Herpetological Review, 2016, 47(3), 403–404.

Preliminary Surveys Fail to Detect *Batrachochytrium dendrobatidis* Infection in the United Arab Emirates and Oman

There is a paucity of data concerning either the presence or absence of *Batrachochytrium dendrobatidis* (*Bd*) in the Middle East and the susceptibility of amphibians in the region to this pathogen. Soorae et al. (2012) skin-swabbed 16 Arabian Toads (*Amietophrynus arabicus*; previously *Bufo orientalis, B. arabicus* and, more recently, *Amietophrynus arabicus*, Portik and Papenfuss 2015) and two Dhofar Toads (*Duttaphrynus dhufarensis*) across five sites in the United Arab Emirates (UAE) and found no evidence for the presence of *Bd* using a commercial, pathogen-specific standard Polymerase Chain Reaction (PCR) analysis. Whereas it is not clear how many animals were skin-swabbed at each site, the sample size was small and would, at best, require a *Bd* infection prevalence of at least 25% (and probably higher, depending on how many animals were sampled per site and the total size of each sampled population) for the pathogen to be detected. Here, we expand on the work of Soorae et al. (2012) in the UAE by examining a larger number of wild animals in the region for the presence of *Bd* infection.

Arabian Toads were sampled from January 2013 to January 2014 at two sites in the UAE and at two sites in Oman (Fig. 1; Table 1). At least 30 toads were sampled per population through the study duration. This sample size was chosen as it would enable the detection of a *Bd* prevalence of 15% with a 99% probability, or a prevalence of 10% with a 95% probability (Cannon and Roe 1982). A population was defined as the same species sharing the same water-body.

Toads were caught by hand and handlers were wearing plastic gloves that were changed between each animal to avoid cross-contamination. Per site, for each animal sampled, the ventral surface of the skin was swabbed with a single dry sterile rayon-tipped swab approximately 35 times. Target areas included the pelvic patch (5 swabs), ventral thighs (5 swabs per thigh), and toe webbing (5 swabs per foot). The swab was air-dried for approximately 5 minutes and then replaced in a plastic sterile sleeve. The swabs were kept at -20°C for up to 6 months before being processed. All the toads screened were in water at the time of sampling and were likely to have been in an aquatic environment for at least one month before being sampled as the site visits took place during the mating season.

DNA was extracted from the swabs and analyzed using a *Bd*-specific real-time PCR assay according to Boyle et al. (2004), modified by the addition of bovine serum albumin to minimize PCR inhibition (Garland et al. 2010). Each sample was tested in duplicate and each PCR plate contained two negative control wells (containing laboratory grade distilled water) and a duplicate set of four positive controls (100, 10, 1, and 0.1 zoospore equivalents).

In total, 67 toads were sampled from the UAE and 60 toads were sampled from Oman. All samples tested were *Bd*-negative. All negative and positive controls gave expected results. Thus, *Bd* is either not present at the sites sampled or is present at a prevalence of less than 10%.

Lack of *Bd* detection could result from several scenarios, including: *Bd* is not native to the area and has not yet reached the sampled amphibian populations; the sampled toads are resistant to *Bd* infection; and the local environmental conditions are not conducive to support the survival of *Bd*. Generally the climate of the UAE and Oman is classified as hyperarid. Limited amounts of fresh water, in combination with extremely high summer temperatures and high evaporation rates, make the Arabian Peninsula a harsh environment for the people, fauna, and vegetation (Böer and Chaudhary 1999). Nevertheless, two of the sites surveyed are known sources of fresh water and sustain aquatic organisms throughout the year. The air temperature in the region ranges from 12°C to more than 49°C and can stay over 30°C for more than 24 h during summer with water temperature in the river-beds over 25°C during summer and is thus higher than the optimal thermal conditions for *Bd*, 17–25°C (Piotrowski et al. 2004). Additionally, *Bd* infection can be "cured" by exposing infected animals to temperatures >25°C for one month (Chatfield and Richards-Zawacki 2011). It is nonetheless important to note that thermal maxima for *Bd* growth are *Bd*-isolate dependent and that the pathogen may exhibit local adaptation (Stevenson et al. 2013).

Although the exact origin of *Bd* has not yet been determined, it has become clear that the global amphibian trade is likely a primary driver for the international spread of *Bd* (Weldon et al. 2004; Schloegel et al. 2010). Dubai Airport is the third busiest cargo hub airport in the world, yet there is no significant amphibian trade in the UAE. Although Soorae et al. (2012) reported finding amphibians for sale in 5 of 16 pet shops visited in the UAE, a 2013 survey of 104 pet shops in the emirates of Dubaï, Sharjah, and Abu Dhabi failed to find any amphibians for

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TABLE 1. Arabian Toad (*Amietophrynus arabicus*) sample sizes per location in the United Arab Emirates (UAE) and Oman for *Batrachochytrium dendrobatidis* analyses.

Site Name	Latitude	Longitude	Elevation (m)	No. samples
Wadi Wurayah – Site 1 (UAE)	25.39583°N	56.23777°E	464	33
Wadi Wurayah – Site 2 (UAE)	25.34805°N	56.24555°E	26	34
Wadi Jazira (Oman)	24.31888°N	56.15000°E	333	30
Wadi Al Hayl Oman)	24.30888°N	56.32916°E	450	30

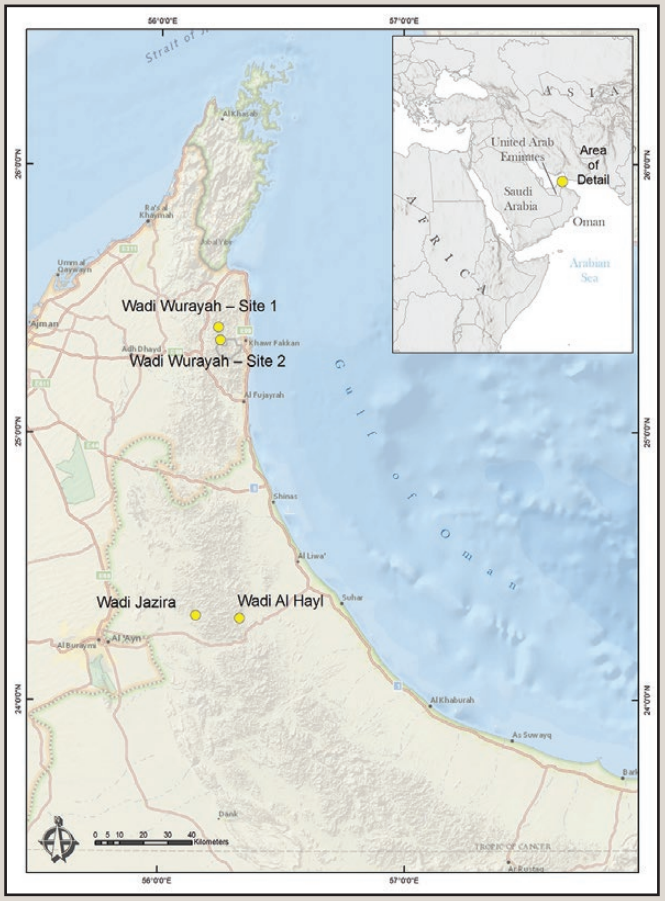

FIG. 1. Sampling locations of Arabian Toads (*Amietophrynus arabicus*) for *Batrachochytrium dendrobatidis* in the United Arab Emirates and Oman.

sale (ALC, unpubl.). However, both public and private amphibian collections are present in the UAE exhibiting species exotic to the region (Soorae et al. 2012). The World Organization for Animal Health's regulations requiring that amphibians be free of *Bd* infection before international shipment to a *Bd*-free country (Schloegel et al. 2010) warrants consideration for any amphibian shipments to the UAE or Oman due to their unique fauna which appears to be *Bd*-free.

In addition, the apparently *Bd*-free native, wild Arabian amphibians suggests that biosecurity of captive animals in the area may be important to consider. It would be prudent to test amphibians currently held in zoological collections in the region for *Bd* infection to address possible pathways of inadvertent environmental contamination. At this time, the susceptibilities to *Bd* of the two amphibian species occurring in the UAE and Oman, *Amietophrynus arabicus* and *Duttaphrynus dhufarensis*, remain unknown. Infection experiments would be useful for informing the degree of risk *Bd* might present native amphibians should it be introduced to the region.

LITERATURE CITED

BÖER, B., AND S. A. CHAUDHARY. 1999. New records for the flora of the United Arab Emirates. Willdenowia 29(1/2):159–165.

BOYLE, D. G., D. B. BOYLE, V. OLSEN, J. A. T. MORGAN, A. D. HYATT, ET AL. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR Assay. Dis. Aquat. Org. 60:141–148.

CANNON, R. M., AND R. T. ROE. 1982. Livestock Disease Surveys: A Field Manual for Veterinarians. Australian Government Publishing Service, Canberra. 32 pp.

CHATFIELD, M. W. H., AND C. L. RICHARDS-ZAWACKI. 2011. Elevated temperature as a treatment for *Batrachochytrium dendrobatidis* infection in captive frogs. Dis. Aquat. Org. 94:235–238.

GARLAND, S., A. BAKER, A. D. PHILLOTT, AND L. F. SKERRATT. 2010. BSA reduces inhibition in a TaqMan assay for the detection of *Batrachochytrium dendrobatidis*. Dis. Aquat. Org. 92:113–116.

PIOTROWSKI, J. S., S. L. ANNIS, AND J. E. LONGCORE. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96(1):9–15.

PORTIK, D. M, AND T. J. PAPENFUSS. 2015. Historical biogeography resolves the origins of endemic Arabian toad lineages (Anura: Bufonidae): Evidence for ancient vicariance and dispersal events with the Horn of Africa and South Asia. BMC Evol. Biol. 15(1):152.

SCHLOEGEL, L. M., P. DASZAK, A. A. CUNNINGHAM, R. SPEARE, AND B. HILL. 2010. Two amphibian diseases, chytridiomycosis and ranaviral disease, are now globally notifiable to the World Organization for Animal Health (OIE): An assessment. Dis. Aquat. Org. 92(2–3):101–108.

SOORAE, P. S, T. AL ABDESSALAAM, C. TOURENQ, M. K. SHURIQI, AND M. AL MEHAIRBI. 2012. Preliminary analyses suggest absence of amphibian chytrid fungus in native and exotic amphibians of the United Arab Emirates. Salamandra 48:173–176.

STEVENSON, L. A, R. A. ALFORD, S. C. BELL, E. A. ROZNIK, L. BERGER, AND D. A. PIKE. 2013. Variation in thermal performance of a widespread pathogen, the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. PloS ONE 8(9):e73830.

WELDON, C., L. H. DU PREEZ, A. D. HYATT, R. MULLER, AND R. SPEARE. 2004. Origin of the amphibian chytrid fungus. Emerg. Infect. Dis. 10(12):2100.