

Correspondence

First report of host co-infection of parasitic amphibian chytrid fungi

STEFAN LÖTTERS¹, NORMAN WAGNER¹, ALEXANDER KERRES², MIGUEL VENCES³, SEBASTIAN STEINFARTZ³, JOANA SABINO-PINTO³, LARISSA SEUFER⁴, KATHLEEN PREISSLER³, VANESSA SCHULZ³ & MICHAEL VEITH¹

¹Trier University, Biogeography Department, Universitätsring 15, 54296 Trier, Germany

²Grünentalstr. 36, 52156 Monschau, Germany

³Braunschweig University of Technology, Zoological Institute, Mendelssohnstr. 4, 38106 Braunschweig, Germany

⁴Ruhr-Universität Bochum, Evolutionsökologie und Biodiversität der Tiere, Universitätsstr. 150, 44780 Bochum

Corresponding author: STEFAN LÖTTERS, e-mail: loetters@uni-trier.de

Manuscript received: 19 September 2018

Accepted: 29 September 2018 by JÖRN KÖHLER

Emerging fungal diseases are increasingly contributing to the global biodiversity crises (FISHER et al. 2012). Among the most blatant are amphibian chytridiomycoses (GARNER et al. 2016), caused by two species of chytrid skin fungi (VAN ROOIJ et al. 2015), *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*). Both fungi are of Asian origin (MARTEL et al. 2014, LAKING et al. 2017, O'HANLON et al. 2018).

Bd parasites species in all three amphibian orders, and it is suggested to be globally responsible for many species declines. It is known to have spread over all continents where amphibians occur (FISHER et al. 2009, VAN ROOIJ et al. 2015). In Europe, *Bd* is present in many countries and host species. Here, many amphibian taxa apparently can coexist with *Bd*, while others have severely suffered from it (GARNER et al. 2005, VAN ROOIJ et al. 2015), including the fire salamander, *Salamandra salamandra* (LINNAEUS, 1758), from the Iberian Peninsula (BOSCH & MARTINEZ-SOLANO 2006).

Bsal was only recently discovered after dramatic population collapses in Dutch fire salamander populations (MARTEL et al. 2013). It is only lethal to many species of the order Caudata, but *Bsal* may survive on anurans, too (NGUYEN et al. 2017, STEGEN et al. 2017). *Bsal* so far is only invasive in Western Europe (MARTEL et al. 2014, SPITZEN-VAN DER SLUIJS et al. 2016), but its spatial potential is much larger (FELDMEIER et al. 2016, BEUKEMA et al. 2018). In Western Europe, the number of recognized outbreaks has increased from 1 to 26 in seven years (SPITZEN-VAN DER SLUIJS et al. 2016, DALBECK et al. 2018). Fire salamanders are highly susceptible to *Bsal*, and at all positive sites remarkable reductions in population size have been noted, occasionally along with rapid mass mortality (MARTEL et al. 2014, STEGEN et al. 2017, DALBECK et al. 2018).

In Asia, *Bsal* occurs in macro-sympatry with *Bd*, but co-occurrence of the two fungi so far has not been found (cf. LAKING et al. 2017). Likewise, SABINO-PINTO et al. (2018), on various occasions, detected both fungi in the same captive amphibian collections in Europe, but never on the same host individual. The question of the existence of *Bd/Bsal* co-infection in the same host individual remains unanswered. We consider this an important question for two reasons, especially in Europe where *Bd* is widespread (GARNER et al. 2005, VAN ROOIJ et al. 2015), *Bsal* is currently emerging (SPITZEN-VAN DER SLUIJS et al. 2016, DALBECK et al. 2018) and both can be harmful (BOSCH & MARTINEZ-SOLANO 2006, STEGEN et al. 2017): First, multi-parasitism may have effects on host susceptibility, infection duration, transmission risk and clinical symptoms (VAUMOURIN et al. 2015, HAFER et al. 2016). Second, horizontal gene transfer (HGT), already known from *Bd* and other fungi, may increase pathogen virulence (SUN et al. 2011).

So far (2015–2018), 15 fire salamander populations in Germany have been tested positive for *Bsal*, i.e. in the Eifel Mountains and the Ruhr District (cf. DALBECK et al. 2018), including six records here reported for the first time (Table 1). From these two regions, we tested 70 *Bsal*-positive and 223 *Bsal*-negative specimens for infection with *Bd*. Furthermore, we examined another 196 individuals for both *Bd* and *Bsal* from various German sites (Table 1) far distant from the known *Bsal* outbreaks (cf. SPITZEN-VAN DER SLUIJS et al. 2016, DALBECK et al. 2018). Field-taken skin swabs were processed using simplex qPCR (for each *Bd* and *Bsal*) in accordance to the protocol of BLOOI et al. (2013, 2016). Each two swabs were taken per individual and analyzed independently in different labs.

Bd/Bsal co-infection was confirmed in one population only: Belgenbach, Eifel Mountains. In December 2015, 22 fire

Correspondence

Table 1. Sites in Germany where fire salamanders (*Salamandra salamandra*) have been studied for *Bsal* and *Bd*. Data refer to the period 2015–2018 and are split into different years for the Belgenbach site only. In addition to the *Bsal* sites reported by DALBECK et al. (2018), those indicated by * are new *Bsal* records for the year 2018. All data refer to metamorphosed salamanders (adults and subadults), whereas data from the Belgenbachtal in 2017 and 2018 refer to larvae only (indicated by ¹). North Rhine-Westphalia is abbreviated NRW.

Site	Coordinates (Latitude, Longitude)	N samples studied for <i>Bd</i> and <i>Bsal</i>	N samples <i>Bd</i> -positive	N samples <i>Bsal</i> -positive	N samples <i>Bsal</i> -positives also <i>Bd</i> -positive
Populations from the Eifel Mountains and the Ruhr District (both NRW), from where <i>Bsal</i> is generally known					
Eifel Mts., Belgenbachtal (2015, 2017 ¹ , 2018 ¹)	50.578169, 6.278448	22, 12, 2	8, 0, 0	21, 0, 0	8, 0, 0
Eifel Mts., Fischbach	50.735368, 6.294149	4	0	4	0
Eifel Mts., Haftenbach	50.613929, 6.431371	3	0	3	0
Eifel Mts., Kallerbach	50.707151, 6.391554	1	0	0	0
Eifel Mts., Lamersiefen	50.756932, 6.321338	1	0	0	0
Eifel Mts., Rosbach	50.692512, 6.435706	10	0	0	0
Eifel Mts., Sauerbach	50.574681, 6.401072	2	0	2	0
Eifel Mts., Solchbach	50.70178, 6.270098	23	0	4	0
Eifel Mts., Simonskall	50.664329, 6.358932	1	0	1	0
Eifel Mts., Vichtbach	50.676448, 6.198452	27	0	0	0
Eifel Mts., Weberbach	50.735089, 6.359651	7	0	7	0
Eifel Mts., Weisse Wehe	50.717116, 6.345695	19	0	5	0
Eifel Mts., Zweifallshammer*	50.682388, 6.423373	24	0	3	0
Ruhr District, Bochum, Botanischer Garten*	51.442199, 7.266148	23	0	1	0
Ruhr District, Bochum Dahlhausen*	51.435073, 7.139507	1	0	1	0
Ruhr District, Bochum, Klosterbusch*	51.442880, 7.270582	2	0	1	0
Ruhr District, Bochum Lottental*	51.440284, 7.256134	4	0	1	0
Ruhr District, Essen, Eschenstraße	51.423689, 7.019866	97	0	8	0
Ruhr District, Essen, Südwestfriedhof	51.431025, 6.968945	8	0	8	0
Total Eifel Mountains, Ruhr District		293	8	70	8
Additional sites from regions where <i>Bsal</i> is unknown (various federal states)					
Bergisches Land, Remscheid, NRW	51.1541, 7.1763	5	0	0	0
Bergisches Land, Wuppertal Barmen, NRW	51.247764, 7.168684	11	1	0	0
Billerbeck, NRW	51.9860, 7.3330	4	0	0	0
Ehrenberg, Kriebstein, Saxony	51.0375, 13.0167	25	0	0	0
Nationalpark Harz, Ilsenburg, Saxony-Anhalt	51.8405, 10.6434	8	0	0	0
Kottenforst near Bonn (various sub-sites), NRW	50.65–50.69, 7.06–70.12	51	0	0	0
Solling (various sub-sites), Lower Saxony	51.80–51.89, 9.49–9.60	45	0	0	0
Tharandt, Saxony	50.9701, 13.5824	25	0	0	0
Waldeck, Thuringia	50.9139, 11.7769	22	0	0	0
Total all sites		489	9	70	8

salamanders were found here, 16 of which dead (SPITZEN-VAN DER SLUIJS et al. 2016). In 21, *Bsal* could be confirmed (SPITZEN-VAN DER SLUIJS et al. 2016), and eight of them we here report to be infected with *Bd*, too. Individual infection loads of *Bd/Bsal* ranged 0.1–0.4/0.14–167.1 GE, i.e. low for *Bd* (cf. BALÁŽ et al. 2013) and low to high for *Bsal* (cf. MARTEL et al. 2014, SPITZEN-VAN DER SLUIJS et al. 2016). Since the observed rapid mass mortality at the Belgenbach, only a few fire salamander larvae have been recorded there. They neither had *Bd* nor *Bsal* (Table 1), but chytrids are suggest-

ed to infect salamander larvae at late developmental stages only (VAN ROOIJ et al. 2015) so that perhaps the studied specimens were not susceptible when sampled.

While our data show that *Bsal*-only infection in fire salamanders is relatively common in the Eifel Mountains and the Ruhr District (SPITZEN-VAN DER SLUIJS et al. 2016, DALBECK et al. 2018), our findings suggest that *Bd*-only infection is rare in this species in Germany. Among the almost 500 studied specimens, just one with *Bd*-only infection was found, i.e. in a population from outside the known

Bsal range (Table 1). Our results are in concert with previous studies which suggest that *Bd* in fire salamanders in Germany is rare (e.g. OHST et al. 2011). In this context it is interesting to note that to the best of our knowledge, fire salamander declines referable to *Bd* are unknown in central Western Europe, in contrast to the rapid declines on the Iberian Peninsula attributed to this pathogen (BOSCH & MARTINEZ-SOLANO 2006).

This is the first report of host co-infection with the two amphibian chytrid fungi *Bd* and *Bsal*. We currently lack information about what co-infection means for the course of a chytridiomycosis outbreak. However, infection with multiple parasites often leads to increased host stress due to conflicts of interest and resource competition of parasites (HAFFER et al. 2016). The result can be a higher susceptibility to either or both pathogens combined with a higher transmission and mortality risk (VAUMOURIN et al. 2015). Besides an alteration of the host manipulation, co-infection can also lead to a suppression of one parasite, so finally pathogen interaction can be synergetic or antagonistic (HAFFER et al. 2016). Moreover, *Bd/Bsal* co-infection is also concerning because of potential HGT. At least *Bd* has acquired virulence effector genes from bacteria and oomycete pathogens (SUN et al. 2011). Hence, HGT between *Bd* and *Bsal* seems expectable when co-existing on the same host individual, hypothetically resulting in new virulent versions of both.

Amphibian chytridiomycoses belong to the globally most dangerous emerging fungal wildlife diseases (FISHER et al. 2012, STEGEN et al. 2017). Mitigating chytrid-related biodiversity loss remains challenging (GARNER et al. 2016), especially as *Bsal* alone severely threatens the entire Western Palearctic salamander fauna (MARTEL et al. 2014, BEUKEMA et al. 2018). Before this background, *Bd/Bsal* co-infection is alarming news. Systematic and intensified field surveys and laboratory studies are necessary to study the potential hazard. As *Bsal* is apparently a novel invasive pathogen in Europe, currently in the process of spreading (cf. SPITZEN-VAN DER SLUIJS et al. 2016, DALBECK et al. 2018), the potential impact of *Bd/Bsal* co-infection, either positive or negative, should not be overlooked and considered in conservation strategies that currently are being developed with the goal to dam amphibian chytridiomycoses.

Acknowledgements

We are grateful to all collaborators from Braunschweig and Trier Universities as well as the Biological Stations of Aachen, Düren and Westliches Ruhrgebiet. We really appreciated the support of the Ruhr-University Bochum, especially of JONAS VIRGO and MATHIAS KRISCH who initiated the data collection in Bochum. In addition, we thank ANDREA KIRZINGER (Nationalpark Harz), ANDREAS NÖLLERT and SUSANNE UHLEMANN for taking skin swabs. Moreover, we would like to thank all authorities who permitted fieldwork and our donors, Federal Agency of Nature Conservation (BfN), Deutsche Bundesstiftung Umwelt (DBU), NIKOLAUS KOCH Stiftung, Stiftung Artenschutz/Verband der Zoologischen Gärten (VDZ).

References

- BALÁŽ, V., J. VÖRÖS, P. CIVIŠ, J. VOJAR, A. HETTYEY, E. SÓS, R. DANKOVICS, R. JEHLE, D. G. CHRISTIANSEN, F. CLARE, M. C. FISHER, T. W. GARNER & J. BIELBY (2013): Assessing risk and guidance on monitoring of *Batrachochytrium dendrobatidis* in Europe through identification of taxonomic selectivity of infection. – *Conservation Biology*, **28**: 123–223.
- BEUKEMA, W., A. MARTEL, T. T. NGUYEN, K. GOKA, D. S. SCHMELLER, Z. YUAN, A. E. LAKING, T.-Q. NGUYEN, C.-F. LIN, J. SHELTON, A. LOYAU & F. PASMANS (2018): Environmental context and differences between native and invasive observed niches of *Batrachochytrium salamandrivorans* affect invasion risk assessments in the Western Palaearctic. – *Diversity & Distributions*, in press.
- BLOOI, M., F. PASMANS, J. E. LONGCORE, A. SPITZEN-VAN DER SLUIJS, F. VERCAMMEN & A. MARTEL (2013): Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. – *Journal of Clinical Microbiology*, **51**: 4173–4177.
- BLOOI, M., F. PASMANS, J. E. LONGCORE, A. SPITZEN-VAN DER SLUIJS, F. VERCAMMEN & A. MARTEL (2016): Correction for BLOOI et al., duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. – *Journal of Clinical Microbiology*, **54**: 246.
- BOSCH, J. & I. MARTINEZ-SOLANO (2006): Chytrid fungus infection related to unusual mortalities of *Salamandra salamandra* and *Bufo bufo* in Peñalara National Park, Spain. – *Oryx*, **40**: 84–89.
- DALBECK, L., H. DÜSSEL-SIEBERT, A. KERRES, K. KIRST, A. KOCH, S. LÖTTTERS, D. OHLHOFF, J. SABINO-PINTO, K. PREISSLER, U. SCHULTE, V. SCHULZ, S. STEINFARTZ, M. VEITH, M. VENCES, N. WAGNER & J. WEGGE (2018): Die Salamanderpest und ihr Erreger *Batrachochytrium salamandrivorans* (*Bsal*): aktueller Stand in Deutschland. – *Zeitschrift für Feldherpetologie*, **25**: 1–22.
- FELDMEIER, S., L. SCHEFCZYK, N. WAGNER, G. HEINEMANN, M. VEITH & S. LÖTTTERS (2016): Present and future high risk zones for the spreading lethal salamander chytrid fungus in its invasive range in Europe using bioclimate and weather extremes. – *PLoS ONE*, **11**: e0165682.
- FISHER, M. C., T. W. J. GARNER & S. F. WALKER (2009): Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. – *Annual Reviews in Microbiology*, **63**: 291–310.
- FISHER, M. C., D. A. HENK, C. J. BRIGGS, J. S. BROWNSTEIN, L. C. MADOFF, S. L. MCCRAW & S. J. GURR (2012): Emerging fungal threats to animal, plant and ecosystem health. – *Nature*, **282**: 186–194.
- GARNER, T. W. J., S. WALKER, J. BOSCH, A. D. HYATT, A. A. CUNNINGHAM & M. C. FISHER (2005): Chytrid fungus in Europe. – *Emerging Infectious Diseases*, **282**: 1639–1641.
- GARNER, T. W., B. R. SCHMIDT, A. MARTEL, F. PASMANS, E. MUTHS, A. A. CUNNINGHAM, C. WELDON, M. C. FISHER & J. BOSCH (2016): Mitigating amphibian chytridiomycoses in nature. – *Philosophical Transactions of the Royal Society London B*, **5**: 371.

- HAFFER, N. (2016): Conflicts over host manipulation between different parasites and pathogens: Investigating the ecological and medical consequences. – *Bioessays*, **38**: 1027–1037.
- LAKING, A. E., H. N. NGO, F. PASMANS, A. MARTEL & T. T. NGUYEN (2017): *Batrachochytrium salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders. – *Scientific Reports*, **7**: 44443.
- MARTEL, A., A. SPITZEN-VAN DER SLUIJS, M. BLOOI, W. BERT, R. DUCATELLE & M. C. FISHER (2013): *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. – *Proceedings of the National Academy of Sciences of the USA*, **110**: 15325–15329.
- MARTEL, A., M. BLOOI, C. ADRIAENSEN, P. VAN ROOIJ, W. M. BEUKEMA, M. C. FISHER, R. A. FARRER, B. R. SCHMIDT, U. TOBLER, K. GOKA, K. R. LIPS, C. MULETZ, K. R. ZAMUDIO, J. BOSCH, S. LÖTTERS, E. WOMBWELL, T. W. J. GARNER, A. A. CUNNINGHAM, A. SPITZEN-VAN DER SLUIJS, S. SALVIDIO, R. DUCATELLE, K. NISHIKAWA, T. T. NGUYEN, J. E. KOLBY, I. VAN BOCXLAER, F. BOSSUYT & F. PASMANS (2014): Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. – *Science*, **346**: 630–631.
- NGUYEN, T. T., T. V. NGUYEN, T. ZIEGLER, F. PASMANS & A. MARTEL (2017): Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. – *Amphibia-Reptilia*, **38**: 554–556.
- O'HANLON, S. J., A. RIEUX, R. A. FARRER, G. M. ROSA, B. WALDMAN, A. BATAILLE, T. A. KOSCH, K. A. MURRAY, B. BRANKOVICS, M. FUMAGALLI, M. D. MARTIN, N. WALES, M. ALVARADO-RYBAK, K. A. BATES, L. BERGER, S. BÖLL, L. BROOKES, F. CLARE, E. A. COURTOIS, A. A. CUNNINGHAM, T. M. DOHERTY-BONE, P. GHOSH, D. J. GOWER, W. E. HINTZ, J. HÖGLUND, T. S. JENKINSON, C.-F. LIN, A. LAURILA, A. LOYAU, A. MARTEL, S. MEURLING, C. MIAUD, P. MINTING, F. PASMANS, D. S. SCHMELLER, B. R. SCHMIDT, J. M. G. SHELTON, L. F. SKERRATT, F. SMITH, C. SOTO-AZAT, M. SPAGNOLETTI, G. TESSA, L. F. TOLEDO, A. VALENZUELA-SÁNCHEZ, R. VERSTER, J. VÖRÖS, R. J. WEBB, C. WIERZBICKI, E. WOMBWELL, K. R. ZAMUDIO, D. M. AANENSEN, T. Y. JAMES, M. T. P. GILBERT, C. WELDON, J. BOSCH, F. BALLOUX, T. W. J. GARNER & M. C. FISHER (2018): Recent Asian origin of chytrid fungi causing global amphibian declines. – *Science*, **360**: 621–627.
- OHST, T., Y. GRÄSER, F. MUTSCHMANN & J. PLÖTNER (2011): Neue Erkenntnisse zur Gefährdung europäischer Amphibien durch den Hautpilz *Batrachochytrium dendrobatidis*. – *Zeitschrift für Feldherpetologie*, **18**: 1–17.
- SABINO-PINTO, J., M. VEITH, M. VENCES & S. STEINFARTZ (2018): Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. – *Scientific Reports*, **8**: 11767.
- SPITZEN-VAN DER SLUIJS, A., A. MARTEL, J. ASSELBERGHS, E. K. BALES, W. BEUKEMA, M. C. BLETZ, L. DALBECK, E. GOVERSE, A. KERRES, T. KINET, K. KIRST, A. LAUDELOUT, L. F. MARIN DA FONTE, A. NÖLLERT, D. OHLHOFF, J. SABINO-PINTO, B. R. SCHMIDT, J. SPEYBROECK, F. SPIKMANS, S. STEINFARTZ, M. VEITH, M. VENCES, N. WAGNER, F. PASMANS & S. LÖTTERS (2016): Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. – *Emerging Infectious Diseases*, **22**: 1286–1288.
- STEGEN, G., F. PASMANS, B. R. SCHMIDT, L. O. ROUFFAER, S. VAN PRAET, M. SCHAUB, S. CANESSA, A. LAUDELOUT, T. KINET, C. ADRIAENSEN, F. HAESBROUCK, W. BERT, F. BOSSUYT & A. MARTEL (2017): Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. – *Nature*, **544**: 353–356.
- SUN, G., Z. YANG, T. KOSCH, K. SUMMERS & J. HUANG (2011): Evidence for acquisition of virulence effectors in pathogenic chytrids. – *BMC Evolutionary Biology*, **11**: 195.
- VAN ROOIJ, P., A. MARTEL, F. HAESBROUCK & F. PASMANS (2015): Amphibian chytridiomycosis: a review with focus on fungus-host interactions. – *Veterinary Research*, **46**: 137.
- VAUMOURIN, E., G. VOURC'H, P. GASQUI & M. VAYSSIER-TAUSSAT (2015): The importance of multiparasitism: examining the consequences of co-infections for human and animal health. – *Parasites & Vectors*, **8**: 545.