

# From incidental findings to systematic discovery: locating and monitoring a new population of the endangered Harlequin Toad

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Abstract.—The scientific and conservation communities recognize toads of genus Atelopus as among the most vulnerable of all amphibian groups, with over 75% of the species assessed as "Critically Endangered" by the International Union for the Conservation of Nature (IUCN) Red List. Atelopus varius, known as the Harlequin Toad, has been thought to be extinct in Costa Rica since the mid-1990s. There have been four rediscovered populations of the species since 2004. This report presents the fifth reappearance of A. varius, this time in the Alexander Skutch Biological Corridor (ASBC) in the Pacific Slope foothills of La Amistad International Park, Costa Rica, which represents a new location. Previously, the pattern of reappearance of this species has been unclear. In this study, the discovery of a new population of A. varius allows us to evaluate the presence of Bd infection and offer critical natural history remarks. In total, 25 different individuals were identified. All samples analyzed for Bd diagnosis were negative. In contrast to other A. varius populations, this one was mostly found high above the riverbed, often in the foliage, tree trunks, and bromeliads, from 1-6 m above the water both during day and night. The absence of Bd infection in these Harlequin Toads, a highly susceptible species, in an area identified as having a high probability of Bd occurrence, suggests that this behavior could have helped this population survive by reducing infection risk. Moreover, the distribution of A. varius may have changed in the last 50 years, by penetrating higher in the montane regions of the Talamanca mountains, a change in distribution that might also help its survival of some environmental stressors. With the discovery of a new locality for A. varius, this study offers an animal behavior argument to account for species recovery in general, as well as a possible expansion of what has been accepted as the historical distribution of this species.

**Keywords.** Atelopus varius, natural history, citizen science, endangered species, chytrid fungus, Alexander Skutch Biological Corridor, Costa Rica

Resumen.—Los sapos del género Atelopus son reconocidos como uno de los grupos de anfibios más vulnerables, con más del 75 por ciento de las especies de este género evaluadas como "En Peligro Crítico" por la Lista Roja de la Unión Internacional para la Conservación de la Naturaleza. Se pensaba que Atelopus varius, conocido como la rana Arlequín, se había extinguido en Costa Rica desde mediados de los años noventa. Después de 2004 ha habido cuatro redescubrimientos de la especie. Este informe presenta la quinta reaparición de A. varius, esta vez en el Corredor Biológico Alexander Skutch (ASBC) en las estribaciones de la vertiente del Pacífico del Parque Internacional La Amistad, Costa Rica como una nueva ubicación. Hasta ahora, el patrón de reaparición de esta especie no ha sido claro. En este estudio, con el descubrimiento de una nueva población de A. varius, ofrecemos importantes observaciones de historia natural y evaluamos la presencia de la infección por Bd. En total, se identificaron 25 individuos diferentes. Todas las muestras analizadas para el diagnóstico de Bd fueron negativas. En contraste con otras poblaciones de A. varius, en nuestro caso se encontró la mayoría de los individuos alto sobre el lecho del río, a menudo en el follaje, troncos de árboles y bromelias, entre 1-6 m sobre el agua, tanto de día como de noche. La ausencia de infección por Bd en estas ranas arlequín, una especie altamente susceptible, en un área identificada como con una alta probabilidad de ocurrencia de Bd, sugiere que los sapos que pasen menos tiempo cerca del río y más tiempo en áreas abiertas, podría haber ayudado a esta población a sobrevivir mediante la reducción al riesgo de infección. Además, hipotetizamos que la distribución de A. varius pudo haber cambiado en los últimos 50 años, penetrando más alto en las regiones montanas de la cordillera de Talamanca, un cambio en la distribución que también podría

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estar ayudando con su supervivencia a ciertos factores ambientales. Con el descubrimiento de una nueva localidad para *A. varius*, nuestro estudio ofrece un argumento de comportamiento animal para explicar la recuperación de especies, así como una posible expansión de lo que se ha aceptado como la distribución histórica de esta especie.

**Palabras clave.** *Atelopus varius*, historia natural, ciencia ciudadana, reaparición de especies amenazadas, rana arlequín, hongo quítrido, Corredor Biológico Alexander Skutch, Costa Rica

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#### Introduction

Over 75% of the species in genus *Atelopus* are assessed as "Critically Endangered" by the International Union for the Conservation of Nature Red List (IUCN 2015). Within this genus, the Harlequin Toad (*Atelopus varius*, in Spanish as "Rana Harlequín"), is the last remaining species in Costa Rica. The other three representatives are believed to be extinct (Barrio-Amorós and Abarca 2016).

This moderately-sized toad is associated with small, fast-moving streams, where it is found along the banks and sitting on rocks in the stream. Endemic to Costa Rica and Panama, at one point there were over 100 known populations in both the Atlantic and Pacific Slope versants of the mountain ranges in Costa Rica and western Panama, reaching up to 2,000 m asl (Savage 2002). In the mid-1990s, A. varius was believed to be extirpated from Costa Rica, following a drastic decline that began in Monteverde in 1988, where it remains absent (Pounds and Crump 1994). Apart from habitat loss, two other leading possible explanations for the Harlequin Toad's disappearance are climate variations and fungal disease. Research so far suggests the possibility that the decline of A. varius has a multifactor explanation (Pounds et al. 2010; Berger et al. 1998) combining both the effect of climate stress and the appearance of the fungus Batrachochytrium dendrobatidis (Bd).

Since its loss during the 1990s, four known rediscoveries of the species have been documented, each in private properties in the Pacific slope in Puntarenas Province, Costa Rica. In 2003, a population was discovered at Fila Chonta, Quepos (Pounds et al. 2010; Ryan et al. 2005). Nine years later, to the South, a new breeding population was found near Las Tablas, near the Panamanian border, at 1,300 m asl (González-Maya et al. 2013). One individual of A. varius was reported near Buenos Aires, at an elevation of 840 m asl (Solano-Cascante et al. 2014). Moreover, there was a recent rediscovery of A. varius by Barrio-Amorós and Abarca (2016) at 400 m asl in a rocky stream located in the Uvita Region. Those authors observed nine adult males, one of which was dead. They tested two live individuals and the one dead specimen for Bd, and the dead specimen was the only one to test positive for Bd (Barrio-Amorós and Abarca 2016).

The pattern of previous reappearances of this species has been unclear. In some cases, it appeared in small secluded creeks (Barrio-Amorós and Abarca 2016), and in others, it appeared in large, open, and fast flowing rivers (Gonzalez-Maya et al. 2013; Solano-Cascante et al. 2014), and at altitudinal ranges from almost sea level to as high as 1,500 m asl. The sites identified thus far are all on the Pacific slope, amid degraded landscapes in unprotected land and fragmented, isolated conditions. This pattern of reappearance makes these populations highly vulnerable to habitat loss and environmental risks, such as the population at one of the known localities in Costa Rica which is under serious threat of landslides (Pounds et al. 2010).

Here a newly discovered population of A. varius is presented, found in the Alexander Skutch Biological Corridor (ASBC), in the Pacific Slope foothills of La Amistad International Park. We present crucial natural history remarks and evaluate the presence of Bd infection in this population. The natural history of amphibian species can hold important clues for their survival, given that the effect of fungal infection can be modified by the host's ecology, behavior, and life history (Woodhams et al. 2008). The exact location of the site is withheld, given that the protection of such sensitive information is of utmost importance in the case of A. varius in Costa Rica. All the re-discovered populations are outside of national protected areas and hence are highly susceptible to commercial trade, collection, and the irresponsible handling that can lead to a decrease in population or increased risk of disease. The IUCN guidelines acknowledge this risk regarding sensitive data access and the publishing of data that identify specific geographic locations (IUCN 2012).

### **Materials and Methods**

The ASBC extends over 6,012.60 ha (Fig. 1) within the canton of Pérez Zeledón in the province of San José. The principal communities of the ASBC include Quizarrá, Santa Elena, Montecarlo, San Francisco, San Ignacio, Santa Marta, Santa María, and Trinidad. The ASBC lies between 650 and 1,650 m asl and contains the first remnant population of *A. varius* outside the province of Puntarenas.

Note that the findings documented by community members and students of the Faculty of Environmental Studies of York University were essential in locating this population of *A. varius*. The incidental sightings came from at least three separate locations along two different

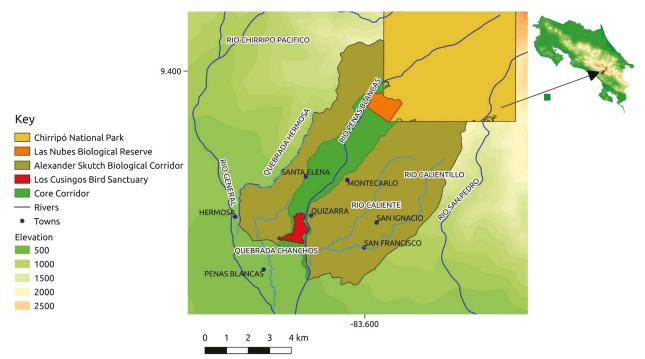


Fig. 1 Location and boundaries of the Alexander Skutch Biological Corridor (ASBC) in the province of San José, Costa Rica.

rivers. The rivers are at least 2.3 km apart in a straight line and separated by mountainous formations. Some observers had seen toads along the same river, but the sightings were 300 m apart. In-depth interviews were conducted with the observers of the Harlequin Toad incidental sightings to locate the population. After the interviews, 18 survey days followed, during which over 65 hrs were spent locating, observing, and sampling *A. varius* in the previously identified locations.

The interviews aimed to determine as accurately as possible the location, date, time, available GPS data (geo-tracking in mobile devices), weather, and relevant information of each sighting. In April and June 2016, two visits to the narrowed-down sites were conducted and systematic searches were performed. These included six independent diurnal explorations ranging from 3–5 h each, mostly starting at 7:00 AM, with night surveys also conducted to try to find individuals sleeping in the foliage. These visits were not successful in locating any individuals of the population. However, in February 2017, another search successfully located the area where *A. varius* resides. After the first individual was encountered, a transect of 1.1 km was established to monitor the identified locality systematically.

The transect was surveyed continuously for six days in February 2017, nine days in June 2017, and three days in January 2018. These months were selected to account for seasonal variation, in order to increase the chances of an encounter. Systematic surveys started early in the morning and lasted for an average of four h, with additional surveys carried out sporadically at night to identify additional locations and sites. The transect was hiked starting on opposite ends alternatively to account for possible time variations of activity of the toads. The search process usually involved 4–6 h of daily effort, walking along the river and searching in caves, rocks, foliage, and

vegetation above the river. Upon encountering the toads, they were swabbed for *Bd* samples, and individuals were measured and photographed both dorsally and ventrally. Upon the conclusion of these procedures, individuals were released in the same location, and each was handled with a separate set of gloves. Fourteen of these individuals were sampled for *Bd*, 10 during the February 2017 surveys and four during June 2017, two of which were juveniles.

Sex and age were also recorded, along with substrate, activity (Rest/sleep, Hide, Bask/Splash, Walk/Climb/Feed), and distance from the river upon first encounter. For simplicity, the behavior labelled as "splash" refers to moisture control, as toads absorb water from wet surfaces in the stream's splash zone (Pounds and Crump 1994). For better data analysis, the substrate Vegetation was classified as either trees, bromeliads, branches, or tree trunks; Soil/Rock refers to either bare ground, crevices, boulders, or mossy regions on top of the bare rock. The distance from the river was recorded with horizontal and vertical distances measured from the water's edge to the perch where the toad was found.

The skin swab and storage protocol by Whitfield et al. (2017) was used, which involves rubbing a sterilized swab (MW-100 cotton-tipped swab) on the dorsum, the venter, the sides, and the limbs. Nucleic acids were extracted from the swabs using Prepman Ultra and Real-Time PCR protocols (Boyle et al. 2004; Kriger et al. 2006) in the Laboratory of Experimental and Comparative Pathology, School of Biology, University of Costa Rica. Positive and negative controls were run in triplicate on each 96-well PCR plate. *Bd* primers and probes (Boyle et al. 2004) were used in a TaqMan® Gene Expression Assay (Applied Biosystems, Carlsbad, California). Samples were run in an Applied BioSystems Prism 7500 Sequence Detection System in Centro de Investigación

en Biología Celular y Molecular (CIBCM), University of Costa Rica. Samples corresponding to 14 animals from the study site are described.

#### Results

## **Open-ended Interviews of Incidental Sightings**

In 2015, two independent incidental sightings of the Harlequin Toad were documented, photographed, and reported by local community members of the ASBC to York University's LNP Director (FM). In March 2016, a York University student spotted and photographed another A. varius specimen in the ASBC. Subsequently, in August 2016, new sightings of the Harlequin Toad were further documented by students and community members. Additional sightings of individuals were also reported by the Tropical Science Center Los Cusingos Administrator Mario Mejía for May, August, and September of 2016. During these two years of incidental sightings, the authors engaged in open-ended interviews with the incidental observers to expand on their information. These interviews provided precious data for reducing the search area and narrowing the times of the day for targeted searches.

#### **Transect Observations**

In February 2017, 13 individuals in a rocky stream within the identified locations in the ASBC were photographed and identifying body patterns were noted. Eleven were males and two were females—no juveniles, eggs, or tadpoles were observed. In June 2017, six new individuals, identified by their unique body patterns, were recorded in the same study area—three females and three juveniles. In August 2017, at least one other individual was observed. In total, with the transect observations and the photographed incidental sightings, at least 25 different individuals have been identified in these locations. The sites where this population resides are along a wide, open, and fast flowing river, with a young riparian forest and some old tall trees, surrounded by a disturbed landscape, with only 10 to 20 m of riparian forest habitat in some areas.

Males had an average snout-vent length of 25.7 mm and females 34.3 mm. Male dorsal patterns appear to include more vellow and green tones, and females have a stronger orange and red coloration (see Fig. 2). This coloration is consistent with the remarks of Savage (1972) on Atelopus populations of Panama and Costa Rica. Juveniles, on the other hand, have no red and tend towards lime green, with patterns that are much more speckled (Fig. 3C,E). All individuals appeared to be in good health, and no external lesions were present; in most cases, the skin was colorful, vibrant, and healthy. Interestingly, the females had very wrinkly cloaca, probably due to oviposition; no males exhibited any similar skin condition. One female was molting (Fig. 2E), from whom skin samples were collected and observed under the microscope, which detected no presence of Bd. In total, 14 individuals were tested for Bd, all of which tested negative for Bd diagnosis.

Just one-quarter of the identified adults were females, and males were sometimes observed in groups. There was no indication of reproduction in February, while in June, juveniles were located, all of them at least 2 m above the river and in the foliage. In contrast to other populations of *A. varius*, the toads in the ASBC were mostly located above the riverbed, often found in the foliage, tree trunks, and bromeliads between 1–6 m above the water, both during day and night. Of the 37 recorded observations, only 11 individuals were seen close to the river or the splash area, and 10 of them were seen in vegetation between 3–6 m high.

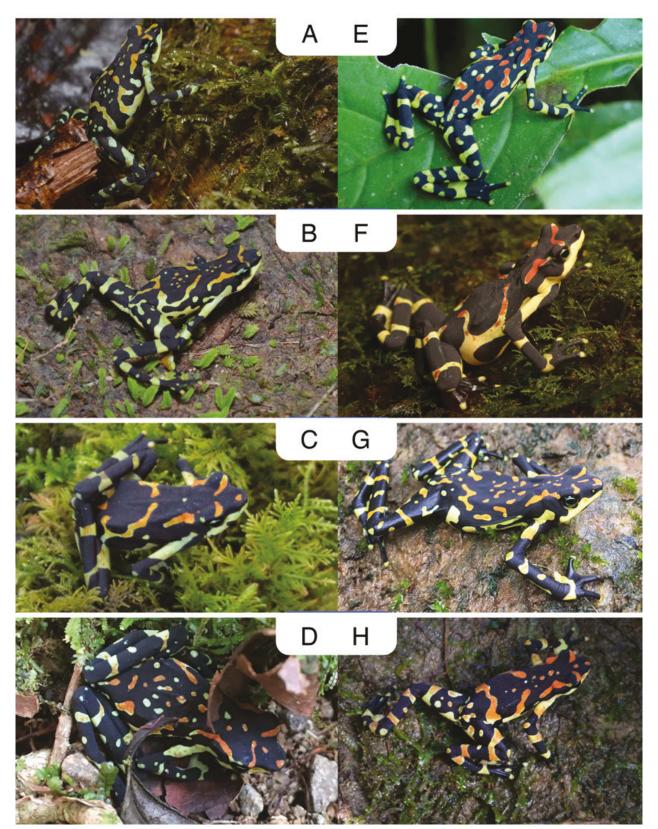
# **Natural History Notes**

During the surveys, there was ample opportunity to observe the natural history of this species, and these observations can help other scientists better locate and identify new sites in the field, especially when there are previously existing reports from community members. Despite the Harlequin Toad's bright coloration, this species can be challenging to locate in the field. For example, during our systematic exploration, the first individuals were found after almost 25 h of searching in the same location where it was later documented.

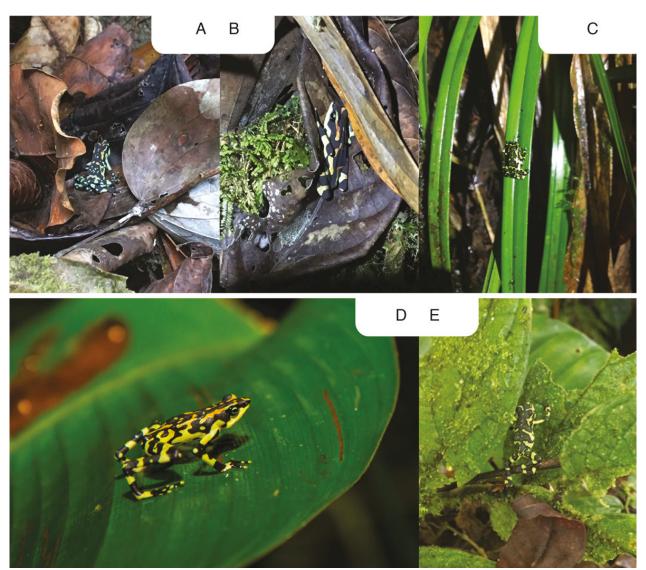
The start of diurnal activities varied among the seasons. During the dry season, toads were easily located very early in the morning; but during the wet season, toads were less frequent, and surveys needed to start much later when the day had warmed up. Nineteen sightings were obtained during January and February, but only six individuals in June, even though more time and effort was spent during this month. Overall, the sightings of females were less abundant, and for both sexes, there were more individuals seen during the dry season. There was a change in sex ratio between the two seasons (Fig. 4, G = 4.216, 1 df, P = 0.040), with proportionately more males detected during the dry season than the wet season.

Regarding the substrate, females were generally absent from the leaf litter and mostly found on the vegetation and river boulders, while males were found on the leaf litter (Fig. 5, G = 9.035, 2 df, P = 0.011). Previous records suggest that this toad is most often found along the banks, sitting on the rocks near the splash zone (Crump and Pounds 1985; Pounds and Crump 1994; Savage 2002). However, in this case, adults were typically found in the vegetation or on the ground, basking on the rocks or foraging along the river bank, and rarely seen in the splash zone (Fig. 6). Juveniles had a stronger preference for vegetation (Fig. 6, G = 8.365, 2 df, P = 0.015), which explains why they were so difficult to locate (Fig. 3E).

The toads were observed basking more during the wet season, on the rocks near the openings in the canopy or the exposed vegetation (Fig. 2E-G). For example, the three females in Fig. 2 are pictured as found: the first was basking, exposed on the vegetation in an open part of the river around 9:00 AM during the dry season; the second and third females were basking on the river rocks during the wet season. Hiding and basking were activities found to be significantly associated with the dry season, while hiding was never seen during the wet season (Fig. 7, G



**Fig. 2** Detail of *Atelopus varius* individuals found during February and June 2017 in the ASBC. The left column includes males; the right column includes females. Males **(B)** and **(C)** were photographed as found, as were females **(E)**, **(G)**, and **(H)**. Note the spread-out basking position of female **(G)**.



**Fig. 3** Males, females, and juveniles of *Atelopus varius* found in the Alexander Skutch Biological Corridor. **(A)** Male found during February surveys. **(B)** Sleeping male in the leaf litter in the same location of a male in Fig. 2(A). **(C)** and **(E)** are juveniles high above the river bank, at least 3–4 m high in the vegetation of the understory, and juvenile **(C)** is sleeping. **(D)** A female Harlequin Toad sleeping on the vegetation five m above the river.

= 13.159, 1 df, P = 0.004). When rains were abundant, the toads were found sleeping or resting high on the vegetation.

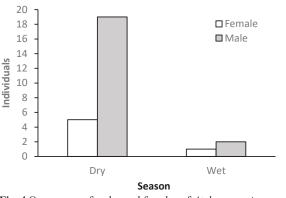
A difference in activity patterns was also observed between males and females of this species (Fig. 8, G = 15.247, 1 df, P = 0.002). During this study, females were mostly seen basking on river boulders, or on top of vegetation above the river; while males were more passive, with significantly predominant activity in the leaf litter and crevices of the riverbed, hiding, or resting (Fig. 3A-B). On the other hand, the activity for juveniles of this species mostly involved sleeping or active movement for feeding. Juveniles were never observed basking or hiding like the adults (Fig. 9, G = 9.934, 1 df, P = 0.019).

The Harlequin Toads studied here seemed to be very faithful to their sleeping grounds. On five separate occasions, three individuals, including a juvenile (Fig. 3C), were seen sleeping on the same leaf during the night. Besides the observed nocturnal feeding behavior, almost

all the individuals seen during the night were sleeping in the vegetation high above the river. Activity started before dawn. One of these individuals was monitored during the night, and its activity started before 4:30 AM. Toads sleeping high in the vegetation (Fig. 3 C,D) made their way down to lower levels of the ground as the morning warmed up, with some of them remaining on the vegetation during the day (Fig. 2E).

#### **Discussion**

Based on Savage's (1972; 2002) reviews of *A. varius* distribution in Costa Rica and Panama, it is clear that most of the recently re-discovered populations have been found in areas of their historical distribution. The report by Ryan et al. (2005) for Fila Chonta, 10 km northwest of Quepos, matches the premontane distribution suggested for southwestern Costa Rica, where the nearest site identified by Savage (1972) is Barú. The account by Barrio-Amorós and Abarca (2016) for the Uvita region



Female

Male

Separate

Soil/rock

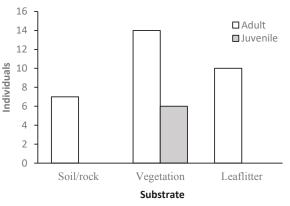
Soil/rock

Soil/rock

Substrate

Fig. 4 Occurrence of males and females of *Atelopus varius* according to season.

Fig. 5 Substrate preference of Atelopus varius according to sex.



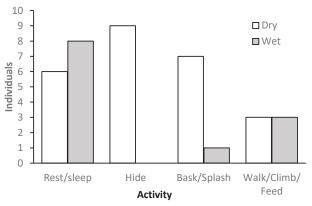
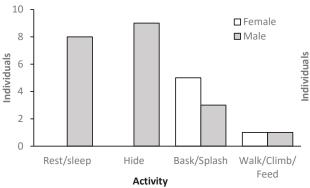


Fig. 6 Substrate preference of Atelopus varius according to age.

Fig. 7 Activity pattern of Atelopus varius according to season.



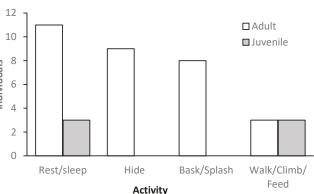


Fig. 8 Activity pattern of Atelopus varius according to sex.

Fig. 9 Activity pattern of Atelopus varius according to age.

matches with that reported by Savage (1972); and finally, the rediscovery in Las Tablas by González-Maya et al. (2013), brings hope for the survival of the toads near Cotón, also previously identified by Savage (1972). These findings confirm that some populations in the Pacific versant have managed to escape the decline while persisting in the same localities, at least so far. However, evidence from our research, with the discovery of a new locality, offers augmented hope for species recovery. We hypothesize that the behavior of this population could have helped to reduce the risk of exposure to *Bd* infection and also to allow a possible change in the historical distribution of this species.

These results, and the similarity between both the scientific and community accounts discussed below, suggest that *A. varius* distribution could have

changed to penetrate higher in the montane regions of the Talamanca Mountain Range in the Pacific Slope. Despite the lack of specimens for the area and the lack of a collection archive, previous evidence has recorded A. varius, specifically this ecomorph, in the San Isidro del General region at 704 m asl (Savage 1972), with no other accounts found by the authors for the ASBC area (from 1,100–1,500 m asl). Furthermore, Savage (1972) refers to this toad as a predominantly premontane frog with penetration into lower montane zones only at six localities in Costa Rica, none of which includes the area of this study—the closest area identified is Pérez Zeledón at 704 m asl (Savage 1972). Taking Savage's (1972) account into consideration, the discovery of this population in montane areas of the ASBC suggests this is an undocumented locality. This scientific account is

congruent with interviews of highly experienced locals who do not report or remember this species being present at elevations higher than 600–700 m asl.

Long-time residents of what is now the Alexander Skutch Biological Corridor remembered that the Harlequin Toad had been abundant in the area some 50 years ago, but that this toad had not been reported again for decades, until now. During initial interviews, local observers showed good competency to immediately identify Harlequin Toads. One of them acknowledged the remarkable altitudinal change for this species over the years, "this frog was very common at 600 m over twenty years ago, now it is over 1,000 m. It was never here, and it has come higher in the mountain. It's the first time I see it again in all these years" (Ramón Mora). This citizen testimony along with Savage's (1972) account could point to a possible geographic displacement. When considering the possible displacement, we acknowledge that reduced biodiversity exploration in the area could explain how this population went under the scientific radar for so long; yet, with no valid reason, this assumption would disregard the confidence, skills, experience, and traditional knowledge of the inhabitants that are quite confident the frog was not present over 700 m asl in the past. If this species were not so easily identified, with such a reduced risk of misidentification in the field, we would consider this possibility. This possible change in distribution might be helping to facilitate its continuing survival through some environmental stressors, such as climate change and habitat loss. It might also increase the risk of Bd exposure, and clearly more research is needed to corroborate this possible explanation, exploring the trade-offs between adaptation to environmental changes and Bd exposure.

The reappearance of *A. varius* in this region could be the result of several possible factors. One hypothesis is that establishing the biological corridor in 2005, and subsequent efforts to enhance ecological connectivity between forest patches, have allowed for the recovery of habitats that previously served as niches for relict populations on the verge of extinction, allowing those populations to recover and relocate, possibly pushed by environmental changes. Another potential explanation has to do with the possible emergence of resistance among these threatened relict populations of *A. varius* to the previously devastating *Bd* fungal disease (Perez et al. 2014).

To expand on the emergence of resistance to the *Bd* infection hypothesis, we present two critical remarks that contribute to the natural history of the species and might be related to this population's survival. First, the toads in the area are seen less often on the splash region of the river, and more often on the vegetation, regardless of seasonality. Second, the typical sedentary behavior in the mossy rocks near the splash zone associated with this species (Pounds and Crump 1994) is not as typical in this location. The toads are not so sedentary, and basking seems to be more important here than capturing moisture in the splash zone. Many authors, including Pounds and Crump (1994), recognize that this species depends on the moisture of the splash zone and individuals tend to aggregate in the waterfall splash with the progression

of the dry season. The experiences of working with this population complement this information. Here, toads are regularly seen in the foliage, tree trunks, and the leaf litter, and not on the splash zone, only seven of the 37 registered encounters were found on the river rocks or crevices. The basking behavior is as important, or perhaps more so, than moisture-seeking behavior. The toads appear to move to open areas on the river bank or the exposed vegetation where there is more solar radiation; and the selection of such areas was more frequent during the warmest time of the morning, especially on colder days. This selection could also explain the difference of activity during dry and wet seasons, where during the wet season the toads were "lazier" in the early hours of the morning when it was cold and they were found closer to the river when the day was warmest. These individuals were much less sedentary than previously reported, engaging in significant daily movements. Anurans sleeping on the vegetation 3–4 m above the river move during the morning down to the river bank; explaining why this species has been hard to locate in the area, as researchers might focus solely on crevices, rocks, and roots as reported by most of the literature. Understanding this was the key to finding the juveniles that were far from the river and much higher than eye-level.

In other words, the natural history remarks noted above suggest that A. varius spends less time closer to the river and more time in open areas basking; which in turn might be linked to available solar radiation and moisture control. Temperature (Woodhams et al. 2003) and moisture (Johnson et al. 2003) have been suggested as two important environmental factors influencing the growth and survival of *B. dendrobatidis*. So this behavior could explain why this toad survived in an area cataloged as highly likely for the existence of Bd (Puschendorf et al. 2009). Furthermore, the negative results of the Bd test support this theory. The documented absence of infection in these Harlequin Toads, a highly susceptible species in an area identified with a high probability for occurrence of Bd, allows us to hypothesize that spending less time closer to the river and more time in open areas basking, could help the toads of this population survive by reducing infection risk. The findings of Woodhams and colleagues (2003) support our hypothesis; they present evidence that short periods of high body temperature can eliminate the pathogen from its hosts, with experiments suggesting that normal thermoregulation can clear frogs of chytrid infection (Woodhams et al. 2003). We hope this account can help provide a better understanding of the behavior of Atelopus, as this behavior might hold clues for the frog's resistance to Bd or climatic changes, and can also help to locate new populations.

#### **Conclusions**

García-Rodríguez et al. (2012) ask "Where are the survivors [of] relict populations of endangered frogs in Costa Rica?" In this research, both community members and scientists have contributed to answering this question. Moreover, with A. varius being among the most endangered of all amphibian species (La Marca et al. 2005), it is certain that the discovery of the Harlequin

Toad in the Alexander Skutch Biological Corridor makes the conservation efforts in this biological corridor of critical importance. This population is particularly relevant because of ongoing attempts by private companies to obtain permits for building a hydroelectric plant that would dam the two major streams in the ASBC, destroying the habitats successfully recovered during a decade of conservation efforts in the corridor. The reappearance of the Harlequin Toad, currently classified as Critically Endangered (IUCN 2015), lends supporting evidence to the effectiveness of this habitat recovery.

The active involvement of citizens in the discovery of A. varius in the ASBC points to the importance of strengthening the citizen science component to further the knowledge base regarding this and other species in the area. The historical role of citizen science in ecological research has been generally overlooked, despite its significant contributions (Miller-Rushing et al. 2012; Dickinson et al. 2012). The opportunistic and widespread nature of these sightings suggests that strengthening citizen science approaches can help to maximize resources and opportunities for encountering this and other rare, endangered species. Citizen science creates a nexus between science and education that expands the frontiers of ecological research and public engagement (Newman et al. 2012). After all, without the enthusiastic and curiosity-filled reports from the community, the reemergence of this rare and endangered species might have gone completely undetected by the scientific community. Moreover, this participatory process can generate new meanings and values for herpetofauna in the community, leading to improved human behaviors directed at the protection of these and other vulnerable species.

In addition to continuing to monitor for the presence of *A. varius* in the ASBC, the community initiative that has already documented the reappearance of the Harlequin Toad in the biological corridor will also be useful in providing further data to help answer pending questions regarding the impacts of climate change, fungal disease, and pesticides on these fragile populations.

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