ASER: Animal Sex Reversal database

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Running title: ASER: Animal Sex Reversal Database

The numbers of words: 4321.

The numbers of figures: 4.

The numbers of tables: 1.

The numbers of supplementary figures: 1.

The numbers of supplementary tables: 3.

The numbers of all the References: 40.

The numbers of References from 2014: 23.

- 30 The counts of letters in the article title: 34.
- 31 The counts of letters in the running title: 34.
- 32 The count of keywords: 5.
- 33 The counts of words in Abstract: 183.

34 **Abstract**

35 Sex reversal, representing extraordinary sexual plasticity during the life cycle, not
 36 only triggers reproduction in animals but also affects reproductive and endocrine
 37 system-related diseases and cancers in humans. Sex reversal has been broadly
 38 reported in animals, however, an integrated resource hub of sex reversal information
 39 is still lacking. Here, we constructed a comprehensive database named ASER by
 40 integrating sex reversal-related data of 18 species from Teleostei to Mammals. We
 41 systematically collected 40,018 published papers and mined the Sex
 42 Reversal-associated Genes (SRGs), including their regulatory networks, from 1,611
 43 core papers. We annotated homologous genes and computed conservation scores for
 44 whole genomes across the 18 species. Furthermore, we collected 206 available
 45 RNA-seq data and investigated the expression dynamics of SRGs during sex reversal
 46 or sex determination processes. In addition, we manually annotated 551 ISH images
 47 of SRGs from the literature and described their spatial expression in the gonads.
 48 Collectively, ASER provides a unique and integrated resource for researchers to query
 49 and reuse organized data to explore the mechanisms and applications of SRGs in
 50 animal breeding and human health. The ASER database is publicly available at
 51 <http://aser.ihb.ac.cn/>.

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53 **KEYWORDS:** Sex reversal; SRGs; Database; Omics; Conservation

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62 **Introduction**

63 Sex determination mechanisms in animals mainly include genetic sex
64 determination (GSD) and environmental sex determination (ESD) [1]. In GSD, the
65 primary sex of organisms is determined by genetics during fertilization, while
66 organisms with ESD remain bipotential gonads until they perceive environmental
67 stress to promote sex differentiation during ontogeny [2]. For many years, it was
68 dogma in vertebrates in the field of sex determination that sex would be fixed for life
69 after primary sex determination. After sex reversal was first reported in *Aplocheilus*
70 *latipes* and natural sex reversal was found in *Monopterus javanensis* [3], it has been
71 widely accepted that sex determination is amazingly plastic in vertebrates, especially
72 in fish. This plasticity shows that sexual fate is not an irreversible process. Indeed,
73 this reversible process leads to sex reversal, a redirection of the sexual phenotype
74 during development [4]. Environmental factors can override genetic factors to redirect
75 sexual fate in fish [5] and reptiles [6]. Sex reversal was found to be driven by diverse
76 factors, such as genes, hormones, temperature, and social changes [7]. Unlike sex
77 change, which implies a transition from the stabilized sex to the opposite sex, sex
78 reversal occurs during gonadal development, including the initiation phase and
79 maintenance phase of sex determination [4].

80 Specifically, sex reversal has been studied in fish, reptiles, birds, amphibians and
81 even in mammals. In fish, gonadal differentiation is roughly divided into two groups:
82 hermaphroditic and gonochoristic [5]. Hermaphroditic species undergo sex reversal
83 during their lifetime and include 3 strategies: female-to-male (protogynous),
84 male-to-female (protandrous), or bidirectional (serial) sex change [8]. Taking
85 *Monopterus albus* as an example, an individual is female from the embryonic stage to
86 first sexual maturity, then enters an intersex state, and later develops into a male [9].
87 Additionally, some hermaphroditic species undergo socially cued female-to-male sex
88 reversal, whereby the removal of the dominant male induces sex reversal in a resident
89 female, such as *Thalassoma bifasciatum* [10]. Among gonochoristic fish, sex reversal

is a synergistic result of both GSD and ESD [11]. For example, *Cynoglossus semilaevis* is a gonochoristic fish with a female heterogametic sex determination system ($ZW^{\ominus}/ZZ^{\ominus}$) characterized by GSD and TSD (a subclass of ESD) [12]. In many reptiles, including *Trachemys scripta*, gonadal sex is determined by the environmental temperature during egg incubation [13]. However, estrogens, including estradiol-17 β , have also been proven to participate in the sex determination of *T. scripta* [14]. Sex reversal in birds such as *Gallus gallus*, is mainly related to alterations in sex steroid hormone action, especially estrogens [15]. Amphibians also show plasticity in sex determination, influenced by estrogens, androgens [16], and sometimes by temperature [17]. Sex determination in mammals has been reported to depend on three processes: chromosome determination (XX or XY), appropriate pathway of gonadal differentiation, and accurate development of secondary sexual characteristics [18]. Disrupting any of these three steps of gonadal differentiation can lead to aberrant sex determination. In *Homo sapiens*, the frequencies of XX and XY sex reversal are 1/20,000 and 1/3,000, respectively, and most of these cases are caused by translocations of the sex-determining *SRY* gene [19]. Although sex reversal has been broadly reported among vertebrates, the molecular events underlying sex reversal remain poorly understood, limited by the lack of integrated omics data across species.

Although there are several reproduction-related resources, such as GUDMAP [20], GonadSAGE [21] and ReproGenomics Viewer [22], an integrated and dedicated database for the community studying sex determination and differentiation is missing. The GUDMAP database is a comprehensive gene expression dataset of the developing genitourinary system in mouse with both *in situ* and microarray data. GonadSAGE is a serial analysis of gene expression database for male embryonic gonad development in mouse. The ReproGenomics Viewer is a cross-species database of omics data such as RNA-seq and ChIP-seq for tissues related to reproduction, such as gametogenesis, in 9 model organisms. Here, we developed the Animal Sex Reversal database (ASER), the first functional genomics hub for sex reversal to our best knowledge. The main works of ASER can be roughly divided as follows: (1) We

120 screened 18 important and typical species with sex reversal phenomena from Teleostei
 121 to Mammalia, including *Betta splendens*, *Cyprinus carpio*, *Cynoglossus semilaevis*,
 122 *Danio rerio*, *Equus caballus*, *Epinephelus coioides*, *Lates calcarifer*, *Monopterus*
 123 *albus*, *Oryzias latipes*, *Oreochromis niloticus*, *Paralichthys olivaceus*, *Thalassoma*
 124 *bifasciatum*, *Xenopus laevis*, *Gallus gallus*, *Bos taurus*, *Mus musculus*, and *Homo*
 125 *sapiens*, and summarized the major inducements of sex reversal or common
 126 approaches used to manipulate sex in these species (**Table 1**). (2) We compiled a list
 127 of the most common genes or drugs related to sex reversal. Then, we collected and
 128 analyzed PubMed literature to mine the Sex Reversal-associated Genes (SRGs) and
 129 obtained their regulatory networks. Meanwhile, we gathered protein-protein
 130 interaction networks related to SRGs from the STRING database. (3) To facilitate
 131 users comparing the homology of SRGs in different species, we collected or
 132 assembled the gene annotations for the 18 species, identified homologous genes,
 133 computed the basewise conservation scores across these species, and identified
 134 conserved motifs for orthologous gene groups. (4) We systematically processed
 135 available RNA sequencing (RNA-seq) data and provided gene expression dynamics
 136 during sex reversal between females and males or different developmental stages. A
 137 user-friendly genome browser was customized to visualize these genome-wide data.
 138 (5) We collected and annotated available *in situ* hybridization and
 139 immunocytochemistry (ISH, FISH, and ICH) data to display the spatial expression of
 140 SRGS in the gonads. In conclusion, our ASER database provides comprehensive and
 141 systemic integration of sex reversal related data, and we believe that this open
 142 resource will greatly promote research on the mechanisms of sex reversal.

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144 **Data collection and database content**

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146 **Framework of ASER**

147 An overview of the ASER database and web server is shown in **Figure 1**. The ASER
 148 database contains information for 18 sex reversal species, SRG regulatory networks,

149 homology alignment, and (fluorescence) *in situ* hybridization and
 150 immunocytochemistry (ISH, FISH, and ICH) images of SRGs. The preprocessed data
 151 was managed with the MySQL database. Django-based applications were developed
 152 to provide a user-friendly interface including an embedded genome browser for
 153 visualizing genome-wide data. The key workflows, tools, and processed data are
 154 summarized in Figure S1A-B and described in detail below.

155

156 **Data sources**

157 SRG information and their regulatory networks were curated from PubMed literature.
 158 All genome sequences and species information used in this database were
 159 downloaded from NCBI public databases. All raw sequencing data were downloaded
 160 from the Sequence Read Archive (SRA) of NCBI. The sets of RNA-seq data were
 161 organized by species, gonad developmental stages, and temperature (Table S1). In
 162 addition, we retrieved images related to sex reversal from the OPENi
 163 (<https://openi.nlm.nih.gov/>) and ZFIN databases [23].

164

165 **SRG mining**

166 We retrieved thousands of articles from PubMed by querying species and functional
 167 keywords (e.g., sex reversal). First, the abstracts and full texts of these articles were
 168 collected by text crawler technology. The full texts of non-open access papers were
 169 obtained through the library portal of Wuhan University. Next, we separated a chunk
 170 of continuous text into separate words, and carried out word stemming to remove
 171 plural and different tenses. Then, we removed stop words such as “the”, “is”,
 172 and “however”. Finally, we counted the frequency of the words from the literature and
 173 manually filtered out some high-frequency but irrelevant words such as
 174 “masculinizing”, “ovotestes”, “pseudomale”, “hermaphrodite”, and “gynogenesis”
 175 into a blacklist until most of the high-frequency words were gene symbols and drug
 176 names. The remaining words related to genes and drugs were manually added to the
 177 wordlist (Table S2).

178 We retained 1,611 papers that contained the words in the wordlist and manually

179 read them with notations about SRG regulation (Table S3). Finally, we found 258
180 SRGs, 6 drugs and 11 hormones, which were validated to be functional in sex reversal
181 in different species, and constructed the regulatory networks of SRGs. We next
182 predicted another 498 genes that were homologous with those SRGs in the 18 species
183 (Figure S1C). Furthermore, protein-protein interaction (PPI) networks of SRGs were
184 extracted from the STRING database [24].

185

186 **RNA-seq data processing**

187 The data quality of the collected RNA-seq data was assessed using FastQC
188 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), and the adapters and
189 low-quality bases in raw reads were removed using Trim Galore
190 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Filtered reads
191 were aligned to the genome using STAR [25] in end-to-end mode. The primary
192 alignments were retained through SAMtools [26]. Gene expression quantification in
193 FPKM (Fragments Per Kb of exon per Million mapped fragments) was computed
194 using StringTie [27]. Differential expression analysis was performed using DESeq2
195 [28].

196

197 **Transcriptome assembly**

198 High-quality reads were *de novo* assembled using StringTie [27] with default
199 parameter settings. The longest ORFs were predicted in assembled transcripts using
200 TransDecoder.LongOrfs (<https://help.rc.ufl.edu/doc/TransDecoder>). DIAMOND [29]
201 was used to collect homologous evidence of identified ORFs from the UniProt
202 database (<https://www.uniprot.org/>). The potential coding regions were further refined
203 by TransDecoder.Predict. Finally, a GFF3 file based on the coding regions of the
204 reference genome was generated through the `cdna_alignment_orf_to_genome_orf.pl`
205 function in TransDecoder.

206

207 **Homology alignment**

208 Orthologous groups of SRGs were identified among all sex reversal species using the

209 BLAST [30] all-v-all algorithm in OrthoFinder [31]. Conserved motifs of orthogroups
210 were predicted using MEME [32]. Comparisons between conserved motifs and
211 known motifs were performed using Tomtom [33]. Species tree was constructed
212 according to the species taxonomy on NCBI. Meanwhile, the evolutionary
213 relationship was verified by OrthoFinder using the STAG [34] algorithm and rooted
214 using the STRIDE [35] algorithm.

215 For the orthologue tracks in a reference species, the homologous genes in other
216 species were mapped to the reference genome using Blat [36]. Alignments with
217 sequence identity larger than or equal to 60% were retained, and the maximum intron
218 size was set to 450,000 bp.

219 For the conservation track, pairwise alignments between genome sequences were
220 built using LASTZ [37], and MULTIZ [38] was then used to construct multiple
221 alignments, based on which the conservation scores were calculated using phyloP
222 from the PHAST package [39].

223

224 **Image collection and annotation**

225 We collected available (fluorescence) *in situ* hybridization and immunocytochemistry
226 (ISH, FISH and ICH) data related to SRGs from the OPENi and ZFIN databases. The
227 images were classified by gene, differentiation status, developmental period, and
228 gender. We manually added descriptions for those images based on the original figure
229 legends and articles.

230

231 **Web interface and usage**

232 ASER is a user-friendly database, and all the contents are interactive and dynamic.
233 There are five main functional modules, including SPECIES, IMAGE, SRG, GENE
234 and BROWSER. In addition, the SEARCH module was developed to display and
235 interconnect different kinds of data in other modules.

236 For the “SPECIES” module, the evolutionary tree constructed for the 18 sex
237 reversal species is displayed on the main page (**Figure 2A**). The reported

238 inducements of sex reversal or common approaches used to manipulate sex in each
239 species are displayed on this page, including natural processes, genetic abnormality
240 (*e.g.*, *amh* overexpression), administration of exogenous hormones or drugs (*e.g.*,
241 17α -methyltestosterone), temperature changes during gonadal differentiation, and
242 manipulation of social factors. The literature supporting this information is also
243 provided. Users can click on any species to obtain detailed descriptions and genome
244 information for this species (**Figure 2B**).

245 For the “IMAGE” module, we summarized the morphological characteristics of
246 zebrafish and mouse ovary at different developmental stages to help users better
247 understand the content of this module (**Figure 2C**). The (fluorescence) *in situ*
248 hybridization and immunocytochemistry (ISH, FISH and ICH) data related to specific
249 SRGs can be queried in different ways by species, gene, differentiative stage, and
250 gonad. Detailed descriptions of images are shown to help users understand the spatial
251 distribution of SRGs in the gonads (**Figure 2D**).

252 The “SRG” module includes word cloud, regulatory and PPI networks, and search
253 pages. The word cloud figure is dynamically presented by species with hyperlinks on
254 the nodes (**Figure 3A**). When the user clicks one node, the original references and
255 additional actions for more information will be shown under the figure. For any
256 validated SRG, ASER allows users to obtain its regulators (including genes, hormones,
257 drugs), targets, and the associated modes of regulation (**Figure 3B**). At the same time,
258 PPI networks of these SRGs in different species are also displayed (**Figure 3C**), in
259 which the colors of the edges are used to distinguish known interactions
260 (experimentally_determined_interaction, database_annotated), predicted interactions
261 (neighborhood_on_chromosome, gene_fusion, phylogenetic_cooccurrence), and other
262 types (homology, coexpression, automated_textmining). In addition, the search page
263 provides an interface for a specific SRG to show its regulatory network and more
264 detailed information, such as tissue, developmental stage, and literature evidence
265 (**Figure 3D–E**).

266 The “GENE” module provides different kinds of data for any annotated gene,
267 some of which are linked to the “BROWSER” module for visualization. The links

corresponding to the query gene are shown in the search page by species and gene symbol (**Figure 4A**). Detailed information for a specific query gene includes its orthogroup in all species, predicted motifs, and similar known motifs (**Figure 4B**). The orthologous genes and 18-way conservation scores for the query gene can be inspected in BROWSER tracks (**Figure 4C**). Detailed alignment information can be obtained and downloaded by clicking on the track. In addition, the gene expression quantifications in FPKM across different stages, tissues, and conditions are shown as bar plots and in detail as tables (**Figure 4D**). The RNA-seq signal profiles are displayed in BROWSER, and the tracks can be customized easily, including color, scale, height, and *etc.* For any species, the available tracks can be dynamically selected or unselected. For example, in *Danio rerio*, a subset of RNA-seq tracks are shown for the *sox9* gene during sex reversal (**Figure 4E**).

Discussion

Studies on sex reversal have been especially useful in helping redefine the concept of sex determination. There are diverse master sex-determining genes reported in different species. In addition, genes previously known to be involved in sex determination or differentiation are emerging as potential key components of sex reversal in other vertebrates [40]. Therefore, studies in different species continue to reveal genes with unexpected roles in sex reversal, and their homologs in other vertebrates also deserve investigation. ASER fills the gap of the sex reversal database by integrating diverse information at different levels for the 18 species with sex reversal, including curated SRGs, RNA-seq data, image data, and conservation data.

For any collected sex reversal species, users can obtain major inducements of sex reversal in this species in the SPECIES module. For any SRG, users can obtain its regulators, targets during sex determination in the SRG module and spatial distribution in different stages in the IMAGE module. For any annotated gene, users can obtain its homologous genes and conserved motifs in 18 species in the GENE module. Furthermore, users can also explore and visualize expression dynamics

297 across different conditions in the GENE or BROWSER modules.

298 In the future, we will continuously select important and typical sex reversal
299 species as their complete genome and omics data from both “female” and “male”
300 samples become available. Hermaphroditic fish such as *Synbranchus marmoratus* and
301 *Amphiprion perideraion* and invertebrates such as *Macrobrachium rosenbergii* and
302 *Venus mercenaria* are candidates. In addition, we will add more omics data, such as
303 sRNA-seq, BS-seq and ChIP-seq data. We expect that the resources in ASER will
304 promote further studies to decode the molecular mechanisms of sex reversal.

305

306 **Data availability**

307 ASER is publicly available at <http://aser.ihb.ac.cn/>.

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309 **CRedit author statement**

310 **Yangyang Li:** Software, Formal analysis, Data curation, Writing - original draft,
311 Writing - review and editing. **Zonggui Chen:** Software, Formal analysis,
312 Visualization, Writing - review and editing. **Hairong Liu:** Investigation, Data curation.
313 Writing - review and editing. **Qiming Li:** Formal analysis. **Xing Lin:** Data curation.
314 **Shuhui Ji:** Data curation. **Rui Li:** Data curation. **Shaopeng Li:** Data curation.
315 **Weiliang Fan:** Visualization. **Haiping Zhao:** Data curation. **Zuoyan Zhu:** Writing -
316 review & editing. **Wei Hu:** Conceptualization, Funding acquisition, Writing - review
317 & editing. **Yu Zhou:** Conceptualization, Project administration, Funding acquisition,
318 Methodology, Writing - review and editing. **Daji Luo:** Conceptualization, Project
319 administration, Funding acquisition, Methodology, Writing - review and editing. All
320 authors read and approved the final manuscript.

321

322 **Competing interests**

323 The authors declare no conflict of interest.

324

325 **Acknowledgements**

326 This work was supported by grants from the National Natural Science Foundation of
327 China (31922085, 31872191 to DL, 31922039 to YZ), the Strategic Priority Research
328 Program of CAS (XDA24010108) to WH and DL, and Natural Science Foundation of
329 of Hubei Province (2020CFA056 to DL and 2020CFA057 to YZ). Part of the
330 computation of this work was done in the Supercomputing Center of Wuhan
331 University and Hydrobiological Data Analysis Center.

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448 **Figure Legends**

449

450 **Figure 1 Schematic diagram of ASER database**

451 **A.** Five main functional modules in ASER, including the 18 sex reversal species, sex
452 reversal genes (SRGs) and their regulatory networks, multiple sequence alignments
453 and conservation scores, gene expression dynamics during sex reversal from
454 RNA-seq data, spatial distribution of SRGs from ISH, FISH and ICH images. **B.** Data
455 sources in ASER database. ASER stores all processed data in a MySQL database with
456 additional indexes and uses the Django framework for interactive queries from the
457 web interface to the backend database. **C.** Overview of the ASER web interface. The
458 main functionalities are provided and organized into six modules.

459

Figure 2

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461 **Figure 2 Species and image modules in ASER**

462 **A.** Evolutionary relationship and sex reversal inducements of the 18 species belonging
463 to Teleostei, Mammalia, Sauropsida, and Amphibia. NAT: Natural; GA: Genetic
464 abnormality; H/D: Hormones or drugs; TEM: Temperature; SF: Social factors. **B.**
465 Detailed description, genome information, and references related to sex reversal in
466 each species. **C.** Examples of gonadal morphology at different developmental stages
467 in mouse and zebrafish. **D.** Image page describing the spatial distribution of SRGs in
468 gonads, with a representative example of cyp26a1 in ovaries.

Figure 4 Gene conservation and expression modules in ASER

A. Search page for any gene in the 18 species. **B.** Homologous genes in the 18 species (top) and motifs corresponding to the query gene (bottom). The conserved motif for these homologous genes was computationally identified by MEME, and known motifs similar to this motif were also presented. **C.** Genome browser tracks of orthologue and conservation scores (18-ways). **D.** Expression dynamics of representative query gene. **E.** Genome browser view of processed RNA-seq signals for a representative query gene (*sox9a* in *zebrafish*).

491 **Tables**

492 **Table 1 Inducements of sex reversal or common approaches used to manipulate**
 493 **sex in 18 species**

Species	Gonadal differentiation	Inducements/Approaches
<i>Cyprinus carpio</i>	gonochoristic	H/D; TEM
<i>Danio rerio</i>	gonochoristic	GA; H/D; TEM
<i>Oryzias latipes</i>	gonochoristic	GA; H/D; TEM
<i>Oreochromis niloticus</i>	gonochoristic	GA; H/D; TEM
<i>Epinephelus coioides</i>	hermaphroditic	NAT; GA; H/D; SF
<i>Thalassoma bifasciatum</i>	hermaphroditic	NAT; SF
<i>Betta splendens</i>	gonochoristic	H/D
<i>Monopterus albus</i>	hermaphroditic	NAT
<i>Lates calcarifer</i>	hermaphroditic	NAT
<i>Paralichthys olivaceus</i>	gonochoristic	H/D; TEM
<i>Cynoglossus semilaevis</i>	gonochoristic	NAT; TEM
<i>Xenopus laevis</i>	gonochoristic	H/D
<i>Trachemys scripta</i>	gonochoristic	H/D; TEM; GA
<i>Gallus gallus</i>	gonochoristic	H/D
<i>Homo sapiens</i>	gonochoristic	GA
<i>Mus musculus</i>	gonochoristic	GA
<i>Bos taurus</i>	gonochoristic	GA
<i>Equus caballus</i>	gonochoristic	GA

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501 **Supplementary material**

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503 **Figure S1 Data processing pipelines and statistics of SRGs**

504 **A.** Workflows for building ASER, including homology alignment, RNA-seq data
505 processing, and transcriptome assembly. **B.** Workflows for SRG mining, and image

506 collection and annotation. **C.** Statistics of validated SRGs and predicted genes
 507 associated with sex reversal in the ASER database.

508

509 **Tables**

510 **Table S1 RNA-seq data used in the ASER database**

511 **Table S2 Wordlist and blacklist in SRG mining**

512 **Table S3 Literature mining of SRGs for the ASER database**

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