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Phylogenetic analyses of the proteins involved in encapsulation signaling pathways in ants

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Abstract. One of the major evolutionary transitions is the shift from solitary to social lifestyle, which involved a plethora of behavioral and physiological changes in social entities. Group living has several advantages as the evolution of collective defense mechanisms. It may also affect the individual immune system either due to the efficiency of social immune defenses or because of the high transmission frequency of pathogens. Individual defense consists of the innate and acquired immune components. In insects, there are two signaling pathways (Toll and Jak/Stat) that result in the expression of specific immune genes, which, in their turn, encode peptides, proteins and activate innate immune responses like encapsulation. The main aim of our study was to verify whether transition to eusocial lifestyle is reflected in proteins involved in immune responses. We carried out phylogenetic analyses of 15 proteins involved in encapsulation signaling pathways in ants. We also included four other social insect groups, bees, sweat bees, social wasps, and termites, and three solitary insect groups, as fruit flies, braconid wasps, and megachilid bees. Ants grouped separately from other insect groups in most cases, however, there were some notable exceptions mostly in the case of pattern recognition proteins, probably correlating

with differences in potential pathogens. No major differences were revealed though between solitary and social insects with respect to proteins involved in encapsulation.

Keywords: eusociality, evolution, immune response, innate immunity, insects, social immunity

Introduction

Multicellular organisms are continuously exposed to different pathogens, like viruses, bacteria, fungi, which can harm the host, may cause behavioural, morphological, and functional changes, or even lead to its death (Schmid-Hempel, 1998; Verble *et al.,* 2012; Csata *et al.,* 2014; Hughes *et al.,* 2016; Csata *et al.,* 2017a, Csata *et al.,* 2017b; Csata *et al.,* 2018; Csősz *et al.,* 2021; Csata et al. 2023). In animals, the innate (non-specific) and acquired immune systems are employed internally against pathogens (Schmidt, 2001). The innate immune response is short-term, general, and inhibits the spread of pathogens within the host's body through physical and chemical defense mechanisms (Cotter and Kilner, 2010). On the other hand, the acquired immune response is long-term, highly specialized, and is based on immune memory since after being challenged by specific pathogens, the organism can recognize these for a longer period (Janeway *et al.,* 2001). While in vertebrates both innate and acquired immune systems are present, invertebrates are considered to lack entirely the acquired component. There are though some notable exceptions as is the case of the *Anopheles gambiae* mosquito (Yan *et al.,* 1997; Kurtz and Armitage, 2006), the fruit fly *Drosophila melanogaster* (Flemming, 2017), and the ant *Lasius neglectus* (Konrad *et al.,* 2012), where a certain degree of immune priming had been shown.

In insects, the innate immune system is made up of physical barriers, cellular and humoral responses (Rosales, 2017). If pathogens or parasites pass the physical barrier (cuticle, peritrophic membrane) then the cellular and humoral immune responses are activated. The cellular immune responses include: phagocytosis, nodulation, coagulation, melanization, and encapsulation. The humoral immune responses are related to some pattern recognition proteins (PRPs), which after the invasion recognize the pathogen-associated molecular patterns (PAMPs) and thus activate immune signaling pathways

(Toll, Imd, Jak/Stat). The activated pathways elicit the synthesis of antifungal and/or antibacterial peptides in the fat bodies, and these peptides will be emitted into the hemolymph (Dubovskiy *et al.,* 2016). Gram-positive bacteria and fungi activate the Toll pathway, Gram-negative bacteria the Imd pathway, while stress/injury activates the Jak/Stat pathway (Broderick *et al.,* 2009; Fig. 1).

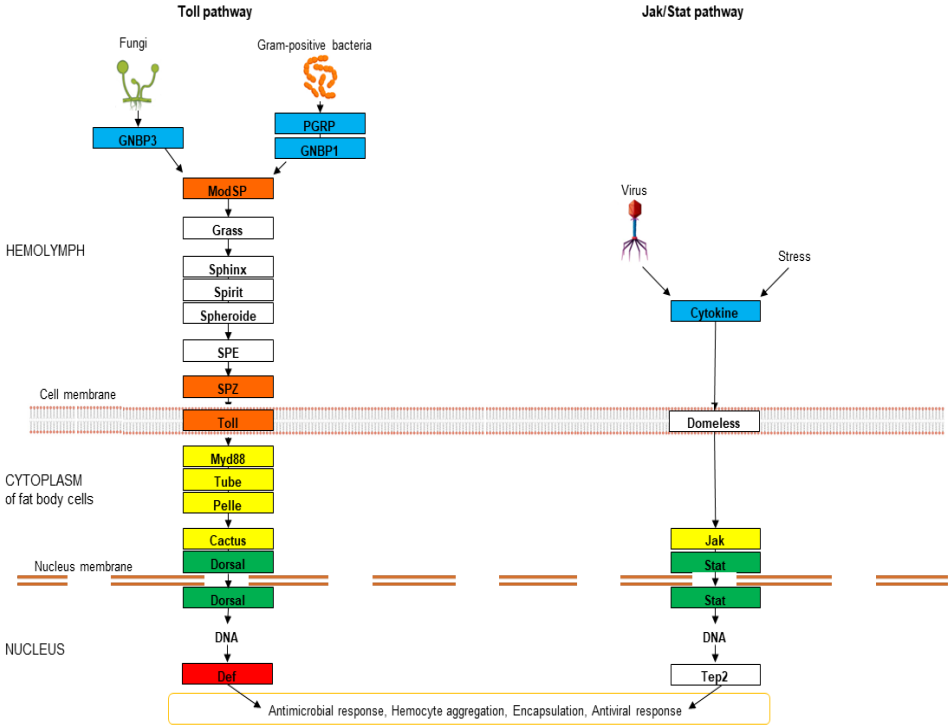

Figure 1. Key proteins of the Toll and Jak/Stat signaling pathways: coloured boxes stand for analyzed proteins; white boxes are for the non-analyzed proteins; dark blue - recognition molecules; orange - the signaling molecules in the haemolymph; yellow - signaling molecules in the cytoplasm; dark green - transcription factor; red - the antimicrobial peptid.

The haemocytes are binding to the target forming a multilayer capsule around the invader, which is melanized by the proPO (prophenoloxidase) cascade (Marmaras and Lampropoulou, 2009; Dubovskiy *et al.,* 2016; Rosales, 2017). The invader within the capsule is then killed and destroyed by asphyxia or by reactive cytotoxic radicals (ROS, RNS) (Nappi *et al.,* 1995; Nappi and Ottaviani, 2000; Carton *et al.,* 2009).

The Toll signaling pathway is regulating the melanization process, and its activation results in the expression of immune genes like drosomycin, and defensin, which encode antimicrobial peptides and prophenoloxidase-activating enzymes. The Jak/Stat pathway's activation results in the expression of immune genes such as Turandot, Tep2 (Rolff and Reynolds, 2009).

While individual defense strategies, both immunological (e.g. encapsulation) and behavioural (e.g. grooming), are quite straightforward to study, in the case of social organisms the social context should also be considered due to the existence of emergent social defensive strategies, which might interfere with the individual immune system. As formulated by Cremer and Sixt (2009) the collective defense of a group comprises all individual defenses of the group members and their interaction. In comparison to solitary lifestyle, there are some mechanisms developed in the social context, particularly in eusocial animals, mostly insects, to fight off parasites, pathogens and/or reduce their spread inside the host body/system: the production of diverse antibiotic secretions, mutual grooming, collective broodcare, removal/exclusion of infected individuals, nest hygienic behaviour (Cremer *et al.,* 2007; Cremer and Sixt, 2009; Meunier, 2015). Two major hypotheses could be formulated as to how sociality could change individual immune responses: (a) individual immunity could be less efficient in social insects due to the compensatory effect of emerging social immunity, (b) or individual immune system should function at a higher level than in solitary animals due to higher risk of infection conferred by high frequency of interactions within the social system (Castella *et al.,* 2008; Cremer and Sixt, 2009; Stroeymeyt *et al.,* 2014). Under both hypotheses switching from solitary lifestyle to sociality could have resulted in evolutionary changes in the level of immune system.

In ants all species are eusocial, and sociality emerged only once during their evolution (Ward, 2006). Therefore, they constitute an intriguing study subject with regard to how sociality might have influenced the immune system. As presented above, we hypothesize there could be differences between solitary and social insects, such as ants, with regard to proteins involved in the immune signaling pathways. Thus, we proposed to analyze the phylogeny of proteins of the Toll and Jak/Stat signaling pathways in ants by considering a wide array of different ant species alongside other social and solitary insects.

Methods

Sequence selection, data sets

Amino acid sequences of the proteins involved in the encapsulation signaling pathways were downloaded from GenomeNet (http://www.genome.jp) and from NCBI (May 2019–October 2020). We used amino acid sequences for the phylogenetic analyses because amino acid sequences are more conserved than the corresponding nucleotide sequences, and they exhibit far less random homoplasy, than DNA sequences. We obtained the amino acid sequences for 15 proteins, key components of the Toll and Jak/Stat signaling pathways, out of a total of 22 proteins (Broderick *et al.,* 2009, Bechsgaard *et al.,* 2016) (Fig. 1). For the other 7 proteins (Grass, Sphinx, Spirit, Speroide, SPE, Domeless, Tep2) the GenomeNet and NCBI research did not get any results, thus we excluded these proteins from our analyses. We used homologue sequences instead of orthologs. After taking a look at OMA (Orthologous matrix) Orthology database (Altenhoff *et al.,* 2021) to determine the orthologs for our species set, we realized the lack of many orthologs. We found that most of the species genomes have not been sequenced, and this is why many of the sequences we use are from transcriptomes rather than genomes. We used the query sequences of the ant species *Atta cephalotes* Linnaeus 1758, for all of 15 proteins to obtain the homologue sequences. Homologues of protein sequences were identified with blastp (protein Blast) search (Altschul *et al.,* 1997), from the non-redundant database. We used it as a common practice and selected the longest isoform annotated from each protein to do the phylogenetic reconstruction. Besides ants (47 species), we also included species from four different social insect families as Apidae (35 species), Halictidae (1 species), Vespidae (2 species) (order Hymenoptera), and Termitidae (15 species) (order Blattodea), and from three solitary insect families as Braconidae (4 species), Megachilidae (2 species) (order Hymenoptera), and Drosophilidae (24 species) (order Diptera) (Table S1). Blast search hits with E-values higher than $1e^{-05}$ were discarded. In the case of the PGRP protein we obtained partial homologous sequences for 8 *Myrmica* ants species, but the query cores (>70%) and the E-values (> $1e^{-05}$) of the sequences were adequate so we used these protein sequences to generate the phylogenetic tree. In the case of the Drosophilidae family, more than 5 homologue sequences were found for each protein, and we chose 5 sequences with the best E-values and query cores (Table 1). For the transmembrane receptor Domeless (Fig. 1), we did not get any results by NCBI protein sequence search.

Phylogenetic trees

Phylogenetic trees were reconstructed by using Phylogeny.fr (http://www.phylogeny.fr), an online web service for analyzing phylogenetic relationships between sequences (Dereeper *et al.,* 2008). We used the Clustal Omega, Muscle, Mafft, and T-coffee softwares for the multiple sequence alignment (Madeira *et al.,* 2019). Then we used the TCS (T-coffee package), a new multiple sequence alignment reliability measure to estimate alignment accuracy and improve phylogenetic tree reconstruction (Chang *et al.,* 2014). From the four multiple sequence alignment programs the T-coffee gave us for each protein the highest score, thus in the following analyses we used the alignments generated by this program. To eliminate the poorly aligned and/or gap positions, such as the nonconserved segments from the alignments we used G-blocks 0.91b (Castresana, 2000; Talavera and Castresana, 2007) with these settings: (i) minimum number of sequences for a conserved position (50% of the number of sequences + 1), (ii) minimum number for a flank position: 85% of the number of sequences, (iii) maximum number of contiguous nonconserved positions: 8, (iv) minimum block length: 10, (v) no gaps in final blocks. Phylogenetic trees were reconstructed using the PhyML 3.0. software (Guindon *et al.,* 2010) based on the Maximum Likelihood method. The default substitution model was LG (Le and Gascuel, 2008). The standard bootstrapping was replaced by a faster approximate Likelihood-ratio test (aLRT), which provides the exact statistical evaluation of branch support and values that bootstrap ones. We used TreeDyn for graphical editing (Chevenet *et al.,* 2006). There are generally accepted values of confidence: 70% or above is considered as statistically significant support (Soltis and Soltis, 2003).

Phylogenetic trees were successfully built for each of the 14 proteins studied with the exception of Defensin, where after multiple alignments and elimination of gap positions, the available amino acid sequences were too short (10 aa positions). The phylogenetic analysis depends on the sequence length, and on the alignment of the sequences. If the amino acid sequence is small, the probability of the wrong alignment is higher and this changes the phylogenetic tree, thus in the case of the Defensin the obtained result was not relevant.

Results

We identified several homologue sequences from a high number of species in the studied insect groups (Table 1, Table S1). The alignments generated by the T-coffee program yielded the best scores, therefore we used these to generate phylogenetic trees.

Table 1. Number of protein homologue sequences identified in five social (Termitidae, Apidae, Halictidae, Formicidae, Vespidae) and three solitary insect families (Megachilidae, Braconidae, Drosophilidae).

Protein	Function	Insect families							
		Termitidae	Apidae	Megachilidae	Halictidae	Formicidae	Vespidae	Braconidae	Drosophilidae
Toll signaling pathways									
PGRP	Recognition	2	28	2	1	30	2	3	5
GNBP1		13	15	2	1	19	2	3	5
GNBP3		15	13	2	1	21	2	3	5
ModSP	Signalling	2	5	2	1	13	2	3	5
Spz		2	11	2	1	20	2	3	5
Toll		2	11	2	1	22	2	3	5
Myd88		2	11	2	1	22	2	3	5
Tube		2	10	2	1	21	2	3	5
Pelle		2	11	2	1	22	2	3	5
Cactus		2	12	2	1	20	2	3	5
Dorsal	Transcription factor	2	10	2	1	22	2	3	5
Def	Antimicrobial response	1	23	2	1	38	2	4	5
Jak/Stat signaling pathway									
Cytokine	Recognition	2	11	2	1	22	2	3	5
Jak	Signalling	2	10	2	1	22	2	3	5
Stat	Transcription factor	2	10	2	1	20	2	3	5

Toll signaling pathway

We reconstructed the phylogenetic trees of the 11 proteins involved in the Toll signaling pathway (Figs 2-3, see supplementary files: Figs S1–S9). The different insect families grouped separately in well-defined clades in most phylogenetic trees (bootstrap proportion 70-99%) (Figs 2-3, see supplementary files: Figs S1–S9).

In the case of Pelle and Dorsal proteins, the phylogenetic tree structure followed the general insect phylogeny with Hymenoptera grouping separately. The different members of the order belonged to a well-supported clade, while species of the other two orders (Blattodea, Diptera) formed outgroups on the phylogenetic trees (Fig. 3, see supplementary files: Fig. S8).

The other proteins of the Toll signaling pathway (PGRP, GNBP1, GNBP3, ModSP, Spz, Toll, Myd88, Tube, Cactus) did not follow the classical insect phylogeny, and they neither did reflect differences in social lifestyle (Fig. 2, see supplementary files: Figs S1–S7, S9).

Ants formed a single well-supported clade in the case of ModSP, Toll, Myd88, Tube, and Dorsal (Fig. 3, see supplementary files: Fig. S3, Figs. S5–S7), whereas in the case of PGRP, GNBP1, GNBP3, Spz, Pelle, and Cactus they did not group together. Phylogenetic trees of recognition proteins as PGRP, GNBP1, and GNBP3 were especially diverse.

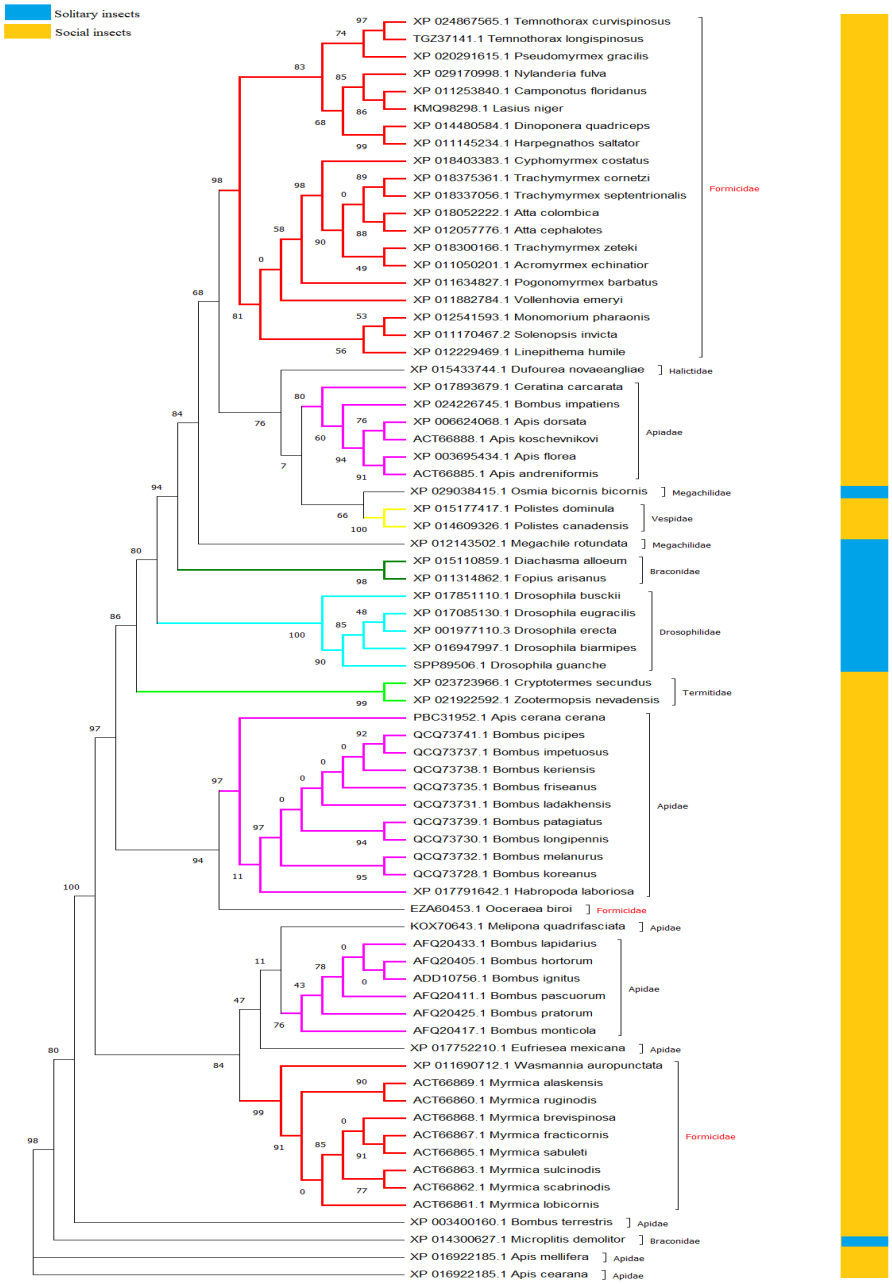

Figure 2. Phylogenetic maximum-likelihood PhyML tree of the peptidoglycan recognition protein (PGRP) based on a LG model. The tree was calculated with 73 sequences and 139 aa positions.

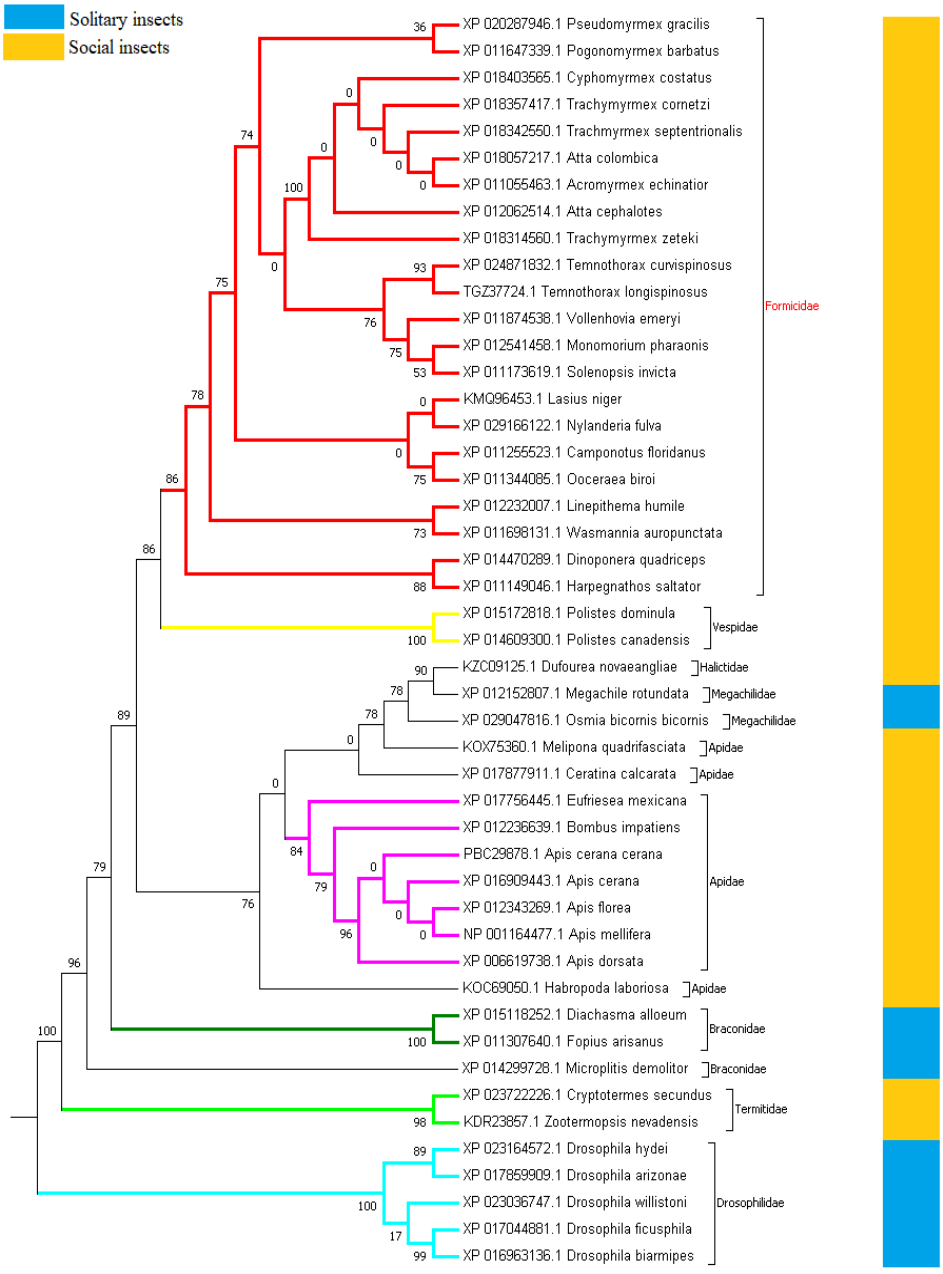

Figure 3. Phylogenetic maximum-likelihood PhyML tree of the Dorsal protein based on a LG model. The Dorsal tree was calculated with 47 sequences and 229 aa position.

Jak/Stat signaling pathway

The different insect families grouped in well-defined separate clades in the majority of phylogenetic trees (Figs. 4-5, see supplementary files: Fig. S10).

In the case of the recognition protein Cytokine receptor, we found that the phylogenetic tree structure followed the general insect phylogeny, thus there was a well-supported clade, of the different social and solitary Hymenoptera species, with two outgroups as Blattodea and Diptera (Fig. 4). The phylogenetic trees of the signaling molecule Jak and the transcription factor Stat did not show the same structure and did not reflect differences with regards to lifestyle either (Fig. 5, see supplementary files: Fig. S10).

All things considered, ants did not group together in a single well-supported clade in any of the studied proteins.

Discussion

The evolutionary success of any species depends, amongst others, on the efficiency of its immune system to properly recognize pathogens and respond adequately (Akira, 2009). Besides species-specific differences, geographical location (Wikelski *et al.,* 2004; Ayres *et al.,* 2010; Matson and Beadell, 2010; Lobato *et al.,* 2017), climatic factors (Jin *et al.,* 2011; Inbaraj *et al.,* 2016; Filipe *et al.,* 2020), and sexual features (Kurtz *et al.,* 2000; Ruggieri *et al.,* 2016) play a major role in shaping the immune responses. Transition from solitary to social life could also account for changes in the immune system of individuals due to e.g., the emergence of efficient social strategies to combat infections.

We studied the phylogeny of proteins involved in immune responses in ants to reveal whether there are ant-specific and sociality specific patterns, respectively. While in many cases proteins grouped according to larger systematic groups, e.g. Formicidae (ants), transition to social life did not seem to shape the phylogeny of these proteins in insects. We did not find evidence that the eusociality or solitary lifestyle affected/explain the separation of the different insect clades, in the studied proteins' phylogenetic trees.

The Toll pathway is activated by infection with Gram-positive bacteria and fungi, whereas the Jak/Stat pathway is activated by stress/injury, viral infection (Broderick *et al.,* 2009). The proteins of both signaling pathways seem to be conserved in the studied solitary and social insects. In the case of PGRP tree, *Myrmica* ants formed a clearly separated, well-defined clade, this separation could be explained by the fact that we had partial protein sequences for these *Myrmica* species, and despite corresponding E-values and query cores, this sequence partiality could result in their separate placement.

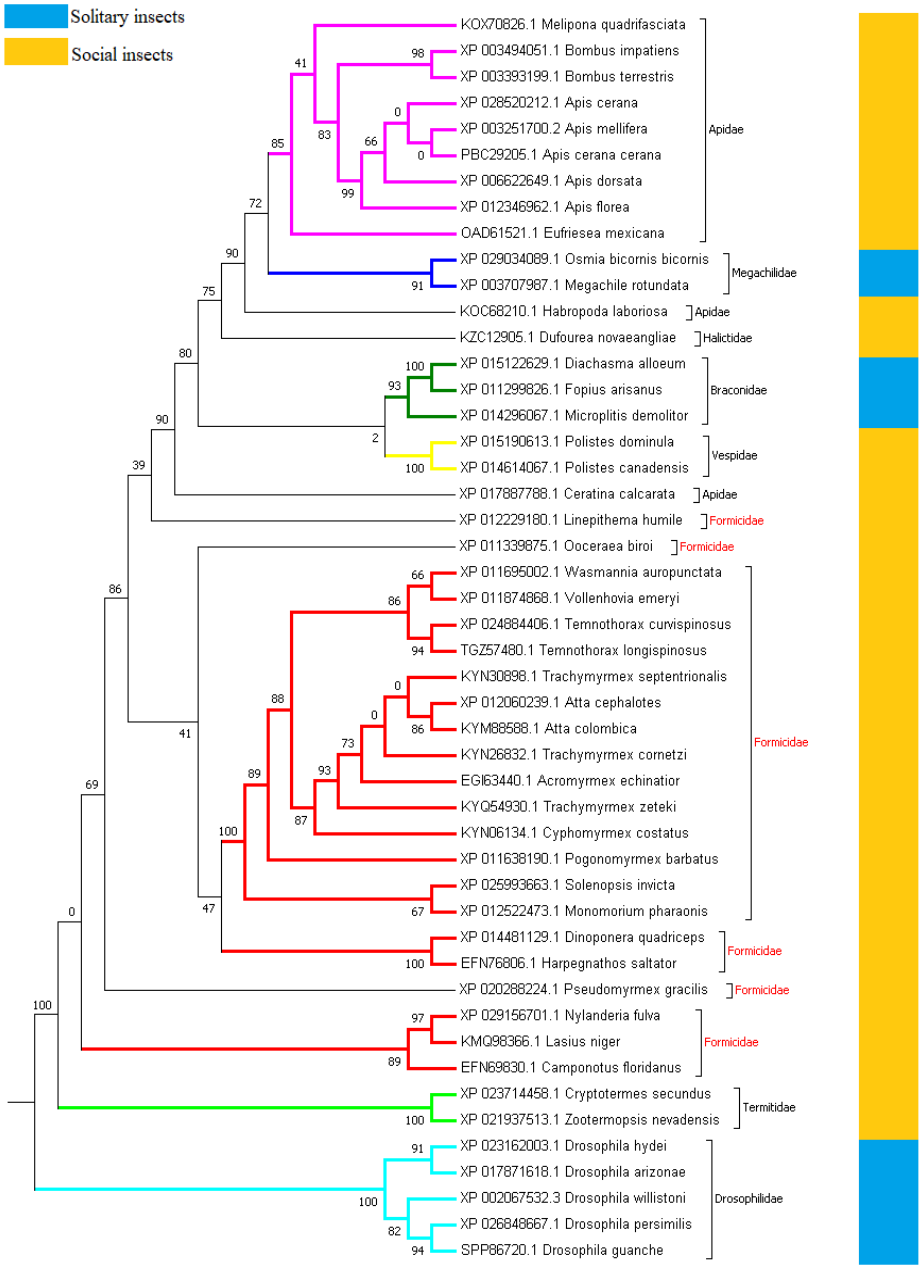

Figure 4. Phylogenetic maximum-likelihood PhyML tree of the Cytokine receptor (Cyt) based on a LG model. The Cyt tree was calculated with 48 sequences and 194 aa positions

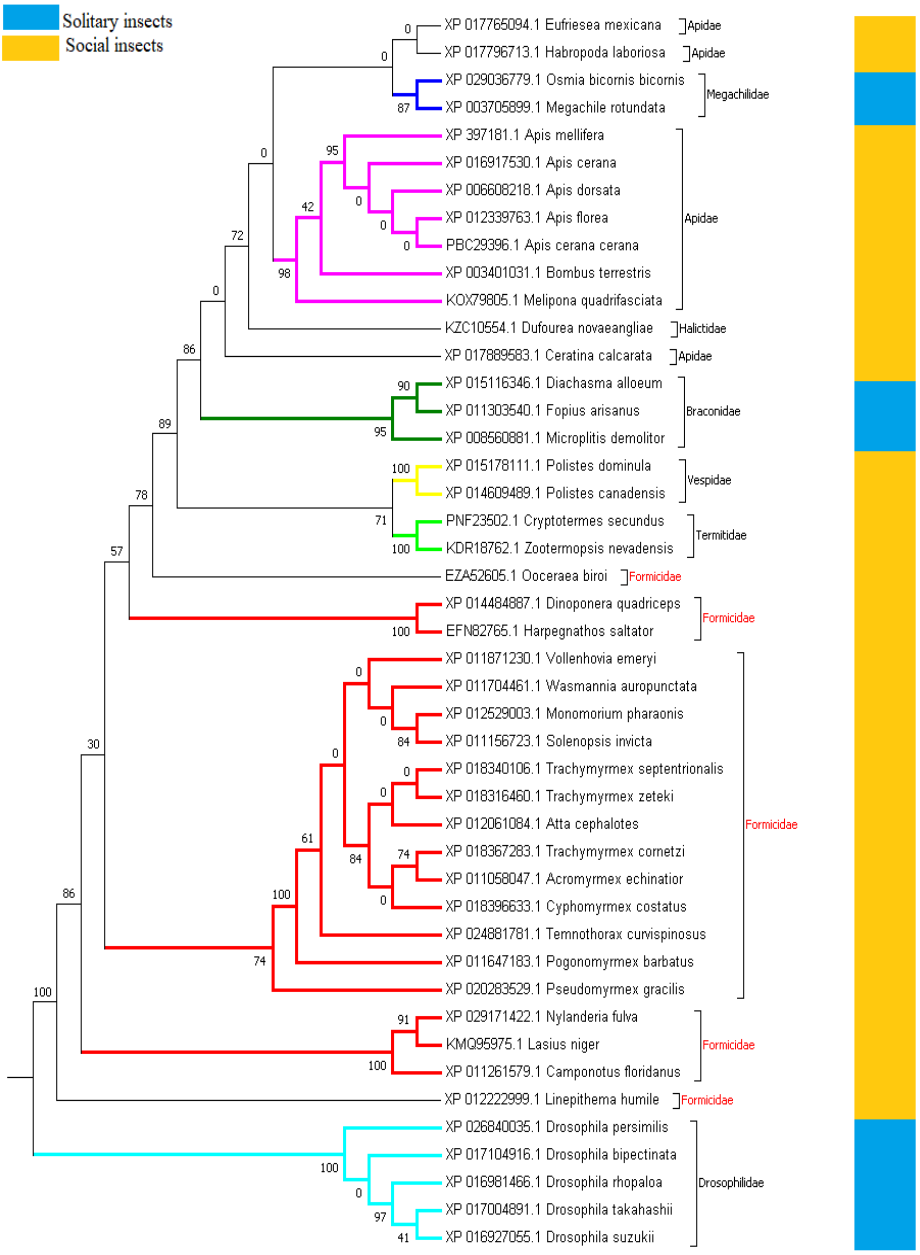

Figure 5. Phylogenetic maximum-likelihood PhyML tree of the signal transducer and activator of transcription protein (Stat) based on a LG model. The Stat tree was calculated with 45 sequences and 426 aa positions.

Recognition proteins (PGRP, GNBP1, GNBP3, Cytokine receptor) show diverse phylogenetic trees in both pathways. Insects do have a very large repertoire of pattern recognition receptors (PRR) (Wang *et al.,* 2019), and the secreted and/or transmembrane PRRs are the most diverse components of the different immune signaling pathways. For example, the cytokine receptor was highly diverse, similar to mammals, where 40 different cytokine receptors are known, suggesting an evolutionary tendency for the diversification of recognition proteins (Murray, 2007). The recognition proteins detect the molecular patterns of the pathogens, and after recognition, they activate the host defense mechanisms (Janeway *et al.,* 2001). Thus, we would expect that species with different lifestyles, with different habitats, should have different pathogens that they need to recognize adequately. There are e.g. several ant-parasitic fungal species with different lifestyles, and each species has a very well-defined set of hosts, which are not necessarily overlapping (see Espadaler and Santamaria, 2012; Csata *et al.,* 2013), therefore differences are expected to occur in the immune system accordingly, and these could present themselves mostly on the level of recognition proteins, but also on the level of immune genes and signaling proteins, transcription factors.

The phylogenetic trees of signaling proteins, and transcription factors showed though less diversity. The same was found by Khush *et al.* (2001) who compared the Toll pathways in *Drosophila* and mammals, and they found that the signal transduction and transcription systems' proteins are conserved. Bechsgaard *et al.* (2016) analyzed the genome of the arthropods and they found that the genes which encode the immune proteins of the Toll and Jak/Stat signaling pathways are conserved. In the case of the cell-type specific signaling pathways, cellular processes like gene expression have the lowest variability. Furthermore, the protein variability decreases from the recognition proteins to the transcription factors. The signal transduction proteins usually have a fundamental role, thus they are conserved, and show less diversity (Schaefer *et al.,* 2014).

Conclusion

There are several factors influencing the immune system of organisms. The building blocks of immune pathways could react differentially, some might show fine-tuned adaptations, while others could be conserved. Exposure to change could be heavily determined by the function of the proteins. Future works should analyze the potential effect of ecological background factors such as geographical distribution, lifestyle, colony structure (e.g. polygyny),

ecological characteristics (e.g. invasiveness), etc. which could, eventually, explain some of the uncovered diversity in ants with regard to their immune system, but also generally in social insects.

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SUPPLEMENTARY MATERIAL
Phylogenetic analyses of the proteins involved in encapsulation signaling pathways in ants

Kincső Orbán-Bakk[1,2]✉, Enikő Csata[3], Bálint Markó[1,4] and Ferencz Kósa[1]

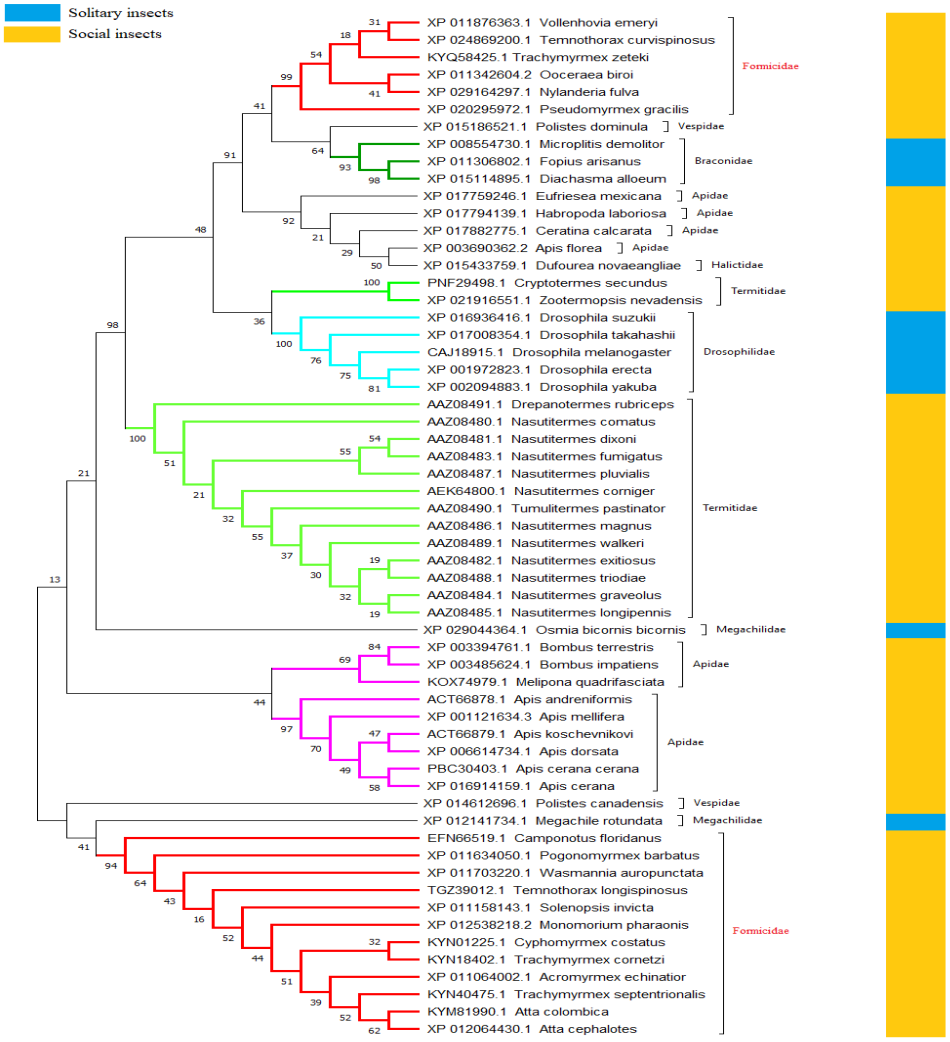

Figure S1. Phylogenetic maximum-likelihood PhyML tree of the Gram-negative binding protein 1 (GNBP1) based on a LG model. The tree was calculated with 59 sequences and 87 aa positions.

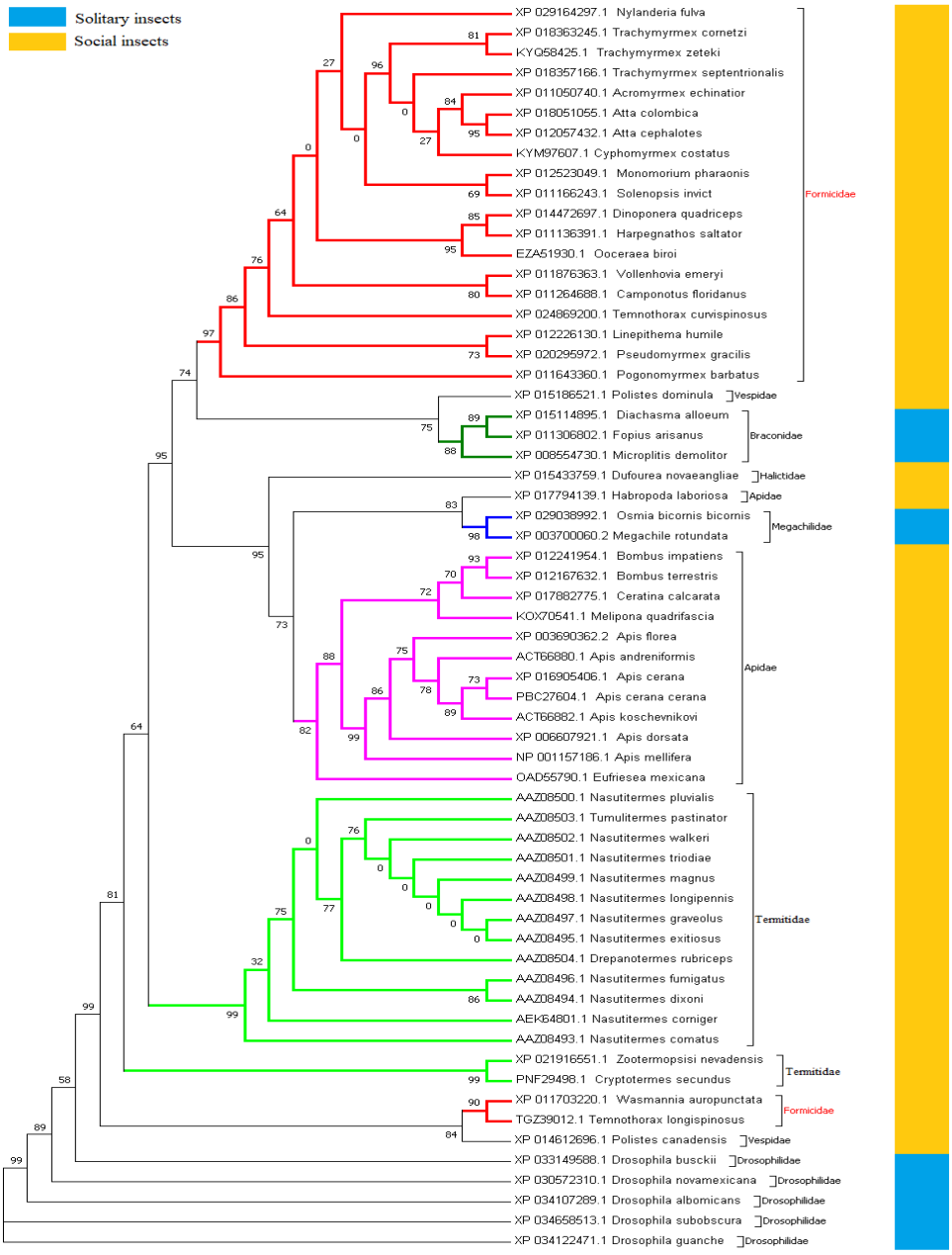

Figure S2. Phylogenetic maximum-likelihood PhyML tree of the Gram-negative binding protein 3 (GNBP3) based on a LG model. The tree was calculated with 62 sequences and 84 aa positions.

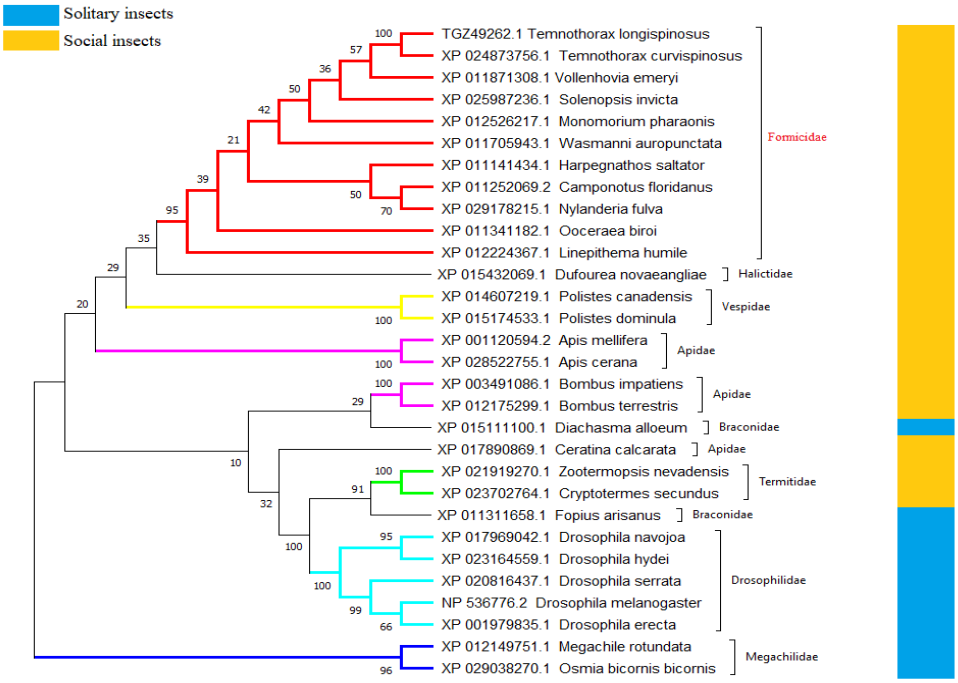

Figure S3. Phylogenetic maximum-likelihood PhyML tree of the modular serine protease (ModSP) based on a LG model. The tree was calculated with 30 sequences and 154 aa positions.

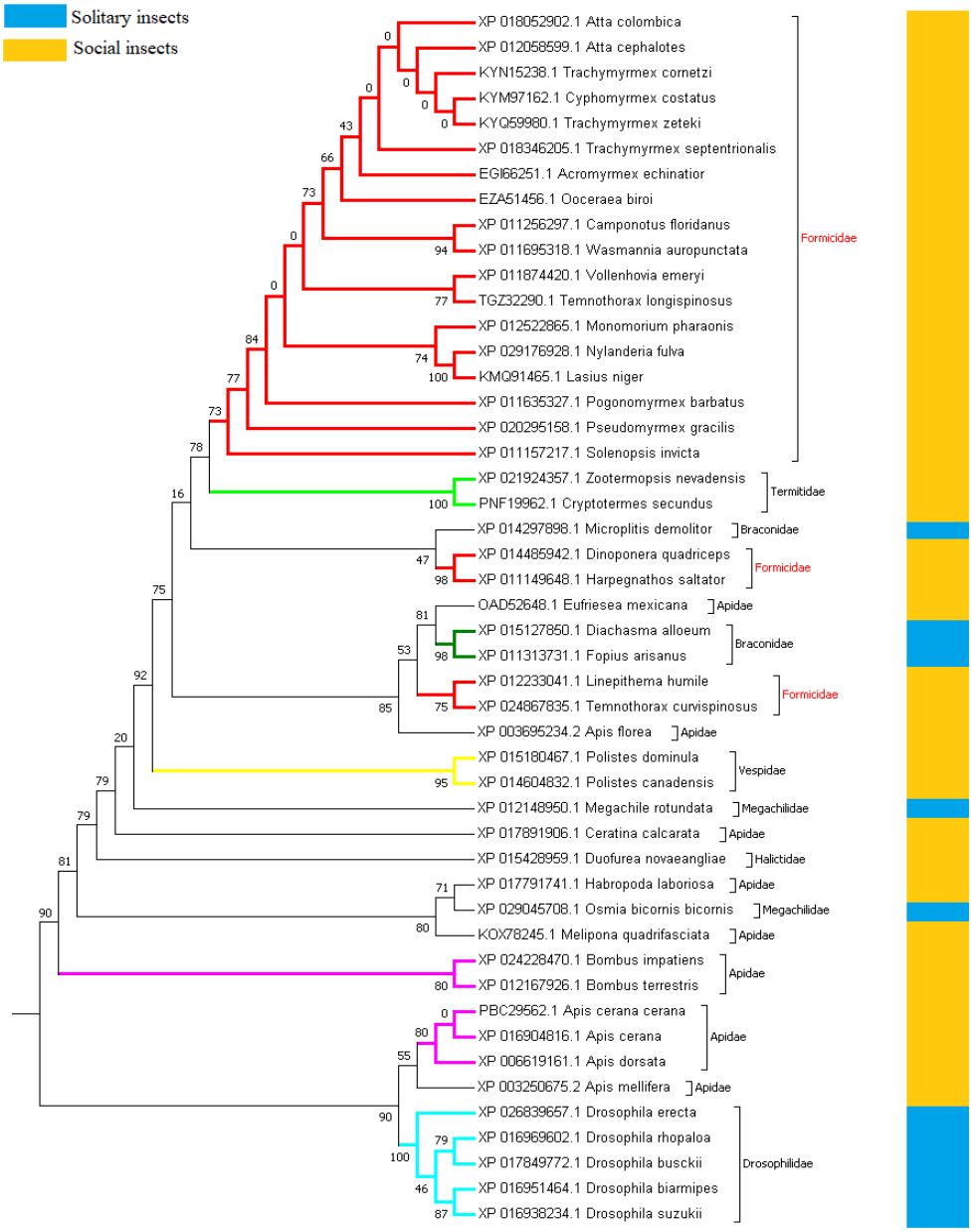

Figure S4. Phylogenetic maximum-likelihood PhyML tree of the Spatzle protein (Spz) based on a LG model. The tree was calculated with 48 sequences and 58 aa positions.

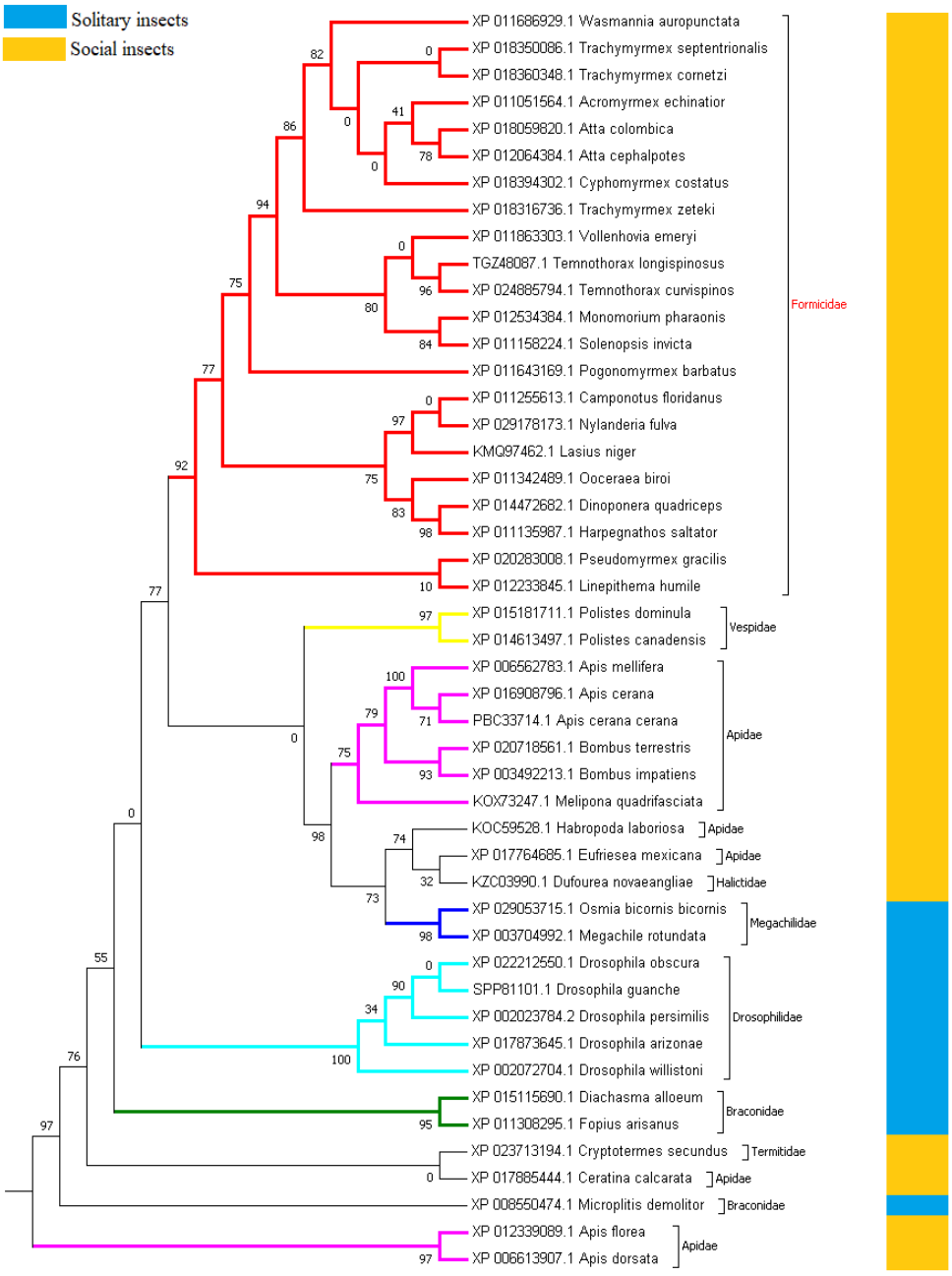

Figure S5. Phylogenetic maximum-likelihood PhyML tree of the Toll protein based on a LG model. The tree was calculated with 47 sequences and 60 aa positions.

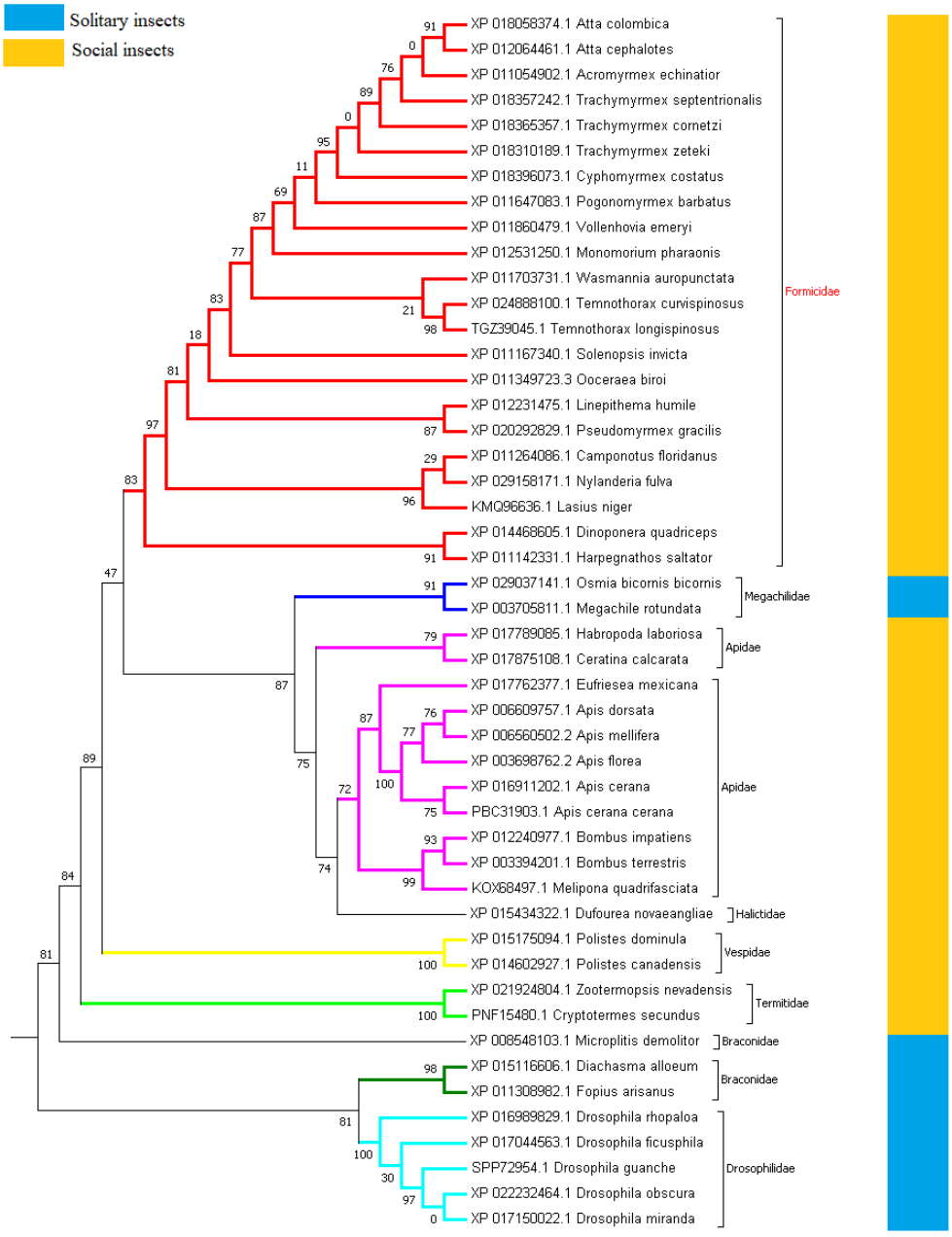

Figure S6. Phylogenetic maximum-likelihood PhyML tree of the Myeloid differentiation primary response 88 protein (MyD88) based on a LG model. The tree was calculated with 48 sequences and 129 aa positions.

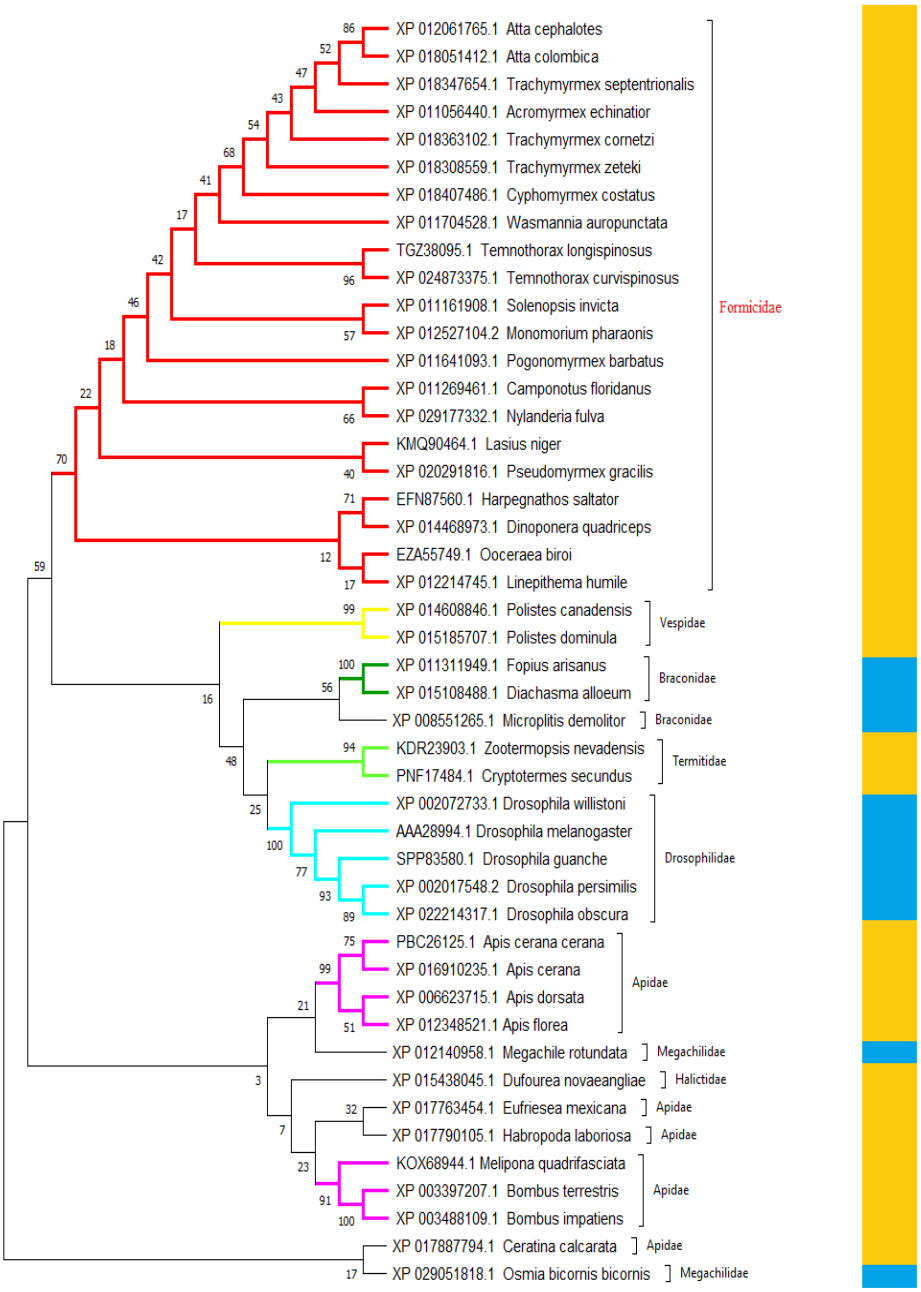

Figure S7. Phylogenetic maximum-likelihood PhyML tree of the Tube protein based on a LG model. The Tube was calculated with 46 sequences and 96 aa positions.

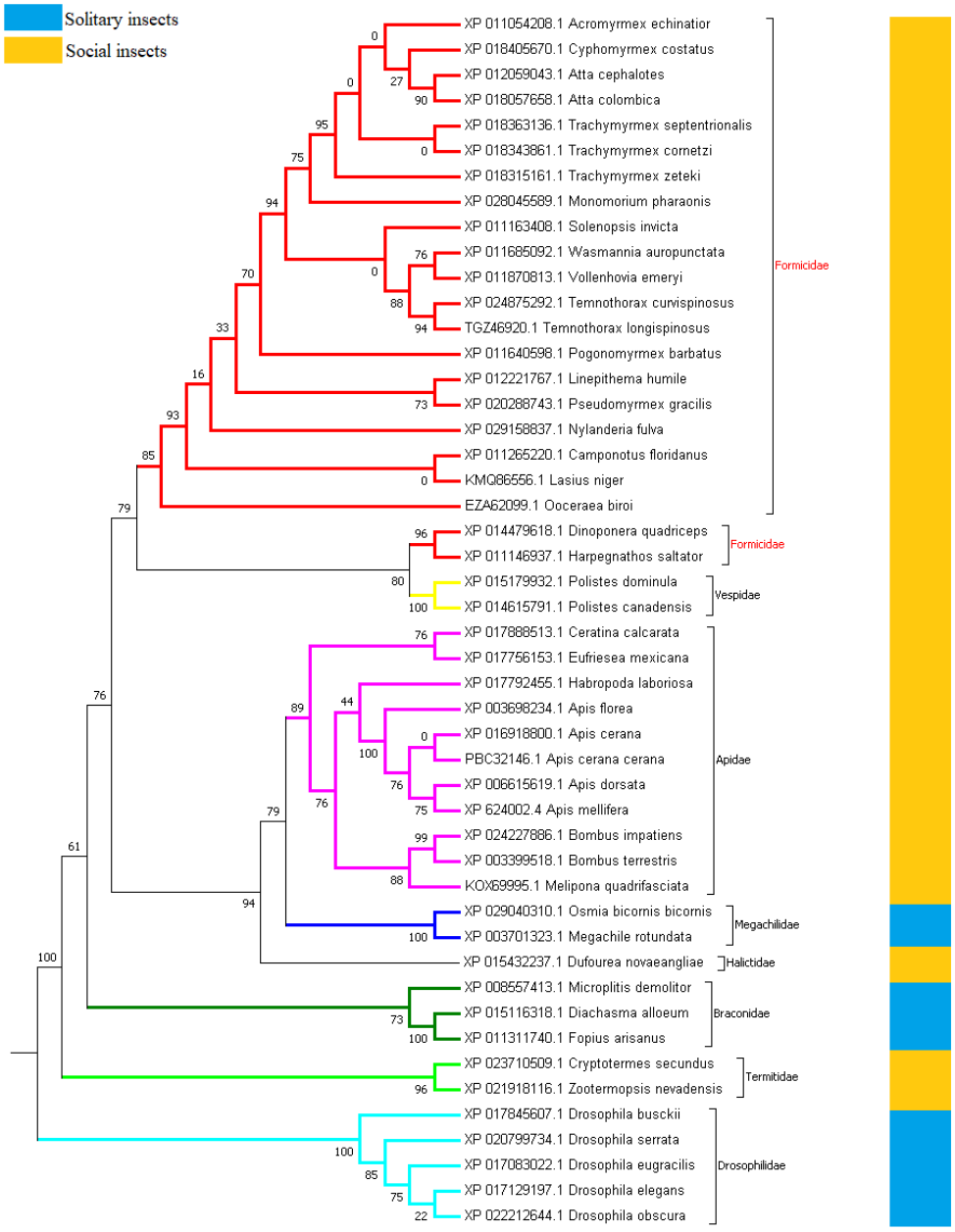

Figure S8. Phylogenetic maximum-likelihood PhyML tree of the serine/threonine-protein kinase Pelle based on a LG model. The Pelle tree was calculated with 48 sequences and 210 aa positions.

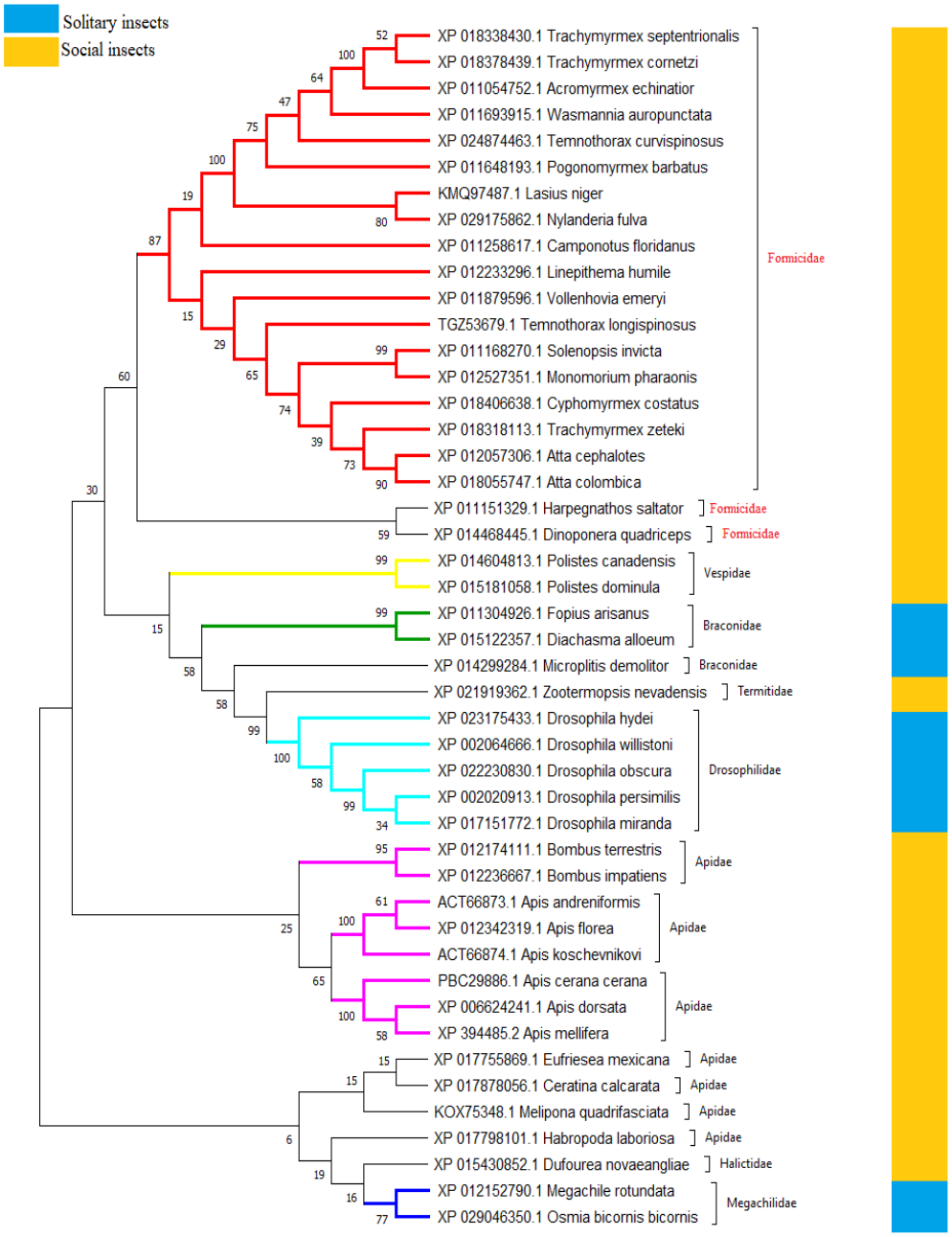

Figure S9. Phylogenetic maximum-likelihood PhyML tree of the Cactus protein based on a LG model. The Pelle tree was calculated with 48 sequences and 172 aa positions.

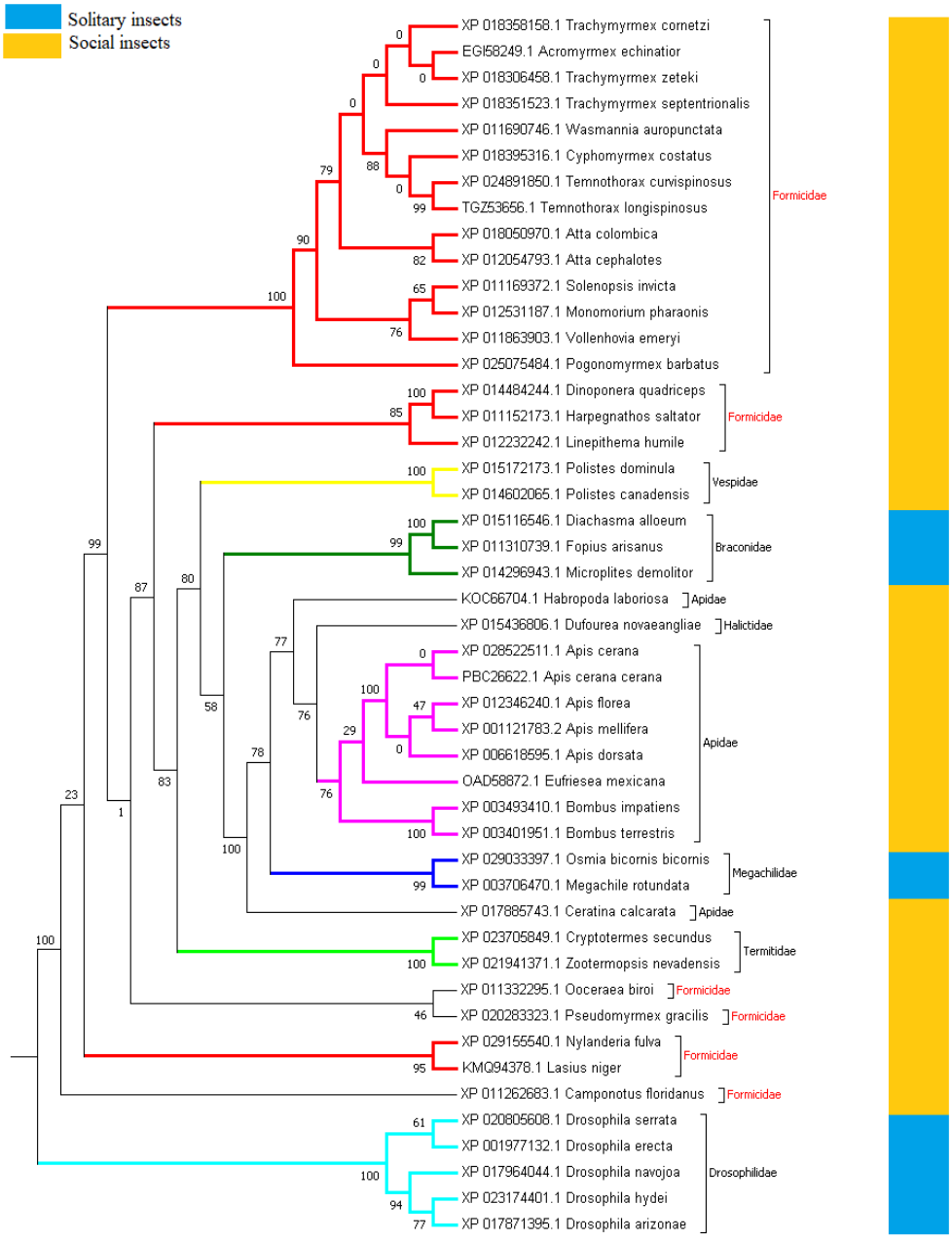

Figure S10. Phylogenetic maximum-likelihood PhyML tree of the Janus kinase (Jak) based on a LG model. The Jak tree was calculated with 47 sequences and 386 aa positions

Table S1. Insect species used in the study and the identified proteins.

Families and species	**Proteins**														
	PGRP	GNBP1	GNBP3	ModSP	Spz	Toll	Myd88	Tube	Pelle	Cactus	Dorsal	Def	Cyt	Jak	Stat
Formicidae															
Acromyrmex echinatior (Forel, 1899)	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Atta cephalotes (Linnaeus, 1758)	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Atta colombica Guérin-Méneville, 1844	+	+	+		+	+	+	+	+	+	+	+	+	+	
Camponotus floridanus (Buckley, 1866)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cataglyphis velox Santschi, 1929												+			
Cyphomyrmex costatus Mann, 1922	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Dinoponera quadriceps Kempf, 1971	+		+		+	+	+	+	+	+	+	+	+	+	+
Harpegnathos saltator (Jerdon, 1851)	+		+	+	+	+	+	+	+	+	+	+	+	+	+
Formica polyctena Foerster, 1850												+			
Formica rufibarbis Fabricius, 1793												+			
Formica sanguinea Latreille, 1798												+			
Formica uralensis Ruzsky, 1895												+			
Lasius austriacus Schödl & Seifert, 2003												+			
Lasius emarginatus (Olivier, 1792)												+			
Lasius flavus (Fabricius, 1782)												+			
Lasius japonicus Santschi, 1941												+			
Lasius lasioides (Emery, 1869)												+			
Lasius niger (Linnaeus, 1758)	+	+			+	+	+	+	+	+	+	+	+	+	+
Lasius sakagamii Yamauchi & Hayashida, 1970												+			
Linepithema humile (Mayr, 1868)	+		+	+	+	+	+	+	+	+	+	+	+	+	+

Families and species	**Proteins**														
	PGRP	GNBP1	GNBP3	ModSP	Spz	Toll	Myd88	Tube	Pelle	Cactus	Dorsal	Def	Cyt	Jak	Stat
Manica rubida (Latreille, 1802)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Monomorium pharaonis (Linnaeus, 1758)	+														
Myrmica alaskensis Wheeler, 1917	+														
Myrmica brevispinosa Wheeler, 1917	+														
Myrmica fracticornis Forel, 1901	+														
Myrmica lobicornis Nylander, 1846	+														
Myrmica rubra (Linnaeus, 1758)	+											+			
Myrmica ruginodis Nylander, 1846	+														
Myrmica rugulosa Nylander, 1849	+											+			
Myrmica sabuleti Meinert, 1860	+														
Myrmica scabrinodis Nylander, 1846	+											+			
Myrmica sulcinodis Nylander, 1846	+														
Notostigma carazzii (Emery, 1895)	+											+			
Nylanderia fulva (Mayr, 1862)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ooceraea biroi (Forel, 1907)	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Pogonomyrmex barbatus (Smith, 1858)	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Polyergus rufescens (Latreille, 1798)	+											+			
Pseudomyrmex gracilis (Fabricius, 1804)	+	+	+		+	+	+	+	+		+	+	+	+	+
Rossomyrmex minuchae Tinaut, 1981	+											+			
Solenopsis invicta Buren, 1972	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Temnothorax curvispinosus (Mayr, 1866)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Temnothorax longispinosus (Roger, 1863)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Families and species	**Proteins**														
	PGRP	GNBP1	GNBP3	ModSP	Spz	Toll	Myd88	Tube	Pelle	Cactus	Dorsal	Def	Cyt	Jak	Stat
Trachymyrmex cornetzi (Forel, 1912)	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Trachymyrmex septentrionalis (McCook, 1881)	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Trachymyrmex zeteki Weber, 1940	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Vollenhovia emeryi Wheeler, 1906	+	+	+	+	+	+	+		+	+	+	+	+	+	+
Wasmannia auropunctata (Roger, 1863)	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Apidae															
Apis andreniformis Smith, 1858	+	+	+							+		+			
Apis cerana Fabricius, 1793	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Apis cerana cerana Fabricius, 1793	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Apis cerana japonica (Radoszkowski, 1877)												+			
Apis dorsata Fabricius, 1793	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Apis florea Fabricius, 1787	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Apis koschevnikovi Enderlein, 1906	+	+	+							+					
Apis mellifera Linnaeus, 1758	+	+	+	+	+	+	+		+	+	+	+	+	+	+
Bombus ardens ardens Smith, 1879												+			
Bombus bohemicus Seidl, 1838												+			
Bombus friseanus Skorikov, 1933	+														
Bombus hortorum (Linnaeus, 1761)	+														
Bombus hypocrita sapporensis Cockerell, 1911												+			
Bombus ignitus Smith, 1869	+											+			
Bombus impatiens Cresson, 1863	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Bombus impetuosus Smith, 1871	+														
Bombus keriensis	+														

Families and species	**Proteins**														
	PGRP	GNBP1	GNBP3	ModSP	Spz	Toll	Myd88	Tube	Pelle	Cactus	Dorsal	Def	Cyt	Jak	Stat
Morawitz, 1887															
Bombus koreanus (Skorikov, 1933)	+														
Bombus ladakhensis Richards, 1928	+														
Bombus lapidarius (Linnaeus, 1758)	+											+			
Bombus longipennis Friese, 1918	+														
Bombus lucorum (Linnaeus, 1761)												+			
Bombus melanurus Lepeletier, 1835	+														
Bombus monticola Smith, 1849	+														
Bombus patagiatus Nylander, 1848	+														
Bombus pascuorum (Scopoli, 1763)	+											+			
Bombus picipes Richards, 1934	+														
Bombus pratorum (Linnaeus, 1761)	+														
Bombus ruderatus corsicola Strand, 1917												+			
Bombus rupestris (Fabricius, 1793)												+			
Bombus terrestris (Linnaeus, 1758)	+	+	+	+	+	+	+	+	+	+		+	+	+	+
Ceratina calcarata Robertson, 1900	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Eufriesea mexicana (Mocsáry, 1897)	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Habropoda laboriosa (Fabricius, 1804)	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Melipona quadrifasciata Lepeletier, 1836	+	+	+		+	+	+	+	+	+	+	+	+		+
Halictidae															
Dufourea novaeangliae (Robertson, 1897)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vespidae															
Polistes canadensis (Linnaeus, 1758)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polistes dominula (Christ, 1791)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Families and species	**Proteins**														
	PGRP	GNBP1	GNBP3	ModSP	Spz	Toll	Myd88	Tube	Pelle	Cactus	Dorsal	Def	Cyt	Jak	Stat
Termitidae															
Cryptotermes secundus (Hill, 1925)	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Drepanotermes rubriceps (Froggatt, 1898)		+	+												
Nasutitermes comatus (Hill, 1942)		+	+												
Nasutitermes corniger (Motschulsky, 1855)		+	+												
Nasutitermes dixoni (Hill, 1932)		+	+												
Nasutitermes exitiosus (Hill, 1925)		+	+												
Nasutitermes fumigatus (Brauer, 1865)		+	+												
Nasutitermes graveolus (Hill, 1925)		+	+												
Nasutitermes longipennis (Hill, 1915)		+	+												
Nasutitermes magnus (Froggatt, 1898)		+	+												
Nasutitermes pluvialis (Mjöberg, 1920)		+	+												
Nasutitermes triodiae (Froggatt, 1898)		+	+												
Nasutitermes walkeri (Hill, 1942)		+	+												
Tumulitermes pastinator (Hill, 1915)		+	+												
Zootermopsis nevadensis (Hagen, 1874)	+	+	+	+	+		+	+	+	+	+		+	+	+
Braconidae															
Cotesia vestalis (Haliday, 1834)												+			

Families and species	**Proteins**														
	PGRP	GNBP1	GNBP3	ModSP	Spz	Toll	Myd88	Tube	Pelle	Cactus	Dorsal	Def	Cyt	Jak	Stat
Diachasma alloeum (Muesebeck, 1956)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fopius arisanus (Sonan, 1932)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Microplitis demolitor Wilkinson, 1934	+	+	+		+	+	+	+	+	+	+	+	+	+	+
Drosophilidae															
Drosophila albomicans (Duda, 1923)			+												
Drosophila arizonae Ruiz, Heed and Wasserman, 1990						+					+		+	+	
Drosophila biarmipes Malloch, 1924	+				+						+				
Drosophila bipectinata Duda, 1923															+
Drosophila busckii Coquillett, 1901	+		+		+				+						
Drosophila elegans Bock and Wheeler, 1972									+						
Drosophila erecta Tsacas and Lachaise, 1974	+	+		+	+									+	
Drosophila eugracilis Bock and Wheeler, 1972	+								+			+			
Drosophila ficusphila Kikkawa and Peng 1938							+				+	+			
Drosophila guanche Monclús, 1977	+		+			+	+	+					+		
Drosophila hydei Sturtevant, 1921				+						+	+		+	+	
Drosophila melanogaster Meigen, 1830		+		+				+				+			
Drosophila miranda Dobzhansky 1935							+			+					
Drosophila navojoa Ruiz, Heed and Wasserman 1990				+										+	
Drosophila novamexicana Patterson, 1941			+												
Drosophila obscura Fallén, 1823						+	+	+	+	+					
Drosophila persimilis						+		+		+			+		+

Families and species	**Proteins**														
	PGRP	GNBP1	GNBP3	ModSP	Spz	Toll	Myd88	Tube	Pelle	Cactus	Dorsal	Def	Cyt	Jak	Stat
Dobzhansky and Epling, 1944 *Drosophila rhopaloa* Bock and Wheeler, 1972					+		+								+
Drosophila serrata Malloch, 1927				+					+					+	
Drosophila subovscura Collin, 1936			+												
Drosophila suzukii (Matsumura, 1931)		+			+							+			+
Drosophila takahashii Hsu, 1949		+										+			+
Drosophila willistoni Sturtevant, 1916						+		+		+	+		+		
Drosophila yakuba Burla, 1954		+													
Megachilidae															
Megachile rotundata (Fabricius 1787)	+	+		+	+	+	+	+	+	+	+		+	+	+
Osmia bicornis bicornis (Linnaeus, 1758)	+	+		+	+	+	+	+	+	+	+		+	+	+