Karyotype and DNA content of *Phractolaemus ansorgei* Blgr. (Teleostei: Gonorynchiformes)[1]

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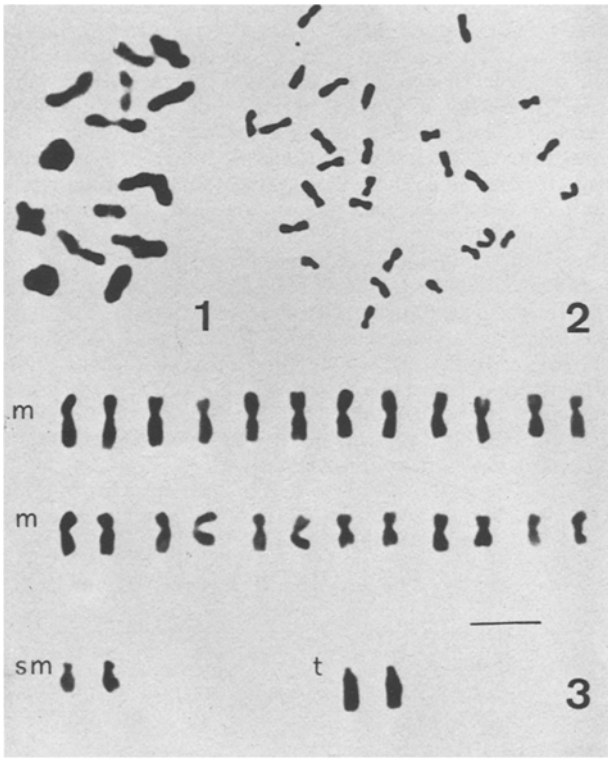
Summary. The diploid DNA content of *Phractolaemus ansorgei spinosus* (Phractolaemidae) is estimated by Feulgen cytophotometry at 3 pg. The karyotype has 28 chromosomes and 54 chromosome arms; it significantly resembles the karyotype of *Chanos chanos* (Chanidae).

P. ansorgei Boulenger, 1901, differentiated in 2 subspecies, is probably the only living species of the teleostean fish family Phractolaemidae[2]. This family has had a checkered taxonomic history[2,3]; relationship with such divergent families as Osteoglossidae, Clupeidae, Cyprinidae and Chanidae has been suggested. Recently, Phractolaemidae were placed with Chanidae, Kneriidae and Gonorynchidae within the Gonorynchiformes[3]. Gosline[4] included gonorynchiform fishes in his order Clupeiformes, but others[5,6] placed them in the Ostariophysi.

In teleostean fishes chromosome numbers, DNA content and taxonomic position are correlated[7]. Only one gonorynchiform species, *C. chanos,* has been previously karyotyped[8]. This paper is the first report of the karyotype and DNA content of *P. ansorgei.*

Materials and methods. 3 males and 2 females of *P. ansorgei spinosus* Pellegrin, 1926, were collected in forest pools at Mpaha, west of lake Tumba, in the central basin of Zaïre. Chromosome preparations were obtained by air-drying cells from gill, kidney and testis[9]. For each specimen, 10 metaphase spreads were analyzed. Nomenclature for centromeric position on chromosomes - determined from arm ratio (long arm/short arm) - follows Levan et al.[10]. Nuclear DNA content was determined from erythrocytes by Feulgen cytophotometry in a Zeiss U.M.P.S. I[11]. Air-dried blood films were fixed in ethanol:acetic acid (3:1) for 30 min, hydrolized in 1 N HCl at 60 °C for 6 min and Feulgen stained following the procedure of de Tomasi[12]. Blood films of 2 fish species with known DNA content, *Ophiocephalus obscurus* and *Parauchenoglanis guttatus,* captured in the same pools as *P. ansorgei spinosus,* were also treated at every stage for use as standards. For each species, 4 preparations were examined, 20 cells from each preparation.



Chromosomes of *Phractolaemus ansorgei spinosus.* 1: Primary meiotic division from testis (n = 14). 2: Metaphase spread from gill epithelium (2n = 28). 3: Karyotype prepared from 2; abbreviations for centromeric position: m (median), sm (submedian), t (terminal); bar = 5 µm.

Results. The haploid chromosome number determined from testis tissue was 14 (figure, 1). Preparations from gill and kidney produced only diploid counts of 28 chromosomes (figure, 2). The diploid complement (figure, 3) consisted of 24 chromosomes with median centromeres (arm ratios ranging from 1 to 1.7), 2 small chromosomes with submedian centromeres (arm ratios about 2.8) and 2 chromosomes with nearly terminal centromeres (arm ratios about 8). The number of chromosome arms was 54. No chromosomal sexual dimorphism was noted.

The mean extinction values, in arbitrary units, with their SE, were as follows: *P. ansorgei spinosus:* 2.16±0.038, *O. obscurus:* 1.37±0.040, *P. guttatus:* 1.55±0.034. Accepting 2 pg DNA per erythrocyte nucleus for *O. obscurus* and 2.2 pg for *P. guttatus*[7], a value of 3 pg was calculated for *P. ansorgei spinosus.*

Discussion. The diploid DNA value of *P. ansorgei spinosus* (3 pg) is higher than the mode for teleostean fishes (2 pg) and also higher than all values known for Osteoglossomorpha but falls within the range of values known for Clupeomorpha, Ostariophysi and other major fish groups[7]. DNA values of other gonorynchiform fishes are not available for comparison. The karyotype of *P. ansorgei spinosus* (with 2n = 28 and 54 chromosome arms) differs greatly from the 'modal' teleostean karyotype which has 2n = 48 and 48 chromosome arms[13,14], but resembles the karyotype of *C. chanos* which has 2n = 32 with 50 chromosome arms[8]. Arai[8] suggested that the karyotype of *C. chanos* could be derived from 50 one-armed chromosomes by centric fusions. More fusions could reduce the 2n to 28 found in *P. ansorgei spinosus.* To explain the difference in arm number between *C. chanos* and *P. ansorgei spinosus,* other mechanisms, e.g. pericentric inversions in 2 pairs of one-armed chromosomes, must be assumed. The probability of a coincidental resemblance is low because a $2n \leq 32$ is rare in the various fish groups suggested to be related to Phractolaemidae. Indeed, for Clupeomorpha, Elopomorpha and Osteoglossomorpha no 2n below 38 were reported[7,15]; for Ostariophysi, where 2n numbers of more than 230 species are reported, only 3 species - one characoid[16] and 2 gymnotoids[7,17] - were found with $2n \leq 32$.

Thus cytogenetic analysis confirms the close relationship between Chanidae and Phractolaemidae indicated by anatomical studies[18,19]. The more derived karyotype condition of *P. ansorgei spinosus* with respect to *C. chanos* agrees with placement of Phractolaemidae after Chanidae within the order Gonorynchiformes[3].

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Biodegradability of Tioctilate

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Summary. The biodegradability of Tioctilate (octylthiobenzoate), a new pesticide, has been examined by means of 5 tests. The compound appears to be susceptible to microbial metabolism.

Tioctilate (octylthiobenzoate) has recently been established as an efficient chemical to eradicate mites, lice and even *Trichophyton.* The compound appears to have a very low oral toxicity (LD_{50} for rats = 5.9 g/kg) and is little or not resorbed through the skin. The purpose of this work was to examine the microbial degradability of Tioctilate in order to evaluate its environmental acceptability.

Materials and method. Tioctilate is a colourless liquid, slightly soluble in water (2 mg/l at 20 °C), stable towards diluted acids and alkali. Water samples are extracted with heptane, the organic layer is evaporated under reduced pressure to dryness and the residue is redissolved in a known quantity of heptane. The latter solution is subsequently analyzed by gas chromatography under the following conditions: column: ⌀ ⅛", 5 foot length, SE 30 10% on chromosorb W-HP 80–100 mesh, 220 °C; carrier gas: nitrogen 30 ml/min; FID; retention time: 7 min.

The biological oxygen demand (BOD_5^{20}-test) was determined by means of the conventional bottle test[2]. The other 4 tests were performed as described in detail by Voets et al.[3]. The 2 aerobic tests were performed according to the OCDE-methods for the biodegradability testing of detergents In the minimal test (MM-test), the biocide is dissolved in a mineral solution with the following composition (g/l): a) KH_2PO_4, 8.5; K_2HPO_4, 21.75; $Na_2HPO_4 \cdot 2H_2O$, 33.4; NH_4Cl, 1.7; b) $MgSO_4 \cdot 7H_2O$, 22.5; c) $CaCl_2$, 27.5; d) $FeCl_3 \cdot 6H_2O$, 0.25. To prepare 1 l of the MM-medium, add 1 ml of each of the solutions a–d to 1 l of distilled water. 1 l of this solution was incubated in a 2.0 l Erlenmeyer flask on a rotary shaker (120 rpm). The flask was inoculated with 1.0 ml of soil extract and incubated open to the air. To prepare the soil extract, 10 g of a fertile field soil were suspended in 100 ml tap water and gently mixed. The suspension was filtered through a 595-Whatman paper filter, the filtrate being used as inoculum. Evaporation losses were regularly adjusted with distilled water. To detect losses of volatile substances, a control flask containing the sterile-filtered biocide solution was also incubated. The synthetic sewage had the following composition (mg/l): peptone, 160; meat extract, 110; urea, 30; NaCl, 7; $CaCl_2 \cdot 2H_2O$, 4; $MgSO_4 \cdot 7H_2O$, 2; tap water 1 l. The synthetic sewage passed through the aeration vessel at a rate of 1 l/h. To start up the activated sludge, the apparatus was fed during a 2-week period with synthetic sewage devoid of biocides. During the actual trials, the concentration of the test chemical was monitored daily in the influent and effluent. Since the test measures the biodegradation of the biocide in a complex organic medium, it is referred to as Organic Medium-test (OM-test).

The media used in the 2 anaerobic tests were the same. However, in the anaerobic MM-test, a 1-l Erlenmeyer flask was filled up to the rim with the MM-solution. The flask was subsequently incubated at 22 °C in an anaerobic chamber (BBL gaspack jar). In the anaerobic OM-test, 1 l of the OM-medium was inoculated with 1 ml of soil extract and incubated in the anaerobic chamber. The chemical oxygen demand (COD) was measured by the dichromate method according to the Standard Methods[5].

Results. The BOD_5^{20}-test revealed that an aqueous solution of Tioctilate with an initial COD of 4.70 mg/l, had a net

Die-away of Tioctilate in mineral medium under aerobic conditions

Days	Percentage remaining					
	Inoculated flasks			Sterile control		
	A	B	Average	A	B	Average
0	100*	100	100	100	100	100
5	1.7	5.5	3.6	–	–	–
10	2.9	2.2	2.5	–	–	–
15	2.5	0.4	1.4	140	65	102
20	0.1	–	0.1	144	72	108

* The percentages refer to the value detected at the onset of the die-away test.