Research Article | Science Technology and Engineering Journal (STEJ) Vol.7, No.1 pages 40-48

Chromosome Study of Slender Walking Catfish (*Clarias nieuhofii*) and African Catfish (*C. gariepinus*) F_1 Hybrid: Revealed by Conventional Staining and Ag-NOR Banding Techniques

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(Received: 3rd January 2021, Revised: 17th May 2021, Accepted: 19th May 2021)

Abstract-The hybrid catfishes *Clarias nieuhofii* (♀) × *C. gariepinus* (♂) were cytogenetically studied using conventional staining and Ag-NOR banding techniques, with the aim to reveal the chromosome complement and chromosome marker of the hybrid. Specimens were kindly provided by faculty of Agricultural Technology, Songkhla Rajabhat University, Songkhla, Thailand. Mitotic chromosomes were prepared from kidney follow that of the standard protocol. The result showed that hybrid catfishes had chromosome diploid of $2n$=61. An intermediate chromosomal pattern incorporating those of the parental species was found in the probable hybrid, confirming its interspecific origin. NORs were observed in one large submetacentric pair and one small subtelocentric pair. The number of diploid chromosome, chromosomal morphology and NOR locations are being cytogenetic evidence to prove that a hybrid occurred from *C. nieuhofii* × *C. gariepinus*.

Keywords: *C. nieuhofii*, *C. gariepinus*, hybrid catfish, karyotype

1. Introduction

Catfishes of the genus *Clarias* Scopoli, 1777 are characterized by their anguilliform body, long dorsal and anal fins, flattened bony head and a broad mouth with 4 pairs of barbels (Hee, 1990). The genus *Clarias* is the most diverse group 56 species within the moderately diverse Old World catfish family Clariidae (113 species in 16 genera) (Ferraris, 2007). Members of the genus are naturally distributed in inland water bodies in both Africa and Asia, with the bulk of the species being found in the former. Nineteen species of Southeast Asian *Clarias* are currently recognized (Ng, 2004). The African catfish (*C. gariepinus*) is considered to have a rapid growth rate (in length and weight), the rate of which strongly depends on ambient conditions and habitat (Britz & Pienaar, 1992; Bruton & Allanson, 1980; Hecht & Appelbaum, 1987). Growth has been found to be positively density dependent (Hecht and Appelbaum, 1987). Individuals have been recorded to reach 200 mm SL within a year (Bruton and Allanson, 1980; Skelton, 2001). In females, the growth rate decreases after 3 years resulting in the males reaching larger sizes (Skelton, 2001). Individuals of this species are known to live for eight or more years (Bruton & Allanson, 1980).

The slender walking catfish (*C. nieuhofii*) command a higher market price compared to other *Clarias*, because of its good taste and stunningly attractive appearance. It is beautifully patterned and eel-like in motion. It is reddish brown in color, with 13 or 14 vertical rows of white spots, which make it a valuable species for aquariums in Thailand. However, their populations and density have decreased significantly during the last 2 decades, mainly due to overexploitation, reduction in habitat area as a result of the reclamation of peat swamp forests, and the injudicious application of insecticides in paddy fields. They have been observed to be rare, and were identified as a vulnerable species by IUCN red list categories and criteria (Pechsiri & Vanichanon, 2015). There have been more than 20 types of catfish hybrids attempted, hybrid catfish has been one of the most popularly culture freshwater fish species in Thailand (Srithong *et al*., 2015).

In *Clarias* species examined to date, diploid chromosome numbers range between $2n$=48 and $2n$=56, with the exception of *C. pachynema, C. nieuhofii* ($2n$=66) and one population of *C. batrachus* ($2n$=100) (Table 1). In this study, we observed remarkable chromosomal dynamism and chromosomal characteristics by conventional staining and Ag-NOR banding techniques. The result obtained not only confirmed the possibility hybridization of *C. nieuhofii* × *C. gariepinus*, but the basic genetic information obtained is also be used for developing of other hybrid catfish in the future.

2. Materials and Methods

2.1 Sample Collection

The *C. nieuhofii* is a native species which inhabit in swamp forest, crossbreeding between *C. nieuhofii* female and *C. gariepinus* male. Ten F_1 hybrid catfishes (five males and five females) were collected from Aquaculture program, Faculty of Agricultural Technology, Songkhla Rajabhat University, Thailand. All specimens were maintained in aerated, flowing freshwater aquaria until analysis.

2.2 Chromosome Preparation

Chromosomes were prepared *in vivo* (Phimphan *et al.*, 2013) as follows. Colchicine (0.1%) solution was injected into the fish's intramuscular and/or its abdominal cavity and then left for one hour. The kidneys were gently minced into small pieces in 0.075 M KCl and were then incubated for 30 min. After centrifugation at 1,200 rpm for 8 min, the cells suspension was fixed in freshly prepared fixative (3 methanol: 1 glacial acetic acid). The fixation step was repeated usually three times. After that the pellet was re-suspended with 1 ml of fixative. The cells were then dropped onto a clean slide and air-dried.

2.3 Chromosome Staining

Conventional staining was done using 20% Giemsa's solution for 30 min. Ag-NOR banding (Howell & Black, 1980) was performed by adding four drops of 50% silver nitrate followed by two drops of 2% gelatin on slides. The slides were then covered with a cover slip and incubated at 60 °C for 5 min. The slides were then washed in distilled water. After air-dried, the slides were stained with 20% Giemsa's solution for 1 min. Mitotic metaphases were observed under a light microscope and were photographed.

2.4 Chromosome Checks

The length of short arms (Ls) and long arms (Ll) of chromosomes were measured and calculated for the length of total arm chromosomes (LT, LT = Ls + Ll). Relative length (RL) and centromeric index (CI) were estimated. CI was also computed to classify the types of chromosomes according to Turpin and Lejeune (1965). Classification of microchromosomes is based on the presence of a size between groups of large chromosomes and smaller chromosomes, all the microchromosomes are very small are often less than 1.5 micrometers (Ezaz & Young, 2013). All parameters were used in karyotyping and idiograming.

This study was carried out in accordance with Ethical Principles and Guidelines for the Use of Animals, National Research Council of Thailand.

3. Results and Discussion

No differences of chromosome complement between male and female were observed in the hybrid catfishes (*C. nieuhofii* × *C. gariepinus*) (Figure 1C). Interestingly, from the metaphase chromosomes plates and karyotype (Figure 2 and 3), they showed a chromosome number of 2*n*=61 and a fundamental number (NF; number of chromosome arm) of 112 which comprised 19 metacentric, 17 submetacentric, 15 subtelocentric and 10 acrocentric chromosomes. Although chromosomal morphology of hybrid is similar to that of parental species, the diploid number is obviously different i.e., *C. nieuhofii* (Figure 1A) and *C. gariepinus* (Figure 1B) had the diploid of 2*n*=66 and 56, respectively (Table 1). Here, we proposed that the diploid chromosome number of the hybrid catfishes are basically assembled from the haploid set of gametes of *C. nieuhofii* (*n*=33) and *C. gariepinus* (*n*=28) resulting in the diploid chromosome number of 2*n*=61 in the hybrid. A half of genome hybridization was also found in other catfish, the natural hybrid from *C. gariepinus* and *C. macrocephalus* (2*n*=54) which produced a new hybrid of 2*n*=55 (Maneechot *et al.*, 2016).

Table 1. Review of available data on 2*n*, karyotypes and sex systems in the genus *Clarias*.

Species	2*n*	NF	Karyotype	Sex system	Locality	Reference
C. albopunctatus	48	75	4m+23sm+21a	ZW	Nigeria	(Eyo, 2005)
	48	74	4m+22sm+22a	ZZ	Nigeria	(Eyo, 2005)
C. anguillaris	56	91	8m+27sm+21a	ZW	Nigeria	(Eyo, 2005)
	56	90	8m+26sm+22a	ZZ	Nigeria	(Eyo, 2005)
	48	-	27m+10sm+3st+8t	-	Nigeria	(Wu *et al*., 1986)
	56	-	33m+12sm+2st+9t	-	Nigeria	(Wu *et al*., 1986)
C. batrachus	100	111	4m+7sm+77a+12m	XY	Thailand	(Cui *et al*., 1991)
	56	-	-	-	China	(Rishi, 1978)
	100	110	4m+6sm+78a+12mc	XY	Thailand	(Cui *et al*., 1991)
	50	88	16m+8sm+14st+12a	-	India	(Prasad, 1998)
	50	96	18m+20sm+8st+4a	-	India	(Hinegarder & Rosen, 1972)
	54	-	-	-	-	(Pandey & Lakra, 1997)
	50	89	16m+11sm+5st+1at	ZW	India	(Nagpure *et al*., 2000)
	50	88	16m+10sm+6st+18a	ZZ	India	(Nagpure *et al*., 2000)
	51	89	16m+11sm+5st+18a+1B-chromosome	ZW	India	(Nagpure *et al*., 2000)
	51	88	16m+10sm+6st+18a+1B-chromosome	ZZ	India	(Nagpure *et al*., 2000)
	50	96	12m+18sm+10st+10t	-	India	(Siraj *et al*., 2009)
	50	90	11m+7sm+2st+34a	-	Malaysia	(Yaqoob *et al*., 2012)
	54	74	12m+18sm+10st+14t	-	India	(Ozouf-Costaz *et al*.,1994)
	104	-	2m+4sm+98st/a	-	Thailand	(Maneechot *et al*., 2016)
C. camerunensis	54	-	-	-	Africa	(Luo *et al*., 1986)
	56	-	22m+20sm+9st+5t	-	Nigeria	(Wu *et al*., 1986)
C. ebriensis	50	-	-	-	Africa	(Luo *et al*., 1986)
	48	77	6m+23sm+19a	ZW	Nigeria	(Eyo, 2005)
	48	76	6m+22sm+20a	ZZ	Nigeria	(Eyo, 2005)
C. fuscus	56	106	18m+24sm+8st+6a	XX	China	(Cui *et al*., 1991)
	56	106	19m+23sm+8st+6a	XY	China	(Cui *et al*., 1991)
	56	106	20m+22sm+8st+6a	XX	China	(Yu *et al*., 1989)
	56	106	20m+22sm+8st+6a	XY	China	(Yu *et al*., 1989)
	56	102	18m+14sm+14st+10a	XX,XY	China	(Arai & Hirano, 1974)
	56	88	32m/sm+24st/a	-	Japan	(Luo *et al*., 1986)
C. gariepinus	56	89	8m+25sm+23a	ZW	Africa, Israel	(Teugels *et al*., 1992)

Table 1. Review of available data on 2*n*, karyotypes and sex systems in the genus *Clarias*. (cont.)

Species	2*n*	NF	Karyotype	Sex system	Locality	Reference
	56	88	8m+24sm+24a	ZZ	Africa, Israel	(Teugels *et al*., 1992)
	56	87	14m+17sm+25a	ZW	Egypt	(Siraj *et al*., 2009)
	56	88	14m+18sm+24a	ZZ	Egypt	(Siraj *et al*., 2009)
	56	102	20m+16sm+10st+10a	-	India	(Siraj *et al*., 2009)
	56	89	8m+25sm+23a	ZW	Nigeria	(Eyo, 2005)
	56	88	8m+24sm+24a	ZZ	Nigeria	(Eyo, 2005)
	56	96	21m+14sm+5st+16a	-	Malaysia	(Yaqoob *et al*., 2012)
	56	-	25m+14sm+14st+3t	-	Nigeria	(Wu *et al*., 1986)
	56	100	28m+6sm+10a+12t	-	Turkey	(Omotayo, 2012)
	56	100	24m+10sm+10a+12t	-	Turkey	(Omotayo, 2012)
	54	98	34m+10sm+10t	-	Nigeria	(Awodiran *et al*., 2014)
	56	102	6m+12sm+28st+10a	-	Nigeria	(Awodiran *et al*., 2014)
	56	98	30m+6sm+6st+14t	-	Thailand	(Donsakul & Magtoon, 1990)
	56	110	18m+20sm+16st+2a	-	Thailand	(Maneechot *et al*., 2016)
C. jaensis	54	-	22m+12sm+5st+15t	-	Nigeria	(Wu *et al*., 1986)
C. macrocephalus	54	104	24m+20sm+6st+4a	-	Thailand	(Cui *et al*., 1991)
	54	98	22m+18sm+4st+10a	-	Malaysia	(Yaqoob *et al*., 2012)
	54	104	22m+16sm+12st+4a	-	Thailand	(Maneechot *et al*., 2016)
C. macromystax	49	-	27 m+10sm+11st+1t	-	Nigeria	(Wu *et al*., 1986)
C. platycephalus	54	-	-	-	Africa	(Luo *et al*., 1986)
C. pachynema	66	-	30m+10sm+16st+10 t	-	Nigeria	(Wu *et al.,* 1986)
C. nieuhofii	66	94	19m +10sm+8st+30a	-	Thailand	(Donsakul & Magtoon, 1992)

Notes: *2n* = diploid chromosome, NF = fundamental number and - not available.

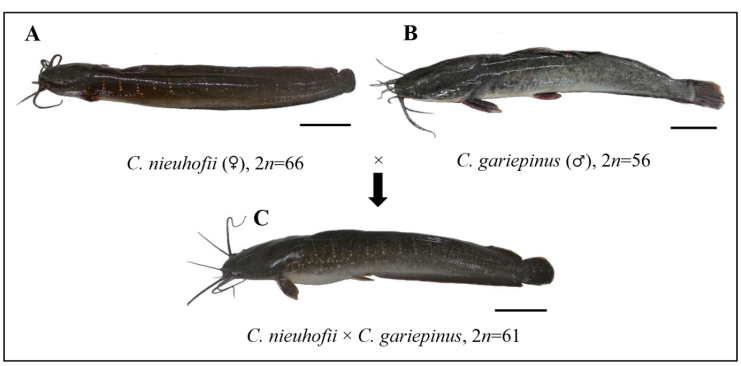

Figure 1. General characteristics of the *Clarias nieuhofii* (♀), 2*n*=66 (A), *C. gariepinus* (♂), 2*n*=56 (B), hybrid catfish *C. nieuhofii* × *C. gariepinus*, 2*n*=61 (C). Scale bars indicate 5 cm.

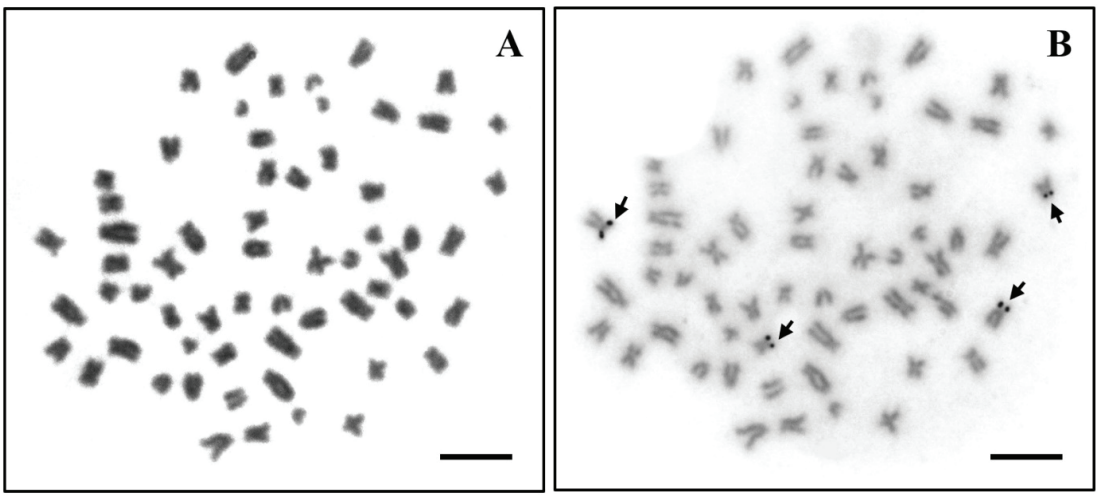

Figure 2. Metaphase chromosome plate of hybrid fish [*Clarias nieuhofii* (♀) × *C. gariepinus* (♂)] 2*n*=61 (A) by conventional staining and Ag-NORs banding (B). Arrows indicate nucleolar organizer regions (NORs). Bar = 5 µm.

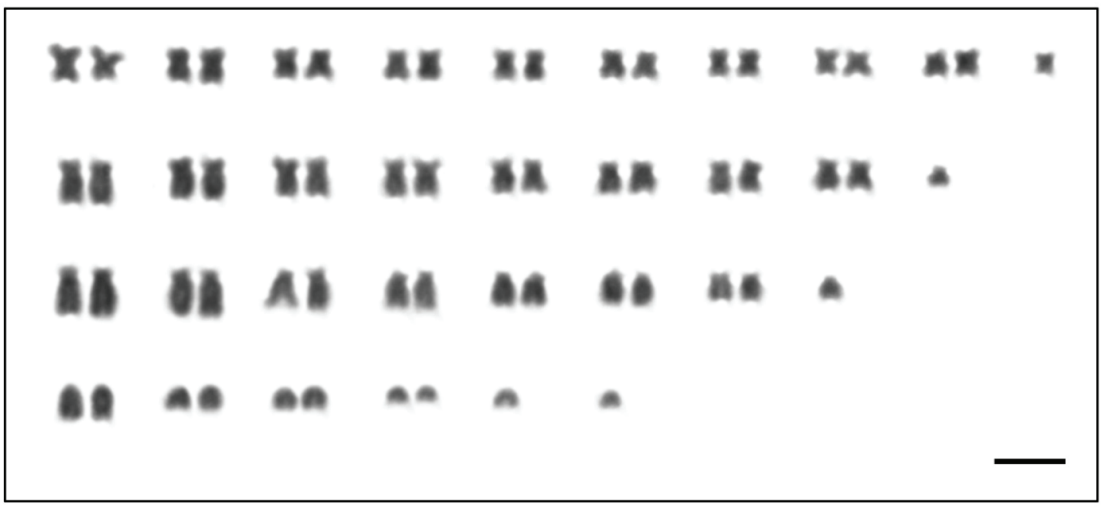

Figure 3. Karyotype of hybrid fish [*Clarias nieuhofii* (♀) × *C. gariepinus* (♂)] 2*n*=61 by conventional staining. Bar = 5 µm.
Note: un = unpaired chromosome.

In the present study, NOR bearing chromosomes were observed in two pairs of the hybrid, one is located in the short arm of large submetacentric pair and the other in the short arm of small subtelocentric pair (Figure 2B). For *C. nieuhofii*, the information of NOR has not yet been reported. However, one reported in *C. gariepinus* in Turkey has been revealed that NOR were observed on 3 or 4 chromosome pairs (Karahan & Ergene, 2011). Moreover, the chromosomes of *C. gariepinus* have been mapped with 5S and 18S rDNA sequences compared to *C. macrocephalus* and their hybrid. Accordingly, the supposed hybrid of *C. gariepinus* x *C. microcephalus* had the intermediate number for both rDNA probes (Maneechot *et al.*, 2016). In the same side, to understand more about genomic hybridization relating to NOR of the hybrid

(*C. nieuhofii* × *C. gariepinus*) and the individual parental species, the molecular cytogenetic approach such as rDNA sequences need to be performed in the future study.

4. Acknowledgment

This work was supported by Faculty of Agriculture and Technology, Rajamangala University of Technology, Isan Surin campus, Thailand.

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