

ANNUAL REPRODUCTIVE ACTIVITY OF A POPULATION OF *Ptychoglossus*
bicolor (SQUAMATA: GYMNOPHTHALMIDAE)

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ESCUELA DE BIOLOGIA

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A mis padres y hermanos por apoyarme y estar siempre a mi lado en esta etapa de mi vida. A mis compañeros y muy especialmente a Fabio por brindarme su amistad.

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CONTENIDO

Pag.

1. LISTA DE FIGURAS.....	VIII
2. LISTA DE TABLAS.....	IX
3. RESUMEN.....	X
4. ABSTRACT.....	XI
5. INTRODUCTION.....	1
6. MATERIALS AND METHODS.....	2
6.1 FIELD AND LABORATORY PROTOCOLS.....	2
6.2 STATISTICAL ANALYSIS.....	4
7. RESULTS.....	5
7.1 SEXUAL DIMORPHISM.....	6
7.2 FEMALE REPRODUCTIVE ACTIVITY.....	6
7.3 MALE REPRODUCTIVE ACTIVITY.....	7
7.4 ABDOMINAL FAT BODIES.....	7
7.5 REPRODUCTION AND RAINFALL.....	8
8. DISCUSSION.....	8
9. LITERATURE CITED.....	13

1. LISTA DE FIGURAS

Pág.

Figure 1. Monthly mean precipitation (solid line, in mm) and maximum and minimum temperatures (broken line, in °C) of the study area. Historical data were taken from the DIVA-GIS database, and current data were recorded from the coffee farm station. 18

Figure 2. Monthly distribution and sizes (SVL) of adult males (white squares), adult females (black circles) and juveniles (gray triangles) of *Ptychoglossus bicolor*. The arrows indicate the minimum size at sexual maturity for each sex. 19

Figure 3. Distribution for sizes (SVL) of the individuals in the different categories considered in the population. Adult males (black bars), adult females (white bars) and juveniles (gray bars). 20

Figure 4. Monthly distribution of reproductive stages of mature females of *Ptychoglossus bicolor*. Non vitellogenic females (white bars), vitellogenic females (black bars), ovigerous females (gray bars), vitellogenic and ovigerous females (striped bars). 21

Figure 5. Mean monthly and standard error of Ln testis mass in *P. bicolor*. Different superscripts indicate statistically significant differences among months. It is observed that the dry months are the months in which the testicular mass was significantly reduced. 22

2. LISTA DE TABLAS

Pág.

Table 1. Sexual dimorphism in body and head size in *Ptychoglossus bicolor*. For the comparative tests we used the transformed morphometrical values and the transformed SVL as the covariate. N = 78 males and 78 females. * Mann-Whitney *U*-test on original data.

23

3. RESUMÉN

TÍTULO: ACTIVIDAD REPRODUCTIVA ANUAL DE UNA POBLACION DE *Ptychoglossus bicolor* (SQUAMATA: GYMNOPTHALMIDAE)*

AUTORES: Eliana Patricia Ramos Pallares, Martha Patricia Ramírez Pinilla, Víctor Hugo Serrano Cardozo**

PALABRAS CLAVES: Lagartos Neotrópicales; plantación de café orgánico; actividad reproductiva; dimorfismo sexual; Gymnophthalmidae

CONTENIDO: Se estudiaron las características reproductivas de una población del lagarto de la familia Gymnophthalmidae, *Ptychoglossus bicolor*. Esta población habita en la hojarasca de una plantación de café orgánico localizada en los flancos occidentales de la Cordillera Oriental de los Andes Colombianos. Machos y hembras alcanzan la madurez sexual a un tamaño corporal similar (aproximadamente 45 mm. de longitud rostro cloaca); sin embargo, los machos adultos son significativamente mas grandes que las hembras en tamaño del cuerpo, masa corporal y tamaño de la cabeza. Todos los machos adultos mostraron epidídimos convolutos y testículos alargados con una actividad espermatogénica y espermiogénica continua durante todo el año. Sin embargo, la masa testicular varió significativamente entre meses y entre estaciones lo cual se encuentra significativamente correlacionado con el régimen de lluvias. Las hembras fueron reproductivamente activas durante todos los meses del año (Vitelogénicas, Ovígeras y Ovígeras y vitelogénicas simultáneamente). El tamaño de postura fue siempre de dos huevos. La masa de los cuerpos grasos abdominales no varía a través del año en machos y hembras. Estos datos junto con la observación de juveniles en la mayoría de los meses del año indica un patrón de actividad reproductiva continua en esta población. Así, en esta población de *Ptychoglossus bicolor* este patrón de actividad reproductiva continua sugiere una continua disponibilidad de alimentos y de recursos que pueden ser suplidos por las condiciones favorables ofrecidas por esta plantación de café orgánico.

* Proyecto de Grado

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4. ABSTRACT

TITLE: ANNUAL REPRODUCTIVE ACTIVITY OF A POPULATION OF *Ptychoglossus bicolor* (SQUAMATA: GYMNOPHTHALMIDAE)*

AUTHORS: Eliana Patricia Ramos Pallares, Martha Patricia Ramírez Pinilla, Víctor Hugo Serrano Cardozo**

KEYWORDS: Neotropical lizard; organic coffee plantation; reproductive activity; sexual dimorphism; Gymnophthalmidae

CONTENT: We studied the reproductive characteristics of a population of the gymnophthalmid lizard *Ptychoglossus bicolor*. This population is found inhabiting the leaf-litter of an organic coffee plantation located on the Western slopes of the Cordillera Oriental of the Colombian Andes. Males and females reached sexual maturity at a similar body size (approximately 45 mm snout vent length); however, adult males are significantly larger than females in body size, body mass, and head size. All adult males showed convoluted epididymides, enlarged testes with continuous spermatogenesis and spermiogenesis along the year. However, testis mass did vary significantly among months and between seasons and it was significantly correlated with the amount of rainfall. Females were reproductively active (vitellogenic, ovigerous, and vitellogenic and ovigerous simultaneously) during all months of the year. Clutch size ever was of two eggs. Abdominal fat bodies did not vary over time, neither in males nor females. These data, plus the observation of juveniles in most of the months show a pattern of continuous reproductive activity and confirms the production of multiple clutches in this population. Thus, in this population of *Ptychoglossus bicolor* this aseasonal reproductive pattern suggests a continuous availability of resources that can be supplied by favorable conditions offered by the organic farming.

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5. INTRODUCTION

New World tropical forests are well known for their biotic diversity (Wilson, 1988; Vitt *et al.*, 2005). However, most of these forests have experienced high deforestation and fragmentation followed by a rapid conversion to agriculture (Perfecto *et al.*, 2007, Vitt and Zani, 2005). In areas where this occurs and coffee is still produced on traditional shade plantations, these plantations are likely to be a critical refuge for the forest biota (Perfecto *et al.*, 1996). In fact, recent studies in traditional shade plantations have demonstrated the potential of these ecosystems as a refuge for biodiversity (Perfecto *et al.*, 1996, 2003, 2007). Therefore it is important to know how species are maintained in these ecosystems (Perfecto *et al.*, 2003).

Tropical lizards exhibit a great diversity of reproductive strategies (Fitch, 1982; Mezquita and Colli, 2003). Currently, it is known that the local environment does not necessarily play an essential role in determining the reproductive phenology of lizards (Walting *et al.*, 2005), since in tropical areas with a seasonal rainfall regime may exist species with seasonal reproductive cycles and species with continuous reproductive cycles (Vitt, 1986; Watling *et al.*, 2005). Therefore, some of these variations are likely to have a historical origin (Dunham and Miles, 1985), variations in such strategies within and between species has been attributed to phylogenetic constraints, adaptive responses to environmental factors, or a combination of both (Vitt, 1992; Wiederhecker *et al.*, 2002).

The family Gymnophthalmidae exhibits a great morphological and ecological diversity which exceeds that of many larger clades of Squamata (Donnelly *et al.*, 2006). However, lizards in this family are poorly known ecologically despite that the family contains about 180 species distributed in 36 genera and their species occupy a wide diversity of neotropical and subtropical habitats in Central and South America (Vitt *et al.*, 2007; Pough *et al.*, 2004; Pellegrino *et*

al., 2001; Castoe *et al.*, 2004). Little is known about reproductive characteristics of the gymnophthalmid species; however, different types of annual reproductive patterns have been described for some of the few studied species (Telford, 1971; Sherbrooke, 1975; Vitt, 1982).

The genus *Ptychoglossus* is composed by small lizards that live in the leaf litter of moist tropical forests in Panama, Costa Rica and the Northwestern quarter of South America from sea level to at least 2190 meters elevation (Harris, 1994). *Ptychoglossus bicolor* inhabits mainly zones of premontane and low montane very humid forests between 1500 and 2100 m elevation, on the Magdalena Valley of Colombia (Harris 1994). Nothing is known about its reproductive biology and other ecological aspects mainly because only few specimens are observed in the field; these lizards are usually hidden in leaf litter or under logs in the forests. During 2005 we found an abundant population of this species inhabiting agroecosystems (large plantation of organic coffee). This discovering gives us the opportunity of study the biology of this species and the impact of organic agrosystems on species, especially in such poorly-known groups as the gymnophthalmids.

In this study we describe the annual reproductive activity of an Andean population of *Ptychoglossus bicolor* and other aspects of its reproductive biology by evaluation of monthly variation in reproductive stages, age composition, sex ratio, clutch size, sexual dimorphism, and the relationship of gonadal activity with rainfall.

6. MATERIALS AND METHODS

6.1 FIELD AND LABORATORY PROTOCOLS

The study was conducted at La Hacienda El Roble, between the municipalities of Los Santos and Piedecuesta, (Santander, Colombia; 06° 52' N; 73° 03' W; altitude 1500 – 1700 m); it is located on the western slope of Cordillera Oriental of the Colombian Andes. The area has a bimodal precipitation regime, with one peak of rains from March to June, and a second peak from August to November, a mean annual precipitation of 1143 mm, and a daily temperature between 8 and 30 °C (Fig. 1). This farm has an organic coffee plantation, characterized by a dominant anthropogenic influence, shade trees, banana trees, and soils covered with a rich leaf-litter layer, and supplied by organic compost.

Specimens of *Ptychoglossus bicolor* were captured manually from the leaf litter deposited on the soils of the coffee plantation. The site was sampled by two collectors monthly from April 2005 to April 2006 between 0900 to 1500 h during two days. Individuals were used for this study, the study of the diet, microhabitat use and temperature preferences (Anaya-Rojas et al., in prep.), and to study skull morphology (Tarazona and Ramírez-Pinilla, 2008). The animals were euthanized by means of a cardiac injection of Lidocain 2%. For each individual, we measured total body mass with an electronic balance (± 0.01 g), and the following morphometric data with a Vernier caliper (± 0.02 mm): snout–vent length (SVL) and total length; head length, width, and height; body height and width. We made a midventral incision, and recorded the following morphometric data by *in situ* observation: for males, longest and shortest diameters of the testes (to calculate testicular volume), and convolution of the epididymides; for females, ovarian diameter, diameter of the largest ovarian follicle, number of vitellogenic follicles, number and diameter of oviductal eggs, and the presence of corpora lutea. In all the individuals the presence and mass of abdominal fat bodies were registered. The left reproductive tracts and abdominal fat bodies were dissected from the abdominal cavity and weighed. Specimens were fixed in 10% formalin, preserved in 70% ethanol, and placed in the herpetological

collection of the Museo de Historia Natural, Escuela de Biología, Universidad Industrial de Santander (UIS-R).

The left reproductive tracts were fixed in 10% buffered formalin. Male reproductive tracts were embedded in paraffin, sectioned at 9 μ m, and stained with hematoxylin-eosin according to Luna (1968). Histological slides were used to determine the reproductive stage according to the classification of Ballinger and Nietfeldt (1989). Males were determined as sexually mature by the presence of sperm in the seminiferous tubules, and in the lumen of the epididymides. The existence of adult females was determined by direct observation of the reproductive tracts, establishing the reproductive condition based on macroscopic gonadal characters. We classified the reproductive condition of the adult females as previtellogenic (without vitellogenic follicles), vitellogenic (with vitellogenic follicles; diameter > 2 mm. and yellowish), ovigerous (with oviductal eggs), vitellogenic and ovigerous (with advanced vitellogenic follicles and oviductal eggs simultaneously).

6.2. STATISTICAL ANALYSIS

Based on data of sexual maturity and reproductive stage, individuals were categorized as juveniles, adult males, and adult females. The minimum size at maturity in males was designated as the body size (SVL) of the smallest male specimen with spermatozoa in testes and ducts; in females, the smallest female specimen with late vitellogenic follicles or oviductal eggs. To inspect for significant differences among months for each category (adult males and females, and juveniles), we employed G-tests or chi-square tests.

We evaluated the existence of sexual dimorphism in the following characteristics: SVL, head length, height and width, body height and total body weight using analysis of covariance (ANCOVA) with the body size (SVL) as

covariate. We used the separate-slope model when covariant had different effects at different levels of a categorical dependent variable (factor). We transformed all variables to satisfy the assumption of normality prior to analyses.

Reproductive stage data for each animal permitted establishing the percentage of males and females in each reproductive stage each month, and throughout the year. This percentage was analyzed to detect intra, and intersexual variation by month (synchrony) and over time (seasonality), we did this by using a G-tests or a chi-square test.

Because gonad mass or volume usually varies with body size, we transformed all variables, and conducted analyses of covariance (ANCOVA) with gonad mass and volume as the dependent variable, SVL as the covariate, and month/season as the class variable to determine whether significant seasonal variation existed. Followed by *a posteriori* tests (Tukey) to determine which month/season differed. To test differences in fat body masses between sexes, among months and among reproductive stages we performed an ANCOVA with SVL as covariate. Clutch size was calculated using the number of oviductal eggs in each female. Body weight and SVL of females were correlated with the eggs' volume and weight. The effects of climatic factors on reproductive activity were tested by means of an Spearman correlation between the monthly scores of rainfall (mm) and the adjusted testicular volume and weight, follicular diameter, and ovarian weight; we did not examined the relationship between temperature and reproduction because the temperature in this area remains almost invariant throughout the year.

7. RESULTS

A total of 170 individuals were collected and categorized as juveniles (14), adult females (78), and adult males (78). Males and females were collected during all months of the year, and juveniles were collected during 10 months (Fig. 2). We did not find any difference in the occurrence of males ($G_{0.05, 12} = 1.35$ $p = 0.99$), females ($G_{0.05, 12} = 4.00$ $p = 0.98$), and juveniles ($\chi^2_{0.05, 12} = 6.4$ $p = 0.89$) throughout the year. This population has a sex ratio of 1:1 (male:female).

7.1. SEXUAL DIMORPHISM

Morphometric comparison of *P. bicolor* showed that males and females were sexually dimorphic in size (SVL), weight, and body shape, with males attaining larger size and mass than females (Table 1). In addition to a larger body size, adult males have a conspicuous orange-red ventral coloration that clearly distinguishes them from females.

7.2. FEMALE REPRODUCTIVE ACTIVITY

The smallest reproductive female was 46.16 mm SVL (Fig. 2); the average SVL of adult females was 55.22 mm (range 46 - 60 mm. Fig. 3). Of the 78 adult females collected 5.1% were previtellogenic and showed signs of a recent oviposition, 64.1% were vitellogenic, 15.4% ovigerous, and 15.4% simultaneously vitellogenic and ovigerous. There were no significant differences in the occurrence of females in each reproductive stage throughout the year: non reproductive ($\chi^2_{0.05, 12} = 15.90$ $p = 0.19$), vitellogenic ($G_{0.05, 12} = 5.07$ $p = 0.95$), ovigerous ($\chi^2_{0.05, 12} = 9.92$ $p = 0.62$), and vitellogenic and ovigerous females ($\chi^2_{0.05, 12} = 18.81$ $p = 0.09$, Fig. 4). The largest follicular diameter and mass of the ovary were not related to SVL ($r^2 = 0.04$ $F_{1, 75} = 3.19$ $p = 0.07$ and

$r^2 = 0.017$ $F_{1, 75} = 1.31$ $p = 0.25$, respectively). No significant differences in the ovarian morphometric variables were found throughout the year (ANCOVA ovarian weight, $F_{12, 76} = 1.39$ $p = 0.19$; follicular diameter, $F_{12, 76} = 1.5$ $p = 0.12$). Clutch size estimated from the number of oviductal eggs always was two. We found no relationship between egg mass, egg volume, and female body mass and size (Sperman $R = 0.09$ $p = 0.66$ and $R = 0.05$ $p = 0.78$, Sperman $R = 0.06$ $p = 0.76$, and $R = -0.24$ $p = 0.24$, respectively).

7.3. MALE REPRODUCTIVE ACTIVITY

The minimum size at maturity for males was estimated at 44.02 mm SVL (Fig. 2); adult males have an average SVL of 56.74 mm SVL (range 45 - 63 mm, Fig 3). All adult males had enlarged testes, and convoluted epididymides. Histological examination of the male gonads throughout the year revealed active spermatogenesis, and spermiogenesis with abundant sperm in the lumen of seminiferous tubules, and sexual ducts. Testis volume and mass were related to SVL ($r^2 = 0.60$ $F_{1, 73} = 45.8$ $p < 0.01$ and $r^2 = 0.77$ $F_{1, 73} = 31.0$ $p < 0.001$ respectively). There was no significant variation in adjusted testis volume among months (ANCOVA, $F_{12, 74} = 0.83$ $p = 0.61$); however, we found a significant variation in testis mass throughout the year (ANCOVA, $F_{12, 74} = 2.36$ $p = 0.01$); the Tukey test revealed the months that differed significantly (Fig. 5).

7.4. ABDOMINAL FAT BODIES

From the mature individuals collected, 15.38% of females did not have detectable fat bodies. We found no significant differences between male and female fat body masses (ANCOVA, $F_{1, 140} = 2.43$ $p = 0.12$), neither when

abdominal fat body masses of females were compared among reproductive stages (ANCOVA, $F_{3, 65} = 1.42$ $p = 0.24$). No significant differences were found in fat body masses during the year in males (ANCOVA, $F_{12, 62} = 1.80$ $p = 0.06$) and females (ANCOVA, $F_{12, 64} = 1.46$ $p = 0.17$).

7.5. REPRODUCTION AND RAINFALL

We did not find significant differences between historical values of mean precipitation and those reported in the year of sampling (*Paired t* $t_{11} = -0.14$ $p = 0.89$). Gonad morphometric variables of females were not affected by rainfall (Spearman, ovarian weight $R = -0.13$ $p = 0.67$; follicular diameter $R = -0.33$ $p = 0.29$). In males, testicular mass was significantly related to rainfall (Spearman $R = 0.58$ $p = 0.044$), and testicular volume was not related to rainfall (Spearman $R = -0.46$ $p = 0.137$). In females gonad morphometric variables did not differ significantly between dry and rainy seasons (ANCOVA, ovarian weight $F_{1, 67} = 1.27$, $p = 0.26$; follicular diameter $F_{1, 68} = 1.52$, $p = 0.22$). However, in males there were significant differences in testicular mass and testicular volume between seasons (ANCOVA, testicular mass $F_{1, 68} = 5.84$, $p = 0.02$; testicular volume $F_{1, 68} = 5.44$, $p = 0.02$), these variables reached the highest values during the rainy season.

8. DISCUSSION

Individuals of the three categories (juveniles, adult males, and adult females) and all sizes can be found during every month of the year. This pattern suggests a continuous reproductive activity in the population, and a constant recruitment of new individuals throughout the year. The low percentage of juveniles reported can be explained by sampling bias, because their small size

and inaccessible habitat (buried in loose soil and under the litter). This same pattern of aseasonal occurrence and continuous reproductive activity has been found in other gymnophthalmids such as *Potamites ecpleopus*, in Perú (Sherbrooke, 1975), and *Vanzosaura rubricauda*, in Brazil (Vitt, 1982). In contrast, other species of gymnophthalmids possess marked seasonal cycles in the size distribution and reproductive categories such as *Leposoma rugiceps* and *Gymnophthalmus speciosus* in Panama (Telford, 1971).

It is common to find a marked sexual dimorphism in head size, and coloration of the body in the Gymnophthalmidae family (Pianka and Vitt, 2003). *Ptychoglossus bicolor* males are bigger and heavier, they have larger heads than females, and in addition to these morphological differences males have a strong ventral dichromatism. As well as in *P. bicolor*, males of *Alopoglossus atriventris* (Vitt et al., 2007), *Cercosaura oshaughnessyi* (Vitt et al., 2003), *Potamites juruazensis* (Vitt and Avila-Pires, 1998) and *Vanzosaura rubricauda* (Vitt, 1982) have relatively larger heads, which is a common characteristic in many taxa of lizards (Vitt et al., 2007). Other gymnophthalmids as *C. oshaughnessyi* (Vitt et al., 2003), *P. juruazensi* (Vitt and Avila-Pires, 1998) and *V. rubricauda* (Vitt, 1982) have a sexual dichromatism biased toward males; this attribute is also present in *P. bicolor*. It has been suggested that sexually dimorphic traits such as the SVL, body weight, relative head size have been selected as a result of male-male interactions that take place when they compete for females, where males with larger heads and bigger usually wins the battles; these attributes probably make them more attractive to females and these males may have a higher reproductive success than their smaller conspecifics (Anderson and Vitt, 1990). Similarly, sexual dichromatism probably has also arisen via sexual selection (Cooper and Vitt, 1988). Although reproductive behavior is unknown for *P. bicolor*, the sexual dimorphism in *P. bicolor* and other gymnophthalmids may be related to sexual selection and territorial defense as it has been proposed for other lizards.

Females have an aseasonal, asynchronous, and continuous reproductive activity pattern. This type of reproductive activity has also been reported for females of *Potamites ecpleopus* (Sherbrooke, 1975) and *Vanzosaura rubricauda* (Vitt, 1982) as well as other Neotropical lizard species. Also, males have an active spermatogenic activity throughout the year and are always available for females in several Neotropical lizards of different families (e.g., Gymnophthalmidae, *Potamites ecpleopus*, Sherbrooke, 1975, Scincidae, *Mabuya* sp., Ramírez-Pinilla *et al.*, 2002, *Sphenomorphus cherriei*, Watling *et al.*, 2005; Teiidae, *Cnemidophorus lemniscatus*, Mojica *et al.*, 2003; Gekkonidae, *Gonatodes albogularis*, Serrano-Cardozo *et al.*, 2007, *Lepidoblepharis xanthostigma*, Watling *et al.*, 2005). In the tropics, rainfall and food resources are known to determine the type of reproductive activity in lizards; in places where the climate and food remain constant throughout the year, it is more likely to find species with aseasonal reproductive cycles, since these conditions favor such reproductive cycles (Sexton *et al.*, 1971, Sherbrooke, 1975, Ballinger, 1977, Fitch, 1982). *P. bicolor* inhabits an organic coffee plantation characterized by a controlled and permanent high humidity, a rich leaf litter layer, and shade provided by the trees of this type of plantation. In addition to those environmental characteristics, *P. bicolor* show a marked microhabitat preference for areas rich in litter; this microhabitat appears to have a continuous availability of food and suitable temperatures for thermoregulation (Anaya-Rojas *et al.*, com. pers.), conditions that could explain their abundance in this microhabitat and the aseasonal reproductive activity found in this species.

Despite the fact that males are reproductive permanently, we found significant differences in the testis mass throughout the year; this pattern of continuous reproductive activity with significant changes in mass and volume of testes have been reported in other tropical lizards (Teiidae, *Cnemidophorus lemniscatus*, León and Cova, 1973; Gekkonidae, *Gonatodes albogularis*, Telford, 1971;

Gymnodactylus geckoides, *Lygodactylus klugei*, Vitt, 1986). In gymnophthalmids, it has been reported for *Potamites ecpleopus* (Sherbrooke, 1975). Licht and Gorman (1970) suggested that testis mass and spermatogenic activity are independent, something that could be an indicative of a slight seasonal pattern within a continuous reproductive cycle. Sherbrooke (1975) suggested that it is probable that a temporal reduction in testis mass may reflect a decrease in spermatogenic activity, so this pattern would represent changes in the reproductive effort of males during a given period of time. However, this hypothesis has not been tested yet. Vitt (1986) suggested a variation in testis mass may be a consequence of water availability. We have observed that the changes in this population of *P. bicolor* are highly correlated with the rainfall, so during months with high rainfall levels, testes were heavier than during months with the lowest levels of rains, simply reflecting the degree of tissue hydration and not a variation in spermatogenic activity and/or reproductive activity.

Clutch size of *P. bicolor* was always of two eggs. Gymnophthalmids have small clutch sizes, ranging from 1 to 3 eggs (mean, 2); clutch size may be genetically determined as in other gymnophthalmids (Fitch, 1970, Sherbrooke, 1975, Vitt, 1982). This pattern is common among Squamata Scleroglossan that are characterized by a reduced clutch size () that is apparently a result of their active mode of foraging, morphology, and its predators escape tactics (Vitt and Price, 1982). Eggs mass and volume were not significantly correlated with mass or SVL of the female in *P. bicolor*, this feature has also been found in other species such as *Podarcis milensis* (Adamopoulou and Valakos, 2000) and in the tropidurid lizard *Eurolophosaurus nanuzae* (Galdino *et al.*, 2003). According to Adamopoulou and Valakos (2000) this could indicate that it has reached an evolutionary optimum between egg volume and clutch size. On the other hand, the occurrence of vitellogenic and ovigerous females simultaneously indicates that females of *P. bicolor* may produce several clutches continuously, resulting in spatially and temporally separated ovipositions. It has been proposed that an

increase in clutch frequency may be the most plausible mechanism to produce more offspring, given that the energy available is not invested to increase the eggs volume or number, but instead of that to increase the clutch frequency (Adamopoulou and Valakos, 2000); this mechanism could also be applicable to *P. bicolor*.

Only a small number of individuals of this population did not accumulate abdominal fat bodies. In the individuals where they were observed, no significant differences were found in their mass through the year, suggesting that in males and females the energy required for reproduction does not depend on abdominal fat body reserves. In fact, this population seems to be not constrained by food since there is continuous food availability in its microhabitat (Anaya-Rojas *et al.*, comp. pers.). In addition to the permanent food availability, species that live in areas with aseasonal climates in the tropics, and that commonly have aseasonal reproductive cycles are not likely to store abdominal body fats (e.g. *Cosymbotus platyurus*, *Hemidactylus frenatus* and *Gehyra mutilata*, Church, 1962). If lizards store fats under such conditions, this storage may be extremely variable and it has no clear relation with reproductive activity (i.e. *Cnemidophorus ocellifer*, Vitt, 1983; *C. lemniscatus*, Mojica *et al.*, 2003). This suggests that they occupy habitats with sufficient environmental resources for reproduction, because the storage of lipids is a direct function of food availability (Derickson, 1976).

This population of *P. bicolor* is found in a habitat which is highly supplied by the organic farming practices, favoring continuous reproductive activity and possibly a densest population than in other habitats. However, Watling *et al* (2005) found that the reproduction of Neotropical lizards with one- and two-egg clutches tends to be continuous. Thus indicating, that although local environmental conditions are important in determining of the type of reproductive activity, phylogenetic effects also play a fundamental role in determining of the

reproductive pattern. Therefore, it is important to establish if this lizard can hold the same reproductive pattern in natural environments.

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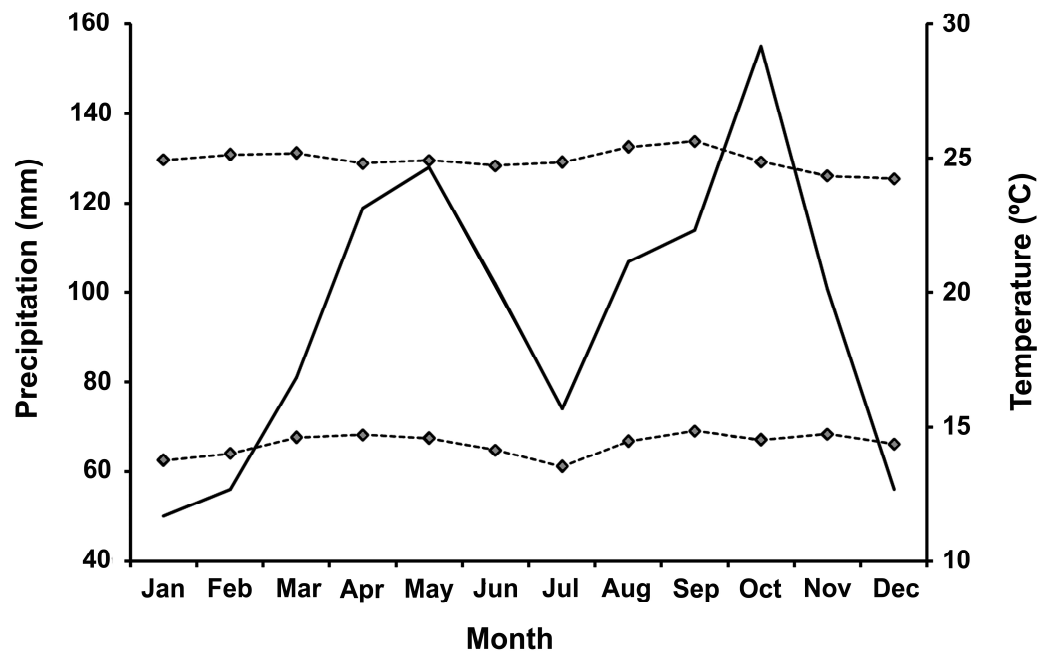


Fig. 1. Monthly mean precipitation (solid line, in mm) and maximum and minimum temperatures (broken line, in °C) of the study area. Historical data were taken from the DIVA-GIS database, and current data were recorded from the coffee farm station.

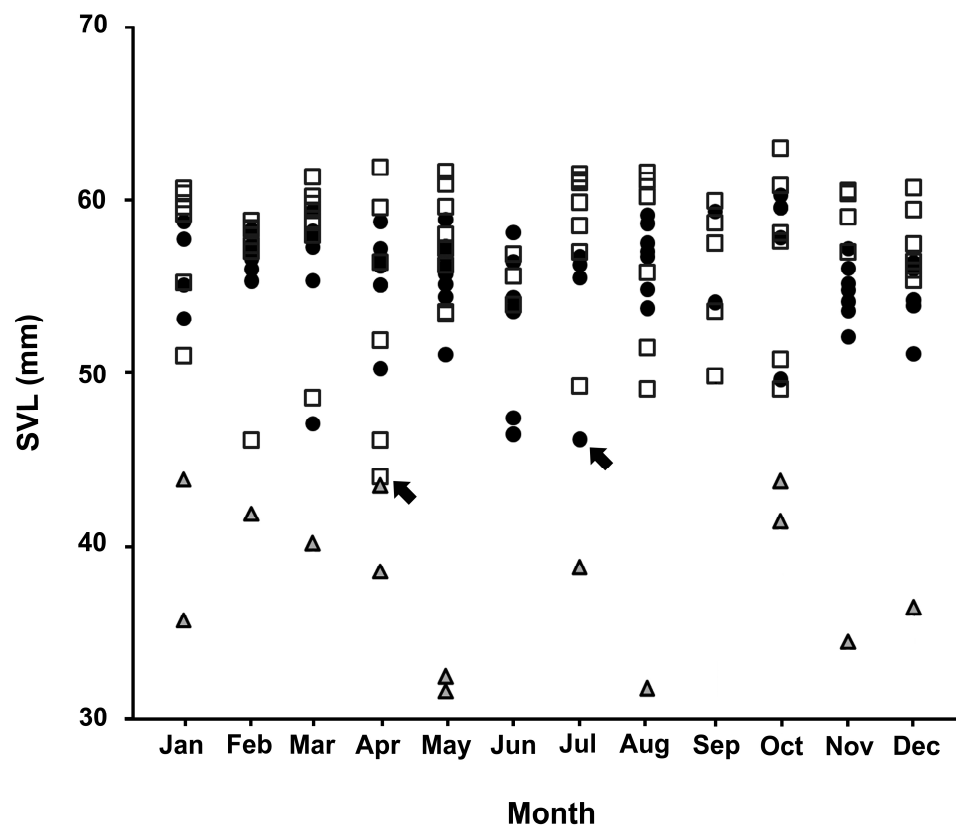


Fig. 2. Monthly distribution and sizes (SVL) of adult males (white squares), adult females (black circles) and juveniles (gray triangles) of *Ptychoglossus bicolor*. the arrows indicate the minimum size at sexual maturity for each sex.

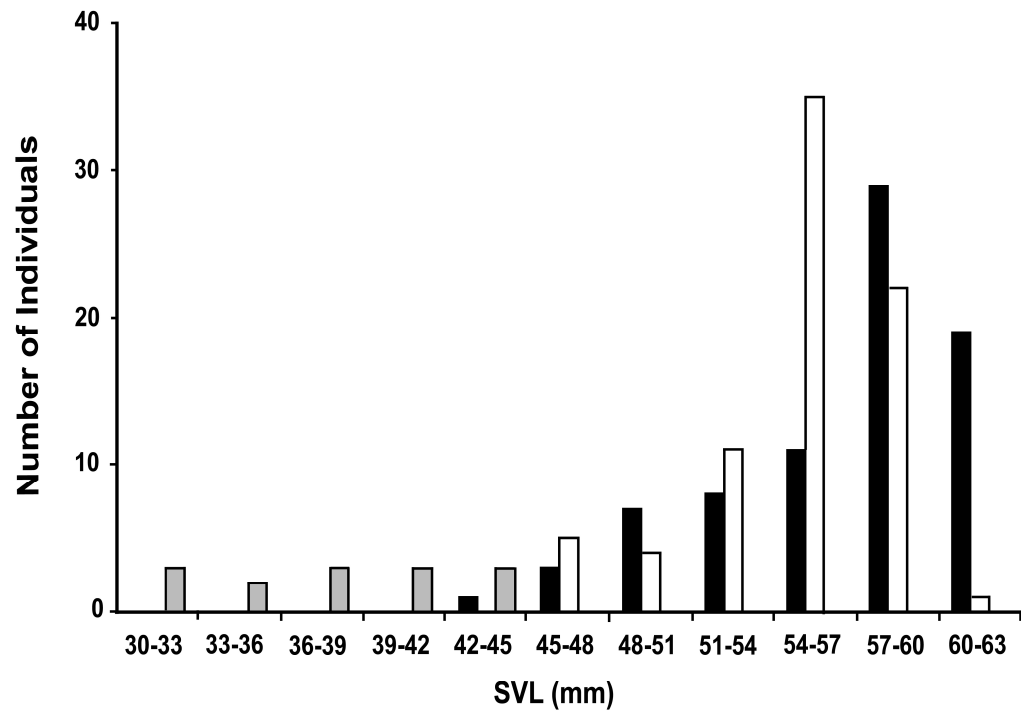


Fig. 3. Distribution for sizes (SVL) of the individuals in the different categories considered in the population. Adult males (black bars), adult females (white bars) and juveniles (gray bars).

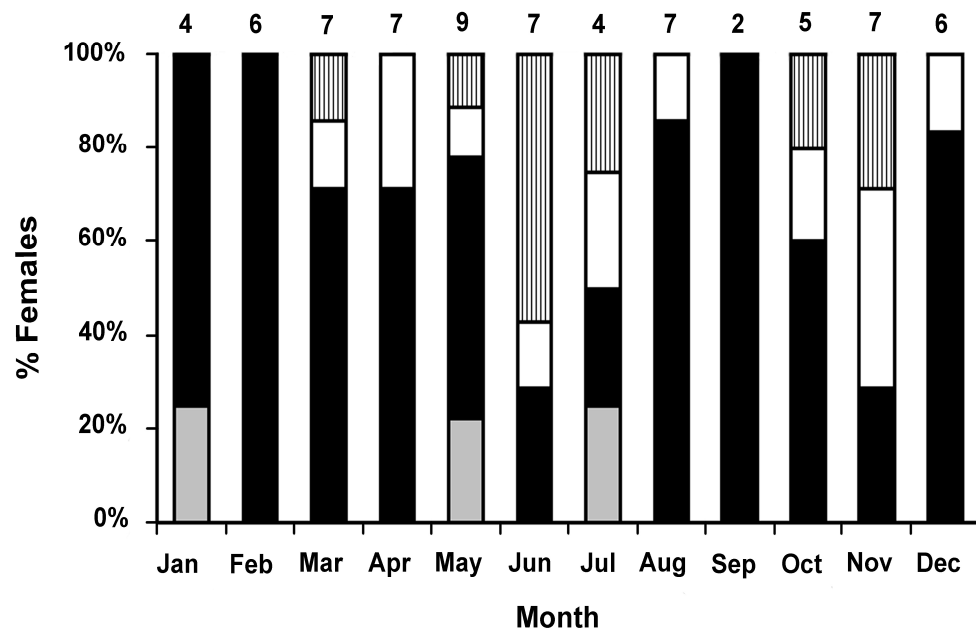


Fig. 4. Monthly distribution of reproductive stages of mature females of *Ptychoglossus bicolor*. Non vitellogenic females (white bars), vitellogenic females (black bars), ovigerous females (gray bars), vitellogenic and ovigerous females (striped bars).

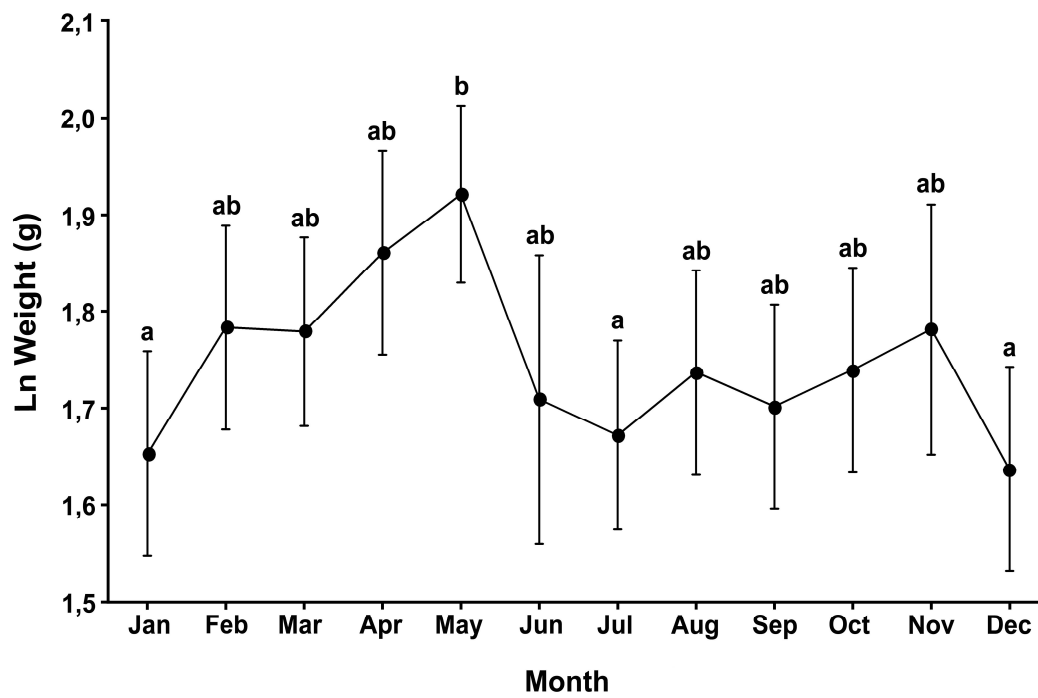


Fig. 5. Mean monthly and standard error of Ln testis mass in *P. bicolor*. Different superscripts indicate statistically significant differences among months. It is observed that the dry months are the months in which the testicular mass was significantly reduced.

Variable	Mean ± SD (min.- max. values)	(n)	Regression data	Statistical Value	Significance
SVL* (mm)					
Males	56,74 ± 4,37 (44,02 - 62,96)	78		z = 3,71	p = 0,0002
Females	55,22 ± 3,23 (46,16 - 60,28)	78			
Weight (g)					
Males	4,08 ± 0,95 (1,80 - 6,10)	78	r² = 0,088 F= 4,46	F = 14,10	p = 0,0002
Females	3,26 ± 0,61 (2,00 - 4,60)	78	p = 0,0008		
Head Height (mm)					
Males	5,88 ± 0,61 (4,06 - 7,24)	78	r² = 0,41 F = 53,20	F = 106,33	p < 0,0001
Females	5,03 ± 0,33 (4,08 - 5,80)	78	p < 0,0001		
Head Length (mm)					
Males	10,72 ± 1,09 (8,04 -14,80)	78	r² = 0,42 F = 27,62	F = 114,43	p < 0,0001
Females	9,16 ± 0,63 (6,86 - 10,68)	78	p < 0,0001		
Head Width (mm)					
Males	8,14 ± 0,93 (4,92 - 9,98)	78	r² = 0,05 F = 4,86	F = 6,23	p = 0,0135
Females	6,78 ± 0,35 (5,76 - 7,46)	78	p < 0,0089		
Body Height (mm)					
Males	6,90 ± 0,72 (5,20 - 8,88)	78	r² = 0,18 F = 17,38	F = 31,91	p < 0,0001
Females	6,29 ± 0,61 (4,00 - 7,70)	78	p < 0,0001		

Table 1. Sexual dimorphism in body and head size in *Ptychoglossus bicolor*. For the comparative tests we used the transformed morphometrical values and the transformed SVL as the covariate. N = 78 males and 78 females. * Mann-Whitney *U*-test on original data.