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Cloning and Expression Profiling of the Gene *vasa* during First Annual Gonadal Development of Cobia (*Rachycentron canadum*)

Qian Ma [1,2], Jiehua Kuang [1], Gang Chen [1,2,*], Jiandong Zhang [1,2], Jiansheng Huang [1,2], Feifan Mao [1] and Qiling Zhou [1]

[1] College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, China; mfm_0624@163.com (Q.M.); kuangjh3@haid.com.cn (J.K.); yzxzjd@126.com (J.Z.); huangjs@gdou.edu.cn (J.H.); ff954366@163.com (F.M.); 18738627206@163.com (Q.Z.)

[2] Southern Marine Science and Engineering Guangdong Laboratory (Zhanjiang), Zhanjiang 524025, China

* Correspondence: cheng@gdou.edu.cn

Abstract: The *vasa* gene is essential for germ cell development and gametogenesis both in vertebrates and in invertebrates. In the present study, *vasa* (*Rcvasa*) cDNA was cloned from cobia (*Rachycentron canadum*) using the RACE amplification method. We found that the full-length cDNA sequence of *Rcvasa* comprises 2571 bp, containing a 5′-UTR of 145 bp, a 3′-UTR of 341 bp, and an open reading frame (ORF) of 2085 bp, encoding a protein of 694 aa. The deduced amino acid sequence contains 8 conserved motifs of the DEAD-box protein family, 7 RGG repeats, and 10 RG repeats in the N-terminal region. Comparisons of the deduced amino acid sequence with those of other teleosts revealed the highest percentage identity (86.0%) with *Seriola quinqueradiata*. By using semiquantitative RT-PCR, *Rcvasa* appeared to be specifically expressed in the testis and ovary, among 13 tissues analyzed. In addition, annual changes in *Rcvasa* expression levels were examined in the gonads by quantitative real-time PCR (qRT-PCR). The expression of *Rcvasa* in the testis first increased significantly at 120 dph (stage II–III), then stabilized as the testis developed from 185 dph (stage III) to 360 dph (stage V). During the development of the ovary (stage I to II), the expression of *Rcvasa* first increased and reached the highest level at 210 dph (stage II), then decreased. Furthermore, the results of chromogenic in situ hybridization (CISH) revealed that *Rcvasa* mRNA was mainly expressed in germ cells and barely detected in somatic cells. In the testis, *Rcvasa* mRNA signal was concentrated in the periphery of spermatogonia, primary spermatocytes, and secondary spermatocytes and was significantly weaker in spermatids and spermatozoa. In the ovary, *Rcvasa* mRNA signal was uniformly distributed in the perinuclear cytoplasm and was intense in early primary oocytes (stage I and II). These findings could provide a reference for understanding the regulatory mechanisms of *vasa* expression during the development of germ cells in cobia.

Keywords: Rachycentridae; *Ddx4*; molecular characterization; gametogenesis; qRT-PCR; mRNA distribution

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1. Introduction

Germ cell development is the basis of vertebrate reproduction and plays a vital role in transmitting species-specific genomic information between generations. In teleosts, germ cells originate from primordial germ cells (PGCs) which were segregated from somatic cells in the early stage of embryogenesis; then, PGCs migrate into the primary gonad and become gonadal germ stem cells (oogonia in the ovary and spermatogonia in the testis) [1]. During gonad maturation, oogonia and spermatogonia undergo meiosis and then transform into ovum and sperm, respectively. The process from formation of PGCs to maturity is regulated by many factors, including genes, hormones, and environment [2]. To date, the mechanism through which germ cells produce either ovum or sperm has been

unclear. Recently, germ cell markers including *vasa* have been widely used in gonadal development studies [3–7], as they could be effectively used to trace the development of germ cells. These studies would facilitate the identification of the mechanisms underlying the specification and development of germ cells.

The *vasa* gene, also called *Ddx4* (DEAD box polypeptide 4), is an ATP-dependent RNA helicase belonging to the DEAD (Asp–Glu–Ala–Asp)-box protein family [8,9]. It also plays an indirect role in the metabolism of RNA [10] and in the regulation of the expression of transcription factors [11]. This gene was initially identified as a maternal-effect gene in *Drosophila*, and mutations in it could hamper the development of germ cells [12]. In the past few decades, several germ cell-specific molecular markers have been discovered and used for identifying the germline in fish [13]. Among these genes, *vasa* is one of the most documented germ cell markers in teleosts [14–19]. Extensive progress has been made in understanding the function of *vasa* in PGC determination; however, little information is available about the function of *vasa* in gonadal tissues. As reported, the *vasa* gene is also expressed in mature gonads, and significant interspecies differences in the expression patterns of *vasa* during gametogenesis were found [20].

Cobia (*Rachycentron canadum*; Rachycentridae, Perciformes) is a migratory pelagic fish widely distributed across tropical and subtropical waters, except in the eastern Pacific [21]. Interest in this species for cage and other intensive aquaculture systems has raised because of its rapid growth, strong disease resistance, and excellent meat quality [22]. To date, the production of cobia in southern China (amounting up to an average of about 40,000 tons per year, according to the China Fishery Statistical Yearbook) has kept increasing and is ranked high among all the fish species maricultured in China. The recent growth of cobia culture in southeast Asia [23] has raised needs for breeding technology development. In order to facilitate the large-scale breeding of cobia and improve the source of germplasm, further research on its gonadal development and gametogenesis is necessary.

In this study, the full-length *vasa* cDNA of cobia (*Rcvasa*) was cloned and identified. We further investigated *Rcvasa* expression patterns by qRT-PCR and detected the localization of *Rcvasa* mRNA by CISH during the first annual gonadal development. These results can help understand the role of *vasa* in spermatogenesis and oogenesis and will ultimately provide a basis for clarifying the mechanisms underlying the migration and differentiation of PGCs in cobia.

2. Materials and Methods

2.1. Biological Samples

From June 2019 to April 2020, cobia individuals at 90 dph (days post hatching), 120 dph, 150 dph, 185 dph, 210 dph, and 360 dph (more than three female and three male fish in each period) were obtained from an indoor aquaculture farm in the town of Mata (Maoming, Guangdong, China). A total of 18 male (weight 230.0–4225.0 g, length 29.2–64.5 cm) and 23 female (weight 215.0–5050.0 g, length 29.8–68.5 cm) cobia were collected. All the fish were reared in metal circular tanks with a diameter of 9 m in 2.5 m-deep water. During the experimental period, water temperature, salinity, and pH were 25.0–30.0 °C, 27.0–30.5, and 7.6–8.0, respectively.

The two-lobed gonads of the same fish were stored separately: one lobe, used for chromogenic in situ hybridization, was fixed in 4% paraformaldehyde (PFA) for 24 h, then stored in 70% ethanol with diethyl pyrocarbonate (DEPC) until histological processing; the other was first rinsed with 0.1% DEPC-treated water, then fixed in RNAlater overnight at 4 °C and stored at −80 °C. As tissue analysis of 150 dph juvenile cobia, 13 tissues (ovary, testis, heart, brain, muscle, liver, gill, intestine, stomach, spleen, kidney, skin, and eye) were extracted and fixed immediately in RNAlater overnight at 4 °C, then stored at −80 °C until used.

2.2. Total RNA Extraction, Cloning, and Phylogenetic Analysis of the vasa Gene

Total RNA from the gonads at different stages during development (90–360 dph) and various tissues of 150 dph juvenile cobia were isolated with the Trizol Reagent (Invitrogen). The concentration and quality of total RNA was determined by a SimpliNano microvolume UV–Vis Spectrophotometer (Biochrom), and its integrity was measured by electrophoresis on a 1.5% agarose gel. First-strand cDNA and 5′/3′ RACE-ready cDNA were synthesized using the EasyScript One-step cDNA Synthesis Kit (TransGen) and SMARTer® RACE 5′/3′ Kit (Clontech), respectively, according to the manufacturer's instructions.

The CDS sequence of the *vasa* gene was extracted from the genome-wide database of cobia, which was obtained previously by our research team (it has not been uploaded to the NCBI database). For amplification of *vasa* cDNA fragments, two pairs of specific primers (vasaF/R, Table 1) were designed from the 5′- and 3′- ends of the CDS sequence. The PCR cycling conditions were as follows: predenaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 64 °C for 30 s, and extension at 72 °C for 3 min; a final extension was performed at 72 °C for 10 min. The PCR products were separated on a 1.5% agarose gel, purified using the Gel Extraction Kit (TransGen, Beijing, China), cloned into the pMD-18T vector (Takara), and propagated in *E. coli* DH-5α (Takara, Dalian, China). The positive clones were sequenced by the sequencing service of Sangon Biotech Co., Ltd. (Shanghai, China). To obtain the upstream and downstream sequences, two forward primers (Rcvasa3′-F1/F2) and two reverse primers (Rcvasa5′-R1/R2) were designed according to fragments of *Rcvasa* (Table 1). Subsequently, nested PCR was performed using the SMARTer® RACE 5′/3′ Kit (Clontech, Mountain View, CA, USA). The products from the first PCR (1 μL each) were used as templates in the following PCR reactions. The PCR conditions for 5′/3′ RACE were as follows: predenaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for 30 s (Rcvasa3′-F1/F2, Rcvasa5′-R1) or at 63 °C for 30 s (Rcvasa5′-R2), and extension at 72 °C for 1 min 30 s; final extension at 72 °C for 10 min.

Table 1. Primer sequences used in this study.

Primer	Sequence (5′–3′)	Usage
Rcvasa-F	ATGAAAAGAGAACCAGGCGCGA	CDS sequence cloning
Rcvasa-R	CTACTCCCATTCTTCATCATCAGCTG	
Rcvasa3′-F1	TGACCTCCCCAACAACATAGACG	3′ RACE
Rcvasa3′-F2	TGGGAGGGCGGTGTCTTTC	
Rcvasa5′-R1	GCCAGCAGAAATGATGGGGAT	5′ RACE
Rcvasa5′-R2	CTCCCTGATCTCCACCTTGTCTG	
Long-UPM	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT	RACE universal primer
Short-UPM	CTAATACGACTCACTATAGGGC	
Rcvasa-F1	GGTTGGCAGGAGCAGTAACT	PCR amplification
Rcvasa-R1	TGTGGTTGTGATCTCCGGTG	
β-actin-F	AGGGAAATTGTGCGTGAC	internal control gene
β-actin-R	AGGCAGCTCGTAGCTCTT	
Rcvasa-Pro	CAGGACGTTACACCCCCGGCATATTTCTC	probe of CISH

Homology comparison and identity analysis of the deduced amino acid sequence of the obtained cDNA were carried out using the NCBI BLAST server. Alignment of the deduced amino acid sequences was performed using the clustalX1.83, and the output of the alignment results through GenDoc. Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method with MEGA 5.0. Bootstrap values were calculated with 1000 replications to estimate the robustness of internal branches.

2.3. Semiquantitative RT-PCR

Total RNA extraction and cDNA synthesis were performed using 13 tissues (ovary, testis, heart, brain, muscle, liver, gill, intestine, stomach, spleen, kidney, skin, and eye). The tissue distribution pattern of *Rcvasa* was examined by semiquantitative RT-PCR. *Rcvasa*

transcripts were amplified by using a pair of specific primers (Rcvasa-F1/R1, Table 1) spanning a 210 bp cDNA fragment. As an internal control, the *β-actin* cDNA was amplified using the primers β-actin-F/R (Table 1). The PCR cycling conditions were as follows: predenaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 20 s; final extension at 72 °C for 10 min. The PCR products were analyzed by 1.5% agarose gel electrophoresis, and the gel was visualized on a Tanon 4100 GEL imaging system.

2.4. Quantitative Real-Time PCR (qRT-PCR)

The expression patterns of *Rcvasa* transcripts during gonadal development (90–360 dph) were determined by qRT-PCR. The total RNA of a gonad (2 μg) was reverse-transcribed into cDNA using the EasyScript One-step cDNA Synthesis Kit (TransGen). The sequences of the specific primers for *Rcvasa* (Rcvasa-F1/R1) and the internal control *β-actin* (β-actin-F/R) are listed in Table 1. The qRT-PCR assays were performed with a SYBR® Premix Ex TaqTM kit (Takara) on an ABI 7500 Real-Time PCR Detection System (ThermoFisher Scientific Inc., Waltham, MA, USA), according to the manufacturer's protocol. The *vasa* expression level was normalized against *β-actin* expression level to generate a ΔCt value, and the relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method described previously [24]. For each data point, triplicate reactions were carried, out and the experiment was repeated three times.

To identify statistically significant differences in *vasa* relative expression by qRT-PCR, one-way ANOVA was employed, followed by a Duncan's multiple range test, using SSPS 19.0 software (IBM). In each case, differences were accepted as statistically significant at $p < 0.05$.

2.5. Chromogenic In Situ Hybridization (CISH)

In situ hybridization by chemical staining with BCIP/NBT substrates on histological sections of gonads from cobia at 120 dph, 210 dph, and 360 dph was performed in accordance with some previously reported procedures [3,17]. The classification of gonad differentiation was based on histological observations of cobia gonadal development [25], and the staging criteria were in accordance with Liu (1993) [26]. A *Rcvasa* oligonucleotide probe (Table 1) labeled with digoxigenin (DIG) was synthesized by Servicebio Biological Technology Co., Ltd. (Wuhan, Hubei, China). Briefly, the testes and ovaries were dissected, fixed in 4% PFA, and then embedded into paraffin, and 6 μm sections were cut. After deparaffinization, hydration, and digestion with proteinase K (20 μg/mL), the samples were hybridized with the probe at 37 °C overnight. The signals were detected by phosphatase-conjugated anti-DIG-AP and NBT/BICP as the chromogenic substrates.

3. Results

3.1. Cloning of Rcvasa cDNA and Phylogenetic Analysis

The full-length cDNA sequence of cobia *vasa* (*Rcvasa*) was cloned by RACE amplification (GenBank accession No. MW436698). The *Rcvasa* cDNA appeared to consist of 2571 bp, comprising an ORF (open reading frame) of 2085 bp, a 5′-UTR (untranslated region) of 145 bp, and a 3′-UTR of 341 bp, and to encode a 694-amino acid protein.

The predicted RcVasa protein contains 8 conserved motifs of the DEAD-box protein family, including motif I (AQTGSGKT), motif Ia (PTRELI), motif Ib (GG), motif II (TPGRL), motif III (DEAD), motif IV (SAT), motif V (RGLD), and motif VI (HRIGRTGR) (Figure 1), and the N-terminal region contains 10 arginine–glycine (RG) repeats and 7 arginine–glycine–glycine (RGG) repeats. The RcVasa protein also has two highly conserved domains, the DEXDc domain (275–486 aa) and the HELICc domain (522–603 aa). Multiple sequence alignment revealed that the deduced amino acid sequence of Rcvasa has 62.6–86.0% identity with those of Rcvasa proteins in other teleosts. The highest identity was found between cobia Rcvasa amino acid sequence and that of the corresponding protein in *Seriola quinqueradiata* (86.0%), followed by *Thunnus thynnus* (84.4%), *Euthynnus affinis* (82.6%), and

Larimichthys crocea (79.5%) proteins, while the lowest identity was found with Rcvasa amino acid sequence in *Danio rerio* (62.6%) as shown in Figure 1.

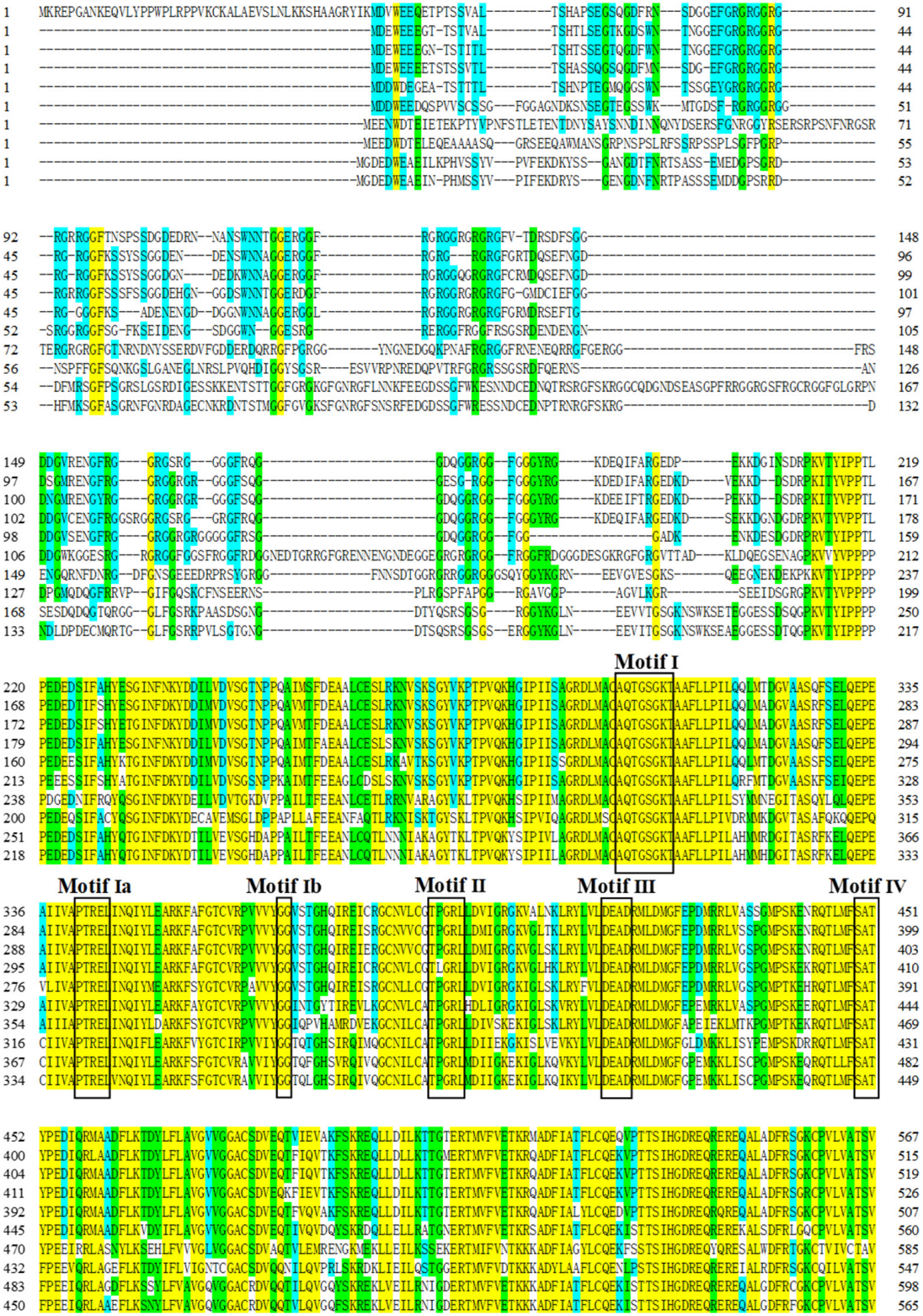

Figure 1. *Cont.*

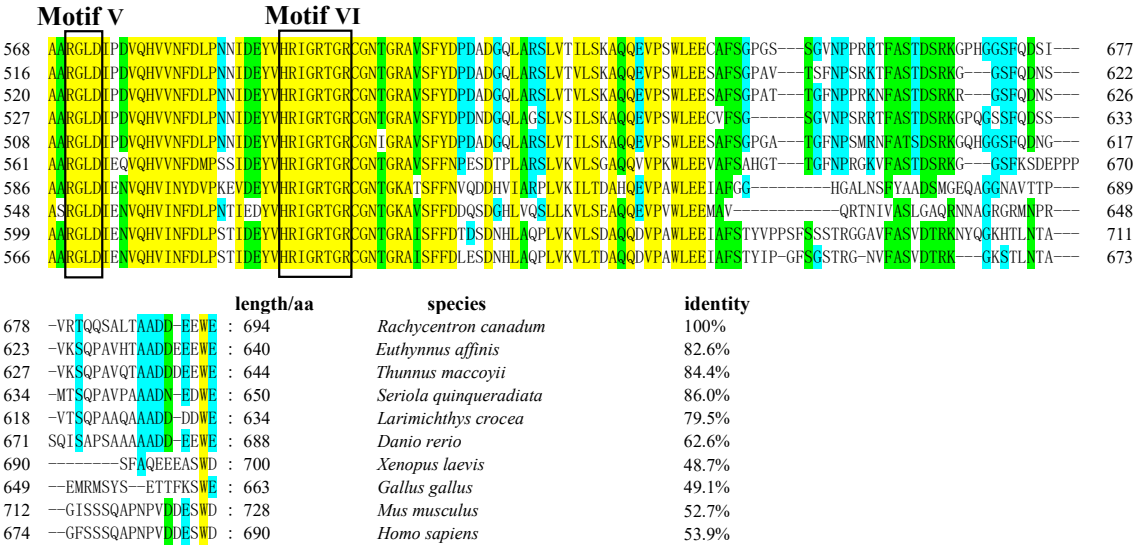

Figure 1. Multiple alignment of *Rcvasa* deduced amino acid sequences. The framed regions indicate the eight conserved functional motifs.

Based on the genetic distances calculated with the Poisson correction model, a phylogenetic tree was constructed by the Neighbor-Joining method to investigate the phylogenetic relationship among different species (Figure 2). The results showed that all the teleost species fall into a lineage, the higher vertebrates, including amphibians, birds, and mammals, fall into another lineage, and *Drosophila melanogaster* forms a single cluster. The teleost cluster comprises 14 species, 7 of which are from the Perciformes order, 3 from the Scombriformes order, and 1 each from the Pleuronectiformes, Scorpaeniformes, Cyprinodontiformes, and Cypriformes orders. Moreover, RcVasa clusters together with *Seriola quinqueradiata* Vasa.

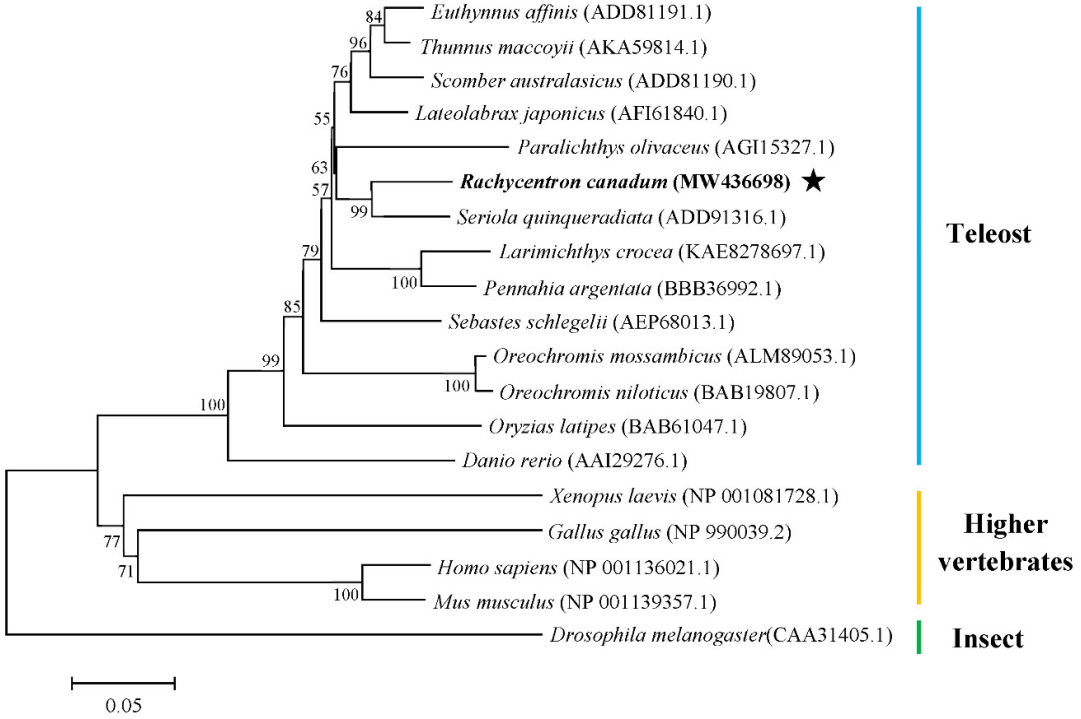

Figure 2. Phylogenetic tree of vasa amino acid sequences based on the Neighbor-Joining (NJ) method. The tree is based on a 1000 bootstrap procedure; the scale bar (0.05 in terms of genetic distance) is indicated below the tree; the asterisk indicates the target species in this study.

3.2. Tissue Distribution Patterns of Rcvasa mRNA

The tissue distribution patterns of *Rcvasa* mRNA in 13 tissues was analyzed by semi-quantitative RT-PCR. The results showed that *Rcvasa* was exclusively expressed in the ovary and testis, whereas almost no expression was detected in other somatic tissues (Figure 3).

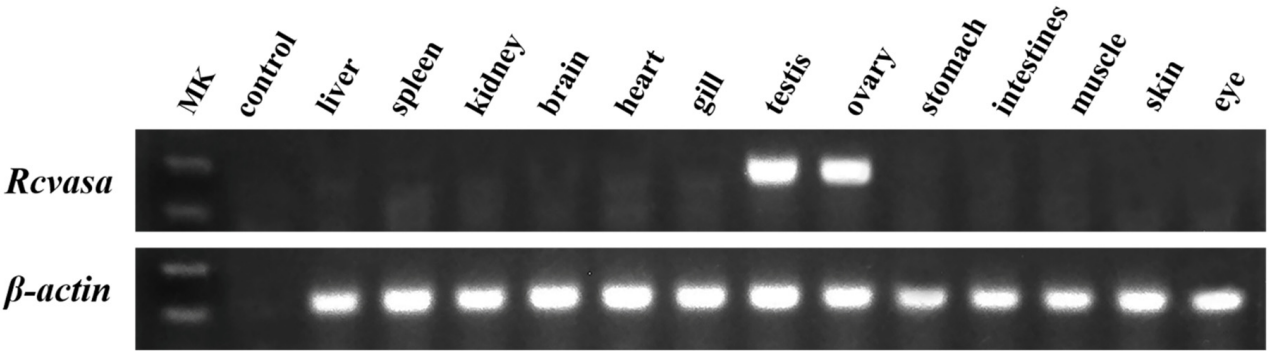

Figure 3. *Rcvasa* mRNA expression in various tissues of cobia. cDNA from various tissues (liver, spleen, kidney, brain, heart, gill, testis, ovary, stomach, intestines, muscle, skin, and eye) was used. *β-actin* was used as an internal control. MK: DNA marker; control: negative control without cDNA template.

3.3. Expression Patterns of Rcvasa mRNA at Different Gonadal Development Stages

Based on histological observation of gonadal differentiation, the testes collected from 90-to-360 dph cobia could be divided into four stages (stage II, III, IV, V). The testes were in the spermatocyte growth stage (stage II) at 90 dph and in stage II–III at 120 dph. Testes at 150 dph and 185 dph were in the spermatocyte mature stage (stage III), while testes at 210 dph and 360 dph were in the spermatid metamorphosis stage (stage IV) and sperm mature stage (stage V), respectively. The first annual ovarian development of cobia was divided into three stages (Stage I, II, III). The ovaries were in the oogonium proliferation-stage (stage I) at 90 dph and developed to stage I–II at 120 dph. Ovaries at 150 dph, 185 dph and 210 dph were in the oocyte primary growth stage (stage II) and entered the oocyte cortical alveolus stage (stage III) at 360 dph.

The expression of *Rcvasa* mRNA was detected in the gonads of both male and female cobia throughout the first annual development. In the testis, the expression level of *Rcvasa* increased significantly after 120 dph; the expression levels at 185 dph (stage III), 210 dph (Stage IV), and 360 dph (Stage V) showed no significant differences. The expression level at 90 dph (Stage II) was the lowest; it then increased significantly, reaching the maximum at 210 dph (Stage IV), which was 2.78 times that at 90 dph (Figure 4).

During the first annual ovarian development, the expression levels of *Rcvasa* first increased and then decreased. The lowest expression level was detected at 90 dph (stage I). The relative expression reached the maximum at 210 dph (stage II), and its value was about 4.97 times that at 90 dph, while the relative expression at 360 dph (stage III) was significantly lower (Figure 4).

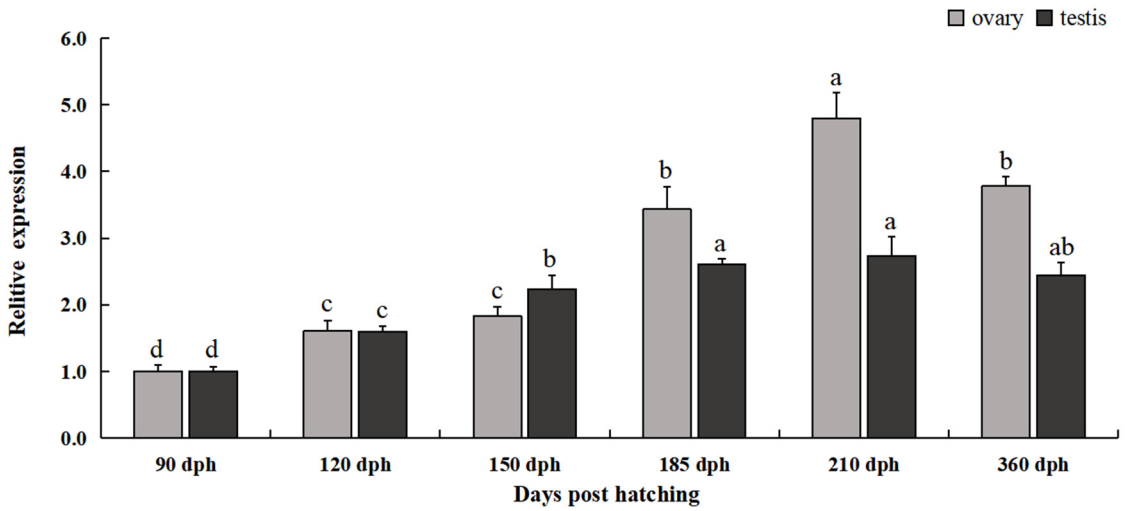

Figure 4. *Rcvasa* mRNA expression patterns during the first annual gonadal development of cobia. The results are presented as the mean ± SD (n = 3), and the values with different letters (a–d) appeared significantly different in pairwise comparisons ($p < 0.05$).

3.4. Localization of Rcvasa mRNA in Germ Cells during Gametogenesis

The localization of *Rcvasa* mRNA in the gonads at different developmental stages was investigated by CISH. *Rcvasa* mRNA signals were restricted to germ cells and were not detected in somatic cells. In the testis of 120 dph cobia, *Rcvasa* mRNA was predominantly detected in the periphery of spermatogonia, primary spermatocytes, and secondary spermatocytes. The expression level of *Rcvasa* was strong in spermatogonia and scarcely detected in spermatids (Figure 5A1). Similar expression patterns were observed in the testis at 210 dph, when *Rcvasa* mRNA signals were also mainly distributed in the periphery of spermatogonia and in spermatocytes (Figure 5B1). In the testis at 360 dph, *Rcvasa* mRNA signal was detected in all germ cells throughout spermatogenesis and were concentrated in spermatocytes; it appeared significantly weaker in spermatids and spermatozoa (Figure 5C1).

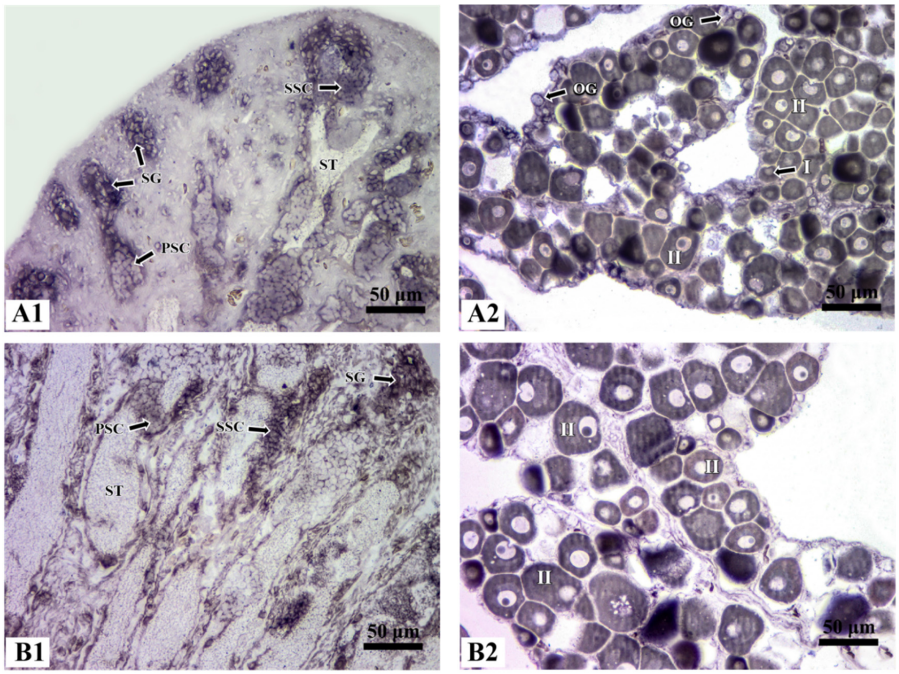

Figure 5. *Cont.*

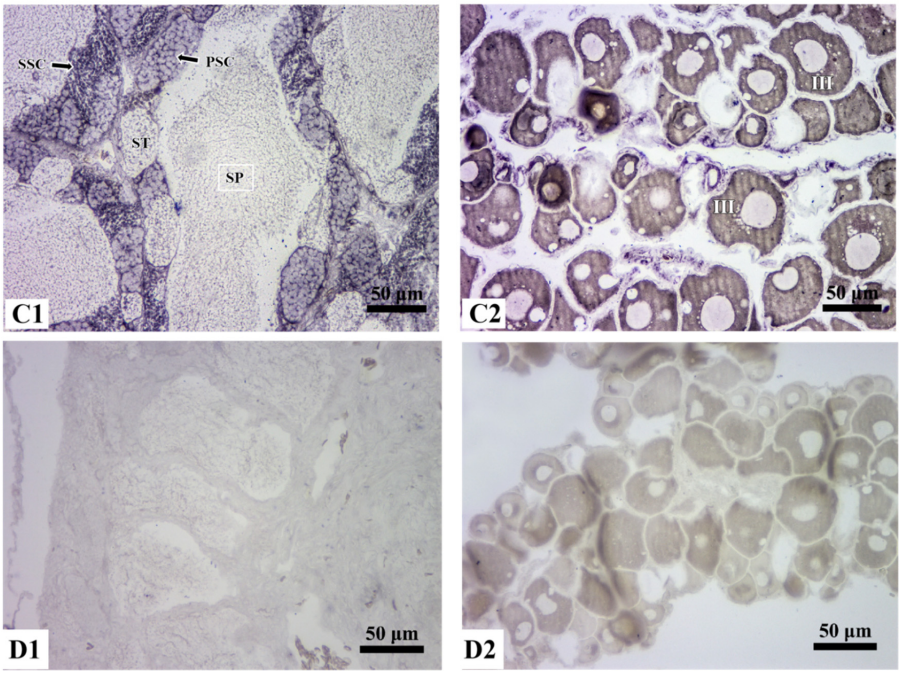

Figure 5. Distribution of *Rcvasa* mRNA at different developmental stages in cobia gonads, analyzed by CISH. (**A1**,**B1**,**C1**), sections of testes at 120 dph, 210 dph, and 360 dph were hybridized with an *Rcvasa* oligonucleotides probe; (**A2**,**B2**,**C2**), sections of ovaries at 120 dph, 210 dph, and 360 dph were hybridized with an *Rcvasa* oligonucleotides probe. (**D1**,**D2**), negative control, sections of testes and ovaries at 210 dph, hybridized with a sense probe. SG: spermatogonia; PSC: primary spermatocyte; SSC: secondary spermatocyte; ST: spermatid; SP: spermatozoa; OG: oogonium (chromatin nucleolar oocyte); I: oocyte at stage I (perinucleolar oocyte); II: oocyte at stage II (previtellogenic oocyte); III: oocyte at stage III (vitellogenic oocyte).

During ovarian development, *Rcvasa* mRNA was specifically expressed in germ cells and evenly distributed in the cytoplasm and nucleoli. In the ovary of 120 dph cobia, *Rcvasa* mRNA was localized in oogonium (chromatin nucleolar stage), stage I and II oocytes (perinucleolar and previtellogenic stage) (Figure 5A2). In the ovary at 210 dph, *Rcvasa* mRNA signal was concentrated in the cytoplasm and nucleoli of stage II oocytes (previtellogenic stage); with the accumulation of oocyte protoplasm, the signals gradually became stronger (Figure 5B2). In the ovary at 360 dph, *Rcvasa* mRNA signal detected in the cytoplasm of stage III oocytes (vitellogenic stage) was significantly weakened (Figure 5C2).

4. Discussion

The present study reported the isolation and characterization of cobia *vasa* homologue. The deduced amino acid sequence of the protein encoded by *Rcvasa* contains eight conserved motifs of the DEAD protein family [27]. RcVasa showed an appreciable identity to the Vasa protein from other marine teleosts, as well as to the Vasa homologue of *Seriola quinqueradiata* (86.0%). In addition, a glycine-rich region in the N-terminal region of RcVasa containing 10 arginine–glycine (RG) repeats and 7 arginine–glycine–glycine (RGG) repeats was observed. This glycine-rich region is regarded as a characteristic of single-stranded nucleic acid-binding proteins, such as RNA helicase, which may regulate the activity of different *vasa* transcripts [28]. Phylogenetic analysis revealed that RcVasa closely aligned to its teleost counterparts. These findings illustrate that the *vasa* gene has been highly conserved in the evolution of teleost, and the conserved motifs of the Vasa protein potentially play an important role in sustaining protein structure and function [29].

The tissue distribution patterns of *vasa* mRNA in 150 dph juvenile cobia showed that *Rcvasa* was specifically and abundantly expressed in testis and ovary. Previous reports

also showed that *Rcvasa* is a gonad-specific gene and may be involved in the regulation of gonadal development [19,20,30,31].

The expression of *Rcvasa* increased significantly when the testis developed from stage I to III, but no significant difference was detected after the late stage III. It has been reported that in the early stage of the testicular development of fish, spermatogonia undergo mitosis and gradually transforms into primary spermatocytes; then, primary spermatocytes divide into secondary spermatocytes [32]. As a result, the expression pattern of *Rcvasa* is positively correlated with the number of spermatocytes, suggesting that *Rcvasa* might play an important role in the regulation of spermatogenesis. Previous studies have reported that the expression levels of catfish (*Clarias gariepinus*) *vasa* gradually increased during testis development from stage I to VI and decreased significantly after stage V [33]. Mu et al. found that the expression of Korean rockfish *vasa* maintained a high level in testicular stages I to III, but *vasa* expression decreased significantly when spermatids started to mature [31]. These results indicate that there are significant interspecies differences in the expression patterns of *vasa* during the testicular development of teleost. The expression levels of *Rcvasa* in the early stage of ovarian development tended to increase gradually. As the ovaries developed to stage II, the oocytes became larger by accumulating protoplasm consisting of carbohydrates, proteins, and nucleic acids [32], and the expression of *Rcvasa* significantly increased. The oocytes began to produce egg yolk in ovarian stage III, and the expression of *Rcvasa* decreased significantly. These results suggest that *Rcvasa* might participate in the accumulation of oocyte protoplasm and in the formation of ovum yolk.

Furthermore, *Rcvasa* mRNA was exclusively expressed in germ cells and not in somatic cells. During testicular development, *Rcvasa* mRNA signal was mainly concentrated in the periphery of spermatogonia, primary spermatocytes, and secondary spermatocytes, and it was found to be weak in spermatids and spermatozoa. Similar results were reported in other fish [34,35]. The localization of *Rcvasa* mRNA during spermatogenesis suggested that this protein might play a major role in the transformation of spermatogonia into primary spermatocytes, but not in the development and maturation of spermatids. As reported, bluefin tuna *vasa* mRNA was only expressed in spermatogonia and not in other testicular cells [19]. This indicated that *vasa* might have a species-specific role in regulating the transformation of spermatogenic cells. The expression pattern of *Rcvasa* during oogenesis was similar to those observed in several teleost species [3,18,36] and revealed that *Rcvasa* mRNA was abundant in early primary oocytes (stage I and II, perinucleolar and previtellogenic stage) and deficient in yolk vesicle (cortical alveolus) formation-stage oocytes (stage III, vitellogenic stage). These findings indicate that *vasa* might regulate oocyte development during the early stage of oogenesis. As for gilthead sea bream (*Sparus aurata*), the *vasa* mRNA level was low in early primary oocytes and high when the oocytes developed to the mature gametes [37]. According to a report in tilapia, vitellogenesis was usually accompanied by the accumulation of *vasa* maternal factors, showing that *vasa* might be involved in regulating the maturation of oocytes [15]. However, a weak *Rcvasa* mRNA signal was detected in stage III oocytes. The reason for this might be related to the fact that the total protein content of these maturating oocytes increased, and as a result, the relative concentration of *Rcvasa* mRNA was diluted. Similar to the expression patterns of vasa during testicular development, a certain interspecies difference was also found in the regulation of *vasa* expression during oogenesis.

5. Conclusions

The *vasa* gene from cobia was cloned and characterized, and further investigation of the expression level and localization of *Rcvasa* mRNA during the first annual gonadal development was performed. The high and exclusive expression of *Rcvasa* mRNA in germ cells indicates the important role of *vasa* in spermatogenesis and oogenesis in this important aquaculture species. The gradually increasing mRNA level along with the growth and development of cobia also provides a basis for clarifying the mechanisms underlying the migration and differentiation of PGCs in teleosts.

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