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Letter to the Editor

Response to "Replies to Fry et al. (Toxicon 2012, 60/4, 434–448). Part A. Analyses of squamate reptile oral glands and their products: A call for caution in formal assignment of terminology designating biological function"

We agree that the definition of "venom" has wide-reaching secondary consequences. Acknowledgement of the fact that many snakes previously considered "non-venomous" are in fact venomous may indeed alter the general public's attitude towards them. We have often been presented, by amateur and professional herpetologists alike, with the argument that the publication of data supporting the classification of these snakes as "venomous" may affect legislation and public opinion. Whilst we acknowledge this possibility, we reject it as justification for withholding our evidence or altering our interpretation of it. We strongly believe it is the duty of scientists to report the evidence their research uncovers along with a balanced interpretation of this evidence. Whilst it is undeniably true that interpretations of evidence may differ and regrettably true that some interpretations may be unpopular and misunderstood, we do not believe that these latter possibilities should deter researchers from publication. We are aware that the presentation of new evidence and the classification of additional species as "venomous" may have legislative consequences, particularly when this classification is misunderstood to mean "dangerous to humans". The example referenced by Weinstein et al. in relation to the study of cone snail toxins is not relevant to this discussion, however, because this legislation restricts conotoxins with particularly low LD_{50} amounts (i.e. those that are particularly toxic). Similar restrictions apply to other biological toxins, including many of bacterial origin. The labelling of a species as "venomous" has nothing to do with this legislation, rather it is the relative toxicity of individual venom (or poison) components that this legislation concerns. We are uncertain if Weinstein et al. (2012) are suggesting that obscuring the biological reality of the presence of toxins in the oral secretions of toxicoferan reptiles would be beneficial to toxinologists by avoiding the possibility that legislation restricting the study of these toxins will be enacted. If the presence of said toxins is not reported, how will toxinologists justify their research interest in these species? Further, a court of law would not be concerned with semantics, they would be looking strictly at relative toxicity. Using semantic justifications to avoid labelling a vial as venom could put a researcher in danger of violating laws such as the IATA regulations surrounding the shipping of toxins and other dangerous goods.

1. Functional morphology and terminology

In re-evaluating the definition of "venom", our intention has not been to prioritise evolutionary homology to the exclusion of functionality, rather it has been to incorporate recognition of the origins and homology of the "venom system" throughout toxicoferan reptiles into our understanding of this key concept. Further, it is our intention to demonstrate and recognise that the variations in the venom system evident throughout toxicoferan reptiles represent points on a continuum, rather than evolutionarily discrete systems. To clarify, the toxin-secreting oral glands of the iguanian lizards and the sophisticated high-pressure venom glands of front-fanged snakes are two points on the same evolutionary continuum. This system, which we call the "venom system", is present in different forms, which may differ functionally and/or mechanically between taxa. Although investigations are far from complete, sampling to date from a wide taxonomic range of species suggests that the venom system is ubiquitous in members of the Toxicofera. In some cases, the system may have functional roles other than prey subjugation and/or self defence, indeed we are investigating this possibility with regard to the "incipient venom system" of iguanian lizards. Iguanian lizards therefore, whilst in possession of a venom system, may not be "venomous" in the traditional sense. We have previously and explicitly made this point by defining the Iguania glands as "incipient venom glands" (Fry et al., 2006).

Our definition of what constitutes venom in the functional sense differs only slightly from the "traditional consensus definition" quoted by Weinstein et al. (2012).

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Our definition endeavours to include the myriad of different ways in which venom is used to facilitate feeding or defence and the myriad of different systems in which venom is produced. We have defined venom 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." (Fry et al., 2009a,b, 2012) Applied to reptiles, in consideration of evidence uncovered principally in the past decade, but also prior to that, this definition extends the classification of "venomous" beyond merely the front-fanged snakes and helodermatid lizards.

Although our knowledge of the dentition and oral secretions of non-front-fanged snakes (former members of the family Colubridae) is far from complete, a large number of taxa from a wide taxonomic range have been investigated in recent years, and our knowledge of the evolution of the venom system in this vast artificial assemblage has increased dramatically. The venom system exists in a variety of states within the non-front-fanged snakes, ranging from an apparently atrophied condition seen in constricting species to a well-developed venom delivery system complete with intraglandular venom storage capacity (i.e. a lumen) and rudimentary compressor musculature in species such as *Dispholidus typus* (Fry et al., 2008). Contrary to the assertion of Weinstein et al. (2012), however, there is little evidence to suggest that "the functions of these glands are notably diverse". It is agreed that the glands are morphologically and thus mechanistically diverse, but this is quite different from their being diverse in function. To repeat, there is little evidence to date that these glands are involved in anything other than the secretion of toxins involved in prey-subjugation, to suggest otherwise is merely speculation at this stage.

Weinstein et al. (2012) highlight three ways in which the oral glands/venom systems of non-front-fanged snakes differ "functionally" (again it is important to highlight that this describes mechanistic differences in function, not differences in functional role) from those of front-fanged snakes. These differences are not as clear-cut as Weinstein et al. claim. As we highlighted in our review, the venom system exists in various states of apparent specialisation in non-front-fanged snakes. The claim that "there is a substantial reservoir of glandular product in venom glands of front-fanged species" is incorrect as the venom glands of many elapid snakes contain no appreciable lumen and most venom storage is intracellular (Kochva et al., 1980; Fry et al., 2008). Indeed the lumina present in the venom glands of some non-front-fanged snakes (notably again *D. typus* – Kochva et al., 1980; Fry et al., 2008) appear to exceed in size those present in the venom glands of many elapid snakes. It is correct that all front-fanged species studied have compressor muscles associated with their venom glands and hollow fangs. However, as highlighted in our review (Fry et al., 2012), compressor muscles have evolved at least six times independently within the advanced snakes and, even with front-fanged clades, there is intra-clade variation in the degree of attachment of this musculature to the venom gland (McDowell and Cogger, 1967). Three of these independent evolutionary events are within the three separate clades of front-fanged snakes (the families Elapidae and Viperidae; and some members of the subfamily Atractaspidinae). It is not surprising that hollow fangs are often associated with compressor muscles as such dentition both requires and can take advantage of a high-pressure venom delivery system – it is likely that each of these two innovations (compressor musculature and hollow fangs) requires the other in order to be of greatest benefit in increasing the efficiency of the venom delivery system. The corequisite nature of these two innovations may help to explain why compressor musculature is rare amongst advanced snakes that do not possess hollow fangs. However, the development of compressor muscles may precede the development of hollow fangs. This possible evolutionary trajectory is suggested by three extant clades possessing rudimentary compressor muscles in the absence of hollow fangs. It is likely that the prior development of compressor muscles is necessary for the development of hollow fangs to be advantageous (i.e., in the absence of compressor muscles, hollow fangs do not increase the efficiency of the venom system, indeed they likely decrease it). This sequence of evolutionary events may explain the fact that hollow fangs apparently never occur in the absence of compressor muscles.

The above discussion pertains to differences in mechanistic function between gland types, not differences in functional role. Weinstein et al. (2012) have stated that low-pressure glands are not inconsistent with venom glands. We agree wholeheartedly and find it interesting therefore that they continue to discuss (mechanistic) "functional differences" between the glands of non-front-fanged and front-fanged snakes whilst disputing our use of the term "venom gland" to describe the former (e.g. Weinstein et al., 2011, 2012).

In their response (Weinstein et al., 2012) to our recent review (Fry et al., 2012), they have again stated that "these notable differences imply likewise different practical use of the respective gland products." This is a speculation with no supporting evidence. We too lament the fact that references to the venom delivery system of non-front-fanged snakes as "inefficient" or "weak" obscure the fact that these systems are in many species evidently efficient enough to aid in prey-subjugation. They are far from "perfect"; however, and may be reasonably described as "less efficient" in relation to the venom delivery systems of front-fanged snakes. This in no way denies their "distinctive nature and conservation". These systems are conserved in non-front-fanged snakes because they confer an evolutionarily-relevant survival advantage to their possessors by facilitating prey-subjugation. That they are not as effective in this role as the venom systems of front-fanged snakes does not "imply" that they have a "different practical use" any more than the fact that the legs of a lion do not allow it to run as fast as the legs of a cheetah implies that the legs of the former are not for running. The assumption that all systems shaped by evolution currently exist in a state that is "perfect" for their intended task is naive. The likelihood is that the mutations that have resulted in the coupling of hollow fangs and high-pressure

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venom glands have not arisen in clades other than the three highlighted above. Since hollow fangs are likely to be less effective (if not useless) in the absence of a high-pressure delivery system and high-pressure delivery is less effective (though not ineffective, as evidenced by *Dispholidus*, *Mehelya*, *Brachyophis* etc) in the absence of hollow fangs, non-front-fanged snakes typically have a low-pressure system with grooved/solid fangs that is "less efficient" that that of front-fanged snakes, but which is efficient enough to aid in prey-subjugation.

We agree that concluding active use in prey capture and/or self-defence requires supporting evidence. We maintain that such evidence exists, as highlighted in our review. Casual and anecdotal field and captive observations of the feeding behaviour of many non-front-fanged snakes have been made for years and have led to the perception of many of these species as "mildly venomous" (as highlighted by Weinstein et al. (2012) and by us in our review, the qualifier of "mildly" refers to the danger this venom poses to human bite victims, not to intended prey animals). This anecdotal evidence has been corroborated by examinations of the dentition (often specialised to some degree for venom delivery e.g., enlarged and/or grooved), and secretory products of the venom glands of these snakes. Pharmacological activity has been determined for some of these compounds, or inferred from homology with known toxins from other snake species (a standard practice in toxinology). It is regrettably true that most of this activity-testing has not been conducted *in vivo* in natural prey items, but this is standard practice in toxinology, where rodents and chicks are often used as proxies for natural prey items.

Again it is worth stating that when a toxin is recovered from the venom of a non-front-fanged snake which is homologous with a toxin known from the venom of another species of snake and when this toxin displays neurotoxic (for example) activity in a standard pharmacological assay, we "assume", based on all available *evidence*, that it is in fact a neurotoxin. When the secretion which contains this (as well as others) toxin is associated with an apparent delivery mechanism (i.e. an enlarged/grooved tooth), we "assume", based on all available *evidence,* and in corroboration with anecdotal reports of prey-handling behaviour, that this neurotoxin is involved in prey subjugation. This is standard practice in toxinology and we maintain that this is all the evidence that exists to support the (currently undisputed) venomous classification of the majority of venomous animals. For example, contrary to the assertion of Weinstein et al. (2012) that "*all* known venomous fish that possess spines and venom glands…*all* actively use these in clearly recognised self-defence", there is no experimental proof to support this statement. We "assume", based on the position of the spines and their association with a gland that secretes compounds of known toxic activity, that all venomous fish use these spines similarly, in self-defence.

2. Do anatomical and developmental origins strictly define "venom"?

Oral glands that secrete venom may indeed have additional functions. The example of the sub-maxillary salivary glands of shrews is appropriate in this respect. It is entirely plausible that the venom glands of snakes, both non-front-fanged and front-fanged, may have functions in addition to the secretion of toxins used in prey subjugation/self-defence. As highlighted by Weinstein et al. (2012), "antibacterial and/or anti-protozoal components have been found in venoms of viperids and elapids", implying that as well as functioning in prey-capture/defence against predators, venom glands may also help defend these snakes against pathogens. In their discussion of shrew submaxillary salivary glands; however, Weinstein et al. (2012) state that "if a gland can be shown to perform a function that that is wholly and solely dedicated to the production of venom, then the term 'venom gland' is functionally and biologically appropriate." These assertions appear to be contradictory. If the venom glands of front-fanged snakes secrete antibacterial compounds, they are not "wholly and solely dedicated to the production of venom." One of the plausible "alternative functions" (*in addition* than the production of venom) proposed by Weinstein et al. (and others) for the oral glands of non-front-fanged snakes (and toxicoferan lizards) is the production of antimicrobial peptides for the maintenance of oral hygiene. If indeed the venom glands of front-fanged snakes share this alternative function, does this suggest that they should no longer be referred to as "venom glands" according to the definition proposed by Weinstein et al.? Clearly not. We propose that a venom gland is a gland whose *primary*, but not necessarily *sole,* function is the production of "venom" (defined above).

It is our expectation that as investigations continue into the evolution of the venom system of toxicoferan reptiles, we will gain further insight into the possible significance of alternate roles (including production of antibacterial peptides) in the evolutionary history of this complex system. Our investigation of the incipient venom system of iguanian lizards is ongoing and is not limited to *Pogona* sp. but includes members of all three extant families currently recognised within this suborder. Contrary to the assertion of Weinstein et al, we did not state in our recent review (Fry et al., 2012) that iguanian lizards are "functionally venomous", rather we stated that they possess an "incipient venom system" (so-called because it is the system of which the venom glands of other toxicoferans are a part) with "no known functional or ecological importance." We stressed that it has no *known* functional role, although as stated our investigations of this system within this clade are ongoing. We do not feel that acknowledgement of the presence of an incipient (i.e. a system in a possibly ancestral condition) venom system in iguanian lizards, which may have a functional role in common with or separate from the homologous venom glands of other toxicoferans, "forces recognition of the 'venomous' nature" of these lizards. It is in no way our intention to "divert attention from other possible roles" of this system, indeed we are actively investigating these possible roles.

3. The komodo monitor, *Varanus komodoensis*

As observed by Auffenberg (1981), stated in our review (Fry et al., 2012) and echoed by Weinstein et al. (2012), the primary effect of komodo dragon bites is physical trauma. Our suggestion that toxins with demonstrated anti-coagulant and hypotensive properties contained in the oral secretions

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of *V. komodoensis* would compound the effects of their bites and result in increased blood loss and incidence of shock in no way contradicts this prior assertion of Auffenberg (1981). Indeed Auffenberg himself noted that goats prey bitten by komodo dragons rapidly went into shock, were unusually quiet and bled profusely for prolonged periods of time. This suggestion is corroborated by pharmacological and morphological data, including the intricate derived compartmentalisation of varanid mandibular venom glands and the capacity for storage of secretion within these glands (similar to the storage capacity of the glands of some front-fanged and non-front-fanged venomous snakes).

The continued references to feline and human saliva made by Weinstein et al. do little other than confuse the issue. Neither cats nor humans typically kill their prey by inflicting bites that result in major trauma and blood loss. The prey of cats is generally subdued by suffocation or by the severing of the spinal cord. This is strongly contrasted with a predator that kills by inflicting massive blood loss – anticoagulant and hypotensive compounds in the saliva of such a predator could not avoid contributing to the effect of its bite. This effect may have initially been the "accidental" result of compounds similar to those found in mammalian saliva, but the unavoidable contribution of these compounds to the subjugation of intended prey items would have resulted in a selection advantage for these lizards. Positive selection would then have acted upon the system, progressing it, over evolutionary time, to the stage (including compartmentalised venom glands with significant storage capacity) in which we find it today. This is an entirely plausible evolutionary scenario based on the best available evidence.

4. Medical relevance

Although Weinstein et al. (2012) state that they agree with our assertion that medical relevance to humans and the biological role of secretions are unrelated, this seems to contradict certain statements made in their otherwise excellent review of bites from non-front-fanged snakes (Weinstein et al., 2011). In the "summary and conclusions" section of the aforementioned review, the authors state that several members of the Dispholidini, as well as the natricids *Rhabdophis tigrinus* and *Rhabdophis subminiatus* are medically important and "meet the criteria for the term 'venomous'". The authors state that these snakes "actively use their venom in prey capture and/or anti predator defence" but do not provide any references in which experimental proof of this statement is provided. No prey-handling experiments demonstrating the active use of venom by these species appear to have been conducted, and thus it appears that this group has been singled out as meeting the "criteria for the term 'venomous'" largely based on their medical relevance to humans.

Elsewhere in the review, the authors discuss the (mechanistic) "functional" differences between "true venom glands" and "Duvernoy's glands". The glands of *Rhabdophis*, according to these criteria, are not "true venom glands", yet contradictorily these snakes "meet the criteria for 'venomous'" because of effects on humans. In our review, we highlighted the inconsistency in these statements. Our discussion of the irrelevance of "medical significance" to the biological role of venom in our recent review (Fry et al., 2012) however, was not directed solely at Weinstein et al. The object of this discussion was to address the continued misconception (amongst laypersons, legislative bodies and many herpetologists) that "venomous" and "dangerous to humans" are synonymous concepts. We take heart from the fact that other toxinologists are working to dispel this misconception (including Weinstein et al., 2012) but feel that some statements in recent reviews such as that highlighted above (Weinstein et al., 2011) may be easily misinterpreted as suggesting that medical significance to humans is relevant to the classification of an organism as "venomous". We appreciate the efforts of Weinstein et al. (2012) in clarifying their position on this matter but we do not consider danger to humans a "secondary consideration" in the classification of the biological role of a toxic secretion; rather, as stated above, we consider it to be wholly irrelevant. To state it quite clearly: our "definition/classification" is based on evolutionary homology and use in prey capture/defence; relative toxicity to humans is not a consideration.

5. Prey specificity and implications of related terminology

We reject the use of the term "prey-specific venom" as a classification for the secretions of certain snakes because, as stated in our review, venom is likely to be "prey-specific" as a general rule, not as an exception. The primary function of venom is prey-subjugation and thus venoms are likely to evolve for the specific subjugation of relevant prey items. As more venoms (and, more specifically, individual toxins) are investigated in this regard, it will likely be revealed that a degree of prey-specificity is common to them all. Rather than use "prey-specific venomous" or "not medically significant" to describe venomous species that are not dangerous to humans, we prefer to use the term "dangerously venomous" for species that are. The majority of venomous animals are not dangerous to humans and therefore those that are the exception to this general rule are deserving of the qualifier "dangerously". This is a question of more than economic use of language, however, because it is our feeling that referring to "harmless" venomous organisms as "prey-specific venomous" reinforces the misconception that "venomous" and "dangerous to humans" are synonymous. It is our duty as scientists to explain that venoms evolve for a specific biological role (i.e. they are selected for based on prey-specific subjugation qualities) that is unrelated to humans. Therefore although the suggestion that we use the qualifier "prey-specific" for non-dangerous (to humans) venoms is well-intentioned, it only serves to confirm amongst laypeople the incorrect assumption that the term "venomous", in the absence of a qualifier, means "dangerous to humans."

6. Biological roles provide evidence of venom functions

Contrary to the assertion of Weinstein et al. (2012) that *Thamnophis elegans* were swallowing rodents "alive and kicking" in a study of 1980 (Gregory et al., 1980), the

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authors of this prey-handling study stated that mice were "alive although often inactive". The full quote is: "the mouse was swallowed alive although often inactive, possibly because of exhaustion. Sometimes a mouse was apparently dead when swallowed, especially if its head had been held in the snake's mouth while struggling." (Gregory et al., 1980) At no point in this paper do the authors refer to mice being swallowed "alive and kicking". As the garter snakes in this study made no attempt to constrict, only to use their bodies in "handling mice" (in a similar fashion to many other venomous species that do not "constrict" but use their bodies to hold struggling prey whilst envenomating them), it is entirely plausible that mice were subdued by the use of venom and not as a result of "exhaustion" (attempting to subdue endothermic prey by exhausting them is unlikely to be a successful strategy for an ectothermic reptile). There has been much speculation in the literature (as well as in Weinstein et al.'s criticism of our review) about snakes "swallowing prey alive." It is highly likely that snakes often swallow prey that is alive, but has been partially subdued by venom/constriction/biting force. However, that snakes would routinely swallow dangerous prey items like a rodents or lizards without subduing them in some way is speculative and seems implausible at best. Not only would this likely lead to injuries for the snake, it begs the question that if such prey is so easy to swallow "alive and kicking", what selection pressure exists for the evolution of complex venom-delivery systems or powerful constriction? Since complex venom delivery systems exist in snakes, as does the facility for powerful constriction (a facility for which a snake must inevitably sacrifice a degree of mobility), we can surmise that swallowing prey "alive and kicking" is not favoured by evolution.

Although Weinstein et al., state that there is a "large body of documented observations" that describe the "biological use of venom by many, although certainly not all, front-fanged species", almost all the references they provide in support of this statement refer to crotaline snakes (pit vipers) alone. The statement that "many, but not all" front-fanged species have been experimentally investigated with regard to the biological role of their venom implies that a majority of front-fanged species has been examined in this way. The truth is that the vast majority of species (particularly those occurring outside the United States of America) have received no such examination. It is simply assumed, based on their possession of venom delivery systems and the presence of toxins in their venoms, and based on anecdotal observation, that these snakes, like the minority that have been investigated experimentally, use their venoms in prey-subjugation.

7. Reliance on venom and other prey capture strategies

It is not surprising that crotaline snakes are unable to capture prey effectively after the removal of their venom system. As stated by Weinstein et al. (2012), crotaline snakes are strike and release predators and therefore rely on venom delivered with the initial strike to subdue their prey. Viperid snakes in general have arguably the most effective venom delivery system of any venomous snake and this strike and release strategy has evolved alongside this system, presumably as a way of minimising the potential for injury to the snake during the subjugation of potentially dangerous prey such as rodents. Not all venomous snakes are strike and release predators; however, and in those species which strike and then hold fast to their intended prey, the option of constriction in addition to envenomation remains viable.

Similar studies to those conducted on crotaline snakes (e.g. Kardong, 1996) have not been conducted on elapid snakes such as *Pseudonaja* which are known to restrain prey bodily whilst envenomating it. If *Pseudonaja textilis*, the snake with the second most toxic venom (in lab mice – Broad et al., 1979) of any species yet tested, were found to be able to subdue prey even after its venom system had been removed, would we revise our classification of them as "venomous"? This appears to be what Weinstein et al. have suggested in their response to our review. They have repeatedly referenced the Rochelle and Kardong (1993) study in which it was apparently demonstrated that *Boiga irregularis* (a non-front-fanged snake) does not rely on oral secretions to subdue its prey, as evidence that the "Duvernoy's secretion" of this (and by extension other non-front-fanged snakes) species should not be considered "venom". As highlighted in our review, there are a number of problems with this frequently cited study. Firstly, as pointed out by Weinstein et al. (2012), the major neurotoxin present in the venom of *B. irregularis* has been demonstrated to be considerably more toxic to birds and lizards than mammals, perhaps even non-toxic to mice (i.e. it is "prey-specific") – this is strong evidence of its importance in prey subjugation (Pawlak et al., 2009). Since the 1993 study used mice as prey animals, this represents an "abstracted reality" in which the snakes may have been forced to utilise constriction to subdue a prey item for which their venom system was ineffective. Secondly, and perhaps equally importantly, it is unlikely that the venom system of the snakes in the 1993 study was effectively occluded. Rochelle and Kardong used an ingenious method of suturing closed the venom duct feeding the rear fangs of the snakes, and then sealing the suture with tissue glue. Whilst this may have effectively closed these ducts, it was subsequently recorded (Fry et al., 2008) that these are not the only ducts through which venom reaches the oral cavity of *Boiga* species. Like a number of other non-front-fanged species, *B. irregularis* possesses venom ducts that open directly into the oral cavity *in addition* to those which open into the fang sheath (Fry et al., 2008); thus, the snakes in the 1993 study may still have been able to envenomate their rodent prey. This fact, in conjunction with the use of a model animal highly resistant to the venom being tested, could account for the lack of difference in "time to death" between the control and experimental groups.

Numerous species of elapid snake have been documented to use "constriction" when subduing prey (Shine and Schwaner, 1985). The speculation presented by Weinstein et al. (2012) that "…highly venomous species likely use constriction for prey that may be less susceptible to their venom toxins" is, however, fanciful. Both *P. textilis* and *Notechis scutatus* have been observed using "constriction" whilst subduing rodents. The respective subcutaneous LD_{50} of these two snakes in lab mice is approximately

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0.041 mg/kg and 0.118 mg/kg (Broad et al., 1979) making them amongst the most toxic snakes to rodents yet examined. The speculation, therefore, that they utilise constriction to kill rodents because these prey animals are "less susceptible to their venom toxins" is implausible. Just as the use of venom has not been experimentally verified in the majority of venomous snakes, the use of constriction to kill prey has not been experimentally verified in these snakes. Indeed, the statement that these snakes use constriction to *kill* prey is an *assumption* based purely on anecdotal observations. Although it is entirely possible that constriction plays a part in the killing of prey animals for these snakes, it is equally likely that what appears to be "constriction" is in fact more akin to securing or holding the prey to allow for more effective delivery of venom. This likelihood was commented upon in the Gregory et al. (1980) study (referred to previously) in relation to garter snakes: "… use of the body in handling mice is almost certainly not true constriction as the snake rarely seems to kill the mouse and never does so quickly." It is possible that snakes with less effective (in relation to those of crotaline snakes) venom delivery systems need to secure their prey using their body in order to facilitate envenomation. This is likely true of many non-front-fanged species and also elapid snakes such as *Pseudonaja* sp., which have extremely short fangs. The fact that *Pseudonaja* sp. have often been observed "constricting" rodents and lizards may relate to the fact that their small fangs have difficulty penetrating the fur of mammals, or the scales and osteoderms of reptilian prey.

It is indisputable that true constriction, as utilised by pythons, boas and colubrids such as *Pituophis melanoleucus*, is a highly effective method of subjugating prey, which is why its evolution has been favoured in some clades above the evolution of a sophisticated venom delivery system. It is worth pointing out, however, that all known constrictors are relatively heavy-bodied, powerful snakes, unlike most *Pseudonaja* or *Boiga*. In many cases, snakes apparently sacrifice mobility in order to become particularly effective constrictors (e.g. pythons, boas) – if a slender elapid snake or non-front-fanged snake is an effective constrictor, what benefit do heavy-bodied constricting snakes gain that offsets their reduced mobility and increased vulnerability to predators? This is the important point implicit in the hypothesis of Savitsky (1980), referenced by Weinstein et al. (2012) that "slender morphology in advanced snakes likely favoured development of prey capture strategies other than constriction."

Contrary to the assertion of Weinstein et al. (2012) that the inappropriately-named *Coluber constrictor* (which in fact does not constrict) possesses "atrophied venom glands" and does not produce venom or "Duvernoy's secretions" we have previously reported the presence of a well-developed serous gland (venom gland) in this species, with a duct that opens directly into the oral cavity (Fry et al., 2008). In addition, we examined the venom of the congener *Coluber rhodorhachis* with LC/MS and noted peaks consistent with the molecular weights of 3FTx and other toxins (Fry et al. 2003a).

Although popular, the speculation that *Drymarchon corais* consistently swallows prey alive has not been experimentally verified and seems unlikely in the case of rodents which could potentially inflict considerable damage on the snake if swallowed "alive and kicking". The oral glands and secretions of *Drymarchon* have not been investigated but from reported observations it seems that plausible that they may subdue their prey by "powerful chewing" – Keegan (1944) reported that *Drymarchon* killed rattlesnakes by holding them down with a coil and "chewing" and "lacerating" the head. This may suggest that *Drymarchon*, and other snakes which have convergently evolved powerful jaws such as *Ptyas carinatus*, kill by inflicting physical damage associated with their large, solid teeth and considerable jaw pressure. This is consistent with dissections by us of *Ptyas* and *Pseudaspis* which revealed very small venom glands but extremely well-developed jaw musculature.

As noted previously, it is correct that certain venom components (e.g., crotamine) have been demonstrated to possess antibacterial qualities. It is therefore possible that one of the roles of the venom system is the maintenance of oral hygiene. The secretions of most non-front-fanged snakes, however, contain numerous toxin types for which no antibacterial activity has been demonstrated. Three-finger toxins (3FTx), for example, are abundant in the venoms of a number of non-front-fanged snakes. These toxins are typically neurotoxins which cause paralysis by interfering with neurotransmitter function. Purified 3FTx from the venom of *Boiga dendrophila* (Pawlak et al., 2006), *B. irregularis* (Pawlak et al., 2009) and *Coelognathus radiatus* (Fry et al., 2003b) plus crude venoms rich in 3FTx from a number of other non-front fanged snake species (Lumsden et al., 2004a,b, 2005) have been demonstrated to be potently neurotoxic to the natural prey (birds and lizards, i.e. they are "prey-specific") of these snakes, strong evidence of their role in prey-subjugation. As 3FTx have no known antibacterial, predigestive or lubricant properties, their presence and abundance in the venoms of many non-front-fanged snakes strongly supports the assertion that these snakes utilise venom in prey subjugation.

8. Conclusion: the need for evidence-based application of terminology

The line that separates "venomous" from "non-venomous" animals has always been indistinct. As we discover more about the evolution of this characteristic in certain clades of animals, our definition of what constitutes a "venomous animal" must be scrutinised. In light of recent discoveries pertaining to the evolution of venom in squamate reptiles, we have attempted to clarify our terminology and adapt it to reflect our current state of knowledge. We would also like to clearly highlight, for fear that it might be misunderstood in the debate over terminology, that we consider the recent review of bites from non-front-fanged snakes (Weinstein et al., 2011) to be a comprehensive and essential work. We welcome the discussion and debate instigated by Weinstein et al.; however, we feel that the repeated references to human saliva being toxic do not add to it, but only serve to confuse certain readers. Whilst it is true that human saliva contains many secretory proteins, it is composed of 99.5% water, with the other 0.5% consists of electrolytes, mucous, glycoproteins, enzymes and antibacterial compounds such as secretory IgA and lysozyme. In

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contrast, a study published in 1997 (Hill and Mackessy, 1997) investigated the protein concentration of the oral secretions of a range of non-front-fanged snakes and found concentrations ranging from 49.8 to 100%. Put another way, the protein concentration of human saliva averages approximately 1.5 mg/ml (Bonilla, 1972; Banderas-Tarabay et al., 1997) whereas concentrations of up to 288 mg/ml were recorded in the aforementioned study of non-front-fanged snakes (Hill and Mackessy, 1997). Thus human saliva is much less concentrated than the secretions produced by Toxicofera venom glands. Additionally, it does not contain hyper-mutated proteins with neo-functionalised activities. Further, as stated previously, humans do not kill their prey by biting. There is no evidence that humans have ever killed their prey this way and they are not part of an evolutionary continuum that includes species with well-recognised and highly specialised venom-delivery systems. Toxicoferan reptiles are equipped with a novel protein-secreting gland system that expresses novel multi-gene families which are evolving under the birth-and-death mode of protein evolution that is a characteristic of venoms; humans are not. We feel therefore that referring to humans as "venomous" would not be analogous to us referring to non-front-fanged snakes and certain Toxicoferan lizards as venomous (see our response to "Part B" for further discussion of this point). Although we expect and welcome continued discussion from our colleagues about the definition of "venom"; we respectfully request that no further reference to the unhelpful and distracting "human saliva analogy" be made.

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