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Captive Maintenance and Venom Extraction of *Tityus serrulatus* (Brazilian Yellow Scorpion) for Antivenom Production

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Abstract

Scorpion envenomation is a public health problem in several tropical and subtropical countries. *Tityus serrulatus* Lutz and Mello, 1922 (Brazilian yellow scorpion) are responsible for approximately 150,000 envenoming cases per year in Brazil, of which 10% require antivenom treatment to reverse life-threatening venom effects. Therefore, thousands of *T. serrulatus* individuals are maintained under controlled captivity conditions for venom extraction, subsequently used in the production of the national supply of scorpion antivenom. Instituto Butantan is the main antivenom-manufacturing laboratory in Brazil, providing about 70,000 vials of scorpion antivenom for the Brazilian health system. Thus, the husbandry protocols and venom extraction methodologies are key points for the success of large-scale, standardized venom production. The objective of this article is to describe the captivity protocols of *T. serrulatus* husbandry, encompassing the husbandry routine and the venom extraction procedures, following good manufacturing practices, and ensuring animal welfare. These practices allow for the maintenance of up to 20,000 animals in captivity, with a routine of 3,000 to 5,000 scorpions milked monthly according to antivenom manufacturing demand, achieving an average of 90% of positive extraction.

Introduction

Scorpions are arthropods belonging to the class Arachnida, order Scorpiones, which comprises approximately 2,621 species[1,2]. These animals have a wide geographical range and are present on all continents except Antarctica[1]. Envenomation caused by scorpions results in the morbidity or death of thousands of people every year globally[3]. In 2019, it was estimated that there were more than 1.2 million accidents and 3,500 annual deaths caused by these animals. In Brazil, the number of cases has been increasing exponentially, reaching more than 100,000 cases per year since 2017[4,5,6]. The uncontrolled urbanization observed in Brazil in the past decades, without adequate sewage treatment and regular collection and disposal of garbage, associated with environmental degradation and

climate changes, has provided conditions for the proliferation of invasive scorpions, such as *T. serrulatus,* increasing the contact with humans and thus resulting in harmful accidents[4,7,8]. There are about 178 scorpion species in Brazil, but stings of medical importance are caused by the genus *Tityus,* with four species (*T. serrulatus, T. bahiensis*, *T. stigmurus,* and *T. obscurus*) being of medical concern, with *Tityus serrulatus* being responsible for the most severe cases and deaths[7,9].

Instituto Butantan is the main antivenom-manufacturing laboratory in Brazil, providing approximately 70,000 vials of scorpion antivenom for the Brazilian health system. Briefly, the steps involved in antivenom manufacturing include the inoculation of venom-derived antigens in equines, the collection and purification of rich-antibodies plasma, resulting in 5 mL vials of scorpion antivenom. Each vial is capable of neutralizing, at least, 1 mg of scorpion venom per mL of antivenom. The Arthropods Bioterium is an integral part of the industrial center, responsible for supplying the source material for the scorpion antivenom.

The Arthropods Bioterium at Instituto Butantan was originally named the Laboratory of Arthropods and was established in 1967. However, it was only in 1995 that the Laboratory moved to an exclusive facility dedicated to housing venomous arthropods[10,11]. At that time, the scorpions were kept in a room of 12 m^2 with no controlled temperature and distributed among approximately 13 enclosures, each housing a maximum of 300 animals[10]. Due to the increasing demand for venom over the years and the consequent need to increase the number of animals, along with improvements in the venom extraction process, a major change occurred in 2016. The Laboratory was integrated into the industrial center of Instituto Butantan and was renamed the Arthropods Bioterium. Additionally, in 2016, the Bioterium moved to a new purpose-built facility. The new scorpion room covers an area of 24 m^2 and contains approximately 48 polypropylene enclosures, each housing up to 300 animals, as shown in **Figure 1**. This results in a total of 10,000 to 20,000 individuals being maintained under controlled captive conditions, with variations throughout the year. The scorpions are carefully managed under strict husbandry protocols to ensure their welfare while meeting the high production demands and adhering to good practices and ethical standards for animal care. For venom extraction, a specific room was designed and constructed, housing two airflow cabinets as shown in **Figure 2A**. These cabinets serve as safety measures to prevent technicians from inhaling venom particles during the extraction procedure. Personal protective equipment (PPE) is mandatory for the technicians, including synthetic aprons and FFP2 masks, to avoid any contamination and ensure their safety.

Figure 1: General view of the scorpion's room of the Arthropods Bioterium. Please click here to view a larger version of this figure.

Figure 2: Working area. (**A**) General view of the venom extraction room of the Arthropods Bioterium. (**B**) Airflow cabinet work surface. All the materials and equipment are prepared and arranged on the top of the airflow cabinet work surface. The black arrow indicates the electrostimulator device, and the red arrow indicates the forceps. Please click here to view a larger version of this figure.

About 90% of the *T. serrulatus* scorpions in the Arthropods Bioterium originate from different sites, collected by municipal health agents as part of a surveillance and control program for these animals. The scorpions are caught in urban areas and then sent to Instituto Butantan as part of a collaborative program. *T. serrulatus* scorpions are parthenogenetic, meaning that birth occurs as a consequence of the development of offspring from unfertilized eggs, without the presence of an individual of the opposite sex[12]. Due to this type of reproduction, some animals are born in captivity in our facility and are maintained until they reach the adult phase, following a similar husbandry protocol as described below. Once they reach the adult stage, they are added to the venom extraction routine. Upon arrival, the scorpions

undergo initial screening, and those in good health conditions are kept in collective enclosures containing a maximum of 350 individuals, divided by their origin. For each enclosure, a control form is filled out daily, providing data on the date of feeding and the type of prey offered (cricket or cockroach), water supply, cleaning procedures, the number of dead animals, and the number of remaining live ones inside the enclosure.

In a pre-scheduled agenda, animals are subjected to electric stimulation, and the resulting venom of hundreds of individuals is then lyophilized, resulting in batches of freeze-dried venom. The standardized venom obtained from this process is destined for horse immunization as part of the manufacturing process, as well as to provide reference material for quality control of the active substance and the final product.

There are very few facilities in the world capable of keeping such a large number of scorpions and performing the volume of venom extractions in accordance with good manufacturing practices and ethical use of the animals, as done in the Arthropods Bioterium of Instituto Butantan. Therefore, our objective is to describe the scorpion maintenance protocols and venom extraction procedures used in *T. serrulatus* husbandry, which successfully provide the necessary amount of venom for the production of scorpion antivenom.

Protocol

Protocols involving invertebrate animals are exempt from Instituto Butantan's Committee on the Use and Care of Animals' approval. However, the maintenance of scorpions described here follows ethical parameters, and animal welfare is respected according to the needs of the species.

1. Housing

1. Keep the scorpions in communal polypropylene containers as enclosures (height 35 cm, width 35.5 cm, length 72 cm) containing a maximum of 350 animals.
2. Maintain the room where the animal enclosures are housed under a controlled temperature of 24 °C (±0.5 °C) in a light cycle of 10 h with lights on and 14 h with lights off.
3. Line the enclosure floor (bottom) of each container with kraft paper fixed with masking tape on the edges on all sides so that the animals cannot get under the paper (**Figure 3A**).
4. Use four cardboard trays (egg trays) as vertical substrates, stacked on one side of the container, with a rigid cardboard sheet placed between each tray (**Figure 3B,C**).
5. Place a shallow polypropylene tray (water tray) on the opposite side of the enclosure, containing soaked cotton as a water source for the animals (**Figure 3B**).
6. Keep the containers without lids to increase the airflow. Place a strip of approximately 10 cm of self-adhesive plastic tape on the top of the internal surface of the enclosure and on the upper corners to make the walls smooth enough to prevent the animals from escaping from the container (**Figure 3A**).

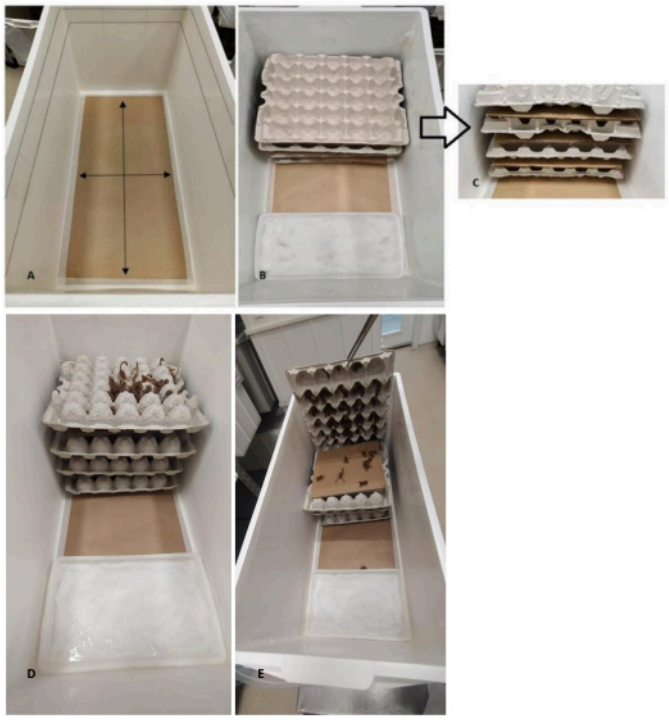

Figure 3: Housing and enclosure preparation. (**A**) Arrows indicate the kraft paper on the bottom, fixed with masking tape on the edges. Dotted lines indicate the self-adhesive plastic tape placed on the top of the internal surface of the enclosure. (**B**) Vertical substrate (egg trays piled on one side of the container) and a shallow polypropylene tray (water tray) placed on the opposite side. (**C**) Vertical substrate detail: egg trays pilled with a rigid cardboard sheet in between two trays. (**D**) Cardboard sheets and egg trays positioned in layers, simulating the natural dark and humid habitat of *T. serrulatus.* (**E**) Animals accommodated in the substrate. Please click here to view a larger version of this figure.

2. Hygiene routine

NOTE: The hygiene routine is divided into two maintenance procedures: complete and partial maintenance. The first one is performed 2 days after feeding the animals or when the enclosure is selected for venom extraction. Partial maintenance is performed when the animals are not fed.

1. Complete maintenance

1. Prepare a clean enclosure with new substrate (egg trays, cardboard, and kraft paper) and a clean water tray, as described in section 1.
2. Utilizing long forceps, remove the water tray from the enclosure. If there are any scorpions on the water tray, remove them and place them in the clean enclosure.
3. Repeat the same procedure described in step 2.1.2 with all egg trays and cardboard sheets, removing

all the scorpions and placing them in the clean enclosure.

4. After the removal of the trays, remove all the scorpions left on the floor of the enclosure and place them in the clean enclosure. Be sure to account for any carcasses and discard them properly. Collect all remaining live prey (insects) and remove them from the enclosure.

5. Remove all the disposable substrates from the enclosure (egg trays, cardboard sheet, and kraft paper) and properly discard them. Send the reusable items (water tray and polypropylene container) for sanitation.

   NOTE: The sanitation of the reusable items is performed with 0.5% hypochlorite combined with a neutral detergent. It is mandatory that the materials are thoroughly and abundantly rinsed with running water after using any sanitation product or solution.

2. Partial maintenance

   NOTE: Partial maintenance consists of inspecting all the substrates (egg trays and kraft paper) with the aim of removing dead scorpion carcasses and other undesirable debris, but without discarding the substrates or changing the enclosure.

   1. Perform the inspection using long forceps to gently lift each egg tray and remove the waste.

   2. If needed, replace one or more egg trays with a clean one.

   3. Completely replace the water tray with a clean one using long forceps. Transfer any scorpions on the water tray to the egg tray in another part of the enclosure.

3. Feeding

1. Feed the animals every 15 days according to the venom extraction schedule. Feed animals that will be milked 7 days prior and 7 days after the venom extraction.

2. For the feeding, supply cockroaches (*Phoetalia pallida*) and crickets (*Gryllus sp.*) alternately.

3. Calculate the amount of food according to the energy intake of the animals; offer 1 prey for every 3 or 5 scorpions in the enclosure.

4. Count the calculated amount of prey items and place them alive inside the enclosure, leaving them for 2 days prior to the complete maintenance (section 2.1).

5. When offering cockroaches, apply a calcium carbonate with alcohol solution around the top of the enclosures. The solution causes the cockroaches to slip and not be able to climb the side surface of the enclosure, thus preventing the insects from escaping.

4. Venom extraction

NOTE: The animals used in the venom extraction routine are adults or pre-adults. Due to the difficulty in determining the complete sexual maturity of the Brazilian yellow scorpion (adult phase), it was determined that animals with a total body length of 5 to 7 cm are eligible for the procedure.

1. Milk each animal every 2-3 months.

2. On the day of venom extraction, count all alive animals in the enclosure and transfer them to a glass container utilizing long forceps (**Figure 4**).

3. Prepare and arrange all the materials and equipment to be used on the top of the airflow cabinet work surface. Plug the electrostimulator power cord into the outlet

and turn the electrostimulator and the airflow cabinet on (**Figure 2B**).

1. Use the following electro stimulator parameters settings: potential (difference of the potential between the electrodes): 540 mV, stress (duration of the electric pulse): 2 ms, action (intermittency to moderate the potency used, which would reduce the heating of the telson organic tissue): 70%, cycle (adjust the cyclic repetition): 1 s, and intensity (electric current that circulates through the animal's muscle): 0.75 mA.

4. Manually restrain each animal in a way that the telson stands still, following the steps:
    1. Hold the metassoma of the animal with straight forceps (**Figure 5A**).
    2. Immobilize the telson with curved forceps (**Figure 5B**).
    3. Restrain the telson with the hand (**Figure 5C,D**).
5. After restraint, place the telson in direct contact with the electrostimulator conductor, without the need to use a conductive solution. The electric shock causes an involuntary muscle contraction of the venom gland and consequent ejection of the venom.
6. Collect the venom drops directly into a plastic microtube placed close to the electrostimulator conductor (**Figure 6**).
7. Next, release the animal back into the glass container, following the steps:
    1. Transfer the animal's telson from the hand to the curved forceps.
    2. Place the animal gently on the bottom of the glass container.
8. Perform steps 4.4-4.7 with each animal in the glass recipient.
9. After all the animals in the glass recipient have been milked, place them back into the enclosure.
10. Store the collected venom at -20 °C until the lyophilization process.

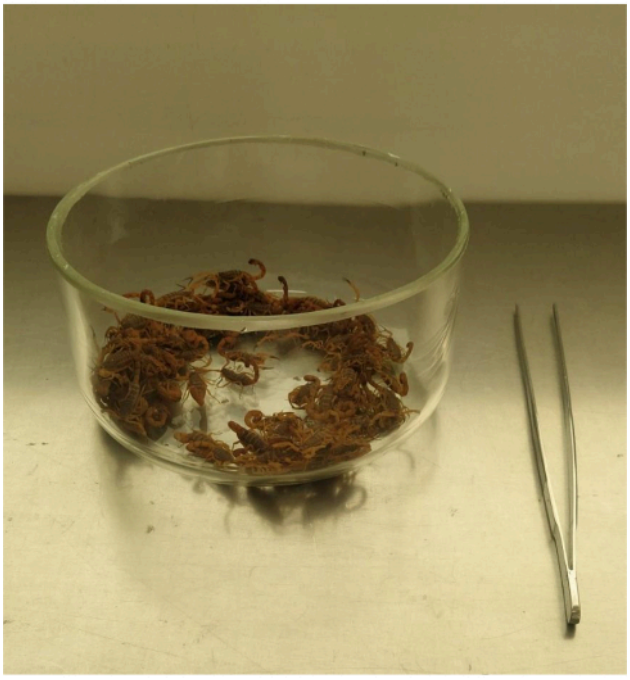

Figure 4: Scorpions in the glass container. Please click here to view a larger version of this figure.

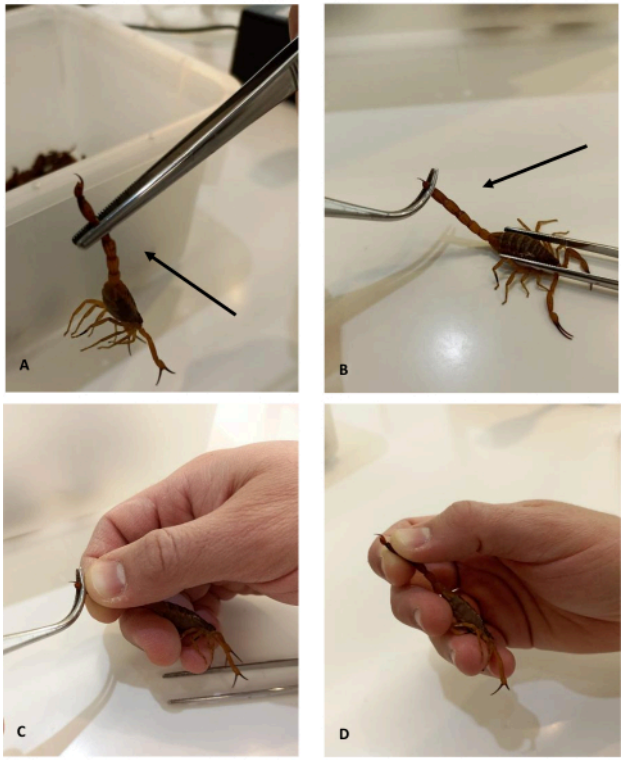

Figure 5: Restraining procedure for venom extraction. (**A**) Metassoma restrained with straight forceps (black arrow). (**B**) Telson immobilized with curved forceps (black arrow). (**C**) Transference of the restrained telson from the forceps to the hand. (**D**) Telson restrained by the hand. It is recommended that the animals be handled with bare hands since when using gloves, the scorpions can grab the material with their claws, making it difficult to release them at the end of the procedure and considerably increasing the risk of accidents. Please click here to view a larger version of this figure.

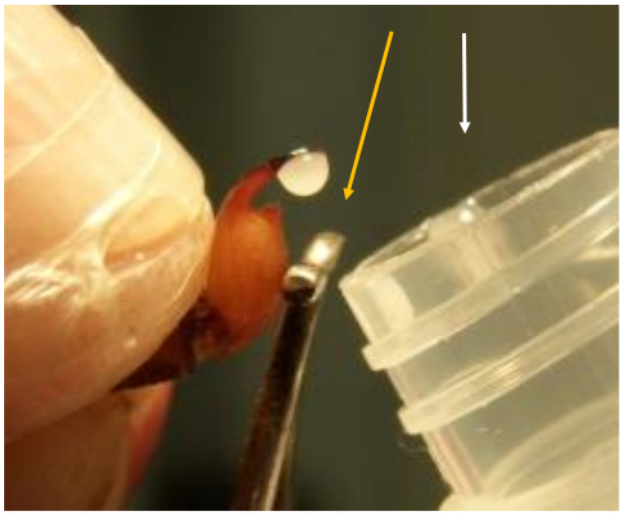

Figure 6: Venom extraction. A plastic microtube (white arrow) placed close to the electrostimulator (yellow arrow). The venom drops are collected directly into the tube maintained at room temperature during the procedure. Please click here to view a larger version of this figure.

Representative Results

Thirty standard operational procedures (SOPs) were developed for each procedure, ensuring the repeatability of the procedures among technicians and compliance with quality parameters. The average mortality rate after the extraction procedure is around 12%, which may be considered low, considering the electrostimulation routine and the captivity environment, where stress factors are successfully reduced by applying the procedures.

To keep the animals healthy, a tray covered with cotton and water is placed at one end of the box, while cardboard sheets and egg trays are positioned in layers on the opposite side, simulating the natural dark and humid habitat of *T. serrulatus* (**Figure 3D,E**). The described substrate has proven to be effective in maintaining a large number of animals in a confined space, providing enough shelter and space, thus ensuring their welfare.

Annually, the *T. serrulatus* venom production surpasses the amount of 80 grams of liquid venom, corresponding to more than 13 grams of freeze-dried venom. We have been collecting production indicators, such as the average venom milked per animal, the number of animals, and the venom amount obtained per month, as shown comparatively in **Table 1**, **Figure 7**, **Figure 8**, and **Figure 9**. The three graphics show a positive trendline, indicating a positive Pearson's correlation coefficient (respectively R= 0.68; R= 0.84; and R= 0.74). About 3,000 to 5,000 scorpions are milked monthly according to the antivenom manufacturing demand, with an average of 90% of positive extractions. Most of the animals are submitted to more than five venom extraction procedures during their productive life in the facility, which reflects the longevity of the animals, the low morbidity or venom gland

damage, and the low mortality rate. It's important to state that there is a decrease in the amount of venom obtained from scorpions that have been extracted more times, but they are still productive.

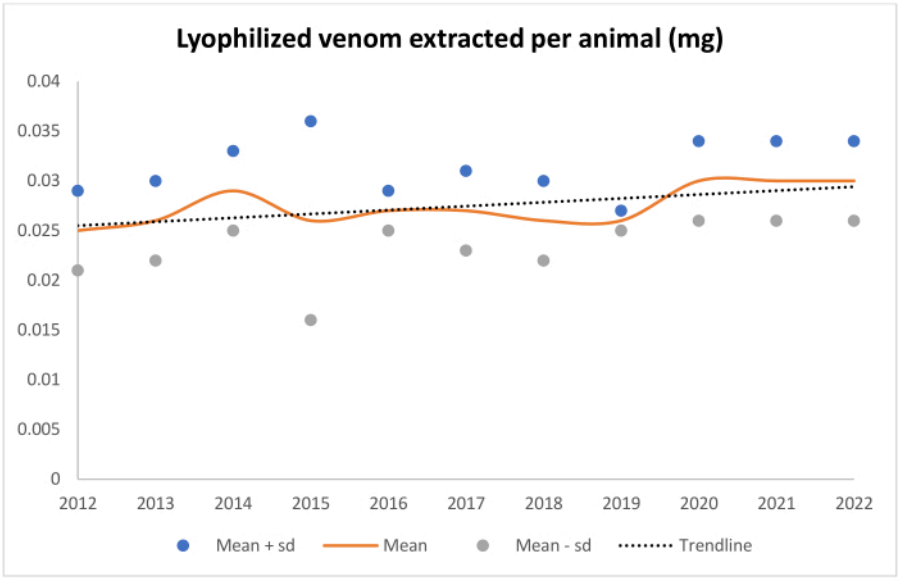

Figure 7: Comparison of lyophilized venom extracted per animal monthly (2012-2022). Please click here to view a larger version of this figure.

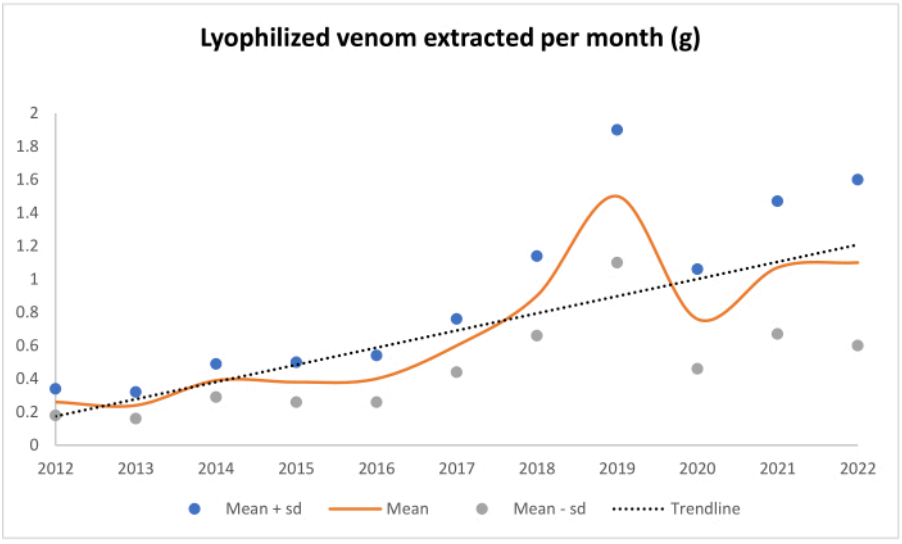

Figure 8: Comparison of lyophilized venom extracted per month (2012-2022) Please click here to view a larger version of this figure.

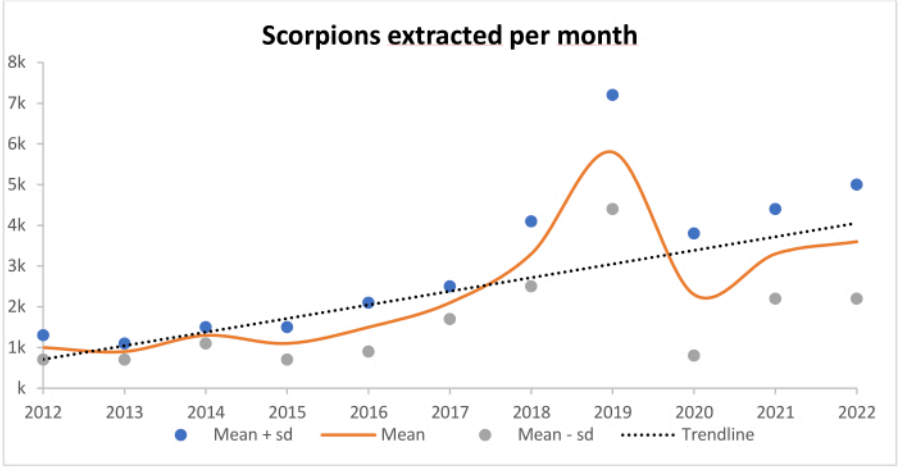

Figure 9: Comparison of the numbers of scorpions extracted per month (2012-2022). Please click here to view a larger version of this figure.

YEAR	Lyophilized venom Extracted per animal / month (mg)	Number of animals extracted / month (x1.000)	Monthly amount of lyophilized venom (g)
2012	0.025 ± 0.04	1.0 ± 0.3	0.26 ± 0.08
2013	0.026 ± 0.04	0.9 ± 0.2	0.24 ± 0.08
2014	0.029 ± 0.04	1.3 ± 0.2	0.39 ± 0.1
2015	0.026 ± 0.1	1.1 ± 0.4	0.38 ± 0.12
2016	0.027 ± 0.02	1.5 ± 0.6	0.4 ± 0.14
2017	0.027 ± 0.04	2.1 ± 0.4	0.6 ± 0.16
2018	0.026 ± 0.04	3.3 ± 0.8	0.9 ± 0.24
2019	0.026 ± 0.01	5.8 ± 1.4	1.5 ± 0.4
2020	0.03 ± 0.04	2.3 ± 1.5	0.76 ± 0.3
2021	0.03 ± 0.04	3.3 ± 1.1	1.07 ± 0.4
2022	0.03 ± 0.04	3.6 ± 1.4	1.1 ± 0.5

Table 1: Comparison of production indicators (2012-2022).

Discussion

The application of the methods described allows us to keep a large number of *T. serrulatus* individuals and gives us consistent predictability of the number of scorpions needed for annual venom production. This way, we are able to provide enough batches of venom in advance to supply the antivenom manufacturing process. At the same time, the development of pre-established schedules for maintenance, feeding, and venom extraction is an essential part of the activities and assists in complying with the described protocols. Therefore, the establishment of a routine is mandatory to maintain continuous production.

To the authors' knowledge, there are no scientifically described protocols regarding the captive husbandry of scorpions with the aim of venom production. Thus, the development and application of the described methods aim to present an efficient and successful protocol for achieving elevated production numbers, as presented in this article. The protocol for venom extraction presented here differs from other previously described methodologies as it was developed with the objective of simplifying the procedure to the maximum extent possible, due to the need to obtain large volumes of venom. Thus, the presented method allows for a large number of animals to be extracted in a short period, consequently obtaining a substantial volume of venom necessary for antivenom production. It is important to highlight that the technicians involved in handling the animals during the venom extraction procedure are rigorously trained.

The graphics illustrate the data presented in **Table 1**, showing that from 2012 to 2022, there was a linear growth in the presented markers due to the strong relationship between the year and the lyophilized venom extracted per animal, lyophilized venom extracted per month, and scorpions extracted per month, respectively. The lyophilized venom extracted per animal showed a small increase over the years, but the amount of venom per month and the number of scorpions extracted increased markedly from 2016 onwards, reflecting the physical improvements in the facility and the development and application of the SOPs. Both production markers decreased in 2020 due to the COVID pandemic but returned to increase in the following year.

Another essential objective accomplished with the application of the described methodology was animal welfare. The protocols were developed taking into account the *T. serrulatus* biology, the necessities of the species in captive conditions, and in a venom production routine, which differs from its needs when free-living[5,12]. The positive results of the methodology, in addition to the success in maintaining a standardized way of a high number of animals while respecting the scorpions' welfare and meeting the demand of venom production, are shown in the long lifespan of the animals, many of which go through more than five venom extraction procedures during their productive life under the described captive conditions.

Regardless of the protocols to be used, the continuous training of the technicians involved in the scorpion's husbandry or venom extraction is extremely important to reduce the stress caused to the animals due to the captivity routine and consequently improve the production parameters. Constant training is also essential to reduce the potential risk of accidents involving the personnel during the manipulation of the scorpions.

The husbandry protocols described here were developed according to the necessity of keeping a large number of *T. serrulatus* individuals in a limited space, and for that, the biology and physiology of the species were of great importance. Besides the specificity of the species being kept, the same protocols can be replicated for several other scorpion genera and species, with minor adjustments. The materials used are easily accessible and inexpensive, making the maintenance viable in the case of large-scale husbandry as presented. Regarding the venom extraction protocols, the electro-stimulator used was specially designed and may be replicable by other antivenom-manufacturing laboratories.

Disclosures

The authors have no conflicts of interest to disclose.

Acknowledgments

None

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