Latitude delineates patterns of biogeography in terrestrial *Streptomyces*

Mallory J Choudoir, James R Doroghazi, and Daniel H Buckley*

School of Integrative Plant Sciences, Cornell University, Ithaca, NY 14853 USA

*** Corresponding Author:** Daniel H Buckley
dbuckley@cornell.edu
School of Integrative Plant Science
Bradfield Hall 705, Cornell University, Ithaca, NY 14853
607-255-1716

Running Title: Biogeography of *Streptomyces*

Conflict of Interest: None

Summary

We examined the biogeography of *Streptomyces* at regional spatial scales to identify factors that govern patterns of microbial diversity within the genus. *Streptomyces* are spore forming filamentous bacteria which are widespread in soil and a major source of antibiotics. We applied population genetic approaches to analyze geographic and genetic population structure in six phylogroups of *Streptomyces* identified from a geographically explicit culture collection. *Streptomyces* strains were isolated from soils associated with perennial grass habitats sampled across a spatial scale of more than 6,000 km. We observed that *Streptomyces* population structure varies at regional spatial scales and that allelic diversity correlates with geographic distance. In addition, patterns of allelic diversity and gene flow are defined by latitude. Finally, we found that nucleotide diversity within phylogroups is negatively correlated with latitude. These results indicate that phylogroup diversification is constrained by dispersal limitation at regional spatial scales. We hypothesize that historical demographic processes have influenced the contemporary biogeography of *Streptomyces*.

Introduction

Patterns of microbial biogeography have been widely documented (Whitaker *et al*., 2003; Vos and Velicer, 2008; Bissett *et al*., 2010; Martiny *et al*., 2011; Gilbert *et al*., 2012; Hatosy *et al*., 2013), and yet we are only beginning to understand the evolutionary forces that generate and maintain these patterns. Explorations of biogeography are valuable because biogeographical patterns illustrate fundamental principles of evolution and ecology. Biogeographical patterns are ultimately governed by rates of dispersal and diversification (Martiny *et al*., 2006; Hanson *et al*., 2012). Since microbial dispersal cannot be observed directly, rates of dispersal are typically inferred from extant

patterns of genetic diversity. It has been hypothesized that microbes disperse ubiquitously due to their small cell size and massive population numbers (Finaly, 2002; Finlay and Fenchel, 2004). Yet endemism and dispersal limitation have been observed for a range of microbes (Cho and Tiedge, 2000; Green and Bohannan, 2006; Telford *et al*., 2006; Boucher *et al.*, 2011), and microbial dispersal limitation has been verified experimentally (Bell, 2010). Evidence in the literature pertaining to microbial dispersal limitation, which initially appears to be contradictory, can be explained by at least two factors: first, dispersal constraints are likely to vary with respect to different species and habitats; and second, methods used to define microbial diversity vary dramatically in their taxonomic and phylogenetic resolution. Each of these factors has been discussed previously (Hanson *et al*., 2012; Choudoir *et al*., 2012), and we will only consider them briefly here.

Patterns of microbial dispersal and gene flow appear to differ between habitats and species. At one end of the spectrum, globally widespread microbes such as *Prochlorococcus* and *Pelagibacter* show little variation in gene content between the Atlantic and Pacific Oceans suggesting that dispersal can homogenize genetic diversity in pelagic systems (Coleman and Chisholm, 2010). At the other end of the spectrum are extremophiles such as *Sulfolobus* and thermophilic *Synechococcus*, which live in island-like volcanic habitats and exhibit strong patterns of allopatric divergence resulting from dispersal limitation (Papke *et al*., 2003; Whitaker *et al*., 2003; Cadillo-Quiroz *et al.*, 2012). Terrestrial microbes fall somewhere between these extremes. For example, soil dwelling microbes such as *Burkholderia pseudomallei*, *Burkholderia mallei*, and *Bacillus anthracis* exhibit biogeographical patterns governed by dispersal limitation at regional spatial scales (Kenefic *et al*., 2009; Pearson *et al*., 2009).

47

48 The phylogenetic resolution at which microbial diversity is defined can have a profound impact
49 on our ability to discern patterns of microbial biogeography (as reviewed Hanson *et al.*, 2012).
50 Surveys of SSU rRNA genes in terrestrial habitats indicate that environmental variables including
51 temperature (Fierer *et al*., 2009; Miller *et al*., 2009), pH (Fierer and Jackson, 2006; Lauber *et al*.,
52 2009; Rousk *et al*., 2010), and salinity (Lozupone and Knight, 2007) are more important than
53 geographic distance or latitude in determining spatial patterns of microbial diversity. However,
54 SSU rRNA gene sequences have an extremely low rate of nucleotide substitution (Ochman *et al*.,
55 1999), and microbes with similar or even identical SSU rRNA genes can have extensive genomic
56 and ecological diversity (Welch *et al*., 2002; Jaspers and Overmann; 2004). Thus, this marker has
57 low sensitivity for detecting neutral processes that drive patterns of biogeography, such as dispersal
58 limitation and genetic drift (Green and Bohannan, 2006; Hanson *et al*., 2012). However, these
59 neutral processes are readily explored using geographically and ecologically explicit culture
60 collections characterized at high genetic resolution.

61

62 *Streptomyces* are ubiquitous across soil habitats, and many species are easily cultured, making this
63 genus an excellent candidate for making a taxon-specific survey of biogeography. Furthermore,
64 *Streptomyces* species have high rates of gene exchange both within and between species
65 (Doroghazi and Buckley, 2010). Hence, this genus is an ideal model to explore dispersal limitations
66 and gene flow in terrestrial systems. *Streptomyces* are gram-positive *Actinobacteria* (Kâmpfer,
67 2006) known for their complex developmental cycle, which entails filamentous growth and the
68 formation of desiccation resistant spores which are readily dispersed (Keiser *et al.*, 2000).
69 *Streptomyces* play a significant role in the terrestrial carbon cycle (McCarthy and Williams, 1992;

Takasuka *et al.*, 2013), represent important agricultural pathogens (Loria *et al.*, 2006; Labeda, 2011), and are prolific producers of antibiotics (Watve *et al.*, 2001). Despite their importance, we lack an evolutionary framework to understand *Streptomyces* biogeography.

Streptomyces diversity varies spatially, though the influence of geographic distance and ecological variation remains poorly resolved. Ecological adaptation constrains the environmental distribution of *Streptomyces* and their genetic and phenotypic diversity can vary in relation to soil characteristics at small spatial scale (1 m – 60 m) in prairie soils (Davelos *et al.*, 2004a and 2004b), and in dune habitats (Antony-Babu *et al.*, 2008). There is also evidence for dispersal limitation at very large (continental) spatial scales with endemic species observed in North America and Central Asia (Wawrik *et al.*, 2007). Remarkably, genetic analysis of *Streptomyces pratensis* has revealed that strains of this species are in linkage equilibrium across a range that spans 1,000 km (Doroghazi and Buckley, 2010 and 2014). One interpretation of this finding is that *Streptomyces* are subject to widespread dispersal and unlimited gene flow at regional spatial scales. However, linkage equilibrium can also be observed for dispersal limited species that have undergone a recent demographic range expansion (Doroghazi and Buckley, 2010 and 2014). Demographic range expansion has previously been implicated as a factor that can explain ancestral patterns of gene exchange in *Streptomyces* clades (Andam *et al*., 2016a). Combined, these data suggest a role for both adaptive and neutral processes in governing the diversity and biogeography of *Streptomyces*, but the evolutionary interpretation of these data depends on the degree to which dispersal limitation drives patterns of diversity in *Streptomyces*.

92 We evaluated *Streptomyces* biogeography to explore the ecological and evolutionary forces that
93 govern diversification within this genus. The most powerful approach for detecting evolutionary
94 patterns that result from dispersal limitation is to examine taxon-specific patterns of biogeography
95 across ecologically similar sites. This approach controls for the effects of selection and provides a
96 powerful test of neutral evolutionary processes (Hanson *et al.*, 2012). We constructed a taxon-
97 specific isolate collection of *Streptomyces* found in ecologically similar grassland sites spanning
98 the United States of America. In a study of *Streptomyces* species-level diversity, which evaluated
99 polymorphism at a single locus (*rpoB*), we observed evidence for dispersal limitation manifesting
100 in a latitudinal diversity gradient (Andam *et al.,* 2016b). The latitudinal diversity gradient is one
101 of the earliest and most well documented patterns of biogeography (Wallace, 1878; Hillebrand,
102 2004), but it is essentially undocumented in terrestrial bacterial systems. The hypothesis of a
103 latitudinal gradient in species diversity makes the prediction that latitudinal patterns of gene flow
104 and genetic diversity should also vary *within* populations. Here we evaluate the hypothesis of a
105 latitudinal diversity gradient in terrestrial *Streptomyces* by employing multilocus sequence analysis
106 (MLSA) to characterize population-level patterns of gene flow and genetic diversity for six
107 phylogroups of *Streptomyces* which are distributed widely across the United States.
108

109 **Results**

110 *Characterization of Streptomyces phylogroups*

111 We identified a total of 308 isolates representing the six targeted phylogroups, and these isolates
112 spanned 13 sites (Table 1). Strains within phylogroups share 99.4-100% SSU rRNA gene sequence
113 identity, and all strains share greater than 97% nucleotide identity in their SSU rRNA gene
114 sequences (Figure S1). Strains within phylogroups also share 97.6%-99.5% average nucleotide

115 identity (ANI) across concatenated MLSA loci, and all strains share greater than 88% ANI (Figure
116 1, Table 2). Phylogroup genetic ancestry is congruent with boundaries defined by nucleotide
117 cutoffs (Figure S2), and this indicates that these phylogroups approximate biological populations.
118 We observed 122 unique MLSA haplotypes, with each phylogroup represented by 13-26
119 haplotypes (Figure 1, Table 2). Good's coverage for each of the six phylogroups ranges from 0.88-
120 1.0 for individual loci and 0.94-1.0 for concatenated MLSA loci clustered at 99% nucleotide
121 identity (Table S1). Hence, allelic diversity and nucleotide diversity are well sampled (Table S1),
122 though unique haplotypes remain under sampled (Figure S3). The per site nucleotide diversity (π)
123 of each phylogroup ranges from 0.0026 to 0.011 (Table 2).
124
125 Four of the phylogroups do not match any *Streptomyces* species described in PubMLST (less than
126 97% ANI across MLSA loci, Figure S4A) (Jolley *et al*., 2004). While the other two, phylogroups
127 WA1063 and MS152 belong to the *S. griseus* species cluster (Rong and Huang, 2010). Isolates in
128 phylogroup WA1063 share greater than 99% MLSA identity with *S. anulatus* and *S. praecox* and
129 form a monophyletic clade with these type strains (Figure S4A). Isolates in phylogroup MS152
130 share greater than 98% MLSA identity with *S. mediolani*, *S. albovinaceus*, and *S. griseinus* and
131 form a paraphyletic clade that includes these type strains (Figure S4A).
132
133 We find evidence of horizontal gene transfer consistent with previous observations of *Streptomyces*
134 species (Doroghazi and Buckley, 2010; Andam *et al.*, 2016a). There is significant phylogenetic
135 incongruence between MLSA loci (Figure S4, Table S2), suggesting that horizontal gene transfer
136 has shaped the phylogeny of these groups. The six phylogroups exhibit evidence of population
137 structure and admixture, with each phylogroup composed of 3.2 ± 0.8 (mean ± s.d.) subpopulations

138 (Figure 1). Evidence of admixture suggests horizontal gene transfer within phylogroups and is
139 consistent with previous evidence of gene exchange in *Streptomyces* (Doroghazi and Buckley,
140 2010). Evidence of recombination within phylogroups MAN196, MS200, MS152, and F34 (PHI
141 test, $p < 0.05$, Table 2) further supports the conclusion of gene exchange within populations. In
142 addition, phylogroups WA1063 and MS152 share two identical *atpD* alleles (Figure S4B), but it is
143 not clear whether these alleles are shared as a result of contemporary horizontal gene transfer or
144 vertical inheritance from the most recent common ancestor of the two clades. The latter
145 explanation is more parsimonious given the low level of polymorphism between the phylogroups
146 WA1063 and MS152.
147
148 *Evidence for dispersal limitation*
149 Strains of the six phylogroups were obtained from soil samples from 13 sites of diverse geographic
150 origin (Table 1). Each phylogroup was detected in 4.2 ± 0.4 sites, and this distribution differs
151 significantly from expectations for a random distribution of strains across sites (permutation test,
152 $p < 0.0005$), thereby rejecting the hypothesis of panmixia. Each phylogroup subpopulation was
153 observed in 2.2 ± 0.9 sites (mean ± s.d.), and this value is lower than expected if subpopulations
154 are randomly distributed across the sites occupied by each phylogroup (permutation test, $p <$
155 0.001). These results indicate that phylogroup distribution is constrained geographically and that
156 phylogroups have subpopulation structure that is also geographically explicit.
157
158 The geographic distribution of *Streptomyces* allelic diversity indicates dispersal limitation.
159 Identical alleles are shared among phylogroup members across each phylogroup's geographic
160 range, which can exceed 5,000 km (Figure 2, Table 1). However, dissimilarity in allele composition

161 increases with geographic distance, and this result is significant (Bray-Curtis dissimilarity, Mantel
162 r = 0.29, p = 0.005) (Figure S5). Hence, alleles are more likely shared between geographically
163 similar sites indicating dispersal limitation with potential for long range dispersal. This result is
164 significant for all individual loci except *recA* (Bray-Curtis dissimilarity, *aptD* Mantel r = 0.31, p =
165 0.004; *gyrB* r = 0.22, p = 0.031; *recA* r = 0.16, p = 0.088; *rpoB* r = 0.27, p = 0.004; *trpB* r = 0.19,
166 p = 0.047). In addition, all MLSA haplotypes (Figure 1, Figure 3) are unique to a single site, with
167 the sole exception being a haplotype from phylogroup MS200 which is observed in both MS and
168 WI (Sun Prairie).
169
170 Analysis of haplotype distribution is consistent with diversification due to dispersal limitation.
171 Nested clade analysis (NCA) establishes significant phylogeographic inferences for phylogroups
172 MAN196, MAN125, WA1063, and MS152 (Figure 3) but not for MS200 and F34. Nested clade
173 phylogeographic inference postulates potential evolutionary and historical demographic processes
174 that support extant patterns of diversity and biogeography. For instance, population subdivision of
175 MAN125 across the Pacific Northwest (Figure 3A) and MAN196 between Maine and the Pacific
176 Northwest (Figure 3B) is consistent with restricted gene flow due to historical long distance
177 dispersal events. Likewise, population subdivision of MS152 and between the Southeast (MS and
178 FL) and CA (Figure 3C) and WA1063 between WI (Brookfield) and OR (Figure 3D) is consistent
179 with allopatric fragmentation.
180
181 *Latitudinal diversity gradient*
182 The distribution and diversity of the phylogroups reveals a latitudinal diversity gradient. Strains
183 from MAN125, MAN196, WA1063 occur mostly north of 40°N latitude, while strains from

184 MS200, MS152, F34 occur mostly south of this latitude (Table 1, Figure 2). This pattern of
185 North/South partitioning is significant for each phylogroup when evaluated against the expectation
186 of a random distribution across sites (permutation test, $p < 0.01$ for each phylogroup after
187 Bonferroni correction). Furthermore, partial Mantel tests were performed to evaluate the latitudinal
188 and longitudinal vector components of geographic distance in relation to the allele composition of
189 sites. There remains a significant relationship between allele composition and geographic distance
190 when we control for longitude, (Mantel r = 0.23, $p = 0.022$), but this relationship is no longer
191 significant when we control for latitude (Mantel r = 0.15, $p = 0.12$). This result indicates that allele
192 composition changes more across latitude than it does across longitude. The latitudinal partitioning
193 of alleles can be readily observed in the pattern of allele sharing between sites (Figure 2). Finally,
194 we also observed a significant relationship between per site nucleotide diversity of phylogroup
195 MLSA loci and the average latitude of sites in which they are found (R = -0.91, $p = 0.012$; Figure
196 4). This result indicates phylogroups recovered from lower latitudes have higher genetic diversity
197 than those recovered from higher latitudes.
198

199 **Discussion**

200 We used population genetic approaches to analyze spatial patterns of genetic diversity for six
201 *Streptomyces* phylogroups isolated from geographically disparate but ecologically similar sites
202 across the United States (Table 1). The distribution of phylogroups is nonrandom and dispersal
203 limited (Figure S5) with phylogroups inhabiting geographic ranges defined by latitude (Table 1,
204 Figure 2). In addition, the genetic diversity of phylogroups is inversely proportional to the latitude
205 from which they were isolated (Figure 4). These findings suggest that there are latitudinal barriers

206 to dispersal, and that patterns of *Streptomyces* biogeography result from dispersal limitation and
207 regional diversification due to genetic drift.
208
209 The phylogroups we describe are coherent phylogenetic groups that approximate biological
210 populations (Figure S2). The members of each phylogroup share a distinguishable geographic
211 range (Table 1, Figure 2), a recent common ancestor (Figure 1), and greater than 97% ANI across
212 MLSA loci (Table 2). Despite geographic and genetic subpopulation structure (Figure 1),
213 phylogroup members frequently share identical alleles across demes. It is worth noting that since
214 all of the strains examined share > 97% SSU rRNA gene identity, these geographic patterns would
215 not be detected through analysis of rRNA genes (Figure S1). We infer the presence of
216 recombination within all phylogroups using both nucleotide polymorphism and phylogenetic
217 methods (Table 2, Table S2).
218
219 Regional patterns of biogeography among phylogroups are consistent with limitations to dispersal
220 and gene flow. Allopatric processes like genetic drift can drive diversification between populations
221 that are geographically isolated. The geographic distribution of our phylogroups is nonrandom,
222 and we find regional subpopulation structure within phylogroups (Figure 1, Figure 3). Although
223 we find identical alleles in sites thousands of kilometers apart (Figure 2, Figure S4), MLSA
224 haplotypes are not shared across sites (with the single exception of a haplotype shared between
225 MS and WI) (Figure 3). We also observe a significant distance decay relationship for MLSA allele
226 composition and geographic distance between sites (Figure S5). This data implies that while gene
227 flow is moderate across the geographic range of a phylogroup, dispersal limitation and genetic
228 drift create appreciable regional population structure.

229

230 *Streptomyces* phylogroup diversity is consistent with a latitudinal diversity gradient. We find that
231 latitude is a significant predictor of gene flow (Figure 2). Furthermore, intra-phylogroup nucleotide
232 diversity has a significant negative relationship with average latitude (Figure 4), which is
233 congruous with the latitudinal diversity gradient observed for diverse macroorganisms (Hillebrand,
234 2004). There is conflicting evidence for latitudinal diversity gradients among microorganisms.
235 Evidence for microbial latitudinal diversity gradients comes from marine systems (Fuhrman *et al*.,
236 2008; Sul *et al*., 2013; Swan *et al*., 2013), with contrary evidence obtained in terrestrial systems
237 (Neufeld and Mohn, 2005; Chu *et al*., 2010). However, most analyses of terrestrial bacterial
238 biogeography are derived from analyses of SSU rRNA genes, and we show that analyses of SSU
239 rRNA genes lack the sensitivity needed to detect the biogeographic patterns that we observe for
240 *Streptomyces* (Figure S1).

241

242 Several hypotheses have been advanced to explain the formation of latitudinal diversity gradients
243 (Wiens and Donoghue, 2004; Mittelbach *et al*., 2007). Ecological hypotheses posit that factors
244 such as carrying capacity, productivity, and niche availability vary across latitude and that these
245 factors impose constraints on biodiversity (Currie *et al.*, 2004; Mouchet *et al.*, 2015). Evolutionary
246 hypotheses invoke the positive relationship between temperature and the kinetics of metabolism
247 to predict that evolutionary rates and cladogenesis correspond with temperature (Allen *et al*.,
248 2002). Historical hypotheses propose that the latitudinal diversity gradient is the product of
249 historical geological, ecological, or demographic events that have influenced dispersal and
250 diversification (Wiens and Donoghue, 2004; Stevens, 2006). For example, the influence of
251 Pleistocene glacial events on the biogeography of diverse species of terrestrial and aquatic plants

252 and animals is well documented (Soltis *et al*., 1997; Bernatch and Wilson, 1998; Conroy and Cook,
253 2000; Milá *et al*., 2006; Maggs *et al*., 2008; Wilson and Veraguth, 2010). There is also evidence
254 that Pleistocene glaciation events have impacted both microbial communities (Eisenlord *et al*.,
255 2012) and populations (Kenefic *et al.*, 2009; Mikheyev *et al*., 2008).
256
257 The biogeography of our *Streptomyces* phylogroups is consistent with historical demography and
258 dispersal limitation resulting in a contemporary latitudinal diversity gradient. For instance,
259 MAN125 is nearly in linkage equilibrium ($I_A = 0.09$) though its members span a geographic range
260 of over 2,000 km across the Pacific Northwest. Similar patterns of recombination have also been
261 observed in a *S. pratensis* population which spanned 1,000 km across sites present in North
262 Carolina and northern New York (Doroghazi and Buckley, 2010 and 2014). The observation of
263 linkage equilibrium indicates that either the population lacks contemporary barriers to gene flow
264 or the population has experienced a recent demographic expansion. Coupled with evidence of
265 limitations to gene flow at regional scales (Figure 2, Figure S5), the most parsimonious explanation
266 for these conflicting observations is a recent demographic range expansion. Conversely, latitudinal
267 gradients in marine bacterial diversity have been attributed to the increased kinetics of metabolism
268 which increase the evolutionary tempo in tropical latitudes (Fuhrman *et al*., 2008). Since latitude
269 is correlated with temperature, we cannot dismiss the influence of kinetics as a cause of the
270 latitudinal diversity gradient in terrestrial *Streptomyces*. Furthermore, it is possible that
271 unappreciated ecological variables, such as the species composition of perennial grass
272 communities, could shape the diversity gradient. Ultimately, it is likely that latitudinal diversity
273 gradients can arise from a combination of ecological, evolutionary, and historical processes that
274 vary in their relative influence with respect to different species and different habitats.

275

276 Evidence for a latitudinal diversity gradient coupled with evidence of demographic range
277 expansion, while not conclusive, is consistent with predictions of phylogenetic niche conservatism
278 (Wiens and Donoghue, 2004; Stevens, 2006) which posits that these biogeographical patterns
279 result from the historical effects of changes in paleoclimate (Hewitt *et al*., 1996, 2000, and 2004).
280 The genetic consequences of glaciation events are described in depth by Hewitt (1996, 2000, and
281 2004), and the population structure we observe in our *Streptomyces* phylogroups is consistent with
282 post-glacial demographic range expansion. One of the expectations of post-glacial expansion is
283 "southern richness versus northern purity" (Hewitt, 2004). This is evident in the negative
284 correlation we observe between latitude and the nucleotide diversity of phylogroups (Figure 4).
285 Williams *et al*. (1998) justifies 40˚N latitude as approximating late Pleistocene glacial and non-
286 glacial regions with respect to species distributions in North America. Hence, the latitudinal
287 delineation of allele distributions for *Streptomyces* phylogroups roughly corresponds to the extent
288 of ice coverage during the late Pleistocene (Figure 2), which suggests historical population
289 expansion from lower to higher latitudes.

290

291 Haplotype distributions of phylogroups MAN125, MAN196, WA1063, and MS152 are consistent
292 with allopatric diversification resulting from dispersal limitation (Figure 3). Haplotype nested
293 clade analysis (NCA) predicts that historical dispersal events and range expansion across northern
294 regions resulted in limits on dispersal during intermediate timescales, allowing genetic drift to
295 create the phylogroup population structures observed today. There is moderate criticism of NCA
296 (Knowles and Maddison, 2002; Nielsen and Beumont, 2009) due to the subjective nature of
297 inferring historical processes and the wide potential for stochastic processes creating similar

298 patterns of biogeography. Yet these tools can provide useful hypotheses. Northern phylogroups
299 MAN125 and MAN196 share a common ancestor with southern phylogroups MS200 and F34
300 (Figure 1). The contemporary population structure of MAN125 and MAN196 is consistent with a
301 historical range expansion from a common ancestor shared by both clades (Figure 1, Figure 3).
302 Further population structure within each phylogroup likely resulted from barriers to gene flow and
303 historical dispersal events across the Pacific Northwest for MAN125 (Figure 3A) and between
304 Maine and the Pacific Northwest for MAN196 (Figure 3B). Analysis of haplotype distribution
305 within WA1063 and MS152 is also consistent with diversification of populations as a result of
306 gene flow limitation between the Midwest and Pacific Northwest for WA1063 (Figure 3D) and
307 between the Southeast and West for MS152 (Figure 3C).
308
309 Phylogroups WA1063 and MS152 share a recent common ancestor (Figure 1, Figure S2) and also
310 share two identical alleles at the *atpD* locus (Figure S4B). These phylogroups have distinct, non-
311 overlapping geographic ranges with WA1063 found in higher latitudes and MS152 in lower
312 latitudes (Table 1, Figure 2). WA1063 and MS152 have 0.0205 net nucleotide substitutions per
313 site across concatenated MLSA loci. We evaluate the possible time range for divergence between
314 WA1063 and MS152 by extrapolating very roughly from the nucleotide substitution rate (μ =
315 4.5×10^{-9}) and generation time (100-300 generations per year) for *E. coli* (Ochman *et al*., 1999),
316 since corresponding values are not available for *Streptomyces* or their relatives. Based upon these
317 gross approximations, we would estimate that WA1063 and MS152 diverged 15,000-50,000 years
318 ago, corresponding to events in the late Pleistocene (Clayton *et al*., 2006). Hence, it is likely that
319 the identical *atpD* alleles found in both WA1063 and MS152 were inherited from a shared
320 ancestral population (Figure S3B) as opposed to inheritance from contemporary gene exchange.

321

322 Through population genetic analysis of six *Streptomyces* phylogroups, we find evidence for
323 dispersal limitation associated with geographically explicit patterns of gene flow which manifest
324 in a latitudinal gradient of nucleotide diversity. Furthermore, these data support the hypothesis that
325 historical demographic processes influence the contemporary biogeography of *Streptomyces*. Due
326 to their spore forming capabilities and potential for long range dispersal, *Streptomyces* are an ideal
327 system for assessing limits on gene flow among terrestrial bacteria. Future research should seek to
328 determine the degree to which dispersal limitation is due to limits on spore mobility, density
329 dependent blocking, or a result of adaptive constraints. A better understanding of *Streptomyces*
330 biogeography and the evolutionary forces that govern *Streptomyces* diversification may ultimately
331 assist in the discovery of novel genetic diversity and possibly novel antibiotics within this genus.

332

333 **Experimental Procedures**

334 *Strain isolation and DNA extraction*

335 We assembled a culture collection of more than 1,000 *Streptomyces* from 15 sites across the United
336 States with soil sampled at 0-5 cm depth (Andam *et al.,* 2016b). Sites were selected to represent a
337 narrow range of ecological characteristics including meadow, pasture, or native grasslands
338 dominated by perennial grasses and having moderately acidic soil (pH: 6.0 ± 1.0; ave. ± s.d.).
339 Strains were isolated using uniform conditions and this will select for strains having similar
340 physiological characteristics. The analysis of physiologically similar strains from ecologically
341 similar sites improves our ability to detect biogeographical patterns that result from drift by
342 minimizing the importance of selection (as reviewed by Hanson *et al.*, 2012). Soil was air dried,
343 and *Streptomyces* strains were isolated on glycerol-arginine agar plates of pH 8.7 containing

344 cycloheximide and Rose Bengal (El-Nakeeb and Lechevalier, 1963; Ottow, 1972) as previously
345 described (Doroghazi and Buckley, 2010). Genomic DNA was extracted for each isolate from
346 purified cultures, which were grown by shaking at 30˚C in liquid yeast extract-malt extract medium
347 (YEME) containing 0.5% glycine (Kieser *et al*., 2000), by using a standard
348 phenol/chloroform/isoamyl alcohol protocol (Roberts and Crawford, 2000).
349
350 The gene encoding the RNA polymerase beta-subunit (*rpoB*) provides a robust, advantageous
351 alternative to the SSU rRNA locus for phylogenetic analyses of the genera *Streptomyces* (Kim *et*
352 *al.*, 2004). We previously assessed genetic diversity of our culture collection using partial *rpoB*
353 sequences clustered at 0.01 patristic distances with RAMI (Pommier *et al.*, 2009), and using this
354 approach we identified 107 species-like phylogenetic clusters, or phylogroups (Andam *et al*.,
355 2016b). We selected six of these phylogroups that represented the geographic and phylogenetic
356 diversity of our culture collection for further analysis. MLSA was performed on 17-47 isolates
357 from each phylogroup. Phylogroup names are capitalized and based on a representative isolate; for
358 example, phylogroup WA1063 is named for *Streptomyces* sp. wa1063. Isolates are identified with
359 a lowercase letter code indicating site of origin followed by a strain number; for example, isolate
360 wa1063 is strain 1063 isolated from Washington (WA) state.
361
362 *Multilocus sequence analysis (MLSA)*
363 We adapted the MLSA scheme developed for *Streptomyces* by Guo *et al*. (2008), which targets the
364 five housekeeping loci *atpD*, *gyrB*, *recA*, *rpoB*, and *trpB* as described in Doroghazi and Buckley
365 (2010). The V2 and V2 regions of SSU rRNA sequences were amplified using the universal primer
366 8F: AGAGTTTGATCCTGGCTCAG. Reactions for Sanger sequencing were performed using

367 forward primers for all loci except *rpoB*, for which the reverse primer was used. Trace files were
368 uniformly screened using CAP3 (Huang and Madan, 1999) interfaced with a Perl script. Sequences
369 were aligned using MUSCLE (Edgar, 2004), trimmed to 431 bp, 415 bp, 446 bp, 557 bp, and 489
370 bp, for genes *atpD*, *gyrB*, *recA*, *rpoB*, *trpB*, respectively, and concatenated consistent with the
371 genomic order in *Streptomyces coelicolor* A3(2). SSU rRNA sequences were trimmed to 357 bp,
372 creating an alignment spanning the V1 and V2 regions. Gene sequences are available on GenBank
373 with accession numbers XXXX-XXXX.
374
375 Good's coverage estimation and haplotype rarefaction was determined using mothur (Schloss *et*
376 *al.*, 2009). DNA polymorphism statistics including: number of segregating sites, nucleotide
377 diversity, and Tajima's D, were determined with DnaSP v5 (Librado and Rozas, 2009) and LDhat
378 (McVean *et al*., 2002). Population scaled mutation rates (Watterson's theta; $\theta_w = 2N_e\mu$),
379 recombination or gene conversion rates ($\rho = 2N_e r/2$), and relative rates of recombination (ρ/θ_w) were
380 estimated using LDhat (McVean *et al*., 2002) and are expressed per nucleotide site. The standard
381 index of association (I_A) was calculated from allelic data with LIAN v3.5 (Haubold and Hudson,
382 2000) using the Monte-Carlo test and 100 iterations. The pairwise homoplasy index (PHI) statistic
383 was determined using PhiPack (Bruen *et al*., 2006), and statistical significance was evaluated
384 under a null hypothesis of no recombination. Sequence identity across phylogroups was calculated
385 with mothur (Shloss *et al*., 2009)
386
387 *Phylogengy reconstruction*
388 Maximum likelihood (ML) trees were constructed from the nucleotide sequences of individual and
389 concatenated MLSA loci using the generalized time reversible nucleotide substitution model

390 (Tavaré, 1986) with gamma distributed rate heterogeneity among sites (GTRGAMMA) supported
391 in RAxML v7.3.0 (Stamatakis, 2006). Bootstrap support was determined for the highest-scoring
392 ML tree of 20 iterations, and the number of bootstrap replicates was determined using the extended
393 majority rule (autoMRE) convergence criteria (Pattengale *et al*., 2010). Root placement is defined
394 by *Mycobacterium smegmatis.* Significant phylogenetic incongruence between loci was
395 determined using the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) implemented
396 in the R package phangorn (Schliep, 2011).
397
398 *Population structure*
399 Concatenated MLSA sequences were analyzed using Structure v2.3.3 (Pritchard *et al*., 2000) to
400 examine population affiliation, subdivision, and admixture within and between phylogroups.
401 Structure was run using an admixture model with a burn-in length of $1.0E^6$ and $3.0E^6$ replicates.
402 The most probable number of sub-populations (k) was evaluated with 10 independent runs and
403 chosen using the Evanno method (Evanno *et al*., 2005), with k = 1 through k = 6 within
404 phylogroups and through k = 8 between phylogroups, implemented by Structure Harvester (Earl
405 and vonHoldt, 2012). After choosing the most probable k-value, the program Clumpp was used to
406 permute outputs of the independent runs (Jakobsson and Rosenberg, 2007).
407
408 *Patterns of dispersal and gene flow*
409 We used permutation tests to evaluate whether phylogroup distribution across sites could be
410 explained by panmixia. We compared the observed distributions to those expected under a random
411 distribution model using 1,000 permutations to assess significance. In addition, correlations
412 between geographic distance and allele composition between sites were assessed using Mantel and

413 partial Mantel tests (Mantel, 1967; Smouse *et al*., 1986). These tests were performed with the R
414 package ecodist (Goslee and Urban, 2007) using the Pearson correlation method and 1,000
415 permutations. Bray-Curtis dissimilarity was calculated from unique allele composition across sites.
416
417 Haplotype networks were created using a statistical parsimony procedure (Templeton *et al*., 1987;
418 Templeton *et al*., 1995) implemented in TCS v1.18 (Clement *et al*., 2000). Nested clade
419 information was used to infer processes that could explain the geographic and genetic distribution
420 of sequences using the program GeoDis v2.2 (Posada *et al*., 2000). Both TCS v1.18 and GeoDis
421 v2.2 were performed in ANeCA (Panchal, 2007).
422

423 **Acknowledgements**

424 This material is based upon work supported by the National Science Foundation under
425 Grants No. DEB-1050475 and DEB-1456821. We would like to thank Ashley Campbell for her
426 help in generating MLSA sequences.
427

428 **References**

429 Allen AP, Brown JH, Gillooly JF. (2002) Global biodiversity, biochemical kinetics, and the
430 energetic-equivalence rule. Science **297**: 1545–1548.
431
432 Andam CP, Choudoir MJ, Nguygen AVT, Park HS, Buckley DH. (2016a) Contributions of
433 ancestral inter-species recombination to the genetic diversity of extant *Streptomyces* lineages.
434 ISMEJ In Press. doi: 10.1038/ismej.2015.230.
435

436 Andam CP, Doroghazi JR, Campbell AN, Kelly PJ, Choudoir MJ, Buckley DH. (2016b). A
437 latitudinal diversity gradient in terrestrial bacteria of the genus *Streptomyces*. mBio In Press.
438
439 Antony-Babu S, Stach JEM, Goodfellow M. (2008) Genetic and phenotypic evidence for
440 *Streptomyces griseus* ecovars isolated from a beach and dune sand system. Antonie Van
441 Leeuwenhoek **94**: 63–74.
442
443 Bell T. (2010) Experimental tests of the bacterial distance-decay relationship. ISME J **4**: 1357–
444 1365.
445
446 Bernatchez L, Wilson CC. (1998) Comparative phylogeography of Nearctic and Palearctic
447 fishes. Mol Ecol **7**: 431–452.
448
449 Bissett A, Richardson AE, Baker G, Wakelin S, Thrall PH. (2010) Life history determines
450 biogeographical patterns of soil bacterial communities over multiple spatial scales. Mol Ecol **19**:
451 4315–4327.
452
453 Boucher Y, Cordero OX, Takemura A, Hunt DE, Schliep K, Bapteste E, *et al*. (2011) Local
454 mobile gene pools rapidly cross species boundaries to create endemicity within global *Vibrio*
455 *cholerae* populations. mBio **2**: e00335–10.
456
457 Bruen TC, Philippe H, Bryant D. (2005) A simple and robust statistical test for detecting the
458 presence of recombination. Genetics **172**: 2665–2681.

459

460 Cadillo-Quiroz H, Didelot X, Held NL, Herrera A, Darling A, Reno ML, *et al.* (2012) Patterns of
461 gene flow define species of thermophilic Archaea. PLoS Biol **10**: e1001265.
462

463 Cho J, Tiedje JM. (2000) Biogeography and degree of endemicity of fluorescent *Pseudomonas*
464 strains in soil. Appl Environ Microbiol **66**: 5448-5456.
465

466 Choudoir MJ, Campbell AN, Buckley DH. (2012) Grappling with Proteus: population level
467 approaches to understanding microbial diversity. Front Microbiol **3**: 336.
468

469 Chu H, Fierer N, Lauber CL, Caporaso JG, Knight R, Grogan P. (2010) Soil bacterial diversity in
470 the Arctic is not fundamentally different from that found in other biomes. Environ Microbiol **12**:
471 2998–3006.
472

473 Clayton L, Attig JW, Mickelson DM, Johnson MD, Syverson KM. (2006) Glaciation of
474 Wisconsin: Wisconsin Geological and Natural History Survey Educational Series 36. Wisconsin
475 Geological and Natural History Survey. Madison, WI.
476

477 Clement M, Posada D, Crandall KA. (2000) TCS: A computer program to estimate gene
478 genealogies. Mol Ecol **9**: 1657–1659.
479

480 Coleman ML, Chisholm SW. (2010) Ecosystem-specific selection pressures revealed through
481 comparative population genomics. Proc Natl Acad Sci U S A **107**: 18634–18639.

482

483 Conroy CJ, Cook JA. (2000) Phylogeography of a post-glacial colonizer: *Microtus longicaudus*
484 (Rodentia: Muridae). Mol Ecol **9**: 165–175.
485

486 Currie DJ, Mittelbach GG, Cornell HV, Field R, Guégan JF, Hawkins BA*, et al.* (2004)
487 Predictions and tests of climate-based hypotheses of broad-scale variation in taxonomic richness.
488 Ecol Lett **7**: 1121–1134.
489

490 Davelos AL, Xiao K, Samac DA, Martin AP, Kinkel LL. (2004a) Spatial variation in
491 *Streptomyces* genetic composition and diversity in a prairie soil. Microb Ecol **48**: 601–612.
492

493 Davelos AL, Kinkel LL, Samac DA. (2004b) Spatial variation in frequency and intensity of
494 antibiotic interactions among *Streptomycetes* from prairie soil. Appl Environ Microbiol **70**:
495 1051–1058.
496

497 Doroghazi JR, Buckley DH. (2014) Intraspecies comparison of *Streptomyces pratensis* genomes
498 reveals high levels of recombination and gene conservation between strains of disparate
499 geographic origin. BMC Genomics **15**: 970.
500

501 Doroghazi JR, Buckley DH. (2010) Widespread homologous recombination within and between
502 *Streptomyces* species. ISME J **4**: 1136–1143.
503

504 Earl DA, vonHoldt BM. (2012) STRUCTURE HARVESTER: a website and program for
505 visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour
506 **4**: 359–361.
507
508 Edgar RC. (2004) MUSCLE: multiple sequence alignment with high accuracy and high
509 throughput. Nucleic Acid Res **32**: 1792–1797.
510
511 Eisenlord SD, Zak DR, Upchurch RA. (2012) Dispersal limitation and the assembly of soil
512 *Actinobacteria* communities in a long-term chronosequence. Ecol Evol **2**: 538–549.
513
514 El-Nakeeb MA, Lechevalier HA. (1963) Selective isolation of aerobic Actinomycetes. Appl
515 Microbiol 11: 75–77.
516
517 Evanno G, Regnaut S, Goudet J. (2005) Detecting the number of clusters of individuals using the
518 software STRUCTURE: a simulation study. Mol Ecol **14**: 2611–2620.
519
520 Fierer N, Carney KM, Horner-Devine MC, Megonigal JP. (2009) The biogeography of
521 ammonia-oxidizing bacterial communities in soil. Microb Ecol **58**: 435–445.
522
523 Fierer N, Jackson RB. (2006) The diversity and biogeography of soil bacterial communities. Proc
524 Natl Acad Sci U S A **103**: 626–631.
525

526 Finlay BJ. (2002) Global dispersal of free-living microbial eukaryote species. Science **296**:
527 1061–1063.
528
529 Finlay BJ, Fenchel T. (2004) Cosmopolitan metapopulations of free-living microbial eukaryotes.
530 Protist **155**: 237–244.
531
532 Fuhrman JA, Steele JA, Hewson I, Schwalbach MS, Brown M V, Green JL, *et al.* (2008) A
533 latitudinal diversity gradient in planktonic marine bacteria. Proc Natl Acad Sci U S A **105**: 7774–
534 7778.
535
536 Gilbert JA, Steele JA, Caporaso JG, Steinbrück L, Reeder J, Temperton B, *et al.* (2012) Defining
537 seasonal marine microbial community dynamics. ISME J **6**: 298–308.
538
539 Goslee SC, Urban DL. (2007) The ecodist package for dissimilarity-based analysis of ecological
540 data. J Stat Softw **22**: 1–19.
541
542 Green J, Bohannan BJM. (2006) Spatial scaling of microbial biodiversity. Trends Ecol Evol **21**:
543 501–507.
544
545 Guo Y, Zheng W, Rong X, Huang Y. (2008) A multilocus phylogeny of the *Streptomyces*
546 *griseus* 16S rRNA gene clade: use of multilocus sequence analysis for streptomycete
547 systematics. Int J Syst Evol Microbiol **58**: 149–159.
548

549 Hanson CA, Fuhrman JA, Horner-Devine MC, Martiny JBH. (2012) Beyond biogeographic
550 patterns: processes shaping the microbial landscape. Nat Rev Microbiol **10**: 497–506.
551
552 Hatosy SM, Martiny JBH, Sachdeva R, Steele J, Fuhrman JA, Martiny AC. (2013) Beta diversity
553 of marine bacteria depends on temporal scale. Ecology **94**: 1898–1904.
554
555 Haubold B, Hudson RR. (2000) LIAN 3.0: detecting linkage disequilibrium in multilocus data.
556 Bioinformatics **16**: 847–848.
557
558 Hewitt GM. (1996) Some genetic consequences of ice ages, and their role in divergence and
559 speciation. Biol J Linnean Soc **58**: 247–276.
560
561 Hewitt G. (2000) The genetic legacy of the Quaternary ice ages. Nature **405**: 907–913.
562
563 Hewitt GM. (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos Trans
564 R Soc Lond B Biol Sci **359**: 183–195.
565
566 Hillebrand H. (2014) On the generality of the latitudinal diversity gradient. Amer Nat **163**: 192–
567 211.
568
569 Huang X, Madan A. (1999) CAP3: a DNA sequence assembly program. Genome Res **9**: 868–
570 677.
571

572 Jakobsson M, Rosenberg NA. (2007) CLUMPP: a cluster matching and permutation program for
573 dealing with label switching and multimodality in analysis of population structure.
574 Bioinformatics **23**: 1801–1806.
575
576 Jaspers E, Overmann J. (2004) Ecological significance of microdiversity: identical 16S rRNA
577 gene sequences can be found in bacteria with highly divergent genomes and ecophysiologies.
578 Appl Environ Microbiol **70**: 4831–4839.
579
580 Jolley KA, Chan MS, Maiden MCJ. (2004) mlstdbNet - distributed multi-locus sequence typing
581 (MLST) databases. BMC Bioinformatics **5**: 86.
582
583 Kâmpfer P. (2006) The family *Streptomycetaceae* Part I: Taxonomy. In: The Prokaryotes,
584 Volume 3. Dworkin, M, Falkow, S, Rosenberg, E, Schliefer, K, & Stackebrandt, E. (eds). New
585 York: Springer, pp. 538–604.
586
587 Kenefic LJ, Pearson T, Okinaka RT, Schupp JM, Wagner DM, Hoffmaster AR, *et al.* (2009) Pre-
588 Columbian origins for North American anthrax. PLoS One **4**: e4813.
589
590 Kieser T, Bibb M, Buttner M, Charter K, Hopwood D. (2000) Practical *Streptomyces* Genetics.
591 Norwich, UK: John Innes Foundation.
592

593 Kim BJ, Kim CJ, Chun J, Koh YH, Lee SH, Hyun JW, *et al.*(2004) Phylogenetic analysis of the
594 genera *Streptomyces* and *Kitasatospora* based on partial RNA polymerase beta-subunit gene
595 (*rpoB*) sequences. Int J Syst Evol Microbiol. **54**: 593–598.
596
597 Knowles L, Maddison W. (2002) Statistical phylogeography. Mol Ecol **11**: 2623–2635.
598
599 Labeda DP. (2011) Multilocus sequence analysis of phytopathogenic species of the genus
600 *Streptomyces*. Int J Syst Evol Microbiol **61**: 2525–2531.
601
602 Lauber CL, Hamady M, Knight R, Fierer N. (2009) Pyrosequencing-based assessment of soil pH
603 as a predictor of soil bacterial community structure at the continental scale. Appl Environ
604 Microbiol **75**: 5111–5120.
605
606 Librado P, Rozas J. (2009) DnaSP v5: a software for comprehensive analysis of DNA
607 polymorphism data. Bioinformatics **25**: 1451–1452.
608
609 Loria R, Kers J, Joshi M. (2006) Evolution of plant pathogenicity in *Streptomyces*. Annu Rev
610 Phytopathol **44**: 469–487.
611
612 Lozupone CA, Knight R. (2007) Global patterns in bacterial diversity. Proc Natl Acad Sci U S A
613 **104**: 11436–11440.
614

615 Maggs CA, Castilho R, Foltz D, Henzler C, Jolly MT, Kelly J, *et al.* (2008) Evaluating
616 signatures of glacial refugia for North Atlantic benthic marine taxa. Ecology **89**: S108–S122.
617
618 Mantel N. (1967) The detection of disease clustering and a generalized regression approach.
619 Cancer Research **27**: 209–220.
620
621 Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, *et al.* (2006)
622 Microbial biogeography: putting microorganisms on the map. Nat Rev Microbiol **4**: 102–112.
623
624 Martiny JBH, Eisen JA, Penn K, Allison SD, Horner-Devine MC. (2011) Drivers of bacterial β -
625 diversity depend on spatial scale. Proc Natl Acad Sci U S A **108**: 7850–7854.
626
627 McCarthy AJ, Williams ST. (1992) Actinomycetes as agents of biodegradation in the
628 environment - a review. Gene **115**: 189–192.
629
630 McVean G, Awadalla P, Fearnhead P. (2002) A coalescent-based method for detecting and
631 estimating recombination from gene sequences. Genetics **160**: 1231–1241.
632
633 Mikheyev AS, Vo T, Mueller UG. (2008) Phylogeography of post-Pleistocene population
634 expansion in a fungus-gardening ant and its microbial mutualists. Mol Ecol **17**: 4480–4488.
635
636 Mila B, Smith T, Wayne R. (2006) Postglacial population expansion drives the evolution of
637 long-distance migration in a songbird. Evolution **60**: 2403–2409.

638

639 Miller SR, Strong AL, Jones KL, Ungerer MC. (2009) Bar-coded pyrosequencing reveals shared
640 bacterial community properties along the temperature gradients of two alkaline hot springs in
641 Yellowstone National Park. Appl Environ Microbiol **75**: 4565–4572.

642

643 Mittelbach GG, Schemske DW, Cornell H V., Allen AP, Brown JM, Bush MB, *et al.* (2007)
644 Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecol
645 Lett **10**: 315–331.

646

647 Mouchet M, Levers C, Zupan L, Kuemmerle T, Plutzar C, Erb K, *et al.* (2015) Testing the
648 effectiveness of environmental variables to explain European terrestrial vertebrate species
649 richness across biogeographical scales. PLoS One **10**: e0131924.

650

651 Neufeld JD, Mohn WW. (2005) Unexpectedly high bacterial diversity in arctic tundra relative to
652 boreal forest soils, revealed by serial analysis of ribosomal sequence tags. Appl Environ
653 Microbiol **71**: 5710–5718.

654

655 Nielsen R, Beaumont MA. (2009) Statistical inferences in phylogeography. Mol Ecol **18**: 1034–
656 1047.

657

658 Ochman H, Elwyn S, Moran NA. (1999) Calibrating bacterial evolution. Proc Natl Acad Sci U S
659 A **96**: 12638–12643.

660

661 Ottow JCG. (2013) Rose bengal as a selective aid in the isolation of fungi and Actinomycetes
662 from natural sources. Mycologia **64**: 304–315.
663
664 Panchal M. (2006) The automation of Nested Clade Phylogeographic Analysis. Bioinformatics
665 **23**: 509–510.
666
667 Papke RT, Ramsing NB, Bateson MM, Ward DM. (2003) Geographical isolation in hot spring
668 cyanobacteria. Environ Microbiol **5**: 650–659.
669
670 Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. (2010) How
671 many bootstrap replicates are necessary? J Comput Biol **17**: 337–354.
672
673 Pearson T, Giffard P, Beckstrom-Sternberg S, Auerbach R, Hornstra H, Tuanyok A*, et al*. (2009)
674 Phylogeographic reconstruction of a bacterial species with high levels of lateral gene transfer.
675 BMC Biol **7**: 78.
676
677 Pommier T, Canback B, Lundberg P, Hagstrom A, Tunlid A. (2009) RAMI: a tool for
678 identification and characterization of phylogenetic clusters in microbial communities.
679 Bioinformatics **25**: 736–742.
680
681 Posada D, Crandall KA, Templeton AR. (2000) GeoDis: A program for the cladistic nested
682 analysis of the geographical distribution of genetic haplotypes. Mol Ecol **9**: 487–488.
683

684 Pritchard JK, Stephens M, Donnelly P. (2000) Inference of population structure using multilocus
685 genotype data. Genetics **155**: 945–959.
686
687 Roberts MA and Crawford DL. (2000) Use of randomly amplified polymorphic DNA as a means
688 of developing genus- and strain-specific *Streptomyces* DNA probes. Appl Environ Microbiol **66:**
689 2555-2564.
690
691 Rong X, Huang Y. (2010) Taxonomic evaluation of the *Streptomyces griseus* clade using
692 multilocus sequence analysis and DNA-DNA hybridization, with proposal to combine 29 species
693 and three subspecies as 11 genomic species. Int J Syst Evol Microbiol **60**: 696–703.
694
695 Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, *et al*. (2010) Soil
696 bacterial and fungal communities across a pH gradient in an arable soil. ISME J **4**: 1340–1351.
697
698 Schliep KP. (2011) phangorn: phylogenetic analysis in R. Bioinformatics **27**: 592–593.
699
700 Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, *et al*. (2009)
701 Introducing mothur: open-source, platform-independent, community-supported software for
702 describing and comparing microbial communities. Appl Environ Microbiol **75**: 7537–7541.
703
704 Shimodaira H, Hasegawa M. (1999) Multiple comparisons of log-likelihoods with applications
705 to phylogenetic inference. Mol Biol Evol **16**: 1114–1116.
706

707 Smouse PE, Long JC, Sokal RR. (1986) Multiple regression and correlation extensions of the
708 Mantel test of matrix correspondence. Syst Zool **35**: 627–632.
709
710 Soltis D, Gitzendanner M, Strenge D, Soltis P. (1997) Chloroplast DNA intraspecific
711 phylogeography of plants from the Pacific Northwest of North America. Plant Syst Evol **206**:
712 353–373.
713
714 Stamatakis A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with
715 thousands of taxa and mixed models. Bioinformatics **22**: 2688–2690.
716
717 Stevens RD. (2006) Historical processes enhance patterns of diversity along latitudinal gradients.
718 *Proc R Soc B Biol Sci* **273**: 2283–2289.
719
720 Sul WJ, Oliver TA, Ducklow HW, Amaral-zettler LA, Sogin ML. (2012) Marine bacteria exhibit
721 a bipolar distribution. Proc Natl Acad Sci U S A **110**: 2342–2347.
722
723 Swan BK, Tupper B, Sczyrba A, Lauro FM, Martinez-Garcia M, González JM, *et al*. (2013)
724 Prevalent genome streamlining and latitudinal divergence of planktonic bacteria in the surface
725 ocean. Proc Natl Acad Sci U S A **110**: 11463–11468.
726
727 Takasuka TE, Book AJ, Lewin GR, Currie CR, Fox BG. (2013) Aerobic deconstruction of
728 cellulosic biomass by an insect-associated *Streptomyces*. Sci Rep **3**: 1–10.
729

730 Tavaré S. (1986) Some probabilistic and statistical problems in the analysis of DNA sequences.
731 Am Math Soc Lect Math Life Sci **17**: 57–86.
732
733 Telford RJ, Vandvik V, Birks HJB. (2006) Dispersal limitations matter for microbial
734 morphospecies. Science **312**: 1015.
735
736 Templeton AR, Boerwinkle E, Sing CF. (1987) A cladistic analysis of phenotypic associations
737 with haplotypes inferred from restriction endonuclease mapping. i. basic theory and an analysis
738 of alcohol dehydrogenase activity in Drosophila. Genetics **117**: 343–351.
739
740 Templeton AR, Routman E, Phillips CA. (1995) Separating population structure from population
741 history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in
742 the tiger salamander, *Ambystoma tigrinum*. Genetics **140**: 767–782.
743
744 Vos M, Velicer GJ. (2008) Isolation by distance in the spore-forming soil bacterium
745 *Myxococcus xanthus*. Curr Biol **18**: 386–391.
746
747 Wallace A. (1878) Tropical nature, and other essays. London: Macmillan and Co.
748
749 Watve M, Tickoo R, Jog M, Bhole B. (2001) How many antibiotics are produced by the genus
750 *Streptomyces*? Arch Microbiol **176**: 386–390.
751

752 Wawrik B, Kutliev D, Abdivasievna UA, Kukor JJ, Zylstra GJ, Kerkhof L. (2007) Biogeography
753 of Actinomycete communities and type ii polyketide synthase genes in soils collected in New
754 Jersey and Central Asia. Appl Environ Microbiol **73**: 2982–2989.
755
756 Welch RA, Burland V, Plunkett G, Redford P, Roesch P, Rasko D, *et al*. (2002) Extensive
757 mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*.
758 Proc Natl Acad Sci U S A **99**: 17020–17024.
759
760 Whitaker RJ, Grogan DW, Taylor JW. (2003) Geographic barriers isolate endemic populations
761 of hyperthermophilic archaea. Science **301**: 976–978.
762
763 Wiens JJ, Donoghue MJ. (2004) Historical biogeography, ecology and species richness. Trends
764 Ecol Evol **19**: 639–644.
765
766 Williams D, Dunkerley D, DeDecker P, Kershaw P, Chappell M. (1998) Quaternary
767 environments. London: Arnold.
768
769 Wilson AB, Veraguth IE. (2010) The impact of Pleistocene glaciation across the range of a
770 widespread European coastal species. Mol Ecol **19**: 4535–53.
771
772
773
774

775 **Table and Figure Legends**

776

777 **Table 1.** A total of 755 *Streptomyces* strains were isolated from 13 sites, and 308 of these strains

778 were found to represent the six targeted phylogroups. The numbers of isolates per site that

779 belonged to each of our target phylogroups is indicated. Isolate names begin with a letter code

780 referring to the sample site.

Site	Code	Latitude	Longitude	Altitude	Isolates/ Site	Isolates from *Streptomyces* sp. phylogroup					
						MAN 125	MAN 196	WA 1063	MS 200	MS 152	F34
Manley Hot Springs, AK	man	63.87˚N	-149.02˚W	452 m	95	51	26	1			
Denali Hwy, AK	den	63.22˚N	-147.68˚W	894 m	40	40					
Bothell, WA	wa	47.73˚N	-122.24˚W	132 m	105	21	5	21			
Astoria, OR	or	46.18˚N	-123.85˚W	40 m	79		8	7			
Kennebunk, ME	me	43.4˚N	-70.54˚W	28 m	85		13				
Sun Prairie, WI	sun	43.17˚N	-89.24˚W	289 m	65	3			3		
Brookfield, WI	b	43.06˚N	-88.13˚W	277 m	22			5			
Palo Alto, CA	st	37.43˚N	-122.17˚W	24 m	34					2	1
Greensboro, NC	gb	36.09˚N	-79.89˚W	276 m	9		1			2	
Troy (Uwharrie), NC	uw	35.71˚N	-79.88˚W	194 m	19		4		7		
Starkville, MS	ms	33.46˚N	-88.8˚W	119 m	90				11	8	2
Austin, TX	t	30.2˚N	-97.77˚W	199 m	42						6
Fort Pierce, FL	f	27.54˚N	-80.35˚W	0 m	70				7	5	48
Total					755	115	57	34	28	17	57

781

782

783

784

785

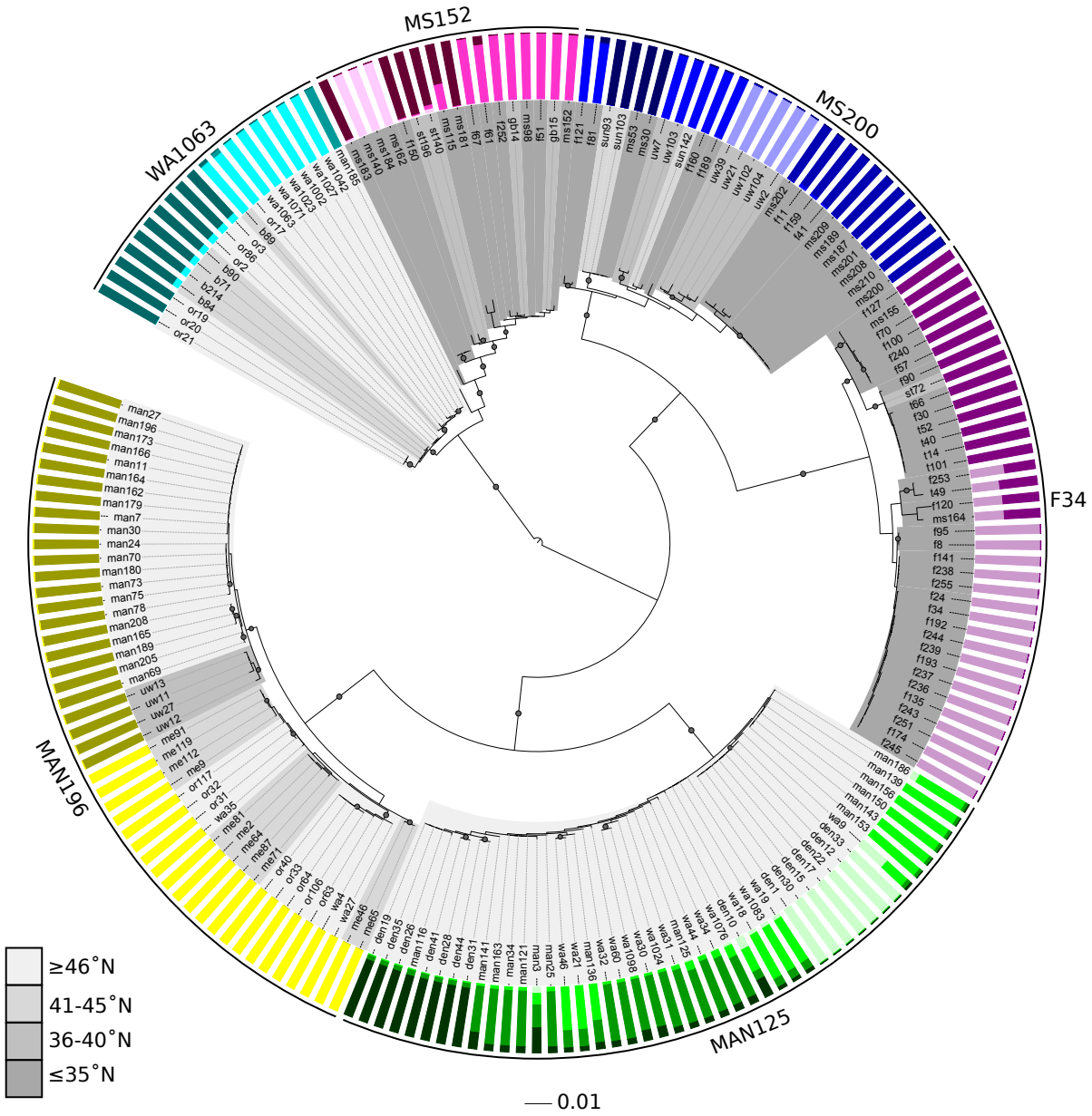
786

787 **Table 2.** MLSA was performed on a total of 17-47 strains from each phylogroup. Summary
788 statistics for concatenated MLSA nucleotide sequences were determined as described in
789 methods. Phylogroup average latitude (Ave Lat) was determined using the total number of
790 isolates per phylogroup provided in Table 1. The standard index of association is zero for a
791 population in linkage equilibrium.

Phylogroup	Ave Lat	n	Haplotypes	Min ANI	S	π	Tajima's D	θ_W	ρ	ρ/θ_W	I_A	Φ_W
MAN125	60.15˚N	45	25	99.5	23	0.0026	0.53	0.0023	0.0094	4.18	0.09	2.95E-01
MAN196	52.84˚N	47	26	98.5	65	0.0075	0.67	0.0063	0.00043	0.068	0.49	4.24E-02*
WA1063	47.20˚N	19	13	98.1	63	0.0052	-1.35	0.0077	0	0	0.43	4.33E-03
MS200	33.58˚N	27	21	98.4	93	0.0088	-0.58	0.0103	0.0017	0.17	0.39	1.33E-03**
MS152	32.50˚N	17	15	97.6	96	0.0107	-0.51	0.012	0.0013	0.11	0.35	1.078E-01**
F34	28.20˚N	36	22	97.7	93	0.0109	0.52	0.0096	0.0011	0.11	0.46	1.79E-01**

Ave Lat: average latitude of phylogroup members
n: number of isolates subsampled for MLSA
Min ANI: minimum percent pairwise average nucleotide identity (ANI) within phylogroup members
S: segregating sites
π: per site nucleotide diversity
θ_W: per site Watterson's theta ($2N_e\mu$)
ρ: per site rate of recombination/gene conversion ($2N_er/2$)
ρ/θ_W: relative rate of recombination to mutation
I_A: standard index of association
Φ_W: pairwise homoplasy index (Phi) statistic with *p*-value < 0.05*, 0.01** under the null hypothesis of no recombination

792
793
794
795
796
797
798
799



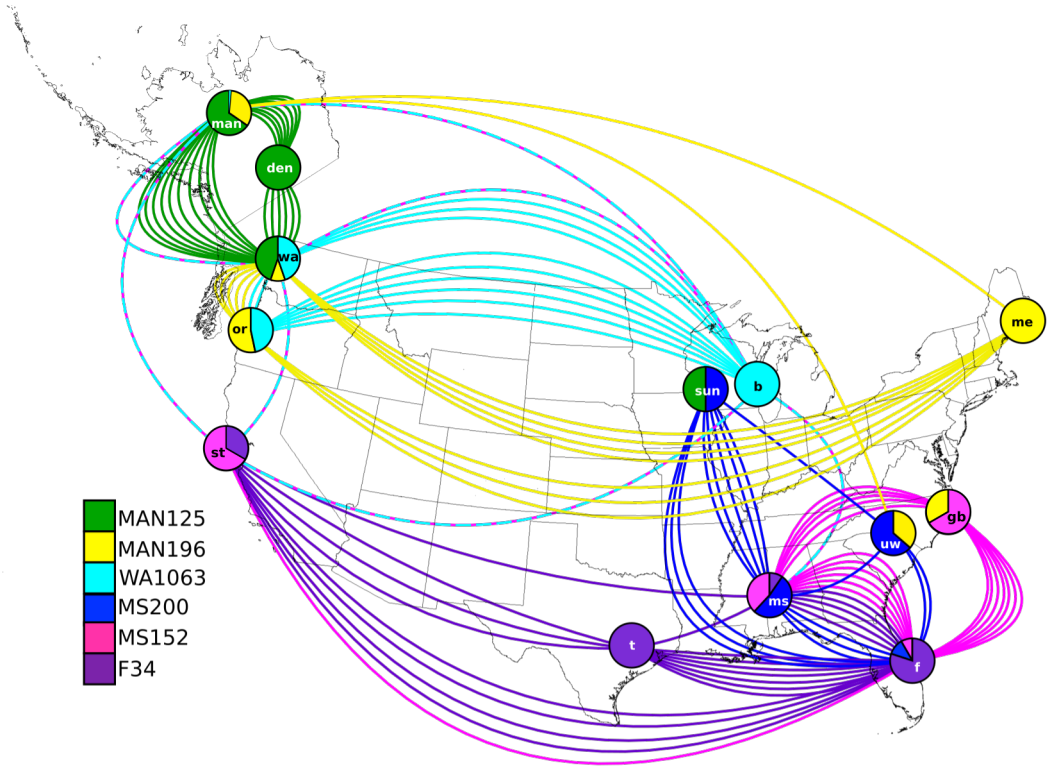
800

801 **Figure 1.** Tree was constructed from concatenated MLSA loci nucleotide sequences using

802 maximum likelihood with a GTRGAMMA evolution model. Scale bar represents nucleotide

803 substitutions per site. The root was defined by *Mycobacterium smegmatis*. Nodes with bootstrap

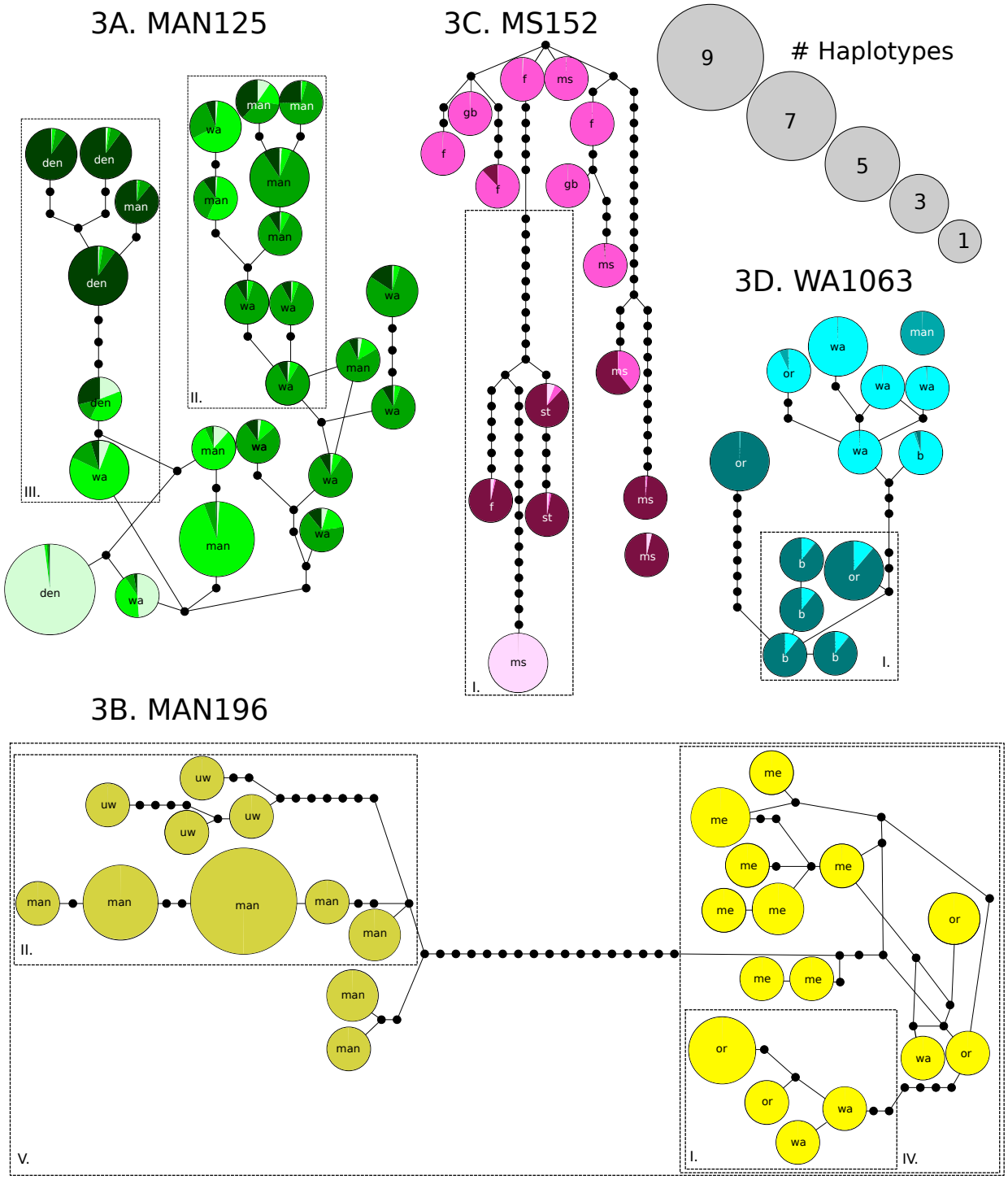
804 confidences > 80 are indicated with gray circles, and precise bootstrap values are found in Figure
805 S4A. The colored bars in the outer ring indicate genetic contributions from different ancestral
806 populations as inferred by Structure analysis. The shading of the inner ring indicates the latitude
807 from which each strain was isolated according to the scale provided. The isolation site for each
808 strain can be determined by isolate names as indicated in Table 1.



809

810 **Figure 2.** Circles depict sample sites and are labeled according to site code (Table 1). The relative
811 abundance of each phylogroup at each site is indicated by color according to the legend, with raw
812 counts provided in Table 1. Solid colored lines represent identical alleles shared by phylogroup
813 members subsampled for MLSA across sites. Dashed multicolored lines depict identical *atpD*
814 alleles shared by strains in phylogroups WA1063 and MS152 (Figure S4B).

815



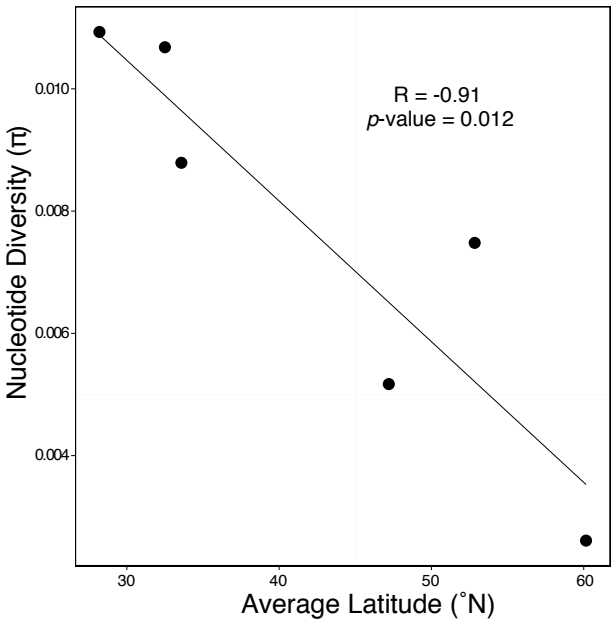
816

817 **Figure 3.** Haplotype networks for phylogroups MAN125 (3A), MAN196 (3B), MS152 (3C),

818 WA1063 (3D). Circles represent unique MLSA haplotypes whose radius is proportional to the

819 number of isolates sharing that haplotype, and colors correspond to strain ancestry and

820 subpopulation affiliation as determined by Structure analysis and defined in Figure 1. Haplotypes
821 are labeled with a letter code referring to sample site location as indicated in Table 1. Black circles
822 represent un-sampled, inferred haplotypes with each circle designating a single nucleotide
823 polymorphism. The length of edges between nodes is uninformative. Dashed rectangles encompass
824 clades that have significant phylogeographic inferences from nested clade analysis, as described
825 in methods. Roman numerals correspond to the following inferences: I. Allopatric fragmentation;
826 II. Long distance colonization and/or past fragmentation; III. Restricted gene flow with isolation
827 by distance; IV. Restricted gene flow but with some long-distance gene flow over intermediate
828 ranges not occupied by the species; or past gene flow followed by extinction of intermediate
829 populations; V. Contiguous range expansion.



830

831 **Figure 4.** Average latitude was determined using a weighted average based on the total number of
832 isolates per site as indicated in Table 1. Nucleotide diversity was calculated using concatenated
833 MLSA loci and is expressed per site.