Morphometric Traits and Karyotypic Features of the African Lungfish (Um Koro) Protopterus annectens annectens (Owen, 1839) and Protopterus aethiopicus aethiopicus (Heckel, 1851) in...
Morphometric Traits and Karyotypic Features of the African Lungfish (Um Koro)

*Protopterus annectens annectens* (Owen, 1839) and *Protopterus aethiopicus aethiopicus* (Heckel, 1851) in Sudan.

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**Abstract**

The purpose of the study was to review the prevalence, the morphometric traits and the karyotypic variation of the African lungfish (*Um koro*) in Sudan. Twenty cocoons containing adults of the African lungfish *Protopterus annectens annectens* were collected from dry ponds of Khor Al-Jogan in Northern Kordofan State. Five adult specimens of the African lungfish *Protopterus aethiopicus aethiopicus* were caught alive at Jebal Aulia dam on the White Nile. Morphometric measurements, including the standard length, depth, distance above the lateral line, distance below the lateral line, peduncle length and peduncle depth were taken. Measurements and ratios performed revealed that *P. aethiopicus aethiopicus* is longer and larger in size than *P. annectens annectens*. The karyotypes of the two species were investigated. *P. annectens annectens* was found to have a diploid number of chromosomes of 2n=34, whereas *P. aethiopicus aethiopicus* demonstrated a diploid number of 2n =28 chromosomes.

**Introduction**

Most of the work performed to classify fishes and to differentiate between the different genera was morphological (Boulenger, 1907; Abu Gideiri, 1984). Recently, the advancement in the methods using genetic information allowed a better analysis of fish species. The African fresh water 'lung' fish *Protopterus* *spp.* belonging to the order dipnoi are a group of osteichthyes fishes whose relationship with tetrapods has been disputed since their discovery. In the past, they were variously considered as being related to actinistsans, tetrapods, and lower actinopterygians; however, nowadays, they are considered as a monophyletic group, the sister group of crossopterygians. Dipnoans first appeared in the geologic record in the Early Devonian, with 50 extinct genera. Those that have survived to date comprise only three genera, *Lepidosiren, Neoceratodus and Protopterus*, and include only six recognized species. Lungfishes (or dipnoans, as they are 'dual breathers') are an archaic group of fishes, characterized by the possession of a 'lung' opening on the ventral side of the esophagus (Chew et al., 2004). Locally known as Um Koro, they are an aggressive predator of frogs and small fish. Lungfishes inhabit brackish freshwater of small rivers and swamps in Senegal, Niger, Gambia, Volta, Chad and Sudan. The fish is known for its ability to breathe air via its 'lungs'. It burrows itself in soft mud and curls up in a chamber lined with mucus, leaving an opening that allows breathing through its mouth. When the fish cocoons itself in such a way, its metabolic rate slows, and the energy necessary for its survival is derived from the breakdown of muscle tissue. The fish has an average lifespan of 4 years, during which time it can reach up to 6.5 inches in length (Kotobuki, 1998).

The present study is an attempt to review the existence and distribution of the lungfish species in the River Nile and in Western Sudan. The study will examine and compare lungfishes using data obtained for morphometric traits and karyotypic features.

Two subspecies of the lungfish, *P. annectens annectens* and *P. aethiopicus aethiopicus*, will be studied using the available criteria, and a comparison will be made between them.
Materials and Methods

Sample Collection

Twenty cocoons containing adult *P. annectens* were collected from dry ponds at Khor Al-Jogan near the town of Umrawaba, Northern Kordofan State, identified by the track left by the fish on the dry mud (collection was performed with permission). A two-headed dagger was used to dig carefully around each cocoon. The removed cocoons with the fish inside were wrapped carefully in sterile cotton and then placed individually inside boxes. Specimens were transferred to the Genetics laboratory, Zoology Department, Faculty of Science, University of Khartoum for further studies. Five adult specimens of *P. aethiopicus* were caught alive at Jebal Aulia dam on the White Nile.

Identification

*Protopterus* spp. were identified using a standard key of morphological characters, as described in the literature (Leveque, 1990; Agbayani, 1999). The morphological descriptions and measurements were carried in this study to stress the specificity of the specimens collected.

Morphometric Measurement

The measurements and ratios were obtained according to the studies of Bishai and Abu Gideiri (1967). The specimens were placed on a wooden measuring board, and the measurements were performed using a ruler and vernier (within 0.01 cm accuracy). Measurements included the following:

1. Standard length (SL) (from the tip of the snout to the origin of the caudal fin) / Depth (D) (the greatest vertical measurement, starting a few millimeters from the origin of the dorsal fin ventral ward) = SL/D.

2. Peduncle length (PL) (the line joining the posterior point of the origin of the anal fin to the point where the lateral line meets the caudal fin) / Peduncle depth (PD) (the narrowest part of the body posterior to the position of the anal fin and anterior to the caudal fin) = PL/PD.

3. Distance Above Lateral Line (DALL) / Distance Below Lateral Line (DBLL) = DALL/DBLL (at the middle position along the lateral line)

Karyotyping

A modification of the karyotyping technique described by Hitotsumachi et al., (1969), Barker (1970) and Cucchi and Baruffaldi (1990) was applied (for both somatic and germ cells).

Each fish was dissected. The liver and the gonads were removed and each organ was squashed vigorously, using a mortar with a glass rod to break the cell clumps. One milliliter of 10% sodium citrate solution [0.5 g sodium citrate + 5 ml distilled water] was added while grinding. After grinding, the mixture was transferred to an Eppendorf tube using a dropper; the tube was marked and labeled. The contents of the tube were centrifuged for 15 minutes at 2000 rpm, and the supernatant fluid was discarded carefully, leaving only the settled, undisturbed cells at the bottom of the tube. One milliliter of freshly prepared Carnoy’s fixative (3 parts absolute methanol: 1 part glacial acetic acid) was added, and then the cells were centrifuged for 10 minutes at 2000 rpm. The last step was repeated three times. The cells were kept in 1.5 ml of the fixative in an Eppendorf tube.

During the fixation of the cells, clean slides were washed with methanol and placed on a slide rack inside a freezer. At the time of use, the slides were removed from the freezer and placed on the bench. Three drops of the cell suspension were dropped from a height of 20 cm onto the slides. The slides were placed on a hot plate; the excess fixative was allowed to evaporate. The slides were then allowed to dry, and then they were stained with Geimsa stain (1 part Geimsa stock solution: 1 part distilled water) for 30 minutes. The slides were then made permanent by passing them through a bath of Xylol for 3-4 minutes. The slides were dried and mounted with cover slips, followed by examination under the microscope.
Slides with well-spread chromosomes were photographed using a digital camera attached to a Leitz Dialux 20 contrast microscope, which was adjusted to the highest magnification; the oil immersion lens was used. Chromosomal counts were performed directly from the slides and were verified using the photographs.

Results

General Observations

Plate (1) shows a cocoon of *P. annectens annectens*. A curved adult specimen can be seen in the cocoon embedded in the dry mud. The color of the dorsal side of the body was light grey, while the ventral side was lighter with red spots. The entire body was covered with thick layers of mucus. When the fishes were freed in water, they began to breathe immediately. The color of the body started to darken, and the red spots disappeared. Plate (2) shows two specimens of *P. annectens annectens* freed from the cocoons. The bodies of these specimens were elongated with a circular cross section and displayed a straight dorsal head profile, as described by Agbayani (1999). The mouth was terminal, with a prominent snout and small eyes. A pair of long and filamentous pectoral fins about three times the head length was a common morphological feature of all of the specimens. The other fins were typical: the pelvic fins were approximately two times the length of the head, and a dorsal fin was continuous with the caudal fin. The scales were cycloid and embedded in the skin.

Plate (1): A specimen of *P. annectens annectens* aestivating in a mucus cocoon within mud. Photograph taken at the Genetics Laboratory, University of Khartoum.
Plate (2): Photograph of *P. annectens annectens* in a water tank at the Genetics Laboratory, University of Khartoum.

Top: *P. annectens annectens* extended on a wooden measuring board.
Bottom: The basal fringe of the pectoral fin is indicated by the arrow.

*P. aethiopicus aethiopicus* specimens collected from the Jebal Aulia dam area were found to comply well with morphological descriptions of Agbayani (1999). Morphological features of *P. aethiopicus aethiopicus* are depicted in Plates (3a and 3b). The five adult specimens collected showed a marble, shiny body color on the dorsal side; the ventral side was lighter in color. The body shape was similar to that of *P. annectens annectens*. Large blackish spots covered the body and fins. *P. aethiopicus aethiopicus* was observed to be very active, aggressive and strong.

Plate (3a): *P. aethiopicus aethiopicus* (the photographed specimens were transferred to the laboratory).
Morphometric Measurements

The results of the morphometric measurements and ratios of *P. annectens annectens* and those of *P. aethiopicus aethiopicus* obtained revealed that *P. aethiopicus aethiopicus* is longer and bigger than *P. annectens annectens* (Table I).

Cytogenetics

The chromosomal number was counted in several mitotic phases in the liver cells of the two subspecies.

The Karyotype

The structure and number of chromosomes of *P. annectens annectens* were arranged in an ideogram according to their sizes, as represented in Plate (4), which shows the karyotype of *P. annectens annectens*, with a diploid number of chromosomes of $2n=34$. The first pair of chromosomes is clearly metacentric, while the remaining 16 pairs are acrocentric. The structures of the chromosomes were not easily determined because of their small size, and moreover, the chromosomes were often found accumulated in a compact form.

The ideogram presented in Plate (5) shows the karyotype of *P. aethiopicus aethiopicus*. The karyotype is characterized by a diploid chromosome number of $2n = 28$. Like *P. annectens annectens*, the chromosomes were not easily determined because of their small size. Chromosome pairs 1, 2 and 3 are distinctly larger than the rest of the chromosomes in the set. Chromosome pairs 2 and 3 are clearly metacentric. There is a clear variation in the sizes of the chromosomes within the set.

Plate (3b): *P. aethiopicus aethiopicus* (the photographed specimens were transferred to the laboratory).
Table I. Comparison between the average morphometric measurements and ratios of *P. annectens annectens* and *P. aethiopicus aethiopicus*, ± the standard error.

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Measurements of <em>P. annectens annectens</em></th>
<th>Measurements of <em>P. aethiopicus aethiopicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth</td>
<td>9.377 ± 0.351</td>
<td>5.351 ± 0.310</td>
</tr>
<tr>
<td>Distance above the lateral line</td>
<td>1.000 ± 0</td>
<td>1.000 ± 0</td>
</tr>
<tr>
<td>Distance below the lateral line</td>
<td>8.018 ± 0.353</td>
<td>3.733 ± 0.417</td>
</tr>
<tr>
<td>Peduncle length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peduncle depth</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Plate (4): Ideogram of a karyotype from *P. annectens annectens* showing a diploid chromosome set of $2n = 34$
Plate (5): Ideogram of a karyotype from *P. aethiopicus aethiopicus* showing a diploid chromosome set of 2n =28. Pairs 1, 2 and 3 are larger than the rest, and pairs 2 and 3 are metacentric.

**Discussion**

The morphometric measurements of the two *Protopterus* spp. revealed that *P. aethiopicus aethiopicus* was longer and larger than *P. annectens annectens* in all ratios: S.L/D of *P. aethiopicus aethiopicus* < S.L/D of *P. annectens annectens* and PL/PD of *P. aethiopicus aethiopicus* < PL/PD of *P. annectens annectens*.

The sizes of the chromosomes were found to be small, similar to the sizes of fish chromosomes cited in the literature (Blaxhall, 1975; Gold, 1979). The chromosomal number of *P. annectens annectens* agreed with that obtained by Morescalchi et al., (2002), 2n = 34. The karyotype of *P. aethiopicus aethiopicus* showed a chromosomal number of 2n=28; however, no referral value for the chromosomal number for this species was found in the literature. The difference in chromosome number between the two subspecies suggests a revision of the taxonomic relationship of the two taxa. It is well established that the chromosome number is uniform within a species, and a deviation from the "standard" set must be explained (sometimes there are apparent chromosomal fusions in one of the species categories). The sizes of the chromosomes of *P. aethiopicus aethiopicus* were found to be distinctly larger than those of *P. annectens annectens*: chromosome pairs 2 and 3 (Plate 5) appear as large metacentric chromosomes. Chromosome fusion is proposed here in to explain the discrepancy in karyotypes.

**Conclusion**

This study reveals useful information about lungfishes in Sudan. It shows that there are two subspecies of lungfishes in Sudan: *P. aethiopicus aethiopicus*, which is found in the River Nile system, and *P. annectens annectens* found in western and central Sudan.

**References**


Leveque, C. 1990, "Morphology Data of *Protopterus annectens annectens*", available at: www.FishBase.com