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ORIGINAL ARTICLE

The Bold and the Beautiful: a Neurotoxicity Comparison of New World Coral Snakes in the *Micruroides* and *Micrurus* Genera and Relative Neutralization by Antivenom

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Abstract Coral snake envenomations are well characterized to be lethally neurotoxic. Despite this, few multispecies, neurotoxicity and antivenom efficacy comparisons have been undertaken and only for the *Micrurus* genus; *Micruroides* has remained entirely uninvestigated. As the USA's supplier of antivenom has currently stopped production, alternative sources need to be explored. The Mexican manufacturer Bioclon uses species genetically related to USA species, thus we investigated the efficacy against *Micrurus fulvius* (eastern coral snake), the main species responsible for lethal envenomations in the USA as well as additional species from the Americas. The use of Coralmyn® coral snake antivenom was effective in neutralizing the neurotoxic effects exhibited by the venom of *M. fulvius* but was ineffective against the venoms of *Micrurus tener*, *Micrurus spixii*, *Micrurus pyrrhocryptus*, and *Micruroides euryxanthus*. Our results suggest that the Mexican antivenom may be clinically useful for the treatment of *M. fulvius* in the USA but may be of only limited efficacy against the other species studied.

Keywords Venom · Neurotoxicity · Antivenom · Micrurus · Micruroides · Coral snake

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Introduction

The New World coral snakes consist of three genera: *Leptomicrurus* (five species), *Micruroides* (one species), and *Micrurus* (approx. 70 species) (Roze 1996). The two less speciose genera are restricted in their geographic range; *Leptomicrurus* is only found in close proximity to the Amazon basin (Campbell and Lamar 1989) while *Micruroides* is restricted in the United States to the southern half of Arizona and extreme southeastern New Mexico, south into the northwestern Mexican states of Sonora, extreme southwestern Chihuahua, and Sinaloa, with an insular population occurring on Isla Tiburón (Campbell and Lamar 2004). In contrast, snakes of the genus *Micrurus* distribution ranges from the southeastern USA to Argentina (Jorge da Silva and Sites 2001).

Bites from most of these snakes, with the exception of the very small *Micruroides*, are considered potentially life-threatening due to deaths reported from species across their geographical range (Bucaretchi et al. 2016; Manock et al. 2008; Morgan et al. 2007). The venom is considered to be

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primarily neurotoxic, and the most common cause of death is paralysis-induced hypoxia (Vital-Brazil 1987) highlighting the importance for each country that contains coral snakes in having access to antivenoms capable of neutralizing the neurotoxic effects of coral snake envenomation. Currently, the most effective treatment for coral snake envenomations is the administration of specific antivenom coupled with direct treatment of symptoms such as pre-synaptic neurotoxicity not neutralized by antivenom (Peterson 2006). As antivenoms are produced by inoculating livestock (generally sheep or horses) against venom from a particular species or selection of species and purifying the resulting antibodies (Angulo et al. 1997; Rojas et al. 1994), antivenoms can be partially or wholly ineffective against the venom from species not included in production (Tanaka et al. 2010; Boyer et al. 2015). Nonetheless, experimental (Bolanos et al. 1975; Silva et al. 2016) as well as clinical evidence of cross-neutralization has been reported even for non-related snake species (Isbister et al. 2014).

The reason antivenoms sometimes fail when treating bites from coral snake species, whose venom was not included in the production of antivenom, has recently become more salient as studies have revealed that the variation in coral snake venoms is deeper and more widespread than previously presumed. In particular, Aird and Jorge da Silva (1991) provided evidence based on enzymatic activities that some coral snake venoms more closely resemble the venoms of more distantly related snakes such as *Bungarus*, *Bothrops*, or *Naja* than other coral snakes. Proteomic and transcriptomic analyses of coral snakes from across the Americas indicate that, broadly speaking, some coral snake venoms contain three-finger toxins (3FTx) as their most abundant components while others are composed primarily of phospholipase A_2 (PLA_2) (Fernandez et al. 2015; Rey-Suarez et al. 2016, Vergara et al. 2014; Lomonte et al. 2016a).

There are currently four antivenoms primarily used in the treatment of New World coral snake bites (Table 1). The production of North American coral snake antivenom (NACSA) was halted in 2008, and remaining stocks have diminished while repeatedly having their effectiveness tested and expiry dates extended (Wood et al. 2013); a single lot was released in 2016 for limited distribution in the USA (Supplementary File 1). The general shortage of coral snake antivenom in the USA has led to the proposal that the Mexican Coralmyn® coral snake antivenom (Bioclon, Mexico) be used in its place. Due to the diversity of coral snakes in each country where they are found being greater than the diversity used in production, testing the cross reactivity of antivenom on species that were not part of its manufacture remains medically important.

The use of commercially available coral snake antivenoms is effective in neutralizing the lethal doses (LD_{50}) of *Micrurus fulvius* and *Micrurus tener* venoms (Sánchez et al. 2008). Sánchez et al. (2008) tested the efficacy of two types of commercially available coral snake antivenom, North American coral snake antivenom (NACSA; Wyeth, United States) and Coralmyn® coral snake antivenom (Bioclon, Mexico), in order to ascertain their ability to neutralize the lethal components in both *M. fulvius* and *M. tener*, two American coral snake species. While the use of Coralmyn® was effective in neutralizing the LD_{50} doses of both *M. fulvius* and *M. tener* venoms, the use of NACSA was ineffective in neutralizing the lethality of three varying doses of venom from *M. tener*. In South America, a study by Camargo et al. (2011) investigated the efficacy of coral snake antivenom against the venom of *Micrurus pyrrhocryptus*. It was shown that both the commercial coral snake antivenom (Instituto Butantan, Brazil) and the specific antivenom raised against the venom of *M. pyrrhocryptus* were effective in neutralizing its neurotoxic effects. However, it remained to be seen whether Coralmyn® coral snake antivenom, which was raised against *Micrurus nigrocinctus nigrocinctus* (black-banded coral snake) venom, would be effective against *M. pyrrhocryptus* venom. Moreover, as the use of Coralmyn® was effective against the venoms of *M. fulvius* and *M. tener* in a murine model, it was hypothesized that the antivenom would be equally as effective in an in vitro preparation. The current study is of clinical significance as the production of NACSA was discontinued by Wyeth in 2008 (Wood et al. 2013) and, thus, it is imperative that the efficacy of other coral snake antivenom is determined.

Historically, murine LD_{50} studies have been performed in order to determine relative neurotoxicity of venoms (WHO 2010). In some circumstances, however, these can be supplemented by in vitro studies such as that of the chick biventer cervicis nerve-muscle preparation (Hodgson and Wickramaratna 2002). Using this preparation, the neurotoxicity of venoms can be examined by observing the time in which the venom/toxins produces 90% inhibition of nerve-mediated twitches (t_{90} values). However, there may be extreme variation between the synapsid (mouse) and diapsid (bird) results if the venoms are evolutionarily shaped to be taxon-specific (Fry et al. 2003; Heyborne and Mackessy 2013; Lumsden et al. 2004a, b, Lumsden et al. 2005a; Utkin et al. 2015). Such specificity has been described in vivo for the venoms of *M. pyrrhocryptus* and *M. spixii* (Jorge da Silva and Aird 2001). There, they determined LD_{50} values of whole venoms on prey and non-prey models and showed that they are, respectively, six and two times more lethal to the snake *Lyophis typhlus* than to mice. The case of *M. tener*, on the other hand, appears to be somewhat different because, even though a few apparently snake specific toxins have been isolated from it, the whole venom has no proven specificity towards snakes (Bénard-Valle et al. 2014).

Chick biventer assays are sometimes utilized in predicting clinical efficacy of antivenoms; however, chick biventer assays are not an antivenom challenge as the antivenom is pre-incubated with the venom prior to the administration. Thus, efficacy under this protocol does not indicate clinical efficacy. However, inability of the antivenom to neutralize under such an ideal scenario is highly likely to be predictive of clinical failure.

Table 1 Currently available coral snake antivenoms

Name	Producer	Country of origin	Venoms used in production
North American coral snake antivenom (NACSA)	Wyeth/Pfizer	USA	*Micrurus fulvius*
Soro Antielapidico	Instituto Butantan	Brazil	*Micrurus corallinus* and *Micrurus frontalis*
Coralmyn	Instituto Bioclon	Mexico	*Micrurus nigrocinctus*
SAC-ICP	Instituto Clodomiro Picado	Costa Rica	*Micrurus nigrocinctus*

In the present study, we examined the neurotoxicity and antivenom cross-reactivity of venoms from *Micruroides euryxanthus*, *M. fulvius*, *M. pyrrhocryptus*, *Micrurus spixii*, and *M. tener* representing a broad phylogenetic diversity of coral snake species (Fig. 1). Of these species, the latter three are found in the USA. We used the Mexican coral snake antivenom, Coralmyn®, in our experiments as it is the most readily available substitute should the remaining supplies of NACSA prove insufficient. Given the phylogenetic distribution of PLA_2 and 3FTx dominated venoms, we would expect Coralmyn® to be more effective against *M. fulvius* and *M. tener* venoms than the other venoms. This is due to *M. fulvius* and *M. tener* belonging, along with *M. nigrocinctus* (the species used in the production of Coralmyn®), to the PLA_2-abundant group (Fernandez et al. 2015).

Methods

Venom Preparation and Storage

M. euryxanthus (AZ, USA collected by CC), *M. fulvius* (FL, USA supplied by Miami Serpentarium), *M. pyrrhocryptus* (IW Surinam captive specimen), *M. spixii* (Brazil, supplied by Miami Serpentarium), and *M. tener* (TX, USA, supplied by Miami Serpentarium) freeze-dried venoms were prepared in MilliQ-H_2O and stored at −80 °C until required.

Drugs and Chemicals

The following drugs and chemicals were used: acetylcholine chloride (Sigma), carbamylcholine chloride (Sigma), potassium chloride (Ajax Finechem), (+)-tubocurarine chloride (Sigma), and Coralmyn® coral snake antivenom (Bioclon, Mexico; Batch #B-2D-06/2004). Stock solutions of drugs were made up MilliQ-H_2O unless otherwise specified.

Neurotoxicity and Antivenom Studies

Male chicks (4–10 days) were sacrificed by CO_2 and exsanguination. Both chick biventer cervicis nerve-muscle preparations were isolated and mounted on wire tissue holders under 1-g resting tension in 5-ml organ baths containing physiological salt solution (NaCl, 118.4 mM; KCl, 4.7 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), maintained at 34 °C, and bubbled with 95% O_2/5% CO_2. Indirect twitches were evoked by electrical stimulation of the motor nerve (supramaximal voltage, 0.2 ms, 0.1 Hz) using a Grass S88 stimulator (Grass Instruments, Quincy, MA). D-Tubocurarine (10 μM) was added, and the subsequent abolition of twitches confirmed selective stimulation of the motor nerve, after which thorough washing with physiological salt solution was applied to re-establish twitches. In the absence of electrical stimulation, contractile responses to acetylcholine (ACh; 1 mM for 30 s), carbachol (CCh; 20 μM for 60 s), and potassium (KCl; 40 mM for 30 s) were obtained prior to the addition of the venoms and at the conclusion of the experiment. Venoms were left in contact with the preparation for a maximum of 3 h to test for slow developing effects. Efficacy of Coralmyn® coral snake antivenom (10 units/mL) was assessed via a 10-min pre-incubation in the organ bath.

Data Analysis and Statistics

Twitch tension was measured from the baseline in 2-min intervals. Responses were expressed as a percentage of twitch tension prior to the addition of the venom. Contractile responses to agonists obtained at the conclusion of the experiment were measured and expressed as a percentage of the response obtained prior to the addition of venom. The time taken to inhibit 90% of twitch contractions (i.e., t_{90}) was measured as a quantitative means of measuring neurotoxicity. Values for t_{90} were measured by the time elapsed to reach 10% twitch tension amplitude following addition of venom. Where indicated, a two-way analysis of variance (ANOVA) or a paired *t* test was used to determine statistical significance of responses. Statistical analysis was performed using the Prism 5 (GraphPad Software, San Diego, CA, USA) software package. Unless otherwise indicated, data are expressed as mean ± S.E.

Animal Ethics

All animal experiments used in this study were approved by the SOBS-B Monash University Animal Ethics Committee.

Phylogenetic Comparative Analyses

A phylogeny was assembled using Lee et al. 2016, as this is currently the most comprehensive phylogeny available for

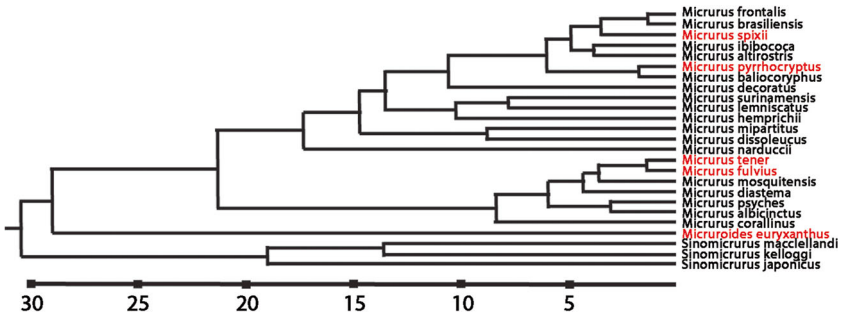

Fig. 1 Phylogenetic relationships of species studied and millions of years of separation (following Lee et al. 2016)

Micrurus and was used for all further analyses conducted in R v3.2.5 (R-Core-Team 2011) using the ape package (Paradis et al., 2004) for general handling of phylogenetic and trait data. Ancestral states were estimated and reconstructed over the tree in order to visualize the changes in neurotoxicity and antivenom efficacy across the phylogeny. Note that we only included five of 76 species of New World coral snakes (~7%) and these were widely dispersed in the phylogeny, so accurate estimates of ancestral states are unlikely to be estimated here. Nevertheless, such reconstructions still provide a visualization of the minimum amount of evolutionary change in these traits across the clade. The continuous functional traits were reconstructed by maximum likelihood in the contMap function in phytools (Revell 2012).

We then fit pGLS models (Symonds and Blomberg 2014) in caper (Orme et al. 2015) to test for relationships. We restricted the venom-antivenom model to a single explanatory variable, and we arcsine square-root-transformed the response variable to improve model fit (since the functional activity values are bound between 0 and 1).

Results and Discussion

The venoms of *M. euryxanthus*, *M. fulvius*, *M. pyrrhocryptus*, *M. spixii*, and *M. tener* (10 μg/mL) caused a concentration-dependent blockade of nerve-mediated twitches in the chick biventer cervicis nerve-muscle preparation (Figs. 2-7, $n = 3$). All venoms (10 μg/mL) significantly inhibited contractile responses to exogenous acetylcholine (ACh, 1 mM) and carbachol (CCh, 20 μM), but not KCl (40 mM) (Figs. 2-7, $n = 3$, $P < 0.05$).

Based on the t_{90} values obtained, the observed rank order of neurotoxicity for the New World coral snakes is *M.tener*>*M.spixii*≥*M.pyrrhocryptus*>*M.euryxanthus*>*M.fulvius* (Table 2; Fig. 2). This contrasts with the rank order of lethality based on the murine LD_{50} values previously reported by Sánchez et al. (2008); *M. spixii* > *M. fulvius* > *M. pyrrhocryptus* > *M. tener*. However, it is important to note the use of t_{90} values as a measure of toxicity differs from that of LD_{50} values in several important ways. Murine LD_{50} values determine the concentration required to kill 50% of a population of mice (i.e., quantity) over an extended period of time (24 h), whereas t_{90} values focus on the time required for the venom to exert its flaccid paralysis effects (i.e., time). As coral snakes mostly consume reptiles (diapsid) and use

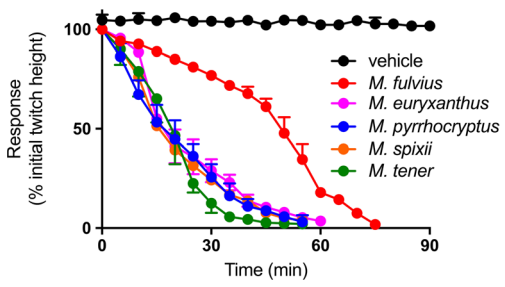

Fig. 2 Comparative effects of *Micruroides euryxanthus*, *Micrurus fulvius*, *Micrurus pyrrhocryptus*, *Micrurus tener*, and *Micrurus spixii* venoms (10 μg/mL) on nerve-mediated twitches in the chick biventer cervicis nerve-muscle preparation ($n = 3$)

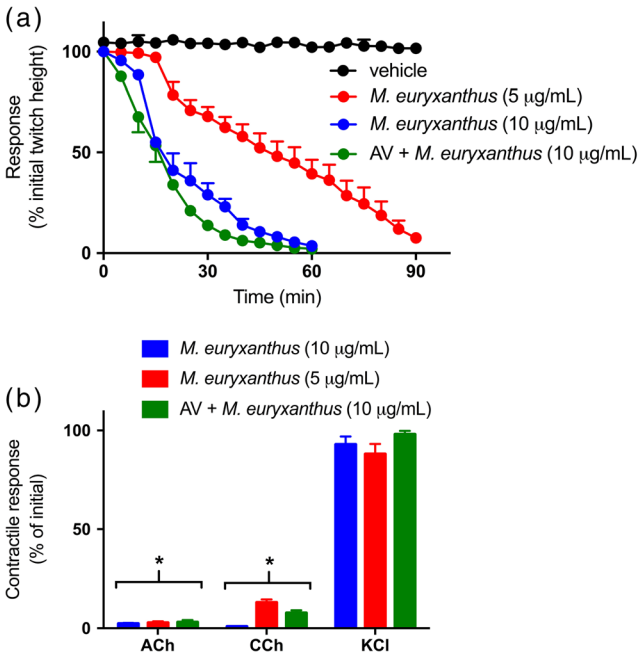

Fig. 3 Effects of *Micruroides euryxanthus* venom (5–10 μg/mL, $n = 3$) on **a** nerve-mediated twitches and **b** responses to exogenous ACh (1 mM), CCh (20 μM), and KCl (40 mM) of the isolated chick biventer cervicis nerve-muscle preparation in the presence and absence of Coralmyn® coral snake antivenom (10 units/mL added prior to 10-μg/mL venom concentration, $n = 3$). *$P < 0.05$, significantly different from contractile response of the initial, paired *t* test

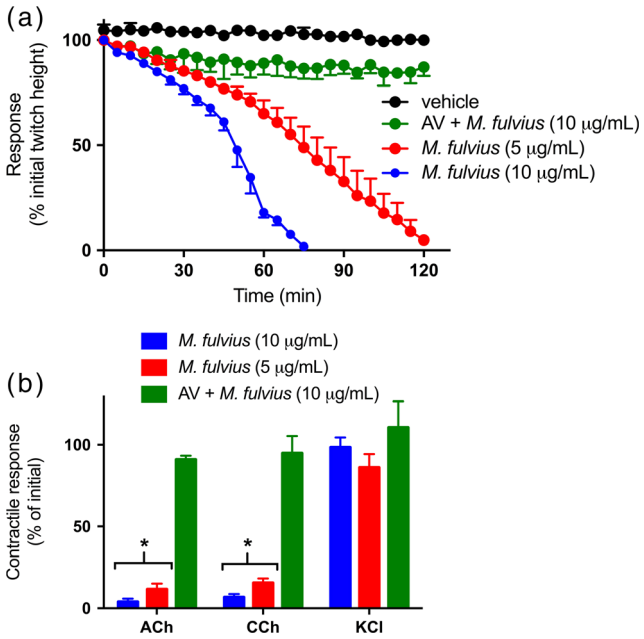

Fig. 4 Effects of *Micrurus fulvius* venom (5–10 μg/mL, n = 3) on **a** nerve-mediated twitches and **b** responses to exogenous ACh (1 mM), CCh (20 μM), and KCl (40 mM) of the isolated chick biventer cervicis nerve-muscle preparation in the presence and absence of Coralmyn® coral snake antivenom (10 units/mL added prior to 10-μg/mL venom concentration, n = 3). *P < 0.05, significantly different from 10-μg/mL venom alone, two-way ANOVA. *P < 0.05, significantly different from contractile response of the initial, paired t test

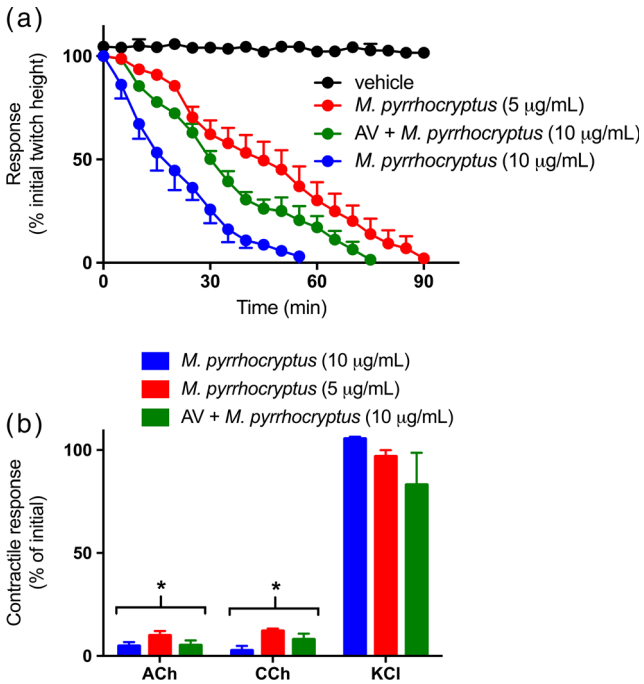

Fig. 5 Effects of *Micrurus pyrrhocryptus* venom (5–10 μg/mL, n = 3) on **a** nerve-mediated twitches and **b** responses to exogenous ACh (1 mM), CCh (20 μM), and KCl (40 mM) of the isolated chick biventer cervicis nerve-muscle preparation in the presence and absence of Coralmyn® coral snake antivenom (10 units/mL added prior to 10-μg/mL venom concentration, n = 3). *P < 0.05, significantly different from contractile response of the initial, paired t test

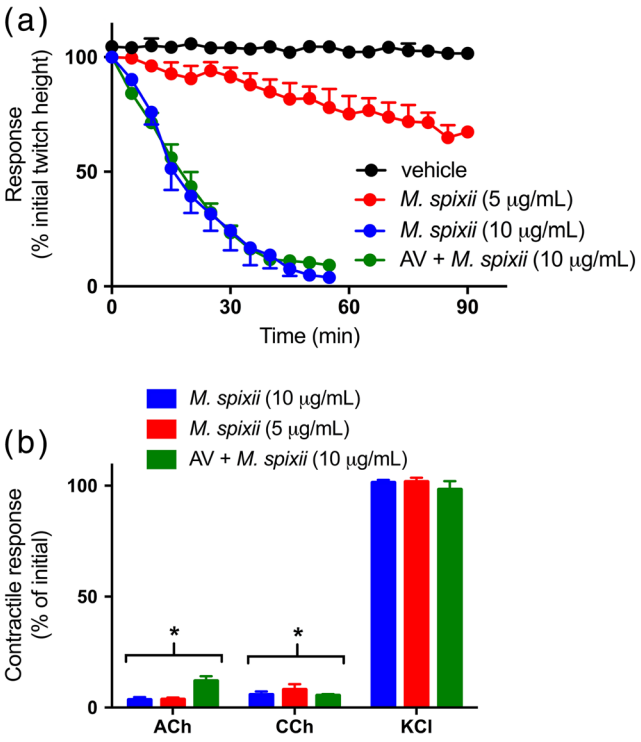

Fig. 6 Effects of *Micrurus spixii* venom (5–10 μg/mL, n = 3) on **a** nerve-mediated twitches and **b** responses to exogenous ACh (1 mM), CCh (20 μM), and KCl (40 mM) of the isolated chick biventer cervicis nerve-muscle preparation in the presence and absence of Coralmyn® coral snake antivenom (10 units/mL added prior to 10-μg/mL venom concentration, n = 3). *P < 0.05, significantly different from contractile response of the initial, paired t test

their venom to rapidly subjugate their prey (Roze 1996), murine (synapsid) LD_{50}s may not be particularly informative from an evolutionary point of view; however, they are likely a better measure of potential lethality to humans (also synapsid) in untreated envenomings (Bénard-Valle et al. 2014). Differences between the two measures of toxicity are likely to be due to the fact that the crude venom possesses other toxic components apart from neurotoxins; such as cardiotoxins, myotoxins, phospholipases, and pro- and anticoagulant factors, all of which act either synergistically or independently on their respective targets to contribute to the lethality of the venom over such a prolonged period of study. This is not the case in t_{90} studies as the in vitro neuromuscular preparations' only factor in neurotoxins and myotoxins, the two types of toxins likely to inhibit twitch height. Despite this, the knowledge obtained from t_{90} values is extremely valuable given that the primary symptom of envenoming by many elapids, including coral snakes, is neuromuscular paralysis.

To the best of our knowledge, this study represents the first analysis of the neuromuscular activity of the venom from *M. euryxanthus*. Here, the relatively low t_{90} value as well as the complete abolition of the response to exogenous ACh and CCh (Fig. 3) suggest a postsynaptic effect.

Prior incubation of Coralmyn® coral snake antivenom (10 units/mL) did not significantly neutralize the neurotoxic

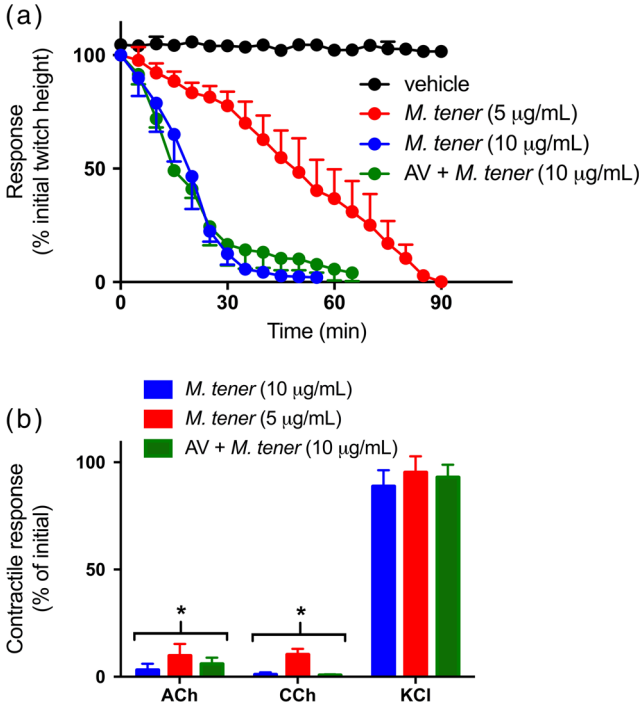

Fig. 7 Effects of *Micrurus tener* venom (5–10 μg/mL, $n = 3$) on **a** nerve-mediated twitches and **b** responses to exogenous ACh (1 mM), CCh (20 μM), and KCl (40 mM) of the isolated chick biventer cervicis nerve-muscle preparation in the presence and absence of Coralmyn® coral snake antivenom (10 units/mL added prior to 10-μg/mL venom concentration, $n = 3$). *$P < 0.05$, significantly different from contractile response of the initial, paired *t* test

effects of *M. euryxanthus* ($P = 0.5662$), *M. pyrrhocryptus* ($P = 0.5747$), *M. spixii* ($P = 0.9520$), and *M. tener* ($P = 0.8218$) venoms (Figs. 3, 4, 5, 6, and 7, $n = 3$). However, Coralmyn® coral snake antivenom significantly neutralized the effects of *M. fulvius* venom ($P = 0.0005$) (Fig. 4a, $n = 3$, $P < 0.05$) and prevented the inhibition of contractile responses to exogenous ACh ($P = 0.0001$) and CCh ($P = 0.0001$) (Fig. 4b, $n = 3$, $P < 0.05$). While it is important to note that pre-incubation of antivenom in an in vitro setting does not necessarily correlate to clinical success, failure to neutralize the neurotoxic effects of these venoms in such ideal conditions may potentially indicate clinical failure.

Against the venoms of the two South American coral snakes (*M. spixii* and *M. pyrrhocryptus*) and the Arizona coral snake (*M. euryxanthus*), Coralmyn® was ineffective in neutralizing their neurotoxicity. Although the response to the venom of *M. pyrrhocryptus* was marginally delayed in the presence of antivenom, the venom was not sufficiently neutralized for it to be deemed effective. However, even adjusting for relative neurotoxicity, the antivenom was clearly most effective against *M. fulvius* than all other species (Fig. 8).

The neutralization of *M. pyrrhocryptus* venom by a South American antivenom raised against *Micrurus frontalis* and *M. corallinus* was tested using also a chick BC preparation and found to be very effective (Camargo et al. 2011). These

Table 2 Snake venom neurotoxicity in the chick biventer cervicis ranked by t_{90} (data from other studies shown for comparison)

Common name	Scientific name	t_{90} @ 10 μg/mL (min)
Curl snake	*Suta suta*	13 ± 1[c]
Malayan krait	*Bungarus candidus*	13 ± 4[e]
Common death adder	*Acanthophis antarcticus*	14 ± 1[a]
Marsh snake	*Hemiaspis signata*	16 ± 1[g]
Northern death adder	*Acanthophis praelongus*	19 ± 2[a]
Tiger snake	*Notechis scutatus*	22 ± 2[b]
Banded krait	*Bungarus fasciatus*	22 ± 5[e]
Inland taipan	*Oxyuranus microlepidotus*	27 ± 3[d]
Rufous beaked snake	*Rhamphiophis oxyrhynchus*	29 ± 7[h]
Texas coral snake	*Micrurus tener*	31 ± 2
Egyptian cobra	*Naja haje*	36 ± 4[f]
Amazon coral snake	*Micrurus spixii*	38 ± 3
Argentinian coral snake	*Micrurus pyrrhocryptus*	40 ± 3
Arizona coral snake	*Micruroides euryxanthus*	46 ± 3
Carpentaria snake	*Cryptophis boschmai*	59 ± 1[g]
Eastern coral snake	*Micrurus fulvius*	67 ± 2

Data shown are represented as mean ± SEM

[a] Wickramaratna and Hodgson 2001

[b] Hodgson et al. 2003

[c] Kuruppu et al. 2007

[d] Crachi et al. 1999

[e] Rusdi Ahmad Rusmili et al. 2014

[f] Kornhauser et al. 2013

[g] Pycroft et al. 2012

[h] Lumsden et al. 2005b

results, together with the ones from the present study, suggest that PLA_2-predominant venoms, such as *M. nigrocinctus*, are not effective immunogens when attempting to neutralize 3FTx-predominant venoms. This scenario has been tested and was shown to be equally ineffective by Lomonte et al. (2016a, b) using an in vivo approach.

The results of the antivenom study with *M. tener* venom were unexpected given that Coralmyn® coral snake antivenom was raised against the venom of *M. nigrocinctus nigrocinctus*, a species that is both phylogenetically and geographically nearby to *M. tener* (Campbell and Lamar 2004). However, note that reptile venom often evolves at very high rates (as reflected in the very broad confidence intervals in Fig. 8), and so it may not be surprising to find that close phylogenetic relatives may still have venoms that differ in important properties for antivenom efficacy.

A likely explanation for the observed disparity in neutralization comes from an analysis of the composition of these two venoms and the immunogen *M. nigrocinctus*. While *M. nigrocinctus* venom has a high proportion of both PLA_2 and 3FTx molecules, a study using an equine monospecific

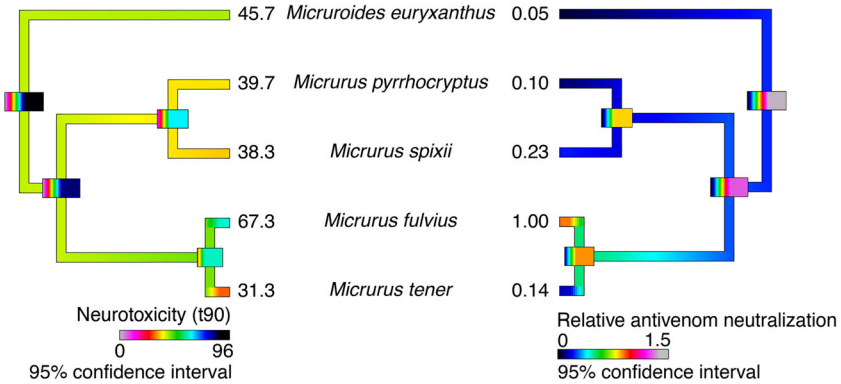

Fig. 8 Ancestral state reconstructions over branches for **a** neurotoxicity t_{90}, whereby *lower values* indicate higher potency and **b** relative antivenom neutralization based upon t_{90} relative shift, whereby *higher values* indicate greater relative antivenom neutralization. *Bars* indicate 95% confidence intervals for the estimate at each node; note that due to the high dynamicity of venom evolution, these quickly become broad as you move down the tree. Phylogeny follows Lee et al. (2016)

antivenom raised against this same venom showed that recognition of antibodies is mainly directed towards PLA_2 (Fernández et al. 2011, Lomonte et al. 2016b). Similarly, Coralmyn® has been shown to have low antibody titers against 3FTx predominant venoms such as *Micrurus surinamensis* (Table 3). Given that the lethality of *M. fulvius* venom is determined by two main neurotoxic PLA_2s and has no lethal 3FTxs (Vergara et al. 2014), neutralization of these molecules is enough to neutralize the lethality of the whole venom. A similarly interesting case has been recently described for the venom of *Micrurus clarki* which is devoid of 3FTx that are lethal to mice and has been proven to be very well neutralized by the antivenom from Clodomiro Picado Institute in Costa Rica which is also raised against *M. nigrocinctus* (Lomonte et al. 2016b). Conversely, *M. tener* has at least two highly lethal 3FTx molecules that have been shown to have a relevant role on the toxicity of the whole venom (Bénard-Valle et al. 2014). A similar case of poor neutralization of coral snake venom due to a highly lethal 3FTx has been described for the venom of *Micrurus laticollaris* (Carbajal-Saucedo et al. 2013).

Table 3 Venom lethality to mice and Coralmyn antibody titers for various coral snake species

	Locality	i.v. LD_{50} μg/mouse	ELISA titer
Micrurus diastema	Chiapas, Mex.	5.06	10,459
M. distans	Jalisco, Mex.	15.0	4612
M. fulvius	FL, USA	11.5	5197
M. laticollaris	Guerrero, Mex.	10.0	5246
M. limbatus	Veracruz, Mex.	19.9	6045
M. nigrocinctus	Guatemala, Gua.	11.5	8178
M. surinamensis	Colombia	ND	189
M. tener	Tamaulipas, Mex.	22.7	4434
Micruroides euryxanthus	Sonora, Mex.	ND	737

Adapted from Bénard-Valle 2009

i.v. LD_{50} intravenous medium lethal dose, *ELISA titer* antivenom dilution at which 50% of colorimetric response is observed

A study by Sánchez et al. (2008) indicated effective neutralization of both *M. fulvius* and *M. tener* venoms with Coralmyn® coral snake antivenom. It was noted that Coralmyn® was more effective in neutralizing *M. fulvius* venom rather than *M. tener* venom on an LD_{50} basis. The venom of *M. fulvius* has been previously described to be well neutralized by Coralmyn and an antivenom against the venom of the tiger snake *Notechis scutatus* (Wisniewski et al. 2003). This was contrary to the results of the current study in which only the venom of *M. fulvius* was neutralized, potentially suggesting that an insufficient dosage of antivenom was utilized for the neutralization of *M. tener* in the present study. The small proportion of 3FTx recognizing antibodies in Coralmyn® could be enough to neutralize the lethal 3FTx of *M. tener* but a high dosage of antivenom would be needed, probably explaining these differences.

As mentioned before, *M. euryxanthus* appears to be a 3FTx predominant venom with a mainly postsynaptic activity. This observation is reinforced by the very low antibody titers observed on Coralmyn® against this venom (Table 3).

This study has shown that the neurotoxic potency of representative species of both North and South American coral snakes varies greatly and that Coralmyn® coral snake antivenom fails to effectively neutralize their neurotoxic effects with the exception of *M. fulvius* venom. These findings suggest that venom variation between coral snake species may be of even greater clinical importance than geographical location and the $3FTx/PLA_2$ venom dichotomy would predict.

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Compliance with Ethical Standards

Conflicts of Interest None declared.

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