

Figure S1

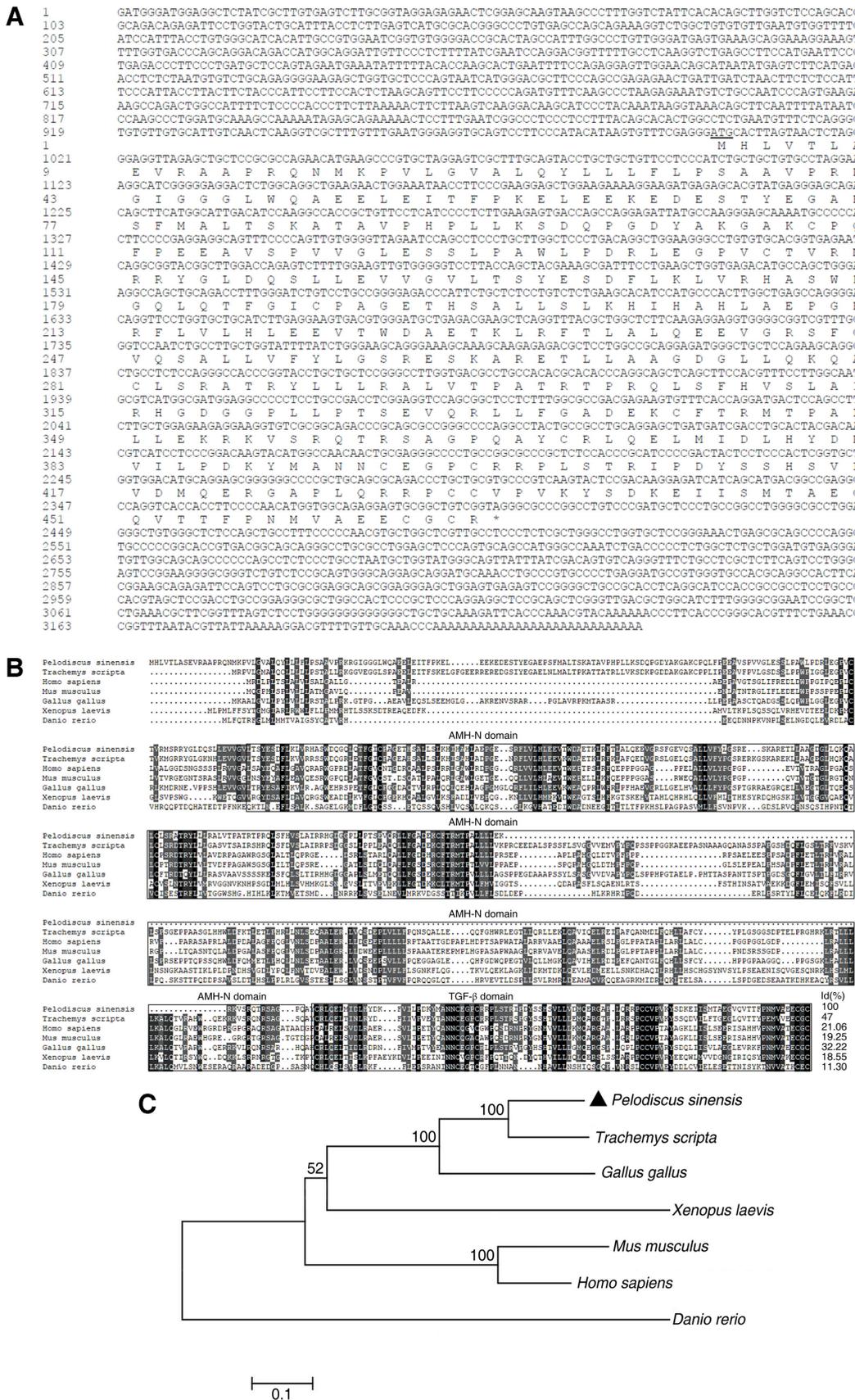


Figure S1. Sequence and phylogenetic analyses of *P. sinensis* Amh. (A) The complete

cDNA sequence of *P. sinensis* *Amh* and deduced amino acid sequence. The start codon ATG was underlined, and the stop codon was indicated by an asterisk. (B) Alignment of amino acid sequence of *P. sinensis* AMH with those from other typical species. The two characteristic functional domains of the TGF- β superfamily, AMH-N and TGF- β domain, were marked. (C) AMH phylogenetic tree from *P. sinensis* and other typical species based on Neighbor-Joining (N-J) method. Numbers at branches were confidence values based on 1000 bootstraps. Each branch length scale in terms of genetic distance was indicated above the tree.

Figure S2

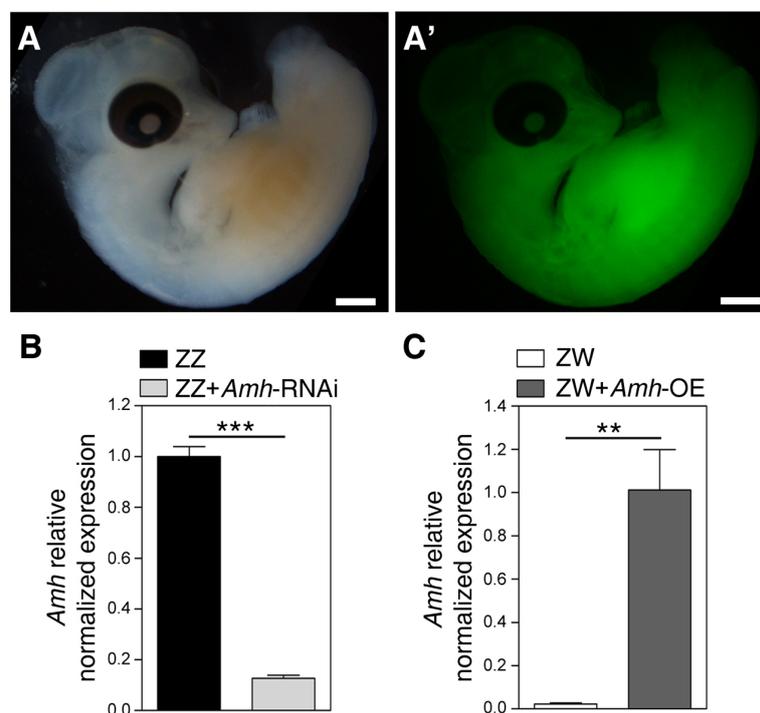


Figure S2. Establishment of *Amh*-Knockdown and -overexpressing turtle model using lentivirus vectors. (A, A') The whole embryos of stage 15 infected with scrambled lentiviral vector (LV-NC) at stage 14 showed widespread GFP expression.

Bright (A) and epifluorescence (A') images. Scale bars are 1 mm. (B, C) qRT-PCR of *Amh* showed >5-fold downregulation in ZZ gonads with LV-*Amh*-shRNA treatment (B) and >50-fold upregulation in ZW gonads with LV-*Amh*-OE treatment (C), respectively. Data are shown as means \pm S.D. $N \geq 3$. **, $P < 0.01$; ***, $P < 0.001$.

Figure S3

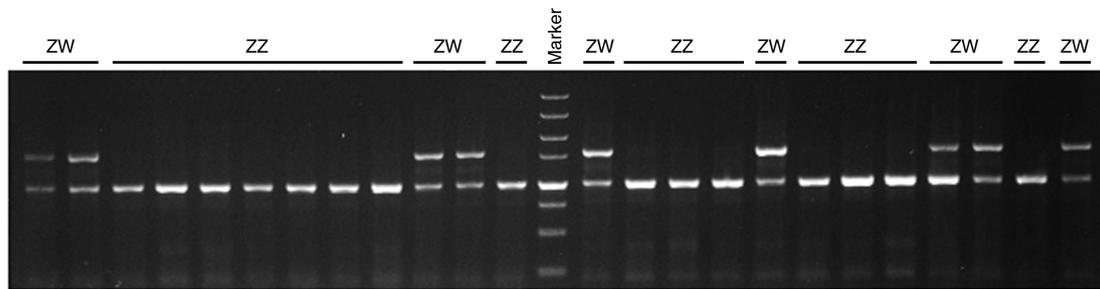


Figure S3. Sex-diagnostic amplification in *Pelodiscus sinensis*. Lower bands represent Z-linked amplified fragments, and higher bands represent W-linked sex-diagnostic fragments. One- and two-band indicated genetic male (ZZ) and female (ZW), respectively.