CYTOGENETIC VARIATIONS IN *Clarias* species (CLARIIDAE: SURULIFROMIS) OF THE ANAMBRA RIVER USING LEUCOCYTES CULTURE TECHNIQUES

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ABSTRACT

Cytogenetic variations among four Clarias species from Anambra river, Nigeria were studied using leucocytes culture techniques. Heterogeneity in chromosome number (2n = 48 in Clarias ebriensis and C. albopunctatus to 2n = 56 in C. anguillaris and C. gariepinus) and Karyotype morphologies occurred among the clariids. The chromosomes were characterized by a high proportion of meta-submetacentric chromosomes and low proportion of acrocentric chromosomes. The females karyotype morphologies exhibited a heteromorphic pair suspected to be the sex chromosome complex. The following formulae were established for the male clariids; C. ebriensis 6m + 22sm + 20a FN =76; C. albopunctatus 4m ± 22sm + 22a FN = 74; C. gariepinus 8m + 24a FN = 88; and C. anguillaris 8m + 26m + 22a FN = 90. The female karyotype morphologies were C. ebriensis 6m + 23sm + 19a FN = 77, C. albopunctatus 4m ± 23sm + 21a FN = 75, C. gariepinus 8m + 25sm + 23a FN = 89 and C. anguillaris 8m + 27sm + 21a FN = 91. A generic chromosomal number of 2n = 54 ± 4 for the clariids was suggested. The almost uniform karyotype morphologies and the closeness of the chromosome numbers around the generic chromosome number may suggest success with which the clariids may hybridize in nature.

Keywords: Cytogenetics, Chromosomes, Karyotype, Idiogram, Clarias, Clariidae, Leucocyte culture

INTRODUCTION

Karyological evidences have often been utilized in solving problems relating to speciation, identity of sex determining chromosome, phyletic relationship, the taxonomic status and identity of both interspecific and even intergeneric hybrids among organisms. Concerning speciation, new evidence suggests that karyotype plays a primary role (White, 1978) contrary to Mayr (1963) original hypothesis that only polyploidy and geographical isolation lead to the formation of new species. Fish comprises polyphyletic group which has undergone an enormous expansion, the number of existing species totals about 20,000 (Lagler *et al.*, 1977). This is about 48.10 % of all the existing vertebrates. The capacity of this group to adapt has led to their colonization of extremely specialized niches. Pisces explosive expansion cannot be explained wholly in terms of geographical isolation since chromosome mechanism probably plays a role in speciation (Sola *et al.*, 1981).

About 1000 to 1500 species have been studied out to the 20,000 species (Lagler *et al.*, 1977). Evidence has been found of evolution by polyploidization in the Cypriniidae (Wolf *et al.,* 1969) and Salmoniidae (Ohno *et al.*, 1969) using karyological techniques. In salmonids currently undergoing expansion, chromosome polymorphisms were shown (Thorgaard, 1977). In the Poecilids, speciation may occur by interspecific hybridization. This apparently applies to *Poecilia formosa* Bloch, 1801; whose hybrid origin is widely accepted based on morphological and karyological characteristics (Prehn and Rasch, 1969).

The teleosts provide good materials for cytologists working on sex determination problems. In most species sex chromosome are absent or not morphologically identified. However, some species are known to exhibit male heterogamete whereas others have female heterogamete. Kallman (1973), reported that allopathic populations of the same species may display female or male heterogamete as shown in *Xiphophorus maculates* Hecke, 1848 in which the sex chromosomes were identified by means of pigment gene marker showing a sex-linked hereditary patterns. Feldber *et al.,* (1987) showed the occurrence of chromosome system for sex determination of ZZ/ZW type in *Semaprochilodus taeniurus* Newton, 1978 (Pisces: Prochilodontidae) which is lacking in *Semaprochilodus insignis* C. and V., 1929. Chromosome W found in the female was the largest in the complement. Chromosome Z was the next largest complement and tentatively considered as pair number one in males. Heaf and Schmid (1984) have shown sex-chromosome differentiation in another poecillid species, *Poecillia sphnops* var. *melonistica* Jordan and Snyder, 1906.

Karyotype evolution and geographical distribution among fishes have been studied. In the genus, *Oryzias;* Oyzidae, Uwa (1986), Uwa and Parenti (1988) and Uwa *et al*., (1988) divided the rice fish into three groups: monoarmed chromosome type (*O. melastigma* Jordan and Starks, 1906 (Uwa and Iwata, 1981) *O. javanicus* Jordan and Starks, 1906 (Uwa and Iwata, 1981), biarmed chromosome type *O. sp. (Oryzias* species) (Uwa and Magtoon, 1986) *O. curvinotus* Jordan and Starks, 1906 (Uwa*, et al.,* 1982), and fused chromosome type (*O. celebensis* Jordan and Seale, 1906 and *O. minutillus* Jordan Starks, 1906 in South and South Eat Asia to the Indian ocean. The biarmed chromosome types are distributed in East Asia and Luzon. The degree of the karyotype evolution by increased of biarmed chromosome number in this group seems to correlate with the geographical distribution from Indo-China to Japan and Luzon to China. Members of the fused arm chromosome types are found in Sulawesi and Thailand.

Cytogenetical investigations among teleosts has revealed inter and intra-specific variations in nucleolar organizing region (NOR). Feldberg and Bertollo (1985) noted among the neo tropical cichlids that the NORs were located on the first chromosome pair in the complement and coincided with secondary constrictions observed there, whereas in *Cichlasoma facetum* Swainson, 1839 and *Geophagus brasilcensis* Heckel, 1840, the NOR were located on another chromosome pair. NORs have been investigated in several fish species, such as *Carassius sp.* Nilsson, 1832 (Funaki *et al.*, 1975; Ojima and Yamano, 1980), *Umbra limi* Gronow, 1763 (Kligerman and Bloom, 1977), cichlid fishes (Kornfield *et al*., 1979), *Fundulus diaphanous* Lacepode, 1803 (Howel and Black, 1979). In the fish species, the NORs were usually located near the telomeric regions of satellite chromosomes except for *F. diaphanous* where they were located on the secondary constriction of the sex chromosomes. The NORs are of obvious importance to geneticists. Kligerman and Bloom (1977) reported that the NORs are sites of prior genetic activities and where satellite chromosomes occur. This chromosome pair could serve as an adequate marker as in *Umbra limi.*

The interests of cytogeneticists and evolutionists are closely connected with that of ichthyologists in the study of hybrids. In the freshwater bony fish, evidences were frequently found in interspecific and even intergeneric hybrids where sympatric populations of different species exist. The development of fish culture has resulted in numerous experiments on hybridization to obtain new strains, which are more disease resistant, faster growing than their relative wild stock. In such case, karyological knowledge allows the parent species to be recognized and give some indication of the likelihood of success in the case of artificial crossbreeding. Furthermore, it may help to make predictions concerning hybrid fertility and the interactions of two parental stocks.

Although it is not possible to draw absolute conclusion about phyletic relationships from karyological comparison of different species, karyotype analyses have supplemented other taxonomic evaluating methods. The systematic definition of species depends on a careful morphological analysis, and the karyotype is a major morphological character among several others.

The phyletic relationship among the siluriform fishes based on karyotype and variation in chromosomal number revealed that, *Bagre marinus* Catesby, 1771 had 2n = 54 chromosomes composed of 12 metacentric, 8 submetacentric and the remainder with terminal or near terminal centromere (Fitzsimons *et al*., 1988). Furthermore, the karyotype of three species of ariid catfishes (*Arius dussumier* C and V, 1840, *Arius felis* C and V 1840 and *Bagre marinus)* indicated the same diploid chromosomal number, but each species had a different arm number. Data for 132 species in 14 families of catfishes indicated a predominance of 56 $\pm$ 2 chromosomes in the diploid set (Fitzsimons *et al*., 1988). According to them, the range in diploid number was most common among the Ariidae, Bagridae, Ictaluridae and Pimelodidae, which together have been suggested from osteological evidence as forming a group close to the ancestral stock from which present day catfish evolved. In a study of chromosomal evolution among the Ictalurid catfishes, LeGrande (1981) observed that a diploid chromosome number of 56 $\pm$ 2 was wide among 70 species of catfishes in 10 families and was especially more frequent in four families, the Ariidae, Bagridae, Ictaluridae and Pimelodidae. In addition, average diploid count of 56 modal count for catfishes were approximated from the averages and / or modal counts of ariids, (all 54), bagrids (mostly between 50 and 60, with a weak mode at 52), clariids (50, 52 and 56), ictalurids (mostly between 56 and 60 with exclusion of divergent karyotype in *Noturus*) and Primelodids (mostly 56). LeGrande (1981) hypothesized an ancestral karyotype of 2n = 56 for Ictalurids and pointed out that the closeness in this number to those reported for the Ariidae, Bagridae and Pimelodidae coincides with Gosline (1975 a, b) suggestion that these families plus Doradidae constitute a group near the ancestral stock from which living catfishes evolved. Considering the Clariids, Teugels *et al*. (1992) reported a standard karyotype of 2n = 56 for *Clarias gariepinus and* 2n = 52 for *Heterobranchus longifilis* Valenciennes, 1840. Their hybrids revealed an intermediate karyotype

of 2n = 54. Furthermore, sex chromosomes were observed as ZW heteromorphic pair in female hybrid karyotypes. The ZZ chromosome pair in male hybrids was similar to that found in male *C. gariepinus* and male *H. longifilis.*

Hartley (1987) divided the species of the sub-order: Salmonidea into two groups (categories A and B) based on chromosome number, chromosome arm number, and the distribution of one-armed and two-armed chromosomes. Category A karyotypes were widespread both in terms of geographic distribution and number of species, while category B karyotypes were restricted to member of the genera *Salmo* and *Oncorhynchus.* In category A, it was assumed that tetraploidy followed arrangement of the diploid ancestral karyotype of 2n = 50, NF = 60 (the lowest diploid and chromosome arm number found in the smolts) and that the resulting ancestral tetraploid salmon had 2n = 100, NF = 120 karyotype. Hartley (1987) observed that a series of pericentric inversion and centric fusions had reduced chromosome number and chromosome arm number through *Brachymystaz lenok* Gunter, 1866, (2n = 94, NF = 116) and *Hucho hucho* Gunter, 1866, (2n = 82, NF = 114) to the category A karyotypes (2n = 80, NF = 100) and finally to the category B karyotypes (2n = 60, NF = 104). He concluded that by the time the category B karyotypes were achieved, centric fusions had played a far greater role in the evolution of the vast majority o the salmonids karyotypes than pericentric inversions, although the latter had played important role in the evolution of the Atlantic salmon karyotype.

The karyotypic evolution of ten Neotropical cichlids showed that the chromosome evolution of the group was more conservative than divergent (Feldberg and Bertollo, 1985). All species had a chromosome number of 2n = 48, though with some differences in chromosome morphology. Pericentric inversions were probably the main event that led to the karyotypes more commonly found in this group. On the basis of fundamental number (FN), four general groups reflected the occurrence of a progressive number of chromosome rearrangements. Group one with FN = 48, (represented by *Chaetobranchopsis australis* Eigenmann and Ward 1907), group two with FN = 50 – 52 (comprising *Geophagus brasiliensis* Heckel, 1840; *G. surinamensis* Heckel, 1840 and *Gymnogeophagus balzanii* Ribeiro, 1918), group three with FN = 54 (represented by *Crenicichla lacustris* Ribeiro, 1918, *C. lepidota* Henkel, 1840 and *C. vittala* Heckel, 1840 and to *Batrachops semifasciatus* Heckel, 1840), group four with FN = 58 – 60 (represented by *Astronotus ocellatus* Swainson, 1839 and *Cichlasoma facetum* Swainson, 1839) (Feldberg and Bertollo, 1985). Despite the constancy of chromosome number, 2n = 48, a few distinctive characteristics which are products of structural rearrangements of chromosomes that occurred during their karyotypic evolution can be seen in some species or group of species.

Ichthyologists and fish taxonomists in the classification of related species have often employ knowledge from karyotype. For instance, the larval forms of most species are morphologically identical and quite different from the adult forms. Because of the resemblance among larval forms, their identification if often difficult. Morphological observations are inadequate *vis-à-vis* the identification of the species, except where the developmental stages can be followed, which is usually difficult and not documented for many fish species. In such cases, Sola *et al.* (1981) noted that the knowledge of the karyotype and their diversified forms might provide a precise diagnostic criterion. Oliveria *et al*. (1988) based the classification of Neotropic Ostrariophysi on karyotypic data and noted that 2n = 50 tend to prevail among Ostrariophysi. This character was also seen among the Otophysi (2n = 50). The Cypriniformes have a second modal number of 2n = 100, often seen among the families of Cyprinidae and Catostanidae. Yu *et al.* (1987) proposed that the cypriniformes might have originated from 2n = 50 ancestors. The characiphysi and characiformes differ from the cypriniforms by having almost 2n = 54. Among the characiformes, several groups may be characterized based on their chromosome number. The characiforms, erythrinidae and lebiasinidae are typified by chromosome number of less than 2n = 54 while the Serrasalminae are characterized by chromosome number of more than 2n = 54.

From the foregoing review, karyotypic information appears to be very important in discriminating species. The technical and interpretation problems often encountered by fish cytologists should not be employed as a base for judging the validity of karyotypic information *vis-à-vis* classification of related and unrelated species. The identification of artificial (diploidy and/or polyploidy) and natural hybrids, and of sex determining chromosomes is currently cytogenetically accomplished. For a proper discrimination of the species, information from fish molecular biology, anatomy, anatomy, physiology ethology and ecology is highly desirable. This study further enriches the information on fish cytogenetic by investigating into the karyotypic variations among members of the Pisces family Clariidae of Anambra river, Nigeria using leucocytes culture techniques.

MATERIALS AND METHODS

Collection and Care of Catfish: Catfish specimens were collected from Anambra river, Nigeria. The fish were captured using set nets (mesh size 70 mm – 120 mm) and long line baited with earthworm and palm fruits. Specimens were also bought from the fishers at the landing site. All catfishes were transported to Fisheries and Hydrobiology wet laboratory, Department of Zoology, University of Nigeria, Nsukka. Identification and classification of fish were done with Lowe-McConnell (1972), Sydenham (1983) and Ezenwaji (1989) (see Eyo, 1997). Live specimens were given 1 ppm potassium permanganate ($KMnO_4$) flush prophylactic treatment for 10 minutes. This was to avoid the introduction of wild ectoparasites and pathogens. Catfishes were acclimatized in nine aquaria tanks (80 x 40 x 40 cm) in groups of eight fish per tank for 14 days. All fishes were fed with 5 % body weight daily with 40 % formulated fish feed (Eyo, 2004a). All other culture conditions were as explained in Eyo and Ezechie (2004).

Phytohaemaglutinin Extraction: Phyto-hemaglutinin (PHA) used for this study was extracted from kidney bean, *Phosealus vulgaris* using a modified method of Rigas and Osgood (1954). Finely ground and sieved red kidney bean seed (250 grams) was digested with one litre of 1 % NaCl for twenty-four hours at 4 ^{0}C refrigeration. The extract was centrifuged at 1400 rpm for 15 minutes using Multex centrifuge. The light yellow supernatant was recovered and the precipitate discarded. The supernatant was adjusted to pH 5.7 and cold ethanol added to final concentration of 30 % ethanol, and centrifuged at 1400 rpm for 15 minutes. The precipitate was rejected and 400 ml of 10 % ethyl ether, absolute ethanol and 75 % ethanol in the ratio (1:1:1) was added to the yellow supernatant. A milky white colour developed upon shaking. The milky white supernatant was re-incubated at 4 ^{0}C refrigeration for 24 hours.

After the re-incubation, the extract was centrifuged at 1400 rpm for 15 minutes. The resulting yellow supernatant was discarded. The milky white precipitate was dissolved in 150 ml of 1 % NaCl. The pH was re-adjusted to 5.7 and the ethanol fractionation repeated by adding 300 ml of 1:1:1 volume of absolute ethanol, 10% ethyl ether and 75% ethanol. The resulting milky white extract was shaken vigorously and re-incubated at 4 ^{0}C for 24 hours. The extract was centrifuged at 1400 rpm for 15 minutes. The resulting light yellow supernatant discarded and the milky white precipitate dissolved in 150 ml of 1% sodium chloride. The ethanol fractionation was repeated by adding 200 ml of 1:1:1 volume of ethanol, 10% ether and 75% ethanol. The extract was vigorously shaken and centrifuged at 2000 pm for 15 minutes. The milky white precipitate was dissolved in 100 ml of 0.1M phosphate buffer pH 8.0 and 40 ml of saturated ammonium sulphate solution added. A light pink colour developed. The light pink extract was centrifuged at 2000 rpm for 15 minutes and the precipitate discarded, 105 ml of saturated ammonium sulphate solution was added to 150 ml of the supernatant, shaken vigorously and centrifuged at 2000 rpm for 15 minutes. The precipitate was dissolved in 100 ml deionized water, shaken vigorously and centrifuged.

The procedure was repeated by adding 50 ml of deionized water and 17.5 ml of saturated ammonium solution was added. The extracted was vigorously shaken and centrifuged at 2000 rpm for 15 minutes. The phytohaemagglutinin (PHA) was freed from the ammonium sulphate by dissolving the light pink precipitate in 50 ml of deionized water, vigorously shaken and centrifuged. This procedure was repeated twice, and the resulting phytohaemagglutinin (2 grams) was freeze-dried. A stock solution of 1 mg ml^{-1} phytohaemagglutinin was made by dissolving 0.1 gram of PHA in 100 ml of 0.85 % sodium chloride solution. The stock solution was stored at 10 $\pm$ 5 ^{0}C.

Blood Sampling: Blood was collected by heart puncture after anaesthezing the catfishes in L/1500 solution of MS-222 (Methanesulfonate salt) for 3 minutes. The abdomen wiped with absolute ethanol and the visceral cavity opened to expose the heart. One ml of blood was obtained using a sterile 2 ml plastic syringe containing 0.2 ml of 0.1 M sodium citrate (an anticoagulant) with a 21 gauge, 1-inch needle. The sampled blood from each catfish was stored in a labeled sterile capped 5 ml plastic tube at 10 $\pm$ 5 ^{o}C until used. Contamination of sampled blood was avoided by dealing with only one sex of a fish species per day. All the equipments were heat sterilized before and after use. Catfishes sampled for blood were 20 males and 18 females of *Clarias gariepinus,* 21 males and 24 females of *Clarias anguillaris,* 30 males and 21 females of *Clarias ebriensis* and 26 males and 30 females of *Clarias albopunctatus.*

Blood Leucocytes Separation: Blood leucocytes were separated by three simple sedimentation methods modified from Blaxhall (1981) and the leucocytes-rich supernatant used as inoculums. The methods were:

1. **Hank's balance salt solution (HBSS) sedimentation:** 1 ml of anticoagulated blood was diluted with 1 ml of HBSS and mixed by gentle inversion in 5 ml sterile

caped plastic test tube. The content was centrifuged at 2,700 rpm for 3 minutes using micro angle centrifuge.

2. **Hank's balance salt solution plus phytohaemagglutinin separation:** 1 ml of HBSS and 0.2 ml of 1 mg ml^{-1} PHA were added to 1 ml of anticoagulated blood. The contents were mixed and centrifuged at 2, 700 rpm for 3 minutes to ensure proper settling out of erythrocytes.

3. **Phytohaemagglutinin separation:** 1 ml of anticoagulated blood was diluted with 0.2 ml of 1 mg ml^{-1} PHA. This was mixed by gentle inversion in a sterile 5 ml capped plastic test tube. The content was centrifuged at 2,700 rpm for 3 minutes. In each case, the leucocyte rich supernatant was used as inoculum.

Blood Leucoyte Culture Techniques: The complete culture medium used composed of 20 ml of HBSS, 2 ml of Eargle's Essential Culture Medium. Laboratory prepared phytohaemagglutinin was added to make up either 5 % of the entire culture medium. Furthermore, freshly prepared rabbit sera were added to make up 20 % of the entire culture medium. The complete medium was aseptically filtered using membrane filter. The complete culture medium (5 ml) was placed in sterile tube. Various glass and plastic sterile test tubes were tried for culturing of fish leuocytes. About 0.05 ml of leucocyte rich supernatant was aseptically placed on the bottom of the culture tube containing 5 ml of the complete culture medium using a sterile 1 ml syringe fitted with 26-gauge 1¼-inch needle. Culture vials were incubated at $36 \pm 1\ ^0C$ for 72 hours.

Chromosome Harvesting and Slide Preparation Techniques: The cultured leucocyte cells were arrested 3 – 6 hours with 0.3 $\mu g\ ml^{-1}$ of colchicine solution before the end of the 72 hours culture period to give good mitotic chromosome spread for karyotyping (Eyo, 1997). At the end of incubation and metaphase arresting period, the cells were harvested by centrifuging at 1000 rpm for 5 minutes. The supernatants were discarded and the white button-like residue given 5 ml of 0.075 M KCl hypotonic treatment for 10 minutes and expirated using a micropipette. The residues were obtained through centrifugation and the supernatants discarded. 2 ml of freshly prepared cold Carnony's fixative (absolute ethanol and glacial acetic acid (3:1)) was added to the residue cells, expirated to disperse the cell pellets and left standing for 15 minutes. The fixation process was repeated twice at 15 minutes intervals. After the final centrifugation, the fixative was decanted leaving about 3 drops in which to re-suspend the cells through expiration. The chromosome spread was accomplished using a modification of Mellman (1965) air-dried technique.

Thoroughly clean grease free slides (commercially pre-cleaned slides) were dipped into deionized water and frozen. The chilled slides were observed for cleanliness by adherence of water film. Excess fluid was shaken off and one drop of cells suspension was placed on the center of the wet slide inclined at an angle of 45^0. A blotter placed underneath the slides removed excess fluid. Drying was completed immediately by blowing the slides to promote evaporation and by gentle warming under 80 watts electric bulb.

The quality of slides was checked using a hand lens. Inadequate spreading was corrected by re-fixation of the cells. Slides were stained in 10 % buffered Giemsa stain for 5 minutes, and dehydrated for 1 min each in two jars of acetone, one jar of acetone and xylene solution (1:1) and finally one jar of xylene. Slides were observed to destain in acetone when shaken and or agitated. Thus, shaking and agitation was avoided. Slides were scanned for metaphase chromosomes using a binocular light microscope at X 1500 magnification. Slides with good metaphase chromosomes were processed into permanent mounts. The chromosomal arm lengths were measured to the nearest micron using ocular micrometer.

Chromosome Analysis Nomenclature: Ten slides per species showing good metaphase chromosome were analysed. The frequency of diploid chromosomes number per species was recorded. The maximum or minimum mean and modal chromosome number were computed for each species. F-LSD was employed to separate the differences in modes. The arm ratio r, (long arm L/ short arm S) and the centromeric index i (100 x short arm/total length of chromosome) were calculated. Furthermore, each chromosome was classified into any of the six groups; M, m, sm, st, t, and T based on Levan *et al.* (1964) classification (Table 1).

Table 1: Chromosome arm nomenclature relevant to Clariid cytotaxonomy

Term	Location	d value	r value
M	Median point	M 00	1.0
m	median region	m 0.0 – 2.5	1.01 -1.7
Sm	Submedian region	Sm 2.5-5.0	1.7-3.0
St	subterminal region	st 5.0-7.5	3.0-7.0
t(a)	terminal region	t 7.5-10	7.0-00
T(a)	Terminal point	T 10-0	00

Based on the nomenclature, the karyotypic difference within and between the *Clarias* species were ascertained.

RESULTS

Cytogenetic Variations among four *Clarias* Species from Anambra River, Nigeria: Tables 2 and 3 illustrates the modal chromosomes and karyotype morphology distribution respectively among the *Clarias* species of Anambra river. The modal metaphase chromosome numbers were 2n = 48 in *C. ebriensis,* 2n = 48 in *C. albopunctatus,* 2n = 56 in *C. gariepinus* and 2n = 56 in *C. anguillaris*. They were characterized by a high proportion of meta-submetacentric chromosomes and low proportion of acrocentric chromosomes. All female karyotypes exhibited a heteromorphic pair suspected to be sex chromosome complex. The following formulae were established for the male clariids; *C. ebriensis* 6m + 22sm + 20a FN =76; *C. albopunctatus* 4m ± 22sm + 22a FN = 74; *C. gariepinus* 8m + 24a FN = 88; and *C. anguillaris* 8m + 26m + 22a FN = 90. The female karyotype morphologies were *C. ebriensis* 6m + 23sm + 19a FN = 77, *C. albopunctatus* 4m ± 23sm + 21a FN = 75, C*. gariepinus* 8m + 25sm + 23a FN = 89 and *C. anguillaris* 8m + 27sm + 21a FN = 91. Furthermore, figures 1 – 4 displays representative metaphase chromosomes of males and females *Clarias* species from Anambra River, Nigeria.

The karyotype distributions and idiograms were developed based on chromosome nomenclature of *Clarias* species using their centromeric indices and presented on figures 5 and 6, 7 and 8, 9 and 10 and 11 and 12 for males *C. ebriensis, C. albopunctatus, C. gariepinus* and *C. anguillaris* respectively. In *C. ebriensis,* six (6) chromosomal length distributions occurred with modal chromosome percentage total length of 7.00 %. Seventy five percent of the modal chromosomes were submedian chromosomes while 12.50 % were both median and terminal chromosomes (Figure 5 and 6).

In *C. albopunctatus,* the chromosome total length percentage distributions exhibiting bimodal length peaks of 9.01 % made up of 50.00 % submedian and 50.00 % terminal chromosomes; and 7.50 % made up of 100% submedian chromosomes (Figures 7 and 8). *C. gariepinus* exhibited chromosome total length percentage distributions of 8.37 % and 3.90 % with modal chromosome percentage length of 5.58 %. Fifty-seven percentage (57.00 %) of the modal chromosome percentage total length distribution were submedian chromosomes while 29.00 % were terminal chromosomes with only one (14.00 %) median chromosome (Figure 9 and 10). Similarly, *C. anguillaris* exhibits chromosome total length percentage distribution of 8.80 % to 4.40 % with bimodal chromosome percentage total length of 6.60 %, made up of 40.00 % median

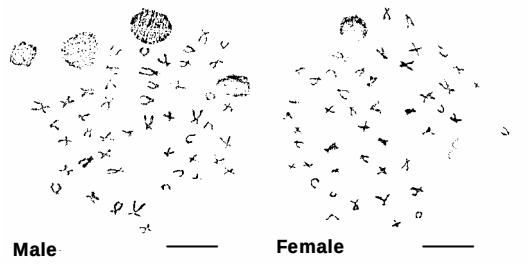

Figure 1: Metaphase chromosomes of *Clarias ebriensis* from Anambra river, Nigeria (bar = 10μ)

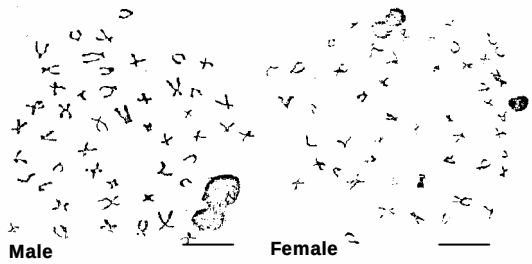

Figure 2: Metaphase chromosomes of *Clarias albopunctatus* from Anambra river, Nigeria (bar = 10μ)

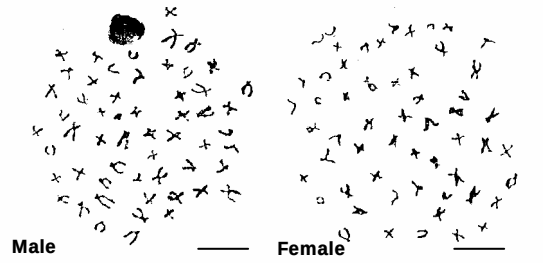

Figure 3: Metaphase chromosomes of *Clarias gariepinus* from Anambra river, Nigeria (bar = 10μ)

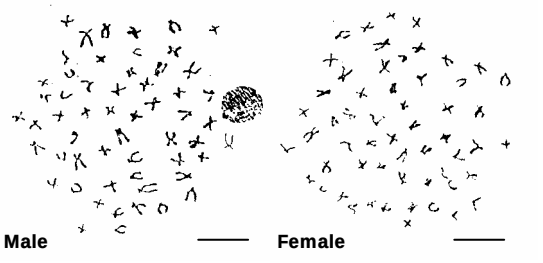

Figure 4: Metaphase chromosomes of *Clarias anguillaris* from Anambra river, Nigeria (bar = 10μ)

chromosomes and 60.00 % terminal chromosomes; and 5.52 %, made up of 100 % submedian chromosomes. The chromosome total length has been categorized into 13 length groups (Figures 11 and 12).

Table 2: Frequency distribution of the diploid chromosome number among *Clarias* species from Anambra River, Nigeria

Species	No. of slides scanned			Diploid chromosome number									
	Male	Female	Total	48	49	50	51	52	53	54	55	56	57	58
C. ebriensis	4	3	7	17	6	-	1	5	8	8	-	10	1	-
C. albopunctatus	2	3	5	15	2	3	2	8	-	14	8	2	-	-
C. gariepinus	4	3	7	7	-	3	3	2	-	10	-	11	-	3
C. anguillaris	2	4	6	6	3	3	-	4	4	6	-	8	-	3

Table 3: Karyotype distribution among *Clarias* species from Anambra River, Nigeria

Species	Karyotype			
	Male	FN	Female	FN
C. ebriensis	6m + 22sm + 20a	76	6m + 23sm + 19a	77
C. albopunctatus	4m + 22sm + 22a	74	4m + 23sm + 21a	75
C. gariepinus	8m + 24sm + 24a	88	8m + 25sm + 23a	89
C. anguillaris	8m + 26sm + 22a	90	8m + 27sm + 21a	19

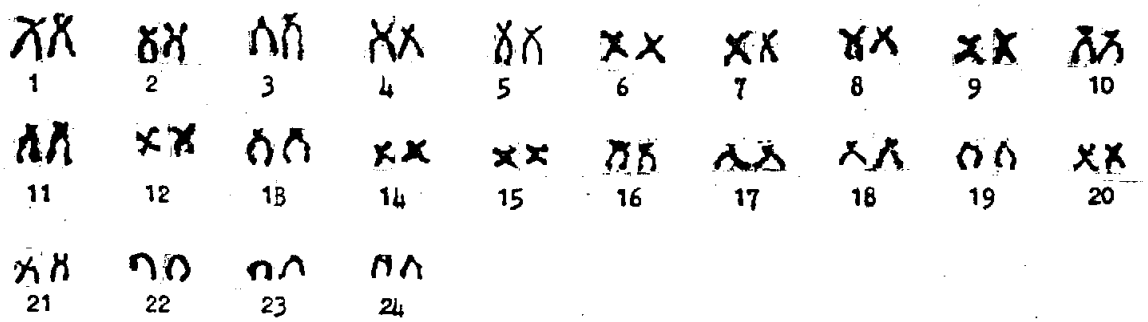

Figure 5: Karyotype of *Clarias ebriensis* from Anambra river, Nigeria

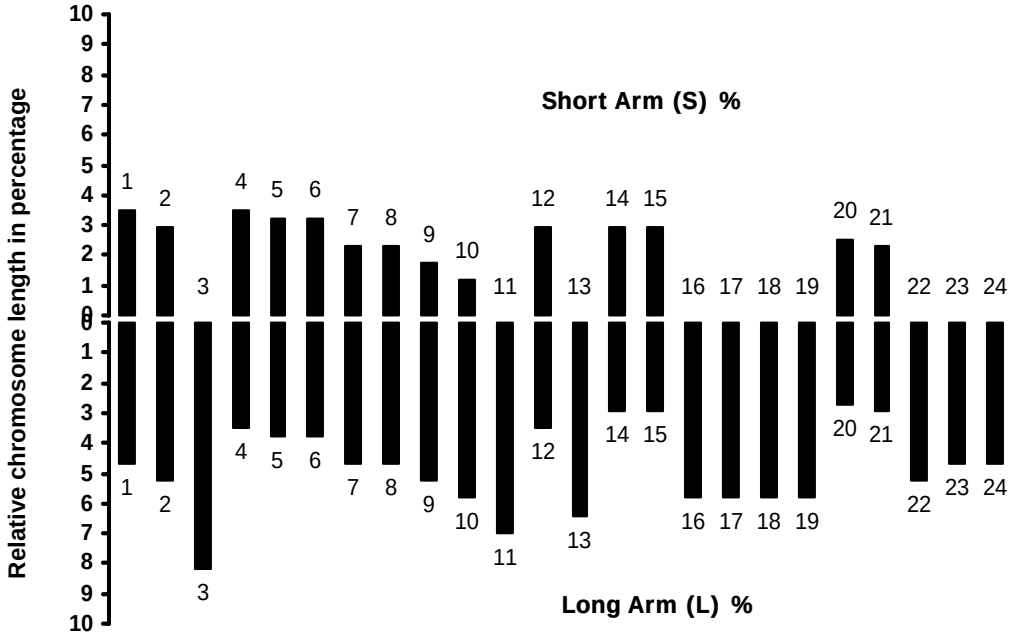

Figure 6: An idiogram of the karyotype of male *Clarias ebriensis* showing the morphology of the chromosomes. The 0 represents the position of the centromere

DISCUSSION

The chromosome number of *Clarias* species of Anambra river, varied from 2n = 48 to 2n = 56 with modal chromosome number of 2n = 56 in *C. gariepinus* and *C. anguillaris.* The observed range and modal chromosome number is in agreement with Ozouf-Costaz *et al.* (1990) for the clariids.

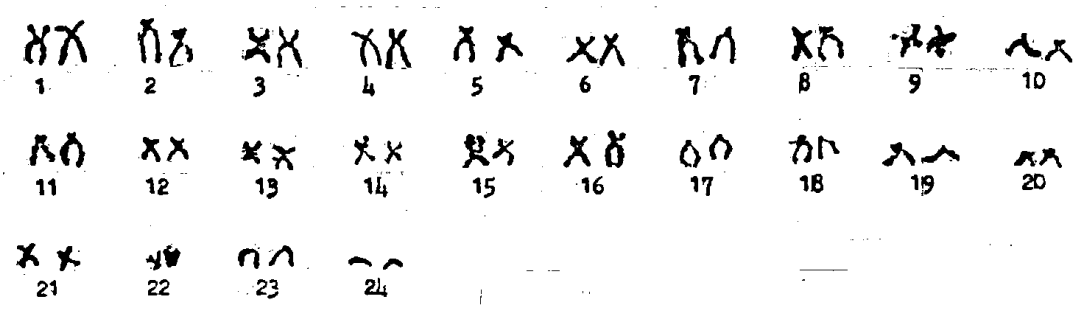

Figure 7: Karyotype of *Clarias albopunctatus* from Anambra river, Nigeria

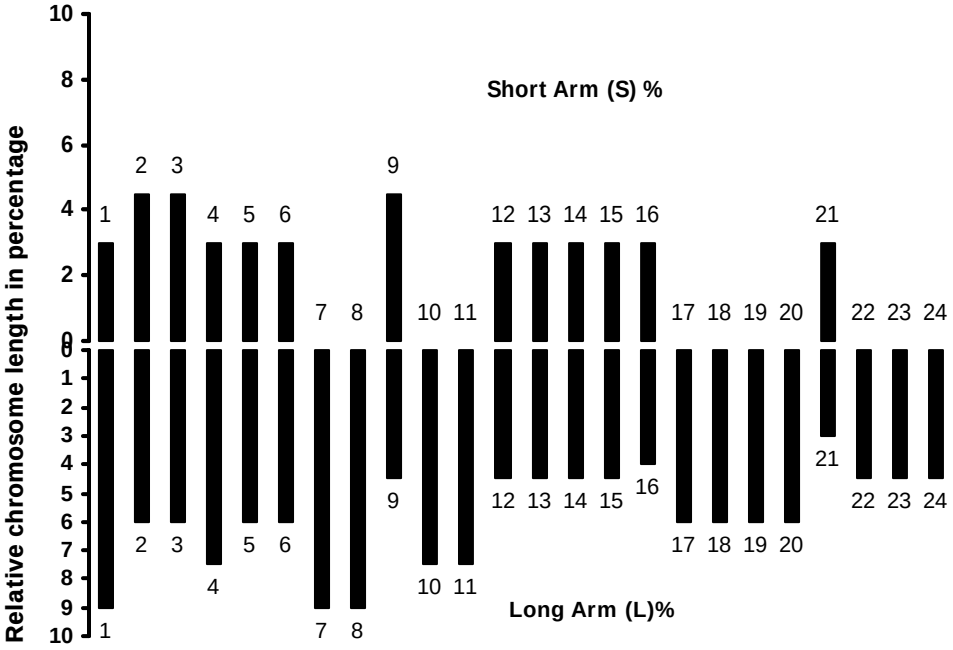

Figure 8: An idiogram of the karyotype of male *Clarias albopunctatus* showing the morphology of the chromosomes. The 0 represents the position of the centromere

Teugels *et al.* (1992) reported 2n = 52 for *Heterobranchus longifilis* Valenciennes, 1840 and 2n = 54 in the hybrid of *C. gariepinus* vs. *H. longifillis.* The result of the present studies indicated that the karyotypes consisted more of submedian chromosomes in *C. ebriensis* and *C. anguillaris* and almost equal number of submedian and terminal chromosomes in *C. gariepinus* and *C. albopunctatus* in which the centromeres were clearly defined. Differences in chromosome sizes were observed, but were very gradual and at no point were the variations greater than error involved in the method of measurement. Similar karyotypes were described for *C. gariepinus* (Ozouf-Costaz *et al.,* 1990) and *H. longifilis* (Teugels *et al.,* 1992) strains used for aquaculture. The observed chromosomal divergence between these clariids species was consistent with their morphological differences (Eyo, 2003). The almost uniformity of chromosome number and morphology within the clariids may also suggest success with which many species will hybridize. Crosses between clariids (*C. gariepinus* vs *H longifilis*) using artificial fertilization method have been reported (Teugels *et al.*, 1992). In nature, the occurrence of chromosome number around modal values among the clariids may suggest that chromosomal changes may be associated with the process of speciation within the group, possibly through high rate of hybridization resulting from communal spawning. Information on the chromosome number among siluriform fishes indicated that diploid chromosome number for catfishes range from the mid-20's to well over 100 with most species having a diploid set in the mid-40's to upper 100's, but no clear modal number have been suggested. An increase in diploid chromosome number is associated with a concomitant change in arm number. In the study of chromosome number among the siluriform fishes, Fitzsimons *et al.* (1988) noted that a diploid chromosome number of 56 ± 2 was widespread

Figure 9: Karyotype of *Clarias gariepinus* from Anambra river, Nigeria

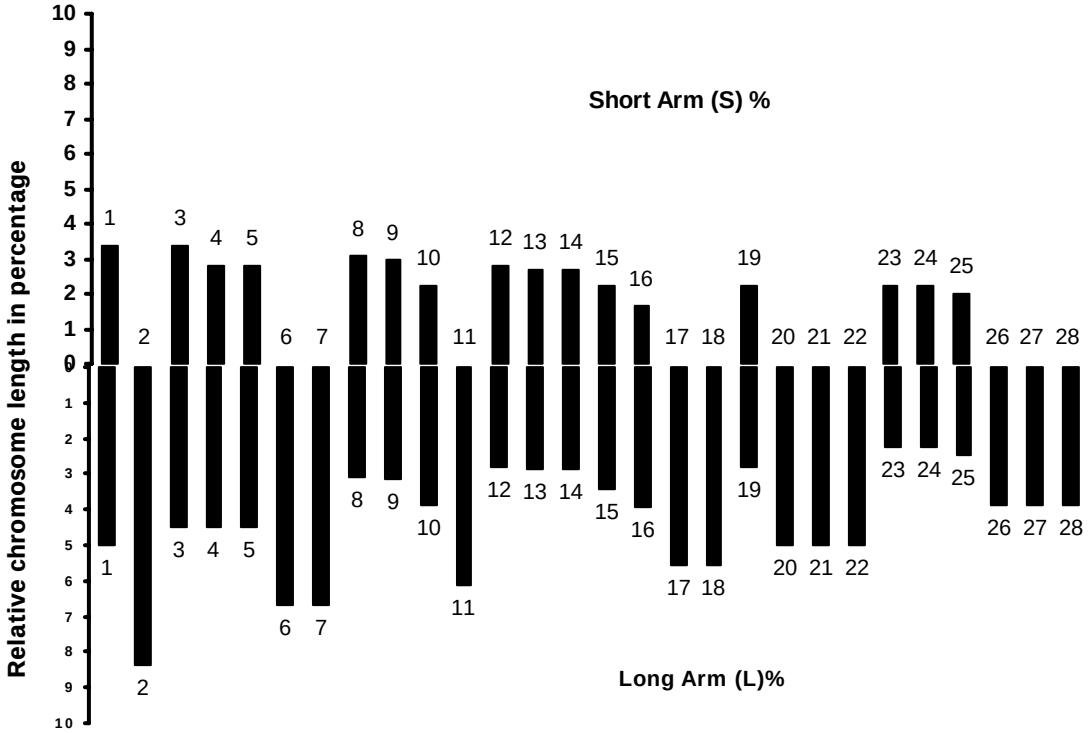

Figure 10: An idiogram of the karyotype of male *Clarias gariepinus* showing the morphology of the chromosomes. The 0 represents the position of the centromere

among 70 species of catfishes in 10 families and was especially frequent in four families, the Ariidae, Bagridae, Ictaluridae and Pimelodidae. The observed range for the clariids 2n = 48 to 2n = 56 fall below the speculated 2n = 56 ± 2 range of Fitzsimons *et al.* (1988). Thus the range for the clariids may be put at 2n = 52 ± 4. The observed similarities in chromosome number although with almost similar morphometric and meristic characters may explain the placement of both *C. anguillaris* and *C. gariepinus* in the *subgenus Clarias (Clarias*) by Teugels (1982). The disparity in chromosome number (Eyo, 1997) along with dissimilar morphometric (Eyo, 2002; 2003) and meristic (Eyo, 2004b) characters of *C. ebriensis* and *C. albopunctatus* from *C. gariepinus* and *C. anguillaris* may equally explain the placement of *C. ebriensis* and *C. albopunctatus* in the subgenera *Clarias (Anguilloclarias)* and *Clarias (Clarioides)* respectively.

Karyological evidences have been employed in solving problems relating to chromosome number, functional arm, phyletic relationship, the taxonomic status as well as possibility of speciation among the studied *Clarias* species. For instance the wide dispersal of chromosome number around modal value (2n = 56) among the clariids suggested possibilities of the species undergoing speciation.

Conclusively, the grouping of *Clarias* either as macroclarias or as megaclarias based on similarities in size and morphological feature should be adopted as a stock management tool. The key diagnostic characters (Eyo, 2003) as well as the relationship between standard length and morphometric characters (Eyo and Inyang, 2003) may be adopted by clariid taxonomist.

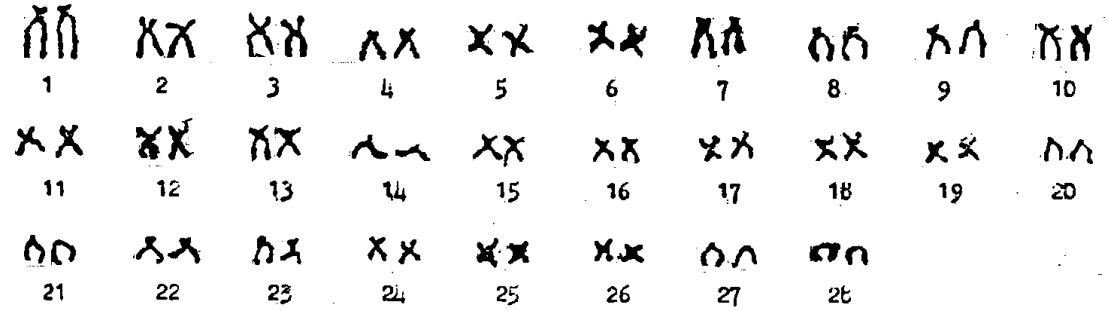

Figure 11: Karyotype of *Clarias anguillaris* from Anambra river, Nigeria

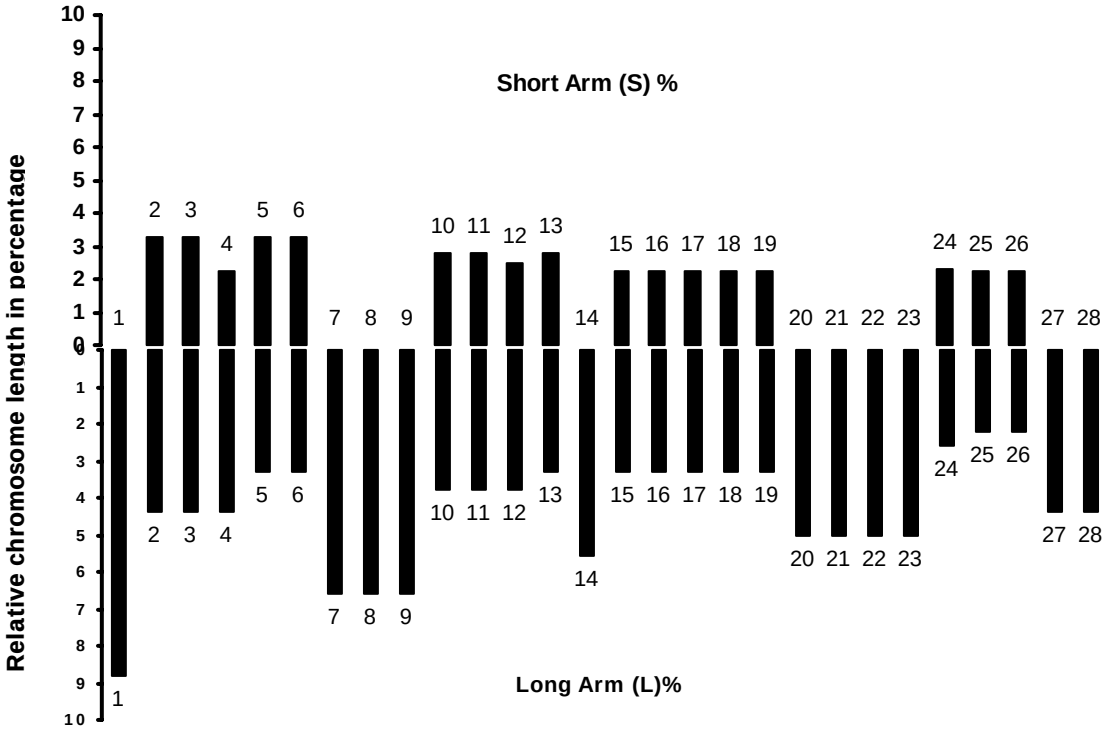

Figure 12: An idiogram of the karyotype of male Clarias anguillaris showing the morphology of the chromosomes. The 0 represents the position of the centromere

The cytotaxonomy of the clariids places them into two chromosomal number types; 2n = 56 represented by C*. anguillaris* and *C. gariepinus,* and 2n < 56 as in *C. ebriensis* and *C. albopunctatus* justifies the managerial designation of the clariids into megaclarias and macroclarias.

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REFERENCES

BLAXHALL, P. C. (1981). A comparison of methods used for the separation of fish lymphocytes. *Journal of Fish Biology, 18:* 177 – 181.

EYO, J. E. (1997). *Morphometric and cytogenetic variations among Clarias species (Clariidae) in Anambra river, Nigeria.* PhD Thesis, Department of Zoology, University of Nigeria, Nsukka. 267 pp.

EYO, J. E. (2002). Conspecific discrimination in ratio morphometric characters among members of the Pisces Genus*: Clarias*

Scopoli, 1777. *The Zoologist, 1(2):* 23 – 34.

EYO, J. E. (2003). Congeneric discrimination of morphometric characters among members of the Pisces Genus *Clarias* (Clariidae) in Anambra River, Nigeria. *The Zoologist, 2(1):* 86 – 103.

EYO, J. E. (2004a). The Effects of Substituting Soyabean Meal for Breadfruit Meal on Diets Acceptability, Growth Response and Cost of Diets Fed to *Heterobranchus bidorsalis* (♂) X *Clarias gariepinus* (♀) Hybrid. *Bio-Research*, *2(2):* 27 – 33.

EYO, J. E. (2004b). Differences in meristic counts of the genus *Clarias* (Pisces: Clariidae) in Anambra river, Nigeria. *Animal Research International, 1(1):* 31 – 35.

EYO, J. E. and EZECHIE, C. U. (2004). The Effects of Rubber (*Havea brasiliensis*) Seed Meal Based Diets on Diet Acceptability and Growth Performance of *Heterobranchus bidorsalis* (♂) *X Clarias gariepinus (♀)* Hybrid. *Journal of Sustainable Tropical Agriculture Research*, *10:*

EYO, J. E. and INYANG, N. M. (2003). Regression Model and *Clarias* species (Clariidae – Scopoli 1777) Taxonomy. *Journal of Science and Technology Research, 2(3): 40 – 49.*

EZENWAJI, H. M. G. (1989). Aspects *of the Biology of some "small" Clarias species in Anambra River Basin, Nigeria.* PhD Thesis, University of Nigeria, Nsukka. 244 pp.

FELDBERG, E. and BERTOLLO, L. A. C. (1985). Karyotypes of 10 species of Neotropical cichlids (Pisces: Perciformes). *Caryologia, 38 (3 - 4):* 257 - 268.

FELBERG, E., BERTOLLO, L. A. C., TOLEDO, L. F. A., FORESTI, F., FICHO, O. M. and SANTOS, A. F. (1987). Biological aspects of Amazonian fish IX. Cytogenetic studies in two species of the genus *Semaprochilodous* (Pisces, Prochilodon-tidae). *Genome, 29:* 1 – 4.

FITZSIMONS, J. M., LEGRANDE, W. H. and KERTH, J. W. (1988). Karyology of the marine catfish Bagre marinus (Ariidae) with an analysis of chromosome numbers among Siluriforms fishes. *Japanese Journal of Ichthyology, 35(2):* 189 – 193.

FUNAKI, K., MATSAI, S. and SASAKI, M. (1975). Location of nucleolar organizers in animal and plant chromosomes by means of an improved N-banding technique. *Chromosoma, 22(4):* 223 – 236.

GOSLINE, W. A. (1975). The palatine-maxillary mechanisms in catfishes, with comments on the evolution and zoogeography of modern Siluriods. *Occasional Papers, California Academy of Science, 120:* 1 – 31.

HAAF, T. and SCHMID, M. (1984). An early stage of ZW/ZZ sex chromosomes differentiation in *Poecilia sphenops* var *melanistica* (Poeciliidae, Cyprinodontiformes). *Chromosoma, 89:* 37 – 41.

HARTHEY, S. E. (1987). The chromosomes of salmonid fishes. *Biological Reviews, 62:* 197 – 214.

HOWEL, W. M. and BLACK, D. A. (1979). Location of the nucleolus organizer regions on the sex chromosomes of the banded killifish, *Fundulus diaphanus. Copeia, 1979:* 544 – 546.

KALLMAN, K. D. (1973). The sex-determining mechanism of the platyfish *Xyphophorus maculatus.* Pages 19 – 28. *In:* SCHRODER, J. H. (Ed). Genetics and mutagenesis of fish. Springer-Verlag, Berlin.

KLIGERMAN, A. D. and BLOOM, S. E. (1977). Rapid chromosome preparations from solid tissues of fishes. *Journal of Fishery Research Board of Canada, 34:* 266 – 269.

KORNFIELD, I, L., RICHLER, U. R. and WAHRMAN, J. (1979). Biochemical and cytogenetical differentiation among cichlid fishes of the sea of Galilee. *Evolution, 33:* 1 – 14.

LAGLER, F. K., BARDACH, I. E., MILLER, R. R. and MAY PASSINO, D. R. (1977). *Ichthyology.* Second Edition, John Wiley and Sons, New York. 506 pp.

LEGRANDE, W. H. (1981). Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae) with particular emphasis on the madtones, *Nototus*. *Copeia, 1:* 33 – 52.

LEVAN, A., FREGDA, K. and SANDBURG, A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas, 52:* 201 – 220.

LOWE-MCCONNELL, R. H. (1972). *Freshwater Fishes of the Volta and Kainji lakes.* Ghana University Press, Accra.

MAYR, E. (1969). *Principles of systematic zoology.* THM Edition, McGraw-Hill Coy, Ltd. Bombay. 428 pp.

MELLMAN, W. J. (1965). Human peripheral blood leucocyte cultures. Pages 22 – 56. *In:* YUNIS, J. J. (Ed). *Human Chromosomes Methodology.* Academic Press, New York.

OHNO, S., MURAMOTO, J., KLEIN, and ATKIN, N. B. (1969). Diploid tetraploid relationship in clupeoid and salmonoid fish. Pages 139 – 147. *In:* DARLINGTON, C. D. and LEWIS, K. R. (Eds.). *Chromosomes Today,* Volume 2, Oliver and Boyd, Edinburgh.

OJIMA, Y. and YAMANO, T. (1980). The assignment of the nuclear organizer in the chromosomes of the fauna (Carassius,

Cyprinidae, Pisces). *Proceedings of the Japanese Academic of Sciences, 56B:* 551 – 556.

OLIVERIA, C., ALMEIDA TOLEDO, L. F., FOREST BRISKI, H. A. and TOLEDO FILHO, S. A. (1988). Chromosome formulae of Neotropical freshwater fishes. *Review of Brazilian Geneticist, 11(3):* 577 – 624.

OZOUF-COSTAZ, C., TEUGELS, G. G. and LEGENDRE, M. (1990). Karyological analysis of three stains of the African catfish *Clarias gariepinus* Clariidae used in aquaculture. *Aquaculture, 87:* 271 – 277.

PREHN. L. M. and RASCH, E. M. (1969). Cytogenetic studies of *Poecilia* (Pisces). 1. Chromosome numbers of naturally occurring poeciliid species and their hybrids from eastern Mexico. *Canadian Journal of Genetics and Cytology, 11:* 880 – 895.

RIGAS, D. A. and OSGOOD, E. E. (1954). Purification and properties of the phytohemagglutinin of *Phaseolus vulgaris*. *Journal of Biological Chemistry, 212:* 607 – 609.

SOLA, L., CATAUDELLA, S. and CAPANNA, E. (1981). New developments in vertebrate cytotaxonomy III. Karyology of bony fishes: A Review. *Genetica, 54:* 285 – 328.

SYDENHAM, D. H. J. (1983). A key to the genus *Clarias* in Nigeria. *Memograph of the Department of Zoology, University of Ibadan, Nigeria.* 2 pp.

TEUGELS, G. G. (1982). A systematic outline of the African species of the genus *Clarias* (Pisces, Clariidae) with annotated biography. *Annales du musee Royal de L' Afrique Centrale, Tervuren, Sciences Zoologyques.* 230 pp.

TEUGELS, G. G., OZOUF-COSTAZ, C., LEGENDRE, M. and PARENT, M. (1992). A karyological analysis of the artificial hybridization between *Clarias gariepinus* Burchell, 1822 and *Heterobranchus longifilis* Valenciennes. 1840 (Pisces: Clariidae). *Journal of Fish Biology, 40:* 81 – 86.

THORGAARD, G. H. (1977). Heteromorphic sex chromosomes in male rainbow trout. *Science, 196:* 900 – 902.

UWA, H. (1986). Karyotype evolution and geographical distribution in the ricefish, genus Oryzias (Oryziidae). Pages 867 - 876. *In:* UYENO, T., ARAI, R., TANIUCHI T. and MATSUURA, K. (eds.) *Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes.* Ichthyological Society of Japan, Tokyo.

UWA, H. and IWATA, A. (1981). Karyotype and cellular DNA content of *Oryzias javanicus* (Oryziidae, Pisces). *Chromosomes Information Service (CIS), 31:* 24 – 26.

UWA, H. and MAGTOON, W. (1986). Description and karyotype of a new rice fish, *Oryzias mekongensis,* from Thailand. *Copeia, 1986(2):* 473 – 478.

UWA, H. and PARENTI, L. R. (1988). Morphometric and meristic variation in ricefish, genus Oryzias: a comparison with cytogenetic data. *Japanese Journal of Ichthyology, 35(2):* 159 – 166.

UWA, H., TANAKA, K. and FORMACION, M. J. (1982). Karyotype and banding analyses of the Hawaiian medaka *Oryzias curvinotus* (Pisces). *Chromosome Information Service (CIS), 33:* 15 – 17.

UWA, H., WANG, R. and CHEN, Y. (1988). Karyotypes and geographical distribution of ricefishes from Yunnan, South Western China. *Japanese Journal of Ichthyology, 35(3):* 332 – 340.

WHITE, M. J. D. (1978). *Modes of speciation.* Freeman and Company, San Francisco. 187 pp.

WOLF, U., RITTER, H., AKIN, N. and OHNO, S. (1969). Polyploidization in the fish family Cyprinidae, order Cypriniformes. I. DNA content and chromosome set in various species of Cyprinidae. *Humangenetik, 7:* 240 – 244.

YU, X., ZHOU, T., LI. K. and ZHOU, M. (1987). On the karyosystematics of cyprinid fishes and a summary of fish chromosomal studies in China. *Genetica, 72:* 225 – 236.