Neurotoxicity Research
https://doi.org/10.1007/s12640-021-00413-2

ORIGINAL ARTICLE

Novel Neurotoxic Activity in *Calliophis intestinalis* Venom

Daniel Dashevsky[1,2] · Jennifer R. Deuis[3] · Irina Vetter[3,4] · Tam Huynh[5] · Wayne C. Hodgson[5] · Choo Hock Tan[6] · Amanda Nouwens[7] · Bryan G. Fry[1]

Received: 6 April 2021 / Revised: 6 August 2021 / Accepted: 2 September 2021

Abstract

In this work, we investigated the in vitro neurotoxicity of *Calliophis intestinalis* venom using chick biventer cervicis neuromuscular preparations and electrophysiological analysis of voltage-gated sodium (Na_V) channels expressed in HEK293 cells. We found that the indirect twitches of the neuromuscular preparations decreased over time when exposed to venom. However, the responses of these preparations to the agonists acetylcholine, carbachol, and potassium chloride were not changed after incubation with the venom. Our electrophysiological experiments show that *C. intestinalis* venom acts as a Na_V channel antagonist—the first known from a vertebrate venom—by decreasing the peak current of $Na_V1.4$ channels without changing the kinetics of activation or inactivation. Our proteomic results accord with earlier analyses and find that the venom contains three-finger toxins, cysteine-rich secretory proteins, kunitz peptides, phospholipase A_2s, snake venom metalloproteases, and vespryns. Some of the three-finger toxins are similar to the δ-elapitoxins from the venom of the closely related *Calliophis bivirgatus*. However, δ-elapitoxins act as Na_V channel agonists in *C. bivirgatus* whereas *C. intestinalis* venom contains Na_V channel antagonists. The toxins and mechanisms responsible for the neuromuscular symptoms remain unclear as does the identity of the Na_V channel antagonists. These aspects of this unusual venom require further study.

Keywords Elapid · Coral snake · Patch clamp · Venomics · Snakebite

Introduction

Of the three families of front-fanged snakes, Elapidae is the most speciose (Uetz et al. 2020) and elapids—especially *Naja* (cobras) and *Bungarus* (kraits)—are responsible for a large proportion of snakebites in Africa and Asia (Kasturiratne et al. 2008; Warrell 2010; Gutiérrez et al. 2017). The most basal extant branch of the family is the genus *Calliophis*, sometimes referred to as Asian coral snakes (Figueroa et al. 2016; Zheng and Wiens 2016; Brown et al. 2018). Within the genus, a distinction is made between the eight short-glanded species which tend to be small, possess brightly colored undersides, and whose bites are fairly innocuous, and the seven long-glanded species which are much larger, often have striking dorsal coloration, with venom glands which can be up to one-third the total length of the body (Bernhard-Meyer 1869; Leviton 1963; Slowinski et al. 2001; Brown et al. 2018; Uetz et al. 2020); the long-glanded species *C. bivirgatus* is responsible for the only documented death from the genus (Institute of Medical Research 1956; Harrison 1957). The unusual glands of the long-glanded *Calliophis* are not the only oddities of their venom system; the toxins they produce are unusual as well (Takasaki et al. 1991). A three-finger toxin (3FTx) known as calliotoxin—the first described δ-elapitoxin and the only known vertebrate toxin which delays the inactivation of voltage-gated sodium (Na_V) channels—was discovered in

✉ Bryan G. Fry
bgfry@uq.edu.au

1 Venom Evolution Lab, School of Biological Sciences, University of Queensland, St Lucia, QLD 4072 Australia

2 Present address: Australian National Insect Collection, Commonwealth Science and Industry Research Organization, Canberra, ACT 2601 Australia

3 Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD 4072, Australia

4 School of Pharmacy, The University of Queensland, Woolloongabba, QLD 4102, Australia

5 Department of Pharmacology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia

6 Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

7 School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia, QLD 4072, Australia

the venom of *C. bivirgatus* (Yang et al. 2016). Rather than the usual flaccid paralysis produced by elapid neurotoxins, these δ-elapitoxins rapidly cause involuntary contractions of the skeletal muscles (Yang et al. 2016; Tan et al. 2016).

Previous research on the proteomic composition of *C. bivirgatus* and *C. intestinalis* venom indicated that 3FTx similar to known cytotoxins from other elapids are a major component of these venoms (Tan et al. 2016; Tan et al. 2019). However, a recent molecular phylogeny of 3FTx from *C. bivirgatus* and other elapids indicates that, despite the sequence similarity, some of the clades of *Calliophis* toxins near the cytotoxins are not cytotoxic at all, but are instead neurotoxic (Dashevsky et al. 2021). This includes the previously described maticotoxins and δ-elapitoxins (Takasaki et al. 1991; Yang et al. 2016). This leaves the possible function of the "cytotoxins" identified by Tan et al. (2019) somewhat unclear. While the bottom-up proteomics that produced these results are not optimal for quantifying the relative abundance of toxins in the overall composition of the venom (Walker et al. 2020; Calvete et al. 2021), they do give us a rough picture of the dominant toxin families. In particular, *C. intestinalis* venom is primarily be composed of phospholipase A2 (PLA2), 3FTx, and kunitz toxins along with minor presence from other toxin families (Tan et al. 2019).

The same research into the proteomics of the venom also tested its in vitro and in vivo activity and found evidence for weak neurotoxicity in geckos and frogs as well as cytotoxic activity. As Tan et al. (2019) noted, this accords with the majority of bite reports which include pain, swelling, and occasional mild neurotoxicity (Meers 1968; Tan et al. 2020). However, an older report indicates that significant systemic neurotoxic symptoms can also occur (Jacobson 1937). Populations of *C. intestinalis* have recently been raised to full species status including *C. bilineata*, *C. nigrotaeniatus*, *C. phillipina*, and *C. suluensis* (Fukuyama et al. 2020), but all the published reports of bites have most likely involved *C. intestinalis* (based on the location of the incidents). This variation in local and systemic manifestations of envenomation—and in the severity of neurotoxicity—may be indicative of further cryptic species or intraspecific venom variation. Despite the uncertainty of these details, all available evidence indicates that the in vitro and in vivo effects of *C. intestinalis* venom are entirely distinct from those of *C. bivirgatus*.

We have previously speculated that one possible explanation for the evolution of the unusually long glands in *Calliophis* might be related to the evolution of the δ-elapitoxins and their unique Na_V toxicity (Yang et al. 2016; Dashevsky et al. 2021). If δ-elapitoxins conferred a selective advantage upon ancestral snakes, but were low potency then there could be evolutionary pressure for these lineages to increase their venom yields. However, since these lineages live in fossorial or otherwise cluttered habitats, it would be to their disadvantage if enlarged venoms glands made their heads larger. Extremely elongated glands could potentially have potentially arisen as a solution that could satisfy both of these competing demands. However, this idea is challenged by the divergent symptoms produced by *C. intestinalis* and *C. bivirgatus* which both possess the long glands. We investigated the in vitro neurotoxic activity of *C. intestinalis* venom and proteomics results combined with a *C. bivirgatus* transcriptome to clarify some of these uncertainties.

Results and Discussion

We used in vitro experiments to compare the biological activity of *C. intestinalis* venom to the published activity of *C. bivirgatus*, which we have previously reported to cause spastic paralysis by inhibiting fast inactivation of $Na_V1.4$ channels (Yang et al. 2016). However, isolated chick biventer cervicis nerve-muscle (CBCNM) preparations that were exposed to *C. intestinalis* venom notably decreased their response to electrical stimulation of the motor neuron compared to controls (Fig. 1A). We also studied the response of CBCNM preparations to various postsynaptic agonists in the presence and absence of *C. intestinalis* venom. ANOVA tests indicated that the venom did not significantly decrease the response of the muscles to acetylcholine ($p > 0.31$), carbachol ($p > 0.27$), or potassium chloride ($p > 0.89$). This suggests that the venom has largely lost its ancestral α-neurotoxicity (i.e., post-synaptic activity) much like *C. bivirgatus* has (Yang et al. 2016), and that there was no evidence of myotoxicity at the concentrations we tested. Not only are these results distinct from those produced by *C. bivirgatus*, but they are also quite different from those produced by most other neurotoxic elapids (Liang et al. 2020).

To investigate the potential mechanism behind the somewhat unusual results of our CBCNM assay, we used more specific electrophysiological methods. In whole-cell patch-clamp experiments on HEK293 cells expressing $Na_V1.4$, we observed activity of *C. intestinalis* venom on voltage-evoked currents. Interestingly, venom from *C. intestinalis* (0.33 mg/mL) inhibited $Na_V1.4$ peak current, but, unlike calliotoxin isolated from *C. bivirgatus* venom, caused no significant delay in the kinetics of fast inactivation (Fig. 2 A, B). In addition, *C. intestinalis* venom had no significant effect on the voltage dependence of activation (V_{50}: control, −28.6±1.3; venom, −27.5±1.9) or voltage dependence of fast inactivation (V_{50}: control, −64.5±2.1; venom, −68.3±1.9), suggesting the venom contains inhibitor(s)

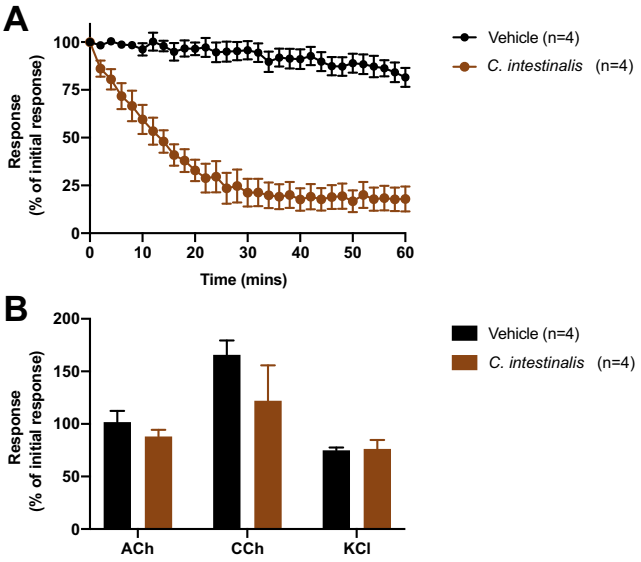

Fig. 1 Skeletal muscle paralysis caused by *C. intestinalis* venom. **A** Time-dependent decrease in twitches of indirectly stimulated CBC preparations incubated with venom (10 µg/mL). Vehicle control preparations were incubated with physiological solution alone (each time point is plotted as the mean ± SEM across the replicates). **B** Contractile responses to the nicotinic cholinergic receptor agonists acetylcholine (ACh, 1 mM) and carbachol (CCh, 20 uM), and to potassium chloride (KCl, 40 mM) were obtained before and after a 60-min incubation with venom or vehicle, with the responses after venom being expressed as a percentage of those before the incubation (taken as 100%). Bar charts show the mean ± SEM across the replicates. Differences between vehicle and venom were non-significant for all three agonists (ANOVA, $p > 0.31$, 0.27, and 0.89 for ACh, CCh, and KCl, respectively)

of voltage-gated sodium channels, without affecting the kinetics of channel activation/inactivation.

We explored the proteomics of *C. intestinalis* venom using shotgun MS/MS (see Supplementary Data) and the *C. bivirgatus* transcriptome as a reference database. While analyzing whole venom proteomics and using a database from a separate species are not ideal (Walker et al. 2020; Calvete et al. 2021), we were able to obtain some results indicating the presence of a variety of toxins including diverse 3FTx (including some that were highly similar to δ-elapitoxins), CRiSP, kunitz, PLA2, SVMP, and vespryn. Without a venom gland transcriptome from the same species (preferably the same individual) that the venom was sourced from, we cannot be confident in the exact sequence of these toxins and without combining both bottom-up and top-down proteomics techniques we cannot reliably estimate the relative abundance of these different toxins.

While δ-elapitoxins in *C. bivirgatus* venom produce spastic paralysis, blockage of Na_V channels like we observe in our electrophysiology experiments could possibly lead to the lowering of muscle tension we observed in our CBCNM results. However, a number of other possible explanations would also be consistent with these findings. We know from previous results and our own analyses that PLA2 enzymes are present in the venom and make up a significant portion of the total protein content (Tan et al. 2019). Other elapid lineages have evolved presynaptic neurotoxic PLA2s which inhibit the release of acetylcholine (Sunagar et al. 2015a) and could decrease a muscle's response to indirect stimulation without changing its ability to respond to direct application of acetylcholine or carbachol. Similarly, other elapids have evolved toxins which target ion channels from other families—such as kunitz or CRiSP toxins—which are found in *C. intestinalis* venom (Sunagar et al. 2015b; Eng et al. 2015).

Further research will also be necessary to clarify the site of action as well as the toxin responsible. While the blockade of $Na_V1.4$ alone could lead to muscle relaxation like we observe in the CBCNM experiments, there are

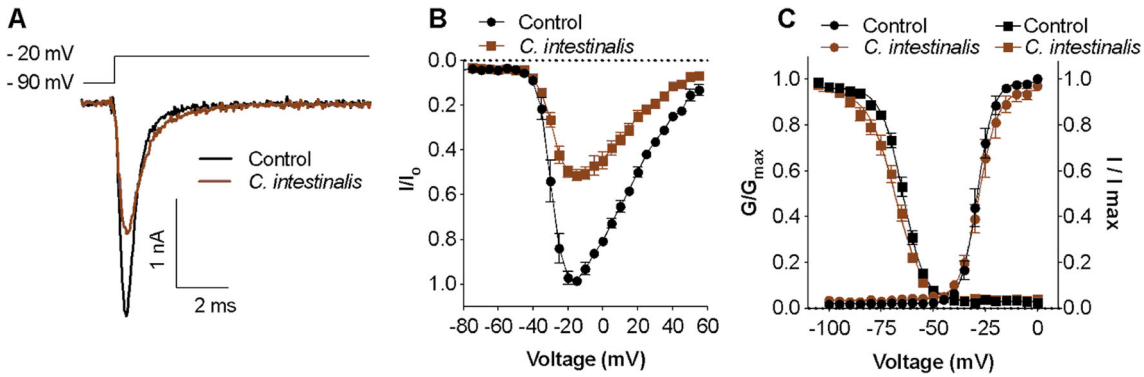

Fig. 2 *Calliophis intestinalis* venom (0.33 mg/mL) blocks $hNav_V1.4$ channels expressed in HEK293 cells without affecting channel kinetics. Sodium currents were measured by whole-cell patch-clamp electrophysiology. **A** Representative current trace before and after addition of *C. intestinalis* venom. Current was elicited by depolarization (50 ms pulse) from −90 to −20 mV and was measured 5 min after venom addition; **B** I-V relationship before and after addition of *C. intestinalis* venom; **C** conductance-voltage curve (circles) and voltage dependence of steady-state fast inactivation (squares) before and after addition of *C. intestinalis* venom. Data are presented as the mean ± SEM, with $n = 5$ cells per data point

a number of presynaptic, postsynaptic, and intracellular targets which could lead to similar symptoms if inhibited. Experiments such as the direct stimulation of curarized CBCNM preparations can help narrow down where the venom is acting.

Even if our hypothesis about δ-elapitoxin homologues inhibiting Na_V channels is correct, a number of questions will remain to be clarified by future research. Our data here do not address which subtypes of Na_V this venom affects or at what potency. Our electrophysiology demonstrates that they inhibit the skeletal muscle subtype ($Na_V1.4$), but the venom may also bind to other subtypes as well, perhaps even more dramatically. More concentrations will also need to be tested to assess the affinity of the toxins in question for their targets and whether the symptoms produced by the whole venom might change in concentration. Some scorpion venoms, for instance, display the paradoxical behavior of enhancing neurotransmitter release at low concentrations but blocking nerve conduction at higher concentrations due to lower potency toxins that preempt the activity seen at lower concentrations (Rita de Cássia et al. 2019). Tests at multiple concentrations will not only improve our understanding of the toxic activity, but will increase our confidence in the results, and better allow us to compare results across different assays.

Conclusions

Bite reports from the two species and in vivo data suggest that the mechanisms of toxicity are quite distinct between the venoms of *C. intestinalis* and its close relative *C. bivirgatus*; our assays confirm this suggestion using in vitro techniques to characterize the neurotoxicity of *C. intestinalis* venom. Specifically, our results show that the venom of *C. intestinalis* causes flaccid paralysis rather than spastic paralysis. Whereas *C. bivirgatus* causes spontaneous contractions, *C. intestinalis* reduced the contractile responses of muscle twitches elicited via electrical stimulation of the motor neuron. However, further assays using the nerve-muscle preparation showed that it did not decrease the response to the post-synaptic agonists acetycholine and carbachol—both of which activate the nicotinic acetylcholine receptors, targets of classic elapid α-neurotoxins—or potassium chloride which would indicate damage to the muscle cells. Electrophysiological measurements in HEK293 cells transfected with skeletal muscle voltage-gated sodium channels ($Na_V1.4$) showed that the venom is able to decrease the peak current of these channels but does not change the kinetics of their activity. This is in contrast to *C. bivirgatus* which delays the inactivation of these same channels. These different actions on sodium channels are one potential explanation for the divergent symptoms we observed in the whole muscle. This is the first vertebrate venom which has been demonstrated to inhibit $Na_V1.4$ channels. Based on the presence of the 3FTxs resembling δ-elapitoxins in *C. intestinalis* venom and its novel Na_V activity, it is possible that these toxins are responsible for the Na_V channel blockage and the effects on nerve-muscle preparations, but there is much research yet needed to clarify the mechanisms behind the effects demonstrated here and to isolate and identify the toxins responsible.

Methods

C. intestinalis venom was provided by Dr. Choo Hock Tan of the University of Malaysia. Venom was imported under Australian Quarantine and Inspection Service permit 0001804439 and subsequent work was undertaken under University of Queensland IBSC Approval #IBC134BSBS2015.

Mass Spectrometry

Venom samples were prepared and analyzed as previously described by Dashevsky et al. (2021). Briefly, 20 µg of *C. intestinalis* venom was reduced, alkylated, and trypsinized. These samples were then analyzed on a Shimadzu Prominence nanoLC system which eluted directly into a TripleTof 5600 instrument (ABSciex) using a Nanospray III interface. Data was searched in ProteinPilot 5.0.2 using the same custom reference database drawn from a *Calliophis bivirgatus* venom gland transcriptome (Dashevsky et al. 2021), all reviewed elapid sequences from UniProt, and a database of common proteomics contaminants (Mellacheruvu et al. 2013; Consortium 2017).

$Na_V1.4$ Whole-Cell Patch-Clamp Electrophysiology

Whole-cell patch-clamp recordings were performed on $hNa_V1.4/\beta1$ heterologously expressed in HEK cells (SB Drug Discovery, Glasgow, UK) using a QPatch-16 automated electrophysiology platform (Sophion Bioscience, Ballerup, Denmark) as previously described (Tay et al. 2019). The extracellular solution contained in mM: NaCl 70, choline chloride 70, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10 and glucose 10; pH 7.4; osmolarity 305 mOsm. The intracellular solution contained in mM: CsF 140, EGTA/CsOH 1/5, HEPES 10 and NaCl 10; pH 7.3 with CsOH; osmolarity 320 mOsm.

Venom from *C. intestinalis* (0.33 mg/mL) was diluted in extracellular solution with 0.1% BSA. Current-voltage

(I-V) curves were obtained with a holding potential of −90 mV followed by a series of 500 ms step pulses that ranged from −110 to +55 mV in 5-mV increments (repetition interval 5 s) before and after a 5-min incubation with venom. Conductance-voltage curves were obtained by calculating the conductance (G) at each voltage (V) using the equation $G = I/(V - V_{rev})$, where V_{rev} is the reversal potential and were fitted with a Boltzmann equation. Voltage dependence of steady-state fast inactivation was assessed using a 10-ms pulse of −20 mV immediately after the 500-ms step (described above) to assess the availability of non-inactivated channels.

Isolated Chick Biventer Cervicis Nerve-Muscle Preparation

Chicks aged 4 to 10 days were euthanized with CO_2. After dissection, the biventer cervicis nerve muscle (CBCNM) preparations were mounted under 1-g tension in 5-mL organ baths containing physiological salt solution (NaCl, 118.4 mM; KCl, 4.5 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; $NaHCO_3$, 25 mM; and glucose, 11.1 mM). Organ baths were maintained at 34 °C and bubbled with carbogen (95% O_2; 5% CO_2). Electrodes were placed around the tendon of the biventer muscle and electrical stimulation applied to the motor nerve (0.2 ms duration, 0.1 Hz, supramaximal V) using a Grass S88 stimulator (Grass Instruments, Quincy, MA, USA) evoked indirect twitches. Selective stimulation of the nerve was confirmed by the abolition of twitches with d-tubocurarine (10 µM), a nicotinic acetylcholine receptor (nAChR) competitive antagonist. Tissues were then washed repeatedly with physiological salt solution to restore twitch responses to nerve stimulation. The stimulation was ceased, and the contractile responses to acetylcholine (ACh, 1 mM for 30 s), carbachol (CCh, 20 µM for 60 s), and potassium chloride (KCl, 40 mM for 30 s) were obtained and recorded. The organ bath was then washed, and electrical stimulation was resumed and maintained for 30 min to allow the preparation to equilibrate. Venom (10 µg/mL) was added to the organ bath and the twitch height was recorded for up to 1 h after venom addition. The stimulator was turned off again and the bath was washed. Contractile responses to ACh, CCh, and KCl were obtained again to compare with responses prior to venom addition. The twitch responses to electrical stimulations and contractile responses to agonists (ACh, CCh, and KCI) were measured using a Grass FT03 force displacement transducer (Grass Instruments, Quincy, MA, USA) and recorded on a PowerLab system (ADInstruments Pty Ltd., Bella Vista, NSW, Australia).

Statistical Analysis

Statistical analysis was conducted using R version 4.1.0 (R Core Team 2021).

Funding DD was funded by a UQ Centennial Scholarship from The University of Queensland, a Research Training Program scholarship from the Australian Government Department of Education and Training, and a CSIRO Early Research Career Postdoctoral Fellowship from the Commonwealth Science & Industry Research Organisation. J.R.D and I.V. are supported by NHMRC fellowships APP1162503 and APP1139961 (J.R.D.). T.M.H. was supported by a PhD scholarship funded from an Australian National Health and Medical Research Council (NHMRC) Centres for Research Excellence Grant (ID:1110343). BGF was funded by Australian Research Council Grant DP210102406.

Data Availability All data are included as supplementary materials along with this publication.

Declarations

Ethics approval Chick biventer cervicis experiments were carried out under Monash University Animal Ethics Committee application number 2257, approved on 18 December 2019.

Conflict of interest The authors declare no competing interests.

References

Bernhard-Meyer A (1869) The poison-glands of *Callophis intestinalis and C. bivirgatus*. Ann Mag Nat History 4(19-24):74

Brown RM, Smart U, Leviton AE, Smith EN (2018) A new species of long-glanded coralsnake of the genus *Calliophis* (squamata: Elapidae) from Dinagat Island, with notes on the biogeography and species diversity of Philippine *Calliophis* and *Hemibungarus*. Herpetologica 74(1):89–104. https://doi.org/10.1655/Herpetologica-D-17-00008

Calvete JJ, Lomonte B, Saviola AJ, Bonilla F, Sasa M, Williams DJ, Undheim EAB, Sunagar K, Jackson TNW (2021) Mutual enlightenment: a toolbox of concepts and methods for integrating evolutionary and clinical toxinology via snake venomics and the contextual stance. Toxicon: X 9–10:100070. https://doi.org/10.1016/j.toxcx.2021.100070

Consortium TU (2017) UniProt: the universal protein knowledgebase. Nucleic Acids Res 45(D1):D158–D169. https://doi.org/10.1093/nar/gkw1099

Dashevsky D, Rokyta D, Frank N, Nouwens A, Fry BG (2021) Electric blue: molecular evolution of three-finger toxins in the long-glanded coral snake species *Calliophis bivirgatus*. Toxins 13(2):124. https://doi.org/10.3390/toxins13020124

Eng WS, Fry BG, Sunagar K, Takacs Z, Jackson TNW, Guddat LW (2015) Kunitz peptides. In: Fry BG (ed) Venomous reptiles & their toxins: evolution, pathophysiology and biodiscovery. Oxford University Press, New York, pp 281–290

Figueroa A, McKelvy AD, Grismer LL, Bell CD, Lailvaux SP (2016) A species-level phylogeny of extant snakes with description of a

new Colubrid subfamily and genus. PLos One 11(9):e0161070. https://doi.org/10.1371/journal.pone.0161070
Fukuyama I, Vogel G, Matsui M, Eto K, Munir M, Hossman MY, Hamidy A, Nishikawa K (2020) Systematics of *Calliophis intestinalis* with the resurrection of *Calliophis nigrotaeniatus* (Elapidae, Serpentes). Zoolog Sci 37(6). https://doi.org/10.2108/zs200100
Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA (2017) Snakebite envenoming. Nat Rev Dis Primers 3(1):1–21. https://doi.org/10.1038/nrdp.2017.63
Harrison JL (1957) The bite of a blue Malaysian coral snake or Ular Matahari. Malayan Nat J 11:130–2
Institute of Medical Research (1956) A death from snake bite. pp 71–72
Jacobson F (1937) A case of snake bite (*Maticora intestinalis*). Bull Raffles Museum 13:77
Kasturiratne A, Wickremasinghe AR, Nd Silva, Gunawardena NK, Pathmeswaran A, Premaratna R, Savioli L, Lalloo DG, HJd Silva (2008) The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLOS Med 5(11):e218. https://doi.org/10.1371/journal.pmed.0050218
Leviton AE (1963) Contributions to a review of Philippine snakes, III. The genera *Maticora* and *Calliophis*. Philipp J Sci 92(4):523–550
Liang Q, Huynh TM, Konstantakopoulos N, Isbister GK, Hodgson WC (2020) An examination of the neutralization of in vitro toxicity of Chinese cobra (Naja atra) venom by different antivenoms. Biomedicines 8(10):377. https://doi.org/10.3390/biomedicines8100377
Meers PD (1968) Snake bite by the malayan coral snake: *Maticora intestinalis*. J R Army Med Corps 114(3):152–153. https://doi.org/10.1136/jramc-114-03-10
Mellacheruvu D, Wright Z, Couzens AL, Lambert JP, St-Denis N, Li T, Miteva YV, Hauri S, Sardiu ME, Low TY, Halim VA, Bagshaw RD, Hubner NC, al Hakim A, Bouchard A, Faubert D, Fermin D, Dunham WH, Goudreault M, Lin ZY, Badillo BG, Pawson T, Durocher D, Coulombe B, Aebersold R, Superti-Furga G, Colinge J, Heck AJR, Choi H, Gstaiger M, Mohammed S, Cristea IM, Bennett KL, Washburn MP, Raught B, Ewing RM, Gingras AC, Nesvizhskii AI (2013) The CRAPome: a contaminant repository for affinity purification mass spectrometry data. Nat Methods 10(8):730–736. https://doi.org/10.1038/nmeth.2557
Rita de Cássia OC, Hyslop S, Dorce VA, Antunes E, Rowan EG (2019) Scorpion venom increases acetylcholine release by prolonging the duration of somatic nerve action potentials . Neuropharmacology 153:41–52. https://doi.org/10.1016/j.neuropharm.2019.04.013
R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org
Slowinski JB, Boundy J, Lawson R (2001) The phylogenetic relationships of asian coral snakes (elapidae: *Calliophis* and *Maticora)* based on morphological and molecular characters. Herpetologica 57(2):233–245
Sunagar K, Jackson TNW, Reeks T, Fry BG (2015a) Group I phospholipase A2 enzymes. In: Fry BG (ed) Venomous reptiles & their toxins: evolution, pathophysiology and biodiscovery. Oxford University Press, New York
Sunagar K, Jackson TNW, Reeks T, Fry BG, Fry BG (2015b) Cysteine-rich secretory proteins. In: Fry BG (ed) Venomous reptiles & their toxins: evolution, pathophysiology and biodiscovery. Oxford University Press, New York, pp 239–246
Takasaki C, Yoshida H, Shimazu T, Teruuchi T, Toriba M, Tamiya N (1991) Studies on the venom components of the long-glanded coral snake, *Maticora bivirgata*. Toxicon 29(2):191–200. https://doi.org/10.1016/0041-0101(91)90103-X
Tan CH, Fung SY, Yap MKK, Leong PK, Liew JL, Tan NH (2016) Unveiling the elusive and exotic: venomics of the Malayan blue coral snake (*Calliophis bivirgata flaviceps*). J Proteomics 132: 1–12
Tan KY, Liew JL, Tan NH, Quah ES, Ismail AK, Tan CH (2019) Unlocking the secrets of banded coral snake (*Calliophis intestinalis*, Malaysia): a venom with proteome novelty, low toxicity and distinct antigenicity. J Proteomics 192:246–257. https://doi.org/10.1016/j.jprot.2018.09.006
Tan MT, Ab Razak MN, Ismail AK (2020) Does the legend speaks the truth? Revisiting the venom profile and the effect of *Calliophis intestinalis* bite to human
Tay B, Stewart TA, Davis FM, Deuis JR, Vetter I (2019) Development of a high-throughput fluorescent no-wash sodium influx assay. PLos One 14(3):e0213751. https://doi.org/10.1371/journal.pone.0213751
Uetz P, Freed P, Hošek J (2020) The reptile database. https://www.reptile-database.org
Walker AA, Robinson SD, Hamilton BF, Undheim EAB, King GF (2020) Deadly proteomes: a practical guide to proteotranscriptomics of animal venoms. Proteomics 20(17-18):1900324. https://doi.org/10.1002/pmic.201900324
Warrell DA (2010) Snake bite. The Lancet 375(9708):77–88. https://doi.org/10.1016/S0140-6736(09)61754-2
Yang DC, Deuis JR, Dashevsky D, Dobson J, Jackson TNW, Brust A, Xie B, Koludarov I, Debono J, Hendrikx I, Hodgson WC, Josh P, Nouwens A, Baillie GJ, Bruxner TJC, Alewood PF, Lim KKP, Frank N, Vetter I, Fry BG (2016) The snake with the scorpion's sting: novel three-finger toxin sodium channel activators from the venom of the long-glanded blue coral snake (*Calliophis bivirgatus*). Toxins 8(10):303. https://doi.org/10.3390/toxins8100303
Zheng Y, Wiens JJ (2016) Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. Mol Phylogenet Evol 94:537–547. https://doi.org/10.1016/j.ympev.2015.10.009