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Chytridiomycosis as a Possible Cause of Population Declines in *Atelopus cruciger* (Anura: Bufonidae)

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Atelopus cruciger is a toad endemic to the lowland rainforest and cloud forest of the Cordillera de la Costa (Lötters 1996), and is the only species of the genus from Venezuela found outside the Andes. As for other *Atelopus* species, *A. cruciger* is diurnal, terrestrial, and breeds along swiftly flowing streams (Lötters 1996; Sexton 1958). Its historical abundance in many localities is evident in museum collections and in the literature (Cocroft et al. 1990; Mebs 1980; Mondolfi 1976; Sexton 1958). With no obvious explanation, the abundance of this species drastically decreased in the mid 1970's and early 1980's (La Marca and Lötters 1997; C. Señaris and J. Manzanilla, pers. comm.), and the last two specimens were collected in 1986 (Fig. 1).

As in many other amphibian species, losses do not seem to be caused by habitat degradation (Laurance et al. 1996; Lips 1999; Pounds and Crump 1994). Recent research has implicated *Batrachochytrium dendrobatidis*, a chytrid fungus, in declines of amphibian populations in Australia, USA, Costa Rica, Panama, Ecuador, Spain, and New Zealand (Berger et al. 1998; Bosch et al. 2001; Bradley et al. 2002; Ron and Merino 2000; Waldman et al. 2001). *Batrachochytrium* is a highly virulent pathogen for many amphibians, and in experimental situations mortality can be 100% (Speare 2001).

To explore the possible chytrid fungus infection of *Atelopus cruciger*, we obtained a complete record of the localities at which *A. cruciger* was collected (Appendix I). Then, we examined histo-

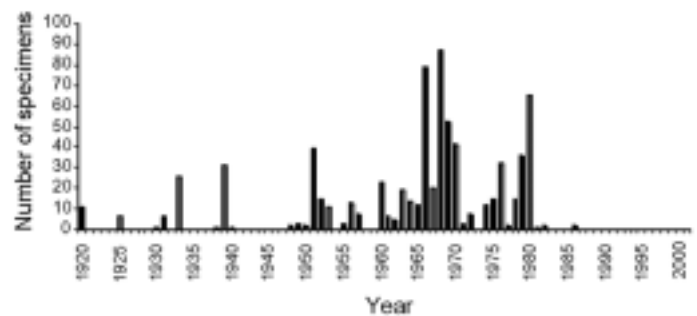


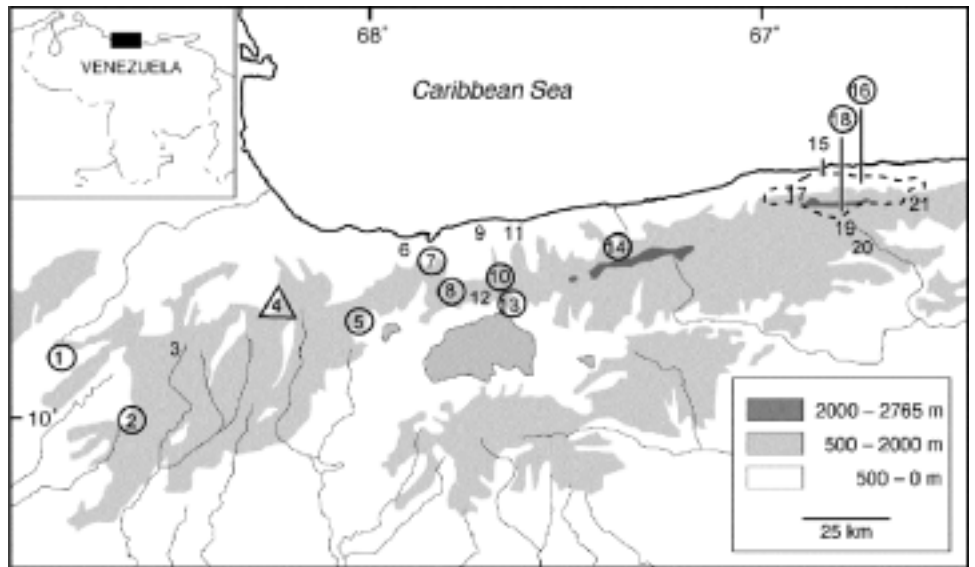
FIG. 1. Number of specimens of *Atelopus cruciger* in 12 herpetological collections (see acknowledgments) from 1920–2001. Note the absence of records after 1986.

logical preparations of specimens collected from 1920–1986 at localities across the historical distribution of the species (Fig. 2; Appendix I).

We removed strips of skin from the ventral surface of Toe IV from 59 specimens preserved in 70% ethanol. Tissue samples were tested for fungal infection following the procedures described in Berger et al. (1999). To remove bias, the histological examination of toad skin was carried out using a blind procedure with the scientist (DM) conducting the examination in the absence of any specimen data.

We found infection with *Batrachochytrium* in histologic sections from one specimen (USNM 259135). The superficial epidermis was hyperkeratotic and many zoosporangia of *B. dendrobatidis* were present (Fig. 3). Although most zoosporangia were empty, some contained characteristic developing stages of the fungus (see Berger et al. 1999). The infected specimen was an adult (SVL = 33.4 mm), collected at Palmichal, 23 km N of Bejuma (1465 m; 110°18' N, 68°13' W), Estado Carabobo, in May 1986. This and another specimen (USNM 259136) collected in the same locality in June 1986, constitute the last individuals of *Atelopus cruciger* to have been collected.

This is the first report of chytridiomycosis for Venezuela, and the third from South America. *Batrachochytrium dendrobatidis* has been reported from Ecuador (Ron and Merino 2000) and Uruguay (Mazzoni 2000), the latter record being from commercially raised American bullfrog (*Rana catesbeiana*). In Ecuador, five frog species were found to be infected (*Atelopus bomolochos*, *A. sp.* [aff. *ignescens*], *Gastrotheca pseustes*, *Hyla psarolaima*, and *Telmatobius niger*) with the earliest record in December 1980 (Ron and Merino 2000). Of the infected species in Ecuador, three (*A. bomolochos*, *A. sp.* [aff. *ignescens*], and *T. niger*) have not been recorded in the wild since 1994 (Ron et al. 2000). Ron and Merino (2000) concluded that chytridiomycosis was a factor involved in frog population declines throughout Ecuador. Because infection by *Batrachochytrium* has been shown to be lethal to other amphibians and to be associated with declines in other countries (Berger et al. 1998; Bosch et al. 2001; Bradley et al. 2002; Nichols et al. 2001; Speare 2001; Waldman et al. 2001), its presence in *A. cruciger* supports the hypothesis of the epidemic disease chytridiomycosis as a possible cause of



- | | | | |
|----------------------|------------------------|------------------|-----------------------------|
| 1. Sortes | 7. Maracay-Ocumare rd | 13. Maracay | 19. Quebrada Caurimare |
| 2. Cerro Azul | 8. Rancho Grande | 14. El Limón | 20. El Hatillo |
| 3. Nirgua | 9. Cuyagua | 15. Caraballeda | 21. Curupao |
| 4. 23 km N of Bejuma | 10. Maracay-Choroni rd | 16. Los Canales | -- Parque Nacional El Avila |
| 5. Barbula | 11. Choroni | 17. El Paraíso | |
| 6. Patanemo | 12. Guamitas | 18. Río Chacaito | |

FIG. 2. Distribution of *Atelopus cruciger*. Numbers = non-overlapping collection localities; numbers in circles = collection localities of specimens examined for chytrid infection; number in triangle = locality where the infected specimen was found.

the population declines.

Species that have shown chytrid associated declines often inhabit high elevation and reproduce in streams (Berger et al. 1998; Bradley et al. 2002; Lips 1999; Ron et al. *in press*). Although the infected specimen of *Atelopus cruciger* was collected at an altitude of 1465 m, the species was distributed from 40–1640 m. Local extinctions of *A. cruciger* have occurred throughout its altitudinal distribution (C. Señaris and J. Manzanilla, pers. comm.), suggesting that chytrid fungus could have affected both highland

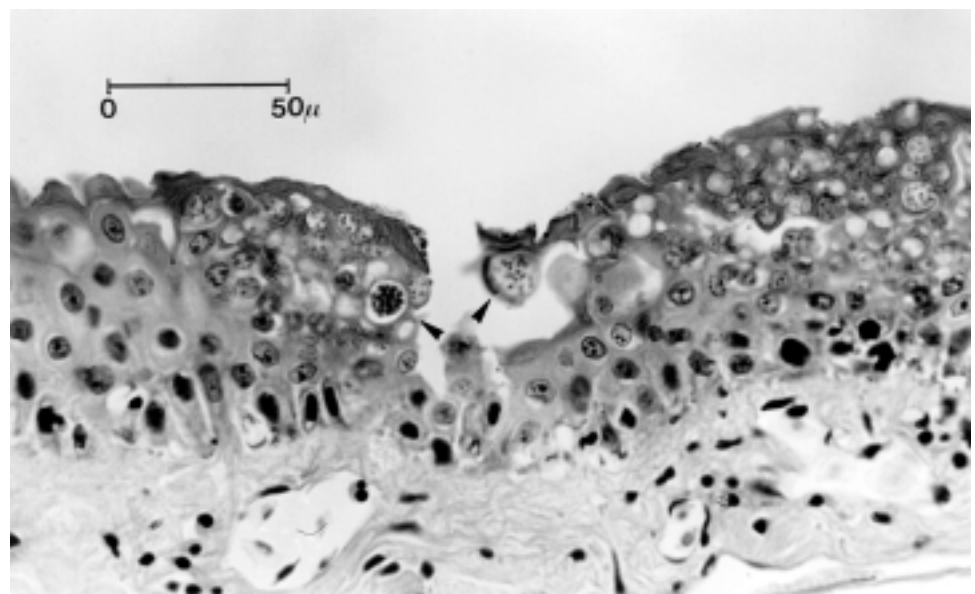


FIG. 3. Photograph of histologic section of toe skin in the specimen USNM 259135 showing zoosporangia of *Batrachochytrium*.

APPENDIX I. Collection localities of *Atelopus cruciger* and specimens examined for chytrid infection.

* Locality	Coordinates	Elevation (m)	Specimens examined	Year of collection
ESTADO YARACUY				
1 Sortes, Chivacoa	10° 11' N, 68° 41' W	500	MHNLS 788–91	1953
3 Nirgua, Quebrada la Chapa	10° 9' N, 68° 34' W	891	—	—
ESTADO COJEDES				
2 Cerro Azul	9° 57' N, 68° 38' W	1300	MHNLS 8135–43, 8452–54, 8459–62	1980
ESTADO CARABOBO				
4 Palmichal, 23 km N Bejuma	10° 18' N, 68° 13' W	1465	USNM 259135–36	1986
5 Bárbula	10° 18' N, 68° 2' W	634	MHNLS 2691–92	1964
6 Patanemo	10° 25' N, 67° 55' W	200–300	—	—
Parque Nacional San Esteban, Quebrada Las Aguas	—	—	UMMZ 55557 (2 specimens)	1920
ESTADO ARAGUA				
7 Km 26 Maracay-Ocumare de la Costa rd	10° 21' 46" N, 67° 43' 39" W	610	—	—
7 Km 29 Maracay-Ocumare de la Costa rd	10° 22' 11" N, 67° 44' 07" W	427	KU 185703–08	1979
7 Km 34 Maracay-Ocumare de la Costa rd	10° 23' 19" N, 67° 44' 47" W	140	KU 166678	1978
8 Parque Nacional Henri Pittier (PNHP), Rancho Grande	10° 21' 10" N, 67° 41' 02" W	1100	MHNLS 622	1952
8 PNHP, Estación Biológica de Rancho Grande	10° 20' 58" N, 67° 41' 04" W	1155	KU 132914–15	1974
8 PNHP, Pico Guacamayo	10° 22' N, 67° 40' W	1524	USNM 142370–71	1960
8 PNHP, Pico Periquito	10° 20' N, 67° 40' W	1494	—	—
8 9 km toward coast from Estación Biológica de Rancho Grande	10° 21' 13" N, 67° 43' 26" W	715	—	—
9 ca. 1 km S Cuyagua, along stream	10° 28' 42" N, 67° 42' 02" W	50–80	—	—
10 Maracay-Choroní rd	10° 21' N, 67° 35' W	1640	—	—
10 Maracay-Choroní rd	—	1300	UMMZ 113931	1952
10 Maracay-Choroní rd	—	1100	—	—
11 Choroní	10° 29' 46" N, 67° 36' 30" W	40	—	—
12 PNHP, Quebrada Guamitas	10° 20' 23" N, 67° 39' 10" W	775	—	—
13 Las Peñas, near Maracay, Hacienda de la Trinidad	10° 16' 39" N, 67° 37' 08" W	473	—	—
13 Maracay	10° 15' 23" N, 67° 34' 47" W	480	USNM 097197–98	1925
ESTADO VARGAS				
14 El Limón	10° 28' N, 67° 17' W	971	MHNLS 4434–35	1967
14 Hacienda El Limón	—	600	MHNLS 4430–31	1965
14 Las llanadas, Hacienda El Limón	—	600	MHNLS 4825–28	1970
14 Cagüita, Hacienda El Limón	10° 31' N, 67° 19' W	465	MHNLS 3662–63	1968
15 Caraballeda	10° 36' N, 66° 50' W	26	—	—
16 Los Canales, Planta Eléctrica de Naguayata	10° 31' N, 66° 45' W	800	USNM 128866–68	1939
DISTRITO FEDERAL				
17 Parque Nacional El Ávila (PNEA) El Paraíso, Río Tocomé	10° 32' 00" N, 66° 49' 00" W	1400	—	—
PNEA, Quebrada Quintero	—	1200	—	—
PNEA	—	**2100	—	—
PNEA	—	1000	—	—
ESTADO MIRANDA				
18 Caracas, Río Chacaíto	10° 31' N, 66° 51' W	ca. 1000	USNM 128879, 128881	1939
18 1 km N of Quebrada Chacaíto	10° 32' N, 66° 51' W	1130–1170	USNM 216506–08	1967
19 Quebrada de Caurimare, Petare	10° 28' 47" N, 66° 49' 50" W	1000	—	—
20 Quebrada de Tusmare, Alto Hatillo	10° 24' 40" N, 66° 50' 10" W	1000	—	—
20 El Hatillo	10° 26' 07" N, 66° 48' 43" W	1200	—	—
20 Urb. Loma Larga, El Hatillo	10° 26' 08" N, 66° 49' 13" W	1200	—	—
Quebarada Los Corutos	—	ca. 1000	MHNLS 760–63	1952
21 Curupao	10° 30' N, 66° 38' W	1100–1300	—	—

* Numbers correspond to those in Fig. 1.

** Questionable locality: this is the only record above 1640 m; because no more specimens have been found at similar elevations (around 2100 m), we question its validity.

and lowland populations. However, additional studies are needed to provide a more comprehensive spatiotemporal profile. Retrospective studies based on museum specimens and prospective studies sampling wild-caught frogs should be conducted in conjunction with ecological investigations to determine the distribution and impact of *Batrachochytrium* in Venezuela.

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Chytrid Fungus in Northern and Western Cape Frog Populations, South Africa

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Chytrid fungus has been found in areas where frog populations are declining (Berger et al. 1998; Bosch et al. 2001; Lips 1999; Muths et al. 2000). Although there have been no reports of amphibian population declines in South Africa, chytridiomycosis has been detected in *Xenopus laevis* (Speare and Berger 2000). The aim of our study was to further assess the occurrence of chytrid fungus in South African frogs, specifically in the Western Cape and Northern Cape Provinces.

The Cape river frog (*Afrana fuscigula*) was chosen as the primary study species because it is widespread throughout the Cape, with populations at high and low elevation. Other species were tested for the presence of chytrid fungus, such as *Afrana vandijki* and *Strongylopus grayii*, when *A. fuscigula* could not be found or when other species were found at the same site.

Sites were chosen opportunistically, to be representative of the full area of the Western Cape Province (N = 17 sites) and as an initial sample of the Goegap Nature Reserve in the Northern Cape (N = 2). Western Cape sites varied from rivers to pools. Both sites in the Goegap Nature Reserve were natural, small, isolated pools.

Nocturnal searches were conducted with flashlights and adult frogs were captured. The fourth toe tip of the right foot was taken from each animal using nail clippers. Each toe tip was placed in a separate vial containing 70% ethanol. The nail clippers were