Supplementary information for

Stage-specific transcriptome of *Bursaphelenchus xylophilus* reveals temporal regulation of effector genes and roles of the dauer-like stages in the lifecycle

Suguru E. Tanaka[1,+], Mehmet Dayi[2,3,+], Yasunobu Maeda[2], Isheng J Tsai[4], Ryusei Tanaka[2], Mark Bligh[2], Yuko Takeuchi-Kaneko[5], Kenji Fukuda[1], Natsumi Kanzaki[6], and Taisei Kikuchi[2]*

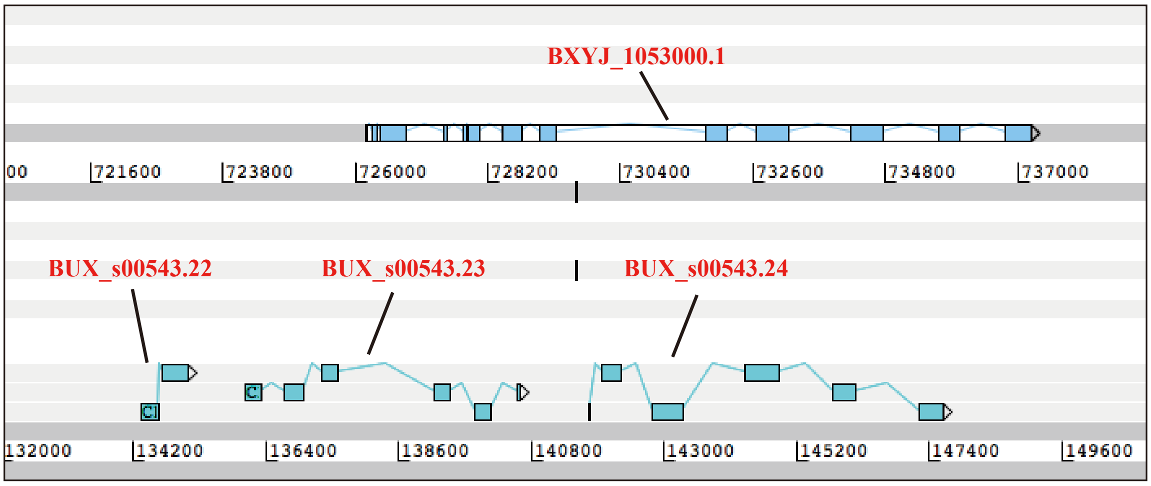

Figure S1. An example of the new (V2.0) and corresponding old (V1.2) gene models. Three old gene models (BUX_s00543.22, 225 aa, BUX_s00543.23, 427 aa and BUX_s00543.24, 686 aa, respectively) were connected into one (BXYJ_1053000.1, 1107 amino acids) in the new gene set.

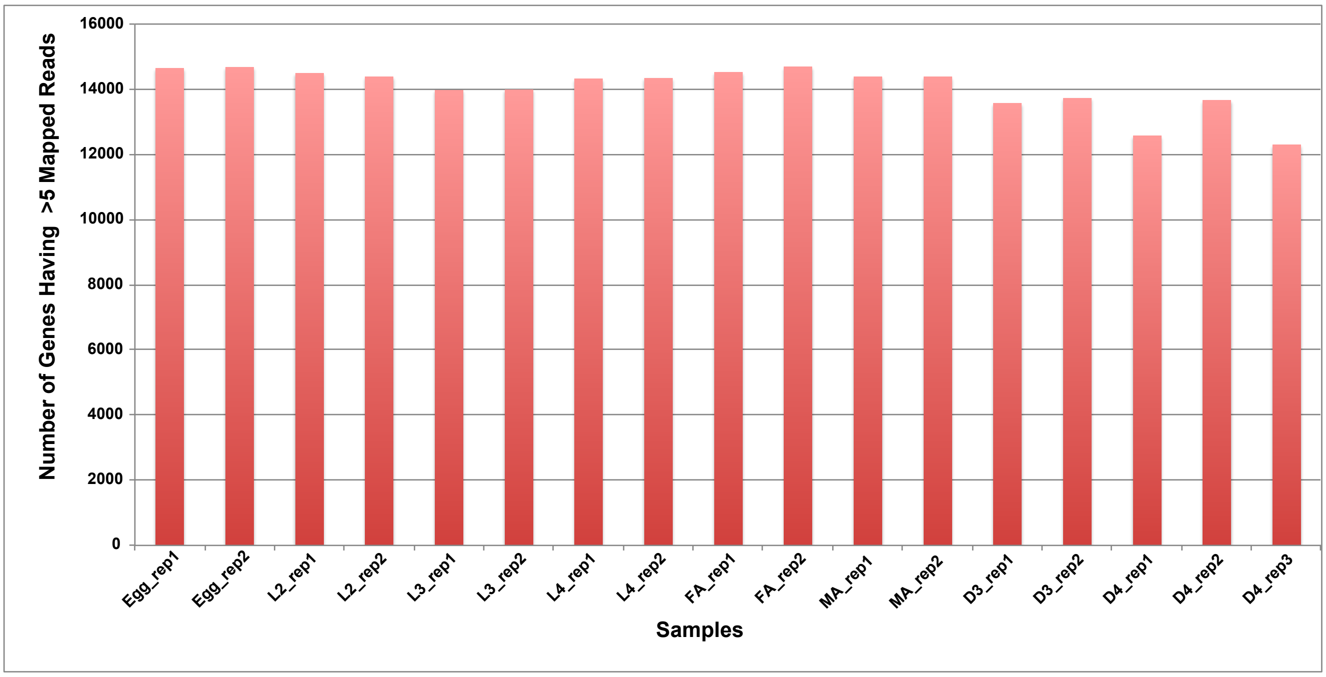

Figure S2. Number of B. xylophilus genes that have over 5 mapped reads in each sample. Each sample has more than 12000 those genes.

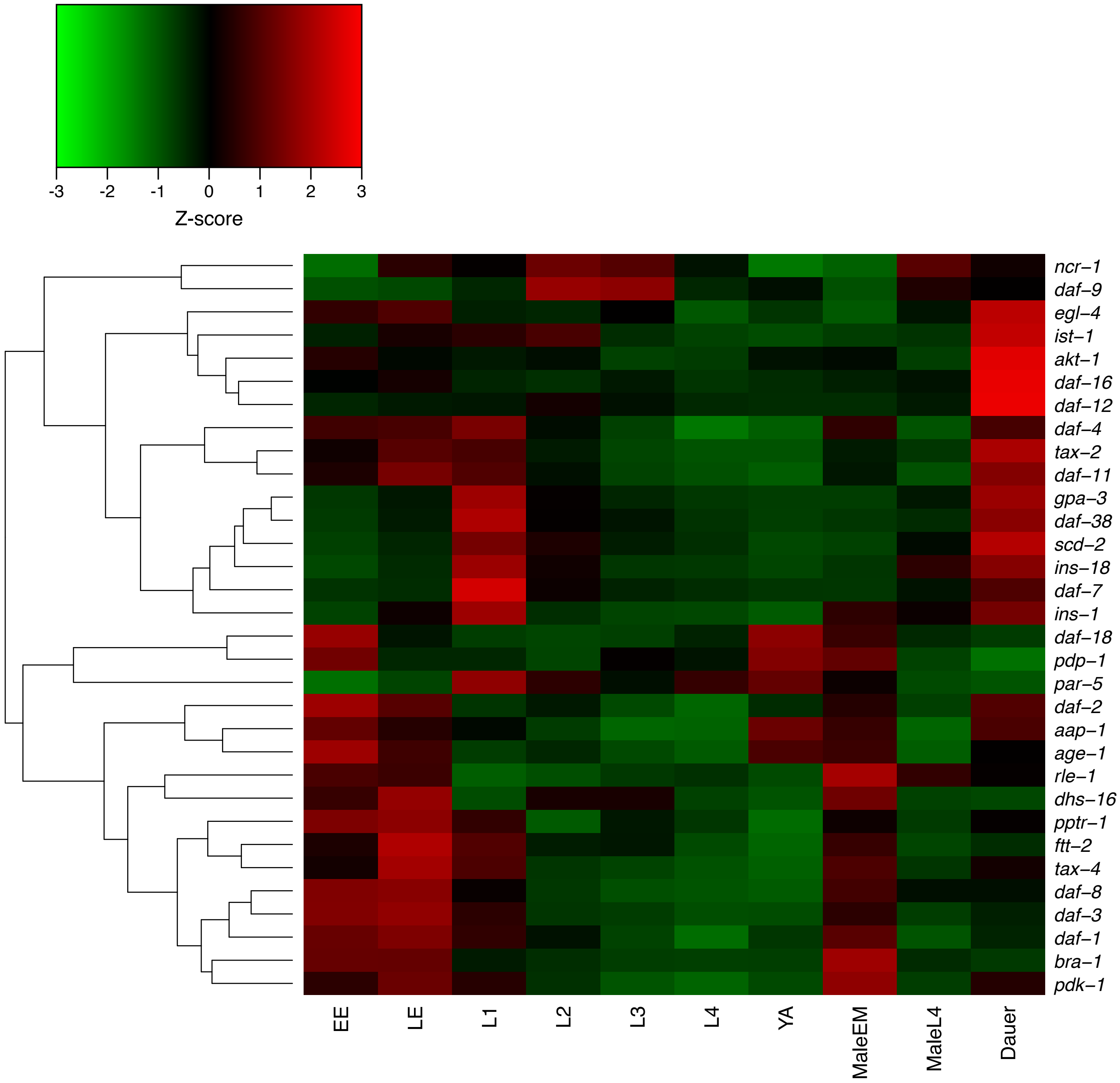

Figure S3. Heatmap showing expression of dauer genes in C. elegans stages. The data was derived from the WormBase (http://www.wormbase.org).

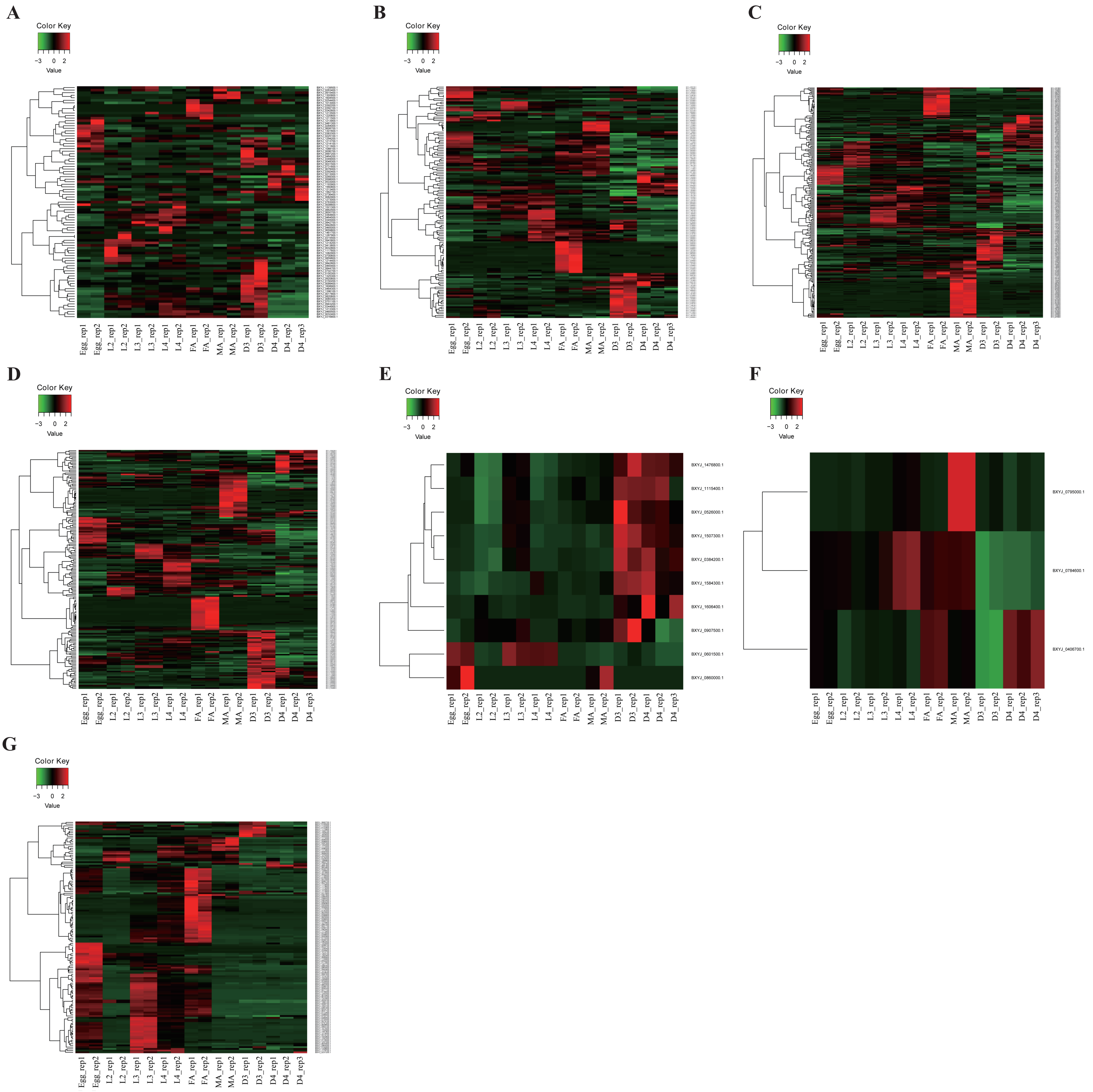

Figure S4. Expression dynamics of peptidase genes in B. xylophilus developmental stages. Heatmaps were generated for each peptidase family; (A) aspartic (91 genes), (B) cysteine (139 genes), (C) metallo (223 genes), (D) serine (171 genes), (E) threonine (10 genes), (F) unknown_32 (3 genes), and (G) unknown_69 (134. genes).

Table S1. Genome and gene model stats for the old (v1.2) and new (v2.0) assemblies.

	Genome version	
	v.1.2	v.2.0
Total size of assemblied sequence (Mb)	74.6	75.9
Number of scaffolds	1,231	501
N50 of scaffolds (kb)	1,158	1,577
Maximum length of scaffold (kb)	3,612	4,119
Gap length (kb)	1,475	42
GC content (%)	40.4	40.4
Completeness by CEGMA (%): (complete/partial)	97/98	98/98
Completeness by BUSCO (%)	78	79
Average CEGMA gene number: (complete/partial)	1.08/1.09	1.18/1.23
Mapped read ratio (%)	98.25	99.09
Proper-pair ratio (%)	91.64	96.4
Number of gene models	18,074	16,346
Number of Genes	18,074	16,346
Number of Transcript	18,074	16,346
Average Transcript Length (bp)	1,572	2,735
Median Transcript Length (bp)	1,214	1,811
Total Transcript Length (bp)	28,429,143	44,709,149
Average Exons per gene	4.51	5.89
Median Exons per gene	4	5
Number of Exon	81,543	96,239
Average Exon Length (bp)	228.88	215.21
Median Exon Length (bp)	183	172
Total Exon Length (bp)	18,663,247	20,711,270
Number of Intron	63,461	79,892
Average Intron Length (bp)	153.02	300.33
Median Intron Length (bp)	69	77
Total Intron Length (bp)	9,710,974	23,994,071

Table S2. (in a separate file) RNA-seq reads and mapping stats and FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values of B. xylophilus genes in life stages

Table S3. Number of differentially expressed genes in pair-wise comparisons.

Stage	Egg	L2	L3	L4	FA	MA	D3	D4
Egg	-	-	-	-	-	-	-	-
L2	2982	-	-	-	-	-	-	-
L3	1641	1232	-	-	-	-	-	-
L4	2316	1088	347	-	-	-	-	-
FA	2606	2764	1852	1367	-	-	-	-
MA	4327	3339	3502	3191	2653	-	-	-
D3	3398	1036	1792	1472	2114	2895	-	-
D4	4260	2567	3121	2618	3172	4141	2112	-

Table S4. (in a separate file) Gene Ontology enrichment of differentially expressed genes in pair-wise comparisons.

Table S5. (in a separate file) Number of DE genes in life stages comparisons and assignment as “flat”, “rising” (rises over time), “oscillating” and “others”.

Pairwise	Flat	Rising	Oscillating	Others	Total
Egg vs L2	431(14.5)	33(1.1)	365(12.2)	2153(72.2)	2982
Egg vs L3	210(12.8)	27(1.6)	74(4.5)	1330(81.0)	1641
Egg vs L4	395(17.1)	35(1.5)	127(5.5)	1759(75.9)	2316
Egg vs FA	359(13.8)	67(2.6)	225(8.6)	1955(75.0)	2606
Egg vs MA	476(11.0)	152(3.5)	410(9.5)	3289(76.0)	4327
Egg vs D3	612(18.0)	45(1.3)	433(12.7)	2308(67.9)	3398
Egg vs D4	904(21.2)	73(1.7)	404(9.5)	2879(67.6)	4260
L2 vs L3	91(7.4)	11(0.9)	320(26.0)	810(65.7)	1232
L2 vs L4	82(7.5)	14(1.3)	240(22.1)	752(69.1)	1088
L2 vs FA	487(17.6)	73(2.6)	214(7.7)	1990(72.0)	2764
L2 vs MA	405(12.1)	156(4.7)	122(3.7)	2656(79.5)	3339
L2 vs D3	182(17.6)	12(1.2)	98(9.5)	744(71.8)	1036
L2 vs D4	514(20.0)	68(2.6)	149(5.8)	1836(71.5)	2567
L3 vs L4	18(5.2)	6(1.7)	69(19.9)	254(73.2)	347
L3 vs FA	150(8.1)	70(3.8)	217(11.7)	1415(76.4)	1852
L3 vs MA	261(7.5)	155(4.4)	377(10.8)	2709(77.4)	3502
L3 vs D3	186(10.4)	22(1.2)	375(20.9)	1209(67.5)	1792
L3 vs D4	442(14.2)	79(2.5)	384(12.3)	2216(71.0)	3121
L4 vs FA	140(10.2)	61(4.5)	63(4.6)	1103(80.7)	1367
L4 vs MA	198(6.2)	152(4.8)	289(9.1)	2552(80.0)	3191
L4 vs D3	146(9.9)	13(0.9)	300(20.4)	1013(68.8)	1472
L4 vs D4	303(11.6)	62(2.4)	310(11.8)	1943(74.2)	2618
FA vs MA	102(3.8)	149(5.6)	229(8.6)	2173(81.9)	2653
FA vs D3	170(8.0)	54(2.6)	267(12.6)	1623(76.8)	2114
FA vs D4	526(16.6)	56(1.8)	281(8.9)	2309(72.8)	3172
MA vs D3	203(7.0)	153(5.3)	106(3.7)	2433(84.0)	2895
MA vs D4	588(14.2)	158(3.8)	190(4.6)	3205(77.4)	4141
D3 vs D4	396(18.8)	46(2.2)	138(6.5)	1532(72.5)	2112
non-DEgene*	3051(45.3)	24(0.4)	207(3.1)	3452(51.3)	6734

*Genes that are not differentially expressed in any pairwise comparisons. Numbers in parentheses are percentage of the total.

Table S6. (in a separate file) Gene Ontology (GO) enrichment results for genes clustered along development. GO analysis of (A) C1, (B) C2, (C) C3, (D) C4, (E) C5, (F) C6, (G) C7, (H) C8, (I) C9, and (J) C10 cluster.

Table S7. (in a separate file) Gene Ontology enrichment results of germline (A)- and somatic-enriched (B) genes.

Table S8. Comparison of numbers of peptidases in genomes of representative nematode species.

Species[1]	**Clade**[2]	**Clade**[3]	**lifestyle**	**Number of peptidases in genome**[4]
Bursaphelenchus xylophilus	IV	10	plant parasitic	808
Meloidogyne incognita	IV	12	plant parasitic	124
Globodera pallida	IV	12	plant parasitic	236
Panagrellus redivivus	IV	10	free-living	416
Caernohabditis elegans	V	9	free-living	435
Brugia malayi	III	8	animal parasitic	402
Strongyloides stercoralis	IV	10	animal parasitic	439
Ascaris suum	III	8	animal parasitic	337
Necator americanus	V	9	animal parasitic	271
Romanomermis culicivorax	I	2	entomoparasitic	218

1 Genome sequences were retrived from wormbase release WS266

2 Blaxter ML et al. (1998) Nature 392:71–75

3 van Megen H et al. (2009) Nematology 11:927–50

4 Peptidases were identified by MEROPS Blast (version 11.0)