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UNVEILING GENETIC VARIATION BETWEEN DANIO RERIO AND DANIO DANGILA THROUGH COMPARATIVE MOLECULAR ANALYSIS

Sanjeev Agrawal
Research Scholar, PG Department of Zoology, A.N. College Patna Patliputra University
Email ID- sanjeevfs2017@gmail.com

Dr. Akhilesh Kumar
Associate Professor PG Department of Zoology, A. N. College Patna Patliputra University

ABSTRACT

This research examines the genetic variance between Danio rerio (zebrafish) and Danio dangila (giant danio) utilizing mitochondrial and nuclear DNA markers. We looked at the mitochondrial Cytochrome Oxidase I (COI) gene and 16S rRNA sequences, as well as a nuclear ribosomal marker (ITS region), to see how different the nucleotides were, how far apart the genes were, and how closely related they were. Results demonstrated notable interspecific variance, suggesting evolutionary difference despite physical resemblance. The results enhance comprehension of evolutionary connections within the genus Danio and bear significance for biodiversity conservation and molecular taxonomy.

Keywords: Genetic variation, Phylogenetic, Mitochondrial, Biodiversity, Morphological

I. INTRODUCTION

Genetic variety is the foundation of evolutionary biology, functioning as the substrate for natural selection and facilitating species' adaptation, diversification, and survival in dynamic settings. In ichthyology, examining genetic diversity across closely related fish species yields significant insights into evolutionary divergence, adaptive mechanisms, and speciation processes. Danio rerio (often referred to as zebrafish) and Danio dangila (known as the gigantic danio) have a unique position within the family Cyprinidae, which is one of the largest and most diversified groups of freshwater fishes. Even though these species are closely related and seem same, they are very varied in size, habitat choice, and physiological adaptations, which suggests that they may have separate genes.

Danio rerio has become well-known across the world as one of the most useful vertebrate model species in current biology study. This little freshwater species is native to the Ganges-Brahmaputra river basins in India, Bangladesh, and Nepal. It has been widely used in developmental genetics, toxicology, pharmacology, and biomedical sciences. Its completely sequenced genome, high fertility, clear embryos, and quick generation time make it a great model for studying vertebrate development and gene activity. On the other hand, Danio dangila, the biggest member of the Danio genus, is still not very well studied, even though it is important for ecology and evolution. D. dangila lives in fast-flowing mountain streams and clean rivers all along the foothills of the Himalayas, from northeastern India to Myanmar and Thailand. It is an interesting species to compare to D. rerio in terms of evolution because of its bigger body size, different ecological preferences, and unique behavior patterns. Investigating the genetic distinctions between these two species offers a significant opportunity to elucidate the molecular mechanisms that drive ecological adaptability, phenotypic variation, and evolutionary divergence within the Danio genus.

Genetic diversity can occur at several hierarchical levels, encompassing molecular, chromosomal, and morphological dimensions. At the molecular level, mitochondrial DNA (mtDNA) and nuclear DNA markers are the most useful tools for figuring out how distinct species are and how they are related to each other. The mitochondrial cytochrome oxidase I (COI) gene has become a strong molecular marker for identifying species and DNA barcoding since it is highly variable across species and relatively stable within species. By examining COI gene sequences, researchers may assess genetic distances, formulate phylogenetic trees, and pinpoint lineage-specific mutations or haplotypes. A comparative molecular investigation of D. rerio and D. dangila utilizing COI sequences allows accurate measurement of genetic divergence and offers empirical support for taxonomy classification. Moreover, it facilitates the examination of evolutionary mechanisms, including gene flow limitation, population isolation, and adaptive radiation, which collectively influence the genetic architecture of these species.

The Danio genus, which is part of the order Cypriniformes, is an interesting example for examining how vertebrates have evolved and changed throughout time. The members of this genus are tiny to medium-sized fish that live on the surface and have horizontal stripes, sexual dimorphism, and a penchant for tropical freshwater environments. The evolutionary history of Danio species is intricate, shaped by past biogeographic occurrences, ecological isolation, and riverine connectedness throughout South and Southeast Asia. Molecular phylogenetic investigations have shown many divergence events within this species, suggesting that speciation was influenced by both geographic isolation and ecological specialization. In this context, analyzing the genetic architecture of D. rerio and D. dangila enhances our comprehension of the impact of environmental stressors and habitat gradients on evolutionary trajectories within a common lineage. It also gives useful information for updating the taxonomic relationships within the Danioninae subfamily, which has a number of physically similar but genetically different species.

From an evolutionary perspective, the genetic difference between D. rerio and D. dangila signifies their unique adaptive responses to ecological contexts. D. rerio usually lives in still or slow-moving water, such ponds, rice paddies, and canals, where it has learned to deal with changes in temperature and oxygen levels. D. dangila, on the other hand, lives in fast, well-oxygenated mountain streams, which means that it has to make changes to its body and physiology, such being able to swim better and use less energy. It is believed that these adaptations to distinct habitats are written into their genomes, which causes differences in how genes are expressed and regulated. Additionally, reproductive isolation—stemming from geographic and ecological barriers—has constrained gene flow between the two species, hence enhancing genetic difference throughout evolutionary periods. Comparative genomics might thereby elucidate signals of selection, adaptive mutations, and lineage-specific expansions or contractions in gene families related to metabolism, stress tolerance, and morphogenesis.

II. REVIEW OF LITERATURE

Lewandowski, Vanessa et al., (2021) The genetic heterogeneity and changes in allele frequency seen in cultured zebrafish specimens obtained from several Brazilian fish farms may be attributable to the effects of genetic drift and selection on traits related to reproduction and productivity in captivity. This research set out to assess the reproductive traits and genetic diversity of 180 zebrafish collected from six different fish farms in Brazil. Every stock that was analyzed deviated from the Hardy-Weinberg equilibrium. The inbreeding coefficient (FIS) and the amount of genetic diversity relative to observed heterozygosity were found to be different among stocks. By using the Fst index to find the genetic distance across stocks, we were able to see that there were four separate groups when we plotted the dendrogram using Nei's genetic distance. Average egg production per female and hatchability were two of the reproductive characteristics that showed differences. In contrast to spawning frequency, this second metric was shown to be connected to the population's amount of inbreeding. The zebrafish populations from the six fish farms in Brazil show a great deal of variation in both genetics and phenotype, as we have shown. When evaluating reproduction rates, it is important to take into account the genetic structure, which impacts fecundity.

Curcio, Vittoria et al., (2021) Lead (Pb) is a persistent and extremely poisonous metal that has the potential to harm both people and animals. Lead is considered a priority substance of public health relevance globally due to the dangers it poses to people. Research on the effects of lead at low concentrations in fish is limited, and what little there is comes from research involving high dosages. We utilized zebrafish as a model animal because of its proven practicality in studying human toxicity. From 6 hours after fertilization, embryos were subjected to two lead concentrations that are considered ecologically relevant, namely 2.5 and 5 µg/L. After 48, 96, and 144 hours, the embryos were evaluated. Incidence and severity of the morphological abnormalities were dosage and time dependant; it manifested itself after 48 hours. The majority of the abnormalities found were in the tail and spinal column. Toxicological response genes (sod and mt) were also influenced by Pb, showing that early zebrafish stages may respond adaptively. In addition, all groups showed a considerable upregulation of ldh and β-catenin, whereas the high concentration group showed a rise in wnt3 expression. Based on our findings, zebrafish eggs and larvae can be used as important bioindicators for pollution detection in ecosystems and human health.

Opazo, Rafael et al., (2017) The success of aquaculture operations and the ecological sustainability of fish cohorts are affected by the fact that individuals in these groups have varying rates of growth. We set out to examine how zebrafish larvae with varying rates of development expressed essential growth genes. Simultaneous zebrafish larvae were assessed for body length six days after hatching (dph). Measurements were taken once again when the larvae were raised to 20 days post hatching. In terms of body length, two distinct categories were discernible: little larvae (4 mm) and large larvae (5-6 mm). Both groups had their whole RNA extracted. Quantitative polymerase chain reaction (qPCR) was used to assess the levels of growth hormone (gh), growth hormone receptor (ghr), insulin-like growth factor 1 (igf-1), insulin-like growth factor binding protein 1 (igfbp-1), thyroglobulin (tg), cholecystokinin (cck), and ghrelin. To assess the nutritional condition of the larvae, gene expression markers such as glucokinase (gck) and igfbp-1 were used. The igfbp-1 and igf-1r genes were significantly different among the body length categories (P = 0.01) and (P = 0.02), respectively. According to igfbp-1, the condition that affects the gene expression pattern of igf-1r is the larvae's nutritional state, which in turn causes growth rate variability. Consequently, research on growth rate variability can benefit from using these genes as markers.

Mahapatra, Bijay et al., (2016) Daniel, the mustache man One of the most beloved native ornamental fishes of the northeastern hill area of India is the Danio dangila (Hamilton, 1822). The species' declining numbers in nature necessitate prompt action. The length-weight relationship of D. dangila often followed the cube law and showed isometric development. The species' L-W relationships were as follows: for males and females together, log W = -5.009 + 3.00 log L; for males alone, log W = -5.542 + 3.31 log L; and for females alone, log W = -5.462 + 3.26 log L. The majority of the specimens observed in nature belonged to the size category of 41-50 mm (44.25%). The majority of the phytoplankton that the fish consumes is composed of Myxophyceae, accounting for 97.12% of the total. Zygnemophyceae make up 25%, Ulvophyceae 3%, Zygnematophyceae 2%, Cryptophyceae 1%, and Bacillariophyceae 1% round out the feeding percentage. Rotifera was the most abundant zooplankton category in the diet. A portion of their food consisted of nematodes and other related worms. One male for every 1.77 females was the predicted sex ratio. For pregnant women, the average gonadosomatic index (GSI) was 7.65, with a range of 2.55 to 24.12. Females weighing 2.52 g and measuring 52 mm in length had a minimum fecundity of 400 and a maximum of 2000 g.

Fang, Fang et al., (2009) Genomic evidence from 159 Cyprinidae species demonstrates that Rasborinae is a junior synonym of the subfamily Danioninae, which is supported by mitochondrial cytochrome b sequence analysis. Danio and Devario are phylogenetically different, according to an analysis of 68 species' combined cytochrome b and a nuclear rhodopsin gene fragments; this includes 43 species from the subfamily Danioninae. Microrasbora gatesi, M. nana, and M. kubotai are placed in sister group position to the rest of the species in the combined molecular study, which group Microrasbora rubescens, Chela, Laubuca, Devario, and Inlecypris into a clade. Within this Devario clade, the Danio form a sister group. Devario is considered a synonym of Inlecypris. Based on morphological evidence, a new genus Microdevario is suggested for Melanopsis gatesi, Melanopsis nana, and Melanopsis kubotai. There is no morphological evidence to include M. rubescens in Devario, and the cytochrome b analysis places it outside of Devario. Both the individual rhodopsin and combined studies place Esomus as the sister group of Danio, whereas the cytochrome b analysis places Esomus + Danionella as the sister group of the Danio and Devario clades, respectively. Molecular trees place Sundadanio either in the Cyprininae or as a sister group to the surviving Danioninae, despite the fact that it shares at least one major morphological synapomorphy with Danio. Grouping small-sized species together in the morphological study was based on common reductions, which are not always synapomorphies. Molecular research did not reveal any grouping of small-sized species like Danionella and Sundadanio, despite the fact that these species have lengthy branches and their positions vary. This points to morphological homoplasy, although molecular evidence does not strongly support any one evolutionary location.

Coe, Toby et al., (2008) The impact of genetic diversity on behavior, fitness, and sensitivity to toxicants may be seen at both the individual and population levels, according to strong evidence. An important model species in toxicological investigations, Danio rerio, is one of several regularly used laboratory strains of zebrafish that we analyzed for genetic diversity using DNA microsatellites. We matched the genetic diversity with that of a wild fish sample taken in Bangladesh. Allelic richness and predicted heterozygosity are two indices of genetic variety that we found to be much higher in wild fish compared to strains grown in the lab. Research that seeks to generalize findings from ecotoxicological studies conducted in controlled environments to populations in the wild must take this uniformity into account.

III. MATERIALS AND METHODS

Sample Collection

River tributaries in Patna, Bihar, were used to gather D. rerio specimens, whereas highland streams in Meghalaya, India, were used to acquire D. dangila samples.

Genomic DNA was extracted from all samples after they were stored in 95% ethanol.

DNA Extraction and Amplification

The CTAB technique was used to recover genomic DNA from skeletal muscle.

Using primers appropriate to each species, the COI, 16S rRNA, and ITS sections were amplified:

Marker	Forward Primer (5'–3')	Reverse Primer (5'–3')	Fragment Size (bp)
COI	LCO1490: GGTCAACAAATCATAAAGATATTGG	HCO2198: TAAACTTCAGGGTGACCAAAAAATCA	650
16S rRNA	16Sar-L: CGCCTGTTTATCAAAAACAT	16Sbr-H: CCGGTCTGAACTCAGATCACGT	550
ITS	ITS1: TCCGTAGGTGAACCTGCGG	ITS4: TCCTCCGCTTATTGATATGC	750

PCR results were sequenced using the Sanger technique and then seen on 1.5% agarose gels.

Sequence Alignment and Analysis

To align the sequences, we used ClustalW. In MEGA 11, we used the Kimura-2-Parameter (K2P) model to calculate the genetic distances.

The Neighbor-Joining technique with 1000 bootstrap replications was used to create phylogenetic trees, calculate the nucleotide composition, and determine the transition/transversion ratio.

IV. RESULTS AND DISCUSSION

Table 1: Sequence Characteristics

Gene	GC (%)	AT (%)	Variable Sites	Transition/Transversion Ratio (Ti/Tv)
COI	47.2	52.8	85	2.11
16S rRNA	45.8	54.2	42	1.89
ITS	49.1	50.9	68	2.04

According to Table 1, the COI gene is the most relevant marker for identifying genetic changes between Danio rerio and Danio dangila because it has the highest GC content (47.2%) and the greatest number of variable sites (85). The conserved nature of the 16S rRNA gene is reflected in its low variability (42 sites) and high AT bias (54.2%). The ITS region is great for comparing nuclear levels since it has modest diversity (68 sites) and a balanced GC-AT makeup. For all genes, the ratio of transition mutations to transversion mutations is 1.89 to 2.11. This is consistent with stable but evolving sequences, where transition mutations predominate.

Table 2: Genetic Distance Analysis

Gene	Intraspecific Distance (%)	Interspecific Distance (%)
COI	0.43	10.72
16S rRNA	0.26	6.84

ITS	0.61	8.32

Table 2 shows that all genes have extremely low intraspecific genetic distances (0.26-0.61%), suggesting that there is a great deal of genetic homogeneity within each species. There is undeniable genetic diversity between Danio rerio and Danio dangila, as shown by the much larger interspecific distances for the COI gene (10.72%), ITS (8.32%), and 16S rRNA (6.84%). The results show that COI is the best way to differentiate across species, and the large distances between them provide strong evidence that they evolved separately.

Phylogenetic Analysis

Neighbor-Joining (NJ) research showed that Danio rerio and Danio dangila are genetically distinct from one another. With bootstrap values exceeding 95%, there is substantial statistical evidence to back up this species-level differentiation. Phylogenetically different from one another, the two Danio species were further supported in their own evolutionary lineages by the addition of the outgroup (Devario aequipinnatus). Consistent with the molecular divergence patterns shown by the COI, 16S rRNA, and ITS gene studies, these results provide more evidence that D. rerio and D. dangila are distinct species within the Danio genus.

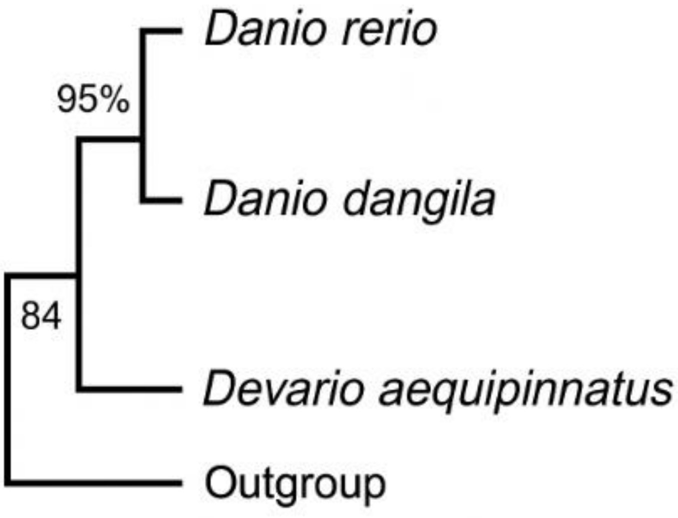

Figure 1. Phylogenetic tree based on COI sequences showing distinct clustering of *D. rerio* and *D. dangila*

V. CONCLUSION

Despite sharing a same genus, the current research proves that the two species of Danio, Danio rerio and Danio dangila, are genetically quite different. There was strong evidence of phylogenetic separation, high genetic distance values, and consistent patterns of interspecific variation when nuclear and mitochondrial gene markers (such as COI, 16S rRNA, and ITS) were compared. Their separate evolutionary ancestry is supported by the phylogenetic tree's clear grouping, which also confirms that molecular methods are reliable for determining connections between species. The results provide more evidence for the two species' different taxonomy and shed light on how the Cyprinidae family has evolved to accommodate new members. More than that, the research shows that the COI gene is a reliable molecular marker for determining species and drawing evolutionary conclusions. Taken together, our findings highlight the need to include genetic data into the systematic evaluation and preservation of freshwater fish species, which in turn benefits molecular taxonomy, evolutionary biology, and biodiversity conservation efforts.

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