Karyotypes and Geographical Distribution of Ricefishes from Yunnan, Southwestern China

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Abstract Morphometric and karyotypic studies were made on two species of ricefishes collected from Yunnan, southwestern China. Oryzias latipes from Yunnan had the same morphological and karyological characteristics as O. latipes collected from eastern China. The Yunnan populations had 2n, 46 chromosomes consisting of 3 metacentric, 8 submetacentric, 2 subtelocentric, and 10 acrocentric pairs, the arm number (NF) being 68 (2n=46, NF=68, 3M+8SM+2ST+10A). The karyotype was characterized by having a "large" metacentric pair and nucleolus organizer regions (NORs) on the short arms of a submetacentric pair. Oryzias minutillus from Yunnan had the same morphological characteristics as O. minutillus from Thailand and Burma, although the karyotype was different from that collected from Thailand. The Yunnan population had 2n, 42 chromosomes consisting of 21 acrocentric pairs, NF being 42 (2n=42, NF=42, 21A). The karyotype was characterized by having NORs at the telomeric regions of an acrocentric pair. Oryzias latipes occurs widely on the eastern Yunnan Plateau where the climate is temperate or subtropical, whereas O. minutillus is found in Xishiangbanna, the southern low mountain areas of Yunnan, where the climate is tropical.

Ricefishes of the genus Oryzias Jordan et Snyder are small atherinomorph fishes found widespread from India to Japan including the Indo-Australian Archipelago. They are common in ponds, ditches and paddy fields, and some species may inhabit brackish water. The eight species for which karyotype data are available can be divided karyologically into three groups: the monoarmed chromosome type, O. melastigma (McClelland) and O. javanicus (Bleeker); the biarmed chromosome type, O. latipes (Temminck et Schlegel), O. curvinotus (Nichols et Pope), O. luzonensis (Herre et Ablan) and O. mekongensis Uwa et Magtoon; and the fused chromosome type, O. celebensis (Weber) and O. minutillus Smith (Uwa et al., 1983; Uwa, 1986). The monoarmed and biarmed chromosome groups have a disjunct distribution, being found in western and eastern parts of Asia, respectively. The fused chromosome group is distributed between these two areas; O. celebensis in Sulawesi and O. minutillus in Thailand and Burma.

In continental Southeast and East Asia, the distribution of *Oryzias* seems to be closely correlated with river systems at their lower drainage areas; O. latipes in the Changjiang and Huanghe, O. curvinotus in the Xijiang and the Songkoi, O. mekongensis in the Mekong, and O. minutillus in

the Chao Phraya (Uwa, 1986; Uwa and Parenti, 1988). These river systems converge at their upper reaches in Yunnan, southwestern China. Therefore, investigation of ricefishes in Yunnan seems to be important in revealing the correlation between phylogeny and geographical distribution of this genus.

Yunnan is situated on the Yunnan-Guizhou Plateau (1,000–2,000 m above sea level) with complex geographical conditions. The land is high in the northwest and low in the southeast with great difference in altitude from over 5,000 m above sea level at high mountains to below 100 m in low river valleylands. The climate is complex and variable; it ranges from temperate or subtropical on the Plateau to tropical in Xishiangbanna, the southern low mountain area.

Yunnan has the upper sections of six river systems: the Jinshajiang (Changjiang), Nanpan-jiang (Xijiang), Yuanjiang (Songkoi), Lancang-jiang (Mekong), Nujiang (Salwin), and branches of Irrawaddy (cf. Fig. 4). The Changjiang, Mekong and Salwin exit from Qinghai-Tibet, run parallel across the high platform, then diverge to the east, southeast and southwest, respectively, at north-western Yunnan, and discharge into the East China Sea, South China Sea and Andaman Sea, respectively.

In the present paper, we describe the relationships of ricefishes in Yunnan on the basis of morphometric, karyological, and geographical data, and discuss the correlation between river systems and distribution of ricefishes in Yunnan and its lower drainage areas.

Materials and methods

Live and/or preserved specimens of two rice-fishes, O. latipes and O. minutillus collected from twelve localities of four river systems in Yunnan were used for morphological study. Preserved specimens collected from eastern China, Thailand and Burma were also examined for comparison. Preserved specimens examined are from the following institutions: KIZ, Kunming Institute of Zoology, Kunming; SU, Stanford University, now at California Academy of Sciences (CAS), San Francisco.

Specimens collected from Yunnan. Oryzias latipes-The Jinshajiang river system: 1) Huahongdong, Kunming, 2,100 m above sea level, coll. Y.-R. Chen, R.-F. Wang and H. Uwa, 1986, KIZ 8691989; 2) Qinghai, Xiangyun, 1604 m, coll. Y.-R. Chen and G.-H. Cui, 1986, KIZ 863950. The Nanpanjiang river system: 3) Yilonghu, Shibing, 1,410 m, coll. Y.-R. Chen and G.-H. Cui, 1985, KIZ 854095; 4) Changqiaohai, Mengze, 1,281 m, coll. Y.-R. Chen and G.-H. Cui, 1984, KIZ 8461001; 5) Mile, Mile, 1,400 m, coll. Y.-R. Chen, 1986, KIZ 8610001. The Yuanjiang river system: 6) Jingdong, Jingdong, 1,400 m, coll. Y.-R. Chen, R.-F. Wang and H. Uwa, 1986, KIZ 8681133. The Lancangjiang river system: 7) Jiangwei, Eryuan, 1,974 m, coll. Y.-R. Chen and G.-H. Cui, 1986, KIZ 8631016; 8) Haidong, Dail, 1,974 m, coll. Y.-R. Chen, R.-F. Wang and H. Uwa, 1986, KIZ 8611021; 9) Yangbi, Yangbi, 1,550 m, coll. Y.-R. Chen and J.-X. Yang, 1986, KIZ 8661104; 10) Pure, Pure, 1,390 m, coll. Y.-R. Chen, R.-F. Wang and H. Uwa, 1986, KIZ 8681122.

Oryzias minutillus—The Lancangjiang river system: 11) Menghai, Xishiangbanna, 1,000 m, coll. Y.-R. Chen, R.-F. Wang and H. Uwa, 1986, KIZ 8681891; 12) Menghan, Xishiangbanna, 600 m, coll. X.-L. Chu and Y.-R. Chen, 1973, KIZ 73527.

Specimens collected from eastern China, Thailand and Burma. *Oryzias latipes*—The Changjiang and Huanghe river systems: Beijing, presented by L.-Y. Liu, KIZ 8681965; Xiangjiang, Hunan, presented by L.-Y. Liu, KIZ 8681970.

Oryzias minutillus—The Chao Phraya and Irrawaddy river systems: Bangkok, Thailand, coll. W. Magtoon and H. Uwa, 1984, KIZ 8404281; Ayuttaya, Thailand,

coll. M. Kottelat, 1985, KIZ, 853383; Rangoon, Burma, coll. A. W. C. T. Herre, 1940, SU 40208.

Straight-line measurements were taken from the tip of the snout to the posterior border of the operculum (head length, HL), anal opening (pre-anal length, PAL), point of flexture of the caudal fin (standard length, SL), posterior extent of the caudal fin (total length, TL), origin of the dorsal fin (predorsal-fin length, PDFL), and origin of the anal fin (preanal-fin length, PAFL) as described by Uwa and Parenti (1988). Measurements were taken under a dissecting microscope to the nearest 0.25 mm with a metric ruler and are reported as a percentage of the SL.

Counts of dorsal-fin rays (DFR), anal-fin rays (AFR), and branched caudal fin rays (br-CFR) were taken on alcoholic specimens and were confirmed, when possible, on cleared and stained specimens according to the counterstaining method of Dingerkus and Uhler (1977).

Live specimens of O. latipes collected from Kunming, Dali, and Jingdong, and O. minutillus from Menghai were used for chromosome study. Chromosome preparations were obtained by cell culture procedures from adult fins or whole embryos (Uwa and Ojima, 1981) and/or direct airdried method from gill arches (Arai, 1973). A simple direct method from the gill epithelium was also applied for the field work according to the methods of Rivlin et al. (1985). Colcemid and colchicin were added to culture medium (10 µg/ml) and aquarium water (50 µg/ml), respectively, 4 to 6 hours prior to the KCl treatment. KCl hypotonization, acetic alcohol fixation and Giemsa staining were carried out as usual (Uwa and Ojima, 1981). For the detection of nucleolus organizer regions (NORs), slides were stained according to the procedure described by Howell and Black (1980).

Results

Morphometric and meristic data of two rice-fishes collected from Yunnan, eastern China, Thailand and Burma are summarized in Table 1. Ricefish collected from ten localities on the Plateau and its western margin in Yunnan had an ordinary body size (20–24 mm SL) at maturation. This species was identified as *O. latipes* on the basis of two morphometric characters; a relatively long abdominal part (55–57% PAL and 58–60%

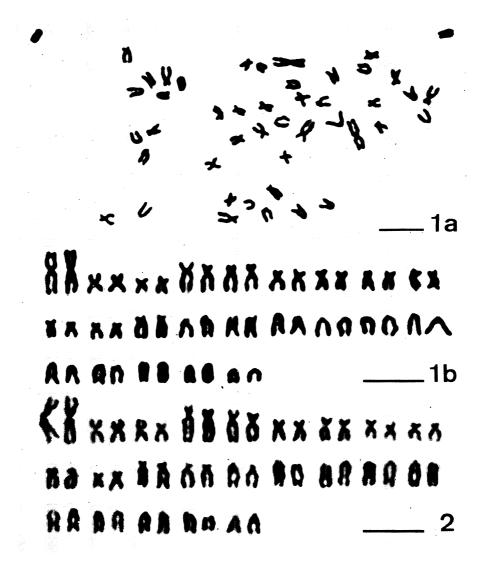


Fig. 1. A metaphase chromosome figure and its karyotype of *Oryzias latipes* from Kunming (1a, b) and a karyotype of *O. latipes* from Dali (2). Bars represent 5 μ m.

PAFL) and an anteriorly situated origin of the dorsal fin (78% PDFL), along with meristic and morphological characters; DFR 6, AFR 16-18, and br-CFR 9, and a parallelogram-shaped anal fin in the male which was formed by the elongation of the posterior rays (Uwa and Parenti, 1988). No notable morphological difference was detected between *O. latipes* from Yunnan and *O. latipes* from Beijing and Hunan, eastern China.

Karyotype study revealed that O. latipes from Kunming and Dali had 2n, 46 chromosomes con-

sisting of 3 metacentric, 8 submetacentric, 2 subtelocentric, and 10 acrocentric chromosome pairs, the arm number (NF) being 68 (Fig. 1). The karyotype had a "large" metacentric pair and NORs were located on the short arms of a submetacentric pair (Fig. 2). *Oryzias latipes* collected from Jingdong also had 2n, 46 chromosomes which was characterized by a "large" metacentric pair.

Ricefish obtained from Menghai and Menghan in Xishiangbanna, southern Yunnan, had a small body size (13–17 mm SL) at maturation. This

Table 1. Proportions and counts of the ricefish genus *Oryzias* from Yunnan and its lower drainage areas. Measurements are given as an average with standard deviation and counts given as a mode with range in parentheses. As for abbreviations, see text. * Eastern China; ** Thailand; *** Burma.

Species and locality	N	SL (mm)	No. of DFR	No. of AFR	No. of br-CFR	HL (%)	PAL (%)	TL (%)	PDFL (%)	PAFL (%)
O. latipes										
Kunming	30	20.1 ± 1.2	6 (6)	18 (16-20)	9 (9–10)	24.7 ± 1.2	54.7 ± 1.3	121 ± 1	77.5 ± 1.3	58.1 ± 1.5
Xiangyun	20	24.2 ± 2.6	6 (6–7)	17 (17–19)	9 (9–11)	24.1 ± 1.2	55.3 ± 2.7	$122\!\pm\!2$	78.3 ± 1.3	59.9 ± 2.9
Yilonghu	7	23.2 ± 2.8	6 (6–7)	18 (16–19)	9 (9–11)	24.4 ± 1.0	56.6 ± 2.8	122 ± 1	78.1 ± 1.3	60.7 ± 2.6
Mengze	7	17.1 ± 1.3	6 (6)	16 (16–18)	9 (9)	25.1 ± 0.9	57.0 ± 1.6	123 ± 1	78.3 ± 1.1	59.9 ± 1.8
Mile	1	15.8	6	17	9	25.0	59.0	127	79.0	62.0
Jingdong	30	22.2 ± 1.8	6 (6–7)	18 (16–20)	9 (9–10)	24.3 ± 1.0	54.7 ± 1.9	$122\!\pm\!2$	77.7 ± 1.4	59.0 ± 1.9
Eryuan	20	22.4 ± 1.4	6 (6–7)	17 (16–19)	9 (8–10)	24.5 ± 1.1	55.6 ± 2.0	123 ± 2	77.7 ± 1.2	58.9 ± 2.2
Dali	30	24.3 ± 2.0	6 (6–7)	18 (16–20)	9 (8–10)	24.3 ± 1.1	55.5 ± 1.5	120 ± 2	77.9 ± 1.2	58.3 ± 1.6
Yangbi	3	23.8	6 (6)	17 (16–17)	9 (9–10)	24.0	56.7	123	78.0	59.0
Pure	11	20.4 ± 1.4	6 (6–7)	18 (17–19)	9 (9–10)	24.5 ± 1.2	55.9 ± 2.3	122 ± 1	78.3 ± 1.4	60.3 ± 2.9
Beijing*	5	25.8 ± 2.1	6 (6)	17 (17–18)	9 (8-9)	22.4 ± 1.1	57.6 ± 2.9	120 ± 3	78.3 ± 1.1	61.8 ± 3.3
Hunan*	2	28.1	6 (6)	18 (18)	_	22.5	57.0	_	78.5	61.0
O. minutillus										
Menghai	30	16.8 ± 1.4	6 (5–6)	19 (18–21)	7 (7–9)	23.5 ± 1.1	50.0 ± 1.4	124 ± 2	80.2 ± 1.0	54.8 ± 1.8
Menghan	4	14.4	6 (6)	20 (17–20)	7 (7–8)	22.3	50.0	126	79.3	54.0
Bangkok**	6	12.0 ± 0.9	6 (5–6)	20 (18–21)	7 (7)	23.3 ± 1.6	49.8 ± 0.8	125 ± 2	82.8 ± 1.9	54.0 ± 1.1
Ayuttaya**	10	11.8 ± 1.2	6 (5–6)	18 (17–19)	7 (7)	24.0 ± 1.6	52.2 ± 2.4	125 ± 4	82.5 ± 2.2	56.7 ± 2.3
Rangoon***	21	15.2 ± 0.9	5 (5-6)	18 (17–20)	7 (6–7)	23.9 ± 1.8	50.9 ± 1.6	$123\!\pm\!2$	81.3 ± 1.8	55.8 ± 1.3

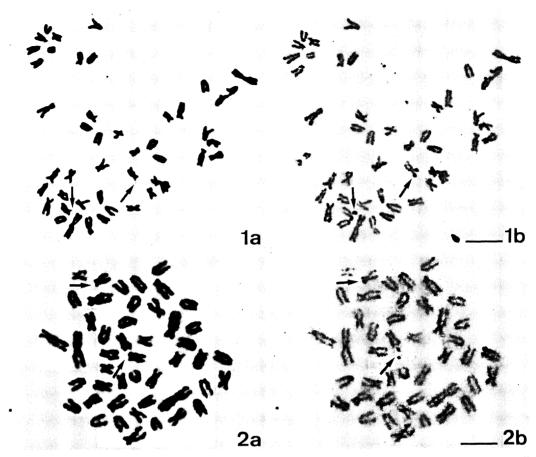


Fig. 2. Metaphases and their silver staining figures of Oryzias latipes from Kunming (1a, b) and Dali (2a, b). NORs are located at the short arms of a metacentric chromosome pair (arrows). Bars represent 5 μm.

small species was identified as *O. minutillus* on the basis of two morphometric characters; a relatively short abdominal part (50% PAL and 54–55% PAFL) and a posteriorly located origin of the dorsal fin (79–80% PDFL), along with meristic and morphological characters; DFR 6, AFR 20, and br-CFR 7, and a right-angled triangle-shaped anal fin in the male which was formed by the elongation of the anterior rays (Uwa and Parenti, 1988). A small number of branched caudal-fin rays (br-CFR 7 in modal) was a derived character for this species. No appreciable morphological difference was detected between *O. minutillus* from Yunnan and *O. minutillus* from Bangkok and Ayuttaya in Thailand, and Rangoon in Burma.

Oryzias minutillus from Menghai had 2n, 42 acrocentric chromosomes and NORs were located

at the telomeric regions of an acrocentric pair (Fig. 3). The karyotype had no "large" biarmed chromosome which is a karyotypical character of *O. minutillus* from Thailand (Magtoon and Uwa, 1985).

Geographical distribution of two ricefishes in Yunnan is shown in Fig. 4. Oryzias latipes occurs in drainage basins of the Jinshajiang (A), Nanpanjiang (B), and Yuanjiang (C); the main part of the Yunnan Plateau where the climate is temperate or subtropical. This species is also found in drainage basins of the Lancangjiang (D) near the western margin of the Plateau; around Erhai Lake and Pure where branches of the Yuanjiang and Lancangjiang become complex. Localities of O. latipes in Yunnan are: Kunming (1), Xiangyun (2), Yilonghu (3), Mengze (4), Mile (5), Jingdong

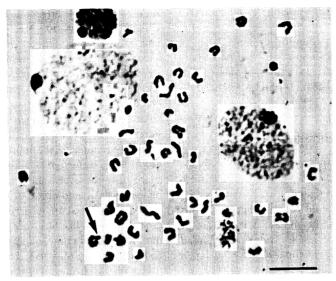


Fig. 3. A silver staining metaphase figure of *Oryzias minutillus* from Menghai, Xishiangbanna. The arrow shows NORs. Bar represents $5 \mu m$.

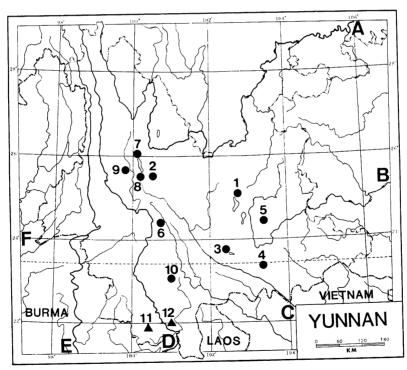


Fig. 4. Geographical distribution of *Oryzias latipes* (solid circles) and *O. minutillus* (solid triangles) in Yunnan. River systems are: A, Jinshajiang (Changjiang); B, Nanpanjiang (Xijiang); C, Yuanjiang (Songkoi); D, Lancangjiang (Mekong); E, Nujiang (Salwin); F, branches of Irrawaddy. Localities are: 1, Kunming; 2, Xiangyun; 3, Yilonghu; 4, Mengze; 5, Mile; 6, Jingdong; 7, Eryuan; 8, Dail; 9, Yangbi; 10, Pure; 11, Menghai; 12, Menghan.

(6), Eryuan (7), Dali (8), Yangbi (9), and Pure (10) as shown in Fig. 4.

Oryzias minutillus is found in drainage basin of the Lancangjiang (D) in southern Yunnan where the climate is tropical. Localities of this species from Yunnan are Menghai (11) and Menghan (12) in Xishiangbanna.

Discussion

In the present study, two species of ricefishes, O. latipes and O. minutillus, are recognized from Yunnan, southwestern China.

Oryzias latipes is found on the Plateau and its western margin where the climate is temperate or subtropical. This species is widely distributed throughout eastern Asia; China, Korea, and Japan (Uwa and Parenti, 1988). Oryzias latipes so far studied can be karyotypically divided into two groups; the Japanese and the East Korean populations (2n=48, NF=68-70) and the Chinese and the West Korean populations (2n=46, NF=68-70; Uwa and Ojima, 1981; Uwa, 1986; Uwa and Jeon, 1987). This division is consistent with divisions suggested by allozymic studies (Sakaizumi, 1986; Sakaizumi and Jeon, 1987). The karyotype of O. latipes from Yunnan (2n=46,NF=68, 3M+8SM+2ST+10A) had the same characteristics as that of O. latipes from eastern China; i.e. 2n, 46 chromosomes and the presence of a "large" metacentric pair. This "large" metacentric pair could have been derived from two pairs of acrocentric chromosomes through centric fusion, and consequently, chromosome number decreased to 46 from the original 48 (Uwa, 1986). From these results, we infer that O. latipes from Yunnan is a member of the Chinese population.

Oryzias minutillus is found from Xishiangbanna, southern Yunnan where the climate is tropical. This small species has been reported from Thailand (Smith, 1945; Magtoon, 1986) and Burma (present study, material collected by Herre). No remarkable difference was detected morphologically among specimens from Yunnan, Thailand and Burma; they share the relatively short abdominal part, posteriorly situated origin of the dorsal fin, and a small number of branched caudal-fin rays.

Magtoon and Uwa (1985) considered O. minutillus from Thailand to be a member of the fused chromosome group, since specimens from

Bangkok (2n=34, NF=44, 4M+1SM+12A) and Chiang Mai (2n=30, NF=44, 6M+1SM+8A)had four and six "large" metacentric pairs, respectively. However, present results showed specimens from Yunnan identified as O. minutillus had 2n, 42 acrocentric chromosomes (2n=42, NF=42, 21A), and no "large" biarmed chromosome. From morphometric data, Uwa and Parenti (1988) have pointed out that O. minutillus is closely related to the monoarmed chromosome group, O. melastigma and O. javanicus; they share the relatively short abdominal part and posteriorly situated origin of the dorsal fin. It has been assumed that 2n, 48 acrocentric chromosomes (2n=48, NF=48, 24A) are to be recognized as the basic model complement of the ricefish genus Oryzias (Uwa et al., 1983; Uwa, 1986) as in many other teleost groups (Ebeling and Chen, 1970; Ojima, 1983). We postulate that the karyotype of specimens from Yunnan is basic for O. minutillus, and consequently, O. minutillus is a member of the monoarmed chromosome group. Although this hypothesis remains to be tested, karyotype evolution by centric fusion may have occurred in parallel in O. minutillus in Thailand and O. celebensis in Sulawesi.

From the freshwater ichthyofauna, Chu (1986) divided six river systems in Yunnan into four subdivisions; the Jinshajiang, Nan-Yuan, Lancangjiang, and Nu-Irrawaddy. The Jinshajiang and Nan-Yuan subdivisions are closely related and the Lanceangjiang and Nu-Irrawaddy are also related. Distribution of ricefishes in Yunnan is consistent with these interrelationships of river systems; O. latipes occurs in the Jinshajiang and Nan-Yuan subdivisions, whereas O. minutillus is found in the Lancangjiang subdivision. Oryzias latipes inhabiting branches of the Lancangjiang around Erhai Lake and Pure may be a remnant population caused by the river capture between the Lancangjiang and Yuanjiang (Li, 1981).

Geographical distribution of ricefishes in Yunnan and its lower drainage areas is correlated with the river system and climate. In the Changjiang river system, O. latipes occurs from the upper section in Yunnan (Jinshajiang) to the lower drainage area in eastern China (Uwa and Parenti, 1988) where the climate is temperate or subtropical. In the Xijiang and Songkoi river systems, O. latipes inhabits their upper sections in Yunnan (Nanpanjiang and Yuanjiang) where

the climate is temperate or subtropical, while O. curvinotus is found in the lower drainage basins in southern China and Vietnam (Uwa and Parenti, 1988) where the climate is tropical. Oryzias curvinotus is morphologically similar to O. latipes but is distinguised by having a posteriorly situated origin of the dorsal fin (Uwa and Parenti, 1988). In the Mekong river system, O. latipes is found in branches of the Lancangjiang in Yunnan where the climate is temperate or subtropical, whereas O. minutillus and O. mekongensis inhabit southern Yunnan and northeastern Thailand (Uwa and Magtoon, 1986), respectively, where the climate is tropical. Orvzias minutillus is common in drainage area of the Chao Phraya (Magtoon, 1986), and is also collected from the lower reach of the Irrawaddy (present study), where the climate is tropical. From these facts Oryzias latipes seems to be adapted to the temperate and subtropical climates, and other species to the tropical climate.

Uwa (1986) suggested that the karyotype evolution in the biarmed chromosome groups of *Oryzias* is well correlated with the geographical distribution of this group in eastern Asia. We postulate that the present geographical distribution of ricefishes in Yunnan and its lower drainage areas was caused by the geological history of river systems and climates in Southeast and East Asia.

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中国雲南省に産するメダカ属魚類の核型と地理的分布 宇和 紘・王 蕊芳・陳 銀瑞

中国南西部の雲南省には2種のメダカ, Oryzias latipes と O. minutillus が, 温帯・亜熱帯性気候の高原地域と, 熱帯性気候の南部低山地域に, それぞれ生息している. 雲南省産 O. latipes の核型は、2n=46, NF=68, 3M+ 8SM+2ST+10A であった. 動原体融合により生じたと 思われる"大型"の中部着糸型染色体が1対あり、核小 体形成部位 (NORs) は次中部着糸型染色体の短腕末端部 に位置していた. 雲南省産 O. minutillus の核型は, 2n= 42, NF=42, 21A で, NORs は端部着糸型染色体の末 端部に位置していた. 雲南省産 O. latipes は形態、核 型ともに中国東部産のものと同じ特徴を持っており、同 じグループに属するものと思われる. 雲南省産 O. minutillus は形態的にはタイ・ビルマ産のものと区別でき ないが、その核型は単腕染色体型で, 染色体融合型であ るタイ国産のものとは異なっていた. この種は、計測的 には単腕型染色体グループに近い、雲南省産 O. minutillus の単腕染色体型の核型がこの種の核型の基本型で, タイ国産 O. minutillus の核型はこれから生じたのでは あるまいか. 雲南省およびその下流域に生息するメダカ 属魚類の地理的分布は, この地域の水系と気候に密接に 関連していると思われる.

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