Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(6): 296-301

Received: 04-09-2019
Accepted: 08-10-2019

Kyaw Lin Maung
(a) State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China
(b) Biotechnology Research Department, Department of Research and Innovation, Ministry of Education (Science and Technology), Kyaukse, Myanmar

Dapeng Jing
College of Plant Protection, Shenyang Agricultural University, Shenyang 110161, China

Tiantao Zhang
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Kanglai He
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Shuxiao Bai
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Zhenying Wang
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Corresponding Author:
Zhenying Wang
State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Structural characteristics of niemann-pick type C2 proteins in *Macrocentrus cingulum* and similarities with olfactory proteins in hymenoptera

Kyaw Lin Maung, Dapeng Jing, Tiantao Zhang, Kanglai He, Shuxiao Bai and Zhenying Wang

Abstract
Niemann Pick type C2 (NPC2) proteins were identified as olfactory related genes such as odorant carriers in arthropods. NPC2 in *Macrocentrus cingulum*'s functional characteristics are similar to odorant binding proteins. Recently, we identified NPC2 protein in *Macrocentrus cingulum* (namely McinNPC2) as a Genbank accession number MK089532.1 at National Center for Biotechnology Information. Comparative evolutionary relationship and primary structures between NPC2 and olfactory proteins such as odorant binding proteins, general odorant binding proteins, pheromone binding proteins (OBPs, GOBPs, PBPs respectively) were performed. Evolutionary relationship results between NPC2 in *Ceratosolen solmsi marchali* (CsolNPC2) and OBP in *Microplitis mediator* (MmedOBP), NPC2 in *Trachymyrmex septentrionalis* (TsepNPC2) and GOBP in *Polistes Canadensis* (PcanGOBP), NPC2 in *Dufourea novaeangliae* (DnovNPC2) and PBP in *Apis cerana* (AcerPBP) were clustered forming one clade as a nearest phylogeny. The identity of CsolNPC2 with MmedOBP, TsepNPC2 and PcanGOBP, DnovNPC2 and AcerPBP are 13.21%, 17.36%, 17.56 % respectively with the similar residues 50.31%, 47.92%, 41.98% respectively. Representative NPC2 alignments in hymenoptera results showed that 6 cysteine consensus amino acids while showing the same 6 cysteine amino acids in an alignment of representative OBPs, GOBPs and PBPs alignments. Unexpectedly, we observed that secondary structure prediction between McinNPC2 and McinOBP1, McinOBP2, McinOBP3, McinOBP4 showed different shade of subphyla. In conclusion, our results reveal that structural characteristics of NPC2 proteins are similar to OBPs, GOBPs and PBPs in hymenoptera.

Keywords: Structural characteristics, *Macrocentrus cingulum*, similarities, hymenoptera

1. Introduction
The NPC2 is the cholesterol binding protein that binds cholesterol with submicromolar affinity at neutral and acidic pH [1]. The function of Niemann–Pick type C2 protein (NPC2) from the antenna of the worker Japanese carpenter ant, *Camponotus japonicus* (CjapNPC2) indicate that it plays crucial roles in chemical communication [2]. The function of a specific NPC2 gene in the moth *Helicoverpa armigera* (HarmNPC2-1) shows that NPC2 proteins support the role of semiochemical carriers [3]. NPC2 in *Pardosa pseudoannulata* are identified as olfactory related genes by transcriptome and expression profile analysis showing putative role of odorant carriers [4].
Odorant binding proteins (OBPs) in *Microplitis mediator* (Hymenoptera: Braconidae) provide insight into the chemosensory functions as Odorant binding proteins (OBPs) are believed to be important for transporting semiochemicals [5]. The suitability of OBPs and odorant receptors (ORs) as pest control targets and their selection toward the discovery of new potent semiochemicals [6]. In *Chilo suppressalis*, pheromone binding proteins (PBPs) enhance the sensitivity of olfactory receptors to sex pheromones even for the relatively simple as of detecting sex pheromones [7]. Hymenoptera is a wide range of insects containing more than 150000 living insects that includes sawflies, wasps, bees and ants [8, 9].
In *Batocera horsfieldi* (Hope), the structure of OBPs show the two disulfide bonds similarities with classic OBPsAgamOBP1 and CquiOBP1, then intermediate structure in evolution of OBPs found in BhorOBPm2 [10]. Most of the predicted structure of PBP/GOBP genes in moths and butterflies showed the chemosensory-based behavior with the additional genomic data of

the lepidoptera [11]. The three dimensional structure of OBPs from the antennae of *bombyx mori* suggests size and shape which vary each other with the three loops that rich content of helix [12]. The predictions of secondary structure of the CSP suggest that two 5-helical CSPs and one 6-helical CSP [13]. The evolutionary relationship of OBPs emerged independently with olfactory receptor (OR) and expanded in the terrestrial insect [14].

In this research, we mainly focused on the structural characterization of McinNPC2 and the structural similarities between NPC2 proteins and olfactory-proteins (OBPs, GOBPs and PBPs) in hymenoptera species. We recently identified McinNPC2, evolutionary relationships were monitored, identity and similar residues were examined, alignments with 6 cysteine structures were compared and the comparative predictions of secondary structure were conducted and the primary and secondary structures were performed. Thus our results revealed that NPC2 Protein's structural characterizations are similar to olfactory proteins as OBPs, GOBPs and PBPs in hymenoptera species.

2. Materials and Methods

2.1 Identification of McinNPC2

Molecular identification of McinNPC2 was conducted by Rapid Amplification cDNA Ends (RACE) technique. Rearing of larval parasitoids, *Macrocentrus cingulum* were carried out, then RNA extraction and cDNA synthesis were performed. The full length sequence of NPC2 proteins in *M. cingulum* were identified by RACE technology. The resulting nucleotide sequence and amino acid sequence was submitted to National Center of Biotechnology Information https://www.ncbi.nlm.nih.gov/nuccore.

2.2 Evolutionary relation NPC2 and olfactory proteins (OBPs, GOBPs, PBPs)

Evolutionary relation of NPC2 and olfactory proteins were assessed by phylogenetic analysis. Phylogenetic tree of NPC2 and olfactory proteins (OBPs, GOBPs, and PBPs) in hymenoptera species was constructed with MEGA-X software using the Maximum-Likehood method based on the JTT matrix-based model [1]. The analysis involved 162 amino acid sequences from the NCBI database https://www.ncbi.nlm.nih.gov/nuccore.

2.3 Alignment of representative NPC2 in hymenoptera

The alignments of representative NPC2 in hymenoptera were examined with DNAMANN 8 software (https://en.bio-soft.net/format/DNAMAN.html) using multiple sequence alignment bases on full alignment methods. Amino acid sequence from the representative hymenoptera species (*Apis mellifera, Nasonia vitripennis, Bombus terrestris, Apis florea, Eufriesea Mexicana, Pardosa pseudoannulata, Camponotus japonicas, Microplitis demolitor, Atta colombica, Fopius arisanus, Neodiprion lecontei, Dufourea novaeangliae, Polistes dominula, Polistes canadensis, Dinoponera quadriceps*) were used from the NCBI database.

2.4 Alignment of OBPs, GOBPs and PBPs in hymenoptera

The representative amino sequence of OBPs, GOBPs and PBPs were selected from the phylogenetic results of the nearest evolutionary relation with McinNPC2. DNAMANN 8 software (https://en.bio-soft.net/format/DNAMAN.html) was used with the multiple sequence alignment bases on full alignment methods. All the amino acid sequence of OBPs, GOBPs, PBPs which is nearest clade of phylogenetic tree are CcosPBP (*Cyphomyrmex costatus*), DallPBP (*Diachasma alloeum*), DnovPBP (*Dufourea novaeangliae)*, FariGOBP (*Fopius arisanus*), FariPBP *(Fopius arisanus)*, HlabGOBP (*Habropoda laboriosa*), MmedPBP (*Microplitis mediator*), MphaPBP (*Monomorium pharaonis*), NlecGOBP (*Neodiprion lecontei*), NvitGOBP (*Nasonia vitripennis*), NvitOBP (*Nasonia vitripennis*), NvitPBP (*Nasonia vitripennis*), OabiPBP (*Orussus abietinus*), PdomGOBP (*Polistes dominula*), TpreGOBP (*Trichogramma pretiosum*) and VemePBP (*Vollenhovia emeryi*) that are used from the NCBI database.

2.5 Structural similarities NPC2 and olfactory proteins (OBPs, GOBPs and PBPs)

The three pair of proteins forming one clade [(1) CsolNPC2 and MmedOBP, (2) TsepNPC2 and PcanGOBP (3) DnovNPC2 and AcerPBP] were selected to perform structural similarities. Identity, similar residue and gaps were conducted with DNAMANN 8 software (https://en.bio-soft.net/format/DNAMAN.html) using two sequence alignment bases on full alignment methods.

2.6 Comparative three dimensional structures between NPC2 and OBPs in *M. cingulum*

McinNPC2, McinOBP1, McinOBP2, McinOBP3 and McinOBP4 that are different proteins and same species of *M. cingulum* were selected to compare the three dimensional structure. The perdition of three dimensional secondary structures of McinNPC2 McinOBP1, McinOBP2, McinOBP3 and McinOBP4 were constructed with Swiss Models https://swissmodel.expasy.org/interactive and edited by Microsoft 10.

3. Results

3.1 Identification of McinNPC2

Niemann-Pick C2 protein in M. cingulum was identified as Genbank accession number MK089532.1 at National Center of Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/nuccore.

3.2 Phylogenetic analysis of NPC2 proteins and olfactory proteins

The three pairs of NPC2 and olfactory proteins such as [(1) CsolNPC2 and MmedOBP, (2) TsepNPC2 and PcanGOBP (3) DnovNPC2 and AcerPBP] were clustered one clade forming as 100% (Fig. 1). McinNPC2 proteins formed near evolution with olfactory proteins such as CcosPBP (*Cyphomyrmex costatus*), DallPBP (*Diachasma alloeum*), DnovPBP (*Dufourea novaeangliae)*, FariGOBP *(Fopius arisanus)*, FariPBP *(Fopius arisanus)*, HlabGOBP *(Habropoda laboriosa)*, MmedPBP *(Microplitis mediator)*, MphaPBP *(Monomorium pharaonis)*, NlecGOBP *(Neodiprion lecontei)*, NvitGOBP *(Nasonia vitripennis)*, NvitOBP *(Nasonia vitripennis)*, NvitPBP *(Nasonia vitripennis)*, OabiPBP *(Orussus abietinus)*, PdomGOBP *(Polistes dominula)*, TpreGOBP *(Trichogramma pretiosum)* and VemePBP *(Vollenhovia emeryi*) in Fig. 1.

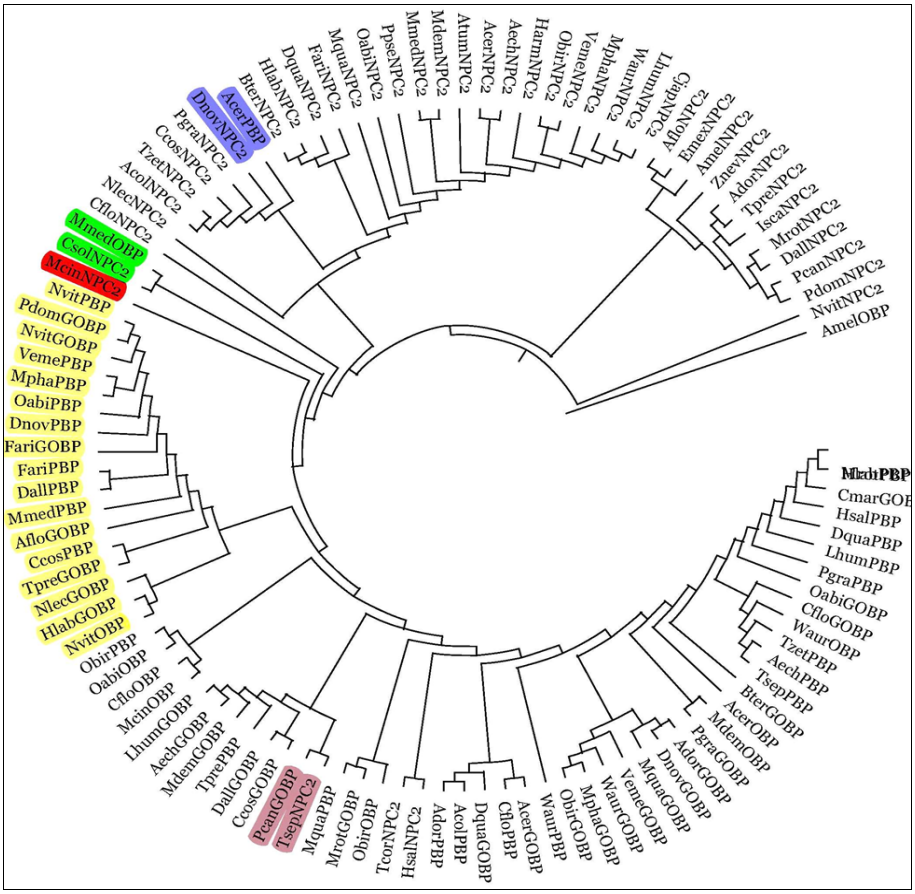

Fig 1: Evolutionary phylogenetic analysis of all candidates NPC2, OBPs, GOBPs and PBPs in hymenoptera species. Green color represents NPC2 and OBP clustered one clade, Brown color represents NPC2 and GOBP clustered one clade, Blue color represent NPC2 and PBP clustered one clade.

3.3 An alignment of NPC2 proteins in hymenoptera

The representative alignment of NPC2 in hymenoptera species such as *(Apis mellifera, Nasonia vitripennis, Bombus terrestris, Apis florea, Eufriesea Mexicana, Pardosa pseudoannulata, Camponotus japonicas, Microplitis demolitor, Atta colombica, Fopius arisanus, Neodiprion lecontei, Dufourea novaeangliae, Polistes dominula, Polistes Canadensis, Dinoponera quadriceps)* showed that the 6 Cysteine consensus sequence and 1 Proline that are shown in Fig. 2.

Fig 2: Amino acid sequences alignment of representative NPC2 protein in hymenoptera was constructed with DNAMANN 8 software using multiple sequence alignment bases on full alignment methods. The red colors represent the consensus sequence of 6 Cysteine and 1 Proline.

3.4 An alignment of olfactory proteins in hymenoptera

The alignment of selected OBPs, GOBPs, PBPs in hymenoptera species such as CcosPBP (*Cyphomyrmex costatus*), DallPBP (*Diachasma alloeum*), DnovPBP (*Dufourea novaeangliae)*, FariGOBP (*Fopius arisanus*), FariPBP (*Fopius arisanus*), HlabGOBP (*Habropoda laboriosa)*, MmedPBP (*Microplitis mediator*), MphaPBP (*Monomorium pharaonis*), NlecGOBP (*Neodiprion lecontei*), NvitGOBP *(Nasonia vitripennis)*, NvitOBP *(Nasonia vitripennis)*, NvitPBP *(Nasonia vitripennis)*, OabiPBP (*Orussus abietinus*), PdomGOBP *(Polistes dominula)*, TpreGOBP (*Trichogramma pretiosum*) and VemePBP (*Vollenhovia emeryi*) were indicated that the 6 Cysteine consensus sequence (Fig. 3).

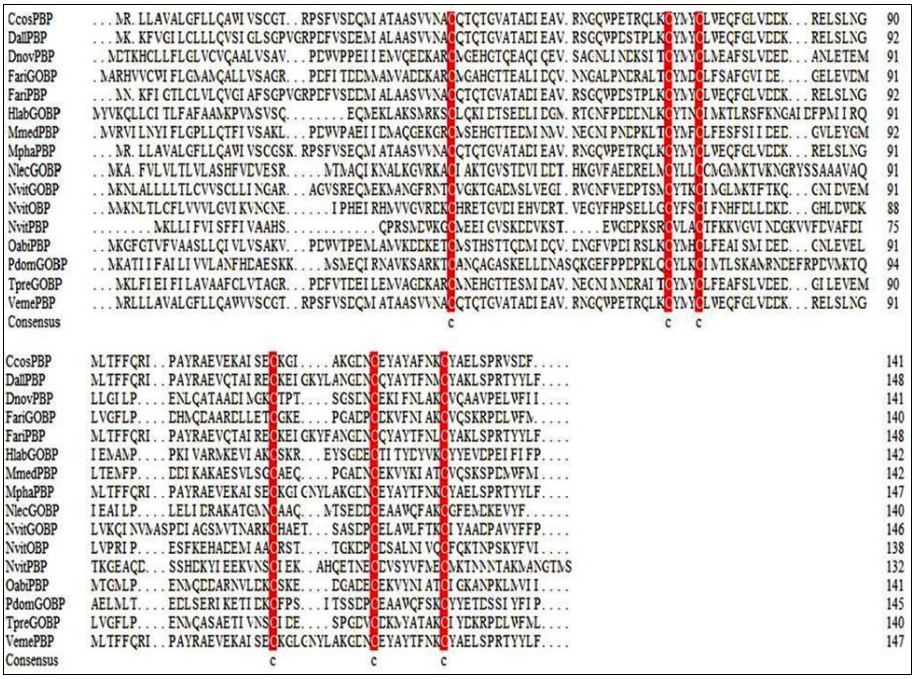

Fig 3: Amino acid sequences alignment of olfactory proteins (OBPs, GOBPs, PBPs) in hymenoptera was constructed with DNAMANN 8 software using multiple sequence alignment base on full alignment methods. The representative olfactory proteins were selected as the results of nearest phylogeny. The red colors align represent the consensus sequence as 6 Cysteine.

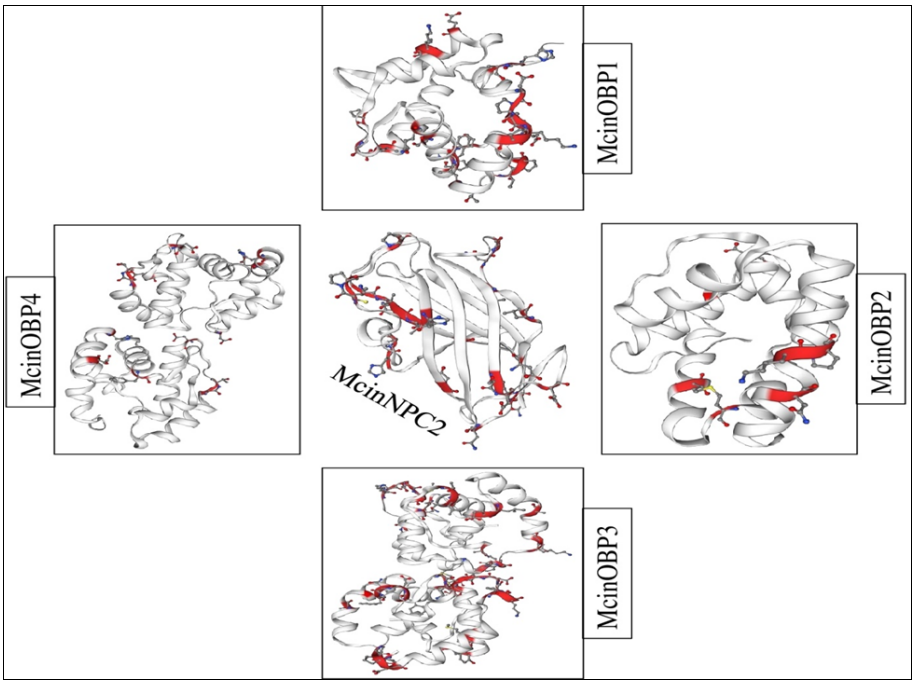

Fig 4: Computational perdition of three dimensional structures comparative analysis between NPC2 and OBP1, OBP2, OBP3, OBP4 in *M. cingulum* was constructed by online software Swiss Models https://swissmodel.expasy.org/interactive.

3.5 Structural similarities of NPC2 proteins and olfactory proteins in hymenoptera

The identity and similar residues of NPC2 and OBP, GOBP, PBP of the selected species were performed as the identity of CsolNPC2 with MmedOBP, TsepNPC2 and PcanGOBP, DnovNPC2 and AcerPBP are 13.21%, 17.36%, 17.56 % respectively with the similar residues 50.31%, 47.92%, 41.98% respectively (Table 1).

Table 1: Structural Similarities between NPC2 proteins and olfactory proteins (OBP, GOBP, PBP) in hymenoptera (identity, similar residues and gaps).

Proteins	Identity	Similar Residues	Gaps
CsolNPC2 and MmedOBP	13.21%(21/159)	50.31%(80/159)	25.00%(53/212)
TsepNPC2 and PcanGOBP	17.36%(25/144)	47.92%(69/144)	9.43%(15/159)
DnovNPC2 and AcerPBP	17.56%(23/131)	41.98%(55/131)	17.09%(27/158)

4. Discussion

Because of the relationship between structure and function, the function of OBPs is similar to chemosensory protein (CSPs) as insecticide resistance anti-inflammatory action and egg shell formation in haematophagous insects due to similar structures [14]. NPC2 protein is an olfactory related protein and NPC2 protein's functional characteristics are similar to odorant binding protein [4]. Thus, NPC2 proteins and olfactory proteins can find the structural similarities because of similar functional similarities in some species.

The soluble olfactory proteins such as OBPs, CSPs and NPC2 proteins have sensing devices for odors with their compact structures, soluble nature and small size [15]. We suggest that NPC2 proteins and some olfactory proteins have similar shape, size and nature in some hymenoptera species because of their sensing devices. PBPs and pheromone receptors (PRs) paring suggested the complexity of olfactory system as a simple task of pheromones with a highly sophisticated combinatorial approach [16]. We selected OBPs, GOBPs and PBPs together as the olfactory proteins with the complexity of olfactory approach.

The structure of AaegOBP1 (*Aedes aegyp*ti) indicated that the common fold of OBPs with six-alpha helices [17]. In this research, the perdition of three dimensional structures of OBPs in *M. cingulum* suggested that OBPs have six-alpha helices and more. AlinOBP14, AlinOBP15, AlinOBP16, AlinOBP17 displayed six highly conserved Cysteine while forming the classic OBP subfamily [18]. We found that some olfactory proteins (OBPs, GOBPs and PBPs) have highly conserved six-Cysteine. The role of OBPs and CSPs in insects performed the classes of soluble proteins and also lipid-transporter proteins, NPC2 in representatives' species of different arthropods [19]. The amino acid composition with complete database basically determine the overall folding of a protein (alpha helices, beta sheets) that can predict the structural class of a protein [20]. The identity and similar residues of amino acid composition between the two proteins determine the structural similarities of overall proteins.

5. Conclusions

Structural characteristics of NPC2 are similar to olfactory proteins (OBPs, GOBPs, PBPs) in hymenoptera species. Because of structure and function relationships, NPC2 proteins and olfactory proteins have similarities such as identity, similar residues, 6-cysteine structures even the difference of three dimensional structures. Prediction of three dimensional secondary structures can perform different shape of alpha helices and beta sheets. Therefore NPC2 is the olfactory-related protein because of their similarities of structures with olfactory proteins (OBPs, GOBPs, PBPs) even it is the cholesterol-binding proteins.

6. References

1. Vanier MT, Millat G. Structure and function of the NPC2 protein. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2004; 1685(1-3):14-21.
2. Ishida Y *et al*. Niemann–Pick type C2 protein mediating chemical communication in the worker ant. Proceeding of the National Academy of Sciences. 2014; 111:3847-3852.
3. Zhu J, Guo M, Ban L, Song L, Liu Y, Pelosi P, Wang G. Niemann-Pick C2 proteins: A new function for an old family, Frontiers in Physiology. 2018; 9:52.
4. Xiu C, Xiao Y, Zhang S, Bao H, Liu Z, Zhang Y. Niemann-Pick proteins type C2 are identified as olfactory related genes of *Pardosa pseudoannulata* by transcriptome and expression profile analysis. Comparative Biochemistry and Physiology - Part D. 2019; 29:320-329.
5. Li K *et al*. Odorant binding characteristics of three recombinant odorant binding proteins in *Microplitis mediator* (Hymenoptera: Braconidae). Journal of Chemical Ecology. 2014; 10:1007. https://doi 10.1007/s10886-014-0458-5
6. Venthur H, Zhou JJ. Odorant receptors and odorant-binding proteins as insect pest control targets: a comparative analysis. Frontiers in Physiology. 2018; 9:1163.
7. Chang H, Liu Y, Yang T, Pelosi P, Dong S, Wang G. Pheromone binding proteins enhance the sensitivity of olfactory receptors to sex pheromones in *Chilo suppressalis*. Scientific Reports. 2015; 5:13093.
8. Mayhew, Peter J. Why are there so many insect species? Perspectives from fossils and phylogenies. Biological Reviews. 2007; 82(3):425-454.
9. Axel J, Seraina K, Lars V, John MH, Michael S, Fredrik R. The hymenopteran tree of life: evidence from protein-coding genes and objectively aligned ribosomal data. PLoS ONE. 2013; 8(8):e69344.
10. Zheng ZH, Li DZ, Zhou A, Yi SC, Liu H, Wang MQ. Predicted structure of a Minus-C OBP from *Batocera horsfieldi* (Hope) suggests an intermediate structure in evolution of OBPs. Scientific Reports. 2016; 6:33981.
11. Vogt RG *et al*. The lepidoptera odorant binding protein gene family: gene gain and loss within the GOBP/PBP complex of moths and butterflies. Insect Biochemistry and Molecular Biology. 2015; 3(003):1-12. http://dx.doi.org/10.1016/j.ibmb.2015.03.003
12. Scaloni A, Monti M, Angeli S, Pelosi P. Structural analysis and disulfide-bridge pairing of two odorant-binding proteins from *Bombyx mori*. Biochemical and Biophysical Research Communications. 1999; 266:386-391.
13. Missbach C, Vogel H, Hansson BS, Wilde EG. Identification of odorant binding proteins and chemosensory proteins in antennal transcriptomes of the jumping bristletail *lepismachilis y-signata* and the firebrat *Thermobia domestica*: evidence for an independent OBP–OR origin. Chemical Senses. 2015; 40:615-626.
14. Pelosi P, Iovinella I, Zhu J, Wang G, Dani FR. Beyond chemoreception: diverse tasks of soluble olfactory proteins in insects. Biological Reiew. 2017; 000-000.

https://doi: 10.1111/brv.12339
15. Pelosi P, Zhu J, Knoll W. Odorant-binding proteins as sensing elements for odour monitoring. Sensors MDPI. 2018; 18:3248.
16. Chang H, Liu Y, Yang T, Pelosi P, Dong S, Wang G. Pheromone binding proteins enhance the sensitivity of olfactory receptors to sex pheromones in *Chilo suppressalis*. Scientific Reports. 2015; 5:13093.
17. Leite NR *et al*. Structure of an odorant-binding protein from the mosquito *Aedes aegypti* suggests a binding pocket covered by a pH-sensitive Lid. PLoS ONE. 2009; 4(11):e8006.
18. Sun L, Wang Q, Wang Q, Dong K, Xiao Y, Zhang YJ. Identification and characterization of odorant binding proteins in the forelegs of *Adelphocoris lineolatus* (Goeze). Frontiers in Physiology. 2017; 8:735.
19. Pelosi P, Iovinella I, Felicioli A, Dani FR. Soluble proteins of chemical communication: an overview across arthropods. Frontiers in Physiology. 2014; 5:320(1).
20. Chou KC, Zhang CT. Prediction of Protein Structural Classes. Critical Reviews in Biochemistry and Molecular Biology. 1995; 30(4):275-349.