Chromosomes of the African Polypterid Fishes, *Polypterus palmas* and *Calamoichthys calabaricus* (Pisces: Brachiopterygii)

From an evolutionary viewpoint, the primitive fishes of the family Polypteridae are among the most interesting of existing fish groups. The family is presently confined to the freshwaters of west and central tropical Africa and contains 2 genera, *Polypterus* and *Calamoichthys*. The genus *Polypterus* contains about 9 species, commonly referred to as 'bichirs' while *Calamoichthys* is represented by a single species, the 'reedfish'. Fossil polypterids are known only from Eocene strata in Egypt and their entire evolutionary history appears to be restricted to Africa[1].

The polypterids represent interesting study material for the systematic zoologist as they have retained many primitive features of their prehistoric ancestors and are truly living fossils. At present, there is much uncertainty concerning the systematic position of the polypterids. Some authors have combined polypterids and crossopterygians into a single subclass, the Crossopterygii (fringe-finned fishes), as distinct from the Actinopterygii (ray-finned fishes) and Dipneusti (lung-fishes)[2-4]. Others feel that polypterids were derived from the ancient palaeoniscoids and should be classified with the subclass Actinopterygii[5-10]. However, some authorities consider polypterids sufficiently distinct from Actinopterygii, Crossopterygii, and Dipneusti to warrant their placement into a separate subclass, the Brachiopterygii[11-13]. Polypterids do differ notably from all known actinopterygians in having double ventral lungs, a well-developed epichordal caudal lobe, and lobed pectoral fins. However, the route of pulmonary circulation differs from that of the crossopterygians and dipneustans and the fins have a different bone pattern. Because of these uncertainties regarding polypterid relationships, it was felt that a cytotaxonomic study of polypterid chromosomes would introduce new data that would be useful in determining the systematic position of polypterids among the major

Table I. Distribution of diploid chromosome counts obtained for *Polypterus* and *Calamoichthys*

Species	No. cells analyzed	*Diploid chromosome counts*		
		35	36	37
P. palmas	43	2	40	1
C. calabaricus	105	3	95	7

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Table II. A Comparative analysis of chromosome pair lengths as a percent of total haploid complement length (% THCL) and L/S arm ratios in 4 metaphase figures each of *Polypterus palmas* and *Calamoichthys calabaricus*

Chromosome pair number	Chromosome length as % THCL				Arm ratio L/S			
	Polypterus Range	x	*Calamoichthys* Range	x	*Polypterus* Range	x	*Calamoichthys* Range	x
m_1	10.8–13.4	11.4	9.6–12.0	10.6	1.1–1.5	1.3	1.0–1.4	1.2
m_2	9.6–10.6	10.0	8.8– 9.8	9.4	1.0–1.3	1.1	1.0–1.2	1.1
m_3	8.6–11.0	9.0	7.8– 9.6	8.8	1.1–1.6	1.3	1.0–1.4	1.2
m_4	7.0– 9.4	8.6	6.6– 9.0	8.0	1.0–1.5	1.3	1.0–1.6	1.3
m_5	5.6– 7.4	6.6	6.8– 8.0	7.2	1.2–1.9	1.5	1.1–1.7	1.5
m_6	5.6– 6.0	5.6	5.4– 6.2	6.0	1.3–1.5	1.4	1.0–1.6	1.4
m_7	4.8– 6.2	5.4	5.4– 6.0	5.8	1.1–1.6	1.4	1.0–1.6	1.3
m_8	4.6– 5.6	5.0	5.2– 6.4	5.6	1.1–1.6	1.3	1.2–1.6	1.3
m_9	3.2– 4.8	4.2	4.8– 5.6	5.0	1.1–1.7	1.4	1.1–1.4	1.3
m_{10}	2.4– 3.8	3.0	4.2– 5.0	4.6	1.0–1.4	1.2	1.1–1.7	1.4
m_{11}	2.4– 3.0	2.8	3.4– 4.8	4.0	1.1–1.4	1.3	1.0–1.5	1.3
m_{12}	2.0– 2.8	2.6	3.2– 4.0	3.6	1.2–1.4	1.3	1.0–1.6	1.3
m_{13}	—	—	3.0– 3.6	3.2	—	—	1.0–1.5	1.2
m_{14}	—	—	2.6– 3.4	3.0	—	—	1.0–1.5	1.4
m_{15}	—	—	2.4– 3.4	2.8	—	—	1.0–1.5	1.2
sm_1	4.6– 7.0	5.6	4.0– 5.8	4.8	1.7– 2.1	1.8	1.8–3.7	2.5
sm_2	4.4– 5.8	5.0	3.8– 4.8	4.2	1.8–2.7	2.2	1.8–3.1	2.4
sm_3	3.8– 4.8	4.4	2.2– 4.2	3.0	1.7–2.4	2.1	1.6–3.6	2.2
sm_4	3.4– 4.6	4.2	—	—	1.6–2.3	1.9	—	—
sm_5	2.6– 4.6	3.4	—	—	1.2–2.4	1.8	—	—
sm_6	2.0– 3.4	2.6	—	—	1.5–1.8	1.7	—	—

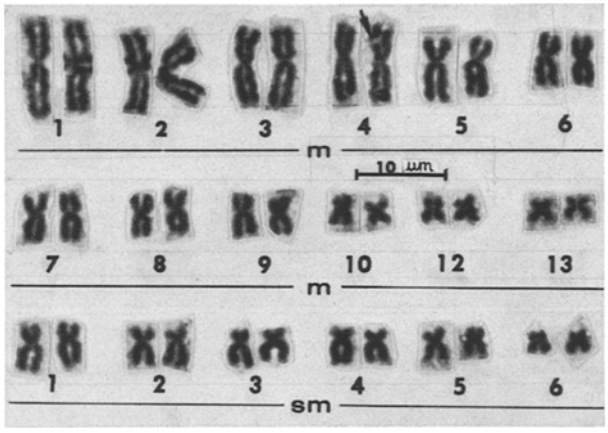

Fig. 1. Photokaryotype of metaphase cell from gill epithelium of *Polypterus palmas*, $2n = 36$. Arrow indicates chromatid gap in chromosome pair No. 4.

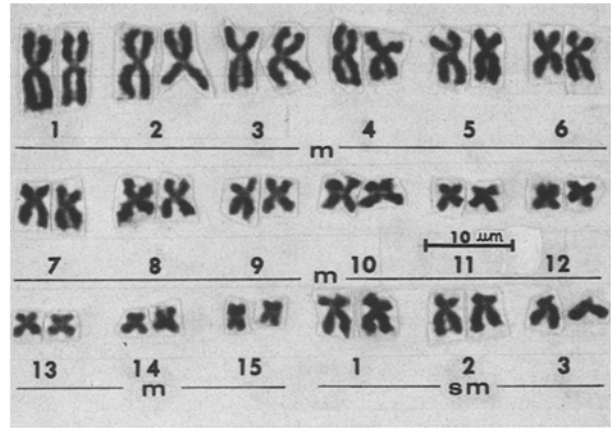

Fig. 2. Photokaryotype of metaphase cell from fin epithelium of *Calamoichthys calabaricus*, $2n = 36$.

living fish groups. Thus, the objective of this study was to determine the chromosome number and morphology of representative polypterids and to compare the results with those obtained by other workers[14–17] who have studied the chromosomes of lungfishes, sturgeons, gars, and bowfins.

Two males and 2 females each of *Polypterus palmas* Ayres and *Calamoichthys calabaricus* Smith were obtained from tropical fish dealers. The specimens received intraperitoneal injections of 0.1 ml of 0.1% sterile, isotonic colchicine 6–8 h before sacrifice. Gill, fin, spleen and kidney tissues were removed, swollen in distilled water for $1^1/_2$ h, fixed in 3:1 methanol glacial acetic acid, dabbed onto a slide, air-dried, and stained[18]. Karyotypes were made from photographic enlargements of metaphase chromosome spreads. Long Arm (L), short arm (S), and total length measurements were obtained from each chromosome to the nearest 0.1 mm using dial calipers. From this data, the L/S ratio was calculated and the total length of each chromosome was expressed as a percent of the total haploid complement length (%THCL). The chromosomes were classified by Levan et al.[19], i.e., the centromere was considered to be median (m) in position if the L/S ratio fell within the range 1.00–1.70 and submedian (sm) if the range was 1.71–3.00.

A diploid chromosome number of 36 was obtained for both *P. palmas* and *C. calabaricus* (Table I). The karyotypes of both species were remarkably similar with *P. palmas* having 24 metacentric and 12 submetracentric chromosomes while *C. calabaricus* had 30 metacentrics and 6 submetacentrics (Table II, Figures 1, 2). 28 of the 36 chromosomes from each specific complement had similar L/S ratios. These consisted of the first 12 pairs of metacentrics and submetacentrics 2 and 3 (Table II). Sex chromosomes were not evident in either species.

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Table III. A comparison of chromosomes between primitive actinopterygian, brachiopterygian and dipneustan fishes

	Characteristics of metaphase complement						
	2N	M	SM	A	Micro-chromosomes	Fundamental number[a]	Length of longest chromosomes (µm)[b]
Actinopterygii							
Chondrostei							
Scaphirhynchus platorhynchus[16]	112	50	—	14	48	114	4
Holostei							
Lepisosteus occulatus[16]	68	28	—	14	26	70	3
Amia calva[16]	46	20	—	26	—	66	3
Brachiopterygii							
Calamoichthys calabaricus	36	30	6	—	—	72	10
Polypterus palmas	36	24	12	—	—	72	12
Dipneusti							
Protopterus annectens[14]	34	(all V-shaped)			—	68	30
Neoceratodus forsteri[14]	32–38	(all V-shaped)			—	64–76	30
Lepidosiren paradoxa[14,15,17]	38	38	—	—	—	76	30

[a] Microchromosomes not included. [b] Except for Brachiopterygii, values were estimated from photokaryotypes[16] or idiograms[14,17].

When the lengths of the chromosomes were compared, *P. palmas* consistently had longer metaphase chromosomes than *C. calabaricus*. In a typical metaphase spread of *P. palmas*, the smallest chromosome averaged 2.3 μm while the longest was 11.2 μm (Figure 1). In *C. calabaricus*, the smallest chromosome averaged 1.8 μm while the longest was 7.8 μm (Figure 2). Thus, it seems that *P. palmas* has more chromosome material than *C. calabaricus*.

Seven of the 105 metaphase cells analyzed for *C. calabaricus* had a chromosome fragment that resembled a small acrocentric chromosome. Since it did not stain as well as the surrounding autosomes, it was considered to contain mostly heterochromatin. No chromosome fragments were found in *P. palmas*. However, in 20 of 43 metaphase cells examined in *P. palmas*, a distinctive chromatid gap was found in a member of chromosome pair number 4 (Figure 1). No gaps were found in *C. calabaricus*. The occurrence of the chromatid gap and the occasional chromosome fragment suggests that some mechanism of karyotypic change is still operating in this group. Except for these two conditions, all karyotype preparations were consistent in both number and structure for both genera.

Most present day classification schemes place the polypterids in the subclass Actinopterygii, superorder Chondrostei, along with the sturgeons and paddlefishes. However, a comparison of polypterid chromosomes with those of the shovelnose sturgeon, *Scaphirhynchus platorhynchus*[16], shows great dissimilarities (Table III).The most obvious differences are the high 2*n* number of 112 and the presence of 48 microchromosomes in the sturgeon. Microchromosomes are characteristic of the sturgeon, gar, reptiles and birds[16], and their absence in polypterids is certainly noteworthy. In addition, the longest chromosome of the sturgeon is only 4 μm while the longest chromosome in polypterids is 12 μm.

Polypterid chromosomes are also very distinct from those reported for the holostean gar, *Lepisosteus productus* (= *oculatus*) and bowfin, *Amia calva*[16] (Table III). The gar, like the sturgeon, has microchromosomes, a much higher 2*n* number, and smaller-sized chromosomes. Thus, the gar and sturgeon are much closer in chromosome morphology than either are the polypterids. The bowfin, like the polypterids, lacks microchromosomes but has a higher 2*n* number (46) and the largest chromosome is only about 3 μm[16].

As already indicated, polypterids have many morphological features in common with fishes of the crossopterygian line and were considered by a few early workers to belong to that group. Unfortunately, the chromosomes of the 2 groups cannot be compared as there have been no chromosome studies done on *Latimeria*, the only living crossopterygian. However, a comparison of polypterid chromosomes with those of Australian, African, and South American dipneustans shows many striking similarities (Table III). The 2*n* numbers range from 32 to 38 for these dipneustans and is in conformity with the 2*n* of 36 for polypterids. In addition, all of the dipneustans have biarmed chromosomes which give a fundamental number of 64–76 which is in agreement with that of 72 in polypterids. The only dissimilarity seems to be in the length of the chromosomes which is up to 30 μm in dipneustans[14,16,17], but only 12 μm in polypterids.

Thus, based upon chromosome morphology as well as body structure, the polypterids seem to have more in common with the dipneustan lungfishes than with the Actinopterygians. However, polypterids differ significantly enough from the dipneustans in chromosome size, route of pulmonary circulation, fin structure, etc., to avoid their placement into this group. Thus, based upon our chromosome data, we agree with others[11–13], that it is justifiable to remove the polypterids from the superorder Chondrostei, subclass Actinopterygii, and place them in a separate group by themselves, the subclass Brachiopterygii. Surely they are distinctive from both groups and seem to occupy a somewhat intermediate phylogenetic position.

Résumé. Une étude de chromosomes des poissons polyptères africains, *Polypterus palmas* et *Calamoichthys calabricus* révèle un nombre diploïde de 36. Ce caryotype a été comparé à celui du Dipneustes, du Holostéens et du Chondrostéens. Il ressort de cette analyse que les Polyptères occupent une situation intermédiaire entre celles des Actinopterygii et des Dipnoi, ce qui légitime le point de vue selon lequel ils doivent être placées dans une sousclasse distincte, les Branchiopterygii.

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Testing of Rifampicin on Possible Genetic Effects on *Drosophila melanogaster* and Human Leukocyte Chromosomes in vitro

Rifampicin, belonging to the rifamycin antibiotics, is the most widely used compound of this class for both clinical and biochemical purposes. Clinically, rifampicin proved to be very helpful, especially for the treatment of tuberculosis. The antibacterial activity of the rifamycin group is due to specific inhibition of bacterial DNA-dependent RNA polymerase. For details on the chemical structures and the actions of the rifamycins see ref.[1] and[2].

Because of the widespread clinical use of rifampicin, we were interested in any mutagenic effects of this substance on *Drosophila* and human leukocyte chromosomes. *Drosophila:* In order to study a possible genetic effect of rifampicin on *Drosophila*, the Basc-technique for the determination of recessive *X*-chromosome lethals was applied. The sodium salt of rifampicin was dissolved in 5% sucrose solution containing phosphate buffer to keep the pH at 7.0. Berlin wild K males, 1–2 days old, were fed with the test solution (concentration 0.5 mg/ml rifamycin) for 3 days, using the adult feeding method described elsewhere[3,4]. Each P-male was crossed to two sc^{S1} In S B w^a sc^8 females (genetic symbols see ref.[5]). The Basc-technique was performed in the usual way with 3 successive broods, each for 3 day's duration. Progeny obtained from

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