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The neurotoxins of the sea snake *Laticauda schistorhynchus*

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Erabutoxins a and b, the major neurotoxins in the venom of the sea snake *Laticauda semifasciata*, were detected in the venom of *Laticauda schistorhynchus*. The identity of the toxins was confirmed on the basis of elution position on CM-cellulose column chromatography, disc electrophoretic mobility, amino acid analysis and toxicity measurement.

The sea snake *Laticauda semifasciata* (Reinwardt, 1837) is distributed along the coasts of the South West Islands of Japan (the Ryu Kyu or Okinawa Islands), the Philippines and the Moluccas, whereas *Laticauda schistorhynchus* (Günther, 1874) was originally described from Savage (=Niue) Island but has since been recorded from a number of islands in the South Pacific. The two species are very similar to each other morphologically, and were long regarded as synonymous; however, on the basis of variation in the number of ventral scales and body bands, Stejneger (1907) suggested that it might prove necessary to recognize a separate race, *Laticauda semifasciata schistorhynchus*, from the South Pacific. Smith (1926), in his classic monograph on sea snakes, referred them to two different species, also based on the fewer ventral scales and body bands in *L. schistorhynchus*, extending the brief observations made by Van Denburgh (1924). Most workers have since adopted Smith's classification. De Rooij (1915) extended the range of *L. schistorhynchus* to Java and the Ryu Kyu Islands, but Smith (1926) attributed the western records to misidentified *L. semifasciata*. Minton (1975) reported on the restricted distribution of *L. schistorhynchus* to Tonga and Samoa. Pernetta (1977), using the data of Smith (1926), agreed with Smith in recognizing *L. semifasciata* and *L. schistorhynchus* as distinct species.

As a part of a review of the comparative morphology and venom chemistry of laticaudid sea snakes, the venoms of these two 'species' have been studied and their principal neurotoxic components compared with those of other species of laticaudids. Further, the morphological criteria, on which their separation has been maintained, have been re-assessed.

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Materials and methods

Sea-snake venoms

L. schistorhynchus venom was collected by one of us (M. L. G.) on the west coast of Niue Island (19°03'S, 169°55'W) in March 1982. *L. semifasciata* venom was collected by one of us (N. T.) on Ikema Island in July 1980 and on Ishigaki Island in August 1982, both in the Ryu Kyu (Okinawa) Islands. The venoms were diluted with equal volumes of 0.2M-acetic acid at the collection sites and brought back to the laboratory, where they were freeze-dried.

Venom and venom component analyses

The CM-cellulose column (1.6cm × 14cm) chromatography of freeze-dried venom preparations (3–6mg) was performed in 0.01M-phosphate (KH_2PO_4/Na_2HPO_4) buffer, pH6.4, as described previously (Tamiya & Arai, 1966). After the elution of the non-retained proteins (46ml), a linear-concentration-gradient elution with 0–0.5M-NaCl in a total volume of 1 litre of buffer was applied. The elution of the proteins was monitored by a Toyo Uvicon UV-750 flow monitor (Toyo Kagaku Sangyo, Tokyo, Japan) at 280nm. The toxicity measurements, the amino acid analysis and disc-gel electrophoresis were performed as described previously (Tamiya & Arai, 1966), except that a JLC 8AS automatic amino acid analyser (JEOL Co., Tokyo, Japan) was used.

Results

The elution patterns of the venom components of *L. schistorhynchus* and *L. semifasciata* are given in Fig. 1. The elution positions of components a and b (Fig. 1*a*) of *L. schistorhynchus* venom agreed with

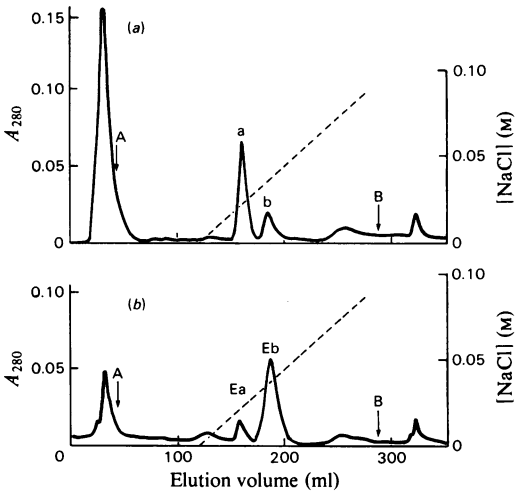

Fig. 1. *Elution patterns of the venom components of Laticauda schistorhynchus (a) and Laticauda semifasciata (b) from a CM-cellulose column*

Dried venom preparation (5.85mg for *L. schistorhynchus* or 3.58mg for *L. semifasciata*) was applied to a column (1.6cm × 14cm) of CM-cellulose in 0.01M-phosphate buffer, pH6.4. After the elution of non-retained proteins (at arrow A), a linear-concentration-gradient elution with 0–0.5M-NaCl in the buffer in a total volume of 1litre was applied. At arrow B, 0.5M-NaCl in the buffer was used for elution. Fractions of volume 9ml were collected at a flow rate of 70ml/h. The elution of proteins was monitored by absorption at 280nm. ——, A_{280}; – – – –, concn. of NaCl. In (*a*) a and b indicate the elution peaks of components a and b respectively, and in (*b*) Ea and Eb indicate those of erabutoxins a and b respectively.

Table 1. *Amino acid contents of components a and b from Laticauda schistorhynchus venom*

Samples (8–12nmol) of components were hydrolysed in 6M-HCl (0.2ml) for 20 or 40h and subjected to automatic amino acid analysis.

Amino acid	Amino acid content (mol of residue/mol) Component a	Erabutoxin a*	Component b	Erabutoxin b*
Lys	4.3	4	4.1	4
His	1.1	1	1.9	2
Arg	3.0	3	2.8	3
Asp	5.0	5	4.0	4
Thr	5.0†	5	5.0†	5
Ser	7.8†	8	7.8†	8
Glu	8.5	8	7.9	8
Pro	4.2	4	4.2	4
Gly	5.0	5	4.9	5
Ala	0.0	0	0.0	0
CyS	6.4‡	8	5.7‡	8
Val	2.3	2	2.5	2
Met	0.1	0	0.0	0
Ile	3.5§	4	3.1§	4
Leu	1.0	1	1.0	1
Tyr	1.1	1	1.2	1
Phe	1.9	2	1.8	2
Trp	1‖	1	1‖	1

* Values obtained by the sequence study (Sato & Tamiya, 1971; Maeda & Tamiya, 1977).

† Corrected for destruction during hydrolysis.

‡ Cystine tends to give low values.

§ The presence of sequence Ile-Ile (36–37) in erabutoxins is probably responsible for the low values.

‖ Components a and b gave the same absorption at 280nm as erabutoxins a and b.

those of erabutoxins a and b (Fig. 1*b*) of *L. semifasciata* venom respectively. The LD_{50} dose values and the migration points on disc-gel electrophoresis at pH4.0 of the components a and b also agreed with those of erabutoxins a and b respectively. The results of amino acid analysis of the components (Table 1) also agreed with those of erabutoxins. All these results indicate that toxins that are the same as erabutoxins a and b are present in the venom of *L. schistorhynchus*.

Because of the small quantity of *L. schistorhynchus* venom available, no attempt was made to detect within it those minor components of *L. semifasciata* venom, such as erabutoxin c (Tamiya & Abe, 1972), toxin Laticauda semifasciata III (Maeda & Tamiya, 1974) and phospholipases (Nishida *et al.*, 1982).

Discussion

Erabutoxins a and b are detected in the venom preparations of *L. semifasciata* from the other parts of the Ryu Kyu Islands and from the Philippines (Tamiya & Takasaki, 1978). Both of the toxins are present in the venom preparations of single specimens of *L. semifasciata*, although their contents vary from preparation to preparation (Tamiya, 1973) and the proportions of their contents vary from specimen to specimen (Takeda *et al.*, 1974).

L. semifasciata and *L. schistorhynchus* differ from other species of the genus *Laticauda* in having the rostral scale divided horizontally. Smith (1926) diagnosed *L. semifasciata* as having 195–205 ventral scales and 30–42 body bands, compared with *L. schistorhynchus* with 187–195 ventral scales and 18–31 body bands. Nine male *L. semifasciata* specimens from Ishigaki Island had 200–207 (mean 204.1) ventral scales and 39–48 body bands (mean 43.1). On the other hand, 26 male *L. schistorhynchus* specimens had 185–197 (mean 193.8) ventral scales and 25–32 (mean 27.3) body bands. The specimens used in the present experiments therefore fall within the described ranges of variation for *L. semifasciata* and *L. schistorhynchus* respectively.

The identity of the venom components shown in the present paper supports the proposition that *L. semifasciata* and *L. schistorhynchus* are conspecific. Although there are some morphological differences that, together with their highly disjunct range, may justify their recognition as subspecies, we believe that there is no justification for treating them as separate species, and we formally propose that *Laticauda schistorhynchus* be relegated to the synonymy of *L. semifasciata*.

The present paper confirms the taxonomic usefulness of protein structure studies.

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