Supplementary material
to

Marxer M, Vollenweider V, Schmid-Hempel P:
"Insect antimicrobial peptides act synergistically to inhibit a trypanosome parasite"

Table S1 Amino acid sequences of the active parts of the three antimicrobial peptides used in this study. The used sequences were - at time of the study - modeled after the case of *B. pascuorum*, but enriched by EST-information from *B. terrestris*. See also Figure S6 for alignments of currently known sequences in different species.

Peptide	Amino acid sequence
Abaecin	FVPYNPPRPGQSKPFPTFPGHGPFNPKIQWPYPLPNPGH
Hymenoptaecin	HADPQGSLVINGKKPLSGPDRRPSLDVDYHQRVYDRNGMNADAYGGLNIRPGQPAQPHLGVQIQREYKNGFIRGYSQAERGPGGRISPSFGVGGGFRF
Defensin	VTCDLLSIKGVAEHSACAANCLSMGKAGGRCENGVCLCRKTNFKDLWDKRF

Table S2. Summary table for peptide concentrations (in µM) of each of the three AMPs applied singly – *Abaecin*, *Defensin* and *Hymenoptaecin*, and which yielded the maximum effect on each of the eight strains tested, and for the three estimated growth parameters – lag phase λ, maximal growth rate µ, and maximal cell numbers, maxA (see Fig.1, main text). Parameters are measured from three replicates of each strain tested.

		Lag phase, λ[a,b]		**Growth rate, µ[a,b]**		**Level, max A[a,b]**	
Strain ID	**AMPs**	**Concentration (µM)**	**Max. Lag**	**Concentration (µM)**	**Min. Grow**	**Concentration (µM)**	**Min. A**
08068	Abaecin	0	0.000	40	0.002	40	0.276
	Defensin	0	*ND*	40	0.004	20	0.217
	Hymenoptaecin	20	0.000	40	0.002	40	0.230
08075	Abaecin	40	0.951	0	0.002	0	0.262
	Defensin	0	*ND*	10	0.002	10	0.242
	Hymenoptaecin	10	1.258	0	0.002	0	0.208
08076	Abaecin	0	0.000	20	**0.001**	20	0.174
	Defensin	0	*ND*	2.5	0.006	10	0.215
	Hymenoptaecin	40	0.000	20	**0.001**	20	0.188
08157	Abaecin	40	25.551	0	0.002	40	0.251
	Defensin	0	*ND*[1]	20	0.004	0	**0.171**
	Hymenoptaecin	0	0.000	40	0.002	40	0.272
08161	Abaecin	0	25.735	20	0.004	20	0.345
	Defensin	0	*ND*	20	0.002	40	0.314
	Hymenoptaecin	0	1.921	20	0.003	20	0.275
08261	Abaecin	0	10.234	0	0.003	0	0.318
	Defensin	0	*ND*	20	0.002	20	0.243
	Hymenoptaecin	20	10.970	0	0.002	0	0.259
10208	Abaecin	20	4.934	40	0.003	20	0.304
	Defensin	0	*ND*	0	0.004	20	0.317
	Hymenoptaecin	0	0.000	40	0.003	40	0.324
10361	Abaecin	20	**38.127**	0	0.003	0	0.302
	Defensin	0	*ND*	0	0.003	0	0.285
	Hymenoptaecin	0	17.313	20	0.002	40	0.242

(a) Bold font indicates the maximal effect.

(b) *ND*: No detectable effect found.

Figures

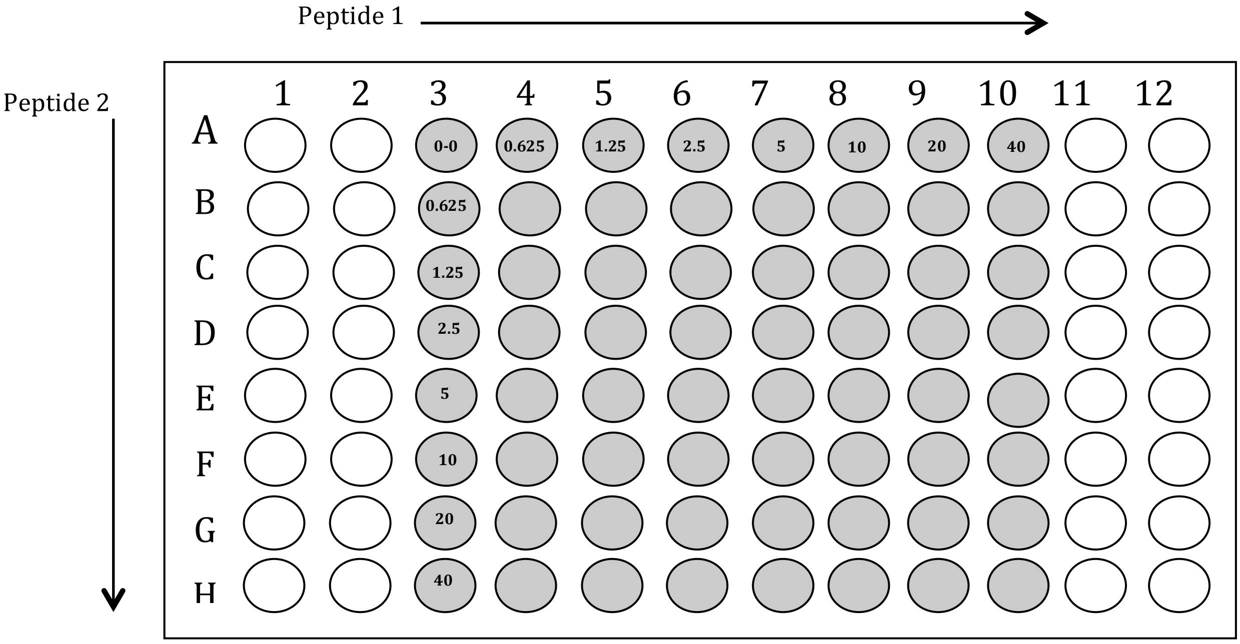


Figure S1. Schematic view of a 96-well plate matrix used for the peptide combination assay of two different antimicrobial peptides inhibiting the growth of *C. bombi* cells.

A treatment matrix was created with a peptide concentration dilution series of the first peptide in rows (0, 0.625, 1.25, 2.5, 5, 10, 20 and 40 µM) and the second peptide dilution series in columns (0, 0.625, 1.25, 2.5, 5, 10, 20 and 40 µM). Hence, a total of 64 distinct peptide combination treatments were generated per plate. Each specific combination/concentration was replicated three times on different plates, and the position of a given combination/concentration rotated on each plate.

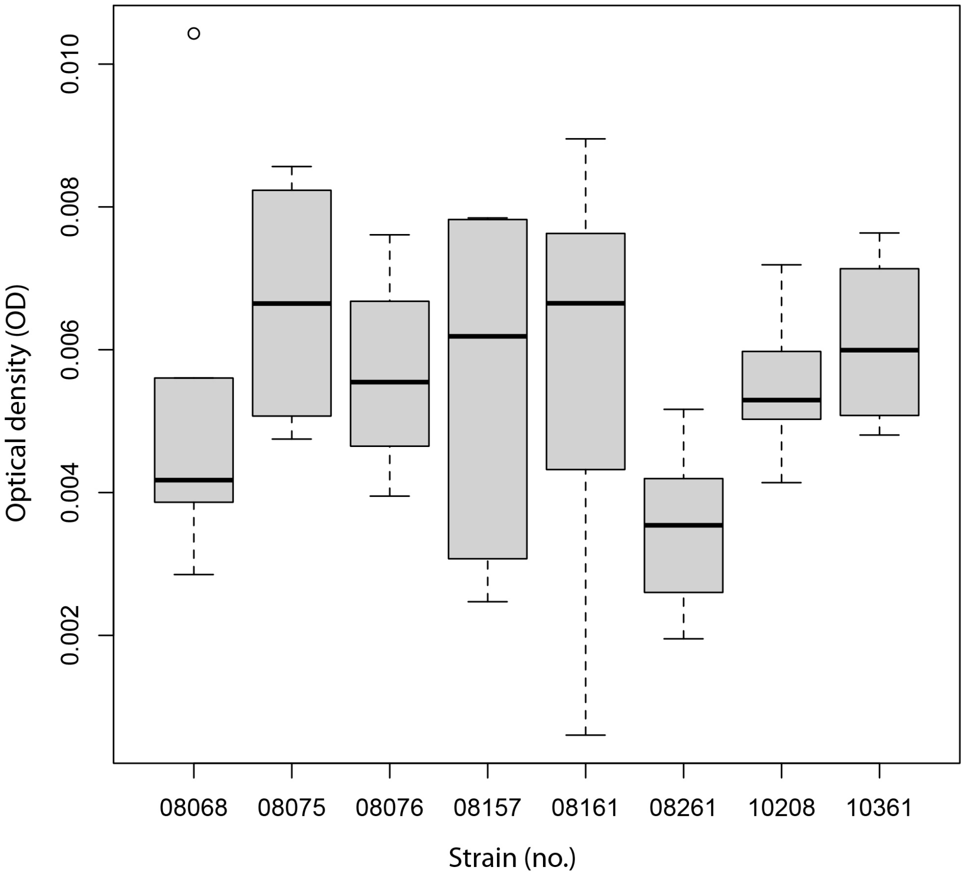
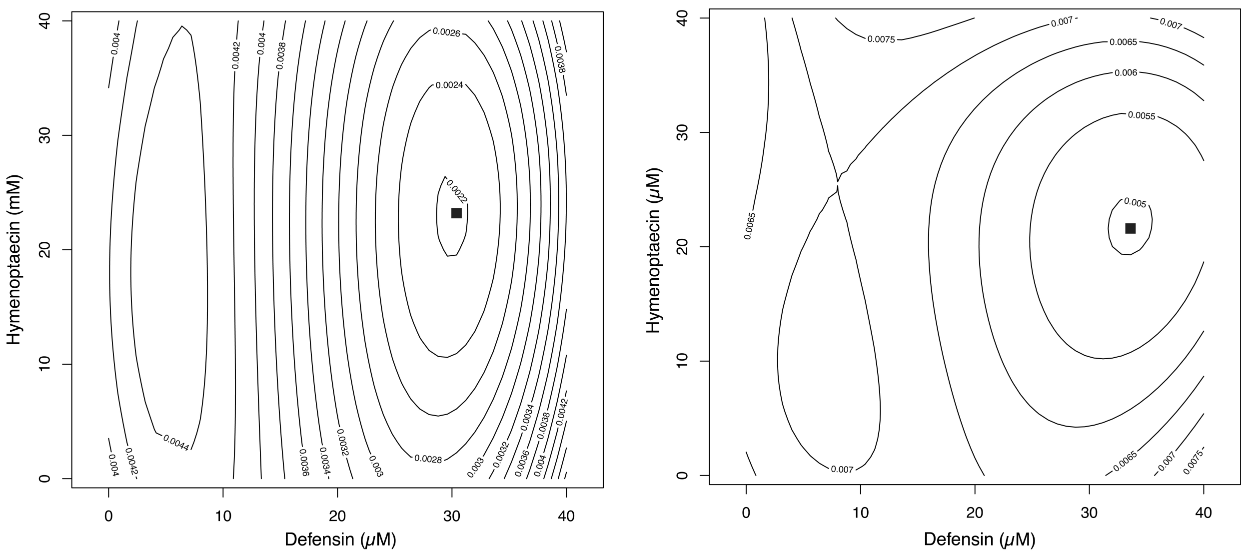


Figure S2. Boxplot of the maximum growth rates (OD), as estimated from fitting a spline with *grofit*(), for the eight tested strains (strain nos.) and under control conditions with no AMPs present (one-way ANOVA for the effect of strain: $F_{7,33}$ =1.06, P = 0.4). Each strain was tested in six replicates.

(A) strain #08068

(B) strain #08076



(C) strain #10208

(D) strain #10361

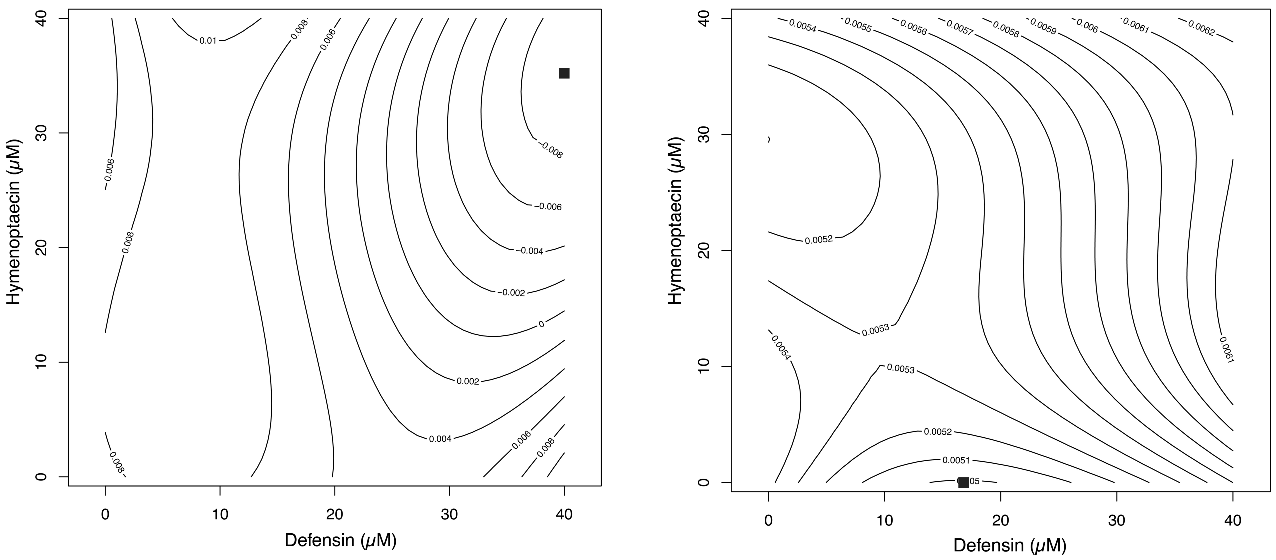
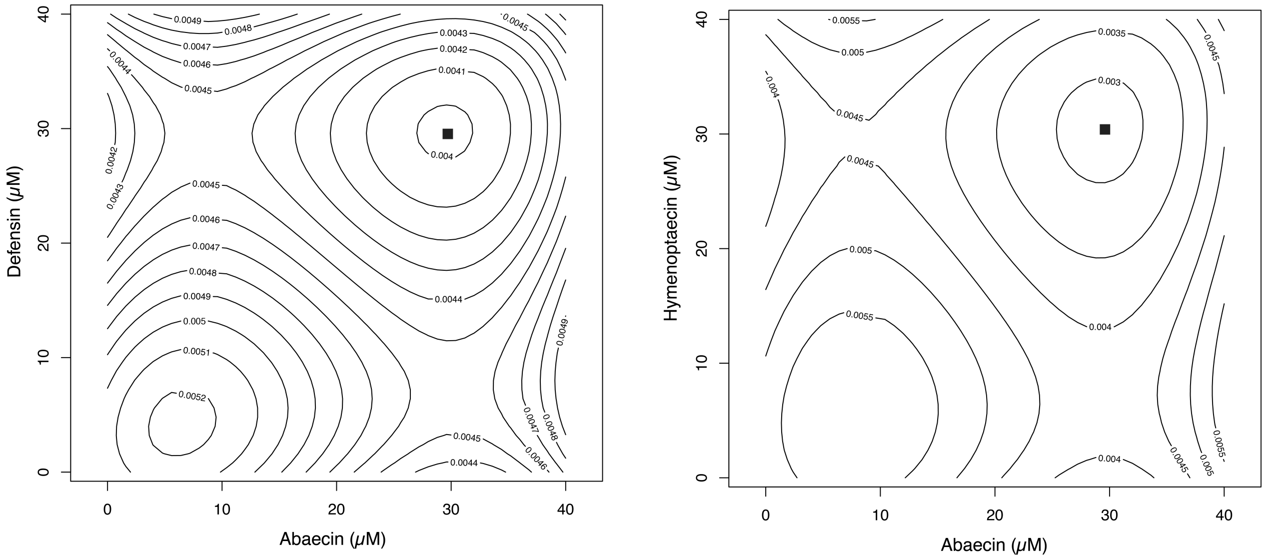


Figure S3. Contour plots representing maximal growth rates (μ; see Fig.1 in text) of four different *C. bombi* strains in the treatment with combinations of *Defensin* and *Hymenoptaecin.*

The observed growth rates are fitted with a least-square surface of 3rd polynomial order, which is shown here as a contour plot landscape. Lines represent the isobars and correspond to the effects of the interaction of both antimicrobial peptides, *Hymenoptaecin* and *Defensin* on maximal growth rate (measured as optical density) against four different *C. bombi* strains – strain nos. 08068 (A), 08076 (B), 10208 (C) and 10361 (D). Concentrations of antimicrobial peptides (x and y-axis) are in units of μM. The black square in each graph marks the optimal combination-concentration needed to reduce this parasite strain's growth rate to a minimum.

(A) Abaecin x Defensin, all strains

(B) Abaecin x Hymenoptaecin, all strains



(C) Defensin x Hymneoptaecin, all strains

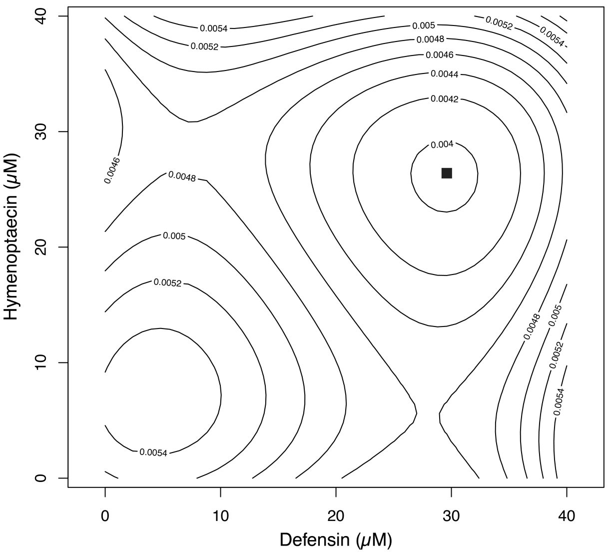


Figure S4. Contour plots representing maximal growth rates (μ; see Fig.1 in text) achieved from the pooled results of testing he eight different *C. bombi* strains. Here, the combination of AMP varies with: (A) *Abaecin* and *Defensin,* (B) *Abaecin* and *Hymenoptaecin*, and (C) *Defensin* and *Hymenoptaecin*. The black square in each graph marks the optimal combination-concentration needed to reduce the parasite growth rate to a minimum. For further explanations, see Fig. S3.

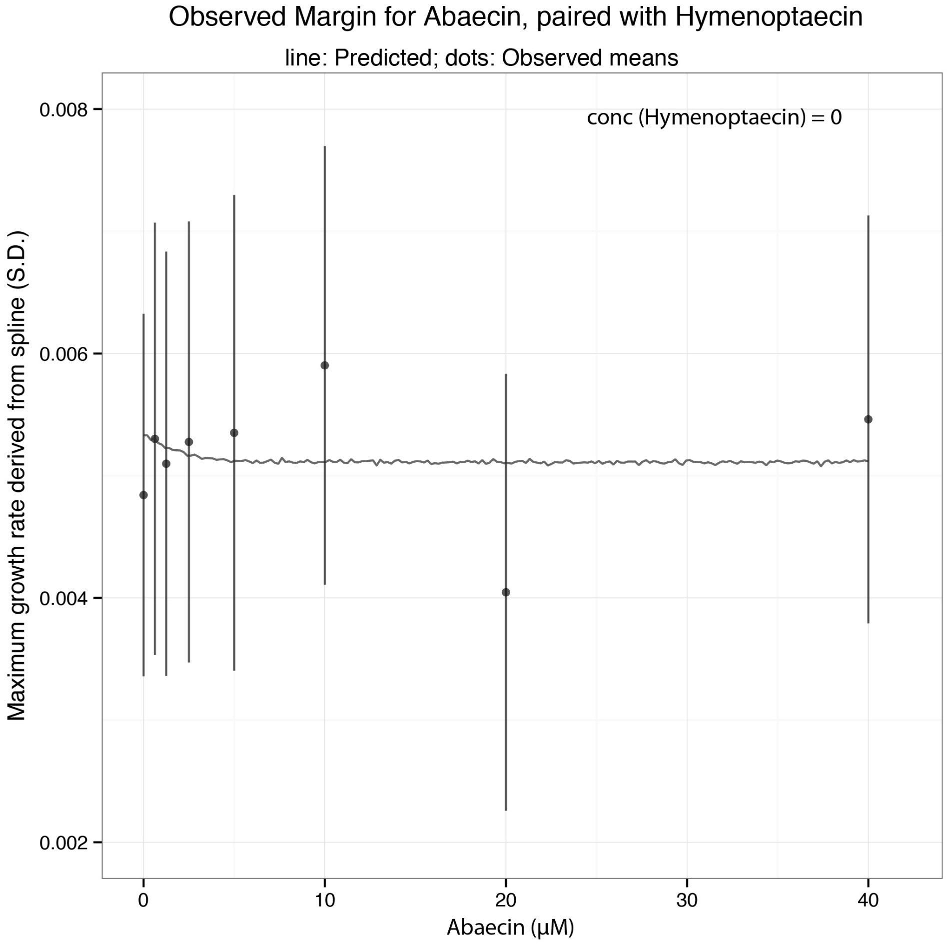
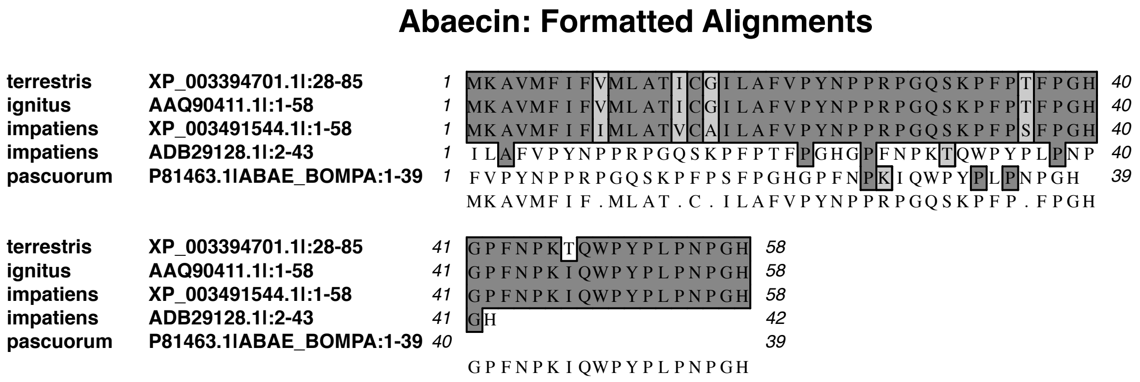


Figure S5. The second "margin" (*c.f.* Fig.3 of main text) of the two-peptide combination assay *Abaecin* x *Hymenoptaecin*. Shown are the observed means (dots, S.D.) of the estimated growth rates from spline when only one AMP (*Abaecin*) is varied across a range of concentrations (0, 0.625, 1.25, 2.5, 5, 10, 20 and 40 µM), and the other one (*Hymenoptaecin)* is not used. The line is the fitted Hill equation as a standard model for the dose-response curve (see Methods). At each concentration, three replicates were measured. The dose-response curve was subsequently used to calculate the expectations for a combined use of the two peptides.

Figure S6. Aligned sequences of bumblebee *Abaecin*, *Defensin,* and *Hymenoptaecin*. *Bombus*-species as indicated; accession number given. Sequences were retrieved by BLAST from NCBI and aligned with MacVector V 14.5.

(1) Abaecin



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impatiens    XP_003491544.1|:1-58        1  MKAVMFIFIMLATVCAILAFVPYNPPRPGQSKPFPSFPGH  40
impatiens    ADB29128.1|:2-43            1   ILAFVPYNPPRPGQSKPFPTFPGHGPFNPKTQWPYPLPNP 40
pascuorum    P81463.1|ABAE_BOMPA:1-39    1   FVPYNPPRPGQSKPFPSFPGHGPFNPKIQWPYPLPNPGH  39
                                            MKAVMFIF.MLAT.C.ILAFVPYNPPRPGQSKPFP.FPGH

terrestris   XP_003394701.1|:28-85      41  GPFNPKTQWPYPLPNPGH  58
ignitus      AAQ90411.1|:1-58           41  GPFNPKIQWPYPLPNPGH  58
impatiens    XP_003491544.1|:1-58       41  GPFNPKIQWPYPLPNPGH  58
impatiens    ADB29128.1|:2-43           41  GH                  42
pascuorum    P81463.1|ABAE_BOMPA:1-39   40                      39
                                            GPFNPKIQWPYPLPNPGH
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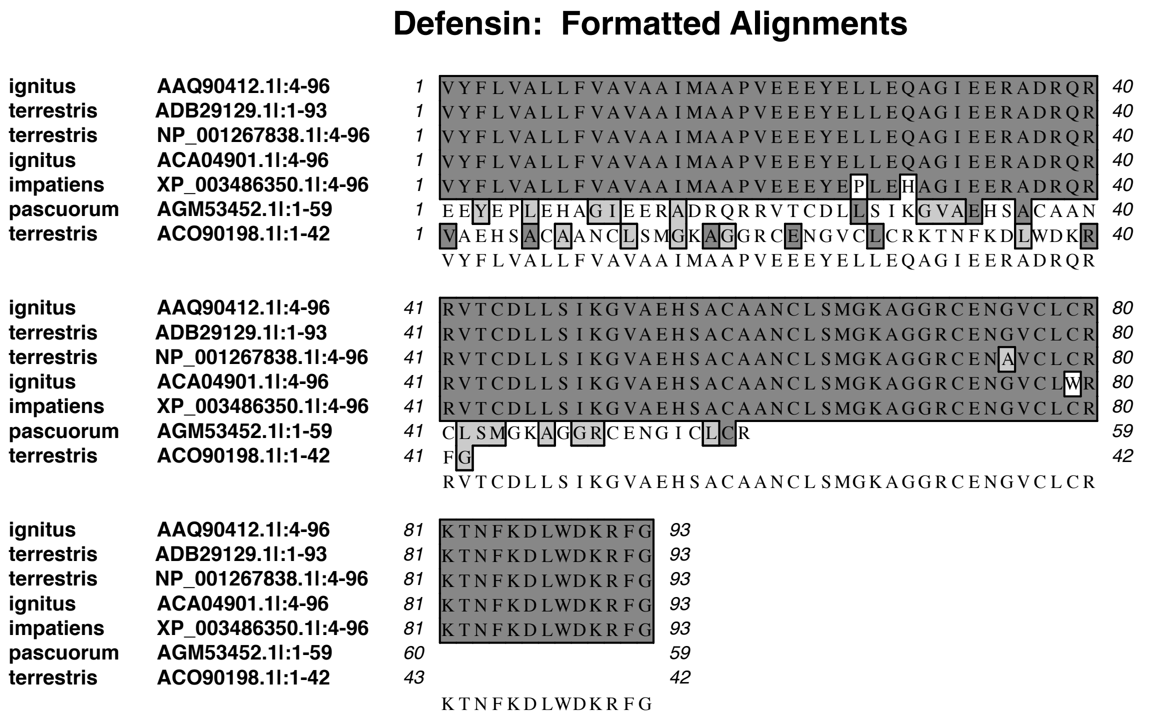
(2) Defensin



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terrestris   ADB29129.1|:1-93         1  VYFLVALLFVAVAAIMAAPVEEEYELLEQAGIEERADRQR  40
terrestris   NP_001267838.1|:4-96     1  VYFLVALLFVAVAAIMAAPVEEEYELLEQAGIEERADRQR  40
ignitus      ACA04901.1|:4-96         1  VYFLVALLFVAVAAIMAAPVEEEYELLEQAGIEERADRQR  40
impatiens    XP_003486350.1|:4-96     1  VYFLVALLFVAVAAIMAAPVEEEYEPLEHAGIEERADRQR  40
pascuorum    AGM53452.1|:1-59         1  EEYEPLEHAGIEERADRQRRVTCDLLSIKGVAEHSACAAN  40
terrestris   ACO90198.1|:1-42         1  VAEHSACAANCLSMGKAGGRCENGVCLCRKTNFKDLWDKR  40
                                         VYFLVALLFVAVAAIMAAPVEEEYELLEQAGIEERADRQR

ignitus      AAQ90412.1|:4-96        41  RVTCDLLSIKGVAEHSACAANCLSMGKAGGRCENGVCLCR  80
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terrestris   NP_001267838.1|:4-96    41  RVTCDLLSIKGVAEHSACAANCLSMGKAGGRCENAVCLCR  80
ignitus      ACA04901.1|:4-96        41  RVTCDLLSIKGVAEHSACAANCLSMGKAGGRCENGVCLWR  80
impatiens    XP_003486350.1|:4-96    41  RVTCDLLSIKGVAEHSACAANCLSMGKAGGRCENGVCLCR  80
pascuorum    AGM53452.1|:1-59        41  CLSMGKAGGRCENGICLCR                      59
terrestris   ACO90198.1|:1-42        41  FG                                       42
                                         RVTCDLLSIKGVAEHSACAANCLSMGKAGGRCENGVCLCR

ignitus      AAQ90412.1|:4-96        81  KTNFKDLWDKRFG  93
terrestris   ADB29129.1|:1-93        81  KTNFKDLWDKRFG  93
terrestris   NP_001267838.1|:4-96    81  KTNFKDLWDKRFG  93
ignitus      ACA04901.1|:4-96        81  KTNFKDLWDKRFG  93
impatiens    XP_003486350.1|:4-96    81  KTNFKDLWDKRFG  93
pascuorum    AGM53452.1|:1-59        60                59
terrestris   ACO90198.1|:1-42        43                42

                                         KTNFKDLWDKRFG
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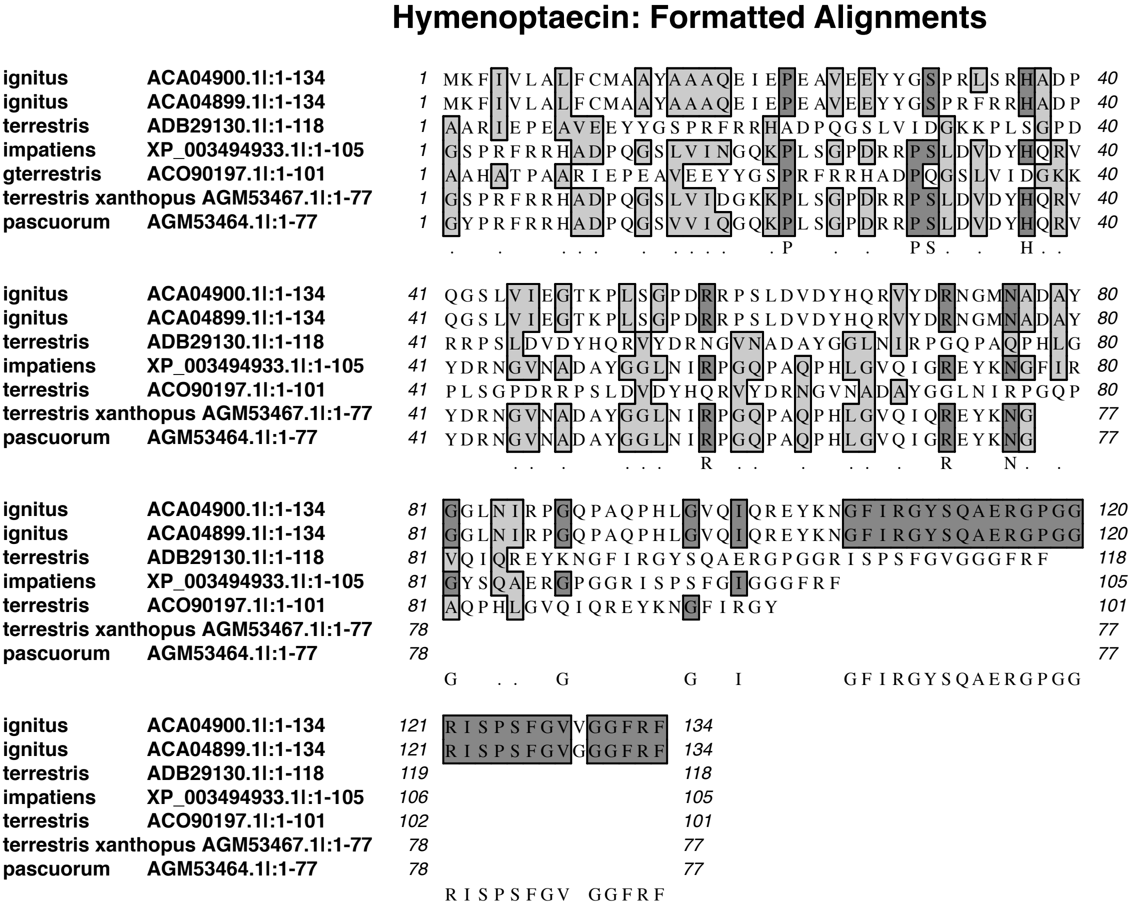
(3) Hymenoptaecin



Hymenoptaecin: Formatted Alignments

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terrestris          ADB29130.1l:1-118         1  AARIEPEAVEEYYGSPRFRRHADPQGSLVIDGKKPLSGPD  40
impatiens           XP_003494933.1l:1-105     1  GSPRFRRHADPQGSLVINGQKPLSGPDRRPSLDVDYHQRV  40
gterrestris         ACO90197.1l:1-101         1  AAHATPAARIEPEAVEEYYGSPRFRRHADPQGSLVIDGKK  40
terrestris xanthopus AGM53467.1l:1-77         1  GSPRFRRHADPQGSLVIDGKKPLSGPDRRPSLDVDYHQRV  40
pascuorum           AGM53464.1l:1-77          1  GYPRFRRHADPQGSVVIQGQKPLSGPDRRPSLDVDYHQRV  40
                                                 .  .   ...  . ....  .P  . .  PS. .  H..

ignitus             ACA04900.1l:1-134        41  QGSLVIEGTKPLSGPDRRPSLDVDYHQRVYDRNGMNADAY  80
ignitus             ACA04899.1l:1-134        41  QGSLVIEGTKPLSGPDRRPSLDVDYHQRVYDRNGMNADAY  80
terrestris          ADB29130.1l:1-118        41  RRPSLDVDYHQRVYDRNGVNADAYGGLNIRPGQPAQPHLG  80
impatiens           XP_003494933.1l:1-105    41  YDRNGVNADAYGGLNIRPGQPAQPHLGVQIGREYKNGFIR  80
terrestris          ACO90197.1l:1-101        41  PLSGPDRRPSLDVDYHQRVYDRNGVNADAYGGLNIRPGQP  80
terrestris xanthopus AGM53467.1l:1-77        41  YDRNGVNADAYGGLNIRPGQPAQPHLGVQIQREYKNG     77
pascuorum           AGM53464.1l:1-77         41  YDRNGVNADAYGGLNIRPGQPAQPHLGVQIGREYKNG     77
                                                     .. .   ...  R ..  .  .. .  R   N. .

ignitus             ACA04900.1l:1-134        81  GGLNIRPGQPAQPHLGVQIQREYKNGFIRGYSQAERGPGG  120
ignitus             ACA04899.1l:1-134        81  GGLNIRPGQPAQPHLGVQIQREYKNGFIRGYSQAERGPGG  120
terrestris          ADB29130.1l:1-118        81  VQIQREYKNGFIRGYSQAERGPGGRISPSFGVGGGFRF    118
impatiens           XP_003494933.1l:1-105    81  GYSQAERGPGGRISPSFGIGGGFRF                 105
terrestris          ACO90197.1l:1-101        81  AQPHLGVQIQREYKNGFIRGY                     101
terrestris xanthopus AGM53467.1l:1-77        78                                            77
pascuorum           AGM53464.1l:1-77         78                                            77
                                                 G  ..  G       G  I      GFIRGYSQAERGPGG

ignitus             ACA04900.1l:1-134       121  RISPSFGVVGGFRF  134
ignitus             ACA04899.1l:1-134       121  RISPSFGVGGGFRF  134
terrestris          ADB29130.1l:1-118       119                  118
impatiens           XP_003494933.1l:1-105   106                  105
terrestris          ACO90197.1l:1-101       102                  101
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pascuorum           AGM53464.1l:1-77         78                   77
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