Correction

MICROBIOLOGY

The authors note that, on page 19964, left column, first paragraph, last sentence, “This desert can be viewed as nearing the cold-arid limit for life, because evidence for microbial activity in inland snow is questionable (see ref. 20)” should instead read as “This desert can be viewed as nearing the cold-arid limit for life, because evidence for microbial activity in inland snow is questionable (see ref. 64).” This error does not affect the conclusions of the article.

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Correction

BIOPHYSICS AND COMPUTATIONAL BIOLOGY

The authors note that, due to a printer error, the legend for Fig. 4 appeared incorrectly. The second sentence, “The red curves are normalized single exponential fits given by \( f = \tau^{-1} \exp(-t / \tau) \), where \( \tau \) is the mean pause lifetime,” should instead appear as “The red curves are normalized single exponential fits given by \( f = \tau^{-1} \exp(-t / \tau) \), where \( \tau \) is the mean pause lifetime.” The figure and its corrected legend appear below.

Fig. 4. Pause lifetimes for Pol I(KF) and \( \phi 29 \) were measured under different conditions for the sample and control sequences. The red curves are normalized single exponential fits given by \( f = \tau^{-1} \exp(-t / \tau) \), where \( \tau \) is the mean pause lifetime. Bins excluded from the fit due to undersampling are shown in white. (A) Pol I(KF) at 23°C with the sample template; (B) Pol I(KF) at 23°C with 1 M betaine with the sample template; (C) Pol I(KF) at 23°C with the control template; (D) \( \phi 29 \) at 23°C with the sample template; (E) \( \phi 29 \) at 23°C with 1 M betaine with the sample template; and (F) \( \phi 29 \) at 23°C with the control template.

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Highly specialized microbial diversity in hyper-arid polar desert


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The McMurdo Dry Valleys in Antarctica are a cold hyperarid polar desert that present extreme challenges to life. Here, we report a culture-independent survey of multidomain microbial biodiversity in McKelvey Valley, a pristine example of the coldest desert on Earth. We demonstrate that life has adapted to form highly-specialized communities in distinct lithic niches occurring concomitantly within this terrain. Endoliths and chasmoliths in sandstone displayed greatest diversity, whereas soil was relatively depauperate and lacked a significant photoautotrophic component, apart from isolated islands of hypolithic cyanobacterial colonization on quartz rocks in soil contact. Communities supported previously unreported polar bacteria and fungi, but archaea were absent from all niches. Lithic community structure did not vary significantly on a landscape scale and stochastic moisture input due to snowmelt resulted in increases in colonization frequency with no significant effects of environmental change to endangered polar land-masses. Here, we present findings from a polyphasic molecular study targeting all domains of life to fully characterize microbial diversity in four distinct microbial niches occurring concomitantly in the dry permafrost of the high inland McKelvey Valley. We identify highly-specialized communities that supported previously unreported polar bacteria and fungi, and demonstrate that landscape-scales and stochastic moisture input had little impact on community structure.

Results

Chasmoliths and endoliths occurred exclusively in above-ground sandstone with a mean frequency of 1 and 3%, respectively (n = 100). Hypoliths occurred exclusively on quartz in soil-contact with a mean frequency of 4.9% (n = 1,260) in typical polygons. A single snowmelt-influenced polygon supported a near 5-fold higher frequency of colonization (22%), and there was no significant difference in available quartz substrate between “dry” and snowmelt-influenced polygons. Microscopy of colonized rock surfaces revealed Chroococcidiopsis-like cyanobacterial morphotypes dominated sandstone substrates, whereas oscillatorian cyanobacterial morphotypes dominated quartz. Copious extracellular polymeric substance was present around colonized areas.

We used real-time quantitative (q)PCR to estimate the absolute and relative abundance of recoverable phylotypes for archaea, bacteria, and eukarya as a proxy for relative biomass (Table 1). Whereas each lithic niche supported both eukaryal and bacterial phylotypes, soil supported bacterial phylotypes only. Presence of a soil-based inhibitor to eukaryal PCR amplification was discounted after successful recovery of amplification from artificially “spiked” soil samples. Eukaryal phylotypes in lithic niches accounted for a relatively low abundance (<5%) of total recoverable phylotypes. The hypolithon supported greatest overall abundance of recoverable phylotypes, with values for surrounding soil several orders of magnitude lower.

Variation in multidomain community structure among all colonized rocks and soil samples was assessed using terminal restriction fragment length polymorphism (t-RFLP) (Fig. 1). The relative contribution of each domain-specific t-RFLP profile to overall community diversity/abundance in any given sample was calculated based on relative abundance obtained from qPCR data. Differences between soil and rock substrates were significant (ANOSIM, Global R = 0.719, P < 0.001). Multiple rank correlations (BEST analysis) of abiotic (Table S1 and S2) and community diversity/abundance data (t-RFLP) revealed that the combination of factors most important in influencing community diversity in soils were soluble salts, K and C (p = 0.274). Combinations including sodium


The authors declare no conflict of interest.

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. FJ490210-490344 and FJ895042-FJ895089).

See Commentary on page 19749.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0908274106/DCSupplemental.
Table 1. Diversity statistics and recovery of microbial phyla from soils, sandstone chamsoliths, sandstone endoliths, and quartz hypoliths in McKeever Valley, McMurdo Dry Valleys, Antarctica

<table>
<thead>
<tr>
<th>Soil</th>
<th>Hypolith</th>
<th>Chamsolith</th>
<th>Endolith</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diversity indices</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shannon’s Index</td>
<td>3.3</td>
<td>2.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Simpson Diversity Index</td>
<td>1</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Pielou’s Evenness</td>
<td>0.9</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Omega diversity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FST bacteria</td>
<td>0.299</td>
<td>0.305</td>
<td>0.301</td>
</tr>
<tr>
<td>FST eukarya</td>
<td>—</td>
<td>0.105</td>
<td>0.083</td>
</tr>
<tr>
<td>AvTD</td>
<td>77</td>
<td>68</td>
<td>84</td>
</tr>
<tr>
<td>VarTD</td>
<td>255</td>
<td>1293</td>
<td>425</td>
</tr>
</tbody>
</table>

| Phylum abundance, %                     |          |            |          |
| Cyanobacteria                           | 0        | 95         | 64       | 56       |
| [Chroococcidiopsis sp.]                 | [0]      | [57]       | [51]     |          |
| [Nostocales]                            | [0]      | [1]        | [5]      |          |
| [Oscillatoriales]                       | [95]     | [0]        | [0]      |          |
| [Unidentified cyanobacterium]           | [0]      | [6]        | [0]      |          |
| Acidobacteria                          | 31       | 2          | 2        | 5        |
| Actinobacteria                          | 33       | 2          | 5        | 4        |
| Bacteroidetes                           | 2        | 0          | 7        | 16       |
| Chloroflexi                             | 3        | 0          | 0        |          |
| Deinococcus-Thermus                     | 6        | 0          | 0        | 2        |
| Gemmatinonadetes                        | 8        | 0          | 0        |          |
| Planctomyces                            | <1       | 0          | 0        | 2        |
| Alpha proteobacteria                    | 0        | 1          | 4        | 4        |
| Beta proteobacteria                     | 0        | 0          | 0        |          |
| Gamma proteobacteria                    | 0        | 0          | 8        | 4        |
| Unidentified bacteria                   | 13       | 0          | 8        | 2        |
| Ascomycota                              | 0        | 0          | <1       | <1       |
| Basidiomycota                           | 0        | 0          | <1       | <1       |
| Chlorophyta                             | 0        | <1         | 2        | 4        |

Alpha diversity: 0, 0, 0, 0; Beta diversity: 3, 3, 3, 3; Gamma diversity: 0, 0, 0, 0.

And moisture content resulted in significant but weaker correlations. For rock substrates, the soluble salts, organic carbon, and moisture content were below detectable limits, and the most important variables determining community structure among quartz and sandstone were CI (μv 0.794) and CI, K, and porosity (μv 0.688).

We were able to assign putative identification to a relatively high percentage (92%) of t-RFLP peaks. These data revealed some general trends among substrates. Chamsoliths and endoliths were dominated by *Chroococcidiopsis* phylotypes, whereas *Leptolyngbya*-like phylotypes dominated hypoliths. Cyanobacterial signals were absent from all but three soil samples, and comprised a very low fraction (<10%) of overall t-RFLP signal. Soil t-RFLP profiles indicated communities dominated by Acidobacteria, Alpha-

![Fig. 1. Nonmetric multidimensional scaling plot of Bray Curtis similarities for bacterial and eukaryal rRNA gene phylotypes recovered from soils, sandstone chamsoliths and endoliths, and quartz hypoliths in McKeever Valley. Dashed line represents statistically significant groupings (ANOSIM, Global R = 0.719, P < 0.1, n = 14).](image-url)

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so may represent a previously uncharacterized lichen mycobiont or
soil communities were markedly more even in terms of taxon contribution to overall abundance than
Our statistical analyses included approaches designed to assess
the calculated diversities were used to illustrate the phylogenetic level between each node. Taxonomic
to show that soil and hypoliths were significantly less diverse than
"microbial" communities. The FST statistic was used to show that bacterial assem-
based on their genetic affiliation to any known phylum. Similarly, unidentified bacteria with little phylo-
and compositional disequilibrium (11021). (B) Taxonomic diversities across soil communities were sig-
getic diversity (Table 1). On the basis of Shannon and Simpson,
vestigated soils supported a relatively large number of
our data to estimate phylogenetic diversity in the different com-
were significantly different. Overall community was significantly differ-
and quartz were not.
the hypoliths, chasmoliths, and endoliths in soil, hypolith, chasmolith, and endolith
topologies are supported by Bayesian posterior probabilities (first number) and Bootstrap values (second number). Scale Bar: 0.1-tt Changes per position.

Fig. 2. Phylogenetic relationships among green bacterial 16S rRNA phylotypes recovered during this study and shown in bold type. Sequence code prefix denotes location. H, hypolith; C, chasmolith; E, endolith.
Blue font denotes Antarctic and glacial phylotypes, orange font denotes phylotypes from nonpolar deserts, black font denotes nondesert phylotypes. Tree}

Blue font denotes Antarctic and glacial phylotypes, orange font denotes phylotypes from nonpolar deserts, black font denotes nondesert phylotypes. Tree
chamosoliths and endoliths. Variation (Var) in TD reflects the level of phylogenetic species differentiation within a community, and clearly showed that soils were depauperate compared with lithic niches.

To address the question of how variable lithic communities are on a landscape scale in the Dry Valleys, we selected hypolithic colonization as an indicator, because this colonization occurred most frequently at all locations and there was no significant difference in colonization frequency for typical polygons within or between locations (P > 0.05). Because bacterial phylotypes accounted for >98% of recoverable phylotypes, we assessed variation in bacterial community t-RFLP profiles for the hypervariable 16S-23S ITS region (n = 61). No significant difference in community structure within or between locations could be delineated based on this highly variable marker (Fig. S4). The inclusion of samples in a polygon atypically experiencing moisture sufficiency (and significantly greater colonization frequency) due to localized snowmelt revealed that, although 14% of rocks displayed apparent differences in diversity/abundance profiles, this pattern was not significantly different from overall community structure on a landscape scale (Fig. S4).

Discussion

Our study represents a complete assessment of terrestrial microbial biodiversity across surface niches at the cold-arid limit for life, and provides insights into ecosystem complexity under extreme stress. These highly-specialized communities face unpredictable effects from climate change. They may be out-competed by invasive species should warming occur, or encounter catastrophic ecosystem shift (18) should aridity increase with further cooling. Given that current global climate-warming trends are more pronounced in polar regions (19) but that increased localized cooling in the Dry Valleys region may also be occurring (5), it is timely to document this endangered biome.

We identified four distinct microbial communities as chamosoliths, endoliths, hypoliths, and in bulk soil that occurred concomitantly in polygonal terrain of McKelvey Valley. Lithic communities were dominated by different cyanobacteria, and overall, diversity spanned 16 phyla. This level of diversity suggests a greater ecosys- tem complexity in the high inland Dry Valleys than previously appreciated. Our data counters the view based on soil studies that cyanobacteria are restricted to wetter, more productive polar locations (16, 20); rather, we demonstrate that, under severe xeric stress, soil becomes too extreme, and the last refuge for life is in lithic niches where distinct communities develop.

The soil biota of McKelvey Valley was dominated by known radiation and desiccation tolerant taxa such as Deinococcus and Rubrobacter, and such adaptation has also been observed for moisture-impacted Dry Valley soils (13, 16). Nonpolar hyperarid soils in the Atacama Desert were instead dominated by Frankia-like actinobacteria at depths of 20–200 mm (21). The relatively low number of microorganisms and taxonomic evenness of the community, plus absence of primary producers, indicate that soil “communities” in McKelvey Valley may possibly represent a transient soil-borne inoculum rather than a stable community. Conversely, the specific occurrence of Chloroflexi in soils, a phylum known to be important in tundra soils (22), suggests a true soil microbial community may indeed exist. We did not investigate whether the deeper subsurface permafrost supported microorganisms.

The absence of Chroococcidiopsis in molecular and morpholog- ical examinations from hypolithon and most soils is surprising given that this cyanobacterium was the dominant taxon in sandstone substrates in our study and has been recorded as the main component of hypolithic communities in nonpolar deserts (23–25). A study focusing on cyanobacteria in soils of Beacon Valley also failed to generate amplicons using cyanobacteria-specific PCR primers, whereas they were commonly retrieved for soils of lower valleys supporting lakes with a presumed aquatic origin for phylotypes (11).

The absence is unlikely to be a result of UV or desiccation stress, because it is one of the most radiation- and desiccation-tolerant organisms known (26). Rather, we suggest, in light of the fact that soils were also the only substrate that did not support algae, that this inland soil presents an environment that somehow precludes significant phototrophic colonization. This finding has major implications for productivity in such inland valleys, because it makes the isolated “islands” of lithic colonization the only significant source of primary production. The reasons for this absence are unclear. We did not identify levels of any soil abiotic variable likely to inhibit photosynthesis per se. We suggest that, in addition to increased exposure to incident light, highly xeric conditions, and lack of thermal buffering in soils, the relative instability of the soil substrate may also hinder colonization given the slow colonization rates for Antarctic microorganisms (27). Therefore, an upward revision of standing biomass and productivity in the Dry Valleys is warranted, because previous estimates have been based largely on soil and aquatic biota (2).

It has been previously shown that Antarctic lakes are a significant source of inoculum in lower lake-bearing valleys, with soils, hylo- liths, and aquatic niches supporting the same cyanobacteria (11). There may also be an aquatic origin for oscillatory hypoliths in our study, because they are common aquatic Antarctic cyanobacteria, although there are no lakes in the immediate vicinity of the study site (the closest, Lake Vashka, is >10 km distant). This finding suggests that lake dispersal reaches deep inland to McKelvey Valley, so why are they not also colonists of soil and sandstone niches? This absence may be explained by viewing these cyanobacteria as opportunists. They were absent from soil and sandstone, yet occurred on quartz in soil where entrapment of wind-dispersed lake-derived organic matter containing cyanobacterial and algal inoculum can be envisaged, and the microenvironment is known to be more favorable than surrounding soil (24, 28). Therefore, relatively rapid colonization under favorable conditions could occur, whereas for sandstone ingress on an exposed substrate and the microclimate may be too challenging (29).

The lichens recorded in our study displayed little similarity in terms of community diversity with sandstone endoliths from Beacon sandstone recovered 24 years ago (30). Both mycobiont and phycobiont phylotypes in our study affiliated with different genera as did cyanobacteria. This identification may indicate the existence of multiple lichen associations in the Dry Valleys; or more specu- latively, our study may indicate Antarctic lichens reflect climatic warming trends and are becoming less unique, because the morphology and cyanobacterial composition of our lichens more closely resembled those recorded for alpine regions of Europe (31).

Nitrogen fixation has been demonstrated by Chroococcidiopsis-dominated communities from alpine endoliths of gyspum (32), whereas others conclude that polar and other endoliths largely used abiotic combined nitrogen sources (27). Data also indicated phylotypes indicating diazotrophic cyanobacterial, alpha proteobacte- rial and actinobacterial taxa. This can be viewed as a useful adaptation in this nitrogen-poor Dry Valleys location. All niches in McKelvey Valley supported a putative heterotrophic bacterial component presumably supported by microbial carbon and nitrogen input.

The lack of significant community variation among samples for a given substrate and between locations reflects the high selective pressure in these high inland sites where environmental stresses are exacerbated compared with lower valleys (6). It also illustrates that our study may be broadly applicable to the inland Dry Valleys in general due to reduced heterogeneity. Across all substrates, salinity-related factors were influential to community structure. It has been suggested that salinity may act as a stressor in certain dry valleys (33), although in our study, soils did not display inhibitory levels of soluble salts. Our multivariate analysis points to a complex interaction of salinity, carbon, moisture, and other variables in this ecosystem.
We used multiple approaches to attempt recovery of archaeal signatures from soils, quartz and sandstone, but all proved unsuccessful. It has been suggested that archaea are unable to tolerate the environmental stress in extreme xeric environments (34), and this inability may explain their absence. Other Dry Valleys studies have not generally focused on archaea, although they were also concluded to be absent from an endolith recovered from the Asgard Range (30). Given the high degree of environmental heterogeneity observed in Antarctic soils (2), their occurrence may yet be recorded elsewhere in the Dry Valleys biome where less moisture stress is experienced.

It has been estimated that polar endoliths are exceptionally persistent over geological time periods (35). Endoliths support the greatest diversity of phylotypes, shared the greatest number of phylotypes with other niches, and they are very long-lived. Therefore, we propose that they act as a reservoir for terrestrial microbiota. This notion is supported by recent isotopic evidence indicating that some organic matter in Dry Valleys soils remote from sources of liquid water has an endolithic origin (36). Weathering of sandstone is accelerated by endolithic colonization (10), and therefore, can be envisaged to disperse endolithic taxa, although in a relatively slow manner. The relatively rare occurrence of endoliths may restrict the volume of inoculum released, but this dispersal may nonetheless represent an important source over time in high inland valleys where hydroterrestrial taxa may also be dispersed but are unable to proliferate. The occurrence of algae from the usually lichenized endolithic Trebouxiophyceae in McKelvey Valley hypolithon without a mycobiont may further indicate local dispersal of endolithic taxa.

Recovery of cyanobacterial phylotypes, including first records for these substrates, allowed comparison with several other deserts worldwide due to ubiquity of this phylum in desert lithic niches. A clear separation among phylogenetic lineages from Antarctic and nonpolar desert locations within the Pleurocapsales, Nostocales, and Oscillatoriales was evident. This pattern may indicate isolated and regionally seeded cyanobacterial populations occur. This idea supports the view that global occurrence among terrestrial soil bacteria is determined primarily by localized factors (37, 38), although the Antarctic is exposed to globally dispersed aerosols that likely disseminate microbial propagules in a near-ubiquitous manner (39). We suggest that extreme xeric low-nutrient environments such as deserts minimize the possible interference of abiotic variables in biogeographic studies, and thus, lithic communities offer a useful model for further testing hypotheses related to biogeography in microbial ecology.

We recovered a limited number of bacterial and fungal phylotypes with low phylogenetic affiliation to any known taxon from other deserts or substrates worldwide. This observation indicates novel diversity at a high taxonomic level in inland Dry Valleys despite the environmental stresses exceeding maritime and lake-influenced Antarctic locations where similar claims for fungi (17) and cyanobacteria (11) have been made. Some records also point to a physiological plasticity among Antarctic taxa, for example, we identified basidiochymic yeasts in endolithic niches yet these taxa also occurred as colonists of archeological wood in the Antarctic (17).

We have characterized highly-specialized communities inhabiting multiple lithic and soil niches in the inland region of the most extreme cold desert on Earth. This diversity is in contrast to apparently more homogeneous communities in the lower, wetter Dry Valleys. The occurrence of location-specific cyanobacterial lineages may be a result of lithic niches acting as local reservoirs for dispersal of microbial biomass. The specialized communities and possible endemism for certain phyla, together with climate-change related threats, emphasize the conservation value of the inland Dry Valleys ecosystem. Ironically, the frequency of hypolithic colonization may have potential as a bioindicator of climate change given that a comparison of colonization frequency in this study with maritime polar (40) and nonpolar deserts (24, 41) suggests that landscape-scale patterns may be closely related to climatic variables. Additional relevance lies with environmental similarities between polar regions on Earth and Mars during recent history and the implications for habitability of Mars (42).

Materials and Methods

Field Sampling. The high inland McKelvey Valley (central valley coordinates 77°26’ S, 161°33’ E) was surveyed during Antarctica New Zealand event K0218 in January 2008. Frost polygons of 40 m² average area were used as in situ quadrats. All sandstone and quartz substrate was surveyed, and frequency of colonization for chasmoliths, endoliths, and hypoliths was recorded. Epilithic lichens were not observed. Soil samples were taken after removing the topmost 2.5 cm of loose soil to minimize transient particles in sampling and be consistent with the average depth of hypolithic colonization. A total of 10 randomly-selected and nonadjacent polygons were surveyed (five on a south-facing slope used in rRNA and ITS studies, plus five additional polygons from a north-facing slope for ITS studies). A single polygon from the southern slope that supported multiple chasmolithic, endolithic, and hypolithic colonization was selected for diversity comparison between niches. Soil and rock samples were sampled aseptically and stored in sterilized plastic containers with no headspace at −80 °C until processed.

Abiotic Variables. A suite of 18 abiotic variables, including moisture content, porosity, pH, Soluble salts, total organic carbon, total nitrogen, and metals, were measured for each substrate. Ambient temperatures remained below freezing throughout the sampling period in January 2008 although solar heating of ground created isolated patches of snowmelt. Long-term climate data are available at the following link: www.scar.org. Moisture content and total organic content in soils and rocks were measured gravimetrically after heating to 100 and 450 °C, respectively. Rock porosity was measured by vacuum displacement. Soluble salts and pH were measured by potentiometric determination. Total carbon and nitrogen were determined using a thermal conductivity detector at 900 °C. All elemental tests were conducted after air-drying and nitric/hydrochloric acid digestion using ICP-MS according to the Environmental Protection Agency 200.2 for soils or using the EDX elemental scanning function during scanning electron microscopy of rock surfaces.

Recovery of Environmental DNA and Target Loci. Recovery of environmental DNA used a protocol optimized for lithic microorganisms (24). PCR amplification of rRNA genes was carried out using domain-specific forward primers for bacteria (43), eukarya (44), and archaea (22), and universal reverse primers (43, 45). Alternative sets of archaea-specific primers were also tested (46). The ITS region was amplified using rRNA gene-specific primers from flanking regions (47, 48).

Real-Time Quantitative PCR. PCR amplification was quantified in real-time (Prism 7000; Applied Biosystems) by fluorometric monitoring with SYBR Green 1 dye (Invitrogen). All standard curves were constructed using plasmids from cloned rRNA genes (Qiagen) separately for archaea, bacteria, and eukarya.

Terminal RFLP. Restriction digests (MspI for 16S rDNA, HaellI for ITS) of FAM-labeled PCR amplicons were subjected to fragment analysis by capillary electrophoresis (3730 Genetic Analyzer; Applied Biosystems). The software Perl and R were used to identify true peaks and bin fragments of similar size (49). A virtual digest using Haelli and MspI was carried out on the sequences retrieved from the bacterial and eukaryal clone libraries. This analysis allowed the assignment of phylogenetic identity to individual peaks.

Clone Library Construction and Sequencing. Samples were selected for clone library construction (PCR Cloning™ kit; Qiagen) based on those with t-RFLP profiles most similar to other samples for a given substrate. Transformants were screened using RFLP (MspI, Haelli, and Cfo I) before automated sequencing (3730 Genetic Analyzer; Applied Biosystems). Phylotypes were delineated on the basis of 97% sequence similarity using the freeware DOTUR (50). All sequences generated by this study have been deposited in the National Center for Biotechnolog Information GenBank database under accession numbers FJ490210-F490344 and FJ895084-FJ895089. Screening for possible chimeric sequences was made using Chimera.Check (http://rdp.cme.msu.edu). Approximate phylogenetic affiliations were then determined by BLAST searches of the National Center for Biotechnology Information GenBank database (http://www.ncbi.nlm.nih.gov). Estimates of clone library sampling effort were made using the freeware EstimateS (51). Sampling effort was assessed by calculation of Coverage and Rarefaction curves, estimates of library richness were made using the nonparametric estimators ACE and Chao 1.
Phylogenetic Analyses. Multiple alignments were created with reference to selected GenBank sequences using BioEdit v.7.0.9.0 (52). The alignments were tested against prescript models of evolution using the softwares PAUP* 4.0b10 (53) and Modeltest v3.0 (54). The criteria described by the most appropriate evolutionary model were input for maximum likelihood analysis using Genetic Algorithm for Random Likelihood Inference (GARLI) Version 0.96 Beta (55). Robustness of furcated branches was supported by both bootstrap values (1,000 replicates) determined using PAUP* 4.0b10 and Bayesian posterior probabilities (56) calculated using Bayes v3.0b4 (57). Values (in percentage) were shown on all branch nodes supported by >50% of the trees.

Statistical Analyses. Alpha diversity indices (Shannon’s Index, Simpsons Diversity Index, and Pielou’s Evenness) were calculated using untransformed data. Phylogenetic Analyses. Divergence of the degree of phylogenetic differentiation between communities was expressed by the FST statistic (58) using the software Arlequin v3.0 (59). TD indices were calculated to reflect phylogenetic diversity within populations (60). Divergence statistics were calculated using one-way and two-way ANOVA, AMOVA, or analysis of similarity (ANOSIM). Multivariate analysis of diversity data were performed on square-root transformed diversity data, and on nontransformed normalized data for environmental variables. Nonmetric multidimensional scaling ordinations (NMDS) were used to visualize Bray Curtis Similarities (diversity data) and Euclidean Distances (environmental data). In BEST analyses, the BIO-ENV procedure was used to maximize the rank correlation between biotic and environmental data; thereby, establishing a ranking (α) for the effects of environmental variables on diversity. All analyses were performed using PRIMER 6.1.6(61). All results stated as significant have a confidence level of P < 0.05 unless stated otherwise.

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