infected than frogs breeding in ephemeral or terrestrial habitats (Kriger and Hero 2007).

Along the Rincón stream, we found most infected individuals near a dirt road crossing, with the exception of one *S. sordida* individual found further downstream. While this concentration of infected frogs may be located near the road for unrelated reasons, the pattern could indicate that a reservoir for this pathogen is located outside of the stream and stream frogs are potentially exposed through the movement of vehicles or animals traveling along the road.

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Occurrence of *Batrachochytrium dendrobatidis* in an Anuran Community in the Southeastern Talamanca Region of Costa Rica

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Soon after the discovery of the amphibian disease chytridiomycosis, caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*, Longcore et al. 1999), it became apparent that *Bd* was a major threat to amphibians resulting in mass die-offs and population declines throughout the world (Berger et al. 1998; Blaustein and Keisecker 2002; Daszak et al. 2003; McCallum 2005; Rachowicz et al. 2006). Evidence suggests that *Bd* infected amphibian populations in Central America as early as the 1980s (Pounds et al. 1997). Work done in Central America has implicated that a wave of *Bd* has moved through the montane regions of Central America and was associated with major declines in amphibian populations and species richness (Lips et al. 2006; Puschendorf et al. 2006). However, in some cases, *Bd* also may occur in amphibian communities with little or no effect on populations (Berger et al. 1998; Brem and Lips 2008; Garner et al. 2006).

We sampled amphibians for the presence of *Bd* in the Kèköldi Indigenous Reserve in the lower elevations of the Talamanca region, Costa Rica, to determine *Bd* infection rates in an area not previously surveyed. Also, we attempted to determine which species might be at greatest risk from *Bd*. Although our study site had not been sampled prior to this study, *Bd* likely reached this region in the early 1990s (see Lips et al. 2006).

We sampled anurans for *Bd* from 6 to 17 January 2008 in the 3538-ha Kèköldi Indigenous Reserve, located in southeastern Costa Rica near Hone Creek in Limon Province (Fig. 1). The habitat in the reserve is mostly secondary forest, underplanted with cacao trees; however some primary lowland tropical rainforest occurs at higher elevations in the reserve. The climate at Kèköldi is hot and

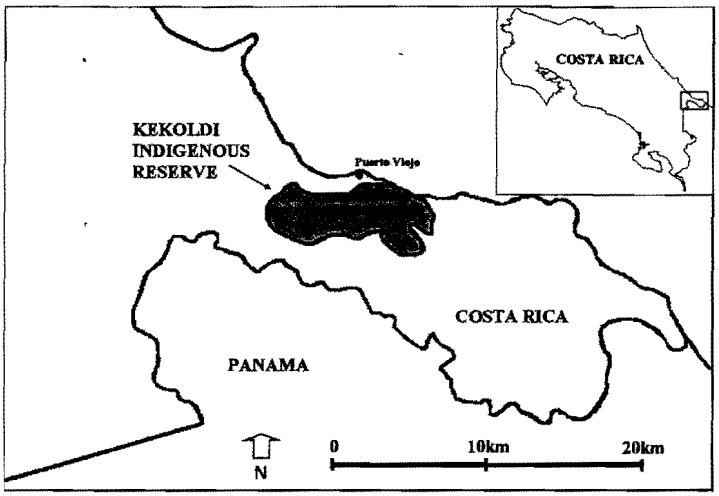

FIG. 1. Study site location, Kèköldi Indigenous Reserve, Costa Rica, where 20 frog species were examined for the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*.

humid year-round, with an annual mean temperature of 26°C and average rainfall of approximately 2500 mm per year.

We searched for adult frogs along the trails and streams in the reserve and captured them by hand. Each individual was handled with a new pair of sterile nitrile gloves. We sampled for *Bd* by rubbing a sterile cotton swab on the dorsum, ventral surfaces and feet of each frog for approximately 30 sec, then the animal was released. The swab was then immediately placed in a sterile microcentrifuge tube containing 1 ml of 70% ethanol and later sent to Pisces Molecular Lab (Boulder, Colorado, USA) for PCR analyses. Global positioning coordinates and elevation were taken at each capture site using a Garmin® GPS unit.

During 12 days of sampling at Kèköldi, no sick or dead frogs were observed. We sampled 126 adult frogs of 20 different species, from 10 different Families. Of these 20 species, only 8 tested positive for *Bd*. Ten of the 126 individuals tested positive for an overall detection rate of 7.9% for the anuran community. Too few individuals were sampled to determine *Bd* prevalence per species. Only one species, *Craugastor crassidigitus*, had more than one individual (3 of 12) test positive for *Bd* (Table 1). Anurans were sampled at elevations ranging from 29 to 150 m. We had too few positive samples to test for effects of elevation on *Bd* detection rates.

Numerous studies have documented the presence of *Bd* in Central America (Brem and Lips 2008; Lips 1998; Lips et al. 2003; Picco and Collins 2007; Puschendorf et al. 2006). Puschendorf et al. (2006) demonstrated through histological examination of museum specimens that the fungus was present in Braulio Carrillo National Park, Costa Rica, by 1986 at almost all elevations sampled. Declining species richness in amphibian communities along a north-to-south transect in Central America has been linked to *Bd* (Lips et al. 2006). According to the timeline in Lips et al. (2006) tracking the epidemic wave of *Bd* in Costa Rica, the fungus probably reached the Kèköldi Reserve in the early to mid 1990s.

Since *Bd* likely moved through Kèköldi 15 to 20 years prior to this study, it is possible that an initial epizootic event took place resulting in declines in amphibian species richness and abundance followed by a rebound to an enzootic state (Brem and Lips 2008). It is also possible that the fungus never reached an epizootic state in the low elevation Kèköldi Reserve because the warm air temperatures are less than optimal for *Bd* growth and infection of amphibians (Kriger and Hero 2006; Longcore et al. 1999; Retallick et al. 2004). Evidence for enzootic *Bd* includes the relative low incidence of detection in PCR samples and the fact that no sick or dead frogs were encountered. Frogs appeared to be very abundant in the reserve; however follow-up population and *Bd* sampling is needed to confirm whether the *Bd* is currently epizootic or enzootic.

TABLE 1. List of anuran species tested for the presence of *Batrachochytrium dendrobatidis* (*Bd*) within Kèköldi Indigenous Reserve, Costa Rica.

Family	Species	No. animals infected / examined
Aromobatidae	*Allobates talamancae*	0/2
Bufonidae	*Incilius coniferus*	0/1
	Rhinella marinus	0/1
Centrolenidae	*Hyalinobatrachyum valerioi*	0/5
Craugastoridae	*Craugastor bransfordii*	1/13
	Craugastor crassidigitus	3/12
	Craugastor gollmeri	0/1
	Craugastor megacephalus	0/12
	Craugastor noblei	1/2
	Craugastor sp.	0/3
Dendrobatidae	*Dendrobates auratus*	1/9
	Oophaga pumilio	1/16
	Phyllobates lugubris	1/4
	Silverstoneia flotator	0/15
Eleutherodactylidae	*Diasporus diastema*	1/16
Hylidae	*Agalychnis callidryas*	0/1
	Smilisca phaeota	0/1
	Smilisca sordida	1/3
Leptodactylidae	*Leptodactylus savagei*	0/6
Ranidae	*Lithobates warszewitschii*	0/2
Strabomantidae	*Pristimantis cerasinus*	0/1

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Detecting *Batrachochytrium dendrobatidis* in the Wild When Amphibians Are Absent

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Once common in the southern Rocky Mountains of North America, sharp declines in Boreal Toad (*Anaxyrus boreas boreas*) populations precipitated their listing as a state endangered species in Colorado, USA (Loeffler 2001) and consideration for listing under the Endangered Species Act (U.S. Fish and Wildlife Service 2005). The amphibian chytrid fungus (*Batrachochytrium dendrobatidis*, hereafter *Bd*) has been implicated in these declines (Livo 2000; Muths et al. 2003; Scherer et al. 2005). Interest in reintroducing *A. b. boreas* into historical habitats (Loeffler 2001) has spurred the need to develop a test for the presence of *Bd*. Reintroduction efforts are time consuming and costly, and their success may hinge on the occurrence of *Bd* at a potential site. As such, it is imperative that disease status be considered when evaluating potential reintroduction efforts.

Currently our ability to detect *Bd* at a site relies on resident amphibians being present, yet they are not at many promising potential reintroduction locations. Since *Bd* can persist at a location even in the absence of amphibian species (Longcore et al. 1999; Rowley et al. 2007; Speare et al. 2001), we suspect that amphibians may not be the only host, and that infection can be maintained through other alternate hosts or environmental reservoirs. We hope that by testing these non-amphibian sources, the *Bd* status at potential reintroduction sites can be evaluated. Rowley et al. (2007) did not detect *Bd* in retreat sites of rain forest stream frogs, while Lips et al. (2006) did find *Bd* DNA on stream boulders but not in filtered water samples. Others have detected *Bd* in filtered water samples (Kirshtein et al. 2007; Walker et al. 2007), but their approaches do not always perform well in waters carrying high organic loads that rapidly clog filters (Cossel and Lindquist 2009) or cause PCR inhibition (Kirshtein et al. 2007). Our initial efforts toward finding alternative *Bd* hosts focused on insects, because they are readily available and chytrid fungi can degrade chitin, a component of aquatic insect exoskeletons (Johnson and Speare 2003; Powell 1993). These early surveys were unable to confirm the presence of *Bd* in samples of Dytiscidae, Coenagrionidae, Hydrophilidae, or Notonectidae from two ponds known to harbor the fungus (Rogers et al. 2004). Samples of Corixidae, algae, snails, and clams taken from a third pond with infected Boreal Chorus Frogs (*Pseudacris maculata*) were also negative for *Bd* DNA (Rogers and Wood 2005). In an effort to establish a more rigorous examination of potential alternate hosts, we initiated a study to explore the feasibility of using sentinel cages and fish following reports that *Bd* could be found on the scales of Fathead Minnows (*Pimephales promelas*) that were exposed to *Bd* in the laboratory (R. Retallick, pers. comm., GHD, Australia). Feathers and keratin were included