The Ethics Committee of the Butantan Institute
Dear colleagues,
On September 12th, I received a notification from *Scientific Reports* (Nature) regarding the retraction of our article, according to the justification below:

nature portfolio

Retraction Form

Manuscript Number (if applicable): 10.1038/s41598-024-59416-6

Journal Name (if applicable): Scientific Reports

Manuscript Title: Study of defensive behavior of a venomous snake as a new approach to understand snakebite

Author Name:

Retraction Statement Text (to be added by editor)

After publication, concerns were raised about the animal work reported in this paper. The Animal Ethic Committee of Butantan Institute confirmed that the approval granted to the Authors for their research on snakes (Bothrops jararaca) did not include newborn snakes or the use of the "soft stepping" method.

[Author A agreed, Author B and C disagreed, etc.]

I reiterate that no restriction on the use of the offspring was made clear, as I will clarify below.
After requesting the use of the offspring (Figure 1), we received a rejection notice due to the executor, João Miguel Alves-Nunes, having a history of ophidic accidents. We promptly complied with the committee's request and informed them that another author of the study, Adriano Fellone, would take over as the executor. After this, we received the approval notice (Figure 2).

butantan *Comissão de Ética no Uso de Animais*

CEUA N: 8444011020

Título: "A influência da temperatura no comportamento defensivo de Bothrops jararaca (serpentes, viperidae)"

Pesquisador: Otávio Augusto Vuolo Marques
Área: Lab. Especial de Ecologia e Evolução

JUSTIFICATIVA (EMENDA) (ID 038716)

Durante o desenvolvimento do projeto, nasceram 29 filhotes de Bothrops jararaca no biotério do laboratório de Ecologia e Evolução. Nosso objetivo, passou a testá-los também.
O número de acidentes botrópicos são mais elevados contra filhotes, então seria muito importante e relevante o teste com este animais.

Serão testados 29 filhotes para que seja possível realizar um modelo estatístico linear misto generalizado.Os animais serão testados da mesma forma como já mencionado anteriormente.
Os experimentos serão realizados no laboratório de ecologia e evolução e após o uso devolvidos para o biotério, como no protocolo anterior.

LISTA DE ANIMAIS

->Animais a serem adicionados

Espécie:	Répteis	Gênero:	Machos e Fêmeas	Peso:	40 e 1600 g	idade:	2 e 10 anos		
Linhagem:	Bothrops jararaca	Instalação:	Caixa	Tipo de forração:	papelão ondulado	N por área:	1 indivíduo por caixa	N:	29

Animais eutanasiados e/ou abatidos: 0 (0,00%)
Origem: Lab. Especial de Ecologia e Evolução
Dimensões da instalação: 45x30x15cm (altura x largura x profundidade, volume, etc...)
Procedência: Biotério de pesquisa e serpentário do Laboratório de Ecologia e evolução
Manutenção: O animal é mantido em uma caixa plástica
Condições de alojamento: Iluminação: Natural
Alimentação: Conforme já realizado pelos técnicos do biotério
Fonte de água: ad libitum
Exaustão de ar: Natural

Fugure 1

11/05/2022 : EMENDA

DESCRIÇÃO: O aluno João Miguel está sendo retirado do projeto como executante

18/05/2022 : APROVADO - (**PARECER CEUA**)

Figure 2

We concluded from the statement above that, with the replacement of the executor, there was authorization for the use of the offspring. This is further supported by the certificate below:

INSTITUTO BUTANTAN
A serviço da vida

Instituto Butantan de São Paulo

Comissão de Ética no Uso de Animais

CERTIFICADO : EMENDA v11/05/2022

Certificamos que a EMENDA (versão de 11/05/2022) da proposta intitulada "A influência da temperatura no comportamento defensivo de Bothrops jararaca (serpentes, viperidae)", CEUA nº 8444011020 (ID 025237), sob a responsabilidade de **Otávio Augusto Vuolo Marques** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos vigentes para sua apresentação, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), sendo assim **APROVADO** pela Comissão de Ética no Uso de Animais da Instituto Butantan de São Paulo (CEUAIB) em 18/05/2022.

Término previsto: 06/2022

ANIMAIS UTILIZADOS

		Total Aprovado	Quantidade Utilizada
Répteis	Machos e Fêmeas	30	0
Répteis	Machos e Fêmeas	124	0

São Paulo, 24 de maio de 2024

Luís Roberto de Camargo Gonçalves
Coordenador da Comissão de Ética no Uso de Animais
Instituto Butantan de São Paulo

Milene Silva Tino
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Instituto Butantan de São Paulo

Please note that the certificate issued by CEUAIB grants approval for the use of 124 specimens* (which corresponds to the initial proposal request), with an addition of 30 specimens.
Who are these 30 specimens approved for the experiments? From my understanding, the system does not differentiate between adults and offspring, nor does it specify whether they are snakes, mentioning only the total number of reptile specimens approved for use.
Thus, I conclude that the addition of 30 reptiles (as described in the certificate) refers to the approval of the use of the offspring (which were actually 29). The number was not exact, and I interpreted this as a mistake or rounding. Note also that the number of 124 specimens issued by CEUAIB is also incorrect, as the initial request was actually for 114 specimens.

Regarding the second item, I acknowledge that the change in procedure from touching the animal with a metal hook to touching it with a leather boot (softly stepped, as described in the article) was not communicated to CEUAIB.

Once again, I emphasize and clarify that touches with the hook or with the foot are similar methods for eliciting the snake's defensive response, which was one of the study's objectives. Both procedures naturally cause some stress to the animal. None of us have concrete, experimentally quantified data to determine which of the two stimuli would cause more stress to the animal. However, the vast experience of most of the article's authors, accumulated over decades of handling thousands of snakes in the field and at the Butantan Institute, does not indicate any difference in this regard.

Considering the premise that both stimuli are similar in triggering stress, we can affirm that the use of boots is less harmful to the snake, since striking a metal apparatus (hook) can cause mouth injuries and lead to the loss of fangs much more easily.

Furthermore, none of the tested animals suffered spinal injuries or died during the experiments, nor in the year following the study.

Appeal to the Ethics Committee

In light of the above, we appeal to this committee to assist in reversing the retraction of the article. A simple communication from this committee to the journal, clarifying these two points, may be sufficient:

1. There was a communication issue during the request for the offspring.
2. Although the requester failed to formally request the change in procedure for stimulating the snake's defensive behavior, we understand that the new stimulus used (*soft steps*) was not more invasive.

Retraction of an article in a scientific journal is usually reserved for extremely serious cases of professional misconduct, such as fraud or plagiarism. We understand the committee's concern in being familiar with each step of the research and the requirement that every modification or adjustment be communicated. However, if our research was not in full compliance with the expected conduct, this was due to a communication failure rather than malicious intent or dishonesty.

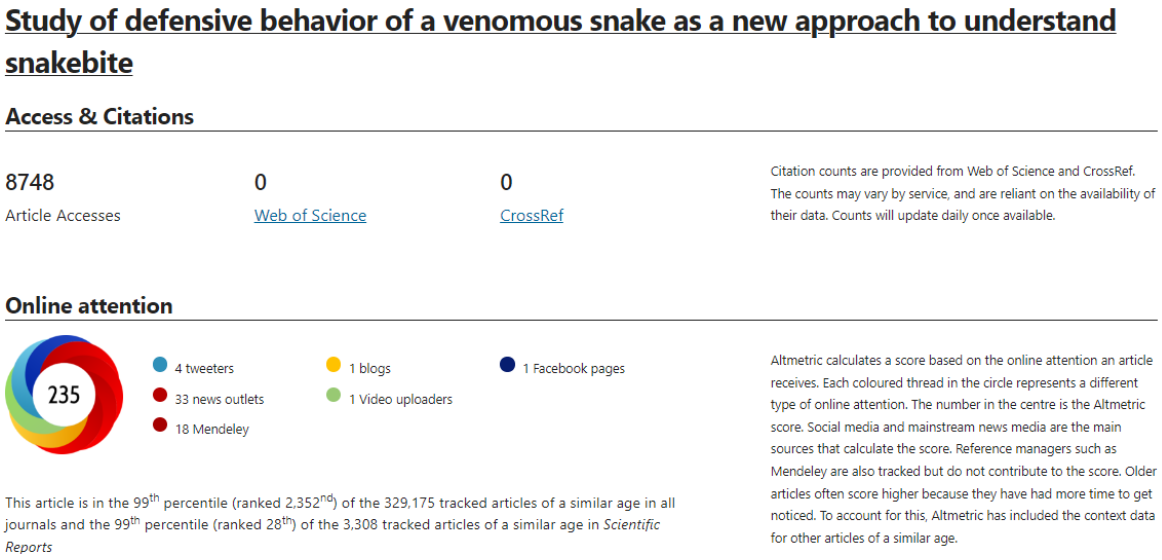
Above all, we emphasize that ethical conduct regarding live animal experimentation—avoiding excessive suffering and minimizing discomfort and the number of specimens used—was upheld. The modifications and adjustments (whether requested or not) made during the study were essential to achieving a meaningful result. Replacing a metal apparatus with the human foot allowed for a more accurate simulation of snakebite incidents involving humans, and the use of offspring provided highly relevant information on ophidic accidents, as discussed in the publication. These modifications did not violate ethical commitments to the animals used, many of which are still housed in our facility today and remain in excellent health.

The Impact of the Retraction

The article's retraction will harm the research and its primary purpose: providing insights to reduce the incidence of snakebites, which is a global public health concern and a priority for us at the Butantan Institute. We play a crucial role in combating ophidic accidents.

To understand the significance and reach of this work, it is essential to highlight some key aspects. First, this is the first study to effectively link animal behavior with the epidemiology of snakebites. The article was published in *Scientific Reports*, the fifth most-cited journal in the world. In less than 30 days after publication, the study was already cited in other articles. The impact of this research is highly significant, with excellent metrics: out of 329,175 articles published in the same period, this article ranks 2,352nd. Furthermore,

among the 3,308 articles published by *Scientific Reports* (Nature), our study holds the 28th position in terms of access and readership.



Study of defensive behavior of a venomous snake as a new approach to understand snakebite

Access & Citations

8748	0	0
Article Accesses	Web of Science	CrossRef

Citation counts are provided from Web of Science and CrossRef. The counts may vary by service, and are reliant on the availability of their data. Counts will update daily once available.

Online attention

235

4 tweeters
33 news outlets
18 Mendeley
1 blogs
1 Video uploaders
1 Facebook pages

This article is in the 99th percentile (ranked 2,352nd) of the 329,175 tracked articles of a similar age in all journals and the 99th percentile (ranked 28th) of the 3,308 tracked articles of a similar age in *Scientific Reports*

Altmetric calculates a score based on the online attention an article receives. Each coloured thread in the circle represents a different type of online attention. The number in the centre is the Altmetric score. Social media and mainstream news media are the main sources that calculate the score. Reference managers such as Mendeley are also tracked but do not contribute to the score. Older articles often score higher because they have had more time to get noticed. To account for this, Altmetric has included the context data for other articles of a similar age.

I also add the professional embarrassment faced by the authors, who are also being penalized for ethical issues related to animal welfare. Many of them are at the end of exemplary careers, always built on prioritizing animal well-being and upholding good scientific conduct.

São Paulo, September 14, 2024

Sincerely,

Otavio A. V. Marques
Laboratory of Ecology and Evolution