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| Title            | Primer Sequences and Cross-species Amplification for Parentage Discrimination of Tanganyikan Cichlid Fishes |
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| Citation         | 北海道大学水産科学研究彙報, 52(3), 131-133   |
| Issue Date       | 2001-12   |
| Doc URL          | <a href="https://hdl.handle.net/2115/21953">https://hdl.handle.net/2115/21953</a>                           |
| Type             | departmental bulletin paper   |
| File Information | 52(3)_P131-133.pdf  |



## Primer Sequences and Cross-species Amplification for Parentage Discrimination of Tanganyikan Cichlid Fishes

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### Abstract

It was done that isolation and cross-species amplification of microsatellite loci for parentage analysis of the Tanganyikan cichlids. The sequences of the primers and the results of cross-species amplification were reported.

**Key words:** Cichlidae, Tanganyika, cross-species amplification, microsatellite loci

Since the late 19th century, many ichthyologists have studied the radiation of cichlid fishes in Lake Tanganyika. There are more than 170 species of cichlids in Lake Tanganyika, most of which are endemic (Kohda et al., 1997), and Lake Malawi may contain even more species (Ribbink et al., 1983). We are studying the evolution of allopaternal care in the Tanganyikan cichlids, *Chalinochromis brichardi*, *Neolamprologus meeli*, *Julidochromis ornatus*, and *Telmatochromis temporalis*.

Microsatellite genotyping has proven to be a useful tool for phylogenetic (Lee and Kocher, 1996; Zardoya et al., 1996; Neff et al., 2000) and parentage analysis (e.g. Kellogg et al., 1995; Parker and Kornfield, 1996) of the cichlids. We designed six primer sets to amplify microsatellites in the four Tanganyikan species to search for genetic markers. Four primer sets (Pze1-4) from the Malawian cichlid fish, *Pseudotropheus zebra*, sequenced by van Oppen et al. (1997) were also tested in these four

species. We described the sequences of the primers and the results of test amplifications.

Pieces of muscle from fish collected in Lake Tanganyika were fixed in 70% ethanol. The genomic DNA for the four cichlid species studied was isolated from this tissue. The DNA was extracted with a standard phenol-chloroform procedure after a reaction in lysis buffer. The procedures for the construction of the genomic libraries, isolation of plasmids containing (GT/CA)<sub>n</sub> regions, and sequencing the plasmids were the same manner as those described in Munehara and Takenaka (2000). Four loci containing perfect repeats of more than (GT/CA)<sub>15</sub> were screened from each genomic library of *C. brichardi* and *N. meeli*, and used to design the PCR primers (Table 1). The level of polymorphism for each locus was estimated using 10-36 individuals from different families in each species. Species and cross-species amplification with our 4 primer

Table 1. Primer sequences of useful microsatellite loci of two cichlid fishes.

| Source                          | Code name | Primer sequence (5'-3') | Accession number |
|---------------------------------|-----------|-------------------------|------------------|
| <i>Chalinochromis brichardi</i> | Chb1      | TTCTTTCAGGCTCTAGCTTTCC  | AB012117         |
|                                 |           | AAGCTCAGAGTCTGCATGTGC   |                  |
| <i>C. brichardi</i>             | Chb2      | ATGTCAATTGAAGGTCCTAACT  | AB012118         |
|                                 |           | TCAGCAAACCTCAACTTAA     |                  |
| <i>Neolamprologus meeli</i>     | Nem1      | TGGTGCAATTCAAAACCTTTCTG | AB012119         |
|                                 |           | CCTTTCCTAACTTCTTGGGG    |                  |
| <i>N. meeli</i>                 | Nem2      | CACAAACCAAGCAGAGGCTTA   | AB012120         |
|                                 |           | CTTAACGAGGTCAAATCTGTTG  |                  |

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Table 2. Polymorphism of some microsatellite loci for three cichlid fishes. Amplifications more than 0.50 in heterozygosity were showed in this table.

| <i>Chalinochromis brichardi</i>  |                      |                             |                |                |          |             |
|----------------------------------|----------------------|-----------------------------|----------------|----------------|----------|-------------|
| Code name                        | Annealing temp. (°C) | Length of PCR products (bp) | No. of alleles | Heterozygosity |          | Sample size |
|                                  |                      |                             |                | observed       | expected |             |
| Chb1                             | 58                   | 88, 92, 110, 114, 116       | 5              | 0.60           | 0.56     | 30          |
| Nem2                             | 52                   | 134, 136, 142               | 3              | 0.70           | 0.57     | 30          |
| <i>Neolamprologus meeli</i>      |                      |                             |                |                |          |             |
| Code name                        | Annealing temp. (°C) | Length of PCR products (bp) | No. of alleles | Heterozygosity |          | Sample size |
|                                  |                      |                             |                | observed       | expected |             |
| Chb2                             | 54                   | 90, 96, 98, 100             | 4              | 0.55           | 0.66     | 30          |
| Nem1                             | 52                   | 101, 111, 113, 115          | 4              | 0.57           | 0.65     | 30          |
| Pze4                             | 54                   | 112, 114, 126, 128          | 4              | 0.70           | 0.62     | 30          |
| <i>Julidochromis ornatus</i>     |                      |                             |                |                |          |             |
| Code name                        | Annealing temp. (°C) | Length of PCR products (bp) | No. of alleles | Heterozygosity |          | Sample size |
|                                  |                      |                             |                | observed       | expected |             |
| Chb1                             | 61                   | range of 96–138             | 15             | 0.56           | 0.66     | 36          |
| Chb2                             | 54                   | range of 97–170             | 17             | 0.69           | 0.83     | 36          |
| Nem1                             | 50                   | range of 103–121            | 8              | 0.58           | 0.49     | 36          |
| Pze3                             | 50                   | range of 314–392            | 17             | 0.81           | 0.82     | 36          |
| <i>Telmatochromis temporalis</i> |                      |                             |                |                |          |             |
| Code name                        | Annealing temp. (°C) | Length of PCR products (bp) | No. of alleles | Heterozygosity |          | Sample size |
|                                  |                      |                             |                | observed       | expected |             |
| Pze2                             | 46                   | range of 152–222            | 20             | 0.88           | 0.93     | 34          |
| Pze4                             | 54                   | range of 122–144            | 11             | 0.76           | 0.84     | 34          |

sets and cross-species amplification with the 4 primer sets from the Malawian cichlid (van Oppen et al., 1997) were performed in a thermocycler, following the manufacturer's recommendation (Perkin-Elmer). An initial denaturation at 95°C for 5 min was performed before the addition of *Taq* DNA polymerase (TaKaRa Co.). The reaction was carried out for 23 or 25 cycles of 30 s at 94°C, 50 s at the annealing temperature (Table 2), and 80 s at 72°C. The procedures described in Munehara and Takenaka (2000) were used to detect PCR products. The length of the PCR products was estimated with 10 bp or 20 bp ladder markers and PCR products from individuals used for primer design.

Cross-species amplification successfully identified heterozygous alleles of *C. brichardi*, *N. meeli*, *J. ornatus*, and *T. temporalis*, and useful primer sets varied with species (Table 2). Successful cross-species amplifications with Pze 2, 3 and 4 suggested that primers from Tanganyikan and Malawian cichlid fishes could provide useful information for parentage analysis.

### Acknowledgments

The study was provided by the facilities for the field research by the Lake Tanganyika Research Unit, Department of Fisheries, Zambia, and financed by Creative Basic Research, a project funded by Ministry of Education, Science, Sports and Culture, Japan. This paper is contribution number 145 from the Usujiri Fisheries Station, Hokkaido University.

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