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Renewing hope: the rediscovery of *Atelopus varius* in Costa Rica

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Abstract. More than 90% of harlequin frog species (*Atelopus* spp.), endemic to the Americas, are currently threatened with extinction. We report the discovery of the only currently known breeding population of the Critically Endangered *A. varius* in Costa Rica. This population was located in 2008 on a private property in Las Tablas Protected Zone near San Vito, Coto Brus at 1300 m elevation. Previously, the only known remaining/remnant population of this species and genus was a single location near Manuel Antonio, Puntarenas, Costa Rica, where two individuals were documented in 2004. Subsequent searches at this location have yielded no additional sightings. Delineating the spatial limits of this population, quantifying demographics and resource use, and implementing conservation actions are necessary to ensure persistence of this population. Conducting additional surveys in this region to ascertain occurrence of additional populations is warranted.

Keywords: *Atelopus varius*, Central America, chytrid, Critically Endangered, harlequin frog, population, Talamanca Mountains, threatened species.

Amphibian species worldwide are declining more rapidly in general than other vertebrate groups, with 31% of known species threatened with extinction (Hoffmann et al., 2010; IUCN, 2011). Decline of amphibians is at the forefront of the global biodiversity crisis and has received considerable attention (Stuart et al., 2004). Many studies have reported amphibian declines and proposed sweeping efforts to save species or populations (e.g., Lips et al., 2003; Stuart et al., 2004). While current research emphasizes understanding and mitigating amphibian declines, less attention has been given to searching for populations and species where they are presumed extirpated or extinct (Bolaños et al., 2008).

Harlequin frogs (Amphibia: Bufonidae; *Atelopus* spp.) are endemic to the Neotropics and range from Costa Rica south to Bolivia and eastward disjunctively through the Amazon Basin into the Guyanas (La Marca et al., 2005). *Atelopus* is the largest genus within the family Bufonidae (Leenders, 2001; Savage, 2002) with 96 recognized species (Lötters, 1996; Frost, 2013). *Atelopus* spp. occur from sea level to 4800 m elevation, but most species occur in highlands above 1500 m with several species restricted to elevations above 3000 m (La Marca et al., 2005).

The decline of *Atelopus* species has been widely studied, and their restricted ranges and habitat associations make them highly vulnerable to population declines (La Marca et al., 2005; Lips et al., 2005; Rohr et al., 2008). Currently, >90% of *Atelopus* species are threatened with extinction and >75% are Critically Endangered (Stuart et al., 2008; IUCN, 2011). These declines are especially prevalent in *Atelopus* species restricted to high elevations; all species with elevational ranges above 1000 m have experienced population declines, and 75%

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of species found only above 1000 m are presumed extinct (La Marca et al., 2005). Before the 1990s, population declines were attributed to large scale collection for the pet trade, habitat loss, invasive species, and climate change (La Marca et al., 2005). However, chytrid fungus is now considered a main cause of amphibian declines (Lips et al., 2003; La Marca et al., 2005; Ron, 2005; Pounds et al., 2008; Lotters et al., 2009; Rodder et al., 2009).

Atelopus varius is terrestrial and found in humid lowland, premontane and lowland montane forests, typically near small, high-gradient streams (Pounds and Crump, 1987; Savage, 2002). During the dry season, this species congregates in remaining moist habitats (Savage, 2002). *Atelopus varius* is listed as Critically Endangered on the IUCN Red List of Threatened Species because it has experienced a population decline >90% over the last three generations (13 years) and almost all populations are now extirpated, presumably because of chytrid fungus (Bolaños et al., 2008; Pounds et al., 2008).

Atelopus varius was known from the Atlantic and Pacific slopes of the Tilarán Mountains and Cordillera Talamanca of Costa Rica and western Panama, including Veraguas, Coclé and Colón provices (Jaramillo et al., 2010), from sea-level to 2000 m (Savage, 1972; Savage, 2002; Pounds et al., 2008). In the 1980s and early 1990s, populations of *A. varius* declined dramatically, resulting in numerous local extinctions, presumably due to chytrid fungus (Santos-Barrera et al., 2008). In 1988, *A. varius* started to disappear from Monteverde, central Costa Rica (Pounds et al., 2008), followed by extirpation from the surrounding Tilarán Mountains between 1990 and 1992 (Pounds and Crump, 1994) and was considered extirpated from Costa Rica by 1996. In less than 20 years, known populations in Costa Rica declined from 100 to one (Pounds et al., 2008). In addition to chytrid fungus and climate change, parasitic fly larvae (Crump and Pounds, 1985) have been reported to feed on harlequin frogs in montane Costa Rica and could represent a threat (Crump and Pounds, 1985; Pounds and Crump, 1987).

In Panamá, it is believed the species also suffered dramatic reductions, and is still considered under severe decline (Hertz et al., 2012). There are records of nine populations in the western portion of Panamá in 2004, including parts of Veraguas, Cocle and Colón provices (Richards-Zawacki, 2009), eight individuals in Coclé (Woodhams et al., 2006), and a more recent record of four individuals from Cerro Negro, Parque Nacional Santa Fé, Veraguas province, between 2008 and 2010 (Hertz et al., 2012). Additional unsubstantiated records exist; however, no literature currently exists supporting these. According to Richards and Knowles (2007) the only known populations in Panama after severe decline are located in Coclé province.

About eight years after the reported extinction in Costa Rica, a population was discovered in 2004 in the Pacific lowlands of Costa Rica 10 km northwest of Quepos in Fila Chonta (Central Pacific; Pounds et al., 2008), and further surveys in 2005 did not find additional populations (Ryan et al., 2005). In November 2008 during routine field surveys, we discovered a different population of *A. varius* on the Pacific foothills of the Talamanca Mountains in Costa Rica, representing the second known population re-discovery for this species in the country. This population was ∼150 km from the population discovered in 2004, on a private property Northeast of San Vito, within the Las Tablas Protected Zone, La Amistad Biosphere Reserve. This location contains primarily regenerating forests with several high-gradient streams and is managed as a conservation preserve. The property is part of the largest remaining contiguous forest in Costa Rica (Rodríguez-Herrera et al., 2012).

Following the initial discovery of the population, four individuals were found in this area with assistance of people from a nearby farming community. We conducted diurnal transect surveys in this same area during March 2009. Each transect was 2 km long and 6 m-wide and was traversed by two researchers in 1.5 h. One transect was searched each of

11 days with overall effort comprising 22 km of transects and 33 person-hours. Overall, we recorded 32 mature individuals (mean ± SD = 2.8 ± 1.3/survey). The mean elevation of observations was 1334 ± 21.2 m. The habitat was typical of montane forest ecosystems (Pounds and Crump, 1987; Savage, 2002), with limited human activity (e.g., cattle grazing) in adjacent areas. We received 15 additional reports of *A. varius* throughout 2009 from the local adjacent community and we reconfirmed species' presence in April 2010 by occasional surveys.

From September 2011 to February 2013 we conducted 16 monthly systematic population surveys, including habitat measurements and qualitative assessments of potential predators and threats. Survey protocol followed the design and sampling recommendations of Lips et al. (2001), Doan (2003), Sutherland (2006) and Heyer et al. (1994). Monthly surveys were conducted on two consecutive days, where two people surveyed 4 km of 6-m wide transects at a rate of 0.25 km/h. Thus, total sampling effort was 64 km and 128 person-hours.

Overall, we obtained 222 sightings of 204 different individuals (identified using their unique dorsal and ventral color patterns; 77% females and 23% males) and we re-sighted 14 individuals on one occasion each and 2 with two re-sightings. The average number of individuals sighted monthly was mean ± SD = 21.50 ± 10.18 during the dry season (November-March) and 6.25 ± 5.44 during the rainy season (April-October). The month with greatest number of sightings during the dry season was March (40 individuals) and during the rainy season was April (16 individuals). The fewest individuals sighted were in June and August (1 individual each) (fig. 1). One amplexus and three calling males were sighted during the reported breeding season (September-October; Savage, 2002), and the continued detection of individuals during the five-year period (2009-2013) suggests that reproduction is occurring (fig. 2). All surveys we conducted followed safety protocols to avoid infection of frogs with chytrid or other diseases. Procedures included clothing and footwear which were exclusively used for this project, restricted access to the population area and septic measures to equipment, personnel and apparel. As chytrid was previously recorded in the area we performed safety and cleaning protocols for each survey.

Our findings fit into the emerging theory that species which have been presumed extinct are beginning to be re-discovered in Costa Rica and elsewhere. After severe declines, often to where zero individuals are detected in the wild, several frog species have been re-discovered in areas of Costa Rica with previous records of chytrid, as well as in areas not sampled previously (Whitfield, 2011). Among recent findings we highlight the records for *Incilius holdridgei* in Central Costa Rica (Abarca et al., 2010), *Isthmohyla rivularis* in Monteverde (Whitfield, 2011) and *Craugastor ranoides* in Northwestern Costa Rica (Puschendorf et al., 2005; Zumbado-Ulate et al., 2011). These findings follow a pattern similar to our finding in that species considered extinct or near-extinct, with recorded massive declines due to chytrid have been rediscovered at previously known locations. Recent analyses suggest that certain areas of Costa Rica have high probabilities of re-discovering populations, and most have not been sampled since 2000 (García-Rodríguez et al., 2012). Our locality is one of these areas which fall within predicted priority regions for surveys and conservation. However, the locality of this study was also identified as a high probability area for chytrid presence (Lips et al., 2003; Puschendorf et al., 2009).

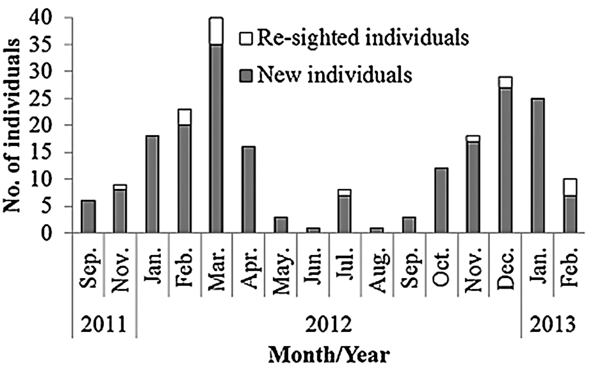

Figure 1. Number of sightings and re-sightings of *Atelopus varius* individuals during systematic surveys in a recently discovered population in Costa Rica (Sep. 2011-Feb. 2013).

Figure 2. An amplexus of *Atelopus varius* from the recent discovered population in the Talamanca mountains of Costa Rica. This figure is published in colour in the online version.

The pattern of presumed extirpations and extinctions of amphibians followed by their rediscovery opens a series of questions about amphibian declines in general, specifically chytrid-caused declines. The recent re-discovery of almost extinct amphibian species is probably due to more intensive surveys and greater sampling efforts in previously known localities and in areas poorly explored. Also, chytrid infection dynamics have shown recently that virulence increased from arrival to spreading southwards across Mesoamerica (Phillips and Puschendorf, 2013). However, to our knowledge no information exists on the prevalence and persistence of chytrid after its primary hosts have disappeared. We propose two hypotheses related to our rediscovery of *A. various*, and potentially other amphibian re-discoveries in the country.

The first hypothesis is that isolated populations of host species may not have come in contact with chytrid fungus. Little is known about the ecology of chytrid fungus outside amphibian hosts, with only a few observations of the fungus surviving in keratine or as a saprobe (Longcore et al., 1999). Also, no information to our knowledge exists on how long the fungus can survive in the environment without hosts and thus where and for how long a reservoir can exist. Given the characteristics of most mountain rocky streams, many small creeks are sometimes isolated and water flow can be a barrier for up-stream dispersal of species. Thus, small habitat pockets on up-stream tributaries of the main rivers could maintain small population relicts that could potentially re-colonize the main rivers after the fungus disappeared.

Our second hypothesis is that it is possible that some individuals within populations experiencing chytrid infection could endure and survive the infection. Though chytrid infection was recorded for *A. varius* collected in 1993 (Lips et al., 2003) and population declines were confirmed in the area in 1996 (Lips, 1998), some individuals may have exhibited resistance to chytrid infection and persisted, as proposed for the other known locality in Costa Rica (Ryan et al., 2005). Each of these potential explanations requires further understanding of the mechanisms of chytrid fungus spread, the interaction of multiple factors (Whitfield, 2011), both environmental and ecological, and population genetics of the fungus and host.

The rediscovery of *A. varius* presents a unique opportunity to conserve a Critically Endangered species and help maintain biodiversity of this region. As the population occurs on land managed as a conservation preserve, there are no immediate threats from land use change.

However, we observed some contamination of the river by upstream land-use practices (e.g., cattle grazing, littering and timber harvest). We suggest the greatest potential threats to this population are chytrid fungus, already reported in this area (Lips et al., 2003), and illegal collection for the pet trade if the location of this population becomes well known. To ensure persistence of this population we recommend assessing its' spatial limits and quantifying demographics, population genetics and habitat use, followed by implementing conservation actions (e.g., preventing trespassing and illegal collection). Also, protocols should be determined and designed to ensure safety of this population. Conducting surveys in this region to ascertain presence of additional populations is warranted, particularly in areas of recent known occurrence. Improved inventory and monitoring efforts, including assessment of chytrid presence, along with effective protection measures (e.g. habitat conservation, protection from illegal collections and measures for reducing outside infection risk), will help ensure the survival of *A. varius* in Costa Rica.

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