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The structural and functional diversification of the Toxicofera reptile venom system

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ABSTRACT

The evolutionary origin and diversification of the reptilian venom system is described. The resolution of higher-order molecular phylogenetics has clearly established that a venom system is ancestral to snakes. The diversification of the venom system within lizards is discussed, as is the role of venom delivery in the behavioural ecology of these taxa (particularly *Varanus komodoensis*). The more extensive diversification of the venom system in snakes is summarised, including its loss in some clades. Finally, we discuss the contentious issue of a definition for "venom", supporting an evolutionary definition that recognises the homology of both the venom delivery systems and the toxins themselves.

1. Introduction

The first two higher-level squamate phylogenetic studies using multiple nuclear genes (C-mos and RAG-1) and broad taxonomic coverage (Vidal and Hedges, 2004; Townsend et al., 2004) suggested that most of the classical phylogeny based on morphology was incorrect. The interrelationships among a large novel clade containing i) snakes, ii) anguimorphs, iii) iguanians, and iv) amphisbaenians, lacertids and teiioids could not be resolved in either of these initial studies. Subsequently, it was demonstrated with the use of nine nuclear genes that venom has been a key evolutionary innovation underlying the diversification of the reptile clade Toxicofera including snakes, anguimorphs and iguanians (Fry et al., 2006; Vidal and Hedges, 2005). It has therefore only recently been determined that the single origin of venom in reptiles occurred approximately 170 million years ago during the Jurassic period (Fry et al., 2006; Vidal and Hedges, 2005). Advances in molecular systematics and venomics have thus provided the vital phylogenetic framework necessary for a reconstruction of the evolutionary history of all components of the reptilian venom-delivery system (Fry et al., 2006; Vidal et al., 2007; Vidal and Hedges, 2004, 2005).

2. Origin of venom proteins

Studies by us have demonstrated that a core set of venom genes were present in the common ancestor of all toxicoferans which subsequently evolved into the complex venoms observed in modern snakes and lizards following

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further toxin recruitment events (Fig. 1) (Fry, 2005; Fry et al., 2003a, 2003b, 2010a, 2008; Fry et al., 2006, 2009c, 2010b, in press). Venoms evolve via a process by which a gene encoding for a normal body protein, typically one involved in key regulatory processes or bioactivity, is duplicated and the copy selectively expressed in the venom gland. Venom proteins were recruited from disparate tissues (Table 1) with diverse ancestral activities (Table 2). Basal toxic activities varied widely too (Table 3) and some classes have mutated to form a myriad of new toxic

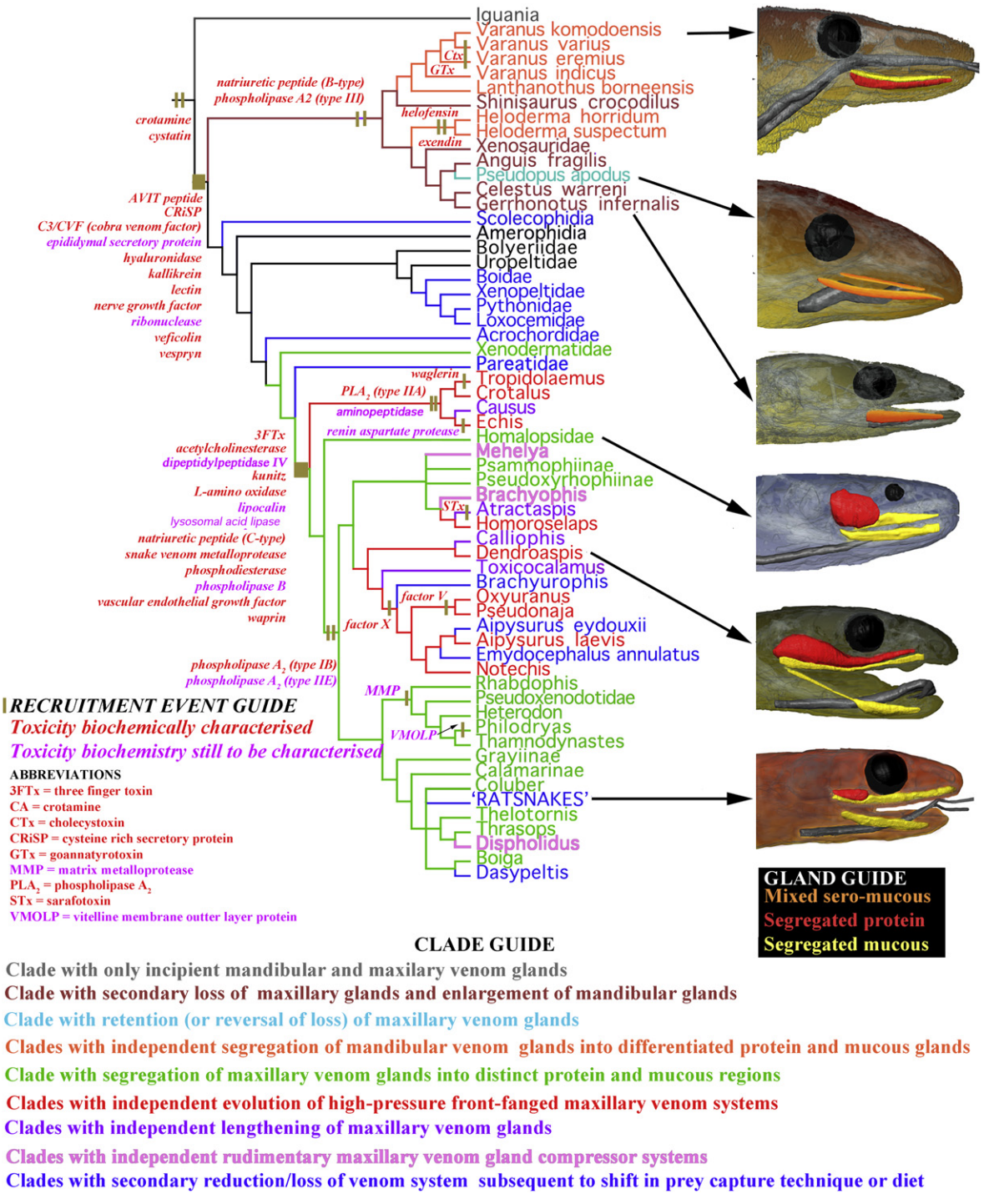

Fig. 1. Cladogram of evolutionary relationships of Toxicofera reptiles (Fry et al., 2006; Vidal and Hedges, 2002, 2004, 2005, 2009) showing relative timing of toxin recruitment events and derivations of the venom system. Magnetic resonance images are shown for representatives as per Fry et al., 2008, 2009a, 2009b, 2009c, 2010b.

Table 1
Secretion locations of nearest non-toxin relations of reptile venom proteins.

Protein type [toxin class if known by specific name]	Tissue type for normal secretion
3FP [3FTx]	Brain
ACN	Muscle
ADAM [SVMP]	Variety of tissues including epididymis, colon, lung, lymph node and thymus
beta-defensin [crotamine]	Brain
beta-defensin [helofensin]	Highly expressed in skin and tonsils, and to a lesser extent in trachea, uterus, kidney, thymus, adenoid, pharynx and tongue. Low expression in salivary gland, bone marrow, colon, stomach, polyp and larynx. No expression in small intestine
C3 [CVF]	Liver
[Celestoxin]	No near matches to any characterized non-venom peptide/protein
Cholecystokinin [cholecystoxin]	Expressed in brain, duodenum and small intestine
CRiSP	Myriad of exocrine tissues including salivary
Cystatin	Restricted to the stratum granulosum of normal skin, the stratum granulosum/spinosum of psoriatic skin, the secretory coils of exocrine sweat glands with low expression levels also found in the nasal cavity
Endothelin [sarafotoxin]	Endothelium
[Exendin]	Nearest match unresolved
Factor V	Liver
Factor X	Liver
Ficolin [veficolin]	Peripheral blood leukocytes. Also detected in spleen, lung, and thymus, may be due to the presence of tissue macrophages or trapped blood in these tissues. Not detected on lymphocytes and granulocytes
Hyaluronidase	Widely expressed
Kallikrein	Variety of exocrine tissues including pancreas as well as the salivary glands
Kunitz	Wide variety of tissues, including brain, conceptus membrane, lung, ovary, placenta, and uterus
LAO	Variety of exocrine and immune tissues
Lectin	Wide-spread
Natriuretic peptide B-type	Brain
Natriuretic peptide C-type	Heart
NGF	Wide variety of tissues including the brain, eye, prostate and salivary glands
Phosphodiesterase	Kidney
PLA_2 – type IB	Pancreas
PLA_2 – type IIA	Synovial fluid
PLA_2 – type III	Expressed in kidney, heart, liver, and skeletal muscle. Also present in placenta and peripheral blood leukocytes
Prokineticin [AVIT]	Expressed at high levels in testis and at lower levels in brain, lung, ovary, spleen, thymus, and uterus
Renin-like aspartic protease	Kidney
SPRY [vespryn]	Hemopoietic lineages
VEGF	Various tissues ranging from the brain to the heart
Veficolin	Peripheral blood leukocytes. Also detected in spleen, lung, and thymus, may be due to the presence of tissue macrophages or trapped blood in these tissues. Not detected on lymphocytes and granulocytes
WAP [waprin]	Variety of tissues including lactating mammary gland, lung, ovary, and testis
[waglerin]	Unknown; no homology to any known peptide
YY peptide [goannatyrotoxin]	Colon mucosa

3FP = three finger peptide; 3FTx = three finger toxin; ADAM = a disintegrin and metalloprotease; C3 = complement 3; CRiSP = cysteine rich secretory protein; CVF = cobra venom factor; LAO = L-amino oxidase; NGF = nerve growth factor; PLA_2 = phospholipase A_2; VEGF = vascular endothelin growth factor; SVMP = snake venom metalloprotase; WAP = whey acidic peptide.

activities (Table 4). A number of frameworks expressed in the venom glands are known only from the mRNA transcripts or corresponding bioactivities remain to be elucidated (Table 5).

3. Ancestral Toxicofera reptile venom system

Although phylogenetic studies based on nuclear genes have thus far failed to resolve the relationships within Toxicofera i.e. the snakes/iguanians/anguimorphs trichotomy, other sources of evidence such as SINEs and morphology favour the clustering of snakes with anguimorphs (Piskurek et al., 2006; Lee, 2009). We therefore follow this arrangement here (Fig. 1). The likely ancestral condition was the possession of relatively simple serous dental glands in both the mandibular and maxillary regions (Fry et al., 2006). These glands produced active substances that were the substrate for the evolution of toxins. Iguania and many other lizards split off while this system was only in an incipient stage.

Consistent with the fact that the venom system has little or no known functional or ecological importance within the Iguania, only trivial further diversification occurred within this lineage and thus this venom system is distinguished from all other Toxicofera reptiles by being 'incipient'. As only one species (*Pogona barbata*) has been studied and with only limited, little is known about relative presence of known toxin types in Iguania glands in general. While only two classes have been sequenced (Fig. 1), it is anticipated that further sequencing will reveal that other toxin types extend to the level of this lineage as well and thus are ancestral to all Toxicofera reptiles.

On the other hand, venom became of tremendous importance within the snakes and also the anguimorph lizards. From common origins, the venom system has evolved dichotomously between the two groups. The maxillary venom glands underwent extensive diversification in snakes, while conversely the mandibular glands were diversified in the anguimorph lizards. Differences are notable in the types of secretory epithelia (serous, sero-

Table 2
Bioactivity of non-toxin ancestors of reptile venom proteins.

Protein type [toxin class if known by specific name]	Normal body function
3FP [3FTx]	Bind to the α7 nicotinic acetylcholine receptor
ACN	Rapidly hydrolyses choline released into the synapse, resulting in less neurotransmitter available for neuromuscular control
ADAM [SVMP]	Enzymatic cleavage of the extracellular matrix
beta-defensin [crotamine]	Unknown
beta-defensin [helofensin]	Antimicrobial but not hemolytic
C3 [CVF]	Central to both classical and alternative complement pathways
[Celestoxin]	No near matches to any characterized non-venom peptide/protein
Cholecystokinin [cholecystoxin]	Hypotensive neuropeptide that binds cholecystokinin receptors
CRiSP	Specific actions largely uncharacterized
Cystatin	Inhibit cysteine proteases such as the cathepsins B, L, and S
Endothelin [sarafotoxin]	Potently vasoconstrictive, modulating the contraction of cardiac and smooth muscle
[Exendin]	Nearest match unresolved
Factor V	Blood cofactor that participate with factor Xa to activate prothrombin to thrombin
Factor X	Vitamin K-dependent glycoproteins that convert prothrombin to thrombin in the presence of factor Va, calcium, and phospholipid during blood clotting
Ficolin [veficolin]	Involved in serum exerting lectin activity. Binds GlcNAc
Hyaluronidase	Random hydrolysis of (1->4)-linkages between N-acetyl-beta-D-glucosamine and D-glucuronate residues in hyaluronate
Kallikrein	Release kinins from circulatory kininogen
Kunitz	Inhibit a diverse array of serine proteinases
LAO	Induce apoptosis in cells by two distinct mechanisms; one rapid and mediated by H_2O_2, the other delayed and mediated by deprivation of L-lysine
Lectin	Hemagglutination activity
Natriuretic peptide – B-type	Produces hypotension mediated by the binding to GC-A with subsequent relaxation of vascular smooth muscle
Natriuretic peptide C-type	Produces hypotension mediated by the binding to GC-B with subsequent relaxation of vascular smooth muscle
NGF	Stimulate division and differentiation of sympathetic and embryonic sensory neurons
Phosphodiesterase	Cleaves a variety of phosphodiester and phosphosulfate bonds including deoxynucleotides, nucleotide sugars, and NAD
PLA_2 – type IB	Release of arachidonic acid from the s_{n-2} position of the plasma membrane phospholipids
PLA_2 – type IIA	Release arachidonic acid from the s_{n-2} position of the plasma membrane phospholipids, involved in inflammatory processes and diseases, such as rheumatoid arthritis and asthma
PLA_2 – type III	Catalyzes the calcium-dependent hydrolysis of the 2-acyl groups in 3-sn-phosphoglycerides. Shows an 11-fold preference for phosphatidylglycerol over phosphatidylcholine (PC). Preferential cleavage: 1-palmitoyl-2-linoleoyl-phosphatidylethanolamine (PE) > 1-palmitoyl-2-linoleoyl-PC > 1-palmitoyl-2-arachidonoyl-PC > 1-palmitoyl-2-arachidonoyl-PE. Plays a role in ciliogenesis
Prokineticin [AVIT]	Constriction of intestinal smooth muscle
Renin-like aspartic protease	Renin is a highly specific endopeptidase, whose only known function is to generate angiotensin I from angiotensinogen in the plasma, initiating a cascade of reactions that produce an elevation of blood pressure and increased sodium retention by the kidney. Cleavage of Leu-|-Xaa bond in angiotensinogen to generate angiotensin I
SPRY [vespryn]	Largely uncharacterized
VEGF	Increase the permeability of the vascular bed
Veficolin	Involved in serum exerting lectin activity. Binds GlcNAc
WAP [waprin]	Inhibit leukoproteinases
[waglerin]	Unknown; no homology to any known peptide
YY peptide [goannatyrotoxin]	This gut peptide inhibits exocrine pancreatic secretion, has a vasoconstrictory action and inhibitis jejunal and colonic mobility

3FP = three finger peptide; 3FTx = three finger toxin; ADAM = a disintegrin and metalloprotease; C3 = complement 3; CRiSP = cysteine rich secretory protein; CVF = cobra venom factor; LAO = L-amino oxidase; NGF = nerve growth factor; PLA_2 = phospholipase A_2; VEGF = vascular endothelin growth factor; SVMP = snake venom metalloprotase; WAP = whey acidic peptide.

mucous, mucous) and their location (gland, duct, transition area); and in the number and physical orientation of the gland compartments as well as the relative encapsulation of the glands.

4. Diversification of the anguimorph lizard venom system

The ancestral anguimorph lizard condition is represented in *Pseudopus apodus* (previously known as *Ophisaurus apodus*) which has retained sero-mucous glands in the maxillary region in addition to the mandibular glands typical of other members of the anguimorph lizards ((Fry et al., 2010b); Fig. 1). The ancestral state is mixed glands with a serous portion occupying the bottom of the glands and a mucous part above it, with the entire arrangement encapsulated by a single thin membrane. There is one gland compartment per tooth, and each compartment has its own duct leading to the base of the tooth. Within the gland compartment, extensive intra-lumen drainage channels are

Table 3
Reptile venom proteins basal toxicity.

Protein type [toxin class if known by specific name]	Basal toxic activity
3FP [3FTx]	α-neurotoxicity, antagonistically binding to the nicotinic acetylcholine receptor
ACN	Bioactivities uncharacterized
ADAM [SVMP]	Tissue necrosis
beta-defensin [crotamine]	Significant neurotoxic activity, modifying voltage-sensitive Na^+ channels, resulting in a potent analgesic effect and myotoxic activities have been reported; which is basal and which is derived remains to be elucidated
beta-defensin [helofensin]	Lethal toxin which possesses an inhibitory effect on direct electrical stimulation of the isolated hemi-diaphragm
C3 [CVF]	Unregulated activation of the complement cascade, causing rapid and significant problems such as anaphylactic-type problems and/or tissue damage via hemolysis/cytolysis
[Celestoxin]	Hypertensive mediated by unknown pathway
Cholecystokinin [cholecystoxin]	Hypotension mediated by binding cholecystokinin receptors
CRiSP	Paralysis of peripheral smooth muscle and induction of hypothermia through blockage of various channels including ryanodine and L-type calcium channels
Cystatin	Inhibition of body defensive enzymes
Endothelin [sarafotoxin]	Potent vasoconstriction resulting in acute hypertension
[Exendin]	Hypotensive
Factor V	Combines with toxic form of factor X to potently convert prothrombin to thrombin
Factor X	Potent conversion of prothrombin to thrombin in the presence of factor V (endogenous or venom forms), calcium and phospholipid
Ficolin [veficolin]	Unknown
Hyaluronidase	Facilitates spread of other venom proteins
Kallikrein	Increase of vascular permeability and production of hypotension in addition to stimulation of inflammation
Kunitz	Inhibition of circulating serine proteinases
LAO	Apoptosis
Lectin	Platelet aggregation mediated by galactose binding
Natriuretic peptide B-type	Potent induction of hypotension leading to loss of consciousness
Natriuretic peptide C-type	Potent induction of hypotension leading to loss of consciousness
NGF	Bioactivities uncharacterized
Phosphodiesterase	Inhibition of platelet aggregation
PLA_2 – type IB	Presynaptic neurotoxicity is either the basal or is basal derivative
PLA_2 – type IIA	Lipase activity resulting in inflammation and tissue destruction
PLA_2 – type III	Prevents platelet aggregation mediated through epinephrine-pathway
Prokineticin [AVIT]	Potent constriction of intestinal smooth muscle, resulting in painful cramping, and induction of hyperalgesia
SPRY [vespryn]	Induces hypolocomotion and hyperalgesia. Unknown which, if either, is basal activity
VEGF	Increase of the permeability of the vascular bed and binding of heparin. Results in hypotension and shock
Veficolin	Bioactivities uncharacterized
WAP [waprin]	Bioactivities uncharacterized other than antimicrobial
[waglerin]	Elicits tachypnea, ocular proctosis, rapid collapse and spasms in mice. The primary cause of death is respiratory failure. Selectively blocks the epsilon subunit of muscle nicotinic acetylcholine receptor
YY peptide [goannatyrotoxin]	Potently hypotensive through relaxation of vascular smooth muscle

3FP = three finger peptide; 3FTx = three finger toxin; ADAM = a disintegrin and metalloprotease; C3 = complement 3; CRiSP = cysteine rich secretory protein; CVF = cobra venom factor; LAO = L-amino oxidase; NGF = nerve growth factor; PLA_2 = phospholipase A_2; VEGF = vascular endothelin growth factor; SVMP = snake venom metalloprotase; WAP = whey acidic peptide.

evident but the major lumen is unstructured. This unstructured, sero-mucous arrangement is retained in the more robust mandibular venom glands of the anguid lizards, as is the arrangement of one compartment per tooth. However, in all anguimorph lizards examined to date other than *P. apodus*, the maxillary gland is entirely lost.

In contrast to the simple, unstructured glands of the other anguimorph lizards, the *Heloderma* and *Lanthanotus*/*Varanus* mandibular venom glands have independently evolved segregated protein and mucous secreting regions and distinct gland types (Fry et al., 2010b). In both cases, the serous protein-secreting glands have well-structured central lumens, and the entire arrangement is encapsulated by thick membranes (Fry et al., 2010b). In these segregated glands, a myriad of mucous lobules are located dorsally and are distinct from the protein glands (Fry et al., 2010b). These two lineages convergently increased lumen storage space by fusing posterior compartments so that in both *Heloderma* and *Varanus* only six compartments remain. Varanid and lanthanotid glands are similar in almost all respects, but may differ in the number of compartments present. *Lanthanotus* may have as few as three compartments, but further investigation is desirable as previous studies have been limited to the examination of poorly-preserved museum specimens. The physical architecture differs between the helodermatid and varanid/lanthanotid clades: the compartments of the *Heloderma* glands have a more layered arrangement than those of varanids/lanthanotids and the varanid/lanthanotid glands are thinner and more tubular. In both clades, extensive intra-lumen drainage channels feed into highly structured lumens. The ducts of helodermatids terminate at the base of the thin, deeply grooved teeth, while those of the varanids/lanthanotids terminate between the large, blade-like teeth.

The anguimorph lizard venom delivery system is less sophisticated than the high-pressure injection mechanism

of the front-fanged advanced snakes, and the vast majority of these species pose trivial direct medical risks to humans. The effects of envenomation from medically important species such as *Heloderma* (for example) however, may be clinically complex with symptoms including extreme pain; acute local swelling; nausea; fever; faintness; myocardial infarction; tachycardia; hypotension; and inhibition of blood coagulation (Bogert and del Campo, 1956; Bouabboud and Kardassakis, 1988; Cantrell, 2003; Hooker and Caravati, 1994, 1995; Miller, 1995; Strimple et al., 1997).

The previous lack of recognition of the venom system of anguimorph lizards has led to fundamental misinterpretations of their predatory ecology, particularly that of *Varanus komodoensis*. Of importance in understanding the current predatory ecology of the of *V. komodoensis* is the litany of changing environmental factors influencing their evolution; not in Indonesia where they are exclusively found today, but in Australia nearly 4 million years ago. *V. komodoensis* evolved in Australia, along with two other giant species of varanid (now extinct), to predate upon the continents now extinct megafauna and their young (Hocknull et al., 2009). It is possible that these giant species of varanid filled alpha-predatory niches in Australia that were filled elsewhere by placental mammals (Eutheria). Sweet and Pianka (2007) have suggested that

Table 4
Reptile venom proteins derived toxicities.

Protein type [toxin class if known by specific name]	Derived toxic activity
3FP [3FTx]	Basal α-neurotoxicity greatly potentiated by the deletion of the C2 and C3 ancestral cysteines. Functional derivations include binding to the postsynaptic muscarinic acetylcholine receptors, presynaptic neurotoxic action upon the L-type calcium channels, cytotoxic interactions, acetylcholinesterase inhibition, and others
ACN	None currently documented
beta-defensin [crotamine]	Significant neurotoxic activity, modifying voltage-sensitive Na^+ channels, resulting in a potent analgesic effect and myotoxic activities have been reported; which is basal and which is derived remains to be elucidated
beta-defensin [helofensin]	None characterized to-date
ADAM [SVMP]	Prothrombin activation a basal derivation. In Viperidae venoms, proteolytic cleavage of C-terminal domains resulted in myriad of other activities including direct-acting fibrinolytic activity. Liberated disintegrin domain inhibits platelets via GP IIb/IIIa integrin receptor
C3 [CVF]	None currently documented
[Celestoxin]	None currently documented
Cholecystokinin [cholecystoxin]	None currently documented
CRiSP	Blockage of cyclic nucleotide gated calcium channels
Cystatin	None currently documented
Endothelin [sarafotoxin]	None currently documented
[Exendin]	Derived VIP-like form is more potently hypotensive and cardiotoxic than the ancestral glucagon derived form
Factor V	None currently documented
Factor X	None currently documented
Ficolin [veficolin]	None currently documented
Hyaluronidase	None currently documented
Kallikrein	Derivations affect the blood, particularly targeting fibrinogen
Kunitz	Derivations include inhibition of plasmin and thrombin and the blockage of L-type calcium channels. Structural derivatives form part of neurotoxic complexes with PLA2 molecules
LAO	Derivations include hemorrhagic effects, not only by affecting platelet aggregation, but also inhibiting blood factor IX
Lectin	Derivations include stimulation of platelet aggregation (binding GPVI, GPIb, GPIa/IIa or VWF), platelet aggregation inhibition (binding GPIb or GPIa/IIa) or anti-coagulant actions by binding blood factors IX, X
Natriuretic peptide B-type	Derivatives contain newly evolved helokinestatin domains located upstream that inhibit bradykinin
Natriuretic peptide C-type	Mutants with C-terminal tail have hypotensive activity mediated by GC-A instead of GC-B receptor (elapid venoms and convergently also in *Cerastes* venom). Upstream of the natriuretic peptide encoding domain viperid venom forms contain multiple proline-rich bradykinin potentiating peptides or brakykinin inhibiting peptides (pit-vipers); other forms have newly derived antiplatelet (*Macrovipera*) or metalloprotease inhibiting peptides upstream (*Echis*)
NGF	None currently documented
Phosphodiesterase	None currently documented
PLA_2 – type IB	Deletion of pancreatic loop facilitated the derivation of a multiplicity of novel, nonenzymatic activities, including antiplatelet and presynaptic neurotoxicity. Some derivatives are parts of neurotoxic complexes
PLA_2 – type IIA	Derivations include neurotoxic and antiplatelet activity. Some derivatives are parts of complexes
PLA_2 – type III	None currently documented
Prokineticin [AVIT]	None currently documented
SPRY [vespryn]	None currently documented
VEGF	None currently documented
Veficolin	None currently documented
WAP [waprin]	None currently documented
[waglerin]	None currently documented
YY peptide [goannatyrotoxin]	None currently documented

3FP = three finger peptide; 3FTx = three finger toxin; ADAM = a disintegrin and metalloprotease; C3 = complement 3; CRiSP = cysteine rich secretory protein; CVF = cobra venom factor; LAO = L-amino oxidase; NGF = nerve growth factor; PLA_2 = phospholipase A_2; VEGF = vascular endothelin growth factor; SVMP = snake venom metalloprotase; WAP = whey acidic peptide.

Table 5
Novel venom proteins scaffolds known only from transcripts or with bioactivities that remain to be characterised.

Molecular scaffold type	Species recovered from	Tissue type of ancestral protein	Bioactivity of ancestral protein	References
Aminopeptidase	*Bitis gabonica, Gloydius brevicaudus, Echis coloratus, Echis carinatus sochureki*	Expressed in epithelial cells of the kidney, intestine and respiratory tract; also found in the vascular endothelium, fibroblasts, granulocytes and monocytes	Broad specificity peptidases, including regulation of blood pressure; release dipeptidases from the N-terminus of peptides such as angiotensin II	Ogawa et al. (2007); Casewell et al. (2009); Vaiyapuri et al. (2010)
Dipeptidylpeptidase IV	*Bothrops jararaca, Gloydius blomhoffi, Lachesis muta, Pseudechis australis, Demansia vestigiata, Tropidechis carinatus*	Widespread expression in most tissues; highest activity identified in the kidney and lung	Regulatory protease; inactivation of bioactive peptides by liberation of dipeptides from the N-terminus	Cidade et al. (2006); Junqueira-de-Azevado et al. (2006); Ogawa et al. (2006); St Pierre et al. (2007)
Epididymal secretory protein	*Liophis poecilogyrus Varanus indicus, Varanus gouldii, Varanus komodoensis*	Epididymis; detected in the epithelial cells of the epididymal duct	Putative role in binding lipids and collagen	Fry et al. (2006, 2010b)
Lipocalin	*Azemiops feae, Dispholidus typus, Rhabdophis tigrinus, Trimorphodon biscutatus*	Preferentially synthesized in nonproliferating cells	Preferentially binds long-chain unsaturated fatty; known allergen	Fry et al. (in press)
Lysosomal acid lipase	*Echis coloratus, Micrurus altirostris, Philodryas olfersii*	Widely expressed in the liver and fibroblasts; since been identified in a variety of tissue and cell types, with the exception of erythrocytes	Degradation of cholesterol esters and triglycerides; modulator of intracellular cholesterol metabolism	Casewell et al. (2009); Corrêa-Netto et al. (2011)
Phospholipase A_2Type IIE	*Leioheterodon madagascarensis, Dispholidus typus*	Restricted to the brain, heart, lung, and placenta	Progression of inflammatory processes	Fry et al. (in press)
Phospholipase B	*Drysdalia coronoides, Pseudechis colletti, Crotalus adamanteus*	Detected in white blood cells (granulocytes and neutrophils), intestinal enterocytes and the epidermis	Removal of fatty acids from both the sn-1 and sn-2 positions of phospholipids	Bernheimer et al. (1987); Chatrath et al. (2011); Rokyta et al. (2011)
Renin-like aspartic protease	*Echis ocellatus, Echis jogeri*	Expression restricted to the kidney	Generation of angiotensin I from angiotensinogen; mediator of extracellular volume and vasoconstriction	Wagstaff and Harrison (2006)
Ribonuclease	*Celestus warreni, Gerrhonotus infernalis, Liophis poecilogyrus, Psammophis mossambicus*	Expressed predominantly in the pancreas	Pyrimidine-specific C-preferring nuclease	Fry et al. (2010b, in press)
Snake venom matrix metalloprotease	*Rhabdophis tigrinus, Thamnodynastes strigatus*	Macrophages and granulocytes	Proteolysis of the extracellular matrix	Ching et al. (2012). *Rhabdophis* UniProt B1Q2M9
Vitelline membrane outer layer protein	*Enhydris polylepis*	Oviduct; component of the outer membrane of the vitelline layer of the egg	Function unknown	Fry et al. (in press)

the absence of eutherian predators in Australia may account for the success of small species of varanid (typically members of the subgenus *Odatria*) on that continent. Sweet & Pianka cite predation rather than competitive exclusion by placental mammal carnivores (Order: Carnivora) as the likely reason for the absence of small varanids elsewhere in the world. However, it seems plausible that a lack competition from eutherian predators may have facilitated the evolution of a clade of giant, alpha-predatory varanids, uniquely in Australia. While the largest member of this clade (*Varanus* (formerly *Megalania*) *prisca*) remained in Australia, the other two radiated outwards to the Lesser Sunda Islands of Indonesia. The second largest fossil species (currently un-named) dispersed to Timor, while smallest of the group (*V. komodoensis*) moved further west to Flores and surrounding islands. The environment on these islands has been marked in recent history by three major faunal turnovers including the extinction of the islands' megafauna 12,000 years ago. After this extinction event, *V. komodoensis* occupied an environment devoid of moderately sized prey until the introduction of the pig from Sulawesi 5000 years later. During the intervening period *V. komodoensis* likely persisted by feeding opportunistically on relatively small prey items, in a manner similar to that of other extant species of varanid. This illustrates their adaptive flexibility. The currently available prey options for adult dragons include mammals (deer, pig and water buffalo) that were introduced by Dutch settlers only a couple of hundred years ago. The komodo dragon is thus living in a 'novel ecosystem'. Encounters between dragons and these potential prey animals are unnatural, man-made interactions which have taken place for far too short a period of time to have influenced the evolution of *V. komodoensis*.

V. komodoensis have light-weight skulls with relatively weak biting force compared to their mass (D'Amore et al., 2011; Fry et al., 2009c; Moreno et al., 2008). Instead, *V. komodoensis* utilises large, serrated teeth as their primary weapon, using a grip-and-rip strategy to inflict deep parallel wounds. Mechanical damage alone may in some cases result in rapid death from blood loss (e.g. slicing the femoral artery). The role of venom is to use anti-coagulant toxins to increase blood loss and other toxin to induce hypotension and shock (Fry et al., 2006, 2010b, 2009c).

V. komodoensis body size has remained stable over the last 900 ka (kiloannum) on Flores. Whilst pigs and deer are of manageable size (40–50 kg), water buffalo (at 400–900 kg) are dramatically larger than any potential prey item that ancestral dragons may have evolved to feed upon. This fact is starkly reflected in the efficacy of predation attempts by dragons. Attacks on pigs and deer are extremely successful (Bull et al., 2010; Fry, personal observation) with an overall kill-rate of approximately 90%. Roughly three-quarters of such predation attempts result in the prey animal dying of blood loss within the first thirty minutes. These rapid deaths are due largely to the mechanical damage resulting from the bite resulting in massive blood loss from a severed major artery. Another ten to fifteen percent of these prey animals succumb within three or four hours. In these cases, death is facilitated by persistent bleeding resulting from the anti-coagulant effects of the venom. This advantage results in significant selection pressure for the maintenance of physiologically-costly venom.

Though dragons seem incapable of bringing down an adult water buffalo, they can inflict deep wounds to the lower hind-quarters of these animals. The water buffalo then seek refuge in water. Unlike the marshes of their native environment, which are regularly flushed clean by rainwater, the only available water in which to seek refuge on the islands is in stagnant tropical water holes. We postulate that the faeces-laden water of these wallows is the actual source of bacterial infection that may result in life-threatening sepsis. Thus, such infections have an environmental source rather than originating in the mouths of komodo dragons. Studies purporting to prove the use of bacteria as weapons by *V. komodoensis* swabbed the mouths of the lizards (Gillespie et al., 2000) but neglected to sample the water sources from which the specimens had been drinking. Thus they did not take into account the fact that any bacteria in the mouths of wild *V. komodoensis* are likely to be transient. The potentially fatal infections contracted by buffalos post-bite are the result of a man-made and unnatural encounter between predator and potential prey that has only taken place for a small fraction of the evolutionary history of either species. Highly imaginative scenarios such as lizard–lizard swapping of 'weaponised bacteria' are evolutionarily implausible (Bull et al., 2010).

5. Diversification of the snake venom system

Venom has had a central role in the evolution of the advanced snakes, under-pinning their extraordinary diversification in the Cenozoic era (Vidal, 2002). Extensive coevolutionary modification of all venom system variables has occurred, including gland morphology, muscles, skull, dentition and biochemical diversification and/or specialisation of the venoms.

Our understanding of the diversification of the snake venom system has been crucially aided by recent molecular phylogenetic studies (Vidal, 2002; Vidal and Hedges, 2002, 2009; Lawson et al., 2005; Vidal et al., 2008a, 2007) which revealed that the non-front-fanged 'colubrid' snakes are not a single, monophyletic group as previously supposed, but represent numerous discrete clades, with the front-fanged Caenophidians nested among them. Furthermore, the front-fanged snakes (*Atractaspis* and *Homoroselaps* [atractaspidines]; elapids; viperids) do not form a monophyletic clade, but consist of three independent lineages within the Caenophidia (viperids occupy a basally-divergent position in this group, whereas elapids and atractaspidines are nested among a clade of African 'colubrid' lineages). Moreover, there is extensive evidence that the venom glands of the front-fanged snakes are homologous with the 'Duvernoy's gland' of non-front-fanged snakes (Kochva, 1978; Jackson, 2003; Vonk et al., 2008). The name 'Duvernoy's gland' was previously assigned to venom glands lacking compressor muscles and associated hollow fangs for reasons based upon a poor understanding of the evolutionary relationships of the snakes themselves. In light of the phylogenetic knowledge described above, the term 'Duvernoy's gland' has been abandoned and the term

'venom gland' should now be used for the toxin-secreting buccal glands of all caenophidians regardless of the degree of anatomical derivation or relative medical importance of human envenomations (Fry et al., 2003b). Supporting the homology of, and thus consistent terminology for these glands, accumulated developmental evidence shows that all venom gland types are derivates of the dental glands, and developed from a common primordium at the posterior end of the dental lamina. 'Dental uncoupling' is responsible for the diversity of fangs in both structure and location on the maxilla (Fry et al., 2008; Jackson, 2003; Kochva, 1963, 1978; Kochva and Gans, 1965; Vonk et al., 2008; Wollberg et al., 1998).

An impressive diversity of maxillary dentition has independently arisen from the evolutionary decoupling of the venom system (Fry et al., 2008; Vonk et al., 2008). Tooth morphology ranges from smooth surface; to surface with a shallow groove; to deep groove present on less than half the length of the tooth; to deep groove running the entire length of the tooth; to fully enclosed venom canal in proteroglyphous and solenoglyphous front-fanged snakes (Fry et al., 2008; Gravlund, 2001; Jackson, 2003; Young and Kardong, 1991, 1996). It has been determined that extensive evolutionary tinkering has produced the impressive diversity of dentition amongst extant species and that the terms aglyph and opisthoglyph are phylogenetically meaningless as such snakes do not group into monophyletic clades (Vidal, 2002; Vidal and Hedges, 2002). The ancestral condition consists of unspecialised teeth lacking any degree of enlargement. In cases where the posterior teeth are not grooved (aglyphous) or enlarged in relation to other teeth, the presence of ridges on the anterior and posterior surfaces of posterior teeth distinguishes them from the anterior teeth (Fry et al., 2008). On numerous independent occasions the posterior teeth have been variably enlarged (opisthodont). In lineages with such enlarged teeth, the introduction of venom into a bite wound is often facilitated by open channels or grooves along the lateral or anterolateral surfaces of the fangs (opisthoglyphous) (Fry et al., 2008; Young et al., 2011).

Snake venom glands also exhibit tremendous variation in all aspects including structure and topography, with countless variants scattered across the taxonomical tree (Fry et al., 2008). The secretory epithelium consists of serous, proteinaceous cells with mucous secreting cells found in some regions, mainly in the ducts. The secretion is stored in the cells and lumina, which are of varying size and shape. The venom glands of vipers, for instance, have very large tubular and centralised lumina and relatively few secretory granules in the cells. The contours range from the ancestral condition of large ovate ducts, to ducts with reduced diameters surrounded by extensive circular connective tissue, through to internal partitions of the venom duct. Vestibules range from absent, to present adjacent to the venom gland, to present at the fang sheath, to in contact with the oral cavity. The location of the gland duct openings ranges from the ancestral condition of opening directly into the oral cavity, to opening near the tooth bases to opening into a fang sheath in front-fanged snakes.

On at least six independent occasions an increase in efficiency and speed of delivery has been accomplished through the evolution of venom gland compressor systems (Fry et al., 2008). The extant genera *Brachyophis, Dispholidus* and *Mehelya* have superficial muscle fibres connected to the venom gland capsule that may be considered a rudimentary compressor system. *Brachyophis* has a purely serous gland (Taub, 1967). *Dispholidus* has a very large venom gland composed of branched tubules covered by a secretory epithelium (Fry et al., 2008). The tubules open into a wide duct lined with mucous cells and the ducts and entire gland are surrounded by extensive circular connective tissue. Vestibules are present adjacent to the venom gland and the enlarged rear fangs have deep grooves running less than half the length. In contrast, *Mehelya* has ungrooved (aglyphous) teeth and a small venom gland of peculiar structure with a relatively wide lumen (Fry et al., 2008). The additional three compressor systems were further developed into high-pressure front-fang venom-delivery systems (Fry et al., 2008): the common ancestor of *Atractaspis* and the sister genus *Homoroselaps* (Vidal et al., 2008b) within the atractaspidine subfamily of the Lamprophiidae; in the Elapidae family; and in the Viperidae family (Fry et al., 2008; Vidal et al., 2007; Vidal and Hedges, 2009). In these snakes, compression of the glands by the compressor muscle (aided by some other muscles) propels venom along the duct and into the enclosed channel running through the shaft of the fangs (Fry et al., 2008; Young et al., 2001, 2004; Young and Kardong, 2007; Young and Zahn, 2001).

Within the Elapidae and within the Viperidae, two of the three discrete lineages of front-fanged snakes, the venom system shows little intra-familial diversity (Fry et al., 2008). The structure of elapid venom glands is a synapomorphy of the family. The venom is stored in the cells, with the lumen being relatively small. This family also has an elongate accessory mucous gland surrounding the venom duct. All elapid venom glands have a vestibule present adjacent to the fang sheath and a venom duct that opens only into the fang sheath. The complex tubular glands of viperids have invaginations that produce several lobes and a large lumen that stores considerable amounts of venom. The primary duct of the gland leads to a globular accessory gland which then connects to the fang via a secondary duct (Kochva, 1978, 1987; Kochva and Gans, 1965). Viper venom glands have a vestibule present adjacent to the fang sheath and venom ducts that open only into the fang sheath. There are no mucous regions in either the Elapidae or Viperidae venom glands except for the accessory glands characteristic to each family. The venom system of *Atractaspis* is completely structurally distinct and resembles none of the venom systems of other front-fanged (or non-front-fanged) snakes. The venom glands of *Atractaspis* have a wide, elongate lumen surrounded by radially arranged secretory tubules that show some branching at their peripheral distal ends. Prominent mucoid regions occupy their luminal areas and there are no separate accessory glands. Vestibules are present adjacent to the fang sheath and venom ducts open only into the fang sheath. Finally, it should be emphasised that the venom glands of *Homoroselaps* are of a pattern entirely different from that of both *Atractaspis* and the other species of the atractaspidine subfamily. Instead they convergently

resemble those of the Elapidae (Kochva et al., 1985; Underwood and Kochva, 1993).

In each of the front-fanged clades, tremendous elongation of the venom gland has occurred independently at least once: in *Atractaspis* (the clade comprised by *Atractaspis engaddensis*, *Atractaspis microlepidota*, *Atractaspis micropholis*, and *Atractaspis scortecci*); twice in the Elapidae (*Calliophis intestinalis* and *Calliophis bivirgata* and then again in *Toxicocalamus buergersi*); and in the Viperidae (*Causus resimus* and *Causus rhombeatus*) (Fig. 1). In these species, the venom glands extend to about a quarter of the body length or even more. The biological advantage conferred by these elongations remains to be elucidated. In the case of *Causus* the change in gland length was not accompanied by a significant shift in venom profile or recruitment of new toxin types (Fry et al., 2008). Rather, they have a typical viperid venom composition with the major toxin types sequenced to date being metalloproteases (SVMP), kallikrein, and Type IIA phospholipases A_2 (PLA_2). It remains to be investigated whether there are significant differences in venom composition between long and short-glanded forms within the genera *Atractaspis*, *Calliophis* or *Toxicocalamus*. Such investigations may shed light on the evolutionary advantage gained by these species. It is notable that each of these three genera is the most slender of their respective families. It may be possible, at least in the cases of *Atractaspis, Calliophis* and *Toxicocalamus*, that the long-glanded condition is an adaptation for a fossorial lifestyle that allows for a decrease in head size and girth whilst maintaining a high venom-yield.

Changes of the venom system also include secondary loss following a shift to a new prey capture technique (constricting) or prey-type ('defenceless' prey, e.g. eggs, worms, snails etc.). In the family Colubridae, the North American 'rat snakes' (typified by *Pantherophis guttatus* [corn snake]) have secondarily evolved a new form of prey capture (powerful constriction) and prey preference (rodents). Subsequently the gland has become greatly atrophied (Fry et al., 2008). This has also occurred independently in some African Lamprophiids such as the powerful-jawed *Pseudaspis cana*, which has a purely mucoid gland (Taub, 1967). Convergently within the basal snakes, the evolution of powerful constriction has resulted in a secondary loss of the venom system. *Acrochord*us also constricts, wrapping around the gills of fish prey to suffocate them (Lillywhite, 1996), and this has also resulted in a secondary complete loss of the venom system (Fry personal observations). The African snake *Dasypeltis scabra*, which feeds exclusively on bird eggs, also has greatly atrophied venom glands (Fry et al., 2008). This 'use it or lose it' evolutionary trajectory is paralleled in the sea snakes *Aipysurus eydouxii* (Li et al., 2005a, 2005b): subsequent to switching from feeding on fish to feeding exclusively on fish eggs, the venom glands of this species have atrophied and significant reduction of the fangs has occurred. These reductions in the venom system of *A. eydouxii* have been accompanied by significant accumulation of deleterious mutations in the toxins still transcribed, indicating that they are no longer subject to selection. *Emydocephalus annulatus*, another sea snake which specialises in fish eggs, has not been investigated yet. It is anticipated; however, that studies will reveal a similar degeneration of the venom system. Similarly, the members of the Australian elapid genus *Brachyurophis* that specialize in feeding on lizard eggs also exhibit significant reduction of the venom system (Fry, personal observations). While the extremely derived Scolecophidia (Anomalepididae, Gerrhopilidae, Leptotyphlopidae, Typhlopidae, and Xenotyphlopidae) (Vidal et al., 2010) have not yet been specifically examined, their morphological uniqueness and specialised diet (e.g. ant and termite eggs and larvae) strongly suggest that they too have undergone a reduction of the venom system secondary to a dietary shift. Finally, the other few lineages displaying an absence or reduction of the maxillary glands are malacophagous (Asian pareatids and some American dipsadids), although evidence suggests that the infralabial/mandibular glands in the 'goo-eating' dipsadid snakes (*Atractus reticulatus*, *Dipsas indica*, and *Sibynomorphus mikanii*) secrete snail specific toxins (de Oliveira et al., 2008) which may help immobilize the molluscs and facilitate removal from the shells (Salomão and Laporta-Ferreira, 1994).

6. An evolutionary definition of venom

The recent insights into the single origin of toxin-secreting buccal glands in toxicoferan reptiles have led to considerable controversy regarding the definition of 'venom', 'fangs' and 'venom glands'. The question of the definition of 'venom' is of more than semantic importance for our attempts to understand the origin and evolution of the venom system in toxicoferans: it may play a key role in guiding the formulation of research questions on the function of the secretions in snake life history and their evolution, as well as in toxinological research and spin-offs such as drug discovery.

Despite the well-established homology of the venom glands of front-fanged and non-front-fanged snakes, some authors continue to insist that the glands are fundamentally different between the two groups and thus that the term 'Duvernoy's gland' should still be used (e.g. Weinstein et al., 2011). Furthermore, these authors insist that the majority of non-front-fanged snakes possessing a venom gland ('Duvernoy's gland'), as well as all toxicoferan lizards other than *Heloderma* sp., are not in fact 'venomous' at all. Broadly speaking, the major divisions of opinion are between those who prefer the traditional, function-based definition of 'venom' (a toxic compound injected into prey or predator to cause rapid death or incapacitation – e.g., Kardong, 1980, 1996, 2002) and those who seek to accommodate and prioritise evidence of evolutionary homology in their definitions (e.g. Fry et al., 2003a, 2009a, 2008, 2006, 2009b, 2003b). Inevitably, these different approaches lead to different conclusions as to how toxicoferan species and/or their secretions should be categorised. Those who prefer a function-based definition assert that the biological role of the secretory toxins of non-front-fanged snakes (and those of toxicoferan lizards other than *Heloderma* sp.) has not been experimentally determined and therefore that it is premature to label them 'venomous'. Additionally, they stress that the glands of non-front-fanged and front-fanged snakes are anatomically distinct from each other and that non-front-fanged snakes,

with few exceptions, have no 'medical significance' to humans (unlike 'truly venomous' species). Medical significance to humans is, however, evolutionarily irrelevant.

One of the difficulties with using function as a criterion for identifying a substance as a 'venom' and a taxon as 'venomous', is the degree of subjectivity involved. The oft-touted criterion of 'rapid prey death' (e.g., Kardong, 1980) has been heavily criticised by (e.g. Rodríguez-Robles, 1994; Fry et al., 2003a, 2003b) as subjective and impractical, but is still being advocated as a criterion for classification as 'venom' (e.g., Kardong, 2002; Weinstein and Keyler, 2009). Adopting this criterion for other taxa would necessitate applying the term 'non-venomous' to bees, platypus, most spiders, some scorpions, and many other clades well-recognised as venomous. The advocates of this position are trying to apply a special-case scenario to toxin-secreting reptiles based largely upon an outdated and poor understanding of the evolution of this clade.

We agree that 'venom' refers to the biological function of a secretion and not merely to its toxicity; however, we assert that 'rapid prey death' is not the only function of a venom. We have previously defined venom (see Fry et al., 2009a) as 'a secretion, produced in a specialised tissue (generally encapsulated in a gland) in one animal and delivered to a target animal through the infliction of a wound (regardless of how tiny it is). A venom must further contain molecules that disrupt normal physiological or biochemical processes so as to facilitate feeding or defence by/of the producing animal.' This definition is not restricted to the venoms of toxicoferan reptiles and applies across all venomous taxa. Additionally, we contend that in some cases a similar amount of evidence exists to support the biological function of toxic secretions in non-front-fanged snakes and non-helodermatid toxicoferan lizards as it does in other traditionally 'venomous' taxa. Those who seek to prioritise functional considerations in their definition of venom often point to the absence of evidence for the biological role of these secretions in non-front-fanged snakes (and non-helodermatid lizards). However, much of this argument rests on solely on this absence of evidence rather than on any evidence of absence: the function of oral secretions has been tested in very few snakes and virtually no lizards, so that no evidence of functionality is available for the majority of toxicoferans, including most of the traditionally 'venomous' taxa. Moreover, some studies purporting to show absence of a function involving prey subjugation and incapacitation are flawed. For instance, Rochelle and Kardong (1991, 1993) found no evidence of envenomation in mice predated upon by *Boiga irregularis*; however, the principal neurotoxin present in the venom of this species has subsequently been demonstrated to be highly specific to avian rather than mammalian prey (Mackessy et al., 2006; Pawlak et al., 2009). The aforementioned study of 1993 was flawed in several other regards including, fundamentally, its criterion for determining the effect (or lack of effect) of envenomation. Other studies were carried out prior to the discovery of the single early origin of venom and therefore did not look for the effects of toxins (e.g., Gregory et al., 1980) – any toxic effects such as accelerated prey death or tranquilisation may thus have been overlooked.

The possibility of multiple alternative functions for the oral secretions of toxicoferans has been suggested (e.g., Kardong, 2002; Weinstein and Keyler, 2009), but there is in fact very little evidence for functions other than prey incapacitation during foraging and a contribution to digestion (Rodríguez-Robles and Thomas, 1992). Although studies that experimentally determine the functionality of the toxic secretions of non-front-fanged snakes are rare, there is a considerable amount of other evidence that strongly supports their role in prey subjugation and thus their status as 'venoms'. For example, a potently neurotoxic peptide would be useful in paralysing prey, but is useless as a lubricant. It is therefore reasonable to surmise that its presence in an oral secretion is in aid of prey subjugation. While laboratory tests of pharmacological mode of action do not absolutely prove the functionality or importance of these secretions, they strongly support it. Furthermore, the very specific mode of action of many toxins (e.g., potent postsynaptic neurotoxicity – Fry et al., 2003a; Lumsden et al., 2004a, 2004b, 2005; Pawlak et al., 2006), including prey specificity (Pawlak et al., 2009), makes a function other than prey subjugation very unlikely. The association of specialised (and highly variable) dentition with the glands (Fry et al., 2008) is also strong evidence of functionality, and although the venom apparatus of non-front-fanged snakes may be unable to inject large quantities of venom in a fraction of a second (Hayes et al., 1993), it is nevertheless capable of inoculating biologically relevant quantities of venom (Young et al., 2011). The fact that rapid degeneration of the venom system almost always occurs following dietary shifts (Fry et al., 2008) (including within 'truly venomous' taxa e.g. *A. eydouxii* – see above; as well as within non-front-fanged taxa such as *Dasypeltis* and *Pareas*) or specialisation towards constriction as the primary mode of prey subjugation (e.g. within the North American 'rat snake' radiation) also supports the role of venom systems, where present, in prey-acquisition. The rapid degeneration of the venom system following these shifts is in stark contrast to the accelerated duplication and diversification of toxin genes observed in many non-front-fanged snakes and non-helodermatid toxicoferan lizards (e.g. Fry et al., 2010a, 2008, 2006, 2010b, 2009c).

Large numbers of anecdotal observations of prey-handling (e.g. lack of constriction; 'chewing' until prey animal stops struggling etc. – see e.g. Endo et al., 2007) exist for many non-front-fanged species. The validity of these anecdotal observations is questionable but, as mentioned above, such observations are all that exists to verify the use of venom in the prey-handling behaviour of the vast majority of venomous taxa (including most elapid and viperid snakes and other venomous groups). If experimental evidence of the use of toxic secretions (and associated systems) in prey subjugation is required before the term 'venomous' can be applied to a taxon (Weinstein et al., 2011), then the majority of elapids and viperids must also be considered 'non-venomous' until proven otherwise (as these snakes also lack such stringent experimental proof). In cases where the biological role of non-front-fanged snake venom has been experimentally verified (e.g. Rodríguez-Robles and Leal, 1993; Thomas and Leal, 1993; Rodríguez-Robles, 1994; Mori, 1998; O'Donnell et al., 2007),

some authors prefer that these species be referred to as 'prey-specific venomous', rather than simply 'venomous', in order to clarify the fact that their venoms are not considered 'medically-significant' to humans (e.g. Weinstein et al., 2011). As previously noted, the effect of a secretion on humans is evolutionarily irrelevant and the vast majority of venomous animals are clinically inconsequential to humans (e.g. almost all spiders – see Sutherland and Tibballs, 2001). Venom is, therefore, likely to be 'prey-specific' to some degree as a general rule (even among front-fanged snakes [e.g., Jorge da Silva and Aird, 2001; Barlow et al., 2009]) and the addition of this qualifier in the case of non-front-fanged snake venoms is superfluous.

The argument for the continued use of the term 'Duvernoy's gland' centres on anatomical distinctions between the toxin-secreting glands of front-fanged and non-front-fanged snakes. This argument contends that, due to their lack of compressor muscles and large lumens for venom storage, the 'low-pressure' toxin-secreting glands of non-front-fanged snakes are functionally distinct from the 'high-pressure' venom systems of front-fanged snakes (e.g. Weinstein and Keyler, 2009). According to this argument, it follows from their functional distinction from 'true venom glands' that these 'low-pressure' systems are not venom systems. This argument is phylogenetically unsound as it obscures the fact that 'high-pressure' venom systems have evolved at least three times independently within the Caenophidia (Vidal and Hedges, 2002). Additionally, rudimentary compressor muscles are present in several non-front-fanged lineages and lumen size shows considerable variation across these taxa (as it does within the front-fanged family Elapidae) (McDowell, 1986; Fry et al., 2008). Regardless, we are unaware of any definition of 'venom' that specifies that the delivery system must be 'high-pressure'; such a restrictive definition would render even the lethal stonefish (*Synanceia horrida*) 'non-venomous'.

This anatomical argument appears to be mutually exclusive with the (also frequently cited as justification for referring to non-front-fanged snakes as 'non-venomous') argument centreing on 'medical significance' to humans. Some species of trivial (or hitherto undetermined) 'medical significance' to humans have 'high-pressure' systems with 'true venom glands'. Conversely, some that have caused fatal or life-threatening bites have 'low-pressure' systems lacking compressor muscles or even grooved fangs. Many Australian members of the front-fanged family Elapidae are widely considered 'harmless' (see e.g. Wilson and Swan, 2010) and have not been involved in any bites to humans with clinically significant sequelae. This is despite the fact that they (like all elapids) possess 'high-pressure' venom systems and are considered 'truly venomous'. On the other hand, the natricids *Rhabdophis tigrinus* and *Rhabdophis subminiatus* have been responsible for fatal (*R. tigrinus*) or life-threatening (*R. subminiatus*) bites (Weinstein et al., 2011), despite the fact that they do not possess even rudimentary compressor muscles and their posteriorly-located fangs lack any significant grooves (Fry et al., 2008; Weinstein et al., 2011). It is important to acknowledge that in most cases the effects of toxic secretions on humans are evolutionarily irrelevant to the animals concerned and that 'venomous' is not synonymous with 'dangerous to humans'. The aforementioned authors concede that the term 'venomous' may be applied to *R. tigrinus* and *R. subminiatus* (as well as *Dispholidus typus* and *Thelotornis kirtlandii* – see Weinstein et al., 2011), yet continue to maintain that 'low-pressure' secretory systems are not 'venom glands'. It is also worth noting that helodermatid lizards, which are traditionally considered venomous, lack compressor muscles and therefore, according to the definition favoured by the aforementioned authors, lack 'venom glands'. We contend that a gland that secretes venom is, *ipso facto*, a venom gland.

The practice of adhering to a definition of venom that is based upon a demonstration of rapid prey death or incapacitation; danger to humans; or the possession of a 'high-pressure' venom system is flawed and outdated on a number of grounds: (i) it is impractical and illogical to use different terms for secretions, based on quantitative differences in their function, when these secretions contain homologous toxins secreted by homologous glands and injected via homologous teeth; (ii) this definition masks the single early evolution of the glands associated with venom delivery, which in turn risks confounding analyses of the importance of venom in toxicoferan evolution; (iii) the misleading contention that venom evolved on multiple occasions is potentially confusing and has the potential to mislead and restrict the process of drug-discovery by eliminating a large number of potentially valuable species of reptile from the enquiry; (iv) the contention that venom is restricted to front-fanged snakes (with few exceptions) has the potential to obscure the possible danger of bites from some species (e.g. *Thrasops*, *Macropisthodon,* large psammophiines etc.) and create a false sense of safety among keepers of many non-front-fanged species, particularly as no specific antivenom treatment is available for any non-front-fanged snakes other than *Rhabophis* and *Dispholidus.*

Instead, the growing realisation of the diversity of venom delivery systems and glands in toxicoferan reptiles constitutes an open invitation to functional morphologists, physiologists and toxinologists to avail themselves of the opportunity to investigate the function of this cornucopia of secretions and delivery systems in the life history of the animals. It is precisely the single origin of these glands and their secretions that renders the evolution of the venom apparatus of toxicoferans such a potential treasure-trove. The recent discoveries on the origins of venom and associated structures in snakes and lizards (Fry et al., 2003a, 2010a, 2008, 2006, 2009b, 2010b, 2009c, 2003b, Fry and Wüster, 2004; Vidal and Hedges, 2002, 2005; Vonk et al., 2008) have led to a paradigm shift in our understanding of the evolution of reptilian venoms. It is only reasonable that such paradigm shifts should also reshape our definition and concept of what constitutes venom. While the majority of these animals are but of trivial human medical importance, the recognition of the greater evolutionary diversity of venomous reptiles that exists brings to light the vast number of unstudied venoms for use in biodiscovery.

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Conflict of interest

None.

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