

Classification and phylogeny of fishes of the genus *Oryzias* and its relatives*

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Introduction

The family Adrainichthyoidei is a small group endemic to Asia. The genera *Oryzias* with 14 species, *Xenopoecilus* with three species, *Adrianichthys* with one species, and *Horaichthys* with one species compose the family Adrainichthyoidei (Yamamoto, 1975; Uwa, 1986; Iwamatsu, 1993; Naruse *et al.*, 1993; Nelson, 1994). These fishes are found in brooks, ponds, canals, paddy fields and lakes. Here I summarize the current classification, taxonomic history, zoogeographical distribution, and phylogenetic relationships, using several character systems, including karyotypes, protein electrophoretic patterns, mitochondrial DNA sequence data and nuclear DNA homology.

Recent status of the classification of medaka and related fishes

Fishes of the genus *Oryzias* are members of the atherinomorpha taxon Beloniformes (Nelson, 1994; Dyer and Chernoff, 1996). Relationships among fishes of the four atherinomorph orders (Atheriniformes, Beloniformes, Mugiliformes and Cyprinodontiformes) are summarized as follows:

Superorder Acanthopterygii

Series "Percomorpha"

[many orders]

Series Atherinomorpha (*sensu lato*)

Order Mugiliformes

Family Mugilidae

Order Atheriniformes

Suborder Bedotioidei

Family Bedotiidae

Suborder Melanotaenioidei

Family Melanotaeniidae

Family Pseudomugilidae

Suborder Atherinoidei

Family Atherinidae

Family Notocheridae (Isonidae)

Family Atherinidae

Family Telmatherinidae

Family Dentatherinidae

Family Phallostethidae

Subfamily Phallostethinae

Subfamily Gulaphallinae

Order Beloniformes

Suborder Belonoidei

Superfamily Scomberescoidea

Family Belonidae

Family Scomberescoidea

Superfamily Exocoetoidea

Family Exocoetidae

Family Hemiramphidae

Suborder Adrianichthyoidei

Family Adrianichthyidae

Subfamily Horaichyinae

Genus *Horaichthys*

Subfamily Oryziinae

Genus *Oryzias*

Subfamily Adrianichthyinae

Genus *Adrianichthys*

Genus *Xenopoecilus*

Order Cyprinodontiformes (Microcyprini)

Suborder Aplocheiloidei

Family Aplocheilidae

Subfamily Aplocheilinae

Subfamily Rivulinae

Suborder Cyprinodontoidei

Family Profundulidae

Family Fundulidae

Family Valenciidae

Family Anablepidae

Subfamily Oxyzygonectinae

Family Poeciliidae

Subfamily Poeciliinae

Subfamily Fluviphylacinae

Subfamily Aplocheilichthyinae

Family Goodeidae

Subfamily Empetrichthyinae

Subfamily Goodeinae

Family Cyprinodontidae

Subfamily Cubanichthyinae

Subfamily Cyprinodontinae

* This paper contributes to the Special Issue "Development of Medaka Biology in Japan."

LES POECILIES. (POECILIA).

1. *Poecilia latipes*, Pl. CIII, fig. 5. Les eaux douces du Japon produisent une espèce de *Poecilia* qui paraît se distinguer de toutes celles connues jusqu'à ce jour, par son anale très-longue, par sa dorsale assez reculée en arrière, et parce que sa membrane branchiostège est pourvue de trois rayons seulement au lieu de cinq, caractères en faveur desquels on pourrait élever cette espèce au rang des sous-genres.

Ce petit poisson atteint une longueur totale d'un pouce et demi à deux pouces. La longueur de la tête égale la hauteur du corps et entre environ cinq fois dans la longueur totale du poisson. La tête est de forme conique, comme d'ordinaire déprimée par devant, et sa ligne supérieure est presque horizontale. Les dents ne diffèrent pas de celles des autres espèces, c'est à dire qu'il y en a une rangée de très-fines dans chaque mâchoire. La bouche forme une fente transversale qui s'ouvre à l'extrémité du museau; la mâchoire inférieure est dirigée vers le haut, lorsque la bouche se trouve fermée. Les yeux sont assez grands, latéraux et rapprochés du sommet de la tête; ils occupent le tiers moyen de la longueur de la tête. Les opercules sont arrondis et lisses. Les écailles sont assez grandes, et leur surface se trouve couverte de stries concentriques très-fines; on en compte environ trente-cinq rangées entre l'opercule et la base de la queue, et dix rangées entre le dos et le ventre. Il ne paraît pas exister de ligne latérale. L'anus s'ouvre immédiatement au devant de l'anale, sur le milieu de la longueur du corps.

Les pectorales naissent au-dessus de l'angle de l'opercule; leur longueur égale presque celle de la tête; elles sont pointues et de forme lancéolée. La dorsale très-petite, étant assez reculée en arrière, elle correspond au dernier tiers de l'anale. Cette nageoire, au contraire, est très-large et de moitié plus basse par derrière que par devant, où sa hauteur fait un peu plus de la moitié du corps aux pectorales. Les ventrales sont pointues, et placées au milieu de la distance comprise entre l'opercule et l'anus qu'elles atteignent lorsqu'elles sont couchées en arrière. La caudale n'est pas très-développée, et est tant soit peu échancrée à l'extrémité. B. 3; D. 6; A. 20; V. 7; P. 14; C. 14.

A l'état frais, ce poisson est d'un brun très-pâle, tirant au bleu sur les flancs, et passant au jaunâtre sur le ventre et vers la base de l'anale. L'anale, la caudale et la dorsale sont quelquefois variées de noirâtre.

On désigne ce poisson au Japon sous le nom de Medaka. Il y est très-commun dans les eaux stagnantes, telles que les champs de riz inondés, notamment en été.

V.

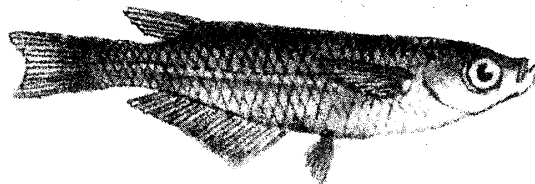


Fig. 1. The plate and the description of the medaka in *Pisces, Siebold's Fauna Japonica* (Temminck and Schlegel, 1846).

Brief history of medaka classification

The medaka (*Oryzias latipes*) was the first species of the Adrianichthyoidei described. It was originally assigned to *Poecilia* by Temminck and Schlegel (1846). Fig. 1 shows the plate and the description of the medaka in *Pisces*, Siebold's Fauna Japonica (1846). Subsequently, the generic position of *O. latipes* has been changed several times. Bleeker (1860) placed it in *Aplocheilus*. Jordan and Snyder (1906) designated the genus *Oryzias* in which to place this species because *Oryzias* has no teeth on the vomer while *Aplocheilus* does it. This was the first time when the generic name *Oryzias* was proposed.

The taxonomic position of *Oryzias* within atheriniform fishes has also been the subject to disagreement. Before 1964 *Oryzias* was widely regarded as a member of the family Cyprinodontidae. Other members of this family include the genera *Cyprinodon*, *Fundulus*, *Aplocheilus* and *Panchax*. Rosen (1964) drastically rearranged the taxonomic relationships of halfbeaks, killifishes and silversides. He proposed the new order Atheriniforms and the new family Oryziatidae (Oryziidae) with one genus *Oryzias*. Three Families (Oryziatidae [Oryziidae], Adrianichthyidae and Horaichthyidae) composed the Superfamily Adrianichthyoidea. The superfamilies Adrianichthyoidea and Cyprinodontidae were members of the Suborder Cyprinodontoidei. At the time medaka and its relatives were related to Cyprinodontiformes. Rosen and Parenti (1981) proposed new taxonomic relationships of *Oryzias* and the group of Atherinomorph fishes. Based on the gill arch skeleton and hyoid apparatus, they assigned *Oryzias* (ricefishes) and their allies to the Belontiiformes (fishes related to halfbeaks). The family Oryziatidae (Oryziidae) was incorporated into the family Adrianichthyidae. Nelson (1994) also agreed with this taxonomic relationships among Atherinomorph fishes.

Distribution of fishes of Adrianichthyoidei

Fishes of the Adrianichthyoidei are widely distributed from India to the Far East. Fig. 2 shows the distribution of these fishes. One lineage of Adrianichthyoidei is represented by a single species, *Horaichthys setnai* (Kulkarni, 1940), which is found along coastal India from near the Gulf of Kutch (northwest India) to Trivandrum near the southern tip (Nelson, 1994). Fishes in the other adrianichthyoid lineage have been assigned

to three nominal genera. There are 14 described species of *Oryzias*. *Oryzias malastigma* (McClelland, 1839) has a wide distribution throughout southern and southeast Asia, ranging from Pakistan, India, and Sri Lanka, to Burma and Thailand. *Oryzias javanicus* (Bleeker, 1854) is found in southern Thailand, the Malaysian Peninsula, Java, Borneo and Sulawesi (Uwa, 1990; Iwamatsu, 1993; Naruse *et al.*, 1993). *Oryzias minutillus* (Smith, 1945) is widely distributed in Thailand, southwestern China (Yunnan), and Burma (Rangoon) (Magtoon, 1991; Magtoon *et al.*, 1992; Magtoon *et al.*, 1995). *Oryzias mekongensis* described by Uwa and Magtoon (1986) is distributed in northeast Thailand. *Oryzias curvintus* (Nicolas and Pope, 1927) is found in south China and Hainan. *Oryzias luzonensis* (Herre and Ablan, 1934) is only distributed in southern part of Luzon. *Oryzias latipes* (Temminck and Schlegel, 1846) is distributed in Japan, Korea and China (Sakaizumi, 1990). *Oryzias celebensis* (Weber and de Beaufort, 1922), *O. nigrimas* (Kottelat, 1990b), *O. matanensis* (Aurich, 1935), *O. marmoratus* (Aurich, 1935), *O. orthognasus* (Kottelat, 1990a), and *O. profundicola* (Kottelat, 1990b), are found in lakes and rivers of central and southern Sulawesi. Three other adrianichthyoid species have been assigned to the genus *Xenopoecilus*: *X. poptae* (Weber and de Beaufort, 1922), *X. saracinorum* (Popta, 1905) and *X. oophorus* (Kottelat, 1990a). These species are also found exclusively in the lakes of central Sulawesi. Another adrianichthyoid, *Adrianichthys kruyti* (Weber, 1913), also inhabits Lake Poso in central Sulawesi. Thus 11 of the 19 species of Adrianichthyoidae are found in Sulawesi (Naruse *et al.*, 1993). Fig. 2b shows the distribution of fishes of Adrianichthyoidei in Sulawesi. With the exception of *O. celebensis*, each adrianichthyoid species in Sulawesi is endemic to a single lake. Despite extensive field surveys in 1991 and 1992, two endemic species, *Xenopoecilus poptae* and *Adrianichthys kruyti*, were not found and may be extinct (Kottelat, 1990a; Naruse *et al.*, 1993). *Oryzias timorensis* (Weber and de Beaufort, 1922) is distributed on the island of Timor, Indonesia. The karyotype and taxonomic relationships of this species are not clear. *Horaichthys* is unique among atherinomorphs in the production of a spermatophore and in possessing internal fertilization (Grier, 1984; Grier and Collette, 1987). *Horaichthys* inhabits both fresh and brackish waters (Nelson, 1994), as do *Oryzias*

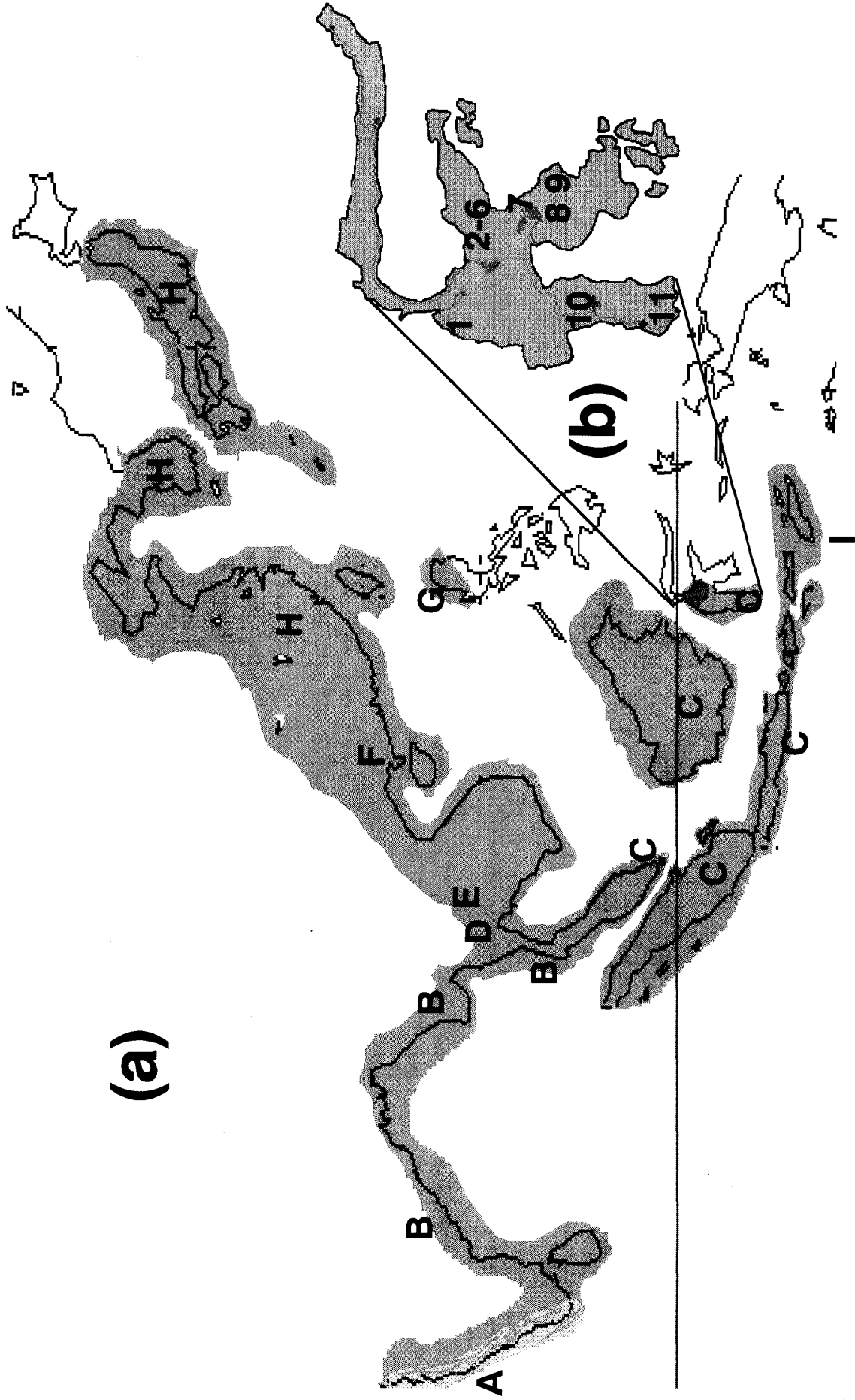


Fig. 2. Distribution of fishes of Adrianichthyoidei
 (a) Distribution of fishes of Adrianichthyoidei in Asia.
 A: *Horrichthys setnai*, B: *Oryzias malastigma*, C: *O. javanicus*, D: *O. minutillus*, E: *O. mekongnensis*, F: *O. curvinotus*, G: *O. luzonensis*, H: *O. latipes*, I: *O. timorensis*.
 (b) Distribution of fishes of Adrianichthyoidei in Sulawesi.
 1: *Xenopoecilus saracinorum*, 2: *X. poptae*, 3: *X. oophorus*, 4: *Adrianichthys kruyti*, 5: *O. nigrimas*, 6: *O. orthognasus*, 7: *O. matanensis*, 8: *O. marmoratus*, 9: *O. profundicola*,
 10: *O. celebensis*, 11: *O. javanicus*.

malastigma and *O. javanicus* (Uwa, 1990). All other adrianichthyoid species are confined exclusively to fresh waters.

Phylogeny of the Adrianichthyoidae

(a) Karyological analysis of fishes in the Adrianichthyoidae

Goodrich (1927) was the first to comment on chromosome number in *Oryzias latipes*, noting that in metaphase plates of spermatocyte divisions varied from 22 to 24. Using testis as material, the precise chromosome number in *O. latipes* was conclusively established by Iriki in 1932, who concluded that the haploid set of chromosomes was 24 (Iriki, 1932a, b). There are several reports on karyotypes of *O. latipes* using direct method (Ojima and Hitostumachi, 1969; Arai, 1973) but detailed karyotypes were not established until 1981. Establishment of cell culture methods have aided the production of detailed karyotypic analysis (Ojima, 1983). Detailed karyotypes of *O. latipes* were determined using cell culture, air dry method, and banding technique (NORs and C banding) (Uwa and Ojima, 1981).

Most karyological studies of adrianichthyoid fishes were done by Uwa and his colleagues (Uwa, 1986, 1990; Formacion and Uwa, 1985; Uwa and Dudgeon, 1988; Uwa and Iwata, 1981; Uwa and Jeon, 1987; Uwa and Ojima, 1981; Uwa and Parenti, 1988; Uwa *et al.*, 1983). Table 1 summarizes karyotypes of fishes in Adrianichthyoidae.

The basic chromosome number of *Oryzias* was determined to be 48 (Uwa, 1986, 1990). Anuploidy and polyploidy have not been observed (Uwa, 1990).

Uwa divided *Oryzias* species into three groups according to their karyotypes. First, there is a "mono-armed" chromosome group, in which the basic karyotype is 48 acrocentric chromosomes (chromosome number is 48, arm number is 48). The members of this group have subtelo- and acrocentric chromosomes. Three species, *O. javanicus*, *O. melastigma* and *O. minutillus*, comprise this group (Magtoon, 1986, 1991; Uwa, 1986, 1990; Uwa and Iwata, 1981; Magtoon *et al.*, 1992, 1995).

The second group is the "bi-armed" chromosome group which is characterized by meta and submetacentric chromosomes. The basic chromosome number of this group is 48 (chromosome number is 48, arm number varied from 58 to 96). These results show that chromosomal change in this group results mainly from peri-centric inversions. The members of this group are *O. latipes*, *O. curvinotus*, *O. luzonensis* and *O. mekongnensis* (Uwa, 1986, 1991a; Uwa and Magtoon, 1986). *Oryzias mekongnensis* was found and described by Uwa and Magtoon in 1986. *Oryzias latipes* is divided into four populations, northern, southern, eastern Korea and China-west Korea, based on allozyme variations (Sakaizumi, 1984, 1986a, b, 1990; Sakaizumi and Jeon, 1987; Sakaizumi *et al.*,

Table 1. Grouping by karyotypes and conservation of OLR1 in fishes of the Adrianichthyoidei

Group name	Species		Karyotypes	ORL1
Mono-armed chromosome group	<i>Oryzias melastigma</i>	48	24A	—
	<i>Oryzias javanicus</i>	48	24A	—
	<i>O. minutillus</i>	28	7M+1SM+6A	—
		30	6M+1SM+8A	—
		32	5M+1SM+10A	—
		34	4M+1SM+12A	—
		40	1M+1SM+18A	—
42	21A	—		
Bi-armed chromosome group	<i>O. mekongnensis</i>	48	1M+4SM+12ST+7A	+
	<i>O. latipes</i>	46	3M+9SM+2ST+9A	++
		48	2M+8SM+1ST+13A	++
	<i>O. curvinotus</i>	48	4M+13SM+5ST+2A	++
	<i>O. luzonensis</i>	48	24M&SM	++
Fused chromosome group	<i>O. celebensis</i>	36	4M+2SM+12A	—
	<i>O. nigrimas</i>	38	3M+2SM+14A	—
	<i>O. marmoratus</i>	42	1M+2SM+1ST+17A	—
	<i>O. matanensis</i>	42	1M+2SM+18A	—
Not classified	<i>X. saracinorum</i>	?	?	—
	<i>X. oophorus</i>	?	?	—

1980, 1987). Fishes of the China-west Korea population have 46 chromosomes (Uwa, 1986, 1990; Chen *et al.*, 1989) compared with 48 observed in fishes of other populations. This difference may have been caused by centric fusion in ancestors of the China-west Korea population.

The third group is the "fused" chromosome group. Fishes of this group possess one to four pairs of large meta- or submetacentric chromosomes in their metaphase plates. The basic chromosome number varies from 36 to 42, and the arm number is 48 (Uwa, 1992; Naruse *et al.*, 1993), indicating that the origin of the fused chromosome may have been mainly caused by centric fusion. The members of this group are *O. matanensis*, *O. nigrimas*, *O. marmoratus* and *O. celebensis*. Karyotypes of *Adrianichthys*, *Xenopoecilus*, *Oryzias timorensis*, *O. profunpicola* and *O. orthognasus* are not well documented. Fig. 3 shows the metaphase plate of *Xenopoecilus saracinorum* which have two pairs of large metacentric chromosomes. According to the criteria used in chromosomal grouping outlined above, *X. saracinorum* should be viewed as a member of the fused chromosome group.

Karyotypic evolution of *Oryzias minutillus* has proved to be a case study in chromosomal evolution. *Oryzias minutillus* was first reported to be a member of the fused chromosome group due to the presence of large metacentric chromosomes (Uwa, 1986; Magtoon and Uwa, 1985). Following field surveys in Thailand and southwestern China, however, karyotype polymorphism were observed. Whereas specimens from peninsular Thailand, the

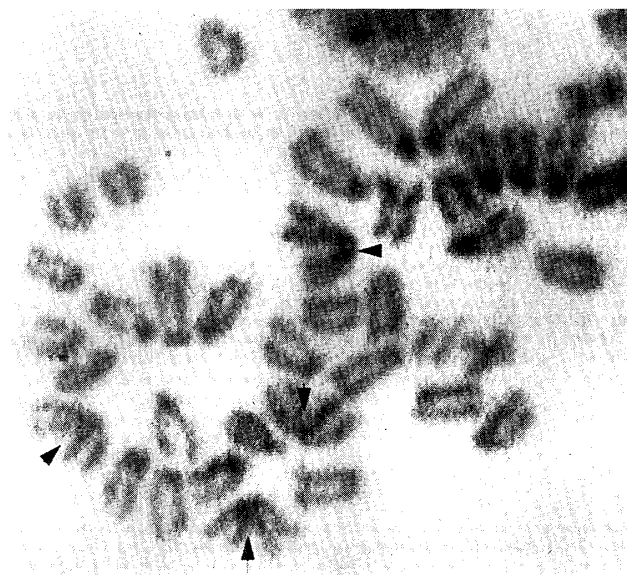


Fig. 3. A metaphase plate obtained from an embryo of *Xenopoecilus saracinorum*. Arrow-heads indicate the large metacentric chromosomes.

Mekong region and southern China (Yunnan) possess 42 acrocentric chromosomes (Uwa 1991b; Magtoon *et al.*, 1992, 1995; Uwa *et al.*, 1988), specimens from central Thailand (the Chao Phraya region) are characterized by the presence of large metacentric and NORs-chromosomes which are presumed to be the result of centric fusion and pericentric inversion. In short, chromosome numbers varied from 28 to 42. In addition, these chromosomal changes have only occurred in populations along the Chao Phraya river. By combining isozyme (Takata, 1991; Takata *et al.*, 1993) and karyotype analyses (Uwa, 1991b; Magtoon *et al.*, 1992), Uwa (1991b) and Magtoon *et al.* (1992) hypothesized the evolutionary pathway of *O. minutillus*. *O. minutillus* bearing the basic karyotype is widely distributed in the Indo-China peninsula. In Thailand this species may have been geographically isolated and differentiated into three subpopulations, the Peninsular, Mae Nam Chao Phraya and Mekong groups. In the Mae Nam Chao Phraya group, several karyotype changes, mainly centric fusion, must have occurred. Populations in the Peninsular Thailand and Mekong region groups have maintained the basic karyotype until now.

(b) Molecular phylogenetic analysis of fishes in the Adrianichthyoidae

Sakaizumi (1985a, b; 1990) has reported the phylogenetic relationships among five species (*O. javanicus*, *O. melastigma*, *O. latipes*, *O. luzonensis* and *O. celebensis*) using allozyme variation and the expression of parvalbumins. These studies indicate that the fishes of *Oryzias* examined are divided into three groups: the *O. javanicus* and *O. melastigma* group, the *O. latipes* and *O. luzonensis* group and the *O. celebensis* group. There are three kinds of parvalbumin bands (MP2, MP3 and MP4) in the fishes of *Oryzias* examined. *O. javanicus* and *O. melastigma* have only the MP2 band, while *O. latipes* and *O. luzonensis* have both MP2 and MP3 bands. *O. celebensis* has both MP4 and MP2 and weakly expressed MP3 bands (Sakaizumi, 1985b, 1990). Expression patterns of parvalbumins suggest the same results as obtained by allozymic variation (Sakaizumi, 1985a, 1990). These groupings coincide with the grouping by karyotype.

DNA sequence data is very useful for investigation of the phylogenetic relationships (Nei, 1987). Phylogenetic relationships among members of Adrianichthyoidae, especially *Oryzias* and *Xeno-*

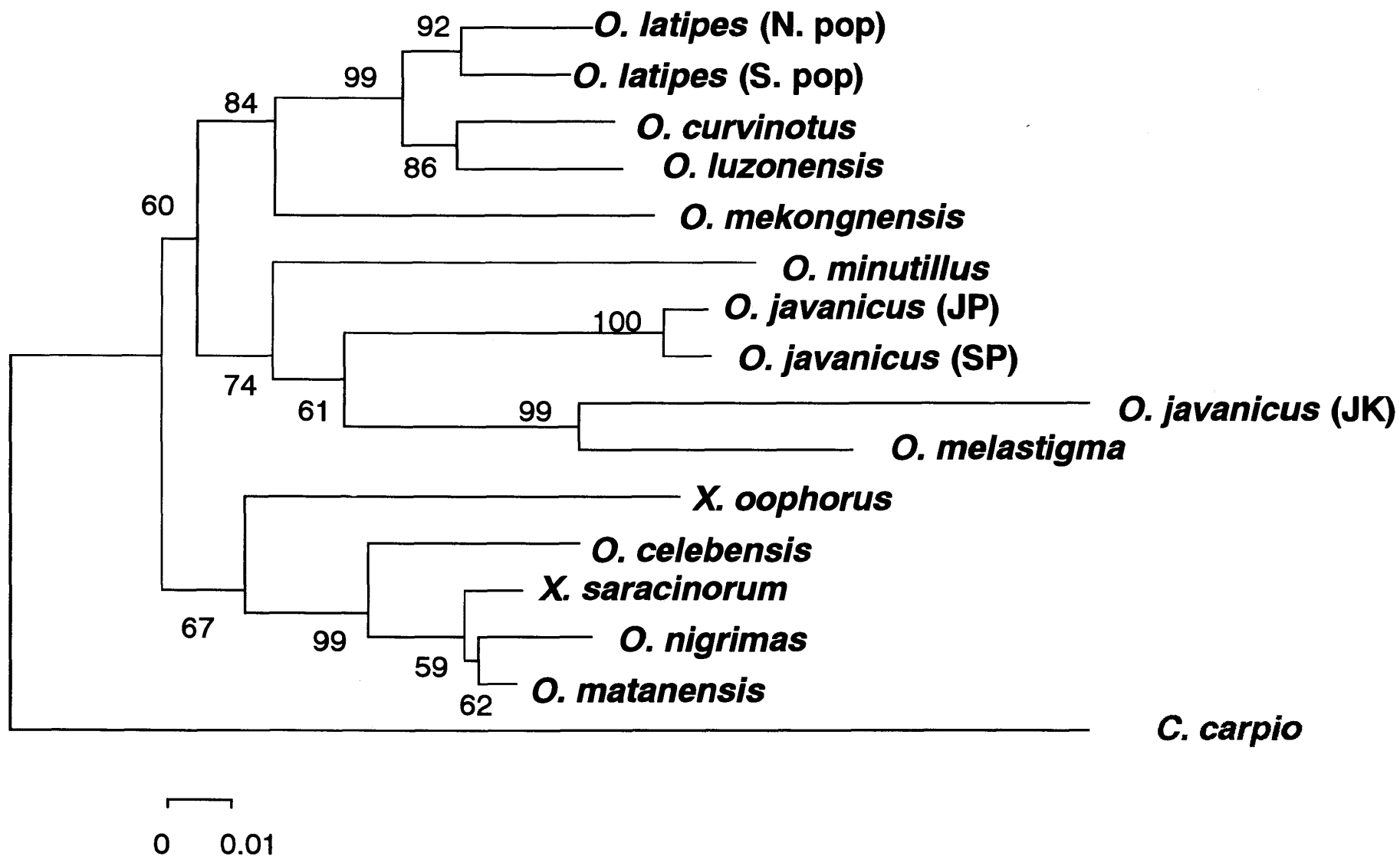


Fig. 4. Phylogenetic tree by neighbor-joining on genetic distance among 12s ribosomal RNA gene sequences for fishes of Adrianichthyoidei. Bootstrap test was performed (1,000 replications). Numbers at each branch represent bootstrap percentages. Scale bar indicates 0.01 unit of tree length. SP means fish collected from Singapore. JK means fish from Jakarta and JP means fish from Jenepono, Sulawesi. Carp is used for an outer group.

poecilus, have been studied using variation in the mitochondrial cytochrome b (Naruse *et al.*, 1993) and 12s ribosomal RNA gene sequences. Fig. 4 shows a phylogenetic tree of 12 species of the Adrianichthyoidae using 12s ribosomal RNA gene sequences by neighbor-joining method (Saitou and Nei, 1987). Three clades are observed in fishes examined, corresponding to the groups identified by karyological analysis. A similar tree topology was obtained by phylogenetic analysis using cytochrome b sequences.

The phylogenetic position of *Xenopoecilus* is especially interesting. Results of phylogenetic analyses indicate that *Xenopoecilus* is a member of the fused chromosome group of *Oryzias*. There are two explanations for this. It is possible that there has been introgression of *Oryzias* mitochondria into *Xenopoecilus*. It is also possible that *Xenopoecilus* is derived from *Oryzias*. We observed large metacentric chromosomes in the metaphase plates in *X. saracinorum*. (see Fig. 3). If the karyotypes reflect the phylogenetic relationship of *X. saracinorum* as shown in the study of *Oryzias* phylogeny, *Xenopoecilus* is not a sister group of the genus *Oryzias*. The sequences related to OLR1 [short interspersed elements from the *O. latipes* nuclear genome (Naruse *et al.*, 1992)] were conserved in the bi-armed chromosome group, and no other fishes of Adrianichthyoidae have OLR1-related sequences (see Table 1). This result supports the monophyly of the bi-armed chromosome group on nuclear DNA basis. Combined analyses of nuclear markers like allozymes and nuclear DNA sequence data, as well as morphology, will be necessary to determine the actual position of fishes of *Xenopoecilus*.

Acknowledgements

I would like to thank Prof. Yamagami and Dr. Yasumasu of Sophia University for help on DNA sequencing and Mr. Z. Wang for help on karyotype analysis. I thank Prof. Shima of University of Tokyo and late Prof. Uwa of Shinshu University for supporting the molecular phylogenetic study. Finally I thank Dr. J. Albert of Nippon Medical School for critical reading of this manuscript. This study is supported by grants-in-aid from the Ministry of Education, Science, Sports and Culture of Japan and Showa Seitoku Memorial Foundation.

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