



Natural history and biological aspects of dipsadidae snakes: *P. olfersii*, *P. patagoniensis* and *P. nattereri*

História natural e aspectos biológicos de serpentes dipsadidae: P. olfersii, P. patagoniensis e P. nattereri

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ABSTRACT. Attacks by venomous snakes are a serious public health problem, accounting for 464 deaths from 2010 to 2013. However, this statistic does not seem to correspond with reality, as some poisonous snakes are not considered poisonous due to anatomic location of their fangs. The genus *Philodryas* belongs to the family Dipsadidae, comprising over 700 species. *Philodryas olfersii*, *P. patagoniensis* and *P. nattereri*, occur in the Caatinga biome and are characterized by diurnal habits, generalist diet and habitat. Although not considered poisonous, the Duvernoy's gland present in these reptiles produces toxic substances that act similar to *B. jararaca* and may cause local and systemic effects.

Keywords: venom effect; biome; reproductive cycle; sexual dimorphism.

RESUMO: Ataques por serpentes peçonhentas é um grave problema de saúde pública, sendo responsável por 464 óbitos de 2010 a 2013. No entanto esse número parece não corresponder com a realidade, já que algumas serpentes venenosas não são consideradas peçonhentas devido a localização anatômica de sua presa inoculadora. O gênero *Philodryas* pertence à família Dipsadidae, constituída por mais de 700 espécies. *Philodryas olfersii*, *P. patagoniensis* e *P. nattereri*, ocorrem na Caatinga e são caracterizadas por hábitos diurnos, dieta e habitat generalista. Embora consideradas não peçonhentas, a glândula de Duvernoy presente nesses répteis produz substâncias tóxicas com ação semelhante ao *B. jararaca*, podendo causar efeitos locais, e sistêmicos.

Palavras-chave: efeito do veneno; bioma; ciclo reprodutivo; dimorfismo sexual.

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INTRODUCTION

Envenomations represent a serious public health problem and snakes are the most frequently responsible for these accidents. In Brazil, 103,422 snakebites were registered with 464 deaths between the years 2010 to 2013. In Ceará State, during the same period, 2,328 attacks were reported (SINAN, 2013). However, this number does not seem to correspond to reality, since some poisonous snakes are not considered poisonous (LEMOS *et al.*, 2009).

This is due to the morphological characteristics of the fangs, since opisthoglyphous snakes have difficulty in injecting the venom due to the location of the fangs in the back of the upper jaw, requiring a longer period of bites to inject their venom into the prey, such as species of Dipsadidae family (MEDEIROS *et al.*, 2010; MENEZES *et al.*, 2013).

The Dipsadidae family is one of the largest families of Colubroides, consisting of more than 700 species, among them *Philodryas* spp. (UETZ, 2013). The

Philodryas genus consists of 20 species and three of these are found in the Northeast region of Brazil: *P. olfersii*, *P. patagoniensis* and *P. nattereri* (UETZ, 2013). Although not considered venomous, studies show that the Duvernoy's gland present in these reptiles produces toxic substances that act similar to the venom of *B. jararaca* and may cause local and systemic effects (Prado-FRANCESCHI *et al.*, 1998; RIBEIRO *et al.*, 1999; PEICHOTO *et al.*, 2006; ZELANIS *et al.*, 2010). However, few studies address the biological, the natural history and the effects of this kind venom, with most targeted species being *P. olfersii* and *P. patagoniensis* due to the more frequent reports of attacks by these species, including a report of a fatality caused by *P. olfersii* (SALOMÃO AND DI-BERNARDO, 1995). Therefore, the present study aimed to carry out a literature review about the biome, reproductive cycle, sexual dimorphism and biological effect of the venoms of *P. olfersii*, *P. patagoniensis* and *P. nattereri*.

HABITAT, SEASON AND DAILY ACTIVITY

P. olfersii, *P. nattereri* and *P. patagoniensis* are found in the Caatinga biome, but have different habitat behaviors (RODRIGUES, 2003; SANTOS *et al.*, 2008; MESQUITA *et al.*, 2013). *P. olfersii* is described as semi-arboreal because it is commonly found in soils and forests, however, when in the woods, it is seen more often on vegetation. *P. nattereri* is a species with generalist habitat behaviors, performing mainly terrestrial activity, although it is also arboreal and fossorial (HARTMANN AND MARQUES, 2005; MESQUITA *et al.*, 2012; MESQUITA *et al.*, 2013). MESQUITA (2010) believes that these snakes also have significant fossorial activity. But it is difficult to prove this behavior because of the difficulties in checking underground environments effectively. In contrast, some researchers claim that this species should be characterized only as terrestrial (VITT, 1980; VITT AND VANGILDER, 1983; CARVALHO AND NOGUEIRA, 1998; Costa, 2006). *P. patagoniensis* seems to be mainly terrestrial, although also perform arboreal activity to prey (HARTMANN AND MARQUES, 2005; PONTES, 2007). Ontological differences in habitat use can also be identified and may be related to survival. Juveniles are mostly found in open areas (CARVALHO AND NOGUEIRA, 1998; PONTES, 2007).

All species have similar seasonal and daily foraging activity, varying the pattern of activity during the year. In rainy months, the amount of active snakes is higher, possibly due to the higher availability of prey and to reproduction purposes. The daily activities of these snakes is exclusively diurnal, being more concentrated in the warmer periods of the day (HARTMANN AND MARQUES, 2005; PONTES, 2007; MESQUITA *et al.*, 2011; MESQUITA *et al.*, 2013).

FEEDING HABITS

The feeding habits of *Philodryas* spp. are quite diverse. Their diet consists of: lizards, frogs, mammals, birds, fish and squamata eggs. However, the preference for certain prey varies according to the species. *P. olfersii* has greater interest in frogs (HARTMANN AND MARQUES, 2005 FRANCE *et al.*, 2008; MESQUITA *et al.*, 2013), although it is common to prey on passerines (CARVALHO AND NOGUEIRA, 1998; HARTMANN AND MARQUES, 2005; OUTEIRAL, 2005; FRANÇA *et al.*, 2008; ALMEIDA AND SANTOS, 2011).

Nevertheless, BALESTRIN (2008) found in a study conducted in a sierra in Southeast of Rio Grande do Sul, that the diet of this snake was exclusively composed of birds. Although the birds are also part of *P. patagoniensis* diet and *P. nattereri*, this type of animal is more frequently preyed upon by

P. olfersii, possibly because this species use more arboreal substrate than the others do. *P. nattereri* displays preference for diets based on lizards, but squamata eggs and snakes are also a part of their diet (CARVALHO AND NOGUEIRA, 1998; MESQUITA AND BORGES-NOJOSA, 2009; MESQUITA *et al.*, 2010; MESQUITA *et al.*, 2011; GODINHO *et al.*, 2012; MESQUITA *et al.*, 2013). Intake of squamata eggs is more likely to happen among snakes that also prey on those adult squamata species (QUEIROZ AND RODRIGUEZ-ROBLES, 2006). *P. patagoniensis* are interested in frogs, but it is common to verify the occurrence of snakes of the same species in their diet. *P. patagoniensis* is possibly the only one of the three species with cannibalistic habits (LÓPEZ, 2003; HARTMANN AND MARQUES, 2005; OLIVEIRA, 2005; HARTMANN *et al.*, 2009; PONTES, 2007).

Conspecific predation is a common event in several families of snakes (Boidae, Colubridae, Elapidae and Dipsadidae) and apparently is related to snakes with generalist and ophiophagous diet (LOURDAIS *et al.*, 2004; HARTMANN AND MARQUES, 2005; FREIRÍA *et al.*, 2006; PONTES, 2007; GÖÇMEN *et al.*, 2008; Hartmann *et al.*, 2009). Quantitative (number of different items consumed) and qualitative (types of items consumed) ontogenetic variation can be observed in these species (HARTMANN AND MARQUES, 2005; PONTES, 2007;

HARTMANN *et al.*, 2009; MESQUITA AND BORGES-NOJOSA, 2009; MESQUITA *et al.*, 2011.).

This variation is influenced by the size of the snake, since juveniles have difficulty to subdue large prey and the availability associated with the use of microhabitat, which explains the higher incidence of birds in *P. olfersii* diet (PONTES, 2007). SAZIMA AND MARTINS (1990) observed that young snakes tend to perform large prey attacks, however, because the size of prey these snakes are forced to regurgitate them and seek smaller prey. MENEZES AND COLLEAGUES (2013) reported the death of *P. nattereri* after ingesting of a large lizard of the species *Tropidurus hispidus*. The authors suggested that the size of the lizard exerted pressure on internal organs hindering lung mechanics, resulting in asphyxiation. Moreover, the extra weight of ingested prey may have affected the mobility of the snake, overextending the time spent the substrate, leading to hyperthermia followed by dehydration.

REPRODUCTION AND DIMORPHISM

Snake reproduction is closely related to the fertile period of females. This period is characterized by the presence of follicles in secondary vitellogenesis and eggs in the oviduct or folded oviducts, indicating recent oviposition (SHINE, 1978). The males are considered mature when the testicles are

turgid and *vas deferens* are compressed (SHINE, 1980; SLIP AND SHINE, 1988).

According to LÓPEZ AND GIRAUDO (2008) the reproductive period of *Philodryas patagoniensis* females is seasonal. The authors noted that this species has vitellogenic period between July and October. However, BALESTRIN (2008) found that the fertile period of this Dipsadidae is longer presenting follicles between the months of October to March. The reproductive cycle of *P. olfersii* is long and occurs between the months of October to April (BALESTRIN, 2008) or between June-December (MESQUITA *et al.*, 2011). MESQUITA *et al.* (2013) reported the presence of vitellogenic follicles in *P. olfersii* throughout the year. Although females present vitellogenic follicles throughout the year, the reproductive cycle of this snake is not continuous because ovulation is clearly seasonal and restricted to November-January. Moreover, males apparently have a period of reproductive dormancy during the colder months (June to September). *P. nattereri*, like the other species, has a long reproductive cycle ranging between February and October, indicating a prolonged breeding season that was not influenced by seasonality (MESQUITA *et al.*, 2011).

All species show similar sexual dimorphism. Females have snout-vent length greater than males, but the latter have relatively longer tails. This difference, however, does not occur in *P.*

olfersii (FOWLER AND SALOMÃO, 1994; MESQUITA *et al.*, 2013). These characteristics can be directly related to the reproductive cycle, since larger females have the capacity to produce more eggs and larger tails accommodate better the hemipenis of males and facilitate intercourse (BALESTRIN, 2008; LOPEZ AND GIRAUDO, 2008; MESQUITA *et al.*, 2011; MESQUITA *et al.*, 2013). The absence of dimorphism between males and females concerning the size of the head is related to food niche, since each sex can prey on different types or sizes. Therefore, this lack of dimorphism suggests that both sexes feed on similar prey (MESQUITA *et al.*, 2011).

BIOLOGICAL VENOM EFFECTS

Snake venoms are complex mixtures of protein and non-protein components which have a wide range of biological activities (ZELANIS *et al.*, 2010). Nevertheless, these components have been widely studied in order to determine these bioactive compounds and their possible therapeutic potential. However, these studies seek substances that can be used in the production of pharmaceuticals for treating various chronic diseases such as hypertension, diabetes mellitus, cancer, etc. Some authors have observed the effect of *Crotalus durissus cascavella*, *Bothrops jararaca* and *Naja naja atra* venoms, and

the possible pharmacological potentials, by observing arterial hypotension, the increase in nitric oxide production, reduced hyperglycemia and cytotoxicity in tumor cells, inducing them to necrosis and/or apoptosis, respectively (EVANGELISTA *et al.*, 2011; JORGE *et al.*, 2011; NUNES *et al.*, 2012). Although *Philodryas* spp. venom has a similar effect to the venom of *Bothrops* spp. there are only a few studies concerning the biological effects of *Philodryas* spp. venom (ACOSTA *et al.*, 2003; PEICHOTO *et al.*, 2005; ROCHA AND FURTADO, 2007).

After inoculating the venom of a species of the family Dipsadidae, the following signs can be observed in the victim: Pain, swelling, bruising, transient bleeding, muscle necrosis and systemic effects such as dizziness and vomiting (ASSAKURA *et al.*, 1992; Prado-FRANCESCHI *et al.*, 1996; 1998; ARAÚJO AND SANTOS, 1997; RIBEIRO *et al.*, 1999; PEICHOTO *et al.*, 2007^A; PEICHOTO *et al.*, 2007^B; COSTA *et al.*, 2008; MEDEIROS *et al.*, 2010; ZELANIS *et al.*, 2010). ARAÚJO AND SANTOS (1997) reported that the bite of *Philodryas olfersii* and *P. patagoniensis* did not cause immediate pain or bleeding. However, 15 minutes after the accident, edema onset was observed that later progressed to the forearm and arm hindering the movement of the affected

limb, and forming two areas of bruises. This edema lasted for 15 days and systemic effects were not identified.

Moreover, PEICHOTO, CÉSPEDEZ AND PASCUAL (2007) observed the presence of burning pain after the bite of *P. olfersii* as well as severe vertigo, nausea and vomiting which lasted for two weeks even after drug intervention in the patient. The presence of transient bleeding, erythema, as well as the clinical signs already mentioned above have been reported but not affecting all cases diagnosed as *P. olfersii* attacks (RIBEIRO *et al.*, 1999). In contrast, bruising after the attack of *P. patagoniensis* is not observed (MEDEIROS *et al.*, 2010).

The secretion of *P. patagoniensis* Duvernoy's gland displays biologically intense edema activity and the increase of this activity is directly proportional to the amount of venom in the inoculated animal, being the minimum dose of edema identified 0.26 µg/mice (PEICHOTO *et al.*, 2004). ROCHA AND FURTADO (2007) showed that edema effect of *P. olfersii* and *P. patagoniensis* venoms was very rapid, occurring between 5 and 10 minutes after inoculation, reaching the maximum effect within 30 min and remained stable until the fourth hour after envenomation.

Some studies showed that the venom from the Duvernoy's gland of *P. patagoniensis* (ACOSTA *et al.*, 2003;

PEICHOTO *et al.*, 2005; ROCHA AND FURTADO, 2007) and *P. olfersii* (ROCHA AND FURTADO, 2007) have high hemorrhagic activity, peaking activity from two to four hours, and minimal bleeding doses of 24µg/mouse for *P. olfersii* venom and 26.9µg/mouse for *P. patagoniensis* venom (ROCHA AND FURTADO, 2007). These data are similar to results obtained in other studies that found a minimum bleeding dose of 0.5µg/mouse for *P. olfersii* venom (ASSAKURA *et al.*, 1992) and 0.27µg/mouse for *P. patagoniensis* venom (PEICHOTO *et al.*, 2007^B). The hemorrhagic activity was probably caused by metalloproteinases that degrade the secretion of basement membrane proteins from the blood vessel wall, resulting in a loss of capillary integrity, thus leading to a local bleeding. Furthermore, the fibrinolytic enzymes reduce plasma fibrinogen by hydrolysis hindering the coagulation (ACOSTA *et al.*, 2003; PEICHOTO *et al.*, 2005).

Philodryas spp. venom have high proteolytic activity, surpassing even *Bothrops alternatus* venom (GAY *et al.*, 2005) and *B. jararaca* venom (ROCHA *et al.*, 2006). This appears to a determining factor, resulting in extensive myonecrosis, characterized by the presence of pyknotic nuclei - condensation of nuclear chromatin (PRADO-FRANCESCHI *et al.*, 1998),

increasing the intensity over time (Prado-Franceschi *et al.*, 1996; 1998; Acosta *et al.* 2003; PEICHOTO *et al.*, 2007^B). ROCHA AND FURTADO (2007) proposed minimum necrotizing doses between 79.1 and 63.5µg/mouse for *P. olfersii* venom and *P. patagoniensis* venom, respectively.

This necrosis is possibly caused by the proteolytic enzymes present in the venom that degrade the extracellular matrix of tissues. This can be seen more prominently on the outskirts of the gastrocnemius muscle inoculated with the venom, where there was degradation of myofibrils, damaging and inhibiting muscle contractions, (PRADO-FRANCESCHI *et al.*, 1998). Another factor in the diagnosis of myonecrosis was increased creatine kinase (CK) in plasma, which is a specific marker for muscle damage (PRADO-FRANCESCHI *et al.*, 1998; PEICHOTO *et al.*, 2004). The Duvernoy's gland secretion of *P. patagoniensis* also presented dermonecrotic activity when tested by intradermal method in rodents, showing an intense effect (15.7µg/mouse), demonstrated by the presence of degeneration of the epidermis and signs of necrosis in the dermis, which was accompanied by an inflammatory infiltrate (PEICHOTO *et al.*, 2004).

Little is known about the biological effects of the *P. nattereri* venom.

ZELANIS *et al.* (2010) conducted a study about biochemical characterization of the *Philodryas* spp. venom and showed that the secretion of *P. nattereri* has a composition similar to the *P. olfersii* and *P. patagoniensis* venoms with high proteolytic action, but with discrete fibrinogenolytic activity. The authors also reported the presence of phospholipase A₂ activity in this genus.

The use of chelating substances have been reported to decrease or inhibit the effects of metalloproteinases present in the venom such as EDTA (ASSAKURA *et al.*, 1992; ACOSTA *et al.*, 2003; Rocha *et al.*, 2006) and the association of Na₂EDTA and Benzamidine (PEICHOTO *et al.*, 2005) or Na₂EDTA and DTT (PEICHOTO *et al.*, 2007^B), respectively. The pH change also shows a decrease in activity at acidic pH (ASSAKURA *et al.*, 1992; PEICHOTO *et al.*, 2007^B) and basic pH (PEICHOTO *et al.*, 2007^B). Therefore, the use of these substances to reduce or inhibit the hemorrhagic, necrotizing and edema activities of the *Philodryas* spp. venom is proposed.

CONCLUSIONS

Therefore, according to the information shown, the snakes of the genus *Philodryas* spp. have generalist habits and may be categorized as terrestrial, arboreal and fossorial. Furthermore, this genus has a wide variety of small animals in their

diet, and preferably frogs or lizards as main preys. Their reproductive cycle, although uncertain, seems long for *P. nattereri* and seasonal for the other two species. Like other snakes, these Dipsadidae members are characterized by females larger than males, but the latter have longer tails. The biological effects from the venoms of these snakes was shown to have potent edema, hemorrhage, as well as myo and dermonecrotic, effects often comparable with *Bothrops* sp venom.

Dessa forma, outros estudos devem ser realizados a fim de se obter mais conhecimento acerca dessas espécies, referente aos efeitos sistêmicos do veneno de *Philodryas*, especialmente *P.nattereri*, devido à escassez de informações na literatura. Portanto, a importância de se estudar e verificar os possíveis efeitos morfofisiológicos e terapêuticos dos componentes do veneno dessas espécies, torna-se de grande relevância para a comunidade científica, na busca de substâncias com potencial fisiofarmacológico.

Thus, further studies should be performed in order to obtain more knowledge about these species, targeting the systemic effects of *Philodryas* venom, especially *P. nattereri* due to lack of information in the scientific literature. Therefore, the importance of studying and verifying the morphophysiological and

therapeutic effects of venom components of these species is of great relevance to the scientific community in the search for substances with physio-pharmacological potential.

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