

A Guide to the Natural History of Freshwater Lake Bacteria†

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INTRODUCTION

The biogeochemical importance of bacteria in freshwater ecosystems was first recognized in the 1940s, when Lindeman

put the “microbial ooze” at the center of a diagram depicting the trophic dynamics in a northern temperate lake, Cedar Creek Bog (121). Since this early recognition of the critical role of bacteria in regenerating and mobilizing nutrients in freshwater food webs, it has become clear that aquatic bacteria drive transformations and the cycling of most biologically active elements in these ecosystems. This key position held in essentially all biogeochemical cycles does not merely stem from the role of bacteria as the principal degraders and min-

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eralizers of organic compounds to their inorganic constituents (30, 36) but also results from their biomass production and trophic coupling to eukaryote predators, which, by fueling the food web, has a profound impact on elemental fluxes and water quality in the ecosystem (154). Given the facts that lakes and other inland waters play a more critical role in the global carbon budget than previously recognized (31) and that lakes have been described as early indicators (i.e., sentinels) of both regional and global environmental change (133, 228), the role of microbes in these processes is of renewed interest.

Despite the recognition that bacteria occupy a prominent role in lake ecosystem processes and greatly impact lake water quality, the bacterial taxa participating in these activities remain largely undescribed. Molecular biology tools now provide us with unprecedented access to the diversity and composition of freshwater lake bacterial communities and have for the first time enabled our field to identify the numerically dominant organisms in these ecosystems and learn much about their distributions in time and space. Although every multilake study has shown that differences in bacterial community composition between lakes can be quite large (e.g., see references 108, 122, 234, and 235), there is a growing body of evidence that many bacterial groups are freshwater specific and have a global distribution (48, 63, 82, 123, 129, 178, 195, 223, 238, 242). These two viewpoints seem to stand in opposition and may be restated as, “each lake has a different bacterial community” and “many lakes have the same bacterial taxa.” This apparent dichotomy arises in part because different research groups inventory bacterial taxa using various levels of phylogenetic or taxonomic resolution, ranging from phylum-level fluorescent *in situ* hybridization (FISH) probes (23, 154, 176) to more narrowly identified phylotypes defined by 16S rRNA gene sequences or rRNA intergenic spacer regions (84, 140, 190, 232, 234). Thus, in order to engage in a meaningful discussion of freshwater lake taxa, we need a consistent and robust vocabulary with which to inventory taxonomic units.

To date, most studies employing molecular methods to examine freshwater lake bacterial taxa have been highly descriptive in nature, offering little more than “species” lists for a scattered set of freshwater ecosystems or the proverbial “stamp collection.” This descriptive approach is arguably a necessary early step in the progression of any natural science, but it cannot provide us with the predictive capabilities needed to create a synthetic understanding of freshwater microbial ecology. In order to continue our maturation as a discipline, we must develop a framework within which to explain taxon or phylotype distribution patterns based on processes and mechanisms. This review, in the form of a natural history guide (or “field guide”), as often compiled for macroscale organisms such as plants and animals, attempts to provide the starting point from which this framework can be developed. We note that previous freshwater bacterial compilations are either now outdated (238), heavily focused on methods and marine water and freshwater comparisons (154), or geared toward highlighting needed avenues of research in modern inland water microbiology (75). While acknowledging the importance of molecular methods for advancing microbial ecology, we refer readers to the many excellent reviews and books dedicated to their description (for example, see references 117, 145, and 154).

In light of the mismatched and overlapping naming structure

for freshwater lake bacterial taxa, our approach to this natural history guide was to gather all published freshwater lake 16S rRNA gene sequences, construct high-quality phylogenies, and propose a new vocabulary that unifies taxon names and provides a platform with which to discuss these important freshwater lake taxa (akin to the approach taken by Zwart and colleagues [238]). We use a classification system that is hierarchical like Linnaean taxonomy but based on phylogenetics. Our classification system is also designed to maintain the phylogenetic context by which freshwater bacterial gene sequences historically have been identified, clustered, and named. With this natural history guide, we are not endorsing “phylogenetic” over “Linnaean” systematization (debate reviewed in reference 57). Instead, we choose a phylogenetic approach, because there are too few defined freshwater lake bacterial genera or species to have meaningful discussions using the Linnaean approach. We expect the number of described freshwater genera and species to grow rapidly, and indeed, this appears to be the trend, as a number of isolates have been described recently (76–79, 81, 113). As the database of described freshwater organisms matures, we envision that the phylogenetic groups described within this guide will be replaced by an organismal naming structure according to the conventions set forth in the bacteriological code. In the interim, this document provides the bridge between former naming conventions and newly described species. Furthermore, the phylogenetic approach allows us to synthesize the available published information on freshwater lake bacterial phylotypes, develop new cross-study comparisons, and discuss these phylotypes in an ecological context.

A natural history guide is a dynamic document, and this is a critical time for a new edition. As larger and larger molecular data sets are gathered through next-generation sequencing (both 16S rRNA gene tag sequencing and metagenomics), this natural history guide will enable cross-study comparisons and syntheses that are currently not feasible. In the following guide, we lay out our new naming structure with the controlled nomenclature of phylum/lineage/clade/tribe. We then review the current information on the ecology, metabolism, and distribution of the defined groups within each phylum. We also highlight previously unidentified bacterial distribution patterns and propose hypotheses to test in future studies. Finally, we review the current knowledge about ecological traits of different groups of freshwater lake bacteria.

Historical Perspectives

As recently as 1980, culture-based techniques led researchers to believe that the organisms inhabiting soil and aquatic environments were quite similar (170). Researchers now appreciate a clear distinction between those bacteria found in the soil and those found in aquatic habitats (130). Molecular methodology has since revealed distinct bacterial communities among aquatic environments that are chemically and physically unique (167, 242). The greatest community composition differences occur along salinity gradients, as manifested in the very distinct bacterial community compositions of the ocean and freshwater lakes (130, 167). Indeed, for other microorganisms (microeukaryotes, archaea, and viruses), evolutionary

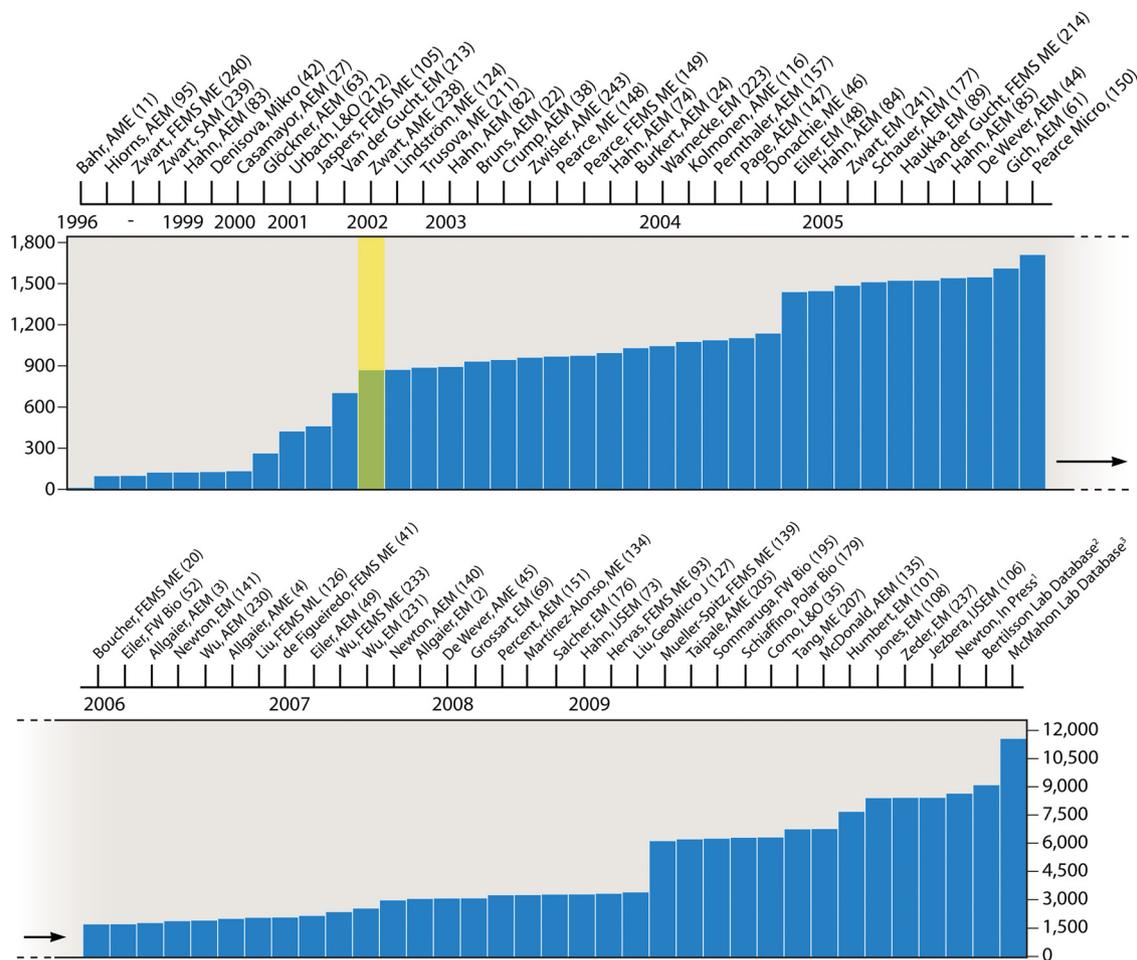


FIG. 1. Timeline of 16S rRNA genes represented in the database. Lead author and journal abbreviation from papers contributing sequences are noted along the timeline. The yellow bar indicates the previous freshwater sequence collection review by Zwart and colleagues (238). Journal abbreviations are as follows: AEM, *Applied and Environmental Microbiology*; AME, *Aquatic Microbial Ecology*; EM, *Environmental Microbiology*; FEMS ME, *FEMS Microbiology Ecology*; FW Bio, *Freshwater Biology*; GeoMicro J, *Geomicrobiology Journal*; Mikro, *Mikrobiologĳia*; L&O, *Limnology and Oceanography*; ME, *Microbial Ecology*; Micro, *Microbiology*; FEMS ML, *FEMS Microbiology Letters*; IJSEM, *International Journal of Systematics and Evolutionary Microbiology*; Polar Bio, *Polar Biology*; SAM, *Systematic and Applied Microbiology*. ¹, manuscript in press at *Environmental Microbiology* (141a) (includes GenBank accession numbers FJ827781 to FJ828505); ², GenBank accession numbers for the sequenced clones are HQ386253 to HQ386631; ³, GenBank accession numbers for the sequenced clones are FJ916807 to FJ916903 and HQ530565 to HQ532908, except HQ530583, HQ531638, and HQ532521. (Based on data from references 2, 3, 4, 11, 20, 22, 24, 27, 35, 38, 41, 42, 44, 45, 46, 48, 49, 52, 61, 63, 69, 73, 74, 82, 83, 84, 85, 89, 93, 95, 101, 105, 106, 108, 116, 124, 126, 127, 134, 135, 139, 140, 141, 147, 148, 149, 150, 151, 157, 176, 177, 179, 195, 205, 207, 211, 212, 213, 214, 223, 230, 231, 233, 237, 238, 239, 240, 241, and 243.)

transitions across the salinity barrier also seem to have been quite rare (128, 129).

In 2002, Zwart and colleagues gathered the reported 16S rRNA gene sequences from all freshwater environments (238), which at the time consisted of 11 lakes and 2 rivers as part of 10 independent studies. This fruitful approach resulted in the collection of 689 bacterial 16S rRNA gene sequences and enabled the identification of 10 freshwater phyla and 34 putative bacterial freshwater clusters, defined as a monophyletic branch of a phylogenetic tree that contained at least two sequences with $\geq 95\%$ gene identity from more than one freshwater environment. Many of the identified clusters were proposed to be common in freshwater and appeared to represent unique bacterial taxa found only in this biome. Over the past 8 years, many more researchers have retrieved and sequenced 16S rRNA genes from freshwater lake systems (Fig. 1), and a great

number of new putative freshwater lake clusters have been proposed. However, the lack of a cohesive collection of known sequences and defined clusters has resulted in a mixture of naming conventions and many overlapping and incongruent clusters.

CREATING A MODERN VIEW OF FRESHWATER LAKE BACTERIA

In this study, we present an overview and meta-analysis of published freshwater lake 16S rRNA gene sequences. To constrain our data set to a more consistent range of habitats, we limited our analysis to sequences collected from the epilimnia of lakes. We acknowledge that bacterial communities may be very different in other freshwater habitats such as wetlands or suboxic hypolimnetic water of stratified lakes. Microbial pop-

ulations and communities in such environments are likely strongly influenced by the overall availability of electron acceptors, steep redox clines, and the release of organics from plant debris or sediments. An in-depth account of the phylogenetic composition and ecology of bacterial communities in these rather poorly characterized microbiomes is needed but lies beyond the scope of this review. We included 68 published papers that represent >8,400 16S rRNA gene sequences and have additionally added >2,600 unpublished sequences from the McMahon laboratory in Wisconsin and >400 unpublished sequences from the Bertilsson laboratory in Sweden (Fig. 1). The majority of these sequences are from projects reported within the last 5 years (Fig. 1). The increasing pace of lake studies containing larger numbers of 16S rRNA gene sequences follows the universal trend of an increasing rate of sequences deposited into the public databases (e.g., the Ribosomal Database [RDB] Project [222]), and with the explosion of next-generation sequencing technologies, the total number of sequences in this database is likely to be surpassed by each new project. We propose that our manually curated data set based largely on nearly full-length sequences generated by traditional Sanger technology will serve as a much-needed backbone for interpretations of the burgeoning collection of such comparatively short sequence reads.

Phylogenetic Tree Reconstructions and Cluster Classification

For each of the 69 published papers included in our synoptic analysis, 16S rRNA gene sequences from lake epilimnia were retrieved and downloaded from GenBank via their accession numbers. Taxonomic assignments were obtained for these published sequences and the unpublished McMahon laboratory and Bertilsson laboratory sequences using the Classifier program at the Ribosomal Database RDP II website (222). Based on the RDP taxonomic assignments, sequences were grouped by phylum and imported into the ARB software package (131), containing a 16S rRNA gene database from Greengenes (January 2007) (43). Sequences were initially automatically aligned by using the FAST_ALIGNER ARB tool before the alignment was heuristically adjusted using primary and secondary rRNA structures as a guide. Sequences that were thought to be chimeras (checked with the software program Mallard [10]) or those that contained many (>10) ambiguous bases were removed from the database. In total, ~200 sequences were removed for either one of these reasons, leaving >11,500 sequences for further analyses.

Phylogenetic inferences were obtained by using only nearly full-length ($\geq 1,300$ -nucleotide [nt]) sequences. A 50% base frequency filter, which excludes highly variable positions, was calculated for the sequence alignment in each phylogenetic analysis. Phylogenetic reconstructions were performed at CIPRES (www.phylo.org) using RAXML (197) maximum likelihood analysis with 100 bootstrap runs and with Bayesian inference in MrBayes v. 3.1.2 (172), using a general time-reversible gamma-distributed rate variation model with three independent Markov chain Monte Carlo analyses, each starting from random trees for each of four simultaneous chains until chain stabilization. Trees recovered before chain stabilization were discarded by using appropriate burn-in values, and a 50%

majority-rule tree was calculated. Consensus trees obtained from the maximum likelihood and Bayesian inference methods were compared manually for the presence of nonstable branching patterns between methods. The final trees illustrated in all figures represent the reconstruction from Bayesian phylogenetic inference but are depicted with both the Bayesian clade credibility values and the maximum likelihood bootstrap values at nodes obtained by both methods. All partial sequences (<1,300 nt) were then added to the consensus trees in ARB using the maximum parsimony criteria with an appropriate 50% base frequency filter and without allowing changes in the tree topology.

Within the 68 published papers used to construct our database, >100 freshwater clusters were previously proposed. We integrated these clusters and many others resulting from our analyses into a single phylogenetic framework using a hierarchical naming structure (phylum/lineage/clade/tribe) illustrating the genetic identity relationships between groups. The phylum is synonymous to the long-established phylum defined by bacterial systematics. The lineage, the first of our phylogenetically defined groups, is the most broadly defined and consists of sequences that cluster as a monophyletic branch of a phylogenetic tree within a phylum (e.g., acI, Luna1, and acIII). Our defined lineages generally share maximum sequence identity differences of between 10 and 15% (calculated including all nucleotide positions over the shared length of the 16S rRNA gene). The clade definition is based upon the cluster definition used previously by Zwart and colleagues (238), thereby providing continuity between the last compiled database and this new rendition. As such, a clade represents a group of sequences clustering as a monophyletic branch of a phylogenetic tree that have $\geq 95\%$ sequence identity to at least one other sequence of that branch (i.e., nearest neighbor). Our clade definition also requires that at least two sequences be obtained from different lakes (e.g., clades acI-A, acI-B, and acI-C). The most refined taxonomic group of our clustering system is the tribe. A tribe also consists of a group of sequences clustering as a monophyletic branch of a phylogenetic tree but requires each sequence to have $\geq 97\%$ sequence identity to another sequence of that branch. As with the clade definition, the tribe definition also requires that at least two sequences originated from different lakes (e.g., tribes acI-B1, acI-B2, and acI-B3). This definition was chosen to avoid the clustering issues created from the large number of sequencing and curation errors contained in sequences deposited in the public databases while also closely representing the most common division (97% 16S rRNA gene identity) used to describe bacterial ecologically relevant groups in nature. When possible, tribes were more narrowly defined than with the minimum allowed 97% sequence identity. The program DOTUR (180) was used to identify suspected clade and tribe groupings observed in the phylogenetic reconstructions. Phyla/lineages/clades were excluded from detailed phylogenetic analyses when they did not contain at least one tribe.

Common Lineages of Freshwater Lake Bacteria

Examination of the database revealed that 21 phyla (based on RDP confidence assignments of $\geq 75\%$) have been recovered from lake epilimnia, with 5 phyla being recovered

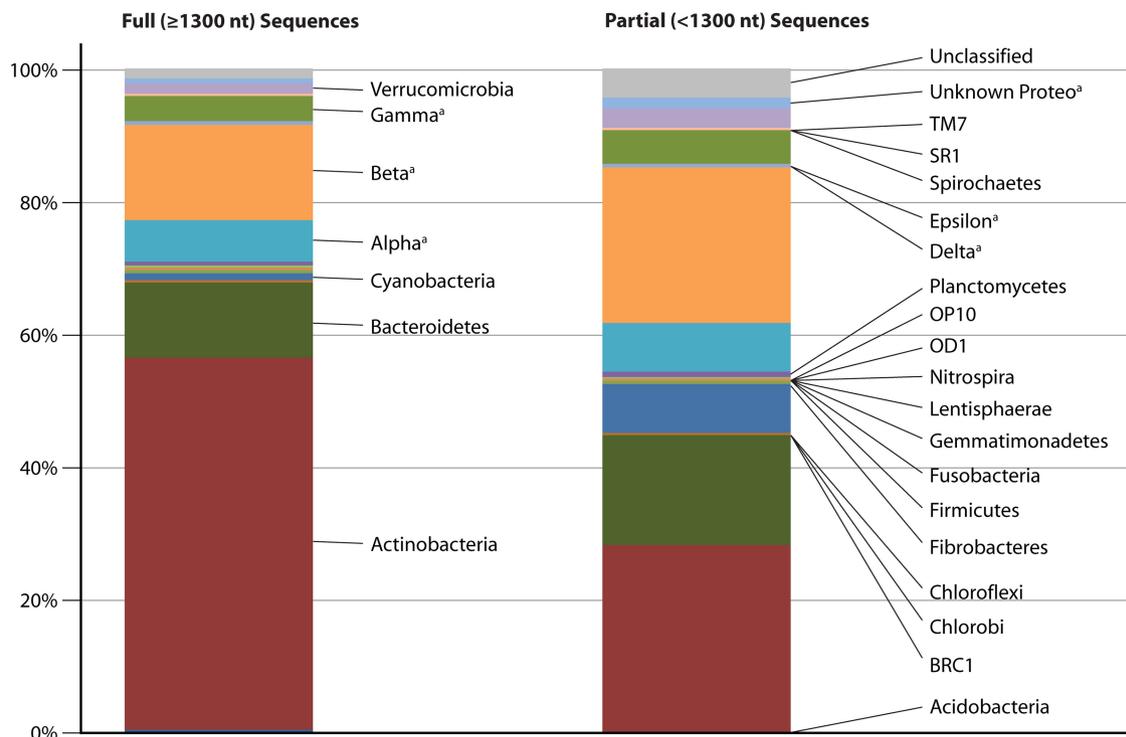


FIG. 2. Stacked bar plot representing the distribution of sequences by phylum in the freshwater lake sequence database. Sequences were taxonomically classified by the RDP classifier (February 2010) (222) and were required to have 75% classifier confidence to be included as a representative of a phylum. Sequences with <75% confidence were termed unclassified. Phyla listed are present in both bar plots but are listed on only one side to aid in the visualization of the plots. ^a, the phylum *Proteobacteria* was split into its representative classes for sequences with a $\geq 75\%$ confidence score at the class taxonomic assignment and unknown *Proteobacteria* for those sequences with confidence scores of <75%.

commonly (*Proteobacteria*, especially *Betaproteobacteria*, with >4,300 and >2,600 sequences, respectively; *Actinobacteria*, with >3,000 sequences; *Bacteroidetes*, with >1,900 sequences; *Cyanobacteria*, with >800 sequences; and *Verrucomicrobia*, with >300 sequences) (Fig. 2 and see Table S1 in the supplemental material). These five phyla were also the most numerous in the database compiled previously by Zwart and colleagues (238) and are in general agreement with the majority of FISH-based studies, which reported the *Actinobacteria* (4, 23, 63, 224) or the *Betaproteobacteria* (62, 93, 139, 153, 230) as being the most abundant bacterial phylum in lake epilimnia. The remaining 16 recovered phyla make up only $\sim 2.6\%$ of the total sequences collected in our database and include *Acidobacteria*, *BRC1*, *Chlorobi*, *Chloroflexi*, *Fibrobacteres*, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Lentisphaerae*, *Nitrospira*, *OD1*, *OP10*, *Planctomycetes*, *Spirochaetes*, *SR1*, and *TM7* (Fig. 2).

A large group of sequences in the data set was termed “unclassified” (~ 500 sequences) (Fig. 2). An unclassified label signifies that a sequence had <75% confidence at the phylum taxonomic level with the RDP Classifier assignment. The majority of these unknown sequences were at least in part the result of the sequence read length being very short (<400 nt) (Fig. 2). It is not clear whether the 15 unclassified nearly full-length sequences represent bacterial 16S rRNA genes from undefined or poorly defined phyla, undetected chimeras, poor sequence reads, or nonspecific amplification. None of these 15 sequences met our criteria to be included in a clade or tribe.

In the following sections we review and summarize the currently available information regarding the ecology and diversity of each major bacterial phylum recovered in freshwater lakes. Along with basic information and the historical context, we present an updated phylogenetic framework that links back to prior findings and use this new framework as an outline for summarizing published studies of the ecology of each phylum and its more finely resolved groups. The length of each section reflects the currently available information and is also a fair proxy for the number of available sequences for each phylum. We conclude each section with a brief summarizing statement of unifying traits for freshwater lake bacteria in each phylum, even if it is clear that there is considerable ecological divergence at this higher taxonomic rank.

Phylum *Actinobacteria*

The phylum *Actinobacteria* (formerly part of the group of high-G+C Gram-positive bacteria) is made up of Gram-positive bacteria with a high mol% G+C DNA composition (generally ranging from 51 to 70% [218]). Historically, soils were considered the primary environment of residence and optimal activity for the *Actinobacteria* (66). The advent of molecular-based studies of aquatic systems has changed this perception. Initial 16S rRNA gene- and FISH-based studies revealed that members of the *Actinobacteria* are common and often numerically important component in a variety of freshwater habitats (42, 63, 95, 238, 239). Also clear from these studies was that the

16S rRNA genes recovered from the epilimnia of lakes were distinct from the “typical” *Actinobacteria* in soils or the more recently discovered *Actinobacteria* in marine systems (166). Further molecular surveys have since shown that these “freshwater” *Actinobacteria* can be found in a variety of limnic systems, such as rivers (37, 238), brackish seas (171), bays (186), and glacial ice (29). The emergent phylogenetic clustering by biome along with evidence that the *Actinobacteria* in freshwater lakes are actively synthesizing DNA (152, 224, 237) and proteins (23, 152) have provided ample evidence that this phylum is an indigenous resident of freshwaters.

***Actinobacteria* 16S rRNA gene-based phylogeny.** Since the first “typical” freshwater *Actinobacteria* 16S rRNA gene sequences were obtained in 1997 (95), >40 phylogenetic clusters have been postulated for this phylum (Fig. 3). In 2004, Warnecke and coauthors combined several previous 16S rRNA gene cluster-naming schemes (95) into a hierarchical classification system that has generally become fixed in the literature (223). Those authors named the most prominent lineages acI, acII, acIII, and acIV and subdivided these lineages into the clades acI-A to -C, acII-A to -D, and acIV-A and -B. The acI to acIII lineages are affiliated with the order *Actinomycetales*, and the acIV lineage is associated with the order *Acidimicrobiales*. Since this naming structure was implemented, numerous studies have contributed additional lineages (82, 233) and additional subdivisions of the acI to acIV lineages (3, 140, 233), many of which are overlapping (Fig. 3B to D).

Our phylogenetic reconstruction yielded nine broadly defined lineages (acI, acTH1, acSTL, Luna1, acIII, Luna3, acTH2, acIV, and acV) (Fig. 3A), all of which were described previously. Six of the nine lineages were divided further into clades and/or tribes. Thirty-two tribes resulted from the reconstructions (Fig. 3B to D). The acI and acIV lineages are particularly abundant among the freshwater lake *Actinobacteria*, with the acI lineage being more abundant in the free-living fraction (2, 207), often comprising $\geq 90\%$ of the identified actinobacterial taxa (224), and with the acIV lineage exhibiting more localized dominance (231). As the most-studied group, the acI lineage was also the most resolved, containing 13 narrowly defined tribes (some with >99% identity). Only one of the 13 acI tribes (Phila) contained a named organism, and this organism, “*Candidatus* Planktophila limnetica,” has been maintained solely in enrichment cultures (106). The acI-A and acI-B clades are nearly encompassed by the popular molecular probes acK-M1 and sta2-30, which were named after two of the first 16S rRNA gene clones recovered for this lineage (95, 238).

The acIV and Luna1 lineages were the next most abundant lineages in our database (Fig. 3C and D). The acIV lineage partitioned into four monophyletic clades (acIV-A, acIV-B, acIV-C, and acIV-D) and six tribes, many of which have received multiple names in the literature (Fig. 3D). The Luna1 lineage partitioned into only a single clade containing four tribes, one of which (Luna1-A2) was highly abundant in the sequence database. The acIV lineage does not contain cultured representatives, while the Luna1 lineage is well represented by previously described organisms (73). Using the same isolation process as that for the Luna2 tribe, Hahn and coworkers isolated or enriched for several bacteria from the Luna1 lineage. Seven species were obtained in these enrichment cultures and were named “*Candidatus* Planktoluna difficilis,” “*Can-*

didatus Aquiluna rubra,” “*Candidatus* Flaviluna lacus,” “*Candidatus* Rhodoluna limnophila,” “*Candidatus* Rhodoluna planktonica,” “*Candidatus* Rhodoluna laticola,” and “*Candidatus* Limnoluna rubra” (73).

The acIII, acV, and acSTL lineages were the remaining three lineages containing clade and tribe divisions. All three of these lineages had a single clade harboring two tribes. The acIII lineage contains the Luna2 tribe, named after Lake Mondsee (meaning moon), the ecosystem from which the first isolate was obtained. This tribe is one of the few tribes to contain multiple isolates (82). The acV lineage is related to bacteria isolated from soils clustering with the defined soil lineage soil II+III but is only distantly related to cultured organisms (data not shown). The acSTL lineage name originates from clones retrieved from Lake Stechlin in Germany and, like many of the other lineages, does not contain cultured representatives.

The final three lineages of the *Actinobacteria*, acTH1, acTH2, and Luna3, are made up of sequences represented by only a single tribe. The acTH1 and acTH2 lineages are named after clone representatives obtained from Lake Taihu in China (231). All sequences obtained for acTH2 were closely affiliated with the genus *Mycobacterium*; therefore, we named the tribe representing this sequence cluster Myco. Unlike lineage acTH2, acTH1 does not contain cultured representatives. Luna3 is closely related to the other Luna lineages but does not have a cultured representative as of yet.

Ecology of the freshwater lake *Actinobacteria*. Many studies employing a variety of methods, including fluorescent labeling of cells (3, 23, 224), PCR-based methods (2, 89, 123, 140, 141, 151, 212, 233, 242), and metagenomic profiling (39), have confirmed and expanded upon the initial observation that the *Actinobacteria* are ubiquitous and abundant in freshwater lakes. In fact, the *Actinobacteria* are often the numerically dominant phylum in lakes, where they can contribute >50% of the bacteria in the surface waters (epilimnion) (63). The freshwater *Actinobacteria* are also present in the bottom waters (hypolimnion) of lakes (20), but their abundance often decreases with decreasing oxygen concentrations (3, 205). This high level of abundance in the epilimnion has proven to be consistent across lake types, as the *Actinobacteria* are common among oligotrophic (101), mesotrophic (39, 101, 237), eutrophic (233), and dystrophic (141) lakes. Likewise, the dominance of the *Actinobacteria* is spread across the globe. Studies have shown high actinobacterial abundances in lakes in North America (140), Europe (63), Africa (44, 45, 101), Asia (82, 142, 230), Australia (84), South America (35), and Antarctica (149, 150).

Despite the ease with which 16S rRNA gene sequences from members of the *Actinobacteria* are recovered from the freshwater environment, the isolation of organisms representative of these sequences has proven difficult. The *Actinobacteria* were one of the first isolated and described groups from the pelagic lake environment (53, 194), but these first isolates were of the chromogenic actinomycete type, often *Micromonospora*-like, a group now recognized as not being particularly abundant in these environments (63). The first time a representative freshwater *Actinobacteria* isolate was available for study was in 2003, when Hahn and coworkers isolated several organisms affiliated with what were termed the Luna clusters (Luna clusters 1 and 2) (82). Nine Luna strains were isolated from lakes

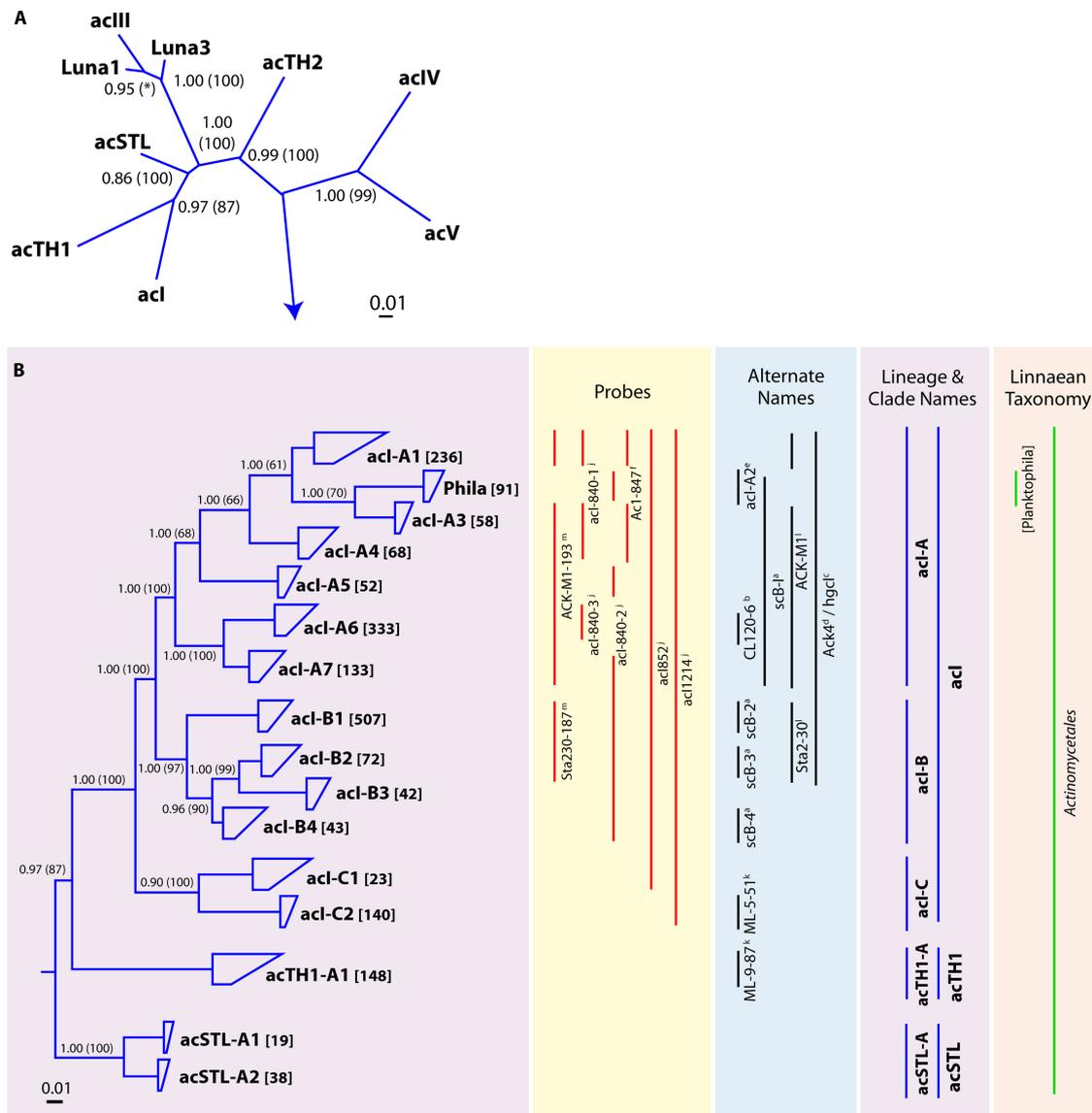


FIG. 3. (A) Radial consensus phylogram of the freshwater lake lineages of the *Actinobacteria*. (B) Consensus phylogram of the tribes of lineages acI, acTH1, and acSTL. (C) Consensus phylogram of the tribes of lineages Luna1, acIII, Luna3, and acTH2. (D) Consensus phylogram of the tribes of lineages acIV and acV. All phylogenetic inference calculations were conducted with nearly full-length sequences (>1,300 nt) representing the lineages/clades/tribes. Frequency base filters were created to mask out highly variable positions, and representative sequences from members of the *Archaea* were used as the outgroup for all tree reconstructions. Bayesian clade credibility values and maximum likelihood bootstrap values (in parentheses) of >0.5 (50) are listed at each node. Tribe names are listed at the end of tree tips (trapezoids), with the number of sequences associated with each tribe listed in square brackets. Tree tips ending in ovals represent phylogenetic clusters that were named previously but did not meet the criteria to be called a tribe (≥ 2 sequences with $\geq 97\%$ identity of $\geq 1,300$ nt isolated from >1 lake). Current lineage and clade names are listed to the right of the phylograms and are highlighted in purple. The Linnaean taxonomy column is based on the RDP classification (222), where all sequences within the classified group must have $\geq 75\%$ assigned confidence for the taxonomic group. Square brackets around a genus name indicate a candidate genus, and an asterisk indicates that the genus is described but not yet covered in the RDP classification. Alternate names from previous studies also covering the defined lineages/clades/tribes are listed. Probe coverage is based upon a perfect match to $\geq 75\%$ of the sequences in a defined lineage/clade/tribe. References to past clades and probes are as follows: ^a, reference 3; ^b, reference 48; ^c, reference 63; ^d, reference 95; ^e, reference 140; ^f, reference 156; ^g, reference 212; ^h, reference 214; ⁱ, reference 223; ^j, reference 224; ^k, reference 233; ^l, reference 238; ^m, reference 242. *, tribe Luna1-A2 is associated with three “*Candidatus*” genera: “*Candidatus* Limnoluna,” “*Candidatus* Flaviluna,” and “*Candidatus* Rhodoluna” (73).

of contrasting trophic states (ranging from oligotrophic to hypereutrophic) located on two continents (Europe and Asia) (82). All strains were very small ($<0.1 \mu\text{m}^3$) with a selenoid cell morphology and thin cell walls, the so-called “ultramicrobacteria” (82). This finding matched previously reported FISH

observations that the freshwater lake *Actinobacteria* were generally free-living single cells of a very small size (63, 224). All isolates were also pigmented, either yellow or red, suggesting that like other soil-dwelling members of the *Actinobacteria*, these taxa might be capable of producing secondary metabo-

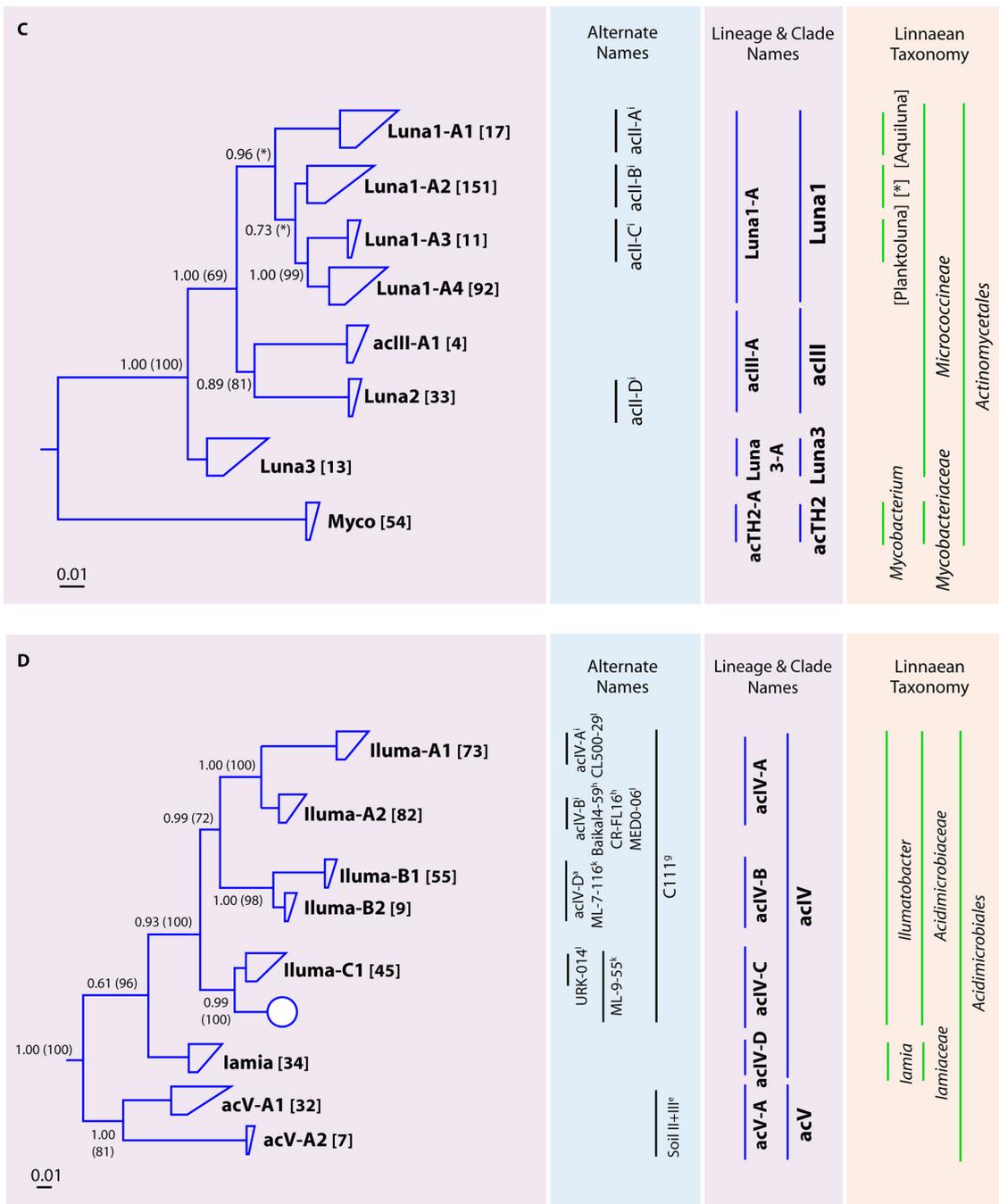


FIG. 3—Continued.

lites. Since this initial isolation, Hahn and coworkers have isolated or enriched several other strains affiliated with the Luna clusters (84), giving several of these enrichments “*Candidatus*” names (73). During the course of isolating organisms from Luna1, those authors noted that they were unable to obtain pure cultures because a small percentage (often <1%) of another bacterial taxon was seemingly required to maintain the *Actinobacteria* in culture, although the identity of the cotaxon was highly variable and thus likely part of a nonspecific relationship (73). The enrichment of members

of the freshwater *Actinobacteria* following the addition of signaling compounds (22) could represent one avenue for the apparent coculture requirement observed by Hahn and colleagues (73).

Although the isolation of the Luna clusters has already revealed many insights into the life-style of the freshwater lake *Actinobacteria*, no isolates have been obtained from the two lineages considered most abundant (lineages acI and acIV). Recently, Jezbera and coworkers established stable cocultures, including a member of the acI lineage, which was named “*Can-*

didatus Planktophila limnetica" (106). Unfortunately, this organism represented $\leq 5.6\%$ of the cocultures; hence, little physiological insight could be attained.

Due to the dearth of freshwater *Actinobacteria* cultures, researchers have turned to molecular methods to gain insight into the ecophysiological roles that these organisms play in their natural habitat, with the majority of studies focusing on the distribution of lineages and clades in time and space. The first comparative study of the distribution of the *Actinobacteria* noted very similar 16S rRNA gene sequences from Europe and North America, suggesting that narrowly defined clades could have a worldwide distribution (239). Numerous studies based on 16S rRNA gene identification since then have confirmed a striking lack of geographic separation for taxa within the dominant *Actinobacteria* clades (84). However, as observed by Hahn and colleagues, strain distribution could be limited by environmental factors (84). In that study, Luna strains isolated from several continents but containing nearly identical rRNA operons exhibited very different adaptations to prevailing local temperatures, suggesting barriers to widespread colonization capabilities (84). It remains to be seen if this apparent lack of a biogeographic signal holds true for strain comparisons, protein-encoding genes, or whole-genome comparisons within some of the narrowly defined *Actinobacteria* clusters.

The cosmopolitan distribution and high level of abundance of actinobacterial 16S rRNA genes in freshwater lakes have prompted several investigations into the environmental factors and cell characteristics contributing to the success of these organisms. Through time, the phylum *Actinobacteria*, especially the acI lineage, appears to have comparatively smaller changes in biomass or prevalence across lake seasons (63), making it the most persistent lineage of freshwater lake bacteria. Those authors suggested that the comparatively minor fluctuations in the abundance of acI taxa point to a consistent source of energy generation for these organisms in spite of the marked seasonal changes observed for bacteria over time in lakes (183). Recently, metagenomic studies of freshwater lakes revealed an abundance of rhodopsin genes (a potential source of light-driven energy generation) in these systems, many of which are now termed actinorhodopsins due to their presence in clones containing freshwater *Actinobacteria* gene signatures (including the acI and Luna lineages [185]). These actinorhodopsin genes are also known to occur in several freshwater isolates (184). This potentially supplemental mode of energy generation may be partly responsible for the success that generates the cosmopolitan distribution and ubiquity of these organisms.

Another major mechanism for the ubiquity of the freshwater *Actinobacteria* seems to be related to their small cell size and cell wall composition. In a study by Pernthaler and coworkers the addition of the size-selective grazer *Ochromonas* (*Potriochromonas*) sp. initiated acI blooms comprising up to 60% of the total bacterial community (156). In 2003, Hahn and coworkers confirmed this observation for the Luna lineages as well (82) but also observed that it was not simply size that reduced *Ochromonas* grazing on their isolates. Those authors posited that the cell wall structure, which included a visually identified S layer, might be an additional factor making these organisms less "edible." In a subsequent study, attempts to disrupt the S layer resulted in increased grazing (4 to 5 times)

upon members of the Luna2 tribe (209). The lack of members of the *Actinobacteria* in the food vacuoles of freshwater bacterivorous flagellates has also provided further evidence for a general grazing protection mechanism among these bacteria (106). The freshwater *Actinobacteria* are not impervious to grazing, however, as the addition of *Cyclidium glaucoma*, a bacterivorous ciliate that efficiently feeds on small prey (163), resulted in equal grazing upon the acI lineage and other freshwater lake lineages (156). Others have also noted correlative decreases in the abundance of the *Actinobacteria* during periods of enhanced grazing pressure (141).

UV stress resistance has also been postulated to be one of the reasons for the success of the *Actinobacteria* in the upper waters of lakes, which often have high UV transparency. Warnecke and colleagues showed a significant positive relationship between *Actinobacteria* abundance and UV transparency in a series of mountain lakes (224). Those authors noted that both pigmentation and a high G+C content have been shown to increase protection from UV damage in bacteria and accordingly suggested that the freshwater *Actinobacteria*, which have a high G+C content and may be pigmented (82), might harbor similar protective mechanisms (224). However, no correlation between the freshwater *Actinobacteria* and UV transparency was observed in a second study involving high mountain lakes, casting doubt on this apparent relationship (230). In addition to UV protection, many members of the *Actinobacteria* are capable of producing spores (reviewed in reference 218), allowing them to survive long periods of desiccation. Although still not observed for the freshwater *Actinobacteria*, strong UV protection and desiccation resistance via spore formation, together with the known small cell size of these organisms, would make the freshwater *Actinobacteria* particularly suitable for aerial dispersal. This could explain their ubiquitous representation in globally dispersed lakes (93). However, to date, these taxa have not been identified in air samples (109).

The abundance and distribution of the freshwater *Actinobacteria* have been studied in relation to the chemical and physical properties of lakes and their resident microbiota. *Actinobacteria* taxa have not been found in physical association with members of the *Cyanobacteria* or other phytoplankton (116) but may become a greater part of the bacterioplankton community during phytoplankton blooms (3, 174, 237). Generally, increased nutrient concentrations select against the freshwater lake *Actinobacteria* (90). This disadvantage does not seemingly stem from an inability to assimilate common substrates, as the *Actinobacteria* have been shown to be capable of incorporating arginine, leucine, and thymidine at rates similar to those of other common freshwater lake taxa (23) or as the most active bacterial fraction for amino acid uptake (174). However, the growth rates for the freshwater *Actinobacteria* often do not match their capability for substrate uptake; generally, this group exhibits growth rates that are average to below average compared to those of other phyla or the bulk freshwater lake bacterioplankton community (188). The various growth and substrate incorporation rates observed for the freshwater *Actinobacteria* may be due to the preferences of individual tribes for different substrate sources. Substrate partitioning among common marine bacteria has been shown (102, 161). Likewise, members of the acI-A taxa of Lake Grosse Fuchskuhle were unable to assimilate acetate, while

members of taxa associated with the acI-B clade readily assimilated this carbon source (23). The distribution of members of the *Actinobacteria* tribes also seems to partition by broad substrate source categories, such as the ratio of allochthonous to autochthonous carbon produced in a lake (108). This resource partitioning between clades and tribes has been postulated as a reason for shifts in the growth rates and biomass changes observed pre- to post-phytoplankton bloom in Lake Zurich (237).

The pH of an ecosystem is often a master driver of bacterial community composition (55) and accordingly has been identified as being one of the major drivers of *Actinobacteria* clade and tribe distribution. In 2005, Lindström and colleagues recognized that members of clade ACK-M1 (similar to clade acI-A) (Fig. 3B) was more prevalent in high-pH lakes, while members of clade Sta2-30 (similar to clade acI-B) was more prevalent in low-pH lakes (123). Similarly, studies by Newton et al. and Taipale et al. found a majority of clade acI-B-related sequences in their humic (low-pH) lakes (141, 205). In an effort to identify more coherent response patterns, Newton and colleagues narrowly defined 11 tribes within clades acI-A and -B and found that individual tribes within the clades showed significant distribution differences based on lake pH (140). It is as yet unknown whether the other major lineages of the freshwater lake *Actinobacteria* show similar distribution differences based on pH.

***Actinobacteria* summary.** The phylum *Actinobacteria* contains several monophyletic lineages that are specialized to limnetic systems. These so-called “freshwater” lineages are highly abundant and ubiquitous in the epilimnia of lakes. The acI lineage appears particularly abundant in these systems and consequently has received the bulk of research attention, directed mainly at its distribution within and among lakes and its relationship to phytoplankton and bacteriophages. The isolation of representative taxa has proven elusive but has been achieved for the Luna lineages. Analysis of these isolates and FISH-based studies has shown that the freshwater *Actinobacteria* are small ($<0.1 \mu\text{m}^3$) with a rod, coccus, or selenoid shape and are pigmented. Biogeographic signals have not been observed for taxa defined by any 16S rRNA gene groupings, but pH differences among lakes and particle attachment and carbon substrate preferences within lakes have been shown to differentiate the clades and tribes. A preliminary examination of metagenomic data has revealed that several members of the freshwater *Actinobacteria* have an overrepresentation of pathways for nucleic and amino acid metabolism (159) and harbor rhodopsins (actinorhodopsins), a potential mechanism for supplemental energy generation by light harvesting. Generally speaking, the freshwater lake *Actinobacteria* are free-living, open-water defense specialists, with possible photo- and heterotrophic energy generation life-styles.

Phylum *Bacteroidetes*

The phylum *Bacteroidetes*, formerly known as the *Cytophaga-Flavobacterium-Bacteroides* (CFB) phylum, exhibits enormous phenotypic and metabolic diversity. The members of this phylum occur in soil, in aquatic environments, or as symbionts of plants, animals, and humans. Most described isolates of the *Bacteroidetes* are chemoorganotrophs, but phototrophic capa-

bilities have been described for two marine isolates (*Polaribacter* sp. strain MED152 and *Dokdonia* sp. strain MED134) that contain a photopigment related to the proteorhodopsin of the *Proteobacteria* (64, 65). Many representatives of the *Bacteroidetes* are known to have close relationships with animal and human hosts, where they can be either synergists or antagonists. Comparative phylogenomic analyses have provided strong support for the phylum *Bacteroidetes* and suggest that it shares many phenotypic characteristics and a common ancestor with the phyla *Fibrobacteres* and *Chlorobi* (72). Within the *Bacteroidetes* there are three distinct classes: *Bacteroidales*, *Flavobacteriales*, and *Sphingobacteriales*. Even if members of the *Bacteroidetes* sometimes dominate freshwater lake bacterial communities (157) and comprise the third most recovered phylum in this lake 16S rRNA gene data set (Fig. 2), few studies have examined the ecology of these organisms and/or defined monophyletic clusters.

***Bacteroidetes* 16S rRNA gene-based phylogeny.** Altogether, five lineages and 23 clades and/or tribes could be identified within the *Bacteroidetes* (Fig. 4). The bacI, bacII (B88), and bacIII lineages of the *Bacteroidetes* contained the majority of 16S rRNA gene sequences in our data set (Fig. 4B and C). The bacII lineage was divided into three tribes made up entirely of the class *Flavobacteria*. The majority of the recovered freshwater lake *Flavobacteria* sequences were not monophyletically related to the isolates from freshwaters (e.g., *Flavobacterium columnare* and *F. limicola*) (data not shown). Likely, there are many more clades and tribes within the bacII lineage, as 24 operational taxonomic units (OTUs) were identified by terminal restriction fragment length polymorphism (tRFLP) patterns in a recent study of their seasonal dynamics in temperate lakes (49).

Another dominant sequence group within the *Bacteroidetes* was the bacI lineage, which is most closely related to the bacIV lineage (Fig. 4B). Both of these lineages are members of the order *Sphingobacteriales*. No studies have focused on the four tribes comprising the better-represented bacI lineage, but several studies have examined the bacIV lineage (157, 177, 178) and previously divided it into 3 clusters (HAL, GKS2-217, and LD2) (Fig. 4B). Two isolates from this lineage have been described, “*Candidatus Aquirestis calciphila*” and “*Candidatus Haliscomenobacter calcifugiens*” (86).

The bacIII and bacVI lineages are also members of the order *Sphingobacteriales*. As with many of the tribes within the *Bacteroidetes*, each of the tribes in these lineages (two each in bacIII and bacVI) contained previously described species. The Algor tribe (*Algoriphagus*-like) of the bacIII lineage was represented by the most sequences among all *Bacteroidetes* tribes. The Flecto (*Flectobacillus*-like) tribe is known to be filamentous and is targeted by the frequently used probe R-FL615 (188, 192). The two bacVI tribes (Muci for *Mucilaginibacter*-like and Pedo for *Pedobacter*-like) were not recovered frequently in our database.

Ecology of the freshwater lake *Bacteroidetes*. In lake epilimnia, the *Bacteroidetes* may comprise a large proportion of particle-associated bacteria (120, 143) and seem to play a particularly important role in the degradation of complex biopolymers (115). In agreement with this inferred niche, several studies using PCR-based clone libraries and/or quantitative PCR (qPCR) have documented an increase in the propen-

sity of the *Bacteroidetes* to occur during periods or at sites characterized by high external dissolved organic carbon (DOC) loading or alga-derived DOC inputs (48, 49, 116, 237). Using batch culture experiments, a relationship between bacterial community composition patterns and humic substance loading revealed that a DOC concentration above 0.54 mM favored tribes bacI-A1 and bacI-A2 of the phylum *Bacteroidetes* (51). Following a humic matter enrichment of lake water in another experiment, members of the *Bacteroidetes* related to *Flexibacter* (*Chitinophaga*) became the most abundant bacterial group, suggesting that they may play a significant role in humic matter degradation (104).

The freshwater lake *Bacteroidetes* are often found in high abundance during periods following cyanobacterial blooms. In one case, a majority of the bacterial community measured using 16S rRNA gene-based tRFLP and qPCR was made up of the *Bacteroidetes* *Flavobacterium*-like lineages B88 (bacII) and B99 (bacV) following the senescence and decline of an intense cyanobacterial bloom (49). It was concluded that many *Flavobacterium*-like populations are favored during periods of high heterotrophic activity and enhanced growth, where resource availability is the main force structuring the distribution of these populations. Furthermore, evidence for this copiotroph life-style (adaptation to high-nutrient conditions) among certain groups of the *Flavobacteria* was provided recently by Zeder and colleagues (237). In dilution cultures, group FLAV2 (within Flavo-A3 and targeted by probe FLAV2-438) (Fig. 4B) exhibited rapid growth. This rapid growth was enhanced even further when samples were obtained and diluted during and after a spring phytoplankton bloom. However, the net increase in FLAV2 cell numbers was much lower in the environment than predicted from the dilution bioassays conducted in the laboratory, suggesting a strong top-down control of the rather large FLAV2 cells by mixotrophic grazers (237).

The influences of microeukaryote predation on the freshwater *Bacteroidetes* have been studied in some detail. Environmental surveys from various freshwater environments have shown an increase in the abundance of members of the *Bacteroidetes* during periods of enhanced grazing (32, 191). However, conflicting results have been reported. In those studies, enrichments of microeukaryote predators resulted in a decrease in the abundance of members of the *Bacteroidetes* (111, 175, 192). In planktonic environments, predator-prey interactions are particularly influenced by the size-structure of microbial prey and predators (71). Size-selective grazing by bacte-

riovorous nanoflagellates allows the smallest bacterial cells to largely avoid or altogether escape predation. Elongated and filamentous morphologies can also provide resistance against grazing, as cells are too large to be ingested by nanoflagellates (70). In fact, a number of field studies and experiments on the occurrence of elongated bacteria during periods of strong protozoan grazing pressure provide strong support for filamentous morphologies as an efficient bacterial defense strategy against predation (70, 83, 112, 118).

In a well-designed study by Pernthaler and coauthors (157), the authors noted a significant increase in numbers of filamentous bacterial cells during the late spring and early summer, which is generally a period of increased grazing by phagotrophic protists, known as the “clear-water” phase (32, 157). During this period, those authors observed a bloom of a phylogenetically narrow cluster ($\geq 99\%$ 16S rRNA gene identity) of members of the *Bacteroidetes*. These filamentous bacteria, identified as LD2 (tribe Aquir; Fig. 4B), made up more than 40% of the total bacterial biomass in the lake water, as measured by using FISH probes. A very interesting hypothesis is that filaments are formed by bacteria capable of a high level of morphological plasticity under high grazing pressure. Strong support for this hypothesis has been provided by independent experimental studies that observed an increase in filamentous morphotypes and a decrease in rod-shaped morphotypes belonging to *Flectobacillus*-like organisms when under strong grazing pressure (33, 34, 83). A phylogenetically broader group of the *Bacteroidetes*, which includes LD2 and has filamentous morphotypes, was described by Schauer and Hahn (177) and was named the SOL cluster (lineage bacIV; Fig. 4B). Although the genus *Flectobacillus* and SOL bacteria represent facultative and obligate filamentous bacteria, they possess highly contrasting growth characteristics, with the latter group having higher growth rates (193). In members of the class *Bacteroidetes* and the family *Saprospiraceae*, three filamentous cell groups within the SOL cluster have been identified: the HAL-A1 tribe (HAL), the HAL-A2 tribe (GKS2-217), and the Aquir tribe (LD2). A population study based on FISH revealed that 84 out of 115 lakes in Europe and Asia contained members of the SOL cluster (177). On average, this lineage made up only 1% of the cells in the epilimnion but contributed a much larger biomass, since the cells were filaments (178). The tribes within the SOL cluster seem to exhibit habitat preferences. The HAL-A2 tribe (GKS2-217) was found only in soft-water lakes ($< 50 \mu\text{S cm}^{-1}$) at a pH range of 6.5 to 7.3, the Aquir tribe

FIG. 4. (A) Radial consensus phylogram of the freshwater lake lineages of the *Bacteroidetes*. (B) Consensus phylogram of the tribes of lineages bacI and bacIV. (C) Consensus phylogram of the tribes of lineages bacII, bacIII, bacV, and bacVI. All phylogenetic inference calculations were conducted with nearly full-length sequences ($> 1,300$ nt) representing the lineages/clades/tribes. Frequency base filters were created to mask out highly variable positions, and representative sequences from members of the *Archaea* were used as the outgroup for all tree reconstructions. Bayesian clade credibility values and maximum likelihood bootstrap values (in parentheses) of > 0.5 (50) are listed at each node. Tribe names are listed at the end of tree tips (trapezoids), with the number of sequences associated with each tribe listed in square brackets. Tree tips ending in ovals represent phylogenetic clusters that were named previously but did not meet the criteria to be called a tribe (≥ 2 sequences with $\geq 97\%$ identity of $\geq 1,300$ nt isolated from > 1 lake). Current lineage and clade names are listed to the right of the phylograms and are highlighted in purple. The Linnaean taxonomy column is based on the RDP classification (222), where all sequences within the classified group must have $\geq 75\%$ assigned confidence to the taxonomic group. Square brackets around a genus name indicate a candidate genus, and an asterisk indicates that the genus is described but not yet covered in the RDP classification. Alternate names from previous studies also covering the defined lineages/clades/tribes are listed. Probe coverage is based upon a perfect match to $\geq 75\%$ of the sequences in a defined lineage/clade/tribe. References to past clades and probes are as follows: ^a, reference 49; ^b, reference 63; ^c, reference 156; ^d, reference 157; ^e, reference 177; ^f, reference 192; ^g, reference 233; ^h, reference 237; ⁱ, reference 238.

(LD2) was found only in hard-water lakes at a pH range of 7.7 to 9.5, and the HAL-A1 tribe (HAL) was found in a variety of lakes with a pH range of 6.7 to 8.6 (177). A pure culture is available for the HAL-A1 tribe (215), and enrichments have been obtained recently for the Aquir and HAL-A2 tribes (86), revealing low growth rates under natural conditions, an obligate aerobic life-style, and often very large (up to 150 μm) filament lengths.

Bacteroidetes summary. Unlike other common freshwater lake groups, the tribes of the *Bacteroidetes* have not exhibited any perceivable seasonal or lake-specific occurrence patterns (49). This finding might be related to their strong dependency on organic matter load or phytoplankton blooms, which are more likely to occur during sporadic and somewhat stochastic disturbance events rather than as part of a predictable seasonal trajectory. Also, the connection between *Bacteroidetes* taxa and grazing pressure is striking (178). Future studies targeting the phylogenetically more narrowly defined tribes could reveal intriguing and possibly diversified roles for members of this phylum in specialized organic matter degradation and in freshwater food webs.

Phylum Cyanobacteria

The conspicuous nature and long-recognized ecological importance of the *Cyanobacteria* have led to a much longer and more complex history of classification than that for the majority of the other bacterial phyla. Common freshwater lake genera include *Microcystis*, *Anabaena*, *Aphanizomenon*, *Oscillatoria*, *Planktothrix*, *Synechococcus*, and *Cyanothece*. Similar to eukaryotic phytoplankton, the freshwater *Cyanobacteria* perform oxygenic photosynthesis but rely only on chlorophyll *a* and an assortment of phycobilins for photosynthesis (132, 198). Some aquatic *Cyanobacteria* contain heterocysts, which are cells dedicated solely to nitrogen fixation (198). These *Cyanobacteria* along with many others that do not contain heterocysts, but fix nitrogen, play a key part in nutrient cycling in lakes. *Cyanobacteria* species are often considered nuisance organisms, as some species form large floating mats and may release toxins into lake waters (100). Numerous studies have been dedicated to an understanding of the environmental factors controlling cyanobacterial blooms and the induction of toxin production in freshwaters (reviewed in references 100, 144, and 146). A more detailed discussion of the large body of knowledge concerning these phototrophic freshwater organisms can be found elsewhere (25, 67, 100, 168, 198, 200).

Cyanobacteria 16S rRNA gene-based phylogeny. The lineages of the freshwater *Cyanobacteria* are much more thoroughly defined and include a vastly greater number of described species than for other freshwater lake bacterial phyla (182). For this reason, we did not wish to recreate the already defined phylogeny but instead point to the many excellent reviews on this subject (see references 182 and 203 and citations therein). More than 800 16S rRNA genes (the majority were <1,300 nt) were obtained from studies included in our data set (Fig. 2). Most of these sequences were closely related to the genera *Cyanothece* and *Microcystis* (data not shown). Two tribes, CyanI-A1 (*Cyanothece* related) and AnaI-A1 (*Anabaena* related) were identified in the data set (see Fig. 7C).

Phylum Proteobacteria

The phylum *Proteobacteria* is a group of Gram-negative bacteria encompassing the majority of recognized agriculturally, industrially, and medically relevant organisms and therefore is the most studied of the bacterial phyla. Six classes of *Proteobacteria* are currently recognized: the *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Epsilonproteobacteria*, *Gammaproteobacteria*, and *Zetaproteobacteria*. The long history of study and wealth of knowledge pertaining to these organisms have led to the independent examination of the proteobacterial classes in most freshwater lake studies. We will follow this convention and discuss them separately below.

Class Alphaproteobacteria

The class *Alphaproteobacteria* has a special role in evolution and microbiology, as eukaryote mitochondria are believed to have originated from an alphaproteobacterial symbiont (8). The *Alphaproteobacteria* are found in all imaginable habitats and display enormous plasticity in their genomes and in their life-styles. The large variation in genome organization and size among members of this class (1 to >9 Mbp) is likely related to their common roles as endosymbionts and intracellular parasites (13). The *Alphaproteobacteria* are also at the hub of the global nitrogen cycle, because symbiotic members of this phylum (e.g., the *Rhizobiales*) often facilitate atmospheric nitrogen fixation by plants. The *Alphaproteobacteria* are numerically dominant in many marine ecosystems (137). In freshwater lakes, the *Alphaproteobacteria* are also ubiquitous although less numerous.

Alphaproteobacteria 16S rRNA gene-based phylogeny. In an early study by Glöckner and colleagues, the phylogeny of the freshwater *Alphaproteobacteria* was broken down into six clusters (alphaI to alphaVI) (63). Since then, eight additional clusters have been described. Our phylogenetic analyses yielded nine tribes from five lineages (Fig. 5B and C). The most widely distributed freshwater lake tribe of the *Alphaproteobacteria* is LD12 (alfV lineage), the freshwater sister group to marine SAR11 (128), and this tribe also contained the most *Alphaproteobacteria* sequences in our database (Fig. 5B). The alfIII lineage, overlapping with the genus *Sphingomonas* sensu stricto (206), contained few sequences, whereas the alfIV lineage was more abundant in our combined freshwater lake data set. This lineage features tribes Novo-A1 and -A2, corresponding largely to the genus *Novosphingobium*, and tribe Pyxis, which overlaps with the genus *Sphingopyxis* (206). The alfI (related to the *Rhizobiales*) and alfII (related to *Caulobacter* and *Brevundimonas*) lineages were also fairly common in freshwater lake clone libraries.

Ecology of the freshwater lake Alphaproteobacteria. Quantitative assays based on FISH have consistently shown that the *Alphaproteobacteria*, at least at the class level, are resistant to predation: their relative abundance increases in response to enhanced microeukaryote grazing (32, 110, 119, 175, 191). The increase in abundance has often been accompanied by a tendency of members of the *Alphaproteobacteria* to form filaments, aggregates, or *Caulobacter*-like stalked cells that sometimes can make up a majority of the *Alphaproteobacteria* population (83, 175, 191). It is currently not known whether

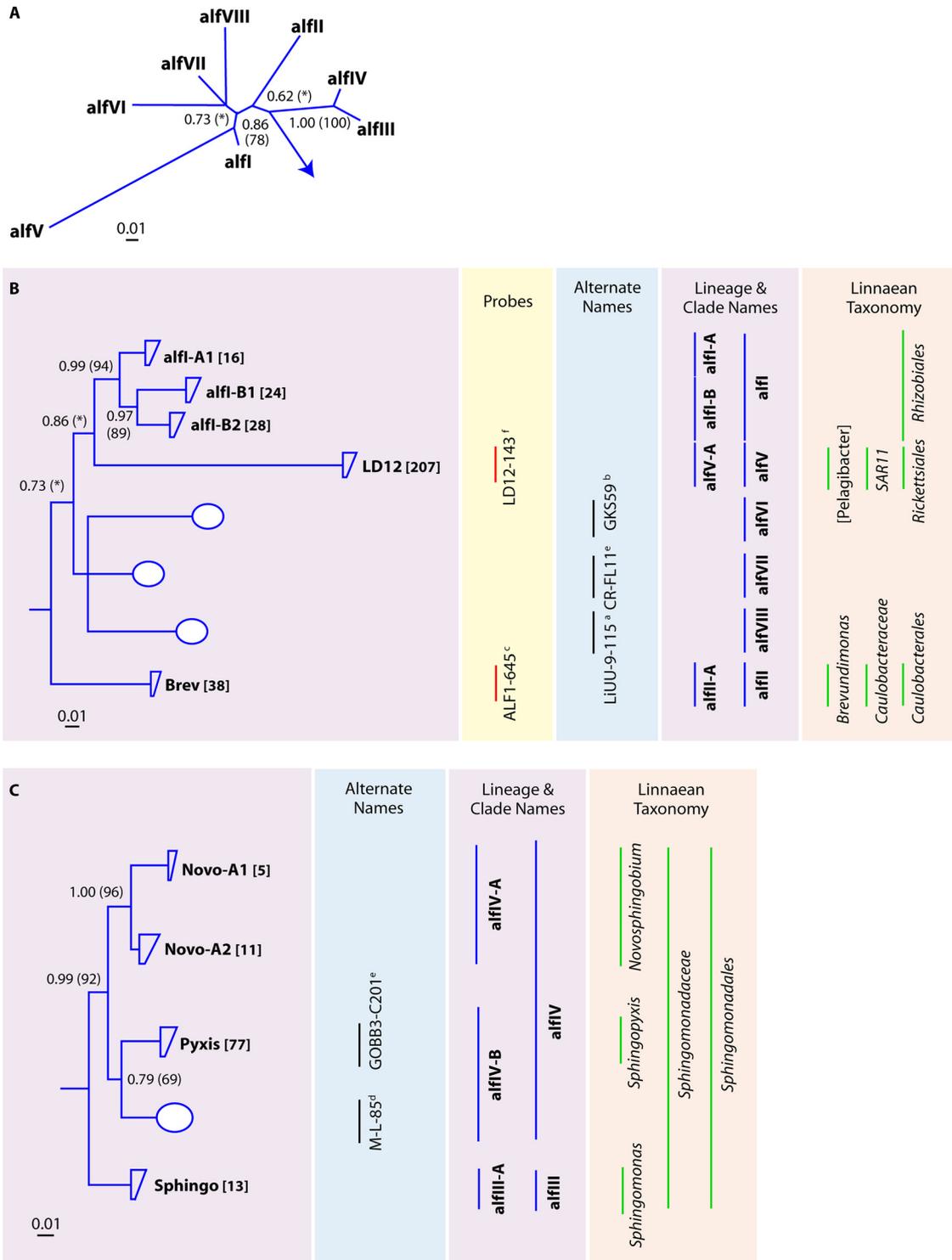


FIG. 5. (A) Radial consensus phylogram of the freshwater lake lineages of the *Alphaproteobacteria*. (B) Consensus phylogram of the tribes of lineages alfi, alfiII, and alfiV. (C) Consensus phylogram of the tribes of lineages alfiIII and alfiV. All phylogenetic inference calculations were conducted with nearly full-length sequences (>1,300 nt) representing the lineages/clades/tribes. Frequency base filters were created to mask out highly variable positions, and representative sequences from members of the *Archaea* were used as the outgroup for all tree reconstructions. Bayesian clade credibility values and maximum likelihood bootstrap values (in parentheses) of >0.5 (50) are listed at each node. Tribe names are listed at the end of tree tips (trapezoids), with the number of sequences associated with each tribe listed in square brackets. Tree tips ending in ovals represent phylogenetic clusters that were named previously but did not meet the criteria to be called a tribe (≥ 2 sequences with $\geq 97\%$ identity of $\geq 1,300$ nt isolated from >1 lake). Current lineage and clade names are listed to the right of the phylograms and are highlighted in purple. The Linnaean taxonomy column is based on the RDP classification (222), where all sequences within the classified group must have $\geq 75\%$ assigned confidence to the taxonomic group. Square brackets around a genus name indicate a candidate genus, and an asterisk indicates that the genus is described but not yet covered in the RDP classification. Alternate names from previous studies also covering the defined lineages/clades/tribes are listed. Probe coverage is based upon a perfect match to $\geq 75\%$ of the sequences in a defined lineage/clade/tribe. References to past clades and probes are as follows: ^a, reference 48; ^b, reference 155; ^c, reference 156; ^d, reference 233; ^e, reference 238; ^f, reference 242.

these changes are due to the phenotypic plasticity of persistent populations or shifts in the *Alphaproteobacteria* community composition. Even if top-down control via predation favors the *Alphaproteobacteria*, members of this class are generally not very abundant, suggesting that unknown mechanisms are keeping the abundance of the *Alphaproteobacteria* low in freshwater lakes. One potential mechanism may relate to the competitive ability of the *Alphaproteobacteria* for organic and inorganic substrates. Indeed, some experiments with lake bacterioplankton suggest that the low availability of organic nutrients, comparable to the nutrient availability characteristic of oligotrophic oceans, favors certain members of the *Alphaproteobacteria* (51, 160).

A second strategy for substrate acquisition among members of the *Alphaproteobacteria* may involve the capacity to degrade recalcitrant organic compounds such as humic substances. This strategy has been suggested for freshwater *Novosphingobium* (clade alfV-A) and *Sphingopyxis* (clade alfV-B) based on the isolation of bacteria in phenol-enriched cultures from humic lakes (104). The physical interaction with primary producers or other organisms that release organic and inorganic nutrients is another strategy used by some bacteria to satisfy their carbon and nutrient demands. This has not been investigated in any detail for the freshwater lake *Alphaproteobacteria* but may be expected in light of the frequent parasitic and symbiotic lifestyles within the class in general (13). Accordingly, some members of the *Alphaproteobacteria* have been identified in freshwater clone libraries from cyanobacterial phycospheres (52). Furthermore, freshwater *Alphaproteobacteria* isolates affiliated with *Brevundimonas* (clade alfII) have been shown to either promote or inhibit the growth of the coexisting blooming *Cyanobacteria*, implying strong functional interactions (15).

Little is known about the ecology and functional role of LD12 (clade alfV-A), which is the freshwater sister group to SAR11 (11). This tribe was first discovered in Toolik Lake (11) but often appears as the most abundant member of the *Alphaproteobacteria* in freshwater lake 16S rRNA libraries (238). It is widely distributed in lakes all over the world (38, 48, 123, 230, 242), and a recent phylogenetic study revealed surprisingly low global diversity within the tribe, indicating either slow diversification or global dispersal between freshwater ecosystems (129). This study also points to an ancient diversification of this tribe from their marine sister group and few historical freshwater-marine water transitions.

***Alphaproteobacteria* summary.** In general, the freshwater *Alphaproteobacteria* are poorly studied, but the available data suggest that their dominant freshwater lake members are (i) resistant to grazing, (ii) competitive under conditions of low nutrient/substrate availability but also capable of degrading complex organic compounds, and (iii) widely distributed in lakes all over the globe.

Class *Betaproteobacteria*

The class *Betaproteobacteria*, like other members of the phylum, is broadly recognized for its morphological and physiological diversity. In contrast to the related *Alphaproteobacteria*, the *Betaproteobacteria* are often the numerically dominant group in freshwater lakes (assessed with FISH [23, 63, 95, 243]) but are in relatively low abundance in the ocean (173). The high abundance and amenability to culturing have contributed to the *Betaproteobacteria* being the best-studied group in freshwater lakes.

***Betaproteobacteria* 16S rRNA gene-based phylogeny.** A number of freshwater lake *Betaproteobacteria*-associated monophyletic clusters have been identified, and many of these clusters are widely distributed among lakes. Following previous efforts to organize the freshwater lake *Betaproteobacteria* into coherent clusters (63, 242), we have divided the subclass into seven lineages, betI, betII, betIII, betIV, betV, betVI, and betVII, six of which are comprised of monophyletic clades containing a total of 16 tribes (Fig. 6B and C). The betVI lineage was reported previously (48); thus, it was included in the tree inference process but did not contain any tribes, so we excluded it from further analyses.

The betI and betII lineages contained the most sequences (Fig. 6B and C). The betI lineage, which is sometimes known as the BAL47 cluster for its affiliation with an initial isolate from the Baltic Sea (*Rhodospirillum* sp. strain BAL47), encompasses two clades (clades betI-A and betI-B), of which clade betI-A has been well studied and further partitioned into tribes (Fig. 6B). Tribe Lhab-A2 is known as the GKS16 cluster based on an original clone retrieved from Lake Gossenköllesee in Austria (63). Both Lhab-A1 and Lhab-A2 are circumscribed by the popular FISH probe R-BT065 (192). Representatives of the betI lineage were recently obtained in pure cultures and systematically described and are now known as members of the genus *Limnohabitans* within the family *Comamonadaceae* (76,

FIG. 6. (A) Radial consensus phylogram of the freshwater lake lineages of the *Betaproteobacteria*. (B) Consensus phylogram of the tribes of lineages betI and betV. (C) Consensus phylogram of the tribes of lineages betII, betIII, betIV, and betVII. All phylogenetic inference calculations were conducted with nearly full-length sequences (>1,300 nt) representing lineages/clades/tribes. Frequency base filters were created to mask out highly variable positions, and representative sequences from members of the *Archaea* were used as the outgroup for all tree reconstructions. Bayesian clade credibility values and maximum likelihood bootstrap values (in parentheses) of >0.5 (50) are listed at each node. Tribe names are listed at the end of tree tips (trapezoids), with the number of sequences associated with each tribe listed in square brackets. Tree tips ending in ovals represent phylogenetic clusters that were named previously but did not meet the criteria to be called a tribe (≥ 2 sequences with $\geq 97\%$ identity of $\geq 1,300$ nt isolated from >1 lake). Current lineage and clade names are listed to the right of the phylograms and are highlighted in purple. The Linnaean taxonomy column is based on the RDP classification (222), where all sequences within the classified group must have $\geq 75\%$ assigned confidence to the taxonomic group. Square brackets around a genus name indicate a candidate genus, and an asterisk indicates that the genus is described but not yet covered in the RDP classification. Alternate names from previous studies also covering the defined lineages/clades/tribes are listed. Probe coverage is based upon a perfect match to $\geq 75\%$ of the sequences in a defined lineage/clade/tribe. References to past clades and probes are as follows: ^a, reference 24; ^b, reference 48; ^c, reference 80; ^d, reference 85; ^e, reference 176; ^f, reference 192; ^g, reference 216; ^h, reference 229; ⁱ, reference 238; ^j, reference 242.

77). The first described species, *Limnohabitans curvus* strain MWH-C5^T, shares 96.6% and 95.7% 16S rRNA gene sequence identities with the previously described species *Curvibacter delicates* and *Rhodiferax fermentans*, respectively (76), and is a member of the Lhab-A3 tribe. Interestingly, its 16S rRNA gene sequence does not match with the popular R-BT065 FISH probe. *Limnohabitans australis* strain MWH-BRAZ-DAM2D^T was described as a very similar species with different substrate utilization characteristics (77). *Limnohabitans planktonicus* strain II-D5^T and *Limnohabitans parvus* strain II-B4^T, both of tribe Lhab-A1, are the first cultured and described members of the well-studied R-BT065 cluster and are targeted by the R-BT065 FISH probe (113). They share only 97.0 to 97.2% 16S rRNA gene sequence identity with *L. curvus*.

The betII lineage includes perhaps the best-studied freshwater lake clade within the *Betaproteobacteria*, Pnec. The name originates from its association with the genus *Polynucleobacter*, which was originally proposed for an obligate symbiont of ciliates (*Polynucleobacter necessarius*) (91). The genus was recently emended to also encompass four strains of a free-living subspecies (*P. necessarius* subsp. *asymbioticus*) (80). The Pnec clade has been subdivided into several phylogenetically refined tribes (tribes PnecA to PnecD). The PnecC tribe is arguably the best studied and includes *P. necessarius* (80). Several clade members were recently obtained in pure cultures from both temperate and subtropical freshwater habitats and systematically identified (80). The first described species of the PnecA tribe, *Polynucleobacter rarus* MT-CBb6A5^T, shares 96.0%, 95.6%, and 96.0% 16S rRNA gene sequence identities with *P. necessarius* subsp. *asymbioticus*, a sequence representing the endosymbiotic *P. necessarius* subsp. *necessarius* E24, and *Polynucleobacter cosmopolitanus*, respectively (81). This species appears to be rare compared to other members of the genus. The first cultured isolate of the PnecB tribe, *Polynucleobacter acidiphobus* MWH-PoolGreenA3^T, shares 97.8%, 97.5%, 97.3%, and 96.7% 16S rRNA gene sequence identities with *P. necessarius* subsp. *asymbioticus*, *P. necessarius* subsp. *necessarius* E24, the type strain of *P. cosmopolitanus*, and *P. rarus* MT-CBb6A5^T, respectively (79).

P. cosmopolitanus is a member of PnecD and shares many phenotypic, chemotaxonomic, and ecological traits with *P. necessarius* (78). Representatives have been cultured from freshwater habitats in Austria, France, Uganda, China, and New Zealand. The only available genome sequence from common freshwater lake bacteria described in this data set (other than *Cyanobacteria*) is derived from a PnecC member (M. W. Hahn, unpublished data).

The betIII lineage encompasses one clade subdivided into two tribes and was originally named the GKS98 cluster based on sequences retrieved from Lake Gossenkollesee (63). Isolates have been obtained from lineage betIII (225, 230). The betIV and betV lineages are each defined by only one clade and tribe. Lineage betIV contains the LD28 tribe, based on a clone recovered from Lake Loosdrecht in the Netherlands (238). No cultured representatives of the tribes in lineages betIV or betV have been reported to date. In contrast, each of the three tribes in lineage betVII are represented by isolates: the betVII-A1 tribe is represented by the genus *Massilia*, Janb is represented by the genus *Janthinobacterium*, and betVII-B1 is represented by the genus *Herbaspirillum*.

Ecology of the freshwater lake *Betaproteobacteria*. The *Betaproteobacteria* are abundant in many different freshwater lake habitats, sometimes constituting up to 60 to 70% of the total number of 4',6-diamidino-2-phenylindole (DAPI)-stained cells, as measured by using FISH probes (63, 176, 187). The relative abundances of various lineages and clades within the *Betaproteobacteria* vary among lakes, within lakes (with depth and horizontally), and with time. Members of the *Betaproteobacteria* are often cocultured with algae such as *Cryptomonas* sp. (156), are associated with the *Cyanobacteria* (52), and are often particle associated (120, 191, 227).

We know the most about the ecology of the betI-A clade and the Pnec clade, based on their distribution patterns in time and space, interaction with other members of the aquatic food web, mesocosm experiments, and available pure cultures. Both clades are considered to be cosmopolitan freshwater lake clades because they are found in nearly all lakes that have been studied. The betI-A clade is most frequently studied by using a 16S rRNA-targeted FISH probe that specifically hybridizes to the Lhab-A1 and Lhab-A2 tribes (together also known as R-BT065; Fig. 6B). This probe-defined cluster was found to be present in 98 of 102 diverse freshwater habitats in Europe that were surveyed for their presence, in abundances of up to 29% of the total number of DAPI-stained bacteria (190). This clade has also been detected by its 16S rRNA gene sequences in nearly every lake studied by using this technique, including Lake Gossenkollesee in Austria (63), Lake Grosse Fuchskuhle in Germany (63), Lake Baikal in Russia (63), deep Patagonian lakes (35), Tibetan lakes (127), eutrophic Swedish lakes (48), shallow eutrophic lakes in Belgium (214), shallow eutrophic Lake Taihu in China (233), oligotrophic Crater Lake in Oregon (212), oligotrophic Toolik Lake in Alaska (38), and a variety of lakes in Wisconsin (this study). The cluster seems to be more abundant under circumneutral to alkaline pH conditions than in acidic or humic habitats and most abundant in small shallow ponds (190). The R-BT065 cluster has also been found distributed throughout stratified water columns (23, 176) but is most abundant at shallower depths (176). A few studies have focused on the substrate assimilation preferences of R-BT065 bacteria using microautoradiography-FISH. In the experimentally partitioned Lake Grosse Fuchskuhle, that group assimilated glucose and leucine but very little acetate (23). R-BT065 also readily used leucine in a reservoir under both P-limiting and P-replete conditions (96). Substantial evidence for both top-down and bottom-up controls on their abundances is available based on both field observations and experiments. The R-BT065 bacteria are known for their relatively short population turnover times and ability to respond rapidly to changing environmental conditions (6, 187, 188, 192). They grow quickly when exposed to nutrient pulses in mesocosms (187, 188), and their abundance was positively associated with watercolor metrics that are proxies for low-molecular-weight alga-derived substrates (190). The latter suggests that they are particularly competitive in photosynthetically active planktonic systems regardless of trophic status (189). Members of the cluster were also highly susceptible to grazing (97, 187, 192) and had higher growth rates in the presence of flagellates (226). Some researchers have proposed that these bacteria are particularly competitive in freshwaters because of their opportunistic life-style, in which they balance

their vulnerability to grazing with fast growth rates (96, 176). We note that very little is known about ecophysiological differences between tribes Lhab-A1 and Lhab-A2 because most studies of the betI-A clade have been conducted by using the R-BT065 FISH probe, which targets both tribes.

The four described *Limnohabitans* species within the betI lineage can be discriminated based on their substrate utilization characteristics as well as their 16S rRNA gene sequences and extent of genomic DNA-DNA hybridization (76, 77, 113). The first described species, *L. curvus*, is a chemoorganotrophic, aerobic, and facultative anaerobe capable of assimilating glucose and a variety of small organic acids such as acetate, pyruvate, and fumarate but not amino acids (76). Notably, *L. parvus* cannot assimilate acetate, while the other three species are capable of doing so (113).

The Pnec clade has also been the subject of a large number of studies directed toward an understanding of their ecophysiology, evolutionary ecology, and role in aquatic food webs. The four tribes, named PnecA to PnecD (*P. rarus*, *P. acidiphobus*, *P. necessarius*, and *P. cosmopolitanus*, respectively) are well delineated phylogenetically (229) and seem to be similarly distinct in their distributions within and among lakes and over time. In two large lakes, Lake Taihu in China and Lake Mondsee in Austria, the FISH-identified PnecB cluster was the most abundant of the four tribes, although the tribe exhibited peak abundances in opposite seasons in the two lakes (232). PnecB exhibited remarkably consistent seasonal dynamics, which mirrored the change in water temperature over 3 years (232). A study of the effect of depth on PnecB abundance showed that its abundance consistently decreased as the depth below the surface increased (232). PnecB and PnecC seem to ecologically partition themselves along a pH gradient, with PnecB being most successful under neutral to alkaline conditions and PnecC being more abundant in more acidic habitats. PnecB was identified with FISH in 51 of 65 lakes that were circumneutral or alkaline but in 0 of 16 lakes that were acidic (230). Likewise, the abundance of PnecB increased with increasing pH, as did the prevalence of PnecA (123). On the other hand, the abundance of PnecC decreased with increasing pH (230). Although PnecC exhibits a preference for low-pH lakes, it is ubiquitous in lake systems (80, 107). A large survey of 137 freshwater lakes and ponds over a 2,000-km² area in the Czech Republic, Austria, and Sweden showed that PnecC could be recovered from lakes with pHs as low as 3.8 and as high as 8 (107).

There is strong evidence that PnecC is particularly competitive in environments rich in humic acids, since they are frequently the dominant betaproteobacterial tribe in dystrophic lakes (24, 69, 85, 107, 141, 205) and are at times known to comprise >50% of the DAPI-stained cells in these lakes (85, 107). PnecC is readily enriched upon exposure to elevated levels of allochthonous DOC (103, 104, 107, 225) and can assimilate small organic acids such as acetate, which are expected to be the breakdown products of humic acids (23). Their substrate incorporation patterns did vary over time and across basins of the partitioned Lake Grosse Fuchskuhle (23). PnecB and PnecC habitats also seem to partition with depth in stratified lakes, with both being present in the epilimnion but only PnecC existing in the hypolimnion of the prealpine oligomesotrophic Piburger See in Austria (176).

Hahn and colleagues have isolated and characterized a large

number of isolates associated with the Pnec clade (genus *Polynucleobacter*), and this has provided valuable insight into substrate utilization preferences, temperature and pH ranges, and the ability to grow in the absence of oxygen (78, 80). The isolation of *Polynucleobacter* often requires a careful filtration-acclimatization method (87) or a dilution-acclimatization method (74), and these cells generally grow poorly on a single carbon source (80). However, a recent study indicated that PnecC could be isolated by using agar plates (225). It remains to be seen if this method is capable of retrieving the diversity of the Pnec clade or if it selects for certain members. Pnec isolates are described as being chemoorganotrophic and aerobic, with some strains being capable of facultative anaerobic growth. The grazing of flagellate bacteriovores upon the Pnec tribes appears to be governed by the size of the Pnec cell (18, 209), as low grazing rates were observed for ultramicrobacterial (cell volume, <0.1 μm³) PnecD organisms, but they increased with increasing cell size (18, 209). Isolates have been described for all Pnec tribes: *P. rarus* for PnecA (81), *P. acidiphobus* for PnecB (79), *P. necessarius* for PnecC (74, 80), and *P. cosmopolitanus* for PnecD (78).

Less is known about the ecology of the betIII, betIV, betV, betVI, and betVII lineages. The betIII lineage (also known previously as the GKS98 cluster) and the LD28 tribe (lineage betIV) were each detected in 12 of 15 lakes in Sweden using reverse line blot hybridization (123). In another survey, the LD28 tribe was found in 59 of 81 lakes across Europe (242) and was present in high mountain Tibetan lakes (230), while the GKS98 cluster was found in both freshwater and hypersaline lakes (230). The betIV lineage in the Piburger See, Austria, was studied by using 16S rRNA gene sequencing and targeted FISH probes (176). This lineage was significantly more abundant in the anoxic hypolimnion than were other members of the *Betaproteobacteria* (i.e., betI and betII).

Betaproteobacteria summary. The *Betaproteobacteria* are by far the most studied and often the most abundant bacteria inhabiting the upper waters of lakes. Two lineages, betI and betII, which are both abundant in lakes and exhibit a cosmopolitan distribution, have received the bulk of the research attention for the freshwater *Betaproteobacteria*. Depth, pH, carbon substrate preferences, and seasonal factors are all known to differentiate closely related organisms within these lineages. Generally speaking, the freshwater lake *Betaproteobacteria* are fast growing and nutrient loving and have a size-dependent vulnerability to grazing pressure. The recent isolation of numerous members of the genera *Limnohabitans* and *Polynucleobacter* has paved the way for a greater understanding of the roles that freshwater bacteria play in these ecosystems.

Class Gammaproteobacteria

The class *Gammaproteobacteria* contains the most studied of all bacterial organisms, the *Enterobacteriales* (commonly known as the enterics). Many enteric organisms, such as *Escherichia coli*, can be found in freshwater lakes; however, they are widely considered transient members dispensed from anthropogenic or zoonotic sources and are generally not seen in 16S rRNA gene libraries made from these environments (238). The *Gammaproteobacteria* are generally more abundant in saltwa-

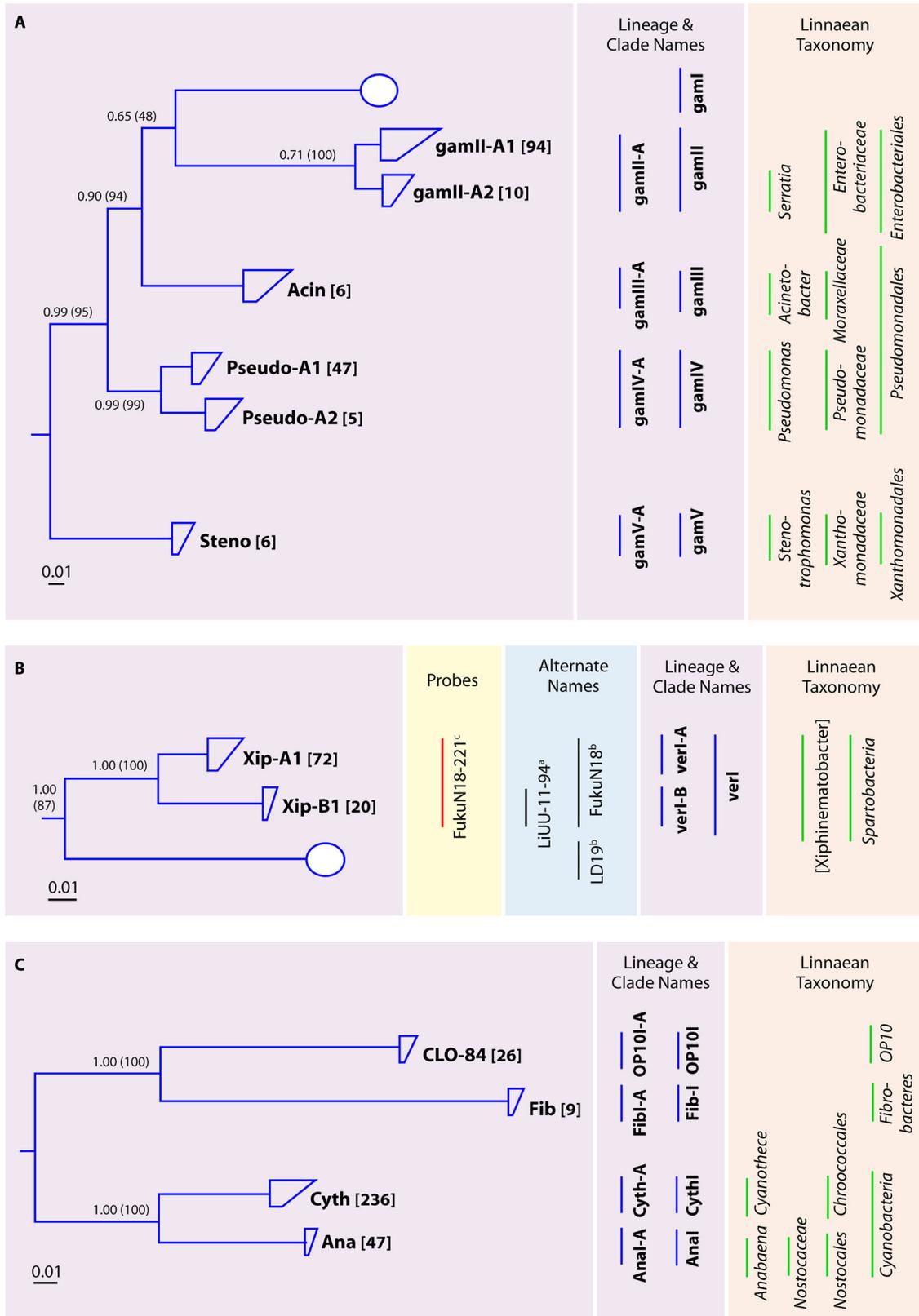


FIG. 7. (A) Consensus phylogram of the freshwater lake lineages of the *Gammaproteobacteria*. (B) Consensus phylogram of the freshwater lake lineages of the *Verrucomicrobia*. (C) Consensus phylogram of the freshwater lake lineages of OP10, the *Fibrobacteres*, and the *Cyanobacteria*. All phylogenetic inference calculations were conducted with nearly full-length sequences (>1,300 nt) representing the lineages/clades/tribes. Frequency base filters were created to mask out highly variable positions, and representative sequences from members of the *Archaea* were used as the outgroup for all tree reconstructions. Bayesian clade credibility values and maximum likelihood bootstrap values (in parentheses) of >0.5 (50) are

ter environments such as the ocean (17, 173) or saline lakes (230) than in freshwater.

Gammaproteobacteria 16S rRNA gene-based phylogeny. The majority of freshwater lake sequence-based studies do not contain data for members of the *Gammaproteobacteria*. Since relatively few of such gene sequences have been recovered from freshwater lakes, only two named clusters had been identified, the gamma I (*Methylobacter psychrophilus*) cluster (238) and the LiUU-3-334 cluster (48) (Fig. 7A). In our phylogenetic analyses we identified six tribes (Fig. 7A). Unlike the majority of freshwater lake clusters in our data set, many of these *Gammaproteobacteria* tribes fit into the described taxonomic framework for this group. For example, the majority of sequences fit into the families *Enterobacteriaceae* and *Xanthomonadaceae* or the orders *Pseudomonadales* and *Legionellales* (Fig. 7A). Many of the bacteria in these *Gammaproteobacteria* groupings were closely related to bacteria commonly cultured in the laboratory or those found associated with soil or animals.

Ecology of the freshwater lake Gammaproteobacteria. The *Gammaproteobacteria*, like the *Alphaproteobacteria*, are abundant in the ocean (17) but are not particularly abundant in freshwater lakes (238). Although the total numbers of sequences recovered in our data set are relatively equal for the *Alphaproteobacteria* and *Gammaproteobacteria*, the diversity among the *Gammaproteobacteria* sequences (i.e., the number of sequences that are not closely [$>97\%$] related to another sequence) is greater (data not shown). This patchy recovery of a wide range of members of the *Gammaproteobacteria* suggests that they may be transient members of lake communities brought in from the surrounding environment (e.g., enterics) (124), or they could be common lake members existing at low abundances, making regular retrieval via clone libraries unlikely. Many *Gammaproteobacteria* strains are regularly used as microbial genetic tools in the laboratories of those researchers carrying out the isolation of DNA from freshwaters, implying that the recovery of their 16S rRNA gene sequences in clone libraries derived from lakes could be a product of methodological contamination.

Zavarzin et al. suggested that the *Gammaproteobacteria* are copiotrophs, which could explain why they are disproportionately isolated in culture-based studies (236). Upholding that hypothesis, members of the *Gammaproteobacteria* grew significantly faster than the average lake bacterioplankton and exhibited even faster growth rates when nitrogen and phosphorus were added to enclosures (59, 188). To our knowledge, few studies have further examined the freshwater lake *Gammaproteobacteria* tribes described here or other members of the *Gammaproteobacteria* as resident members of the upper waters in lakes (for notable exceptions, see references 164 and 221). There is, however, vast literature on the use of members of the

Gammaproteobacteria, specifically members of the *Enterobacteriaceae*, in the source tracking of pollutants in surface waters (reviewed in reference 201).

Phylum Verrucomicrobia

Members of the *Verrucomicrobia* were identified and cultured as early as the 1970s (genus *Prostheco bacter*) (196), but the phylum, which took its name from its first aerobic freshwater isolate (*Verrucomicrobia spinosum*), was not created until 1997 (92). *Verrucomicrobia* species have been identified in lakes, soil, oceans, and human feces and even as ecto- and endosymbionts of eukaryotes (220). Recently, methane-oxidizing representatives of the *Verrucomicrobia* have been isolated from extremely low-pH, thermophilic environments (181). The *Verrucomicrobia* have an elevated degree of phylogenetic relationship to members of the *Planctomycetes* and *Chlamydiae*. Some representatives of these three groups also have compartmentalized intracellular structures (220).

Verrucomicrobia 16S rRNA gene-based phylogeny. In our phylogenetic reconstructions, one lineage, two clades, and two tribes were defined (Fig. 7B). Despite being ubiquitous, little is known about freshwater lake *Verrucomicrobia* taxa. The three previously described clusters of the freshwater *Verrucomicrobia* are FukuN18, subdivision 3, and CL120-10 (*Opitutaceae*). These three clusters each harbor many sequences, suggesting that a finer resolution of clusters could reveal more phylogenetic structures within each lineage. In our database, only lineage verI (FukuN18) could be further divided (Fig. 7B) into clades (clades verI-A and verI-B) and tribes (tribes Xip-A1 and Xip-B1).

Ecology of the freshwater lake Verrucomicrobia. Members of the *Verrucomicrobia* do not seem particularly abundant in lakes, ranging between $<1\%$ and 6% in abundance based on clone recovery. However, this may reflect poor primer coverage or PCR bias, since a recent study based on newly developed FISH probes revealed abundances of up to 20% of the total bacterial community in a humic lake (9). Still, this phylum does have a cosmopolitan distribution in lakes. In a study by Zwart and colleagues (242), the freshwater cluster CL0-14 was found in 73 of 81 studied lakes in Europe. The prevalence of this cluster was negatively correlated with pH and positively correlated with hydraulic retention time and temperature (123). Members of the *Verrucomicrobia* have also been observed in both surface and hypolimnetic waters, suggesting a variety of metabolic strategies within the group (45, 125). Some members of the phylum seem to be associated with high-nutrient environments or algal blooms (48, 90, 116).

listed at each node. Tribe names are listed at the end of tree tips (trapezoids), with the number of sequences associated with each tribe listed in square brackets. Tree tips ending in ovals represent phylogenetic clusters that were named previously but did not meet the criteria to be called a tribe (≥ 2 sequences with $\geq 97\%$ identity of $\geq 1,300$ nt isolated from >1 lake). Current lineage and clade names are listed to the right of the phylograms and are highlighted in purple. The Linnaean taxonomy column is based on the RDP classification (222), where all sequences within the classified group must have $\geq 75\%$ assigned confidence to the taxonomic group. Square brackets around a genus name indicate a candidate genus, and an asterisk indicates that the genus is described but not yet covered in the RDP classification. Alternate names from previous studies also covering the defined lineages/clades/tribes are listed. Probe coverage is based upon a perfect match to $\geq 75\%$ of the sequences in a defined lineage/clade/tribe. References to past clades and probes are as follows: ^a, reference 48; ^b, reference 238; ^c, reference 242.

Minor Freshwater Lake Phyla

Sixteen phyla, the *Acidobacteria*, BRC1, *Chlorobi*, *Chloroflexi*, *Fibrobacteres*, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Lentisphaerae*, *Nitrospira*, OD1, OP10, *Planctomycetes*, *Spirochaetes*, SR1, and TM7, were recovered infrequently by the studies included in our database (Fig. 2). Of these phyla, members of the *Planctomycetes* and the *Firmicutes* were recovered most often. Few studies examining the presence, distribution, or activity of any of the 16 minor phyla have been conducted for the epilimnion of freshwater lakes, although a few studies have identified putative monophyletic freshwater clusters (48, 212, 233). Notably, members of the *Acidobacteria* and *Firmicutes* are commonly found in freshwater sediments, and members of the *Chloroflexi* (green nonsulfur bacteria) and *Chlorobi* (green sulfur bacteria) are commonly present in the metalimnia or hypolimnia of deeper lakes.

16S rRNA gene-based phylogenies of minor phyla. Two of the minor phyla in our data set, OP10 and the *Fibrobacteres*, were represented by tribes (Fig. 7C). The OP10 tribe CL0-84 was described previously (238), while the *Fibrobacteres* tribe was not. Certainly, all 16 minor phyla would show greater phylogenetic structures with further sequencing efforts.

Ecology of minor freshwater lake phyla. In this section we attempt to summarize the little that is known about the more prevalent and/or more studied freshwater lake representatives from the minor phyla listed above. In particular, we will focus on the *Fibrobacteres*, *Chloroflexi*, and *Planctomycetes* and the candidate division OP10. Studies of bacterial 16S rRNA gene clone libraries generated from geographically and geochemically distinct freshwater lake samples have reported sequences related to each of these phyla (48). Even though isolates have been obtained for three of these phyla and several are known to exhibit unusual functional traits and/or suspicious intracellular structures, they have not received much attention from freshwater studies.

Using a *Fibrobacteres*-specific quantitative PCR assay, McDonald and colleagues (135) showed a depth-dependent distribution of the freshwater *Fibrobacteres* never exceeding 1% of the total bacterioplankton. Interestingly, those authors were able to enrich members of the *Fibrobacteres* in a biofilm that developed on cotton strings incubated in a eutrophic lake. Since all cultured strains assigned to the *Fibrobacteres* are capable of cellulose hydrolysis, those authors suggested that they could play a role in cellulose hydrolysis in freshwater environments. However, the freshwater lake *Fibrobacteres* sequences fall into the narrow clade FibI-A, which is not closely related to any of the described isolates (data not shown). Members of the *Fibrobacteres* are more common in sediments, which is consistent with the obligate anaerobic physiology of all known cultured members of the phylum.

The majority of *Chloroflexi* 16S rRNA gene sequences belong to the cluster called SAR202, which are commonly recovered from soils, sediments, and deep-subsurface terrestrial environments. *Chloroflexi* SAR202-related bacterioplankton also seems to be common in the meso- and bathypelagic areas of the open ocean (138) and has been identified in the hypolimnia and epilimnia of freshwater systems (212). However, of the 39 *Chloroflexi* sequences recovered in our database, only the two sequences recovered from Crater Lake by Urbach and col-

leagues (212) fell into the SAR202 clade. Since there are currently no cultured representatives of the *Chloroflexi* that are closely related to the obtained environmental sequences and since very little is known about their distribution in aquatic environments, our understanding of their ecology and physiology is purely speculative.

The *Planctomycetes* are understudied despite the fact that they are common in freshwater lake environments and are able to provide important ecosystem functions. Part of the reason for the lack of studies examining this phylum stems from the fact that the *Planctomycetes* are underrepresented in 16S rRNA gene clone libraries, as many members have mismatches to commonly used universal primers (219). In fact, members of the *Planctomycetes* may be very abundant in freshwater lakes. In one study of an acidic bog lake using FISH probes to quantify the major phyla, the *Planctomycetes* were the most abundant phylum (40). In the field of biotechnology, research on the *Planctomycetes* has recently gained increased notoriety because of the group's general ability to carry out the anaerobic ammonium oxidation (anammox) reaction (202). Furthermore, genomic and proteomic analyses of *Planctomycetes* isolates suggest that they can degrade phytoplankton-derived carbohydrates (165). For more details on the ecology of members of the *Planctomycetes*, we refer the readers to two excellent reviews (58, 220).

"*Candidatus*" phylum OP10 lacks cultured representatives, and its existence is generally verified only by 16S rRNA gene sequences. Sequences representing the OP10 phylum have been reported for a wide diversity of habitats (162) but very rarely make up $\geq 5\%$ of the sequences from 16S rRNA gene libraries. Since representative isolates are lacking and their dynamics have not been a focus of any freshwater lake studies, the abundance and role of OP10 in freshwater lakes are uncertain. A first hint at their ecological role was provided by observations that some taxa follow or are associated with phytoplankton blooms (58). OP10 members have also been implicated in metal cycling, as a narrow but abundant phylogenetic cluster was retrieved from iron- and manganese-rich particles in the hypolimnion of a reservoir during late-summer stratification (199).

Summary of minor phyla. Studies involving the less abundant phyla of freshwater lakes represent a particular avenue of research that is understudied and should be explored. Very little is known about the diversity of these phyla or the roles that members of these phyla play in lakes. Some phyla, such as the *Planctomycetes*, may even be very abundant in particular freshwater lake systems (40). Members of this so-called "rare biosphere" could prove to be important contributors to crucial biochemical reactions and ecosystem functions or may harbor unique properties amenable to biotechnology applications (75). The advent of cheaper sequencing technologies may for the first time provide a way to access the potentially incredible bacterial diversity sustained by freshwater lakes.

META-ANALYSIS: LIFE HISTORY PATTERNS OF FRESHWATER LAKE BACTERIA

Natives, Vagabonds, or Tourists?

Freshwater lakes are intimately linked to the terrestrial and marine biomes via the hydrological cycle and other modes of

dispersal. Hence, it is relevant to address the question of whether the putative freshwater lake tribes that we have defined with phylogenetic tools represent true indigenous populations characteristic of the freshwater biome (natives), if they have a wider distribution and/or niche in the biosphere (vagabonds), or if they are just there by chance (tourists). To do this, we used blastn to map the distribution of representative sequences from each tribe to GenBank sequences from terrestrial, freshwater, and marine habitats (Fig. 8). This synoptic analysis clearly shows that the majority of putative freshwater lake tribes identified within the *Actinobacteria*, *Bacteroidetes*, *Alphaproteobacteria*, and *Betaproteobacteria* appear to be restricted (natives) to lake epilimnia. However, there are some notable exceptions within these phyla and classes that will be discussed below. In particular, the *Gammaproteobacteria* tribes found in freshwater lake ecosystems appear to be abundant in terrestrial ecosystems with an emphasis on organism-associated habitats (Fig. 8), pointing to their potential role as “tourists” in freshwater lakes.

Although the identified *Actinobacteria* tribes are found almost exclusively in freshwaters, two tribes feature a partly terrestrial signal, the Myco (*Mycobacterium*) and acV-A2 tribes. The former group contains many parasitic and pathogenic representatives, which is possibly reflected in the higher incidence of organism-associated sources for this tribe. Tribe acV-A2 is also a member of the “soil II+III cluster,” and hence, it is not surprising that it is well represented in terrestrial habitats. It was surprising, however, that tribe acV-A1, the sister group to tribe acV-A2, which also groups with the “soil II+III cluster,” has not been found in terrestrial habitats. Three actinobacterial tribes (tribes Luna3, Luna1-A1, and acIII-A1) have a strong saltwater/marine signal (approximately 50% of BLAST hits). Among these, Luna1-A1 was postulated to be an indigenous freshwater lake bacterial group with a planktonic life-style (73), but our analysis suggests that members of this group are found in estuaries, which may suggest that this tribe can tolerate more saline environments (Fig. 8). Tribes Luna-A1 and acIII-A1 have similar freshwater-marine signals, but the closely related tribes Luna-A2 and Luna2 appear to be more exclusive freshwater groups, pointing to substantial diversification with regard to habitat preferences among related phylotypes.

None of the *Bacteroidetes* tribes described for freshwater lakes match a large proportion of sequences originating from marine habitats (<10% occurrence [previously noted in reference 5]). Instead, 5 of the 14 tribes have a terrestrial signal, pointing to a broader distribution across the terrestrial-aquatic continuum (Fig. 8). In particular, the closely related tribes Muci (*Mucilaginitobacter*) and Pedo (*Pedobacter*) frequently match sequences from terrestrial systems. Both of these taxa are often detected and isolated in soils and wetlands and were also quite rare in the freshwater lake 16S rRNA gene data set.

Unlike the other major phyla present in freshwater lakes, the *Proteobacteria* have tribes that are seemingly less restricted to the freshwater biome. A strong freshwater-terrestrial distribution pattern is evident for several *Alphaproteobacteria* tribes: the Brev (*Brevundimonas*), Sphingo (*Sphingomonas*), and alfi-BI (within the *Rhizobiales*) tribes (Fig. 5B and C). Notably, the freshwater sister group of the marine SAR11 (LD12) clade appears to be an exclusively freshwater tribe. Few tribes within

the *Betaproteobacteria* appear to be restricted to a single biome. Many of the groups commonly identified as “typical freshwater bacteria” (*Polynucleobacter* tribes, LD28, members of the genes *Limnohabitans*, and tribe betIII-A1 within the GKS98 cluster) frequently match sequences retrieved from marine ecosystems, particularly estuaries, pointing to a wider distribution than previously assumed. Some tribes also appear to be distributed across the aquatic-terrestrial interface; e.g., tribe betIII-A2 and all three tribes within the betVII lineage, known as the *Oxalobacteraceae*, almost exclusively match sequences from the terrestrial environment (Fig. 8). Hence, none of the putative freshwater lake tribes within the *Burkholderiales* appear to be indigenous freshwater bacteria but rather seem to have broad and divergent habitat preferences (vagabonds). This habitat divergence is also apparent for the *Janthinobacterium* (tribe JanB), where >50% of the GenBank matches are from organism-associated habitats coupled mainly to the terrestrial biome, although this genus has been implicated as a common contaminant in clone libraries (208). This result points to a physical association between this tribe and other organisms, where notably >60% of the closest matches in GenBank are from the human microbiome and another 38% are linked to miscellaneous terrestrial animals.

An even more pronounced association with other organisms is evident for the *Gammaproteobacteria*, where all but one (tribe Pseudo-A2) of the putative freshwater lake tribes have matching sequences associated mainly with other organisms from terrestrial habitats. Most of these tribes (tribe gamII-A2/*Serratia*, *Stenotrophomonas*, and *Acinetobacter*) contain mainly sequences from the human biome, whereas the more abundant freshwater lake tribe gamII-A1 is associated with sequences from other terrestrial animals (50% of the nearest GenBank matches). Hence, these tribes also seem to represent “tourists” rather than indigenous freshwater bacteria. This finding contrasts with the two *Verrucomicrobia* tribes, the OP10 tribe (CLO-84), and the *Fibrobacteres* (all minor components of freshwater lake bacterial communities), which match only freshwater sequences and hence likely represent true freshwater groups.

The emergent picture is that most putative freshwater lake tribes identified in our phylogenetic study are found exclusively in this biome. However, our results also point to considerable variation across phyla and families, where some groups are dominated by tribes with alternative native habitats. This alternative habitat is most often terrestrial, which is consistent with the dominant flow of water (and bacteria) from soils via freshwater and then further into the oceans. The stronger connection between the freshwater and terrestrial environments also makes sense in light of the salinity difference between lakes and marine habitats, which acts as an apparent habitat and evolutionary barrier for microorganisms (128). The absence of such a salinity barrier between the terrestrial and freshwater biomes may allow a more extensive exchange of microflora between these environments.

Biogeography of the Freshwater Lake Tribes

The extent to which “the environment selects” is a critical question in microbial ecology. We know that many biological, physical, and chemical features of lakes can influence the distribution of ecologically coherent bacterial populations. Previ-

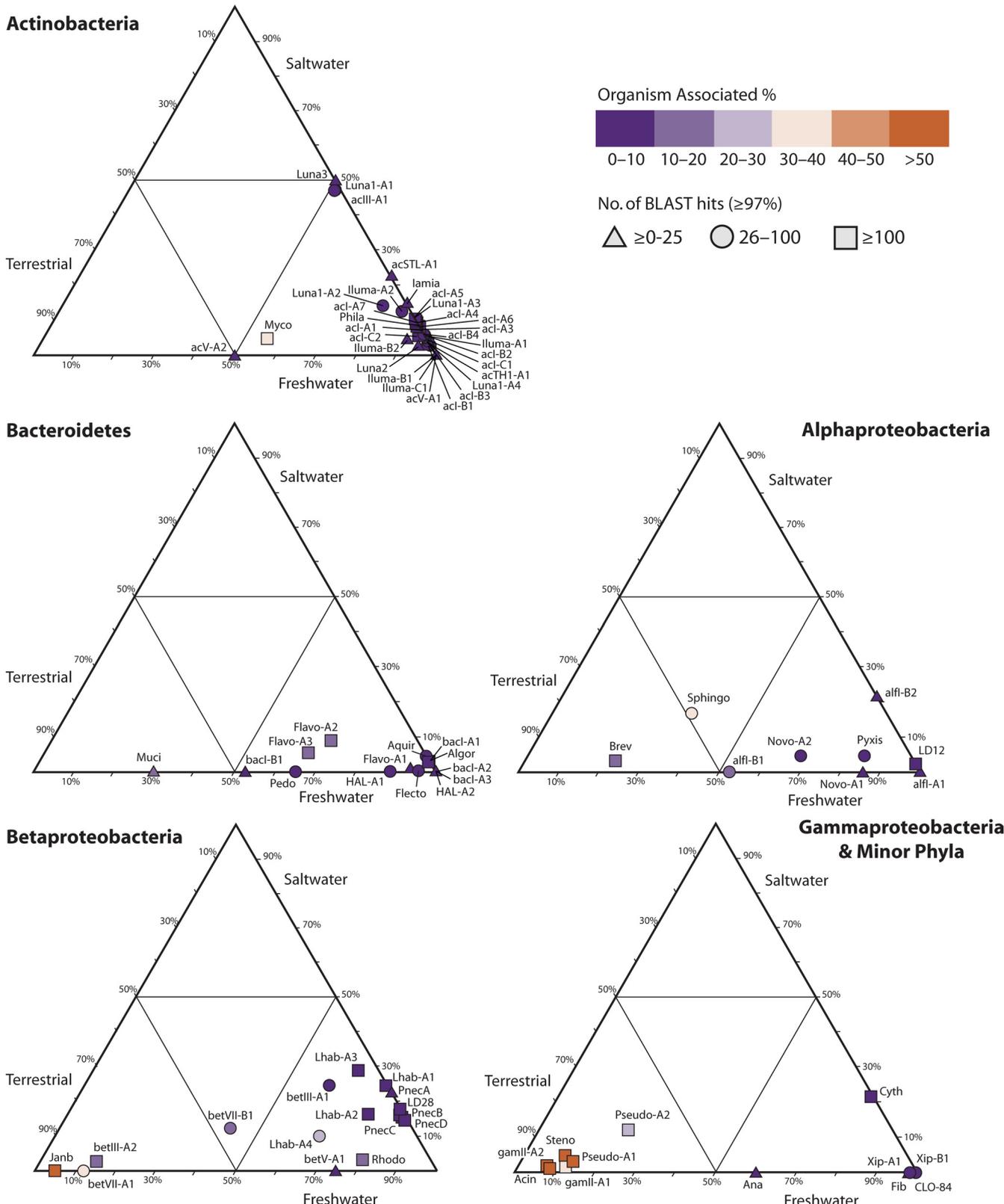


FIG. 8. Habitat specificity of putative freshwater lake bacterial tribes. Two nearly full-length (>1,300 nt) 16S rRNA gene sequences from each tribe were chosen as representatives for a subsequent blastn analysis against the NCBI nonredundant (nr) database. Each tribe's sequence representatives were chosen based on obtaining a maximum or near-maximum within-tribe phylogenetic distance between the two representatives. The top 5,000 blastn hits were returned for each sequence representative, and the database sequence records with $\geq 97\%$ identity were retained. The "isolation source" field was parsed from each sequence record, allowing categorization by the biome from which the sequence was obtained

ous bacterial 16S rRNA gene sequence database surveys making freshwater-marine comparisons (12) and comparisons across a diverse set of microbial systems (28) have observed strong compositional contrasts across ecosystems and have related these contrasts to environmental conditions. We undertake an approach similar to those of the studies described above to identify factors that correlate with tribe compositional differences among freshwater ecosystems. Biotic interactions that are likely to structure freshwater bacterial communities include predation (114), viral lysis (210), and competition for resources (93a). Specifically, the carbon source appears to be an important biological regulator of community composition (51). Physical features like landscape position (234) and lake retention time (123) have also been shown to strongly influence community structure. Finally, pH stands out as the strongest chemical determinant of freshwater lake bacterial community composition (123).

To date, the majority of freshwater microbial community ecology studies have focused on single groups (e.g., see reference 84), a limited number of systems (238), or a confined geographic range (234). In the phylogenetic framework presented in this review, we identified many narrow monophyletic freshwater groups, such as the described tribes (97% to 99.5% sequence similarity), which allowed us to explore their biogeographic patterns. To synthesize the patterns of the most common freshwater lake tribes, we gathered as much environmental information as possible about the lakes used to generate our freshwater lake sequence database. We used our environmental database along with tribe occurrence patterns and multivariate statistics to conduct a broad, cross-system comparison and reveal a more general picture of patterns of distribution along environmental gradients. By correlating the occurrence patterns of taxa to gradients under environmental conditions, we can approximate the niches of our tribes. The gradient analysis approach has been used with plant and animal systems for decades (e.g., see reference 60), but only recently have they been applied to microorganisms (123). We used three environmental state variables, pH and total phosphorus (TP) and dissolved organic carbon (DOC) concentrations, as well as the latitudinal position of the lakes. DOC and TP were most significant in explaining tribe distribution when considered as a ratio (DOC/TP ratio) that summarizes the relative importance of external (terrestrial) and internal (algal/cyanobacterial) carbon substrate supplies. In addition, we considered absolute latitude (AbsLat) as an indicator of climate. We initially compared the relative strengths of geographic and local environmental conditions using a partial Mantel test approach (19). Although both geographic ($r = 0.24$; $P = 0.001$ [partial Mantel test]) and environmental ($r = 0.39$; $P = 0.001$ [partial Mantel test]) variables were significantly correlated with bacterial

community composition, the environmental effect was relatively stronger, especially when considering the limited number of environmental parameters examined in this analysis. In subsequent analyses we focused on local environmental characteristics in an attempt to characterize the environmental preferences of various bacterial tribes. However, we do remind the reader that geographic patterns could still play a weak but significant role in the structuring of global freshwater lake bacterial compositional patterns.

The tribe occurrence matrix that we used to characterize freshwater lake bacterial biogeographic patterns was populated by 79 freshwater lake bacterial tribes represented by 4,012 sequences collected from 47 lakes. Sites in the data set included lakes from all around the globe, such as Lake Taihu (China), Lake Mendota (Wisconsin), and Lake Grosse Fuchskuhle (Germany). Only one lake (Heywood Lake, Antarctica) is located in the southern hemisphere, and only seven lakes (six reservoirs in Burkina Faso and a Chinese lake) are located in the tropics. The majority of lakes were located in North America and Europe. Data sets were selected based on the amount of available sequence data and associated environmental-contextual data, which, with few exceptions, included pH, TP, DOC, global positioning system (GPS) coordinates, and trophic status. A study was included only when at least 40 clones were randomly sequenced, with the exception of a few cases where we used studies that provided clone counts based on $\geq 97\%$ sequence similarity clusters. The choice of environmental variables was rather arbitrary, as they represent the most commonly measured environmental state variables. However, these variables are often thought to be the most significant drivers of bacterioplankton community composition. We used TP, which is a good indicator of nutrient availability and system productivity; lake pH, which has been repeatedly related to bacterial community composition and diversity patterns (140); and DOC, which provides information on the trophic status of the system, as high-DOC systems usually represent net heterotrophic humic lakes.

We used both correspondence analysis (CA) (94) and detrended correspondence analysis (DCA) (94) to visualize patterns of tribe distribution and community composition. CA and DCA are multivariate statistical techniques widely used by community ecologists to identify the main factors and/or gradients in species-rich but sparsely populated data matrices. They are both similar to principal-component analysis (PCA) (98) but assume Gaussian, rather than linear, responses along environmental gradients. DCA also incorporates a rescaling step to overcome distortions ("the arch effect") commonly encountered with correspondence analyses (94). In general, DCA ordinations perform better with simulated data than do CA ordinations. Prior to conducting the CA and DCA, the

(freshwater, saltwater, or terrestrial) and by organism association (whether or not the sequence was isolated from a bacterium living on or within another organism). Two environments required special biome characterization: a brackish isolation source was considered half freshwater and half saltwater, and a sediment isolation source was considered half terrestrial and half aquatic (either freshwater or saltwater). Following categorization, the means of the biome and organism association results were calculated for each tribe. The percentage of sequences associated with each biome category for each tribe is visualized in ternary plots. Triangle vertices indicate a 100% relative abundance of the indicated biome for the plotted tribe. The symbol color for each tribe indicates the percentage of GenBank hits for the tribe that were noted as having been associated with another organism when the sequences were obtained (e.g., human skin or algal phycosphere). The symbol shape for each tribe reflects the mean number of GenBank hits with $\geq 97\%$ sequence identity to the tribe reference sequences.

library composition from each lake was scaled to a sum of 1 to control for sampling differences across studies. In addition, environmental variables were standardized to a mean of zero and scaled by their standard deviations (Z score).

To display the general abundance and occurrence patterns of all tribes, we plotted the relative recoveries of each tribe (number of tribe sequences/number of total sequences) from all lakes and their prevalence (number of lakes from which sequences from a tribe were recovered/number of lakes in the meta-analysis) in the 47 lakes (Fig. 9). Lineage acI appears to be the most common bacterioplankton lineage, with small changes in prevalence across lakes. Our findings suggest that the acI lineage is also the most persistent freshwater lake bacterial lineage, and this is in agreement with previous documentation of its low temporal dynamics over seasons (e.g., see reference 63). Other very abundant and widespread freshwater bacterial groups are the betaproteobacterial lineages *Polynucleobacter* (Pnec), where PnecC represents the most common tribe in this lineage, and Lhab, belonging to the *Comamonadaceae*. Tribe LD12, a freshwater lineage of the SAR11 group, showed the highest prevalence and relative recovery of all alphaproteobacterial lineages. However, tribe LD12 does not seem to be as ubiquitous and abundant in freshwater systems as its sister group SAR11 is in marine environments, where it was previously shown to dominate most bacterioplankton communities (50, 137).

In addition, Spearman rank correlation coefficients between the relative recoveries of each tribe from each lake and environmental state variables (pH, DOC/TP ratio, and AbsLat) were determined. In Fig. 10, positive (blue) and negative (red) correlations between each tribe and each of the three variables are indicated. The size of the symbol indicates the strength of the correlation. In general, a conservation of environmental responses at higher taxonomic levels (i.e., phylum) was rare, as suggested previously by others (108). However, many examples of conservation within more refined taxonomic groups can be observed (e.g., Iluma lineage response to pH, acI-B lineage response to pH and DOC/TP, and Flavo lineage response to DOC/TP). Coherent patterns in the environmental response appear to be more common in the phylum *Actinobacteria*, where tribes could be narrowly defined as a result of previous phylogenetic work and a large number of available sequences (140).

DCA and CA resulted in highly similar plots (for DCA, see Fig. 11; for CA, see the supplemental material); thus, we will discuss only DCA. The axis that explained the most variation in freshwater lake bacterial tribe distribution in our DCA was highly correlated with pH. Numerous studies have shown that pH is a major environmental covariate of taxon distribution patterns of zooplankton and phytoplankton (21) and was shown more recently to correlate strongly with biogeographic patterns of freshwater lake bacterioplankton (123). In a more detailed analysis of tribes within the phylum *Actinobacteria*, distribution patterns along pH gradients corresponded very well with our meta-analysis (140). Tribes belonging to the acI-B lineage seem to be more common in acidic lakes with high DOC concentrations, except for tribe acI-B1. This tribe and the tribes belonging to the acI-A lineage (except tribe acI-A1) seem to be more common in lakes with high pHs and low DOC concentrations. Tribes within the ubiquitous *Polynucleobacter*

lineage revealed some clear distinctions. The abundance of PnecB, which has been suggested to be circumneutral or alkaline (230), likewise increased with increasing pH. On the other hand, the abundance of cluster PnecC was negatively correlated with pH and could be associated with acidic lakes. Such pH-dependent biogeography has also been described for members of the *Bacteroidetes*, where the HAL-A2 tribe (GKS2-217) was found only in soft-water lakes at a pH range of 6.5 to 7.3, the Aquir tribe (LD2) was found only in hard-water lakes at a pH range of 7.7 to 9.5, and the HAL-A1 tribe (HAL) was found in a variety of lakes at a pH range of 6.7 to 8.6 (178). Our DCA corroborates that HAL-A2 is more common in more acidic lakes than is Aquir. Other tribes that seem to be associated with acidic lakes are the bacI-A2, bacI-A3, bacI-B1, Flavo-A2, Flecto (R-FL615), and HAL-A2 (HAL) tribes, whereas the Flavo-A1, Algor, Aquir (LD2), and Pedo tribes are typical representatives in alkaline lakes (Fig. 11).

In previous studies, resource availability has been linked to the distribution of freshwater lake bacterial tribes. The ratio of water color to chlorophyll a was recently suggested to be a useful way to summarize the relative contributions of within-lake (autochthonous) primary production versus terrestrial (allochthonous) organic matter (26, 108) supporting heterotrophic growth. In this meta-analysis the DOC-to-TP ratio appears to serve a similar purpose, where low ratios are indicative of autochthonous-dominated systems and high ratios are indicative of allochthonous systems. The *Flavobacteriales* especially seem to be favored in autochthonous systems, where phytoplankton blooms often occur (49). A metabolic capability to degrade recalcitrant organic compounds such as humic substances was suggested for *Bacteroidetes* tribes bacI-A1 and bacI-A2 (51) as well as for freshwater *Novosphingobium* (clade alfIV-A) and *Sphingopyxis* (clade alfIV-B) (104). These and numerous other lineages were associated with high DOC/TP ratios in the DCA plots, which suggested that these lineages are competitive under conditions where humic organic matter is abundant and possibly points to their role in the degradation of this large pool of recalcitrant (mostly allochthonous) organic carbon. Tribes within the betaproteobacterial lineage Lhab had strong negative correlations with the DOC/TP ratio, supporting their previously documented responses to nutrient enrichment (187). The two major tribes within the *Verrucomicrobia* appeared to contrast in the majority of their environmental responses, including their distribution along the gradient in DOC/TP.

Our meta-analysis provides a number of suggestive descriptions of the responses of tribes to environmental gradients. However, these relationships are correlative and may be confounded by correlation among other environmental variables. Hence, there is clearly a need for more comprehensive environmental sampling, including a higher resolution of the quality and quantity of resources, like nitrogen, phosphorus, trace elements, and organic compounds, but also biotic interactions, such as predation by grazers and viruses. Experimental approaches should be employed to make more mechanistic links between environmental drivers and tribe responses. Even though the defined tribes represent phylogenetic groups with broad ecological properties that seem to be congruent among the environmental variables used in our meta-analysis, we need to consider that in some cases, organisms with (almost) iden-

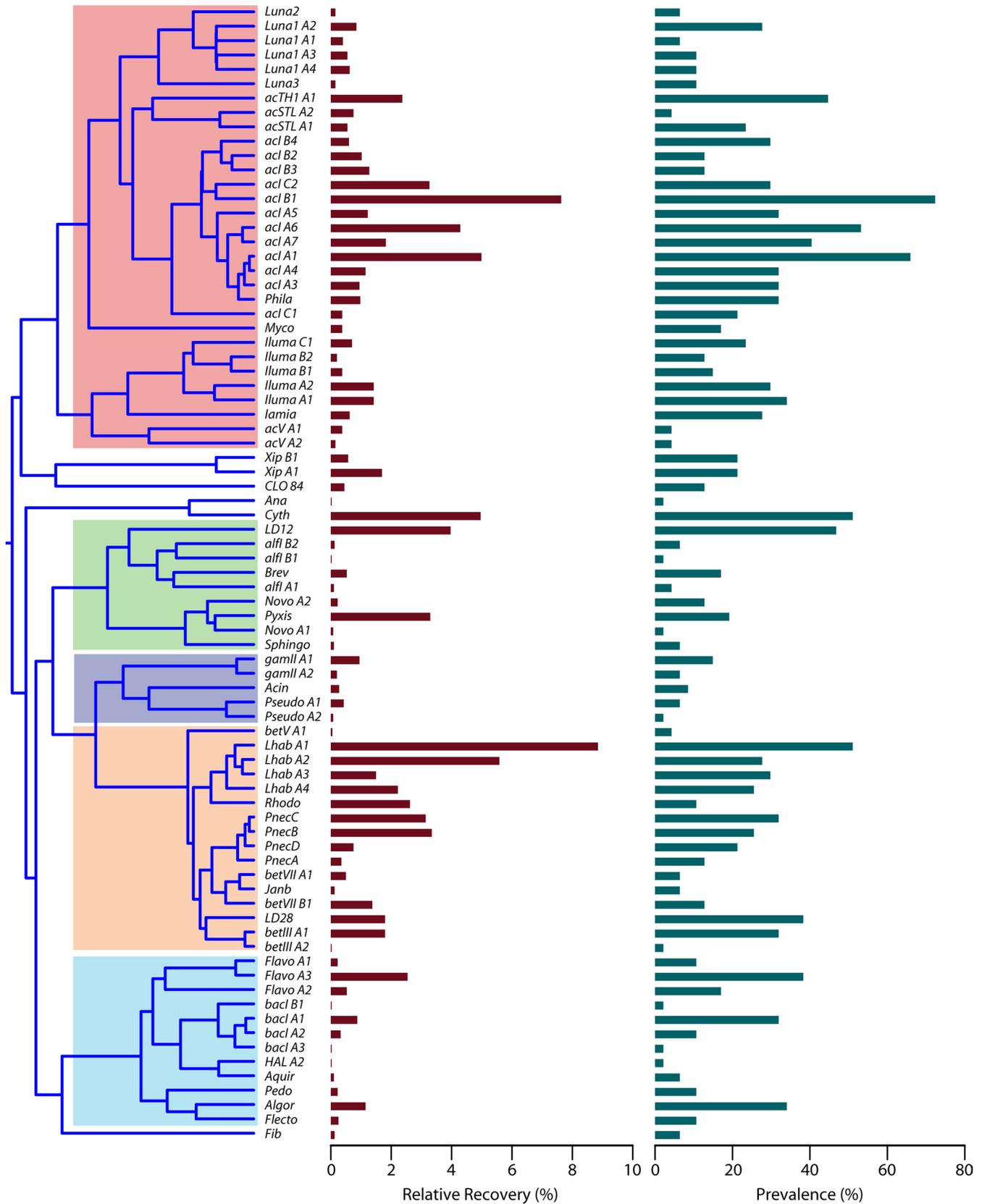


FIG. 9. Bar plots indicating the relative recovery (number of tribe sequences/number of total sequences) of each tribe from all lakes and their prevalence (number of lakes from which sequences from a tribe were recovered/total number of lakes) in the 47 lakes. For reference, bars are aligned with an ultrametric tree of all freshwater lake tribes observed for the 47 lakes.

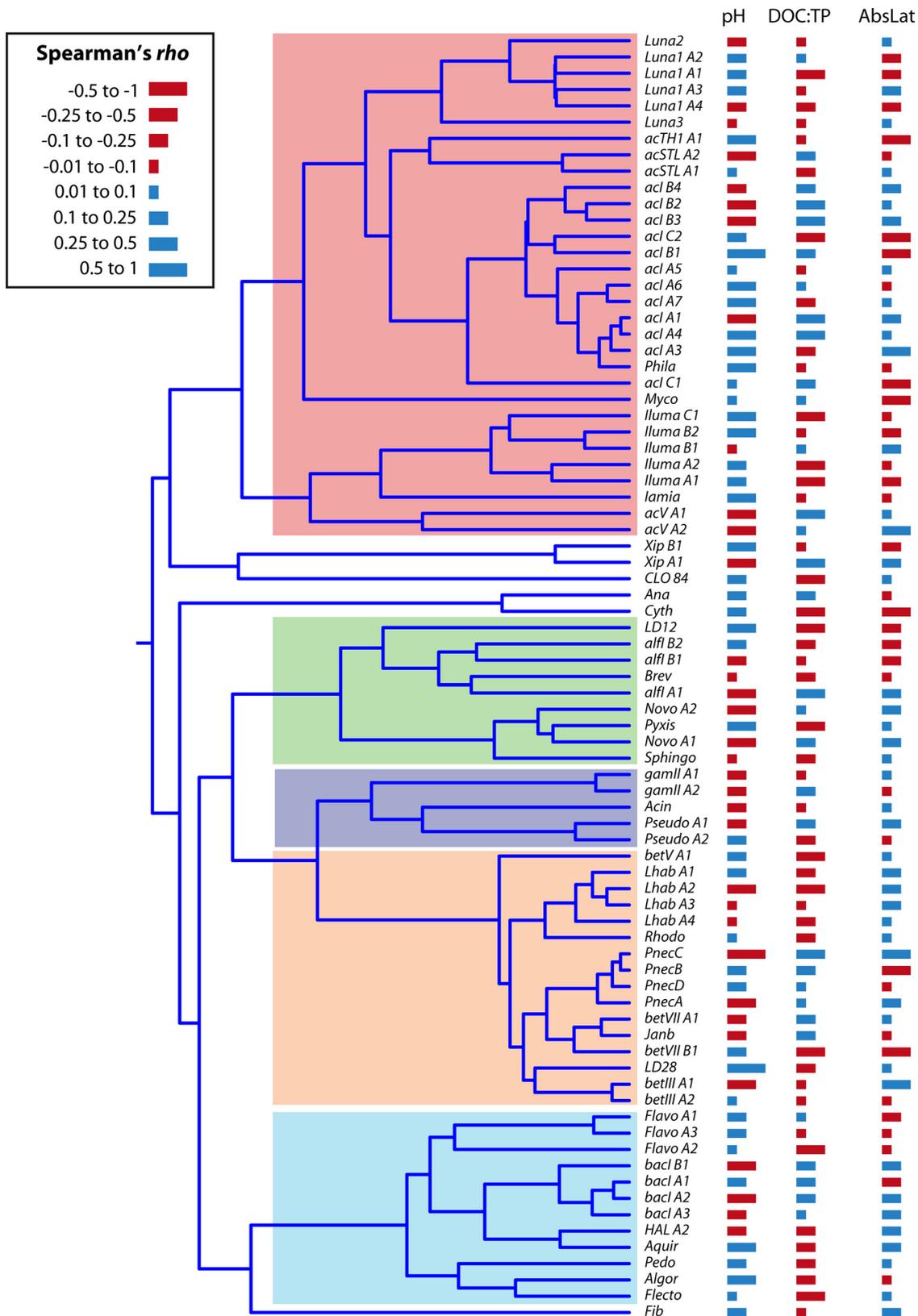


FIG. 10. Sign and magnitude of Spearman rank correlation rho between the relative recovery of each tribe from each lake and environmental conditions (pH, the ratio between dissolved organic carbon and total phosphorus concentrations [DOC/TP], and absolute latitude [AbsLat]). Positive (blue) and negative (red) correlations between each tribe and each of the three variables are indicated. The size of the symbol indicates the magnitude of Spearman's rho. Environmental correlations are aligned with an ultrametric tree of all freshwater lake tribes observed for the 47-lake data set used for this analysis.

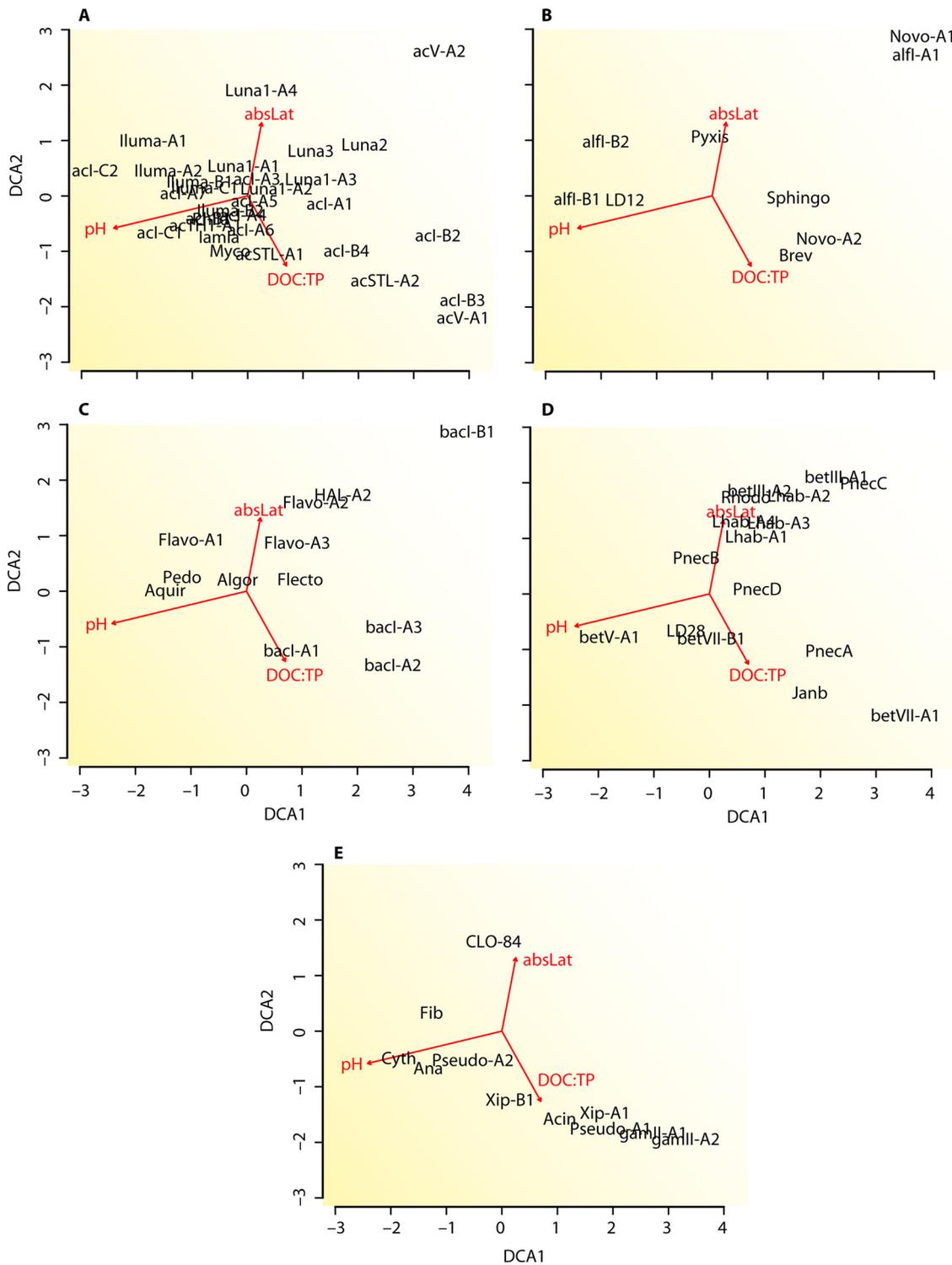


FIG. 11. Detrended correspondence analysis (DCA) (94) to visualize patterns of tribe distribution along environmental gradients (pH, ratio between dissolved organic carbon and total phosphorus concentrations [DOC/TP], and absolute latitude [AbsLat]). The first axis explained ~12% of the variance, and the second axis explained an additional 6%. All tribes were included in a single DCA, but each panel presents a limited number of tribes for clarity. (A) Actinobacteria; (B) Alphaproteobacteria; (C) Bacteroidetes; (D) Betaproteobacteria; (E) minor phyla.

tical 16S rRNA genes have rather divergent genomes, allowing them to inhabit different environmental niches (e.g., see reference 88). There is clearly a need to verify the level of resolution defining ecologically coherent units among the freshwa-

ter lake bacteria. Having said that, we observe many promising and suggestive biogeographic patterns within the freshwater lake bacterial groups examined in this review and encourage others to test the hypotheses presented here. We have a long

way ahead of us before we reach a robust understanding of the traits of freshwater lake bacterial groups and incorporate this understanding into a theoretical framework allowing predictive microbial ecology.

OUTLOOK: HOW TO USE THIS GUIDE

Lakes are foci for ecological processes and human activities in global landscapes (47). The resident bacterial communities of these ecosystems play a central role in many hydrological and biogeochemical cycles, thereby providing critical ecosystem services to humankind (136). Recently, lakes have been described as early indicators (i.e., sentinels) of both regional and global environmental change (228). Much of this sensitivity and responsiveness to environmental change can be attributed to the resident microorganisms, but with respect to bacteria, this has not been explored to its full potential (1). Given the importance of bacteria to lake ecosystems, few studies have been able to link bacterial identity to function in this environment. Most would agree that this research gap results largely from a lack of an understanding of the characteristics of the major taxa present in lake systems, which stems from the tendency of abundant freshwater lake bacteria to escape cultivation. Hence, a continued strategy to gather enrichments and pure cultures of the abundant groups of freshwater lake bacteria for further ecological and metabolic characterization is necessary, especially when in complement with the cultivation-independent identification of ecophysiological traits of these organisms in nature. The framework developed in this review provides a useful guide for this ongoing effort.

To date, much of the work carried out on connecting lake bacterial taxa to biogeochemical processes and other ecological traits has been done at the phylum level (reviewed in reference 154). Phylum-based analyses have certainly proven useful as an initial assessment of the types and abundances of bacteria that may be present in an environment and as a way to broadly partition the drivers and functions of bacterial taxa in their particular environments. However, combining a multitude of taxa into a single group, as is done when studying phyla in lakes, creates the unrealistic expectation that all taxa within a phylum behave similarly. Thus, the phylum level of taxonomic identity is a rather blunt tool with which to decipher the ecology of freshwater bacterioplankton and, as such, is more likely to generate conflicting data among studies. For freshwater lake bacteria, both the reviewed literature and synoptic phylogenetic analyses suggest substantial ecological divergence within phyla (for a visual representation of the ecological divergence of tribes within phyla, see Fig. 11 and 12). We do not altogether discard the possibility that there is also some level of basic ecological coherence at higher taxonomic ranks of bacteria (158). However, given the data at hand, this seems unlikely for the majority of bacterial phyla that dominate in freshwater lake ecosystems. The spatial and temporal separation of phyla and other higher taxonomic ranks has been observed repeatedly for freshwater lakes and many other environments. One interpretation of such patterns is that this reflects ecological coherence at this higher taxonomic level (158). We believe that it is much more likely that these apparent distribution patterns for phyla reflect the niches of particularly competitive lineages, clades, or tribes within a phylum

that occasionally dominate the community, as previously observed for brackish bacterial communities in the Baltic Sea (7) and *Actinobacteria* in freshwater lakes (Fig. 12).

A few studies have addressed the question of whether or not community composition and, therefore, diversity are related to the functional traits of a community (e.g., see references 14, 16, 54, 56, 68, and 204). Those studies have provided conflicting results, thereby fueling the ecosystem diversity debate with arguments about extensive functional and ecological redundancy in bacterial communities but without fully acknowledging the diverse ecological and physiological properties of the individual populations within the studied communities. In fact, even the understanding and appreciation of mechanisms causing temporal and spatial biogeography patterns, like seasonal succession in freshwater lake bacterioplankton, are hampered by our currently very limited knowledge about the ecology and physiology of even the most abundant bacterial taxa in this ecosystem. During yearly cycles, lake bacterial communities undergo dramatic shifts in community composition (e.g., see reference 183), at least partly in response to disturbance events. It has been argued that such disturbances may alter community composition, thereby preventing competitive exclusion and the maintenance of community diversity (169). Diversity may also be maintained and community composition may be structured by internal community processes such as predation and concurrent competition between bacterial (species) tribes for multiple resources (99). However, without detailed genomic and experimental studies of the abundant bacterioplankton tribes, our understanding of these processes will remain uncertain. Again, the phylogenetic framework presented here provides an outline for gathering and synthesizing such information.

Now that many of the abundant pelagic lake bacteria have been identified and organized into phylogenetically narrow groups, we can focus on studying them in more detail using genomic and experimental approaches. We propose an emphasis on these defined freshwater groups instead of the previous emphasis on phyla, with the idea that these groups may be more similar at the level of biological organization to that of "species." This will lead to more coherent responses and the development of more precise and more robust predictive models for their distribution patterns over time and space as well as for ecosystem-level functioning. Studies employing this more narrowly defined level of focus have already provided many insights into the ecology and physiology of common freshwater bacteria (as reviewed here). We do not suggest that all of the narrowly defined phylogenetic groups (i.e., tribes) represent congruent ecological units, as ecological differentiation within tribes has been shown previously. For example, there are known differences in habitat types among members of the PnecC tribe (217) and in salinity tolerance among members of the beIII-A1 tribe (230). This guide does, however, provide the framework within which questions about the identification of bacteria to the species level and ecological differentiation in freshwater bacterioplankton can be asked and explored while allowing for the continued refinement of the lineages, clades, and tribes until genera and species are properly described. We also note that our database has a major overrepresentation of lakes from northern latitudes, especially Europe and North America (see the supplemental material).

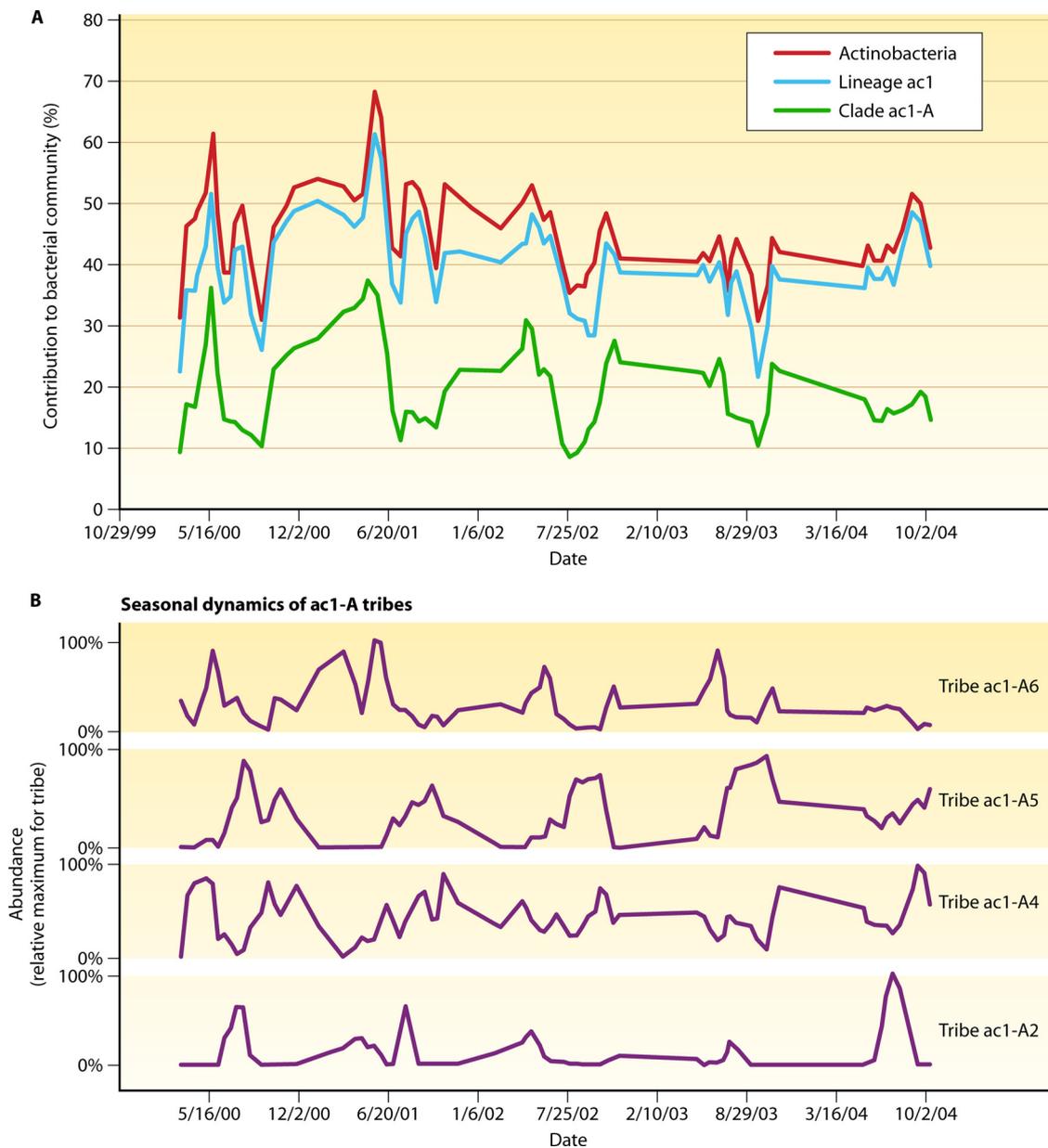


FIG. 12. (A) Time course of the relative contributions by the acI lineage and acI-A clade (A) and the acI-A2, acI-A4, acI-A5, and acI-A6 tribes (B) of the phylum *Actinobacteria* to the bacterial community of Lake Mendota (by percentage of total fluorescence), inferred from automated ribosomal intergenic spacer analysis (ARISA) according to methods described previously (141). Sampling was conducted biweekly for 4 years during the ice-off period of the lake. The curves are constructed as a moving average ($n = 2$) of the relative contribution data. For comparison, the tribe plots are scaled to the maximum fluorescence observed for each tribe.

In order to fully characterize the bacteria of freshwater lakes and further our understanding of their roles in this ecosystem, we emphasize the need to obtain more information on bacteria from tropical lakes and lakes of the southern hemisphere.

The results presented here illustrate the powerful nature of comparative cross-study analysis. Many new putative freshwater lake clusters were identified, but probably more importantly, the large numbers of described bacterial 16S rRNA clusters spread across the literature were brought together into a single phylogenetic framework. Phylogenies are always evolving as more sequences are obtained and added; thus,

the phylogenies presented here are not intended to be static or constraining but provide a temporary structure for an ever-expanding collection of freshwater sequences. The results here also further support the notion that bacteria commonly found in freshwater lakes are not there accidentally but are the result of a suite of environmental forces that define freshwater lakes as a unique habitat that acts upon the global bacterial community, selecting for the taxa found living there.

The natural history guide to freshwater lake bacteria provides a sorely needed knowledge base and controlled vocabulary for future freshwater studies to build upon and explore.

Our data set should provide a good starting point for further comparative studies among different chemically and physically defined epilimnia or between different freshwater environments, since many freshwater habitats (e.g., lake hypolimnia and littoral zones, rivers, and groundwater) were not included here. In addition, comparisons between more distantly related habitats such as the ocean or various terrestrial environments should now be more feasible. Those interested in learning more about lake microflora could benefit greatly from a synoptic table of shared ecological and metabolic traits for tribes, clades, lineages, and phyla. Our understanding of lake bacteria is still too superficial to enable the preparation of such a table, but the use of our phylogenetic framework to structure sequences from the growing number of surveys and experiments that employ next-generation sequencing might take us to that point in the near future. It is our hope that this effort spurs many new studies aimed at these potentially more ecologically relevant clusters so that the freshwater research community can learn more about the relationships between freshwater bacterial phylotypes and taxa, their habitat preferences, and their functions in these ecosystems.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences generated for this study have been deposited in GenBank under accession numbers FJ916807 to FJ916903, HQ386253 to HQ386631, and HQ530565 to HQ532908, except HQ530583, HQ531638, and HQ532521. The 16S rRNA gene ARB database used in this study is available upon request from R.J.N. (rjnewton@gmail.com) or the corresponding author.

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