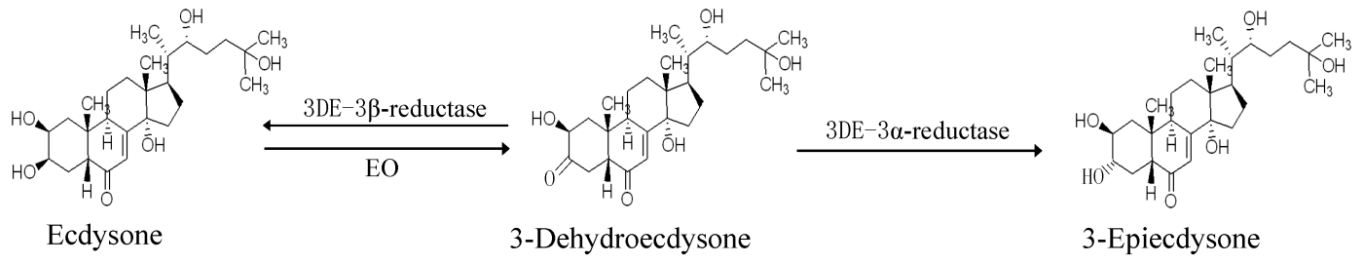
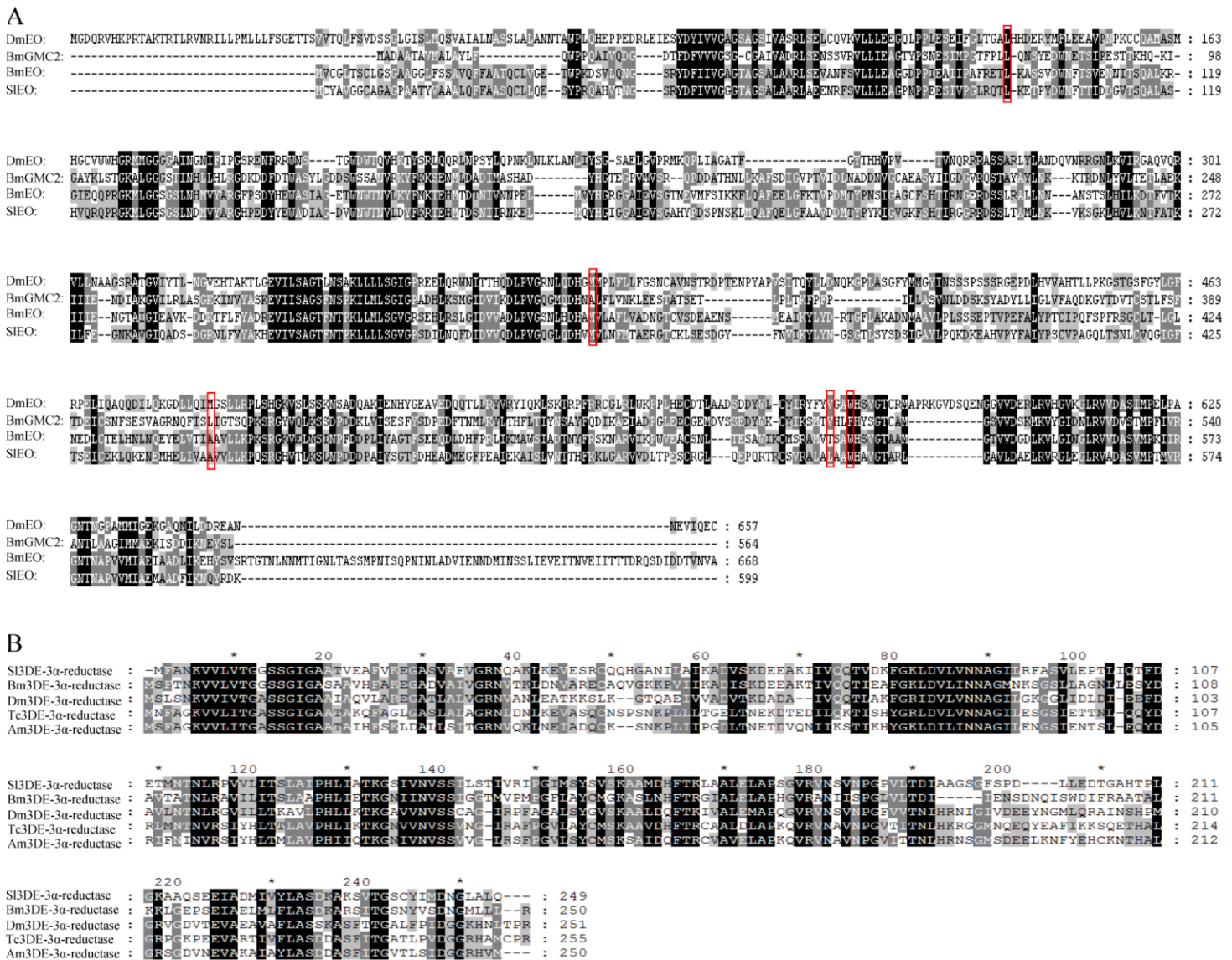


## Supplementary materials

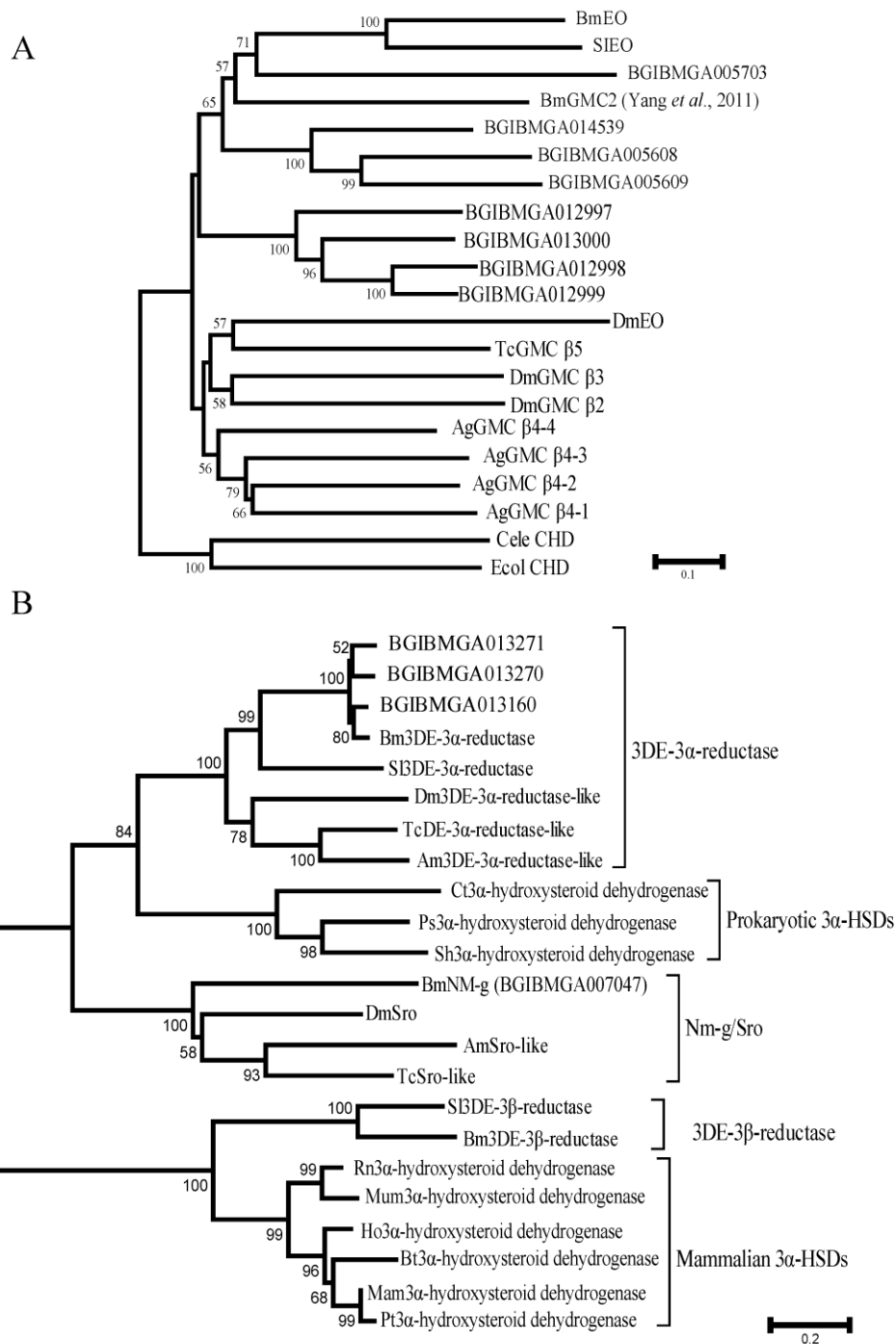
Wei Sun et al. Molecular cloning and characterization of *ecdysone oxidase* and *3-dehydroecdysone-3 $\alpha$ -reductase* involved in the ecdysone inactivation pathway of silkworm, *Bombyx mori*



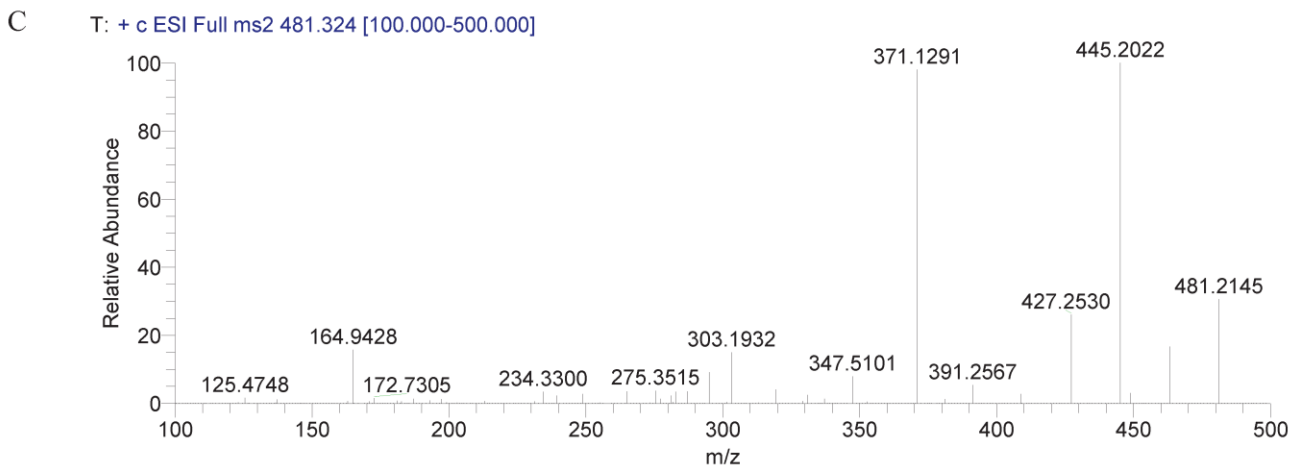
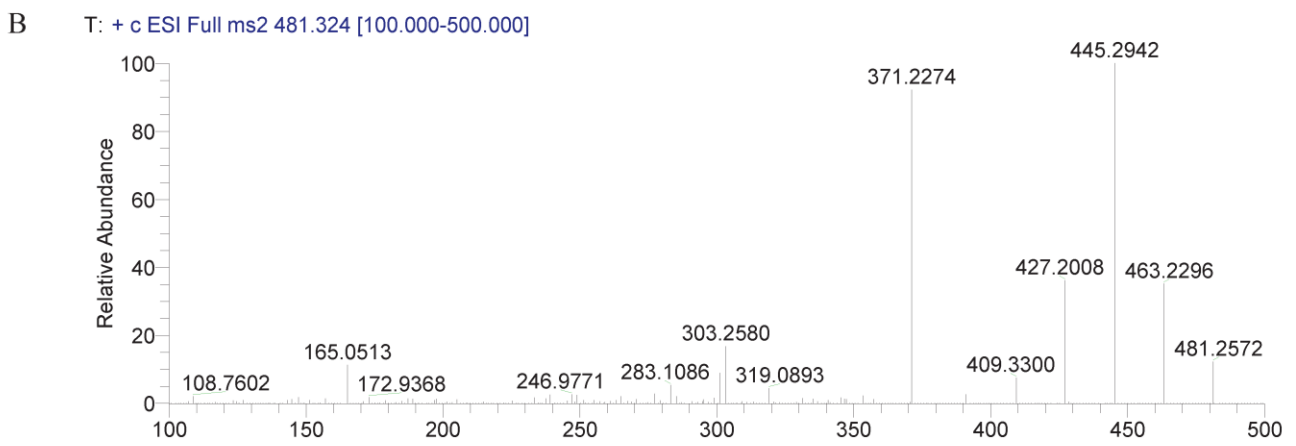
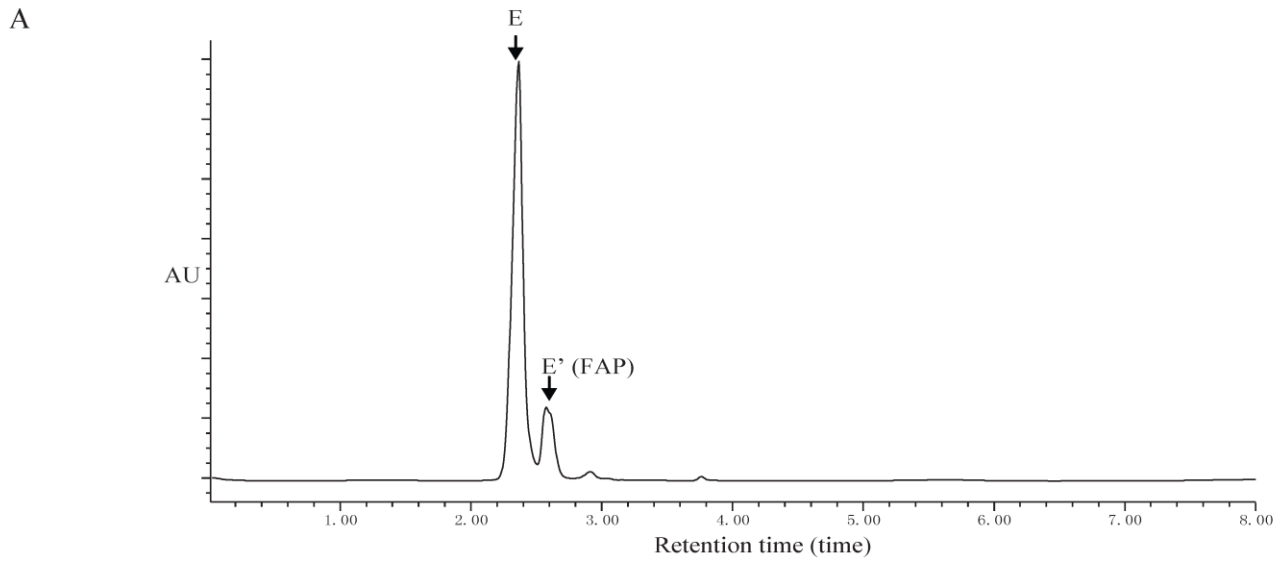
**Figure S1.** Enzymic interconversions of ecdysone, 3-dehydroecdysone and 3-epiecdysone. EO represents the Ecdysone Oxidase; 3DE-3 $\beta$ -reductase represents the 3-dehydroecdysone-3 $\beta$ -reductase; 3DE-3 $\alpha$ -reductase represents the 3-dehydroecdysone-3 $\alpha$ -reductase



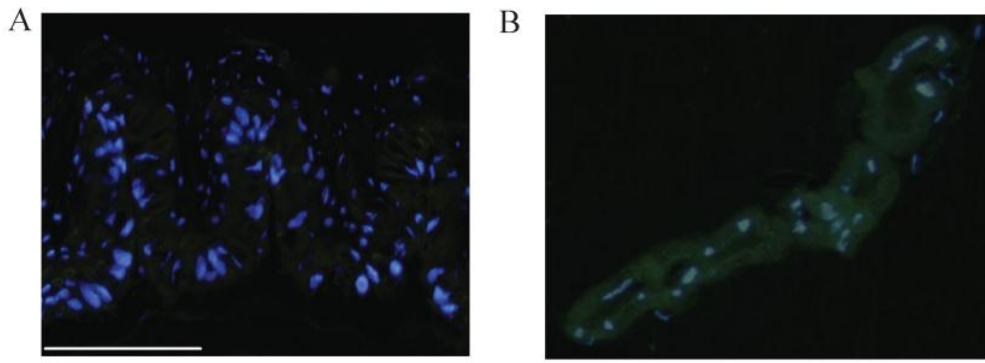
**Figure S2.** Multiple sequence alignment of EO and 3DE-3DE-3 $\alpha$ -reductase proteins. (A) Multiple sequence alignment of EO. The red box means conserved ecdysone binding residues. (B) Multiple sequence alignment of 3DE-3 $\alpha$ -reductase. Identical and similar residues have been highlighted in black and grey, respectively. SIEO represents the Ecdysone Oxidase from the *S. littoralis*; DmEO represents the Ecdysone Oxidase from the *D. melanogaster*. SI3DE-3 $\alpha$ -reductase represents the 3DE-3 $\alpha$ -reductase from the *S. littoralis*.



**Figure S3.** Phylogenetic tree of EO and 3DE-3DE-3 $\alpha$ -reductase in insects. (A) The phylogenetic tree of EO. (B) The phylogenetic tree of 3DE-3 $\alpha$ -reductase. Abbreviations: Dm: *D. melanogaster*; Ag: *Anopheles gambiae*; Cele: *Caenorhabditis elegans*; Ecol: *Escherichia coli*; Tc: *Tribolium castaneum*; Am: *Apis mellifera*; Sl: *Spodoptera littoralis*; Ct: *Comamonas testosteroni* S44; Ps: *Pseudomonas sp.* B-0831; Sh: *Shewanella halifaxensis* HAW-EB4; Rn: *Rattus norvegicus*; Mum: *Mus musculus*; Bt: *Bos taurus*; Ho: *Homo sapiens*; Mam: *Macaca mulatta*; Pt: *Pan troglodytes*. GMC: Glucose-Methanol-choline oxidoreductases family; CHD: choline dehydrogenase; SDR: short-chain dehydrogenases/reductases (SDR) superfamily; Sro: Shroud; 3 $\alpha$ -HSDs: 3 $\alpha$ -hydroxysteroid dehydrogenases.



**Figure S4.** HPLC and Mass spectrum of the ecdysone and 3-epiecdysone (FAP). (A) the HPLC of the ecdysone and 3-epiecdysone (FAP). (B) the mass spectrum of the ecdysone. (C) the mass spectrum of the 3-epiecdysone (FAP). FAP means the first additional peak in the Figure 3D.



**Figure S5.** Negative control of Immunohistochemical analysis. (A) Immunohistochemical analysis in the midgut using control antiserum. (B) Immunohistochemical analysis in the Malpighian tubule using control antiserum. Bar, 200  $\mu\text{m}$ . The control antiserum was collected from the mice immunized with PBS. Blue fluorescence represents the nuclei.

**Table S1.** List of primers

Gene	Sequence (5' to 3')	Purpose
<i>BmEO</i>	S: <sup>a</sup> <u>GGATCC</u> ATGGTTTGC GGGTTG	Probe synthesis for RT-PCR and prokaryotic expression
	AS: <sup>a</sup> <u>GCGGCCG</u> CTCATGCGACATTGAC	
	S: <sup>a</sup> <u>GAATTC</u> ATGGTTTGC GGGTTG	Probe synthesis for eukaryotic expression
	AS: <sup>a</sup> <u>GCGGCCG</u> CTCATGCGACATTGAC	
	S: CGTTTCGCAGCGGGTGTC	Probe synthesis for quantitative RT-PCR
	AS: CCGATTTGGGTTTCAGCAGG	
	S: TGGATCTCCGCCCGCCTCCAGTAGGAG	Probe synthesis for 5'RACE
	AS: CTCGAGAGTCTGGCGGCGAGCGCAG	
	S: TGGCATTCCGTGGGTACTGCCGCT	Probe synthesis for 3'RACE
	AS: GCACCGGTTGTGATGATCGCGGAA	
<i>Bm3DE-3<math>\alpha</math>-reductase</i>	S: <sup>a</sup> <u>GGATCC</u> ATGAGTTTCACAAAT	Probe synthesis for prokaryotic expression
	AS: <sup>a</sup> <u>GCGGCCG</u> CTCATCTGAGCAGCAG	
	S: <sup>a</sup> <u>GAATTC</u> ATGAGTTTCACAAAT	Probe synthesis for eukaryotic expression
	AS: <sup>a</sup> <u>GCGGCCG</u> CTCATCTGAGCAGCAG	
	S: AATGCCAGGGTTTCTGGCATACT	Probe synthesis for RT-PCR and quantitative RT-PCR
	AS: CTTCAACGCCGTGGCAGCCCTA	
	S: TGAGCACATCTAGCTTCCCGAAGGC	Probe synthesis for 5'RACE
	AS: CCGACTTGCGCACATTCCCGGGCCAC	
	S: TGTCAGAGCAAACATCATCAGTCCAGGTCT	Probe synthesis for 3'RACE
	AS: GACATTTTTAGGGCTGCCACGGCGTTGAAG	

<sup>a</sup> Restriction sites are underlined; S: sense primer; AS: antisense primer.

**Table S2.** The Genbank Accession Numbers of the proteins used in the phylogenetic analysis

	Protein name	Accession No.	Species
a*	DmEO	CG9504	<i>Drosophila melanogaster</i>
	DmGMC $\beta$ 2	CG9509	<i>Drosophila melanogaster</i>
	DmGMC $\beta$ 3	CG9512	<i>Drosophila melanogaster</i>
	AgGMC $\beta$ 4-1	AAAB01008844	<i>Anopheles gambiae</i>
	AgGMC $\beta$ 4-2	AAAB01008844	<i>Anopheles gambiae</i>
	AgGMC $\beta$ 4-3	AAAB01008844	<i>Anopheles gambiae</i>
	AgGMC $\beta$ 4-4	AAAB01008844	<i>Anopheles gambiae</i>
	SIEO	AY035784	<i>Spodoptera littoralis</i>
	BmGMC2	BGIBMGA000158 <sup>#</sup>	<i>Bombyx mori</i>
	Cele CHD	NP_495846	<i>Caenorhabditis elegans</i>
	Ecol CHD	NP_414845	<i>Escherichia coli</i>
b*	DmSDR	NP_610235	<i>Drosophila melanogaster</i>
	DmSDR 1	NP_647839	<i>Drosophila melanogaster</i>
	DmSDR 2	NP_729808	<i>Drosophila melanogaster</i>
	DmSDR 4	NP_001163844	<i>Drosophila melanogaster</i>
	Dm-3DE-3 $\alpha$ -reductase-like	NP_569875	<i>Drosophila melanogaster</i>
	DmSro-like	CG12068	<i>Drosophila melanogaster</i>
	TcSDR 1	XP_974493	<i>Tribolium castaneum</i>
	TcSDR 2	XP_969456	<i>Tribolium castaneum</i>
	TcSDR 3	XP_970029	<i>Tribolium castaneum</i>
	TcSDR 4	XP_969383	<i>Tribolium castaneum</i>
	Tc-3DE-3 $\alpha$ -reductase-like	XP_974115	<i>Tribolium castaneum</i>
	TcSro-like	GA11371	<i>Tribolium castaneum</i>
	AmSDR 1	XP_624401	<i>Apis mellifera</i>
	AmSDR 2	XP_624353	<i>Apis mellifera</i>
	Am-3DE-3 $\alpha$ -reductase-like	XP_394891	<i>Apis mellifera</i>
	AmSro-like	XP_001120169	<i>Apis mellifera</i>
	Sl-3DE-3 $\alpha$ -reductase	AF255341	<i>Spodoptera littoralis</i>

\*a represents the protein sequences used in the phylogenetic analysis of the ecdysone oxidase;

\*b represents the protein sequences used in the phylogenetic analysis of the 3DE-3 $\alpha$ -reductase.

# represents the Accession No. of ecdysone oxidase identified by Yang et al (2011) in SilkDB.