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ORIGINAL PAPER

Larvae of a marine gastropod and a marine bivalve share common gene expression signatures during metamorphic competence

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Abstract

Many aquatic invertebrates undergo an indirect development, a biphasic life cycle which encompasses the transformation of free-swimming larvae into benthic juveniles via settlement and metamorphosis. During this transition, metamorphic competence is a crucial developmental stage that allows larvae to swim and feed in the planktonic realm while retaining the ability to settle and metamorphose in response to environmental cues. Although there have been substantial efforts to decipher the molecular mechanisms underlying this event in several molluscan species, the conserved biological pathways that are crucial to enable this transition across species are not well understood. Here, we performed a comparative analysis of the developmental transcriptomes between bivalve *Crassostrea gigas* and gastropod *Rapana venosa*. We particularly explored the common gene expression signatures that may underlie their larval competence. We showed that, although the developmental transcriptomes differed remarkably between *C. gigas* and *R. venosa*, they likely shared a plethora of genes ($n = 690$) that exhibited similar expression signatures during their larval competence. Gene Ontology enrichment and expression analyses further indicated that competent larvae of both species exhibited up-regulation of pathways associated with response to stimuli, metal ion binding and transport, and neuronal development, but showed down-regulation of pathways that were mainly involved in cilium assembly and organ development. Using oyster and whelk as models, our study suggests that regulation of these conserved pathways is crucial for their subsequent settlement and metamorphosis and may represent a universal mechanism that enables the pelagic-to-benthic transition in a broader range of marine invertebrates.

Keywords Marine gastropods · Bivalves · Metamorphic competence · Comparative transcriptomics

Background

The development of most benthic marine invertebrates involves a biphasic lifecycle (Jackson et al. 2004). Within a relatively short period of time after fertilization, larvae typically search for a suitable settlement surface and start to metamorphose, a fundamental bioprocess that transforms free-living larvae into predominantly benthic juveniles (Hadfield 1986; Jackson et al. 2004; Bishop et al. 2006a). During this transition, the major morphological changes in larvae include re-absorption of larval tissues (e.g., velum), extension of the pre-formed juvenile structures (e.g., foot), and shell growth (Lv et al. 2019). Accompanied by these alterations are the complex metabolic, physiological, and developmental changes that enable larvae to initiate a sedentary benthic lifestyle. Although metamorphosis is considered to have independently evolved multiple times across the tree of animals, one remarkable similarity in the development of marine invertebrates is the concept of metamorphic competence, a developmental stage that directly precedes settlement (Medina 2009; Hadfield et al. 2001). Metamorphic competence is characterised by the ability of developing larvae to commence settlement and complete morphogenetic transformations into the adult benthic stage (Bishop et al. 2006b). It usually occurs, in most marine invertebrates, when the development of juvenile structures is all or nearly complete. For totally lecithotrophic species such as most Porifera, Cnidaria, and Ascidiacea, larvae become competent at the time of hatching, while for other marine invertebrates,

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larvae usually spend a period of time in the planktonic realm before achieving metamorphic competence (Hadfield et al. 2001). The transformation from planktonic larvae into benthic juveniles usually requires reception of external environmental cues, which are typically chemicals released by conspecific adults or present around settlement sites, and substrate-derived physical cues that indicate the quality of habitat (Rittschof et al. 1998; Rodriguez et al. 1993). Many marine invertebrate larvae can postpone metamorphosis and retain competence for extensive periods of time until specific cues are encountered (Bishop et al. 2006b; Pechenik 1999). Given that metamorphic competence is pervasively observed in the development of diverse animal phyla, this plasticity may represent a crucial convergent adaptation that enables larvae to survive until favourable habitats are found.

Over the past few decades, substantial efforts have been made to investigate the molecular mechanisms of the biphasic transition in many marine invertebrates, with a particular focus on molluscs such as oyster *Crassostrea angulata* (Qin et al. 2012), scallop *Patinopecten yessoensis* (Wang et al. 2020), clam *Meretrix meretrix* (Huan et al. 2012), abalone *Haliotis diversicolor* (Huang et al. 2012), sea hare *Aplysia california* (Fiedler et al. 2010), and whelk *Rapana venosa* (Song et al. 2016). These studies have revealed a number of molecular pathways that may underlie the genetic basis of larval metamorphic competence in molluscs. These pathways include transmembrane receptor signalling (Vogeler et al. 2014; Kaur et al. 2015), neuronal development (Qin et al. 2012; Zhou et al. 2013), neuroendocrine-immune crosstalk (Balseiro et al. 2013; Vogeler et al. 2014), and shell formation (Yu et al. 2016). However, large variation exists among different species in the degree to which larvae can maintain metamorphic competence without the presence of cues and the degree to which specific cues are required for metamorphic changes over time (Bishop et al. 2006b). In addition, it was previously thought that competent larvae exhibit decreased transcriptional activity and low metabolism and growth (Hadfield et al. 2001), but recent studies have uncovered a different scenario. For example, a study on the developmental transcriptomes of abalone *Haliotis asinina* revealed that the genes highly expressed in the competent stage are known to suppress metamorphosis, indicating their possible involvement in the maintenance of metamorphic competence (Williams et al. 2009). Sedanza et al. (Sedanza et al. 2022) compared the transcriptomes between competent larvae and post-larvae of oyster *Crassostrea gigas*. This study discovered that genes up-regulated in competent larvae mainly encode chemoreceptors and neurotransmitter receptors that enable larvae to sense and transduce environmental signals (Sedanza et al. 2022). Some of these genes also show up-regulation in the competent larvae of more distant-related polychaete *Capitella teleta* (Burns and Pechenik 2017) and coral *Acropora millepora* (Strader et al. 2018). These results suggest the complexity of the larval transcriptomes of marine invertebrates at the competent stage, and that understanding the genetic basis of such an evolutionary event requires comparative investigation across taxa.

To explore the common molecular mechanisms underlying larval competence, a comparative study on developmental transcriptomes between species which undergo the biphasic transition is required. Ideally, the taxa should display remarkable disparities in physiological and morphological changes during larval development and have comprehensive sets of developmental transcriptome data available. Comparing two species exhibiting such striking differences throughout their development potentially avoids the inclusion of common gene expression signatures which are not crucial to metamorphic competence across a range of species. As far as we are aware, time-series transcriptomes that cover the entire larval development are currently not available for most molluscan species, except for the veined rapa whelk *Rapana venosa* (Song et al. 2016) and the Pacific oyster *Crassostrea gigas* (Zhang et al. 2012). While the availability of data is clearly a determining factor in the choice of species for this study, the disparities in the development of *R. venosa* and *C. gigas* render them as ideal taxa to investigate the common molecular pathways underlying metamorphic competence in Mollusca.

R. venosa and *C. gigas* are from the two large molluscan groups (gastropods and bivalves) which diverged approximately 500 million years ago (Smith et al. 2011). Both species are important shellfish in China due to their high economic and medicinal value. However, artificial aquaculture has been limited by high larval mortalities, especially for *R. venosa*, due to extremely low settlement and metamorphosis rates of competent larvae (Yu et al. 2020). Although not fully understood yet, differences have been observed in metamorphic competence between *R. venosa* and *C. gigas*. Their retention periods vary, and specific signals are required to effectively trigger settlement and metamorphosis in respective species (Bishop et al. 2006b; Hadfield et al. 2001). For example, acetylcholine chloride and $CaCl_2$ are active inducers of *R. venosa* larval metamorphosis (Yang et al. 2015; Joyce and Vogeler 2018), whereas EPI (epinephrine) and L-DOPA (L-3,4-dehydroxyphenylalanine) are more effective to trigger the biphasic transition of *C. gigas* larvae (Coon et al. 1985). From a physiological and ecological perspective, one of their major differences lies in the fact that *R. venosa* is carnivorous and needs to undergo diet shift from herbivorous to carnivorous during metamorphosis, compared to the lifelong herbivorous *C. gigas*. Despite these notable disparities, their competent larvae exhibit common developmental traits, such as settlement behaviours and loss of larval characteristics (Hadfield et al. 2001). A comparative transcriptomic study on the development of *C. gigas*

and *R. venosa* will, therefore, enable us to detect common molecular mechanisms underlying these traits that are essential to their successful biphasic transition. This will provide great insights into the key molecular mechanisms of larval metamorphic competence that are universal in molluscs, with potential benefits for the aquaculture industry.

In this study, we conducted a comparative analysis of the developmental transcriptomes between gastropod *R. venosa* and bivalve *C. gigas*. We particularly investigated the changes of molecular pathways when their larvae became metamorphically competent and were readily adaptive to a benthic lifestyle, a relatively comparable developmental stage that occurs in the development of both species. Although the developmental transcriptomes differed strikingly between oyster and whelk, we showed that both species shared a wealth of genes ($n = 690$) that exhibited similar expression signatures during larval competence. Gene Ontology (GO) enrichment analyses further indicated that these common genes were enriched in multiple bioprocesses. The genes that exhibited up-regulation in competent larvae of both species were mainly associated with response to organic and inorganic substances, metal ion transport, and neuronal development, while the down-regulated genes were enriched in cilium assembly and organ development. Our results suggest that the molecular changes in these common pathways are crucial for the adaptation to a benthic lifestyle of competent larvae in both *R. venosa* and *C. gigas*.

Materials and methods

The developmental transcriptomes of *C. gigas* and *R. venosa*

This study was performed using publicly available sequencing data and did not require any ethical approval or permits. The transcriptome data of *C. gigas* ($n = 30$) and *R. venosa* ($n = 18$) from different developmental stages were obtained from the National Centre for Biotechnology Information (NCBI) under the BioProject PRJNA146329 (Zhang et al. 2012) and PRJNA288999 (Song et al. 2016), respectively. The oyster transcriptomes cover the stages including early gastrula (7.5 hpf), gastrula (8.5 hpf), trochophore (12.5 hpf), early D-shape (16 hpf), D-shape (1 dpf), early umbo (5.7 dpf), umbo (10.3 dpf), late umbo (15.3 dpf), pediveliger (competent, 18 dpf), spat (22.2 dpf), and juvenile (215 dpf), while the whelk transcriptomes were sequenced from larvae at 1-spiral whorl stage (1 dpf), 2-spiral whorl stage (5 dpf), early 3-spiral whorl stage (12 dpf), late 3-spiral whorl stage (20 dpf), 4-spiral whorl stage (competent, 30 dpf), and post-larval stage (33 dpf), respectively. The detailed information of the samples is available in Supplementary Table 1.

Prediction of putative single-copy orthologous genes between *C. gigas* and *R. venosa*

To elucidate the common molecular pathways underlying larval competence of *C. gigas* and *R. venosa*, their putative orthologous genes were identified. To achieve this, we extracted the coding sequences (CDS) from the genome of *C. gigas* (Zhang et al. 2012) and the transcriptome assembly of *R. venosa* (Song et al. 2016), respectively. Using these CDS, a reciprocal tBLASTx (v2.2.27) search (Altschul et al. 1990) was performed between *C. gigas* and *R. venosa*. The best reciprocal blast hits (RBH), with E-value $< 10^{-5}$, sequence coverage $> 70\%$ and sequence identity $> 70\%$, were considered as putative orthologous genes. These putative orthologs between *C. gigas* and *R. venosa* were further annotated by comparing them against the Uniprot database (release 2021_02) using BLAST (v2.2.27) (Altschul et al. 1990), with the same criteria above.

Transcriptomic analysis across developmental stages

Focusing on the orthologs, we investigated the overall transcriptomic shift across the different developmental stages in both species. Prior to ortholog quantification, the adaptor sequences and low-quality bases (base score < Q30) in each sample were removed using Cutadapt (v2.9) (Martin 2011). For each species, we mapped the high-quality reads of each sample to the corresponding orthologs using Salmon (v1.2.0) (Patro et al. 2017). Within each species, the expression counts of the orthologs were normalized using the Trimmed Mean of M-values (TMM) method, and further $\log_2$-transformed. The orthologs that did not express in either species were removed from downstream analyses. Based on the normalized expression data, pairwise Spearman's correlation coefficient tests and principal component analyses (PCA) were performed using the R packages *cor* and *prcomp*, respectively (v3.0). To further explore the expression patterns of these orthologs across the development of *C. gigas* and *R. venosa*, we implemented a weighted gene co-expression network analysis for each species using the R package WGCNA (v1.63) (Langfelder and Horvath 2008). In brief, the orthologous genes were clustered into different modules based on their expression levels across different stages, and the eigengene that represents the expression pattern of each module was identified. It is noteworthy that, due to the limited number of biological replicates for oyster, we grouped the oyster samples from a few developmental stages for PCA, WGCNA, and the downstream differential gene expression analyses (Supplementary Table 1).

Differential gene expression and pathway analysis

This study aims to identify the common transcriptomic signatures which are shared in metamorphically competent larvae of both oyster and whelk. However, it is challenging to compare transcriptomes of complex developmental processes, because the developmental stages in *R. venosa* and *C. gigas* do not align and the samples from these two species were sequenced with different platforms, methodologies, and depths. Instead of directly comparing developmental transcriptomes between *C. gigas* and *R. venosa*, we established an analysis pipeline that detected differentially expressed genes in the competent larvae within each species. Then, we obtained the overlapping up- and down-regulated genes common to both oyster and whelk. To achieve this, for each species, we performed differential gene expression analyses between different developmental stages. In particular, we compared the competent stages (pediveliger for oyster, 18 dpf; 4-spiral whorl stage for whelk, 30 dpf) with all other stages using DESeq2 (v1.6.3) (Love et al. 2014), respectively. Only the genes which exhibited differential expression (FDR < 0.05) between the competent stage and at least three other stages were considered specifically expressed (up-regulation or down-regulation) in competent larvae. By intersecting the gene lists, we obtained the common genes which were up-regulated and down-regulated in competent larvae in both species. Gene Ontology (GO) enrichment analyses were performed, respectively, on the common up-regulated and down-regulated genes using Fisher's exact tests through Metascape (Zhou et al. 2019). The putative orthologous genes identified between *C. gigas* and *R. venosa* were used as background for GO enrichment analyses, and the enriched GO terms with FDR < 0.05 were considered statistically significant. We further explored the overall expression of these enriched GO terms, which are specific to competence, across the developmental stages. Briefly, for each species, we collected all the genes belonging to each enriched GO term, summed up their normalized expression values, and further converted them to Z-scores. We used these Z-scores to represent the expression of each enriched GO term across different developmental stages. Z-scores were visualized using heatmap.

Results

Identification and quantification of orthologs

Using our pipeline, we identified 9863 putative orthologous genes between *C. gigas* and *R. venosa*, and used them as references for comparative transcriptomic analyses. In total, 446.8 million reads and 226.3 million reads were obtained from 30 oyster samples and 18 whelk samples, respectively (Supplementary Table 1). For *C. gigas*, on average, 52.71% ± 3.86% of reads per sample mapped to the references, whereas the average mapping rate is 63.46% ± 2.94% per sample for *R. venosa*. We removed the genes that were functionally unknown or not expressed. This led to two matrices containing expression data of 5427 genes across different developmental stages in *C. gigas* and *R. venosa*, respectively.

Overview of developmental transcriptomes in *C. gigas* and *R. venosa*

To gain a global view of transcriptomes, based on the gene expression matrices, we performed pairwise Spearman's correlation tests across different developmental stages in both *C. gigas* and *R. venosa* (Fig. 1a, b). Not surprisingly, the correlation coefficients between the samples from the same developmental stages ($\rho_{oyster} = 0.96 \pm 0.02$; $\rho_{whelk} = 0.98 \pm 0.02$) were significantly higher ($P < 0.00001$, Mann–Whitney *U* test) than those between the samples from different stages ($\rho_{oyster} = 0.86 \pm 0.08$; $\rho_{whelk} = 0.82 \pm 0.12$). In addition, PCA showed that the samples were clustered by species rather than the comparable developmental stages (e.g., metamorphic competence; Fig. 2), suggesting that the larval developmental transcriptomes differed remarkably between *C. gigas* and *R. venosa* (Fig. 2). Next, we performed weighted gene co-expression analyses (WGCNA) to explore the dynamic expression patterns of these orthologs across different developmental stages. We identified 23 and 22 modules that exhibited distinct expression patterns in *C. gigas* and *R. venosa*, respectively. The raw expression counts used for the expression analyses are available in Supplementary Table 2.

Common transcriptomic signatures of metamorphic competence

To further identify the common genes which may underlie larval competence in both *C. gigas* and *R. venosa*, we conducted differential gene expression analyses (see Materials and methods). We predicted a large number of differentially expressed genes (DEGs) between competent stages and other developmental stages (Fig. 3a, b). This indicated that each stage exhibited a distinctive transcriptomic signature likely responsible for their developmental bioprocesses. Expectedly, more DEGs were found in the comparisons of more distant stages. For example, in *C. gigas*, only 163 genes (78 up-regulated and 85 down-regulated) were detected as DEGs between competent larvae and post-larvae, while 3565 DEGs (1803 up-regulated and 1762 down-regulated) were found between competent stage and gastrula (Fig. 3a, b). In addition, we considered that genes had distinctive expression signatures in the competent stage only if they

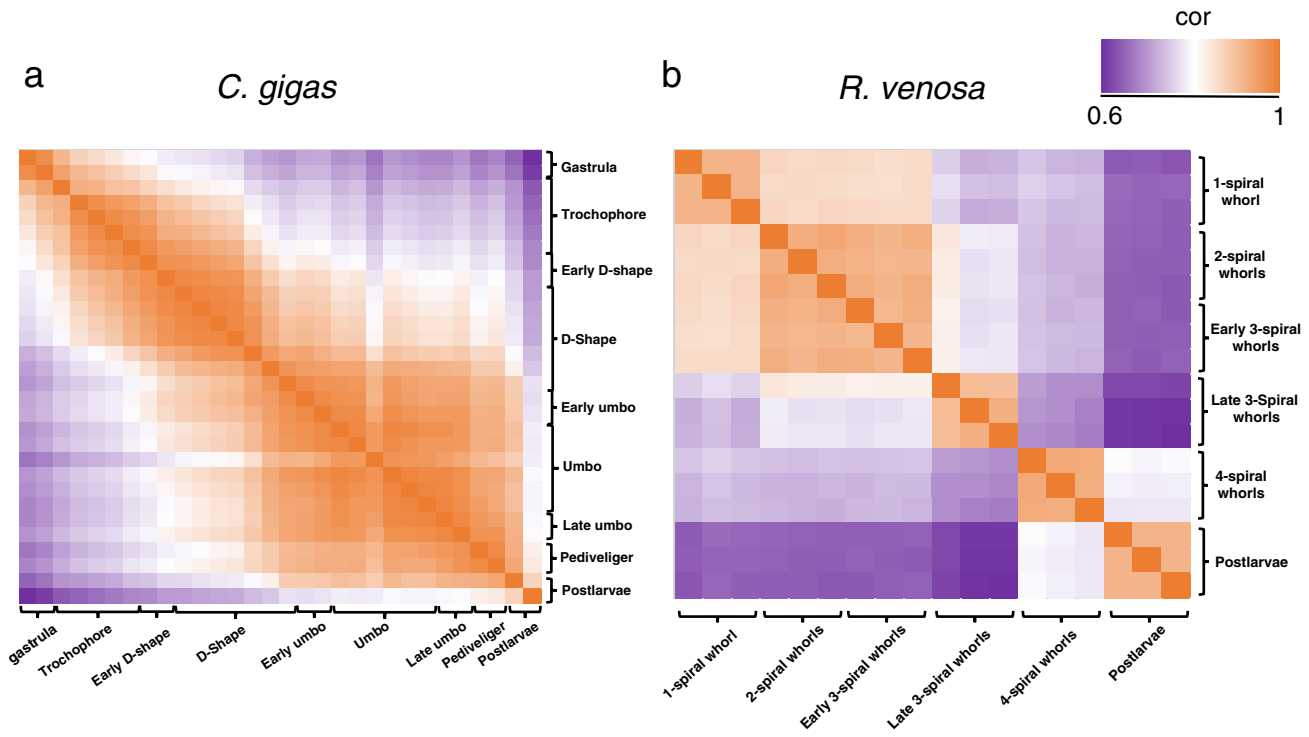

Fig. 1 Pairwise Spearman's correlation coefficients between each sample based on the normalized expression of 5427 orthologs. The colour key indicates correlation levels. Orange: high; white: median; purple: low. **a** *C. gigas*. **b** *R. venosa*

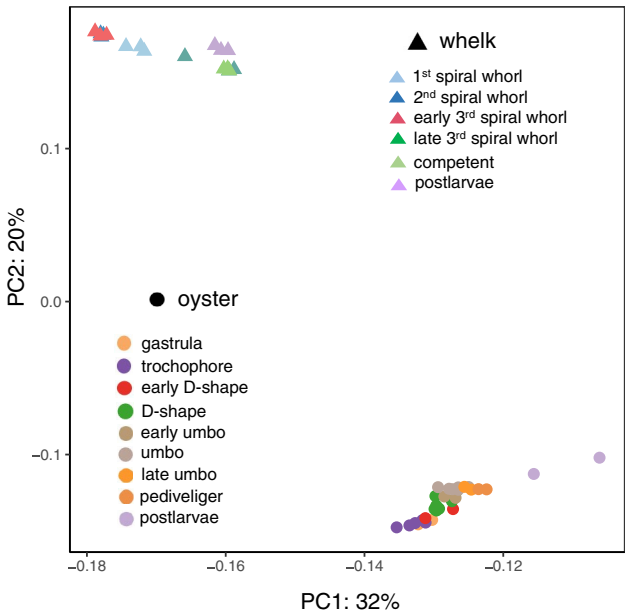

Fig. 2 Principal component analysis (PCA) of the larval developmental transcriptomes. PCA was performed using the normalized expression of 5427 orthologs across different developmental stages of *C. gigas* and *R. venosa*. PC1 and PC2 explain 32 and 20% of variation, respectively

showed differential expression with at least three other developmental stages. Based on this criterion, 2260 genes (1275 up-regulated and 985 down-regulated) and 2330 genes (1,062 up-regulated and 1268 down-regulated) were distinctively expressed in competent larvae of oyster and whelk, respectively. By intersecting the gene lists, we obtained 690 common genes (333 up-regulated and 357 down-regulated) that exhibited distinctive expression signatures in competent larvae of both species (Supplementary Table 3).

Pathway enrichment and expression analyses

To further explore the biological processes in which these 690 common genes were enriched, we performed GO enrichment analyses (see Materials and Methods). The common up-regulated genes in the competent larvae of both *R. venosa* and *C. gigas* were enriched in a variety of developmental processes, such as response to stresses (GO:0010035, GO:0009725, GO:0009410, GO:0009314), ion transport (GO:0030001, GO:0006814), and neuronal development (GO:0021954, GO:0099177). The common down-regulated genes were mainly involved in cilium assembly (GO:0060271), organ development (GO:0007420, GO:0007423, GO:0060541), and cell division (GO:0051301). A full list of the enriched GO terms is available in Supplementary Table 4. Consistent in both *C.*

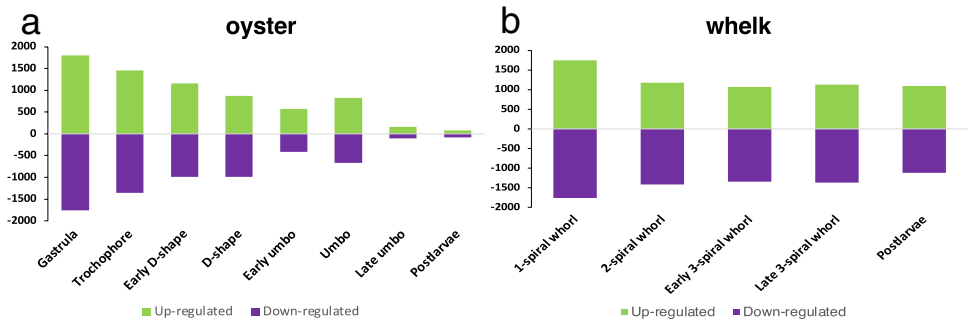

Fig. 3 The number of differentially expressed genes (DEGs) detected between the competent stage and any other stage. **a** *C. gigas*. **b** *R. venosa*

gigas and *R. venosa*, the overall expression levels of these GO terms differed dramatically between the competent stage and other developmental stages (Fig. 4a, b).

Discussion

The life cycle of marine gastropod and bivalve species involves a transition from planktonic larvae to benthic juveniles during development (Hadfield et al. 2001; Jackson et al. 2004). Uncovering the underlying biological pathways shared by both lineages can shed light on the mechanisms of settlement and metamorphosis in marine invertebrates from an evolutionary perspective. However, it is a grand challenge to conduct a comprehensive comparison of developmental transcriptomes between these lineages due to their idiosyncratic developmental processes (Rodriguez et al. 1993; Joyce and Vogeler 2018; Pechenik 1999). Therefore, a direct comparison between developmental transcriptomes of two species is not feasible. In this study, we developed a strategy to address this issue and performed a comparative analysis of developmental transcriptomes between *C. gigas* and *R. venosa*, the species representing Bivalvia and Gastropoda, respectively, with a particular focus on the competent stages.

From a global perspective, our analyses showed that both species experienced dynamic gene expression changes during larval development, reflected by correlation analyses, WGCNA, and differential gene expression analyses (Fig. 1, 3). This is not surprising, as this phenomenon has been observed in a wide range of taxa, including cnidarians (Leclère et al. 2019), sponges (Conaco et al. 2012), and barnacles (Chen et al. 2011). The unique gene expression signatures in each stage may correspond to its specific developmental changes. Interestingly, the principal component analysis showed that the developmental transcriptomes differed remarkably between *C. gigas* and *R. venosa* (Fig. 2). This result appears to reflect the taxonomic disparities in physiology, morphology, and behaviour during development

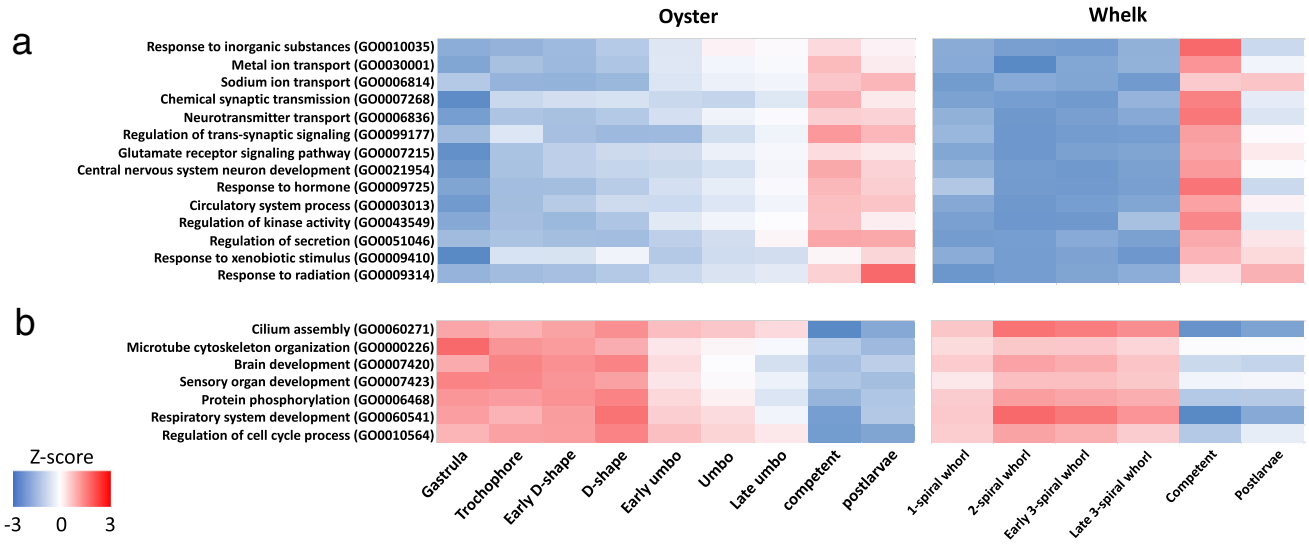

Fig. 4 The overall expression of common GO terms underlying larval metamorphic competence in *C. gigas* and *R. venosa*. **a** GO terms that exhibited up-regulation in the overall expression during larval competence in both species. **b** GO terms that exhibited down-regulation in the overall expression during larval competence in both species

[e.g., torsion uniquely seen in gastropods (Page 2003)] between these two species.

Despite these differences, the development of both *C. gigas* and *R. venosa* involves metamorphic competence, a convergent adaptation which enables free-swimming larvae to transition to benthic juveniles. Although variation exists, we showed that competent larvae of both species exhibited common gene expression signatures, which were enriched in several fundamental bioprocesses (Fig. 4a, b, Supplementary Table 4). The genes that were up-regulated were mainly enriched in the GO terms 'response to metal ion', 'response to hormone', 'metal ion transport', 'regulation of hormone levels', and 'neuronal development' (Fig. 4a, Supplementary table 4). Not surprisingly, up-regulation of some of these GO terms has also been reported in competent larvae of the sea snail *Babylonia areolata* (Shen et al. 2018), and the oyster *C. gigas* (Sedanza et al. 2022). Competent larvae typically have almost developed juvenile traits and are prepared to settle and metamorphose in response to favourable chemical signals. These signals are represented by a wide range of stimuli such as metal ions (e.g., Ca^{2+}, Mg^{2+}, and K^{+}) and neurotransmitters (e.g., serotonin, L-DOPA). Besides the reception of external cues, internal regulation of hormones is also indispensable to initiate larval settlement and metamorphosis. We found that the genes involved in hormone regulation (e.g., *ACE*, *CRHR1*, *PTPRN2*) showed up-regulation in the competent stage of *C. gigas* and *R. venosa*. Recent studies indicated that hormones have pleiotropic effects on the development of marine invertebrates and are involved in regulation of larval and juvenile morphogenesis and apoptosis (Heyland and Moroz 2006). One of the most prominent examples is that thyroid hormones (THs) were verified to promote differentiation of juvenile characteristics while simultaneously deconstructing larval traits in competent larvae of ascidians, echinoderms, and molluscs (Heyland et al. 2004; Patricolo et al. 2001; Fukazawa et al. 2001). Likewise, some juvenile hormones such as methyl farnesoate (MF) were reported to have identical effects in some barnacle species (Yamamoto et al. 1997). These results might indicate that distantly related marine invertebrates inherited the same basic molecular receptive and regulatory mechanisms from their most recent common ancestor during larval competence.

Noticeably, we found that several genes encoding cadherin proteins such as CDH1 and FAT1 were highly expressed in competent larvae of *C. gigas* and *R. venosa*. Cadherins are a family of transmembrane receptors that regulate cell–cell adhesion in animals (Takeichi 1988). For example, competent larvae of most marine invertebrates exhibit a substratum-testing manner to explore suitable habitats by foot extension. This process involves tissue morphogenesis regulated by cadherins, which includes cell size and shape changes and formation of junctions to bind cells together within tissues. Up-regulation of cadherins has been documented during larval competence in diverse marine invertebrates including the oyster *C. angulata* (Di et al. 2020), the coral *Acropora gemmifera* (Yuan et al. 2018), and the polychaete *Boccardia wellingtonensis* (Figueroa et al. 2021). These results suggest that cadherins and their associated pathways may represent a universal mechanism in tissue remodelling during larval competence in marine invertebrates. In addition to cadherins, we found another gene encoding a transmembrane protein, epidermal growth factor receptor (EGFR), that was highly expressed in the competent larvae of *C. gigas* and *R. venosa*. EGFR is a member of the receptor tyrosine kinase superfamily. In mammals, EGFR is activated by a variety of polypeptide ligands and regulates cell adhesion, proliferation, differentiation, and apoptosis by signalling transduction (Wee and Wang 2017). These cellular processes are highly associated with the morphological changes during larval settlement and metamorphosis, such as loss of velums, foot extension, and shell formation. During the development of *C. angulata*, *EGFR* exhibited an elevated expression from the onset of metamorphic competence (Qin et al. 2012). In addition, inhibition of EGFR signalling was reported to prevent settlement and metamorphosis of the barnacle *B. amphitrite* larvae (Okazaki and Shizuri 2000). In addition to *EGFR*, we noticed that several genes involved in the EGFR signalling pathway (e.g., *NEDD4*, *PLD1*, and *STMN1*) were up-regulated in competent larvae of both *C. gigas* and *R. venosa*. These results indicate that morphogenesis may occur at the competent stage ahead of larval settlement in *C. gigas* and *R. venosa*, and this process is likely mediated by EGFR signalling.

Similar to *C. gigas* and *R. venosa*, the GO term 'metal ion transport' was also enriched by the genes that were up-regulated in competent larvae of the eastern oyster *Crassostrea virginica* (Prytherch 1934) and a more phylogenetically distant species, the annelid *C. teleta* (Burns and Pechenik 2017). On top of its involvement in the process of receiving environmental cues, ion transport appears to play crucial roles in biomineralization and the nervous system during metamorphic competence of many marine invertebrates. For example, the transport of calcium and bicarbonate ions to the extracellular calcification space results in shell formation in molluscs (Clark et al. 2020). This process involves calcium ATPase and Na^{+}/Ca^{2+} exchangers, whose activation is driven by the transmembrane sodium gradient (Clark et al. 2020). In many molluscan species, the metamorphic transition involves rewiring of the central nervous system, and metal ion channels and transport are pivotal for neurons to transmit signals, process information, and conduct larval morphogenetic changes. In parallel, we also observed that the GO terms related to neuronal development and synapse were up-regulated in competent larvae of *C. gigas* and *R. venosa* (Fig. 4a). This is not surprising as molluscan species

usually have extensive larval nervous systems, beginning to develop from trochophore larvae (Croll and Dickinson 2004). For example, the competent larvae of the sea hare *A. californica* will remain planktonic until the complete adult nervous system is formed (Heyland et al. 2011). In pediveliger larvae of the mussel *Mytilus trossulus*, more neurons appear in the ganglia and neurites, which will later form the adult central nervous system (Voronezhskaya et al. 2008). This similar developmental process was also seen in more phylogenetically distant lineages, such as the annelid *C. teleta* (Meyer et al. 2015). Interestingly, besides its functions mentioned above, the gene *EGFR* also regulates differentiation and functions of neurons and neuroglia in animals (Romano and Bucci 2020). This suggests that EGFR signalling is implicated in remodelling of the central nervous system in competent larvae, such as degeneration of larval nervous system.

A major characteristic of metamorphosis in marine invertebrate larvae is loss of larvae-specific structures, such as ciliated velum (Bonar 1976). In molluscs, the velum develops from the ciliary lobe (prototroph), and larvae at this stage feed, swim and exchange gas by means of velums. However, they shrink and degenerate in metamorphosed larvae (Hadfield et al. 2001). Interestingly, we found that a wealth of genes ($n = 52$) enriched in cilium assembly and microtube cytoskeleton organization were down-regulated in competent larvae of oyster and whelk (Fig. 4b, Supplementary Table 4), suggesting that degeneration of larvae-specific characteristics may begin from late larval stages prior to the onset of settlement and metamorphosis. Down-regulation of these bioprocesses was also reported in competent larvae of the peanut worm *Sipunculus nudus* (Cao et al. 2020) and the demosponge *Amphimedon queenslandica* (Conaco et al. 2012). These morphogenetic changes can also be ascribed to the up-regulation of bioprocesses associated with hormone regulation and apoptosis, possibly mediated by EGFR signalling. In particular, we noticed that a large number of bioprocesses engaged in organ development were also down-regulated (Fig. 4b, Supplementary Table 4). This phenomenon is also seen in other molluscan species, and aligns with a previous review on metamorphic competence of marine invertebrates, which argued that in marine invertebrates, metamorphic competence typically occurs when nearly all required juvenile characteristics are present in the larvae prior to settlement (Hadfield et al. 2001). Indeed, we did notice that these bioprocesses specifically identified in competent larvae exhibited similar expression signatures in post-larvae, relative to larvae from other stages (Fig. 4a, b). These results further indicate that competent larvae in these two species possess some juvenile characteristics, and metamorphosis is mainly restricted to loss of larvae-specific structures, physiological processes, and behaviours (Hadfield et al. 2001).

Although variations in morphology, physiology, and behaviour exist in competent larvae across marine invertebrates, by searching current literatures, we found that some of the transcriptomic signatures identified in molluscan competent larvae are also seen within a broader phylogenetic bracket, including corals (Meyer et al. 2009), molluscs (Song et al. 2016; Qin et al. 2012; Shen et al. 2018), barnacles (Chen et al. 2011), and many others (Conaco et al. 2012; Burns and Pechenik 2017; Cao et al. 2020; Mok et al. 2009). These signatures include up-regulation of pathways associated with response to organic and inorganic substances, sodium ion transport and neuronal development, and down-regulation of bioprocesses related to cilium assembly and organ development. This suggests that the transcriptomes of competent larvae are highly dynamic, and that different marine invertebrates share some conserved molecular mechanisms that are critical for larvae to enable a successful pelagic-to-benthic transition. It is worth mentioning that some of these pathways, such as metal ion transport and EGFR signalling, may have pleiotropic functions in morphogenesis during this biphasic transition. These results indicate the complexity of larval development in marine invertebrates. In the future, a time-series analysis on different phases of competent larvae is required, because the length of competent stage varies significantly across species (Hadfield et al. 2001). For example, competence period can last as short as a few days in many invertebrate larvae while it can exceed several months in other species under laboratory conditions, such as the sea hare *Aplysia juliana* (Kempf 1981) and the coral *Pocillopora damicornis* (Richmond 1987). Therefore, it is necessary to sample multiple time points during larval competence and increase the sample size to gain a refined comparison across species.

Collectively, using comparative transcriptomics, we showed that marine gastropod and bivalve species likely share some common molecular pathways during metamorphic competence. These pathways exhibit specific expression signatures in competent larvae and may form a complex gene regulatory network that orchestrates larval settlement and metamorphosis. These findings contribute to our knowledge of the evolution of metamorphic competence in molluscan species. A systematic comparison is required, in the future, to determine if these conserved bioprocesses are also employed by competent larvae of other molluscan species and a wider range of lineages, including sponges, corals, and barnacles.

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Author contributions Z.H: devised the study. Z.H and Z.Z: performed the data analysis. Z.H, Z.Z, C.J, Q.T, and B.T: interpreted the results. Z.H and Z.Z: wrote the first draft. All the authors edited and approved the final manuscript.

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Data availability The raw RNA-Seq data are available via the National Centre for Biotechnology Information (NCBI) under the BioProject PRJNA146329 and PRJNA288999. Supplementary Tables S1-4 are available via GitHub https://github.com/huangzixia/marine_gastropods_and_bivalves.

Declarations

Conflict of interest The authors declare no competing interests.

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