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Research Article

Comparative Genomics Reveals Autophagy-Lysosome Pathway Expansion and Adaptive Gene Selection in Anguillid Eels

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Anguillid eels have a catadromous life cycle, transitioning from deep-sea larvae to adults in estuaries and freshwater environments. It is well-established that adult eels cease feeding and experience gut degeneration during their spawning migration. However, the evolutionary mechanisms behind the removal and recycling of damaged tissues in eels during this process remain unclear. This study aims to identify the genes that may play a role in the adaptation of Anguillid eels to their unique, once-in-a-lifetime deep-sea migration. To investigate these mechanisms, we conducted a comparative genomic analysis using high-quality genomes from four Anguillid eels. We specifically examined genes that exhibited significant expansion or were subject to positive selection. Among the expanded gene families in four eel species, we observed notable enrichment in the endocytosis pathway ($p < 0.05$). Additionally, using the branch-site model, we detected 65 positively selected genes (PSGs) in the Anguillid eel branch ($p < 0.05$). Interestingly, we found PSGs are associated with autophagy-lysosome pathways (ALPs), suggesting their role in energy production during periods of nutrient scarcity. This study provides valuable insights into the adaptive mechanisms of Anguillid eels, enhancing our understanding of their survival strategies during migration, despite their being in a nonfeeding state.

Keywords: Anguilla; apoptosis; autophagy-lysosome pathway (ALP); comparative genomics; genomic adaptation; phagocyte

1. Introduction

The eel industry remains dependent on collecting wild-caught glass eels, as full-life cycle cultivation of this species remains elusive [1, 2]. Eels are highly valuable aquaculture resources, with ~277,103 metric tons cultivated in 47 countries [3]. Despite significant efforts over the years, eel populations continue to decline due to climate change and overexploitation of juvenile eel resources [4, 5]. Currently, *Anguilla anguilla* is classified as "Endangered" by the International Union for Conservation of Nature Red List (https://www.iucnredlist.org/search?query=anguilla&searchType=species). Additionally, *Anguilla japonica*, *Anguilla dieffenbachii*, and *Anguilla rostrata* are listed as "Endangered," while *Anguilla bicolor*, *Anguilla bengalensis*, *Anguilla australis*, and *Anguilla mossambica* were categorized as "Near Threatened" and *Anguilla borneensis* and *Anguilla luzonensis* as "Vulnerable." As a result, eel industries face eel stock restrictions and rising prices [6]. Consequently, comprehensive research into molecular adaptation mechanisms is crucial for making full-life cycle aquaculture a viable option.

Anguilla eels, with their catadromous life cycle, undergo significant environmental adaptations. They start as larvae (leptocephalus) in saltwater, transition to brackish water as juveniles (glass eel), and eventually inhabit freshwater as adults [7, 8]. After maturing, they migrate to the deep ocean to spawn

[7]. During their once-in-a-lifetime spawning migration, eels experience significant degeneration of digestive organs [9]. Remarkably, they do not feed during the journey but maintain muscle mass [10]. This suggests that they rely on resorption for the breakdown of lipid-rich muscles to fuel migration [11, 12]. However, no study has elucidated the genetic mechanisms underlying this breakdown process.

Currently, genomic sequences are available for seven eel species, with chromosome-level genome assemblies completed for *A. japonica* [13], *A. rostrata* (GCA_018555375.3), and *A. anguilla* [14]. Recently, we constructed a high-quality chromosome-level genome of *A. bicolor pacifica* [15]. Additionally, the genomes of Anguilla *megastoma*, *Anguilla marmorata*, and *Anguilla obscura* have been assembled at the scaffold level [16]. In this study, we performed a comparative genomic analysis using four high-quality genomes and identified several genes associated with apoptosis, phagocytosis, and lysosomal pathways in Anguillid eels. Understanding these degenerative pathways provides valuable insights into the efficient recycling of energy by eels during periods of inevitable starvation.

2. Materials and Methods

2.1. Data Collection. Genome, protein-coding nucleotide sequences, and amino acid sequences were obtained from the National Center for Biotechnology Information (NCBI) (Table S1 for species information).

2.2. Comparative Genomics and Phylogenetic Analysis Using Four Anguillid Genomes. We used OrthoFinder2 (ver. 2.5.4) [17] to identify orthologous genes among the 14 species using amino acid sequences. To cluster the orthologous genes from the 573,255 genes across these 14 species, we employed Diamond (ver. 2.0.15) [18]. Subsequently, MAFFT (ver. 7.475) [19] was used to align multiple amino acid sequences using default parameters.

Next, we used MCMCtree in the PAML package (ver. 4.10.5) [20] to estimate divergence times, employing an independent clock rate model by setting "clock = 2." For the input tree file, we used a maximum likelihood (ML) tree inferred from OrthoFinder2. Based on the TimeTree database [21], a total of three calibrations were used as follows: *A. japonica–Gymnothorax javanicus* (89.3–211 million years ago [MYA]), *Amphiprion ocellaris–Cololabis saira* (99.5–116.7 MYA), and *A. japonica–Mobula hypostoma* (495.2 MYA). Tree topologies and divergence times, along with 95% confidence intervals, were visualized using FigTree (ver. 1.4.4; http://tree.bio.ed.ac.uk/software/figtree).

2.3. Gene Family Evolution Analysis. Gene families may expand or contract owing to natural selection, often exhibiting similar patterns among closely related species. To investigate gene gain or loss while accounting for phylogenetic history, we utilized Computational Analysis of Gene Family Evolution (CAFE, ver. 5.0.0) [22]. CAFE requires two sets of input data: a matrix containing gene counts in each orthologous group, and an ultrametric tree. Matrix and Ultrametric tree files generated using Orthofinder2 and MCMC_tree, respectively, were used in this study. Before conducting statistical tests, we trimmed our data by removing gene families containing more than 100 genes and those in which at least five species had zero genes [23]. Gene families were considered significantly expanded or contracted if the Viterbi *p*-value was less than 0.05. As our primary focus was on significantly expanded gene families in the eel group, we performed a *t*-test to determine whether there was a significant difference between the means of two fish groups: those living in ocean, estuary, and freshwater environments (four eels) and those living exclusively in ocean environments (the remaining species). Next, we used eggNOG-mapper (ver. 5.0.2) [24] to identify the functions of each gene family. Finally, we used KOBAS [50] to perform KEGG enrichment analysis using Gene IDs inferred from the eggNOF-mapper.

2.4. Positive Selection Analysis. We investigated evidence of positive selection, driven by adaptation through allele alterations favored by natural selection. To detect positively selected genes (PSGs), we used an ML tree and 237 single-copy orthologous genes inferred from OrthoFinder2. First, we unrooted the ML tree and removed the branch lengths. The amino acid sequences of the 237 single-copy orthologous genes were aligned using MAFFT, followed by alignment of the corresponding nucleotide sequences using PAL2NAL (ver. 14) [25]. Next, a branch-site model was used to identify PSGs using codeml within the PAML package. The likelihood ratio test (LRT) was carried out to compare two models: a null model by setting "model = 2, NSsites = 2, fix_omega = 1" and an alternative model by setting "model = 2, NSsites = 2, fix_omega = 0." The best-fitting model was selected using the chi-squared (χ^2) distribution. Genes were considered positively selected based on the following criteria: $p < 0.05$, $d_N/d_S > 1$ [26, 27].

We employed AlphaFold2 (ver. 2.3.1) [28] and PyMOL (ver. 4.6) [29] to predict the three-dimensional structure and visualize the PSG, respectively.

3. Results

3.1. Evolutionary History and Gene Family Dynamics in Anguillid Eels. Our analysis identified 25,867 orthogroups encompassing 573,255 genes, with 97.9% of these genes successfully clustered and assigned to orthogroups. We constructed a species tree, using 260 single-copy orthologous genes (Figure 1).

We analyzed significantly expanded gene families among the 14 actinopterygian species ($p < 0.05$) (Figure 1). Among the 25,867 orthogroups, 749 orthogroups were significantly expanded in the Anguillid eel group. Among the four eels, *A. bicolor pacifica* exhibited the smallest significantly expanded gene families, whereas *A. rostrata* showed the most. This variation in gene family expansion may reflect different evolutionary pressures or ecological adaptations within eel lineages. Subsequently, a *t*-test was used to identify gene families that were exclusively enriched in the Anguillid eel lineage compared to the 10 actinopterygian species. To investigate the functional categories of the expanded gene families, we annotated them using the KEGG database. Among the expanded gene families, the necroptosis, mitophagy, ferroptosis, endocytosis, apoptosis,

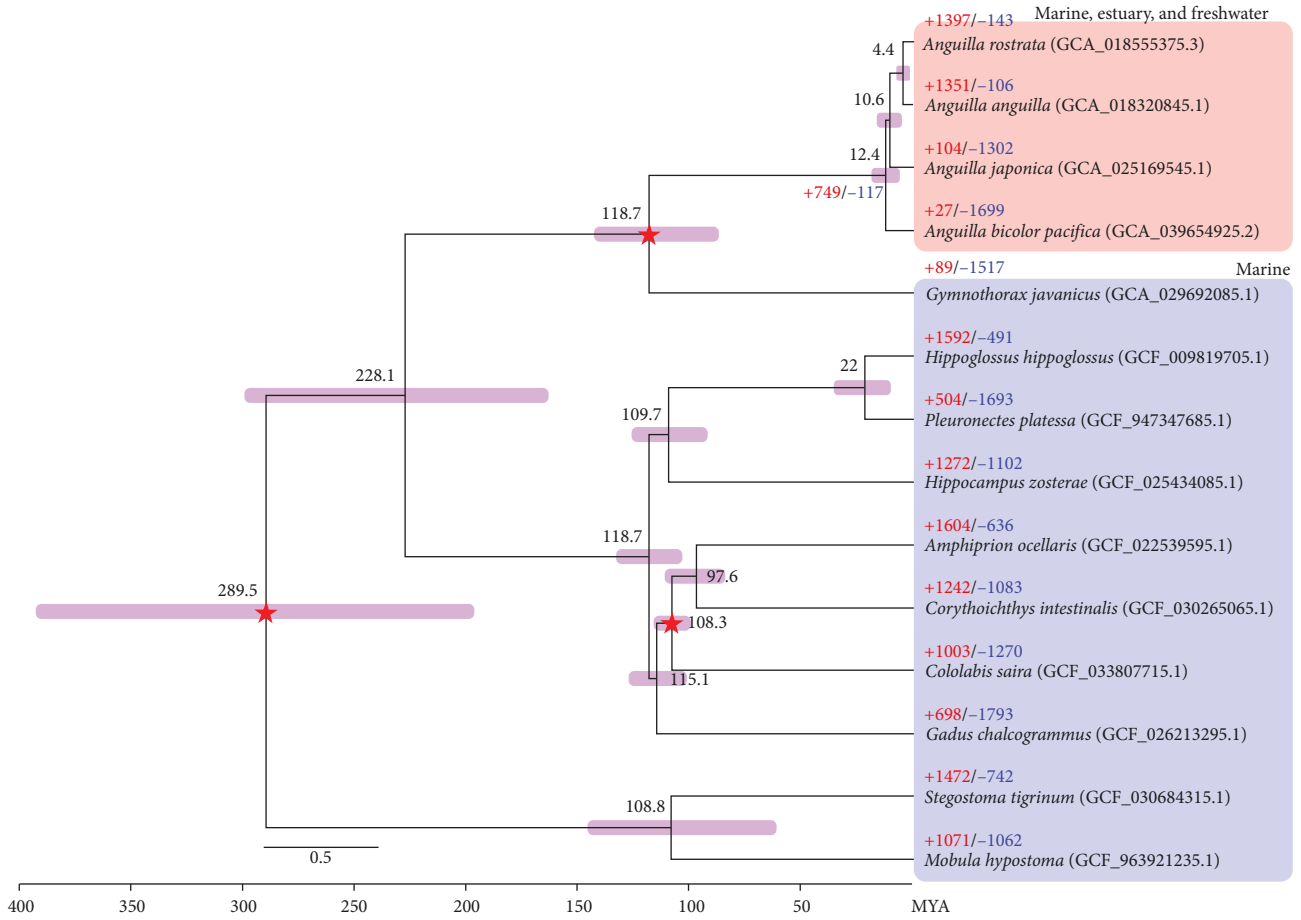

FIGURE 1: Maximum-likelihood (ML) tree reconstructed using 260 single-copy orthologous genes from 14 actinopterygian species. The numbers at each node indicate divergence times. The red and blue numbers above the species name represent significantly expanded and contracted gene families, respectively. Purple bars denote the 95% confidence intervals for evolutionary divergence times. The three red stars represent the calibration points used in this study. The time scale, represented in million years ago (MYA), is displayed under the tree.

phagosome, lysosome, and efferocytosis pathways were significantly enriched in the genomes of Anguillid eels (Figure S1). To gain deeper insights into cellular degradation and recycling mechanisms, we further investigated the gene families associated with the autophagy-lysosome pathway (ALP) (Table S2). Furthermore, we observed the expansion of tumor protein (*TP53*), a gene known for its role in responding to cellular stress stimuli, including oncogene activation and DNA damage, thereby promoting apoptosis [30, 31]. The BCL2 family, which plays a critical role in regulating the apoptosis process, was also enriched in the genomes of the Anguillid eels, specifically through the expansion of the *BCL2L14* gene family [32]. Furthermore, monocyte to macrophage differentiation-associated 2 (*MMD2*), a gene involved in apoptosis signaling pathways, was expanded as well [33].

3.2. PSGs Along the Anguillid Eel Lineage. Using the branch-site model, we identified 65 PSGs in the four Anguillid eels. The functions of PSGs were inferred through KEGG functional annotation. Among the pathways identified, necroptosis and endocytosis were consistently detected across all four Anguillid eel species, whereas phagosome and peroxisome pathways were specifically observed in the genomes of *A. anguilla*, *A. japonica*, and *A. bicolor pacifica* (Figure S2). Among PSGs, the *malonyl-CoA decarboxylase* (*MLYCD*) was noted, with its function annotated as "peroxisome (hsa04146)" in the KEGG pathway (Figure S2 and Table 1). The *MLYCD* gene showed significant support for the alternative model over the null model ($p = 0.03$), exhibiting a notably elevated d_N/d_S ratio on the Anguillid eel lineage ($d_N/d_S = 9.61$). Among the five positively selected sites, one site (Ala310) displayed a convergent amino acid substitution (Figure 2a and Table 1). This positively selected site is located within the active site of the MCD domain, which is critical for MLYCD activity (Figure 2b,c) [34].

4. Discussion

The remarkable once-in-a-lifetime migratory behavior of anguillid eels has been the focus of extensive research over many decades [35, 36, 8, 9]. However, a fundamental limitation lies in the inaccessibility of spawning grounds, making it extremely difficult to obtain specimens during their migratory phase. As a result, direct investigations of genome-wide expression dynamics across different environmental stages have not been feasible. To overcome this challenge, the

TABLE 1: Statistic results for the *malonyl-CoA decarboxylase* (*MLYCD*) gene obtained from the branch-site model with the Anguillid eel branch designated as the foreground branch.

Branch	**Eel branch (foreground)**		
Null model (np)	−9911.068820 (29)		
Alternative model (np)	−9908.826303 (30)		
LRTs (*p*-value)	4.49 (0.03)		
Site class	**Proportion**	**Foreground ω**	**Background ω**
0	0.87	0.06	0.06
1	0.12	1	1
2a	0.008	9.61	0.06
2b	0.001	9.61	1
Positively selected sites (BEB)	68 R (0.878), 87 A (0.573), 310 A (0.636), 414 S (0.505), 434 Q (0.82)		

present study was designed to provide new insights into the evolutionary and adaptive processes of anguillid eels from a genomic perspective. To investigate the evolutionary history and patterns of gene family expansion and contraction of Anguillid eels, a comparative genomic analysis of 14 actinopterygian species was conducted (Table S1). Divergence time for the four Anguillid eels was estimated to be between four and 12 MYA, indicating that these four eels diverged relatively recently compared to the other 10 actinopterygian species. During the Miocene epoch (23-5 MYA), the Earth underwent a significant period of global cooling and drying, resulting in a decline in sea levels [37]. This environmental shift posed substantial challenges for aquatic species, as habitat availability and connectivity were altered. However, the ecological adaptability of Anguillid eels, characterized by their capacity to thrive in both freshwater and marine environments, likely provided them with a survival advantage. Additionally, the reduction in sea levels led to the fragmentation of ocean regions, potentially acting as a driver of speciation during this period.

The ALP is a central catabolic system responsible for the degradation and recycling of cytoplasmic materials, thereby maintaining cellular homeostasis [38]. ALP plays a crucial role in the degradation of intracellular macromolecules [39]. Previous studies have documented intestinal degradation in migrating eels, which may be attributed to the evolutionary expansion of the acquisition of genes involved in ALP [9]. Interestingly, our analysis revealed the significant expansion of four genes associated with lysosome function and apoptosis in Anguillid eel species. Among them, the ATPase H+ transporting V1 subunit H (*ATP6V1H*) gene was notably expanded across the genomes of all four eel species. V-ATPases play a crucial role in maintaining the acidic environment of lysosomes by actively pumping protons, a process essential for proper lysosomal function [40]. Since autophagic degradation cannot be completed without lysosomal acidification, the gene expansion of *ATP6V1H* in eels may enhance their ability to regulate the cellular degradation process [41]. Furthermore, we have detected significant expansion of *MMD2*, a gene which involved in apoptosis signaling pathways. This suggests that under stressful or damaged conditions, MMD2 may stimulate programed cell death to remove compromised cells. This process may help maintain cellular health by recycling components, ensuring energy efficiency during their once-in-a-lifetime migration to the deep sea. Likewise, necroptosis, mitophagy, ferroptosis, endocytosis, apoptosis, phagosome, lysosome, and efferocytosis, which may be related to the ALP, were significantly expanded in the genomes of the four Anguillid eels. The core components of the ALP include mitophagy, endocytosis, phagosome, and lysosomal degradation, which directly mediate the clearance of damaged organelles [42]. In contrast, apoptosis and necroptosis represent genetically programed cell death pathways that are not canonical components of the ALP, but share extensive molecular crosstalk with autophagy [43]. Ferroptosis is mechanistically distinct, driven by iron-dependent lipid peroxidation, yet is partially regulated by autophagy-related processes such as ferritinophagy [44, 45]. Similarly, efferocytosis—the phagocytic clearance of apoptotic cells—does not belong to the ALP per se, but functionally converges on lysosome-dependent degradation [46]. Taken together, these results suggest that while only a subset of these pathways (mitophagy, endocytosis, phagosome, and lysosome) constitutes the ALP proper, other forms of programed cell death (apoptosis, necroptosis, and ferroptosis) and clearance (efferocytosis) extensively interact with the ALP, highlighting its role as a central hub coordinating both survival and death programs. Therefore, the significant expansion of those pathways in Anguillid eels suggests that their genomes have adapted to meet the physiological demands of their unique life-history traits, such as long-distance migration and the need for efficient energy recycling. These findings provide valuable insights into the genetic mechanisms underlying the resilience and adaptability of Anguillid eels and provide a foundation for further studies on the functional implications of these genetic expansions in the context of their ecological and evolutionary success.

Using the branch-site model, we found the MLYCD gene was positively selected. The function of MLYCD in peroxisomes contributes to maintaining the metabolic balance between lipid and glucose oxidation [47]. Therefore, MLYCD-mediated fatty acid metabolism may play a critical role in facilitating adaptive benefits in Anguillid eels. Furthermore, this convergent mutation may be associated with the expansion of ALP genes in the eel lineage mentioned above, suggesting a potential link between MLYCD and the ALP's role in the unique physiological processes of Anguillid eels. Despite

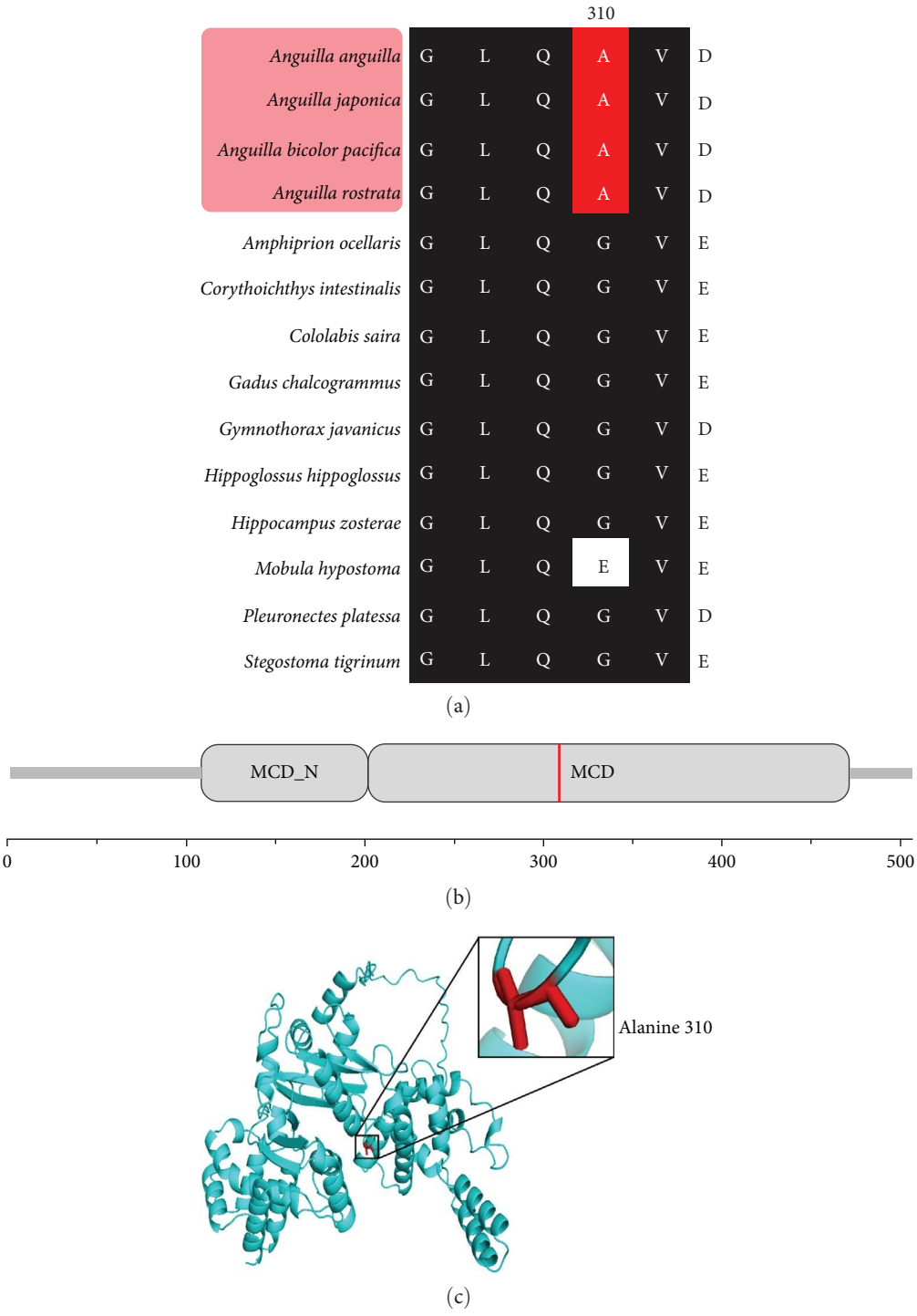

FIGURE 2: (a) Multiple sequence alignment (MSA) of the *MLYCD* gene across 14 actinopterygian species. The four Anguillid eels are highlighted with red on the left side of the alignment. Within the MSA, regions with a black background denote highly conserved sequences, while regions with a red background indicate positively selected residues. (b) Domain architecture of MLYCD is illustrated, highlighting the MCD_N and MCD domains. The red line represents the position of the positively selected residue (Ala 310) within the MCD domain. (c) The three-dimensional structure of malonyl-CoA decarboxylase (MLYCD) from *Anguilla bicolor pacifica* is depicted in a ribbon diagram. The positively selected site is highlighted in red.

the significance of MLYCD for cellular stability, its specific function in aquatic vertebrates, including Anguillid eels, remains largely unknown. Given the importance of MLYCD in the adaptive evolution of these species, it is crucial to conduct further functional studies to determine its precise role in aquatic environments. Such research could provide deeper insights into the molecular mechanisms supporting the survival and adaptability of Anguillid eels, particularly

during the challenging migratory life stages. Understanding the MLYCD functions may also have broader implications for studying lysosomal stability and autophagy pathways in other vertebrates, potentially revealing new aspects of cellular resilience in diverse ecological contexts.

In this study, we performed a comparative genomic analysis of 14 actinopterygian species to identify lineage-specific genomic patterns in Anguillid eels. Because these eels spawn in the deep ocean, collecting samples from migratory or reproductive stages is virtually impossible, making direct validation of gene expression under natural conditions unfeasible. Even today, in the twenty-first century, the genetics of Anguillid eels remain enigmatic. Although artificial maturation and spawning have been achieved, the essential environmental factors of deep-sea migration, such as extreme hydrostatic pressure, strong currents, and limited food availability, cannot be reproduced experimentally [48, 49]. This limitation precludes direct functional testing of our genomic predictions. Nevertheless, the unique physiological traits of Anguillid eels, including intestinal degradation and the cessation of feeding during migration, provide a compelling biological framework for interpreting the genomic signatures identified here. Our findings reveal both the challenges and the potential of genomic approaches to eel biology, suggesting critical insights into the evolutionary processes that shape the Anguillid lineage's remarkable life history.

5. Conclusions

In summary, this study provides a comparative genomic perspective on the evolutionary history of Anguillid eels, highlighting lineage-specific expansions of gene families linked to the ALP and the identification of PSGs. Our findings suggest a potential role for these genes in supporting the physiological challenges of long-distance migration and prolonged nonfeeding states. However, the lack of direct functional validation due to the inaccessibility of migratory-phase samples remains a significant limitation. Consequently, the interpretations presented here should be regarded as hypotheses that establish a genomic framework for future studies rather than definitive evidence of functional adaptation. Nevertheless, by linking genomic signatures with known physiological traits, such as intestinal degradation and cessation of feeding during spawning migration, this work offers valuable insights into the adaptive strategies of Anguillid eels. Further experimental and functional investigations will be essential to validate these predictions and to further elucidate how eels accomplish their remarkable life history strategy.

Data Availability Statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics Statement

The authors have nothing to report.

Consent

All authors consent to the publication of this study.

Conflicts of Interest

The authors declare no competing interests.

Author Contributions

Hyeongwoo Choi: conceptualization, software, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization. **Hyeongju Choi:** sampling, formal analysis. **Junil Ko:** software, formal analysis. **Yeongjin Gwon:** investigation, writing – review and editing. **Jeong-Kyu Kim:** data curation, writing – review and editing. **Young-Jin Seo:** formal analysis, writing – review and editing. **Seong-il Eyun:** conceptualization, writing – review and editing, supervision, project administration.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*) Figure S1: KEGG pathway enrichment analysis results for significantly enriched in Anguillid eels (PSGs). Figure S2: KEGG pathway enrichment analysis results for PSGs. Table S1: Information on the 14 genomes used for comparative analysis in this study. Table S2: Comparison of expanded gene families in four Anguillid eel groups with 12 actinopterygian species.

References

[1] I. C. Liao, Y. K. Hsu, and W. C. Lee, "Technical Innovations in Eel Culture Systems," *Reviews in Fisheries Science* 10, no. 3-4 (2010): 433–450.

[2] H. Tanaka, "Progression in Artificial Seedling Production of Japanese Eel *Anguilla japonica*," *Fisheries Science* 81, no. 1 (2015): 11–19.

[3] Y. Yuan, Y. Yuan, Y. Dai, Y. Gong, and Y. Yuan, "Development Status and Trends in the Eel Farming Industry in Asia," *North American Journal of Aquaculture* 84, no. 1 (2022): 3–17.

[4] C. G. J. Belpaire, G. Goemans, C. Geeraerts, et al., "Decreasing Eel Stocks: Survival of the Fattest?" *Ecology of Freshwater Fish* 18, no. 2 (2009): 197–214.

[5] B. Guhl, F. J. Stürenberg, and G. Santora, "Contaminant Levels in the European Eel (*Anguilla anguilla*) in North Rhine-Westphalian Rivers," *Environmental Sciences Europe* 26, no. 1 (2014): 26.

[6] H. Shiraishi and V. Crook, "Eel Market Dynamics: An Analysis of Anguilla Production," (2015).

[7] T. Arai, "Ecology and Evolution of Migration in the Freshwater Eels of the Genus *Anguilla Schrank*, 1798," *Heliyon* 6, no. 10 (2020): e05176.
[8] R. M. Wright, A. T. Piper, K. Aarestrup, et al., "First Direct Evidence of Adult European Eels Migrating to Their Breeding Place in the Sargasso Sea," *Scientific Reports* 12, no. 1 (2022): 15362.
[9] K. Tsukamoto, S. Chow, T. Otake, et al., "Oceanic Spawning Ecology of Freshwater Eels in the Western North Pacific," *Nature Communications* 2, no. 1 (2011): 179.
[10] M. Freese, L. Y. Rizzo, J. D. Pohlmann, et al., "Bone Resorption and Body Reorganization during Maturation Induce Maternal Transfer of Toxic Metals in Anguillid Eels," *Proceedings of the National Academy of Sciences of the United States of America* 116, no. 23 (2019): 11339–11344.
[11] M. Freese, R. Sühring, L. Marohn, et al., "Maternal Transfer of Dioxin-Like Compounds in Artificially Matured European Eels," *Environmental Pollution* 227 (2017): 348–356.
[12] Y. Ozaki, H. Koga, T. Takahashi, S. Adachi, and K. Yamauchi, "Lipid Content and Fatty Acid Composition of Muscle, Liver, Ovary and Eggs of Captive-Reared and Wild Silver Japanese Eel *Anguilla japonica* during Artificial Maturation," *Fisheries Science* 74, no. 2 (2008): 362–371.
[13] H. Wang, H. T. Wan, B. Wu, et al., "A Chromosome-Level Assembly of the Japanese Eel Genome, Insights Into Gene Duplication and Chromosomal Reorganization," *GigaScience* 11 (2022): giac120.
[14] E. Parey, A. Louis, J. Montfort, et al., "Genome Structures Resolve the Early Diversification of Teleost Fishes," *Science* 379, no. 6632 (2023): 572–575.
[15] H. Choi, J. Nam, S. Yang, and S. Eyun, "Highly Contiguous Genome Assembly and Gene Annotation of the Short-Finned Eel (*Anguilla bicolor Pacifica*)," *Scientific Data* 11, no. 1 (2024): 952.
[16] J. M. I. Barth, C. Gubili, M. Matschiner, et al., "Stable Species Boundaries Despite Ten Million Years of Hybridization in Tropical Eels," *Nature Communications* 11, no. 1 (2020): 1433.
[17] D. M. Emms and S. Kelly, "OrthoFinder: Phylogenetic Orthology Inference for Comparative Genomics," *Genome Biology* 20, no. 1 (2019): 238.
[18] B. Buchfink, C. Xie, and D. H. Huson, "Fast and Sensitive Protein Alignment Using DIAMOND," *Nature Methods* 12, no. 1 (2015): 59-60.
[19] K. Katoh, K. Misawa, K. I. Kuma, and T. Miyata, "MAFFT: A Novel Method for Rapid Multiple Sequence Alignment Based on Fast Fourier Transform," *Nucleic Acids Research* 30, no. 14 (2002): 3059–3066.
[20] Z. Yang, "PAML 4: Phylogenetic Analysis by Maximum Likelihood," *Molecular Biology and Evolution* 24, no. 8 (2007): 1586–1591.
[21] S. Kumar, G. Stecher, M. Suleski, and S. B. Hedges, "TimeTree: A Resource for Timelines, Timetrees and Divergence Times," *Molecular Biology and Evolution* 34, no. 7 (2017): 1812–1819.
[22] F. K. Mendes, D. Vanderpool, B. Fulton, and M. W. Hahn, "CAFE 5 models Variation in Evolutionary Rates among Gene Families," *Bioinformatics* 36, no. 22-23 (2020): 5516–5518.
[23] H. Choi, Y. K. An, C. J. Lee, et al., "Genome Assembly, Gene Content, and Plastic Gene Expression Responses to Salinity Changes in the Brackishwater Clam (*Corbicula japonica*) from a Dynamic Estuarine Environment," *Journal of Hazardous Materials* 483 (2025): 136627.
[24] C. P. Cantalapiedra, A. Hernández-Plaza, I. Letunic, P. Bork, and J. Huerta-Cepas, "EggNOG-Mapper v2: Functional Annotation, Orthology Assignments and Domain Prediction at the Metagenomic Scale," *Molecular Biology and Evolution* 38, no. 12 (2021): 5825–5829.
[25] M. Suyama, D. Torrents, and P. Bork, "PAL2NAL: Robust Conversion of Protein Sequence Alignments Into the Corresponding Codon Alignments," *Nucleic Acids Research* 34 (2006): W609–W612.
[26] H. Choi, O. H. Yu, and S. Eyun, "Evolutionary Insights Into Adaptation of Hemocyanins From Deep-Sea Hydrothermal Vent Shrimps," *Marine Pollution Bulletin* 215 (2025): 117872.
[27] E.-J. Kim, H. Doh, J. Yang, and S.-i. Eyun, "The Occurrence of Positive Selection on BicA Transporter of *Microcystis aeruginosa*," *Ecotoxicology and Environmental Safety* 283 (2024): 116795.
[28] J. Jumper, R. Evans, A. Pritzel, et al., "Highly Accurate Protein Structure Prediction With AlphaFold," *Nature* 596, no. 7873 (2021): 583–589.
[29] L. Schrödinger and W. DeLano, "PyMOL (Version 2.4.0)," 2020, http://www.pymol.org/pymol.
[30] J. D. Amaral, J. M. Xavier, C. J. Steer, and C. M. Rodrigues, "The Role of p53 in Apoptosis," *Discovery Medicine* 9, no. 45 (2010): 145–152.
[31] B. J. Aubrey, G. L. Kelly, A. Janic, M. J. Herold, and A. Strasser, "How Does p53 Induce Apoptosis and How Does This Relate to p53-Mediated Tumour Suppression?" *Cell Death and Differentiation* 25, no. 1 (2018): 104–113.
[32] M. L. Hartman and M. Czyz, "BCL-G: 20 Years of Research on a Non-Typical Protein From the BCL-2 Family," *Cell Death and Differentiation* 30, no. 6 (2023): 1437–1446.
[33] Y. Bu, Y. Liu, R. Wang, et al., "CRISPR/Cas9-Mediated Deletion of Mmd2 Gene Affects the Growth of *Exopalaemon carinicauda*," *Aquaculture* 599 (2025): 742127.
[34] H. Park, V. K. Kaushik, S. Constant, et al., "Coordinate Regulation of Malonyl-CoA Decarboxylase, Sn-Glycerol-3-Phosphate Acyltransferase, and Acetyl-CoA Carboxylase by AMP-Activated Protein Kinase in Rat Tissues in Response to Exercise," *The Journal of Biological Chemistry* 277, no. 36 (2002): 32571–32577.
[35] J. Aoyama, S. Watanabe, M. J. Miller, et al., "Spawning Sites of the Japanese Eel in Relation to Oceanographic Structure and the West Mariana Ridge," *PLoS ONE* 9, no. 2 (2014): e88759.
[36] M. Béguer-Pon, M. Castonguay, S. Shan, J. Benchetrit, and J. J. Dodson, "Direct Observations of American Eels Migrating across the Continental Shelf to the Sargasso Sea," *Nature Communications* 6, no. 1 (2015): 8705.
[37] M. Steinthorsdottir, H. K. Coxall, A. M. de Boer, et al., "The Miocene: The Future of the Past," *Paleoceanography and Paleoclimatology* 36, no. 4 (2021): e2020PA004037.
[38] N. Mizushima and M. Komatsu, "Autophagy: Renovation of Cells and Tissues," *Cell* 147, no. 4 (2011): 728–741.
[39] S. Finkbeiner, "The Autophagy Lysosomal Pathway and Neurodegeneration," *Cold Spring Harbor Perspectives in Biology* 12, no. 3 (2020): a033993.
[40] M. Forgac, "Vacuolar ATPases: Rotary Proton Pumps in Physiology and Pathophysiology," *Nature Reviews Molecular Cell Biology* 8, no. 11 (2007): 917–929.
[41] C. H. Lo and J. Zeng, "Defective Lysosomal Acidification: A New Prognostic Marker and Therapeutic Target for Neurodegenerative Diseases," *Translational Neurodegeneration* 12, no. 1 (2023): 29.
[42] L. Galluzzi, E. H. Baehrecke, A. Ballabio, et al., "Molecular Definitions of Autophagy and Related Processes," *The EMBO Journal* 36, no. 13 (2017): 1811–1836.
[43] X. Huang, H. Yan, Z. Xu, B. Yang, P. Luo, and Q. He, "The Inducible Role of Autophagy in Cell Death: Emerging

Evidence and Future Perspectives," *Cell Communication and Signaling* 23, no. 1 (2025): 151.
[44] S. J. Dixon, K. M. Lemberg, M. R. Lamprecht, et al., "Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death," *Cell* 149, no. 5 (2012): 1060–1072.
[45] W. Hou, Y. Xie, X. Song, et al., "Autophagy Promotes Ferroptosis by Degradation of Ferritin," *Autophagy* 12, no. 8 (2016): 1425–1428.
[46] D. R. Green, T. H. Oguin, and J. Martinez, "The Clearance of Dying Cells: Table for Two," *Cell Death and Differentiation* 23, no. 6 (2016): 915–926.
[47] L. Zhou, Y. Luo, Y. Liu, et al., "Fatty Acid Oxidation Mediated by Malonyl-CoA Decarboxylase Represses Renal Cell Carcinoma Progression," *Cancer Research* 83, no. 23 (2023): 3920–3939.
[48] H. Izumi, K. Gen, P. M. Lokman, et al., "Maternal Transcripts in Good and Poor Quality Eggs From Japanese Eel, *Anguilla japonica*—Their Identification by Large-Scale Quantitative Analysis," *Molecular Reproduction and Development* 86, no. 12 (2019): 1846–1864.
[49] R. Sudo, T. Asakura, T. Ishikawa, et al., "Transcriptome Analysis of the Japanese Eel (*Anguilla japonica*) During Larval Metamorphosis," *BMC Genomics* 25, no. 1 (2024): 585.
[50] D. Bu, H. Luo, P. Huo, et al., "KOBAS-i: Intelligent Prioritization and Exploratory Visualization of Biological Functions for Gene Enrichment Analysis," *Nucleic Acids Research* 49, no. W1 (2021): W317–W325.