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Epidemiological surveillance and amphibian assemblage status at the Estación Experimental de San Lorenzo, Sierra Nevada de Santa Marta, Colombia

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***Abstract.*—Amphibian population declines and extinctions have occurred in conserved sites or protected areas far from anthropogenic activities as a result of emerging infectious diseases such as chytridiomycosis. Regular epidemiological surveillance, monitoring of key species, and the implementation of biosecurity protocols are fundamental actions for the *in-situ* conservation of amphibian fauna. Since 2008 biosecurity protocols have been implemented for all personnel that enter the Estación Experimental de San Lorenzo, a partly mountainous protected and conserved area of the Sierra Nevada de Santa Marta with a high diversity of endemic and endangered amphibians. Semiannual disease screenings of amphibians were carried out, as well as an amphibian inventory and a survey of species of the genus *Atelopus*. To-date no mass mortality events have been reported and *Bd* has not been detected. Nevertheless, some individuals of *Ikakogi tayrona* and *Pristimantis megalops* showed symptoms of disease, the latter of which included individuals affected with skin tumors. Deformities in individuals of *Atelopus* were also observed. The implementation of epidemiological surveillance, monitoring of key amphibian species, and biosecurity protocols are important strategies for the conservation management of the endemic amphibians within the protected area of the Sierra Nevada of Santa Marta.**

Key words. Anura, *Atelopus*, *Pristimantis*, tumors, chytridiomycosis, disease screening, mortality events, health, disease, *Batrachochytrium dendrobatidis*, *Bd*

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Introduction

During the last decades amphibian population declines and extinctions have been observed around the world, causing concern from academic, scientific, and governmental entities (Gascon et al. 2007; Mendelson et al. 2006; Stuart et al. 2008). Close to 41% of amphibian species worldwide are categorized under some level of threat (Baillie et al. 2010; IUCN 2014) and additionally about a quarter of amphibian species are classified as Data Deficient (DD, IUCN 2014), which makes it more difficult to determine the actual status of populations. Colombia harbors approximately 215 threatened amphibian species, which represent slightly more than a fourth of its entire amphibian fauna (Acosta-Galvis 2014), making Colombia the country with the

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greatest number of threatened amphibian species in the world (IUCN 2014). Nevertheless, some species have experienced serious declines while others remain stable. In addition entire lineages have been affected almost to the brink of extinction, as reported for the species of the genus *Atelopus* (La Marca et al. 2005; Lötters 2007), which in Colombia 76% (33 of 43) of the species are categorized as Endangered and Critically Endangered (IUCN 2014; Acosta-Galvis 2014).

Although habitat destruction continues to be the main cause of population declines and extinctions worldwide (IUCN 2014), it is puzzling that many species have disappeared in well conserved, remote areas such as primary forests in protected refuges (Crawford et al. 2010; Crump et al. 1992; Lips et al. 2003; Pounds et al. 2006). We now have a better understanding of the pathogenic microscopic fungus *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al. 1999), which causes the disease known as chytridiomycosis, one of the leading factors behind mass mortality events previously considered enigmatic. This fungus interferes with the process of osmoregulation and affects electrolyte balance, which often leads to the death of susceptible individuals (Voyles et al. 2009). Furthermore, *Bd* seems to inhibit the immune response of its hosts (Fites et al. 2013) which may present symptoms such as lethargy, abnormal postures, and hyperemia (Berger et al. 2000; Daszak et al. 1999).

In Colombia, there are about 565 protected areas (RUNAP 2011), of these, 58 are administered by the Parques Nacionales Naturales de Colombia, and constitute approximately 11% of the continental territory (PNNC 2015). These areas safeguard the country's biodiversity and represent a refuge for amphibian assemblages, including endemic species or species with narrow distributions, which may be susceptible to the loss and fragmentation of their habitat. However, these areas are still vulnerable to the threat imposed by *Bd* and other emerging diseases. For example, it is known that for the Parque Nacional Natural Gorgona, *Bd* has been present for at least eight years (Flechas et al. 2012), however there is no evidence of declines. Additionally, amphibians are vulnerable to diseases produced by aquatic pathogens, due to their dependency on aquatic environments. (Bosch 2003). For this reason authorities and administrators of protected areas in Colombia consider emerging diseases (especially chytridiomycosis) a challenge to the protection of threatened amphibians.

The implementation of recurrent epidemiological surveillance, monitoring of key amphibians species, and biosecurity protocols, become fundamental to the *in situ* conservation of amphibian assemblages in protected areas. This way, early alerts are generated and can be used to implement and carry out the best management practices in a timely manner, thus reducing *Bd* (or disease) outbreaks and transmission. This paper shows the implementation of a pilot program (the first program of its kind in the country) of these actions (surveillance, monitoring, and protocols for amphibian species implemented for disease control) set in a protected area, the Parque Nacional Natural Sierra Nevada de Santa Marta, considered one of the principle centers for amphibian endemism in Colombia (Lynch et. al. 1997).

Materials and Methods

Study Area

The Sierra Nevada de Santa Marta (SNSM) was declared a reserve of the Biosphere in 1979 by United Nations Educational Scientific and Cultural Organization (UNESCO). Situated within three departments of the Colombian Caribbean (Fig. 1, 2A.); 383,000 hectares belong to the protected area (PNNC 2015). This area, comprises mul-

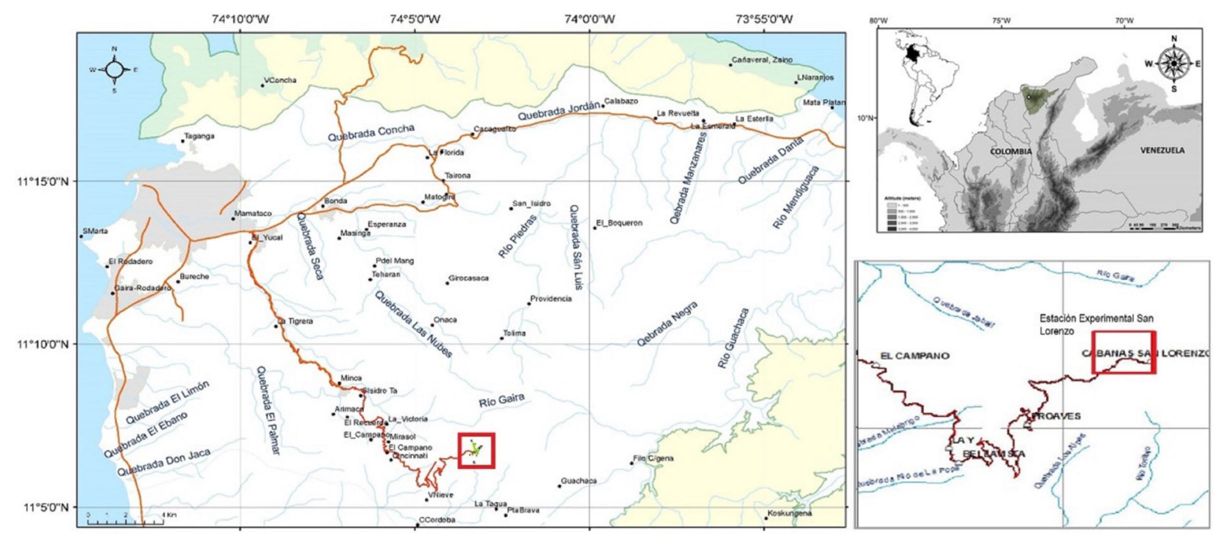

Fig. 1. Map of the Serrania de San Lorenzo, Sierra Nevada de Santa Marta, Colombia. Red square area highlights the Estación Experimental de San Lorenzo to 2,200 meters.

Fig. 2. Serrania de San Lorenzo (**B**) Querbrada San Lorenzo (**A**) Sierra Nevada de Santa Marta, Colombia. *Photographs by Luis Alberto Rueda Solano.*

tiple ecosystems, including dry and wet tropical forests, sub-Andean and Andean forests, moors, and zones with perpetual snow cover (PNNC 2015; ProSierra 2015). With approximately 17 species of amphibians, 12 of reptiles, 14 of birds and one mammal, all of them endemic to the area (PNNC 2015; ProSierra 2015), it is considered one of the greatest centers of endemism in the country and one of the irreplaceable protected areas of the world (Le Saout et al. 2013; Lynch et al. 1997).

The study site is the Estación Experimental de San Lorenzo (11° 6' 41.61" N 74° 3' 17.13" W), located in the Serranía de San Lorenzo, on the northwestern slope of the SNSM, department of Magdalena, Colombia (Fig. 2A) at 2,200 m and comprising an area of 400 ha. The surrounding vegetation is comprised of well conserved partially mountainous primary and secondary Andean forest, which include tropical and subtropical rainforests of the isomesothermic jungles (14 °C to 24 °C) (Hernández-Camacho and Sánchez-Páez 1992). The site's native flora includes woody species of *Gustavia speciosa*, *Sloanea* sp. and some palm species of the genus *Geonoma* sp. and *Chamaedorea* sp. (Cleef and Rangel 1984; Rangel and Garzon 1995). Nevertheless, some hectares of non-native vegetation are present. Coniferous forests with species of *Pinus patula* and *Cupressus lusitanica* introduced in the early 90s, may influence the amphibian assemblage that inhabit this area (Camero and Chamorro 1999). This sector presents one rainy season, between April through November, with a dry period between December through March (Tamaris-Turizo et al. 2007). The mean annual temperature is 12.8 °C, the mean annual precipitation is 2,446 mm and the relative humidity oscillates between 73–98% (Tamaris-Turizo and López-Salgado 2006).

Epidemiological Surveillance

Since 2008, population census have been performed employing the visual encounter survey (VES) method (Rodda et al. 2001; Rueda et al. 2006; Heyer et al. 1994) in determining numbers of animals with clinical signs of diseases, present deformities and/or individuals found dead in the surrounding areas of the Estación Experimental San Lorenzo. Epidemiological surveillance has been carried out through programmed visits every six months by researchers and biology students from the Universidad de Magdalena and through scheduled or nonscheduled visits by staff of the Parque Nacional Natural SNSM. We looked for anurans presenting lethargy, macroscopic lesions, abnormal postures, hyperemia, ulcers or the presence of fungi or other corporal anomaly, such as deformities. Data was collected for all individuals presenting clinical signs of a potential *Bd* infection. Swab samples from individuals were obtained following the protocol described by Hyatt et al. (2007). Frogs were captured using fresh disposable nitrile gloves and held individually in bags until the sample was taken. Each animal was swabbed by running a cotton swab ten times over the ventral surface, the inner thigh area, and the plantar surface for a total of 50 strokes. Cotton swabs were kept dry and then stored at -20 °C until processing of future laboratory analysis.

One single specimen of *Pristimantis megalops* with clinical disease signs was analyzed using histological methods. The samples of the skin areas affected with lesions were fixed in FAA (Formalin alcohol-acetic) for 24–48 hr at 6 °C. Subsequently, dehydrated in graded series of alcohols (30, 40, 50, 60, 70, 80, 90, 95, and 100%) and two cleared steps in xylene for two hours, then embedded in Paraplast Plus (Mc Cormick®) for 12 hr at 55 °C (Luna 1968; Suvarna et al. 2012). Transverse and longitudinal sections were obtained with a rotary microtome Leica ® model (RM2125) set to 4–5µm thick. These were stained with hematoxylin-eosin for general descriptions and were previously stained using the Van Gieson technique with alcian blue and Gill III hematoxylin (changes made by the authors) to demonstrate collagen in the connective tissue. The sections were examined under a light microscope Nikon Eclipse Ni-U® equipped with differential interference contrast (CDI). The photographs were obtained with DS-Fi2® Nikon digital camera using the NIS Elements of Nikon software version 3.07. The image processing was performed with Image-Pro Analyzer 6.3 program (Media Cybernetics). These analysis were carried out in the biology laboratory of the Universidad de

Antioquia (Medellin, Colombia) and photographs taken in the Laboratory of Biotechnology of the Sede de Investigacion Universitaria (SIU-UdeA).

Furthermore, a single specimen of *Ikakogi tayrona* with clinical disease signs was analyzed using conventional Polymerase Chain Reaction (PCR) to determine *Bd* presence. DNA was extracted from swabs using GeneReleaser® (Bioventures Inc., Carlsbad, California, USA). We used the primers developed by Annis et al. (2004) to amplify the ITS1-ITS2 region specifically in *B. dendrobatidis*: *Bd*1a (5'-CAGTGTGCCATATGTCACG-3') and *Bd*2a (5'-CATGGTTCATATCTGTCCAG-3'). Amplifications were performed in an MJ Research Peltier Thermal Cycler (PTC-200), as follows: an initial two minute denaturation at 95 ºC followed by 35 cycles of DNA amplification (i.e., 45 sec at 95 ºC, 45 sec at 55 ºC, and one min at 72 ºC). A final extension at 72 ºC for 10 min completed the amplifications. Each reaction consisted of 0.5 µL of each primer (1 M), 3.0 µL of doubly distilled DNA-free water, 6 µL of GoTaq® Green Master Mix (1X; Promega), and 2 µL of the DNA extract. The amplified fragments were separated by electrophoresis through 1% agarose gels. These analysis were carried out in the genetic laboratory of the Universidad de los Andes (Bogotá, Colombia).

Biosecurity Protocols

To date the presence of *Bd* has not been reported at Serranía de San Lorenzo. To prevent and reduce the risk of transmission of *Bd*, since 2008 park administrators from SNSM have been implementing a biosecurity protocol for all foreign and national personnel that enter the Estación Experimental de San Lorenzo. The protocol consists of disinfection of field equipment (boots, nets, measuring devices) used as much by researchers as by tourists who visit the protected area (Phillott et al. 2010).

Nevertheless, as a preventive measure *in situ*, field boots are washed with approximately 200 mL of a commercial sodium hypochlorite solution diluted in three liters of water.

Amphibian Assemblage

To describe the amphibian assemblage from the San Lorenzo sector, an inventory was carried out during seven surveys throughout the months of October and November of 2008, March of 2009, April and October of 2013, and April and November of 2014. No surveys were made in the years 2010–2012. Each field trip had a duration of four days, and the study site was surveyed for eight hours daily, employing the VES method in diurnal periods (9:00–12:00 and 15:00–17:00 hrs) and nocturnally (18:00–21:00 hrs). During the surveys, for each individual data associated with the habitat and time of day of the observation were registered. The number of researchers varied among surveys (from two to seven), and thus each survey had different effort levels (between 16 and 80 hours × person). To determine the relative abundance (RA) of different species in the sector, a classification of

Table 1. Epidemiological surveillance from the year 2008 to 2014 for each of the reported amphibians within the sector of the experimental station of San Lorenzo, 2,200 m altitude, Sierra Nevada of Santa Marta, Northern Colombia. Very rare (VR); Rare (R); Common (C); Abundant (A); Very Abundant (VA).

Species	*n*	RA	Microhabitat	Habit	Nº diseased individuals	Disease type	Nº deformed individuals	Nº dead individuals	Year of reported sick individual
Atelopus laetissimus	128	VA	Terrestrial/ Shrubs	Nocturnal	1	Cutaneous ulcers	1	0	2013
Atelopus nahumae	10	R	Terrestrial/ Shrubs	Diurnal/ Nocturnal	0	—	1	0	2013
Ikakogi tayrona	26	C	Shrubs	Nocturnal	1	Undetermined	0	0	2008
Pristimantis delicatus	13	R	Shrubs	Nocturnal	0	—	0	0	—
Pristimantis carmelitae	23	C	Terrestrial	Nocturnal	0	-	0	0	—
Pristimantis cristinae	5	VR	Shrubs	Nocturnal	0	-	0	0	—
Pristimantis insignitus	4	VR	Terrestrial	Nocturnal	0	-	0	0	—
Pristimantis megalops	477	VA	Terrestrial	Diurnal/ Nocturnal	9	Fibropapilloma (tumors)	0	0	2008; 2013; 2014
Pristimantis ruthveni	15	Rare	Shrubs	Nocturnal	0	—	0	0	—
Pristimantis sanctaemartae	211	VA	Shrubs	Nocturnal	0	—	0	0	—
Pristimantis tayrona	48	A	Phytotelmata	Nocturnal	0	—	0	0	—
Pristimantis sp. nov. 1	29	C	Shrubs	Nocturnal	0	—	0	0	—
Pristimantis sp. nov. 2	21	C	Terrestrial/ Shrubs	Nocturnal	0	—	0	0	—
Bolitoglossa savagei	44	A	Shrubs/Phytotelmata	Nocturnal	0	—	0	0	—

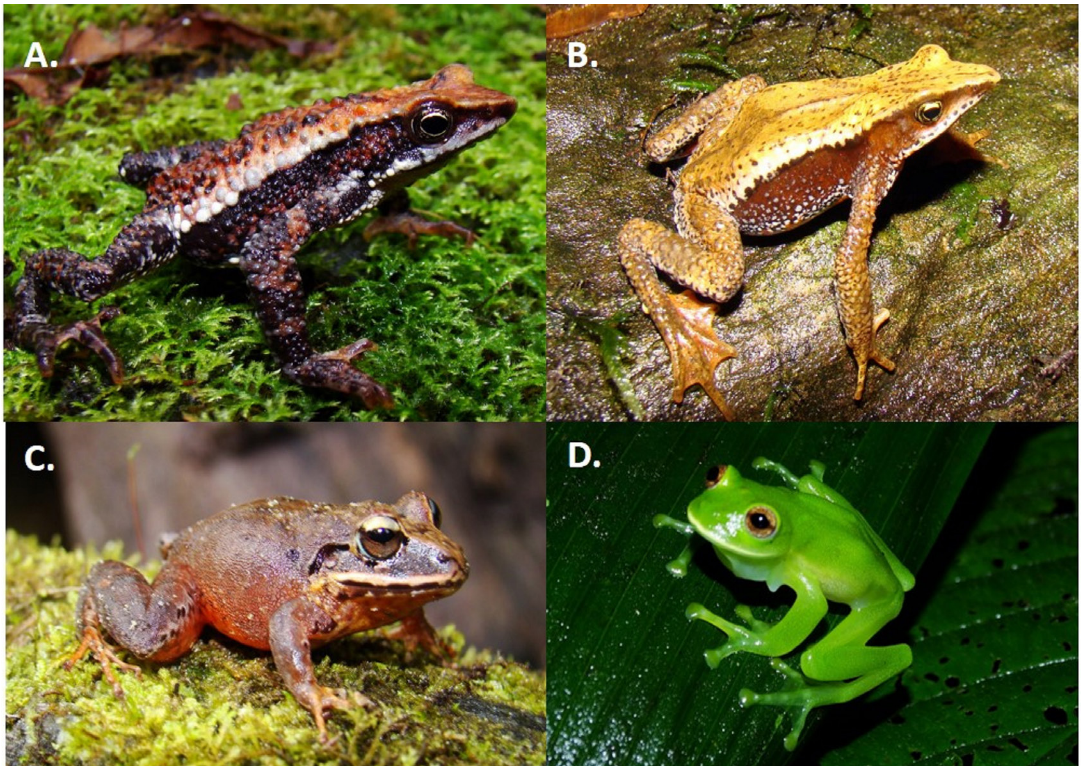

Fig. 3. Healthy individuals of *Atelopus laetissimus* (**A**) (Bufonidae); *Atelopus nahumae* (**B**) (Bufonidae); *Pristimantis megalops* (**C**) (Craugastoridae); and *Ikakogi tayrona* (**D**) (Centrolenidae). *Photographs by Luis Alberto Rueda Solano.*

very rare, rare, common, abundant and very abundant, according to the of individuals (ind.) recorded during all the surveys was established. Species were very rare, if it was observed equal to or less than nine ind.; rare, if it was observed between 10–20 ind.; common, if it was observed between 21–30 ind.; abundant if it was observed between 31–50 ind.; and very abundant, if it was observed over 50 ind. in the total of all surveys. For identification of the species belonging to the genus *Pristimantis* we employed the synopsis of Lynch and Carranza (1985) and for the remaining species we used the information provided by the American Museum of Natural History from its online reference Amphibian Species of the World (Frost 2014).

Monitoring of *Atelopus laetissimus* and *Atelopus nahumae*

Due to the importance represented by the species of *Atelopus*, for being one of the most affected genus for *Bd* (La Marca et al. 2005; Lotters 2007), we monitored both species reported at this locality, *A. laetissimus* and *A. nahumae* (Ruiz-Carranza et al. 1994). These species were monitored during the same months of the amphibian inventories of the sector. Nevertheless, these surveys were done during two additional days. Employing a low lying transect of 50 m in length and five m wide over the stream known as "La Quebrada San Lorenzo" located about 500 m north of the Estación Experimental de San Lorenzo at 2,100 m (Fig. 2B). Surveys were done twice daily for individuals of *Atelopus*, in the morning (9:00–12:00 hrs) and at night (18:00–00:00 hrs). For individuals of *Atelopus*, SVL, weight, and sex have been systematically recorded since 2013. Each individual was handled with a new pair of gloves as part of the biosecurity protocols to prevent the transmission of *Bd*.

RESULTS

Epidemiological Surveillance

To date no mass mortality events have been reported, or individuals with field evidence of *Bd* infections in the assemblage of the 15 endemic species of amphibians at the San Lorenzo sector, corresponding to 1,375 ind. mostly healthy individuals of Bufonidae, Craugastoridae, Centrolenidae, and Plethodontidae (Table 1, Fig. 3). However, 13 sick individuals of the species *Ikakogi tayrona*, *Atelopus laetissimus*, *A. nahumae*, and *Pristimantis megalops* were recorded (Table 1, Fig. 4).

The sick individual of *Ikakogi tayrona* presented symptoms similar to those of chytridiomycosis, such as lethargy, pale skin, and hyperemia of the ventral skin (Fig. 4A, B). Nevertheless, the conventional PCR laboratory analysis yielded negative results for *Bd*. *Pristimantis megalops*, presented the greatest number of sick individuals (Table 1), they were affected by white colored tumors, bulgy and with a hard consistency (Fig. 4C, D). Healthy skin of *P. megalops* is smooth and dark-brown in color (Fig. 4, 5), while the area associated with the tumor was hyperplastic with white coloration (Fig. 5A). The

Fig. 4. Sick individuals of *Ikakogi tayrona* (**A**, **B** same individual); *Pristimantis megalops* (**C, D**), and malformation in *Atelopus nahumae* (**E**) and *Atelopus laetissimus* (**F**) found in epidemiological surveillance 2008–2014 in La Estación Experimental de San Lorenzo (2,200 meters), Sierra Nevada de Santa Marta, North of Colombia. *Photographs by Luis Alberto Rueda Solano* (**A**, **B**, **D**), *Cesar Molina* (**C**)*; Andres Rocha Usuga* (**E**, **F**).

epidermis of the areas that are not associated with tumors is thin with few cell layers and is delimited by a layer of strongly pigmented skin cells (Fig. 5B). The dermis is thick and has large collagen layers that are joined closely to the underlying striated muscle through connective tissue (Fig. 5B). Neoplasia histologically corresponds to a skin fibropapilloma or fibroma, formed mainly of fibroblasts and marked anisocariosis characterized to be heavy vascularization (Fig. 5C, D). The fibropapilloma is white in color because the pigmented layer of the dermis present in healthy skin disappears completely (Fig. 5E, F). Also, dermal collagen layers are less compact, unorganized, and not associated with the muscle due to the profuse growth of fibroblasts in the tumor (Fig. 5F). The epidermis associated with neoplasia can present areas of few cell layers similar to healthy skin or otherwise be hyperplastic (Fig. 5F). Despite these individuals presenting cutaneous disease, they did not present symptoms of lethargy or malnutrition. No other similar characteristics pertaining to these tumors were observed in other species of the assemblage.

Regarding *Atelopus laetissimus* only one individual presented a cutaneous ulcer on the abdomen, and two individual of *A. laetissimus* and *A. nahumae* were found with malformations of the extremities and face (Fig 4 E, F). Skin swab samples were collected for future *Bd* analysis for *Atelopus* species. In 2013 we collected 11 samples for *A. laetissimus* and one sample for *A. nahumae* and in 2014, 13 samples for *A. laetissimus* and four for *A. nahumae* were collected. These samples were deposited dry and cool (-20 °C) at the biology laboratory of the Universidad de Magdalena.

Amphibian Assemblage

All species for which epidemiologic surveillance was carried out at the sector of San Lorenzo are endemic to the SNSM. The majority of individuals (61.5%) belong

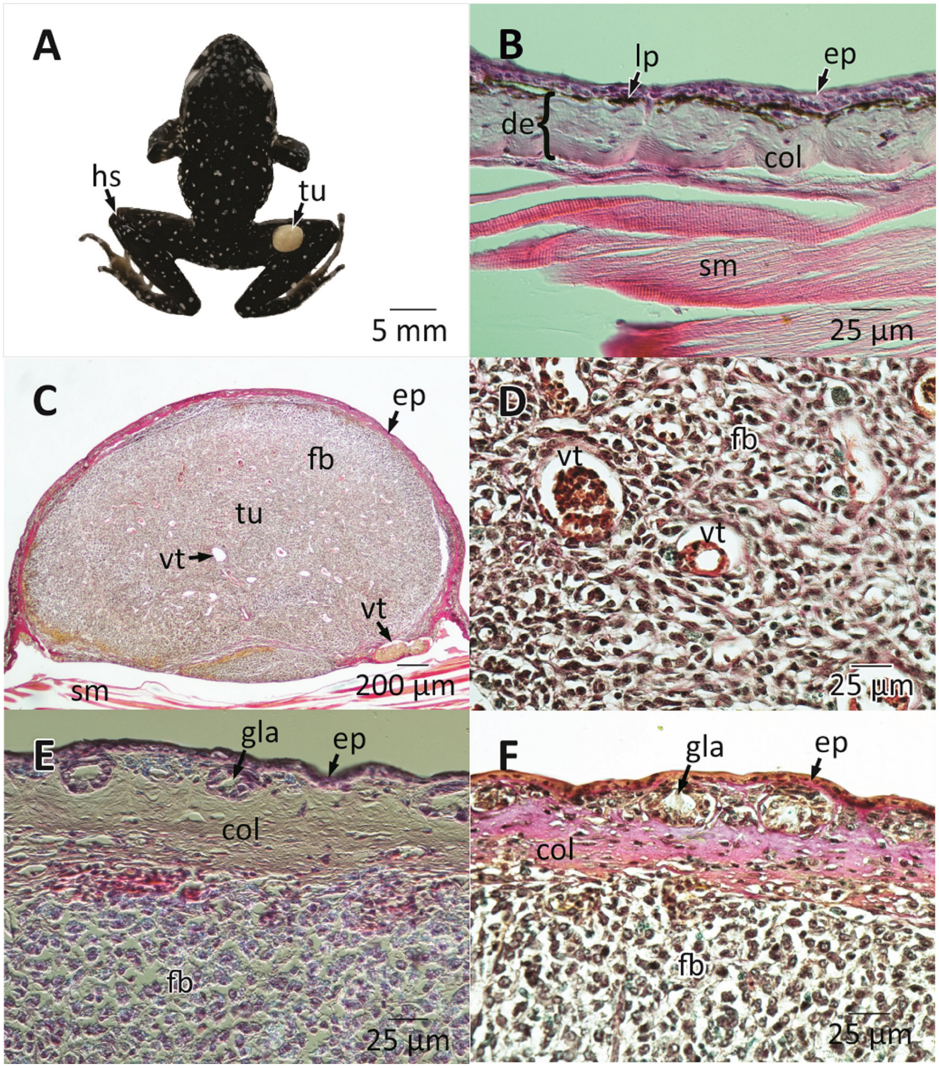

Fig. 5. Healthy skin and fibropapilloma in *Pristimantis megalops* (**A**). Cross section of the healthy skin of *Pristimantis megalops* (**B**): The epidermis is thin, can be seen pigmented layer and layers of collagen in the dermis. Cross section of Fibropapilloma (**C**). Detail of fibroblasts forming fibropapilloma (**D**): the tissue is highly vascularized. Detail of the epidermis and collagen layers covering fibropapilloma (**E–F**). Histochemical staining in Fibropapilloma (**F**): The layers of collagen in the dermis can be seen in magenta color. **fb**: fibroblasts; **col**: layers of collagen; **de**: dermis; **ep**: epidermis; **gla**: dermal glands; **hs**: healthy skin; **lp**: pigmented layer; **sm**: striated muscle tissue; **tu**: tumor or fibropapilloma; **vt**: vascular tissue. *Photographs by Edgar Javier Rincón Barón.*

to the genus *Pristimantis*, with ten species (of 15 in total); dominating in abundance, two of these are in the process of being described (Table 1). *Pristimantis megalops* and *P. sanctaemartae* were the most abundant. In this sector both genera of endemic and monotypic species *Ikakogi tayrona* and *Geobatrachus walkeri* were recorded. Only one species of salamander was observed, *Bolitoglossa savagei* with a moderate abundance (Table 1). Similarly, both described species of *Atelopus* for this sector were reported, *A. laetissimus* are more abundant; conversely individuals of *A. nahumae* were rarely observed (Table 1).

Monitoring of *Atelopus laetissimus* and *Atelopus nahumae*

In relation to the monitoring of the *Atelopus* species at the San Lorenzo stream, 128 records of *A. laetissimus* were obtained over the course of all the surveys, with an annual mean of 18 ind. (n = 7 surveys SD ± 5.46). In 2008 a mean of 19.5 ind. (n = 2 surveys; SD ± 2.12) (Fig. 6), in 2009 only one survey was done in which nine ind. were recorded (Fig. 6). From the year 2010 through 2012 no monitoring was done at the San Lorenzo stream. In 2013 a mean of 16 ind. (n = 2 surveys SD ± 1.41) of *A. laetissimus*, making this the year with the fewest number of records in comparison to other years (Fig. 6). Finally, in the year 2014 the greatest mean was recorded with 24 ind. (n = 2 surveys; SD ± 2.82) (Fig. 6). Although the sequence of years is incomplete, the tendency in the past two years has been a slight increase in the number of observations of *A. laetissimus* individuals (Fig. 6).

Around 90% of the records of *A. laetissimus* observed have been males, principally in the night hours perched on leaves. Males had a snout-vent-length (SVL) and average weight of 4.05 cm (n = 80 SD ± 0.46) and 4.87 g

(n = 80 SD ± 1.65) respectively. Only 11 females were observed, with SVL and weight averages of 5.0 cm (n = 11 SD ± 0.46) and 6.5 g (n = 11 SD ± 1.23) respectively. For *A. nahumae*, 10 individuals were recorded, two male individuals in the year 2008, one female in the year 2013 and three males and four females in 2014. Due to the scarce observations it was not possible to discern a population trend.

Discussion

Epidemiological surveillance in the amphibian assemblage of the San Lorenzo sector showed a low number of individuals with symptoms of disease and no dead individuals were found over the years 2008, 2009, 2013, and 2014. This contrasts with other localities in protected areas and nonprotected areas of the American continent in countries such as Canada (Greer et al. 2005), United States (Green et al. 2002), Costa Rica (Crump et al. 1992; Lips and Papendick 2003), Panama (Crawford et al. 2010; Lips et al. 2006; Lips 2003), Venezuela (Bonaccorso, Guayasamin, Méndez, and Speare 2003), and Ecuador (Bustamante et al. 2005; Merino-Viteri et al. 2005), where mass mortalities and population and amphibian assemblage collapses have been reported due to emergent diseases.

Only one mass mortality event has been reported in Colombia in the year 1997 in Serranía de los Paraguas between the departments of Choco and Valle del Cauca (Lynch and Grant 1998), nevertheless anecdotal information shows the absence of some amphibian species in other localities which were previously more diverse and abundant, for example the Parque Nacional Natural Chingaza (A. Amézquita and C. Navas, pers. comm.). This would not be the case for the amphibian assemblage of the protected area of the San Lorenzo sector, given that all the species reported have historical type localities from this mountain range (Lynch and Carranza 1985) including the species of *Atelopus* (Ruiz-Carranza et al. 1994) which would be vulnerable to declines due to chytridiomycosis (Lips et al. 2003). Additionally, we did not find evidence that any of these species are currently experiencing population declines. On the contrary, in this study new species were reported increasing the diversity of endemic amphibians for this locality and for the biogeographic region. Although, some anuran species still inhabit this mountain range, we could not survey them occurring in remote locations with restricted access; this is the case of *Atelopus arsyecue*, *A. walkeri*, and *A. carrikeri* (Rueda-Solano 2008). Therefore, our conclusions only focus on the populations of the protected area of the Serranía de San Lorenzo, for other localities we do not know the conservation status of amphibian assemblages. Although biosecurity protocols are an important aspect in the conservation of endemic amphibians, this study does not formally test or validate their decontamination protocols in this protected area.

In the special case of sick individuals found throughout the epidemiologic surveillance, the signs of illness of the individual of *Ikakogi tayrona* were very similar to those presented by individuals affected by chytridiomycosis (Daszak et al. 1999). Nevertheless, with negative *Bd* results the possibility exists that the signs detected in the field may be misinterpreted as typical of chytridiomycosis, when in reality are part of the symptomatology of other diseases which have not been previously diagnosed. However, our results are limited mainly to the detection of signs of chytridiomycosis in the field, it is al-

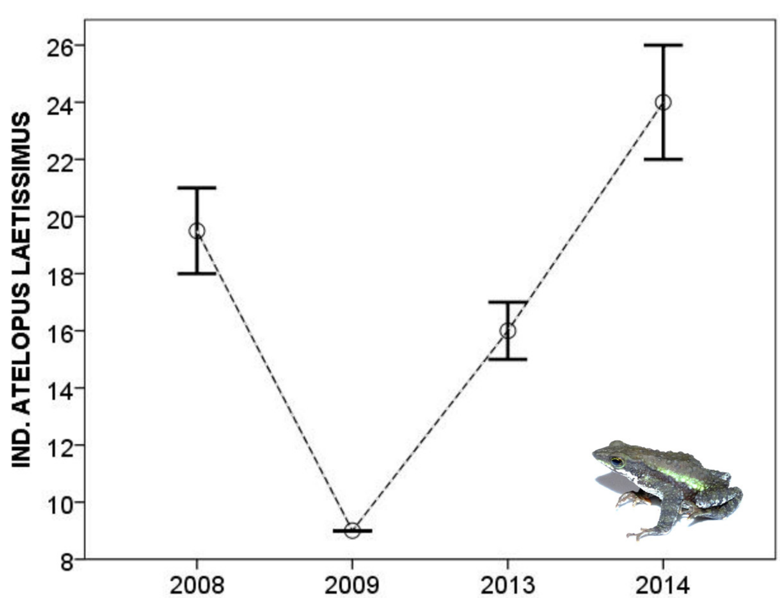

Fig. 6. Monitoring of *Atelopus laetissimus* through the years in the Quebrada San Lorenzo Serrania de San Lorenzo, SNSM. Circle = average number obtained from individuals in each year (n = 2 samples for the years 2008, 2013 and 2014) (n = 1 sample for 2009); Error Bars = maximum and minimum individual in each year. Dotted line = trend in the number of individuals over time.

ways recommended to corroborate the field observations with laboratory analysis for accurate detection of *Bd*.

In respect to the sick specimens of *Pristimantis megalops*, we are unaware of any reference regarding the symptoms presented by these individuals in Colombia, however similar neoplastic diseases are documented in others amphibian species (Green and Harshbarger 2001; Khudoley and Mizgireuv 1980). The origin of these spontaneous neoplasms are still unclear and often limited to specific species or populations (Stacy and Parker 2004), as a matter of fact; *P. megalops* was the only species in the assemblage that presented this cutaneous anomaly. Similarly, we are unaware if these tumors may be lethal for these individuals, above all because the individuals reported to have these tumors did not appear to have affected locomotion or nutritional state. It is necessary to conduct more studies about the tumor effects on these individuals, at the physiological, behavioral, and ecological level. Additionally, it should be corroborated whether this disease is unique to *P. megalops* or it can be found among populations of amphibians at different localities.

Historically the populations of *Atelopus laetissimus* and *A. nahumae* found at the Serranía de San Lorenzo have been abundant (Carvajalino-Fernandez et al. 2008; Granda-Rodriguez et al. 2008, 2012; Ruiz-Carranza et al. 1994). Nevertheless, at our study site, *A. laetissimus* is predominantly abundant, while *A. nahumae* is ecologically rare, it may be due to our surveys coincide with the upper extreme of its altitudinal distribution. This is corroborated at lower localities (approx. 1,500 m) where this species is predominantly abundant and *A. laetissimus* is ecologically rare (Carvajalino-Fernandez et al. 2013; Rueda-Solano, pers. observ.). In relation to the abundance of *A. laetissimus* at the San Lorenzo stream, we do not have sufficient data to estimate a population trend, nevertheless, in the last two years of monitoring (2013 and 2014) we have found a similar abundance, which may suggest a stable population for this species in the sector of the Sierra Nevada de Santa Marta. This conclusion coincides with previous studies which show relative abundances of *A. laetissimus* and *A. nahumae* (Carvajalino-Fernandez et al. 2008; Granda-Rodriguez et al 2008, 2012). At present *A. laetissimus* and probably *A. nahumae* have stable and abundant populations, this strongly contrast with the problematic declines and extinctions of the entire *Atelopus* clade in the neotropics principally those which inhabit high elevations (La Marca et al. 2005; Lotters 2007). Nevertheless, we are unaware of the historical population trend for these species, especially during the years where reported declines were in Colombia (Lynch and Grant 1998), where *Bd* may have caused declines in these species, however it was not documented and may currently coexist with *Bd*, as occurs for other species of *Atelopus* (Flechas et al. 2012; Tarin et al. 2014), or in other amphibian communities where there is a higher prevalence of *Bd* and no evidence it affects the natural populations (Guayasamin et al. 2014). We suggest retrospective studies including historical demography and *Bd* diagnosis using museum specimens (Rodriguez et al. 2014; Cheng et al. 2011).

Similarly, the majority of records for *Atelopus laetissimus* correspond to male individuals found active during nocturnal surveys, perched on leaves of bushes adjacent to streams. These observations coincide with historical data, in similar proportions (Granda-Rodríguez et al. 2008). In respect to females of these species, we can only deduce they possess different home ranges than males and encounters occur during reproductive seasons. Our results provide strong evidence about the nocturnal habits of the males of this species, which would employ leaves to perch and forage from (Rueda-Solano, pers. observ.). These observations are inconsistent with almost all the species of the genus *Atelopus*, which are diurnal (Lotters 1996), with one exception previously described for *Atelopus nocturnus* (Bravo-Valencia and Rivera-Correa 2011).

Conclusion

The implementation of epidemiological surveillance, monitoring of key amphibian species, and biosecurity protocols at the San Lorenzo area have been constituted as important strategies for the conservation management of the endemic amphibians within the protected area of the Sierra Nevada of Santa Marta. It is expected that these actions be sustained and replicated at other protected areas in Colombia and the world, with amphibian assemblages susceptible to *Bd*. At a minimum they should serve as a baseline in establishing amphibian conservation methods and best management practices for *in-situ* programs. In like manner these actions should be complemented with the utilization of laboratory methods for the detection of *Bd* and other diseases.

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