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SHORT NOTE

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Segmental muscle twitching behaviour in the flanks of lancehead vipers *Bothrops* spp in response to human approach

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ABSTRACT - *Bothrops* species are known to have a wide range of defensive behaviours against potential threats. Herein we show a frequently observed, but to date undocumented, behaviour in male and female *Bothrops* spp of varying sizes in newly arrived specimens from the wild, short-or long-term captives as well as in captive born individuals. The behaviour consists of irregular, synchronous or asynchronous muscular twitches in segments of the body flanks in otherwise motionless snakes. We named this 'Segmental Muscle Twitching Behaviour' (SMTB). We observed fifteen *Bothrops* spp from six species groups for incidence of this behaviour and made videos of snakes during the 'alertness' and 'threatening' phases of response to human approach. We found the behaviour in only five species, these belong to the monophyletic *Bothrops jararacussu* and *Bothrops atrox* species groups, suggesting a single evolutionary origin of this behaviour. Macroscopically, SMTB varied in the number of segments involved and the twitches had uneven intensities. Although recurrent and often replicable in similar situations during human approach, the behaviour was not displayed consistently. In addition, SMTB can be interrupted, stay inactive for quite some time and then restart. Hypotheses to explain this behaviour are suggested.

INTRODUCTION

The Neotropical pitviper genus *Bothrops* comprises forty six species (Uetz & Hosek, 2021) within six recognised species groups (Werman, 1992; Wüster et al., 2002; Salomão et al., 1997; Alencar et al., 2016). They occur in all the main ecosystems east of the Andes in South America (Hoge & Romano, 1972; Martins et al., 2001; Campbell & Lamar, 2004).

The expected passive or active antipredator displays described for *Bothrops* spp are easily witnessed during routine interactions with these snakes and include immobility/freezing, fleeing, dorso-ventral body compression/flattening, long duration tongue flicks, tail vibration, body thrash, S-coil, head elevation, strike, and bite (Sazima, 1988; 1992; Greene, 1988; 1997; Araújo & Martins, 2006). Here we document a frequently observed, but to date unreported, behaviour in two species groups *Bothrops* spp., that consists of irregular, synchronous or asynchronous muscular contractions (twitches) in segments of the body flanks in otherwise motionless snakes which we have named 'Segmental Muscle Twitching Behaviour' (SMTB).

METHODS

During reception and/or routine maintenance at the snake laboratory or public exhibition cages in the Butantan Institute, São Paulo, Brazil (IBSP), fifteen *Bothrops* spp from six different species groups and four different Brazilian biomes (Amazon, Atlantic forest, Cerrado, and Caatinga) were investigated. The lineages and species observed were as follows: *Bothrops alternatus* group (including *B. alternatus*, *B. fonsecai*), *Bothrops atrox* group (including *B. atrox*, *B. leucurus*, *B. moojeni*), *Bothrops jararaca* group (including *B. jararaca*, *B. insularis*, *B. sazimai*), *Bothrops jararacussu* group (including *B. jararacussu*, *B. brazili*), *Bothrops neuwiedi* group (including *B. neuwiedi*, *B. erythromelas*, *B. marmoratus*, *B. pauloensis*) and *Bothrops taeniatus* group (only *B. bilineatus*).

The specimens were either donated by the general public, collected by IBSP staff during fieldwork (Project 'Scales of Biodiversity' - SB/LCZ or Museu Biológico IBSP - MB) or born in captivity. Their defensive behaviours were videoed during alertness and threatening phases in response to human approach at 09:00 h to 15:00 h with a Nikon D5300 or Sony DSC-HX 300 camera (15 or 29 frames per second). Apart from SMTB, the behavioural responses listed follow the terminology of Greene (1988) and Araújo & Martins (2006).

The snakes were categorised in three groups: newly arrived, captive and captive-born. Details of these snakes are given in Table 1S (see Supplementary Material). The newly arrived specimens were studied immediately from the wild without quarantine, they had not been fed although water was freely available. They were kept individually in plastic cages of different dimensions according to their size (Grego et al., 2021). After their arrival, the subject was gently placed with a snake hook, unrestrained, on the bare ground outside the laboratory, in order to video-record it immediately at a safe distance. Air temperature during video-recording ranged from 22–31 °C. Relative humidity of the air was not measured. The captive group had been collected from

Table 1. Defensive behaviours, including Segmental Muscle Twitching Behaviour (SMTB), and a physiological adjunct exhibited by *Bothrops* spp of the *B. atrox* and *B. jararacussu* groups during human approach

Species	[1]Status	SMTB	Immobility	Head & neck elevation	Head hiding	S-coil	Flattening	Tongue flicking	Tail vibration	Body thrashing	[2]Hyper-ventilation	Strike
B. atrox♀	CW	X	-	X	-		X	X	X	X	-	-
B. atrox♂	CW	X	X	X	-	X	X	X	-	-	-	X
B. atrox♀	CB	X	X	X	-	X	-	X	-	-	X	-
B. moojeni♀	NA	X	X	-	-	X	-	X	-	-	-	-
B. moojeni♀	CW	X	-	-	-		-	-	-	-	-	-
B. moojeni♀ Juvenile	CW	X	X	-	X		X	-	-	-	X	-
B. moojeni♂	CB	X	X	X	-	X	-	-	X	-	-	-
B. leucurus♀	CB	X	X	X	-	X	-	X	-	-	X	-
B. jararacussu♀	NA	X	X	X	-	X	-	X	-	-	X	-
B. jararacussu♀	CW	X	X	X	-	X	-	X	X	-	-	-
B. brazili♂	NA	X	X	X	-	X	-	X	X	-	-	-
B. brazili♂	NA	X	X	X	-	X	-	-	X	-	X	-

[1]CW- captive wild caught, CB- captive born, NA- new arrival

[2]Physiological adjunct

Table 2. Defensive behaviours observed in *Bothrops* species of four lineages that otherwise have not been observed to display SMTB

Species	[1]Status	Immobility	Head & neck elevation	Head hiding	S-coil	Flattening	Tongue flicking	Tail vibration	Body thrashing	[2]Hyper-ventilation	Strike
B. alternatus♂	NA	X	X	-	-	X	X	-	-	X	-
B. alternatus♂	CW	-	X	-	-	X	X	-	-	-	-
B. fonsecai♀	CW	-	X	-	-	-	X	-	-	-	-
B. jararaca♂	CB	X	X	-	-	-	X	-	-	-	-
B. insularis♀	CB	X	X	-	X	X	X	-	-	-	-
B. sazimai♂	NA	-	X	-	-	-	X	X	-	X	X
B. sazimai♂	CW	-	X	-	X	-	X	-	-	-	X
B. neuwiedi♂	CW	X	X	-	X	X	X	-	-	-	-
B. erythromelas♀	CW	-	X	-	X	X	X	-	-	-	-
B. erythromelas♂	CB	-	X	-	X	-	X	-	-	-	-
B. marmoratus♂	CW	-	X	-	X	X	X	X	-	-	-
B. pauloensis♀	CB	X	X	-	X	X	X	X	X	-	X
B. bilineatus♂	CW	-	X	-	X	-	X	X	-	-	-

[1]CW- captive wild caught, CB- captive born, NA- new arrival

[2]Physiological adjunct

the wild, subject to double quarantine, and were housed individually in cages made of impermeable transparent plastic material, free from fissures, inert to disinfectants and cleaning chemicals with corrugated cardboard substrate and water freely available (Grego et al., 2021). The only exceptions were a specimen of *B. atrox* and *B. moojeni* that were kept in wooden cages (56 x 37 x 24 cm) with a glass door (Costa et al., 2005). Rooms temperatures were maintained between 23–26 °C with relative humidity around 60 % monitored with thermo-hygrometers, and a light/dark cycle of 12 h (more details in Grego et al., 2021). They had their behaviour recorded in their individual cage, upon a table, or in a transitory box during sanitising procedures. The captive born group had been kept under the same protocols as the captive group (Grego et al., 2021). Their behaviours were recorded during sanitising procedures. All those snakes in

lineages which showed SMTB had their behaviour recorded between 2005–2019, while those which did not display SMTB were recorded between January–March 2022.

RESULTS & DISCUSSION

Five *Bothrops* spp from the *B. atrox* and *B. jararacussu* species groups showed SMTB during the typical alertness and threatening phases in response to human approach (Table 1; **BHS video, 2022a**) as well as other defensive behaviours categorised as 'threatening' (Greene, 1988; Araújo & Martins, 2006), including hyperventilation (a physiological adjunct) during the alertness phase (Table 1). Macroscopically, SMTB varied in the number of segments involved, and the twitches appear to have uneven intensities. Four other *Bothrops* species groups, represented by ten other species, did not display SMTB but were recorded expressing other defensive behaviours (Table 2; **BHS video, 2022b**).

When the video footage (shot at 29 frames/sec) is viewed at half speed, the posteroanterior sense of SMTB is revealed and the number of body segments involved. Likewise, better visualised in slow motion video, subtle SMTB has been detected close to the snake´s head, and certainly anterior to the location of the heart (e.g. *B. brazili*, *B. jararacussu*, and *B. leucurus* - **BHS video, 2022a**). SMTB may or may not occur concomitantly with hyperventilation (*B. jararacussu* and *B. leucurus* - **BHS video, 2022a**). SMTB cannot be misinterpreted as a byproduct of hyperventilation, a phenomenon witnessed in all six *Bothrops* species groups, as the twitches occur along the flanks only in small segments of otherwise motionless snakes, without volumetric expansion of the snake body as consequence of lung inflation. Although recurrent and often replicable in similar situations during human approach, macroscopically the twitching behaviour is not displayed consistently either in captivity or in the wild. In addition, SMTB can be interrupted, suspended, and then restarted.

Vipers are shy and secretive creatures that rely heavily on crypsis but, when moving, crypsis may be lost so that they may be more likely to bite (Glaudas, 2021). When dealing with species whose behaviours in the field are poorly known, it is impossible to know whether behaviours recorded in the laboratory are typical of those exhibited under natural conditions (Cundall, 1987). Nevertheless, most *Bothrops* spp are endowed with a well-known and predictable defensive repertoire in response to human approach. To date, in the four *Bothrops* lineages in which we did not observe SMTB (i.e. the *B. alternatus*, *B. jararaca*, *B. neuwiedi* and *B. taeniatus* groups) this behaviour has not been observed by other researchers in either wild or captivity (Werman, 1992; Salomão et al., 1997; Alencar et al., 2016; Sazima, 1988; 1991; 1992; Araújo & Martins, 2006). It would therefore appear that this innate behavior is strongly linked to the *B. atrox* and *B. jararacussu* species groups. These two groups are monophyletic which suggests that there is just a single evolutionary origin of this behaviour.

In the case of long-term captive *B. moojeni* and *B. leucurus* kept in our laboratory, SMTB has been displayed routinely in response to human approach, apparently without any detectable degree of accommodation over time. The plethora of defensive behaviours displayed by pitvipers, particularly in *Bothrops* spp are easily perceived even by laypeople. Perhaps this may be the reason why SMTB has until now not been formally recorded in these two species groups. Because SMTB may be either explicit or subtle, several hypotheses can be suggested as to its purpose: i) as a possible distraction strategy before retaliation, ii) a warning display, iii) propelling blood to the cranial region in order to improve perception of the surroundings, or iv) an action to ensure better anchorage for a strike. In the case of a possible distraction function, it is conceivable that fast-moving, highly reactive predators, such as certain small mammals or birds, could perceive these twitches as strike precursors, and be confused by the large number of 'false alarms' generated by this behaviour.

Further research into SMTB could address whether the behaviour is displayed in darkness, the frequency and intensity of twitches under different conditions, and the impact of twitches on blood vessels flux. Furthermore, the distraction hypothesis could be tested in a staged encounter experiment with natural predators.

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