Itraconazole treatment of Batrachochytrium dendrobatidis (Bd) infection in captive caecilians (Amphibia: Gymnophiona) and the first case of Bd in a wild neotropical caecilian

Article · November 2015

CITATIONS
11

READS
377

6 authors, including:

Matthew Rendle
Association of Zoo and Exotic Veterinary Nurses
16 PUBLICATIONS **72** CITATIONS
SEE PROFILE

Benjamin Tapley
ZSL
79 PUBLICATIONS **404** CITATIONS
SEE PROFILE

Matthew W Perkins
Zoological Society of London
39 PUBLICATIONS **980** CITATIONS
SEE PROFILE

Gabriela Bueno Bittencourt-Silva
Natural History Museum, London
26 PUBLICATIONS **206** CITATIONS
SEE PROFILE

JZAR JOURNAL OF ZOO AND AQUARIUM RESEARCH
www.jzar.org

Research article

Itraconazole treatment of *Batrachochytrium dendrobatidis* (*Bd*) infection in captive caecilians (Amphibia: Gymnophiona) and the first case of *Bd* in a wild neotropical caecilian

Matthew Rendle[1], Benjamin Tapley[1], Matthew Perkins[1], Gabriela Bittencourt-Silva[2], David J. Gower[3] and Mark Wilkinson[3]

[1]*Zoological Society of London, Regents Park, London NW1 4RY, UK*
[2]*Department of Environmental Science (Biogeography), University of Basel, Klingelbergstrasse 27, 4056 Basel, Switzerland*
[3]*Department of Life Sciences, The Natural History Museum, London, SW7 5BD, UK*
**Correspondence: Matthew Rendle; Matthew.Rendle@zsl.org*

Keywords:
chytridiomycosis, *Geotrypetes seraphini*, *Potomotyphlus kaupii*

Article history:
Received: 16 November 2014
Accepted: 16 October 2015
Published online: 30 October 2015

Abstract
Batrachochytrium dendrobatidis (*Bd*) is the causative agent of the disease amphibian chytridiomycosis, one of the factors driving amphibian population declines. *Bd* infections are treatable in at least some cases, but in the Gymnophiona has been little reported, and restricted to heat treatment in the form of increased environmental temperature. We report the successful treatment of *Bd* infection in the terrestrial African caecilian *Geotrypetes seraphini* and the prophylactic treatment of the aquatic neotropical caecilian *Potomotyphlus kaupii*, using 30 minute immersions in a 0.01% solution of the antifungal itraconazole over a period of 11 days. Previously only recorded in wild African Gymnophiona, our report of *Bd* in *P. kaupii* is not only the first record of infection in a wild aquatic caecilian but also in a caecilian of neotropical origin. To improve our understanding of the impact of *Bd* on caecilians, *Bd* isolates should be obtained from wild caecilians in order to ascertain what lineages of *Bd* infect this order. In addition, more wild individuals should be subjected to *Bd* diagnostic surveys, including in Asia where caecilians have not yet been subject to such surveys.

Introduction

The causes of global amphibian population declines are varied and often synergistic (e.g. Alford and Richards 1999; Stuart 2004). The amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has become a major cause of decline (Fisher et al. 2009; Crawford et al. 2010) as the causative agent of the potentially lethal infectious disease chytridiomycosis. *Bd* is not host specific and is known to infect and potentially kill species of all three extant orders of amphibians, including Gymnophiona (caecilians: Doherty-Bone et al. 2013; Gower et al. 2013).

To date, only African caecilians have tested positive for *Bd* in the field (Doherty-Bone et al. 2013; Gower et al. 2013). Raphael and Pramuk (2007) used qPCR to diagnose *Bd* infection in swabbed captive *Typhlonectes natans*, an aquatic caecilian native to South America, but it is unknown whether these animals acquired infection in captivity or at the locality of origin (Gower et al. 2013). Churgin et al. (2013) reported *Bd* in two groups of *T. natans*, one a customs seizure of 24 animals, and the other, two captive-bred individuals at a zoological institution in the United States. Again, the source of infection in these cases is uncertain. Gower et al. (2013) reported swabbing over 80 wild-caught terrestrial neotropical caecilians (three families, nine species) but none was positive for *Bd* following qPCR assays.

Maintaining caecilians in captivity provides opportunities to study life history, behaviour and reproductive biology, and to investigate and to develop treatment protocols for disease, including chytridiomycosis (Wake, 1994; O' Reilly, 1996; Churgin et al. 2013; Tapley et al. 2014). *Bd* infections are treatable but treatments are somewhat species, life-stage, and context specific (Woodhams et al. 2012). Heat therapy (elevated ambient temperature) has been shown to clear *Bd* infection in anurans (Woodhams et al. 2003), as have antifungal treatments,

including itraconazole and voriconazole (Garner et al. 2009; Martel et al. 2011). Negative side effects have been documented for itraconazole treatment in anurans, including the depigmentation of larvae and mortality of post-metamorphs (Garner et al. 2009; Pessier and Mendelson 2009; Branelly 2014). Itraconazole has been the most widely used treatment of *Bd* infection in captive amphibians (Branelly 2014) but daily 10 minute immersion in a 0.01% solution over a period of seven days did not clear *Bd* infection in the caecilian *T. natans* (Churgin et al. 2013).

To date, only heat treatments have been reported as effective in clearing *Bd* infection in caecilians. Temperatures of 32.2° C for 72 hours cleared *Bd* infection in *T. natans* (Churgin et al. 2013). However, heat treatment may not be appropriate for all amphibians because thermal tolerances vary (Bury 2008; Catenazzi et al. 2013) and the high temperatures required for the duration of the treatment period may prove lethal to some species.

Here we report the first *Bd* infection in a wild neotropical caecilian, *Potomotyphlus kaupii.* We also report the first successful antifungal treatment of *Bd* infection in the African caecilian *Geotrypetes seraphini* and the prophylactic treatment of *Bd* in *P. kaupii*, with itraconazole immersions.

Methods

The African dermophiid *Geotrypetes seraphini* (Duméril, 1859) is a widely distributed, viviparous terrestrial caecilian, found from Guinea to Angola (Scholz et al. 2010). This species is one of the caecilians most commonly collected from the wild for the international pet trade (Gower and Wilkinson 2005; Gower et al. 2013) and is maintained by several zoological collections, including the Zoological Society of London (ZSL), London Zoo.

On 13 November 2012, the Institute of Zoology, ZSL acquired 19 pet trade *G. seraphini* that had been recently imported directly to the UK from Cameroon by a licensed importer (see also Gower et al. 2013). Specimens were housed individually in enclosures containing a coir substrate in a climatically controlled room at 25° C. On 14 November 2012, all specimens were swabbed for *Bd*. Wearing clean nitrile powder-free gloves, one person held the caecilian by the head and posterior end and attempted to straighten the body, while another used a different swab to separately sample the head (all surfaces), anal disc, dorsal and ventral surface of the body. Gloves were changed between each specimen.

Potomotyphlus kaupii (Berthold, 1859) is an aquatic typhlonectid caecilian known from the drainage basins of the Amazon and Orinoco rivers of South America (Marty et al. 2007; Oliveira et al. 2012). Over two nights during 2013 (28–30 April) GB-S, DG and MW trapped 14 *P. kaupii* in 17 baited bait fish traps traps from Saut Maripas on the Oyapock river, French Guiana (see Kupfer et al. 2006). At the trapping locality water temperature was 26–27° C with a pH of 6.8. Our trapping method differed somewhat from that used for typhlonectid caecilians by Kupfer et al. (2006). Traps were baited with cooked shrimp or raw fish, used without floats, fully submerged on the river bed close to the shore, and checked every one or two hours through the night to ensure that both target and non-target organisms were removed from the traps frequently and that caecilians did not drown. This is the first report of trapping of this species. Captured animals were kept together and transported in a single-species plastic aquarium (ambient temperature, no filtration) refreshed with water from a forest stream at Camp Patawa in the Kaw Mountains. On 3 May 2013 the animals were shipped to the UK and eight specimens immediately transferred to ZSL London Zoo where they underwent strict quarantine for 180 days. Specimens were housed in two groups of four individuals at 27° C with mature filtration systems that were established using an ammonium chloride dosing regime. At no time did these captive animals, their enclosures, life support systems or furnishings come into contact with other amphibians. Specimens were swabbed for *Bd* 47 days post-arrival, this being an observed period of acclimatisation and confirmed regular feeding. Swabbing followed the protocol outlined above.

DNA was extracted from swabs following the protocol given by Boyle et al. (2004). Samples were subjected to quantitative real time polymerase chain reaction (qPCR) diagnostic assay, using *Bd* primers specific to the ribosomal ITS-1/5.8S region (Boyle et al. 2004). Positive controls of known concentration of *Bd* DNA (100, 10, 1 and 0.1 *Bd* zoospore genomic equivalents, GE) were run as standards along with the samples, as were negative controls. Samples were run in duplicate on PCR plates and, if necessary, were repeated until both wells for each sample gave the same (positive or negative) result. Bovine serum albumin (BSA) was included in PCR reactions to reduce amplification inhibition (Garland et al. 2010) for all DNA extracts, but a subset was run initially without BSA. Running the samples with and without BSA had no impact on the C_t value or the number of positive or negative results within the subsample.

Specimens of *Geotrypetes seraphini* and *Potomotyphlus kaupii* were treated for *Bd* in individual 5.0 L plastic containers containing a 3.0 cm deep itraconazole solution of 0.01% made with aged tap water at room temperature (25° C). Solutions were prepared 30 minutes before treatment to ensure that beads of intraconazole contained in the Sporanox (Janssen Pharmaceutica N.V., Beerse B-2340, Belgium) capsules had fully dissolved. Animals were immersed for 30 minutes per day over 11 days after which they were returned to their enclosures until treatment the following day. The substrate in enclosures housing *G. seraphini* was changed and tanks disinfected on day 1 and day 11 of the itraconazole treatment programme. *Potomotyphlus kaupii* enclosures were not disinfected between the 11 treatments because the biological filtration systems were mature (supported colonies of bacteria responsible for biological filtration) and it was not deemed appropriate to maintain this species without biological filtration during the itraconazole treatment period.

All individuals were swabbed for *Bd* immediately after the 11 day treatment and again after one month, following the method described above. *Bd* treatment was considered successful if both swabs tested negative for *Bd*.

Results

From the initial screening, three *G. seraphini* tested positive for *Bd* (GE scores 0.3±0.15, 0.62±0.11, 7.10±1.35). Three specimens, including the specimen with the highest GE score and two that had not been positive for *Bd*, died before the swabs were analysed. Histological samples from these three specimens showed skin disease consistent with chytridiomycosis (Gower et al. 2013). Surviving specimens were re-swabbed three weeks later and four specimens tested positive for *Bd*, including two which had previously not been positive (GE scores 3.92±0.55, 111.74±1.45, 0.22±0.16, 78.33±16.8). All were subsequently treated for infection using the method described above.

A single *Bd* infection was confirmed from one of the dorsal swabs within the group of *P. kaupii* (GE score 0.42±0.25). Two specimens died between the dates that the swabs were taken and samples analysed. Histological samples and *Bd* isolates could not be obtained post mortem due to rapid autolysis in the warm aquarium conditions, thus it could not be confirmed that the two specimens that died were infected with *Bd.* The remaining six specimens underwent a prophylactic itraconazole treatment following the procedure outlined above. Specimens were screened twice for *Bd* post treatment. All individuals were swabbed for *Bd* immediately after the 11 day treatment and again after one

month. *Bd* was not detected post-treatment in either *P. kaupii* or *G.seraphini* and all remaining individuals were assimilated into the caecilian collection at ZSL London Zoo.

Discussion

Itraconazole successfully cleared *Bd* infection in *Geotrypetes seraphini*. With this first reported case of successful treatment of caecilians with itraconazole there are now two proven treatments: temperature elevation (Churgin et al. 2013) and itraconazole immersion. Temperature elevation has been successful in clearing *Bd* infection in *Typhlonectes natans* (Churgin et al. 2013) but may not be a suitable treatment for all species because thermal tolerances vary (Bury, 2008; Catenazzi et al. 2013) and the high temperatures required for the duration of the treatment period may prove lethal to some species. *Potomotyphlus kaupii* has several anatomical features that suggest a greater reliance upon cutaneous gas-exchange (Wilkinson and Nussbaum 1997), the potential for which would be reduced by temperature increase.

Bd infection has been found in wild *Potomotyphlus kaupii.* Although *Bd* is known from the Guiana shield and has been reported in frogs from French Guiana (Courtoise et al. 2012), this is the first confirmed report of *Bd* in a neotropical caecilian as well as the first case of *Bd* infection in a wild aquatic caecilian. From our data it is not possible to determine whether or not *Bd* infection in *P. kaupii* was cleared using itraconazole baths, but the lack of negative side effects during prophylaxis indicate that this is a potentially viable treatment.

Bd infection is normally associated with the ventral surfaces of anuran and caudatan amphibians (e.g. Berger et al. 2004; Piotrowski et al. 2004; Hyatt et al. 2007; Van Rooij et al. 2011). *Bd* was detected from the dorsal surface of *P. kaupii*, and Gower et al. (2013) reported *Bd* infection in *G. seraphini* on the head, dorsal surface and anal disc but not the ventral surface. Sample sizes for zonal swabbing are currently small, but this warrants further investigation.

To improve our understanding of the impact of *Bd* on caecilians, more wild individuals should be subjected to *Bd*-specific disease screening, especially in Asia where caecilians have not yet been subject to *Bd* screening (Swei et al. 2011). *Bd* isolates should be obtained from wild caecilians in order to ascertain what lineages of *Bd* infect this amphibian order. The death of the *G. seraphini* with the highest GE during the treatment might have been due to advanced chytridiomycosis rather than to adverse reaction to itraconazole. However, deaths during treatment in this and another study (Churgin et al. 2013), highlight that future research into the treatment of *Bd* in caecilians should investigate using lower dosages of itraconazole. Brannelly (2014) found that itraconazole solutions of 0.01–0.0025% and a reduced treatment duration of five minutes over six days cleared *Bd* infection in anurans. These lower dosages could be trialled on caecilians infected with *Bd*.

In light of our findings we recommend that captive caecilians, and new acquisitions in particular, are subject to rigorous *Bd* screening and that histological samples, especially areas including skin lesions are preserved to facilitate future research on *Bd* infection and chytridiomycosis in caecilian amphibians. Duration of quarantine is determined by a risk assessment that incorporates a number of factors, including the geographic origin of the animal and the likelihood of carrying pathogens of concern. We suggest a minimum 90-day quarantine period for caecilians to allow for pathogen screening and for any clinical symptoms of disease to be exhibited before specimens are assimilated into established collections. Quarantine is best conducted in dedicated facilities. During quarantine, caecilians should be swabbed a minimum of twice, preferably at the beginning and towards the end of the quarantine period, given that detectable *Bd* infections may arise from previously low and undetectable infection levels (Gower et al. 2013). If *Bd* infection is detected in captive caecilians, the *Bd* positive animals or group should be maintained in isolation and only assimilated into established collections once they have been treated and have tested negative for *Bd* post treatment on a minimum of two occasions .

Acknowledgements

Permits for export of samples were provided by Direction de l'Environment de l'Aménagement et du Logement and the Direction des Services Vétérinaires de la Guyane, Cayenne, French Guiana. We are grateful to Myriam Virevaire and Le Comité Scientifique Régional du Patrimonie Naturel for supporting our research in French Guiana. For companionship and/or practical assistance in organizing and executing laboratory and fieldwork we thank Elodie Courtoise, Antoine Fouquet, Philippe Gaucher, Fausto Starace and family, and Jeannot and Odette (Camp Patawa). For help with the captive care and treatment of the caecilians at the Zoological Society of London we thank Toni Beadle, Joanna Korn, and Heather Macintosh. We are grateful to Felicity Wynne for her assistance with the initial screening of the caecilians.

References

Alford R.A., Richards S.J. (1999) Global amphibian declines: a problem in applied ecology. *Annual Reviews in Ecology and Systematics* 30: 133–165.

Berger L., Speare R., Hines H.B., Marantelli G., Hyatt A.D., McDonald K.R., Skerratt L.F., Olsen V., Clarke J.M., Gillespie G., Mahony M., Sheppard N., Williams C., Tyler, M.J. (2004) Effects of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinary Journal* 82: 434–439.

Boyle D.G., Boyle D.P., Olsen V., Morgan J.A.T., Hyatt A.D. (2004) Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real time Taqman PCR assay. *Diseases of Aquatic Organisms* 60: 141–148.

Brannelly L.A. (2014) Reduced itraconazole concentration and durations are successful in treating *Batrachochytrium dendrobatidis* infection in amphibians. *Journal of Visualized Experiments* 85, e51166, doi:10.3791/51166.

Bury R.B. (2008) Low thermal tolerances of stream amphibians in the Pacific Northwest: Implications for riparian and forest management. *Applied Herpetology* 5: 63–74.

Catenazzi A., Lehr E., Vredenburg V.T. (2014) Thermal physiology, disease, and amphibian declines on the eastern slopes of the Andes. *Conservation Biology* 28: 509–517.

Churgin S.M., Raphael B.L., Pramuk J.B., Trupkiewicz J.G., West G. (2013) *Batrachochytrium dendrobatidis* in aquatic caecilians (*Typhlonectes natans*): A series of cases from two institutions. *Journal of Zoo and Wildlife Medicine* 44: 1002–1009.

Courtoise E.A., Pineau K., Villette B., Schmeller D.S., Gaucher P. (2012) Population estimates of *Dendrobates tinctorius* (Anura: Dendrobatidae) at three sites in French Guiana and first record of chytrid infection. *Phyllomedusa* 11: 63–70.

Crawford A.J., Lips K.R., Bermingham E. (2010) Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama. *Proceedings of the National Academy of Sciences of the United States of America* 107: 13777–13782.

Doherty-Bone T.M., Gonwouo N.L., Hirschfeld M., Ohst T., Weldon C., Perkins M., Kouete M.T., Browne R.K., Loader S.P., Gower D.J., Wilkinson M.W., Rödel M.O., Penner J., Barej M.F., Schmitz A., Plötner J., Cunningham A.A. (2013) *Batrachochytrium dendrobatidis* in amphibians of Cameroon, including first records for caecilians. *Diseases of Aquatic Organisms* 102: 187–194.

Fisher M.C, Garner T.W.J., Walker S.F. (2009) Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. *Annual Review of Microbiology* 63: 291–310.

Garland S., Baker A., Phillott A.D., Skerratt, L.F. (2010) BSA reduces inhibition in a TaqMan assay for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 92: 113–116.

Garner T., Garcia G., Carroll B., Fisher, M. (2009) Using itraconazole to clear *Batrachochytrium dendrobatidis* infection, and subsequent depigmentation of *Alytes muletensis* tadpoles. *Diseases of Aquatic*

Organisms 83: 257–260.

Gower D.J., Wilkinson M. (2005) The conservation biology of caecilians. *Conservation Biology* 19: 45–55.

Gower D.J., Doherty Bone T, Loader S.P., Wilkinson M., Kouete M., Tapley B., Orton F., Daniel O.Z., Wyne F., Flach E., Muller H., Menegon M., Stephen I., Browne R.K., Fisher M.C., Cunningham A.A., Garner T.W.J. (2013) *Batrachochytrium dendrobatidis* infection and lethal chytridiomycosis in caecilian amphibians Gymnophiona. *EcoHealth,* DOI: 10.1007/s10393-013-0831-9.

Hyatt A.D., Boyle D.G., Olsen V., Boyle D.B., Berger L., Obendorf D., Dalton A., Kriger K., Hero M., Hines H., Phillott R., Campbell R., Marantelli G., Gleason F., Colling A. (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73: 175–192.

IUCN, Conservation International and NatureServe (2006) *Global Amphibian Assessment*. www.globalamphibians.org (accessed on 11 December 2008).

Kupfer A., Gaucher P., Wilkinson M., Gower, D.J. (2006) Passive trapping of aquatic caecilians (Amphibia: Gymnophiona: Typhlonectidae). *Studies on Neotropical Fauna and Environment* 41: 93–96.

Loader S., Rödel M., Wilkinson, M. (2004) *Geotrypetes seraphini*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. www.iucnredlist.org (accessed 11 February 2014).

Marty C., Ravet E., Bordage D., Lescure J. (2007) Redécouverte de *Potomotyphlus kaupii* (Berthold, 1859) (Amphibia, Gymnophiona, Typhlonectidae) en Guyane française. *Bulletin Societé Herpétologique de France* 121: 35–36.

Martel A., Van Rooij P., Vercauteren G., Baert K., Van Waeyenberghe L., Debacker P., Garner T.W.J., Woeltjes T., Ducatelle R., Haesebrouck F., Pasmans F. (2011) Developing a safe antifungal treatment protocol to eliminate *Batrachochytrium dendrobatidis* from amphibians. *Medical Mycology* 49: 143–149.

Oliveira U.S.C., Meneghelli D., Messias M.R., Gomes I.B.S.R., Coragem J.T. (2013) First record of *Potomotyphlus kaupii* (Berthold, 1859) (Gymnophiona: Typhlonectidae) for the state of Rondônia, Brazil. *Herpetology Notes* 5: 155–156.

O'Reilly J.C. (1996) Keeping caecilians in captivity. *Advances in Herpetoculture* 1: 39–45.

Pessier A.P., Mendelson J.A. (2009) *Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs*. Apple Valley, Minnesota: IUCN/SSC Conservation Breeding Specialist Group.

Piotrowski J.S., Annis S.L., Longcore J.E. (2004) Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96: 9–15.

Raphael B.L., Pramuk J. (2007) Treatment of chytrid infection in *Typhlonectes* spp. using elevated water temperatures. In: *Proceedings of Amphibian Declines and Chytridiomycosis Conference*, Tempe, AZ, 5–7 November 2007, 37. Available at: http://www.parcplace.org/images/stories/pdf/bd_program_post-final.pdf

Scholz S., Orlik M., Gonwouo L.N., Kupfer A. (2010) Demography and life history of a viviparous Central African caecilian amphibian. *Journal of Zoology* 280: 17–24.

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

Swei A., Rowley J.J.L., Rodder D., Diesmos M.L.L., Diesmos A.C., Briggs C.J., Brown R., Cao T., Cheng T.L., Chong R.A., Han B., Hero J.-M., Hoang H.D., Kusrini M.D., Le T.T.D., McGuire J.A., Meegaskumbura M., Min M.-S., Mulcahy D.G., Neang T., Phimmachak S., Rao D.-Q., Reeder N.M., Schoville S.D., Sivongxay N., Srei N., Stock M., Stuart B.L., Torre, L.S., Tran D.T.A., Tunstall T.S., Vieites D., Vredenburg V.T. (2011) Is chytridiomycosis an emerging amphibian disease in Asia? *PLoS ONE* 6: e23179.

Tapley B., Bryant Z., Grant S., Kother G., Feltrer Y., Masters N., Strike T., Gill I., Wilkinson M., Gower D.J. (2014) Towards evidence based husbandry for caecilian amphibians: Substrate preference in *Geotrypetes seraphini* (Amphibia: Gymnophiona: Dermophiidae). *Herpetological Bulletin* 129: 15–18.

Van Rooij P., Martel A., Nerz J., Voitel S., Van Immerseel F., Haesbrouck F, Pasmans F. (2011) Detection of *Batrachochytrium dendrobaitids* in Mexican Boliglossinae salamanders using an optimal sampling protocol. *Ecohealth*, DOI: 10.1007/s10393-011-0704-z.

Wake M.H. (1994) Caecilians (Amphibia: Gymnophiona) in captivity. In: Murphy J.B., Alder K., Collins J.T. (eds). *Captive Management and Conservation of Amphibians and Reptiles.* London: Society for the Study of Amphibians and Reptiles, 223–228.

Wilkinson M., Nussbaum R.A. (1997) Comparative morphology and evolution of the lungless caecilian *Atretochoana eiselti* (Taylor) (Amphibia: Gymnophiona: Typhlonectidae). *Biological Journal of the Linnean Society* 62: 39–109.

Woodhams D.C., Alford R.A., Marantelli G. (2003) Emerging disease of amphibians cured by elevated body temperature. *Diseases of Aquatic Organisms* 55: 65–67.

Woodhams D.C., Geiger C.C., Reinert L.K., Rollins-Smith L.A., Lam B., Harris R.N., Briggs C.J., Vredenburg V.T., Voyles J. (2012) Treatment of amphibians infected with chytrid fungus: learning from failed treatments with itraconazole, antimicrobial peptides, bacteria, and heat therapy. *Diseases of Aquatic Organisms* 98: 11–25.