

Short communication

# Further studies on pumiliotoxin **251D** and hydroquinone content of the skin secretion of *Melanophryniscus* species (Anura, Bufonidae) from Uruguay

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## Abstract

In whole animal ethanolic extracts from adult specimens of *Melanophryniscus atroluteus* (27 specimens) and *M. devincenzii* (16 specimens) as well as of two egg clutches and four tadpole samples from the latter species, the major alkaloid pumiliotoxin (PTX) **251D** and hydroquinone were assayed quantitatively by gas chromatography/mass spectrometry. All toad extracts contained high concentrations of PTX **251D** and hydroquinone and exhibited considerable variation in the content of these compounds among individual specimens. The extracts of the eggs and tadpoles were entirely free of alkaloids as well as hydroquinone, pointing to a dietary origin of these compounds.

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## 1. Introduction

The small toads of the genus *Melanophryniscus* (family: Bufonidae) are distributed in the southern part of South America, i.e. in Brazil, Uruguay, Paraguay, Argentina and Bolivia (Kwet et al., 2005). This genus includes 20 species (Di Bernardo et al., 2006), from which only in three species (*Melanophryniscus devincenzii*, *Melanophryniscus montevidensis* and *Melanophryniscus stelzneri*) the skin secretions were studied (Cei et al., 1968; Daly

et al., 1984; Garraffo et al., 1993; Mebs et al., 2005). In these *Melanophryniscus* species various alkaloids such as pumiliotoxins, decahydroquinolines, indolizidines, pyrrolizidines and quinolizidines besides remarkable concentrations of hydroquinone have been identified (Daly et al., 1984; Garraffo et al., 1993; Mebs, 2003; Mebs et al., 2005). A high variability in the levels of the major alkaloid, pumiliotoxin **251D** (PTX **251D**), has been observed in several populations of *M. montevidensis* from southern Uruguay (Mebs et al., 2005). A dietary source for the skin alkaloids has been proposed (Mebs et al., 2005), but the original producer has not yet been identified. Moreover, nothing is known about the ontogeny of the skin secretions.

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In the present paper, further analytical data of the PTX **251D** and hydroquinone content of whole body extracts from individual specimens of two other *Melanophryniscus* species, *M. atroluteus* and *M. devincenzii*, from northern Uruguay, as well as of extracts of eggs and tadpoles from *M. devincenzii* are reported.

## 2. Methods and materials

### 2.1. Collection of the animals

Adult specimens of *M. atroluteus* (27) were collected at Parque Gran Bretaña in April 2005 and of *M. devincenzii* (16), two clutches of eggs (50 eggs in each sample) and four samples of tadpoles (each sample consisted of five tadpoles in stage 37 (Gosner, 1960), were collected in nature and sacrificed immediately) at Cuchilla Negra in September 2005, in both cases in Departamento Rivera, northern Uruguay. Each toad was sacrificed by immersing into 70% ethanol (about 20 ml) and extracted for several days. The collected toads were deposited thereafter in the Sección Zoología Vertebrados, Facultad de Ciencias, UDELAR, Montevideo, Uruguay.

### 2.2. Analysis of the extracts

The ethanolic extracts were evaporated to dryness with a stream of air at 25 °C and were dissolved in 200 µl chloroform. One microliter was subjected to gas chromatography/mass spectrometry (GC/MS) as described previously (Mebs et al., 2005). Hydroquinone (Merck, Darmstadt, Germany) and synthetic pumiliotoxin **251D** (Sudau et al., 2002), kindly provided by Prof. Nubbemeyer, University of Mainz, Germany, were used as standard compounds.

## 3. Results

Besides hydroquinone and the alkaloid PTX **251D**, the major compounds in the whole body extracts of the toads, various other alkaloids of the pumiliotoxin class (PTX **237A**, **265D**, **267C**, **307A**, **309A**, **323A**, for mass spectral data see Mebs et al., 2005) were detected, but only in trace amounts. The quantitative assay of hydroquinone and of PTX **251D** in the extracts revealed very high concentrations of both compounds, particularly in extracts from *M. devincenzii* (Fig. 1). Moreover, high

variability in the levels of both compounds was observed among individual specimens.

In contrast, neither hydroquinone nor PTX **251D** or other alkaloids were found in the extracts of the two eggs and the four tadpole samples of *M. devincenzii*.

## 4. Discussion

The results of the present study on body extracts from two other Uruguayan *Melanophryniscus* species, i.e. *M. atroluteus* and *M. devincenzii*, confirm previous investigations on six populations of *M. montevidensis* toads (Mebs et al., 2005) suggesting three trends in the content of their skin secretions: (1) PTX **251D** is the major alkaloid in the extracts beside high levels of the non-alkaloidal compound hydroquinone; (2) there exists high variability not only among populations (as reported by Mebs et al., 2005), but also among individuals belonging to the same population in the levels of both compounds, and (3) the hydroquinone concentration is not related to that of PTX **251D**. The lack of these compounds and of other alkaloids in eggs and tadpoles, which were collected at the same time and location as the adult toads of *M. devincenzii*, indicate that neither alkaloids nor hydroquinone are transferred to the eggs during oogenesis or during spawning or fertilization. Consequently, the developing tadpoles are entirely free of alkaloids and hydroquinone, which otherwise may have provided some protection from predators. Other amphibian eggs, such as from newts of the genus *Taricha* (Mosher et al., 1964) and from the toad *Atelopus chiriquiensis* (Pavelka et al., 1977) contain the highly toxic tetrodotoxin present in high concentrations in the adult amphibians. However, the high variability of the PTX **251D** content in various *Melanophryniscus* species and populations, its lack of occurrence in eggs and tadpoles support the assumption that alkaloids and other compounds such as hydroquinone are of dietary origin as suggested for frogs of the family Dendrobatidae (Jones et al., 1999; Daly et al., 1997, 2000, 2002; Saporito et al., 2004).

Pumiliotoxins have been identified in mixed leaf-litter collections of arthropods from Isla Bastimentos (Panama) where *Dendrobates pumilio* frogs contain high levels of these alkaloids (Daly et al., 2002). Some formicine ants (*Brachymyrmex* and *Paratrechina* spp.) containing PTX **307A** and **323A** have been suggested to be a putative source for

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