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Poor alkaloid sequestration by arrow poison frogs of the genus *Phyllobates* from Costa Rica

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ABSTRACT

Frogs of the genus *Phyllobates* from Colombia are known to contain the highly toxic alkaloid batrachotoxin, but species from Central America exhibit only very low levels or are entirely free of this toxin. In the present study alcohol extracts from 101 specimens of *Phyllobates lugubris* and *Phyllobates vittatus* and 21 of three sympatric species (*Dendrobates pumilio, Dendrobates auratus, Dendrobates granuliferus*) from Costa Rica were analyzed by gas chromatography-mass spectrometry. Whereas the extracts of the *Dendrobates* species exhibited typical profiles of toxic alkaloids, those of the two *Phyllobates* species contained low levels of few alkaloids only, batrachotoxin was not detected. Although the feeding pattern of the *Dendrobates* and *Phyllobates* species are similar as revealed by examination of their stomach content (mainly ants and mites), the *Phyllobates* species are poorly sequestering alkaloids from their food source in contrast to the *Dendrobates* frogs.

1. Introduction

To poison their arrows for hunting Indians of northern South-America use the skin secretion of frogs of the genus *Phyllobates* (Myers et al., 1978). These frogs are known to contain one of the most powerful alkaloids in their skin, batrachotoxin and its homologues. It was first identified in the skin of *Phyllobates aurotaenia* from Colombia by Märki and Witkop (1963). Later on extremely high concentrations of batrachotoxin have been detected in a frog named *Phyllobates terribilis* (Myers et al., 1978). More than 1 mg of the toxin was found to be present in a single specimen, whereas the other two species *Phyllobates bicolor* and *P. aurotaenia* contained 100–200 μg per frog (Daly et al., 2005, 1980). Batrachotoxin specifically acts on the voltage-gated Na^+-channel by preventing its inactivation (Khodorov, 1985). The toxin became an important tool in ion channel research.

Interestingly, nerve and muscle cells of *P. terribilis* and *P. aurotaenia* are virtually insensitive to batrachotoxin probably due to minor changes at the site controlling channel-activation and thus preventing toxin binding (Daly et al., 1980).

Despite the high toxicity of the skin secretion from these species, in specimens of *Phyllobates lugubris* from Panama batrachotoxin levels are very low (Daly et al., 1987). In one specimen of *Phyllobates vittatus* from Costa Rica the toxin was undetectable. Although frogs of the genus *Phyllobates* may contain other alkaloids, these were found to be present only in trace amounts.

It is well accepted that alkaloids in the skin of Dendrobatidae, but also of Mantellidae, Myobatrachidae frogs and of toads of the genus *Melanophryniscus* are of dietary origin, deriving from their food source, i.e. ants, mites, beetles and other arthropods (Daly et al., 2000, 2002). Alkaloids such as pyrrolidine, pyrrolizidine, piperidine,

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indolizidine, quinolizidine and decahydroquinoline derivatives have been detected in Myrmicinae (Daly et al., 1994a,b, 2000; Jones et al., 1999; Clark et al., 2005) and pumiliotoxins in Formicinae ants (Saporito et al., 2004) as well as in the mites of the family Oribatidae (Takada et al., 2005; Saporito et al., 2007a). Since *P. terribilis* reared in captivity were entirely free of batrachotoxin (Daly et al., 1980; Mebs unpublished), it must also be of exogeneous, perhaps dietary origin. It is interesting to note that batrachotoxin and its homologues have likewise been detected in skin and feathers of two passerine birds from New Guinea (*Pitohui* and *Ifrita* spp.), where a beetle (*Choresine pulchra*, Melyridae) was found to be the potential source of the toxin (Dumbacher et al., 1992, 2004).

In the present study ethanolic extracts of *P. lugubris* and *P. vittatus* specimens from Costa Rica were analyzed for alkaloids as well as extracts of the frogs *Dendrobates pumilio*, *Dendrobates auratus* and *Dendrobates granuliferus* which live sympatrically in the same habitat with the *Phyllobates* species. Moreover the stomach content of all species was examined for food items.

2. Methods and materials

2.1. Frogs, collection and treatment

A total of 101 specimens of the two *Phyllobates* species (*P. vittatus* – 51, *P. lugubris* – 50) were collected in 11 locations of Costa Rica as well as 21 specimens of the *Dendrobates* species: *D. pumilio* (formerly *Oophaga*, but renamed *Dendrobates* according to Santos et al. (2009)) (7, location 1 and 5), *D. auratus* (7, location 1 and 7) and *D. granuliferus* (7, location 7 and 10) as shown in Fig. 1. They were sacrificed by injection of tetracaine and each specimen was preserved in 50 ml 70% ethanol. The stomach content was obtained by dissection and analyzed microscopically and by scanning electron microscopy (SEM). The dried content was glued to an alumina holder, sputtered with gold and analyzed with a Hitachi SEM (model S-4500) at an accelerating voltage of 5 kV (cold-field emission electron source).

2.2. Alkaloid identification

Alkaloids were identified by gas chromatography combined with mass spectrometry (GC/MS). Extracts were evaporated to dryness with a stream of air at 25 °C and were dissolved in 100 or 200 μl chloroform. One μl was subjected to GC/MS, performed on an Agilent Technologies (Waldbronn, Germany) HP6890 GC equipped with an autosampler HP6890 ALS and interfaced to a HP5973 MSD. A Factor Four MS capillary column (CP 8982, 30 m × 0.25 mm I.D., 0.25 μm film thickness) from Varian (Darmstadt, Germany), which was protected by a guard column (1.5 m of deactivated [diphenyltetramethyldisiloxane] glass capillary [0.25 mm I.D.] from BGB Analytik AG [Anwil, Switzerland]), was used with helium (1.0 ml/min) as carrier gas. Splitless injection was performed at 230 °C injection port temperature and a temperature program from 80 °C, which was held for 2 min and increased with 12 °C/min to 310 °C, held for 6.5 min, was applied. The MS transfer line was maintained at 280 °C, the ion source at 250 °C, and was operated with 70 eV ionization energy. Mass spectra were recorded in full scan mode from *m/z* 43 to *m/z* 550. Data analysis was performed using the HP ChemStation software (Rev. B.01.00).

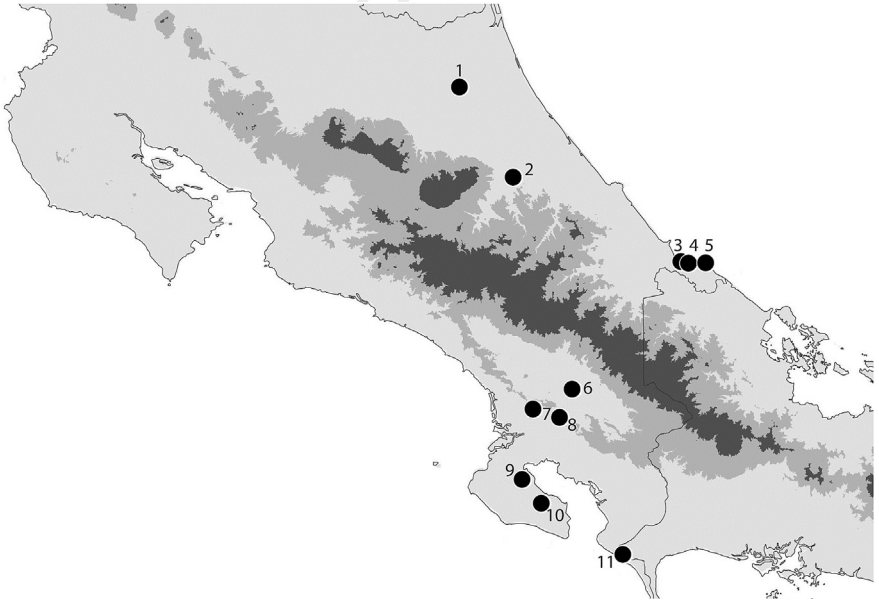

Fig. 1. Map of Costa Rica with the collection sites of *Phyllobates lugubris* (site 1–5), *P. vittatus* (site 6–11), *Dendrobates pumilio* (site 1 and 5), *D. auratus* (site 1 and 7), and *D. granuliferus* (site 7 and 10). Locations: 1 – La Suerte Limón; 2 – Bajo Vacas, Limón; 3 – Pan Dulce, Limón; 4 – Calle Margarita, Limón; 5 – Manzanillo, Limón; 6 – Buenos Aires, Puntarenas; 7 – Cataratas, Puntarenas; 8 – El Progreso, Puntarenas; 9 – Reserva Los Patos, Puntarenas; 10 – Rio Tigre, Puntarenas; 11 – Rio Coco, Puntarenas.

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