

Karyotype and Nucleolar Organizer Regions in the Pomacentrid Fish *Neoglyphidodon melas*

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Abstract.

Karyotype and nucleolar organizer regions (NORs) in the pomacentrid fish *Neoglyphidodon melas* (Perciformes) were studied. The chromosome number was observed to be $2n = 48$, and the karyotype consisted of 3 pairs of metacentric, 13 pairs of submetacentric, and 8 pairs of subtelocentric or acrocentric chromosomes. The fundamental number was 80. Ag-positive NORs were located at terminal regions of the short arms of a pair of large subtelocentric chromosomes. The present results suggest that the karyotype of this species is highly differentiated mainly because of pericentric inversions, and *Neoglyphidodon* is karyologically one of most differentiated genera.

Key words: karyotype, NORs, fundamental number, Pomacentridae

1. Introduction

The family Pomacentridae of the order Perciformes comprises about 348 species, which are widely distributed in tropical seas of the world¹⁾.

Cytogenetic investigations of Pomacentridae have revealed the karyotypes of 30 species²⁻⁹⁾. The chromosomal locations of nucleolar organizer regions (NORs) have been

reported in 23 species⁵⁻⁹⁾. These studies have provided interesting findings that help in understanding karyotypic evolution and phylogenetic relationships.

The present study deals with the karyotype and NORs in a species of Pomacentridae, *Neoglyphidodon melas* (also known as “kuro-suzumedai”).

2. Materials and methods

Four specimens of *N. melas* obtained from a fish dealer of Osaka, Japan, were used for this study.

Chromosome preparations were made according to the direct method with PHA and colchicine injection⁶⁾. Specimens were injected intraperitoneally with 0.01 ml/g (body weight) of PHA-M (Difco Laboratories) and 0.1 µg/g (body weight) of colchicine. After 5 to 6 h, kidney tissue was removed, placed on a Petridish with a small amount of culture medium (about 0.5 ml) such as Eagle's MEM, and crushed with tweezers having flat heads. After about 4 ml of the medium was added, sufficient pipetting was done to obtain more liberated cells, and then tissue fragments were removed. The cells thus obtained were hypotonized with 0.068M KCl for 20 min and fixed with a mixture of acetic acid and methyl alcohol (1:3) freshly prepared. After dropping of the cell suspension on clean slides, the latter were air-dried and stained with 2% Giemsa solution diluted with 1/15M phosphate buffered saline.

Following microscopic observations, the slides were destained with 70% alcohol, and then Ag-staining was carried out according to the one-step method of Howell and Black¹⁰⁾. Conventional and silver staining chromosomes on the same metaphase

plates were analyzed according to the chromosome nomenclature established by Levan et al.¹¹⁾. For the calculation of fundamental number (NF), two were assigned to meta- and submetacentrics and one to subtelo- and acrocentrics.

3. Results and discussion

As shown in Figure 1, *N. melas* had a diploid chromosome number of $2n = 48$, and the karyotype consisted of 3 pairs of metacentric, 13 pairs of submetacentric, and 8 pairs of subtelo- or acrocentric chromosomes (NF = 80). The chromosomes showed a gradual change in size. Faintly stained secondary constrictions (SCs) were observed at terminal regions of the short arms of a pair of large subtelocentric chromosomes. Ag-positive NORs were located in SCs. No heteromorphic chromosome pair was observed.

We previously reported that the karyotype of *N. melas* consisted of a pair of metacentric and 23 pairs of acrocentric chromosomes⁵⁾. However, we later learned that the species we studied was wrongly identified as *N. melas*. Therefore, we report the karyotype of *N. melas* for the first time in the present study.

Teleostean fishes have a noticeable karyotypic feature that many other species possess 48 diploid chromosomes,

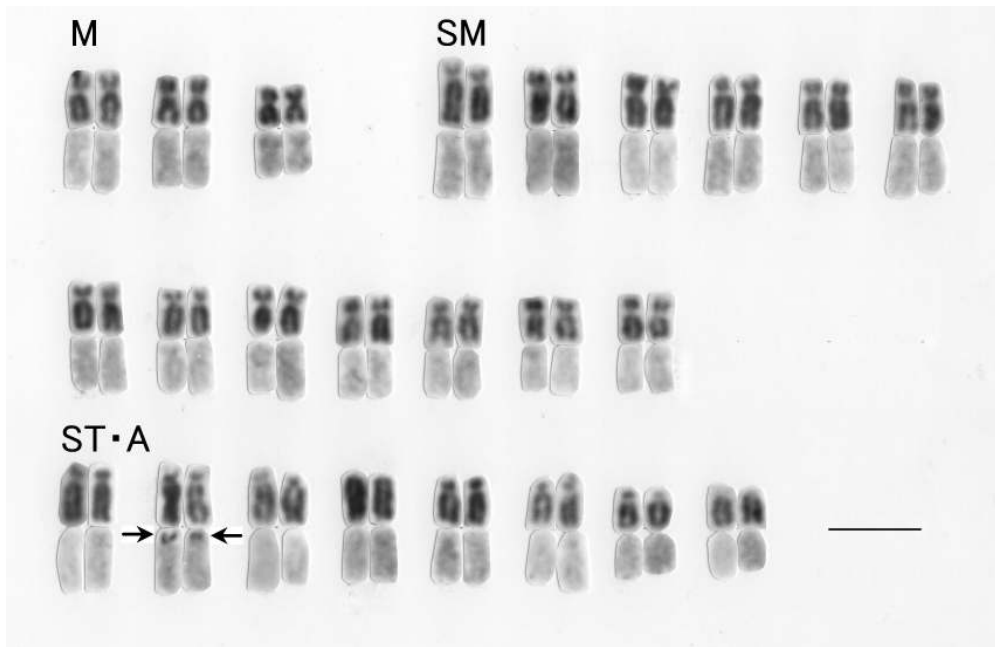


Figure 1. Conventional and silver staining karyotypes of *Neoglyphidodon melas* ($2n=48$, $NF=74$). M, metacentric; SM, submetacentric; ST, subtelocentric; A, acrocentric. Arrows indicate Ag-positive NORs. Bar scale represents 5 μ m.

and the karyotypes of many of the species consist of entirely or almost entirely acrocentric chromosomes. This feature is conspicuous in highly evolved groups, in particular in the order Perciformes¹²⁻¹³. According to Ohno¹², the karyotype consisting of only 48 acrocentric chromosomes (48A karyotype) may have been ancestral in fish evolution, and this presumed ancestral karyotype was also ancestral in many fish groups at family and order levels. Pomacentridae has several species with the 48A karyotype^{4,5,7}.

Therefore, we believe that the 48A karyotype was also ancestral in Pomacentridae⁵.

Thus far, karyotypes of many pomacentrid fish have been characterized as having a constant chromosome number of $2n = 48$ and widely ranging NFs between 48 and 88. These results suggest that karyotype differentiation in pomacentrid fishes has progressed mainly because of pericentric inversions⁵⁻⁷. This theory may be applicable to *N. melas* in this study. Conversely, three *Dascyllus* species and two

Chrysiptera species have karyotypes with large bi-armed chromosomes because of Robertsonian rearrangements^{4,8}. Thus, karyotypes of Pomacentridae may have diversified not only because of pericentric inversions but also Robertsonian rearrangements, resulting in increased NF and decreased chromosome number compared to the ancestral 48A karyotype.

In the genus *Neoglyphidodon*, karyotypes of two species, *N. nigroris* and *N. oxyodon*, have been reported to have high NFs (82 and 70, respectively)⁶. NF (80) of *N. melas* in this study was also high. Thus far, it is evident that *Neoglyphidodon* is a karyologically highly differentiated genus. In other genera, *Amphiprion* and *Chrysiptera* species have high NFs. Conversely, *Dascyllus* and *Abudefduf* species have low NFs, ranging between 48 and 52. NFs of *Pomacentrus* species range from low to high (48–84). Further karyological studies may provide interesting findings related to phylogenetic relationships.

The present study revealed that NORs of *N. melas* were located on a single pair of chromosomes, at the terminal region of the short arms. This is a usual state in Pomacentridae. Occurrence of active chromosomal changes has been demonstrated in

Pomacentridae¹⁴, however, it is an interesting phenomenon that the number of NORs have been conserved throughout these changes.

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(和文要旨)

スズメダイ科魚類の一種 *Neoglyphidodon melas* の核型と核小体形成部位

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スズキ目のスズメダイ科魚類の一種 *Neoglyphidodon melas* (和名: クロスズメダイ) の核型と核小体形成部位 (NORs) を明らかにした。本種は $2n=48$ の染色体数を有し、3 対の metacentric、13 対の submetacentric、8 対の subtelocentric または acrocentric 染色体で構成され、基本数(NF) は 80 であった。NORs は一対の大型の subtelocentric 染色体の短腕部に認められた。本種の核型は両腕間逆位により分化してきたことが示唆され、本種を含む *Neoglyphidodon* 属の魚類は核型的に最も分化したグループのひとつであると考えられる。