Jun 15, 2023

A comparative cytogenetic study of Hypsibarbus malcolmi and H. wetmorei (Cyprinidae: Tribe Poropuntiini)

DOI
https://dx.doi.org/10.17504/protocols.io.36wgq3r8klk5/v1

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Protocol Citation: Sudarat Khensuwan, alotan 2023. A comparative cytogenetic study of Hypsibarbus malcolmi and H. wetmorei (Cyprinidae: Tribe Poropuntiini). **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq3r8klk5/v1

Protocol status: Working
We use this protocol and it's working

Created: June 15, 2023

Last Modified: June 15, 2023

Protocol Integer ID: 83442

Keywords: chromosomal characteristics of hypsibarbus malcolmi, comparative cytogenetic study, molecular cytogenetics, chromosomal characteristic, freshwater perciform fish, hypsibarbus species, diploid chromosome number, cyprinidae, chromosome, karyotype, cytogenomic different pattern, genus osteochilus, cytogenomic different patterns across the genome, microsatellite, females fish, using different microsatellite motif, different microsatellite motifs as probe, telomeric regions of all chromosome, short arm of chromosome, cyprininae, hypsibarbus malcolmi, markers for specific evolutionary differentiation, signals on all chromosome, tribe poropuntiini, strong hybridization pattern in the centromeric region

Funders Acknowledgements:
National Research Council of Thailand (NCRT):
Grant ID: NRCT-RGJ63003-068

Abstract

The cyprinids, freshwater perciform fish belong to the subfamily Cyprininae. Among them, the genus *Osteochilus* contains 11 recognized valid species. Here, karyotype and chromosomal characteristics of *Hypsibarbus malcolmi* and *H.wetmorei* were examined applying conventional and nucleolar organizing region (NORs) staining with molecular cytogenetics.The diploid chromosome number (2*n*) of *H.malcolmi* was 50, fundamental number (NF) 62, and the karyotype displayed 8m +4sm +38 a with NORs being located at a centromeric and telomeric position of the short arms of chromosome pairs 1 and 2 ,respectively. 2*n* of *H.wetmorei* was 50, NF 67, karyotype 14m +14sm +22 a with the NORs at the telomeric position of the short arm of chromosome pairs 2. 2n and NF in males and females fish were identical. Fluorescence in situ hybridization using different microsatellite motifs as probes also showed substantial genomic divergence between both studied species. In *H. wetmorei* (CAG)n and (CAC)n microsatellites accumulated in the telomeric regions of all chromosomes, while in *H. malcolmi* they had scattered signals on all chromosome. Besides, the (GAA)n microsatellites were distributed along all chromosomes of *H. malcolmi*, but a strong hybridization pattern in the centromeric region of a single pair in *H. malcolmi,* but gave a strong hybridization pattern in the centromeric region of a single pair in *H. wetmorei.* These cytogenomic different patterns across the genomes of these *Hypsibarbus* species are markers for specific evolutionary differentiation inside these two species.

Classical and molecular Cytogenetics

1 **Animals**

Individuals of *H. malcolmi* (12♂ and 6♀) and *H. wetmorei* (8♂ and 8♀) were collected in the Mekong river basin (Thailand)(Fig 1.). Fishes were transferred to the laboratory and identified according to morphological criteria of Rainboth et al. (2012). Experiments were performed in accordance with ethical protocols, and anesthesia using clove oil (Eugenol 3%) prior to the euthanasia, as approved by the Ethics Committee of Khon Kaen University (Record No. IACUC-KKU-105/63). The specimens were deposited in the fish collection of the Cytogenetic Laboratory, Department of Biology Faculty of Science, Khon Kaen University (Thailand).

2 **Chromosome preparation, solidandNOR staining**

Mitotic chromosomes were obtained from the anterior kidney, following the drop onto microscopic slides and air-drynmethod to the visualize chromosomes (Bertollo2015). Conventional/solid staining was done by applying5% Giemsa solution in phosphate buffer (pH 6.8) to the spread chromosomes for 10 min. In addition, the distribution of NORs was visualized according to the standard protocol using silver (Ag) staining (Howell and Black 1980).

3 **Probe preparation and FISH experiments**

We used three microsatellite motif as probes: (CAG)10 (GAA)10, and (CAC)10. These sequences were directly labeled by Cy3 at the 5′endduring synthesis (Sigma, St. Louis, MO, USA) as described by (Kubat et al. 2008). FISH was performed under high stringency conditions and hybridization occurred overnight in a moist chamber at 37 °C (Sassi et al. 2023). Chromosomes were counterstained with 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI,1.2 µg/ml) mounted in antifade solution (Vector, Burlingame, CA, USA,).

4 **Image processing**

At least 20 metaphase spreads per individual were analyzed to confirm the diploid number, karyotype structure and FISH data. Images were captured using an Axioplan II microscope (Carl Zeiss Jena GmbH, Germany) with CoolSNAP and processed using Image Pro Plus 4.1 software (Media Cybernetics, Silver Spring, MD, USA). Chromosomes were classified according to centromere position as metacentric (m), submetacentric (sm) and subtelocentric (st) and acrocentric (a)(Levan et al. 1964). For the chromosomal arm number (NF;fundamental number) m+sm were scored as bi-armed while st + as mono-armed.