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Sex Pheromone Receptors of Lepidopteran Insects

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The sex pheromone receptors (SPRs) of Lepidopteran insects play important roles in chemical communication. In the sex pheromone detection processes, sex pheromone molecule (SPM), SPR, co-receptor (Orco), pheromone binding protein (PBP), sensory neuron membrane protein (SNMP), and pheromone degradation enzyme (PDE) play individual and cooperative roles. Commonly known as butterfly and moth, the Lepidopteran insects are widely distributed throughout the world, most of which are pests. Comprehensive knowledge of the SPRs of Lepidopteran insects would help the development of sex lure technology and the sex communication pathway research. In this review, we summarized SPR/Orco information from 10 families of Lepidopteran insects from corresponding studies. According to the research progress in the literature, we speculated the evolution of SPRs/Orcos and phylogenetically analyzed the Lepidopteran SPRs and Orcos with the neighbor-joining tree and further concluded the relationship between the cluster of SPRs and their ligands; we analyzed the predicted structural features of SPRs and gave our prediction results of SPRs and Orcos with Consensus Constrained TOPology Prediction (CCTOP) and SwissModel; we summarized the functional characterization of Lepidopteran SPRs and SPR-ligand interaction and then described the progress in the sex pheromone signaling pathways and metabotropic ion channel. Further studies are needed to work out the cryo-electron microscopy (EM) structure of SPR and the SPR-ligand docking pattern in a biophysical perspective, which will directly facilitate the understanding of sex pheromone signal transduction pathways and provide guidance in the sex lure technology in field pest control.

Keywords: sex pheromone receptor, structure, signal transduction, Lepidopteran insects, evolution, function

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INTRODUCTION

Belonging to general odorant receptors, insect sex pheromone receptors (SPRs) are expressed by olfactory sensory neurons (OSNs) and can detect volatile sex pheromones or other chemical signals to coordinate their social behaviors such as mating, reproduction, and alarming (Fleischer and Krieger, 2018). In the sex pheromone detection processes, sex pheromone molecule (SPM), SPR, co-receptor (Orco), pheromone binding protein (PBP), sensory neuron membrane protein (SNMP), and pheromone degradation enzyme (PDE) play individual and cooperative roles (Leal, 2013).

Usually, SPM was released from female insects and then sensed by specific SPR of conspecific male insects; meanwhile, the specific PBP surrounding the SPR transports the SPM to SPR and increases the magnitude of SPR response to SPM (Grosse-Wilde et al., 2006; Krieger et al., 2009); Orco was expressed in the same OSN of SPR and had been reported to increase the response strength of SPR to SPM through an ionotropic channel or a metabotropic channel (Nakagawa et al., 2005). After a series of elusive signal transduction pathways, the PDE contributes to the signal inactivation through degrading the SPM (Leal, 2013). Above all, the native mechanism of sex pheromone reception and signal transduction has not been fully elucidated until present.

Commonly known as butterfly and moth, the Lepidopteran insects are widely distributed throughout the world. In recent years, many odorant receptors had been characterized following the public annotation of *Drosophila melanogaster* odorant receptor family based on the whole-genome data (Wang and Anderson, 2010). Importantly, several SPRs have been deorphanized in *Bombyx mori* and other insect species according to the analysis of antennal transcriptome data (Nakagawa et al., 2005). As a consequence, sex attractants have been used as sex lures to wipe out pests without using chemical insecticides (Witzgall et al., 2010), and this environment-friendly pest control policy had been growing up gradually.

In this review, we summarized SPR and Orco information from 10 families (i.e., Bombycidae, Plutellidae, Sphingidae, Saturniidae, Geometridae, Nymphalidae, Noctuidae, Tortricidae, Pyralidae, and Crambidae) of Lepidopteran insects (Krieger et al., 2004, 2005; Sakurai et al., 2004; Miura et al., 2005; Nakagawa et al., 2005; Grosse-Wilde et al., 2007, 2010, 2011; Mitsuno et al., 2008; Jordan et al., 2009; Patch et al., 2009; Zhang et al., 2009, 2010, 2013, 2014, 2015; Wanner et al., 2010; Legeai et al., 2011; Wang et al., 2011; Yasukochi et al., 2011; Zhan et al., 2011; Bengtsson et al., 2012; Carraher et al., 2012; Leary et al., 2012; Liu et al., 2012, 2014; Montagne et al., 2012; Xu et al., 2012, 2015; Liu C. et al., 2013; Liu Y. et al., 2013; Sun et al., 2013; Wu et al., 2013; Zhang and Lofstedt, 2013; Jiang et al., 2014; Corcoran et al., 2015; De Fouchier et al., 2015; Feng et al., 2015; Garczynski and Leal, 2015; Lin et al., 2015; Steinwender et al., 2015; Chang et al., 2016; Ge et al., 2016; Jia et al., 2016; Walker et al., 2016; Zhang D. D. et al., 2016; Zhang Y. N. et al., 2016; Gonzalez et al., 2017; Li et al., 2017; Wicher et al., 2017; Yang S. et al., 2017; Du et al., 2018; Grapputo et al., 2018; Rojas et al., 2018; **Table 1**). Among all the Lepidopteran SPRs, several of them have been characterized to be sex pheromone sensing receptors. First of all, we reviewed the phylogenetic analyses of Lepidopteran SPRs, and the evolution of the summarized Lepidopteran SPRs was analyzed through MEGA X (Whelan and Goldman, 2001; Kumar et al., 2018). Second, we reviewed the transmembrane predictions of Lepidopteran SPRs, and the protein structure of Lepidopteran SPRs was predicted by online software Consensus Constrained TOPology Prediction (CCTOP) (Dobson et al., 2015) and SwissModel (Bertoni et al., 2017; Bienert et al., 2017; Waterhouse et al., 2018; Guex et al., 2019; Studer et al., 2020). Third, the interaction of Lepidopteran SPM and SPR was reviewed. Finally, the research status of downstream signaling responses and ligand-gated ion channels by the coupling of SPR and Orco was depicted.

Evolution of Lepidopteran Sex Pheromone Receptors

The olfactory receptor (OR) repertoire of several Lepidopteran species was usually phylogenetically analyzed, and SPRs always belong to the same clade. The neighbor-joining tree of sequences of all identified ORs of *Heliothis virescens* revealed a very high degree of diversity, i.e., a group that comprises 6 SPRs has at least 40% of their amino acids in common (Krieger et al., 2004). The neighbor-joining tree of *H. virescens* OR repertoires and BmOR1/3/4/5/6 receptors showed apparent relatedness of SPRs in *B. mori* and *H. virescens* (Krieger et al., 2005). The phylogenetic tree of *B. mori* and *H. virescens* ORs and also PxylOR1/3/4 and DindOR1/3 showed that pheromone receptors were clustered and were different from that of the Or83b family (Mitsuno et al., 2008; Patch et al., 2009). The phylogenetic analysis of *B. mori*, *Manduca sexta*, *Helicoverpa armigera*, and *H. virescens* ORs and PxylOR1/3/4/5/6/7 shows that the 6 candidate SPRs cluster together in the group of SPRs (Sun et al., 2013). SPRs formed a single subgroup in a phylogenetic tree by the ORs of *B. mori*, *H. armigera*, *H. virescens*, and *Plutella xylostella*, and the Orcos of the four species form a clade (Yang S. et al., 2017). The phylogenetic analysis of *B. mori*, *H. armigera*, and *H. virescens* ORs and PxylOR1/3/4/5/6/7/8/41/45 shows that the 8 candidate SPRs cluster together in the group of SPRs, and they are phylogenetically distinct from general odorant receptors (Liu et al., 2018). Neighbor-joining tree based on MUSCLE multiple sequence alignment of MsexOR1-5 and *B. mori* and *H. virescens* ORs shows that MsexOR1-4 belongs to the subgroup of SPRs, and a highly conserved Or83b group was indicated (Grosse-Wilde et al., 2010, 2011). The neighbor-joining tree including the ORs of *B. mori*, *H. virescens*, and MsexOR1 and ApolOR1/AperOR1 revealed that ApolOR1/AperOR1 are categorized in the subfamily of the candidate and functionally verified SPRs (Forstner et al., 2009). A phylogenetic analysis that was performed using candidate SexiOR and OR repertoires from *H. armigera*, *Helicoverpa assulta*, *Spodoptera littoralis*, and *B. mori* revealed a highly conserved Orco that was clustered with orthologs from all four of these species, and another group of relatively conserved SexiOR6/11/13/16 belongs to the same clade as SPRs (Du et al., 2018).

The phylogenetic tree that contained 21 OR sequences from *Sesamia inferens*, 43 from *M. sexta*, 21 from *H. virescens*, and 60 from *B. mori*, SinfOR2 was clustered with other Lepidopteran Orco sequences, and three SinfOR21/27/29 were clustered in the Lepidopteran SPR clade (Zhang Y. N. et al., 2016). A phylogenetic tree using a dataset containing all AlepOR sequences and all HassOR, HarmOR, HvirOR, and BmOR sequences revealed that AlepOrco was clustered with other Lepidopteran Orco sequences, and four AlepOR3/4/5/6 with full-length open reading frame (ORFs) were clustered in the Lepidopteran SPR clade (Zhang Y. N. et al., 2016). Multiple alignments of *H. armigera* SPRs and their homologs in *H. virescens* showed that the orthologous SPRs in these two insects had a high similarity. The phylogenetic analyses

TABLE 1 | The CCTOP results of SPRs and Orcos from 10 families in Lepidopteran insects.

Family	Species	Receptors	Genbank ID	CCTOP	HMMTOP	Memsat	Octopus	Philius	Phobius	Pro	Prodiv	Scampi	ScampiMsa	TMHMM
Bombycidae	*Bombyx mori* (Sakurai et al., 2004; Krieger et al., 2005; Nakagawa et al., 2005)	BmOR1	NP_001036875.1	I-6-I	I-7-O	I-8-I	I-7-O	I-8-I	I-6-I	I-8-I	I-9-O	I-6-I	I-6-I	O-7-I
		BmOR3	NP_001036925.1	I-4-I	I-5-O	I-7-O	I-6-I	I-4-I	I-4-I	I-6-I	I-7-O	I-6-I	I-6-I	I-4-I
		BmOR4	NP_001036926.1	I-6-I	O-4-O	O-7-I	O-5-I	I-7-O	I-6-I	I-6-I	I-7-O	O-6-O	I-7-O	I-6-I
		BmOR5	NP_001036927.1	I-7-O	I-7-O	I-8-I	I-7-O	I-5-O	I-6-I	I-6-I	I-7-O	I-6-I	I-7-O	I-8-I
		BmOR7	NP_001106227.1	I-7-O	O-8-O	I-8-I	I-6-I	I-4-I	I-5-O	I-6-I	I-7-O	I-6-I	I-7-O	I-5-O
		BmOR9	NP_001116805.1	I-8-I	O-6-O	I-8-I	O-4-O	I-7-O	I-7-O	I-6-I	I-6-I	I-7-O	I-7-O	I-6-I
		BmOR2*	CAD88206.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Bombyx mandarina (Zhang et al., 2009)	BmanOR1	ACT34880.1	I-6-I	O-4-O	I-8-I	I-7-O	I-8-I	I-6-I	I-8-I	I-9-O	I-6-I	I-6-I	O-7-I
		BmanOR3	ACT34882.1	I-6-I	I-5-O	I-7-O	I-6-I	I-4-I	I-4-I	I-6-I	I-7-O	I-6-I	I-6-I	O-4-O
		BmanOrco*	XP_028043387.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
Plutellidae	*Plutella xylostella* (Mitsuno et al., 2008; Sun et al., 2013; Yang S. et al., 2017; Liu et al., 2018)	PxylOR1	AGK43824.1	I-4-I	I-6-I	O-7-I	I-6-I	I-4-I	I-4-I	I-4-I	I-6-I	I-5-O	I-6-I	I-4-I
		PxylOR3	AGK43825.1	I-6-I	I-7-O	I-7-O	I-6-I	I-6-I	I-6-I	I-4-I	I-7-O	I-6-I	I-6-I	I-6-I
		PxylOR4	AGK43826.1	I-6-I	I-7-O	I-7-O	I-6-I	I-6-I	I-6-I	I-4-I	I-9-O	I-6-I	I-6-I	I-6-I
		PxylOR5	AGK43827.1	I-6-I	I-7-O	I-6-I	O-5-I	I-6-I	O-7-I	I-4-I	I-6-I	I-7-O	I-6-I	I-6-I
		PxylOR6	AGK43828.1	I-4-I	O-4-O	I-6-I	O-5-I	I-7-O	I-4-I	I-2-I	I-7-O	I-6-I	I-7-O	I-4-I
		PxylOR7	AGK43829.1	I-4-I	O-4-O	I-8-I	O-5-I	I-4-I	I-4-I	I-4-I	I-8-I	I-7-O	I-6-I	O-3-I
		PxylOR8	ASA39901.1	I-6-I	I-7-O	I-7-O	O-5-I	I-7-O	I-6-I	I-4-I	I-7-O	I-7-O	O-8-O	O-5-I
		PxylOR41	ASA39902.1	I-6-I	I-7-O	I-8-I	I-5-O	I-6-I	I-4-I	I-4-I	I-7-O	O-5-I	I-6-I	I-4-I
		PxylOR45	ASA39903.1	I-6-I	I-7-O	I-5-O	I-5-O	I-6-I	O-7-I	I-4-I	I-7-O	O-4-O	I-5-O	I-6-I
		PxylOr83b*	NP_001296031.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
Sphingidae	*Manduca sexta* (Patch et al., 2009; Grosse-Wilde et al., 2010, 2011; Wicher et al., 2017)	MsexOR1	CUQ99387.1	I-6-I	I-8-I	I-7-O	I-6-I	I-4-I	I-4-I	I-6-I	I-7-O	I-6-I	I-7-O	I-5-O
/		MsexOR4	CUQ99388.1	I-6-I	O-2-O	O-7-I	I-6-I	I-6-I	I-4-I	I-4-I	I-7-O	I-6-I	I-6-I	O-4-O
		MsexOrco*	CUQ99422.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-8-I	I-7-O	I-7-O
Saturniidae	*Antheraea pernyi* (Forstner et al., 2009; Li et al., 2020)	AperOR1	CBH19583.1	I-6-I	I-6-I	I-6-I	I-7-O	I-6-I	I-6-I	I-6-I	I-7-O	I-7-O	I-6-I	I-8-I
		AperOR2*	CAD88205.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	O-8-O
	Antheraea polyphemus (Forstner et al., 2009; Li et al., 2020)	ApolOR1	CBH19582.1	I-7-O	I-8-I	I-8-I	I-7-O	I-8-I	I-6-I	I-6-I	I-7-O	I-7-O	I-7-O	I-8-I
		ApolOR2*	CAD88205.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	O-8-O
Geometridae	*Operophtera brumata* (Zhang D. D. et al., 2016; Li et al., 2018)	ObruOR1	AJF20961.1	I-6-I	I-6-I	I-8-I	I-6-I	I-4-I	I-6-I	I-5-O	I-7-O	I-6-I	I-6-I	I-5-O
		ObruOrco*	AJF20962.1	I-7-O	O-8-O	I-8-I	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Ectropis grisescens (Li et al., 2017, 2018)	EgriOR31												
		EgriOrco*												
Nymphalidae	*Danaus plexippus* (Zhan et al., 2011)	DpleOR1	OWR49463.1	O-5-I	O-4-O	I-8-I	O-6-O	I-6-I	O-5-I	I-4-I	I-7-O	O-8-O	I-7-O	O-5-I
		DpleOR2*	OWR42934.1	I-7-O	I-7-O	I-8-I	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	

(Continued)

TABLE 1 | (Continued)

Family	Species	Receptors	Genbank ID	CCTOP	HMMTOP	Memsat	Octopus	Philius	Phobius	Pro	Prodiv	Scampi	ScampiMsa	TMHMM
Noctuidae	*Mythimna separata* (Mitsuno et al., 2008; Jiang et al., 2019, 2020; Tang et al., 2020)	MsepOR2	QEI49013.1	I-6-I	I-6-I	I-8-I	O-8-O	I-5-O	O-5-I	I-4-I	I-7-O	O-7-I	O-7-I	I-3-O
		MsepOR3	QEI49012.1	I-7-O	I-6-I	I-8-I	I-7-O	I-5-O	I-7-O	I-4-I	I-7-O	I-6-I	I-7-O	I-6-I
		MsepOrco*	QEI49014.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Heliothis virescens (Krieger et al., 2004; Grosse-Wilde et al., 2007; Wang et al., 2011)	HvirOR6	CAD31948.1	I-6-I	I-8-I	I-8-I	I-6-I	I-8-I	I-6-I	I-6-I	I-8-I	I-6-I	O-7-I	I-6-I
		HvirOR11	CAG38112.1	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	I-5-O	I-7-O	I-6-I	I-6-I	I-6-I
		HvirOR13	CAG38114.1	I-5-O	I-4-I	I-7-O	O-5-I	I-5-O	I-5-O	I-4-I	I-7-O	O-8-O	I-7-O	I-5-O
		HvirOR14	CAG38115.1	I-6-I	I-8-I	I-7-O	I-5-O	I-6-I	I-4-I	I-4-I	I-7-O	I-5-O	I-6-I	I-5-O
		HvirOR15	CAG38116.1	O-5-I	O-6-O	O-8-O	I-7-O	I-6-I	O-5-I	I-6-I	O-8-O	O-7-I	O-7-I	I-4-I
		HvirOR16	CAG38117.1	I-6-I	O-7-I	I-7-O	I-6-I	I-5-O	I-4-I	I-6-I	I-7-O	I-6-I	O-7-I	I-3-O
		HvirOR2*	CAD31851.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	O-8-O	I-7-O	I-7-O
	Spodoptera littoralis (Legeai et al., 2011; Montagne et al., 2012; De Fouchier et al., 2015)	SlitOR6	ACL81183.1	I-6-I	I-9-O	I-7-O	I-6-I	I-2-I	O-3-I	I-4-I	I-7-O	I-4-I	I-6-I	O-2-O
		SlitOR11	ACL81180.1	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	O-4-O	I-7-O	I-6-I	I-6-I	O-7-I
		SlitOR13	ACL81181.1	I-7-O	I-6-I	I-7-O	I-6-I	I-7-O	I-7-O	I-4-I	I-7-O	I-7-O	I-7-O	I-5-O
		SlitOR16	ACL81182.1	I-6-I	O-8-O	O-9-I	I-6-I	I-5-O	O-5-I	I-6-I	O-9-I	I-6-I	I-6-I	I-4-I
		SlitOR83b*	ACJ06648.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Spodoptera exigua (De Fouchier et al., 2015; Du et al., 2018; Zhang et al., 2018)	SexiOR6	AGH58119.1	I-4-I	O-5-L	O-6-O	I-5-O	I-5-O	I-4-I	I-2-I	I-8-I	I-6-I	I-6-I	I-5-O
		SexiOR11	AGH58120.1	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	O-4-O	I-7-O	I-6-I	I-6-I	O-7-I
		SexiOR13	AGH58121.1	I-7-O	I-6-I	I-7-O	I-6-I	I-7-O	I-7-O	I-4-I	I-7-O	I-6-I	I-7-O	I-5-O
		SexiOR16	AGH58122.1	I-6-I	O-9-I	O-9-I	I-6-I	I-6-I	O-5-I	O-6-I	I-7-O	I-6-I	I-6-I	I-4-I
		SexiOR2*	AAW52583.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Sesamia inferens (Zhang et al., 2013, 2014)	SinfOR21	AGY14579.2	I-6-I	O-7-I	I-8-I	O-5-I	I-6-I	I-8-I	I-6-I	I-7-O	I-6-I	O-7-I	I-3-O
		SinfOR27	AGY14585.2	I-4-I	I-8-I	I-7-O	O-6-O	I-4-I	I-4-I	I-6-I	I-7-O	I-6-I	I-7-O	I-5-O
		SinfOR29	AGY14587.2	I-4-I	I-5-O	I-7-O	I-7-O	I-4-I	O-5-I	I-4-I	I-7-O	O-7-I	O-7-I	I-4-I
		SinfOR2*	AGY14565.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Athetis lepigone (Zhang Y. N. et al., 2016, Zhang et al., 2019)	AlepOR3	AOE48008.1	I-6-I	I-6-I	I-8-I	I-7-O	I-8-I	I-4-I	I-6-I	I-7-O	I-6-I	I-6-I	I-6-I
		AlepOR4	AOE48009.1	I-6-I	I-8-I	O-6-O	O-6-O	I-6-I	I-4-I	I-6-I	I-7-O	I-6-I	I-8-I	I-4-I
		AlepOR5	AOE48010.1	I-6-I	I-6-I	I-7-O	I-6-I	I-6-I	O-5-I	I-6-I	I-7-O	O-7-I	O-8-O	I-4-I
		AlepOR6	AOE48011.1	I-6-I	I-8-I	I-8-I	I-6-I	I-6-I	I-6-I	I-4-I	I-7-O	I-6-I	I-6-I	I-8-I
		AlepOrco*	AOE48007.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Helicoverpa armigera (Zhang et al., 2010; Liu et al., 2012, Liu Y. et al., 2013; Liu et al., 2014; Jiang et al., 2014; Chang et al., 2016)	HarmOR1	ACS45304.1	I-7-O	I-5-O	I-8-I	O-5-I	I-5-O	O-6-O	I-4-I	I-7-O	I-6-I	I-7-O	O-4-O
		HarmOR2	ACS45305.1	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	I-5-O	I-7-O	I-6-I	I-6-I	I-6-I
		HarmOR3	ACS45306.1	I-6-I	O-7-I	I-7-O	O-5-I	I-5-O	I-4-I	I-6-I	I-7-O	I-6-I	O-7-I	O-4-O

(Continued)

TABLE 1 | (Continued)

Family	Species	Receptors	Genbank ID	CCTOP	HMMTOP	Memsat	Octopus	Philius	Phobius	Pro	Prodiv	Scampi	ScampiMsa	TMHMM
		HarmOR6	AIG51854.1	I-8-I	O-9-I	I-8-I	I-6-I	I-7-O	I-8-I	I-6-I	I-7-O	I-6-I	I-6-I	I-8-I
		HarmOR11	AIG51859.1	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	I-5-O	I-7-O	I-6-I	I-6-I	I-6-I
		HarmOR13	AIG51861.1	I-7-O	I-5-O	O-8-O	O-6-O	I-5-O	O-6-O	I-2-I	I-7-O	I-6-I	I-7-O	O-4-O
		HarmOR14	AJG42377.1	I-4-I	O-4-O	I-7-O	I-6-I	I-4-I	I-4-I	O-5-I	I-7-O	I-6-I	I-6-I	I-4-I
		HarmOR15	AIG51863.1	I-6-I	O-9-I	I-7-O	I-7-O	I-6-I	I-4-I	I-6-I	I-7-O	I-6-I	I-6-I	I-4-I
		HarmOR16	QLF97404.1	I-6-I	O-8-O	I-8-I	I-6-I	I-5-O	I-4-I	I-6-I	I-7-O	I-6-I	O-7-I	I-3-O
		HarmOR6-1	AGK90000.1	I-8-I	I-8-I	I-8-I	I-7-O	I-7-O	I-8-I	I-6-I	I-7-O	I-6-I	I-6-I	I-5-O
		HarmOR14a	AGK90005.1	I-4-I	I-4-I	I-7-O	I-7-O	I-4-I	I-4-I	I-6-I	I-7-O	I-5-O	O-7-I	I-4-I
		HarmOR14b	AGK90006.1	I-4-I	O-2-2	I-6-I	I-6-I	I-4-I	I-4-I	I-6-I	I-7-O	I-6-I	I-8-I	I-4-I
		HarmOr83b*	ADQ13177.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	O-8-O	I-7-O	I-7-O
	Helicoverpa assulta (Zhang et al., 2010; Jiang et al., 2014; Xu et al., 2015; Chang et al., 2016)	HassOR1	ACS45307.1	I-7-O	I-7-O	I-8-I	O-6-O	I-5-O	O-6-O	I-2-I	I-7-O	I-6-I	I-7-O	O-7-I
		HassOR2	ACS45308.1	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	I-5-O	I-7-O	I-6-I	I-6-I	I-6-I
		HassOR3	ACS45309.1	I-6-I	I-6-I	I-7-O	I-6-I	I-5-O	I-4-I	I-6-I	I-7-O	I-6-I	I-6-I	O-4-O
		HassOR6	AGK90014.1	I-6-I	I-8-I	I-8-I	I-6-I	I-6-I	I-6-I	I-6-I	I-7-O	O-7-I	I-6-I	I-8-I
		HassOR11	AJD81549.1	I-6-I	I-7-O	I-7-O	I-6-I	I-6-I	I-6-I	I-5-O	I-7-O	I-6-I	I-6-I	I-6-I
		HassOR13	AJD81551.1	I-7-O	I-5-O	I-8-I	O-6-O	I-5-O	I-7-O	I-2-I	I-7-O	I-6-I	I-7-O	O-7-I
		HassOR14	AHI44516.1	I-4-I	O-3-I	I-7-O	I-6-I	I-4-I	I-4-I	I-4-I	I-7-O	I-5-O	O-7-I	I-4-I
		HassOR14b	AGK90019.1	I-4-I	O-7-I	I-7-O	I-7-O	I-4-I	I-4-I	I-6-I	I-7-O	I-5-O	I-6-I	I-4-I
		HassOR15	AJD81553.1											
		HassOR16	AJD81554.1	I-6-I	I-6-I	I-7-O	I-7-O	I-4-I	I-4-I	I-6-I	I-7-O	I-6-I	I-6-I	O-4-O
		HassOr83b*	ABU45983.2	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	O-8-O	I-7-O	I-7-O
	Agrotis segetum (Zhang and Lofstedt, 2013)	AsegOR1	AGS41441.1	I-5-O	I-6-I	O-8-O	I-7-O	I-5-O	O-5-I	I-4-I	I-7-O	O-7-I	O-8-O	I-4-I
		AsegOR3	AGS41442.1	I-6-I	O-6-O	I-7-O	O-5-I	I-6-I	O-5-I	I-4-I	I-7-O	I-6-I	I-6-I	O-7-I
		AsegOR4	AGS41443.1	I-7-O	I-7-O	I-8-I	I-6-I	I-5-O	O-4-O	I-6-I	I-7-O	I-5-O	I-6-I	O-6-O
		AsegOR5	AGS41444.1	I-4-I	I-8-I	I-8-I	I-6-I	I-6-I	O-5-I	I-4-I	I-7-O	I-6-I	O-7-I	I-4-I
		AsegOR6	AGS41445.1	I-6-I	I-8-I	O-8-O	I-6-I	I-6-I	O-5-I	I-4-I	I-7-O	O-7-I	O-7-I	O-4-O
		AsegOR7	AGS41446.1	I-4-I	O-9-I	I-8-I	I-7-O	I-5-O	I-4-I	I-6-I	I-7-O	O-7-I	I-7-O	I-4-I
		AsegOR8	AGS41447.1	I-7-O	I-8-I	O-8-O	I-7-O	I-6-I	O-5-I	I-4-I	I-7-O	O-7-I	O-8-O	I-5-O
		AsegOR9	AGS41448.1	I-6-I	O-7-I	I-7-O	I-7-O	I-6-I	I-5-O	I-4-I	I-7-O	O-7-I	O-8-O	I-5-O
		AsegOR10	AGS41449.1	O-7-I	I-8-I	I-7-O	I-5-O	O-7-I	O-5-I	O-7-I	O-9-I	O-7-I	O-7-I	I-4-I
		AsegOrco*	AGS41440.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Spodoptera litura (Wu et al., 2013; Feng et al., 2015; Lin et al., 2015; Zhang et al., 2015)	SlituOR6	AGI96748.1	I-6-I	I-9-O	I-7-O	I-6-I	I-3-O	O-3-I	I-4-I	I-7-O	I-4-I	I-6-I	O-2-O
		SlituOR11	AGI96749.1	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	I-5-O	I-7-O	I-6-I	I-6-I	O-7-I
		SlituOR13	AGI96750.1	I-7-O	I-6-I	I-7-O	I-6-I	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-5-O
		SlituOR16	AGI96751.1	I-4-I	O-9-I	O-9-I	I-7-O	I-5-O	O-5-I	I-6-I	I-7-O	O-7-I	O-7-I	I-4-I
		SlituOrco*	XP_022831582.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O

(Continued)

TABLE 1 | (Continued)

Family	Species	Receptors	Genbank ID	CCTOP	HMMTOP	Memsat	Octopus	Philius	Phobius	Pro	Prodiv	Scampi	ScampiMsa	TMHMM
Tortricidae	*Ctenopseustis obliquana* (Steinwender et al., 2015; Grapputo et al., 2018)	CoblOR1	AIT71977.1	I-5-O	I-1-O	O-7-I	I-4-I	I-5-O	I-5-O	I-2-I	I-7-O	I-5-O	I-6-I	O-4-O
		CoblOR6	AIT71981.1	I-3-O	I-1-O	O-7-I	I-4-I	I-3-O	I-2-I	I-2-I	I-7-O	I-3-O	I-6-I	I-1-O
		CoblOR7	AIT71982.1	I-4-I	I-1-O	O-7-I	I-4-I	I-5-O	I-4-I	I-5-O	I-6-I	I-5-O	I-7-O	I-4-I
		CoblOR22	AIT71991.1	I-5-O	O-5-I	O-7-I	I-5-O	I-5-O	O-6-O	I-4-I	O-7-I	I-5-O	I-5-O	I-5-O
		CoblOR45a	AIT72004.1	I-6-I	I-2-I	O-7-I	I-7-O	I-3-O	I-2-I	I-2-I	I-6-I	I-7-O	I-6-I	I-1-O
		CoblOR45b	AIT72005.1	I-4-I	I-3-O	I-8-I	O-5-I	I-5-O	I-2-I	I-2-I	I-8-I	I-7-O	I-6-I	I-4-I
		CoblOrco*	AIT72022.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Epiphyas postvittana (Jordan et al., 2009; Corcoran et al., 2015)	EposOR1	ACJ12927	I-6-I	O-2-O	O-7-I	O-5-I	I-4-I	I-2-I	I-2-I	I-6-I	I-3-O	I-6-I	I-3-O
		EposOR6	JAI18060.1	I-4-I	I-5-O	I-6-I	I-5-O	I-4-I	I-4-I	I-4-I	I-7-O	I-6-I	I-6-I	I-4-I
		EposOR7	JAI18059.1	I-6-I	O-5-I	I-8-I	I-5-O	I-4-I	I-5-O	I-4-I	I-7-O	I-7-O	I-6-I	I-6-I
		EposOR21	JAI18051.1	I-6-I	I-7-O	O-7-I	I-6-I	O-7-I	O-7-I	I-6-I	I-7-O	I-7-O	I-6-I	I-6-I
		EposOR22	JAI18050.1	I-5-O	O-8-O	I-7-O	I-5-O	I-5-O	I-5-O	I-4-I	I-7-O	I-6-I	I-6-I	I-5-O
		EposOR41	JAI18032.1	I-6-I	I-1-O	I-8-I	O-6-O	I-5-O	I-3-O	I-2-I	I-7-O	I-7-O	I-6-I	I-4-I
		EposOR43	JAI18030.1	I-6-I	I-1-O	O-6-O	O-6-O	I-6-I	I-6-I	I-3-O	I-8-I	I-6-I	I-6-I	I-6-I
		EposOR45	JAI18028.1	I-6-I	I-2-I	I-6-I	I-4-I	I-4-I	I-6-I	I-4-I	I-8-I	I-4-I	I-6-I	I-6-I
		EposOR2*	ACJ12928.2	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Cydia pomonella (Bengtsson et al., 2012; Walker et al., 2016; Tian et al., 2020)	CpomOR1	AFC91714.1	I-6-I	O-4-O	I-8-I	I-7-O	I-6-I	O-5-I	I-4-I	I-6-I	O-6-O	O-7-I	I-5-O
		CpomOR2a	AFC91715.2	I-6-I	O-4-O	I-7-O	I-6-I	I-5-O	I-6-I	I-6-I	I-7-O	I-6-I	I-6-I	I-5-O
		CpomOR2b	JAP38462.1	I-5-O	O-5-I	I-7-O	I-6-I	I-5-O	I-5-O	I-6-I	I-7-O	I-6-I	I-6-I	I-5-O
		CpomOR2c	JAP38461.1	I-6-I	I-5-O	I-8-I	I-6-I	I-4-I	I-4-I	O-7-I	I-7-O	I-6-I	I-6-I	I-5-O
		CpomOR3	AFC91713.2	I-6-I	I-7-O	I-7-O	O-6-O	I-6-I	I-6-I	I-6-I	I-7-O	I-6-I	I-6-I	I-6-I
		CpomOR4	AFC91716.2	O-7-I	O-4-O	O-8-O	I-7-O	O-5-I	O-5-I	O-7-I	O-8-O	I-5-O	I-6-I	O-6-O
		CpomOR5	JAP38459.1	I-6-I	I-2-I	I-8-I	I-7-O	I-6-I	O-7-I	I-6-I	I-7-O	I-6-I	I-6-I	I-7-O
		CpomOR6a	AFC91711.2	I-6-I	I-6-I	O-7-I	I-3-O	I-5-O	O-5-I	I-3-O	I-7-O	I-6-I	I-6-I	I-3-O
		CpomOR6b	JAP38458.1	I-7-O	I-7-O	I-7-O	I-4-I	I-5-O	O-6-O	I-3-O	I-7-O	I-7-O	I-7-O	I-5-O
		CpomOR7	JAP38457.1	I-6-I	I-7-O	I-8-I	O-5-I	I-7-O	I-6-I	I-4-I	I-7-O	I-7-O	I-6-I	I-7-O
		CpomOR8	JAP38456.1	I-6-I	I-6-I	I-7-O	I-7-O	I-6-I	I-6-I	I-6-I	I-7-O	I-7-O	I-6-I	I-7-O
		CpomOR9	JAP38455.1	I-6-I	I-6-I	I-8-I	I-3-O	I-6-I	I-6-I	I-2-I	I-6-I	I-7-O	I-7-O	O-4-O
		CpomOR21	JAP38451.1											
		CpomOR22	AFC91723.2	I-6-I	O-6-O	I-7-O	I-6-I	I-5-O	I-5-O	I-6-I	I-7-O	I-5-O	I-6-I	I-6-I
		CpomOrco*	AFC91712.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-5-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Cydia fagiglandana (Gonzalez et al., 2017)	CfagOR1	AST36293.1	I-6-I	I-6-I	I-8-I	I-6-I	I-6-I	I-5-O	I-6-I	I-7-O	I-5-O	I-6-I	I-6-I
		CfagOR2.1	AST36294.1											
		CfagOR2.2	AST36295.1											
		CfagOR3	AST36296.1	I-5-O	I-6-I	I-7-O	I-5-O	I-5-O	I-4-I	I-5-O	I-7-O	I-6-I	I-6-I	I-5-O
		CfagOR4	AST36297.1	O-4-O	I-6-I	O-7-I	O-6-O	I-3-O	O-4-O	I-4-I	I-7-O	I-6-I	I-8-I	O-4-O
		CfagOR5.1	AST36298.1	I-6-I	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	I-7-O	I-5-O	I-6-I	I-6-I
		CfagOR5.2	AST36299.1											

(Continued)

TABLE 1 | (Continued)

Family	Species	Receptors	Genbank ID	CCTOP	HMMTOP	Memsat	Octopus	Philius	Phobius	Pro	Prodiv	Scampi	ScampiMsa	TMHMM
		CfagOR6	AST36300.1	I-6-I	I-6-I	O-6-O	I-4-I	I-5-O	O-5-I	I-4-I	I-7-O	I-7-O	I-6-I	O-4-O
		CfagOR7	AST36301.1	I-6-I	I-7-O	I-7-O	O-5-I	I-6-I	I-6-I	I-4-I	I-7-O	I-6-I	I-6-I	I-6-I
		CfagOR8	AST36302.1	I-4-I	O-5-I	I-8-I	O-6-O	I-4-I	I-4-I	I-2-I	I-7-O	I-6-I	I-6-I	I-5-O
		CfagOrco*	AST36341.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Ctenopseustis herana (Steinwender et al., 2015; Grapputo et al., 2018)	CherOR1a	AIT69867.1	I-6-I	I-3-O	O-7-I	I-6-I	I-4-I	I-6-I	I-2-I	I-9-O	I-6-I	I-6-I	I-5-O
		CherOR1b	AIT69868.1	I-5-O	I-3-O	O-7-I	O-4-O	I-5-O	I-5-O	I-2-I	I-7-O	I-5-O	I-6-I	I-5-O
		CherOR6	AIT69872.1	I-3-O	I-1-O	O-7-I	I-4-I	I-3-O	I-2-I	I-2-I	I-7-O	I-3-O	I-6-I	I-1-O
		CherOR7	AIT69873.1	I-4-I	I-2-I	I-7-O	I-6-I	I-5-O	I-4-I	I-3-O	I-5-O	I-5-O	I-6-I	I-4-I
		CherOR45	AIT69894.1	I-6-I	O-3-I	O-7-I	O-6-O	I-5-O	I-4-I	I-2-I	I-8-I	I-7-O	I-6-I	I-6-I
		CherOrco*	AIT69913.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Planotortrix octo (Steinwender et al., 2016; Grapputo et al., 2018)	PoctOR1	AJF23780.1	I-5-O	I-1-O	I-8-I	O-4-O	I-5-O	I-5-O	I-5-O	I-7-O	I-5-O	I-6-I	I-5-O
		PoctOR6	AJF23784.1	I-5-O	I-1-O	I-8-I	I-4-I	I-5-O	I-3-O	I-2-I	I-8-I	I-4-I	I-6-I	I-1-O
		PoctOR7	AJF23785.1	I-4-I	I-1-O	I-6-I	I-4-I	I-5-O	I-4-I	I-4-I	I-7-O	I-5-O	I-7-O	I-4-I
		PoctOR21	AJF23792.1	I-6-I	I-5-O	I-8-I	I-5-O	I-4-I	I-6-I	I-2-I	I-7-O	I-5-O	I-6-I	I-6-I
		PoctOR22	AJF23793.1	O-6-O	O-7-I	O-7-I	I-5-O	O-6-O	O-6-O	O-5-I	O-7-I	O-7-I	O-6-O	I-5-O
		PoctOR45	AJF23806.1	I-6-I	I-2-I	I-8-I	O-5-I	I-5-O	I-4-I	I-4-I	I-6-I	I-7-O	I-6-I	I-3-O
		PoctOrco*	AJF23826.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Planotortrix excessana (Steinwender et al., 2016; Grapputo et al., 2018)	PexcOR1	AJE25866.1	I-5-O	I-3-O	I-8-I	O-4-O	I-5-O	I-5-O	I-5-O	I-7-O	I-5-O	I-6-I	I-5-O
		PexcOR7	AJE25869.1	I-5-O	I-2-I	I-8-I	I-5-O	I-5-O	I-4-I	I-4-I	I-5-O	I-6-I	I-7-O	I-5-O
		PexcOR22	AJE25877.1	I-5-O	O-5-I	I-8-I	I-5-O	I-5-O	I-5-O	I-4-I	I-6-I	I-6-I	I-6-I	I-4-I
		PexcOR45	AJE25890.1	I-7-O	O-4-O	I-7-O	I-6-I	I-5-O	I-6-I	I-4-I	I-7-O	I-7-O	I-7-O	I-6-I
		PexcOrco*	AJE25910.1											
	Hedya nubiferana (Grapputo et al., 2018)	HnubOR2.1	AST36245.1	I-6-I	I-7-O	I-7-O	I-4-I	I-6-I	I-6-I	I-4-I	I-8-I	I-7-O	I-7-O	I-6-I
		HnubOR2.2	AST36246.1	I-6-I	O-6-O	I-8-I	I-4-I	I-6-I	I-8-I	I-4-I	I-7-O	I-7-O	I-6-I	I-8-I
		HnubOR3	AST36247.1	I-5-O	I-8-I	I-8-I	I-5-O	I-5-O	I-5-O	I-3-O	I-8-I	I-7-O	I-6-I	I-8-I
		HnubOR6	AST36248.1											
		HnubOR8.1	AST36249.1	I-7-O	I-6-I	O-7-I	I-7-O	I-5-O	I-5-O	I-4-I	I-7-O	I-6-I	I-7-O	I-6-I
		HnubOR8.2	AST36250.1	I-5-O	I-5-O	O-7-I	I-5-O	I-5-O	I-4-I	I-5-O	I-6-I	I-5-O	I-7-O	I-5-O
		HnubOrco*	AST36292.1											
	Cydia nigricana (Gonzalez et al., 2017)	CnigOR1	AST36373.1	I-6-I	I-6-I	O-7-I	I-6-I	I-6-I	I-5-O	I-6-I	I-7-O	I-5-O	I-6-I	I-5-O
		CnigOR2	AST36374.1	I-6-I	I-5-O	I-8-I	I-7-O	I-4-I	O-5-I	I-6-I	I-7-O	I-5-O	I-6-I	I-5-O
		CnigOR5	AST36376.1	I-6-I	I-6-I	O-8-O	I-7-O	I-6-I	I-6-I	I-6-I	I-7-O	I-5-O	I-6-I	I-6-I
		CnigOR6	AST36377.1	O-6-O	I-6-I	O-6-O	O-6-O	I-5-O	O-3-I	I-4-I	I-7-O	I-7-O	I-7-O	O-4-O
		CnigOR7	AST36378.1	I-7-O	O-5-I	I-8-I	I-5-O	I-5-O	I-7-O	I-2-I	I-7-O	I-7-O	I-6-I	I-7-O
		CnigOR8	AST36379.1	I-7-O	I-6-I	I-7-O	O-6-O	I-5-O	I-6-I	I-4-I	I-7-O	I-6-I	I-7-O	I-5-O
		CnigOR9	AST36380.1	I-6-I	O-8-O	I-7-O	O-5-I	I-4-I	I-4-I	I-4-I	I-7-O	I-7-O	I-6-I	O-4-O
		CnigOrco*	AST36420.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O

(Continued)

TABLE 1 | (Continued)

Family	Species	Receptors	Genbank ID	CCTOP	HMMTOP	Memsat	Octopus	Philius	Phobius	Pro	Prodiv	Scampi	ScampiMsa	TMHMM
	Lobesia botrana (Rojas et al., 2018)	LobOR1	AXF48756.1	I-6-I	O-5-I	I-8-I	O-5-I	I-6-I	O-7-I	I-6-I	I-7-O	I-9-O	O-7-I	I-6-I
		LobOR2.1	AXF48757.1											
		LobOR2.2	AXF48758.1	O-4-O	O-4-O	I-6-I	O-3-I	I-4-I	I-4-I	I-4-I	I-4-I	I-3-O	I-4-I	I-4-I
		LobOR2.3	AXF48759.1	I-5-O	I-5-O	I-5-O	I-5-O	I-6-I	I-6-I	I-4-I	I-6-I	I-5-O	I-5-O	I-5-O
		LobOR2.4	AXF48760.1	I-6-I	I-8-I	I-7-O	I-6-I	I-4-I	I-4-I	I-6-I	I-7-O	I-6-I	I-6-I	I-6-I
		LobOR3.1	AXF48761.1											
		LobOR4.2	AXF48764.1											
		LobOR6	AXF48766.1											
		LobOR38.2	AXF48785.1											
		LobOR76	AXF48812.1											
		LobOrco*	AXF48755.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Planotortrix notophaea (Carraher et al., 2012)	PnotOR1	AET06153.1											
		PnotOR2*	AET06159.1											
Pyralidae	*Amyelois transitella* (Xu et al., 2012; Garczynski and Leal, 2015)	AtraOR1	AFP54146.1	I-6-I	I-5-O	I-8-I	O-4-O	I-4-I	I-6-I	I-4-I	I-6-I	I-6-I	I-6-I	I-4-I
		AtraOR3	AFP54147.1	I-5-O	I-7-O	I-8-I	O-4-O	I-5-O	O-6-O	I-4-I	I-8-I	O-6-O	O-5-I	I-4-I
		AtraOR4	AFP66948.1	I-6-I	I-7-O	I-8-I	I-7-O	I-6-I	I-6-I	I-6-I	I-7-O	I-6-I	I-6-I	I-6-I
		AtraOR4A	AFP66949.1	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-6-I	I-7-O	I-7-O	I-6-I	I-7-O	I-6-I
		AtraOrco*	AFP54145.1	I-7-O	O-8-O	I-8-I	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
Crambidae	*Diaphania indica* (Mitsuno et al., 2008)	DindOR1	BAG71417.1	I-6-I	O-6-O	I-8-I	O-5-I	I-8-I	O-5-I	I-4-I	I-7-O	I-7-O	I-7-O	I-6-I
		DindOR3	BAG71424.1	I-6-I	I-5-O	I-7-O	I-6-I	I-6-I	I-7-I	I-6-I	I-7-O	O-8-O	I-8-I	I-5-O
		DindOR2*	BAG71418.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Ostrinia latipennis (Miura et al., 2009)	OlatOR1	BAH57981.1	I-7-O	I-7-O	I-8-I	I-7-O	I-4-I	I-7-O	I-4-I	I-7-O	I-7-O	I-6-I	I-4-I
		OlatOR3	BAI66617.1											
		OlatOR4	BAI66618.1											
		OlatOR5a	BAI66619.1											
		OlatOR5b	BAI66620.1											
		OlatOR7	BAI66621.1											
		OlatOR8	BAI66622.1											
		OlatOR2*	BAH57974.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Ostrinia nubilalis (Miura et al., 2009, 2010; Wanner et al., 2010; Yasukochi et al., 2011; Leary et al., 2012)	OnubOR1	BAH57980.1											
		OnubOR3	BAI66623.1											
		OnubOR4	BAI66624.1											
		OnubOR5	BAI66625.3											
		OnubOR6	BAI66626.1											
		OnubOR7	BAI66627.1											
		OnubOR8	BAJ61934.1	I-7-O	I-7-O	I-7-O	O-5-I	I-5-O	I-7-O	I-5-O	I-7-O	I-7-O	I-7-O	O-7-I

(Continued)

TABLE 1 | (Continued)

Family	Species	Receptors	Genbank ID	CCTOP	HMMTOP	Memsat	Octopus	Philius	Phobius	Pro	Prodiv	Scampi	ScampiMsa	TMHMM
		OnubOR2*	ADB89179.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Conogethes punctiferalis (Ge et al., 2016; Jia et al., 2016)	CpunOR1	ARO76407.1	I-6-I	I-7-O	I-7-O	I-6-I	I-6-I	I-4-I	I-4-I	O-9-I	I-6-I	I-7-O	I-4-I
		CpunOR3	ARO76409.1	I-4-I	O-4-O	I-5-O	I-4-I	I-4-I	I-2-I	I-4-I	I-5-O	I-4-I	I-4-I	O-4-O
		CpunOR4	ARO76410.1											
		CpunOR5	ARO76411.1	I-7-O	I-6-I	O-7-I	I-7-O	I-7-O	I-6-I	I-4-I	I-7-O	I-8-I	I-9-O	I-4-I
		CpunOR6	ARO76412.1											
		CpunOR7	ARO76413.1	I-6-I	O-6-O	I-6-I	O-8-O	I-5-O	I-6-I	I-6-I	I-6-I	I-8-I	O-8-O	O-7-I
		CpunOR8	ARO76414.1	I-6-I	I-5-O	I-8-I	I-6-I	I-6-I	O-5-I	I-4-I	I-7-O	I-7-O	I-7-O	I-6-I
		CpunOR9	ARO76415.1	I-6-I	O-5-I	I-8-I	I-6-I	I-5-O	O-6-O	I-4-I	I-7-O	I-5-O	I-6-I	I-6-I
		CpunOR2*	ARO76408.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Ostrinia furnacalis (Miura et al., 2009, 2010; Leary et al., 2012)	OfurOR1	BAH57982.1	I-5-O	I-6-I	I-8-I	O-6-O	I-5-O	I-5-O	I-5-O	I-7-O	I-7-O	I-7-O	I-5-O
		OfurOR3	AFK30395.1	I-7-O	O-4-O	I-7-O	O-5-I	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	O-6-O
		OfurOR4	AFK30397.1	I-6-I	I-6-I	O-7-I	I-7-O	I-5-O	I-8-I	I-6-I	I-7-O	I-6-I	I-6-I	I-5-O
		OfurOR5	BAI66613.1											
		OfurOR6	AFK30403.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-3-O	I-7-O	I-7-O	I-6-I	O-7-I
		OfurOR7	AGG91649.1	I-4-I	O-4-O	O-9-I	I-4-I	I-4-I	O-5-I	I-2-I	I-9-O	I-7-O	I-7-O	I-2-I
		OfurOR8	AGG91650.1	I-5-O	I-7-O	I-7-O	O-4-O	I-5-O	I-7-O	I-5-O	I-7-O	I-7-O	I-7-O	I-5-O
		OfurOR2*	AGG91643.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Ostrinia scapulalis (Miura et al., 2009, 2010)	OscaOR1	BAH57975.1	I-5-O	I-7-O	I-8-I	I-7-O	I-5-O	I-5-O	I-2-I	I-7-O	I-6-I	I-7-O	I-5-O
		OscaOR3	BAI66604.1	I-7-O	I-6-I	O-7-I	I-7-O	I-5-O	I-9-O	I-6-I	I-7-O	I-6-I	I-6-I	I-5-O
		OscaOR4	BAI66605.1	O-6-O	O-6-O	O-6-O	O-5-I	I-7-O	I-7-O	I-4-I	I-7-O	I-7-O	I-7-O	O-6-O
		OscaOR5	BAI66607.1	I-7-O	I-7-O	I-7-O	I-7-O	I-7-O	I-5-O	I-5-O	I-9-O	I-7-O	I-7-O	I-7-O
		OscaOR6	BAI66608.1	I-7-O	I-7-O	O-6-O	I-7-O	I-7-O	I-7-O	I-5-O	I-7-O	I-7-O	I-7-O	I-7-O
		OscaOR7	BAI66609.1	I-6-I	O-6-O	O-8-O	I-6-I	I-6-I	O-5-I	I-3-O	I-6-I	I-7-O	O-8-O	I-6-I
		OscaOR8	BAI66610.1	I-7-O	I-7-O	I-7-O	O-5-I	I-5-O	I-7-O	I-5-O	I-7-O	I-7-O	I-7-O	O-7-I
		OscaOR2*	BAH57973.1	I-7-O	O-8-O	I-7-O	I-7-O	I-7-O	I-7-O	I-6-I	I-7-O	I-7-O	I-7-O	I-7-O
	Ostrinia palustris (Miura et al., 2009, 2010)	OpalOR1	BAH57978.1	I-5-O	I-7-O	I-8-I	I-7-O	I-5-O	I-5-O	I-4-I	I-7-O	I-6-I	I-7-O	I-3-O
		OpalOR3	BAI66634.1											
		OpalOR4	BAI66635.1											
		OpalOR7	BAI66636.1											
		OpalOR8	BAI66637.3											
		OpalOR2*	BAJ23262.1											
	Ostrinia ovalipennis (Miura et al., 2009, 2010)	OovaOR1	BAH57979.1	I-7-O	I-7-O	I-8-I	I-7-O	I-4-I	I-7-O	I-4-I	I-7-O	I-7-O	I-6-I	I-4-I
		OovaOR3	BAI66629.3											
		OovaOR4	BAI66630.1											
		OovaOR5	BAI66631.1											
		OovaOR7	BAI66631.1											
		OovaOR8	BAI66633.1											

(Continued)

TABLE 1 | (Continued)

Family	Species	Receptors	Genbank ID	CCTOP	HMMTOP	Memsat	Octopus	Philius	Phobius	Pro	Prodiv	Scampi	ScampiMsa	TMHMM
		OovaOR2*	BAJ23264.1											
	Ostrinia zaguliaevi (Miura et al., 2009, 2010)	OzagOR1	BAH57976.1	I-5-O	I-6-I	I-8-I	I-8-O	I-5-O	I-5-O	I-4-I	I-7-O	I-6-I	I-7-O	O-4-O
		OzagOR3	BAI66638.1											
		OzagOR4	BAI66639.1											
		OzagOR5	BAI66640.1											
		OzagOR7	BAI66642.1											
		OzagOR8	BAI66643.1											
		OzagOR2*	BAJ23265.1											
	Ostrinia zealis (Miura et al., 2009, 2010)	OzeaOR1	BAH57977.1	I-5-O	I-6-I	O-7-I	O-6-O	I-5-O	I-5-O	I-3-O	I-7-O	I-7-O	I-7-O	O-4-O
		OzeaOR3a	BAI66644.1											
		OzeaOR3b	BAI66645.1											
		OzeaOR4	BAI66646.1											
		OzeaOR6	BAI66647.1											
		OzeaOR7	BAI66648.1											
		OzeaOR8	BAI66649.1											
		OzeaOR2*	BAJ23260.1											

"" represents the Orco of each species.*

I/O-n-I/O: "n" represents the transmembrane number of certain PR predicted by CCTOP et al; "I" means Intracellular, "O" means Extracellular; the "I/O" in front of "n" means the N terminus location, and the "I/O" behind "n" means the C terminus location.

showed that these SPRs were clustered together with other *B. mori* and *H. virescens* SPRs separated from general odorant receptors (Liu Y. et al., 2013). All identified chemosensory receptors of *H. armigera* and *H. assulta* were used to construct a phylogenetic tree with the known ORs of *H. virescens*, HvOrco, HarmOrco, and HassOrco which were grouped together in a single lineage named the Orco subfamily. OR6/11/13/14/15/16 from the three species were clustered in the SPR subfamily, which included the functionally identified SPRs in *H. virescens* (Jiang et al., 2014). Notably, 10 OR sequences were used in the phylogenetic analysis with another 41 *H. assulta* ORs identified, 68 *B. mori* ORs, and 60 *H. armigera* ORs. One group of ORs which was formed by 7 *B. mori* SPRs, 7 *H. armigera* SPRs, and 7 HassORs was identified as the SPR group (Xu et al., 2015).

In a neighbor-joining tree including OR repertoires of *Ctenopseustis obliquana*, *Ctenopseustis herana*, and *Epiphyas postvittana*, of which the clade predicted to contain the SPRs of many moth species is well supported by the bootstrap analysis (Steinwender et al., 2015). The phylogenetic analysis of the EposORs was performed against comprehensive OR datasets from *B. mori*, *H. virescens*, and *C. pomonella*, in which 8 receptors (EposOR1/6/7/21/22/41/43/45) fall into a well-supported clade that contains SPRs from other moth species, including BmOR1/3 and HvirOR6/13/14/16 (Corcoran et al., 2015). In the sequence similarity analysis of the *C. pomonella* ORs, the OR repertoires of *B. mori*, *H. virescens*, *M. sexta*, *S. littoralis*, and several ORs, Cpom1/3/4/5/6 are grouped in a conserved clade containing Lepidopteran SPRs, and Orco forms a clade (Bengtsson et al., 2012).

The *C. pomonella* ORs are presented phylogenetically within the context of other tortricid moth species (*C. obliquana*, *C. herana*, and *E. postvittana*) from which large OR repertoires have been published, along with *B. mori* ORs serving as a Lepidopteran out-group, Orco clade, and SPR clade (Walker et al., 2016). The phylogenetic analyses of odorant receptors from *Planotortrix octo*, *Planotortrix excessana*, *C. obliquana*, *C. herana*, and *E. postvittana* showed that the tree is rooted with Orco, and the SPR clade is formed (Steinwender et al., 2016). The phylogenetic relationships of odorant receptors from *L. botrana* and other insects, such as *C. pomonella*, *E. postvittana*, *B. mori*, *Ostrinia nubilalis*, *Spodoptera exigua*, *S. littoralis*, *P. xylostella*, *H. armigera*, and *H. assulta*, indicated that 10 LbotOR sequences were predicted to be closely related to the SPR clade proposed for *C. pomonella*, *B. mori*, *M. sexta*, *S. littoralis*, and *O. nubilalis* (Rojas et al., 2018). In the phylogenetic tree of odorant receptors, orthologous OR1s of the genus *Ostrinia* formed a clade, and the OR1 group was included in a single lineage of the SPR subfamily (Miura et al., 2009). In the phylogenetic tree of ORs constructed using the sequences of 162 ORs from *B. mori*, *Ostrinia furnacalis*, and *Conogethes punctiferalis*, the OR sequences were clustered into SPRs, Orco, and other divergent ORs (Ge et al., 2016). In a neighbor-joining tree of 130 OR sequences built from three different Lepidoptera species, including *C. punctiferlis*, *B. mori*, and *O. furnacalis*, the Orco was clustered with other Lepidoptera Orco sequences (Jia et al., 2016).

The SPRs of Lepidopteran insects had been phylogenetically analyzed in several studies. The phylogeny of Lepidopteran

SPRs shows four orthologous clades, in which a cluster only contains candidate SPRs of noctuid species, and paralogous SPRs from Cluster I differ dramatically in ligand selectivity and sensitivity (Zhang Y. N. et al., 2016). The neighbor-joining analysis of highly conserved Noctuidae SPRs and their ligands had been summarized (Jiang et al., 2019, 2020). In a maximum-likelihood phylogeny of Lepidopteran candidate SPRs from Yponomeutoidea, Pyraloidea, Tortricoidea, Papilionoidea, Bombycoidea, and Noctuoidea, SPRs were grouped into 5 different paralogous lineages, i.e., each containing SPRs from different Lepidopteran superfamilies (De Fouchier et al., 2015). The phylogenetic tree showed that the SPRs of different moths were clustered into four branches; while the moth Orcos were clustered into one branch that was separated from the SPRs (Zhang et al., 2014, Zhang Y. N. et al., 2016). Phylogenetic analysis constructed with 10 SPRs from *Cydia fagiglandana*, 7 SPRs from *Hedya nubiferana*, 8 SPRs from *B. mori*, 6 SPRs from *E. postvittana*, and 14 SPRs from *C. pomonella* revealed that the four genes (i.e., CpomOR1, CpomOR2a, CpomOR5, and CpomOR7) were clustered with Lepidopteran SPRs (Tian et al., 2020). In the phylogenetic tree of SPR subfamily proteins, OR1/3/4/5/6/7/8 of different *Ostrinia* species, respectively, formed a clade (Miura et al., 2010). The phylogenetic relationship of OnOR1-6 with SPRs from the superfamily of Bombycoidea, Noctuoidea, Pyraloidea, Yponomeutoidea, and Tortricoidea suggests that there is no clear relationship between the phylogeny of SPRs and their ligand (Wanner et al., 2010). In a phylogenetic tree, the OR4,5,8 genes of several *Ostrinia* moths and the OnOr6 gene formed a definite clade, which did not include any known SPRs of other Lepidoptera (Yasukochi et al., 2011). The neighbor-joining phylogenetic tree of SPRs of 8 *Ostrinia* species forms 5 orthologous lineages, i.e., each with 100% bootstrap support, belonging to the Lepidopteran SPR lineage (Leary et al., 2012).

In our summary, the phylogenetic tree of 256 SPRs and Orcos showed some similar information as former reports (**Figure 1**). The Orcos of 40 species from 10 families of Lepidoptera form a clade with a bootstrap value of 100 distinct from SPRs, indicating the high conservation of Orcos, implied the fixed function of Orcos (Zhang et al., 2019). In the Orco clade, only the 10 Orcos from Noctuidae are in a single branch; the Orcos from Tortricidae are distributed in two branches, with one branch clustered with Orcos from Crambidae and Plutellidae. As to SPRs, SPRs from Noctuidae, Tortricidae, and Crambidae are with the major number, and there is no strict affiliation between SPRs and families.

We then summarized some SPRs and their corresponding ligands (**Figure 2**), and the branch position of each SPRs are the same as in **Figure 1**. The 10 SPRs share the same ligand Z11-16:Ald; AtraOR3, MsepOR3, HvirOR13, HassOR1/13, and HarmOR1/13 are in a branch with a bootstrap support value of 99; and PxylOR1, HvirOR16, HassOR14b, and DindOR1 are in different clusters. Z11-16:Ald is the pheromone component of *H. armigera*, *H. assulta*, *H. virescens*, and *P. xylostella.* SexiOR13/SlitOR13/SlituOR13/SinfOR27 and SlitOR6/SlituOR6 are tuned to the ligand Z9,E12-14:OAC, which is the pheromone component of the 3 corresponding species. E10,Z12-16:Ald is the ligand of BmOR3 and MsexOR1 on the same branch with a bootstrap value of 99; AlepOR3 and AlepOR4 are both tuned to Z7-12:Ac, which is their corresponding pheromone component. HarmOR16, HvirOR16, and SinfOR21 are dispersed on different branches but share one ligand Z11-16:OH. PxylOR4/41/AlepOR4, OnubOR1/3/OfurOR3, and OnubOR5/OscaOR3, respectively, tune to their corresponding major ligands Z9-14:Ac, E12-14:OAc, and Z12-14:OAc. OscaOR1/OlatOR1/OovaOR1/OscaOR3 are tuned to E11-14:OH. As the SPRs from Tortricidae, CpomOR2a, and CpomOR5 are tuned to E8,E10-12:Ac, CoblOR7 and CherOR7 both tuned to Z8-14:OAc. Similar to former research (Wanner et al., 2010), there is no clear relationship between the phylogeny of SPRs and their ligands.

Structure of Lepidopteran Sex Pheromone Receptors

Several studies have predicted the transmembrane and topology of Lepidopteran SPRs and Orcos. The sequence analysis of PxylOR1/3/4 and DindOR1/3 by transmembrane domain prediction tools TMpred and TMHMM indicated that proteins coded by the genes possess the seven transmembrane domains that are the characteristics of the G protein-coupled receptors (GPCR) superfamily (Mitsuno et al., 2008). TMHMM2.0 was used for the prediction of transmembrane domains of PxylOR8/41/45, and these SPRs contain seven putative transmembrane domains (Liu et al., 2018). As with ORs from other insects, SexiOR3 hypothetically contains seven transmembrane domains with a predicted intracellular N-terminus and an extracellular C-terminus by TMHMM Server version 2.0 (Liu C. et al., 2013). SinfOR21/27/29 predicted to have the typical characteristics of an OR, including seven putative transmembrane domains, an intracellular N-terminus, and an extracellular C-terminus by TMHMM2.0 (Zhang et al., 2014). Predicted by the Phobius and MANSAT3, the SlituOR3 has seven transmembrane domains (Lin et al., 2015). According to TMBase and the SFINX package, SlituOR6/11/13/16 were predicted to possess 7 transmembrane domains (Zhang et al., 2015). The predicted transmembrane topology of CoblOR7 and CherOR7 was using SPLIT 4.0 at the transmembrane prediction server (Steinwender et al., 2015). Through TMHMM2.0, TMAP, and TMpred, EposOR1 was predicted to contain 7 transmembrane domains and an intracellular N-terminus with the exception of TMpred (Jordan et al., 2009). SlitOrco and other Noctuidae of the Orcos of Lepidoptera insects were predicted with seven transmembrane domains by TMHMM2.0 (Wu et al., 2013).

The transmembrane topology of the SPRs and Orcos of Lepidopteran insects was predicted by online software CCTOP (Dobson et al., 2015), and the structural features of these receptors according to these predictions were speculated (**Table 1**). All the SPRs showed no significant common features (such as transmembrane numbers) according to the

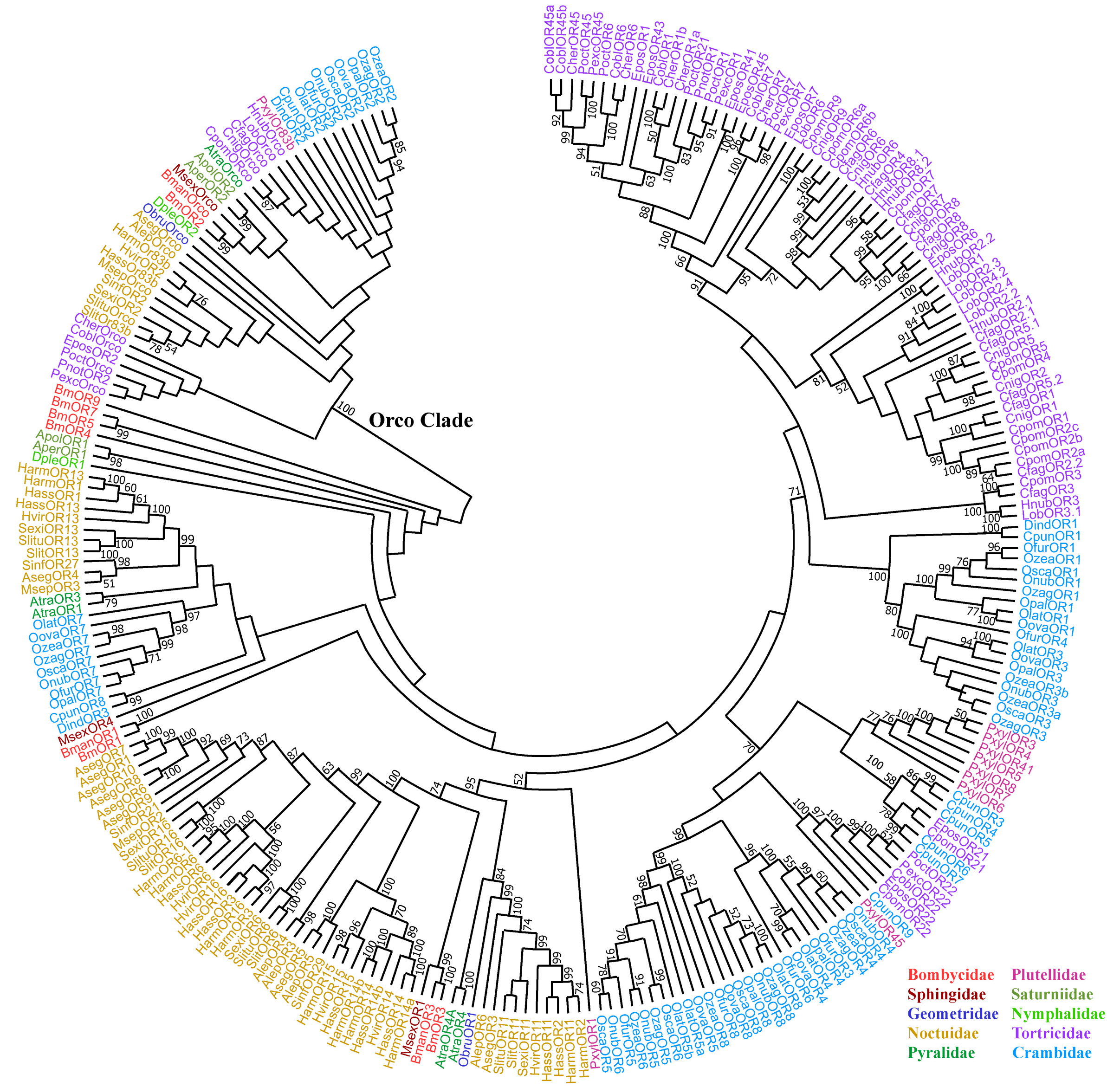

FIGURE 1 | Phylogenetic tree of candidate sex pheromone receptors (SPRs) and Orcos of Lepidopteran insects. Lepidopteran SPR labels and their corresponding family name (at the bottom right corner) are in the same color. Node support was assessed with 1,000 bootstrap replicates, and values greater than 50% are shown. The model used was JTT according to the model prediction by MEGA X, and the neighbor-joining method was used. All the GenBank IDs of receptors are shown in **Table 1**.

CCTOP prediction results, but the intracellular N-terminus and extracellular C-terminus locations of these SPRs were similar to the previous reports of Lepidopteran insects (Mitsuno et al., 2008). The single-particle cryo-electron microscopy (EM) structure of an Orco homomer from the parasitic *fig wasp* at 3.5 Å resolution had been reported, which confirmed the predicted topology of the Orco in Lepidopteran insects (Butterwick et al., 2018). Through SwissModel online predictions, the cryo-EM structures of the Orco mentioned earlier (SMTL ID: 6c70.1) were the best templates of all the SPRs according to sequence identities, and thus, all the receptors have similar structural characteristics with 7 transmembrane helixes, of which BmOR3 is shown in **Figure 3** as a sample.

Characterization and Interaction of Sex Pheromone Receptors and Specific Sex Pheromone Molecules

The functional characterization of SPRs in Lepidopteran insects can be divided into two types, namely, *in vivo* and *in vitro*. Almost all the Lepidopteran SPRs were characterized through classical *in vitro* electrophysiological recording (i.e., two-electrode voltage-clamp) of heterologous expression system on

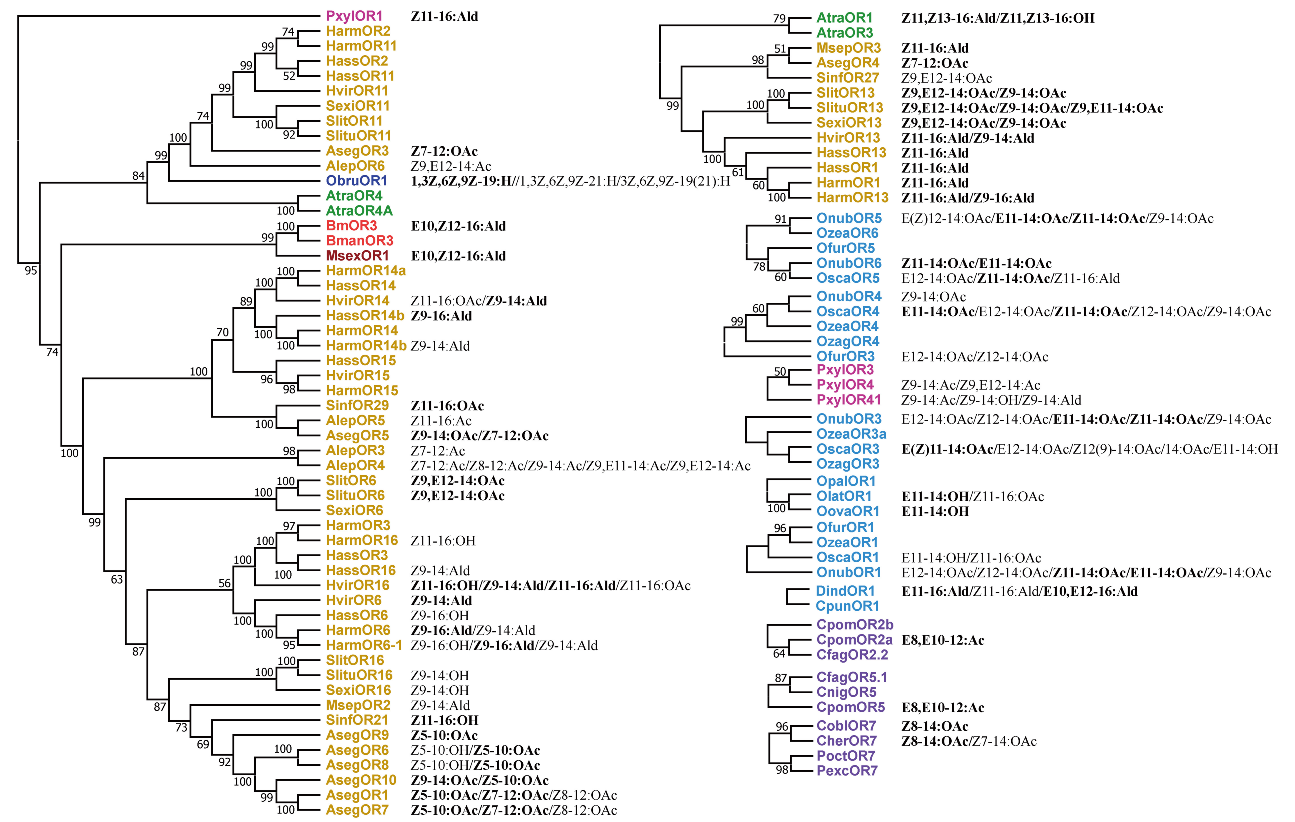

FIGURE 2 | Lepidopteran SPRs and their ligands in the form of phylogenetic branches. Lepidopteran SPR labels and their corresponding family name are in the same color as shown in **Figure 1**. The ligands of SPRs are on the right side of each SPR, among which the chemical compound in bold is the sex pheromone molecule (SPM) of the corresponding species.

SPM activation. The two-electrode voltage-clamp recording was conducted in Xenopus oocytes coinjected with complementary RNAs encoding BmOR1 and BmOR2 to test the bombykol-inducing current response (Nakagawa et al., 2005). The similar method was used in a specific pheromone detection of PxylOR1/4/8/41/45 (Sun et al., 2013; Liu et al., 2018), MsexOR1 (Wicher et al., 2017), EgirOR1 (Li et al., 2017), MsepOR2/3 (Jiang et al., 2019, 2020), HvirOR6/13/14/16 (Wang et al., 2011), SexiOR13/16 (Liu C. et al., 2013), SinfOR21/29 (Zhang et al., 2014), AlepOR3/4/6 (Zhang et al., 2019), HarmORs/HassORs (Liu Y. et al., 2013; Liu et al., 2014; Jiang et al., 2014; Xu et al., 2015), AsegOR1/4/5/6/9 (Zhang and Lofstedt, 2013), SlituOR6/13/16 (Zhang et al., 2015), CpomOR2a/5 (Tian et al., 2020), AtraOR1/3 (Xu et al., 2012), and OlatOR1/OovaOR1/OscaOR1/3/4/5 (Miura et al., 2009, 2010). HEK293/sf9 cell calcium assay verified the sex pheromone component of ApolOR1, HassOR13, CoblOR7, and CherOR7 (Forstner et al., 2009; Jordan et al., 2009; Liu et al., 2014; Steinwender et al., 2015; Xu et al., 2015). In calcium imaging, calcium indicator Fluo-AM was used to detect the ion flow response induced by a specific ligand. In sf9 cells, endogenous Orco was provided to SPRs. Recent research adopted the Bioluminescence Resonance Energy Transfer (BRET)-based calcium sensor CalfluxVTN to detect ligand (Gu et al., 2009). *In vivo*, it means that the heterologous expression system is *Drosophila* antennae. In the characterization of *S. littoralis* SPRs, SlitOR6/SlitOR13 was expressed in a majority of *Drosophila* OSN in addition to endogenous receptors, and the responses to SPM stimuli were monitored by electroantennography, or *Drosophila* OR67d was replaced with SlitOR6 and the response was monitored by single sensillum recordings (Montagne et al., 2012; De Fouchier et al., 2015).

The interaction of SPR and specific SPM can be classified into receptor-ligand interaction in the perspective of biophysics, and the affinity between receptor and ligand is usually quantified by receptor-ligand complex dissociation constant Kd. According to **Figure 2**, most SPRs are tuned not only to the SPMs of their corresponding species but also to the SPMs of sibling species/analogs and antagonists, with different response amplitudes or different SPR-ligand affinities. In some researches, amino acid mutations of SPRs are responsible for the alteration of ligands. HarmOR14b and HassOR14b share 90% identities, and F232I + T355I are the key mutations that alter HassOR14b tuning to Z9-16:Ald to HarmOR14b tuning to Z9-14:Ald (Yang K. et al., 2017), which indicates that 232 and 355 are key residues in SPR and ligand docking. In *Ostrinia* species, OR3 amino acid mutation A148T in TM3 domain alters the pheromone recognition pattern by selectively reducing

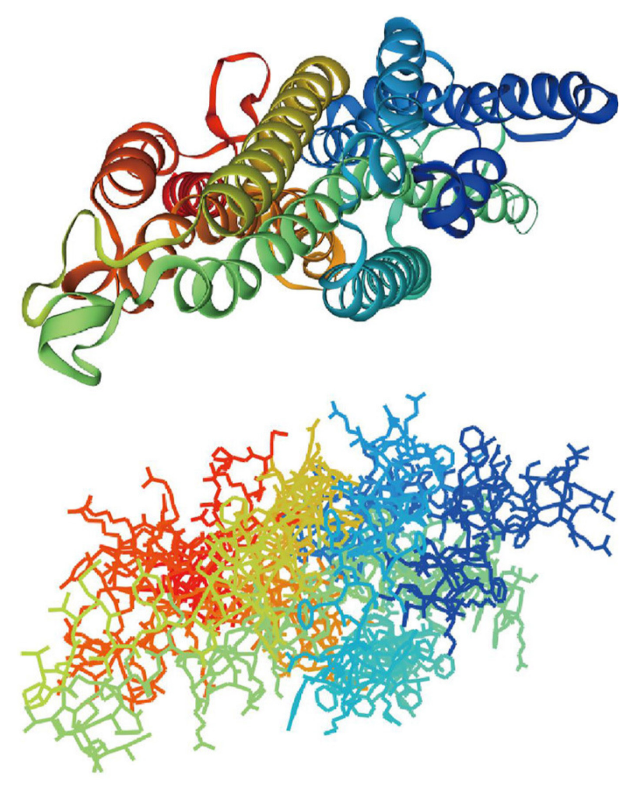

FIGURE 3 | Predicted structure of Lepidopteran SPRs BmOR3 and Orcos through SwissModel. In total, 7 transmembrane helixes were colored rainbow in the form of silk ribbon (Cartoon) and lines.

the E11-14:OAc response (EC50 of the dose-response curve) approximately 14-fold (Leary et al., 2012). Until present, no researches about SPR mutation and SPM recognition in a species have been reported. From the abovementioned studies, we can speculate that SPR and ligand docking pattern and the structure of SPR-ligand will be the future direction in SPR-ligand interaction studies.

Downstream Signaling Pathways of Sex Pheromone Receptors

Early research revealed the presence of G-protein, belonging to the Gαq family, in antennal preparations (especially the pheromone-sensitive sensilla trichodea) of *B.mori and Antheraea pernyi*, implied a participation of G-protein of the Gαq family in the signal transduction of OR cells in moths (Laue et al., 1997; Nakagawa et al., 2005). The bombykol stimulation of *Xenopus laevis* oocytes expressing BmOR-1 and BmGαq elicited robust dose-dependent inward Ca^{2+}-dependent Cl^- currents on two-electrode voltage-clamp recordings, demonstrating that the binding of bombykol to BmOR-1 leads to the activation of a BmGαq-mediated signaling cascade (Sakurai et al., 2004). MsexOR1 and MsexOrco coexpressed in HEK293 and CHO cells caused bombykal-dependent increases in the intracellular free Ca^{2+} concentration, and inhibitor evidence showed that phospholipase C (PLC) and protein kinase C (PKC) activities are involved in the bombykal-receptor-mediated Ca^{2+} signals of hawk moths. It could be hypothesized that MsexOrs couple to Gαq proteins, requiring the activation of PLC for pheromone transduction (Wicher et al., 2017). Immunocytochemistry research showed that anti-Gαq and anti-Gαs antisera stained the inner and outer dendritic segments of the putative OR neuron in male and female antennae, which suggested that each subunit mediates a subset of the odorant response (Miura et al., 2005). In addition, a computational model of the insect pheromone transduction cascade had been used to calculate the presence of the G-protein pathway in pheromone detection (Gu et al., 2009). Furthermore, recent research showed that in HEK293A cells expressing BmOR3 and human Gαi, the dose-dependent coupling of BmOR3 and Gαi on bombykal stimulation was detected through BRET (Lin et al., 2021). From the biophysical perspective, a conservation residue W103 in transmembrane 2 of BmOR3 is the key that determines receptor-Gi coupling (Lin et al., 2021). Pretreatment with specific Gi inhibitor PTX had no significant effects on bombykal-induced BmOR3-BmOrco complex formation or complex-regulated calcium influx, suggesting that Gi coupling and BmOrco coupling are the two independent processes in the case of BmOR3 (Lin et al., 2021).

The GPCRs usually direct the recruitment, activation, and scaffolding of the cytoplasmic signaling complexes *via* two multifunctional adaptor and transducer molecules, β-arrestins 1 and 2, and arrestins also function to activate signaling cascades independently of G-protein activation or mediate receptor desensitization (Lefkowitz and Shenoy, 2005; DeWire et al., 2007). Individual arrestins had been reported to function in both olfactory and visual pathways in Dipteran insects (Merrill et al., 2001) but not in Lepidopteran insects. Recent research reported that bombykal robustly stimulated the recruitment of human α-arrestin-1/2 and *B. mori* intrinsic arrestin to BmOR3 in HEK293A cells in a concentration-dependent manner, and the arrestin, in turn, regulated BmOR3 internalization (Lin et al., 2021). Bombykal also induced downstream kinase (i.e., ERK, SRC, AKT, and JNK) activation (phosphorylation) through arrestin (Lin et al., 2021). These results confirmed the arrestin-mediated signaling downstream of BmOR3. The knockdown of β-arrestins significantly reduced bombykal-induced calcium influx through BmOR3-BmOR2, which was accompanied by the collapse of the receptor complex, suggesting that the α-arrestins mediate Ca^{2+} response mainly by regulating the structural and functional integrity of the BmOR3-BmOR2 complex (Lin et al., 2021). The summarized researches show that insect pheromone receptors may both have G-protein and arrestin downstream pathways (**Figure 4**).

Metabotropic Ion Channel by the Coupling of Lepidopteran Sex Pheromone Receptors and Orcos

BmOR1 and BmOR3 of *B. mori* are mutually exclusively expressed in a pair of adjacent pheromone-sensitive neurons of male antennae, and both of which are coexpressed

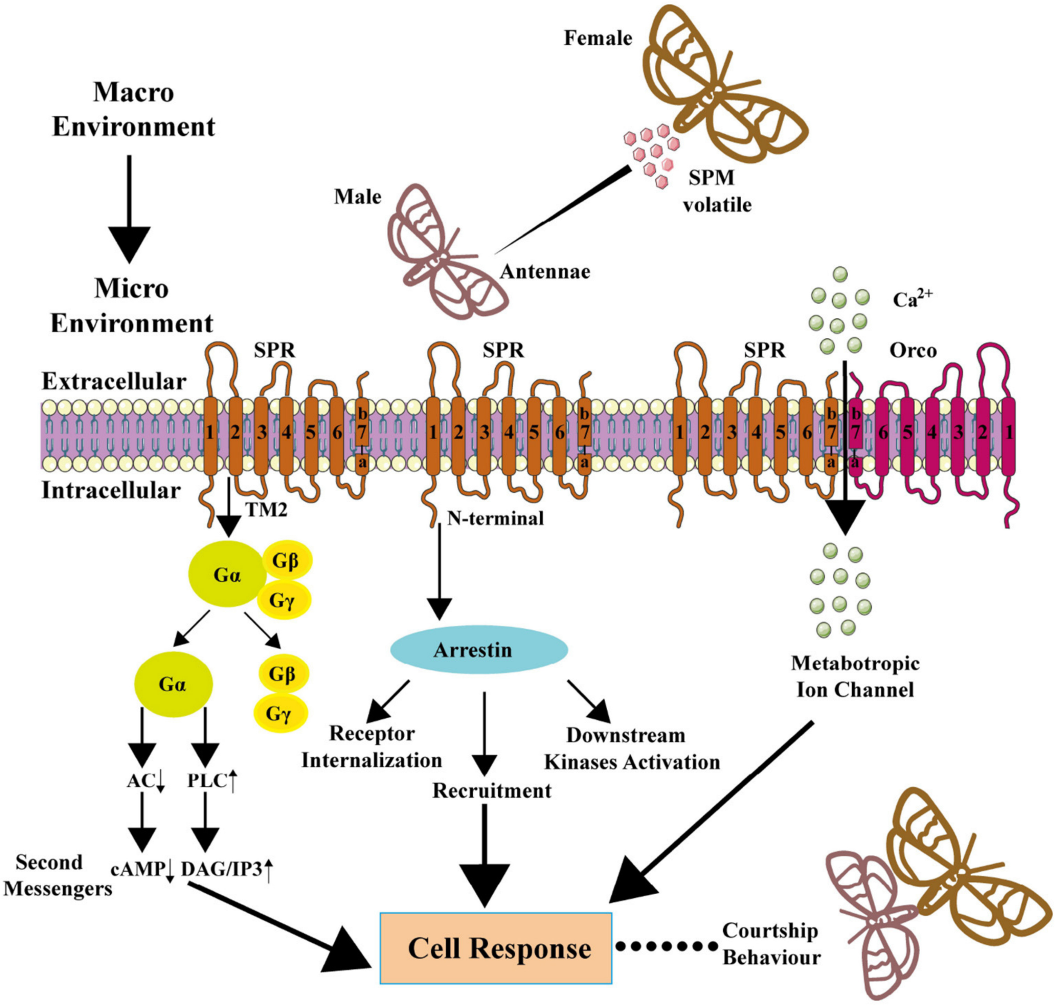

FIGURE 4 | Downstream signaling pathway of SPR and metabotropic channel of SPR-Orco. In macro environment, male insects sense the SPM released by female insects through an olfactory sensory neuron in antennae, and SPM works as an agonist to SPR, and signal transduction leads to ultimate mating behavior.

with the highly conserved insect Orco. Heterologous cells coexpressing BmOR2 can greatly enhance the sensitivity of BmOR1 to bombykol, and the current-voltage analysis showed that bombykol activated a non-selective cation channel in oocytes expressing BmOR1 and BmOR2, which is different from Ca^{2+}-activated Cl^{-} channel through BmGαq, and the non-selective cation channel activity in response to bombykol was also observed when BmOR1 was coexpressed with HvirOR2 or Or83b (Whelan and Goldman, 2001; Nakagawa et al., 2005; **Figure 4**). As reported in MsexOR1, the PLC/PKC activity is a prerequisite to bombykal-receptor-mediated Ca^{2+} signals in HEK293 and CHO cells, and it could be hypothesized that MsexOR1 and MsexOrco need to be phosphorylated before they can be gated by bombykal as an ionotropic odor receptor-ion channel complex (Wicher et al., 2017).

In a recent study, the BmOR3-BmOR2 combination elicited a response to bombykal and showed similar channel properties, and the coupling of BmOR3 and BmOR2 forms a cation channel with the detection of calcium influx (Lin et al., 2021). From the view of biophysics, there was also physical interaction between BmOR3 and its Orco BmOR2. On bombykal stimulation, the cytoplasmic parts intracellular loop 1 (ICL1), ICL2, and ICL3 moved away from the N-terminus, while the C-terminal helical kink moved close to the N-terminus of BmOR3. On the contrary, the lower part of loop7a-7b moved away from the N-terminus in BmOR2. ICL1, ICL2, and ICL3 also moved away from the N-terminus of BmOR2 (Lin et al., 2021). The replacement of transmembrane 7 in both receptors confirmed its indispensable role in BmOR3-BmOrco coupling for ionotropic functions (Lin et al., 2021). Several key motifs determine the BmOR3-BmOR2 coupling, the charged residue pair of BmOR3-E403 and BmOrco-K437 represents an important "ionic lock" in regard to mediating BmOR3-BmOrco coupling, and the hydrophobic patches F428/F433 of BmOR3 and zipper Y464/V467/L468/L471 of BmOrco are spatially close to each other, suggesting that they might form hydrophobic interactions (Lin et al., 2021). These reports suggest that the coupling of both SPR and Orco plays a vital role in sex pheromone signal detection and transduction.

There is some evidence that the SPR-Orco channel is metabotropic but not ionotropic. In *M. sexta*, the agonist induced SPR to activate the Gq-signaling pathway (Nakagawa et al., 2005; Nolte et al., 2013, 2016; Wicher et al., 2017). In *B. mori*, the SPM elicited G-protein and arrestin pathway, and arrestin knockdown had an effect on ion influx (Lin et al., 2021). Thus, we presume that the SPR-Orco coupling forms a metabotropic channel as before (Fleischer and Krieger, 2018). Both the metabotropic channel and the downstream signaling of SPR may be teamwork in sex pheromone signal transduction (**Figure 4**).

FUTURE DIRECTIONS

By using the mature technology in transcriptome sequencing and bioinformatic analyses, more sex pheromone components and SPRs of Lepidopteran insects are needed to be identified and characterized, which will help the development of sex lure technology and its usage in pest control.

Further researches are needed to work out the cryo-EM structure of SPR and the SPR-ligand docking pattern in a biophysical perspective, which will directly facilitate the understanding of sex pheromone signal transduction pathways and provide guidance in the sex lure technology in field pest control.

AUTHOR CONTRIBUTIONS

JS and XY proposed the title of the review and provided funding required. CY wrote the original draft, performed the data processing, and prepared figures. JC, JL, and YZ contributed to review editing. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.