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Description of Chemosensory Genes in Unexplored Tissues of the Moth *Spodoptera littoralis*

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Illumina-based transcriptome sequencing of chemosensory organs has become a standard in deciphering the molecular bases of chemical senses in insects, especially in non-model species. A plethora of antennal transcriptomes is now available in the literature, describing large sets of chemosensory receptors and binding proteins in a diversity of species. However, little is still known on other organs such as mouthparts, legs and ovipositors, which are also known to carry chemosensory sensilla. This is the case of the noctuid *Spodoptera littoralis*, which has been established as a model insect species in molecular chemical ecology thanks to the description of many—but not all—chemosensory genes. To fulfill this gap, we present here an unprecedented transcriptomic survey of chemosensory tissues in this species. RNAseq from male and female proboscis, labial palps, legs and female ovipositors allowed us to annotate 115 putative chemosensory gene transcripts, including 30 novel genes in this species. Especially, we doubled the number of candidate gustatory receptor transcripts described in this species. We also evidenced ectopic expression of many chemosensory genes. Remarkably, one third of the odorant receptors were found to be expressed in the proboscis. With a total of 196 non-overlapping chemosensory genes annotated, the *S. littoralis* repertoire is one of the most complete in Lepidoptera. We further evaluated the expression of transcripts between males and females, pinpointing sex-specific transcripts. We identified five female-specific transcripts, including one odorant receptor, one gustatory receptor, one ionotropic receptor and one odorant-binding protein, and one male-specific gustatory receptor. Such sex-biased expression suggests that these transcripts participate in sex-specific behaviors, such as host choice for oviposition in females and/or mating partner recognition in both sexes.

Keywords: RNAseq, chemosensory transcriptome, *Spodoptera littoralis*, proboscis, palps, legs, ovipositor

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INTRODUCTION

Chemosensation—olfaction and taste—are at the basis of insects' environmental perception, triggering vital behaviors such as feeding, mating, ovipositing and avoiding dangers and enemies (Stocker, 1994; Chapman, 2003; Dahanukar et al., 2005; Nishino et al., 2005). Several organs for chemical reception are found on the insect body. In moths, the head bears the antennae, the proboscis and the labial palps. The thorax and the abdomen also harbor sensory organs,

such as the tarsi and the female ovipositor. Antennae have been the most studied because of their important role in olfaction, but they are also involved in other senses such as taste and mechano/hydroreception (Altner et al., 1977; Keil, 1999). Mouth parts are known for their role in taste but labial palps are also involved in sensing carbon dioxide. The tarsi and the abdomen extremities are also crucial for partner, food and oviposition site recognition and approval by insects (Fan et al., 1998; Chapman, 2003; Calas et al., 2007; Hansson and Stensmyr, 2011; Seada, 2015; Seada et al., 2018). These chemosensory organs contain plethora of different types of innervated hair structures, the sensilla, adapted to detect a wide range of molecules. Electrophysiological observations in combination with immunostaining and behavioral studies showed functional differences of the different types of chemosensory sensilla (Blaney and Simmonds, 1988, 1990; Marion-Poll et al., 1992; Marion-Poll and Van Der Pers, 1996; Laue and Steinbrecht, 1997; Calas et al., 2009; De Brito Sanchez et al., 2014; Seada, 2015; Seada et al., 2016, 2018). For instance, sensilla chaetica are devoted to the detection of soluble molecules, trichoid/basiconic sensilla to volatiles, coeloconic sensilla to acids and amines (Yao et al., 2005; Vosshall and Stocker, 2007; Hill et al., 2010).

Olfactory and gustatory cues are detected with these sensilla by chemoreceptor proteins embedded in the membrane of the neurons. After interaction with chemoreceptors, the chemical signals are transduced into electrical signals that are transmitted to the brain through the primary neuronal axons, where they are integrated, eventually resulting in a behavioral response. In insects, there are three main families of chemoreceptors: the odorant receptors (ORs), the gustatory receptors (GRs)k, and the ionotropic receptors (IRs), the latter being likely involved in both olfaction and taste (Benton et al., 2009; Fleischer et al., 2018). ORs and IRs function as complexes with co-receptors, such as Orco for ORs and IR8a/IR25a for IRs (Fleischer et al., 2018). Whether GRs also function with co-receptor(s) is not clearly established, but it is known that several GR genes are co-expressed within the same neuron, suggesting they form multimeric complexes as well (Freeman and Dahanukar, 2015; Xu and Anderson, 2015; Ning et al., 2016; Dweck and Carlson, 2020; Xu et al., 2020). Sensory neuron membrane proteins (SNMPs) are transmembrane proteins, some of which found expressed in olfactory neurons and possibly involved in pheromone detection (Benton et al., 2007; Leal, 2013; Suh et al., 2014; Lemke et al., 2019). Families of small soluble proteins are also found within chemosensory sensilla: the odorant-binding proteins (OBPs) and the chemosensory proteins (CSPs). They have been proposed to facilitate the diffusion of hydrophobic stimulants within the sensillum lymph up to the neuronal membrane (Pelosi et al., 2018).

Sequences of ORs, IRs, GRs, SNMPs, OBPs, and CSPs have been accumulated in the recent years for various insect species thanks to the progress in sequencing technologies. Most of the transcriptomic studies focused on antennae, describing a plethora of OBPs and CSPs thanks to their high expression facilitating their detection, and of ORs because of their role in long distance chemical communication. Larger repertoires of chemosensory genes have come from genome analyses, including vast arrays of GRs and IRs. For instance, recent genomic data from Lepidoptera revealed that Noctuidae genomes contain an incomparable high number of GR genes compared to ORs, with up to 250 GRs (and only 70–80 ORs) in polyphagous species such as *Helicoverpa armigera*, *Spodoptera frugiperda,* and *S. litura* (Cheng et al., 2017; Gouin et al., 2017; Pearce et al., 2017). However, little is known on their expression pattern, because transcriptomic data from taste tissues is scarce (Xu et al., 2016; Cheng et al., 2017; Guo et al., 2018).

Among Noctuidae, the cotton leafworm *S. littoralis* has been established for several years as a model in molecular chemical ecology. The sex pheromone and many plant volatiles detected by the antennae and triggering specific behaviors have been identified. Transcriptomic data have been accumulated on chemosensory organs such as male and female antennae (Legeai et al., 2011; Jacquin-Joly et al., 2012; Poivet et al., 2013), larval antennae and palps (Poivet et al., 2013), and adult non-chemosensory tissues such as brain, body and proboscis (Walker et al., 2019), leading to the description of a substantial number of expressed chemosensory genes in a Lepidoptera. A total of 60 OR transcripts have been described, and the recent functional characterization of one third of them has been a landmark in Lepidoptera olfaction (de Fouchier et al., 2017). However, only 17 GR transcripts could be reconstituted in this species (Poivet et al., 2013; Walker et al., 2019), probably due to the lack of taste organs in the tissues investigated.

The aim of the present study was to fulfill this gap, by performing in-depth RNA sequencing of different tissues known to carry taste sensilla but never investigated before. We generated new RNAseq data from male and female adult labial palps and forelegs as well as female ovipositors, and completed previous data on proboscis. We identified new candidate chemosensory genes in *S. littoralis*, and expanded coding sequences of previously described transcripts. Altogether, these transcriptomic data offer incomparable resources to explore the molecular mechanisms of olfaction and taste in a single model and revealed ectopic expression of most chemosensory gene families.

MATERIALS AND METHODS

Insect Rearing

All *S. littoralis* individuals were from an inbred colony reared in the laboratory at 24°C, 70% relative humidity and under a 16 h:8 h light:dark photoperiod. Larvae were fed on a semi-artificial diet (Poitout and Bues, 1974) and adults were provided with sugar water. Males and females were sexed as pupae and kept in separate rooms, since it has been shown that smelling the sex pheromone can impact chemosensory gene expression in this species (Guerrieri et al., 2012).

Tissue Dissections, RNA Extractions, and Sequencing

Thirty proboscis and 15 pairs of labial palps and forelegs were dissected from both males and females. For females, 30 ovipositors were also dissected. Adults were 2–3 days

old upon dissection and all dissections were conducted between the second and third hours of the scotophase. Proboscis RNAs (one sample from males, one sample from females) were extracted separately from the rest of the tissues, which were pooled together, and formed two samples (one for males and one for females). These tissue mix samples (labial palps, forelegs plus ovipositors for the females) are further designed as "palp/leg" tissue mix to facilitate description. RNAs were extracted using TRIzol™ Reagent (Thermo Fisher Scientific, Waltham, MA, United States) and quality was checked using a NanoDrop™ ND-2000 spectrophotometer (Thermo Fisher Scientific). About 5 μg of total RNA per sample were used for cDNA paired-end library construction using the Truseq RNA Stranded Sample Preparation Illumina Kit at the Institut de Génomique Fonctionnelle, Plateforme Montpellier GenomiX IBiSA (MGX). Illumina HiSeq2000 sequencing (2 × 100 bp) was performed at MGX.

Transcriptome Assembly

MGX provided raw reads cleaned by removing 3′ adaptors and poly-A/T tails. Data were further processed using Galaxy Version 2.8.3. FastQC was run on each sample and low-quality reads (<30) and sequences shorter than 20 bp were trimmed using Trimmomatic (Bolger et al., 2014). Ribosomal RNAs were removed using Ribopicker (Schmieder et al., 2011). A reference transcriptome was assembled with all the data generated in this study using Trinity (Grabherr et al., 2011) with default parameters. After reconstruction, the transcriptome was filtered using two criteria: (1) transcripts with no read support (expected count < 1) were identified using Bowtie (v1.0.0) (Langmead et al., 2009) and discarded, (2) CD-HIT-EST was used to remove transcript redundancy with a similarity threshold of 0.90 and a word size of 8. Open reading frames (ORFs) encoding proteins of more than 50 amino acids were predicted by TransDecoder (Haas et al., 2013). We used the Benchmarking Universal Single-Copy Orthologs tool (BUSCO v3.0.2) (Simão et al., 2015) against the Insecta odb10 dataset (1,367 reference genes) to assess the completeness of the assembled transcriptome.

Identification of Chemosensory Genes

To identify putative chemosensory genes (ORs, GRs, IRs, OBPs, CSPs, and SNMPs), we used amino acid sequences of proteins previously annotated in *S. littoralis* and the related species *S. frugiperda* and *S. litura* (Legeai et al., 2011; Jacquin-Joly et al., 2012; Poivet et al., 2013; Cheng et al., 2017; Gouin et al., 2017; Walker et al., 2019) as queries in a blastp search on the translated ORFs predicted from the reference transcriptome. Annotations of all retrieved sequences were confirmed by blastp on the NCBI non-redundant database (NR). Sequences described for the first time in *S. littoralis* were named according to their ortholog in *S. frugiperda* (Gouin et al., 2017), except IRs that were named following orthology with *S. litura* (Zhu et al., 2018). For sequences previously described we followed the nomenclature of Walker et al. (2019), except for *SlitGR70* that was renamed *SlitGR272*.

Phylogeny Construction

Phylogenetic analyses were performed using the newly identified protein sequences (ORs, GRs, IRs and OBPs) together with previously annotated amino acid sequences in *Spodoptera* spp. (*S. littoralis, S. frugiperda, S. litura*), sequences from other Lepidoptera (*Bombyx mori, Helicoverpa armigera, Heliconius melpomene*) and from species from other insect orders when relevant (*Drosophila melanogaster, Tribolium castaneum,* and *Apis melifera*). MAFFT v7 (Katoh and Standley, 2013) was employed for amino acid sequence alignment and maximum-likelihood phylogenies were constructed using PhyML 3.0 (Guindon et al., 2010) with default parameters. The best-fit model of amino acid substitution was determined by SMS (Lefort et al., 2017). Node support was estimated via SH-like aLRT (Anisimova and Gascuel, 2006). Trees were visualized with FigTree v1.4.3 and images were edited with Adobe Illustrator software.

RESULTS AND DISCUSSION

Transcriptome Description

The *de novo* transcriptome presented in this paper was generated from four libraries: proboscis from each sex, labial palps and forelegs from males, and labial palps, forelegs and ovipositors from females. A total of 418 million raw reads were obtained and deposited at the NCBI Sequence Read Archive (BioProject ID PRJNA693435, Biosamples SAMN17386877, SAMN17386878, SAMN17386879, SAMN17386880). After cleaning, a total of 325 million reads were pooled and assembled to generate a *de novo* transcriptome of 234,643 transcripts. Removing transcripts with no read support led to an assembly of 203,642 transcripts. Last, CD-HIT-EST was applied to remove transcript redundancy, leading to a final assembly of 154,302 transcripts. Out of the transcripts, 47,853 ORFs could be identified. All metrics are provided in **Supplementary Material 1**. BUSCO analysis revealed that the *de novo* transcriptome contained 89.9% of complete sequences, with 78.6% as single copy and 11.3% as duplicated, thus reflecting a good representative transcriptome (**Supplementary Material 1**).

Identification of Candidate Chemosensory Genes and Tissue Distribution

We sequenced previously unexplored tissues (adult labial palps, legs and ovipositors) from *S. littoralis*. These tissues have been selected as surface contact tissues and candidate tissues for close range chemoreception (taste), without neglecting their potential long range (olfaction) chemosensory capacities. These tissues all carry chemosensory sensilla and are expected to play a role in assessing the quality and toxicity of food and oviposition substrates, as well as in close range mate detection. We thus expected to extend our current knowledge on *S. littoralis* chemosensory transcripts. This proved to be rewarding since we identified a total of 115 candidate transcripts from the three chemosensory receptor families (GRs,

TABLE 1 | Summary of *S. littoralis* chemosensory genes identified in this study.

	ORs	GRs	IRs	CSPs	OBPs	SNMPs	Total
Total (this study)	26	34	13	10	31	1	115
New	4	21	5	0	0	0	30
Expressed in proboscis only	3	11	1	0	0	0	15
Expressed in palp/leg only	5	1	2	0	1	0	9
Expressed in female only	1‡	2‡	1††	0	1††	0	5
Expressed in male only	0	1†	0	0	0	0	1
Total (Walker et al., 2019, this study)	64	38	22	21	49	2	196

‡*Proboscis;* †*palps/legs;* ††*palps/legs/ovipositor.*

ORs and IRs), the pheromone detection associated SNMPs and the two lipid transporter families (OBPs and CSPs), including 30 new chemosensory transcripts compared to the latest published repertoire (Walker et al., 2019; **Table 1**). We also identified full-length sequences for 13 previously published sequences (**Supplementary Material 2**). In addition, we got insight into the presence of *S. littoralis* chemosensory transcripts according to tissue and sex, as summarized in **Figure 1**.

Details per family are described below.

Gustatory Receptors

Great Extension of the SlitGR Repertoire

We identified 34 candidate GRs in our *S. littoralis* gustatory transcriptome and we obtained complete ORFs for 14 of them (**Supplementary Material 2**). We could retrieve 13 out of the 17 GRs already known from previous work on this species (Jacquin-Joly et al., 2012; Poivet et al., 2013; Walker et al., 2019) and identified 21 new GRs (**Table 1** and **Supplementary Material 2**). A total of 38 GRs are now described for this species (*SlitGR158* and *SlitGR206*, previously described in Walker et al. (2019), as expressed in the proboscis, were not found here).

The number of 38 GR genes found expressed in the different *S. littoralis* transcriptomes is far behind the numbers of GR genes found in the genomes of Noctuidae (Cheng et al., 2017; Gouin et al., 2017; Pearce et al., 2017). For instance, 231 GRs have been annotated in the genome of *S. frugiperda* and it has been hypothesized that this extended number of GRs could be linked to the acquisition of polyphagy. With only 38 GR transcripts described by now in *S. littoralis*, we can speculate that the vast majority of GRs have probably not been identified yet in this species. GRs are known to be expressed at very low levels and their identification may need very deep sequencing. Alternatively, some may be expressed in tissues not investigated here, for example the gut, or at different periods of the life cycle, for example in larvae as already demonstrated for *S. litura* (Cheng et al., 2017). Further genomic data will help to establish a complete set of GRs in *S. littoralis*. Anyhow, SlitGRs were found in each of the major clades in the GR phylogeny (**Figure 2**). We extended the candidate sugar receptor subfamily to six GRs (one more than previous studies), all having orthologs in *S. frugiperda*, and we identified one of the two candidate fructose receptor orthologs found in the genome of *S. frugiperda* (Gouin et al., 2017). We found the three candidate CO_2 receptors *SlitGR1, 2* and *3* (Erdelyan et al., 2012). All other candidate *SlitGRs* clustered in the so-called "bitter" GR subfamily.

Tissue Distribution of Sugar and CO_2 Receptors

SlitGR6, *12*, *13,* and *14* (candidate sugar GRs) were expressed both in the proboscis and the palps/legs. This suggests their importance for sugar detection during food intake but also palps/legs implication in sugar detection in addition to proboscis and antennae (Walker et al., 2019).

Three candidate CO_2 receptors *SlitGR1*, *2* and *3* were expressed both in the proboscis and in the palp/leg samples (**Supplementary Material 2**). This is in accordance with the observation that the labial palps—and more specifically the labial pit organ—are involved in CO_2 detection in Lepidoptera (Bogner et al., 1986; Kent et al., 1986). Previous studies have revealed that *SlitGR2* and *3* are also highly expressed in antennae (Jacquin-Joly et al., 2012; Walker et al., 2019) and that *SlitGR1* is enriched in female ovipositor (Legeai et al., 2011). This may suggest that most chemosensory organs participate in CO_2 sensing in *S. littoralis*, although functional studies on orthologous CO_2-sensing GRs in *H. armigera* have shown that co-expression of GR1 and 3 is necessary and sufficient for CO_2 detection (Xu and Anderson, 2015; Ning et al., 2016).

Tissue Distribution of the So-Called "Bitter" Receptors

More than half (57%) of the *S. littoralis* candidate bitter GRs were specifically expressed in the proboscis (**Supplementary Material 2**) and have not been described in previous studies (Jacquin-Joly et al., 2012; Poivet et al., 2013; Walker et al., 2019). The remaining candidate bitter GRs were expressed in both proboscis and palps/legs at the exception of *SlitGR16*, only found in male palps/legs. Two other candidate bitter GRs were sex-biased: *SlitGR79* and *149* were exclusively found in the female proboscis, which may be related with the need for females to eat for their sexual maturation or to find adequate surface for oviposition and progeny nutrition. Indeed, it is known that *S. littoralis* premating behavior consists in female foraging for flower nectar while postmating females are more attracted to larval host odors (Ahmed et al., 2012). Almost no functional data are available for moth candidate bitter GRs, but impressive expansions of these GRs have been described in Noctuidae (Cheng et al., 2017; Gouin et al., 2017; Pearce et al., 2017).

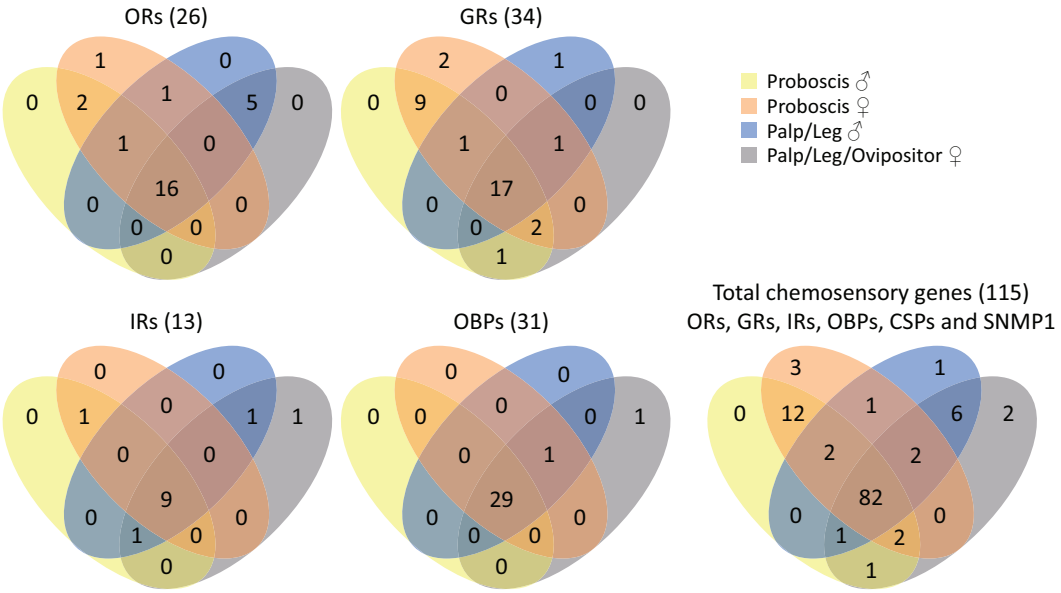

FIGURE 1 | Venn diagrams showing the expression of chemosensory transcripts in the different tissues and sexes.

Low Expression of GRs in the Palp/Leg Tissue Mix

Although legs are known to harbor taste sensilla and play an important role in gustation, *SlitGRs* were surprisingly poorly represented in the palp/leg tissue mix (**Supplementary Material 2**). We did not evidence any female-specific GRs in this tissue mix, although the female palp/leg mix also included ovipositors. This is intriguing since female moths are known to use their tarsi and ovipositor to taste oviposition sites before egg laying. Sensilla chaetica have been found at the external distal border of the ovipositor papillae in *S. littoralis* (Seada et al., 2016). They are uniporous (a hallmark of taste sensilla) and contain four gustatory sensory neurons responding mainly to salt, sugars, caffeine and water (Seada et al., 2016). Although several molecules have been shown to promote or deter oviposition in moths and several chemosensory genes seem to be specifically expressed in the ovipositor, no chemoreceptor/molecule association has been established (Qiu et al., 1998; van der Goes van Naters and Carlson, 2006; Xu et al., 2017; Chen et al., 2019).

Odorant Receptors

An Extended Repertoire of ORs in *S. littoralis*

Sixty *S. littoralis* ORs (SlitORs) have been previously described in the literature (Legeai et al., 2011; Jacquin-Joly et al., 2012; Poivet et al., 2013; Walker et al., 2019). We identified 22 of these in our transcriptome, including the co-receptor *SlitOrco*. In addition, we annotated four ORs that have never been described in *S. littoralis* before (**Table 1**). We named these new ORs as *SlitOR61, SlitOR65, SlitOR68*—according to their orthologs in the *S. frugiperda* genome (Gouin et al., 2017)—and *SlitOR70* (no identified ortholog in *S. frugiperda*). We obtained complete ORFs for 7 SlitORs (**Supplementary Material 2**). As a result, the *S. littoralis* OR family reached a total of 64 expressed ORs in this species. This number is close to the number of OR genes annotated in the genomes of other noctuid species (69 in *S. frugiperda*, 73 in *S. litura*, 84 in *H. armigera* and 82 in *H. zea*) (Cheng et al., 2017; Gouin et al., 2017; Pearce et al., 2017), suggesting we have identified most—if not all—of the ORs expressed in *S. littoralis*. The phylogenetic analysis (**Figure 3**) showed that ORs expressed in the mouth parts, legs and ovipositor are found in the vast majority of lepidopteran OR clades, thus mirroring the wide diversity of ORs expressed in taste tissues. Interestingly, there was no representative in the classical pheromone receptor clade (**Figure 3**).

An Unexpected Distribution of SlitORs in Non-antennal Tissues

Out of the 64 SlitORs, we found that one third (21 ORs) were expressed in the proboscis (**Table 1** and **Figure 1**). We found a set of 22 ORs expressed in the palp/leg samples of both males and females (**Table 1** and **Figure 1**), some of which being also expressed in the proboscis. Seven ORs described as antennal specific in Walker et al. (2019) and 19 others described as enriched in the antennae (with only one or two reads in the brain and/or body) were not retrieved in our transcriptome, supporting their antennal specific expression or enrichment.

This tissue distribution of ORs in a moth is surprising (half of the SlitORs expressed in the proboscis, one third in the palps/legs) and suggests that these organs participate in odorant sensing. Out of the 83 known ORs in adult *H. armigera* (Pearce et al., 2017), only 4 and 3 ORs, including Orco, have been found expressed in proboscis and palps, respectively (Guo et al., 2018). One type of multiporous sensilla (sensilla styloconica) was found in the proboscis of *H. armigera*, suggesting a role in olfaction (Guo et al., 2018). Few studies on *S. littoralis* have investigated the sensilla on mouthparts, tarsi and ovipositor (Chadha and Roome, 1980;

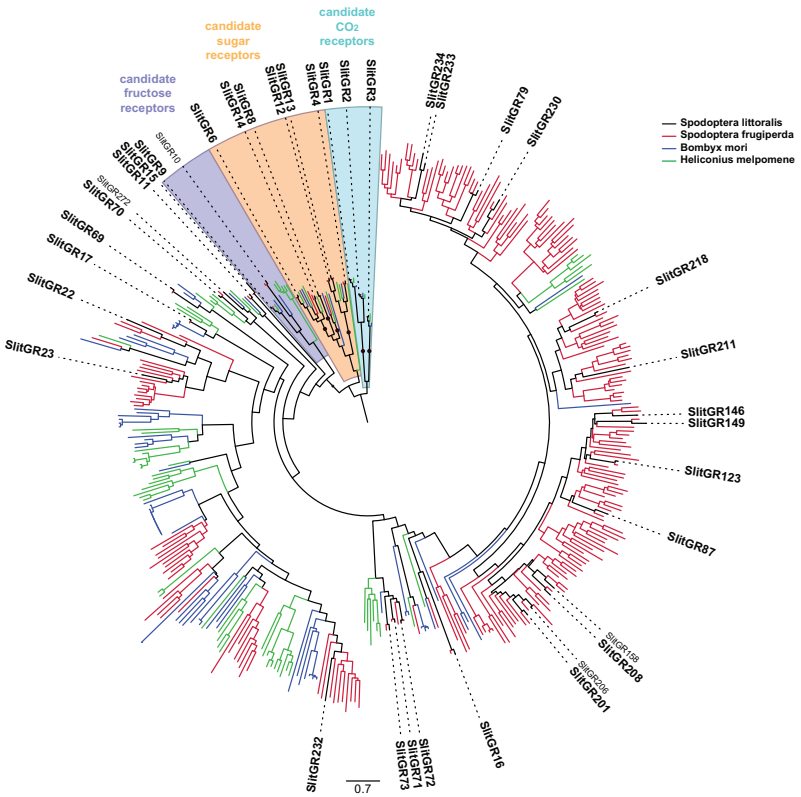

FIGURE 2 | Phylogenetic position of *Spodoptera littoralis* gustatory receptors (SlitGRX, black). The phylogeny was built using GR repertoires of the moths *S. frugiperda* (red) and *Bombyx mori* (blue), and the butterfly *Heliconius melpomene* (green). SlitGRs found in the present transcriptome are written in bold. Candidate CO_2, sugar and fructose receptor clades are pictured with color shades. The CO_2 receptor clade was used as an outgroup. Clades supported by an aLRT value over 0.9 are indicated by a black dot.

El-Degwi and Gabarty, 2015; Seada et al., 2018, 2016) but none has described their numbers or their morphology in a resolution that would permit to distinguish uniporous from multiporous sensilla. Overall, the number of ORs described here likely exceeds the number of olfactory sensilla carried by these organs. This suggests that they might be transcribed but that not all of them are functional in these tissues.

Comparison Between Tissues and Sexes

SlitOrco was found expressed in the proboscis and in the palp/leg tissue mix of both sexes. This was expected in view of its role as a co-receptor for ORs and it predicts that at least some of the ORs found in these gustatory tissues should form functional olfactory ligand gated ion channels.

SlitOR45, 61, and *68* were found to be expressed in the proboscis samples but not in the palps/legs (**Supplementary Material 2**). On the contrary, *SlitOR20, 28, 58, 60,* and *70* were found exclusively expressed in the palp/leg tissue mix.

We found one OR, *SlitOR37*, expressed in the female proboscis but not in male ones, with no expression in palp/leg mixes (**Supplementary Material 2**). This transcript was not previously found expressed in the proboscis (Walker et al., 2019) but was found in both male and female antennae with a female bias. Interestingly, *SlitOR37* clustered in the phylogeny with *BmorOR19* and *MsexOR5*, which were found to be female-specific (Anderson et al., 2009; Grosse-Wilde et al., 2010; Koenig et al., 2015). *BmorOR19* has been functionally characterized as responding to linalool (Anderson et al., 2009), an oviposition cue for many female moths (Rostelien et al., 2005) and a male pheromone component in some Noctuidae, e.g., *Mamestra brassicae* (Heath et al., 1992). The *SlitOR37* ortholog in *Helicoverpa assulta, HassOR62*, also exhibited the same female-biased expression pattern (Zhang et al., 2015). Although *SlitOR37* has not been functionally characterized yet in *S. littoralis*, its expression pattern suggests it may play a role in the detection of suitable oviposition sites or in male mate recognition. *SlitOR38* was clearly male-biased in palps/legs, as it is in antennae (Walker et al., 2019), suggesting that this receptor presents a general male-enriched expression. Additionally, *SlitOR38* belongs to a recently described candidate pheromone receptor clade (Bastin-Héline et al., 2019), which highlights an eventual role in female-pheromone recognition.

A Sex Pheromone Receptor Is Expressed in All Tissues Examined

According to the OR phylogeny, none of the SlitORs retrieved from our transcriptome belonged to the classical pheromone receptor group (**Figure 3**). However, we detected

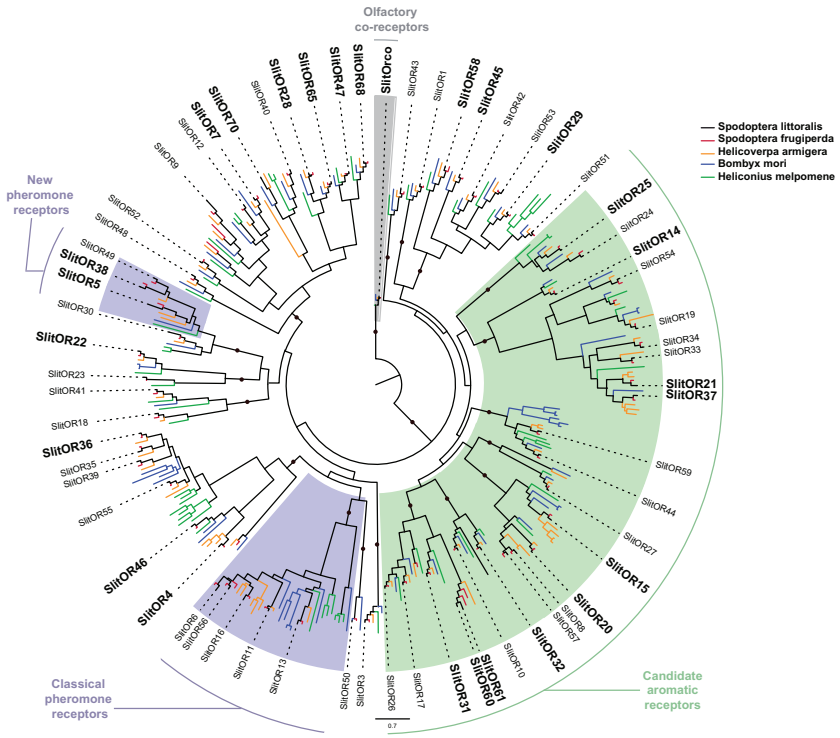

FIGURE 3 | Phylogenetic position of *S. littoralis* odorant receptors (SlitORX, black). The phylogeny was built using OR repertoires of the moths *S. frugiperda* (red), *H. armigera* (yellow) and *B. mori* (blue), and the butterfly *H. melpomene* (green). SlitGRs found in the present transcriptome are written in bold. Clades of candidate receptors for pheromones and aromatics are shown with purple and green color shades, respectively. The odorant receptor coreceptor (Orco) clade is shaded in gray and was used as an outgroup. Clades supported by an aLRT value over 0.9 are indicated by a black dot.

SlitOR5 expressed in all tissues examined from both sexes (**Supplementary Material 2**). This receptor does not belong to the classical pheromone receptor clade but has been recently described as highly expressed in male antennae (Walker et al., 2019) and functionally characterized as an unconventional pheromone receptor tuned to the major component of the *S. littoralis* female sex pheromone (Bastin-Héline et al., 2019). *SlitOR5* expression in antennal and non-antennal tissue suggests that other tissues than the antennae may be involved in sex pheromone detection. However, its low expression levels in the non-antennal tissues calls for further investigation of its precise expression pattern before any assumptions and interpretations could be given on its function in these tissues. Interestingly, we found *SlitSNMP1*, encoding a membrane protein probably involved in pheromone delivery to pheromone receptors (Vogt et al., 2009), in the present transcriptome (**Table 1** and **Supplementary Material 2**). Detailed observations of *S. littoralis* premating behavior are also needed to investigate if mouthpart and/or leg contacts occur during courtship and if courtship and mating are disturbed when these appendices are blocked. Alternatively, sex pheromone cues may be used in non-sexual behaviors. For instance, females may detect these cues via mouthparts, legs or ovipositor in order to regulate their pheromone production (as proposed in the moth *Heliothis virescens*, Widmayer et al., 2009) or to detect traces of sex pheromone from other females in oviposition sites to avoid over-oviposition on the same plant and high competition between larvae for the same food resources.

Ionotropic Receptors

New Candidate Taste IRs in *S. littoralis*

Thirteen IR transcripts were identified in our transcriptome (**Figure 1** and **Table 1**)and complete ORFs were obtained for five of them (**Supplementary Material 2**). Among these 13 IRs, we found the three candidate IR coreceptors (*SlitIR8a*, *25a,* and *76b*), 4 members of the so-called antennal IR clades (*SlitIR41a*, *64a*, *75d,* and *75p.2*) supposedly involved in olfaction, and 6 divergent IRs (*SlitIR7d.1*, *7d.4*, *60a*, *85a*, *100b,* and *100i*) more likely to be involved in taste (**Figure 4**). Five of these divergent IRs are described for the first time as they were not found in previous *S. littoralis* transcriptomes. The total number of IRs found expressed in *S. littoralis* is now 22. This is approximately half of the number of IR genes described in the genomes of *S. frugiperda* (43 IRs, Gouin et al., 2017) and *H. armigera* (39 IRs, Liu et al., 2018). Out of these 22 SlitIRs, 17 have been found to be expressed in the antennae (Walker et al., 2019), 14 in the proboscis (Walker et al., 2019 and our study) and 12 were described, for the first time in this paper, in the palps/legs of *S. littoralis*. Of the 5 new *S. littoralis* divergent IRs discovered here (thus not previously found expressed in antennae), 4 were

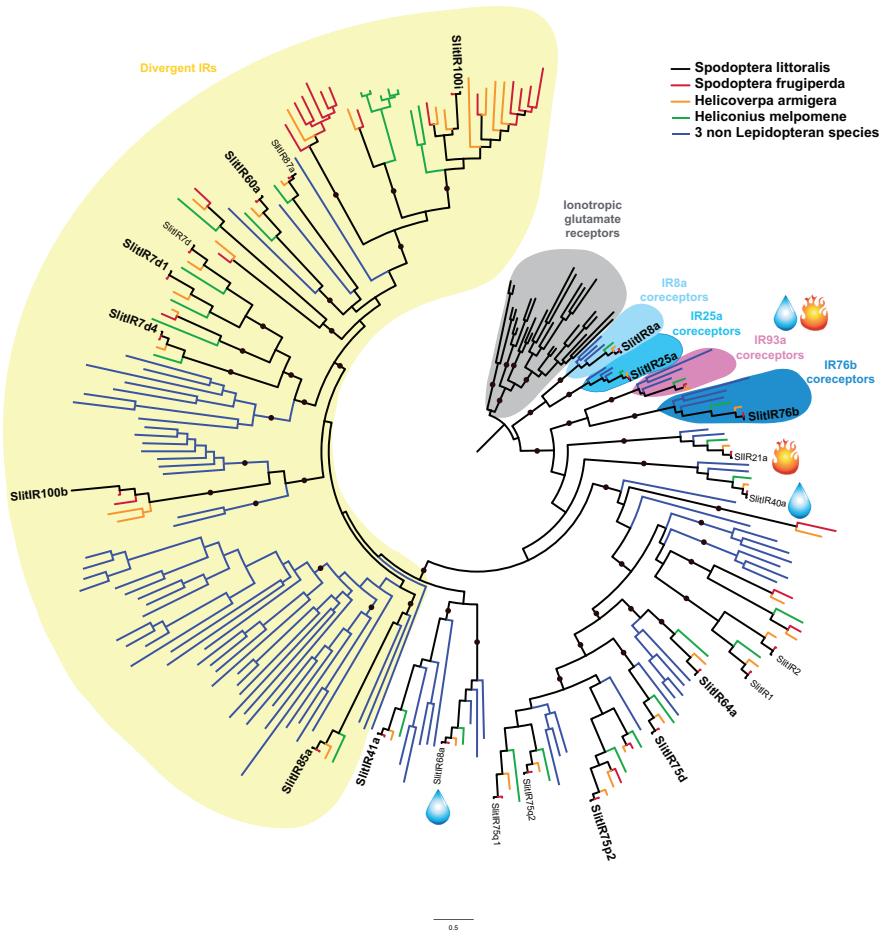

FIGURE 4 | Phylogenetic position of *S. littoralis* ionotropic receptors (SlitIRX, black). The phylogeny was built using IR repertoires of the moths *S. frugiperda* (red), *H. armigera* (yellow) and the butterfly *H. melpomene* (green), and three non-Lepidoptera species (blue): *Drosophila melanogaster*, *Apis melifera,* and *Tribolium castaneum*. SlitIRs found in the present transcriptome are written in bold. Three coreceptor clades are pictured with blue color shades and the coreceptor IR93a for hygro- and thermosensation is pink shaded. The ionotropic glutamate receptor clade is shaded in gray and was used as an outgroup. Candidate ionotropic receptors for hygrosensation and thermosensation according to their homology with *Drosophila* IRs are designated with the accolade of a self-explaining pictogram. Clades supported by an aLRT value over 0.9 are indicated by a black dot.

expressed in both the proboscis and the palp/leg tissue mix and one (*SlitIR7d.4*) was exclusively expressed in the proboscis of both males and females. This expression pattern, together with their phylogenetic position within divergent IRs, makes these SlitIRs good candidate taste receptors.

Expression of Antennal IRs Outside Antennae

As already observed in *H. armigera* (Liu et al., 2018), we showed that expression of the so-called antennal IRs was not restricted to antennae (**Supplementary Material 2**), as four antennal IRs were expressed either in the proboscis and the palp/leg tissue mix (*SlitIR41a*, *64a*, *75d*) or specifically in the female palp/leg/ovipositor tissue mix (*SlitIR75p.2*). As this latter IR was not expressed in the male palp/leg mix, one could hypothesize that it is expressed in the ovipositor, playing a potential role in host quality evaluation for oviposition. Deeper studies of its expression pattern would be needed to precisely identify in which tissues of the palp/leg/ovipositor mix this IR is expressed.

Looking at the three candidate IR coreceptor transcripts, *SlitIR25a* appeared expressed in the proboscis and in the palp/leg tissue mix from both sexes, *SlitIR76b* was also expressed in all tissues examined here, and *SlitIR8a* was found only in the palp/leg tissue mix (**Supplementary Material 2**). All have been previously found highly expressed in the antennae (Olivier et al., 2011; Walker et al., 2019). The expression pattern of these coreceptors is in line with previous observation in insects. They are usually described as highly expressed in all insect taxa in which they have been described. Most are expressed in more than one tissue and the different expression patterns between different taxa suggests that insect sensing is differently distributed between the chemosensory tissues among taxa (Leal et al., 2013; Sparks et al., 2014; van Schooten et al., 2016; Walker et al., 2019). Following knowledge on *Drosophila* IR coreceptors, our results are not surprising since DmelIR8a has been associated to olfactory neurons in the antennae and palps and DmelIR76b has been associated to gustatory neurons which are more prominent

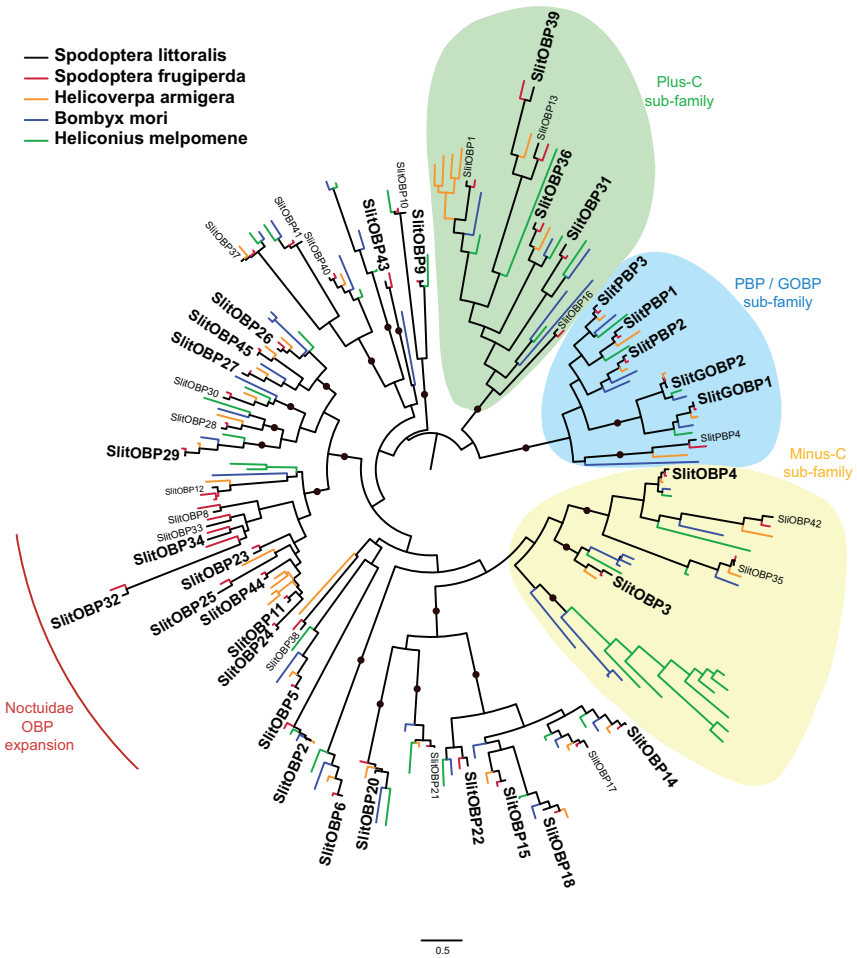

FIGURE 5 | Phylogenetic position of *S. littoralis* odorant-binding proteins (SlitOBPX, black). The midpoint rooted phylogeny was built using OBP repertoires of the moths *S. frugiperda* (red), *H. armigera* (yellow) and *B. mori* (blue), and the butterfly *H. melpomene* (green). SlitOBPs found in the present transcriptome are written in bold. The General OBP (GOBP) and Pheromone-Binding Protein (PBP) clade is designated with a light blue color shade. Plus and minus-C clades are highlighted with green and yellow shades, respectively. The noctuidae-specific OBP expansion is indicated by a red line. Clades supported by an aLRT value over 0.9 are indicated by a black dot.

in the *Drosophila* antennae, labellum and legs (Benton et al., 2009; Croset et al., 2010; Abuin et al., 2011; Zhang et al., 2013; Koh et al., 2014; Hussain et al., 2016; Rimal and Lee, 2018). IR25a is known to be a more generally and highly expressed coreceptor, which could be considered as the equivalent of Orco (Benton et al., 2009; Croset et al., 2010; Abuin et al., 2011; Rimal and Lee, 2018).

Interestingly, the IR phylogeny showed that we found no SlitIR in the clades housing IRs involved in hygrosensation and thermosensation (**Figure 4**). The cool temperature sensing candidate *SlitIR21a* and the humidity sensing candidates *SlitIR40a* and *SlitIR68a* (Knecht et al., 2017, 2016) have been found previously in *S. littoralis* antennae (Olivier et al., 2011; Poivet et al., 2013; Walker et al., 2019), yet the candidate coreceptor involved in hygrosensation and thermosensation, IR93a (Benton et al., 2009; Corey et al., 2013; Rytz et al., 2013; Groh-Lunow et al., 2015) was not retrieved in our transcriptome nor in previous *S. littoralis* antennal transcriptomes (Olivier et al., 2011; Poivet et al., 2013; Walker et al., 2019). All together, these observations suggest that proboscis, palps and legs are not involved in hygro and thermosensation in *S. littoralis*.

Chemosensory Proteins

CSPs are known to be largely expressed in a variety of chemosensory and non-chemosensory tissues in insects, including Noctuidae (Zhang et al., 2015; Guo et al., 2018; Walker et al., 2019). Our results comfort these observations, since among the 22 *SlitCSPs* previously identified in *S. littoralis* antennal transcriptomes (Poivet et al., 2013; Walker et al., 2019), 10 were found and they were all expressed in both the proboscis and palps/legs (**Supplementary Material 2**). The current hypothesis is that CSPs would play a general role in hydrophobic molecule transport (Pelosi et al., 2018). It is thus difficult for now to propose any specific function of CSPs in olfaction and/or taste in *S. littoralis*.

Odorant-Binding Proteins

Thirty one OBP transcripts were found in the present transcriptome, all previously described in adult antennae and larval tissues (Legeai et al., 2011; Jacquin-Joly et al., 2012; Poivet et al., 2013; Walker et al., 2019). Twenty-seven had a full-length ORF (**Supplementary Material 2**). Among the 31 SlitOBPs, we retrieved the two OBPs classified as general OBPs (GOBPs) and three of the four candidate pheromone-binding proteins (PBPs), as shown in **Figure 5**. At the exception of *SlitOBP8* and *SlitOBP33*, we also found all OBPs belonging to the Noctuidae-specific OBP expansion.

First studies on OBPs have suggested that, contrary to CSPs, they would be antennae-specific and only involved in olfaction, with some (the PBPs) being sex-specific and involved in pheromone transportation in the sensillum lymph. Here, we found 31 SlitOBPs expressed in proboscis and palps/legs (**Supplementary Material 2**) *SlitOBP6* was expressed only in the female palp/leg/ovipositor tissue mix. This OBP has been previously detected in female adult antennae and caterpillar antennae (Poivet et al., 2013), suggesting that *SlitOBP6* may be female-specific but not tissue-specific. Whether this OBP is involved in female specific behaviors, such as oviposition site selection, remains to be investigated.

Four OBPs expressed in the proboscis (i.e., *SlitOBP11*, *25*, *32,* and *34*) belong to the Noctuidae-specific expansion mentioned above. Interestingly, some of these have also been found to be more expressed in the proboscis than in antennae (Walker et al., 2019). Expression of these OBPs in gustatory tissues has been observed also in *H. assulta* (five out of seven OBPs of this clade are also expressed in legs; Zhang et al., 2015) and in *H. armigera* (all OBPs of this clade are also expressed in caterpillar mouthparts; Chang et al., 2017). Whether these OBPs from the Noctuidae OBP expansion play a role in olfaction has not yet been determined.

Interestingly enough and rather surprisingly, we found three *SlitPBPs* expressed in all tissues tested in this study. This expression pattern was unexpected for PBPs that are believed to bind volatile hydrophobic pheromone molecules and to be expressed in antennae. In addition to the expression of *SlitOR5* and *SlitSNMP1* in all samples (see upper), expression of PBPs revealed that all the machinery for sex pheromone detection is present in proboscis and palp/leg tissues. This reinforces our hypothesis that *S. littoralis* may use sensory appendages such as proboscis, palps and/or legs to evaluate mating partners via the detection of long range sex pheromone and/or close range pheromonal cues yet unidentified.

CONCLUSION

S. littoralis is a model species in chemical ecology. It is crucial to combine long term knowledge and accumulate data on this model for further advances in deciphering the mechanisms of chemosensation in moths. Our study adds a large amount of data on chemosensory genes and their expression specificities in this species, revealing unexpected expression of many. We pinpointed interesting genes for further functional studies that will help understanding the intimate link of the different sensory appendages to olfaction and taste. Also, we contribute additional evidence that the sex pheromone communication may be more complex than previously thought in this species, with involvement of a probable close range communication. *S. littoralis* is also an economically important agricultural pest and a representative of the *Spodoptera* genus that contains some of the most dangerous invasive pest species, such as the fall armyworm *S. frugiperda*. Chemosensation drives many detrimental behaviors in pests, such as food, oviposition site and mate choice. A better understanding of the chemoreception pathways will lead to identifying key molecular targets to disturb such chemical communication in a context of sustainable pest management.

DATA AVAILABILITY STATEMENT

The data generated in this study have been deposited in NCBI Sequence Read Archive, (BioProject ID PRJNA693435, Biosamples SAMN17386877, SAMN17386878, SAMN17386879, and SAMN17386880).

AUTHOR CONTRIBUTIONS

EJ-J conceived the research. FAK performed the laboratory work and the transcriptomic data analysis. FAK, CMo, and M-CF annotated the transcripts. CMe helped with the transcriptomic data analysis and NM with the phylogenetic analysis. FAK, NM, CMe, and EJ-J contributed to the writing and reviewing of the manuscript. DS was responsible for transcriptomic sequencing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.678277/full#supplementary-material

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