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Short communication

Infection of an invasive frog *Eleutherodactylus coqui* by the chytrid fungus *Batrachochytrium dendrobatidis* in Hawaii

Karen H. Beard [a,*], Eric M. O'Neill [b]

[a] *Department of Forest, Range, and Wildlife Sciences and Ecology Center, Utah State University, 5230 Old Main Hill, Logan, UT 84322-5230, USA*
[b] *Department of Biology, Utah State University, Logan, Utah 84322-5305, USA*

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Abstract

The chytrid fungus *Batrachochytrium dendrobatidis* has contributed to declines and extinctions of amphibians worldwide. *B. dendrobatidis* is known to infect the frog *Eleutherodactylus coqui* in its native Puerto Rico. *E. coqui* was accidentally introduced into Hawaii in the late 1980s, where there are now hundreds of populations. *B. dendrobatidis* was being considered as a biological control agent for *E. coqui* because there are no native amphibians in Hawaii. Using a DNA-based assay, we tested 382 *E. coqui* from Hawaii for *B. dendrobatidis* and found that 2.4% are already infected. We found infected frogs in four of 10 study sites and on both the islands of Hawaii and Maui. This is the first report of *B. dendrobatidis* in wild populations in Hawaii. As the range of *E. coqui* expands, it may become a vector for the transmittance of *B. dendrobatidis* to geographic areas where *B. dendrobatidis* does not yet exist.

Keywords: Amphibian declines; Biocontrol; Chytridiomycosis; *Eleutherodactylus coqui*; Hawaii; Emerging diseases; Frog; Invasion; Puerto rico

1. Introduction

Nearly one-third of all amphibians are threatened with extinction (Stuart et al., 2004). Chytridiomycosis, a disease caused by the pathogenic fungus *Batrachochytrium dendrobatidis*, has been identified as a causal agent of amphibian declines in the Americas, Europe, and Australia (e.g., Bell et al., 2004; Berger et al., 1998; Bosch et al., 2001; Lips et al., 2004; Muths et al., 2003), and has been found on every continent with amphibians, except Asia (Weldon et al., 2004). *B. dendrobatidis* is a waterborne pathogen that primarily infects keratinized tissues in the epidermis of amphibians and spreads through colonization by motile, aquatic zoospores (Longcore et al., 1999). Because *B. dendrobatidis* does not survive desiccation (Johnson and Speare, 2003), amphibians are thought to be the primary means by which the disease is transported to new areas (Daszak et al., 2003; Hanselmann et al., 2004; Weldon et al., 2004).

Some invasive amphibians (e.g., *Rana catesbeiana*) are relatively resistant to chytridiomycosis, yet are efficient carriers of the pathogen (Daszak et al., 2004). The Puerto Rican terrestrial frog, *Eleutherodactylus coqui*, is a notable amphibian invader that has not been tested for *B. dendrobatidis* outside of its native range. *E. coqui* has invaded Florida and several islands in the Caribbean, and was accidentally introduced to Hawaii via nursery plants in the late 1980s (Kraus et al., 1999). Direct development and year-round breeding are thought to contribute to its rapid spread. There are now over 250 known populations on the islands of Hawaii and Maui, located mostly in lowland forests on the windward sides (from 0

* Corresponding author. Tel.: +1 435 797 8220; fax: +1 435 797 3796.
E-mail addresses: karen.beard@usu.edu, kbeard@cc.usu.edu (K.H. Beard).

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to 1100 m altitude), with new populations being reported weekly (Kraus and Campbell, 2002).

In Hawaii, *E. coqui* appears to establish populations that have greater densities than those in their native range (20,000 frogs/ha on average in Puerto Rico, Stewart and Woolbright, 1996; K. Beard, unpublished data). The invasion threatens Hawaii's unique ecological communities because *E. coqui* predates upon endemic invertebrates, which comprise the large majority of Hawaii's endemic fauna (Beard and Pitt, 2005). The invasion also threatens Hawaii's multi-million dollar floriculture and nursery industries due to quarantine restrictions and frog de-infestation measures (Kraus and Campbell, 2002). Likewise, property value and tourism are threatened because of its loud (80–90 dBA at 0.5 m) mating calls.

Numerous methods for managing *E. coqui* populations have been developed in Hawaii; yet, there has been no report of a successfully eliminated population. Biological control based on amphibian diseases is considered an attractive option because Hawaii has no native amphibians. *B. dendrobatidis* has been found to infect *E. coqui* in Puerto Rico dating back to 1978 and is thought to contribute to declines at high elevations (Burrowes et al., 2004). Thus, it has been suggested that *B. dendrobatidis* could be used to control *E. coqui* (Hawaii State Department of Agriculture, 2004). Our objective was to determine whether *B. dendrobatidis* is already present in *E. coqui* populations in Hawaii.

2. Materials and methods

E. coqui were collected from seven locations on the island of Hawaii and three locations on Maui in May and August 2004, respectively (Table 1). Locations were selected to maximize diversity in forest-type, elevation, and geological history. For one night at each location, subadult [snout-vent length (SVL) < 24 mm (Woolbright, 1985)] and/or adult frogs [SVL ⩾ 24 mm] were collected by slowly and systematically walking in a 20 × 20 m plot between 2000 and 2200 h. For each frog, SVL and perch height were recorded. Frogs were collected using standard protocols for testing for *B. dendrobatidis* infection [as outlined in O'Neill et al. (in review)] and were preserved in 70% ethanol. *E. coqui* demonstrated no overt clinical signs of chytridiomycosis when collected, such as unusual sloughing of the skin or mortality.

We tested 175 subadults and 207 adults for *B. dendrobatidis* using the DNA-based assay described by Annis et al. (2004). This assay uses species-specific primers (*B. dendrobatidis*1a and *B. dendrobatidis*2a) located within ITS1 and ITS2 to amplify the 5.8S region of nuclear rDNA. Tissue samples ranged from a whole foot (subadults) to a half toe (adults). DNA was extracted using the protocol from Schizas et al. (1997) with the following modifications: the digestion reaction contained 20–30 µl Te (10 mM Tris, 0.1 mM EDTA), and 1.0 µl Proteinase K (20 mg/ml). Samples were digested and periodically vortexed for 3 h at 55 °C. PCR protocols were the same as those described in Annis et al. (2004) including the use of Platinum® *Taq* DNA Polymerase (Invitrogen Corporation, Carlsbad, California, USA).

Positive controls were both pure *B. dendrobatidis* DNA extracted from culture (Joyce Longcore, unpublished data) and DNA extracted from *Rana muscosa* that had previously tested positive for *B. dendrobatidis* (Jessica Morgan, unpublished data). Negative controls consisted of purified water in the PCR reaction and reanalyses of DNA from animals that previously tested negative for *B. dendrobatidis*. PCR products were visualized on a standard 1.4% agarose gel. Samples that contained a band at 330 base pairs (BP) in length were presumed to be positive for *B. dendrobatidis* infection (Annis et al., 2004). Three samples resulted in a faint band at 330 BP. In these cases, samples were PCR amplified a second time using the first PCR product as the template. To create comparable negative controls in

Table 1
Number of *Eleutherodactylus coqui* examined and diagnosed with the chytrid fungus *Batrachochytrium dendrobatidis* from 10 locations in Hawaii

Location	Island	Coordinates	Elevation (m)	(No. with fungus/No. examined)	
				Subadults	Adults
Hawaiian Paradise Park	Hawaii	N19°36′W154°59′	50	1/42	–
Humane Society	Hawaii	N19°36′W155°01′	135	0/13	–
Kurtistown	Hawaii	N19°36′W154°05′	310	1/18	–
Lava Tree State Park	Hawaii	N19°29′W154°54′	180	4/26	1/75
Manuka Natural Area Reserve	Hawaii	N19°07′W155°50′	560	0/24	–
Puainako/Safeway	Hawaii	N19°42′W155°04′	45	0/4	–
Waipio Overlook	Hawaii	N20°07′W155°35′	305	0/7	–
Kihei Nursery	Maui	N20°44′W156°27′	400	–	0/51
Maliko Gulch	Maui	N20°52′W156°19′	440	2/41	0/75
Miles Makawao	Maui	N20°53′W156°19′	20	–	0/6
Total				8/175	1/207

Frogs were collected in the summer of 2004.