Identification and Expression Analysis of G Protein-Coupled Receptors in the Miridae Insect Apolygus lucorum

Han Gao, Yanxiao Li, Miao Wang, Xiaowen Song, Jing Tang, Fan Feng and Bin Li*

Jiangsu Key Laboratory for Biodiversity and Biotechnology, College of Life Sciences, Nanjing Normal University, Nanjing, China

G protein-coupled receptors (GPCRs) are the largest and most versatile family of transmembrane receptors in the cell and they play a vital role in the regulation of multiple physiological processes. The family Miridae (Hemiptera: Heteroptera) is one of the most diverse families of insects. Until now, information on GPCRs has been lacking in Miridae. Apolygus lucorum, a representative species of the Miridae, is an omnivorous pest that occurs worldwide and is notorious for causing serious damage to various crops and substantial economic losses. By searching the genome, 133 GPCRs were identified in A. lucorum. Compared with other model insects, we have observed GPCR genes to be remarkably expanded in A. lucorum, especially focusing on biogenic amine receptors and neuropeptide receptors. Among these, there is a novel large clade duplicated from known FMRFamide receptors (FMRFaRs). Moreover, the temporal and spatial expression profiles of the 133 genes across developmental stages were determined by transcriptome analysis. Most GPCR genes showed a low expression level in the whole organism of A. lucorum. However, there were a few highly expressed GPCR genes. The highly expressed LW opsins in the head probably relate to nocturning of A. lucorum, and the expression of Cirl at different times and in different tissues indicated it may be involved in growth and development of A. lucorum. We also found C2 leucine-rich repeat-containing GPCRs (LGRs) were mainly distributed in Hemiptera and Phthiraptera among insects. Our study was the first investigation on GPCRs in A. lucorum and it provided a molecular target for the regulation and control of Miridae pests.

Keywords: identification, GPCRs, Apolygus lucorum, expansion, phylogenetic analysis

1 INTRODUCTION

G protein-coupled receptors (GPCRs) are in a large family of protein cell surface receptors that detect molecules outside the cell and activate cellular responses (1, 2). GPCRs are found only in eukaryotes, namely, yeast, choanoflagellates, and animals (3). Based on sequence homology and functional similarity, GPCRs can be grouped into six families (4, 5): Family-A (rhodopsin-like); Family-B (secretin receptor family); Family-C (metabotropic glutamate/pheromone); Family-D
(fungal mating pheromone receptors); Family-E (cyclic AMP receptors); and Family-F (frizzled/smoothened). These receptors are involved in a wide variety of physiological processes (6), namely, visual sensation (7), taste (8), smell sensation (9), behavioral and mood regulation (10), regulation of immune system activity and inflammation (11, 12), and autonomic nervous system transmission (10, 13). Because of their crucial roles in the regulation of multiple physiological processes, GPCRs are an important drug target (14) and approximately 34% (15) of all Food and Drug Administration (FDA) approved drugs target 108 members of this family.

With the continuous innovation of next-generation sequencing technology and bioinformatics, systematic identification research about GPCRs has been reported in several insects (16–21). Among Hemiptera, it has been reported in Acyrthosiphon pisum (22), Aphis craccivora (23), Cimex lectularius (24), Diaphorina citri (25), Nilaparvata lugens (26), and Rhodnius prolixus (27). The family Miridae (Hemiptera: Heteroptera), which includes plant bugs, leaf bugs, or grass bugs, is one of the most diverse families of insects, including over 11,000 species in more than 1,300 genera (4, 28). It is the largest family of true bugs belonging to Hemiptera and new members of Miridae are being described constantly. Mirids exhibit a wide range of food preferences and behaviors, including phytophagy, carnivory, and omnivory. Some mirids exhibit significant economic impacts and some are pests of food and fiber crops, whereas others are beneficial species used as biological control agents (29). Although Miridae is the largest family of Hemiptera and exhibits a complex habit, there has been little information reported on GPCRs.

Apolygus lucorum (Miridae) is an omnivorous pest that occurs worldwide and is notorious for the serious damage it causes in various crops and its substantial economic losses (30, 31). Recently, the genome of A. lucorum has been reported, which provided convenient in-depth studies of this pest (32). In the present research, using bioinformatics analysis, we screened the genes encoding GPCRs from the genome of A. lucorum. The expression profiles of all GPCRs were also determined by using public transcriptome data. These results allowed us to make comparisons of GPCR systems in different insect species and to provide relevant information for further functional studies in A. lucorum. Our study was the first investigation of GPCRs in A. lucorum, which may become the basis for further investigation of the function of miridae GPCRs.

2 MATERIALS AND METHODS

2.1 Identification of A. lucorum GPCRs

A. lucorum protein sequences were retrieved from the NCBI Genome database (https://www.ncbi.nlm.nih.gov/assembly/GCA_009739505.2) (32). Based on previous studies and records in Flybase (http://www.flybase.org/) (33), the GPCRs of Drosophila melanogaster (34), A. pism (22), Bombyx mori (17), Tribolium castaneum (16), and Pediculus humanus humanus (18) were collected. By using D. melanogaster GPCRs as references and A. lucorum protein sequences as queries, BLASTP searches (35) were performed with a cut-off e-value of 1e-5 to look for all GPCR candidates. Then, seven-transmembrane (7TM) domain and annotation information was adopted as the basic criteria for all GPCR candidates. The GPCR candidates in which the number of 7TM domains was more than four or the annotation information indicated it was a GPCR were retained. The remaining GPCR candidates were also confirmed by means of BLASTX analysis in the UniProtKB/Swiss-Prot database. Using all GPCRs that we collected, pre-phylogenetic analysis with the maximum likelihood method was the final criteria to remove non-GPCRs from candidate pools. If a candidate showed fewer genetic relationships with known GPCRs by phylogenetic analysis and the hit sequences in BLASTX analysis indicated they were not GPCRs, they were classified as a non-GPCR and removed from our analysis.

2.2 Structural Analyses, Annotation Information, and Gene Locations of GPCRs

The 7TM domains for all GPCR candidates were predicted with the server TMHMM (v2.0) (36) from the Centre for Biological Sequence Analysis (http://www.cbs.dtu.dk/services/TMHMM/). Functional annotations of the target proteins were done using InterProScan (37). In addition, the chromosomal location of each GPCR candidate was extracted from the genome annotation file of A. lucorum.

2.3 Phylogenetic Analysis

Partial GPCRs of R. prolixus and C. lectularius that also belonged to Heteroptera were also obtain based on previous study (24, 27). GPCRs from D. melanogaster, A. pism, C. lectularius, and R. prolixus were assigned to a family/subfamily according to previous results (22, 24, 27, 34). Putative A. lucorum GPCRs were classified into different families/subfamilies according to the families to which their orthologous proteins were assigned. Amino acid sequences of the putative A. lucorum GPCRs in each family/subfamily were aligned with receptors of the same family/subfamily in D. melanogaster, A. pism, C. lectularius, and R. prolixus using MAFFT v7 (38). Phylogeny tests were accomplished using the bootstrap method with 1,000 replications to reconstruct maximum likelihood (ML) trees using IQ-TREE (39) and the best-fit tree model was determined with ModelFinder (40). It should be noted that the GPCRs of R. prolixus and C. lectularius were uncompleted, which were composed of opsins, biogenic amine receptors, and neuropeptide GPCRs. For the Drosophila sequences, the name of the GPCRs were used, while for A. pism and R. prolixus, the protein names were same as in previous work (22, 27), and for C. lectularius, the accession numbers in NCBI were used. The GPCRs of A. lucorum identified in this work were numbered according to their families.

2.4 Expression Analysis

To study the expression profiles of the GPCRs, a total of 39 transcriptome data of A. lucorum were downloaded from the genome project of A. lucorum (Accession: PRJNA526332) in the
NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra/) (30, 41), which included egg and different tissues (leg, head, body, mouthpart, wing, and gut) of nymphs and adults. Each tissue contained three biological replicates. In detail, we downloaded the SRA data first and then we used an SRA-Toolkit to split the paired-end reads. Clean reads were obtained from the raw data using Trimmomatic (42) to remove reads with quality scores lower than 10 and adapter sequences. To analyze gene expression profiles, clean reads of each sample were mapped to A. lucorum gene sets using hisat2 (43), and then the TPM value (44) of each putative GPCR gene was calculated with featureCounts (45). These TPM expression values were scaled and served to generate a cross-sample normalized trimmed mean of the M-values (TMM) gene expression matrix (46). Finally, the heatmap was drawn in ITOL (https://itol.embl.de) (47) using the normalized matrix. The value used for each sample was the mean of three independent biological replicates.

2.5 Classification of Gene Duplication Types
MCScanX (48) was used to classified the duplication types of different duplicate GPCR genes. First, the homology with different genes in the genome of A. lucorum was determined by a whole-genome BLASTP analysis with a max target seq of 5 and a cut-off e-value of 1e^{-5}. Then, the homology with different genes and the chromosomal location were combined and all genes were classified into various types, including the segmental duplication, and tandem duplication. Finally, the duplication types of GPCRs were extracted based on these results. All visualized works were accomplished in TBtools (49).

3 RESULTS
A total of 133 putative GPCRs were identified in A. lucorum. These GPCRs were classified into four families and included 98 family-A members, 21 family-B members, 10 family-C members, and four family-F members (Tables 1–4 and Table S1). Based on the protein sequences, phylogenetic trees were reconstructed for each GPCR family/subfamily of A. lucorum, R. prolites, A. pisum, and D. melanogaster. All GPCRs were quantified with the TPM values obtained from transcriptomic data. The expression profile of each GPCR across developmental stages was also present in the phylogenetic trees of each GPCR family/subfamily (Figures 1–3, and Figures S1–S3). The chromosomal locations of all GPCRs are shown in Figure 4.

3.1 Family-A GPCRs
Insect family-A GPCRs include opsins, biogenic amine receptors, neuropeptide and protein hormone receptors, and purine GPCRs (17, 18, 22). In this study, 98 family-A GPCRs were identified in the genome of A. lucorum, and these receptors were composed of seven opsins, 30 biogenic amine receptors, 58 neuropeptide and protein hormone receptors, and three purine GPCRs (Tables 1, 2).

3.1.1 Opsins
Color vision in insects is based on the expression of different opsins in photoreceptor cells. Opsins are members of the family-A GPCRs and are coupled to light-sensitive chromophores in animal photoreceptors (50). Three groups of opsins have been reported in D. melanogaster: one related to long-wavelength (LW) vision (including Rh1, Rh2, and Rh6), another group related to short-wavelength (SW) vision (Rh3, Rh4, and Rh5), and a third group including only Rh7 (34, 51). A fourth group of invertebrate opsins, named pteropsins, has been found in Apis mellifera (50) and R. prolites (27), which was missing from the genome of D. melanogaster and A. pisum.

In this study, seven putative opsins were identified in A. lucorum. The phylogenetic analysis suggested that A1 and A2 are related to the LW opsin, A3 is related to the SW opsin, A4 and A5 belong to a third group, and A6 and A7 are close to pteropsins (Figure 1). Four groups of invertebrate opsins were also identified in the A. lucorum genome. According to the expression profile, opsins were expressed at the highest levels in the head and mouthpart tissue, which is corresponding to their biological function. Among the four types of opsins detected in A. lucorum, the A1 showed the highest expression in the head tissue of adults with a transcripts per kilobase of exon model per million mapped reads (TPM) of 28,787 (Figure 5) followed by A3 with a TPM of 770.

3.1.2 Biogenic Amine Receptors
The known biogenic amines that act as ligands for GPCRs in insects contain acetylcholine, dopamine, serotonin, octopamine, and tyramine (27). Here, we identified 30 biogenic amine receptors in A. lucorum. Based on phylogenetic analysis and sequence similarity, A8–11 are receptors for acetylcholine; A12–16 are dopamine-like receptors; A17–24 are orthologs of the octopamine receptors; A26–33 and A35–36 were identified as the serotonin-like receptors; and A25 is the GPCR that could be stimulated by two structurally related endogenous ligands, octopamine and tyramine (Figure S1). Additionally, A34 and A37 are orphan receptors of this subfamily in A. lucorum, and are orthologs of RPRC011175 and CG13579, respectively. However, two tyramine receptors (TyrR and TyrRII) are likely to be missing in all three heteropteran insects. A25 is the only tyramine receptor in A. lucorum. Compared with opsins, the expression level of biogenic amine receptors is much lower. A36 showed the highest expression in gut tissues of adults with a TPM of 11. In FlyBase (33), we found 5-HT7, the ortholog gene of A36 in D. melanogaster, was also expressed in the digestive system.

3.1.3 Neuropeptide and Protein Hormone Receptors
The rhodopsin-like neuropeptide and protein hormone receptors are the largest subfamily in the rhodopsin-like family (17, 22, 52). In this subfamily, 58 putative A. lucorum sequences were identified. Like other insects, A. lucorum rhodopsin-like neuropeptide and protein hormone receptors can be classified into 25 groups based on their ligands; i.e., adipokinetic hormone receptors (AKHR), AKH/corazonin-related peptide (ACP)
| No. | Accession number | Putative Endogenous ligand | Orthologue of D. melanogaster | Orthologue of A. pismum | Predicted TMHs | Annotation by InterProScan | Homology search in Swissport (blastp) |
|-----|------------------|-----------------------------|-----------------------------|------------------------|----------------|---------------------------|-----------------------------------|
|     |                  |                             |                             |                        |                |                           | E-value | Description | Species                      |
| Opsin |                 |                             |                             |                        |                |                           |         |             |                              |
| A1   | KAF6206346.1     | Orphan                      | Rh6                         | ACYPI009332            | Complete       | (IPR0000276) GPCR, rhodopsin-like; (IPR001760) Opsin; (IPR001391) Opsin lateral eye type | 0       | Opsin-1      | Schistocerca gregaria         |
| A2   | KAF6206345.1     | Orphan                      | Rh6                         | ACYPI009332            | Complete       | (IPR0000276) GPCR, rhodopsin-like; (IPR001760) Opsin; (IPR001391) Opsin lateral eye type | 0       | Opsin        | Sphodromantis sp.             |
| A3   | KAF6207755.1     | Orphan                      | Rh3, Rh4                    | ACYPI002544, ACYPI004442 | Complete       | (IPR0000276) GPCR, rhodopsin-like; (IPR001760) Opsin; (IPR001391) Opsin lateral eye type | 1.00E-165 | UV-sensitive opsin | Apis mellifera                 |
| A4   | KAF6207831.1     | Orphan                      | Rh7                         | ACYPI001006, ACYPI005074 | Complete       | (IPR0000276) GPCR, rhodopsin-like | 1.60E-77 | Opsin-2       | Manduca sexta                 |
| A5   | KAF6207832.1     | Orphan                      | Rh7                         | ACYPI001006, ACYPI005074 | Complete       | (IPR0000276) GPCR, rhodopsin-like; (IPR001760) Opsin | 1.88E-73 | Opsin Rh3     | D. melanogaster               |
| A6   | KAF6205310.1     | Orphan                      | na                          | na                      | 5              | (IPR0000276) GPCR, rhodopsin-like; (IPR001760) Opsin | 4.83E-48 | Pinopsin      | Columba livia                 |
| A7   | KAF6208054.1     | Orphan                      | na                          | na                      | 6              | (IPR0000276) GPCR, rhodopsin-like; (IPR001760) Opsin | 2.01E-70 | GQ-rhodopsin    | Musnopsecten yessoensis       |
| Biogenic amine receptors |            |                             |                             |                        |                |                           |         |             |                              |
| A8   | KAF6211999.1     | Acetylcholine mACHR-A       | ACYPI005180                 | Complete               | 4.10E-160      | mACHR DM1                 | D. melanogaster               |
| A9   | KAF6206451.1     | Acetylcholine mACHR-B       | ACYPI001255                 | 6                      | (IPR0000276) GPCR, rhodopsin-like | 2.84E-57 | mACHR gar-2    | Caenorhabditis elegans        |
| A10  | KAF6206450.1     | Acetylcholine mACHR-B       | ACYPI001255                 | 2                      | (IPR0000276) GPCR, rhodopsin-like | 1.28E-27 | mACHR gar-2    | Caenorhabditis elegans        |
| A11  | KAF6202800.1     | Acetylcholine mACHR-C       | na                          | Complete               | 1.07E-23       | D(1B) DopR               | D. melanogaster               |
| A12  | KAF6209068.1     | Dopamine Dopr1R1            | ACYPI006935                 | Complete               | 4.69E-164      | DopR1R2                  | D. melanogaster               |
| A13  | KAF6217029.1     | Dopamine Dopr1R2            | ACYPI007241                 | 6                      | (IPR0000276) GPCR, rhodopsin-like; (IPR001671) | 2.03E-61 | Dopr2R        | D. melanogaster               |
| A14  | KAF6204820.1     | Dopamine Dopr2R             | ACYPI007145                 | 2                      | (IPR0000276) GPCR, rhodopsin-like | 2.20E-103 | Dopr2R        | D. melanogaster               |
| A15  | KAF6204823.1     | Dopamine Dopr3R             | ACYPI007145                 | 2                      | (IPR0000276) GPCR, rhodopsin-like | 5.89E-24 | G-protein coupled receptor 52 | Mus musculus                 |
| A16  | KAF6201362.1     | Dopamine, Ecdysteroids DopcR | ACYPI005538                 | Complete               | 5.20E-109      | Omb                      | D. melanogaster               |
| A17  | KAF6209377.1     | Octopamine Oamb             | ACYPI005578                 | 5                      | (IPR0000276) GPCR, rhodopsin-like | 2.03E-29 | Omb           | D. melanogaster               |
| A18  | KAF6209376.1     | Octopamine Octbeta1R        | ACYPI005578                 | 3                      | (IPR0000276) GPCR, rhodopsin-like | 2.90E-104 | Octbeta1R      | D. melanogaster               |
| A19  | KAF6209222.1     | Octopamine Octbeta2R        | ACYPI004685                 | 5                      | (IPR0000276) GPCR, rhodopsin-like | 4.18E-91 | Octbeta2R      | D. melanogaster               |
| A20  | KAF6209522.1     | Octopamine Octbeta2R        | ACYPI004685                 | 3                      | (IPR0000276) GPCR, rhodopsin-like | 4.37E-54 | Octbeta2R      | D. melanogaster               |
| A21  | KAF6209465.1     | Octopamine Octalpha2R       | ACYPI0010155                | 4                      | (IPR0000276) GPCR, rhodopsin-like | 6.10E-76 | Octbeta2R      | D. melanogaster               |
| A22  | KAF6209394.1     | Octopamine Octbeta3R        | ACYPI004685                 | 4                      | (IPR0000276) GPCR, rhodopsin-like | 6.00E-100 | Octbeta3R      | D. melanogaster               |
| A23  | KAF6209913.1     | Octopamine Octbeta3R        | ACYPI0010025                | 5                      | (IPR0000276) GPCR, rhodopsin-like | 2.09E-44 | Octbeta3R      | D. melanogaster               |
| A24  | KAF6209915.1     | Octopamine Octbeta3R        | ACYPI0010025                | 3                      | (IPR0000276) GPCR, rhodopsin-like | 0       | OctR          | Heliosis virescens            |
| A25  | KAF6199206.1     | Octopamine/ Tyramine Oct-TyrR | ACYPI007379                | Complete               | 4.80E-107      | 5-HT receptor          | Bombyx mori                  |
| A26  | KAF6209660.1     | Serotonin, 5-HT1A, 5-HT1B   | XP_001949725                | 4                      | (IPR0000276) GPCR, rhodopsin-like | 8.60E-103 | 5-HT receptor | Heliosis virescens            |
| A27  | KAF6210003.1     | Serotonin, 5-HT1A, 5-HT1B   | XP_001949725                | 4                      | (IPR0000276) GPCR, rhodopsin-like | 1.71E-51 | 5-HT receptor | Heliosis virescens            |
| A28  | KAF6208615.1     | Serotonin, 5-HT1A, 5-HT1B   | XP_001949725                | 2                      | (IPR0000276) GPCR, rhodopsin-like | 1.06E-41 | 5-HT receptor | Heliosis virescens            |
| A29  | KAF6209646.1     | Serotonin, 5-HT1A, 5-HT1B   | XP_001949725                | 2                      | (IPR0000276) GPCR, rhodopsin-like | 5.75E-29 | 5-HT-2C        | Canis lupus familiaris        |
| A30  | KAF6204179.1     | Serotonin, 5-HT2A           | ACYPI008969                 | 2                      | (IPR0000276) GPCR, rhodopsin-like |                      |         |                      |                              |
### TABLE 1 | Continued

| No. | Accession number | Predicted TMDs | Orthologue of D. melanogaster | Orthologue of D. pismum | Putative Endogenous ligand | Species | Description | E-value | Homology search in Swissport (blastp) |
|-----|------------------|----------------|-----------------------------|-----------------------|---------------------------|---------|-------------|---------|--------------------------------------|
| A31 | KAF6204181.1     | 5-HT-2B        | Senorin                     | Senorin               | 5-HT-2B                   | Mus musculus | serotonin 5-HT2A | 4.66E-25 | 2.15E-19 |
| A32 | KAF6204453.1     | 5-HT-2B        | Senorin                     | Senorin               | 5-HT-2B                   | Homo sapiens | serotonin 5-HT2B | 9.00E-24 | 9.00E-12 |
| A33 | KAF6204455.1     | 5-HT-2B        | Senorin                     | Senorin               | 5-HT-2B                   | Mus musculus | serotonin 5-HT2B | 3.57E-02 | 3.57E-12 |
| A34 | KAF6204452.1     | 5-HT-2A        | Senorin                     | Senorin               | 5-HT-2A                   | D. melanogaster | serotonin 5-HT2B | 9.00E-24 | 9.00E-12 |
| A35 | KAF6205718.1     | Complete        | Senorin                     | Senorin               | 5-HT-2A                   | D. melanogaster | serotonin 5-HT2B | 9.00E-24 | 9.00E-12 |
| A36 | KAF6200010.1     | Complete        | Senorin                     | Senorin               | 5-HT-2A                   | D. melanogaster | serotonin 5-HT2B | 9.00E-24 | 9.00E-12 |

*Note: na, not annotated or not applicable, Complete means there is a complete 7TM structure.

receptors, allatotropin receptor (AT-R), allatostatin-A receptors (AstA-R), allatostatin-B receptors (AstB-R), allatostatin-C receptors (AstC-R), bursion receptor, corazonin receptors (CrzR), neuropeptide F receptors (NPFR), short neuropeptide F receptors (sNPFR), proctolin receptors (Proc-R), pyrokinin receptors (PK-R), leukokinin receptors (Lkr), cholecystokinin-like receptors (CCKLR), tachykinin receptors (TkR), CAPA receptors (CapaR), crustacean cardioactive peptide receptors (CCAP-R), CNMamide receptors (CNMaR), CCHamide receptors (CCHA-R), ecysis triggering hormone receptors (ETHR), FMRFamide receptors (FMRFaR), GPA2/GPB5 receptors, SIFamide receptors (SIFaR), relaxin receptors, RYamide receptors (RYa-R), and several orphan GPCRs (Figure 2). Most of these neuropeptide receptors displayed one-to-one orthologous relationships between A. lucorum, R. prolixus, C. lectularius, A. pismum, and D. melanogaster, and all subtypes of leucine-rich repeat-containing GPCRs (LGR) were observed in A. lucorum (Figures 2 and 6). However, several duplications and losses of neuropeptide receptor genes were also observed in A. lucorum. It is worth mentioning that as many as nine A. lucorum GPCRs (A54–62) displayed strong evidence of an evolutionary kinship with the FMRFaRs of R. prolixus, C. lectularius, A. pismum, and D. melanogaster, indicating that a large clade may have duplicated from FMRFaRs in A. lucorum (Figures 2 and 7). Duplications of eight neuropeptide receptor genes (CapaR, CCAP-R, CNMaR, ETHR, Lkr, NPFR, PK1-R, and SIFaR) were identified in A. lucorum, and duplications of Lkr, PK1-R, ETHR, CCAP-R, NPFR, and SIFaR were also observed in R. prolixus or C. lectularius. The trapped in endoderm 1 (Tre1) receptors, trissin receptors (TrissinR), myosuppressin receptors (MsR), and other six orphan receptors were not found in the genome of A. lucorum. Instead, we found six orphan receptors (A89–92 and A94–95) that have not been reported in A. lucorum. The expression levels of neuropeptide and protein hormone receptors were higher than in biogenic amine receptors. The expression of A65 (LGR) in the bodies of nymphs was the highest in this subfamily with a TPM of 41. Moreover, A50 (CCAP-R) and A86 (moody) showed high expression levels in multiple tissues (TPM >10 at least in five tissues).

### 3.1.4 Purine GPCRs

Only one receptor in this subfamily, adenosine receptor (AdoR), has been previously classified in this subfamily (17, 53). Here, three putative A. lucorum GPCRs (A96–98) were identified as AdoR, whereas there was only one member in D. melanogaster and A. pismum (Table 2). Purine GPCRs are activated by the binding of purine nucleotides or their derivatives (principally adenosine or ADP/ATP) (54, 55). Duplication of AdoR suggests that purinergic neural transmission may play a more important role in A. lucorum.

### 3.2 Family-B GPCRs

Family-B GPCRs play vital roles in many biological processes, including growth, development, and reproduction. They are characterized by long N-terminal domains, and they form a small group of receptors that are structurally and functionally divergent from other groups of GPCRs (56). Within this family,
| No. | Accession number | Putative endogenous ligand | Orthologue of D. melanogaster | Orthologue of A. pisum | Predicted TMHs | Annotation by InterProScan | Homology search in Swissport (blastp) |
|-----|------------------|-----------------------------|-----------------------------|-----------------------|----------------|-----------------------------|----------------------------------|
|     |                  | Neuropeptide and protein hormone receptors |                      |                      |                | (IPR000276) GPCR, rhodopsin-like; (IPR000405) Galanin receptor family | 4.74E−31 GnRHR II C. gariepinus |
|     |                  |                          |                      |                      |                | (IPR027417) P-loop containing nucleoside triphosphate hydrolase; | 5.09E−49 GnRHR II C. gariepinus |
| A38 | KAF6210560.1     | AKH/corazonin-related peptide |                      |                      | 4 (IPR000276) GPCR, rhodopsin-like; (IPR000405) Galanin receptor family | 5.02E−41 Asta-R B. mori |
| A39 | KAF6216586.1     | Adipokinetic hormone AkhR | ACYPI002471 Complete |                      | 4 None predicted | (IPR000276) GPCR, rhodopsin-like; (IPR005390) Neuromedin U receptor | 2.45E−79 Asta-R B. mori |
| A40 | KAF6198962.1     | Allostastatins-A AstA-R1, AstA-R2 | ACYPI008623 4 |                      | 5.02E−41 Asta-R B. mori |
| A41 | KAF6198963.1     | Allostastatins-A AstA-R1, AstA-R2 | ACYPI008623 4 |                      | 5.02E−41 Asta-R B. mori |
| A42 | KAF6206708.1     | Allostastatins-C AstC-R1, AstC-R2 | ACYPI002528 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR002131) Glycoprotein hormone receptor family; (IPR036055) LDLreceptor-like superfamily; (IPR032675) Leucine-rich repeat domain superfamily | 1.27E−81 Asta-R B. mori |
| A43 | KAF6202370.1     | CAPA CapaR | ACYPI007245 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 1.17E−85 CapaR D. melanogaster |
| A44 | KAF6216164.1     | CAPA CapaR | ACYPI007245 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR019427) 7TM GPCR, serpentine receptor class w (Srw) | 6.22E−102 Cap2bR D. melanogaster |
| A45 | KAF6210431.1     | CCHamide CCHa2-R | ACYPI004781 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 7.23E−131 CCHa1-R D. melanogaster |
| A46 | KAF6201633.1     | CNMamide CNMaR | ACYPI008027 2 |                      | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | NA NA NA |
| A47 | KAF6216331.1     | CNMamide CNMaR | ACYPI008027 2 |                      | (IPR000276) GPCR, rhodopsin-like | 9.79E−14 FMRFaR D. melanogaster |
| A48 | KAF6205178.1     | Corazonin CrzR | ACYPI002471 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 3.69E−59 GnRHR T. vulguris |
| A49 | KAF6213085.1     | Crustacean cardioactive peptide CCAP-R | ACYPI002528 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR005390) Neuromedin U receptor | 3.69E−158 CCAP R. mori |
| A50 | KAF6211684.1     | Crustacean cardioactive peptide CCAP-R | ACYPI002528 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 1.9E−156 CCAP R. mori |
| A51 | KAF6209917.1     | ETH ETHR | BK008727 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 7.23E−131 CCHa1-R D. melanogaster |
| A52 | KAF6209916.1     | ETH ETHR | BK008727 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 4.21E−16 TRH R. mori |
| A53 | KAF6209916.1     | ETH ETHR | BK008727 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 4.21E−16 TRH R. mori |
| A54 | KAF6209916.1     | FMRFamides FMRFaR | ACYPI006053 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 1.75E−130 FMRFaR D. melanogaster |
| A55 | KAF6209916.1     | FMRFamides FMRFaR | ACYPI006053 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 5.96E−45 FMRFaR D. melanogaster |
| A56 | KAF6200840.1     | FaRP | ACYPI004597 Complete |                      | (IPR000276) GPCR, rhodopsin-like; (IPR019427) 7TM GPCR, serpentine receptor class w (Srw) | 4.53E−40 FaRP R. norvegicus |
| A57 | KAF6215133.1     | FaRP | ACYPI004597 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 5.5E−45 FaRP R. norvegicus |
| A58 | KAF6202705.1     | FaRP | ACYPI004597 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 2.25E−31 FaRP R. norvegicus |
| A59 | KAF6190962.1     | FaRP | ACYPI004597 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 9.69E−35 FaRP R. norvegicus |
| A60 | KAF6212897.1     | FaRP | ACYPI004597 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 1.13E−33 FaRP R. norvegicus |
| A61 | KAF6203114.1     | FaRP | ACYPI004597 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 3.58E−26 FaRP R. norvegicus |
| A62 | KAF6204912.1     | FaRP | ACYPI004597 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 2.55E−29 FaRP R. norvegicus |
| A63 | KAF6216165.1     | GPA2/GPB5 Lgr1 | ACYPI004597 Complete |                      | (IPR000276) GPCR, rhodopsin-like | 2.6E−103 LH/CG-R M. musculus |

(Continued)
| No. | Accession number | Putative endogenous ligand | Orthologue of *D. melanogaster* | Orthologue of *A. pisum* | Predicted TMHs | Annotation by InterProScan | Homology search in Swissport (blastp) |
|-----|-----------------|-----------------------------|-----------------------------|-------------------------|----------------|-----------------------------|--------------------------------------|
|     |                 |                             |                            |                         |                |                             | E-value | Description | Species |
| A64 | KAF6216698.1    | Bursicon                     | rk                         | ACYP000221              | Complete       | (IPR008365) Prostanoid receptor | 7.46E−112 | LGR5         | Rattus norvegicus |
| A65 | KAF62111436.1   | Insulin-like peptide 7 and 8 | Lgr3                       | ACYP0008291             | 5              | (IPR000276) GPCR, rhodopsin-like | 2.26E−116 | Relaxin receptor | Mus musculus |
| A66 | KAF6202512.1    | Insulin-like peptide 7 and 8 | Lgr4                       | na                      | Complete       | (IPR000276) GPCR, rhodopsin-like | 0        | GPCR, GRL101 | Lymnaea stagnalis |
| A67 | KAF6211616.1    | Leucokinin                   | Lkr                        | ACYP010083, ACYP000762  | 5              | (IPR000276) GPCR, rhodopsin-like; (IPR001681) Neurokinin receptor | 5.04E−40 | TkR99D        | D. melanogaster |
| A68 | KAF6205586.1    | Leucokinin                   | Lkr                        | ACYP010083, ACYP000762  | 5              | (IPR000276) GPCR, rhodopsin-like | 4.36E−27 | DmNPFR1       | D. melanogaster |
| A69 | KAF6212176.1    | Neuropeptide F               | NPFRR                      | ACYP007664              | 4              | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 1.14E−101 | PK1-R         | D. melanogaster |
| A70 | KAF6209720.1    | Neuropeptide F               | NPFRR                      | ACYP007664              | 2              | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 9.86E−16 | NPFR          | D. melanogaster |
| A71 | KAF6212186.1    | Neuropeptide F               | NPFRR                      | ACYP007664              | 2              | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 4.33E−117 | PK1-R         | D. melanogaster |
| A72 | KAF6213141.1    | Pyrokinin-1                  | PK1-R                      | ACYP000735, ACYP005805  | Complete       | (IPR000276) GPCR, rhodopsin-like; (IPR002120) Thyrotropin-releasing hormone receptor | 4.33E−117 | PK1-R         | D. melanogaster |
| A73 | KAF6213123.1    | Pyrokinin-1                  | PK1-R                      | ACYP000735, ACYP005805  | 3              | (IPR000276) GPCR, rhodopsin-like; (IPR000611) Neuropeptide Y receptor family | 2.91E−42 | PK1-R         | D. melanogaster |
| A74 | KAF6213143.1    | Pyrokinin-2                  | PK2-R2                     | na                      | Complete       | (IPR000276) GPCR, rhodopsin-like; (IPR019427) 7TM GPCR, serpentine receptor class w (Srw) | 8.96E−16 | NPFR          | D. melanogaster |
| A75 | KAF6205859.1    | Proctolin                    | Proc-R                     | ACYP030716              | Complete       | (IPR027417) P-loop containing nucleoside triphosphate hydrolase | 3.98E−25 | FMRFaR        | D. melanogaster |
| A76 | KAF6207650.1    | Ryamide                      | RyFaR                      | ACYP002886              | Complete       | (IPR000832) GPCR, family 2, secretin-like | 1.52E−122 | RyFa-R        | D. melanogaster |
| A77 | KAF6211457.1    | SIFamide                     | SiFaR                      | ACYP008341, BK008728    | 3              | (IPR000276) GPCR, rhodopsin-like; (IPR002131) Glycoprotein hormone receptor family; (IPR002675) Leucine-rich repeat domain superfamily | 8.27E−107 | SiFaR         | D. melanogaster |
| A78 | KAF6213872.1    | SIFamide                     | SiFaR                      | ACYP008341, BK008728    | 3              | (IPR00276) GPCR, rhodopsin-like; (IPR019427) 7TM GPCR, serpentine receptor class w (Srw) | 4.69E−101 | SiFaR         | D. melanogaster |
| A79 | KAF6209074.1    | short neuropeptide F         | sNPFR-R                    | ACYP005474              | Complete       | (IPR000276) GPCR, rhodopsin-like; (IPR002131) Glycoprotein hormone receptor family; (IPR002675) Leucine-rich repeat domain superfamily | 1.15E−62 | NPY2-R        | Homo sapiens |
| A80 | KAF6215350.1    | Allatostatin-C               | AstC-R                     | ACYP003290              | Complete       | (IPR000276) GPCR, rhodopsin-like; (IPR002131) Glycoprotein hormone receptor family; (IPR002675) Leucine-rich repeat domain superfamily | 3.65E−147 | SPR           | D. melanogaster |
| A81 | KAF6198907.1    | Sulfakinin                   | CCKLR-17D1, CCKLR-17D3     | na                      | 3              | (IPR000276) GPCR, rhodopsin-like | 3.51E−29 | CCK-XLR       | Xenopus laevis |
| A82 | KAF6213665.1    | Tachykinin                   | TkR86C                     | ACYP011103              | 3              | (IPR000276) GPCR, rhodopsin-like | 4.57E−50 | TkR86C        | D. melanogaster |
| A83 | KAF6210188.1    | Tachykinin                   | TkR99D                     | ACYP002917              | 4              | (IPR000276) GPCR, rhodopsin-like | 2.1E−93 | TkR99D        | D. melanogaster |
| A84 | KAF6211953.1    | Orphan                       | CG4313                     | ACYP005234              | 5              | (IPR000276) GPCR, rhodopsin-like | 2.38E−55 | moody         | D. melanogaster |
| A85 | KAF6198896.1    | Orphan                       | CG32547                    | ACYP000671              | 3              | (IPR000276) GPCR, rhodopsin-like; (IPR001817) Vasopressin receptor | NA      | NA            | NA          |
| A86 | KAF62111940.1   | Orphan                       | moody                      | ACYP006293              | 6              | (IPR000276) GPCR, rhodopsin-like | 5.19E−143 | moody         | D. melanogaster |
| A87 | KAF6198907.1    | Orphan                       | na                         | ACYP04167               | Complete       | (IPR000276) GPCR, rhodopsin-like | 1.52E−14 | NPY2-R        | Mus musculus |
| A88 | KAF6216499.1    | Orphan                       | na                         | ACYP038121              | Complete       | (IPR000276) GPCR, rhodopsin-like | 1.14E−10 | Melatonin receptor type 1A | Gallus gallus |
| No. | Accession number | Putative endogenous ligand | Orthologue of D. melanogaster | Orthologue of A. pisum | Predicted TMHs | Annotation by InterProScan | Homology search in Swissport (blastp) |
|-----|------------------|-----------------------------|------------------------------|------------------------|---------------|---------------------------|-------------------------------------|
|     |                  |                             |                              |                        |               |                           |                                      |
| A89 | KAF6200805.1     | Orphan                      | na                           | na                     | 6             | (IPR000276) GPCR, rhodopsin-like | 1.02E−12 Melatonin receptor type 1A G. gallus |
| A90 | KAF6202756.1     | Orphan                      | na                           | na                     | 2             | (IPR000276) GPCR, rhodopsin-like | 1.17E−13 Somatostatin receptor type 5 H. sapiens |
| A91 | KAF6205087.1     | Orphan                      | na                           | na                     | Complete      | (IPR000276) GPCR, rhodopsin-like | 5.87E−12 Prostaglandin E2 receptor EP4 subtype RYa-R B. taurus |
| A92 | KAF6208108.1     | Orphan                      | na                           | na                     | Complete      | (IPR000276) GPCR, rhodopsin-like; (IPR001556) Bombesin receptor-like | 1.75E−41 Orexin receptor type 2 D. melanogaster |
| A93 | KAF6215130.1     | Allatotropin                | na                           | na                     | Complete      | (IPR000276) GPCR, rhodopsin-like; (IPR005390) Neuromedin U receptor | 8.144E−89 Orexin receptor type 2 M. musculus |
| A94 | KAF6215334.1     | Orphan                      | na                           | na                     | 5             | (IPR000276) GPCR, rhodopsin-like | 1.53E−53 TRHR G. gallus |
| A95 | KAF6215529.1     | Orphan                      | na                           | na                     | Complete      | (IPR000276) GPCR, rhodopsin-like | 2.03E−13 Cadherin EGF M. musculus |
|     |                  |                             |                              |                        |               |                           |                                      |
| Purine receptor |
| A96 | KAF6207107.1     | Adenosine                   | AdoR                         | ACYPI24713             | 3             | (IPR000276) GPCR, rhodopsin-like; (IPR001817) Vasopressin receptor | 1.34E−29 AdoR A2a E. caballus |
| A97 | KAF6207108.1     | Adenosine                   | AdoR                         | ACYPI24713             | 6             | None predicted            | 1.46E−57 AdoR A2a E. caballus |
| A98 | KAF6207109.1     | Adenosine                   | AdoR                         | ACYPI24713             | 3             | (IPR000276) GPCR, rhodopsin-like | 4.05E−21 AdoR A2a G. gallus |

*na*, not annotated or applicable. Complete means there is a complete 7TM structure.
| No. | Accession number | Putative endogenous ligand | Orthologue of D. melanogaster | Orthologue of A. pisum | Predicted TMHs | Annotation by InterProScan | Homology search in Swissport (blastp) |
|-----|-----------------|---------------------------|-------------------------------|------------------------|----------------|----------------------------|-----------------------------------|
|     |                 |                           |                               |                        |                |                            | E-value                           |
|     |                 |                           |                               |                        |                |                            | Description                      |
|     |                 |                           |                               |                        |                |                            | Species                           |
| SUBFAMILY B1 |                 |                           |                               |                        |                |                            |                                   |
| B1  | KAF6209407.1    | Diuretic hormone 31       | Dh31-R                        | ACYP0007222, ACYP001361 | Complete       | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily | 4.8E−74 Calcitonin gene-related peptide type 1 receptor |
| B2  | KAF6209957.1    | Diuretic hormone 44       | Dh44-R1, Dh44-R2              | ACYPi54924             | 5              | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily; (IPR000201) GPCR, family 2, diuretic hormone receptor | 5.79E−68 DH-R A. domesticus |
| B3  | KAF6209955.1    | Diuretic hormone 44       | Dh44-R1, Dh44-R2              | ACYPi54924             | 2              | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily | 5.51E−46 DH-R A. domesticus |
| B4  | KAF6198455.1    | Pigment-dispersing factor | Pdfr                          | ACYPi54924             | 2              | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, diuretic hormone receptor | 6.6E−49 PDF receptor D. melanogaster |
| B5  | KAF6198460.1    | Pigment-dispersing factor | Pdfr                          | ACYPi54924             | 2              | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, diuretic hormone receptor | 6.82E−26 PDF receptor D. melanogaster |
| B6  | KAF6210210.1    | Diuretic hormone 31       | hec                           | ACYPi009569             | 4              | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily | 1.12E−52 Calcitonin receptor Oryctolagus cuniculus |
| B7  | KAF6210211.1    | Diuretic hormone 31       | hec                           | ACYPi009569             | 3              | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily | 6.2E−21 Corticotropin-releasing factor receptor 1 Mus musculus |
| B8  | KAF6210212.1    | Diuretic hormone 31       | Dh31-R                        | ACYPi0007222, ACYP001361 | Complete       | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily | 4.18E−84 Calcitonin gene-related peptide type 1 receptor D. rerio |
| B9  | KAF6204739.1    | Parathyroid hormone       | na                            | ACYPi0007222, ACYP001361 | Complete       | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily | 6.86E−75 PTH2 receptor Homo sapiens |
| SUBFAMILY B2 |                 |                           |                               |                        |                |                            |                                   |
| B10 | KAF6216792.1    | α-latrotoxin              | Cirl                          | ACYPi005705             | Complete       | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily; (IPR043159) D-galactoside/L-rhamnose binding SUEL lectin domain superfamily; (IPR031234) Latrophilin-1 | 0 Latrophilin Drosophila ananassae |
| B11 | KAF6198557.1    | Orphan                    | stan                          | ACYPi001529             | Complete       | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily; (IPR013783) Immunoglobulin-like fold; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily | 1.59E−80 stan D. melanogaster |
| B12 | KAF6198871.1    | Orphan                    | CG15744                       | ACYPi001529             | Complete       | (IPR000832) GPCR, family 2, secretin-like; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily; (IPR013783) Immunoglobulin-like fold; (IPR036445) GPCR family 2, extracellular hormone receptor domain superfamily | 2.6E−111 Adhesion GPCR A3 D. rerio |
| SUBFAMILY B3 |                 |                           |                               |                        |                |                            |                                   |
| B13 | KAF6217262.1    | Orphan                    | mthl5                         | ACYPi003439             | Complete       | None predicted             | 1.2E−128 Mth-like 5 D. melanogaster |
| B14 | KAF6207251.1    | Orphan                    | mthln                         | ACYPi0003439             | Complete       | None predicted             | 2.45E−12 Probable Mth-like 4 D. melanogaster |
| B15 | KAF6215469.1    | Orphan                    | mthln                         | ACYPi0003439             | Complete       | None predicted             | 1.91E−50 Mth2 Drosophila simulans |
| B16 | KAF6197298.1    | Orphan                    | mthln                         | ACYPi0003439             | Complete       | None predicted             | 3.21E−26 Mth2 Drosophila yakuba |
| B17 | KAF6207182.1    | Orphan                    | mthln                         | ACYPi0003439             | Complete       | None predicted             | 6.91E−21 Probable Mth-like 3 D. melanogaster |

(Continued)
family-B GPCRs can be further subdivided into three subfamilies: B1–B3, which are greatly divergent in both function and structure. In total, 21 family-B GPCRs were identified from the genome of *A. lucorum* in this study, which consisted of nine B1 subfamily members, three B2 subfamily members, and nine B3 subfamily members (Table 3 and Figure 3).

The B1 subfamily is made of largely classical hormone receptors. It comprises three types of hormone receptors in *D. melanogaster*: diuretic hormone 31 receptor (DH31-R/hector), CRF-like diuretic hormone 4 (DH44-R), and pigment dispersing factor receptor (Pdfr) (57–60). In our study, all three types of hormone receptors were identified. The parathyroid hormone receptor (PTHR), which is involved in the calcium and phosphate homeostasis and bone growth in vertebrates, is also a subfamily-B1 GPCR (58). There are two PTHRs in mammals, which are involved in calcium and phosphate homeostasis and bone growth (61, 62). In insects, PTHR-like (PTHRL) have been identified from *T. castaneum*, *A. mellifera*, *P. h. humanus*, and *N. lugens* (18, 26, 57), but its counterparts in *D. melanogaster*, *B. mori*, *A. pisum*, and *A. gambiae* are not found (17, 22, 34, 63). *T. castaneum* has two distinct PTHRLs (57), *N. lugens* possesses a pair of homologous PTHRLs (26), and *A. mellifera* only has one PTHRL (57). In our study, we also identified one PTHRL, B9, which shared a low e-value (1e−104 and 1e−111) with two PTHRLs in *N. lugens*. These results showed that genes coding for PTHR are divergent among insects.

The B2 subfamily is characterized by a long extracellular N-terminal domain and a GPCR proteolytic site (57, 58, 64). Based on phylogenetic analysis and sequence similarity, three receptors (B10–12) were classified in the B2 subfamily, which correspond to a calcium-independent receptor for *α*-latrotoxin (Cirl), starry night (stan), and CG15744, respectively. However, the orthologs for CG11318 and CG15556 were not identified in the genome of *A. lucorum*.

There is only one group of receptors in the B3 subfamily; i.e., Methuselah (mth)/Methuselah-like (mthl) (57, 58). This gene family is involved in the modulation of life span and stress responses. No counterpart for the mth gene family has been identified in vertebrates (57). In insects, the number in the B3

| TABLE 3 | Continued |
|---|---|
| Accession number | Species |
| B18 | KAFF60317.1 |
| B19 | KAFF603244.1 |
| B20 | KAFF603028.1 |
| B21 | KAFF603037.1 |

| TABLE 4 | The number of *A. lucorum* GPCRs of each family in comparison with the other four insects. |
|---|---|---|---|---|---|
| | *A. lucorum* | *A. pisum* | *D. melanogaster* | *T. castaneum* | *B. mori* |
| Family-A | 98 | 62 | 73 | 68 | 89 |
| Opsin | 7 | 5 | 7 | 2 | 6 |
| Biogenic amine receptors | 30 | 18 | 21 | 20 | 16 |
| Neuropeptide and protein hormone receptors (contained the purine GPCRs) | 58 | 39 | 45 | 46 | 47 |
| Family-B | 21 | 10 | 26 | 21 | 12 |
| Family-C | 10 | 7 | 10 | 10 | 9 |
| Family-F | 4 | 3 | 5 | 4 | 3 |
| Total | 133 | 82 | 113 | 103 | 93 |
subfamily is highly variable (18, 57). In *D. melanogaster*, this subfamily can be divided into two groups based on their structure, 12 mth ectodomain-positive members (*mth*, *mthl2*, *mthl3*, *mthl4*, *mthl6*, *mthl7*, *mthl8*, *mthl9*, *mthl10*, *mthl11*, *mthl12*, and *mthl13*) and four mth ectodomain-negative members (*mthl1*, *mthl5*, *mthl14*, and *mthl15*) (65). In our study, nine receptors (B13–21) were identified in this family. Based on phylogenetic analysis, B13 and B20 may belong to the
mth ectodomain-positive group, and others may be members of the mth ectodomain-negative group. At the mRNA level, B13, B18, and B21 showed a higher expression level than other mth members, which indicated these three members of the B3 subfamily may play a more important role in A. lucorum.

3.3 Family-C GPCRs

Family-C GPCRs possess a large ligand-binding extracellular domain and form constitutive dimers (34, 66–68). There are three types of GPCRs in family-C, the glutamate and γ-amino butyric acid (GABA-B) receptors, the bride of sevenless (boss-type) receptors, and the metabotropic glutamate (mGlu) receptors. Until now, nine family-C GPCRs from D. melanogaster and seven from A. pism have been reported. By using these reference sequences, 10 family-C members (Table S1 and Figure S2) of A. lucorum were identified here.

Like A. pism (22), there are two GABA-B receptors (C1 and C2) in A. lucorum. C1 shares a 78% sequence similarity with D. melanogaster GABA-B-R1, while C2 has a 44% sequence identity with D. melanogaster GABA-B-R2. The orthologous gene to D. melanogaster GABA-B-R3 has not been found in two Hemiptera insects. The boss-type receptor was first identified as a ligand for sevenless tyrosine kinase, which was involved in eye differentiation in D. melanogaster. Subsequently, boss has been implicated in the glucose-response (69, 70). It has been reported in D. melanogaster (34), A. gambiae (63), A. pism (22), and T. castaneum (16), but not in B. mori (17), A. mellifera, N. vitripennis, and P. humanus corporis. Here, C3 was identified as the orthologous gene to boss. These results indicated that boss has been randomly lost in insects during their evolutionary process. Moreover, three mGlu receptors (C4, C5, and C6) were found in A. lucorum, whereas there are only two mGlu receptors in D. melanogaster (34) and one mGlu receptor in A. pism (22). Small expansions of A. lucorum mGlu receptors have been observed. There are some unclassified receptors in this family. C7 was the orthologous gene to smog. C8 and C9 showed 53 and 38% sequence identities with their counterparts in D. melanogaster, respectively. As shown in Figure S2, C9 showed a high expressed level in egg, adult head, and all nymph tissues, except leg, while the function of its orthologous gene is unclear. C10 was an orphan receptor that has not been reported in D. melanogaster and A. pism (22, 34).

3.4 Family-F GPCRs

Family-F GPCRs comprise the frizzled gene family and the smoothened gene (34, 71). In this study, four putative A. lucorum GPCRs were identified in the frizzled/smoothened GPCR family, which are orthologous to D. melanogaster fz, fz2, fz3, and smo (Table S1 and Figure S3). Our results indicated that orthologs for D. melanogaster fz4 were missing in A. lucorum. Among family-F, F4 (smo) showed the highest expression in the egg with a TPM of 61.

4 DISCUSSION

In this study, we systematically identified 133 GPCRs from A. lucorum. Compared with other model insects, we also found the GPCR genes remarkably expanded among the biogenic amine receptors, neuropeptide and protein hormone receptors, and the B1 subfamily (Table 4). Some of them had been reported in R. prolitus or C. lectularius (24, 27), such as the duplications of 5-HT7, Lkr, PK1-R, ETHR, CCAP-R, NPFR, SIFaR, and DH31-R. However, missing TyrR, TreI, TrissinR, MsR, and some orphan receptors has also been observed in the genome of A. lucorum (Tables S2, S3). All these predicted GPCRs were quantified by transcriptome data. Although most GPCR genes showed a low expression level in A. lucorum, there were a few highly expressed GPCR genes, such as the long-wavelength opsin and Cirl. By comparative analysis, we also found C2 LGR types were widely distributed in Hemiptera. All these aspects will be discussed in detail below.

4.1 GPCRs Gene Expansion Occurred in A. lucorum

Compared with other well-studied insects, we noticed that the number of genes coding for GPCRs is obviously larger than for other insects, especially expanded among the biogenic amine
receptors, neuropeptide and protein hormone receptors, and B1 subfamily (Table 4). There were 26 GPCRs duplicated in *A. lucorum*. Twenty-three of them were classified into the three subfamilies mentioned above. By MCScanX analysis, we found six tandem duplication events occurred among Rh6, Rh7, AstA-R, ETHR, FMRFaR, and AdoR, while most GPCR genes duplicated dispersely. Considering the location of GPCR genes on chromosomes, it suggested that the duplication of GPCR genes mainly occurred as independent duplications and transitions (Figure 4).

The duplicate biogenic amine receptors in *A. lucorum* included 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B, 5-HT7, Dop2R, mAChR-B, Oamb, Octbeta2R, and Octbeta3R. These biogenic amine receptors can regulate many behaviors including flight and fight, learning and memory, sleep and wakefulness, feeding, and social and reproductive behaviors (72–74). For example, 5-HT1A was related to locomotor activity in *B. mori*. Injecting the antagonist of the Bm5-HT1A receptor into larvae caused slow or weak motility, and adults had lowered courtship vitality or moving speed (75). mAChRs have also been reported to be critical in regulation of locomotory behavior in *Drosophila* (76). In addition, 5-HT1B mediates hemocyte phagocytosis and serotonergic signaling performs critical modulatory functions in immune systems. Moreover, 5-HT7 and Dop2R were shown...
to be associated with learning ability (77, 78) and octopamine receptors were required for ovulation in D. melanogaster (79). The ligands of neuropeptide and protein hormone receptors and the B1 subfamily belong to neuropeptides, which also play an important role in the regulation of development, reproduction, feeding, courtship, aggression, olfaction, locomotor activity, circadian rhythm, and many other physiological processes in insects (21, 80, 81). The gene expansion of these three subfamilies indicated that A. lucorum had a more complex peptidergic signaling system.

Expansions of genes associated with omnivorousness and mesophyll feeding, such as those related to digestion, chemosensory perception, and detoxification, were also observed in A. lucorum (32). Gustatory receptors (Grs) and odorant receptors (Ors) are thought to be the most important chemosensory receptors. Like GPCRs, Ors, and Grs are seven-transmembrane domain receptors but belong to the chemosensory 7tm receptor superfamily (82, 83). It has been suggested that the cause of gene expansion in GPCRs might be similar to that of chemosensory receptors, also to better adapt to the environment (32). A. lucorum is found in natural and agricultural ecosystems throughout the world (30), and many of them are generalists, exhibiting diverse feeding habits or preferences (e.g., feeding on leaf, stem, inflorescences, nectar, pollen, and fruit) (32). These results indicated the complex peptidergic signaling system is more favorable for A. lucorum to adapt to multiple living environments and multiple hosts.
4.2 A. lucorum Appears to Have Evolved From a Novel Large Clade of Known FMRFaRs

FMRFamide (FMRFa) is a cardioexcitatory peptide that was first isolated from the nervous system of the clam, *Macaraster nimbosa* (84), and is active as a tetrapeptide only in mollusks and annelids. Since the discovery of FMRFa, peptides with extended length at the N-terminal portion have been reported, such as myosuppressin (Ms) (85). Here, nine receptors (A54–62) displayed a certainly evolutionary kinship with the FMRFaR of *D. melanogaster*, *A. pisum*, *C. lectularius*, and *R. prolixus*, while the MsR is missing in *A. lucorum* (Figure 2). By reconstruction of the phylogenetic tree of MsR and FMRFaR with more species (17, 86), we found these receptors were closer to the known FMRFaRs (Figure 7). Among these, A54 and A55 were clustered in a single clade with the FMRFaRs that had been identified in other insects, whereas the other seven receptors are clustered on the other single clade. The most similar proteins in UniProtKB/Swiss-Prot of these receptors are both *D. melanogaster* FMRFaRs (CG2114). Among these receptors, A54 was the ortholog to the insect FMRFaRs with the smallest e-value of 1.75E−130, and A55, which was adjacent located on chromosome 9, is a tandem duplication that occurred at the beginning of this receptor expansion. However, the other seven receptors (A56–62) were scattered across six chromosomes, which indicated these seven receptors might have arisen from transposition.

By searching in the genome of other heteropteran insects, we found there are only one or two FMRFaRs in each heteropteran species. We suggest A54 and A55 should be classified as FMRFaRs, while the others (A56–62) were named as FMRFaR-like for the moment. This branch might be another unknown GPCR or even contain the MsR. Recent research had found that FMRFaR stimulates intracellular calcium signaling through the IP3R and helps maintain neuronal excitability in a subset of dopaminergic neurons for positive modulation of LW opsin (95). Most opsin genes are expressed in photoreceptors, but there are opsins expressed in other tissues, suggesting some nonvisual functions (96, 97). In *A. lucorum*, opsin genes (A1–3) were expressed highly not only in head but also in leg, wing, and mouthpart, indicating these opsins may execute some nonvisual functions (Figure 1). Among these, the LW opsin (A1) showed the highest expression levels in the head tissue of adults (TPM = 28,787, at least 20 times more than other GPCR; Figure 5). The peak absorbance of the LW opsin is 500–600 nm, which corresponds to yellow-green light. As night sets in, the natural ambient light is increasingly dominated by longer wavelengths (98, 99). The importance of LW opsin had been reported in many nocturnal insects (100, 101). The adults of the *A. lucorum* were mainly active from dusk to early morning (102). High expression levels of LW opsin may help the organism adapt to a low light environment. We found B10, orthologous to Cirl, is the most widely expressed GPCR gene, which can be tested in all tissues (Figure 5). Cirl belongs to a unique branch of GPCRs and, specifically, is an adhesion GPCR (103, 104). The orthologs of Cirl have been discovered in almost all animals from invertebrates to vertebrates, including humans (105). There are three homologs of Cirl in most vertebrates (Cirl-1, Cirl-2, and Cirl-3) and two in birds and worms, whereas there is only one homolog in insects—which is most homologous to vertebrate Cirl-2 (103, 106). The expression pattern of insects *Cirl*, which had been reported to be expressed in multiple tissues (103, 107), was also like vertebrate *Cirl-2* (108). Although there is only one *Cirl* member in insect species, *Cirl* is still involved in multiple physiological processes, which can regulate sensory, developmental, reproductive, and immune functions in insects (104, 109). Here, B10 had been detected in all transcriptomic samples and its distribution range is wider than in *T. castaneum* and *D. melanogaster* (103, 106). This kind of expression pattern suggested B10 is crucial in the development of *A. lucorum*.

4.4 Type C2 LGRs Are Mainly Distributed in Hemiptera and Phthiraptera Insects

Within the neuropeptide and protein hormone receptor subfamily, LGRs are a distinct subgroup with important functions in development and reproduction (110). Three distinct types of LGRs have been defined based on their structural characteristics and they are distinguished by the number of leucine-rich repeat (LRR) motifs, the absence or presence of a low density lipoprotein receptor domain class A (LDLa) motif, and their type-specific hinge region. Generally, type B LGRs have about twice the number of LRRs compared to the other two types. An exclusive feature of the type C LGRs is the presence of at least one LDLa motif in the ectodomain. The more general type containing only one LDLa will be referred to as type C1, whereas type C2 contained more than one LDLa (111). Type C2 LGRs were first discovered in echinoderms, mollusks, and in one insect species (*Pediculus humanis corporis*). In our study, we found it existed in all hemipteran insects that we studied. Combining recent work, we mentioned that type C2 LGRs are reported in many hemipteran insects and *P. h. humanus* (18, 23, 25, 26), and are lost in other orders of insects (16, 17, 63, 86) (Figure 6). Until now, the presence of type C2 LGRs have been found in all Hemiptera insects in which their GPCRs have been identified.
Among insects, Phthiraptera is one of the orders most closely related to Hemiptera (112). Type C2 LGRs may be present in the common ancestor of these two orders. To clarify the distribution of type C2 LGRs in insects, we checked all protein sequences in the non-redundant protein sequences database (nr) of NCBI. The result showed, except for Hemiptera and Phthiraptera, type C2 LGRs were also found in Zootermopsis nevadensis of Blattodea. In terms of functionality, LGRs have important functions like peptide 8 and they coordinate organ growth in type C1 LGRs are the receptors of insulin-like peptide 7 and insulin-type C2 LGRs in Phthiraptera need to be explored in future work. The function of type C2 LGRs and the existence of type C2 LGRs were also founded in NCBI. The result showed, except for Hemiptera and Phthiraptera, sequences in the non-redundant protein sequences database (nr) of most closely related to Hemiptera (112). Type C2 LGRs may be further explored in future work.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

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**AUTHOR CONTRIBUTIONS**

The majority of the work described here was carried out by HG. This work was also assisted by YL, MW, XS, JT, and FF. BL designed the study and crucially revised the manuscript for important intellectual content and data analysis. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

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