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# Genome-Wide Identification and Expression Profiling of the *COMMD* Gene Family in Four Bivalve Molluscs

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The *COMMD* (copper metabolism gene MuRR1 domain) gene family, highly conserved among multicellular eukaryotic organisms, plays important roles in a variety of biological processes, ranging from copper homeostasis, ionic transport, protein trafficking, NF-κB-mediated transcription, and cell proliferation. However, systematic identification, spatiotemporal expression, and stress-responsive patterns of *COMMD* genes remain obscure in molluscs. Here, we analyzed the characteristics of the *COMMD* gene family in four bivalve molluscs based on both genome and extensive transcriptomic resources. Firstly, we investigated the genomic signatures, functional domains, and phylogenetic relationships, and ten single-copy members were identified in Yesso scallop (*Patinopecten yessoensis*), Zhikong scallop (*Chlamys farreri*), Pacific oyster (*Crassostrea gigas*), and dwarf surf clam (*Mulinia lateralis*), respectively. Strong purifying selection was revealed for *COMMD4*. Higher expressions of most *COMMD*s were observed in the hepatopancreas, besides which a different tissue preference of *COMMD*s' expression was found among four bivalves. Moreover, in the dwarf surf clam, the responses of *COMMD* members under stresses were found more sensitive in the hepatopancreas than in the gill, and *MlCOMMD9* and *MlCOMMD4* might be the good candidate stress indicator genes respectively for copper ion stress and *V. Anguillarum* infection. Our study would contribute to a better understanding for the evolution of the *COMMD* gene family and provide valuable information for their innate immune roles in bivalve molluscs.

**Keywords: bivalve, *COMMD*, expression profiling, phylogenetic analysis, innate immune**

## INTRODUCTION

The *COMMD* (copper metabolism gene MuRR1 domain) family includes ten evolutionarily conserved proteins, namely, COMMD1–10, in the extreme carboxyl terminus of which they share a unique motif known as the COMM domain (Burstein et al., 2005). The COMM domain, with 70–85 amino acids in length and being rich in tryptophan, proline, and leucine, not only defines the gene family but also provides a critical interface for protein–protein interactions with

each other (Maine et al., 2007). The hydrophobic residues located in the COMM domain could form two conserved nuclear export signals (NES1 and NES2) (Muller et al., 2009). Except COMMD6, other COMMD members possess an amino terminal region, which is divergent among the subfamilies but is highly conserved within the ortholog proteins (Burstein et al., 2005). *COMMD1* is the first identified member, which was initially termed as *Murr1* due to its proximity to the *U2af1-rs1* locus in mice, and other *COMMD* members were identified through homologous screening (Nabetani et al., 1997; van De Sluis et al., 2002). A total of ten subfamilies were found in the vast majority of vertebrates (Burstein et al., 2005), while in invertebrates, including insects, worms, and molds, only several *COMMD* members were reported, and none of the *COMMD*s were found in unicellular eukaryotic organisms or bacteria (Nabetani et al., 1997). The wide existence as well as the highly conservative characteristic of *COMMD* homologues imply their critical roles during the metazoan evolution (Riera-Romo, 2018).

As the best-characterized member, *COMMD1* may represent a prototype of the family (Burstein et al., 2005). *COMMD1* was reported to be able to participate in two distinct activities, control of copper metabolism and regulation of the transcription factor NF-κB (Riera-Romo, 2018). Researchers found that mutations of *COMMD1* are responsible for copper toxicosis in Bedlington terrier dogs, resulting in excessive copper accumulation in the liver (Tao et al., 2003). In human, biochemical findings had indicated a direct role for *COMMD1* in biliary copper transport, and *COMMD1* defect could impair the copper excretion (Tao et al., 2003; Riera-Romo, 2018). Besides *COMMD1*, various functions were found for other *COMMD* genes in vertebrates. COMMD4, as one of the protein kinase A targets, was able to interact with myomegalin and inhibit NF-κB activity (Uys et al., 2011). The COMMD3 and COMMD8 complex could selectively recruit GRK6, which induced GRK6-mediated phosphorylation of the receptor and activated the β-arrestin-mediated signaling (Nakai et al., 2019). *COMMD3* and *COMMD9*, which are endogenous regulators, regulate $Na^+$ transport through altering ENaC cell surface expression (Liu et al., 2013). *COMMD5* affects cell proliferation (Solban et al., 2000), and *COMMD6, 7* participates in the invasion and migration regulation of a variety of cancer (You et al., 2017; Yang et al., 2019). Research has reported that *COMMD10* is related to phagosomes in murine macrophages (Dill et al., 2015), and in myeloid cells, deficiency in *COMMD10* can cause increased NF-κB activation and then aggravate lipopolysaccharide systemic sepsis (Naugler and Karin, 2008; Mouhadeb et al., 2018). In comparison, only the functions of several *COMMD* genes were reported in invertebrates. For example, *COMMD4* is found ubiquitously expressed in amphioxus, with the highest level in gonad, and lipopolysaccharide injection could induce its expression (Jin et al., 2012). Wang et al. have cloned *COMMD1* in *Crassostrea hongkongensis*, and the transcription level of *COMMD1* was increased significantly in the gill and hemolymph after salinity stimulation (Wang et al., 2017a).

As benthic filter feeders, bivalve molluscs are well adapted to highly dynamic oceans and freshwater environments since the early Cambrian. Along with intensified human activities in recent decades, the bivalve habitats are subject to various biotic/abiotic stressors, and pathogenic microbes and heavy metals are two of the main stressors. The *COMMD* genes play critical roles in many vital functions, and exploration of whether these genes participate in heavy-metal or bacterial resistance may help gain a better understanding for the outstanding adaptability of bivalve molluscs. In the present study, a systematic identification and characterization of *COMMD* genes in four bivalves were conducted, namely, Yesso scallop, Zhikong scallop, Pacific oyster, and dwarf surf clam. Detailed genic structure comparison and spatiotemporal expression analysis provided insights into the potential function of *COMMD* genes in bivalve molluscs. Further, transcription patterns of different *COMMD* members under copper ion stress and after *Vibrio Anguillarum* infection were investigated in the gill and hepatopancreas of *M. lateralis*. This is the first comprehensive research of the *COMMD* family genes in bivalves, which notably provided helpful information regarding the classification, evolution, and function of these genes. These findings may assist a better understanding of the bivalves' adaption to adverse heavy-metal pollutions and bacterial challenge.

# MATERIAL AND METHODS

## Genome-Wide Identification of *COMMD* Genes

To identify *COMMD* genes, the whole genomes and transcriptomes of four bivalves (Zhang et al., 2012; Li et al., 2017; Wang et al., 2017b) were searched against the available COMMD protein sequences from representative vertebrates (*Homo sapiens*, *Mus musculus*, *Xenopus laevis*) and Cephalochordata (*Branchiostoma belcheri*). The orthologous COMMD proteins were used as query sequences for whole-genome and transcriptome-based blasts, and the threshold of the E value was 1E-5. Sequence analysis was performed by the HMM searching method (http://www.ebi.ac.uk/Tools/hmmer/search/phmmer) to ensure the integrity of *COMMD*s. BLASTN was used to confirm their genomic structure, and the ORF Finder program (https://www.ncbi.nlm.nih.gov/orffinder/) was used to predict the open reading frame. Further, to ensure the completeness of *COMMD*s, the translated sequences were submitted to the SMART tool (http://smart.embl-heidelberg.de/) and ProtParam tool (https://web.expasy.org/protparam/) to ensure the presence of the conserved COMM_domain and to predict the isoelectric point (*pI*), molecular weight, instability index, and grand average of hydropathicity (GRAVY) values, which were illustrated for all potential bivalve *COMMD*s shown in **Table 1**. Then, the drawing of the protein structure was done by IBS 1.0.3. Finally, we identified and counted *COMMD* family genes of 26 metazoan species, namely, the deuterostomes *Homo sapiens*, *Oryzias latipes*, *Danio rerio*, *Oreochromis niloticus*, *Xenopus laevis*, *Ciona intestinalis*, *Branchiostoma floridae*, and *Strongylocentrotus purpuratus*, the protostomes *Drosophila melanogaster*, *Daphnia pulex*, *Caenorhabditis elegans*, *Tribolium castaneum*, *Lingula anatina*, *Capitella teleta*,

**TABLE 1** | Sequence characteristics of the *COMMD* gene family of four bivalve molluscs.

| Gene | Intron number | Genomic position | Protein length | *COMM* domain position | *PI* | Molecular weight (Da) | GRAVY | Instability index |
|---|---|---|---|---|---|---|---|---|
| *PyCOMMD1* | 1 | 2137.16:679703-680902 = 1200 | 135 | 61–134 | 5.91 | 15,765.54 | -0.713 | 49.20 |
| *PyCOMMD2* | 4 | 5989.10:450020-460247 = 10228 | 199 | 121–189 | 6.09 | 23,379.78 | -0.374 | 50.83 |
| *PyCOMMD3* | 7 | 10911.5:103350-117222 = 13873 | 196 | 122–194 | 5.01 | 21,617.27 | -0.189 | 34.89 |
| *PyCOMMD4* | 8 | 9739.4:207199-226481 = 19283 | 200 | 128–200 | 6.10 | 22,355.46 | -0.136 | 25.68 |
| *PyCOMMD5* | 6 | 2433.4:97391-108491 = 11101 | 220 | 145–212 | 7.72 | 25,079.12 | -0.080 | 43.06 |
| *PyCOMMD6* | 4 | 11181.36:885386-891755 = 6370 | 80 | 11–80 | 6.53 | 8,894.22 | -0.134 | 24.39 |
| *PyCOMMD7* | 8 | 4359.39:1265516-1276033 = 10518 | 201 | 132–201 | 5.76 | 22,393.30 | -0.228 | 25.26 |
| *PyCOMMD8* | 4 | 9685.21:829055-837430 = 8376 | 185 | 116–185 | 4.94 | 21,048.57 | -0.368 | 52.68 |
| *PyCOMMD9* | 5 | 7491.23:709119-722211 = 13093 | 198 | 120–197 | 5.93 | 22,260.12 | -0.289 | 36.16 |
| *PyCOMMD10* | 7 | 2329.50:1883135-1896660 = 13526 | 198 | 127–198 | 5.72 | 22,886.02 | -0.429 | 43.66 |
| *MlCOMMD1* | 2 | 12840.10:78282-82291 = 4010 | 186 | 113–184 | 5.00 | 21276.63 | -0.658 | 56.19 |
| *MlCOMMD2* | 6 | 45.74:1970118-1985717 = 15600 | 240 | 170–237 | 6.06 | 27,717.89 | -0.175 | 42.79 |
| *MlCOMMD3* | 8 | 12040.29:507160-516075 = 8916 | 198 | 122–193 | 5.05 | 22,323.13 | -0.220 | 38.87 |
| *MlCOMMD4* | 8 | 12703.15:299462-305380 = 5919 | 201 | 128–200 | 5.45 | 22,428.40 | -0.219 | 27.39 |
| *MlCOMMD5* | 6 | 11059.62:859122-866779 = 7658 | 220 | 145–212 | 7.72 | 25,072.87 | -0.159 | 41.06 |
| *MlCOMMD6* | 6 | 10801.218:3510817-3516483 = 5667 | 182 | 113–182 | 5.24 | 20,562.56 | -0.051 | 43.55 |
| *MlCOMMD7* | 6 | 12762.5:96783-102258 = 5476 | 202 | 133–202 | 4.98 | 22,642.41 | -0.249 | 32.27 |
| *MlCOMMD8* | 4 | 12846.13:145184-150718 = 5535 | 183 | 113–183 | 5.57 | 20,484.17 | -0.185 | 39.12 |
| *MlCOMMD9* | 5 | 12715.132:1758244-1793803 = 35560 | 197 | 119–196 | 6.73 | 22,066.33 | -0.127 | 30.39 |
| *MlCOMMD10* | 6 | 12002.12:113626-123104 = 9479 | 197 | 127–197 | 5.00 | 22,767.92 | -0.351 | 46.04 |
| *CfCOMMD1* | 2 | 22817.34787729-790581:=2853 | 187 | 113–186 | 5.34 | 21,825.27 | -0.781 | 50.26 |
| *CfCOMMD2* | 2 | 44835.8:183117-188074 = 4958 | 151 | 73–141 | 6.97 | 17,606.36 | -0.163 | 66.00 |
| *CfCOMMD3* | 7 | 33479.9:142988-153101 = 10114 | 196 | 122–194 | 4.99 | 21,623.27 | -0.171 | 33.32 |
| *CfCOMMD4* | 2 | 723733.1:101-1790 = 1690 | 105 | 27–105 | 9.10 | 9,625.85 | -0.379 | 33.73 |
| *CfCOMMD5* | 6 | 15543.1:12696-26178 = 13483 | 220 | 145–212 | 8.69 | 25,138.19 | -0.095 | 41.58 |
| *CfCOMMD6* | 4 | 55811.12:168214-191931 = 23718 | 80 | 11–80 | 7.87 | 8,896.21 | -0.193 | 19.53 |
| *CfCOMMD7* | 8 | 53827.18:384544-393259 = 8716 | 201 | 132–201 | 5.28 | 22,290.20 | -0.201 | 22.97 |
| *CfCOMMD8* | 35 | 13299:134558-189797 = 55240 | 1339 | 116–180 | 5.62 | 151,426.29 | -0.264 | 46.52 |
| *CfCOMMD9* | 5 | 64623.27:808604-820076 = 11473 | 198 | 120–197 | 5.62 | 22,258.06 | -0.275 | 35.40 |
| *CfCOMMD10* | 9 | 63763.53:945408-1095753 = 150346 | 215 | 127–189 | 8.63 | 24,776.25 | -0.368 | 46.48 |
| *CgCOMMD1* | 2 | NW_011935966.1:87568-88717 = 1150 | 191 | 117–190 | 5.52 | 22,229.83 | -0.741 | 54.41 |
| *CgCOMMD2* | 4 | NW_011936396.1:515939-521925 = 5987 | 199 | 121–189 | 5.90 | 23,336.65 | -0.347 | 49.02 |
| *CgCOMMD3* | 5 | NW_011936388.1:20012-24869 = 4858 | 160 | 122–158 | 4.81 | 17,907.22 | -0.193 | 48.20 |
| *CgCOMMD4* | 8 | NW_011935883.1:64603-67169 = 2567 | 199 | 128–199 | 5.63 | 22,629.06 | -0.255 | 31.13 |
| *CgCOMMD5* | 5 | NW_011934779.1:876616-887615 = 11000 | 190 | 145–189 | 9.04 | 21,364.73 | -0.109 | 46.65 |
| *CgCOMMD6* | 2 | NW_011936252.1:55195-56300 = 1106 | 138 | 69–138 | 6.57 | 15,283.34 | -0.469 | 39.87 |
| *CgCOMMD7* | 7 | NW_011935176.1:337529-362408 = 24880 | 198 | 129–198 | 6.60 | 22,213.42 | -0.013 | 20.75 |
| *CgCOMMD8* | 5 | NW_011936271.1:100615-105861 = 5247 | 184 | 114–183 | 5.14 | 21,193.77 | -0.478 | 39.28 |
| *CgCOMMD9* | 5 | NW_011937700.1:144762-154995 = 10234 | 199 | 121–198 | 6.96 | 22,440.55 | -0.340 | 43.82 |
| *CgCOMMD10* | 6 | NW_011937034.1:445720-452824 = 7105 | 198 | 127–198 | 5.36 | 22,889.05 | -0.418 | 43.62 |

*Helobdella robusta*, *Lottia gigantea*, *Elysia chlorotica*, *Biomphalaria glabrata*, *Octopus bimaculoides*, *Patinopecten yessoensis*, *Chlamys farreri*, *Crassostrea gigas*, and *Mulinia lateralis*, and the non-bilaterians *Nematostella vectensis*, *Stylophora pistillata*, and *Amphimedon queenslandica* (**Supplementary Table 1**).

## Multiple Alignment and Phylogenetic Analysis

MEGA7.0 (Sudhir et al., 2016) was used to construct phylogenetic analysis to determine which *COMMD* subfamily the bivalve *COMMD* genes belong to. The whole amino acid sequences of COMMD proteins from Human (*H. sapiens*), zebrafish (*D. rerio*), medaka fish (*O. latipes*), African clawed frog (*X. laevis*), ciona (*C. intestinalis*) and Stylophora (*S. pistillata*), were downloaded from the Ensemble genome browser database. The whole amino acid sequences of COMMD proteins from Nile tilapia (*O. niloticus*), notoacmea (*L. gigantea*), Biomphalaria (*B. glabrata*), and sea snail (*E. chlorotica*) were downloaded from the Uniport database. The whole amino acid sequences of COMMD proteins from amphioxus (*B. belcheri*) and octopus (*O. bimaculoides*) were downloaded from the NCBI database. Multi-sequence alignment was performed through ClustalW (Larkin et al., 2007) and was edited by GeneDoc software (Nicholas et al., 1997), then the phylogenetic analyses based on the neighbor-joining (NJ) method and maximum likelihood (ML) method with a bootstrap of 1,000 replicates, both including all the amino acids from the COMMDs. 154 amino acids across 16 animals were involved in this analysis. The accession numbers of 154 COMMDs are listed in **Supplementary Table 2**.

## Selective Pressure Analysis

*COMMD* gene sequences were aligned based on codons, using Muscle (codons) implemented in MEGA7.0 (Sudhir et al., 2016). MEGA7.0 was used to build the alignment result into a tree file,

and a Newick format file was formed. To explore selective pressure between *COMMD* gene sequences, after removing the gap, a strict statistical analysis was performed using the software EasyCodeML1.2 (Gao et al., 2019). Based on the Preset Site Model, the ratios of non-synonymous (dN) and synonymous (dS) substitutions for 40 *COMMD* genes among four bivalve were calculated. The LRT was used to test whether the selected model is significant ($P < 0.05$). Two likelihood ratio tests were performed to detect positively selected sites—M1a (neutral) vs. M2a (positive selection), M7 (β) vs. M8 (β and ω), and M0 (one-ratio) vs. M3 (discrete)—and the site-specific model was used for comparison. If the tests produced a significant result, then the empirical Bayes method was used to identify individual positively selected codon sites (Yang et al., 2005).

## Expression Analysis

The TPM (reads per kilobase million) values were summarized from the published RNA-seq datasets of Yesso scallop (Wang et al., 2017b), Zhikong scallop (Li et al., 2017), and Pacific oyster (Zhang et al., 2012) and from our unpublished data for dwarf surf clam. During development, eleven embryo/larval developmental stages were chosen to perform expression analysis, including zygotes; multi-cells; blastula; gastrula; trochophore; D-shaped larvae; early-, mid-, and late-term umbo larvae; metamorphosis larvae; and juvenile. For adults, six tissues were chosen to perform expression analysis, namely, muscle, hepatopancreas, mantle, gill, male gonad, and female gonad. The expressional heatmaps were displayed by the heatmap package in R environment.

## Copper Ion Stress and *V. anguillarum* Infection Experiment

Healthy adult dwarf surf clams were obtained from a laboratory breeding population. Dwarf surf clams were cultured in filtered and aerated seawater at 20°C–25°C. For the copper ion stress experimental group, the clams were acclimated in the sterilized seawater with a final copper ion concentration of 100 μg/l (from the anhydrous copper sulfate) (Zhang et al., 2012). For both control and experimental groups, five random clams were sampled at 0 h, 12 h, and 9 days, from which gill and hepatopancreas tissues were collected for RNA extraction. For the bacterial challenge group, gram-negative bacteria (*Vibrio Anguillarum*) were cultured in liquid 2216 E broth at 28°C to an OD600 of 0.2 and were harvested by centrifugation at 2,000 × g for 5 min. Then, the cell precipitates were suspended in filtered seawater and adjusted to $1 \times 10^7$ CFU/ml (Zhou et al., 2019) to challenge clams. For both control and experimental groups, five random clams were sampled at 0, 3, 6, 12, and 24 h, from which gill and hepatopancreas tissues were collected for RNA extraction.

## RNA Isolation and Quantitative Real-Time PCR Analysis

Total mRNA was extracted from the gill and hepatopancreas of the sampled clams by using the conventional guanidinium isothiocyanate method (Chomczynski and Sacchi, 2006). The cDNA was synthesized using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA). Primers of *MLCOMMD*s were designed using Primer Premier 5 software; the sequences of primers are listed in **Supplementary Table 3**. All reactions were repeated in triplicate. The transcription of target genes was standardized according to the transcription of two internal reference genes (namely, *RS23* and *NDUS4*). For the comparisons of the *COMMD* transcription changes between control experimental groups, statistical analysis of the data was performed using t-test with statistical significance at $P < 0.05$.

# RESULTS

## Identification and Characterization of *COMMD* Genes in Four Bivalve Molluscs

A total of 10 single-copy *COMMD* genes were identified in Yesso scallop, Zhikong scallop, Pacific oyster, and dwarf surf clam, respectively represented as *Py*COMMD*s*, *Cf*COMMD*s*, *Cg*COMMD*s*, and *Ml*COMMD*s*. The length of most bivalve COMMDs ranged from 150 to 240 aa, except four relatively short COMMDs (namely, *Py*COMMD1, 6, and *Cf*COMMD4, 6, which possess less than 135 aa) and *Cf*COMMD8 which was obviously longer (1,339 aa). The COMMD family shared the conserved COMM domain (**Figure 1**). Two highly conserved nuclear export signal regions were located at the COMM domain, namely, NES1 and NES2, which were mainly composed of the well-conserved hydrophobic amino acids L, I, V, M, and F (**Figure 2**). Besides, the COMMD1 proteins possessed an additional COMMD1_domain (PF17221) at the N-terminal, and a specific Glyco_hydro_15 domain (PF00723) was found at the C-terminal of *Cf*COMMD8 (**Figure 1**). Consistent with human COMMD6 (de Bie et al., 2006), the *Py*COMMD6 and *Cf*COMMD6 lacked a variable amino terminal, while *Cg*COMMD6 and *Ml*COMMD6 contained an extended amino terminal portion. A low-complexity region was located at the amino terminal of *Py*COMMD4, 8, *Cg*COMMD10, and *Ml*COMMD10. In comparison with *CgCOMMD*s and *MlCOMMDs*, a higher similarity of gene structure was revealed between *COMMD* orthologs from two scallops (**Table 1**). A subfamily-specific conserved intron number was found for scallop *COMMD3*, *5*, *6*, *7*, *9*, which comprised 7, 6, 4, 8, and 5 introns, respectively. Of note, the 5-intron pattern was also found in *CgCOMMD9* and *MlCOMMD9*, making *COMMD9* as the only member which showed the most conservative exon–intron structure in bivalves.

## Phylogenetic and Evolutionary Analysis of the *COMMD* Family

In the present study, besides the *COMMD* family gene identification from four bivalve molluscs, we also identified *COMMD*s from 22 additional animal species, across the major representative groups in Metazoa (**Figure 3**). It revealed that five groups, namely, Deuterostomia (except Urochordata), Mollusca, Brachiopoda, Cnidaria, and Sponge, have a full set of ten *COMMD* subfamilies, while in Ecdysozoa, Annelida, and Urochordata, they usually lack several *COMMD* subfamilies (up to 9). Especially, we noted that *COMMD1, 6, 9* subfamilies were

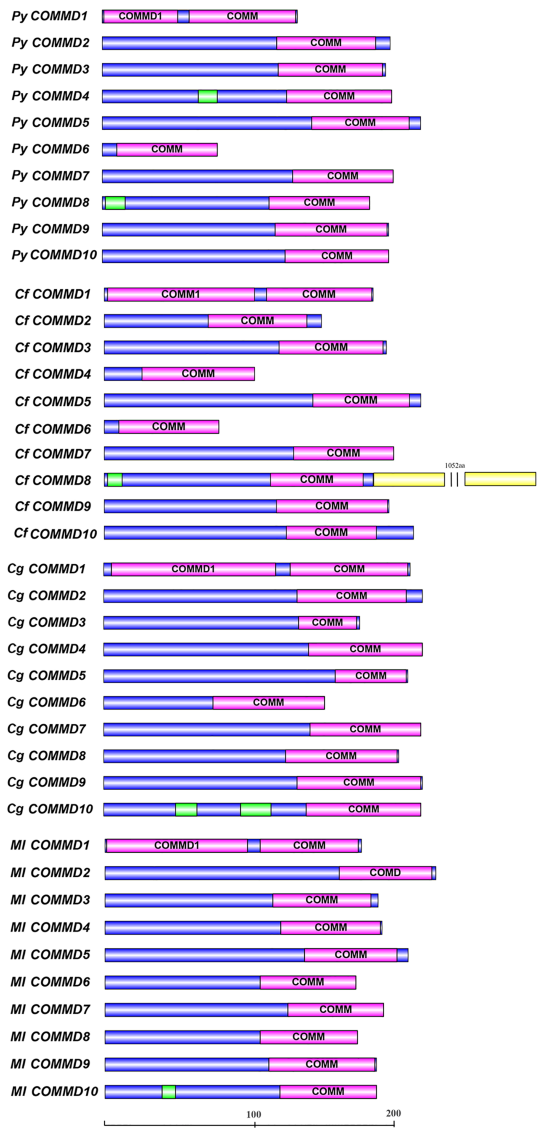


**FIGURE 1** | The structure of COMMD proteins in four bivalves. The purple boxes indicate the conserved COMM domains. The green boxes indicate the low-complexity regions, and the yellow boxes indicate the Glyco_hydro_15 Pfam domain.

absent in all investigated ecdysozoans, and within Lophotrochozoa, molluscs and brachiopods have more complete *COMMD* family members than annelids. We further investigated the evolutionary relationship of bivalve *COMMD*s. Phylogenetic analysis showed that all COMMD proteins were subdivided into ten subfamilies, including COMMD1-10, and consistent topologies were revealed based on both the NJ method (**Figure 4A**) and ML method (**Figure 4B**). For each subfamily, *COMMDs* from Zhikong scallop, Yesso scallop, and Pacific oyster are always grouped together first, then clustered together with *COMMDs* from the dwarf surf clam and other bivalves, which is in line with their assured phylogenetic relationship.

## Positive Selection Analysis

To explore the selective pressure of the *COMMD* genes, the CODEML program in the EasyCodeML1.2 software was further used. Results show that six subfamilies were detected with positive selection sites by the M7 vs. M8 model (**Table 2**). According to the M8 model, *COMMD4* possessed 8 positive sites, including two highly positively selected sites ($P > 0.95$), followed by *COMMD1* (5), *COMMD7* (5), and *COMMD10* (4). Only one and two positive sites were respectively detected in *COMMD3* and *COMMD6*. Overall, a total of 25 sites under potentially positive selection were identified in four bivalve *COMMDs*.

## Temporal and Spatial Expression of the *COMMDs* From Four Bivalve Molluscs

The TPM (reads per kilobase million) calculated from the RNA sequence data are displayed as a heat map (**Figure 5**, **Supplementary Tables 4** and **5**). As shown in **Figure 5A**, the embryo expression profiles of *COMMD*s in four bivalves can be parted into three groups. Firstly, most of the *COMMD1, 3, 4* subfamilies were detected at the beginning of fertilization and maintained high transcriptions until the multicellular stage; a similar transcription pattern was also found in *PyCOMMD6*, *CgCOMMD2, 7*, and *MlCOMMD5, 8, 10*, suggesting their maternal origin to play protective roles. *COMMD5, 8, 9, 10* in two scallops started increasing the transcription levels during blastula, and their high transcriptions were maintained until the D-shaped veliger stage. Except the abovementioned, other members of *COMMD* genes, including *PyCOMMD2, 7*, *CfCOMMD3, 7*, *CgCOMMD1, 3, 5, 6, 8, 9, 10*, and *MlCOMMD2, 6, 7, 9*, were enhanced exponentially from the D-shaped veliger stage and sustained their high level of transcription during late larval development.

According to the spatial expression pattern of *COMMD*s in six organs/tissues (**Figure 5B**), we found that most of the *COMMD2, 3, 4, 5, 7, 8, 10* subfamilies were predominantly expressed in the hepatopancreas in four bivalves. Besides, most of the *COMMD1, 6* members were highly expressed in the female gonads, and *COMMD2, 9* of two scallops were detected with high levels in male gonads. Moreover, we noticed that *COMMD*s' transcription showed a certain tissue preference among different bivalves. For example, most *MlCOMMD*s and *CfCOMMD*s were highly expressed in the hepatopancreas and gonad, respectively, while most *CgCOMMD*s and *PyCOMMD*s were highly expressed in the hepatopancreas, mantle, and gill. Overall, organ/tissue transcription patterns of *COMMD* genes in four bivalves may imply their diverse cellular functions.

## Responses of *MlCOMMD* Genes Under Copper Ion Stress and Bacterial Stress

Taking the advantages of laboratory-standardized breeding and cultivation, we investigate the potentially biological functions and defensive mechanism of *COMMD* genes in the dwarf surf clam. First, we explored the responses of *MlCOMMD* genes in the gill and hepatopancreas under copper ion stress (**Figure 6**). After the copper ion stress, *MlCOMMD1, 4, 9* showed significant responses in both tissues, with *MlCOMMD9* being remarkably upregulated, while *MlCOMMD1* and *MlCOMMD4* were

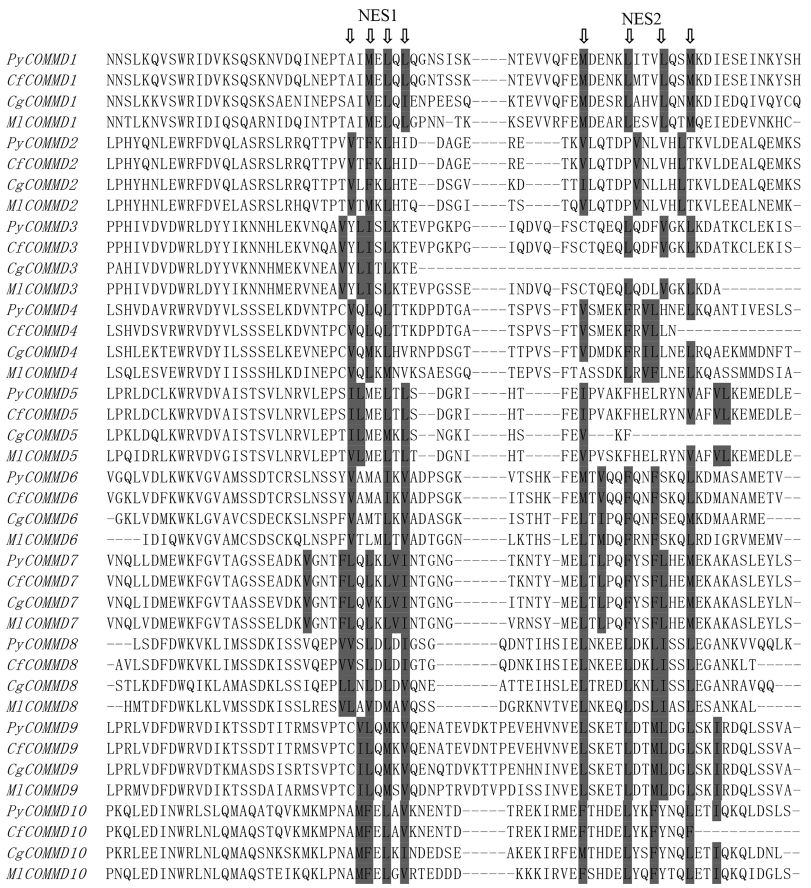


**FIGURE 2** | Alignment of deduced COMM domain amino acid sequences. The two nuclear export signals (NES1 and NES2) are indicated in gray shades and by arrows pointing at conserved hydrophobic residues.

significantly suppressed. Of note, a more acute and intensive response of *MlCOMMD9* was shown in the hepatopancreas (12 h, >6-fold, $P < 0.001$) than in the gill (day 9, >2-fold, $P < 0.05$). Besides, *MlCOMMD4* and *MlCOMMD7* were found significantly upregulated on day 9 respectively in the hepatopancreas and gill. In the next scenario, the temporal responses of *MlCOMMD* genes after *V. Anguillarum* infection were as shown in **Figure 7**. In the gill, only three *COMMD* members showed significant responses after infection, with *MlCOMMD4*, *8* being found to be significantly upregulated after 12 h and *MlCOMMD7* being suppressed at 6 h. In comparison, responses of *COMMD*s in the hepatopancreas seems more ubiquitous, with six members showing significant transcription alternations. After infection, transcription of *MlCOMMD4* was significantly elevated after 6 and 12 h, and *MlCOMMD7* was significantly upregulated at 6 h. In the meantime, *MlCOMMD5* was found to be acutely suppressed after 3 h and *MlCOMMD6*, *8*, *9* showed a significantly lower transcription at 6 h. Notably, *MlCOMMD4* was the only member that showed consistent significant induction in both the gill and hepatopancreas after infection, and similar to copper ion stress, a more acute and intensive response of *MlCOMMD4* was found in the hepatopancreas (6 h, >9-fold, $P < 0.05$) than in the gill (12 h, >3-fold, $P < 0.05$). Above all, we found that responses of *COMMD* members were more sensitive in the hepatopancreas than the gill, in which *MlCOMMD9* and *MlCOMMD4* might be good candidate stress indicator genes respectively for copper ion stress and *V. Anguillarum* infection.

## DISCUSSION

In this study, the *COMMD* family in four bivalve molluscs was identified and characterized based on the genomic and transcriptomic data. Similar to the human COMMD proteins, these bivalve COMMD proteins contain the conserved COMM domain in the extreme carboxyl terminus, which could mediate the interaction of COMMD-COMMD proteins and the formation of the COMMD polymer (Burstein et al., 2005). The amino terminal of COMMDs shares a low homology among members of the family but has highly conserved sequences with their ortholog proteins, which may contribute to the functional diversity of different subfamilies (Maine and Burstein, 2007). Besides four bivalve molluscs in our study, a 5-intron pattern of

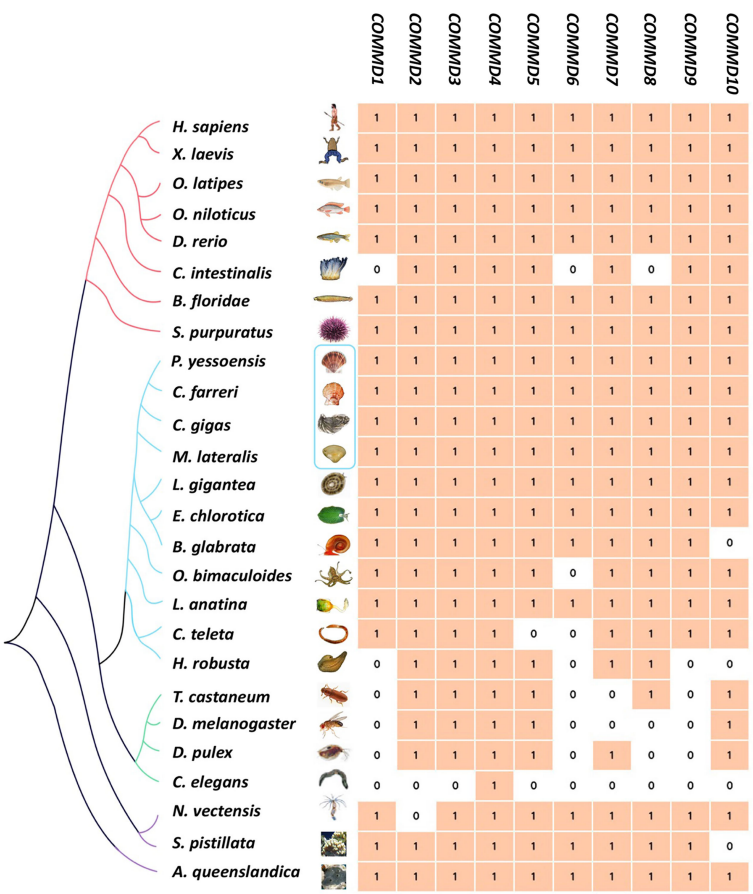


**FIGURE 3** | Genome-wide identification of *COMMD* gene families of 4 bivalve molluscs (in blue box) and 22 other metazoan species. Different colors of branches represent different metazoan groups (red, Deuterostomia; blue, Lophotrochozoa; green, Ecdysozoa; purple, non-Bilateria).

*COMMD9* was also discovered in human and zebrafish, which may imply that *COMMD9* has retained the ancestral exon–intron structure during the evolution. Other *COMMD* subfamilies of four bivalve molluscs have inconsistent intron patterns. Previously, researchers have found that intron insertion and loss may be associated with selective splicing, encoding the untranslated RNAs and enhancing the levels of mRNA transcription (Jin et al., 2012). The frequent occurrence of intron insertion and deletion among the *COMMD* family except for *COMMD9* may be to a certain extent related to the function of introns.

Phylogenetic analysis showed that four bivalve mollusc *COMMD* genes always clustered together in the invertebrate clades, consisting of their evolution status and conservativeness. Previous findings show that *COMMD* genes are highly conserved throughout vertebrate evolution, and *COMMD1* and *COMMD9* seem to be restricted to vertebrates (Burstein et al., 2005). In the present study, we further retrieved *COMMD* genes from 26 metazoan species (**Figure 3**). We found that both *COMMD1* and *COMMD9* could be identified in the Lophotrochozoa, Deuterostomia, Cnidaria, and Sponge groups, while they were absent in the Ecdysozoa group. Besides *COMMD1, 9*, *COMMD6, 7, 8* were also absent in most ecdysozoans and that nematodes only have the *COMMD4* subfamily, which may attribute to the fact that the Ecdysozoa genomes are rapidly evolving (Telford et al., 2008). Besides, *COMMD1, 6, 8* have been lost in ciona (Jin et al., 2018), while 10 intact single-copy *COMMD* genes were found in amphioxus as well as in most vertebrates. Species in the Mollusca, Deuterostomia (except Urochordata), Brachiopoda, Cnidaria, and Sponge groups almost have ten intact single-copy *COMMD* family genes; only several species were found to have loss of one the *COMMD* subfamily members. However, it remains unclear whether this phenomenon is due to genome assembly fragmentation or they have been lost during evolution. An across-Metazoa comparison implied that *COMMD* members diverged from each other at early stages of evolution and the integrated *COMMD* family may already exist in the metazoan last common ancestors.

Evolutionary analysis have shown that purifying selection dominated the evolution of *COMMD* genes (Jin et al., 2018). For four bivalves, a total of 25 sites under potentially positive selection were identified which may provide a support for the structural and functional diversity of the *COMMD* family members. Similarly, the selective pressure analyses of *COMMD*

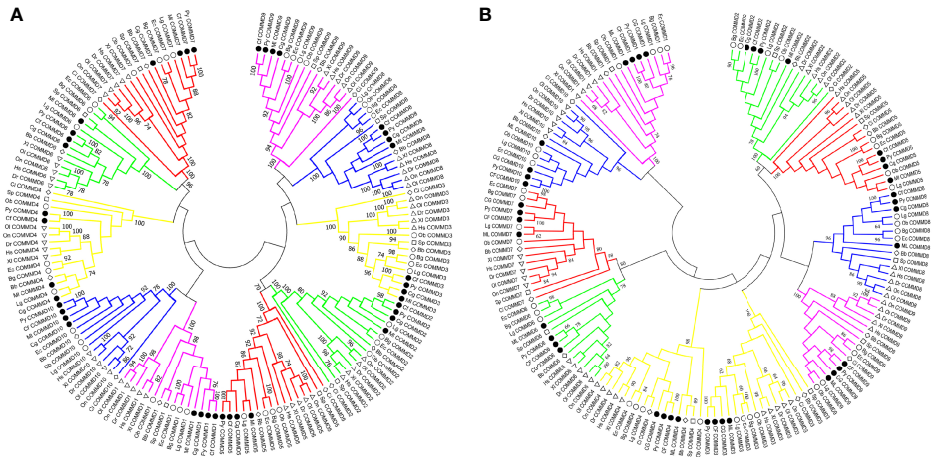


**FIGURE 4** | Phylogenetic analyses of *COMMD* gene families involving 154 amino acid sequences across 16 animals were conducted based on the NJ method and ML method. **(A)** Phylogenetic analysis of COMMD proteins using the NJ method, with a bootstrap of 1,000 replicates. **(B)** Phylogenetic analysis of COMMD proteins using the ML method, with a bootstrap of 1,000 replicates. Numbers in the trees represent the confidence interval. Different symbols were used to represent different clades of animals: the circles label the molluscs, the diamonds label the chordates, the triangles label the vertebrates, and the squares label the cnidarians. Four bivalve COMMDs were specially emphasized by solid color inside the symbol. Hs, *Homo sapiens*; Xl, *Xenopus laevis*; Dr, *Danio rerio*; Ol, *Oryzias latipes*; On, *Oreochromis niloticus*; Ci, *Ciona intestinalis*; Bb, *Branchiostoma belcheri*; Sp, *Stylophora pistillata*; Py, *Patinopecten yessoensis*; Cf, *Chlamys farreri*; Cg, *Crassostrea gigas*; Ml, *Mulinia lateralis*; Ec, *Elysia chlorotica*; Ob, *Octopus bimaculoides*; Lg, *Lottia gigantean*; Bg, *Biomphalaria glabrata*.

family genes in amphioxus showed that there were 16 positive selective sites detected, although the *COMMD* family genes have undergone very strong purifying selection during evolution (Jin et al., 2018).

In previous studies, the expression profile of *COMMD* family genes has been reported in vertebrates (van De Sluis et al., 2002; Klomp et al., 2003). However, such research remains lacking in invertebrates. To better understand the characteristic and function of *COMMD* genes in molluscs, extensive transcriptome resources were used to profile the temporal and spatial expression patterns of *COMMD* genes in four bivalve molluscs (**Figure 5**). The *COMMDs* were reported to play a vital role during mouse embryonic development, and *COMMD*-knockout mice are embryonically lethal and die at different stages of embryogenesis (Semenova et al., 2003; van de Sluis et al., 2007; Bartuzi et al., 2013). Besides, researchers found that *COMMD1* has a regulatory role in the cell cycle of HEK293 cells (Jiang et al., 2019). Our results showed that bivalve *COMMD1* is highly expressed during multicellular cleavage and its transcription level declines rapidly from blastula. It may also implicate that *COMMD1* is involved in the regulation of cell proliferation. Bivalve *COMMD7*s were found to enhance their transcription exponentially from the D-shaped veliger stage and sustained a high level of transcription during late larval development. Previous studies have found that *COMMD7* promoted cell proliferation, migration, and invasion processes but suppressed cell apoptosis (Devlin et al., 2003; Zheng et al., 2018). Therefore, we speculate that *COMMD7* may be involved

**TABLE 2** | Likelihood values and parameter estimates of computing position selection site by site model for the COMMD family members.

| Gene name | Model (Name of parameters) | lnL | Likelihood ratio test P-value | Positively selected sites |
|---|---|---|---|---|
| *COMMD1* | M8(10) | -1,262.595448 | 0.000001065 | 1 M 0.987*, 2 W 0.979*, 3 F 0.805, 97 S 0.907, 98 I 0.598 |
| | M7(8) | -1,276.348083 | | |
| *COMMD3* | M8(10) | -1,686.029432 | 0.013882920 | 44 R 0.941 |
| | M7(8) | -1,690.306528 | | |
| *COMMD4* | M8(10) | -1,096.111503 | 0.000165421 | 32 A 0.671, 88 K 0.751, 89 Q 0.784, 90 A 0.572, 91 N 0.909, 95 E 0.929, 96 S 0.953*, 98 S 0.980* |
| | M7(8) | -1,104.818519 | | |
| *COMMD6* | M8(10) | -821.039991 | 0.002870954 | 7 I 0.527, 9 D 0.698 |
| | M7(8) | -826.893102 | | |
| *COMMD7* | M8(10) | -1,821.686106 | 0.031505820 | 3 S 0.584, 32 R 0.638, 36 A 0.669, 61 S 0.607, 91 V 0.637 |
| | M7(8) | -1,825.143689 | | |
| *COMMD10* | M8(10) | -2,032.127041 | 0.050129732 | 50 T 0.635, 186 E 0.957*, 190 K 0.904, 191 Q 0.638 |
| | M7(8) | -2,035.120182 | | |

*For 1 M 0.987*, 1 means the number of amino acid, M means abbreviations of amino acid, 0.987 means posterior possibility (P), and * means that P > 0.95 by LRT test of Bayes empirical Bayes analysis.*

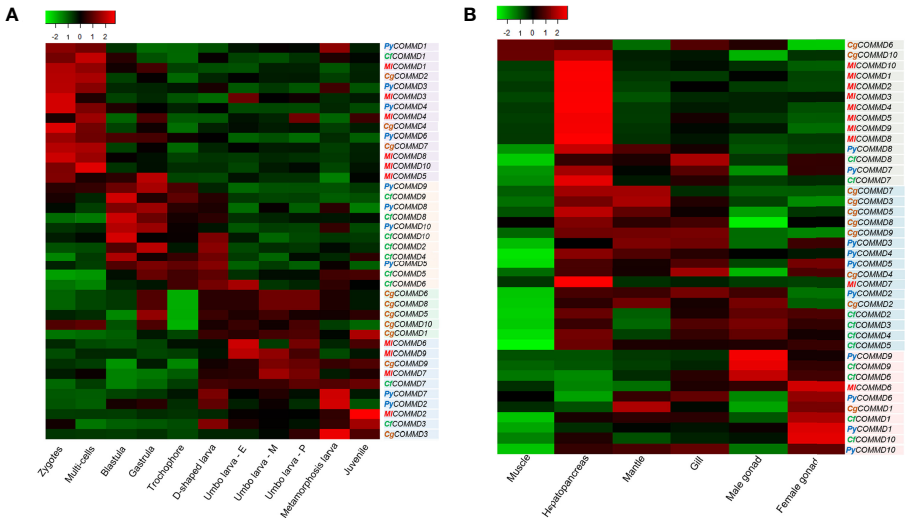


**FIGURE 5** | Heatmap of *COMMD* expression profiles (TPM) in embryonic developmental stages and different tissues in four bivalves. The color varies from green to red, representing the scale of the relative expression level. **(A)** Expression of *COMMD*s during embryonic development. **(B)** Expression of *COMMD*s in adult tissues of four bivalves.

in the regulation of organogenesis during embryonic larval formation. Among different adult tissues, high transcription levels of most *COMMD*s were found in the hepatopancreas. As filter-feeding animals that mainly feed on microalgae, bivalves could accumulate hazardous substance produced through diet, and the hepatopancreas is the main organ for processing and accumulating the incoming hazardous substance (Lian et al., 2019). Therefore, the relatively higher transcription levels of *COMMD*s in the hepatopancreas may assist with toxin tolerance in bivalves. *COMMD1, 9, 10* are involved in the regulation of cell proliferation, migration, and cell-cycle progression (Yang et al., 2017; Zhan et al., 2017). We found that *COMMD1, 6, 10* from two scallops were highly expressed in the female gonads, suggesting that these three *COMMD* members may contribute to the ovarian cell homeostasis maintenance to assist with oogenesis in scallops.

The *COMMD* genes play key roles in regulating copper homeostasis and innate immune response (Bartuzi et al., 2013; Jin et al., 2018; Mouhadeb et al., 2018). To investigate the possibly biological functions of the *MlCOMMD*s, their mRNA expression levels were measured at different time points under copper ion stress and bacterial stress. *MlCOMMD1, 4, 9* transcriptions exhibited a significant alteration after copper ion stress in both assayed organs of dwarf surf clam, and

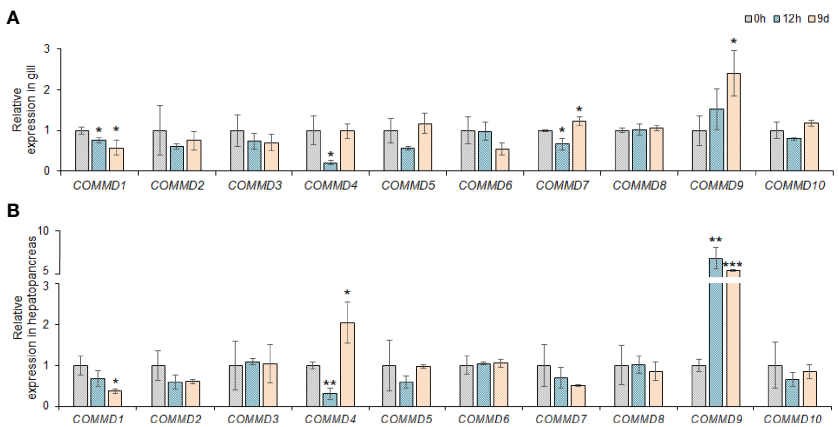


**FIGURE 6** | Relative expression of COMMD genes in M. lateralis gill and hepatopancreas under copper ion stress. **(A)** Relative expression of MLCOMMDs in gill under copper ion stress. **(B)** Relative expression of MLCOMMDs in hepatopancreas under copper ion stress. The fold changes compared with the control group for each test point are shown as a bar chart (significance: ***P < 0.001; **P < 0.01; *P < 0.05). The gray, light blue and light orange boxes respectively indicate 0 h, 9h and 9d under copper ion stress.

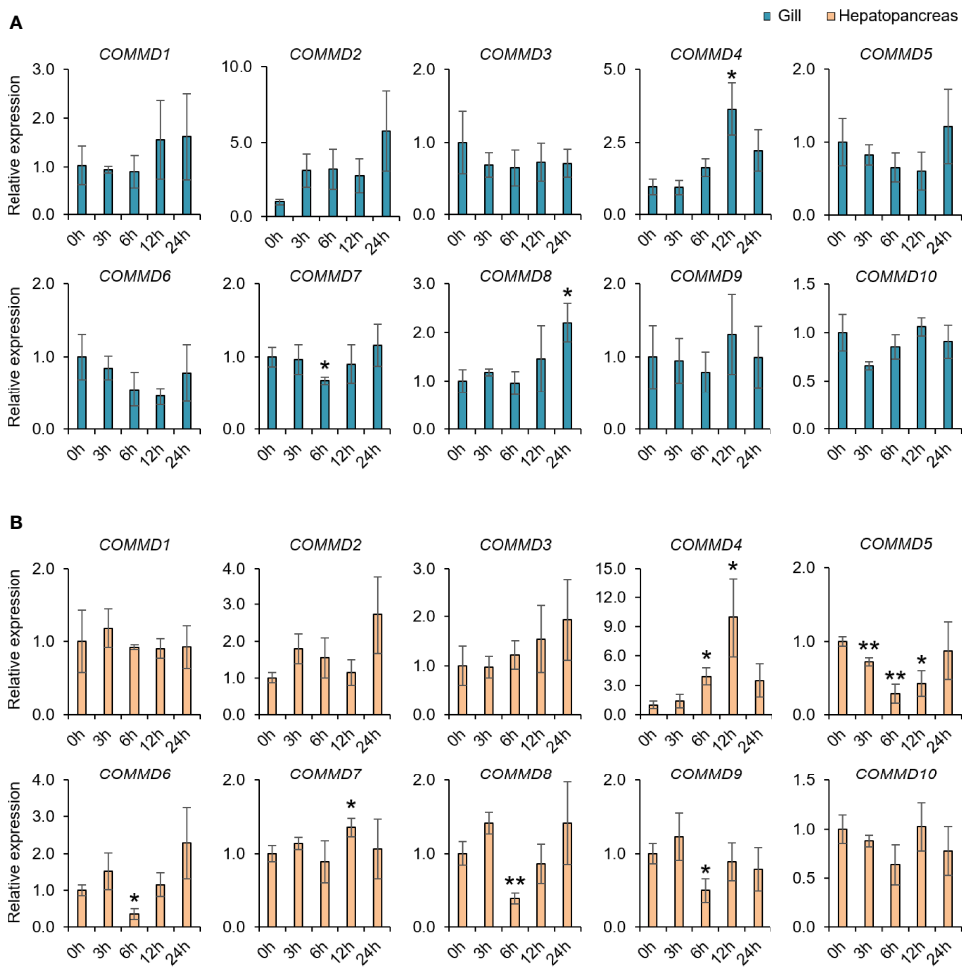


**FIGURE 7** | Relative COMMD genes expression in M. lateralis gill and hepatopancreas after V. anguillarum infection. **(A)** Relative expression of MLCOMMDs in gill after V. anguillarum infection. **(B)** Relative expression of MLCOMMDs in hepatopancreas after V. anguillarum infection. The relative fold changes compared with control group for each test point are shown as a bar chart (significance: ** $p < 0.01$; * $p < 0.05$). The blue boxes indicate gill and the light orange boxes indicate hepatopancreas.

*MlCOMMD9* showed the most drastic upregulation, suggesting their functional relation with cellular copper ion metabolism. It was reported that *COMMD1* regulates the endosomal sorting of the copper transporter (Phillips-Krawczak et al., 2015), and *COMMD9* may be an endogenous regulator of the epithelial sodium channel (ENaC) to regulate $Na^+$ transport, which could indirectly alter intracellular Cu flux (Handy et al., 2002; Liu et al., 2013). Besides, it was previously found that deficiency of *COMMD1* or *COMMD9* can result in hepatic copper accumulation under high-copper diets (Singla et al., 2021). Our *V. Anguillarum* infection experimental results revealed that *MlCOMMD4* was significantly upregulated in both the gill and hepatopancreas of dwarf surf clam. Previous studies reported that *COMMD4* has the ability to inhibit NF-κB, the key regulator of both innate and adaptive immune responses (de Bie et al., 2006; Maine and Burstein, 2007; Hayden and Ghosh, 2008; Naugler and Karin, 2008);, while whether bivalve *COMMD4* can regulate NF-κB needs further more detailed studies.

## CONCLUSIONS

In this study, 10 *COMMD* genes were respectively identified from the four bivalves, namely, Yesso scallop, Zhikong scallop, Pacific oyster, and dwarf surf clam. They possessed conserved COMM domains and comprised ten subfamilies. Purifying selection of six subfamilies was revealed, with the strongest selection on *COMMD4*. The expression profiling during embryonic development and in adult organs provided valuable implications for exploring the function of the bivalve *COMMD* gene. After exposure to two different stresses, *MLCOMMD*s exhibited different regulation patterns in different tissues or organs. The responses of *COMMD* members under stresses were found more sensitive in the hepatopancreas than in the gill, and *MlCOMMD9* and *MlCOMMD4* might be the good candidate stress indicator genes respectively for copper ion stress and *V. Anguillarum* infection. This study comprehensively describes the first genome-wide characterization of the *COMMD* gene family in

bivalves, and our work will be helpful in better understanding the function and evolution of *COMMD* family bivalve molluscs.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

## AUTHOR CONTRIBUTIONS

SL and JH conceived and designed the study. CX and NH performed the experiments. LL and FS participated in the data analysis. XC, SL, LZ, SW, and ZB wrote the manuscript. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.884991/full#supplementary-material

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