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Comparative Genomic Analysis of *Caridina pseudogracilirostris* and Model Organisms: Insights into Phylogenetic Relationships and Gene Evolution

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ABSTRACT:

Objectives:

To compare *Caridina pseudogracilirostris* with other model organisms based on orthologous features and understand the evolutionary changes by identifying conserved genes and gene families.

Methods:

Total protein sequences of eleven different species (*Caenorhabditis elegans, Danio rerio, Daphnia pulex, Drosophila melanogaster, Dugesia japonica, Gallus gallus, Hydra vulgaris, Mus musculus, Strongylocentrotus purpuratus, Xenopus laevis*), including *C. pseudogracilirostris* were retrieved from NCBI. The orthology and comparative genomics were performed using orthofinder tool which uses Diamond, MCL, mafft and FastTree algorithms.

Findings:

A total of 5,62,515 (75.9%) genes were involved in ortho-groups representing conserved genes/gene families across different species. *C. pseudogracilirostris* has a total of 14,101

genes out of which 9,843 (69.8%) were identified to be in orthologues with other species. This analysis revealed that *C. pseudogracilirostris* have a close relationship with *D. japonica*, and had unique rapidly evolving conserved protein families like Trypsin-like protease try-5, Acyl-CoA synthetase 7, Receptor-type tyrosine-protein phosphatase dep-1, Probable vesicular glutamate transporter eat-4, and Receptor-type guanylate cyclase gcy-25.

Conclusion and Novelty:

Comparing the genomes of *C. pseudogracilirostris* with other model organisms provided information on its relationship and rapidly evolving conserved protein families across different species. We herewith first report that *C. pseudogracilirostris* can be used as a potential model system to study environmental pollution, climate change, and metabolism pathways based on the conserved protein sequence.

Keywords: *Caridina pseudogracilirostris*, vertebrate and invertebrate, model organism, comparative genomics, Orthogroups, shrimp.

1. INTRODUCTION:

Model organisms are species that are used in scientific research to help understand biological processes. The genome of model organisms is well-characterized so it helps to study the effects of genetic mutations on various biological processes. They typically have a short life cycle, which allows understanding of the biological processes along with multiple generations in a relatively short period of time [1]. There are model organisms like *Caenorhabditis elegans* to study ageing, development, and neurobiology, *Daphnia pulex* for ecological and evolutionary research. *Drosophila melanogaster*, a classic model organism used in genetics, neuroscience, and developmental biology, *Dugesia japonica* is used to study regeneration and in stem cell biology, *Hydra vulgaris* for studying regeneration and cellular differentiation and *Strongylocentrotus purpuratus* used in developmental biology. Similarly, vertebrates like *Danio rerio, Gallus gallus are* used in studying embryogenesis, *Xenopus laevis* for developmental biology and *Mus musculus* a common model for biological research. The limitations in each model system include lack of organs, limited tissue differentiation, limited evolution, behavioural repertoire and immune system [2]. These multiple model organisms have unique features that are common to all or specific to one. Comparison of the common features with respect to humans can provide insight into how these model organisms can be used. Comparing *C. pseudogracilirostris* with these models will help understand the proteins and their evolutionally across species and their usefulness in understanding the biological processes underlying it.

Comparative genomics provides a powerful tool for studying evolutionary changes among organisms and helps to identify genes that are conserved or common among species, as well as genes that give each organism its unique characteristics[3]. Phylogenetic comparison of vertebrate and invertebrate genomes provides information on the evolution of different animal groups and the genetic basis of their traits and behaviors [1]. Phylogeny infers evolutionary relationships between homologous genes. The human genome and the fruit fly genome on comparison reveal that about sixty per cent of genes are conserved and shared core set of the same genes. Cross-species sequence comparisons are to identify

biologically active regions of a genome since the functional proteins are frequently conserved between evolutionarily distant species [4].

C. pseudogracilirostris was chosen for this study based on its benefits, though it is one of the brackish tolerant species adaptation for survival is comparatively high than the survival range of other shrimp species. The size of the animal is small enough to maintain in small-scale lab conditions [5]. The duration of embryonic development is also less when compared to other shrimp species, which helps in accomplishing the research work easily, and the main advantage is the size and transparency of the embryo similar to that of zebrafish [6]. To use *C. pseudogracilirostris* as a model organism, the basic features to rely on involve protein and pathway similarities among different species of invertebrate and vertebrate model systems. In this study phylogenetic comparison of invertebrate and vertebrate model systems were compared along with *C. pseudogracilirostris* for understanding the overlying evolutionary relationship with other model organisms and how this can be useful for coining *C. pseudogracilirostris* as a model system in future.

2. METHODOLOGY

2.1. Shrimp Collection and Rearing

Live shrimps were collected from different locations of the sampling site at Rajakkamangalam estuary located in Kanyakumari district, Tamilnadu, India (8°07'17.9"N, 77°22'19.3"E).89. Fresh animals were collected and euthanized using tricaine (MS-222) and fixed in 4% paraformaldehyde. Shrimp identification has been carried out based on morphological features [6]. Further, a purposive sampling method was followed for the collection of *C. pseudogracilirostris* and to avoid other types of shrimps. Samples were collected, using a long aquarium net (30.5 cm x 30.5 cm) and packed immediately in large polyethylene bags containing fresh water. Packed samples were brought to the lab within 20 hours and transferred to laboratory tanks at Aquaculture Facility (Aquaneering USA). Further DNA extraction, sequencing and data analysis were performed and have been previously reported [6].

2.2. Comparative and Phylogenetic studies

This study was carried out using the OrthoFinder Software package (https://github.com/davidemms/OrthoFinder) [6,7,8], which provides high-accuracy ortho-groups inference to provide a phylogenetic inference of orthologs, rooted gene trees, gene duplication events, the rooted species tree, and comparative genomics statistics. The software consists of packages like BlastX, Diamond, MCL, FastME, MAFFT and FastTree. The protein sequence of the species *Caenorhabditis elegans, Danio rerio, Daphnia pulex, Drosophila melanogaster, Dugesia japonica, Gallus gallus, Hydra vulgaris, Mus musculus, Strongylocentrotus purpuratus, Xenopus laevis* along with the sequence of *C. pseudogracilirostris* was retrieved from NCBI and used for the phylogenic and orthologous studies.

Phylogenetic analysis was performed within the 10 different species above and the analysis yielded a rooted species tree presented based on the STAG species tree (species tree inference from all genes) derived from all ortho groups, STAG support values are indicated at internal nodes and STRIDE (species tree root inference from gene duplication events). This

tree was visualized and exported using the online tool ITOL (Interactive Tree Of Life) [9,10] https://itol.embl.de/.

3. RESULTS AND DISCUSSION

Vertebrates and invertebrates are two major groups of animals that differ in a number of ways, including their body structure, behaviour, and genetics. Vertebrates, which include fishes, birds, and mammals, are characterized by the presence of a backbone, which is a structure that runs along the length of the body and supports the body weight. In contrast, invertebrates, which include insects, worms, and molluscs, do not have a backbone and are supported by a more flexible exoskeleton. When it comes to genetics, vertebrates and invertebrates also have some notable differences. Vertebrates generally have larger and more complex genomes than invertebrates, with more genes and a greater variety of gene types. For example, the human genome contains around 25,000 genes [11], while the genome of the fruit fly (*D. melanogaster*), which is a common model organism for studying genetics, contains only around 14,000 genes [12]. Despite these differences, both vertebrates and invertebrates share many fundamental genetic pathways and processes, such as those involved in the regulation of gene expression and the development of the body's structure. The current paper focuses towards identifying the relationship between *C. pseudogracilirostris* and other model systems using orthologues data interpretation.

Initially, the live shrimps were collected from the Rajakkamangalam estuary located in the Kanyakumari district and cultured in the laboratory condition at Sathyabama Institute of Science and Technology at 28°C. The DNA was isolated from the tissue sample and library preparation was carried out using the Illumina TruSeq Nano DNA Library (350bp PE insert) and sequenced using NovaSeq6000 (2x150bp paired-end read length). The raw data were submitted to the SRA database (PRJNA847710) [6].

3.1. Comparative phylogenetic analysis

The complete protein sequences of selected vertebrates and invertebrate models were retrieved from NCBI. A total of 10 different species have been commonly used as model organisms, *C. elegans, D. rerio, D. pulex , D. melanogaster, D. japonica, G. gallus, H. vulgaris, M. musculus, S. purpuratus, X. laevis,* along with *C. pseudogracilirostris* were analysed. A total of 5,62,515 genes were involved in ortho groups representing conserved genes/gene families across different animals. The percentage of genes included in ortho-groups was 4,26,744 (75.9%) among the total genes. The proportion of unassigned genes was about 1,35,771 representing 18.1 %. A total of 37,053 ortho groups were shared by all the species involved in this study. The highest number of species-specific ortho-groups was found to be 20,207. The number of genes involved in species-specific ortho-groups was 1,02,202 (Table. 1). The percentage of *C. pseudogracilirostris* genes involved in ortho groups was about 69.8%. The number of gene duplications for *C. pseudogracilirostris* is minimum (2,418) and maximum duplication was seen in *Danio rerio* (57,068). Gene duplication is an important mechanism for organisms to acquire new genes and create genetic novelty [13]. Many new gene functions have evolved as a result of gene duplication, which has greatly aided the evolution of developmental proteins in *D. rerio* and the result shows that *C. pseudogracilirostris* was less evolved. The branch length of *C. pseudogracilirostris* with

0.525 is higher than *D. rerio* with 0.193, Generally, branch lengths indicate genetic change; the longer the branch, the greater the genetic change or divergence [14]. Phylogenetic analysis of all 11 species showed *D. japonica* to be the closest and sister species to *C. pseudogracilirostris* (Figure 1). The average number of nucleotide or protein substitutions per site is estimated to determine the extent of genetic change and these substitutions are used to calculate the likelihood of phylogenetic trees using multiple sequence alignment data. Based on the branch length we can conclude that *C. pseudogracilirostris* is evolving for long period but at a very lower rate.

Number of species	11
Number of genes	562515
Number of genes in orthogroups	426744
Number of unassigned genes	135771
Percentage of genes in orthogroups	75.9
Percentage of unassigned genes	24.1
Number of orthogroups	37053
Number of species-specific orthogroups	20207
Number of genes in species-specific orthogroups	102202
Percentage of genes in species-specific orthogroups	18.2
Mean orthogroup size	11.5
Median orthogroup size	5
G50 (assigned genes)	25
G50 (all genes)	15
O50 (assigned genes)	3922
O50 (all genes)	7416
Number of orthogroups with all species present	98
Number of single-copy orthogroups	0

Table 1: Comparative orthogroup analyses for all species.

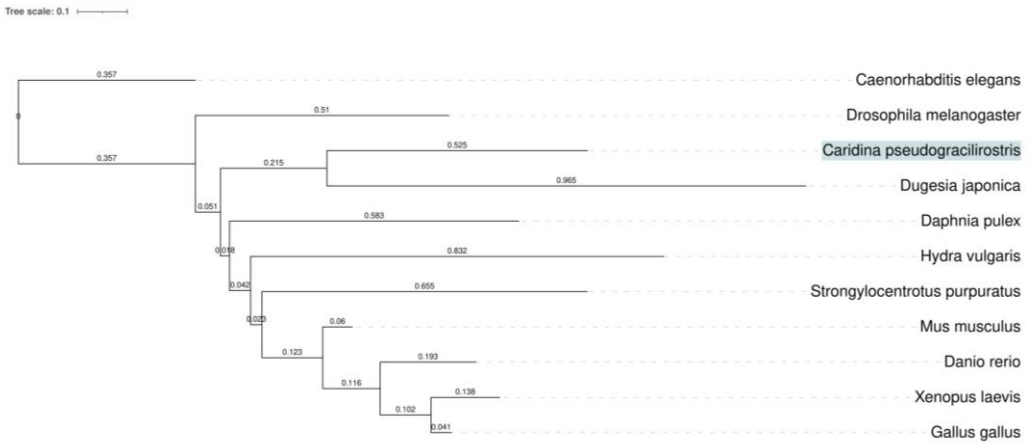

Figure 1: Phylogenetic orthology inference tree based on comparative genomics

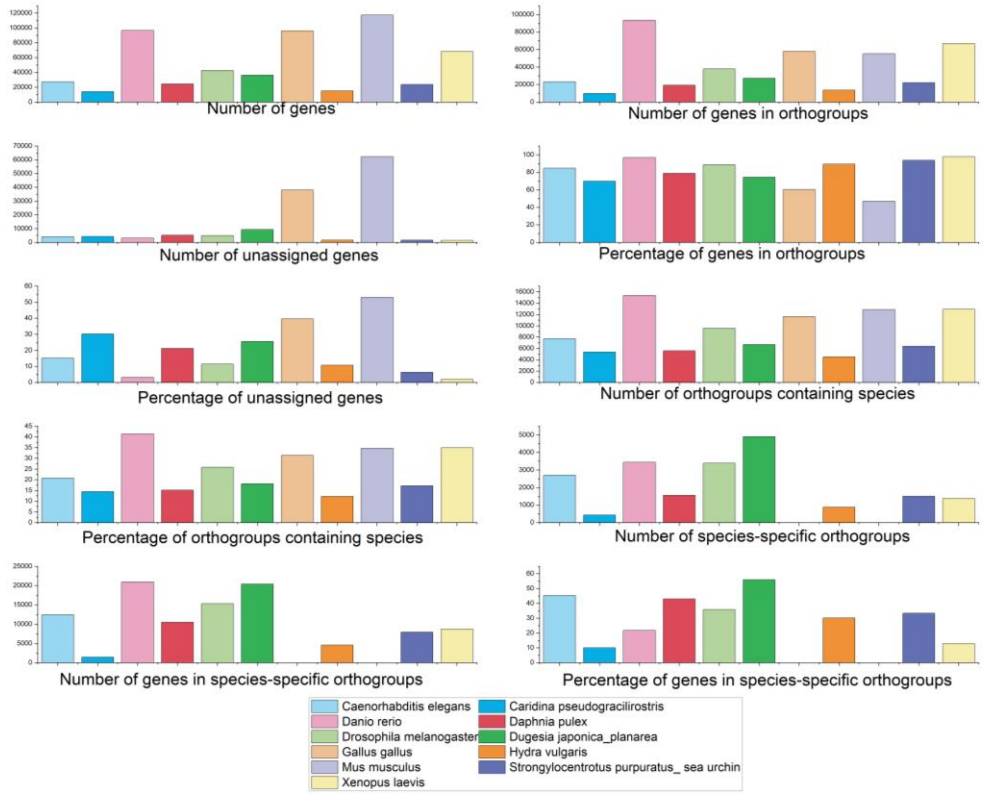

Figure 2: Graph showing statistic per species for 1) Number of genes 2) Number of genes in orthogroups 3) Number of unassigned genes 4) Percentage of genes in orthogroups 5) Percentage of unassigned genes 6) Number of orthogroups containing species 7) Percentage of orthogroups containing species 8) Number of species-specific orthogroups 9) Number of genes in species-specific orthogroups 10) Percentage of genes in species-specific orthogroups

3.2. Comparative Genomics

Crustaceans, which include animals such as shrimp, lobsters, and crabs, are often used as model organisms in scientific research. Crustaceans have a relatively simple anatomy and physiology, which makes them easier to study than more complex animals. Many species of crustaceans are found in diverse environments and are relatively easy to collect and breed in the laboratory [5]. Crustaceans have short lifespans, which help in studying the entire lifecycle of an animal within a relatively short period. While the genomes of crustaceans are quite different from those of humans, they do share many fundamental biological processes and pathways. Shrimp are used in a variety of research areas, including the study of genetics, development, and environmental pollution.

The number of genes ranges from 3,050 for *D. pulex* to 117,518 for *G. gallus* (Figure 2). The percentage of genes in orthogroups ranges from 47.0% for *M. musculus* to 96.8% for *D. rerio*. Interestingly, the sea urchin *S. purpuratus* has a very high percentage of genes in orthogroups (93.6%) while having a relatively small number of genes (23,755). The data also reveals the number of orthogroups containing genes from multiple species, as well as the

number of species-specific orthogroups. *D. rerio* has the highest number of orthogroups containing genes from multiple species (15,346), while *G. gallus* has the highest number of genes in orthogroups (55,187). On the other hand, *D. japonica* has no species-specific orthogroups, while *M. musculus* has the highest percentage of genes in species-specific orthogroups (45.3%). *C. pseudogracilirostris* has 14,101 genes, which is the second lowest among the 11 species in the dataset. The species with the lowest number of genes is *D. pulex* with 24,376 genes, while the species with the highest number of genes is *G. gallus* with 96,087 genes. *C. pseudogracilirostris* has a lower number of genes compared to most of the other species in the dataset, but this may not necessarily reflect its biological complexity. In terms of orthogroups, *C. pseudogracilirostris* has 9,843 genes that are part of shared gene families, which is the second lowest percentage among the 11 species. The species with the lowest percentage of genes in orthogroups is *M. musculus* with 47%, while the species with the highest percentage is *S. purpuratus* with 93.6%. This suggests that *C. pseudogracilirostris* has a moderate level of genetic similarity with other species in the dataset. *C. pseudogracilirostris* has 5,375 orthogroups containing genes from multiple species, which is the second lowest number of orthogroups among the 11 species. The species with the lowest number of orthogroups is *C. pseudogracilirostris*, while the species with the highest number is *Gallus gallus* with 11,648 orthogroups. This indicates that *C. pseudogracilirostris* may have a relatively unique set of shared genes with other species. In terms of species-specific orthogroups, *C. pseudogracilirostris* has 433, which is the third lowest number among the 11 species. The species with the lowest number of species-specific orthogroups is *Gallus gallus* with none, while the species with the highest number is *Dugesia japonica* with 4,908 orthogroups. This suggests that *C. pseudogracilirostris* has some unique genetic features, but not as many as some other species.

Further, on comparing the proteome of *C. pseudogracilirostris* with other vertebrate and invertebrate models, 99 conserved orthologous proteins were identified. Among these the highly clustered orthologous proteins with rapidly evolutionary were found, to be Trypsin-like protease try-5, Acyl-CoA synthetase 7, Receptor-type tyrosine-protein phosphatase dep-1, Probable vesicular glutamate transporter eat-4, and Receptor-type guanylate cyclase gcy-25. Try-5 has mainly been investigated in the worm *C. elegans*. Try-5 is expressed in the pharynx of this organism and aids in the breakdown of the bacteria that the worm consumes and also been demonstrated to play a role in *C. elegans* lifespan control [15] and in zebrafish, the try-5 has a role in blood coagulation and found that it was necessary for the activation of factor X in the developing embryo [16]. Acyl-CoA synthetase 7 (Plays a role in ascaroside pheromones biosynthesis [17] which regulates development and behaviour), supports the effective oxidation of fatty acids and their incorporation into cellular membranes, ACSL7 is expressed in a variety of tissues in mammals, including the liver, brain, adipose tissue, and skeletal muscle [18] and in invertebrates ACSL7 is involved in regulating lipid metabolism and energy homeostasis, as well as the development of the nervous system [19]. Receptor-type tyrosine-protein phosphatase dep-1 (Phosphatase which can dephosphorylate receptor let-23 and cellular regulation underlying diverse physiological events [20], PTPRJ has been shown to regulate a variety of signalling pathways, including the insulin receptor, growth factor receptor, and integrin signalling pathways. PTPRJ has also been implicated in the

regulation of angiogenesis and tumour progression in vertebrates, in drosophila PTPRJ is involved in regulating the growth of sensory neuron axons, as well as in the formation of neuromuscular junctions and is important for a variety of cellular processes including cell proliferation, differentiation, and survival. Probable vesicular glutamate transporter eat-4 (Required for glutamatergic synaptic transmission) [21], In vertebrates, three VGLUT isoforms (VGLUT1-3) have been identified, and they are expressed in different neuronal populations. VGLUT1 is primarily expressed in the cerebral cortex, hippocampus, and cerebellum, while VGLUT2 is found in the thalamus, hypothalamus, and brainstem. VGLUT3 is expressed in a subset of neurons in the cortex, striatum, and limbic system. The function of VGLUTs in vertebrates is critical for synaptic transmission, neuronal excitability, and plasticity [22], but in invertebrates, the function of VGLUTs is less well understood. However, studies in *C. elegans* have shown that eat-4/ VGLUT3 is involved in regulating synaptic transmission at both inhibitory and excitatory synapses. Receptor-type guanylate cyclase gcy-25 (Guanylate cyclase involved in the production of the second messenger cGMP) [23] also was predicted to be a rapidly evolving protein family, generally, in vertebrates, GCY-25 is known as the retinal guanylate cyclase 1 (GUCY2D) and is primarily expressed in the photoreceptor cells of the retina. GCY-25 plays a critical role in vision by regulating the levels of cGMP in the photoreceptor cells, in *C. elegans* GCY-25 is expressed in the sensory neurons and is involved in regulating the behaviour of the worm in response to environmental cues, such as temperature and oxygen levels [24] (Supplementary 1). The higher degree of similarity of *C. pseudogracilirostris* with rapidly evolving protein families in these species may suggest that *C. pseudogracilirostris* shares similar biological processes with these species. The findings from this study provide valuable insights into the evolutionary history of *C. pseudogracilirostris* and its relationship with other species. Future studies can investigate the functional roles of these rapidly evolving protein families in *C. pseudogracilirostris* and other related species to better understand the biological processes that shaped their evolution.

4. CONCLUSION

In conclusion, this study analyzed the complete protein sequences of 10 different model organisms and *C. pseudogracilirostris* to identify conserved genes and gene families. The results revealed that *C. pseudogracilirostris* shared many ortho-groups with the other species but also had a substantial number of species-specific ortho-groups. The comparative phylogenetic analysis revealed that *C. pseudogracilirostris* is closely related to *Dugesia japonica*, an invertebrate model organism. However, the analysis also showed that *C. pseudogracilirostris* has fewer genes involved in ortho-groups and a lower number of gene duplications compared to other model organisms such as *Danio rerio*. The branch length analysis suggests that *C. pseudogracilirostris* has been evolving for a longer period but at a lower rate. The study also identified several rapidly evolving protein families that are highly similar in *C. pseudogracilirostris* and other species, including Trypsin-like protease try-5, Acyl-CoA synthetase 7, Receptor-type tyrosine-protein phosphatase dep-1, Probable vesicular glutamate transporter eat-4, and Receptor-type guanylate cyclase gcy-25. These rapidly evolving proteins may be important targets for natural selection in different species

and could contribute to adaptations in reproductive strategies, development, behaviour, and neuronal signalling. Overall, these findings provide important insights into the molecular basis of evolution across different species and highlight the need for further research to elucidate the functional significance of these rapidly evolving proteins.

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6. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

7. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

8. DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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10. AUTHORS' CONTRIBUTIONS

Nandhagopal Soundharapandiyan, and Rajesh Kannan Rajaretinam made substantial contributions to conception and design. Nandhagopal Soundharapandiyan made acquisition of data, Carlton Ranjith Wilson Alphonse did analysis and interpretation of data; Nandhagopal Soundharapandiyan took part in drafting the article, All authors was revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and All the authors are agreed to be accountable for all aspects of the work.

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