UNIVERSIDAD REGIONAL AMAZÓNICA IKIAM

FACULTAD DE CIENCIAS DE LA VIDA

CARRERA DE INGENIERÍA EN BIOTECNOLOGÍA

Detection of the lethal fungus *Batrachochytrium dendrobatidis* (Chytridiomycota) and molecular characterization of cultivable skin bacteria associated with three critically endangered species of *Atelopus* (Anura: Bufonidae) in Ecuador.

Proyecto de investigación previo a la obtención del Título de:

INGENIERA EN BIOTECNOLOGÍA

AUTORA: JOMIRA KATHERINE YÁNEZ GALARZA

TUTOR: PhD. HUGO MAURICIO ORTEGA ANDRADE

COTUTOR: PhD. LEOPOLDO ANTONIO NARANJO BRICEÑO

Napo – Ecuador

2022

DECLARACIÓN DE DERECHO DE AUTOR, AUTENTICIDAD Y RESPONSABILIDAD

Yo, Jomira Katherine Yánez Galarza con documento de identidad N° 0928912229, declaro que los resultados obtenidos en la investigación que presento en este documento final, previo a la obtención del título Ingeniera en Biotecnología son absolutamente inéditos, originales, auténticos y personales.

En virtud de lo cual, el contenido, criterios, opiniones, resultados, análisis, interpretaciones, conclusiones, recomendaciones y todos los demás aspectos vertidos en la presente investigación son de mi autoría y de mi absoluta responsabilidad.

Tena, 09 de febrero de 2022

Firma:

Jomira Katherine Yánez Galarza
C.I: 0928912229

CERTIFICADO DE DIRECCIÓN DE TRABAJO DE INTEGRACIÓN CURRICULAR

Certifico que el trabajo de integración curricular titulado: “Detection of the lethal fungus *Batrachochytrium dendrobatidis* (Chytridiomycota) and molecular characterization of cultivable skin bacteria associated with three critically endangered species of *Atelopus* (Anura: Bufonidae) in Ecuador”, en la modalidad de: artículo original en inglés, fue realizado por: Jomira Katherine Yánez Galarza, bajo mi dirección.

El mismo ha sido revisado en su totalidad y analizado por la herramienta de verificación de similitud de contenido; por lo tanto, cumple con los requisitos teóricos, científicos, técnicos, metodológicos y legales establecidos por la Universidad Regional Amazónica Ikiam, para su entrega y defensa.

Tena, 21 de febrero de 2022

Firma:

Firmado electrónicamente por:
HUGO MAURICIO ORTEGA ANDRADE

Hugo Mauricio Ortega Andrade

C.I: 1717270811

Firma:

Firmado electrónicamente por:
LEOPOLDO ANTONIO NARANJO BRICENO

Leopoldo Antonio Naranjo Briceño

C.I: 1758579294

AGRADECIMIENTOS

A la Universidad Regional Amazónica Ikiam, su creación fue una puerta abierta a los sueños que tuve desde niña. A sus maestros, por brindarme las herramientas para descubrir el camino que quiero seguir.

Aunque no existen palabras suficientes para expresar toda mi gratitud, al Dr. Mauricio Ortega Andrade. Por transmitirme su pasión por los anfibios, el campo, la ciencia. Por permitirme crecer como profesional trabajando en sus proyectos de investigación y apoyar mi anhelo de realizar este proyecto desde el día uno, cuando le hablé de él en el páramo de la RBCC. Por ser un gran amigo y no dejarme marchitar cuando creí que ya no podía más.

Al Dr. Leopoldo Naranjo Briceño por designarme una tarea de clases que despertó tanta curiosidad en mí que no dejé de hablar de ello hasta hoy. Por aceptar guiarme en este proyecto compartiéndome su sabiduría e impulsándome a explotar mis capacidades.

A MSc. Andrea Carrera Gonzalez por haber sido mi maestra de laboratorio y enseñarme que es posible mostrar excelencia hasta en los más mínimos detalles. Por su invaluable apoyo técnico y logístico durante esta investigación. Por su paciencia y empatía.

DEDICATORIA

A mis padres, Oliveros Yánez y Alexandra Galarza, por no cortar mis alas cuando quise seguir mis sueños lejos de casa. Por todos los sacrificios que han hecho por mi hermano y por mí. Por su amor y apoyo incondicional.

A mi Pinina, por siempre alegrarse al verme volver. Por irme a saludar cuando estaba cansada, y por haber sido la mejor compañía para mis papás. Te atesoraremos por toda la eternidad.

A mis amigos por escucharme y darme ánimo en los momentos de quebranto.

A mi familia por su cariño y consejos.

A mi abuelito Víctor Yánez por protegerme y consentirme tanto.

A mi abuelita Flor Román, cuyos ojos azules desearía me estuvieran viendo hoy, a ella le dedico mi trabajo, mi alegría y mis lágrimas. Por tener la certeza de que lo lograría.
Algún día volveremos a estar juntas.

TABLA DE CONTENIDO

ÍNDICE DE TABLA

ÍNDICE DE FIGURAS

ÍNDICE DE INFORMACIÓN SUPLEMENTARIA

RESUMEN

La quitridiomicosis es una enfermedad que ha devastado poblaciones de anfibios alrededor del mundo y es causada por el hongo *Batrachochytrium dendrobatidis* (Bd). Sin embargo, existen bacterias que habitan la piel de las ranas que son capaces de inhibir el crecimiento de Bd y evitar sus efectos letales. En esta investigación, se evalúa la presencia de Bd y las bacterias cutáneas cultivables asociadas a comunidades nativas de *Atelopus balios*, *A. bomolochos*, y *A. nanay* en los Andes centrales y la costa sur del Ecuador. Mediante PCR convencional, se detectó la presencia de Bd en el 91.7% (11/12) de los anfibios muestreados. A través de secuenciación del gen 16S rRNA y análisis filogenético, se encontró que las bacterias aisladas pertenecen a 10 géneros: *Pseudomonas* (31.4%), *Stenotrophomonas* (14.3%), *Acinetobacter* (11.4%), *Serratia* (11.4%), *Aeromonas* (5.7%), *Brucella* (5.7%), *Klebsiella* (5.7%), *Microbacterium* (5.7%), *Rhodococcus* (5.7%), y *Lelliottia* (2.9%). Calculando el índice de similitud de Jaccard (J), se determinó que las comunidades bacterianas se asemejan según el hábitat y la especie hospedera, pero no por el estado de infección de los especímenes. Además, los hábitats de tierras bajas mostraron un mayor número de géneros bacterianos en comparación al páramo. La prevalencia de Bd y la composición bacteriana encontrada podría estar determinada por reservorios de Bd, la biología de *Atelopus*, y las condiciones ambientales intrínsecas de su habitad. Esta investigación puede servir como base para futuras estrategias integradas de conservación de *Atelopus* utilizando consorcios de bacterias cutáneas como herramientas de biocontrol.

Palabras clave: Amphibia, Bufonidae, hongo quitridio, bacterias cutáneas, Andes centrales.

ABSTRACT

Chytridiomycosis is a fungal disease that has resulted in massive amphibian die-offs worldwide and is caused by the fungus *Batrachochytrium dendrobatidis* (Bd). However, there are bacteria inhabiting frog's skin (skin-bacteria) that inhibit Bd growth and avoid its lethal effects. This research evaluates the Bd presence and cultivable skin bacteria associated to native populations of *Atelopus balios*, *A. bomolochos*, and *A. nanay* in the central Andes and coastal southern of Ecuador. Through conventional PCR, Bd was detected in 91.7% (11/12) of sampled amphibians. Sequencing of 16S ARNr and phylogenetic analysis allowed to assign the isolated skin-bacteria to 10 genera: *Pseudomonas* (31.4%), *Stenotrophomonas* (14.3%), *Acinetobacter* (11.4%), *Serratia* (11.4%), *Aeromonas* (5.7%), *Brucella* (5.7%), *Klebsiella* (5.7%), *Microbacterium* (5.7%), *Rhodococcus* (5.7%), and *Lelliottia* (2.9%). were calculated for comparisons in bacterial communities. Calculating Jaccard similarity indexes (J), it was determined that the bacterial communities are similar according to the habitat and the host species, but not according to the specimen's infection status. Bd prevalence and cutaneous bacterial composition could be influenced by Bd reservoirs, *Atelopus* biology, and intrinsic environmental conditions. This research can serve as the basis for future integrated conservation programs of endangered Amphibian species using Skin-Bacteria consortiums as powerful biocontrol tools.

Keywords: Amphibia, Bufonidae, chytrid fungi, skin bacteria, central Andes.

Detection of the lethal fungus *Batrachochytrium dendrobatidis* (Chytridiomycota) and molecular characterization of cultivable skin bacteria associated with three critically endangered species of *Atelopus* (Anura: Bufonidae) in Ecuador.

Introduction

A fungal pandemic has devastated amphibians since the 90s, when the first cases of massive die-offs on pristine areas were reported [1]. Multidisciplinary investigations showed that amphibian declines were caused by the fungus of Asian origin *Batrachochytrium dendrobatidis* (hereafter, Bd) [2]. This is the best-known species related to the development of Chytridiomycosis, a lethal disease for frogs, toads, and salamanders [3,4]. Bd reproduces using asexual zoospores with a single flagellum that can move in aquatic environments. The infection is unleashed when zoospores contact the host's keratinized tissues (mouthparts in tadpoles and skin in adults) [5,6]. Clinical signs of Chytridiomycosis are lethargy, abnormal posturing, seizures, severe cutaneous disorders, and even sudden death [6–8]. Nevertheless, there is evidence that some amphibian species are more tolerant to Bd than others, avoiding sickness development. This resilience is linked with their skin microbiota [9,10].

The amphibians' skin has a superficial layer composed of microorganisms such as viruses, bacteria, and fungi known as cutaneous microbiota, which establishes symbiotic relationships of commensalism or mutualism [10]. On the way to understand the role of cutaneous microbiota in the development of chytridiomycosis, it was found that skin bacteria was able to produce secondary metabolites capable of inhibiting Bd growth [9]. Among them, the molecules 2,4-diacetylploroglucinol produced by *Lysobacter gummosus*, indole-3-carboxaldehyde, and violacein produced by

Janthinobacterium lividum. Evidence show that Bd inhibition is determined by metabolites action and not because of competition between microorganisms [11,12].

Until now, chytridiomycosis treatment is based on chemical products that can be harmful for the amphibian skin [13]. For this reason, anti-Bd bacteria have raised expectations about their potential to be used as amphibian protectors and, therefore, as Bd bio-controllers [14]. Flechas *et al*. [15] indicate that bacteria such as *Janthinobacterium lividum* effectively prevent the disease on species such as *Rana muscosa*, while not on others, such as *Atelopus zeteki*. The specificity of the bacterial strain-pathogenic fungus interaction could indicate that the proposed treatment should be carried out with indigenous bacteria of the target hosts. Efforts must be increased to of threatened toads and frogs. Although Ecuador is one of the countries with the highest number of amphibian species [16], it has few studies characterizing amphibian skin bacteria. For instance, Bresciano *et al*. [17] report the anti-Bd bacteria *J. lividum*, *Pseudomonas fluorescens* and *Serratia* sp., isolated from *Gastrotheca riobambae*.

Several studies have recorded Bd presence in the provinces of Carchi, Azuay, Chimborazo, Pichincha, Imbabura, El Oro, Napo and Orellana [17–23]. These data show that Bd is distributed in the three continental regions of Ecuador, indicating that the fungus has adapted to different environmental conditions. Bd has been detected in amphibian species of the families Bufonidae, Centrolenidae, Hylidae, Hemiphractidae, Craugastoridae, Leptodactylidae. However, Bufonidae is the largest family of threatened species in Ecuador with dramatic population declines possibly related with Bd, among others [16]. Twenty-five (44.6%) of these species belong to the *Atelopus* genus.

Amphibians of the *Atelopus* genus are organisms with diurnal activity and have a larval stage. Their eggs are deposited in water bodies, the tadpoles of this group are

rheophilic (preference for flowing waters), whereby they occupy a unique niche associated with streams [24]. Tadpoles have an abdominal suction cup located behind the oral disc and with which they can adhere to submerged rocks, from where they feed [25] and can also be a Bd substrate [3]. The mentioned ecological characteristics, as well as *Atelopus* species life history, home range, habitat type, and threats [26] can help understand the genus vulnerability to Bd.

The first case of chytridiomycosis in South America corresponds to a specimen of the endemic Ecuadorian species *Atelopus bomolochos* that was collected in 1980 [18]. This evidence shows that the fungus was already found in wild populations before these amphibian declines, observed in the late 1980s. *A. bomolochos* was believed extinct for 28 years, and it is categorized as critically endangered by the IUCN [27]. To date, no populations have been found outside the Área de Conservación Municipal y Uso Sustentable (ACMUS) Cordillera Oriental of Azuay province [28].

Atelopus nanay, another species declared critically endangered, lives only in the Cajas National Park, Azuay [29]. In the last five years, Bd cases have been reported in this population [20,30], indicating the persistence of the pathogen. Other populations of endangered species may be affected by the fungus; which could be the situation of the critically endangered species *Atelopus balios*. Its distribution is restricted to a threatened area of 55 km^2 [31], where Bd detection is suspected [32].

This study aims to (1) detect the presence of Bd in native populations of *Atelopus balios*, *A. bomolochos*, and *A. nanay* in the central Andes and coastal southern of Ecuador, (2) molecularly identify their cultivable skin bacteria, and (3) determine the differences of the cultivable bacterial communities characterized between the sampling sites, the three *Atelopus* species, and the infected/uninfected specimens. Being Bd possibly implicated in declines of amphibian populations [33], detecting the fungus in

the mentioned populations is expected. Based on previous approaches [17,34], it is hypothesized that the *Atelopus* cutaneous cultivable bacteriome is composed at least of the genera *Janthinobacterium*, *Pseudomonas,* or *Serratia,* and this composition differs between the habitat, host specie and infected/uninfected sampled individuals [35,36]. This study is expected to serve as the basis for a future integrated strategy in conservation programs for habitat protection, monitoring, and biocontrol of this fungal pathogen in critically endangered amphibian species.

Methods

Study species and sampling sites

Four populations of three species of *Atelopus* were included in the present study. The first two correspond to lowland population of *A. balios* from Guayas province, one is located in Cerro Las Hayas (S 02.72452, W 79.61892) and the other in Estero Arenas (S 02.75077, W 79.61269). The other two correspond to highland populations of two *Atelopus* species from Azuay province, *A. bomolochos* from Cerro Negro (S 03.15675, W 78.84538), and *A. nanay* from Cajas National Park (S 02.88337, W 79.30685) (see Figure 1). The three *Atelopus* species are considered as Critically Endangered (CR) by the IUCN [16].

Site	T (°C)	RH (%)	Elevation (m a.s.l.)	Latitude	Longitude
Cerro Las Hayas	23.9	90.5	107	-2.72452	-79.61892
Estero Arenas	24.9	85.3	203	-2.88337	-79.30685
Cerro Negro	12.2	82.1	3064	-3.15675	-78.84538
CNP	8.4	80.8	3800	-2.75077	-79.01269

Figure 1. Sampling sites in the province of Azuay and Guayas, central Andes of Ecuador. Studied species are shown: (a) *A. balios*, (b) *A. bomolochos*, (c), and *A. nanay*. T = temperature, RH = relative humidity, CNP = Cajas National Park.

Sampling Strategy

Amphibians were sampled by visual encounters on longitudinal transect surveys near permanent streams. To avoid transient bacteria, the specimens were captured using fresh disposable nitrile gloves and rinsed with sterilized ultrapure water [15]. Swabbing was performed by duplicate with CITOSWAB SERIES® Microbiological Collection and Transport System Amies Charcoal Gel. Individuals were swabbed fivefold on their belly, groin, legs, feet, and hands [37]. The first duplicate was immediately stored on its transport system according to manufacturer's specifications for bacteria isolation. The second sample was preserved in 1.5 mL cryovial containing 400 µL lysis buffer (Tris-HCl 0.18 M; EDTA 10 mM, SDS 1 %, pH 8.2), refrigerated at -4°C and processed for DNA isolation within 24 h after sampling.

Bacterial isolation

One swab duplicate was plated through the streaking method in Luria-Bertani Agar (37 g/L) in duplicate and incubated at 30°C for two days. Bacterial morphotypes were defined according to the macroscopic characteristics of the obtained colonies (i.e., color and form). Single colonies of each bacterial morphotype by sample were subcultured on fresh LB Agar to obtain axenic cultures. Each isolate was cryopreserved in Mueller Hinton Broth (21 g/L) with 30% glycerol at -80°C.

DNA isolation and PCR amplification

Bd detection

Genomic DNA isolation was performed using the Wizard® Genomic DNA Purification Kit with modifications. First, swabs were shaken in the lysis buffer, where they were stored using sterile forceps. Then, 5µL of Proteinase K (50 µg/µL) was added, and

maceration was performed by pressing a sterile pistil against the micro tube walls. After vortexing for 20 s at maximum speed (Personal Vortex V-1 plus), it was incubated in a thermoblock at 65°C for 30 min. 2.5 µL RNAse was added and the solution was mixed by pipetting. Later, it was incubated at 37 ° C for 30 min. Next, 100 µL of protein precipitation solution was added, vortexed for 20 s, and incubated for 10 min on ice. Samples were centrifugated at 12000 rpm for 5 min, then the supernatant was recovered and transferred to a 1.5 mL clean microtube. Later, 40 µL of sodium acetate (3M, pH 5.2) and 1000 µL of absolute ethanol were added. It was centrifuged for 5 min at 12000 rpm. The supernatant was discarded, and the remaining drops were removed with the help of a micropipette after a spin. The microtube was dried in a thermoblock at 60°C until no liquid residues were observed. Finally, the DNA was resuspended in 25 µL of autoclaved MilliQ H_2O.

The specific primers ITS1-3 Chytr-F (5'-CCTTGATATAATACAGTGTGCCATATGTC-3') and 5.8S Chytr-R (5'-AGCCAAGAGATCCGTTGTCAA-3') were used [38], which result in a 146 bp amplicon. Reactions contained 7.5 µL of autoclaved MilliQ H_2O, 1 µL of each primer at a concentration of 10 mM, 12.5 µL of TaqMan Environmental Master Mix 2.0, and 3 µL of DNA for a total volume of 25 µL per reaction. The thermal cycle was programmed in the miniPCR App v2.0 software with the following conditions: initial denaturation at 95°C for 120 s, 35 cycles of denaturation at 95°C for 60 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, and final extension at 72°C for 300 s.

Molecular identification of bacteria

Bacterial DNA isolation was performed following the protocol for isolating genomic DNA from gram-positive and gram-negative bacteria of the modified Promega

Wizard® genomic DNA purification kit [39]. PCR amplification was performed using a miniPCR ™ mini16 thermal cycler. The 16S bacterial rRNA was amplified using the primers 16s-F (5'-GGAGGCAGCAGTAGGGAATA-3 ') and 16s-R (5'-TGACGGGCGGTGAGTACAAG-3') [40]. PCR Master Mix contained 15.5 µL of sterile MilliQ H_2O, 2.5 µL of Invitrogen's 10X Buffer Green, 0.75 µL of $MgCl_2$, 0.5 µL of dNTPs, 0.5 µL of each primer (10 µM), 0.1 µL of Invitrogen's Platinum Taq DNA Polymerase, and 5 µL of DNA (25 ng/µL) for a final volume of 25 µL per reaction. PCR conditions were as follows: initial denaturation at 95°C for 300 s, 34 cycles of denaturation at 94°C for 60 s, annealing at 54°C for 45 s and extension at 70°C for 60 s, and final extension at 70°C for 480 s.

PCR products were evaluated by electrophoresis in a blueGel ™ electrophoresis with built-in transilluminator equipment, using 2% agarose gel in TBE 1X with 1X GelGreen ™ Nucleic Acid stain. The 100 bp DNA Ladder (Promega) was used to confirm the size of the amplified products. Bd (146 bp) and bacteria (1062 bp) amplicons were purified from agarose gel using the Wizard® SV Gel and PCR Clean-Up System [41].

Sequencing data processing

Purified products were sent to Macrogen Co. Ltd. (South Korea) for Sanger DNA sequencing. Sequences were trimmed, edited, and assembled using Geneious v.5.4.7 software. Finally, 16S rRNA sequences were identified using BLAST/n against the complete GenBank nucleotide database with default parameter settings [15].

Taxonomy and phylogenetic analysis

The 16S rRNA consensus sequences obtained and additional 135 sequences (>1000 bp) from bacteria from GenBank were aligned using Geneious v.5.4.7. In addition, the

Synechococcus elongatus sequence (AB871649) was employed as outgroup.

Mesquite v3.0 was used to export the aligned matrix in NEXUS format for MrBayes with the default parameterization. jModelTest2 software was used to test the best nucleotide substitution model on the CIPRES platform <http://www.molecularevolution.org/index>.

Once the best nucleotide substitution model was stablished, the phylogenetic analysis was performed with Bayesian methods on the aligned matrix in MrBayes v3.2.2 in CIPRES. The following parameters were configured: two parallel sections of the Metropolis coupled Monte Carlo Markov chain, two independent runs, 20 million generations, with three hot chains (temperature 0.2), saving a tree and its statistics every 1000 generations; and a burn fraction of 25% of the trees.

Tracer v1.7.1 software was used to validate the phylogenetic models based on the distribution pattern and stability of the likelihood values evaluated from the Effective Sample Size (ESS> 200) parameter estimates across generations.

The taxonomic assignation was based on the Basic Local Alignment Search Tool (BLAST/n) similarity and the phylogenetic position of each sample to bacterial lineages. Unconfirmed genetic samples were labeled with *affinis* (aff.) and *confer* (cf.) to refer to similar or comparative taxonomic identities.

Community composition

A similarity analysis was performed to compare the composition between cultivable bacterial communities among species and positive/negative samples with the Jaccard coefficient (J) in PAST software [42]. In addition, a hierarchical clustering routine by unweighted pair group average (UPGMA) algorithm based on J was performed in PAST to qualitatively asses the distribution of bacterial communities by sampling site.

Results

Bd detection

A total of 12 specimens were sampled from *A. balios* (n=7), *A. bomolochos* (n=2), and *A. nanay* (n=3). One specimen of *A. nanay* (sample code: HMOA 2399) was found dead in a decomposition state under a rock in Cajas National Park, which was positive for Bd. No chytridiomycosis clinical signals were observed in the other toads. The fungus was detected in the four sampling sites and the three *Atelopus* species, showing a prevalence of 91.7% (11/12). In addition, one negative specimens of *A. nanay* was identified (Table 1, Figure S1-S4).

Table 1. Bd prevalence on sampled specimens of *Atelopus*.

Site	Sample code HMOA	Specie	Bd Status
Cerro Negro	2392	*A. bomolochos*	Positive
	2395	*A. bomolochos*	Positive
Cajas National Park	2397	*A. nanay*	Positive
	2398	*A. nanay*	Negative
	2399	*A. nanay*	Positive
Cerro de Hayas	2415	*A. balios*	Positive
	2416	*A. balios*	Positive
	2417	*A. balios*	Positive
	2418	*A. balios*	Positive
	2419	*A. balios*	Positive
San Miguel	2420	*A. balios*	Positive
	2421	*A. balios*	Positive

Bacterial taxonomy and phylogeny

Identification of 16S rRNA from a total of 35 bacterial morphotypes through BLAST/n search yielded 11 genera (*Acinetobacter*, *Aeromonas*, *Brucella*, *Klebsiella*, *Lelliottia*, *Microbacterium*, *Pantoea*, *Pseudomonas*, *Rhodococcus*, *Serratia,* and *Stenotrophomonas*) belonging to 10 families, 6 orders, 3 classes, and 2 phyla (Tables S1-S2). However, maximum scores, percentage of query coverage, and percentage

identity of genetic samples coincided with multiple sequences from different species. One hundred seventy sequences were downloaded from GenBank to recover the phylogeny, based on BLAST/n similitude for the 16S rRNA gene.

The best topology (log-likelihood -16777.01) was obtained from a matrix with an extended set of 1349 characters (Figure 2, Figure S5). It allowed to identify 22 clades belonging to 10 genera: *Pseudomonas* (31.4%), *Stenotrophomonas* (14.3%), *Acinetobacter* (11.4%), *Serratia* (11.4%), *Aeromonas* (5.7%), *Brucella* (5.7%), *Klebsiella* (5.7%), *Microbacterium* (5.7%), *Rhodococcus* (5.7%), and *Lelliottia* (2.9%) (Figure 3 and Table S3). *Pantoea* was renamed as *Klebsiella* based on the phylogenetic position (Figure 2). Non-attachment of this study bacterial sequences to BLAST/n suggested species was observed as well as Bayesian posterior probabilities < 0.7 (Figure S1). Hence, a total of 12 (34.3%) samples were assigned as *affinis* (aff.), whereas 17 (48.6%) were assigned as *conferring* (cf.).

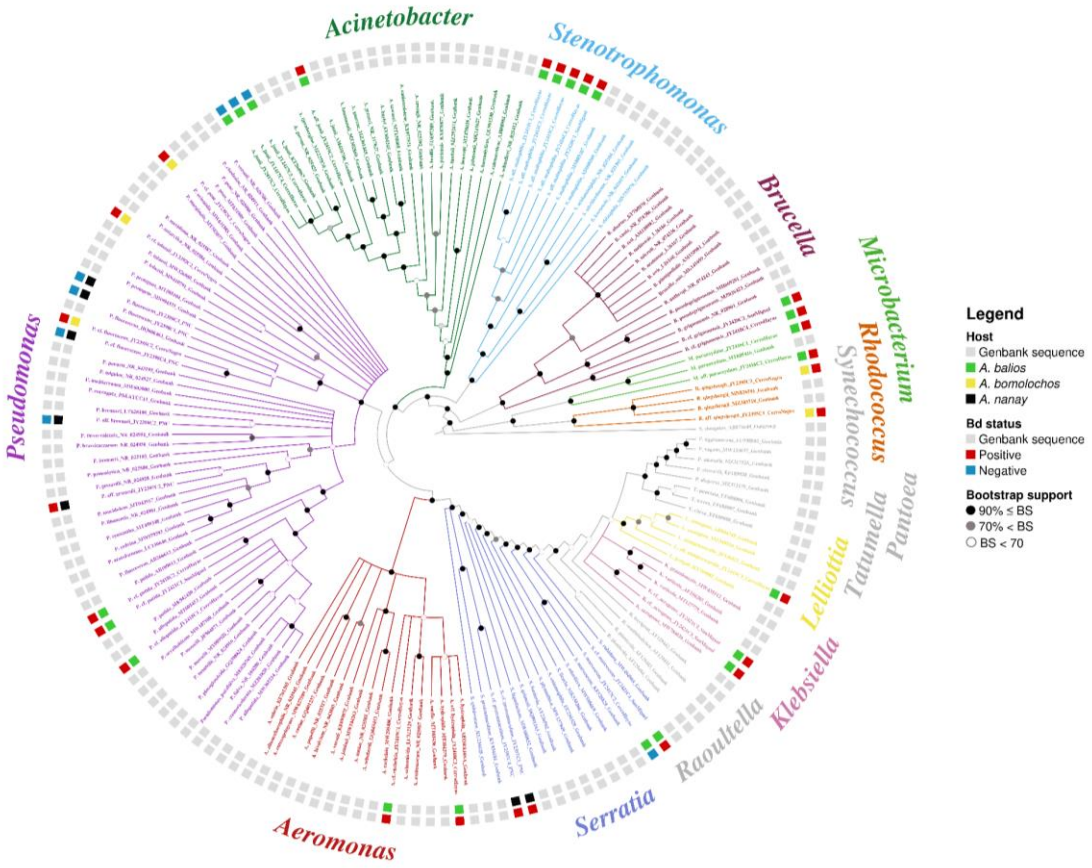


Figure 2. Optimal maximum likelihood tree from the 16s rRNA gene of a matrix (log-likelihood = -16777.01; 1349 aligned sites) showing the phylogenetic relationships of 35 bacteria isolates sequences joined to 135 GenBank sequences. The color per clade label indicates genera. The two rings in the outer circle correspond to the character state: the host species from which the bacteria were isolated (inner ring), and the host species Bd status (outer ring). Supporting values of non-parametric bootstrap (colors on the nodes) are shown.

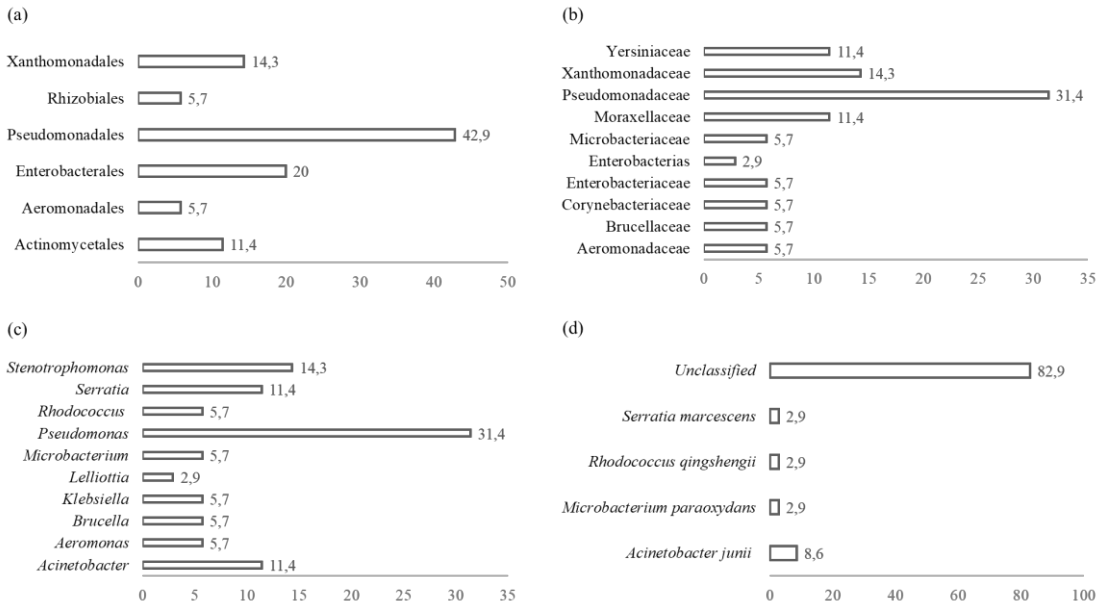


Figure 3. Prevalence of bacterial (a) order, (b) family, (c) genus, and (d) species isolated from the cutaneous microbiome of the sampled *A. balios*, *A. bomolochos*, and *A. nanay*.

Comparison between cultivable bacteria communities

Clustering by the UPGMA hierarchical method (see Figure 4a and 4b) show two major clusters, one composed by the cultivable bacterial communities of the highlands and the other of the lowlands. In addition, Cerro Las Hayas and Estero Arenas exhibited a larger bacterial genera number (n=8 and n=5, respectively) than Cerro Negro and Cajas National Park (n=2 each) (see Figure 4c). A similar pattern can be observed when comparing the cultivable bacterial community among the lowland species *A. balios* and the highland species *A. nanay* and *A. bomolochos* (see Figure 5a). Results from the Jaccard similarity coefficient (J) show that bacterial genera were less similar in *A. bomolochos* and *A. balios* (J= 0.10), while the most significant similarity corresponded to *A. bomolochos* and *A. nanay* (J= 0.33). Only *A. bomolochos* and *A. nanay* shared bacterial species (J=0.13) at a specific phylogenetic level (Table 2).

Table 2. Jaccard similarity coefficient (J) of *Atelopus* species by identified (a) bacteria genera and (b) bacteria clades.

(a) Species compared by bacterial communities at genera level			
Specie	*A. balios*	*A. bomolochos*	*A. nanay*
A. balios	-	-	-
A. bomolochos	0.10	-	-
A. nanay	0.22	0.33	-
(b) Populations compared by bacterial communities at clade-species level			
Specie	*A. balios*	*A. bomolochos*	*A. nanay*
A. balios	-	-	-
A. bomolochos	0.00	-	-
A. nanay	0.00	0.13	-

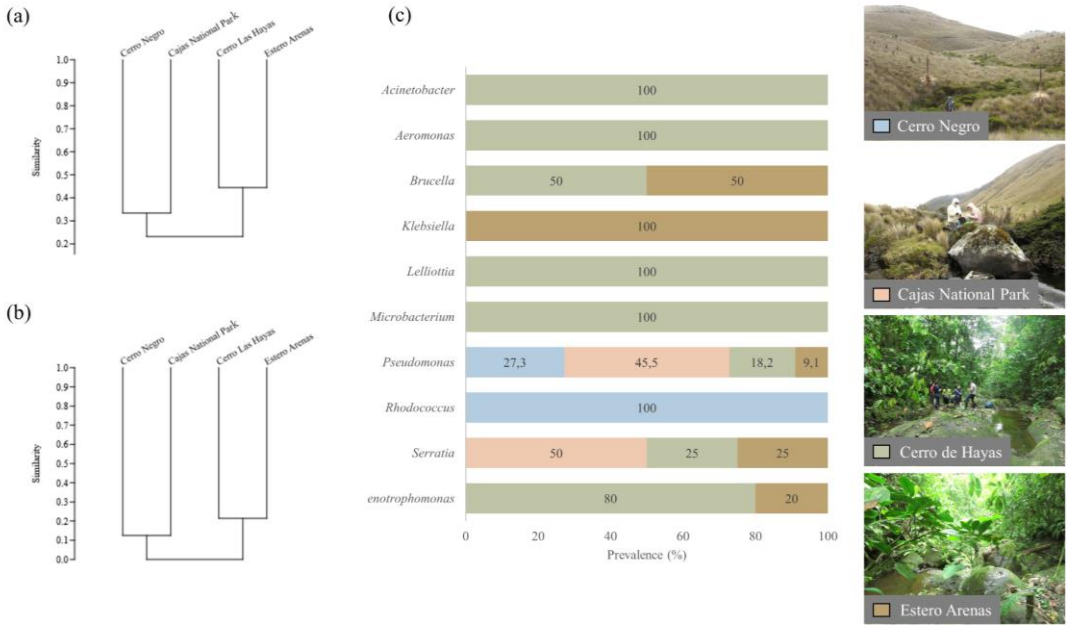


Figure 4. UPGMA dendrograms showing the relationship between bacterial communities of four sampling sites at (a) genera and (b) clade-species level, based on Jaccard Similarity index. (c) Genera prevalence by sampling site.

Cultivable bacterial communities of specimens diagnosed with positive (n=10) or negative (n=1) Bd share a value of 0.1 J at genus and 0.04 J at species-clade level. *Pseudomonas* was the only genus found in both infected and uninfected individuals. In contrast, *Acinetobacter, Aeromonas*, *Brucella*, *Klebsiella*, *Lelliottia*, *Microbacterium*,

Rhodococcus Serratia, and *Stenotrophomonas* were only found in infected toads (Figure 5b). Internal communities' comparison results in high values for shared bacterial genera in *A. nanay* (J= 0.50). All specimens from *A. balios* and *A. bomolochos* were infected.

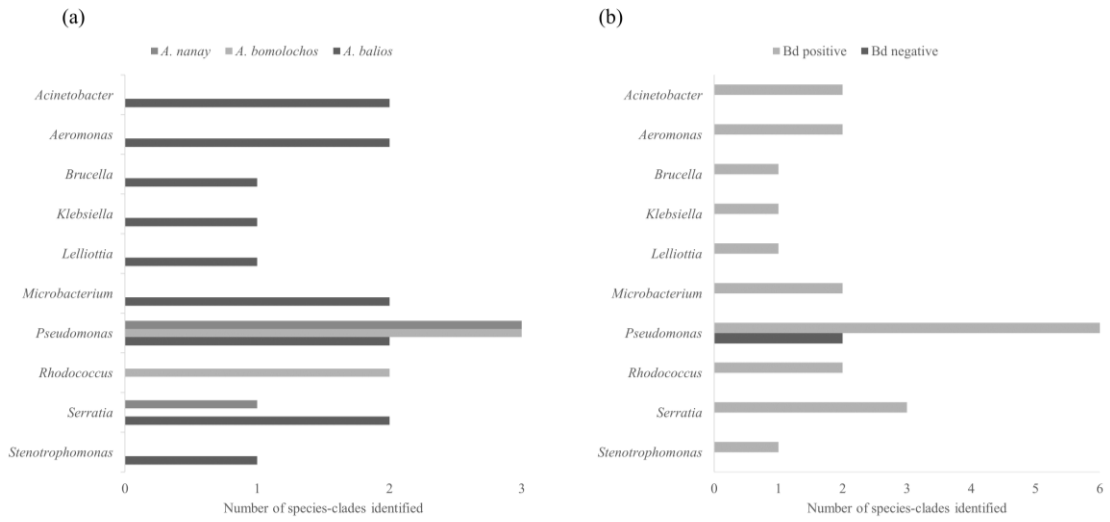


Figure 5. Distribution of bacteria species-clades in (a) the three *Atelopus* species and (b) infected/uninfected toads.

Discussion

Bd presence and skin cultivable bacteria communities were analyzed in native populations of three Critically Endangered *Atelopus* species in Central Andes and coastal Ecuador. The decline of *A. bomolochos* has been related to Bd infection after the histological diagnosis of a museum specimen collected in 1980 [18]. Since its rediscovery, no reports of the fungi associated with this population have been published. According to Siavichay [28], ACMUS Cordillera Oriental conserve favorable population conditions for *A. bomolochos* survival; however, the present study shows that specimens assessed in Cerro Negro are infected by Bd.

In the case of *A. nanay*, Cáceres [30] analyses the species population in Cajas National Park in 2014 to detect Bd by conventional PCR tests, resulting in a 9.1%

infection rate. Despite this report shows a low prevalence value for those populations, this finding is possibly related to the mortality caused by the fungus since most of the infected specimens could have died before the sampling [30]. Although the specimen HMOA 2399 was found dead and tested positive in our sampling, it is not possible to confirm that chytridiomycosis is the direct cause. The present Bd detection on 2/3 (66.6%) *A. nanay* individuals indicates a higher fungus prevalence and its persistence in the zone.

Bd persistence in Cajas National Park could be due to a "latent" stage of Bd that allows it to survive outside the hosts [43], biotic and/or abiotic suitable conditions for the fungus development [44]. It is known that the presence of non-declining amphibian species could act as the Bd reservoirs [45]. Among the Cajas National Park amphibian community, *Gastrotheca* and *Pristimantis* genera have been detected with Bd [30]. It has been observed that amphibians of pond breeding and those of direct development, respectively, are less likely to be infected than stream breeding (i.e., *Atelopus* toads) [46]. These populations may lead to continued exposure of the fungus to species in the community. On the other hand, studies have shown positive Bd growth patterns at cooler temperatures at higher tropical elevations [1,44], the conditions for Cajas National Park and Cerro Negro (Figure 1).

A. balios is a lowland rainforest inhabitant species. This is the first report of Bd infection on the sampled populations in Guayas [32]. However, according to the UICN assessment (2018), specimens collected in 1992 at Río Patul on the border of Azuay and Cañar (350 m a.s.l.), a population 35.7 km apart from our sample sites in Guayas, were infected by the fungus [31]. It could indicate that Bd spread to nearby areas, eventually reaching Cerro Las Hayas and Estero Arenas. Even though that Bd has been particularly prevalent in high elevation in the tropics, it has been detected in amphibian species of

high, mid, and low altitude in Ecuador [19,21–23,46,47]. Cerro Las Hayas (107 m a.s.l.) is perhaps the lowest elevation site where Bd presence has been reported in Ecuador to date.

Phylogeny demonstrates disagreements by presenting isolates sequences that do not fall into the taxonomic name, denoted by the similitude values recovered from BLAST/n. Koski & Golding [48] showed that the closest BLAST/n hit does not imply phylogenetic proximity. Some hypotheses could emerge to explain this pattern. Data from National Center for Biotechnology Information (NCBI) could be outdated, taxonomic names for species currently reclassified, or bacterial species not yet described. Besides, misidentification for close species, which returns multiple compatibility hits in similitude results. Therefore, further studies could subject the isolated strains to a polyphasic approach of bacterial classification [49]. Results from this work resemble a potential wide underestimation of bacterial diversity in the skin of *Atelopus* toads.

Previous studies described the isolation of *Acinetobacter* [50–52], *Aeromonas* [17,50,52–54], *Brucella* [55], *Microbacterium* [50,52], *Klebsiella* [50,51], *Pseudomonas* [17,34,50,52], *Rhodococcus* [52,56], *Serratia* [34,52] and *Stenotrophomonas* [50,52] from the skin of different hosts inhabiting different environments. There are no reports of *Lellitotia* isolations from amphibian skin. Cluster analysis indicates a tendency for isolates to group according similar habitats. There is more similarity among the bacterial communities of Cerro Negro and Cajas National Park highlands (3064-380 m.a.s.l), and Cerro Las Hayas and Estero Arenas lowlands (107-203 m.a.s.l.). These patterns are consistent with Catenazzi *et al*. [34] who maintain that bacteria community composition can be affected by elevation.

Furthermore, *Pseudomonas* was the only genus distributed in a wide range of elevations. It was found in the four sampling sites and the three *Atelopus* species. *Serratia* occurred in the two habitats of *A. balios* and Cajas National Park. This shows the *Pseudomonas* and *Serratia* presence in a wide range of elevations in both paramo and tropical environments. On the other hand, while *Rhodococcus* was only found in *A. bomolochos* in the paramo conditions of Cerro Negro, the other seven genera are only associated with *A. balios* in lowlands. This last can be compared with the also lowland toads *Atelopus aff. limosus*, *A. spurrelli*, and *A. elegans*.

Flechas *et al*. [15] found eight cultivable skin bacteria genera distributed in the three species. Six isolated from *A. elegans* (n=82), five from *A. aff. limosus* (n=80), and six from *A. spurelli* (n=78). Three of these bacteria are shared with *A. balios* (*Acinetobacter*, *Pseudomonas*, *Stenotrophomonas*). Later, Flechas *et al.* [50] found 22 genera distributed in the same *Atelopus* species in the wild. Nineteen isolated from *A. elegans* (n=5), four from *A. aff. limosus* (n=8), and five from *A. spurelli* (n=5). Six of them shared with *A. balios* (*Pseudomonas*, *Acinetobacter, Stenotrophomonas, Microbacterium*, *Klebsiella*, *Aeromonas*). *Janthinobacterium* was not found in our samples. It has been isolated from captive specimens of *Atelopus zeteki* [46] but not from other *Atelopus* toads.

Cultivable bacterial communities diverged between *Atelopus* species as expected. Although the species sampled belong to the same genus, they have their own biological characteristics (i.e., host immunity, skin toxin production, frequency of skin shedding) and inhabit particular environments that could determine their microbiota composition. Thus, this study is consistent with previous evidence that amphibian skin bacterial communities tend to be host species-specific [36,57–59]. The high similarity value between *A. bomolochos* and *A. nanay* could be linked to their biogeography

restricted to highland forests and paramos in the central Andes of Ecuador. *A. balios* is distributed in a completely different environment, in tropical conditions. Previous studies have demonstrated that this kind of habitats favors bacterial abundance [17] and richness [60].

Regarding the Bd effect over the skin microbiota composition, contradictory evidence can be found. Belden *et al*., [61] , Becker *et al*., [62] and Kruger [59] found no variation in skin microbiota caused by Bd. In contrast, Jani and Brigg [35] show disturbance caused by Bd in laboratory and field surveys. Walke *et al*., [63] stand that Bd alters both structure and function of the skin microbiota. The present report provides additional evidence that Bd positive and Bd negative individuals harbor distinct cultivable bacterial communities. Despite the relatively small number of uninfected toads (2/12), Kruger's results suggest that sampling roughly equal numbers of Bd positive/negative members does not interfere in the trend found [59].

Although the strains identification could not be confirmed at the species level, some isolates belong to known anti-Bd genera such as *Acinetobacter*, *Pseudomonas*, *Serratia,* and *Stenotrophomonas* [15,17]. Among the probable species present is *Serratia marcescens*, the potent antifungal metabolite prodigiosin producer. This bacterium had shown up to 100% inhibition against Bd [52]. Also, experiments with *Stenotrophomonas maltophilia* demonstrate its capability to inhibit Bd *in vitro* [64] and modulate the host's ability to survive to chytridiomycosis at certain temperature conditions [65]. *Pseudomonas* is one of the top anti-Bd genera because it has several species with antifungal potential (i.e., *P. entomophila*, *P. azotoformans*, *P. fluorescens*). This group includes non-identified species with high inhibition percentage [34]. Thus, further studies must test the strain's isolated anti-Bd potential.

While beneficial bacteria could be found, others were reported as amphibian pathogens. *Brucella* is a bacterial genus able to produce the zoonotic disease brucellosis. However, since 2008, case reports have been published about bacteria isolated from amphibians (most captive-bred) cataloged as 'atypical' *Brucella*. Their zoonotic potential was recently demonstrated after the first case report [66]. These isolates differ from 'classical' *Brucella* by phylogenetic distance and constitute a different clade of early-diverging strains [55]. Clinical signals (e.g., subcutaneous abscesses, corneal lesions, spinal arthropathy) were observed in some frog individuals but not in all, suggesting that they may be opportunistic pathogenic microorganisms [67]. Finding *Brucella* in two *A. balios* specimens Bd-positive, from two distant sites in our study area, showed a possible coinfection with unknown consequences. A synergistic interaction may compromise the host immune responses, intensifying population declines as predicted for Bd and *B. salamandrivorans* coinfection in newts [68].

Other genera probably play a synergetic role within the skin microbiota. *Microbacterium* genus has been shown to produce nutritive compounds promoting Bd growth [69]. On the other hand, *Aeromonas hydrophila* has broadly been known for causing the 'red leg' disease [70], but it was recently associated with severe pathological clinical signs in eggs and adult frog individuals of *Pelophylax ridibundus* in Saudi Arabia [54]. Despite *A. hydrophila* was previously isolated from the *Atelopus aff. limosus* skin [50], no pathological signs were associated with the bacterium. Last, *Lelliottia* bacteria are motile, non-spore-forming microorganisms [71] with no wild vertebrate hosts reports. Species of *L. nimipressuralis* have been isolated from plants, trees, drinking water, and air [72], organisms and geographical sites unrelated to the *A.*

balios habitat or Ecuador. Therefore, it is hard to predict implications for this strain and toads' symbiosis.

Finally, bacteria communities assessed could be emerging pathogens or promising amphibian probiotics to face Bd; other likely beneficial uses must be considered. For instance, *Acinetobacter junii* produces lipopeptides with antimicrobial and anti-biofilm activity [73]. *Aeromonas encheleia* has been confirmed to rAeduce selenite to elemental selenium (Se). This species has a high bioremediation potential for selenite-contaminated soil, which is responsible for a large-scale Se intoxication of humans and livestock [74]. *Microbacterium paraoxydans* produce antioxidant enzymes and bio-accumulate the carcinogenic compound hexavalent chromium [75], making it a candidate for wastewater treatment. *Pseudomonas brenneri* is the source of thermo-solvent-stable lipase proposed as a biocatalyst for biodiesel production and bioremediation of oil in the environment [76]. The mentioned bacteria are close species to this study isolates, so the examples illustrate the biotechnologically application of those interesting strains detected in *Atelopus* toads.

Conclusion

This is the first skin microbial exploration on critically endangered *A. balios*, *A. bomolochos,* and *A. nanay*. Evaluation over 12 specimens found that 83% of them were infected with Bd (85.7% *A. balios*, 100% *A. bomolochos*, 66.6% *A. nanay*), showing the fungus as a current threat that must be considered to prioritize conservation actions. Besides, ten cultivable skin bacteria genera and 22 species-clades were identified, providing promising strains for disease mitigation that need to be tested in further approaches. Bacterial communities clustered according similar sampling sites, suggesting that *Atelopus* skin bacteria composition could be shaped by habitat

conditions. In addition, most bacteria (7/10 genera) were only found in *A. balios* at tropical sites, while one was only found in *A. bomolochos* at paramo. *Pseudomonas* was the only genus occurring in all sampled sites. Assessment of this genus is highly recommended because of its high prevalence shared on the three *Atelopus* species in both high and lowlands, and its Bd-inhibition rate previously reported. The identified genera also reveal the presence of probably emerging pathogens (whose monitoring should be expanded) and constitute an alert to limit direct contact with amphibians to prevent possible bacterial zoonosis diseases.

Last, it was found that bacterial genera differed between infected and uninfected toads. All genera present in Bd-negative specimens are reported as anti-Bd bacteria. On the other hand, the skin community of Bd-positive specimens is composed of both potential anti-Bd and pathogenic bacteria. Interestingly, this finding could suggest that toads infected with the fungus face opportunistic bacteria as their immune system is depressed. Or otherwise, as no clinical signs were observed, toads and pathogens could be coexisting after a critical period of natural selection. To complement this work and further studies, it is indispensable to increase the sampling size to understand the skin microbiome-amphibian interactions better. Metagenomic analysis from environmental samples (e.g., water and soil) from toads' habitats are also suggested to increase the bacteria spectrum of probable biocontrollers. It is essential to focus research efforts on monitoring emerging diseases as these are the last populations of *A. balios*, *A. nanay,* and *A. bomolochos*. Conservation strategies should be strengthened to protect their habitats, emphasizing the *A. balios* habitats that are strongly threatened by anthropogenic activities, and increasing support to *in-situ* and *ex-situ* programs.

Acknowledgments

This work was supported by the projects “Biomonitoring of aquatic environments in the Amazon using environmental DNA (eDNA)”, “DNA-based monitoring for assessing the effect of invasive species on aquatic communities in the Amazon basin of Ecuador”, “Conservación de Anfibios y Uso Sostenible de Recursos Genéticos (PARG).”, and “Creación de la Unidad de Identificación Molecular de Agentes Infecciosos como Apoyo Médico Científico al Hospital José María Velasco Ibarra ubicado en la ciudad de Tena, Provincia de Napo, Ecuador.” Thanks to G. Morán, N. Espinosa de los Monteros, K. Apunte, and S. Sislema for their practical contributions. I gratefully acknowledge the field assistance and logistic support of F. Siavichay, J. Cáceres, J. Webster, L. Oyagata, E. Ocampo, J. Arpi, C. Parra, A. Paredes, and A. Ocampo. The Ministerio de Ambiente from Ecuador provided research permit number MAATE-DBI-CM-2021-0177.

References

[1] Piotrowski JS, Annis SL, Longcore JE. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia. 2004;96:9–15.

[2] Hanlon SJ, Rieux A, Farrer RA, et al. Recent Asian origin of chytrid fungi causing global amphibian declines. Science. 2018;360:621–627.

[3] Bradley PW, Brawner MD, Raffel TR, et al. Shifts in temperature influence how *Batrachochytrium dendrobatidis* infects amphibian larvae. PLoS ONE. 2019;14:1–13.

[4] Dillon MJ, Bowkett AE, Michael J, et al. Tracking the amphibian pathogens *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* using a highly specific monoclonal antibody and lateral-flow technology. Microbial Biotechnology. 2016;10:381–394.

[5] James TY, Toledo LF, Rödder D, et al. Disentangling host, pathogen, and environmental determinants of a recently emerged wildlife disease: lessons from the first 15 years of amphibian chytridiomycosis research. Ecology and Evolution. 2015;5:4079–4097.

[6] Romero-Zambrano GL, Bermúdez-Puga SA, Sánchez-Yumbo AF, et al. Amphibian chytridiomycosis, a lethal pandemic disease caused by the killer fungus *Batrachochytrium dendrobatidis*: New approaches to host defense mechanisms and techniques for detection and monitoring. Bionatura. 2021;6.

[7] Marcum R, St-Hilaire S, Murphy P, et al. Effects of *Batrachochytrium dendrobatidis* infection on ion concentrations in the boreal toad *Anaxyrus* (*Bufo*) *boreas boreas*. Diseases of Aquatic Organisms [Internet]. 2010;91:17–21. Available from: http://www.int-res.com/abstracts/dao/v91/n1/p17-21/.

[8] van Rooij P, Martel A, Haesebrouck F, et al. Amphibian chytridiomycosis: a review with focus on fungus-host interactions. Veterinary Research. 2015;46:1–22.

[9] Woodhams DC, Ardipradja K, Alford RA, et al. Resistance to chytridiomycosis varies among amphibian species and is correlated with skin peptide defenses. Animal Conservation. 2007;10:409–417.

[10] Woodhams DC, Brandt H, Baumgartner S, et al. Interacting Symbionts and Immunity in the Amphibian Skin Mucosome Predict Disease Risk and Probiotic Effectiveness. PLoS ONE. 2014;9:e96375.

[11] Brucker RM, Harris RN, Schwantes CR, et al. Amphibian chemical defense: Antifungal metabolites of the microsymbiont *Janthinobacterium lividum* on the salamander *Plethodon cinereus*. Journal of Chemical Ecology. 2008;34:1422-1429.

[12] Brucker RM, Baylor CM, Walters RL, et al. The identification of 2,4-diacetylphloroglucinol as an antifungal metabolite produced by cutaneous bacteria of the salamander *Plethodon cinereus*. Journal of Chemical Ecology. 2008;34:39-43.

[13] Garner TWJ, Garcia G, Carroll B, et al. Using itraconazole to clear *Batrachochytrium dendrobatidis* infection, and subsequent depigmentation of *Alytes muletensis* tadpoles. Diseases of Aquatic Organisms. 2009;83:257–260.

[14] Bletz MC, Loudon AH, Becker MH, et al. Mitigating amphibian chytridiomycosis with bioaugmentation: characteristics of effective probiotics and strategies for their selection and use. Ecology Letters. 2013;16:807–820.

[15] Flechas SV, Sarmiento C, Cárdenas ME, et al. Surviving chytridiomycosis: Differential anti-*Batrachochytrium dendrobatidis* Activity in Bacterial Isolates from Three Lowland Species of *Atelopus*. PLoS ONE. 2012;7:e44832.

[16] Ortega-Andrade HM, Rodes Blanco M, Cisneros-Heredia DF, et al. Red List assessment of amphibian species of Ecuador: A multidimensional approach for their conservation. PLoS ONE. 2021;16:1–28.

[17] Bresciano JC, Salvador CA, Paz-y-Miño C, et al. Variation in the Presence of Anti-*Batrachochytrium dendrobatidis* Bacteria of Amphibians Across Life Stages and Elevations in Ecuador. Ecohealth. 2015;12:310–319.

[18] Ron SR, Merino A. Declinación de anfibios del Ecuador: información general y primer reporte de chytridiomycosis para Sudamérica. Froglog. 2000;42:2–3.

[19] Sáenz V. Genética de poblaciones y prevalencia del hongo patógeno *Batrachochytrium dendrobatidis* en anfibios de Papallacta y Guamaní, Ecuador. [Bachelor's thesis]. Quito (Ecuador): Pontifica Universidad Católica del Ecuador; 2011.

[20] Torres NG. Implementación y evaluación de un ensayo de PCR para la detección del agente causal de la quitridiomicosis [Bachelor's thesis]. Cuenca (Ecuador): Universidad de Cuenca; 2019. Available from: https://dspace.ucuenca.edu.ec/bitstream/123456789/33383/1/trabajo%20de%20titulaci%C3%B3n.pdf.

[21] Proaño-Bolaños C, Merino-Viteri A, Peña-Loyola P, et al. A midaltitude report of *Batrachochytrium dendrobatidis* in Ecuador. Froglog. 2007;3–4.

[22] Guayasamin JM, Mendoza ÁM, Longo A, et al. High prevalence of *Batrachochytrium dendrobatidis* in an Andean frog community (Reserva Las Gralarias, Ecuador). Amphibian & Reptile Conservation. 2014;8:33–44.

[23] McCracken S, P. Gaertner J, Forstner M, et al. Detection of *Batrachochytrium dendrobatidis* in amphibians from the forest floor to the upper canopy of an Ecuadorian Amazon lowland rainforest. Herpetological Review. 2009;40:190-195.

[24] Peters JA. The Frog Genus *Atelopus* in Ecuador. Smithsonian Contributions to Zoology. 1973;145:1–49.

[25] Marcillo-Lara A, Coloma LA, Álvarez-Solas S, et al. The gastromyzophorous tadpoles of *Atelopus elegans* and *A. palmatus* (Anura: Bufonidae), with comments on oral and suction structures. Neotropical Biodiversity [Internet]. 2020;6:1–13. Available from: https://doi.org/10.1080/23766808.2019.1709378.

[26] Esperanza D. *Batrachochytrium dendrobatidis* (Bd) Prevalence: An Analysis of the *Atelopus* Species Population Decline in Panama and Costa Rica [dissertation]. Olympia (U.S.): The Evergreen State College; 2020.

[27] IUCN SSC Amphibian Specialist Group. *Atelopus bomolochos*. The IUCN Red List of Threatened Species. 2019.

[28] Siavichay FR. Diagnóstico de la comunidad de Anfibios para el Manejo y Gestión del Área de Conservación Municipal y Uso Sustentable Cordillera Oriental, del cantón Sígsig, Azuay [master's thesis]. Cuenca (Ecuador): Universidad de Cuenca; 2018.

[29] IUCN SSC Amphibian Specialist Group. *Atelopus nanay*. The IUCN Red List of Threatened Species. 2018.

[30] Cáceres-Andrade JF. Análisis Exploratorio de datos para desarrollar propuestas de conservación de la comunidad de Anfibios referentes al *Batrachochytrium dendrobatidis* en el Parque Nacional Cajas, Cuenca-Ecuador [master's thesis]. Cuenca (Ecuador): Universidad de Cuenca; 2014.

[31] IUCN SSC Amphibian Specialist Group. *Atelopus balios*. The IUCN Red List of Threatened Species. 2018.

[32] Pérez-Lara MB, Ramírez-Jaramillo SM. Aportes al conocimiento de la ecología de *Atelopus balios* (Anura: Bufonidae) en el Naranjal, Ecuador. Revista Colombiana de Ciencia Animal RECIA. 2020;13:e774.

[33] Bonaccorso E, Guayasamin JM, Méndez D, et al. Chytridiomycosis as a Possible Cause of Population Declines in *Atelopus cruciger* (Anura: Bufonidae). Herpetological Review. 2003;34:331–334.

[34] Catenazzi A, Flechas S v, Burkart D, et al. Widespread Elevational Occurrence of Antifungal Bacteria in Andean Amphibians Decimated by Disease: A Complex Role for Skin Symbionts in Defense Against Chytridiomycosis. Frontiers in Microbiology. 2018;9:465.

[35] Jani AJ, Briggs CJ. The pathogen *Batrachochytrium dendrobatidis* disturbs the frog skin microbiome during a natural epidemic and experimental infection. Proceedings of the National Academy of Sciences. 2014;111:E5049–E5058.

[36] Kueneman JG, Parfrey LW, Woodhams DC, et al. The amphibian skin-associated microbiome across species, space and life history stages. Mol Ecol. 2013;23:1238–1250.

[37] Hyatt AD, Boyle DG, Olsen V, et al. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms. 2007;73:175–192.

[38] Boyle DG, Boyle DB, Olsen V, et al. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Diseases of Aquatic Organisms [Internet]. 2004 [cited 2022 Feb 21];60:141–148. Available from: https://www.int-res.com/abstracts/dao/v60/n2/p141-148/.

[39] PROMEGA. Wizard genomic DNA purification kit: technical manual. [Internet]. Promega Corporation Madison; 2017 [cited 2022 Feb 14]. Available from: https://ita.promega.com/-/media/files/resources/protocols/technical-manuals/0/wizard-genomic-dna-purification-kit-protocol.pdf.

[40] Persson S, Olsen KEP. Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. Journal of Medical Microbiology. 2005;54:1043–1047.

[41] PROMEGA. Wizard® SV Gel and PCR Clean-Up System [Internet]. 2019 [cited 2022 Feb 14]. Available from: https://worldwide.promega.com/-/media/files/resources/protocols/technical-bulletins/101/wizard-sv-gel-and-pcr-clean-up-system-protocol.pdf?rev=39cbdfc6eec849db9f6dd9915d714930&sc_lang=en.

[42] Hammer Ø, Harper DAT, Ryan PD. Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica [Internet].

2001;4:9. Available from: http://palaeo-electronica.orghttp://palaeo-electronica.org/2001_1/past/issue1_01.htm.

[43] Mitchell KM, Churcher TS, Garner TWJ, et al. Persistence of the emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction. Proceedings of the Royal Society B: Biological Sciences. 2008;275:329–334.

[44] Lambertini C, Becker CG, M. Belasen A, et al. Biotic and abiotic determinants of *Batrachochytrium dendrobatidis* infections in amphibians of the Brazilian Atlantic Forest. Fungal Ecology. 2021;49:100995.

[45] Hudson MA, Griffiths RA, Martin L, et al. Reservoir frogs: seasonality of *Batrachochytrium dendrobatidis* infection in robber frogs in Dominica and Montserrat. PeerJ. 2019;2019:1–21.

[46] Jervis P, Pintanel P, Hopkins K, et al. Post-epizootic microbiome associations across communities of neotropical amphibians. Molecular Ecology. 2021;30:1322–1335.

[47] Bresciano JC, Salvador CA, Paz-y-Miño C, et al. Variation in the Presence of Anti-*Batrachochytrium dendrobatidis* Bacteria of Amphibians Across Life Stages and Elevations in Ecuador. Ecohealth. 2015;12:310–319.

[48] Koski LB, Golding GB. The closest BLAST hit is often not the nearest neighbor. Journal of Molecular Evolution. 2001;52:540–542.

[49] van den Beld MJC, Reinders E, Notermans DW, et al. Possible misidentification of species in the *Pseudomonas fluorescens* lineage as *Burkholderia pseudomallei* and *Francisella tularensis*, and emended descriptions of *Pseudomonas brenneri*, *Pseudomonas gessardii* and *Pseudomonas proteolytica*. International Journal of Systematic and Evolutionary Microbiology. 2016;66:3420–3425.

[50] Flechas SV, Blasco-Zúñiga A, Merino-Viteri A, et al. The effect of captivity on the skin microbial symbionts in three *Atelopus* species from the lowlands of Colombia and Ecuador. PeerJ. 2017;5:e3594.

[51] Barra D, Simmaco M, Boman HG. Gene-encoded peptide antibiotics and innate immunity. Do "animalcules" have defence budgets? FEBS Letters. 1998;430:130–134.

[52] Becker MH, Brophy JAN, Barrett K, et al. Genetically modifying skin microbe to produce violacein and augmenting microbiome did not defend Panamanian golden frogs from disease. ISME Communications. 2021;1:1–10.

[53] Kanchan C, Panchai K, Imjai P, et al. Effects of antibiotics and medicinal plants extracts against *Aeromonas hydrophila* isolated from *Rana rugulosa* in Thailand. Aquaculture, Aquarium, Conservation & Legislation. 2021;14:1222–1230.

[54] Khalifa AYZ, AlMalki MA, Bekhet GM. Pathological and mortality findings associated with *Aeromonas hydrophila* from frog eggs in Al-Ahsa region of Saudi Arabia. Aquaculture Research. 2021;52:1227–1236.

[55] Latheef S, Keyburn A, Broz I, et al. Atypical *Brucella* sp in captive Australian green tree frogs (*Litoria caerulea*): clinical features, pathology, culture and molecular characterization. Australian Veterinary Journal. 2020;98:216–221.

[56] Bates KA, Clare FC, O'Hanlon S, et al. Amphibian chytridiomycosis outbreak dynamics are linked with host skin bacterial community structure. Nature Communications. 2018;9:693.

[57] McKenzie VJ, Bowers RM, Fierer N, et al. Co-habiting amphibian species harbor unique skin bacterial communities in wild populations. ISME Journal. 2012;6:588–596.

[58] Walke JB, Becker MH, Hughey MC, et al. Dominance-function relationships in the amphibian skin microbiome. Environmental Microbiology. 2017;19:3387–3397.

[59] Kruger A. Frog Skin Microbiota Vary With Host Species and Environment but Not Chytrid Infection. Frontiers in Microbiology. 2020;11:1330.

[60] Nottingham AT, Fierer N, Turner BL, et al. Microbes follow Humboldt: temperature drives plant and soil microbial diversity patterns from the Amazon to the Andes. Ecology. 2018;99:2455–2466.

[61] Belden LK, Hughey MC, Rebollar EA, et al. Panamanian frog species host unique skin bacterial communities. Frontiers in Microbiology. 2015;6:1171.

[62] Becker MH, Walke JB, Cikanek S, et al. Composition of symbiotic bacteria predicts survival in Panamanian golden frogs infected with a lethal fungus. Proceedings of the Royal Society B: Biological Sciences. 2015;282:20142881.

[63] Walke JB, Becker MH, Loftus SC, et al. Community structure and function of amphibian skin microbes: An experiment with bullfrogs exposed to a chytrid fungus. PLoS ONE. 2015;10:e0139848.

[64] Robak MJ. Effects of temperature on amphibian resistance and susceptibility to chytridiomycosis. [New Orleans, L.A.]; 2016.

[65] Robak MJ, Richards-Zawacki CL. Temperature-dependent effects of cutaneous bacteria on a frog's tolerance of fungal infection. Frontiers in Microbiology. 2018;9:410.

[66] Rouzic N, Desmier L, Cariou M-E, et al. First Case of Brucellosis Caused by an Amphibian-type *Brucella*. Clinical Infectious Diseases. 2021;72:e404–e407.

[67] Mühldorfer K, Wibbelt G, Szentiks CA, et al. The role of 'atypical' *Brucella* in amphibians: are we facing novel emerging pathogens? Journal of Applied Microbiology. 2017;122:40–53.

[68] McDonald CA, Longo A v., Lips KR, et al. Incapacitating effects of fungal coinfection in a novel pathogen system. Molecular Ecology. 2020;29:3173–3186.

[69] Becker MH, Walke JB, Murrill L, et al. Phylogenetic distribution of symbiotic bacteria from Panamanian amphibians that inhibit growth of the lethal fungal pathogen *Batrachochytrium dendrobatidis*. Molecular Ecology. 2015;24:1628–1641.

[70] Densmore CL, Green DE. Diseases of Amphibians. ILAR Journal [Internet]. 2007;48:235–254. Available from: https://doi.org/10.1093/ilar.48.3.235.

[71] Wu W, Zong Z. Genome analysis-based reclassification of *Lelliottia aquatilis* as a later heterotypic synonym of *Lelliottia jeotgali*. International Journal of Systematic and Evolutionary Microbiology. 2019;69:998–1000.

[72] Heinle CE, Junqueira ACM, Uchida A, et al. Complete genome sequence of *Lelliottia nimipressuralis* type strain SGAir0187, isolated from tropical air collected in Singapore. Genome Announcements. 2018;6:e00231-18.

[73] Ohadi M, Forootanfar H, Dehghannoudeh G, et al. Antimicrobial, anti-biofilm, and anti-proliferative activities of lipopeptide biosurfactant produced by *Acinetobacter junii* B6. Microbial Pathogenesis. 2020;138:103806.

[74] Gan X, Huang JC, Zhang M, et al. Remediation of selenium-contaminated soil through combined use of earthworm *Eisenia fetida* and organic materials. Journal of Hazardous Materials. 2021;405:124212.

[75] Singh V, Mishra V. Microbial removal of Cr (VI) by a new bacterial strain isolated from the site contaminated with coal mine effluents. Journal of

Environmental Chemical Engineering [Internet]. 2021;9:106279. Available from: https://www.sciencedirect.com/science/article/pii/S2213343721012562.

[76] Priyanka P, Kinsella GK, Henehan GT, et al. Isolation and characterization of a novel thermo-solvent-stable lipase from *Pseudomonas brenneri* and its application in biodiesel synthesis. Biocatalysis and Agricultural Biotechnology. 2020;29:101806.

Supplementary information

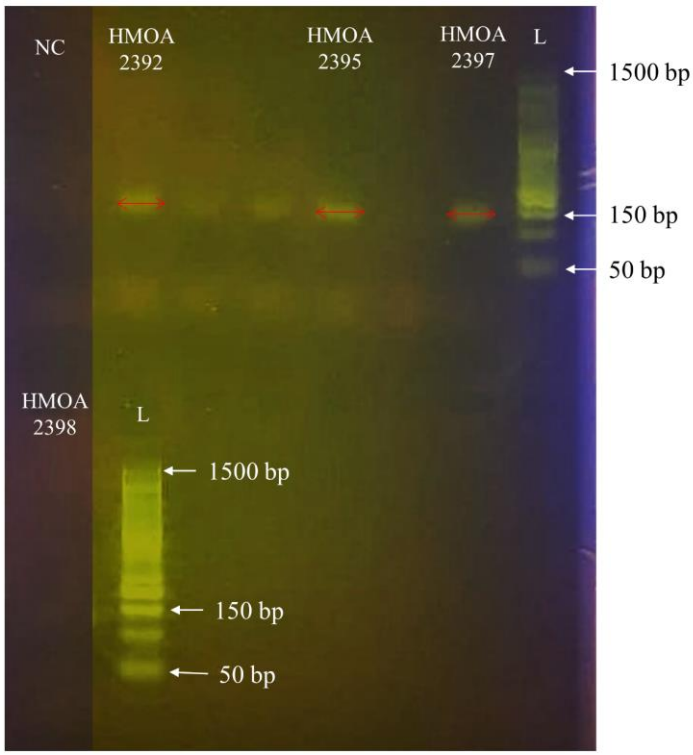


Figure S1. PCR amplification for Bd detection with ITS 1-3 Chytr/5.8 Chytrid primers (amplicon size ~146 bp). Lane L: 50 bp DNA Ladder. Lane NC: negative control. Lanes HMOA 2392-2395 (positive for Bd) belong to *A. bomolochos* specimens. Lanes HMOA 2397 (positive for Bd) and HMOA 2398 (negative for Bd) belong to *A. nanay* specimens.

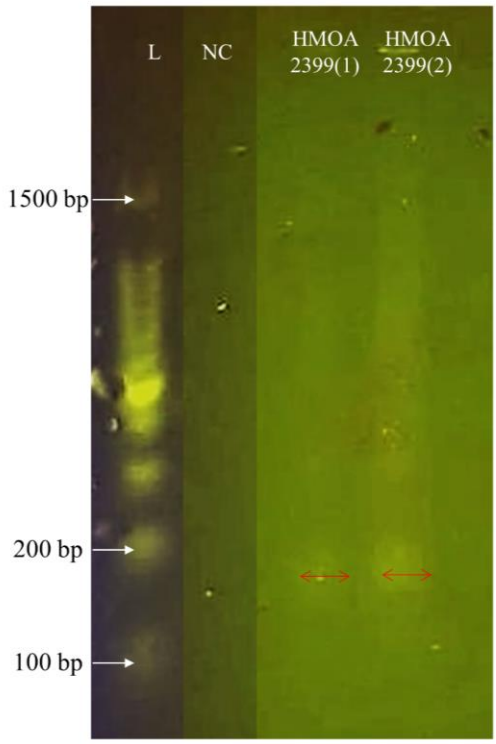


Figure S2. PCR amplification for Bd detection with ITS 1-3 Chytr/5.8 Chytrid primers (amplicon size ~146 bp). Lane L: 100 bp DNA Ladder. Lane NC: negative control. Lanes HMOA 2399(1)-2399(2), positive for Bd, are repetitions of an *A. nanay* specimen.

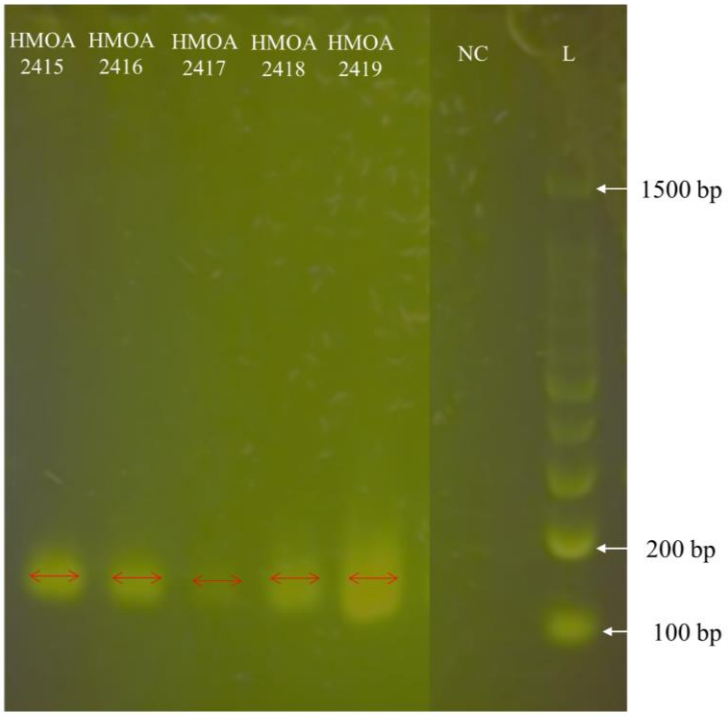


Figure S3. PCR amplification for Bd detection with ITS 1-3 Chytr/5.8 Chytrid primers (amplicon size ~146 bp). Lane NC: negative control. Lane L: 100 bp DNA Ladder. Lanes HMOA 2415-2419 (positive for Bd) belong to *A. balios* specimens.

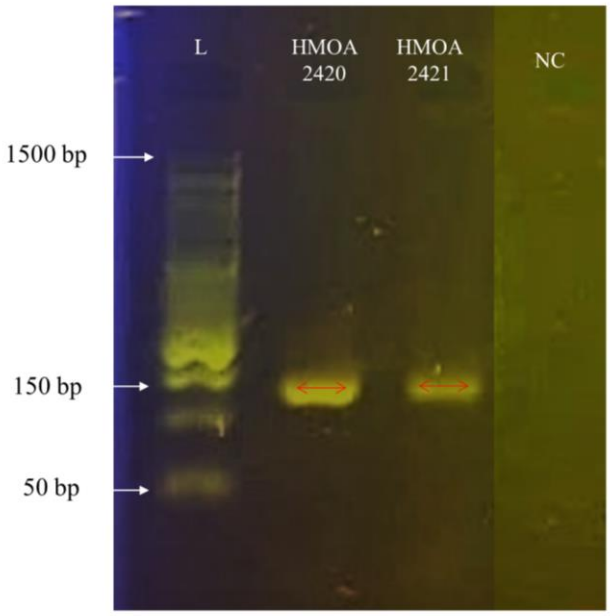
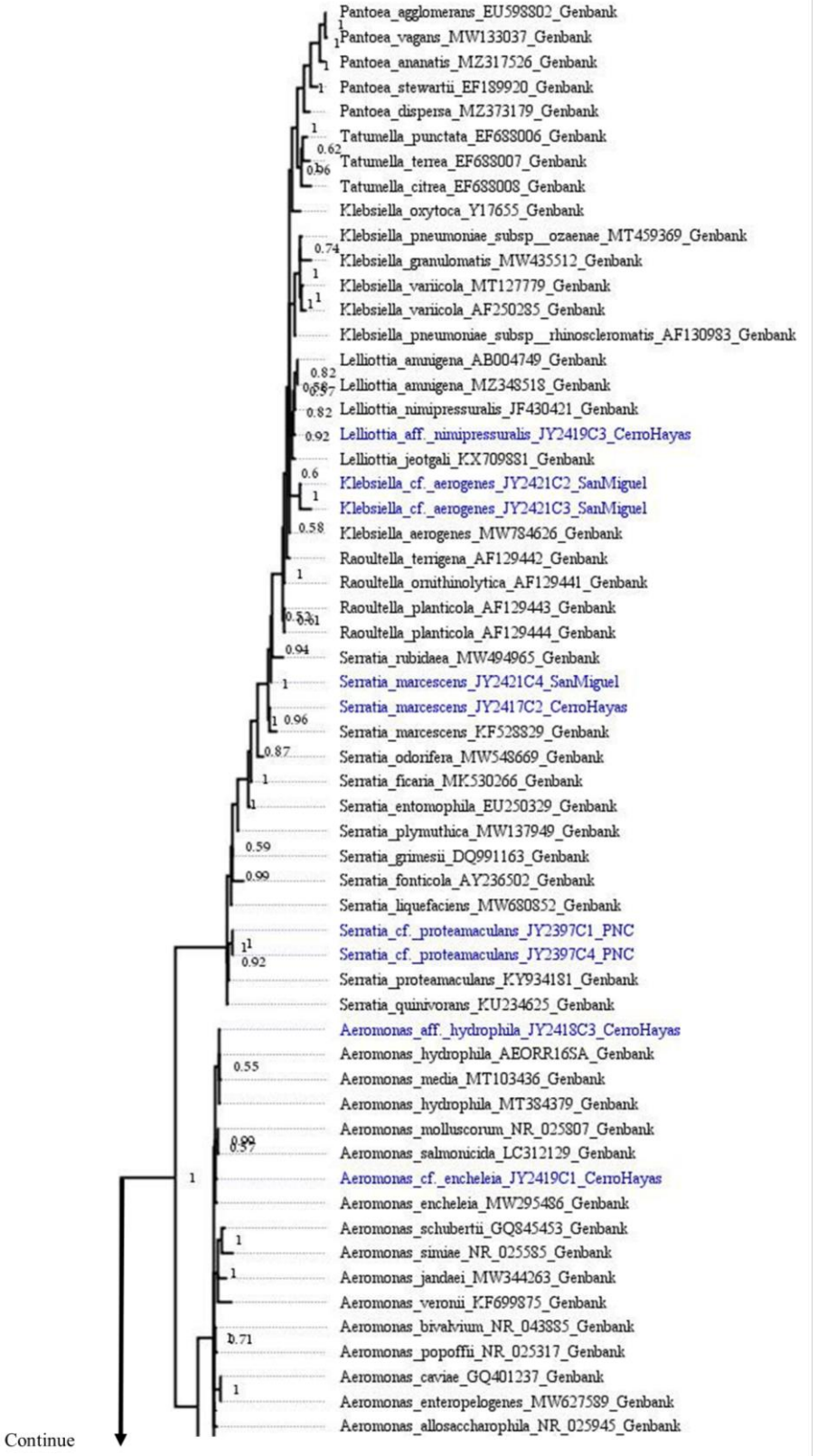
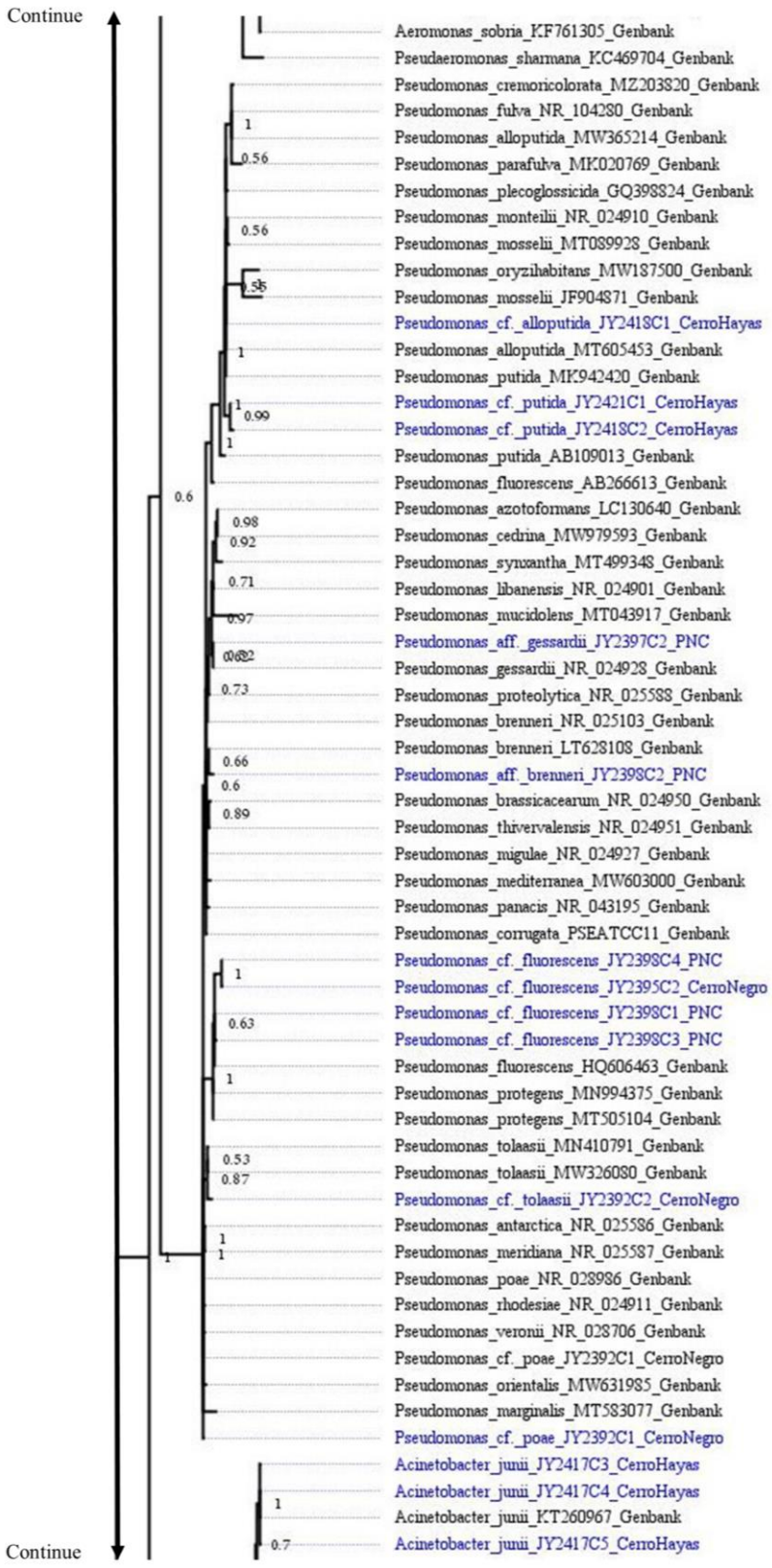


Figure S4. PCR amplification for Bd detection with ITS 1-3 Chytr/5.8 Chytrid primers (amplicon size ~146 bp). Lane NC: negative control. Lane L: 50 bp DNA Ladder. Lanes HMOA 2420-2421 (positive for Bd) belong to *A. balios* specimens.


Pantoea_agglomerans_EU598802_Genbank
Pantoea_vagans_MW133037_Genbank
Pantoea_ananatis_MZ317526_Genbank
Pantoea_stewartii_EF189920_Genbank
Pantoea_dispersa_MZ373179_Genbank
Tatumella_punctata_EF688006_Genbank
Tatumella_terrea_EF688007_Genbank
Tatumella_citrea_EF688008_Genbank
Klebsiella_oxytoca_Y17655_Genbank
Klebsiella_pneumoniae_subsp__ozaenae_MT459369_Genbank
Klebsiella_granulomatis_MW435512_Genbank
Klebsiella_variicola_MT127779_Genbank
Klebsiella_variicola_AF250285_Genbank
Klebsiella_pneumoniae_subsp__rhinoscleromatis_AF130983_Genbank
Lelliottia_amnigena_AB004749_Genbank
Lelliottia_amnigena_MZ348518_Genbank
Lelliottia_nimipressuralis_JF430421_Genbank
Lelliottia_aff._nimipressuralis_JY2419C3_CerroHayas
Lelliottia_jeotgali_KX709881_Genbank
Klebsiella_cf._aerogenes_JY2421C2_SanMiguel
Klebsiella_cf._aerogenes_JY2421C3_SanMiguel
Klebsiella_aerogenes_MW784626_Genbank
Raoultella_terrigena_AF129442_Genbank
Raoultella_ornithinolytica_AF129441_Genbank
Raoultella_planticola_AF129443_Genbank
Raoultella_planticola_AF129444_Genbank
Serratia_rubidaea_MW494965_Genbank
Serratia_marcescens_JY2421C4_SanMiguel
Serratia_marcescens_JY2417C2_CerroHayas
Serratia_marcescens_KF528829_Genbank
Serratia_odorifera_MW548669_Genbank
Serratia_ficaria_MK530266_Genbank
Serratia_entomophila_EU250329_Genbank
Serratia_plymuthica_MW137949_Genbank
Serratia_grimesii_DQ991163_Genbank
Serratia_fonticola_AY236502_Genbank
Serratia_liquefaciens_MW680852_Genbank
Serratia_cf._proteamaculans_JY2397C1_PNC
Serratia_cf._proteamaculans_JY2397C4_PNC
Serratia_proteamaculans_KY934181_Genbank
Serratia_quinivorans_KU234625_Genbank
Aeromonas_aff._hydrophila_JY2418C3_CerroHayas
Aeromonas_hydrophila_AEORR16SA_Genbank
Aeromonas_media_MT103436_Genbank
Aeromonas_hydrophila_MT384379_Genbank
Aeromonas_molluscorum_NR_025807_Genbank
Aeromonas_salmonicida_LC312129_Genbank
Aeromonas_cf._encheleia_JY2419C1_CerroHayas
Aeromonas_encheleia_MW295486_Genbank
Aeromonas_schubertii_GQ845453_Genbank
Aeromonas_simiae_NR_025585_Genbank
Aeromonas_jandaei_MW344263_Genbank
Aeromonas_veronii_KF699875_Genbank
Aeromonas_bivalvium_NR_043885_Genbank
Aeromonas_popoffii_NR_025317_Genbank
Aeromonas_caviae_GQ401237_Genbank
Aeromonas_enteropelogenes_MW627589_Genbank
Aeromonas_allosaccharophila_NR_025945_Genbank
Continue


Continue
Aeromonas_sobria_KF761305_Genbank
Pseudaeromonas_sharmana_KC469704_Genbank
Pseudomonas_cremoricolorata_MZ203820_Genbank
Pseudomonas_fulva_NR_104280_Genbank
Pseudomonas_alloputida_MW365214_Genbank
Pseudomonas_parafulva_MK020769_Genbank
Pseudomonas_plecoglossicida_GQ398824_Genbank
Pseudomonas_monteilii_NR_024910_Genbank
Pseudomonas_mosselii_MT089928_Genbank
Pseudomonas_oryzihabitans_MW187500_Genbank
Pseudomonas_mosselii_JF904871_Genbank
Pseudomonas_cf._alloputida_JY2418C1_CerroHayas
Pseudomonas_alloputida_MT605453_Genbank
Pseudomonas_putida_MK942420_Genbank
Pseudomonas_cf._putida_JY2421C1_CerroHayas
Pseudomonas_cf._putida_JY2418C2_CerroHayas
Pseudomonas_putida_AB109013_Genbank
Pseudomonas_fluorescens_AB266613_Genbank
Pseudomonas_azotoformans_LC130640_Genbank
Pseudomonas_cedrina_MW979593_Genbank
Pseudomonas_synxantha_MT499348_Genbank
Pseudomonas_libanensis_NR_024901_Genbank
Pseudomonas_mucidolens_MT043917_Genbank
Pseudomonas_aff._gessardii_JY2397C2_PNC
Pseudomonas_gessardii_NR_024928_Genbank
Pseudomonas_proteolytica_NR_025588_Genbank
Pseudomonas_brenneri_NR_025103_Genbank
Pseudomonas_brenneri_LT628108_Genbank
Pseudomonas_aff._brenneri_JY2398C2_PNC
Pseudomonas_brassicacearum_NR_024950_Genbank
Pseudomonas_thivervalensis_NR_024951_Genbank
Pseudomonas_migulae_NR_024927_Genbank
Pseudomonas_mediterranea_MW603000_Genbank
Pseudomonas_panacis_NR_043195_Genbank
Pseudomonas_corrugata_PSEATCC11_Genbank
Pseudomonas_cf._fluorescens_JY2398C4_PNC
Pseudomonas_cf._fluorescens_JY2395C2_CerroNegro
Pseudomonas_cf._fluorescens_JY2398C1_PNC
Pseudomonas_cf._fluorescens_JY2398C3_PNC
Pseudomonas_fluorescens_HQ606463_Genbank
Pseudomonas_protegens_MN994375_Genbank
Pseudomonas_protegens_MT505104_Genbank
Pseudomonas_tolaasii_MN410791_Genbank
Pseudomonas_tolaasii_MW326080_Genbank
Pseudomonas_cf._tolaasii_JY2392C2_CerroNegro
Pseudomonas_antarctica_NR_025586_Genbank
Pseudomonas_meridiana_NR_025587_Genbank
Pseudomonas_poae_NR_028986_Genbank
Pseudomonas_rhodesiae_NR_024911_Genbank
Pseudomonas_veronii_NR_028706_Genbank
Pseudomonas_cf._poae_JY2392C1_CerroNegro
Pseudomonas_orientalis_MW631985_Genbank
Pseudomonas_marginalis_MT583077_Genbank
Pseudomonas_cf._poae_JY2392C1_CerroNegro
Acinetobacter_junii_JY2417C3_CerroHayas
Acinetobacter_junii_JY2417C4_CerroHayas
Acinetobacter_junii_KT260967_Genbank
Acinetobacter_junii_JY2417C5_CerroHayas
Continue

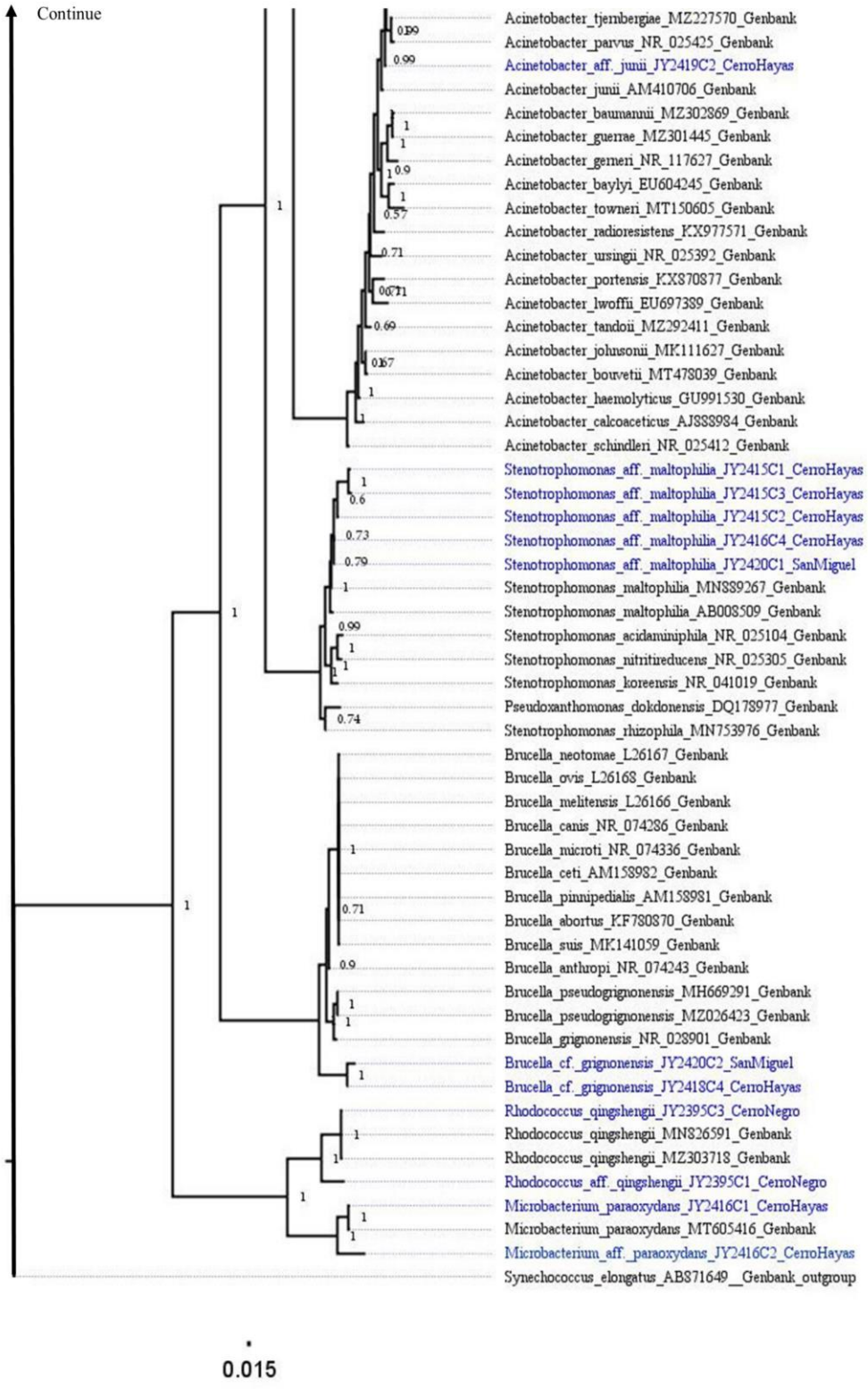


Figure S5. Distance-based phylogenetic tree hypothesized from the 16sRNAr gene of a matrix (log likelihood = -16777.01; 1349 aligned sites) showing the phylogenetic

relationships of 35 bacteria morphotypes sequences (blue) joined to multiple GenBank sequences (black)

Table S1. Preliminary information of the bacterial isolates was given by BLAST/n analysis. Isolate code is composed by author initials (JY), followed by the *Atelopus* sample code HMOA (2392-2395: *A. bomolochos* from Cerro Negro, 2397-2398: *A. nanay* from Cajas National Park, 2415-2419: *A. balios* from Cerro Las Hayas, 2420-2421: *A. balios* from Estero Arenas), and finally the colony/morphotype name.

Isolate code	Phylum	Class, Order	Family	Genus	Probable species	Maximun % Identity
JY2395C1	Actinobacteria	Actinobacteria, Actinomycetales	Corynebacteriaceae	*Rhodococcus*	*R. qingshengii*	100
JY2395C3						100
JY2416C1			Microbacteriaceae	*Microbacterium*	*M. paraoxydans*	99.80
JY2416C2						100
JY2418C4	Proteobacteria	Alphaproteobacteria, Rhizobiales	Brucellaceae	*Brucella*	*B. anthropi*	99.90
JY2420C2					*B. pseudogrignonensis*	99.90
JY2418C3		Gammaproteobacteria, Aeromonadales	Aeromonadaceae	*Aeromonas*	*A. hydrophila*	100
JY2419C1					*A. encheleia*	99.80
JY2417C2		Gammaproteobacteria, Enterobacterales	Yersiniaceae	*Serratia*	*S. marcescens*	99.90
JY2421C4						100
JY2397C1					*S. proteamaculans*	99.60
JY2397C4						99.59
JY2419C3			Enterobacteriaceae	*Lelliottia*	*L. amnigena*	99.70
JY2421C2				*Klebsiella*	*K. aerogenes*	99.60
JY2421C3			Enterobacterias	*Pantoea*	*P. agglomerans*	99.80
JY2417C3		Gammaproteobacteria, Pseudomonadales	Moraxellaceae	*Acinetobacter*	*A. junii*	99.90
JY2417C4						99.90
JY2417C5						99.80
JY2419C2					*A. calcoaceticus*	99.80
JY2418C1			Pseudomonadaceae	*Pseudomonas*	*P. alloputida*	99.80
JY2421C1						100
JY2398C2					*P. brenneri*	99.90
JY2418C2					*P. mosselii*	99.90
JY2397C2					*P. fluorescens*	100
JY2398C1						99.80
JY2398C3						99.70
JY2398C4						99.80
JY2392C1					*P. poae*	99.41
JY2395C2					*P. protegens*	100
JY2392C2					*P. tolaasii*	99.80
JY2415C1		Gammaproteobacteria, Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*	*S. maltophilia*	99.80
JY2415C2						99.70

JY2415C3	99.41
JY2416C4	99.80
JY2420C1	100

Table S2. Summarized similarity measures of the identified bacterial species using 16S rRNA gene by BLAST/n analysis. Isolate code is composed by author initials (JY), followed by the *Atelopus* sample code HMOA (2392-2395: *A. bomolochos* from Cerro Negro, 2397-2398: *A. nanay* from Cajas National Park, 2415-2419: *A. balios* from Cerro Las Hayas, 2420-2421: *A. balios* from Estero Arenas), and finally the colony/morphotype name.

Isolate code	Probable species	Maximum score	Total score	% Query cover	E- value	Maximum % Identity	GenBank Accession Number
JY2395C1	*R. qingshengii*	828	828	100	0	100	MN826591.1
JY2395C3		1783	1783	100	0	100	MN826591.1
JY2416C1	*M. paraoxydans*	1829	1829	100	0	99.80	MT605416.1
JY2416C2		1840	1840	100	0	100	MT605416.1
JY2418C4	*B. anthropi*	1792	1792	99	0	99.90	MT534544.1
JY2420C2	*B. pseudogrignonensis*	1818	1818	99	0	99.90	MH669291.1
JY2418C3	*A. hydrophila*	1845	1845	99	0	100	MT384379.1
JY2419C1	*A. encheleia*	1864	1864	100	0	99.80	MN999980.1
JY2417C2	*S. marcescens*	1858	1858	99	0	99.90	MT538443.1
JY2421C4		1803	1803	100	0	99.60	MK530287.1
JY2397C1	*S. proteamaculans*	1783	1783	100	0	99.59	MK530287.1
JY2397C4		1796	1796	100	0	100	MT101739.1
JY2419C3	*L. amnigena*	1818	1818	100	0	99.70	MH669129.1
JY2421C2	*K. aerogenes*	1799	1799	100	0	99.60	MN177204.1
JY2421C3	*P. agglomerans*	1805	1805	100	0	99.80	MT367857.1
JY2417C3	*A. junii*	1866	1866	100	0	99.90	KT260967.1
JY2417C4		1866	1866	100	0	99.90	KT260967.1
JY2417C5		1862	1862	99	0	99.80	KT260967.1
JY2419C2	*A. calcoaceticus*	1851	1851	99	0	99.80	MG011543.1
JY2418C1	*P. alloputida*	1853	1853	100	0	99.80	MT605453.1
JY2421C1		1851	1851	100	0	100	MT605453.1
JY2398C2	*P. brenneri*	1869	1869	99	0	99.90	LT628108.1
JY2418C2	*P. mosselii*	1862	1862	99	0	99.90	MT089928.1
JY2397C2	*P. fluorescens*	1821	1821	100	0	100	MN715320.1
JY2398C1		1855	1855	100	0	99.80	HQ606463.1
JY2398C3		1858	1858	100	0	99.70	HQ606463.1
JY2398C4		1866	1866	100	0	99.80	HQ606463.1
JY2392C1	*P. poae*	1230	1230	100	0	99.41	MT631989.1
JY2395C2	*P. protegens*	1786	1786	100	0	100	MT505104.1
JY2392C2	*P. tolaasii*	1858	1858	99	0	99.80	MN410791.1

Isolate code	Probable species	Maximum score	Total score	% Query cover	E- value	Maximum % Identity	GenBank Accession Number
JY2415C1	*S. maltophilia*	1871	1871	100	0	99.80	MN006523.1
JY2415C2		1868	1868	99	0	99.70	MT256163.1
JY2415C3		1844	1844	100	0	99.41	MT256163.1
JY2416C4		1857	1857	99	0	99.80	MN006523.1
JY2420C1		1864	1864	99	0	100	MN889267.1

Table S3. Bacterial identification by phylogenetic analysis, distance percentages between the sequences of the isolates, and the DNA sequences of the possible species from GenBank. Isolate code is composed by author initials (JY), followed by the *Atelopus* sample code HMOA (2392-2395: *A. bomolochos* from Cerro Negro, 2397-2398: *A. nanay* from Cajas National Park, 2415-2419: *A. balios* from Cerro Las Hayas, 2420-2421: *A. balios* from Estero Arenas), and finally the colony/morphotype name.

Isolate code	Genus	Probable species	% Distance	Clade
JY2419C2	*Acinetobacter*	*Acinetobacter aff. junii*	98,72	Clade 1
JY2417C3		*Acinetobacter junii*	100	Clade 2
JY2417C4			99,90	
JY2417C5			99,80	
JY2418C3	*Aeromonas*	*Aeromonas aff. hydrophila*	99,90	Clade 3
JY2419C1		*Aeromonas cf. encheleia*	99,61	Clade 4
JY2418C4	*Brucella*	*Brucella cf. grignonensis*	93,77	Clade 5
JY2420C2			93,51	
JY2421C2	*Klebsiella*	*Klebsiella cf. aerogenes*	97,16	Clade 6
JY2421C3			95,93	
JY2419C3	*Lelliottia*	*Lelliottia aff. nimipressuralis*	99,63	Clade 7
JY2416C2	*Microbacterium*	*Microbacterium aff. paraoxydans*	93,04	Clade 8
JY2416C1		*Microbacterium paraoxydans*	100	Clade 9
JY2398C2	*Pseudomonas*	*Pseudomonas aff. brenneri*	99,01	Clade 10
JY2397C2		*Pseudomonas aff. gessardii*	99,92	Clade 11
JY2418C1		*Pseudomonas cf. alloputida*	99,90	Clade 12
JY2395C2		*Pseudomonas cf. fluorescens*	98,6	Clade 13
JY2398C1			100	
JY2398C3			99,70	
JY2398C4			98,81	
JY2392C1		*Pseudomonas cf. poae*	100	Clade 14
JY2418C2		*Pseudomonas cf. putida*	98,42	Clade 15
JY2421C1			99,10	
JY2392C2		*Pseudomonas cf. tolaasii*	99,01	Clade 16
JY2395C1	*Rhodococcus*	*Rhodococcus aff. qingshengii*	91,54	Clade 17
JY2395C3		*Rhodococcus qingshengii*	100	Clade 18
JY2421C4	*Serratia*	*Serratia cf. marcescens*	98,97	Clade 19

Isolate code	Genus	Probable species	% Distance	Clade
JY2397C1		*Serratia cf. proteamaculans*	99,29	Clade 20
JY2397C4			99,28	
JY2417C2		*Serratia marcescens*	97,64	Clade 21
JY2415C1	*Stenotrophomonas*	*Stenotrophomonas aff. maltophilia*	97,13	Clade 22
JY2415C2			99,12	
JY2415C3			97,13	
JY2416C4			99,60	
JY2420C1			99,90	