MORPHO-MERISTIC CHARACTERIZATION AND KARYOMORPHOLOGICAL VARIATIONS IN *CHANNA PUNCTATA* (BLOCH, 1793) FROM MEIKTILA LAKE, MEIKTILA, MANDALAY REGION

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Abstract

Assessment on the variations of morpho-meristic and cytotaxonomic characteristics due to the variation of karyo-morphology in some *Channa punctata* from Meiktila Lake, Mandalay Region was conducted during July 2022 to January 2023. Blood samples were treated using 0.50 % colchicine solution and incubated with the saturated NaCl for various durations: 1 hr 15 mins, 1 hr 30 mins, 1 hr 45 mins, 2 hrs, 2 hrs 15 mins, 2 hrs 30 mins, 2 hrs 45 mins, 3 hrs, 3 hrs 15 mins and 3 hrs 30 mins, respectively. A drop of each sample was put onto the slide and stained with undiluted Giemsa stain. The best optimal checkpoint for metaphase chromosomes was at 2 hrs 30 mins and were not clearly discerned on morphometric changes. Model numbers of metaphase chromosomes were octaploidy 2n = 8X = 128 having 3 submetacentric (sm) + 6 acrocentric (ac) + 7 telocentric (t) with fundamental arm number (NF = 304). Thus, *C. punctata* from Meiktila lake possess the diverged karyotypic patterns indicating that they are asymmetric species and can be used as cytogenetic marker for their population.

Keywords: *Channa punctata*, morpho-meristic, cytotaxonomic, octaploidy, asymmetric

Introduction

Myanmar's fishery sector plays an important role in Myanmar's economy and culture, and provide at least 60 % of Myanmar's animal protein consumption. Presently, 36385 valid species in which 300 new species in 2022 are reported in the Eschmeyer fish catalogue and new ones are discovered yearly, mainly from the tropical and subtropical areas (Fricke *et al.*, 2022). In addition, Froese and Pauly (2022) reported that among 1135 species, only 24 species of fishes are still remained to confirm their species status in fishery resource management.

To designate the species, not only the taxonomic aspect but also cytogenetic analysis is critically important in every research area. For that reason, understanding on the genome function is incomplete without the basic knowledge of genome organization at the chromosome level. Such chromosome-scale genome assemblies provide new opportunities for both cytogenetic and genome research (Sharakhova and Trifonov, 2021).

Channa punctata locally known as (Nga-yant-panaw) in Myanmar is distributed throughout India, Iran, Afghanistan, Bangladesh, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka and Thailand. In order to validate, the karyo-morphology of *Channa punctata* from Meiktila Lake, the present research was conducted with following objectives: to collect, identify and record the *C. punctata* in Meiktila Lake; to investigate the effect of mitotic inhibitor on metaphase checkpoint of the species identified; and to confirm through the erected hypothesis: H_0 : Karyomorphology cannot be referred as cytogenetic marker in the morpho-meristic variations in *C. punctata* and H_1 :Karyomorphology can be referred as cytogenetic marker in the morpho-meristic variations in *C. punctata.*

Materials and methods

Study area and study site

The present study was conducted at Meiktila Lake for fish specimen collection, locating in the center of Meiktila town between North latitude 20˚50′ 0″ and 20˚56′ 0″N and East longitude between 95˚49′ 30″ E and 95˚52′ 0″E (Plate 1).

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Study period

This study was conducted from July 2022 to January 2023.

Sample collection

Fifteen fishes of *Channa punctata* were collected from Meiktila Lake. After identification, the fish specimens were kept in separate aquaria at Department of Zoology, Meiktila University.

Plate 1 Location map of Meiktila Lake, (Source: Google Earth 2022)

Identification of species

The identification of collected fish species *Channa punctata* was done according to Tawlar and Jhingran (1991) and Froese and Pauly (2022).

Preparation of solution

Stock solution 0.50 % of colchicine, the saturated hypotonic solution NaCl, and Carnoy fixative (3 methanol:1 acetic acid) were prepared. Giemsa stain was also prepared by mixing 0.78 g mixed with 500 mL methanol and 500 mL glycerol.

Preparation of mitotic division

One milliliter of blood samples were extracted from caudal vasculature by using syringe and placed into 10 mL anti-coagulant tube, treated with 0.50 % colchicine solution and then incubated with saturated hypotonic solution NaCl at room temperature for various durations: 1 hr 15 mins, 1 hr 30 mins, 1 hr 45 mins, 2 hrs, 2 hrs 15 mins, 2 hrs 30 mins, 2 hrs 45 mins, 3 hrs, 3 hrs 15 mins and 3 hrs 30 mins. The blood sample mixtures were vigorously shaken for 5 mins and fixed with 1 mL Carnoy's fixative solution, and stored at room temperature.

Slide preparation and staining

One mililiter of Carnoy fixative was added to the treaded cells and mixed thoroughly by shaking the tube back and forth. One or two drops of sample solution were placed on a clean slide and dried at room temperature. The slides were immersed in undiluted Giemsa stain for 15

mins and destained by rinsing with distilled water, and then dried overnight. Permanent slides were made by mounting Canada balsam and covered with cover slip.

Examination on microphotograph

Microphotographs were taken with Olympus CX-21FS1 camera attached to microscope (x1000) with Magvision camera attachment software.

Karyological study

The metaphase chromosome spreads (n = 8 - 10) from each specimen were examined by using immersion oil. The recorded michrophotographs were generated with SmartType3 (SDB-459) and arranged by decending order according to their size. The chromosomal numbers were recorded by Image.J (1.52a, USA).

Characteristics of chromosome patterns were classified according to Levan *et al.* (1964). The fundamental arm numbers (NF) were assessed and also the metacentric and sub-metacentric chromosomes were designated as biarmed chromosome, and acrocentric and telocentric chromosomes were denoted as uniarmed chromosome.

Statistical Analysis

All the data recorded of *Channa punctata* was analyzed by Microsoft Excel 2019.

Results

A total of 15 specimens of *Channa punctata* were collected from Meiktila Lake and identified the species confirmation. Their morpho-meristic characterization and karyo-morphological variations were investigated in detailed studies (Table 1).

Description of *Channa punctata* (Bloch, 1793)

Common name - Spotted snakehead,

Vernacular name - Nga-yant-pa-naw

The body is elongated and slightly rounded. Eyes are moderate and mouth is large. Pectoral fin is extended to anal fin. Pelvic fin is about 75 % of pectoral fin length. Caudal fin is rounded. Pre dorsal scales 13 - 14 and lateral line scales 37 - 42 present. Body color varies from black to light green on dorsal side and flanks; ventral side is white to pale yellow, sometimes with a reddish tinge; several dark blotches are on flanks; numerous black spots with a reddish tinge and paired fins are pale orange (Plate 2).

Plate 2 Lateral view of *Channa punctata*

Karyomorphological analysis of Snakehead *Channa punctata*

The blood samples of study fish species were treated with 0.50 % colchicine solution to block the mitotic check point of metaphase chromosomes and incubated with saturated NaCl solution at room temperature for various durations. The optimal check point of metaphase spread chromosomes was observed at 2 hrs 30 mins. The shape and size of chromosomes were identified by staining with undiluted Giemsa. The unique chromosomal characteristics and the unusual characters of chromosome spread were observed (Plate 3).

Percent and Frequency Distribution

The highest percentage of chromosome counts was 128 (54.67 %) with 88 frequencies followed by 126 (14.67 %) with 22 frequencies, 125 (12.00 %) with 18 frequencies, 129 (7.33 %) with 11 frequencies, 127 (6.0 %) with 9 frequencies and the lowest percent was found in 130 (5.33 %) with 8 frequencies (Table 2 and Fig. 1).

Karyotype

A total of 150 metaphase chromosome spreads of snakehead *Channa punctata* was 2n = 8X = 128, autotetraploid having 3 submetacentric (sm) + 6 acrocentric (a) + 7 telocentric (t) with constant fundamental arm number NF = 38 and the advanced fundamental arm number (NF) was 304. The distinct chromosomal patterns were found in chromosome set number 3 with extra-rectangular pattern and 6 with ring-shaped chromosome. Asymmetric chromosomal shape and size appeared in each set of chromosomes. The V - shaped chromosomes were observed in chromosome set number 1, 3, 4, 5 and 7 (Table 3, Plate 3)

The secondary constriction of chromosomes was found in chromosome number 1, 2, 3 and 4. The J - shaped chromosomes were found in chromosome set 1 and 7. The rod-shaped chromosomes were observed in 6, 7, 8, 10,11 and 12. The triangular patterns were found in chromosome set number 10 and 12. The last chromosome set number 16 represents the male sex character of spotted snakehead (Table 3, Plate 3).

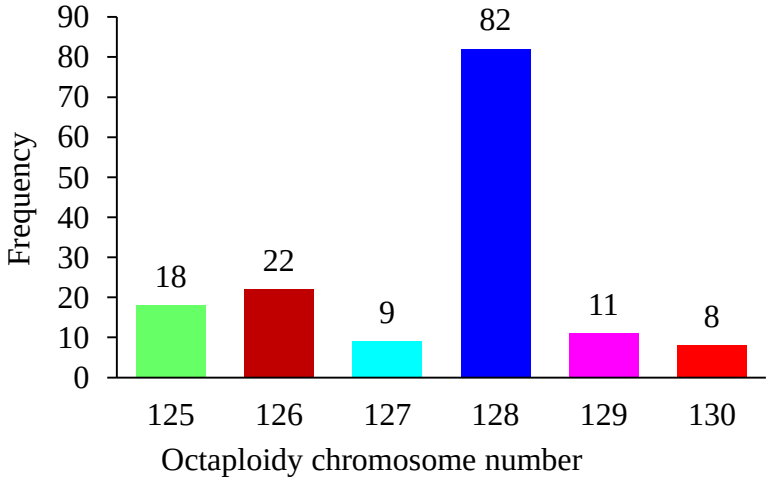

Figure.1. Frequency distribution of octaploidy chromosome number in *Channa punctata*

Table 1. Meristic counts of *Channa punctata* (n = 15) from Meiktila Lake

Meristic characters	Numbers
Dorsal fin rays (DF)	33-34
Pectoral fin rays (PecF)	17-19
Pelvic fin rays (PelF)	4-5
Caudal fin rays (CF)	13-15
Anal fin rays (AF)	22-24
Scales above lateral line (SaLL)	4.5
Scales below lateral line (SbLL)	8.5-9.5
Lateral line scales (LLS)	37-42
Pre dorsal scales (PDS)	**13-14**

Table 2.Percent and frequency distribution of octaploidy *Channa punctata* in Meiktila Lake

Chromosome counts	Frequency	Percent	Valid percent	Cumulative percent
125	18	12.00	12.00	12.00
126	22	14.67	14.67	26.67
127	9	6.00	6.00	32.67
128	82	54.67	54.67	87.33
129	11	7.33	7.33	94.67
130	8	5.33	5.33	100
Total	150	100	100	

Table 3. Karyotypical analysis of Channa punctata (NF = fundamental arm numbers)

Species	Chromosome sets				Chromosome types				NF	
	Haploid number (n)	Diploid (2n)	Ploidy (> 2n)	Chromosome numbers	Metacentric (m)	Sub-metacentric (sm)	Acrocentric (ac)	Telocentric (t)	Constant	Advanced
C. punctata	16	32	8X	128	0	3	6	7	38	304

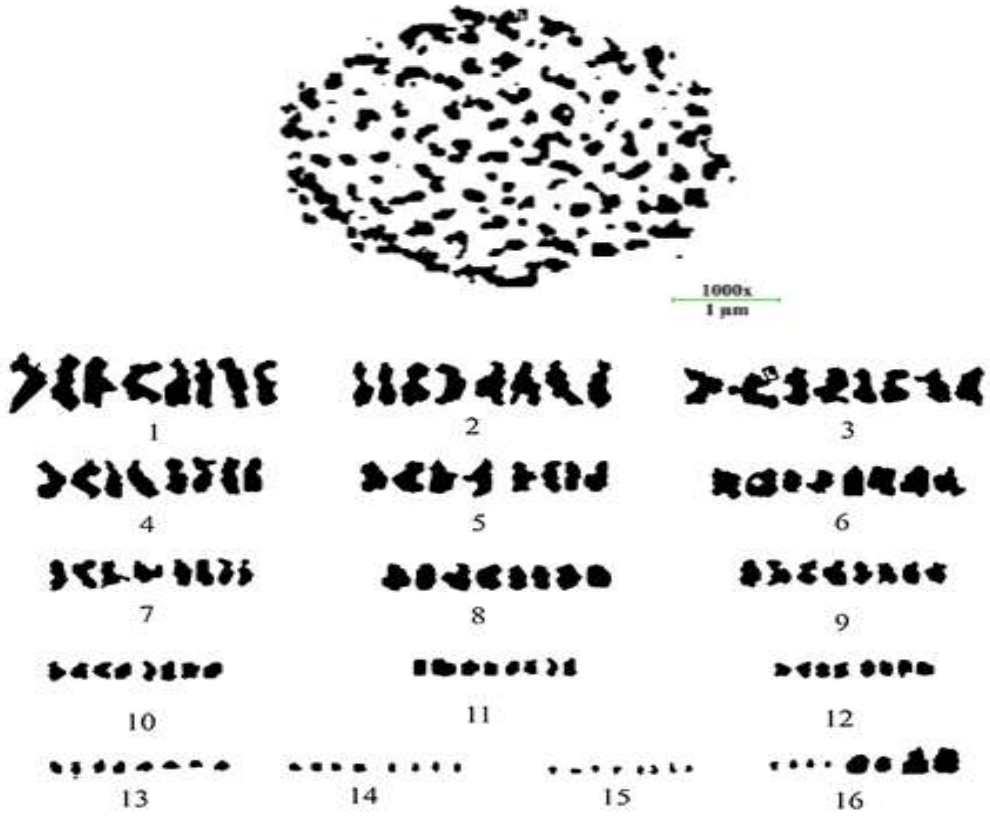

Plate 3. Metaphase chromosome plate (Upper) and karyotype (Below) of *Channa punctate* (1000x).

Discussion

The morpho - meristic characterization and karyo-morphological variations of *Channa punctata* from Meiktila Lake were investigated from July 2022 to January 2023. Some of morphological characters of *Channa punctata* are consistent with Talwar and Jhingran (1991), Plamoottil (2017), Widodo *et al.* (2020), Paunikar and Panwar (2021), and Froese and Pauly (2022) whereas the meristic counts of *Channa punctata* such as the pelvic fin rays (PelF), dorsal fin rays (DF) and pre dorsal scales (PDS) were different.

In nature, every organism has its own unique karyotype. However, there was lack of phylogenetic analysis in *Channa punctata* including morphological and cytogenetic data (Naorem and Bhagirath, 2006). With respect to the cytogenetic data, Kumar *et al.* (2019) suggested that the process of evolution in *Channa punctata* is complicated due to the polyploidization, duplication, rearrangement, fusion and loss of chromosomes. However, there is no evidence of a fixed pattern found in *Channa punctata*. Furthermore, Lawce and Brown (2017) reported that chromosome number can vary due to a number of errors in cell division, that is, during meiosis, fertilization, or mitosis.

In this study, *Channa punctata* are asymmetrical species having more acrocentric and telocentric chromosomes with variable chromosomal characteristics. Thus, the cytotaxonomic study on *Channa punctata* could be based not only on their centromeric position but also on their size and morphology of chromosomes. The best optimal check point of metaphase chromosomes was observed at a duration of 2 hrs 30 mins compared to other durations. The highest percentage

of chromosome counts 128 (54.67 %) with 88 frequencies and the lowest percentage of chromosome counts 130 (5.33 %) with 8 frequencies in *C. punctata*.

The karyological formula of *Channa punctata* was 3 sm + 6 ac + 7 t with fundamental arm number (NF) = 304 indicating that the fish possesses the autotetraploid 2n = 8X = 128 instead of constant diploid n = 16.

To sum up, the study on the morpho-meristic characters and karyograms revealed variable morpho-meristic characters with the divergence of karyotypic patterns in *Channa punctata* from Meiktila Lake. Therefore, the present karyomorphological results revealed the diverged karyotypic patterns indicating that *C. punctata* is asymmetric species and the present karyomorphological results can be referred as cytogenetic marker for *Channa* species.

Therefore, the erected hypothesis, H_1 is accepted and H_0 is rejected. The alternative hypothesis – "karyomorphology can be referred as cytogenetic marker in the morpho-meristic variations in *Channa punctata*" is accepted. The null hypothesis – "karyomorphology cannot be referred as cytogenetic marker in the morpho-meristic variations in *Channa punctata*" is rejected.

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