Short Communication

Rediscovery of *Atelopus cruciger* (Anura: Bufonidae): current status in the Cordillera de La Costa, Venezuela

Argelia Rodríguez-Contreras, J. Celsa Señaris
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Abstract The genus *Atelopus* (Anura: Bufonidae) has suffered one of the most drastic declines recorded in the Neotropics. Nine of 10 Venezuelan species are categorized as Critically Endangered on the IUCN Red List. None of these species had been observed since the mid 1980s until recently, when an adult female of *Atelopus mucubajiensis* and several adults of *A. cruciger* were rediscovered. To assess the current distribution and status of *A. cruciger* we explored 15 locations where it was formerly known. Two populations were detected. Historical and current distribution maps were constructed based on these field explorations and data from museum collections. Using PCR assays we detected infection with *Batrachochytrium dendrobatidis* in one morbid and three live specimens. Our surveys suggest *A. cruciger* populations from lower altitudes on the northern slope of the Cordillera de La Costa are recovering, although the presence of *B. dendrobatidis* could jeopardize their long-term survival unless active conservation strategies are adopted.

Keywords Amphibian decline, *Atelopus cruciger*, *Batrachochytrium dendrobatidis*, Critically Endangered, harlequin frog, Venezuela.

The Neotropical genus *Atelopus* (Anura: Bufonidae) has declined dramatically; 70% of 113 species have suffered population crashes (Lötters *et al.*, 2004; La Marca *et al.*, 2005; Pounds *et al.*, 2006; IUCN, 2007). There are 10 endemic *Atelopus* species in Venezuela (La Marca & Reinthaler, 1991). Nine of these are categorized as Critically Endangered and one is possibly extinct (Rodríguez & Rojas-Suárez, 1995; IUCN, 2007). Despite intensive sampling in their former habitats, most species had not been observed since the 1980s (La Marca & Lötters, 1997; Manzanilla & La Marca, 2004). Although the causes of many amphibian population declines are not yet well understood, chytridiomycosis, a disease caused by the pathogenic fungus *Batrachochytrium dendrobatidis*, has been frequently associated with *Atelopus* declines (Berger *et al.*, 1998; Lips, 1998; Bonaccorso *et al.*, 2003; Lampo *et al.*, 2006).

Once common, *Atelopus cruciger* disappeared for almost 20 years and was believed to be extinct (Manzanilla & La Marca, 2004). However, recent sightings of *A. cruciger* at the Cordillera de La Costa in the Venezuelan coastal range and *A. mucubajiensis* at the Cordillera de Mérida in the Venezuelan Andes indicated they may yet be extant (Eliot, 2003; Barrio-Amorós, 2004). We therefore evaluated the current distribution of *A. cruciger* and the presence of *B. dendrobatidis* in its remnant populations.

To construct a historical distribution map of the species we examined data from five national (Museo Estación Biológica de Rancho Grande, MEBRG; Museo de Biología de la Universidad Central de Venezuela, MBUCV; Museo de Historia Natural La Salle, MHNLS; Museo del Instituto de Zoología Agrícola de la Universidad Central de Venezuela, MIZA; Dr. Manuel González Sponga's private collection) and eight US museum collections (California Academy of Sciences, CAS; Carnegie Museum of Natural History, CM; Field Museum of Natural History, Chicago, FMNH; Museum of Natural History, University of Kansas, KU; Museum of Comparative Zoology, Harvard University, Cambridge, Massachussets, MCZ; Museum of Vertebrate Zoology, University of California, Berkeley, MVZ; University of Michigan Museum of Zoology, UMMZ; National Museum of Natural History, Washington, DC, USNM), and reviewed literature on sightings and collections (Sexton, 1958; Bonaccorso *et al.*, 2003; Manzanilla & La Marca, 2004). From the historical distribution map we selected 15 locations for field exploration, based on accessibility, safety, and likely persistence of the original habitats (Fig. 1, Table 1).

We found 627 museum records of *A. cruciger* from 33 locations distributed over most of the Cordillera de La Costa (Fig. 1). The data indicated that *A. cruciger* was once abundant, especially in streams along the road between Maracay and Choroní (mostly inside the Parque Nacional Henri Pittier), and on the southern slope of the Parque

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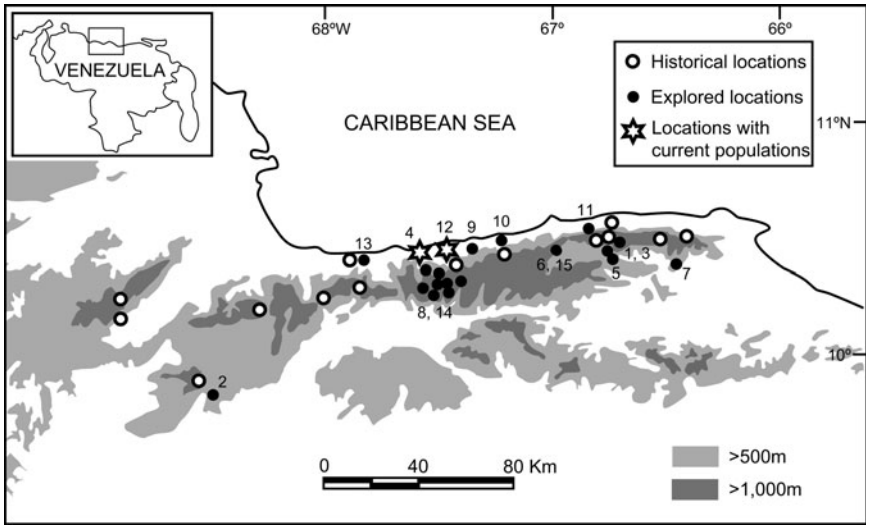

FIG. 1 Known historical distribution of *Atelopus cruciger* based on museum records, with the locations that we explored and the current distribution of the species (for further details see Table 1). Inset shows the location of the main map in Venezuela.

Nacional El Avila, next to Caracas. The altitudinal distribution of the species was 0-2,400 m but most records were over 500-2,000 m in gallery, cloud and semi-deciduous forests and their ecotones with littoral vegetation and agricultural areas.

We searched for *A. cruciger* using visual encounter survey methods (Heyer *et al.*, 1994; Lips *et al.*, 2004) between 08.00 and 16.00 to maximize the probability of sighting this diurnal species (Sexton, 1958). Each individual encountered was photographed. We sampled 23 individuals for *B. dendrobatidis* using swabs and then released them (Kriger *et al.*, 2006). Biosecurity protocols were followed to avoid pathogen dispersal and sample contamination (Aguirre & Lampo, 2006). For detection of *B. dendrobatidis* and zoospore quantification we extracted nucleic acids from swabs with PrepMan Ultra and conducted real time Taqman PCR assays (Boyle *et al.*, 2004). Standard curves were constructed by using 100, 10, 1 and 0.1 *B. dendrobatidis*

TABLE 1 Locations explored in the search for *Atelopus cruciger* (see numbered locations on Fig. 1).

Location	Altitude (m)	Date	Search effort (h per person)
1. Quebrada Anauco, Parque Nacional (PN) El Avila, Distrito Capital	1,350	6 May 2004	6.3
	1,500	1 June 2004	7.5
2. Río Llano, Cojedes	450	12-13 May 2004	11
3. Quebrada Chacaito, PN El Avila, Distrito Capital	980-1,100	22-23 May 2004	10.5
		4-5 Dec. 2004	9
4. Río Cata 2.3 km S. of Cata, N. slope of PN Henri Pittier, Aragua	120-220	15-18 June 2004	22
		14-15 July 2004	24.3
		18-19 May 2005	24
		10-12 Oct. 2005	20
5. Quebrada del Cerro El Volcán, El Hatillo, Miranda	1,220	31 June 2004	6
6. Permanent streams (without name), Colonia Tovar, Aragua	1,800	16, 18, 22 Aug. 2004	7.5
7. Quebrada Curupao - Quebrada Santa Rosa, Miranda		17, 20 Sep. 2004	Interviews*
8. Río El Limón (Aguacatal - Puerto Cruz road), several streams, Aragua-Vargas	566	15-16 Sep. 2004	3
	965	15 Apr. 2005	6
9. Río Choroní, Aragua	10-410	9-10 Nov. 2004	5.5
10. Río Chuao, Aragua	0-50	15-16 Nov. 2004	23.6
11. Quebrada Caurimare, S. slope PN El Avila, Distrito Capital	1,675	30 Nov. 2004	9
12. Río Cuyagua, Aragua	322	18-19 Jan. 2005	16
13. Río San Miguel and affluents, Aragua	20-40	21-23 Jan. 2005	16
14. Several streams on the S. slope of PN Henri Pittier, Aragua	1,075-1,100	Feb. 2005	14
15. Quebradas Hierbabuena y La Negra, affluents to Embalse Lagartijo, sub-basin of Río Tuy, Miranda	1,770	7 Apr. 2005	5.2
Total search effort			246.4

*We did not explore this location because it is unsafe but interviews with local people suggested the species is not present.

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zoospore quantification standards provided by the Australian Animal Health Laboratory.

After a total of 246 person hours of intensive searching (Table 1) we found *A. cruciger* at only two locations (4 and 12; Table 1, Fig. 1) at altitudes of 220 and 322 m, respectively, on the northern slope of the Parque Nacional Henri Pittier. This suggests that *A. cruciger* persists on the northern slope of the Cordillera de La Costa but in a more restricted area than formerly. We encountered 83 individuals including juveniles and adult males and females (Table 2; Plate 1). The presence of juveniles suggests these two populations may be reproducing and viable, although larval stages were not detected. One adult male was found morbid, at Río Cata (location 4 in Fig. 1). A DNA analysis of tissue samples from this male revealed high levels of infection (244,484 zoospores in 1-3 mg of tissue). Excessive skin sloughing indicated that chytridiomycosis was probably implicated (Berger *et al.*, 1999). Infection was also detected in three out of 23 apparent healthy adults (from location 4), although zoospore loads were very low (1.0-3.9 zoospores in 1-3 mg of tissue).

The apparent synchrony of rediscovery of a few *Atelopus* species in different regions (Eliot, 2003; Barrio-Amorós, 2004; Black, 2006) suggests that recent climatic conditions could be favouring population recoveries. It is also possible that this synchronized reappearance is a consequence of the relative synchrony in the disappearance of species phylogenetically related and with similar life history traits. *A. cruciger* was rediscovered within a year of the detection of one adult female *A. mucubajiensis* in the Andes (Barrio-Amorós, 2004). Furthermore, a third population of *A. cruciger* was rediscovered in February 2006 in Río El Duro, Edo, Aragua (J. Valera & M. Frontado, pers. comm.), also on the northern slope of the Parque Nacional Henri Pittier. According to the chytrid-thermal-optimum hypothesis, a decrease in the maximum daily temperatures over the last decade promoted the growth of *B. dendrobatidis* in amphibians, which led to high mortalities (Pounds *et al.*, 2006). The fact that all *A. cruciger* populations detected are at $<$350 m is consistent with this hypothesis. Maximum temperatures at these sites are greater than at highland altitudes, although other non-exclusive explanations are also possible. Dry conditions, for instance, could have also favoured pathogen transmission rates triggering epidemic events that led to population crashes (Lampo *et al.*, 2006). It is possible that *A. cruciger* populations are now experiencing a post epidemic recovery, with low prevalence and infection loads of *B. dendrobatidis*. However, the discovery of a severely infected morbid frog also suggests that other infected frogs could develop the disease and die. The climate-linked hypothesis predicts that, unless regional climatic trends are reversed, new epidemics are likely to occur where the pathogen persists endemically.

PLATE 1 *Atelopus cruciger* adult male sighted at Río Cata (location 4 on Fig. 1) on 11 October 2005. Photo: F. Rojas.

To ensure management in any case of new epidemics that could jeopardize the long-term survival of *A. cruciger,* we need to monitor the prevalence of *B. dendrobatidis* infection and the sizes of remnant populations. As long as treatments cannot be effectively applied in the field, *ex situ* management will remain the only available conservation strategy. However, *in situ* conservation could be a feasible alternative in the short-term. Close monitoring of climatic, ecological and epidemiological variables, which we are now conducting, are fundamental for the identification of threshold indicators that could help us decide when and where to apply management strategies.

TABLE 2 *Atelopus cruciger* individuals found in two locations at the Parque Nacional Henry Pittier (see numbered locations on Fig. 1).

Date	Location	No. of individuals		
		Males	Females	Juveniles
15-18 June 2004	4	1	1	1
14-15 July 2004	4	22	1	2
18-19 May 2005	4	9	2	1
10-12 Oct. 2005	4	29	2	0
18-19 Jan. 2005	12	12	0	0

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Biographical sketches

Argelia Rodríguez-Contreras is interested in the conservation of threatened amphibian species and the link between amphibian declines, chytridiomycosis and climate change. J. Celsa Señaris, the Director of the Museo de Historia Natural La Salle, works on the systematics and taxonomy of the Venezuelan herpetofauna. Margarita Lampo has a broad interest in population ecology and epidemiology, specializing in amphibians. Ramón Rivero is the curator of the herpetological section of the Museo Estación Biológica de Rancho Grande, and researches the taxonomy of Venezuelan amphibians.

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