Epidemiological screening of captive salamanders reveals current absence of *Batrachochytrium salamandrivorans* in private collections throughout the federal state of Hesse (Germany)

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Abstract. The infamous chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*) recently led to the collapse of European fire salamander populations (*Salamandra salamandra*) in The Netherlands. Currently, the pathogen has been rapidly expanding its range and threatens salamander populations throughout Europe, including Germany. Here, *Bsal* is known from wild and captive amphibians and has mostly been reported from the federal state of Northrhine-Westphalia. Due to the geographical proximity of infected areas, its dispersal into neighbouring states is possible. A *Bsal* taskforce was therefore recently formed in the state of Hesse that aims to implement preparative measures for *Bsal* mitigation at different levels. Based on the known *Bsal* susceptibility of salamanders in captivity and their inherent threat potential towards natural populations, an epidemiological screening for *Bsal* prevalence in private amphibian collections throughout the state of Hesse was conducted. We analysed a total of 174 samples from nine private collections of different urodelan species via qPCR and did not detect *Bsal*. We discuss our results and their implications for salamander conservation relative to other surveys of this kind and underscore the importance of tight cooperation between private keepers and conservation scientists in order to protect wild amphibians from the lethal *Bsal* fungus.

Key words. Amphibia, Caudata, *Salamandra salamandra*, *Bsal*, chytrid fungus, emerging infectious diseases, disease monitoring, European fire salamander, herpetoculture.

Introduction

Amphibians are considered the most endangered group of vertebrates (Stuart et al. 2004, Hoffmann et al. 2010). Besides habitat loss and fragmentation, emerging infectious diseases (EIDs) are largely responsible for population declines in amphibians. In particular, the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) (Longcore et al. 1999) has imperilled amphibian communities on a global scale and even caused species extinctions (Fisher et al. 2012). Recently, a newly discovered chytrid fungus, *Batrachochytrium salamandrivorans* (*Bsal*) (Martel et al., 2013), has become a major concern for conservationists in Europe. *Bsal* is notorious for infecting urodelans in a pandemic often referred to as the 'salamander plague'.

According to present knowledge, the European fire salamander, *Salamandra salamandra* (Linnaeus, 1758), is the most susceptible species for *Bsal*. Here, this fungus causes an unexceptionally lethal, dose-independent course of disease with characteristic lesions and ulcerations of the skin transmitted via skin contact with infected individuals or contaminated materials (Martel et al. 2013, 2014, Stegen et al. 2017). The inherent threat that *Bsal* poses to the European fire salamander became obvious in The Netherlands: Here, population levels have decreased by 96% due to *Bsal* outbreaks, driving the European fire salamander to the brink of extinction throughout the country (Spitzenvan der Sluijs et al. 2013). However, mass extinction processes have also been taking place in Belgium and Germany (Martel et al. 2014, Stegen et al. 2017, Dalbeck et al.

2018, Schulz et al. 2018, Wagner et al. 2019). Germany is in fact currently considered the 'hot spot' of *Bsal* infections with almost 50 affected sites in the Eifel Mountains as well as the Ruhr District in the federal states Northrhine-Westphalia and Rhineland-Palatinate (Dalbeck et al. 2018, Schulz et al. 2018, 2020 in this issue, Wagner et al. 2019, Lötters et al. 2020 in this issue).

Among the federal states of Germany, Hesse is a state of pivotal importance for salamander conservation: Its borders are located less than 150 km distant from the current *Bsal* epicentre in the Eifel and different models suggest that dispersal of the fungus into Hesse is a likely scenario (Fig. 1) (Feldmeier et al. 2016, Beukema et al. 2018). Hesse probably hosts the highest density of European fire salamander populations with a significant biomass of this species, as it is centrally located in the distribution range of the European fire salamander in Germany (Klewen 1988, Thiesmeier 2004, Deutsche Gesellschaft für Herpetologie und Terrarienkunde 2018). These circumstances have led to the founding of a task force for the protection of European fire salamanders in Hesse in 2018 (Ziemek 2019). This task force aims to develop preventative means in preparation for the arrival of *Bsal* in Hesse and to design strategies to efficiently mitigate its spread within the state.

Recent studies have suggested that *Bsal* was originally introduced to Europe from infected Asian urodelans imported for the pet trade (Martel et al. 2014, Laking et al. 2017, Nguyen et al. 2017, Yuan et al. 2018). This implies that private terrarium collections of amphibians may represent a reservoir for this biodiversity-threatening disease from which novel infections could emerge. Hence, considerable effort was invested into epidemiological tracing of *Bsal* throughout privately kept urodeles across Europe in the recent past (Cunningham et al. 2015, Sabino-Pinto et al. 2015, 2018, Fitzpatrick et al. 2018). These studies

have highlighted the infestation of such collections in, e.g., Sweden, Germany, Spain, and the United Kingdom (Cunningham et al. 2015, Sabino-Pinto et al. 2015, 2018, Fitzpatrick et al. 2018), and one of them detected *Bsal*-positive salamanders in one collection in Hesse (Sabino-Pinto et al. 2015, 2018).

This finding has made us ask to what extent the pathogen may be distributed in private collections within the federal state of Hesse. We opted for a qPCR-based tracing strategy and screened salamander stocks of hobbyists, zoos, universities and professional breeders in order to derive insights about the fungus prevalence in Hesse and to infer hypotheses about the threat potential such collections may pose to European fire salamander populations in the wild.

Material and methods

Throughout 2019, skin swabs of captive urodelans were collected from nine private collections across Hesse (Fig. 1). Animals to be sampled were randomly selected from the keepers' enclosures. First, the habitus of all animals was checked for obvious signs of infections, such as ulcerations and lesions. For the handling of animals, nitrile gloves (one set of gloves per individual) were used in order to avoid potential transmissions of *Bsal*.

Skin swabs were collected as previously described (Hyatt et al. 2007, van Rooij et al. 2011, Blooi et al. 2013): Briefly, the ventral side of each individual was wiped ten times utilizing two sterile cotton swabs (A- and B-samples for data validation), which then were stored separately in sterile Eppendorf tubes. If possible, at least three individuals per enclosure were included in this study, however, if an enclosure housed fewer individuals all available animals were analysed. Negative controls at each locality were generated by opening an unused cotton swab and placing it into a separate tube midway through the sampling process. Used swabs were stored at -20°C until further processing.

For molecular *Bsal* screening, genomic DNA was extracted by using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, DE) following the manufacturer's instructions after a pretreatment with lysozyme. Quantitative polymerase chain reaction (qPCR) was performed as published previously (Blooi et al. 2013, 2016) with the alterations of using a *Bsal*-specific FAM-labelled probe (biomers, Ulm, DE) and the TaqMan™ Fast Universal PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, US) following the manufacturers' instructions as to temperature and cycling conditions. PCR was performed on a RotorGene System (Qiagen, Hilden, DE). Prior to sample analysis, the PCR protocols detection limit was determined by testing a ten-fold dilution series of the positive control with 1000 GE/5 ml. The lower detection limit was demonstrated to be 0.1 GE/5 ml, which conforms with Blooi et al. (2013). The subsequent initial screening of all samples was performed at the Hospital for Birds, Reptiles, Amphibians and Fish of the Justus Liebig University Gießen.
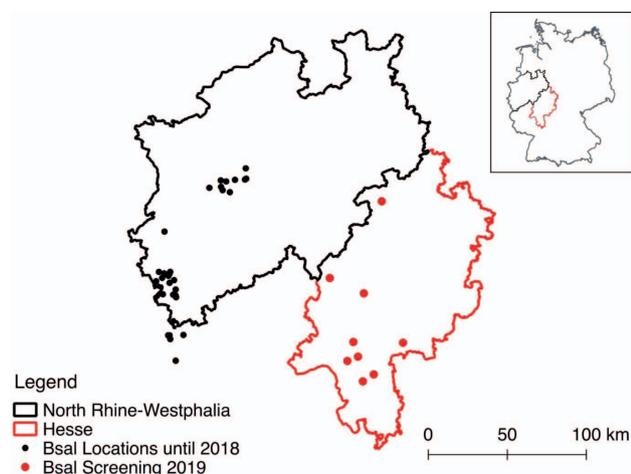

Figure 1. The federal state of Hesse (red) borders Northrhine-Westphalia (black) where Bsal outbreaks in the wild have become known (black spots, following Dalbeck et al. 2018, Schulz et al. 2018). We examined the prevalence of the pathogen in private urodelan collections throughout the state (red spots).

From all obtained samples, 20 B-samples were randomly selected, extracted and analysed a second time in an independent laboratory at the Zoological Institute of the Technische Universität Braunschweig for data validation using the protocol of Blooi et al. (2013). Here, gBlock fragments were used as a standard. The threshold for the ITS copies was set to 100 ITS based on a comparison of Ct values between both standards (1 GE = 33 cycles/100 ITS copies = 35 cycles). Each qPCR plate contained two replicates of samples, two replicates of *Bsal* standard (10-fold dilution scale from 100–10,000,000 ITS copies), and two negative controls. All runs were performed on a CFX96 Real-Time System (Bio-Rad Laboratories Inc., Hercules, CA, US).

Results

For the epidemiological screening of *Bsal* in captive urodelans within Hesse, we sampled animals from nine different localities across the federal state (Fig. 1). Our sampling included 28 taxa, 20 belonging to the family Salamandridae including several subspecies of the European fire salamander as well as the Alpine salamander, *Salamandra atra* Laurenti, 1768, and several newt species (Table 1). The remaining eight taxa included in this study represented the genus *Ambystoma*, family Ambystomatidae (Table 1).

In total, we collected skin swabs from 174 specimens and analysed all of these by means of qPCR. None of the examined animals exhibited any obvious symptoms of the salamander plague, indicating that the pathogen was either recently introduced and had not yet caused any physical symptoms, or was absent from the studied private collections. Our phenotypical examinations were in accordance with our molecular genetic analysis for *Bsal* prevalence in that all analysed skin swabs tested negative (Table 1).

Discussion

As an emerging infectious disease, the salamander plague has been spreading rapidly in Europe and has already caused several local mass extinction events in European fire salamanders (e.g., Spitzen-van der Sluijs et al. 2016, Dalbeck et al. 2018, Schulz et al. 2018, 2020 in this issue, Wagner et al. 2019, Lötters et al. 2020 in this issue). Previous studies suggested that captive urodelans may represent a reservoir for *Bsal* as indicated by the abundance of *Bsal*-positive specimens that were reported from private collections in Germany, Spain, Sweden and England (Cunningham et al. 2015, Sabino-Pinto et al. 2015, 2018, Fitzpatrick et al. 2018). Apparently, *Bsal* has been introduced to the European amphibian fauna via infected Asian newts imported for the pet trade. It has also been reported that infections of *Bsal* may be asymptomatic and therefore difficult or even impossible to detect (Sabino-Pinto et al. 2018). Recent studies found that captivity as an exter-

Table 1. List of all sampled species and subspecies, number of samples per taxon at each locality (N), and resulting *Bsal* status as identified via qPCR. A-samples were analysed from all specimens, specimens with analysed B-samples are indicated with an asterisk. Coll = Collection.

Coll	Species	N	*Bsal* status
Coll 1	*Salamandra salamandra terrestris**	9	neg.
	Ichthyosaura alpestris	3	neg.
	Neurergus crocatus	3	neg.
	Neurergus kaiseri	3	neg.
Coll 2	*Salamandra salamandra alfredschmidti*	3	neg.
	Salamandra salamandra bernardezi	3	neg.
	Salamandra salamandra gigliolii	3	neg.
	Salamandra salamandra terrestris	6	neg.
	Tylototriton shanjing	3	neg.
	*Salamandra atra**	1	neg.
Coll 3	*Salamandra salamandra bernardezi*	4	neg.
	Salamandra salamandra salamandra	10	neg.
Coll 4	*Salamandra salamandra salamandra*	3	neg.
	Ambystoma andersoni	1	neg.
	Ambystoma macrodactylum	7	neg.
	*Ambystoma maculatum**	5	neg.
	Ambystoma mavortium melanostictus	4	neg.
	Ambystoma mavortium nebulosum	6	neg.
	Ambystoma opacum	5	neg.
Coll 5	*Ambystoma talpoideum*	4	neg.
	*Ambystoma tigrinum**	10	neg.
	*Salamandra salamandra gallaica**	5	neg.
	Salamandra salamandra salamandra	2	neg.
	*Salamandra salamandra terrestris**	7	neg.
	Tylototriton kweichowensis	5	neg.
	*Tylototriton shanjing**	4	neg.
Coll 6	*Salamandra salamandra**	8	neg.
	*Ichthyosaura alpestris**	3	neg.
	Lissotriton vulgaris	3	neg.
	Salamandra atra	3	neg.
	*Salamandra salamandra bernardezi**	3	neg.
Coll 7	*Salamandra salamandra terrestris*	3	neg.
	Taricha granulosa	4	neg.
	*Triturus cristatus**	3	neg.
	Triturus marmoratus	3	neg.
Coll 8	*Salamandra salamandra**	1	neg.
	Ichthyosaura alpestris	3	neg.
	Lissotriton italicus	3	neg.
	Salamandra salamandra bernardezi	3	neg.
Coll 9	*Salamandra salamandra gallaica*	3	neg.
	Salamandra salamandra terrestris	3	neg.
	Triturus carnifex	3	neg.
	Triturus dobrogicus	3	neg.

nal factor negatively influences the microbial communities within the mucosome (i.e., the complex micro-ecosystem of amphibian skin poisons plus their microbial symbionts), which is an important anti-infectious line of defence in amphibians (e.g., Woodhams et al. 2014, Bates et al. 2018, 2019, Lüddecke et al. 2018). As a result of this negative effect, captive animals are probably much more receptive to colonization by pathogens such as *Bsal* (e.g., Bates et al. 2019). On the other hand, animals in private collections are usually kept well fed and in good general condition. In addition, they usually receive good health monitoring so that clinical diseases are detected and can be treated early. In such collections, latent pathogen infections without obvious clinical disease are nevertheless possible (e.g., Sabino-Pinto et al. 2018). Therefore, captive collections might represent a reservoir of pathogens that may cause fatal disease if introduced into naïve free-ranging populations (e.g., Martel et al. 2014, Sabino-Pinto et al. 2015, 2018, Fitz-patrick et al. 2018, Martel et al. 2020).

All these aspects in their totality highlight the importance of close monitoring for *Bsal* in private and public collections as a protective measure, because animals kept thus have an increased likelihood of either harbouring the disease, or are at least at higher risk of being infected if the pathogen is introduced. Since any biological material that is transferred from such collections to the environment (e.g., organic waste, water or soil) constitutes a potential infectious agent, it is of pivotal importance to know the infection status of these. This urgency becomes especially obvious when the potential treatment is taken into account: *Bsal*-infected salamanders can often be treated by keeping the animal at higher temperatures (25°C) for an extended period of time (Blooi et al. 2015). This therapy often results in the eradication of *Bsal*, and many salamanders recover from it when conducted correctly and monitored well. While this therapy may be useful for captive collections with only a limited number of animals being affected, it is not practicable for wild salamander populations due to their sheer numbers and legislative obstacles (Kuz-min et al. 2009, AGAR & FENA 2010). Heat treatment does furthermore not alter the salamander immune system and therefore provides no long-term protection against *Bsal* (Stegen et al. 2017).

In their exploratory study that assessed infection rates in captive salamandrids in Germany and Sweden, Sabino-Pinto et al. (2018) detected several infected animals, with the most severely affected collection being one in Hesse. We therefore expected that several of the screened collections would also contain infected animals. However, none of our qPCR screenings recovered the presence of *Bsal* in any of the analysed localities and consequently, apart from the case documented by Sabino-Pinto et al. (2018), no *Bsal*-positive salamander has been reported in Hesse in 2019.

That said, it is obvious that neither our study across the federal state, nor the study by Sabino-Pinto et al. (2018) are of holistic nature. It should also be kept in mind that our study, like that by Sabino-Pinto et al. (2018), is based on data obtained from collections that voluntarily participated. It may be presumed that collections experiencing clinical problems more regularly are not supportive of such screenings. However – if the pathogen is widely distributed in captive collections in Hesse, it is likely that it would have been detected also in this study – as animals are commonly exchanged between collections. In total, both studies analysed twelve private collections, but this unfortunately represents only a marginal fraction of all such collections, as urodeles and the European fire salamander in particular are widely kept in terraria (e.g., Seidel & Gerhardt 2016). Since even a single infected animal in one of such collections has the potential of causing a massive outbreak of *Bsal* if spores are released into the environment, it remains of critical importance to further monitor such collections and include previously unexamined ones as well (e.g., Martel et al. 2020). Such an initiative, however, can only be successful if scientists studying *Bsal* and private keepers cooperate tightly. Without providing scientists access to the kept animals for subjecting these to analysis, it will be virtually impossible to infer the distribution of *Bsal* in private collections, may it be in one federal state (e.g., Hesse) or across Germany. Furthermore, it is important to conduct epidemiological screenings on a regular basis to the benefit of collections, most importantly when new animals are added, wherever they may come from, or before animals are exchanged (e.g., for breeding attempts). It should also be made mandatory that positive *Bsal*-screening results be reported to a data collection centre so that the distribution and possible spread of *Bsal* can be monitored.

The possible consequences of not sticking to such a dense monitoring for *Bsal* are a serious threat to any native salamander population that happens to exist in proximity to a captive collection and need to be avoided at all costs.

Future Perspectives

Understanding the distribution and prevalence of *Bsal* in captive collections remains an important task for the future. Sampling to this end needs to be continued and even expanded if possible. The *Bsal* task force in Hesse plans to proceed in this critical aspect of disease monitoring and aims to expand its scope by including wild salamander populations. This will be done in tight cooperation with local conservationists as well as forest authorities, utilizing a combined approach of qPCR screening of wild individuals linked to dense monitoring of larval population dynamics in order to detect cryptic population declines.

Acknowledgements

References

AGAR & FENA (2010): Rote Liste der Amphibien und Reptilien Hessens, 6. Fassung. – Hessisches Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz, Arbeitsgemeinschaft Amphibien- und Reptilienschutz in Hessen e.V. und Hessen-Forst Servicestelle Forsteinrichtung und Naturschutz, Fachbereich Naturschutz, Wiesbaden.

BATES, K. A., F. C. CLARE, S. O'HANLON, J. BOSCH, L. BROOKES, K. HOPKINS, E. MCLAUGHLIN, O. DANIEL, T. W. J. GARNER, M. C. FISHER & X. A. HARRISON (2018): Amphibian chytridiomycosis outbreak dynamics are linked with host skin bacterial community structure. – Nature Communications, **9**: 693.

BATES, K. A., J. M. G. SHELTON, V. L. MERCIER, K. P. HOPKINS, X. A. HARRISON, S. O. PETROVAN & M. C. FISHER (2019): Captivity and infection by the fungal pathogen *Batrachochytrium salamandrivorans* perturb the amphibian skin microbiome. – Frontiers in Microbiology, **10**: 1834.

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 and Distributions, **24**: 1788–1801.

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., A. MARTEL, F. HAESEBROUCK, F. VERCAMMEN, D. BONTE & F. PASMANS (2015): Treatment of urodelans based on temperature dependent infection dynamics of *Batrachochytrium salamandrivorans*. – Scientific Reports, **5**: 8037.

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.

CUNNINGHAM, A. A., K. BECKMANN, M. PERKINS, L. FITZPATRICK, R. CROMIE, J. REDBOND, M. F. O'BRIEN, P. GHOSH, J. SHELTON & M. C. FISHER (2015): Emerging disease in UK amphibians. – Veterinary Record, **176**: 468.

DALBECK, L., H. DÜSSEL-SIEBERT, A. KERRES, K. KIRST, A. KOCH, S. LÖTTERS, 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.

Deutsche Gesellschaft für Herpetologie und Terrarienkunde (2018): Verbreitungsatlas der Amphibien und Reptilien Deutschlands. – DGHT, Mannheim. Available at www.feldherpetologie.de, accessed 10 February 2020.

FELDMEIER, S., L. SCHEFCZYK, N. WAGNER, G. HEINEMANN, M. VEITH & S. LÖTTERS (2016): Exploring the distribution of the spreading lethal salamander chytrid fungus in its invasive range in Europe – a macroecological approach. – PLoS ONE, **11**: e0165682.

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, **484**: 186–194.

FITZPATRICK, L. D., F. PASMANS, A. MARTEL & A. A. CUNNINGHAM (2018): Epidemiological tracing of *Batrachochytrium salamandrivorans* identifies widespread infection and associated mortalities in private amphibian collections. – Scientific Reports, **8**: 13845.

HOFFMANN, M., C. HILTON-TAYLOR, A. ANGULO, M. BÖHM, T. M. BROOKS, S. H. BUTCHART, K. E. CARPENTER, J. CHANSON, B. COLLEN, N. A. COX, W. R. T. DARWALL, N. K. DULVY, L. R. HARRISON, V. KATARIYA, C. M. POLLOCK, S. QUADER, N. I. RICHMAN, A. S. L. RODRIGUES, M. F. TOGNELLI et al. (2010): The impact of conservation on the status of the world's vertebrates. – Science, **330**: 1503–1509.

HYATT, A. D., D. G. BOYLE, V. OLSEN, D. B. BOYLE, L. BERGER, D. OBENDORF, A. DALTON, K. KRIGER, M. HERO, H. HINES, R. PHILLOTT, R. CAMPBELL, G. MARANTELLI, F. GLEASON & A. COLLING (2007): Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. – Diseases of Aquatic Organisms, **73**: 175–192.

KUZMIN, S., T. PAPENFUSS, M. SPARREBOOM, I. H. UGURTAS, S. ANDERSON, T. BEEBEE, M. DENOËL, F. ANDREONE, B. ANTHONY, B. SCHMIDT, A. OGRODOWCZYK, M. OGLIESKA, J. BOSCH, D. TARKHNISHVILI & V. ISHENKO (2009): The IUCN Red List of Threatened Species. *Salamandra salamandra*. – IUCN, Gland etc. Available at https://dx.doi.org/10.2305/IUCN.UK.2009.RLTS.T59467A11928351.en, accessed 10 February 2020.

KLEWEN, R. (1988): Die Landsalamander Europas, Teil 1. – A. Ziemsen, Wittenberg Lutherstadt.

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.

LÖTTERS, S., N. WAGNER, G. ALBALADEJO, P. BÖNING, L. DALBECK, H. DÜSSEL, S. FELDMEIER, M. GUSCHAL, K. KIRST, D. OHLHOFF, K. PREISSLER, T. REINHARDT, M. SCHLÜPMANN, U. SCHULTE, V. SCHULZ, S. STEINFARTZ, S. TWIETMEYER, M. VEITH, M. VENCES & J. WEGGE (2020): The amphibian pathogen *Batrachochytrium salamandrivorans* in the hotspot of its European invasive range: past – present – future. – Salamandra, **56**: 173–188 [this issue].

LÜDDECKE, T., S. SCHULZ, S. STEINFARTZ & M. VENCES (2018): A salamander's toxic arsenal: review of skin poison diversity and function in true salamanders, genus *Salamandra*. – The Science of Nature, **105**: 56.

MARTEL, A., A. SPITZEN-VAN DER SLUIJS, M. BLOOI, W. BERT, R. DUCATELLE, M. C. FISHER, W. BOSMAN, K. CHIERS, F. BOSSUYT & F. PASMANS (2013): *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. – Proceedings of the National Academy of Sciences, **110**: 15325–15329.

MARTEL, A., M. BLOOI, C. ADRIAENSEN, P. VAN ROOIJ, W. 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. PASMANS (2014): Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. – Science, **346**: 630–631.

Martel, A., M. Vila-Escale, D. Fernández-Giberteau, A. Martinez-Silvestre, S. Canessa, S. van Praet, P. Pannon, K. Chiers, A. Ferran, M. Kelly, M. Picart, D. Piulats, Z. Li, V. Pagone, L. Pérez-Sorribes, C. Molina, A. Tarragó-Guarro, R. Velarde-Nieto, F. Carbonell, E. Obon, D. Martínez-Martínez, D. Guinart, R. Casanovas, S. Carranza & F. Pasmans (2020): Integral chain management of wildlife diseases. – Conservation Letters: e12707.

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.

Sabino-Pinto, J., M. Bletz, R. Hendrix, R. B. Perl, A. Martel, F. Pasmans, S. Lötters, F. Mutschmann, D. S. Schmeller, B. R. Schmidt, N. Wagner, M. Vences & S. Steinfartz (2015): First detection of the emerging fungal pathogen *Batrachochytrium salamandrivorans* in Germany. – Amphibia-Reptilia, **36**: 411–416.

Sabino-Pinto, J., M. Veith, M. Vences & S. Steinfartz (2018): Asymptomatic infection of the fungal pathogen *Batrachochytrium salamandrivorans* in captivity. – Scientific Reports, **8**: 11767.

Schulz, V., S. Steinfartz, A. Geiger, K. Preissler, J. Sabino-Pinto, M. Krisch, N. Wagner & M. Schlüpmann (2018): Ausbreitung der Salamanderpest in Nordrhein-Westfalen. Aktueller Kenntnisstand. – Natur in NRW, **4**: 26–30.

Schulz, V., A. Schulz, M. Klamke, K. Preissler, J. Sabino-Pinto, M. Müsken, M. Schlüpmann, L. Heldt, F. Kamprad, J. Enss, M. Schweinsberg, J. Virgo, H. Rau, M. Veith, S. Lötters, N. Wagner, S. Steinfartz & M. Vences (2020): *Batrachochytrium salamandrivorans* in the Ruhr District, Germany: history, distribution, decline dynamics and disease symptoms of the salamander plague. – Salamandra, **56**: 189–214 [this issue].

Seidel, U. & P. Gerhardt (2016): Die Gattung *Salamandra*. – Edition Chimaira, Frankfurt am Main.

Spitzen-van der Sluijs, A., F. Spikmans, W. Bosman, M. D. Zeeuw, T. van der Meij, E. Goverse, M. Kik, F. Pasmans & A. Martel (2013): Rapid enigmatic decline drives the fire salamander (*Salamandra salamandra*) to the edge of extinction in the Netherlands. – Amphibia-Reptilia, **34:** 233–239.

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. Haesebrouck, W. Bert, F. Bossuyt & A. Martel (2017): Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. – Nature, **544**: 353–356.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. Rodrigues, D. L. Fischman & R. W. Waller (2004): Status and trends of amphibian declines and extinctions worldwide. – Science, **306**: 1783–1786.

Thiesmeier, B. (2004): Der Feuersalamander. – Laurenti Verlag, Bielefeld.

Van Rooij, P., A. Martel, J. Nerz, S. Voitel, F. van Immerseel, F. Haesebrouck & F. Pasmans (2011): Detection of *Batrachochytrium dendrobatidis* in Mexican bolitoglossine salamanders using an optimal sampling protocol. – Eco-Health, **8**: 237–243.

Wagner, N., V. Schulz, S. Steinfartz, T. Reinhardt, M. Vences, S. Lötters, L. Dalbeck, H. Düssel-Siebert, M. Guschal, K. Kirst, D. Ohlhoff, J. Wegge & M. Veith (2019): Aktuelle Erkenntnisse zum Status der Salamanderpest in Deutschland. – Natur und Landschaft, **94**: 463–471.

Woodhams, D. C., H. Brandt, S. Baumgartner, J. Kielgast, E. Küpfer, U. Tobler, L. R. Davis, B. R. Schmidt, C. Bel, S. Hodel, R. Knight & V. McKenzie (2014): Interacting symbionts and immunity in the amphibian skin mucosome predicts disease risk and probiotic effectiveness. – PLoS ONE, **9**: e96375.

Yuan Z., A. Martel, J. Wu, S. Van Praet, S. Canessa & F. Pasmans (2018): Widespread occurrence of an emerging fungal pathogen in heavily traded Chinese urodelan species. – Conservation Letters, **11**: e12436.

Ziemek, H.-P. (2019): Der "Salamanderfresser" breitet sich aus! – DATZ, **3**: 18–21.