

## Reviewer Report

**Title: Adaptive venom evolution and toxicity in octopods is driven by extensive novel gene formation, expansion and loss**

**Version: Original Submission**    **Date: 6/10/2020**

**Reviewer name: Bryan Fry**

### Reviewer Comments to Author:

A truly excellent paper that was a pleasure to read. My comments are very minor:

- TTX resistance in *Thamnophis* species of snakes should be referenced in the sentence "How resistance to TTX has been acquired at the genetic level, remains a large unknown, with TTX resistance studied in only in a few select species (i.e. pufferfish<sup>13</sup>, newts<sup>14,15</sup> and gastropods<sup>16</sup>)". This is cited later (ref 58) but it would be appropriate for inclusion in this sentence too.
- For the PSG specific genes, calculations of the relative rates of evolution would be informative as this would be suggestive of adaptive evolution eg are the abundant serine proteases *C. minor* showing signs of accelerated evolution seen in other venomous lineages such as snakes? Previous work has shown that the sites on the molecular surface are undergoing episodic diversification when compared across a wide range of lineages. In this case, it would be interesting to see what the evolutionary patterns are for *C. minor*, in that is the extensive duplication accompanied by signs of diversification?
- The M12B metalloprotease type in snake venom has a wide range of demonstrated activities, both anticoagulant (fibrinogenolytic) but also procoagulant (Factor X activating [Atractaspis and Daboia venoms] and prothrombin activating (Bothrops, Echis, and Dispholidus/Thelatornis venoms]) prothrombin activating metalloproteases from *Dispholidus typus* (boomslang) and *Thelatornis mossambicanus* (twig snake)." *Comp Biochem Physiol C Toxicol Pharmacol*: 108625. Oulion, B., J. S. Dobson, C. N. Zdenek, K. Arbuckle, C. Lister, F. C. P. Coimbra, B. Op den Brouw, J. Debono, A. Rogalski, A. Violette, R. Fourmy, N. Frank and B. G. Fry (2018). "Factor X activating Atractaspis snake venoms and the relative coagulotoxicity neutralising efficacy of African antivenoms." *Toxicol Lett* 288: 119-128. Rogalski, A., C. Soerensen, B. Op den Brouw, C. Lister, D. Dashevsky, K. Arbuckle, A. Gloria, C. N. Zdenek, N. R. Casewell, J. M. Gutierrez, W. Wuster, S. A. Ali, P. Masci, P. Rowley, N. Frank and B. G. Fry (2017). "Differential procoagulant effects of saw-scaled viper (Serpentes: Viperidae: Echis) snake venoms on human plasma and the narrow taxonomic ranges of antivenom efficacies." *Toxicol Lett* 280: 159-170. Sousa, L. F., C. N. Zdenek, J. S. Dobson, B. Op den Brouw, F. Coimbra, A. Gillett, T. H. M. Del-Rei, H. M. Chalkidis, S. Sant'Anna, M. M. Teixeira-da-Rocha, K. Grego, S. R. Travaglia Cardoso, A. M. Moura da Silva and B. G. Fry (2018). "Coagulotoxicity of Bothrops (Lancehead Pit-Vipers) Venoms from Brazil: Differential Biochemistry and Antivenom Efficacy Resulting from Prey-Driven Venom Variation." *Toxins (Basel)* 10(10): 411.

## Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

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