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Karyotype of the Australian nurseryfish, *Kurtus gulliveri* (Kurtidae : Perciformes)

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Abstract. We studied the chromosomes of *K. gulliveri* in order to describe its karyotype and investigate its ploidy. We determined a modal diploid chromosome number 2n=44 in both sexes of *K. gulliveri*. The chromosomes were very small, and only the largest three pairs were distinguishable morphologically. The largest chromosome pair was submetacentric while the rest were telocentric or acrocentric.

Keywords : Chromosomes, *Kurtus gulliveri*, nurseryfish, Meiosis, DAPI

Introduction

The perciform family Kurtidae comprises two species. The nurseryfish, *Kurtus gulliveri* Castelnau 1878, occurs in coastal rivers of northern Australia and southern New Guinea (Berra, 2003), and *K. indicus* Bloch 1786, occurs from India to Borneo (Berra, 2001). The nurseryfish is remarkable for its unique method of egg brooding, in which males carry the eggs on a supraoccipital hook (Berra and Humphrey, 2002) (Fig. 1). Recent fieldwork in the Adelaide River of the Northern Territory of Australia has begun to reveal the unique life history of this poorly known species (Berra *et al.*, 2004). The purpose of this paper is to describe the cytogenetic feature of *K. gulliveri*.

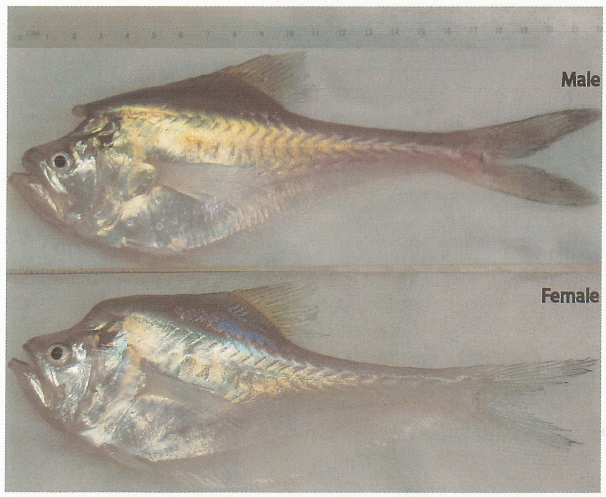

Figure 1. Photograph of live male and female *Kurtus gulliveri*, approximately 175 SL, male showing supraoccipital hook.

The reported diploid number of chromosomes in fishes ranges from 2n=12 (*Gonostoma bathyphilum*, Gonostomidae) to 2n=446 (*Diptychus dipogon*, Cyprinidae) (Bond, 1996 ; Klinkhardt *et al.*, 1995) with a majority having 2n=44-52 (Gold, 1979). Most perciforms have 2n= 46-48 (Denton, 1973 ; Gold *et al.*, 1980 ; Klinkhardt *et al.*, 1995). In this study, we describe the karyotype of *Kurtus gulliveri* based on specimens collected from the Adelaide River, Northern Territory, Australia. We compare the chromosomal numbers and karyotype of *K. gulliveri* with possibly related species and speculate on their phylogenetic relationship.

Materials and methods

Nurseryfish were collected by cast netting from the Adelaide River at the boat ramp at the Arnhem Highway bridge c. 65 km southeast of Darwin, Northern Territory, Australia. Additional specimens were collected by gill netting approximately 2.5 km upstream from the boat ramp in Marrakai Creek (12°40.950′S, 131°20.030′E), a major freshwater tributary to the Adelaide River. A map of the study area can be found in (Berra, 2003). Live fish were packaged in plastic bags containing river water, inflated with oxygen, placed inside a styrofoam box and transported to Canberra, Australia by overnight air-freight.

Mitotic metaphase chromosomes were prepared from

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solid tissues (kidney and spleen) from six males and five females *K. gulliveri* following the protocol described by Kligerman and Bloom (1977) with minor modifications. Fish were euthanized by overdose of 10% benzocaine (Sigma) solution and were dissected after the cessation of opercular movement. The tissues were cut into small pieces using a pair of fine scissors and placed in a hypotonic solution (0.075M KCl solution) and incubated for 45 min at room temperature. After 45 min of hypotonic treatment, the solution was discarded and the tissues were fixed with two changes of freshly prepared 3 : 1 methanol acetic acid.

For slide preparation, a small piece of tissue was removed from the fixative, blotted on absorbent paper and placed in the well of a depression slide. Two to three drops of 50% glacial acetic acid were added and the tissue was minced with fine forceps for about 1 min. Unsuspended tissue was returned to the fixative and a few more drops of 50% glacial acetic acid were added to the suspension. Using a P200 Gilson micropipette one drop of cell suspension was placed on to a clean slide heated to 45°C on a heat block. The cell suspension was left for about 8 s and then withdrawn back into the pipette leaving a ring of cells approximately 1 cm in diameter. Air-dried slides were incubated in 2X SSC for 3 min then stained with DAPI (4′-6-diamidino-2-phenylindole ; 80 ng/ml in 2X SSC) for one min and mounted with anti-fade medium Vectashield (Vector Laboratories).

Meiotic chromosomes from testicular tissue were prepared following the method described previously by Ezaz *et al.* (2005). Testes were dissected out on to a Petri dish and the testicular tunica was removed in calcium- and magnesium-free phosphate buffered saline and the seminiferous tubules cut into small pieces using a sterile scalpel blade. These tissues were incubated in 0.075M KCl for 30–45 min at 37°C or overnight at room temperature, and then fixed in 3 : 1 methanol : acetic acid. Cell suspension was prepared by dissolving a piece of tissue in equal volumes of freshly prepared 3 : 1 methanol : acetic acid and distilled water. The slides were prepared as described earlier.

Results and discussion

The DAPI stained mitotic karyotypes of four females

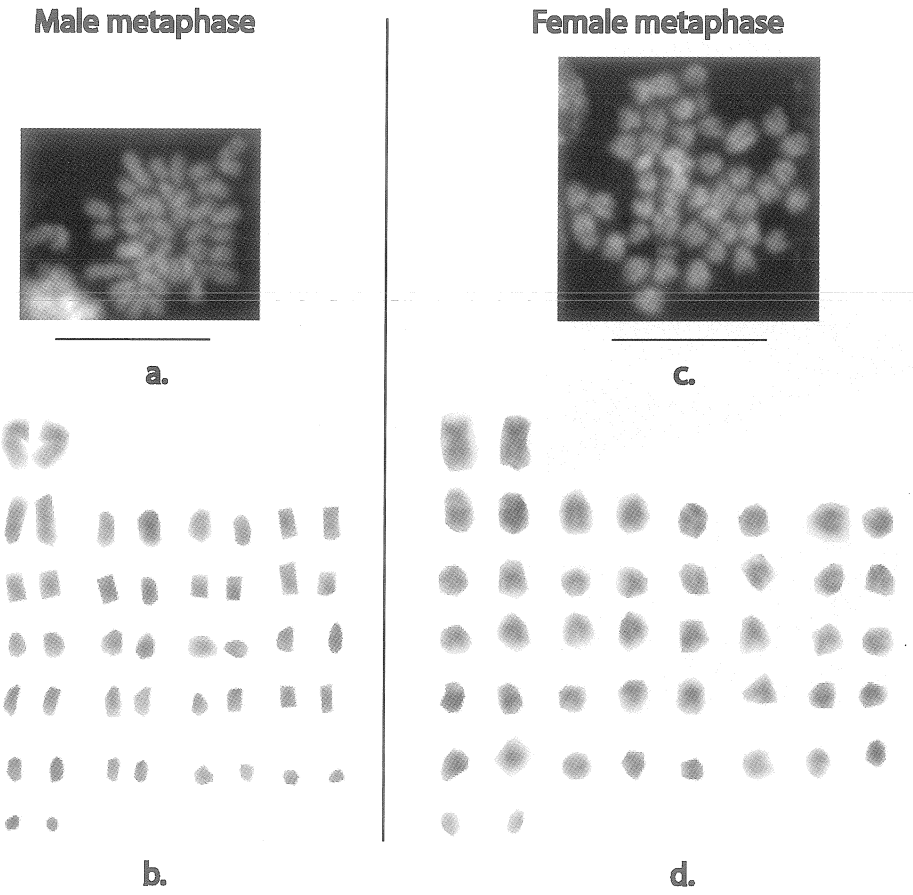

Figure 2. Chromosomes of *Kurtus gulliveri*. a : DAPI stained male metaphase chromosome spread ; b : male karyotype from the reversed image of the same metaphase ; c : DAPI stained female metaphase chromosome spread ; d : female karyotype from the reversed image of the same metaphase. Scale bar represents 10 μm.

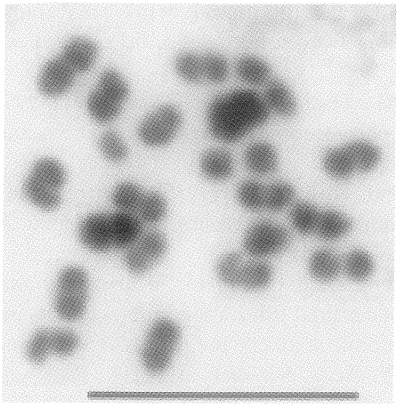

Figure 3. Male meiosis I (diakinesis) of *Kurtus gulliveri* showing 22 bivalents. Scale bar represents 10 μm.

and three males were examined. For each individual, a total of 15-20 mitotic chromosome spread at metaphase were examined. A modal diploid chromosome number of 2n=44 was detected in both sexes of *K. gulliveri*. The chromosomes were very small (the largest pair was approximately 4 μm while the smallest pair was only 0.5 μm), and only the largest three pairs were distinguishable morphologically. The largest chromosome pair was submetacentric while the rest were telocentric or acrocentric (Fig. 2). Comparison of the DAPI stained karyotypes from males and females did not reveal the presence of any morphologically differentiated sex chromosomes in either sex (Fig. 2).

The first meiotic division (diakinesis) was examined in a total of 20 cells from testis of two *K. gulliveri* males. All showed 22 bivalents, as expected, representing the 22 pairs of homologous chromosomes. No unpaired chromosome was observed, nor any bivalent in which chromosomes were conspicuously unpaired over part of their length, as might be expected for differentiated sex chromosomes (Fig. 3).

Although several studies of the morphology and anatomy of nurseryfish have been published, nothing was known about the chromosome morphology or ploidy status of this unique Australian fish. We have analysed the chromosomes of this species in order to understand the genome evolution. The taxonomic position of *Kurtus* is not yet clearly understood, but it has been suggested that it is close to the cardinalfishes, the family Apogonidae (Johnson, 1993), and molecular data for perciforms supports that phylogenetic position (Smith and Wheeler, 2006).

The seven apogonids that have been karyotyped so far have 2n=34-46 (Klinkhardt *et al.*, 1995; Ojima and Kojima, 1985: Rivlin and Dale, 1986; Rivlin *et al.*, 1987; Rivlin *et al.*, 1988) which suggests that the diploid number is not constant within the family. The most common diploid chromosome number in this group is 2n=36, however *Apogon nubilis* and *A. moluccensis* have 2n=46 (Klinkhardt *et al.*, 1995). It has been proposed that *A. nobilis* has a more ancestral perciform karyotype (2n=48), while the rest of the species have more derived karyotypes (Rivlin and Dale, 1986). The diploid chromosome complement 2n=44 in nurseryfish thus may represent a more primitive karyotype than that of some cardinalfishes. A comparative chromosome analysis would be required to establish that the karyotype of nurseryfish and species of the Apogonidae evolved from the same ancestral karyotype.

Smith and Wheeler (2006) indicated that *K. gulliveri* is sister group to a clade consisting of *Apogon atradorsatus* (Apogonidae), *Odontobutis potamophila* (Odontobutidae), and *Ratsiraki legendrei* (Eleotridae). Klinkhardt *et al.* (1995) cited four studies that listed 2n=44 for *Odontobutis obscurus*, and several studies showing other eleotrids with 2n=46. Our report that *K. gulliveri* has a diploid chromosome complement of 2n=44, combined with the recent molecular analysis of Smith and Wheeler (2006), brings us closer to an understanding of the evolutionary history of this bizarre species, and suggests that future phylogenetic work should examine the kurtid-apogonid-gobioid connection.

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