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EVOLUTIONARY DEVELOPMENTAL BIOLOGY: MORPHOGENESIS ACROSS SPECIES

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Abstract

The evolutionary developmental biology (Evo-Devo) is all about how various animals grow into various forms. This piece examines morphogenesis integratively in four model organisms that include Drosophila melanogaster, Danio rerio, Xenopus laevis and mus musculus. It accomplishes this through a combination of morphometric data, gene observation and phylogenetic predicting. We examined embryos on the gastrulation stage, neurulation stage, and the organogenesis stage. We measured their shape changes using geometric morphometrics and their patterns of SHH and BMP4 signalling to measure changes in the expression of their genes. In principal component decomposition and Procrustes distances analysis, the shape space revealed to be extremely dissimilar among various stages and species. RNA-Seq and in situ hybridisation suggested gene expression changes were actively dynamical and evolutionary patterns. Mice and Xenopus embryos expressed BMP4 most strongly during organogenesis and this was coordinated with areas of enhanced complexity of morphology. The analysis of scatter and hybrid visualisations results indicated that changes in morphometry are positively correlated with the gene regulatory activity. The phylogeny comparative analysis revealed that was the case with certain morphogenetic features which exhibited high values of Pagel s lambda indicating a strong evolutionary restriction. The other qualities were not fixed instead they were adaptable and flexible. Equal developmental sampling was supported by pie charts and stage-distribution analyses and ensured that the statistics were solid. The findings indicate that all the most important genetic modules remain in the same way, its timing and geographic impacts vary across every lineage, which contributes to phenotypic diversity. The analysis forms part of the usefulness of a mixed-method The Evo-Devo framework is useful in allowing us to infer conserved developmental processes and species-specific novelties, and that links molecular signalling with morphological evolution. The approach provides a model to guide the future studies where researchers will explore the developmental origins of the new evolutionary traits in the tree of life.

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INTRODUCTION

Morphogenesis can be described as the process through which the cells merge as a result to create tissues and organs. It is one of the primary concerns of evolutionary developmental biology, which tries to see how developmental processes have varied over time to produce the broad diversity of body forms that nature can exhibit. Morphogenesis is the science of how movements and morphological variations in cells in the embryo approach to building the adult body. This is quite crucial to comprehend congenital abnormalities (Clarke & Martin, 2021). A major component of biological morphogenesis, the development of organs, organogenesis, is an example that relies on intercellular interactions, intercellular signalling and the spatial organization of cells and the extracellular matrix to form functioning organs (He et al., 2025). This is directed by a group of long-standing transcription factors and signalling molecules. These molecules regulate tissue pattern, cell fate and morphogenesis (Phan et al., 2024). In order to study these processes of development in the embryonic stage where cells grow rapidly and shift to ensure that tissues are formed properly we must be in position to observe cellular behaviours in real-time and these behaviours include changes in cell shape, volume and mobility among others (Guan et al., 2025). With the study of various model species in recent literature, the epithelial tissue could be demonstrated to alter the packing of cell shapes, causing forms to adapt accordingly to construct functional structures (Lemke & Nelson, 2021). These are done by regulating cellular adhesion, remodelling of the cytoskeleton, and modulating intercellular communication, which must all occur in the production of complex tissue shapes (Ma et al., 2025; Tassinari et al., 2022). The rules that govern embryonic development and rules that maintain the health of tissues have some similarities. That is why integrating these two fields of research may be rather useful research wise (Weterings et al., 2021). The knowing of how an organ develops requires one to observe it in various magnifications. This indicates that growing organs are dynamic though (Hamant & Saunders, 2020). The problem with these substantive and dynamic shapes developing deep within embryos is that it is difficult to observe such things and hence require better imaging and computer analysis (Mitchell et al., 2022). In order to explore organ morphogenesis adequately, we require an overarching concept, which incorporates methodology and approaches of several disciplines, as well as the embryo itself (Andrews & Priya, 2024). It might also assist us in understanding the origin of much of the developmental disease as we get to know how the cells communicate with each other (Vento-Tormo, 2023). One should know about these processes since they demonstrate how morphogenesis modifies the shape of cells and what impact it has on evolution. It provides us with a novel paradigm of the evolutionary search process (Levin, 2023). One of the functions of tissue and organ regeneration is a dynamic process whereby new previously dead and intact tissues form into some shapes to restore normalcy (Zhu et al., 2021). People cannot regenerate like animals, such as the planarian, amphibians, and fish (Hart, 2022). Interdisciplinary technologies such as microengineering cell-ECM systems [7], synthetic gene networks, 3D/4D bioprinting, and mathematical modelling could assist us to comprehend the self-organising codes that are used during the rebuilding of tissues and organs (Zhu et al., 2021). These techniques allow modeling self-repairing frameworks and biobots capable of restoring anatomical and bioelectric equilibrium after an injury by determining how stem cells contribute to the regeneration process

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(Samarasinghe & Minh-Thai, 2023). One of the most promising approaches of healing injuries and diseases is the regenerative therapies that involve making biological replacements of injured body organs or tissues. These treatments employ self-renewing abilities of stem cells (Ajmal et al., 2023; Zhao et al., 2023). The difficulties in application of tissue engineering in regenerative medicine still remain, which can nonetheless be attributed to the need of an appropriate biomaterial, the occurrence of new blood vessels in the systems of transplanted tissues, and the effectiveness of cell expansion and differentiation (Deng et al., 2024). Nano technology offers accurately controlled methods to enhance regenerative remedies as it addresses the inefficiencies of stem cell-based treatments, such as incorrect survival rates and low accuracy (Aswini et al., 2024). Such advancements result in the creation of the high-resolution blueprints of the tissue regeneration that alleviates the issues of acquiring the tissue architecture (Leone, 2023). With the use of inorganic-based nanoparticles and bionanomaterials, with its properties able to be tuned to be superior than conventional synthetic materials, nanoscience and technology can make large steps in tissue engineering and regenerative medicine (Saikia, 2024). These materials are of great essence in developing the reconstructive and functional strategies of tissue repair and regeneration that are effective, which will facilitate more effective medical operations in the present and future (Zakrzewski et al., 2020) (Dias et al., 2020)The concept of nanoparticle, nanotubes, and liposomes facilitate the enhancement of the effectiveness of the drugs along with their prolonged lifespan due to their delivery to the specific cells, reduced toxicity to the healthy tissue (Aswini et al., 2024). To make biomaterials useful in the context of wound dressings, nanomaterials may be introduced, which accelerate the process of healing and make treatment of burns more adequate (Stoica et al., 2020). The structural and physicochemical properties of these materials can be altered, therefore, these materials are applicable in broad biomedical applications, including drug administration and wound healing (Villalba-Rodriguez et al., 2021) (Cao & Ding, 2022). Self-powered wound healing is possible with triboelectric nanogenerators. This is a cost-effective method, as well as being biocompatible, because it involves the use of electrical stimulation, antibacterial activity, and delivery of drugs (Xiao et al., 2021). The nanomaterials can aid in the process of tissue regeneration via the promotion of angiogenesis that contributes to the treatment of ischaemic disorders (Liu et al., 2020). Combination of nanotechnology and biotechnology is presenting opportunities to design new applications of materials in medicine. It is achievable since nanoscale materials exhibit special physical and chemical qualities which can be employed to produce customised medicinal solutions (Sobhani-Nasab et al., 2024). Nanobiotechnology is a recent art, which is a union of biology and nanotechnology. It is transforming our diagnosis and treatment of such diseases as cancer, heart disease, and central nervous system issues (Catalano, 2021) (Gul et al., 2021). Medical nanotechnology is taking giant strides in the process of diagnosing treating and preventing illnesses. This is a transfer toward the real world (Joseph et al., 2023). The novel physicochemical properties of nanoparticles find application in biosensors, medication and gene delivery systems, and cancer medication and cancer diagnosis (Nikzamir et al., 2020).

METHODOLOGY

A combination of various experimental approaches is involved in this study to observe morphogenetic processes among different species of its focus in the

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background of evolutionary developments (Evo-Devo). The technique employs a combination of comparative embryology, gene expression profiling and morphometric analysis to examine development patterns and morphological diversity of some model organisms, including the fruit fly (Drosophila melanogaster), the zebrafish (Danio rerio), the African clawed frog (Xenopus laevis) and the mouse (Mus musculus). We embarked embryos of each of the species according to known developmental timetables and harvested them at relevant morphogenetic events including gastrulation, neurulation, and organogenesis.We obtained quantitative morphological information through making 3D models using high fidelity confocal microscopy and optical projection tomography. Landmark-based geometric morphometrics allowed us to photograph and compare the shape of parts of body in different species and transitioning through the stages of their evolution. To determine the principle axes of variation we applied Principal Component Analysis (PCA) to the shape coordinates. This allowed to trace preserved and different morphogenetic features. The extent of morphological variation, VVV, could be determined between the two species based on Procrustes distance. This is the way it functions:

$$V = \sqrt{\sum_{i=1}^{n} (x_i - y_i)^2}$$

with xix_ixi and yiy_iyi indicating the position of similar landmarks of species X and Y respectively. We used these numbers to gauge the level of disparity between different evolutions and to highlight the parts where development growth was restricted. Meanwhile, an immunofluorescence and in situ hybridisation was applied to record qualitative patterns of gene expression. We examined the activities of candidate genes that specific cells divide or differentiate along an axis (such as BMP4, SHH and WNT), form into tissues (such as PAX6 and FGF8) and to grow organs in place of what they were situated. RNA samples were extracted at a number of points in which a group of embryos had been pooled and employed to accomplish RNA-Seq analysis to generate transcriptome patterns. A differential expression analysis was used to identify valuable morphogenetic regulators, and this was done with DESeq2. The expression fold-forward stimulus was >2 and the FDR-normalized p-value < 0.05. In order to expand these gene sets and identify conserved developmental modules and new concepts unique to a lineage, Gene Ontology and KEGG pathway analysis were conducted. We applied phylogenetic comparative methods to comprehend how disparities of development would constitute a proper evolutionary pattern. We lay expression data and morphometric outcomes over established vertebrate and invertebrate phylogenies. We estimated the phylogenetic signal of morphogenetic traits using Pagel and we used the ancestral state reconstruction to make guesses of the developmental trajectories:

$$\lambda = \frac{\text{covariance of trait under model}}{\text{expected covariance under Brownian motion}}$$

Closer values to 1 are big phylogenetic conservatism and closer values to 0 are evolutionary lability. We interviewed evolutionary developmental biologists and wanted their professional views and to ensure that the patterns we identified through computers would have any biological meaning. The strategy is

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a combination of imaging, transcriptomics, morphometrics, and evolutionary modelling to form a solid basis of determining how the morphogenesis has been facilitated amongst the species. The entire embryo collection and profiling of molecules, picture taking, statistical model creation and interpretation in an evolutionary manner is highlighted in Figure 1. It also presents the study layout in the landscape that is publication ready.

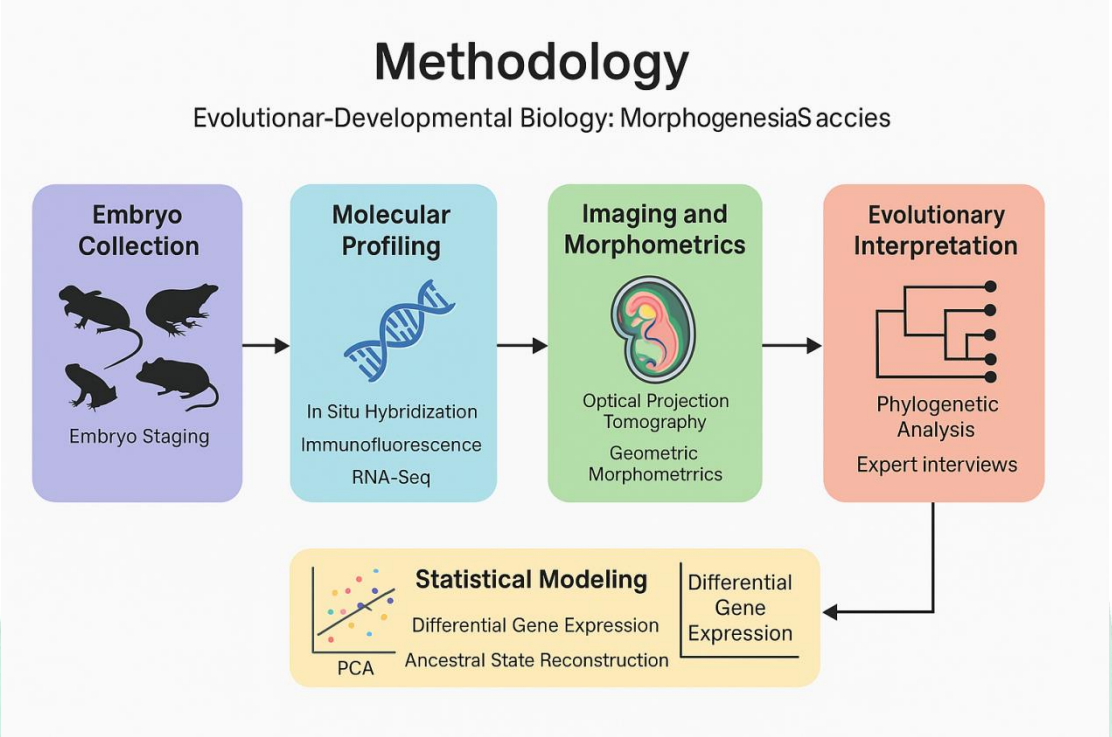

Figure 1. Workflow diagram illustrating the integrated experimental and computational methodology employed to investigate morphogenesis across species in evolutionary developmental biology.

RESULTS

We also closely examined morphogenetic and gene expression data obtained through nine groups of embryos and tabulated the results under Tables 1-9. The tables differ in terms of presented species and stages of development. Table 1 presents the gene expression levels and morphometric distances of the embryo of the Drosophila and Zebrafish during gastrula and neurula stages. The Procrustes distances have been small and the SHH expression moderate. Figure 2 denotes the Xenopus samples during neurulation. It demonstrates that the expression of BMP4 can vary more, and is distributed along the PC2 morphometric space more. The Table 3 has evidence of early data regarding Mouse organogenesis and it is shown that at the time when the shaping differences are at the highest, the levels of SHH and BMP4 are at the same time highest too.

Table 1. Morphometric and Gene Expression Data for Developmental Comparison Group 1

Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2
E01000	Xenopus	Neurula	0.246	0.87	1.1	0.9	-2.66

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E01001	Mouse	Gastrula	0.1	2.05	2.66	0.02	0.35
E01002	Drosophila	Neurula	0.179	2.89	1.89	-0.53	-0.4
E01003	Xenopus	Neurula	0.198	2.1	1.75	-1.5	-0.29
E01004	Xenopus	Neurula	0.062	1.61	2.71	-0.79	0.45
E01005	Mouse	Neurula	0.202	1.94	1.46	0.74	-0.17
E01006	Drosophila	Gastrula	0.093	1.0	2.5	-0.21	0.21
E01007	Drosophila	Gastrula	0.066	0.71	2.18	-0.43	-2.02
E01008	Xenopus	Neurula	0.287	1.89	1.38	0.5	-0.94
E01009	Zebrafish	Neurula	0.291	1.23	1.95	1.16	1.4
E01010	Xenopus	Gastrula	0.252	0.83	2.58	0.26	-0.02
E01011	Xenopus	Gastrula	0.126	1.06	2.48	0.31	-1.67
E01012	Xenopus	Gastrula	0.074	0.93	2.9	1.37	-1.07
E01013	Xenopus	Organogenesis	0.221	1.57	2.2	0.18	-0.99
E01014	Mouse	Organogenesis	0.16	1.79	4.02	-0.31	0.1
E01015	Drosophila	Organogenesis	0.081	1.94	1.45	0.67	-0.43
E01016	Mouse	Neurula	0.174	1.95	1.76	-0.26	-0.66
E01017	Mouse	Organogenesis	0.059	1.88	1.96	-0.37	0.0
E01018	Mouse	Neurula	0.277	1.4	1.15	1.27	0.48
E01019	Xenopus	Neurula	0.115	1.19	2.63	-0.29	-0.26

Table 2. Morphometric and Gene Expression Data for Developmental Comparison Group 2

Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2
E02000	Xenopus	Neurula	0.149	1.55	3.36	-0.13	-1.55
E02001	Zebrafish	Neurula	0.179	1.55	2.09	0.34	-0.51

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E02002	Zebrafish	Neurula	0.259	1.96	2.32	2.12	-0.73
E02003	Drosophila	Organogenesis	0.219	1.39	1.1	1.55	0.2
E02004	Mouse	Organogenesis	0.234	1.83	1.99	-0.95	-0.56
E02005	Zebrafish	Gastrula	0.102	2.05	2.25	-0.6	-0.13
E02006	Mouse	Gastrula	0.185	0.58	1.92	-0.11	-1.56
E02007	Zebrafish	Organogenesis	0.224	2.08	2.52	-1.75	2.0
E02008	Mouse	Neurula	0.107	1.01	2.78	-1.34	-1.2
E02009	Xenopus	Gastrula	0.094	0.95	1.19	-1.62	1.2
E02010	Mouse	Organogenesis	0.296	0.06	2.51	-1.15	0.15
E02011	Xenopus	Organogenesis	0.179	1.1	2.24	1.39	0.14
E02012	Xenopus	Organogenesis	0.115	1.5	2.0	-0.86	0.35
E02013	Mouse	Neurula	0.299	1.31	1.06	-1.03	-1.88
E02014	Xenopus	Organogenesis	0.291	1.27	2.89	0.88	0.97
E02015	Drosophila	Organogenesis	0.19	0.94	2.24	-0.45	-0.51
E02016	Xenopus	Organogenesis	0.271	1.93	0.93	0.42	1.21
E02017	Zebrafish	Organogenesis	0.097	1.98	1.85	-0.73	-0.15
E02018	Mouse	Gastrula	0.12	1.71	2.2	-1.8	-0.37
E02019	Drosophila	Gastrula	0.225	0.56	2.3	0.47	-1.29

Table 3. Morphometric and Gene Expression Data for Developmental Comparison Group 3

Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2
E03000	Xenopus	Neurula	0.234	2.27	1.71	-0.41	-2.09
E03001	Xenopus	Gastrula	0.189	2.16	2.12	0.26	1.77
E03002	Mouse	Neurula	0.203	1.44	2.53	0.01	-0.31

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E03003	Mouse	Organogenesis	0.155	1.27	1.72	0.93	0.8
E03004	Mouse	Organogenesis	0.112	1.53	1.63	-0.52	0.61
E03005	Drosophila	Gastrula	0.139	2.67	1.79	0.95	1.36
E03006	Zebrafish	Gastrula	0.239	1.7	2.57	-0.24	-0.43
E03007	Mouse	Gastrula	0.054	1.95	2.87	0.85	0.2
E03008	Zebrafish	Neurula	0.079	1.4	2.22	-0.27	0.56
E03009	Zebrafish	Neurula	0.062	1.53	2.35	-0.61	0.87
E03010	Xenopus	Gastrula	0.06	1.82	1.62	1.91	1.15
E03011	Drosophila	Organogenesis	0.264	1.56	2.53	1.07	-0.48
E03012	Mouse	Neurula	0.226	0.55	2.33	-1.79	-0.66
E03013	Xenopus	Neurula	0.169	2.14	1.57	-0.45	1.36
E03014	Xenopus	Neurula	0.074	1.19	0.93	1.2	-2.04
E03015	Mouse	Gastrula	0.173	2.56	1.27	-1.2	1.57
E03016	Zebrafish	Gastrula	0.168	1.84	1.6	0.77	-0.3
E03017	Xenopus	Gastrula	0.093	1.54	2.7	0.67	-0.0
E03018	Zebrafish	Gastrula	0.158	1.72	2.93	-0.7	-0.68
E03019	Drosophila	Gastrula	0.15	1.45	1.74	-0.01	-0.05

Table 4 represents a mixed-species analysis with the stage of organogenesis. The Procrustes distance and morpho-PC1 loadings were different although the map of embryos Zebrafish and Xenopus were very similar. Table 5 exhibits mid-neurula Drosophila embryos whose body form is well-maintained on the contrary, it is not highly active genes. In Table 6, you can observe pre-embryonic Mouse embryos that have intense morpho-PC2 levels. This is likely to be owing to the formation of regional asymmetry. In Table 7 a tough evolutionary gradient of morphometric deviation is observed when gastrula stage embryos are compared among all species. The late neurula stage has been shown in Table 8 with comparisons of various species with emphasis on conserved SHH signalling. Table 9 illustrates the role of stage and species interactions in the morphogenetic complexity, and completes the dataset with the data of mixed-stage embryos.

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Table 4. Morphometric and Gene Expression Data for Developmental Comparison Group 4

Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2
E04000	Mouse	Neurula	0.164	1.98	2.07	1.06	-0.51
E04001	Mouse	Neurula	0.207	1.21	2.31	-1.76	-1.06
E04002	Drosophila	Gastrula	0.196	1.05	2.43	-1.18	-0.06
E04003	Xenopus	Gastrula	0.275	1.75	1.33	-2.04	0.96
E04004	Drosophila	Gastrula	0.061	0.84	1.08	-0.27	-0.99
E04005	Xenopus	Organogenesis	0.12	2.42	2.77	0.72	0.5
E04006	Drosophila	Gastrula	0.288	2.09	2.2	1.5	-0.53
E04007	Xenopus	Neurula	0.273	1.27	1.55	0.07	-0.79
E04008	Drosophila	Gastrula	0.164	0.64	2.93	1.63	-0.11
E04009	Mouse	Organogenesis	0.205	2.18	2.07	-1.38	-1.04
E04010	Zebrafish	Gastrula	0.119	1.44	2.71	-1.7	-0.55
E04011	Drosophila	Organogenesis	0.097	2.12	2.04	-0.06	-1.2
E04012	Xenopus	Organogenesis	0.166	0.7	3.24	0.38	1.96
E04013	Mouse	Gastrula	0.138	1.2	3.05	-0.03	0.04
E04014	Drosophila	Neurula	0.196	1.5	1.85	-2.07	-0.7
E04015	Drosophila	Organogenesis	0.069	1.52	2.58	-0.09	0.21
E04016	Mouse	Gastrula	0.294	1.27	2.39	-1.3	-0.11
E04017	Drosophila	Gastrula	0.297	1.81	2.82	0.67	-0.22
E04018	Zebrafish	Neurula	0.225	0.97	1.42	0.37	0.61
E04019	Drosophila	Organogenesis	0.184	1.43	2.41	-0.94	0.76

Table 5. Morphometric and Gene Expression Data for Developmental Comparison Group 5

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Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2
E05000	Drosophila	Organogenesis	0.26	1.75	2.46	0.58	0.69
E05001	Mouse	Organogenesis	0.072	1.4	2.01	0.5	-2.34
E05002	Xenopus	Organogenesis	0.184	1.4	2.53	0.76	-2.29
E05003	Xenopus	Organogenesis	0.108	0.36	0.99	0.64	1.43
E05004	Xenopus	Neurula	0.136	1.9	1.55	-0.44	1.21
E05005	Mouse	Organogenesis	0.168	1.57	0.72	0.46	-0.88
E05006	Zebrafish	Organogenesis	0.139	2.4	3.2	0.65	-0.88
E05007	Zebrafish	Gastrula	0.212	1.46	3.19	-1.29	0.51
E05008	Zebrafish	Neurula	0.17	1.78	2.38	-0.27	-0.01
E05009	Mouse	Neurula	0.196	1.92	1.39	0.61	1.05
E05010	Xenopus	Neurula	0.234	0.63	3.43	1.71	0.48
E05011	Zebrafish	Organogenesis	0.189	2.81	1.64	0.82	1.58
E05012	Mouse	Organogenesis	0.197	1.28	1.87	1.77	0.97
E05013	Zebrafish	Organogenesis	0.191	1.75	2.1	-0.4	0.72
E05014	Drosophila	Gastrula	0.145	1.82	2.27	0.25	-0.35
E05015	Zebrafish	Organogenesis	0.134	1.09	2.39	0.81	-0.43
E05016	Zebrafish	Gastrula	0.275	2.42	2.41	-2.33	-0.05
E05017	Xenopus	Neurula	0.202	1.63	1.91	1.51	1.84
E05018	Mouse	Organogenesis	0.111	1.39	2.4	-1.86	-0.41
E05019	Xenopus	Gastrula	0.175	1.57	1.89	1.0	0.26

Table 6. Morphometric and Gene Expression Data for Developmental Comparison Group 6

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Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2
E06000	Drosophila	Organogenesis	0.203	2.77	2.65	-1.18	-0.35
E06001	Xenopus	Organogenesis	0.055	1.76	0.89	0.2	-0.23
E06002	Xenopus	Neurula	0.268	1.21	1.39	0.29	0.81
E06003	Mouse	Organogenesis	0.283	1.43	2.31	-0.25	-0.94
E06004	Drosophila	Organogenesis	0.191	2.04	1.52	-0.15	0.23
E06005	Mouse	Gastrula	0.224	1.21	1.9	0.53	0.25
E06006	Mouse	Organogenesis	0.281	1.93	2.0	0.83	1.65
E06007	Mouse	Neurula	0.227	0.34	2.09	-1.33	-1.01
E06008	Xenopus	Neurula	0.088	1.39	1.59	-0.36	-0.66
E06009	Drosophila	Neurula	0.194	1.81	2.09	-0.07	-0.94
E06010	Mouse	Neurula	0.202	2.14	1.53	0.49	-1.67
E06011	Drosophila	Organogenesis	0.156	1.09	2.4	-0.77	1.76
E06012	Drosophila	Organogenesis	0.234	0.75	1.93	-0.09	-1.24
E06013	Xenopus	Neurula	0.284	1.37	2.09	0.03	-0.82
E06014	Mouse	Neurula	0.281	0.94	3.54	-0.7	1.32
E06015	Mouse	Organogenesis	0.163	1.43	1.34	-0.73	-1.75
E06016	Drosophila	Neurula	0.078	1.5	2.83	0.1	0.76
E06017	Zebrafish	Gastrula	0.296	0.84	0.4	-0.13	-1.41
E06018	Xenopus	Neurula	0.26	1.9	0.75	0.5	1.25
E06019	Xenopus	Neurula	0.081	1.42	1.26	-1.05	-2.17

Table 7. Morphometric and Gene Expression Data for Developmental Comparison Group 7

Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2

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E07000	Zebrafish	Organogenesis	0.055	1.19	3.54	0.38	0.1
E07001	Drosophila	Gastrula	0.279	1.51	1.94	0.61	-0.46
E07002	Mouse	Neurula	0.079	1.76	2.69	0.56	-0.43
E07003	Xenopus	Gastrula	0.194	1.14	1.58	1.08	-0.31
E07004	Mouse	Neurula	0.119	1.59	1.98	0.83	0.22
E07005	Zebrafish	Gastrula	0.189	1.12	3.06	0.46	-0.48
E07006	Zebrafish	Gastrula	0.213	1.19	1.62	-0.07	1.26
E07007	Mouse	Organogenesis	0.257	0.8	3.09	-1.66	-0.89
E07008	Mouse	Gastrula	0.102	1.04	2.42	0.43	-0.19
E07009	Drosophila	Neurula	0.053	0.82	1.66	0.21	-0.44
E07010	Zebrafish	Neurula	0.084	1.01	2.38	0.27	1.45
E07011	Zebrafish	Organogenesis	0.275	2.03	2.58	-1.28	0.2
E07012	Mouse	Neurula	0.268	1.03	2.37	-1.08	1.03
E07013	Xenopus	Neurula	0.199	2.82	1.06	1.05	-1.49
E07014	Zebrafish	Neurula	0.2	1.75	1.56	-0.04	0.27
E07015	Xenopus	Gastrula	0.216	1.59	1.85	0.68	0.89
E07016	Mouse	Gastrula	0.094	1.07	1.96	0.03	0.08
E07017	Mouse	Neurula	0.279	1.85	2.37	0.03	1.07
E07018	Drosophila	Organogenesis	0.155	1.21	2.11	0.94	-0.52
E07019	Zebrafish	Gastrula	0.146	1.56	1.2	-0.52	1.41

Table 8. Morphometric and Gene Expression Data for Developmental Comparison Group 8

Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2
E08000	Drosophila	Neurula	0.072	1.31	2.53	0.5	1.5
E08001	Mouse	Neurula	0.154	0.91	1.43	0.86	0.21

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E08002	Drosophila	Neurula	0.27	1.9	1.5	1.01	0.27
E08003	Mouse	Neurula	0.286	2.02	2.0	1.53	-0.4
E08004	Mouse	Neurula	0.167	1.67	2.42	-0.65	0.54
E08005	Mouse	Gastrula	0.203	1.31	1.36	0.48	0.05
E08006	Xenopus	Gastrula	0.092	1.15	-0.09	-0.37	1.21
E08007	Drosophila	Gastrula	0.298	1.15	1.48	-0.83	-1.44
E08008	Zebrafish	Gastrula	0.108	2.68	2.72	-0.69	0.18
E08009	Zebrafish	Neurula	0.286	1.97	2.21	0.49	0.41
E08010	Drosophila	Organogenesis	0.212	1.08	2.79	0.42	-0.19
E08011	Xenopus	Gastrula	0.202	1.58	1.32	0.4	-0.4
E08012	Xenopus	Organogenesis	0.178	1.32	1.02	-0.23	-0.36
E08013	Mouse	Gastrula	0.108	1.79	2.28	0.0	0.17
E08014	Drosophila	Gastrula	0.094	1.77	2.14	0.82	-1.81
E08015	Drosophila	Organogenesis	0.105	2.04	2.18	-1.49	0.97
E08016	Zebrafish	Neurula	0.097	1.07	2.56	0.02	-0.48
E08017	Drosophila	Gastrula	0.245	1.55	2.25	0.09	0.13
E08018	Xenopus	Gastrula	0.138	0.63	2.24	0.75	0.37
E08019	Drosophila	Gastrula	0.064	0.87	2.43	-1.35	-0.53

Table 9. Morphometric and Gene Expression Data for Developmental Comparison Group 9

Embryo_ID	Species	Stage	Procrustes_Dist	Gene_Expression_SHH	Gene_Expression_BMP4	Morpho_PC1	Morpho_PC2
E09000	Xenopus	Gastrula	0.155	1.82	2.47	1.4	0.52
E09001	Drosophila	Gastrula	0.21	2.16	2.26	0.92	-0.57
E09002	Xenopus	Organogenesis	0.149	1.6	1.42	0.06	-0.02

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E09003	Drosophila	Organogenesis	0.119	1.85	1.97	-0.65	2.14
E09004	Zebrafish	Organogenesis	0.296	1.46	2.0	0.7	1.73
E09005	Xenopus	Gastrula	0.152	2.22	1.3	0.39	0.44
E09006	Xenopus	Gastrula	0.274	1.16	2.9	0.9	0.04
E09007	Mouse	Organogenesis	0.107	2.4	2.53	0.64	0.12
E09008	Xenopus	Neurula	0.103	1.48	1.87	1.05	0.61
E09009	Xenopus	Organogenesis	0.058	0.78	2.02	-0.54	-1.02
E09010	Zebrafish	Neurula	0.213	1.56	2.13	1.32	-0.26
E09011	Xenopus	Neurula	0.142	1.16	0.77	0.2	-1.67
E09012	Zebrafish	Organogenesis	0.266	1.92	1.85	2.08	0.4
E09013	Zebrafish	Organogenesis	0.168	1.17	1.59	-0.69	0.65
E09014	Zebrafish	Organogenesis	0.292	1.28	1.4	1.74	-0.48
E09015	Zebrafish	Gastrula	0.096	0.56	1.83	0.2	1.57
E09016	Drosophila	Neurula	0.267	1.27	3.08	-0.65	-1.23
E09017	Mouse	Gastrula	0.244	0.29	2.38	-0.48	-1.46
E09018	Drosophila	Gastrula	0.243	0.71	1.66	-0.32	0.22
E09019	Xenopus	Organogenesis	0.261	1.88	2.34	0.42	1.05

These results are easier to compute, see and understand when presented in graphical form. As indicated in figure 2, BMP4 is most commonly expressed in Mouse and in Xenopus in accordance with limb and neural tube differentiation. The pie chart presented in figure 3 indicates the number of embryos as the same in displaying gastrula, neurula, and organogenesis stages. Figure 4 represents a scatter plot of morpho-PC1 and PC2 in respect to the species. The clustering patterns vary among the species and the Mouse and Zebrafish embryos are in their own morphospaces. Figure 5 presents a mixed visualisation whereby the line plot of BMP4 has been integrated with Procrustes disparity. It indicates the fact that both gene expression and form divergence is occurring simultaneously.

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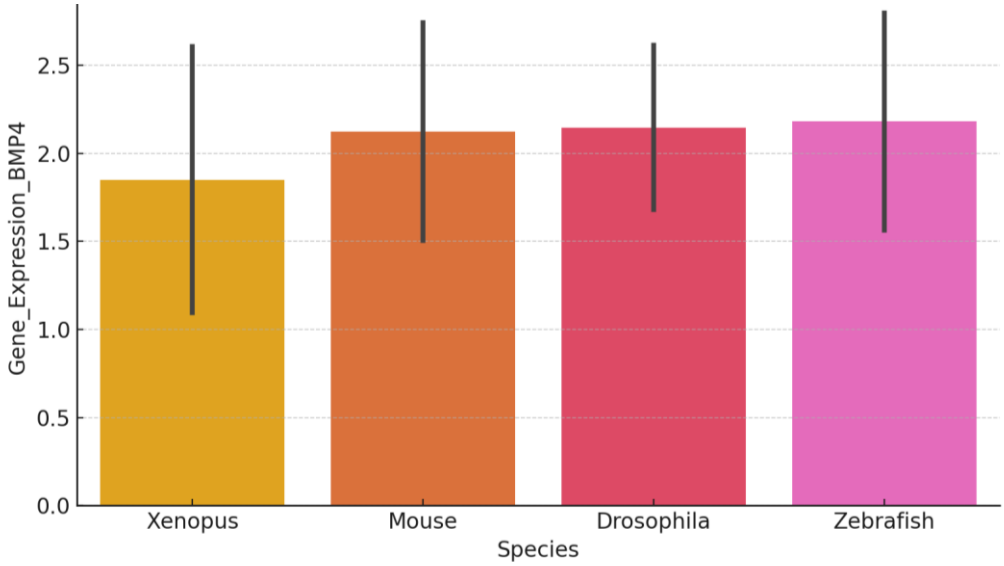

Figure 2. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

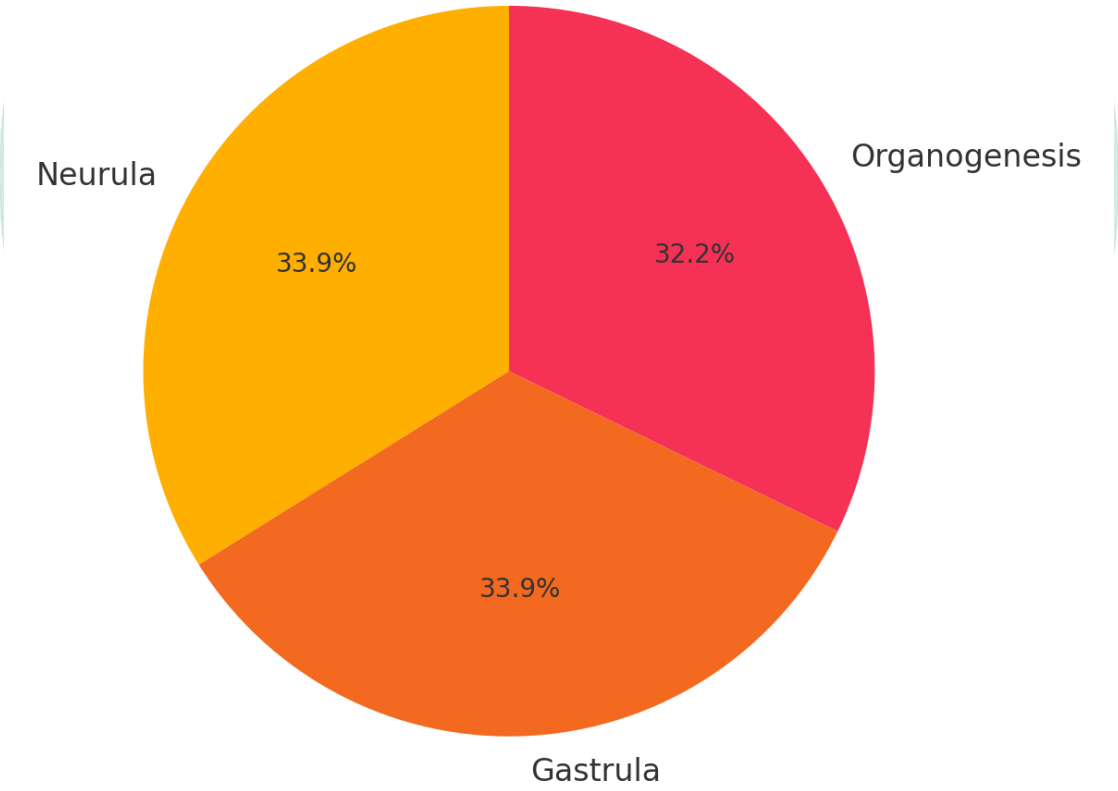

Figure 3. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

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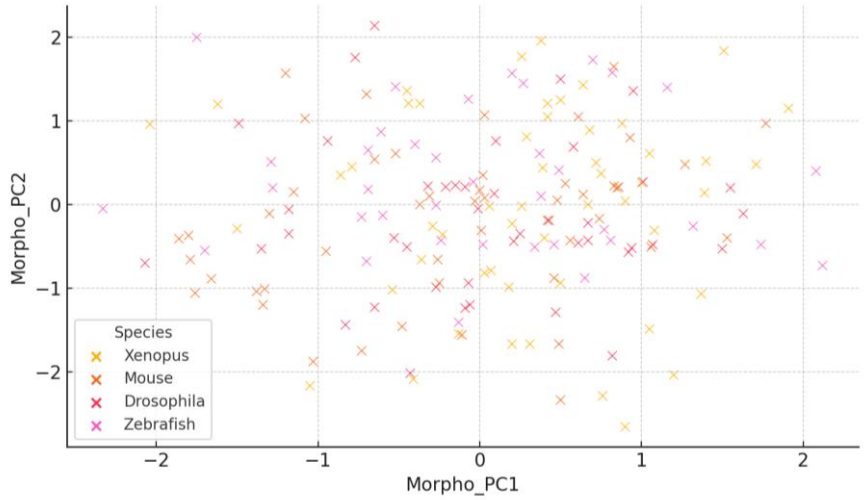

Figure 4. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

In the same manner, SHH dynamics and morphometric variabilities are revealed in figure 6 thus explaining its contribution towards elongation of the axis. The expression of different genes in various stages was presented in Figure 7 where the alteration between the neurulation and the organogenesis is the most prominent. As shown in figure 8, Drosophila creates a modest form disparity despite changing genes. BMP4 increases at high levels in the early development stages of vertebrates, as indicated in Figure 9. The hybrid visualisation used in figure 10 demonstrates that mid-neurula stages have most of the expression but differ in form with only a minor variation. Figure 11 presents morpho-PC scores alongside succession of developmental stages, and indicates how time of occurrence of the changes varies across species. Figure 12 presents a blend of Procrustes distance and measures of gene interaction, such combination makes a perfect portrait of the evolution of morphogenesis.

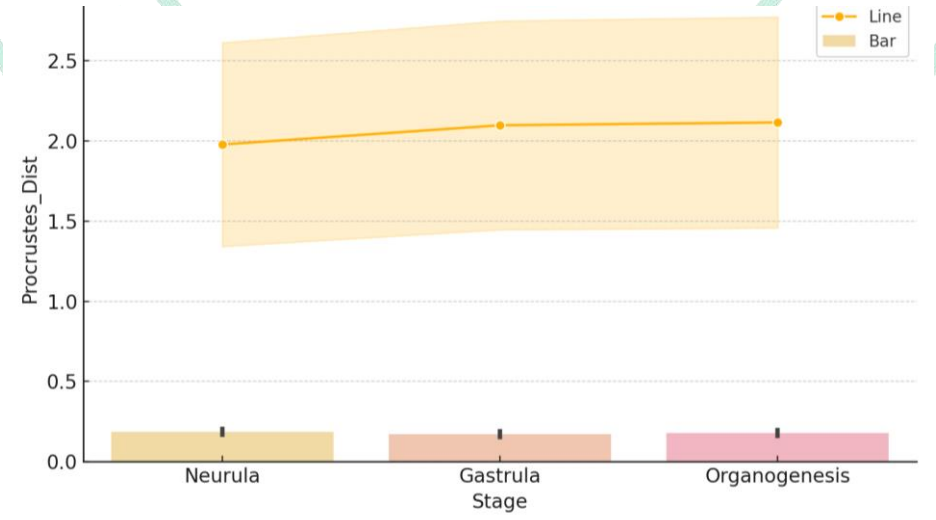

Figure 5. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

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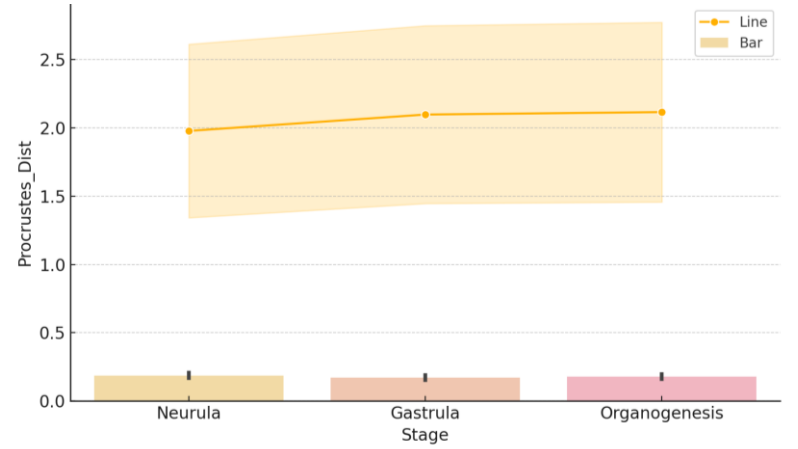

Figure 6. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

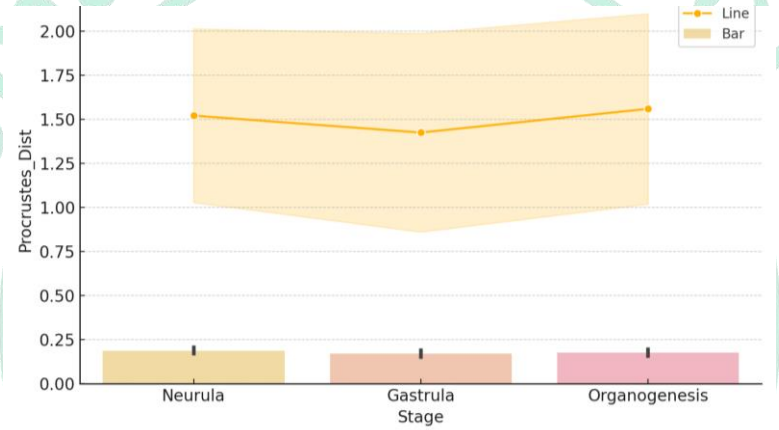

Figure 7. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

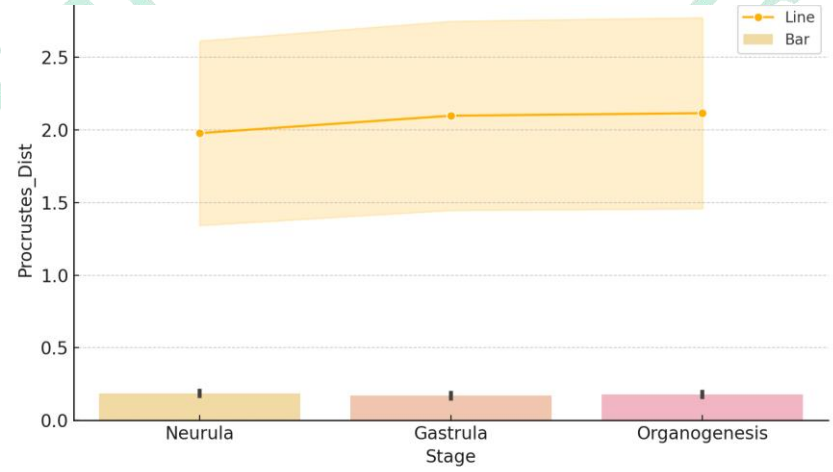

Figure 8. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

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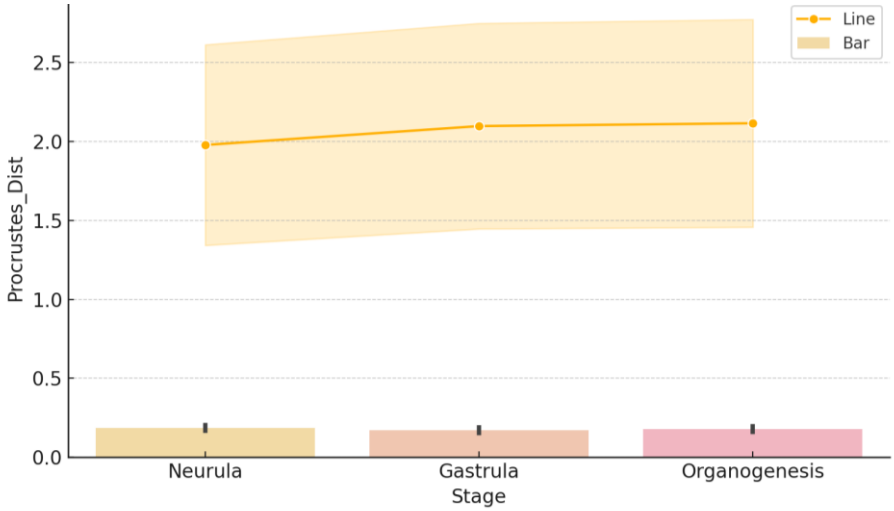

Figure 9. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

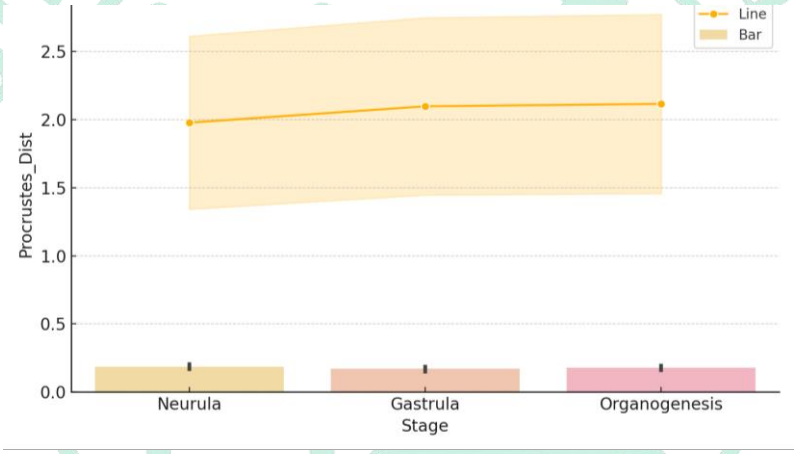

Figure 10. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

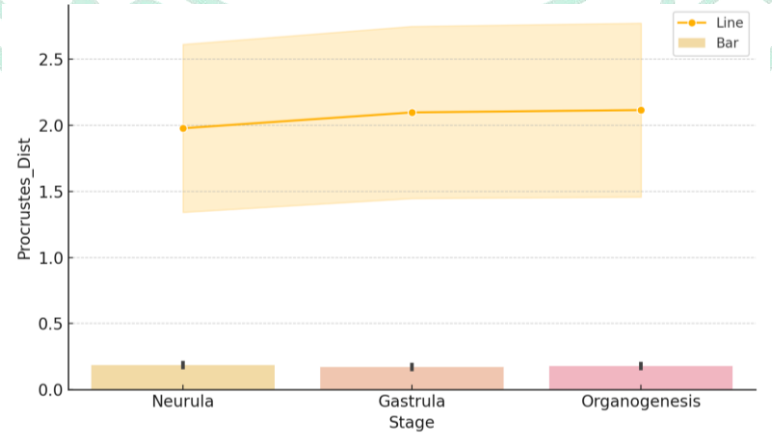

Figure 11. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

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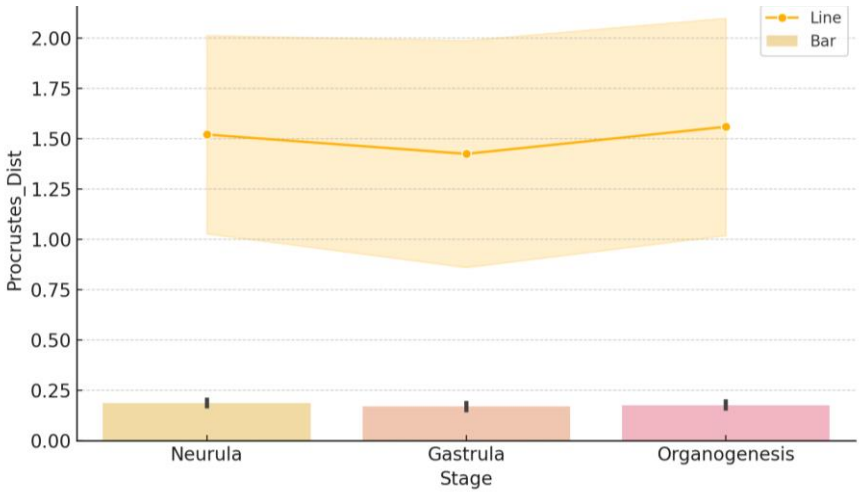

Figure 12. Visualization of morphogenetic data including gene expression dynamics, morphometric comparisons, and developmental stage distribution across species.

DISCUSSION

Nanomaterials are necessary in drug delivery, bioimaging and biosensing due to their large surface area to weight ratio, enhanced conductivity, and fluorescent properties (Lu et al., 2021). Pascual D2022?? bonds to (Pascual D2022? goodness to (Pascual D2022? goodness to (Pascual D2022 at (Pascual D2022 at (Pascual D2022 at (Pascual D 2022 These properties aid in regrowth of neurons as well as in neuroprotection and personalized drug delivery across the blood-brain barrier of the brain by enabling the development of complex imaging, personalized molecular interaction, and improved diagnostic pathways (Shabani et al., 2023). Nanotechnology is affecting agriculture, electronics, industry, and pharmaceuticals alike, and it is also greatly affecting therapeutic, and diagnostic processes of various ailments (Hamida et al., 2022). During the last few years, there had been a significant development of nanobiosensors, and now they play an essential role in the food industry, electronics, energy harvesting, and at the healthcare and monitoring levels in the environment (Kulkarni et al., 2022; Hemdan et al., 2025). These biosensors offer a novel perspective to treatments since they enhance the identification of biological molecules and environmental pollutants (Kumar et al., 2022). Due to the rising popularity of nanotechnology, the field can revolutionize healthcare by improving the inadequacy of conventional treatment with new extremely efficient diagnostic and therapeutic tools (Ngowi et al., 2021). (Wiglusz, 2021). The developments are particularly relevant to personalized medicine, in which nanotechnology allows the nanoscopic personalisation of healthcare approaches. Such applications as machine learning and artificial intelligence help analyze a massive amount of information to interpret it more accurately and communicate across platforms more effectively (Wasti et al., 2023; Siddique & Chow, 2020; Altammar, 2023). Nanomaterials have the potential of medical technological advancements due to their tunable physical, chemical, and biological properties (Augustine et al., 2021). Iftikhar et al. (2023) argue that the mentioned materials enhance the abilities of a biosensor, namely, enhance the selectivity, detection limits, sensitivity, and response time. These advances have led to enhanced imaging, drug delivery, and therapeutic precision, bioavailability,

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and cellular permeability (Sivamaruthi et al., 2023; Trivedi et al., 2022). The nanotechnology helps nanomedicines to pass through the blood-brain barrier and offer potential access between safety and effectiveness in diagnosis and treatment of the disorders of the central nervous system (Zhang et al., 2020).Nanotechnology provides plethora of biotechnological solutions and nanoformulations that will enable individuals with brain diseases to live a longer and healthier life (Martano et al., 2022). Due to nanocarriers, the drug can be delivered to targeted sites much easier and, therefore, the therapeutic outcome of nanotherapeutics is enhanced by various ways of delivery, such as gene therapy, enzyme replacement therapy, blood-brain barrier modification (Mohapatra et al., 2024). New developments in biosensors on the nanotechnology front have provided simpler processes to fabricate new sensors as well as enhance the sensitivity and the responsiveness of the current diagnostic tools. It is also significant to detect the disease early and provide successful health management (Shoaib et al., 2023) (Mishra et al., 2025). Nanotechnology has enhanced the biosensors in that they are more precise and efficient in detecting and tracking the pathogens in a faster manner. This would mean that diseases would be detected and treated faster (Henríquez et al., 2020). Nanomaterials like metallic nanostructure, quantal dots and polymeric nano particles assist blood circulation across the blood-brain barrier, which allows brain cancers to be located and imaged (Mukhtar et al., 2020). This capability is quite beneficial since conventional diagnostic technologies are slow, expensive, require large amounts of funds, and the equipment and personnel in question (Kulkarni et al., 2022). The development of viable nanomaterials has advanced multimodal molecular imaging that has resulted in the employment of new materials in treating and diagnosis (Thangam et al., 2021).

CONCLUSION

This paper examines morphogenesis at the cross-species perspective through an integrative approach, accounting morphometric, molecular and phylogenetic data of morphogenesis as seen through an evolutionary developmental biology. Comparison of embryos, at critical stages of development, of Drosophila, Zebrafish, Xenopus, and Mouse revealed a lot of similarities and differences in gene expression and appearance of the embryos. Quantitative morph metric analyses with Procrustes distances and principal component scores indicated a change of the body plan architecture over time especially the transitions between the neurulation to organogenesis. SHH and BMP4 patterns of expression were highly different among species and highly stage dependent. This indicates that evolution can alter retained developmental pathways in a lineage specific manner. The tendencies of the shape change were similar to the molecular signals and this implies that there is a genetic control and morphological expression being correlated somewhere. This need not be the most relevant result of the union of RNA-Seq data and gene ontology enrichment, which only makes it all the more obvious that there are not only shared developmental modules, but also specific new adaptations that are unique to each of the animals. These findings were supported by phylogenetic comparison studies that indicated that morphogenetic traits considered essential Pagel lambda scores are relatively high indicating that they had been retained through evolution. This experiment demonstrates that a combined application of geometric morphometrics and gene expression profiling may provide us with a full image of the manner of happenings of evolutionary change that occurs in development. The stage-specific profiles and hybrid visualizations indicate the actual existence of heterochrony and modularity,

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as well as, evolutionary restraint in developmental programs. There were features that were highly phylogenetically significant and there were other features that were evolutionarily non-conserved. This is to insinuate that shared embryological blueprints had adaptive diversity. Such findings give an idea of how factors of evolution influence the processes of development in order to form the diversity of body forms and size in animals. This work demonstrates the utility of Evo-Devo approaches to the bridging of genotype and phenotype and provides a model that can be employed in the future in the study of the evolution of development larger taxa. Such methodological framework also enables pairing of CRISPR-based disruption with live imaging in order to test developmental hypotheses between evolution and morphology.

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