Toxicon xxx (2012) 1–12

Contents lists available at SciVerse ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Letter to the Editor

Response to Jackson et al. (2012)

To the Editor,

We note the response of Jackson et al. (2012) to our collective replies (*Toxicon* 60, 954–963 and 60, 964–966) to their paper entitled, "The structural and functional diversification of the Toxicofera reptile venom system", Fry, B.G., Casewell, N.R., Wüster, W., Vidal, N., Young, B., Jackson, N.W.J. 2012; *Toxicon* 60, 434–448. Although their response contains numerous misinterpretations of our comments, as well as further speculation, we are only addressing a few select points that were germane to our reply to their criticisms of the terminology used in our book (Weinstein et al., 2011). Further opinionated debate on this subject can interminably clutter future issues of *Toxicon* with our respective interpretations without a mutually agreeable resolution. Therefore, our final comments are restricted only to the important issues, and this necessarily truncated response should not imply agreement with other comments made by Jackson et al. (2012), because as stated above, it is not desirable to perpetuate ongoing, lengthy debates without probable resolution.

It is important to note that we certainly feel, as we stated in our Replies, that Fry et al. (2012) have produced interesting data that warrant further research and independent confirmation. In this, we have not, as Jackson et al. (2012) have stated, "instigated" (a term with a negative connotation) this discussion. Rather, we originally included in our book (Weinstein et al., 2011) critical consideration of the terms "venom" and "venomous", and our expressed opinion that these terms were *currently* unsupported when used for many non-front-fanged colubroid snakes (NFFC) and some lizards (e.g. *Pogona barbata*). This was included because in numerous cases these produce oral products that have unclear biological roles. Thus, we stressed patience and the need for more precise use of these terms while fully recognizing that it is likely that many NFFC will eventually fit the biological definition of "venomous". Therefore, we opined that use of the term "venomous" is premature for many species where little is known about their natural history and/or oral products, as well as for those that do not seem to rely on "venom" neither for capturing/subjugating prey nor for self defense.

Fry et al. (2012) then included a number of critical comments about our views in their paper, and we responded with our Replies (Weinstein et al., 2012). We have considered this an interesting exchange of views on a topic that begs re-examination due to the expanding knowledge of the evolution, molecular biology and behaviour of many squamate reptiles. Our argument is not with the efforts of Fry et al. to bring new data on which emerging hypotheses can be developed and tested. Rather, our concern is with how their interpretation of their data is advanced prematurely as a "shifted paradigm", with their expressed opinion seemingly beyond critical discussion. Thus, as stated numerous times in our Replies: we have no objection to a change in the defined consensus of "venom", "venom gland" and "venomous" as long as there is reasonably sufficient verified, reproducible data encompassing the natural history of the relevant species that supports the change. We agree with the comments expressed by Fry et al. (2012) and Jackson et al. (2012) about the often *assumed* biological role assigned to many biologically active substances secreted by some animals. This practice should not be perpetuated any further especially with the risk of misapplication of information obtained using increasingly sensitive methods. Below, we have listed some specific comments and related discussion as our final response to those of Fry et al. (2012) and Jackson et al. (2012):

- Contrary to the assertions of Jackson et al. (2012), we have not, and never would, advocate the "withholding of evidence", and it is puzzling how they interpreted this from our comments. We strongly support the publication of original findings of scientific enquiry. Each of us has done the same and would not expect any researcher to act otherwise. However, our remarks did criticize the widespread presentation of *hypothesis* derived from those data in the guise of confirmed fact. There have been many published studies of NFFC Duvernoy's secretions/venoms that preceded the papers of Fry et al., in which the terminology used for these products has been inconsistent. Many of the related issues were

0041-0101/$ – see front matter
http://dx.doi.org/10.1016/j.toxicon.2012.11.005

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

delineated in our previous reply. It is our view that while there is an increasing body of data about these oral products, it is still *premature* to declare hypothetical interpretations as a new "paradigm". If further and preferably independent information verifies these interpretations, we would have no issue at all with the change in terminology and it's associations. The essence of our argument is the need for patience and accumulation of combined and independently verified evidence and its associated interpretations in order to synthesize the most accurate model encompassing these complex features of squamate evolution.

- The authors have clearly missed the point of our reference to *Conus* spp. toxins intended as an example of the consequence of statutory control that may result from viewing a given species as "venomous" or dangerous to humans. This was presented as an example of what can be an over-reaching interpretation of danger as there are many substances (including some venoms and toxins with low LD_{50}) that do not carry such restrictions. Many popular Internet fora already are replete with inaccurate comparisons of the medical risks between some front-fanged and non-front-fanged colubroid snakes (e.g. timber rattlesnakes, *Crotalus horridus*, and false water cobras, *Hydrodynastes gigas*) based wholly on the presence of toxins and experimental lethal potencies. Like it or not, legislators commonly follow popular information and impression when enacting laws involving animals. This can be significantly impacted by the premature presentation of hypotheses as fact prior to pragmatic evidence of associated/implied hazards. Again, we never stated or implied that any researchers, including the authors, should withhold or "obscure" evidence of toxins in the oral products of any squamate reptile. What we stated was that it is one thing to report finding toxins, and yet another to imply their biological role(s), or "basal" functions/toxicity without some verified evidence.
- Jackson et al. (2012) state that in "their re-evaluating the definition of venom" they have not sought to "prioritize evolutionary homology to the exclusion of functionality". Of course, we disagree with this wholeheartedly as this foundation is a major basis for their describing an entire clade of squamates as the "Toxicofera". Wouldn't it be preferable to manifest a measure of patience when assessing and interpreting the data? As we have already stated, contrary to the comments of Jackson et al. (2012), we noted the interesting nature of the authors' collective data and never stated that it shouldn't be published. We also commented that it is every scientist's right to *argue* in favour of a new consensus, but not to declare *imposition* of that consensus without patient and thorough consideration from a broad interdisciplinary review. Shifting models, paradigms, which result from the acquisition of new knowledge, constitute the very nature of scientific endeavour. But, the fact that the authors insist in advocating the presence of "venom" in Iguanian lizards without any reasonable evidence of biological role suggests strongly that they are using the simple presence of toxins and/or their transcripts and phylogeny as their basis for inclusion under the definition of "venom". We agree that the identification of biological roles/functions of the Iguanian oral products would be most valuable and could clarify the use of a natural historically and evolutionarily correct terminology. Also, as we indicated, we welcome the considered efforts of Fry et al. (2009a,b, 2012) to more accurately describe the defining features of "venom". However, their additional defining criteria do not improve the use of the term any more than that of the traditional definition. Inclusion of the statement, "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, 2012), doesn't distinguish the effects of venom on the feeding process anymore than that caused by trauma inflicted by a feeding colubrine snake that swallows it's prey alive (e.g. *Coluber constrictor* [Eastern or black racer], or *Drymarchon corais couperi* [Eastern indigo snake]), regardless of the presence of Duvernoy's glands ("venom glands") in these species. Hemorrhagic effects of physical trauma and shock obviously "disrupt normal physiological or biochemical processes" and can be identified in humans as damage-associated molecular patterns ("DAMPs"; e.g. Diebel et al., 2012). A prey item such as a frog, small rodent, lizard or snake seized in the jaws of a snake attempting to ingest it would receive proportionally significant physical trauma. In some cases, as Jackson et al. (2012) noted, the snake may preferentially grasp the head of the prey and repeatedly advance its jaws (see ahead). Just the substantial local trauma from this alone likely induces marked "disruption in normal physiological or biochemical processes". Fry et al. (2009a) also identify the oral secretions of some hematophagous animals such as desmodontine phyllostomid bats (vampire bats) as "venom". As this discourse demonstrates, perhaps the issue that is the crux of our respective dissent is the limitations of our language in its attempt to harness a broad and variable natural phenomenon. This emphasizes the need for renewed attention to the accuracy of such descriptions and the possible need for interdisciplinary re-evaluation of how the terminology is used.
- Jackson et al. (2012) misunderstood our comments regarding the diversity of function of the low-pressure glands of NFFC and those of front-fanged colubroids. Our point was that these glands *function* differently and this is apparent from their functional morphology that we then outlined. Contrary to the comments of Jackson et al. (2012), we stated that the biological role(s) of the products of the majority of these glands is (are) currently unknown. Therefore, it is speculative to assume prey-subjugation in these without supporting evidence. Jackson et al. (2012) suggest a kind of Epimenides paradox in that they are *assuming* prey-subjugation functions for toxins secreted by *all* of these glands, and view any other consideration as "speculation" even though their stated assignment of function is speculation as well. As we have stated repetitively, *we have no doubt that with further study many NFFC taxa will probably prove to be functionally "venomous"*. However, in order to assign this potentially

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

indelible label accurately into the foreseeable future, a reasonable body of supporting evidence should be provided that identifies the use(s)/role(s) of the oral product in the natural history of the relevant species, or at least representative members of a given genus.

- We agree that there are examples of some elapids that have comparatively limited venom reservoir capacity to those of others, but these species still possess high-pressure venom glands that are associated with fully enclosed, canaliculated dentition. This obviously sets them apart from almost all of the low-pressure glands of NFFC that are all associated with non-canaliculated dentition. Of course we recognize their shared evolutionary development and homology, but this doesn't clarify their different functional realities. We also indicated, as did Jackson et al. (2012), that there is also, unlike almost all other NFFC, the presence of appreciable storage capacity in the venom gland of *Dispholidus typus.* In his extensive study of the cephalic glands of 120 genera (180 species) of NFFC, Taub (1967) remarked that the glands of *D. typus* were different and distinctive from all others examined. One can find exceptions that likely represent evolutionary "tinkering" within many taxa. However, as we observed, many selective pressures influence these processes and thus phylogenetic investigations often provide "windows" into a given process, and thus commonly reach variable conclusions. This is why further confirmation of the authors' hypotheses should be evident prior to supplantation of "venom" and "venom gland" as traditionally defined, as well as inclusion of a saurian with "incipient venom glands" into a clade largely founded on the presence of shared classes of orally-derived toxins, their transcripts or venoms. The putative association of prior evolution of compressor muscles with hollow dentition does not establish a basis for the authors' premature terminology, as this is part of the basis of their hypothetical organization. Again, presumed function does not accomplish established functional realities. This is also why it is an anthropomorphic judgement to refer to the low-pressure glands of the NFFC that possess them as "less efficient", "inefficient" or "weak", in comparison to the high-pressure glands of front-fanged species, as low-pressure glands have been conserved in numerous lineages and thus appear to serve their function(s) adequately. Such terminology can be useful if qualified carefully (see ahead). Again, the authors' collective hypotheses are interesting and certainly contribute to a growing understanding of squamate evolutionary adaptation, but prematurely assert associations without greater functional evidence and independent confirmation of their hypotheses. With such confirmation and additional evidence, the basis for changed consensus definition would be biologically supported and would likely qualify as the new standard of reference.
- We clearly and repetitively stated that our objection in using the term "venom gland" for the low-pressure gland present in many NFFC is due to the *unknown biological role(s) of their products.* We have suggested that as there are notable differences in functional morphology between NFFC and front-fanged species, there is an implied difference in function(s) of the oral products, and that it is premature to name something by association, rather than by known function. This is not consistent with the scientific method. We never implied, or would have stated, "all systems shaped by evolution currently exist in a state that is "perfect" for their intended task". While it is obvious that "less than perfect" systems occur in nature, we cited Gans and Elliott (1968) specifically in order to establish that "less than perfect" systems occur within the relevant species under discussion, but that it is also judgemental and anthropomorphically subjective to use such terms as "less efficient", "inefficient" or "weak" without defined qualification (see ahead for an example of qualified use that makes biological sense). The conservation of these glands among many NFFC taxa suggests that these glands aid fitness of the species that possess them by providing their particular biological roles, and thus meet the bioenergetic cost needed to support their function(s). This may be prey lubrication, inhibition of pathogens present on prey, pre-digestion and/or subjugation/immobilization, etc, and the presence of ducts from these glands that open into the general buccal cavity (e.g. in *Heterodon* spp.), or open in the proximity of modified maxillary dentition, as well as the buccal cavity (e.g. in *Boiga irregularis*) can suggest any of these biological roles. These roles are unclear and undefined for many species, including some that are already common in the commercial trade/private collections. Again, the authors accuse us of speculation, when their entire interpretation is in itself speculative and our differences present on the basis of our refusal to *assume* the same function among all essentially on the basis of toxin presence without evidence of biological role(s). The comparison of the role of the legs of two great cats presented by Jackson et al. (2012) is unworthy of this otherwise intelligent discussion. On the aside, it is unclear what Jackson et al. (2012) are implying in their reference to the lamprophiine lamprophiids, *Mehelya* spp. (*Gonionotophis* spp., Kelly et al., 2011; African file snakes) and the monotypic aparallactine lamprophiid, *Brachyophis revoili* (Revoil's short snake). A wide buccal gaping capacity, multiple, deeply grooved and further modified posterior maxillary teeth and the semi-folded gland epithelium that provides some storage space for venom aid the venom delivery of *D. typus*. Aside from the possible presence of rudimentary muscle fibers on the glands of *Gonionotophis* spp. and *B. revoili* (e.g. Kochva and Wollberg, 1970; Underwood and Kochva, 1993), if Fry et al. (2008) and Jackson et al. (2012) are suggesting that these taxa have delivery systems comparable to that of *D typus*, the authors should also detail this evidence of similar functional utility in prey capture by these species.
- Our response to the comments of Fry et al. (2012) in which they compared the low-pressure glands of a *Synanceja* spp. to our comments about low-pressure glands in NFFC focussed on their inappropriate comparison between these. We emphasized the fact that all the known venom glands from venomous fish

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

functioned as low-pressure systems, while some colubroid snakes (front-fanged) have high-pressure systems, and others (NFFC) don't. While we agree that in some cases there is limited information of the biological role(s) of these systems in some fishes, well-studied scorpaenids, tetrarogids, trachinids and others commonly orient and erect their spines towards approaching animals (including humans). This has been noted by many observers (including one of the authors [SAW]) and recorded by numerous authors (e.g. Halstead, 1970; Williamson et al., 1996; Sutherland and Tibbals, 2001; Mebs, 2002) under wild, as well as aquarium conditions, and the experiences of victims of stings from many species have attested to the active use of these armaments in defensive modes. There are also reports of possible offensive use as well (e.g. in the air-sac catfish, *Heteropneustes fossilis* [fossil catfish]; and the trachinid, *Trachinus vipera* [lesser weeverfish] Halstead, 1957, 1970).

- The fact for example *in vitro* nerve-twitch assay may contribute to the characterization of a given component as a "neurotoxin" is fine and good. But, this still does not indicate what the actual role may be in the natural history of the animal possessing the toxin. Human saliva contains α-kynurenic acid, an excitatory neurotoxin that is a metabolic product of tryptophan metabolism. Do we humans use this in prey subjugation? Obviously not, but we do pre-digest our foods, and, while we agree that collectively snakes obviously use oral products in ways that differ from humans, some ophidian species do not show any active use of venom as the prey is swallowed alive, and often struggling. This includes some of the species that Jackson et al. (2012) and Fry et al. (2012) identify as "venomous", and additionally assert or assume the use of their venoms in prey subjugation and/or self-defense. The conservation of shared toxins between some NFFC and front-fanged colubroids may not consistently indicate their use/role, and/or may suggest prey capture strategies for different types of prey. We have already addressed this in our Replies and will not reiterate it here. But we will later briefly discuss further the objection of Jackson et al. (2012) regarding our use of these comparisons. This is not to say that we disagree about the prey-specific roles of some NFFC toxins (as we also noted in our Replies), and our objection, again, is in the over-reaching assumptions and impatience inherent in asserting shared functions for all identified oral product/venom components for all species, and assigning "basal activities" (Fry et al., 2012) to some of these based only on their known roles in human physiology. This impatience and "rush to judgement" is a major contributor to the premature assignment of the "incipient venom system" to Iguanian lizards on the basis of investigating a single species, *P. barbata* that has been noted to have several classes of toxins (including crotamine) or their transcripts in its sub-maxillary glands and/or oral products ("venom") (Fry et al., 2006). The authors should review our previous comments (Weinstein et al., 2012) more carefully, as we never stated that Fry et al. (2012) referred to the iguanian lizard "incipient venom system" as "functionally venomous". Rather, we challenged the use by Fry et al. (2012) of the term "incipient venom system", as there is no evidence of biological role; therefore, it shouldn't be termed a "venom system"! Thus, to use the term, "incipient venom system", inherently forces recognition of these lizards as having a "venomous nature", "incipient", or not.
- We did not "echo" anyone in our comments regarding the effects reported to date of the bites of Komodo dragons, *Varanus komodoensis*; rather, we cited the work of Auffenberg (1981) who reported the results of his extended observations of these lizards. While Jackson et al. (2012) remark that Auffenberg (1981) commented about the rapid prostration of animals bitten by *V. komodoensis*, this is not unusual for animals profusely bleeding due to laceration/traumatic rupture of major blood vessels, and the concomitant sequestration of blood in vital organs. At no time did Auffenberg (1981) express any opinion that this might be due to anything other than massive haemorrhage. This obviously will often lead to hypovolemic shock. The comments of Jackson et al. (2012) completely miss the point about our noting the presence of potent depressor effects of feline or human saliva injected into experimental animals. The intended point was that the oral products and/or glands of many animals, venomous and non-venomous, contain biologically active components that share properties. Their properties do not automatically indicate how they are used. How cats kill their prey has nothing to do with our central point; feline saliva contains depressor properties, as does human saliva, and that is the point. Fry et al. (2009b, 2012) emphasized their findings of hypotensive effects of *V. komodoensis* oral products ("venom") when administered to anesthetized rats. It is purely speculation to call *V. komodoensis* "venomous" on the basis that having such components in their oral products in the setting of inflicting massive physical trauma on prey justifies the classification of these lizards as "venomous". Tangible evidence is needed of "venom"-induced pharmacological effects in prey capture, regardless of their possessing shared genetic loci with related squamates. It is purely speculation to make such an assignment based on the presence of phylogenetically shared toxins and observations of bleeding induced by substantial traumatic injuries. Again, Jackson et al. (2012) miss our point in providing these examples and those of other biologically active components (some found in venoms) present in human saliva. Humans and felines are obviously non-venomous, and that is the point: using the presence of toxins or other biologically active components of oral secretions as a sole indicator of the "venomous" nature of a given oral secretion, "incipient" or not, can be patently misleading. In part due to the common origins of many genes and their subsequent duplication, the increasing sensitivity of genomic and transcriptome-based investigations suggests that one must also be increasingly cautious when interpreting these data. It emphasizes the need for a synthesized knowledge of the relevant animal's encompassing natural history, as well as its biochemistry and

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

molecular biology. This will most likely provide reliable evidence of *how the oral components are used*, and we feel that this is still essential especially in the case of relatively poorly studied, distinctive and endangered species such as *V. komodoensis*. There is limited knowledge of *V. komodoensis* ecology and ethology, and thus, further evidence of the specific role of these oral products in the life history of these lizards should be procured prior to applying the indelible label, "venomous". As we stated in our Replies, with such evidence, *V. komodoensis* would then merit recognition as a "venomous lizard".

- Our comments regarding the lack of medical relevance to the definition of "venom" were derived from the works of Minton (1974), Minton and Minton (1980), Russell (1980), Kardong (1996) and Mebs (2002), but it is clear that Fry et al. (2012) and Jackson et al. (2012) share this view. We never stated that the active use of venoms of the Dispholidines (e.g. *D. typus*, *Thelotornis* spp.), and the natricines, *Rhabdophis tigrinus* and *R. subminiatus*, was based solely on experimental data; rather, we stated that active use in some NFFC species has been observed and/or experimentally recorded. For instance, in our book (Weinstein et al., 2011) we included an observed example of a wild *Thelotornis usambaricus* clearly subjugating a chameleon with venom. We stated in our Replies our additional views on the mixed use of a term such as "venom" by Fry et al. (2012) in regard to perceived vs. proven risks of NFFC, and the lack of correlation between the given name of a given substance and its lethal potency for humans. We agree that it is desirable that toxinologists, herpetologists, physicians, and other medical professionals universally recognize the lack of relevance of medical effects in humans when considering the definition of "venom".
- We also agree with Jackson et al. (2012) that the term "venomous" is commonly misinterpreted to mean "dangerous to humans" among diverse popular, legislative, and even scientific circles. However, this is a reality that is not going to change anytime soon. Perhaps with the introduction of a selectively used term such as, "prey-specific venom", this will gradually change. This also would clarify that there are notable differences among the toxicities and hazards of venomous animals, as well as taxa-specific susceptibilities to the action of some of their toxins and that they shouldn't be equated. Our relevant reply was more directed at the insufficient comparison of this term as might be used for some NFFC with that for spiders as presented by Fry et al. (2012). Therefore, as we stated in our Replies, we do agree more than disagree with Fry et al. (2012) and Jackson et al. (2012) on this point, and thus will not belabour it further.
- Jackson et al. (2012) emphasized some of the observations of Gregory et al. (1980) in order to support their contention of venom use in *Thamnophis elegans* (mountain garter snake). Our point emphasized that the mice were alive when swallowed and struggled vigorously through the entire process, thus leading many of the snakes to attempt to control the grasped mouse with a loose "coil". Thus, *some* of the mice were "exhausted" and "inactive", as Gregory et al. (1980) commented, when swallowed, and Gregory et al. (1980) emphasized this in the sentence partially quoted by Jackson et al. (2012), "Most often, even when the snake had used its body to restrain the mouse, the mouse was swallowed alive although often inactive, possibly because of exhaustion" (Gregory et al., 1980). There was no association suggested with "envenomation" because the preponderance of the observations described a vigorous, ongoing struggle between the mice and the snakes that grasped them, often repeatedly prior to successful ingestion. Although we were simply employing a commonly used descriptive phrase for a struggle, "alive and kicking", Gregory et al., 1980 did indicate in their Materials and Methods that they used "kicking" and "breathing movements" as signs of murine survival during the restraint and swallowing efforts performed by the snakes in their experiments. We did not anticipate that use of this popular term would be confusing, but we recognize that it is important to stress precise terminology within this relevant discussion.

It is noteworthy that Gregory et al. (1980) also commented in the introduction section of their paper that, "…garter snakes usually just seize their prey and swallow it alive. As might be expected, garter snakes feed upon mammals only rarely". This is concordant with the observations of many other field investigators including some of us. Interestingly, one of us (SAW) chose to originally use this reference in our book and in the Internet discussion of this topic as mentioned in our Replies due to the higher proportion of mammal prey included in the diet of some populations of *T. elegans* ssp., and their behaviour that is associated with capture of such prey. Other *T. elegans* populations (e.g. some in Colorado) exhibit a form of constricting behaviour that is: employed when feeding on small rodents; lacks parallel coils such as are seen in powerful constrictors, and often succeeds in asphyxiating the seized rodent (De Queiroz and Groen, 2001). The constricting behaviour of *T. elegans* has been compared to that of *Pituophis catenifer* (gopher snake), and the constriction by *T. elegans* described as "inconsistent and inefficient" (De Queiroz and Groen, 2001). However, those authors carefully qualified the term "inefficient" in terms of "relative ability to reduce feeding costs" (De Queiroz and Groen, 2001). In our view, as this is a carefully qualified expression, this presents a tangible biological quantity that is directly relevant to the current considerations. De Queiroz and Groen (2001) reported that all of the mice in their study were killed by constriction and the protracted struggles between the snakes and the mice often resulted in the snakes being "chaotically thrown about". This is concordant with the observations of one of the authors (SAW) who noted similar behaviour in several captive specimens (3) of *T. e. vagrans* (wandering garter snake) maintained as part of a large colony of this species. Specimens fed neonate ("pinkie") mice rapidly seized and swallowed the mice alive, and, yes, kicking. On several occasions when these snakes were offered weanling ("hopper") mice, the mice were often readily seized and

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

a protracted struggle lasting for several minutes ensued. These snakes were also "thrown about", frequently rolling over multiple times. On several occasions, the mice escaped the grasp of the snake after a few minutes, and aside from mild physical trauma, appeared unaffected. These responses to a larger prey item led to discontinuation of inclusion of live "hopper" mice in the diet of these specimens.

Cundall and Greene (2000) considered prey restraint in snakes that used jaw adduction with or without loose coils and/or sections of the body along with physical trauma induced by impacting the grasped prey against nearby objects. They noted that success of this underived strategy depends on the ability of the ophidian adductor muscles to resist fatigue longer than the prey. As mentioned previously, this behaviour is exhibited by a significant number of species. The prey type also infers potential handling costs, and "Type II" prey (elongate, limbless and relatively heavy) may incur increased costs due to the greater difficulty in accomplishing subjugation (Cundall and Greene, 2000). Some advanced snakes feed frequently on a variety of small prey (Greene, 1983), and thus metabolic costs associated with capture of larger prey may be a contributing factor for the preference.

Thus, this is an example of a species (*T. elegans*) that can on occasion exploit a regionally plentiful food source by using what may be an early form of constriction. While Jackson et al. (2012) may "surmise that swallowing prey 'alive and kicking' is not favoured by evolution", there are many species that do this such as some thamnophiines (*Thamnophis* spp.) and colubrines (e.g. *C. constrictor*, *D. corais couperi* [Eastern indigo snake]). For exerting evolutionary perspectives, Jackson et al. (2012) profess a relatively restricted view, as although many ophidian species have evolved venom and it likely provides a greater biomass return (due to facilitating procurement of larger, heavier prey without severe retaliatory injury and/or protracted struggle) than might be its required expenditure in metabolic energy (e.g. theoretically needed to support the complex protein synthesis of all of the constituent components of venoms, the precise costs and selective implications of which may be taxa-specific and are still controversial, e.g. see Pintor et al., 2010), other species appear to select prey within their morphological proportions, swallow it alive and thus seek to more frequently eat a larger number of relatively smaller prey items. A number of these species are not surprisingly fast moving and physically strong. This prey size preference is complex as it has been also observed in some highly venomous snakes such as *Pseudechis porphyriacus* (red-bellied black snake), and in this elapid the smaller prey preference was postulated to be associated with either prey encounter rates, active selection of prey size and/or gape limitation (Shine, 1991).

Some colubroids use powerful jaw muscles such as the *intermandibularis* and adductors in order to retain the grip on struggling prey, possibly specifically targeting the head, and thereby potentially deprive the prey of oxygen during the struggle/deglutition process. It is worth mentioning here that we are well aware of the report by Keegan (1944) as cited by Jackson et al. (2012) in which Keegan described the feeding behaviour used by *D. c. couperi* when preying on crotaline viperids. In the brief report, Keegan (1944) focused on the *D. c. couperi* tendency to seek the head of the intended prey snake and inflict repeated 'chewing'. In some cases, the seized snakes escaped the grasp of the *D. c. corais* and were re-grasped. This behaviour has been noted in other reptile-eating species and has been considered to be possibly due to avoidance of injury (e.g. Maschio et al., 2010), directional deglutional stimulus via scale overlap (Greene, 1976), or generalization of a response to prey that are more difficult to swallow (De Queiroz and De Queiroz, 1987). We see no evidence in this of "venom" use, and the authors' employ their own speculation in inferring otherwise. While the metabolic cost of this prey–predator interaction may seem costly, these snakes physically overpower their prey as other ophidian species do as well. It is worth noting that there is no apparent justification for the presumed metabolic cost of maintaining an Iguanian "incipient venom system" without identifiable function. Again, we addressed this in our previous Replies and will not reiterate it further here.

As we have stated repeatedly, we are *not* denying that many NFCC may be producing oral products that could be consistent with "venoms". We have already acknowledged some that are venomous. We are stressing the need for increased caution and care when applying the term venomous for all of the reasons repetitively stated previously, and here again. For a brief example relevant to the discussion of *T. elegans*, again, we specifically chose the example of this taxon because among the *Thamnophis*, it is one of the few species that includes a proportionally greater amount of rodent prey in their diet. While there is no convincing evidence to date of "venom" function in *T. elegans* when feeding on rodents, it is certainly worth further investigation, as is the possible role of Duvernoy's secretions ("venoms") in capturing molluscs, a favoured prey item of a few species of *Thamnophis* such as some populations of *T. elegans* and *Thamnophis ordinoides* (Northwestern garter snake). As some malacophagous NFFC (e.g. *Dipsas* spp., *Sibynomorphus* spp., *Pareas* spp., etc.) are morphologically/behaviorally adapted for preying on molluscs such as slugs or snails (Gans, 1972; De Oliveira et al., 2008), and produce oral gland extracts that immobilize/kill slugs under experimental conditions (Salomão and Laporta-Ferreira, 1994), there may be evidence of "venom" function in some snakes that prey on this less-exploited food source, partly because they have adaptations that facilitate capture of these difficult to grasp and digest molluscs (Britt et al., 2009; Arnold, 1993). Some specimens of both *T. elegans* and *T. ordinoides* possess posterior maxillary teeth that have posterior ridges, a trait absent in *Thamnophis* from generalist feeding populations (including some *T. elegans* and *Thamnophis couchii*), that has been hypothetically ascribed to their malacophagy (Britt et al., 2009). Further research of the feeding habits of these species and their specified prey handling behaviours may illuminate some of the hypothetical functions of their oral products.

- As we commented, contrary to the comments of Fry et al. (2012) and Jackson et al. (2012), there are quite a few authors who have identified the biological use of

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

venom by front-fanged colubroids in prey capture/subjugation, and these have included viperids and elapids. We also qualified our statement in noting that these reports/comments certainly did not include all venomous snakes. Many classic works contain relevant observations and/or comments: e.g. a few others in addition to those cited in our Replies, Krefft, 1869 (sea snakes); Fitzsimons, 1912 (vipers); Ditmars, 1922 (elapid, specifically, *Micrurus* (*Elaps* Ditmars, 1922) *fulvius*, Eastern coral snake), as well as others. As we stated, there is a prominent emphasis on venomous taxa that are medically important. However, this unfortunate disparity doesn't alter the need for caution and avoidance of premature assignments of inference laden terms.

- Jackson et al. (2012) raise the scenario of rendering a specimen of the highly venomous *Pseudonaja textilis* (Eastern brown snake) venomoid and then question whether we would then revise its classification as "venomous". This is a specious argument as the whole basis of our example of *B. irregularis* (brown tree snake) rested on its use of its oral product during prey capture. The example was used in a discussion of *how* venoms/oral secretions are used differently by different species, especially those with canaliculated front-fangs and associated high-pressure glands, compared with those with mid- or posterior-maxillary teeth that may or may not be enlarged or modified (in the case of *B. irregularis*, they are both enlarged and deeply grooved) and associated with low-pressure glands. Therefore, we purposely chose to compare *two species that* Weinstein et al. (2011) *referred to as venomous* (*B. irregularis* and *Crotalus* spp.; we also referred to them as such) because these both use venoms but *rely* on them very differently.

The venomoid scenario raised by Jackson et al. (2012) also speaks to our brief discussion of the use of constriction by highly venomous elapids, a behaviour several of us have witnessed as well. We clearly presented the possible species-specific application of prey handling by these elapids (e.g. venom vs. constriction, or both) as speculation, and it was not "fanciful", just a clearly identified possible consideration of an atypical elapid behaviour. Jackson et al. (2012) also specifically interpreted our comments as distinctively separating the use of constriction for either rodents or other prey. However, we never said that constriction would exclusively be used for non-mammalian prey, or that it was used to "kill rodents". Rather, the intention was to suggest that the subjugation of poikilothermic prey such as anuran amphibians could be substantially more *reliant* on constriction, while this wouldn't have the *same* role when capturing rodents. It is obvious that the venoms of these species, *P. textilis* and *Notechis scutatus* (common tiger snake) are highly toxic to rodents, and rodents seized by these snakes often expire very rapidly; we never implied otherwise.

- We acknowledge an editing error that remained in our Replies that was contained in our comments about *C. constrictor*. While we stated that Fry et al. (2012) had described this colubrine species as having an "atrophied venom gland", we mistakenly named *C. constrictor* instead of the intended species, *Pantherophis guttatus* (Eastern corn, or red rat snake; incorrectly identified in the text of Fry et al. (2008), undoubtedly also due to an editing error, as "*Pituophis guttatus*"), that Fry et al. (2008) describe as having an "atrophied venom gland". We regret our retained typo, but we were well aware of the well-developed gland in *C. constrictor* for as we mentioned in our book (Weinstein et al., 2011), Taub (1967) described a well-developed Duvernoy's gland in *C. constrictor*, and Fry et al. (2008) further described it as well. But, this is another species that uses physical strength and body compression to "pin" its prey while simultaneously swallowing it alive and struggling (Ditmars, 1907, 1922; Conant, 1975; authors' personal observations). This doesn't change our intended point: many species without venom or constriction physically overcome a wide variety of prey. Having venom-producing capacity in snakes has been associated with an ability to successfully overcome larger, stronger prey without injurious reprisal along with the increased biomass benefit of a larger prey item that supplies a net advantage to the venomous snake (Greene, 1983). However, *C. constrictor* and some other studied NFFC (e.g. some thamnophiines) will feed opportunistically on larger prey, but most often rely on intake of numerous small prey items that are, again, overcome with physical force and forms of restraint (also see previous section regarding *T. elegans*) while being swallowed alive, and yes, often kicking. Detection in "venom" of *Platyceps* (*Coluber* Lanza, 1990) *rhodorhachis* of chromatographic and mass spectroscopic peaks with molecular mass "consistent with the molecular weights of 3-finger-fold toxins and other toxins" doesn't provide any evidence of how these putative toxins are used, and if their presence alone should qualify the oral product (s) of this species as "venom". Also, as *P. guttatus* clearly captures prey with powerful constriction, is it functionally accurate, or useful to refer to its maxillary gland as an "atrophied venom gland"? By this definition of Fry et al. (2008), it is no longer functioning as a "venom gland", regardless of its possible phylogenetic origin, and thus, wouldn't it best be differently named while recognizing its shared phylogenetic origins? This again speaks to the basis of our call for patience and further supporting evidence before assigning the term, "venom gland", "venom", etc. to these and other NFFC.
- While we agree that the line separating "venomous" from "non-venomous" animals has long been indistinct, premature interpretations of interesting phylogenetically based data further diminish any palpable separation between those animals with "venom" and those without. In this it must be noted again that we have not "instigated" this essentially convivial discussion/debate; rather, we noted and discussed in our recent book an emerging effort to redefine terms relevant to our analysis of NFFC bites. This resulted in the critical comments of Fry et al. (2012) that then led to our respective responses and the present exchange. Rather than possibly serving to "confuse certain readers", comparisons between orally-occurring toxins/venoms

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

of squamate reptiles and properties of saliva in mammals including humans may reveal important issues in presupposition and definition of "toxic saliva", "mild venom", and the nature of "venomousness". It is understandable that Jackson et al. (2012) object to this example as it suggests what can happen when over-emphasising the presence of toxins/biologically-active in a phylogenetically-based definition without some supporting information describing their fundamental biological roles. As we commented in our previously published Replies, we fully agree that humans are certainly not venomous, neither are cats, mice, guinea pigs or rats, and that is the very point we made. All of these mammals have salivary glands and/or saliva that contain either/and potent depressor agents such as kinins, proteolytic enzymes, bacteriostatic peptides, platelet-activating factor and its inhibitor, and in the case of humans, excitatory neurotoxins. However, as these are not used in a manner consistent with "venom", their presence alone definitely does not qualify the animals possessing them as "venomous". Although we also do not wish to berate the issue, Jackson et al. (2012) made several incorrect comments regarding some of the associated features of this comparison. We will only point out a few of these in the following bullet point.

- The protein content of human saliva, like that of many other animals, is quite variable. There is a wide range of protein content and associated salivary viscosity that is dependent on factors such as: orally-stimulated or non-stimulated salivary states, hydration status, constitutional condition, presence of medical co-morbidities, prescription medications, diet and use of street drugs and/or alcohol (Rudney, 1995; Banderas-Tarabay et al., 1997; personal clinical observations of SAW, JW). The authors of one of the papers cited by Jackson et al. (2012) clearly stated this in relation to the salivary samples measured from members of their Mexican population: "These findings could be associated to degree of nutrition, genetic characteristics and level of oral disease in our population" (Banderas-Tarabay et al., 1997). Ranges of variation in salivary protein concentrations are typically extensive, even when salivary source, stimulation status, flow rate, and assay methods are carefully controlled (Rudney, 1995). Studies evaluating the role of saliva in caries incidence among defined geographic populations would be advised to consider factors that may influence variation within sample populations (Rudney, 1995). As noted, laboratory determinations of human salivary protein content yields results that are influenced by methods and standards used. Measured protein levels in pooled human saliva can range between 0.74 and 65.5 mg/ml (Jenzano et al., 1986), thus reflecting notable differences in protein content of individual samples. Thus, it is perfectly acceptable to present an average value of human salivary protein content as long as one recognizes that this is a very loose point of reference due to its marked variability.

Jackson et al. (2012) also compare the protein contents of some NFFC Duvernoy's secretions/venoms with that of human saliva per their reference. Although they reference the protein content range of NFFC Duvernoy's secretions/venoms published by Hill and Mackessy (1997), they omitted other relevant published data. In an earlier study of chromatographic analysis and immunological properties of Duvernoy's secretions/venoms of several NFFC, Weinstein and Smith (1993) also reported the protein content of several taxa, and this ranged between 16.5 and 100%. Due to this notable variation, lethal potency studies of the crude secretions/venoms were conducted with both samples calculated by measured protein content, as well as by weight/volume for comparative purposes. The variability of Duvernoy's secretion/venom protein content among several species of NFFC was demonstrated by comparison of reported levels for a number of taxa (Weinstein and Kardong, 1994). Intraspecific protein content variability has been well documented for *B. irregularis*. Vest et al. (1991) reported 22.8% protein in the venom of *B. irregularis*; Weinstein et al. (1991) documented 100%, while Weinstein et al. (1993) reported a snake size-related range between 66% (large adult) and 100% (small), and Mackessy et al. (2006) further demonstrated this size- or ontogentically-related variability and reported a range between 47% (neonate) and 90% (large adult). Therefore, protein content exhibits marked variability among diverse species. Of course, we are detailing this in response to some of the incomplete comments of Jackson et al. (2012), and those remarks still missed our point in using these comparisons, as we indicated at the beginning of this section. Although it is unessential to continue on this further, we will point out, as an aside, that study of many human salivary proteins is far from complete, and we have not characterized the full spectrum of our oral proteins, nor whether any of these have undergone a wide variety of selection and recruitment. Certainly, several genes encoding common salivary proteins (e.g. amylase) are widespread among animals and in humans have undergone notable molecular variation (even if not hypermutation) due to evolutionary juxtaposition of inserted elements (Samuelson et al., 1996). It is well established that human pancreatic and salivary amylase genes are highly related, with similar intron/exon boundaries and 98% nucleotide sequence identity over their open-reading frames (Horii et al., 1987). The structure of these genes (e.g. differences in promoters and 5′ untranslated ends) are consistent with the theory that all five copies arose during evolution from a single ancestral gene through a series of duplications, with subsequent divergence of the promoter regions leading to differences in tissue-specific expression (Samuelson et al., 1996). This is an example of a human salivary protein that has been subject to notable mutation and has been expressed/recruited in different tissues.

Again, we used these comparisons as an example to highlight that patently non-venomous animals like humans still have oral products that have properties that can be alternatively interpreted without considering biological function. But, it is also fair to indicate that, as has been discussed, some venoms may have what is apparently a primarily defensive function. Jackson et al. (2012) correctly emphasize that humans do not, and likely have not, killed their prey using oral secretions,

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

although we have no definitive way of knowing if, in our distant past, humans ever used such behaviour in gathering prey or in active intraspecific aggression. Human oral toxins are present in our saliva, and some of these have unknown roles. As two of us (SAW and JW) (and many of our colleagues) have treated quite a few human bites, it must be observed that humans aggressively biting other humans in purported self-defense are all too common, and as we mentioned in our Replies, are among the more medically serious bites that commonly present. Also, humans *do* have modified dentition (e.g. incisors, molars) and salivary flow occurs in the immediate vicinity of the gingival sulci of the dentition, as well in the general oral cavity. Although the additional modifications appear to be for processing of different food types (e.g. molars for grinding hard shelled foodstuffs), perhaps incisors played other roles in our distant past aside from aiding mastication of foods using shearing forces. Modifications of feeding habits and/or a developing reliance on tool use are believed to be major contributing factors that resulted in reduction of tooth size in the family Hominidae (Molnar, 1971; Simons and Ettel, 1970; Washburn, 1959). As mentioned in our Replies, protein constituents may vary according to their respective human oral gland source/type (Rudney, 1995). In fact, it is a bit puzzling why Jackson et al. (2012) protest vehemently to these examples that are intended only to demonstrate the need for consideration of biological role (s), as well as phylogenetic relationship. It is relevant to consider that one of the authors (BGF) of Fry et al. (2012) and of Jackson et al. (2012) has advanced the idea that some primates (the prosimians, *Nycticebus coucang*, *Nycticebus bengalensis* [slow lorises] and *Nycticebus pygmaeus* [pygmy loris]) are "venomous" (the "only venomous primate" see: http://www.venomdoc.com/venomdoc/Venomdoc.html and http://nyexotics.blogspot.com.au/2012/05/dr-bryan-grieg-fry-venom-useless-pickup.html) based on the secretoglobins present in their brachial glands, whose secretion is anointed onto the comb teeth of these taxa (Hagey et al., 2007; one of the authors of this paper is BGF). These teeth are commonly used for grooming, and possibly to stimulate glandular secretion of pheromones and apply related odorants (Rosenberger and Strasser, 1985). Interestingly, Krane et al. (2003) reported extensive sequence similarity between a major component (18 kDa) component of the brachial gland secretion of *N. coucang* and two chains of Fel d1, the major allergen from the domestic cat (*Felis catus*), and concluded that this major protein in *N. coucang* secretion is an allergen. In discussing the proteins (particularly a 17.6 kDa species) they isolated from *N. pygmaeus* and *N. bengalensis* secretions, Hagey et al. (2007) noted that, "Similar to the dual functionality of cat allergen, a loris glandular secretion likely evolved as a communication molecule, and it is a *toxin* only for certain (incidentally) susceptible species, like humans" (our emphasis). They also stated, "In addition to being a *defensive toxin reservoir*, the strong-smelling secretion displays all of the components necessary for it to play an important functional role in olfactory communication" (our emphasis; Hagey et al., 2007). Thus, there is an intentionally inferred implication that the gland secretion is used defensively as a "venom" even though the natural history of most prosimians, including lorises, is notably incomplete. In this, Hagey et al. (2007) also appropriately cited several references that reported that loris bites did not prevent predation by snakes, birds of prey or great apes (orangutans, e.g. Utami and Van Hooff, 1997). They also noted the presence of possible pseudogenes among the human and ape (chimpanzee) genomes that may represent remnants of the loris brachial gland protein ("toxin") (Hagey et al., 2007). This could be interpreted as evidence of previous functionality of this protein, or a related molecular species, in other non-human and human primates. Hagey et al. (2007) also reference Wilde (1972) who reported a victim bitten by a *N. coucang* that experienced life-threatening anaphylaxis, but did not conclude that they were "venomous". Relevant to the secondary medical considerations associated with the term, "venom", Hagey et al. (2007) added that humans "have even died" from such bites, although there has been formal documentation (published as a brief abstract) of only one other loris bite (inflicted by *N. pygmaeus*), and it caused only mild, local effects (Kalimullah et al., 2008). The available evidence suggests that incidental hypersensitivity to an allergenic protein constituent of the gland is responsible for these very rare cases. It must also be noted that similarly serious anaphylactic responses have occurred after exposure to countless animal-derived products, and this obviously does not denote envenoming. For one example among many, anaphylaxis has occurred after a gerbil (*Meriones unguiculatus*) bite (Trummer et al., 2004). As we noted in our Replies, many animals, including some rodent genera, have kallikreins and other serine proteases, as well as other numerous biologically active proteins in their saliva and/or sub-maxillary salivary glands. Also, all of the reported "human deaths" from loris bites are derived from anecdotal reports without any substantive documentation. This is reminiscent of some cases of NFFC bites that have anecdotally and incorrectly been perpetuated in the literature as "fatal" (Weinstein et al., 2011). Such reports require careful evidence-based, clinically qualified analysis in order to determine their medical accuracy, prior to their perpetuation in the published literature.

Therefore, we never intended our human saliva example as a system directly comparable to that of squamates, but instead were emphasizing the need for careful consideration of biological role before applying the inference-laden term, "venom" and "venomous". The protests of Jackson et al. (2012) regarding this example missed our very basic point that was directed at simply calling for care in the use of specific terminology. Review of some of their work noted above suggests the importance of carefully using terminology and the avoidance of prematurely labelling any species as "venomous" without a clearer understanding of the animal's biology, life cycle and relevant natural history. Due to some of the puzzling remarks of Jackson et al. (2012) it is important to again emphasize that just because we are commenting that there is no current evidence that some animals (e.g. any primates) are venomous, this should not be interpreted to

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

mean that further research of this question is invalid and shouldn't be done!

Lastly, as we mentioned, other mammals produce oral secretions (venoms) that in some species (e.g. the short-tailed shrew, *Blarina brevicauda*) assist in the procurement of prey. Interestingly, as Fry et al. (2009a,b, 2012) are aware, there is a well-established structural and functional link between several venom toxins of *B. brevicauda* and that of the venomous helodermatid lizards, the Gila monster (*Heloderma suspectum*), and beaded lizard (*Heloderma horridum*; Kita et al., 2004; Ligabue-Braun et al., 2012). In particular, blarina toxin from *B. brevicauda* venom and gilatoxin from *H. suspectum* venom, both appear to be structurally similar toxic kallikreins that are derived from non-toxic kallikrein precursors, an example of convergent evolution (Aminetzach et al., 2009) involving recruitment of specialized serine proteases. We mention this very interesting example of functional evolutionary convergence in order to reinforce that closely similar toxins may develop in divergent animals, and the knowledge of how these are used is central in assigning terminology that reflects their respective function (s). It is important to remain aware that several human human glands/organs (e.g. pancreas, kidneys, apocrine glands, etc.), including the salivary glands (that alone express >12 forms of kallikreins), express human kallikrein 1, as well as over 15 other kallikrein and kallikrein-like species. Although it typically exerts its biological activity through release of lysyl-bradykinin (kallidin) from kininogen, its functional roles are biologically complex (e.g. regulation of vascular tone, coagulation, inflammatory mediation, enamelization, etc.), and may be tissue-specified (Yousef and Diamandis, 2001; Simmer et al., 2011).

Finally, we thankfully acknowledge the amiable comments of Jackson et al. (2012) about our book (Weinstein et al., 2011). Likewise, as we have stated, the collective works of Fry et al. are important and interesting contributions to the growing knowledge of squamate oral gland/venom evolution. Our collegial debate addresses a difference in the approach to the application and contextual use of emerging information that adds to the existing synthesis of knowledge about venoms and the condition of "venomousness". As Russell (1935) opined, there is no question that scientific conclusions and their associated "ingrained" realities are provisional and subject to dramatic change. Our main tenet remains that prior to broad adoption, these inevitable changes require repetitive observation, reasonable independent reproducibility, and, if applicable, broader synthesis with other phylogenetic investigations. It is important to approach a proposed paradigm shift with patience, diligence and a receptive attitude to critical discussion and formal review. It is preferable to have an interdisciplinary review (e.g. including evolutionary biologists, systematic herpetologists, toxinologists and physicians, etc.) because the information reaches conclusions about multiple aspects of squamate biology with broad implications. There already is good reason to conduct an informed interdisciplinary review of the terminology defining "venom" and "venomous" as discussed in our interesting debate. The importance of this is clear because of the contemporarily rapid integration of information into multiple levels of academic, private and public access through the plethora of print and digital media. This is only going to expand in the near and distant future. Therefore, it is important, as Kardong (2012) indicated in our collective Replies, not to prematurely advance terminology that may be destined for rapid obsolescence. Rather, it is best to carefully, methodically, and most of all, patiently, gather the most complete information possible and then synthesize the new defined model for the relevant natural phenomena. We look forward to the future information that Fry and colleagues, as well as other investigators will undoubtedly contribute to this essential feature of squamate evolution.

Acknowledgements

We thank Dr. David J. Bates for his helpful comments.

References

Aminetzach, Y.T., Srouji, J.R., Kong, C.Y., Hoekstra, H.E., 2009. Convergent evolution of novel protein function in shrew and lizard venom. Curr. Biol. 19, 1925–1931.

Arnold, S.J., 1993. Foraging theory and prey size-predator size relations in snakes. In: Seigel, R.A., Collins, J.T. (Eds.), Snakes: Ecology and Behavior. McGraw-Hill, New York, pp. 87–115.

Auffenberg, W., 1981. The Behavioural Ecology of the Komodo Monitor. University of Florida Press, Gainesville, p. 406.

Banderas-Tarabay, J.A., González-Begné, M., Sánchez-Garduño, M., Millán-Cortéz, E., López-Rodríguez, A., Vilchis-Velázquez, A., 1997. The flow and concentration of proteins in human whole saliva. Salud Publica. Mex. 39, 433–441.

Britt, E.J., Clark, A.J., Bennett, A.F., 2009. Dental morphologies in garter-snakes (*Thamnophis*) and their connection to dietary preferences. J. Herpetol. 43, 252–259.

Conant, R., 1975. A Field Guide to the Reptiles and Amphibians of Eastern and Central North America, second ed. Houghton Mifflin, Boston, p. 429.

Cundall, D., Greene, H.W., 2000. Feeding in snakes. In: Schwenk, K. (Ed.), Feeding: Form, Function and Evolution in Tetrapod Vertebrates. Academic Press, San Diego, pp. 293–333.

De Oliveira, L., Jared, C., da Costa Prudente, A.L., Zaher, H., Antoniazzi, M. M., 2008. Oral glands in dipsadine "goo-eater" snakes: morphology and histochemistry of the infralabial glands in *Atractus reticulatus, Dipsas indica*, and *Sibynomorphus mikanii*. Toxicon 51, 898–913.

De Queiroz, A., De Queiroz, K., 1987. Prey-handling behavior of *Eumeces gilberti* with comments on head first ingestion in squamates. J. Herpetol. 21, 57–63.

De Queiroz, A., Groen, R.R., 2001. The inconsistent and inefficient constricting behavior of Colorado western terrestrial garter snakes, *Thamnophis elegans*. J. Herpetol. 35, 450–460.

Diebel, L.N., Liberati, D.M., Ledgerwood, A.M., Lucas, C.E., 2012. Changes in lymph proteome induced by hemorrhagic shock: the appearance of damage-associated molecular patterns. J. Trauma Acute Care Surg. 73, 41–50.

Ditmars, R.L., 1907. The Reptile Book: A Comprehensive Popularised Work on the Structure and Habits of the Turtles, Tortoises, Crocodilians, Lizards and Snakes Which Inhabit the United States and Northern Mexico. Doubleday, New York, p. 472.

Ditmars, R.L., 1922. Reptiles of the World: Tortoises and Turtles, Crocodilians, Lizards and Snakes of the Eastern and Western Hemispheres. MacMillan, New York, p. 373.

Fitzsimons, F.W., 1912. The Snakes of South Africa: their Venom and the Treatment of Snake Bite. T.M. Miller, Capetown, p. 547.

Fry, B.G., Vidal, N., Norman, J.A., Vonk, F.J., Scheib, H., Ramjan, S.F.R., Kuruppu, S., Fung, K., Hedges, S.B., Richardson, M.K., Hodgson, W.C., Ignjatovic, V., Summerhayes, R., Kochva, E., 2006. Early evolution of the venom system in lizards and snakes. Nature 439, 584–588.

Fry, B.G., Scheib, H., van der Weerd, L., Young, B., McNaughtan, J., Ramjan, S.F., Vidal, N., Poelmann, R.E., Norman, J.A., 2008. Evolution of an arsenal: structural and functional diversification of the venom

system in the advanced snakes (Caenophidia). Mol. Cell. Proteomics 7, 215–246.

Fry, B.G., Roelants, K., Champagne, D.E., Scheib, H., Tyndall, J.D., King, G.F., Nevalainen, T.J., Norman, J.A., Lewis, R.J., Norton, R.S., Renjifo, C., de la Vega, R.C., 2009a. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annu. Rev. Genomics Hum. Genet. 10, 483–511.

Fry, B.G., Wroe, S., Teeuwisse, W., van Osch, M.J., Moreno, K., Ingle, J., McHenry, C., Ferrara, T., Clausen, P., Scheib, H., Winter, K.L., Greisman, L., Roelants, K., van der Weerd, L., Clemente, C.J., Giannakis, E., Hodgson, W.C., Luz, S., Martelli, P., Krishnasamy, K., Kochva, E., Kwok, H.F., Scanlon, D., Karas, J., Citron, D.M., Goldstein, E.J., McNaughtan, J.E., Norman, J.A., 2009b. A central role for venom in predation by *Varanus komodoensis* (Komodo Dragon) and the extinct giant *Varanus (Megalania) priscus*. Proc. Natl. Acad. Sci. U. S. A. 106, 8969–8974.

Fry, B.G., Casewell, N.R., Wüster, W., Vidal, N., Young, B., Jackson, N.W.J., 2012. The structural and functional diversification of the Toxicofera reptile venom system. Toxicon 60, 434–448.

Gans, C., 1972. Feeding in *Dipsas indica* and Dunn's paradox. Am. Zool. 12, 730.

Gans, C., Elliott, W.B., 1968. Snake venoms: production, injection, action. Adv. Oral Biol. 3, 45–81.

Greene, H.W., 1976. Scale overlap, a directional sign stimulus for prey ingestion by ophiophagous snakes. Z. Tierpsychol. 41, 113–120.

Greene, H.W., 1983. Dietary correlates of the origin and radiation of snakes. Am. Zool. 23, 431–441.

Gregory, P.T., Macartney, J.M., Rivard, D.H., 1980. Small mammal predation and prey handling behavior by the garter snake *Thamnophis elegans*. Herpetologica 36, 87–93.

Hagey, L.R., Fry, B.G., Fitch-Snyder, H., 2007. Talking defensively, a dual use for the brachial gland exudate of slow and pygmy lorises. Primate Anti-Predat. Strat. 2, 253–272.

Halstead, B.W., 1957. Weever stings and their medical management. US Armed Forces Med. J. 8, 1441–1451.

Halstead, B.W., 1970. Poisonous and Venomous Marine Animals of the World. In: Vertebrates, vol. 3. US Government Printing Office, Washington, D.C, p. 1006.

Hill, R.E., Mackessy, S.P., 1997. Venom yields from several species of colubrid snakes and differential effects of ketamine. Toxicon 35, 671–678.

Horii, A., Emi, M., Tomita, N., Nishide, T., Ogawa, M., Mori, T., Matsubara, M., 1987. Primary structure of human pancreatic α-amylase gene: its comparison with human salivary α-amylase gene. Gene 60, 57–64.

Jackson, N.W.J., Casewell, N.R., Fry, B.G., 2012. Reply to: Weinstein, S.A., Keyler, D.E., White, J. 2012. Replies to Fry et al. (Toxicon 2012 60/4, 434–448). Part A. Analyses of squamate oral glands and their products: a call for caution in formal assignment of terminology designating biological function. Toxicon 60, 954–963.

Jenzano, J.W., Hogan, S.L., Noyes, C.M., Featherstone, G.L., Lundblad, R.L., 1986. Comparison of five techniques for the determination of protein content in mixed human saliva. Anal. Biochem. 159, 370–376.

Kalimullah, E.A., Schmidt, S.M., Schmidt, M.J., Lu, J.J., 2008. Beware the pygmy slow loris? Clin. Toxicol. 46, 602.

Kardong, K.V., 1996. Snake toxins and venoms: an evolutionary perspective. Herpetologica 52, 36–46.

Kardong, K.V., 2012. Replies to Fry et al. (Toxicon 2012, 60/4, 434–448). Part B. Properties and biological roles of squamate oral products: the "venomous lifestyle" and preadaptation. Toxicon 60, 964–966.

Keegan, H.L., 1944. Indigo snakes feeding upon poisonous snakes. Copeia, 59.

Kelly, Christopher M.R., Branch, William R., Broadley, Donald G., Barker, Nigel P., Villet, Martin H., 2011. Molecular systematics of the African snake family Lamprophiidae Fitzinger, 1843 (Serpentes: Elapoidea), with particular focus on the genera Lamprophis Fitzinger 1843 and *Mehelya* Csiki 1903. Mol. Phylogenet. Evol. 58, 415–426.

Kita, M., Nakamura, Y., Okumura, Y., Ohdachi, S.D., Oba, Y., Yoshikuni, M., Kido, H., Uemura, D., 2004. Blarina toxin, a mammalian lethal venom from the short-tailed shrew *Blarina brevicauda*: isolation and characterization. Proc. Natl. Acad. Sci. U. S. A. 101, 7542–7547.

Kochva, E., Wollberg, M., 1970. The salivary glands of Aparallactinae (Colubridae) and the venom glands of *Elaps* (Elapidae) in relation to the taxonomic status of this genus. Zool. J. Linn. Soc. 49, 217–224.

Krane, S., Itagaki, Y., Nakanishi, K., Weldon, P.J., 2003. "Venom" of the slow loris: sequence similarity of prosimian skin gland protein and Fel d 1 cat allergen. Naturwissenschaften 90, 60–62.

Krefft, G., 1869. The Snakes of Australia; an Illustrated and Descriptive Catalogue of All the Known Species. Govt. Printer, Sydney, p. 125.

Lanza, B., 1990. Amphibians and reptiles of the Somali Democratic Republic: check list and biogeography. Biogeographia 14, 407–465.

Ligabue-Braun, R., Verli, H., Carlini, C.R., 2012. Venomous mammals: a review. Toxicon 59, 680–695.

Mackessy, S.P., Sixberry, N.M., Heyborne, W.H., Fritts, T., 2006. Venom of the Brown Treesnake, *Boiga irregularis*: ontogenetic shifts and taxa-specific toxicity. Toxicon 47, 537–548.

Maschio, G.F., Lúcia da C. Prudente, A., da S. Rodrigues, F., Hoogmoed, M.S., 2010. Food habits of *Anilius scytale* (Serpentes: Aniliidae) in the Brazilian Amazonia. Zoologia 27, 184–190.

Mebs, D., 2002. Venomous and Poisonous Animals. CRC Press, Boca Raton, FL, p. 360.

Minton, S.A., 1974. Venom Diseases. Thomas Publishing, Springfield, Illinois, p. 235.

Minton, S.A., Minton, M.R., 1980. Venomous Reptiles. Scribners, New York, p. 308.

Molnar, S., 1971. Human tooth wear, tooth function and cultural variability. Am. J. Anthropol. 34, 175–190.

Pintor, A.F., Krockenberger, A.K., Seymour, J.E., 2010. Costs of venom production in the common death adder (*Acnathophis antarcticus*). Toxicon 56, 1035–1042.

Rosenberger, A.L., Strasser, E., 1985. Toothcomb origins: support for the grooming hypothesis. Primates 26, 73–84.

Rudney, J.D., 1995. Does variability in salivary protein concentrations influence oral microbial ecology and oral health? Crit. Rev. Oral Biol. Med. 6, 343–367.

Russell, B., 1935. Religion and Science. Paperback Ed., 1997. Oxford University Press, New York, p. 272.

Russell, F.E., 1980. Snake Venom Poisoning. Lippincott, Philadelphia, p. 562.

Salomão, M.G., Laporta-Ferreira, I.L., 1994. The role of secretions from the supralabial, infralabial, and Duvernoy's glands of the slug-eating snake *Sibynomorphus mikani* (Colubridae: Dipsadinae) in the immobilization of molluscan prey. J. Herpetol. 28, 369–371.

Samuelson, L.C., Phillips, R.S., Swanberg, L.J., 1996. Amylase gene structures in primates: retroposon insertions and promoter evolution. Mol. Biol. Evol. 13, 767–779.

Shine, R., 1991. Why do larger snakes eat larger prey? Funct. Ecol. 5, 493–502.

Simmer, J.P., Richardson, A.S., Smith, C.E., Hu, Y., Hu, J.C., 2011. Expression of kallikrein-related peptidase 4 in dental and non-dental tissues. Eur. J. Oral Sci. 119 (Suppl. 1), 226–233.

Simons, E., Ettel, P.C., 1970. Gigantopithecus. Sci. Am. 222, 77–85.

Sutherland, S.K., Tibbals, J., 2001. Australian Animal Toxins: the Creatures, Their Toxins and the Care of the Poisoned Patient, second ed. Oxford University Press, Melbourne, p. 880.

Taub, A.M., 1967. Comparative histological studies on Duvernoy's gland of colubrid snakes. Bull. Am. Mus. Nat. Hist. 138, 1–50.

Trummer, M., Komericki, P., Kränke, B., Aberer, W., 2004. Anaphylaxis after a Mongolian gerbil bite. J. Eur. Acad. Derm. Venerol. 18, 634–635.

Underwood, G., Kochva, E., 1993. On the affinities of the burrowing asps *Atractaspis* (Serpentes: Atractaspididae). Zool. J. Linn. Soc. 107, 3–64.

Utami, S., Van Hooff, J.A.R.A.M., 1997. Meat-eating by adult female Sumatran orangutans (*Pongo pygmaeus abelii*). Am. J. Primatol 43, 159–165.

Vest, D.K., Mackessy, S.P., Kardong, K.V., 1991. The unique Duvernoy's secretion of the brown tree snake (*Boiga irregularis*). Toxicon 29, 532–535.

Washburn, S.L., 1959. Speculations on the interrelations of the history of tools and biological evolution. Hum. Biol. 31, 21–31.

Weinstein, S.A., Kardong, K.V., 1994. Properties of Duvernoy's secretions from opisthoglyphous and aglyphous colubrid snakes: a critical review. Toxicon 32, 1161–1185.

Weinstein, S.A., Smith, L.A., 1993. Chromatographic profiles and properties of Duvernoy's secretions from some boigine and dispholidine colubrids. Herpetologica 49, 78–94.

Weinstein, S.A., Chiszar, D., Bell, R.C., Smith, L.A., 1991. Lethal potency and fractionation of Duvernoy's secretion from the brown tree snake, *Boiga irregularis*. Toxicon 29, 401–408.

Weinstein, S.A., Stiles, B.G., McCoid, M.J., Smith, L.A., Kardong, K.V., 1993. Variation of lethal potencies and acetylcholine receptor binding activity of Duvernoy's secretions from the brown tree snake, *Boiga irregularis* Merrem. J. Nat. Toxins 2, 187–198.

Weinstein, S.A., Warrell, D.A., White, J., Keyler, D.E., 2011. Venomous Bites from Non-venomous Snakes: a Critical Analysis of Risk and

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005

Management of "Colubrid" Snake Bites. Elsevier Science, Oxford, p. 364.
Weinstein, S.A., Keyler, D.E., White, J., 2012. Replies to Fry et al. (Toxicon 2012 60/4, 434–448). Part A. Analyses of squamate oral glands and their products: a call for caution in formal assignment of terminology designating biological function. Toxicon 60, 954–963.
Wilde, H., 1972. Anaphylactic shock following bite by a 'slow loris', *Nycticebus coucang*. Am. J. Trop. Med. Hyg. 21, 592–594.
Williamson, J.A., Fenner, P.J., Burnett, J.W., Rifkin, J.F., 1996. Venomous and Poisonous Marine Animals: a Medical and Biological Handbook. University of New South Wales Press, Sydney, p. 504.
Yousef, G.M., Diamandis, E.P., 2001. The new human tissue kallikrein gene family: structure, function and association to disease. Endocr. Rev. 22, 184–204.

Scott A. Weinstein*, Julian White
Department of Toxinology, Women's and Children's Hospital, 72 King William Street, North Adelaide, South Australia 5006, Australia

Daniel E. Keyler
Department of Experimental and Clinical Pharmacology, College of Pharmacy, University of Minnesota, 308 Harvard Street, SE, Minneapolis, MN 55455, USA

Kenneth V. Kardong
School of Biological Sciences, Washington State University, Pullman, WA 99164, USA

* Corresponding author. Tel.: +61 08 8 161 4088.
E-mail addresses: herptoxmed@msn.com, venfraction@yahoo.com (S.A. Weinstein)

21 September 2012
Available online xxx

Please cite this article in press as: Weinstein, S.A., et al., Response to Jackson et al. (2012), Toxicon (2012), http://dx.doi.org/10.1016/j.toxicon.2012.11.005