Field notes on findings of threatened amphibian species in the central mountain range of western Panama

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Abstract.—During field work along a transect in the Cordillera Central of western Panama between 2008 and 2010, we detected several populations of amphibian species which are considered as "Endangered" or "Critically Endangered" by the IUCN. Some of these species had suffered from serious population declines, probably due to chytridiomycosis, but all are generally threatened by habitat loss. We detected 53% of the Endangered and 56% of the Critically Endangered amphibian species that have previously been reported from within the investigated area. We report on findings of species that have not been found in Panama for many years, and provide locality data of newly discovered populations. There is a need to create a new protected area in the Cerro Colorado area of the Serranía de Tabasará, where we found 15% of the Endangered and Critically Endangered amphibian species known to Panama.

Resumen.—Durante trabajo de campo en un transecto a lo largo de la Cordillera Central en el oeste de Panamá entre 2008 y 2010, encontramos varias poblaciones de anfibios que son considerados "En Peligro" o "En Peligro Crítico" por la UICN. Algunas de estas especies habían sufrido serias disminuciones de sus poblaciones, probablemente causadas por la quitridiomicosis, pero todas se encuentran amenazadas por pérdida de hábitat. Detectamos el 53% de las especies En Peligro y el 56% de las especies En Peligro Crítico que se habían reportado previamente en el área de estudio. Reportamos hallazgos de especies que no se habían avistado en Panamá por muchos años, así como localidades de poblaciones descubiertas por primera vez. Se requiere crear una nueva área de protección en los alrededores del Cerro Colorado en la Serranía de Tabasará, donde se han encontrado 15% de los anfibios En Peligro y En Peligro Crítico de todo Panamá.

Key words. Anura, *Batrachochytrium dentrobatidis* (*Bd*), decline, Panama, Serranía de Tabasará, Serranía de Talamanca, Urodela

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Introduction

Amphibian populations are declining or disappearing around the globe at an alarming rate, and are the most threatened vertebrate class on earth (Daszak et al. 1999; Stuart et al. 2004; Gascon et al. 2007). The most obvious threat to tropical amphibians and to biodiversity in general, comes from anthropogenic activities such as deforestation, habitat modification, and contamination. In addition, from the late 1980s on, even amphibian populations in pristine, undisturbed habitats have begun to decline enigmatically. These enigmatic declines have been especially severe at upland sites of Australia (Campbell 1999) and the Neotropics (Young et al. 2001, 2004; Lips et al. 2006). In particular, lower central America has suf-

fered from multiple amphibian population declines that were well documented (e.g., Crump et al. 1992; Young et al. 2001; Lips 1999; Lips et al. 2006; Ryan et al. 2008).

After several experts have conducted research regarding the causes, these enigmatic amphibian die-offs and population declines could be clearly associated with, an emerging infectious disease (EID) (Daszak et al. 2000) caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) soon known as chytridiomycosis (Berger et al. 1998; Daszak et al. 1999; Ryan et al. 2008). The mortality rate of infected individuals can be as high as 100% in some populations (Lips et al. 2003b), but not all species have been affected equally. Due to the physiology of *Bd*, which grows best at temperatures between 17-28 °C (Piotrowski et al. 2004), populations in tropical moun-

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tainous habitats are more likely to be affected than those in tropical lowlands (Longcore et al. 1999; Andre et al. 2008). Montane riparian or lentic amphibian species are at highest risk to *Bd* infection and population decline (Lips et al. 2003b; Kriger and Hero 2007). Furthermore, degree of disease susceptibility of species depends on their particular immunologic defence in terms of quantity and quality of antimicrobial skin peptide mixtures (Woodhams et al. 2006).

Chytridiomycosis first appeared in western Panama between 1993 and 1994 and spread in a wave-like manner south-eastward, crossing the Panama Canal in 2007 (Lips et al. 2008; Woodhams et al. 2008). The disease has caused population declines and loss of amphibian diversity wherever it has been detected (Crawford et al. 2010a). Since *Bd*'s arrival, many formerly abundant species have disappeared or become rare. Only recently, reports on rediscoveries or newly discovered populations of amphibian species, which were thought to be lost, have been reported (Puschendorf et al. 2005; Lotzkat et al. 2007; Rodríguez-Contreras et al. 2008; Kolby and McCranie 2009; Abarca et al. 2010; McCranie et al. 2010), but none from Panama.

Our objective is to report on amphibian species found in the central mountain range of western Panama facing a high (IUCN category: Endangered, EN) to extremely high (IUCN category: Critically Endangered, CR) risk of extinction in the wild. Most species mentioned in this paper are highly susceptible to *Bd* infection and have suffered dramatic population declines in Costa Rica and Panama, although their distribution areas comprise well-protected areas like national parks and protected forests (IUCN 2011). Others have always been rare and are only known from a few specimens. Almost all of these species have not been reported for many years and most of them are the only persisting populations known. We also inspected some of the collected voucher specimens for chytrid infection by either histological examination of skin scrapings or PCR testing of skin swabbings. Moreover, we provide information on localities and contexts of our findings and add brief ecological field notes on every species.

Material and methods

Between 2008 and 2010, we collected amphibian species along a transect which covered the continental divide mainly above 1000 m a.s.l., from the Costa Rican-Panamanian border to about 81 degrees of western longitude. Only two findings were made prior to this project (see *Isthmohyla angustilineata* and *Oedipina grandis* accounts) in 2006. An overview of the investigated area, with all localities sampled, is shown in Fig. 1 below.

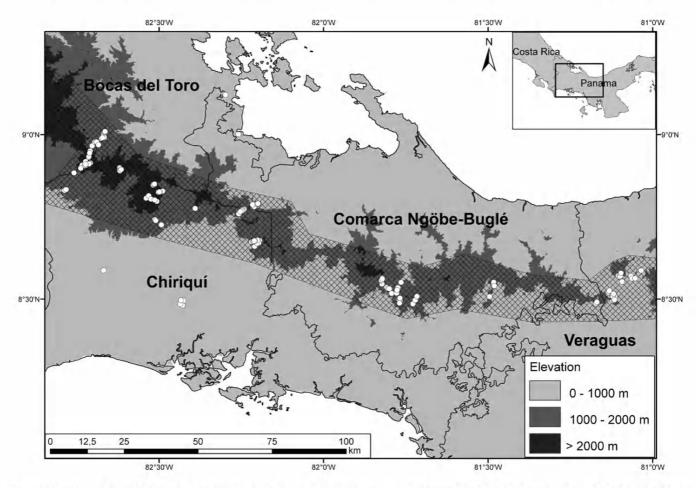


Figure 1. Sampled localities in the central mountain range of western Panama (Cross hatched area = transect, white dots = collecting sites).

We collected all specimens (adults and larvae) during opportunistic searches mostly at night. We obtained coordinates and altitude of the study sites using a Garmin GPS Map 60 CSx GPS receiver or alternatively a Garmin Etrex Summit, both with integrated barometric altimeters. For preservation and preparation of voucher specimens we follow the recommendations of Köhler (2001). We assigned tadpoles to Gosner stages (Gosner 1960) prior to identification. We identified specimens to the species level using the dichotomous keys of Köhler (2011), Savage (2002), and Duellman (2001), and if necessary original species descriptions. We deposited voucher specimens (listed in Appendix I) in the collection of the Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Germany (SMF), those labelled with AH field numbers will be included into the Museo Herpetológico de Chiriquí (MHCH), the herpetological collection of the Universidad Autónoma de Chiriquí, Davíd, Panama.

We created all maps using ArcGIS 10, and calculated mean distances of populations to the next Protected Area with the "Near" tool in the "Proximity" folder. We obtained map layers from the map server of the Smithsonian Tropical Research Institute (STRI) (URL: http://mapserver.stri.si.edu/geonetwork/srv/en/main.home). All maps and coordinates are in the geographic coordinate system and WGS 1984 datum. We rounded coordinates to the second decimal place; elevations are rounded to the next tenth.

Some of the detected specimens have been tested for infection with Batrachochytrium dentrobatidis. Therefore, we gently rubbed a cotton swap over the ventral surfaces of the pelvic patch and inner thighs following the sampling guidelines of Hyatt et al. (2007). Testing for Bd was performed by real-time Taqman PCR assay following the protocol of Boyle et al. (2004). Because this technique was not available to us prior to 2010, we did not take PCR samples from all specimens. Further, we examined 10 specimens collected before 2010 by histological examination of stained skin scrapings, for detection of Bd thalli and sporangia. We took skin scrapings from the ventral surface of the inner left thigh of preserved animals and stained them with 0.01% solution of Congo Red dye dissolved in PBS buffer (pH 7.4) and stained for 20 minutes (Briggs and Burgin 2003, 2004). Stained scrapings were examined with bright-field microscopy using a Bresser Biolam optical microscope.

Since the most recently published list of amphibian species known to occur in Panama (Jaramillo et al. 2010), ongoing investigation has led to some changes and additions to this list. To get an updated list (Appendix II) of the amphibian species that are currently known to be native in Panama we updated and revised the list of Jaramillo et al. (2010). First we included all species that have been added to the Panamanian herpetofauna after 2010. These are (with references in parentheses): *Agalychnis annae* (Hertz et al. 2011), *Craugastor evanesco* (Ryan et al. 2010b), *Incilius karenlipsae* (Mendelson and Mulca-

hy 2010), Pristimantis adnus (Crawford et al. 2010b) and P. educatoris (Ryan et al. 2010a). Moreover, we included Bolitoglossa pygmea and B. robinsoni (Bolaños and Wake 2009) not found in Jaramillo et al. (2010). Because of taxonomic changes we exchanged Rhinella granulosa for R. centralis (Narvaes and Rodriguez 2009), and excluded Agalychnis lithodryas that we treat as a synonym of A. spurelli (Ortega-Andrade 2008). We follow Köhler 2011 and list Lithobates taylori in place of L. pipiens complex. Although Hyalinobatrachium vireovittatum might be a synonym of H. talamancae (Kubicki 2007; Hertz et al. 2011) we decided to list both species until further investigation has been conducted. The same decision was taken for Relictivomer pearsei that might be a synonym of Elachistocleis ovalis, and both taxa are listed as present in Panama. Further, we excluded the three introduced species Eleutherodactylus antillensis, E. johnstonei, and E. planirostris (Crawford et al. 2011).

In the following text we use the abbreviation PA for "Protected Areas" and abbreviations for Panamanian PAs (in parentheses) reflect their original names in Spanish: Parque Internacional La Amistad (PILA); Parque Nacional Volcán Barú (PNVB); Reserva Forestal La Fortuna (RFLF); Bosque Protector Palo Seco (BPPS); Parque Nacional Santa Fé (PNSF). Abbreviations of IUCN categories follow IUCN (2011).

Results

We now count 206 native species of amphibians known to occur in Panama. Of these, 23 species (11%) are considered Endangered (EN) and 22 (11%) Critically Endangered (CR) as defined by the IUCN (Fig. 2).

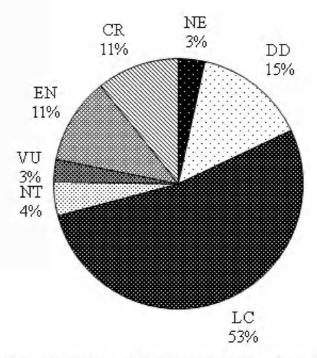


Figure 2. Percentage of Panamanian amphibian species in each IUCN category.

In total 33 (73%) of those 45 species, in detail 17 (74%) of the 23 species in the EN and 16 (73%) of the 22 species in the CR category, are known to occur within the investigated area (Appendix II). During this study we found 18 (55%) of these 33 species, on which we report below. We further present the positive results of analysis of skin swabbings and histological examination for presence of *Bd* illustrated in an overview map (Fig. 3).

Individual species accounts of species categorized as Endangered (EN)

We found a total of nine EN species within the investigated areas representing 4% of all amphibian species known to occur in Panama. Furthermore, 39% of the Panamanian species in the EN category, and 53% of the EN species that are known to occur within the transect. We made all but three findings within the boundaries of PAs, except one at Santa Clara (*Ptychohyla legleri*), and two in the Cerro Colorado region (*Agalychnis annae* and *Pristimantis museosus*). The former two species could not be reported from any of Panama's PAs (Appendix II).

Agalychnis annae (Duellman 1963): This species, formerly considered as a Costa Rican endemic, has been only recently recorded from Panama (Hertz et al. 2011). Between 1980 and 1990, Agalychnis annae disappeared from all PAs in Costa Rica and was subsequently listed as EN. In Costa Rica, it is only found near heavily polluted streams in the metropolitan region of San José. Pounds et al. (2008) hypothesized that the frog and its tadpoles may be less susceptible to water pollution than the chytrid fungus, so it survives only at disturbed and polluted sites. We found a single female during daytime surveys inactive on a leaf by the side of a dirt road. The area is inhabited and fit for agricultural use, though streams in the area did not appear to be heavily polluted. Admittedly, we did not conduct chemical analysis. We furthermore cannot tell whether a reproductive population exists. Thus, further monitoring is strongly needed.

Ecnomiohyla fimbrimembra (Taylor 1948): This species is associated with primary humid montane forest. It is an obligatory canopy dweller that lives and reproduces principally in tree crowns. Ecnomiohyla fimbrimembra is very rarely observed and collected, and there are no dependable data on its population status (Savage 2002). However, it is presumed to have a decreasing population trend, because it is strongly associated with mature primary forests, which are endangered by human activities. The first and formerly only specimen from Panama was an adult male collected in 1982 at Cerro Horqueta, PILA, Chiriquí (Ibáñez et al. 1991).

We collected an adult male during a rainy night on the north-eastern slopes of Volcán Barú. This expands the known distribution of *E. fimbrimembra* in Panama to a second PA, the PNVB, and represents the second male specimen in a scientific collection (Savage 2002; Mendelson et al. 2008).

Ptychohyla legleri (Taylor 1958): This species is considered moderately common in appropriate habitats (Solís et al. 2008). We found only a single froglet near a small creek at Santa Clara in extreme western Panama, where the first Panamanian specimens were collected in 1966 (Duellman 2001). Despite intensive search efforts we carried out at different times of the year at this site, it remained the only encountered specimen. Even though it was found on an organic farm, the surroundings are dominated by intensive coffee plantations nowadays. As a stream breeder it faces a potential risk of chytrid infection, but there are no reports on population declines of this species due to chytridiomycosis yet. Santos-Barrera et al. (2008) recorded P. legleri from the San Vito region in Costa Rica, near Santa Clara, but found no evidence for Bd in that area. However, former studies have proved the presence of Bd at this site (Lips et al. 2003a; Picco and Collins 2007). In addition, we collected a male glass frog (Cochranella granulosa) at the same creek in Santa Clara, which tested positive for Bd by PCR.

Pristimantis museosus (Ibáñez, Jaramillo, and Arosemena 1994): This species can be characterised as uncommon. We found it on the Caribbean slopes along the central mountain range wherever pristine forest was present. Ibáñez et al. (1994) collected the type material at pristine premontane wet forest and lower montane rainforest life zones (Holdridge 1967). Likewise, we never found it in degraded habitats and therefore suspect it of being very sensitive to deforestation.

Bolitoglossa magnifica Hanken, Wake, and Savage 2005: The type material of this species was collected on the lower slopes of Volcán Barú, Chiriqui, Panamá in 1975. This species was first assigned to B. nigrescens until the revision of the B. nigrescens complex by Hanken et al. (2005). It has not been collected or observed since. It is listed as EN because of its small known distribution range and a general loss of habitat. In fact, inadequate data allow only a restrained assessment of this species. We collected four individuals during the day only a few kilometres away from the species' type locality at PNVB while ascending from the town of Boquete to the peak by car. In a relatively short period of time (approximately 15 min), we found all four individuals under a pile of wood by the side of the road. Further research is needed, but it seems to be common at certain sites.

Bolitoglossa compacta Wake, Brame, and Duellman 1973: This is a rather uncommon species (Savage 2002) with a small distribution between the Costa Rican-Panamanian border and Volcán Barú. The type material was collected on the northern slope of Cerro Pando between 1920 and 1970 m a.s.l. in undisturbed cloud forest (Wake et al. 1973). It was first recorded from Costa Rica by Lips (1993), who did not give much information on its habitat, but described the vegetation at this site as lower montane

rainforest in a later work (Lips 1998). Although little is known about the species' environmental demands, it is assumed to inhabit relatively undisturbed lower montane rainforest, and therefore is threatened by habitat loss due to logging.

We found a single specimen on the south-eastern slope of Volcán Barú, within the boundaries of PNVB, under a rotten trunk by the side of the road.

Bolitoglossa marmorea (Tanner and Brame 1961): This species has almost the same distribution as Bolitoglossa compacta, but inhabits slightly higher elevations. Specimens collected during this study were, just as the type material, found in montane rainforest at the edge of the timberline and pluvial paramo above timberline. The ability to live in open habitats is probably the reason why it persists also in degraded habitats. There is a need for further studies as these are the first records of the species in more than ten years (IUCN 2010).

Still it seems to be a common species at the peak of Volcán Barú. In only one morning, we collected five specimens by turning a couple of rocks near the summit of the volcano.

Bolitoglossa minutula Wake, Brame, and Duellman 1973: This seems to be still a quite common species where unspoiled forest exists. Nevertheless, its known distribution area is small and it is assumed to be threat-

ened by deforestation. We collected at least nine specimens of *B. minutula* at PILA and PNVB. Especially near the continental divide on both southern and northern slopes of Cerro Pando (PILA), it appears to be the most common salamander.

Oedipina grandis Brame and Duellman 1970: This elongate, fossorial salamander was easily found in the early 1990s, but then decreased in abundance for unknown reasons (Lips 1998). We detected a single, adult female in January 2006 at night in Jurutungo on the edge of PILA. The specimen was crawling between recently cut *Heliconia* leaves, on a small trail leading to a water intake point of a small stream. It remained the only individual taken during this study.

Individual species accounts of species categorized as Critically Endangered (CR)

We found a total of nine CR species within the investigated area. That is 4% of all Panamanian amphibian species, 41% of all Panamanian amphibian species in the CR category, and 56% of all Panamanian CR amphibians that are known to occur within the transect. We made most of the records within the boundaries of PAs, except three species (*Isthmohyla debilis*, *I. graceae*, and *I. tica*) from

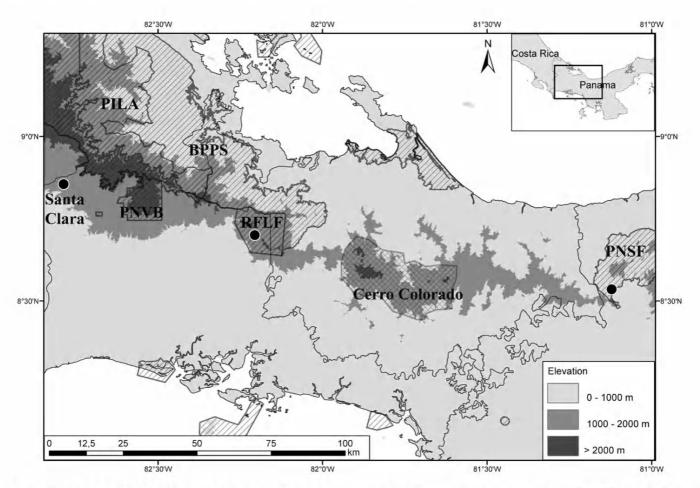


Figure 3. Map of localities referred to in Table 1. (hatched areas = PAs; cross hatched area = Cerro Colorado region, with Cerro Saguí massive in the western and Cerro Santiago in the eastern portion; black dots indicate positive *Bd* results).

the Cerro Colorado region. We could not find the former two in any of Panamas PAs, while the latter one was also present in PILA (Appendix II).

Atelopus varius (Lichtenstein and von Martens 1856): This eye-catching harlequin toad once was a common species in Costa Rica and Panama, but has suffered dramatic population declines throughout its range (Crump et al. 1992; Pounds and Crump 1994; Lips 1998, 1999; Lips et al. 2003b; La Marca et al. 2005). Although there have been sporadic sightings of this species, it is believed to be still in serious decline. We observed four individuals, all at Cerro Negro (PNSF), Veraguas. All adults were found sleeping on low riparian vegetation and a single tadpole (Gosner stage: 36) was found in a mountain stream. None of the collected individuals appeared to be sick. We conducted histological examination of skin scrapings from two adults, which yielded no evidence for Bd. These specimens are probably part of a small population that still persists at Cerro Negro.

Agalychnis lemur (Boulenger 1882): This used to be a quite common species in Costa Rica and Panama, but has become rare in recent years, probably due to chytridiomycosis. It is suspected that A. lemur is more resistant to Bd than is other species (Woodhams et al. 2006). There is no doubt that deforestation is a major threat to this species as well. We collected a single specimen at Cerro Negro (PNSF), Veraguas. The frog was found in a small creek where it was sitting on a rock. Although we visited Cerro Negro between 2008 and 2009 seven times at different seasons of the year, this remained the only detected specimen.

Duellmanohyla uranochroa (Cope 1875): This species was once common in the RFLF, but has become rare after severe declines (Lips 1999; Pounds et al. 2008). We collected the first four tadpoles (Gosner stages: 26-29) from a small creek on the south-western slope of Cerro Pata de Macho (RFLF) in 2008, where several more tadpoles were present in slow-moving water puddles along the creek. In the following years, tadpoles have been observed in this creek during several times of the year. In 2009, we found two adults: one from vegetation near the collection site of the tadpoles, and a second at BPPS, Comarca Ngöbe-Buglé, only a few km from the former adult. This individual was also arranged in riparian vegetation, about three m above the ground. The latter one appeared meagre and feeble and we found it to be parasitized by a large nematode that moved under its skin. Both adults tested negative for the presence of Bd. All of the collected tadpoles showed mouthpart deformations ranging from slight folding of the oral disc to complete loss of keratine in denticles and beak. A skin scraping we took from a tadpole's oral disc tested positive for Bd by histological examination. D. uranochroa is currently known from Monteverde and Tuis de Turrialba, Costa Rica (IUCN 2010). Our records represent the only recent findings of this species in Panama.

Hyloscirtus colymba (Dunn 1931): This species has undergone drastic population declines in western and central Panama (Lips 1999; Lips et al. 2006; Crawford et al. 2010a). We made the first record in 2008, collecting four tadpoles from a fast-moving stream near Alto de Piedra, Veraguas. A few weeks later, we found a single adult male, and in 2009 we encountered an adult female. Then, in 2010 we collected four adult specimens, two males and two females, in both forest and streams, and some more tadpoles. One male was calling from riparian vegetation, and we heard several additional males calling along the stream. Although field work has been carried out in Alto de Piedra between 1998 and 2004, e.g., by Brem and Lips (2008), this population was only recently discovered in the course of this project (Hertz et al. 2011). One of the individuals we collected in 2010 at first sight appeared to be sick tested positive for Bd by PCR. This confirms that Bd is still present within the habitat. All other collected specimens were also tested, but appeared to be healthy and we did not find any evidence for a Bd infection.

Isthmohyla angustilineata (Taylor 1952): This has always been an uncommon species (Savage 2002). There are only sparse data available on geographic distribution and population status. The most recent record comes from Costa Rica at Braulio Carillo National Park near Volcán Barva (Nishida 2006). At Monteverde it has declined drastically but is still found sporadically, whereas there are no recent records from Cerro Chompipe and Tapantí (IUCN 2010). In Panama there is little information on its distribution and population status. The species was first reported from Panama by Arosemena and Ibáñez (1991), who collected three specimens in 1990 at Cerro Horqueta, PILA, the only published record for Panama until now. In 2006, we collected an adult female at almost the same locality; the frog was sitting in a shrub in an inundated pasture. The current population status at this site is unknown.

Isthmohyla debilis (Taylor 1952): This small frog is an uncommon species collected at a few locations (Savage 2002). In 1996 and 1997, the populations of this species collapsed at RFLF (Lips 1999). In Panama, I. debilis was last detected in 1998 from neighboring BPPS (Hofer and Bersier 2001; IUCN 2010). In Costa Rica it had equally declined, and there are no recent findings in this country. We collected two calling males at La Nevera on the western slopes of Cerro Santiago, Comarca Ngöbe-Buglé, in 2008. The frogs were only traceable by following their cricket-like calls, as they sat in very dense vegetation overhanging a stream. In 2009, we collected another male near Llano Tugrí on the eastern slopes of Cerro Santiago, Comarca Ngöbe-Buglé. This one was not hidden in vegetation nor was it calling, but sat exposed on a rock in a mountain stream. In the same year, we collected two more male specimens, one of which was found calling in bushes at the margin of a mountain

stream at La Nevera; the other one sat in the same bush, moving towards its calling conspecific. Only two days later we found an additional calling male, but for conservation reasons refrained from collecting it. The frog cowered well-concealed between the leaves of a fallen tree overhanging a mountain stream. Our findings represent the only recent records of this species within its native range. Accordingly, there are no known lingering populations within the boundaries of any PA. These records also extend the known geographic distribution of the species about 30 to 40 km to the east from its nearest collecting site at Río Chiriquí, RFLF, Chiriquí (Myers and Duellman 1982).

Isthmohyla graceae (Myers and Duellman 1982): Since there were drastic declines of the species reported from RFLF, Chiriqui (Lips 1999) this Panamanian endemic has disappeared from all well-known sites. Despite many search efforts, it is uncertain if any of these populations now survive (IUCN 2010). Although the Cerro Colorado area is the type locality of this species, previous search efforts concentrated mainly on PAs. In 2010, at the south-eastern slopes of Cerro Sagui, Comarca Ngöbe-Buglé, close to the species' type locality, we detected various calling males and tadpoles at a marshy headwater of Río Cricamola between pasture and forest. We collected four adult males and one tadpole as representative samples. All four collected adults were tested for Bd using swab samples for PCR; three with negative and one with a uncertain result(s). Like in the previous species, this represents the only known population that so far persists, and there are no recent records from any PA.

Isthmohyla rivularis (Taylor 1952): This species once was very common (Savage 2002), but declined throughout its range in the 1990s. In Panama, the last records date back to 1996 (IUCN 2010). In 2007, it was rediscovered at Monteverde, Costa Rica (Andrew Gray, pers. comm. 2011), where it was last seen in 1989. We collected a total of six adult males and one adult female around Cerro Pando, PILA, Chiriquí and Bocas del Toro. Six specimens were obtained from the Pacific slopes and one from the Caribbean slopes. The first individual was detected in 2008 by coincidence at night near a small mountain stream. In one of the ensuing nights, after heavy rain falls, we located three more males by the side of an unpaved road by following their calls. Calling site was very dense vegetation, intermingled with leaf litter that was overhanging a small creek. The calling males were sitting near ground level, well-hidden between plant material. In the same night, we heard more males calling from different creeks in the surrounding area. In 2009, we collected another calling male from the bank of a mountain creek, where it was perched on a leaf near a small cascade. In the same night, and only about 100 m away from the latter specimen, we detected a female on a broad-leaved plant in a waterless anabranch. In November 2009, we found another individual, when crossing over the continental divide to the Caribbean slopes of Cerro Pando.

It was sitting in a bush, about 1.5 m above ground level, near a fast-moving mountain creek. The abundance of this species in the surroundings of Cerro Pando indicates that there is a surviving population. None of the collected specimens appeared to be sick. Histological examination of skin scrapings did not provide any evidence of a chytrid infection.

Isthmohyla tica (Starrett 1966): This stream-breeder has also been a common species in Costa Rica and Panama (Savage 2002), until it dramatically declined at all known sites. In Panama, it had been collected at multiple sites between PILA and RFLF in the 1980s and 90s (Tejera and Dupuy 2003). These populations collapsed in 1997 and there were no recent sightings in Costa Rica or Panama. In July 2010, we collected a male specimen at Río Changena, northern slope of Cerro Pando, PILA, Bocas del Toro. The cricket-like call drew our attention to the frog that was sitting well-hidden about three m above the water line in a bush. We heard another male from the opposite side of the river, but could not find it. Later in the same month, we found a specimen at the upper reaches of Río Hacha, Comarca Ngöbe Buglé. This individual was not calling, but sitting about 3.5 m above the water in a small tree.

Discussion

The Panamanian Central Cordillera is an area where Bd driven amphibian declines have been especially severe (Young et al. 2001) and where declines were exceptionally well documented (Lips 1998, 1999; Lips et al. 2006). At all of these sites certain species, mainly hylids, bufonids, and stream-associated craugastorids, have not been found since these documented decline events. While in neighboring Costa Rica recent surveys have led to several rediscoveries of lost species (García-Rodruíguez et al. 2012), upland sites in western Panama were not frequently visited by herpetologists. The majority of present amphibian research in Panama is carried out east of El Copé following the Bd wave and little attention has been paid to post decline sites. Accordingly, to date the present paper is the only information on relict populations of rare amphibians, and shall serve as a basis for future studies. There is a paucity of data for many species in general, as indicated by the high number of species listed as Data Deficient by the IUCN. In Panama, there are 34 species (more than 16% of all Panamanian amphibians; Fig. 2) for which more information is required to assess their population status and conservation trend.

The absence of chytrid at a certain site could explain why a population persists. We took chytrid samples from several specimens and various localities. Though, the sample size presented here is not large enough to prove the absence of Bd at an investigated site, and we cannot identify refuge areas free of Bd; this will require further investigation. However, our presence data together

Table 1. Visited areas, protection status, and percentage of all EN/CR species found.

Name of area	Land cover (ha)	Protection status	Number of EN species found	Number of CR species found	Percentage of EN/CR species in transect
PILA	207,000 (Panama only)	Transboundary Protected Area; UNESCO World Heritage Site	3	3	18%
BPPS	167,410	Conservation Forest	1	1	7%
PNSF	72,636	National Park	1	3	12%
RFLF	19,500	Forest Reserve	0	1	3%
PNVB	14,322	National Park	5	0	15%
Cerro Colo- rado Area	at least 14,000	unprotected	2	3	15%
Santa Clara	N.A.	unprotected	1	0	3%

with other studies (e.g., Kilburn et al. 2011) show that at a large-scale this pathogen is still present at sites in Panama where chytridiomycosis once emerged (Fig 3). Further research on chytridiomycosis in wild amphibian populations should focus on populations at post-decline sites.

Altitudinal distribution of a species may also be a reason for a species to persist. There is evidence that populations of species that inhabit a vast altitudinal range may at least persist at lower altitudes where mean temperature is higher (Berger et al. 2004), or could even actively keep *Bd* infections low by exploration to warm microclimate (Daskin et al. 2011). The same is imaginable with species having wide horizontal distributions, with populations persisting in climatic refuges, e.g., drier and warmer regions (Puschendorf et al. 2005). Unfortunately, lowland forests are facing a higher deforestation risk due to a better accessibility and higher agricultural value. For example, nowadays there are almost no remainders of pristine pacific lowland forest in western Panama.

Notwithstanding, habitat loss by means of modification, fragmentation, and destruction is still the biggest threat to amphibians and wildlife in general (Gardner et al. 2007; Young et al. 1999, 2004). About 44% of Panama's land mass is still covered by forests. Compared to other Central American countries, Panama showed a relatively low deforestation rate of -1.2% in the period between 1990 and 2000 (total Central America -1.6%), and an even lower -0.4% between 2000 and 2010 (total Central America -1.2%; data taken from FAO 2011). But deforestation rates in Panama are not equally distributed, and some forests are under higher anthropogenic pressure than others. By far the highest deforestation rate among Panamanian provinces is found in the Comarca Ngöbe-Buglé (-21.8%, 1992-2000) followed by the central-eastern provinces of Darién (-13.9%), and Panamá (-12.2%) (ANAM 2009). A great portion of the Cordillera Central, almost the whole part known as Serranía de Tabasará, is located within the limits of the Comarca Ngöbe-Buglé. In addition, the Cordillera Central is home to 73% of the EN and CR amphibian species in Panama. Consequently, there is a general need for more well-protected areas in this mountain range. During this study, we identified the Cerro Colorado region as one of the most important unprotected areas for amphibian conservation in Panama. The minimum convex polygon drawn around our collection points would comprise around 14,000 ha and could include at least 15% of Panamas EN and CR amphibian species (Table 1). Two Critically Endangered species, Isthmohyla debilis on the slopes of Cerro Santiago and I. graceae on the slopes of Cerro Saguí, both of which had not been detected since the late 1990s, do occur here. These are the only known populations of those two species that now persist. Additionally, it is the only place in Panama where the Endangered Agalychnis annae is known, and beside La Amistad, the only additional place across its whole distribution area where the Critically Endangered I. tica can still be found. As presumed by the restricted time we spent in field, these findings are better understood as only a limited sample of an estimated apparent higher species diversity, including certainly many undescribed species, signifying important diversity of other non-herpetological organisms possibly as well. Unfortunately, Cerro Colorado is under increased anthropogenic pressure, especially through international mining companies that wish to establish a copper mine at this site. At present time, the current Panamanian government is attempting to ease the solicitation process for foreign countries to get concessions (Nakoneczny and Whysner 2010). From all that we know, mining at Cerro Colorado will cause severe environmental damage and reduce Panamanian amphibian fauna once more. Thus, we strongly recommend to Panamanian authorities, both the government of Panama and traditional authorities of Ngöbe-Buglé, to assign the Cerro Colorado area, including Cerro Santiago, Cerro Saguí, and the whole mountain ridge in between, an area of approximately 65,400 ha (Fig. 3), as PA in order to preserve this highly diverse area for future generations.

Unfortunately, even PAs are not always successful in their ability to control deforestation. This is especially severe in PILA and BPPS, where deforestation of mature forest was high, despite the protection status (Oestreicher et al. 2009). Currently, the discussion on new roads through remote areas of different PAs, including PILA, PNVB, and BPPS, has come up again. The Panamanian government expects an increase of tourism and trade to be triggered by these projects. However, costs to build and maintain roads in tropical mountainous forests are usually high, making it doubtful that economic aims will be achieved (Reid and Hanily 2003). Beside other effects these roads will exacerbate deforestation by facilitating the access to formerly well-protected sites (Young 1994; Chomitz and Gray 1996; Nelson and Hellerstein 1997). It is further questionable if new roads in PAs rather annoy tourists, who are predominantly looking for pure nature, than stimulating them to make a visit, in particular if there is no forest left to see when driving through a park. There is a general need for comprehensive management plans, better demarcation of PAs, and year-round personnel to stop ongoing deforestation in areas that are supposed to be protected.

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Appendix I

Voucher specimens

Anura: Agalychnis annae: Comarca Ngöbe-Buglé: Cerro Colorado, N8.53, W81.81, 1600 m a.s.l.: SMF 89791; Agalychnis lemur: Veraguas: PNSF, Cerro Negro, N8.57, W81.10, 690 m a.s.l.: SMF 89959; Atelopus varius: Veraguas: PNSF, Cerro Negro, N8.57, W81.10, 730-1090 m a.s.l.: SMF 89822-3, 89917, AH 218; Duellmanohyla uranochroa: Chiriquí: RFLF, western slopes of Cerro Pata de Macho, N8.67, W82.20. 1420 m a.s.l.: SMF 89824, 89826, AH 195; Comarca Ngöbe-Buglé: BPPS, continental divide, street to Petroterminal, N8.79, W82.21, 1060-1080 m a.s.l.: SMF 89825, 89827; Ecnomiohyla fimbrimembra: Chiriquí: Boquete, Bajo Mono, north-eastern slopes of Volcán Barú, N8.83, W82.50, 1820 m a.s.l.: SMF 89857; Hyloscirtus colym-

ba: VERAGUAS: Alto de Piedra, N8.51, W81.12, 880-940 m a.s.l.: SMF 89794, 89828-32, AH 059, AH 547; Isthmohyla angustilineata: CHIRIQÍ: PILA, Cerro Horqueta, N8.85, W82.42, 1800 m a.s.l.: MHCH 483; Isthmohyla debilis: Comarca Ngöbe-Buglé: La Nevera, western slopes of Cerro Santiago, N8.50, W81.77, 1540-1650 m a.s.l.: SMF 89833, 89835, AH 166, AH 347; Llano Tugri, eastern slopes of Cerro Santiago, N8.50, W81.72, 1570 m a.s.l.: SMF 89834; Isthmohyla graceae: Comarca NGÖBE-BUGLÉ: Cerro Saguí, Finca Alto Cedro, N8.56, W81.82, 1710 m a.s.l.: SMF 8936-8, AH 477, AH 478; Isthmohyla rivularis: CHIRIQUÍ: PILA, Jurutungo, southern slopes of Cerro Pando, N8.91, W82.72, 1870-2070 m a.s.l.: SMF 89839-43, AH 138, AH 139; northern slopes of Cerro Pando near continental divide, N8.93; W82.71, 2290 m a.s.l.: SMF 89843; Isthmohyla tica: Bocas Del Toro: PILA, Río Changena, northern slopes of Cerro Pando, N8.98, W82.69, 1620 m a.s.l.: SMF 89845; Co-MARCA NGÖBE-BUGLÉ: Caribbean slopes of Cordillera de Tabasará, valley of Río Hacha, N8.52, W81.79, 1220 m a.s.l.: SMF 89846; Pristimantis museosus: Bocas DEL Toro: PILA, Río Changena, northern slopes of Cerro Pando, N8.98, W82.69, 1650 m a.s.l.: SMF 89859; Private Reserve Willi Mazu, N8.79, W82.20, 690 m a.s.l.: SMF 89860; Comarca Ngöbe-Buglé: La Nevera, western slopes of Cerro Santiago, N8.50, W81.77, 1590 m a.s.l.: SMF 89858; VERAGUAS: Alto de Piedra, N8.51, W81.12, 940-1000 m a.s.l.: SMF 89861-2, AH 555; PNSF, Cerro Negro, N8.57, W81.10, 770 m a.s.l.: SMF 89864; Ptychohyla legleri: Chiriquí: Santa Clara, N8.83, W82.78, 1200 m a.s.l.: SMF 89863.

CAUDATA: Bolitoglossa compacta: CHIRIQUÍ: PNVB, Volcán Barú, road from Boquete to summit, N8.80, W82.52, 3000 m a.s.l.: SMF 89849; Bolitoglossa magnifica: CHIRIQUÍ: PNVB, Volcán Barú, road from Boquete to summit, N8.80, W82.51, 2350 m a.s.l.: SMF 89847-8, AH 323, AH 325; Bolitoglossa marmorea: Chiriquí: PNVB, Volcán Barú, summit region of volcano, N8.81, W82.54, 3340-3400 m a.s.l.: SMF 89850-2, AH 328, AH 329; Bolitoglossa minutula: Chiriquí: PILA, Jurutungo, southern slopes of Cerro Pando, N8.91, W81.72, 1960-2030 m a.s.l.: SMF 89854, AH 141; northern slopes of Cerro Pando near continental divide, N8.93, W82.71, 2320 m a.s.l.: SMF 89855, AH 277; PNVB, Sendero Los Quezales, northern slope of volcano, N8.85, W82.51, 2130 m a.s.l.; SMF89856; PNVB, Volcán Barú, road from Boquete to summit, Camp Mamecillos, N8.80, W82.51, 2600 m a.s.l.: SMF 89853; *Oedipina grandis*: CHIRIQUÍ: PILA, Jurutungo, southern slopes of Cerro Pando, N8.54, W82.43, 2060 m a.s.l.: SMF 85076.

APPENDIX II
List of amphibians known to occur in Panama

Amphibian species reported for Panama	IUCN Category	EN/CR known from transect	EN/CR detected during this study	Distance of detected population to nearest PA (km)	Name of nearest PA
Order Anura					
Family Aromobatidae					
Allobates talamancae	LC				
Family Bufonidae					
Atelopus certus	EN				
A. chiriquiensis	CR	X			
A. glyphus	CR				
A. limosus	EN				
A. varius	CR	X	X	0	PNSF
A. zeteki	CR				
Crepidophryne epiotica	LC				
Incilius aucoinae	LC				
I. coniferus	LC				
I. fastidiosus	LC				
I. karenlipsae	NE				
I. melanochlorus	LC				
I. peripatetes	CR	X			
I. signifer	LC				
Rhaebo haematiticus	LC				
Rhinella acrolopha	DD				
R. alata	DD				
R. centralis	NE				
R. marina	LC				
Family Centrolenidae					
Cochranella euknemos	LC				
C. granulosa	LC				
Espadarana prosoblepon	LC				
Hyalinobatrachium aureoguttatum	NT				
H. chirripoi	LC				
H. colymbiphyllum	LC				
H. fleischmanni	LC				
H. talamancae	LC				
H. valerioi	LC				
H. vireovittatum	DD				
Sachatamia albomaculata	LC				
S. ilex	LC				
Teratohyla pulverata	LC				
T. spinosa	LC				
Family Craugastoridae					
Craugastor azueroensis	EN				
C. bransfordii	LC				
C. catalinae	CR	X			
C. crassidigitus	LC				

Hertz et al.

Amphibian species reported for Panama	IUCN Category	EN/CR known from transect	EN/CR detected during this study	Distance of detected population to nearest PA (km)	Name of nearest PA
C. emcelae	CR	X			
C. evanesco	NE				
C. fitzingeri	LC				
C. gollmeri	LC				
C. gulosus	EN	X			
C. jota	DD				
C. longirostris	LC				
C. megacephalus	LC				
C. melanostictus	LC				
C. monnichorum	DD				
C. noblei	LC				
C. obesus	EN	X			
C. opimus	LC				
C. podiciferus	NT				
C. polyptychus	LC				
C. punctariolus	EN	X			
C. raniformis	LC				
C. ranoides	CR	X			
C. rhyacobatrachus	EN	X			
C. rugosus	LC				
C. stejnegerianus	LC				
C. tabasarae	CR				
C. talamancae	LC				
C. taurus	CR				
Family Dentrobatidae					
Ameerega maculata	DD				
Colostethus latinasus	DD				
C. panamansis	LC				
C. pratti	LC				
Dendrobates auratus	LC				
Hyloxalus chocoensis	DD				
Oophaga arborea	EN	X			
O. granulifera	VU	2000			
O. pumilio	LC				
O. speciosa	EN	X			
O. vicentei	DD				
Phyllobates lugubris	LC				
Ranitomeya claudiae	DD				
R. fulgurita	LC				
R. minuta	LC				
Silverstoneia flotator	LC				
S. nubicola	NT				
Family Eleutherodactylidae	111				
Diasporus diastema	LC				
D. hylaeformis	LC				
D. quidditus	LC				
D. vocator	LC				

IUCN Category	EN/CR known from transect	EN/CR detected during this study	Distance of detected population to nearest PA (km)	Name of neares
EN	X			
LC				
NT				
EN	X	X	30	BPPS
LC				
CR	X	X	0	PNSF
LC				
EN	X			
CR	X	X	0	BPPS; RFLF
EN	X	X	0	PNVB
VU				
	X	X	0	PNSF
	X	X	0	PILA
		X	36	BPPS
				BPPS
			<u>-</u> ,	BITS
	Y	Y	0	PILA
				PILA
	Α	A	Ü	TILA
	v	v	7	PILA
	Λ	Λ	1	FILA
LC				
	EN LC NT LC CR LC LC LC LC LC CR CR CR	EN X LC NT EN X LC CR X LC LC LC LC LC LC LC LC EN X EN X VU CR X EN X VU CR X EN X LC LC LC LC LC LC LC CR X EN X CR X EN X VU CR DD CR X LC	Category known from transect EN/CR detected during this study EN X LC X NT X EN X LC X EN X LC X CR X CR X CR X CR X CR X X X CR X CR X CR X CR X	Category Category Category Category Category Iransect EN/C detected during this study with study of the category cat

Hertz et al.

Amphibian species reported for Panama	IUCN Category	EN/CR known from transect	EN/CR detected during this study	Distance of detected population to nearest PA (km)	Name of nearest PA
S. sila	LC				
S. sordida	LC				
Trachycephalus venulosus	LC				
Family Leiuperidae					
Engystomops pustulosus	LC				
Pleurodema brachyops	LC				
Family Leptodactylidae					
Leptodactylus bolivianus	LC				
L. fragilis	LC				
L. fuscus	LC				
L. melanonotus	LC				
L. poecilochylus	LC				
L. savagei	LC				
Family Microhylidae					
Chiasmocleis panamensis	LC				
Elachistocleis ovalis	LC				
Nelsonophryne aterrima	LC				
Relictivomer pearsei	LC				
Family Pipidae					
Pipa myersi	EN				
Family Ranidae					
Litobathes taylori	LC				
L. vaillanti	LC				
L. vibicarius	CR	X			
L. warszewitschii	LC				
Family Strabomantidae					
Pristimantis achatinus	DD				
P. adnus	NE				
P. altae	NT				
P. caryophyllaceus	NT				
P. cerasinus	LC				
P. cruentus	LC				
P. educatoris	NE				
P. gaigei	LC				
P. moro	LC				
P. museosus	EN	X	X	0	PILA; BPPS; PNSF
P. pardalis	NT				
P. pirrensis	DD				
P. ridens	LC				
P. taeniatus	LC				
Strabomantis bufoniformis	LC				
S. laticorpus	DD				
Order Caudata					
Family Plethodontidae					
Bolitoglossa anthracina	DD				
B. biseriata	LC				

Amphibian species reported for Panama	IUCN Category	EN/CR known from transect	EN/CR detected during this study	Distance of detected population to nearest PA (km)	Name of nearest PA
B. bramei	DD				
B. colonnea	LC				
B. compacta	EN	X	X	0	PNVB
B. copia	DD				
B. cuna	DD				
B. gomezi	DD				
B. lignicolor	VU				
B. magnifica	EN	X	X	0	PNVB
B. marmorea	EN	X	X	0	PNVB
B. medemi	VU				
B. minutula	EN	X	X	0	PILA; PNVB
B. phalarosoma	DD				
B. pygmaea	DD				
B. robinsoni	DD				
B. robusta	LC				
B. schizodactyla	LC				
B. sombra	VU				
B. taylori	DD				
Oedipina alfaroi	VU				
O. alleni	LC				
O. collaris	DD				
O. complex	LC				
O. cyclocauda	LC				
O. fortunensis	NE				
O. gracilis	EN				
O. grandis	EN	X	X	0	PILA
O. maritima	CR				
O. pacificensis	LC				
O. parvipes	LC				
Order Gymnophiona					
Family Caeciliidae					
Caecilia isthmica	DD				
C. leucocephala	LC				
C. nigricans	LC				
C. volcani	DD				
Dermophis glandulosus	DD				
D. gracilior	DD				
D. parviceps	LC				
Gymnophis multiplicata	NE				
Oscaecilia elongata	DD				
O. ochrocephala	LC				
Total 206		33	18		

APPENDIX III

Representative species



Agalychnis annae (SMF 89791), Cerro Colorado, Comarca Ngöbe-Buglé. *Photo by SL*.



Agalychnis lemur (SMF 89959), Cerro Negro, PNSF, Veraguas. Photo by AC.



Atelopus varius (AH 218), Cerro Negro, PNSF, Veraguas. *Photo by AH*.



Bolitoglossa compacta (SMF 89849), Volcán Barú, PNVB, Chiriquí. Photo by AH.



Bolitoglossa magnifica (AH323), Volcán Barú, PNVB, Chiriquí. *Photo by AH*.



Bolitoglossa marmorea (SMF 89850), summit of Volcán Barú, PNVB, Chiriquí. *Photo by AH*.



Bolitoglossa minutula (SMF 89855), valley of Jurutungo, southern slope of Cerro Pando, PILA, Chiriquí. *Photo by AH*.

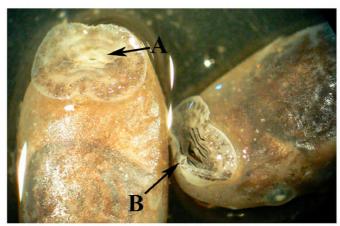


Duellmanohyla uranochroa (SMF 89824), western slope of Cerro Pata de Macho, RFLF, Chiriquí. *Photo by AH*.

Threatened amphibian species in western Panama



Back view of *Duellmanohyla uranochroa* (SMF 89825) with large nematode under the skin, BPPS, Comarca Ngöbe-Buglé. *Photo by AH*.



Tadpoles of Duellmanohyla uranochroa (AH 195) showing complete loss of keratinized mouthparts (A) and deformation of oral disk (B), western slope of Cerro Pata de Macho, RFLF, Chiriquí. Photo by AH through dissecting microscope.



Ecnomiohyla fimbrimembra (SMF 89857), Bajo Mono, eastern slope of Volcán Barú, PNVB, Chiriquí. *Photo by AH*.



Hyloscirtus colymba (AH 547), Alto de Piedra, PNSF, Veraguas. *Photo by AH*.



Hyloscirtus colymba (SMF 89831) infected with chydridiomycosis, note reddish coloration through vascular dilatation, Alto de Piedra, PNSF, Veraguas. *Photo by AH*.



Isthmohyla angustilineata (MHCH 483), El pianista, PILA, Bocas del Toro. *Photo by MP*.



Isthmohyla debilis (AH 347), La Nevera, Comarca Ngöbe-Buglé. *Photo by AH*.



Isthmohyla graceae (SMF 89837), Cerro Saguí, Comarca Ngöbe-Buglé. *Photo by AH*.

Hertz et al.



Isthmohyla rivularis (SMF 89843), northern slope of Cerro Pando, PILA, Bocas del Toro. *Photo by AH*.



Isthmohyla tica (SMF 89846), Río Hacha, Comarca Ngöbe-Buglé. Photo by AH.



Oedipina grandis (SMF 85076), valley of Jurutungo, southern slope of Cerro Pando, PILA, Chiriquí. *Photo by GK.*



Pristimantis museosus (SMF89859), Río Changena, northern slopes of Cerro Pando, PILA Bocas del Toro. *Photo by AH*.



Ptychohyla legleri froglet (SMF 89863), Santa Clara, Chiriquí. *Photo by AH*.



View of the Cerro Colorado area looking westward along the continental divide, from La Nevera. Note road from San Felix and cone-shaped peak of Cerro Saguí in the background (cp. Fig. 1 in Myers and Duellman 1982). *Photo by SL*.

Threatened amphibian species in western Panama



Andreas Hertz



SEBASTIAN LOTZKAT



Arcadio Carrizo



MARCOS PONCE



GUNTHER KÖHLER



Bruno Streit



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