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Morphological Differentiation and Karyotypic Studies of the African Common Toad *Sclerophrys* (*Bufo*) *Regularis* (Reuss, 1833)

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Abstract

The study analyzed the morphological differentiation and karyotype among populations of the common toad, *Sclerophrys (Bufo) regularis* from six Local Government Areas in Ondo state. Variations in fifteen morphometric and qualitative characters in one hundred and seventy-one animals from six population samples were analyzed using univariate and multivariate statistics. Descriptives of sexes and correlation between morphological characters was also analyzed. Female toads had larger mean values of most of the morphological characters studied with weight contributing the largest variance. The chromosomes of twenty-four specimens of the toad were also studied using standard bone marrow smear techniques. A chromosome complement of 2n=20 was observed for this species. The chromosomes consists of nine pairs of metacentric and one pair of submetacentric chromosomes. The chromosomes based on centromeric position could be grouped into two - metacentric and submetacentric - and based on size, into three metacentric chromosomes; 1-4 which were large, metacentric chromosomes; 5-6 medium sized metacentric chromosomes, while 7-9 were small sized, and the last pair (10), submetacentric chromosomes were small. The fundamental number of this species is 40 and with no sex chromosomes or satellites reported, while bi-armed chromosomes were observed. Findings also showed that female individuals of the species weighed more than the male.

Keywords: *Sclerophrys regularis;* Morphometric; Differentiation; Karyotype; Ondo state.

1. Introduction

The African common toad, *Sclerophrys (Bufo) regularis* [1] also known as square-marked toad is a widely distributed species in the Sub-Saharan Africa, with its range extending to the oasis in Algeria and Libya as well as to northern Nilotic Egypt [1]. It is widely distributed in Africa and widespread in Nigeria [2]. Toads in general have been the object of various morphometric studies [3-7]. However, data from the southern part of the range on the Balkan Peninsula were mainly limited to demographic parameters [8-11]. There exists a dearth of information on such morphometric study for this species in Nigeria.

Karyotype is the chromosomal characteristics (number, size and shape) of the chromosomes of an individual species [12]. Parameters used in describing the karyotype of an organism include diploid (2n) number, position of the primary constriction (centromere) in relation to the total length of the chromosome, presence or absence of structural aberrations, supernumerary or B chromosomes and fundamental number. Karyotype reveals chromosomal aberrations, cellular function, taxonomic relationship and past evolutionary events. Karyotypic study is the standard initial step in the investigation of individual species identity & evolutionary relationship among related species and genera [13, 14]. Information on any form of cytogenetic work (karyotypic in particular) on Anurans in Ondo state is scarce in literature.

The aim of this study is to carry out morphometric investigation on *S. (B.) regularis* by comparing the morphometrics of body parts of both sexes with and to determine the body parts which contribute majorly to variations within the population. Result will also establish the karyotype (chromosome number, structure and other characteristics) of individuals making up the population of this species found within the study area.

2. Materials and Methods

2.1. Morphometric Studies

Morphometric analysis was conducted using a total of 171 mature individuals belonging to six populations of *S.* (*B.*) *regularis* collected from six LGAs of Ondo state (Table 1 & Fig. 1). More female toads (140) were captured compared with the male (31). The animals were collected by hand, protected with gloves, and transported per

individual in separated labelled holding containers to the laboratory where they were weighed (recorded as Wg) almost immediately to prevent weight loss and then kept in empty aquarium tanks. Morphometric analysis was performed on fifteen (15) traits which determine the size of this tailless amphibian (Table 2). Morphometric measurements were achieved with the aid of a digital calliper. The fifteen (15) different body parameters measured were as modified from Simons [15] and eSilva, *et al.* [4]. To avoid bias, all measurements were taken with the digital calliper to a precision of 0.01mm [3]. Identification and sex determination was carried out using the keys of Rödel [16]. In some of the females, eggs were observed through the transparent skin on the ventral side. In exceptional cases where sex could not be determined from the taxonomic characters, dissection of the toads was carried out as a means of definitive determination of their sex. At the end of the morphometric analysis, only twenty-nine animals were retained for the cytogenetic studies while the rest were released back into the wild.

2.2. Cytogenetic Studies

Karyotypic studies were carried out on twenty-four animals using the metaphase stage of mitosis when chromosomes are at the maximum state of condensation and the chromatid arms are clearly visible for measurement and other morphological observations; two male and female from each population following the protocol of Schmid [17] from bone marrow fluid as follows; animals were injected intraperitoneally with 0.03ml/g of body weight of 0.1% Colchicine solution for six hours before sacrificing. The animals were anaesthesized, dissected and the femur and humerus were removed and cleaned of all flesh. The bone marrow was then flush into 10ml centrifudge tubes with freshly prepared 0.56% Potassium chloride solution pre-warmed to 36^0C. It was left to stand for 30 – 45 minutes and centrifuged at 1,000rpm for 10 minutes. The supernatant was removed leaving iml of the hypotonic solution and ice cold Glacial acetic acid-Methanol (fixative) was then added drop wise with vigorous agitation. The cell suspension was then centrifuged again at 1,000rpm for 10 minutes, the supernatant removed, leaving 1ml of the fixative. The cells were re-suspended by agitating the centrifuge tube and the cells then spread on cold, new and clean microscope slides stored in 70% Methanol from a height of 7 – 10cm. The slides were then air dried at room temperature. Prepared slides were then stained with Giemsa in Phosphate buffer (pH 6.8), air dried, scanned for good spreads and photomicrographs taken with a binocular photomicroscope at x40 and x100 oil immersion objectives. Determination of arm ratios, centromeric position of chromosomes, and fundamental number were then carried out.

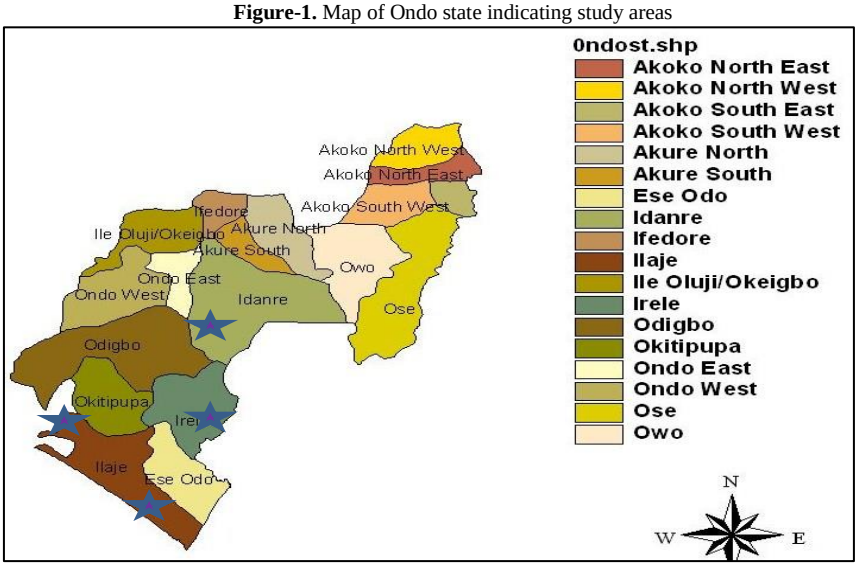
Table-1. Local Government Areas, the Headquarters, GPS coordinates and total number of *Sclerophrys (Bufo) regularis* collected.

LGA	Headquarters	GPS coordinates	Number collected (%)
Akure South	Akure	7.2571°N; 5.2058°E	100 (58.5)
Akure North	Iju/Itaogbolu	7.4000°N; 5.2500°E	09 (5.3)
Ondo East	Bolorunduro	7.1657°N; 4.9633°E	11 (6.4)
Idanre	Owena	7. 0914°N; 5.1484°E	26 (15.2)
Ilaje	Igbokoda	6.3697°N; 4.7847°E	10 (5.8)
Okitipupa	Okitipupa	6.5025°N; 4.7795°E	15 (8.8)
Total			**171 (100)**

Table-2. Standardized morphometric measurements taken on all specimens

Measurement	Abbreviation	Description
Weight	Wg	Weight of specimen
Snout- vent length	SVL	Tip of snout to the posterior margin of vent
Manus width (hand)	MW	Width of an out stretched pes
Manus length	ML	Proximal end of carpal to tip of longest finger
Femur length	FL	Cloaca to knee
Pes width (foot)	PW	Width of an outstretched pes
Pes length (foot)	PL	Tarsal/metatarsal joint to tip of longest bone
Head width	HW	Distance between mean edges of head
Anterior interorbital distance	AID	Distance between anterior edges of eyes
Eye-nostril distance	END	Distance between anterior edges of eyes and posterior edges of nostril
Snout-nostril distance	SND	Distance between anterior edge of snout and posterior edges of nostril
Tibia length	TL	Knee to heel
Head length	HL	Distance between anterior and posterior edge of head
Interorbital distance	ID	Shortest distance between eye sockets
Tympanum diameter	TD	Maximum distance between rim of tympanum

Figure-1. Map of Ondo state indicating study areas



2.3. Data Analysis

Data analysis of morphometric characters included all 171 individuals collected. Descriptive statistics (mean, standard error and minimal and maximal values) were calculated for non-transformed morphometric characters based on sex factor using ANOVA. Biometric parameters which had the most impact or contribution to morphological variation were also identified using the Principal Component Analysis (PCA). All statistical analyses were performed using the computer package *(Statistical Package for Social Sciences (SPSS®) version 20 for Windows).

3. Results

3.1. Morphometric Characters

Descriptive statistics of non-transformed morphometric characters of male and female animals for the population groups are presented in Table 3. The table shows female animals having greater means in most of the characters studied (Wg, SVL, MW, ML, END, SND, TL, FL, PW, HW, AID, PL) while the male had greater mean in a few (HL, ID, TD), indicating male had larger head size when compared with the female.

The total variance explained presented in Table 4 showed the individual components by eigenvalue and variance was accounted for, retaining only components with eigenvalue greater than 1 (Kaiser criterion/K1 Rule) namely: weight, snout-vent length and manus width. Data were explained in three components for the data analyzed explaining 74.15% in total; the first component (weight) explaining 59.75%, second component (snout-vent length) explaining 7.31% and the third component (manus width) explaining 7.09%.

Existence of strong positive correlation among the morphological characters studied and analyzed as shown in Table 5. The only exception is the interorbital distance (ID) which showed a negative correlation with other characters. The three morphometric characters that define the total variance as possible i.e. weight, snout-vent length and manus length were used to generate the component matrix (Table 6).

Table-3 Descriptive statistics of morphometric characters (male and female *S.* (*B.) regularis*) examined from population groups

	Male (N= 31)				**Female (N =140)**			
Trait	Mean	SE	Min	Max	Mean	SE	Min	Max
Wg	39.95	4.00	5.80	96.90	47.67	3.07	2.90	156.30
SVL	6.67	0.23	3.81	9.28	7.00	0.17	2.95	11.28
MW	1.70	0.16	0.12	5.82	1.76	0.50	0.14	5.00
ML	1.62	0.06	0.95	2.24	1.68	0.04	0.13	2.90
END	0.46	0.02	0.24	0.62	0.50	0.01	0.20	0.78
SNL	0.51	0.03	0.21	0.83	0.60	0.01	0.11	1.07
TL	2.57	0.12	0.75	3.72	2.63	0.06	0.60	4.43
HL	2.15	0.07	1.28	3.19	2.13	0.04	0.71	3.26
FL	2.73	0.11	1.26	3.72	2.89	0.08	0.82	8.30
PW	1.86	0.08	0.82	2.60	1.86	0.04	0.68	3.20
HW	2.53	0.10	1.08	3.34	2.66	0.06	1.20	4.12
AID	0.92	0.03	0.57	1.23	0.95	0.02	0.30	2.00
ID	1.59	1.01	0.35	0.86	0.64	0.02	0.16	1.20
PL	2.78	0.11	1.34	3.81	2.84	0.06	1.05	4.53
TD	0.69	0.12	0.25	3.63	0.56	0.18	0.22	2.25

Sample size (N), Mean value (cm), Standard error (SE), and range (Min & Max).

Table-4. Total variance explained for the data analyzed using the principal component analysis extraction method

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
1	10.160	59.762	59.762	10.160	59.762	59.762
2	1.283	7.546	67.308	1.283	7.546	67.308
3	1.164	6.850	74.158	1.164	6.850	74.158
4	.973	5.726	79.884			
5	.713	4.195	84.079			
6	.511	3.007	87.086			
7	.430	2.528	89.614			
8	.367	2.160	91.774			
9	.308	1.811	93.585			
10	.244	1.436	95.021			
11	.201	1.182	96.203			
12	.185	1.090	97.293			
13	.126	.742	98.034			
14	.109	.639	98.673			
15	.095	.559	99.233			
16	.073	.432	99.665			
17	.057	.335	100.000			

Table-5. Correlation matrix for morphometric characters studied

Correlation Matrix

	Wg (gm)	SVL (cm)	MW (cm)	ML (cm)	END (cm)	SND (cm)	TL (cm)	HL (cm)	FL (cm)	PW (cm)	HW (cm)	AID (cm)	ID (cm)	PL (cm)	TD (cm)
Wg (gm)	1.000	.881	.598	.755	.562	.678	.801	.861	.819	.720	.835	.705	-.038	.818	.298
SVL (cm)	.881	1.000	.686	.828	.608	.744	.819	.902	.837	.816	.902	.762	-.076	.907	.330
MW (cm)	.598	.686	1.000	.622	.467	.538	.628	.651	.595	.677	.665	.568	-.053	.690	.199
ML (cm)	.755	.828	.622	1.000	.627	.654	.754	.812	.839	.824	.838	.721	-.072	.865	.329
END (cm)	.562	.608	.467	.627	1.000	.578	.594	.672	.596	.626	.651	.619	-.055	.658	.256
SND (cm)	.678	.744	.538	.654	.578	1.000	.716	.770	.715	.705	.746	.596	-.099	.749	.249
TL (cm)	.801	.819	.628	.754	.594	.716	1.000	.850	.777	.759	.831	.679	-.081	.838	.353
HL (cm)	.861	.902	.651	.812	.672	.770	.850	1.000	.841	.819	.891	.749	-.081	.915	.353
FL (cm)	.819	.837	.595	.839	.596	.715	.777	.841	1.000	.784	.850	.783	-.057	.862	.292
PW (cm)	.720	.816	.677	.824	.626	.705	.759	.819	.784	1.000	.850	.714	-.085	.879	.325
HW (cm)	.835	.902	.665	.838	.651	.746	.831	.891	.850	.850	1.000	.778	-.086	.916	.343
AID (cm)	.705	.762	.568	.721	.619	.596	.679	.749	.783	.714	.778	1.000	-.067	.777	.308
ID (cm)	-.038	-.076	-.053	-.072	-.055	-.099	-.081	-.081	-.057	-.085	-.086	-.067	1.000	-.104	-.055
PL (cm)	.818	.907	.690	.865	.658	.749	.838	.915	.862	.879	.916	.777	-.104	1.000	.346
TD (cm)	.298	.330	.199	.329	.256	.249	.353	.353	.292	.325	.343	.308	-.055	.346	1.000

Table-6. Component matrix showing the three components derived from the data with its respective loadings

Component Matrix[a]			
	Component		
	1	2	3
Wg (gm)	886	-.072	-.074
SVL (cm)	.944	-.087	-.080
ML (cm)	.893	.043	.055
END (cm)	.714	.400	.271
SND (cm)	.806	.123	-.109
TL (cm)	.887	-.067	-.024
HL (cm)	.946	-.051	.030
FL (cm)	.907	-.033	-.043
PW (cm)	.894	.062	.111
HW (cm)	.949	-.025	-.032
AID (cm)	.831	-.029	-.008
ID (cm)	-.096	-.247	.520
PL (cm)	.959	-.038	-.015
TD (cm)	.383	-.182	.386
MW (cm)	.729	-.015	-.025
Extraction Method: Principal Component Analysis.			
a. 3 components extracted.			

3.2. Cytogenetic Studies

The photomicrographs (Plates 1 & 2) indicate a diploid number (2n) of 20 chromosomes for *S. (B.) regularis* [1]. The karyotype of this species can be classified into two major groups according to the nomenclature proposed by Green and Sessions [18]. Group 1 consists of 9 pairs of metacentric chromosomes, 1-4 are large chromosomes, 5-6 are medium size and 7-9 are small chromosomes. Group 2 consist of 1 pair of small size submetacentric chromosomes. This is shown in Table 7 by comparing the measurement obtained from the short arm and the long arm. The size of the chromosomes and type of centromeres are determined by using the results obtained from the arm ratios. The Fundamental Number (FN) was determined to be 40 (Table 8). There were no micro chromosomes, sex chromosomes or satellites observed in all specimens cytogenetically analyzed with all the chromosomes being bi-armed (possessing both short ***p*** and long ***q*** arms). The various categories of chromosomes are shown in Table 9.

Plate-1. Photomicrograph of the metaphase chromosomes of female *S. (B.) regularis* showing 2n = 20 chromosomes. Mag. x4000

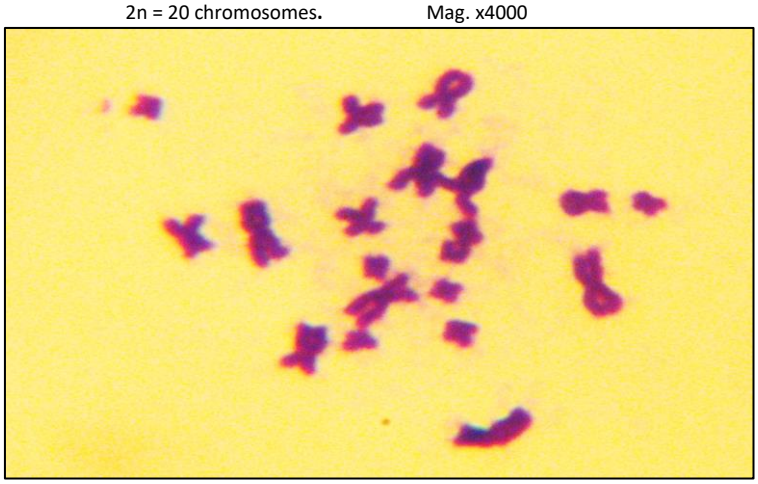


Plate-2. Photomicrograph of the metaphase chromosomes of male *S. (B.) regularis* showing 2n = 20 chromosomes. Mag. x4000

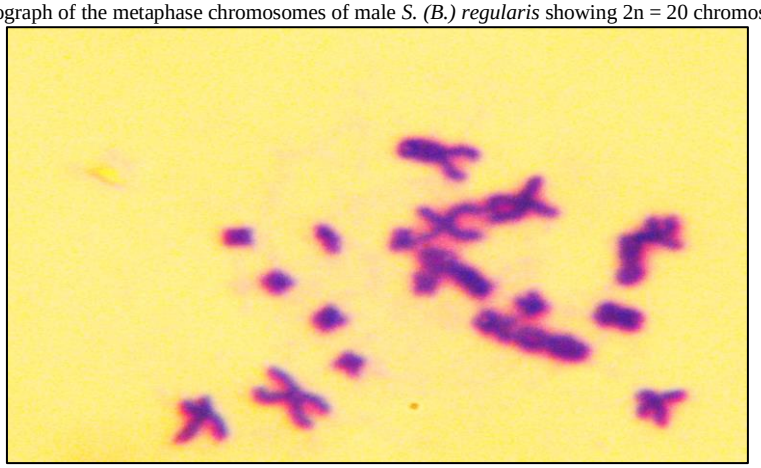


Table-7. Arm ratios and type of centromeres of *S. (B.) regularis*

Chromosome number	Short arm p(µm)	Long arm q(µm)	Total length q + p(µm)	Arm ratio q/p	Type of centromere
1	0.69	0.75	1.44	1.09	M
2	0.45	0.74	1.19	1.64	M
3	0.44	0.68	1.12	1.55	M
4	0.43	0.50	0.93	1.16	M
5	0.42	0.49	0.91	.17	M
6	0.34	0.48	0.82	1.69	M
7	0.33	0.38	0.71	1.15	M
8	0.27	0.32	0.59	1.19	M
9	0.22	0.24	0.46	1.09	M
10	0.16	0.38	0.54	2.38	SM

M: *metacentric*; SM: *submetacentric.*

Table-8. The fundamental number for *S. (B.) regularis*

Type of centromere	*Haploid No. N*	*Diploid No. 2N*	*Fundamental No. FN*
M	9	18	36
SM	1	2	4
Total	10	20	40

Table-9. Size of chromosome of *S. (B.) regularis* and position of centromere

Chromosome number	*Size*	*Position of centromere*
1	Large	Metacentric
2	Large	Metacentric
3	Large	Metacentric
4	Large	Metacentric
5	Medium	Metacentric
6	Medium	Metacentric
7	Small	Metacentric
8	Small	Metacentric
9	Small	Metacentric
10	Small	Submetacentric

4. Discussion

A high degree of female-biased sexual size dimorphism was observed, which is also known for other populations of the common toad [7, 9, 19-21]. The most common explanation of the sexual dimorphism in the body size is the advantage that is conferred on bigger females in having the capacity to produce a greater number of eggs [19, 22, 23]. Positive correlation was revealed in terms of size between different body parts under study, along with Snout-vent length and manus width, the only exception being the interorbital distance which had a negative correction; meaning the larger or greater the value of a morphological character of a specimen of the *S. (B.) regularis*, the lesser the value of the interorbital distance. This can also be interpreted that interorbital distance is wider in smaller specimens of the toad. Three morphometric attributes (Wg, SVL and MW) contribute the greatest to the variance observed and can therefore be inferred to be the most important parameters that aid/contribute to the positive identification of the species and sex determination.

The detected size differences between the population groups may be caused by environmental factors and reflect phenotypic plasticity. Factors such as climatic conditions, trophic resources, predatory effect, intra- and interspecific competition could have complex influence on size variation. Head and body size were the two major variables that contributed to significant differences related to morphometric trait as observed for other groups of the toad [4, 24-27]. eSilva, *et al.* [4], studied morphometric differentiation among populations of another toad species, *Eupemphix natteteri* from central Brazil and the results they obtained from their analyses was similar to the result of this research. eSilva, *et al.* [4], detected differences among the species population mainly for Snout-vent length, head width, head length, femur length, foot length (pes length) and tibia length. Therefore, we can conclude that morphological variation among most toad species in the world is strongly influenced by head length, head width, snout-vent length and femur length of the individual members of the toad species.

S. (B.) regularis from this study showed a 2n = 20. This is in accordance with what has been reported by Bogart [14], Schmid [17], Alasoadura [28], Cunningham and Cherry [29] and Al-Shehri and Al-Saleh [30]. Bogart [14], also reported a diploid chromosome number of 2n = 20 for some North and South America *S. (B.) regularis*. Beckert and Doyle [31], confirmed that *S. (B.) regularis* has a diploid complement of only twenty chromosomes from an African population in Nairobi, Kenya. Alasoadura [28], carried out karyotypic analysis on *S. (B.) regularis* population on the campus of the Obafemi Awolowo University, Ile-Ife, Nigeria and reported a diploid number of twenty chromosomes. Cunningham and Cherry [29] also reported a diploid number of twenty chromosomes for *S. (B.) regularis* in the course of carrying out molecular systematic on Africa species group. Al-Shehri and Al-Saleh [30], also reported that the diploid number of twenty chromosomes in specimens analysed in the Arabian Peninsula (Saudi Arabia).

The grouping of *S. (B.) regularis* into two groups is in accordance with the result obtained from Al-Shehri and Al-Saleh [30] in which the first group consists of 9 pairs of metacentric chromosomes and the second group of 1 pair of submetacentric chromosome. Among these twenty chromosomes, four pairs of the chromosomes are large, two pairs of the chromosomes are medium and the remaining four pairs of the chromosomes are small. Bogart [14], suggested that if twenty chromosomes complement were the original number in the genus, and twenty two the derived number, then Africa would be the origin of the toad.

The abundance of this *S. (B.) regularis* in the six LGAs surveyed and their immediate environment is being revealed for the first time. The abundance may be as a result of streams, undrained gutters, water logged area, bushes and grassland which provide good habitat and food sources. However, as more buildings, roads and other anthropological activities are carried out, these can cause reduction in their habitat and will further threaten the population of this species. Therefore, a well-planned structural arrangement should be put in place to protect this natural mosquito "predator". This study does not only re-confirm the reported existence of diploid number of twenty chromosomes earlier reported for this species in several African populations but also shows a total absence of distinct morphological markers within the karyotype such as Nucleolar Organizer Regions (NORs)/ secondary constriction and a lack of clearly identified sex chromosomes (homomorphic or heteromorphic). This species studied is evidently a member of the 20-chromosome group of African toads [14, 31], made up of around 28 species, approximately one-third of African bufonids, and is distributed throughout the continent [29].

5. Conclusion

This research work has clearly shown that the following morphometric parameters of weight (wg), snout-vent length (SVL) and manus width (MW) are the most distinctive parameters of body form for the positive

morphological identification of the Ondo state population of the square-marked toad, *S. (B.) regularis* [1]. It evidently aids in the separation of this species from other member species of the Genus *Sclerophrys* which in the African tropical environment is rich and diverse. Karyotypic studies have also confirmed this species as a member of the *Sclerophrys* African 20-chromosome group. This population lacked homomorphic or heteromorphic sex chromosomes as well as satellites/nucleolar organizer regions (NOR).

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