THE POPULATION DECLINE OF *ATELOPUS QUIMBAYA* (ANURA: BUFONIDAE) IN THE ANDES OF COLOMBIA

EL DECLIVE DE LAS POBLACIONES DE *ATELOPUS QUIMBAYA* (ANURA BUFONIDAE) EN LOS ANDES DE COLOMBIA

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Abstract.— The Quimbaya toad, *Atelopus quimbaya*, is a small bufonid endemic to the Central Andes in Colombia and it is categorized as Critically Endangered by the International Union for Conservation of Nature -IUCN-. Here, we report historical sightings and recent extensive surveys conducted by us in three historical localities where the Quimbaya toad was reported. The last observations of the Quimbaya toad were between 1994 and 1997 in these three localities. Although we conducted three extensive surveys during several years (2003–2004, 2010–2011, and 2011–2012), we could not detect any individual, nor eggs or tadpoles. We discuss some potential drivers of population declines and suggest that this species be declared as Possibly Extinct. However, we believe that additional surveys should be conducted with the aim to find new populations in other nearby localities and also to test for the presence and prevalence of chytrid fungus in the region. Finally, we do not have sufficient evidence to firmly establish which factors drove the decline of this species.

Keywords.— Colombia, Extinction, Harlequin frog, Amphibians, Andes.

Resumen.— El sapo Quimbaya, *Atelopus quimbaya*, es un bufónido pequeño endémico de los Andes en Colombia y categorizado como Críticamente Amenazado por la Unión Internacional para la Conservación de la Naturaleza -IUCN-. Aquí, reportamos los avistamientos históricos y muestreos recientes realizados por nosotros en tres localidades donde el sapo Quimbaya fue registrado en el pasado. Los últimos registros del sapo Quimbaya fueron en 1994–1997 en estas tres localidades. Aunque realizamos muestreos extensivos recientemente (años 2003–2004, 2010–2011 y 2011–2012) ningún sapo Quimbaya fue detectado, así como tampoco huevos o renacuajos. Discutimos factores de riesgo de extinción potenciales que condujeron al declive poblacional de esta especie en la región y sugerimos que esta especie sea declarada como probablemente extinta. Sin embargo, recomendamos que los monitoreos biológicos continuen en la región con el fin de registrar poblaciones en localidades nuevas, asimismo para confirmar la presencia y prevalencia del hongo quitridio en la región. No tenemos evidencia sustancial para establecer los factores que probablemente condujeron al colapso poblacional de esta especies.

Palabras clave.— Colombia, Extinción, Rana arlequín, Anfibios, Andes.

INTRODUCTION

The Neotropical toad genus *Atelopus* (Bufonidae), with 96 currently recognized species, is one of the most diverse genera of vertebrates and is globally threatened as a group (Frost, 2017; IUCN, 2017). According to the IUCN Red List, there are 65 *Atelopus*

species classified as Critically Endangered with declining population trends, as well as 13 Endangered and four Vulnerable (IUCN, 2017). Population declines and extinction risk in *Atelopus* are caused to the combination of multiple stressors including habitat loss, fragmentation, pathogens (e.g., *Batrachochytrium dendrobatidis -Bd-*), and climate change (Pounds, 2001; La Marca et al., 2005; Ron et al,. 2003). However, it is unclear why a few species remain stable whereas many others have not been seen in many years in sites with similar anthropic pressures and presence of *Bd* (Flechas et al., 2012; 2017; Rueda-Solano et al., 2015; 2016).

The conservation status of *Atelopus* species in Colombia is critical because approximately 80% of the 44 species occurring in the country face a serious extinction risk (Gómez-Hoyos et al., 2014; IUCN, 2017). Forty-one of 44 *Atelopus* species occur in the Andes region, but relatively recent field surveys have been able to obtain very few records for a few species (La Marca et al., 2005). Some of these recent occurrence records for Andean *Atelopus* in Colombia are restricted to the Sierra Nevada de Santa Marta (*Atelopus carrikeri*, *A. laettisimus*, and *A. nahumae*; Rueda-Solano et al., 2015, 2016; Pérez et al., 2017) and the eastern Andes (*A. subornatus*; Enciso-Calle et al., 2017). Despite extensive fieldwork in some of the type localities for Andean *Atelopus*, recent surveys have not resulted in recent sightings or discovery of new populations (e.g. *Atelopus quimbaya*; Gómez-Hoyos et al., 2017).

The Quimbaya toad, *Atelopus quimbaya*, is endemic to the Andes of Colombia and is known from only three localities on the western slope of the Central Andes. This species has been categorized as Critically Endangered by the IUCN due to its reduced geographical range, the potential presence of *Bd* in the area, and the confirmed presence of the invasive rainbow trout (*Oncorhynchus mykiss*) (IUCN SSC Amphibian Specialist Group, 2017). Despite intensive effort to find A. quimbaya in these three localities we have no recent records, and the last records date back to 1994-1997 (J. D. Lynch and M. C. Ardila field notes; GHK unpublished data).

Here, we document the historical sightings and surveys conducted by us in the three localities where the Quimbaya toad was recorded in the past. We discuss potential extinction drivers in the region including the presence of the invasive rainbow trout and the potential presence of the chytrid fungus (*Bd*). We recommended that this species should be declared as possibly extinct. We also suggest that it is necessary to conduct further surveys in nearby localities with the aim to discover potential undetected populations of this species and test for the presence and prevalence of the chytrid fungus in this region. Also, we recommend a systematic survey for other Andean *Atelopus* species with similar characteristics (e.g., *A. chocoensis*, *A. pictiventris*) that lack information about their current population status.

MATERIAL AND METHODS

Study area

We conducted an extensive literature review and specimen checking to compile all localities where *A. quimbaya* has been reported. Two localities are located within the buffer area of the Los Nevados National Park, and the other one is located 11 km north (Fig. 1). These localities are: La Montaña Nature Reserve (henceforth La Montaña) in the Quindío River watershed (Municipality of Salento, Quindío; between 4.45044°N, 75.6422°W and 4.65063°N, 75.45684°W), Ucumarí Regional Natural Park (henceforth Ucumarí) in the Otún River watershed (Municipality of Pereira, Risaralda; between 4.7007°N, 75.53469°W and 4.71694°N, 75.48767°W), and Río Blanco Forest Reserve, in the Hoyo Grande River adjacent to Las Palmas in La Navarra, Río Chinchiná watershed (Municipality of Manizales, Caldas; 5.090588°N and 75.395538°W), between 2200 and 2900 m of elevation (Ruiz-Carranza and Osorno-Muñoz, 1994; Kattan, 2005) (Fig. 1).

Historical and recent surveys

We compiled historical records and ecological data from voucher specimens of the Quimbaya toad deposited in the herpetological collection in the Instituto de Ciencias Naturales (ICN) at the Universidad Nacional of Colombia. These specimens were collected between 1989 and 1994 at La Montaña and Ucumarí, the type localities for the Quimbaya toad. During a trip between January and October 1996, additional sightings were made by GHK in Ucumarí. These sightings were made in the context of an ornithological study, thus the data on the presence of the Quimbaya toad were obtained opportunistically.

In the last two decades, we conducted three intensive systematic surveys directed to obtain records of the Quimbaya toad in the three historical localities: Ucumarí, La Montaña, and Río Blanco (Fig. 1). The first survey was conducted from September 2003 to February 2004 (except during December 2003) in Ucumarí. We conducted surveys each month in six streams to search for adult individuals and tadpoles. In addition, we conducted diurnal visual encounter surveys in the forest and installed 120 pitfall traps at 2200 m, 2400 m and 2600 m of elevation (40 traps at each elevation). These traps were distributed in the forest (20 traps in each elevation) and near streams (20 traps at each one). The second systematic survey was conducted in four localities (Ucumarí, La Montaña, La Picota, and Acaime;

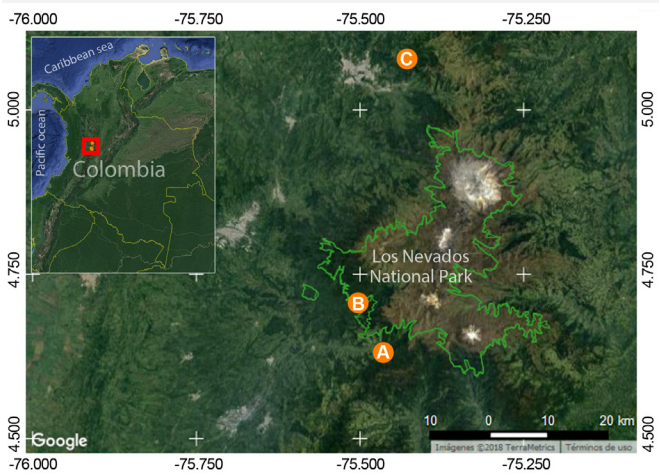

Figure 1. Historical distribution of the Quimbaya toad (*Atelopus quimbaya*) in the Central Andes of Colombia. A) La Montaña Nature Reserve, Río Quindío watershed (Salento, Quindío); B) Ucumarí Regional Natural Park, Río Otún watershed (Pereira, Risaralda); C) Río Blanco Forest Reserve, Río Chinchiná watershed (Manizales, Caldas).

Figura 1. Distribución histórica del sapo Quimbaya (*Atelopus quimbaya*) en los Andes Centrales de Colombia. A) Reserva Natural La Montaña, cuenca del Río Quindío (Salento, Quindío); B) Parque Regional Ucumarí, cuenca del Río Otún (Pereira, Risaralda); C) Reserva forestal Río Blanco Forest, cuenca del Río Chinchiná (Manizales, Caldas).

Fig. 1) between August 2010 and February 2011 in an elevational range between 2200–2800 m. In each locality we searched in twelve linear transects of variable length (mean 202 m ± 53.08) along eleven streams and used visual encounter surveys. The third survey was conducted between November 2011 and May 2012 in Río Blanco, as well as in other rivers in the adjacent area (La Guerra, Las Delicias, La Navarra, Pinares, Boquerón, Hoyo Grande and Martinica). We established several linear transects in these rivers, and we conducted visual encounter surveys in nearby wooded forested areas between 2600 and 3200 m of elevation. In all localities, we conducted detailed searches during the day (7:30 h - 17:30 h), checking under fallen trunks and rocks on edges of streams, as these correspond to microhabitats where Quimbaya toads have been found in the past (Ruíz-Carranza and Osorno-Muñoz, 1994).

Historical climate

To analyze historical climatic trends related to temperature, cloud cover and precipitation in the geographic range of the Quimbaya toad (Fig.1), we compiled data from the CRU-TS 3.10.01 Historic Climate Database for GIS (Harris et al., 2014; http://www.cgiar-csi.org/data/uea-cru-ts-v3-10-01-historic-climate-database) between 1948 and 1997. CRU TS3.10 contains monthly observations from meteorological stations across the world (Harris et al., 2014). We extracted the climatic information for the entire polygon of the distribution of the Quimbaya toad. We preferred to use this coarse-grain time-series database because we are more interested in evaluating regional trends in climatic conditions instead of more fine-resolution temporal trends from the CHELSEA database. We analyzed five variables: precipitation, cloud cover, maximum temperature, minimum temperature, and diurnal temperature range. Temperature variables are in °C*10. We downloaded the raster for each variable and extracted the data. We plotted box-and-whisker plots for each variable per year or decade (i.e., ten years prior to the last occurrence record of the Quimbaya toad: 1988-1997; five decades prior the last record: from 1948–1597 to 1988–1997) (data analysis available on: https://github.com/biodiego88/Material_suplementario_pub/tree/master/Quimbaya_toad_extinction_RevistaLatAmHerpetologia). Notches were drawn to detect differences among years or decades per variable (Chambers et al., 1983). We conducted data analyses using raster and rgdal packages for R language (R Core Team, 2017).

RESULTS

Historical and recent surveys

During five field trips to Ucumarí between 1989 and 1994, a total of 35 Quimbaya toad individuals were collected (Fig. 2 and 3). In two surveys conducted in 1990 and 1991, the relative abundance of the Quimbaya toads was 0.33 and 1.67 individuals per hour per person, respectively (based on field notes deposited in the herpetological collection of the ICN by J. H. Restrepo). Additionally, 18 specimens were obtained in one field trip to the Quindío River watershed in 1990. Tadpoles of the species were uncommon, with records at Las Delicias River in Ucumarí (ICN35776 and ICN35772, 23 June 1994; ICN35777, 26 June 1994), and La Pastora creek, a tributary of Otún River (ICN27301, 15 February 1991; ICN33217, 24 January 1993). Occasionally, some eggs were found in Monteloro River in Salento (ICN35774, undated) and Otún River tributaries (ICN27300; 19 November 1990; ICN27302, 15 February 1991).

In opportunistic observations at Ucumarí between 1994 and 1996 (GHK, *pers. obs.*), toads were recorded in 18 out of 34 monthly visits. All these records were obtained between 2200 and 2600 m. In four months between January and October 1996, one to six amplectant pairs were observed near streams (Fig. 2A), and females had prominent abdominal egg masses. Additionally, some egg masses were observed in the water (Fig. 2B). Other non-breeding records involved solitary individuals spread throughout the forest floor. The last record of the Quimbaya toad was on May 27, 1997, when an individual was found in the Río Blanco reserve, Las Palmas, Navarra farm near El Hoyo Grande

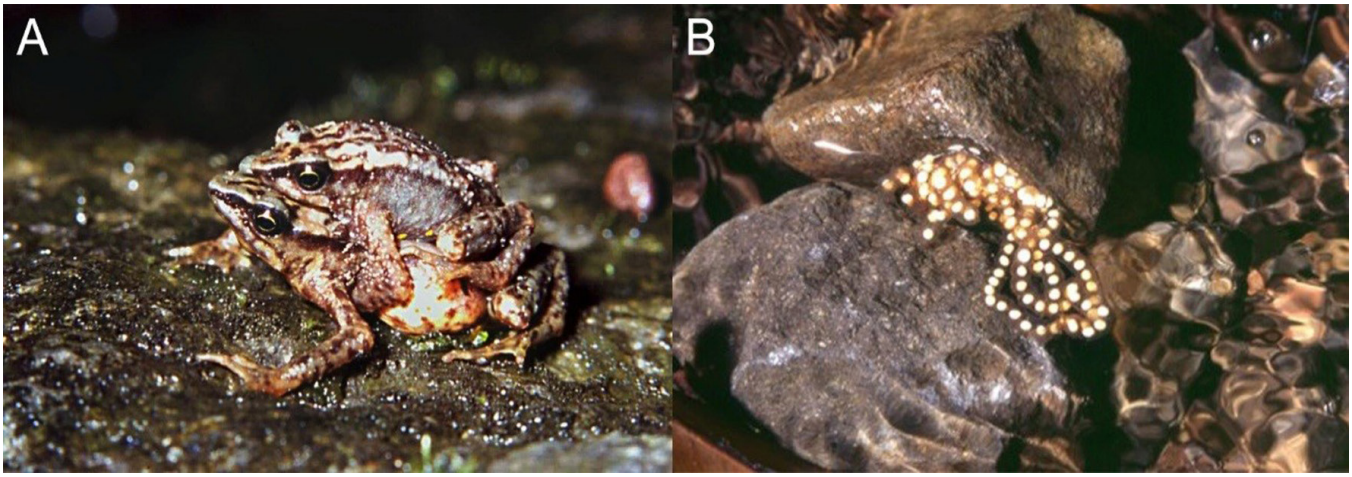

Figure 2. Amplectant pair (A) and eggs (B) of the Quimbaya toad (***Atelopus quimbaya***) recorded at Ucumarí Regional Natural Park (Las Peñas, trail between El Cedral and La Pastora, 2200 m) during 1996 (photos: Gustavo Kattan)..

Figura 2. Pareja en amplexo (A) y huevos (B) del sapo Quimbaya (***Atelopus quimbaya***) registrado en el Parque Natural Ucumarí (Las Peñas, camino entre El Cedral y La Pastora, 2200 m) durante 1996 (fotos: Gustavo Kattan)..

River (Fig. 3D).

In the surveys conducted between September 2003 and February 2004 we did not find toads or tadpoles. Furthermore, in surveys conducted between 2010 and 2011, we accumulated a sampling effort of 143 hours/person (with two persons sampling in each site), but no Quimbaya toads were detected, nor eggs or tadpoles. Although we did not record *A. quimbaya* during these surveys, we detected 10 amphibian species including *Pristimantis thectopternus*, *P. permixtus*, *P. uranobates*, *P. boulengeri*, *P. piceus*, *Pristimantis* sp., *Dendropsophus columbianus*, *Hyloscirtus larinopygion*, and *Colostethus fraterdanieli*. Moreover, surveys carried out between November 2011 and May 2012 in Río Blanco were unsuccessful in finding the Quimbaya toads, but other amphibians were reported: *P. permixtus*, *P. uranobates*, P. *boulengeri*, *P. piceus*, *P. maculosus*, *Centrolene buckleyi*, and *Hyloscirtus larinopygion*. Another species that was reported sympatric with *A. quimbaya* was *P. alalocophus* (abundant at that time), but our recent sampling has also failed to find it at any of these localities.

Historical climatic variability

We found no evidence of changes in precipitation during the last ten years and five decades before the last record of the Quimbaya toad (1996–1997; Fig. 4A, B). Cloud cover did not change significantly before the last decade (Fig. 4C), but we detected an increase in the last three decades (Fig. 4D). By contrast, we noted a decrease in maximum temperature (Fig. 4E, F) and an increase of minimum temperature (Fig. 4G, H), resulting in a decrease in temperature range. The range of diurnal temperature also decreased during the last ten years and five decades prior to the last record of the Quimbaya toad (Fig. 4I, J).

DISCUSSION

Current population status

Atelopus quimbaya is categorized as Critically Endangered in the IUCN red list due to its restricted distribution, presence of invasive species in its natural habitat and the presence of *Bd* in the Andean region (IUCN SSC Amphibian Specialist Group 2017). Historical data for *A. quimbaya* are scarce and therefore do not allow us to estimate population size with any confidence. However, historical data suggest that the Quimbaya toad was relatively common in some localities and that it was possible to record it in incidental visits to these sites. We note that the last records of Quimbaya toad occurred at about the same time in two of the three localities (1997 in Río Blanco and 1996 in Ucumarí). After our extensive sampling in the only three known localities and other nearby localities with similar habitat type, we recommend that this species should be declared as possibly extinct.

Potential extinction drivers for the Quimbaya toad
Presence of the invasive rainbow trout

In the Otún and Quindío River basins there are two major rainbow trout farms, Pez Fresco S.A and Truchas Cocora, respectively. The rainbow trout was introduced in Quindío Department in 1953, in the Natural Reserve Navarco both in artificial ponds (for

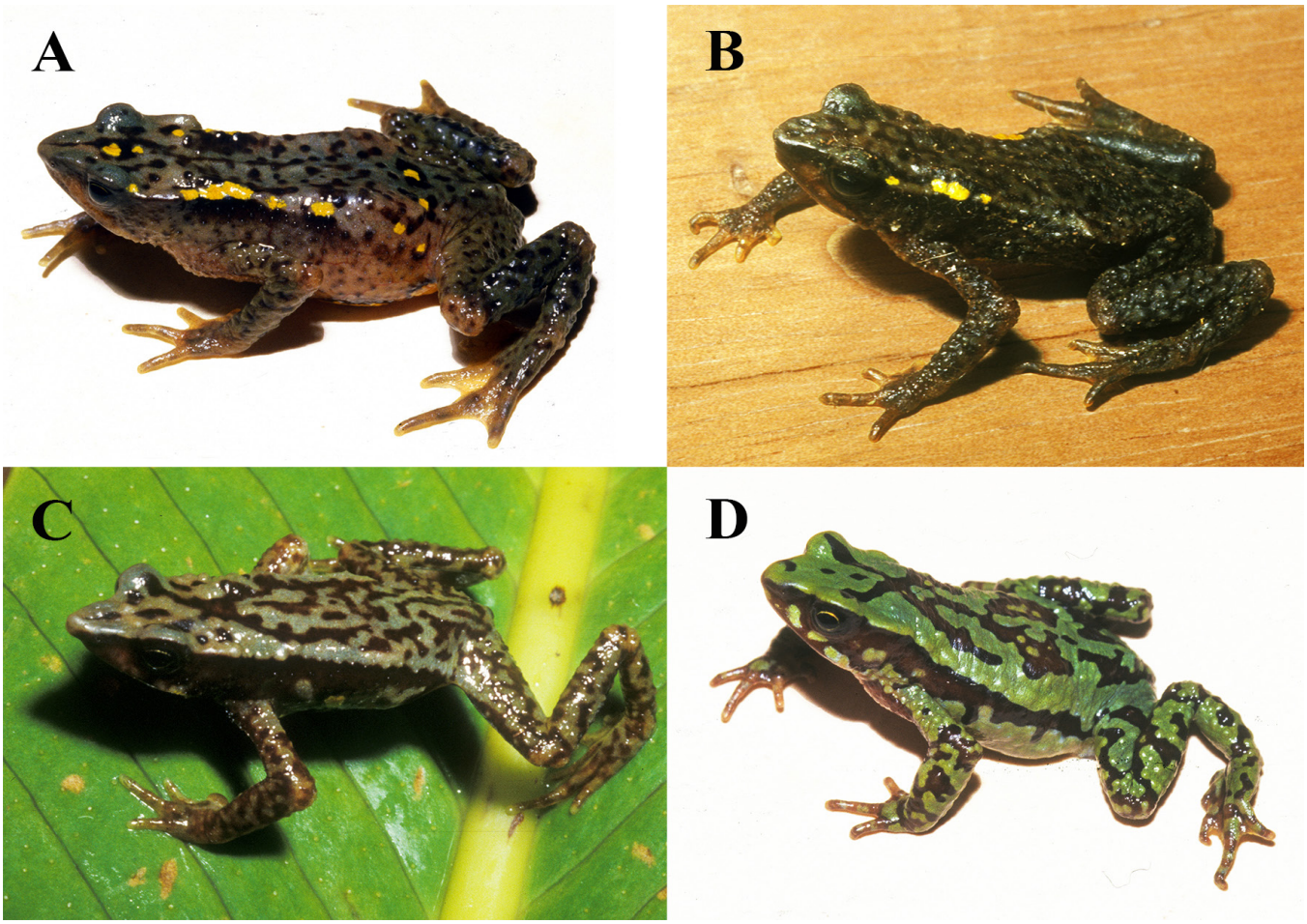

Figure 3. Record of the the Quimbaya toad. A) ICN13771 adult female (SVL 35.7; unspecified date) from Río Quindío watershed, Salento, Quindio (photo: John D. Lynch). B) ICN36142 adult female (SVL 33.3) collected at June 1994 in Ucumarí Natural Regional Park, Pereira, Risaralda (photo: John D. Lynch). C) ICN36145 adult male (SVL 27.5) collected at June 1994 in Ucumarí Natural Regional Park, Pereira, Risaralda (photo: J.D.Lynch). D) ICN38001 adult male (SVL 25.3; unspecified date) from Manizales, Caldas (photo: María Cristina Ardila-Robayo)..

Figura 3. Registro del sapo Quimbaya. A) ICN13771 hembra adulta (SVL 35.7; fecha no específica) de la cuenca del Río Quindío, Salento, Quindio (foto: John D. Lynch). B) ICN36142 hembra adulta (SVL 33.3) y C) ICN36145 macho adulto (SVL 27.5) colectados en junio 1994 en el Parque Natural Regional Ucumarí, Pereira, Risaralda (fotos: J.D.Lynch). D) ICN38001 macho adulto (SVL 25.3; fecha no específica) de Manizales, Caldas (foto: María Cristina Ardila-Robayo)..

aquaculture) and rivers (Maya Gómez, 1984). Fish farming and sport fishing activities are tourist attractions in the Quindío and Otún river basins for both locals and foreigners. During our field surveys we detected the presence of rainbow trout in even small streams in both basins. We suggest that rainbow trout may be involved in the population decline of *A. quimbaya* through predation of mainly eggs and possibly tadpoles. One of us (GK) occasionally observed that *A. quimbaya* laid strings of eggs in small streams in the Otún river basin which would be vulnerable to predation by rainbow trout. The rainbow trout is also widespread in Río Blanco (GAGD *pers. obs.*). The potential negative effect of rainbow trout has been proposed for *Atelopus* species and documented for other amphibians (Ron et al., 2003; La Marca et al., 2005; Martín-Torrijos et al., 2016). Although the timing of trout introduction does not coincide with the population declines, we suspect that trout population expansion occurred steadily to reach the toad's habitat only in the 1990s.

Land use change and land degradation

Historical localities were subject to intensive agricultural activities, mainly cattle ranching. After this habitat degradation, the stressor factors were stopped and localities are currently protected at the regional level (Vásquez and Serrano, 2009). We do not have any data on how cattle ranching and agriculture affected habitat quality in this region, and how these stressors affected the Quimbaya toad populations. Also, the species was

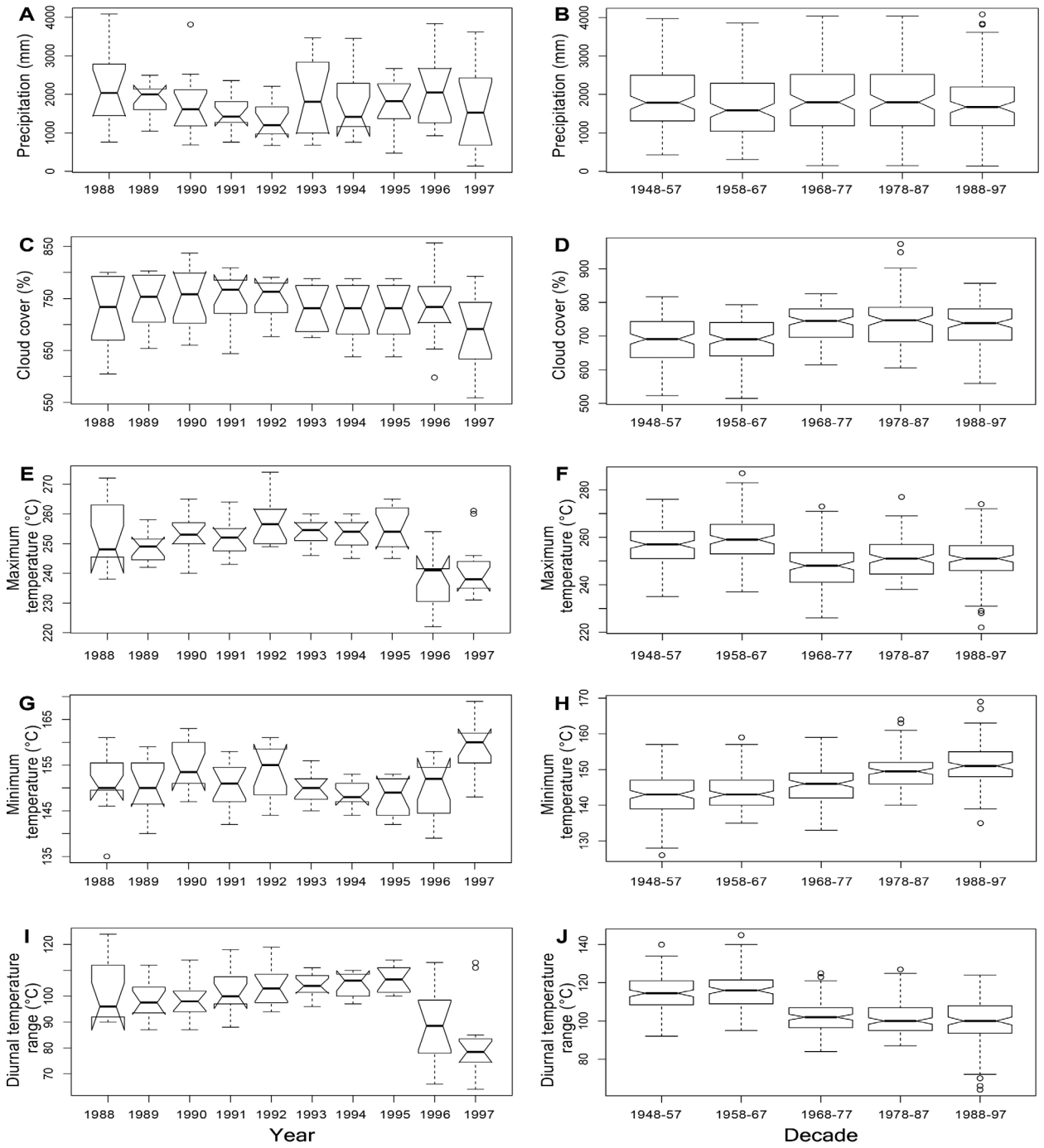

Figure 4. Box-and-whisker plots of historical temperature, cloud cover and precipitation trends within of the geographical range of the Quimbaya toad during the 10 years (left) and five decades (right) prior to the last observation of this species (1997). Climatic data were taken from the CRU-TS 3.10.01 (see main text for details).

Figura 4. Diagramas de cajas y bigotes de la temperatura histórica, nubosidad y tendencias de precipitación dentro del área de distribución del sapo Quimbaya durante los 10 años (izquierda) y cinco décadas (derecha) previos a la última observación de esta especie (1997). Los datos climáticos se tomaron de la CRU-TS 3.10.01 (ver el texto principal para más detalles).

reported close to these reforestation sites (Ruíz-Carranza and Osorno-Muñoz, 1994) and we assume that site protection could positively affect the Quimbaya toad. Therefore, past land use changes and land degradation were not likely related to the population declines and extinction of this species (La Marca et al., 2005).

Chytridiomycosis

The fungal disease chytridiomycosis has been related to population extirpations and extinction of many amphibian species all over the world. The genus *Atelopus* is considered one of the most susceptible groups and the vanishing of many species has been associated to the presence of *Batrachochytrium dendrobatidis* (La Marca et al., 2005). For Colombia, the earliest record of *Bd* is from an individual of *Hyloxalus fascianigrus* collected in 1994 in the Valle del Cauca (Velásquez et al., 2008). For *Atelopus* species, Ruíz and Rueda-Almonacid (2008) examined specimens of at least 37 species distributed in Colombia to detect presence of *Bd* using histological techniques. Only nine specimens of *A. quimbaya*, two collected in 1987 and seven in 1991, were included in the survey and they did not find evidence of *Bd* infection. More recently, skin swab samples from 14 specimens collected between 1979 and 1994 were analyzed using quantitative PCR. Again, we did not find signs of chytridiomycosis (SVF unpublished data). The reduced number of samples in both surveys (histology and molecular analysis) does not allow us to establish if *Bd* was involved in the decline of this species. Another species -*Pristimantis alalocophus*- associated with streams and micro-sympatric with *A. quimbaya* was not detected during our surveys and we believe this species is also possibly extirpated from these localities (Gómez-Hoyos et al., 2017). We suggest that an exhaustive examination of museum specimens and field surveys will be crucial to establish the timing of arrival and prevalence of the chytrid fungus in the region. Such studies would help to clarify the role of *Bd* in the population decline of the Quimbaya toad and its current impact on other amphibian species in the region.

Climate change effects

Changes in climatic conditions are indirect stressors causing declines in amphibians by acting synergistically with other factors such as emergent diseases and habitat degradation (Pounds, 2001; Pounds et al., 2006; Blaustein et al., 2010). Changes in historical climatic trends are a potential explanation of the decline and extinction of *Atelopus ignescens* in Ecuador; the year previous to the last record was unusually warm and dry (Ron et al., 2003). We found that some climatic conditions including cloud cover, decrease of maximum temperature, increase of minimum temperature, and reduction of the range of diurnal temperature, changed during the last year before the last observation of *A. quimbaya*. Although cloud cover was initially related with the thermal optimum for *Bd* growth (Pound et al., 2006), when more robust statistical methods were used with the same data this relationship disappeared (Rohr et al., 2008). As we were unable to detect the presence of the *Bd* in the region where *A. quimbaya* occurred (but *Bd* presence has been confirmed in Colombia in other highland elevations; see Flechas et al., 2017), we cannot rule out the potential link between Bd and regional climate variability as a potential extinction driver for the Quimbaya toad. Furthermore, we cannot rule out a synergistic effect of multiple stressors driving amphibian declines (Pounds, 2001; Ron et al., 2003; Pounds et al., 2006; Sodhi et al., 2008; Blaustein et al., 2010), but we currently have no evidence. Therefore, we recommend an exhaustive survey of chytrid fungus combining information from museum specimens deposited in Colombian herpetological collections (e.g., ICN; UVC; MHUA; IAvH) and extensive field surveys to detect the presence and prevalence of *Bd*.

CONCLUSIONS

Here, we conclude that the Quimbaya toad, *Atelopus quimbaya*, should be declared as possibly extinct. We suggest that additional surveys should be conducted for other very rare Andean *Atelopus* with similar characteristics that have not been detected in the last 30 years (e.g., *A. choconesis* and *A. pictiventris*). We are unable to establish the causes of the presumed extinction of *A. quimbaya*, but we suspect that a combination of stressors including rainbow trout, undetected *Bd* in this region, and changes in climatic variability may have played a role in the extinction/decline of this species. We consider that an extensive survey of chytrid fungus and rainbow trout should be conducted in these known localities to establish the scope of the impact of these factors and how they are affecting other amphibian species. For example, a population of *Hyloscirtus larinopygion* still survives at El Cedral (Ucumarí, 2100 masl), but *Colostethus fraterdanieli* is the only common species in the Otún drainage between 1800 and 2100 masl (GHK *pers. obs.*). A long-term monitoring plan for amphibian species in the Andean region should be an integral part of any conservation efforts (e.g., *ex-situ* rescue programs and creation of additional local protected areas).

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