represents the first extensive data of *G. destructans* occurrence in Austria. In 2011 and 2012 we visited 102 hibernacula and found five sites with 16 *G. destructans*-affected bats. At least one bat per site was analysed by molecular methods while bats too difficult to reach were registered visually. In 2011 in two bat hibernacula *G. destructans* was not visible detected on bats but in the same hibernacula in 2012 *G. destructans* was confirmed genetically on at least one bat. Transmission pathways and reasons for differences in *G. destructans* occurrence between bats and bat populations still remain unclear. The origin and distribution of *G. destructans* in Europe is still subject of interest and our study contributes to this necessary research across national borders.

Keywords: *Geomyces destructans*, fungus, *Myotis* spp., Austria

1. Introduction:

Since the discovery of White-nose syndrome (WNS) in Howes Cave, New York State, in 2006 (Blehert et al. 2009), bat species in North America have been seriously affected by the causative fungal pathogen (Lorch et al. 2011). The psychrophilic fungus *Geomyces destructans* (Gd) was first described in 2009 (Gargas et al. 2009) and it is responsible for a dramatic population decline of hibernating bats in eastern North America (Blehert et al.

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2009). At some sites losses reach up to 100 % and formerly stable hibernacula populations are likely to face regional extinction (Frick et al. 2010). So far six species are known to succumb to WNS: *Myotis lucifugus, M. sodalis, M. septentrionalis, M. leibii, Perimyotis subflavus* and *Eptesicus fuscus*. Additionally, Gd was confirmed in three further species without evidence of disease or mortalities: *M. velifer, M. austroriparius* and *M. grisescens*.

The optimal growth rate of the cold-loving fungus, *Geomyces destructans*, ranges between 5° and 15°C (Blehert et al. 2009), a temperature which can be found in cavernous humid hibernacula (e.g. caves, adits, cellars) of many bat species. The fungus' mycelium appears as white powdery substance on nose, ears and wings of the affected bats and hyphae penetrating into the skin can cause severe ulcerative epidermal lesion. Associated with the mass mortalities of WNS are abnormal hibernation behaviour such as multiple early arousals in mid winter and outdoor day flights. It is assumed that water balance and thermoregulatory functions of these bats are markedly disturbed and as a fatal consequence body fat reserves decrease before the end of hibernation resulting in fatal starvation or weakness (Cryan et al. 2010).

It is thought that Gd is spread amongst bats by using the same swarming and hibernating sites, but the exact transmission mechanisms are still unclear. Puechmaille et al. (2011) reported the isolation of Gd from the cave wall where a few days before a bat covered with Gd was hibernating. In Europe the presence of Gd was first genetically confirmed in 2009 (Puechmaille et al. 2010), sampled from a hibernating *M. myotis* in France. In the United States WNS is directly linked to mass mortality, but no mass mortality has been recorded for Europe (Puechmaille et al. 2011). The first evidence for European bats to survive the colonisation by the fungus was made in Hungary where an affected banded *M. myotis* was captured in 2009 and recaptured in 2010 (Wibbelt et al. 2010). In total, so far 7 European bat species are confirmed for Gd colonisation: *M. myotis, M. oxygnathus (*former *M. blythii), M. nattereri, M. daubentonii, M. dasycneme, M. mystacinus* and *M. brandtii*. One additional species, *M. escalerei*/sp. A is classified as Gd-suspect via photographic documentation (Puechmaille et al. 2011). Up to now the fungus was recorded in 15 European countries either by molecular methods (Belgium, Czech Republic, Estonia, France, Germany, Hungary, The Netherlands, Poland, Slovakia, Switzerland and Ukraine) or by photographic/visual detection (Austria, Denmark, Romania and Turkey) (Martínková et al. 2010; Puechmaille et al. 2011; Wibbelt et al. 2010).

Such findings underline the importance of international communication for a better understanding of the ecology of the fungus. The first indication for the occurrence of Gd in Austria was a photograph taken in February 2007 in Styria showing a *M. myotis / oxygnathus* with the characteristic growth pattern of Gd around its nose (Gebhardt 2010, Puechmaille et al. 2011). Following this suspicious photograph as well as serial evidence of bats in Europe colonized by Gd, a group of Austrian volunteers started a monitoring programme in February 2011 to confirm and document the appearance and distribution of Gd in Austria. Here we report the first results of this study covering a broad area of Austria.

2. Material and methods:

Between mid February and mid April 2011 and 2012 we visited 102 hibernacula (84 once, 16 twice and 2 three times), in six federal states of Austria including Carinthia, Lower Austria, Upper Austria, Salzburg, Styria and Vorarlberg. The only data from western Austria derive from two caves in Vorarlberg (Fig. 1), other hibernacula are rarely known in the West. The investigated hibernacula were partly known as wintering sites of *Myotis* spp. but also included new sites suitable for the presence of *Myotis* spp. in roosts potentially used between summer and winter.

During the hibernacula inspection in 2011 and 2012 hibernating bats were visually examined for suspicious fungal growth in order to collect samples. Fungus was sampled from the nose of eight live bats and one dead bat in Carinthia, Lower Austria and Styria by touch imprints with adhesive tape which was subsequently adhered on a microscope slide and stored in a sterile plastic tube. For each bat new disposable gloves were used to avoid contamination. This sampling technique is a common and widely-used method and does not harm the bat (Wibbelt et al. 2010). All samples were sent to the Leibniz Institute of Zoo and Wildlife Research (IZW), Berlin, Germany, for further analyses.

Permission numbers from the department of nature conservation for Carinthia (VK3-NS-724/2010(002/2010), VL3-NS-1308/2006(008/2010), SV19-NAT-522/2009-4), Lower Austria (RU5-BE-517/004-2010), Upper Austria (0055325/2009 ABA Nord 501/N093052, N-104912/22-2012-Has/Gre), Salzburg (21301-RI-7/133-2007), Styria (6-6-1/2010-4a, 6.00-13/2009, 6.0-160/2009, 6.0 95 – 2004/16) and Vorarlberg (BHBR-I-7100.00-210/0050).

Fungus identification:

Glass slides with adhesive tape samples were examined by light microscopy to search for fungal hyphae and conidia with morphologic characteristics of Gd. Areas containing high numbers of conidia were marked and after disinfecting the tapes' surfaces with 70% ethanol the respective areas were cut out with a sterile scalpel blade. One part of these tape samples was used for culture isolation on Saboreoux agar, the other part was submerged in PrepMan Ultra reagent (Applied Biosystems, Darmstadt, Germany) and subjected to molecular analysis as described earlier (Wibbelt et al. 2010). Additionally, samples of successful culture isolation were equally investigated by molecular analysis.

3. Results:

Out of 102 visited bat hibernacula 78 sheltered 2283 different bats during the study period. In total, 721 individuals from the *Myotis* genus were found and out of these 16 were visibly affected by fungal growth on nose, ears and/or wings (Table 1). In four out of five hibernacula Gd was confirmed from six out of nine sampled bats identified as *Myotis myotis/oxygnathus* (Table 1). For the protection of hibernating bats, sampled individuals were not handled for exact species identification.

As shown in figure 1 Gd was isolated at four locations of low altitude (between 300 and 712 m a.s.l.) from the North, East and South of Austria. The first Gd evidence for Austria was verified for two individuals of *M. myotis/oxygnathus* sampled in Hermann's Cave (627 m a.s.l., the largest show cave of Lower Austria) in 2011. In the same year a suspected individual was photographed in Schachern Cave (Fig. 2B) but could not be genetically confirmed for Gd colonisation (Table 1, Fig. 1, northern black triangle). In 2012 a further Gd-positive sample was derived from a *M. myotis/oxygnathus* in the Aqueduct in

Hainburg in Lower Austria. In Styria the first Gd evidence for Austria was recorded by a photograph in 2007 (GEBHARDT 2010; PUECHMAILLE et al. 2011) (Fig. 1, southern black triangle). In 2011 Gd could not be verified at this location as bats were absent from this cave, but in spring 2012 two bats (*M. myotis/oxygnathus*) were tested positive for Gd in direct distance of 50 km at Adolf-Mayer-Cave. On one dead bat sampled from Adolf-Mayer-Cave Gd was not present. The southernmost finding of Gd was located in Carinthia, less than 10 km from the border to Slovenia (Table 1).

In addition to hibernacula sheltering Gd-negative bats (Fig. 1, white dots) 24 hibernacula without bats are included in the data set but these sites are not shown on the map due to overlapping of symbols.

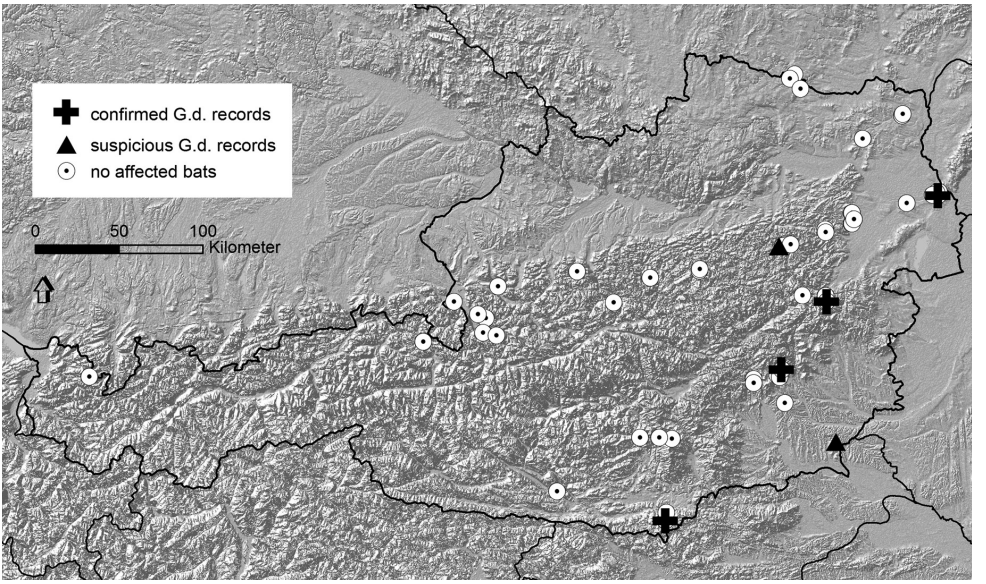

Fig. 1: Map of Austria with hibernacula investigated for bats colonized by *Geomyces destructans* in 2011 & 2012.

Fig. 2: Photographic records A: of a Gd-confirmed bat (ID-number: Gd-NOE-002) from Hermann's Cave, Lower Austria on 1st of April 2011 (© L. Plan), B: of a suspicious Gd-affected bat from Schachern Cave, Lower Austria, March 2011 (© M. Schröder) and C: from Adolf-Mayer-Cave in Styria, March 2012 (© O. Gebhardt).

Table 1: Records of *Geomyces destructans* on sampled *M. myotis/oxygnathus* (ID-Number) in Austria.

Federal state	Location	Date	ID-Number	total No of suspected bats	Gd	analyses
Lower Austria	Hermann's Cave	01/04/2011	NOE-Gd-002	5	positive	light microscopy + molecular
Lower Austria	Hermann's Cave	01/04/2011	NOE-Gd-003		positive	light microscopy + molecular
Lower Austria	Aqueduct Hainburg	18/02/2012	NOE-Gd-004	1	positive	light microscopy + molecular
Lower Austria	Schachern Cave	27/03/2011	-	1	suspect	photograph
Lower Austria	Schachern Cave	24/03/2012	NOE-Gd-006	2	negative	light microscopy
Styria	Adolf-Mayer-Cave	18/03/2012	STMK-Gd-001	5 (+1)	positive	light microscopy + molecular
Styria	Adolf-Mayer-Cave	18/03/2012	STMK-Gd-002		negative	light microscopy
Styria	Adolf-Mayer-Cave	18/03/2012	STMK-Gd-004		positive	light microscopy + molecular
Styria	Adolf-Mayer-Cave	18/03/2012	STMK-Gd-005†		negative	light microscopy
Carinthia	Türkenkopfstollen	10/04/2012	OT-K-001	1	positive	light microscopy

† dead *M. myotis* ( ) number of dead bats

Table 2: Bat species in caves where *Geomyces destructans* was present in 2011 or 2012. (Bbarb = *Barbastella barbastellus*, Mbech = *Myotis bechsteinii*, Mdau = *Myotis daubentonii*, Mema = *Myotis emarginatus*, Mmyo/oxy = *Myotis myotis/oxygnathus*, Mmys/bra = *Myotis mystacinus/brandtii,* Mnat = *Myotis nattereri*, Rhip = *Rhinolophus hipposideros*, Rfer = *Rhinolophus ferrumequinum*).

Bat species	Location	Year	No of bats	Gd
Rhip (61), Rfer (1), Mmyo/oxy (9), Mdau (1), *Myotis* sp. (1), unidentified (1)	Hermann's Cave	2011	74	+
Rhip (321), Bbarb (1), Mmyo/oxy (2)	Hermann's Cave	2012	324	-
Mmyo/oxy (16), Mnat (1)	Aqueduct Hainburg	2011	17	-
Mmyo/oxy (14), Mnat (1)	Aqueduct Hainburg	2012	15	+
Rhip (86), Mmyo/oxy (25), Mema (83), Mnatt (4), Mbech (1), *Myotis* sp. (1)	Schachern Cave	2011	200	+*
Rhip (61), Mmyo/oxy (31), Mema (106), Mmys/bra (1), *Myotis* sp. (1)	Schachern Cave	2012	199 (+1)	+*
Mmyo/oxy (202), Rhip (34), Rfer (1), Mmys/bra (6)	Adolf-Mayer-Cave	2012	242 (+1)	+
Rhip (39), Mmys/bra (1)	Türkenkopfstollen	2011	40	-
Rhip (24), Mmyo/oxy (1)	Türkenkopfstollen	2012	25	+

() number of dead bats * photographic record (Fig.2B, BÜRGER & SCHRÖDER 2011)

4. Discussion:

This study represents the first confirmed records of Gd in Austrian hibernating bats (Fig. 1, black crosses). The popular show cave, Hermann's Cave, in Lower Austria is one of the best documented larger caves in regard to bat species and numbers of hibernating

bats in Austria. As published in Baar et al. (1986) first records of bats in Hermann's Cave date back to 1836 and since 1945 annual counting sessions were conducted between December and February. From 1969 to 1999 bats were directly handled to specifically separate *M. myotis* and *M. oxygnathus* by morphological features (Baar et al. 1986; Hermann's Cave responsibles – personal communication, personal records from A. Mayer). Since 1970 additional census counts were performed in March, coincidently the ideal time to recognize Gd by its white mycelium on hibernating bats (Puechmaille et al. 2011), but fungus-like structures on any part of the bats have so far never been registered. Beside lesser horseshoe bats (*R. hipposideros*), the most abundant species in the cave, *M. myotis* is a permanent resident with fluctuating numbers (census counts in March since 1970: 1-28) (Hermann's Cave responsibles – personal communication, personal records from A. Mayer).

In comparison to other cave systems Hermann's Cave is highly frequented by tourists in summer and despite the occasional visits in winter and spring (e.g. repair of guiding pathway, bat census, etc.) fungal growth on bats has never been reported before (H. Mrkos – personal communication).

In 1958 *M. myotis* individuals were banded at Hermann's Cave and one year later a female was found 113 km southeast in Tekenye, Kom. Zala in Ungarn, (Baar et al. 1986). Interestingly, at sites very close to this recapture location (about 44 km, pers. comm., T. Görföl) *M. myotis* and *M. oxygnathus* with Gd were detected (Wibbelt et al. 2010, Puechmaille et al. 2011) allowing the speculation that spreading of fungal spores between cross-national roosts may be considered.

Visible appearance of the fungus depends on different factors like regional climate, ambient weather conditions, the cave's microclimate, arousal behaviour and the origin of the bats. As the first evidence for Gd was verified in Hermann's Cave in April 2011 (Table 1, Fig. 2A), in mid February 2012 fungal growth was not noticed on bats. At the same time Gd was detected on a bat in a hibernacula in the warmer eastern part of Austria, the Aqueduct of Hainburg. Hermann's Cave is located in a cooler region and the number of *M. myotis/oxygnathus* individuals increase in March therefore we can expect visible fungus later in the season. At the end of March we found evidences for Gd in Schachern Cave (Fig.1, northern black triangle) located 44 km westward to Hermann's Cave which allows bats to use this cave for possible roost changes. In Schachern Cave, which mainly inhabited *R. hipposideros* and *M. emarginatus* (Table 2), Gd-like fungal growth was visibly detected on three individuals of *M. myotis/oxygnathus* (Fig. 2B). Unfortunately, molecular analyses could not verify the presence of Gd.

Adolf-Mayer-Cave is a cave with the highest numbers of *Myotis myotis/oxygnathus* (Table 2) forming clusters up to 20-30 individuals tightly stacked together, but only 6 visible Gd colonised bats in different clusters as well as solitary (Fig. 2C) could be noticed. Before our survey no data are available from this cave. Lorch et al. (2011) emphasized the transmission of Gd between bats occurs via direct contact and assumes that Gd is not transferred through the air and thus, bats in further distance are not infected. Low abundance of Gd but various other *Geomyces* species were detected in soil samples from WNS affected

sites in North America (Lindner et al. 2011) indicating a minor importance in non-bat contact transmissions. Nonetheless, Gd was isolated from a cave wall in Estonia a few days after a Gd-colonised bat had left. Thus, resting places can also be considered as a contact site for transmission (Puechmaille et al. 2011). This may explain the random occurrence of Gd-affected bats in Adolf-Mayer-Cave assuming that they change hanging sites during winter, but the question why only a few bats are colonised with visible fungus still remains to be answered.

It is intriguing that in our investigations visible fungal growth was only detected on *Myotis myotis/oxygnathus* although other *Myotis* species were found in the same location of Gd confirmed bats (Table 2). Bat species with Gd of other European countries are listed in Puechmaille et al. (2011) and the questions remain on transmission pathways and susceptibility of single individuals as well as different species in Austria and in other European countries.

Our investigations raise the question whether the occurrence of Gd is a recent phenomenon in Austrian hibernacula or whether it has been associated with our hibernating bats for some time already. Whatever the right answer might be, the small number of bats found with Gd colonization gives us the great opportunity to document the distribution and possible spread of Gd amongst the different hibernacula in Austria in longitudinal studies.

White fungal structures on the nose of bats were recognized in the early eighties in Germany (Feldmann 1984, Ohlendorf et al. 2011), but without further attention till the devastating impact of Gd in North America. Severe ulcerative wing damages caused by Gd have not yet been recorded in European Gd-affected bats and associated mass mortality has also not been found in Europe (Puechmaille et al. 2011), prompting the assumption, that European bats are resistant or more tolerant to Gd colonisation (Warnecke et al. 2012). In contrast, seven bat species in North America are fatally affected by this infectious disease. The virulence of the fungus and fast velocity of fungal spread into intact and naive ecosystems in North America is in concordance with the occurrence of a novel pathogen, and researchers keep pursuing their search for the origin of the fungus and its transmission pathways in nature. In a recent study Pikula et al. (2012) describe two dead bats and one live bat from Czech Republic associated with Gd. Although they could proof that Gd was causing typical superficial lesions in the outer layers of the skin of the live bat, it seems critical in this case to use the term WNS instead of Gd-infection, because the typical WNS criteria like changes in hibernation behaviour or mass mortality were not found.

Further investigations, especially in the western part of Austria, are necessary to make a more detailed statement on the geographic distribution of Gd in Austria. But due to the limited number of known winter roosts of *Myotis* species in this part of the country and the difficulties to reach potential hibernacula under the harsh winter conditions acquiring additional data will be challenging.

However, in cooperation with our international colleagues from different European countries we will continue our efforts to gain further knowledge on Gd distribution as well as transmission pathways across borders.

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