

Investigating snake venom variation to mitigate snakebite envenomation in Indonesia

Syahfitri Anita^{1*}, Kelvin Octavianus², Mulyadi¹, Wahyu Trilaksono¹, Herjuno Ari Nugroho³, Bambang Kiranadi², Reinhard Pinontoan², and Amir Hamidy¹

¹Laboratory of Herpetology, Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Jalan Raya Bogor Cibinong Km. 46, 16911, Indonesia.

²Fakultas Sains dan Teknologi, Universitas Pelita Harapan, Jl. M. H. Thamrin Boulevard 1100 Lippo Village Tangerang 15811 – Indonesia.

³Research Center for Applied Microbiology, National Research and Innovation Agency (BRIN), Jalan Raya Bogor Cibinong Km. 46, 16911, Indonesia.

Abstract. Snakebite envenoming is one of the neglected tropical diseases and is still rarely studied in Indonesia. The high diversity of venomous snakes in Indonesia is one of the challenges. To overcome this, we consider that snake venom research guided by phylogenetic relationships can serve valuable information that may contribute to snakebite mitigation. Here, we briefly introduce our recent study using HPLC-MS/MS to analyse the venom composition and variation across four Indonesian *Trimeresurus*. This adds more information on venom variation among *Trimeresurus* species within the close geographic origin, which congruent with their phylogenetic relationships. We also conducted a preliminary study to detect intraspecific variation between the venom of males and females of *T. puniceus* using SDS-PAGE. There is an addition of protein with a molecular weight of 13 kDa in the venom of male *T. puniceus*, while a protein with a molecular weight of 16 kDa is only detected in female venom. We summarize recent studies showing different factors that can affect venom variation between and within snake species. Finally, we discuss the importance of transdisciplinary research to understand snake venom variation and suggest future directions, particularly from a herpetological view, to mitigate human-snake conflict in Indonesia.

1 Introduction

Snakes are an important component in the ecosystem so their existence must be preserved. The presence of snakes and humans in the same habitat poses a risk for both, in which the encounters can cause humans to kill snakes or snakes to bite humans. Humans are not prey for snakes, but human envenoming may happen because of defensive bites inflicted by snakes when they have an unexpected encounter in the shared natural environment. Globally, over 5.8 billion people are at risk of encountering a venomous snake, almost 7400 people every day are bitten by snakes, and up to 2.7 million cases of envenoming and 81,000–

* Corresponding author: syafiet@gmail.com

138,000 deaths a year [1]. Snakebite envenomation was recognized as a neglected tropical disease by the World Health Organization, associated with poverty and kill more people than other tropical diseases and even though the victim can be saved, it usually leaves permanent physical disabilities [1, 2, 3]. Snake venom is a product of evolutionary innovation that allowed snakes to transition from a mechanical mechanism (constriction) to a chemical mechanism (venom) to subdue prey and defend themselves quickly [4, 5]. More than 90% of snake venom components are proteins, and each species' venom can contain hundreds of types of proteins in the form of enzymes, non-enzymatic polypeptides and non-toxin proteins. Study shows that there are at least 63 protein families comprising the venom of 132 types of snakes [6].

Compare to other countries, Indonesia has a high diversity of venomous snakes, which include Elapidae (57 species) and Viperidae (22 species) families [7]. Snakes in the Elapidae and Viperidae families are classified into categories of medical importance, widely distributed and causing high levels of mortality and morbidity [8]. Of the various medically important venomous snakes, vipers and pit vipers are commonly known to cause snakebite in Asia. These include the Asiatic lance-headed pit vipers (subfamily: Crotalinae) of the *Trimeresurus* complex. Here, we summarize our recent findings on the venom composition and variation across four Indonesian *Trimeresurus*. We then presented the preliminary study to detect intraspecific variation between the venom of males and females of *T. puniceus* (recently confirmed as *Craspedocephalus puniceus*). We then described the factors affecting venom variation between and within snake species. Finally, we discuss the importance of transdisciplinary research to understand snake venom variation and suggest future directions, particularly from a herpetological view, to mitigate human-snake conflict in Indonesia.



Fig. 1. The female (A) and male (B) of *Trimeresurus puniceus* (recently confirmed as *Craspedocephalus puniceus*) collected from Central Java, Indonesia.

2 Methodology

2.1 Venom composition of *Trimeresurus* from Indonesia

Fifteen species of *Trimeresurus* are distributed in Indonesia [7]. Bites from these snake groups, especially *T. albolabris* and *T. insularis*, are frequently happened and known to cause fatalities in Indonesia. The bite can cause intense pain, mild to severe local swelling, and spontaneous bleeding [9]. Indonesia still does not have antivenom to treat bites from this group of snakes, but the Thailand antivenom, Thai Green Pit Viper Antivenom (GPVAV), can be used to treat *Trimeresurus* bites. Study shows that GPVAV, a horse antivenom produced against Thailand *T. albolabris* venom, have cross-reactivity and neutralization capability against Indonesian *T. insularis*, *T. purpureomaculatus*, *T. hageni* and *T. puniceus* venoms [10]. Several species of *Trimeresurus* snakes are commonly found in Java and Sumatra islands, where most of the Indonesian population lives. Thus, snakebite incidents by

these species are unavoidable in these islands, particularly in Java. Therefore, understanding the venom composition of the *Trimeresurus* snake species and estimating its impact on health is essential for snakebite treatment and future antivenom development. In our previous study, we characterized the venom of *T. albolabris* (Ta), *T. insularis* (Ti), *T. purpureomaculatus* (Tpur), and *T. puniceus* (Tpun), which were collected from different regions of Indonesia [11]. We characterized the venom using LC-MS/MS analysis and compared the venom protein between each species using clustering analysis. The study revealed a common protein pattern of *Trimeresurus* venom but indicated inter-species variation of the toxin composition. The proteomic analysis identified 65 proteins in Tpur; 64 proteins in Ta; 58 proteins in Tpun; and 48 proteins Ti. Among all the proteins detected, there were 11 protein families identified in all snake venoms, and the four proteins identified in dominant amounts were snake venom metalloproteinase, type C lectin, snake venom serine protease, and phospholipase A2. Hierarchical cluster analysis of all identified proteins indicated that Ta, Tpur and Ti venoms were more similar, meanwhile, the Tpun venom has the highest number of unique proteins and exhibited the most distinct composition. These proteomes clustering also reflected the immunoreactivity pattern of Thai Green Pit Viper Antivenom, in which there was different activity of GPVAV in cross-neutralizing the Tpun venom [10]. The result indicates that Tpun toxins are more distinct from the other. The venom proteome clustering of those four species is also parallel with the species' phylogenetic relationships, in which the clade of the Tpun species is distantly separated from the other three species. The venom analysis is congruent with the recent systematic study that revealed the presence of South Asian radiation of the clade *Craspedocephalus*, and confirmed *T. puniceus* as a member of the genus *Craspedocephalus* [12]. Altogether, our findings showed how snake venom study is related to the taxonomy relationship and may serve as initial information for the effective snakebite treatment and to the development of the optimum heterologous antivenom.

Table 1. Number and identity of snakes use as venom source and the mean weight of extracted venom.

	SVL range (cm)	Body weight range (g)	Mean(SE) (g)	
			Raw venom	Dried venom
Female (n=6)	52.6-85.4	121.5-494.1	0.209(0.05)	0.161(0.04)
Male (n=6)	56.4-77.2	163.4-362.4	0.095(0.03)	0.084(0.03)

3 Result and discussion

3.1 Variation of *Trimeresurus puniceus*

Trimeresurus puniceus, or Flat-nose pitviper, is an endemic snake in Indonesia that belongs to the Viperidae family and is distributed in Sumatra, Java and Bali [7]. The species is characterized by its bright background color, composed of shades of grey or yellowish-brown. The coloration is ornamented with irregular dark blotches with cream and dark dots, giving an “ashy” or lichen-like appearance, but it is less contrasted in females (Fig 1.) [13]. This terrestrial snake mainly lives in trees, and females prefer semi-arboreal areas. The snake can reach up to 1.5 meters long, and males are generally smaller than females. The *Trimeresurus* complex envenomation, particularly by *T. puniceus*, can cause hemotoxicity, localized pain, swelling, bruising, and thrombocytopenia [14]. It is indicated that *T. puniceus* venom is highly procoagulant and can induce significant haemorrhage [15]. The venom of *T.*

puniceus is composed of 12 protein families and the major toxin detected in the venom are related to the snakebite effect of haemorrhage and coagulopathy [11, 14].

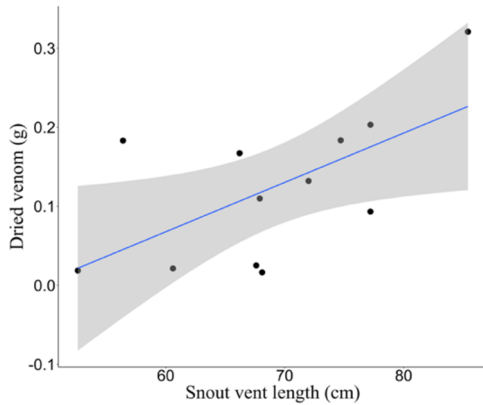


Fig. 2. Linear model between snout-vent length (SVL) and weight of freeze-dried venom extracted from each individual of *T. puniceus*. The weight of dry venom is tended to increase with the length of snake. The shaded area around the line represents the 95% confidence interval.

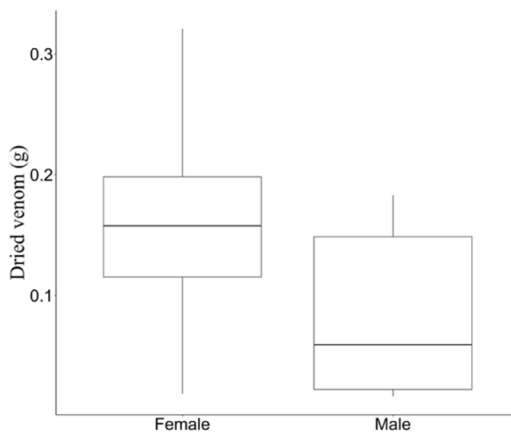


Fig. 3. Box-plot of dry venom weight according to sex differences. The median is represented by the middle horizontal line in the box plot. Edges of the boxes represent 25% and 75% quartiles, the range is represented by whiskers.

To investigate the sexual differences on venom variation, we use venom from 12 adult individuals (six males and six females) of *T. puniceus* collected from central parts of Java, Indonesia (Table 1). The snake is stimulated to bite a rubber band attached to the top of a sterile container and the fluid released from its fangs is collected as a venom sample. The venom stored in a cool box during transportation to laboratory. Once in the laboratory, samples were lyophilized and stored at -20°C before further analysis. Dry weight mean of female venom is 0.161 g and male venom is 0.083 g (Table 1). Statistical analysis using linear model indicated that the venom dry weight is positively correlated with their length ($P < 0.05$, R-squared: 0.3795, Fig. 2). However, there is no significant difference observed between males and females dry venom weight (T-test, $P > 0.05$, Fig. 3). The total protein in the venom was quantified using the biuret method. Measurement indicated that protein concentration in male snake venom is $0.018 \pm 0.00013 \text{ mg/dL}$, while in females is $0.016 \pm 0.00011 \text{ mg/dL}$. There is significant difference of protein concentration between females and males (T-test, $P < 0.05$).

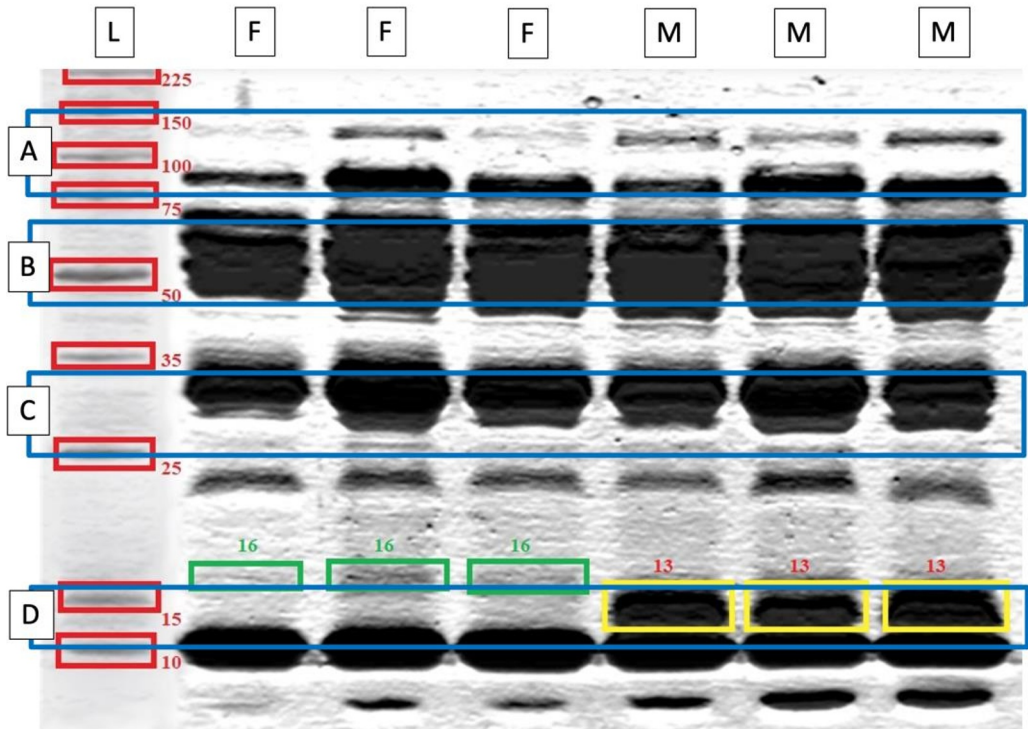


Fig. 4. SDS-PAGE analysis of the venom protein profiles of *T. puniceus*. Whole venom (12.5 µg) from three females and three males of snake were resolved on a linear gradient (12–18%) SDS gel and visualized by Coomassie Brilliant Blue staining. F: Female, M: Male, L: Protein ladder, A: Nucleases, B: Metalloproteinase, C: Serine protease, D: PLA2, 16: Protein with molecular weight of 16 kDa, 13: Protein with molecular weight of 13 kDa.

Dry venom of each individual was further separated based on their molecular weight using SDS-PAGE. The protein profile on SDS-PAGE gel indicates almost similar bands for each venom, and the related proteins can be predicted based on their molecular weight (Fig. 4). Venom of both sexes contained nucleases and L-amino acid oxidase (53 kDa–150 kDa), metalloproteinase (20 kDa–85 kDa), serine protease (31 kDa–15 kDa), phospholipase A2 (13 kDa–15 kDa) [16]. Protein band differences between female and male venom were observed. A protein with a molecular weight of 13 kDa is detected only in male venom, while in female venom, a 16 kDa protein was observed. The protein with a molecular weight of 16 kDa is known to be the PLA2 enzyme, while the protein with a molecular weight of 13 kDa is still unidentified. The role of PLA2 is associated with venom-induced coagulopathy and can cause local edema [17]. The sexual difference in venom composition has been observed in several species and may indicate differences in biological activity of the venom. Relatively higher content of PLA2 in male snake venom is exhibited by *Bothrops pauloensis* [18], while study on the venom of *Bothrops leucurus* exhibit less evidence of sexual variations [19]. In contrast, biological characterization of venoms of each sex in *Bothrops asper* and *Crotalus simus* did not show significant qualitative differences [20]. These results indicate that selecting snakes as venom donors for antivenom production may not be related to sexual variations, at least in the species that have been studied.

3.2 Factors influencing venom variation within and between snake species

Recently, with the development of 'omic' technologies, the variation in venom composition between or within species of venomous snakes has been increasingly revealed. The mixture of toxins in snake venom can vary greatly within species, between populations, and between age classes or body sizes. Although snakes can release their venom in a defensive bite, the primary function of venom is more related to diet and assists them with the acquisition of prey. Venom is considered to be an evolving ecological trait, and it is possible that the same species but differing in their food ecology, may also have differences in venom composition [21]. For example, juveniles and adults of the same species may have differences in prey type, foraging strategies or prey handling behavior, and these differences may be reflected in the composition of their venom. This is demonstrated by several studies investigating ontogenetic changes in venom composition within species [18, 19, 22-26]. They suggested that the venom variation is related to diet changes, such as from specialists as juveniles to more generalists as adults. Furthermore, several studies also suggest that variations in venom composition in the same snake species may also reflect differences in their geographic origins and implicated in differences of venom toxicity levels [27-31].

Many factors may interact and influence venom variability, but dietary factors play an important role as the main function of venom is to immobilize and digest prey. Daltry et. al. [32] found that variation in the venom of the pitviper *Calloselasma rhodostoma* was related to diet and geographic variation, but they suggested that diet was the controlling factor, as venom composition reflected natural selection for local prey. Despite geographical isolation, the venom composition of *Trimeresurus insularis* from adjacent islands in the east of Indonesia shows significant differences, but indicate high similarities when it compared to the venom from their congeneric [33]. More recent studies of venom variation within species suggest that differences in composition may related to prey availability and correlated with abiotic factors such as temperature and geographic patterns [34]. Broad differences can exist in venom compositions at multiple phylogenetic levels, within each family, between genera, and between species within each genus [11, 35-38]. However, similarity of the venom proteome between species may also presence, but the differences may implicated in their level of toxicity. For instance, the venom proteome of pit vipers genus *Agkistrodon* is similar, but differences were observed in the hemorrhagic and myotoxic activities [39].

Possible mechanisms behind intra- or interspecific variations in snake venom have been discussed in many studies. Many ecological variables may interplay and shape the degree of variability in snake venom. At the molecular level, genetic structure along with transcription and translation mechanisms can contribute to phenotypic variation in toxins [40, 41]. However, Zacolli et al. [41] also emphasized that individual toxin genes can correlated with different environmental factors, which indicating that different selective pressures may act on individual loci independently of their co-expression patterns or genome proximity. The relationship between venom and diet composition in snake suggests prey availability as a major biotic determinant of venom composition and variation. Furthermore, studies on Australasian elapid snakes described how feeding ecology, such as foraging strategy, prey type and condition, prey handling behavior and venom delivery systems, may be related to differences of venom composition [42]. Recent research involving biotic and abiotic factors found that food availability and temperature-related abiotic factors correlated with geographic trends in venom composition [43]. Overall, these studies show that information on diet, related genetic and ecological variables is also important to understand variations in snake venom composition and their evolutionary history.

3.3 Further perspectives to mitigate human-snake conflict in Indonesia

Differences in venom composition between or within species, can cause a variety of pathologies in human snakebite victims and possibly result in treatment failure [21]. One case of venom variation is demonstrated by the venom of the Mojave rattlesnake (*Crotalus scutulatus*), which varies geographically, resulting in different toxicities between regions, from hemotoxic to neurotoxic [27, 41]. Venom variation also makes prediction of anti-venom efficacy problematic thus presents a significant challenge to the management of snakebite envenomation and the development of snakebite therapeutics. This can happen because the different composition of toxins may cause variation in the toxic syndrome, and moreover, the toxins may have different antigenicity that can cause ineffective treatment using standard antivenom [28, 30, 39, 44]. Therefore, it is crucial to have a comprehensive understanding of snake venom variation (at inter- or intraspecific levels), and the correlation of ecological and genetic factors to this variation, and the consequences it has on snakebite envenomation and treatment [2].

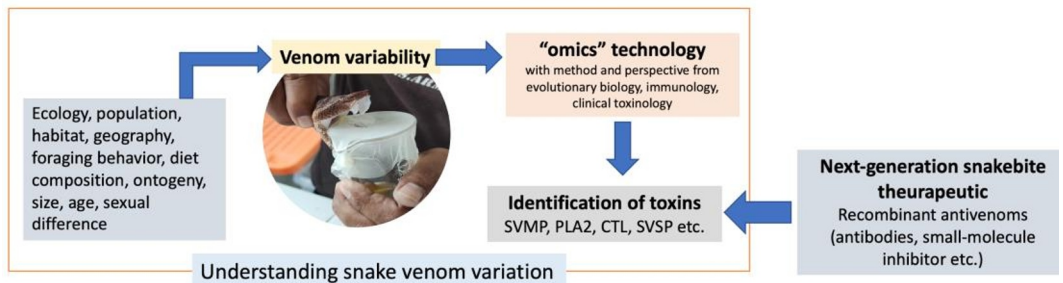


Fig. 5. A transdisciplinary approach can be taken to understand snake venom variations comprehensively in order to create effective prevention and mitigation programs.

Snakebite envenomation is one of the most complex neglected tropical diseases, and requires a transdisciplinary approach to prevent, manage, and treat it [45]. Of many approaches that can be taken to understand variations in snake venom, increasing the herpetological knowledge base is essential, particularly for Indonesia. There is a significant knowledge gap between the number of venomous snakes in Indonesia and information on their ecology and venom. The study conducted by Malhotra et al. [46], revealed that Indonesian Archipelago is a high-priority region for studying venomous species to mitigate snakebite envenomation. This is based on the number of snake species classified as “the greatest threat to public health”, available demographic information, and the quality of available data on snakebite incidence. The study suggests that increasing knowledge of snake biology aspect which currently remains understudied, can improve our understanding of why snakebites envenomation occur. This includes understanding venomous snake behaviour, spatial ecology and activity patterns, distribution, and population demography. According to the study, information on snake behavior can be used to understand why snakes bite. Data on snake distribution and snake ecology may help to understand the overlap between snake activity and human activity, and further can be used to estimate risk factor of human-snake conflict.

We believe that these approaches are important and urgent to apply in Indonesia, considering the differences in the biogeographic character of the Indonesian archipelago and the distinct venomous species between these geographic regions, which possibly have an impact on the venom composition and toxicity. The approach can be started by improving documentation related to herpetology aspects, such as snake distribution and demographic, diet, foraging strategies, behavior, activity patterns, habitat, weather and spatial information,

and other related ecology variables, followed by toxin identification and toxicity analysis (Fig. 5). In a broader perspective, information from this approach can help us understand the correlation between variations in snake venom and Indonesia's geographical characteristics, which can be utilised as a base for determining suitable snakebite treatment and antivenom supply for certain geographic areas in Indonesia. This herpetological information is also important in determining biological criteria of snake that will be used as a source of venom in the production of antivenom suitable for different Indonesian region. In community level, by knowing the activities and foraging patterns of venomous snakes, we can build public awareness by providing information on when and where potential danger may occur and how to take appropriate preventive measures, especially if the foraging area overlaps with human settlement. These are just a few suggestions on how information of snake herpetology possible to be utilized in the formulation of human-snake conflict mitigation. We believe that many effective snakebite mitigation ideas may be created once new information on the herpetological aspects of venomous snakes in Indonesia is revealed. Then hopefully, the coexistence between snakes and humans may happen and does not always end in loss for both.

4 Conclusion

Our previous study showed how *Trimeresurus* venom composition is related to the taxonomy relationship of the group and may serve as initial information for the effective snakebite treatment. Further, we identified venom variation of *Trimeresurus puniceus*; in particular, the protein band differences between female and male venom. Many biotic and abiotic factors may interact and influence snake venom composition and variability. Therefore, it is very important to have a comprehensive understanding of the correlation of these factors with snake venom composition which can now be facilitated by the latest methods and technologies. Increasing knowledge of herpetological aspects of venomous snakes may benefit human-snake conflict mitigation.

5 Acknowledgment

We thank the anonymous reviewer who provided comments on the initial manuscript. This study was supported in part by the RIIM grant to SA.

References

1. World Health Organization (WHO). Snakebite envenoming - A strategy for prevention and control (2019)
2. J. J. Calvete, *Biochem.* **41**, 6 (2019)
3. J. P. Chippaux, *J. Venom. Anim. Toxins. Incl. Trop. Dis.* **23**, 1 (2017)
4. V. Bels, K. V. Kardong, T. L. Kiene, *Neth. J. Zool.* **47**, 4 (1996)
5. S. P. Mackessy, *Handbook of Venoms and Toxins of Reptiles.* **3** (2021)
6. T. Tasoulis, G. Isbister, *Toxins.* **9**, 9 (2017)
7. P. Uetz, P. Freed, J. Hošek, *The Reptile Database.* <http://www.reptile-database.org> (accessed 2023-10-16).

8. World Health Organization (WHO). Guidelines for the Management of Snakebites, Regional Office For South-East Asia (2016)
9. R. de Lang, *The Snakes of Java, Bali and Surrounding Islands*, Edition Chimaira: Frankfurt Am Main (2017)
10. C. H. Tan, J. L. Liew, N. H. Tan, I. A. Khaldun, T. Maharani, S. Khomvilai, V. Sitprija, *Toxicon*. **140** (2017)
11. S. Anita, A. R. Sadjuri, L. Rahmah, H. A. Nugroho, Mulyadi, W. Trilaksono, W. Ridhani, N. Safira, H. Bahtiar, Maharani, A. Hamidy, A. Azhari, J. Venom. Anim. Toxins. Incl. Trop. Dis. **28** (2022)
12. A. K. Mallik, A. N. Srikanthan, S. R. Ganesh, S. P. Vijayakumar, P. D. Campbell, A. Malhotra, K. Shanker, *Vertebr. Zool.* **71** (2021)
13. P. David, G. Vogel, S. P. Vijayakumar, N. A. Vidal, *Zootaxa*. **1**, 1 (2006)
14. L. P. Lee, K. Y. Tan, C. H. Tan, *Comp. Biochem. Physiol. Part D Genomics Proteomics*. **40** (2021)
15. L. P. Lee, K. Y. Tan, C. H. Tan, *Toxicon*. **185** (2020)
16. S. P. Mackessy, *Handbook of Venoms and Toxins of Reptiles*, Crc Press: Boca Raton (2010)
17. Y. M. Wang, H. F. Peng, I. H. Tsai, *FEBS Journal*. **272**, 12 (2005)
18. L. J. Tasima, D. M. Hatakeyama, W. da S. Aguiar, E. O. V. Lima, J. Miyamoto, A. K. Tashima, S. S. Sant'Anna, K. F. Grego, K. de Moraes-Zani, A. M. Tanaka-Azevedo, *Toxicon*. **214** (2022)
19. J. R. M. Braga, K. de Moraes-Zani, D. D. S. Pereira, S. S. Sant'Anna, N. da Costa Galizio, A. M. Tanaka-Azevedo, A. R. Gomes Vilarinho, J. L. Rodrigues, M. M. Teixeira da Rocha, *Toxicon*. **184** (2020)
20. A. Gómez, A. Segura, G. Solano, D. Chacón, G. Corrales. *Toxicon* **204** (2021)
21. N. R. Casewell, T. N. W. Jackson, A. H. Laustsen, K. Sunagar, *Trends. Pharmacol. Sci.* **41**, 8 (2020)
22. C. M. Modahl, A. K. Mukherjee, S. P. Mackessy, *Toxicon*. **119** (2016)
23. V. Cipriani, J. Debono, J. Goldenberg, T. N. W. Jackson, K. Jackson, J. Dobson, I. Koludarov, B. Li, C. Hay, N. Dunstan, L. Allen, I. Hendrikx, H. F. Kwok, B. G. Fry, *Comp. Biochem. Physiol. C. Toxicol Pharmacol.* **197** (2017)
24. J. Durban, L. Sanz, D. Trevisan-Silva, E. Neri-Castro, A. Alagón, J. J. Calvete, J. Proteome. Res. **16**, 9 (2017)
25. I. Avella, J. J. Calvete, L. Sanz, W. Wüster, F. Licata, S. Quesada-Bernat, Y. Rodríguez, F. Martínez-Freiría, *J. Prot.* **263** (2022)
26. X. Nie, Q. Chen, C. Wang, W. Huang, R. Lai, Q. Lu, Q. He, X. Yu, *Toxins*. **14**, 9 (2022)
27. D. J. Massey, J. J. Calvete, E. E. Sánchez, L. Sanz, K. Richards, R. Curtis, K. Boesen, *J. Prot.* **75**, 9 (2012)
28. B. Kalita, S. P. Mackessy, A. K. Mukherjee, *Expert Rev. Proteomics*. **15**, 10 (2018)
29. M. E. Girón, V. Padrón, M. Ramos, E. E. Sánchez, B. Guerrero, A. García, N. L. Uzcátegui, L. F. Navarrete, A. Rodríguez-Acosta, *Toxicon*. **144** (2018)
30. D. Pla, L. Sanz, S. Quesada-Bernat, M. Villalta, J. Baal, M. A. W. Chowdhury, G. León, J. M. Gutiérrez, U. Kuch, J. J. Calvete, *J. Prot.* **207** (2019)

31. L. N. da Silva-Júnior, L. de S. Abreu, C. F. B. Rodrigues, N. da C. Galizio, W. da S. Aguiar, C. Serino-Silva, V. S. dos Santos, I. A. Costa, L. V. F. Oliveira, S. S. Sant'Anna, K. F. Grego, A. M. Tanaka-Azevedo, L. N. da S. Rodrigues, K. de Morais-Zani, J. Venom. Anim. Toxins. Incl. Trop. Dis. **26** (2020)
32. J. C. Daltry, W. Wüster, R. S. Thorpe, Nature. **379**, 6565 (1996)
33. B. K. Jones, A. J. Saviola, S. B. Reilly, A. L. Stubbs, E. Arida, D. T. Iskandar, J. A. McGuire, J. R. Yates, S. P. Mackessy, J. Proteome. Res. **18**, 5 (2019)
34. C. F. Smith, Z. L. Nikolakis, K. N. Ivey, B. W. Perry, D. R. Schield, N. R. Balchan, J. Parker, K. C. Hansen, A. J. Saviola, T. A. Castoe, S. P. Mackessy. BMC Biol. **21**, 1 (2023)
35. D. Pla, L. Sanz, M. Sasa, M. Acevedo, Q. Dwyer, J. Durban, A. G. Pérez, Y. Rodríguez, B. Lomonte, J. J. Calvete. J. Prot. **152** (2017)
36. C. C. Liu, C. Lin, Y. C. Hsiao, P. J. Wang, J. Yu. J, Proteomics. **187** (2018)
37. R. R. S. Laxme, S. Khochare, H. F. de Souza, B. Ahuja, V. Suranse, G. Martin, R. Whitaker, K. Sunagar, PLoS Negl. Trop. Dis. **13**, 12 (2019)
38. J. van Thiel, L. L. Alonso, J. Slagboom, N. Dunstan, R. M. Wouters, C. M. Modahl, F. J. Vonk, T. N. W. Jackson, J. Kool, Toxins. **15**, 1 (2023)
39. B. Lomonte, W. C. Tsai, J. M. Ureña-Díaz, L. Sanz, D. Mora-Obando, E. E. Sánchez, B. G. Fry, J. M. Gutiérrez, H. L. Gibbs, M. G. Sovic, J. J. Calvete, J. Proteomics. **96** (2014)
40. D. R. Amazonas, J. A. Portes-Junior, M. Y. Nishiyama-Jr, C. A. Nicolau, H. M. Chalkidis, R. H. V. Mourão, F. G. Grazziotin, D. R. Rokyta, H. L. Gibbs, R. H. Valente, I. L. M. Junqueira-de-Azevedo, A. M. Moura-da-Silva, J. Proteomics. **181** (2018)
41. G. Zancolli, J. J. Calvete, M. D. Cardwell, H. W. Greene, W. K. Hayes, M. J. Hegarty, H. W. Herrmann, A. T. Holycross, D. I. Lannutti, J. F. Mulley, L. Sanz, Z. D. Travis, J. R. Whorley, C. E. Wüster, W. Wüster, Proc. Royal Soc. B P ROY SOC B-BIOL SCI. **286**, 1898 (2019)
42. T. N. W. Jackson, I. Koludarov, S. A. Ali, J. Dobson, C. N. Zdenek, D. Dashevsky, B. Op den Brouw, P. P. Masci, A. Nouwens, P. Josh, J. Goldenberg, V. Cipriani, C. Hay, I. Hendrikx, N. Dunstan, L. Allen, B. G. Fry, Toxins. **8**, 11 (2016)
43. C. F. Smith, Z. L. Nikolakis, K. N. Ivey, B. W. Perry, D. R. Schield, N. R. Balchan, J. Parker, K. C. Hansen, A. J. Saviola, T. A. Castoe, S. P. Mackessy, BMC Biol. **21**, 1 (2023)
44. R. R. S. Laxme, S. Khochare, S. Attarde, V. Suranse, A. Iyer, N. R. Casewell, R. Whitaker, G. Martin, K. Sunagar, PLoS Negl. Trop. Dis. **15**, 3 (2021)
45. R. R. de Castañeda, I. Bolon, J. M. A. Gutiérrez, Toxicon. **13** (2022)
46. A. Malhotra, W. Wüster, J. B. Owens, C. W. Hodges, A. Jesudasan, A.; C. Gnaneswar, A. Kartik, P. Christopher, J. Louies, H. Naik, V. Santra, S. R. Kuttalam, S. Attre, M. Sasa, C. Bravo-Vega, K. A. Murray, Toxicon. **12** (2021)