A historical overview of *Batrachochytrium dendrobatidis* infection from specimens at the National Zoological Collection Suriname

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Abstract

The amphibian skin disease chytridiomycosis, caused by the pathogenetic fungus *Batrachochytrium dendrobatidis* (Bd) has become one of the major contributors to global amphibian population declines and extinctions. This fungus has spread globally and has caused mortalities in nearly every continent. In South America, Suriname, Guyana and Paraguay are among the remaining three countries where Bd has not been detected to date. To complete the assessment of the possible presence of Bd in Suriname, 205 specimens from the Zoological Collection of Suriname, compromising 6 frog families and 15 genera were sampled for chytrid fungus. No specimens were found to be infected by this fungus and as such the outcome strengthens the previous result of field sampling that there is no support that Bd has spread to Suriname.

Introduction

Amphibians are generally regarded as good indicators for habitat or ecosystem health due to their dependency on both terrestrial and aquatic habitats for their life history [1]. With 40% of global amphibian species currently at risk of declines or extinctions [https://www.iucn-amphibians.org/; accessed 8 June 2020], concerns for amphibian threats are increasing, with human activities at the forefront of risks [2]. The decline in amphibian populations is not something occurring locally but was demonstrated by [3, 4] to be on a more global scale than once thought. In addition to rapid habitat change from anthropogenic factors, a top contributor to amphibian losses includes the human-mediated introduction of invasive alien species throughout the world including emerging infectious diseases such as chytridiomycosis caused by *Batrachochytrium dendrobatidis* (Bd) [5]. Originating in South Korea [6] this disease-causing skin fungal pathogen has taken proportions of a global epidemic with disease affecting species on all continents with amphibians. In South America, Bd has been detected in amphibian communities within all the countries apart from Suriname, Guyana and Paraguay (Bd-maps.net, accessed December 2019). In Suriname, tests for Bd presence were initially conducted by sampling *Atelopus hoogmoedi* at the Brownsberg Nature Park [7]. In a second study, *Pipa pipa* frogs collected from Suriname and deposited in museums abroad were also been tested for Bd
by Soto-Azat et al. [8]; this research was done to verify the possibility of African *Xenopus* spp. as a source of Bd, and also tested other frog species in the family Pipidae from Guyana, Venezuela, French Guiana, and Africa [8]. No Pipidae specimens of these South American countries collected between 1844 and 1994 tested positive for Bd [8]. Although Bd has not been detected in Suriname to date, it has been detected in many places in French Guiana [9] with one location being approximately 7 km away from the border of Suriname where frog infection rates ranged between 1–5% [9]. Though Bd has just recently gathered a lot of attention by the international community, the existence of this fungus can be found in museum specimens dating back as far as the 1970’s [10]. Knowledge of verified gaps in Bd occurrence is of paramount importance, as enhanced biosecurity may forestall inadvertent human-mediated transmission, aiding amphibian conservation. Although initial investigations of selected taxa including one field study [7] and one museum study [8] have not supported Bd presence in Suriname, additional sampling is warranted for broader spatial and taxonomic coverage. Herein, we report findings of a broader geographic and taxonomic study in Suriname, conducted by checking museum specimens housed at the National Zoological Collection Suriname (NZCS) for the presence of Bd. The advantages of testing museum specimens for the presence of Bd includes a provision of an historical overview of the time that the fungus is present in a particular species and in a particular location. Despite the fact that most museum specimens are formalin fixed, successful studies have still been able to check for the presence of Bd [11–14]. The few museum specimens from Suriname tested by Soto-Azat et al. [8] in a single family support the efficacy to test more species using this approach. Additionally, it allowed us to examine the Bd presence across widespread locations that could be logistically constrained today due to budgetary and time limitations for sampling species in the wild. The research presented herein gives an overview of specimens tested for Bd stored at the NZCS in 6 families and 15 genera compromising 205 specimens.

**Materials and methods**

The NZCS houses approximately 2500 specimens of several amphibian species and is the only zoological institute in Suriname equipped to store specimens and disseminate knowledge on the diverse amphibian taxa present in Suriname. Species that were selected from the database for Bd testing were in the following families; Bufonidae, Dendrobatidae, Aromobatidae, Leptodactylidae, Ranidae and Pipidae. These families were selected due to past studies showing their members can be vulnerable to Bd infection (e.g., Bufonidae [15, 16] Dendrobatidae [17, 18] and Aromobatidae [9]) or have associations with water resources for parts of their life cycle (Leptodactylidae [19, 20] Ranidae [21] and Pipidae [8]), hence could encounter flagellated zoospores of the aquatic fungal pathogen Bd if it were present [22]. Temporal and spatial considerations also affected selection for sampling (Fig 1 and Table 1). Of the species that were selected in the families mentioned above some of the oldest specimens were collected in 1905 and consisted of 2 *Leptodactylus* specimens. Some specimens were chosen from just a few years back (2014 to 2016) targeting the location where collected. Waypoints on the map were selected based on their singularity of depicting a new geographic area. Although it might not be evident on the map for some areas, a selection for depicted waypoints was done using a minimum distance of 3 km from the nearest waypoint. The main purpose for this was to present all the different geographic areas sampled instead of cluttering the map with waypoints of every specimen. All species mentioned in the table are sorted starting with the oldest specimens collected on top.
Fig 1. Bd sampling locations. Overview of all the locations (red dots) where *Batrachochytrium dendrobatidis* museum specimens were sampled in Suriname.

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Table 1. Table 1 gives an overview of the museum specimens from Suriname that were sampled for *Batrachochytrium dendrobatidis*. Specimens in the table are sorted on date collected in the field in ascending order.

| NZCS # | Family          | Genus       | Species       | Location                                      | Date Collected | No of specimens |
|--------|-----------------|-------------|---------------|-----------------------------------------------|----------------|-----------------|
| A572   | Leptodactylidae | Leptodactylus | fuscus        | Paramaribo neighbourhoods                     | May 17, 1905   | 1               |
| A631   | Leptodactylidae | Leptodactylus | rhodomystax   | Paramaribo and neighbourhoods                 | May 17, 1905   | 1               |
| A571   | Leptodactylidae | Leptodactylus | fuscus        | Charlesburg, Paramaribo                        | April 8, 1957  | 1               |
| A435 & A438 | Dendrobatidae | Dendrobates   | tinctorius    | Kappel Savanna                                | March 22, 1958 | 2               |
| A425, A427 | Dendrobatidae | Dendrobates   | tinctorius    | Basecamp Wilhelmina mountain                   | July 23, 1959  | 2               |
| A433 & A434 | Dendrobatidae | Dendrobates   | tinctorius    | Coeroeni island                               | August 12, 1959 | 2             |
| A568   | Leptodactylidae | Leptodactylus | bolivianus    | Coeroeni island                               | August 12, 1959 | 1               |
| A613   | Leptodactylidae | Leptodactylus | mystaceus     | Coeroeni island, expedition to Coeroeni       | September, 1959 | 1               |
| A632 & A633, A 634 | Leptodactylidae | Leptodactylus | rhodomystax   | Coeroeni island, expedition to Coeroeni       | September, 1959 | 3               |
| A220   | Leptodactylidae | Leptodactylus | bolivianus    | Kabelstation (flooded by creation of Brokopondo Lake) | May 17, 1961   | 1               |
| A607 & A608 | Dendrobatidae | Dendrobates   | longirostris  | Kabelstation                                   | May 17, 1961   | 2               |
| A423 & A424 | Dendrobatidae | Dendrobates   | tinctorius    | On path heading towards Juliana top           | August, 1963   | 2               |
| A428 & A429 | Dendrobatidae | Dendrobates   | tinctorius    | Anapaie kondre, Lawa                          | November 18, 1963 | 1           |
| A431 & A432 | Dendrobatidae | Dendrobates   | tinctorius    | Bakhuis mountain                              | January, 1963   | 2               |
| A219   | Leptodactylidae | Leptodactylus | bolivianus    | Mammadam soela                                | December 11, 1963 | 1           |
| A620   | Leptodactylidae | Leptodactylus | bolivianus    | Camp Lucie river                              | August, 1963   | 1               |
| A602   | Leptodactylidae | Leptodactylus | knudseni      | Camp Lucie rivier, expedition to Wilhelmina mountain | September, 1963 | 1               |
| A614   | Leptodactylidae | Leptodactylus | mystaceus     | Camp Lucie river, expedition to Wilhelmina mountain | September, 1963 | 1               |
| A615   | Leptodactylidae | Leptodactylus | mystaceus     | Pokigron                                       | December 12, 1963 | 1           |
| A621   | Leptodactylidae | Leptodactylus | pentadactylus | In line with Lucie river                      | August 12, 1963 | 1               |
| A622   | Leptodactylidae | Leptodactylus | pentadactylus | Adampada expedition                           | September, 1964 | 1               |
| A426   | Dendrobatidae   | Dendrobates   | tinctorius    | Raleighvallen, Central Suriname Nature Reserve | September, 1971 | 1               |
| A570   | Leptodactylidae | Leptodactylus | bolivianus    | Baruba kreek, km 113, Kabalebo area district Nickerie | September 29, 1980 | 1           |
| A573 to A575 | Leptodactylidae | Leptodactylus | fuscus        | Kopieweg, the first turning on the road, district Suriname | February 9, 1980 | 3           |
| A179   | Pipidae         | Pipa         | pipa          | Avanavero falls                               | May 24, 1980    | 1               |
| A347   | Bufonidae       | Atelopus     | hoogmoedi     | Road to Amotopo, km 80.5, district Nickerie    | May 13, 1981    | 1               |
| A348   | Bufonidae       | Atelopus     | hoogmoedi     | Road to Amotopo, km 39, 2000 m in line in North Western direction, district Nickerie | June 1, 1981 | 1 | |
| A566 & A567 | Leptodactylidae | Leptodactylus | bolivianus    | Road to Amotopo, km 25, Kabalebo area, district Nickerie | May 29, 1981 | 2  |
| A600   | Leptodactylidae | Leptodactylus | knudseni      | Celos camping ground, Kabo, district Saramacca | February 4, 1981 | 1  |
| A601, A605 | Leptodactylidae | Leptodactylus | knudseni      | Road to Celos proefcentrum Kabo, district Saramacca | February 7, 1981 | 2  |
| A604   | Leptodactylidae | Leptodactylus | knudseni      | Road to Wittagron, 25 km from Celos proefcentrum Kabo, district Saramacca | February 6, 1981 | 1  |
| A606   | Leptodactylidae | Leptodactylus | knudseni      | Road to Amotopo km 108.5, district Nickerie    | May 8, 1981     | 1               |
| A609 & A610 | Leptodactylidae | Leptodactylus | longirostris  | Celos camping Tonka, Kabo area, district Saramacca | February 4, 1981 | 2  |
| A616 & A617 | Leptodactylidae | Leptodactylus | mystaceus     | Drempekanaallijn, km 215–217.5, road to Amotopo | May 25, 1981 | 2  |
| A569   | Leptodactylidae | Leptodactylus | bolivianus    | Camping ground G.M.D, Alaskakondre, Marowijne | August 28, 1982 | 1  |
| A440   | Aromobatidae    | Allobates    | femoralis     | Alaskakondre, camp G.M.D, Lawa, Marowijne     | September 13, 1982 | 1  |
| A603   | Leptodactylidae | Leptodactylus | knudseni      | Alaskakondre, district Marowijne              | July 17, 1982   | 1               |
| A635 & A636 | Leptodactylidae | Leptodactylus | rhodomystax   | Alaskakondre, upper Lawa, district Marowijne  | July 17, 1982   | 2               |
| A32    | Aromobatidae    | Allobates    | femoralis     | 91 km upstream of Maratukka River, approx. 3 km from camp | November 12, 1987 | 1  |

(Continued)
Table 1. (Continued)

| NZCS # | Family         | Genus         | Species      | Location                                      | Date Collected       | No of specimens |
|--------|----------------|---------------|--------------|-----------------------------------------------|----------------------|-----------------|
| A33    | Leptodactylidae| Leptodactylus | bolivianus   | 40 km upstream of Maratakka River             | November 15, 1987    | 1               |
| A34    | Leptodactylidae| Leptodactylus | bolivianus   | 91 km upstream of Maratakka River             | November 6, 1987     | 1               |
| A35    | Leptodactylidae| Leptodactylus | bolivianus   | 125 km upstream of Maratakka River            | November 8, 1987     | 1               |
| A178   | Leptodactylidae| Leptodactylus | fuscus       | University of Suriname                        | October 9, 1987      | 1               |
| A578 to A580 | Leptodactylidae | Leptodactylus | fuscus       | Dampoentong (near Groningen), district Saramacca | June 27, 1987        | 3               |
| A59    | Pipidae        | Pipa          | pipa         | Dampoentong near Groningen, in recently deforested plots / agricultural land | June 27, 1987        | 1               |
| A43 & A44 | Dendrobatidae | Ameerega     | trivittata   | Dirt road to boat landing at Coesewijne River | April 13, 1988       | 2               |
| A67    | Dendrobatidae  | Ameerega     | trivittata   | Sipaliwini River, Mouth of Akalapi creek     | August 20, 1988      | 1               |
| A75 t/m A77 | Dendrobatidae | Dendrobates  | tinctorius   | Vier Gebroeders creek, basecamp               | August 28, 1988      | 3               |
| A10    | Aromobatidae   | Allobates    | femoralis    | Zuid River, 15 km upstream from Kayser Mts. Airstrip | February 9, 1988     | 1               |
| A11    | Aromobatidae   | Allobates    | femoralis    | Zuid River, 9 km downstream of Kayser Mountains Airstrip, on line transect 0.5 km North of river | February 13, 1988    | 1               |
| A98    | Ranidae        | Rana         | palmipes     | Blanche Marie Falls                           | October 13, 1988     | 1               |
| A1     | Leptodactylidae| Leptodactylus | bolivianus   | Zuid River, 9 km upstream of Kayser Mountains Airstrip | February 11, 1988    | 1               |
| A2 & A3 | Leptodactylidae | Leptodactylus | bolivianus   | Kayser Mountains Airstrip                      | February 5, 1988     | 2               |
| A69    | Leptodactylidae| Leptodactylus | bolivianus   | Vier Gebroeders creek, basecamp               | August 22, 1988      | 1               |
| A89 to A92 | Leptodactylidae | Leptodactylus | bolivianus   | Vier Gebroeders Mountains                      | August 28, 1988      | 4               |
| A73    | Leptodactylidae| Leptodactylus | fuscus       | Sipaliwini savanna, Sipaliwini creek           | August 25, 1988      | 1               |
| A289, A297 & A298 | Leptodactylidae | Leptodactylus | fuscus       | Zanderij 1                                     | June 25, 1988        | 3               |
| A5, A20, A23, A24 | Leptodactylidae | Leptodactylus | longirostris | Kayser Mountains Airstrip                      | February 5, 1988     | 4               |
| A19, A22, A25 | Leptodactylidae | Leptodactylus | longirostris | Savanna 3.5 km South-East of Kayser Mountains Airstrip | February 17, 1988    | 3               |
| A68    | Leptodactylidae| Leptodactylus | mystaceus    | Sipaliwini River, Mouth of Apiego creek       | August 18, 1988      | 1               |
| A112   | Pipidae        | Pipa          | pipa         | Coesewijne River, km 19                       | November 1, 1988     | 1               |
| A283   | Leptodactylidae| Lithodytes    | lineatus     | South-East side of the Apalagadi, 350 m       | August 16, 1989      | 1               |
| A235, A262 | Dendrobatidae | Dendrobates  | tinctorius   | South side of Apalagadi, Koewini creek, 300 m  | August 14, 1989      | 2               |
| A241   | Dendrobatidae  | Dendrobates  | tinctorius   | Palaime creek, (Sipaliwini savanna)           | August 10, 1989      | 1               |
| A237   | Dendrobatidae  | Dendrobates  | tinctorius   | 2 km North-East of Sipaliwini airstrip         | August 19, 1989      | 1               |
| A263, A282 | Aromobatidae | Ameerega     | trivittata   | South-East side of the Apalagadi, 480 m       | August 16, 1989      | 2               |
| A284, A285 | Aromobatidae | Allobates    | femoralis    | Koewini creek, 300 m at the South side of the Apalagadi | August 17, 1989      | 2               |
| A266   | Dendrobatidae  | Dendrobates  | tinctorius   | South-East side of the Apalagadi, 480 m       | August 15, 1989      | 1               |
| A147 to A149 | Aromobatidae | Allobates    | femoralis    | Nickerie River, 159 km upstream of Wageningen, in line (transect) | March 11, 1989       | 3               |
| A261, A264 | Dendrobatidae | Dendrobates  | tinctorius   | South side of the Apalagadi, 350 m            | August 14, 1989      | 2               |
| A254   | Leptodactylidae| Leptodactylus | mystaceus    | Upper Coeroeni km 22, South Suriname          | August 2, 1989       | 1               |
| A258   | Leptodactylidae| Leptodactylus | mystaceus    | Koewini creek, 300 m at the South side of the Apalagadi | August 12, 1989      | 1               |
| A340   | Leptodactylidae| Leptodactylus | bolivianus   | Kabori creek, West Suriname                   | September, 1990      | 1               |
| A308   | Leptodactylidae| Leptodactylus | fuscus       | Para, Hannover                                | October 28, 1990     | 1               |
| A305   | Leptodactylidae| Lithodytes    | lineatus     | District Para, Loebeek                        | July 23, 1990        | 1               |
| A317   | Pipidae        | Pipa          | pipa         | Coropina creek, district Para, between Para river en Prinsie | October 24, 1990     | 1               |
| A333 t/m A335 | Aromobatidae | Allobates    | femoralis    | Kabori creek, West Suriname                   | September, 1990      | 3               |
| A599   | Pipidae        | Pipa          | Pipa         | Prinsie, Coropina creek, district Para        | October 8, 1990      | 1               |

(Continued)
Table 1. (Continued)

| NZCS #   | Family            | Genus        | Species      | Location                                      | Date Collected | No of specimens |
|----------|-------------------|--------------|--------------|-----------------------------------------------|----------------|-----------------|
| A874     | Leptodactylidae   | Leptodactylus| pentadactylus| 75 km upstream of Tepoe, between camp 3 and camp 4 | September 1, 1991 | 1               |
| A920 & A921 | Bufonidae      | Ateolopus    | hoogmoedi    | Tepoe, camp 5                                  | September 4, 1991 | 2               |
| A779 to A781 | Dendrobatidae | Dendrobates  | tintorius    | Tepoe, Awalape creek, km 68                    | September 11, 1991 | 3               |
| A906     | Dendrobatidae     | Dendrobates  | tintorius    | Tepoe, camp 6, in line                         | September 8, 1991 | 1               |
| A894a,b; A901a,b | Dendrobatidae | Dendrobates  | tintorius    | Tepoe, camp 5                                  | September 3, 1991 | 4               |
| A931a,b,c,d | Dendrobatidae    | Dendrobates  | tintorius    | Tepoe, Awalapa creek km. 68                    | September 11, 1991 | 4               |
| A875     | Dendrobatidae     | Dendrobates  | tintorius    | 101.5 km. upstream of Tepoe, by the mouth of the Peluli creek | September 4, 1991 | 1               |
| A902, A911 | Dendrobatidae    | Dendrobates  | tintorius    | Tepoe, line at the North side of camp 5        | September 5, 1991 | 2               |
| A744, A749 & A750 | Bufonidae      | Ateolopus    | hoogmoedi    | Camp 5, 101.5 km upstream of Tepoe, at mouth of Peluli creek | September 5, 1991 | 3               |
| A928     | Leptodactylidae   | Leptodactylus| pentadactylus| Tepoe, camp 5, 101.5 km. upstream               | September 2, 1991 | 1               |
| A929     | Leptodactylidae   | Leptodactylus| pentadactylus| Tepoe, camp 7, Ogoime                          | September 11, 1991 | 1               |
| A664     | Pipidae           | Pipa         | pipa         | Zanderij 1, Savanna                            | July 22, 1992   | 1               |
| A679     | Aromobatidae      | Allobates    | femoralis    | Palumeu airstrip, in the village               | March 2, 1993   | 1               |
| A764     | Leptodactylidae   | Leptodactylus| bolivianus   | Akinto soela, Mapane area                      | June 27, 1993   | 1               |
| A730, A731, A732 | Leptodactylidae | Leptodactylus| bolivianus   | Royal Hill                                     | September 19, 1994 | 3               |
| A693     | Leptodactylidae   | Leptodactylus| fuscus       | Royal Hill, Gros Rosabel area in deep hole (5 m) | June 26, 1994   | 1               |
| A694     | Leptodactylidae   | Leptodactylus| fuscus       | Gross Savanna, North of Roma                   | June 24, 1994   | 1               |
| A698     | Leptodactylidae   | Leptodactylus| knudseni     | Koolhoven                                      | June 20, 1994   | 1               |
| A737     | Leptodactylidae   | Leptodactylus| knudseni     | Maikaboeka creek                               | September 20, 1994 | 1               |
| A717 to A721 | Leptodactylidae | Lithodytes   | lineatus     | Royal Hill                                     | August 10, 1994 | 3               |
| A695     | Leptodactylidae   | Leptodactylus| longirostris | On the confluence of the Mindrineti river and the Maikaboeka creek | June 21, 1994 | 1               |
| A696     | Leptodactylidae   | Leptodactylus| longirostris | Royal Hill, in mine shaft                      | June 17, 1994   | 1               |
| A697     | Leptodactylidae   | Leptodactylus| longirostris | Mayo                                            | June 24, 1994   | 1               |
| A733     | Leptodactylidae   | Leptodactylus| longirostris | Royal Hill                                      | August 10, 1994 | 1               |
| A738     | Leptodactylidae   | Leptodactylus| pentadactylus| Royal Hill                                      | August 10, 1994 | 1               |
| A716     | Aromobatidae      | Allobates    | femoralis    | Koolhoven                                      | September 26, 1994 | 1               |
| A736     | Leptodactylidae   | Leptodactylus| rhodomystax  | On the confluence of the Mindrineti river and the Maikaboeka creek | June 21, 1994 | 1               |
| A736     | Leptodactylidae   | Leptodactylus| rhodomystax  | Royal Hill                                      | August 10, 1994 | 1               |
| A759     | Leptodactylidae   | Leptodactylus| knudseni     | Wane Hill 2, on the road                       | September 25, 1995 | 1               |
| A888     | Aromobatidae      | Allobates    | femoralis    | Wane hill 2 (camp)                             | March 31, 1995  | 1               |
| A739     | Leptodactylidae   | Leptodactylus| longirostris | Wane Hill 2 (slope)                            | March 29, 1995  | 1               |
| A760     | Leptodactylidae   | Leptodactylus| longirostris | Wane Hill, camp                                 | September 24, 1995 | 1               |
| A767 to A769 | Leptodactylidae | Leptodactylus| longirostris | Wane Hill 2, line AB                           | July 14, 1995   | 3               |

(Continued)
Specimen sampling

Amphibian specimens used for this study were selected by querying the NZCS database.

To swab for Bd we followed the same procedure as Rodriguez et al. [23] where each individual frog was first rinsed with clean uncontaminated 70% ethanol and allowed to dry on a clean sheet of tissue paper. We then swabbed each specimen using Medical Wire swabs as per the standard swabbing methods described by in Hyatt et al. [24] and Cheng et al. [13]. We swabbed and pooled up to eight specimens to minimize the cost of lab analyses keeping swabs of the same species in one vial as much as possible. Pooling swabs or swabbing multiple specimens has become an accepted method of verifying Bd in presence absence studies [24, 25]. After swabbing the specimens, swabs were cut to desired length and placed in sterile 1.5ml screw top centrifuge tubes with O rings. Swabs were allowed to dry completely before

Table 1. (Continued)

| NZCS # | Family         | Genus            | Species      | Location                              | Date Collected | No of specimens |
|--------|----------------|------------------|--------------|---------------------------------------|----------------|-----------------|
| A879   | Leptodactylidae| Leptodactylus    | rhodomystax | Wane Hill 2                           | March 29, 1995 | 1               |
| A908   | Leptodactylidae| Leptodactylus    | knudseni    | Berlijn                               | May, 1997      | 1               |
| A786   | Leptodactylidae| Leptodactylus    | bolivianus  | Sipaliwini Airstrip                   | April 11, 1997 | 1               |
| A794, A341 | Leptodactylidae| Leptodactylus | fuscus    | Sipaliwini Airstrip                   | April 15, 1997 | 2               |
| A803, A805 & A806 | Aromobatidae | Allobates | femoralis | Sipaliwini airstrip | April 14, 1997 | 3               |
| A784   | Leptodactylidae| Leptodactylus    | rhodomystax | Sipaliwini Airstrip                   | April 16, 1997 | 1               |
| A908   | Leptodactylidae| Leptodactylus    | knudseni    | Berlijn                               | May, 1997      | 1               |
| A844   | Aromobatidae   | Allobates        | femoralis   | Ulemari, 99 km upstream of confluence with the Litani | April 12, 1998 | 1               |
| A843   | Leptodactylidae| Leptodactylus    | knudseni    | Ulemari, 13 km upstream of confluence with the Litani | April 5, 1998  | 1               |
| A860   | Dendrobatidae  | Dendrobates      | tinctorius  | Ulemari                               | April 22, 1998 | 1               |
| A851   | Aromobatidae   | Allobates        | femoralis   | Ulemari, 39 km upstream of confluence with the Litani | April 30 & May 1, 1998 | 1               |
| A863 & A864 | Dendrobatidae | Dendrobates      | tinctorius  | Oranje mountain, left tributary, Ulemari | April 15, 1998 | 2               |
| A873   | Leptodactylidae| Leptodactylus    | rhodomystax | Ulemari                               | April 22, 1998 | 1               |
| A932a,b,c | Dendrobatidae | Dendrobates | tinctorius | Sipaliwini, Tepec  | August, 1999   | 3               |
| A904   | Pipidae        | Pipa             | pipa        | Mayakabuka tributary                  | February 10, 1999 | 1               |
| A985   | Leptodactylidae| Leptodactylus    | bolivianus  | Brownsberg, on the mazaroni road      | January 28, 2009 | 1               |
| A1016  | Dendrobatidae  | Ameerega         | trivittata  | Sipaliwini Apalagadi                  | April 19, 2014 | 1               |
| A1034  | Dendrobatidae  | Ameerega         | hahneli     | Sipaliwini                            | June 27, 2014  | 1               |
| A1063  | Dendrobatidae  | Ameerega         | hahneli     | Sipaliwini Apalagadi                  | April 18, 2014 | 1               |
| A1064  | Dendrobatidae  | Ameerega         | trivittata  | Sipaliwini community                  | April 15, 2014 | 2               |
| A1076 & A1077 | Dendrobatidae | Ameerega | trivittata | Nassau trail 7 | December 15, 2014 | 2               |
| A1000a, A1000b | Leptodactylidae| Leptodactylus    | longirostris| Sipaliwini                            | April 22, 2014 | 2               |
| A1122  | Dendrobatidae  | Ameerega         | trivittata  | Bakhuis Mountains, near camp          | April 26, 2015 | 1               |
| A1139 & A1140 | Dendrobatidae | Dendrobates | tinctorius | Bakhuis Mountains, Low creek          | April 28, 2015 | 2               |
| A1175 & A1176 | Pipidae | Pipa | pipa | Voltzberg, raleighvallen | January 24, 2016 | 2               |
| A430   | Dendrobatidae  | Dendrobates      | tinctorius  | Basecamp on the foot of the Emma mountains, appr. 350 m high | date unknown. | 1               |
the tubes were closed and stored in a refrigerator with a temperature of 4˚C until shipped to be processed.

Sample preparation

All swabs were processed using the following procedure. One ml of 70% ethanol diluted to 70% final concentration with deionized H₂O was added in the lab to each sample tube. After vigorous mixing the liquid to dislodge any zoospores/skin tissue from the swabs, the entire volume from each sample was transferred in two different microfuge tubes. The tubes were spun in a microcentrifuge at ~16,000 x G for 3 minutes. Next, the supernatant was drawn off and discarded since B.d zoospores are negatively buoyant in 70% ethanol and therefore will pellet upon centrifugation. Lysis buffer (180 ul of Qiagen ATL buffer + 20 ul Qiagen Proteinase K) was added to the tubes and any pellet present was resuspended by vortexing. Ten μg of carrier DNA was added to the lysis buffer. Total DNA was extracted from all samples using a silica membrane spin-column DNA purification procedure (Qiagene DNeasy, blood and tissue kit).

qPCR assay. The sample DNAs were assayed for the presence of the *Batrachochytrium dendrobatidis* ribosomal RNA Intervening Transcribed Spacer (ITS1) region by 45 cycle PCR amplification using a qPCR assay developed at Pisces and a Stratagene MX4000 real-time PCR instrument. Primer and probe base sequence are as follow:

Primer Forward: 5’ TGGATGGGAGTTTTATTGATGTGTA
Reverse: 5’ TCGTGACATATGGCACACTGTATT
and the probe 5’-FAM -TGG AAT GAC CCA TTG TT-BHQ1 plus. The reaction master mix contains ROX as passive reference dye for normalizing variations in individual reaction total volumes, and a VIC-labeled internal positive control (IPC) (Life Technologies TaqMan Positive Control, catalog #4308323) to detect PCR inhibition. The detection sensitivity of this assay is three target sequence molecules (approximately 0.02 zoospore equivalents). Each PCR run included the following controls: Positive DNA: DNA prepared from a plasmid constructed at Pisces containing the *B. dendrobatidis* ribosomal RNA Intervening Transcribed Spacer (ITS1) region. Serial ten-fold dilutions of this plasmid DNA from 2.9 x 10⁶ to 2.9 x 10⁰ molecules per reaction were used to generate the standard curve. Serial dilutions controls were done since these spanned the Ct values observed for all positive samples observed while using this assay.

Control. Water in place of template DNA. This reaction remains uncapped during addition of sample DNA to the test reactions, and serves as a control to detect contaminating DNA in the PCR reagents or carryover of positive DNA during reaction set-up.

Results

Using the 0.02 threshold for any sample that gave clearly observable, exponential (log-linear) fluorescent signal increase we found no museum specimens to be positive for the chytrid fungus. However, any weak or questionably positive samples were retested in a second independent qPCR run. Only samples which retested positive in this second run were scored as positive. In the study herein all the samples were tested negative. The specimens used for swabbing also showed no aberrant skin condition to indicate a possible chytrid infection.

Discussion

The negative results for the museum specimens tested in this study supports that Bd has not infected any frogs in Suriname. Many South American countries have had specimens tested positive for this fungus including countries bordering Suriname. The data presented herein and the specimens sampled in the field by the author gives a first country wide overview of this chytrid fungus sampling and as such serves as a baseline study for future Bd presence/absence
research. The inclusion of museum specimens in this chytrid sampling overview for Suriname, apart from the historical point of view enabled us also to sample specimens that were not sampled in the field survey due to a lack of funds to be able to visit those places. The research presented herein of the 15 different species sampled added to the number of species sampled during the field survey gives us a total of 52 frogs species sampled for Suriname and a total of 555 specimens. One particular reason for the Bd free status of Suriname could probably be due to the relatively high mean temperature of Suriname which varies between 26.2˚C for the coldest month to 28.2˚C for the warmest time period [26] thus making conditions not favorable for Bd to get established [27, 28]. However, the inhibition by temperature should not be ruled out completely as lowland sites have also been reservoirs of species carrying the pathogen [29, 30] so chances are that in due time and with few existing precautions chytridiomycosis caused by Bd might eventually set foot in Suriname. The wide eastern and western river border of Suriname could be yet another reason that this chytrid fungus has not yet reached here. Evidence that Bd infection spreading into different countries seems to be mostly coupled with human activities [31] and that possibly infected animals or substrate with the zoospores could make it into Suriname. Although this fungus has spread due to the pet trade in many countries [32] it is highly unlikely that the amphibian pet trade in Suriname will develop to such an extent that frogs will be imported from other countries to keep as pets. In the same vein it is not envisaged that frogs will be imported from other countries to be consumed or bred here to serve as part of the Surinamese diet. Ornamental fish however are imported in Suriname and have shown to be carriers of this chytrid fungus [33].

Regular surveys sampling for Bd in Suriname should be a mandatory activity to constantly monitor the status of this pathogen.

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References
1. Welsh HH Jr, Ollivier LM. Stream amphibians as indicators of ecosystem stress: a case study from California’s redwoods. Ecological applications. 1998 Nov; 8(4):1118–32.
2. Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, Fischman DL, et al. Status and trends of amphibian declines and extinctions worldwide. Science. 2004 Dec 3; 306(5702):1783–6. https://doi.org/10.1126/science.1103538 PMID: 15486254
3. Houlihan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL. Quantitative evidence for global amphibian population declines. Nature. 2000 Apr; 404(6779):752–5. https://doi.org/10.1038/35008052 PMID: 10783886
4. Alford RA, Dixon PM, Pechmann JH. Global amphibian population declines. Nature. 2001 Aug; 412 (6846):499–500. https://doi.org/10.1038/35087658 PMID: 11484041
5. Bishop PJ, Angulo A, Lewis JP, Moore RD, Rabb GB, Moreno JG. The Amphibian Extinction Crisis-what will it take to put the action into the Amphibian Conservation Action Plan? SAPI EN. S. Surveys and Perspectives Integrating Environment and Society. 2012 Aug 23(5.2).
6. O’hanlon SJ, Rieux A, Farrer RA, Rosa GM, Waldman B, Batalille A, et al. Recent Asian origin of chytrid fungi causing global amphibian declines. Science. 2018 May 11; 360(6389):621–7. https://doi.org/10.1126/science.aar1965 PMID: 29748278
7. Luger M, Garner TW, Ernst R, Hödl W, Lötters S. No evidence for precipitous declines of harlequin frogs (Atelopus) in the Guyanas. Studies on neotropical fauna and environment. 2008 Dec 1; 43 (3):177–80. https://doi.org/10.1080/01650520802239531
8. Soto-Azat C, Clarke BT, Poynton JC, Cunningham AA. Widespread historical presence of Batrachochytrium dendrobatidis in African pipid frogs. Diversity and Distributions. 2010 Jan; 16(1):126–31. https://doi.org/10.1111/j.1472-6462.2009.00618.x

9. Courtois EA, Gaucher P, Chave J, Schmeller DS. Widespread occurrence of Bd in French Guiana, South America. PloS one. 2015; 10(4). https://doi.org/10.1371/journal.pone.0125128 PMID: 25902035

10. Scheele BC, Pasmans F, Skerratt LF, Berger L, Martel A, Beukema W, et al. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. 2019 Mar 29; 363(4643):1459–63. https://doi.org/10.1126/science.aav3079 PMID: 30923224

11. McLeod DS, Sheridan JA, Jiaungkoorskul W, Khonsue W. A survey for chytrid fungus in Thai amphibians. Raffles Bulletin of Zoology. 2008 Feb 29; 56(1):199–204.

12. Soto-Azat C, Clarke BT, Fisher MC, Walker SF, Cunningham AA. Non-invasive sampling methods for the detection of Batrachochytrium dendrobatidis in archived amphibians. Diseases of Aquatic Organisms. 2009 Apr 6; 84(2):163–6. https://doi.org/10.3354/dao02029 PMID: 19476287

13. Cheng TL, Rovito SM, Wake DB, Vredenburg VT. Coincident mass extirpation of neotropical amphibians with the emergence of the infectious fungal pathogen Batrachochytrium dendrobatidis. Proceedings of the National Academy of Sciences. 2011 Jun 7; 108(23):9502–7. https://doi.org/10.1073/pnas.1105538108 PMID: 21543713

14. Richards-Hrdlicka KL. Extracting the amphibian chytrid fungus from formalin-fixed specimens. Methods in Ecology and Evolution. 2012 Oct; 3(5):842–9.

15. Bonaccorso E, Guayasamin JM, Mendez D, Speare R. Chytridiomycosis as a possible cause of population declines in Atelopus cruciger (Anura: Bufonidae). Herpetological Review. 2003 Dec 1; 34(4):331–3.

16. La Marca E, Lips KR, Lötters S, Puschendorf R, Ibañez R, Rueda-Almonacid JV, et al. Catastrophic population declines and extinctions in Neotropical harlequin frogs (Bufo: Atelopus). 1 Biota tropica: The Journal of Biology and Conservation. 2005 Jun; 37(2):190–201.

17. Pessier AP, Nichols DK, Longcore JE, Fuller MS. Cutaneous chytridiomycosis in poison dart frogs (Dendrobates spp.) and White’s tree frogs (Litoria caerulea). Journal of Veterinary Diagnostic Investigation. 1999 Mar; 11(2):194–9. https://doi.org/10.1177/104063879901100219 PMID: 10098698

18. Courtois EA, Pineau K, Villette B, Schmeller DS, Gaucher P. Population estimates of Dendrobates tinctorius (Anura: Dendrobatidae) at three sites in French Guiana and first record of chytrid infection. Phylomedusa: Journal of Herpetology. 2012 Jun 18; 11(1):63–70.

19. Herrera RA, Steciow MM, Natale GS. Chytrid fungus parasitizing the wild amphibian Leptodactylus ocellatus (Anura: Leptodactylidae) in Argentina. Diseases of aquatic organisms. 2005 May 20; 64(3):247–52. https://doi.org/10.3354/dao064247 PMID: 15997823

20. Ghirardi RO, Lescano JN, Longo MS, Robledo GE, Steciow MM, Perotti MG. Batrachochytrium dendrobatidis in Argentina: first record in Leptodactylus gracilis and another record in Leptodactylus ocellatus. Herpetological Review. 2009; 40(2):175–6.

21. Olson DH, Aanensen DM, Ronnenberg KL, Walker SF, Bielby J, et al. Mapping the global emergence of Batrachochytrium dendrobatidis, the amphibian chytrid fungus. PloS one. 2013; 8(2). https://doi.org/10.1371/journal.pone.0056802 PMID: 23463502

22. Piotrowski JS, Annis SL, Longcore JE. Physiology of Batrachochytrium dendrobatidis, a chytrid pathogen of amphibians. Mycologia. 2004 Jan 1; 96(1):9–15. PMID: 21148822

23. Rodriguez D, Becker CG, Pupin NC, Haddad CF, Zamudio KR. Long-term endemism of two highly divergent lineages of the amphibian-killing fungus in the Atlantic Forest of Brazil. Molecular Ecology. 2014 Feb; 23(4):774–87. https://doi.org/10.1111/mec.12615 PMID: 24471406

24. Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, et al. Diagnostic assays and sampling protocols for the detection of Batrachochytrium dendrobatidis. Diseases of aquatic organisms. 2007 Jan 18; 73(3):175–92. https://doi.org/10.3354/dao073175 PMID: 17307377

25. Sabino-Pinto J, Krause ET, Bletz MC, Martel A, Pasmans F, Steinfartz S, et al. Detectability vs. time and costs in pooled DNA extraction of cutaneous swabs: a study on the amphibian chytrid fungi. Amphibia-Reptilia. 2019 Jan 1; 40(1):29–39. https://doi.org/10.1163/15685381-20181011

26. Amatali MA. Climate and surface water hydrology. In The freshwater ecosystems of Suriname 1993 (pp. 29–51). Springer, Dordrecht.

27. Chatfield MW, Richards-Zawacki CL. Elevated temperature as a treatment for Batrachochytrium dendrobatidis infection in captive frogs. Diseases of Aquatic Organisms. 2011 May 9; 94(3):235–238. https://doi.org/10.3354/dao02337 PMID: 21790070

28. Johnson ML, Berger L, Philips L, Speare R. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid Batrachochytrium dendrobatidis. Diseases of aquatic organisms. 2003 Dec 29; 57(3):255–260. https://doi.org/10.3354/dao057255 PMID: 14960039
29. Rebollar EA, Hughey MC, Harris RN, Domangue RJ, Medina D, Ibáñez R, et al. The lethal fungus Batrachochytrium dendrobatidis is present in lowland tropical forests of far eastern Panama. PloS one. 2014; 9(4). https://doi.org/10.1371/journal.pone.0095484

30. Rodríguez-Brenes S, Rodríguez D, Ibáñez R, Ryan MJ. Spread of amphibian chytrid fungus across lowland populations of túngara frogs in Panamá. PLoS One. 2016; 11(5). https://doi.org/10.1371/journal.pone.0155745 PMID: 27176629

31. Rohr JR, Halstead NT, Raffel TR. Modelling the future distribution of the amphibian chytrid fungus: the influence of climate and human-associated factors. Journal of Applied Ecology. 2011 Feb; 48(1):174–6. https://doi.org/10.1111/j.1365-2664.2010.01891.x

32. Kolby JE, Smith KM, Berger L, Karesh WB, Preston A, Pessier AP, et al. First evidence of amphibian chytrid fungus (Batrachochytrium dendrobatidis) and ranavirus in Hong Kong amphibian trade. PloS one. 2014; 9(3). https://doi.org/10.1371/journal.pone.0090750 PMID: 24599268

33. Liew N, Moya MJ, Wierzbicki CJ, Hollinshead M, Dillon MJ, Thornton CR, et al. Chytrid fungus infection in zebrafish demonstrates that the pathogen can parasitize non-amphibian vertebrate hosts. Nature Communications. 2017 Apr 20; 8(1):1–0.