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## Genome sequence of the phylogenetically isolated spirochete *Leptonema illini* type strain (3055<sup>T</sup>)

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Keywords: Gram-negative, flexible, motile, cytoplasmatic tubules, non-sporulating, axial flagella, aerobic, chemoorganotrophic, *Leptospiroaceae*, GEBA

*Leptonema illini* Hovind-Hougen 1979 is the type species of the genus *Leptonema*, family *Leptospiroaceae*, phylum *Spirochaetes*. Organisms of this family have a Gram-negative-like cell envelope consisting of a cytoplasmic membrane and an outer membrane. The peptidoglycan layer is associated with the cytoplasmic rather than the outer membrane. The two flagella of members of *Leptospiroaceae* extend from the cytoplasmic membrane at the ends of the bacteria into the periplasmic space and are necessary for their motility. Here we describe the features of the *L. illini* type strain, together with the complete genome sequence, and annotation. This is the first genome sequence (finished at the level of Improved High Quality Draft) to be reported from a member of the genus *Leptonema* and a representative of the third genus of the family *Leptospiroaceae* for which complete or draft genome sequences are now available. The three scaffolds of the 4,522,760 bp draft genome sequence reported here, and its 4,230 protein-coding and 47 RNA genes are part of the *Genomic Encyclopedia of Bacteria and Archaea* project.

### Introduction

Strain 3055<sup>T</sup> was isolated from urine of a clinically healthy bull [1], and was first mentioned in the literature as a new *Leptospira* serotype, serovar *illini* [2,3], but as no name was proposed, it was not validly published. This occurred in the comparative study of Hovind-Hougen [4] who found morphological differences between '*Leptospira illini*' strain 3005 and other members of *Leptospira*, i.e. the presence of cytoplasmatic tubules and the structure of the basal complex of the flagella. These differences, together with the finding of a higher DNA

base composition and growth behavior [5] were used as criteria to taxonomically separate strain 3055 from *Leptospira* as *Leptonema illini* with strain 3055<sup>T</sup> (= DSM 21528 = NCTC 11301) as the type strain. This species is the only species of the genus. The family *Leptospiroaceae* was created in the same publication [4], although the name was proposed before, though not effectively published [J Pilot, Ph D Thesis, University of Paris, Paris, France 1965]. Despite a description in the International Journal of Systematic Bacteriology the name

*Leptonema* was not included in the Approved List of Bacterial Names [6]. The omission of this name was not in accordance with the *Bacteriological Code* (1990 Revision) Rule 24a, Note 1, but was corrected in Validation List N° 10 [7].

The phylogenetic relatedness among spirochetes and the isolated position of *L. illini* was first elucidated by 16S rRNA cataloguing [8] and then by comparative sequence analysis of reverse-transcribed 16S rRNA sequences [9] and by rDNA analyses [10,11]. The moderate similarity values between *L. illini* and strains of *Leptospira* were later supported by the absence of significant DNA-DNA hybridization values between members of the two genera [12-14], 16S rRNA restriction fragment analysis [15] and PCR amplification of the 16S-23S ribosomal DNA spacer [16]. Application of a 16S rRNA gene real-time PCR assay to leptospires [17] confirmed the presence of *L. illini* strains in kidneys of Indian rats and bandicoots. Here we present a summary classification and a set of features for *L. illini* strain 3055<sup>T</sup> together with the description of the complete genomic sequencing and annotation. The rationale for sequencing the genome of this non-pathogenic strain is based on its isolated position within the phylum *Spirochaetes*.

## Classification and features

### 16S rRNA gene sequence analysis

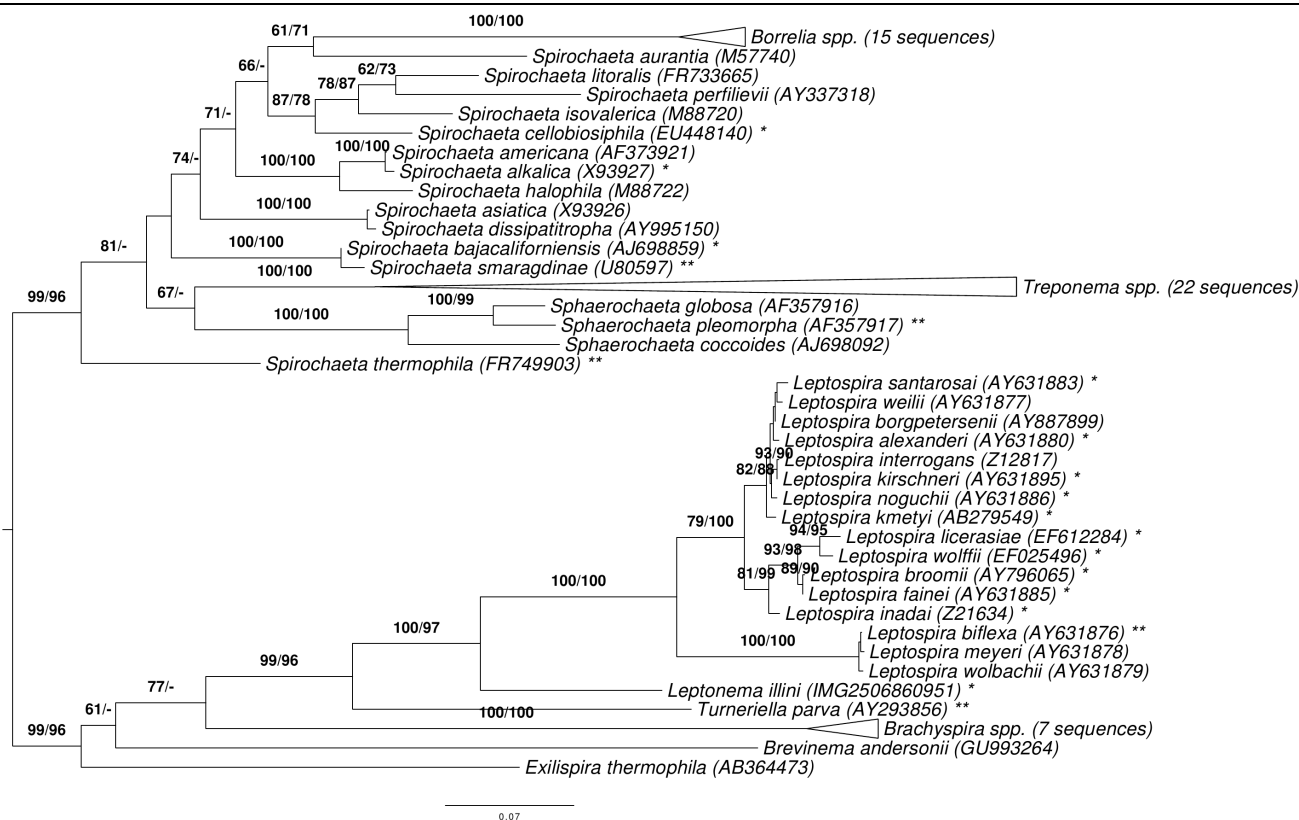
The single genomic 16S rRNA gene sequence of *L. illini* 3055<sup>T</sup> was compared using NCBI BLAST [18,19] under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [20] and the relative frequencies of taxa and keywords (reduced to their stem [21]) were determined, weighted by BLAST scores. The most frequently occurring genera were *Leptospira* (53.4%), *Anaeromyxobacter* (31.6%), *Leptonema* (11.5%), *Turneriella* (1.3%) and *Desulfomonile* (0.8%) (96 hits in total). Regarding the three hits to sequences from members of the species, the average identity within HSPs was 99.7%, whereas the average coverage by HSPs was 97.4%. Among all other species, the one yielding the highest score was *Leptospira wolbachii* (AY631890), which corresponded to an identity of 86.4% and an HSP coverage of 76.8%. (Note that the Greengenes database uses the INSDC (= EMBL/NCBI/DDBJ) annotation, which is not an authoritative source for nomenclature or classification.) The highest-scoring environmental sequence

was EF648066 (Greengenes short name 'dynamics during produced water treatment aerobic activated sludge clone HB63'), which showed an identity of 99.2% and an HSP coverage of 98.4%. The most frequently occurring keywords within the labels of all environmental samples which yielded hits were 'microbi' (5.2%), 'soil' (2.3%), 'anaerob' (2.3%), 'industri' (2.0%) and 'ecolog' (1.4%) (154 hits in total). The most frequently occurring keywords within the labels of those environmental samples which yielded hits of a higher score than the highest scoring species were 'microbi' (4.5%), 'cell' (3.1%), 'prmr' (3.0%), 'sediment' (3.0%) and 'coral' (3.0%) (12 hits in total). None of these keywords provides useful information about the close relatives of strain 3055<sup>T</sup> in the environment.

Figure 1 shows the phylogenetic neighborhood of *L. illini* in a 16S rRNA based tree. The sequence of the single 16S rRNA gene copy in the genome does not differ from the previously published 16S rRNA sequence (AY714984).

### Morphology and physiology

The unicellular cells of strain 3055<sup>T</sup> stain Gram negatively and are of helical shape (13-21 µm long and 0.1 µm wide) [4] [Figure 2]. Most cells have hook-shaped ends and display a typical leptospiral morphology [46]. The wavelength of the coils within the helix is about 0.6 µm with an amplitude of about 0.1 µm. A single flagellum is inserted at each pole and in well-preserved cells the flagellum is entwined with the helical body within the periplasmic cell for about four to six turns of the helix (not visible in Figure 2). Rotation of the flagella by a flagellar motor induces changes in the cell morphology that drives motility [47]. In cells treated with Myxobacter AL-1 protease [48] bundles of three to four cytoplasmic tubules are observed which originate close to the insertion point of each of the two flagella. The bundles are located close to the inner site of the cytoplasmic membrane just underneath the flagellum. As bundles and flagella are shorter than the total length of the cell, the middle part is devoid of both. Flagella, released by the AL-1 protease, are often found as spirals. Each flagellum consists of a core (diameter 10 nm), covered by a sheath (diameter 16 nm). One of the arguments to classify strain 3055 as the type of a new genus was the structure of the insertion part of the flagellum, similar to those of Gram-positive bacteria in *L. illini* while other leptospires possess the Gram-negative type insertion [4].



**Figure 1.** Phylogenetic tree highlighting the position of *L. illini* relative to the type strains of the other species within the phylum *Spirochaetes*. The tree was inferred from 1,325 aligned characters [22,23] of the 16S rRNA gene sequence under the maximum likelihood (ML) criterion [24]. Rooting was done initially using the midpoint method [25] and then checked for its agreement with the current classification (Table 1). The branches are scaled in terms of the expected number of substitutions per site. Numbers adjacent to the branches are support values from 550 ML bootstrap replicates [26] (left) and from 1,000 maximum-parsimony bootstrap replicates [27] (right) if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [28] are labeled with one asterisk. Those also listed as 'Complete and Published' with two asterisks (see [29-35] and CP003155 for *Sphaerochaeta pleomorpha*, CP002903 for *Spirochaeta thermophila*, CP002696 for *Treponema brennaborensis*, CP001841 for *T. azotonutricium*). The collapsed *Treponema* subtree contains three species formerly assigned to *Spirochaeta* that have recently been included in the genus *Treponema*, even though those names are not yet validly published [34].

Serum and long-chain fatty acids are required for growth, no serum is required in trypticase soy broth. The organism is chemoorganotrophic and aerobic. Long-chain fatty acids (>14 carbons) are used as source of carbon and energy. Ammonia, in the form of inorganic salts rather than amino acids is used as a nitrogen source. Purines, but not pyrimidines, are utilized. Strain 3005<sup>T</sup> is non-pathogenic for hamsters, mice, gerbils, guinea pigs and cattle [15], although it may cause opportunistic infections, as it has been isolated from the blood of a HIV-infected patient [43].

### Chemotaxonomy

No data are available for fatty acids, quinones or polar lipids. The G+C content of the DNA was previously reported with 51-53 mol% [49], which is

below the value inferred from the genome sequence (see genome statistics table).

## Genome sequencing and annotation

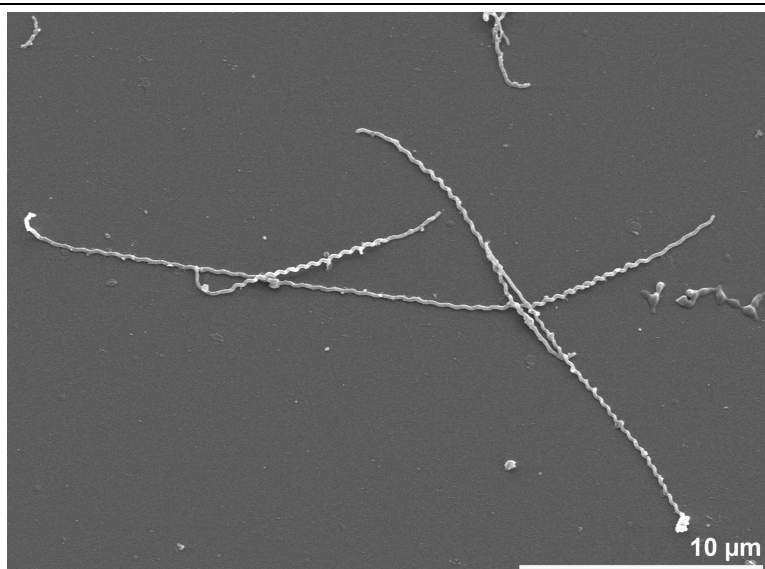
### Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position [50], and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project [51]. The genome project is deposited in the Genomes OnLine Database [28] and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI) using state of the art sequencing technology [52]. A summary of the project information is shown in Table 2.

**Table 1.** Classification and general features of *L. illini* 3055<sup>T</sup> according to the MIGS recommendations [36].

MIGS ID	Property	Term	Evidence code
		Domain <i>Bacteria</i>	TAS [37]
		Phylum <i>Spirochaetes</i>	TAS [38]
		Class <i>Spirochaetes</i>	TAS [39,40]
	Current classification	Order <i>Spirochaetales</i>	TAS [41,42]
		Family <i>Leptospiraceae</i>	TAS [4,14,42]
		Genus <i>Leptonema</i>	TAS [4,7]
		Species <i>Leptonema illini</i>	TAS [4,7]
MIGS-7	Subspecific genetic lineage (strain)	3055 <sup>T</sup>	TAS [4]
MIGS-12	Reference for biomaterial	Hovind-Hougen, 1979	TAS [4]
	Gram stain	negative	TAS [4]
	Cell shape	helical rods	TAS [4]
	Motility	motile	TAS [4]
	Sporulation	non-sporulating	TAS [4]
	Temperature range	mesophile	TAS [4]
	Optimum temperature	29° C	TAS [4]
	Salinity	not reported	
MIGS-22	Relationship to oxygen	aerobe	TAS [4]
	Carbon source	long-chain fatty acids	TAS [4]
	Energy metabolism	chemoorganotroph	TAS [4]
MIGS-6	Habitat	not specified	
MIGS-6.2	pH	not reported	
MIGS-15	Biotic relationship	free living	TAS [4]
MIGS-14	Known pathogenicity	opportunistic infections	TAS [43]
MIGS-16	Specific host	<i>Bos taurus</i> (cow)	TAS [4]
MIGS-18	Health status of host	healthy	TAS [4]
	Biosafety level	1	TAS [44]
MIGS-19	Trophic level	not reported	
MIGS-23.1	Isolation	urine of a bull	TAS [4]
MIGS-4	Geographic location	Iowa	TAS [5]
MIGS-5	Time of sample collection	1965	TAS [1]
MIGS-4.1	Latitude	not reported	
MIGS-4.2	Longitude	not reported	
MIGS-4.3	Depth	not reported	
MIGS-4.4	Altitude	not reported	

Evidence codes - TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). Evidence codes are from the Gene Ontology project [45].



**Figure 2.** Scanning electron micrograph of *L. illini* 3055<sup>T</sup>

**Table 2.** Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Improved high quality draft
MIGS-28	Libraries used	Three genomic libraries: one 454 pyrosequence standard library, two 454 PE library (13 kb insert size), one Illumina library
MIGS-29	Sequencing platforms	Illumina GAii, 454 GS FLX Titanium
MIGS-31.2	Sequencing coverage	1,276.9 × Illumina; 35.5 × pyrosequence
MIGS-30	Assemblers	Newbler version 2.3, Velvet 1.0.13, phrap version SPS - 4.24
MIGS-32	Gene calling method	Prodigal 1.4, GenePRIMP
	INSDC ID	AHKT00000000
	GenBank Date of Release	January 24, 2012
	GOLD ID	Gi04604
	NCBI project ID	60435
	Database: IMG	2506783010
MIGS-13	Source material identifier	DSM 21528
	Project relevance	Tree of Life, GEBA

### Growth conditions and DNA isolation

*L. illini* strain 3055<sup>T</sup>, DSM 21528, was grown in DSMZ medium 1113 (*Leptospira* Medium) at 30°C. DNA was isolated from 1-1.5 g of cell paste using MasterPure Gram-positive DNA purification kit (Epicentre MGP04100) following the standard protocol as recommended by the manufacturer with modification st/DL for cell lysis as described in Wu *et al.* 2009 [51]. DNA is available through the DNA Bank Network [53].

### Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [54]. Pyrosequencing reads were assembled using the Newbler assembler (Roche). The initial Newbler assembly consisting of 140 contigs in tree scaffolds was converted into a phrap [55] assembly by making fake reads from the consensus, to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (5,940

Mb) was assembled with Velvet [56] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 179 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20. The Phred/Phrap/Consed software package [55] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution [54], Dupfinisher [57], or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F. Chang, unpublished). A total of 103 additional reactions and one shatter library were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI [58]. The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided  $1,312.4 \times$  coverage of the genome. The final assembly contained 488,975 pyrosequence and 75,603,747 Illumina reads.

## Genome annotation

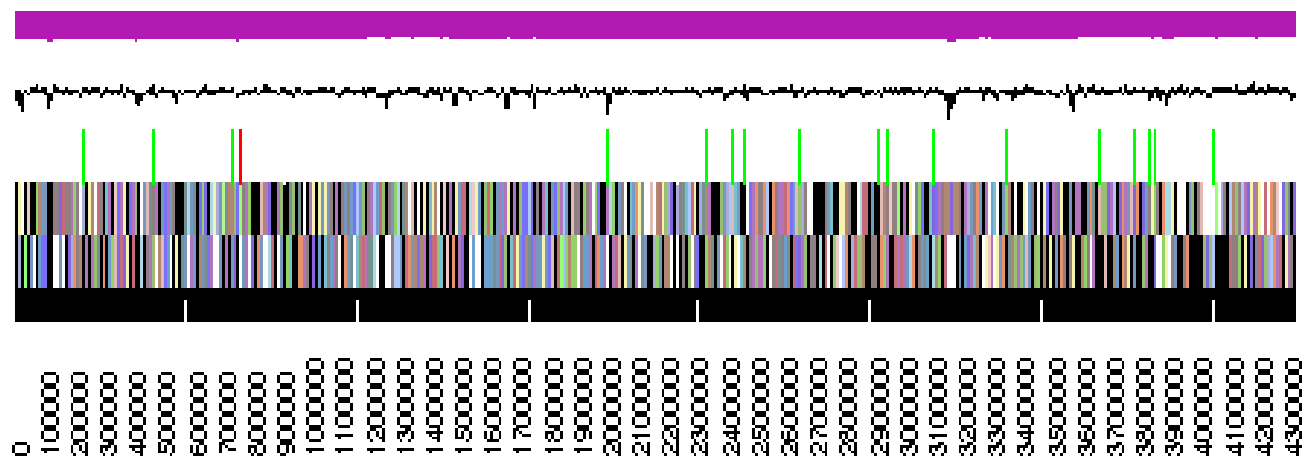
Genes were identified using Prodigal [59] as part of the DOE-JGI genome annotation pipeline [60], followed by a round of manual curation using the JGI GenePRIMP pipeline [61]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [62].

## Genome properties

The genome statistics are provided in Table 3 and Figure 3. The assembly of the draft genome sequence consists of three scaffolds with 4,325,094 bp, 184,087 bp and 13,579 bp length, respectively, and a G+C content of 54.3%. Of the 4,277 genes predicted, 4,230 were protein-coding genes, and 47 RNAs; 69 pseudogenes were also identified. The majority of the protein-coding genes (60.3%) were assigned a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

**Table 3.** Genome statistics

Attribute	Value	% of Total
Genome size (bp)	4,522,760	100.00
DNA coding region (bp)	4,079,818	90.21
DNA G+C content (bp)	2,453,341	54.26
Number of scaffolds	3	
Extrachromosomal elements	unknown	
Total genes	4,277	100.00
RNA genes	47	1.10
rRNA operons	1	
tRNA genes	41	0.96
Protein-coding genes	4,230	98.90
Pseudo genes	69	1.61
Genes with function prediction	2,579	60.30
Genes in paralog clusters	1,764	41.24
Genes assigned to COGs	2,805	65.58
Genes assigned Pfam domains	2,865	66.99
Genes with signal peptides	1,481	34.63
Genes with transmembrane helices	1,089	25.46
CRISPR repeats	0	



**Figure 3.** Graphical map of the largest scaffold. From bottom to the top: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew (purple/olive).

**Table 4.** Number of genes associated with the general COG functional categories

Code	Value	%age	Description
J	156	5.0	Translation, ribosomal structure and biogenesis
A	0	0.0	RNA processing and modification
K	201	6.5	Transcription
L	194	6.3	Replication, recombination and repair
B	4	0.1	Chromatin structure and dynamics
D	34	1.1	Cell cycle control, cell division, chromosome partitioning
Y	0	0.0	Nuclear structure
V	61	2.0	Defense mechanisms
T	303	9.8	Signal transduction mechanisms
M	226	7.3	Cell wall/membrane/envelope biogenesis
N	108	3.5	Cell motility
Z	0	0.0	Cytoskeleton
W	0	0.0	Extracellular structures
U	74	2.4	Intracellular trafficking, secretion, and vesicular transport
O	119	3.8	Posttranslational modification, protein turnover, chaperones
C	160	5.2	Energy production and conversion
G	111	3.6	Carbohydrate transport and metabolism
E	189	6.1	Amino acid transport and metabolism
F	60	1.9	Nucleotide transport and metabolism
H	139	4.5	Coenzyme transport and metabolism
I	131	4.2	Lipid transport and metabolism
P	128	4.1	Inorganic ion transport and metabolism
Q	43	1.4	Secondary metabolites biosynthesis, transport and catabolism
R	401	12.9	General function prediction only
S	260	8.4	Function unknown
-	1,472	34.4	Not in COGs



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