

Microprotein Hit Finding Library Uncovers Bee Venom PLA2 Inhibited by Spider Venom

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Introduction

Phospholipase A2 (PLA2) enzymes are a large and diverse family of enzymes which are involved in the metabolism of potent inflammation mediators such as prostaglandins, leukotrienes and platelet activating factor (Huang *et al.*, 1999). PLA2 enzymes have a role in inflammatory conditions such as atherosclerosis and rheumatoid arthritis. PLA2 inhibition is therefore considered an attractive target for therapeutic application in such inflammatory conditions.

Toxic forms of PLA2 enzymes are present in many venoms, particularly those of Hymenoptera (Bees, Wasps and Ants). More recently, PLA2 enzymes have been discovered for the first time in venom of Theraphosidae (tarantula spiders) (Ferreira *et al.*, 2016). PLA2 inhibitors have been documented in snake serum, presumably as protection against accidental envenomation (Dunn & Broady, 2001), but no such inhibitors have been discovered thus far in spiders.

We therefore set out to investigate the potential presence of novel PLA2 inhibitors in theraphosid venom using the Targeted-Venom Discovery Array™ hit finding strategy. Venom libraries contain a diversity of pharmacological actives, including microproteins and peptides, which deliver hits on nearly all targets, especially those difficult to hit with small molecule libraries.

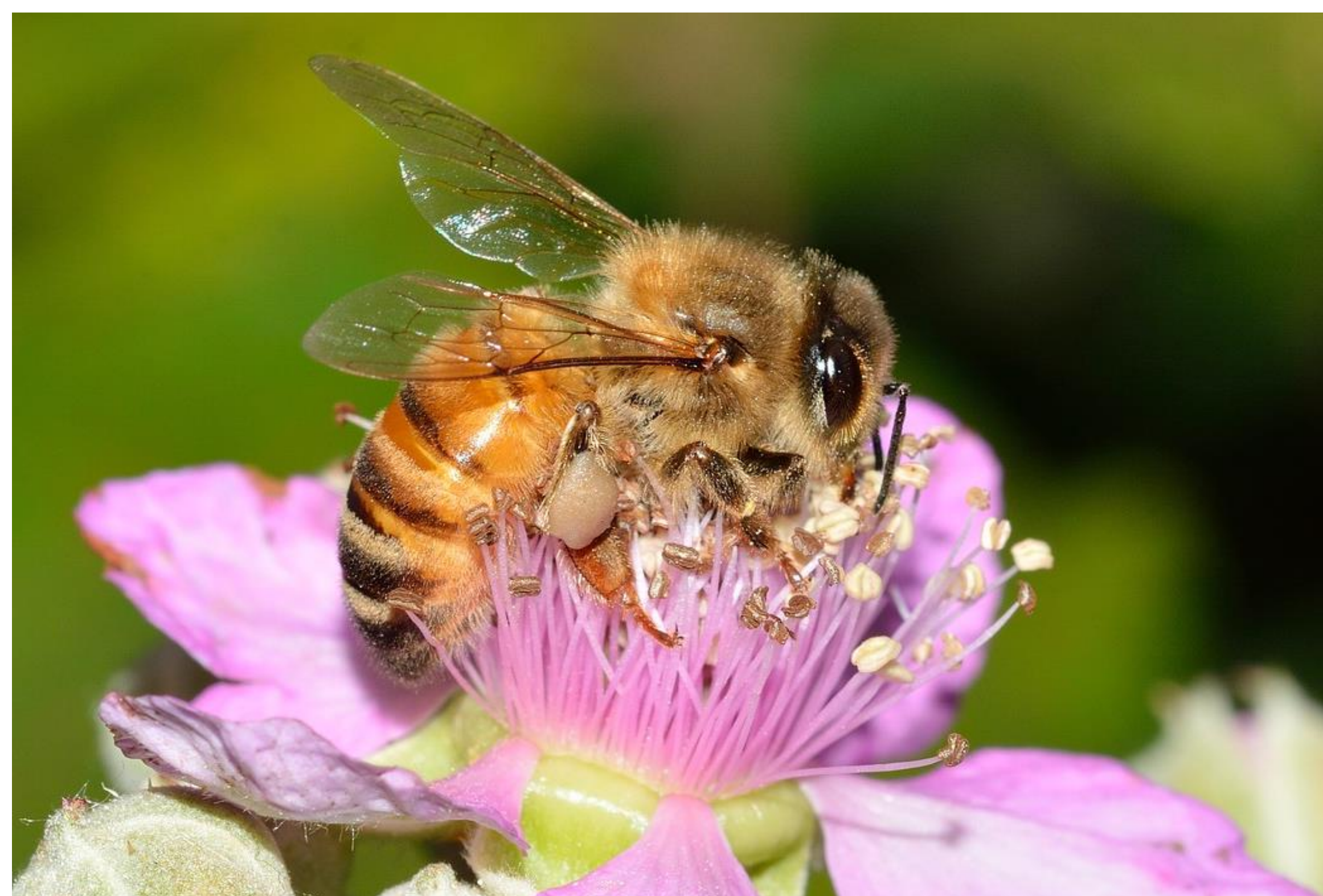
Materials and Methods

Venoms were selected to provide a wide coverage of genera of Theraphosidae (commonly known as tarantulas). EnzChek® Phospholipase A2 Assay Kit (Molecular Probes®, Invitrogen) was used to study PLA2 activity. The assays were performed in 384 well format using a reaction volume of 25 µl per well. Honey bee PLA2 supplied with the kit was prepared at 5 Unit/ml. Substrate-liposome mix was prepared by slowly adding 25 µl of Lipid mix to 5ml of 1 x PLA2 reaction buffer whilst vortexing.

Protein concentrations of test venoms were measured using the 'protein' function on a DS-11 spectrophotometer (Denovix, Wilmington, USA). Test venoms were prepared at a concentration of 200 µg/ml for screening either from frozen or lyophilised stocks. 6.25 µl of 200 µg/ml venom was added to 6.25 µl of 5 Unit/ml Bee venom PLA2 and incubated for 10 minutes at 25°C. 12.5 µl of prepared substrate-liposome mix was added to each well and the plate incubated at 25°C in the dark for a further 10 minutes. The plate was read using a FLUOstar Galaxy plate reader (BMG LABTECH, Ortenberg, Germany) with an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The same protocol was performed but with the addition of reaction buffer rather than bee venom PLA2 to investigate intrinsic PLA2 activity of the venoms.

For the dose response curve, *Acanthoscurria geniculata* (A.gen) venom was thawed and a 2-fold serial dilution was performed from 4 mg/ml. The PLA2 assay was repeated as above for each dilution.

Species codes are as follows: A.gen: *Acanthoscurria geniculata*, H.mac: *Heteroscodra maculata*, H.gig: *Hysterocrates gigas*, H.lat: *Hysterocrates laticeps*, L.klu: *Lasiadora klugi*, M.rob: *Megaphobema robustum*, M.lam: *Monocentropus lambertoni*, P.reg: *Poecilotheria regalis*, T.pru: *Thrixopelma pruriens*, G.pul: *Grammostola pulchra*.



Results

The theraphosid venoms tested show varying degrees of fluorescence in the PLA2 assay without the addition of bee venom PLA2 (Fig 1), suggesting intrinsic PLA2 enzyme activity in the venoms, particularly in *Lasiadora klugi* (L.klu).

The venoms also showed various levels of bee venom PLA2 inhibition (Fig 2). In particular, *Acanthoscurria geniculata* (A.gen) venom inhibited bee venom PLA2 by approximately 70%. A dose response assay was performed to investigate the effect of concentration of venom on inhibitory activity.

The *Acanthoscurria geniculata* dose response curve (Fig 3) shows that higher doses of venom lead to increased levels of PLA2 inhibition, up to a maximum of around 70%. Intrinsic PLA2 activity could be contributing to the inhibition plateaus observed.

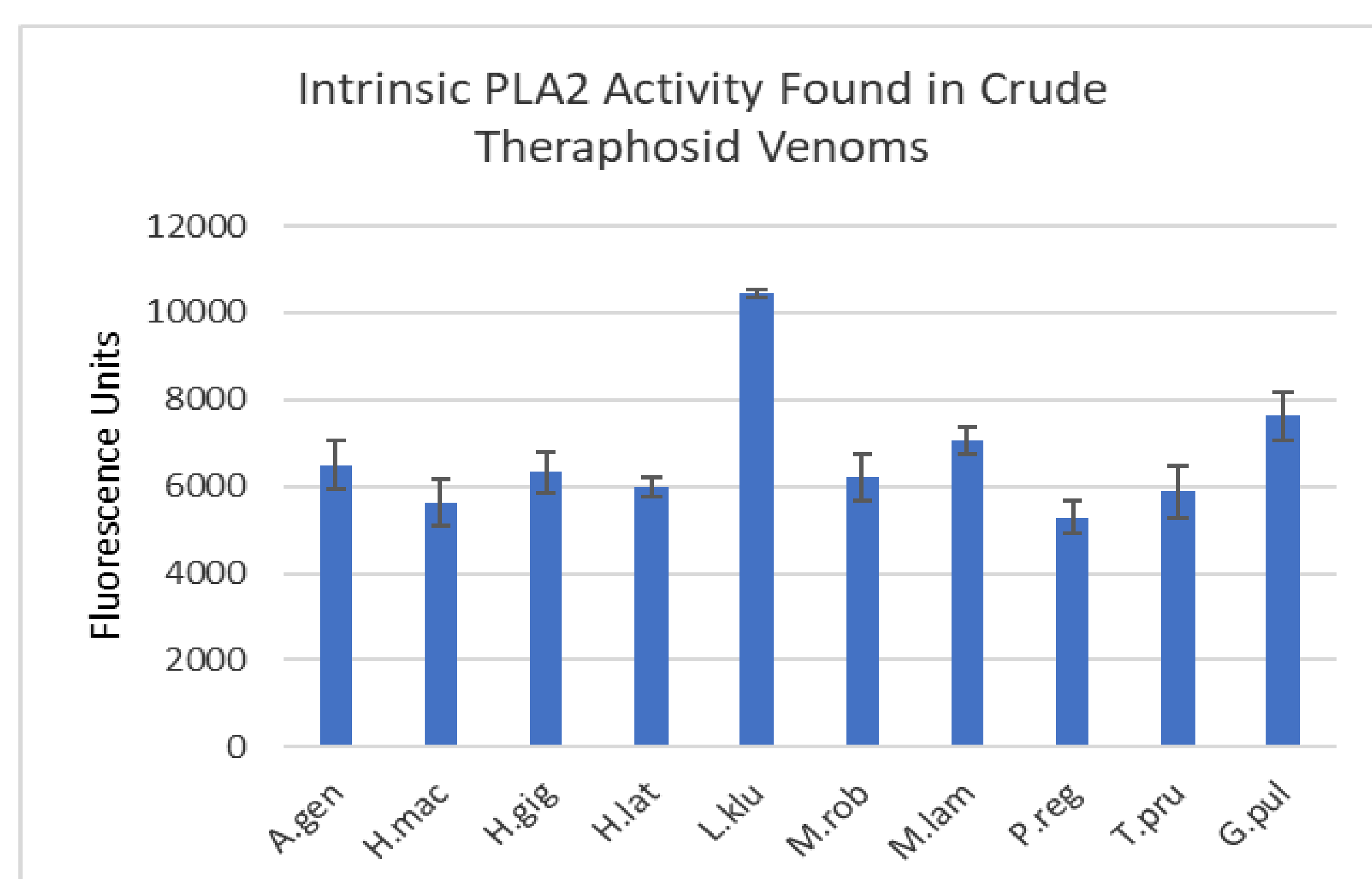


Figure 1. Intrinsic PLA2 activity of crude theraphosid venoms.

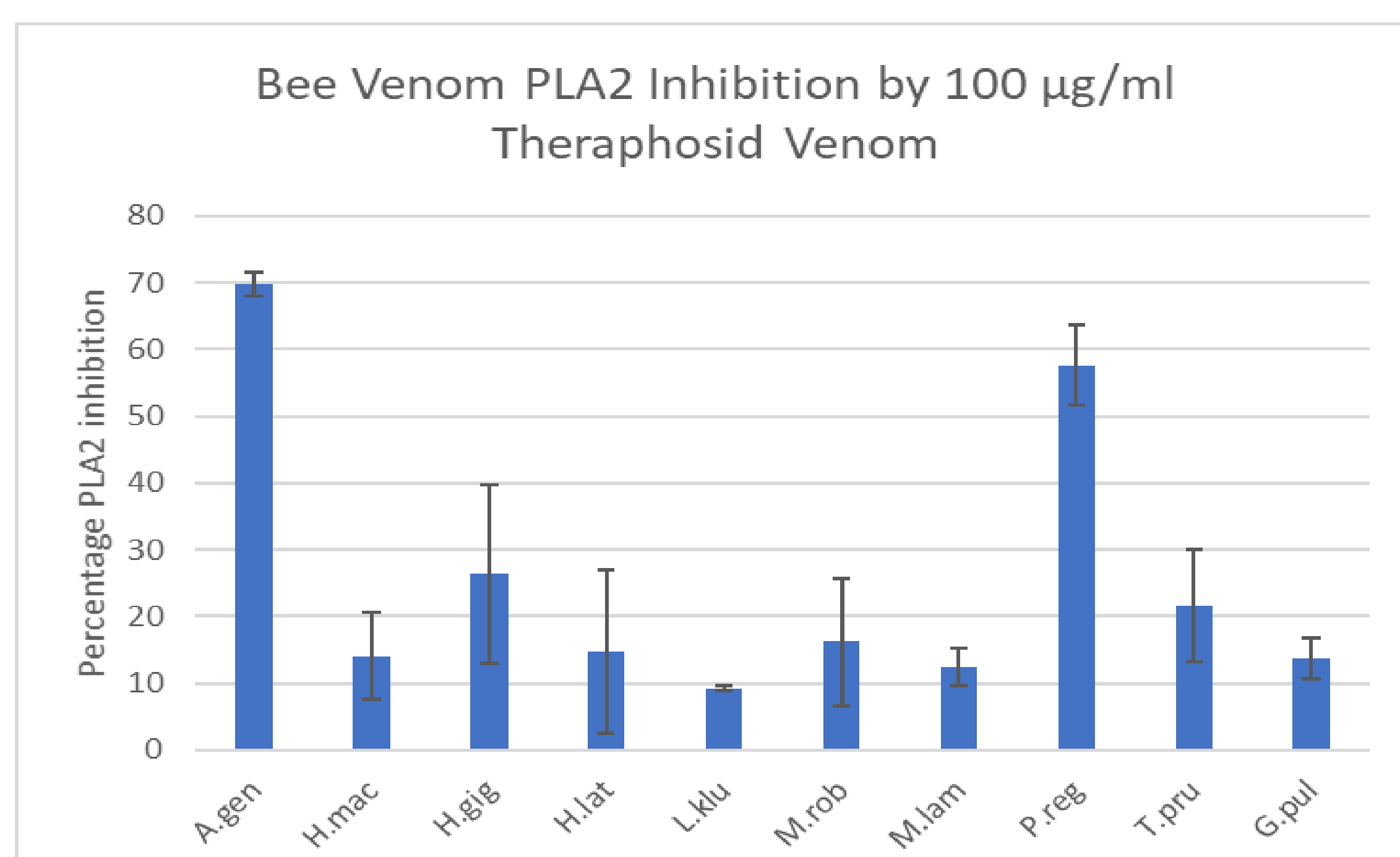


Figure 2. Percentage inhibition of bee venom PLA2 by the different theraphosid venoms at 100 µg/ml concentration.

PLA2 Inhibition after Agen Venom Exposure

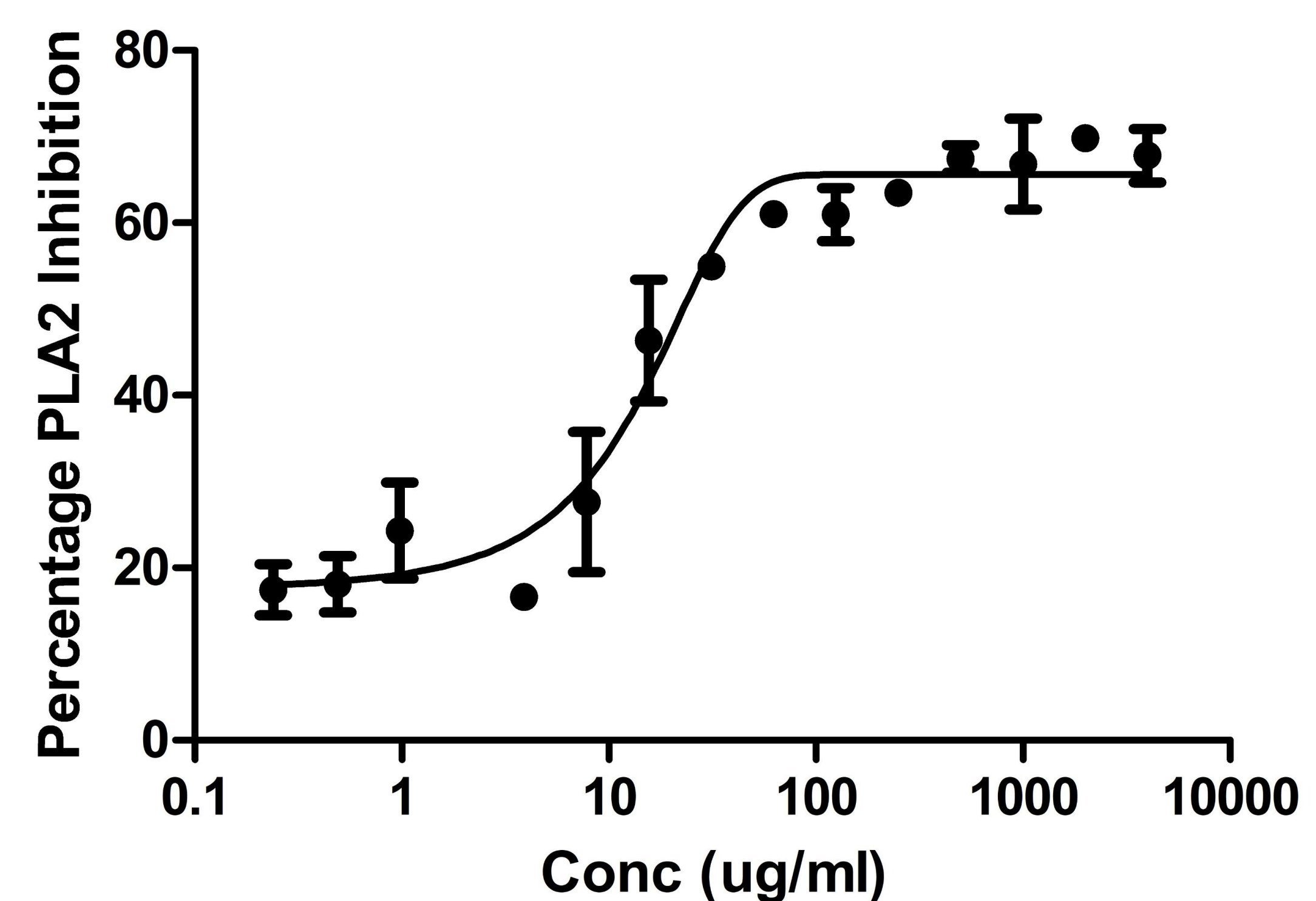
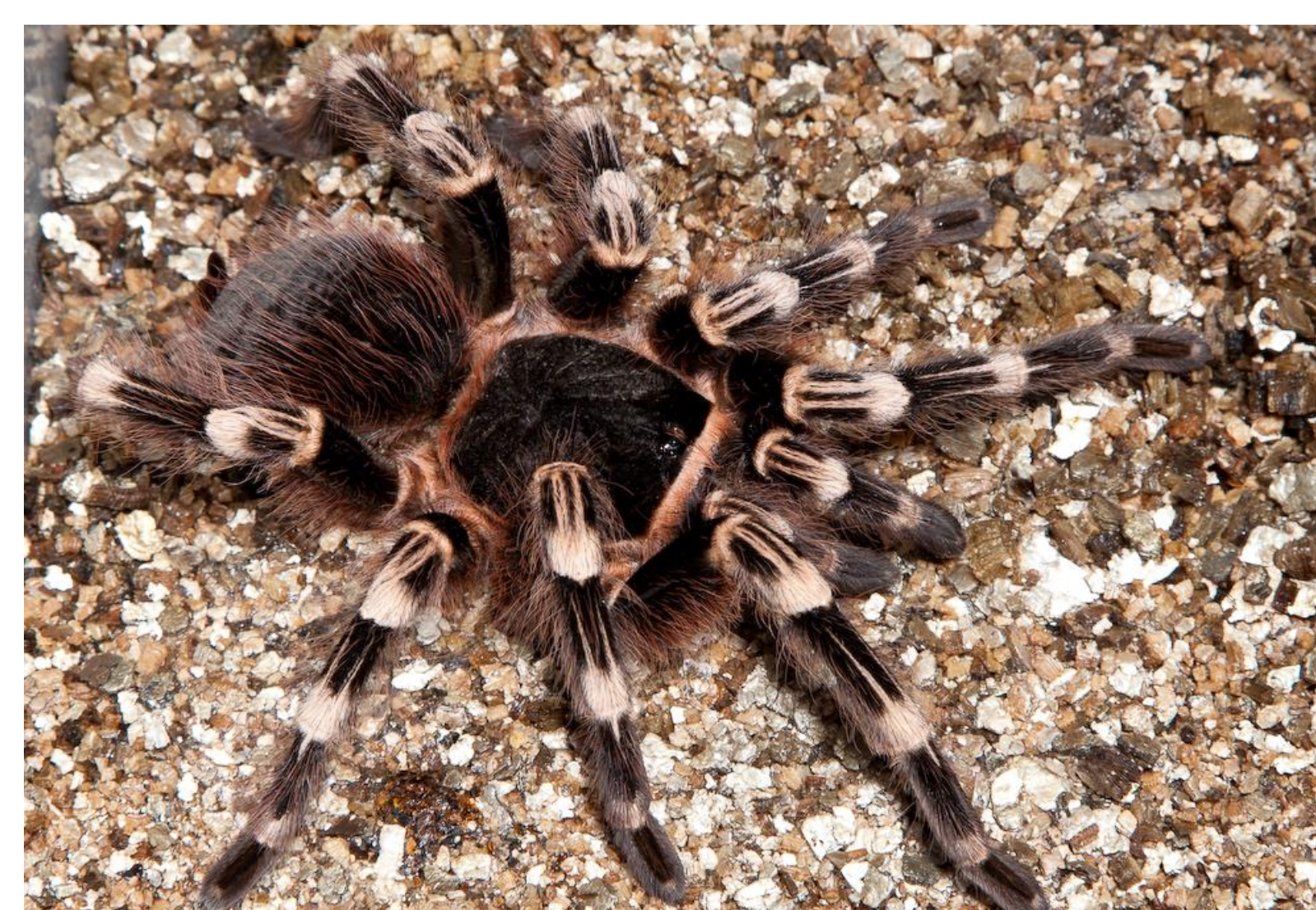


Figure 3. Dose response curve for bee venom PLA2 inhibition by serial dilution of *Acanthoscurria geniculata* (A.gen) venom.

This preliminary study compares intrinsic PLA2 activity of theraphosid venoms for the first time. It also is the first study to discover the presence of PLA2 inhibiting compounds in theraphosid venom. The venom of *Acanthoscurria geniculata* was studied further and showed a dose response between venom concentration and PLA2 inhibition.



Acanthoscurria geniculata

Photo by Stuart Thomson Photography, taken at Venomtech Ltd.

Future work includes fractionation of *Acanthoscurria geniculata* venom to determine active fraction(s), followed by identification of compound(s) by mass spectroscopy. This is the first study showing theraphosid venoms have activity against bee venom PLA2. Further work could include testing theraphosid venoms for selectivity over other PLA2 enzymes such as from snake venoms and relevant human enzymes. If identified to have activity against human PLA2 or other disease relevant enzymes, theraphosid venoms could provide an exciting untapped resource for potential treatment of inflammatory conditions.

References

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