

Sexual maturity and sexual dimorphism in a population of the rocket-frog *Colostethus aff. fraterdanieli* (Anura: Dendrobatidae) on the northeastern Cordillera Central of Colombia

Madurez y dimorfismo sexual de la ranita cohete *Colostethus aff. fraterdanieli* (Anura: Dendrobatidae) en una población al este de la Cordillera Central de Colombia

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Abstract

The minimum size of sexual maturity and sexual dimorphism are important life history traits useful to study and understand the population dynamics of any species. In this study, we determined the minimum size at sexual maturity and the existence of sexual dimorphism in a population of the rocket-frog, *Colostethus aff. fraterdanieli*, by means of morphological and morphometric data and macro and microscopic observation of the gonads. Females attained sexual maturity at 17.90 ± 0.1 mm snout-vent length (SVL), while males attained sexual maturity at 16.13 ± 0.06 mm SVL. Females differed from males in size, shape and throat coloration. Males were smaller than females and had a marked and dark throat coloration that sometimes extended to the chest, while females lacked this characteristic, with a throat either immaculate or weakly pigmented. In this study, we describe some important aspects of the reproductive ecology of a population of *C. aff. fraterdanieli* useful as a baseline for other more specialized studies.

Key words: Amphibian, Andes, gonads, histology, morphometry, reproduction

Resumen

El tamaño mínimo de madurez sexual y el dimorfismo sexual son importantes características de historia de vida, útiles para estudiar y comprender la dinámica poblacional de cualquier especie. En este estudio, determinamos el tamaño mínimo de madurez sexual y la existencia de dimorfismo sexual en una población de la ranita cohete, *Colostethus aff. fraterdanieli*, a través de datos morfológicos y morfométricos y de observaciones macro y microscópicas de las gónadas. Las hembras alcanzan la madurez sexual a los $17,90 \pm 0,1$ mm de longitud rostro cloaca (SVL), mientras que los machos alcanzan la madurez sexual sobre los $16,13 \pm 0,06$ mm (SVL). Las hembras difirieron de los machos en tamaño, forma y coloración de la garganta. Los machos fueron más pequeños que las hembras y tuvieron una marcada y oscura coloración gular que algunas veces se extiende hasta el pecho, mientras que las hembras carecen de esta característica, con una garganta inmaculada o débilmente manchada. En este estudio, describimos algunos aspectos importantes de la ecología reproductiva de una población de *C. aff. fraterdanieli* útiles como línea base para otros estudios más especializados.

Palabras clave: Andes, anfibios, gónadas, histología, morfometría, reproducción

INTRODUCTION

Reproductive characteristics are critical components in the life history of any organism, and constitute an important

aspect of the population dynamics of any species (Morrison et al. 2004, Watling and Donnelly 2002). The minimum size of sexual maturity (MSSM), the size at which an individual has all the morphological and physiological conditions to

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begin to breed; Morrison and Hero 2003, Stearns 1992) along with sexual dimorphism (SD, the existence of morphological differences between sexes; Shine 1979), are important life history traits useful to study and understand the population changes through time.

Different theories have been suggested to explain the evolutionary mechanisms behind the MSSM and SD (Lovich et al. 2014, Shine 1979). Life history theory explains the variation in MSSM between sexes, populations and species through natural selection mechanisms mainly related with adult mortality rates (Roff 1992, Stearns 1992). Furthermore, SD is usually explained through two processes: sexual selection (e.g., intrasexual competition for mates, epigametic selection; Lovich and Gibbons 1992) and natural selection (differential interactions of each sex with its environment; Lovich and Gibbons 1992). Both processes can operate independently or in a combination, working together or sequentially to promote a specific degree or type of SD (Shine 1989). However, ecological differences may also be forced as a consequence of sexually selected dimorphism or vice versa (Lovich et al. 2014). Other non-adaptive theories have been suggested, including body-size scaling, genetic correlations between male and female body size, and phylogenetic constraints/inertia (Lovich et al. 2014).

Amphibians and particularly anurans display an extraordinary diversity of life histories and reproductive strategies, which include since species with unattended eggs deposited in water bodies to develop into aquatic larvae until species with terrestrial eggs and direct development, passing by a wide range of mating and parental behaviors (Duellman and Trueb 1994). This diversity has limited the finding of clear patterns within life history of amphibians, and the ability to generate hypotheses about its occurrence. However, has been found that most amphibian females have larger body sizes than males and thus females usually reach sexual maturity at larger sizes (90% of evaluated species; Shine 1979, Woolbright 1983). Several explanations have been suggested for this pattern: the first one is related to a higher fertility in larger females (Shine 1989, Woolbright 1983). The second one refers to energetic constrains in males because territorial defense, agonistic behavior, and advertising behavior may require energy that would otherwise be used for growth (Woolbright 1983). Another explanation is usually appealed in species where males are larger than females and refers to the existence of physical male combats for mating because large males are more likely to win combats (Katsikaros and Shine 1997, Shine 1979, Wells 1978).

Some studies show the existence of chromatic SD in some amphibian species, where males and females exhibit different colorations patterns in particular body areas like dorsum (Buchanan 1994, Rojas and Endler 2013), upper jaw (Buchanan 1994), vocal sac (Buchanan 1994), hidden surfaces of forelimbs (e.g., *Pristimantis bicolor*, Rueda-Almonacid and Lynch 1983; *Pristimantis erythropleura*, Lynch 1996) and fingers (*Allobates caeruleodactylus*, Lima et al. 2002). However, because these traits are not conservatives across different taxa have received little attention. A more conservative coloration SD between different anuran species is the throat coloration, which is dark, or dark spotted in males whiles is clear or immaculate in females (Grant and Castro 1998, Greenberg 1942, Wells 1978).

In this study, we describe some aspects of the reproductive ecology of *Colostethus* aff. *fraterdanieli*, specifically we focus on the following objectives: **1)** Determine the minimum size at sexual maturity in males and females and **2)** establish if exist some sexual dimorphism in this species. Keeping in mind the general trends of anurans respect these two life history traits, we predicted that females would reach MSSM at larger sizes than males. Additionally, we expected that this species present sexual dimorphism with females larger than males and a darker throat coloration pattern in males.

MATERIALS AND METHODS

Study area. The population is located in the site known as El Edén, a secondary forest in “vereda San Antonio”, Alejandria municipality, Antioquia, Colombia (6,36664 N, 75,02746 W), in the buffer area of the Jaguas hydroelectric project. The area corresponds to pre-montane wet forest (according to the system of Holdridge 2000) and is located at 1400 m. a. s. l. at the northeastern of the Cordillera Central in Colombia. Temperature varies between 18 and 24 °C, with an average relative humidity of 82.2%, and annual rainfall between 2000 and 4000 mm.

Study species. *Colostethus* aff. *fraterdanieli* is an undescribed species sister to *C. fraterdanieli* with 13% of genetic distance in the mitochondrial gene COI (Daza et al. unpublished). The genus *Colostethus* is a trans-Andean clade, extending from eastern Central America to northwestern Ecuador, with most species occurring in cloud forests in the western Andes (Grant et al. 2006). *Colostethus* aff. *fraterdanieli* is a leaf-litter dwelling frog inhabiting forest fragments with small streams. Like

its congeners, this species is extremely fast and agile, responding to danger by hopping a short distance and hiding underneath the leaf litter. Males call constantly from leaf litter. The advertisement call is composed by one note and in some cases; males emit calls with three notes. Adults seem to be territorial and males have been observed carrying 10 to 11 tadpoles in their backs.

Field work. Frogs were collected between March and May 2013 using active search (Crump and Scott 2001). Although it could be useful to collect the animals throughout the year to record the temporal and seasonal variation in reproductive status of the frogs, we had to restrict the fieldwork to March and May due to some logistical difficulties. All collected individuals were euthanized using lidocaine 3%, fixed with formaldehyde 10% and stored in the herpetological collection of the Museo de Herpetología de la Universidad de Antioquia (MHUA; vouchers MHUA-A 7878-7898, 7900-7929, 7931-7933, and 7940-7984).

Minimum size of sexual maturity (MSSM). All individuals were dissected under a stereomicroscope to examine gonadal characteristics and to determine the sex and the reproductive stage. The sex of the individuals were established by the presence of ovaries or testes. The females were classified according to the following reproductive stages: previtellogenic (without vitellogenic follicles), vitellogenic (with brown follicles), and gravid (with oviductal eggs; Méndez-de la Cruz et al. 2013). The females were considered matures when presented vitellogenic follicles, eggs, or when presented at the same time previtellogenic follicles and convoluted oviducts (Prado et al. 2004, Wake and Dickie 1998). We prepared histological slides of testes sections stained with the hematoxylin-eosin (Luna 1968) to classified males like matures or immature. We used the presence of spermatozoa in the seminiferous tubules to identify adult males (Prado et al. 2004, Wake and Dickie 1998). We inferred the MSSM on each sex as the size of the mature individual with the lowest SVL.

Sexual dimorphism (SD). Prior to dissection, frogs were photographed and measured to determine the presence of sexual dimorphism. Only mature individual were included in the analysis of SD. All measurements were taken three times at the stereomicroscope with a digital caliper (0.1 mm precision) and then were averaging to reduce type II errors (Yezerinac et al. 1992). Characters measured were: snout-vent length (SVL), head length (HL), head width (HW) measured at the level of the posterior part of the tympanum, inter-orbital diameter (IOD), inter-nostril distance (IND), eye diameter (ED), disc diameter of the

third digit (DD3), disc diameter of the fourth toe (DF4), eye-nostril distance (END), tympanum diameter (TD), length of the humerus (LHU), forearm length (FOL), thigh length (THL), tibia length (TL), and foot length (FL) (figure 1). These characters correspond to the typical measures used to determine morphometric differences in anurans (e.g., Felgueiras-Napoli 2005).

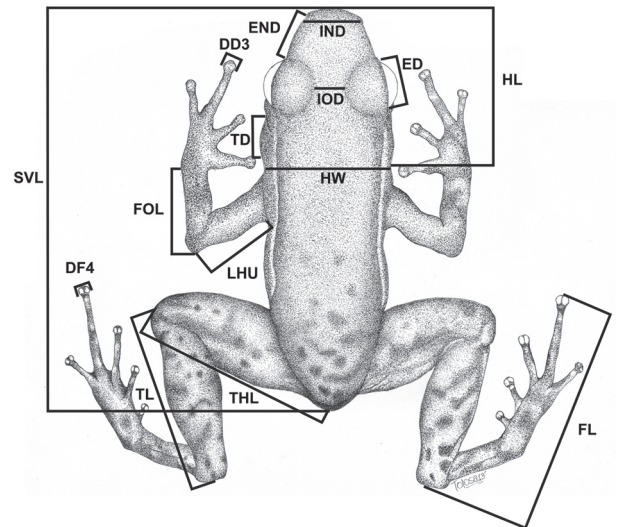


Figure 1. Morphometric measurements used for the analysis of sexual dimorphism in *Colostethus aff. fraterdanieli* (Anura: Dendrobatidae) on the northeastern Cordillera Central of Colombia (SVL = snout-vent length; HL = head length; HW = head width; IOD = inter-orbital diameter; IND = inter-nostril distance; ED = eye diameter; DD3 = disc diameter of the finger three; DF4 = disc diameter toe four; END = eye-nostril distance; TD = tympanum diameter; LHU = length of the humerus; FOL = forearm length; THL = thigh length; TL = tibia length; FL = foot length)

We used a principal components analysis on the covariance matrix of all morphological measurements to reduce the total morphometric variation in few dimensions using the software Past v.3.01 (Hammer et al. 2001). The first principal component (PC1) calculated from a set of morphometric measurements generally expresses body size variation when all traits load largely and in the same direction (Reyment et al. 1984). Remaining variance describes relative shape differences that are not due to body size (and these are expressed in subsequent PCs). The relative contribution of the original variables to each principal components were determined from the matrix of factor-variable correlations (table 1). We evaluated sexual dimorphism in size (PC1) and shape (PC2) using one-way ANOVAs.

Table 1. Factor-variable correlations between morphological variables and the first two principal components: body size (PC1) and body shape (PC2) for a population of *Colostethus* aff. *fraterdanieli* (Anura: Dendrobatidae) on the northeastern Cordillera Central of Colombia

Variable	PC1	PC2
SVL	0.311	-0.09523
LC	0.2818	-0.02747
AC	0.3143	0.007738
DIO	0.2159	-0.133
DIN	0.2562	-0.1063
DO	0.2359	0.09878
DD3	0.1763	0.5648
DDP4	0.1403	0.62
DON	0.2681	0.1194
DT	0.1951	0.09748
LH	0.2159	0.05085
LAB	0.2458	-0.3765
LM	0.3155	-0.02916
LT	0.3074	0.0382
LP	0.3087	0.05409

In addition to morphometric variation, we classified all mature individuals according to throat coloration. Individuals with spots covering less than 20% of the throat were classified as “*immaculate*” and the remaining individuals were classified as “*spotted*”. We used a chi-square test (χ^2) to test for differences between sexes in this feature.

RESULTS

Minimum size of sexual maturity (MSSM). We examined 101 frogs and based on external observation of the gonads and histological evaluation of the testes: We found 22 matures females, 24 immature females, 53 matures males and two immature males. MSSM in females was at 17.90 ± 0.1 mm. All mature females had between 8-10 brown vitellogenic follicles and convoluted oviduct. We did not find any female with eggs in the oviduct (figure 2A). The MSSM in males was 16.13 ± 0.06 mm, and all showed seminiferous tubules with cysts that had most of the stages of spermatogenesis (spermatogonia, spermatocytes, spermatids and sperm). All mature males had free spermatozoa in the seminiferous tubules (figure 2B). The right testis was larger than the left one, and in some individuals, testes had dark pigmentation surrounding the seminiferous tubules (figure 2C). This pigmentation seems not related with the reproductive status of individual.

Sexual dimorphism (SD). PCAs showed that the subspaces defined by the first two principal components had the same orientation in both sexes and can be used as size and shape variables. Specifically, the sums of the eigenvalues (S) were

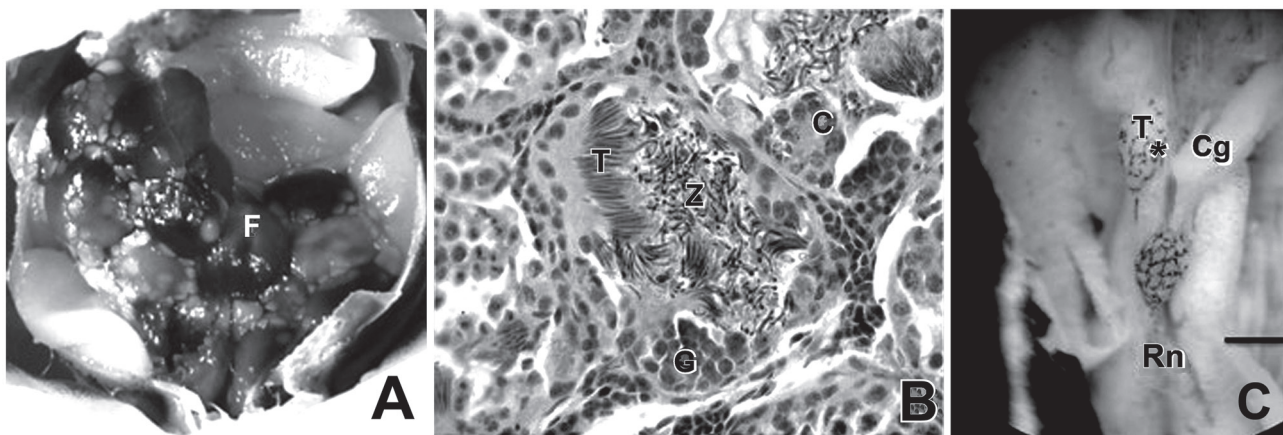


Figure 2. Macroscopic and microscopic view of the gonads of *Colostethus* aff. *fraterdanieli* (Anura: Dendrobatidae) on the northeastern Cordillera Central of Colombia. **A.** Female ovaries with brown developed follicles (F); **B.** Histological section of the testis showing: spermatogonia (G), spermatocytes (C), spermid (T*), sperm (Z). **C.** Male testes with dark pigmentation surrounding the seminiferous tubules: testicle (T), kidney (Rn), fat bodies (Cg)

0.023 of a possible range of 0 to 2, where 2 indicates identical subspaces (Krzanowski 1979). The morphometric analysis showed differences in size and shape between males and females (size: $df = 1$, $F = 24.33$, $p < 0.001$; shape: $df = 1$, $F = 109.9$, $p < 0.001$). Females were bigger than males (figure 3). We also found differences between sexes in the throat coloration ($df = 1$, $\chi^2 = 41.57$, $p < 0.001$). Most of the mature males had the *spotted* pattern (90%). The two immature males lacked this coloration. All females had an *immaculate* pattern on the throat (figure 4).

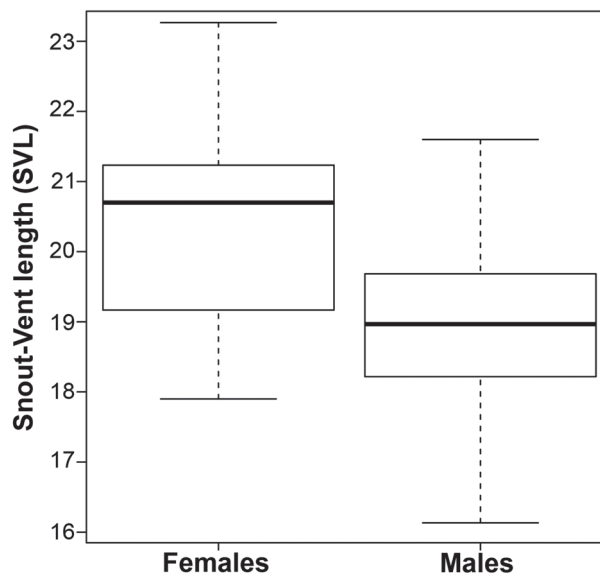


Figure 3. Difference in snout-vent length (SVL) between sexes of *Colostethus* aff. *fraterdanieli* (Anura: Dendrobatidae) on the northeastern Cordillera Central of Colombia

DISCUSSION

In this study we describe some aspects of the reproductive ecology of a population of *C. aff. fraterdanieli*, we show that females and males differ in MSSM, size, shape, and throat coloration. Females reach sexual maturity around 17.9 mm while males reach it at a smaller size of about 16.1 mm. Due to the great diversity of life histories and reproductive strategies recorded to the dart-poison frogs, this particular group of anurans have attracted the attention of numerous researchers evaluating different reproductive and behavioral characteristics. An important part of these studies has been conducted in species belonging to what was formerly known as *Colostethus* (before Grant et al. 2006) and that now corresponds to several taxa with



Female
MHUAA-7952



Male
MHUAA-7924

Figure 4. Throat coloration in males and females of *Colostethus* aff. *fraterdanieli* (Anura: Dendrobatidae) on the northeastern Cordillera Central of Colombia

independent evolutionary histories (Grant et al. 2006). Most of these works showed that like *C. aff. fraterdanieli*, in species as *Colostethus beebei* (Bourne et al. 2001; now *Anomaloglossus beebei*; see Grant et al. 2006), *Colostethus inguinalis* (Wells 1980; now *Colostethus panamensis*; see Grant 2004), *Colostethus subpunctatus* (Fandiño et al. 1997; now *Hyloxalus subpunctatus*, see Grant et al. 2006), *Mannophryne oblitterata* (Dixon and Rivero-Blanco 1985) and *Colostethus trinitatis* (Cummins and Swan 1995, now *Mannophryne trinitatis*, see Grant et al. 2006) females reach sexual maturity at a larger size than males (table 2).

Table 2. Minimum size of sexual maturity (MSSM) and sexual dimorphism (SD) in some *Colostethus* species (before Grant et al. 2006) (“–” indicate not reported data)

Species	MSSM (mm)		SD	References
	Female	Male		
<i>Allobates caeruleodactylus</i>	15.4	–	–	Lima et al. 2002
<i>Allobates marchesianus</i>	16.4	–	–	Juncá 1998
<i>Allobates marchesianus</i>	15.7	15.7	No	Lima and Keller 2003
<i>Anomaloglossus beebei</i>	18.1	15.3	♂ smaller	Bourne et al. 2001
<i>Anomaloglossus stepheni</i>	15.4	–	–	Juncá 1998
<i>Colostethus aff. fraterdanieli</i>	17.9	16.1	♂ smaller	This study
<i>Colostethus panamensis</i>	25	22	♂ smaller	Wells 1980
<i>Hyloxalus subpunctatus</i>	22.4	21	♂ smaller	Fandiño et al. 1997
<i>Mannophryne herminae</i>	21.2	–	–	Dixon and Rivero-Blanco 1985
<i>Mannophryne obliterata</i>	32	31	♂ smaller	Dixon and Rivero-Blanco 1985
<i>Mannophryne trinitatis</i>	19.5	18.5	♂ smaller	Cummins and Swan 1995

Age and size at sexual maturity is a critical component of life history studies because of their importance in determining the fitness (Roff 1992, Stearns 1992). Observed differences between sexes in MSSM, can relate to patterns of mating and parental effort (Howard 1981). For their reproductive role, females should produce and host the greatest possible number of eggs to maximize their fitness, which favors that females reach a larger size (Howard 1978, 1981, Woolbright 1983). Meanwhile, male fitness is not necessarily related to their body size depending on the mating system display for particular taxa (Howard 1981, Shine 1979).

We found that the ration of females and males in the population was very similar (1 female: 1.3 male), however the proportion of mature individuals differed between sexes, with a huge proportion of mature male (96%) compared with a median percentage of mature females (48%). These differences could be explained by variation in detectability of mature and immature males. Adult males usually display territorial and calling behaviors, which make them more conspicuous to the researchers. Unlike immature males that have more cryptic behavior because they are usually transients (Bailey et al. 2004, Mazerolle et al. 2007, de Solla et al. 2005).

Our results suggest sexual size dimorphism in *Colostethus aff. fraterdanieli*, where females are larger than males, as has been reported in the most of anuran species (Shine 1979) and particularly in other species of *Colostethus* (table 2, before Grant et al. 2006). Sexual dimorphism is the result of divergent selective pressures between sexes that promote morphological and behavioral differences (Howard 1981). However, regardless of the selection pressures and evolutionary forces that originated the differences in size between females and males, these seems to be the reflection of divergence in size at sexual maturity because in amphibians both sexes usually have the same body growth rates (Howard 1981). One the most known life history trade-offs is that between size and age at maturity that imply that to reduce time for growth, and decrease age at maturity might result in a decrease of adult body size (Roff 2000).

In this study, we found an evident dimorphism in throat coloration with males having a “spotted” pattern and females with “immaculate” throats. This kind of chromatic sexual dimorphism is relatively generalized between anurans and is related to a differential distribution of dermal chromatophore units between sexes with a higher number of melanophores per unit area in males (Buchanan 1994,

Greenberg 1942). Differences in color saturation seems to be a secondary result of elevated testosterone levels in males (Buchanan 1994, Grant and Castro 1998, Greenberg 1942) therefore, immature males lack this character. Although not quantified here, we also evidenced that the third finger, as in many *Colostethus* species, is swollen in adult males (see figure 4).

The minimum size of sexual maturity is a critical parameter to understand the population dynamic of any species (Morrison and Hero 2003) and its knowledge is essential to make inferences about evolutionary history, population trends, and conservation status. Despite the importance of this parameter, most of studies in these kind of topics make inferences about it without a direct evaluation in the populations generating inaccuracy in their estimates and conclusions. For this reason, studies as this are valuable and constitute a baseline to other more specialized studies.

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