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Research Article

Differences in plasmatic butyrylcholinesterases (BChE) values between Pacific and Caribbean populations of terciopelo (*Bothrops asper*) in Costa Rica

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ABSTRACT

The terciopelo (*Bothrops asper*) inhabits human-modified environments such as agricultural areas, becoming more prone to be exposed to organophosphate insecticides. These chemicals can inhibit plasmatic butyrylcholinesterases (BChE) activity in *B. asper*. Caribbean and Pacific populations of *B. asper* belong to two divergent lineages that exhibit robust genetic partitioning; however, differences across versants in biochemistry and hematology have not yet been examined, especially in BChE variations. This study aims to evaluate the differences of BChE plasmatic values, hematology parameters, and some biochemical analytes as biomarkers in the presence of organophosphates between the Pacific and Caribbean population of *B. asper* in Costa Rica. A total of 89 snakes (41 Pacific and 48 Caribbean) were used, and hematology parameter, albumin, aspartate aminotransferase (AST), total protein and BChEs were evaluated. Differences in hemoglobin content, thrombocytes, white cell count, AST, and BChE values were found between both versants. Intrinsic genetic factors might influence the variation found in BChE and AST values in the snakes sampled from both versants; moreover, understanding this variation in BChE and AST values across the *B. asper*'s distribution can be useful in future ecotoxicology, biomonitoring, genetic and other clinical/health studies.

1. Introduction

The terciopelo (*Bothrops asper*) is a snake species distributed from Mexico to Colombia and Ecuador. The Caribbean distribution of *B. asper* extends continuously along the coast of the Gulf of Mexico, the Yucatan Peninsula, Central America, Panama, the Caribbean coast and inter-Andean valleys of Colombia, thence eastward across Northern and Central Venezuela (Savage, 2002; Campbell & Lamar, 2004; Solórzano, 2004; Alape-Girón et al., 2009). The Pacific distribution of *B. asper* includes the region from the Central and South part of Costa Rica, through Panama, continuing along Western Colombia, Northern Ecuador and the Northeastern extreme of Peru (Savage, 2002; Campbell & Lamar, 2004; Solórzano, 2004; Alape-Girón et al., 2009). The Caribbean and Pacific populations belong to two divergent lineages that exhibit robust genetic partitioning and account for at least ten distinct phylogroups with no mitochondrial admixture between the Central American lineages (Saldarriaga-Córdoba et al., 2017). Moreover, the Caribbean and Pacific populations of *B. asper* from Costa Rica and Panama have been separated since the late Miocene or early Pliocene (8–5 Mya) by the mountain ridge constituted by the Cordillera Volcánica Central, Cordillera de Talamanca, Cordillera de Chiriquí and Serranía de Tabasará, which represent a barrier to the dispersal of this species (Campbell & Lamar, 2004).

On the other hand, the habitat of terciopelo comprises human-modified environments where rat populations have propagated, making this species linked to agriculture and farming (Savage, 2002; Campbell & Lamar, 2004; Solórzano, 2004). Additionally, Costa Rica has suffered an increase in the intensive – extensive soil use mainly by pineapple and banana crops in both versants; and therefore, increasing

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the pesticide use in the plantations (Sánchez-Hernández, 2001; Badii and Varela, 2008). Furthermore, the terciopelo is the most abundant pit-viper wherever it occurs, with high odds of human encounters when compared to other snakes inhabiting agricultural zones (Campbell & Lamar, 2004; Solórzano, 2004).

Habitat use and population density of *B. asper* increase the probability of exposure to chemical pesticides used in agricultural areas, such as organophosphates (OP) and carbamates (CB). Organophosphate compounds are within the insecticides more widely used in agriculture, affecting several wildlife species that occur near plantations (Sánchez-Hernández, 2001). Reptiles, especially snakes, have been the least studied animals in ecotoxicology. Snakes can be profoundly affected by pesticides because of their long sedentary periods and low metabolic rate (Hopkins, 2006).

Mechanisms from which this species can be intoxicated deserve further investigation, but due to its terrestrial habits, the most likely way of exposure is dermic by soil contact (either by bioaccumulation or runoff waters) or by aerial dispersal of insecticides. Moreover, organic pesticides can adhere and be retained at the organic matter through 26 weeks (Badii and Varela, 2008) and dermic absorption of OP compound has been demonstrated in *B. asper* (Arguedas et al., 2018). Therefore, a non-destructive method to determine whether the snakes have been in contact with OPs is needed to assess their health and the inhibition of cholinesterase activity has been tested as a biomarker in ecotoxicology studies using terciopelo snakes (Arguedas et al., 2018).

Vertebrates present two types of enzymes with cholinesterase activity classified according to their substratum: acetylcholinesterase (AChE) and butyrylcholinesterases (BChE) (Nunes, 2011). In reptiles, around 75–80% of the cholinesterase activity in plasma is due to butyrylcholinesterase (Sánchez-Hernández & Moreno-Sánchez, 2002; Bain et al., 2004) while over 80% of the acetylcholinesterase activity occurs in the brain (Schmidt, 2003). The inhibition of cholinesterases is commonly used as an index in biomonitoring programs (Sánchez-Hernández, 2003) and BChE has been used as an exposition biomarker to evaluate the effect of organophosphates (Hopkins, 2006; Mitchelmore et al., 2006).

It has been demonstrated that BChE activity can be inhibited in *B. asper* when exposed to organophosphate compounds (Arguedas et al., 2018). Nonetheless, the inhibition itself should not be considered as an indicator of exposure, mainly because natural BChE levels are highly variable and might be influenced by factors such as circadian cycles, sex, species, age and the route of exposure to the toxic (Basso et al., 2012). Therefore, the natural variation in BChE that occurs in response to environmental and biological factors must be considered to avoid misinterpretation of normal values in the species assessed (Forbes et al., 2006).

In addition to BChE evaluation, a clinical assessment should be performed in the animals to obtain the most valuable information regarding the health status of the snakes (Gómez et al., 2016; Arguedas et al., 2018). Thus, complete hematology analysis and at least some biochemical analytes such as albumin, aspartate aminotransferase (AST) and total protein (TP) content, should be tested (Gómez et al., 2016). Moreover, these biomarkers must be measured to accomplish an integral evaluation of the species considering possible physiologic alterations caused by a toxic effect of the pesticides or by other environmental pollutants (Andreadis et al., 2014).

As mentioned before, the BChEs may be influenced by several factors, including biogeographically separated populations of the same species. Therefore, the potential use of BChE as biomarker against intoxications by OP in separated populations within the same species is relevantly important to study.

Thus, this work aims to evaluate the differences of BChE plasmatic values, hematology parameters and differences in albumin, aspartate aminotransferase and total protein content, between the Pacific and Caribbean population of *B. asper* in Costa Rica as biomarkers in the presence of organophosphates.

2. Materials and methods

The animal use was approved by the "Comité Institucional para el Cuido y Uso de Animales de Laboratorio (CICUA)," of Costa Rica University (CICUA 065-17). All procedures comply with the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 1986) and meet the ARRIVE guidelines (Kilkenny et al., 2011).

2.1. Snakes selection and maintenance

Bothrops asper, as an animal model, was chosen because of its peculiar distribution near rural human settlements and highly association to agricultural plantations (Campbell & Lamar, 2004; Solórzano, 2004). Moreover, *B. asper* is likely being exposed to chemical pesticides such as OP and CB, which are a threat to wild animals due to the well-known toxic effects (Mitchelmore et al., 2006). Since *B. asper* is frequently found near crops, the evaluation of ecotoxicological and health aspects area rapidly applied. Lastly, this snake species is kept in captivity at the Clodomiro Picado Institute' Serpentarium, which makes possible conducting microcosm studies in ecotoxicology.

The following criteria were followed to consider the use of the animals: recently received animals in quarantine time (≤one week), clinically healthy animals, no internal or external parasites, and ≥1 m of total length. A total of 89 snakes were used in the course of 2 years (2016–2018), 41 of which arrived from locations of the Pacific versant and 48 from the Caribbean versant of Costa Rica. The most common localities from the Caribbean slope were: Siquirres (Limón), Río Cuarto (Grecia, Alajuela) and La Virgen (Sarapiquí, Heredia); whereas the most common localities from the Pacific slope were: Parrita (Puntarenas), Candelarita (Puriscal), Quepos (Puntarenas), and Bahía Ballena (Osa, Puntarenas) (Fig. 1). All snakes were kept in acrylic terraria within temperature-controlled rooms (26–28 °C) and controlled humidity (80–90%). Feeding was done every 15 days with laboratory mice (*Mus*

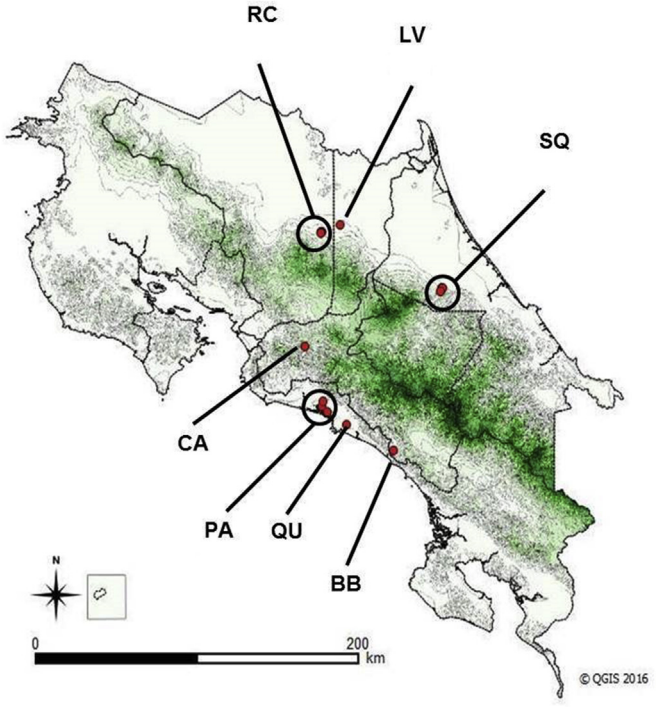

Fig. 1. Map of Costa Rica showing collection sites near agricultural plantations. Each red dot represents an individual of *Bothrops asper* (n = 14). RC (Río Cuarto, Grecia, Alajuela), LV (La Virgen, Sarapiquí, Heredia), SQ (Siquirres, Limón), PA (Parrita, Puntarenas), QU (Quepos, Puntarenas), BB (Bahía Ballena (Osa, Puntarenas), CA (Candelarita, Puriscal, San José).

musculus, CD-1 strain), and water was provided ad libitum. The sex ratio was 1.1 : 0.8 females (n = 47) to males (n = 42).

2.2. Localities

In the Pacific slope, Parrita (23 masl), Quepos (5 masl) and Bahía Ballena (0 masl) are categorized as Lowland Pacific Wet Forest. This habitat is by far the richest in terms of species diversity and biotic interactions; very rich herpetofauna is represented here (Savage, 2002; Solórzano, 2004). It is characterized by tall, multistratal, evergreen forest; a few canopy tree species are briefly deciduous, but this does not change the overall evergreen aspect of the forest. Canopy trees are 45–55m tall, with round to umbrella-shaped crowns, and have clear boles to 30m and attaining 100–200cm dbh (diameter to breast high). Smooth, thin, light-colored bark and high buttresses are common. Subcanopy trees are 30–40m tall, with narrow conical crowns and slender boles often twisted or crooked, usually with smooth dark bark. Stilt-rooted palms are often abundant. The shrub layer is 1.5–2.5m tall with abundant dwarf palms, unbranched treelets and giant broad-leaved herbs are occasionally. The ground layer is sparse with a few ferns. Woody lianas are not common, and epiphytic shrubs and strangling trees are rare (Savage, 2002; Solórzano, 2004), whereas Puriscal (483 masl) is categorized as a premontane wet forest. This kind of forest is found along the lower slopes of the mountains where there is a long dry season because of the northern Pacific regime of rainfall or a rain shadow. This type of forest supports a wide variety of amphibians and reptiles. Because this life zone lies in the foothill regions, many lowland species range upward into the premontane zone, and a number of lower montane forms range downward into the zone as well (Savage, 2002; Solórzano, 2004).

Regarding the Caribbean slope, Siquirres (62 masl) and Sarapiquí (292 masl) are categorized as Lowland Atlantic Wet Forest. This is the richest habitat for amphibia and reptiles on the Atlantic versant and is comparable in species diversity to the lowland Pacific wet forest (Savage, 2002; Solórzano, 2004), and it is similar to the lowland Pacific wet forest in terms of vegetal composition. While Grecia (1000 masl) is categorized as a premontane wet forest. This forest is medium to tall, semi-evergreen forest with two or three strata, with a few canopy species dry-season deciduous. Canopy trees are mostly 30–40m tall, with mostly round to spreading crowns and relative short, clear boles. Buttresses are common but small. The bark is mostly brown or grey, moderately thick and flaky or fissured. Leaves are often clustered at the twig ends. Understory trees are 10–20m tall with deep crowns and smooth, often dark bark. Stilt roots and long strap-shaped leaves are common. Tree ferns are occasional. The shrub layer is 2–3m tall and often dense. The ground layer is generally bare except for ferns. Epiphytes are present but not conspicuous. Climbing herbaceous vines are abundant (Savage, 2002; Solórzano, 2004).

2.3. Blood sampling and processing

Body condition and physical examination, according to Divers (1999), was conducted before the blood withdrawal. Additionally, the veterinary medical experience in wild animals, especially venomous snakes, was considered as a selection criterion for assessment. Lastly, all the snakes were examined for apparent abnormalities or lesions.

Blood was obtained from the coccygeal vein using a 23G needle attached to a 3.0 ml syringe and transferred to heparin tubes. Blood samples were centrifuged to obtain plasma, which was collected in Eppendorf® tubes and transported at 4 °C to the Laboratory for Clinical Analysis of the University of Costa Rica. The hematology analysis was performed following Salakij et al. (2002). Briefly, a total blood smear was made and dyed with Wright dye after air-dried. The differential count was made using a 100x magnification. Leukocytes were classified in heterophils, eosinophils, basophils, monocytes, and lymphocytes, according to Campbell (2015). The plasmatic cellular volume (PCV) was determined using capillary tubes and centrifuged at 10000G for 5 min. Hemoglobin concentration was quantified by the cyanmethemoglobin method, centrifuged to remove all nuclei debris before absorbance (540 nm) was recorded. The cellular counts were quantified by a Neubauer chamber using a 1/200 blood dilution with Natt-Herrick solution and leaving the solution 5 min before reading using 40x magnification. The red blood count (RBC) was measured according to the technique described by Salakij et al. (2002).

Because the liver is the main organ where BChE is produced, biochemistry analytes that could detect liver problems (aspartate aminotransferase, total protein, albumin), as well as the BChE, were quantified. Approximately 10μL of plasma per individual were used for the analysis. A variation in Ellman et al. (1961) spectrophotometry method for BChE detection was used. Tests were performed by the ACE automatized (Alfa-Wassermann, Amsterdam, Holanda) that uses photometry for the results and using Alfa-Wassermann (San José, Costa Rica) reagents.

2.4. Statistical analysis

A one-way ANOVA test assessed the differences between Caribbean and Pacific versants regarding hematological parameters as well as the biochemical analytes and BChEs values. Normality and homogeneity of variances were tested by Shapiro-Wilks and Levene's tests, respectively. Values of hematological parameters and biochemical analyses were expressed as mean ± se, and values of $P < 0.05$ were considered statistically significant. The map was elaborated with QGIS Las Palmas version 2.18.0 (Open Source Geospatial Foundation Project, 2016), layer from Atlas digital de Costa Rica (2008).

3. Results

After a physical examination of all individuals, no evidence of lesions, injuries, secretions, or infections were detected on the eyes, nostrils, loreal pits, and oral cavity. No evidence of articular injuries in the body and tail; the skin appeared healthy, and none of the females were gravid based on abdomen palpation. Lastly, cloacal mucosa looked healthy in all the animals, and no abnormalities were observed on the cloaca.

For hematology (Table 1), total protein, and albumin (Table 2), similar values were found between Caribbean and Pacific populations, excepting for hemoglobin content, thrombocytes, white cell count and AST values where Caribbean and Pacific versants were statistically different. Additionally, the mean snout-vent length (SVL) of the animals was 128.6 ± 16.8cm, the mean total length (TL) was 145.9 ± 20.0cm, and the average weight was 1046.2 ± 341.3g (Table 3).

The BChE values of the Caribbean population sampled was 10055.56 ± 1037.25 U/L with a range of 48.18–25626.23 U/L, whereas the Pacific population sample was 2552.20 ± 390.00 U/L with a range of 29.82–9348.16 U/L; which are statistically different ($F= 40.68$, $df= 1; 87$, $P < 0.0001$).

4. Discussion

We performed a clinical assessment based on hematology and some biochemical parameter values because blood evaluation is one of the most objective ways to assess health status in animals. Individuals in our study seemed to be clinically healthy, and no significant differences were found in hematology (Table 1) and biochemical analytes (Table 2) when compared with reference values (Gómez et al., 2016). Nevertheless, we found significant variations in the hemoglobin content, thrombocytes, white cells count, BChE, and AST values of *B. asper* when both versants of Costa Rica (Caribbean and Pacific) were compared.

The variations in hemoglobin, thrombocytes and white cells count seem not to be insightful to elaborate differences in terms of the health status of the snake studied and should be considered and tested against interval reference values (Gómez et al., 2016). Moreover, hemoglobin variation is not always related to any anemic conditions (lowest values)

Table 1
Hematological parameter values of *B. asper* from both versants of Costa Rica.

Variable (units)	Versant	
	Pacific (n = 41)	Caribbean (n = 48)
	Mean ± se (range)	Mean ± se (range)
Hemoglobin (g/dL) *	7.18 ± 0.52 (2.70–19.65)	8.80 ± 0.51 (3.45–19.02)
TRBC (μL)	438964.39 ± 27547.29 (120600.00–986910.00	489979.37 ± 23365.76 (211050.00–944700.00)
Thrombocytes (μL) *	6569.26 ± 1323.26 (0.00–35175.00)	2491.55 ± 277.88 (502.50–9045.00)
WCC (μL) *	14008.71 ± 2656.26 (2010.00–75375.00)	7736.39 ± 682.14 (2512.50–26130.00)
PCV (%)	17.32 ± 0.75 (5.00–26.00)	16.42 ± 0.71 (4.00–30.00)
Lymphocytes (μL)	6489.60 ± 1047.15 (844.20–35034.30)	5408.77 ± 515.97 (361.80–18552.30)
Monocytes (μL)	1965.64 ± 299.12 (261.30–9165.60)	1783.97 ± 241.21 (125.63–8321.40)
Heterophils (μL)	360.20 ± 95.69 (0.00–2412.00)	184.24 ± 32.15 (0.00–1195.95)
Eosinophils (μL)	189.60 ± 70.69 (0.00–2532.60)	582.26 ± 469.35 (0.00–22612.00)
Basophils (μL)	198.82 ± 93.30 (0.00–3859.20)	117.24 ± 22.33 (0.00–783.90)
Lymphocytes (%)	69.70 ± 3.27 (9.00–91.00)	71.58 ± 2.58 (4.00–95.00)
Monocytes (%)	24.04 ± 3.00 (4.00–87.00)	22.95 ± 2.40 (4.00–92.00)
Heterophils (%)	2.87 ± 0.58 (0.00–19.00)	2.27 ± 0.29 (0.00–8.00)
Eosinophils (%)	1.63 ± 0.39 (0.00–10.00)	1.54 ± 0.30 (0.00–9.00)
Basophils (%)	1.73 ± 0.29 (0.00–8.00)	1.58 ± 0.22 (0.00–6.00)

TRBC total red blood cells, *WCC* white cell count, *PCV* plasma cellular volume, *se* standard error, *$P < 0.05$.

Table 2
Biochemical analyte values of *B. asper* from both Versants of Costa Rica.

Variable (units)	Versant	
	Pacific (n = 41)	Caribbean (n = 48)
	Mean ± se (range)	Mean ± se (range)
Albumin (g/dL)	1.30 ± 0.03 (0.41–1.82)	1.28 ± 0.03 (0.64–1.81)
AST (μL) *	63.32 ± 16.39 (3.20–653.00)	28.73 ± 4.54 (2.70–174.00)
TP (g/dL)	4.26 ± 0.12 (2.40–6.50)	4.29 ± 0.11 (2.20–6.20)
Cholinesterase *	2552.20 ± 390.00 (29.82–9348.16)	10055.56 ± 1037.25 (48.18–25626.23)

AST aspartate aminotransferase, *TP* total protein, *se* standard error, *$P < 0.05$.

Table 3
Morphometric data of the animals used, divided by versants and according to sex.

Variable (units)	Versant			
	Pacific (n = 41)		Caribbean (n = 48)	
	Sex			
	Female (n = 22)	Male (n = 19)	Female (n = 25)	Male (n = 23)
	Mean ± sd			
Weight (g)	1160.4 ± 455.3	740.0 ± 263.5	1242.3 ± 106	966.0 ± 328.7
SVL (cm)	134.8 ± 13.6	119.3 ± 12.6	138.6 ± 10.1	119.5 ± 23.5
TL (cm)	152.5 ± 16.3	137.0 ± 12.5	157.1 ± 10.6	134.8 ± 30.3

SVL snout-vent length, *TL* total length.

(Saggese, 2009), or considered higher due to altitude variations in reptiles (González-Morales et al., 2017); since in both versants, Pacific and Caribbean, the biogeographical distribution of *B. asper* is similar. The health status, leucocytes, and thrombocytes may vary due to stress when animals were captured, age, environmental factors, season, diet, and even between captive and wild populations (Gómez et al., 2016).

However, considering that the animals studied came from both versants, different diets and submitted to the same handling stress when collecting the blood sample, the variations in hemoglobin, thrombocytes, and white cells count might be related to intrinsic metabolic or genic differences (Sasa et al., 2009; Saldarriaga-Córdoba et al., 2017).

The BChE has a role in detoxication of poisons that are eaten or inhaled (Lockridge, 2015). Examples in humans are physostigmine (eserine) in the Calabar bean and cocaine in the leaves of the coca plant that are naturally occurring toxicants and hydrolyzed by BChE to inactive products (Lockridge, 2015). Organophosphorus esters, including nerve agents, pesticides, and a neurotoxic anticholinesterase called anatoxin-a(s), are scavenged and destroyed by BChE. Other naturally occurring organophosphorus esters produced by *Streptomyces* bacteria and the marine sponge *Ulosa ruetzleri* have been identified as inhibitors of AChE and presumed to inhibit BChE (Lockridge, 2015).

Exposure to organophosphate insecticides have an effect in BChE plasmatic activity in reptiles (Fossi et al., 1995; Sánchez et al., 1997; Khan, 2003; Schmidt, 2003; Bain et al., 2004; Mitchelmore et al., 2006), including *B. asper* (Arguedas et al., 2018). Although Arguedas et al. (2018) reported significant inhibition of BChE values when *B. asper* was experimentally exposed to an OP (chlorpyrifos), we suggest that the animals from this study were not exposed acutely (at least 22 days before sampling) to OP insecticides, a condition necessary to detect exposure.

In addition, morphological, genetic, behavioral and ecological differences (Solórzano, 2004; Sasa et al., 2009), as well as differences in the venom proteome (Alape-Girón et al., 2009; Lomonte et al., 2014), have been described previously. Therefore, it should not be surprising that physiological differences also occur in both versant populations. Moreover, BChE values can be very variable for different reasons, including genetic variation, routes of exposition to pesticides, the health of the animal, nutritional state, diet, age, sex, seasonality, and conditions of sample storage (Arguedas et al., 2018).

Other reported causes of BChE variation in vertebrates that are not probably causing an effect in the study are stress, health impairment, and sex. However, BChE values of vertebrates can be altered by environmental or physiological stress (Rattner and Fairbrother, 1991); likewise, handling of individuals can cause stress and should be considered a source of variation. Although hematology analysis did not show acute stress in our samples (see also Arguedas et al., 2018). Health status has been studied as an important cause of variation in BChE values since the presence of several diseases can significantly alter these values (Greig et al., 2002; Soliday et al., 2010; Mikecin et al., 2013; Grosset et al., 2014).

The genetic divergence has been referred to as an important factor of variation in BChE values (Pantuck, 1993). Significant differences in BChE values within families of vertebrates have been detected in fish (Chuiko et al., 2003). In humans, a single gene with nucleotide alterations is responsible for several variants of BChE (Pantuck, 1993) and values can range between 0-70% among ethnicities (Soliday et al., 2010). *Bothrops asper* exhibits robust genetic partitioning that accounts for at least ten distinct mitochondrial phylogroups included in two separate lineages (Saldarriaga-Córdoba et al., 2017). The groups occupy different geographic regions and show private haplotypes, indicating a clear division among them (Saldarriaga-Córdoba et al., 2017). Therefore, a genetic component might play a role to account for the BChE differences detected between Caribbean and Pacific populations of Costa Rica, although this requires further research.

Bothrops asper populations also show remarkable differences in natural history traits (e.g., search and selection of prey items) and venom composition (Savage, 2002; Campbell & Lamar, 2004; Solórzano, 2004; Alape-Girón et al., 2009; Lomonte et al., 2014; Saldarriaga-Córdoba et al., 2017). Venom composition could be a result of diet in vipers (Daltry et al., 1996). As the number of prey items consumed by terciopelo (i.e., anurans, reptiles, birds, mammals, and some large arthropods) can be very variable across its range (Sasa et al., 2009; Wasko and Sasa, 2012), diet could have an effect on venom composition and cause variation in BChE values. The link between diet and BChE values have been

observed for other vertebrate species. For example, low BChE values have been reported in rats that consume cod oil (Narvaez et al., 2015) and differences have been detected between granivore (higher BChE values) and insectivore and carnivore (lower BChE values) passerine birds (Van Lith et al., 1992).

Because BChE has a role in detoxification of different chemical compounds, an increase in the consumption of organisms secreting toxins (e.g., amphibians and some arthropods) would increase BChE values. This could be the case for Caribbean populations of *B. asper* in Costa Rica, where higher BChE values were detected. Although no significant differences in prey items have been reported for Pacific and Caribbean populations of *B. asper* in Costa Rica (Sasa et al., 2009; Lomonte et al., 2014), this requires more detailed analysis in the light of the BChE variation detected in this study. Additionally, the increasing soil use for intensive – extensive crops, such as banana and pineapple in the Caribbean side of Costa Rica, has enhanced the use of pesticides with the related pollution of rivers and impacting the surrounding micro-environments (Sánchez-Hernández, 2001; Badii and Varela, 2008).

Lastly, the variation found in AST values from both versants might be a casual finding, since high variations in AST may occur due to manipulation and tail puncture leading to a rise in AST values (Dallwig et al., 2011; Arguedas et al., 2018). The AST is produced in various tissues including skeletal muscle and liver (Campbell, 2015); moreover, differences in liver metabolism and enzyme production are still unknown and, as BChEs are produced in the liver, deserve further research to understand these differences.

The liver function evaluation in reptiles is usually performed using AST because of its synthesis in the hepatic tissue and skeletal muscle (Campbell, 2015). Although not determinant of liver damage, the use of other enzymes such as alkaline phosphatase (ALP) and alkaline transaminase (ALT), deserves precaution. The ALP is widely distributed in the reptilian body, and plasma activity is not considered to be organ-specific. Little information is available concerning the interpretation of increased plasma ALP activity in reptiles; however, increased activity may reflect osteoblastic activity rather than a hepatobiliary disease (Thrall et al., 2006; Campbell, 2015).

Furthermore, the ALT is not considered to be organ-specific in reptiles and may not be reliable in the detection of hepatocellular disease (Thrall et al., 2006; Campbell, 2015). Nevertheless, enzymes such as AST should be interpreted with precaution when used for reptile health diagnosis.

5. Conclusions

In conclusion, the differences observed between *B. asper* populations from the Pacific and Caribbean slopes might have an intrinsic origin such as genetic or biochemical, due to the ancestral separation of both races. The use of biomarkers, such as BChEs and AST, is essential to determinate whether an inhibition due to OP or CB is occurring, and therefore, an intoxication of the animal is undergoing. However, establishing normal values, especially in BChE, may be demanding since a considerable variation has been documented (Gómez et al., 2016; Arguedas et al., 2018), and its interpretation and further clinical implications should be addressed carefully.

Finally, we demonstrate that there are significant differences between Caribbean and Pacific *B. asper* BChE and AST values, hence knowing normal values can be very useful in future ecotoxicology, biomonitoring, genetic or other health studies for this species.

Declarations

Author contribution statement

Randall Arguedas, Aarón Gómez: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Paloma Alcázar, Marco D. Barquero: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Danilo Chacón, Greivin Corrales: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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