Venomics of *Tropidolaemus wagleri*, the sexually dimorphic temple pit viper: Unveiling a deeply conserved atypical toxin arsenal

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*Tropidolaemus wagleri* (temple pit viper) is a medically important snake in Southeast Asia. It displays distinct sexual dimorphism and prey specificity, however its venomics and inter-sex venom variation have not been thoroughly investigated. Applying reverse-phase HPLC, we demonstrated that the venom profiles were not significantly affected by sex and geographical locality (Peninsular Malaya, Insular Penang, Insular Sembr) of the snakes. Essentially, venoms of both sexes share comparable intravenous median lethal dose (LD\(_{50}\)) (0.55–0.63 μg/g) and cause neurotoxic envenomation in mice. LCMS/MS identified six wagleri forms as the predominant lethal principles, comprising 38.2% of total venom proteins. Fourteen other toxin-protein families identified include phospholipase A\(_2\), serine proteinase, snakes: and metalloproteinase. In mice, HPLC fractions containing these proteins showed insignificant contribution to the overall venom lethality. Besides, the unique elution pattern of approximately 34.5% of non-lethal, low molecular mass proteins (3–5 kDa) on HPLC could be potential biomarker for this primitive crotalid species. Together, the study revealed the venom proteome of *T. wagleri* that is atypical among many pit vipers as it comprises abundant neurotoxic peptides (wagleriins) but little hemotoxic proteinases. The findings also revealed that the venom is relatively well conserved intraspecifically despite the drastic morphological differences between the sexes.

Venom is a critical innovation in the evolution of advanced snakes. It represents a trophic adaptive trait crucial for the foraging success of the venomous snakes\(^1\). As complex biological products, snake venoms are known to vary across different taxa suited for diverse ecological niches\(^2\). With the recent advent in proteomic study of snake venoms, the variability in snake venoms is increasingly recognized even within a single species. Intraspecific snake venom variability could be attributed to individual or ontogenic differences\(^3\); however environmental factors (e.g. geographical locale, season) and endogenous factors (e.g. age and sex of the snake) are all possible sources of venom variation within the species\(^4\). The primary cause underlying snake venom variability is usually diet and feeding\(^3\). Diet driven phenotypic variations are most apparent between adult and juvenile snakes, or between male and female specimens as they have been reported to show differences in venom profiles\(^5\). Presumably, the distinction is due to differences in the body and/or head sizes, where the juvenile and the male (of sexually dimorphic snake) usually feed on smaller prey compared with their counterparts. Unveiling the intraspecific variability of snake venom is hence a crucial component to understand the evolvability and diversity of snake venom toxins. In this context, the Wagleri or temple pit viper, *Tropidolaemus wagleri* from Southeast Asia is perhaps a model species with intriguing underling sex-based venom divergence in view of its remarkable sexual dimorphism.

The genus *Tropidolaemus* (Family: Viperidae; Subfamily: Crotalinae) first coined by Wagler in 1830\(^6\), represents a clade of primitive pit vipers of the Old World. It was subsequently merged into the Asian lance-headed pit viper complex (*Trimeresurus sensulato*) for a long period of time (perhaps since 1892 by Gray)\(^7\). Morphologically, the genus *Tropidolaemus* appears rather different from the rest of *Trimeresurus* species, in which *Tropidolaemus* is characterized by several unique features including the absence of a nasal pore, upper surfaces of the snout, strongly gular keels, head covered with distinctly keeled small scales and the hemipenis type that is closer to...