Supplementary Figure 1. Phylogenetic tree of insulin receptor protein sequences identified across Animalia, including selected representatives of Insecta. *Sycon raphanus* InR sequences were set as outgroup. Duplication events in non-insect phyla are marked with arrowheads. The topology and branching supports were inferred using RAxML maximum likelihood algorithm with WAG + F model (-ln = 131220.705824), the bootstrap values calculated from 1000 replicates are shown for nodes represented in more than 50% of trees. InR, insulin receptor; IRR, insulin receptor-related receptor; IGF1R, insulin-like growth factor 1 receptor.

**Phylogenetic tree of insulin receptor protein sequences identified across Animalia, including selected representatives of Insecta.**
Supplementary Figure 2. Phylogenetic tree and nomenclature of insect insulin receptors and decoy of insulin receptors identified in the Cluster II in 98 species from 23 orders. The tree represents the full version of the simplified tree given in Figure 1 of the main text. Orange arrow marks the Cluster II duplication within Polynoeptera. Red arrow (DR2) marks the loss of the tyrosine kinase domain giving rise to the decoy of insulin receptor gene DR2 (red) in Mecopterida, a group of advanced Holometabola including Diptera, Lepidoptera, Trichoptera and Mecoptera. Asterisks mark the duplication identified in Tribolium castaneum and the multiple duplications in Auchenorrhyncha. The topology and branching supports were inferred using RAxML maximum likelihood algorithm with WAG+Γ model (-ln = 285839.374747) the bootstrap values calculated from 500 replicates are shown for nodes represented in more than 50% of trees. Black dots in the right part of the figure indicate the insect orders included in phylegetic analyses in previous studies, the boxes give the nomenclature used by previous studies for the identified InR genes. Bold marking in brackets shows the InR nomenclature used in this study for P. simplex and P. apterus InRs.
Supplementary Figure 3. Phylogenetic tree and nomenclature of insect insulin receptors and decoy of insulin receptors identified in the Cluster I in 98 species from 23 orders. The tree represents the full version of the simplified tree given in Figure 1 of the main text. Green arrow marks the Cluster I duplication in Gerromorpha, blue arrow (SDR) marks the loss of the tyrosine kinase domain in Muscomorpha, giving rise to the secreted decoy of insulin receptor gene SDR (blue). Asterisks mark the duplication identified in Pyrrhocoridae and in Thermobia domestica. The topology and branching supports were inferred using RAxML maximum likelihood algorithm with WAG + Γ model (-ln = 285839.37447) the bootstrap values calculated from 500 replicates are shown for nodes represented in more than 50% of trees. Black dots in the right part of the figure indicate the insect orders included in phylogenetic analyses in previous studies, the boxes give the nomenclature used by previous studies for the identified InR genes. Bold marking in brackets shows the InR nomenclature used in this study for P. simplex and P. apterus InRs.
Supplementary Figure 4. Phylogenetic tree of insect insulin receptor genes and two homologous decoy receptor genes identified in 98 species from 23 orders. The numbers in condensed branches indicate the numbers of species. Orange arrow marks the Cluster II duplication within Polynoptera, red arrow (DR2) marks the loss of the tyrosine kinase domain in advanced Holometabola, giving rise to the decoy of insulin receptor gene DR2 (red). Green arrow marks the Cluster I duplication in Gerromorpha, blue arrow (SDR) marks the loss of the tyrosine kinase domain in Muscomorpha, giving rise to the secreted decoy of insulin receptor gene SDR (blue). The topology and branching supports are based on Bayesian inference (PhyloBayes v4.1).
Supplementary Figure 5. Detailed phylogenetic tree of insulin receptor gene sequences identified in eight species of Blattodea and five species of Phasmatodea. The topology and branching were inferred using RAxML maximum likelihood algorithm with WAG + Γ model (-ln = 44434.953962), the bootstrap values calculated from 500 replicates are shown for nodes represented in more than 50% of trees.
Supplementary Figure 6. Detailed phylogenetic tree of insulin receptor gene sequences identified in five species of Orthoptera and major insect lineages for comparison. Orthopteran representatives are marked in red. The topology and branching were inferred using RAxML maximum likelihood algorithm with WAG + Γ model (-ln = 103575.553052; WAG + Γ model), the bootstrap values calculated from 500 replicates are shown for nodes represented in more than 50% of trees.
Supplementary Figure 7. Detailed phylogenetic tree of insulin receptor gene sequences identified in five species of Orthoptera and major insect lineages for comparison. Orthopteran representatives are marked in red. The topology and branching were inferred using Bayesian inference (WAG + Γ model; chain length = 1 million; MrBayes).
Supplementary Figure 8. Expression pattern of three InRs in somatic tissues and gonads of sterile workers (pseudergates) and neotenic reproductives of both sexes 10 days after their moult from workers in the termite *Prorhinotermes simplex*. The graphs show qRT-PCR values of the three genes relative to those of the *rp49* reference gene and log-transformed to reduce heteroscedasticity. Bars show means, whiskers standard deviations. The transformed data was subjected to two-way analysis of variance with sex and tissues as factors. Gonads (absent in workers) were compared separately between males and females using a t-test. 1-3 asterisks denote significant expression differences at p<0.05, p<0.01, and p<0.001, respectively, between different tissues of each caste/sex. Columns marked with different letters indicate significant inter-caste or inter-sex expression differences at p<0.05 for individual tissues.

For all three InRs, the analyses were evaluated as highly significant, especially due to the significant contribution of the tissues to the total variance (p<10^{-4} for all three InRs) and the interaction effects (p<7×10^{-3} for all three InRs). The contribution of caste was retrieved as highly significant for InR3 (p<10^{-4}), while being marginally non-significant for InR2 (p=0.056) and non-significant for InR1 (p=0.12). Post-hoc comparison highlighted several trends in InR expression among tissues. First, InR2 is highly upregulated in the digestive tube of all castes, while the other two InRs show an opposite pattern, having the smallest expression in the digestive tube, except for workers and InR1. This points to an eventual differential role of the InRs in the nutrient sensing. Last but not least, all InRs had larger expression in the body cavity than in heads in workers and in males (except for InR2 in males).

Intercaste differences included significantly higher expressions of all three InRs in heads of females (InR2 also in males) when compared to workers. The same applied for InR3 and the digestive tube. Separate evaluation of InR expression in gonads of reproductives revealed a dramatic difference in InR3 expression in favor of female gonads when compared to very low values in male testes, making the InR3 a female biased gene in all tissues but body cavity. InR1 had high expression values and InR2 moderate expression in gonads of both sexes without striking inter-sex differences.
Supplementary Figure 9. Protein domains and alignments of InRs and decoys of insulin receptors in selected insect taxa. A. Protein domains recognized in a typical InR (*D. melanogaster*). B. Detail of protein alignment covering Furin-like cystein-rich domain. Red asterisks indicate cysteine residues identified as InR1-specific by Xu et al. (2015), the orange asterisk highlights additional residue unique to Cluster I InRs and SDRs. C. Detail of protein alignment covering transmembrane domain identified in InRs and DR2s. Small black asterisk indicates C terminus in DR2 proteins. N-terminal part of protein tyrosine kinase domain is highlighted in InRs.
Supplementary Figure 10. Protein domains and alignments of InRs and decoys of insulin receptors in selected insect taxa compared to epidermal growth factor receptor (EGFR). A. Protein domains recognized in a typical InR (*D. melanogaster*). B. L1 receptor L-domain. C. L2 receptor L-domain. D. Fibronectin type III superfamily in InRs and Growth factor receptor cysteine-rich domain superfamily in EGFRs.
Supplementary Figure 11. Detailed phylogenetic tree of insulin receptor gene sequences identified in 15 species of Hymenoptera. The topology and branching were inferred using RAxML maximum likelihood algorithm with WAG + Γ model (-ln = 59986.190587; WAG + Γ model), the bootstrap values calculated from 1000 replicates are shown for nodes represented in more than 50% of trees.
Supplementary Figure 12. Expression pattern of InR and decoy receptor genes SDR and DR2 in somatic tissues and gonads of adult fruit flies Drosophila melanogaster. The graphs show qRT-PCR values of the three genes relative to those of the control reference gene rp49 and log-transformed to reduce heteroscedasticity. Bars show means, whiskers standard deviations. The transformed data was subjected to two-way analysis of variance with sex and tissues as factors. 1-3 asterisks denote significant expression differences at probability values p<0.05, p<0.01, and p<0.001, respectively, between different tissues of each sex. Columns marked with different letters indicate significant inter-sex differences at p<0.05 for individual tissues.

All three genes are expressed in all studied tissues of both sexes and have similar expression patterns with low values in the digestive tube and higher expression in the head and gonads. Another general trend is a male-biased expression, most pronounced in the case of DR2. Two-way analysis of variance with sex and tissue as considered factors revealed that for InR and SDR, the tissue differences are the main driving force for the total significance of the tests (p<10^{-4} and p<5×10^{-4}, respectively), while the inter-sex differences are of secondary importance (p<4×10^{-3} and p=0.21, interaction p=0.15 and 0.84, respectively). By contrast, the male-female difference was evaluated as the main contributor to the overall significance in DR2 (p<4×10^{-4}), compared to the tissues (p<10^{-2}, interaction p=0.7).
Supplementary Figure 13. Expression pattern of three InRs in somatic tissues and gonads of adult linden bugs Pyrrhocoris apterus. The graphs show qRT-PCR values of the three genes relative to those of the control reference gene rp49. Bars show means, whiskers standard deviations. The data was subjected to two-way analysis of variance with sex and tissues as factors. 1-3 asterisks denote significant expression differences at $p<0.05$, $p<0.01$, and $p<0.001$, respectively, between different tissues of each sex. Columns marked with different letters indicate significant inter-sex differences at $p<0.05$ for individual tissues.

Both InR1a and InR1b genes are expressed in adults of both sexes, just as it is the case for the Cluster II InR (KX087105, hereafter InR2). InR1a shows the highest transcript abundances in all tissues of both sexes, while InR1b the lowest. Expression patterns for all three genes are tissue-specific, the most pronounced differences are observed for InR1b, which show a downregulation in the digestive tube. Some tissues also show a significant effect of sex on the InR expression.
Supplementary Figure 14. Pyrrhocoris apterus InR transcripts, design of dsRNA and RNAi efficiencies. **A.** *P. apterus* InR transcripts with highlighted open reading frames and positions, for which gene-specific dsRNAs were designed. **B.** Sequence similarity between dsRNA and the corresponding regions in paralogous transcripts agrees with the close relationship between *InR1a* and *InR1b*. **C.** Expression levels (mean±SD) of *InR1a* (left) and *InR1b* (right) indicate gene-specific targeting in heads of the fourth instar larvae after injection of *egfp* (control), *InR1a* or *InR1b* dsRNA. 1-3 asterisks denote significant differences at p<0.05, p<0.01, and p<0.001, respectively, in expression levels resulting from one-way ANOVA followed with Dunnett posthoc test (*egfp* set as control) on log-transformed data.
Supplementary Figure 15. *Pyrrhocoris apterus* insulin-like peptide (*Pilp*) genes. A. *Pilp* transcripts with highlighted open reading frames and positions, for which dsRNAs were designed. B. Sequence similarity between dsRNA and the corresponding region in paralogous transcripts agrees with the close relationship between *Pilp2* and *Pilp3*. C. Expression levels of *Pilp1* (left graph) and *Pilp2*+*3* (right graph) in heads of the fourth instar larvae after injection of *egfp* (control), *Pilp1* or *Pilp2*+*3* dsRNA. 1-3 asterisks denote significant differences at p<0.05, p<0.01, and p<0.001, respectively, in expression levels resulting from one-way ANOVA followed with Dunnett posthoc test (*egfp* set as control) on log-transformed data.
**Supplementary Figure 16.** Gene structures of two *Tribolium castaneum* InR paralogs identified in the Cluster II compared to representative DR2 genes and Cluster II InR of *Apis mellifera*. A. Schematic depiction of exon (boxes) and intron (dotted lines) structure and homologous intron-exon boundaries (gray vertical lines). White, dark grey and blue color in exons correspond to exon coding in panel B. Regions coding for protein tyrosine kinase (dark orange) and transmembrane domain (light orange) are highlighted. B. Detail of amino acid sequence alignment with exons indicated as color coded boxes under each sequence.
Supplementary Materials and Methods

Expression of *D. melanogaster* InR, DR2 and SDR

White eye (*w1118*) *D. melanogaster* strain flies were grown on standard corn meal diet at 25 °C. Five days after adult eclosion male and female flies were CO₂-anesthetized and heads, digestive tubes, and gonads were dissected. RNA isolation, reverse transcription and qRT PCR were performed as in *P. apterus* experiments, with *Drosophila*-specific primers (Supplementary table 3, Supplementary Material online). The analysis was performed with three biological replicates, each consisting of pooled tissues from 25 individuals. The quantified transcripts were normalized to the level of the reference gene *rp49* (Bazalova and Dolezel 2017), the results log-transformed to reduce heteroscedasticity and analyzed using two-way ANOVA (tissue and sex as predictors) in GraphPad 5.00.

Expression of InRs in the termite *P. simplex* and its caste and tissue specificity

The three *InR* genes identified in *Prorhinotermes simplex* (Rhinotermitidae) were investigated with respect to mRNA levels and eventual caste and tissue-specific expression. For this purpose, we compared their expression in workers (pseudergates), ten-day-old neotenic males and females. The neotenics were obtained from orphaned groups of 100 workers, kept together with 15 soldiers in 9 cm Petri dishes on moistened sand and offered with blocks of spruce wood (permanent darkness, 26°C). In such groups, the workers start to differentiate into neotenics within 10 days. Groups were controlled every 12h, freshly molted neotenics removed and held for ten days in new groups of 50 workers.

Four biological replicates were prepared for each caste and tissue, each of them from pooled tissues of four individuals. Total RNA was isolated using TRI Reagent® (Sigma Aldrich) following the manufacturer's protocol. RNA isolates were treated with RQ1 RNase-Free DNase (Promega) to eliminate contaminant DNA. cDNA template was generated from 0.7 μg of the respective total RNA using the SuperScript III First-Strand Synthesis System (Invitrogen by Life Technologies) and random hexamers. The sequences of specific primers used for amplification are given in supplementary table 3 (Supplementary Material online). qRT-PCR was performed as published earlier (Jirošová et al. 2017). The resulting data was log-transformed to reduce heteroscedasticity and analyzed using two-way ANOVA (tissue and caste/sex as predictors) in GraphPad 5.00.

Expression of *P. apterus* InRs and its sex and tissue specificity

Adults of *Pyrrhocoris apterus* strain Oldrichovec (Pivarciova et al. 2016) were kept in 0.5 liter glass jars on linden seeds and water *ad libitum* in long photoperiod (18 hrs light: 6 hrs dark) at 25 °C. Brain, fat body, digestive tube and gonads were dissected in RNAse-free Ringer's solution from males and females 10 days after adult eclosion, anesthetized by CO₂. Four biological replicates for each tissue and organ were prepared, each from pooled tissues of five individuals. Total RNA was isolated with the Trizol reagent (Invitrogen). After Turbo DNase (Ambion/ThermoFisher) treatment, 1 μg of total RNA was used for cDNA synthesis using the SuperScript III reverse transcriptase (Invitrogen). Relative transcript levels were measured by quantitative PCR using the qPCR 2x SYBR Master Mix (Top Bio) and the C1000 Thermal Cycler (Bio-Rad). Primers sequences are listed below in supplementary table 3. All measured transcripts were normalized to the level of the reference gene *rp49* (Dolezel et al. 2007). The data was analyzed using two-way ANOVA (tissue and sex as predictors) in GraphPad 5.00.

Identification of *P. apterus* insulin-like peptide (*Pilp*) transcripts

Insect insulin-like peptides (ILPs) from *Drosophila melanogaster* (Dilp1-8), *Bombyx mori* (bombyxin A1-10, B1-12, C1-2, E1, F1, G1) and *Nilaparvata lugens* (NlILP1-4) were used as a query in BLAST searches in our in-house *P. apterus* transcriptomic databases. Candidate hits were validated by reciprocal BLAST searches in NCBI database, protein alignments with insect ILPs, and by position of characteristic Cysteine residues in the preprohormone. The mRNA sequences of three identified *P. apterus insulin-like peptide* (*Pilp*) transcripts were confirmed by PCR and Sanger sequencing.
RNAi-mediated silencing of InRs and Pilps in P. apterus

For each InR, gene-specific fragment was designed within the open reading frame in regions where stretches of identity between P. apterus paralogs where the shortest. In case of the closely related paralogs InR1a and InR1b, specificity of silencing was confirmed by qRT-PCR from whole head extracts of 4th instar larvae three days after RNAi. The protocol was identical to tissue-specific quantification, with the exception that RNA was isolated from individual heads and processed separately for RNAi efficiency assessments. Because Pilp2 and Pilp3 only differ by several SNPs, both genes were targeted by one common dsRNA. By contrast, Pilp1 is clearly distinct from Pilp2+3. Therefore, one Pilp1-targeting and one Pilp2+3-targeting fragment were designed within the open reading frame and 5’ untranslated region. The resulting primers are given in supplementary table 4 (Supplementary Material online).

Specific fragments were PCR amplified from head total cDNA by PPP Master Mix (Top Bio, Czech Republic), PCR products were purified by QIAquick PCR Purification Kit (Qiagen), ligated into the pGEM-T Easy vector (Promega) and verified by Sanger sequencing. Templates for dsRNA in-vitro synthesis were prepared from pGEM-T Easy clones by PCR using M13 forward and pGEM-RNAi reverse 5’-TAATACGACTCATAAGGGACACTATAAGATCT-3’ primer replacing SP6 to T7 promoter. Double-stranded RNA was synthesized using MEGAscript T7 Transcription Kit (Ambion/ThermoFisher) following the manufacturer’s protocol. dsRNA was then precipitated by adding 0.1 volume of sodium acetate (pH = 5) and 2.5 volume of 100 % ethanol and after spinning and washing it was dissolved in Ringer's solution. As a negative control, 720bp long egfp ORF in pEGFP-N1 (Clontech) was digested with SalI and NotI restriction enzymes and subcloned to pBlueScript KS (-) plasmid. T3 promoter in pBlueScript plasmid was replaced by T7 promoter in in vitro dsRNA transcription template by using M13F 5’- GTAAAACGACGGCCAGTG - 3’ and Blue-RNAi-R 5’-AATACGACTCATAAGGGACACAAAG - 3’ primers.

One-day-old 4th-instar P. apterus larvae of both strains were CO2-anesthetized, attached using a double-sided tape to a small tray and injected ventrolaterally into the abdomen under the stereomicroscope using a micromanipulator (Narishige, Japan) equipped with a borosilicate glass capillary needle. 1 µl of 3-4 µg/µl dsRNA dissolved in Ringer’s solution was administered to each larva, which was then transferred to a glass jar supplemented with linden seeds, water, and a folded filter paper. Adult animals were CO2-anesthetized and scored for wing length. The obtained proportions of long-winged vs. short-winged adult phenotypes were compared with the control treatment using an equivalent of Dunnett test adjusted for proportion data (Zar 1999).
### Supplementary Table 1. Insect taxa studied in phylogenetic analyses of InRs and decoy of InRs

| Higher unit | order | species | acc. number | note |
|-------------|-------|---------|-------------|------|
| **Ametabola** | Zygentoma | Lepisma saccharina | KX087106 |  |
|  |  | Thermobia domestica | GASM02067420 |  |
|  |  |  | GASM02067910 |  |
| **Palaeoptera** | Ephemeroptera | Ecdyonurus insignis | GCLC01041336 |  |
|  |  | Ephemerana | GCLC01095620 |  |
|  |  | Eurylophella sp. | GAKU01018742 |  |
|  |  |  | GAKU01018485 |  |
|  |  |  | GAZ00105571 |  |
|  | Odonata | Cordulegaster boltonii | GAY001000057 |  |
|  |  | Epiphielia superstes | GAVV01013590 |  |
|  |  | Megaloprepus caerulatus | GAVY01062158 |  |
| **Polyneoptera** | Plecoptera | Leuctra sp. | GAUF01084507 |  |
|  |  |  | GAUF01012297 |  |
|  |  | Perla marginata | GAVT01011223 |  |
|  | Zoraptera | Zorotypus caudelli | GAYA00104618 |  |
|  |  |  | GAYA0030434 |  |
|  | Orthoptera | Ceuthophilus sp. | GAU002040867 |  |
|  |  | Gryllus bimaculatus | GFMG01298068 |  |
|  |  | Laupla cerasina | GG001005618 |  |
|  |  | Tettix subulata | GAS002008536 |  |
|  |  |  | GAS002009651 |  |
|  |  |  | GSO00101905 |  |
|  |  | Xyla variegata | GCP01033443 |  |
|  |  |  | GCP01052609 |  |
|  | Mantophasmatodea | Tanzaniophasma sp. | GAX80105547 |  |
|  |  |  | GAX802030434 |  |
|  | Phasmatodea | Aretoa asperimus | GAWC01082819 |  |
|  |  |  | GAWC010105012 |  |
|  |  | Extatosoma tiaratum | GAWS001046894 |  |
|  |  |  | GAWG01048820 |  |
|  |  |  | GAWG01064895 |  |
|  | Medauroidea extradentata |  | GAWD001037141 |  |
|  |  |  | GAWD001049541 |  |
|  |  |  | GAWD001065334 |  |
|  |  |  | GAWD002032211 |  |
|  |  |  | GAWD002044630 |  |
|  |  |  | GAWD002032289 |  |
|  |  |  | GFRG01028988 |  |
|  |  |  | GFRG010121056 |  |
|  |  |  | GFRG01012227 |  |
|  | Blattodea | Blattella germanica | HG581866 |  |
|  |  |  | KN196784_1 |  |
|  |  |  | KN196784_2 |  |
|  | Cryptocercus wrighti |  | GA2N00045874 |  |
|  |  |  | GA2N00040031 |  |
|  |  |  | GA2N00048104 |  |
|  | Cryptoterme sequana |  | XP_023702637 |  |
|  |  |  | XP_023702527 |  |
|  | Embrimaterme neotenicus |  | MN000103 |  |
|  |  |  | MN000104 |  |
|  |  |  | MN000105 |  |
|  | Lamprobatta albipalpus |  | GCP010144670 |  |
|  |  |  | GCP010150496 |  |
|  |  |  | GCP010150204 |  |
|  | Panchlora nivea |  | GGLV01034977 |  |
|  |  |  | GGLV0105863 |  |
|  |  |  | GGLV01061540 |  |
|  | Prothrinotermes simplex |  | MHS60589 |  |
|  |  |  | MHS60588 |  |
|  |  |  | MHS60587 |  |
|  | Zoortermopsis nevadensis |  | KDR13786 |  |
|  |  |  | KDR21367 |  |
|  |  |  | KDR21366 |  |
| **Paraneoptera** | Thysanoptera | Frankliniella occidentalis | GP002677828 |  |
|  |  | Philaethripidae gen. sp. | GCP010249007 |  |
|  | Sternorrhyncha | Acrystosiphon pisum | XP_001942660 |  |
|  |  |  | XP_001859197 |  |
|  |  | Adelges tsuga | GBX01018946 |  |
|  |  |  | GBX01018510 |  |
|  |  | Aphidius rufus | ARD07922 |  |
|  |  | Bemisia tabaci | XP_018897134 |  |
|  |  | Daktulosphaira vitifoliae | GDEB01056903 |  |
|  |  | Diaphorina citri | GDEB01025952 |  |
|  |  |  | GDEB01020876 |  |
|  |  |  | XP_00879213 |  |
|  |  |  | GAC01009847 |  |
|  |  |  | XP_015363980 |  |
|  |  |  | XP_015379515 |  |
## Supplementary Table 1, continued

| higher unit order | species | acc. number | note |
|-------------------|---------|-------------|------|
| Paraneoptera | Myzus persicae | XP_022180848, XP_022180005 | |
| Paraneoptera | Pachysyrelfa venusta | GAAP01019620, GAAP01066619+21 |
| Paraneoptera | Phenacoccus solenopsis | GGT01015335, GGT01013183 |
| Paraneoptera | Campylodactyla latipes | GCVW01051784 |
| Paraneoptera | Clastoptera arizonana | GDC01010167, GDC01006489 |
| Paraneoptera | Cuerna arida | GEC01003109 |
| Paraneoptera | Diceroprocta seminicta | GGHY01012960 |
| Paraneoptera | Euscelidius varieatus | GFTU01000789, GFTU01000338, GFTU01001093 |
| Paraneoptera | Graminella nigrifrons | GAQX01001714, GAQX01006206, GAQX01002061 |
| Paraneoptera | Graphocephala atrapunctata | GBQ01020846_03005496 |
| Paraneoptera | Homalodisca luteola | GECU01004804, GECU01010637 |
| Paraneoptera | Homalodisca viripennis | HVIT002229_PA, HVIT005314_PA, HVIT005312_PA, HVIT016189_PA |
| Paraneoptera | Mapuche sp. | GCXU01004447_1045662 |
| Paraneoptera | Neotibicen dorsatus | GCYV01005367, GCYV01004607, AY24639 |
| Paraneoptera | Nilaparvata lugens | GCXU01002061 |
| Paraneoptera | Alydus pilosus | GCVW01036674, GCWY01047286 |
| Paraneoptera | Anasa tristis | XP_014256336, XP_014242611 |
| Paraneoptera | Aquarius paludum | INR1, INR1-like |
| Paraneoptera | Cimex lectularius | XP_014256336, XP_014242611 |
| Paraneoptera | Gerris buenoi | INR1, INR1-like |
| Paraneoptera | Halymorpha halys | XP_014217440, XP_014273515 |
| Paraneoptera | Hebrus sp. | INR2, INR1, INR1-like |
| Paraneoptera | Hydrometra cumata | INR2, INR1, INR1-like |
| Paraneoptera | Jadera haematoloma | AVT56265, AVT56264 |
| Paraneoptera | Largus californicus | GCXW01035597, GCXW01053901 |
| Paraneoptera | Limnopus dissoritis | INR2, INR1, INR1-like |
| Paraneoptera | Lygus hesperus | JAG02168, JAG020929 |
| Paraneoptera | Mesovelia furcata | INR2, INR1, INR1-like |
| Paraneoptera | Microvelia longipes | INR2, INR1, INR1-like |
| Paraneoptera | Oncapeltus fasciatus | AVT56270 |
| Paraneoptera | Pagasa sp. | GCXW01035597, GCXW01053901 |
| Paraneoptera | Podisus maculiventris | GFUB01078628, GFUB01066485 |
| Paraneoptera | Pyrrhocoris apterus | KKO87104, KKO87103, KKO87105 |
| Paraneoptera | Rhagovelia antillea | INR2, INR1, INR1-like |
| Paraneoptera | Rhodnius prolixus | GECX01013918, GECX01019799 |

Sequences available in Armisén et al. (2018) whole genome shotgun.
| Higher unit | Order       | Species                          | acc. number       | note                                      |
|-------------|-------------|----------------------------------|-------------------|-------------------------------------------|
| Paraneoptera| Psocodea    | 
|             |             | Coccinella sanguinea             | GDFG001033096     |                                           |
|             |             | Sericinus undulatus               | GCDX00101296      |                                           |
|             |             | Syntarsus capitatus              | GCVW00102679      |                                           |
|             |             | Pedicululus humanus corporis     | GAYV00100422      |                                           |
|             |             |                                  | XMJ00043015       |                                           |
| Holometabola| Hymenoptera | Acromyrmex echinatior            | XP_011062671      | used only in detailed analysis (Figure S8) |
|             |             | Apis mellifera                   | XP_011051377      | used only in detailed analysis (Figure S8) |
|             |             |                              | NP_001233506      |                                           |
|             |             | Athalia rosae                    | LDC105688867      |                                           |
|             |             | Bombus terrestris                | XP_02255985       |                                           |
|             |             | Cephus cinctus                   | XP_022560956      |                                           |
|             |             | Cephalthera woodsia              | XP_011444410      | used only in detailed analysis (Figure S8) |
|             |             | Copidosoma floridanum            | XP_014208001      | used only in detailed analysis (Figure S8) |
|             |             | Fopius arisanus                  | XP_0113000057     |                                           |
|             |             | Nasonia vitripennis              | XP_008203941      |                                           |
|             |             | Neodiprion lecontei              | XP_01518357       | used only in detailed analysis (Figure S8) |
|             |             | Solenopsis invicta               | JF304723          |                                           |
|             |             | Trachymyrmex zeteki              | XP_0218305319     | used only in detailed analysis (Figure S8) |
|             |             | Trichogramma pretiosum           | XP_014228758      | used only in detailed analysis (Figure S8) |
|             |             | Trichomalous sarcoptae           | XP_011882700      | used only in detailed analysis (Figure S8) |
|             |             | Vollenhovia emeryi                | XP_011864732      | used only in detailed analysis (Figure S8) |
|             |             | Wasmannia auropunctata           | XP_011696536      | used only in detailed analysis (Figure S8) |
| Holometabola| Coleoptera  | Dendroctonus ponderosae          | XP_019755880      |                                           |
|             |             | Leptinotarsa decemlineata       | XP_023030119      |                                           |
|             |             | Pogonius chalcoides              | XP_02301333       |                                           |
|             |             | Tribolium castaneum              | XP_01829423       |                                           |
| Holometabola| Raphidioptera| Xanthostigma xanthostigma        | GAUX00204510      |                                           |
|             |             | Chrysopa pallens                 | GAUX00204470      |                                           |
| Holometabola| Neuroptera  |                                                              | AVKX0043099       |                                           |
|             |             |                                                              | AVKX0043099       |                                           |
| Holometabola| Lepidoptera | Bombyx mori                      | NP_001037011      |                                           |
|             |             |                                  | XP_00925605       | DR2                                       |
|             |             | Danaus plexippus                 | ENH505707         | DR2                                       |
|             |             | Plutella xylostella              | XP_011567916      | DR2                                       |
|             |             |                                  | XP_011567028      | DR2                                       |
| Holometabola| Trichoptera | Anulapilia sp.                    | GATX01009528      | DR2                                       |
|             |             | Platycerthys radians             | GATX01012065      | DR2                                       |
|             |             | Ceratophylus gallinae            | GAXC00202916      | DR2                                       |
|             |             | Ctenocladus felis                | GAXC00202878      | DR2                                       |
| Holometabola| Siphonaptera|                                                                | GAUX002019316     |                                           |
|             |             | Aedes aegypti                    | Q39105            |                                           |
|             |             | Anopheles sinensis               | FAA011010         | DR2                                       |
|             |             | Ceratitis capitata               | KF84958           | DR2                                       |
|             |             | Clunio marinus                   | KF849143          | DR2                                       |
|             |             | Drosophila melanogaster          | AAF452722         | DR2                                       |
|             |             | Liogma simplicicornis            | AAF452727         | DR2                                       |
|             |             | Musca domestica                  | NP_635048         | SDR                                       |
|             |             | Nyssomyia neivai                 | NP_724517         | DR2                                       |
|             |             | Sarcoptes peregrina              | GJAY006400        | SDR                                       |
| Holometabola| Diptera     | Aedes aegypti                    | Q39105            |                                           |
|             |             | Anopheles sinensis               | FAA011010         | DR2                                       |
|             |             | Ceratitis capitata               | KF84958           | DR2                                       |
|             |             | Clunio marinus                   | KF849143          | DR2                                       |
|             |             | Drosophila melanogaster          | AAF452722         | DR2                                       |
|             |             | Liogma simplicicornis            | AAF452727         | SDR                                       |
|             |             | Musca domestica                  | NP_635048         | SDR                                       |
|             |             | Nyssomyia neivai                 | NP_724517         | DR2                                       |
|             |             | Sarcoptes peregrina              | GJAY006400        | SDR                                       |

**Supplementary Table 1, continued**
Supplementary Table 2. Accession numbers of genomic sequences used for gene structure analysis

| higher unit          | order                  | species                  | acc. number | note                                      |
|----------------------|------------------------|--------------------------|-------------|-------------------------------------------|
| Crustacea            | Cladocera              | Daphnia pulex            | FLTH02000001.1 | Cluster I                                |
|                      |                        |                          | FLTH02000008 | Cluster II                                |
| Palaeoptera          | Ephemeroptera          | Ephemeria danica         | AYNO2013259.1 | Cluster I                                |
| Polyneoptera         | Phasmatodea            | Prophorimorphus simplex  | MH560587    | Cluster I (InR1 in this study)            |
|                      |                        |                          | MH560588    | Cluster II (InR2 + InR3 in this study)    |
| Polyneoptera         | Blattodea              | Geras buenoi             | KZ651042.1  | Cluster I (InR1)                          |
|                      |                        |                          | JHBY02104847.1 | Cluster I (InR1-like)                     |
|                      |                        |                          | KZ651190.1  | Cluster II                                |
|                      |                        | Palaeoptera              | MN987938    | Cluster I (InR1a in this study)           |
|                      |                        |                          | MN987939    | Cluster II (InR2 in this study)           |
|                      |                        | Paraneoptera             | CM009478.1  | Cluster I                                |
|                      |                        |                          | PGTA01000614 | Cluster II                                |
|                      |                        | Paraneoptera             | DS238541.1  | Cluster I                                |
|                      |                        |                          | NC_037639.1 | Cluster I                                |
|                      |                        |                          | NC_037646.1 | Cluster II                                |
|                      |                        | Holometabola             | KZ651042.1  | Cluster I                                |
|                      |                        |                          | JHBY02104847.1 | Cluster I (InR1-like)                     |
|                      |                        |                          | KZ651190.1  | Cluster II                                |
|                      |                        | Paraneoptera             | KZ651042.1  | Cluster I                                |
|                      |                        |                          | JHBY02104847.1 | Cluster I (InR1-like)                     |
|                      |                        |                          | KZ651190.1  | Cluster II                                |
|                      |                        | Holometabola             | NW_004582016.1 | Cluster I                                |
|                      |                        |                          | NW_004581734.1 | Cluster II (DR2)                         |
|                      |                        | Paraneoptera             | KX087101    | Cluster I (InR1)                          |
|                      |                        |                          | KX087104    | Cluster II (DR2)                         |
|                      |                        |                          | KX087105    | Cluster I (InR1-like)                     |
|                      |                        |                          | GDF101014277 | Cluster I                                |
|                      |                        | Drosophila melanogaster  | KX087103    | Cluster I (InR1)                          |
|                      |                        |                          | KX087104    | Cluster II (DR2)                         |
|                      |                        |                          | KX087105    | Cluster I (InR1-like)                     |
|                      |                        |                          | NM_170460   | Cluster I                                |

Supplementary Table 3. Primers used for qRT PCR analyses of InRs and decoy of InR

| species               | gene   | accession number | forward primer (5’-3’)        | reverse primers (5’-3’)          |
|-----------------------|--------|------------------|-------------------------------|----------------------------------|
| Prophorimorphus simplex | InR1  | MH560587         | CTGCCAGGCTTACAGACGCT          | ATGGTGCCGTGTCACTCAT               |
|                       | InR2  | MH560589         | CCCCGGCTAGCAGGAGATTTTGG       | AGCCAGACTCACATATTTCAGGATT         |
|                       | InR3  | MH560588         | TCCCGCGATGCTTGGATG            | TCTCGAGGACCATCCATG               |
|                       | rp49  | GASE02006626     | CTGGTGCATAACGGAAGGACT          | CAGGGAGGACCTAGCATTTGTGA          |
| Pyrrhocoris apterus   | InR1a | KX087103         | TGTAATGTCGATGCCAGCAAG         | GTGACACTGAGAAAACGACCC            |
|                       | InR1b | KX087104         | TCCTGAGGTAGGGAAGTAC           | CGTCAAGGGTTCTACTAAGAGGA          |
|                       | InR2  | KX087105         | GGGCTGCGAGGACCTGTAGG          | CTGCGAGGACTCTAGCCTGAG            |
|                       | rp49  | GDF101014277     | CCGATATGAAAACCTAGGAGAAAAC     | GAGAGTCTGCTGCCGTTTTT             |
| Drosophila melanogaster| InR  | CG18402; AAF55903 | AAGTCTGCGGTTAGCACT             | TCTCGGCGAGAGACCTAGAT             |
|                       | SDR   | CG3837; NP_650408 | CATGAGAAGTGGTGATG             | CTGCGAGGACTCTAGCCTGAG            |
|                       | DR2   | CG10702; NP_724157 | GATGTGAGAAGCCTCAT             | CACCAAAACGAGAACAGAG             |
|                       | rp49  | NM_170460        | GATATGAAAGTGGTGTCACA          | CACCAAAACGAGAACAGAG             |

Supplementary Table 4. Primers used for dsRNA templates in P. apterus

| gene | accession number | forward primer (5’-3’) | reverse primers (5’-3’)          |
|------|------------------|-------------------------|----------------------------------|
| InR1a| KX087103         | CTGTAGAATGGCCTACCAA     | GCATGCAAATTTGTCCTCAT             |
| InR1b| KX087104         | GGATAGATTGGGGATTGGA     | AGCAGCGACCTTGCTCAGTAG            |
| InR2 | KX087105         | TTTACGCTACCTACCAAAAGAC | GTTAGACGAGCAGACGAGTACCA          |
| Pilp1| MN200106         | ACTGTGTTTACAGGAGCTCC    | ACCTGTGTAACCTCTCAGTTCTAGAG       |
| Pilp2/3| MN200107/MN200108 | AGAAGAGCATTGGAGCAGCA    | CACCGCTGAGAGCAGAGG               |
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