A Study on Morphology, Cytogenetics and Mitochondrial DNA Sequences of Ricefish, *Oryzias* in Thailand

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Introduction

Ricefish in the genus *Oryzias* are small mostly freshwater fish commonly found in the ponds, ditches, and paddy fields associated with rice paddy ecoagroecological systems. They are endemic in Asia and 28 species have been reported from India to Japan and throughout the Indo-Australian Archipelago [1, 2]. *Oryzias* has become one of the important model organisms due to its short life cycle, small size, oviparity and breeding capacity in laboratories and artificial ponds. *Oryzias* also provides good material for studies of species differentiation and geographical distribution of freshwater fishes in Asia because various species of *Oryzias* are distributed from tropics to the temperate regions. The first species of the genus *Oryzias* to be described was *O. latipes* (or medaka in Japan) which has been studied in many areas of biological research by Japanese and European scientists. Recently, other species of *Oryzias* have been employed to examine morphology, embryology and development, karyotype, isozyme, nucleotide sequences as well as sex determination and geographical distribution.

Thailand is located in the tropical region and has excellent biodiversity. Smith [3] and Vidthayanon *et al.* [4] studied freshwater fishes in the country and found 17 orders, 56 families and 570 species. Taxonomists, including Parenti, Kotellat and Roberts [2, 5, 6] identified specimens mainly based on morphological characters.
Table 1. Ranges and modes of selected morphometric characters in ricefish, *Oryzias* species in Thailand.

<table>
<thead>
<tr>
<th>Species</th>
<th>SL</th>
<th>*HL</th>
<th>*PAFL</th>
<th>*PDFL</th>
<th>*LAFB</th>
<th>*CPD</th>
<th>*BD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. javanicus</em></td>
<td>22.3-30.9 (26.6)</td>
<td>21.2-34.2 (27.3)</td>
<td>48.2-63.0 (58.1)</td>
<td>65.7-84.0 (80.6)</td>
<td>22.0-36.7 (31.2)</td>
<td>10.1-18.5 (12.2)</td>
<td>18.0-29.5 (25.5)</td>
</tr>
<tr>
<td><em>O. dancena</em></td>
<td>21.6-35.6 (28.4)</td>
<td>15.1-33.3 (27.6)</td>
<td>52.5-69.9 (58.8)</td>
<td>65.5-92.1 (80.5)</td>
<td>29.7-39.2 (34.1)</td>
<td>10.7-15.9 (13.1)</td>
<td>16.7-35.5 (28.6)</td>
</tr>
<tr>
<td><em>O. haugiangensis</em></td>
<td>13.4-19.7 (16.6)</td>
<td>23.6-29.6 (25.5)</td>
<td>49.0-64.5 (54.3)</td>
<td>80.5-101.4 (82.9)</td>
<td>29.9-39.7 (32.6)</td>
<td>10.2-12.7 (11.0)</td>
<td>20.1-29.0 (23.4)</td>
</tr>
<tr>
<td><em>O. minutillus</em></td>
<td>8.7-12.9 (10.2)</td>
<td>20.1-25.2 (23.2)</td>
<td>46.6-54.1 (50.8)</td>
<td>76.6-84.6 (79.9)</td>
<td>28.4-34.9 (31.6)</td>
<td>8.3-10.6 (9.1)</td>
<td>16.2-21.0 (18.6)</td>
</tr>
<tr>
<td><em>O. mekongensis</em></td>
<td>12.6-20.4 (16.1)</td>
<td>20.9-25.9 (23.5)</td>
<td>53.4-60.1 (55.9)</td>
<td>74.1-80.5 (77.0)</td>
<td>19.0-29.5 (25.1)</td>
<td>8.1-11.5 (9.8)</td>
<td>17.4-23.6 (19.8)</td>
</tr>
<tr>
<td><em>O. songkhramensis</em></td>
<td>12.9-19.0 (15.8)</td>
<td>17.5-29.4 (25.0)</td>
<td>42.1-69.9 (57.9)</td>
<td>57.8-82.7 (77.1)</td>
<td>19.4-30.0 (25.6)</td>
<td>6.2-13.4 (9.8)</td>
<td>9.6-22.3 (17.2)</td>
</tr>
</tbody>
</table>

Remarks: SL, standard length (mm); HL, head length; PAFL, preanal fin length; PDFL, predorsal fin length; LAFB, length of anal fin base; CPD, caudal peduncle depth; BD, body depth. *Proportions were expressed in percentage of standard length (SL).

Table 2. Ranges and modes of selected meristic characters in ricefish, *Oryzias* species in Thailand.

<table>
<thead>
<tr>
<th>Species</th>
<th>A</th>
<th>D</th>
<th>Pel</th>
<th>Pec</th>
<th>Pc</th>
<th>Ca</th>
<th>BR</th>
<th>SLS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. javanicus</em></td>
<td>18-26 (23)</td>
<td>6-8 (7)</td>
<td>5-6 (6)</td>
<td>11-12 (11)</td>
<td>10-11 (10)</td>
<td>17-19 (19)</td>
<td>5</td>
<td>27-30</td>
</tr>
<tr>
<td><em>O. dancena</em></td>
<td>22-25 (24)</td>
<td>7-8 (7)</td>
<td>6</td>
<td>10-12 (12)</td>
<td>10-11 (11)</td>
<td>17-19 (19)</td>
<td>5</td>
<td>27-28</td>
</tr>
<tr>
<td><em>O. haugiangensis</em></td>
<td>19-22 (21)</td>
<td>7</td>
<td>6</td>
<td>10-11 (10)</td>
<td>10-11</td>
<td>17-19 (19)</td>
<td>5</td>
<td>27-31</td>
</tr>
<tr>
<td><em>O. minutillus</em></td>
<td>17-21 (19)</td>
<td>5-6 (6)</td>
<td>4-5 (5)</td>
<td>7-9 (7)</td>
<td>9-11 (10)</td>
<td>14-17 (15)</td>
<td>3-5 (4)</td>
<td>26-29 (28)</td>
</tr>
<tr>
<td><em>O. mekongensis</em></td>
<td>14-17 (14)</td>
<td>6-7 (6)</td>
<td>5-6 (6)</td>
<td>7-8 (7)</td>
<td>10-12 (11)</td>
<td>15-17 (16)</td>
<td>3-4 (4)</td>
<td>29-32 (30)</td>
</tr>
<tr>
<td><em>O. songkhramensis</em></td>
<td>15-16 (15)</td>
<td>5-7 (6)</td>
<td>5-6 (6)</td>
<td>7-8 (7)</td>
<td>11-12 (12)</td>
<td>16-17 (16)</td>
<td>4</td>
<td>27-29 (27)</td>
</tr>
</tbody>
</table>

Remarks: A, anal-fin rays; D, dorsal-fin rays; pel, pelvic-fin rays; Pec, pectoral-fin rays; Pc, abdominal vertebrae; Ca, caudal vertebrae; BR, brachiostegal rays; SLS, scale in lateral series.
Figure 1  Illustration of Oryzias in Thailand: A, Oryzias javanicus; B, O. dancena; C, O. haugiangensis; D, O. minutillus; E, O. mekongensis and F, O. songkhramensis. Scale bars indicate 5 mm. (Adapted from Magtoon and Termvidchakorn, 2009 [9]).
In Thailand six species of *Oryzias* have been recorded. They are *O. minutillus* Smith 1945; *O. mekongensis* Uwa and Magtoon, 1986; *O. javanicus* (Bleeker, 1854); *O. dancena* (Hamilton, 1822); *O. huagianensis* Robert, 1998 and *O. songkhramensis* Magtoon, 2010 [7, 8]. Based on maximum adult body size these species were divided into two groups. The first group comprising *O. minutillus, O. mekongensis, O. songkhramensis* and *O. huagianensis* had standard lengths (SL) smaller than 26 mm while the second group containing *O. dancena* and *O. javanicus* had larger body size [2]. In addition, *Oryzias* in the first group are usually found in lowlands or flood plains inhabiting ponds, brooks, ditches and mangrove forests in the southeastern Thailand whereas those in the second group are found in brackish water or tidally influenced areas of mangrove forests in Peninsular Thailand. Cytogenetic data and mitochondrial DNA sequences may also be used to distinguish ricefish species.

The objectives of this article are (i) to explain the interspecific variation of *Oryzias* and intraspecific variation of *O. minutillus* (ii) to describe the cytogenetics of *Oryzias* and (iii) to examine mitochondrial DNA (mtDNA) control region of *Oryzias* in Thailand.

**Morphological analysis of *Oryzias***

Mature *Oryzias* species in Thailand have similar proportion in the relative standard length (SL) or head length (HL). The six species occurring in Thailand (Fig. 1) were separated by adult body sized into two groups, one comprising *O. minutillus, O. mekongensis, O. songkhramensis* and *O. huagianensis*, and the other containing *O. dancena* and *O. javanicus*. Specimens in the former group are smaller than 26 mm SL while those in the latter are larger than 26 mm SL, and the ranges of the two groups showing no overlap (Table. 1). The two groups are called small-sized and large-sized groups, respectively, hereafter. No differences in the body size were observed between males and females within each species.

Variation of five meristic counts for the six species are shown in Table. 2. For *O. minutillus, O. mekongensis* and *O. songkhramensis*, the modal number of dorsal-fin rays was 6 without any individual variation, whereas for *O. dancena, O. javanicus*, and *O. huagianensis* the modal numbers of dorsal-fin rays were 7 or 8 rays. The anal-fin ray count was subject to more or less great variations in each of six species. The modal number of anal-fin ray count was smallest in *O. mekongensis* (14), followed by *O. songkhramensis* (15), *O. minutillus* (19), *O. huagianensis* (21), *O. javanicus* (23) and *O. dancena* (24). The modal number of pectoral-fin ray count was 12 in *O. dancena*, 11 in *O. javanicus*, 10 in *O. huagianensis*, and 7 in *O. songkhramensis, O. mekongensis* and *O. minutillus*. The modal number of caudal vertebral count was smallest in *O. minutillus* (15), followed by *O. mekongensis* and *O. songkhramensis* (16), and *O. huagianensis, O. javanicus* and *O. dancena* (19). For the
branchiostegal counts in six species of *Oryzias* the results were as follows: 4 in *O. mekongensis*, *O. songkramensis* and *O. minutillus*, 5 in *O. javanicus*, *O. dancena* and *O. haugianensis*.

Interestingly, intraspecific variation of *Oryzias minutillus* populations within the various regions of Thailand were observed. The canonical discriminant analysis (CDA) of *O. minutillus* based on 22 morphometric proportions being almost entirely separated into different areas (Fig. 2). A dendrogram revealing the relationships among *O. minutillus* was constructed on the basis of the morphometric characters (Fig. 3). From the dendrogram *O. minutillus* was clearly divided into three groups, namely Chao Phraya, Mekong and Peninsular groups.

**Figure 2**  Plot of discriminant scores on the first and second canonical (CAN) axes based on 22 morphometric characters of Chao Phraya population (solid circles); Peninsular population (open circles); and Mekong population (open square).

**Figure 3**  Dendrogram of eight species of *Oryzias minutillus* in Thailand by paired group average method based on 22 morphometric characters.
For the meristic counts, significant differences of *O. minutillus* were found in four meristic characters, including the number of anal-and dorsal-fin rays, and abdominal and caudal vertebrae. This fish in the Mekong and Peninsular basins had a fewer anal-fin rays [16-20(17) and 16-19 (18), respectively] than the Chao Phraya population [17-21(19)], differing significantly from one another. On the other hand, the modal number of dorsal-fin rays of *O. minutillus* in the Chao Phraya and Mekong basins was 6, which was different from that of the Peninsular basin (5). The mode of caudal vertebrae counts of the Chao Phraya, Peninsular and Mekong basins were 15, 16, and 17 respectively, also differing significantly from one another. The mode of abdominal vertebrae of the Chao Phraya basin was 10, which differed from that of the Peninsular and Mekong basins (9).

**Karyological analysis of *Oryzias***

Karyotypes of five known *Oryzias* in Thailand are summarized in Table 3.

*Oryzias javanicus* from Ranong province in the Peninsular region had 2n = 48 chromosomes. The karyotype consisted of 1 subtelocentric and 23 acrocentric chromosome pairs. The arm number was 48. Nucleolus organizer regions (NORs) were located on the secondary constriction of an acrocentric chromosome pair.

**Table 3 Chromosome constitution of *Oryzias* in Thailand**

<table>
<thead>
<tr>
<th>Region</th>
<th>Species</th>
<th>Locality</th>
<th>2n</th>
<th>NF</th>
<th>Constitution (pairs)</th>
<th>NORs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peninsular</td>
<td><em>O. dancena</em></td>
<td>Bangban Ranong</td>
<td>48</td>
<td>48</td>
<td>24A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td><em>O. javanicus</em></td>
<td>Bangban Ranong</td>
<td>48</td>
<td>48</td>
<td>1ST+23A</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td><em>O. minutillus</em></td>
<td>Narathiwat</td>
<td>42</td>
<td>42</td>
<td>21A</td>
<td>A</td>
</tr>
<tr>
<td>Mekong</td>
<td><em>O. minutillus</em></td>
<td>Phimai</td>
<td>42</td>
<td>42</td>
<td>21A</td>
<td>A</td>
</tr>
<tr>
<td>Southeastern</td>
<td><em>O. mekongensis</em></td>
<td>Kalasin</td>
<td>48</td>
<td>58</td>
<td>1M+4SM+12ST+7A</td>
<td>SM</td>
</tr>
<tr>
<td></td>
<td><em>O. minutillus</em></td>
<td>Chachoengsao</td>
<td>42</td>
<td>44</td>
<td>1SM+20A</td>
<td>SM</td>
</tr>
<tr>
<td>Chao Phraya</td>
<td><em>O. haugianensis</em></td>
<td>Trat</td>
<td>48</td>
<td>52</td>
<td>2ST+22A</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>O. minutillus</em></td>
<td>Chai Nat</td>
<td>30</td>
<td>44</td>
<td>6M+1SM+8A</td>
<td>SM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ayuthaya</td>
<td>30</td>
<td>44</td>
<td>6M+1SM+8A</td>
<td>SM</td>
</tr>
</tbody>
</table>

**Remarks**: 2n, diploid chromosome number; NF, arm number; NORs, nucleolus organizer regions
*Oryzias dancena* from Bangban, Ranong province in the Peninsular region had 2n = 48 chromosomes. The karyotype consisted of 24 acrocentric chromosome pairs. The arm number was 48. NORs were located on the secondary constriction of an acrocentric chromosome pair.

*Oryzias mekongensis* from Kalasin province in the Mekong region showed 2n = 48 chromosomes. The karyotype consisted of 1 metacentric, 4 submetacentric, 12 subtelocentric and 7 acrocentric chromosome pairs. The arm number was 58. NORs were located on the satellited region of an acrocentric chromosome pair.

*Oryzias haugianensis* from Trat province in the Southeast region had 2n = 48 chromosomes. The karyotype consisted of 2 submetacentric and 22 acrocentric chromosome pairs. The arm number was 52.

*Oryzias minutillus* exhibited great diversity of karyotype associated with the geographical distribution.

Specimens of *O. minutillus* from Narathiwat province in the Peninsular region had 2n = 42 chromosomes. The karyotype consisted of 21 acrocentric chromosome pairs. The arm number was 42. NORs were located at telometric position of an acrocentric chromosome pair.

*Oryzias minutillus* from Phimai, Nakhon Ratchasima province had 2n = 42 chromosomes. The karyotype consisted of 21 acrocentric chromosome pairs. The arm number was 42. NORs were located at telometric position of an acrocentric chromosome pair.

*Oryzias minutillus* from Chachengsao province in the Southeastern region possessed 2n = 42 chromosomes. The karyotype consisted of 1 submetacentric and 20 acrocentric chromosome pairs. The arm number was 44. NORs were located at a submetacentric chromosome pair. In the same region, a specimen from Rayong basin had 2n = 40 chromosomes. The karyotype consisted of 1 metacentric, 1 submetacentric, and 18 acrocentric chromosome pairs. NORs were located on the submetacentric chromosome pair.

Specimens from Ayuthaya and Chinat provinces in the Chao Phraya region showed 2n = 30 chromosomes. The karyotype consisted of 6 metacentric, 1 submetacentric and 8 acrocentric chromosome pairs. The arm number was 44. NORs were located on a submetacentric chromosome pair.

*Oryzias* species thus far studied can be divided karyotypically into three groups: those with monoarmed chromosomes (subtelo-and acrocentric), biarmed chromosomes (meta-and submetacentric) and fused chromosomes (“large” meta-and submetacentric) [10]. According to this hypothesis, the monoarmed type is considered to be basic among *Oryzias*, and the biarmed and fused types seem to have developed from the monoarmed type through pericentric inversion and centric fusion, respectively. The karyotype of *O. javanicus* from Ranong province, in the
Peninsular region of Thailand was similar to the specimens from Singapore. The subtelocentric pair of chromosomes in *O. javanicus* from these localities seemed to have occurred by pericentric inversion from acrocentric chromosomes. Moreover, the chromosome number and the morphological characters indicated that *O. javanicus* has a close relationship with *O. dancena*. The karyotype analysis revealed that *O. dancena* and *O. javanicus* belonged to the monoarmed type, which was characterized by abundance of subtelo-and acrocentric chromosomes. Based on its karyotype *O. mekongensis* belonged to the biarmed type, which was characterized by abundance of meta-and submetacentric chromosomes. Other species include in this type were *O. curvinotus, O. sinensis,* and *O. luzonensis*.

A hypothetical karyotype evolution of *O. minutillus* in Thailand is illustrated in Fig. 4. Karyotypes of this species can be divided into two major categories: the basic and evolved types. The basic karyotype consisted of 2n = 42 acrocentric chromosomes. The arm number was 42 and NORs were of the acrocentric chromosome type (NF = 42, NORs-A), while the evolved karyotype had different arm number and NORs chromosome type (NF = 44, NORs-SM). The evolved karyotype can be subdivided into two stages depending on the chromosome rearrangements: more primitive stage (2n = 42-40, 1-2 large metacentric chromosomes) and more developed stage (2n = 34-28, 8-14 large metacentric chromosomes). The karyotype of *O. minutillus* (2n = 28, NF = 44) from Salween region belonged to the group of a more developed stage.

Figure 4  Dendrogram shows karyotype evolution of *O. minutillus* in Thailand. Karyotype evolution seems to be caused by pericentric inversion and centric fusion. Chromosomal rearrangements are: 1) pericentric inversion of NORs-chromosome pair, 2) centric fusion. S, South; NE, Northeast; SE, Southeast; N, North; C, Central (Magtoon *et al.*, 1992 [11]).
The first chromosome rearrangement that occurred in this species seemed to be a pericentric inversion of NORs-chromosome from acrocetric type to submetacentric type, which brought an increase in NF from 42 to 44 (2M + 40A). Centric fusion seemed to have occurred subsequently, resulting in the decrease in diploid number (2M + 2SM + 36A). These karyotypes were observed in the populations from the Bang Pakong and Ranong basins in the Southeastern region. Centric fusion, then, may have reoccurred, and other series of karyotype were formed (8M + 28SM + 24A to 14M + 2SM + 12A). These karyotypes were found in the population from the Chao Phraya region. The karyotype evolution of *O. minutillus* occurred in the drainage area of the Chao Phraya region and collaterals (Southeastern region), and developed in the Chao Phraya basin. However, the basic karyotype has been preserved allopatrically in the Peninsular region and the basin of the Mae Num Mun (a tributary of the Mekong) in the Mekong region.

**Molecular phylogenetic analysis of Oryzias**

The detailed electrophoretic studies of allozymes and muscle protein by Sakaizumi [12] indicated that the phylogenetic relationships among five species, *O. latipes, O. javanicus, O. dancena, O. luzonensis* and *O. celebensis* can be divided into three groups: the *O. javanicus*, *O. dancena* group, the *O. latipes* and *O. luzonensis* group and *O. celebensis* group. Therefore, these groups coincided with the three chromosomal groups proposed by Uwa [13]. The result of Naruse’s study [14] also supported these groups with moderate bootstrap support (BS = 67-84%) in the mtDNA phylogeny. Takehana et al. [15] further investigated molecular relationships among 13 species of *Oryzias* and 2 species of *Xenopoecilus* using nuclear and mitochondrial DNA sequences (Fig. 5). The results of this study showed that *Oryzias* species can be divided into three major groups, namely Lapipes, Javanicus and Celebensis. These three groups corresponded with the results previously reported based on karyotypic analysis. (i.e., monoarmed, biarmed and fused chromosome groups)

A recent phylogenetic analysis by Smitthikunanon et al. [16] based on the mitochondrial control region (domain II and III) of these five *Oryzias* using *Cololabis saira* as out group revealed a close relationship between *O. javanicus* and *O. dancena* (BS = 100%) with *O. minutillus* as a sister group. A study phylogenetic relationships based on nucleotide sequences of the partial mitochondrial control region (domain I) of *O. minutillus* from 11 localities in Thailand and one locality from Cambodia using *O. dancena* and *O. celebensis* as outgroup revealed two important clades. Clade I (BS = 100%) comprises *O. minutillus* from Tonle Sap basin (Surin, Sakaeo and Cambodia) and from the eastern Peninsular basin (Songkla and Nakhon Si Thammarat). Clade II (BS = 100%) consists of *O. minutillus* from the Chao Phraya basin (Suphanburi, Nakhonsawan, Saraburi and Ayuthaya), Phechaburi, Prachuaphirikhan and the upper Mekong basin (Chiang Rai) (Fig. 6).
**Figure 5** Hypothesis of relationships among 13 species of ricefishes based on a maximum parsimony analysis of nuclear and mitochondrial sequence data. The outgroup taxa are *Cololabis saira* and *Cypselurus pinnatibarbatis japonicus*. (Adapted from Takehana *et al.*, 2005 [15]).

**Figure 6** Single most parsimonious tree based on domain I sequences of *O. minutilius* from 12 localities. Numbers above branches indicate branch lengths and numbers in parentheses show bootstrap values of 1000 replicates (BS%). S, South; NE, Northeast; N, North; C, Central. (Adapted from Smittikunananon *et al.*, 2009 [16]).
Conclusions

Six species of the genus *Oryzias*, *O. javanicus*, *O. dancena*, *O. haugiangensis*, *O. minutillus*, *O. mekongensis* and *O. songkramensis* have been described in Thailand. The morphometric characters of *Oryzias* could be divided into two groups: small and large groups. *Oryzias javanicus* and *O. dancena* belonged to the large species and inhabited brackish water whereas *O. minutillus*, *O. mekongensis*, *O. songkramensis* and *O. haugiangensis* were smaller and inhabited freshwater except *O. haugiangensis* which inhabited brackish water. The canonical discriminate analyses (CDA) of the morphometric characters of *O. minutillus* showed that the populations within each river basin were clearly separated. Therefore, the dendrogram of the morphometric characters of *O. minutillus* populations were clearly divided into three groups, namely Chao Phraya, Mekong and Peninsular groups.

Information on karyotypes of *Oryzias* species could be divided into three chromosomal groups: the monoarmed chromosome group, *O. javanicus*, *O. dancena* and *O. minutillus*, with 2n = 48 and 2n = 42 acrocentric or subtelocentric chromosomes; the biarmed chromosome group, *O. mekongensis*, with 2n = 48 including metacentric and submetacentric chromosomes; and the fused chromosomes group, *O. cellebensis*, with 2n = 36 including “large” metacentric or submetacentric chromosomes [13]. Karotypical polymorphism of *O. minutillus* was first reported by Magtoon and Uwa [17]. They documented both isozyme [18] and karyotype analyses [19, 20] and hypothesized the evolutionary pathway of *O. minutillus*. In Thailand this species may have been geographically isolated and differentiated into three subpopulations; the Chao Phraya, Mekong and Peninsular groups.

A recent phylogenetic analysis based on the mitochondrial control region (domains II and III) of five *Oryzias* species, *O. dancena*, *O. javanicus*, *O. minutillus*, *O. mekongensis* and *O. cellebensis* yielded similar grouping. The analysis included *O. minutillus* from 11 localities of in Thailand and Cambodia. *Oryzias minutillus* can be divided into two clades. Clade I (BS = 100%) consisted of *O. minutillus* from Tonle Sap basin (Surin, Sakaeo and Cambodia). Clade II (BS = 100%) comprised *O. minutillus* from Chao Phraya basin (Suphan Buri, Nakhon Sawan, Saraburi and Ayuthaya), Phetchaburi, Prachuap Khiri Khan and upper Mekong basin (Chiang Rai). This phylogenetic study showed a tendency to classify *O. minutillus* according to their patterns of chromosomes as primitive type (2n = 40-42) in clade I and developed type (2n = 28-34) in clade II.
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References


