

Supplementary materials

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

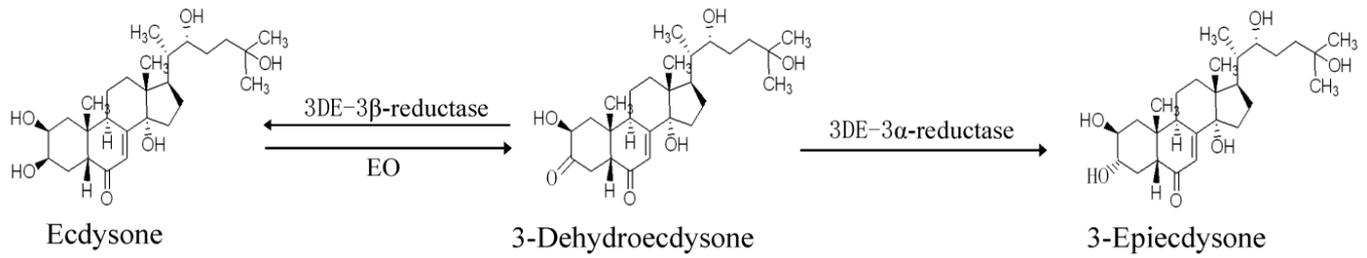


Figure S1. Enzymic interconversions of ecdysone, 3-dehydroecdysone and 3-epiecdysone. EO represents the Ecdysone Oxidase; 3DE-3 β -reductase represents the 3-dehydroecdysone-3 β -reductase; 3DE-3 α -reductase represents the 3-dehydroecdysone-3 α -reductase

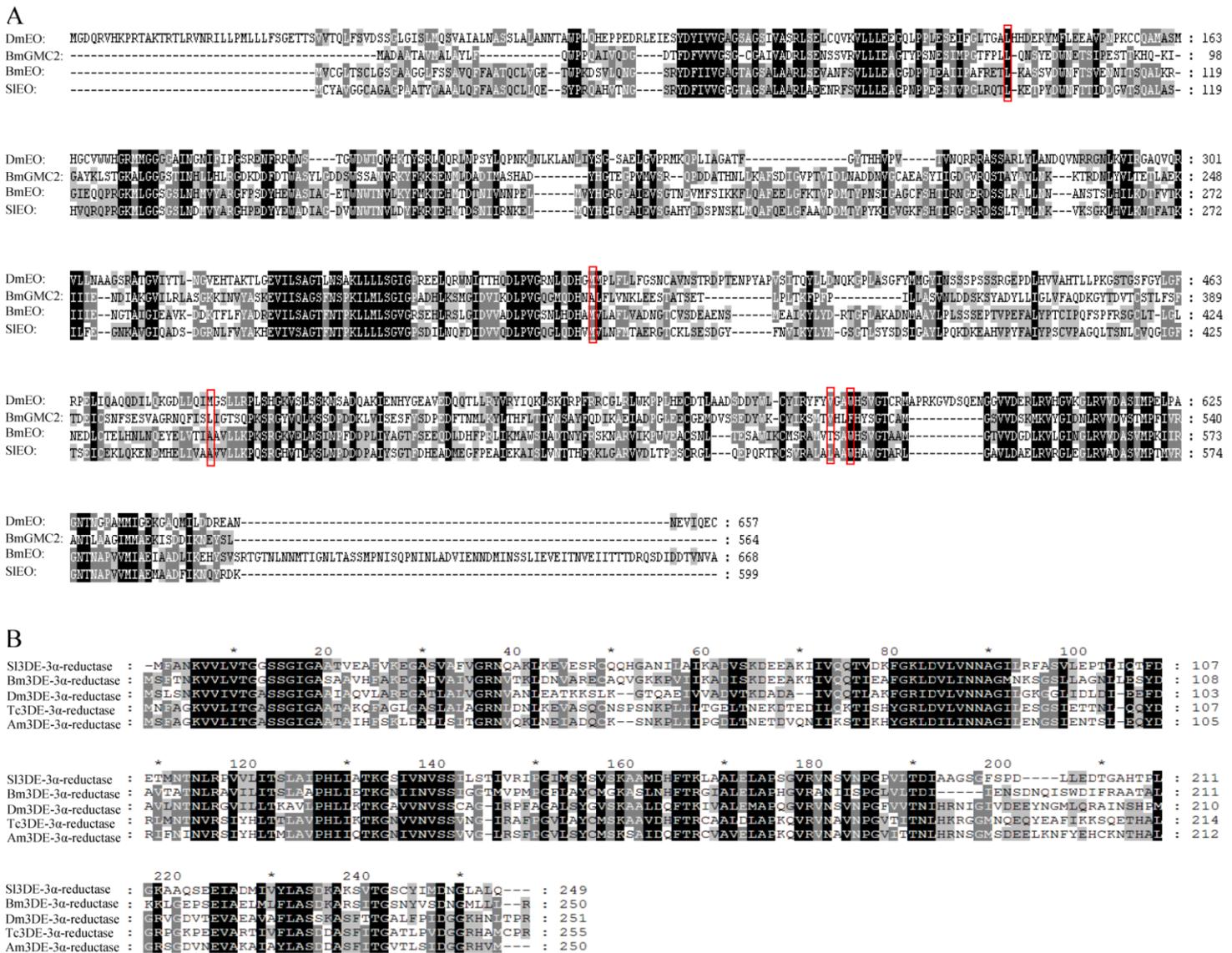


Figure S2. Multiple sequence alignment of EO and 3DE-3DE-3 α -reductase proteins. (A) Multiple sequence alignment of EO. The red box means conserved ecdysone binding residues. (B) Multiple sequence alignment of 3DE-3 α -reductase. Identical and similar residues have been highlighted in black and grey,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

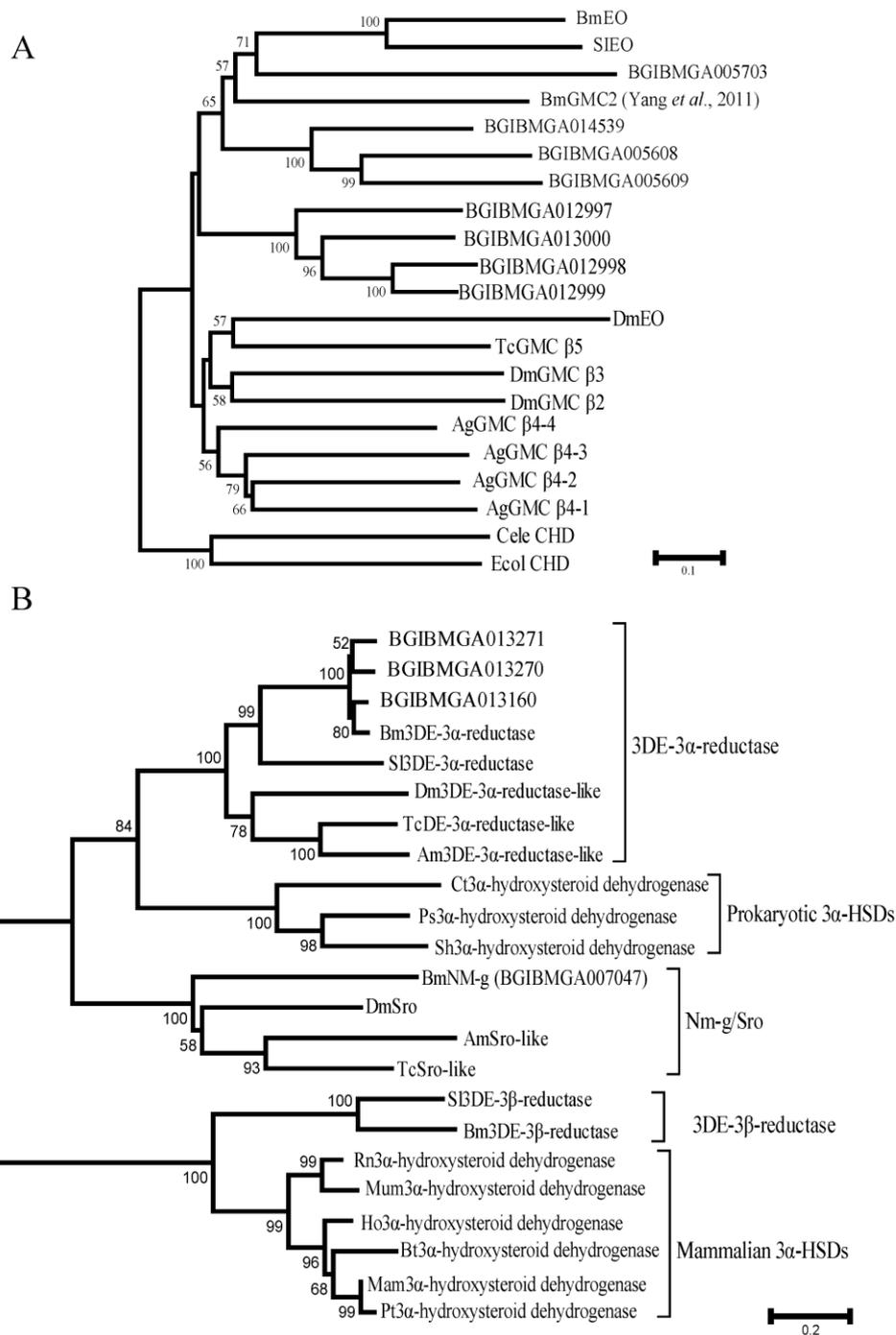


Figure S3. Phylogenetic tree of EO and 3DE-3DE-3 α -reductase in insects. (A) The phylogenetic tree of EO. (B) The phylogenetic tree of 3DE-3 α -reductase. Abbreviations: Dm: *D. melanogaster*; Ag: *Anopheles gambiae*; Cele: *Caenorhabditis elegans*; Ecol: *Escherichia coli*; Tc: *Tribolium castaneum*; 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 α -HSDs: 3 α -hydroxysteroid dehydrogenases.

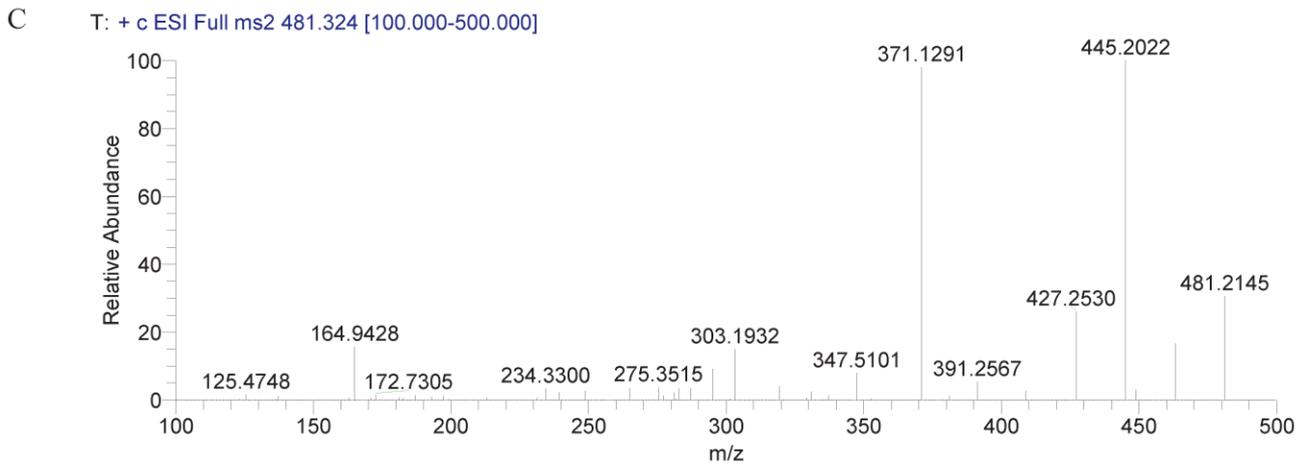
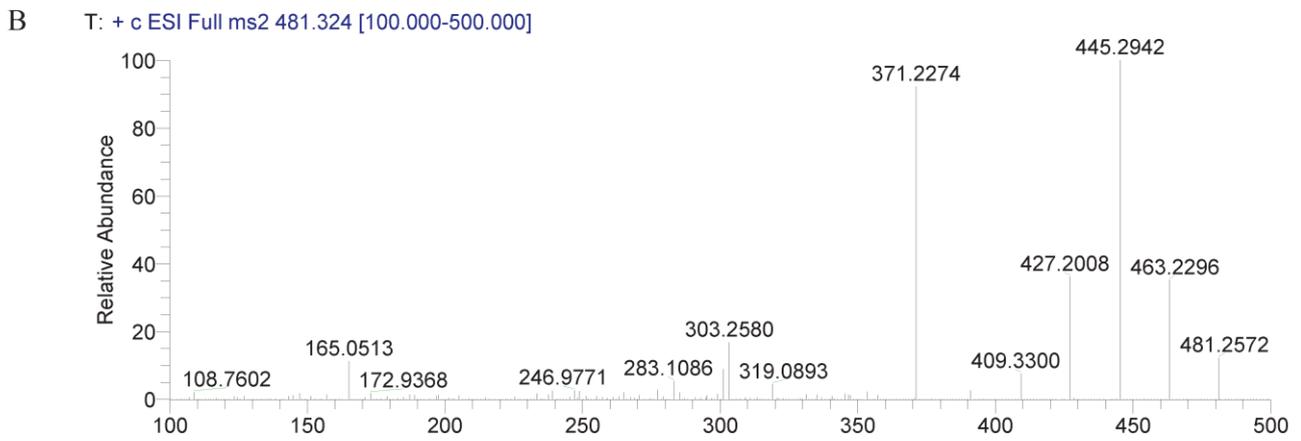
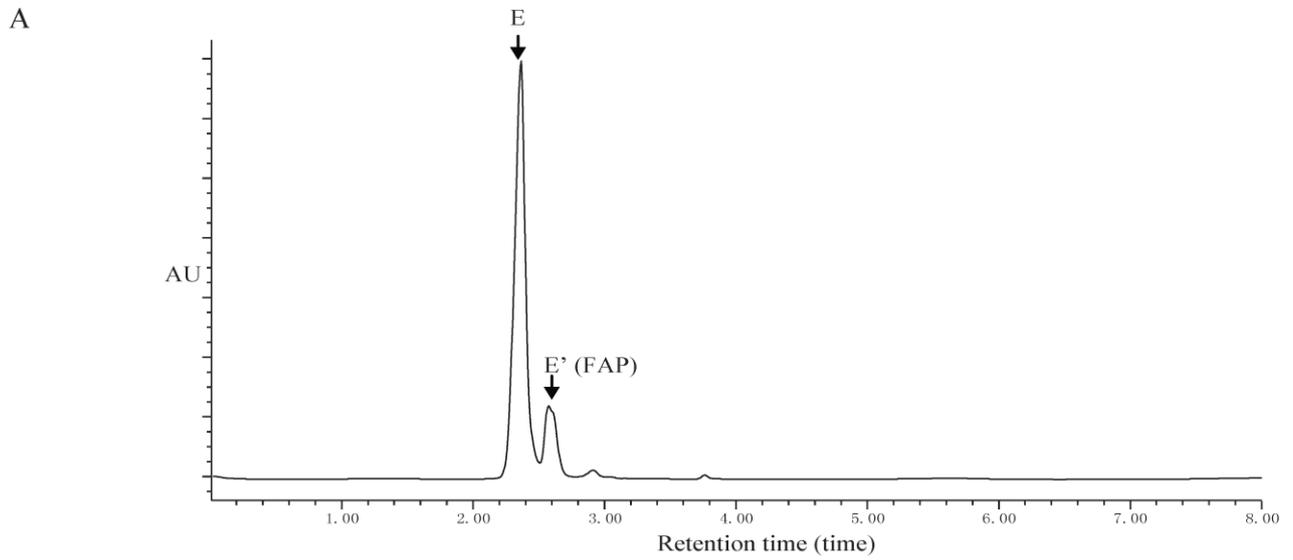


Figure S4. HPLC and Mass spectrum of the ecdysone and 3-epiecdysone (FAP). (A) the HPLC of the ecdysone and 3-epiecdysone (FAP). (B) the mass spectrum of the ecdysone. (C) the mass spectrum of the 3-epiecdysone (FAP). FAP means the first additional peak in the Figure 3D.

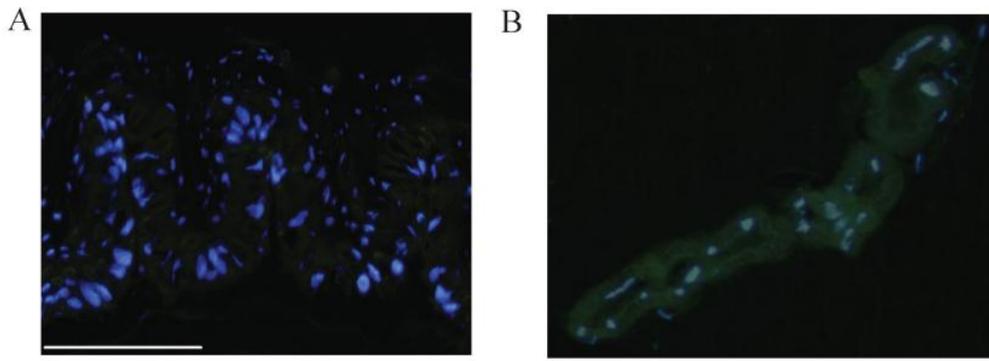


Figure S5. Negative control of Immunohistochemical analysis. (A) Immunohistochemical analysis in the midgut using control antiserum. (B) Immunohistochemical analysis in the Malpighian tubule using control antiserum. Bar, 200 μm . The control antiserum was collected from the mice immunized with PBS. Blue fluorescence represents the nuclei.

Table S1. List of primers

Gene	Sequence (5' to 3')	Purpose
<i>BmEO</i>	S: ^a <u>GGATCC</u> ATGGTTTGCGGGTTG	Probe synthesis for RT-PCR and prokaryotic expression
	AS: ^a <u>GCGGCCGCT</u> CATGCGACATTGAC	
	S: ^a <u>GAATTC</u> ATGGTTTGCGGGTTG	Probe synthesis for eukaryotic expression
	AS: ^a <u>GCGGCCGCT</u> CATGCGACATTGAC	
	S: CGTTTCGCAGCGGGTGTC	Probe synthesis for quantitative RT-PCR
	AS: CCGATTTGGGTTTCAGCAGG	
	S: TGGATCTCCGCCCGCCTCCAGTAGGAG	Probe synthesis for 5'RACE
	AS: CTCGAGAGTCTGGCGGCAGCGCAG	
	S: TGGCATTCCGTGGGTACTGCCGCT	Probe synthesis for 3'RACE
	AS: GCACCGGTTGTGATGATCGCGGAA	
<i>Bm3DE-3α-reductase</i>	S: ^a <u>GGATCC</u> ATGAGTTTCACAAAT	Probe synthesis for prokaryotic expression
	AS: ^a <u>GCGGCCGCT</u> CATCTGAGCAGCAG	
	S: ^a <u>GAATTC</u> ATGAGTTTCACAAAT	Probe synthesis for eukaryotic expression
	AS: ^a <u>GCGGCCGCT</u> CATCTGAGCAGCAG	
	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: GACATTTTATAGGGCTGCCACGGCGTTGAAG	

^a 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 β 2	CG9509	<i>Drosophila melanogaster</i>
	DmGMC β 3	CG9512	<i>Drosophila melanogaster</i>
	AgGMC β 4-1	AAAB01008844	<i>Anopheles gambiae</i>
	AgGMC β 4-2	AAAB01008844	<i>Anopheles gambiae</i>
	AgGMC β 4-3	AAAB01008844	<i>Anopheles gambiae</i>
	AgGMC β 4-4	AAAB01008844	<i>Anopheles gambiae</i>
	SIEO	AY035784	<i>Spodoptera littoralis</i>
	BmGMC2	BGIBMGA000158 [#]	<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 α -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 α -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 α -reductase-like	XP_394891	<i>Apis mellifera</i>
	AmSro-like	XP_001120169	<i>Apis mellifera</i>
	Sl-3DE-3 α -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 α -reductase.

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