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Variability of alkaloid profiles in *Oophaga pumilio* (Amphibia: Anura: Dendrobatidae) from western Panama and southern Nicaragua*

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Abstract. Ethanol extracts from *Oophaga pumilio* specimens collected in western Panama (14 populations) and southern Nicaragua (two populations) were analyzed for their alkaloid composition by gas chromatography-mass spectrometry. High variability in the alkaloid profiles among the various populations as well as among individual specimens from Panama and Nicaragua were observed. Since alkaloids in dendrobatid frogs and other anurans are of dietary origin, the various alkaloid profiles found are not representative for certain populations, but are indicative for the availability of prey (mites, ants and other arthropods) containing these chemical compounds selected as food by the frogs in their habitats.

Key words. Amphibia, Anura, Dendrobatidae, *Oophaga pumilio*, alkaloid profiles, chemical variability.

Introduction

The strawberry poison frog *Oophaga pumilio* O. Schmidt, 1857 (previously: *Dendrobates pumilio*) exhibits a considerable diversity in colour patterns. According to our current knowledge, these belong to the same, polymorphic species known to range from southern Nicaragua into western Panama (e.g. Summers et al. 1997, Batista & Köhler 2008).

Skin secretions of *O. pumilio* contain numerous alkaloids (Daly et al. 1987, 2005, Saporito et al. 2006, 2007a) which are used in chemical defence against potential predators as well as microorganisms (Brodie & Tumbarello 1978, Macfoy et al. 2005). More than 800 different alkaloids have been identified in skin extracts of Dendrobatidae frogs from Central and South America, Bufonidae from South America, Mantellidae from Madagascar and Myobatrachidae from Australia (Daly et al. 1984, 2005, Garaffo et al. 1993, Smith et al. 2002). Populations of *O. pumilio* exhibit alkaloid profiles in their skin extracts, which vary individually as well as among populations (Saporito et al. 2006). Since these alkaloids derive from the frogs´ diet, i.e alkaloid-containing arthropods, spatial and temporal variations of the availability of these arthropods as food source are considered to contribute to the distinct alkaloid profiles in the frogs´ skin (Saporito et al. 2007a).

In the present paper, the alkaloid profiles of *O. pumilio* specimens from 14 populations in western Panama and two from southern Nicaragua were analyzed by gas chromatography-mass spectrometry to supplement the studies of Batista & Köhler (2008). The data obtained underline the high chemical variability in skin secretion on both the population and the individual level.

Materials and methods

Ethanol (70%) extracts, used for the preservation of 138 specimens (single or up to 12 individuals per sample) from 14 populations

* This paper is dedicated to the memory of the late John W. Daly.

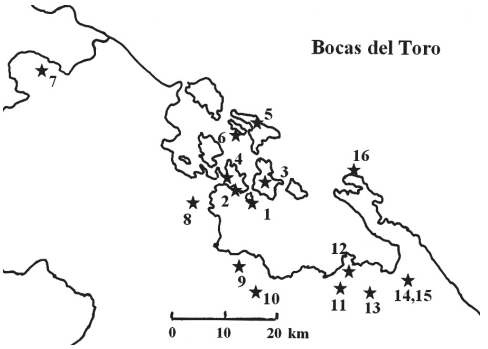


Fig. 1. Map indicating the collection sites of Panamanian *Oophaga pumilio*. The numbers correspond to the localities as shown in Table 1.

(Fig. 1) allocable to 10 colour morphs from the Province Bocas del Toro, western Panama (for GPS coordinates and colour morph descriptions and voucher specimens see Batista & Köhler 2008), and two from southern Nicaragua were used for chemical analysis: (i) Cerro Musum: north side (13°00'41.0" N, 85°14'11.6" W, 620 m elevation), SMF (Senckenberg-Museum Frankfurt) 87885, south side (12°57'18.8" N, 85°13'51.2" W, 630 m elevation), SMF 87890, 87888; (ii) Río San Juan: El Alemendro (10°59'43.9" N, 84°16'37.5" W, 70 m elevation), SMF 87887, Boca de Bartola (10°58'18.3" N, 84°20'23.1" W, 20 m elevation), SMF 87886, Boca de San Carlos (10°47'25.7" N, 84°11'37.7" W, 40 m elevation), SMF 87894, 87895, 87893, Dos Bocas de Rio Indio (11°02'54.8" N, 83°52'48.4" W, 20 m elevation), SMF 87896, 87897.

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 8912, 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). Synthetic pumiliotoxin **251D** (Sudau et al. 2002), kindly provided by U. Nubbemeyer, University of Mainz, was used as standard compound. Identification of the major alkaloids was achieved by comparison with published data (Daly & Spande 1986, Daly et al. 1993) and with MS-spectra provided by the software NIST/EPA/NIH Mass Spectral Database (Rev. C.00.00).

Code names were assigned to the alkaloids in the manner of Daly et al. (1987, 2005). Bold faced numbers indicate the nominal mass, the bold-faced letter for identification of the alkaloids exhibiting the same nominal mass according to the recent tabulation of anuran alkaloids.

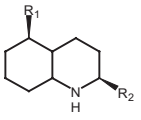
Results

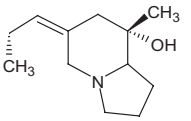
From the ethanolic extracts of 27 samples of *Oophaga pumilio* frogs from the 14 populations in western Panama, a total of 37 alkaloids were identified by GC/MS. Up to 14 different alkaloids were detected in single samples (ranging from 2 to 14 alkaloids), either as major or minor components. Among them, seven alkaloids were found to be most abundant such as 2,5-disubstituted decahydroquinoline **195A** (DHQ **195**), 5,8-disubstituted indolizidines (IND **205A, 207A, 235B**) and pumiliotoxins (aPTX **267A, 323A**) (Table 1).

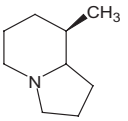
DHQ **195A** and IND **205A** were the predominant alkaloids in 15 of the 29 samples,

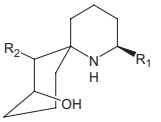
Tab. 1. Most abundant alkaloids in extracts from *Oophaga pumilio* samples from western Panama and southern Nicaragua. In the Panamanian samples, the first number indicates the collection localities in Figure 1. The number of specimens analysed is given parentheses after the locality name. Abbreviations: DHQ – 2,5-Disubstituted decahydroquinoline, PTX – Pumiliotoxins, aPTX – Allopumiliotoxins, IND – 5,8-Disubstituted indolizidines, HTX – Histrionicotoxins, PYR – 3,5-Disubstituted pyrrolizidine.

Alkaloids









	DHQ	PTX			aPTX		IND				HTX		
	195A	**251D**	**307A**	**323A**	**267A**	**323B**	**205A**	**207A**	**233D**	**235B**	**283A**	**285C**	**291A**
Panama													
1. Loma Partida (2)			+	+	+	+							
same (1)			+	+	+	+		+			+		
same (1)	+				+		+						
same (3)	+				+		+						
2. Aguacate (2)				+	+			+	+		+		
same (1)	+		+	+			+	+		+			
3. Isla Popa (11)	+						+	+		+			
4. Cero Brujo (9)	+						+			+			
5. Isla Bastimentos(6)	+						+						
same (10)	+			+									
6. Isla Solarte (10)	+						+			+			
7. Cerro Tebata (4)							+		+				
same (7)							+		+				
8. Rio Uyama (8)							+				+	+	+
9. Quebrada Gloria(1)	+												+
same (1)	+												+
same (1)	+												+
same (2)	+							+					+
10. Rambala (1)				+	+								
same (1)							+			+			
same (1)			+				+						
same (3)			+							+			
11. Queb. Kedami (10)	+												
12. Rio Krikamola (10)								+					
13. Rio Canaveral (2)							+	+	+	+			
same (2)	+	+		+									
14. Kusapin (11)					+					+			
Nicaragua													
Río San Juan (12)	+				+		+				+	+	IND **181A**
Cerro Musum (5)	IND **233D, 277E**, PYR **251**												

but they were found to be unevenly distributed within the populations (Table 1). Evaluation of the data confirms the existence of a considerable variability in the alkaloid composition of the various populations. This also applies to individual specimens from the same location.

Of the two samples from Nicaragua, one (Cerro Musum) exhibited a totally different alkaloid profile, compared to Panamanian specimens, in which the pyrrolizidine (PYR) **251** and the indolizidines (IND) **233D** and **277E** alkaloids were predominant. In the Río San Juan sample, besides common alkaloids like those found in the Panama samples, an indolizidine, IND **181A**, was detected.

This is certainly not an exhaustive analysis and many more alkaloids present as minor constituents might have been identified. For instance, Saporito et al. (2006) identified 153 alkaloids in skin extracts of *O. pumilio* frogs from various locations of the Isla Bastimentos, western Panama. However, based on available spectral data, the present analysis was undertaken to provide an overview of the occurrence or absence of major alkaloid classes in the frog extracts. In a study of specimens throughout Costa Rica and Panama over 30 years, Saporito et al. (2007a) detected 232 alkaloids of 21 classes.

When alkaloid profiles of the present study are compared to those of Daly et al. (1987) and Saporito et al. (2006, 2007a) no common or even similar alkaloid patterns are observed in specimens from the same population. This strongly suggests that there exists high temporal and geographical variability in the alkaloid composition of *O. pumilio* populations.

Discussion

This study of alkaloid profiles in *Oophaga pumilio* populations from western Panama and southern Nicaragua demonstrates that their chemical profiles, e.g. alkaloid composition, vary significantly among populations as well as individually from specimen to specimen. For instance, in some individuals of *Oophaga pumilio* from the same collection site, e.g. Loma Partida (Fig. 1), the alkaloid DHQ **195A** was detected, which has not been detected in another specimen from the same locality, which, on the other hand, contained more pumiliotoxins (**267A, 307A, 323A**). Despite the close proximity of the collection sites of specimens from Rambala and Rio Uyama (Fig. 1), the alkaloid profiles of these frogs differed drastically. These results are consistent with studies of Daly et al. (1987) and Saporito et al. (2006) indicating that alkaloid profiles vary spatially among populations being less than 1 km apart and temporally across time periods such as during dry and wet seasons. However, geographic proximity may favour more similar alkaloid profiles which is illustrated by *O. pumilio* populations from southern Nicaragua exhibiting a quite distinct alkaloid composition characterized by the presence of indolizidines and pyrrolizidines which were not found in the Panama populations. Recently, hydroquinone has been identified in extracts of the South American toads of the genus *Melanophryniscus*, which also exhibit numerous alkaloids in their skin (Mebs et al. 2005, 2007). This compound has not been found in the extracts of *O. pumilio* confirming previous analyses of skin extracts from dendrobatids (Daly et al. 1987).

It is well accepted that alkaloids in the skins of Dendrobatidae, Mantellidae, Myobatrachidae frogs and *Melanophryniscus* toads are of dietary origin and derive from their food sources such as ants, mites, beetles and other arthropods (e.g. Daly et al. 2000, 2002). For instance, pyrrolidine, pyrrolizidine, piperidine, indolizidine, quinolizidine and decahydroquinoline alkaloid derivatives have been detected in ants of the subfamily Myrmicinae (Daly et al. 1994, 2000, Jones et al. 1999, Clark et al. 2005) and pumiliotoxin alkaloids in ants of the subfamily Formicinae from Panama (Saporito et al. 2004). Coccinelline alkaloids and some structurally re-

lated tricyclic alkaloids have been found in coccinellid beetles (Ayner & Browne 1977) and spiropyrrolizidine alkaloids in siphonotid millipedes (Saporito et al. 2003, Clark et al. 2005). Mites, which belong to the major food items of many Dendrobatidae frogs (e.g. Donnelly 1991, Biavita et al. 2004), seem to considerably contribute to the alkaloid profiles of the frogs. Recently, pumiliotoxins and several other alkaloids have been identified in mites of the family Oribatidae (Takada et al. 2005, Saporito et al. 2007b). Forty of the 80 alkaloids detected in extracts of oribatid mites also occurred in *O. pumilio* from the same site where the mites had been collected (Saporito at al. 2007b).

As a main reason for the variability in the alkaloid profiles among the frogs, the availability of a certain spectrum of alkaloid-containing arthropods can be assumed. Their presence or absence in a particular area as well as changes in their abundance may significantly influence the highly variable alkaloid patterns of a frog population. Moreover, frogs of the genus *Dendrobates* have been found to modify some dietary alkaloids such as converting pumiliotoxin **251D** into the allopumiliotoxin **267A** by hydroxylation (Daly et al. 2003) suggesting that the latter alkaloid detected in a population of *O. pumilio* from Loma Partida may be a product of such an enzymatic reaction.

It has been suggested that the ability of certain anurans to uptake and sequester alkaloids is under genetic control (Myers et al. 1995), which could also explain the variation in the alkaloid profiles. However, Summers et al. (1997) demonstrated low degrees of mitochondrial DNA divergence among *O. pumilio* populations in the Boca del Toro region of Panama, including populations on the Isla Bastimentos. These data rather support the conclusion that genetic factors are rather unlikely to contribute to the high variability of the alkaloid profiles (Saporito et al. 2006).

Alkaloids accumulate in the frogs over time and the results of the analysis of their composition in a frog may reflect an age- and, perhaps, also gender-dependent profile in an individual from a certain population (Donnelly 1991, Saporito at al. 2007a). The fact that alkaloids in dendrobatid frogs are of dietary source, the absence of these compounds in the F2-generation raised in captivity (Daly et al. 1997) clearly indicates that alkaloid profiles are not species-specific and are not characteristic for certain populations as demonstrated in the present paper and in studies of Saporito at al. (2006, 2007a).

Toxic alkaloids, particularly the pumiliotoxins (Daly & Myers 1967, Daly et al. 1978, 2003) may play an important role in chemical defence. The bitter taste of alkaloids, at least their unpalatability (Daly et al. 1987) may deter potential predators, but they may also provide protection from ectoparasites (Weldon et al. 2006) or from infections by microorganisms (Macfoy et al. 2005). The impressive colour patterns of many dendrobatid frogs is considered to have an aposematic effect and may serve as a strong signal towards potential predators such as birds. However, there is still a need for more realistic studies on the ability of natural predators to learn from unpleasant experience with poisonous frogs and on the role colour-signals may play than few experiments with one-month-old domestic chickens used to prove mimicry in "less toxic" frogs (Darst & Cummings 2006).

In conclusion, alkaloid profiles of Dendrobatidae frogs and most likely of other alkaloid containing anurans as well are not representative for certain species or populations, but are indicative for the prey containing these chemical compounds the frogs and toads find and select as food in their habitat.

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