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INTRASPECIFIC VARIATION OF *Bothrops pubescens* (COPE, 1869) VENOM IN URUGUAY (SERPENTES: VIPERIDAE)

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ABSTRACT: In Uruguay, there was no information about the variations degree in *Bothrops pubescens* venoms until the present work, in which we investigated intraspecific venom variation using polyacrylamide gel electrophoresis (SDS-PAGE). We found some differences in the venom protein profile; however, they were not related to the parameters studied (geographic distribution, weight, sex, and captivity time). Moreover, we distinguished two different groups in relation to band densities at 49 and 57 kDa. Specimens with predominant density in the 49kDa band tend to be predominantly females. Weight distribution in this group extended for all the range (150-1500 g) with an average weight of 720 g. The other group (57kDa predominant band) showed restricted weight range (150-400 g) with an average weight of 280 g. Cluster analysis was also performed. The variability observed in the venom profile probably corresponds to genetic variations.

KEY WORDS: venom, *Bothrops pubescens*, Uruguay, antivenom production, electrophoretic pattern.

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INTRODUCTION

Venoms are complex mixtures of components which have a diverse array of actions both on prey and human victims. These components are mainly biologically highly active proteins and peptides whose primary function is to kill or immobilize prey and also to assist in the digestion of that prey (5, 8).

Variation in snake venom composition was observed at the beginning of the 20th century by Vital Brazil (2). Intraspecific level variation has also been reported and associated with diverse factors, such as the snake geographic origin, sex and age (4, 11). Knowledge of venom variability would allow more efficacious treatment of snakebite victims due to a more rational venom selection for effective antivenom production (1, 9, 15, 16).

Proteomic tools make possible the study of individual venom composition and variation (14). Some authors have examined venoms electrophoretic patterns as an auxiliary tool for taxonomic and intraspecific variation studies (7, 10, 13, 14).

In recent years, the complex *B. neuwiedi* was divided into 7 species (12). *Bothrops neuwiedi pubescens* was included in the synonymy of *Bothrops pubescens*. This new systematic arrangement reduces the species distribution to Rio Grande do Sul (Brazil) and Uruguay.

Bothrops pubescens is one of the species implicated in human envenomation in Uruguay (3), where antivenom production includes venoms from different localities to minimize geographic variation. It is the most important parameter for venom pool design. However, investigation about differences in venom composition has not been performed so far.

The aim of the present study was to investigate the existence of differences in *B. pubescens* venoms based on whole protein profile and considering: geographic distribution, weight, sex, and captivity time.

MATERIALS AND METHODS

Animals

Thirty-nine *B. pubescens* specimens were captured from different localities in Uruguay (Table 1) between 1995 and 2003 and kept in optimal captivity conditions according to the national regulations established by CHEA (“Comisión Honoraria de Experimentación Animal”). Feeding during captivity period consisted of mice (*Mus musculus*) from the animal facility of Instituto de Higiene. The geographic distribution

depends on the captured snake locality: north or south of Rio Negro, which divides the country into two regions.

Venoms

Milking from both venom glands was performed under aseptic conditions. Venom was immediately frozen until use.

SDS-PAGE

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to the method of Laemmli (6). Venom samples (30 μg) were loaded onto 15% acrylamide gel and stained with Coomassie Brilliant Blue. Bicinchoninic acid method (Pierce, IL USA) was used to determine protein concentration.

Statistical analysis

Chi-square test was used for sex and geographic comparisons. Weight and captivity time data were contrasted by the Student's t-test.

Gel bands evaluation was made using Quantity One software (Biorad, PA, USA).

For cluster analysis, matrix was generated using bands between 116 and 31 kDa. The resulting absence-presence matrix (35 columns X 60 rows) was analyzed using Statistica 5.0 (StatSoft, OK, USA). Tree diagrams were obtained using single linkage and Euclidean distances.

RESULTS

All venoms showed similar band profiles. A 40kDa band was observed in seven venoms only, but this variation did not correspond to any of the parameters studied (Figure 1).

On the other hand, there was a visible difference in the distribution of protein concentration, especially in protein bands with molecular weight above 20 kDa. A relevant variation occurred in bands corresponding to 49 and 57kDa proteins (Figure 1). In some venoms, one of these protein bands was more concentrated, while in others it was quite the opposite. The relation between this variation and the parameters studied is shown in Table 2. This different distribution of protein concentration was strongly correlated with sex and weight but no relation was found

with geographic distribution and captivity time. Venoms with 49kDa predominant protein band corresponded to female snakes and weight distribution in this group extended for all the range (150-1500 g) with an average weight of 720 g, while the other group (57kDa predominant band) showed restricted weight range (150-400 g) with an average weight of 280 g.

The shortest distance in the cluster analysis was found between specimens 106 and 195 (Figure 2). On the other hand, we observed that specimens 106, 195, 196 and 179 were grouped by a distance of 2.0. The same was observed for the following groups: 3, 198-136, 214-142, 102. Groups 106, 195, 179, 196, 180, 204, 199, 268, 111, 112, 49-3, 198, 163-136, 214, 142, 102, 331, 54 were observed between distances of 2.2 and 2.4. No correlation was found between the resulting groups and the parameters studied.

Table 1: Specimen data for the parameters studied.

Specimen	Captivity time (years)	Locality (Uruguay)	Sex	Weight (g)	Predominant band (49 or 57 kDa)
N3	>10	Tacuarembó (N)	F	665	49
N4	>10	Treinta y Tres (SE)	F	920	49
N49	10	Tacuarembó (N)	F	188	57
N50	10	Treinta y Tres (SE)	F	1325	49
N54	9	Canelones (S)	Not identified	375	57*
N93	9	Treinta y Tres (SE)	F	1475	49
N102	9	Treinta y Tres (SE)	M	945	49
N106	9	Rivera (N)	F	1465	49
N109	9	Canelones (S)	F	1200	49
N111	9	Canelones (S)	M	265	57*
N112	9	Canelones (S)	M	265	57*
N127	8	Canelones (S)	M	195	49
N128	8	Rocha (S)	F	1330	49
N132	8	Rocha (S)	M	395	57
N136	8	Canelones (S)	Not identified	235.3	57
N140	8	Montevideo (S)	M	360	57*
N142	8	Montevideo Zoo (S)	M	905	49
N146	8	Montevideo (S)	Not identified	850	49
N163	7	Montevideo Zoo (S)	M	217.9	57
N179	6	Canelones (S)	M	269.7	57
N180	6	Canelones (S)	F	325	57
N195	6	Rivera (N)	M	269	57
N196	6	Canelones (S)	Not identified	Not determined	49
N198	5	Canelones (S)	F	680	49
N199	5	Canelones (S)	F	575	49
N203	4	Maldonado (S)	Not identified	191	57*
N204	4	Maldonado (S)	Not identified	Not determined	57*
N205	4	Maldonado (S)	Not identified	283.1	49
N206	4	Maldonado (S)	M	158.7	57
N208	4	Maldonado (S)	Not identified	275	49
N210	4	Maldonado (S)	M	307.8	49
N214	4	Maldonado (S)	F	236	57*
N215	4	Maldonado (S)	M	341	57*
N219	4	Canelones (S)	M	320.7	57
N222	4	Rivera (N)	F	400	49
N268	3	Canelones (S)	F	278.1	49
N289	2	Maldonado (S)	F	600	49
N331	1	Not determined	M	190	49
N332	9	Canelones (S)	F	288	49

57*- Specimens with similar concentration in 49 and 57 kDa bands.
Groups 57* and 57 did not show significant differences in relation to the studied parameters.
N – north
S – south
SE – southeast
F – female
M – male

Table 2: Statistical analysis between predominant band groups and the parameters studied.

	p value	Correlation
Sex	<0.01	Strong
Geographic variations	0.9	No
Captivity time	0.5	No
Weight	<0.001	Very strong

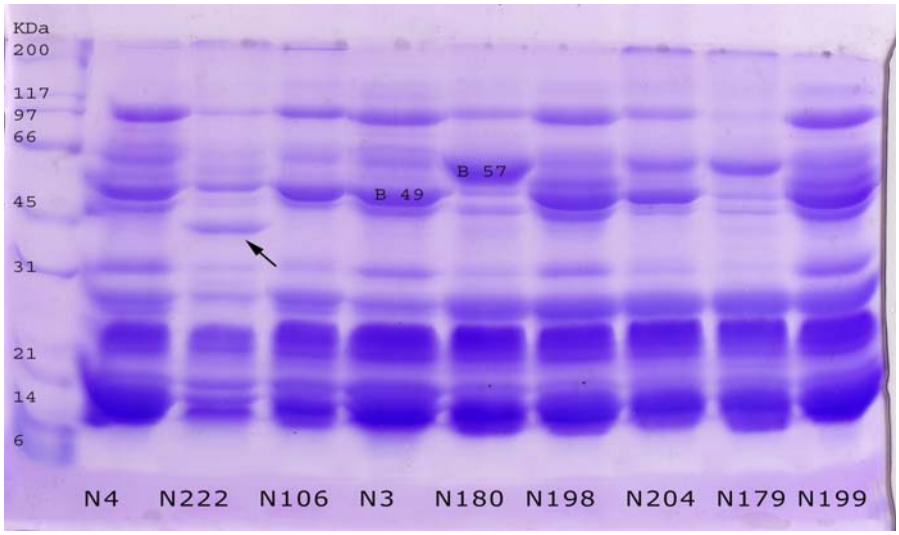

Figure 1: SDS-PAGE (15%) of individual venom profiles. The arrow shows the 40kDa band.

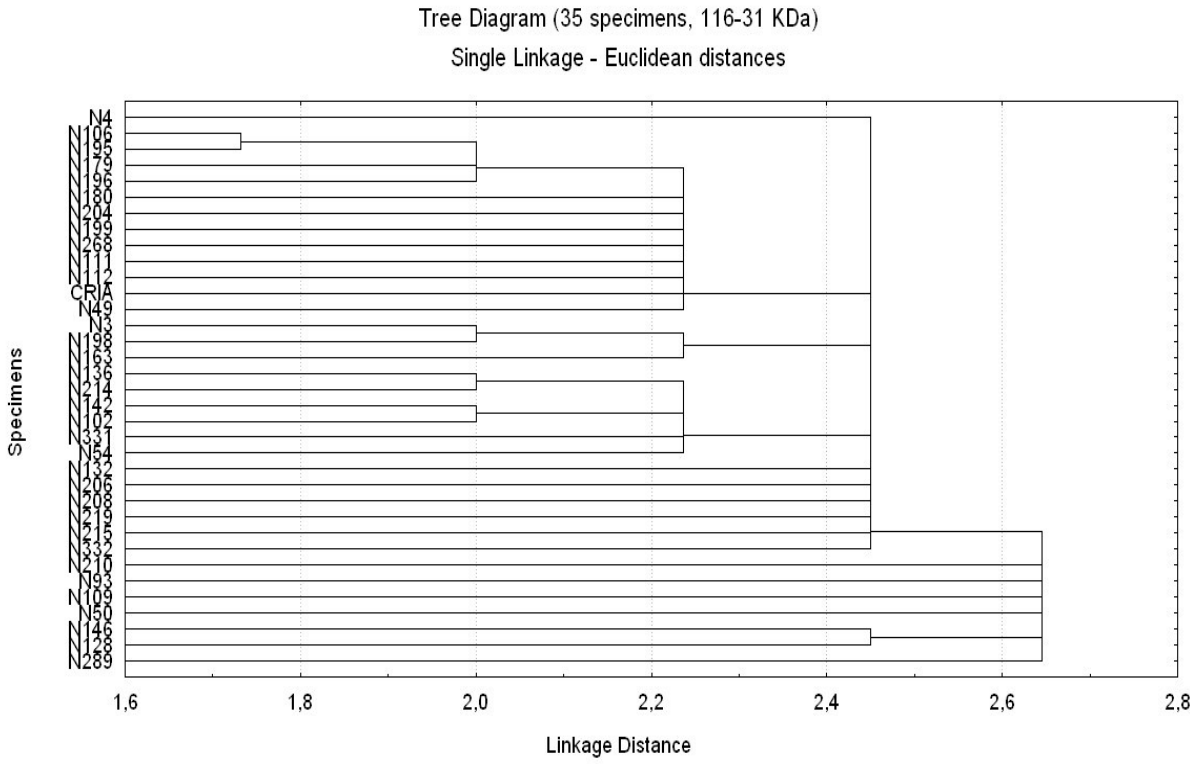

Figure 2: Cluster analysis including all specimens studied.

DISCUSSION

The existence of considerable variability at intraspecific level may be debatable and perhaps reliant, to some extent, on the interpretation of similarity.

Our results show that individual variation in the protein profiles did not correspond to the parameters studied. Relative quantity differences in 49 and 57 kDa bands are strongly related to weight and must be considered for further protein identification and analysis.

The groups proposed by the cluster analysis do not bring additional information. Genetic variation could explain the analysis results.

Knowledge of intraspecific variations is very important for the design of reference venom pool to obtain snake antivenom.

Traditionally, *B. pubescens* antivenom production in Uruguay has used venoms from different localities to minimize geographic variations, although in Uruguay there are not important geographic features or long distances.

According to our results, there are no evidences of geographic intraspecific variation. Venoms from different localities of Uruguay may be unnecessary for antivenom production. Further analysis, such as cross-neutralization assays, will be required to confirm this point. This result is very relevant in relation to serpentarium management and finally to economical cost of antivenom production.

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