Research Article

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# Karyotype of Four Mouth-Brooding Betta Fishes (Betta Bleeker, 1850) in Thailand

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**Abstract -** The karyotype and chromosomal characteristics of nucleolar organizing regions (NORs) of four mouth-brooding Betta from Thailand: Betta pi Tan, 1998, B. prima Kottelat, 1994, B. pugnax (Cantor, 1849), and B. simplex Kottelat, 1994, were reported. The results exhibited that the diploid chromosome number (karyotype formula) and fundamental number (NF) of each species are; B. pi, 2n=34 (4m+4sm+18a+8t) NF=60, B. prima, 2n=38 (6m+12a+20t) NF=56, B. pugnax, 2n=40 (4m+2sm+14a+20t) NF=60 and B. simplex, 2n=46 (2m+2sm+4a+38t) NF=54. All species revealed one pair of NOR-bearing chromosomes located on the short arm (p) of the acrocentric chromosome. In addition, the different sizes and morphology of Ag–NOR were observed in these four Betta fishes.

**Keywords**: Mouth–brooding betta, betta, karyotype, chromosome

## 1. Introduction

Wild fighting fish species in the genus Betta are native to ASEAN countries. Two groups of fighting fish in Thailand can be found: bubble nester and mouth-brooder. The bubble nesting Betta distributes throughout Thailand, while mouth-brooding Betta fish is mainly found in southern Thailand (Panijpan *et al.*, 2020). There are 12 wild-type species been found in Thailand, of which five species are bubble nester, and the other seven species are mouth-brooder Betta (Panijpan *et al.*, 2017). These 12 species are divided into five groups: B. picta group, B. pugnax group, B. waseri group, B. smaragdina group, and B. splendens group (Panijpan *et al.*, 2014)

The bubble nester is more comfortable identifying with the fins and body color, and habitat based on the general appearance. However, the mouth-brooder is challenging to identify (Monvises *et al.*, 2009) with a similar body shape, mostly brown with darker stripes body, and even the habitat except for B. simplex in Krabi province. Some studies adapted the DNA analysis techniques, for example, COI (cytochrome c oxidase subunit I) barcoding, 16SrRNA (16S ribosomal RNA), ITS (internal transcribed spacer), RAG (recombination activating gene) genes to identify the Betta fishes (Panijpan *et al.*, 2020). This technique is reasonably acceptable, but it takes time and cost for species identification.

Cytogenetics has become popular in fish classification, such as in the cyprinids fish group (Yang *et al.*, 2015). Cytogenetics provides the necessary information of chromosomes such as number, size, and morphology, which can help determine the variety of animal evolution and may permit the detection of the change and modification of karyotype from an ancestor to their newline (Winkler *et al.*, 2004). Karyological studies of fishes provide the comprehensive knowledge to solve the problem in many areas (Alsabti, 1985), such as taxonomy, systematic, phylogenetic relationship (Campiranont, 2003), evolution (Tanomtong *et al.*, 2014; Cioffi *et al.*, 2015), and environmental toxicology (Klinkhardt, 1993). Moreover, karyotype also provides the chromosome complement such as the number, chromosome type, and size of individuals, species, and a related group of individuals.

Studies of cytogenetics are essential in the aquaculture context in which using chromosome manipulation techniques, including polyploidy, gynogenesis, androgenesis, and inter-or intra-species hybridization (Campiranont, 2003). Nevertheless, the cytogenetic information of Betta fishes in Thailand is scarcely known. There are only four species that have been performed. This study aims to establish cytogenetic knowledge regarding the issues mentioned earlier, including diploid number, karyotype formula, and Ag-NOR of four mouth-brooding Betta are B. pi, B. prima, B. pugnax, and B. simplex. This information may facilitate the hybridization between different populations in the future for strain improvement of Betta fish. Moreover, this information may be a valuable tool for the taxonomy revision in this group.

## 2. Materials and methods

**Table 1** Sources of the examined ornamental Betta fishes.

| No. | Scientific name | Common name | Collection sites |
|---|---|---|---|
| 1 | Betta pi Tan, 1998 | | *6 .0746°N, 101.9585°E*<br>*Pru Toh Daeng, a peat swamp in Narathiwat Province* |
| *2* | *B. prima Kottelat, 1994* | | *14 .0539°N, 102 .0445°E Hauisamong, Na Di district, Prachinburi Province* |
| *3* | *B. pugnax (Cantor, 1849)* | *Penang Betta* | *8.4000°N, 99.5700°E*<br>*Chawang District, Nakhon Si Thammarat Province* |
| *4* | *B. simplex Kottelat, 1994* | *Krabi mouth–brooding Betta* | *7.9226°N, 99.2600°E*<br>*Khlong Thom District, Krabi Province* |

The mouth-brooding Betta fishes, i.e., B. prima Kottelat (1994) and B. simplex Kottelat (1994) were selected as the representative of the B. picta group while B. pugnax (Cantor, 1849) and Betta pi Tan, 1998 were selected as the of B. pugnax group and B. waseri group, respectively (Panijpan *et al.*, 2014). Species identification of the mouth-brooding Betta fishes in this study was identified according to Tan and Ng (2005). The five male and female fishes of each species were obtained from the different natural sources throughout Thailand as shown in (Table 1). The chromosome preparation method had been accomplished after Sarasan *et al.* (2019) and Juntaree and Supiwong (2000). Detection of the NOR was following the silver staining method of Howell and Black (1980) with slight modification. The chromosome lengths of 20 cells (males and females) were measured on their short and long arms. The length of the short arm (Ls) and the long arm (Ll) were calculated for the length of the chromosome (LT) and centromeric index (CI). The CI was computed to classify the types of chromosomes according to Turpin and Lejeune (1965). All parameters were used in karyotyping (Reungsing *et al.*, 2000).

## 3. Results

Betta pi: Karyological analysis of B. pi revealed 2n=34 and NF=60. Karyotype of B. pi consisted of four metacentric, four submetacentric, 18 acrocentric, and eight telocentric chromosomes, which could be deduced as 2n (diploid) 34=4m+4sm+18a+8t. The NOR region is located at the telomeric position of the short arm of the acrocentric chromosome pair 9. There is only one active NORs in the metaphase cell, which was coincided with the interphase NOR in this sample. The metaphase chromosome and karyotype by conventional and silver-staining of B. pi are shown in (Figure 1A and 2A) respectively. There is only stick-like Ag-NOR structure was observed in B. pi (Figure 3)

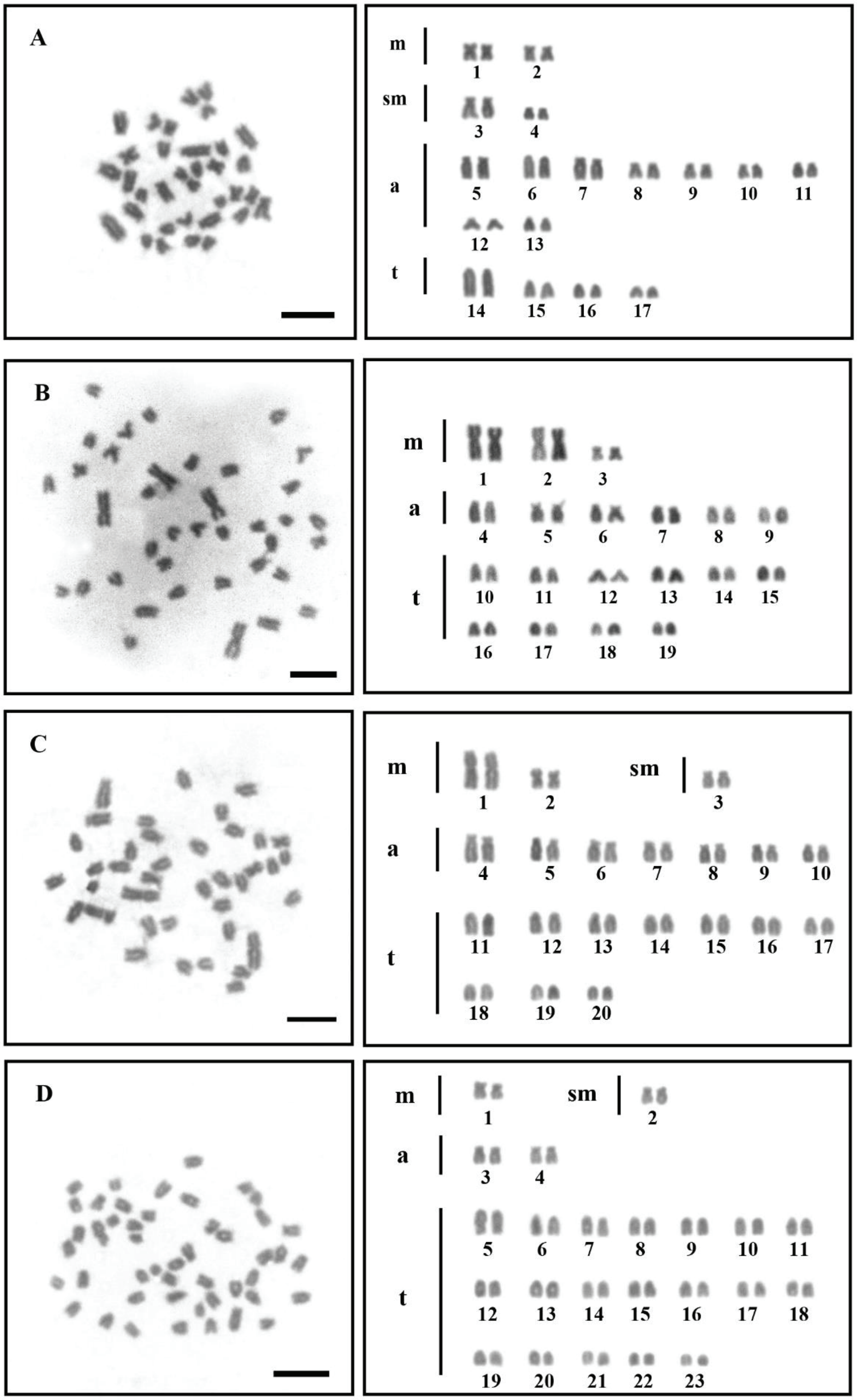

**Figure 1.** Metaphase chromosome plates and karyotypes by conventional staining of B. pi, 2n=34 (A), B. prima, 2n=38 (B), B. pugnax, 2n=40 (C) and B. simplex, 2n=46 (D). Scale bars=5 µm.

Betta prima: Chromosome of B. prima possessed 2n=38 and NF=56. Karyotype comprises six metacentric, 12 acrocentric, and 20 telocentric chromosomes which could be deduced as 2n (diploid) 38=6m+12a+20t. Besides, distinctive secondary constriction was observed on short arms of chromosome pair 5, which is coincided with the Ag-NOR, determining a size heteromorphism between homologous chromosomes. There are two active NORs in the metaphase cell. The metaphase chromosomes and karyotypes by a conventional and silver straining technique of B. prima are shown in (Figure 1B and 2B, respectively. Two types of Ag-NOR shapes were observed in B. prima; pair structure and stick-like structures (Figure 3)

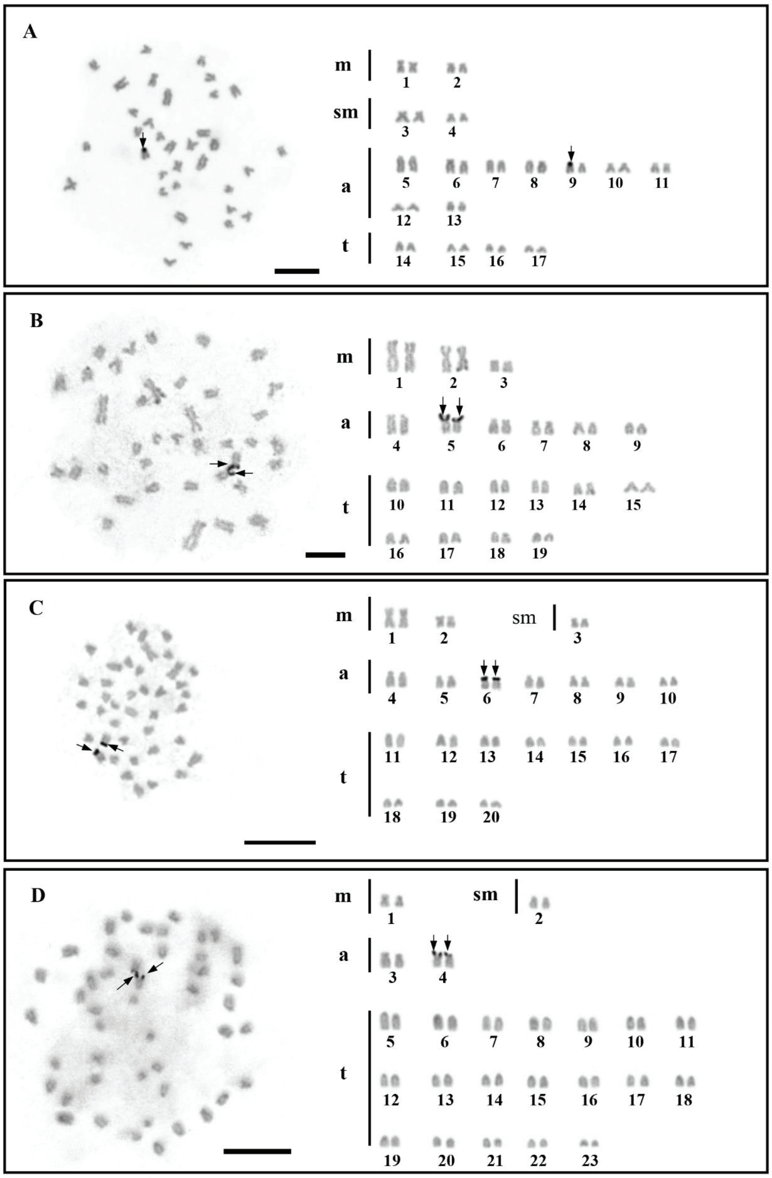

**Figure 2.** Metaphase chromosome plates and karyotypes of B. pi (A), B. prima (B), B. pugnax (C) and B. simplex (D) by Ag–NOR straining technique, arrows indicate Ag–NOR. Scale bars=5 μm.

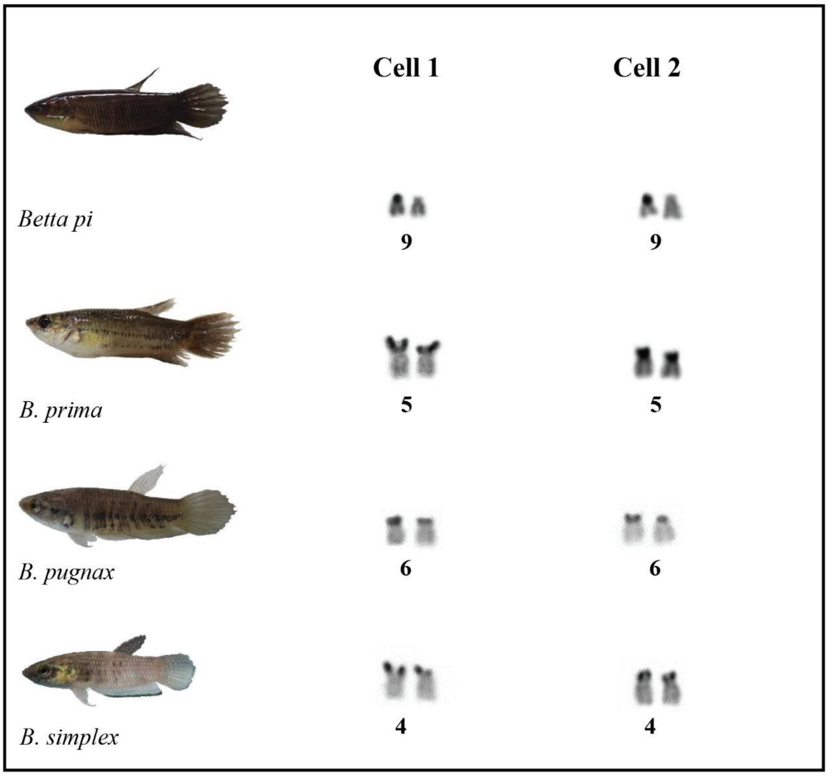

**Figure 3.** Ag–NOR shapes of the examined mouth–brooding Betta fishes. Number indicate chromosome pair.

Betta pugnax: Karyological analysis of B. pugnax revealed 2n=40 and NF=60. Karyotype of B. pugnax comprises four metacentric, two submetacentric, 14 acrocentric, and 20 telocentric chromosomes, which could be deduced as 2n (diploid) 40=4m+2sm+14a+20t. One pair of NOR-bearing chromosome was observed in B. pugnax. The NORs regions are located at the telomeric position of the short arm of the acrocentric chromosome pair 6. There are two Ag-NORs per cell, both interphase and metaphase cells. The metaphase chromosomes and karyotypes by conventional and Ag-NOR straining technique of B. pugnax are shown in (Figure 1C and 2C) respectively. Both; pair and stick-like structures of Ag-NOR were observed in B. pugnax (Figure 3).

B. simplex: Karyological analysis revealed 2n=46 and NF=54. The karyotype comprises two metacentric, two submetacentric, four acrocentric, and 38 telocentric chromosomes deduced as 2n (diploid) 46=2m+2sm+4a+38t. One pair of NOR-bearing chromosome was observed in B. simplex. The NORs regions are located at the telomeric position of the short arm of the acrocentric chromosome pair 4. Two Ag-NORs were observed both in interphase and metaphase cells. The metaphase chromosomes and karyotypes by conventional and Ag-NOR straining technique of B. simplex are shown in (Figure 1D and 2D) respectively. There are two types of Ag-NOR structure of B. simplex; pair structure and stick-like structures (Figure 3)

We could not find the 2n and NF differences between males and females of all these four mouth-brooding Betta fishes.

**Table 2** The comparative karyotypes of the examined fishes in the genus Betta.

| Species | 2n | NF | Karyotype formula | Ag-NORs (location) | Reference |
|---|---|---|---|---|---|
| Betta pi | 34 | 60 | 4m+4sm+18a+8t | 1 (9p) | The present study |
| B. prima | 34 | 42 | 4m+4sm+4st+22t | | Magtoon *et al.* (2007) |
| | 38 | 56 | 6m+12a+20t | 2 (5p) | The present study |
| B. pugnax | 40 | 60 | 4m+2sm+14a+20t | 2 (6p) | The present study |
| B. simplex | 44 | 52 | 4m+4sm+36t | | Donsakul *et al.* (2009) |
| | 46 | 54 | 2m+2sm+4a+38t | 2 (4p) | The present study |

**Remarks**: NF=fundamental number, m=metacentric, sm=submetacentric, a=acrocentric and t=telocentric chromosomes.

## 4. Discussion

Four species of the mouth-brooding Betta fish are included in the present study: B. pi, B. prima, B. pugnax, and B. simplex. Under cytogenetic viewpoint, karyotype report in the genus Betta is scarce, especially in the mouth-brooding Betta. The B. pugnax and B. pi's chromosomal data were reported here for the first time. The conspicuous diversification of diploid number and karyotype was found among mouth–brooding Betta species. The diploid number ranges from 2n=34 to 2n=46 with the NF=54 to NF=60. The results revealed that all four species have different diploid numbers and karyotypes. The diploid number of B. pi, B. prima, B. pugnax, and B. simplex are 34, 38, 40, and 46 chromosomes, respectively See (Table 2)

Cytogenetic information of B. prima and B. simplex reported herein is different from those of the previous studies reported by Magtoon *et al.* (2007) and Donsakul *et al.* (2009), respectively. In the present study, B. prima showed a diploid number of 38 chromosomes for both sexes and a fundamental number (NF) of 56 while Magtoon *et al.* (2007) found that this species had had 2n=34, NF=42. The present study results found that B. simplex had 2n=46, NF=54, while the study of Donsakul *et al.* (2009) found that this species had 2n=44, NF=52. The present result and previous reports suggest that the variation in diploid number at the intraspecific level is found in genus Betta. Nonetheless, the variation in diploid number among the individuals of the same population was not observed. The variation of diploid numbers observed in Betta may be due to several causes, such as chromosome preparation method and the precise chromosome measurement (Zhang & Reddy, 1991). For example, a chromosome can be constricted at a high degree when chromosomes were overexposure to colchicine. More fish samples from the same population and different populations are needed to confirm the Betta fishes' karyotype. In addition, the karyotype diversification of Betta can be caused by chromosomal evolution as found in other Perciform fishes (Molina *et al.*, 2014; Rishi & Haobam, 1990 ; Singh & Barman, 2013; Almeida *et al.*, 2017). Interestingly, karyotype analysis of the genus Betta revealed that there are very large metacentric chromosomes in B. prima and B. pugnax. Moreover, all species of Betta fishes show 2n<48, but the number of large metacentric chromosomes is not compatible with the reduction of diploid values, indicating the simultaneous occurrence of tandem fusions or major deletions in the chromosome evolution of this group (Almeida *et al.*, 2017).

The NOR data of these four mouth-brooding Betta fishes were obtained herein for this first time. One pair of NOR-bearing chromosome was observed in all these fishes. The polymorphic NOR was found in B. pi. The Ag-NOR can be detected only on one chromosome of pair 9 (9a). The NORs of four the mouth-brooding Betta fishes were located terminally on the short arm (p) of the acrocentric chromosome. The different sizes of Ag–NOR were observed in Betta fish. In addition, the metaphase Ag-NORs of Betta fishes exhibited different morphologies (Figure 3). Two types of NOR structures that can be observed are sticky-like structure and pair structure. Pair and stick-like Ag-NOR structures have been described by Ploton *et al.* (1994). The example of a sticky-like structure and pair structure found in the genus Betta is shown in (Figure 3) B. pi showed only sticky-like structure while another six species revealed both sticky-like structure and pair structure of NORs. The different Ag-NOR shapes reflect both the number of ribosomal genes carried by each chromosome and the differential recruitment of active ribosomal genes in each NOR cluster (Héliot *et al.*, 2000). However, the size of the NOR did not measure in this present study.

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