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EVOLUTIONARY CONSERVATION AND FUNCTIONAL DIVERGENCE OF GLUTATHIONE PEROXIDASE (GPX) GENES IN GALLIFORM BIRDS: *GALLUS GALLUS*, *MELEAGRIS GALLOPAVO*, AND *COTURNIX JAPONICA*

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ABSTRACT

The glutathione peroxidase (GPX) gene family plays a crucial role in maintaining cellular redox homeostasis and protecting against oxidative stress. In this study, we performed a comprehensive comparative analysis of GPX genes in three galliform species: *Gallus gallus* (chicken), *Meleagris gallopavo* (turkey), and *Coturnix japonica* (Japanese quail). Genomic structure, chromosomal localization, phylogenetic relationships, conserved motifs, subcellular localization, synteny, and selection pressure analyses were conducted to investigate the evolutionary conservation and functional divergence of the GPX family. Our findings revealed strong conservation of gene structure and chromosomal positioning, particularly for GPX1, GPX2, GPX3, and GPX7, as well as species-specific expansions and alternative splicing events in genes such as GPX4 and GPX8. Phylogenetic and Ka/Ks analyses indicated that most GPX genes are under purifying selection, with notable instances of positive selection suggesting adaptive functional divergence. Conserved motifs and subcellular localization patterns further supported the core redox-protective roles of GPX proteins, while highlighting their compartment-specific and extracellular functions. Overall, this study enhances our understanding of the evolutionary and functional landscape of the GPX gene family in birds and provides a foundation for future research into avian oxidative stress adaptation and redox signaling.

Keywords: Glutathione Peroxidases, Synteny, Galliform species, Phylogenetic, Chromosomal localization.

INTRODUCTION

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the capacity of antioxidant defense systems, posing a significant threat to cellular integrity and overall organism health. Among the primary enzymatic antioxidants, glutathione peroxidases (GPXs) play a pivotal role in detoxifying hydrogen peroxide and lipid hydroperoxides, thereby protecting cells from oxidative damage (Prabhakar *et al.,* 2005; Lubos *et al.,* 2011; Pizzino *et al.,* 2017). These

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selenoproteins are crucial for maintaining redox homeostasis and are involved in diverse physiological processes, including immune function, reproduction, and development. Given their central biological roles, the evolutionary dynamics of *GPX* genes have garnered increasing interest, particularly in exploring how these genes have adapted to varied physiological demands and environmental pressures across species (Bae *et al.,* 2009; Ufer & Wang, 2011; Tian *et al.,* 2021; Dai *et al.,* 2023; Pei *et al.,* 2023).

Birds represent a highly diverse and ecologically versatile clade, making them excellent models for exploring the evolutionary dynamics of antioxidant defense mechanisms (Saino *et al.,* 2011; Castiglione *et al.,* 2020; McWilliams *et al.,* 2021). Within this group, galliform birds, namely chickens (*Gallus gallus*), turkeys (*Meleagris gallopavo*), and Japanese quail (*Coturnix japonica*), are particularly valuable due to their agricultural significance, well-annotated genomes, and distinct ecological adaptations (Barros *et al.,* 2022; Haqani *et al.,* 2023; Degalez *et al.,* 2024; Hajibarat *et al.,* 2024). Despite their close phylogenetic relationships, these species experience different environmental pressures and physiological demands, providing a unique opportunity to study how GPX gene families have diversified and adapted (Wang *et al.,* 2012; Wu *et al.,* 2018; Morris *et al.,* 2020; Eleiwa *et al.,* 2024).

Understanding the evolution of GPX genes in galliforms has broader implications for elucidating the molecular underpinnings of stress resilience in vertebrates. Domesticated birds, in particular, frequently encounter environmental challenges such as temperature extremes, hypoxia, and pathogen exposure, conditions that elevate oxidative stress and may drive adaptive changes in antioxidant gene networks (Bae *et al.,* 2009; Costantini & Verhulst, 2009; Abbasi *et al.,* 2017; Akinyemi & Adewole, 2021; Tian *et al.,* 2021; Oke *et al.,* 2024). Comparative analyses can reveal evolutionary mechanisms, such as gene duplication, alternative splicing, or positive selection that enhance GPX functionality in response to these challenges (Kondrashov, 2012; Song *et al.,* 2021; Pei *et al.,* 2023). Such insights have potential applications in selective breeding programs aimed at improving oxidative stress resistance in poultry, with relevance to both agricultural sustainability and avian conservation (Tizard *et al.,* 2019; Oleforuh-Okoleh *et al.,* 2023; Lin & Chen, 2024; Nawaz *et al.,* 2024).

This study presents a comprehensive comparative analysis of the *GPX* gene family in three economically and biologically important galliform species: *Gallus gallus*, *Meleagris gallopavo*, and *Coturnix japonica*. Through an integrated approach combining genomic characterization, phylogenetic reconstruction, chromosomal localization, synteny mapping, conserved motif analysis, and selection pressure estimation, we investigate the evolutionary conservation and functional diversification of *GPX* genes in these birds. Special emphasis is placed on gene structure variability, alternative isoform expression, and subcellular localization patterns. Our findings offer novel insights into the evolutionary forces shaping antioxidant gene families in avian species and enhance our understanding of how molecular defense mechanisms adapt to environmental and physiological challenges.

MATERIALS AND METHODS

Database Mining and Sequence Retrieval

To identify GPX gene sequences, a genome-wide search was conducted on the protein datasets of chicken, turkey, and Japanese quail using HMMER3 (version 3.3.1) (http://hmmer.org/) on a Linux platform. The Hidden Markov Model (HMM) profile corresponding to the glutathione peroxidase domain (PF00255) was downloaded from the PFAM database (http://pfam.xfam.org/) and used as a query. The hmmsearch tool in HMMER3 (http://hmmer.org/) was employed to scan each species' protein database against the HMM profile using BlastP methods with a cut-off E-value <10−5. For each candidate

gene, detailed genomic annotations were compiled from multiple database sources, including the NCBI database (https://www.ncbi.nlm.nih.gov/) and the Ensembl database (https://www.ensembl.org/index.html). This multi-platform approach ensured comprehensive characterization of conserved and variant GPX isoforms across the studied avian species.

Comparative Phylogenetic Analysis of GPX Proteins

Multiple sequence alignment of full-length GPX protein sequences from the three species and *Mus musculus* (house mouse) was performed using the MUSCLE algorithm. A maximum likelihood (ML) phylogenetic tree was constructed using MEGA 11.0 with 1000 bootstrap replicates to assess the statistical reliability of the branching patterns. The *Mus musculus* GPX sequences were used as an outgroup. Clustering patterns and bootstrap support values were analyzed to infer evolutionary relationships among GPX family members.

Chromosomal Mapping, Synteny, and Ka/Ks of GPX Analysis

The genomic characteristics of GPX genes, including gene size, coding sequence (CDS) length, protein length, and exon count, were analyzed across three avian species. Chromosomal positions of GPX genes were determined using the MG2C online tool (http://mg2c.iask.in/mg2c_v2.1/), while syntenic relationships were assessed by comparing gene arrangements among species. Additionally, structural variations, such as alternative splicing events, were examined in different isoforms.

For synteny analysis, MCScanX in TBtools was used to detect gene duplication events and conserved genomic regions. Genome data, including chromosomal lengths and sequences (in GFF3 and FASTA formats), were retrieved from the NCBI genome database (https://www.ncbi.nlm.nih.gov/datasets/genome/) accessed on 15 January 2025. The results were visualized using the TBtools Dual Synteny Plot function to identify conserved blocks and gene pairs across chicken, turkey, and Japanese quail genomes.

The ratio of nonsynonymous (Ka) to synonymous (Ks) substitution rates was calculated to evaluate the evolutionary selection pressures acting on GPX genes, a widely recognized indicator of selection pressure on protein-coding genes (Hanada *et al.,* 2007). Protein-coding sequences of GPX paralogs were aligned, and Ka/Ks ratios were computed using the Neighboring method implemented in MEGA 11.0. Paralog pairs with Ka/Ks > 1 were considered under positive selection, while those with Ka/Ks < 1 were considered under purifying selection.

Identification of Conserved Motifs and Prediction of GPX Proteins Subcellular Localization

To identify conserved motifs within the GPX protein sequences, a MEME (Multiple EM for Motif Elicitation) analysis was performed using the MEME Suite (version 5.5.7; http://meme-suite.org/tools/meme) (Bailey *et al.,* 2009). Parameters were set to detect motifs with a width of 6 to 50 amino acids, and the maximum number of motifs to be identified was set to 10. The analysis yielded a series of conserved motifs represented as colored boxes aligned with the protein sequences. The position information and motif sequences were used to assess conserved functional regions across the species. The results were visualized using TBtools (Chen *et al.,* 2020). The subcellular location of each wheat protein was predicted using WoLF PSORT with default built-in parameters (https://wolfpsort.hgc.jp/) (Horton *et al.,* 2007). Prediction consensus was predicted based on the majority of the result probabilities.

RESULTS

Genomic Analysis of GPX Genes in Chickens, Turkeys, and Quail

A comparative overview of the genomic features of GPX genes identified in the three species is summarized in Table (1). This dataset includes detailed information such as gene symbols, reference sequence IDs, protein IDs, chromosomal locations, exon counts, genomic start and end positions, gene sizes, coding sequence (CDS) lengths, and corresponding protein lengths (in amino acids).

GPX Genes identified a high degree of conservation in gene structure across the three avian species. For instance, the *Gpx1* gene is characterized by similar CDS lengths (588 bp in *G. gallus* and *M. gallopavo*, 597 bp in *C. japonica*), resulting in proteins of comparable lengths (195, 195, and 198 amino acids, respectively). The chromosomal locations of GPX genes are consistent among species, with genes such as *Gpx8* being mapped to the Z chromosome in all cases (Table 1).

Gene size and CDS length variations are evident among isoforms of *Gpx4* and *Gpx8*. In *G. gallus*, *Gpx4_v1* and *Gpx4_v2* differ in CDS length (585 bp and 609 bp) and protein size (194 and 202 amino acids), highlighting the presence of alternative splicing. Similarly, *Gpx8_v1* and *Gpx8_v2* in all species exhibit substantial differences in CDS lengths (633 bp and 276 bp) and protein sizes (210 and 91 amino acids), reflecting isoform-specific characteristics**.**

Among the three species, *C. japonica* exhibits some of the largest gene sizes, such as *Gpx7* (3080 bp) and *Gpx8_v1* (1283 bp), compared to their counterparts in *G. gallus* and *M. gallopavo*. These differences may be attributed to variations in intronic or non-coding regions. Exon counts range from 2 in *Gpx1* and *Gpx2* to 7 in *Gpx4* and 6 in *Gpx7* across species, indicating conserved gene architecture (Table 1).

The proteins encoded by GPX genes vary slightly in length across species, with most differences arising from alternative splicing or subtle variations in CDS. For example, *Gpx3* encodes a protein of 219 amino acids in all species, demonstrating strong evolutionary conservation in this enzyme structure. However, isoforms like *Gpx8_v2* encode shorter proteins (91 amino acids), possibly reflecting specialized functions.

Comparative Phylogenetic Analysis of GPX Proteins

The phylogenetic analysis of full-length glutathione peroxidase (GPX) protein sequences from *Gallus gallus*, *Meleagris gallopavo*, *Coturnix japonica*, and *Mus musculus* was performed using a maximum likelihood (ML) approach with 1000 bootstrap replicates. Multiple sequence alignment was conducted using MUSCLE, and the phylogenetic tree was constructed using MEGA 11.0. The resulting tree (Figure 1) revealed distinct clustering patterns among the GPX protein sequences, with each GPX family member forming well-defined clades.

The tree reveals that GPX proteins cluster primarily by subfamily (GPX1-8) rather than by species, indicating that functional divergence of these antioxidant enzymes preceded the speciation events separating these organisms. The color-coded branches (green for GPX8, light blue for GPX7, red for GPX4, blue for GPX3/5/6, and yellow for GPX1/2) highlight distinct evolutionary lineages. At the same time, the high bootstrap values (many exceeding 90%) demonstrate strong statistical support for the major branch points. Within each GPX subfamily, avian proteins typically cluster together separately from mouse homologs, reflecting expected evolutionary relationships, while multiple variants (v1, v2, v3) of specific GPX proteins suggest gene duplication events. This pattern of organization emphasizes that the functional conservation of these critical antioxidant enzymes outweighs species-specific adaptations, characteristic of proteins serving essential biological functions across diverse organisms.

Table 1: Genomic Features of GPX Genes Identified in Chicken, Turkey, and Japanese quail

Gene Symbol	RefSeq_ID	Protein_ID	Chromosome	No of Exons	start	End	Gene_size	CDS_Size	Protein (a)
Gg_Gpx1	NM_001277853.3	NP_001264782.3	12	2	3029728	3030641	837	588	195
Gg_Gpx2	NM_001277854.3	NP_001264783.2	5	2	530082	530888	736	576	191
Gg_Gpx3	NM_001163232.3	NP_001156704.1	13	5	12661488	12663434	981	660	219
Gg_Gpx4_v1	NM_204220.3	NP_989551.2	28	7	3370734	3372272	817	585	194
Gg_Gpx4_v2	NM_001346449.2	NP_001333378.1	28	7	3370734	3372272	841	609	202
Gg_Gpx7	NM_001163245.2	NP_001156717.1	8	3	24131723	24140316	1257	633	210
Gg_Gpx8_v1	XM_423834.8	XP_423834.1	Z	3	17236443	17239616	1234	633	210
Gg_Gpx8_v2	XM_015277569.4	XP_015133055.1	Z	3	17236443	17239616	972	276	91
Mg_Gpx1	NM_001308652.1	NP_001295581.1	14	2	2377076	2377998	853	588	195
Mg_Gpx2	XM_010710801.3	XP_010709103.2	5	2	4863657	4864493	768	576	191
Mg_Gpx3	NM_001308655.1	NP_001295584.1	15	4	13423927	13425131	829	580	219
Mg_Gpx4	NM_001308651.1	NP_001295580.1	30	6	2216471	2217828	736	504	192
Mg_Gpx7	XM_003208870.4	XP_003208918.1	10	3	22901172	22909103	1179	633	210
Mg_Gpx8 v1	XM_010725414.2	XP_010723716.1	Z	3	15294539	15298910	1269	633	210
Mg_Gpx8 v2	XM_019610395.2	XP_019465940.1	Z	3	15294539	15298910	1010	276	91
Cj_Gpx1	XM_015874588.1	XP_015730074.1	12	2	1695958	1696951	911	597	198
Cj_Gpx2	XM_015863087.2	XP_015718573.1	5	2	493794	494637	773	576	191
Cj_Gpx3	XM_015875840.1	XP_015731326.1	13	5	5011535	5013460	1017	660	219
Cj_Gpx7	XM_015870585.2	XP_015726071.1	8	3	21819761	21828816	3080	633	210
Cj_Gpx8_v1	XM_015848738.2	XP_015704224.1	Z	3	18248182	18252372	1283	633	210
Cj_Gpx8_v2	XM_015848739.2	XP_015704225.1	Z	3	18248182	18252372	1036	276	91

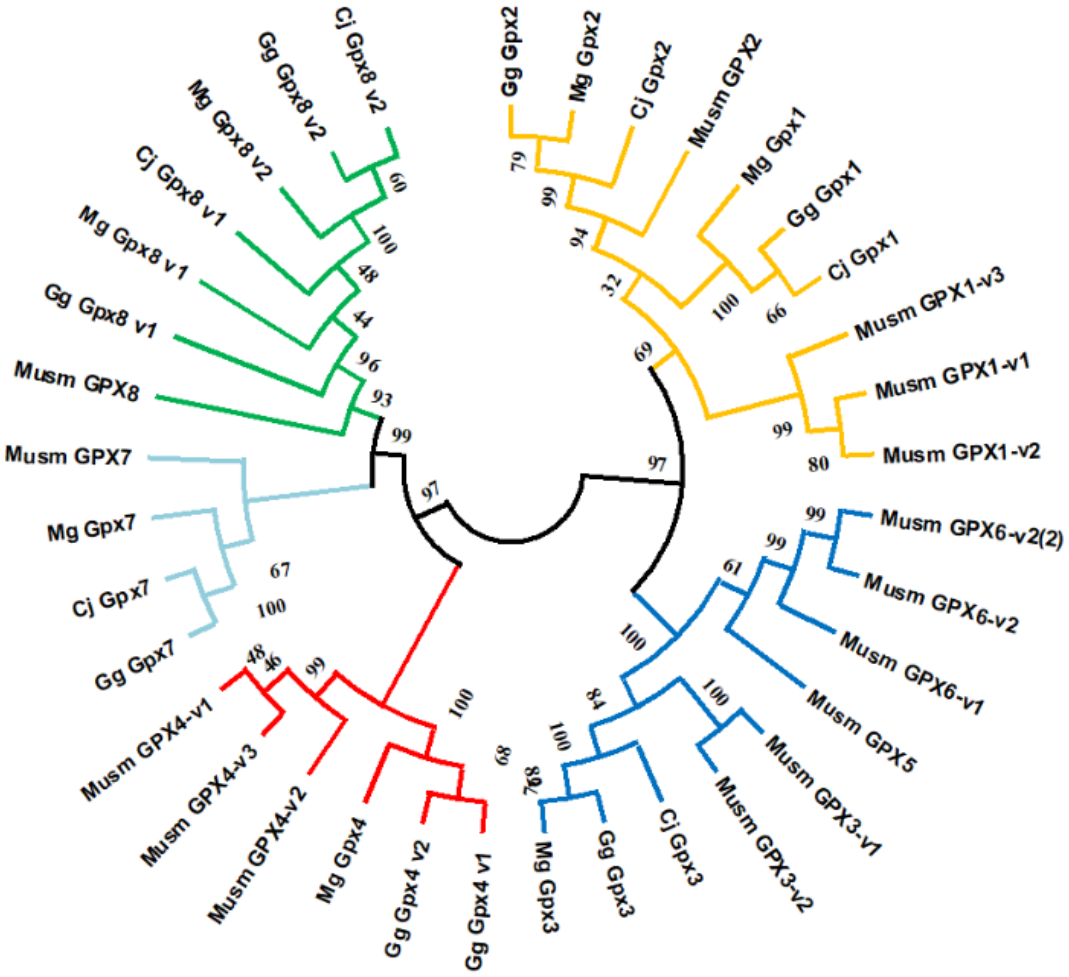

Figure 1: Phylogenetic analysis of full-length GPX protein sequences from *Gallus gallus, Meleagris gallopavo, Coturnix japonica,* and *Mus musculus*.

Chromosomal Localization and Evolutionary Conservation of *GPX* Genes

The chromosomal localization of glutathione peroxidase (*GPX*) gene family members was comprehensively mapped across three avian species: *Gallus gallus*, *Meleagris gallopavo*, and *Coturnix japonica* (Figure 2).

Notably, the *GPX* gene family exhibited distinct chromosomal distribution patterns in each species, varying lengths and positions across different chromosomes. In *Gallus gallus*, *GPX* genes were distributed across several chromosomes: *Gg_Gpx2* on chromosome 5, *Gg_Gpx1* on chromosome 12, *Gg_Gpx3* on chromosome 13, *Gg_Gpx4* on chromosome 28, and *Gg_Gpx8* on chromosome Z. Additionally, *Gg_Gpx7* was observed on chromosome 8. In *Meleagris gallopavo*, a similar distribution pattern was observed with *Mg_Gpx2* on chromosome 5, *Mg_Gpx7* on chromosome 10, *Mg_Gpx1* on chromosome 14, *Mg_Gpx3* on chromosome 15, *Mg_Gpx4* on chromosome 30, and *Mg_Gpx8* on chromosome Z. For *Coturnix japonica*, the distribution included *Cj_Gpx2* on chromosome 5, *Cj_Gpx7* on chromosome 8, *Cj_Gpx1* on chromosome 12, *Cj_Gpx3* on chromosome 13, and *Cj_Gpx8* on chromosome Z. The chromosomal positions ranged from approximately 0 to 90 Mb in length across all species, with precise positions marked in green adjacent to their respective chromosomes.

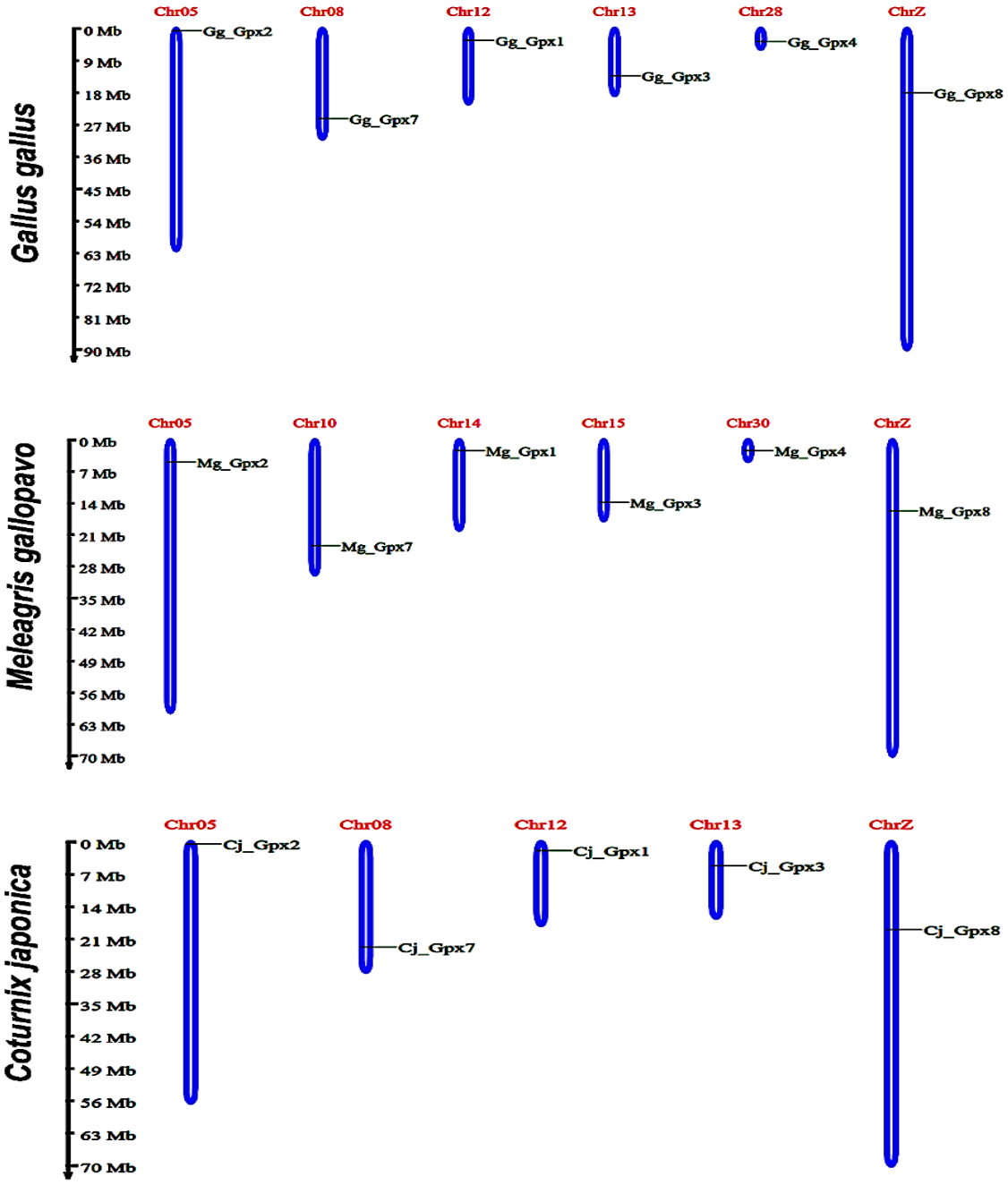

Figure 2: Chromosomal localization of the *GPX* gene family members of *Gallus gallus, Meleagris gallopavo, and Coturnix japonica* chromosomes. The blue bars represent the chromosomes. Chromosome numbers are shown on the top. *Camelus GPX* genes are marked green to the right of the chromosomes. The scale bar on the left indicates the length of the chromosome.

Syntenic Conservation of GPX Gene Family Members Across Three Species

Synteny analysis was conducted to investigate the evolutionary relationship of GPX genes among three avian species: Chicken, Turkey, and Japanese quail (Figure 3). The study revealed significant conservation of GPX gene syntenic relationships across the species examined. In particular, syntenic blocks containing GPX genes were identified and depicted as gray lines connecting aligned genomic regions between the paired genomes. In contrast, red lines clearly distinguished the syntenic GPX gene pairs.

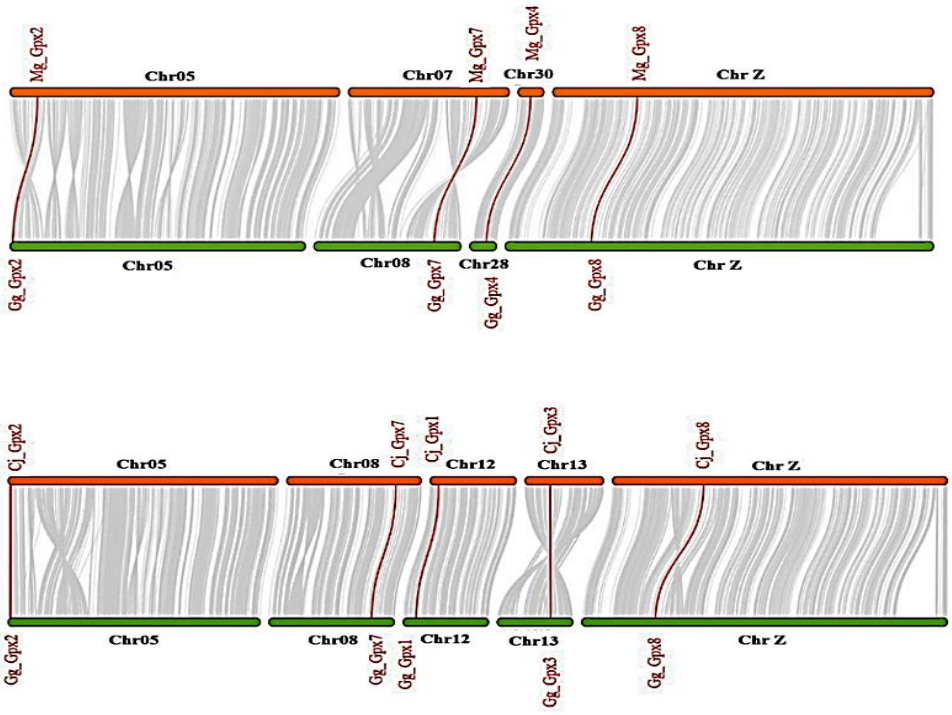

Figure 3: Synteny analysis of *Meleagris gallopavo and Coturnix japonica* with *Gallus gallus*. The gray lines represent aligned blocks between the paired genomes, and the red lines indicate syntenic *GPX* gene pairs.

In the comparison between *Meleagris gallopavo* and *Gallus gallus* (upper panel), syntenic relationships were observed for multiple *GPX* genes: *Mg_Gpx2* on chromosome 5 showed synteny with *Gg_Gpx2* on the same chromosome, *Mg_Gpx7* on chromosome 7 aligned with *Gg_Gpx7* on chromosome 8, *Mg_Gpx4* on chromosome 30 displayed synteny with *Gg_Gpx4* on chromosome 28, and *Mg_Gpx8* on chromosome Z exhibited syntenic relationship with *Gg_Gpx8* on the same sex chromosome. Similarly, the synteny analysis between *Coturnix japonica* and *Gallus gallus* (lower panel) demonstrated conserved syntenic blocks for *Cj_Gpx2* on chromosome 5 with *Gg_Gpx2*, *Cj_Gpx7* on chromosome 8 with *Gg_Gpx7*, *Cj_Gpx3* on chromosome 13 with *Gg_Gpx3*, *Cj_Gpx1* on chromosome 12 with *Gg_Gpx1*, and *Cj_Gpx8* on chromosome Z with *Gg_Gpx8*. The syntenic blocks, represented by gray lines, showed varying degrees of genomic rearrangements while maintaining the positional conservation of *GPX* genes.

Ka/Ks Analysis of GPX Gene Evolution in the Three Species

The evolutionary selection pressures acting on glutathione peroxidase (*GPX*) genes were analyzed in three avian species: Chicken, Turkey, and Japanese quail. The analysis employed the ratio of non-synonymous substitution rates (Ka) to synonymous substitution rates (Ks), a widely recognized indicator of selection pressure on protein-coding genes.

In Turkey, analysis of six paralog pairs revealed a predominantly purifying selection, with Ka/Ks ratios below 1 for four pairs (66.7%). Two paralog pairs exhibited positive/diversifying selection: Gpx4-Gpx3 (Ka/Ks =1.87) and Gpx3-Gpx2 (Ka/Ks =1.05). The strongest purifying selection was observed between Gpx4-Gpx1 (Ka/Ks =0.52), indicating strong functional constraint

In chicken, analysis of eight GPX paralog pairs revealed that five pairs (62.5%) evolved under purifying selection (Ka/Ks <1), indicating strong functional constraint (Table 2).

Purifying selection occurred in Gpx7-Gpx4 (Ka/Ks = 0.27) and Gpx7-Gpx3 (Ka/Ks = 0.35), suggesting critical conserved roles. Three pairs (37.5%) exhibited positive selection (Ka/Ks ≥ 1), with the strongest signal in Gpx4-Gpx3 (Ka/Ks =1.63) and Gpx3-Gpx2 (Ka/Ks =1.34), implying potential functional divergence. The Gpx3-Gpx1 pair (Ka/Ks = 1.05) showed weak positive or near-neutral evolution. Moderate purifying selection was observed in Gpx4-Gpx2 (Ka/Ks =0.68), Gpx4-Gpx1 (Ka/Ks =0.66), and Gpx2-Gpx1 (Ka/Ks =0.46), reflecting varying degrees of evolutionary constraint among these paralogs.

Table 2: Ka/Ks calculation and the type of mutation for the duplicated GPX Gene pairs

Paralog1	Paralog 2	Ks	Ka	Ka/Ks	Type of mutation or Evoluation
Mg_Gpx4	*Mg_Gpx3*	0.510	0.954	1.869955637	Positive or Diversifying
Mg_Gpx4	*Mg_Gpx2*	0.737	0.631	0.855990507	Negative or purifying
Mg_Gpx4	*Mg_Gpx1*	1.323	0.688	0.519579252	Negative or purifying
Mg_Gpx3	*Mg_Gpx2*	0.724	0.759	1.048280572	Positive or Diversifying
Mg_Gpx3	*Mg_Gpx1*	0.870	0.821	0.943625609	Negative or purifying
Mg_Gpx2	*Mg_Gpx1*	1.062	0.568	0.534713879	Negative or purifying
Gg_Gpx7	*Gg_Gpx4*	2.151	0.591	0.274979245	Negative or purifying
Gg_Gpx7	*Gg_Gpx3*	2.283	0.798	0.349613781	Negative or purifying
Gg_Gpx4	*Gg_Gpx3*	0.565	0.923	1.631370933	Positive or Diversifying
Gg_Gpx4	*Gg_Gpx2*	0.947	0.648	0.684166095	Negative or purifying
Gg_Gpx4	*Gg_Gpx1*	1.194	0.792	0.663158067	Negative or purifying
Gg_Gpx3	*Gg_Gpx2*	0.555	0.746	1.342894251	Positive or Diversifying
Gg_Gpx3	*Gg_Gpx1*	0.744	0.781	1.049998206	Positive or Diversifying
Gg_Gpx2	*Gg_Gpx1*	1.080	0.502	0.464484535	Negative or purifying
Cj_Gpx7	*Cj_Gpx3*	2.675	0.784	0.293091779	Negative or purifying
Cj_Gpx3	*Cj_Gpx2*	0.773	0.798	1.032671982	Positive or Diversifying
Cj_Gpx3	*Cj_Gpx1*	0.914	0.837	0.915386694	Negative or purifying
Cj_Gpx2	*Cj_Gpx1*	1.102	0.497	0.450861439	Negative or purifying

In Japanese quail, analysis of four GPX paralog pairs revealed that three pairs (75%) evolved under purifying selection (Ka/Ks < 1), indicating functional conservation. The strongest purifying selection was observed in Gpx7-Gpx3 (Ka/Ks = 0.29), suggesting critical structural or functional constraints. One pair (Gpx3-Gpx2, Ka/Ks = 1.03) exhibited weak positive or near-neutral selection, potentially reflecting relaxed functional constraints or minor adaptive divergence. The remaining purifying selection signals were moderate, with Gpx3-Gpx1 (Ka/Ks = 0.92) and Gpx2-Gpx1 (Ka/Ks = 0.45) further supporting evolutionary conservation across these paralogs.

Conserved Motifs and Subcellular Localization of GPX Proteins

Using a MEME analysis (Figure 1), we analyzed the conserved protein motifs identified in GPX proteins across three galliform species: chicken, turkey, and Japanese quail. The motifs are represented as colored boxes aligned along each protein sequence (Figure 1 and supplementary data), indicating their positions within the amino acid chains. Several motifs, such as Motifs 1, 2, 3, and 7, are consistently present across all species, suggesting conserved functional regions essential for GPX activity.

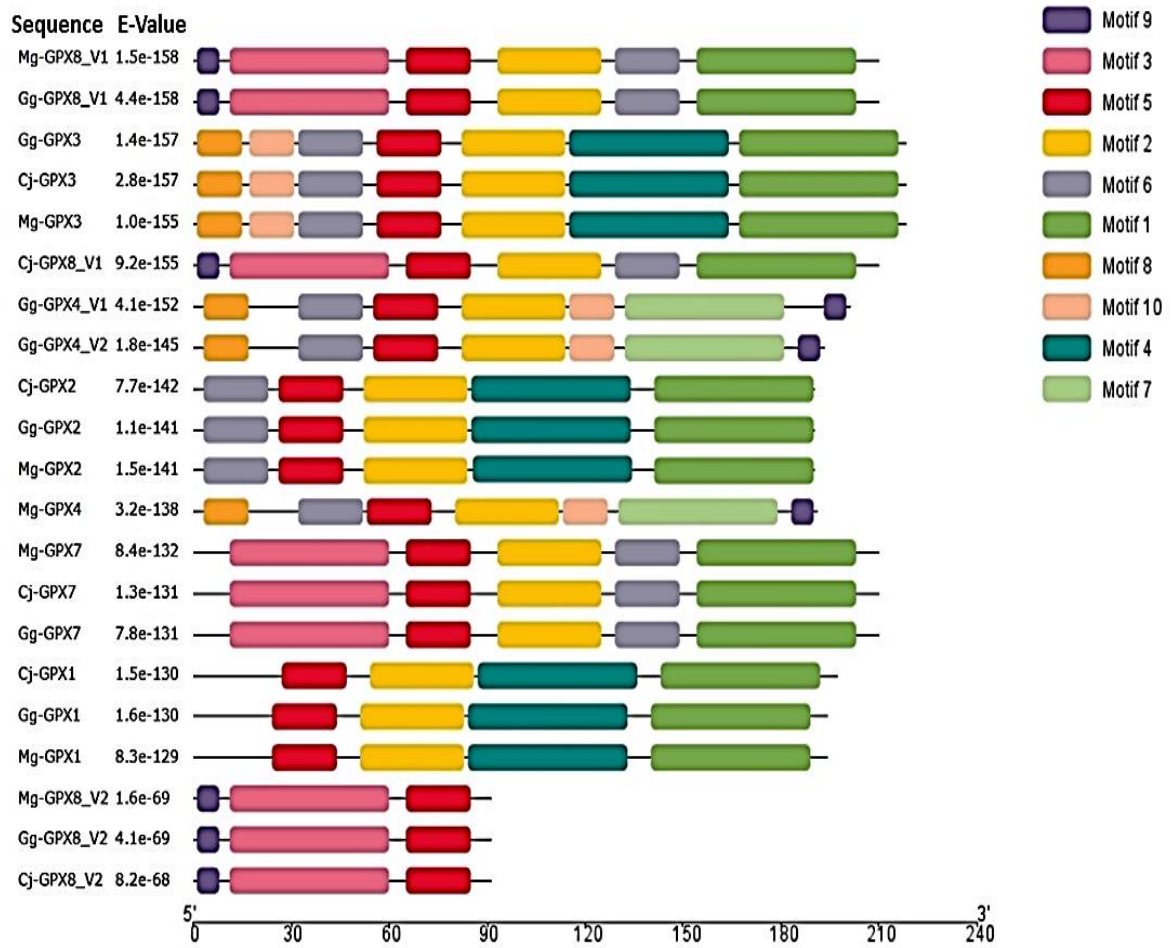

Figure 4: Alignment of conserved motifs identified in GPX protein sequences across the three species. Gg for *Gallus gallus*, Mg for *Meleagris gallopavo*, and Cj for *Coturnix japonica* via MEME analysis. The sequences are shown with their respective motif numbers and amino acid positions. The motifs are represented as colored boxes labeled with their motif number, with the corresponding amino acid sequences provided for each motif at the right. The numbers at the beginning of each row indicate the E-value of each protein sequence.

Interestingly, the GPX8-v1 protein sequence in the Japanese quail showed a slightly different motif structure than *Gallus gallus* and *Meleagris gallopavo*. Similarly, the GPX4 protein in Turkey exhibited a slightly different motif arrangement. Variations in the presence and position of other motifs highlight potential divergence among isoforms or species-specific adaptations. At the same time, the overall shared pattern underscores the evolutionary conservation of key regions within the GPX family.

The predicted subcellular localizations of GPX proteins from *Gallus gallus* (Gg), *Meleagris gallopavo* (Mg), and *Coturnix japonica* (Cj) were assessed using WoLF PSORT. GPX1 proteins in all three species showed high scores for mitochondrial and cytoplasmic localization, with additional minor predictions for extracellular and peroxisomal compartments. GPX2 isoforms were predominantly localized to the cytoplasm (21–21.5), with notable secondary predictions for nuclear and cytoplasmic/nuclear localization, indicating potential dual localization. GPX3 was strongly predicted to be extracellular in all species, with minor cytoplasmic and lysosomal signals, consistent with its classification as an extracellular protein.

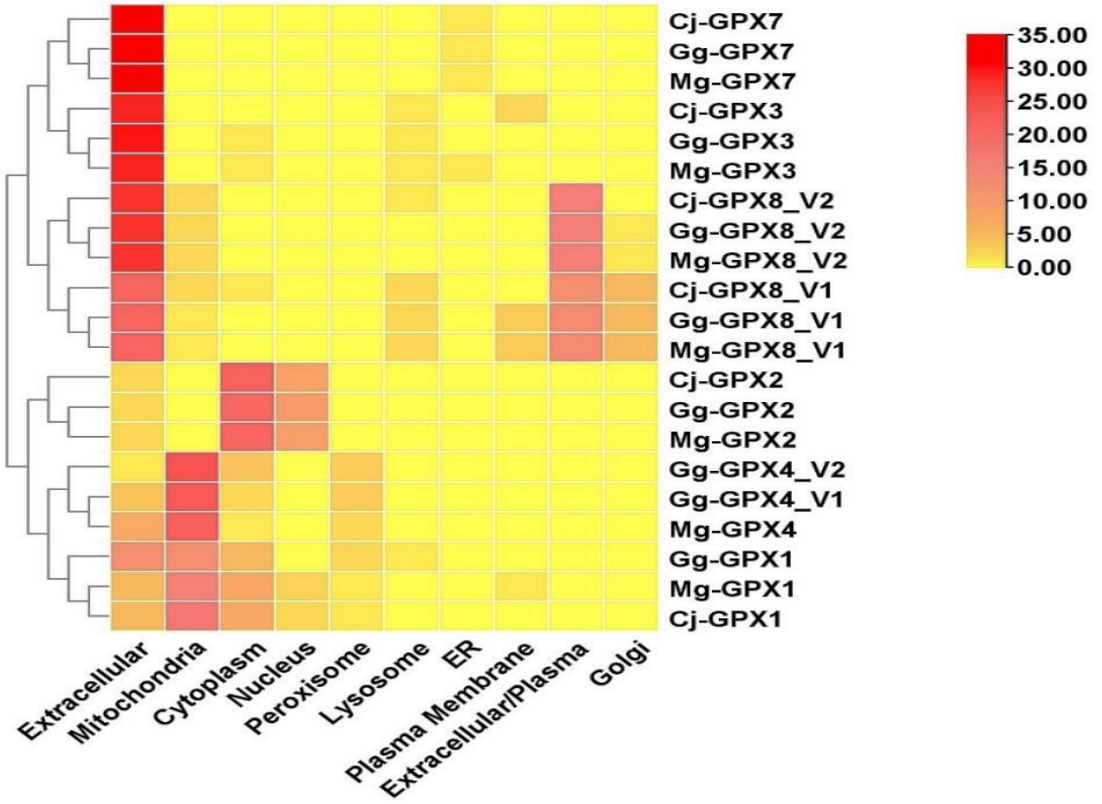

Figure 5: Predicted subcellular localization of GPX protein isoforms in chicken (Gg), turkey (Mg), and Japanese quail (Cj) using WoLF PSORT. The table displays the localization scores across different cellular compartments, including extracellular space, mitochondria, cytoplasm, nucleus, peroxisome, lysosome, endoplasmic reticulum (ER), plasma membrane, and Golgi apparatus. Higher scores indicate stronger localization predictions. These data highlight potential functional divergence and compartmental specialization among GPX isoforms across species.

GPX4 variants (Gg-GPX4_V1/V2 and Mg-GPX4) were primarily predicted to localize to the mitochondria, with secondary predictions for extracellular, peroxisomal, and cytoplasmic compartments. GPX7 proteins across species exhibited dominant extracellular localization scores (31), accompanied by low ER-associated scores. Both GPX8_V1 and GPX8_V2 variants displayed complex localization profiles. In all species, these variants were most strongly predicted to be extracellular and associated with plasma membranes. Additional predictions included the Golgi apparatus, lysosomes, mitochondria, and, to a lesser extent, the cytoplasm.

DISCUSSION

The comparative genomic and evolutionary analysis of the glutathione peroxidase (GPX) gene family across *Gallus gallus*, *Meleagris gallopavo*, and *Coturnix japonica* reveals a complex yet highly conserved gene architecture, underscoring the critical antioxidant functions preserved across avian lineages. The strong conservation of exon-intron structures and protein lengths, particularly in core isoforms such as GPX1 and GPX3, suggests these genes have been subject to purifying selection to maintain essential redox-regulatory roles (Griffin *et al.,* 2008; Betts *et al.,* 2001; Movassat *et al.,* 2019). This evolutionary constraint is further supported by conserved chromosomal synteny and the localization of key genes, such as *GPX8* on the Z chromosome, implicating potential roles in sex-linked physiological traits and avian genomic stability (Sazanov *et al.,* 2006; O'Connor *et al.,* 2024).

Gene structure analysis revealed notable diversification, particularly in *GPX4* and

GPX8, where alternative splicing generates multiple isoforms. These variations may enable tissue-specific or developmentally regulated functions, as observed in mammals (Yang *et al.,* 2016; Kliesmete *et al.,* 2023). For instance, the size and coding differences between *Gpx4_v1* and *Gpx4_v2* in chickens may reflect subcellular specialization, potentially aligning with roles in lipid peroxide detoxification or spermatogenesis (Zhong *et al.,* 2021; Maiorino *et al.,* 2018). Furthermore, the presence of truncated isoforms like *Gpx8_v2* (91 aa) invites speculation about their substrate specificity and enzymatic efficiency, which might be tailored to specific oxidative stress contexts (Yap & Makeyev, 2016).

Species-specific differences in GPX gene size, especially in *C. japonica*, likely stem from intronic expansions or regulatory sequence divergence. These regions often host transcriptional enhancers or splicing signals, which could mediate adaptive responses to environmental pressures or physiological demands (Rose, 2018; Dai *et al.,* 2022). Despite such differences, synteny analysis revealed consistent gene order and positioning for *GPX2*, *GPX7*, and *GPX8*, underscoring the evolutionary pressure to preserve functionally essential genomic configurations (Barros *et al.,* 2022; Skinner *et al.,* 2009). Minor syntactic block rearrangements suggest lineage-specific genomic adaptations without disrupting core gene functions (Castiglione *et al.,* 2020).

Phylogenetic reconstruction grouped GPX genes into functionally coherent clades, reaffirming evolutionary relationships among paralogs. High bootstrap support for *GPX1*, *GPX2*, *GPX7*, and *GPX8* clades suggests robust divergence and functional specialization within the GPX family. Interestingly, *GPX3* and *GPX6* exhibited close phylogenetic clustering, while *GPX4* branched independently but closely, supporting its intermediary evolutionary status between peroxidase classes (Bae *et al.,* 2009; Trenz *et al.,* 2021). These patterns align with the established roles of GPX4 in membrane lipid protection, particularly within reproductive and neuronal tissues (Li *et al.,* 2020; Ma *et al.,* 2022).

Selection pressure analysis (Ka/Ks) further supports the functional importance of these genes. Most GPX paralog pairs showed evidence of strong purifying selection (Ka/Ks < 1), particularly *GPX7*, emphasizing its vital role in ER protein quality control and cellular redox balance (Yoboue *et al.,* 2018). However, instances of positive selection, such as between *GPX3* and *GPX2*, suggest adaptive divergence, possibly reflecting differences in tissue expression or physiological function (Pei *et al.,* 2023). The elevated Ka/Ks ratio observed in the *GPX4–GPX3* pair in *G. gallus* (1.63) may result from artificial selection during domestication, particularly in traits linked to fertility and oxidative stress resilience (Schmidt *et al.,* 2023; Abdel-Hafez Elnoomany *et al.,* 2023). Moderate purifying selection in *GPX1* further supports its role as a ubiquitously expressed cytosolic enzyme, essential for basal antioxidant defense (Hou *et al.,* 2020).

The conserved motif analysis provided additional insight into the structural and functional preservation of GPX enzymes. Motifs 1, 2, 3, and 7 were universally conserved across species, likely corresponding to key catalytic and stabilizing regions (Brigelius-Flohé & Maiorino, 2013). Nevertheless, species- and isoform-specific divergence—such as in *GPX8_v1* of *C. japonica* and *GPX4* in *M. gallopavo*—indicates subtle lineage-specific adaptations that may optimize function in distinct oxidative niches.

Finally, subcellular localization predictions revealed conserved and divergent patterns that likely reflect functional diversification. *GPX1* and *GPX4* were primarily cytosolic and mitochondrial, consistent with their roles in detoxifying intracellular ROS. In contrast, *GPX2* showed dual cytoplasmic and nuclear localization, possibly mediating oxidative protection and redox signaling (Lubos *et al.,* 2011). Extracellular predictions for *GPX3*,

GPX7, and *GPX8* reinforce their roles in extracellular fluid protection, with *GPX8* also showing membrane localization, suggesting involvement in ER stress response and protein folding (Kanemura *et al.,* 2020; Buday and Conrad, 2021). The diversity in subcellular targeting underscores the functional specialization of GPX isoforms, likely shaped by cellular demands and evolutionary pressures unique to avian species.

In conclusion, the integrative genomic, phylogenetic, and functional analyses of GPX genes across galliform birds underscore a dynamic interplay between evolutionary conservation and functional innovation. While core redox functions remain tightly preserved, isoform variation, positive selection, and subcellular localization patterns highlight adaptive refinements that may enhance organismal resilience to oxidative stress in species-specific ecological contexts. These findings establish a foundation for future functional studies and comparative genomics in avian antioxidant defense systems.

CONCLUSION

This study presents an integrated genomic and evolutionary analysis of the glutathione peroxidase (GPX) gene family across three economically and biologically significant galliform species. The findings reveal a high degree of evolutionary conservation in gene structure, motif composition, and chromosomal localization, particularly among core isoforms implicated in both intracellular and extracellular antioxidant defense. Notably, the consistent localization of *GPX8* on the Z chromosome across species suggests a conserved role in sex-linked regulatory mechanisms. While overall patterns reflect purifying selection and phylogenetic clustering, variation in isoform structure and genomic positioning—especially in *GPX4* and *GPX8*—indicates functional diversification potentially shaped by tissue-specific requirements and ecological adaptation. Moreover, signals of positive selection in specific gene pairs suggest ongoing functional innovation, possibly influenced by domestication pressures or environmental stressors. Collectively, these results provide valuable insights into the evolution of avian antioxidant systems and establish a foundation for future functional studies of GPX isoforms in oxidative stress responses, with implications for avian health, breeding, and resilience to environmental change.

Authors, contribution

The authors contributed equally to the present investigation.

Conflict of interest

The authors declare no conflict of interest**.**

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الحفظ التطوري والإختلاف الوظيفي لجينات بيروكسيداز الجلوتاثيون(GPX) في الدجاج (Gallus gallus) ، الديك الرومي (Meleagris gallopavo) ، والسّمان الياباني (Coturnix japonica)

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تلعب عائلة جينات جلوتاثيون بيروكسيديز (GPX) دورًا حيويا في الحفاظ على التوازن التأكسدي داخل الخلية والحماية من الإجهاد التأكسدي. في هذه الدراسة، أجرينا تحليلًا مقارنًا شاملًا لجينات GPX في ثلاثة أنواع من الطيور والتى تتبع رتبة طيور الدجاجيات (Galliformes) ومنها الدجاج (Gallus gallus) ، والديك الرومي (Meleagris gallopavo)، والسمان الياباني(Coturnix japonica). تم إجراء تحليلات للخريطة الجينومية، واماكنها علي الكروموسومات، والعلاقات التطورية، وتسلسل الانماط المحفوظة للبروتينات، والتوطين الخلوي الفرعي، والتماثل الجيني، والتاثيرالانتقائى وذلك من اجل البحث والتحقيق في العلاقة التطورية المحفوظة والتباين الوظيفي لعائلة جلوتاثيون بيروكسيديز (GPX) .أظهرت النتائج وجود تشابه تطوري ملحوظ في بنية الجينات ومواقعها الكروموسومية، وبالأخص الجينات GPX1 ، وGPX2، وGPX3، وGPX7 .علاوة على ذلك، كشفت الدراسة عن توسعات جينية خاصة بالأنواع وأحداث طرز بديلة في جينات أخرى مثل GPX4 و GPX8 وقد أوضحت التحليلات التطورية وتحليلات نسبة Ka/Ks أن غالبية جينات GPX تخضع للتأثيرالانتقائى، مع وجود حالات ملحوظة من الانتقاء الإيجابي تشير إلى تباين وظيفي تكيفي. دعمت أنماط البروتينات المحفوظة والتموضع الخلوي الفرعي الأدوار الأساسية لبروتينات GPX في الحماية من الأكسدة مع تسليط الضوء على وظائفها الخاصة بالعضيات الخلوية ووظائفها خارج الخلوية. بشكل عام، تعزز هذه الدراسة فهمنا للمشهد التطوري والوظيفي لعائلة جينات GPX في الدجاجيات وتوفر أساسًا للبحوث المستقبلية في مجال تكيف الطيور مع الإجهاد التأكسدي وإشارات التوازن التأكسدي.

الكلمات المفتاحية: بيروكسيداز الجلوتاثيون، التزامن الجيني، الدجاجيات، علم الوراثة التطوري، الموقع الكروموسومي.