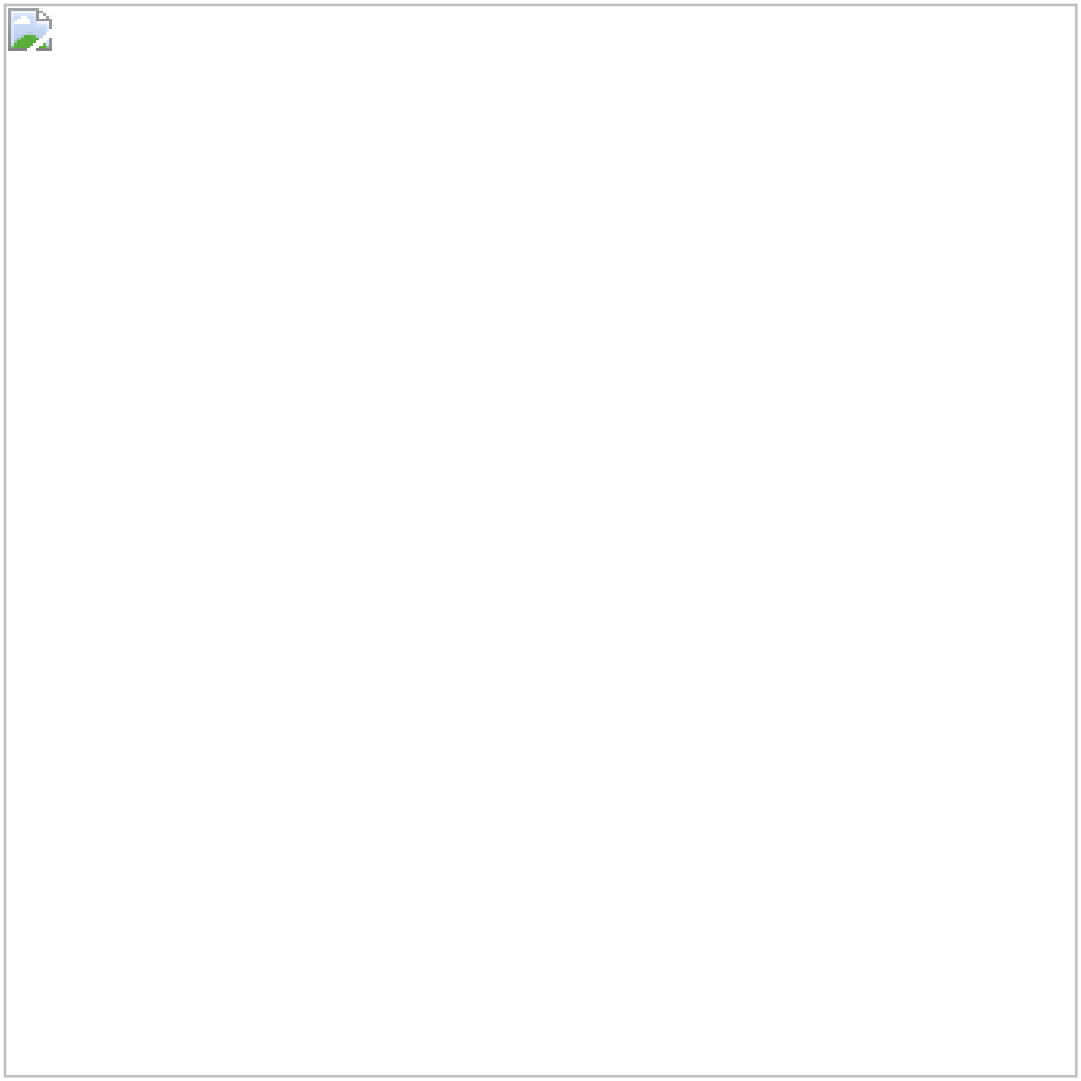

Rediscovery of the critically endangered streamside frog, Craugastor taurus (Craugastoridae), in Costa Rica

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Research Article

Rediscovery of the critically endangered streamside frog, *Craugastor taurus* (Craugastoridae), in Costa Rica

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Abstract

In 1987 the amphibian decline crisis reached its apex in Costa Rica when at least 17 species experienced population crashes and subsequently went undetected for decades. The amphibian declines in Costa Rica were relatively well documented and came to exemplify the current global amphibian decline crisis. The Mesoamerica endemic frog clade, the *Craugastor punctariolus* species group, is one of most severely affected anuran clades, experiencing a loss of 26 out of 33 species throughout Mesoamerica. Eight species of *C. punctariolus* group frogs occur in Costa Rica, and all declined following the 1987 die-off; despite intensive surveys over the last 14 years, most remain undetected. To date, only one species in this group, the stream-breeding frog *C. ranoides*, in known to have a stable population, and only in the Santa Elena Peninsula. Here we document the rediscovery of another species, the South Pacific streamside frog *C. taurus*, in southeastern Costa Rica, representing the first sighting after fifteen years of searching. We discovered two previously unknown populations in Punta Banco, the driest section within the historical range, in an area representing only 4% of the historical distribution. Our findings add to the short but growing list of recently rediscovered amphibian species in Costa Rica and provide encouraging news in an otherwise discouraging situation for amphibian conservation. Additional research and monitoring are urgently needed to develop long-term management plans for this and other Critically Endangered species

Key Words: *Craugastor taurus*, *Craugastor punctariolus* group; amphibian declines, Punta Banco; relict populations

Resumen

En 1987, la disminución y desaparición de las poblaciones de anfibios alcanzó su punto más crítico en Costa Rica cuando las poblaciones de al menos 17 especies se tornaron indetectables por varias décadas. Las disminuciones de poblaciones de anfibios en Costa Rica están bien documentadas y ejemplifican la crisis mundial actual que sufren los anfibios. En Mesoamérica, el grupo de especies de anuros del Clado *Craugastor punctariolus* es uno de los más afectados, experimentando disminuciones dramáticas en 26 de las 33 especies a lo largo de Mesoamérica. De las especies de este grupo, ocho se distribuyen en Costa Rica, y todas disminuyeron a partir de 1987 y en su mayoría no han sido detectadas a pesar de búsquedas intensivas durante los últimos 14 años. Actualmente, solamente la especie *C. ranoides* cuenta con poblaciones estables y únicamente en la Península de Santa Elena. En este estudio, documentamos la reaparición de otra especie de este grupo, la rana de quebrada del Pacífico sur, *C. taurus*, en el sureste de Costa Rica, representando el primer registro después de 15 años sin ser detectada. Encontramos dos poblaciones no registradas anteriormente de *C. taurus* en Punta Banco, la zona más seca de su rango histórico y representa sólo el 4% de la distribución histórica. Nuestro hallazgo se suma a la lista de especies recientemente redescubiertas en Costa Rica, una noticia alentadora en medio de un preocupante escenario global para conservación de los anfibios. Destacamos la necesidad de más investigación así como monitoreo adicional para desarrollar planes de gestión a largo plazo para esta especie en peligro crítico.

Palabras Clave: *Craugastor taurus*, grupo *Craugastor punctariolus*; disminuciones de anfibios, Punta Banco; poblaciones relictas

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Introduction

The last few decades have seen the decline of approximately 40% of all amphibian species worldwide [1] and the extinction of an approximately 200 species since 1980 [2]. There are many causes of amphibian declines, which include, but are not limited to, climate change, disease, introduced species, and habitat loss and degradation [3, 4]. The most severe and rapid declines of Central American amphibian species have been attributed to the fungal disease, chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (*Bd*) [5]. Species most susceptible to *Bd* in Central America occur in altitudes > 500 m, live along streams, have large body size and small geographic range, and are phylogenetically clustered [6-8]. Population declines and extirpations that have occurred over the last several decades in Costa Rica were not always obvious because few amphibian populations were monitored during the die-offs. We believe that in Costa Rica, the collapse of amphibians is likely due to complex factors that vary in importance by region. For example, some population declines and extirpations have occurred at low elevation sites where *Bd* has not been identified as a major threat, making some disappearances truly enigmatic [9, 10].

Two Neotropical frog groups exemplify the current amphibian decline crisis, the harlequin frogs in the genus *Atelopus* [11], and the stream-breeding frogs in the *Craugastor punctariolus* species group [12, 13]. Both groups are restricted to riparian habitats, and most species occur at elevations greater than 500 m, making them especially vulnerable to *Bd* [11, 14]. The declines of multiple species of harlequin frogs have been well documented, with 90% of the 96 described species either in decline, extirpated, or threatened with extinction [1, 11]. Much less information is available for the Central American endemic *C. punctariolus* species group, but at least 27 of 34 species are critically endangered, endangered, or vulnerable [1]. The best available evidence suggests that the entire clade has experienced severe declines and population extinctions throughout Central America [12, 15-17], with only rare observations of these animals over the last ten years despite significant survey efforts [18-20]. Both harlequin frogs and the *C. punctariolus* species groups from low- and mid-elevation populations have declined and been extirpated. While the mid-elevation population declines fit the typical pattern of decline caused by epizootic outbreaks of highly virulent *Bd* [14], the low elevation declines cannot be as easily described by the effects of *Bd* epizootics. For example, while numerous studies match *Bd* outbreaks with collapse of amphibian fauna at mid and high elevations [8, 11 ,14], there have been no documented low elevation *Bd* caused die-offs [5], yet *Bd* has been detected in low elevation populations [10, 21]. Thus the role of *Bd* in low elevation declines

remains unknown, and other factors may be responsible for low elevation population declines [3]. Low elevation populations of the *C. punctariolus* clade have declined in a manner similar to their mid-elevation counterparts.

Costa Rica is home to eight species of the *Craugastor punctariolus* species group [22]; one species is listed as Extinct (*C. escoces*), five as Critically Endangered, and two as Endangered [23]. Historically, pre-1985, all species except one (*C. rhycobatrachus*) were considered abundant where they occurred, were easily detected, and were not likely to be overlooked (Jay M. Savage and Norman Scott, pers. comm.). There has been considerable effort since 2000 to locate extant populations of these species in Costa Rica, due to their highly threatened status, their historic abundance, their once easy detection, and their rapid declines in the late 1980s and early 1990s.

Survey efforts for frogs of the *Craugastor punctariolus* species group have been focused in those areas where extant populations are most likely to be found [24]. To select those sites, we reviewed historic museum records and field notes to identify previously occupied streams where frogs were noted to be abundant [19, 25]. We combined these data with ecological niche modeling [17] that predicted 88% of relict frog populations in Costa Rica should occur in seasonal environments with relatively low precipitation and high temperatures. This approach has been successfully used to rediscover two other species of the *Craugastor punctariolus* group. Since 2005 several breeding populations of the stream-breeding frog *C. ranoides* were discovered in the Santa Elena Peninsula [18, 26, 27], an area predicted to be unsuitable for *Bd* [28, 29]. In 2010 a single individual of the stream-breading frog *C. fleischmanni* was found on Volcan Barva near San Jose [19]. This species had not been observed since since 1986, and was presumed to be extinct (Federico Bolaños, pers. comm.). Despite the ongoing efforts, the remaining six missing species of the *C. punctariolus* species group have still not been observed in Costa Rica since the historic die-offs that began in 1987.

The South Pacific streamside frog, *Craugastor taurus*, is restricted to southeastern Costa Rica and extreme southwestern Panama, with an elevation range from 20-525 m [12]. It is a moderately sized frog with males reaching 44 mm snout-to-vent length (SVL) and females 80 mm SVL [21]. Little is known about this species' basic ecology, and like all members of the *C. punctariolus* clade, it is a riparian specialist that reproduces by direct development and perches on boulders in streams at night [12, 22]. It is defined as a Critically Endangered species [23] because estimated population declines were greater than 80% over the last three generations [30]. This species was last observed in 1997, and its decline is considered enigmatic because population collapses were apparently simultaneous and occurred in protected and unprotected areas at low elevations.

Herein we report the rediscovery of two relict populations of the South Pacific streamside frog *Craugastor taurus* in extreme southeastern Costa Rica and add evidence that both relict populations represent breeding populations which persist with high prevalence of the fungal pathogen *Bd*. These findings are the first reports since 1997. We also document the non-detection of this species from 34 historically occupied streams that were continuously monitored between 2000 and 2012. Furthermore, we provide a new geographic range for this species, based on its current distribution and its absence from historic streams.

Methods

Monitoring program

In 2000 we began an ongoing effort to locate extant populations for this once-common stream frog species. We reviewed museum specimen records (UCR, LACM, KU, and CAS) and identified 34 historically occupied streams that were inhabited by the species between 1963-1990 (Fig.1). Between 2000 and 2012, we spent 720.94 (4.90 ± 3.95 hours/stream) person hours sampling 47 streams and tributaries (34 historically occupied and 13 unoccupied streams) within the range of *C. taurus*. The authors, in conjunction with occasional field courses from the Universidad de Costa Rica and University of New Mexico, conducted the range-wide surveys (Fig 1).

Field survey of rediscovery and *Bd* sampling

In addition to the historic sites, on December 2, 2011 we also conducted a survey at Punta Banco, in extreme southeastern Costa Rica,, where we sampled two streams: an unnamed stream (08°21'25'' N, 83°08'03'' W, 47 m asl) and Quebrada Banco (08°21'41'' N, 83°08'56'' W, 24 to 36 m asl). We used standardized nocturnal visual encounter surveys and recorded duration (minutes) and distance (meters) for each survey [31]. In every stream we estimated density as the number of individuals encountered per 50-meter stream stretch. We also determined sex and age class, and recorded basic habitat use information. We collected two voucher specimens, one from each stream (Fig 2), which are housed in the Museo de Zoología at Universidad de Costa Rica (UCR 21492−21493). Because frogs of the *C. punctariolus* species group are notoriously difficult to identify [12], vouchers of this Critically Endangered frog were collected in order to verify the species identity. By comparing our vouchers with other museum specimens we are sure of proper species identification and can proceed with effective conservation efforts [32].

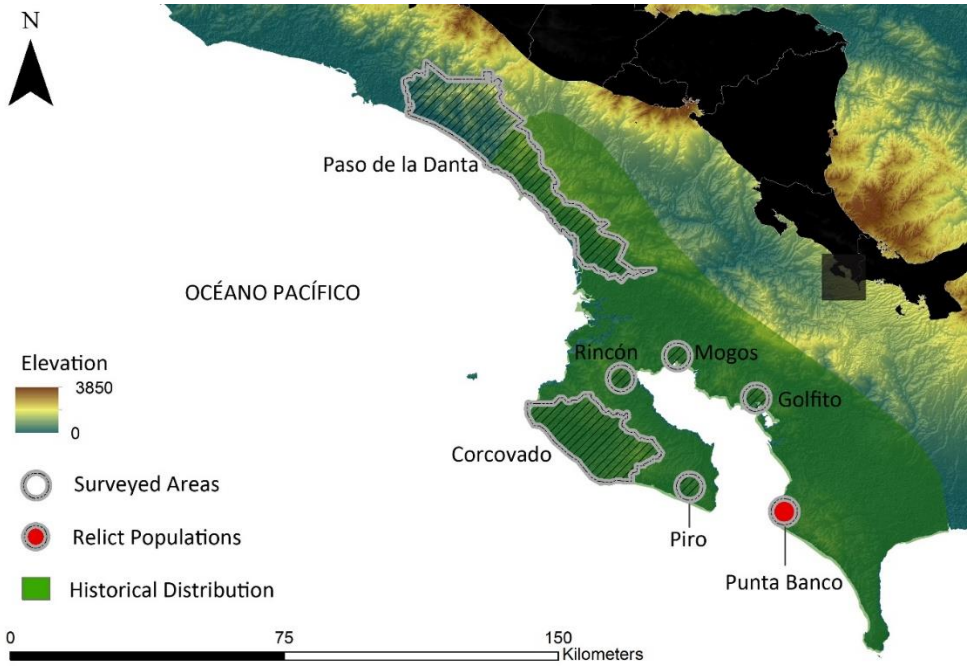

Fig 1. Map of Costa Rica showing historical distribution and surveyed areas since 2000 of the South Pacific streamside frog *Craugastor taurus.*

Based on the new records and historical distribution of the South Pacific streamside frog *C. taurus*, we established the previous and current range of distribution by forming polygons which joined the most external points of these localities [33]. The area of polygons was measured with Diva-GIS software [34]. We designed a plot of environmental space comparing the pre-decline and post-decline environmental distribution of this species in Costa Rica using annual air temperature and annual precipitation. These data were obtained as environmental layers at 1km resolution from the WorldClim (version 1.4) dataset [35]. We used these environmental variables because they are the most useful for defining areas of *Bd* occurrence in Costa Rica [28].

We captured fifteen frogs using powder-free latex gloves and placed individuals in new plastic bags for processing. We swabbed frogs for *Bd* [36]. Swabs were stored dry and frozen at -20°C until extraction. DNA extraction and diagnostic real-time PCR was conducted at the Vredenburg Lab at San Francisco State University following standardized procedures [37], with the following exception: (1) the nucleic acids were extracted using 50 µl PrepMan and (2) a negative control (H2O), and all the samples were run in triplicate to ensure the integrity of results. We determined a sample as positive when the target sequence amplified in the three replicate wells of the assay. We estimated prevalence of *Bd* at 95% binomial confidence intervals.

Results

In our limited sampling on December 2, 2011, we observed sixteen frogs of *Craugastor taurus* in the unnamed stream (eight adult frogs, four males, and four females; 0.46 frogs/50 meters; 2.81 person hours; 864 m). At Quebrada Banco we observed fourteen individuals (eight males, three females, and four juveniles; 1.63 frogs/50 meters; 2.45 person hours; 459 m).

Fig. 2. Adult male of the South Pacific streamside frog *Craugastor taurus* collected at Punta Banco (Photographed by Gerardo Chaves).

Based on: (1) distribution and climatic data from specific localities of these two new populations; (2) the non-detection of *Craugastor taurus* at historically known occupied sites, and (3) extrapolation of the actual distribution to the farthest point in south Costa Rica, we estimated the current range as 97.59 km2 (3.71% of the pre-decline geographic distribution, estimated at 2,962.27 km2). The two newly discovered populations of *C. taurus* occurred in an area with a drier environment (annual precipitation of 3,000 mm; annual temperature of 26 °C) than those where historic populations of this species have been described in Costa Rica (3,784 mm; 25.3 °C) (Fig. 3). We detected *Bd* on twelve of fifteen swabbed frogs (prevalence 80%; C.I. 51.9—95.67%) and intensity of infection varied from 6.52 to 63,861 genomic equivalents.

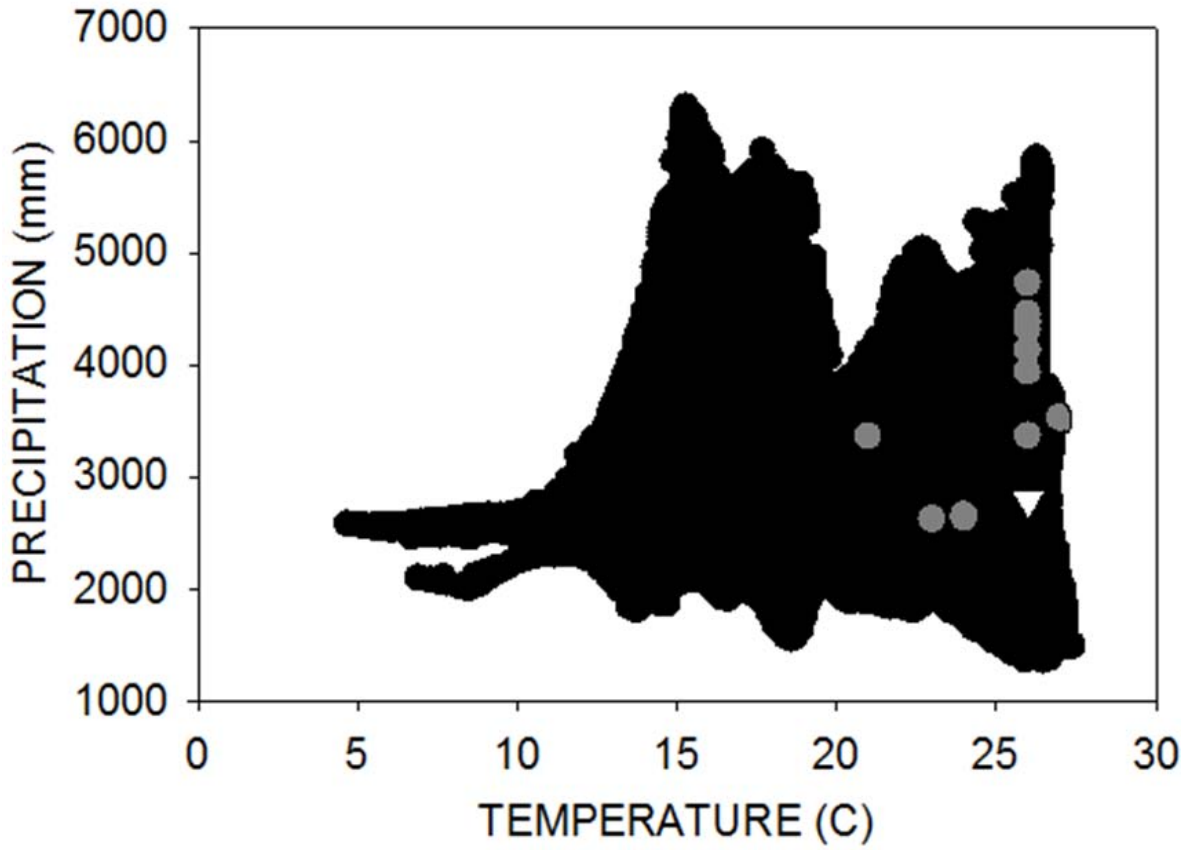

Fig. 3. Plot of environmental space available (black surface) for the South Pacific streamside frog *Craugastor taurus* in Costa Rica. Values for declined (gray dots) and current populations (white triangle) show the distributional changes of this species due to the amphibian declines that affected this species since the 1990s.

Discussion

Here we present the rediscovery of a once-common streamside frog *Craugastor taurus* in southeastern Costa Rica after 17 years of no sightings for the species. Additionally, we found that the populations appear to be persisting with a high prevalence of *Bd.* This is one of eight species of lost Costa Rican frog species that have been rediscovered over the last six years [17]. Prior to 1997, 34 populations of *C. taurus* had been documented in Costa Rica [12]. To date, this species remains missing from historical localities, but no other stream-breeding frog species have declined in the same localities (G Chaves and MJ Ryan, unpublished). The recently discovered populations of *C. taurus* are significant for three reasons: (1) they represent new locality records; (2) they are the only known extant populations of this Critically Endangered species; and (3) these populations are persisting with an apparently high prevalence and infection of *Bd*.

The combination of threats from habitat loss and occurrence of *Bd* in the newly discovered populations warrants immediate and continued study of the Punta Banco populations. The clear and fast-flowing streams where the new populations were found are surrounded by small patches of primary or secondary forest adjacent to rural dirt roads. Much of the original forest cover around the occupied streams has been converted to palm oil and cattle production and rural development that represent persistent threats to this species. We are not able to estimate local deforestation rates, but continued habitat loss and degradation clearly represent a serious threat to the habitat of these small populations.

A clear picture of the host/pathogen dynamics is needed to understand the true threat of *Bd* in these populations. Despite our limited sampling of only one night, we found a high prevalence of *Bd* in the two populations. We did not observe dead or moribund frogs, which would be an indicator of a disease outbreak and a subsequent massive die-off [5]. As stated previously, *Bd* has been implicated in the decline of many *C. punctariolus* species group populations in cool moist regions > 500 m [8, 16, 17], yet the role of *Bd* in low elevation population declines is unknown. *Bd* has been found on low elevation terrestrial and riparian amphibian species [10], but despite population losses, dead frogs and loss of up to 40% of local amphibian species characteristic of montane mass die-offs [5] have not been reported at low elevations. This is supported by our observation that no other low-elevation frog species disappeared in this region.

One possible explanation for the persistence of populations of *C. taurus* with high prevalence of *Bd* in southeastern Costa Rica is that local climate conditions are not conducive to a large scale outbreak of chytridiomycosis. The Punta Banco region is drier than the very humid ecosystems of Golfo Dulce region just to the northwest. Therefore, the dry conditions in Punta Banco may allow the frogs to coexist with *Bd*. This pathogen has exhibited high mortality under dry conditions and temperatures above 30°C in laboratory conditions [38] [39]. Thus *Bd* has a relatively narrow thermal and desiccation tolerance that may preclude the disease from reaching epidemic levels at low elevations [40]. Experimentally infected frogs experienced 100% mortality at 23°C [40] with mortality decreasing by 50% at 27°C [39] suggesting that temperature can limit prevalence of *Bd*. The mean annual temperature at Punta Banco is 26°C, which is warmer than the 100% mortality temperature [40].

This is the second case of a proposed environmental refuge from *Bd* for a species of the *Craugastor punctariolus* group in Costa Rica. The first case was the stream-breeding frog *C. ranoides*, which was

once widespread in Costa Rica and is now restricted to the tropical dry forests of the Santa Elena Peninsula in northern Costa Rica, where the dry environment and high temperatures have presumably reduced the capacity of *Bd* to grow on the frogs' skin and produce disease [29]. For the case of *C. taurus*, the newly discovered populations occur at sites that are drier than historic sites where the previously known and declined populations were found. We hypothesize that this species may occur further south on the nearby Punta Burica (in the border between Costa Rica and Panama) and in Puerto Armuelles in Panama because these locations have dry conditions similar to Punta Banco. Future collaborative surveys with Panamanian researchers will test this hypothesis.

Additional reports of frogs from *Craugastor punctariolus* clade frogs in dry environments include the stream-breeding frogs *C. laevissimus* from Honduras [41] and *C. azueroensis* from the Azuero Peninsula in Panama [42, 43]. Regarding this last example, monitored populations of *C. azueroensis* occur at higher elevation and milder climatic conditions than *C. ranoides* and *C. taurus* in Costa Rica. However, the historic distribution of *C. azueroensis* is restricted in part by dry and hot lowlands, which together with an exclusive and isolated hydrographic system in Azuero Peninsula might prevent the occurrence of *Bd* and possible outbreaks of chytridiomycosis [43]. Hundreds of species appear to have been affected by *Bd* epizootic events, and yet some species appear unaffected. Many hypotheses have been proposed to explain why some species or populations survive. The environmental refuge hypothesis is debatable, in part due to a lack of examples, but the new populations described here may well be explained by the climate envelope in which they were found.

Implications for Conservation

The rediscovery of *Craugastor taurus* is one of eight recent rediscoveries of lost frogs in Costa Rica [17], which include the endemic and believed to be extinct Holdridge's toad (*Incilius holdridgei*) [44]. The non-detection of *C. taurus* in historical streams is troubling, but the discovery of two populations in a new location is encouraging news. Continuous sampling within the historic range might result in discovery of additional populations. We emphasize that much more fieldwork needs to be done in order to explain the uniqueness of these two extant populations. The significance of the recent frog rediscoveries in Costa Rica and elsewhere is not clear. The mounting evidence warrants a rethinking of amphibian conservation to include regular resampling of historic habitats and coordinated efforts by governmental agencies and conservation organizations to promote monitoring, identification, and protection of the recently rediscovered populations. Only with continuous monitoring programs and coordinated efforts will it be possible to understand why some amphibian species have recovered from population declines.

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