1. **piRNA genes**

To identify piRNA clusters, we used our previously sequenced small RNA-Seq data (Yoshida et al., 2017). Sequenced reads were mapped to known non-coding RNA sequences of *H. exemplaris* (tRNA, rRNA, miRNA from Yoshida et al. (2017)), and reads that were not mapped were used as follows. After removal of redundant and low-complexity reads using scripts from proTRAC (Rosenkranz and Zischler, 2012), we mapped the remaining reads to piRBase (Wang et al., 2019) to identify piRNA sequences from *C. elegans* and *D. melanogaster*. The reads that did not map to piRBase were further mapped to the *H. exemplaris* genome, and proTRAC v. 2.4.2 and PILFER (downloaded at 2018.11.05) were used for piRNA cluster identification (Ray and Pandey, 2018; Rosenkranz and Zischler, 2012). File conversion was conducted using BEDtools v. 2.25.0, SAMtools and G-language Genome Analysis Environment v 1.9.1 (Arakawa et al., 2010; Arakawa et al., 2003; Arakawa and Tomita, 2006; Li et al., 2009; Quinlan and Hall, 2010), and read mapping was conducted using Bowtie2 v. 2.2.9 (Langmead and Salzberg, 2012).

We observed that the expression of small RNA-related genes (Argonaute, OQV17169.1/ OQV21162.1, g3665.t1) was induced at the developmental stages, which supports the implication of piRNA existence suggested in previous studies. We used our previously sequenced small RNA-Seq data and observed that 1.5M reads mapped to the *H. exemplaris* genome, but only 27K reads to known piRNA sequences from *C. elegans* and *D. melanogaster* in piRbase. Using these piRNA candidates, PILFER and proTRAC, predicted 894 and 187 piRNA clusters, respectively (**Supplementary Data S3**, **Supplementary Data S4**). We designated the 201 regions that were identified in both methods as putative piRNA clusters in *H. exemplaris*, which overlapped with 490 genes (504 transcripts), of which 385 transcripts were hypothetical genes (**Supplementary Data S5**, **Supplementary Data S6**).

Using our previously sequenced *H. exemplaris* small RNA-Seq data　(Yoshida et al., 2017), we predicted piRNA clusters in the *H. exemplaris* genome, consistent with previous studies (Sarkies et al., 2015). Most of these piRNA clusters showed no homology with *D. melanogaster* nor *C. elegans* piRNAs, suggesting most of these piRNAs may have been acquired after divergence from the common ancestors of Arthropoda and Nematoda.

1. **Anhydrobiosis related genes**

We observed that several copies of CAHS and SAHS genes were expressed exclusively in the developmental stages (**Supplementary Figure S4AB**, **Supplementary Table S2**). Additionally, we have found that the anti-oxidative stress-related thioredoxin was also induced in Egg 3d in both species (**Supplementary Figure S5**). In comparison, glutathione S-transferase and superoxide dismutase were most expressed during the early juvenile stages, and catalase at late juvenile to adult stages.

Tardigrades are famous for their tolerance capabilities, such as desiccation tolerance (Keilin, 1959). Although we found several CAHS and SAHS orthologs to have relatively high expression during the embryonic stages in both species (Yamaguchi et al., 2012), we found one SAHS ortholog that showed particularly high expression in both embryonic stages, suggesting that the regulation of an embryo-specific ortholog may be conserved between Hypsibidae and Ramazzottidae. Along with the expression profiles of antioxidative genes, these data suggest the fundamental anhydrobiosis mechanism during the embryonic stages in Hypsibidae and Ramazzottidae. Additional analysis of the developmental stages in *Milnesium inceptum*, a terrestrial species capable of anhydrobiosis in the embryonic stages (Schill and Fritz, 2008), may further support our findings.