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Letter to the Editor

Replies to Fry et al. (*Toxicol* 2012 XX, 1–15). Part A. Analyses of squamate reptile oral glands and their products: A call for caution in formal assignment of terminology designating biological function

To the Editor:

We read with interest the contribution entitled, “The structural and functional diversification of the Toxicofera reptile venom system” by B. G. Fry and colleagues. This review article recounts the previous contributions of the authors, presents the authors’ views of the terminology regarding “venom” and Duvernoy’s glands as well as the relevant application of the authors’ interpretations of their phylogenetically based data. The paper also includes dismissal of our *concerns* and/or recommended *cautions* about the premature, broad use of the term “venomous” when not supported by evidence of function. In their paper, the authors included some of our published views in total, while others were only partly stated and taken out of context, not mentioned, or in our view, misinterpreted.

While refutation of the views presented in the paper requires detailed argument, there are some fundamentals that arise from the paper. Firstly, at the centre of contention is the definition of “venom” and, by extension, “venom glands”. Not only is such definition central to the science of toxinology, but it also has important secondary consequences. If an animal is labelled as “venomous” this can affect the way it is considered by society, the restrictions that are placed on it and the study of the animal, and the attitude, at a community level, towards conservation of the animal. As some readers of *Toxicol* will know, at the governmental level there are increasing restrictions on the movement and study of anything labelled as a toxin or venom, most recently affecting the study of toxins from cone snails. Although the biological/functional definition of “venom” (see ahead) has nothing to do with medical relevance, the conversely inaccurate labelling of an animal as “non-venomous” can carry serious secondary consequences. These are practical considerations providing a meaningful perspective that cannot be ignored.

Secondly, there is the concept of science and the scientific method versus speculation or opinion that can become

embedded as scientifically accepted “fact”. As “evidence” will always be selective and partial, it is subject to interpretation based on both the views of the investigators and the selective data being considered by them. Due to these considerations, we recognize that the use of the term, “evidence”, including our employment of it here, has inherent limitations that require further information in order to ascertain a complete objective fact or concept.

Thirdly, there is the issue of experimental confirmation, the concept that any new discovery, before formal acceptance, should stand the test of independent experimental confirmation, and be reproducible. Consensus will evolve and change over time, as new information and understanding become available and individual scientists exercise their right to argue in favour of a new consensus. However, they should not, in our opinion, declare they have unilaterally developed a new imposed consensus and “abandoned” specific terminology without broad formal acceptance.

In our opinion the paper by Fry et al. (2012) is both premature and non-consensual in its attempt to redefine “venom”, based on its origin (i.e. phylogenetics) rather than by function. Many genes are common, in whole or part, across diverse taxa, doubtless reflecting evolutionary origins, but it is the way each organism has utilised the gene product, in a functional sense, that has traditionally determined the definition of venom and what is “venomous”. The limits we place on a definition clearly affect inclusion/exclusion criteria and rightly are the subject of scientific debate and consensus. Without such care in terminology, humans might be defined as “venomous” (see ahead).

Here, we present in detail our disagreement with Fry et al. (2012) regarding the definition of “venom” and “venomous”:

1. Functional morphology and terminology

There has long been controversy and animated debate among the scientific and medical communities regarding

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the definition of venom, venom glands, fangs and venomous snakes (e.g. Nicolson, 1874; Quelch, 1893; Stejneger, 1893; Ditmars, 1948; Minton and Minton, 1980; Minton, 1990; Kardong, 1996a; and many others). Many of these discussions have focussed on members of the former taxonomically artificial family, Colubridae, which incorrectly included approximately 65% of the world's extant snake species. This family is in the process of being re-evaluated and many taxa already have been re-assigned to different families and sub-families. There is little information about the dentitional features and oral secretion properties of a majority of these snakes, termed, non-front-fanged colubroids. As noted by Fry et al. (2012), the developmental homology between the respective high-pressure and low-pressure glands of front-fanged (elapids, viperids and the lamprophiids, *Atractaspis* spp. and *Homoroselaps* spp.) and some non-front-fanged colubroid snakes has been increasingly recognized for over 40 years. However, while these authors assume shared functions for both types of glands, the functions of these glands are notably diverse, and in many cases, are unclear or unestablished (Weinstein et al., 2011; also see Part B for further discussion of this integral issue). Some of the major differences are: (i) there is a substantial reservoir of glandular product in venom glands of front-fanged species, while those ("Duvernoy's glands") of almost all non-front-fanged species studied to date lack any comparably appreciable storage; (ii) all front-fanged species have notable muscle insertion in their venom glands (high-pressure systems) which is lacking in the majority of non-front-fanged colubroid glands (low-pressure systems); (iii) all high-pressure venom glands in front-fanged species are associated with hollow, or canaliculated fangs that function akin to a hypodermic needle, while the glands of those non-front-fanged colubroids previously termed, "rear-fanged" or opisthoglyphous "colubrids", are associated with only solid non-canalculated maxillary teeth that may or may not be grooved and are positioned variably mid- or posterior in the maxilla (Taub, 1967; McKinstry, 1983; Minton and Weinstein, 1987; Weinstein and Smith, 1993; Weinstein and Kardong, 1994; Kardong, 1996a, 2002; Mackessy, 2002; Weinstein et al., 2010, 2011).

The gland pressures differ markedly between front-fanged and non-front-fanged snakes, although in a handful of non-front-fanged colubroid species studied there is some slight augmentation of secretion pressures exerted by nearby anatomical structures. Some front-fanged species can produce intra-glandular pressures in excess of 30 psi, while the limited number of non-front-fanged species studied to date produce below 5 psi (Kardong, 2009). These notable differences imply likewise different practical use of the respective gland products. Gans and Elliott (1968) considered that Duvernoy's glands might represent an imperfect specialization for a current and possibly unknown function. The low-pressure system of non-front-fanged colubroids has on occasion, unfortunately, been termed, "inefficient", or "weak". This is a subjective judgement that denies the distinctive nature and conservation of the low-pressure gland in a large number of snake species. This may contribute to its true function(s) being overlooked. These terms have also been inappropriately

applied in relation to the ability of a given colubroid species to inflict a significant bite resulting in medical effects in humans. Thus, again the function has been mistakenly related to potential medical consequences to humans rather than a biological and physiological function for survival.

If evidence accumulates supporting the term, "venom", for a growing number of non-front-fanged species, and as has been stated in a recently published book (Weinstein et al., 2011) as well as previously published works that it likely will, it would be more functionally accurate to differentiate between high-pressure and low-pressure venom glands. The situation in colubroid snakes is notably different from that of other organisms that produce venoms as there is a marked difference in the way the gland products are stored and delivered among these snakes, and in some cases it isn't clear that they are delivered to grasped prey, or used in self-defence. Weinstein and Kardong (1994) commented that, in relation to probable prey-specific toxins in these secretions, "possibly, even shallow biting movements could produce immobility". Therefore, there is recognition of these possible subtle functions even in some of the non-front-fanged species with unmodified dentition (previously called, "aglyphous"). However, as has been repeatedly emphasized, concluding active use in prey capture or self-defence requires supportive evidence in the form of reproduced field or/and lab observations and *not* assumptions.

It should be stressed that there is full understanding of the position Fry et al. (2012) in advocating recognition of these glands as two variants of the same evolutionary processes, and that there is no rejection of or resistance to the use of the term "venom gland" to include both high-pressure and low-pressure systems if there were sufficient supporting evidence. Rather, we are advocating caution and patience regarding application of this term for the glands of non-front-fanged colubroids when the functions of the oral products of the majority of species have not been established.

The traditional consensus definition of venom is: "a complex substance produced in a specialized gland and delivered by an associated specialized apparatus that is deleterious to other organisms in a given dosage and is actively used in the subjugation and/or digestion of prey and/or in defense" (Minton, 1974; Minton and Minton, 1980; Russell, 1980; Mebs, 2002). According to this definition, biological function, that is how it is used, is a crucial defining factor. It is agreed as Fry et al. (2012) state that there have been very few studies assessing biological functions of oral gland products from non-front-fanged colubroid snakes (see Weinstein et al., 2011 for a review of some of these studies).

Contrary to the assertions of Fry et al. (2012), at no time was it stated that low-pressure glands were inconsistent with venom glands. Rather, it was stated that the *function of the products* (if known) should be taken in context with the gland morphology when assigning such a biologically robust, inference-laden term such as "venom gland" (Weinstein et al., 2011). In arguing that low-pressure should not be used as a characteristic relative to defining glandular function, Fry et al. compared the low-pressure

systems of non-front-fanged colubroids with that of a scorpaenid fish, *Synanceja* spp. (stonefish). The above comparison is inappropriate as *all* known venomous fish that possess spines and venom glands have low-pressure systems and *all* actively use these in clearly recognized self-defence. As *some* colubroid snakes use high-pressure systems and *others don't*, the functional implications are clearly different. As the functions of the oral products of many non-front-fanged colubroids are currently unknown or unverified, it is an *assumption* to essentially call all of these, “venomous”.

It is noteworthy that even studies of some proposed functions (e.g. pre-digestion of prey) of venoms of front-fanged species such as the Western diamondback rattlesnake (*Crotalus atrox*) have reached different conclusions (Thomas and Pough, 1979; McCue, 2007). This accentuates that speculation or assignment of function by simple association should be deemed unacceptable.

2. Do anatomical and developmental origins strictly define “venom”?

There is no inconsistency in applying the term, “venom”, to products that function per the traditional consensus definition regardless of the glandular source as there are other precedents of glands that are not universally called “venom glands”, but produce products considered venoms. For example, some insectivorous mammals (including those which feed on other mammals as well) such as shrews and the seriously endangered solenodons (all of the Order Soricomorpha) have sub-maxillary salivary glands that are sometimes called “venom glands”. Some shrews such as the short-tailed shrew (*Blarina brevicauda*) certainly produce venom effective in immobilizing insects and mammals (Tomas, 1978; Martin, 1981). However, these oral glands likely perform other functions such as those of typical salivary glands (maintenance of oral hygiene, etc.) and to identify them with a restrictive title denies the totality of their function.

Further, the embryological processes that resulted in these glands are likely closely similar to those for other mammalian salivary glands. However, it is the recruitment of several specific proteins and the way they are used that confers the additional functions of this particular mammalian oral product. Therefore, if an oral product is shown to function as “venom”, it should be termed as such without denying the other functions that the secreting gland may possess as would occur if using specific and generally restrictive terminology. If a gland can be shown to perform a function that is wholly and solely dedicated to the production of venom, then the term “venom gland” is functionally and biologically appropriate.

According to Fry et al. (2012) some members of the sub-order Iguania exhibit an “incipient venom system”, but the authors’ studies of the Iguania (which contains approximately 1600 + taxa; Uetz, www.reptile-database.org) are limited to only *Pogona* spp. While the detection of protein classes of components found among snake venoms or their transcripts in oral glands of bearded dragons (*Pogona* spp.) is interesting from an evolutionary perspective, it does not automatically classify these lizards as “venomous”.

Importantly, there is no clear understanding of the role(s) of these components in the oral secretions of these omnivorous lizards, and in many cases they have a strong vegetarian preference. Thus, to date, there is no evidence that these lizards employ these secretions in prey capture, pre-digestion, etc., and no natural historical data exist that might indicate such functions. Tangible evidence of function must be provided before formal assignment of the indelible label, “venom”. Despite this, the authors apply this label as they forthrightly state: “...the venom system has little or no known functional or ecological importance within the Iguania, only trivial diversification occurred within this lineage and thus this venom system is distinguished from all other Toxicofera reptiles...” (Fry et al., 2012). Yet, they later remarked that, “One of the difficulties with using function as a criterion for identifying a substance as ‘venom’ and a taxon as ‘venomous’, is the degree of subjectivity involved”. While it is agreed that there is subjectivity involved, the authors’ interpretations carry broad speculation as well as contradictory subjectivity. Unconditional acceptance of the authors’ hypothesis forces recognition of the “venomous” nature of a number of Iguanian and Anguimorphan lizards when there is no clear supporting evidence of oral secretion use consistent with the definition of venom (aside from the thoroughly documented venomous condition of the Anguimorphan family, Helodermatidae).

It is premature to insist on classifying these saurian oral glands as “venom glands”. Rather, it is possible that these glands are exapted (meaning, preadapted; producing recruited proteins that have previous functions newly adapted for developing roles; Gould and Vrba, 1982; Gould, 2002), and their products may actually have an unsuspected function notably different from that of “venom”. It is concerning that prematurely labelling such glands and their products without further corroborative evidence of their meaning in the totality of the organism’s natural history (as well as with a greater body of relevant molecular data), may be diverting attention from their other possible roles. The studies by Fry (2005) and Fry et al. (2006, 2007, 2009, 2012) offer useful and interesting glimpses into these features of squamate evolution, but these are just that; glimpses into a broader phenomenon with a more encompassing breadth. Many phylogenetic investigations reach differing conclusions partly due to the “window” defined by the methods and analyses used. Therefore, while these investigations are interesting and valuable, they are as a window looking out onto a larger “landscape”, and a given investigation does not provide an unobstructed view of the landscape in totality. Such an encompassing profile of a natural phenomenon should be assembled with diligent patience, and preferably by synthesis of multiple independent investigations that provide several perspectives, “windows”, of the greater landscape. Therefore, the authors’ collective data are interesting and valuable to the growing understanding of squamate glandular evolution, including that of venom systems, but their interpretations are premature and lack independent corroboration and analysis. These latter requirements are important features of ambitious scientific endeavours that require academic rigor in seeking to “shift

the paradigm” and incorporate new knowledge into the literature.

3. The Komodo monitor, *Varanus komodoensis*: Interesting data, but premature conclusions

Fry et al. (2009) described toxins and toxin transcripts in oral products of *V. komodoensis* and reported hypotensive effects of *V. komodoensis* oral secretions administered i.v. to anesthetized rats. This has led Fry et al. (2009, 2012) to speculate that these macropredators use “anti-coagulant toxins to increase blood loss and other toxins to induce hypotension and shock” in bitten prey. However, it must be noted that some early research demonstrated depressor effects of i.v. administered mammalian saliva in some experimental animals. For instance, feline saliva injected i.v. into cats had a potent depressor effect, and this may have been due to either salivary components or colloidal compounds (Gibbs, 1935). Similarly, filtered and centrifuged human saliva intradermally injected into rabbits resulted in rapidly increased capillary permeability (Levy and Appleton, 1942). Further, tissue kallikreins and renin have been isolated from various mammalian sub-maxillary glands (Bhoola and Ogle, 1966; Erdős et al., 1968; Chiang et al., 1968). Many tissue kallikreins liberate kinins (e.g. bradykinin) from kininogens, and thereby play an integral role in blood pressure regulation, while the renin-angiotensin-aldosterone system plays a major role in regulating systemic vascular resistance and blood volume.

Therefore, although the limited animal data of Fry et al. (2012) do not prove venom function in *V. komodoensis*, they can be included in an overall evaluation of the function of these products conducted with a measure of patience in order to correctly assign the label “venom”. Thus, the authors must test their *hypothesis* further before assigning an unproven function to the oral secretions of these lizards, as similar pharmacological activities can be found in the oral products of some animals that are clearly non-venomous by any reasonable definition. Also, the authors’ descriptions and imaging studies of the cranium and cephalic glands of these enigmatic lizards are interesting findings that are certainly worthy of further investigation especially regarding their functional morphology.

In regard to possible functions of these oral products, Fry et al. (2012) suggest that three-quarters of prey attacked by *V. komodoensis* succumb to exsanguination within “the first thirty minutes”. However, the authors then state that “another ten to fifteen percent of prey animals succumb within three to four hours” and the deaths of these animals are “facilitated by persistent bleeding from the anti-coagulant effects of the venom. This advantage results in significant selection pressure for the maintenance of physiologically-costly venom” (Fry et al., 2012). These are entirely speculative comments that require a good deal more confirmatory evidence both biochemically and observationally. For instance, assessment is required of possible susceptibility of bovine blood and its constituent coagulation factors to the procoagulant effects ascribed to these oral products (remaining cognizant of the possible low specificity of some of these assays), and sufficient observation of predator-prey

interactions is needed to support the effects as hypothesized in prey capture.

These authors also reject the popular notion (which was also almost wholly speculation) of *V. komodoensis* bite-delivered sepsis as a form of prey capture (Montgomery et al., 2002). It is agreed that this “microbial weapon” theory of prey capture by *V. komodoensis* is likely incorrect. However, Fry et al. (2012) assign possible life-threatening sepsis in retreating bitten adult buffalos that occurs as a consequence of extended submersion in fecally contaminated water because *V. komodoensis* “seem incapable of bringing down an adult water buffalo” (Fry et al., 2012). As noted by Bull et al. (2010), there is a paucity of natural historical information about these imposing lizards, and previous extended observations (Auffenberg, 1981) largely supported physical trauma and associated blood loss with prey capture without the implied venom-induced enhancement. It doesn’t require, as inferred by Fry et al. (2012), prior knowledge of the putative presence of venom in the species to have a trained field biologist recognize signs of an animal succumbing to a possible venom- or toxin-induced effect. With their large and serrated teeth analogous to a steak knife, bites from these lizards often inflict severe damage to major blood vessels. Interestingly, two bites inflicted on human victims by 1.0–1.2 m specimens caused uncomplicated, aseptic wounds (Auffenberg, 1981). Human fatalities from these lizards are very rare, but to date the scant handful of recorded cases have consisted of massive bleeding associated with severe trauma (see Auffenberg, 1981 and *The Guardian*, June 4 2007).

The predominantly speculative nature of assigning prey capture functions to the oral products of *V. komodoensis* does not provide evidence characterizing the tentative role of these substances in the natural history of these endangered lizards. Although the notable challenges in procuring oral secretions from these lizards are certainly recognized, characterization of these substances as “venom” that contains “procoagulant toxins” as well as other biologically active toxins requires further biomedical confirmation and well-documented observations of use in capturing prey, not simply speculation. The presentation of such data could establish a solid factual basis supporting recognition of *V. komodoensis* as “venomous”.

4. Medical relevance

In regard to the authors’ citation of our comments about “medical insignificance” of most of these non-front-fanged species, it is important to note that we have repeatedly and vigorously stressed that clinical relevance has nothing to do with the biological definition of “venom” (e.g. Kardong, 1996a, 2002; Weinstein and Keyler, 2009; Weinstein et al., 2010, 2011). It is necessary to reiterate, clinical effects have no basis in the primary definition of venom; medical effects are an accidental consequence of the intersection between humans and animals that produce secretions, which circumstantially prove to have toxicity for *Homo sapiens*. Therefore, this is only a secondary consideration distantly relative to the primary criterion. In relation to the question of medical relevance of many non-

front-fanged colubroid species, again, it has been thoroughly emphasized that most were not assessable due to a lack of objective and/or well-documented clinical data (Weinstein et al., 2011).

Fry et al. (2012) seem confused regarding their own repeated proviso that mirrored those of previous authors (Kardong, 1996a, 2002; Weinstein and Keyler, 2009; Weinstein et al., 2010, 2011) about the lack of medical relevance to the definition of venom. After repeating their exhortation, Fry et al. (2012) comment, "...the contention that venom is restricted to front-fanged snakes (with few exceptions) has the potential to obscure the possible danger of bites from some species (*Thrasops*, *Macropisthodon*, large psamophiines, etc.) and create a false sense of safety among the keepers of many non-front-fanged species...". Issue is taken with several aspects of this comment. In the recent book analysing the possible risks of bites from non-front-fanged colubroids, it was repetitively addressed that there is a need for caution with those species for which there were insufficient data available amenable to an evidence-based risk analysis (Weinstein et al., 2011). It was also clearly delineated that as the term "venom" is not determined by clinical relevance to humans, neither should another term such as "oral secretion", or Duvernoy's secretion be considered innocuous by default. Medical risks of any natural or manmade substance are not determined by terminology. In fact, many reptile hobbyist Internet fora and popular magazines contain much greater volume of the reverse, that is a greater use of disproportionate, or over-reaching exaggeration of perceived risks of common species that have no evidence-based hazard to humans per "toxic saliva", or "mild venom" (e.g. *Thamnophis* spp., *Hierophis* spp., and others; Weinstein et al., 2011). This is increasingly due to the functionally indistinctive use of the terms, "venom" and "venomous". Weinstein et al. (2011) also provided clear warnings about the need for caution in handling a number of taxa for which there were mixed quality or purely anecdotal information (including genera such as *Macropisthodon* and *Thrasops*), as well as due to the very limited body of reliable data regarding the medical risks of the vast majority of non-front-fanged colubroids. Unfortunately, non-medically qualified authors have published a large number of reports (primarily in non-clinically refereed journals with limited circulation) describing bites from some of these species, and this has definitely resulted in misinterpretation of the possible hazards posed by some taxa. Terming as "venomous" every snake species solely on the presence of prematurely defined oral glands and/or detectable toxins or toxin transcripts, doesn't provide a functionally accurate definition, or secondarily, a sound medical risk assessment.

Additionally, in regards to medical relevance, there are a number of other speculative comments included in Fry et al. (2012). As a single example among many, they identify C3 (complement component 3) of the cobra venom factor (CVF) "toxin class" as a venom protein with "basal toxicity" that causes "unregulated activation of the complement cascade, causing rapid and significant problems such as anaphylactic-type problems and/or tissue damage via hemolysis/cytolysis" (Fry et al., 2012). C3 of the complement cascade is activated by C3 convertase to C3a

(one of several anaphylatoxins generated in the activated cascade) and C3b (e.g. an opsonin, as it may bind to microbial membrane proteins thereby facilitating phagocytosis). C3a also is an auto-convertase, and it certainly plays a role in anaphylaxis as well as inflammation/neutrophil recruitment in human homeostasis. CVF plays a role in complement depletion in envenoming; however, the role of venom C3 in clinical envenomation is unestablished. In a study of *in vitro* activities of several *Micrurus* spp. venoms, Tanaka et al. (2012) suggested that the vasodilatory effects of anaphylatoxins resulting from venom-induced complement activation may assist in the spread of other venom components. However, this is speculation as is the putative assignment of "anaphylactic-type problems" to venom components of unknown clinical importance. Until and if supportive information about biological function and clinical data are obtained, such hypothetical assignments based on known roles of these substances in human physiology should be presented as purely conjecture, and not as an identified "basal toxicity".

5. Prey specificity and implications of related terminology

Fry et al. (2012) reject the proposed use of the term, "prey-specific venoms" as suggested by Weinstein et al. (2011) who considered the need for terminology that reflects the prey organism (e.g. lizards, birds, invertebrates, etc.) targeted characteristics of some studied non-front-fanged colubroid toxins. Although it is agreed that this may not be uniformly necessary, in some species (including some popular in private collections), this may be desirable in order to avoid incorrect perceptions of the lack of evidence-based medical importance of some non-front-fanged colubroids. In many cases, a qualifying appellation is already applied, such as the frequently amended statement, "not medically significant to humans", etc. Use of the more precise term would obviate the need for such variable qualifiers.

Among those species that have received some attention, whether from field studies or laboratory investigations, there are data that support prey-specific (especially lizards and/or birds) functions of oral secretions in some of these snakes. For example, field and laboratory studies have shown that the ringneck snakes (*Diadophis* spp.) produce venom that is used in the capture of ophidian prey (O'Donnell et al., 2007). Although medical considerations are not defining factors in this discussion, it is of growing importance for statutory as well as popular scientific purposes to emphasize the prey-specificity of non-front-fanged colubroid species for which sufficient evidence has accumulated (not assumed by association) to identify their low-pressure glandular product as "venom".

We disagree with the analogy offered by Fry et al. (2012) that compares terminology regarding "prey-specific venoms" used for spiders with that provisionally proposed for non-front-fanged colubroids. Although a number of spider species are maintained in captivity (the vast majority of these are mygalomorphs that generally are capable of inflicting only mild local envenoming), there are comparatively many more species of non-front-fanged

colubroids entering private collections. By specifying the prey-targeted nature of their oral products, this suggested term addresses both a biological property of many species that have had confused perceptions of the potential hazard associated with their bites, and their concomitant lack of danger to humans. It has been previously stated clearly that accumulating evidence of prey-specific use of oral secretions will support the applicability of the term “venom” for some well-studied species (see ahead; Weinstein et al., 2011). However, unlike other venomous organisms such as spiders in which all have closely similar venom apparatus with the major differences present in the position of fang deployment (vertical in mygalomorphs, horizontal in araneomorphs), the glands and delivery systems of non-front-fanged and front-fanged colubroid taxa are substantially morphologically different. Again, it is important to reinforce that there is no rigid resistance to any change of terminology; rather, emphasis is on the notable difference in function and the lack of clear evidence supporting the uniform use of non-front-fanged gland products among the vast majority of species. As Fry et al. correctly note, there is accepted recognition that several dispholidine and natriicine species are “venomous”. As was detailed in Weinstein et al. (2011), this is due to the active use of the gland secretions of these species in prey subjugation and/or self-defence. Contrary to the comments by Fry et al. (2012), neither Weinstein and Keyler (2009), nor Weinstein et al. (2011) ever stated that “rapid prey death” was a necessary, or even desirable, component in the definition of “venom”. However, there are alternative insights of this perspective relative to the academic discussion of snake venoms alone (see Part B and Weinstein et al., 2010). Scientific analyses require reproducible evidence supportive of a given hypothesis, not assumption. We obviously realize that it is unrealistic to procure proof of prey subjugation/defence for every species found with orally derived toxins, but it is unacceptable to assume evidence in the absence of any. Such an assumption is not consistent with the scientific method.

6. Biological roles provide evidence of venom functions

As stated previously, it should be re-emphasized that a growing list of non-front-fanged colubroid oral secretions will probably be recognized as “venoms” as more data are accumulated about the biological roles played by them. At present, sufficient information is available to demonstrate that a number of non-front-fanged species use oral secretions in a manner consistent with the robust consensus of venoms as presently defined. These include: ringneck snakes (*Diadophis* spp.), the Puerto Rican racer (*Borikenophis [Alsophis] portoricensis*), the green vine snake (*Oxybelis fulgidus*), and probably the Mangrove snake (*Boiga dendrophila*), as well as the brown tree snake (*Boiga irregularis*) [see Weinstein et al., 2011 for a brief review of the evidence for these]. In none of these cases, is “rapid prey death” a relevant criterion; instead, there are accumulating well-recorded observations in field and/or lab studies that report subjugation/tranquilization of seized/grasped prey. In some cases, such as that of *B. dendrophila* and *B.*

irregularis, there are prey-specific toxins that have been characterized from their respective venoms. However, the presence of these toxins in some squamate reptiles does not automatically endow them with the same use in all species. Fry et al. (2012) cite a study of *Thamnophis elegans* (mountain garter snake) prey handling by Gregory et al. (1980), as did Weinstein et al. (2011). As stated earlier, we wholeheartedly disagree with Fry et al. (2012) that just because the study by Gregory et al. (1980) pre-dated some of the present knowledge of venom evolution, that they would have reached any other conclusion than they did: *T. elegans* in their study were swallowing their small rodent prey alive and kicking. Some of the specimens observed also attempted to throw a loose “coil” over the seized mice in order to aid their deglutition. Thus, *T. elegans* showed no evidence of actively subjugating prey by using an oral product. In fact, this comment in the paper by Fry et al. (2012) appears derived from a presentation of this reference by one of us (SAW) in a forum discussion on this topic with one of the authors (WW) of Fry et al. (2012) [see <http://www.fieldherpforum.com/forum/viewtopic.php?f=2&t=7572&hilit=severe+garter+snake+bite&start=25>].

Also, the possibility of “venom” in *Thamnophis* spp. and many other common “colubrid” species was under consideration long before the contributions of Fry et al. By the mid-1970’s active discussion was already underway about the potential toxicity and possible associated medical significance of oral secretions of many of these snakes (Minton, 1976; McKinstry, 1978; also see references in section 1). Taub (1967) had also previously described a serous Duvernoy’s gland in three taxa of *Thamnophis*, and Kochva (1965) concluded that there was a primordium common to the “venom gland” and posterior maxillary teeth of the Mediterranean or European cat snake (*Telescopus fallax*), and that of comparable structures in *Thamnophis* spp.

One paper by Finley et al. (1994) suggested possible depilation of a vole (*Microtus* spp.) while being swallowed by a wandering garter snake (*T. elegans vagrans*). This could be interpreted as possible pre-digestion. Thus, further observations and confirmatory evidence could provide some clues regarding the use of the Duvernoy’s secretion of this species. However, to our knowledge, and in our own combined experiences, there are no documented data that show any “venom” effects induced in prey by a *Thamnophis* spp., or that have been used in defence. This includes personal observations by two of us (SAW, DEK) of prey handling by several thamnophiines in which both were well aware of the theoretical use of oral products in subtle toxin-induced assistance in prey capture/ingestion, but observed no indication of this. Yet, according to Fry et al. (2012), *Thamnophis* would be considered venomous as are some iguanian lizards that have “venom systems” with, as previously noted, “...little or no known functional or ecological importance...” (Fry et al., 2012). Or could it be that the terminology is premature and the authors’ interesting body of data should be subject to further testing, independent studies, and considered interpretation by other groups?

Science advances with formulation and testing of hypotheses, followed by independent reproduction and testing of the results. It does not move forward by singular

opinion/interpretation that includes assumption and speculation without further verification, no matter how relevant it may seem to other information. Again, it is not being stated that the terminology describing venom over time will remain unchanged. In fact, it may be desirable to modify the consensus definition of venom as greater knowledge of its evolutionary history and associated functions are accumulated. But, it is most important not to prematurely use terms that are biologically unproven especially as these carry profound functional, and secondarily, medical and legal implications. In contrast with the assertions of Fry et al. (2012), there is a large body of documented observations that have historically described the biological use of venom by many, although certainly not all, front-fanged species, and of course most of these are coincidentally medically important (e.g. Mitchell, 1861; Stejneger, 1893; Ditmars, 1907; Wall, 1921; Klauber, 1956; Minton, 1969; Minton and Minton, 1980; many others). Some of these observations, such as those of *Crotalus* spp. by the well-known Philadelphia physician, Silas Weir Mitchell (1829–1914), also described fang deployment and venom delivery in fine detail (Mitchell, 1861). Thus, this is a result of increased attention paid to those more commonly encountered taxa that impact human health. Although this is an unfortunate disparity, it does not change the need for methodical and scientifically supported terminology.

7. Reliance on venom and other prey capture strategies

For a stark example of the reliance of some ophidian species on venom function, one may consider prey capture strategies of some pit vipers (family Viperidae, subfamily Crotalinae). Disruption of the venom delivery system of a strike and release venomous snake such as a rattlesnake species (genera *Crotalus* and *Sistrurus*), leads to an inability of the snake to capture prey and thereby results in a marked selective disadvantage and probable loss of fitness (Kardong, 1996b). By contrast, disruption of the Duvernoy's gland ("venom gland") of a non-front-fanged colubroid species such as the brown tree snake (*B. irregularis*) does not prevent this species from procuring prey (Rochelle and Kardong, 1993). Thus, the relative roles of venom in the natural history of these species are obviously quite different and in the latter taxa, adaptation of constriction provides a means of prey capture absent in the aforementioned crotaline genera. Some Australian elapid snakes such as *Pseudonaja* spp. and *Notechis* spp. (brown snakes and tiger snakes, respectively) utilize constriction for selected prey (Shine and Schwaner, 1985), but certainly do not show any detectable "atrophy" in their venom glands as Fry et al. (2012) suggest has happened to some squamate clades. These highly venomous species likely use constriction for prey that may be less susceptible to their venom toxins, while venom is used for those with greater sensitivity. Therefore, relative risk of injurious reprisal to the snake is minimized with utilization of a prey-specific strategy. Some of the ophidian species with purported "atrophied venom glands" (e.g. *Coluber constrictor*, Eastern racer, or black racer) do not constrict or produce venom or

Duvernoy's secretions and yet overcome a wide variety of prey animals including small mammals, amphibians and other reptiles. Other non-venomous species such as the Eastern Indigo snake (*Drymarchon corais couperi*) simply use physical force exerted by pressing their body against large prey (including small mammals and snakes) and swallowing it alive. Thus, the absence of a toxic oral product, or lack of active use of such a product, has not been detrimental to the survival of a significant number of extant snakes and other squamates.

As has been hypothesized by Savitzky (1980), increasingly slender morphology in advanced snakes likely favoured development of prey capture strategies other than constriction. But, it is also premature to label glands of unknown function as "atrophied venom glands" in clades that now may or may not utilize powerful constriction. In fact, some studies of individual herpetological communities have found that non-venomous constrictors such as *Pituophis melanoleucus* (pine snake) were equally successful in feeding on prey such as the kangaroo rat (*Dipodomys merriami*) as were highly venomous species such as *C. scutulatus* (Mojave or green rattlesnake) (Reynolds and Scott, 1982).

The attempt to uncover and accurately report natural phenomena follows the finest tradition of the scientific method that was formulated over millennia by insightful scholars such as Aristotle, Al-Biruni, Francis Bacon, Galileo and many others, and into the present day with the contributions of Popper, Feyerabend and Kuhn. The development of hypothesis, testing and subsequent verification not only provides a standard that seeks to minimize personal belief and recognize objective reality; it also stimulates consideration of alternative theories. This can result in testing different explanations for a given phenomenon, and thereby lead to additionally useful information. For example, Duvernoy's gland products have been hypothesized to possibly neutralize microbes and their toxins (Shivik, 2006), thereby aiding scavenging behaviour, a practice that has been retained in numerous extant ophidian species (DeVault and Krochmal, 2002). Antibacterial and/or antiprotozoal components have been found in venoms of viperids and elapids as well as oral secretions of non-front-fanged colubroids such as *Thamnophis* spp. and *Philodryas* spp. (e.g. Stiles et al., 1991; Nair et al., 2007; Jansen, 1983; Peichoto et al., 2011). These may be used identically, similarly or differently. This is determined by the specific biological application of these components in the life history of the respective ophidian species. It is essential not to assume or judge what the functions of these components may be in a given species when there is little known about so many. Again, this example stresses the need for methodical investigation the results of which must be analysed with patience and a desire for the objective facts relevant to a species of interest, and not for a premature supposition.

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

Following the defining criteria of Fry et al. (2012) there is an indistinct line that separates many animal species on

Planet Earth as “venomous” from those that are not. Humans possess modified dentition, salivary ducts that variably open into the buccal cavity or in the vicinity of the teeth, and multiple glands of varying morphology and function. The parotid glands are salivary glands as are the sub-mandibular, sub-maxillary and sublingual glands. An important functional adaptation of the parotids is associated with their assumption of resting salivary secretory capacity following the gradual inevitable ontogenetic loss of salivary parenchymal acinar cells (Fischer and Ship, 1999; Scott et al., 1987; Drummond et al., 1995). Therefore, during the aging process concomitant with the decreased function of other salivary glands, the parotids maintain a level of salivary secretion that is essential for oral hygiene, dental mineralization and anti-microbial effects. Thus, although these glands share function, some may perform them in variable ways and offer complementary roles that enhance the odds of survival of the organism by altered functions of related structures. It is important to comprehend these variable roles in order to place the gland function in the appropriate context and inclusive of the organism's entire life cycle. Consider that human saliva contains at least 309 proteins (Hu et al., 2005), and these include: platelet-activating factor and its inhibitor (Smal and Baldo, 1991); numerous proteases of several classes; mucins; alpha-kynurenic acid (a neuroexcitatory product of tryptophan metabolism) (Kuc et al., 2006); amylase; anti-microbial peptides such as the anti-fungals, histatins (Situ and Bobek, 2000), and many other components, some that remain uncharacterized. Human saliva is also toxic (Bonilla et al., 1971), and among bites inflicted by proportionally sized mammals, those from humans are among the most clinically serious. Although this describes constituents consistent with “venom”, humans do not use their oral secretions in the subjugation of prey, and most of us do not use them for self-defence. However, we humans do partly pre-digest our foods and share many of these properties with other mammals. As mentioned earlier, several mammals such as some shrews and solenodons, as well as the platypus (*Ornithorhynchus anatinus*; Order Monotremata), are venomous as they either use their oral venoms in prey capture/self defence (shrews and solenodons), or rear limb spur-delivered venom (male platypus) in self-defence, and possibly for intraspecific combat, although some research has suggested that for the platypus, this may also be a premature assertion (Whittington et al., 2008a, 2008b). As mentioned earlier, shrews actively use their sub-maxillary salivary (“venom”) glands in the procurement and subjugation of prey and humans do not, although we have a wide variety of biologically active proteins, including toxins, in our saliva.

If the presence of toxins alone as well as shared evolutionary origins are sufficient criteria for assignment of the term, “venomous”, then basic biological functions as well as functional morphological realities have been prematurely devalued in place of interesting molecular data that require further development and expansion for contextual comprehension. The hypotheses of Fry et al. (2012) may eventually be bolstered by a wider spectrum of data, but their current interpretation requires broader supportive evidence and independent confirmation. The authors of

this commentary welcome the further contributions by Fry and colleagues as well as those of independent researchers that address the collective meaning of these fascinating evolutionary adaptations of squamate reptiles.

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Conflict of interest statement

None to declare.

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