

Asian Hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae), Venom Obtention Based on an Electric Stimulation Protocol [†]

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Abstract: The yellow-legged Asian hornet (AH) (*Vespa velutina* Lepeletier 1836 (Hymenoptera: Vespidae)) is naturally distributed in China, Southeast Asia and India; however it was detected outside of its native area, confirmed as being established in South Korea, Europe and Japan. Health risks and deaths caused by AH stings have become a public health concern. In the present work, a straightforward, quick and inexpensive method for obtaining AH venom by electric stimulation is described. The availability of AH venom will lead to improved diagnostic and therapeutic methods, mainly by venom immunotherapy (VIT) in patients allergic to this invasive species.

Keywords: Asian hornet; *Vespa velutina*; Venom; Electrical; Stimulation; Allergy; Stings; Invasive species; Venom Immunotherapy (VIT); Hymenoptera

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1. Introduction

Invasive alien species (IAS) are plants, animals, pathogens and other organisms that are introduced and/or spread outside of their natural geographic range and which may cause severe ecological, economic and social impacts on the invaded environments.

Several IAS insect species have migrated in the last decade to Galicia, located on the northwestern end of the Iberian Peninsula, and successfully colonized and spread, resulting in a broad range of consequences to recipient ecosystems and, thereby, human society [1]. Of these IAS, the yellow-legged Asian hornet (*Vespa velutina* Lepeletier 1836 (Hymenoptera: Vespidae)) was detected in Galicia in 2012. It was soon recognized as a pan-European threat after being detected in the province of Navarra, northern Spain (2010), in the north-western province of Minho in Portugal (2011), Belgium (2011), Italy (2012), Germany (2014), the Netherlands (2018), Majorca in the Balearic Islands (2015), England and the Channel Islands (2016). The species has become a major concern to apiculture and pollination, given that the diet of these hornet colonies is predominantly based on honeybees and other insects [1,2].

However, *Vespa velutina* is not only a problem for beekeepers and their industrious flying insects, since other agricultural sectors such as fruit production and viticulture are also impacted; nevertheless the medical-veterinary potential of *Vespa velutina* should also be outlined. Due to its habits, abundance and wider distribution, the risk that the IAS *Vespa velutina* represents for human health is incomparable with other native species of Hymenoptera [3]. The medical community is requesting *Vespa velutina* venom extracts to aid diagnosis and treat allergy and/or anaphylaxis through immunotherapy [4]. Venom immunotherapy is the standard of care for people with severe reactions and has

been shown to reduce the risk of future anaphylactic events and the risk of death [5-7]. In the present work, a straightforward, quick and inexpensive method for obtaining *Vespa velutina* venom by electric stimulation is described.

2. Methods

Adult female *Vespa velutina* specimens were obtained while using an active collecting net method at an apiary (Figure 1a). An electrical venom collection device (IGK Electronics, Varna, Bulgaria) was used to harvest the insect venom (Figure 1b). Once transported to the lab, the dried venom was removed from the glass plate (Figure 1c). Insects were identified using their external morphological characteristics. In brief, *Vespa velutina* averages about 2 to 3 cm in length, head is black with face and mouthparts orange, the antennae are brown dorsally and orange ventrally. The thorax is dark brown, almost black. Metasomal terga brown, with a thin yellow band on segment 1st and a thin orange band on segments 2nd and 3rd; metasomal segment 4th orange; metasomal segments 5th and 6th orange-brown. Legs are brown, with yellow tarsi and the wings are brownish hyaline.

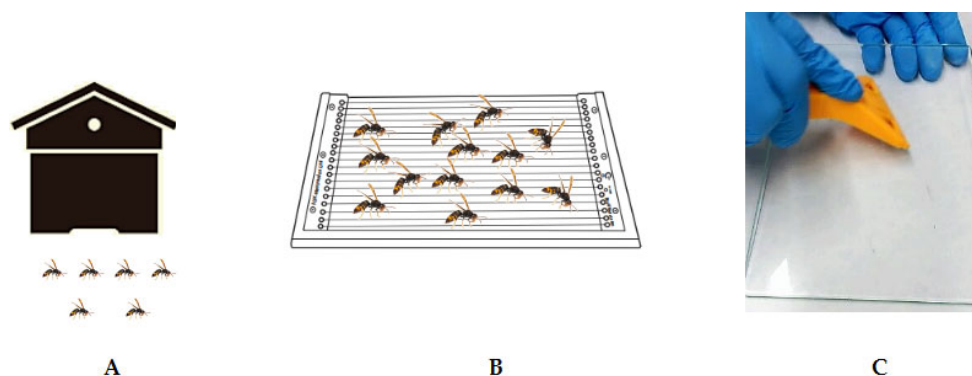


Figure 1. Sequential steps for obtaining *Vespa velutina* venom, based on an electric stimulation protocol: (a) Collection of *Vespa velutina* specimens in the apiary ; (b) *Vespa velutina* electric stimulation and (c) scraped of the *Vespa velutina* venom.

3. Results and Discussion

A prerequisite to studying the nature of the venoms is the development of methods for their collection. The distinctive *Vespa velutina* honey bee-capture behaviour allows the pinpointing of apiaries as a suitable place, to catch enough live specimens for posterior venom extraction. The target insect is not easily confused with any other hymenopteran species of hornet, bees and wasps normally present at the apiaries in different areas of Europe, such as: the European hornet (*Vespa crabro* Linnaeus, 1758); the Oriental hornet (*Vespa orientalis* Linnaeus 1771); *Bombus* spp, *Vespula* spp and/or *Polistes* spp. The *Vespa velutina* can be clearly differentiated because of its unique dark colour pattern, which is mostly black. Moreover, the *Vespa velutina* has the 4th abdominal segment almost entirely orange-yellow, is smaller than the native European hornet and possesses yellow tipped legs.

At a lab-scale level, there are well tested and widely accepted techniques for obtaining venom from several hymenopterans. They are based on death, frozen insects, where the whole sting apparatus needs to be dissected, using microsurgical forceps, and the obtained venom sacs are basically: (i) manually extracted from the separated venom sacs, i.e. by gentle squeezing, or (ii) homogenized and/or just pooled to collect the liquid fraction by centrifugation. The microdissection of the venom reservoir, usually by the precise manipulation of specialized needles and requires a high degree of operator skills. This makes the manual extraction of venom a tedious, labori-

ous and time-consuming task. In the present work, an electrical venom collection device designed to harvest honeybee venom was used. The device consists of a solid wooden frame with a removable glass plate, under the electrical wires. Once the venom-harvesting is complete, the glass plate is removed and scrapped with a razor at the lab for *Vespa velutina* venom obtention.

4. Conclusions

The *Vespa velutina* is an invasive alien species with medical importance on the health of members of the public. We described a straightforward, quick and inexpensive method for obtaining *Vespa velutina* venom, based on an electric stimulation protocol. The availability of *Vespa velutina* venom will lead to improved diagnostic and therapeutic methods, mainly by venom immunotherapy (VIT), in patients allergic to this invasive species.

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Institutional Review Board Statement: The Asian hornet, *Vespa velutina* Lepeletier 1836 (Hym.: Vespidae) is not a regulated invertebrate. Therefore, no ethical use approval is necessary.

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Conflicts of Interest: The authors declare no conflict of interest.

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