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Computational analysis and physico chemical character ization of phospholipase A2 protein invarious poisonous snakes

Nandini N Trivedi[1], Jeel A Ladhani[1] and Pratibha Parihar*[2]

[1]ResearchStudent
[2]AssistantprofessorinBioinformatics
Department of Biotechnology, Genetics and Bio informatics, N.V. Patel college of Pureand Applied Sciences, CVMU niversity, Vallabh Vidyanagar-388120,Gujarat,India.
*Correspondingauthoremailaddress:pratibhaparihar@gmail.com

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ABSTRACT

Snake venoms are known for their extensive potential in clinical and medical sciences due tothepresenceofpharmacologicallyactiveproteins.Thepresentworkincludedthe *insilico* study of phospholi paseA2 protein sequence and structure from twenty different venomous snake species. These are predictions of various physical and chemical properties,domain scanning, and structural analysis. The average molecular weights of protein found indifferent snake species were calculated as 15.229kDa, most of the proteins were basic and ther mostable. Prot Comp 9.0 tool predicts the location of phospholipase A2 protein in the extracel lularregionor plasmamembrane. CYS_REC tool recognizes7-16cysteine residuesin phospholipase A2 protein sequences and a probable disulfide bond ranges from 4-7. The clustering algorithm in the phylogenetic tree grouped all the species in 3 clusters and showedthat the PLA2 of Inland Taipan and Coastal taipan are the most recent and closest neighbors'inthecluster and are found distant from the rest of the snakes pecies.

Key words: Venom,phospholi paseA2,physio chemical properties,phylogeneticstree

Venom is a poisonous or sometimes lethal secretion containing zootoxins secreted by thefangs of an animal.It assists defense against terrorizations by immobilizationand digestionof prey (Bauchot, 1994). The production of zootoxins is mainly due to the modification ofparotid salivary glands which are encapsulated in a muscular sheath generally situated on thesidesofthe head, below,and behind theeye.

Venoms are composed of around twenty different compounds, mostly proteins (enzymes), polypeptides, lipids, steroids, amino polysac charides, amines, quinines, neurotransmitters (Bottrall et al, 2010) which helps in the digestion of prey. These compounds play a vital rolein various biochemical functions such as the transmission of nervous or muscular impulses, coagulation of blood, regulation of blood pressure.It canalso beused as adefenseme chanism and as a pharm acological ordiagnostic tool (Bauchot,1994).Different poison ous snake contains venoms are rich in pharmacologically active proteins such as proteolyticenzymes (metalloproteases and serine proteases), cysteine-rich secretory proteins, argin in eesterhy drolase, thrombin- like enzymes, Kunitz peptides, shortneurotoxin,longneurotoxinhyaluronidase, phospholipaseA2,three-finger toxins, acetyl cholinesterase,nucleases (RNase, DNase, and Phosphodiesterase), and L-amino-acid oxidase (Kang et al. 2011; Modahletal. 2016). These components bind orinter fere with the affinity and mechanism of a large number of targets such as ion channels or membrane receptors, andhemostatic pathways (Beraud et al. 2011; Tasouliset al. 2017). The venoms present insnakes, spiders, honey bees, wasps, reptiles, scorpions, cone snails, and amphibians may

beuseful in the medical and biotechnological or clinical potential. Phospholipase A2 (PLA2s)are enzymes that break down and release fatty acids or phospholipids and thus produce glycerolly sophospholipid. These enzymes perform many pharma cological activities such ascardiotoxic, neurotoxic, anticoagulant,myotoxic, and hemorrhagic effects.

The enzyme is rich in α-helix. There are sixresidues in the active region: His-48, Asp-49,Tyr-28, Gly-30, Gly-32, and Asp-99. During the catalytic activity of PLA2s, the conservedresidue, Asp49, plays an important role in coordinating Ca2+, thus helping in intermediate transition state stabilization. Thus, for the catalytic activity, Ca2+ acts as a co-factor (Ojeda etal.2017).

In the present work, we studied twenty various venomous species sequences with the help ofbioinformatics tools and available databases. It involved retrieval of sequences, physical and chemical properties prediction, domain scanning, and structural analysis. Further, the sefindings could beuseful for designing various peptide-based drugs designing for the treatment of the chronicdi sorder.

Materials and Methods Sequence retrieval

Twenty snakes were selected based on theirpoisonous property and the availability of theirprotein sequences in the database. The UniProt database (https://www.uniport.org/) was usedto retrieve their protein sequences. The sequences were saved in the FASTA file along with their accessionl Dsgivenin table 1.

Physio chemical Analysis

The physicochemical properties of protein like aliphatic index (AI), amino acid composition, atomic composition, estimated half-life, extinction coefficient (EC), grand average of hydropathicity(GRAVY),instability index(II), molecular weight (MW), theoretical isoelectric point(pI), were calculated with the Prot Param tool (https://www.expasy.org/) (Artimoet al., 2012).

Domain Analysis

As reviewed by El-Gebali et al., 2019, Pfam is a curated database of classification of proteinfamilies and domains. It is used further for the multiple sequence alignment, identification ofdistant homology, and annotation of protein sequences generated by profile hidden Markovmodels (HMM).

SecondaryStructurePrediction andanalysis

The Self Optimized Prediction method With Alignment (SOPMA) (https://npsa prabi. ibcp. fr/cgi-bin/npsa_ automat. pl? page= /NPSA/ npsa_sopma. html)(Geourjonetal.,1995) tool was used to predict the secondary structures of all the protein sequences.It usesthe homolog method to form homologous secondary structures. The accuracy of SOPMA is69.5%. It gives detailed information for all three states of secondary structure (α-helix, βsheet, and coil) in a wholes equence. CYS_REC (http:// linux 1. softberry. com/berry.phtml/topic) tool was usedto predict the sulfi debond patterns (SS) and linkages.

Homology modeling and validation:

SWISS MODEL SERVER was used to model the structures of all the species and the predicted structures were validated using the PROCHECK tool. Homology modeling methods is a highly reliable and accurate method to built models for evolutionarily relatedproteinsusingthe homologous experimental protein structures.

Phylogenetics analysis

The phylogenetic tree was constructed for Phospholipase A2 toxin of all snake species tostudy the evolutionary history of snake venom proteome. Distance score was calculated usingthe Kimura 2- parameter model and clustering were done using the neighbor-joining (NJ)methodusingthe software package CLC genomic work bench.

Result and analysis Retrieval of sequences:

All the sequences were downloaded from the UniProtKB protein database and were saved inFASTA format in the notepad. The sequence length was found to range between 118-152 aaand the average length 137 aa. The detailed information of accession number, sequencelength, and protein nameareshown in table1.

Table 3 Relationship between productivity and agricultural inputs

Speciesnames	UniprotKBID	Length (inaa)
Acanthophisantarcticus(DEATHADDER)	P81236	119
Notechisscutatusscutatus (MainlandTigersnake)	P00609	119
Oxyuranusmicrolepidotus (InlandTaipan)	Q45Z42	146
Pseudonajatextilis (Easternbrownsnake)	P23026	145
OphiophagusHannah (KingCobra)	Q9DF33	146
Oxyuranusscutellatusscutellatus (Coastaltaipan)	Q45Z47	146
Dispholidustypus (Boomslang)	I0BWR7	150
Agkistrodonpiscivoruspiscivorus (Easterncottonmouth)	P51972	123
Echiscarinatus (Sawscaledviper)	Q7T3S7	136
Daboiasiamensis (EasternRussell'sviper)	Q7T2R1	138
Najasumatrana (Malayanspittingcobra)	Q92084	146
Many-bandedkrait (Bungarusmulticinctus)	P00617	147
Bungaruscaeruleus (Indiankrait)	Q9DF52	152
Crotalusdurissusterrificus (SouthAmericanrattlesnake)	P62022	138
Enhydrinaschistose (Beakedseasnake)	P00610	119
Najaatra (Chinesecobra)	P00598	146
Bitisgabonica (Gaboonviper)	P00620	118
Agkistrodoncontortrixlaticinctus (Broad-bandedcopperhead)	P49121	137
Bothropsasper (Fer-de-lance)	P20474	138
Laticaudacolubrine (Yellow-lippedseakrait)	P10116	118

Physiochemical Analysis

The various physical and chemical parameters for Phospholipase A2 are shown in Table 2.The molecular weight for various venomous snake Phospholipase A2 protein is ~12-16 kDaand the average molecular weight is 15.229 kDa. At the isoelectric point (pI), proteins arestable and compact.The computed pI value of most of Phospholi pase A2 protein sequences in the poisonous snake was found to be pI> 7 indicating their basic nature while some of theviper, cobra, and krait species show a slightly acidic nature of Phospholipase A2 protein. Theutility of calculation of computed isoelectric point (pI) of a protein is the identification ofcharged nature of the protein, analyzing antibody behavior and in the purification of theproteins (Gasteiger et al., 2005). The extinction coefficient (EC) of Phospholipase A2 protein at 280nm ranges from12,000 to 38,000M-1cm-1 for the concen tration of Cys,Trp, and Tyr.The highest EC value was found in South American rattle snake and Eastern brown snake which indicates the presence of a high concentration of Trp, Tyr, and Cys while the lowest EC value was found in the Yellow-lipped seakra it. The computed EC values are useful in the concentration of protein, quantitative analysis of protein-ligand, and protein-protein interactions (Gill and Von Hippel, 1989). Instability index (II) is a measure of the invitro stability of proteins. The instability index value less than 40 is considered as a stable protein while a higher than 40 value shows unstable protein in *in-vitro* condition. Phospholipase A2 sequences howed highstability for most snakes pecies. The in stability index value is related to in vivo half-life of the protein. Unstable protein with more than 40instabilityindexvaluesshowsaninvivohalf-life of fewerthan 5 hours, whereas *in-vitro* stable protein shows an in vivo half-life of more than 16 hours. Protein Aliphatic Indexis the relative amount of aliphatic side chains present in the protein-like Alanine, Valine, Leucine, and Isoleucine.The higher value ofthe Aliphatic Index shows higher ther mal stability of globular proteins. Aliphatic

amino acids showed a higher hydrophobic nature ofamino acids. The Aliphatic Index value ranges between 66.5 to 84.33 for cytotoxins protein. 37.2-71.1The higher value of the aliphatic index shows the thermostability of protein over awide temperature range. Many-banded krait and Indian krait showed the highest AliphaticIndex. The presence of both hydrophobic and hydrophilic (charged) residues within proteinshows the amphipathic nature of cytotoxins found in Indian krait. It is an important criterionfor a molecule for biological membrane perturbation. The Grand Average of Hydropathicity(GRAVY) (Kyte and Doolittle, 1982) index indicates the solubility of the proteins. Thepositive value of GRAVY indicates the hydrophobic nature of protein while the negativevalue shows the polar nature of the protein. The result shows proteins have negative GRAVY values for all the snakes pecies.

M.W.-molecular weight, pI- theoretical pI, EC-extinction coefficient, estimated half-life, II-instability index, Aialiphaticindex, grand average of hydropathicity(GRAVY), AA-aminoacid Domainanalysis

Proteins are made of one or many motifs or functional and/or structural regions that mayassociate with a diverse range of protein families, termed as domains. Phospholipase A2 hasthedomain PhospholipaseA2 in all the species.Theclan shows four families PLAG12,Phospholip_A2_1,

Phospholip_A2_2 and Phospholip_A2_3. Phospholipases A2 (PLA2s)are enzymes with ECnumberas3.1.1.4whichhydrolysesthefattyacidbetweenthesecondfattyacid"tail"andthe glycerol molecule. PLA2 is stimulated by melittin present in the venom of the snake. Inour analysis, the domains of all the above species were predicted from the Pfam database andthedata areshown in the table below.

Secondary structure prediction:

The secondary structure element like an alpha helix, extended strand, and random coil of allthe protein sequences was predicted by the SOPMA tool. The result shows that most of theregions in the sequences were occupied by random coils than an alpha helix, extended strand,and beta-turn. Phospholipase A2 showed a higher percentage of the alpha helix which ishighest in Indian krait and Broad-banded copperhead. High

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Homo logy Modeling

The 3D structures of all the species were modelled by using SWISS MODEL server given in Fig.1 and predicted models were validated using PROCHECK tool. The template and organism name with its identity scores are shown in table 6. Most of the structures showed high homologywiththe template structure. The Ramachandran plots core was obtained for all the species was found to be above 85% for all the structures. The Generated models were visualizedin RasMol as shown in table 6.

TABLE 2: Physio chemical analysis of PHOSPHOLI PASE A2

Species name	M.W. (kDa)	pI	AI	II	Half life(in hours)	EC	Freq.ofapolar AA	Freq.ofpolar AA	Highfreq.AA	lowfreq. AA	Gravy
Deathadder	12.8	8.55	44.3	34.80 Stable	11	25,590	0.420	0.361	(C),(G)	(H)	-0.428
MainlandTigersnake	13.67	8.56	42.68	50.38 unstable	1.4	25,020	0.353	0.361	(C)	(M), (W)	-0.720
Inland Taipan	16.32	8.8	61.50	48.56 Unstable	30	22,520	0.432	0.336	(C)	(W)	-0.286
Easternbrownsnake	16.68	9.04	59.86	34.44 Stable	30	38,450	0.414	0.359	(Y)	(M)	-0.388
kingCobra	15.90	**4.86**	68.83	36.31 Stable	30	22,520	0.425	0.390	(C)	(M), (W)	-0.132
Coastal taipan	16.10	8.81	64.86	41.69 Unstable	30	21,240	0.445	0.322	(C)	(W)	-0.275
Boomslang	16.34	8.65	69.66	38.34 Stable	30	23,860	0.447	0.353	(C), (L)	(M)	-0.156
Easterncottonmouth	13.98	8.92	42.03	24.49 Stable	1.4	29,430	0.366	0.366	(K)	(H)	-0.658
saw scaledviper	15.5	**5.44**	64.48	38.71 Stable	30	36,400	0.404	0.331	(C)	(H)	-0.393
eastern Russell's viper	15.39	**4.92**	60.07	32.19 Stable	30	23,800	0.413	0.377	(C)	(P), (W)	-0.162
Malayan spitting cobra	16.18	7.34	62.94	34.69 Stable	30	29,490	0.438	0.377	(A), (C)	(E), (H), (W)	-0.269
Many-bandedkrait	16.22	8.05	71.76	21.08 Stable	30	19,330	0.442	0.327	(C)	(W)	-0.183
Indiankrait	15.76	**5.42**	68.69	23.53 Stable	30	18,110	**0.428**	**0.428**	(C)	(W)	-0.079
SouthAmerican rattlesnake	15.90	8.98	60.79	48.43 Unstable	30	37,680	0.435	0.312	(C)	(H), (Q)	-0.260
Beakedseasnake	13.44	8.54	45.96	43.01 Unstable	1.4	16,200	0.328	0.395	(C)	(W)	-0.716
Chinese cobra	16.01	**6.34**	62.26	33.22 Stable	30	29,490	0.445	0.384	(A), (C)	(H)	-0.208
Gaboon viper	13.37	**5.29**	37.20	32.82 Stable	1.1	24,900	0.339	0.424	(G)	(H), (L), (P),(W)	-0.696
Broad-bandedcopperhead	15.77	9.15	66.93	26.34 Stable	30	28,150	0.372	0.321	(K)	(Q)	-0.482
Fer-de-lance	15.75	8.98	63.62	38.94 Stable	30	25,020	0.420	0.304	(C),(K)	(H)	-0.329
Yellow-lipped seakrait	13.35	8.72	46.35	42.38 Unstable	1.4	12,360	0.373	0.398	(C)	(W)	-0.483

Table 3: Pfam results

Speciesname	Domain	Start	End	Accession	Score	E-value	Description
DeathAdder	Phospholip_A2_1	2	110	PF00068.20	120.0	6.6E-35	PhospholipaseA2
Mainland Tiger snake	Phospholip_A2_1	2	110	PF00068.20	110.5	5.8E-32	PhospholipaseA2
InlandTaipan	Phospholip_A2_1	29	137	PF00068.20	112.7	1.3E-32	PhospholipaseA2
Eastern brown snake	Phospholip_A2_1	29	136	PF00068.20	104.6	4E-30	PhospholipaseA2
KingCobra	Phospholip_A2_1	29	138	PF00068.20	119.1	1.3E-34	PhospholipaseA2
Coastaltaipan	Phospholip_A2_1	29	137	PF00068.20	115.7	1.5E-33	PhospholipaseA2
Boomslang	Phospholip_A2_1	25	130	PF00068.20	119.4	1.1E-34	PhospholipaseA2
Eastern cottonmouth	Phospholip_A2_1	2	108	PF00068.20	111.8	2.3E-32	PhospholipaseA2
SawScaledviper	Phospholip_A2_1	18	124	PF00068.20	113.4	7.7E-33	PhospholipaseA2
EasternRussell's viper	Phospholip_A2_1	18	124	PF00068.20	109.1	1.7E-31	PhospholipaseA2
Malayanspitting cobra	Phospholip_A2_1	29	137	PF00068.20	117.3	4.7E-34	PhospholipaseA2
Many-banded krait	Phospholip_A2_1	29	138	PF00068.20	104.8	3.5E-30	PhospholipaseA2
Indiankrait	Phospholip_A2_1	29	136	PF00068.20	120.9	3.5E-35	PhospholipaseA2
SouthAmerican rattlesnake	Phospholip_A2_1	18	124	PF00068.20	109.0	1.7E-31	PhospholipaseA2
Beaked seasnake	Phospholip_A2_1	2	110	PF00068.20	108.2	3.1E-31	PhospholipaseA2
Chinesecobra	Phospholip_A2_1	29	137	PF00068.20	118.8	1.6E-34	PhospholipaseA2
Gaboonviper	Phospholip_A2_1	2	105	PF00068.20	102.6	1.7E-29	PhospholipaseA2
Broad-banded copperhead	Phospholip_A2_1	18	124	PF00068.20	104.7	3.7E-30	PhospholipaseA2
Fer-de-lance	Phospholip_A2_1	18	124	PF00068.20	110.4	6.4E-32	PhospholipaseA2
Yellow-lipped seakrait	Phospholip_A2_1	2	109	PF00068.20	107.7	4.4E-31	PhospholipaseA2

Table 4: Result of secondary structure of all the species predicted

Speciesname	Alphahelix(Hh)	Extendedstrand(Ee)	Randomcoil(Cc)
Deathadder	29.41%	19.33%	49.58%
Mainlandtigersnake	33.61%	15.97%	48.74%
InlandTaipan	33.61%	11.64%	44.52%
Easternbrownsnake	39.04%	11.64%	44.52%
kingCobra	31.51%	11.64%	44.52%
Coastaltaipan	39.73%	10.27%	48.63%
Boomslang	39.33%	12.00%	44.67%
Easterncottonmouth	35.77%	16.26%	46.34%
SawScaledviper	36.96%	14.49%	43.48%
EasternRussell'sviper	32.19%	15.75%	48.63%
Malayanspittingcobra	31.97%	14.97%	48.98%
Many-bandedkrait	35.17%	17.24%	46.21%
Indiankrait	44.93%	13.77%	39.86%
SouthAmericanrattlesnake	44.93%	13.77%	39.86%
Beaked seasnake	36.13%	15.97%	41.18%
Chinesecobra	31.51%	16.44%	50.00%
Gaboonviper	33.05%	18.64%	45.76%
Broad-bandedcopperhead	45.26%	16.79%	32.12%
Fer-de-lance	42.03%	15.94%	37.68%
Yellow-lippedseakrait	36.44%	15.25%	44.92%

Table 5: Location of disulphidebridgein the Phospholipase A2 structure

Species Name	Positions of cysteinesresidues	Probablepattern of disulphide bond formation
Deathadder	1127 29 44 45 51 60 71 78 85 90 9299118	11-71,29-45,44-99,51-90,60-85,78-92
MainlandTigersnake	1127 29 44 45 51 60 71 78 85 90 9299118	27-60,29-45,44-99,51-92,71-85,78-90
InlandTaipan	1338 54 56 71 72 78 88 99106112 117119127146	54-88,56-72,71-106,78-112,99-117
Easternbrownsnake	1338 54 56 71 72 78 87 98105111 116118125144	54-111,56-72,71-125,78-118,87-98,105-116
kingCobra	1338 54 56 71 72 78 88 99106113 118120130149	54-106,56-72,71-130,78-99,88-113,118-120
Coastaltaipan	1338 54 56 71 72 78 88 99106112 117119126145	38-106,56-72,71-126,78-119,88-112,99-117
Boomslang	616 48 50 65 66 71 72 81 99105 110112119137144	48-144,50-66,65-119,71-105,72-81,99-110, 112-137
Easterncottonmouth	2628 43 44 49 50 57 75 81 86 88 95116123	26-116,28-44,43-95,50-88,57-81,75-86
sawscaled viper	4244 59 60 65 66 73 91 97102104 111129136	42-129,44-60,59-111,66-104,73-97,91-102
easternRussell'sviper	1042 44 59 60 65 66 73 91 97102 104111131138	42-131,44-60,59-111,66-104,73-97,91-102
Malayanspittingcobra	1338 53 55 70 71 77 87 98105112 117119126145	38-98,55-71,70-126,77-119,87-112,105-117
Many-bandedkrait	1342 54 56 71 72 78 88106113118 120127146	42-54,56-72,71-127,78-120,88-113,106-118
Indiankrait	1338 52 54 69 70 76 86 97104111 116118125144	52-97,54-70,69-125,76-118,86-111,104-116
SouthAmericanrattlesnake	4244 59 60 65 66 73 91 97102104 111131138	42-97,44-60,59-111,66-104,73-131,91-102
Beaked seasnake	1127 29 44 45 51 60 71 78 85 90 9299118	11-71,29-45,44-99,51-92,60-85,78-90
Chinesecobra	1338 53 55 70 71 77 87 98105112 117119126145	38-98,55-71,70-126,77-119,87-112,105-117
Gaboonviper	2426 41 42 47 48 73 83 85 92112 118	24-112,26-42,41-92,48-85,73-83
Broad-bandedcopperhead	4244 59 60 65 66 73 91 97102104 111131137	44-60,59-111,65-131,66-104,73-97,91-102
Fer-de-lance	4244 59 60 65 66 73 91 97102104 111131138	42-131,44-60,59-111,66-104,73-97,91-102
Yellow-lippedseakrait	11272944455160	11-51,27-44,29-45

Secondary structure prediction:

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Table 6: 3-Dstructure detail for Phospholipase A2 for all the snakes pecies.

SPECIESNAME	TEMPLATE AND ORGANISMNAME	TEMPLATEIDENTITY
Acanthophisantarcticus (DeathAdder)	5p2p.1.A	54.70%
Notechisscutatusscutatus(MainlandTigersnake)	2not.2.A	100%
Oxyuranusmicrolepidotus(InlandTaipan)	1psh.1.A	55.93%
Pseudonajatextilis(Easternbrownsnake)	2not.1.A	65.25%
OphiophagusHannah(kingCobra)	1gp7.2.A	80.82%
Oxyuranusscutellatusscutellatus(Coastaltaipan)	3v9m.1.A	68.64%
Dispholidustypus (boomslang)	5wzs.1.A	57.02%
Agkistrodonpiscivoruspiscivorus(Easterncottonmouth)	5wzs.1.A	57.02%
Echiscarinatus(SawScaled Viper)	1oz6.1.A	100%
Daboiasiamensis(EasternRussell'sviper)	1oqs.1.A	89.34%
Najasumatrana (Malayanspittingcobra)	1yxh.1.A	88.10%
Bungarusmulticinctus (Many-bandedkrait)	1bun.1.A	99.17%
Bungaruscaeruleu (Indiankrait)	1dpy.1.A	91.53%
Crotalusdurissusterrificus (SouthAmericanrattlesnake)	6tmy.2.C	99.18%
Enhydrinaschistose (Beakedseasnake)	2not.2.A	78.15%
Najaatra (Chinesecobra)	1yxh.1.A	90.48%
Bitisgabonica (Gaboonviper)	1vpi.1.A	54.28%
Agkistrodoncontortrixlaticinctus(Broad-bandedcopperhead)	1s8g.1.A	100%
Bothropsasper(Fer-de-lance)	5tfv.1.A	99.18%
Laticaudacolubrine(Yellow-lippedseakrait)	2not.2.A	66.10%

Fig.1:3 Dstructure of PhospholipaseA2 for all the snakes pecies

1.DEATHADDER



2. MainlandTigersnake



3. Inland Taipan



4.Easternbrownsnake



5. kingcobra



6. Coastaltaipan



7. Boomslang



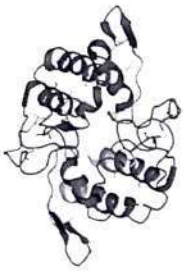
8. Easterncottonmouth



9. Sawscaledviper



10. EasternRussell'sviper



11. Malayan spitting cobra



12. Many-bandedkrait



13. Indiankrait



14. S. American rattle snake



15. Beaked sea snake



16.Chinesecobra



17. Gaboonviper



18. Broad-bandedcopperhead

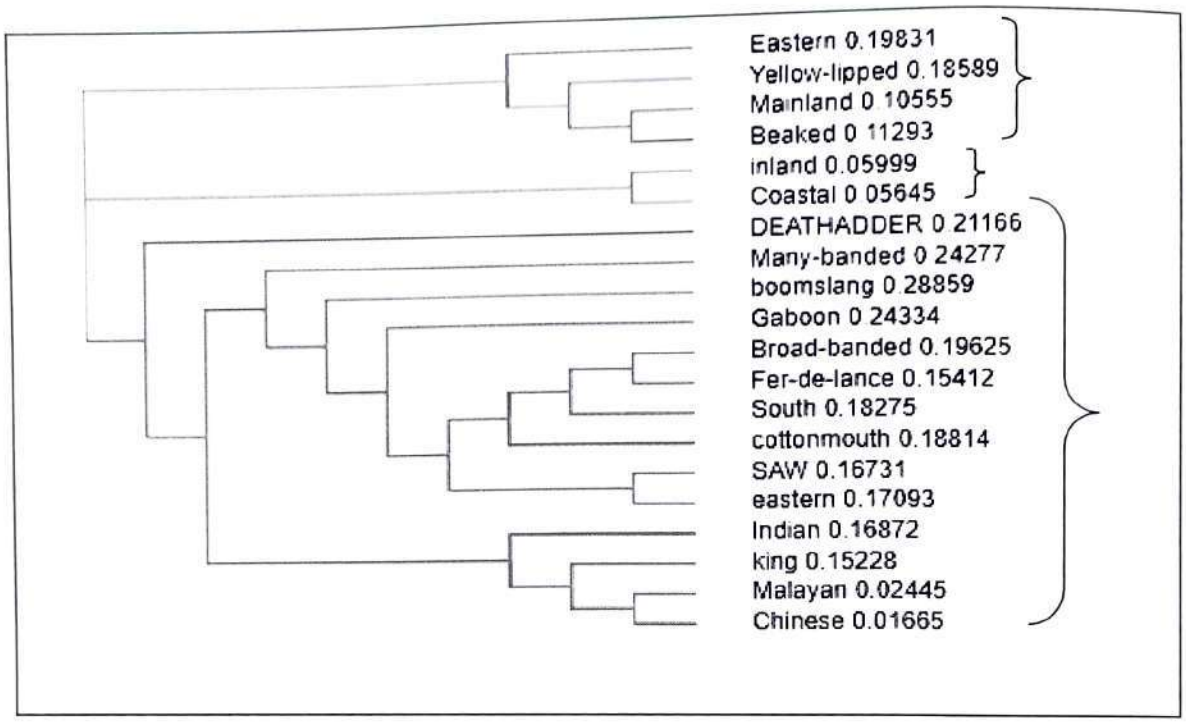


19. Fer-de-lance



20. Yellow-lippedseakrait





Conclusion

Primary structure analysis revealed that Phospholipase A2 protein of most of venomoussnake has an average molecular weight 15.229 kDa, basic and hydrophobic in nature. Thesecondary structure analysis confirmed random coils dominated followed by an alpha helix and beta strand. The presence of disulfidebridges further established stability of protein and thus aiding in formulating their uses. Further, phylogenetics study shows that the evolution isthe major factor that plays in the physio chemical properties of protein in the snake species. Apheno type of any organisms influence from both its genotype and environmental condition.Present analysis revealed the toxic phospholipase A2 in present in different organism weremoreorless related to oneanother and may have originated from the samean cestor.

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