fishes

MDPI

Article

Cloning and Expression of Sox2 and Sox9 in Embryonic and Gonadal Development of *Lutraria sieboldii*

Min Lu [1,†], Zenghou Xing [1,†], Yurui Zhou [1], Youhou Xu [1], Huijing Peng [2], Jie Zou [2], Solomon Felix Dan [1], Zhicai She [1], Pengliang Wang [1], Jinfeng Liu [3], Shaomin Qin [3], Jialin Yang [1,*] and Peng Zhu [1,*]

[1] Guangxi Key Laboratory of Beibu Gulf Marine Biodiversity Conservation, Beibu Gulf University, Qinzhou 535011, China
[2] Guangxi Institute of Oceanology, Beihai 536000, China
[3] Guangxi Key Laboratory of Veterinary Biotechnology, Guangxi Veterinary Research Institute, Nanning 530001, China
* Correspondence: yangjialin@bbgu.edu.cn (J.Y.); zp2017@bbgu.edu.cn (P.Z.)
† These authors contributed equally to this work.

Abstract: The Sox family plays essential roles as transcription factors in vertebrates; however, little is known about the Sox family in *Lutraria sieboldii*. *L. sieboldii* are pleasant to eat with a short growth cycle and have become one of the best bottom-seeded enrichment species in Guang Xi. In this study, Sox2 (named LsSox2) and Sox9 (named LsSox9) from *L. sieboldii* were cloned, and their expression patterns were analyzed. The length of the *LsSox*2 gene coding sequence was 1011 bp, encoding 336 amino acids, and *LsSox*9 was 1449 bp, encoding 482 amino acids. *LsSox*2 had its highest expression levels in the ovary, which were 356 times those in testis, whereas *LsSox*9 presented higher expression in testis, which was 6 times more highly expressed than in the ovary. *LsSox*2 exhibited the highest expression during the morula stage, which was 20 times that of the D-shaped larvae or zygote. *LsSox*9 exhibited two expression peaks, one at the four-cell stage and the other at the trochophore stage, while the lowest expression was in the zygote. *LsSox*9 was 73 times more highly expressed in the four-cell stage than in the zygote stage. During gonadal development, *LsSox*2 presented the highest expression in the mature ovary, which was 756 times more highly expressed than in mature testis. *LsSox*9 presented higher expression in testis at the emission stage which was 6 times more highly expressed than in the ovary. These results indicate that *LsSox*2 and *LsSox*9 may play important roles in embryonic and gonadal development.

Keywords: *Lutraria sieboldii*; LsSox2; LsSox9; expression; cloning

Citation: Lu, M.; Xing, Z.; Zhou, Y.; Xu, Y.; Peng, H.; Zou, J.; Dan, S.F.; She, Z.; Wang, P.; Liu, J.; et al. Cloning and Expression of Sox2 and Sox9 in Embryonic and Gonadal Development of *Lutraria sieboldii*. *Fishes* **2022**, *7*, 392. https://doi.org/10.3390/fishes7060392

Academic Editor: Alberto Pallavicini

Received: 26 November 2022
Accepted: 13 December 2022
Published: 16 December 2022

1. Introduction

The sex determining region Y-box (*Sox*) gene family is involved in sex differentiation and embryonic development. Its main features are a conserved High Mobility Group (HMG) motif that binds specifically to DNA sequences and has more than 50% sequence identity in the same family [1]. The *Sox* gene family consists of more than 20 members that mediate DNA binding through the HMG domain [2], which has been divided into eight groups (sex determining region Y-box A to sex determining region Y-box H, SoxA–SoxH) [3] that play essential roles in development, cell division, and differentiation [4].

Sex determining region Y (SRY) is the only member in the SoxA group [5]. Although SRY plays a regulatory role in sex determination, little information is available on the sequences surrounding the HMG structure. The SoxB group is subdivided into SoxB1 and SoxB2. The SoxB1 group includes Sox1, Sox2, and Sox3, while Sox14 and Sox21 are part of the SoxB2 subgroup. SoxB1 members play major roles in nervous system development and architecture, while SoxB2 has a C-terminal trans-inhibitory domain but not a trans-activating domain [6]. SoxE contains Sox8, Sox9, and Sox10, which are similar in structure

and contain a unique dimerization domain in the HMG structure [7]. These 20 *Sox* genes are currently found only in animals, including invertebrates, but not in plants.

The Sox transcription factor is a fundamental molecule that plays a key role in almost all stages of embryonic development and differentiation of many cell types [4]. A study of the *Sox* gene family in cyprinid fish revealed that the *Sox* gene family is expressed at different levels and duplicated in cyprinid fish with different ploidy after a whole-genome duplication even [8]. Studies on metamorphosis in the African clawed toad have shown that many members of the Sox family play important roles in the formation and proliferation of intestinal stem cells and play an active role in the intestinal epithelium [9]. Sox2, a member of the SoxB1 subfamily [10], has also been implicated in intestinal development [11]. Early studies showed that, like other members of the SoxB1 subfamily, Sox2 promotes neural cell differentiation during vertebrate embryonic development, stem cell maintenance [12], and is required for maintenance of central nervous system stem cells [12,13]. Lc-Sox2 is zygotically expressed in *Larimichthys crocea* embryos, with high levels starting at the gastrulation stage [14]. Sox2 is not maternally inherited in Japanese flounder, and the transcripts are present from the high blastula stage onwards, with the highest level at the mid-gastrula stage [14].

Sox9, a member of the SoxE subfamily, is an important transcription factor in the development of many tissues, particularly in sex determination and chondrogenesis. The Sox9 expression pattern changes throughout the reproductive cycle of male *Astyanax altiparanae* and is significantly downregulated after spermatogenesis [15]. The *Sox*9 gene is conserved in mammals and birds, and its structure and function are preserved in teleost fish. Two *Sox*9 genes, Sox9a and Sox9b have been identified in *Paralichthys olivaceus*, and quantitative real-time polymerase chain reaction (qRT-PCR) indicated that PoSox9a and PoSox9b were expressed at higher levels in testis than in ovaries of adults [14,16]. On the other hand, Sox9a is preferentially expressed in the brain and ovary of *Oryzias latipes*, while Sox9b is expressed more in the testis than in the ovary [17].

Mollusks are the second largest group in the animal kingdom after arthropods; however, the phylogenetic characterization of the *Sox*2 gene family is very rare in mollusks, particularly in *Lutraria sieboldii* which are pleasant to eat, have a short growth cycle, and have become one of the best bottom-seeded enrichment species in Guang Xi, with an annual output of about 76,000 tons (4.5–13.68 billion Yuan). Here, we report a study of the *Sox*2 and *Sox*9 genes in the *L. sieboldii*. The qPCR results suggest that these transcription factors have evolved potentially functions during embryogenesis.

2. Materials and Methods

2.1. Tissue and Embryo Collection

L. sieboldii were purchased from a culture farm in BeiHai, Guangxi Province, China. Tissues, such as the gills, mantle, pipe, visceral mass, digestive gland, gonads, muscle, and genital duct, were collected from three individuals. RNA was extracted with the RNAiso Plus reagent (Takara, Dalian, China). The RNA was used for cloning and tissue expression analyses of the *LsSox*2 and *LsSox*9 genes.

Jiao et al. reported that embryonic development of *L. sieboldii* can be divided into cleavage, blastocyst, gastrulation, dandruff larvae, and disc larvae stages [18]. In our study, embryos were collected from adult females and males after artificial insemination. Furthermore, 46 *L. sieboldii* gonads were collected during gonadal development and paraffin sections of the gonads were prepared. Gonadal development in *L. sieboldii* peaks from October to April of the following year, three samples were collected at each development stage [19].

2.2. Gene Isolation and Cloning

RNA was isolated and cDNA was synthesized following a protocol previously established in our laboratory [20]. The coding sequences (CDSs) of the *LsSox*2 and *LsSox*9 genes were cloned by RT-PCR. Two pairs of primers were designed for PCR amplification

and qRT-PCR of *LsSox*2 and *LsSox*9 based on the results of *L. sieboldii* transcriptome data previously generated in our laboratory. PCR, sequencing, and sequence assembly were performed following a protocol previously established in our laboratory [20], and sequences were submitted to NCBI under Accession numbers OM243913 and OM243914.

2.3. Bioinformatics Analysis

Bioinformatics analysis was performed following a protocol previously established in our laboratory [21]. The online websites (http://www.ncbi.nlm.nih.gov (accessed on 6 June 2022) and http://www.expasy.org (accessed on 6 June 2022)) were used to analyze the nucleotide sequences, deduced amino acid sequences, and open reading frames. The BLAST tool (http://www.ncbi.nlm.nih.gov, accessed on 6 June 2022) was used to compare the *LsSox*2 and *LsSox*9 sequences of the homologs with other CDSs. Signal peptides were predicted at http://www.cbs.dtu.dk/services/signalp/ (accessed on 6 June 2022). Protein functional loci were detected by Prosite, Motif Scan, and Protter. The cloned *LsSox*2 and *LsSox*9 sequences were aligned using DNAMAN software, and phylogenetic analysis of bootstrap resampling (with 1000 pseudocopies) was performed with MEGA10 using the neighbor-joining method to construct the evolutionary tree [22].

2.4. Tissue Distribution and LsSox2 and LsSox9 Expression Profiles in Adults and Embryos

The expression profiles of the two genes in *L. sieboldii* were determined using the CFX96^{TM} Real-time System (Bio-Rad Laboratories, Hercules, CA, USA), as described previously [23]. The gene-specific primers (*LsSox*2 and *LsSox*9) are shown in Table 1. Each treatment was carried out in triplicate with a reaction volume of 20 µL, and independently repeated three times.

Table 1. Primer sequences.

Primers	Sequence (5′-3′)	Amplified Length (bp)	Tm (°C)	Note
LsSox2F1	CAAGCACTGTTTTTGAGAGG	1574	56	CDS cloning
LsSox2R1	TTCATTTCACGATTTAACGG			
LsSox2dlF1	TTTACCCGTTAAATCGTGAAAT	92	59	qRT-PCR
LsSox2dlR1	TGAAAGTTGAGAATCGCCTCT			
LsSox9F1	AGCGAACTTGTTTGGACATA	1504	57	CDS cloning
LsSox9R1	ACCGTTTCTCAGCGTCTAAT			
LsSox9dlF1	AAAGTAAGAGACGACGCAG	123	58	qRT-PCR
LsSox9dlR1	GTAACGAGACACAGAGGGG			
LsEf1dlF1	CTGTCTTAGATTGTCACACTGCC	104	65	qRT-PCR
LsEf1dlR1	CGTCTTAGGGTTGTCTTCCAA			

2.5. Statistical Analysis

The fold-change in the relative mRNA expression of the target genes was determined using the $2^{-\Delta\Delta ct}$ method. The results were analyzed using one-way analysis, and mean comparisons of the treatments were determined using Duncan's method with SPSS statistical 19 software (IBM Corp., Armonk, NY, USA). The significant difference standard was set as $p < 0.05$, and the highly significant difference was set as $p < 0.01$.

3. Results and Analysis

3.1. Cloning and Sequencing Analysis of the LsSox2 and LsSox9 Genes

The specific bands of the *LsSox*2 and *LsSox*9 genes were 1574 and 1504 bp via PCR amplification, respectively (Figure 1A,B).

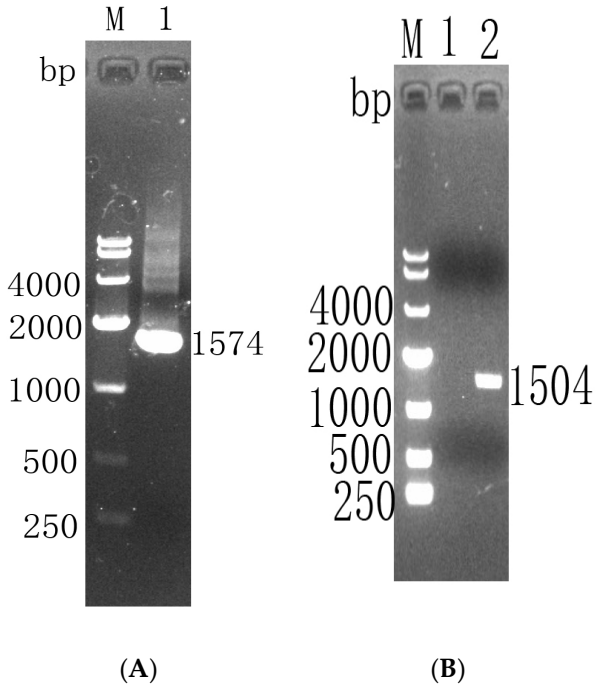

Figure 1. Cloning of the *LsSox*2 and *LsSox*9 genes of *Lutraria sieboldii*. (**A**) Cloning of the *LsSox*2 gene, M: DL10000 DNA marker, 1 *LsSox*2: amplification product. (**B**) Cloning of the *LsSox*9 gene, M: DL10000 DNA marker, 1: water, 2: *LsSox*9 amplification product.

3.2. Phylogenetic Analysis

The homology analysis by Blast2GO showed that the *LsSox*2 (OM243913) CDS was 85.00% homologous for *Corbicula fluminea*, 71.33% for *Sinanodonta woodiana* and 62.39% for *Crassortrea gigas*. The homology of the *LsSox*9 (OM243914) CDS was 88.17% for *C. fluminea* and 45.60% for *Mytilus edulis*. The amino acid sequences of the Sox2 and Sox9 proteins were used to construct a phylogenetic tree among *L. sieboldii* with 30 other *Sox* genes, including 10 in *Lamellibranchia*, 10 in *Mammalia*, and 10 in *Pisces* (Figure 2). The phylogenetic analysis showed that the *L. sieboldii Sox*2 and *Sox*9 genes were clustered with their respective counterparts from *C. fluminea* and *Sinonovacula constricta*. The most striking result to emerge from the tree is that Sox2 and Sox9 were conserved in the *Sox* gene family, and our phylogenetic tree provided strong evidence for correctly naming the *L. sieboldii Sox* genes.

3.3. Bioinformatics Analysis

The CDSs of the *LsSox*2 and *LsSox*9 genes were 1011 and 1449 bp, encoding 336 and 482 amino acids, respectively. *LsSox*2 was predicted to contain cAMP- and cGMP-dependent protein kinase phosphorylation sites, N-glycosylation sites, and other sites (Figure 3A), while *LsSox*9 was predicted to contain casein kinase II phosphorylation sites, N-myristoylation sites, and other sites (Figure 3B). The SMART program predicted that *LsSox*2 has an HMG domain at residue sites 68–141 aa (Figure 3C), while LsSox9 has HMG domains at residue sites 77–147 aa (Figure 3D).

The formula, theoretical Mw, and pI of *LsSox*2 were $C_{1557}H_{2437}N_{479}O_{487}S_{30}$, 36.62 kDa, and 9.67, respectively. No signal peptide was detected, but it was predicted to be nuclear with a score of 9.0. The formula, theoretical Mw, and pI of *LsSox*9 were $C_{2301}H_{3547}N_{695}O_{745}S_{27}$, 53.73 kDa, and 6.53, respectively. It had no signal peptide and was predicted to be nuclear with a score of 7.2 and plasma membrane with a score of 1.7. *LsSox*2 was predicted to have a total of 13 α-helices, 24 β-folds, 23 T-turns, and 26 irregular coils (Figure 4A), while *LsSox*9 had a total of 15 α-helices, 13 β-folds, 43 T-turns, and 41 irregular coils (Figure 4B). The tertiary structures of *LsSox*2 and *LsSox*9 are shown in Figure 4C,D.

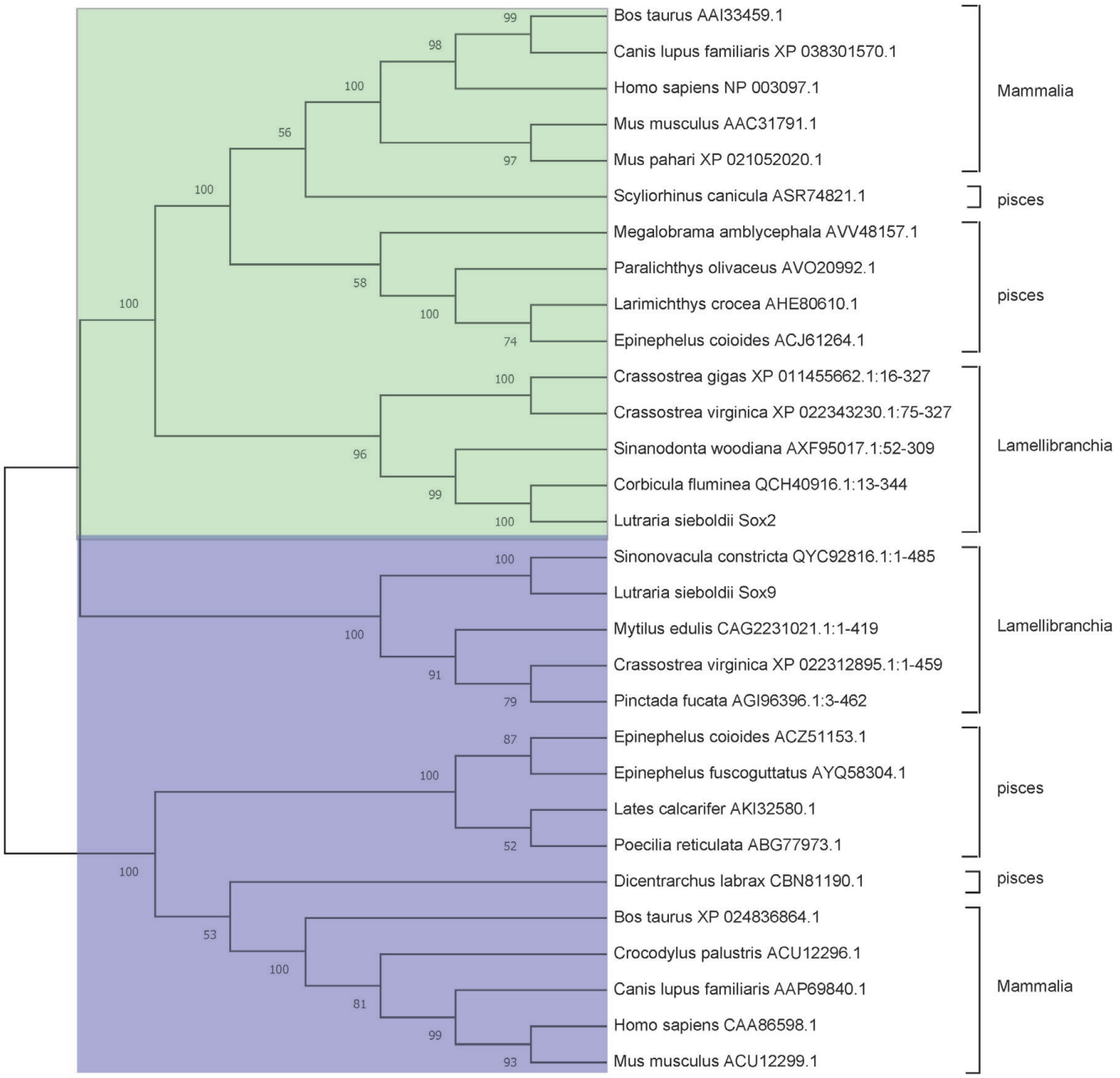

Figure 2. Phylogenetic analyses of the (green) *LsSox*2 and (purple) *LsSox*9 proteins.

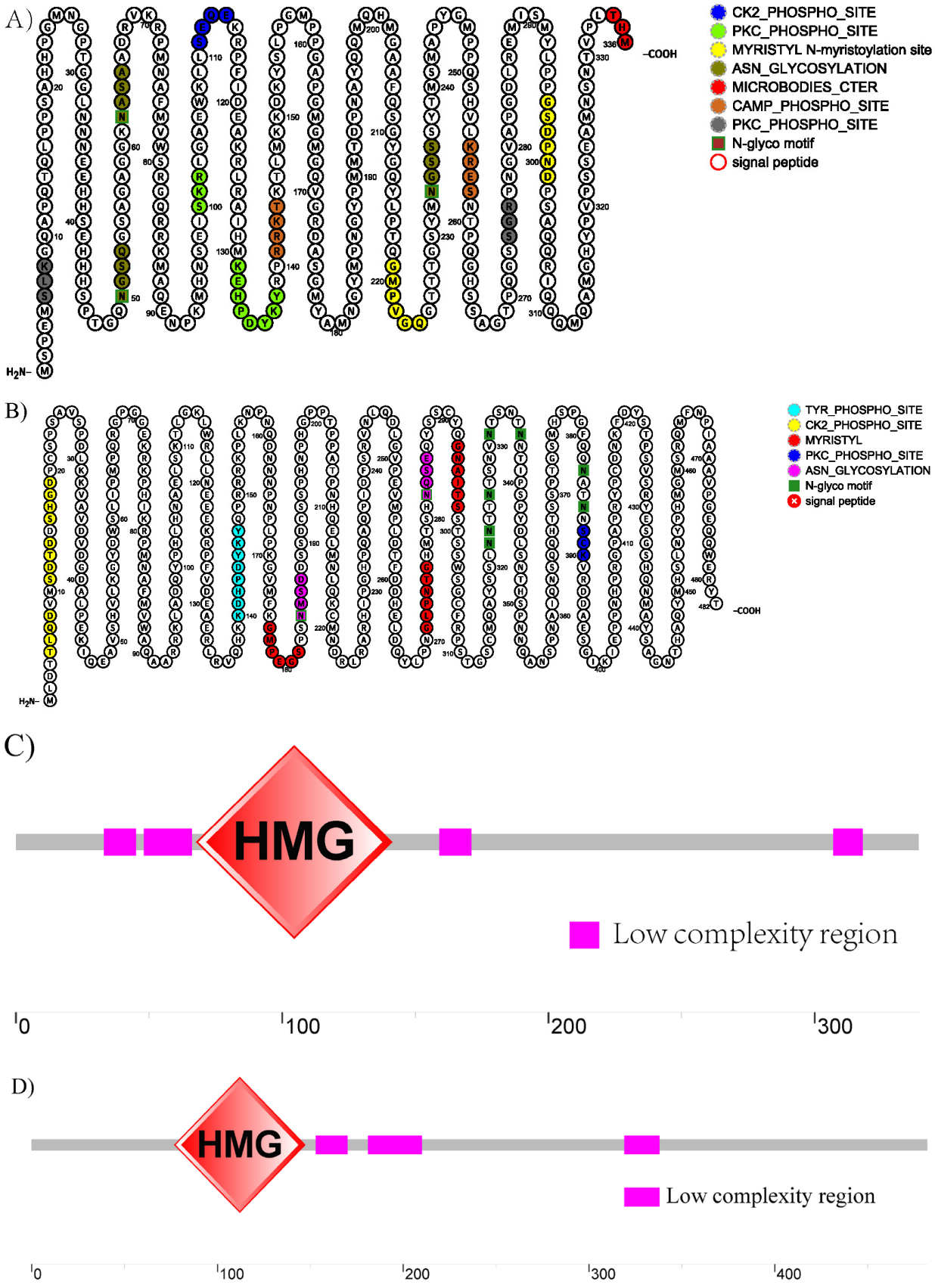

Figure 3. Sequence analysis of *LsSox*2 and *LsSox*9. (**A**) *LsSox*2 motif sites. (**B**) *LsSox*2 protein domain structures. (**C**) *LsSox*9 motif sites. (**D**) *LsSox*9 protein domain structures.

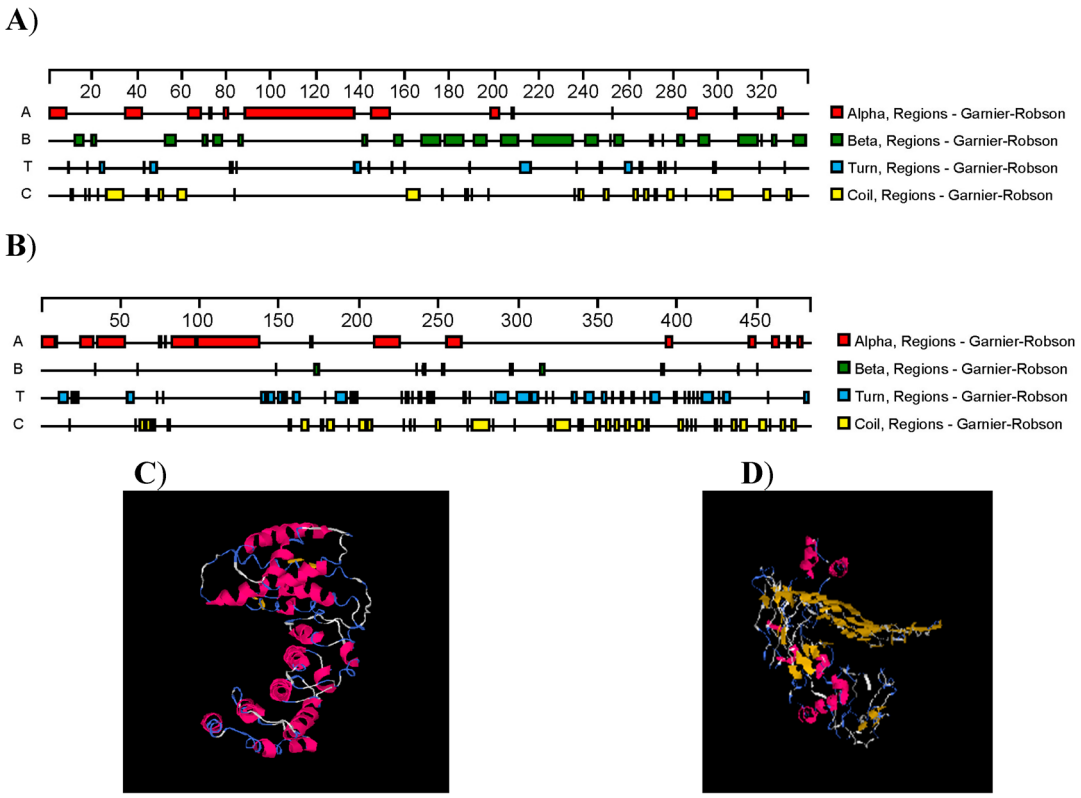

Figure 4. Tertiary structures of (**A**) *LsSox*2 and (**B**) *LsSox*9; secondary structures of (**C**) *LsSox*2 and (**D**) *LsSox*9.

3.4. Tissue Distribution of LsSox2 and LsSox9 mRNA

The quantitative real time-polymerase chain reaction (qRT-PCR) results suggested that *LsSox*2 was abundantly expressed in the ovary of females, moderately expressed in the gill, mantle, pipe, and genital duct, and weakly expressed in muscle, the visceral mass, and the digestive gland, *LsSox*2 was 250 times more highly expressed in the ovary than the visceral mass. In males, *LsSox*2 was abundantly expressed in the mantle, moderately expressed in the gill and pipe, and weakly expressed in muscle, the visceral mass, the digestive gland, and the testis, *LsSox*2 was 51 times more highly expressed in the mantle than in the testis. *LsSox*2 was more highly expressed in female tissues than in males (Figure 5A).

In males, *LsSox*9 was abundantly expressed in the muscle, pipe, gill, testis, digestive gland, and genital duct and moderately expressed in the mantle and visceral mass. *LsSox*9 was six times more highly expressed in testis than in the mantle. In females, *LsSox*9 was abundantly expressed in the mantle, ovary, and genital duct; moderately expressed in the visceral mass, muscle, and gill, and weakly expressed in the pipe and digestive gland. *LsSox*9 was 32 times more highly expressed in the ovary than in the pipe. *LsSox*9 was more highly expressed in male tissues than females (Figure 5B).

We next investigated *Sox* gene expression during embryogenesis by collecting cells from *L. sieboldii* zygotes after artificial insemination, including dividing, blastocyst stage, and D-shaped larvae. During embryogenesis, *LsSox*2 mRNA initially increased, and then decreased, exhibiting the highest expression at the multicellular stage (morula stage) and the lowest expression in the zygote and D-shaped larvae. The former was 20 times that of the D-shaped larvae (Figure 5C). During embryogenesis, *LsSox*9 expression exhibited two peaks. One peak occurred at the four-cell stage and the other peak occurred at the trochophore stage. The lowest expression was observed during the early stages of embryonic development (zygote stage), and it was 73 times more highly expressed at the four-cell stage than in the zygote stage (Figure 5D).

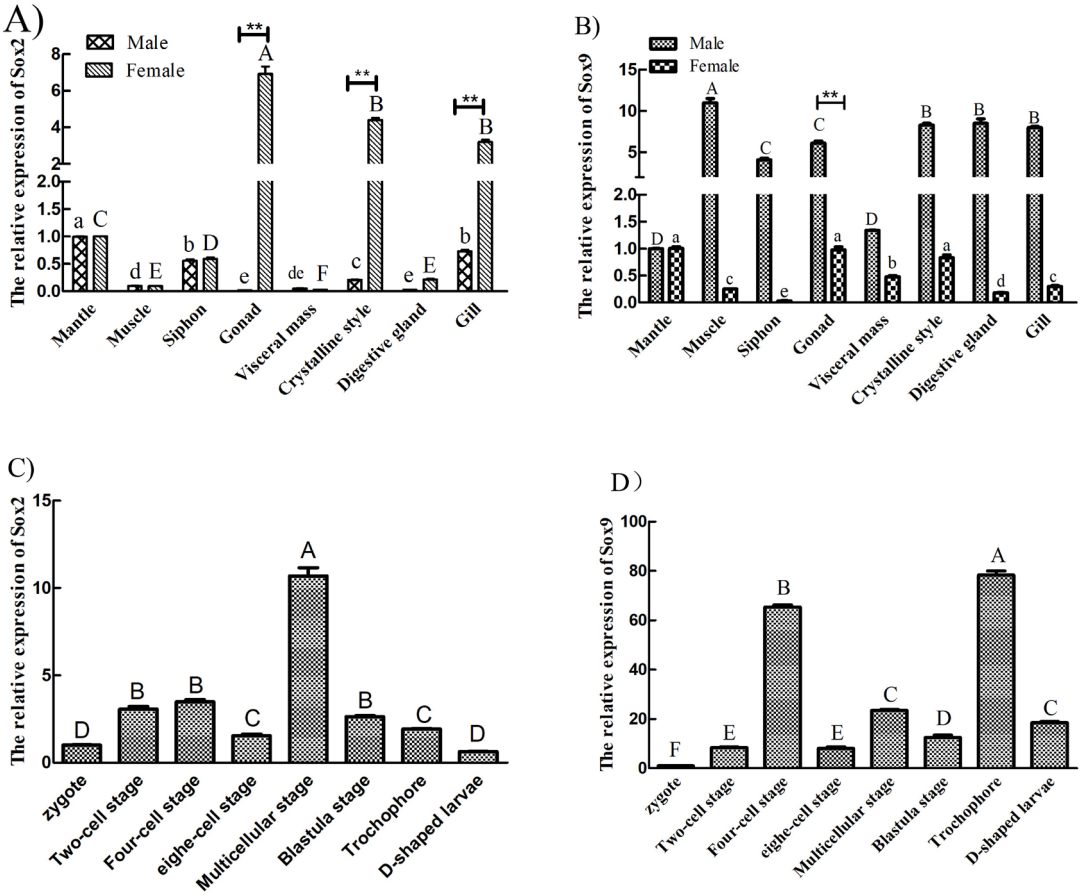

Figure 5. Relative expression of *LsSox*2 and *LsSox*9 in tissues of *Lutraria sieboldii*. (**A**) *LsSox*2 and (**B**) *LsSox*9. Expression of *LsSox*2 and *LsSox*9 at different stages of development. (**C**) *LsSox*2 and (**D**) *LsSox*9. Each bar represented mean ± SEM (n = 3), asterisks above the bars indicate the results of Duncan's test at ** $p < 0.01$; Different letters indicate a significant difference.

3.5. Gene Expression Analysis of L. sieboldii Sox Genes in Gonads

L. sieboldii gonads were collected and paraffin sections were prepared to monitor gonadal development according to previous studies. In the growth stage, the germ cells of males presented in cysts, and some were in advanced stages of spermatogenesis (Figure 6A). Mature sperm filled the vesicles during the maturation stage, indicating that these animals were sexually mature (Figure 6B). Mature sperm were expelled during the emission stage (Figure 6C). Primary oocytes were recognized in females during the growth stage (Figure 6D). Then, the entire follicular cavity was filled with mature eggs and oocytes during the late growth stage, and the irregularly shaped mature eggs were squeezed together in the cavity (Figure 6E). The follicles developed cavities of different sizes during the emission stage, but there were still immature oocytes remaining on the follicle wall (Figure 6F).

Notably, our results show that LsSox2 was significantly higher in the ovary than in the testis and reached its highest expression during gonadal maturation ($p < 0.05$) (Figure 6G). LsSox2 was 756 times more highly expressed in the ovary than in the testis during this stage (Figure 6G). Similarly, the *LsSox*9 gene was significantly more expressed in males than in females and was twice as high during the emission period (Figure 6H), suggesting that Sox9 plays a unique role during gonadal development in *L. sieboldii*.

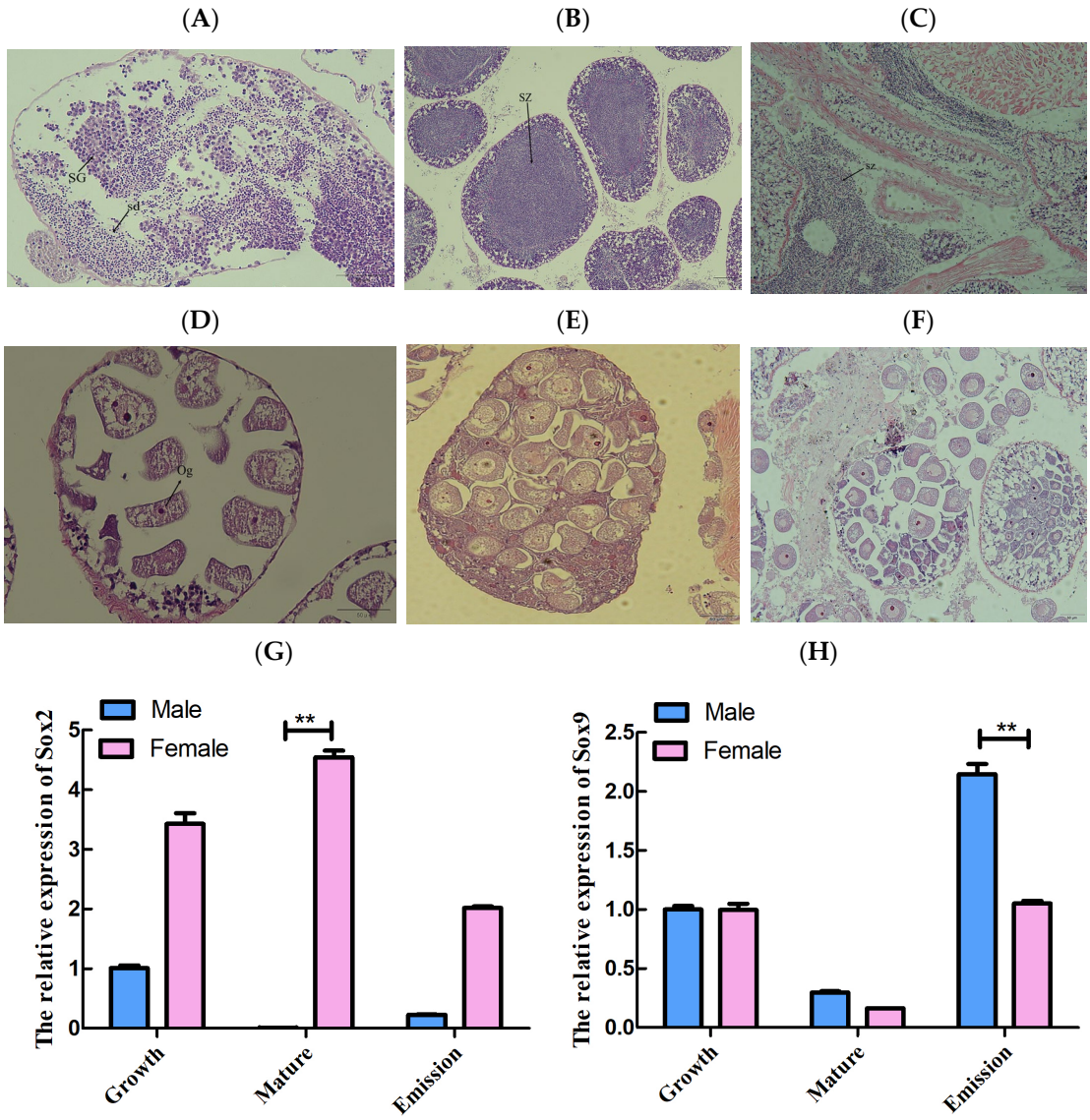

Figure 6. Paraffin sections of the *Lutraria sieboldii* gonad at different stages of development. (**A**) Growth stage, (**B**) maturation stage, and (**C**) emission stage of male *L. sieboldii*. (**D**) Growth stage, (**E**) maturation stage, and (**F**) emission stage of female *L. sieboldii*. (**G**) *LsSox*2 and (**H**) *LsSox*9. Expression of *LsSox*2 and *LsSox*9 at different stages of gonadal development in *L. sieboldii*. Each bar represented mean ± SEM (n = 3), asterisks above the bars indicate the results of Duncan's test at ** $p < 0.01$. sd: spermatids; SG: spermatogonia; sz: spermatozoa. Og: oogonia.

4. Discussion

Mollusks are the second largest group in the animal kingdom after arthropods; however, expression analysis and functional characterization of the *Sox* gene family are very rare in mollusks. In the present study, the length of the *LsSox*2 gene CDS was 1011 bp, encoding 336 amino acids. It contained cAMP- and cGMP-dependent protein kinase phosphorylation sites, N-glycosylation sites, and an HMG domain, which are typical structural features of the Sox2 family [16]. The HMG domain of *LsSox*2 was highly similar to that of *Anodonta woodiana* [16] and *Larimichthys crocea* [24]. *LsSox*9 was 1449 bp, encoding 482 amino acids, and containing casein kinase II phosphorylation sites, N-myristoylation sites, and an HMG domain. Alignment of the LsSox9 protein sequence with sequences from other species indicated that the *LsSox*9 HMG box was highly conserved across taxa, consistent with earlier reports [19]. The highly conserved HMG domain of *LsSox*2 and *LsSox*9 with other species, assumed that the HMG box domain plays an important role in

the recognition of specific target DNA elements, and suggested that the two genes likely functions as an important player in the gonadal tissue of *L. sieboldii* [25].

As reported in previous studies, many *Sox* genes have been associated with gonadal development [26,27], and are widely expressed during different stages of embryogenesis [28]. Herein, we report that Sox2 was highly expressed in the gonads and gills of normal *L. sieboldii* tissues, which was consistent with a previous study that the *A. woodiana Sox*2 gene is highly expressed in the gonads and gills [16]. While LsSox9 was more highly expressed in tissues of males than females, and it was six-fold more highly expressed in testis than in ovary, which was consistent with previous study that Sox9 was mainly expressed in the gonad, eyestalk, and cerebral ganglion of *Scylla paramamosain* [17], or with the highest expression levels in the brain and testis of *Cyprinus carpio* [18,19,29].

*LsSox*2 gene was more highly expressed during maturation of the *L. sieboldii* gonads, particularly during oocyte maturation, when it was significantly more expressed than in sperm. This finding is consistent with a study on *Paralichthys olivaceus* [14] but contrasted with one on *A. woodiana* [16]. Sox9 is a 'hub' gene of gonadal development, regulated positively in males and negatively in females [30]. *LsSox*9 was more highly expressed in tissues of males than females. *LsSox*9 was initially expressed at the same level during gonadal development in both sexes, but then declined sharply at the onset of ovarian differentiation and was expressed at the average level during spermatogonial emission stage. We suspect that this occurred due to the large number of sperm released to fill the spermathecae, making *LsSox*9 expression significantly higher in males than in females during the emission phase [31,32]. Similarly, the massive division and differentiation of oocytes at maturity results in much higher expression of Sox2 in the ovary than in testis. The differential expression patterns of *LsSox*2 and *LsSox*9 genes in the ovary and testis suggest that they may be involvement in the regulation of sex determination of *L. sieboldii*.

Previous studies have shown that the SoxB1 genome is critical for early embryogenesis, particularly as Sox2 promotes neural cell differentiation [15]. Okuda et al. provided evidence that the *SoxB*1 gene is highly redundant in early zebrafish embryos and that their encoded proteins are functionally interchangeable during early embryonic development by knocking out Sox2/3/19a/19b [33]. In the present study, *LsSox*2 exhibited the highest expression during the *L. sieboldii* morula stage and its lowest expression in D-shaped larvae. The results of these experiments indicate that Sox2 is a key transcriptional regulator in the oocyte-to-embryo transition and show that Sox2 expression was associated with the number of cell divisions, suggesting that Sox2 also plays a role in the embryonic development of *L. sieboldii*, which is consistent with previous studies [14]. For example, White et al. showed that Sox2-DNA binding predicts the fate of mammalian cells as early as the four-cell stage [34]. Pan et al. reported that *Sox*2, which is localized in the nucleus, is first expressed during the two-cell stage and that its expression dramatically increases between the morula and blastocyst stages, indicates that asymmetric distribution of Sox2 before the morula stage is essential for lineage segregation [35,36]. *Sox*9 gene, acts as a potent transcriptional regulator, involved in sex determination, gonad development and embryo development [29]. During embryo development, Spsox9 was highly expressed in 5 pairs of appendages, 7 pairs of appendages, and eyepigment formation stage [17]. Herein, *LsSox*9 gene exhibited two expression peaks, one at the four-cell stage and the other at the trochophore stage, it had been reported that during embryonic development, MnSoxE1 was mainly expressed in the gastrula stage, implicating its involvement in tissue cell differentiation and formation [37].

5. Conclusions

The *Sox* gene family plays a unique role in development. In this study, gene cloning and expression analyses were used to characterize Sox2 and Sox9 in *L. sieboldii*. The LsSox2 and LsSox9 expression patterns in normal tissues during gonadal development and embryogenesis suggest that the *Sox*2 and *Sox*9 genes play unique roles in *L. sieboldii* growth and development, and maybe sex-determining genes.

Author Contributions: P.Z. and J.Y. designed this project. M.L., Z.X. and Y.Z. cultured the experimental *Lutraria sieboldii*. M.L., Z.X., H.P. and J.Z. collected samples, extracted RNA and qRT-PCR. Y.X., J.L., M.L. and S.Q. performed date analysis and drafted the manuscript. Z.S., P.W. and M.L. participated in the sequence alignment and performed the statistical analysis. S.F.D., P.Z. and J.Y. revised the manuscript. P.Z. and J.Y. conceived the study, participated in its design and coordination. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Guangxi innovation driven development special funds (Grant no. AA19254008-3), Guangxi Natural Science Foundation (Grant No. 2019GXNSFBA245081), Marine Science Program for Guangxi First-Class Discipline, Beibu Gulf University (Grant nos. DRB005, DTB001) and College Students' innovation training program (202211607104, S202111607045 and Beike 202082030).

Institutional Review Board Statement: This article does not contain any studies with human participants. All animals used in this study were purchased from market, and the experimental protocols adhered to the the Guidelines of Animal Care by the Animal Management and Ethics Committee of BeiBu Gulf University.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Grimm, D.; Bauer, J.; Wise, P.; Krüger, M.; Simonsen, U.; Wehland, M.; Infanger, M.; Corydon, T.J. The role of SOX family members in solid tumours and metastasis. *Semin. Cancer Biol.* **2020**, *67*, 122–153. [CrossRef]
2. She, Z.-Y.; Yang, W.-X. SOX family transcription factors involved in diverse cellular events during development. *Eur. J. Cell Biol.* **2015**, *94*, 547–563. [CrossRef]
3. Schepers, G.E.; Teasdale, R.D.; Koopman, P. Twenty Pairs of Sox: Extent, Homology, and Nomenclature of the Mouse and Human Sox Transcription Factor Gene Families. *Dev. Cell* **2002**, *3*, 167–170. [CrossRef]
4. Hou, L.; Srivastava, Y.; Jauch, R. Molecular basis for the genome engagement by Sox proteins. *Semin. Cell Dev. Biol.* **2017**, *63*, 2–12. [CrossRef]
5. Kashimada, K.; Koopman, P. Sry: The master switch in mammalian sex determination. *Development* **2010**, *137*, 3921–3930. [CrossRef]
6. Miyagi, S.; Kato, H.; Okuda, A. Role of SoxB1 transcription factors in development. *Cell. Mol. Life Sci.* **2009**, *66*, 3675. [CrossRef]
7. Bernard, P.; Tang, P.; Liu, S.; Dewing, P.; Harley, V.R.; Vilain, E. Dimerization of SOX9 is required for chondrogenesis, but not for sex determination. *Hum. Mol. Genet.* **2003**, *12*, 1755–1765. [CrossRef]
8. Huang, X.; Wu, C.; Gong, K.; Chen, Q.; Gu, Q.; Qin, H.; Zhao, C.; Yu, T.; Yang, L.; Fu, W.; et al. Sox Gene Family Revealed Genetic Variations in Autotetraploid *Carassius auratus*. *Front. Genet.* **2020**, *11*, 804–804. [CrossRef]
9. Fu, L.; Shi, Y.-B. The Sox transcriptional factors: Functions during intestinal development in vertebrates. *Semin. Cell Dev. Biol.* **2017**, *63*, 58–67. [CrossRef]
10. Bowles, J.; Schepers, G.; Fau-Koopman, P.; Koopman, P. Phylogeny of the SOX family of developmental transcription factors based on sequence and structural indicators. *Dev. Biol.* **2000**, *227*, 239–255. [CrossRef]
11. Kuzmichev, A.N.; Kim Sk Fau-D'Alessio, A.C.; D'Alessio Ac Fau-Chenoweth, J.G.; Chenoweth Jg Fau-Wittko, I.M.; Wittko Im Fau-Campanati, L.; Campanati, L.; Fau-McKay, R.D.; McKay, R.D. Sox2 acts through Sox21 to regulate transcription in pluripotent and differentiated cells. *Curr. Biol.* **2012**, *22*, 1705–1710. [CrossRef] [PubMed]
12. Archer, T.C.; Jin, J.; Casey, E.S. Interaction of Sox1, Sox2, Sox3 and Oct4 during primary neurogenesis. *Dev. Biol.* **2011**, *350*, 429–440. [CrossRef] [PubMed]
13. Hoffmann, S.A.; Hos, D.; Fau-Küspert, M.; Küspert, M.; Fau-Lang, R.A.; Lang Ra Fau-Lovell-Badge, R.; Lovell-Badge, R.; Fau-Wegner, M.; Wegner, M.; Fau-Reiprich, S.; et al. Stem cell factor Sox2 and its close relative Sox3 have differentiation functions in oligodendrocytes. *Development* **2014**, *141*, 39–50. [CrossRef] [PubMed]
14. Gao, J.; Wang, Z.; Shao, K.; Fan, L.; Yang, L.; Song, H.; Liu, M.; Wang, Z.; Wang, X.; Zhang, Q. Identification and characterization of a Sox2 homolog in the Japanese flounder Paralichthys olivaceus. *Gene* **2014**, *544*, 165–176. [CrossRef]
15. Cui, C.-P.; Zhang, Y.; Wang, C.; Yuan, F.; Li, H.; Yao, Y.; Chen, Y.; Li, C.; Wei, W.; Liu, C.H.; et al. Dynamic ubiquitylation of Sox2 regulates proteostasis and governs neural progenitor cell differentiation. *Nat. Commun.* **2018**, *9*, 4648. [CrossRef]
16. Xia, X.; Guan, C.; Chen, J.; Qiu, M.; Qi, J.; Wei, M.; Wang, X.; Zhang, K.; Lu, S.; Zhang, L.; et al. Molecular characterization of AwSox2 from bivalve Anodonta woodiana: Elucidating its player in the immune response. *Innate Immun.* **2020**, *26*, 381–397. [CrossRef]

17. Wan, H.; Liao, J.; Zhang, Z.; Zeng, X.; Liang, K.; Wang, Y. Molecular cloning, characterization, and expression analysis of a sex-biased transcriptional factor sox9 gene of mud crab *Scylla paramamosain*. *Gene* **2021**, *774*, 145423. [CrossRef]
18. Takamatsu, N.; Kanda, H.; Ito, M.; Yamashita, A.; Yamashita, S.; Shiba, T. Rainbow trout SOX9: cDNA cloning, gene structure and expression. *Gene* **1997**, *202*, 167–170. [CrossRef]
19. Du, Q.Y.; Wang, F.Y.; Hua, H.Y.; Chang, Z.J. Cloning and study of adult-tissue-specific expression of Sox9 in *Cyprinus carpio*. *J. Genet.* **2007**, *86*, 85–91. [CrossRef]
20. Liao, Y.; Liu, K.; Ren, T.; Zhang, Z.; Ma, Z.; Dan, S.F.; Lan, Z.; Lu, M.; Fang, H.; Zhang, Y.; et al. The characterization, expression and activity analysis of three superoxide dismutases in Eriocheir hepuensis under azadirachtin stress. *Fish. Shellfish. Immunol.* **2021**, *117*, 228–239. [CrossRef]
21. Lu, M.; Yang, J.; Wang, Z.; Song, J.; Hu, Y.; Wang, P.; Zhang, H.; Xu, Y.; Zhu, P. Cloning and expression of the ChGstα and ChGstκ genes in the gills of Crassostrea hongkongensis under nanoparticulate and ionic Zn stress. *Comp. Biochem. Physiology. Toxicol. Pharmacol. CBP* **2021**, *244*, 109007. [CrossRef] [PubMed]
22. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef] [PubMed]
23. Xu, Y.; Pan, H.; Lu, M.; Liu, Q.; Shafique, L.; Peng, J.; Ahmed, T.; Wang, R.; Zhang, H.; Wang, Q.; et al. Analysis of transcripts and splice isoforms in red claw crayfish (*Cherax quadricarinatus*) using single-molecule long-read sequencing. *Aquaculture* **2021**, *541*, 736828. [CrossRef]
24. Jiang, Y.-H.; Han, K.-H.; Wang, S.-H.; Chen, Y.; Wang, Y.-L.; Zhang, Z.-P. Identification and expression of transcription factor sox2 in large yellow croaker *Larimichthys crocea*. *Theriogenology* **2018**, *120*, 123–137. [CrossRef]
25. Kurtz, S.; Lucas-Hahn, A.; Schlegelberger, B.; Göhring, G.; Niemann, H.; Mettenleiter, T.C.; Petersen, B. Knockout of the HMG domain of the porcine SRY gene causes sex reversal in gene-edited pigs. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2008743118. [CrossRef]
26. Jeng, S.R.; Wu, G.C.; Yueh, W.S.; Kuo, S.F.; Dufour, S.; Chang, C.F. Gonadal development and expression of sex-specific genes during sex differentiation in the Japanese eel. *Gen. Comp. Endocrinol.* **2018**, *257*, 74–85. [CrossRef]
27. Kanda, H.; Kojima, M.; Miyamoto, N.; Ito, M.; Takamatsu, N.; Yamashita, S.; Shiba, T. Rainbow trout Sox24, a novel member of the Sox family, is a transcriptional regulator during oogenesis. *Gene* **1998**, *211*, 251–257. [CrossRef]
28. Kamachi, Y.; Uchikawa, M.; Kondoh, H. Pairing SOX off: With partners in the regulation of embryonic development. *Trends Genet. TIG* **2000**, *16*, 182–187. [CrossRef]
29. Adolfi, M.C.; Carreira, A.C.; Jesus, L.W.; Bogerd, J.; Funes, R.M.; Schartl, M.; Sogayar, M.C.; Borella, M.I. Molecular cloning and expression analysis of dmrt1 and sox9 during gonad development and male reproductive cycle in the lambari fish, *Astyanax altiparanae*. *Reprod. Biol. Endocrinol. RBE* **2015**, *13*, 2. [CrossRef]
30. Vining, B.; Ming, Z.; Bagheri-Fam, S.; Harley, V. Diverse Regulation but Conserved Function: SOX9 in Vertebrate Sex Determination. *Genes* **2021**, *12*, 486. [CrossRef]
31. Raghuveer, K.; Senthilkumaran, B. Isolation of sox9 duplicates in catfish: Localization, differential expression pattern during gonadal development and recrudescence, and hCG-induced up-regulation of sox9 in testicular slices. *Reproduction* **2010**, *140*, 477–487. [CrossRef] [PubMed]
32. Degani, G. Expression of SOX3 and SOX9 Genes in Gonads of Blue Gourami. *Adv. Biol. Chem.* **2014**, *4*, 2162–2183. [CrossRef]
33. Okuda, Y.; Ogura, E.; Kondoh, H.; Kamachi, Y. B1 SOX coordinate cell specification with patterning and morphogenesis in the early zebrafish embryo. *PLoS Genet.* **2010**, *6*, e1000936. [CrossRef]
34. White, M.D.; Angiolini, J.F.; Alvarez, Y.D.; Kaur, G.; Zhao, Z.W.; Mocskos, E.; Bruno, L.; Bissiere, S.; Levi, V.; Plachta, N. Long-Lived Binding of Sox2 to DNA Predicts Cell Fate in the Four-Cell Mouse Embryo. *Cell* **2016**, *165*, 75–87. [CrossRef] [PubMed]
35. Pan, H.; Schultz, R.M. Sox2 modulates reprogramming of gene expression in two-cell mouse embryos. *Biol. Reprod.* **2011**, *85*, 409–416. [CrossRef]
36. Lee, M.; Choi, K.H.; Oh, J.N.; Kim, S.H.; Lee, D.K.; Choe, G.C.; Jeong, J.; Lee, C.K. SOX2 plays a crucial role in cell proliferation and lineage segregation during porcine pre-implantation embryo development. *Cell Prolif.* **2021**, *54*, e13097. [CrossRef]
37. Hu, Y.; Jin, S.; Fu, H.; Qiao, H.; Zhang, W.; Jiang, S.; Gong, Y.; Xiong, Y.; Wu, Y. Functional analysis of a SoxE gene in the oriental freshwater prawn, Macrobrachium nipponense by molecular cloning, expression pattern analysis, and in situ hybridization (de Haan, 1849). *3 Biotech.* **2019**, *10*, 10. [CrossRef]