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| A screenshot of a computer  Description automatically generated  **A B C** |  | A group of colorful letters  Description automatically generated with medium confidence |

**Figure 1.** **(A) Multiple Sequence Alignment, (B) Phylogenetic Tree, and (C) WebLogo Sequence Conservation Analysis of *Pseudechis australis* basic phospholipase A2-PA5 orthologs.**

1. Panel A displays a multiple sequence alignment (MSA) of 15 phospholipase A2-PA5 orthologs, visualized in ESPript. Absolutely conserved residues are shown in white letters against red backgrounds, with strongly conserved residues shown in blue-rimmed boxes.
2. Panel B displays a phylogenetic tree constructed using MABL, based on the previously aligned sequences. The tree illustrates the evolutionary relationships among the phospholipase A2 (PLA2) proteins in different species. Values in red are bootstrap support values, which indicate the statistical confidence of each node. Higher values (closer to 1.0) suggest stronger support for that branch. The scale bar, with a value of 0.2 represents the evolutionary distance, specifically the substitutions per site in the sequence alignment.
3. Panel C displays a sequence conservation analysis using WebLogo, which represents the conservation of amino acids at each position in the aligned sequences. The height of each letter represents the degree of conservation at that position, with larger letters indicating highly conserved residues. Color coding distinguishes amino acid properties: green for polar, blue for basic, red for acidic, and black for hydrophobic residues. Conserved regions likely correspond to functionally important residues, while variable regions suggest sites under diversifying selection or structural flexibility in phospholipase A2 enzymes.

**Species Included in the Analyses:** *Pseudechis australis* (UniProt: P20252), *Pseudonaja textilis* (NCBI: P23026.3), *Bungarus caeruleus* (NCBI: Q9DF52.1), *Bungarus flaviceps* (NCBI: Q7T1R0.1), *Bungarus multicinctus* (NCBI: P00606.2), *Crotalus adamanteus* (NCBI: KAK9391663.1), *Hemachatus haemachatus* (NCBI: P00595.1), *Hydrophis hardwickii* (NCBI: Q8UW30.2),  
*Laticauda colubrina* (NCBI: Q8UUH9.1), *Laticauda laticaudata* (NCBI: Q8UUI2.1), *Laticauda semifasciata* (NCBI: Q9I842.1),  
*Micropechis ikaheca* (NCBI: 1PWO\_A), *Micrurus mipartitus* (NCBI: C0HKB9.1), *Micrurus tener* (NCBI: AET85561.1), *Naja pallida* (NCBI: P14556.1), *Naja sagittifera* (NCBI: P60043.2), *Notechis scutatus* (NCBI: P08873.1), *Oxyuranus microlepidotus* (NCBI: Q45Z42.1), *Oxyuranus scutellatus* (NCBI: P00614.1), *Pseudechis rossignolii* (NCBI: BAJ07181.1), *Austrelaps superbus* (NCBI: Q9PUH5.1).

Sequences were obtained from UniProt (for Pseudechis australis) and NCBI standard databases (for all other sequences).

**Homolog with a resolved crystal structure:** Micropechis ikaheca (PDB: 1PWO\_A).

**Functional characterization of PLA2 enzymatic activity has been confirmed in**: Pseudonaja textilis (P23026.3)

The P23026.3 sequence encodes a basic phospholipase A2 neurotoxin (Lawrence Berkeley National Laboratory, n.d.). This snake venom exhibits strong presynaptic neurotoxicity, with Chain A having low toxicity while still being essential for neurotoxic effects (Lawrence Berkeley National Laboratory, n.d.). It has low enzymatic activity and catalyzes the calcium-dependent hydrolysis of 2-acyl groups in 3-sn-phosphoglycerides (Lawrence Berkeley National Laboratory, n.d.). The protein functions as a heterohexamer, with different chain compositions, and requires Ca²⁺ as a cofactor (Lawrence Berkeley National Laboratory, n.d.). Functional annotations were derived from a PaperBLAST literature search.

**References:**Lawrence Berkeley National Laboratory. (n.d.). PaperBLAST: Find papers about a protein or its homologs. Retrieved February

3, 2025, from <https://papers.genomics.lbl.gov/cgi-bin/litSearch.cgi?query=MHPAHLLVLLGVCVSLLGASDIPPLPLNLVQFSYLIRCANKYKRPGWHYANYGCYCGSGGRGTPVDDVDRCCQAHDKCYEDAEKLGCYPKWTTYYYYCGANGPYCKTRTKCQRFVCNCDVVAADCFASYPYNRRYWFYSNKKRCR%0D%0A&Search=Search>