



A new locality, range extension and record of *Batrachochytrium dendrobatidis* in the endangered terrestrial breeding frog *Pristimantis katoptroides* Flores, 1988 (Anura: Craugastoridae) in Peru

Juan C. Cusi^{1*}, Andy C. Barboza², Vance Vredenburg^{3,4} and Rudolf von May⁴

1 Departamento de Herpetología, Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Av. Arenales 1256, Jesús María, Apdo. 14-0434, Lima 14, Peru

2 División de Herpetología, CORBIDI (Centro de Ornitología y Biodiversidad). Santa Rita 117, Huertos de San Antonio, Surco, Lima, Peru

3 Department of Biology, San Francisco State University, San Francisco, CA 94132-1722, USA

4 Museum of Vertebrate Zoology, University of California, Berkeley, 3101 Valley Life Sciences Bldg., Berkeley, CA 94720, USA

* Corresponding author. E-mail: jcarloscusim@gmail.com

Abstract: We provide a new record of the poorly known and endangered *Pristimantis katoptroides* from the Cordillera Central in Peru, based on a specimen collected in the Alto Mayo Protected Forest, San Martin Department. We provide notes on the morphology and coloration of the species and two other specimens previously collected in Peru, as well as photographs and an updated map indicating the known localities of *P. katoptroides*. This record represents an extension of 210 km in the southern limit of the geographic range. Additionally, we report the presence of the chytrid fungus in this species.

Key words: Andes, chytrid fungus, *Pristimantis katoptroides*, San Martin, Peru

INTRODUCTION

Pristimantis katoptroides Flores, 1988 was described from a site located 1 km west of Puyo, Pastaza Province, on the eastern slopes of the Andes in Amazonian Ecuador (01°28' S, 078°00' W, 1,000–1,500 m above sea level [a.s.l.]). This species was initially assigned to the *crucifer* assembly (Lynch and Duellman 1980; Flores 1988) and, more recently, it was assigned to the *Pristimantis unistigratus* species group (Hedges et al. 2008). The type locality is a low montane rainforest consisting of a mixture of heavily disturbed forest, a cultivated hillside, and less disturbed second growth forest (Flores 1988). Coloma et al. (2014) pointed out that *P. katoptroides* is a very rare species and that additional fieldwork is required to determine its precise geographic distribution. In Ecuador, the distribution of this species

spans Reserva Ecológica Río Zúñag, Sucumbios, Napo, Pastaza, Morona-Santiago, Zamora-Chinchi and Tungurahua provinces (Yáñez-Muñoz et al. 2013; Reyes-Puig et al. 2013). The first record of this species in Peru was made by Catenazzi and Venegas (2012), who collected specimens at the upper part of the Katerpiza creek, Santiago River basin (04°02'33.4" S, 077°32'26.2" W), during a rapid biological inventory in the Kampankis Mountain, Loreto department, northern Peru. To date, no additional records of this species are known from Peru.

Chytridiomycosis is an infectious disease caused by the chytrid fungus, *Batrachochytrium dendrobatidis* (Bd), which has contributed to amphibian population declines and extinction (Berger et al. 1998). In Peru, the chytrid fungus has been reported in diverse regions throughout the country (Seimon et al. 2005, 2007; Venegas et al. 2008; Catenazzi et al. 2010; Kosch et al. 2012), including one natural protected area (Manu National Park; Catenazzi et al. 2011). Despite the widespread occurrence of the chytrid fungus in Peru, many areas in the country have never been surveyed for this pathogen.

In a recent herpetological expedition conducted in the Alto Mayo Protected Forest (AMPF), San Martin department, Peru, we found one specimen of *Pristimantis katoptroides* in a montane forest located on the south-east part of the preserve. The study was conducted under permit number RJ N° 001-2014-SERNANP-BPAM-JEF issued by Ministerio del Ambiente, SERNANP, and the specimen captured was deposited in the herpetological collection of the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM) in Lima, Peru. The voucher specimen is an adult female (MUSM 33661)

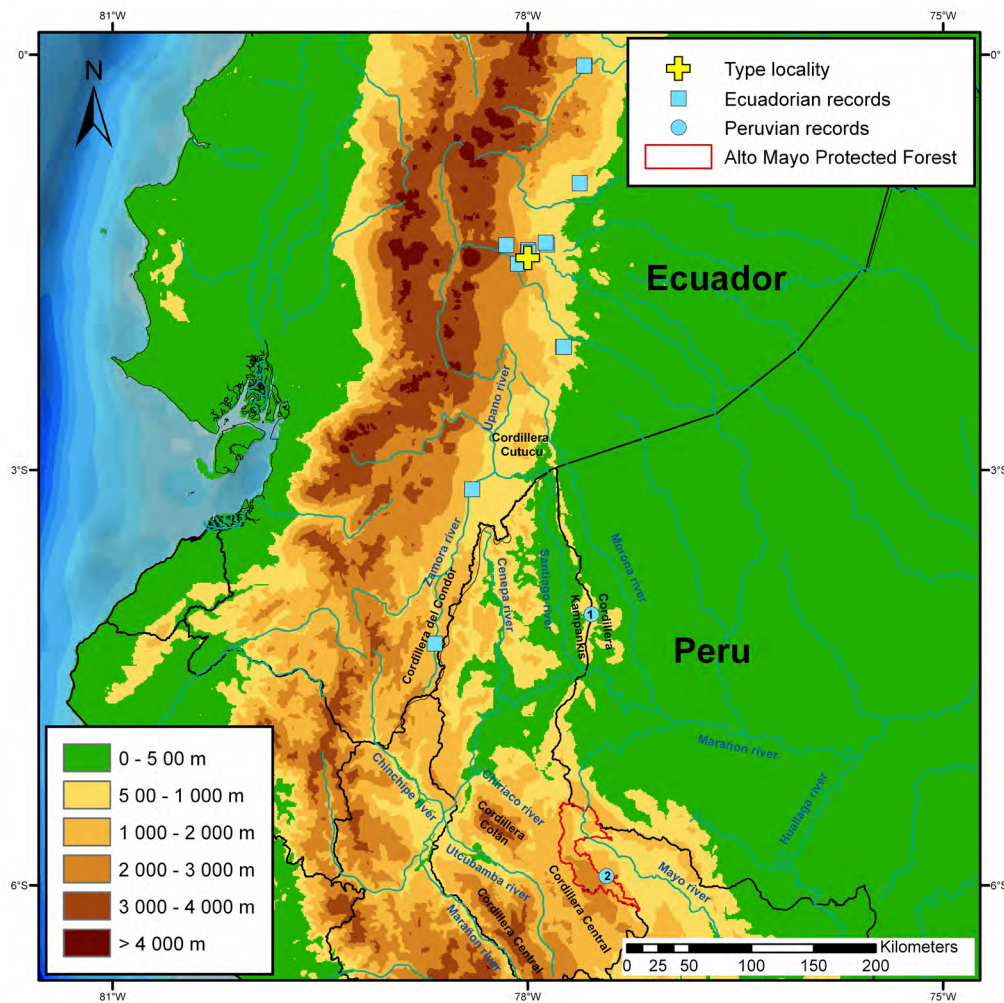


Figure 1. Map showing the distribution of *Pristimantis katoptroides* from Ecuador and Peru. Type locality from Near Puyo, Pastaza, Ecuador (yellow cross); additional Ecuadorian localities (sky-blue squares), Katerpiza creek, Kampankis Mountain, Loreto, Peru (sky-blue circle, number 1) and Tailandia stream, Awajun, Rioja, San Martin, Peru (sky-blue circle, number 2). Map by Juan C. Cusi.

and was found at nighttime, on 3 July 2014, sitting on fern leaves at 1.5 m above the ground along the Tailandia stream, Awajun District, Rioja Province, San Martin (05°56'06.3" S, 077°25'54.2" W, 1,644 m a.s.l.; Figure 1). This stream is located 4.97 km south from the town of Sol de Oro, and can be accessed on foot through a narrow trail that connects Sol de Oro and the property of a local settler (Mr. Ascón). Following the protocol from an ongoing study on the thermal physiology of amphibians in the AMPF, we recorded the body temperature of the frog in the field, as well as the air and substrate temperatures, using a handheld infrared thermometer (RayTek MiniTemp MT6) prior to capturing the specimen for further study. We recorded a body temperature of 19°C, air temperature of 17.2°C, and substrate (leaves) temperature of 18.2°C. In addition, a skin tissue sample was taken in field using an MW113-Advantage Bundling sterile cotton swab to swab the abdomen, thighs and hind limbs of the specimen and then it was stored dried in 1.5 ml tubes. The swab sample was tested for *Bd* using quantitative real time PCR (Boyle et al. 2004; Hyatt et al. 2007). We compared the voucher specimen we collected

with the two voucher specimens collected by Catenazzi and Venegas (2012) to verify the identity of the species. (These were the only specimens of *P. katoptroides* available for comparison in a Peruvian collection.) The morphometric measurements were taken with a digital caliper to the nearest 0.01 mm following Duellman and Lehr (2009) (Table 1).

Table 1. Morphometric measurements (mm) of three Peruvian specimens of *Pristimantis katoptroides*.

	CORBIDI 9451 ♀	CORBIDI 9455 ♀	MUSM 33661 ♀
Snout-vent length (SVL)	33.02	29.61	26.78
Tibia length (TL)	17.07	15.20	13.66
Foot length (FL)	15.90	14.67	12.90
Head length (HL)	10.86	10.13	9.80
Head width (HW)	13.44	12.28	10.57
Interorbital distance (IOD)	3.41	2.22	3.76
Upper eyelid width (EW)	2.50	2.56	2.70
Internarial distance (IN)	2.59	2.33	2.31
Eye-Nostril distance (EN)	3.70	3.79	2.97
Eye diameter (ED)	3.88	3.91	3.02
Tympanum diameter (TY)	1.51	1.31	0.84

The three Peruvian specimens from San Martín (MUSM 33661; this study) and Loreto (CORBIDI 9451, 9455; Catenazzi and Venegas 2012) agree with diagnostic characters of *P. katoptroides* by Flores (1988): snout long (EN about equal ED) anteroventrally-sloping

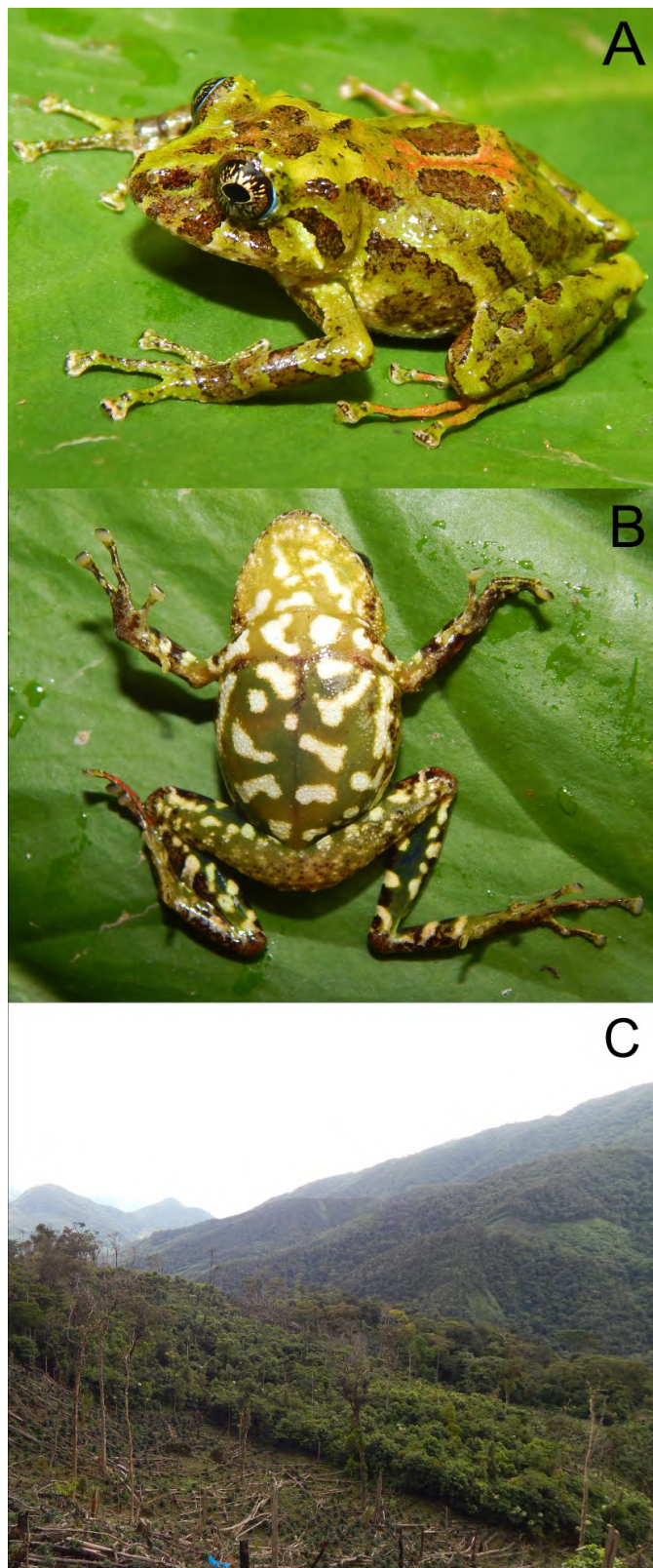


Figure 2. Dorsolateral (A) and ventral (B) views of *Pristimantis katoptroides* (MUSM 33661). (C) Panoramic view of the disturbed montane forest in the new locality record of the species. Photos by Juan C. Cusi.

dorsally in profile, rounded ventrally; upper eyelid bearing many conical tubercles; rounded concave canthus rostralis; flared lips; vague and narrow lateral keels on the fingers; apically truncate digital pads; two conical tubercles on the heel and one on the knee; dorsum ground grass green with coppery brown markings, underside off-white with greenish-brown reticulations and groin and inside of calves deep greenish-blue. *Pristimantis katoptroides* is easily distinguishable from similar species (*P. galdi*, *P. eriphus*, *P. crucifer* and *P. roni*) by the absence of cranial crests, a partially concealed tympanum, creamy yellow iris with black radiated reticulations, ulnar tubercles non-conical, and outer edges of forelimbs and hind limbs bearing non-conical tubercles, ventral coloration off-white with greenish-brown reticulations, and a greenish-blue groin in female (Figure 2A and 2B). Of these, *P. roni* is the most similar to *P. katoptroides* but markedly distinctive by its greenish cream belly and throat finely dotted with many gray spots in females and gray with white markings on males. The three Peruvian specimens of this study are all females with snout-vent lengths (SVL) of 33.02–26.78 mm (Table 1). The result of the molecular diagnostic assay (real time PCR) detected the presence of *Bd* in the only one Peruvian specimen of *Pristimantis katoptroides* collected on 2014. The place where we collected the specimen *Bd*+ is located approximately 136.2 km ESE of Santa Rosa, Jaén, Cajamarca and 137.3 km west-northwest of Ahuashiyacu, La Banda de Shilcayo, San Martín, San Martín, which were the nearest *Bd*+ sites reported (Kosch et al. 2012).

The new locality record reported here represents an extension of 210.1 km from the southern limit of the geographic range of *P. katoptroides* (Catenazzi and Venegas 2012). The new locality is located 499.8 km south from the type locality (Flores 1988) and 231.6 km north-northwest from Zumi, Las Orquídeas, Tepuy, Zamora Chinchipe, southern most record of Ecuador (Ron et al. 2014) (Figure 1). The northern versant of the Cordillera Central of Peru is dissected by the Chiriaco, Utcubamba, and Mayo rivers. This results in several isolated mountain ranges such as the Cordillera Colán. These mountain ranges are interconnected with the Cordillera Cóndor, Cordillera Kampankis and Cordillera de Cutucú, and are home to amphibian species that are present in more than one mountain range (i.e., shared species) as well as species that are endemic to each mountain range (Duellman and Pramuk 1999; Catenazzi and Venegas 2012). Our observations suggest that *P. katoptroides* is associated with streams and can inhabit secondary forest, given that we captured the voucher specimen in a secondary forest surrounded by coffee crops and clear-cut areas (Figure 2C). *P. katoptroides* is currently

categorized as Endangered B1ab (iii) by the IUCN Red List of Threatened Species (www.iucnredlist.org) because the extent of its habitat has been dramatically reduced as a result of agriculture expansion, logging, and development of human settlements in the region (Coloma et al. 2004). Furthermore, our record of *Bd* in *Pristimantis katoptroides* from AMPF suggests that the fungus could be present in other amphibian populations in the region. The presence of *P. katoptroides* within the boundaries of the AMPF is relevant for the conservation of this threatened species because large areas of potentially suitable habitat have been lost outside of the preserve. Given that many threatened amphibian species in Peru are known to occur only outside natural protected areas (von May et al. 2008), is it essential to carry out additional field surveys focusing on target species and determine the conservation status of their habitats inside and outside protected areas.

ACKNOWLEDGEMENTS

We thank Conservation International Proyecto BioCuenas for providing support for this study. We thank Dr. Ulla Helimo for her encouragement and valuable suggestions on our research plan, and Jesús Córdova for providing access to Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM). We also thank Pablo Venegas and Alessandro Catenazzi for providing information and access to Centro de Ornitología y Biodiversidad (CORBIDI), Mauricio Ortega-Andrade for verifying our species identification and Santiago Ron for his comments and records of occurrence of *P. katoptroides*. Gustavo Montoya and Ivonne Paico of the AMPF office kindly helped with collecting permits and Sergio Villacorta (park ranger) helped us in the field during the expeditions to the Sol de Oro sector, Alto Mayo.

LITERATURE CITED

- Berger, L., R. Speare, P. Daszak, D. Green, A. Cunningham, C. Goggin, R. Slocumbe, M. Ragan, A. Hyatt, K. McDonald, H. Hines, K. Lips, G. Marantelli and H. Parkes. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proceedings of the National Academy of Sciences of the USA* 95: 9031–9036. doi: [10.1073/pnas.95.15.9031](https://doi.org/10.1073/pnas.95.15.9031)
- Boyle, D., D. Boyle, V. Olsen, J. Morgan and A. Hyatt. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms* 60: 141–148. doi: [10.3354/daoo60141](https://doi.org/10.3354/daoo60141)
- Catenazzi, A., V. Vredenburg and E. Lehr. 2010. *Batrachochytrium dendrobatidis* in the live frog trade of *Telmatobius* (Anura: Ceratophryidae) in the tropical Andes. *Diseases of Aquatic Organisms* 92: 187–191. doi: [10.3354/daoo2250](https://doi.org/10.3354/daoo2250)
- Catenazzi, A., E. Lehr, L. Rodríguez and V. Vredenburg. 2011. *Batrachochytrium dendrobatidis* and the collapse of anuran species richness and abundance in the Upper Manu National Park, Southeastern Peru. *Conservation Biology* 25: 382–391. doi: [10.1111/j.1523-1739.2010.01604.x](https://doi.org/10.1111/j.1523-1739.2010.01604.x)
- Catenazzi, A. and P. Venegas. 2012. Anfíbios y Reptiles, pp. 106–117, in: N. Pitman, E. Ruelas, D. Alvira, C. Vriesendorp, D. Moskovits, A. del Campo, T. Wachter, D. Stotz, S. Noningo, E. Tuesta and R. Smith (eds). Perú: Cerros de Kampankis. Rapid Biological and Social Inventories Report 24. Chicago: The Field Museum.
- Coloma, L., S. Ron, D. Almeida and F. Nogales. 2004. *Pristimantis katoptroides*. The IUCN Red List of Threatened Species. Version 2014.3. Accessed at <http://www.iucnredlist.org>, 22 November 2014.
- Duellman, W. and J. Pramuk. 1999. Frogs of the genus *Eleutherodactylus* (Anura: Leptodactylidae) in the Andes of northern Peru. *Scientific Papers, Natural History Museum, University of Kansas* 13: 1–78. doi: [10.5962/bhl.title.16169](https://doi.org/10.5962/bhl.title.16169)
- Duellman, W. and E. Lehr. 2009. Terrestrial-breeding frogs (Strabomantidae) in Peru. Münster: Nature und Tier-Verlag. 382 pp.
- Flores, G. 1988. Two new species of Ecuadorian *Eleutherodactylus* (Leptodactylidae) of the *E. crucifer* assembly. *Journal of Herpetology* 22: 34–41. doi: [10.2307/1564354](https://doi.org/10.2307/1564354)
- Hedges, S., Duellman W. and M. Heinicke. 2008. New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa* 1737: 1–182.
- Hyatt, A., D. Boyle, V. Olsen, D. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, R. Phillott, R. Campbell, G. Marantelli, F. Gleason and A. Colling. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73: 175–192. doi: [10.3354/daoo73175](https://doi.org/10.3354/daoo73175)
- Kosch, T., V. Morales and K. Summers. 2012. *Batrachochytrium dendrobatidis* in Peru. *Herpetological Review* 43(2): 150–159.
- Lynch, J. and W. Duellman. 1980. The *Eleutherodactylus* of the Amazonian slopes of the Ecuadorian Andes (Anura: Leptodactylidae). *Miscellaneous Publication, Museum of Natural History, University of Kansas* 69: 1–86. doi: [10.5962/bhl.title.16222](https://doi.org/10.5962/bhl.title.16222)
- Ron, S., Guayasamin J., Yanez-Muñoz M., Merino-Viteri A., Ortiz D. and D. Nicolalde. 2014. AmphibiaWebEcuador. Version 2014.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador. Accessed at <http://zoologia.puce.edu.ec/Vertebrados/anfibios>, 14 January 2015.
- Seimon, T., G. Hoernig, P. Sowell, S. Halloy and A. Seimon. 2005. Identification of chytridiomycosis in *Telmatobius marmoratus* at 4450 m in the Cordillera Vilcanota of southern Peru. In: *Studies on the Andean frogs of the genera Telmatobius and Batrachophrynus*. *Monografías de Herpetología* 7: 275–283.
- Seimon, T., A. Seimon, P. Daszak, S. Halloys, L. Schloegel, C. Aguilar, P. Sowell, A. Hyatt, B. Konecky and J. Simmons. 2007. Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biology* 13: 288–299. doi: [10.1111/j.1365-2486.2006.01278.x](https://doi.org/10.1111/j.1365-2486.2006.01278.x)
- Venegas, P., A. Catenazzi, K. Siu Ting and J. Carrillo. 2008. Two new harlequin frogs (Anura: Bufonidae: *Atelopus*) from the Andes of northern Peru. *Salamandra* 44: 163–176.
- von May, R., A. Catenazzi, A. Angulo, J. Brown, J. Carrillo, G. Chávez, J. Córdova, A. Curo, A. Delgado, M. Enciso, R. Gutiérrez, E. Lehr, J. Martínez, M. Medina-Müller, A. Miranda, D. Neira, J. Ochoa, A. Quiroz, D. Rodríguez, L. Rodríguez, A. Salas, T. Seimon, A. Seimon, K. Siu-Ting, J. Suárez, C. Torres and E. Twomey. 2008. Current state of conservation knowledge on threatened amphibian species in Peru. *Tropical Conservation Science* 1: 376–396.
- Yáñez-Muñoz, M., C. Frenkel, Varela-Jaramillo A. and S. Ron. 2013. *Pristimantis katoptroides*. AmphibiaWebEcuador. Version 2014.0. Museo de Zoología, Pontificia Universidad Católica del Ecuador.

Accessed at <http://zoologia.puce.edu.ec/vertebrados/anfibios/FichaEspecie.aspx?Id=1424>, 23 November, 2014.

Yáñez-Muñoz, M., P. Bejarano-Muñoz, J. Brito and D. Batallas. 2014. Terrestrial frog of the Southeastern Andes of Ecuador II: A new species of spiny green *Pristimantis* of montane forests of Sangay National Park Anura: Craugatoridae. *Avances en Ciencia e Ingenierías* 6(2): B63–B77.

Authors' contribution statement: JCC and AB conducted the fieldwork, analyzed the data, and wrote the manuscript. RVM reviewed all drafts and contributed significantly with manuscript preparation. VV made the real time PCR analysis. All authors reviewed, edited, and approved the final version.

Received: February 2015

Accepted: February 2015

Editorial responsibility: Natan Maciel