NATURE NOTES

Amphibia: Anura

Another surviving population of the Critically Endangered *Atelopus varius* (Anura: Bufonidae) in Costa Rica

Neotropical harlequin toads of the genus *Atelopus* are among the most threatened anurans on the planet. A substantial amount of research has been conducted to raise awareness about declining populations of amphibians worldwide, and members of this genus have become flagship species for conservation initiatives, and also have been used in several environmental and pathological studies (La Marca et al., 2005; Rueda Almonacid et al., 2005). Currently, 96 species of *Atelopus* are recognized (Frost, 2015), and most taxa have been assessed into the following categories by the International Union for the Conservation of Nature (IUCN, 2015): three = Extinct (EX); 72 = Critically Endangered (CR); eight = Endangered (E); five = Vulnerable (VU); and three = Data Deficient (DD).

The following four species of *Atelopus* have been reported from Costa Rica (Savage, 2002; Savage and Bolaños, 2009; Köhler, 2011): *A. chirripoensis* (CR), *A. chiriquiensis* (CR), *A. senex* (CR), and *A. varius* (CR). *Atelopus chirripoensis* is known from a single specimen collected in Costa Rica in 1980, and has not been seen since; *A. chiriquiensis*, with a distribution in Costa Rica and Panama, has not been encountered in Costa Rica since 1996, and no records from Panama are available since the late 1990s; and *A. senex*, a Costa Rican endemic, has not been found in the country since 1986; thus, these three species appear to be extinct (IUCN, 2015). The distribution of *A. varius* was more extensive, however, as this species was known to occur from northwestern Costa Rica to western Panama; in Costa Rica, it was an abundant element of the lowland and midland forests in all the cordilleras of the Pacific and Caribbean versants at elevations from 16 to 2,000 m (Savage 1972, 2002). Over 100 populations of *A. varius* were known from Costa Rica (Pounds et al., 2008), but in the 1980s and early 1990s populations began to decline dramatically, including from such known well-known localities as Monteverde (Pounds and Crump, 1994). Lips and Papendick (2003) and Lips et al. (2006) noted a wave of the highly pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) as the cause, and indicated that it was heading eastward from Costa Rica to Panama; soon after, many populations of several species of amphibians, including *A. varius*, were in severe decline or had vanished in the region. By 1996 *A. varius* was considered extirpated from Costa Rica, but in 2004 a glimmer of hope came when a population was discovered in the Pacific lowlands at Fila Chonta, ca. 10 km NW of Quepos, Provincia de Puntarenas (Pounds et al., 2008). Surveys in 2005, however, yielded no additional populations (Ryan et al., 2005). More recently, González-Maya et al. (2013) reported the rediscovery of a breeding population of *A. varius* on private property at the Las Tablas Protected Zone near San Vito, Coto Brus, Provincia de Puntarenas, at an elevation of 1,300 m; interestingly, surveys conducted from 2002 to 2004 had failed to find this species in the area (Santos-Barrera et al., 2008). Solano-Cascante et al. (2014) then reported finding an individual of *A. varius* close to Buenos Aires, Provincia de Puntarenas, at an elevation of 840 m; the individual, however, either died or was sacrificed to examine its stomach contents. In Panama, Hertz et al. (2012) reported seeing four individuals of *A. varius* at Cerro Negro, Provincia de Veraguas; none appeared to be sick, and a histological examination showed no evidence of *Bd*. Subsequently, Perez et al. (2014) found *A. varius* persisting at multiple sites in western Panama, although evidence of *Bd* was found on other anuran species.

Herein we report the discovery of another surviving population of *A. varius* from the Pacific versant of Costa Rica, at an elevation of ca. 400 m; we indicate the general area as Uvita de Osa, Provincia de Puntarenas, but do not provide the exact locality to discourage visitors to the area because of the dangers from illicit collecting or the potential introduction of disease.

In early June of 2015, one of us (CLBA) was told that some "yellow and black frogs" had been sighted along a stream in the vicinity of Uvita de Osa. After interacting with residents from the area and showing them photographs, CLBA contacted the guard in charge of the private reserve where the animals were seen and asked for permission to visit the property. On 26 June 2015 at ca. 1300 h, accompanied by a friend and the guardian of the reserve, after walking for 90 min in rainforest without following a trail we arrived at a stream used to provide water for the property (Fig 1); several high waterfalls prevented us from hiking to the site by walking upstream. Soon after reaching the stream, we observed nine adult male *A. varius*, as well as one dead, decomposing individual lying on a wet rock (Figs. 2–6). CLBA collected the carcass and performed a skin swab on one live individual; each individual was handled with a new set of gloves. He later sent the specimen and skin swab to JA to conduct a test for *Bd*. A number of similar streams and creeks are found in the area of Costa Ballena and Corridor Paso de la Danta, but despite intensive fieldwork in the area CLBA has not encountered another population of *A. varius*.

Ecomorphs and Variation

As its specific name implies, *Atelopus varius* is among the most variable of anurans. Savage (1972), in his seminal work on Costa Rican *Atelopus*, recognized 13 ecomorphs of *A. varius*, of which the one at Uvita de Osa approaches patterns A and H (p. 85: fig. 8), which later were modified as patterns F and J (p. 192: fig 7.19) in Savage (2002). The color pattern of the Uvita de Osa population is as follows: ground color pale to vivid yellow, with a dorsal pattern of black blotches, inverted and sometimes interrupted chevrons, and spotting (Figs. 2A, C, D, E, F, and 3A, B, C); a single individual contained irregular red-orange dorsal markings on the yellow ground color (Fig. 3D); upper arm yellow, followed by a long black band connecting the upper and lower arms, in which a spot on the elbow is yellow; another black band extends from above to the lower portion of the wrist; the upper portion of the hand is yellow, and the fingers mostly are black but with varying amounts of yellow; the black banding on the thighs and legs is more evenly dispersed, although the bands often are irregular in shape and contain yellow spotting; and, the venter of one individual (Fig. 2B) is white except for isolated black or brown spots of varying sizes, with the undersides of the hands, feet, vent, and posteroventral portion of the thighs orange. For individual variation in color pattern, see Figs. 2 and 3.

Fig. 1. Habitat of the population of *Atelopus varius* along a short and narrow (< 5 m) stream in Uvita de Osa on the Pacific versant of the Cordillera Brunca, Provincia de Puntarenas, Costa Rica.

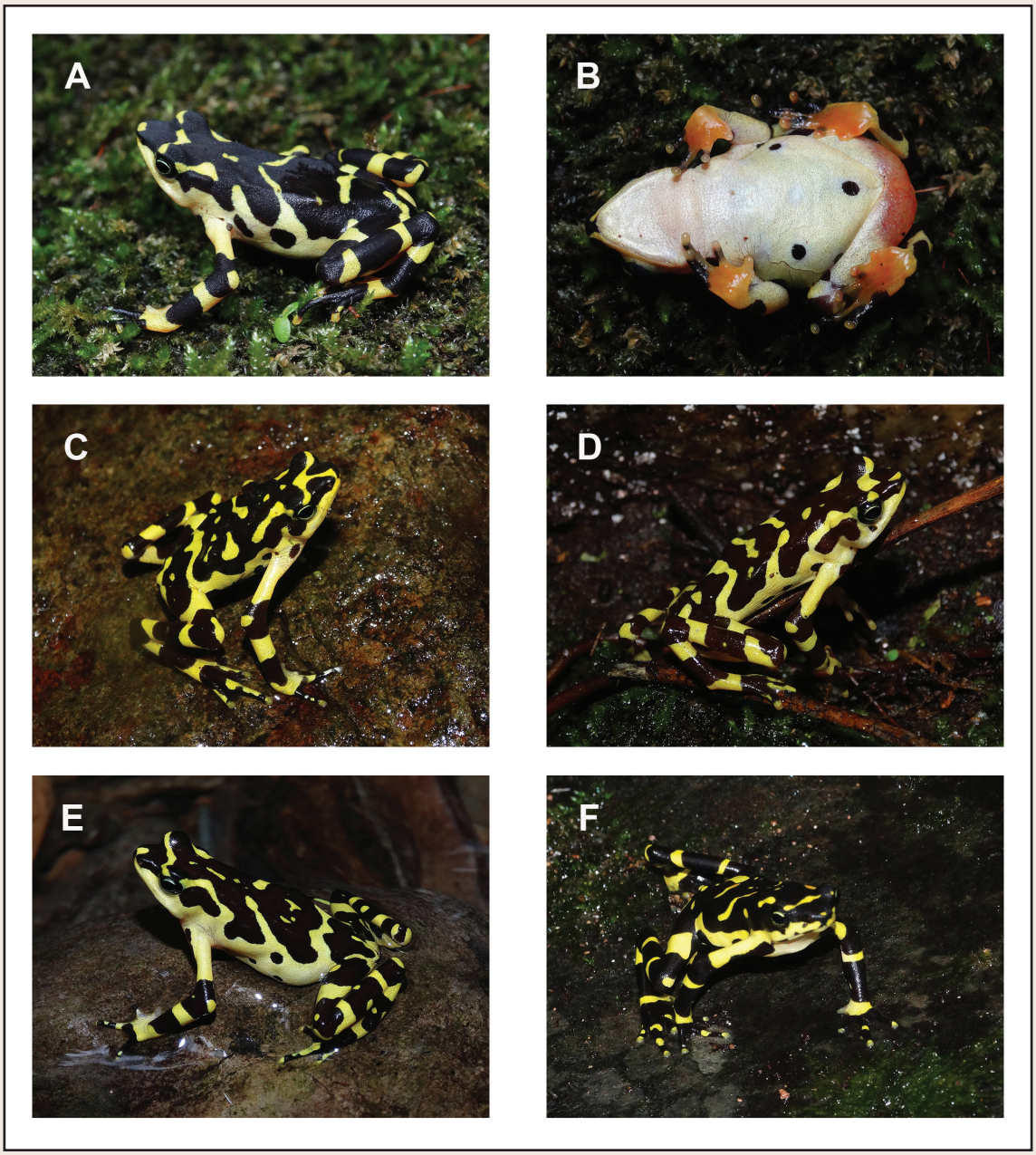

Fig. 2. Interpopulational variation in the population of *Atelolus varius* at Uvita de Osa.
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Laboratory Results

We tested two *Atelopous varius* for the presence of *Bd*, the one found dead (Fig. 5) and the body placed in 96% ethanol, and the live individual from which a skin swab was taken before it was released. We used a cotton swab, following the protocols of Kriger et al. (2006). Following the methods outlined by Hyatt et al. (2007), we extracted DNA from the swab with PrepMan Ultra (Applied Biosystems, Carlsbad, California), and analyzed the samples using the standard real-time quantitative polymerase chain reaction assay. The DNA extraction and diagnostic real-time PCR was conducted at the at the Centro de Investigación en Estructuras Microscópicas, Universidad de

Costa Rica, following the standardized procedures in Boyle et al. (2004) with the following exception: the nucleic acids were extracted using 50 µl PrepMan, a negative control (H2O), a positive control swab dipped in a broth of *Batrachochytrium dendrobatidis* culture from CostaRican strainJGA01. For the PCR reaction we used a TaqMan® Gene Expression Assay (Applied Biosystems, Carlsbad, California) mix that contained TaqMan MGB probe mark with FAM™ and the two primers ITS1-3 Chytr and 5.8S Chytr.

To ensure the integrity of the results, we ran the samples in triplicate. We determined a sample as positive when the target sequence amplified in the three replicate wells of the assay. We used an internal positive control (IPC) to detect inhibitors (Hyatt et al., 2007), and an Applied BioSystems Prism 7500 Sequence Detection System to test for the presence of *Bd*. This PCR was run with samples of other projects, but positive extraction of *A. varius* was maintained at -20°C, for posterior triplicate reruns of positive samples, due to limited sampling. When more samples are available, the quantification of DNA equivalent zoospores numbers will be performed. The IPC was running well in the six replicates, three replicates of the dead specimen sample tested positive for *Bd*, and the three replicates from the living specimen tested negative. So the prevalence of 50% is due to the low sample size.

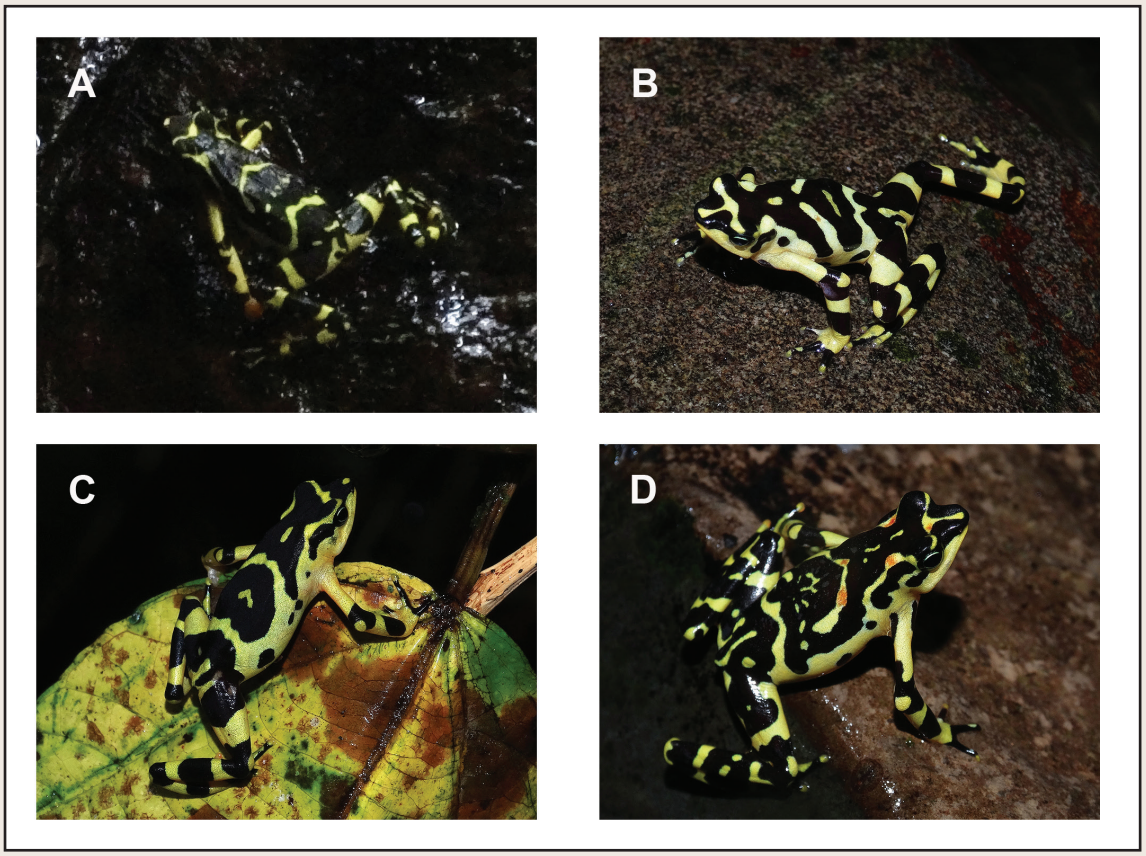

Fig. 3. Interpopulational variation in the population of *Atelolus varius* at Uvita de Osa. © César L. Barrio-Amorós

Observations of the Natural Habitat

The stream where this population of *Atelopus varius* was found is located within a private reserve where no one is allowed to enter without permission. This situation offers some guarantee of protection, at least against threats by humans, as the owner is not planning on developing the area and strongly defends it against unwanted visitors.

The stream is located in primary forest. We walked a distance of about 300 m up and down the stream, from 1430 to 1600 h. We observed only one *A. varius* under direct sunlight (Fig 4; the same individual as in Fig 2C), and the remaining individuals were found in the shade, but mostly exposed; five individuals were situated on moss-covered boulders in the stream, and three were somewhat camouflaged because they were lying on wet, black rocks and seen only when they jumped away. The level of crypsis shown by this morph was notable, especially when animals on the wet, black boulders remained still. Curiously, individuals become visible as soon as they attempt to escape, after which they walked away slowly or quickly, or took short hops. We saw no females, tadpoles, or juveniles.

Fig. 4. An *Atelopus varius* from Uvita de Osa basking in sunlight. To obtain this photograph the individual was handled with gloves and photographed in the spot where it was originally seen. © César L. Barrio-Amorós

The presence of a dead toad that tested positive for *Bd* among apparently healthy individuals (one of which tested negative) makes us suspect that at this elevation and environmental conditions the immune system of sick individuals can be suppressed but later attacked by the *Bd*, which can become a deadly pathogen (as reported by Ellison et al., 2014). The typical pattern of skin epidermal infection caused by the effects of *Bd* is shown in Fig. 5, so it is plausible that *Bd* was the cause of death. A *Bd* test on all the amphibian species in the area might reveal valuable data on the presence or absence of *Bd*, and how this pathogen affects other species.

The surviving populations of *A. varius* in Panama (Hertz et al., 2012; Perez et al., 2014) and Costa Rica (indicated here) make this species an excellent candidate for studying and monitoring the prevalence of *Bd* and other diseases in these anuran populations.

Fig. 5. An individual of *Atelopus varius* found dead and rotting at Uvita de Osa, and collected for further analysis.
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Conservation Remarks

Savage's (2002) map (p.189) shows 66 populations of *Atelopus varius* in Costa Rica, and Pounds et al. (2008) were aware of over 100. This population of *A. varius* is the fourth known to have survived in Costa Rica, but only the second viable one after the one at Las Tablas (González-Maya et al., 2013). No information on the survival of the Quepos population has been reported since the publication of Ryan et al. (2005), or on the survival of the Buenos Aires population (Solano-Cascante et al., 2014). Concerted efforts are necessary to study the surviving populations of *A. varius*, as well as to search for others. *Atelopus varius* is assessed as Critically Endangered by the IUCN, and therefore can be regarded as a flagship species in attempting to conserve the general areas where it survives.

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