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Rediscovery of the nearly extinct longnose harlequin frog *Atelopus longirostris* (Bufonidae) in Junín, Imbabura, Ecuador

Elicio Eladio Tapia[a], Luis Aurelio Coloma[a,b]*, Gustavo Pazmiño-Otamendi[a] and Nicolás Peñafiel[c]

[a]*Centro Jambatu de Investigación y Conservación de Anfibios, Fundación Otonga, Quito, Ecuador;* [b]*Universidad Regional Amazónica IKIAM, Tena, Ecuador;* [c]*Laboratorio de Biología Molecular, Centro de Investigación de la Biodiversidad y Cambio Climático, Universidad Tecnológica Indoamérica, Machala y Sabanilla, Quito, Ecuador*

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Abstract

We report the recent finding of four adults of *Atelopus longirostris*, a Critically Endangered species that was last seen in 1989, when catastrophic *Atelopus* declines occurred. The rediscovery of *A. longirostris* took place in a new locality, Junín, 1250–1480 m asl, Provincia Imbabura, Ecuador, on 28–31 March 2016. The four frogs were found in two isolated small patches of native forest in a fragmented area heavily modified for agriculture and livestock; one patch protected by the Junín Community Reserve, and another non-protected private patch near the reserve. We found high prevalence of *Batrachochytrium dendrobatidis (Bd)* in the amphibian community of Junín, but *A. longirostris* tested negative. The finding of *A. longirostris* after 27 years is surprising and fits an apparent pattern of mild conditions that might be promoting either the recovery or persistence in low numbers of some relict amphibian populations. The frogs are the first founders of an *ex situ* assurance colony in Jambatu Research and Conservation Center. Expansion of the Junín Community Reserve is urgently needed to add the currently non-protected patch of forest, where *A. longirostris* also occurs. The restoration of the forest in degraded areas between both forest patches and in the related river margins is also necessary. This restoration will grant the connectivity between both isolated metapopulations and the normal movement of individuals to the breeding sites in the Chalguayacu and Junín River basins. The latter should be protected to prevent any kind of water pollution by the opencast copper exploitation of the mining concession Llurimagua, which is underway. *Atelopus longirostris* belongs to a group of at least 29 species of Ecuadorian *Atelopus* that are critically endangered, 15 of which remain unsighted for at least one decade, and most of them might be extinct. Further synchronous, multidisciplinary and integrative research is needed, aiming to understand the most aspects of the biology of species of *Atelopus* to support *in situ* and *ex situ* conservation actions.

Keywords: *Atelopus longirostris*; Bufonidae; Ecuador; extinction; rediscovery

Reportamos el reciente hallazgo de cuatro adultos de *Atelopus longirostris*, una especie en Peligro Crítico, la misma que fue vista por última vez en 1989, cuando se produjeron declives catastróficos de *Atelopus*. El redescubrimiento de *A. longisrostris* tuvo lugar en una nueva localidad, Junín, 1250–1480 msnm, Provincia de Imbabura, Ecuador, entre el 28–31 de marzo de 2016. Las cuatro ranas se encontraron en dos pequeñas parcelas de bosque natural en un área fragmentada y densamente modificada para agricultura y ganadería, la una parcela forma parte de la Reserva de la Comunidad Junín y la otra está en un área privada no protegida cercana a la reserva. Encontramos alta prevalencia de *Batrachochytrium dendrobatidis (Bd)* en la comunidad de anfibios de Junín, aunque no se encontró en *A. longirostris*. Su hallazgo después de 27 años es sorprendente y se ajusta a un patrón aparente de condiciones benignas que estarían promoviendo sea la recuperación o persistencia de poblaciones relictas de algunas especies de anfibios. Estas ranas son los primeros fundadores de una colonia de manejo *ex situ* en el Centro Jambatu de Investigación y Conservación de Anfibios. Se necesita con urgencia la expansión de la Reserva de la Comunidad de Junín para incluir todos los bosques en donde *A. longisrostris* habita. Es también necesaria la restauración de los bosques en las áreas destruidas que quedan entre los parches de bosque y en la rivera de los ríos. Esta restauración garantizará la conectividad entre metapoblaciones aisladas y también el desplazamiento normal de individuos a los sitios de reproducción en las cuencas de los ríos Chalguayacu y Junín. Estas cuencas deben ser protegidas para evitar cualquier tipo de contaminación en el agua producida por la explotación de cobre a cielo abierto de la concesión minera Llurimagua, la cual está en ejecución. *Atelopus longirostris* pertenece a un grupo de no menos de 29 especies de *Atelopus* de Ecuador que están Críticamente Amenazadas, 15 de las cuales no han sido vistas en al menos una década y la mayoría de ellas podrían estar extintas. Se requiere ejecutar más investigaciones simultáneas, multidisciplinarias e integrales para entender la mayoría de aspectos de la biología de las especies de *Atelopus*, y las cuales apoyen a los programas de conservación *in situ* y *ex situ*.

Palabras claves: *Atelopus longirostris*; Bufonidae; Ecuador; extinción; redescubrimiento

*Corresponding author. Email: lcoloma@otonga.org

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Introduction

Frogs of the genus *Atelopus* are distributed across tropical forests, cloud forests and the páramos of Central and South America. The genus is the largest in the family Bufonidae, with 96 species described to date [1] plus 30–70 undescribed [2]. *Atelopus* has been affected by catastrophic declines and extinctions; all species restricted to elevations above 1000 m have declined and about 75% have disappeared [3]. *Atelopus* represent about 15% of the 528 amphibian species that are currently categorized as Critically Endangered (CR) in the IUCN Red List of Threatened Species [4]. In Ecuador, these severe extinction processes have hit a hot spot of *Atelopus* diversity given the relatively large number of species (25 described and at least 7 undescribed, Table 1) known to occur to date [5]. Twenty-four of the described species are included in categories of threat in the IUCN Red List and none in low or no threat categories; among them 11 are considered Critically Endangered (tagged as Possibly Extinct [6]). Thus, the conservation status of species of *Atelopus* in Ecuador is of major concern. The causes of these sudden declines and extinctions of *Atelopus* species, mostly noticed by the end of the eighties and first half of the nineties, have been a matter of debate (e.g. [7,8]). Several stressors seem to be the culprit, most importantly climate change and pathogens [9–12].

The Longnose harlequin frog, *Atelopus longirostris* (Cope 1868), is endemic to the Chocoan region of Ecuador. It used to inhabit the lowlands and subtropics in premontane and montane forests in the Cordillera Occidental de los Andes of Northwestern Ecuador, at altitudes between 900 and 1925 m asl. It was last seen in 1989 in Río Esmeraldas (1557 m asl), San Francisco de Las Pampas, Provincia Cotopaxi, Ecuador. Its historical records come from 20 localities in the provinces of Imbabura, Cotopaxi, Pichincha, and Santo Domingo de los Tsáchilas encompassing an area of extent of occurrence (measured by a minimum convex polygon that contains all the sites of occurrence) of about 1746 Km^2 in 20 localities from Provincia de Imbabura to Cotopaxi (Figure 1). The populations of Carchi and Esmeraldas are excluded because they are in need of a taxonomical revision [13].

Atelopus longirostris is a diurnal species of terrestrial and semiarboreal habits. Its activity is associated with

Table 1. Species of *Atelopus* of Ecuador indicating their political endemicity to Ecuador (E), Red List category (RL), date of most recent sighting in Ecuador (DS) for possibly extinct species, *Ex situ* assurance colony (ES).

Species	E	RL	DS	ES
Atelopus angelito	NE	CR	22 July 1988	–
Atelopus arthuri	E	CR	1 February 1988	–
Atelopus balios	E	CR	Recent	Yes*
Atelopus bomolochos	E	CR	Recent	Yes
Atelopus boulengeri	E	CR	July 1984	–
Atelopus coynei	E	CR	Recent	No
Atelopus elegans	NE	CR	Recent	Yes*
Atelopus exiguus	E	CR	Recent	Yes
Atelopus guanujo	E	CR	10 April 1988	–
Atelopus halihelos	E	CR	28 August 1989	–
Atelopus ignescens	E	CR	Recent	Yes
Atelopus longirostris	E	CR	Recent	Yes
Atelopus lynchi	NE	CR	30 May 1977	–
Atelopus mindoensis	E	CR	7 May 1989	–
Atelopus nanay	E	CR	Recent	Yes*
Atelopus nepiozomus	E	CR	Recent	Yes
Atelopus onorei	E	CR	21 April 1990	–
Atelopus orcesi	E	CR	May 1988	–
Atelopus pachydermus	NE	CR	September 1985	–
Atelopus palmatus	E	CR	Recent	No
Atelopus pastuso	NE	CR	29 June 1993	–
Atelopus petersi	E	CR	8 November 1996	–
Atelopus planispina	E	CR	October 1987	–
Atelopus podocarpus	NE	CR	1 December 1994	–
Atelopus spumarius	NE	EN	Recent	Yes
Atelopus sp. (Limón)	NE	CR	Recent	Yes*
Atelopus sp. (Cóndor)	E	EN	Recent	Yes
Atelopus sp. (Carchi)	E	CR	Recent	No
Atelopus sp. (Azuay)	E	CR	Recent	Yes
Atelopus sp. (Sangay)	E	CR	Recent	No
Atelopus sp. (Chimborazo)	E	CR	24 April 2002	–
Atelopus sp. (Pastaza)	NE	DD	Recent	No

Notes: An asterisk (*) indicates that breeding has occurred. CR = Critically Endangered, EN = Endangered, DD = Data deficient, Recent = seen at any date between 2008 and 2016.

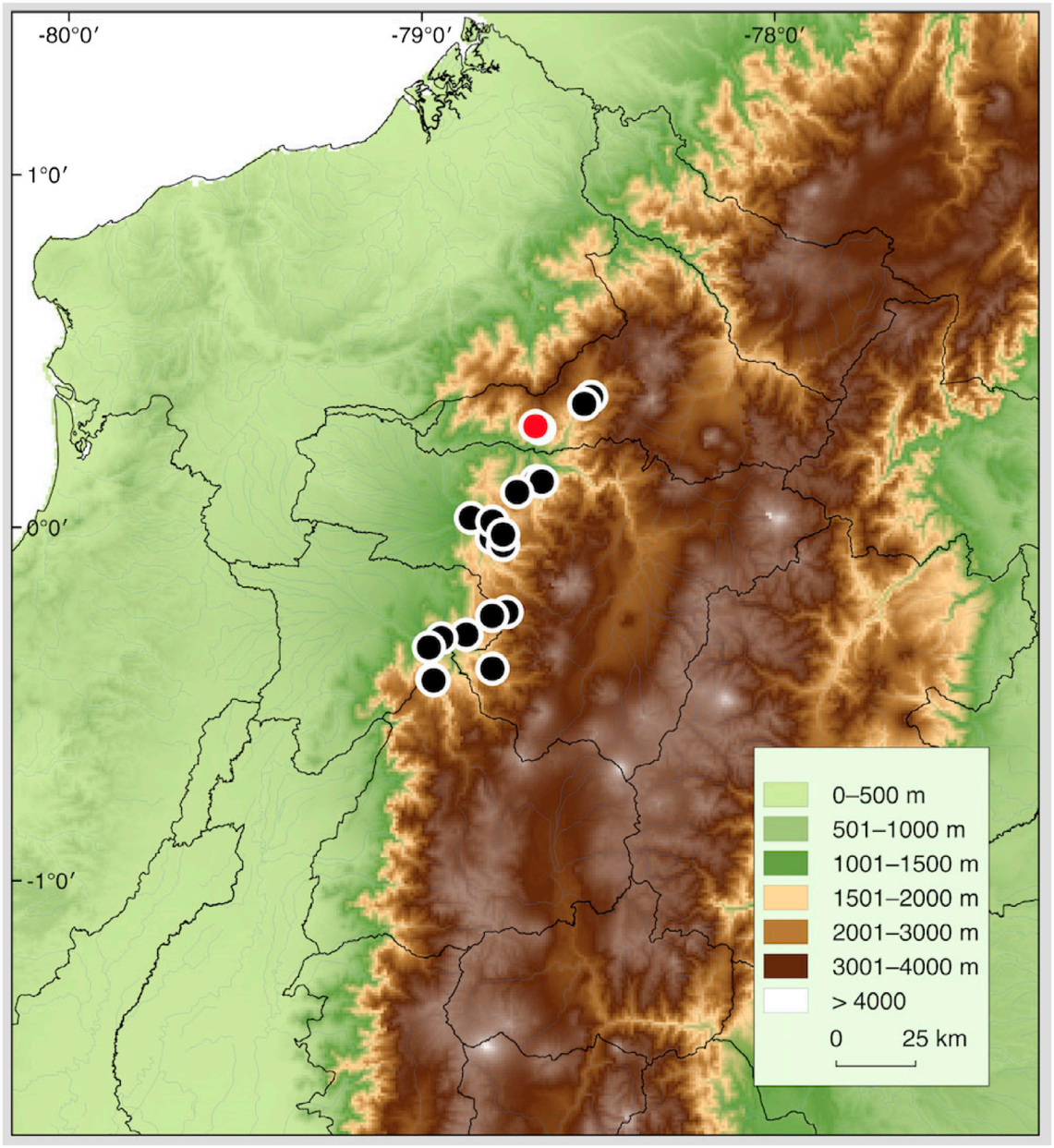


Figure 1. Localities of ocurrence of *Atelopus longirostris* in Ecuador. Historical records (black), new record (red).

water streams during the day, where it can be found walking in opened rocky shores of evergreen forests; by night it hides under rocks or sleeps on leaves near the ground. It is a stream breeding species [14]. An amplexus was reported in 1959 during the end of the rainy season, and according to the author the female was heavy with eggs [14]. Besides this, nothing is known about its biology.

As part of an ongoing inventory of amphibians in the reserve of the Junín community, Intag, Provincia Imbabura, Ecuador, we did extensive searches in the area from 28 March to 6 April 2016. Among 16 species found, we report the rediscovery of *Atelopus longirostris,* provide additional biological information and discuss about its conservation.

Methods

The study region was located at the reserves of the Junín community, Cabañas EcoJunín, and also surrounding private areas, Cantón Cotacachi, Provincia Imbabura, Ecuador, where we sampled areas between 1159 and 2560 m asl in foothill, lower montane, and montane cloud forests from 28 March to 6 April 2016. A second survey focused on *Atelopus longirostris* habitat, where we recorded them previously, was made on 3–4 December 2016. The first field trip surveys were conducted every day between 07:00 and 18:00 h during the day, and between 18:30 and 02:00 h at night, using Visual Encounter Surveys to record as many amphibians as possible. *Atelopus longirostris* total frog search effort was

114:30 h. It was done during seven nights (five nights for a total of 104:30 h in the first survey and two nights for a total of 10:00 h in the second) time spent in the potential *Atelopus* habitat around rivers and streams. These efforts were divided in the first survey as follows: three nights-three persons (from 18:30 to 02:00 h), one night-two persons (from 18:30 to 02:00 h), one night-six persons (from 18:30 to 22:00 h). In the second field trip, search effort was two nights-five persons (20:00 to 21:00 h). During the first survey, tadpole searches were done during day and night in about two hundred meters along the river, at the same sites where adults were found. Clear plastic containers were used as underwater visors. Also, stones were removed manually to look for tadpoles at the undersides. Tadpole search effort was done during two days (from 07:00 to 18:00 h) and one night (18:30 to 02:00 h) for a total of 29:30 h.

We sampled different types of land cover: forests (native and secondary), farmlands, grasslands, mixed areas of agricultural and grasslands, riverbanks (large rivers and smaller streams), and native bamboo areas. Information collected in the field included: geographic positions of each encounter, air and water temperature (°C), time of encounter (24 h), perch height (cm), sex (when possible), and age class (froglet, juvenile, adult). Geographic information was recorded using a GPS GARMIN GPSmap 62s; ph data, water and air temperature were taken with a HANNA pHep 5 waterproof pH tester, and with a New RadioShack 22-170 Infrared Thermometer Pistol Grip Design 10.1 Range. In the field, each individual of *Atelopus longirostris* was collected and handled with a plastic bag, in which it was placed. These living individuals were transferred to the *ex-situ* conservation program named Life Bank 'Arca de los Sapos' of Jambatu Center of Amphibian Research and Conservation (CJ). Once deposited in the laboratory, each individual was handled with a fresh pair of latex gloves to prevent transferring pathogens such as amphibian chytrid fungus (*Batrachochytrium dendrobatidis; Bd*), and underwent quarantine. Tests for the presence/absence of chytrid fungus were done using skin swabs of *Atelopus longirostris* and pieces of pelvic patch (stored in ethanol 75%) of other amphibians. Tests were performed following the standard procedures in Hyatt et al. [15]; dry swabs were stored in −4 °C until analysis. DNA from swabs and tissue samples was extracted with a protocol that uses SDS and Proteinase K for cellular lysis, guanidine isothiocyanate for protein precipitation and isopropanol for DNA precipitation. *Bd* presence was tested by Polymerase Chain Reaction (PCR) designed to isolate a 300 bp region of the fungal rDNA using primers Bd1a (5′-CAGTGTGCCATATGTCACG-3′) and Bd2a (5′-CATGGTTCATATCTGTCCAG-3′) developed by Annis et al. [16]. Each PCR reaction contained a final concentration of 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.05 U/μL *Taq* DNA polymerase (Invitrogen) and 0.5 μM of each primer in a 25 μL total volume. PCR protocol followed Annis et al. [16], except that 35 cycles were performed. When the PCR product retrieved was insufficient or dubious, an additional PCR was carried out, using a 1:50 dilution of the cleaned-up product from the first PCR as template. The conditions of this second PCR were the same as the first one, but fewer cycles were performed. Two controls: a negative control, containing water instead of DNA, and a positive control – a sample previously tested positive for *Bd* – were used in every PCR. The presence/absence of *Bd* was determined via electrophoresis in 1.5% agarose gels. We estimated point prevalence of *Bd* within each anuran species as the number of frogs that tested positive for *Bd,* divided by the total number of sampled frogs for that particular species in our sample.

Results

Study site

An ecological characterization of the Reserve of the Community of Junín is provided by Peñafiel Cevallos et al. [17]. Annual mean temperature varies between 17 and 20 °C, and annual mean humidity varies between 50 and 75%. Mean annual precipitation is 2000–3000 mm, and the rainy season extends from December to April whereas the dry season is from May to November. The sampling area (1159–2560 m asl) belongs to the Foothill Cloud Forest and Montane Cloud Forest [18] in the subtropical and temperate zoogeographical zones *sensu* Albuja et al. [19]. Vegetation at the site is described by Pazmiño-Otamendi et al. [20]. The lower parts (about 1159–2000 m asl) are highly disturbed by human activities, with villages, agricultural areas and pastures for livestock (Figure 2(A)). In the lower portion, hilltops are usually deforested because the flat terrain is optimal for human activity. Despite the impact, there are some patches of native forest, usually on the slopes of hills along rivers and streams (Figure 2(B) and (C)). One of these patches (about 15 hectares) is protected and is part of Cabañas EcoJunín, which belong to the Junín community. At higher altitudes, fewer disturbances occur; however, there are also large deforested fragments, paddocks, and pastures. Areas between 2000 and 2560 m asl belong to the Junín Community Reserve [17]. These areas are in much better condition, with large zones of native forest, even on hilltops.

Atelopus longirostris

An unexpected finding in two sites of the lower area of the sampled region was the presence of 4 adult individuals (two males and two females) of *Atelopus longirostris.* They were in two small patches of native forest (site 1 of about 21 hectares and site 2 of about 45 hectares, Figure 2(A) and (B)) associated with the Chalguayacu and Junín river basins. All the individuals were found at night between 19:45 h and 22:00 h on 28, 29, and 31 March 2016. Three (one female, two males) of the four

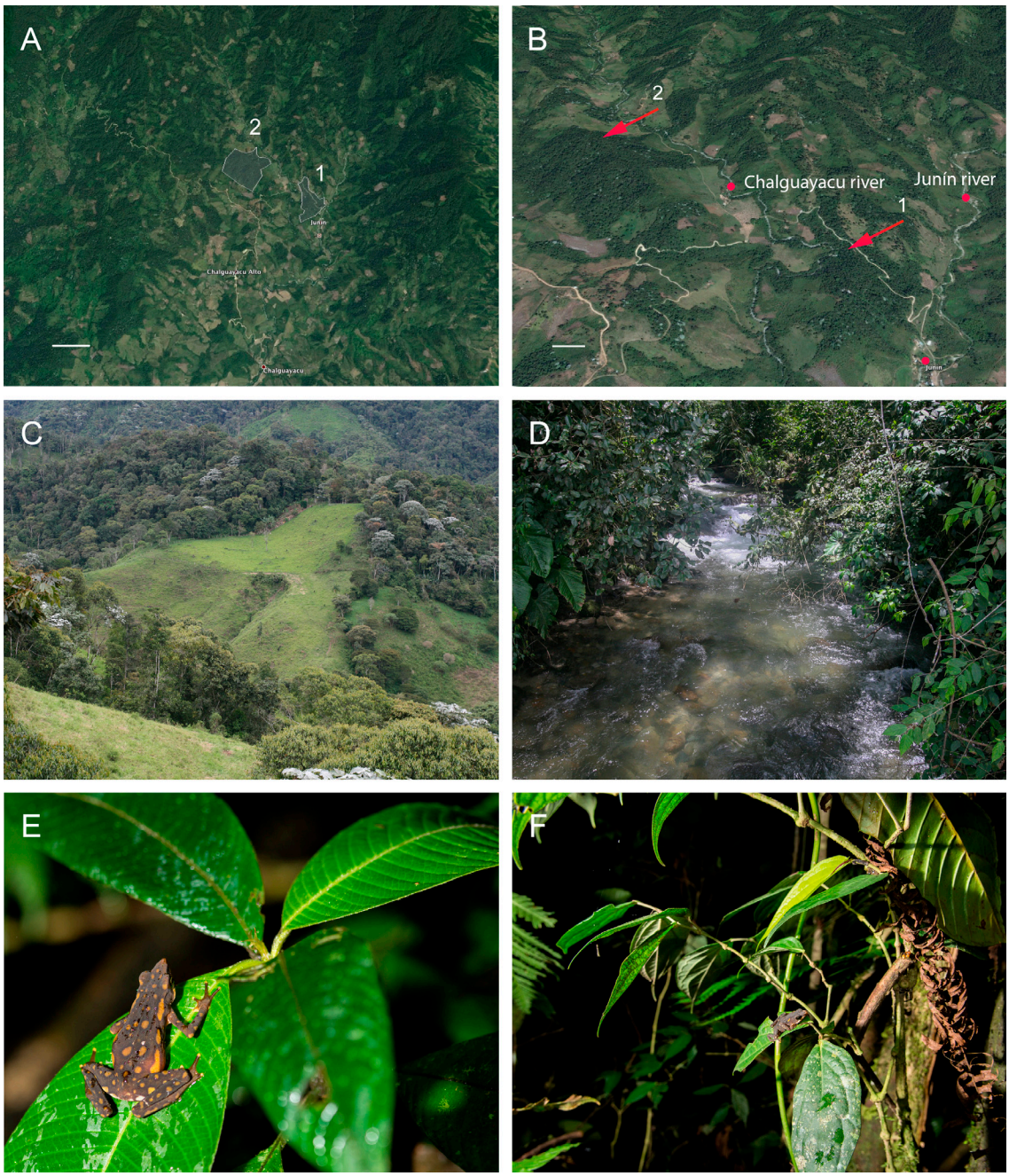


Figure 2. *Atelopus longirostris* habitat at Junín, Provincia Imbabura: (A–B) Aerial views from an altitude of 11.09 and 2.89 km, taken from Google, digital Globe; red arrows indicate collection sites at site 1, the reserve of Cabañas EcoJunín (right) and site 2, a private property (left), scale = 667 and 108 m in A and B, respectively. In figure B note the nearly complete disconnection between the private forest and Chalguayacu river caused by forest clearing, (C) forest at reserve of Cabañas EcoJunín, (D) Chalguayacu river, (E) female CJ (sc 5521) on a leaf of Rubiaceae, (F) male CJ (sc 5582) on a leaf of Piperaceae. Photos C–D by EET, E–F by GPO.

individuals were found on site 1, a protected forest of the reserve of Cabañas EcoJunín, at 1250–1300 m asl. One female was 15 m from the top of the hill at about 80 m from the Chalguayacu river, whereas the other two males were 40–50 m from the Chalguayacu river, all of them on a slope of 60–75%. Another female was found on site 2 at the top of the hill at about 410 m from the Chalguayacu river and 200 m from the small river Argentina (with a river bed about 5 m wide, 50 cm depth) in a non-protected private property, at 1480 m asl. The four individuals were found at night resting on leaves at 40–60 cm above the ground: female CJ (sc 5521) was on a leaf (16 × 6 cm) of Rubiaceae, female CJ (sc 5583) was on an *Anthurium* sp. leaf (25 × 15 cm), male CJ (sc 5522) on a leaf (25 × 20 cm) of *Anthurium* sp. growing on a stone (100 × 70 cm) covered by moss, and male CJ (sc 5582) was on a leaf (9 × 3.5 cm) of *Piper* sp. in a bush 2 m high. They were in a

patch of secondary forest mixed with fallen trees and branches. The four frogs were collected, transported to *ex situ* breeding facilities of Jambatu Center, and maintained in a quarantine period. Two females and one male survive to date 12 May 2017 in healthy condition. One male died for undetermined reasons. Latest updates of their survival status are provided in Centro Jambatu web page [21].

The pH of the Chalguayacu river was 7.5 and the water temperature was 20 °C, taken at 22:00 h on 28 March 2016. Air temperature was 19.4 °C taken at 21:00 h on 29 March 2016; 18 °C taken at 20:30–22:00 h on 31 March 2016, and 20 °C taken at 07:30 h on 31 March 2016. The river-bed is about 15 m width, and water depth can reach about 4 m during the rainy season, whereas during the dry season stream depth is about 0.8 m.

Prevalence of Batrachochytrium dendrobatidis *in amphibians*

Pelvic patch (56) and skin swabs (4 of *Atelopus longirostris*) of 60 frogs of 16 species were tested for *Bd,* and a third (20 frogs) of those (belonging to nine species) were positive for *Bd* infection (Table 2). The chytrid analysis of *Atelopus longirostris* tested negative.

Morphology

The SVL (snout-vent length) and weight (taken on 28 October 2016) of *Atelopus longirostris* are as follows: male CJ (sc 5582) 31.6 mm, 2.2060 g; gravid female CJ (sc 5521) 36.8 mm, 3.4636 g; female CJ (sc 5583) 39.4 mm, 2.7927 g. External morphology features, patterns and color in life of male CJ (sc 5582) and female CJ (sc 5521) are depicted in Figure 3.

Sympatric species

During the surveys we recorded observations of 16 species of amphibians, seven of which were found at the same site where we found *Atelopus longirostris* (Table 2). Some of them, for example *Espadarana prosoblepon* (Boettger 1892), *Dendropsophus carnifex* (Duellman 1969), *Hyloscirtus alytolylax* (Duellman 1972), and *Hyloxalus awa* (Coloma 1995) occurred at the same collecting site or near *Atelopus longirostris* microhabitat, inside the forest associated with rivers or water streams.

Discussion

The four specimens of *Atelopus longirostris* have a SVL within the known range, a swollen gland at tip of snout, and white pustulae on lateral sides. Thus, they fit well with previous descriptions of the species [14,22–24].

The species found in sympatry with *Atelopus longirostris* are nocturnal and mainly arboreal, except for *Hyloxalus awa*, which is diurnal, terrestrial, and associated to streams as *Atelopus longirostris* [14], but *H. awa* has been found to occupy much more open areas, rather than native forest areas.

It is interesting that males of *Atelopus longirostris* were found relatively far (40–50 m) from the river, unlike what has been reported in other species of *Atelopus*, where some males can be found at the edge of the breeding sites in both the dry and rainy seasons [25,26]. If *A. longirostris* breeds in the Chalguayacu and or Junín rivers, the absence of males at the river shore

Table 2. Species of amphibians encountered at Cabañas EcoJunín, the Junín Community Reserve, and surrounding areas between 1149 and 2560 m asl, Intag, Provincia Imbabura, Ecuador.

Family	Species	Endemic	RL	*Bd* (n = 60)
Bufonidae	*Atelopus longirostris**	Yes	CR	– (4)
Centrolenidae	*Espadarana prosoblepon**	No	LC	+ (3/8)
Craugastoridae	*Pristimantis achatinus**	No	LC	+ (4/11)
Craugastoridae	*Pristimantis appendiculatus*	No	NT	– (1)
Craugastoridae	*Pristimantis dissimulatus*	Yes	EN	– (4)
Craugastoridae	*Pristimantis laticlavius*	No	DD	– (1)
Craugastoridae	*Pristimantis leoni*	No	LC	– (3)
Craugastoridae	*Pristimantis pahuma*	Yes	EN	+ (1/4)
Craugastoridae	*Pristimantis pteridophilus*	Yes	EN	– (1)
Craugastoridae	*Pristimantis walkeri**	Yes	LC	+ (3/6)
Craugastoridae	*Pristimantis w-nigrum*	No	EN	+ (3/5)
Dendrobatidae	*Hyloxalus awa**	Yes	VU	+ (1/4)
Hemiphractidae	*Gastrotheca plumbea*	Yes	VU	Not analyzed
Hylidae	*Dendropsophus carnifex**	No	LC	+ (2/3)
Hylidae	*Hyloscirtus alytolylax**	No	NT	+ (1/2)
Leptodactylidae	*Leptodactylus ventrimaculatus**	No	LC	+ (2/3)

Notes: Their political endemicity to Ecuador and Red List category (RL) are indicated. CR = Critically Endangered, EN = Endangered, VU = Vulnerable, NT = Near Threatened, DD = Data Deficient, LC = Least Concern, n = sample size. An asterisk (*) indicates sympatric species found with *Atelopus longisrostris.* Results of *Batrachochytrium dendrobatids (Bd)* analyses are given as positive or negative (number of positives/total number analyzed).

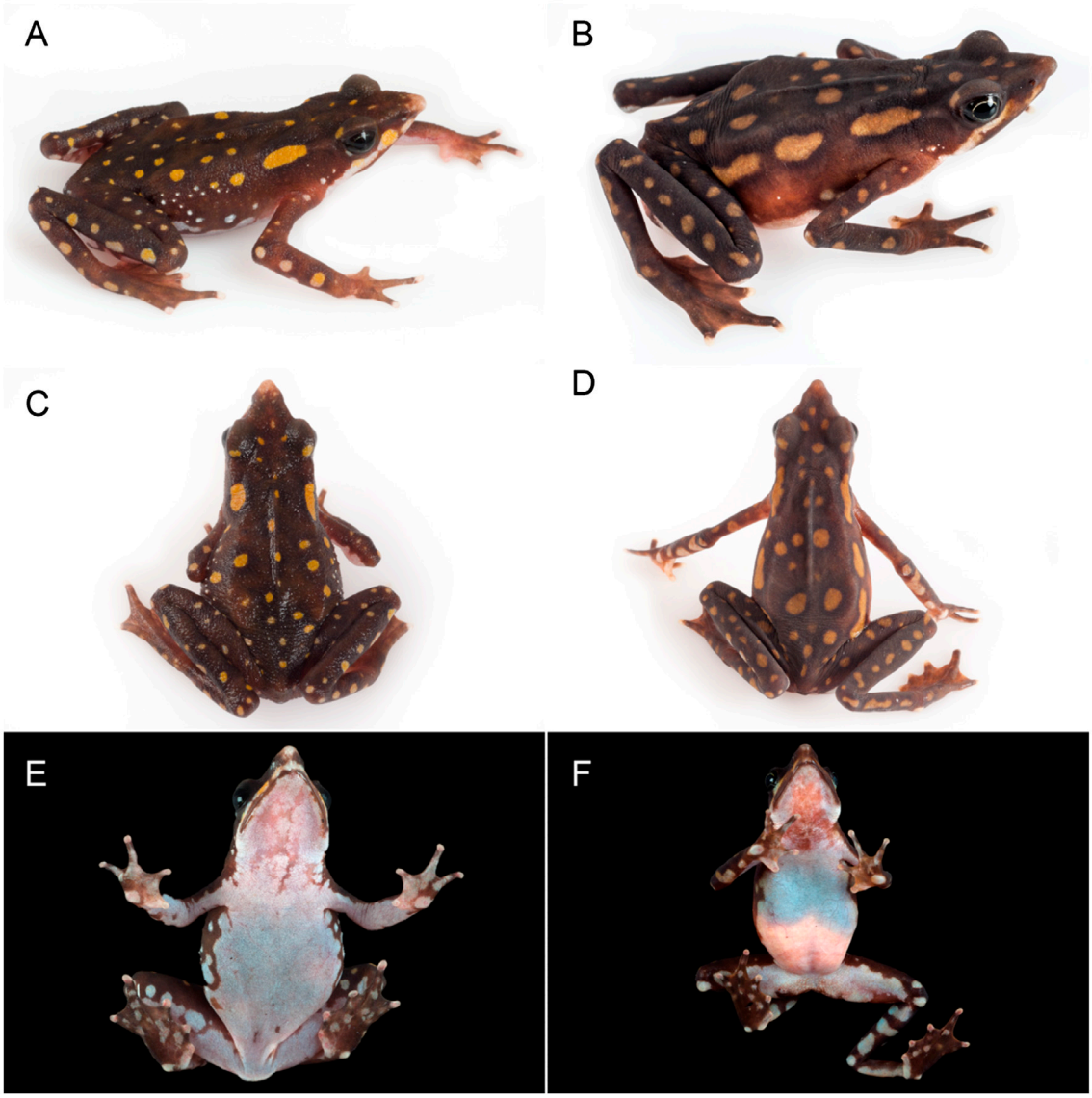


Figure 3. Live adults of *Atelopus longirostris*: (A, C, E) CJ (sc 5582), male, SVL = 31.6 mm, (B, D, F) CJ (sc 5521), female, SVL = 36.8 mm. Photos by LAC.

might occur because they avoid large rivers that increase greatly their water level and current speed during the rainy season.

Conservation

Previous to our report, *Atelopus longirostris* was sighted 27 years ago in May 1989 at San Francisco de Las Pampas, Provincia Cotopaxi. At that time, it was known from an area of extent of occurrence (measured by a minimum convex polygon that contains all the sites of occurrence) of about 1746 Km^2 in 20 localities from Provincia de Imbabura to Cotopaxi (Figure 1). Since 1989, San Francisco de Las Pampas and the protected forest surrounding it (e.g. Bosque Integral Otonga, BIO) have been the subject of numerous inventories and studies of flora and fauna [27,28], including amphibians [29,30], but *A. longirostris* has not been found [EET, pers. obs.]. Thus, this population might be extirpated. Search effort at Las Pampas and BIO have been immense; however, it is difficult to quantify it given that hundreds of students, researchers, and reserve guards that have been in the area, many of them doing amphibian searches during about 3 decades.

Additionally, Bustamante et al. [31] report its absence in a monitoring study in Río Faisanes, Provincia Pichincha, a locality where it was recorded until the mid-eighties. Also no recent records exist from the Mindo region, Provincia Pichincha, a zone commonly visited by ecotourists and scientists working on biodiversity [13]. Based on this information, the categorization of *Atelopus longirostris* in the IUCN Red List has changed slightly over time. Chronologically, it has been considered either Critically Endangered [32,33], Extinct

[34], or Critically Endangered (Possibly Extinct) [35]. Currently, it fits the Critically Endangered category and we eliminated the Possibly Extinct tag, which has been developed to identify those critically endangered species that are likely already extinct, but for which confirmation is required [6].

The western slopes of Cordillera Occidental de los Andes of Ecuador harbors at least four species of *Atelopus* (*A. coynei* Miyata 1980, *A. longirostris*, *A. lynchi* Cannatella 1981, and *A. mindoensis* Peters 1973). Among them, a population of *A. coynei* was found in 2012 by Andreas Kay [36], whereas *A. lynchi* and *A. mindoensis* are missing from Ecuador since 1977 and 1989, respectively.

The rediscovery of *Atelopus longirostris* suggests that some of the species that were thought as Possibly Extinct have actually survived strong bottlenecks and are somehow persisting, even though their populations seem quite small. In recent years, a relict population of *Rhaebo olallai* Hoogmoed 1985 was found [37], and at least four species of Ecuadorian *Atelopus* previously thought to be Critically Endangered (Possibly Extinct) have been rediscovered. They are *A. nepiozomus* Peters 1973 [38], *A. palmatus* Andersson 1945 [38], *A. bomolochos* Peters 1973 [39], and *A. ignescens* (Cornalia 1849) [40]. These patterns of rediscoveries and persistence and/or recoveries have been discussed for *Atelopus* by Lötters et al. [41], and are discussed for other taxa in Central America and North America (e.g. [42,43]). Whether these patterns of apparent favorable conditions reported in distant and unrelated places in America reflect common causes or are independent events requires further investigation. These *Atelopus* findings in Ecuador of either small or seemingly small populations of *Atelopus* can be explained by an increase of awareness of the amphibian extinction problem allied to the increase of batrachologists or naturalists exploring new areas. Nonetheless, why these populations of a few species persisted in only a single or few sites of their once historical more widespread distribution is a matter of further research. Major potential culprits of the sudden amphibian die-offs and declines such as climate change and/or pathogens or their interaction [7,11] seem to be, at least temporally, not acting strongly at sites where nearly extinct populations have been rediscovered. If these factors were responsible for the sudden declines, their persistence suggests that they have changed to mild conditions. Our findings of a high point prevalence of chytrid in the amphibian community at Junín reveals a similar pattern of *Bd* prevalence recently described for Las Gralarias (a locality 33 km South West of the *A. longirostris* site in Junín) in the western Cordillera de los Andes [44]. Absence of *Bd* in *A. longirostris* might be explained as a sampling artifact, given the small sample size ($n = 4$). At both sites no evidence of mortality due to *Bd* was found. In others sites in Ecuador (e.g. Tarvin et al. [26]) in the Amazonian region, no evidence of mortality due to *Bd* has been found either. Recent data of the presence of *Bd* in the Neotropics since historical times (e.-g. as early as 1863 in the Andes of Bolivia [45]) challenges previous hypothesis about *Bd* as the main culprit of the drastic and enigmatic amphibian declines especially occurred in the late 1980s and early 1990s; thus further research is needed. Several hypotheses have already been discussed [44–49], among which interactions between pathogens (e.g. chytrid strains, ranavirus), host's evolutionary history, and environmental factors (e.g. climate change, dry seasons) might be involved.

Nonetheless, it is clear that the genus *Atelopus* continues to be highly endangered and that the conservation of relict populations and species of *Atelopus* remains a challenging multidisciplinary task of *in situ* and *ex situ* actions, as has been discussed elsewhere [50,51]. For example, in Ecuador 29 species of *Atelopus* are Critically Endangered, thus nearly extinct, 15 species have not been sighted in at least 10 years and most of them probably are extinct, and for none there are genetically viable populations, although 3 species have been successfully bred (Table 1).

The reappearance of *Atelopus longirostris* in the Intag region of Ecuador constitutes a unique and possibly unrepeatable opportunity to save this endemic species from extinction. Pragmatic emergency actions, both *ex situ* and *in situ*, are required to accomplish this objective [50,52–54]. *Atelopus longirostris* is a priority species recommended for *ex situ* rescue by the Amphibian Conservation Needs Assessment workshop for Ecuador, done in May 21–24 of 2012 [55]. For that purpose, the captive assurance colony we initiated is a first step that would avoid the impacts of current *in situ* threats that the species currently suffers, such as: chytrid presence and high prevalence, deforestation, predation, pollution, rising rivers, habitat degradation and fragmentation, trout presence on the rivers, and mining exploration. Other threats such as other diseases (e.g. ranaviruses) and climate change might also be affecting them, but not data at the site are available. Our initial survey and sampling effort revealed much fewer individuals than we would expect for a healthy *Atelopus* population, thus we suspect that the population numbers are extremely low and that a bottleneck occurred and survival *in situ* is far from assured. Certainly the three surviving frogs at the *ex situ* program do not grant a genetically viable population either, and efforts should be taken to increase the number of founders, especially with a focus on catching tadpoles, bringing them through metamorphosis in laboratory conditions, and releasing most of them as frogs, when they might be able to persist better, while some frogs would be retained as founders. McGregor Reid and Zippel [56] summarize and discuss criticism to *ex-situ* programs. We have chosen a rapid response [57], especially when considering the serious threat of opencast mining activity that is underway. Also, recent progress by Centro Jambatu, in developing technologies of maintenance and breeding *Atelopus* in captivity [58] let us to

be optimistic that we are doing the right choice in this particular case. In contrast, some cases of rediscovered *Atelopus,* in low numbers, resulted in the documentation of their population extirpation or the species possible extinction [26,59,60].

Under the assumption that the individuals we found represent only a portion of a population still existing, expansion of the reserve of the Junín Community to include the patch of currently non-protected forest where *Atelopus longirostris* occurs is pivotal, as is the restoration of the habitat between them, to grant the connectivity among this isolated putative metapopulations. Also, the restoration of associated river shores is critical to allow the normal movement of individuals to the breeding sites in the Chalguayacu and Junín river basins. The fact that we found females up to 410 m in a straight line from the river, and males between 40 and 50 m from it, suggests that females go up to the top of the hills to mature, and then go back down to the river banks for breeding. For this shift to occur it is necessary the forest to be in good condition, from the banks of the river up to the top of the hills.

Current mining activities, which are in the advanced mining exploration phase, for opencast copper exploitation of the mining concession Llurimagua at the headwaters of the Chalguayacu and Junín rivers are of high environmental impact [59]. Deforestation to built trails and well drilling is active at this time. Land slices in the headwaters of the Chalguayacu and Junín rivers are causing erosion, resulting in increased sedimentation on the rocks of these bodies of water. The sedimentation presumably will affect growth of algae, which are the main food of *Atelopus* tadpoles. Additionally, current water contamination by non-treated thermal waters from well drilling and other chemicals (e.g. high levels of arsenic as reported by Knee and Encalada [62]) are a serious threat to *Atelopus longirostris* tadpoles. If mining activity continues, it will cause serious forms of water pollution [61]. Because of these factors, it is of great urgency to stop mining and forest destruction in the area and to prevent the disposal of any kind of pollutants into the rivers and streams. Current and potential threats related to mining activities [63] should be discouraged. An *in situ* controlled management program is also essential. For this, it is a priority to initiate a census and monitoring program of the species. It is also necessary to further explore other sites where the species could potentially exist, especially in Cordillera of Intag and other areas of its distribution that have not been explored yet. Simultaneously, it is essential to start studies of the biology of the species, with emphasis on its reproductive biology and behavior. Climate, pathogens, and both physiological and genetic variables associated with the survival of this population need to be evaluated. This way we might be able to gain a better understanding of how this population has survived the environmental and disease impacts that have been mentioned, whereas other populations of this and other *Atelopus* species did not survive.

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Author contributions

EET and GPO collected specimens, wrote sections of the MS, and revised the MS. LAC identified species and wrote the MS. NP did the chytrid analyses and revised the MS.

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ORCID

Luis Aurelio Coloma http://orcid.org/0000-0003-0158-2455
Gustavo Pazmiño-Otamendi http://orcid.org/0000-0002-8788-7542

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