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SEXUAL DIMORPHISM IN THE FROG *AUBRIA SUBSIGILLATA* (DUMÉRIL, 1856) (ANURA: PYXICEPHALIDAE) IN BENIN REPUBLIC, WEST AFRICA

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Sexual dimorphism in the frog *Aubria subsigillata* (Duméril, 1856) (Anura: Pyxicephalidae) in Benin republic, West Africa.

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Abstract

Sexual dimorphism, when present in a given species, is an important adjunct when studying reproductive strategies in amphibian populations. In this study we investigated biometric and qualitative characters for sexing the frog *Aubria subsigillata*. The objective of this study was to determine from these data the criteria for accurately distinguishing males and females of the frog *Aubria subsigillata*. Multivariate analysis of variance was used to compare the means of the collected variables between the sexes and canonical discriminant analysis was used to identify notable dimorphic traits. The results reveal that males have a fairly long humerus (mean 13.49 mm), abdomen and throat generally heavily mottled with black. They lack femoral glands on the femora. In contrast, females have a shorter humerus (mean 13.01 mm), a whitish abdomen and throat and a protruding femoral gland. Females are larger in size (mean 86.19 mm) and are heavier (mean 38.63 g) than males (means 80.19 mm & 30.25 g respectively). For sexing of *A. subsigillata* individuals, it is important to take into account all these morphological criteria.

Key words

Anuran, sexual differentiation, dimorphic criteria, Ouémé Valley, Benin

Introduction

Sexual dimorphism is the set of more or less marked morphological, structural or behavioural differences between male and female individuals of the same species (Peters & Aulner, 2000). In many vertebrates, this observed sexual dimorphism is indeed the work of a natural genetic selection in which males differentiate from females through a great divergence in physiological, morphological and behavioural traits (Kupfer 2007; Petrović *et al.* 2017). In the anuran community, sexual dimorphism can be seasonal or permanent (Angèl 1946). To this end, many aspects are taken into account in the process of identifying the phenotypic traits at the origin of a notable difference in a population of anuran species. Indeed, anuran males are generally characterised by their aggressiveness resulting from their capacity to defend their territory and seize females during amplexus (Wells 1977; Peters & Aulner 2000). Males of anuran species have powerful forelimbs that enable them to cling to females during mating. Forelimbs are also used to repel other males when males grapple with each other or when a male wants to remove another male in amplexus (Peters & Aulner 2000). They are also used by terrestrial frogs to support the body during movement on land (Peters *et al.* 1996).

Similarly, some males have hooked and highly swollen thumbs with very spiny rough fingers that are copulatory brushes used to grip females during amplexus. This is the case of the males of the frogs *Pelobates spp., Bombina sp.* and *Rana dalmatina* Fitzinger in Bonaparte, 1839, in which copulatory brushes appear on the toes, under the arms and sometimes on the belly during the breeding period and are used to hold females during the amplexus (Angel 1946; Howard & Kluge 1985; Petrović *et al.* 2017). Males of other species, on the other hand, can be distinguished from females by the presence of vocal sacs of varying size, which may be internal or external and which are used to invite females to the amplexus. During the mating season of anurans, variability in sex coloration, horny skin secretions, ridge development, webbing and tympanum are also observed. In contrast to males, receptive females in nuptial livery are large with a distended abdomen and a very high body mass index (Nali *et al.* 2014; Quiroga *et al.* 2015; Blain *et al.* 2015; Kamath & Sreekar 2016).

Not all of these traits can be observed in all anuran species, however, and contradictory morphometric traits remain in some anuran populations. This is the case for the frog *Aubria subsigillata*. Indeed, *A. subsigillata* is an aquatic frog belonging to the family Pyxicephalidae. It has a maximum length extending from the snout to urostyle (SUL) that varies with sex. Males observed in the Banko National Park in Côte d'Ivoire have a SUL of 73 mm while females of this species observed have a SUL of 78 mm (Assemian *et al.* 2006). Apart from size dimorphism, this frog has a large and developed femoral gland placed in the middle of each femur. This femoral gland is light red in colour. Perret (1994) and Parker (1936) found that this gland is more developed in females (6.6 to 8.5 mm diameter) while it is less developed or absent in males (5.5 to 6.5 mm diameter). Ohler & Kasadi (1990) noted, however, that this gland was not a distinctive feature. Parker (1936) had made his observation in Liberia on a female specimen of *A. subsigillata* which he mistook for a male. As for Perret (1994), he made his observations on a small number of samples (N= 31) even though these samples came from various West African countries (Ivory Coast, Ghana, Nigeria, Cameroon and Gabon).

Furthermore, sexual dimorphism in size does not allow for sexing of individuals at a younger age. In order to successfully conserve the *Aubria subsigillata* population through the implementation of techniques aimed at reproducing the species in captivity; it is crucial to be able to differentiate male and female of these individuals. Once sexual differentiation has been achieved, breeding groups (i.e. assigning a certain number of males to a certain number of females) between the sexes can be selected, as can hormonal induction (i.e. the use of hormones to complete oocyte maturity and trigger egg-laying in frogs) at doses defined according to the sex (Kouba *et al.* 2012; Arregui & Bosch 2023).

The present study is therefore initiated to determine from biometric and qualitative data the notable dimorphic criteria for distinguishing males and females of the *A. subsigillata* frog with a view to its successful sexing.

Furthermore, with the high level of human malnutrition in Africa, some authors (Paixão & Bressan 2009; Oliveira *et al.* 2017) have shown that frogs are a better food because they contain low levels of lipids and are rich in protein and amino acids that are essential for the human diet. This makes frogs a good source of protein to replace fish in the diet. Indeed, in Benin, it is not at all surprising to see fish consumers queuing up to gorge themselves on frog meat during times of fish shortage (Houénafa A. C. Gansa, pers. obs.). Two species of fish are commonly farmed in Benin (the tilapia *Oreochromis niloticus* (Linnaeus, 1758) and the catfish *Clarias gariepinus* Burchell, 1822). But these fish species are characterised by low productivity due to the strains used, which have declined in genetic performance over the years of production (Tapsoba 2017). Faced with this situation, frog farming with *Aubria subsigillata* may appear to be an excellent solution, as these frogs are known for their ease of handling, high fertility, rapid growth, prolificacy and large size (Lutz & Avery 1999; Cribb *et al.* 2013). The species, therefore, is a good potential candidate for being a significant contribution to food security (Afonso *et al.* 2017).

Methods

Study environment

Sampling of the frog *Aubria subsigillata* was conducted in the area between 6°36′00″ N and 6°39′00″N; and between 2°31′ 30″ E and 2°33′00″E in the Commune of Dangbo in the South-East of Benin (Fig. 1). The habitat of *A. subsigillata* in the Commune of Dangbo comprises the River Ouémé and its floodplain, a wet meadow colonised by the plant species *Andropogon gayanus* Kunth*, Mitragyna inermis* (Willd.) Kuntze*, Paspalum notatum* Flüggé*, Aeollanthus pubescens* Benth., *Panicum maximum* Jacq.*, Calopogonium mucunoides* Desv. and *Tridax procubens* L. (Gansa *et al.* 2023). During the wet season from August to October the river overflows its normal channel across the floodplain. During the dry season (December to March), the floodplain has numerous pools, marshes and swamps. In the dry season local people grow food crops such as chili peppers (*Capsicum frutescens* L.), maize (*Zea mays* L.), cassava (*Manihot utilissima* (Pohl) Link), tomato (*Solanum lycopersicum* L.) and okra (*Hibiscus esculentus* L.) in the study site (I.N.S.A.E 2016). These farmers make many irrigation canals that are used by *A. subsigillata.* These canals are abandoned by at the end of the cultivation season (Gansa *et al.* 2021).

Materials

Over a period of 12 months, 116 males and 218 females of *A. subsigillata* were purchased from frog catchers. Biometric data were collected and morphological descriptions were made on each individual.

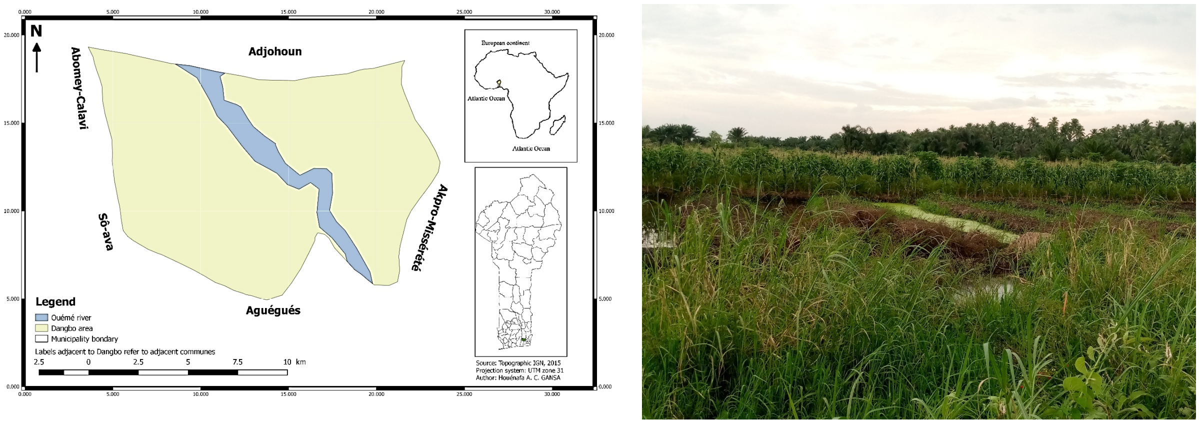


Figure 1. Commune of Dangbo (left) and habitat sampled for *A. subsigillata* (right).

Morphometric traits

A total of 394 dead individuals of *A. subsigillata* (278 females and 116 males) were purchased from frog catchers in the Commune of Dangbo during 12 months (June 2021 to May 2022). The purchased frogs were individually weighed on a scale (0.001g sensitivity) and then fixed in 5% formalin and preserved in 70% ethanol before being sent to the

Zootechnics and Breeding Systems Research Unit of Laboratory of Fisheries and Animals Sciences (LaSAH) of the National University of Agriculture (UNA). In the laboratory, the sampled individuals were rinsed with water and then each individual was observed both with the naked eye and with a magnifying glass for a description of the following phenotypic traits: throat markings (black spotted or not), abdominal markings (black spotted or not), femoral glands (protuberant or not). Next, numerous measurements were taken on each individual using a caliper. These were: snout to urostyle length, head with, forearm length, hand length, upper arm length, thigh length, tibia length, foot length, sacrum width, and the sacrum shaft extension length (Table 1). Finally the body was cut open with scissors in the ventral midline and inspected for the presence of testes or ovaries.

Table 1. Types of measurements made on individuals of *Aubria subsigillata* (Watters *et al.* 2016).

Types of measures	Definition
Head width (HW)	The HW was measured between the left and right angles of the palate and more precisely at the widest points.
Forearm length (FLL)	The FLL was measured from the angle of the elbow to the basal part of the palmar tubercle.
Hand length (HAL)	The HAL was measured from the base of the external palmar tubercle to the tip of the finger in position III (longest finger).
Upper arm length (UAL)	The UAL was measured between the angle of the elbow and the body of the frog.
Thigh length (THL)	The THL was measured from the angle of the knee to the level of the belly.
Tibia length (TL)	The TL was measured from the outer surface of the knee angle to the heel (the tibiotarsal inflection angle).
Foot length (FL)	The FL was measured between the basal part of the inner metatarsal tubercle and the tip of the toe in position IV (longest toe).
Snout to urostyle length (SUL)	The SUL was measured from snout to the urostyle
Sacrum width (SW)	The SW is the measurement of the base of the large triangle of hip bone located at the level of the ninth vertebra.
Sacrum shaft extension length (SSEL)	The SSEL was measured from the junction between the femur and the body to the junction between the upper arm and the body.

Statistical analysis

The values of the measured parameters were subjected to a descriptive statistical analysis and the normality of the distribution of these variables was confirmed by the Shapiro-Wilk test. Also, all the parameters were compared simultaneously according to gender using the multivariate analysis of variance (MANOVA) method based on the fixed-crossed model. In view of the significant differences observed between the various parameters, a canonical discriminant analysis (CDA) was carried out to identify the most discriminating characteristics between the sexes. The IBM SPSS Statistics Version 25 software was used to carry out the descriptive statistics, MANOVA and DCA. Prior to the execution of MANOVA, the conditions of multivariate normality and homogeneity of variances were checked using the Mardia and Levene test (Zar 1999). A correlation matrix was performed to visualize the most correlated variables and then a principal component analysis is performed to spatially represent the correlations between the variables.

Table 2. Homogeneity test of variances based on frog sex.

Variables	Levene test based on the mean	df_1	df_2	Sig.
Snout to urostyle length (mm)	6.585	1	392	0.005
Total weight (g)	0.002	1	392	0.967
Upper arm length (mm)	0.918	1	392	0.339
Forearm length (mm)	3.453	1	392	0.064
Hand length (mm)	0.298	1	392	0.586
Thigh length (mm)	1.338	1	392	0.248
Tibia length (mm)	0.692	1	392	0.406
Foot length (mm)	6.514	1	392	0.011
Sacrum width (mm)	2.046	1	392	0.153
Sacrum shaft extension length (mm)	0.420	1	392	0.518
Head width (mm)	0.355	1	392	0.552
Total weight (mass) over Snout to urostyle length	3.157	1	392	0.076

*Significant at the α = 0.05 threshold; df = degree of freedom

Table 3. Homogeneity test of variances based on the aspect of femoral gland.

Variables	Levene test based on the mean	df_1	df_2	Sig.
Snout to urostyle length (mm)	2.931	2	391	0.055
Total weight (g)	1.749	2	391	0.175
Upper arm length (mm)	0.419	2	391	0.658
Forearm length (mm)	0.093	2	391	0.912
Hand length (mm)	30.859	2	391	0.022
Thigh length (mm)	0.291	2	391	0.748
Tibia length (mm)	1.623	2	391	0.199
Foot length (mm)	2.846	2	391	0.059
Sacrum width (mm)	1.634	2	391	0.196
Sacrum shaft extension length (mm)	1.626	2	391	0.198
Head width (mm)	0.396	2	391	0.673
Total weight (mass) over Snout to urostyle length	2.454	2	391	0.087

*Significant at the α = 0.05 threshold; df = degree of freedom

Table 4. Homogeneity test of variances based on the abdominal aspect of frogs.

Variables	Levene test based on the mean	df_1	df_2	Sig.
Snout to urostyle length (mm)	1.619	2	391	0.200
Total weight (g)	1.716	2	391	0.181
Upper arm length (mm)	1.827	2	391	0.128
Forearm length (mm)	1.498	2	391	0.225
Hand length (mm)	0.490	2	391	0.613
Thigh length(mm)	1.942	2	391	0.131
Tibia length (mm)	0.180	2	391	0.835
Foot length (mm)	1.463	2	391	0.233
Sacrum width (mm)	1.338	2	391	0.264
Sacrum shaft extension length (mm)	1.504	2	391	0.224
Head width (mm)	1.309	2	391	0.271
Total weight (mass) over Snout to urostyle length	1.542	2	391	0.215

*Significant at the α = 0.05 threshold; df = degree of freedom

Table 5. Homogeneity test of variances based on the throat aspect of frogs.

Variables	Levene test bas on the mean	df_1	df_2	Sig.
Snout to urostyle length (mm)	1.303	2	391	0.273
Total weight (g)	0.942	2	391	0.391
Upper arm length (mm)	0.201	2	391	0.818
Forearm length (mm)	1.891	2	391	0.152
Hand length (mm)	0.476	2	391	0.622
Thigh length (mm)	1.505	2	391	0.223
Tibia length (mm)	1.102	2	391	0.333
Foot length (mm)	5.134	2	391	0.006
Sacrum width (mm)	0.299	2	391	0.742
Sacrum shaft extension length (mm)	0.897	2	391	0.409
Head width (mm)	1.874	2	391	0.155
Total weight (mass) over Snout to urostyle length	2.784	2	391	0.063

*Significant at the α = 0.05 threshold; df = degree of freedom

Table 6. Statistical description of morphometric variables in the frog *Aubria subsigillata*.

Biometric variables	sex	Mean	Minimum	Maximum	Standard deviation	Number of individuals	Sig.
Snout to urostyle length (mm)	Male	80.19	63	94	6.88	116	0.0001**
	Female	86.19	72	98	4.89	278	
Total weight (g)	Male	30.25	10.53	52.23	7.24	116	0.0001**
	Female	38.63	17.15	62.66	9.10	278	
Upper arm length (mm)	Male	13.49	10	17	1.97	116	0.029*
	Female	13.01	10	17	1.99	278	
Forearm length (mm)	Male	12.76	9	17	2.33	116	0.0001**
	Female	12.79	9	17	2.08	278	
Thigh length (mm)	Male	27.25	18	34	3.05	116	0.047*
	Female	26.47	18	34	3.73	278	
Foot length (mm)	Male	49.86	32	55	4.01	116	0.025*
	Female	48,67	32	55	5,09	278	

Significant at the threshold of α = 0.05

Results

Tables 2 to 5 present the tests of homogeneity of variance performed according to the categories sex, presence of femoral gland, aspect of abdomen and aspect of throat. For all the homogeneity of variance tests performed, the variance of the variables (snout to urostyle length, total weight, upper arm length, forearm length, hand length, thigh length, tibia length, foot length, sacrum width, sacrum shaft extension length, head width, total weight (mass) over snout to urostyle length) were homogeneous in the categories of sex, presence of femoral glands, aspect of abdomen and aspect of throat ($P > 0.05$). Thus, the MANOVA test is applicable to the different variables under study.

Morphometric traits

Table 6 presents a statistical description of the different biometric data collected in the *Aubria subsigillata* population. The MANOVA test applied to the different morphometric variables of males and females reveals a highly significant difference ($p < 0.001$) for the following variables:
Snout to urostyle length: (male = 80.19 mm; female = 86.19 mm),
Total weight (male=30.25 g; female = 38.63 g)
Forearm length (male = 12.76 mm; female = 12.79 mm)
Upper arm length ($p < 0.01$).

Differences were also significant between males and females for the variables:
Distal part of the foot (male = 49.86 mm; female = 48.67 mm),
Length of the thigh (male = 27.25 mm; female = 26.47 mm), and
Upper arm length (male =13.49 mm; female = 13.01 mm)
Forearm ($p < 0.05$).

No significant differences were observed ($p > 0.05$) for the variables:
Distal part of the forearm (male = 17.59 mm; female = 17.07 mm),
Tibia length (male = 27.03 mm; female = 26.97 mm),
Sacrum width (male =14.97 mm; female = 14.91 mm),
Sacrum shaft extension length (male = 28.63 mm; female = 28.03 mm),
Head width (male = 25.41 mm; female = 24.9 mm) and
Total weight (mass) over snout to urostyle length male = 41.53% mm; female = 41.89% mm).

In general, females of *A. subsigillata* are larger than males in terms of snout to urostyle length (86.19 mm), total weight (38.63 g), forearm length (12.79 mm) and thigh length (26.47 mm). Males, on the other hand, differ from females in the larger size of their upper arm (13.49 mm) and the foot (49.86 mm).

Table 7 presents the results of the tests of equality of means between the different qualitative traits between males and females of *A. subsigillata*. The Wilks' lambda values varied between 0.634 and 1. The value of 1 recorded for the variables forearm length, tibia length, sacred width and total weight (mass) over snout to urostyle length reflect the equality of means for these variables in both sexes. On the other hand, the Wilk's values = 0.634 recorded for the throat aspect variable at a risk of $\alpha < 0.05$ indicates low variability for this variable within each sex and therefore high inter-sex variability and obviously different group means.

Table 8 shows the eigenvalue associated with the linear discriminant function, which allows us to judge the discriminant power of the function. Thus, the eigenvalue of the function is 0.86 represents the inter-class variance of the linear discriminant function of the same rank. Moreover, it explains 100% of the inter-class variability.

Table 9 presents the correlation matrix of quantitative and qualitative traits recorded in *A. subsigillata* individuals. In fact, the correlation matrix makes it possible to explain the links existing between all the variables under study for the identification of the sexes.

According to this table, the correlation is positive and high between femoral gland and snout to urostyle length (0.52), throat aspect and abdominal aspect (0.68), foot and hand (0.60), extension of sacrum diaphysis and length of upper arm (0.65). On the other hand, it is strong but negative between throat aspect and snout to urostyle length (-0.54). This indicates that between the variables femoral gland, snout to urostyle length, throat aspect and abdomen aspect, foot part and hand part and between extension of the sacrum diaphysis and upper arm length, any increase in one variable leads to an increase in the other variable. On the other hand, for the variables throat aspect and snout to urostyle length, any increase in one variable leads to a decrease in the other.

Table 7. Tests for equality of means between males and females.

	Wilks' Lambda	df	Sig.
Snout to urostyle length (mm)	0.804	1	0.068
Total weight (g)	0.835	1	0.071
Femoral gland	0.817	1	0.069
Abdomen appearance	0.826	1	0.073
Throat appearance	0.634	1	0.048*
Length of Upper arm (mm)	0.988	1	0.089
Forearm length (mm)	1.000	1	0.191
Hand (mm)	0.992	1	0.097
Thigh length (mm)	0.990	1	0.095
Length of tibia (mm)	1.000	1	0.112
Distal part of the foot (mm)	0.987	1	0.093
Sacred Width (mm)	1.000	1	0.098
Sacrum shaft extension (mm)	0.996	1	0.097
Head width (mm)	0.993	1	0.098
Total weight (mass) over Snout to urostyle length	1.000	1	0.131

*Significant at the $\alpha = 0.05$ threshold; df = degree of freedom

Table 10 shows the total variance explained for all 15 different variables collected on the males and females of *A. subsigillata*. The total variance explained measures the dispersion of the 15 different variables collected around the mean. In this way, it can be used to detect the variability existing between the 15 variables collected so that the complexity of all these variables can be reduced to a few components while minimising the loss of information.

According to the table, the 15 variables were grouped into 10 components. Among these 10 components, only the first 5 components are principal and these explain 69.58% of the total information held by the different variables. These are component 1, which accounts for 21.11% of the total information, followed by component 2, which accounts for 18.41% of the information, components 3 and 4, which account for 13.27% and 9.13% of the information respectively, and finally component 5, which accounts for 7.65% of the total information

Table 11 presents the correlations between the 5 main components and the different variables. The 5 main components representing the 15 variables collected on the frogs are not linearly correlated with each other. The component matrix shows the degree of correlation between each of the 15 variables collected and the 5 main components.

The analysis of the table shows that the variables foot (0.77), head width (0.66), hand (0.63), forearm length (0.63), sacrum width (0.59), total weight (mass) over snout to urostyle length (0.58) and thigh length (0.58) are all positively correlated with component 1.

Table 8. Eigenvalues.

Function	Eigenvalue	% of variance	Cumulative % of variance	Canonical correlation
1	0.86[a]	100.0	100.0	0.68

a. The first canonical discriminant functions were used for the analysis.

Component 2, on the other hand, is negatively correlated with the variables throat aspect (-0.80), abdomen aspect (-0.71) and positively correlated with the variables snout to urostyle, snout to urostyle length (0.77), femoral gland (0.62) and total weight (0.59). Component 3 is positively correlated with the variables length of upper arm (0.79), extension of sacrum diaphysis (0.74). The variables foot (-0.58) and tibia length (0.76) are correlated with components 4 and 5 respectively.

Fig. 2 shows the variables in space along three main axes holding 47.88% of all the information. Thus, all the quantitative variables are positively and strongly correlated with components 1 and 3 than with component 2. Unlike components 1 and 3, component 2 is strongly correlated with the qualitative variables. Thus, the variables abdominal aspect and throat aspect are strongly opposed to femoral gland aspect, total weight of individuals and snout to urostyle length. Therefore, individuals with a very mottled throat and abdomen are small and have a low weight. These individuals do not have protruding femoral glands. On the other hand, individuals characterised by a large size, high body weight and protruding femoral glands do not have a heavily spotted throat and abdomen.

Table 12 presents the coefficients of the standardised canonical discriminant function and the structure matrix giving the combined intra-group correlations between the variables and the discriminant function on the other hand. The linear discriminant function reveals that the variables snout to urostyle length (0.021), throat aspect (0.75), upper arm length (0.14), forearm length (0.01), hand (0.202) and thigh length (0.39 contrast with the variables total weight (-0.37), femoral gland (-0.34), aspect of abdomen (-0.01), tibia length (-0.03), foot (-0.28), sacrum width (-0.24) and sacrum diaphysis extension (-0.07). The contributions of the variables aspect of abdomen, length of snout to urostyle length, tibia and extension of sacrum diaphysis to the discriminant function are, however, very minor compared to the variable aspect of throat. the combined intragroup correlations between the discriminant variables and the standardised canonical function variables show that the variable most correlated with the discriminant function is throat aspect (0.81) followed by snout to urostyle length (-0.53) and femoral gland aspect (-0.51).

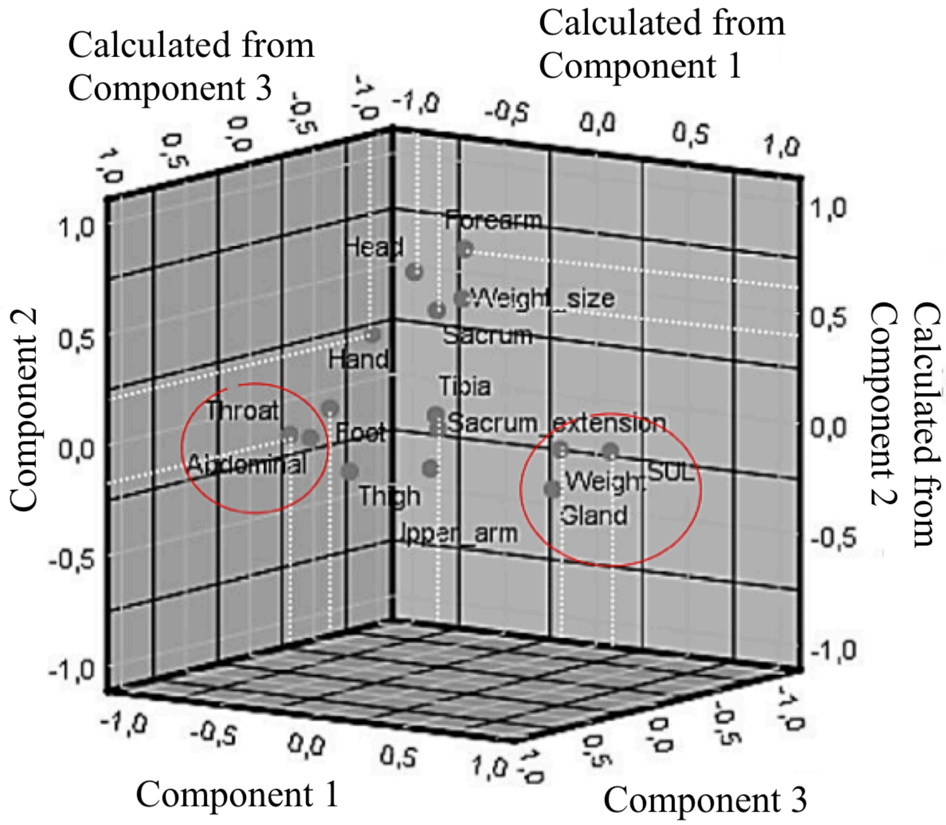


Fig. 2. Factor map of variables. (SUL: snout to urostyle length, Gland: femoral gland aspect, Weight: total weight, Weight-size: weight to size ratio, Forearm: forearm length, Hand: hand length, Head: head width, Foot: foot length, Thigh: thigh length, Sacrum: sacrum width, Sacrum extension: extension of the sacrum diaphysis, Upper arm: upper arm length, Abdominal: aspect of the abdomen, Throat: aspect of the throat.)

Table 13 presents the scores for classifying the variables within the groups. Based on the study of intra- and inter-class covariances and on the optimal Bayesian classifier in the sense of the probability of error, the highest scores allowing to discriminate the sexes are observed at the level of the variable Throat aspect (male = 11.49, female = 9.23) and Femoral gland (male = -7.17, female = -6.06).

The ranks and logarithms of the printed determinants of the group covariance matrices presented in Table 14 indicate that males (Determinant Log = 22.25) appear very significantly ($F = 2.854$; $P < 0.01$) to be the sex with the most variability, while females (Determinant Log = 21.92) are more homogeneous.

The results of the DISCRIM ranking procedure according to the Bayesian assignment rule presented in table 15 show that 1.2% of the time females are incorrectly assigned male status and 3.5% of the time males are assigned to the female group.

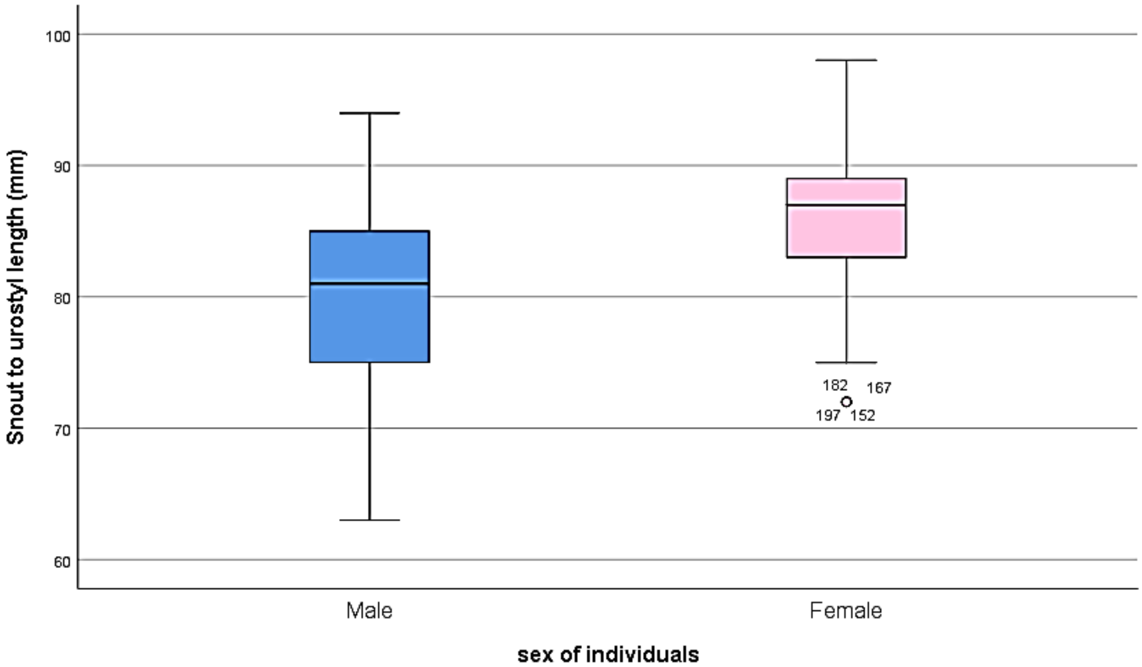


Fig. 3. Growth performance between sexes. (A) Difference in snout to urostyle length between sexes. (B) Viewpoint difference in total weight between sexes. (C) Difference in upper arm length between sexes.

Figs. 3 A-C present whisker boxes showing the results of the median; the first, second and third quartile as well as the minimum and maximum values recorded for the main morphometric traits of sexual differentiation in *A. subsigillata.*

In terms of snout to urostyle length, females have a longer SUL (median = 87 mm). Thus, 50% of females have an SUL of 87 mm, with a range varying between 72 mm (minimum) and 98 mm (maximum). Furthermore, 25% of the females sampled had an SUL of 83 mm and 75% of the females had an SUL of 89 mm. However, it was not surprising to observe very young females in the sample with a SUL less than 75 mm. As for the males characterised by a short SUL (median= 81 mm), the differences in SUL varied between 63 mm (minimum) and 94 mm (maximum). Also, 25% of the males sampled have an SUL of 75 mm and 75% of the males have an SUL of 85 mm.

In parallel with snout to urostyle length, the total weight of females (median = 38.44 g) is also greater than that of males, with a range between 17.15 g (minimum) and 62.66 g (maximum). Among the females, 25% of individuals had a total weight of 32.01 g; 50% of individuals had a total weight of 38.44 g and 75% of females had a total weight of 44.77 g. On the other hand, males weighing less (median = 29.18g) than females had a total weight ranging from 15.8 g (minimum) to 45.7 g (maximum). For 25% of the males sampled the total weight is 25.29 g; for 50% of the males the total weight is 29 g and for 75% of the males sampled the total weight is 34.86 g. It is therefore not surprising to find very large males with a total weight of over 35 g and very young males weighing less than 26.2 g.

Table 9. Correlation matrix of quantitative and qualitative traits recorded in individuals of *A. subsigillata.*

	Snout-cloaca length (mm)	**Total weight (g)**	**Femoral gland**	**Abdominal appearance**	**Throat appearance**	**Length of humerus (mm)**	**Length of Radioulna (mm)**	**Distal part of the forearm (mm)**	**Length of the femur (mm)**	**Tibia (mm)**	**Distal part of the foot (mm)**	**Sacral width (mm)**	**Extension of the sacral diaphysis (mm)**	**Width of the mouth (mm)**	**Weight to size ratio**
Snout-cloaca length (mm)	1														
Total weight (g)	0.45	1													
Femoral gland	**0.525**	0.278	1												
Abdominal appearance	-0.411	-0.283	-0.263	1											
Throat appearance	**-0.539**	-0.323	-0.398	**0.682**	1										
Length of humerus (mm)	-0.062	-0.323	-0.127	0.087	0.102	1									
Length of Radioulna (mm)	0.040	-0.051	-0.016	-0.048	-0.077	-0.134	1								
Distal part of the forearm (mm)	-0.060	-0.001	-0.034	0.006	.051	-0.095	0.354	1							
Length of the femur (mm)	-0.055	0.018	0.101	0.037	0.057	0.259	0.075	0.237	1						
Tibia (mm)	0.114	-0.003	-0.046	0.030	0.012	0.155	0.126	-0.013	0.205	1					
Distal part of the foot (mm)	-0.076	0.045	0.051	0.032	0.071	0.024	0.209	**.598**	0.757	0.290	1				
Sacral width (mm)	-0.001	0.011	-0.044	0.047	0.045	0.300	0.389	0.327	0.262	0.154	0.166	1			
Extension of the sacral diaphysis (mm)	-0.005	0.011	-0.050	0.043	0.044	**0.650**	0.141	-0.007	0.350	0.069	0.100	0.297	1		
Width of the mouth (mm)	-0.042	.021	-0.126	0.043	0.010	-0.139	0.484	0.418	0.038	0.180	0.425	0.324	-0.038	1	
Weight to size ratio	0.084	-0.046	0.026	0.019	-0.057	-0.169	**0.589**	0.159	0.115	0.376	0.302	0.216	-0.057	0.428	1

Values in bold in the table are highly correlated ($R^2 > 0.50$)

Table 10. Total explained variance.

	Initial eigenvalues			Sums extracted from the square of the loadings			Rotation sums of the square of the loadings		
Component	Total	% of the variance	% cumulative	Total	% of the variance	% cumulative	Total	% of the variance	% cumulative
1 Distal part of the foot	3.167	21.115	21.115	3.167	21.115	21.115	2.709	18.059	18.059
2 Head width	2.762	18.414	39.528	2.762	18.414	39.528	2.438	16.255	34.314
3 Hand	1.991	13.272	52.801	1.991	13.272	52.801	2.036	13.573	47.887
4 Forearm length	1.369	9.130	61.930	1.369	9.130	61.930	1.961	13.072	60.959
5 Sacrum width	1.147	7.648	69.579	1.147	7.648	69.579	1.293	8.619	69.579
6 Weight to height ratio	0.864	5.757	75.335						
7 Thigh length	0.788	5.256	80.591						
8 Throat appearance	0.639	4.260	84.852						
9 Snout to cloaca length	0.548	3.656	88.508						
10 Abdominal appearance	0.460	3.065	91.572						
11 Femoral gland	0.369	2.459	94.032						
12 Total weight	0.327	2.183	96.215						
13 Length of upper arm	0.287	1.916	98.131						
14 Sacrum shaft extension	0.226	1.505	99.635						
15 Tibia	0.055	0.365	100.000						

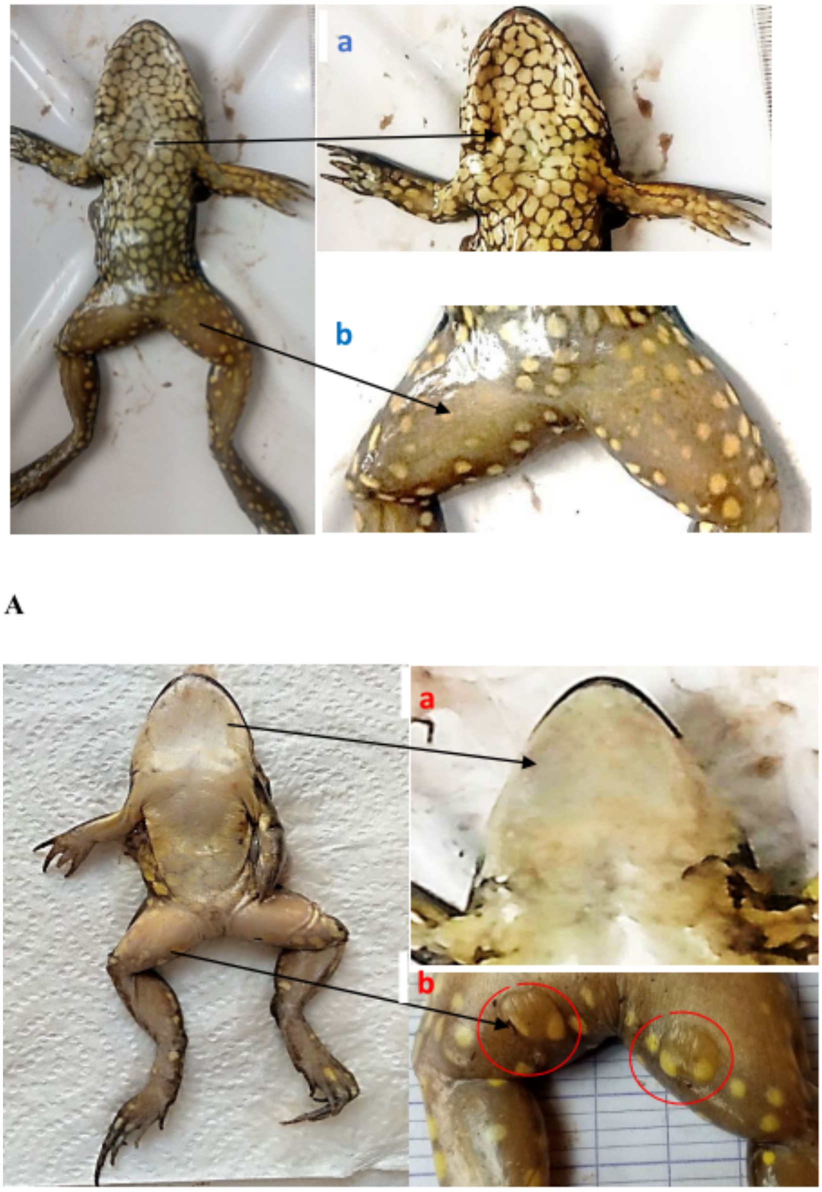
Table 11. Component matrix.

	1	2	3	4	5
Distal part of the foot (mm)	**0.769**	0.042	0.069	**-0.584**	0.085
Head width(mm)	**0.658**	0.104	-0.395	0.154	-0.112
Hand (mm)	**0.635**	0.058	-0.196	-0.315	-0.449
Forearm length (mm)	**0.634**	0.216	-0.279	0.421	-0.159
Sacrum width (mm)	**0.588**	-0.012	0.226	0.370	-0.288
Weight to height ratio	**0.583**	0.287	-0.322	0.290	0.367
Thigh length (mm)	**0.577**	-0.046	0.479	-0.505	0.110
Throat appearance	.132	**-0.800**	-0.145	-0.066	0.143
Snout to cloaca length (mm)	-.107	**0.777**	0.218	0.067	0.089
Abdominal appearance	.105	**-0.712**	-0.122	-0.038	0.184
Femoral gland	-.104	**0.627**	0.198	-0.262	0.014
Total weight (g)	-.038	**0.587**	0.146	-0.030	0.058
Length of upper arm (mm)	.139	-0.293	**0.792**	0.264	-0.018
Sacrum shaft extension (mm)	.284	-0.181	**0.738**	0.284	-0.158
Tibia (mm)	.405	0.086	0.120	0.149	**0.762**

Values in bold are those strongly correlated with the main component ($R^2 > 0.5$)

As far as upper arm length is concerned, the differences are as great in females (minimum = 10 mm; maximum = 17mm) as in males (minimum = 10 mm; maximum = 17mm). However, the length of the upper arm was shorter in females (median = 12 mm) than in males (median= 14 mm). In fact, 25% of females sampled had an upper arm length of 11 mm, 50% of females sampled had an upper arm length of 12 mm and 75% of females had an upper arm length of 15 mm. In males, 25% of individuals have an upper arm length of 12 mm; 50% of individuals have an upper arm length of 14 mm and 75% of individuals have an upper arm length of 15 mm.

Fig. 4 presents some sexual dimorphic criteria between sexes in *A. subsigillata* individuals.



A

B

s

Fig. 4. Some sexual dimorphic criteria between sexes in *A. subsigillata* individuals.
(**A**) A male *Aubria subsigillata* showing a throat and abdomen with quadrangular black spots (**a**) with femora lacking femoral glands (**b**). (**B**) A female *Aubria subsigillata* showing a whitish throat and abdomen (**a**) with two protruding femoral glands on the femora (**b**).

Table 12. Coefficients of the canonical standardised discriminant functions and correlations between predictor variables and the linear discriminant function.

	Function 1	
	Coefficients	**R^2**
Variables	0.021	**-0.531**
Snout to urostyle length (mm)	-0.375	-0.478
Total weight (g)	-0.342	**-0.510**
Femoral gland	-0.012	0.494
Aspect of abdomen	0.750	**0.817**
Throat appearance	0.144	0.119
Length of upper arm (mm)	0.010	-0.007
Length of Forearm (mm)	0.202	0.097
Distal part of forearm (mm)	0.395	0.108
Length of thigh (mm)	-0.031	0.108
Tibia (mm)	-0.283	0.122
Distal part of foot (mm)	-0.244	0.014
Sacrum Width (mm)	-0.072	0.067
Sacrum diaphysis extension (mm)	0.192	0.087
Width of head(mm)	0.060	-0.022
Total weight (mass) over Snout to urostyle length		

Values in bold are those that are highly correlated with the discriminant function ($R^2 > 0.5$)

Discussion

Sexual dimorphism, if present in any given species, is an important adjunct when studying reproductive strategies in amphibian populations. To this end, the identification of phenotypic traits distinguishing male and female individuals of *Aubria subsigillata* revealed that out of a total of fifteen (15) different criteria taken into account for the study of sexual dimorphism, only six (6) criteria made it possible to sex the individuals of this species. These were snout to urostyle length, total weight, length of the upper arm, aspect of the femoral glands, aspect of the throat and aspect of the abdomen.

From morphological criterion view, the aspect of the throat is one of the main criteria identified by the discriminant function to distinguish males from females. This is due to the black quadrangular spots on the throat of males. These spots also extend to the abdomen in males. Females, on the other hand, do not have these spots on their throat. Generally the abdomen of females (58% of females) is also free of these black spots. However, these traits are not systematically present in all individuals, which could require the consideration of other traits such as biometric traits. Indeed, it is not surprising to find females with quadrangular black spots on the throat and abdomen (2%) and females spotted exclusively on the abdomen (22%). Similarly to females, males with unblemished throats have been observed (13%). Perret (1994) found no noticeable difference in the abdominal parts of females and males of *A. subsigillata*.

Table 13. Coefficients of the ranking function.

	sex	
	Male	**Female**
Snout to urostyle length (mm)	3.775	3.767
Total weight (g)	-0.245	-0.157
Femoral gland	-7.175	-6.061
Aspect of abdomen	2.437	2.468
Throat appearance	11.492	9.234
Length of upper arm (mm)	3.276	3.129
Length of forearm (mm)	1.129	1.120
Distal part of forearm (mm)	-0.640	-0.796
Length of thigh (mm)	-0.259	-0.485
Tibia (mm)	-0.060	-0.042
Distal part of foot (mm)	2.160	2.280
Sacrum width (mm)	1.002	1.269
Sacrum diaphysis extension (mm)	0.033	0.066
Width of head (mm)	2.502	2.350
Total weight (mass) over Snout to urostyle length	-0.250	-0.265
(Constant)	-261.719	-259.936

Table 14. Ranks and logarithms of the printed determinants of the group covariance matrices.

Sex of individuals	Rank	Determinant Log
Male	15	22.250
Female	15	21.926
Intra-group combined	15	22.942

Females of *Aubria subsigillata* are significantly larger in size (86.19 mm) than males (80.19 mm). A similar result was observed by Assemian *et al.* (2006) in males (73 mm) and females (78 mm) of *A. subsigillata* in Banko National Park, Côte d'Ivoire. This sexual size dimorphism in favour of females is generally observed in nearly 90% of anuran species (Shine 1979). Indeed, Caldart *et al.* (2019) also found that females of the anuran species *Crossodactylus schmidti* Gallardo, 1961 in Brazil are larger in size (27.68 mm) than males (25.03 mm). Similarly Székély & Nemes (2002) also made the same observations in males and females of the anuran species *Pelobates fuscus* (Laurenti, 1768) in Germany, Australia and Romania. Liao et al. (2012) noted a positive correlation between female size and fecundity, leading us to believe that large size of females *A. subsigillata* may be explained by continuous development of large numbers of oocytes". As far as the males are concerned, the low size may be linked to an evolutionary advantage in being small and agile.

Regarding the total weight of the *A. subsigillata* frog, it is also much higher in females than in males of *A. subsigillata* with an average difference of 8.38 g. This result confirms the size difference between males and females. The females of *A. subsigillata* sampled during this study were mostly pregnant (96% of females) (Houénafa A. C. Gansa, pers. obs.) and therefore need to feed abundantly and constantly to ensure egg development (Caldart *et al.* 2019); this could be responsible for the large difference in total weight observed in favour of females. It has also been observed that the abdominal cavity of females has one organ more than that of males (the oviducts) and its weight increases as females feed and approach their spawning time. However, based on the criteria of total weight and SUL length, sexual differentiation between virgin females and mature males is unlikely.

In terms of upper arm length, males of *A. subsigillata* have a significantly longer upper arm (13.49 mm) than females (13.01 mm). This observation is also made by Peters and Aulner (2000) in the edible frog *Rana catesbeina* (Shaw, 1802) in the USA that showed powerful forearms. Similarly, Petrović *et al.* (2017) reported similar information in males of many anuran species (*Pelophylax kl. esculentus* Linnaeus, 1758, *Bufotes viridis* (Laurenti, 1768), *Rana temporaria* Linnaeus, 1758, *Hyla arborea* (Linnaeus, 1758), *Rana graeca* Boulenger, 1891, *Bombina variegata* (Linnaeus, 1758), and *R. dalmatina* Fitzinger in Bonaparte, 1839, *Pelobates fuscus* (Laurenti, 1768) and *P. syriacus* Boettger, 1889) in Serbia. The high length of the upper arm in *A. subsigillata* is certainly related to the mating pattern of this species. Indeed, it has been observed on the breeding station that before the egg-laying and oocyte fertilization phase, males fight over the female by clinging to her with their forearms on their backs in order to hold the monopoly. Thus, the forearm muscle is highly used in intra-species competitions (Wells 1979; Petrović *et al.* 2017). As a result, due to natural selection, the male of *A. subsigillata* has a rather long and powerful upper arm and a much larger hand (17.59 mm) than females (17.07 mm).

Table 15. Ranking results.

			Membership of the expected group		
		sex of individuals	**Male**	**Female**	**Total**
Original	Number	Males	89	27	116
		Females	45	233	278
	Percentage	Males	76.7%	23.3%	100.0%
		Females	16.2%	83.8%	100.0%

In contrast to the aspects of the throat and abdomen, the absence of femoral glands is one of the most stable dimorphic criteria. Indeed, small size individuals without femoral glands were systematically males (100%). However, many large males were observed with protruding femoral glands (23%) or only slightly protruding ones (14%). Females with slightly protruding femoral glands were also found in the population (17%).

These observations are in contrast to those of Perret (1994 who found that males differed from females not only in the absence of femoral glands but also in the low protuberance of femoral glands on the femurs. Similarly, Vences *et al.* (2007) in their studies on the evolutionary process of the femoral glands in certain amphibian species reported that the femoral glands serve to secrete hormones during reproduction. This leads us to wonder why this gland is present in a protuberant manner in some males of *A. subsigillata* and absent in other males.

Other authors such as Crook & Tyler (1981), Clarke (1997) and Fontana *et al.* (2006) find that populations of anurans with femoral glands use them for the secretion of mucoproteins that lubricate and moisten their skin in order to rid themselves of parasites or to produce toxins of a peptide nature. But if this is the case with the frog *A. subsigillata*, it would also be obvious to ask what causes the absence of this gland in some individuals. It would therefore be of great interest for further investigations to analyse microscopically and chemically the femoral glands of the frog *A. subsigillata* before and after reproductive activity in order to understand the real functions of these glands in this species.

On the other hand, the presence of femoral glands in both males and females of *A. subsigillata* in varying proportions and the total absence of these glands in some males in the population could suggest that there are actually cryptic species in the population of *A. subsigillata*. More detail would be revealed with the genetic analysis of these individuals. Furthermore, these observations made on *A. subsigillata* frogs need to be extended to specimens of *A. subsigillata* from other West African countries to eliminate subjectivity.

For sexing of *A. subsigillata* individuals, it is important to remark that males have a fairly long humerus, lack femoral glands and have abdomen and throat generally heavily mottled with black. In contrast, females have a shorter humerus, a whitish abdomen and throat and a protruding femoral gland. Females are larger in size (mean 86.19 mm) and are heavier (mean 38.63 g) than males (means 80.19 mm & 30.25 g respectively)

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