The ISME Journal (2012) 6, 610–618
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www.nature.com/ismej

ORIGINAL ARTICLE

An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea

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Reference phylogenies are crucial for providing a taxonomic framework for interpretation of marker gene and metagenomic surveys, which continue to reveal novel species at a remarkable rate. Greengenes is a dedicated full-length 16S rRNA gene database that provides users with a curated taxonomy based on *de novo* tree inference. We developed a 'taxonomy to tree' approach for transferring group names from an existing taxonomy to a tree topology, and used it to apply the Greengenes, National Center for Biotechnology Information (NCBI) and cyanoDB (Cyanobacteria only) taxonomies to a *de novo* tree comprising 408 315 sequences. We also incorporated explicit rank information provided by the NCBI taxonomy to group names (by prefixing rank designations) for better user orientation and classification consistency. The resulting merged taxonomy improved the classification of 75% of the sequences by one or more ranks relative to the original NCBI taxonomy with the most pronounced improvements occurring in under-classified environmental sequences. We also assessed candidate phyla (divisions) currently defined by NCBI and present recommendations for consolidation of 34 redundantly named groups. All intermediate results from the pipeline, which includes tree inference, jackknifing and transfer of a donor taxonomy to a recipient tree (tax2tree) are available for download. The improved Greengenes taxonomy should provide important infrastructure for a wide range of megasequencing projects studying ecosystems on scales ranging from our own bodies (the Human Microbiome Project) to the entire planet (the Earth Microbiome Project). The implementation of the software can be obtained from http://sourceforge.net/projects/tax2tree/.

The ISME Journal (2012) **6,** 610–618; doi:10.1038/ismej.2011.139; published online 1 December 2011
Subject Category: evolutionary genetics
Keywords: evolution; phylogenetics; taxonomy

Introduction

A robust universal reference taxonomy is a necessary aid to interpretation of high-throughput sequence data from microbial communities (Tringe and Hugenholtz, 2008). Taxonomy based on the 16S rRNA gene (16S) is the most comprehensive and widely used in microbiology today (Pruesse *et al.*, 2007; Peplies *et al.*, 2008), but has yet to reach its full potential because numerous microbes belong to taxa that have not yet been characterized and because numerous sequences that could be reliably classified remain unannotated. For example, two thirds of 16S sequences in GenBank are only classified to domain (kingdom), that is, Archaea or Bacteria, by the National Center for Biotechnology Information (NCBI) taxonomy: this taxonomy is likely the most widely consulted 16S-based taxonomy, despite its disclaimer that it is not an authoritative source, in part because classifications are maintained up to date through user submissions. Most of the un(der)classified sequences are from culture-independent environmental surveys; these

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Received 10 May 2011; revised 23 August 2011; accepted 25 August 2011; published online 1 December 2011

sequences can swamp BLAST searches, leaving users baffled about the phylogenetic affiliation of their submitted sequences. This shortcoming has been addressed by several dedicated 16S databases, including the Ribosomal Database Project (Cole *et al.*, 2009), Greengenes (DeSantis *et al.*, 2006), SILVA (Pruesse *et al.*, 2007) and EzTaxon (Chun *et al.*, 2007), that classify a higher proportion of environmental sequences. However, improvements are still needed because many sequences remain unclassified and numerous classification conflicts exist between the different 16S databases (DeSantis *et al.*, 2006). Moreover, the emergence of large-scale next-generation sequencing projects such as the Human Microbiome Project (Turnbaugh *et al.*, 2007; Peterson *et al.*, 2009) and TerraGenome (Vogel *et al.*, 2009), and the availability of affordable sequencing to a wide range of users who have traditionally lacked access, mean that the need to integrate new sequences into a consistent universal taxonomic framework has never been greater.

The Greengenes taxonomy is currently based on a *de novo* phylogenetic tree of 408 135 quality-filtered sequences calculated using FastTree (Price *et al.*, 2010). *De novo* tree construction is among the most objective means for inferring sequence relationships, but requires either generation of new taxonomic classifications or transfer of existing taxonomic classifications between iterations of trees as the 16S database expands. Previously, we developed a tool that automatically assigns names to monophyletic groups in large phylogenetic trees (Dalevi *et al.*, 2007), which is useful for naming novel (unclassified) clusters of environmental sequences. Here we describe a method to transfer group names from any existing taxonomy to any tree topology that has overlapping terminal node (tip) names. We used this ‘taxonomy to tree’ approach to annotate the 408 135 sequence tree with the NCBI taxonomy as downloaded in June 2010 (Sayers *et al.*, 2011), supplemented with the Greengenes taxonomy from the previous iteration (Dalevi *et al.*, 2007) and CyanoDB (http://www.cyanodb.cz). Explicit rank information, prefixed to group names, was incorporated into the Greengenes taxonomy to help users orient themselves and to improve the consistency of the classification. We assessed the consistency of the resulting classification with the NCBI taxonomy including currently defined candidate phyla (divisions), and present recommendations for consolidation of 34 redundantly named groups and exclusion of one on the basis that its sole representative is chimeric.

Materials and methods

16S data compilation and de novo *tree inference*

We obtained 16S sequences from the Greengenes database, which extracts these sequences from public databases using quality filters as described previously (DeSantis *et al.*, 2006). We only used sequences that had <1% non-ACGT characters. The sequences were checked for chimeras using UCHIME (http://www.drive5.com/uchime/) and ChimeraSlayer (Haas *et al.*, 2011). We only removed sequences from named isolates if they were classified as chimeric by both tools; we removed other sequences if they were classified as chimeric by either tool or if they were unique to one study, meaning that no similar sequence (within 3% in a preliminary tree) was reported by another study. Quality filtered 16S sequences were aligned based on both primary sequence and secondary structure to archaeal and bacterial covariance models (ssu-align-0.1) using Infernal (Nawrocki *et al.*, 2009) with the sub option to avoid alignment errors near the ends. The models were built from structure-annotated training alignments derived from the Comparative RNA Website (Cannone *et al.*, 2002) as described in detail previously (Nawrocki *et al.*, 2009). The resulting alignments were adjusted to fit the fixed 7682 character Greengenes alignment through identification of corresponding positions between the model training alignments and the Greengenes alignment. Hypervariable regions were filtered using a modified version of the Lane mask (Lane, 1991). A tree of the remaining 408 135 filtered sequences, (tree_16S_all_gg_2011_1) was built using FastTree v2.1.1, a fast and accurate approximately maximum-likelihood method using the CAT approximation and branch lengths were rescaled using a gamma model (Price *et al.*, 2010). Statistical support for taxon groupings in this tree was conservatively approximated using taxon jackknifing, in which a fraction (0.1%) of the sequences (rather than alignment positions) is excluded at random and the tree reconstructed. We use these support values to help guide selection of monophyletic interior nodes for group naming during manual curation.

For evaluation of NCBI-defined candidate phyla, we added 765 mostly partial length sequences, that failed the Greengenes filtering procedure but were required for the evaluation, to the alignment using PyNAST (Caporaso *et al.*, 2010; based on the 29 November, 2010 Greengenes OTU templates) and generated a second FastTree (tree_16S_candiv_gg_2011_1) using the parameters described above.

Transferring taxonomies to trees (tax2tree)

Having constructed *de novo* trees, the next key step was to link the internal tree nodes to known, named taxa. We used the NCBI taxonomy (Sayers *et al.*, 2011) as the primary taxonomic source to annotate the trees, supplemented by the previous iteration of the Greengenes taxonomy (Dalevi *et al.*, 2007) and cyanoDB (http://www.cyanodb.cz). This taxonomic annotation used a new algorithm called tax2tree. Briefly, tax2tree consists of the following steps:

(1) Input consists of a flat file containing the donor taxonomy and an unannotated (no group names)

newick format recipient phylogenetic tree with common sequence (tip) identifiers. The tax2tree donor taxonomy is in a very simple format (Supplementary Figure S1) comprising a unique ID followed by a taxonomy string with rank prefixes and was derived from the NCBI taxonomy (ftp://ftp.ncbi.nih.gov/pub/taxonomy/) using a custom Python script. The tax2tree algorithm first filters out non-informative taxonomic assignments from the donor taxonomy strings including the words 'environmental', 'unclassified', as described previously (Dalevi *et al.*, 2007). After filtering, the remaining assignments at each taxonomic level from domain to species are added to each tip with empty placeholders at levels that are missing taxon names. The result of this phase is a tree in which some of the tips have taxonomic information at some or all ranks, imported directly from a donor taxonomy. In addition, each node in the phylogenetic tree is augmented with a tip start and stop index corresponding to a list that contains tip taxonomy information. This structure allows for rapid lookups of the taxon names present at all of the tips that descend from any internal node.

(2) Precision and recall values necessary for the F-measure calculation (see step 5) are calculated and stored on the internal nodes. This caching markedly improves performance on large trees.

(3) Each taxonomic rank at each internal node is determined if it is safe to hold a name. A taxonomic rank is considered safe if (a) there exists a name at that taxonomic rank on the tips that descend that is represented on $\geqslant$50% of those tips and (b) the parent taxonomic rank (for example, phylum to class) is also safe. These names are decorated onto the tree at this point resulting in a phylogenetic tree containing many duplicate names on internal nodes.

(4) The F-measure ($F = 2 \times$ ((precision $\times$ recall)/(precision + recall))) (van Rijsbergen, 1979) is then calculated for each internal node for each name at each taxonomic rank in order to determine the optimal internal node for a name. The F-measure is defined as the harmonic mean of precision and recall, and balances false positives and false negatives (precision is the fraction of informative tips with a given name under a given node out of the total count of informative tips under the node; recall is the fraction of informative tips descending from a given node out of all the informative tips of the entire tree containing the same name). Node references and F-measure scores are then cached in a 2-dimensional Python dictionary external to the tree keyed by both the rank level and by the taxon name. After all nodes are scored, the 2-dimensional hash table is iterated over and for each unique name, the internal node with the highest F-measure score for each name is retained. Each name will only be saved on an internal node once; all other internal nodes with that name will be stripped of the name. If a tie is encountered, the internal node with the fewest tips is kept. The result of this phase is that the phylogenetic tree contains many names on the internal nodes with each name occurring at most a single time. Gaps in the taxa names decorated onto the tree are likely as the result of polyphyletic groups.

(5) Backfilling is used to fill taxonomic gaps in the unique taxon names left on the phylogenetic tree from the F-measure process. For this procedure, the input taxonomy is transformed into a tree and a Python dictionary is constructed that is keyed by the taxon name and valued by its corresponding node. A gap is defined as missing taxonomy rank name information in the phylogenetic tree between a named interior node and its nearest named ancestor (for example, having phylum and order names but without a name for the intervening class rank). For each internal node and nearest named ancestor pair in the phylogenetic tree in which a gap occurs, the taxon name of the node farthest from the root is identified in the input taxonomy. The input taxonomy is traversed until the nearest ancestor's taxa name is found. The names of the nodes traversed in the input taxonomy tree are then appended to the node farthest from the root of the phylogenetic tree. Following the backfilling procedure, it is possible for the phylogenetic tree to have duplicate taxa names.

(6) A back-propagation procedure identifies redundant taxon names in the phylogenetic tree that can be collapsed into a single clade. Here, we test whether any internal node has nearest named descendants at a given rank (for example, phylum) that all share the same name. If so, the name can be removed from the descendants and propagated to the internal phylogenetic node being interrogated.

Secondary taxonomies were then applied manually to the annotated recipient tree, tree_16S_candiv_gg_2011_1, in ARB (Ludwig *et al.*, 2004) using the group tool. For the Greengenes taxonomy, this was achieved by displaying the Greengenes taxonomy field at the tips of the tree and assigning group names missed in the automated taxonomy transfer (mostly higher level ranks associated with candidate phyla). For the cyanoDB taxonomy, manual assignments were based on type species (http://www.cyanodb.cz/valid_genera; 405 instances in tree_16S_candiv_gg_2011_1; Supplementary Table S1). The manually supplemented taxonomic assignments were then exported from the curated tree as a flat file (Supplementary Figure S1) using functionality in the tax2tree pipeline and reapplied to tree_16S_all_gg_2011_1 ensuring manual updates were efficiently propagated to both trees. The tax2tree software is implemented in the Python programming language using the PyCogent toolkit (Knight *et al.*, 2007), and is available under the open-source General Public license at http://sourceforge.net/projects/tax2tree/.

Taxonomy comparisons

The NCBI and Greengenes taxonomies were compared for each of the 408 135 sequences in tree_16S_all_gg_2011_1 making use of the explicit rank designations. The lowest classified rank for each sequence was determined and compared (Figure 2a) to estimate overall improvements in classification. Note that only contiguous classifications were used in this estimate, that is, all ranks leading to the lowest named rank also had to have names. Taxonomic similarities and differences for each sequence at each rank were also assessed by dividing sequences into five categories, (i) the two taxonomies had equal values (same name) at a given taxonomic rank, (ii) the two taxonomies had unequal values at a given rank, (iii) and (iv) one of the taxonomies lacked a value at a given rank and (v) both taxonomies lacked a value at a given rank. This provided an indication of the type of changes that had occurred between the NCBI and Greengenes taxonomies (Figure 2b).

Results and discussion

The rapid accumulation of sequence data from across the tree of life is a boon for molecular taxonomy, but also presents a major barrier to sequence-based taxonomy curators as it is essentially impossible to manually curate trees comprising hundreds of thousands of sequences from scratch. We overcame this difficulty by developing an automated procedure based on F-measures (van Rijsbergen, 1979) for transferring any (donor) taxonomy (in a standard flat text format, Supplementary Figure S1) to any unannotated (recipient) tree (in Newick format) given common sequence identifiers. The F-measure is most often used to measure the classification performance (precision and recall) of information retrieval processes such as database searches (van Rijsbergen, 1979). This approach also has the potential to provide an assessment of the quality of fit between a taxonomy and a tree, which could be used to screen multiple taxonomies and/or trees before manual curation efforts.

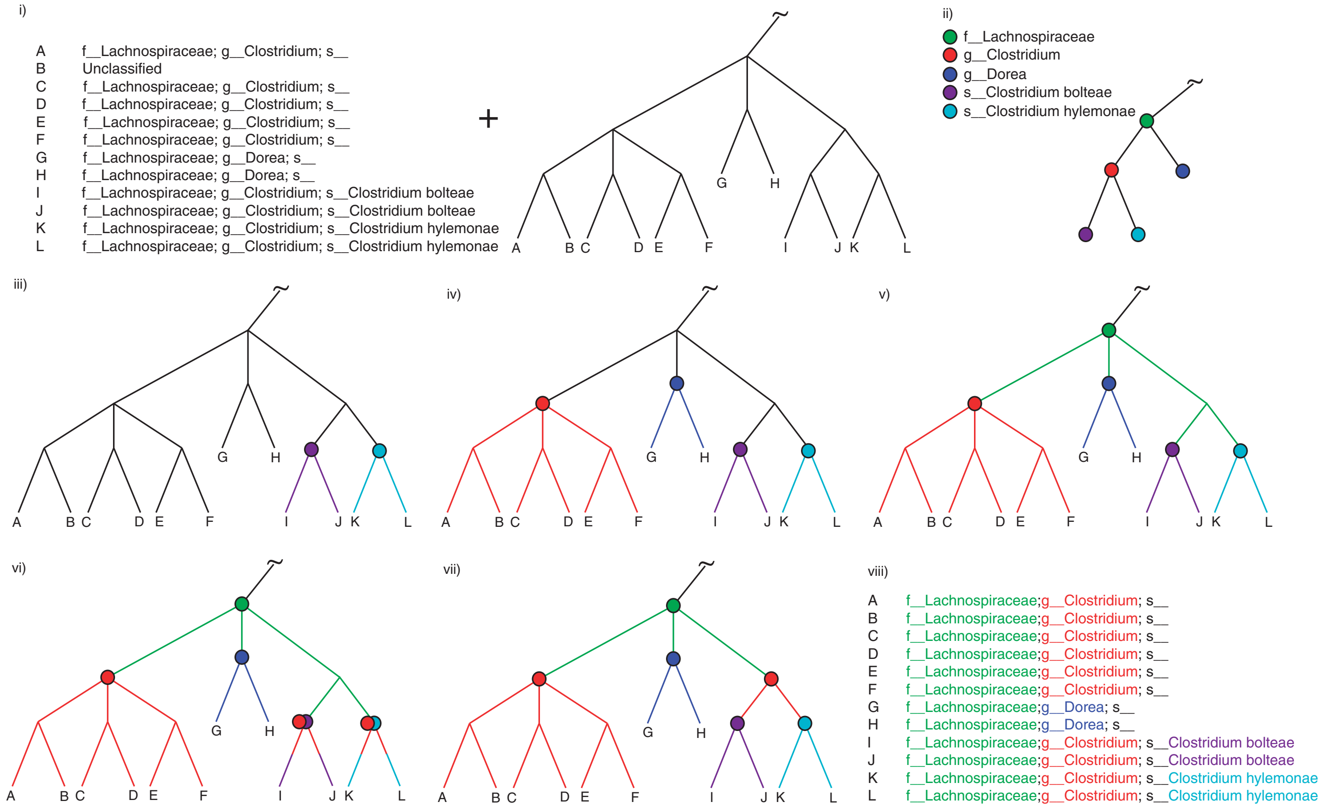
Construction of the rank-explicit Greengenes taxonomy

Using quality-filtered sequences from Greengenes (DeSantis *et al.*, 2006) aligned with the secondary structure-aware infernal package (Nawrocki *et al.*, 2009), we constructed a phylogenetic tree using FastTree2 (Price *et al.*, 2010) containing 408 135 sequences (tree_16S_all_gg_2011_1). We inferred confidence estimates using taxon jackknife resampling (in which sequences, rather than positions in the alignment, are resampled) as it provides a conservative guide to group monophyly, which we found greatly assists manual group name curation between tree iterations (see below). We then applied NCBI classifications to this topology using the tax2tree algorithm (Figure 1 and methods) also taking advantage of the explicit rank designations provided by NCBI to include rank prefixes to group names (for example, p(hylum)__, c(lass)__, o(rder)__). Most sequences (69%; 280 488 of 408 135) had uninformative NCBI classifications, that is, no rank information below domain (kingdom; Figure 2a); of these, most were environmental clones designated as ‘unclassified Bacteria’. The remaining 127 647 sequences with informative classifications were then applied to the tree. This ‘unamended’ approach alone resulted in an improved classification, to at least phylum-level, of nearly all of the taxonomically uninformative sequences (280 452 of 280 488) because most belong to known phyla but were simply deposited without classifications.

We then overlaid additional taxonomic information onto the NCBI-annotated tree by manual group name curation in ARB. This information consisted primarily of candidate phyla and other rank designations for environmental clusters imported from previous iterations of Greengenes (either assigned manually or by GRUNT (Dalevi *et al.*, 2007)), and taxonomic information for the Cyanobacteria obtained from cyanoDB (http://www.cyanodb.cz/). This resulted in more informative classifications for 75% of sequences by at least one rank up to six ranks. These increases in classification depth are graphically shown in Figure 2a by rank.

Changes in sequence classifications between NCBI and Greengenes at each rank are summarized in Figure 2b. Most changes were from uninformative (domain/kingdom name only) in NCBI to informative in Greengenes again reflecting the classification of the large fraction of unclassified environmental sequence in NCBI. The percentage of changes to informative NCBI classifications were relatively low (<7% for all ranks), indicating the degree of congruence between NCBI and Greengenes classifications achieved in part by accommodating polyphyletic groups (see below). Of these type of changes, many occurred at the higher ranks particularly in the candidate phyla where Greengenes is manually curated most intensively (see below). A similar comparison with SILVA or RDP was not possible because of a lack of explicit ranks in these taxonomies. However, by comparing the group name immediately following the domain (kingdom) name (either Bacteria or Archaea) in the SILVA taxonomy, we estimated that only 28% of the 408 135 sequences lacked phylum-level classifications in SILVA, as opposed to 68% in NCBI.

Further, the updated Greengenes taxonomy performed well in a test of reference taxonomies using a naïve Bayesian classifier (Werner *et al.*, 2011). In this paper, we found that retraining the RDP classifier (Wang *et al.*, 2007) using taxa from the



A	f__Lachnospiraceae; g__Clostridium; s__
B	Unclassified
C	f__Lachnospiraceae; g__Clostridium; s__
D	f__Lachnospiraceae; g__Clostridium; s__
E	f__Lachnospiraceae; g__Clostridium; s__
F	f__Lachnospiraceae; g__Clostridium; s__
G	f__Lachnospiraceae; g__Dorea; s__
H	f__Lachnospiraceae; g__Dorea; s__
I	f__Lachnospiraceae; g__Clostridium; s__Clostridium bolteae
J	f__Lachnospiraceae; g__Clostridium; s__Clostridium bolteae
K	f__Lachnospiraceae; g__Clostridium; s__Clostridium hylemonae
L	f__Lachnospiraceae; g__Clostridium; s__Clostridium hylemonae

A	f__Lachnospiraceae;g__Clostridium; s__
B	f__Lachnospiraceae;g__Clostridium; s__
C	f__Lachnospiraceae;g__Clostridium; s__
D	f__Lachnospiraceae;g__Clostridium; s__
E	f__Lachnospiraceae;g__Clostridium; s__
F	f__Lachnospiraceae;g__Clostridium; s__
G	f__Lachnospiraceae;g__Dorea; s__
H	f__Lachnospiraceae;g__Dorea; s__
I	f__Lachnospiraceae;g__Clostridium; s__Clostridium bolteae
J	f__Lachnospiraceae;g__Clostridium; s__Clostridium bolteae
K	f__Lachnospiraceae;g__Clostridium; s__Clostridium hylemonae
L	f__Lachnospiraceae;g__Clostridium; s__Clostridium hylemonae

Figure 1 Overview of the tax2tree workflow. (**i**) The inputs to tax2tree; a taxonomy file that matches known taxonomy strings to identifiers that are associated with tips of (that is, sequences within) a phylogenetic tree. To simplify the diagram, only the family, genus and species are used, although the full algorithm uses all phylogenetic ranks. (**ii**) The input taxonomy represented as a tree and a taxon name legend for the figure. (**iii**, **iv**) Nodes chosen by the F-measure procedure at each rank; (**iii**) species, (**iv**) genus and (**v**) family. In this example, the genus *Clostridium* is polyphyletic, and the F-measure procedure picked the 'best' internal node for the name (uniting tips A–F). However, as unique names at a given rank can only be placed once on the tree, this leaves tips I–L without a genus name placed on an interior node. (**vi**) The backfilling procedure detects that tips I–L have an incomplete taxonomic path (species to family) and (**vi**) prepends the missing genus name (obtained from the input taxonomy) to the lower rank because this step of the procedure examines only ancestors but not siblings. (**vii**) The common name promotion step identifies internal nodes in which all of the nearest named descendants share a common name. In this example, the node that is the lowest common ancestor for tips I–L has immediate descendants that all share the same genus name, *Clostridium*. This name can be safely promoted to the lowest common ancestor (interior node) uniting tips I–L. (**viii**) The resulting taxonomy. Note that the sequence identified as B was unclassified in the donor taxonomy but is now classified as f__Lachnospiraceae; g__Clostridium; s__.

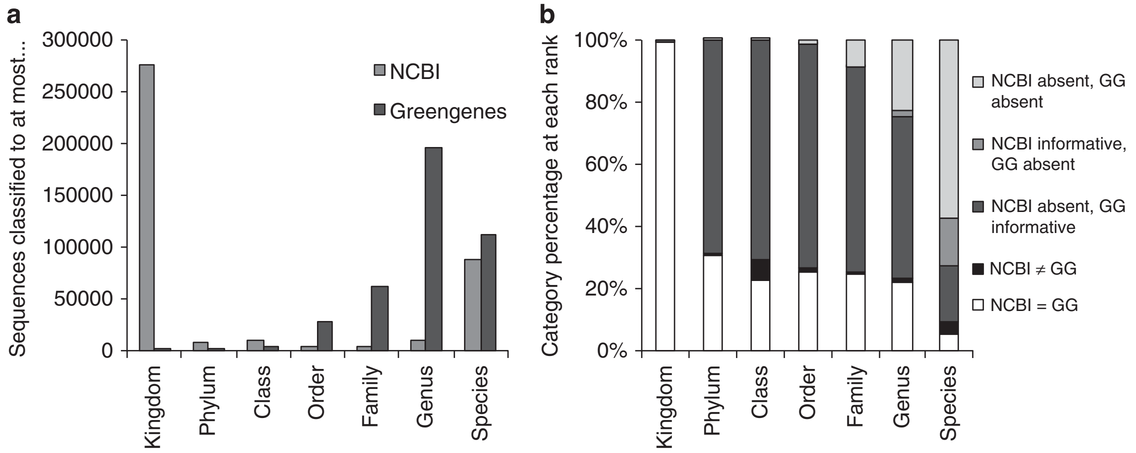


Figure 2 A comparison of the NCBI taxonomy to the updated Greengenes taxonomy for sequences in tree_16S_all_gg_2011_1. (**a**) Lowest taxonomic rank assigned to each sequence; (**b**) taxonomic differences between NCBI and Greengenes at each rank, showing the percentage of sequences classified to each of five possible categories (see inset legend; GG, Greengenes) highlighting cases where NCBI and Greengenes differ.

new Greengenes taxonomy resulted in increased classification resolution relative to SILVA or RDP for a range of environments (human body habitats, snake and mouse gut and soils).

The value of accommodating polyphyletic groups in a 16S rRNA-based taxonomy

In principle, every taxon should correspond to a single monophyletic group in the 16S rRNA-based taxonomy, but practical considerations make relaxing this constraint very useful. In our approach, the back-filling step in the tax2tree (see methods) allows multiple groups to be given the same rank name. This feature is important for taxonomic groups that are well-established in the literature but polyphyletic in the reference 16S rRNA tree. A prominent example is the class Deltaproteobacteria, which rarely forms a monophyletic group in large 16S rRNA topologies and comprises five groups in the current tree_16S_all_gg_2011_1. This result may indicate that the Deltaproteobacteria do not form an evolutionary coherent grouping and will need to subdivided and reclassified. Alternatively, the Deltaproteobacteria may be a monophyletic group not resolved in 16S rRNA trees due to tree inference artifacts, chimeric sequences and/or to limits in the phylogenetic resolution of trees constructed from the 16S rRNA molecule alone. This issue can best be addressed using ‘whole genome’ tree approaches that have greater phylogenetic resolution than single-gene topologies. Trees based on a concatenation of 31 conserved near ubiquitous single-copy gene families indicate that the small subset of Deltaproteobacteria with genome sequences are monophyletic (Ciccarelli *et al.*, 2006; Wu *et al.*, 2009). A second example, also based on concatenated conserved marker genes, indicate that the first genome sequence representative of the family Halanaerobiales (*Halothermothrix orenii*) is a member of the Firmicutes phylum (Mavromatis *et al.*, 2009); which is its (contested) classification based on 16S rRNA trees (Ludwig and Klenk, 2001). Indeed, the Halanaerobiales is separate from the Firmicutes in the current Greengenes topology and is only classified as such because of the back-filling procedure.

Similarly, a number of phylum-level associations have been suggested based on concatenated gene topologies including a relationship between the class Deltaproteobacteria and phylum Acidobacteria (Ciccarelli *et al.*, 2006). We predict that at least a subset of the currently defined candidate phyla will coalesce with other phyla once they are adequately represented by genome sequences and whole-genome trees can be constructed. Therefore, current estimates of the number of 16S-based candidate phyla (>50) should only be used as an approximation and may well drop as candidatus genome sequences accrue. However, regardless of absolute number of phyla, there is a strong need for consistent delineation of taxonomic groups between public databases, particularly candidate phyla. Thus, by retaining polyphyletic groups as sets of monophyletic taxa with the same name, we can accommodate the uncertainty in our present knowledge about both the tree and taxonomy and easily propagate whole-genome-based classification improvements in subsequent iterations of *de novo* 16S rRNA-based trees.

Reconciliation of NCBI and Greengenes-defined candidate phyla

At the time of writing, the NCBI taxonomy lists 71 candidate phyla (divisions) of which 30 are represented only by partial (<1200 nt) sequences. Therefore, in order to address the classification of these groups we amended tree_16S_all_gg_2011_1 with 765 mostly partial length sequences from GenBank and generated a new *de novo* tree using FastTree; tree_16S_candiv_gg_2011_1. We found some NCBI candidate phyla to be polyphyletic in tree_16S_candiv_gg_2011_1 because of a small number of submitter misclassifications or chimeric artifacts. In these instances, we reconciled NCBI and Greengenes designations using the majority classification for a given NCBI group. On the basis of tree_16S_candiv_gg_2011_1, we resolved the 71 candidate phyla into 45 monophyletic groups and one chimeric artifact (Table 1). Many proposed phyla appear to belong to well-established lineages including the Proteobacteria, Firmicutes, Bacteroidetes, Chloroflexi and Spirochaetes. In cases where two or more NCBI candidate phyla were combined and did not cluster with more established groups, we gave priority to either the oldest and/or the largest group. For example, we reclassified candidate phylum kpj58rc (Kelly and Chistoserdov, 2001) as OP3 (Hugenholtz *et al.*, 1998) because of the priority of OP3 in the literature and larger number of representative OP3 sequences in the public databases. We also compared our classifications to SILVA and RDP and in many instances saw consistencies. For example, Greengenes and SILVA both classify candidate phylum CAB-I as Cyanobacteria and Greengenes and RDP both classify KSA1 in the Bacteroidetes. Conversely, in some instances we saw disagreements, such as candidate phylum GN02 (Ley *et al.*, 2006) being classified as BD1-5, and WS5 (Dojka *et al.*, 1998) as WCHB1-60 by SILVA (Table 1). This points to the need to consolidate classifications and also to give priority to published group names where possible.

Final comments and prospectus

The new NCBI-reconciled Greengenes taxonomy rescues over 200 000 environmental sequences from unclassified oblivion. Moreover, the tax2tree pipeline will assist in reconciling information among the various 16S rRNA resources (Greengenes, SILVA,

Table 1 Greengenes classifications of NCBI-defined candidate phyla (divisions) based on tree_16S_candiv_gg_2011_1. SILVA_106 and RDP classifications are included for reference

Candidate bacterial divisions (phyla) in the NCBI taxonomy[a]	*Number of NCBI representative sequences; full (partial)*[b]	*Consensus phylum-level classification*[c]		
		Greengenes	*SILVA*	*RDP*
AC1	6 (7)	p__AC1[d]	TA06	
OS-K	3 (7)	p__Acidobacteria[d]	Acidobacteria	Acidobacteria
OP10	69 (279)	p__Armatimonadetes	OP10	OP10
KSA1	0 (2)	p__Bacteroidetes[d]		Bacteroidetes
KSB1	13 (23)	p__Caldithrix	Deferribacteres	
MSBL5	0 (1)	p__Chloroflexi		Chloroflexi
NT-B4	0 (1)	p__Chloroflexi		
CAB-I	7 (59)	p__Cyanobacteria	Cyanobacteria	Cyanobacteria
OP2	1 (25)	p__Elusimicrobia[e]	Thermotogae	
GN01	10 (12)	p__GN01	Spirochaetes	
GN02	4 (10)	p__GN02	BD1-5	
GN10	3 (4)	p__GN02	BD1-5	
GN11	3 (0)	p__GN02	BD1-5	
GN07	0 (4)	p__GN02		
GN08	0 (1)	p__GN02		
GN04	7 (7)	p__GN04	TA06	
GN12	0 (2)	p__GN04		
GN15	0 (2)	p__GN04		
GN13	0 (2)	p__GN13		
GN14	0 (2)	p__GN14		
GN06	1 (2)	p__KSB3	Proteobacteria	
NC10	6 (27)	p__NC10	Nitrospirae	Firmicutes
NKB19	4 (11)	p__NKB19	BRC1	
KB1 group	7 (20)	p__OP1	EM19	
OP1	10 (38)	p__OP1	EM19	
MSBL6	0 (5)	p__OP1		
Sediment-3	0 (1)	p__OP1		
MSBL4	0 (3)	p__OP3		
kpj58rc	0 (1)	p__OP3		
OP8	36 (390)	p__OP8	Nitrospirae	
JS1	26 (89)	p__OP9	OP9	Firmicutes
VC2	0 (2)	p__Proteobacteria[d]		
Marine group	0 (2)	p__SAR406		
SBR1093	9 (1)	p__SBR1093	Proteobacteria	
SPAM	8 (1)	p__SPAM	Nitrospirae	
GN05	4 (9)	p__Spirochaetes[d]	Spirochaetes	
WWE1	3 (2)	p__Spirochaetes[d]	Spirochaetes	
OP4	1 (1)	p__Spirochaetes[d]	Spirochaetes	
MSBL2	0 (6)	p__Spirochaetes[d]		
KSA2	0 (1)	p__Spirochaetes[d]		
Sediment-4	0 (3)	p__Spirochaetes[d,f]		
Sediment-2	0 (2)	p__Spirochaetes[d];p__SAR406[g]		
GN09	6 (4)	p__TG3	Fibrobacteres	
TG3	41 (40)	p__TG3	Fibrobacteres	
MSBL3	0 (1)	p__Verrucomicrobia[d]		
Sediment-1	0 (3)	p__WS3		
GN03	0 (27)	p__WS3		
KSB4	0 (1)	p__WS3		WS3
WS5	1 (2)	p__WS5	WCHB1-60	
WWE3	116 (0)	p__WWE3	OD1	
ZB3	11 (0)	p__ZB3	Cyanobacteria	
TG2	4 (0)	p__ZB3	Cyanobacteria	
SAM	1 (0)	Chimera[h]	Chloroflexi	

Abbreviation: NCBI, National Center for Biotechnology Information.

[a] The following candidate phyla are not shown because they were consistent between NCBI, Greengenes, SILVA and RDP (where classifications were available): BRC1, KSB2, KSB3, OD1, OP11, OP3, OP6, OP7, OP9, SR1, TM6, TM7, WS1, WS2, WS3, WS4, WS6 and WYO.

[b] Full-length representatives ⩾1200 nt, partial length <1200 nt, not all sequences are 16S rRNA. Phylogenetic placements based only on partial sequences should be considered probationary until full-length or genomic sequence data become available.

[c] Name of phylum that encompasses the majority of the NCBI representative sequences, except where specifically noted. Gaps indicate no classification.

[d] Not robustly supported as a monophyletic group in tree_408135 (jackknife <70%).

[e] On the basis of the position of the single full-length representative after which the group was originally named, the 25 partial length representatives are not affiliated with the full-length sequence and belong to the Chlorobi.

[f] On the basis of the longest representative of this proposed group (AF142890), the two shorter sequences are members of the Firmicutes.

[g] One representative belongs to each phylum; AF142866—Spirochaetes, AF142828—SAR406.

[h] Between Planctomycetes and Chloroflexi.

RDP and EZ-Taxon) with phylogenetic trees built using different methods, and will, we hope, make it easier for users to reconcile taxonomic classifications of large data sets obtained using different taxonomic schemes. This is especially important because which taxonomy is used can have a larger effect on the results than which assignment method is used (Liu *et al*., 2008). The new Greengenes taxonomy, along with all intermediate data products including the tree, can be downloaded from the Greengenes web site at http://greengenes.lbl.gov/.

This work, by automating the process of improving the tree and allowing import of taxonomic knowledge from elsewhere, provides the first step toward an automated pipeline that will immensely improve our ability to link organisms to environment and to understand the evolutionary change associated with phenotypic changes such as adaptation to a new host, switching to a new habitat or adapting to use a new substrate. By providing the foundation for organizing microbial knowledge, these expanded taxonomies will greatly expand our ability to understand the microbes that pervade all aspects of life on the Earth.

Acknowledgements

We thank Adam Arkin and Sean Eddy for supporting this study. The work conducted by ENIGMA was supported by the Office of Science, the Office of Biological and Environmental Research, of the US Department of Energy under Contract no. DE-AC02-05CH11231. This work was supported in part by the Sloan Indoor Environment program, the National Institutes of Health (grants UH2/UH3CA140233 and U01-HG004866), the Bill and Melinda Gates Foundation and the Howard Hughes Medical Institute.

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Supplementary Information accompanies the paper on The ISME Journal website (http://www.nature.com/ismej)