

GQ Female



SY Female



GQ Male



SY Male

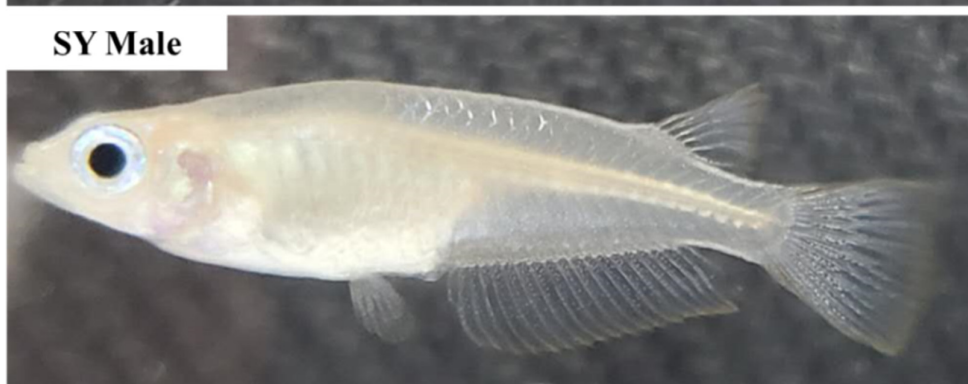


Figure S1. *O. curvinotus* form Gaoqiao and Sanya

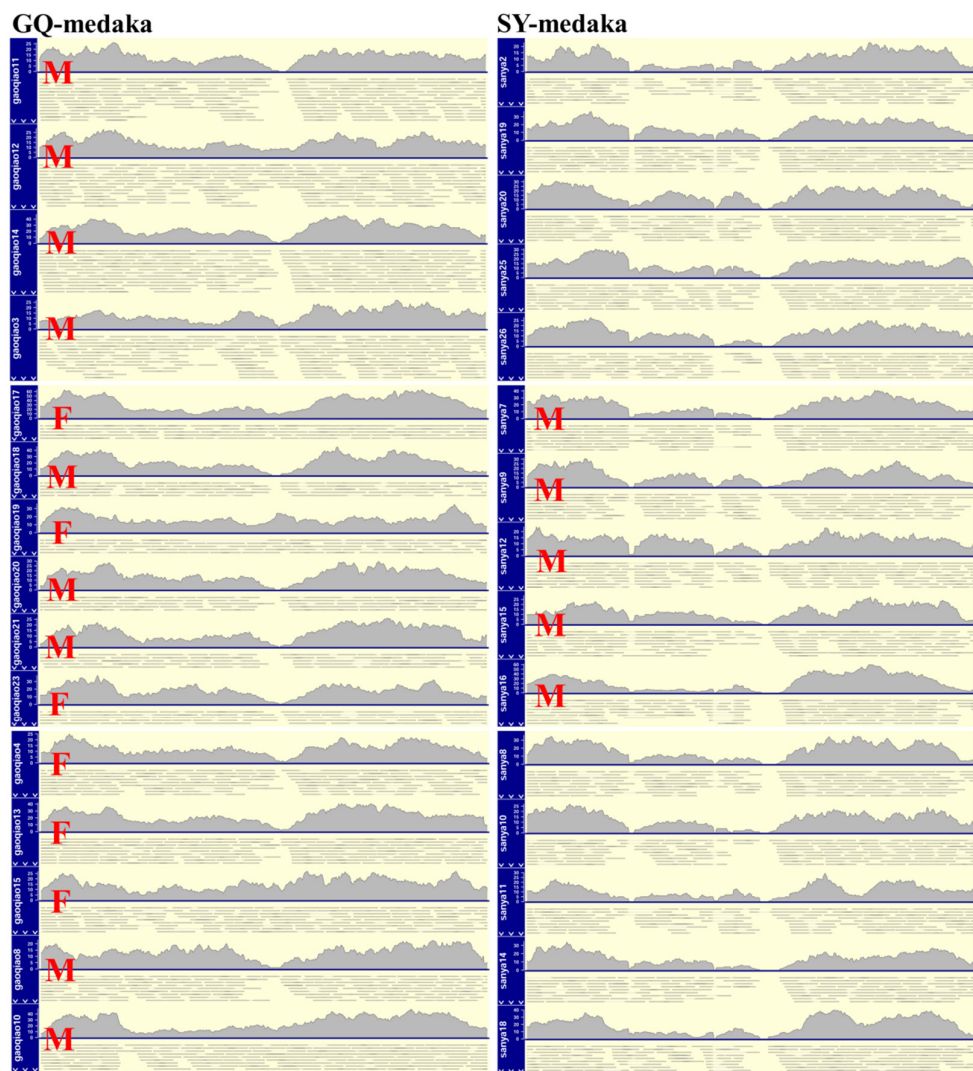


Figure S2. Coverage of *dmrt1* by whole genome re-sequencing reads of GQ-medaka and SY-medaka. M indicates males and F indicates females.

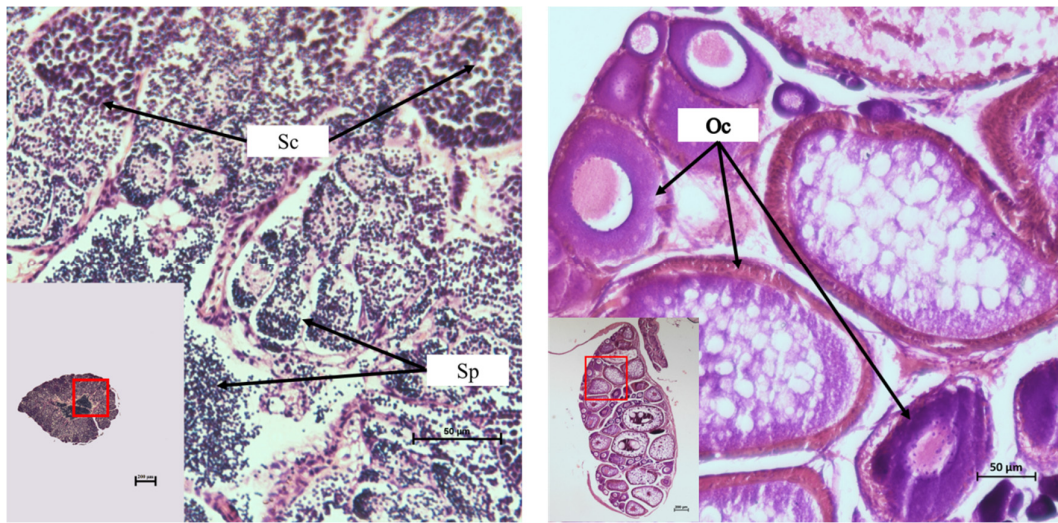


Figure S3. Histological section of testis and ovary of SY-medaka. SC, spermatocytes Sp, spermatids; OC, oocytes.

Species distribution

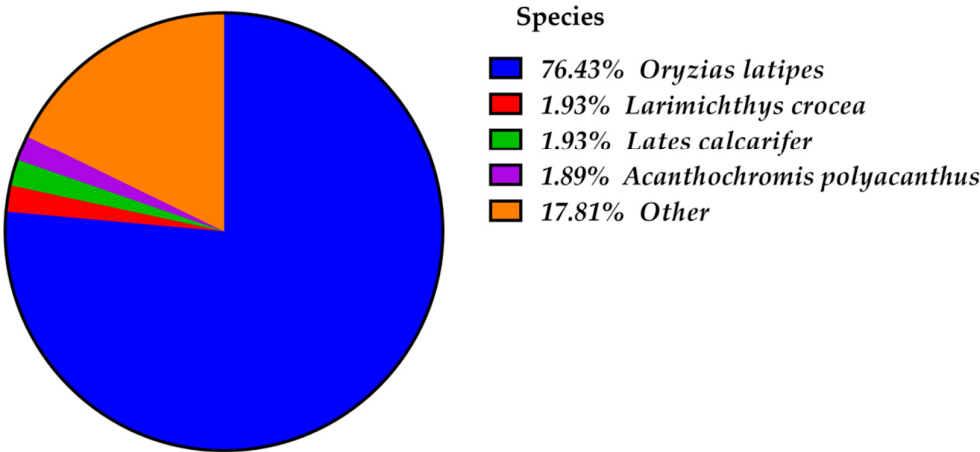


Figure S4. Distribution of homologous species of SY-medaka unigenes

Table S1. The sequences of primers used in this study

Primer name	Sequence 5'-3'	purpose
Co1-F	TCAACCAACCACAAAGACAATGGCAC	Species identification
Co1-R	TAGACTTCTGGGTGGCCAAAGAATCA	
Ocsex-F	ATGGTAACGCAGCCTTTCC	Genetic sex determination
Ocsex-R	GCCACATTCTTCTCAGGCA	
<i>cbpa1</i>	F: CTTCACCACGAGCAATGTC, R: GGGAGTGTCCAGATTCAAAGTG	qPCR
<i>odf3a</i>	F: TCAGGGTGTAACCTGGTGCC, R: TCTTTGGCTGGGAGACTTCC	qPCR
<i>cmbp4b</i>	F: CGAAGACGCTACTCCTGATGTATC, R: CCTTTCTGTCCGCCTCAAAC	qPCR
<i>saca4</i>	F: TGTAACGAGGCACCTGAATCC, R: CACACACAGTCAAAGGCTTCTTC	qPCR
<i>bmp15</i>	F: CAAACTTCACTCCGTCACCC, R: GACAGTCTCCTTGGCAGTATCG	qPCR
<i>h2a1a</i>	F: TAGCACCACGCCACATCTTG, R: AGCAGCACCGTCTTCTTTTCG	qPCR
<i>zp4</i>	F: TCTTGGAGGTCAACCCTGTTC, R: TCTACACAACCCTTTGCGAGAC	qPCR
<i>horn</i>	F: CATCAGAAACCTACAGGACAATCC, R: TCATCGTCGTCGTCACCATC	qPCR
<i>loc101162755</i>	F: GCTGAACCTTTGCCCTGATG, R: AGTGAGCGAGGAGGACCAAG	qPCR
<i>dmrt1</i>	F: CTTCAATTCTTACCACCACCTTC, R: CGGAGCGTCAGAGAGTCAT	qPCR
<i>zglp1</i>	CCCAGAGAAGAAGCCAAGTTC, R: ATGGTGGTCAGCATCGTCAG	qPCR
<i>loc101175599</i>	F: TGTCAAACAAGAGAATGGGAGC, R: TCTGAAGTGGATGGAAGTGTAGC	qPCR
<i>spc11</i>	F: CATCAGGTCCATCAAGGCTTC, R: GAGGAGTGTGAAACCGTAGAGC	qPCR
<i>nanos3</i>	F: ACGGAGGTCAGTTTCGGATG, R: TAATGAACGGGTGAAGCGTG	qPCR
<i>gsdf</i>	F: TAGCCTATCTTGACGGACAGCC, R: GCTCTTCCCTTGATGGATGC	qPCR
<i>insl5</i>	F: GTCAGCCCGTCAACCAGAATC, R: CAAACATCTCACAGGAAAGGAGG	qPCR
<i>cyp19a</i>	F: CTCTTCTGGGTGTTCCTGTTG, R: TCTTGTGCCTCTGATGAATCC	qPCR
<i>izumo1</i>	F: GGTCTCAGCAGCAGGGATAAAG, R: AGGAAATCATCAGAACCCAGAGTG	qPCR
<i>mlf1</i>	F: GCTTCAGACTCATCCAGGTTC, R: GCCACTACATCCAAGACAGAGG	qPCR
<i>foxl2</i>	F: TTCAAGACAGAAGCAGCAGTATTC, R: GCCGATGACACCTTTATCTCC	qPCR
<i>ee1b</i>	F: GATGATGACATTGACCTGTTTGG, R: GATGGAGGACTTGGCGATG	qPCR
<i>rps4x</i>	F: CGAAACACTGGATGCTGGATAAG, R: ACTTCAGGCGGTTCTCAGG	qPCR

Table S2. The COI sequencing results of 15 SY-medaka

Name	Sequence 5'-3'
COI_1#	AAAAATGTTGTATTAGGTTCCGATCTGTGAGAAGCATGGTGATACCTGCAGCTAGAACAGGAAGAGA GAGTAGAAGTAATACTGCGGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGGGAAATGG CTGGAGGTTTATATTAATAATAGTTGTGATGAAATTAATGGCCCTAGGATAGAAGAAATCCGGCCAG GTGAAGAGAGAAAATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGACAAGGGGGGA TATACGGTTCATCCTGTTCGGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGAAGGAAAGAAAGG GGGCAGGAGTCAAAAGCTTATATTATTTATCCGGGGGAAGGCCATGTCGGGGGCTCCAATCATTAAAG GGATTAATCAGTTGCCAAACCCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTACGAAAGCAT GTGCAGTTACAATTACGTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTTCTGCCCC AATGAGTAGACTTAAGGCCGTCCCTACCATTCCCGCCAGGCACCAAGATTAGATACAGGGTGCCAT TGCTTTGTGGTT
COI_2#	TCCGATCTGTGAGAAGCATGGTGATACCTGCAGCTAGAACAGGAAGAGAGAGTAGAAGTAATACTGC GGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGGGAAATGGCTGGAGGTTTATATTAAT AATAGTTGTGATGAAATTAATGGCCCTAGGATAGAAGAAATCCGGCCAGGTGAAGAGAGAAAATGG TTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGACAAGGGGGGATATACGGTTCATCCTGTTC CGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGAAGGAAAGAGGGGGCAGGAGTCAAAAGC TTATATTATTTATCCGGGGGAAGGCCATGTCGGGGGCTCCAATCATTAAAGGGATTAATCAGTTGCCAA ACCCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTACGAAAGCATGTGCAGTTACAATTACGTT ATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTTCTGCCGAATGAGTAGACTTAAGGC CGTCCCTACCATT
COI_3#	TTCCGATCTGTGAGAAGCATGGTGATACCTGCAGCTAGAACAGGAAGAGAGAGTAGAAGTAATACTGC GGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGGGAAATGGCTGGAGGTTTATATTAAT AATAGTTGTGATGAAATTAATGGCCCTAGGATAGAAGAAATCCGGCCAGGTGAAGAGAGAAAATGG TTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGACAAGGGGGGATATACGGTTCATCCTGTTC CGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGAAGGAAAGAGGGGGCAGGAGTCAAAAGC TTATATTATTTATCCGGGGGAAGGCCATGTCGGGGGCTCCAATCATTAAAGGGATTAATCAGTTGCCAA ACCCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTACGAAAGCATGTGCAGTTACAATTACGTT ATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTTCTGCCGAATGAGTAGACTTAAGGC CGTCCCTACCATTCCCGCCAGGCACCAAGATTAGATACAGGGTGCCATTGTCTT
COI_4#	AAGAGAGAGTAAAAGTAATACTGCGGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGGG AAATGGCTGGAGGTTTATATTAATAATAGTTGTGATGAAATTAATGGCCCCAGGATAGAAGAAATTC CGGCCAGGTGAAGAGAGAAAATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGACAA GGGGGATATACGGTTCATCCTGTTCGGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGAAGGA AAGAAGGGGGCAGGAGTCAAAAGCTTATATTATTTATCCGGGGGAAGGCCATGTCGGGGGCTCCAATC ATTAAAGGGATTAATCAGTTGCCAAACCCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTACGA AAGCATGTGCAGTTACAATTACGTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTTC TGCCGAATGAGTAGACTTAAGGCCGTCCCTACCATTCCCG
COI_5#	AACAGGAAGAGAGAGTAGAAGTAATACTGCGGTAATTAGTACAGCTACACAAATAAAGGGGTTTGAT ATTGGGAAATGGCTGGAGGTTTATATTAATAATAGTTGTGATGAAATTAATGGCCCTAGGATAGAAGA AATTCCGGCCAGGTGAAGAGAGAAAATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTG ACAAGGGGGGATATACGGTTCATCCTGTCCCGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGA AGGAAAGAAGGGGGCAGGAGTCAAAAGCTTATATTATTTATCCGTGGGAAGGCCATGTCGGGGGCTCC AATCATTAAAGGAATTAATCAGTTGCCAAACCCCCCAATTATGATTGGCATTACTATAAAGAAAATTATT ACGAAAGCATGTGCAGTTACAATTACGTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTT AGTTCTGCCGAATGAGTAGACTTAAGGCCGTCCCAACCATTCCCGCCAGGCACCAAGATTAGATA CAGGGTGCCATTGCTTTGTGGTTTG
COI_6#	TGAGAAGCATGGTGATACCTGCAGCTAGAACAGGAAGAGAGAGTAGAAGTAATACTGCGGTAATTAGT ACAGCCACACAAATAAAGGGGTTTGATATTGGGAAATGGCTGGAGGTTTATATTAATAATAGTTGTG ATGAAATTAATGGCCCTAGGATAGAAGAAATCCGGCCAGGTGAAGAGAGAAAATGGTTAAATCTAC GGAGGCCCTGCGTGTGCCAAATTACCTGACAAGGGGGGATATACGGTTCATCCTGTTCGGGCACCAG CTTCTACACCAGATGAGGCCAATAATAGAAGGAAAGAGGGGGCAGGAGTCAAAAGCTTATATTATTT ATCCGGGGGAAGGCCATGTCGGGGGCTCCAATCATTAAAGGGATTAATCAGTTGCCAAACCCCCCAAT TATGATTGGCATTACTATAAAGAAAATTATTACGAAAGCATGTGCAGTTACAATTACGTTATAGATCTGG TCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTTCTGCCGAATGAGTAGACTTAAGGCCGTCCCTACC ATTCCCGCCAGGCACCAAGATTAGATACAGG
COI_7#	AGGAAGAGAGAGTAGAAGTAATACTGCGGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATAIT

	GGGAAATGGCTGGAGGTTTATATTAATAATAGTTGTGATGAAATTAATGGCCCCAGGATAGAAGAAA TTCCGGCCAGGTGAAGAGAGAAAAATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGAC AAGGGGGGATATACGGTTCATCCTGTTCGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGAAG GAAAGAAGGGGGCAGGAGTCAAAAGCTTATATTATTTATCCGGGGGAAGGCCATGTCGGGGGCTCCAA TCATTAAGGGGATTAATCAGTTGCCAAACCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTAC GAAAGCATGTGCAGTTACAATTACGTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGT TCTGCCCGAATGAGTAGACTTAAGGCCGTCCCTACCATTCCCGCCCAGGCACCAAAGA
COI_8#	TAGGTTCCGATCTGTGAGAAGCATGGTGATACCTGCAGCTAGAACAGGAAGAGAGAGTGAAGTAATA CTGCGGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGGGAAATGGCTGGAGGTTTATAT TAATAATAGTTGTGATGAAATTAATGGCCCTAGGATAGAAGAAATTCCGGCCAGGTGAAGAGAGAAA ATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGACAAGGGGGGATATACGGTTCATCCT GTTCGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGAAGGAAAGAAGGGGGCAGGAGTCAAA AGCTTATATTATTTATCCGGGGGAAGGCCATGTCGGGGGCTCCAATCATTAAAGGGATTAATCAGTTGCC AAACCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTACGAAAGCATGTGCAGTTACAATTAC GTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTCTGCCCGAATGAGTAGACTTAAG GCCGTCCCTACCATTCCCGCCCAGGCACCAA
COI_9#	GGTCGAAAAATGTTGTATTTAGGTTCCGATCTGTGAGAAGCATGGTGATACCTGCAGCTAGAACAGGA AGAGAGAGTAGAAGTAATACTGCGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGGGA AATGGCTGGAGGTTTATATTAATAATAGTTGTGATGAAATTAATGGCCCTAGGATAGAAGAAATCCG GCCAGGTGAAGAGAGAAAAATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGACAAGG GGGGATATACGGTTCATCCTGTTCGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGAAGGAAA GAAGGGGGCAGGAGTCAAAAGCTTATATTATTTATCCGGGGGAAGGCCATGTCGGGGGCTCCAATCAT TAAAGGGATTAATCAGTTGCCAAACCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTACGAA AGCATGTGCAGTTACAATTACGTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTTCT GCCCGAATGAGTAGACTTAAGGCCGTCCCTACCATTCCCGCCCAGGCACCAAAGATTAGATACAGGGT GCCATTGTC
COI_10#	GGTCGAAAAATGTTGTATTTAGGTTCCGATCTGTGAGAAGCATGGTGATACCTGCAGCTAGAACAGGA AGAGAGAGTAGAAGTAATACTGCGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGGGA AATGGCTGGAGGTTTATGTTAATAATAGTTGTGATGAAATTAATGGCCCTAGGATGGAAGAAATTC GGCCAGGTGAAGAGAGAAAAATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGACAAG GGGGATATACGGTTCATCCTGTTCGGCACCAGCTTCTACACCAGATGAGGCCAATAATAGAAGGAA AGAAGGGGGCAGGAGTCAAAAGCTTATATTATTTATCCGTGGGAAGGCCATGTCGGGGGCTCCAATCA TTAAAGGGATTAATCAGTTGCCAAACCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTACGA AAGCATGTGCAGTTACAATTACGTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTTC TGCCCGAATGAGTAGACTTAAGGCCGTCCCTACCATTCCCGCCCAGGCACCAAAGATTAGATACAGGG TG
COI_11#	CGAAAAATGTTGTATTTAGGTTTCGATCTGTGAGAAGCATGGTGATACCTGCAGCTAGAACAGGAAGG GAGAGTAGAAGTAATACTGCGTAATTAGTACAGCTCACACAAATAAAGGGGTTTGATATTGGGAAAT GGCTGGGGGTTTATGTTAATAATAGTTGTGATGAAATTAATGGCCCTAGGATAGAAGAAATCCGGC CAGGTGAAGAGAGAAAAATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCTGACAAGGGG GGATATACGGTTCATCCTGTTCGGCACCAGCTTCTACACCAGATGAGGCCAATAAAGAAGGAAAGA AGGGGGCAGGAGTCAAAAGCTTATATTATTTATCCGTGGGAAGGCCATGTCGGGGGCTCCAATCATTAA AGGAATTAATCAGTTGCCAAACCCCCAATTATGATTGGCATTACTATAAAGAAAATTATTACGAAAGC ATGTGCAGTTACAATTACGTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAGTTCTGCC CGAATGAGTAGACTTAAGGCCGTCCCTACCATTCCCGCCCAGGCACCAAAGA
COI_12#	GGGTCGAAAAATGTTGTATTTAGGTTTCGATCTGTGAGAAGCATAGTGATACCTGCAGCTAGAACAGG AAGAGAGAGTAGGAGTAATACTGCGTAATTAGAACAGCTCACACAAATAAAGGGGTTTGATATTGGG AGATGGCTGGAGGTTTATGTTAATAACTTGTGAATGAAATTAATGGCCCTAGAATAGAAGAAATTC GGCCAGGTGAAGAGAGAAAAATGGTTAAATCTACGGAGGCCCTGCGTGTGCCAAATTACCGGACAAG GGGGATATACGGTTCATCCTGTCCCGCACCAGCTTCTACACCAGATGAGGCCAATAAAGAAGGAA AGAAGGGGGCAGGAGTCAAAAGCTTATATTATTTATCCGGGGGAAGGCCATGTCGGGAGCTCCGATCA TTAAAGGAATTAATCAGTTGCCGAACCTCCAATTATAATTGGCATTACTATAAAGAAAATTATTACGAA AGCATGTGCAGTTACAATTACGTTATAGATCTGGTCGTCTCCTAATAGGGAGCCTGGTTGACTTAATTCT GCCCGAATGAGCAGACTTAAGGCCGTCCCTACTATTCCCGCCCAGGCACCAA
COI_13#	GTATTTAGGTTCCGATCTGTGAGAAGTATGGTGATTCTGCAGCTAGAACAGGAAGAGAGAGTAGAAG TAATACTGCGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGAGAAATGGCTGGAGGTTT CATGTTAATAATTGTTGTGATGAAATTAATGGCCCTAAGATGGAAGAAATTCCTGCCAGGTGAAGAGA

	GAAAAATGGTTAAGTCTACGGATGCCCCCTGCGTGTGCCAAATTACCTGACAAGGGGGGATATACGGTTC ATCCTGTTCGGGCACCAGCTTCTACACCAGATGAAGCCAATAAAAGAAGGAAAGAAGGGGGCAGGAG TCAAAAAGCTTATATTATTTATCCGAGGGAAGGCCATGTCTGGGGCTCCAATCATTAAAGGGATTAATCA GTTGCCAAACCCCCCAATTATGATTGGCATTACTATAAGAAAATTATTACGAAAGCATGTGCAGTTAC AATTACGTTATAAATCTGGTCGTCTCCTAATAGAGAGCCTGGTTGACTTAATTCTGCCCCG
COI_14#	TGTATTTAGGTTCCGATCTGTGAGAAGTTGGTGATTCTGCAGCTAGAACAGGAAGAGAGAGTAGAAG TAATACTGCGGTAATTAGTACAGCCACACAAATAAAGGGGTTTGATATTGGGAAATGGCTGGAGGTTT CATGTTAATAATTGTTGTGATGAAATTAATGGCCCTAAGATGGAAGAAATTCCTGCCAGGTGAAGAGA GAAAAATGGTTAAGTCTACGGATGCCCCCTGCGTGTGCCAAATTACCTGACAAGGGGGGATATACGGTTC ATCCTGTTCGGGCACCAGCTTCTACACCAGATGAGGCCAATAAAAGAAGGAAAGAAGGGGGCAGGAG TCAAAAAGCTTATATTATTTATCCGGGGGAAGGCCATGTCTGGGGCTCCAATCATTAAAGGGATTAATCA GTTGCCAAACCCCCCAATTATGATTGGCATTACTATAAGAAAATTATTACGAAAGCATGTGCAGTTAC AATTACGTTATAAATCTGGTCGTCTCCTAATAGAGAGCCTGGTTGACTTAATTCTGCCCCG
COI_15#	CCTGCGGGGTCGAAGATGTTGTATTTAGGTTCCGATCTGTGAGAAGTATGGTGATTCTGCAGCTAGAA CAGGAAGGGAGAGTAGAAGTAAACTGCGGTAATTAGTACAGCTCACACAAATAAGGGGGTTTGATA TTGGGAAATGGCTGGGGGTTTCATGTTAATAATTGTTGTGATGAAATTAATGGCCCTAAGATGGAAGA AATTCCTGCCAGGTGAAGAGAGAAAATGGTTAAGTCTACGGATGCCCCCTGCGTGTGCCAAATTCCTG ACAAGGGAGGATATACGGTTCATCCTGTTCGGGCACCAGCTTCTACACCAGATGAAGCCAATAAAAGA AGGAAAGAAGGGGGCAGGAGTCAAAAGCTTATATTATTTATCCGAGGGAAGGCCATGTCTGGGGCTCC AATCATTAAAGGAATTAATCAGTTGCCAAACCCCCCAATTATGATTGGCATTACTATAAGAAAATTATT ACGAAAGCATGTGCAGTTACAATTACGTTATAAATCTGGTCGTCTC

Table S3. Estimates of Evolutionary Divergence between Sequences

	SY-medaka	Ocu	Olu	Ola	Osi	Oja	Omi	Oda	Ome	Oce	Osa	Oma	Dre
SY-medaka													
Ocu	0.005												
Olu	0.095	0.094											
Ola	0.135	0.135	0.144										
Osi	0.137	0.137	0.145	0.107									
Oja	0.184	0.185	0.191	0.186	0.188								
Omi	0.204	0.204	0.214	0.205	0.206	0.187							
Oda	0.187	0.186	0.200	0.191	0.187	0.169	0.181						
Ome	0.185	0.185	0.200	0.189	0.187	0.169	0.180	0.010					
Oce	0.189	0.189	0.199	0.192	0.188	0.195	0.199	0.184	0.184				
Osa	0.192	0.191	0.198	0.194	0.189	0.199	0.208	0.193	0.193	0.096			
Oma	0.188	0.187	0.196	0.191	0.188	0.198	0.204	0.190	0.189	0.093	0.070		
Dre	0.266	0.265	0.272	0.272	0.267	0.275	0.272	0.267	0.267	0.263	0.266	0.263	

Ocu, *O. curvinotus*; *Olu*, *O. luzonensis*; *Ola*, *O. latipes*; *Osi*, *O. sinensis*; *Oja*, *O. javanicus*; *Omi*, *O. minutillus*; *Oda*, *O. dancena*; *Ome*, *O. melastigma*; *Oce*, *O. Celebensis*; *Osa*, *O. sarasinorum*; *Oma*, *O. marmoratus*; *Dre*, *D. rerio*. The mitochondrial sequences ID of those fish are shown in Figure. 1B.

Table S4. Summary statistics of testes and ovaries transcriptome data of *O. curvinotus*

Sample	ovary_SY1	ovary_SY2	ovary_SY3	testis_SY1	testis_SY2	testis_SY3
Raw Reads(M)	63.23	62.86	63.15	62.85	63.53	63.27
Clean Reads(M)	61.01	60.92	61.14	61.2	61.59	61.08
Clean Reads Q20(%)	98.35	98.53	98.38	98.57	98.68	98.39
Clean Reads Q30(%)	94.25	95.06	94.21	95.03	95.41	94.25
GC content (%)	49.23	49.03	48.89	49.16	48.32	49.01
Transcripts number	64,959	66,276	72,248	75,928	98,055	87,829
Unigenes number	49,135	49,232	52,952	55,270	61,902	60,810

Table S5. *O. curvinitus* transcriptome reference assembly and annotation statistics

Database	Number	Percentage (%)
Assembly		
Total base (bp)	94,333,707	
Number of unigenes	84,484	
Average length (bp)	1,116	
N50 (bp)	2,384	
GC content		48.67
Annotation		
Annotated in NR	46,377	54.89
Annotated in NT	78,492	92.91
Annotated in KEGG	37,636	44.55
Annotated in SwissProt	37,843	44.79
Annotated in KOG	33,324	39.44
Annotated in GO	10,221	12.10
Overall	79,298	93.87

Table S6. The expression patterns of some DEGs related to gonad development and reproduction

Gene	Gene annotation	Probabilit	Nr ID	log2FC(ovary/testi
<i>wnt17b</i>	wingless-type MMTV integration site family, member 7B	1	XP_020569989.	-7.39518
<i>wnt4b</i>	wingless-type MMTV integration site family member 4b	1	NP_001153912.	-8.82761
<i>wnt2</i>	wingless-type MMTV integration site family member 2	0.999605	XP_020492875.	-7.49185
<i>trd7b</i>	Tudor domain-containing protein 7B	0.999051	XP_004079778.	-2.67008
<i>theg</i>	Testicular haploid expressed gene protein	0.999995	XP_020569141.	-6.79008
<i>tdr15</i>	Tudor domain-containing protein 15	1	XP_018550215.	-3.03149
<i>star9</i>	StAR-related lipid transfer protein 9	0.998506	XP_020569428.	-2.44163
<i>spef2</i>	Sperm flagellar protein 2	1	XP_020561437.	-8.77329
<i>spef1</i>	Sperm flagellar protein 1	1	XP_004077227.	-5.21739
<i>spc11</i>	Speriolin-like protein	1	XP_020565798.	-14.1918
<i>spat7</i>	Spermatogenesis-associated protein 7	0.999657	XP_011488521.	-2.11963
<i>sox9</i>	SRY (sex determining region Y)-box 9	1	BAH05019.1	-4.37676
<i>smc1b</i>	Structural maintenance of chromosomes protein 1B	1	XP_017285706.	-8.81513
<i>smc3</i>	structural maintenance of chromosomes protein 3	0.99944643	XP_004066378.	-2.00674226
<i>rec8</i>	Meiotic recombination protein REC8 homolog	1	XP_004081264.	-5.22288
<i>r3hd4</i>	R3H domain-containing protein 4	0.999294	XP_004068025.	-7.89785
<i>pol5</i>	Retrovirus-related Pol polyprotein from transposon opus	0.999049	XP_008293872.	-3.43296
<i>pol4</i>	Retrovirus-related Pol polyprotein from transposon 412	0.995518	XP_019221790.	-3.55639
<i>pol3</i>	Retrovirus-related Pol polyprotein from transposon 17.6	0.993254	XP_008283279.	-2.49442
<i>pk3cb</i>	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit beta	0.982855	XP_011481352.	-2.70834
<i>mlf1</i>	Myeloid leukemia factor 1	1	XP_011481028.	-8.19251
<i>kat7</i>	Histone acetyltransferase KAT7	1	XP_020561190.	-5.26325
<i>kat5</i>	Histone acetyltransferase KAT5	0.998634	XP_011481726.	-3.41885
<i>kapca</i>	cAMP-dependent protein kinase catalytic subunit alpha	0.999524	XP_008434985.	-5.11088
<i>izumo1</i>	Izumo sperm-egg fusion protein 1	1	XP_020561433.	-8.92296
<i>itpr3</i>	Inositol 1,4,5-trisphosphate receptor type 3	0.998897	XP_020557263.	-4.1838
<i>itpr1</i>	Inositol 1,4,5-trisphosphate receptor type 1	0.986726	XP_020559132.	-2.36535
<i>hsp70</i>	Heat shock cognate 70 kDa protein	0.999753	NP_001098385.	-7.06286
<i>hsd17b</i>	hydroxysteroid 17-beta dehydrogenase 7	0.998642	XP_017280664.	-4.72247
<i>hs90a</i>	Heat shock protein HSP 90-alpha	0.998449	XP_004083819.	-2.81199
<i>hdac8</i>	Histone deacetylase 8	0.999995	XP_011485972.	-2.53244
<i>hdac7</i>	Histone deacetylase 7	0.999047	XP_011473874.	-2.90754
<i>hdac3</i>	Histone deacetylase 3	1	XP_004073599.	-2.75168
<i>gtd2b</i>	General transcription factor II-I repeat domain-containing protein 2B	1	XP_004077286.	-9.38298
<i>gsdf</i>	gonadal soma derived factor	0.999904	NP_001171213.	-2.92127
<i>gphb5</i>	Glycoprotein hormone beta-5	0.998848	XP_020567290.	-7.88874
<i>gnrr2</i>	gonadotropin-releasing hormone receptor 2	1	NP_001098392.	-9.34725
<i>gnaq</i>	Guanine nucleotide-binding protein G(q) subunit alpha	0.99888	XP_020556761.	-2.18813
<i>gna14</i>	Guanine nucleotide-binding protein subunit alpha-14	0.998538	XP_004074607.	-2.21025
<i>gin1</i>	Gypsy retrotransposon integrase-like protein 1	0.996027	XP_016415952.	-4.12928
<i>gata5</i>	Transcription factor GATA-5	0.998401	XP_011475114.	-3.54386
<i>fgfr3</i>	Fibroblast growth factor receptor 3	0.995207	XP_004082745.	-2.50714
<i>dmrt1</i>	Doublesex- and mab-3-related transcription factor 1	1	AA591465.1	-9.7535
<i>dhcr7</i>	7-dehydrocholesterol reductase	0.998121	XP_022071062.	-2.6881
<i>dhc24</i>	Delta(24)-sterol reductase	1	XP_018525864.	-8.27922
<i>dh12b</i>	Very-long-chain 3-oxoacyl-CoA reductase-B	0.982641	XP_011485867.	-2.36457
<i>cpeb4</i>	Cytoplasmic polyadenylation element-binding protein 4	0.999635	XP_011481506.	-4.70769
<i>cp4b1</i>	cytochrome P450 4B1-like	0.999993	XP_004078318.	-6.35814
<i>cp26b</i>	cytochrome P450, family 26, subfamily B	0.998745	NP_001265750.	-5.564
<i>cp11b</i>	cytochrome P450 11beta	1	NP_001098570.	-7.91754
<i>cfa70</i>	Cilia- and flagella-associated protein 70	1	XP_020567678.	-8.26835
<i>cfa54</i>	Cilia- and flagella-associated protein 54	0.99999	XP_020564512.	-6.77196
<i>cb5d1</i>	cytochrome b5 domain-containing protein 1	1	XP_004086762.	-7.56122
<i>amh</i>	anti-Mullerian hormone	0.984112	NP_001098198.	-1.0285
<i>akt3</i>	RAC-gamma serine/threonine-protein kinase	0.999764	XP_007570539.	-3.84082
<i>zp4</i>	ona pellucida sperm-binding protein 4	1	XP_011472723.	4.20555243
<i>zp3</i>	ona pellucida sperm-binding protein 3	1	XP_011488823.	12.42472542
<i>zp1</i>	ona pellucida sperm-binding protein 1	1	XP_004071858.	4.428162622
<i>zglp1</i>	zinc finger, GATA-like protein 1	1	XP_004065851.	7.585234067

<i>zar1</i>	Zygote arrest protein 1	1	XP_004067611.	9.217390254
<i>wnt9b</i>	wingless-type MMTV integration site family, member 9B	0.99999958	XP_020560833.	7.686500527
<i>wnt9a</i>	wingless-type MMTV integration site family member 9a	0.99698495	XP_004079455.	4.041963293
<i>wnt5b</i>	wingless-type MMTV integration site family, member 5B	0.99752726	XP_011489302.	3.348832279
<i>wnt5a</i>	wingless-type MMTV integration site family, member 5A	0.99941565	XP_022059770.	4.371022644
<i>wnt4a</i>	wingless-type MMTV integration site family member 4a	0.99840374	NP_001153911.	2.032069901
<i>wnt16</i>	wingless-type MMTV integration site family, member 16	0.99881709	XP_020557844.	5.32309109
<i>wnt2bb</i>	wingless-type MMTV integration site family, member 2Bb	0.99158103	XP_011475046.	2.704871964
<i>wif1</i>	Wnt inhibitory factor 1	0.99970288	XP_020558359.	5.764871591
<i>tf7l2</i>	Transcription factor 7-like 2	0.99546189	XP_022046618.	2.036951136
<i>tesk1</i>	Dual specificity testis-specific protein kinase 1	0.99980362	XP_011487062.	2.161067814
<i>sx17a</i>	SRY (sex determining region Y)-box 17	0.99785707	NP_001158345.	2.515945327
<i>strbp</i>	Spermatid perinuclear RNA-binding protein	0.99693893	XP_018525986.	2.205448658
<i>spat1</i>	Spermatogenesis-associated protein 1	0.99378780	XP_011472245.	2.683194338
<i>sp130</i>	Histone deacetylase complex subunit SAP130	1	XP_011472693.	3.005244672
<i>sox7</i>	SRY (sex determining region Y)-box 7	0.99889622	XP_011490013.	2.484079144
<i>sox10</i>	SRY (sex determining region Y)-box 10	0.99951725	NP_001158343.	5.294620749
<i>sox6</i>	SRY (sex determining region Y)-box 6	0.94845405	XP_019213816.	1.131911676
<i>sox17</i>	SRY (sex determining region Y)-box 17	0.96333333	NP_001158345.	2.515945327
<i>sx18a</i>	SRY (sex determining region Y)-box 18	0.92435533	XP_011475950.	1.071622928
<i>sox5</i>	SRY (sex determining region Y)-box 5	0.98125103	BAO57768.1	1.413397141
<i>sox4</i>	SRY (sex determining region Y)-box 4	0.99488196	XP_020555578.	1.876193798
<i>rspo3</i>	R-spondin-3	0.99966952	XP_015250864.	6.942514505
<i>rarga</i>	Retinoic acid receptor gamma-A	0.99748218	XP_004069175.	4.611024797
<i>pthd1</i>	Patched domain-containing protein 1	0.99858132	XP_011487428.	4.442943496
<i>ptc1</i>	Protein patched homolog 1	0.99540125	XP_011485123.	2.329214449
<i>nr6a1</i>	Nuclear receptor subfamily 6 group A member 1-A	0.99977779	XP_007557810.	4.218834602
<i>nr5a2</i>	Nuclear receptor subfamily 5 group A member 2	1	NP_001098298.	3.715420433
<i>nr4a1</i>	Nuclear receptor subfamily 4 group A member 1	1	XP_004070930.	5.448553828
<i>nr2f6</i>	Nuclear receptor subfamily 2 group F member 6	0.99965987	XP_004078947.	2.972767361
<i>nr1d2</i>	Nuclear receptor subfamily 1 group D member 2	0.99849059	XP_011492490.	2.150889227
<i>nanos3</i>	Nanos homolog 3	0.99946184	NP_001116300.	4.778718695
<i>lef1</i>	Lymphoid enhancer-binding factor 1	0.99665026	XP_011474499.	4.026472211
<i>kdm6b</i>	lysine-specific demethylase 6B-like	0.99881383	XP_011482001.	2.468659977
<i>kdm6a</i>	lysine-specific demethylase 6A isoform X1	1	XP_020568543.	2.953526973
<i>kat6b</i>	Histone acetyltransferase KAT6B	0.99368946	XP_020561845.	3.36923381
<i>hdac7</i>	Histone deacetylase 7	0.99737741	XP_004070312.	2.523867903
<i>hdac4</i>	Histone deacetylase 4	0.99794789	XP_011488255.	2.327989848
<i>gper1</i>	androgen receptor alpha	0.98747173	XP_004071380.	2.266786541
<i>gon3</i>	Progonadoliberin-3	0.99864504	NP_001098142.	8.537747862
<i>gnrr1</i>	gonadotropin-releasing hormone receptor 1	0.99488797	NP_001098352.	3.101719921
<i>gdf9</i>	Growth/differentiation factor 9	1	XP_004073386.	4.092295995
<i>fzd9</i>	Frizzled-9	0.99999877	XP_004075813.	3.723658827
<i>fzd7</i>	Frizzled-7	0.99142416	XP_018521153.	2.060473859
<i>fxp1b</i>	Forkhead box protein P1-B	0.99511063	XP_020558754.	2.643653692
<i>fstl4</i>	Follistatin-related protein 4	0.99974672	XP_022046629.	6.242493627
<i>foxl2</i>	Forkhead box protein L2	1	BAH05020.1	10.0247703
<i>foxk2</i>	Forkhead box protein K2	1	XP_004080716.	3.740955396
<i>foxh1</i>	Forkhead box protein H1	0.99984307	NP_001153943.	3.971122923
<i>foxf1</i>	Forkhead box protein F1	0.99117670	XP_004067264.	2.300917602
<i>figla</i>	factor in the germline alpha isoform X1	1	XP_011477803.	5.814649649
<i>fgfr2</i>	Fibroblast growth factor receptor 2	0.99472363	XP_011483053.	2.046820857
<i>fgfr1a</i>	Fibroblast growth factor receptor 1-A	0.99999999	BAF49180.1	2.187471297
<i>fgf3</i>	Fibroblast growth factor 3	0.99945322	XP_011485317.	5.856066337
<i>fgf16</i>	Fibroblast growth factor 16	0.99945682	XP_004073393.	4.962267353
<i>fgf13</i>	Fibroblast growth factor 13	0.99511659	XP_011478253.	3.532269607
<i>fgf12</i>	Fibroblast growth factor 12	0.99952503	XP_020556550.	3.398233681
<i>dvl1</i>	Segment polarity protein dishevelled homolog DVL-1	0.99968349	XP_008276717.	2.612405897
<i>dhbp7</i>	5.6e-122/3-keto-steroid reductase	0.99951301	XP_004068242.	2.63599101
<i>cp3ar</i>	Cytochrome P450 3A27	0.99999997	XP_004077330.	2.413655224
<i>cp2k1</i>	Cytochrome P450 2K1	0.99999999	AGN04293.1	3.747955999

<i>cp26a</i>	cytochrome P450, family 26, subfamily A,	1	NP_001265772.	6.845619377
<i>cp24a</i>	1,25-dihydroxyvitamin D(3) 24-hydroxylase	1	XP_020558883.	6.074992301
<i>cp19b</i>	cytochrome P450 19b	0.99949942	XP_020559313.	5.466978428
<i>cp19a</i>	cytochrome P450 19A1-like	1	NP_001265808.	11.51191734
<i>cp191b</i>	cytochrome P450 19b	0.99949942	XP_020559313.	5.466978428
<i>bmr1b</i>	Bone morphogenetic protein receptor type-1B	0.99766148	XP_020561516.	2.259910442
<i>bmp7</i>	Bone morphogenetic protein 7	0.99809437	XP_004070841.	2.184590389
<i>bmp15</i>	Bone morphogenetic protein 15	1	XP_004079992.	10.84999144
<i>arb</i>	androgen receptor beta subtype	1	BAI58984.1	4.132855278
<i>ara</i>	Androgen receptor alpha	0.99958649	AHF58586.1	3.936856017