**Supplemental Figure S1.** A closed inland culture system. (A-C) Animals were cultured in 20L tank (A), polypropylene 5L beaker (B) and 2L aquarium tank (C). (D) The bar shows the survival rate of animals. (E) The bar shows the proportion of animals having sperm. n shows the numbers of individuals.

**Supplemental Figure S2.** Genotyping of F1 juveniles at marker 2 gene locus. (A) PCR was done with primers designed at marker 1 and marker 2 gene loci [(Suzuki *et al.* 2005)](https://paperpile.com/c/x8DmRw/m4Kj) from genomic DNAs of F0 *C. robusta* and *C. intestinalis* sperm. (B) Three F1 juveniles in each type were analyzed by PCR with marker 2 primers. (C) PCR was done with marker 2 primers with genomic DNAs collected from oral siphon and sperm of F1 *C. robusta*, RxI hybrid, IxR hybrid and *intestinalis* mature animals. (D) Sequences were read from the PCR products.

**Supplemental Figure S3.** Genotyping of F1 juveniles at *Myl2/5/10* gene locus. (A, B) PCR was done from genomic DNA of F0 *C. robusta* (A) and *C. intestinalis* (B) sperm. (C) Alignment of *Myl2/5/10* sequence between *C. robusta* and *intestinalis*. Cyan shows exon sequence. Arrow shows sequence primer. Underlines show the region of sequence in Figures 5C, S2D and S3E. (D) Three F1 juveniles in each strain were analyzed by PCR with *Myl2/5/10* primers. (E) Sequences were read from the PCR products.