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Maturity stage categorization of endemic Sri Lankan Green Pit Viper (*Peltopelor trigonocephalus*) in Sri Lanka

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Abstract

The Sri Lankan Green Pit Viper (GPV), *Peltopelor trigonocephalus* (Donndorff, 1798), is a medically important snake species in Sri Lanka. It is a high-potential venomous species endemic to Sri Lanka. Data on sex-specific morphological differences are scarce. A visual encounter survey was utilized to observe GPV and obtain their morphometric measurements. The morphometric data was analyzed using Principal Component Analysis (PCA), which identified three distinct clusters that were divided into the three maturity phases of adult, sub-adult and juvenile. Positive values for all parameters were represented on the Principal 1 (PC1) axis, which showed a high variance percentage (90.1%) and related proportional relationships between the values, showing that when one parameter increases, the other parameters follow suit. The morphometric parameter with the greatest Dim 1 value (0.979) was found as total length (TL), which had significant impacts on the PC1 axis. TL was used as the primary measurement to visually categorize maturity stages. Adult females had the highest values for all of the variables evaluated. According to the findings of this study, the maturity stages of GPV species were successfully categorized using morphometric data.

Keywords: Morphometric, *Peltopelor trigonocephalus*, Principal Component Analysis, Sri Lankan Green Pit Viper

Introduction

Most studies have examined sexual size dimorphism in snakes (Solórzano and Cerdas, 1989; Rivas and Burghardt, 2001; Krause et al., 2003; Furtado et al., 2006; Pinto et al., 2008). Nevertheless, only a few studies have examined sexual shape dimorphism (Gregory, 2004; Vincent et al., 2004a; Smith and Collyer, 2008; Tomovic et al., 2010). The evolutionary transition towards viviparity is often correlated with a consistent shift towards sexual size dimorphism, where females tend to exhibit larger body size relative to males (Shine, 1994). Apart from sexual dimorphism, snakes can exhibit a range of changes throughout their lifespan. Juvenile and adult snakes may differ in terms of body size, as well as various behavioral, morphological, and physiological traits, which can result in ontogenetic shifts in diet, coloration, foraging behavior, habitat preferences, and venom composition (Sazima, 1991; Lind and Welsh, 1994; Vincent et al., 2004a, b, 2006; Zelanis et al., 2007). Snakes of the genus *Peltopelor* are all arboreal or semi-arboreal and nocturnal (Strine, 2014). They camouflage themselves in trees and lush vegetation during daytime and have slender elongated bodies than most terrestrial pit vipers to facilitate movement through trees and bushes above the ground (Malhotra et al., 2011). The current study concentrated on the morphometries of the endemic *Peltopelor trigonocephalus* since recording the ecological requirements and interactions of different maturity stages within a population helps to better understand the macroscale habitat requirements and microhabitat requirements of a species.

Peltopelor trigonocephalus is commonly known as the Sri Lankan Green pit viper or ‘Palapolaga’. It is widely distributed in all three climatic zones of Sri Lanka, except higher hills and arid zones. Three primary climatic zones, wet zone, intermediate zone, and dry zone have been established based on the amount and distribution of annual rainfall received. The wet zone encompasses a region with a reasonably high mean annual rainfall of over 2,500 mm and no significant dry spells. The Dry Zone is defined as a region with an average annual rainfall of less than 1,750 mm and a distinct dry season that lasts from May to September. The Intermediate zone denotes a region with an average annual rainfall of 1,750 mm to 2,500 mm and a brief but noticeable dry season. Despite the fact that these climatic zones were defined by the amount and distribution of annual rainfall, other physical variables such as soil, topography, height, vegetation, and land use had a significant role in their formation (Pathmarajah,2016).

Moreover, this species is relatively more common in the wet zone and rainforest areas and is found occasionally in plantations of cardamom, cocoa, coffee and tea spanning over altitudes from 153 to 1,000 m (De Silva,2009).

One of the most colorful and attractive snakes that can be observed in Sri Lanka is *P. trigonocephalus*. From head to tail, the dorsal surface is mottled and variegated with black patterns. On either side, a black stripe runs from the eye to the angle of the jaw (De Silva and Aloysius, 1983). Between the eye and the snout, there are two loreal pits on either side of the head (Rathnayaka et al., 2017; De Silva and Aloysius, 1983). It is bright green on the ventral side, with yellowish lateral margins. It features a long, thin body, a large, triangular head, a neck that is clearly constricted, and a short, prehensile tail. Despite their sexual dimorphism, males and females cannot be distinguished based on appearances.

In this paper, we present preliminary results of the morphological analyses of *P. trigonocephalus* populations. A forthcoming paper will present detailed morphological variation of all populations of Green Pit Vipers currently known as *P. trigonocephalus*, compared to data provided by mtDNA analyses.

Material and methods

Fieldwork was carried out during a one-year period, between April 2021 and March 2022, encompassing the wet zone, intermediate zone, and dry zone (Fig. 1).

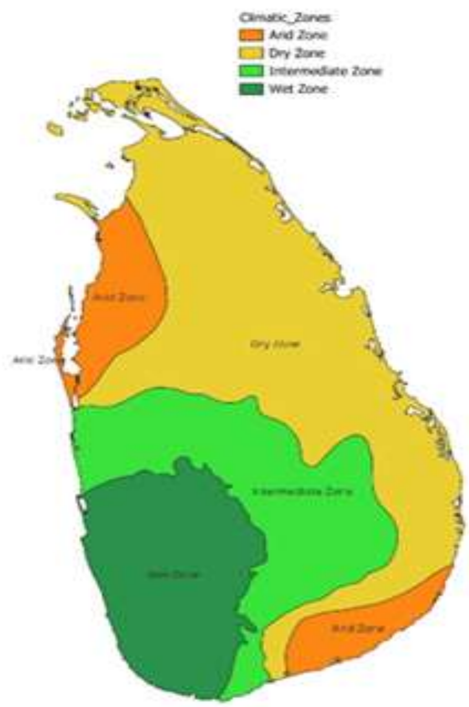

Figure 1. Map of climatic zones in Sri Lanka

The visual search method (TLVS; Campbell and Christman, 1982) was applied, which consists of slowly walking along a transect while searching for snakes visually exposed in the environment. Head length (HL), head width (HW), snout-vent length (SVL), and tail length (TaL) were measured with a venire-caliper to the nearest 0.1 mm.

"Hemipenial popping" method was used to identify gender when required (Stebbins, 2003). The probe was inserted into the cloaca and gently pushed towards the tail. It barely penetrated from 1-3 subcaudals in females and from 8- 16 subcaudals in males. GPVs were released back to their natural habitat after taking the measurements within a short period of time. PCA analysis was performed on the collected morphometric data. Other descriptive analyses were also performed using "R 4.1.2 version" statistical software.

Results

Maturity stage categorization based on PCA

A total of 68 *P. trigonocephalus* were measured in the wet, dry, and intermediate zones. According to the results of Principal Components Analysis (PCA), the external measurements of *P. trigonocephalus* showed five different clusters (Fig. 2). This result was used to separate them into three maturity stages; adult, sub-adult and juvenile. Additional information gathered during the study was used for further clarification of these categories. The first principal component (Dim.1) has an eigenvalue of 4.505, which explains 90.1% of the variance in the original data (Table 1). This is a high amount of variance explained, indicating that Dim.1 is a strong principal component that captures most of the important information in the original data. The second principal component (Dim.2) has an eigenvalue of 0.279, explaining an additional 5.6% of the variance in the data. Overall, these results suggest that the first principal component (Dim.1) is the most important component, explaining the majority of the variance in the original data, while the latter components explain relatively less variance. These results can help determine which principal components are most important and should be retained for further analysis (Table 2).

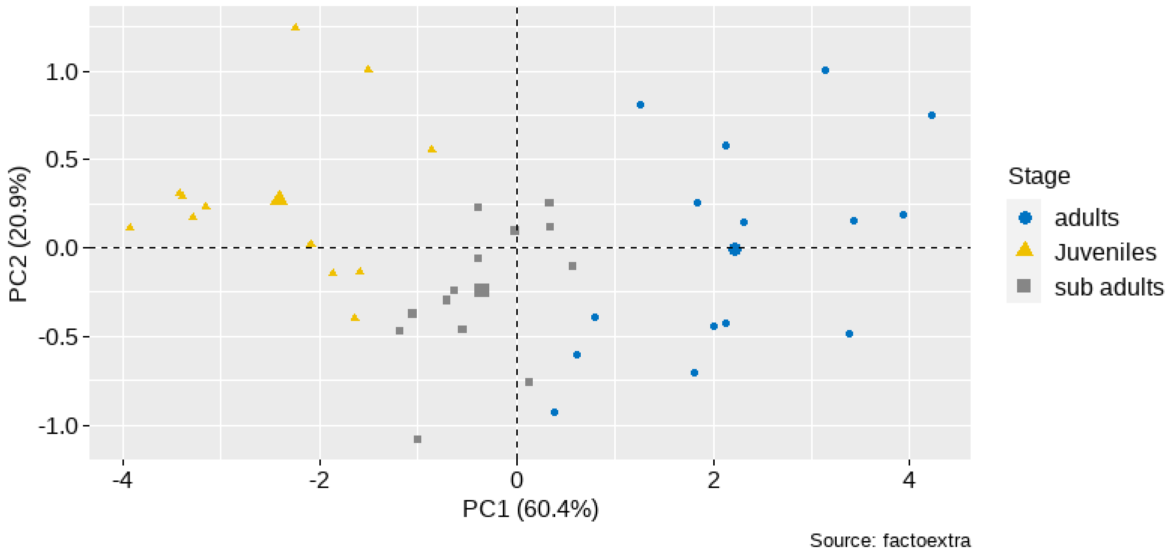

Figure 2. Scatter plot using principal component analysis (PCA) showing three maturity stage clusters-adult male, Juvenile, sub-adult female and juvenile.

Table 1. The Summary of Results Obtained from Principal Component Analysis of Morphometric Characters (Variance Extracted from the First Three Axes)

	Dim.1	**Dim.2**	**Dim.3**	**Dim.4**	**Dim.5**
eigenvalue	4.505	0.279	0.137	0.075	0.002
variance percent	90.107	5.589	2.759	1.502	0.041
cumulative variance percent	90.107	95.696	98.456	99.958	100

Table 2. Factor loadings on the first five principal component (PC) axes on the five morphometric measurement variables used to distinguish age class

Variable	**Dim.1**	**Dim.2**	**Dim.3**	**Dim.4**	**Dim.5**
SVL	0.963	-0.177	-0.192	-0.051	0.027
TL	0.979	-0.171	-0.093	-0.037	-0.035
Tal L	0.932	-0.217	0.281	0.055	0.006
HL	0.926	0.326	0.088	-0.166	0.001
HW	0.943	0.254	-0.070	0.200	-0.000

Percentages of the Sri Lankan Green Viper's different maturity stages

A total of 68 individual GPVs were encountered throughout the study. The maturity stage "adult female" was the highest recorded accounting for 30% of total individuals. Adult males were 8%. Sub-adult males and females observed were respectively 15% and 17.5%. The juvenile percentage was the lowest with a value of 20% of females. Adults made up 62.5% of the total individuals and it was a little less than half the total of Sub-adults and juveniles percentage (Fig. 3)

All stages did not show any external characteristics to determine gender. Morphometric measurements varied between the five age classes. Among adults, (Fig. 4) *P. trigonocephalus*, females were larger than males in all measurements. SVL of adult males ranged from (482.0 -740.9) mm. For adult females, it was (482.0-709.0) mm. Sub-adult males (Fig. 5) tended to be larger than sub-adult females. Sub-adult males and females also differed in SVL length [sub-adult male – (514.3 ± 64.8) mm, sub-adult females – (482.0 ± 31.0) mm]. Juveniles (Fig. 6) were very small with an SVL between (277.8 ± 91.9) mm and did not show any external characteristics to determine gender.

Moreover, the second parameter, TL (Total Length), represents the overall length of the reptile from the snout to the tip of its tail. Similar to SVL, the table provides mean measurements and standard deviations for each category. Adult males have an average TL of 868.7 mm (SD: 102.6 mm), while adult females measure 882.3 mm on average (SD: 130.3 mm). Sub-adult males and females exhibit average TLs of 617.7 mm (SD: 72.3 mm) and 586.57 mm (SD: 21.23 mm) respectively. Juveniles have a mean TL of 332.1 mm with a standard deviation of 110.8 mm. Since TL had the highest PC1 value, it can be used as a base for this maturity stage categorization; these individuals by maturity stage, with specific ranges for adults, sub-adult, and juveniles. TL of Adult Males> 98 cm, Adult Females> 110 cm, Sub-adult Males 60-98 cm, Sub-adult Females 60-110 cm, Juveniles < 60 cm.

Table 3. Average morphometric measurement values for different age classes of *P. trigonocephalus*

morphometric measurements	Adults		Subaults		Juvenile
	male	female	male	Female	
	mean ± SD	mean ± SD	mean ± SD	mean ± SD	mean ± SD
SVL(mm)	740.9 ± 73.7	709.0 ± 92.0	514.3 ± 64.8	482.0 ± 31.0	277.8 ± 91.9
TL(mm)	868.7 ±102.6	882.3±130.3	617.7 ± 72.3	586.57 ±21.23	332.1 ± 110.8
TaL(tail) (mm)	135.59±24.73	173.3 ± 39.3	103.33 ±20.97	104.57 ±21.42	54.33 ± 20.54
HL(mm)	35.80 ± 9.04	33.35 ± 7.77	24.15 ± 4.96	22.25 ± 5.50	16.45 ± 5.00

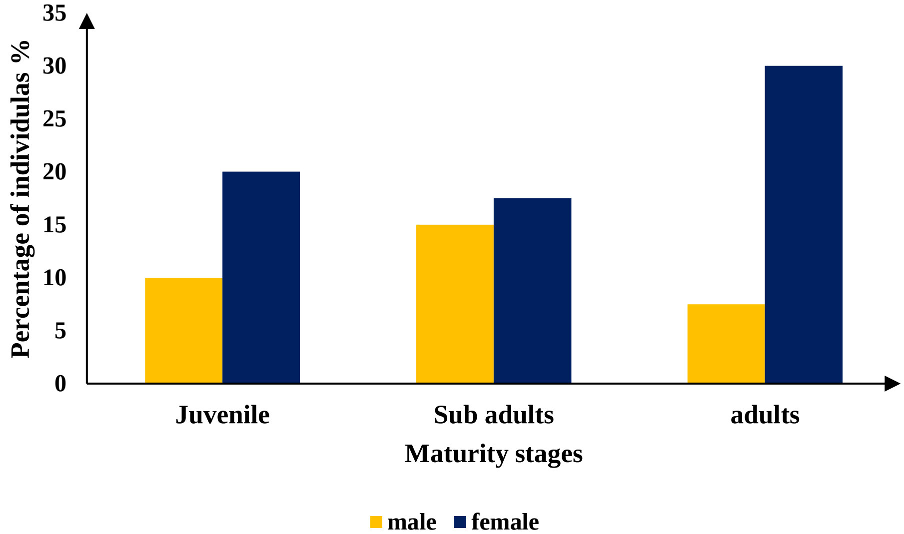

Figure 3. Chart showing the percentages of different maturity stages

Figure 4. *P. trigonocephalus* adult

Figure 5.0 *P. trigonocephalus* sub adult

Figure 6. *P. trigonocephalus* juvenile

Discussion

Sexual dimorphism in animals is thought to have evolved through sexual selection via female choice or male-male fighting. Although several research has established variations in overall size between male and female snakes (Shine, 1991), few studies have specifically proven that differences in consumed prey type or prey shape are connected to differences in head shape (Camilleri & Shine, 1990; Houston & Shine, 1993). *P. trigonocephalus* is a pit viper species with sexual dimorphism. With the "hemipenial popping" method, secondary sexual characteristics could be utilized to detect the gender of snakes. We revealed that adult male and adult female GPVs are sexually dimorphic not only in body size but also in several features of head shape. This result was used to separate them into three maturity stages; adult, sub-adult and juvenile, considering the gender and maturity. In a previous study by Jestrzemski and Kuzyakova (2019), another viper species named *Macrovipera lebetina lebetina* (Blunt-nosed viper) has been categorized into three maturity stages and they used gender for further categorization.

However, this is the first time that three different stages of maturity for the Sri Lankan green pit viper have been categorized based on morphometric data.

Adult females were larger in all parameters considered since they have bulky bodies than adult males. However, there was no significant morphometric difference in appearance between female and male individuals. The maturity stage structure of *P. trigonocephalus* shows that the total juvenile and sub-adult percentage was little more than half the adult population. The explanation for this result is that adults were observed to have lower rates of encounters in areas with high canopy levels. The SVL is useful for determining the diameter of the perch needed to capture prey items and utilize them in behavior. Therefore, larger snakes were discovered on thicker perches, and pit vipers with recently consumed prey items ought to select larger limbs compared to their SVL, according to Shine (2002).

Furthermore, the results of the present study indicate that morphometric data can be used as a successful tool for the categorization of the maturity stage of this pit viper species. Therefore, the management, ecology, and conservation of this species will benefit from this classification of maturity stages.

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