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Preliminary analyses suggest absence of the amphibian chytrid fungus in native and exotic amphibians of the United Arab Emirates

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The amphibian disease chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*), is known to have impacted on more than 350 species of amphibians worldwide and is found on all continents expect Antarctica (Fisher et al. 2009). It has further been found that *Bd* associated with amphibians consists of three deeply diverged lineages with one strain being found across five continents and is considered hypervirulent for amphibians (Farrer et al. 2011). There is also a lack of data on sampling and *Bd* detection within the Arabian Peninsula as can be seen on http://www.spatialepidemiology.net/datasets/, which shows which countries have been sampled and the number of *Bd*-positive samples per country. Therefore this preliminary analysis aims to provide information from this region where there is a lack of data. The United Arab Emirates (UAE) covers an area of approximately 89,000 km² including offshore islands. The UAE is bordered to the west and south by Saudi Arabia, Oman to the east, and the Arabian Gulf to the north, and is situated in the north of the Arabian Peninsula. Wild populations of native toads are mainly found in the northern parts of the UAE, which are mountainous and have 'wadis' (i.e., valleys of seasonal rivers in mountainous areas) (Fig. 1).

The UAE is home to two species of native amphibians, namely the Arabian toad, *Duttaphrynus arabicus* (Heyden, 1827), and Dhofar toad, *Duttaphrynus dhufarensis* (Parker, 1931), which are both regional endemics (Soorae et al. 2010). A *Bd* survey was conducted simultaneously both on native (wild and captive populations in zoological institutions) and exotic amphibians (in zoological institutions and pet trade) in the UAE to ensure that assessment data for both categories would be relative to the same point of time. This type of first survey, i.e., where there are no records or information of previous infection, has previously been conducted in the high biodiversity hotspot of the Western Ghats of India where opportunistic sampling actually found the presence of *Bd* in *Indirana* frogs during 2008–2010 (Nair et al. 2011).

For the native toad survey, a total of eight sites were sampled in the northern UAE. Toads were found only at five sites (62.5%), and the details of the sampled sites are provided in Table 1. Out of the eight sites sampled in the northern Emirates, 16 Arabian toads were found at four sites and two Dhofar toads at only two sites. Large wild assemblages of toads are usually only encountered during breeding periods in the UAE, when surface water becomes available in the 'wadis' from the short rains, mainly during the winter months, otherwise toads are seen at very low densities in the wild. In some cases, even during winter seasons with little rain, toads were seemingly absent, but when conditions are right, there will be frantic breeding activity. Finding these toads is therefore dependent on these factors, rendering sampling difficult, as their presence or absence cannot be predicted easily.

For the non-native (exotic) species, our *Bd* survey was based on sampling a total of 16 pet shops, out of which only five (31%) had amphibians in stock, and these pet-shops were located in Abu Dhabi, Dubai, Sharjah and Ajman Emirates. The two zoological gardens sampled both kept native and exotic toads and frogs and were located in Abu Dhabi and Sharjah Emirates.

There were mainly two types of swab samples collected, i.e. single and pooled ones. Single swabs were sealed in a single tube while pooled swabs could take up to eight swabs from a single location in one sealed tube. Therefore single swabs were taken when toads were encountered at

low density and pooled swabs when a breeding assemblage was encountered. Out of 36 swab samples taken, 56% were single swabs and 44% were pooled swabs, and all results from this survey were negative for the chytrid fungus.

Sampling for the chytrid fungus involved taking a skin swab of mainly the ventral face of the legs. The swabs were then sealed in a sample tube that was kept in a cooling-box whilst in the field. The samples were then shipped by courier to the Pisces Molecular LLC lab in Colorado, USA, for chytrid fungus detection using PCR analysis. Their method of detection was as follows: the sample DNA(s) prepared was/were assayed for the presence of the *Bd* ribosomal RNA Intervening Transcribed Sequence (ITS) region by 45 cycle single-round PCR amplification using an assay developed by Seanna Annis and modified at Pisces for greater specificity (65°C annealing temperature, instead of 60°C) and sensitivity (75 mM KCl in the reaction instead of 50 mM, 3.5 mM Mg^{2+} instead of 1.5 mM, 45 cycles of amplification instead of 30 cycles). This assay has been tested against DNA extracts from a wide variety of Chytridiomycota species other than *Bd* as well as environmental samples and shows no cross-reactivity; additionally the sensitivity of the assay has been shown to be ≤ 0.1 *Bd* zoospore-equivalent (both J. Wood, unpublished data). Combining multiple swabs into one pre-pooled sample in ethanol would not be expected to have any deleterious effect on the detection sensitivity compared with single swabs in ethanol, because DNA was extracted from all of the (biological) material on each swab: 70% ethanol, sufficient to cover the swabs (approximately 5 ml), was added to the tube containing the pooled swabs, then vortexed vigorously. The ethanol and any particles were then transferred to a clean tube with a micropipettor, spun at 1000 × G for 5 minutes (*Bd* zoospores in ethanol pellet at 1000 × G; J. Wood, unpublished data).

After centrifugation, the supernatant liquid was carefully pipetted off and discarded, before the remaining volume of liquid (≤ 0.5 ml) and any pellet were transferred to a clean microcentrifuge tube and spun at 16,000 × G for 3 minutes. Again, the supernatant was gently removed with a pipettor and discarded. Tissue lysis buffer (Qiagen DNeasy ATL + Proteinase K) was added to the remaining liquid (> 50 µL) and any pellet, then vortexed and incubated at 55°C, before total DNA extraction (Qiagen DNeasy 96, mouse-tail protocol). Given the lack of stand-

Figure 1. Typical 'wadi' habitat in the UAE.

Table 1. Details of wild and captive amphibian species sampled in the UAE. Legend: ACF – African clawed frog (*Xenopus* sp.); AT – Arabian toad (*Duttaphrynus arabicus*); DT – Dhofar toad (*Duttaphrynus dhufarensis*); FF – Floating frog (*Occidozyga lima*); GT – Green toad (*Bufo viridis*); HR – *Hoplobatrachus rugulosus;* SF – Skittering Frog (*Euphlyctis ehrenbergii*). All species are classified as Least Concern in the IUCN Red List (IUCN 2010).

Location	GPS location	Species*	Number and type of swab sample(s)		Presence of *Bd*
			single	pooled	
Captive collections (16 pet shops and 2 zoological collections) June, July, August 2009		DT, AT, ACF, HR, FF, SF, GT	13	5 (44)	negative
Wadi Wurayah October 2009	25.39508° N, 056.26792° E	AT	1	1 (8)	negative
Wadi ham'd dam October 2009	25.13148° N, 056.30056° E	No specimens recorded	0	0	negative
Wadi Safad October 2009	25.22021° N, 056.31116° E	No specimens recorded	0	0	negative
Wadi Shi March 2010	25.34649° N, 056.32168° E	No specimens recorded	0	0	negative
Wadi Mahda-Mirbah Dam March 2010	25.271390° N, 056.349551° E	AT & DT	5	0	negative
Wadi Showka March 2010	25.09476° N, 056.06489° E	AT	0	7 (7)	negative
Wadi Al Baeh March 2010	25.79463° N, 056.07918° E	DT	1	0	negative
Wadi Al Ghayl March 2010	25.41047° E, 056.09378° E	AT	0	3 (3)	negative
Total number of swabs			20	16	total of 36 swab samples

ardized, reproducible skin swab samples that accurately reflect what is captured on a skin swab from a real animal in the environment (*Bd* zoospores from a lab culture spiked onto a swab are not an ideal surrogate sample), a direct test of the detection sensitivity from pooled vs. individual swab samples is not possible. However, the efficacy of this pooling procedure has been empirically demonstrated across thousands of pooled samples by comparing the detection sensitivity of individual swab samples in ethanol versus pooled samples using a variation of this procedure, but not applied to the samples described in this paper: Starting with between three and eight individual swab samples in ethanol (1 ml each), only ½ of the liquid volume from each individual sample (3-8 × 0.5 ml, total, described as a "½ volume pooled sample") was combined before proceeding with the centrifugation and DNA extraction steps described above. PCR tests of DNA extractions from the individual swab samples, with the DNA extract of the "1/2 volume pooled sample" of the same individual samples, indicate no loss of detection sensitivity in the pooled sample DNA extract versus the corresponding individual sample DNA extracts, with 8 or fewer individual swab samples combined and 1 or more of the individual samples being *Bd* PCR positive (J. Wood, unpublished data).

This first baseline survey of *Bd* in the UAE has fortunately not detected any presence of *Bd* both in wild populations and captive collections. The possibility exists, though, that *Bd* could enter the UAE be via the trade in imported exotic amphibians that come in from various countries, mainly from South East Asia. In the wild, the *Bd* fungus would also be challenged for survival, as summer temperatures can reach 50°C and surface water becomes very limited indeed. A possible route of transmission to wild populations could be the release of infected aquatic specimens into 'wadi' systems.

The Arabian and Dhofar toads already live in a very harsh environment that is highly arid, marked by unpredictable rainfall and water availability for breeding, and any additional stress factors, like *Bd,* would certainly put additional strain on these species. We plan on conducting periodical testing for *Bd* so as to be on the lookout for this potentially devastating amphibian fungus.

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References

Farrer, R. A., L. A. Weinert, J. Bielby, T. W. J. Garner, F. Balloux, F. Clare, J. Bosch, A. A. Cunningham, C. Weldon, L. H. Du Preez, L. Anderson, S. L. K. Pond, R. Shahar-Golan, D. A. Henk & M. C. Fisher (2011): Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. – Proceedings of the National Academy of Sciences of the USA, **108**: 18732–18736.

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.

IUCN (2010): IUCN Red List of Threatened Species. Version 2010.1. – Gland, IUCN, www.iucnredlist.org, accessed 25 May 2010.

Nair, A., O. Daniel, S. V. Gopalan, S. George, K. S. Kumar, J. Merila & A. J. F. Teacher (2011): Infectious disease screening of *Indirana* frogs from the Western Ghats biodiversity hotspot. – Herpetological Review, **42**: 554–557.

Soorae, P. S., M. Quarqaz & D. Gardner (2010): An overview and checklist of the native and alien herpetofauna of the United Arab Emirates. – Herpetological Conservation & Biology **5**: 529–536.