Supporting Information

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SI Experimental Procedures

Insects. Female specimens of 19 *Philanthus* species and subspecies from North America, Germany, Turkey, and South Africa; five *Trachypus* species from South America; and one *Philanthinus* species from Turkey were collected (Table S4). Adult females were paralyzed by placing them into a freezer for 30 min (if available) and killed by decapitation. Antennae were immediately transferred to ∼500 μL methanol and kept in methanol until extracts could be processed in the laboratory. If cocoons were available through excavation of beewolf nests (*Philanthus triangulum*, *Philanthus gibbosus*, and *Trachypus elongatus*), they were either frozen or extracted immediately after removal from the brood cells after removal of the larva from the cocoon. For *Philanthinus quattuordecimpunctatus* and all *Philanthus* species from the United States, extracts from different individuals of the same species were pooled before analysis due to low concentrations of secondary metabolites.

HPLC-ESI-MS/MS Analysis. The extracts were concentrated to 100 μL, and 5 μL were subjected to UHPLC-ESI-MS/MS analysis on a Dionex UltiMate 3000 UHPLC (Thermo Fisher Scientific) coupled to an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific) equipped with an ESI source. Substances were separated by reverse-phase chromatography using 0.1% aqueous formic acid (A) and acetonitrile containing 0.1% formic acid (B) at a flow rate of 0.3 mL/min at 50 °C and with UV detection at 230 nm (gradient of solvent B: 0–12 min, hold at 45%; 12–32 min, 45–95%; 32–36 min, hold at 95%; symmetry, 3.5 μm C18 phase, 100 × 2.1 mm i.d.; Waters; symmetry guard column, 3.5 μm C18, 10 × 2.1 mm i.d.; Waters). Full scan data were acquired at a resolution of 30,000 and a mass range of *m/z* 100–1,500. MS/MS data were acquired with normalized collision energy of 35.0%. In the data-dependent acquisition mode, the five most abundant ions from the full scan were subjected to fragmentation. Fragmented ions were excluded from additional MS/MS experiments for 30 s to increase the number of fragmented and scanned precursor ions. Raw data files were recalibrated postacquisition with the RecalOffline software (Thermo Fisher Scientific) using the constantly present diisooctylphthalate (*m/z* 391.28429) as a reference mass.

Screen for Unique Compounds. Mass spectra of previously described secondary metabolites from *Streptomyces* bacteria were obtained from Antibase 2005 (1), and the beewolf extracts were screened for corresponding exact masses using the ToxID software (Version 2.1.1; Thermo Fisher Scientific). Additionally, we searched for possible modifications of the known compounds piericidin A1 and streptochlorin. For a more comprehensive screen for piericidin and streptochlorin derivatives, we used Mass Frontier 7.0 and MetWorks1.3 (both Thermo Fisher Scientific) to create candidate parent and fragment masses of piericidin, actinopyrone, and Mer-A2026 modifications and screened all extracts with ToxID again. First, we performed an isotope search of MetWorks 1.3 to screen for chlorine-containing compounds by searching the full scan for the characteristic isotope pattern of one and two chlorine atoms. Second, we used the fragment ion search of Mass Frontier 7.0 to detect derivatives of piericidin A1 based on MS/MS spectra that included the pyridine ring ($[C9H12NO3]^+$; *m/z* 182.08172). In contrast, the fragmentation pattern of all actinopyrones did not include an indicative ring fragment and thus, could not be detected via the MS/MS spectra. Compounds were identified based on MS/MS spectra obtained during data-dependent acquisition and by additional targeted MS/MS scans. We only performed relative quantification of the reported compounds, as the absolute amount of antibiotics was highly variable and likely depended on the species' size and the filling status of the antennal gland reservoirs upon collection. As external standards were not available for most compounds, we used the peak areas as proxies for the relative amounts of compounds, which may be biased due to different ionization efficiencies. Peak areas of all substances were quantified in Xcalibur software (Version 2.1; Thermo Fisher Scientific) with a mass tolerance of 3 ppm and transformed into relative peak areas.

Data Analysis. For visualization of the antibiotic composition across beewolf species, we calculated the decadic logarithm of the relative amount of the mean of each compound in single or pooled extracts of a species. The MultiExperiment Viewer software (2) was used to construct heat maps. As the relative peak areas of a sample are not statistically independent, the dataset was transformed before statistical analysis. We used the centered log ratio transformation according to Aitchison (3), which allows us to include peaks that are absent in some samples (4).

A discriminant analysis (DA) was performed with, in total, 242 extracts (including 220 single and 22 pooled extracts) to investigate whether the antibiotic profiles of the sampled species cluster according to the sampling location. Due to the large number of quantified substances, we reduced the number of variables using a principal component analysis before the DA (varimax rotation; eigenvalues > 1). Both analyses were performed with SPSS 23 (IBM). For the DA, the samples were grouped according to host species and tissue type (antenna or cocoon), and the first two discriminant functions were plotted to observe geographic patterns in the dataset. Additionally, a DA was performed with the samples grouped according to their geographic origin (South America, North America, Europe/Africa).

Procrustes analyses and Mantel and partial Mantel tests as well as Blomberg's *K* statistic and PGLS models were applied to assess correlations between the chemical composition of the antibiotic mixture, the host and symbiont phylogenies, and the environmental influence represented by the sampling location. Diversity measures [compound richness, evenness (Shannon's *E*), Shannon's *H*, Simpson's λ] of the chemical composition were calculated from absolute peak areas, and a Euclidean distance matrix was calculated from the centered log ratio-transformed dataset of relative peak areas that was also used for the heat map visualization using SPSS 23. The multigene alignments of the already established cophylogeny (5, 6) were reduced to match the species sampled in this study. For *Trachypus fulvipennis*, partial host gene sequences for arginine kinase, elongation factor 1-alpha, long-wavelength rhodopsin, wingless, cytochrome oxidase I, and 28S rRNA [National Center for Biotechnology Information (NCBI) accession numbers KU759557–KU759562] were obtained as described previously (5). Additionally, partial sequences of the symbiont genes gyrase A, gyrase B, elongation factor G, and Tu (including the intergenic spacer) and 16S rRNA were obtained for the symbionts of *T. fulvipennis*, and elongation factor G and Tu for *T. flavidus* (NCBI accession numbers KU759552–KU759556) (methods are in ref. 5). Phylogenetic trees for hosts and symbionts were reconstructed using approximately maximum likelihood analysis (FastTree 2.1) (7, 8) and Bayesian Inference (MrBayes 3.1.2) based on partitioned alignments (details are in ref. 5). The approximate maximum

likelihood trees used for the comparative displays were visualized with FigTree (tree.bio.ed.ac.uk/software/figtree/), and distance matrices for the Mantel tests were computed from the FastTree trees using the T-REX package via the webserver interface (9) (www.trex.uqam.ca). The geographic distances were calculated from the GPS coordinates of the sample locations using a web-based solution (www.koordinaten.de) and log-transformed. The Procrustes analysis and Mantel and partial Mantel tests were computed with the R software (Version 3.0.1) using the packages "permute" and "vegan" (Version 2.0–8), respectively. Spearman rank correlations were used, as not all datasets were normally distributed. The dendrograms based on the geographic and chemical distance matrices were calculated in PHYLIP (10) using the neighbor-joining algorithm.

To test for a phylogenetical signal of both the host and symbiont phylogeny on the composition of the antibiotic mixtures, we calculated Blomberg's K (11–13) for the different diversity measures and tested against a random change (P_R) as well as against expected evolutionary changes under a Brownian motion model (P_{BM}). A Blomberg's K value of one indicates that investigated traits in related taxa resemble each other according to a Brownian motion model of trait evolution, a value higher than one means that taxa resemble each other more than under this model, values lower than one indicate that they resemble each other less, and a value of zero indicates random change of the trait (no phylogenetic signal). Blomberg's K and P_R were calculated using R studio Version 1.0.143 and the command phylosig in the package "phytools," with 10,000 randomizations. A Brownian motion model of trait evolution was established, and P_{BM} calculated using the commands brownie.lite and fastBM in the package "phytools," with 10,000 randomizations.

Finally, we tested for an influence of geography by computing PGLS (14, 15) using the log-transformed Universal Transverse Mercator coordinates of sampling locations as input variables and correcting for host and symbiont phylogeny. PGLS using either a Brownian motion model or an Ornstein–Uhlenbeck model of trait evolution (16) was computed using the command gls of the R package "nlme." We used Akaike's Information Criterion to select the better fitting model of evolution. The significant values of diversity indices tested for phylogenetic and geographic influences were corrected for multiple testing using the Bonferroni procedure of Benjamini and Hochberg (17).

Sequencing of the PKS Gene Cluster. Total DNA was extracted from *Streptomyces philanthi* biovar *triangulum* strain tri23Af2 grown in Grace's medium as described before (6). Briefly, bacteria were lyzed in a Tris-EDTA-sucrose buffer (25 mM Tris, pH 8.0, 25 mM EDTA, pH 8.0, 0.3 M sucrose) with lysozyme (2 mg/mL), proteinase K (20 mg/mL), and SDS (0.6%); proteins were precipitated from the lysate using Protein Precipitation Solution (Qiagen) followed by centrifugation at >16,000 × g for 10 min at 4 °C. Nucleic acids were precipitated with an equal volume of isopropanol followed by centrifugation at ≥16,000 × g for 10 min. The pellet was then washed twice with EtOH (70%), air-dried, and resuspended in elution buffer; later, the extracted nucleic acid solution was treated with RNase A (Epicentre), and the DNA was precipitated using isopropanol as described above.

Genomic DNA from *S. philanthi* biovar *triangulum* was sequenced using 454 technology from shotgun (LGC Genomics) and 8-kb paired end libraries (Eurofins MWG Operon). De novo genome assembly was performed using Newbler software package v 2.7 (454 Life Sciences; Roche) that resulted in one scaffold of 261 contigs and 2 unassembled contigs. Gene prediction was done using the software GeneMark.hmm (exon.gatech.edu/GeneMark/gmhmmp.cgi); predicted genes were annotated using Blast2go 2.8 (18). The piericidin biosynthesis gene cluster (NCBI accession number KX098584) was identified by analyzing possible products from revealed PKS gene clusters using NP.searcher (dna.sherman.lsi.umich.edu/). Later, prediction was verified using the SEARCHPKS program (19, 20) and the piericidin gene cluster from *Streptomyces piomogenus* (21). The substrate specificity of the AT domains was assessed with the NRPS-PKS tool of the "Structure Based Sequence Analysis of Polyketide Synthases" program (22, 23).

Antibiotic Interaction Assays. To assess synergistic and antagonistic interactions among the beewolf symbiont-produced antibiotics, we tested the bioactivity of combinations of the three major components of the antibiotic mixture in agar diffusion assays focusing on differential activities of the combination of two vs. single antibiotic and three vs. two. Piericidin A1 was purchased from Enzo LifeSciences, piericidin B1 was synthesized from piericidin A1 following the protocol by Schnermann et al. (24), and streptochlorin was synthesized de novo as described previously (25). For the inhibition assays, we chose *Aspergillus oryzae* (DSM1147) as a filamentous mold fungus and the yeasts *Candida guilliermondii* (*Meyerozyma guilliermondii* DSM6381) and *Yarrowia lipolytica* (DSM1345). Piericidin A1 and B1 and streptochlorin were all dissolved in methanol. In a preliminary test series, we established working concentrations of all three antibiotics and controlled for any effects of the pure solvent. In our final tests, we tested 1 μg of piericidin A1, 1 μg of piericidin B1, 20 μg of streptochlorin, and additive mixtures of all possible combinations of two or three of the compounds (e.g., 1 μg piericidin A1 plus 20 μg streptochlorin) in eight replicates. The test organisms were pregrown in liquid culture and streaked on potato-dextrose agar using cotton swabs. After drying the plates, holes with 5-mm diameters were punched in the agar and filled with 20 μL methanol containing the different antibiotic mixtures. After 24 h of incubation at 30 °C, we measured inhibition zones to the closest millimeter for the yeasts and after 48 h at 30 °C for *A. oryzae*. Measurement was done blind (i.e., the person measuring the zones was unaware of each field's treatment). Square root-transformed inhibition zones were compared in a one-way ANOVA with Tukey post hoc tests. Data were tested for normal distribution and variance homogeneity with the Shapiro–Wilk test and Levene test, respectively. As all except the square root-transformed piericidin B1 *Y. lipolytica* inhibition zones were normally distributed, we used a one-way ANOVA, performing the *Y. lipolytica* test with and without the piericidin B1 group to compare global test results, and complemented the global and two "missing" post hoc tests involving the piericidin B1 group with nonparametric Kruskal–Wallis and Mann–Whitney U tests and subsequent correction for multiple testing following the Bonferroni correction by Benjamini and Hochberg (17). All tests were performed with SPSS 23.

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Table S1. Secondary metabolites with demonstrated or putative antibiotic activity identified in the antennal and cocoon extracts of beewolf digger wasps (Hymenoptera, Crabronidae: Philanthini)

No.	Compound	Formula [M]	Molecular mass [u]	Substance verification	Bioactivity	Ref(s).
Streptochlorin derivatives						
1	**Streptochlorin (SF2583A)**	**C11H7ClN2O**	**218.02469**	**N, S, IF**	**AB, AF**	1
2	Pimprinine (SF2583B)	C11H8N2O	184.06366	IF	AB, AF	1
3	Pentenyl-Streptochlorin	C16H15ClN2O	286.08729	IF		—
Piericidin derivatives						
4	**Piericidin A1**	**C25H37NO4**	**415.27226**	**N, S, IF**	**AB, AF**	2,3
5	**Piericidin B1**	**C26H39NO4**	**429.28791**	**N, S, IF**	**AB, AF**	4
6	**Piericidin A5**	**C26H39NO4**	**429.28794**	**IF**		5
7	**Piericidin B5**	**C27H41NO4**	**443.30359**	**IF**	**no AB, no AF**	6
8	12-Propyl-Piericidin (Piericidin A3)	C27H41NO4	443.30356	IF		7
9	**IT143B**	**C28H41NO4**	**455.30356**	IF	**AB, AF**	8
10	**Piericidin C1a**	**C25H37NO5**	**431.26717**	IF		7
11	Piericidin C1b	C25H37NO5	431.26717	IF		—
12	Dehydro-Piericidin A1a	C25H35NO4	413.25661	IF		—
13	Dehydro-Piericidin A1b	C25H35NO4	413.25661	IF		—
14	Dehydro-Piericidin A1c	C25H35NO4	413.25661	IF		—
15	Dehydro-Piericidin A1d	C25H35NO4	413.25661	IF		—
16	Dehydro-Piericidin A1e	C27H39NO4	441.28791	IF		—
17	Dehydro-Piericidin B1a	C26H37NO4	427.27226	IF		—
18	Dehydro-Piericidin B1b	C26H37NO4	427.27226	IF		—
19	11-Dihydro-12-Propyl-Piericidin	C27H43NO4	445.31921	IF		—
20	11-Dihydro-12-Butyl-Piericidin	C28H45NO4	459.33486	IF		—
21	11-Dihydro-12-Pentyl-Piericidin	C29H47NO4	473.35051	IF		—
22	3-deMe-Piericidin A1	C24H35NO4	401.25664	IF		—
23	7-deMe-Piericidin A1	C24H35NO4	401.25661	IF	AB, AF	9
24	**9-deMe-Piericidin A1**	**C24H35NO4**	**401.25661**	IF		5
25	11-deMe-Piericidin A1	C24H35NO4	401.25661	IF	AB	10
26	Hydroxy-Piericidin C1	C26H37NO6	459.26209	IF		—
27	Nitro-Piericidin C1	C25H38N2O7	478.26790	IF		—
28	**Glucopiericidin A1a**	**C31H47NO9**	**577.32508**	IF	**AB, AF**	5,11
29	Glucopiericidin A1b	C31H47NO9	577.32508	IF	AB, AF	11
30	Glucopiericidin A5a	C32H49O9N	59134073	IF	AB	10
31	Glucopiericidin A5b	C32H49O9N	591.34073	IF		—
32	*N*-Acetyl-Glucosamine-Piericidin A1	C33H50N2O9	618.35163	IF		—
33	GlucoPhospho-Piericidin A1	C31H48NO12P	657.29142	IF		—
34	Phospho-Piericidin A1	C25H38NO7P	495.23859	IF		—
35	AnhydroGlucoPhospho-Piericidin A1	C31H46NO11P	6392.8085	IF		—
36	5,6-Dimethoxy-3-methyl-2-hydroxymethylpyridin-4-ol	C9H13NO4	199.08446	IF		12
37	5,6-Dimethoxy-3-methyl-2-hydroxypropylpyridin-4-ol	C11H17NO4	227.11576	IF		—
38	306.16998 @ 18.55 min	C17H23NO4	305.16271	F		—
39	334.1650 @ 10.0 min	C18H23NO5	333.15762	F		—
40	348.21689 @ 20.8 min	C20H29NO4	347.20966	F		—
Mer-A2026 derivatives						
41	Mer-A2026-0	C23H33NO3	371.24604	IF		
42	10-MeO-Mer-A2026-0	C24H35NO3	385.26169	IF		13 for Mer-A2026-A and -B
43	10-MeO-Mer-A2026-B	C25H37O3N	399.27734	IF		
44	x-Me-Mer-A2026-B	C25H37O3N	399.27734	IF		—
45	x-Me-10MeO-Mer-A2026-B	C26H39NO3	413.29299	IF		—
Actinopyrone derivatives						
46	Actinopyrone A	C25H36O4	400.26136	S, F	AB	14
47	10-MeO-Actinopyrone A	C26H38O4	414.27701	F		—
48	10-MeO-Actinopyrone B	C25H36O4	400.26136	F		—

Table S1. Cont.

No.	Compound	Formula [M]	Molecular mass [u]	Substance verification	Bioactivity	Ref(s).
Nigericin derivatives						
49	Nigericin	C40H68O11	724.47616	S	AB (AF)	15

Compounds described previously from cocoons of the European beewolf (*P. triangulum*) (5) are highlighted in bold. Methods used to verify substance identities and structures are indicated by abbreviations (N, NMR; S, synthetic standard; IF, highly informative fragmentation patterns; F, simple fragmentation patterns) (Fig. S1). Known bioactivities are included as antibacterial (AB) and antifungal (AF), and all previously described compounds are referenced.

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Table S2. Detailed statistical results for tests of phylogenetic influence on richness, evenness, and diversity of secondary metabolites in beewolf cocoon extracts, using phylogenetically independent contrasts (PICs) as well as phylogenetic generalized least squares models (PGLS)

Output variables	Richness	Evenness	Shannon's *H*	Simpson's λ
Corrected for symbiont phylogeny				
PICs				
F	2.9	17.4	7.3	10.3
p	0.05995	0.0001	0.0018	0.0003
df		3 and 20		
Intercept				
Estimate ± SE	−38.2 ± 46.4	−0.02 ± 0.8	−0.6 ± 2.2	0.3 ± 0.8
t	−0.825	−0.024	−0.273	0.313
p	0.04194	0.9809	0.776	0.7572
Log_easting				
Estimate ± SE	−37.3 ± 16.1	0.5 ± 0.2	0.4 ± 0.8	−0.4 ± 0.3
t	−2.315	1.992	0.476	−1.35
p	0.0314	0.0602	0.6393	0.1920
Log_northing				
Estimate ± SE	27.5 ± 53.2	−0.1 ± 0.8	1.9 ± 2.6	−0.8 ± 1.0
t	0.518	−0.162	0.735	−0.838
p	0.6105	0.8728	0.471	0.4121
Interaction				
Estimate ± SE	−9.3 ± 11.1	−0.6 ± 0.2	−1.8 ± 0.5	0.7 ± 0.2
t	−0.841	−3.729	−3.394	3.41
p	0.4102	0.0013	0.0029	0.0028
PGLS—Brownian motion model				
AIC	181.4	−10.3	39.2	−4.1
Intercept				
Estimate ± SE	3,294.6 ± 3,158.0	−22.7 ± 68.3	−506.2 ± 184.0	235.8 ± 77.3
t	104.2200	−3.2888	−2.7513	3.0510
p	0.3092	0.0035	0.0120	0.0061
Log_easting				
Estimate ± SE	−616.1 ± 561.6	40.4 ± 12.1	89.8 ± 32.7	−41.5 ± 13.7
t	−1.0972	3.3253	2.7446	−3.0184
p	0.2850	0.0032	0.0121	0.0065
Log_northing				
Estimate ± SE	472.1 ± 466.9	32.9 ± 10.1	74.3 ± 27.2	−34.3 ± 11.4
t	−1.0112	3.2532	2.7314	−3.0054
p	0.3234	0.0038	0.0125	0.0067
Interaction				
Estimate ± SE	89.0 ± 83.0	−5.9 ± 1.8	13.1 ± 4.8	6.1 ± 2.0
t	1.0725	−3.2837	−2.7185	2.9784
p	0.2957	0.0035	0.0129	0.0072
PGLS—Ornstein–Uhlenbeck model			Model not converged	
AIC	180.3	−25.7		−20.37
Alpha	35.3	516.8		6655.2
Intercept				
Estimate ± SE	3,599.5 ± 3,078.5	−179.5 ± 58.0		135.0 ± 64.4
t	1.1692	−3.0957		2.0968
p	0.2554	0.0055		0.0483
Log_easting				
Estimate ± SE	−668.9 ± 546.5	32.7 ± 10.4		−23.9 ± 11.5
t	−1.2240	3.1560		−2.081
p	0.2345	0.0048		0.0499
Log_northing				
Estimate ± SE	−517.5 ± 455.8	26.3 ± 8.5		−19.7 ± 9.5
t	−1.1350	3.0710		−2.073
p	0.2691	0.0058		0.0506
Interaction				
Estimate ± SE	96.9 ± 80.9	−4.8 ± 1.5		3.5 ± 1.7
t	1.1970	−3.1240		2.064
p	0.2446	0.0051		0.0515
Corrected for host phylogeny				
F	2	3.2	1.8	4.8
p	0.146	0.046	0.1752	0.0109

Table S2. Cont.

Output variables	Richness	Evenness	Shannon's *H*	Simpson's λ
df		3 and 20		
Intercept				
Estimate ± SE	−26.0 ± 12.3	0.08 ± 0.2	−0.5 ± 0.4	0.01 ± 0.2
t	−2.085	0.494	−1.066	0.058
p	0.0501	0.6265	0.299	0.9546
Log_easting				
Estimate ± SE	−23.9 ± 12.3	0.4 ± 0.2	0.4 ± 0.4	−0.4 ± 0.2
t	−1.95	2.226	0.901	−2.271
p	0.0654	0.0377	0.378	0.0343
Log_northing				
Estimate ± SE	−13.5 ± 36.0	−0.7 ± 0.5	−0.8 ± 1.3	0.08 ± 0.5
t	−0.375	−1.403	−0.648	0.155
p	0.7119	0.176	0.524	0.878
Interaction				
Estimate ± SE	30.0 ± 19.6	0.3 ± 0.3	1.0 ± 0.7	−0.4 ± 0.3
t	1.53	1.138	1.527	−1.545
p	0.1417	0.2686	0.142	0.138
PGLS—Brownian motion model				
AIC	196.7	−15.6	29.7	−10.3
Intercept				
Estimate ± SE	−2,417.6 ± 4846.5	−121.1 ± 69.3	−270.0 ± 171.6	111.8 ± 77.2
t	−0.4988	−1.7462	−1.5738	1.4480
p	0.6231	0.0954	0.1305	0.1623
Log_easting				
Estimate ± SE	388.0 ± 858.8	22.6 ± 12.3	49.1 ± 30.4	−19.8 ± 19.7
t	0.4519	1.8368	1.6138	−1.4485
p	0.6560	0.0804	0.1215	0.1623
Log_northing				
Estimate ± SE	385.7 ± 717.1	17.6 ± 10.3	39.8 ± 25.4	−16.1 ± 11.4
t	0.5378	1.7153	1.5685	−1.4119
p	0.5964	0.101	0.1317	0.1726
Interaction				
Estimate ± SE	−61.8 ± 127.1	−3.3 ± 1.8	−7.2 ± 4.5	2.9 ± 2.0
t	−0.4865	−1.8000	−1.6018	1.4174
p	0.6317	0.0862	0.1241	0.1710
PGLS—Ornstein–Uhlenbeck model				
AIC	194.6	−24.6	22	−20.5
Alpha	41.8	259.4	119.3	235.3
Intercept				
Estimate ± SE	984.1 ± 4653.9	−174.2 ± 59.2	−289.5 ± 148.9	134.7 ± 64.1
t	0.2115	−2.9440	−1.9441	2.0947
p	0.8346	0.0077	0.0654	0.0485
Log_easting				
Estimate ± SE	−222.3 ± 831.4	31.9 ± 10.5	51.7 ± 26.6	−23.7 ± 11.4
t	−0.267	3.024	1.948	−2.077
p	0.792	0.0065	0.0649	0.0503
Log_northing				
Estimate ± SE	−122.3 ± 687.4	25.5 ± 8.7	42.7 ± 21.9	−19.6 ± 9.4
t	−0.178	2.924	1.947	−2.071
p	0.861	0.0081	0.065	0.0509
Interaction				
Estimate ± SE	29.2 ± 122.8	−4.7 ± 1.6	−7.6 ± 3.9	3.5 ± 1.7
t	0.238	−2.997	1.944	2.06
p	0.814	0.0069	0.0655	0.052

AIC, Akaike's Information Criterion.

Table S3. Substrate specificity of the piericidin PKS elongating AT domains

Organism	Polyketide module	Substrate-specifying amino acid motif													Specificity	Detected incorporation
S. philanthi biovar *triangulum*	PiericidinAl_AT_002.seq	Q	Q	G	H	S	Q	G	R	S	H	N	N	V	Methylmalonate/malonate	99.7% mmal
S. philanthi biovar *triangulum*	PiericidinA2_AT_001.seq	Q	Q	G	H	S	Q	G	R	S	H	N	N	V	Methylmalonate/malonate	99.2% mmal
S. philanthi biovar *triangulum*	PiericidinA2_AT_002.seq	Q	Q	G	H	S	Q	G	R	S	H	N	N	V	Methylmalonate/malonate	99.9% mmal
S. philanthi biovar *triangulum*	PiericidinA4_AT_001.seq	Q	Q	G	H	S	Q	G	R	S	H	N	N	V	Methylmalonate/malonate	99.1% mmal
S. philanthi biovar *triangulum*	PiericidinA6_AT_001.seq	Q	Q	G	H	S	Q	G	R	S	H	N	N	V	Methylmalonate/malonate?	N.d.
S. philanthi biovar *triangulum*	PiericidinA3_AT_001.seq	Q	R	G	V	G	D	T	~	~	~	~	~	~	Malonate	100% mal
S. philanthi biovar *triangulum*	PiericidinA5_AT_001.seq	Q	Q	G	H	S	V	G	R	F	H	N	H	V	Malonate	N.d.
S. philanthi biovar *triangulum*	PiericidinA6_AT_002.seq	Q	Q	G	H	D	V	G	R	F	H	N	H	V	Malonate	N.d.
S. piomogenus var *hangzhouwanensis*	PiericidinAl_AT_002.seq	Q	Q	G	H	S	Q	G	R	D	H	T	N	V	Methylmalonate	
S. piomogenus var *hangzhouwanensis*	PiericidinA2_AT_001.seq	Q	Q	G	H	S	Q	G	R	S	H	T	N	V	Methylmalonate	
S. piomogenus var *hangzhouwanensis*	PiericidinA2_AT_002.seq	Q	Q	G	H	S	Q	G	R	S	H	T	N	V	Methylmalonate	
S. piomogenus var *hangzhouwanensis*	PiericidinA4_AT_001.seq	Q	Q	G	H	S	Q	G	R	S	H	T	N	V	Methylmalonate	
S. piomogenus var *hangzhouwanensis*	PiericidinA6_AT_001.seq	Q	Q	G	H	S	Q	G	R	S	H	T	N	V	Methylmalonate	
S. piomogenus var *hangzhouwanensis*	PiericidinA3_AT_001.seq	S	R	A	P	G	A	G	~	~	~	~	~	~	Malonate	
S. piomogenus var *hangzhouwanensis*	PiericidinA5_AT_001.seq	Q	Q	G	G	S	I	G	R	F	H	N	H	V	Malonate	
S. piomogenus var *hangzhouwanensis*	PiericidinA6_AT_002.seq	Q	Q	G	H	S	I	G	R	F	H	N	H	V	Malonate	
Streptomyces noursei	Nystatin_002_AT_001.seq	Q	Q	G	H	S	Q	G	R	S	H	T	N	V	Methylmalonate	
Streptomyces nodosum	Amphotericin_004_AT_003.seq	Q	Q	G	H	S	Q	G	R	S	H	T	S	V	Methylmalonate	
Streptomyces caelestis	Niddamycin_003_AT_001	Q	Q	G	H	S	Q	G	R	S	H	T	N	V	Methylmalonate	
Stigmatella aurantiaca	Myxothiazol_001_AT_003.seq	Q	Q	G	S	S	I	G	R	S	H	T	N	V	Methylmalonate	
Streptomyces sp. CK4412	Tautomycetin_001_AT_004.seq	Q	Q	G	H	S	Q	G	R	S	H	S	N	V	Methylmalonate	
Streptomyces platensis	Pladienolide_003_AT_001.seq	Q	Q	G	H	S	Q	G	R	S	H	S	N	V	Methylmalonate	
Streptomyces natalensis	Pimaricin_003_AT003.seq	Q	Q	G	H	S	Q	G	R	S	H	S	N	V	Methylmalonate	
Streptomyces halstedii	Vicenistatin_004_AT_002.seq	Q	Q	G	H	S	V	G	R	F	H	N	H	V	Malonate	
Streptomyces noursei	Nystatin_004_AT_004.seq	Q	Q	G	H	S	V	G	R	F	H	N	H	V	Malonate	
Streptomyces nanchangensis	Nanchangmycin_003_AT_001.seq	Q	Q	G	H	S	V	G	R	F	H	N	H	V	Malonate	
Streptomyces sp. FR-008	FR008_005_AT_003.seq	Q	Q	G	H	S	V	G	R	F	H	N	H	V	Malonate	
Streptomyces antibioticus	Chlorothricin_006_AT_001.seq	Q	Q	G	H	S	V	G	R	F	H	N	H	V	Malonate	
Streptomyces nodosus	Amphotericin_004_AT_004.seq	Q	Q	G	H	S	V	G	R	F	H	N	H	V	Malonate	
Streptomyces hygroscopicus	Herbimycin_003_AT_001.seq	Q	Q	G	H	S	I	G	R	F	H	N	H	V	Malonate	
Saccharopolyspora spinosa	Spinosad_004_AT_002	Q	Q	G	H	S	V	G	R	F	H	T	Q	V	Malonate	
Streptomyces hygroscopicus	Geldanamycin_003_AT_001.seq	Q	Q	G	H	S	I	G	R	F	H	N	H	V	Malonate	

Shown are substrate-specifying motifs of the piericidin AT modules of *S. philanthi*, *S. piomogenus*, and the most similar modules of other characterized polyketide synthases. Amino acid residues (given in the IUPAC one-letter abbreviation; ~ indicates amino acids not present in the corresponding AT domain) typical for different substrate-specifying motifs are colored based on described substrate specificity from several polyketide synthases (1) (for methylmalonate, green; for malonate, magenta). The detected substrate incorporation of methylmalonate vs. malonate on cocoons of *P. triangulum* is based on the metabolites detected in the HPLC-MS/MS data (N.d. refers to metabolites that could not be determined by applied methods). For the AT domains of piericidin modules A5 and A6, we could not determine if unspecific incorporation of malonyl-CoA (mal) or methylmalonyl-CoA (mmal) occurred, as our screening protocol did not include modifications of the pyridine ring.

1. Yadav G, Gokhale RS, Mohanty D (2003) Computational approach for prediction of domain organization and substrate specificity of modular polyketide synthases. *J Mol Biol* 328:335–363.

Table S4. Sampling locations of beewolf specimens

Species	Sampling location
Philanthinus quattuordecimpunctatus	Horasan, Erzurum Province, Turkey
Philanthus albopilosus	San Rafael Desert, Utah, United States
Philanthus barbiger	San Rafael Desert, Utah, United States
Philanthus bicinctus	Lake Creak, Wyoming, United States
Philanthus bilunatus	Andover, New Hampshire, United States
Philanthus capensis	Riet River Mouth, Eastern Cape Province, and Simon's Town, Western Cape Province, South Africa
Philanthus crabroniformis	Deadmens's Bar and Lake Creak, Wyoming, United States
Philanthus fuscipennis	Patterson, Eastern Cape Province, South Africa
Philanthus gibbosus	Madison, Wisconsin, United States; Andover, New Hampshire, United States; and San Rafael Desert, Utah, United States
Philanthus histrio	Franschhoek, Western Cape Province, South Africa
Philanthus loefflingi	Swellendam and Simon's Town, Western Cape Province, South Africa
Philanthus multimaculatus	Sandy, Utah, United States
Philanthus multimaculatus	San Rafael Desert, Utah, United States
Philanthus parkeri	San Rafael Desert, Utah, United States
Philanthus politus	Andover, New Hampshire, United States
Philanthus psyche	San Rafael Desert, Utah, United States
Philanthus pulcher	Deadmens's Bar, Wyoming, United States
Philanthus rugosus	Riet River Mouth, Eastern Cape Province, South Africa
Philanthus triangulum diadema	Patterson, Eastern Cape Province, and Simon's Town, Western Cape Province, South Africa
Philanthus triangulum triangulum	Erzurum, Erzurum Province, Turkey
Philanthus triangulum triangulum	Erlangen and Berlin, Germany
Philanthus ventilabris	Sandy, Utah, United States
Philanthus ventilabris	San Rafael Desert, Utah, United States
Philanthus venustus	Isparta, Isparta Province, Turkey
Trachypus boharti	Bauru and Ribeirão Preto, São Paulo Province, Brazil
Trachypus elongatus	São Simão and São Paulo, São Paulo Province, Brazil
Trachypus flavidus	Bauru and Ribeirão Preto, São Paulo Province, Brazil
Trachypus fulvipennis	Bauru and Ribeirão Preto, São Paulo Province, Brazil
Trachypus patagonensis	Ribeirão Preto, São Paulo Province, Brazil

Fig. S1. Predicted structures of beewolf symbiont-produced secondary metabolites described in Table S1 based on MS/MS fragmentation patterns. Proposed metabolite structures that also served as a basis for structure prediction by interpretation of fragmentation patterns are printed in red if confirmation is based on NMR and are italicized if confirmation is based on coinjection of synthetic standards (Fig. S2). Informative fragmentation sites are highlighted in the structures, and observed exact masses from the experimental data are indicated. Additional spectroscopic data would be needed for structural confirmation of predicted compounds.

Fig. S1

Fig. S2. Substance verification by synthetic standards. Ion traces ($[M + H]^+$ adducts) of a representative extract of a beewolf antenna (*T. elongatus*; red) compared with pure synthetic standards (black) and coinjections (blue).

Fig. S2

Fig. S3. Clustering of beewolf antennal and cocoon extracts by geographic location. (*A*) DA highlighting beewolf species with both antennal and cocoon samples available. The DA is based on the 10 principal components extracted from the chemical composition of the symbiont-produced antibiotic mixture in antennal (circles) and cocoon extracts (triangles) based on the different beewolf species ($n = 242$ extracts, Wilk's $\Lambda = 7.6 \times 10^{-5}$, df = 270, $P < 0.001$). (*B*) DA based on the 10 principal components of the chemical composition of the symbiont-produced antibiotic mixture based on their geographic origin ($n = 242$ extracts, Wilk's $\Lambda = 0.049$, df = 20, $P < 0.001$).

Fig. S3

Fig. S4. Molecular basis of diversity in the synthesis of the piericidin derivatives in the beewolf symbiont-produced antibiotic mixture (piericidin A1 biosynthesis modified after refs. 1 and 2). (*A*) Structure and organization of the piericidin clusters of *S. piomogenus* var *hangzhouwanensis* and *S. philanthi* biovar *triangulum* and their similarity on the amino acid sequence level. (*B*) Tailoring steps of the immature polyketide after its release from the PKS enzyme complex are shown underneath the polyketide modules, and additional enzymatic modifications after the release from the polyketide synthase complex are also shown. Most of the variation in the antibiotic mixture of the beewolves arises from (*i*) different starting units, (*ii*) incorporation of different acyl-CoA (especially malonyl-CoA instead of methylmalonyl-CoA) units during elongation of the polyketide backbone, or skipped enzymatic steps as well as post-PKS modifications, like (*iii*) a missing reduction or later introduced oxidation of the polyketide side chain, (*iv*) ring formation without prior aminotransferase reaction, (*v*) modifications of hydroxyl groups, and (*vi*) additional introduction of hydroxyl groups or an epoxide function by oxygenases. Blue structural features indicate the changes introduced by the preceding enzymatic step. Magenta structural features indicate deviations from the major product of the polyketide cluster (piericidin A1). ACP, acyl carrier protein; AT, acyltransferase; DH, dehydratase; ER, enoylreductase; KR, ketoreductase; KS, ketosynthase; mal, malonyl-CoA; mmal, methylmalonyl-CoA; TE, thiolesterase.

Fig. S4

1. Liu Q, et al. (2012) Elucidation of Piericidin A1 biosynthetic locus revealed a thioesterase-dependent mechanism of α-pyridone ring formation. *Chem Biol* 19:243–253.
2. Ansari MZ, Yadav G, Gokhale RS, Mohanty D (2004) NRPS-PKS: A knowledge-based resource for analysis of NRPS/PKS megasynthases. *Nucleic Acids Res* 32:W405–W413.

Fig. S5. Annotation of piericidin biosynthesis genes and their products from the genome of *S. philanthi* biovar *triangulum* strain 23Af2 in comparison with *S. piomogenus* PKS cluster HQ840721 (schematic below). G, gap; I, identity; P, positive.

Fig. S5

Dataset S1. Observed retention times and fragmentation patterns of metabolites described in Table 1 on MS/MS high-energy collision dissociation fragmentation

Dataset S1

Indicative fragments for molecule structures are printed in bold. Electron impact fragmentation patterns published by Yoshida et al. (1) of piericidin A1, B1, and C1 are highlighted in green if they also occurred after ESI ionization/HCD fragmentation, and they are highlighted in magenta if the fragments were absent.

1. Yoshida S, Yoneyama K, Shiraishi S, Watanabe A, Takahashi N (1977) Chemical structures of new piericidins produced by Streptomyces pactum. *Agric Biol Chem* 41:855–862.