# Apparent Absence of Chytrid Infection in Trinidad's Frogs

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**ABSTRACT**

Frogs were sampled for the presence of the pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* at nine sites across Trinidad. No chytrid was detected amongst 245 individuals, of 15 species sampled. These results, together with the negative findings from Greener *et al.* (2017) who sampled 116 stream frogs, *Mannophryne trinitatis* from six sites in the Northern Range, suggest that the low level chytrid infection detected in stream frogs in 2007 and 2009 may have died out, or at least is not spreading through Trinidad's frog populations. Possible explanations are discussed and the need for continued vigilance stressed.

**Key words:** amphibians, chytridiomycosis, Trinidad

## INTRODUCTION

The Global Amphibian Assessment found 32.5% of species threatened with extinction compared to 12% of birds and 23% of mammals (Stuart *et al.* 2004). Although habitat loss and change were identified as major problems for amphibians, as for other groups, a particular concern for amphibians was the high proportion of 'enigmatic' cases where populations were reported to be declining in what appeared to be good quality habitat. One of the factors driving these enigmatic declines has been identified as the chytrid fungus *Batrachochytrium dendrobatidis (Bd)* which infects and damages amphibian keratinised skin, often leading to fatal electrolyte imbalances (Kilpatrick *et al*. 2010). Although *Bd* infections have proved fatal to many individuals and may have been the principal cause of some amphibian extinctions, some individuals and species show either resistance or tolerance to the infection. Infected but tolerant individuals can pose a danger to others, as carriers (Venesky *et al.* 2014).

In Trinidad and Tobago, *Bd* infections have been reported by Alemu *et al.* (2013, 2008). Their observations were as follows: In Tobago, *Bd* was present in 21/84 individual *Mannophryne olmonae* sampled from several locations in 2006, though no individuals showed signs of clinical disease; Alemu *et al*. (2008) also tested 36 other individuals from four species (mainly *Rhinella marina*) but none were found to be positive for *Bd*; in Trinidad (sampled in 2007, with a follow-up in 2009), *Bd* infection was found in two out of 12 *Mannophryne trinitatis* populations (11 in the Northern Range, one in the Central Range; infections found only in the Northern Range). The *M. trinitatis* surveys included 120 individuals tested in 2007 and 60 in 2009, with positive samples being found in 8/40 and 2/40 over the two years at the two sites. No other Trinidad frog species were tested by Alemu *et al.*(2013).

As part of an evaluation of the conservation status of *M. trinitatis*, Greener *et al*. (2017) re-assessed this species for the presence of *Bd*. They tested 116 individuals from six populations, including one population on the Blanchisseuse Road where Alemu *et al*.(2013) had found *Bd* present, but obtained no evidence for the continuing presence of *Bd*.

In this report, we present results from *Bd* sampling of 15 other Trinidad frog species. We sampled from sites where single species predominated and from sites where several species occurred together, to test the possibility that *Bd* can be spread between individuals where multiple species occur, or the alternative 'dilution effect' where the presence of resistant species reduces transmission in multispecies communities (Venesky *et al.* 2014).

## METHODS

### *Site and species selection*

Chytrid swabbing was conducted in Trinidad between June and mid-August 2014. A total of nine sites were visited (Table 1; Fig. 1). At four of these sites only a single species was sampled and at five sites more than one species was sampled (mixed species and breeding assemblage sites).

### *Single species sample sites*

The four single species sampling sites were known sites for amphibians (Downie, personal observations). These sites included drainage ditches along the Caura Valley specifically to swab *Hypsiboas crepitans*, University of West Indies (St. Augustine Campus) to swab *Rhinella marina*, Simla Research Station (Arima valley) to swab *Phyllomedusa trinitatis* and Austin Trace, Cedros to swab *Scarthyla vigilans*.

### *Mixed species and breeding assemblage sample sites*

Sites known to contain multiple species or breeding

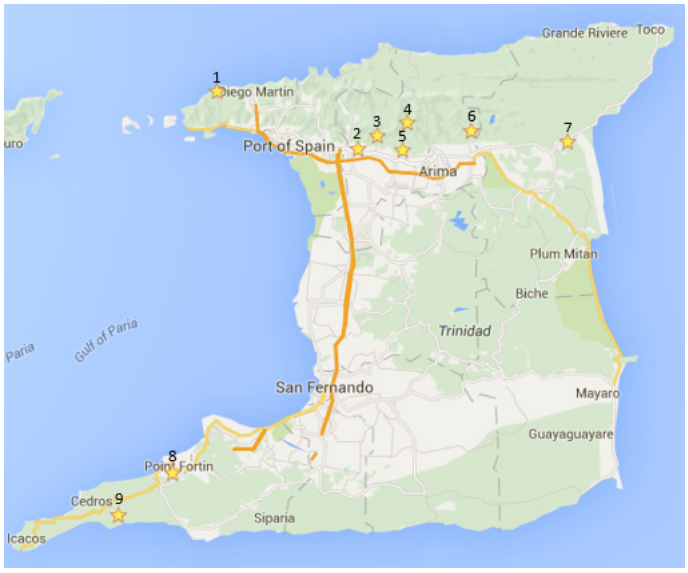


**Fig. 1.** Map showing the sample sites, numbered as in Table 1.

assemblages (Downie and members of the Trinidad and Tobago Field Naturalists' Club, personal communications) were also sampled. The aim was to collect samples widely across the island so as to sample from a broad range of species and populations. The sites sampled included Point Fortin (south-west), the Bamboo Cathedral, Chaguaramas (north-west), Toco Main Road (north-east) and two sites in the Northern Range – a site close to a Chicken farm in the Lopinot Valley and the garden of an abandoned house in Lopinot village.

### Sampling methods

The frog species sampled in this study are all nocturnally active and are most easily found around breeding sites (ponds, ditches) on nights following wet days. Frogs were caught by hand or with the aid of hand nets and transferred to individual polythene bags. The collection team were all trained in frog identification by JRD and had a copy of Murphy (1997) available whenever there was any doubt. Frogs were sampled for chytrid close to the collection site so that they could be immediately returned to their habitat. To allow effective sampling at night, we set up a sheltered illuminated work table in the field.

Amphibians were caught and swabbed following the standard chytrid protocol (Brem *et al*. 2007)**.** The swabs used were clinical grade sterile Deltalab single-packed swabs, routinely used for the collection of microbiological samples that are later subjected to PCR analysis. All equipment was sanitised in 0.5% sodium hypochlorite bleach solution following each site visit or, for nets, after each use. Samples were stored at -20 °C in 95% ethanol in Trinidad, transported to the UK on ice, and transferred to -80 °C storage thereafter. DNA was extracted using Phenol-Chloroform extraction (Sambrook and Russell 2001). A standardised PCR analysis was then used to look for the presence or absence of *Bd* in each sample. The primers used were BOB5 and BOB6 (Boyle *et al*. 2004) and a positive control DNA for *Bd* was supplied by Prof Andrew Cunningham, Institute of Zoology, London.

## RESULTS

Out of the 245 amphibians sampled, no chytrid- positive individuals were detected at any single species site (*H. crepitans, R. marina, P. trinitatis* or *S. vigilans*), mixed species, or breeding assemblage site. In addition, we saw no individuals visibly suffering from the symptoms of chytrid infection. A sample PCR gel is illustrated in Fig. 2. DNA quality control using 16s rDNA universal primer

**Table 1.** Results from swabs of single species samples and mixed species and breeding assemblage sites showing the total number swabbed at each site. No positive individuals were found for any species or site.

| Site and GPS co-ordinates | Species | Total number swabbed |
|---|---|---|
| Caura Royal Road (3) 10°40'18.4"N, 61°22'03.5"W | *Hypsiboas crepitans* | 19 |
| University of West Indies Campus (2) 10°38'33.1"N 61°24'02.0"W | *Rhinella marina* | 20 |
| Simla Research Station, Arima Valley (6) 10°40'45.5"N, 61°13'29.0"W | *Phyllomedusa trinitatis* | 19 |
| Austin Trace, Cedros (9) 10°05'53.3"N, 61°45'52.1"W | *Scarthyla vigilans* | 7 |
| La Fortunee Dam, Point Fortin (8) 10°09'41.9"N, 61°40'54.7"W | *Scarthyla vigilans* | 5 |
| | *Dendropsophus microcephalus* | 7 |
| | *Hypsiboas punctatus* | 8 |
| Bamboo cathedral, Chaguaramas (1) 10° 43'08.5''N 61° 37' 29.0''W | *Pristimantis urichi* | 5 |
| | *Rhinella marina* | 8 |
| | *Dendropsophus microcephalus* | 1 |
| Chicken farm, Lopinot Valley (5) 10°38'59.9"N 61°19'48.4"W | *Engystomops pustulosus* | 12 |
| | *Leptodactylus fuscus* | 13 |
| | *Trachycephalus typhonius* | 4 |
| | *Leptodactylus validus* | 1 |
| | *Hypsiboas crepitans* | 2 |
| | *Scinax ruber* | 11 |
| | *Dendropsophus microcephalus* | 27 |
| | *Rhinella marina* | 5 |
| Abandoned house, Lopinot (4) 10°41'27.0"N 61°19'19.9"W | *Engystomops pustulosus* | 10 |
| | *Leptodactylus fuscus* | 5 |
| Toco Main Road (7) 10°39'55.4"N, 61°04'21.1"W | *Dendropsophus microcephalus* | 17 |
| | *Elachistocleis ovalis* | 1 |
| | *Hypsiboas punctatus* | 2 |
| | *Leptodactylus fuscus* | 4 |
| | *Rhinella beebei* | 4 |
| | *Sphaenorhynchus lacteus* | 1 |
| | *Scinax ruber* | 25 |
| | *Rhinella marina* | 2 |

pair and chytrid-specific primer pair control are shown in Fig. 3 and 4 respectively.

***Single species sample sites***

At each single species sample site (rows 1-4 Table 1) a target of 20 individuals were sampled, to ensure that chytrid would be detected, if present. At three locations 19 or 20 individuals were successfully sampled, but at Austin Trace, Cedros, only seven *Scarthyla* were swabbed due to time constraints.

***Mixed species and breeding assemblage sites***

Results from mixed species sites and breeding assemblages can be found in Table 1 showing species and numbers caught at each site.

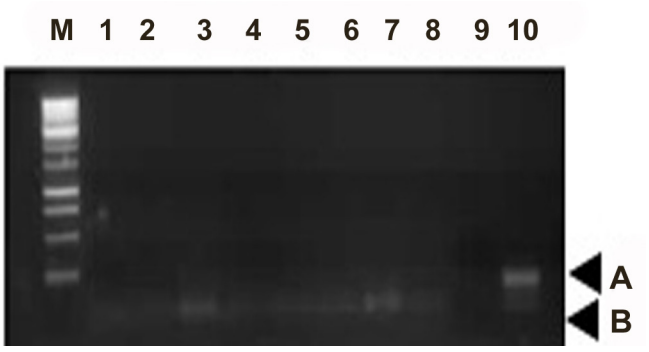


**Fig. 2.** Gel electrophoresis of swab samples using Chytrid-specific primer pair. Lane 1 to 8 show swab extractions showing no positives. The negative control is present in lane 9 and positive 2 ng *B. dendrobatidis* DNA control is present in lane 10. Lane M shows the 100 bp Markers (Promega).

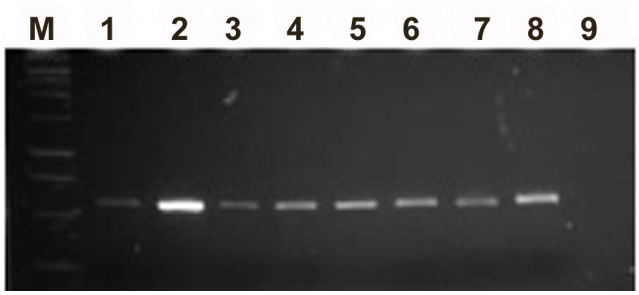


**Fig. 3.** DNA Extraction and PCR quality DNA Control 16s rDNA Universal primer pair. Lane 1 to 8, taken from 8 randomly selected samples, show positive results for the presence of PCR quality frog DNA that can be amplified by PCR. Negative control is present in lane 9. Lane M shows the 100 bp Markers (Promega).

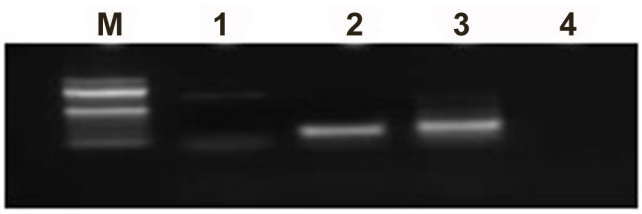


**Fig. 4.** Chytrid-specific primer pair control. Lane 1: *Mannophryne trinitatis* DNA, lane 2: 2 ng *B. dendrobatidis* DNA, lane 3: 10 ng *B. dendrobatidis* DNA and lane 4: negative control. Lane M shows the 100 bp Markers (NEB).

**DISCUSSION**

We found no positives for chytrid (*Batrachochytrium dendrobatidis*), out of the 245 individuals from 15 species sampled.

All protocols, including collecting skin swabs, storing swabs, extracting DNA and PCR analysis were followed rigorously and controls were included in the analysis. Furthermore, as no visibly diseased amphibians were found at any site and the recommended minimum sample size (about 20) for detection of chytrid at single species swabbing sites was achieved in most cases, it is highly unlikely that chytrid was present in the samples collected.

At some of the mixed species/breeding assemblage sites the sample size for some species was very small (in six species, five or fewer sampled). In such species we cannot be sure that chytrid is not present. However, as there were no positive individuals for chytrid at any site, and Greener *et al*.(2017) found no chytrid infection in a sample of 116 *Mannophryne trinitatis*, where chytrid was detected in 2007 and 2009 (Alemu *et al*. 2013), the likelihood of one species having chytrid and not another at the same site is small. Seasonal variation is unlikely to be a factor since Alemu *et al*.'s samples were taken at the same time of year as ours.

Although chytrid infection has been reported from many Caribbean islands (Olson *et al.* 2013) and from nearby Venezuela (Hanselmann *et al*. 2004), the danger posed by chytrid to Caribbean amphibians is less clear. Recent work emphasises that although some species are devastated by the arrival of chytrid, in other cases chytrid has been present for decades without causing significant mortality, because species are tolerant or resistant to the infection (Venesky *et al*. 2014). Alemu *et al*.(2008, 2013) found chytrid present in the *Mannophryne* species endemic to Tobago and Trinidad respectively, but not at high rates of prevalence and with no signs of clinical disease, possibly indicating resistance. In Trinidad, but not in Tobago, chytrid infection appeared to be associated with populations at the highest altitude sampled. In Trinidad, we have found no chytrid some years after Alemu *et al*.'s survey either in *M. trinitatis* (Greener *et al*. 2017), including at one of the higher altitude sites sampled by Alemu *et al*.(2013), or in 15 other species. It would be surprising if all these species were resistant. Perhaps the environmental conditions that favour the spread of chytrid are currently absent in Trinidad. Whatever the underlying cause of our results, there is no reason for complacency. Regular sampling for chytrid in both Trinidad and Tobago should be instituted and care taken, through biosecurity measures

as detailed in the sampling methods section, to avoid any spread of the disease.

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