



Reproductive ecology of the Amaral's Blind Snake *Trilepida koppesi* in an area of Cerrado in south-eastern Brazil

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Supplementary data – Data 1. Data used in the R script for Sexual Size Dimorphism determination

id,sex,dissected,svl,tail.length,body.circumference,head.length,head.width,head.height,body.width,body.mass

312,Female,YES,225,15,28.5,6.15,4.41,3.19,6.41,6.75

290,Female,YES,262,18,26.7,5.7,3.35,3.4,6.45,10.75

381,Female,YES,280,20,23.59,8.2,5.64,4.53,7.73,15

457,Female,YES,285,20,29.56,6.04,5.32,4.03,7.98,13.5

383,Female,YES,290,17,28.3,7.88,5.9,4.42,8,16

382,Female,YES,290,20,NA,8.09,6.01,4.95,8.35,17

546,Female,NO,298,19,21.85,7.59,5.72,4.13,8.79,14

318,Female,YES,304,18,22,6.4,5.7,3.8,NA,11.9

355,Female,YES,305,22,36.46,8.19,6.32,5.77,8.12,13.5

292,Female,YES,311,20,26,7.57,5.23,4.75,7.7,13.75

311,Male,YES,198,20,21.33,6.79,4.16,2.93,5.61,4.75

313,Male,YES,209,20,25.61,5.86,4.6,3.38,6,5.5

308,Male,YES,220,20,28.27,5.16,6.16,3.36,6.59,7.5

327,Male,YES,221,20,19,6.8,5,3.8,NA,6.5

323,Male,YES,224,20,17,5.6,4.5,3.9,NA,6.5

328, Male, YES, 231, 19, 20, 7.4, 4.7, 4, NA, 6.5

310, Male, YES, 237, 20, 23.52, 6.72, 5.1, 3.01, 6.39, 7.5

314, Male, YES, 238, 20, 35.29, 6.12, 4.96, 3.27, NA, 7.25

309, Male, YES, 239, 24, 26, 5.65, 4.43, 3.14, 5.86, 6

294, Male, YES, 249, 24, 20.09, 7.49, 5.08, 4.6, 6.23, 7.5

307, Male, YES, 250, 25, 25.04, 6.28, 4.65, 3.68, 6.53, 9.5

321, Male, YES, 254, 20, 25, 7.9, 4, 3.3, NA, 3.5

320, Male, YES, 254, 23, 25, 6.56, 4.7, 3.5, NA, 7.75

Supplementary data – Script 1. R script used in Sexual Dimorphism analysis:

Data analysis from “Reproductive ecology of the Amaral’s Blind Snake *Trilepida koppesi* in an area of Cerrado in south-eastern Brazil”

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March, 31, 2020

Load required R packages

```
library(car)
library(dplyr)
library(kableExtra)
library(lattice)
```

Testing for sexual dimorphism

Load, check, and visualise the data

Load data

```
confirmed.data <- read.csv("data_morphology_trilepida_confirmed.csv", header = TRUE)
attach(confirmed.data)
```

Check data structure

```
str(confirmed.data)

## 'data.frame': 23 obs. of 8 variables:
## $ id      : int 312 290 381 457 383 382 546 318 355 292 ...
## $ sex     : Factor w/ 2 levels "Female","Male": 1 1 1 1 1 1 1 1 1 1 ...
## $ svl     : int 225 262 280 285 290 290 298 304 305 311 ...
## $ tail.length: int 15 18 20 20 17 20 19 18 22 20 ...
## $ head.length: num 6.15 5.7 8.2 6.04 7.88 8.09 7.59 6.4 8.19 7.57 ...
## $ head.height: num 3.19 3.4 4.53 4.03 4.42 4.95 4.13 3.8 5.77 4.75 ...
## $ body.width : num 6.41 6.45 7.73 7.98 8 8.35 8.79 NA 8.12 7.7 ...
## $ body.mass  : num 6.75 10.75 15 13.5 16 ...
```

View data

```
table_data <- kable(head(confirmed.data, 100), "pipe")
table_data
```

id	sex	svl	tail.length	head.length	head.height	body.width	body.mass
312	Female	225	15	6.15	3.19	6.41	6.75
290	Female	262	18	5.70	3.40	6.45	10.75

id	sex	svl	tail.length	head.length	head.height	body.width	body.mass
381	Female	280	20	8.20	4.53	7.73	15.00
457	Female	285	20	6.04	4.03	7.98	13.50
383	Female	290	17	7.88	4.42	8.00	16.00
382	Female	290	20	8.09	4.95	8.35	17.00
546	Female	298	19	7.59	4.13	8.79	14.00
318	Female	304	18	6.40	3.80	NA	11.90
355	Female	305	22	8.19	5.77	8.12	13.50
292	Female	311	20	7.57	4.75	7.70	13.75
311	Male	198	20	6.79	2.93	5.61	4.75
313	Male	209	20	5.86	3.38	6.00	5.50
308	Male	220	20	5.16	3.36	6.59	7.50
327	Male	221	20	6.80	3.80	NA	6.50
323	Male	224	20	5.60	3.90	NA	6.50
328	Male	231	19	7.40	4.00	NA	6.50
310	Male	237	20	6.72	3.01	6.39	7.50
314	Male	238	20	6.12	3.27	NA	7.25
309	Male	239	24	5.65	3.14	5.86	6.00
294	Male	249	24	7.49	4.60	6.23	7.50
307	Male	250	25	6.28	3.68	6.53	9.50
321	Male	254	20	7.90	3.30	NA	3.50
320	Male	254	23	6.56	3.50	NA	7.75

Does adult SVL differ between sexes?

Checking the assumptions for the parametric test both graphically and statistically.

- a) Checking normality for each of the two groups (male and female) using Shapiro-Wilk normality test

```
tapply(svl, sex, shapiro.test)
```

```
## $Female
##
## Shapiro-Wilk normality test
##
## data: X[[i]]
## W = 0.84882, p-value = 0.05625
##
##
## $Male
##
## Shapiro-Wilk normality test
##
```

```
## data: X[[i]]
## W = 0.94101, p-value = 0.4701
```

CONCLUSION: Normality assumption is met.

- b) Checking homogeneity of variances between groups using Levene's test with one independent variable

```
leveneTest(svl ~ sex, data = confirmed.data)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 1  0.1796  0.676
##      21
```

CONCLUSION: Homogeneity of variances are met.

Run independent 2-group t-test

```
svl.t.test <- t.test(svl ~ sex, var.equal = TRUE)
svl.t.test
```

```
##
## Two Sample t-test
##
## data: svl by sex
## t = 5.852, df = 21, p-value = 8.273e-06
## alternative hypothesis: true difference in means between group Female and group Male is not equal to 0
## 95 percent confidence interval:
##  33.76888 71.00035
## sample estimates:
## mean in group Female mean in group Male
##      285.0000      232.6154
```

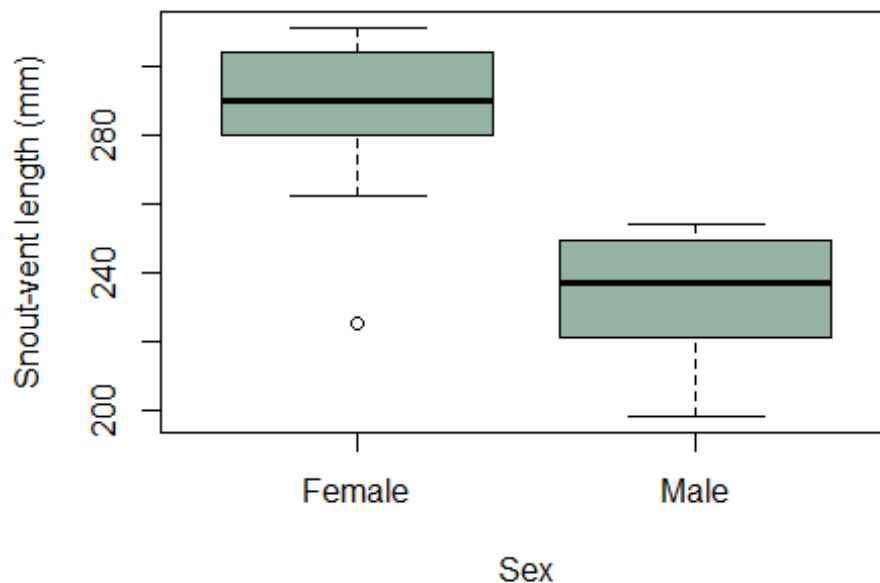
Compute summary statistics for SVL

```
group_by(confirmed.data, sex) %>% summarise(count = n(),
      mean = mean(svl, na.rm = TRUE),
      sd = sd(svl, na.rm = TRUE),
      min = min(svl, na.rm = TRUE),
      max = max(svl, na.rm = TRUE))
```

```
## # A tibble: 2 x 6
##   sex count mean sd min max
##   <fct> <int> <dbl> <dbl> <int> <int>
## 1 Female  10 285  25.4 225 311
## 2 Male   13 233  17.6 198 254
```

A simple box plot of SVL

```
boxplot(svl ~ sex, data = confirmed.data,
  col = rgb(0.3,0.5,0.4,0.6),
  ylab = "Snout-vent length (mm)", xlab = "Sex")
```



CONCLUSION: Adult females are significantly larger than adult males.

What is the Sexual Size Dimorphism (SSD) index?

- The SSD index is calculated as the mean SVL of the larger sex divided by the mean SVL of the smaller sex minus one. This index is conventionally expressed as positive when females are the larger sex and negative when males are larger (Lovich & Gibbons, 1992. Growth, Development and Aging 56, 269-281).

```
mean.female.SVL <- with(confirmed.data, mean(svl[sex == "Female"]))
mean.female.SVL
```

```
## [1] 285
```

```
mean.male.SVL <- with(confirmed.data, mean(svl[sex == "Male"]))
mean.male.SVL
```

```
## [1] 232.6154
```

- Calculate SSD index

```
mean.SVL.larger.sex = mean.female.SVL
mean.SVL.smaller.sex = mean.male.SVL
SSD = (mean.SVL.larger.sex/mean.SVL.smaller.sex) - 1
round(SSD, digits = 3)
```

```
## [1] 0.225
```

Does tail length, head length, head height, body width, and body mass differ between sexes?

- We will compute a one-way Analysis of Covariance (ANCOVA) because those variables are affected by SVL and differ between sexes.
- We will use the recommended method of type III sums of squares for unbalanced design (unequal numbers of subjects in each group).
- Change the default way R codes factors from its default.

```
options(contrasts = c("contr.sum", "contr.poly"))
```

First, we will check ANCOVA assumption of equality of slopes interaction

```
result.ancova.tail.length <- aov(tail.length ~ svl + sex + svl:sex, data = confirmed.data)
Anova(result.ancova.tail.length, type = "III")
```

```
## Anova Table (Type III tests)
##
## Response: tail.length
##          Sum Sq Df F value    Pr(>F)
## (Intercept)  2.837  1  1.0588 0.316413
## svl         33.070  1 12.3414 0.002326 **
## sex          0.240  1  0.0897 0.767822
## svl:sex       0.263  1  0.0982 0.757443
## Residuals   50.912 19
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
result.ancova.head.length <- aov(head.length ~ svl + sex + svl:sex, data = confirmed.data)
Anova(result.ancova.head.length, type = "III")
```

```
## Anova Table (Type III tests)
##
## Response: head.length
##          Sum Sq Df F value    Pr(>F)
## (Intercept)  0.5308  1  0.7604 0.39408
## svl          3.1486  1  4.5104 0.04704 *
## sex          0.0573  1  0.0821 0.77763
## svl:sex       0.0338  1  0.0484 0.82821
## Residuals   13.2635 19
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
result.ancova.head.height <- aov(head.height ~ svl + sex + svl:sex, data = confirmed.data)
Anova(result.ancova.head.height, type = "III")
```

```
## Anova Table (Type III tests)
##
## Response: head.height
##          Sum Sq Df F value    Pr(>F)
## (Intercept)  0.0017  1  0.0064 0.93717
## svl          1.7796  1  6.5801 0.01894 *
## sex          0.3881  1  1.4349 0.24569
## svl:sex       0.3846  1  1.4221 0.24775
## Residuals    5.1385 19
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
result.ancova.body.width <- aov(body.width ~ svl + sex + svl:sex, data = confirmed.data)
Anova(result.ancova.body.width, type = "III")
```

```
## Anova Table (Type III tests)
##
## Response: body.width
##      Sum Sq Df F value    Pr(>F)
## (Intercept) 0.58496  1  2.9005 0.114286
## svl      2.02987  1 10.0651 0.008031 **
## sex      0.27444  1  1.3608 0.266058
## svl:sex   0.36728  1  1.8211 0.202089
## Residuals  2.42010 12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
result.ancova.body.mass <- aov(body.mass ~ svl + sex + svl:sex, data = confirmed.data)
Anova(result.ancova.body.mass, type = "III")
```

```
## Anova Table (Type III tests)
##
## Response: body.mass
##      Sum Sq Df F value    Pr(>F)
## (Intercept) 3.582  1  1.1080 0.305725
## svl      27.671  1  8.5593 0.008676 **
## sex      3.376  1  1.0441 0.319697
## svl:sex   6.187  1  1.9137 0.182592
## Residuals 61.426 19
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

CONCLUSION: Interaction is not significant (the slope across groups is not different). We can remove interaction terms from final models.

Run final ANCOVAs

```
result.ancova.tail.length.1 <- aov(tail.length ~ svl + sex, data = confirmed.data)
Anova(result.ancova.tail.length.1, type = "III")
```

```
## Anova Table (Type III tests)
##
## Response: tail.length
##      Sum Sq Df F value    Pr(>F)
## (Intercept) 3.099  1  1.211 0.284200
## svl      33.417  1 13.060 0.001732 **
## sex      61.701  1 24.114 8.444e-05 ***
## Residuals 51.175 20
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
result.ancova.head.length.1 <- aov(head.length ~ svl + sex, data = confirmed.data)
Anova(result.ancova.head.length.1, type = "III")
```

```
## Anova Table (Type III tests)
##
## Response: head.length
##      Sum Sq Df F value    Pr(>F)
## (Intercept) 0.5061  1  0.7613 0.39328
## svl      3.4653  1  5.2120 0.03351 *
## sex      0.2009  1  0.3022 0.58857
```



```
## Residuals 13.2973 20
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

result.ancova.head.height.1 <- aov(head.height ~ svl + sex, data = confirmed.data)
Anova(result.ancova.head.height.1, type = "III")

## Anova Table (Type III tests)
##
## Response: head.height
##      Sum Sq Df F value    Pr(>F)
## (Intercept) 0.0012  1  0.0043 0.948217
## svl         2.2773  1  8.2465 0.009431 **
## sex         0.0038  1  0.0138 0.907802
## Residuals   5.5231 20
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

result.ancova.body.width.1 <- aov(body.width ~ svl + sex, data = confirmed.data)
Anova(result.ancova.body.width.1, type = "III")

## Anova Table (Type III tests)
##
## Response: body.width
##      Sum Sq Df F value    Pr(>F)
## (Intercept) 0.3842  1  1.7917 0.203659
## svl         3.1862  1 14.8600 0.001989 **
## sex         0.3449  1  1.6088 0.226904
## Residuals   2.7874 13
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

result.ancova.body.mass.1 <- aov(body.mass ~ svl + sex, data = confirmed.data)
Anova(result.ancova.body.mass.1, type = "III")

## Anova Table (Type III tests)
##
## Response: body.mass
##      Sum Sq Df F value    Pr(>F)
## (Intercept) 4.899  1  1.4492 0.242709
## svl        35.525  1 10.5083 0.004088 **
## sex        24.529  1  7.2557 0.013971 *
## Residuals  67.612 20
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

CONCLUSION: Only tail length and body mass differed between the sexes.

Checking other ANCOVA assumptions

- The residuals from the linear regression of Y on X from our fitted ANCOVA model should:
 - (i) be normally distributed
 - (ii) have similar variances between groups.

- Checking for normality using Shapiro-Wilk's tests (extract the residuals and then run the tests)

```

ancova.tail.length.1.residuals <- residuals(object = result.ancova.tail.length.1)
shapiro.test(x = ancova.tail.length.1.residuals)

##
## Shapiro-Wilk normality test
##
## data: ancova.tail.length.1.residuals
## W = 0.96508, p-value = 0.573

ancova.head.length.1.residuals <- residuals(object = result.ancova.head.length.1)
shapiro.test(x = ancova.head.length.1.residuals)

##
## Shapiro-Wilk normality test
##
## data: ancova.head.length.1.residuals
## W = 0.91841, p-value = 0.06159

ancova.head.height.1.residuals <- residuals(object = result.ancova.head.height.1)
shapiro.test(x = ancova.head.height.1.residuals)

##
## Shapiro-Wilk normality test
##
## data: ancova.head.height.1.residuals
## W = 0.96352, p-value = 0.5381

ancova.body.width.1.residuals <- residuals(object = result.ancova.body.width.1)
shapiro.test(x = ancova.body.width.1.residuals)

##
## Shapiro-Wilk normality test
##
## data: ancova.body.width.1.residuals
## W = 0.97628, p-value = 0.9272

ancova.body.mass.1.residuals <- residuals(object = result.ancova.body.mass.1)
shapiro.test(x = ancova.body.mass.1.residuals)

##
## Shapiro-Wilk normality test
##
## data: ancova.body.mass.1.residuals
## W = 0.95617, p-value = 0.3906

```

CONCLUSION: Normality is met.

- Homogeneity of variances using Levene's test with one independent variable

```

leveneTest(tail.length ~ sex, data = confirmed.data)

## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 1  0.0722 0.7907
##      21

leveneTest(head.length ~ sex, data = confirmed.data)

```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 1  0.477 0.4973
##      21
```

```
leveneTest(head.height ~ sex, data = confirmed.data)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 1  2.2572 0.1479
##      21
```

```
leveneTest(body.width ~ sex, data = confirmed.data)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 1  1.1572 0.3002
##      14
```

```
leveneTest(body.mass ~ sex, data = confirmed.data)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##      Df F value Pr(>F)
## group 1  1.6065 0.2189
##      21
```

CONCLUSION: All passed homogeneity of variances.

Supplementary data – Table S1. Variation in testis lobes and volume in males and variation in females' follicles count, sizes and vitellogenesis
All data is from rainy season.

Males				Females		
	Larger lobe volume			Follicles range		Follicles in secondary
	Number of lobes	(mm ³)	Testis volume (mm ³)	Number of follicles	length (mm)	vitellogenesis (>6mm)
Right side	4 – 7	6.46 – 51.34	26.89 – 63.57	5 – 20	3.1 – 19.62	1 – 3
(n=13)	(5.23 ± 1.09)	(17.48 ± 11.33)	(40.53 ± 10.25)	(11.4 ± 4.76)	(10.24 ± 6,24)	(2 ± 0.81)
Left side	3 – 7	6.47 – 26.08	14.35 – 71.78	6 – 14	3.5 – 21,4	1 – 4
(n=14)	(4.78 ± 1.18)	(14.49 ± 5.21)	(38.38 ± 15.23)	(11.5 ± 6.29)	(10.79 ± 6.37)	(2.13 ± 0.83)

Supplementary data – Table S2. Histological measurements from seminiferous tubules (ST), sexual segment of the kidney (SSK) and *ducti differentia* (DD) from males in spermiogenesis and early regression.

	Spermiogenesis (n=3)	Early regression (n=5)
ST diameter (μm)	445.18 – 503.69 (469.00 ± 30.73)	401.07 – 527.07 (456.66 ± 47.53)
ST epithelium thickness (μm)	72.14 – 114.86 (89.79 ± 22.30)	61.45 – 93.75 (80.65 ± 15.53)
SSK diameter (μm)	183.62 – 284.23 (240.99 ± 51.76)	224.68 – 280.64 (261.71 ± 21.70)
SSK epithelium thickness (μm)	42.84 – 71.31 (54.68 ± 14.82)	48.24 – 51.92 (50.22 ± 1.35)
DD diameter (μm)	219.69 – 333.04 (287.85 ± 60.06)	240.47 – 356.8 (292.47 ± 44.6)
DD epithelium thickness (μm)	10.76 – 19.29 (14.77 ± 4.38)	12.24 – 31.73 (19.38 ± 7.47)

Supplementary data – Table S3: Histological measurements from infundibulum and glandular uterus epithelium.

	Infundibulum (µm)	Glandular uterus (µm)
Dry season (n=5)	42 – 108 (75.53 ± 23.7)	77 – 117 (96.58 ± 16.76)
Rainy season	60.35 – 127.91 (95.22 ±	115.63 – 179.73
(n=3)	34.16)	(142.08 ± 33.48)