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SHORT COMMUNICATION

Limb abnormalities in *Peltophryne florentinoi* (Anura: Bufonidae) from Cuba

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Abnormal individuals in natural populations of amphibians have been reported for more than 400 species from around the World (Henle *et al.* 2017a, Haas *et al.* 2018). Several forms of externally visible abnormalities have been described, according to the types and location (Meteyer *et al.* 2000, Lannoo 2008, Henle *et al.* 2017b). Skeletal duplications, absence and reduction of skeletal elements, and other skeletal abnormalities (bony bridges, rotated limbs, truncated vertebral column, and torsion of the tail or body) are probably the most common in adults. However, many other non-skeletal morphological anomalies, such as duplication of the eyes and the spiraculum, absence of the tympanum, microphthalmy and anophthalmy, edema, tumors and several color abnormalities have been also reported (Henle *et al.* 2017b). Malformed mouthparts have been widely documented in tadpoles of several species (Lannoo 2008, Henle *et al*. 2017a). Recently, malformed adult individuals, tadpoles with abnormal mouthparts and anomalously colored frogs have been documented in Cuba (Alonso Bosch *et al.* 2017, García-Padrón and Alonso Bosch 2017, 2019). According to these authors, such abnormalities may indicate degraded environmental health or genetic disorders.

The Zapata Toad, *Peltophryne florentinoi* (Moreno and Rivalta, 2007), is a Cuban toad restricted to the coastal microphyllous evergreen forest on limestone landscapes, near Playa Girón

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and Guasasa, Zapata Swamp, Mantanzas Province (Moreno and Rivalta 2007, Alonso Bosch and Cobos 2016). Recently, one individual of this species was located in Guajimico Villagein, Cienfuegos Province (Díaz *et al*. 2019). Based on its reduced geographical distribution (Figure 1) and the threat that sea level rise would represent for this species, *P. florentinoi* was categorized as Critically Endangered (CR), according to the criteria of the IUCN (Rivalta 2008). Its habitat is being transformed by small-scale agriculture, the extraction of forest elements and dumping of solid waste (Alonso Bosch and Cobos 2016). Warmer and drier conditions, occasional inundations and saltwater intrusion may represent additional stressors in the near future (Cobos and Alonso Bosch 2018).

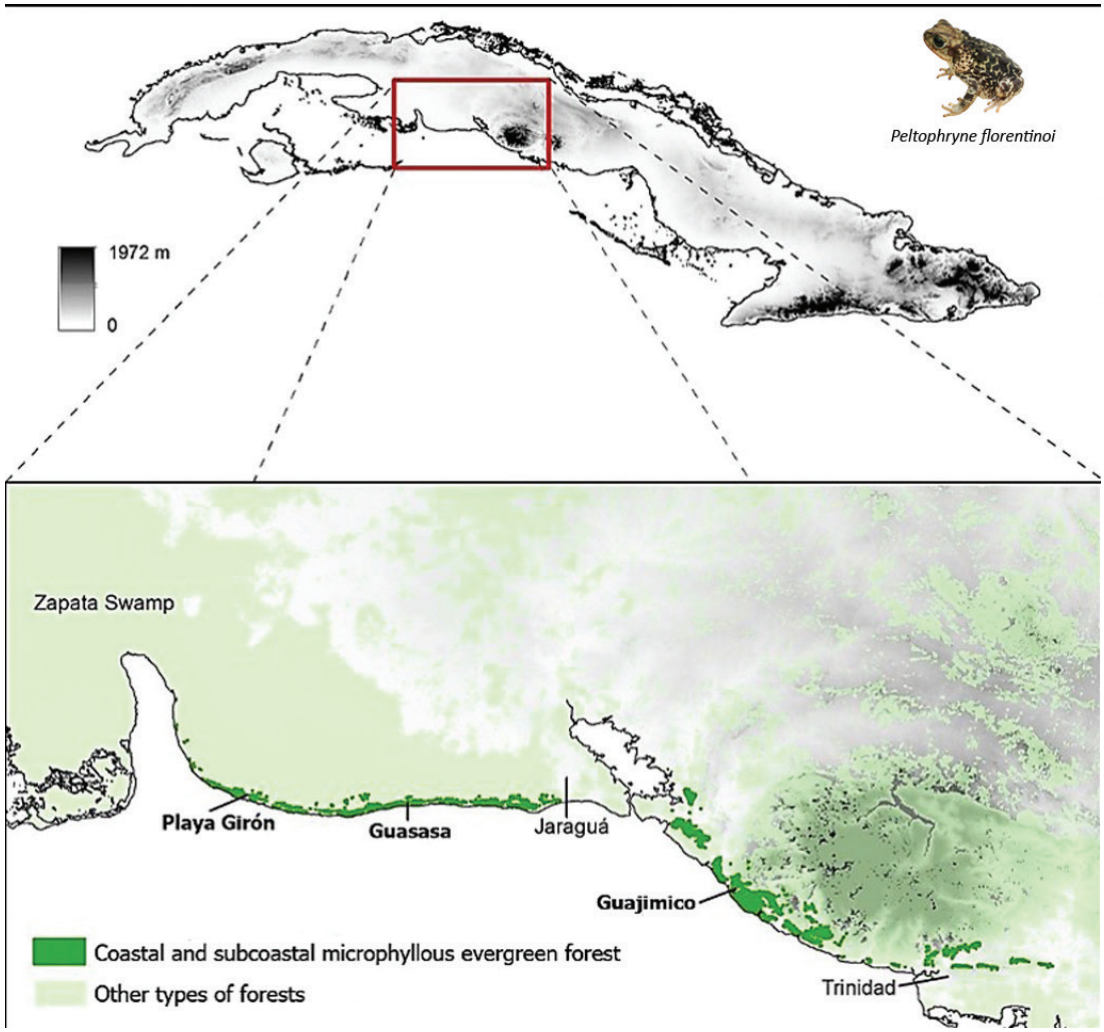

Figure 1. Geographic distribution of *Peltophryne florentinoi* in the Microphyllous Evergreen Forest of the southern coasts of Matanzas and Cienfuegos provinces of Cuba (courtesy of Marlon Cobos). The names of known localities of *P. florentinoi* are highlighted in bold.

During an ongoing amphibian-monitoring initiative focused on the study of the abundance, phenology and habitat quality of *P. florentinoi* and its environment, adult individuals with limb abnormalities were observed. We visited the coastal Microphyllous Evergreen forests surrounding the type locality, Playa Girón (22°4'27.91" N, 81°2'73" W; 7 m a.s.l.) and the vicinity of Guasasa town, 20 km E of the type locality (22°3'28.74" N, 80°47'56.81" W; 4 m a.s.l.). At the beginning (May) and the middle (August) of the rainy season, we visited both areas in 2016 and 2018. We also inspected a third area near the type locality during May–June 2019. Via a combination of visual and acoustic encounter surveys (Crump and Scott 1994, Zimmerman 1994), we inspected forest patches, using headlamps, searching active animals and in breeding sites between 21:00-24:00 h, during three consecutive nights. Each captured individual was marked by a unique combination of color points (using permanent markers: black-blue-red) placed on ante-orbital crests and then released at the site of collection.

We detected externally visible skeletal abnormalities in adult individuals from both localities during the three years of the study. The frequency of abnormalities was slightly similar between localities. We caught 61 adult individuals from Playa Girón, 10 of them (16.4%) exhibited at least one type of abnormality. We identified three abnormal individuals (17.6%) among a sample of 17 animals captured at Guasasa. In total, thirteen adult specimens of both sexes had one or more abnormalities. Fore and hind limb abnormalities of adults were observed. Based on the nomenclature suggested by Henle *et al.* (2017b), we recognized at least six types of abnormalities in the present study (Figure 2). Brachydactyly was the most frequently observed abnormality, but the combination of mixed abnormalities (N = 5), usually included brachydactyly too. We observed amely, ectropody and syndactyly only once each. Most limb abnormalities were unilateral (N = 1 bilateral); abnormal fore and hind limbs in the same individual were rarely observed (N = 1).

Although the relationship between the causative agents and the observed abnormalities in the environment is still poorly understood, physicochemical environmental stress factors, such as ultraviolet radiation and pollution, have been suggested to explain such observations in wild amphibian populations (Blaustein and Johnson 2003, Linzey *et al.* 2003, Henle *et al.* 2017c). On the other hand, biological stress factors such as predation, pathogen infections, hybridization and inbreeding have also been considered among the possible causes (Johnson *et al.* 1999, 2001a, b, Bowerman *et al.* 2010). Some authors have proposed synergistic interactions between some environmental or biological factors may exist, as well (Ouellet 2000, Bancroft *et al*. 2008, Reeves *et al*. 2010, Lunde and Johnson 2012).

The causes for the abnormalities recorded in the Zapata's Toad remain unknown. Even though we should not rule out any of the aforementioned biotic or abiotic factors, some seem unlikely to apply to this system. Small-scale agriculture has been identified as threat to habitat quality of the Zapata's Toad (Alonso Bosch and Cobos 2016), but chemical pesticides or fertilizers are not widely used here. No evidence of predation or parasitic infection have been found. Limb anomalies have been observed in laboratory experiments that evaluated the exposure to UV radiation, yet the overall existing literature indicates that it is an unlikely cause for limb anomalies observed in natural populations (Henle *et al.* 2017c). The phenology of this species (reproductive activity exclusively during the wettest days of the rainy season), and the particular features of its breeding sites, suggest that the adverse effects of UV radiation should be monitor carefully in the future. These ephemeral breeding sites, usually located in areas with poor or no vegetation cover, are particularly vulnerable to the impacts of solar radiation, high temperatures and high rates of evaporation (Cobos and Alonso Bosch 2018).

Figure 2. Some limb abnormalities exhibited by adult *Peltophryne florentinoi* toads from Playa Girón and Guasasa, Zapata Swamp, Matanzas, Cuba. (**A**) Amely in adult male. (**B**) Ectropody in adult female. (**C**) Brachydactyly + syndactyly. (**D**) Both abnormal hindlimbs, schizodactyly is shown on the right side of the photo, with ectromely on the left side. (**E-G**) Brachydactyly. (**H**) Ectromely shown on the left side of the photo. Photos by L. Gómez Castillo.

UV-B radiation is rapidly attenuated in aquatic ecosystems, often within a few centimeters (Diamond *et al.* 2002), but the reproductive activity and larval development of *P. florentinoi* take place exclusively in small and very shallow depressions of karstic soil typical of the region, that temporarily accumulate rainwater (Díaz and Cádiz 2008).

Henle *et al.* (2017c) discard inbreeding as a probable cause of abnormalities in a population of *Bufotes viridis* from southern Germany. These authors considered that inbreeding would lead to the same types of anomalies in all affected individuals, not to the heterogeneous observed. However, the high frequency of malformations detected in insular populations of toads from Brazil, ranging from mouthpart anomalies, limb reduction to loss of eyes, has been associated with genetic structure and inbreeding (Toledo and Ribeiro 2009, Tolledo and Toledo 2015, Bessa-Silva *et al.* 2016, Reboucas *et al.* 2019). The small population of Zapata's Toad is restricted to a few localities from the eastern part of the Zapata region, Matanzas province to the east of Cienfuegos City, Cienfuegos Province, associated with coastal microphyllous evergreen forest with different level of human perturbation. Due to the specific ecological requirements, these toads have a small population and a distribution restricted to perturbed habitats and a limited availability of breeding sites (Alonso Bosch and Cobos 2016, Cobos and Alonso Bosch 2018). Coupling these factors with apparent site fidelity may increase the probability for consanguineous mating, the consequent loss of genetic diversity, and exposure of deleterious recessive mutations. Further studies are needed to understand the causes and consequences of this phenomenon along the distribution of this endemic and highly threatened Cuban toad.

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