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Distribution and Chromosomal Diversity of Thai-medaka, *Oryzias minutillus* in Thailand

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INTRODUCTION

Ricefishes of the genus *Oryzias* Jordan and Snyder 1906, consisted of 14 species and distribute widely in fresh and brackish waters from India eastward through Southeast Asia, China, the Korean Peninsula, and Japan, and southward to Sulawesi and Timor crossing Wallace's line (Yamamoto, 1975; Labhart, 1978). Four species of *Oryzias*, *O. minutillus*, *O.mekongensis*, *O. javanicus*, and *O. melastigma* are found in Thailand. The *O. minutillus* are widely distributed in the Chao Phraya region, Mekong region, Salween region and Peninsular Thailand (Smith, 1945; Scheel, 1969; Magtoon and Uwa, 1985; Magtoon, 1986; Magtoon *et al.*, 1992; Takata *et al.*, 1993), while *O. mekongensis* are only distributed in the Mekong region (Uwa and Magtoon, 1986). *O. javanicus* and *O. melastigma* are distributed in the mangrove forest from the peninsular Thailand (Magtoon, 1986; Magtoon and Uwa, 1986).

The chromosomal polymorphism in *O. minutillus* was the first reported with specimens from Bangkok (2n=34; NF=44), central and Chiang Mai (2n=30; NF=44), north, from the Chao Phraya region in Thailand (Magtoon and Uwa, 1985). Specimens of *O. minutillus* from Yunnan in the Mekong region in China had 2n=42 and NF=42 acrocentric chromosomes (Uwa *et al.*, 1988). Uwa *et al.* (1988) and Magtoon *et al.* (1992) suggested the chromosome of this species with 2n=42 (NF=42) is to be basic karyotype, based on comparison among karyotypes of several species of *Oryzias*.

Uwa (1986) suggested that genus *Oryzias* evolved from the monoarmed chromosome group to the biarmed and fused chromosome groups by centric fusion and pericentiric inversion in South East Asia. In the present study, we examined whether the same mechanism on chromosome changes in the course of speciation in the genus *Oryzias* had also occurred in intraspecific karyotype polymorphism

of *Oryzias minutillus* or not.

METHODS

The sampling localities of *O. minutillus* examined in the present study were as follows: a) The Salween: (1) Mae Sariang, Mae Hong Son, b) The Chao Phraya: (2) Uthai, Phra Nakhon Si Ayuthaya, c) The Chachoengsao: (3) Phan Thong or Ban Khai, d) The Mekong: (4) Nong Wua So, Udon Thani, e) The Peninsula: (5) Maung, Narathiwat (Fig.1). We added other 18 localities (6-23) which had already examined by Magtoon *et al.* (1992) for comparison. The specimens were kept at Srinakharinwirot University and Shinshu University as laboratory stocks and/or preserved specimens.

Chromosome preparation was carried out through the direct method of Kligerman and Bloom (1977). Colchicine treatment, KCl hypotonization, enthonal acetic acid (3:1) fixation, dissociation in 50% acetic acid and air drying were executed. Air-dried preparation was performed as usual, and then stained with Giemsa solution. In detecting nucleolar organizer regions (NORs), slides were stained according to the procedure described by Howell and Black(1980). Nomenclature of chromosomes followed the system of Levan *et al.*(1964). Metacentrics and submetacentrics were described as two-arm chromosomes, while subtelocentrics and acrocentrics as one-arm chromosomes.

RESULTS AND DISCUSSION

Karyotypes of *O. minutillus* in Thailand are summarized in Table 1. *O. minutillus* exhibited polymorphic karyotypes in the present study. Specimens of *O. minutillus* from Mae Hong Son (1) in the Salween region had 2n=28 chromosomes. The karyotype comprised of 8 metacentric, 1 submetacentric and 5 acrocentric chromosome pairs (Fig.2). The arm number was 44 . Specimens of *O. minutillus* from Ban Khai (3) in the Chachoengsao region had 2n= 42 chromosomes. The karyotype comprised of 1 submetacentric and 20 acrocentric chromosome pairs (Fig.3). NORs were located at a submetacentric chromosome pairs (Fig.4). Specimens of *O. minutillus* from Ayuthaya (2) in the Chao Phraya region possessed 2n= 30 chromosomes. The karyotype consisted of 6 metacentric, 1 submetacentric and 8 acrocentric chromosomes pairs (Fig.5). NORs were located at a submetacentric chromosomes. Specimens of *O. minutillus* from Udon Thani (4) in the Mekong region possessed 2n= 32 chromosomes. The karyotype had 5 metacentric, 1 submetacentric and 10 acrocentric chromosomes pairs (Fig.6). NORs were located at a submetacentric chromosomes.

Specimens of *O. minutillus* from Narativat (5) in the peninsula region had 2n=42 chromosomes. The karyotype consisted of 21 acrocentric chromosome pairs (Fig.7). The arm number was 42. NORs were located at a acrocentric chromosomes.

Magtoon et al (1992) suggested that the karyotypes of *O. minutillus* can be divided into two major categories: the basic and the evolved types. The basic karyotype consists of 2n=42 acrocentric chromosomes. The arm number of the basic karyotype is 42 and NORs are located on acrocentric chromosomes (NF=42, NORs-A), while the evolved karyotype has different arm numbers and NORs-chromosomes are located on submetacentric chromosomes (NF=44, NORs-SM). The evolved karyotype can be subdivided into two stages depending on the chromosome rearrangements: more primitive (2n=42-40, 1-2 large metacentric chromosomes) and more developed (2n=34-28, 8-14 large metacentric chromosomes) stage. According to this classification, Narativat (5) in the peninsula region belongs to the basic type. Although Ban Khai (3) in the Chachoengsao region had 42 chromosomes, this population possessed one pair of submetacentric chromosomes which characterize more primitive stage in the evolved type. Mae Hong Son (1) in the Salween region, Ayuthaya (2) in the Chao Phraya region and Udon Thani (4) in the Mekong region belong to developed stage in the evolved type.

Uwa *et al.* (1988) proposed the basic karyotype of *O. minutillus* is 2n=42 acrocentric chromosomes (42A) based on comparison of karyotypes among other *Oryzias* species. Although no evidence for decrease in diploid number from 48, basic for the genus *Oryzias*, to 42, has not been obtained, the proposed basic karyotype is found in the populations from the Mekong and Peninsula regions. The population from the Mekong region had the same chromosome number to the basic type, but, a small chromosome rearrangement (NF=44). This suggest the first chromosome rearrangement step in this species seems to be a pericentric inversion of NORs-chromosomes from acrocentric type to submetacentric types, which could provide an increase in the NF from 42 to 44 (2SM+40A) (Fig.8).

After the first step chromosome rearrangement, centric fusion seem to occur subsequently. The centric fusion of two chromosomes may be also caused the formation of large metacentric chromosomes and the decrease in diploid number (2M+2SM+36A). These karyotypes were observed in the populations from the Bang Pakong and Rayong basins in the Chachoengsao region. The centric fusion must had occurred several times in the course of intraspecific differentiation, because populations which have different karyotypes with change of chromosome numbers (8M+2SM+24A to 14M+2SM+12A) are distributed mainly in the Chao Phraya region. This fact also suggest chromosome rearrangement had occur in the Chao Phraya region and the basic karyotype has been

preserved allopatrically in the Peninsula region and the basin of the Mae Nam Mun (a tributary of the Mekong) in the Mekong region. The basic karyotype is also found in a tributary of the Mekong in Xishiangbanna, Yannan, China (Uwa *et al.*, 1992).

Mae Hong son in the Salween region and Udon Thani (4) are not located in the Chao Phraya region, though they had the evolved karyotype. The local population from Chiang Rai belongs to the Mekong region also had the evolved karyotype and was grouped with the Mae Num Chao Phraya subpopulations based on the genetic analysis and karyotypic study (Magtoon and Uwa, 1985; Magtoon *et al.*, 1992; Takata *et al.*, 1993).

Kottelat (1989) described that there had been many change in the river course affecting the freshwater fish fauna in Indo-china. This indicated that the Chiang Rai and Udon populations may be the remnant populations caused by the river capture between the Chao Phraya and Mekong Rivers, judged from the geographic circumstances. Local populations of *O. minutillus* from Salween region is also a member of the evolved type group and the chromosome number (2n=28) of this locality is similar to populations from Suphan Buri and Ratchaburi (Chao Phraya region). These chromosomal data suggest that the tributary of the Salween region had once connected to the Chao Phraya River system. Before the two river systems disconnected again, a part of population which had the evolved karyotype in the Chao Phraya region may have colonized into the Salween River.

No remarkable change was found in DNA value through their karyotype evolution, since no significant difference was detected on 2 C value specimens of *O. minutillus* from Bangkok and Chiang Mai in the Chao Phraya region (Magtoon and Uwa, 1985) and from Phuket in the Peninsula region (Uwa, 1986). Estimated erythrocyte DNA was 1.5 pg could breed each other (Ashida and Uwa, 1987), and the pairing of miotic chromosomes occurred normally and spermatogenesis of F1 hybrids was confirmed (Tsuyuki and Uwa, 1988). These strongly suggest that *O. minutillus* is not regarded as a species complex with different chromosome numbers but a single species regardless of its remarkable geographical karyotype variation.

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Table 1. Karyotype of *Oryzias minutillus* from Thailand. Localities with numbers in parenthesis are shown in Fig. 1.
NF=arm number; M=metacentric; SM=submetacentric; ST=subterocentric; A=acrocentric;LM=large metacentric chlomosome

Locality	2n	NF	Karyotype	LM	NORs	Reference
Peninsula Thailand						
Chumphon	42	42	42A	0	A	Magtoon et al., 1992
Surat Thani	42	42	42A	0	A	Magtoon et al., 1992
Songkhla	42	42	42A	0	A	Magtoon et al., 1992
Phuket	42	42	42A	0	A	Tsuyuki & Uwa 1988
Narativat	42	42	42A	0	A	Present study
Mecong region						
Phayakkhaphum Phisai	42	42	42A	0	A	Magtoon et al., 1992
Bua Yai	42	42	42A	0	A	Magtoon et al., 1992
Phimai	42	42	42A	0	A	Magtoon et al., 1992
Pak Chong	28(30)	44	14M+2SM+12A	14	SM	Magtoon et al., 1992
Chiang rai	32	44	10M+2SM+20A	10	SM	Magtoon et al., 1992
Udon thani	32	44	10M+2SM+20A	10	SM	Present study
Chachoengsao region						
Chachoengsao	42	44	2SM+40A	0	SM	Magtoon et al., 1992
Prachin Buri	42	44	2SM+40A	0	SM	Magtoon et al., 1992
Rayong	40(39,41)	44	2M+2SM+36A	2	SM	Magtoon et al., 1992
Chonburi	42	44	2SM+40A	0	SM	Present study
Chao Pharaya region						
Bangkok	34	44	8M+2SM+24A	8	SM	Magtoon & Uwa 1985
Chiang Mai	30	44	12M+2SM+16A	12	SM	Magtoon & Uwa 1985
Saraburi	30	44	12M+2SM+16A	12	SM	Magtoon et al., 1992
Suphan Buri	28	44	14M+2SM+12A	14	SM	Magtoon et al., 1992
Ratcha Buri	28	44	14M+2SM+12A	14	SM	Magtoon et al., 1992
Ayuthaya	30	44	12M+2SM+16A	14	SM	Present study
Salween region						
Mae Hong Son	28	44	16M+2SM+10A	16	---	Present study

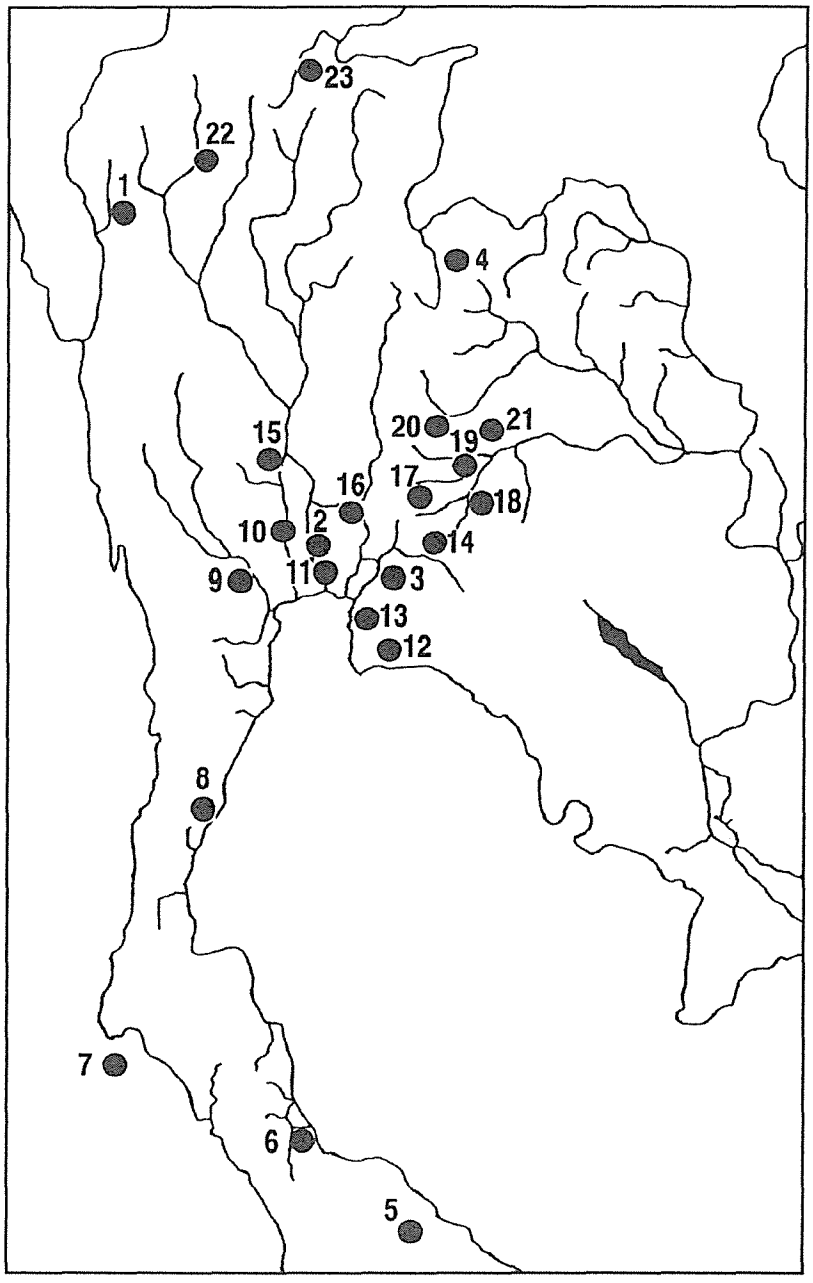

Fig. 1. Map showing the sampling sites of Oryzias minutillus in Thailand. For locality names: 1. Mae Hong Son; 2. Ayuthaya; 3. Chachoengsao; 4. Udon Thani; 5. Narativat; 6. Songkhla; 7. Phuket; 8. Chumphon; 9. Ratchaburi; 10. Suphan Buri; 11. Bangkok; 12. Rayong; 13. Chonburi; 14. Prachin Buri; 15. Chai Nat; 16. Saraburi; 17. Pak Chong; 18. Chok Chai; 19. Phimai; 20. Bua Yai; 21. Phayakkhaphum Phisai; 22. Chiang Mai; 23. Chiang Rai.

Fig. 2. Mitotic metaphase chromosomes and karyotype of Oryzias minutillus from Mae Hong son, the Salween region. Scale indicates 5 μ m.

Fig. 3. Mitotic metaphase chromosomes and karyotypes of Oryzias minutillus from Ban Khai, the Chachoengsao region. Scale indicates 5 μ m.

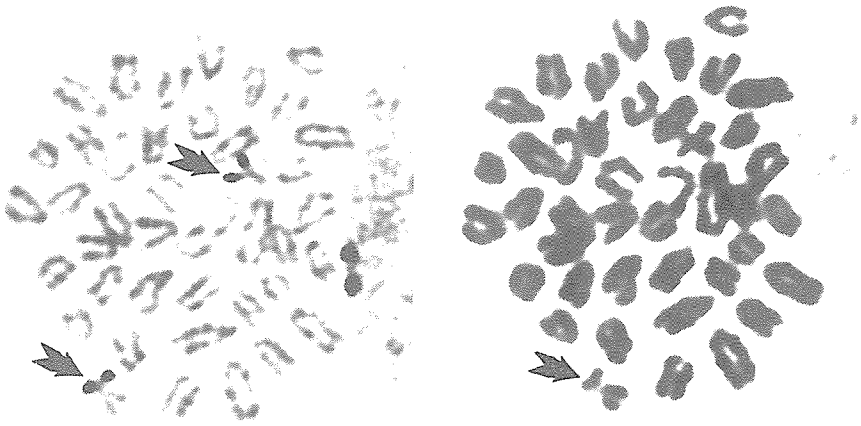

Fig. 4. Mitotic metaphase chromosomes and its silver-staining figure in Oryzias minutillus from Ban Khai, the Chachoengsao region. Arrow heads show the silver-positive nucleolar organizer regions (NORs).

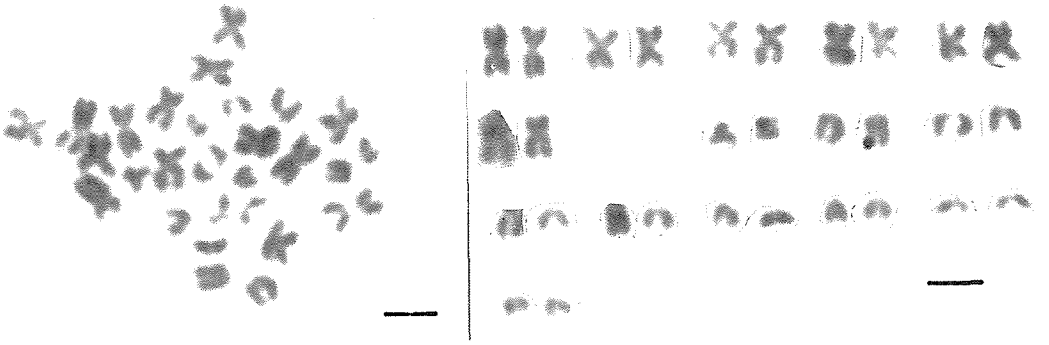

Fig. 5. Mitotic metaphase chromosomes and karyotype of Oryzias minutillus from Auythaya, the Chao Phraya region. Scale indicates 5 μ m.

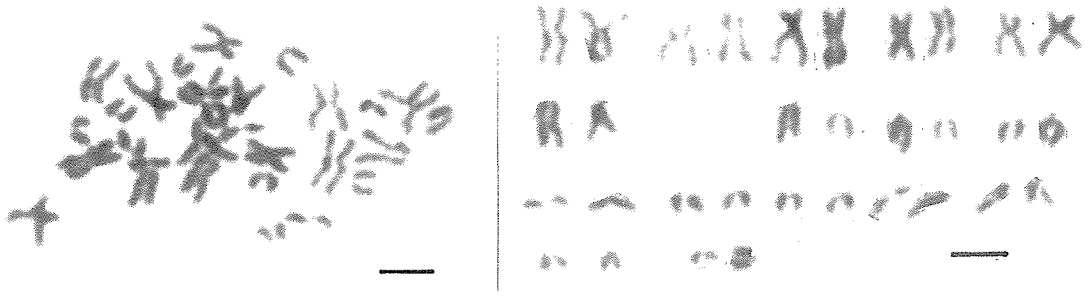

Fig. 6. Mitotic metaphase chromosomes and karyotype of Oryzias minutillus from Udon Thani, the Mekong region. Scale indicate 5 μ m.

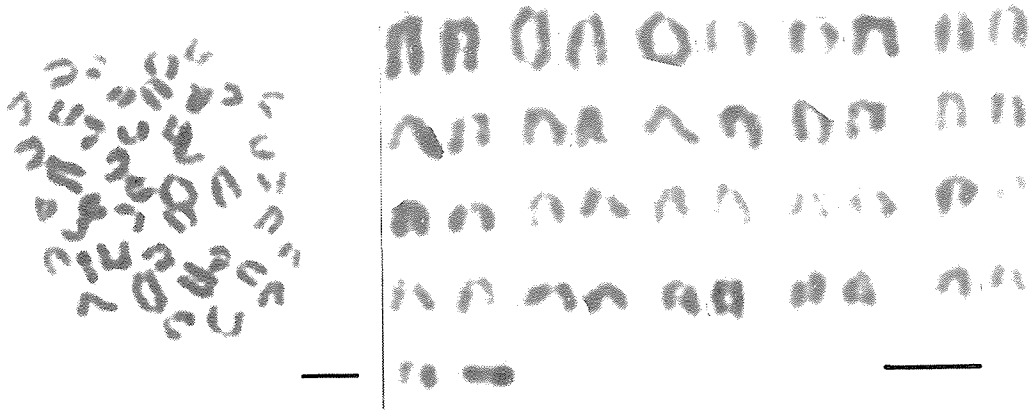

Fig. 7. Mitotic metaphase chromosomes and karyotype of Oryzias minutillus from Narativat, the Peninsula region. Scale indicate 5 μ m.

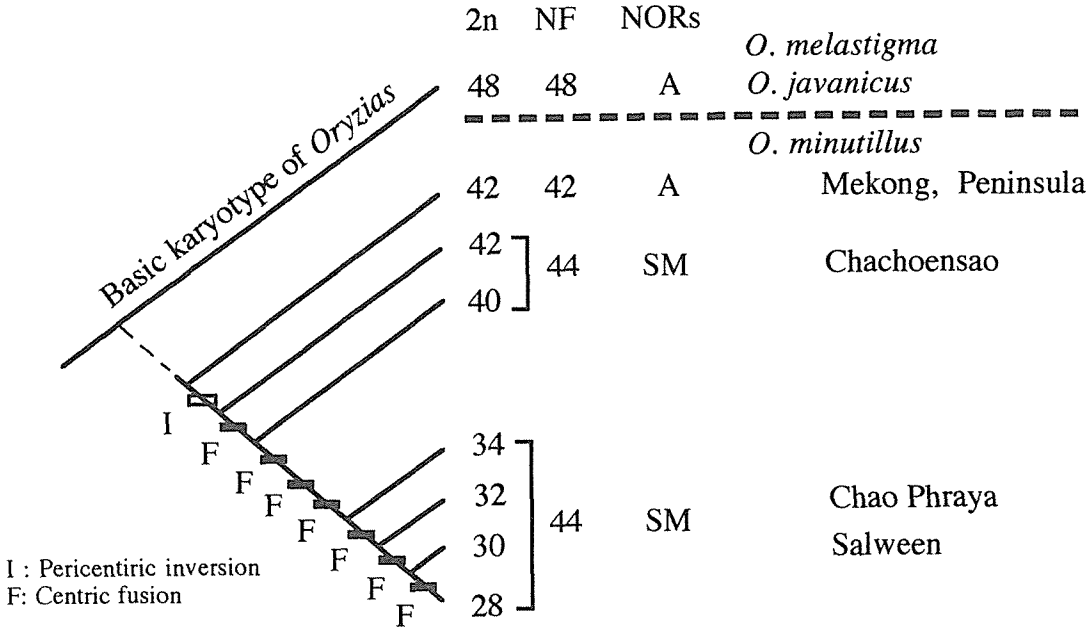

Fig. 8. Schematic figure shows karyotype differentiation of Oryzias minutillus in Thailand.
I:Pericentric inversion of NORs-chromosome, F:centric fusions