

SHORT COMMUNICATION

An easy method for handling the genus *Phoneutria* (Araneae, Ctenidae) for venom extraction

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Abstract. This paper describes an easy, cheap, and safe method of capturing and handling the medically important spider *Phoneutria* for venom extraction. The method does not injure or kill the spider and allows the extraction of pure venom.

Keywords: Armed spider, venomous spiders, vivarium

The venomous ctenid spiders of the genus *Phoneutria* (*P. nigriventer* Keyserling 1891) (Figure 1a) are medically important due to their aggressiveness (Fig. 1a), their great speed, and the dangerous effects of their venom (Bücherl 1953a). Consequently, researchers are interested in obtaining pure venom from these spiders so that the medical significance of its components can be explored. Traditionally, spiders have been immobilized using anesthesia (CO₂, chloroform, ether, or other substances) or with cold temperatures, but these techniques can cause mortality or alter the behavior and physiology of the animal (Harris et al. 1965; Randall 1982), with immediate or latent harmful effects (Nicolas & Sillans 1989). Furthermore, venom extraction following these handling methods has often entailed the excision and maceration of the entire venom gland, a procedure that produces impure venom (Bücherl 1953a). Bücherl (1953a) describes a technique that avoids these problems. It consists of irritating the spider so that it attacks and envenomates a device (two pipettes connected with an elastic surgical tube) from which the venom can be extracted. This may be the safest method for the extractor and does not cause the animal's death, but the process is laborious and does not produce a sufficient amount of venom.

Here we propose an easy, cheap method for capturing and handling *Phoneutria* for venom extraction that is safe for both the extractor and the spider. The handling device consists of a transparent or semi-transparent 2-liter plastic (PET) bottle (i.e., empty soft drink bottle) that has been cut transversely across the middle. Only the top half of the bottle is retained for use as a handling chamber (Fig. 1b). An 8-cm longitudinal slit is cut into the side of the handling chamber. Then, while holding onto the top of the bottle mouth (with lid), the chamber is placed over the spider, imprisoning it. At this point, the spider typically attempts to climb upward toward the neck of the bottle. Filter paper should be used as a floor to capture any venom released by the spider; the venom can be recovered later by washing the filter paper with an organic solvent (e.g., acetonitrile). The spider is forced away from the bottle wall or lid and onto the filter paper by gently tapping the chamber against the extraction bench. Once the spider is on the filter paper, a glass stirring rod is inserted through the

longitudinal slit and pushed down onto the spider in the area between the cephalothorax and the abdomen. This presses the spider onto the filter paper and immobilizes it (Figure 1c).

While continuing to press the animal down with the glass rod, the handling chamber is removed and the sides of the animal's cephalothorax are grasped between the index finger and thumb (Figure 1d). Once the spider is firmly grasped, the glass rod can be removed and the animal carried away for the venom extraction (Figure 1e). Venom extraction consists of placing electrodes against the region of the cephalothorax lying above the venom glands and then stimulating the spider with 6 V (Bücherl 1953b). The venom is collected from the chelicera in a small capillary tube (Smith & Micks 1968; Morris & Russell 1975) or on a glass plate.

LITERATURE CITED

- Bücherl, W. 1953a. Novo processo de obtenção de veneno seco, puro, de *Phoneutria nigriventer* (Keyserling, 1891) e titulação da LD₅₀ em camundongos. *Memórias do Instituto Butantan* 25:153–174.
- Bücherl, W. 1953b. Escorpiões e escorpionismo no Brasil. I. Manutenção dos escorpiões em viveiros e extração de veneno. *Memórias do Instituto Butantan* 25:53–82.
- Harris, R.L., R.A. Hoffman & E.D. Frazar. 1965. Chilling vs. other methods of immobilizing flies. *Journal of Economic Entomology* 58:379–380.
- Morris, J.J. & R.L. Russell. 1975. The venom of the brown recluse spider *Loxosceles reclusa*: composition, properties and an improved method of procurement. *Federation Proceedings* 34:225.
- Nicolas, G. & D. Sillans. 1989. Immediate and latent effects of carbon dioxide on insects. *Annual Review of Entomology* 34:97–116.
- Randall, J.B. 1982. Surgical restraint apparatus for living spiders. *Journal of Arachnology* 10:91.
- Smith, C.W. & D.W. Micks. 1968. A comparative study of the venom and other compounds of three species of *Loxosceles*. *American Journal of Tropical Medicine and Hygiene* 17:651–656.

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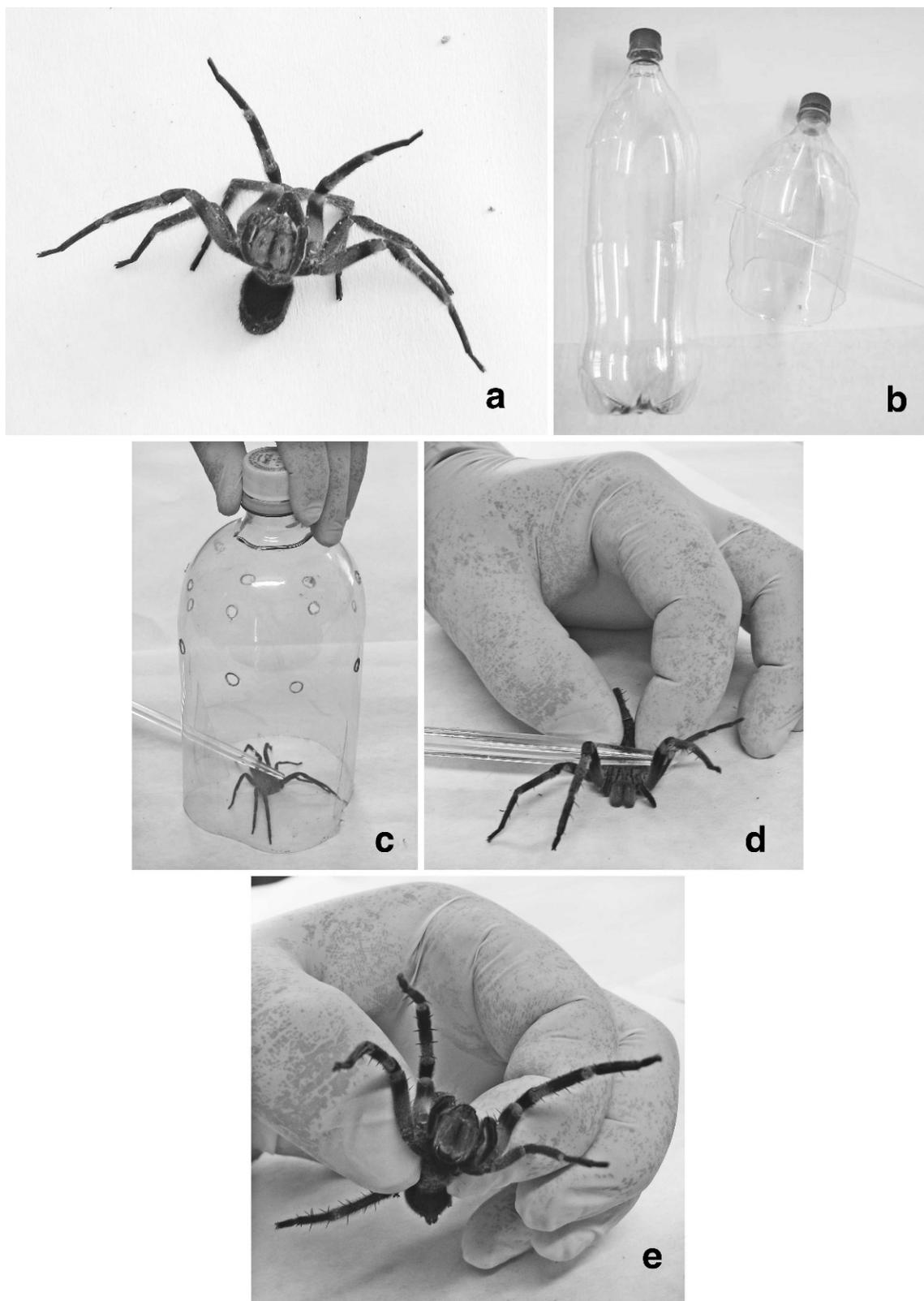


Figure 1.—The step-by-step handling method for venom extraction of *Phoneutria nigriventer*. a. The spider in an aggressive position; b. Left side of an empty 2-liter plastic bottle and right side of a bottle with a transverse cut and a glass stick inserted in the perpendicular cut; c. The trapped animal in the bottle being immobilized with the glass stick; d. Grasping the spider with fingers; e. Spider ready for venom extraction.