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Original Research Paper

Karyotype description of the African weakly electric fish *Campylomormyrus compressirostris* in the context of chromosome evolution in Osteoglossiformes

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ABSTRACT

Karyotyping is a basic method to investigate chromosomal evolution and genomic rearrangements. Sixteen genera within the basal teleost order Osteoglossiformes are currently described cytogenetically. Our study adds information to this chromosomal dataset by determining the karyotype of *Campylomormyrus compressirostris*, a genus of African weakly electric fish that has not been previously examined. Our results indicate a diploid chromosome number of 2n = 48 (4sm + 26m + 18a) with a fundamental number of FN = 72. This chromosome number is identical to the number documented for the sister taxon of the genus *Campylomormyrus*, *i.e.*, *Gnathonemus petersii* (2n = 48). These results support the close relationship of *Campylomormyrus* and *Gnathonemus*. However, the karyotype formula of *C. compressirostris* is different from *Gnathonemus petersii*, thereby confirming the high variability of karyotype formulae within the Mormyridae. We infer that the differences in chromosome number and formula of *Campylomormyrus* relative to other mormyrids may be caused by Robertsonian fusion and pericentric inversion. In addition to the karyotype description and classification of *Campylomormyrus*, a ChromEvol analysis was used to determine the ancestral haploid chromosome number of osteoglossiform taxa. Our results indicate a relatively conservative haploid chromosome number of n = 24 for the most recent common ancestor of Osteoglossiformes and for most of the internal nodes of osteoglossiform phylogeny. Hence, we presume that the high chromosome variability evolved recently on multiple independent occasions. Furthermore, we suggest that the most likely ancestral chromosome number of Mormyridae is either n = 24 or n = 25. To the best of our knowledge this is the first attempt to determine and classify the karyotype of the weakly electric fish genus *Campylomormyrus* and to analyze chromosomal evolution within the Osteoglossiformes based on Maximum Likelihood and Bayesian Inference analyses.

1. Introduction

Campylomormyrus, a genus of African weakly electric fish, has been intensively studied with regard to evolution, electric discharge, electric organ anatomy, and behavior (Feulner et al., 2007, 2009; Lamanna et al., 2015, 2016; Paul et al., 2015). The genus belongs to the ancient teleost order Osteoglossiformes. An understanding of phylogenetic relationships within Osteoglossiformes has been hampered by the evolutionary history of this order, its morphological heterogeneity, and its relatively low species diversity (in comparison to its sister taxon, the Elopocephala; Nelson, 1969; Bonde, 1996; Li et al., 1997; Hilton, 2003). Relationships within Osteoglossiformes were first inferred from anatomical and morphological traits (Greenwood et al., 1966; Nelson, 1969), while more recent studies have utilized genetic markers (e.g., Lavoué and Sullivan, 2004; Lavoué et al., 2011). Several phylogenetic hypotheses have been advanced for the order, suggesting different relationships within Osteoglossiformes (Bonde, 1996; Kumazawa and Nishida, 2000; Hilton, 2003). In the most recent phylogenetic analyses (Lavoué and Sullivan, 2004; Lavoué et al., 2011), there is strong support for the hypothesis that the family

Abbreviations: a, acrocentric; CI, centromeric index; CRND, Constant Rate with No Duplication; FN, Fundamental Number; m, metacentric; n, haploid chromosome number; p, short chromosome arm; q, long chromosome arm; PP, posterior probability; sm, submetacentric; 2n, diploid chromosome number.

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Pantodonidae is the sister group of the two osteoglossiform suborders Osteoglossoidei (Osteoglossidae) and Notopteroidei, the latter consisting of the families Notopteridae, Mormyridae, and Gymnarchidae. *Campylomormyrus*, a taxon that has recently diversified in an adaptive radiation (Feulner et al., 2007; Tiedemann et al., 2010), is part of the Mormyridae, the family with by far the highest species diversity (~180 species), while all other families are relatively species-poor (ranging from one species in Pantodonidae and Gymnarchidae to ten species in Notopteridae).

To date, 20 species from 16 genera of osteoglossiform fishes are described cytogenetically (Uyeno, 1973; Ozouf-Costaz et al., 2015; Table 1), but the karyotype of *Campylomormyrus* has not hitherto been described. Furthermore, previous studies of osteoglossiform karyotypes have mainly focused on chromosome morphology and karyotype formulae, but do not specifically address karyotype evolution (Suzuki et al., 1982; Marques et al., 2006; Krysanov and Golubtsov, 2014). Uyeno (1973) describes the chromosome formulae of nine osteoglossiform fishes, and hypothesizes a diploid chromosome number of 2n = 48 for the most recent common ancestor of Osteoglossiformes. This is supported by later studies in which the ancestral chromosome number of Teleostei is proposed to be 2n = 48–50 (Jaillon et al., 2004; Mank and Avise, 2006; Kohn et al., 2006; Nakatani et al., 2007). Here, we describe the first karyotype of a *Campylomormyrus* species (*C. compressirostris*) and compare it to the karyotype information from other Mormyrids as well as non-electrogenic osteoglossiforms. Additionally, we estimate the most likely ancestral chromosome number of Osteoglossiformes using Maximum Likelihood and Bayesian Inference methods to discuss patterns of chromosome number evolution. As this analysis requires a phylogenetic tree as input, we reconstructed a phylogeny of those Osteoglossiforms for which karyotypes are available, based on mitochondrial genomes.

2. Material and methods

2.1. Laboratory procedures

Chromosome preparations were obtained from embryonic tissue of *Campylomormyrus compressirostris*. The samples were provided by the department of Biology and Ecology of Fishes (Prof. Kirschbaum, HU Berlin) and the protocol was adapted from Shao et al. (2010) and Karami et al. (2015). First, freshly hatched fish embryos (less than 24 hours old) were exposed to colchicine solution. After incubation, they were put on ice until vital signals were no longer detected. The yolk sac and lipid membrane were removed. The remaining tissue was incubated in a hypotonic solution for 30 min. To yield the best chromosome spreads, the preparation parameters were altered as follows: Two colchicine concentrations (0.02% or 0.05%) were tested for two different incubation times (3 h or 5 h) with two alternative hypotonic solutions (0.4% KCL or ddH_2O). In total, this resulted in eight different preparations. Henceforth, each preparation was treated equally. First, an incubation in Carnoy's solution (ethanol:glacial acetic acid; 3:1) for 20 min was performed to ensure the fixation of cell components. This step was repeated twice. The fixed embryonic tissue was then ground with a pestle in 50% acetic acid, and 10 µl of the cell suspension was pipetted onto a clean slide. Subsequently, the steps as described in Karami et al. (2015) were performed. To visualize the chromosomes, each slide was stained either with Giemsa (G-Banding) or with DAPI. For G-Banding, the slides were first swivelled briefly in a mixture of 2.5%Trypsin and 0.9% NaCl solution, washed twice with 0.9% NaCl solution, and incubated in 6% Giemsa staining for 12 min. Afterwards, the slides were washed with Gurr's buffer and conserved using DPX. Regarding the DAPI staining, slides were covered with 0.001% DAPI solution and incubated for 15 min in the dark, followed by a washing step with phosphate buffer and the conservation with DPX. The slides were stored at 4 °C in the dark until they were viewed. All slides were scanned manually under 1000× magnification (oil immersion) using a Leica DM4000 B microscope. Chromosome spreads were photographed with a Leica DFC480 digital camera and saved as TIFF-files.

Among all treatments, the highest number of chromosome spreads per preparation was observed on slides treated with higher colchicine concentration (0.05%), an incubation of 5 h, and KCl as hypotonic solution. Chromosomes generally clustered together so that a complete chromosome set could be expected. In single cell preparations, we counted diploid chromosome numbers ranging from 40 to 48. For all other treatments, most cells were either not bloated so that chromosomes could not be distinguished or chromosome spreads were not visible. DAPI staining worked

Table 1
Available karyotypes in the teleost order Osteoglossiformes.

Family	Species	2n	Karyotype formula	Reference
Osteoglossidae	*Arapaima gigas*	56	4m, 12sm, 40a	Hinegardner and Rosen (1972)
			28m, 28a	Marques et al. (2006)
	Heterotis niloticus	40	26m, 10sm, 4a	Hirata and Urushido (2000)
	Osteoglossum bicirrhosum	56	1sm, 55a	Uyeno (1973)
			56a	Suzuki et al. (1982)
	Osteoglossum ferreirai	54	2m, 4sm, 48a	Suzuki et al. (1982)
	Scleropages formosus	50	4m, 46a	Hirata and Urushido (2000)
	Scleropages jardini	48	16m, 6sm, 26a	Hirata and Urushido (2000)
	Scleropages leichardti	44	16m, 8sm, 20a	Hirata and Urushido (2000)
Pantodontidae	*Pantodon buchholzi*	48	12m, 12sm, 24a	Uyeno (1973)
Mormyridae	*Gnathonemus petersii*	48	10m, 6sm, 32a	Uyeno (1973)
			18m, 2sm, 28a	Ozouf-Costaz et al. (2015)
	Marcusenius brachistius	48	1m, 4sm, 43a	Uyeno (1973)
	Marcusenius moori	50	4sm, 46a	Ozouf-Costaz et al. (2015)
	Ivindomyrus opdenboschi	50	10m, 2sm, 38a	Ozouf-Costaz et al. (2015)
	Brienomyrus sp.	50	2m, 6sm, 42a	Ozouf-Costaz et al. (2015)
	Stomatorhinus wallkeri	50	2sm, 48a	Ozouf-Costaz et al. (2015)
	Petrocephalus microphtalamus	50	2sm, 48a	Ozouf-Costaz et al. (2015)
	Pollimyrus cf. nigricans	40	2m, 38a	Krysanov and Golubtsov (2014)
	Campylomormyrus compressirostris	48	4sm, 26m, 18a	This study
Notopteridae	*Chitala chitala*	42	42a	Uyeno (1973)
	Notopterus notopterus	42	42a	Rishi and Singh (1983)
	Papycranus afer	34	4sm, 30a	Uyeno (1973)
	Xenomystus nigri	42	42a	Uyeno (1973)

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