



# INTERNATIONAL SOCIETY ON TOXINOLOGY

## NEWSLETTER

December 2016

### UPCOMING MEETINGS

The next IST World Congress will be in Haiku, Hainan, China, October 24th to 30th, 2017. See later in this newsletter for further details. A website for this congress is available at [www.ist2016.com](http://www.ist2016.com).

IST Council has agreed to a changed schedule for IST congresses, commencing in 2015 with the World Congress held every second year, rotating between the 3 regions, so the following IST World Congress will be in 2019, somewhere in the Pan-American region, hosted by the Pan-American Section of IST. IST Council welcomes bids from within this region to host this congress.

The next Clinical Toxinology Short Course will be held in Adelaide, Australia, November 28th to December 6th, 2017. The 2017 course will be rather special, with an expanded program and faculty in celebration of 20 years of courses since the first course in November 2017.

The next European Association of Poisons Control and Clinical Toxicology Congress will be in Basel, Switzerland, May 16-19, 2017.

The next North American Congress of Clinical Toxicology will be in Vancouver, Canada, October 11-15, 2017.

The next Oxford Venoms meeting will be August 29-30, 2017.

### FROM THE IST EXECUTIVE

2017 will see the next IST World Congress, to be held in Haiku, Hainan, China, October 24th to 30th. There is still much planning to complete for this important meeting for our Society, the first time we have had a World Congress hosted in China, though we have had Asia-Pacific Section congresses hosted in China, the most recent in Changsha in 2014.

I hope many members will be able to participate in this meeting, both to support our Society and to learn about new developments in toxinology in both toxin research and clinical toxinology.

The Pan-American Section congress, held in Miami Beach in September 2016, was a great success. I hope members will join me in thanking the hard working organisers, the two Franks, senior (Frank Mari) and junior (Frank Bosmans). I hope to have a report on this congress in a future newsletter. For those of you wondering who won the great paella competition, it was Juan Calvete with his traditional Valencian recipe, by a very small margin against the challenger, Richard Lewis, with his exciting new interpretation of paella. Well done to both our champion cooks!

While yet to be confirmed, it seems increasingly likely the next European section congress will be hosted in Yerevan, Armenia, in late 2018. An exciting prospect!

Lastly, I wish all members who celebrate Christmas a Very Merry Christmas and a Happy New Year. I will send out notices about IST dues for 2017 in January.

Julian White AM, Secretary/Treasurer, IST

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## MESSAGE FROM THE PRESIDENT (I.S.T)

Greetings and Happy Holidays Fellow IST Members: This has been a remarkable year for the IST in my opinion based on the many outstanding scientific works I have observed published by our members. Also noteworthy was the excellent Pan American Sectional meeting hosted by Frank Mari and Frank Bosman in Miami. One thing for certain, if the IST has any more paella cook-offs much larger pans are needed!

I am pleased to say there has been a tremendous amount of effort on the part of the IST Council over this past year in terms of thoughtful discussions and actions to strengthen the Society and grow our membership. To that end we are finalizing memoranda of understanding with a number of national societies that share our interests. These include the Toxinological Society of India, The Brazilian Society of Toxinology and the Toxicological Association of Argentina. Furthermore, we have very positive discussions ongoing with the French Toxinological Society and the Toxinology Society of Nigeria. Several other societies have also reached out to us and we are also in discussion with them as well. We hope these MOUs will be mutually beneficial and will synergize our activities and membership. I wish to offer my thanks to Dr. Kini for taking the lead on this and all the work of the Council making this at last a reality.

We are in the process of developing an active student membership in the IST hoping to attract more young scientists to our field. I fully expect our student members will play a more predominant role in our meetings and have some voice in the future of the IST. I would encourage each of our members to nominate at least one student for membership in the new year!

Other activities of the Council have included supporting the organization of our society meeting, the next which will be in the fall of 2017. This is following the not debatably wonderful meeting held in Oxford in 2015. If you have not attended an IST World or Sectional Congress in the past few years I would strongly encourage you to do so. They are outstanding for the science, networking and collegiality.

The Council has been discussing the concept of moving to an annual World Congress, hosted by our Sections; revisiting the meaning and costs of corporate membership in the IST and has established a Life Member category. There are many other important topics under consideration and I would ask you to seek out your Sectional representative to understand the full scope of these activities and offer your input on the topics.

A final thanks to our Council and our Secretary/Treasurer Dr. Julian White. Julian somehow manages to keep us informed and the communication amongst us all open and active. Julian will be reaching out to members for support with some of our initiatives and I hope you will say "yes" if asked.

Finally, at year's end it is timely to reflect on our individual blessings and on those less fortunate than ourselves. Scientists, through good times and bad, have a wondrous and in some ways perhaps even sacred role in this world of discovery and ultimately through those discoveries benefiting the human condition. Regardless of the experiment or lecture or perhaps odious task at hand we must never lose sight our ultimate charge of helping our fellow humans through our scientific pursuits.

I wish you all a Happy and Prosperous New Year and I hope to see you all at IST 2017! All the best,  
Jay W. Fox, IST President

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# INTERNATIONAL SOCIETY ON TOXINOLOGY COUNCIL MEETING

September 21st, 2016

Meeting held at the Pan-American Section Congress, Miami

Meeting held in person during IST Congress, Miami

## SUMMARY OF MINUTES

1. Those participating: J Fox, J White, A Harvey, G King, F Mari, JM Gutierrez, D Tambourgi, J Calvete
2. Apologies: D Warrell, S Luo, S Liang, Y Cury, M Kini, J Tytgat, R Harrison
3. Minutes of last meeting: Approved
4. Business arising:
  - 4.1 IST incorporation:

Jay Fox has found an attorney in Virginia who can investigate and advise regarding incorporating IST in the US, at a fee of up to US\$250. Council approved this expenditure.
  - 4.2 Developing and promoting IST:

A standing item for Council. A general discussion occurred and it was agreed to pursue affiliating region/national toxinology societies with IST, if the revised constitution is passed at the AGM (it was passed), starting with the Toxinology Society of India. An appropriate MOU was ready for this purpose.
  - 4.3 Review of relationship with Toxicon:

The contract between Elsevier (publisher and owner of Toxicon) and IST is due for renewal in 2017 and the Chief Editor position changes, with Alan Harvey retiring and Glenn King being the replacement favoured by Elsevier. Jay Fox suggested it is time to consider the IST relationship with Toxicon and if the interests of IST can better be met through a contract with a rival journal. Considerable discussion ensued. Jay Fox agreed to seek an improved contract from Elsevier and also to see what an interested rival publisher might offer. Council moved and passed a motion of thanks to Alan Harvey as Editor of Toxicon and similarly recommended Glenn King as the new Editor.
5. Officer bearers reports

Reports were presented by the President, the Secretary/Treasurer, and Section executives and related Council subcommittees. The IST finances are sound (refer to Secretary/Treasurers report to the AGM in Miami, elsewhere in this newsletter). Of particular note, from the European Section, was a bid by Dr. Naira Ayvazyan to host the 2018 section congress in Yerevan, Armenia. Naira presented a comprehensive bid to Council and subject to clarification of several matters, this was accepted. Council thanks Naira for this initiative. Council also noted the success, to date, of the Miami IST Pan-American Section Congress and thanked Frank Mari and Frank Bosmans for their excellent work in organising this congress.
6. New business:
  - 6.1 Regional congress programs:

Council discussed the tendency of sectional congresses to become mini-world congresses.

The MOUs between Council and congress organisers will be reviewed and, if necessary, tightened, to ensure IST has a reasonable level of control over future congresses.

Julian White  
Secretary/Treasurer, IST

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## IST Nomenclature Committee

At the last IST World Congress held in Recife, Brazil in March 2009, a symposium devoted to the topic of toxin nomenclature received significant interest from IST members. The IST Council subsequently decided to form a nomenclature committee to examine the issue of toxin naming standards and recommend possible solutions. The mandate of this committee was to propose a nomenclature system, with interim reports to IST Council and a "final" report to be delivered at the IST World Congress in 2012. This deadline was not met, but it is hoped progress will be made in the following triennium. If you have any comments or suggestions on toxin nomenclature, could you please send them to a member of the nomenclature committee, which is currently comprised of the following members:

Dr Gerardo Corzo, Mexico (Email: [corzo@ibt.unam.mx](mailto:corzo@ibt.unam.mx))

Dr Florence Jungo, Switzerland (Email: [Florence.Jungo@isb-sib.ch](mailto:Florence.Jungo@isb-sib.ch))

Dr Evanguedes Kalapothakis, Brazil (Email: [ekalapo@icb.ufmg.br](mailto:ekalapo@icb.ufmg.br))

Prof. Glenn King, Australia (Chairman; Email: [glenn.king@imb.uq.edu.au](mailto:glenn.king@imb.uq.edu.au))

Prof. Manjunatha Kini, Singapore (Email: [dbskinim@nus.edu.sg](mailto:dbskinim@nus.edu.sg))

Prof. Graham Nicholson, Australia (Email: [graham.nicholson@uts.edu.au](mailto:graham.nicholson@uts.edu.au))

Prof. Toto Olivera, USA (Email: [olivera@biology.utah.edu](mailto:olivera@biology.utah.edu))

Prof. Jan Tytgat, Belgium (Email: [jan.tytgat@pharm.kuleuven.be](mailto:jan.tytgat@pharm.kuleuven.be))

### ***ArachnoServer spider toxin database***

ArachnoServer is a manually curated database that provides detailed information about proteinaceous toxins from spiders. Key features of ArachnoServer include a new molecular target ontology designed especially for venom toxins, the most up-to-date taxonomic information available, and a powerful advanced search interface. Toxin information can be browsed through dynamic trees, and each toxin has a dedicated page summarising all available information about its sequence, structure, and biological activity. ArachnoServer currently manages 567 protein sequences, 334 nucleic acid sequences, and 51 protein structures. ArachnoServer is available online at [www.arachnoserver.org](http://www.arachnoserver.org).

*The IST has established a special wiki site for members of this Nomenclature Committee to use to both communicate and develop information and recommendations. Members of the committee will soon receive an email detailing how they may access this site.*

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## IST Snake Taxonomy Advisory Group

Keeping up with changes in taxonomy for venomous animals is always a challenge for toxinologists, but it is important to do so, if published research is to maintain viability longer term, as taxonomy evolves. To improve dissemination of information on taxonomic changes the IST has invited Assoc. Prof. Scott Weinstein (Australia/USA) to chair the snake taxonomy committee with a view to generation of regular taxonomy updates which can be made available to members.

We will consider making these updates available through the newsletter and, possibly, the IST website.

Julian White AM, Secretary IST

# International Society on Toxinology

## Annual General Meeting Thursday September 22nd, 2016 Deauville Beach Resort, Miami Beach, USA

### MINUTES

**Meeting opened:** by Prof. Jay Fox, IST President & Prof. Julian White, IST Secretary/Treasurer. 28 members present (well above the quorum minimum required by the IST constitution).

**Apologies:** None were tendered

**President's Report:** delivered by Prof. Fox

Prof. Fox provided a general overview of IST activities over the last year, including development of Memorandum of Understanding templates for congresses and for relationships with national toxinology societies. The latter will come into operation if the proposed revised constitution is ratified as this will include new provisions for relationships with such societies. Prof. Fox noted that while IST membership remains stable, there is a need to grow membership and this will be assisted by providing increased value of membership. IST Council is investigating a number of initiatives to achieve this. Investigation into incorporation of IST is progressing and if successful may open new opportunities for IST activities and sponsorship of congresses by outside organisations.

**Toxicon Editor's Report:** delivered by Prof. Alan Harvey, Editor in chief

Prof. Harvey informed those present that there have been 439 manuscripts received by Toxicon this year so far, about a 30% increase on recent years. He noted that the journal appeared "in good shape". He also noted that he will retire as Editor-in-Chief at the end of this year and that Prof. Glenn King from Brisbane, Australia has agreed to take over as Editor-in-Chief from 2017, a choice endorsed by IST Council. The meeting thanked Prof. Harvey for his work in this role over many years.

**Secretary/Treasurer's Report:** delivered by Prof. Julian White

The past year, since the IST World Congress in Oxford, UK, has been one of consolidation for the Society. Annual dues have remained unchanged at US\$55.00 per year. Notice for payment of 2016 annual fees to IST was sent out by email to members in late January, with several follow ups, the most recent being late August. As of 7-9-16 220 members have paid their 2016 dues. In 2015 223 members paid their 2015 fees. By comparison, in 2014, only 149 members paid their dues, though several have now paid back dues for past years (mostly 2015 & 2014) this year, while paying 2016 dues. There are a further 15 in the "special" category currently unable to pay dues due to technical issues. There are 82 student members currently listed as "active" (ie contactable and student status confirmed).

As of September 7th, 2016, the IST bank accounts held Aus\$9,829.73, compared to Aus\$15,487.21 held in early January 2016. In January \$533.51 was paid to the StHilda's College, Oxford, for catering for the IST Business Meeting last year. In February \$752.37 was paid from these accounts to settle remaining invoices related to the IST Oxford World Congress. In May \$4371.60 (US\$3,000.00 + US\$25.00 in US bank fees) was paid to the Miami Congress organisers. Since I last presented accounts to Council, the Aus\$ has varied significantly in value and continues to be worth substantially less than the US\$. These currencies remain volatile. Based on the exchange rate today this amount equates to about US\$7,538.06, compared to US\$10,669.64 in January.

As of September 7th the IST PayPal account held about Aus\$76,970.66, compared to Aus\$68,231.55 in January. This is an approximate amount because funds were collected (from IST annual fees payments) in US\$, so PayPal holds funds in US\$. The precise US\$ amount currently is US\$60,638.06, compared to US\$48,069.20 in January. Several members continue to have prob-

lems using the online payment system via PayPal, but I do not have another viable alternative payment route to suggest to Council at this time.

This means the approximate (allowing for exchange rate variations) amount of funds held by IST in all accounts is currently US\$68,176.12, compared to US\$58,738.84 in January 2016 (in January 2015 it was US\$57,550.90 and in February 2014 the comparable figure was US\$43,477.81). A substantial amount of the increase in funds in the last few months is 2016 annual dues collected via the IST website, although a portion, compared to 12 months ago, can be ascribed directly to exchange rate fluctuations in recent times, particularly affecting funds held in Aus\$.

The Society is at least as financially sound as 12 months ago and arguably more sound. Nevertheless, there is a clear need to improve both Society income and membership numbers and retention. Our President, Prof. Jay Fox is proposing a number of initiatives to address these issues.

A Newsletter was issued in December 2015 and in September 2016. Articles etc for the next Newsletter are sought from members. It would be good to release another newsletter in the next month or so, after the Miami Congress.

The most important change for the Society will be the revised Constitution, if adopted by members at this Business Meeting. The revised Constitution, approved by IST Council and sent to members more than 90 days prior to this meeting, with a reminder in the September 2016 Newsletter, introduces several important changes, particularly allowing the Society to establish formal links with national toxinology societies. I recommend members approve this revised Constitution today.

Motion: That Treasurer's report be accepted.

Moved: Prof. Glenn King

Seconded: Dr. Angel Yanagihara

Motion carried

Discussion about the report followed.

Prof. Carl Vogel questioned why the IST needed a substantial financial reserve, as listed in the report and why this was not used to assist congress organisers who overran budgets. He also requested details be provided of all income and expenditure, in tabular form.

Prof. White explained that all income and expenditure was, in fact, listed in the report. He noted that the IST has needed to build a healthy balance sheet to ensure enough funds are available to help underwrite IST congresses in future, something IST has been unable to do because funds were insufficient. However, funds are now approaching a sufficient amount to allow for such underwriting, which will enable IST to be a more active participant in the organising, budgeting and control of congresses.

Prof. Fox explained that some of the funds may be used to establish some form of enterprise or innovation fund within IST to help further promote IST activities. The development of Memorandums of Understanding with future congress organisers will also assist in ensuring IST oversight of congress organisation and budgeting.

Prof. Leslie Boyer suggested that IST needs to further encourage student membership and participation in congresses and that perhaps a set amount could be made available for each congress to assist student attendance/involvement.

Prof. Fox noted and agreed with this suggestion which can be considered by IST Council.

#### **Annual dues:**

Motion: That the Annual Dues for the Society be maintained at US\$55.00

Moved: Prof. Julian White

Seconded: Prof. Frank Mari

Motion carried

Prof. Juan Calvete noted the problems and irritations encountered in using the online payment portal for IST annual dues, using PayPal. He requested that some alternative system be considered.

Prof. White explained the reasons for using the current system which include both convenience

for many members and the Treasurer, and also the ability to audit payments. He noted that direct interbank transfers were unlikely to be a viable alternative because of the substantial bank charges levied. He undertook to further investigate alternatives.

Prof. Frank Mari expressed agreement about the impracticality of using interbank transfers, because of levied fees.

Prof. Fox noted that if IST can be incorporated, this may also open up some alternatives.

### **The next IST World Congress, 2017:**

The congress organiser, Prof. Sulan Luo, Hainan, China, was not present as she had been unable to obtain a visa to attend the Miami congress. However, she had chosen dates in October 2017.

Prof. Glenn King noted that the chosen dates directly clashed with the peptides meeting in Australia and that this was unacceptable because it would prevent most Australian research toxinologists attending the IST congress. It would be necessary to move the congress dates back by at least 2 weeks to avoid clashes with the peptides meeting and a similar meeting immediately following it. He undertook to contact Prof. Luo and inform her of this issue.

### **The next IST European Section Congress, 2018:**

Dr. Naira Ayvazyan gave a short presentation on her bid to host the next European Section IST congress in Yerevan, Armenia, in September 2018. She explained that she and her colleagues had considerable experience and resources for running the congress and that they had located a suitable well equipped venue at a substantially reduced rate.

This proposal is being considered by IST Council and has been provisionally accepted, with thanks.

### **Other business:**

**9.1 Adoption of a revised IST Constitution**, as previously circulated to all financial members more than 90 days prior to this meeting:

Motion: That this meeting approve the revised constitution, as presented.

Moved: Prof. Jay Fox

Seconded: Prof. Julian White

Motion carried (almost unanimously; one vote opposed)

There was some discussion about aspects of the constitution:

Prof. Carl Vogel considered that for the new "Life Member" category the constitution should include a specific multiplier factor, not leave this to be determined by IST Council, as the current wording allows. He also noted that the "Honorary Membership" category should be only for the most distinguished retired members and that there should be a separate "Emeritus" category for members now retired who may no longer be able to pay annual dues.

Prof. White noted that placing such a multiplier for Life Membership in the constitution was unwieldy and that it was far more appropriate to leave this to IST Council. He noted that the Honorary category was available for emeritus members and that adding a new category at this time was not practical.

### **9.2 Any other business:**

Motion: That this meeting congratulate and thank Prof. Frank Mari and Prof. Frank Bosmans, organisers of the IST Miami Congress, for their outstanding efforts and a successful congress.

Moved: Prof. Julian White

Seconded: Prof. Jay Fox

Motion carried unanimously.

Meeting closed:

Julian White

Secretary/Treasurer IST

# **THIS IS THE NEW, REVISED CONSTITUTION OF IST, APPROVED AT THE AGM HELD DURING THE MIAMI IST CONGRESS, SEPTEMBER 2016.**

## **CONSTITUTION AND BY-LAWS OF THE INTERNATIONAL SOCIETY ON TOXINOLOGY**

### **ARTICLE I**

#### **NAME**

The name of this organization shall be the INTERNATIONAL SOCIETY ON TOXINOLOGY (IST).

### **ARTICLE II**

#### **OBJECT**

The object of the Society is to advance knowledge on the properties and clinical aspects of poisons, toxins and antitoxins derived from animals, plants and microorganisms, and antivenoms and other treatments for toxin-induced illness, and to bring together those scholars and clinicians interested in these substances and their effects through a common Society and to support training and credentialing of medical doctors in the specialty of clinical toxinology.

### **ARTICLE III**

#### **NOT FOR PROFIT CLAUSE**

The assets and income of the organisation shall be applied solely in furtherance of its above-mentioned objects and no portion shall be distributed directly or indirectly to the members of the organisation except as bona fide compensation for services rendered or expenses incurred on behalf of the organisation.

### **ARTICLE IV**

#### **MEMBERS**

- Section 1. The Society shall consist of Members, Associate Members, Student Members, Honorary Members and Corporate Members.
- Section 2. Persons who have conducted and published original investigations in toxinology shall be eligible for Membership in the Society. On payment of annual Society dues they will be Financial Members for that year and entitled to vote at Society meetings.
- Section 3. Persons who do not qualify for Membership but are working or interested in the field of toxinology shall be eligible for Associate Membership. On payment of annual Society dues they will be entitled to vote at Society meetings.
- Section 4. Persons who are registered students studying an aspect of toxinology shall be eligible for Student Membership. On payment of any annual fee which will be determined annually by Council, they will become registered Student Members for that Society financial year, entitled to any rights that may be defined, from time to time, by Council.
- Section 5. Persons eligible for full Membership of the Society and who opt to pay a one-time fee which shall be determined annually by Council shall be Life Members and shall not have to pay further annual dues, but shall be thereafter entitled to all the rights and privileges of Membership, except that if the Life Member indulges in activities that Council consider bring the reputation of the Society into disrepute, Council may, at its sole discretion, suspend or terminate the Life Membership, in accordance with the principles provided in Section 14 (below) of this Constitution.
- Section 6. Persons who have, in the view of Council or the Society, made a special or unique contribution to toxinology, shall be eligible for Honorary membership in the Society. Honorary Members shall be exempt from paying annual dues to the Society, but shall have the same rights to vote as Financial Members.

- Section 7. Organizations contributing to toxinology and the Society, which provide a regular Financial contribution to the Society, determined by Council, shall be eligible for Corporate Membership. Corporate Members shall be required to pay annual dues in an amount to be determined, year to year, by Council, but shall not have voting rights in the Society.
- Section 8. Organizations related to and contributing to toxinology and the Society which provide a regular Financial contribution to the Society, determined by Council, shall be eligible for Affiliate Organisation Membership. Affiliate Organisation Members shall be required to pay annual dues in an amount to be determined, year to year, by Council, but shall not have voting rights in the Society. Council has the right to negotiate with an Affiliate Organisation in regard to any further fees that might be applied to some or all members of that organisation in return for any specific privileges that might then apply to these members in regard to the Society and its activities and services, if any. Council shall have the right to revoke Affiliate Organisation Membership at any time.
- Section 9. Council shall have the right to establish subclasses of Membership as required to further the Objects of the Society. All such subclasses of Membership may attract annual dues as determined by Council which may be additional to any dues required for the main classes of Membership (Article IV, Sections 2-6) and shall only be open to Members of the Society or members of organisations covered by Corporate Membership, or Affiliate Organisation Membership who meet criteria approved by Council for the subclass of Membership.
- Section 10. A. Applications for Membership on behalf of the applicant shall be made by a Member of the Society on forms provided by the Secretary. The Member proposing a candidate must upon request submit to Council a letter in support of the candidate.
- B. Applications for Associate Membership shall be submitted by the applicant on forms provided by the Secretary. Nomination by a Member of the Society is not required.
- C. Applications for Student Membership shall be submitted by the applicant on forms provided by the Secretary. Nomination by a Member of the Society is not required, but proof of student status is required. Student membership status is only permissible while the person is a bona fide student.
- D. A person shall be considered by Council for Honorary Membership if requested in writing by at least ten Financial Members. Honorary Membership shall be recommended by the Council of the Society, by a two thirds majority at secret ballot of the Council, conducted by the Secretary/Treasurer and shall become conferred following a simple majority vote of Financial Members at a General or Special Meeting of the Society.
- Should a vote of Financial Members of the Society at a general or special meeting fail to attain a simple majority, then the person proposed for Honorary Membership shall not be eligible for reconsideration for such membership for a period of two years after the first failed vote and four years after a second or subsequent failed vote.
- E. Applications for Life Membership of the Society shall be made by the Member, to the Secretary, and shall only be open to current Financial Members. Such applications shall then be submitted to Council for determination of an appropriate fee. On payment of such fee the Member will become a Life Member, subject to the provisions of Sections 5 and 14 of this constitution.
- F. Applications for Corporate Membership shall be made to Council. An organization shall be considered by Council for Corporate Membership, by a two thirds majority at secret ballot of the Council, conducted by the Secretary/Treasurer and shall become conferred following a simple majority vote of Financial Members at a general or special meeting of the Society. Corporate Membership, once conferred, shall be reconsidered by Council every four years and reconfirmed following a simple majority vote of Financial Members at a general or special meeting of the Society.
- Should a vote of Financial Members of the Society at a general or special meet-

ing fail to attain a simple majority, then the organization proposed for Corporate Membership shall not be eligible for reconsideration for such membership for a period of two years after the first failed vote and four years after a second or subsequent failed vote.

G. Applications for Affiliate Organisation Membership shall be made to the Secretary by organisations meeting the provisions of Section 8 of this constitution and interested in seeking such Membership. The Secretary shall place such applications before Council, or such other subcommittee of Council as Council may, from time to time, determine, to decide on whether to accept, or in the case of a subcommittee, recommend to Council on acceptance, the application and to set terms and conditions in relation to granting Affiliate Organisation Membership for the organization so applying, in accordance with provisions of Section 8 of this constitution. An organization shall be accepted for Affiliate Organisation Membership, by at least a two thirds majority vote of the Council.

- Section 11. The Secretary will, on request of a Council Member, circulate details of all applications for Membership, Associate Membership and Student Membership to all Council Members. After consideration Council Members may effect election and the Secretary will then inform the Member of such election. The Secretary may be delegated by Council to determine, on Council's behalf, if an application can be approved and effect election. A list of all members, including associate and student members, will be made available to all Members of the Society, not less than once a year, by such means, including electronic, as Council may, from time to time, deem appropriate. Applications for Corporate Membership and proposals for Honorary Membership shall be distributed to all Council Members, in accordance with Sections 5 and 6 (above).
- Section 12. Failure of a Member or Associate Member to pay the annual assessment (dues) for two successive years constitutes forfeiture of membership. The Member may be reinstated either at the discretion of the Secretary, or by majority vote of the Council, upon payment of the full amount of the assessment due.
- Section 13. Dues. Each Member and Associate Member shall pay annual dues as be prescribed by the Council, and as approved by the membership at a general meeting or special meeting.
- Section 14. A Member in any category who behaves or undertakes activities that may bring the field of toxinology, or the Society, into disrepute or in other ways acts to the detriment of the Society, or it's Members, may be subject to discipline by majority decision of the Council. Members may petition Council to consider disciplinary action against a member, but Council has the sole right to determine if disciplinary action should be considered. Such discipline will be at the sole discretion of Council and may include suspension of Membership, or consideration of termination of Membership, except that where termination of Membership is recommended by Council, in a majority vote, it must be confirmed by a majority vote of Members present at a General Meeting or Special General Meeting of the Society and until such time as termination of Membership is so confirmed, the Member will have their Membership suspended. On suspension or termination of Membership, any dues paid to the Society shall be forfeit and not refundable to the Member or ex-Member.

## ARTICLE V

### ANNUAL MEETING

There shall be a regular, formal "annual" meeting of the Society (referred to in this constitution as a "General Meeting") which, whenever possible, shall include a meeting of the Council (as determined in By-Laws Article II). The meeting shall be scheduled to occur once each year, except where Council decides that for practical reasons given the international membership of the Society, a meeting must be delayed beyond one year, such a delayed meeting must be held within two years of the previous meeting. The meeting will be held at a time and place prescribed in the ByLaws, or by notice

communicated by electronic or other distribution means to each Member at least 90 days before the date of the meeting; the notice shall state the time, place, and agenda for the meeting. The Council has the right to postpone or cancel the Annual Meeting for one year if deemed necessary.

## ARTICLE VI

### COUNCIL

- Section 1. The Council shall consist of the elected Executive Officers (President, Secretary/Treasurer, President Elect), the Immediate Past President, the current presidents and secretaries of the Regional Sections, the Editor-in-chief of Toxicon, the Chairperson or their proxy of any Board or other membership subclass governing body established by Council, and Financial Members elected from the membership. The individuals elected by the membership shall include two Members from each Region of the Society. The President shall act as Chairman of the Council or in the absence of the President, the following, in order of preference, shall act as Chairman; President Elect, Secretary/Treasurer, Immediate Past President.
- Section 2. The purpose of the Council shall be to act as an administrative and governing body to further the activities and interests of the Society, on behalf of Members. The Council is authorized to accept any donations of cash or property, voluntarily made to further the purpose of the Society.
- Section 3. The Council shall meet at least once each year at a time and place designated by the President and shall recommend the dues of the membership. The meeting may be at a physical place, or virtual place such as through a teleconference or similar electronic meeting system. Greater than one half of the Members of the Council, but including at least one Executive Officer (President, Secretary/Treasurer, or President Elect) shall constitute a quorum for all purposes.
- Section 4. The Members of the Council, excluding the Executive Officers, shall serve for a term of two years, with a maximum of two consecutive terms, and shall not be eligible for re-election for the two years immediately following their term of office. The Council is empowered to appoint Members to fill any vacancy that may occur.
- Section 5. The Council is empowered to co-opt any Member, including an Associate or Student Member, to serve on Council for a specific purpose, up to a maximum of 3 such co-opted Members, but co-opted Members shall not have voting rights on Council.

## ARTICLE VII

### ELECTED EXECUTIVE OFFICERS

- Section 1. Number. The Executive Officers of the Society shall be President, President Elect, and Secretary/Treasurer.
- Section 2. Election. The Executive Officers of the Society shall be elected by a majority vote of the Financial Members in a ballot by electronic or other distribution means, and will serve for a term of 4 years for the President and 4 years for the Secretary/Treasurer. Their term of office will begin immediately after the first World Congress (International Congress) following their election.  
The President Elect shall assume the office of the President at the end of the regular term of office or at any time the office of the President becomes vacant. The President may serve for a maximum of two consecutive terms. The Secretary-Treasurer may be re-elected for further terms as agreed by Council.
- Section 3. Vacancies. Any vacant office of the Society, other than that of the President, may be filled by a Financial Member elected at the next Society Meeting. During the intervals between meetings of the Society, the Council may elect a Financial Member to fill a vacant office, and the Executive Officer so elected shall serve until the next Meeting of the Society.

## **ARTICLE VIII**

### **BOARD OF CLINICAL TOXINOLOGY**

- Section 1. Council shall have the right to establish and modify a Board of Clinical Toxinology, for the purpose of furthering the development of expertise in the medical field of clinical toxinology.
- Section 2. Council shall determine the constitution of and by-laws controlling function of the Board of Clinical Toxinology and shall have the right to modify the constitution and by-laws for the Board.
- Section 3. Membership of the Board of Clinical Toxinology shall be restricted to registered medical practitioners who meet criteria established by the Board and by Council.
- Section 4. The functions and activities of the Board of Clinical Toxinology shall be determined by the Board and by Council and supervised by Council in accordance with the constitution and by-laws of the Board as established by Council.

## **ARTICLE IX**

### **COMMITTEES**

- Section 1. Credentials Committee. Council is empowered to create and dissolve, as it determines, a Credentials Committee to undertake assessment of applications for Membership on behalf of the Secretary and Council. The Committee shall be elected by the Council and shall serve for a term of two years.
- Section 2. Other Committees. Other Committees of the Society may be constituted for the promotion of the objectives of the Society, and shall consist of a limited number of Financial Members, with their number, jurisdiction, and tenure determined by Council.

## **ARTICLE X**

### **AMENDMENT OF CONSTITUTION**

This Constitution may be amended at any Meeting of the Society by the affirmative vote of a majority of the Financial Members present in person or represented by proxy provided that notice of the proposed amendment is given to the membership at least 90 days before the Meeting at which the amendment is offered.

## **ARTICLE XI**

### **ADOPTION AND AMENDMENT OF BY-LAWS**

By-Laws may be adopted, amended, or rescinded at any Meeting of the Society by the affirmative vote of a majority of the Financial Members present or represented by proxy provided that notice of the proposed actions is given to the Membership at least 90 days before the Meeting where such action is to be considered.

## **ARTICLE XII**

### **THE EFFECTIVE DATE OF THE CONSTITUTION**

This Constitution will be deemed to be effective from the date of most recent revision. The most recent revision was approved on September 22nd, 2016, in accordance with the rules and articles of this Constitution and the Society.

## **ARTICLE XIII**

### **DISSOLUTION CLAUSE**

In the event of the Society being dissolved, the amount that remains after such dissolution and the satisfaction of all debts and liabilities shall be transferred to another organisation with similar purposes and which has rules prohibiting the distribution of its assets and income to its members.

## BY-LAWS-ARTICLE I

### MEMBERS AND MEETINGS OF MEMBERS

- Section 1. General meeting. The General Meeting shall be held at a time and place approved by the Society during a previous General Meeting, regularly at the World Congresses or Regional Congresses, or if a time or place for the Meeting has not been prescribed, it will be determined by the Council. The Council shall have power to change the time or place of a Meeting when circumstances so require.
- Section 2. Special meetings. Special Meetings of the Society may be called at any time by the President at the request of the Council, or on receipt of a written request of not less than one-third of the Financial Members.
- Section 3. Notice of meetings. Notice of the time, place, and purpose or purposes of General and Special Meetings of the Society shall be given to the Financial Members by electronic or other distribution means at least 90 days before the Meeting.
- Section 4. Quorum. At any General or Special Meeting of the Society, fifteen Financial Members must be present or represented by proxy to constitute a quorum. A Meeting may be adjourned by vote of a majority of the Financial Members present.
- Section 5. Voting. At every Meeting of the Society each Financial Member shall be entitled to one vote in person or represented by proxy. The proxy shall be duly appointed by instrument in writing subscribed by the Financial Member appointing the same and bearing date not more than 11 months prior to the Meeting.
- Section 6. Business of the general meeting. The business of the General Meeting of the Society shall be:
- (a) to elect Executive Officers and Members of the Council as prescribed by the Constitution;
  - (b) to determine the time and place for the next General Meeting;
  - (c) to fix the annual dues;
  - (d) to consider the annual Financial statement and balance sheet presented by the Council and to arrange for any action therewith as seems appropriate;
  - (e) to consider reports of the Council and Committees, and motions relating to the adoption of such reports, either in whole or in part, and to arrange for such action to be taken thereon, if appropriate and
  - (f) to consider any resolutions that can properly be considered to affect the purpose of the Society and its Membership.
- Section 7. Scientific Congresses of the Society. The Society exists for the principal purpose of furthering the science of toxinology and to that end shall promote interchange of ideas and research in toxinology through scientific meetings of the Society which shall be designated as Society Congresses.

The Executive Officers and Council shall work with the officers and members of Society Regional Sections to ensure regular Society Congresses are scheduled. By-Law XI governing Regional Sections of the Society shall guide the Executive Officers and Council in determining a schedule for Society Congresses.

The Society, through the Executive Officers and Council, shall have final say on the place, timing, budget, scientific and social programs for all Society Congresses.

All Members of the Society, from all categories, shall be entitled to attend Society Congresses, providing they pay any Congress fees that may be set for a Congress, except where such fees are waived, such as for invited speakers in some cases. Any person involved in toxinology, even though not a member of the Society, may be permitted to attend, at the discretion of the Congress organizers and Council, provisional on payment of any fees that may be set, but as a general principle the regular fees for attending a Society Congress shall be higher for a non-member or a Member who is

non-Financial, than for a Financial Member or Financial Associate Member or a Student Member.

## **BY-LAWS-ARTICLE II**

### **COUNCIL**

- Section 1. Meetings. The Council shall meet at each General Meeting for the purpose of trans-action of business, and if a majority of the Council be present, no prior notice of such Meeting need be given. Special Meetings of the Council may be held at the call of the President, or upon the written request of four Members of the Council, and shall be called by the Secretary/Treasurer. Meetings of Council, held either in person or through electronic communication, may be called by the Secretary/Treasurer to discuss and, where appropriate, decide on action in response to matters concerning the Society as may arise from time to time and no notice is required if the meeting shall be held by electronic communication except that Councilors shall have at least 48 hours to respond as part of the electronic communication process.
- Section 2. Notice of meetings. Notice of all meetings of the Council shall be given by electronic or other distribution means at least 15 days before the meeting, except where the Secretary/Treasurer calls an electronic communication meeting as noted in Section 1 (above), or in regard to General Meetings of the Society where a Meeting of Council is a requirement as noted in Section 1 (above).
- Section 3. Chairman. At all in-person meetings of the Council the President, or in his absence the President-Elect, shall preside.
- Section 4. Quorum. At all Meetings of the Council the act of a majority of those Members present shall be the act of the Council. In the absence of a quorum of the Council at a regular Meeting of the Society and when it becomes apparent that decisions vital to the Society are necessary, the Financial Members on the Editorial Board of Toxicon, in coordination with the Council Members, shall make the necessary decisions.

## **BY-LAWS-ARTICLE III**

### **ANNUAL AND Financial REPORTS**

The Council shall submit annually to the Society for adoption and approval a report on the general state and proceedings of the Society for the past year(s), a balance sheet and Financial statement for the past year(s).

## **BY-LAWS-ARTICLE IV**

### **CUSTODY OF PAPERS, ADDRESSES AND REPORTS**

All papers, addresses and reports read before the Society, or accepted by the Society, shall be lodged with the President and become the property of the Society. Publications of these reports in the official Journal of the Society, or in any other way, may be recommended by the Council or appropriate Committee.

## **BY-LAWS-ARTICLE V**

### **EXECUTIVE OFFICERS OF THE SOCIETY**

- Section 1. Duties. The Executive Officers of the Society shall perform the duties usually performed by such officers, together with such duties as shall be prescribed by the Constitution and ByLaws or by the Society or Council.
- Section 2. The President. The President shall preside at all Meetings of the Society and Meetings of the Council. The President shall be an ex officio member of all committees, except the Nomination Committee. The President shall have general charge and supervision of the business and affairs of the Society.
- Section 3. The President-Elect. At the request of the President, or in the event of his absence or disability, the President-Elect may perform any or all duties of the President.

Section 4. The Secretary-Treasurer shall: 1) attend to the giving of all notices of the Society; 2) have custody of all of the Society's funds and securities, subject to such regulations as may be imposed by the Council; 3) make such payments on behalf of the Society, subject to the control of the Council; 4) enter regularly into the records of the Society full and accurate account of all money received and paid, or obligations incurred on behalf of the Society, and shall exhibit such records at all reasonable times to any Financial Member of the Society on written request to the office of the Society; 5) provide a report on the Financial situation of the Society at each General Meeting; 6) provide a report to Council on new membership applications; 7) may be required to give bond for the faithful performance of his /her duties should the Council advise.

## **BY-LAWS-ARTICLE VI**

### **LIABILITY OF COUNCIL MEMBERS AND EXECUTIVE OFFICERS**

Each Council member or Executive Officer, or former Council member or Executive Officer of the Society, shall be indemnified by the Society against expenses actually and necessarily incurred by him/her in connection with the defense of any action, suit or proceeding in which he/she is made a party by reason of his/her being or having been a Council member or an Executive Officer of the Society, except in relation to matters as to which he/she shall be adjudged in such action, suit or proceeding to be liable for negligence or misconduct in the performance of his/her duties as such Council member or Executive Officer.

## **BY-LAWS-ARTICLE VII**

### **CONTRACTS**

The Council, except as provided in the By-Laws may authorize any officer or officers, agent or agents, to enter into any contract or execute and deliver any instrument in the name of and on behalf of the Society, and such authority may be general or confined to specific instances; and unless so authorized by the Council, no officer, agent or employee shall have power or authority. to bind the Society by any contract or engagement or to pledge its credit or render it liable Financially for any purpose or amount.

## **BY-LAWS-ARTICLE VIII**

### **FISCAL YEAR**

The fiscal year of the Society shall begin on the first day of January in each year and shall end on the thirty-first day of the following December.

## **BY-LAWS-ARTICLE IX**

### **PUBLICATIONS**

The official journal of the Society is Toxicon. Council shall recommend to the publisher of Toxicon (Elsevier) who should be appointed as Editor-in-chief, but the publisher shall have final say on who is appointed. Tenure for this position is not defined and is determined by the publisher. Society Newsletters will be edited by the Secretary/Treasurer of the Society in order to inform the membership on current Society affairs.

Council may determine, by majority vote, to recommend to the membership of the Society that a further publication or publications be designated as official publications of the Society, but such a recommendation shall only come into effect if a majority of Financial Members vote in favor of the recommendation at a properly constituted General or Special Meeting of the Society.

## **BY-LAWS-ARTICLE X**

### **REDI AWARD**

In recognition of distinguished work in the field of toxinology the Society confers the Redi Award at successive international meetings as determined by Council. The Redi Award consists of a framed award describing the merits of the awardee and a Financial contribution to help cover expenses as-

sociated with attendance at the meeting.

The recipient is selected by the Redi Award Committee (R.A.C.) which consists of the Editor of Toxicon (chairman), past and present Executive Officers of the Society and former Redi awardees. It is the duty of the chairman to request that members of the Committee propose nominations one year in advance of the next presentation. A list of all persons previously nominated and those being currently proposed is then sent to all members of the R.A.C., who then select three names, noting their first, second and third choices.

The chairman will award 3 points for 1st place votes, 2 points for 2nd place and 1 point for third place. The awardee is chosen on the basis of the largest number of points, but must receive 25% of the total points counted.

If no candidate reaches this level there shall be a second ballot between the three highest candidates. (Or: If 2 or more candidates receive approximately (within three votes) the same number of votes, a second ballot will be circulated. In the event two or more candidates receive the same number of votes in the final ballot, or a difference of less than three, the award will be shared equally.)

## **BY-LAWS-ARTICLE XI**

### **REGIONAL SECTIONS**

To promote the aims of the Society and to encourage local participation in the discipline of toxinology Regional Sections may be established. These must be approved by the Council. At Regional Meetings a Regional President, a Regional Secretary (and if necessary up to three additional members as officers) may be selected. They will serve a fixed term, but can be re-elected. Council shall determine the length of the fixed term and shall modify this fixed term, as necessary, to fit with meeting schedule rosters, but the fixed term shall not be less than 2 years and not more than 4 years.

Council will determine the meeting schedule roster between Regions, such that each Region is regularly responsible for organizing both Regional and World Congresses of the Society, according to the roster.

A Regional Section of the Society encountering difficulty in organizing their rostered meeting may apply to Council to have their roster position deferred and Council can determine to allow such deferment, providing another Regional Section can appropriately agree to swap roster positions with the Region seeking deferment.

At the time of adoption of this Constitution the Society has three Regional Sections; European, Pan-American and Asia-Pacific Sections. The roster for meetings of the Society is based on the number of Regional Sections, and their ability to regularly host full Sectional and World Congresses. Council shall determine the roster and shall amend the roster, as necessary, depending on prevailing circumstances, including the addition of new Sections and the dissolution of current Sections.

The Officers of a Regional Section will be responsible for organizing scientific meetings (Congresses) of the Society in those years in which they are rostered to do so, by direction of Council. Regional Sections may not collect fees or other monies for the Section. All Financial affairs will be the responsibilities of the Secretary/Treasurer of the Society. Exceptions due to special circumstances should be discussed with and agreed by the Council.

The Society does not indemnify any officer of a Regional Section, or Executive Officer of the Society, or any Member of the Society, who incurs any debt or obligation in the course of organizing a meeting of and on behalf of the Society, except where such indemnity is agreed by Council under By-Law VII, but requires that the Executive Officers and Council of the Society be involved in the planing, budgeting and scientific and social programs for the meeting. Notwithstanding the lack of indemnity, the Society, as determined by the Executive Officers and Council may, at their discretion, agree to provide Society funds in support of the meeting, with such conditions and guarantees as may be determined in each such case. The use to which such funds may be put shall be at the sole discretion of the Executive Officers and Council.

Members of IST are automatically members of a Regional Section in their specific region, provided their Financial obligations to the Society are current.

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## Getting to Know Us

In the previous issue of the newsletter we introduced a new section called Getting to Know Us. The aim of this section is to introduce either an individual toxinology laboratory or an institute with a focus on venoms or toxins to the toxinology community. Each article will cover the history, major areas of interest, and primary research methods of the laboratory or institute. In this way we hope to foster collaborations between toxinology laboratories around the world.

We are delighted that Dr Robert Harrison agreed to write the inaugural article for this section covering the history and scientific objectives of the Alastair Reid Venom Research Unit at the Liverpool School of Tropical Medicine in Liverpool, England. You will find his fascinating article in the previous issue of the newsletter.

If you would like to contribute an article to this section on your laboratory or institute, or you have a suggestion about who we should contact for submissions, please email Glenn King at [glenn.king@imb.uq.edu.au](mailto:glenn.king@imb.uq.edu.au).

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**REPORT ON THE INTERNATIONAL SYMPOSIUM ON CORAL SNAKES**

**Prof. Julian White  
Toxinology Dept. WCH**

**Introduction**

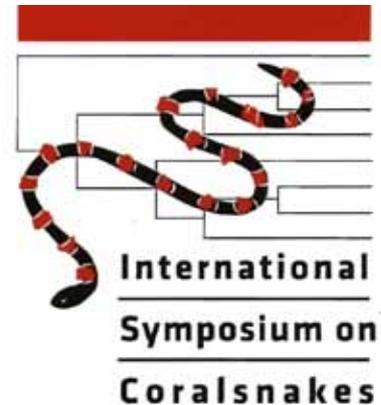
This symposium was devised to both bring together researchers in many fields working on coral snakes and to honor an important contributor to coral snake research, Prof. Janis Roze. The principal organiser was Prof. Nelson Jorge da Silva Jr., a zoologist at the major private catholic university in Goiania, Pontificia Universidade Católica de Goiás (PUC Goiás). In Brazil there is a strong catholic university system, of which this university is part. It has a large campus and student body in Goiania which is Brazil's 11th largest city with about 2 million people, situated in the savanna region of central inland Brazil.

Coral snakes are Elapid (cobra-like) snakes, the same family as Australia's dangerous venomous snakes. I was invited to attend to give 2 lectures covering Elapid snakes in the rest of the world, outside of the Americas. While I knew several of the other international invited speakers, I did not know the local organisers of this symposium, nor had I been to Goiania previously.

The symposium covered all aspects of coral snake biology, zoology, taxonomy, biogeography, toxinology and clinical effects of bites, plus covered a broader review of snake venom, particularly Elapid venom toxinology and clinical effects. My lectures covered Elapid snake taxonomy, venom summary, clinical effects of envenoming and treatment for Elapid snakes in Africa, Asia, Australia, New Guinea and the Pacific, including sea snakes.

The symposium was held at a large lecture hall in PUC Goiás, Goiania, October 17-21, 2016. There were over 300 people registered for the symposium. The symposium was originally advertised as "international" and in English, but most of those registered were from Latin America, especially Brazil and unfortunately many spoke no English. Therefore, and to my consternation, many of the lectures and most post-lecture discussions, were in Portuguese, or less commonly, Spanish. Fortunately most lecture slides were in English and I and some other English speaking invited lecturers had the services of local translators sitting immediately behind us to summarise what was being said. I sympathise with the main symposium organiser, Prof. da Silva, who had two conflicting language groups to please, ultimately being forced to accept that many Latin American speakers chose to speak in their native tongue, even though requested originally to speak in English. Despite this difficult language issue, the symposium presented a large amount of valuable information and a number of expert speakers, so was certainly worthwhile, quite apart from the very valuable opportunities to network with colleagues during breaks.

I also had the opportunity to visit the PUC Goiás snake collection facility and see and photograph a range of local medically important snakes.



**17 a 21**  
Outubro  
2016

Auditório da área  
IV, Campus I da  
PUC – Goiás



Report on the International Symposium on Coral Snakes, Goiania, Brazil, October 2016



Lecture theatre at PUC Goiás



Symposium organiser, Prof. Nelson Jorge da Silva

**Summary of Presentations**

**The relationship between complexity, variability and toxicity in North American coral snakes**

Dr. Mark Margres (Florida State University, USA)

Mark, a grad student, discussed (in English) his studies on the genetic basis of adaptive traits, noting the difficulty in finding relevant traits and genes. He then posited that venom provided a good tool, a view which I would question, since previous research has shown, in my opinion, a poor linkage between venom diversity/complexity and adaptation to varying microenvironmental and niche requirements. Mark’s studies concentrated on comparison of *Micrurus fulvius* (Florida coral snake) versus *Crotalus adamanteus* (eastern diamondback rattlesnake), particularly focussing on PLA<sub>2</sub> and 3-finger-toxins (3FTx).



**Coral snakes of Brazil**

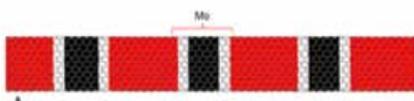
Prof. Nelson Jorge da Silva (PUC Goias, Brazil)

Nelson gave a comprehensive lecture (in English) covering the known species of coral snakes in the Americas, with an emphasis on those in South America, where there is a vast diversity of species, most within just a single genus, *Micrurus*. The previously documented distribution, as provided by Campbell and Lamar in their classic book, was updated using comprehensively sourced museum records which helped to highlight collection bias caused by inaccessibility of certain regions. Checking these museum records took 12 years, so a major study. What is striking was the diversity of body coloration and ring patterning, or for some species, lack thereof. Coral snakes in the Americas are commonly characterised by their striking multi-colored ring patterns along the body, classically variations on reddish, yellowish and black rings.



It appears that the distinctive coloration of coral snakes may not necessarily be a deterrent for predators, especially since this classic pattern is not seen on all species, though is distinct within each species. I therefore queried why it had evolved so consistently, for which there is no current answer it seems. Clearly an area for further research, likely at PhD or postdoc level.

The symposium also coincided with release of a new textbook on coral snakes and two identification charts, using the species-specific distinctive coloration to differentiate between very similar species. Of those coral snakes with classic ring patterning, there are two major types; those with a bi-color ring pattern (“mondal”) and those with a tricolor pattern (“triadal”). According to this comprehensive global revision, there are 77 species in *Micrurus* (47 spp. mondal; 30 spp. triadal, with 5 bicolor, 2 spp. in Central America and 24 spp. in South America), plus 4 species in *Leptomicrurus* and one species in *Micruroides*.



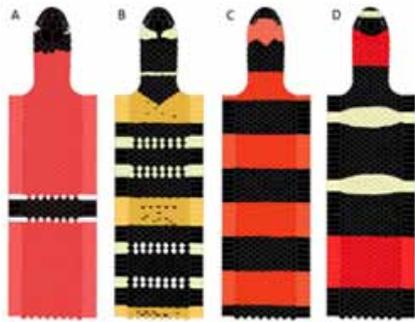
Mondal system pattern



Triadal system pattern

Illustration courtesy Prof. Nelson da Silva

Report on the International Symposium on Coral Snakes, Goiania, Brazil, October 2016



- A. Mondal
- B. Central American Triadal
- C. Bicolor
- D. South American Triadal

Illustration courtesy Prof. Nelson da Silva





Photos of diverse species of *Micrurus* illustrating the wide scope for diversity in coloration and patterning within the described mondal/triadal pattern schema. Note that some species have no “warning” coloration.

Illustrations courtesy Prof. Nelson da Silva

**Taxonomic revision of Argentinian coral snakes**

*Dr. Alejandro Giraudo (Instituto Nacional de Limnologia, Argentina)*

(Lecture presented in Spanish) Alejandro’s group examined 500 snakes from 23,000 museum records to determine the taxonomic and biogeographic status of coral snakes in Argentina. *M. frontalis altirostris* was elevated to full species, *M. altirostris*, thus there are now 8 *Micrurus* spp. in Argentina (*M. pyrrhocryptus*, *M. frontalis*, *M. altirostris*, *M. crallinus*, *M. lemniscatus*, *M. baliocoryphus*, *M. silviae*, *M. tricolor*), while previously only 5 species were listed. This was clearly a detailed and definitive taxonomic study.



*M. pyrrhocryptus*      *M. baliocoryphus*      *M. frontalis*      *M. altirostris*      *M. lemniscatus*      *M. silviae*      *M. corallinus*      *M. tricolor*

Illustration courtesy of Dr. Alejandro Giraudo

## Natural history of the New World Elapidae: what we know and what we do not know

Dr. Otávio Augusto Vuolo Marques (Instituto Butantan, Brazil)

(Lecture presented in Portuguese) A comprehensive presentation. Some coral snakes can live up to 16 years, all lay eggs which hatch around February/March and there is sexual dimorphism. In monodals females are longer, while in triadals, males are longer. Microhabitat varies between species, but these are generally fossorial snakes, hiding under leaf litter or, in some species, underground. They occur across most habitats, from rainforest to arid areas and some (e.g. *M. surinamensis*, *M. lemniscatus*) are aquatic. There are diurnal species, though many may be nocturnal. Diet varies with species and with region/habitat; *M. corallinus* has a diet varied between lizards, snakes (mostly blind snakes), amphisbaenians and caecilians depending on geographic region. Some species feed on other snakes, some on caecilians, some on onychophorans (velvet worms). These are active foraging snakes, preferring long thin prey (snakes, reduced-legged lizards, amphisbaenians, caecilians, onychophorans), usually eaten head-first, but with long swallowing times (40+ minutes) during which time they are vulnerable. Predators include birds, opossums and boars. It appears the patterning may offer disruptive benefits, improving camouflage in their common leaf litter habitat. The oft quoted “warning” coloration of distinctive rings may, in fact, be mimicry by coral snakes (and some other snakes) of more primitive invertebrates that are poisonous. An example given was the flat worm, *Bipalius* sp. and apparent mimicry by *Sinomicrourus japonicus*. Defensive behaviors include tail curling, head hiding, emitting “popping” sounds, erratic body movements, feigning death, and cloacal discharges. All are egg layers, but at least some species use sperm storage in males. Male-male combat occurs during the mating season.



## The intense sexual activity in the genus *Micrurus*: mating aggregations, male-male fights, courtship, sperm storage, and sperm competition

Dr. Selma Maria de Almeida-Santos (Instituto Butantan, Brazil)

(Lecture presented in Portuguese) This lecture, which included several videos of male-male combat and of mating, focussed on reproductive behaviour and the split into two distinct strategies. The monadal group where females are usually larger, male-male combat does not occur and vitellogenesis (production of yolk, prior to egg formation) occurs in spring synchronous with mating. This group relies on a mating aggregation strategy with multiple males courting each female. This may then favor sperm competition in the female, obviating the need for male-male combat to select the fittest mate. An example from this group is *M. corallinus*. In triadal species males are larger, male-male combat occurs, there is a broader season of vitellogenesis and oviposition, while mating occurs in autumn synchronous with spermatogenesis and vitellogenesis. In this group a single mating by a dominant male determined by combat is the apparent strategy. Examples from this group include *M. frontalis*, *M. altirostris*, *M. ibibiboca*, *M. lemniscatus*.



Illustration courtesy of Dr. Selma Maria de Almeida-Santos

### Herpetological collections: a vision of the future

*Prof. Ana Lucia da Costa Prudente (Museo Paraneze Emilio Goeldi, Brazil)*

(Lecture presented in Portuguese) Ana, as Director of the herpetological section at the museum in Belem, one of the major “public” collections (Museo de Zoologica, University of Sao Paulo, Sao Paulo; Museo Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro; Museo Paraneze Emilio Goeldi, Belem), presented a possibly controversial view that the many (>40) private museum collections in Brazil should have their collections registered with information available online and they should allow external researchers to work with their material. She considered the challenges for the future centre around complete accessible information which will include attention to curation, geographic coverage, data availability, public policy and physical and data security. The issue of physical security was highlighted by the catastrophic fire at Instituto Butantan in 2013 which destroyed their entire, massive and irreplaceable specimen collection which was centered on snakes (77,000 specimens) and other venomous animals.



### The role of museum herpetological collections as guardians of coral snake diversity

*Dr. Patrick Campbell (British Museum of Natural History, UK)*

Dr. Campbell, who has been at the Natural History Museum, London for 30 years, presented (in English) an overview of how the NHM was founded, grew, is organised, and how it acts as a vital resource of information and research material for the animal kingdom, including the large herpetological collection of 175,000 specimens. Specimens are housed mainly in alcohol (95% ethanol, 5% methanol, diluted to 80% in water). In 2011 a new spirit room was opened with a controlled temperature environment (13°C), improved fire prevention (oxygen exclusion) and better OHS-based facilities. Famous contributors to the herpetological collection include Gray, Gunther, O’Shaughnessy, Boulenger, Grandison, Procter, Parker, Underwood, Arnold, McCarthy. Patrick emphasised that the curatorial staff, which he leads in herpetology, are not researchers, but custodians of the collection with a primary role in maintaining the viability of specimens in the collection and ensuring appropriate expansion of the collection. They also have an active role in education and in assisting external researchers. They can conduct research themselves, but as a secondary function when time permits. The NHM has major in-house research facilities including molecular labs and Xray equipment (even a CT scanner) to assist researchers, both external and those employed specifically for research by the NHM. The NHM is now the 3rd most visited UK tourist attraction. Entry is free. It is not immune to controversy, the most recent public issue being the plans to move the *Diplodocus* dinosaur skeleton from the main entrance hall and replace it with a whale skeleton. The NHM had its origins in the private collection of “curiosities” collected by Sir Hans Sloane (1660-1753) and donated to the nation, then housed and expanded through a progression of buildings, now in the current building which was opened in 1880. This building is too small for the vast NHM collections, so much is stored off-site, such as at the NHM premises at Tring. In regard to coral snakes, the NHM has a number of important specimens which are available for research.



### The taxonomic status of the *Micrurus lemniscatus* species complex

Dr. Matheus Godoy Pires (PUC Goiás, Brazil)

(Lecture presented in Portuguese) Matheus detailed his studies into the *M. lemniscatus* complex. He examined 976 specimens morphologically, including basic scalation, color pattern, hemipenes, skulls etc. All known type specimens were examined. Species included were *M. lemniscatus* (subspecies *lemniscatus*, *carvalhoi*, *helleri*), *M. diutius*, *M. potyguara*, *M. frontifasciatus*, *M. serranus*. As a result of this research, *M. lemniscatus helleri* was subsumed into *M. lemniscatus*; *M.l. carvalhoi* was raised to full species level as *M. carvalhoi*; *M. diutius* and *M. potyguara* are confirmed as distinct species within the *M. lemniscatus* complex; *M. serranus* is removed from this complex; *M. frontifasciatus* remains uncertain and is considered a synonym of *M. lemniscatus*, subject to confirmation once more specimens become available for examination.



### The taxonomic status of the Amazonian coral snake, *Micrurus spixii*

Lywouty Nascimento (PUC Goiás, Brazil)

(Lecture presented in Portuguese) An overview of a research project looking at the taxonomy of this snake. The main conclusions were that *M. spixii* subspecies *martiusi* and *principis* are not valid and should be subsumed into *M. spixii*. *M.s. obscurus*, on the other hand, should be elevated to full species, *M. obscurus*.



### A first insight into the systematics and spatio-temporal evolution of old world coral snakes (genera *Calliophis* and *Sinomicrurus*)

Uptal Smart (University of Texas at Arlington, USA)

An interesting lecture (in English!). Old world (Asia in this case) coral snakes comprise 16 species in 2 genera, *Calliophis* and *Sinomicrurus*. This lecture provided a summary of complex studies on evolution of these snakes. First he discussed *Calliophis*, separated into the Indian (*C. bibroni*, *C. melanurus*, *C. maculiceps*, *C. castoe*, *C. nigrescens*) and Sundaland (SEAsia) (*C. bivirgata*, *C. intestinalis*) taxa. The more diverse genus *Sinomicrurus*, had competing hypotheses developed and tested, some found disproved. It appears that the main phylogenetic driver for *Sinomicrurus* evolution was a pre-pleistocene diversification on ancestral taxa; these latter occupied a locus on Ryukyu, Taiwan and mainland (all joined by land bridges during glacial period), with divergence during Miocene followed by multiple dispersals and accumulation of endemic diversity was a result of allopatric speciation. This corresponds with similar findings in other taxa (geckos, Natricine snakes, spiders, scorpions).



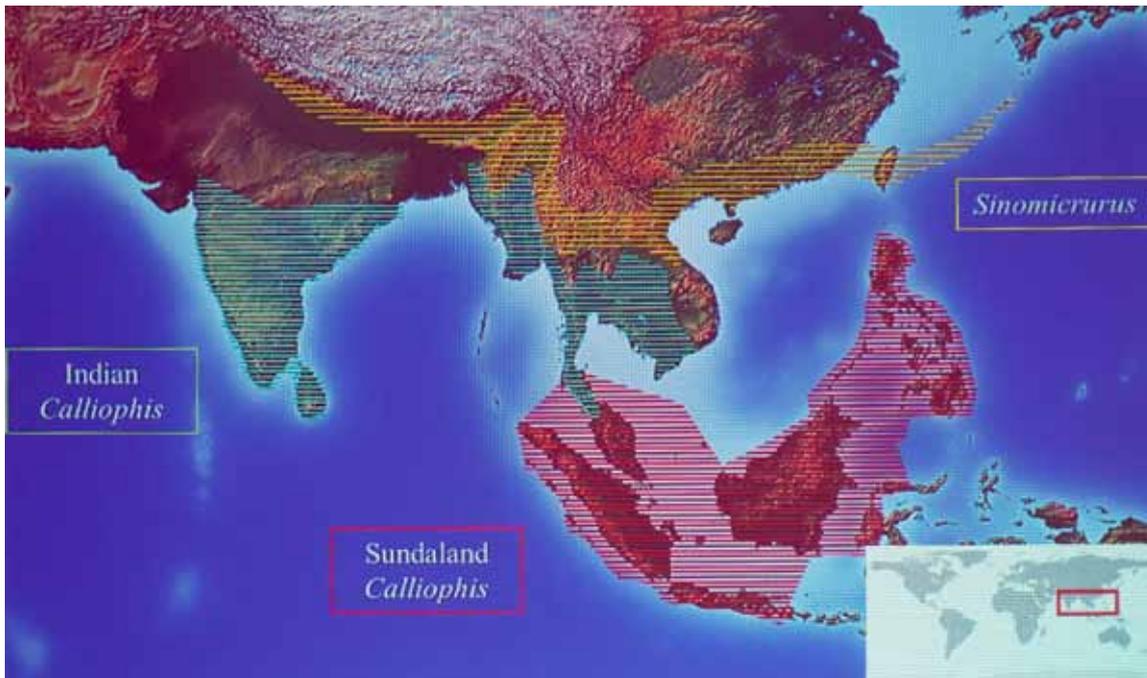


Illustration courtesy of Uptal Smart

### Phylogeography of *Micrurus surinamensis* and *Micrurus lemniscatus*

Renan Bosque (PUC Goias, Brazil)

Another student lecture in English, discussing the warning coloration and mimicry in coral snakes versus “colubrids”, with plenty of background information and details on methodology, but unfortunately no results available. The aim of the research is to test model signal diversity (e.g. snake color pattern) on predator learning and then relate this to possible evolution of pattern diversity, with an ultimate aim of linking this to molecular/genetic changes/evolution. In the models developed, *Micrurus* (*M. surinamensis* and *M. lemniscatus*) will be compared to the “colubrid” *Oxyrhopus*.



### Mimicry and local variations in coral snakes: the example of *Erythrolamprus false coral species*

Dr. Felipe Franco Curcio (Universidade Federal do Mato Grosso, Brazil)

Felipe presented (in Portuguese) an overview of mimicry, noting it was a complex involving the model, the mimic and the dupe, the latter being the animal, usually a predator, dissuaded from attacking the mimic because of its similarity to the model (dangerous) species. The two main systems, Batesian and Mullerian mimicry were discussed. However, a number of the likely predators for coral snakes and their mimics are mammals that do not see colours and hunt at night when colour becomes irrelevant, notable since many coral snakes are nocturnal. Birds and monkeys do see colour and hunt diurnally, so possibly these are the dupes for mimicry. As mentioned in some previous lectures, the use of coloured plasticine models to mimic either coral snake patterns, or no patterns, are extensively used to test for effectiveness of mimicry. Another issue is the lethality of the model species; if it is likely to kill a predator then no learning is possible, so no benefit in a “warning” appearance. A number of predators, notably birds, appear to have innate recognition of coral snake colouration/patterns.

### Venomomics of Brazilian coral snakes

Dr. Steven Aird (Okinawa Institute of Science and Technology, Japan)

Steven presented an overview (in English), pointing out much was still to be discovered and that there was a need to develop toxin assays in natural prey such as lizards, amphisbaenians, fish etc. He noted that *M. surinamensis* eats electric fish.

Transcriptomes of 6 *Micrurus* spp. have been examined, revealing a wide range of components and major differences between species. He then concentrated on some specific toxin classes, commencing with the 3-finger-toxins (3FTx). To date >300 3FTx have been isolated from *Micrurus* spp., with varying structures. The muscarinic 3FTx subgroup bind to the muscarinic ACh receptor and may also target the equivalent glutamate receptor in arthropods. The Kunitz Serine Protease Inhibitors (KSPIs), which include dendrotoxins from mamba venom are larger than 3FTx and often target K<sup>+</sup> ion channels, though may not share this target for *Micrurus* spp. toxins. Their role in pain causation after bites is uncertain. Vascular endothelial growth factors (VEGFs) which enhance vascular permeability and vasodilation, thereby inducing hypotension, occur in *Micrurus*, though their clinical relevance is uncertain.

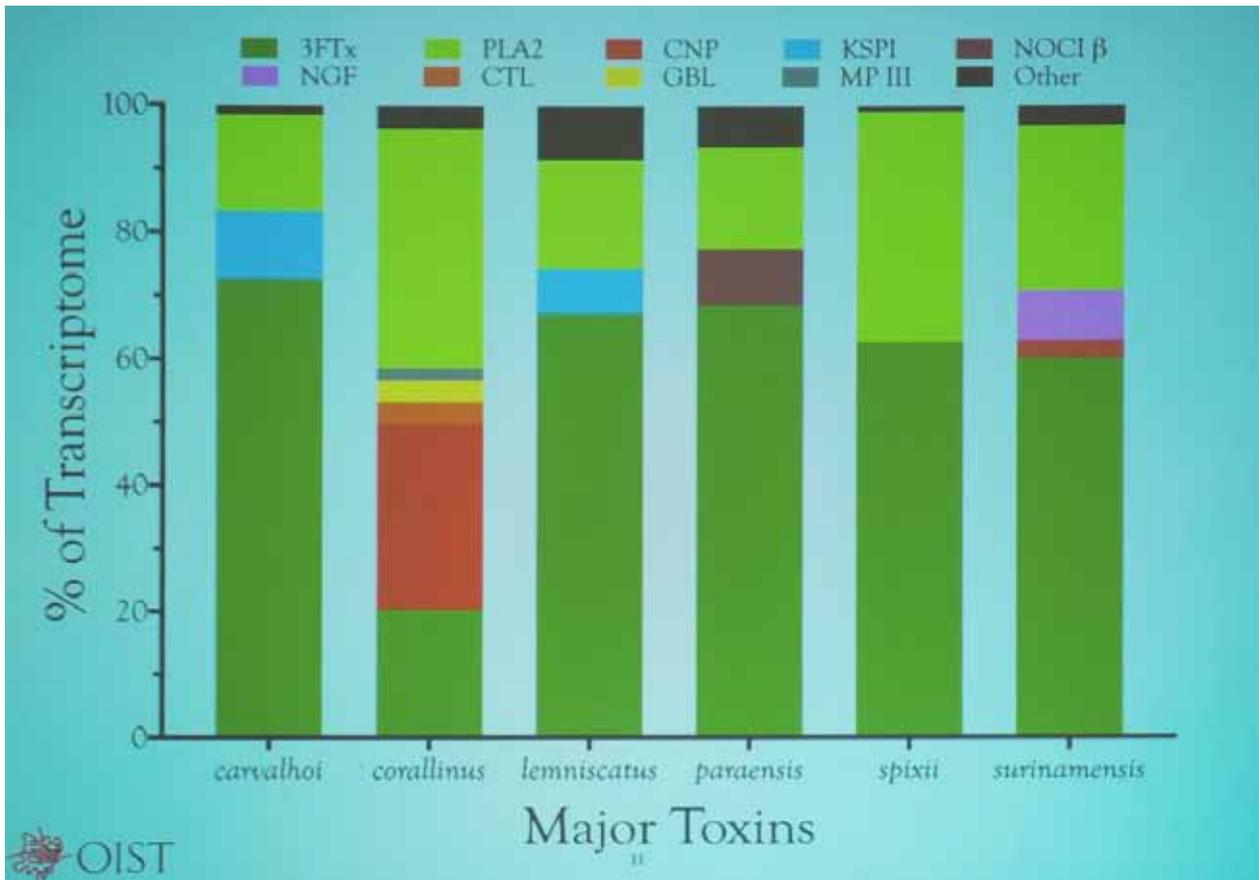
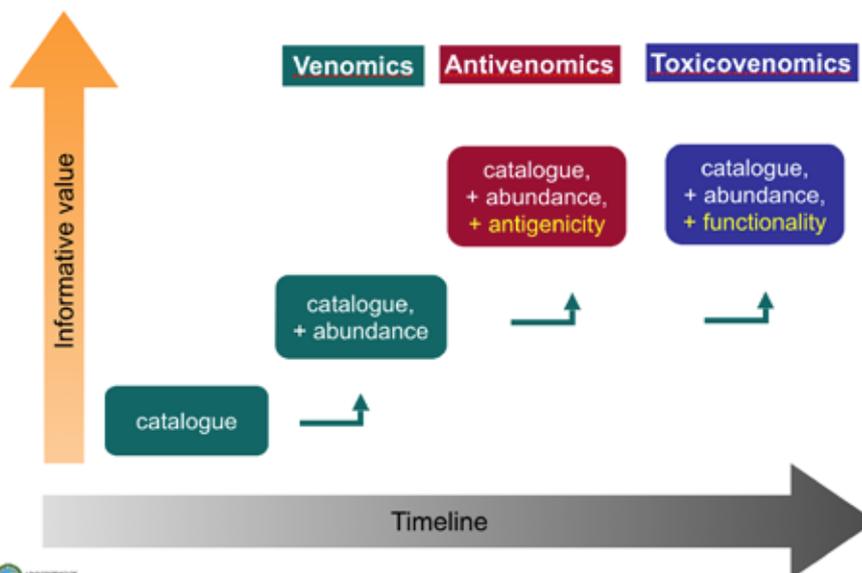
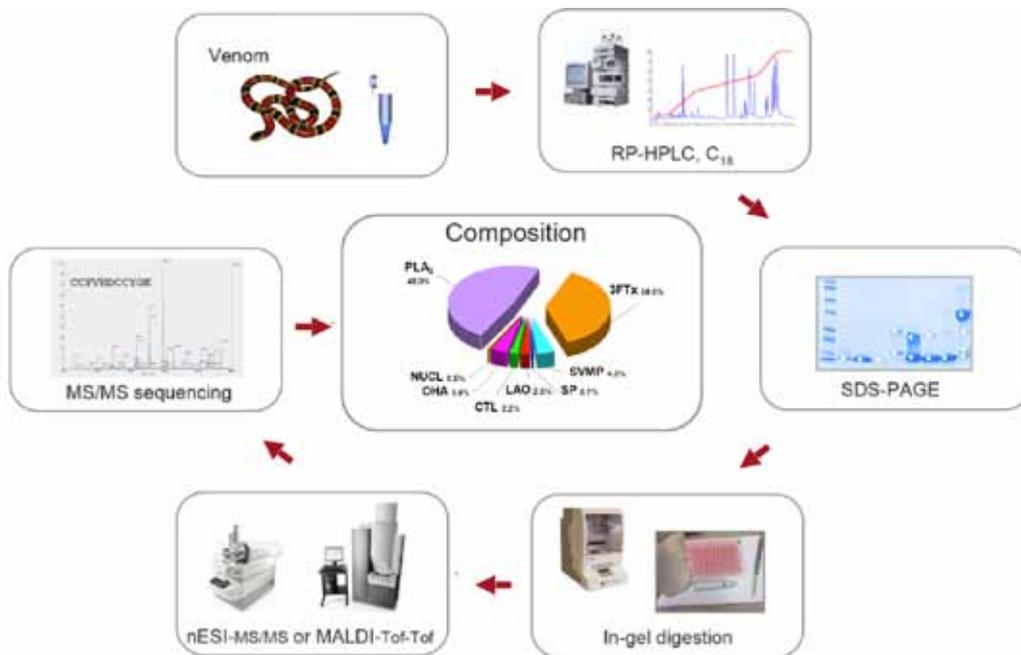


Illustration courtesy of Steven Aird

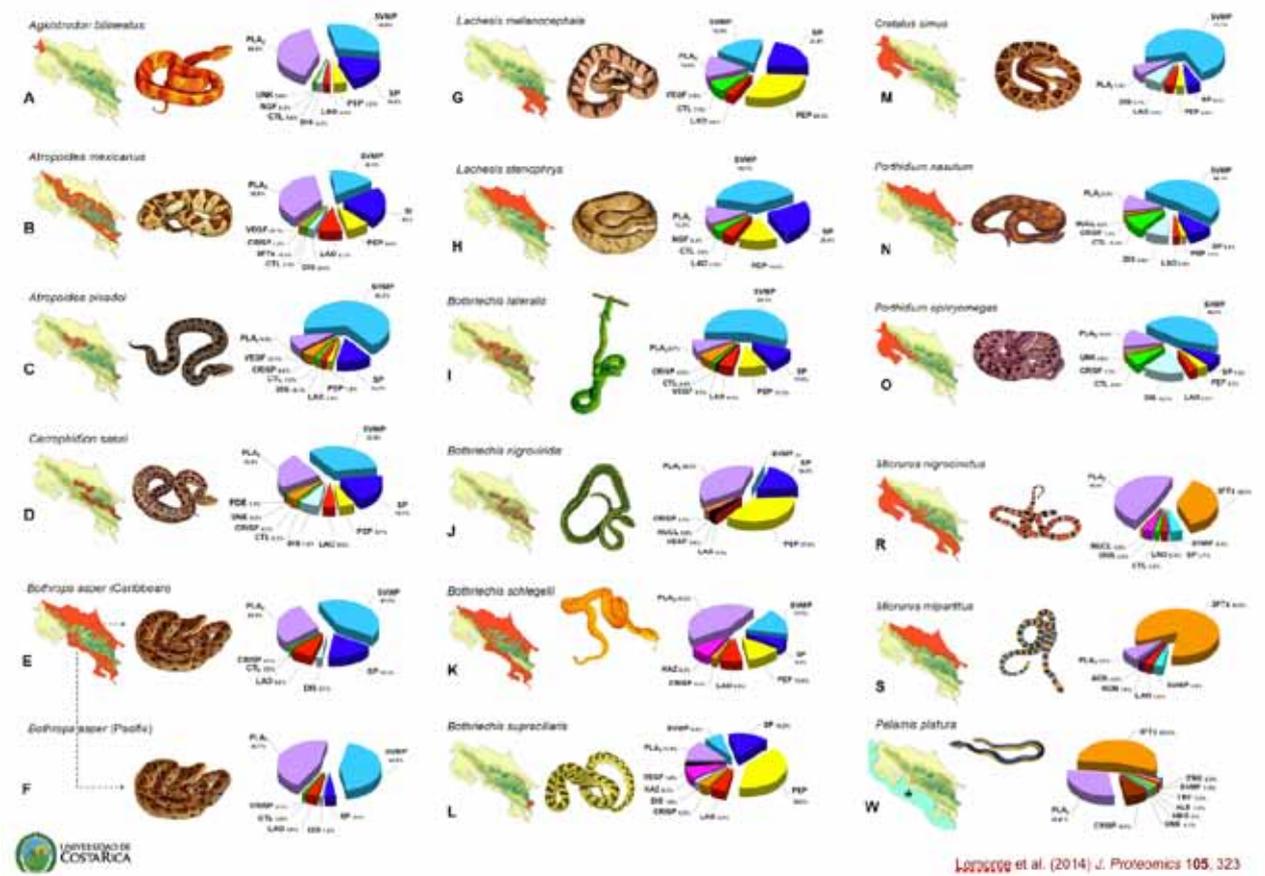
### Venoms of *Micrurus* coral snakes: evolutionary trends in compositional patterns emerging from proteomic analysis

Dr. Bruno Lomonte (Instituto Clodomiro Picado, Costa Rica)

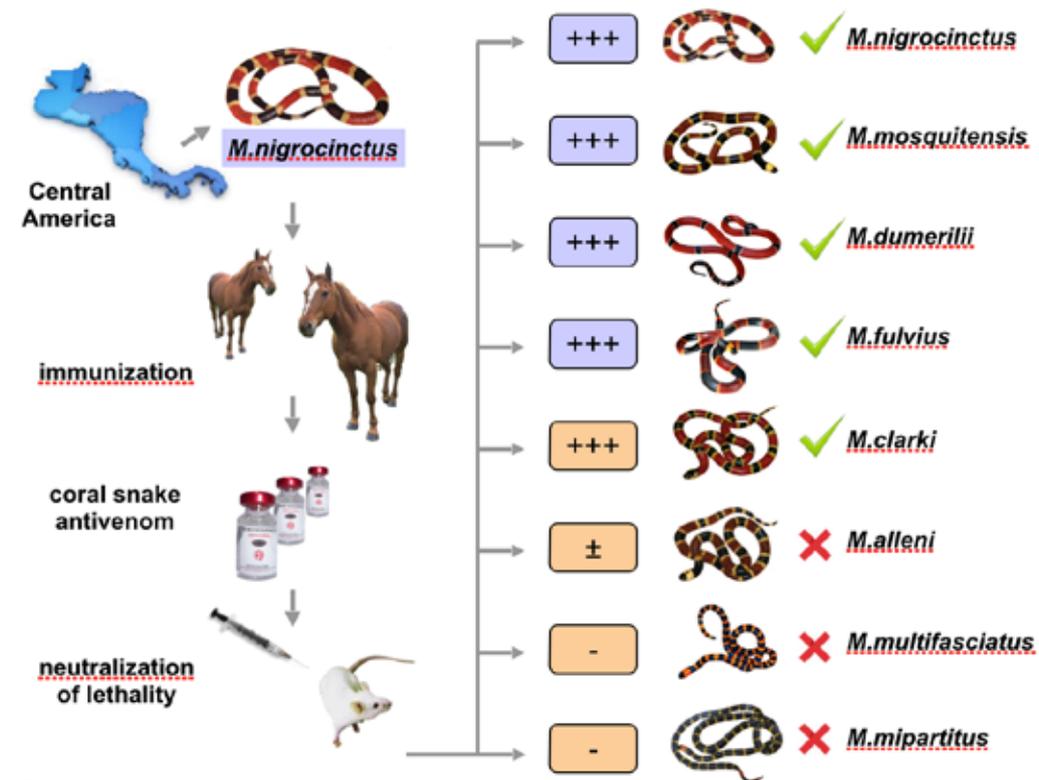
Bruno provided a comprehensive overview (in English) of *Micrurus* venom proteomics and how this relates to presumed evolution of these snakes. Only about 20% of *Micrurus* spp. have had this level of proteomic analysis, therefore theories around evolution may change once more species are covered. He detailed the proteomic discovery process used, but noted that further work is needed to elucidate the structure and function of components identified during the proteomic survey. His team use a combined gel-based and LC-based approach. He also mentioned the relationship of venomomics, proteomics, antivenomics and toxicovenomics. One of the problems he noted was the limitations on obtaining *Micrurus* samples because of their poor survival in captivity. Here he noted development by his colleagues of a successful captive program for *Micrurus* using fish as the primary diet.



Proteomic profiles of the venoms of Costa Rican viperid snakes



Implications for serotherapy



Illustrations courtesy of Bruno Lomonte

### Venom yields of Brazilian coral snakes

*Prof. Nelson Jorge da Silva (PUC Goias, Brazil)*

Nelson detailed (in English) results from 613 individual venom extractions, of which 277 were accompanied by snake body measurements. For those with body measurements, the largest snakes were *M. frontalis*, closely followed by *M. surinamensis*, with the latter yielding the most venom (52.67mg), while the smallest was *M. decoratus*, with close to the lowest venom yield (8.06mg - the lowest was *M. albicinctus* with 8.04mg), though it did have the lowest average yield. *M. surinamensis* consistently produced substantially more venom than other tested species.



### Elapid snakebites in Africa and Asia

*Prof. Julian White (Women's & Children's Hospital, Adelaide, Australia)*

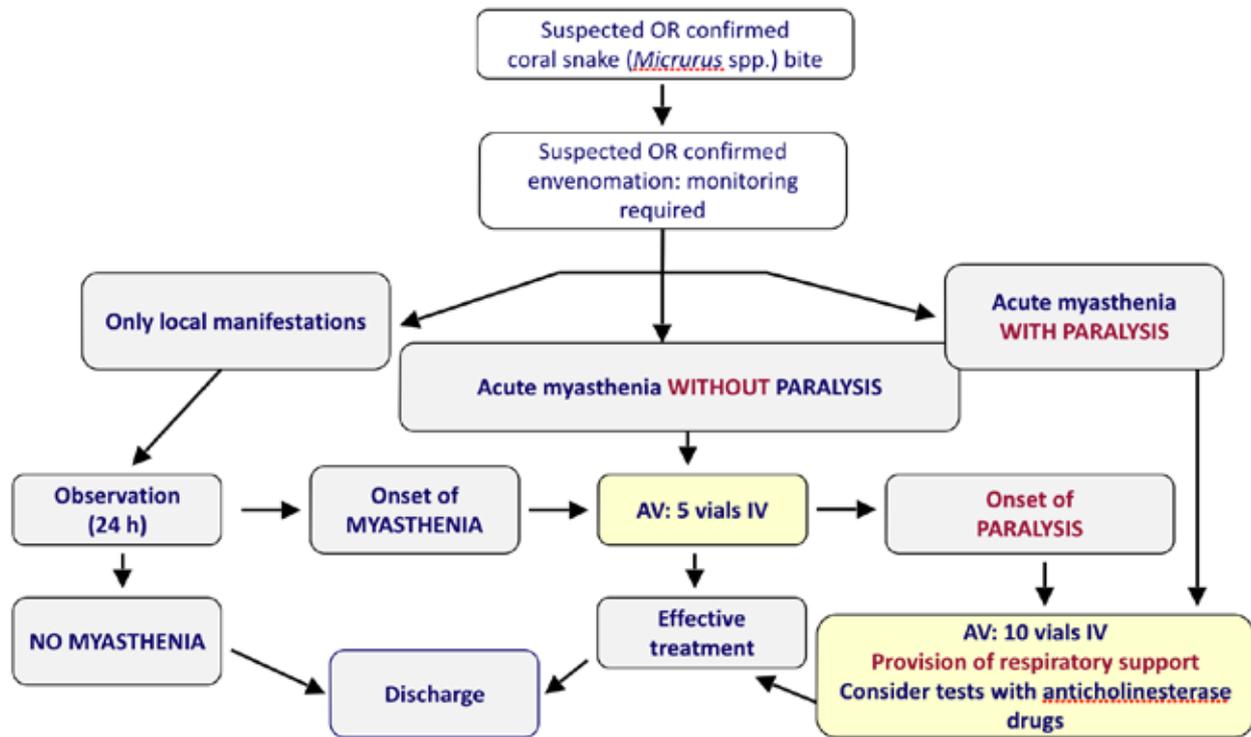
Prof. White presented (in English) an overview of Elapid snakes globally and a selection of species from Africa and Asia, including African cobras (spitting, non-spitting, non-necrotic), mambas, minor African elapids, Asian cobras (spitting, non-spitting, non-necrotic), kraits, minor Asian elapids, listing medical problems encountered in envenoming and an overview of the treatment pathway.

### Coral snake bites in Brazil

*Prof. Fábio Bucarechi (Iniversidade Estadual de Campinas, Brazil)*

Fabio presented (in Portuguese) his studies documenting reports of coral snake bites in Brazil since 1867. This yielded 30 reports covering 194 cases, with usable information on 150 cases in 25 reports, the majority published in Portuguese, so not widely accessible. He noted a further 74 cases have since been published from a separate study at Instituto Butantan and Hospital Vital Brazil. The large majority of cases where the snake was identified were ascribed to bites by either *M. corallinus* (24%), or *M. frontalis* (8%), with >60% of cases with either no confirmation of snake ID, or only to genus level (15%). Most bites occurred on distal limbs, with fingers/hands nearly twice as common as feet. Parasthesia and local pain were the most prominent clinical features. Paralytic features, manifest as at least ptosis, were seen in nearly 60% of cases. 20% showed only local effects. Myalgia was reported in just under 10% of cases, but CK rise was only noted in 3 cases (range 500 to 1766 IU/l). Fatality was a rare outcome. Dry bite rate was 14%. 77% received antivenom, mean 10 vials. Only 5 cases required ventilation (3x *M. corallinus*, 1x *M. surinamensis*, 1x no ID). Neostigmine was trialled in 9 cases, with a positive response in 5 (2x *M. frontalis*, 3x no ID); the positive response in *M. frontalis* cases is consistent with Vital-Brazil's research using monkeys. Amongst the few fatal cases, mostly old cases from the first half of the 20th century, complete paralysis was the common theme, usually without an option for mechanical ventilation.





**Algorithm for the treatment of patients bitten by coral snakes in Brazil**  
(Brazilian Ministry of Health, 2014).

Illustration courtesy of Fabio Bucarechi and the Brazilian Ministry of Health

### **Coral snake bites: historical aspects and the clinical experience of Instituto Butantan**

*Dr. José Yamin Risk (Instituto Butantan, Brazil)*

This lecture was presented in Portuguese, with slides in Portuguese and appeared to be a recapitulation of an historical study previously published. I was unable to gain anything useful from this presentation.

### **Coral snake bites in Argentina**

*Dr. Adolfo Rafael De Roodt (Universidad de Buenos Aires, Argentina)*

Adolfo (presenting in English) first noted the epidemiology of envenoming fatalities in Argentina, where hymenopteran stings cause the most deaths (26%; allergy), followed by snakes (23%), scorpions (20%), spiders (14%) and miriapods (5%). However, recent data from mandatory reporting of cases of envenoming accidents (not just deaths) indicates that scorpion stings are far more common (77%) than spiderbite (14%), or snakebite (9%). The rate of snakebites appears to have fallen since 2006, but is now steady and is maximal in the poorer northern regions. Of the approximately 1,000 snakebites cases/yr, only 0.2% are by coral snakes and these cause very few deaths and many bites are in rural workers (55%), so an occupational hazard. Where clinical data was available, local pain occurred in 69% of cases, oedema in 33% and only 9% developed any signs of respiratory neurotoxicity. The government produces about 2,000 vials of antivenom /yr for coral snake bites with a recommended dose of 10 vials. Adolfo finished his lecture by noting that published studies show that Australian (CSL/Seqirus) snake antivenom, particularly Tiger Snake AV, is effective against South American *Micrurus* venoms and possibly more effective and with greater coverage than local AVs.



### Coral snake bites in Colombia

Dr. Rafael Otero-Patino (Universidad de Antioquia, Colombia)

Rafael, though an important documenter of envenoming in Colombia, chose to speak in Spanish and grossly overran his allotted time. He noted that the number of snakebites reported in Colombia is steadily rising, currently >4,000/yr (9 per 100/000 pop./yr). 90% of cases are due to *Bothrops* spp., 2% to *Lachesis muta*, 1% to *Crotalus* spp. and only 0.4-0.8% due to *Micrurus* spp.. Of those *Micrurus* causing bites, 40% are *M. dumerilii*, 37% *M. mipartitus*, 7% *M. nigrocinctus*, 7% *M. isozonus*, with the rest causing few or no cases. The Antioquia region has 60% of cases, with a scattered few cases in other regions. Unlike Brazil, the majority of bites are to the feet (53%), with 30% to hands. 63% developed at least ptosis as evidence of neurotoxicity and 53% developed respiratory paralysis requiring mechanical ventilation. Antivenoms were sourced from several nations, particularly Costa Rica (50%) and Brazil (30%).



### Coral snake bites in Central America

Prof. José María Gutiérrez (Instituto Clodomiro Picado, Costa Rica)

Chema (presented in English) noted that of the approximately 5,000 snakebites in Central America per year, only 1-2% are due to *Micrurus*. A wide range of *Micrurus* spp. occur in the region, but most have limited geographic distribution. The geographic distribution of venom types in *Micrurus* shows a predominance of PLA2 toxins in venoms from Central America through into the USA, with consequent presynaptic neurotoxicity, while 3FTx (post synaptic neurotoxins) dominate venoms in South America, with pockets in Central America, especially around Costa Rica. Antivenom made in Costa Rica is raised against *M. nigrocinctus* venom, a PLA2 dominant venom, thus is likely to be effective widely in the region and in the USA. This echoes the previous lecture from Bruno Lomonte. The Costa Rica guidelines state that AV should be given (5 vials) only in cases where a coral snake has been identified and if local symptoms (parasthesiae) are present, but without waiting for signs of neurotoxicity.



### Coral snake bites in the United States

Dr. Tamas Peredy (Florida Poisons Control Center, USA)

Tamas, a clinical toxicologist and EP, presented (in English) data on coral snake bite reported to the Florida PIC system, which averages 40 cases/yr, with only 1 fatality (in 2006) since 1967. The local species is *Micrurus fulvius* (*M. tener* is found in Texas). A 4 yr retrospective review found 82 cases, 90% with local pain, but only 7% with system symptoms. 39 patients received AV and 5 had adverse reactions. Tamas pointed out that erroneous identification of snakes (either another type of venomous snake, or a coral snake mimic) occurs and can muddle statistics. The AV appears to assist with pain and he presented a case where recurrent venom levels post AV responded to a repeat AV dose over 24hrs post-bite. He also presented preliminary data on a new experimental AV (INA2013) indicating it may prove effective. Overall coral snake envenoming occurs in <50% of cases and far fewer develop neurotoxicity, with pain the most common feature and mild rhabdomyolysis possible, but not haemolysis or AKI. Given the acute shortage of supply of the coral snake AV (stop-press - about to go back into production) in the US, there has been an issue of whether to give AV to all patients, or



await evidence of envenoming. A study in 2010 in Florida with 387 cases over 5 yrs, 252 given AV initially, showed no difference in outcome between AV treated or non-treated cases. Therefore the evidence for AV use, when and in which patients to use it, remains incomplete.

### **Elapid snakebites in Australia and Papua New Guinea**

*Prof. Julian White (Women's & Children's Hospital, Adelaide, Australia)*

Prof. White presented (in English) an overview of the medically important Elapid snakes of Australia, New Guinea and adjacent Pacific regions, including a listing of major species within each subgroup, clinical effects and treatment response. The New Guinea snake fauna shares many genera, even some species, with Australia. In consequence the Australian snake antivenoms are effective for New Guinea snakebite. However, a new taipan-specific antivenom made in Costa Rica is undergoing clinical trial in PNG and current data indicates it may be a cheaper and equally effective AV compared to the current Australian AV, though it will only cover taipan bites, not the many other species in PNG, so will not be a total replacement for the Australian AV.

### **Coral snake venoms: toxic properties, immunogenicity, antivenoms cross reactivity and neutralisation potential**

*Dr. Denise Tambourgi (Instituto Butantan, Brazil)*

Denise (presented in English) an overview of issues surrounding treatment response for coral snake envenoming in Brazil. Her data indicated only 213 cases of coral snake bite, compared to 19,287 bites by *Bothrops* spp. and 1,895 bites by *Crotalus* spp., 831 bites by *Lachesis* spp.. The coral snake AV is made using a 1:1 mix of *M. frontalis* and *M. corallinus* venoms, in horses, with snakes collected from a limited geographic area. Her concern is that this AV might be ineffective against other *Micrurus* spp. in Brazil, so this was tested using cross reactivity studies, plus comparative venom studies. The latter showed quite significant variation between species in venom composition/activity and similar variability in toxicity using LD50. The cross neutralisation studies indicated that the current AV will not be particularly effective against some other *Micrurus* spp., particularly (of those tested), *M. lemniscatus* and *M. altirostris* and, to a lesser extent, *M. ibiboboca* and *M. spixii*. She recommended the introduction of a greater range of *Micrurus* spp. venoms into the immunising mix for coral snake AV in Brazil. However, she then presented data that showed achieving this in a production situation proved difficult, particularly in achieving neutralisation of lethal activity for *M. lemniscatus*, *M. altirostris* and *M. surinamensis* venoms.



### **Monoclonal-based antivenomics and biological activities revealing high variability in coral snake venoms**

*Dr. Carlos Correa Netto (Instituto Vital Brazil, Brazil)*

Presented research into the venomomics and antivenomics of Brazilian *Micrurus* venoms, starting with a comparison of *M. corallinus* and *M. altirostris* venoms. This included use of "second generation" antivenomics using monoclonal Ab (MAb)-based methods. One focus was anti-PLA2 activity, noting the variability in PLA2 activity between different venom pools from the same species (*M. altirostris*) and the effect of captive care of snakes on venom profiles (in *M. corallinus*).



**Identification of epitopes for the development of a new antivenom against coral snakes (*Micrurus*)**

*Dr. Carlos Chavez-Olortegui (Universidade Federal de Minas Gerais, Brazil)*

Presented (in Portuguese) noting the ongoing production issues for Brazilian coral snake AV relating to difficulty in obtaining sufficient venom for immunisation. To overcome this his group have investigated new immunogens, specifically developing artificial epitopes starting with individual toxins from *M. corallinus* venom, determining the AA sequence, then spot synthesis, through isolation of sequences of linear epitopes, ending up with synthesised peptides of known epitope characterisation that can reliably be used in larger scale immunisation systems. It appears this remains to be tested in commercial production models, but has the potential to avoid the raw venom supply bottleneck.



**Rational design for mapping of linear B-cell epitopes in toxins**

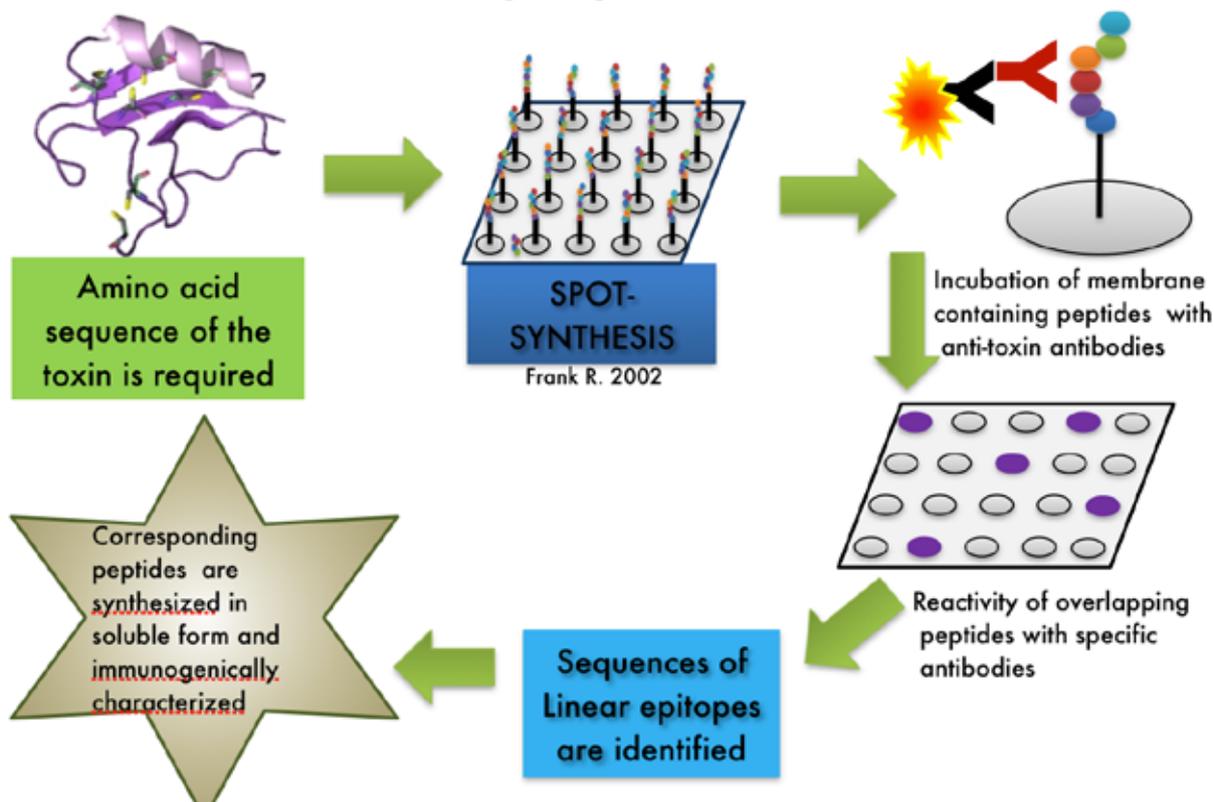


Illustration courtesy of Carlos Chavez-Olortegui

**Towards a universal antielapidic serum**

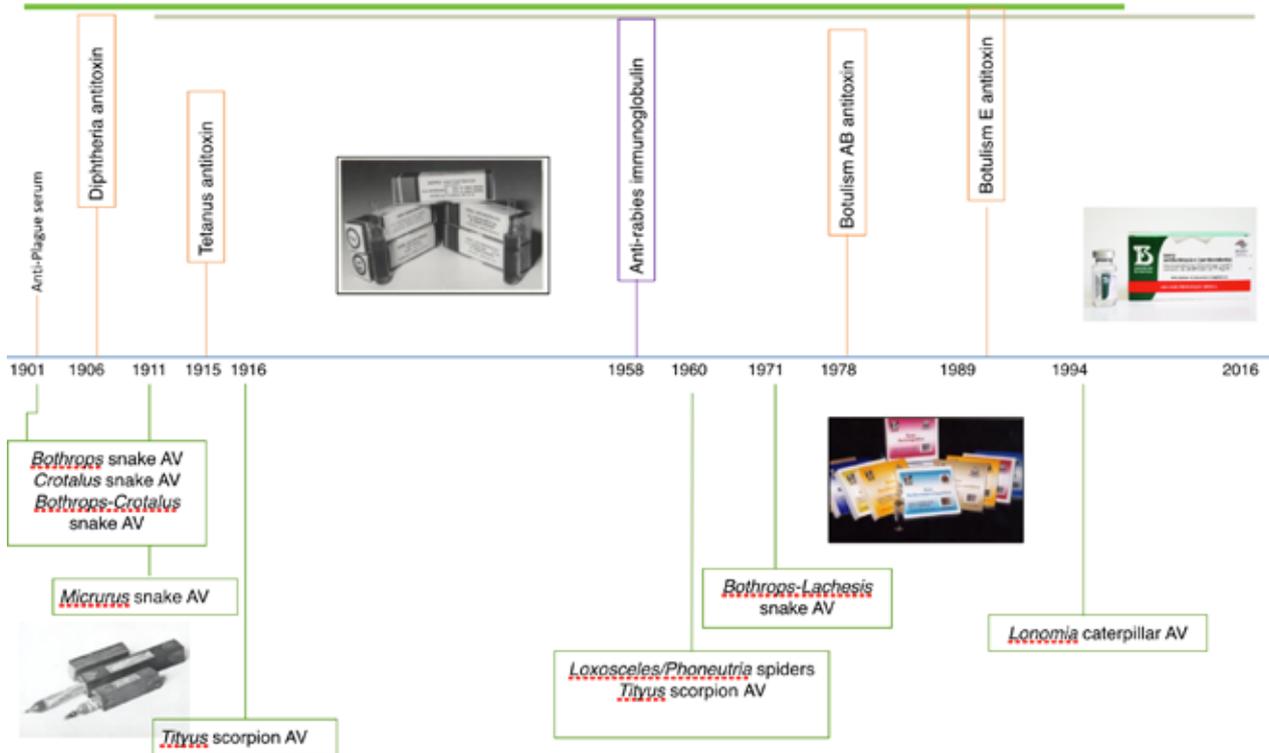
*Dr. Paulo Lee Ho (Instituto Butantan, Brazil)*

This research (presented in English) first gave an overview of Instituto Butantan antivenoms, their history and production, with current AVs. He then detailed comparative studies between Brazilian and Australian CSL AVs in neutralising *Micrurus* venom. He reiterated earlier papers, that the bottleneck for AV production was often availability of immunising venom, particularly for anti-

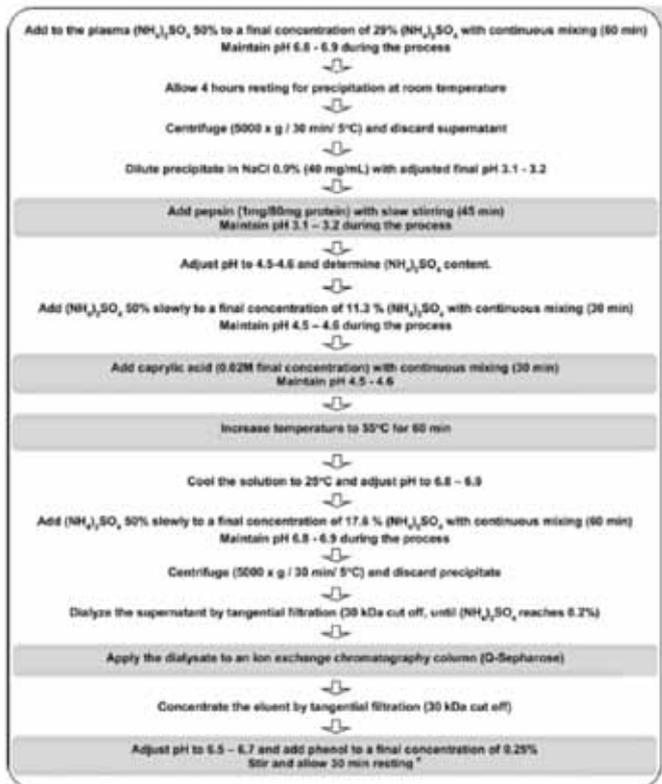




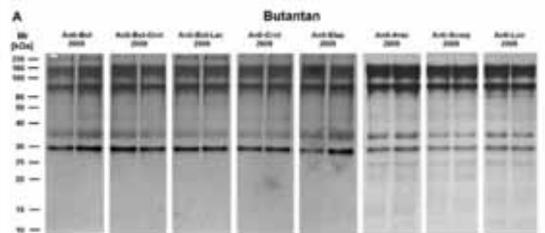
# Horse hyperimmune sera produced at Instituto Butantan



## "Hyperimmune serum" production process (fração F(ab')<sub>2</sub>)



### Reliable and well defined process SDS-PAGE



Am. J. Trop. Med. Hyg., 90(3), 2014, pp. 574-584

### Western-Blot anti-Horse IgG

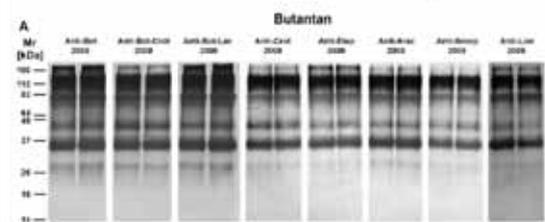


Figure 1. Process flowchart to obtain purified SAI. (a) The bulk product is further filter sterilized and held for at least 21 days (quality control testing period). After the formulation (0.11% phenol concentration), the final product is packed (ampoules).

Biotechnol. Prog., 2013, Vol. 29, No. 4

Report on the International Symposium on Coral Snakes, Goiania, Brazil, October 2016

**Table 1.** Neutralization assay against different *micrurus* spp. snake venoms.

Snake venom	Serum used for venom neutralization				
	Serum from naïve mice	Butantan antielapidic serum	CSL antielapidic serum	OsPa serum	OsPaNs serum
<i>M. corallinus</i>	5/5	0/5	0/5	0/5	0/5
<i>M. frontalis</i>	5/5	0/5	0/5	0/5	0/5
<i>M. fulvius</i>	5/5	0/5	0/5	0/5	0/5
<i>M. nigrocinctus</i>	5/5	0/5	0/5	0/5	0/5
<i>M. pyrrhocryptus</i>	5/5	0/5	0/5	0/5	0/5
<i>M. altirostris</i>	5/5	5/5	0/5	0/5	0/5
<i>M. ibiboboca</i>	5/5	5/5	0/5	3/5	0/5
<i>M. lemniscatus</i>	5/5	5/5	0/5	5/5	2/5
<i>M. spixii</i>	5/5	5/5	4/5	4/5	<sup>a</sup>

OsPa: *Oxyuranus scutellatus* + *Pseudechis australis* antivenom; Ns: *Notechis scutatus*.

<sup>a</sup>Neutralization of OsPaNs antivenom was not performed against *M. spixii* venom, as CSL serum itself did not provide protection.

Results are shown as total deaths/number of animals per group. For this assay, before intraperitoneal injection, 21 LD<sub>50</sub> of venom diluted in 700 µL saline were incubated, for 30 min, with the same volume of serum. Each animal was injected with 200 µL of this mixture. Animals were monitored every 6 h for a total of 48 h.

Illustrations courtesy of Dr. Paulo Lee Ho

### Debate: Towards a continental *Micrurus* antivenom: is it feasible?

A number of invited experts were involved.



This interesting and at times controversial debate, essentially between clinicians, researchers and antivenom production experts on one side and a Brazilian Government representative on the other side, debated the issues surrounding provision of AV for treating coral snake bites. The production side contended that they are no longer able to meet AV demand because of government regulation that was impeding development and production.

The government representative essentially said he didn't believe the experts since Health Dept. statistics indicated no deaths from coral snakes in areas where AVs were claimed not to work.

This was followed by discussion around whether AVs were even needed for coral snake bites, given that paralysis was the main clinical risk and this could be managed by intubation/ventilation in ICU. This question remained unanswered.

Prof. Gutierrez suggested that what was needed was a political solution, not a scientific one, and this should be considered from a regional (Latin American) perspective, not just a Brazilian perspective.

However the government seems determined to down-regulate production of AV, reducing total output by more than 50%.

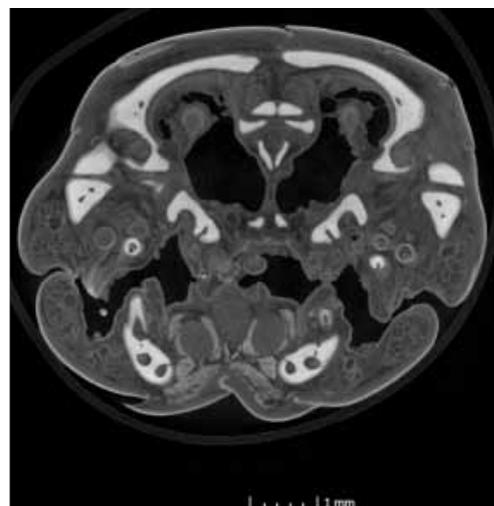
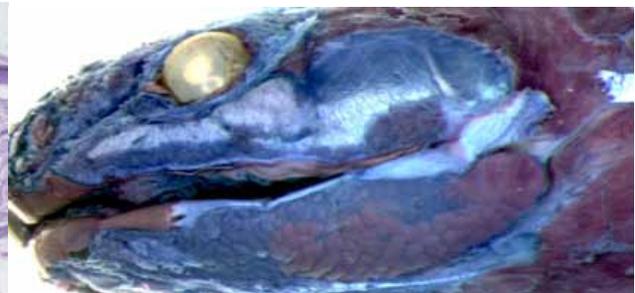
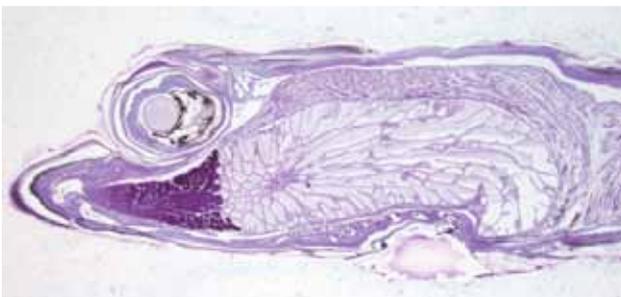
It was my impression there was no meeting of minds on this issue, with the government at odds with all their experts in toxinology. Nevertheless, Prof. Gutierrez suggested more such round table meetings were required to more effectively discuss this and other antivenom and envenoming management issues within the region because bringing together experts from multiple disciplines and locations provides a broader perspective and may allow more robust recommendations to emerge.

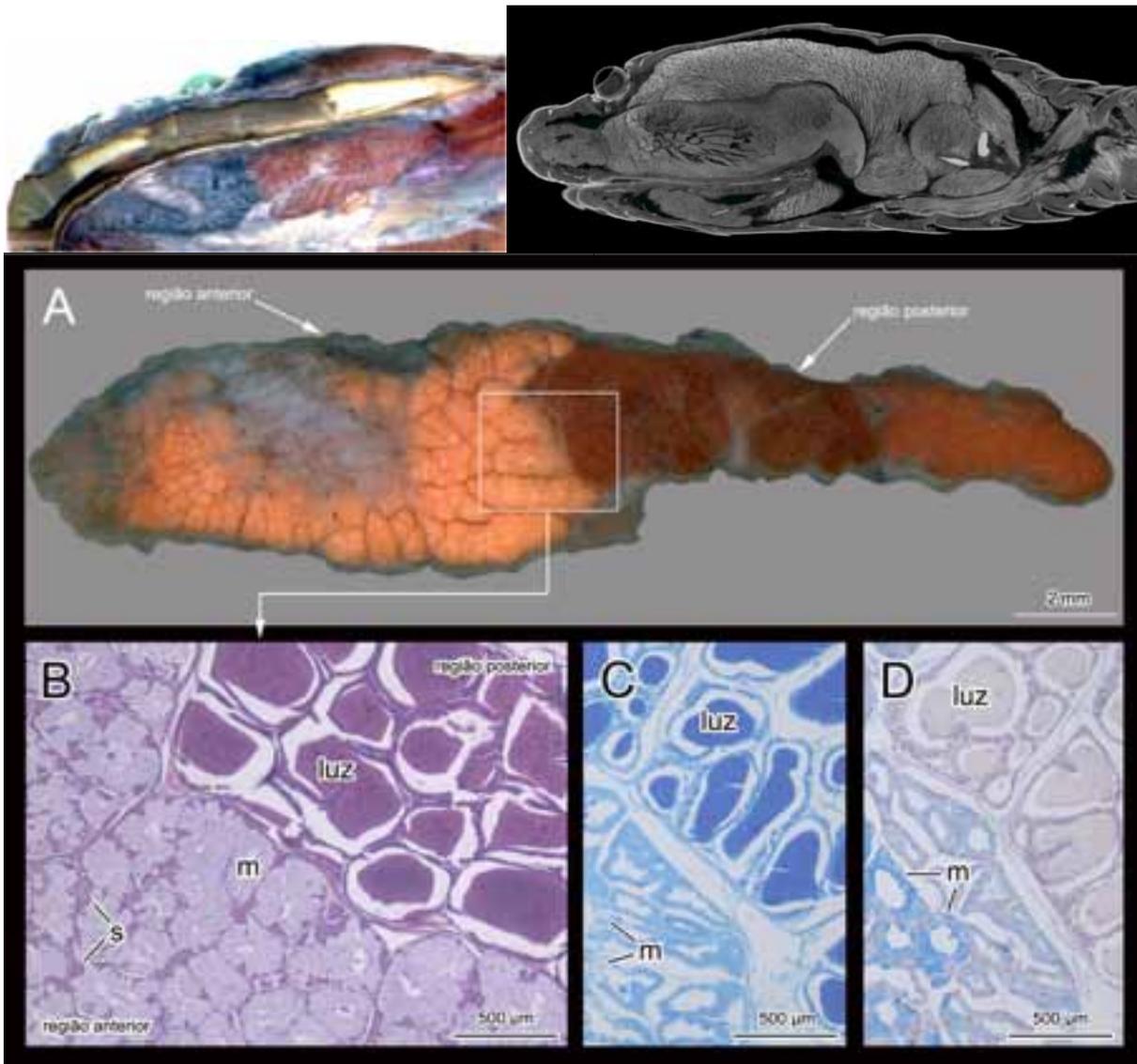
**Comparative study of coral snake cephalic glands**

*Dr. Leonardo de Olivera (Museo de Zoologia da Universidade de Sao Paulo, Brazil)*

(Presented in Portuguese)

Commenced with an historical perspective of the evolution and origin of snake venom and related oral glands noting the work of Phisalix, Kochva, then mentioning the Toxicofera theory, followed by support for the separate evolution of venom glands multiple times, citing Hargreaves et al. He then discussed details of the venom glands in *Micrurus* spp., based on dissections, staining with iodine and use of CTscans and serial whole-head sections, also citing previous studies by Rosenberg and Roze. Accessory glands might help venom flow, or possibly activate venom immediately prior to discharge. He then touched on supralabial glands, including the harderian glands (supra-orbital) which have no venom function, and submandibular glands, the latter being consistently present across all studied *Micrurus*, with 2 distinct staining regions and connecting near the rear of the mouth via a duct. The fang, in the maxilla, on mouth closure, appears to connect into a space in this gland, distant from duct, but the function, if any, is unknown. It secretes a waxy substance here, quite different from the posterior ductal discharge. The rictal gland discharges well away from teeth and the role is unclear.





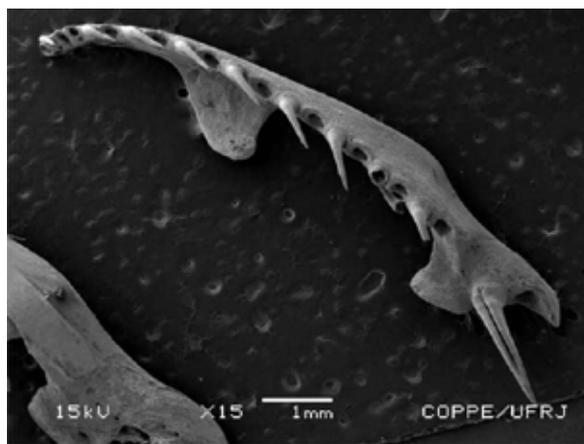
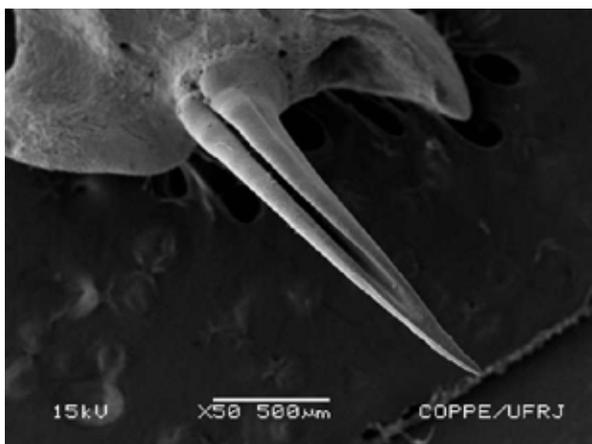
Illustrations courtesy of Dr. Olivera

### Mechanism of venom inoculation in coral snakes

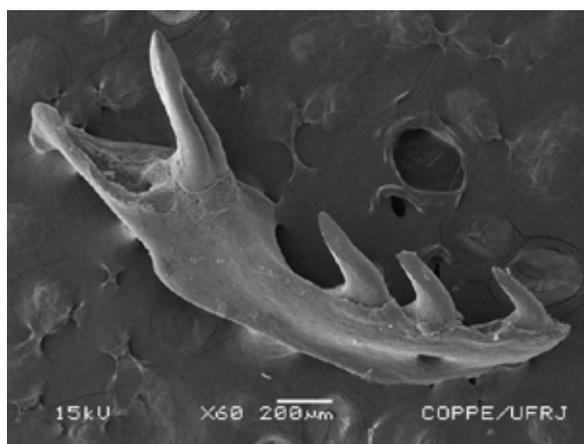
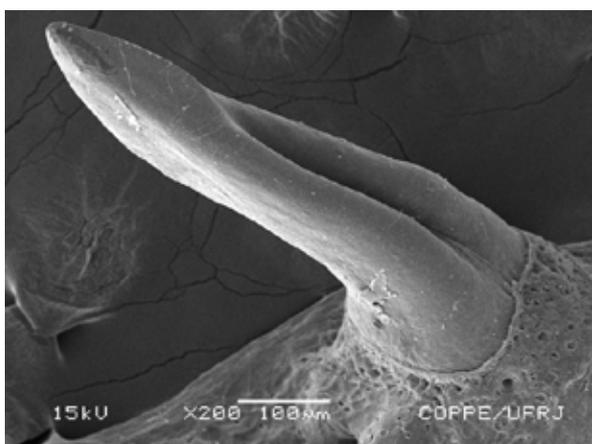
*Dr. Anibal Rafael Melgarejo Gimenez (Instituto Vital Brazil, Brazil)*

Anibal presented (in Portuguese) a rather rambling lecture on the mechanics of envenoming by coral snakes. He commenced with an overview of the evolution of the venom apparatus, illustrated with many SEMs, including the deep grooves on the “fangs” of *Thamnodynastes* sp. and *Elapomorphus quinque-lineatus*. He then discussed viperid snake fang architecture and biting mechanics. After this he moved to proteroglyphs - Elapid snakes, particularly coral snakes. These latter snakes have small fangs (generally <math><2.5\text{mm}</math>) and limited mouth opening (

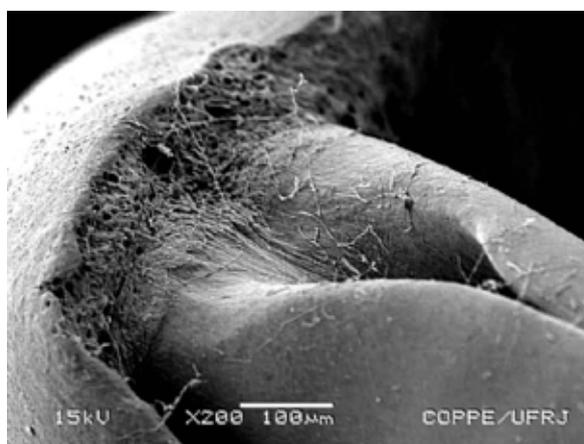
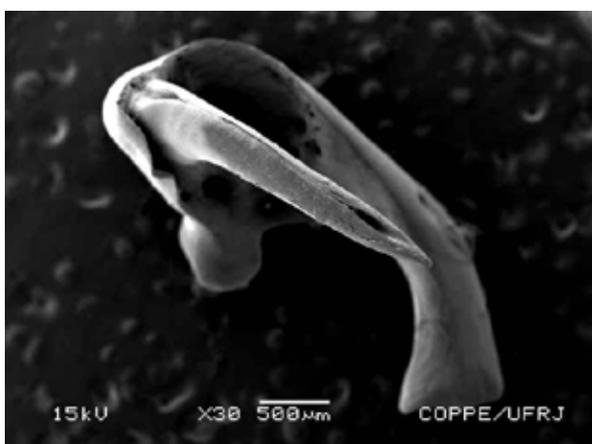




*Thamnodynastes* sp. deeply grooved posterior “fang”



*Elapomorphus quinquelineatus* deeply grooved posterior “fang”



*Micrurus* sp. fangs showing enclosed groove

Photos courtesy of Dr. Melgarejo

### **The Mechanism of action of coral snake (*Micrurus*:*Elapidae*) venoms**

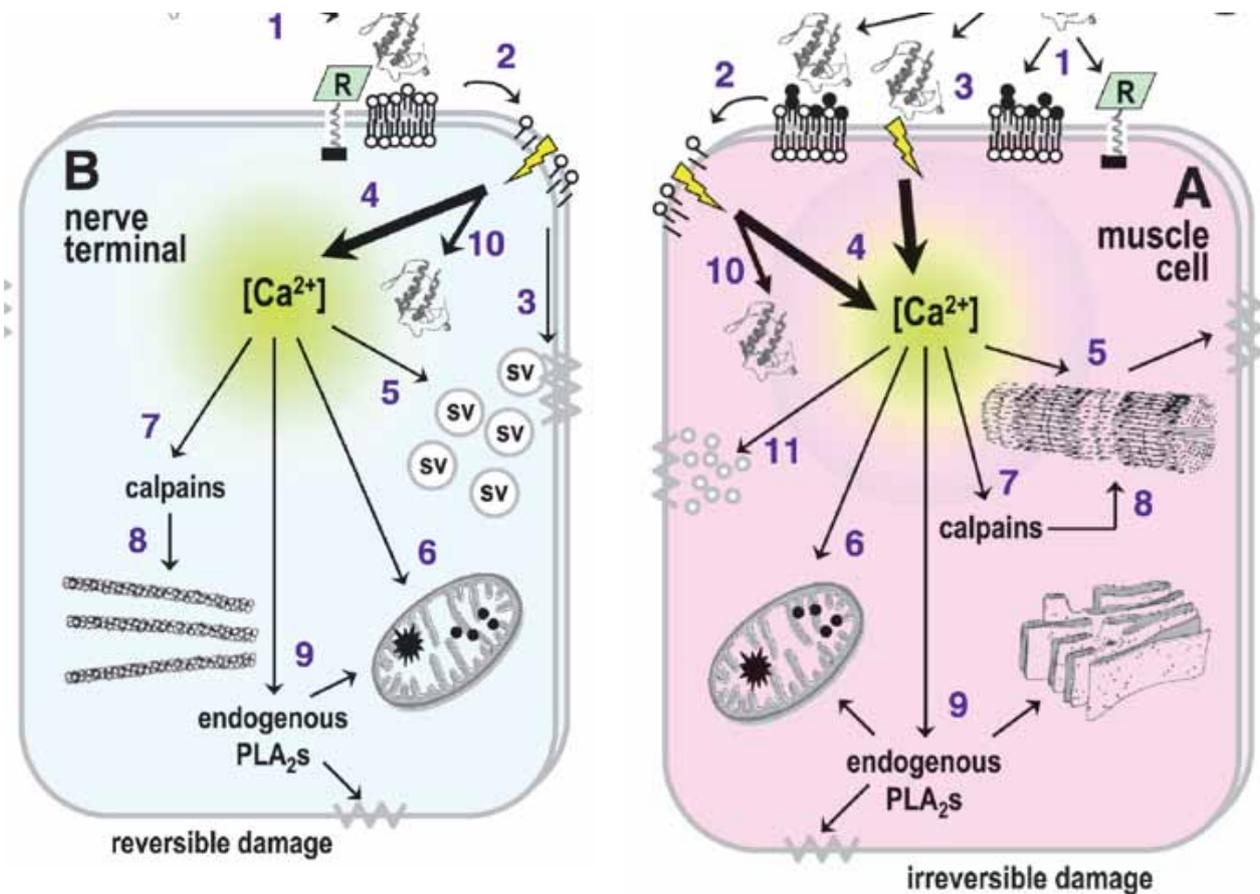
*Prof. José María Gutiérrez (Instituto Clodomiro Picado, Costa Rica)*

This excellent overview lecture (in English) covered the diversity of venom components, the major types of clinical significance and explanations of their mode of action as understood at this time. As noted earlier by Bruno Lomonte, Chema's co-worker, *Micrurus* venoms fall into 2 main types; 3FTx-rich and PLA<sub>2</sub>-rich. The former predominate in South American *Micrurus* while the latter predominate in Central and North American species.

Bites are often to the fingers with subcutaneous venom injection causing local pain and parasthesiae. Heteromeric pain-inducing toxin complexes have been discovered in the venom, including both PLA<sub>2</sub> and Kunitz-type toxins, which bind to acid-sensing Ca<sup>++</sup> ion channels. Chema also noted that studies had shown most venom was absorbed/transported via the lymphatic system. Neuromuscular paralysis is the major risk with coral snake envenoming and is a classic flaccid descending paralysis first affecting cranial nerves. The 3FTx neurotoxins act post-synaptically at the NMJ, binding to the Acetylcholine receptor (mainly to the  $\alpha$ -subunit, thereby blocking ion flow), while the PLA<sub>2</sub> neurotoxins act pre-synaptically, entering the terminal axon via PLA<sub>2</sub> hydrolysis of the cell membrane or by activating the synaptosome invagination process, cause Ca<sup>++</sup> influx into the cell, and disrupting the intracellular structure including mitochondria, with consequent cessation of synaptosome production and therefore no further release of neurotransmitter (Acetylcholine). The Ca<sup>++</sup> influx activates calpains which results in intracellular degradation. The PLA<sub>2</sub> toxins also generally are myotoxic. This effect is more easily seen in mice where the venom load is higher than in humans where in most cases insufficient venom is injected to cause detectable myolysis. When it occurs the myolysis involves intracellular destruction, including mitochondria, with hypercontraction of the muscle fibres, all of which may be secondary to Ca<sup>++</sup> influx as a result of PLA<sub>2</sub> damage to the cell membrane. In viperid venoms the myotoxic activity is largely from locally acting general cytotoxins/myotoxins that bind to many cell types, leaving little to reach the systemic circulation and distant targets. In elapid venoms the far more specific myotoxins bind only to mature muscle cells and so are not bound locally at the bite site, thus are available to bind systemically to muscle. The lack of myotoxicity in humans bitten by *Micrurus* spp. may be due to insufficient venom volume being injected to cause significant damage. These venoms also have pro-inflammatory actions whose role in envenoming is uncertain. Some *Micrurus* venoms (e.g. *M. fulvius*) have a haemolytic action in mice, though not seen in humans or dogs, possibly due to the different composition of RBC membranes in these species (more phosphatidylcholine versus sphingomyelin), compared to mouse, horse, or rabbit RBC. The role of physical strain in the circulation, affecting haemolysis rates, may also be important. Chema speculated that coral snakes may use myotoxicity in prey acquisition/digestion, while the 3FTx's that may be of low potency in mice, because they target mainly GABA receptors rather than ACh receptors, they may be highly potent in a natural prey, such as worms, which have predominantly GABA receptors at their NMJ. It is therefore important to test for toxin actions in relevant prey species as this can elucidate the function of specific toxins.



Report on the International Symposium on Coral Snakes, Goiania, Brazil, October 2016



Illustrations courtesy of Prof. Chema Gutierrez

## Origin and evolution of Elapids and coral snakes

Dr. Felipe Graziotin (Instituto Vital Brazil, Brazil)

Felipe was an entertaining presenter (in English) who discussed the origin of Elapids in general and coral snakes in particular. He defined Elapids as proteroglyphous (fang structure), with no loreal scale (between preocular and postnasal scales), possessing a bifurcated sulcus spermaticus on their hemipenes, having a venom gland compressor muscle (derived from the adductor mandibulae externus superficialis), and an accessory gland anterior to the main venom gland. He also mentioned that the “type” genus, *Elaps*, from which the family Elapidae is named, is no longer recognised. He then discussed the diversity within Elapidae and the various taxonomic arrangements proposed, fixing on a version of Pyron et al 2013 as the basis for higher level taxonomy. Within Elapidae, looking at coral snakes, the Asian genus *Calliophis* is basal phylogenetically and *Sinomicrurus* is the sister group to American coral snakes (*Micrurus*, *Micruroides*, *Leptomicrurus*). This same view places Australian terrestrial Elapidae as part of the hydrophiine (sea snake) clade. He recognises 87 coral snake species in 4 genera (*Micrurus* 79; *Micruroides* 1; *Leptomicrurus* 1; *Sinomicrurus* 5), which therefore excludes the Asian *Calliophis* spp.. He then discussed evolutionary time scales for Elapids, including discussion of the Australian fauna



### What is an Elapidae?

- Proteroglyphous fangs



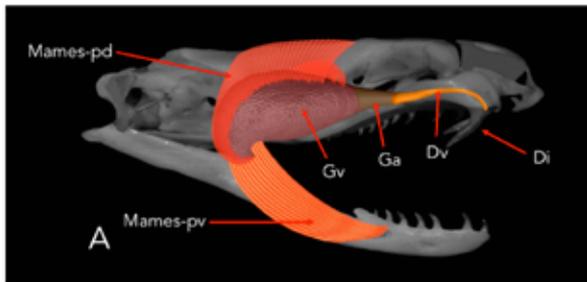
- Absence of loreal scale



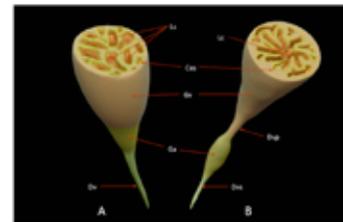
- *Sulcus spermaticus* bifurcated



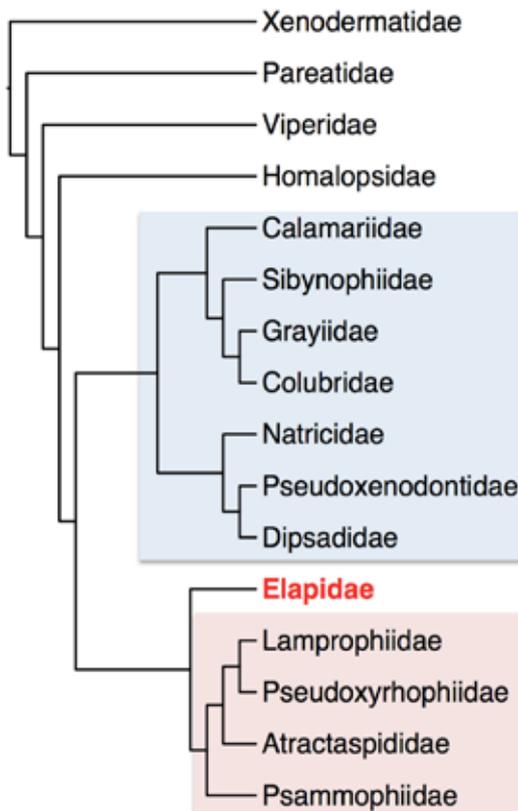
- Muscle that compresses the gland derived from *adductor mandibulae externus superficialis*



- Accessory gland



(Underwood, 1967; Smith *et al.*, 1977; McCarthy, 1985)

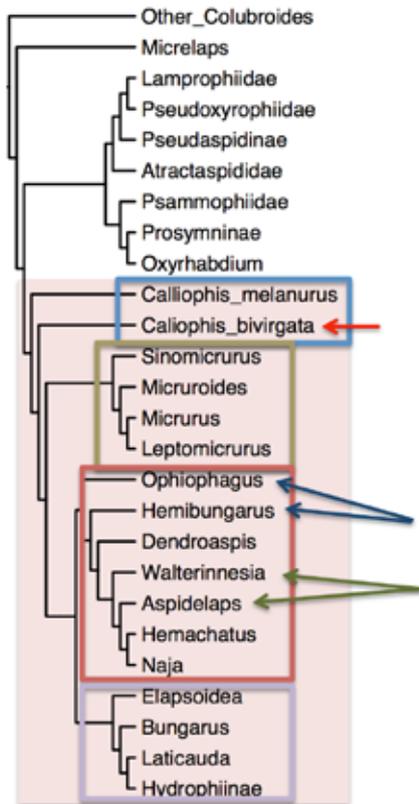


Pyron <i>et al.</i> , 2013	This study
4. Colubroidea	1. Colubroidea
5. Xenodermatidae	5. Xenodermatidae
5. Pareatidae	2. Colubriiformes
5. Colubridae	5. Pareatidae
6. Calamariinae	3. Endoglyptodonta
6. Colubrinae	5. Viperidae
6. Natricinae	6. Viperinae
6. Grayiinae	6. Azemiopinae
6. Dipsadinae	6. Crotalinae
6. Pseudoxenodontinae	5. Homalopsidae
6. Sibynophiinae	4. Colubroidea
5. Elapidae	5. Calamariidae
5. Lamprophiidae	5. Colubridae
6. Lamprophiinae	6. Ahaetullinae
6. Pseudoxyrhopiinae	6. Colubrinae
6. Atractaspidae	5. Natricidae
6. Aparallactinae	5. Dipsadidae
6. Psammophiinae	6. Xenodontinae
6. Pseudaspidae	6. Dipsadinae
6. Prosymninae	6. Carphophiinae
5. Homalopsidae	5. Pseudoxenodontidae
5. Viperidae	5. Sibynophiidae
6. Azemiopinae	5. Grayiidae
6. Crotalinae	4. Elapoidea
6. Viperinae	5. Elapidae
	5. Lamprophiidae
	5. Pseudoxyrhopiidae
	5. Atractaspidae
	5. Psammophiidae

What is the main difference?

## Phylogenetic Relationships

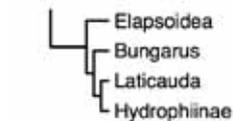
Zaher et al., 2016



### Concordant results:

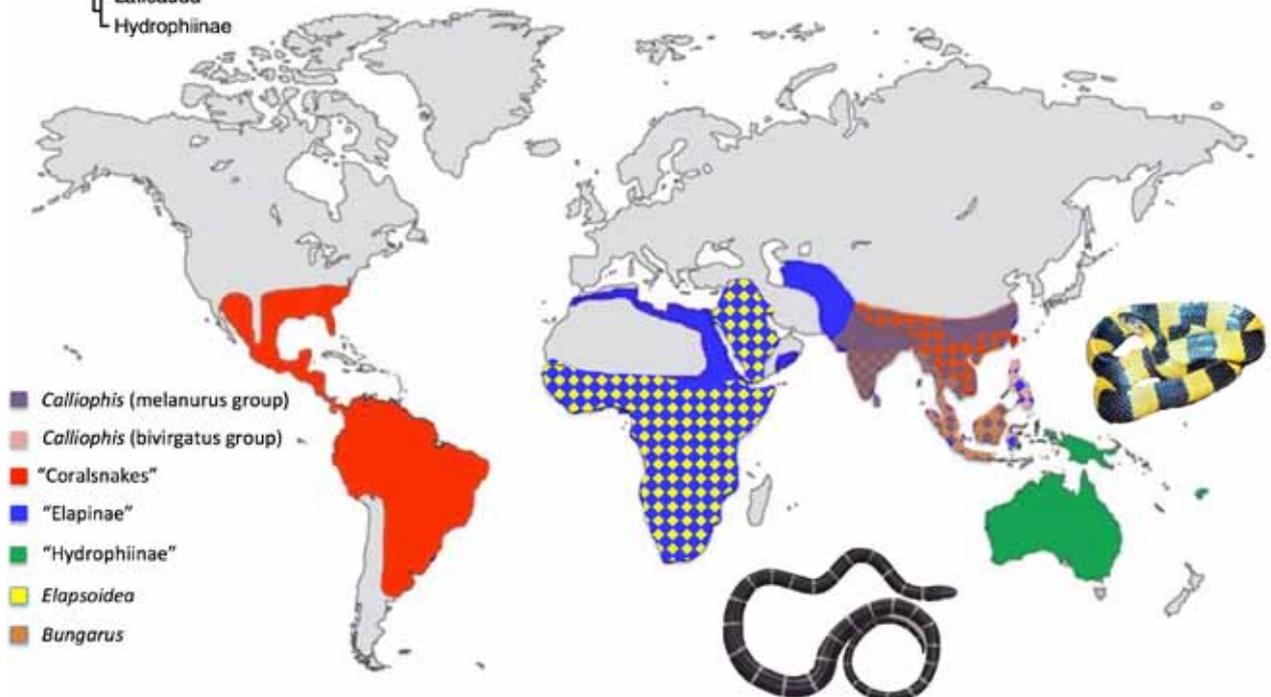
- Basal position of *Calliophis*
- Uncertainty about *C. bivirgata* groups
- *Sinomicrurus* is the sister group of SA coralsnakes
- Afrotropical and Palearctic elapids grouped in a clade
- Uncertainty about *Hemibungarus* and *Ophiophagus*
- Uncertainty about *Walterinnesia* and *Aspidelaps*
- *Elapsoidea* and *Bungarus* successive sister groups of Hydrophiinae
- Hydrophiinae is monophyletic

## Distribution

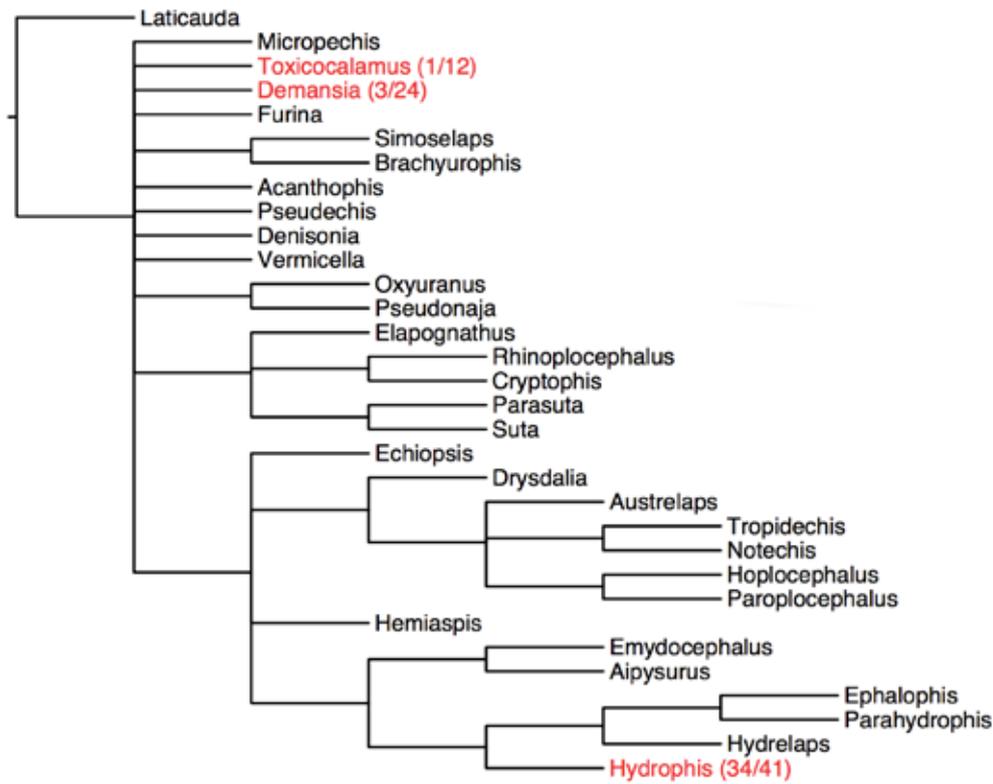


### Biogeographic Realms:

- Neotropic
- Neoartic
- Afrotropic
- Palaartic
- Indomalaya
- Australasia



### Australasian Elapids



Illustrations courtesy of Dr. Grazziotin

### Inauguration Ceremony for Prof. Janis Roze

Linked directly to this symposium was an official ceremony, quite spectacular, inaugurating Prof. Roze as an honorary “Doutor Honoris Causa” of the university, presided over by many university academic dignitaries, lead by the local Archbishop and the University President. While this was all in Portuguese, it was an interesting ceremony and certain parts were understandable, notably where Prof. Roze discussed the origins of diversity and the Archbishop responded with “Deus Amor” (God’s love), an amicable interchange of views. Prof. Roze was only the 20th person in the history of the university to be awarded this title and he was clearly delighted by the honor bestowed upon him.



Report on the International Symposium on Coral Snakes, Goiania, Brazil, October 2016



## Instituto Butantan

by Prof. Yara Cury and Ligia Farias-Cardon



Aerial view of Instituto Butantan, 1930s



Vital Brazil



Aerial view of Instituto Butantan, 2015

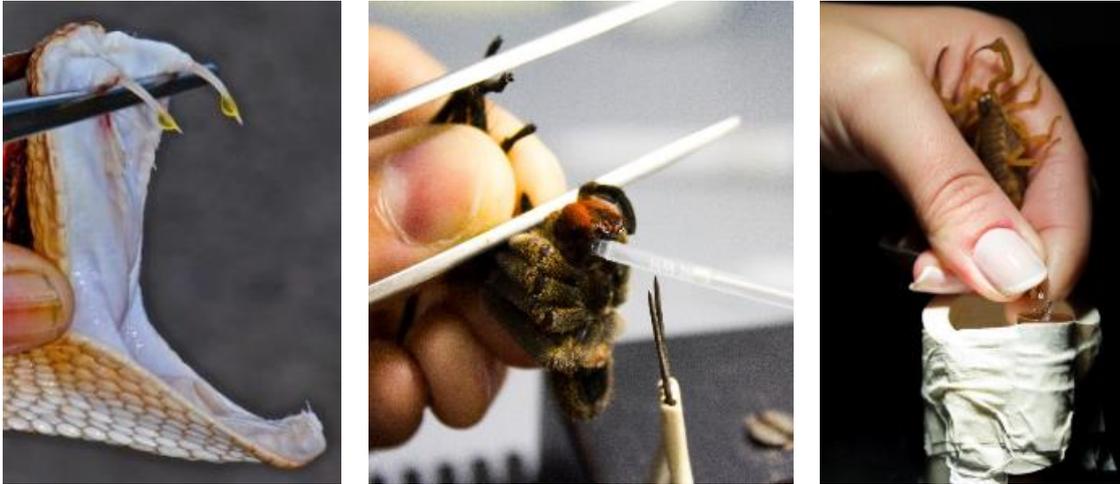
## Introduction

Instituto Butantan was established in 1901 as a Serum Therapy Institute, with the immediate responsibility of producing a serum to be used in combating the epidemic of bubonic plague afflicting the country. The institute's first director, Dr. Vital Brazil, was also interested in human envenomations, a big public health issue, and he started research on the production of antivenoms. He soon started dealing with the specificity of snake antivenoms, one of his most important contributions to the advance of this aspect of medical science and public health. Dr. Vital Brazil also had a wide understanding about how to share knowledge with the public and create an efficient knowledge dissemination and education strategy so that, even nowadays, people around the world strongly associate the word Butantan with the study of snakes. Dr. Vital Brazil went even further, studying other features the venoms. Thanks to Dr. Vital Brazil, Butantan soon became a reference for research into, and production of, sera against snake, spider and scorpion venoms.

Currently, Instituto Butantan is one of the most prestigious scientific institutions in Brazil. Internationally recognized for its work on venomous animals, it is an outstanding biomedical research center that integrates scientific and technological research, production of immunobiologicals, and dissemination of technical-scientific information. Connected to the State of São Paulo Secretary of Health, it avails itself of

approximately 2,000 employees and more than 30 laboratories. Butantan is responsible for the large-scale production of vaccines, anti-animal venoms and anti-bacterial toxins, as well as other biopharmaceuticals for the benefit of public health.

Butantan's facilities – spread out over more than 750,000 m<sup>2</sup> of preserved green space and officially recognized as part of the historic heritage of the city of São Paulo – are home to laboratories, production plants and museums, representing the three main areas of the Institute: research and development, production and cultural. In addition to these facilities, Instituto Butantan also has a reference hospital in envenomation, a library, a public health museum outside its campus and educational areas.



## Research and development

### Milking – snake, spider and scorpion

During the last five years, Butantan researchers published around 200 articles/year in scientific journals and were cited more than 4,500 times in 2015 alone.

The Instituto Butantan laboratories are dedicated to animal biology, toxinology, vaccinology and development. Development of fundamental research takes place in 19 laboratories and at the Vital Brazil Hospital using interdisciplinary approaches. The Biotechnology Center is dedicated to research and development of processes using modern technology for vaccine and biopharmaceutical production in close partnership with the manufacturing arm and with the support of Butantan's research areas. The institution's diligence in matching national science leaders with young researchers produces excellent results in the most diverse scientific areas, mainly those related to the systematic biology of serpents, arthropods and parasites, as well as the biochemistry and pharmacology of venoms and their components, the pathophysiology of the venoms, the immunology in response to venoms and pathogenic microorganisms, the genetic basis for the immune response, the cytogenetics and genetics of venomous animals, among other areas.

Instituto Butantan promotes new knowledge through research and trains human resources with science and technology skills, decisive stages for innovation in health. 150 researchers who are in constant dialogue with the 400 graduate students connected to Institute lead the scientific research. The graduate courses in Toxinology and Biotechnology (the latter in partnership with the Institute for Technology Research [Instituto de Pesquisas Tecnológicas – IPT] and the University of São Paulo [USP]) deepen the fundamental research and strengthen the exchange and training of skilled researchers. In order to reaffirm the bond between fundamental and applied science, Instituto Butantan offers an MBA in Health Innovation

Management. The only such program in Brazil, Butantan's MBA enables professionals to transform scientific research into innovative products in the field of health. Support for research comes from national agencies in the form of grants and scholarships and from large institutional projects like the Center for Research on Toxins, Immune Response and Cell Signaling (CeTICS), Centre of Excellence in New Target Discovery (CENTD) and the National Institute of Science and Technology for Toxins (INCTTox).

### Innovation and Development



Äkta Avant chromatography

Until very recently, the study of toxins and bioactive compounds advanced side-by-side in the institutional history. The main approach is directly related to the effects triggered by the venoms and the symptoms observed in patients. The results, despite being of a more phenomenological character, have allowed us to understand, at a certain level, the compounds' mechanisms of action. The new tools and approaches available nowadays have made it possible to expand the previous approach, with the use of those venom molecules and their sub-products to identify potential molecular targets, enabling not only the understanding of the mechanisms of action, but also the rational design of new therapeutic entities.

### Toxins and Antigens



ClonePix<sup>FL</sup> - for mAbs cell line generation

The whole venom or isolated compounds have been extensively studied to understand the local and systemic effects of envenomation, as well as the associated pathophysiology and the molecular and genetic mechanisms involved. Among the most serious consequence of envenomation by venomous animals are local effects at the bite site. In this sense, isolated compounds and molecules obtained from venoms (peptides, native and recombinant proteins, enzymes) have been used as pharmacological tools to understand the local reactions induced by these venoms, their mechanisms of action and how to neutralize them. Systemic effects such as neurotoxicity, myotoxicity, interference with coagulation, hemorrhagic

activity, renal toxicity, cardiac toxicity, induced by the animals' venoms are also analyzed in the studies developed by Instituto Butantan.

The cloning, expression and sequencing of bioactive peptides isolated from venoms, annotation and functional characterization of genetic sequences of secreted bioactive peptides, their biological multifunctionality and therapeutic potential are also goals of our studies. According to the specificity and selectivity of these molecules to their targets, they can also be used as tools for the understanding of physiological and pathophysiological processes. For example, some toxins isolated from animal venoms have been used as experimental models in the study of neurological and other disorders, such as epilepsy and arthritis.

In addition, antibodies, antibody fragments, toxins, in both native and recombinant forms, have been explored in order to improve antivenoms and immunodiagnosis.

In parallel to the research developed in the cited labs, Vital Brazil Hospital is a Reference Center for the treatment of patients injured by venomous animals. From 2011 renovation works at the hospital building have been performed and new medical equipment was acquired and installed. Recently, a new Telemedicine project was implemented which provides a national network to support the care of those injured by venomous animals in Brazil.

### **Transcriptome and Biopharmaceuticals**

Important lines of research have been under way for some time now. We believe that the expertise associated and acquired with these research lines is important for the Instituto Butantan. The goal of the Transcriptome line is to contribute, indirectly (through transcript description), to a precise description of animal venoms and to understand the vectors of pathogens; the goal of the Biopharmaceutical line is to develop new techniques to produce biopharmaceuticals.

### **Cell and Animal Biology Laboratories**

This area conducts morphological and structural studies through high-resolution analysis of the organs, tissues and animal cells producing poisons, as well as studies on the molecular and genetic basis of action of the toxins isolated from venoms. Several aspects of animal and cell biology have been explored by these groups, such as:

- Studies in the areas of Natural History, Morphology, Taxonomy, Systematics, Biogeography, Cytogenetics, Molecular Biology, Biodiversity, Conservation, Ecology and Evolution of vertebrates (with emphasis on serpents, small mammals and spiders), zoological inventories and arthropod, reptile and amphibian molecular biology.
- Studies on species of urticating Lepidoptera with an emphasis on *Lonomia obliqua*.
- Traditional and molecular taxonomy, biology and ecology of ticks (Acari: Ixodidae). Biochemical studies of the glands and substances produced by ticks and cultures of embryonic cells of some species.
- Research on population genetics with disease vectors of the family Culicidae and on taxonomy and biology of Solifugae (Arachnida).

These laboratories also have as their responsibility, to receive and identify venomous animals (newly available computerized and networked); to identify antigens for production of Antilonomic serum, which has the capacity to revert hemorrhagic disturbances in humans caused by this caterpillar; to maintain the public Serpentarium of Instituto Butantan; to establish lists of endangered species and to consolidate the tissue bank; to provide a public service to citizens through identification of insects and ticks of medical importance, and guidance on preventing contact with these arthropods; housing the Arachnological, Acarological, Entomological, Herpetological and Myriapodological and other educational activities.

## Research funds

One of the most important research resources at the Instituto Butantan are research grants from both individual and collective efforts. Research platforms have played an important role in the development of institutional projects, with the participation of pharmaceutical or biotechnology companies and partially supported by the BNDES (Brazilian Development Bank) or Finep (Brazilian Innovation Agency), as well.

A platform for Innovation and Development was built in order to scale up the production of recombinant proteins and perform proof-of-concept studies conforming to GLP regulations. Such a platform would not have been possible without the financial support provided by research foundations such as FAPESP (São Paulo Research Foundation). Recently, a research program plan for a Centre of Excellence for Research In Target Discovery was approved.

## Production



Spider venom extraction at the new Arthropods Laboratory



New facility for antivenom production





Production of Instituto Butantan's Dengue Vaccine (currently in Phase 3 clinical trials)

Instituto Butantan is currently responsible for production of half of Brazil's sera and vaccines, which are distributed free of charge through the Unified Health System (Sistema Único Saúde, SUS) to the entire Brazilian population. Instituto Butantan's Division of Technological Development and Production (Divisão de Desenvolvimento Tecnológico e Produção, or DDTP) masters the technology for producing 13 types of sera and 6 vaccines (Influenza trivalent, adsorbed hepatitis B– recombinant, inactivated human rabies vaccine, Diphtheria, Tetanus and Pertussis adsorbed vaccine for adults and children and Tetanus adsorbed vaccine). Instituto Butantan is the first domestic public producer to possess a complete production line certified by the Brazilian Health Surveillance Agency (Agência Nacional de Vigilância Sanitária, or Anvisa) for Good Manufacturing Practices. Recently, new agreements have been signed for the development and production of vaccines against human papillomavirus (HPV) and hepatitis A, as well as an acellular pertussis (whooping cough) vaccine and the domestic production of three monoclonal antibodies.

In 2016, Butantan began Phase 3 clinical trials for a new dengue vaccine, based on attenuated viruses, that is effective against all four dengue serotypes. 17,000 volunteers will be vaccinated during this stage of the research, an unprecedented study in Brazil. The Production and Technological Development Division (DDTP) comprises several Laboratories and Production Plants.

In order to supply the Ministry of Health with the sera demanded, Butantan holds a horse farm for the production of hyperimmune plasma (São Joaquim Farm), a hyperimmune plasma processing plant and a formulation, filling and packing line. The production from the São Joaquim Farm, with around 800 horses, reaches 20,000 liters of plasma each year. A new plasma processing plant is re-starting operations at this moment. This plant was completed refurbished to comply with GMP conditions and will increase production capacity from 400,000 to 700,000 vials/year. The antivenom and antitoxins produced by Instituto Butantan are: *Loxosceles* and *Phoneutria* spiders, and *Tityus* scorpion antivenom (or spider-scorpion antivenom); *Bothrops-Crotalus* snake antivenom (or pit viper-rattlesnake antivenom); *Bothrops-Lachesis* snake antivenom (or pit viper-bushmaster snake antivenom); *Crotalus* snake antivenom (or rattlesnake antivenom); *Bothrops* snake antivenom (or pit viper snake antivenom); *Micrurus* snake antivenom (or coral snake antivenom); *Tityus* scorpion antivenom (or scorpion antivenom); *Lonomia* caterpillar antivenom (or caterpillar antivenom); Botulism antitoxin AB (bivalent); Botulism antitoxin E; Diphtheria antitoxin; Antirabies immunoglobulin and Tetanus antitoxin.

## Innovation

Since 2013, Butantan signed six Productive Development Partnerships (PDP) for vaccines against the following: HPV, Hepatitis A and acellular dT<sub>p</sub>, in addition to the production of monoclonal antibodies (mAb). Collaboration with both Brazilian and international institutions are underway, such as the partnership with the National Institutes of Health (NIH) related to the dengue vaccine and the zika virus.

With more than 40 patents, the pipeline of Instituto Butantan includes 19 products, 5 of which are discoveries made from toxins as follows:

- Amblyomin-X, an anticancer medicine obtained from Cayenne tick saliva.



- Crotalphine, a long-lasting (2–5 days) analgesic from *Crotalus durissus terrificus* snake venom.
- 
- Crotamin, a cell penetrating peptide to introduce genetic material into cells.
- Lopap, a prothrombin activator, with anti-apoptotic activity and other pharmaceutical formulations obtained from *Lonomia obliqua* caterpillar.



- Immunosuppressor, a peptide to prevent or treat conditions that require immunosuppression, obtained from *Lachesis muta* snake venom.



## Culture

Since 1901, Instituto Butantan has been bringing research and production together with general scientific dissemination, encouraging the development of scientific knowledge in wide and varied ways. Through its Cultural Development Center (CDC), the institute holds educational programs — conferences, courses, art and science activities, historical publications, prevention guides — to arouse the greater public's interest in scientific knowledge and especially to strengthen the role that the Institute plays as an innovator and its importance for the development of science in Brazil. Butantan is home to three museums — the Biological Museum, the Historical Museum and the Microbiology Museum. The Emilio Ribas Museum, in the Bom Retiro neighborhood (downtown São Paulo), is also part of the institute, which receives approximately 300,000 visitors per year. The CDC also organizes the Institute's historical collection, promotes the publication of the journal *Cadernos de História da Ciência* (History of Science journal) and constantly searches for new ways to connect with society, translating the knowledge produced into language that is accessible and of general interest. Instituto Butantan's Library, placed in a historical building, is suited for individual or group study involved in research activities, as well as catalogs, databases and journals consultation. It offers customized services to meet the demand of its public. The collection consists of approximately 15,000 items (including books, theses and dissertations) and 200,000 titles of scientific journals in the fields of toxins, biotechnology and biodiversity.

**Biological Museum.** The Biological Museum, whose collection started to be organized in 1912, is housed in a building originally used as a stable for immunizing horses and reconfigured as an exhibit space in the 1960s. The objective of its long-term exhibit is to spread knowledge related to biodiversity and zoological conservation, being recognized as one of the only museums in the world to feature a display with live animals, such as snakes, lizards, iguanas, frogs, spiders and scorpions.

**Historical Museum.** The Historical Museum's exhibit shows old objects that were used for research activities and the production of sera and vaccines at Butantan. Its objective is to preserve, investigate and disseminate the history of the Institute. The museum is located in a refurbished building where Vital Brazil established his first laboratory. The original floors and part of the building walls are part of the exposition.

**Microbiology Museum.** In the Microbiology Museum, visitors get to know more about the world of microbes, microscopic bacteria and other microscopic beings with hands-on displays and observation of live microorganisms. Inaugurated in 2002, the museum fosters curiosity for science in youth and brings the public and science closer together.

**Emilio Ribas Public Health Museum.** Located in the neighborhood of Bom Retiro, the Emilio Ribas Museum is located in the old Central Disinfection Ward. A longterm exhibit about the history of health is open to the public, and many academic and knowledge dissemination activities are offered throughout the museum's

events calendar. Its collection contains important documents relating to the history of health in Brazil, available for consultation by appointment only.



History Museum



Biological Museum



Microbiology Museum



Library – main room



Memórias do Instituto Butantan collection bound with snake skin

Photos: Camilla Carvalho and Antonio COR Costa / Instituto Butantan Collection

## Collaborative publications, 2005–present.

Almeida C de S, Andrade-Oliveira V, Câmara NO, Jacysyn JF, Faquim-Mauro EL. Crotoxin from *Crotalus durissus terrificus* is able to down-modulate the acute intestinal inflammation in mice. *PLoS One*, 2015, **10**:e0121427.

Baldo C., Lopes D.S., Faquim-Mauro E.L., Jacysyn J.F., Niland S., Eble J.A., Clissa P.B., Moura-da-Silva A.M. Jararhagin disruption of endothelial cell anchorage is enhanced in collagen enriched matrices. *Toxicon*, 2015, **108**:240–248.

Carvalho, Daniela Cajado, Kuniyoshi, Alexandre K., Kodama, Roberto T., Oliveira, Ana K. , Serrano ,Solange M.T., Tambourgi, Denise V., Portaro, Fernanda V. Neuropeptide Y family-degrading metallopeptidases in

- the *Tityus serrulatus* venom partially blocked by commercial antivenoms. *Toxicological Sciences*, 2014, **142**:418–426.
- Chacur M, Gutiérrez JM, Milligan ED, Wieseler-Frank J, Britto LR, Maier SF, Watkins LR, Cury Y. Snake venom components enhance pain upon subcutaneous injection: an initial examination of spinal cord mediators. *Pain*, 2004, **111**:65–76.
- Chudzinski-Tavassi, Ana Marisa; Carrijo-Carvalho, Linda C.; Waismam, Kaline; Farsky, Sandra H.P. ; Ramos, Oscar H.P. ; Reis, Cleyson V. . A lipocalin sequence signature modulates cell survival. *FEBS Letters*, 2010, **584**:2896–2900.
- Correa PG, Oguiura N. Phylogenetic analysis of  $\beta$ -defensin-like genes of *Bothrops*, *Crotalus* and *Lachesis* snakes. *Toxicon*, 2013, **69**:65–74.
- Costa, E.S., Faiad, O.J., Landgraf, R.G., Ferreira, A.K., Brigatte, P., Curi, R., Cury, Y., Sampaio, S.C. Involvement of formyl peptide receptors in the stimulatory effect of crotoxin on macrophages co-cultivated with tumour cells. *Toxicon*, 2013, **74**: 67–178.
- Cunha Neto R dos S, Vigerelli H, Jared C, Antoniazzi MM, Chaves LB, da Silva Ade C, Melo RL, Sciani JM, Pimenta DC. Synergic effects between ocellatin-F1 and bufotenine on the inhibition of BHK-21 cellular infection by the rabies virus. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, 2015, **21**:50.
- Dale, C.S.; Gonçalves, L.R.C.; Juliano, L.; Juliano, M.A.; Moura da Silva, A.M.; Giorgi, R. The C-terminus of murine S100A9 inhibits hyperalgesia and edema induced by jararhagin. *Peptides*, 2014, **25**:81–89.
- Della-Casa MS, Junqueira-de-Azevedo I, Butera D, Clissa PB, Lopes DS, Serrano SM, Pimenta DC, Magalhães GS, Ho PL, Moura-da-Silva AM. Insularin, a disintegrin from *Bothrops insularis* venom: inhibition of platelet aggregation and endothelial cell adhesion by the native and recombinant GST-insularin protein. *Toxicon*, 2011, **57**:125–133.
- Favoretto BC, Silva SR, Jacysyn JF, Câmara NO, Faquim-Mauro EL. TLR2- and 4-independent immunomodulatory effect of high molecular weight components from *Ascaris suum*. *Molecular Immunology*, 2014, **58**:17–26.
- Favoretto BC, Ricardi R, Silva SR, Jacysyn JF, Fernandes I, Takehara HA, Faquim-Mauro EL. Immunomodulatory effects of crotoxin isolated from *Crotalus durissus terrificus* venom in mice immunised with human serum albumin. *Toxicon*, 2011, **57**:600–607.
- Fernando F, Joly ST, de Abreu Marcelo E, Gondo HH. Fed-batch production of tetanus toxin by *Clostridium tetani*. *Biotechnology Progress*, 2010, **26**:88–92.
- Frezzatti R, Silveira, PF. Allopurinol reduces the lethality associated with acute renal failure induced by *Crotalus durissus terrificus* snake venom: comparison with probenecid. *PLoS Neglected Tropical Diseases*, 2011, **5**:e1312.
- Gonçalves-Machado L, Pla D, Sanz L, Jorge RJ, Leitão-De-Araújo M, Alves ML, Alvares DJ, De Miranda J, Nowatzki J, de Moraes-Zani K, Fernandes W, Tanaka-Azevedo AM, Fernández J, Zingali RB, Gutiérrez JM, Corrêa-Netto C, Calvete JJ. Combined venomomics, venom gland transcriptomics, bioactivities, and antivenomics of two *Bothrops jararaca* populations from geographic isolated regions within the Brazilian Atlantic rainforest. *Journal of Proteomics*, 2016, **135**:73–89.
- Jared C, Antoniazzi MM, Jordão AEC, Silva JRMC, Greven H, Rodrigues MTU. Parotoid macroglands in toad (*Rhinella jimi*): their structure and functioning in passive defence. *Toxicon*, 2009, **5**:197–207.

- Jared C, Mailho-Fontana PL, Antoniazzi MM, Mendes VA, Barbaro KC, Rodrigues MT, Brodie Jr. ED. Venomous frogs use heads as weapons. *Current Biology*, 2015, **25**:2166–2170.
- Kimura, LF, Prezotto-Neto JP, Távora BCLF, Antoniazzi MM, Knysak I, Guizze SPG, Santoro ML, Barbaro KC. Local inflammatory reaction induced by *Scolopendra viridicornis* centipede venom in mice. *Toxicon*, 2013, **76**:239–246.
- Kimura, LF, Prezotto-Neto JP, Antoniazzi MM, Jared SG, Santoro ML, Barbaro KC. Characterization of inflammatory response induced by *Potamotrygon motoro* stingray venom in mice. *Experimental Biology and Medicine*, 2014, **239**: 01–609.
- Kodama RT, Cajado-Carvalho D, Kuniyoshi AK, Kitano ES, Tashima AK, Barna BF, Takakura AC, Serrano SM, Dias-Da-Silva W, Tambourgi DV, Portaro FV. New proline-rich oligopeptides from the venom of African adders: Insights into the hypotensive effect of the venoms. *Biochimica et Biophysica Acta – General Subjects*, 2015, **1850**:1180–1187.
- Leiguez E, Zuliani JP, Cianciarullo AM, Fernandes CM, Gutierrez JM, Teixeira C. A group IIA-secreted phospholipase A2 from snake venom induces lipid body formation in macrophages: the roles of intracellular phospholipases A2 and distinct signaling pathways. *Journal of Leukocyte Biology*, 2011, **90**:155–166.
- Lima-Dos-Santos I, Della-Casa MS, Portes-Junior JA, Calabria PA, Magalhães GS, Moura-da-Silva AM. Characterization of Neuwiedin, a new disintegrin from *Bothrops neuwiedi* venom gland with distinct cysteine pattern. *Toxicon*, 2015, **104**:57–64.
- Lima TS, Cataneo SC, Iritus AC, Sampaio SC, Della-Casa MS, Cirillo MC. Crotoxin, a rattlesnake toxin, induces a long-lasting inhibitory effect on phagocytosis by neutrophils. *Experimental Biology and Medicine*, 2012, **237**:1219–1230.
- Lopes DS, Faquim-Mauro E, Magalhães GS, Lima, Iara C, Baldo C, Fox JW, Moura-da-Silva AM, Clissa PB. Gene expression of inflammatory mediators induced by jararhagin on endothelial cells. *Toxicon*, 2012, **60**:1072–1084.
- Luz D, Chen G, Maranhão AQ, Rocha LB, Sidhu, Sachdev P, Roxane MF. Development and characterization of recombinant antibody fragments that recognize and neutralize *in vitro* Stx2 Toxin from Shiga toxin-producing *Escherichia coli*. *PLoS One*, 2015, **10**:e0120481.
- Machado FC, Zambelli VO, Fernandes AC, Heimann AS, Cury Y, Picolo G. Peripheral interactions between cannabinoid and opioid systems contribute to the antinociceptive effect of crotalpine. *British Journal of Pharmacology*, 2014, **171**:961–972.
- Magalhães GS, Caporrino MC, Della-Casa MS, Kimura LF, Prezotto-Neto JP, Fukuda DA, Portes-Junior JA, Neves-Ferreira AG, Santoro ML, Barbaro KC. Cloning, expression and characterization of a phospholipase D from *Loxosceles gaucho* venom gland. *Biochimie*, 2013, **95**:1773–1783.
- Magalhães GS, Novo JB, Clissa PB, Della Casa MS, Butera D, da Silva AM. Engineered mammalian vector to express EGFP-tagged proteins as biomarkers. *Molecular Biotechnology*, 2012, **51**:119–127.
- Mariano DO, Yamaguchi LF, Jared C, Antoniazzi MM, Sciani JM, Kato MJ, Pimenta DC. *Pipa carvalhoi* skin secretion profiling: absence of peptides and identification of kynurenic acid as the major constitutive component. *Comparative Biochemistry & Physiology — Part C: Toxicology & Pharmacology*, 2015, **167**:1–6.
- Martins AN, Nencioni ALA, Dorce ALC, Paulo MEV, Frare EO, Dorce VAC. Effect of maternal exposure to *Tityus bahiensis* scorpion venom during lactation on the offspring of rats. *Reproductive Toxicology*, 2016, **59**:147–158.

- Moura-da-Silva AM, Butera D, Tanjoni I. Importance of snake venom metalloproteinases in cell biology: effects on platelets, inflammatory and endothelial cells. *Current Pharmaceutical Design*, 2007, **13**:2893–2905.
- Nencioni ALA, Lourenço GA, Lebrun I, Florio JC, Dorce VAC. Central effects of *Tityus serrulatus* and *Tityus bahiensis* scorpion venoms after intraperitoneal injection in rats. *Neuroscience Letters*, 2009, **463**:234–238.
- Nunes FP, Zychar BC, Della-Casa MS, Sampaio SC, Gonçalves LR, Cirillo MC. Crotoxin is responsible for the long-lasting anti-inflammatory effect of *Crotalus durissus terrificus* snake venom: involvement of formyl peptide receptors. *Toxicon*, 2010, **55**:1100–1106.
- Oguiura N1, Collares MA, Furtado MF, Ferrarezzi H, Suzuki H. Intraspecific variation of the crotoxin and crotoxin genes in *Crotalus durissus* rattlesnakes. *Gene*, 2009, **446**:35–40.
- Oguiura N, Kapronezai J, Ribeiro T, Rocha MM, Medeiros CR, Marcelino JR, Prezoto BC. An alternative micromethod to access the procoagulant activity of *Bothrops jararaca* venom and the efficacy of antivenom. *Toxicon*, 2014, **90**:148–154.
- Pacheco MT, Berra CM, Morais KL, Sciani JM, Branco VG, Bosch RV, Chudzinski-Tavassi AM. Dynein function and protein clearance changes in tumor cells Induced by a Kunitz-type molecule, Amblyomin-X. *Plos One*, 2014, **9**:e111907.
- Pedroso A, Matioli SR, Murakami MT, Piddé-Queiroz G, Tambourgi DV. Adaptive evolution in the toxicity of a spider's venom enzymes. *BMC Evolutionary Biology*, 2015, **15**:290.
- Piddé-Queiroz G, Magnoli FC, Portaro FC, Serrano SM, Lopes AS, Paes Leme AF, van den Berg CW, Tambourgi DV. P-I snake venom metalloproteinase is able to activate the complement system by direct cleavage of central components of the cascade. *PLoS Neglected Tropical Diseases*, 2013, **7**:e2519.
- Ribeiro C, Santos J, Silva J, Godoi P, Magalhães M, Spadafora-Ferreira M, Fonseca S and Pfrimer IAH. *Crotalus durissus collilineatus* venom induces TNF- $\alpha$  and IL-10 production on human peripheral blood mononuclear cells. *ISRN Inflammation*, 2014, **2014**:563628.
- Rizzi CT, Carvalho-de-Souza JL, Schiavon E, Cassola AC, Wanke E, Troncone LR. Crotoxin inhibits preferentially fast-twitching muscles but is inactive on sodium channels. *Toxicon*, 2007, **50**:553–562.
- Rocha LB, Luz DE, Moraes CT, Caravelli A, Fernandes I, Guth BE, Horton DS, Piazza RM. Interaction between Shiga toxin and monoclonal antibodies: binding characteristics and in vitro neutralizing abilities. *Toxins*, 2012, **4**:729–747.
- Sampaio SC, Santos MF, Costa EP, Rangel-Santos AC, Carneiro SM, Curi R, Cury Y. Crotoxin induces actin reorganization and inhibits tyrosine phosphorylation and activity of small GTPases in rat macrophages. *Toxicon*, 2006, **47**:909–919.
- Santoro ML, Carmo T, Cunha BHL, Alves AF, Zelanis A, Serrano SMT, Grego KF, Sant'Anna SS, Barbaro KC, Fernandes W. Ontogenetic variation in biological activities of venoms from hybrids between *Bothrops erythromelas* and *Bothrops neuwiedi* snakes. *Plos One*, 2015, **10**:e0145516.
- Silva NG, Sampaio SC, Gonçalves LRC. Inhibitory effect of *Crotalus durissus terrificus* venom on chronic edema induced by injection of bacillus Calmette-Guérin into the footpad of mice. *Toxicon*, 2013, **63**:98–103.
- Sousa-e-Silva MCC, Tomy SC, Tavares FL, Navajas L, Larsson MHMA, Lucas SRR, Kogika MM, Sano-Martins IS. Hematological, hemostatic and clinical chemistry disturbances induced by *Crotalus durissus terrificus* snake venom in dogs. *Human & Experimental Toxicology*, 2003, **22**:491–500.

Sousa LF, Nicolau CA, Peixoto PS, Bernardoni JL, Oliveira SS, Portes-Junior JA, Mourão RHV, Lima-dos-Santos I, Sano-Martins IS, Chalkidis HM, Valente RH, Moura-da-Silva AM. Comparison of phylogeny, venom composition and neutralization by antivenom in diverse species of *Bothrops* complex. *PLoS Neglected Tropical Diseases*, 2013, **7**:e2442.

Tavares FL, Peichoto ME, Marcelino JR, Barbaro KC, Cirillo MC, Santoro ML, Sano-Martins IS. Platelet participation in the pathogenesis of dermonecrosis induced by *Loxosceles gaucho* venom. *Human & Experimental Toxicology*, 2015, **34**:1–11.

Villas Boas IM, Pidde-Queiroz G, Magnoli FC, Gonçalves-de-Andrade RM, van den Berg CW, Tambourgi DV. A serine protease isolated from the bristles of the Amazonian caterpillar, *Premolis semirufa*, is a potent complement system activator. *PLoS One*, 2015, **10**:e0118615.

Yamasaki SC, Mendes MT, Alpointi RF, Silveira PF. Efficacy of parenteral administration of bee venom in experimental arthritis in the rat: a comparison with methotrexate. *Toxicon*, 2015, **98**:75–88.

Yamashita KM, Alves AF, Barbaro KC, Santoro ML. *Bothrops jararaca* venom metalloproteinases are essential for coagulopathy and increase plasma tissue factor levels during envenomation. *PLoS Neglected Tropical Diseases*, 2014, **8**:e2814.

Yamashita KM, Nogueira TO, Senise LV, Cirillo MC, Gonçalves LRC, Sano-Martins IS, Giorgi R, Santoro ML. Involvement of circulating platelets on the hyperalgesic response evoked by carrageenan and *Bothrops jararaca* snake venom. *Journal of Thrombosis and Haemostasis*, 2011, **9**:2057–2066.

Yonamine CM, Troncone LR, Camillo MA. Blockade of neuronal nitric oxide synthase abolishes the toxic effects of Tx2-5, a lethal *Phoneutria nigriventer* spider toxin. *Toxicon*, 2004, **44**:169–172.

Zambelli VO, Fernandes AC, Gutierrez VP, Ferreira JC, Parada CA, Mochly-Rosen D, Cury Y. Peripheral sensitization increases opioid receptor expression and activation by crotalphine in rats. *PLoS One*, 2014, **9**:e90576.

Zambelli VO, Sampaio SC, Sudo-Hayashi LS, Greco K, Britto LR, Alves AS, Zychar BC, Gonçalves LR, Spadacci-Morena DD, Otton R, Della-Casa MS, Curi R, Cury Y. Crotoxin alters lymphocyte distribution in rats: Involvement of adhesion molecules and lipoxygenase-derived mediators. *Toxicon*, 2008, **51**:1357–1367.

Zambelli VO, Pasqualoto KF, Picolo G, Chudzinski-Tavassi AM, Cury Y. Harnessing the knowledge of animal toxins to generate drugs. *Pharmacological Research*, 2016, in press (doi: 10.1016/j.phrs.2016.01.009).

Zanchet EM, Longo I, Cury Y. Involvement of spinal neurokinins, excitatory amino acids, proinflammatory cytokines, nitric oxide and prostanoids in pain facilitation induced by *Phoneutria nigriventer* spider venom. *Brain Research*, 2004, **1021**:101–111.

Zuliani J, Fernandes C, Zamuner S, Gutierrez J, Teixeira C. Inflammatory events induced by Lys-49 and Asp-49 phospholipases A<sub>2</sub> isolated from snake venom: role of catalytic activity. *Toxicon*, 2005, **45**:335–346.

Zychar BC, Dale CS, Demarchi DS, Gonçalves LRC. Contribution of metalloproteinases, serine proteases and phospholipases A<sub>2</sub> to the inflammatory reaction induced by *Bothrops jararaca* crude venom in mice. *Toxicon*, 2010, **55**:227–234.

## **Toxinology – past, present and future. Some reflections**

**Alan Harvey, University of Strathclyde, Glasgow G4 0NR, UK**

**a.i.harvey@strath.ac.uk**

### **Toxins and toxinology**

Scientists and clinicians studying venoms and toxins have made notable contributions to knowledge. In terms of contributions to medicine, such studies have been important in diverse areas as anti-venoms (of course), adjuncts to anaesthesia (tubocurarine and similar agents that induce selective muscular paralysis without the need for dangerously high levels of general anaesthetic agents), drugs used in high blood pressure and heart failure (captopril and other ACE inhibitors, and integrin antagonists), analgesics (ziconotide) and migraine (perhaps most unexpectedly – botulinum toxin). Apart from the clinical arena, toxins have been widely used as experimental tools in physiology and pharmacology to elucidate mechanisms in many areas, including: synaptic transmission, receptor isolation, auto-antibodies, ion channel subtypes, transmitter release, and functional deconvolution of complex signalling pathways.

Such advances were made by many different types of specialists, including anaesthetists, biochemists, chemists, clinicians, immunologists, neuroscientists, physiologists and pharmacologists. What about ‘toxinologists’? What about ‘toxinology’?

Toxinology is still a relatively recent discipline, if, indeed, it can be regarded as a separate discipline. It might be better thought of as drawing on a much wider range of disciplines under the common banner of an interest in the study of venoms and toxins.

### **The IST and Toxicon**

The first major international conference on venoms appears to have been organised by the American Association for the Advancement of Science in California in 1954. The journal *Toxicon* was planned in 1960, and first published in October 1962 by Pergamon Press. The International Society on Toxinology was founded in 1962 with 79 members. It held its 1st World Congress of IST on Animal, Plant & Microbial Toxins in Atlantic City, New Jersey in 1966. Despite attempts to avoid a clash of dates, another major conference was held in the same year: the International Symposium on Animal Venoms at Instituto Butantan, Brazil. Both meetings attracted a reasonable number of enthusiasts from several countries and many disciplines.

From its beginnings in 1962 with 79 members drawn from 23 countries, the IST has grown to around 400 members (although not all paid up at any one time) from 55 countries. Curiously, the original membership fee of USD 10 per annum in 1962 is equivalent to about USD 80 today, while the actual IST membership fee is USD 55 – a bargain, presumably!

*Toxicon* has grown even more strongly since its launch. The first volume had 256 pages, whereas currently *Toxicon* has over 2000 pages annually. And of course, the availability of *Toxicon* has increased dramatically with more than 500,000 downloads of full-text articles each year. IST members can also get a free digital subscription to *Toxicon*.

### **Current topics in toxinology**

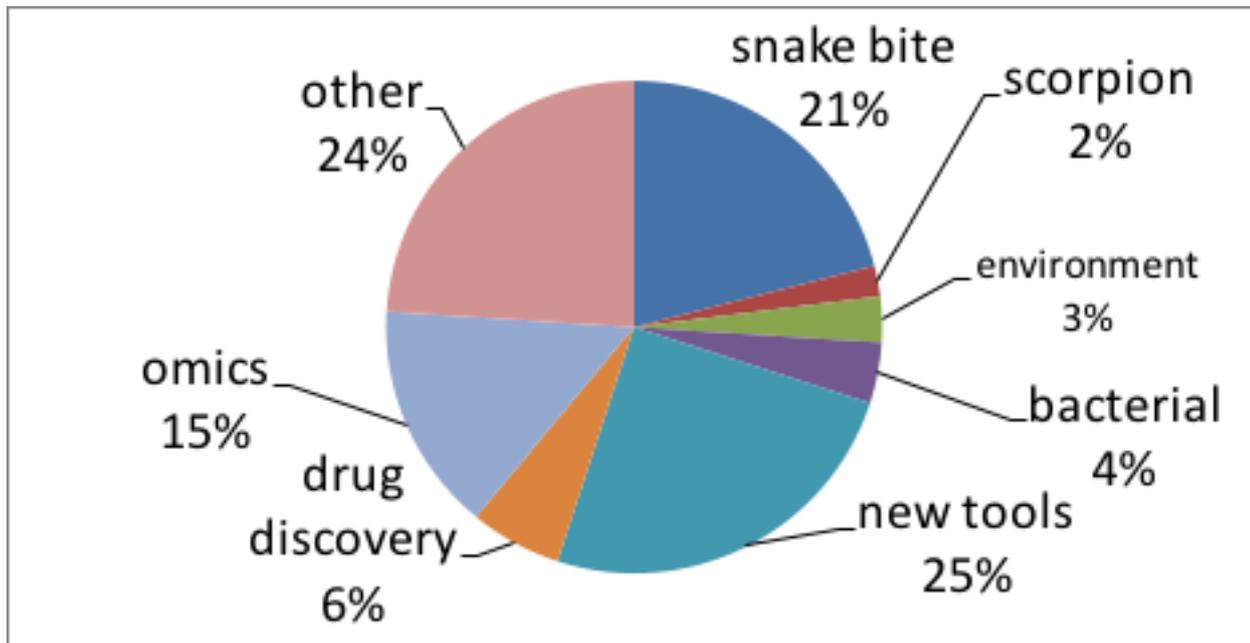
A scan of titles in *Toxicon* and other journals suggests that the outstanding issues in toxinology are the following:

- treating victims of snake envenoming
- treating victims of scorpion stings
- dealing with toxins in the environment (mycotoxins, cyanobacterial toxins, etc)
- dealing with dangerous bacterial pathogens
- finding and characterising new pharmacological tools
- contributing to drug discovery

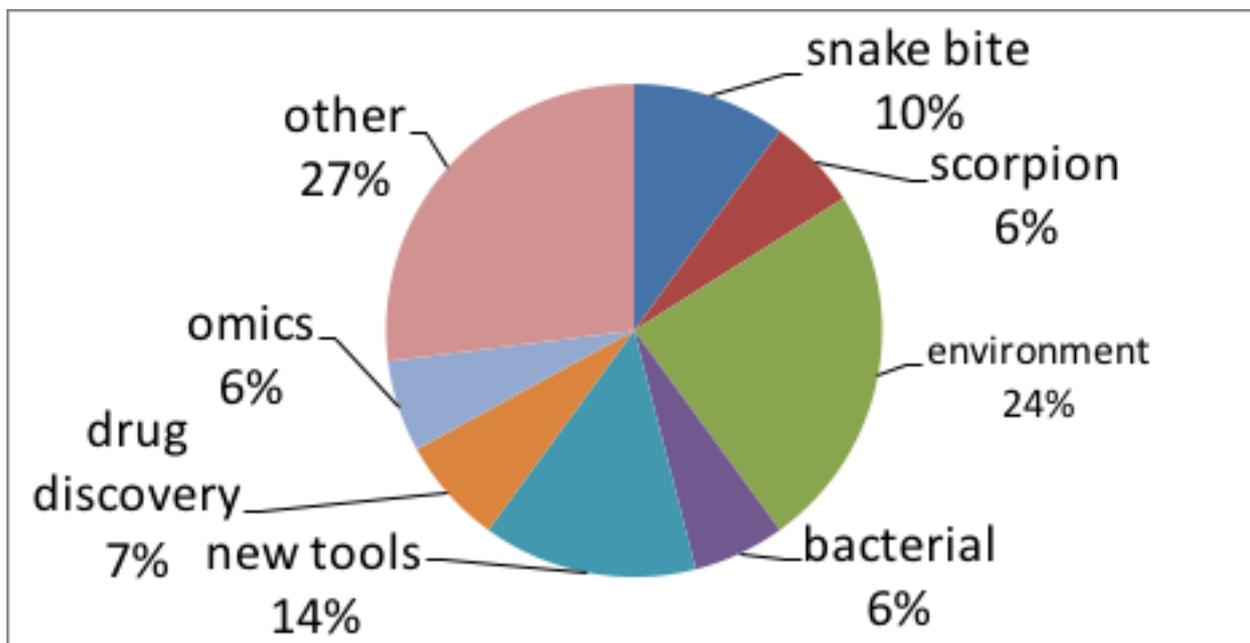
The level of interest from IST members in these major topics can be gauged by looking at the coverage in presentations at the recent 18th World Congress of the IST in Oxford in September

2015 and from articles appearing in Toxicon. The information is summarised in the following diagrams.

Level of interest – Oxford (percentage of abstracts relating to a particular topic):



Level of interest – from articles in Toxicon in 2015:



It might not be valid to draw conclusions from such simple snapshots, but it does seem that the toxinology community represented by the IST continues to contribute to knowledge about snake envenoming and to the discovery of potentially novel experimental tools. Studies on environmental toxins are a mainstay in Toxicon, although this was not a major topic at the IST Congress. The increasing number of publications and presentations based on the use of various 'omics' technologies probably is the latest manifestation of how toxinologists readily adapt and adopt scientific developments to help study venoms and toxins.



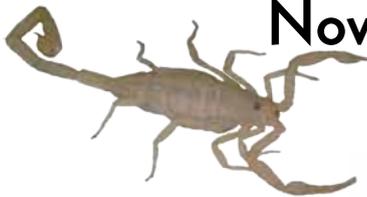
University of Adelaide  
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# CLINICAL TOXINOLOGY SHORT COURSE 2017

Women's & Children's Hospital  
Adelaide, Australia

November 28th to December 6th,  
2017



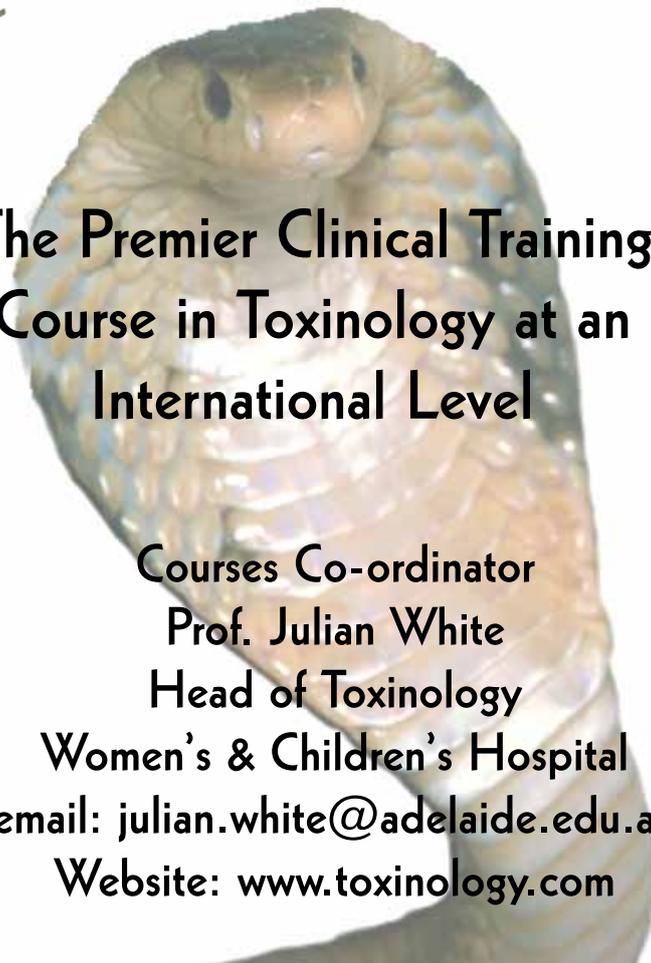
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Website: [www.toxinology.com](http://www.toxinology.com)



## IMPORTANT COURSE INFORMATION

### THE 2017 COURSE IS SPECIAL!

The first Clinical Toxinology Short Course was held in November 1997. No similar comprehensive course in clinical toxinology existed. A maximum of 30 course registrants was allowed and the course was full. In 1999 the course was again offered, but with additional segments including poisonous plants and mushrooms and a true international scope and coverage. Since then the course has been held about every 2 years (1997, 1999, 2001, 2003, 2005, 2008, 2010, 2012, 2014, 2016), the most recent being in March 2016. Nearly every course is full (current maximum registrants is 50) and doctors and other health professionals, and occasional herpetologists have attended over the last 19 years, from about 40+ countries, from the Pacific, Asia, Africa, South & North America, and Europe.

The 2017 course is being held almost exactly 20 years after that first course in November 1997. The course program has been slightly expanded to accommodate the increased faculty, including 2 members of the original 1997 faculty who have since retired, though they remain active in toxinology. We expect this to be a very special course, not to be missed.

In addition it may count as the first module in a proposed new Diploma in Clinical Toxinology which may commence in 2018 (to be confirmed).

### COURSE RELATED QUESTIONS:

#### **Who is this course designed for?**

Primarily for doctors/health professionals requiring detailed and practical information on snakebite, spiderbite, scorpion stings, marine envenoming, poisonous plants & mushrooms and related topics with a global and Australian perspective. It is particularly relevant for those working in emergency medicine, toxicology, intensive care, or in rural practice. Throughout there will be an emphasis on practical clinical issues and development of clinically relevant skills. It will also be of interest to poisons information pharmacists and graduate nurses in emergency medicine and toxinology scientists. You should be fluent in English, as no language translation will be available.

#### **When and where are the courses held?**

The course runs over 7 days; Tuesday November 28th to Wednesday December 6th, 2017, with a 2-day break for the weekend. The venue is the Women's and Children's Hospital, North Adelaide, SA, Australia

#### **What does the course cover?**

We cover terrestrial & marine animals, plants & mushrooms, including extensive sessions on venomous snakes by region. Detailed sheets on course content will be available on the web at <http://www.toxinology.com>.

#### **Is the course accredited in any way?**

The course is a University of Adelaide postgraduate training course. We are seeking formal accreditation of continuing education points with relevant colleges and possible incorporation within some college specialist training schemes.

#### **How many people can attend the course?**

The maximum course capacity is 50 registrants, to ensure a chance for interactions with faculty. Previous courses filled early, so early registration is advisable.

#### **How much does the course cost and what does this cover?**

The course costs are yet to be finalised, but for 2016 were Aus\$2,200 (+GST for Australians only); the fee covers the full course, course notes, field trip, morning and afternoon teas and light lunches. It does not cover the course dinner or accommodation.

**Are there any course notes or reading material available prior to the course?**

We produce course notes for registrants prior to the course, which will include recommended textbooks and reading list. You are still strongly advised to take notes during all sessions. (The 2016 Course Handbook exceeded 500 pages.)

**What sort of practical clinical sessions are included?**

The programme includes many interactive sessions discussing “clinical evolving problems” (CEPs) to develop registrant’s understanding of clinical skills in toxinology and test those skills in a group setting. These are all based on real patients contributed by faculty members, drawn from their own clinical experience.

**Is there any formal evaluation of my performance on the course?**

Yes! Faculty will be evaluating all registrants on their interactions, especially during the clinical evolving problem sessions. On the Saturday there will be a written examination.

**For further information** check the Course pages on [www.toxinology.com](http://www.toxinology.com), or contact Prof. White ([julian.white@adelaide.edu.au](mailto:julian.white@adelaide.edu.au)).

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3<sup>rd</sup> International Symposium

# Venoms 2016

05<sup>TH</sup> – 06<sup>TH</sup> September 2016

Vernon Harcourt Room, St Hilda's College  
Oxford OX4 1DY, United Kingdom



**Email:** [VenomsOxford@gmail.com](mailto:VenomsOxford@gmail.com)

**Web:** <http://lpmhealthcare.com/venoms-2016>

**Twitter:** @LPMHealthcare | @VenomsOxford | **Hashtag:** #VenOx16



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# GENERAL INFORMATION

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<b>Event Dates:</b>	05 <sup>th</sup> - 06 <sup>th</sup> September 2016
<b>Event Website:</b>	<a href="http://lpmhealthcare.com/venoms-2016">http://lpmhealthcare.com/venoms-2016</a>
<b>Venue:</b>	Vernon Harcourt Room, St Hilda's College, Cowley Place, Oxford, OX4 1DY, England, UK Tel: +44 (0) 1865 276884, Website: <a href="http://www.st-hildas.ox.ac.uk">http://www.st-hildas.ox.ac.uk</a>
<b>Registration Desk:</b>	Located outside the meeting hall
<b>Name Badges:</b>	The College requests all delegates to wear name badges while on the premises to avoid any confusion.
<b>Refreshments/Lunch:</b>	College dining hall
<b>Mobile Phones:</b>	As a courtesy to speakers and participants, please switch off your mobile phone during oral presentations.
<b>Speaker Presentations:</b>	We will not be distributing speaker presentations. Therefore, if you interested in presentation slides of any speakers, please get in touch with them directly.
<b>Internet access:</b>	Please use edurom if you can. Otherwise, WiFi Code and instructions for internet access via your laptop/mobile device can be obtained at the time of registration.
<b>Health and Safety:</b>	Please do not leave your belongings unattended or in passageways and familiarise yourself with emergency exits.
<b>Smoking:</b>	In addition to any local venue regulations, UK no-smoking regulations apply on the College premises.

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## INFORMATION FOR PRESENTERS

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### SPEAKERS:

- Presentation standard will be data projection from a central PC. Your presentation is best brought in a memory stick.
- Macintosh will not be available. Therefore, if you are a Macintosh user please bring your own.
- As a courtesy to other speakers and attendees please finish your talk within your allocated time slot. (Guide: For a 20 minute talk, prepare 12-14 slides maximum ; for a 30 minute talk, prepare 20-22 slides maximum; and for a 40 minute talk, prepare 25-30 slides; allow 3-4 minutes for questions). Please check the agenda below for your presentation schedule.

### POSTER DISPLAY:

- Please leave your poster at the registration desk when you register.
- There is no specific poster session. The posters will be displayed in the registration/refreshment area for full duration of the meeting.

## INSURANCE AND LIABILITY:

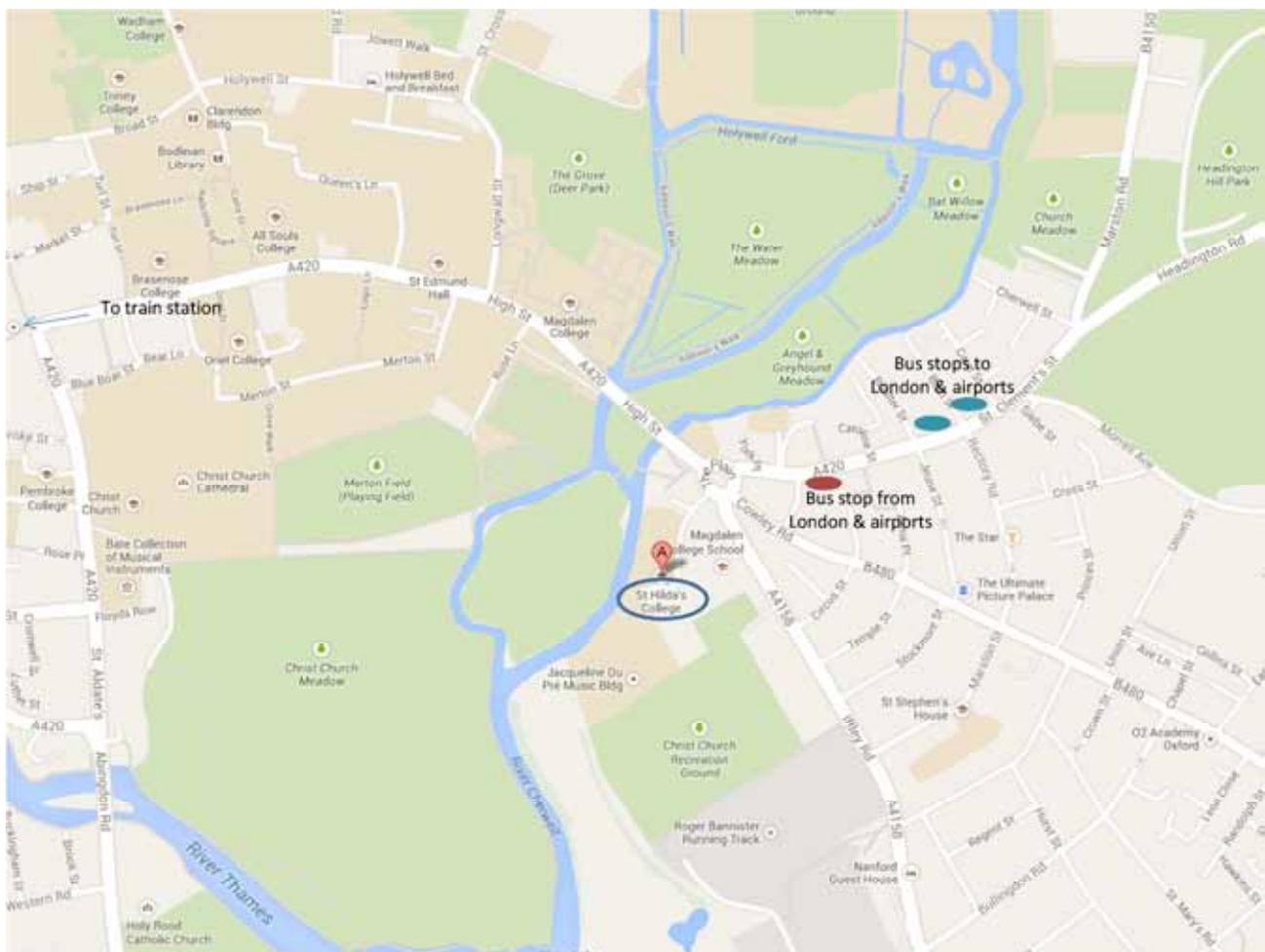
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# PODIUM AGENDA *(subject to change)*

Monday 05<sup>th</sup> September 2016 | Vernon Harcourt Room, St Hilda's College

- 0830:** Registration, networking and welcome coffee  
**0925:** Welcome and housekeeping  
**0930:** **Plenary-1 Professor David Warrell**, University of Oxford, UK  
**1000:** **Life-time achievement award to Professor David Theakston**, Liverpool School of tropical Medicine, UK

## Session 1: Venom Evolution - Chair Dr Nicholas Casewell

- 1020:** **Professor Juan Calvete**, Instituto de Biomedicina de Valencia, CSIC, Spain  
*Proteomic analysis of venom variability and ontogeny across genus Bothriechis supports an adaptationist view for the evolution of arboreal palm-pitvipers*  
**1040:** **Dr Anita Malhotra**, Bangor University, UK  
*Mutation, duplication and gene conversion in the evolution of pitviper phospholipase A<sub>2</sub> toxins*  
**1100:** **Refreshment Break**  
**1130:** **Dr Ronald A Jenner**, The Natural History Museum of London, UK  
*The evolution of toxins in carnivorous crustaceans and venomous bloodworms*

## Session 2: Drugs from Toxins - Chair Dr Denis Servent

- 1150:** **Miss Andrea Martos**, Technical University of Denmark, Denmark  
*Making Trypanosoma brucei for ever go to sleep with snake venom toxins*  
**1210:** **Miss Laura Droctové**, CEA, France  
*Crystal structure and functional domains of the Mambaquearin-1, a vasopressin type 2 receptor peptide inhibitor to treat kidney cysts*  
**1230:** **Professor Peter Strong**, Sheffield Hallam University, UK  
*Scorpion venom antimicrobial peptides: mechanism of action of antimicrobial peptides from the Egyptian scorpion, Scorpio maurus palmatus*  
**1250:** **Professor Jeroen Kool**, VU University Amsterdam, Amsterdam, The Netherlands  
*Post-column flow cytometry analytics for discovery of venom peptides targeting the nicotinic acetylcholine receptor*  
**1310:** **Lunch Break**  
**1400:** **Plenary-2 Professor Cesare Montecucco (Keynote)**, University of Padova, Italy  
*Intra- and inter-cellular signalling during nerve terminal degeneration and regeneration induced by presynaptic animal neurotoxins*

## Session 3: Neurotoxins - Chair Professor Cesare Montecucco

- 1430:** **Dr Denis Servent**, CEA, France  
*Three-finger fold toxins as multipotent structural fold to modulate various physiological functions*  
**1450:** **Dr Ian Mellor**, University of Nottingham, UK  
*Analogues of a toxin from solitary wasp venom as tools for dissecting elements of excitatory neurotransmission and as potential insecticides*  
**1510:** **Dr Edward Rowan**, University of Strathclyde, UK  
*Pre- and Post-synaptic Activities of Tityus bahiensis Scorpion Venom on Avian Neuromuscular Preparation*  
**1530:** **Refreshment Break**

#### Session 4: Snake-bite and antivenom-1 - Chair Dr Robert Harrison

- 1600: Dr Robert Harrison**, Liverpool School of Tropical Medicine, UK  
*Recent progress to substantially and sustainably reduce the mortality, morbidity and socioeconomic burden of tropical snakebite*
- 1620: Dr Maya Gopalakrishnan**, All India Institute of Medical Sciences, India  
*Circulatory shock and mortality in viper envenomation: A prospective observational study from India*
- 1650: Dr Nicholas Casewell**, Liverpool School of Tropical Medicine, UK  
*The procoagulant effect of snake venoms and their neutralisation by antivenom*
- 1710: Ms Fiona Bolton**, Liverpool School of Tropical Medicine, UK  
*Analgesia to implement 3Rs into the preclinical assessment of venom toxicity and antivenom efficacy*
- 1730: Professor Nelson Jorge da Silva Jr**, Pontificia Universidade Católica de Goiás, Brazil  
*Comparative venom yield of coralsnakes and the paradigm of anti-venom production and use in Brazil*
- 1750: Close**
- 1900: Networking Dinner** (by invitation or prior booking only - entry by ticket)

**Tuesday 06<sup>th</sup> September 2016** | Vernon Harcourt Room, St Hilda's College

- 0925:** Welcome and housekeeping
- 0930: Plenary-3 Professor Dr Dietrich Mebs (Keynote)**, University of Frankfurt, Germany  
*Toxic newts – where does tetrodotoxin come from?*

#### Session 5: Snake-bite and antivenom-2 - Chair Dr Edward Rowan

- 1000: Dr Mikael Engmark**, Technical University of Denmark, Denmark  
*High-throughput epitope profiling of snake venom toxins – unveiling the complexity of antigen-antibody interactions of polyvalent antivenoms*
- 1020: Dr Julien Potet**, Policy Advisor (Neglected Tropical Diseases, Vaccines), Médecins Sans Frontières, France  
*Addressing the antivenom supply crisis in Africa: Médecins Sans Frontières' multi-pronged approach*
- 1040: Refreshment Break**
- 1100: Plenary-4 Professor Julia Prado Franceschi, UNICAMP, Brazil**  
*The transcendence of Vital Brazil's work*
- 1130: Professor Philippe Billiard**, Museum National d'Histoire Naturelle, Paris, France  
*Design of humanized antibody fragments that neutralize the venom of *Loxosceles* spiders: Structural and functional characterization*
- 1150: Dr Andreas Hougaard Laustsen**, Technical University of Denmark & University of Copenhagen, Denmark  
*Discovery of human IgGs targeting the medically important toxins from the venom of the black mamba (*Dendroaspis polylepis*)*
- 1210: Dr Robert Harrison**, Liverpool School of Tropical Medicine, UK  
*The burden of snakebite and snakebite treatment on rural African health infrastructures: a Burkina Faso case study*
- 1230: Lunch and close**

# PODIUM ABSTRACTS

## Snake-bite: a tale of two countries - Nigeria and Myanmar

David Warrell

University of Oxford, UK

**Nigeria:** Within a few months of arriving in Zaria, northern Nigeria, in 1970, I admitted a 35-year-old Hausa man to Ahmadu Bello University Hospital, 5 hours after he was bitten by a “kububuwa” snake while farming. He was bleeding from his mouth, nose and urinary tract and, unexpectedly (because I was naïve about snake-bite in those days), his blood taken for cross-matching for blood transfusion would not clot in a glass test tube. No antivenom was available, none of my doctor colleagues could advise me on treatment and, despite repeated blood transfusions he bled to death 18 hours after being bitten. This was my shocking and tragic introduction to saw-scaled/carpet viper (*Echis ocellatus*) envenoming in West Africa. Subsequent discussions with government and missionary doctors working in the North quickly convinced me of the importance of snake-bite, the depth of ignorance about medically-important species and the clinical effects of their venoms, and the lack of effective antivenoms. Although many patients brought the dead snake responsible, in the majority (as in published reports) the aetiology was unknown, prompting the development of immune-diagnosis, initially by simple immunodiffusion or immune-electrophoresis (H Whittle and BM Greenwood) and later by EIA (RDG Theakston). Zaria, Gombe, Bambar and especially Kaltungo proved ideal sites over the next 35 years for clinical studies of snake-bite envenoming, carried out with the help of HA Reid, RDG Theakston, HM Pope, NMCD Davidson, CRM Prentice and others. and others, culminating in 2005-7 in a large RCT, helped by Abdulrazak Habib, Saidu Ballah Abubakar, Abdulsalami Nasidi and others. Two whole IgG antivenoms raised against Nigerian *E. ocellatus* venom were compared. Both had demonstrated effectiveness in pre-clinical tests and a Phase I safety/dose-finding study employing a novel 3 x 3 escalating dosage protocol. *Echis* species are now recognised as the most important causes of snake-bite across supra-equatorial Africa. The 20 minute whole blood clotting test, developed for bed-side detection of venom-induced consumption coagulopathy in Nigeria, has been widely implemented in Africa, Asia, Oceania and Latin America. Black-necked spitting cobra (*Naja nigricollis*) and its congeners elsewhere in Africa proved capable of debilitating local necrosis without the expected neurotoxicity, while burrowing asps (*Atractaspis* species) were revealed as common causes of envenoming. Despite the barbaric activities of Boko Haram, especially since 2009, Kaltungo General Hospital (SB Abubakar) has retained its pre-eminence as a snake-bite treatment centre, attracting thousands of patients each year, nation-wide and from adjacent countries.

**Myanmar:** Reading Swaroop & Grab’s classic 1954 paper “Snakebite mortality in the world”, I was struck by the statement: “As compared with other countries of the world, the incidence of snakebite in Burma (1936-40) is very high, the average rate being 15.4 per 100,000 population (Sagaing 36.8, Meiktila 34.0, Magwe 32.5, Tharawaddy 31.4.....) >2,000 deaths/year”. Surely, Burma was where I should be working! Within a year of moving to Thailand in 1979, I had visited Myanmar and started to persuade the Department of Medical Research (DMR), under its dynamic DG Aung-Thant-Batu, to set up a clinical research unit at Tharawaddy General Hospital, 120 km north of Yangon, to study malaria and snake-bite. Russell’s viper (*Daboia siamensis*) was the dominant cause of snake-bite. We discovered the high case-fatality to be attributable to coagulopathy/bleeding, shock, acute kidney injury (AKI), acute pituitary-adrenal failure and generalised increase in capillary permeability. Given early, “Burma Pharmaceutical Industry” monospecific Russell’s viper antivenom was effective in reversing anti-haemostatic effects and shock, but it did not prevent evolution of multi-factorial AKI. Pathophysiological mechanisms of *D. siamensis*-induced cardiovascular collapse, coagulopathy, micro-vascular obstruction, nephrotoxicity and renal ischaemia, acute/chronic pituitary failure and capillary permeability were revealed by clinical and autopsy studies. We were helped by a network of local and UK-based collaborators, notably Tun-Pe, RE Phillips, RA Hutton, N Francis, CW Burke and PJ Ratcliffe. Several decades of snake-bite research followed, initiated by Tun-Pe and his DMR colleagues. In 2015, international collaboration resumed with Australian Department of Foreign Affairs and Trade (DFAT) and Government Partnerships for Development (GPDF) funding of a project “Improving the health outcomes for snakebite patients in Myanmar” led by Chen Au Peh of the University of Adelaide.

## Proteomic analysis of venom variability and ontogeny across genus *Bothriechis* supports an adaptationist view for the evolution of arboreal palm-pitvipers

Davinia PLA<sup>1,\*</sup>, Libia SANZ<sup>1,\*</sup>, Mahmood SASA<sup>2</sup>, Manuel E ACEVEDO<sup>3</sup>, Quetzal DWYER<sup>4</sup>, Alicia PÉREZ<sup>1</sup>, Yania RODRIGUEZ<sup>1</sup>, Bruno LOMONTE<sup>2</sup>, Juan J CALVETE<sup>1,&</sup>

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<sup>4</sup> Parque Reptilandia, Puntarenas, Costa Rica

*Bothriechis* is a genus of eleven currently recognized slender and arboreal venomous rattleless snakes, commonly called palm-pitvipers, that range from southern Mexico to northern South America. Despite dietary studies suggesting that palm-pitvipers are generalists with an ontogenetic shift toward endothermic prey, venom proteomic analyses have revealed remarkable divergence between the venoms of the Costa Rican species, *B. lateralis*, *B. schlegelii*, *B. supraciliaris*, and *B. nigroviridis*. To achieve a more complete picture of the venom landscape across *Bothriechis*, the venom proteomes of adult specimens of the northern Middle American highland palm-pitvipers, *B. thalassinus*, *B. aurifer*, and *B. bicolor* from Guatemala, *B. marchi* from Honduras, and neonate Costa Rican *B. lateralis* and *B. schlegelii*, were investigated. *B. thalassinus* and *B. aurifer* venoms are comprised by similar toxin arsenals dominated by SVMs (33-39% of the venom proteome), CTLs (11-16%), BPP-like molecules (10-13%), and CRISPs (5-10%), and are characterized by the absence of PLA<sub>2</sub> proteins. Conversely, the predominant (35%) components of *B. bicolor* are D49-PLA<sub>2</sub> molecules. The venom proteome of *B. marchi* is, like those of *B. aurifer* and *B. thalassinus*, rich in SVMs and BPPs, but unlike them, contains appreciable amounts (14.3%) of PLA<sub>2</sub>s. The major toxin family found in the venoms of both neonate *B. lateralis* and *B. schlegelii*, is serine proteinase (SVSP), comprising about 20% of their toxin arsenals. The venom of neonate *B. schlegelii* is in addition characterized by being the only palm-pitviper venom where relative high amounts of Kunitz-type (6.3%) and  $\gamma$ PLA<sub>2</sub> inhibitors (5.2%) have been identified. Despite notable differences between their proteomes, neonate venoms are more similar to each other than any of them to the adult of its same species. However, the ontogenetic changes taking place in the venom of *B. lateralis* strongly differ from those that occur in the venom of *B. schlegelii*. Overall, genus-wide venomomics illustrate the high evolvability of palm-pitviper venoms. Integrated into a phylogenetic and biogeographic framework, the pattern of venom variation across *Bothriechis* is consistent with the view that divergence was driven by selection for efficient resource exploitation in arboreal 'islands', and thereby contributed to the ecological speciation of the genus.

## Mutation, duplication and gene conversion in the evolution of pitviper phospholipase A<sub>2</sub> toxins

Anita Malhotra

School of Biological Sciences, Bangor University, Gwynedd LL57 2UW, UK

Toxins represent one of the fastest evolving types of protein to be found in animal systems, sharing many of their features with other protein families that respond to extrinsic factors, such as those involved in immunity, and detecting and responding to the environment in which they live. Several molecular features acting on toxin gene have been stressed in the toxinological literature, i.e., their hypervariability, accelerated (Darwinian) evolution, apparent focal mutagenesis centring on the active site of the toxins, and underlying birth-and-death model of gene family evolution. However, the evolution of a gene family depends on a balance between different types of recombination (reciprocal/non-reciprocal) and point mutation rates. In this talk I use data from phospholipase A<sub>2</sub> whole gene sequences from pitvipers to explore the relative importance of mutation, duplication, and gene conversion.

## The evolution of toxins in carnivorous crustaceans and venomous bloodworms

Ronald A Jenner

Department of Life Sciences, Natural History Museum, Cromwell Road, London, UK

Rampant convergent evolution is a hallmark of venoms. Yet, the composition of venom cocktails is far from capricious. Striking similarities occur even between distantly related taxa, resulting from the recruitment of homologous toxins and convergent evolution of similar toxins. In this talk I will focus on our ongoing research on the venoms of remiped crustaceans and polychaetes. Our combined transcriptomic and proteomic analyses reveal the enormous complexity of these venoms, as well as the extraordinary degree to which convergent evolution has shaped venom composition. The inclusion of data from non-venom gland tissues and non-venomous species allow us to begin to address the question of the evolutionary origins of venom toxins, a fascinating issue for which contrasting models are currently being debated. Moreover, selection analyses for selected toxins shed some initial light on the possible biological roles of different toxins in the venom cocktail of bloodworms.

## Making *Trypanosoma brucei* for ever go to sleep with snake venom toxins

Andrea Martos<sup>1</sup>, Mark Carrington<sup>2</sup>, Andreas H Laustsen<sup>1</sup>

<sup>1</sup>Department of Systems Biology, Technical University of Denmark

<sup>2</sup>Department of Biochemistry, University of Cambridge, UK

As part of its lifecycle, the protozoan pathogen *Trypanosoma brucei* infects and proliferates in humans and animals causing African trypanosomiasis. It resides exclusively in the extracellular environment in the mammalian host, and the external face of the plasma membrane is covered by a densely packed monolayer of a single polypeptide, the variant surface glycoprotein (VSG), which is the basis for antigenic variation. One VSG gene is transcribed from a repertoire of ~2000 VSG genes, switching from one VSG to another allowing the *T. brucei* population to avoid the adaptive immune response. This remarkable antigenic variation, together with its ability to cross the blood brain barrier, underpin the pathology of the disease. Better understanding of the biology of the cell surface of *T. brucei* biology may provide new approaches and targets. Snake venoms represent one of Nature's most diverse arsenals of potent bioactive compounds. The toxins present in snake venoms target a myriad of different physiological processes with high specificity and selectivity. In our work, we investigated the effect of a number of different elapid and viperid venoms against *T. brucei* and discovered that the enzymatically active venoms of *Bothrops asper*, *Naja nigricollis*, and *Naja mossambica* had potent lethal activities. In contrast, a number of elapid venoms with no or low enzymatic activities did not seem to affect *T. brucei*. The enzymatically active venoms demonstrated potent toxic effects, which is speculated to be due to the presence of metalloproteinases (*B. asper*) and phospholipase A<sub>2</sub>s (all venoms). Further investigations are undergoing with the aim of elucidating the molecular mechanism behind the observed venom-induced lethality of *T. brucei* and possibly developing molecular tools that may aid future drug discovery efforts against trypanosome related diseases.

## Crystal structure and functional domains of the Mambaquaretin-1, a vasopressin type 2 receptor peptide inhibitor to treat kidney cysts

Laura Droctové<sup>1</sup>, Justyna Ciolek<sup>1</sup>, Manon Lancien<sup>1</sup>, Enrico A. Sture<sup>1</sup>, Laura Vera<sup>1</sup>, Nicolas Floquet<sup>2</sup>, Ralph Witzgall<sup>3</sup>, Bernard Mouillac<sup>4</sup>, Christiane Mendre<sup>4</sup>, Gilles Mourier<sup>1</sup>, Denis Servent<sup>1</sup>, Nicolas Gilles<sup>1</sup>

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Mambaquaretins are a group of toxins from mamba venoms, containing seven members until now, which target the vasopressin type 2 receptor (V2R). V2R, primarily expressed in the kidney, belongs to the G-protein coupled receptor family and regulates fluid osmotic pressure and diuresis through the vasopressin hormone. The Mambaquaretin-1, the very first identified member of the Mambaquaretin group, antagonizes V2R and this property gives it potential therapeutic applications to treat disorders such as hyponatremia, syndrome of inappropriate antidiuretic hormone secretion (SIADH) or polycystic kidney diseases (PKD). PKD are severe genetic diseases which affect 1 in 1,000 people in the world. However, therapeutic strategies are very limited. An animal trial was conducted in a rodent model of PKD and demonstrated the efficacy of Mambaquaretin-1 to slow down the disease progression after a 100 days treatment, validating the toxin as a novel therapeutic strategy. The pharmacodynamics of the toxin was further investigated in

healthy rats to estimate the best dose to inject regarding duration of action and side effects occurrence. To define the molecular mode of interaction between V2R and Mambaquaretin-1, a structure activity relationship study was completed. X-ray diffraction approach confirmed that the toxins adopt a Kunitz fold reticulated by three disulphide bridges. Comparing the natural SAR of the seven active Mambaquaretins and exploring the V2R activity of six toxins from databases very close in sequence, interesting positions were mutated in order to determine the pharmacophore of the Mambaquaretin-1. Consequently, we could advance a receptor-toxin complex model in which the toxin interacts both with the upper part of the orthosteric site and the extracellular loops. Thanks to these results, engineered toxins are being designed to enhance its in vivo characteristics, such as blood stability or speed of elimination in the aim to propose the best therapeutic candidate.

## Scorpion venom antimicrobial peptides: mechanism of action of antimicrobial peptides from the Egyptian scorpion, *Scorpio maurus palmatus*

Peter N. Strong<sup>1</sup>, Patrick L. Harrison<sup>1</sup>, George R. Heath<sup>2</sup>, Benjamin R. G. Johnson<sup>2</sup>, Mohamed M. Tawfik<sup>1</sup>, Mohamed A. Abdel-Rahman<sup>1,3</sup>, Stephen D. Evans<sup>2</sup> & Keith Miller<sup>1</sup>

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<sup>3</sup>Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

Antimicrobial peptides (AMPs) represent an ancient and universal host defence mechanism of the innate immune system in the animal kingdom. Their predominant mechanism of action is to disrupt the structure and function of microbial cell membranes. Because of their selectivity for prokaryotes and their membrane-disruptive mechanisms for which microbes have little natural resistance, they offer an attractive approach to the development of novel antibiotics. AMPs have been found in the venoms of all venomous species examined. Abdel Rahman and colleagues (2013) have recently identified several novel alpha-helical AMPs, from the venom of the North African scorpion *Scorpio maurus palmatus*. Smp-24 and Smp-43 have broad spectrum antimicrobial activity. Structure-function studies of Smp43 highlight the beneficial effect of a helical-hinge-helical conformation in promoting prokaryotic selectivity, as well as the importance of examining a wide range of mammalian cell types in cytotoxicity testing. We have used atomic force microscopy, quartz crystal microbalance-dissipation and liposomal leakage assays to study the mechanism of Smp24. Our data provides direct evidence that Smp24 has multiple mechanisms of action, dependent on lipid composition. This peptide forms toroidal pores in model prokaryotic membranes but induces hexagonal, non-lamellar phase structures and causes phase segregation in model eukaryotic membranes.

## Post-column flow cytometry analytics for discovery of venom peptides targeting the nicotinic acetylcholine receptor

Reka A Otvos<sup>1</sup>, Manjunatha R Kini<sup>2</sup>, August B Smit<sup>1</sup>, Govert W Somsen<sup>1</sup>, Jeroen Kool<sup>1</sup>

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<sup>2</sup>National University of Singapore, Singapore

This presentation discusses the combination of a functional cell-based assay with LC and MS in order to obtain biological cellular responses of eluting venom components after chromatography. The analytical technique developed includes a mammalian cellular assay performed in post-column continuous flow format with flow cytometry as readout. This novel flow cytometry hyphenation is performed directly on-line after LC with parallel MS analysis via a flow split and allows simultaneous assessment of biological activity and identity of eluting venom toxins. The  $\alpha 7$ -nicotinic Acetylcholine Receptor ( $\alpha 7$ -nAChR) is a target for many different venom toxins. A functional calcium-flux assay with human neuroblastoma SH-SY5Y cells over-expressing the  $\alpha 7$ -nAChR was used to screen for bioactive toxins in venoms and other natural extracts. The analytical system was first optimized and pharmacologically validated in agonist, allosteric modulator and antagonist setup. Next, various types of natural extracts were screened, such as a tobacco extract in agonist mode as a proof of principle, and then snake venoms in mixed antagonist and agonist analysis mode. Eluting bioactive peptides could directly be correlated to their respective accurate mass. The developed methodology opens up the possibility for direct and fast post column cellular screening of venoms for toxins targeting the  $\alpha 7$ -nAChR. It is

anticipated that other types of fast cell-based assays (e.g. ion flux assays in general) are also applicable to the here presented analytical technique.

## Mitochondrial Alarmins and ATP are Key Intercellular Signals in the Degeneration & Regeneration of the Neuromuscular Junction

Cesare Montecucco<sup>1</sup>, Michela Rigoni<sup>1</sup>, Elisa Duregotti<sup>1</sup>, Samuele Negro<sup>1</sup>, Bryan C. Dickinson<sup>2</sup>, Christopher J. Chang<sup>2</sup>

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<sup>2</sup> Department of Chemistry and Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

The neuromuscular junction is one of the few human tissues capable of complete regeneration after major damages (1). We have set up a reliable model of acute degeneration of the motor axon terminals followed by complete recovery of function based on the use of  $\alpha$ -latrotoxin or  $\beta$ -bungarotoxin (2). We have found that alarmins (Hydrogen Peroxide, mt-DNA and cyt c) are released by mitochondria of the degenerating nerve terminal (2). In addition motor axon terminals right after damage release ATP which activates perisynaptic Schwann cells (3). Stimulated by mitochondrial alarmins by ATP, these cells are activated, become phagocytes and release signals that act retrogradely on the nerve terminal inducing its regeneration. As an example we will show the effect of a chemokine which was identified via a specific transcriptomics analysis. This chemokine strongly stimulates axonal growth and recovery of function after nerve terminal degeneration. Other retrograde signals may be identified using imaging and transcriptomics methods.

1. Sanes M & Lichtman JW. Development of the vertebrate neuromuscular junction. *Annu Rev Neurosci* (1999) 22:389-442

2. Duregotti E, Negro S, Scorzeto M, Zornetta I, Dickinson BC, Chang CJ, Montecucco C, Rigoni M. Mitochondrial alarmins released by degenerating motor axon terminals activate perisynaptic Schwann cells. *Proc Natl Acad Sci U S A*. (2015) 112:497-505

3. Negro S, Bergamin E, Rodella U, Duregotti E, Scorzeto M, Jalink K, Montecucco C, Rigoni M. ATP Released by Injured Neurons Activates Schwann Cells. *Front Cell Neurosci*. (2016) 10:134. doi: 10.3389/fncel.2016.00134

## Three-finger fold toxins as multipotent structural fold to modulate various physiological functions

Guillaume Blanchet, Gilles Mourier, Nicolas Gilles, Denis Servent

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Despite their extraordinary diversity, animal toxins belong to a limited number of structural superfamilies from which the three-finger fold is probably the most common in snake venoms. Three-finger toxins (3FTx) have a molecular mass within the range of 6000- 8000 Da, they contain four disulphide bridges conserved in all members, located in the small globular core from where three  $\beta$ -strands emerge. Members of 3FTx family show a wide array of pharmacological effects by targeting different receptors, enzymes and ion channels with often high specificity and affinity. We have focused our studies on 3FTx from mambas that display the unique property to interact with various GPCRs. Indeed, in addition, to the well-known muscarinic toxins, we have identified several toxins active on  $\alpha$ -adrenoceptors as well as on dopamine D3 subtype, highlighting the multipotent interacting property of 3FTx for aminergic GPCRs. These toxins may display either absolute selectivity for one receptor subtype or a polypharmacological property for various aminergic receptors. Moreover, we have studied the mode of action of some of these toxins (MT7 and p-Da1a) on their respective targets (muscarinic M1 and  $\alpha$ 1<sub>A</sub>-adrenoceptor), by pharmacological and structural approaches. Our results highlight that both toxins interact in a complete different way with GPCRs. Based on these results, toxin's engineering using a loop permutation strategy was used in order to design new three-finger toxins with original pharmacological profiles.

Finally, phylogenic analyzes of these 3FTx show that muscarinic, adrenergic and dopaminergic toxins may be pooled in one family called aminergic toxins. We recently apply the ancestral protein resurrection strategy to aminergic toxins in order to pinpoint important functional mutations which have probably occurred during their evolution and analyze them to modulate their binding properties on various GPCRs.

## Analogues of a toxin from solitary wasp venom as tools for dissecting elements of excitatory neurotransmission and as potential insecticides

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Spiders and solitary wasps have developed low molecular weight toxins that contain a polyamine moiety, usually linked to an aromatic moiety, and these toxins are capable of causing paralysis of their prey by targeting ion channels involved in excitatory neurotransmission. One of the best studied examples is philanthotoxin-433 found in the venom of the Egyptian Digger Wasp, *Philanthus triangulum*, and its many analogues. These have potent inhibitory actions on ionotropic glutamate receptors (IGRs) and nicotinic acetyl-choline receptors (nAChRs); in their insect prey these are found at the neuromuscular junctions and ganglia respectively. However, they are also effective against vertebrate IGRs and nAChRs, but here the potency of the toxin depends on the receptor subtype. For example, philanthotoxins potently inhibit the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid subtype of IGRs (AMPA) that contain GluA1, GluA3 and/or GluA4 subunits but are almost inactive against those containing GluA2 subunits. Based on their use-dependence and dependence on membrane potential it is thought that philanthotoxins are open channel blockers. Our recent work with philanthotoxins has focused on nAChR subtypes in order to understand better their interactions with nAChRs and to try and identify more potent and selective analogues. Philanthotoxin-343, a close synthetic relative of the naturally occurring philanthotoxin-433 (both triamines), inhibited vertebrate neuronal nAChRs in a strongly subunit-dependent manner, strongly inhibiting those containing  $\beta$ 4 subunits but with weak potency at muscle type receptors. The monoamine analogue philanthotoxin-12 was less subtype selective but it potently inhibited muscle type nAChRs. We identified new synthetic analogues of philanthotoxin-343 with inhibitory potencies in the pico-molar range at  $\alpha$ 3 $\beta$ 4 nAChRs. Finally, we have identified a hydrophobic analogue that is highly potent at insect nAChRs and may be a good lead for insecticide design.

## Pre and Postsynaptic Activities of *Tityus bahiensis* Scorpion Venom on Avian Neuromuscular Preparation

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In this study, we performed a pharmacological study to investigate the pre and post-synaptic effects of *T. bahiensis* crude venom (1-30  $\mu$ g/mL) on chick *biventer cervicis* preparations (BCP). The BCP were submitted to indirect (nerve-evoked contraction) or direct stimulation [after dTubocurarine pre-incubation, 0mM Ca bath solution (avoiding the acetylcholine release) or after the muscles being 24h on low temperatures (to eliminate the nerve ending, 24h-LT)]. Exogenous KCl (40nM) and Ach (1mM), dTubocurarine (dTc, 5 $\mu$ g/mL), Tetrodotoxin (TTx, 20nM) and Botulinum Toxin (BoTox, 4 $\mu$ g/mL) were also used. Under indirect stimulation, *T. bahiensis* venom promoted a persistent neuromuscular facilitation (within 120min-incubation) and a complete blockade (~10min, reversible after 2h-washing) at 1 and 30 $\mu$ g/mL respectively. The exogenous KCl- and Ach-induced contractures were intensified after venom incubation (1-10 $\mu$ g/mL) and unaffected at 30 $\mu$ g/mL. Under direct stimulation (curarized preparations, 0mM Ca solution and 24h-LT), the venom caused a concentration dependent facilitation (TTx-reversible) and partial blockade (60% of blockade, 120min-incubation) at 1 and 30 $\mu$ g/mL respectively; it was observed delay on twitches decay-time, reversible by TTx (30 $\mu$ g/mL). The highest concentration (30 $\mu$ g/mL) also promoted a muscle contracture under indirect and direct (0mM Ca and 24h-fridge) stimulations, on absence of stimulation and after BoTox incubation; these contractures were reversed by dTc and was absent on curarized preparations. These results indicate that *T. bahiensis* venom contains substances that can promote concentration-dependent pre- and post-synaptic facilitation/blockade. The decay-time delay is TTx-reversible demonstrate a post-synaptic sodium channels action. The contracture observed on all protocols (including BoTox-incubated and non-stimulated muscles) and reversible by dTc, suggests that *T. bahiensis* has a compound nicotine receptor agonist. Financial support: UNICAMP, CNPq. (Committee for Ethics in Animal Use: CEUA/UNICAMP no. 4068-1).

## Recent progress to substantially and sustainably reduce the mortality, morbidity and socioeconomic burden of tropical snakebite

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The Wellcome Trust funded a Hinxton Retreat in September 2015 entitled '**Mechanisms to reverse the public health neglect of snakebite victims**'. This 36 participant workshop was organized to combine the breadth of knowledge, opinion, and experience of key physicians, scientists and non-academics working on tropical snakebite in Africa (Nigeria, Kenya, Senegal, Angola), Asia (India, Sri Lanka, Nepal, Bangladesh, Vietnam), Latin America (Costa Rica, Brazil, Colombia), Oceania (Australia and Papua New Guinea), and Europe (UK, the Netherlands, Spain and Switzerland) with the the fiscal, political and advocacy power of civil society groups and funding agencies - Médecins Sans Frontières (MSF), Drugs for Neglected Diseases initiative (DNDi), Global Snakebite Initiative (GSI), Health Action International (HAI), AMREF Health Africa, the WHO and the Wellcome Trust. The participants were tasked with identifying key strategic, and achievable, interventions to:

- (i) reduce snakebite incidence and increase access to effective health care
- (ii) improve the clinical management of snakebite before, during and after hospital care
- (iii) apply scientific innovation to devise novel effective, affordable and safe snakebite therapies and rapid diagnosis
- (iv) implement an effective snakebite advocacy initiative

This presentation will describe the priority objectives identified during the workshop, and the progress achieved since the retreat or prior exemplars of effective practice in these key areas.

## Circulatory shock and mortality in viper envenomation: A prospective observational study from India

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Snake bite is a neglected and serious occupational health problem. Development of circulatory shock in viper envenomation is multifactorial due to direct action of venom toxins, myocardial depression, capillary leak syndrome and sepsis. Reports of acute pituitary insufficiency represent yet another facet of shock. We aimed to identify the clinical risk-factors predicting the development of shock and mortality in viper envenomation. Patients with viper envenomation confirmed through snake identification or applying a syndromic approach comprising of clinical features and 20 minute Whole Blood Clotting Time estimation, admitted to a tertiary care hospital in Southern India from September 2011 to August 2013 were included. Patients received polyvalent Anti-Snake Venom (ASV, Serum Institute of India, Pune) according to standard institute protocol along with supportive care. The frequency of shock, capillary leak syndrome, disseminated intravascular coagulation and acute kidney injury was estimated in these patients. Bite to ASV time was documented. In those with a fatal outcome, post-mortem histopathological examination of adrenal, pituitary and kidney tissues was performed. Risk factors, were determined by using univariable logistic regression analysis followed by multivariable logistic regression with "Death" or "Shock" as the dependent variable. Of the 248 patients studied, in-hospital mortality was 23% while shock prevalence was 19%. Acute adrenal insufficiency was found in 63% of whom serum cortisol could be measured. Necrosis of adrenal or pituitary was reported in 54% of postmortem specimens. Presence of shock, capillary leak syndrome, disseminated intravascular coagulation, bleeding and renal dysfunction significantly predicted mortality while time to receipt of ASV did not. The prevalence of shock and consequently mortality is quite high in viper envenomation. We establish acute adrenal insufficiency, a potentially correctable problem, as a contributor to shock.

## The procoagulant effect of snake venoms and their neutralisation by antivenom

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The composition of snake venom varies from species to species, resulting in extensive variation in pathologies observed in human victims, and fundamental limitations in the use of antivenom immunotherapies to treat paraspecific snakebites. Many snake venoms interfere with haemostasis; some are procoagulant and cause venom-induced consumption coagulopathy in human victims. Here we show that calcium-independent procoagulant venom activities have evolved convergently in snakes on a number of occasions. Using a range of *in vitro* assays, we show that this bioactivity is mediated by venom toxins that activate prothrombin (some vipers, elapids and “colubrids”) and/or degrade fibrinogen in a thrombin-like manner (some vipers). We find no evidence for snake venoms requiring the activation of clotting factors upstream of prothrombin to cause clotting in the absence of additional calcium. Despite extensive inter-specific variation among the venoms tested, we find that the monospecific saw-scaled viper antivenom EchiTABG neutralises the *in vitro* procoagulant activity of the majority of the venoms tested, including from the “colubrid” snakes *Dispholidus typus* and *Rhabdophis subminiatus*, despite their divergence ~62 million years ago. Surprisingly, the monospecific SAIMR boomslang antivenom also exhibits *in vitro* neutralising cross-reactivity with many viper venoms, although the CSL polyspecific antivenom was found to only be effective at neutralising venoms from the Australian elapid snakes used in its manufacture. Finally, we demonstrate that antibodies raised against ecarin, a prothrombin activating snake venom metalloproteinase from the saw scaled-viper, are capable of neutralising venom-induced clotting of human plasma by both saw-scaled viper (*Echis spp.*) and boomslang (*D. typus*) venoms. These results hold much promise for the future design of specific antibodies capable of neutralising venom activities from distinct snake species.

## Incorporating the 3Rs into the preclinical assessment of venom toxicity and antivenom efficacy

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Antivenom is the most effective treatment of snakebite and is immunoglobulin from venom-immunised horses or sheep. Globally, there are in excess of 45 manufacturers making over 120 snake antivenoms. It is a regulatory requirement that the venom-neutralising efficacy of antivenom is pre-clinically tested prior to human administration. The WHO-recommended preclinical tests of antivenom efficacy are the LD<sub>50</sub> and ED<sub>50</sub> murine assays which cause substantial pain and suffering to the murine subjects and use death/survival as the metric. With NC3R-funding, we sought to apply the ‘Refine, Reduce and Replace’ principals of animal experimentation to these murine preclinical assays. Pain is a near-universal symptom of snake envenoming, and one of our objectives was to identify an effective analgesic that could be utilised without invalidating the assay results. The Mouse Grimace Scale and Activity scores were used to measure pain. We examined the effects of two opioid analgesics, Buprenorphine and Morphine, on mice in a range of venom LD<sub>50</sub> and venom/antivenom ED<sub>50</sub> assays. Both were effective at reducing pain scores, but death rates were higher in groups of mice which had received Buprenorphine. We also identified humane endpoints for a number of venom and venom/antivenom combinations - with the result that we were able to reduce the duration of the LD<sub>50</sub> and ED<sub>50</sub> assays from 24 to 6 hours. We also implemented a ‘dose-staging’ element into the experimental design (in which multiple doses are prepared for the assays, one dose given and the next dose(s) selected based on the results of the previous dose) to reduce the number of mice required for these assays.

## Comparative venom yield of coralsnakes and the paradigm of anti-venom production and use in Brazil

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This study reports coralsnake sizes and venom yields, and evaluates the production and use of antielapidic serum, based upon the size, inoculation capacity, and variability between coralsnake species in different regions of Brazil. Diversity and geographic distribution data were based on current taxonomy. Epidemiological data for elapidic accidents were obtained from the Disease Report Information System of the Ministry of Health. Data regarding extracted venom volumes and snake sizes were obtained in the field and the laboratory from 1986 to 2010, and were stored in the venom database of the Center for Studies and Biological Research of the Pontifical Catholic University of Goiás. Two data sets were analyzed: venom samples with specimen total lengths (N=277) and venom samples without specimen data (N=336). The greatest diversity of coralsnakes occurs in the Amazon region. However, the greatest incidence of *Micrurus* accidents is in northeastern Brazil. Most species of coralsnake are of small or medium size. Linear regression analysis showed a strong correlation between body size and extracted venom volume. Despite the diversity of coralsnakes in Brazil (33 species) the present antielapidic serum is produced mainly from two species from southeastern Brazil. Based on rare reported clinical cases, the Brazilian Ministry of Health recommends that all accidents be treated as severe and that 10 ampoules of antivenom be administered (one ampoule neutralizes 15 mg of venom). Venom yields of the studied species ranged from 8,04 to 54,38 mg. Accordingly, the amount of antivenom necessary should be only one to four ampoules. Due to their small size, which results in low inoculation capacity, the policy to administer high doses of antielapidic serum in coralsnake accidents should be revised. However, the diverse venom chemistry of coralsnakes raises concerns about the effectiveness of the current antielapidic serum and confounds simplistic solutions.

## Toxic newts – where does tetrodotoxin come from?

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Tetrodotoxin (TTX), a deadly neurotoxin, was first discovered in pufferfish (Tetraodontidae), but was later found to occur in a wide range of marine as well as terrestrial animals. Its ultimate biological origin is still a matter of discussion. Whereas bacteria have been suggested to be involved in TTX biosynthesis in marine organisms, so far no conclusive data exist regarding the toxin's origin in terrestrial vertebrates such as newts, frogs and toads. When breeding fire belly newts of the genus *Cynops*, i.e. *C. pyrrhogaster* from Japan and *C. orientalis* from South-China, which generally contain high TTX-concentrations, the larvae are entirely toxin-free and the semi-adult offspring remain non-toxic even after one year in captivity. Among various populations of North-American eastern newts, *Notophthalmus viridescens*, and of the western newts of the genus *Taricha*, *T. granulosa* and *T. torosa*, high intraspecific variability in their TTX-content is observed ranging from marginal to very high toxin levels. Recent studies on Chinese newts also indicate that specimens of *Pachytriton labiatus* and of *Paramesotriton sinensis* contain TTX. Skeletal muscles of TTX-bearing newts are unaffected by TTX which results from the expression of toxin-resistant variants of the voltage-gated sodium channel Na<sub>v</sub> 1.4. Whether the newts generally lack the capability of TTX-biosynthesis, as the breeding experiments may suggest, or they sequester the toxin from the environment, or even develop their ability to produce the toxin during lifetime, is an open question and needs in-depth studies.

## High-throughput epitope profiling of snake venom toxins – unveiling the complexity of antigen-antibody interactions of polyvalent antivenoms

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Antivenom against snakebite is a 120 years old invention based on polyclonal mixtures of antibodies (or fragments thereof) purified from the blood of hyper-immunized animals. A deeper understanding of toxin antibody recognition has the potential to provide explanations on the molecular level for antivenom cross-reactivity and predict further para-specificity. Such knowledge can be employed for the improvement of the therapeutic value of antivenoms. We have identified and characterized linear B-cell epitopes from hundreds of snake venom toxins and employed a high throughput methodology, based on custom designed high-density peptide microarrays, to analyze a range of polyvalent antivenoms raised in horses immunized with venoms from African *Naja*, *Dendroaspis*, *Echis*, and *Bitis* snake species. The peptide microarray was synthesized to include overlapping peptides spanning the complete sequences of all relevant toxins available in the UniProtKB database. By combining data on antibody-peptide interactions with multiple sequence alignment of homologous toxin sequences and protein modelling, we determined a vast number of conserved antibody binding sites for the most pathologically important toxin families. Combined with results from antivenomic experiments, this novel approach sheds light on the recognition patterns of the investigated antivenoms and provides insights into the equine immune system. Funding: The Novo Nordisk Foundation (NNF13OC0005613), Augustinus fonden (15-4174), Knud Højgaards Fond (15-02-6348) and Ministerio de Economía y Competitividad, Madrid, Spain (BFU2013-42833-P)

## Addressing the antivenom supply crisis in Africa: Médecins Sans Frontières' multi-pronged approach

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For decades, Médecins Sans Frontières/Doctors Without Borders (MSF) has provided treatment of snakebite in many of the hospitals it manages in resource-limited settings. However, MSF had to take specific actions to mitigate the impact of Sanofi-Pasteur's decision to cease the manufacture of its African polyvalent antivenom product, FavAfrique, three years ago. MSF adopted a multi-pronged approach (operations, research and advocacy) to improve access to quality antivenoms in Sub-Saharan Africa. Now, between 150 and 350 cases of envenomings are treated annually at each of MSF's flagship projects in Africa (Ethiopia, CAR and South Sudan). MSF has also increased the capacity of its research teams and has conducted two research projects; an observational study in Paoua (CAR) to document the clinical outcomes and safety of an antivenom product, and an epidemiological survey in Agok (South Sudan) to estimate the local burden of snakebite. At the global level, MSF advocates for the World Health Organisation (WHO) to be given a stronger mandate to address snakebite, and has funded the on-going WHO assessment of snake antivenoms intended for use in Sub-Saharan Africa. MSF also supports the intention of some WHO Member States to submit a resolution on snakebites at the next World Health Assembly.

## The transcendence of Vital Brazil work

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Vital Brazil (1865- 1950) was one of the most preeminent builders of Brazilian medical toxinology. Since his early beginning as a physician interested in the solution of the social problem represented by ophidism, he started a prolific correspondence with foreign scientists, sharing with them his observations on the biology of Brazilian snakes and their venomous properties. His many outstanding contributions to different fields like immunology, public health and herpetology required not only a very high level of observational, deductive and practical ability, but also an unswerving intuition. As a medical scientist he was the first to demonstrate the specificity of antivenomous serum and produced the

first polyvalent antivenom. As a humanitarian, he campaigned successfully to bring the antivenom produced by Instituto Butantan to the farms in the countryside. In addition, he founded two institutes of public health: Butantan (São Paulo) and Vital Brazil (Niteroi). In order to summarize his results, we can say that due to his efforts the mortality rate associated with snakebites estimated at 20% had declined to 0.7%. Besides his fight against ophidism, he studied also scorpion and spider venoms. To obtain enough venom to prepare the serum, a great number of snakes were needed. At the Instituto Butantan, he established an exchange system, delivering one ampoule of serum for each specimen sent by farmers. The railroads carried them for free. He wrote numerous didactic papers and gave many lectures to diffuse knowledge about serum therapy against poisons. His book "A defesa contra o ofidismo", when published in French, attained international recognition with three editions. The characterization as well as the physiological actions of the main active components of ophidian venoms were successfully set in the last century. Nowadays, we can say that a multitude of workers have given their brilliant contribution to these fields.

## Design of humanized antibody fragments that neutralize the venom of *Loxosceles* spiders: Structural and functional characterization

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*Loxosceles* spider bites often lead to a necrotizing-hemolytic syndrome considered as the most important form of arachnidism in South America. Despite global efforts, no definite therapy has yet been established. In such a context, it is of interest to consider an antibody-based targeted therapy. We have previously prepared murine monoclonal antibodies that bind to *L. intermedia* venom, some of them being capable to cross-react with the venom of other dangerous spiders belonging to the same genus. Among these antibodies, LiMab7 binds to 32-35 kDa shingomyelinase isoforms of the venom and neutralizes the dermonecrotic activity. Recently, we re-engineered LiMab7 into a recombinant diabody that proved to be efficient at neutralizing sphingomyelinase and hemolytic activities of the crude venom. However, the dimeric structure of the recombinant molecule was unstable, the kinetic constants were slightly altered and this antibody fragment remained potentially immunogenic due to its rodent origin. Here, a concise humanization strategy combined with an optimized production method was successfully employed to generate humanized antibody fragments derived from LiMab7. We report the structural and functional characterization of these molecules and discuss their potential for a next generation antivenom.

## Discovery of human IgGs targeting the medically important toxins from the venom of the black mamba (*Dendroaspis polylepis*)

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The notorious black mamba (*Dendroaspis polylepis*) is responsible for most snakebite related deaths in South Africa, and it is feared as a ferocious marauder across the East African region. The venom of *D. polylepis* exerts its potent neurotoxic effects through the combined action of dendrotoxins targeting potassium channels and  $\alpha$ -neurotoxins targeting the nicotinic acetylcholine receptor. The only current effective treatment against envenoming from this snake consists of animal-derived antisera that are associated with a high degree of immunogenicity, high cost, and batch-to-batch variation. In the future, however, snakebite envenoming therapy could be based on novel biotechnological approaches within monoclonal antibodies and recombinant DNA technology. Here, we report the discovery of several hundreds human antibodies targeting the key toxins from the venom of *D. polylepis*. Based on a combined toxicovenomics and

phage display selection approach, approx. 400 human scFv binders targeting dendrotoxins and  $\alpha$ -neurotoxins were isolated from a phage display library. These scFvs were expressed in *E. coli*, sequenced, and their cross-reactive binding patterns to other selected toxins were investigated. The most promising scFvs were selected for conversion to IgG format to be expressed in CHO cells. Further preclinical evaluation is ongoing in order to design an oligoclonal mixture of recombinantly expressed human IgGs that can effectively neutralize the lethal effects of the venom of *D. polylepis*.

## The burden of snakebite and snakebite treatment on rural African health infrastructures: a Burkina Faso case study

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The socioeconomic impact of snakebite upon rural tropical communities is often misunderstood and under-estimated. A recent study in 16 West African countries described that snakebite-induced mortality and morbidity imposes a disease burden (319,874 DALYs) that equals or exceeds that of regional NTDs such as Buruli ulcer, Echinococcosis, Leishmaniasis, Trachoma and Trypanosomiasis. In this study we sought to identify the burden of snakebite treatment upon rural tropical hospitals in sub-Saharan Africa. Snakebite affects the same rural African communities that suffer from several NTD infections. In an example of cross-NTD cooperation, we exploited the infrastructure developed to control lymphatic filariasis (LF) in Burkina Faso by having medical personnel in hospitals involved in LF MDA programs to complete a simple prospective form capturing snakebite admissions and treatment data. We will describe the number of hospital beds occupied by snakebite victims in three states in Burkina Faso over a 17 month period, the availability of antivenom in those hospitals, the outcome of treatment and the subsequent cost to the health infrastructure of hospital treatment of snakebite victims.

## POSTER ABSTRACTS

### Investigating Britain's most venomous spider: what can we learn from the venom of the False Noble Widow *Steatoda nobilis*?

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The spider subfamily Latrodectinae includes the genera *Latrodectus* ("true" widows) and *Steatoda* (false widows) of which many have medically significant bites. It is assumed that Latrodectinae of medical importance possess a neurotoxic venom containing variations of  $\alpha$ -latrotoxin which targets specifically the nervous system of vertebrates. Reported symptoms range from local pain to systemic envenomations which, when left untreated, may lead to paralysis and death in humans. The false noble widow *Steatoda nobilis* (Theridiidae: Latrodectinae) has established thriving and expanding populations around the world including the UK and Ireland after stowing away from its native range in the Canary Islands. In recent years, sensationalist news reports in the UK and Ireland have focused on alleged bites from *Steatoda nobilis*, with members of the public reporting symptoms ranging from cardiac arrhythmia to intense pain, swelling and necrotic lesions. While most of these reports can be dismissed on the basis of misinformed self-diagnosis, the toxicity of *Steatoda nobilis* venom has never been thoroughly investigated. We present here the results of our first toxicological and proteomic assays based on the venom of *Steatoda nobilis* collected from the inner city of Dublin, Republic of Ireland. Results demonstrate very high toxicity of the venom with significant lytic and apoptotic effects.

### Spider-bite telephone enquiries to the United Kingdom Poisons Information Service

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The epidemiology of presumed spider bite cases that present to medical services within the United Kingdom is not well described. The National Poisons Information Service (NPIS) database was interrogated for telephone enquiries concerning spider bites between January 2011 and December 2015. Anonymised data were collected on patient demographics, source of enquiry, location and circumstances of exposure, spider type, clinical features and poisoning severity score (PSS). A total of 212 cases were reported with an annual incidence of 30 to 60 cases. The median age range was 30-39 years. 56% of cases were male and 44% female. In 52% of cases the identity of the spider was unknown, 25% were identified as false widow spiders, 15% tarantulas and 2% recluse spiders. 75% of exposures occurred within a domestic environment. Fifteen (7%) involved pet spiders, all of which were various species of tarantula. The majority of enquiries (51%) were received from hospitals; 25% of cases were received from general practitioners or primary care services and 14% of cases from NHS telephone triage services. There were 147 reports of localized features of erythema (52 cases), visible bite mark(s) (49 cases), oedema (45 cases) or necrosis (1 case). Systemic features reported included: sensory disturbance (24 cases), cardiovascular features (16 cases), myalgia (13 cases) and gastrointestinal upset (10 cases). Twenty-four cases (11%) were asymptomatic. One hundred and sixty cases (75%) were classified as "minor" PSS, 20 were "moderate" (9%) and 1 case was "severe". NPIS recommended either further treatment or referral to an appropriate medical facility in 164 (77%) cases. In conclusion, the NPIS received spider bite enquires at a rate of less than one a week over a five year period. The majority of cases reported were minor; however, more serious clinical features may occur in a minority of cases.

## Cobra venoms as drug discovery tools

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Elapidae venoms, specifically those of the genus *Naja*, have a diverse range of utilities in drug discovery programmes. The well characterised Nicotinic AcetylCholine blocking activity is the dominant mechanism, however a diverse range of other activities have been discovered. Divergent evolution across the genus has produced an amazing diversity of stable peptides and protein tools. Screening venoms from closely related species allows for the production of Structural Activity Relationship (SAR) tables to guide peptide engineering to maximise drug efficiency. Presented here are some of the diverse range of drug discovery activities discovered from the genus *Naja* along with insights into further drug discovery potential. Such activities demonstrated here include infection control, haemodynamic and oncology.

## Development of an ELISA to detect Indian red scorpion (*Hottentotta tamulus*) antigenaemia

Kirsti A Newton<sup>1</sup>, Tim Winks<sup>1</sup>, Khuyen T K Vo<sup>1</sup>, Dinh Ng<sup>1</sup>, Jade Kilty<sup>1</sup>, Keeley M Berry<sup>1</sup>, Rose Wanjiru<sup>1</sup>, Sandeepan Mukherjee<sup>3</sup>, Nakul Shah<sup>3</sup> Abhay Chowdhary<sup>3</sup> Vivek Natu<sup>2</sup> & Peter N Strong<sup>1</sup>

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The Indian red scorpion is the medically most important scorpion on the Indian sub-continent and a sting to young children can often be fatal. Although the alpha-adrenergic blocker prazosin has been the conventional method of treating individuals stung by this scorpion, specific antivenom has recently been shown to be very effective and in many cases, is now being used as the treatment of choice. At present, the amount of either prazosin or antivenom given to the patient depends on the severity of the clinical symptoms. Here we are trying to establish an ELISA to detect red scorpion venom antigenaemia in envenomed patients, with the goal of being able to more accurately predict an effective dose and thereby hopefully avoiding any complications arising from an excessive dose of foreign IgG. Using a direct ELISA, we can detect venom at a level of 0.3 microgram /ml (carbonate buffer) using horse antivenom biotinylated F(ab)2 (Haffkine Institute, Mumbai) and streptavidin- horseradish peroxidase (HRP). In efforts to mimic the serum of an envenomed patient, we can presently detect venom at a level of 40 microgram / ml using venom- spiked human serum. The sensitivity of the assay increased x20 fold using a sandwich format, where the ELISA plate was coated with horse antivenom F(ab)2 as capture antibody and biotinylated F(ab)2 was used as the detection antibody, with streptavidin-HRP. Here we could detect 8ng/ml venom (carbonate buffer) and 15ng /ml (serum spiked venom). Western blots of Haffkine antiserum clearly show that the majority of the antibodies recognize high molecular weight proteins. Ion channel peptides (responsible for the majority of the venom's pathology) are not so antigenic.

## Discovery of human antibodies against black cobra toxins

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<sup>1</sup>Department of Biotechnology and Biomedicine, Technical University of Denmark

<sup>2</sup>Department of Drug Design & Pharmacology, Faculty of Health & Medical Sciences, University of Copenhagen, Denmark

Snakebite envenoming represents a major health threat in tropical parts of the developing world, where shortage of antivenom is currently the cause of morbidity and a high number of fatalities. In sub-Saharan Africa only 1-2% of snakebite victims are treated with animal-derived antisera, which currently constitute the only effective treatment option. Unfortunately, serum-based antivenoms are associated with severe side effect, since they are not compatible with the human immune system due to their heterologous nature. The black cobra, *N. melanoleuca*, is the largest cobra species in Africa and among the snakes of the highest medical importance according to the World Health Organization. The toxicity of *N. melanoleuca* venom is derived from potent type I and II  $\alpha$ -neurotoxins that target nicotinic acetylcholine

receptors, causing inhibition of neuromuscular transmission. The clinical manifestations of envenomings caused by *N. melanoleuca* therefore include systemic neurotoxicity and flaccid paralysis. In our work, we aim at discovering human antibodies that target the medically most important toxins from *N. melanoleuca* venom using phage display technology. These human antibodies will be evaluated based on their ability to bind to and neutralize toxins *in vitro* and *in vivo* and their ability to cross-recognize homologous toxins both from other cobra species and the related neurotoxic mamba species. We hope that this work will advance our development of the first polyvalent recombinant snakebite antivenom based on oligoclonal mixtures of human antibodies with high efficacy and low immunogenicity in human recipients.

## **Synthesis and expression of antimicrobial peptides derived from *Scorpio maurus palmatus* venom**

**Kirstie M Rawson**, Melissa Lacey, Peter N Strong and Keith Miller

Biomolecular Sciences Research Centre, Sheffield Hallam University, Sheffield, S1 1WB UK

Antibiotic resistant bacteria are becoming an increasing threat globally. The challenges of treating multi-drug resistant strains are already having a significant impact on patient morbidity and mortality. Antimicrobial peptides (AMPs) are being viewed as potential alternatives to traditional antibiotics, due to a rapid killing mechanism and low resistance potential. AMP mechanism of action is thought to be influenced by their amphipathic nature. The majority of AMPs are cationic and interact preferentially with the weakly anionic bacterial membrane. The binding mechanisms are multi-site and are typically more complex than a simple cation-anion binding reaction and other domains, for example charged or hydrophobic regions, have been implicated. This study seeks to identify and characterise the critical residues for antimicrobial activity and eukaryotic cytotoxicity. Venoms provide a rich source of antimicrobial peptides. We have previously characterised Smp24 an AMP from the venom of the scorpion *Scorpio maurus palmatus*. A synthetic gene coding for Smp24 was fused to a sequence encoding the signal peptide of *E. coli* (STII). A plasmid (pET22b/STII/Smp24) was expressed in pET22b between the NdeI and HindIII restriction sites. Two independent serine to lysine substitutions (S15K and S24K) have been introduced to the peptide in order to investigate the relationship between structure, function and toxicity to inform future antimicrobial drug development strategies.

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**MicroPharm Limited** is a developer and manufacturer of therapeutic polyclonal antibodies for human and veterinary use. The Company's core expertise lies in the raising of ovine antisera containing high levels of specific antibodies directed against antigens such as toxic molecules or viruses and the subsequent purification and modification of such antibodies to produce a range of immunotherapeutic products for

clinical use. All are designed to treat acute, life-threatening emergencies, have been developed at the request of the medical profession and are required urgently either because no alternative exists or because any alternative is ineffective and/or unsafe. MicroPharm currently produces two antivenoms, ViperaTAB<sup>®</sup> for the treatment of envenomation by the European adder and EchiTABG<sup>™</sup> for the treatment of the carpet viper, *Echis ocellatus*. The Company has a number of products in development including: PolyCAb for the treatment of severe *C. diff.*; ColchiBIND for the treatment of colchicine poisoning; EBOTAB for the treatment of Ebola Virus Disease and ViperaVet<sup>™</sup>, for the treatment of dogs envenomed by one of the four medically important species of *Vipera* (adder) found throughout Western Europe. MicroPharm's collaborators include Public Health England, University of Oxford, University of Leeds and the University of Edinburgh.





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## Southwest Venoms

### CATALOGUE OF INSECT VENOMS (2012-2013)

Prices in U.S. dollars. All venoms are pure venoms (not venom sac or apparatus homogenates) collected according to the methods of Schmidt (1986. *In: Venoms of the Hymenoptera* [T. Piek, ed.], pp. 425-508. Academic Press: London.).

Prod. No.	VENOM	(LD <sub>50</sub> mg/kg, mice)	VENOM PRICE			
			1 mg	5 mg	25 mg	100 mg
<b>SOCIAL WASPS</b>		(LD <sub>50</sub> )				
Yellowjackets -- <i>Vespula</i>						
W-10	<i>V. pensylvanica</i>	(6.4)	50	225	1000	*
W-19	other species**		*			
Hornets -- <i>Vespa</i>						
W-20	<i>V. mandarina</i>	(4.1)	50	225	1000	*
W-21	<i>V. tropica</i>	(2.8)	50	225	1000	*
W-29	others **		*			
Paper wasps -- <i>Polistes</i>						
W-30	<i>P. comanchus navajoe</i>	(5)	40	180	800	*
W-31	<i>P. flavus</i>	(3.8)	40	180	800	*
W-32	<i>P. canadensis</i>	(2.5)	50	225	*	
W-33	<i>P. erythrocephalis</i>	(1.5)	50	225	*	
W-39	<i>Polistes</i> sp. as available**		30	135	600	2100
New World Polybiine wasps						
W-40	<i>Brachygastra mellifica</i>	(1.5)	60	270	1200	*
W-50	<i>Synoeca septentrionalis</i>	(2.7)	60	270	1200	*
W-60	<i>Parachartergus fraternus</i>	(5)	70	300	1400	*
W-70	<i>Polybia sericea</i>	(6)	80	350	*	
W-71	<i>P. simillima</i>	(4.1)	80	350	*	
W-72	<i>P. occidentalis</i>	(5)	100	*		
W-80	<i>Agelaia myrmecophila</i>	(5.6)	140	*		
Old World Polybiine wasps						
W-90	<i>Belonogaster juncea colonialis</i>	(3)	80	350	*	
<b>SOCIAL BEES</b>						
Honey bees -- <i>Apis</i>						
B-10	<i>A. mellifera</i>	(2.8)	20	90	400	1400
B-11	<i>A. mellifera</i> Africanized bees	(2.8)	20	90	400	1400
B-12	<i>A. mellifera</i> queens		40	180	800	2800
B-13	<i>A. dorsata</i>	(2.8)	50	225	1000	3500
B-14	<i>A. cerana</i>	(3.1)	55	245	*	
B-19	others ( <i>A. florea</i> , etc.)**		*			
Bumble bees -- <i>Bombus</i>						
B-20	<i>B. sonorus</i>	(12)	50	225	1000	*
B-21	<i>B. impatiens</i>	(12)	50	225	*	
B-29	other species**		30	*		

Prod. No.	VENOM	(LD <sub>50</sub> mg/kg, mice)	VENOM PRICE			
			1 mg	5 mg	25 mg	100 mg
<b>ANTS -- FORMICIDAE</b>		(LD <sub>50</sub> )				
Pogonomyrmex -- harvester ants						
A-10	<i>P. barbatus</i>	(0.6)	50	225	1000	3500
A-11	<i>P. maricopa</i>	(0.12)	60	270	1200	4200
A-12	<i>P. occidentalis</i>	(0.5)	70	315	1400	*
A-13	<i>P. rugosus</i>	(0.7)	50	225	1000	3500
A-15	<i>P. desertorum</i>	(0.7)	160	*		
A-19	<i>Pogonomyrmex</i> sp. as available		45	200	900	3200
Myrmecia -- bull ants						
A-20	<i>M. gulosa</i>	(0.18)	60	270	1200	4200
A-21	<i>M. tarsata</i>	(0.18)	60	270	1200	*
A-22	<i>M. browningi</i>	(0.18)	70	315	*	
A-23	<i>M. rufinodis</i>	(0.35)	70	315	*	
A-24	<i>M. simillima</i>	(0.21)	70	315	*	
A-25	<i>M. pilosula</i>	(5.7)	100	*		
A-30	<i>Pachycondyla (Neoponera) villosa</i>	(7.5)	60	270	*	
A-31	<i>P. (Neoponera.) apicalis</i>	(> 16)	70	*		
A-32	<i>P. crassinoda</i>	(2.8)	80	*		
A-33	<i>P. (Megaponera) foetens</i> (Metabele ant)	(130)	70	315	*	
A-34	<i>P. (Paltothyreus) tarsatus</i> (stink ant)	(64)	50	225	1000	3500
A-35	<i>P. (Bothroponera) strigulosa</i>	(9)	70	*		
A-36	<i>Termitopone commutata</i>	(10)	70	315	1400	*
A-40	<i>Platythyrea lamellosa</i>	(11)	70	315	*	
A-50	<i>Diacamma</i> sp.**	(35)	100	450	*	
A-60	<i>Dinoponera gigantea</i>	(11)	60	270	1200	4200
A-70	<i>Paraponera clavata</i> (bullet ant)	(6.0)	60	270	1200	4200
A-80	<i>Ectatomma tuberculatum</i>	(1)	60	270	*	
A-81	<i>E. quadridens</i>	(17)	60	270	*	
A-90	<i>Odontomachus</i> sp.**	(33)	60	275	*	
A-110	<i>Tetraponera</i> sp.**	(.35)	140	600	*	
A-120	<i>Streblognathus aethiopicus</i>	(8.0)	80	360	*	
<b>SOLITARY WASPS AND BEES</b>						
Spider wasps -- Pompilidae						
SW-10	<i>Pepsis</i> sp.**	(65)	60	270	1200	4200
Mutillid wasps -- Mutillidae						
SW-20	<i>Dasymutilla</i> sp.**	(71)	70	315	1400	*
SW-39	Other wasps (Scoliidae, Tiphiidae, Sphecidae, Eumenidae, etc.)**		*			
Carpenter bees -- <i>Xylocopa</i>						
SB-10	<i>X. californica</i>	(21)	50	225	1000	*
SB-11	<i>X. veripuncta</i>	(33)	55	245	*	
SB-20	<i>Proxycopa rufa</i>	(11)	100	450	*	
SB-39	Other bees**		*			

\*Inquire for prices and availability.

\*\*Available species provided; exact determinations usually included.

# Natural Toxins Research Center (NTRC)

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## VENOM QUALITY GUARANTEE

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Maximum Biological Activity • Our Venom is Never Pooled**

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The Natural Toxins Research Center (NTRC) at Texas A&M University-Kingsville is dedicated to providing high quality snake products for biomedical research. We are committed to the procurement and distribution of venoms, venom fractions and tissue for biomedical research. Venoms from the same species can be different, and therefore extracted venoms are never pooled. Each vial contains venom from a single snake, and venoms of the same species are never mixed. The vials are labeled with the snakes' scientific and common names, ID tag number and sex. The ID tag number can be traced back to the NTRC Internet Database ([ntrc.tamuk.edu/cgi-bin/serpentarium/snake.query](http://ntrc.tamuk.edu/cgi-bin/serpentarium/snake.query)) for additional information about each snake.

Southern Copperhead - <i>Agkistrodon contortrix contortrix</i> .....	\$75 <sup>00</sup> /1g .....	\$50 <sup>63</sup> /500mg		
Broad-Banded Copperhead - <i>Agkistrodon contortrix laticinctus</i> ..	\$100 <sup>00</sup> /1g .....	\$67 <sup>50</sup> /500mg		
Northern Copperhead - <i>Agkistrodon contortrix mokasen</i> .....	\$50 <sup>00</sup> /1g .....	\$33 <sup>75</sup> /500mg		
Trans-Pecos Copperhead - <i>Agkistrodon contortrix pictigaster</i> .....	\$75 <sup>00</sup> /1g .....	\$50 <sup>63</sup> /500mg		
Florida Cottonmouth - <i>Agkistrodon piscivorus conanti</i> .....	\$60 <sup>00</sup> /1g .....	\$40 <sup>50</sup> /500mg		
Western Cottonmouth - <i>Agkistrodon piscivorus leucostoma</i> .....	\$56 <sup>00</sup> /1g .....	\$37 <sup>80</sup> /500mg		
Eastern Diamondback Rattlesnake - <i>Crotalus adamanteus</i> .....	\$50 <sup>00</sup> /1g .....	\$33 <sup>75</sup> /500mg		
Western Diamondback Rattlesnake - <i>Crotalus atrox</i> .....	\$45 <sup>00</sup> /1g .....	\$30 <sup>38</sup> /500mg		
Sonoran Sidewinder - <i>Crotalus cerastes cercobombus</i> .....	\$125 <sup>00</sup> /1g .....	\$84 <sup>38</sup> /500mg		
Timber Rattlesnake - <i>Crotalus horridus</i> .....	\$70 <sup>00</sup> /1g .....	\$47 <sup>25</sup> /500mg		
Mottled Rock Rattlesnake - <i>Crotalus lepidus lepidus</i> .....	\$125 <sup>00</sup> /1g .....	\$84 <sup>38</sup> /500mg		
Blacktail Rattlesnake - <i>Crotalus molossus molossus</i> .....	\$400 <sup>00</sup> /1g .....	\$270 <sup>00</sup> /500mg	\$72 <sup>90</sup> /100mg .....	\$49 <sup>21</sup> /50mg
Great Basin Rattlesnake - <i>Crotalus oreganus lutosus</i> .....	\$125 <sup>00</sup> /1g .....	\$84 <sup>38</sup> /500mg		
Grand Canyon Rattlesnake - <i>Crotalus oreganus abyssus</i> .....	\$250 <sup>00</sup> /1g .....	\$168 <sup>75</sup> /500mg	\$45 <sup>56</sup> /100mg .....	\$30 <sup>75</sup> /50mg
Texas Coral Snake - <i>Mircrurus tener tener</i> .....	\$2000 <sup>00</sup> /1g			
Florida Coral Snake - <i>Mircrurus fulvius</i> .....	\$1800 <sup>00</sup> /1g			
Southern Pacific Rattlesnake - <i>Crotalus oreganus helleri</i> .....	\$400 <sup>00</sup> /1g .....	\$270 <sup>00</sup> /500mg	\$72 <sup>90</sup> /100mg .....	\$49 <sup>21</sup> /50mg
Northern Pacific Rattlesnake - <i>Crotalus oreganus oreganus</i> .....	\$400 <sup>00</sup> /1g .....	\$270 <sup>00</sup> /500mg	\$72 <sup>90</sup> /100mg .....	\$49 <sup>21</sup> /50mg
Mohave Rattlesnake - <i>Crotalus scutulatus scutulatus</i> (A).....	\$250 <sup>00</sup> /1g .....	\$168 <sup>75</sup> /500mg	\$45 <sup>56</sup> /100mg .....	\$30 <sup>75</sup> /50mg
Mohave Rattlesnake - <i>Crotalus scutulatus scutulatus</i> (B).....	\$1000 <sup>00</sup> /1g .....	\$675 <sup>00</sup> /500mg	\$182 <sup>25</sup> /100mg .....	\$123 <sup>02</sup> /50mg .....
Prairie Rattlesnake - <i>Crotalus viridis viridis</i> .....	\$70 <sup>00</sup> /1g .....	\$47 <sup>25</sup> /500mg		
Red Spitting Cobra - <i>Naja pallida</i> .....	\$100 <sup>00</sup> /1g .....	\$67 <sup>50</sup> /500mg		
Desert Massasauga - <i>Sistrurus catenatus edwardsii</i> .....	\$1000 <sup>00</sup> /1g .....	\$675 <sup>00</sup> /500mg	\$182 <sup>25</sup> /100mg .....	\$123 <sup>02</sup> /50mg .....
Western Massasauga - <i>Sistrurus catenatus tergeminus</i> .....	\$1000 <sup>00</sup> /1g .....	\$675 <sup>00</sup> /500mg	\$182 <sup>25</sup> /100mg .....	\$123 <sup>02</sup> /50mg .....
Bushmaster - <i>Lachesis muta muta</i> .....	\$2000 <sup>00</sup> /1g .....	\$1350 <sup>00</sup> /500mg	\$364 <sup>50</sup> /100mg .....	\$246 <sup>04</sup> /50mg .....

(A) - neurotoxic venom  
(B) - non-neurotoxic venom  
\*Subject to availability

Venom is collected under stringent laboratory conditions using disposable labwear for each extraction. Venom is collected in new, non-reusable plastic cups with parafilm coverings. Snakes are allowed to bite into the parafilm diaphragm and the venom glands are not massaged. Immediately following collection, each venom sample is clarified by centrifugation at 500 x g for 5 minutes to remove cellular debris and frozen at -90° C until lyophilized.

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<i>Naja kaouthia</i>	\$60	\$250
<i>Naja melanoleuca</i>	\$50	\$200
<i>Naja mossambica</i>	\$60	\$250
<i>Naja siamensis</i>	\$60	\$250
<i>Notechis ater humphreysi</i>	\$350	\$1,600
<i>Notechis ater niger</i>	\$350	\$1,600
<i>Notechis ater serventyi</i>	\$350	\$1,600
<i>Notechis scutatus</i>	\$300	\$1,445
<i>Ophiophagus hannah</i>	\$200	\$850
<i>Oxyuranus microlepidotus</i>	\$300	\$1,300
<i>Oxyuranus scutellatus</i>	\$260	\$1,250
<i>Oxyuranus scutellatus canni</i>	\$400	\$1,500
<i>Pseudechis australis</i>	\$110	\$520
<i>Pseudechis butleri</i>	\$160	\$700
<i>Pseudechis colletti</i>	\$110	\$500
<i>Pseudechis guttatus</i>	\$110	\$500
<i>Pseudechis porphyriacus</i>	\$140	\$650
<i>Pseudechis papuanus</i>	\$288	\$1,380
<i>Pseudonaja affinis</i>	\$800	\$3,900
<i>Pseudonaja aspidorhyncha</i>	\$800	\$3,990
<i>Pseudonaja inframacula</i>	\$800	\$3,990
<i>Pseudonaja nuchalis</i>	\$800	\$3,990
<i>Pseudonaja textilis</i>	\$760	\$3,700
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*Latrodectus hasseltii*

\$500/50 sac contents.

**Bee Venom**Pure bee venom (*Apis mellifera*)

250mg	\$58
(1-5gm)	\$130/gm
(6-10gm)	\$116/gm
(60gm and over)	\$95/gm

**Amphibian Venoms***Bufo marinus*

\$95/200mg    \$450/gm

5% discount will apply for all orders over 5 gm and 7% will apply to orders over 15gm for venoms produced at Venom Supplies Pty Ltd.

**Medtoxin Venom Laboratories**  
**2710 Big John Drive**  
**Deland, Florida 32724**  
**Phone: 386-734-3049**  
**386-740-9143**  
**Fax: 386-734-4163**  
**elapid33@aol.com**  
**www.Medtoxin.com**

## VENOM PRICELIST SPRING/SUMMER 2009

Dendroaspis polylepis	\$550.00
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Dendroaspis viridis	\$750.00
Naja nivea	\$205.00
Naja melanoleuca	\$205.00
Naja nigricollis (Tanzania)	\$205.00
Naja nigricollis (Ghana)	\$205.00
Naja h. annulifera	\$125.00
Naja kaouthia	\$205.00
Naja naja (Pakistan)	\$250.00
Ophiophagus hannah	\$150.00
Micrurus f. fulvius	\$2100.00
Bitis arietans	\$150.00
Bitis g. gabonica	\$150.00
Bitis g. rhinoceros	\$150.00
Crotalus adamanteus	\$150.00
Crotalus atrox	\$150.00
Crotalus h. atricaudatus	\$150.00
Crotalus h. horridus	\$150.00
Crotalus s.scutulatus	\$450.00
Crotalus d. terrificus	\$450.00
Sistrurus m. barbouri	\$450.00
Agkistrodon c.contortrix	\$190.00
Agkistrodon c. laticinctus	\$190.00
Agkistrodon c. mokasen	\$100.00
Agkistrodon p. conanti	\$100.00

Many other venoms available in limited quantity, please inquire  
 Special orders to meet research needs

Exact locality data on most species available, Species are guaranteed

Prices are quoted per gram in U.S. dollars, subject to change without notice

Payment terms net 30 days check, money order, or wire transfer

Shipping is free in the U.S. may be extra for international orders

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<b>Bothrops alternatus</b>	<b>1440,00 US\$</b>
<b>Bothrops jararaca</b>	<b>220,00 US\$</b>
<b>Bothrops jararacussu</b>	<b>264,00 US\$</b>
<b>Bothrops moojeni</b>	<b>300,00 US\$</b>
<b>Bothrops neuwiedi</b>	<b>340,00 US\$</b>
<b>Crotalus durissus terrificus</b>	<b>220,00 US\$</b>
<b>Crotalus durissus collineatus</b>	<b>300,00 US\$</b>

**Lachesis muta muta** 600,00 US\$

**Bufo marinus / schneideri** 264,00 US\$

**All venoms collected in a sterile manner**

**Blood cells and freeze dried blood plasma from snakes**

**We have also other proteins, aminoacids and toxin polyclonal antibodies from Brazilian snakes**

**We trade or sale our products only with CITES from the IBAMA (Brazilian Environment Agency & Wildlife)**

**Prices quoted per gram in US\$. Transport FOB**

**Brazilian Contact:**

**Sanmaru Serpentarium,**

**Rod. Brig. Faria Lima km 365**

**14765-000 Taquaral SP, Brazil**

**[herpetoscience@hotmail.com](mailto:herpetoscience@hotmail.com)**

**[taquaral@gmail.com](mailto:taquaral@gmail.com)**

**Fone (55) 14 9731 2436**

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## Kentucky Reptile Zoo offers only the highest quality venoms for sale!

Our venoms are collected only from healthy individuals and our non-invasive technique insures that all individuals are unharmed. Snakes used for extraction have passed quarantine, feed voluntarily, and produce viable offspring (if breeding is intended). They are kept in clean, sanitary conditions and we have had individuals live longer than their average life span.

- All venoms are collected in a sterile manner and frozen at  $-70^{\circ}\text{C}$  before lyophilization.
- Locale information available for many species.
- DNA samples in the form of scale clippings available; please inquire about tissue samples
- Other venoms available upon request; contact us for more information.
- CITES permits available for all CITES listed species. Extra cost for permits.
- KRZ makes every effort to stay current regarding nomenclature and taxonomy. Our listing reflects current trends, with former names in parentheses. If you have any questions, feel free to contact us.



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## VENOM PRICE LIST



### **Crotalidae**

<b>Species Name</b>	<b>Price (US\$) Per Gram</b>
<i>Agkistrodon bilineatus</i>	\$300.00
<i>Agkistrodon contortrix</i> (fmr. <i>A. c. contortrix</i> , <i>A. c. mokasen</i> )	\$150.00
<i>Agkistrodon laticinctus</i> (fmr. <i>A. c. laticinctus</i> , <i>A. c. phaeogaster</i> , <i>A. c. pictogaster</i> )	\$150.00
<i>Agkistrodon leucostoma</i>	\$75.00
<i>Agkistrodon piscivorus</i>	\$75.00
<i>Bothrops alternatus</i>	\$200.00
<i>Bothrops atrox</i> (Columbia origin)	\$250.00
<i>Bothrops atrox</i> (Surinam origin)	\$250.00
<i>Bothrops moojeni</i>	\$250.00
<i>Calloselasma rhodostoma</i>	\$200.00
<i>Crotalus adamanteus</i>	\$120.00
<i>Crotalus atrox</i>	\$120.00
<i>Crotalus durissus durissus</i>	\$200.00
<i>Crotalus durissus terrificus</i>	\$225.00
<i>Crotalus horridus</i>	\$150.00
<i>Crotalus scutulatus scutulatus</i>	\$175.00
<i>Crotalus viridis viridis</i>	\$100.00
<i>Protobothrops flavoviridis</i>	\$200.00
<i>Sistrurus catenatus tergeminus</i>	\$300.00

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**Helodermatidae**

**Species Name**

- Heloderma horridum*
- Heloderma suspectum*

**Price (US\$) Per Gram**

- \$600.00
- \$600.00



**Viperidae**

**Species Name**

- Bitis arietans*
- Bitis gabonica*
- Bitis rhinoceros*
- Deinagkistrodon acutus*
- Echis carinatus sochureki*
- Echis pyramidium*

**Price (US\$) Per Gram**

- \$225.00
- \$225.00
- \$225.00
- \$300.00
- \$400.00
- \$400.00

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### Elapidae

#### **Species Name**

#### **Price (US\$) Per Gram**

<i>Dendroaspis angusticeps</i>	\$450.00
<i>Dendroaspis polylepis</i>	\$500.00
<i>Naja annulifera</i>	\$80.00
<i>Naja haje</i>	\$80.00
<i>Naja kaouthia</i>	\$100.00
<i>Naja kaouthia</i> (Suphan province)	\$100.00
<i>Naja melanoleuca</i>	\$150.00
<i>Naja mossambica</i>	\$100.00
<i>Naja naja</i> (India origin)	\$150.00
<i>Naja naja</i> (Pakistan origin)	\$150.00
<i>Naja nigricollis nigricollis</i>	\$80.00
<i>Naja nivea</i>	\$150.00
<i>Naja pallida</i>	\$100.00
<i>Naja philippinensis</i>	\$300.00
<i>Naja samarensis</i>	\$300.00
<i>Naja siamensis</i>	\$80.00
<i>Ophiophagus hannah</i>	\$120.00
<i>Oxyuranus scutellatus scutellatus</i>	\$500.00
<i>Pseudechis colletti</i>	\$320.00



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