Contrasting patterns of genome-level diversity across distinct co-occurring bacterial populations

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Abstract

To understand the forces driving differentiation and diversification in wild bacterial populations, we must be able to delineate and track ecologically relevant units through space and time. Mapping metagenomic sequences to reference genomes derived from the same environment can reveal genetic heterogeneity within populations, and in some cases, be used to identify boundaries between genetically similar, but ecologically distinct, populations. Here we examine population-level heterogeneity within abundant and ubiquitous freshwater bacterial groups such as the acI Actinobacteria and LD12 Alphaproteobacteria (the freshwater sister clade to the marine SAR11) using 33 single cell genomes and a 5-year metagenomic time series. The single cell genomes grouped into 15 monophyletic clusters (termed “tribes”) that share at least 97.9% 16S rRNA identity. Distinct populations were identified within most tribes based on the patterns of metagenomic read recruitments to single-cell genomes representing these tribes. Genetically distinct populations within tribes of the acI actinobacterial lineage living in the same lake had different seasonal abundance patterns, suggesting these populations were also ecologically distinct. In contrast, sympatric LD12 populations were less genetically differentiated. This suggests that within one lake, some freshwater lineages harbor genetically discrete (but still closely related) and ecologically distinct populations, while other lineages are composed of less differentiated populations with overlapping niches. Our results point at an interplay of evolutionary and ecological forces acting on these communities that can be observed in real time.

Introduction

Bacteria represent a significant biomass component in almost all ecosystems and drive most biogeochemical cycles on Earth. Yet we know little about the population structure of bacteria in natural ecosystems and have yet to find and define the boundaries for ecological populations. Cohesive temporal dynamics and associations inferred from distribution patterns have been documented for many habitats and these observations are consistent with the notion of such populations as locally coexisting members of a species (Shapiro and Polz 2014). The most compelling cases are from collections of closely related isolates (Hanage et al 2005, Luo et al 2011, Shapiro and Polz 2014), but cultured species represent only a very small portion of the bacteria populating the Earth (Amann et al 1995, Hug et al 2016, Kaeberlein et al 2002), and thus we still know little about the most abundant lineages. Therefore it is critical to study microorganisms in their natural environments (Little et al 2008), in order to test if and how their population-level heterogeneity differs from the established models based on isolates. The advent of culture-independent approaches, such as single-cell genomics and metagenomics, provides an opportunity for gaining new insights about genome-level diversity at the population level for organisms that are currently difficult or impossible to culture.

The delineation of ecologically differentiated lineages within complex microbial communities remains controversial because direct evidence for such differentiation is usually sparse (Hunt et al 2008). Additionally, the appropriate level of phylogenetic resolution defining ecologically equivalent groups has not yet been established and likely varies across different groups (Fuhrman et al 2015). Past explorations for defining such groups have used genome-wide average nucleotide identity (gANI) across shared regions of isolate genome sequences (Konstantinidis and Tiedje 2005, Varghese et al 2015). These studies have found that gANI greater than 94-96% unites past classical species definitions and separates known

89 sequenced strains into consistent and distinct groups. Genetically distinct populations have
90 been identified in microbial communities using metagenomics by mapping reads against
91 reference genomes and noting a coverage gap at 90-95% identity (Bendall et al 2016, Caro-
92 Quintero and Konstantinidis 2012, Kashtan et al 2014, Konstantinidis and DeLong 2008, Oh
93 et al 2011). Reads mapping with identities above the coverage discontinuity have been
94 defined as originating from a 'sequence discrete population' (SDP) of genetically nearly
95 identical cells that are distinct from other cells whose sequences map with identities below
96 the coverage discontinuity (Bendall et al 2016). For the remainder of the manuscript, we will
97 use the terms 'population' and 'sequence-discrete population' interchangeably.

98 We used a combination of time-series metagenomics and single cell genomics to
99 define genetic diversification within ubiquitous and abundant freshwater lineages such as acI
100 and tribe LD12. The term "tribe" was previously coined to delineate these groups using 16S
101 rRNA gene sequences, where tribes are defined by monophyly and >97.9% within-clade 16S
102 rRNA gene sequence identity (Newton et al 2007, Newton et al 2011). Freshwater microbial
103 ecology researchers generally discuss and track these tribes as coherent units that are
104 ecologically distinct from one another. A primary motivation for the present study was the
105 challenge of moving beyond 16S rRNA sequence identity to delineate ecologically relevant
106 taxonomic units given observed patterns of population-level heterogeneity, using shared
107 genomic content. This study includes thirty-three Single Amplified Genomes (SAGs)
108 representing fifteen phylogenetically coherent groups (i.e. freshwater "tribes").

109 The SAGs in this study originated from four lakes geographically isolated from one
110 another and represent a rich source of reference genomes that can be used to recruit
111 metagenomic reads in order to study population-level heterogeneity and dynamics through
112 time in naturally assembled communities. Two of the lineages featured in the present study
113 are the abundant and ubiquitous freshwater Actinobacteria acI and Alphaproteobactera alfV

114 containing the freshwater SAR11 sister-clade, tribe LD12. Members of these lineages are
115 intriguing in their own right, as they represent groups of free-living ultramicrobacteria that
116 dominate many freshwater ecosystems (Ghai et al 2014, Glöckner et al 2000, Heinrich et al
117 2013, Rösel et al 2012, Salcher et al 2010, Salcher et al 2011, Warnecke et al 2005, Zwart et
118 al 2002). They differ markedly with respect to within-lineage diversity: LD12 is the sole tribe
119 defined within the freshwater alfV lineage, while the acI lineage is comprised of 13 tribes
120 (Newton et al 2011). The acI and alfV are not easy to cultivate in monocultures (Kang et al
121 2017) (though see (Henson et al unpublished data)) and share a large number of genomic and
122 cellular traits. First, both lineages have genomes with GC content values lower than 40% and
123 estimated sizes of about 1.5 Mb or less (Garcia et al 2013, Ghylin et al 2014, Kang et al 2017,
124 Zaremba-Niedzwiedzka et al 2013). These genome characteristics are all the more striking
125 since most cultivated species in the Alphaproteobacteria and Actinobacteria have GC-rich
126 genomes up to 10 Mb in size. Second, both lineages have evolved by massive gene loss
127 (Zaremba-Niedzwiedzka et al 2013). Third, the fraction of gained genes is only about 10% of
128 the lost genes. Fourth, both groups of bacteria have small cell volumes (Heinrich et al 2013,
129 Salcher et al 2011). However, acI and alfV seem to employ different substrate niche
130 specialization. While acI is thought to primarily use polyamines, oligopeptides and
131 carbohydrates, alfV specializes in carboxylic acids and lipids (Eiler et al 2016, Ghylin et al
132 2014, Salcher et al 2013).

133 By combining genome information from twenty-one previously published (Ghylin et
134 al 2014, Zaremba-Niedzwiedzka et al 2013) and twelve new SAGs from different freshwater
135 lineages and an extensive five-year time series of lake metagenomes (94 samples), we
136 investigated the population-level heterogeneity of such ubiquitous freshwater bacteria for the
137 first time. Our results confirm the existence of coherent sequence-discrete populations within
138 these ubiquitous freshwater bacterial groups in natural communities and we could trace the

abundance and gANI of these populations over monthly to seasonal time scales. Our work demonstrates the power of combining time-series metagenomics and single cell genomics for studying bacterial diversification and for describing ecologically meaningful population-level heterogeneity within communities inhabiting natural ecosystems.

Results

The SAG collection represents multiple clades within cosmopolitan freshwater lineages

We analyzed 33 SAGs from four different freshwater lakes. Twenty-one of these SAGs were previously analyzed for their genomic features and phylogenetic relationships (Eiler et al 2016, Garcia et al 2013, Ghylin et al 2014, Zaremba-Niedzwiedzka et al 2013). The 33 SAGs had total assembly sizes between 0.33 and 2.42 Mbp and were organized into 8 to 103 contigs with GC contents between 29.1% and 51.7% (**Table 1**). Estimated genome completeness, calculated using two different methods, ranged between 30% and 99%. Throughout the paper we will use mostly the shorter name version to facilitate reading, for example, M14 in place of AAA027-M14.

The 33 SAGs in the study represent fifteen different previously defined freshwater "tribes" (that are each monophyletic and defined by >97.9% within-clade 16S rRNA gene sequence identity, measured across the nearly full-length 16S rRNA gene) (Newton et al 2007, Newton et al 2011). Ten tribes are represented by only one SAG each, while four tribes (LD12, acI-A1, acI-A7 and acI-B1) have more than one SAG representative in our dataset. In addition to their classification based on 16S rRNA genes, the nine SAGs that were the only representatives of their lineage were classified using protein coding marker genes and PhyloSift (Darling et al 2014) (**Table S1**). To illustrate phylogenetic and taxonomic placement of the LD12 and acI SAGs, we used the PhyloPhlAn pipeline (Segata et al 2013) to generate a multi-gene tree (**Figure 1A and 1B**). The tree topology was consistent with

164 previous phylogentic reconstructions for LD12 (Zaremba-Niedzwiedzka et al 2013) and acI
165 (Ghylin et al 2014, Newton et al 2007). The tree supported the 16S rRNA gene-based tribe
166 designations but did not reveal a clear biogeographic pattern, in agreement with previous
167 analyses, i.e. members of the same tribes were found in different lakes (Zaremba-
168 Niedzwiedzka et al 2013). However, our SAG collection was not designed to explore
169 biogeography and much deeper sampling of each population would be needed to address this
170 question rigorously.

171

172 *Genome-wide nucleotide identity is consistent with phylogeny*

173 Although multi-locus phylogenies supported the 16S rRNA gene based phylogeny, we
174 wondered whether gANI could similarly be used to demarcate one tribe from another. To this
175 end, we determined the pairwise gANI for genomes in the set of four tribes that each
176 contained more than one SAG representative. This general approach has been proposed as a
177 way to compare genome pairs using a single metric that robustly reflects phylogenetic and
178 taxonomic groupings obtained using other polyphasic methods (Konstantinidis and Tiedje
179 2005, Varghese et al 2015). We asked whether all genome pairs from the same tribe shared a
180 consistent minimum gANI. Most SAGs shared gANI of at least 78% and alignment fractions
181 greater than 40% with other members of the same tribe (**Figure 1C and Table S2**). Most
182 pairs from the same tribe that were also recovered from the same lake shared at least 84%
183 gANI, but some pairs were much more similar (gANI above 95%). gANIs between pairs
184 belonging to different tribes but still within the same lineage were markedly lower and
185 typically below 74% (e.g. acI-A1 vs acI-B1) (**Figure 1C and Table S2**).

186 Although gANI is a useful univariate metric for comparing genome pairs, it masks the
187 differences in sequence similarity of individual genes or genome regions that arise due to
188 varying rates of divergence across loci. This variation can be visualized by plotting the

189 frequency distribution of nucleotide identities calculated using a sliding window across the
190 genome (Konstantinidis and Tiedje 2005). We asked whether different homologous genomic
191 regions from two SAGs would have markedly different nucleotide identities even if they
192 were from the same tribe. We used the most complete SAGs from the acI-B1 and LD12 tribes
193 as reference genomes and calculated nucleotide identity using a sliding window with other
194 SAGs from the same respective tribe and visualized the results as a frequency distribution
195 (**Figure 2 and Figure S1**). The acI-B1 SAGs featuring the highest gANI (L06 and A23) were
196 both from Lake Mendota and shared nucleotide identity consistently greater than 95% with a
197 peak at 99-100%, suggesting they belong to the same SDP. The acI-B1 SAG P03 recovered
198 from a lake in Germany had a frequency distribution with a peak more near 97% and a
199 distinctly different shape. Other acI-B1 SAGs shared genomic regions with primarily 80-85%
200 nucleotide identity. This was even true for J17, which was also collected from Lake Mendota
201 and shared an average gANI of 79% with L06/A23 (**Table S2**), suggesting that cells
202 belonging to the same tribe (acI-B1) and living in the same environment can have substantial
203 genetic differences. The LD12 SAGs, which all belonged to the same tribe, also displayed
204 three distinct patterns, with one peak near 85%, several near 91%, and two near 97%. Lake
205 origin did not appear to explain these differences. That is, some LD12 cells from Lake
206 Mendota were more similar to LD12 cells from Sparkling Lake than to other LD12 cells from
207 Lake Mendota.

208

209 *Diversity of wild populations inferred using SAGs*

210 The variety of patterns observed in **Figure 2** indicated substantial within-tribe
211 variability even among cells recovered from the same lake. This made us wonder if tribes
212 were composed of genetically and ecologically distinct populations coexisting in the same
213 environment. SAGs can serve as relevant reference points to study the diversity of abundant

214 populations sampled using shotgun metagenomics by recruiting metagenomic reads and
215 examining the extent of nucleotide identity for each aligned read (Stepanauskas 2012). The
216 results can also be used to identify sequence-discrete populations whose boundaries are
217 revealed by recruitment patterns and specifically the dramatic drop in coverage observed
218 around 95% sequence identity (Bendall et al 2016, Caro-Quintero and Konstantinidis 2012,
219 Konstantinidis and DeLong 2008). We asked whether such SDPs could be identified using
220 metagenomic reads from Lake Mendota, WI, USA, by mapping them to the 33 SAGs, 19 of
221 which were collected from this lake.

222 Each of the SAGs was first used to recruit reads from a single metagenomic dataset
223 collected from Lake Mendota on 29 April 2009 (**Figure S2**). This time point was chosen
224 because it was the sample collected closest to the date on which the single cells were
225 collected (12 May 2009). Frequency distribution plots of the same data (**Figure 3 and Figure**
226 **S3**) revealed patterns that were similar to those obtained with SAG pairs (**Figure 2**). The five
227 acI-SAGs from Lake Mendota (J17, L06, A23, M14 and I14) recruited more reads than the
228 acI-SAGs from other lakes, with many reads recruiting at nucleotide identity greater than
229 97.5% (**Figure 3A**). All of the acI-SAGs also recruited many reads at 60 – 90% identity
230 (**Figure 3A and D**), creating the characteristic bimodal distribution observed in previous
231 work (Caro-Quintero and Konstantinidis 2012). Based on these results, we hereafter consider
232 reads sharing > 97.5% nucleotide identity as coming from the same, operationally defined
233 *population* (i.e. SDP) as the reference SAG. Thus, the acI lineage in Lake Mendota on 29
234 April 2009 was composed of multiple SDPs. Interestingly, the acI-B1 tribe in Lake Mendota,
235 a subset of the acI lineage, appeared to be composed of at least two coexisting and genetically
236 distinct populations, one represented by SAG J17 and the other by SAGs A23 and L06,
237 consistent with the pairwise gANI observed using only the SAGs (**Figure 2**).

238 To determine if we recovered representative SAGs from all acI populations in Lake

239 Mendota, we next performed recruitments competitively, allowing each read to only map to
240 the SAG with the greatest percent identity (**Figure S4**). Since the patterns in **Figure 3** were
241 generated by non-competitive recruiting, some reads mapping with 100% identity to one
242 SAG might for example also have mapped with 60-90% identity to SAGs from different
243 SDPs. Under competitive recruiting conditions the resulting frequency distributions changed
244 and the fraction of reads recruiting with 60-90% identity to each acI SAG dropped
245 dramatically (**Figure S4**). However, a secondary peak around 80% identity still remained in
246 most cases, and it is possible these reads originated from cells belonging to other acI
247 populations lacking a representative SAG.

248 LD12 SAGs collected from Lake Mendota (C06, J10, L15, C07 and D10) also had a
249 distinctive peak of recruited reads at >97.5% sequence identity (**Figure 3B**), although the
250 overall shape of the recruitment patterns differed dramatically from those of the acI lineage.
251 For example, LD12 SAGs had a secondary recruitment peak at ~92% identity whereas the acI
252 SAGs had secondary peaks at ~75% with non-competitive recruiting (**Figure S4**). This
253 suggests the SDPs within the LD12 tribe were more similar genetically than populations
254 comprising the acI-B1 tribe. In fact, the populations were sufficiently similar that the
255 hallmark coverage discontinuity below 97% similarity was not particularly pronounced
256 (**Figure 3B**). Under competitive recruiting conditions, the LD12 recruitment distribution
257 plots had remarkably different shapes (**Figure S4B and D**), as compared to the uncompetitive
258 recruiting conditions (**Figure 3B**), and each SAG had only a single peak at >97.5% identity.
259 This suggests the majority of LD12 cells in Lake Mendota belong to SDPs represented by the
260 SAGs in our collection.

261 All but one (I06) of the other freshwater SAGs in this study that were collected from
262 Lake Mendota generated the distinctive read recruitment frequency peak above 97.5%
263 identity (**Figure 3C**) that was observed for acI (**Figure 3A**). A negligible number of reads

264 recruited to the SAGs collected from other lakes under the competitive recruiting conditions
265 (data not shown).

266 Four complete acI genomes recovered from Lake Soyang in Korea were recently
267 published, and we included these in our recruitment analysis (**Figure 3F**). Three of the SAGs
268 exhibited recruitment frequency distributions analogous to those obtained using acI SAGs
269 from Sparkling Lake and Damariscotta Lake (**Figure 3D**), with very few reads mapping
270 above 90% ANI. The distribution from one SAG (IMCC19121) was remarkably similar to
271 that obtained from SAG N04, which was recovered from Damariscotta Lake in Maine. Both
272 IMCC19121 and N04 are members of the acI-A7 tribe and share 89.8% ANI with each other.

273

274 *Are sequence-discrete populations within a tribe ecologically discrete too?*

275 Results from a single metagenome sample suggested that individual tribes were
276 composed of multiple genetically distinct populations that could be delineated and tracked
277 using metagenomic read recruitment. Next we hypothesized that these populations might also
278 be ecologically distinct and fill different realized niches. If so, we might expect these
279 populations to display different temporal abundance patterns. We followed changes in
280 population abundance through time by recruiting reads from a five-year metagenomic time-
281 series applying a nucleotide identity cutoff of 97.5%, using only those SAGs derived from
282 Lake Mendota. SAGs from the LD12 tribe recruited more reads than all of the acI SAGs
283 summed together, on almost all sample dates (**Figure S5**).

284 Using the relative number of reads recruited as a proxy for abundance, we found the
285 J17 population, which belonged to the acI-B1 tribe, to be the most abundant acI population in
286 almost every sample (**Figure 4A and 5A**). The abundance of the J17 population was poorly
287 correlated over time with the other acI-B1 population represented by L06 (maximum
288 Spearman rank correlation = 0.256), indicating each population had a different temporal

289 abundance pattern.

290 In contrast to the acI-B1 tribe, the populations comprising the LD12 tribe had highly
291 similar abundance patterns. (**Figure 4B and S6**). The abundances of J10, L15, and C06
292 populations were strongly correlated (Spearman rank correlation = 0.996-0.999) (**Figure S8**
293 **and Table S5**) and tended to peak both in Spring and Fall (**Figure S6**). The D10 population
294 was the most abundant in the dataset but its abundance was not as strongly correlated to the
295 other LD12 populations (Spearman rank correlation = 0.705-0.725) (**Figure S8 and Table**
296 **S5**). The C07 population was the least abundant but was also correlated to both the J10-L15-
297 C06 populations and the D10 population (Spearman rank correlation = 0.850-0.873).

298

299 *Does the genetic diversity of populations change over time?*

300 We also examined the extent to which within-population diversity varied through time
301 by quantifying changes in population-wide ANI, i.e. the average identity of all reads mapping
302 with at least 97.5% identity (**Figure 5B**). For this purpose, we only recruited reads to SAGs
303 recovered from Lake Mendota. More abundant populations (such as LD12 and acI-B1 J17)
304 generally had lower population-wide ANI variance through time compared to some less
305 abundant populations (such as acSTL-A1-D23 and acI-A6-I14). For example, the SAG bacI-
306 A1 G08 population had relatively high population-wide ANI in June 2009, around the time
307 when the sample was collected for SAG library collection, but had markedly lower ANI on
308 all other dates. One interesting exception to this observation was a significantly lower ANI
309 for the relatively abundant acI-B1 L06-A23 population in 2012, as compared to 2007-2011
310 (Mann-Whitney U test p=1.4e-06).

311

312 ## Discussion

313 Comparative genomics can reveal the diversity and structure of bacterial populations.

314 This approach is particularly powerful when applied using single cells recovered from
315 environmental samples (SAGs) and shotgun metagenomes from the same or similar
316 ecosystems. Here we used a combination of 33 SAGs and 94 metagenomes collected over
317 five years to ask the following questions: 1) How well do individual SAGs represent the
318 population-level diversity found in natural communities? 2) Do common freshwater bacterial
319 groups have similar patterns of population-level diversity? and 3) How stable is population
320 abundance and diversity through time? We used the answers to these questions to gain insight
321 into the population-level diversity and ecology of the cosmopolitan and abundant freshwater
322 bacteria, alfV-LD12 (Alphaproteobacteria) and acI (Actinobacteria).

323 Sequence-discrete populations could be delineated in the Lake Mendota metagenome
324 using our 33 SAGs as references, as has previously been demonstrated in other lakes using
325 genomes assembled from metagenomes (Bendall et al 2016, Caro-Quintero and
326 Konstantinidis 2012). We interpret the occurrence of these populations in the context of
327 previously defined phylogenetically coherent and ostensibly ecologically distinct "tribes"
328 composed of cells with >97.9% 16S rRNA identity (Newton et al 2011). We conclude that the
329 freshwater tribes can contain multiple sequence-discrete populations. The converse is, of
330 course, not true: sequence-discrete populations can never represent multiple tribes because
331 tribes are by definition more distantly related to one another than genomes sharing a
332 minimum of 97.5% gANI.

333 Pair-wise gANI analysis of SAGs and metagenomic read recruitment indicated that
334 cells belonging to the same tribe but inhabiting different lakes were usually genetically
335 distinct. For example, SAGs collected from other lakes generally recruited very few reads
336 from Lake Mendota at ANI >97.5% while many recruited a substantial number of reads in the
337 89-92% range (**Figure 3**). However there were two prominent exceptions: LD12 N17 and
338 L09, both of which are from Sparkling Lake. N17 and L09 share 97% gANI with Mendota

339 SAG D10, which is substantially higher than the average (88%) and median (90%) within-
340 tribe gANI (**Table S2**). These SAGs also recruited roughly the same number of reads with
341 >97.5% identity as did the LD12 SAGs from Lake Mendota, though around 17% (L09) and
342 23% (N17) of the base pairs in the genomes did not recruit any reads. This implies that some
343 gene content was present in the Sparkling Lake populations but missing in Lake Mendota.
344 However, 10% of the base pairs in the D10 genome also did not recruit any reads, even
345 though it was from Lake Mendota. We examined the phylogenetic distribution of low-
346 coverage contigs and did not discern any evidence of contamination. This rare genome
347 content could represent flexible or low frequency genes in the population, or contamination
348 in the SAG preparation (Blainey 2013). However, it could also represent systematic coverage
349 bias, a phenomenon that we are not able to rule out with the data at hand.

350 In Lake Mendota, acI cells are organized into genetically discrete populations, but the
351 forces creating this organization remain unknown. The consistent lack of coverage around
352 90-97% identity in recruitment plots indicates Lake Mendota lacks acI genotypes sharing this
353 degree of sequence similarity with our SAGs, or at least that these putative genotypes were
354 consistently at much lower abundances than their close relatives over the five years surveyed.
355 The P03 SAG from Stechlin Lake shares gANI of 96% with acI-B1 SAGs from Mendota,
356 indicating that genotypes within this locally excluded sequence space do exist, at least as long
357 as they are from different environments. We infer the persistence of the coverage
358 discontinuity between populations to be less a factor of dispersal limitation and more likely
359 the result of competitive exclusion and barriers to recombination within Mendota
360 populations. Additional SAG and metagenomic studies are necessary to determine if similar
361 coverage discontinuities are observed in other phylogenetic groups and in different
362 environments. However, we do note that others have observed similar population-level
363 diversity in other lakes (Bendall et al 2016, Caro-Quintero and Konstantinidis 2012) and

364 marine ecosystems (Konstantinidis and DeLong 2008).

365 We know that both acI tribes and LD12 vary in abundance over seasonal and annual
366 time-scales, based on previous work using 16S rRNA gene sequencing, quantitative PCR, and
367 FISH (Allgaier and Grossart 2006, Eiler et al 2012, Heinrich et al 2013, Salcher et al 2011).
368 Here we used our SAGs to track such populations at monthly intervals over five years
369 (**Figure 4 and Figure S5**). The results confirmed prior work that showed acI tribes and LD12
370 are among the most abundant non-cyanobacterial groups in Lake Mendota (Newton et al
371 2011) but also revealed dynamics at unprecedentedly high phylogenetic resolution. Based on
372 our extensive comparison of how SAGs recruited relative to one another, we are confident
373 that our metagenomic recruitment filters allowed us to delineate discrete populations that
374 would not be possible to resolve using more traditional and widely used methods (e.g. 16S
375 rRNA gene sequencing or FISH). However, we do note that our acI SAG collection to date
376 does not seem to fully capture the full diversity of acI populations in the lake, as evidenced
377 by the residual peak of reads matching our SAGs at ~70-80% ANI, even under competitive
378 recruiting conditions. For example, we roughly estimate that our acI SAGs captured only
379 12% of the resident acI metagenome on 29 April 2009, as compared to 50% of the LD12
380 metagenome (**Table S3**). Thus, we cannot completely rule out the possibility that we missed
381 strong correlations among other acI populations that we could not detect.

382 However, the most striking finding of our study was that metagenomic recruitments to
383 LD12 SAGs yielded dramatically different patterns compared to the acI lineage. We
384 discovered that LD12 populations were not as strongly genetically separated as acI
385 populations; pair-wise gANIs between SAGs were higher and recruitment plots showed
386 secondary peaks between 90-95% identity (**Figure 3B**), the same range where coverage of
387 acI SAGs was at a minimum (**Figure 3A**). Under a competitive recruitment analysis, wherein
388 each read is counted only once and attributed to the best match SAG, the secondary peaks

389 disappear (**Figure S4**), indicating the LD12 SAGs represent highly similar, but still
390 genetically discrete, populations. Temporal abundance patterns of these LD12 populations
391 were strongly correlated over five years, whereas acI populations showed much lower
392 correlation within tribes **(Figure S8**). This suggests that the acI-B1 populations are
393 ecologically distinct (i.e. occupying temporally discrete niches) while LD12 populations are
394 less differentiated with respect to niche dimensions, leading to co-occurrence and
395 synchronization of temporal abundance patterns. LD12 is a particularly fascinating group
396 because it is also a subclade of the broader SAR11 clade, with hypothesized ancient transition
397 from marine to freshwater (Logares et al 2010) followed by specialization through gene flux
398 and mutation, with comparatively low recombination rates (Zaremba-Niedzwiedzka et al
399 2013). Over time, low recombination rates and relatively low selection levels should lead to
400 large genetic divergence among coexisting populations. Thus, we propose that LD12
401 populations are simply at earlier stages of differentiation as compared to acI populations,
402 although we cannot exclude that something fundamental about their lifestyle is "holding" the
403 populations together genetically and ecologically. This is particularly interesting in light of
404 recent reports of unusually high recombination rates in LD12 (Zaremba-Niedzwiedzka et al
405 2013), pointing to the need to further investigate contrasting population structures and what
406 these structures mean for the ecophysiology of the organisms. We do note that it is also
407 possible that the highly correlated LD12 populations are each occupying unidentified distinct
408 niches that are unrelated to the temporal correlation, allowing these slightly genetically
409 differentiated populations to co-occur while being ecologically distinct. In any case, the lack
410 of coherence among acI-B1 populations challenges our concept of tribes as ecologically
411 coherent units and suggestions that freshwater microbial ecologists re-examine conventions
412 for tracking these units through space and time. Taken together, these observations suggest
413 fundamental differences in evolutionary history and/or lifestyles among these abundant and

414 ubiquitous freshwater bacteria.

415 The metagenomic recruitments allowed us to also examine the extent to which
416 diversity varied within and among populations as well as how diversity changed over time.
417 We calculated the population-wide ANI for reads that recruited only above 97.5% and found
418 the resulting value was remarkably stable through time for most of the abundant populations
419 (**Figure 5B**). This was particularly true for the LD12 populations. However, one striking
420 contrast was the acI-B1 population represented by L06/A23, which had consistent
421 population-wide ANI of 99.3% during 2008-2011 but 99.0% during 2012 (Mann-Whitney U
422 test p=1.4e-06). Similar shifts were observed previously in sequence-discrete populations
423 inhabiting Trout Bog Lake, indicating this could be a common phenomenon among
424 freshwater clades (Bendall et al 2016). Unlike the genome-wide selective sweep observed in
425 one *Chlorobium* population from Trout Bog Lake, the distribution of single nucleotide
426 polymorphisms within the L06/A23 population before and after 2012 exhibited no clear
427 pattern of gene- or genome-wide sweep (data not shown). That is, it seems that the increase in
428 population-wide gANI resulted in a change in the relative abundance of individual genotypes,
429 rather than a single new genotype overtaking the population. It is difficult or impossible to
430 separate genotypes within sequence-discrete populations using short-read shotgun
431 sequencing, so further work using long-read technologies will be needed to link SNPs in
432 populations to individual genomes. This kind of approach will likely be required to tease
433 apart the paths leading to diversification within and among populations.

434

435 ## Methods

436 *Single amplified genomes (SAGs)*

437 Water samples (1-ml) were collected from the upper 0.5m to 1m of each of four lakes
438 (Mendota, Sparkling, Damariscotta, Stechlin) and cryopreserved, as previously described

439 (Garcia et al 2013, Martinez-Garcia et al 2011). These lakes were originally selected because
440 they represent different freshwater trophic status (eutrophic, oligotrophic, mesoeutrophic, and
441 oligotrophic, respectively) and geographic regions (Wisconsin and Maine, USA, and
442 Germany). Bacterial single amplified genomes (SAGs) were generated by fluorescence-
443 activated cell sorting (FACS) and multiple displacement amplification (MDA), and identified
444 by PCR-sequencing of their 16S rRNA genes at the Bigelow Laboratory Single Cell
445 Genomics Center (SCGC; http://scgc.bigelow.org). Thirty-two SAGs from lakes Mendota,
446 Sparkling and Damariscotta were selected for sequencing based on the previously sequenced
447 16S rRNA gene as well as the kinetics of the MDA reactions (Martinez-Garcia et al 2011).
448 The one SAG from Lake Stechlin was selected from a separate library because its 16S rRNA
449 gene was 100% identical to an acI-B1 SAG previously analyzed (AAA027-L06) (Garcia et al
450 2013). In the present study we analyze 21 previously published and 12 new SAGs. All 33
451 SAGs were analyzed (**Table 1**) after genome sequencing, assembly, contamination removal
452 and annotation as previously described (Ghylin et al 2014). Estimation of completeness was
453 done using CheckM (Parks et al 2015) and the gene markers from a recent study examining a
454 large collection of draft environmental genomes (Rinke et al 2013).
455
456 *Tree construction, Average Amino acid and Average Nucleotide Identity (AAI, ANI)*

457 A phylogenomic analysis was conducted using PhyloPhlAn (Segata et al 2013). ANI
458 was calculated by using the method described in (Konstantinidis and Tiedje 2005) with
459 fragment size of 1000, minimum alignment length of 700 bp, percent identity of 70, and e-
460 value of 0.001. AAI was calculated by averaging the identity of the reciprocal best hits from
461 the BLASTP searches of the predicted proteins for each pair of genomes. 16S rRNA gene
462 similarity for each pair was calculated using the overlapping region in an alignment created
463 using a multiple alignment (default options) in Geneious Version R6 (Kearse et al 2012).

464 Additional classifications were carried out using PhyloSift version 1.0.1, which examines 37
465 conserved single copy marker genes and places them into a phylogenetic reference tree
466 (Darling et al 2014).
467
468 *SAG-to-SAG recruitments*

469 SAG pairs from the same tribe were used to examine the frequency distribution of
470 nucleotide identities across homologous regions of the two genomes. In order to create a
471 sliding window for comparison, the contigs of all SAGs were shredded into 301bp fragments
472 with 150 bp overlap. Two SAGs were selected as reference genomes: L06 as the most
473 complete from the tribe acI-B1 and C06 as the most complete LD12. The contigs of each of
474 the two selected SAGs were used as a reference for recruiting from the shredded SAGs using
475 Blast 2.2.28 (Camacho et al 2009). Ribosomal RNAs were masked from the SAGs prior to
476 performing blast.
477
478 *Five-year time series metagenome data: sampling, sequencing and recruitments*

479 Samples were collected from Lake Mendota over the course of five years, as
480 previously described (Kara et al 2013, Shade et al 2007). Lake Mendota, Madison,
481 Wisconsin, (N 43°06, W 89°24) is one of the most well-studied lakes in the world, and is a
482 Long Term Ecological Research site affiliated with the Center for Limnology at the
483 University of Wisconsin Madison (Carpenter et al 2006). It is dimictic and eutrophic with an
484 average depth of 12.8 m, maximum depth of 25.3 m, and total surface area of 3938 ha. Depth
485 integrated water samples were collected from 0 to 12 m of the epilimnion (upper mixed layer)
486 at 94 different time points during ice-free periods from summer 2008 to summer 2012, and
487 filtered onto 0.2 μm pore-size polyethersulfone filters (Supor, Pall) prior to storage at -80°C.
488 DNA was later purified from these filters using the FastDNA kit (MP Biomedicals). DNA
489 sequencing was performed at the Department of Energy Joint Genome Institute using

490 standard protocols (Walnut Creek, CA, USA). DNA from the 94 samples was used to
491 generate libraries that were sequenced on the Illumina HiSeq 2000 platform. Paired-end
492 sequences of 2 X 150bp were generated for all libraries. Adapter sequences, low quality reads
493 (i.e. ≥80% of bases had quality scores <20), and reads dominated by short repeats of ≥3 bp
494 were removed. The remaining high quality reads were merged with the Fast Length
495 Adjustment of Short Reads (Magoc and Salzberg 2011) with a mismatch value of ≤0.25 and a
496 minimum of ten overlapping bases from paired sequences, resulting in merged read lengths of
497 150 to 290 bp (**Table S4**). Metagenomes were pooled by month to reduce the time-series data
498 to 30 observations and increase coverage. Original records can be found as a group in JGI's
499 Genome Portal: http://genome.jgi.doe.gov/Mendota_metaG.
500
501 All contigs from each of the 33 SAGs were used as a reference to recruit reads from
502 the Mendota metagenomes using blastn. Metagenome reads that recruited to the SAGs were
503 filtered and only alignments 200bp or longer were considered. An additional filter requiring
504 an alignment percent identity of at least 97.5% was applied when analyzing the metagenome
505 time series. Ribosomal RNAs were masked from the SAGs prior to performing the
506 recruitments. Relative abundance was calculated by normalizing the number of basepairs that
507 recruited to each SAG by the genome and pooled metagenome size and multiplying all by the
508 average pooled metagenome size. When appropriate to the research question, recruitment was
509 conducted "competitively", meaning that if a read recruited to more than one SAG it was
510 only counted for the best hit SAG. In this case, if a read recruited equally well to both SAGs,
511 it was counted for both. In some cases we applied an even stricter definition of "competitive"
512 and did not count any read that recruited equally well to more than one SAG. For **Figure 3**,
513 recruitment was conducted "non-competitively", meaning that reads could be counted for
514 multiple SAGs as long as the hits met the filtering criteria. We note that this a commonly
515 used approach developed by other researchers (Konstantinidis and Tiedje 2005,

Konstantinidis and DeLong 2008). The figure and table legends contain the information necessary to discern which kind of recruitment criteria were applied for that specific analysis.

Statistics, Visualization, Reproducible Methods

Datasets were analyzed and results were visualized using custom scripts written in R (R Core Team 2014) and python. Pipeline and scripts for analysis can be found at https://github.com/McMahonLab/blast2ani.

Supplementary information is available at the ISME Journal's website.

Conflict of interest Statement: The authors declare no conflict of interest.

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Author contribution

SLG, SLRS, RM, SB and KDM conceived the research. RM, MMG, TW and SGT conducted experiments and generated the data. SLG, SLRS and KDM analyzed the data.

SLG, SLRS and BC prepared the figures. SLG, SLRS, RM, SB and KDM wrote the manuscript. All authors participated in revision of the manuscript.

Additional Information

The raw shotgun metagenome reads and SAGs are publicly available in the JGI Genome Portal and via IMG/MER. The access number for each SAG and metagenome can be found in **Table 1** and **Table S4**.

Figure and table legends

Figure 1. A. Phylogenetic tree of acI SAGs based on conserved single copy genes selected by PhyloPhlAn. Amino acid sequences from 400 genes were aligned. The tree topology is consistent with 16S rRNA gene-based phylogenies (Ghylin et al 2014). SAGs L06 and A23 are part of the same sequence discrete population (SDP) as defined in the text and further based on data shown in Figure 2. B. Phylogenetic tree of LD12 SAGs based on conserved single copy genes selected by PhyloPhlAn, representing 400 genes. The tree topology was consistent with prior work that provided evidence for finer-scale groups within the LD12 tribe (Zaremba-Niedzwiedzka et al 2013). C. Genome-wide nucleotide identity (gANI) versus 16S rRNA gene identity for pairs of SAGs. Alignment fractions for homologous genomic regions and 16S rRNA genes are given in **Table S2**. Shapes indicate the lake the tribe is from, if same, otherwise different lake is indicated. Colors indicate the tribe a pair is from, if same, otherwise different tribe is indicated. The arrow denotes the L06-A23 pair.

Figure 2. Nucleotide identity density plots for SAG versus SAG genome-wide comparison using a sliding window. Results are shown for two reference SAGs representing the most complete genomes from the most thoroughly sampled tribes. All SAG pairs were from the same tribe. Nucleotide identity was calculated with blastn using 301 bp fragments that overlapped by 150 bp. A. acI-B1 SAGs and other selected acI SAGs vs L06. Note that the purple line (D18) is hidden underneath the orange (I18) and red (J17) lines. B. selected LD12 SAGs vs C06. Note the dark blue line (L09) is hidden under the light green (N17) line. Group designations match those shown in **Figure 1B**, as proposed previously (Zaremba-Niedzwiedzka et al 2013). An expanded multi-panel version of the same data is shown in **Figure S1**, for clarity.

780

781 **Figure 3.** Mapping metagenomic reads from Lake Mendota to SAGs and four genomes from
782 Lake Soyang (Kang et al 2017). The x-axis represents nucleotide identity of the recruited
783 reads. The metagenome sample was collected from Lake Mendota on 29 April 2009. Reads
784 were only counted if they aligned over a minimum of 200 bp. Recruitments were not
785 competitive, meaning that each read could recruit to multiple SAGs. Analogous competitive
786 recruitments that required each read to recruit to only one SAG are presented in **Figure S4**.
787 The noncompetitive recruitment showed the close relationship of the LD12 populations that
788 is not visible in the competitive recruitment. An expanded multi-panel version of the same
789 data is presented in **Figure S3** for clarity. Each panel represents a different sub-set of the
790 SAGs: A. acI from Mendota, B. acI not from Mendota, C. LD12 from Mendota (group
791 members demarcated in legend), D. LD12 not from Mendota (group members demarcated in
792 legend), E. other freshwater groups from Mendota, F. genomes from Lake Soyang, Korea.
793 Regarding the other freshwater groups from Mendota, since each of these SAGs represent
794 just one tribe, it is not appropriate to infer any general conclusions for these populations or
795 tribes, but we present them here to show the intriguing diversity of recruitment patterns. We
796 finally underscore the need to more deeply sample individual population members using
797 SAGs, to better capture and describe the range of variation in population heterogeneity.

798

799

800 **Figure 4.** Sequence-discrete population abundance in Lake Mendota over time, as measured
801 by the relative number of reads recruited to each SAG using blastn. All SAGs and samples
802 are from Lake Mendota. Timepoints are pooled by month. Filtering criteria: ≥97.5% ANI and
803 ≥200 bp alignment length. Recruitment was done using the most strict definition of
804 competitive described in the methods, meaning any read that matched equally well to more

than one SAG was not counted at all. Colors for each SAG are the same as in Figures 2 and 3. Relative abundance was calculated by normalizing the number of basepairs that recruited to each SAG by dividing by the genome size and the pooled metagenome size. The normalized number was then multiplied by the average pooled metagenome size. A. Relative abundance for each acI-B1 SAG. B. Relative abundance for each LD12 SAG. Membership in the groups defined in Figure 1B and by (Zaremba-Niedzwiedzka et al 2013) are denoted in the legend.

Figure 5. A. Metagenomic read recruitment using the SAGs from Lake Mendota. SAGs are in rows with bubbles representing all metagenomes from a particular month recruited against SAG. Filtering criteria: ≥97.5% ANI and ≥200 bp alignment length. Color scale indicates the ANI of the recruited metagenome reads. Bubble size represents the average coverage per base in the reference SAG divided by the size of the metagenome, multiplied by the average size of all metagenomes (1.34 Gigabases). Grey bubbles indicate that fewer than 200 reads recruited to the SAG in that month. Note that the resulting values do not represent a true measure of absolute abundance, but allow for quantitative comparison of month-to-month variation in population-level abundance. Recruitments were performed competitively, meaning that each read was counted for only one SAG, unless the read hit two SAGs equally well in which case it was counted for both SAGs. B. Variation in ANI for each SAG, across all 30 metagenomes from throughout the five years. Variation was not calculated for a SAG unless at least ten months recruited more than 200 hits each. The data underlying these plots can be found in Table S6.

830 **Tables**

831

832 Table 1. Metadata for the 33 SAGs and genomes from (Kang et al 2017). The Genome OID
833 is the object identifier for the genome record in the Joint Genome Institute's IMG/MER
834 Database. Estimated Genome Completeness was calculated using CheckM as described in the
835 main text and (Parks et al 2015).

836

Table 1

Genome name	Genome OID in IMG/MER	Phylum/Class	Tribe	Lake	Collection date (M/D/Y)	Assembly size (Mb)	Est. Genome Comp. (checkm)	Number of contigs	GC content (%)	Citation
AAA278-O22	2236661007	Actinobacteria	acI-A1	Damariscotta	09/18/09	1.14	74.4	43	47.6	Ghylin et al (2014)
AAA027-M14	2236661003	Actinobacteria	acI-A1	Mendota	12/5/09	0.82	43.1	22	47.3	Ghylin et al (2014)
IMCC25003	2602042019	Actinobacteria	acI-A1	Soyang	Jun-13	1.35	NA	1	49.1	Kang et al (2017)
IMCC26103	2602042020	Actinobacteria	acI-A4	Soyang	Apr-14	1.46	NA	1	47.0	Kang et al (2017)
AAA028-I14	2619618809	Actinobacteria	acI-A6	Mendota	12/5/09	0.78	39.66	54	45.2	This paper
AAA044-N04	2236661005	Actinobacteria	acI-A7	Damariscotta	04/28/09	1.29	79.59	23	45.6	Ghylin et al (2014)
AAA041-L13	2519899769	Actinobacteria	acI-A7	Damariscotta	04/28/09	1.38	74.14	103	44.2	This paper
AAA024-D14	2264265190	Actinobacteria	acI-A7	Sparkling	05/28/09	0.78	48.4	82	45.4	Ghylin et al (2014)
AAA023-J06	2236661001	Actinobacteria	acI-A7	Sparkling	05/28/09	0.70	34.48	98	45.1	Ghylin et al (2014)
IMCC19121	2602042021	Actinobacteria	acI-A7	Soyang	Oct-11	1.51	NA	1	45.5	Kang et al (2017)
AB141-P03	2236876028	Actinobacteria	acI-B1	Stechlin	05/25/10	0.66	45.98	66	40.8	Ghylin et al (2014)
AAA278-I18	2236661006	Actinobacteria	acI-B1	Damariscotta	09/18/09	0.94	63.73	54	41.4	Ghylin et al (2014)
AAA028-A23	2236661004	Actinobacteria	acI-B1	Mendota	12/5/09	0.83	57.56	64	41.5	Ghylin et al (2014)
AAA027-L06	2505679121	Actinobacteria	acI-B1	Mendota	12/5/09	1.16	76.59	75	41.7	Garcia et al (2013)
AAA027-J17	2236661002	Actinobacteria	acI-B1	Mendota	12/5/09	0.97	65.26	81	42.1	Ghylin et al (2014)
AAA023-D18	2236661009	Actinobacteria	acI-B1	Sparkling	05/28/09	0.75	44.22	67	39.6	Ghylin et al (2014)
AAA044-D11	2619618811	Actinobacteria	acI-B4	Damariscotta	04/28/09	1.15	66.18	30	44.2	This paper
IMCC26077	2606217181	Actinobacteria	acI-C1	Soyang	Apr-14	1.55	NA	1	51.3	Kang et al (2017)
AAA027-D23	2524023172	Actinobacteria	acSTL-A1	Mendota	12/5/09	0.94	44.01	18	48.0	This paper
AAA028-N15	2619618810	Actinobacteria	acTH1-A1	Mendota	12/5/09	0.83	45.98	19	38.0	This paper
AAA487-M09	2236347068	Alphaproteobacteria	LD12	Damariscotta	09/18/09	0.63	53.15	97	29.1	Zaremba-Niedzwiedzka et al (2013)
AAA280-P20	2236876029	Alphaproteobacteria	LD12	Damariscotta	09/18/09	0.72	65.06	65	29.6	Zaremba-Niedzwiedzka et al (2013)
AAA280-B11	2236876032	Alphaproteobacteria	LD12	Damariscotta	09/18/09	0.67	51.24	47	29.8	Zaremba-Niedzwiedzka et al (2013)
AAA028-D10	2236347069	Alphaproteobacteria	LD12	Mendota	12/5/09	0.93	81.64	57	29.6	Zaremba-Niedzwiedzka et al (2013)
AAA028-C07	2236661008	Alphaproteobacteria	LD12	Mendota	12/5/09	0.85	74.12	32	29.5	Zaremba-Niedzwiedzka et al (2013)
AAA027-L15	2236876031	Alphaproteobacteria	LD12	Mendota	12/5/09	0.72	68.68	56	29.4	Zaremba-Niedzwiedzka et al (2013)
AAA027-J10	2236876030	Alphaproteobacteria	LD12	Mendota	12/5/09	0.79	69.56	82	29.8	Zaremba-Niedzwiedzka et al (2013)
AAA027-C06	2264265094	Alphaproteobacteria	LD12	Mendota	12/5/09	0.78	82.29	90	29.6	Zaremba-Niedzwiedzka et al (2013)
AAA024-N17	2236876027	Alphaproteobacteria	LD12	Sparkling	05/28/09	0.33	30.19	45	30.1	Zaremba-Niedzwiedzka et al (2013)
AAA023-L09	2236661000	Alphaproteobacteria	LD12	Sparkling	05/28/09	0.77	68.1	76	29.4	Zaremba-Niedzwiedzka et al (2013)
AAA027-G08	2619618806	Bacteroidetes	bacI-A1	Mendota	12/5/09	1.32	59.36	36	35.5	This paper
AAA027-N21	2619618807	Bacteroidetes	Flavo-A2	Mendota	12/5/09	2.21	92.44	36	33.1	This paper
AAA027-K21	2619618803	Betaproteobacteria	betIII-A1	Mendota	12/5/09	1.38	42.1	21	51.5	This paper
AAA028-K02	2619618804	Betaproteobacteria	LD28	Mendota	12/5/09	0.56	34.48	8	37.5	This paper
AAA027-I06	2619618802	Betaproteobacteria	Lhab-A1	Mendota	12/5/09	1.52	39.38	79	50.9	This paper
AAA027-C02	2619618801	Betaproteobacteria	PnecC	Mendota	12/5/09	1.27	61.93	49	43.7	This paper
AAA027-I19	2619618805	Verrucomicrobia	Opiputaceae	Mendota	12/5/09	2.42	54.58	63	51.7	This paper

NA = Not applicable

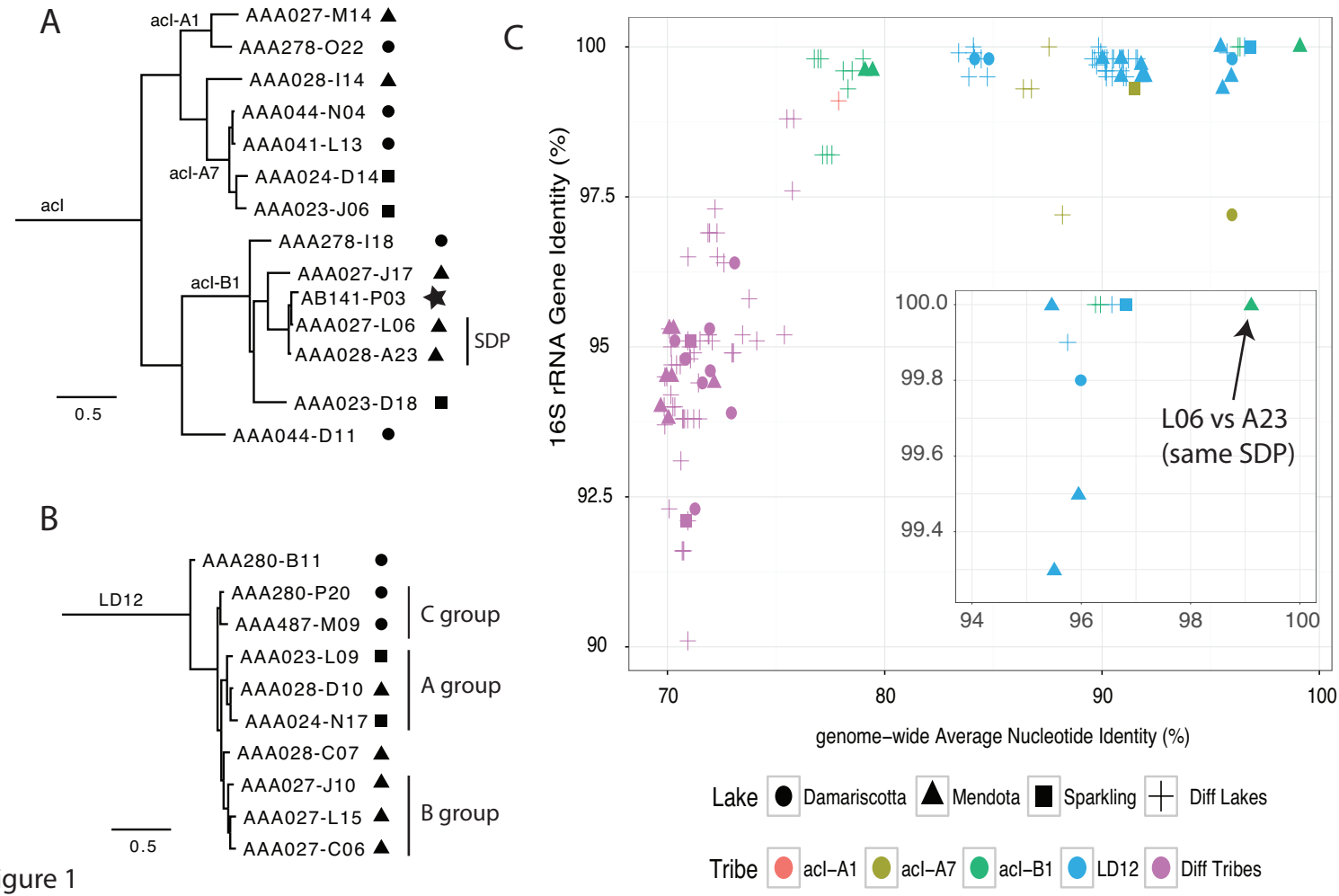

A
acl-A1
AAA027-M14
AAA278-O22
AAA028-I14
AAA044-N04
AAA041-L13
acl-A7
AAA024-D14
AAA023-J06
acl
AAA278-I18
acl-B1
AAA027-J17
AB141-P03
AAA027-L06
AAA028-A23
SDP
AAA023-D18
0.5
AAA044-D11
B
AAA280-B11
LD12
AAA280-P20
AAA487-M09
C group
AAA023-L09
AAA028-D10
AAA024-N17
A group
AAA028-C07
AAA027-J10
AAA027-L15
AAA027-C06
B group
0.5
C
16S rRNA Gene Identity (%)
100
97.5
95
92.5
90
70
80
90
100
genome–wide Average Nucleotide Identity (%)
100.0
99.8
99.6
99.4
94
96
98
100
L06 vs A23
(same SDP)
Lake
Damariscotta
Mendota
Sparkling
Diff Lakes
Tribe
acl–A1
acl–A7
acl–B1
LD12
Diff Tribes

Figure 1

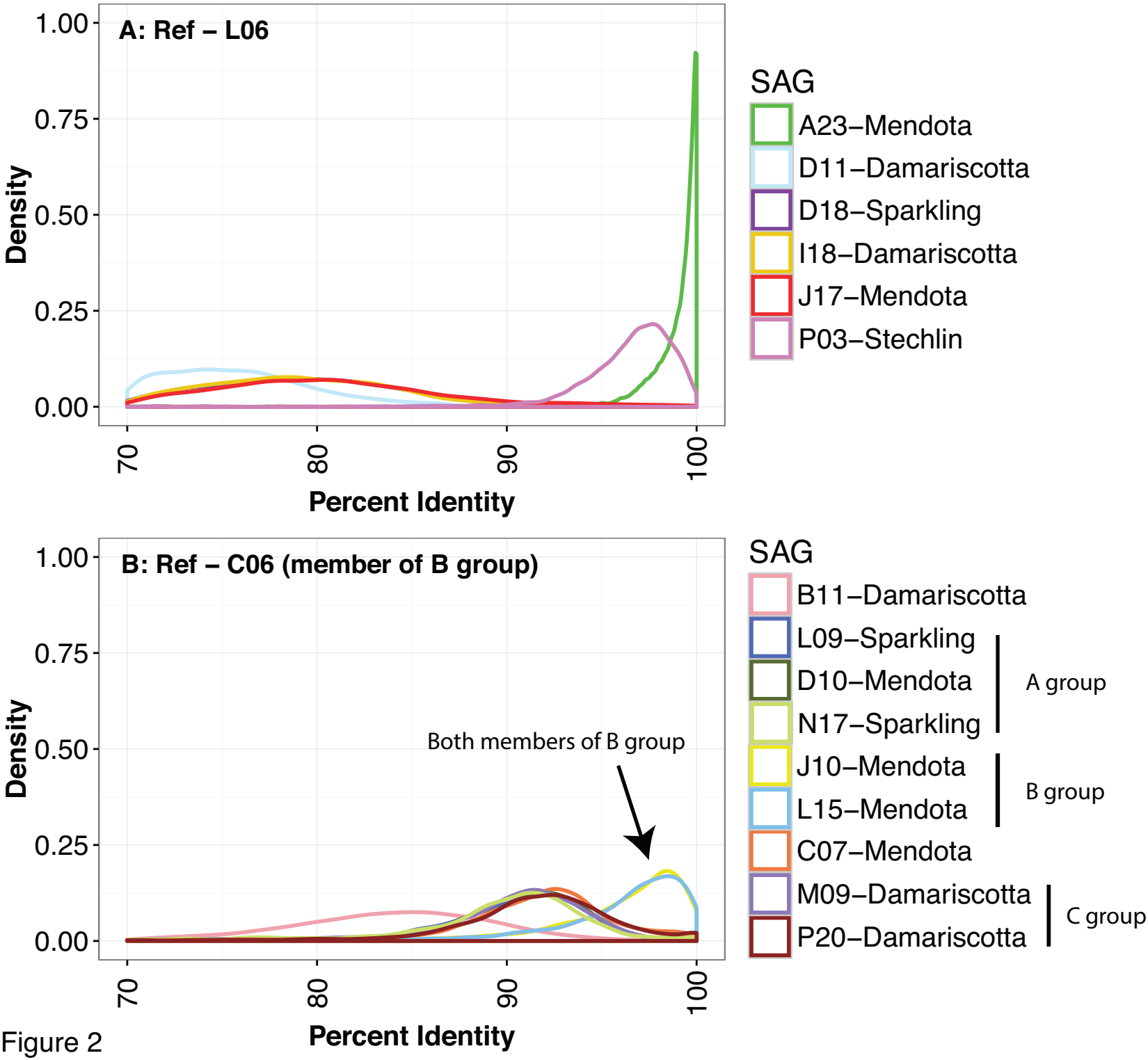

A: Ref – L06
Density
1.00
0.75
0.50
0.25
0.00
70
80
90
100
Percent Identity
SAG
A23–Mendota
D11–Damariscotta
D18–Sparkling
I18–Damariscotta
J17–Mendota
P03–Stechlin
B: Ref – C06 (member of B group)
Both members of B group
SAG
B11–Damariscotta
L09–Sparkling
D10–Mendota
N17–Sparkling
A group
J10–Mendota
L15–Mendota
B group
C07–Mendota
M09–Damariscotta
P20–Damariscotta
C group

Figure 2

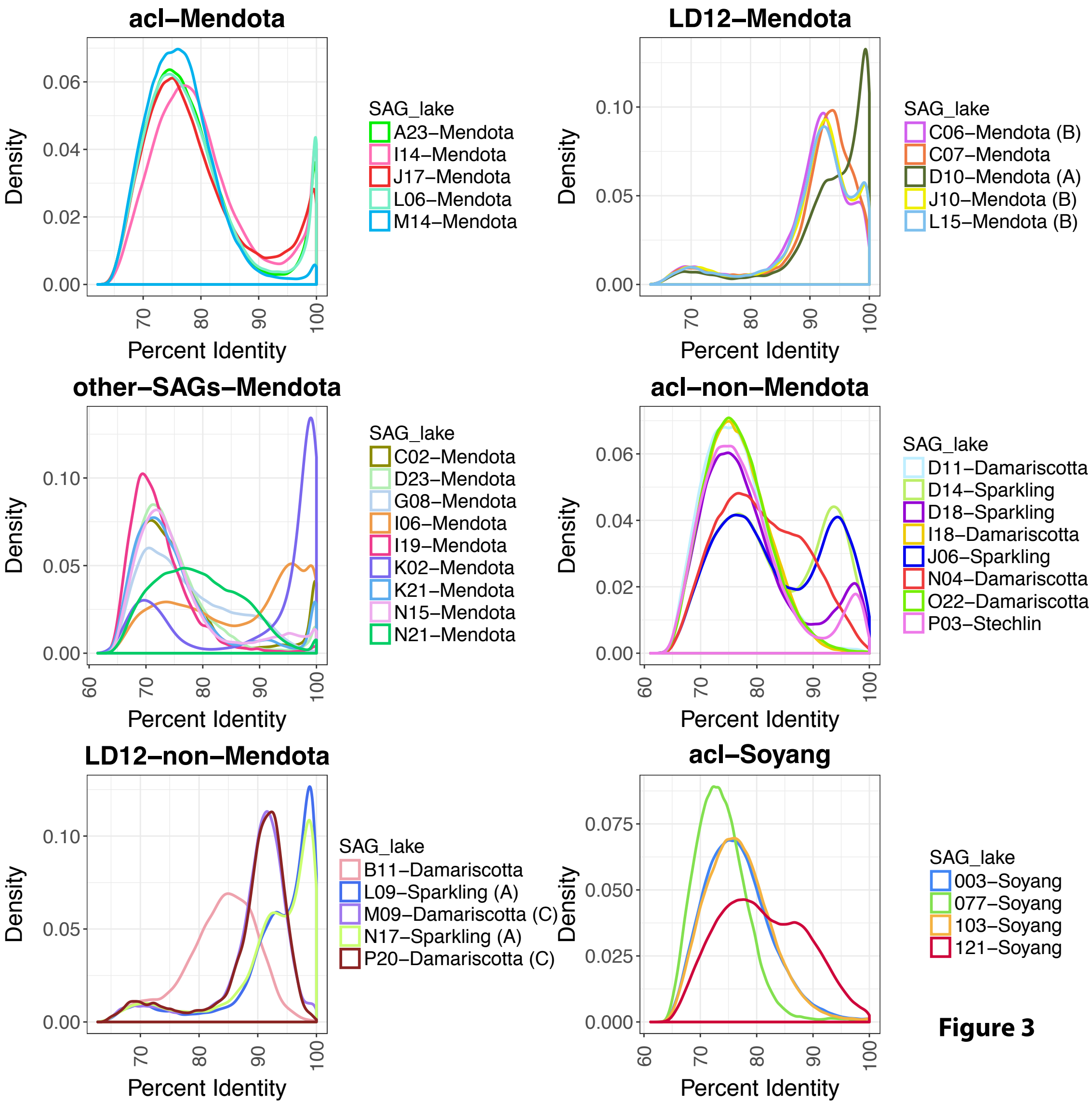
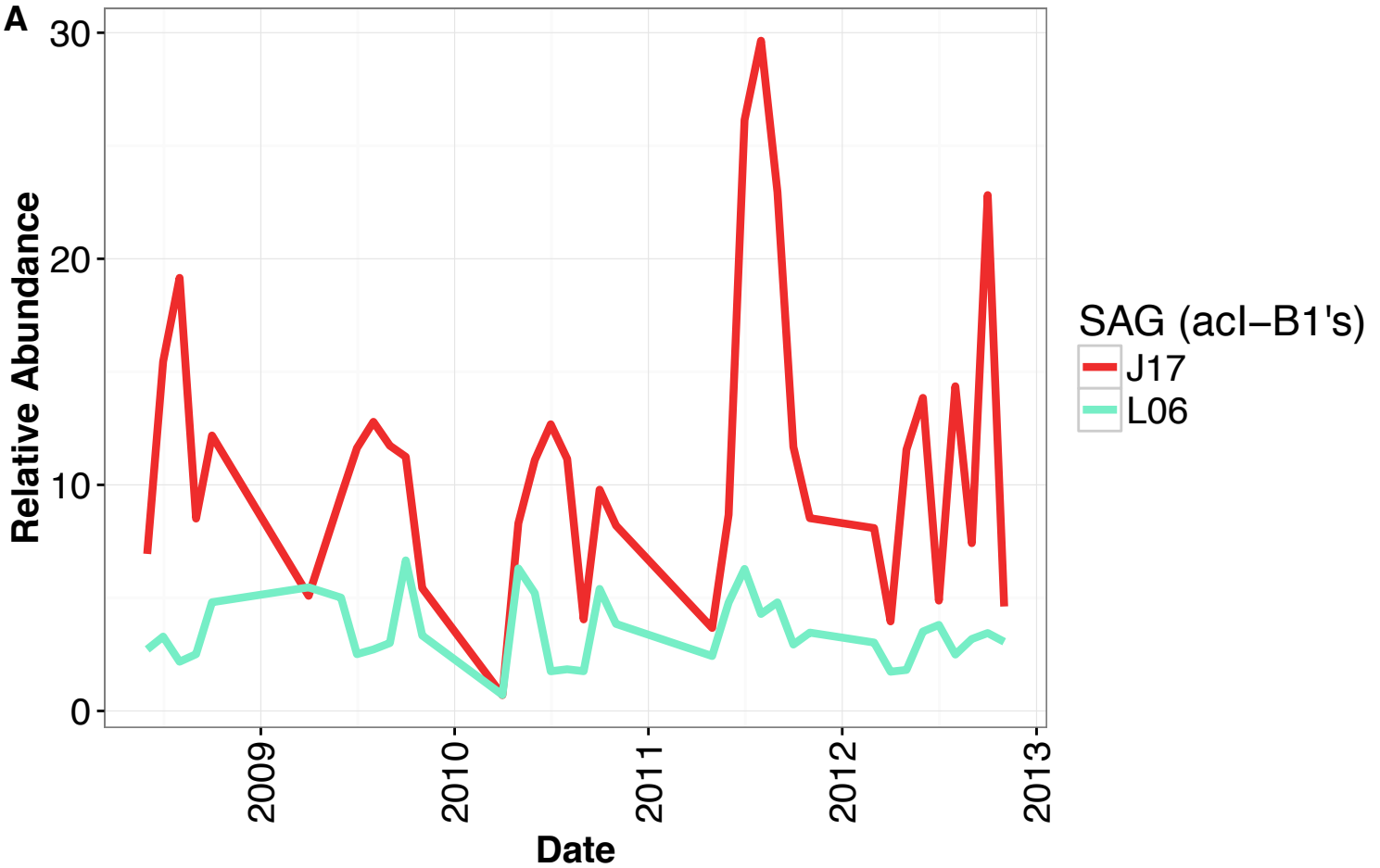
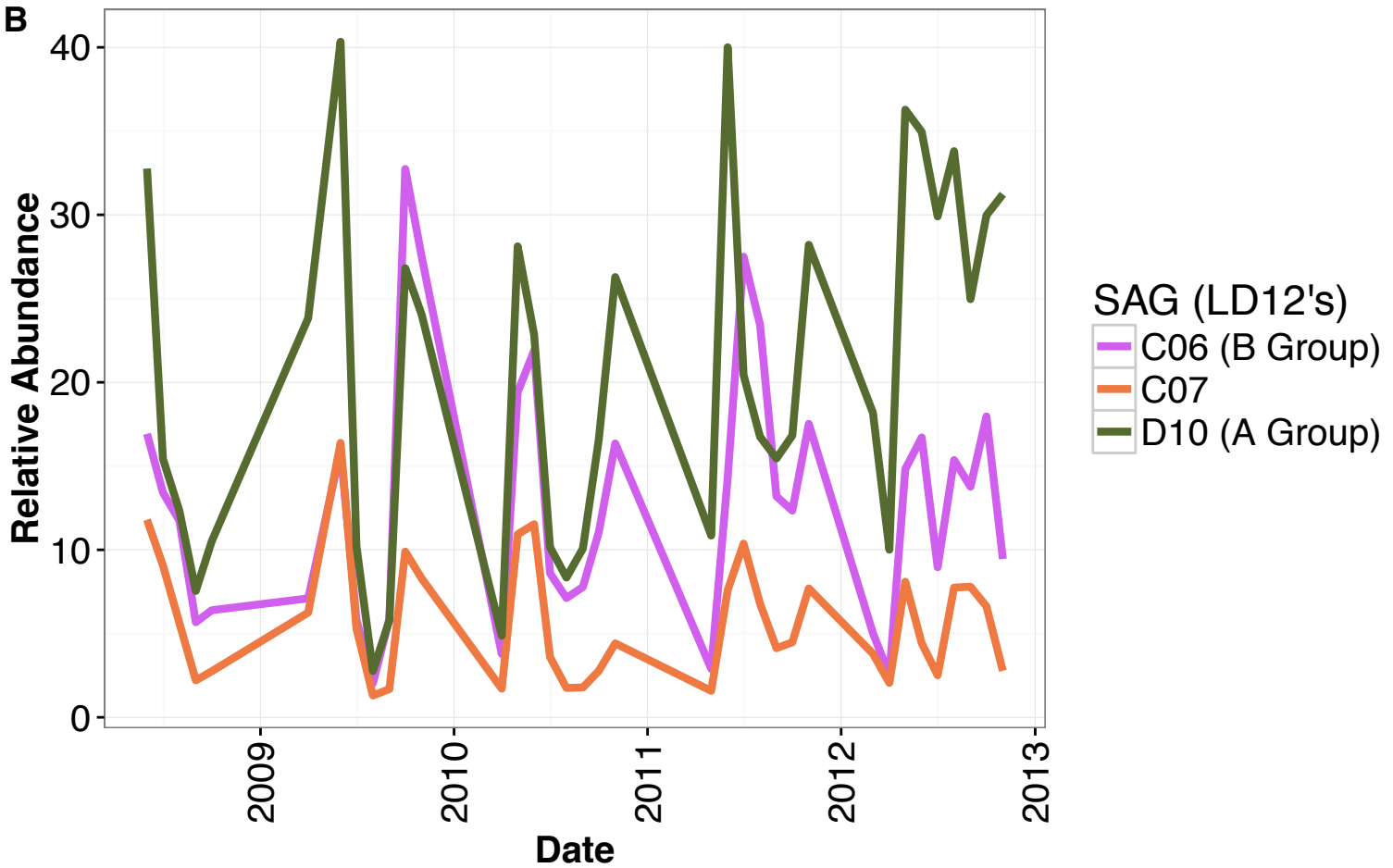

acl−Mendota
Density
0.06
0.04
0.02
0.00
70
80
90
100
Percent Identity
SAG_lake
A23−Mendota
I14−Mendota
J17−Mendota
L06−Mendota
M14−Mendota
LD12−Mendota
Density
0.10
0.05
0.00
70
80
90
100
Percent Identity
SAG_lake
C06−Mendota (B)
C07−Mendota
D10−Mendota (A)
J10−Mendota (B)
L15−Mendota (B)
other−SAGs−Mendota
Density
0.10
0.05
0.00
60
70
80
90
100
Percent Identity
SAG_lake
C02−Mendota
D23−Mendota
G08−Mendota
I06−Mendota
I19−Mendota
K02−Mendota
K21−Mendota
N15−Mendota
N21−Mendota
acl−non−Mendota
Density
0.06
0.04
0.02
0.00
60
70
80
90
100
Percent Identity
SAG_lake
D11−Damariscotta
D14−Sparkling
D18−Sparkling
I18−Damariscotta
J06−Sparkling
N04−Damariscotta
O22−Damariscotta
P03−Stechlin
LD12−non−Mendota
Density
0.10
0.05
0.00
70
80
90
100
Percent Identity
SAG_lake
B11−Damariscotta
L09−Sparkling (A)
M09−Damariscotta (C)
N17−Sparkling (A)
P20−Damariscotta (C)
acl−Soyang
Density
0.075
0.050
0.025
0.000
60
70
80
90
100
Percent Identity
SAG_lake
003−Soyang
077−Soyang
103−Soyang
121−Soyang

Figure 3


A
Relative Abundance
30
20
10
0
2009
2010
2011
2012
2013
Date
SAG (acI–B1's)
J17
L06


B
Relative Abundance
40
30
20
10
0
2009
2010
2011
2012
2013
Date
SAG (LD12's)
C06 (B Group)
C07
D10 (A Group)

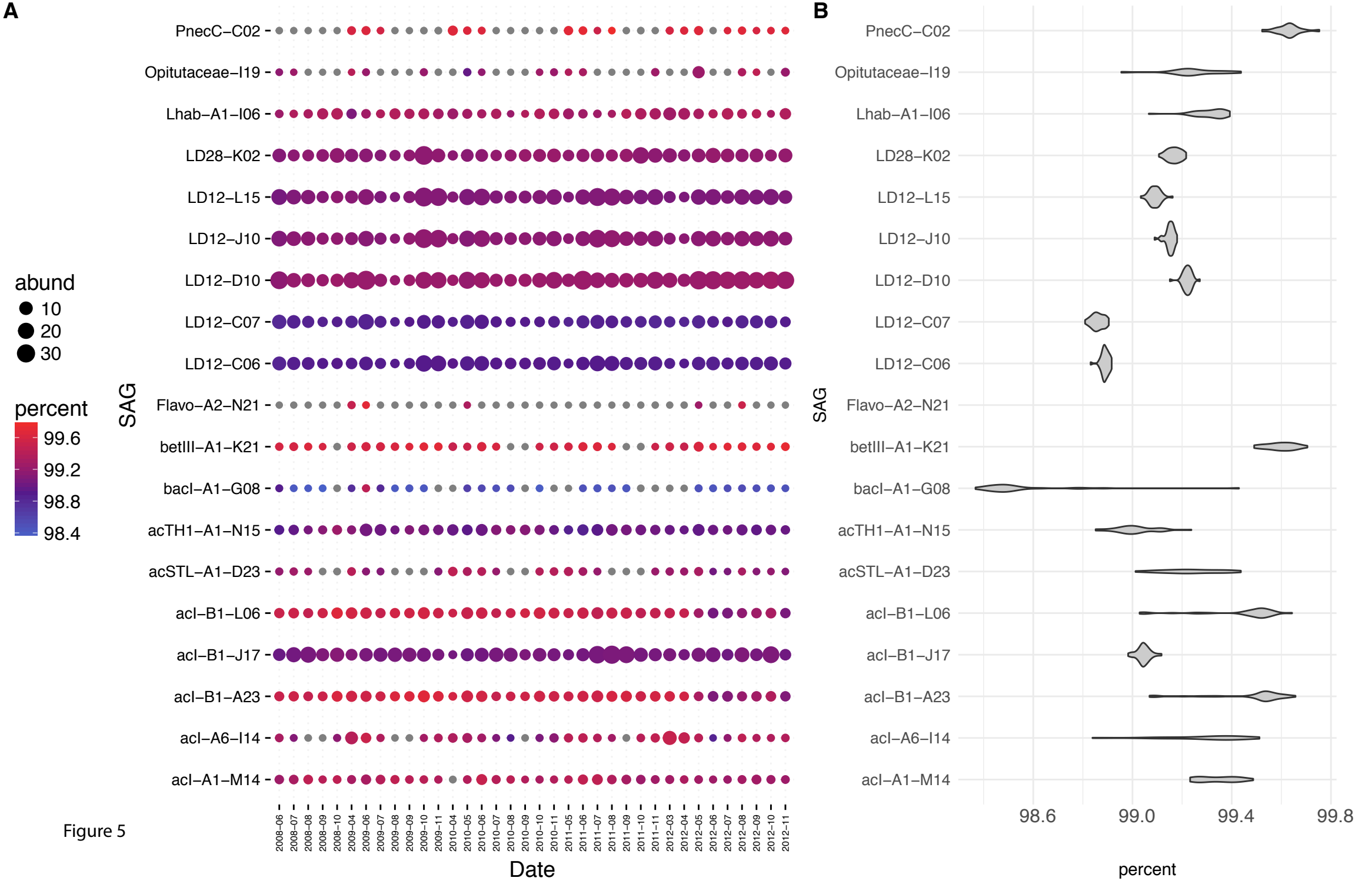

A
B
abund
10
20
30
percent
99.6
99.2
98.8
98.4
SAG
PnecC–C02
Opitutaceae–I19
Lhab–A1–I06
LD28–K02
LD12–L15
LD12–J10
LD12–D10
LD12–C07
LD12–C06
Flavo–A2–N21
betIII–A1–K21
bacI–A1–G08
acTH1–A1–N15
acSTL–A1–D23
acI–B1–L06
acI–B1–J17
acI–B1–A23
acI–A6–I14
acI–A1–M14
2008–06
2008–07
2008–08
2008–09
2008–10
2009–04
2009–06
2009–07
2009–08
2009–09
2009–10
2009–11
2010–04
2010–05
2010–06
2010–07
2010–08
2010–09
2010–10
2010–11
2011–05
2011–06
2011–07
2011–08
2011–09
2011–10
2011–11
2012–03
2012–04
2012–05
2012–06
2012–07
2012–08
2012–09
2012–10
2012–11
Date
98.6
99.0
99.4
99.8
percent

Figure 5