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Evidence of species recruitment and development of hot desert hypolithic communities

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Summary

Hypoliths, photosynthetic microbial assemblages found underneath translucent rocks, are widely distributed within the western region of the Namib Desert and other similar environments. Terminal restriction fragment length polymorphism (T-RFLP) analysis was used to assess the bacterial community structure of hypoliths and surrounding soil (below and adjacent to the hypolithic rock) at a fine scale (10 m radius). Multivariate analysis of T-RFs showed that hypolithic and soil communities were structurally distinct. T-RFLP-derived operational taxonomic units were linked to 16S rRNA gene clone libraries. Applying the ecological concept of 'indicator species', six and nine indicator lineages were identified for hypoliths and soil, respectively. Hypolithic communities were dominated by cyanobacteria affiliated to Pleurocapsales, whereas actinobacteria were prevalent in the soil. These results are consistent with the concept of species sorting and suggest that the bottom of the quartz rocks provides conditions suitable for the development of discrete and demonstrably different microbial assemblages. However, we found strong evidence for neutral assembly processes, as almost 90% of the taxa present in the hypoliths were also detected in the soil. These results suggest that hypolithons do not develop independently from microbial communities found in the surrounding soil, but selectively recruit from local populations.

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Introduction

The Namib Desert in South West Africa is considered to be the world's most ancient desert and has substantially varied ecotopes including gravel plains, dunes, inselbergs, escarpments and playas (Eckardt and Drake, 2011). This desert spans a longitudinal distance of over 200 km, stretching from the western coastline to the eastern mountains along the Tropic of Capricorn. The Namib has been classified as an arid zone with some regions demonstrating hyperarid characteristics (Eckardt *et al.*, 2012). The desert surface is subject to wide temperature fluctuations (from 0°C to as high as 50°C) with a general increase from the coast inland. Rainfall patterns within this desert are scant and erratic, with long periods of aridity (Eckardt *et al.*, 2012).

The undersides of rocks in climatically extreme deserts, such as the Namib, act as a refuge for microorganisms (defined as 'hypoliths') and their community (the 'hypolithon') (Chan *et al.*, 2012; Pointing and Belnap, 2012). The overlying rock creates a favourable sub-lithic microhabitat where microorganisms benefit from greater physical stability, desiccation buffering, increased water availability and protection from UV fluxes (Pointing *et al.*, 2009; Cowan *et al.*, 2010). As they are typically dominated by primary producers (Cockell and Stokes, 2004; Wood *et al.*, 2008) hypolithic communities are thought to be significant contributors to regional carbon and nitrogen inputs (Burkins *et al.*, 2001; Cowan *et al.*, 2011).

Previous studies have suggested that hypolithons develop independently from surrounding soil communities (Warren-Rhodes *et al.*, 2006; Pointing *et al.*, 2007; Davila *et al.*, 2008; Tracy *et al.*, 2010). However, data on the mechanisms of community assembly leading to site-to-site variations (beta diversity) in community composition in deserts remain scant. Recently, Caruso and colleagues (2011) reported that deterministic and stochastic processes interact in the assembly of hypolithons on a global scale. However, the drivers of bacterial beta diversity are known to depend on both spatial (Martiny *et al.*, 2011) and temporal scales (Langenheder *et al.*, 2012; Lindström and Langenheder, 2012). For example, dispersal limitation was found to drive Nitrosomondales beta diversity at the

scale of an individual marsh (Martiny *et al*., 2011). In direct contrast, the environment was the most important factor in explaining differences between these communities across regional and continental scales (Martiny *et al*., 2011). These differences highlight the need to identify the patterns and mechanisms that shape bacterial community composition in different habitat types and at different spatial scales.

Here, we apply the ecological concept of 'indicator species' (Dufrene and Legendre, 1997) to interrogate the process behind hypolithic community assembly at a microscale (10 m radius), and present strong evidence that in the Namib Desert recruitment from soil sources supports hypolithic community assembly. We predict that if deterministic processes are strong, hypoliths and surrounding soil should demonstrate greatly dissimilar bacterial communities (specialists). If the effect of the environment is limited, both hypolith and surrounding soil should contain similar bacterial communities (generalists).

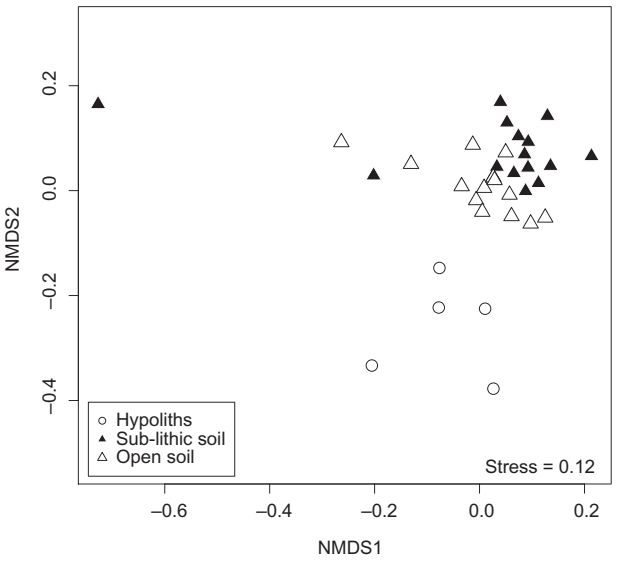

Fig. 1. NMDS ordination plot (Bray–Curtis distance matrix) of T-RFLP profiles for soil- and hypolith-derived samples. The quality of the ordination is indicated by a low-stress value.

Results and discussion

The comparative bacterial composition of hypolithic and nearby soil samples at a desert site in the hyperarid Namib Desert was assessed using terminal restriction fragment length polymorphism (T-RFLP) analysis and clone libraries (see *Supporting information* for materials and methods). A total of 98 T-RFs were obtained, ranging from 23 to 44 operational taxonomic units (OTUs) for the individual samples. When averaged for the different sample types, hypoliths and surrounding soil contained similar numbers of OTUs, with values of 22.0 [± 4.7 (SD)], 25.5[± 7.2 (SD)] and 30.3 [± 6.7 (SD)] for hypoliths, open soil and sub-lithic soil, respectively. Shifts in OTU composition (beta diversity) revealed that five OTUs were unique to the hypoliths, 10 were unique to the open soil and 29 were unique to sub-lithic soil (Fig. S1). In total, 38 OTUs (38% overlap) were shared between hypolith and soil samples.

When bacterial community patterns were visualized by non-metric multidimensional scaling (NMDS) of Bray–Curtis similarities, communities grouped separately according to their habitat (Fig. 1). Similar results were obtained after accounting for the unequal number of samples by applying a random resampling procedure (Fig. S2). When habitat type, depth and the interaction between both factors were assessed in an *adonis* model (PERMANOVA analysis), habitat was found to have a significant effect ($F_{2,28} = 4.82$, $P = 0.001$). Each group was clearly distinct (hypoliths vs. sub-lithic soil $R^2 = 0.26$, $P = 0.001$; hypoliths vs. open soil $R^2 = 0.30$, $P = 0.001$; sub-lithic vs. open soil, $R^2 = 0.08$, $P = 0.02$); that is, the overlying quartz rocks not only influenced the hypolithon but also the soil bacterial community below the rock. Although differences between hypolithic and soil bacterial community structure have been reported in polar deserts (Pointing *et al*., 2009; Khan *et al*., 2011), similar observations have not been reported for hot desert communities. In contrast to previous studies of microbial communities (Zhou *et al*., 2002; Ge *et al*., 2008), no spatial variation on vertical axes was observed, although these studies were performed on a broader scale and bacterial community patterns are known to depend on both spatial and resource factors (Zhou *et al*., 2002; Martiny *et al*., 2011).

In order to relate OTU abundance and habitat type, a multivariate regression tree (MRT) analysis was performed. Habitat type alone explained 10% of the variation observed. Indicator OTUs identified using the IndVal indexes were mainly responsible for the topology of the tree (Fig. 2a) suggesting that these specialist lineages represented ecological indicators of the prevailing environmental. Overall, six and nine OTUs were found to be statistically significant indicators of the hypoliths and surrounding soil respectively ($P < 0.05$) (Fig. 2b).

Clone libraries yielded a total of 85 unique, non-chimeric sequences, of which 33 and 52 clones were sequenced from hypolith and soil, respectively (Table S1). Phylogenetic analysis of the clone libraries was consistent with multivariate analysis of the T-RFLP profiles. Both F_{ST} and *P*-tests were significant (not shown), indicating a lower genetic diversity within each community than for two communities combined and that the different communities harboured distinct phylogenetic lineages (Martin, 2002). Rarefaction curves and Chao 1 estimates indicated that sampling had approached an asymptote only for hypoliths (Fig. S3). In spite of the relatively low number of clones

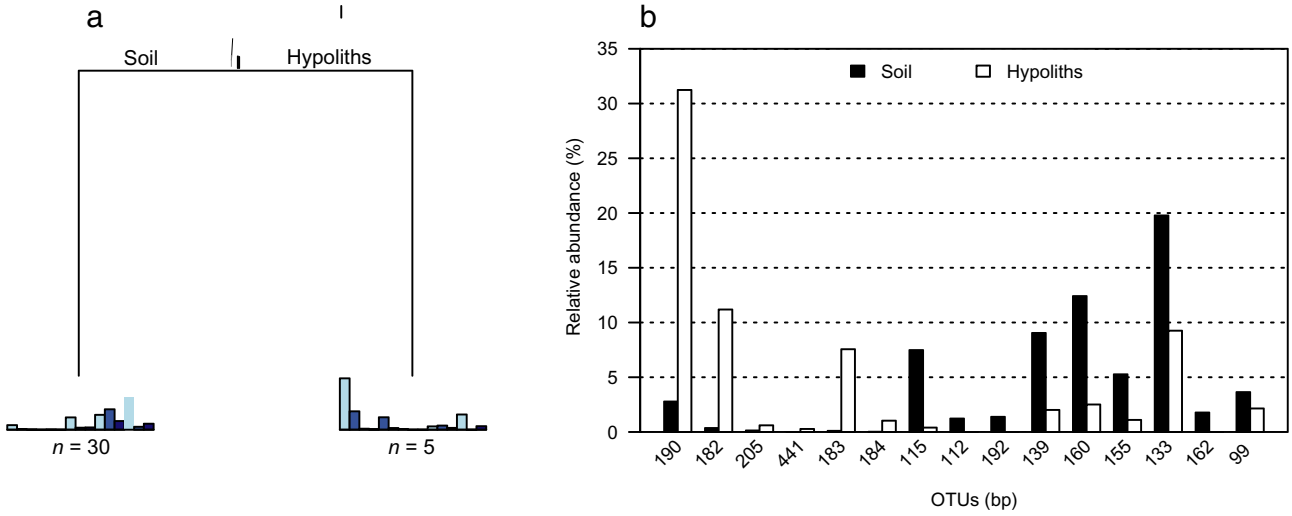

Fig. 2. Multivariate regression tree (MRT) analysis (a). The model explained 10% of the variance in the whole data set. Bar plot under each leaf (magnified in b) shows the contribution of the different indicator species. The number of samples included in the analysis is shown under bar plots.

sampled, this is not unexpected since previous studies have shown low phylogenetic diversity in hot desert ecosystems (Wong *et al*., 2010). The majority of the clones displayed homology to sequences retrieved from hot hyperarid deserts (Table S1). Nonetheless, only six OTUs showed identity values higher than 97%, indicating that the majority of sequences might represent novel taxa.

Soil samples were dominated by the phyla Actinobacteria (49%) and Proteobacteria (21%). Acidobacteria, Cyanobacteria, Bacteroidetes and Chloroflexi phylotypes were detected in lower numbers (Figs S4 and S5). Members of these phyla are generally among the most common inhabitants of soils (Fierer and Jackson, 2006; Jones *et al*., 2009; Lauber *et al*., 2009). Clones derived from hypoliths were affiliated to the phylum Cyanobacteria (85%) dominated by *Chroococcidiopsis* lineages (order Pleurocapsales), although members of the orders Oscillatoriales, Stigonematales and Chroococcales were also observed. *Chroococcidiopsis* has been identified as one of the common primary producers occurring in both hot and cold deserts (Tracy *et al*., 2010; Bahl *et al*., 2011; Caruso *et al*., 2011; Lacap *et al*., 2011). Other phyla represented in the hypolithic clone library included Acidobacteria (2.9%), Proteobacteria (2.9%), Actinobacteria (2.9%) and unclassified bacteria (3%). A total of 60 (out of 98) T-RFLP-defined OTUs were matched to 16S rRNA gene sequences resulting in an overall assignment of 61%.

We found that hypolithic and surrounding soil indicator species were identified as cyanobacteria and actinobacteria, respectively. If indicator lineages play a pivotal ecological role within the habitat (Auguet *et al*., 2010), these results support the view that cyanobacteria are among the most important functional groups in hypoliths (Cowan *et al*., 2011). Cyanobacteria are ubiquitous in most terrestrial habitats and have central ecological roles in energy transduction, nitrogen fixation and as pioneer species (Whitton and Potts, 2000).

Only five OTUs were exclusive to hypolithic samples and the most abundant OTUs were present in both soil and hypolithic samples. This is somehow consistent with neutral theory predictions (Hubbell, 2001) that assume species are ecologically equivalent. Thus, the compositions of local communities are regulated only by chance without considering deterministic factors (intra-specific competition or niche differentiation). Although these assumptions are still controversial, there is empirical evidence that both deterministic and stochastic processes shape the structure of microbial communities (Dumbrell *et al*., 2010; Ofiteru *et al*., 2010; Caruso *et al*., 2011; Langenheder and Szekely, 2011). Notably, a global-scale study of hypolithic communities found that neutral models failed to show evidence of deterministic processes when cyanobacteria and heterotrophic bacteria were analysed separately, whereas species co-occurrence was non-random when both groups were analysed together (Caruso *et al*., 2011). The global study of Caruso and colleagues identified demographic stochasticity as a major factor influencing community assembly, and here we present evidence that stochasticity also plays a pivotal role in local community assembly. Since 88% of the OTUs observed in hypolithic community samples were also found in soil it is most likely that a great proportion of taxa that 'seeded' hypolithons were recruited from the surrounding soil. It is also possible that a common source (e.g. aeolian transport) seeded both soil and hypolithic communities. In any case, under the assumptions of

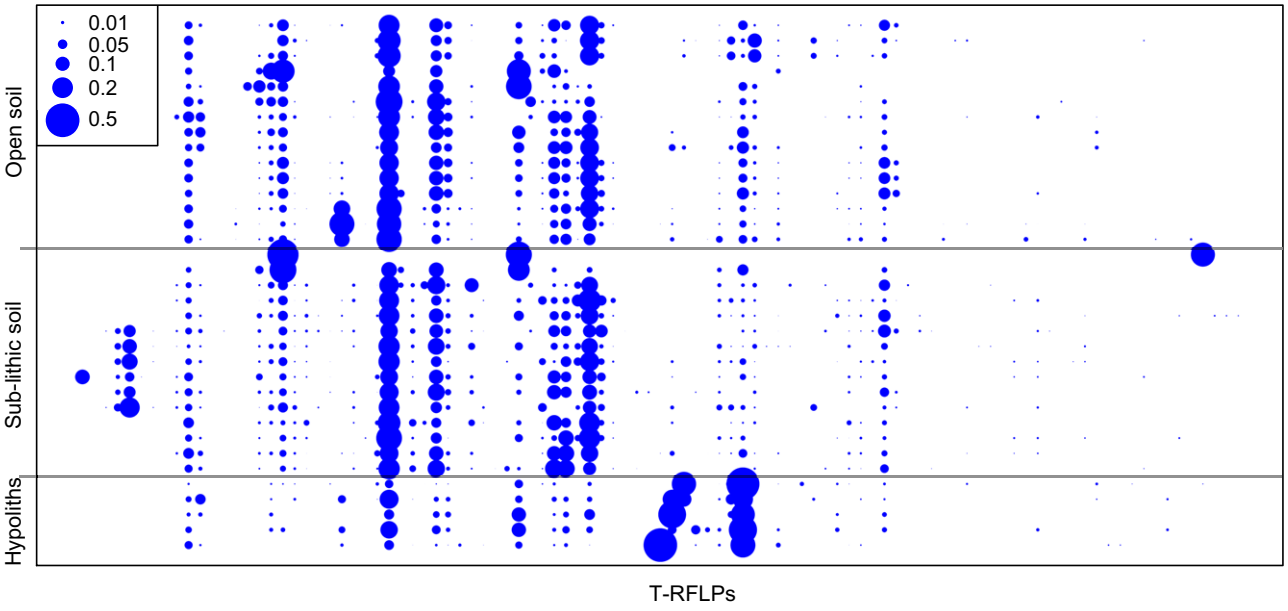

Fig. 3. Bubble plot. Each bubble represents one T-RF (columns) and is sized according to its relative abundance in the sample (rows).

neutral theory it might be expected that taxa composition and abundance should be approximately the same in hypoliths and in soil (Sloan *et al*., 2006; Ostman *et al*., 2010). As has been observed previously in rock pools seeded by rainfall water (Langenheder and Szekely, 2011) or lakes seeded by soils (Crump *et al*., 2012), we found that most abundant taxa in the soil were also present in hypoliths albeit in lower abundance (Fig. 3). Nevertheless, this was not always the case as demonstrated by the presence of indicator species (Fig. 2b). Consequently, the neutral theory failed to explain all the variation found in the bacterial community structure. In fact, cyanobacteria and actinobacteria were overrepresented in hypoliths and surrounding soil, respectively, suggesting that deterministic processes (habitat filtering) are also important.

We suggest three non-exclusive reasons for the relatively weak deterministic effect. First, it could reflect a limitation of the technique (i.e. T-RFLP), as it is well known that fingerprinting methods only target the most abundant taxa (Bent and Forney, 2008). Second, critical deterministic elements of local environmental conditions in hypoliths and surrounding soil at the Namib study site may not differ significantly (temperature and % relative humidity values are shown in Fig. S6). Finally, high dispersal rates (source-sink dynamics) (Cottenie, 2005) could buffer the effect of selection by continued homogenization of the communities involved. Indeed, there was a high degree of overlap between the soil and hypolithic communities (Figs 3 and S1). It is important to note, however, that non-neutral processes such as intra-species interactions, invariance under assemblage or the complexity of ecological interactions and the 'melting' of competitive hierarchies can generate neutral patterns (Alonso *et al*., 2006). Clearly, more focused research is required in order to explain the differences in microbial community structure between hypoliths and soil.

Metacommunity studies typically relate assembly processes to the entire community and do not take into account different categories of species. However, it has been shown for aquatic bacteria that habitat specialists and generalists have different population dynamics (Shade *et al*., 2010). Co-occurrence patterns were also found for soil microbial communities (Barberan *et al*., 2011). More important, habitat generalist and specialist have been shown to differ in their respective contributions to ecosystem functioning (Gravel *et al*., 2011).

In conclusion, the presence of generalist lineages indicates that Namib hypolithic bacterial communities did not develop independently from the surrounding soil. This is in contrast to some hyperarid Antarctic hypoliths where cyanobacteria-dominated hypolithon occurs in soils where cyanobacterial signatures were undetectable by sequence analysis of environmental clone libraries (Pointing *et al*., 2009). Similarly, in the hyperarid Atacama Desert hypoliths occur in soils devoid of recoverable cyanobacteria, although other reservoirs of cyanobacteria exist in this desert within deliquescent minerals (Davila *et al*., 2008). The significant fog moisture input to our Namib study site may be a factor affecting microbial diversity in soil reservoirs, and the extent to which aridity affects this will be a fruitful area for future work. In our study we provide empirical evidence that cyanobacteria are indicator species (specialists) for hypoliths,

suggesting that both habitat filtering and stochastic processes shaped the assembly of hypolithic bacterial communities in the Namib. Since specialist assemblages seem to be more productive (Gravel *et al*., 2011) and more susceptible to extinction than generalists when habitat conditions are altered (Tilman *et al*., 1994), these results have implications for habitat conservation in drylands that support hypoliths. Our study suggests that future investigations of hypoliths could exploit our finding that cyanobacteria are indicator taxa and focus more closely on this component to infer ecological patterns.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Supplementary materials and methods.

Fig. S1. Venn diagram comparing the distribution of bacterial T-RFLP fragments between hypolith and soil samples.

Fig. S2. NMDS generated from random 'resampling'. This was applied in order to demonstrate that the effect of sample size does not influence the overall structure of hypolithic and soil bacterial communities. Code indicators: circles (hypolithons), filled triangles (sub-lithic soil) and empty triangles (open soil). Twelve randomizations are depicted.

Fig. S3. Hypolithic (A) and soil (B) clone library coverage (Good's and CACE) and estimator (Schao1 and SACE) plots.

Fig. S4. Clone libraries distribution for both hypoliths and soil communities.

Fig. S5. Maximum likelihood tree of eubacteria (A) and cyanobacteria (B). Phylotypes recovered during this study are shown in bold type. NCBI GenBank accession tree topologies are supported by Bayesian posterior probabilities (first number) and bootstrap values for 1000 replications (second number). Code indicators: triangles (hypolithons), squares (soil).

Fig. S6. Temperature (A) and % relative humidity (RH) (B) over a 6 month period at the sampling location. Data were acquired at 5 min intervals.

Table S1. BLASTN results against the NCBI database.

Supplementary Material for

Evidence of species recruitment and development of hot desert hypolithic communities

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This pdf contains:

Materials and Methods

Field site, sample collection, and soil chemical analysis

The study was conducted close to the Gobabeb Training and Research Centre. Samples were collected within a 10 m radius site (S 23°32.031', E 015°01.813'). At each of the 5 discrete sampling points, one hypolith and 6 soil samples, at 0 - 10, 10 - 20 and 20 - 30 cm below the hypolith (hereafter, sub-lithic) and at 0 - 10, 10 - 20 and 20 - 30 cm one meter from the hypolith (hereafter, open soil) (see figure below), were aseptically collected. Hypolithic biomass was recovered by scraping adherent material from the rock sub-surface. Samples (5 hypolithic, 15 sub-lithic and 15 open soil), were transported to the laboratory, homogenized with a sterile spatula, transferred into 2 ml tubes and frozen at -80°C until further use.

The annual mean rainfall at Gobabeb (from 1962 to 2010) was 25 mm (Eckardt *et al.*, 2012), and fog events, which are common in a zone from the coast to ca. 60 km inland (Eckardt *et al.*, 2012), are thought to be the dominant source of bioavailable water in the region (Budel et al., 2009).

Rocks were generally small (40-80 mm) and thin (20-60 mm), and transmission values across the visible spectrum ranged from 0.4 to 14%.

The physico-chemical properties of soil from which sampling was conducted were as follows:

Soil Type	Sand
pH (KCl)	7.0
% C	0.09
% N	0.016
Na^+ (mg/kg)	160.93
K^+(mg/kg)	164.21
Ca^+ (mg/kg)	2793.44
Mg^+ (mg/kg)	93.22

Values are presented as means of five samples.

Field measurements of micro climatic data

In situ micro-environmental data [relative humidity [(%RH) and temperature (ºC)] were recorded, using Thermochron/Hygrochron iButtons (model DS1923, Embedded Data Systems). iButtons were positioned beneath hypolithic quartz rocks at the soil surface. Measurements were recorded automatically every 5 min over a 6-month period at different depths of (i.e. 0 - 10, 10 - 20, and 20 - 30 cm) (see supplementary figure 6).

DNA extraction

Metagenomic DNA was extracted from 0.5 g aliquots of hypolith and soil samples using the PowerSoil™ DNA Isolation Kit (MoBio, West Carlsbad, CA, USA) and following manufacturer's instructions. Concentrations of DNA yield were determined using a Nanodrop ND-1000 UV-Vis spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

T-RFLP analysis

Terminal restriction fragment length polymorphism (T-RFLP) analysis was conducted using 16S rRNA gene primers 341F-FAM (5′-CCTACGGGAGGCAGCAG-3′; tetrahydrochloro 6-carboxyfluorescein) (Ishii and Fukui, 2001) and 908 R (5'-CCGTCAATTCCTTTRAGTTT -3' (Lane et al., 1985). PCR reactions were carried out in a Thermo Hybrid (Ashford, GB) in a standard 50 µl reaction containing 1 X PCR buffer [(10 X being 200 mM Tris pH 8.8, 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 1 % (w/v) Triton X-100)], 0.2 mM each dNTP, 0.5 µM of each primer, 0.2 U of Dream*Taq* polymerase (Fermentas, USA) and 10 ng of template DNA. Thermal cycling conditions were as follow; 5 min denaturation at 94 ºC followed by 30 cycles with denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, and elongation at 72 ºC for 105 s with a final elongation at 72 ºC for 10 min. Products were purified using the NucleoSpin Kit™ (Clonetech, Japan) and digested using HaeIII (Fermentas, USA). After a second purification, electrophoretic separation of restriction fragments was conducted using an ABI3130XL (Applied Biosystems, USA). T-RFLP profiles were analyzed using Peak Scanner 1.0 (Applied Biosystems, available online (https://products.appliedbiosystems.com). True peaks and fragments of similar size were identified and binned using the software R and Perl (Abdo *et al.,* 2006).

Statistical analyses

T-RFLP data reflecting relative OTU abundance were Hellinger-transformed (Legendre and Gallagher, 2001) and used to calculate Bray-Curtis dissimilarity matrices, which were further visualized using non-metric multidimensional scaling (NMDS). To account for unequal number of samples (5 hypoliths vs. 15 each soil type) we also performed a resampling procedure, taking 5 samples of each habitat type to achieve 100 randomly generated nMDS plots. Permutational multivariate analysis of variance (PERMANOVA), function *adonis* (vegan package for R), was performed to test for significant differences between sample groups (hypoliths, sub-lithic soil and open soil). MRT analysis (De'Ath, 2002) was used to determine correlations between bacterial community composition and habitat parameters (e.g. type and depth) (mvpart package for R). Indicator species analysis (IndVal index) (Dufrene and Legendre, 1997), which combines relative abundance and relative frequency of occurrence, was used to identify the species that were statistically significant indicators of the habitat type (labdsv package for R).

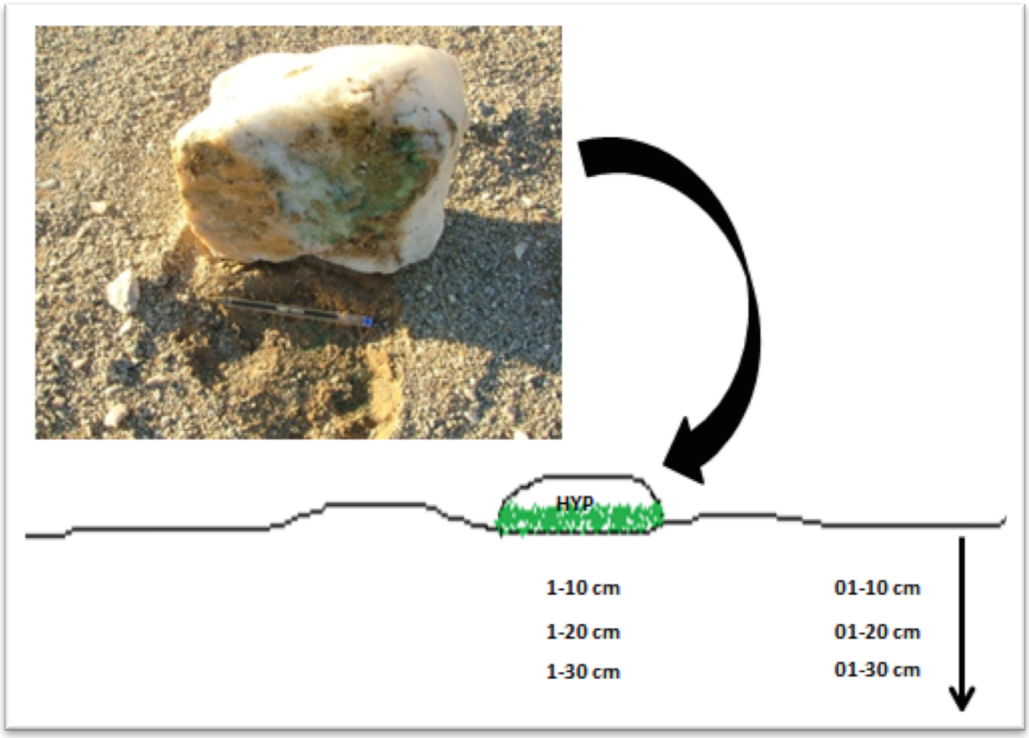
16S rRNA gene Clone library construction

Two clone libraries were constructed using primers 341F, without FAM-labeling, and 908R (conditions as above) for pooled hypolith and surrounding soil, respectively. Purified PCR amplicons were ligated to the pGEM-T Easy Vector System® (Promega Corporation, Madison, WI, USA), and transformed into Gene Hoggs® cells. The resulting plasmid vectors were isolated and purified using the Qiagen Miniprep kit (Qiagen, Valencia, USA) following the manufacturer's instructions. For each library, 200 clones were screened using ARDRA (RsaI and AluI, Fermentas) and the de-replicated clones were sequenced at the University of Stellenbosch Sequencing Facility (South Africa). Chimeric sequences were checked using the Chimera slayer implementation in Mothur (Schloss *et al.,* 2009).

Phylogenetic analysis

Phylogenetic affiliations of representative OTUs were determined using the Classifier tool (Wang *et al.,* 2007) at a confidence interval of 80 % (Ribosomal Database Project II, http://rdp.cme.msu.edu) (Cole *et al.,* 2009). Nucleotide sequences were aligned with references from GenBank database using ClustalX v.1.8.1 (Thompson *et al.,* 1997). Maximum Likelihood trees were constructed using Paup*4.0b10 (Posada, 2003) and GARLI (Genetic Algorithm for Rapid Likelihood Inference) (Swofford, 2003) as described previously (Lacap *et al.,* 2011). Arlequin v3.0 (Excoffier and Scheider, 2005) and Unifrac (Lozupone et al., 2006) were used to assess the phylogenetic differences between communities using the F_{ST} and P tests, respectively. Phylogenetic OTUs at a similarity level of 97 % were determined using CD-HIT (http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi?cmd=h-cd-hit-est). Diversity estimates

(Chao1) were calculated using an online tool (http://www.aslo.org/lomethods/free/2004/0114a.html) (Kemp and Aller, 2004). *In silico* predictions of terminal restriction fragments (T-RFs) were performed using TRF-CUT (Ricke et al., 2005). Sequence data have been submitted to NCBI GenBank database (accession numbers JN714842 - JN714926). All other analyses were conducted using R (http://www.R-project.org).



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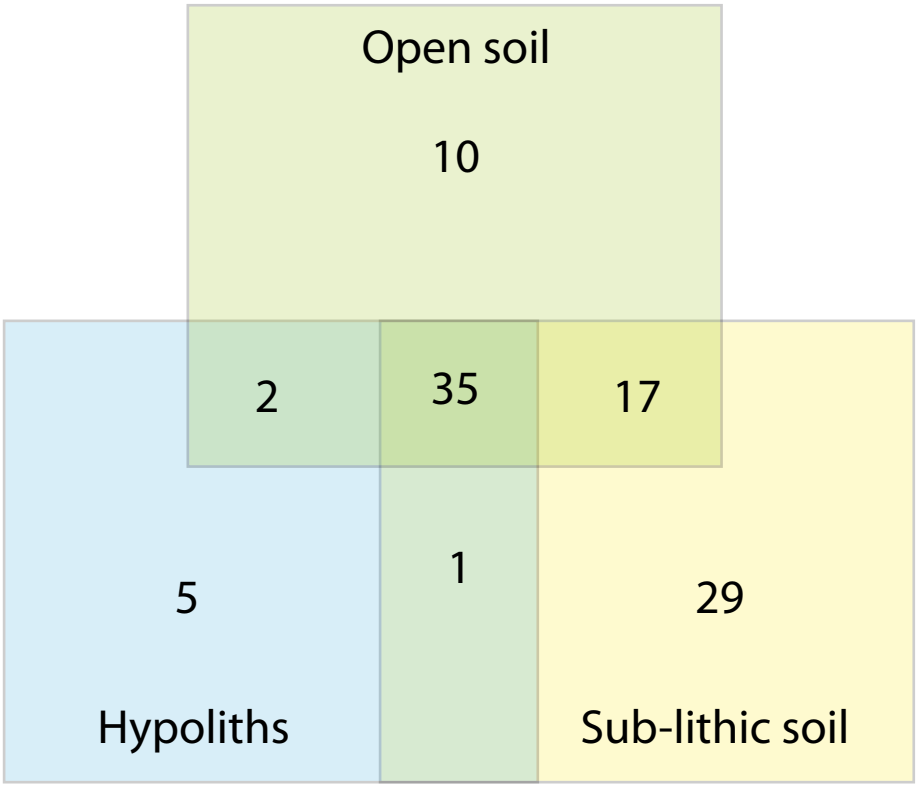
S1



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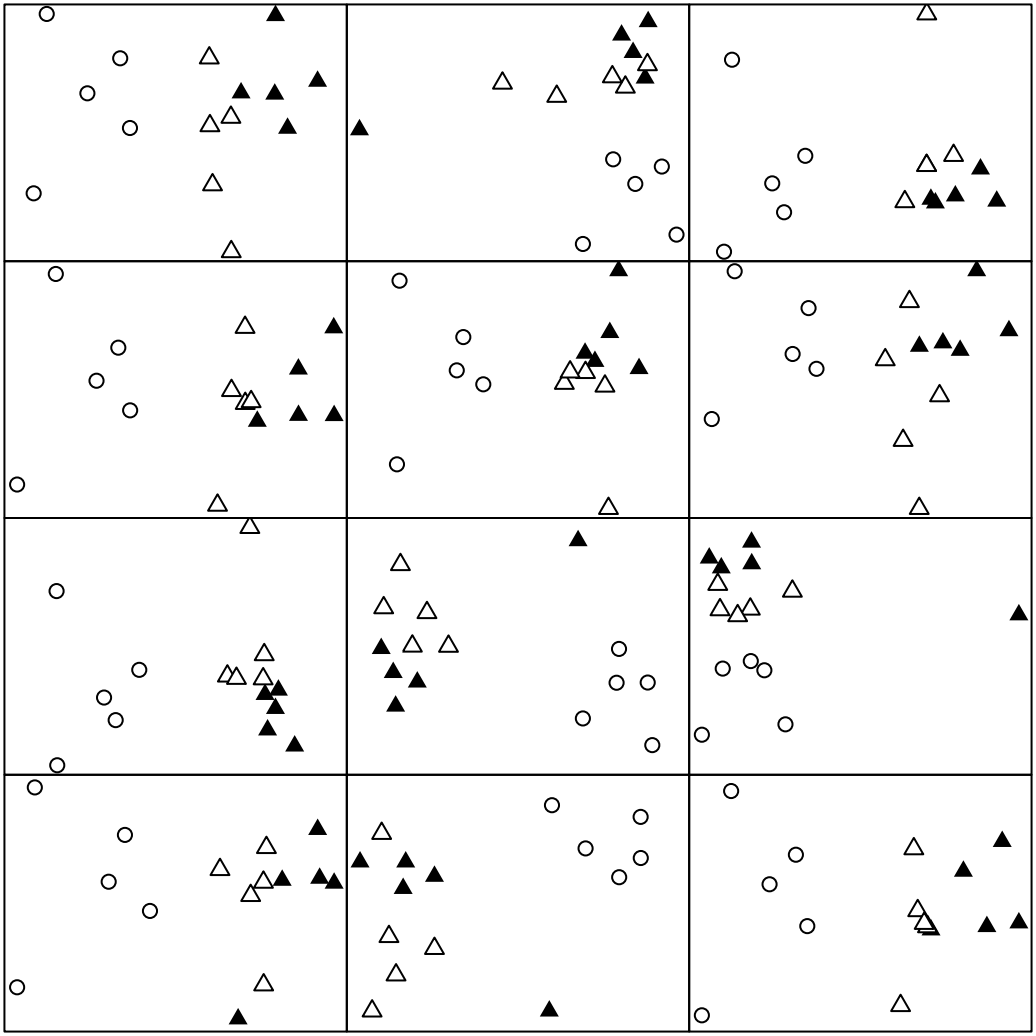

S3

A

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Number of phylotypes observed	30
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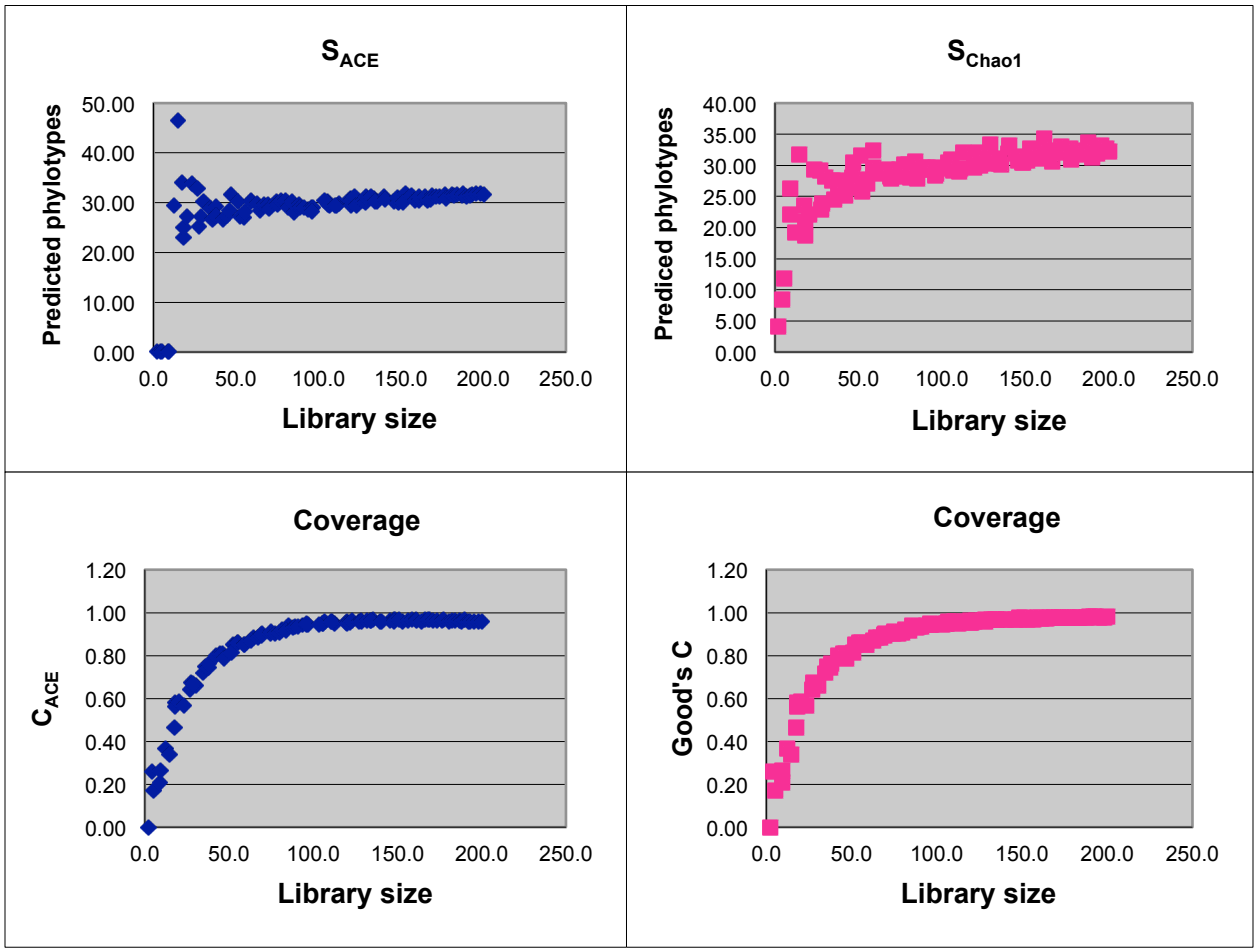
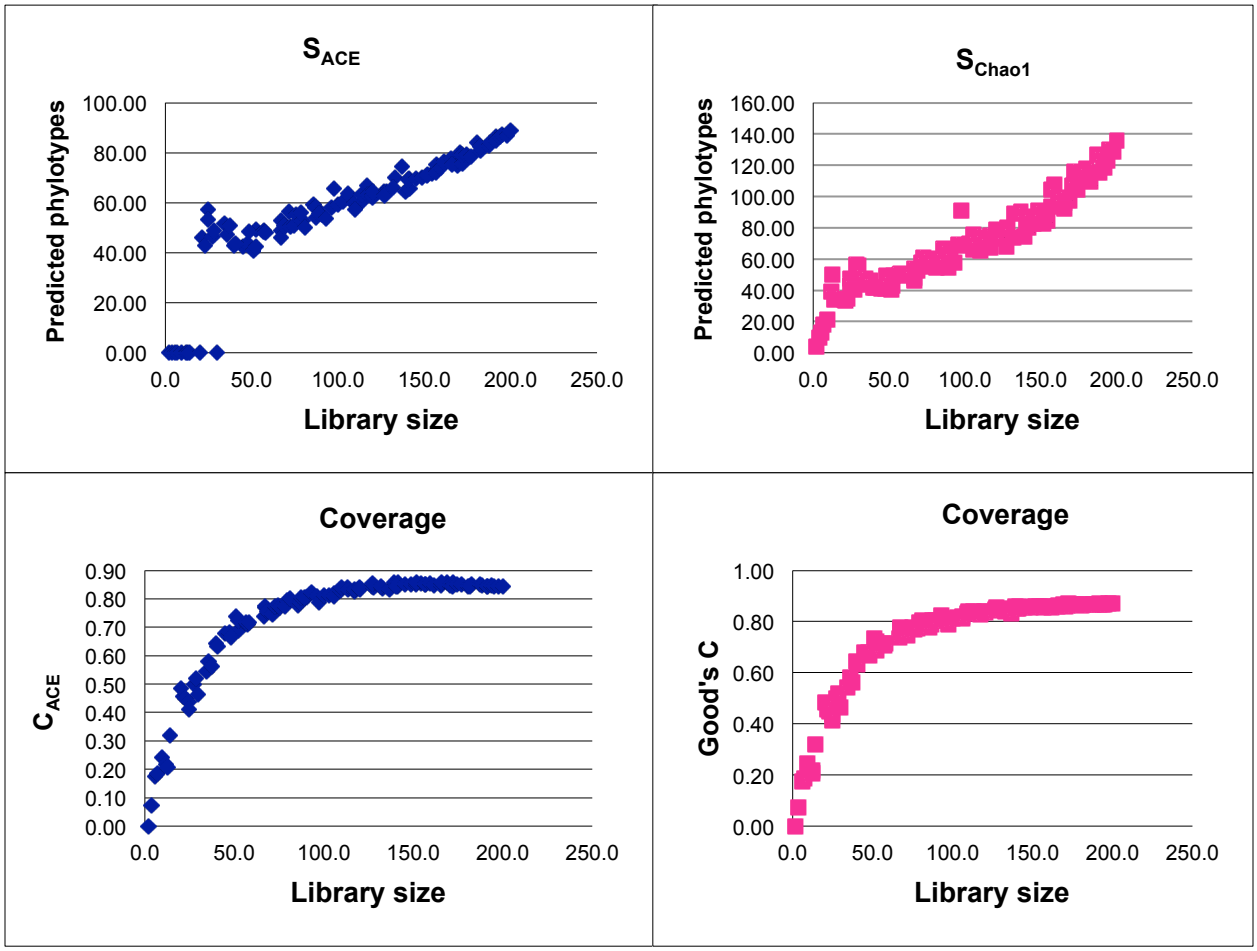
Predicted value of S_{Chao1}	32.39386
Observed phylotypes / predicted S_{ACE}	0.956043
Observed phylotypes / predicted S_{Chao1}	0.924231



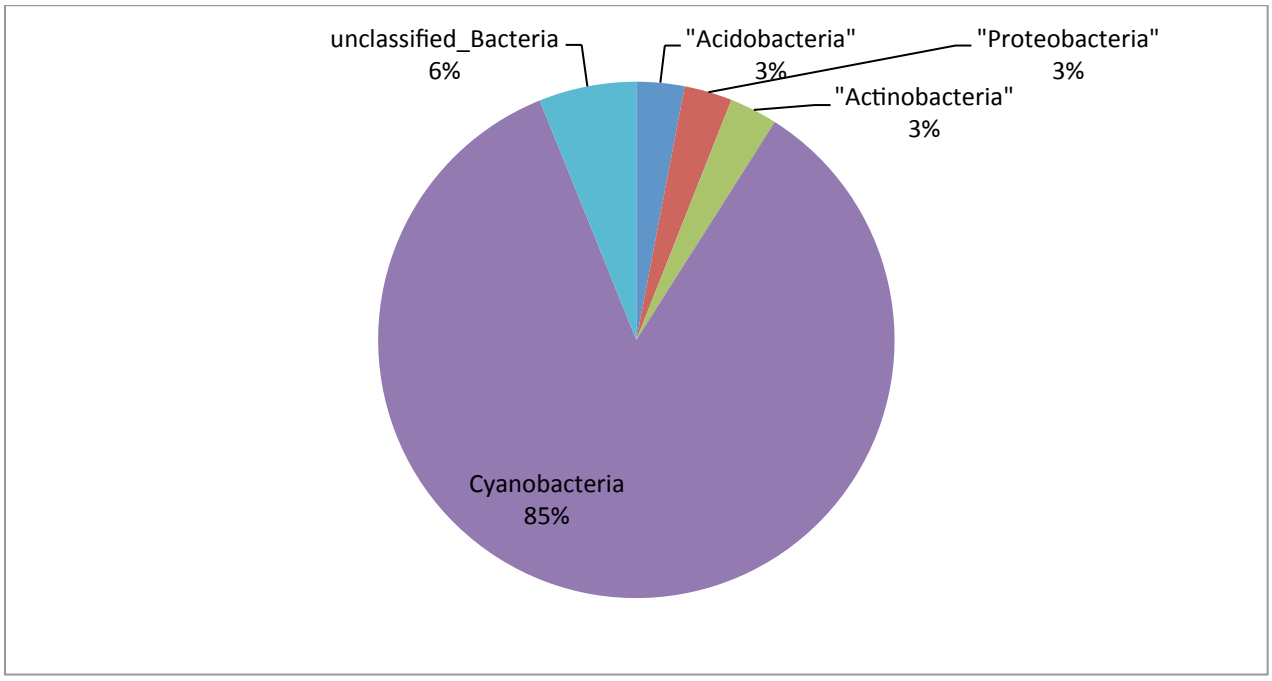
B

Number of clones in library	200
Number of phylotypes observed	54
Predicted value of S_{ACE}	86.87694
Predicted value of S_{Chao1}	127.2837
Observed phylotypes / predicted S_{ACE}	0.668097
Observed phylotypes / predicted S_{Chao1}	0.493066



S5

A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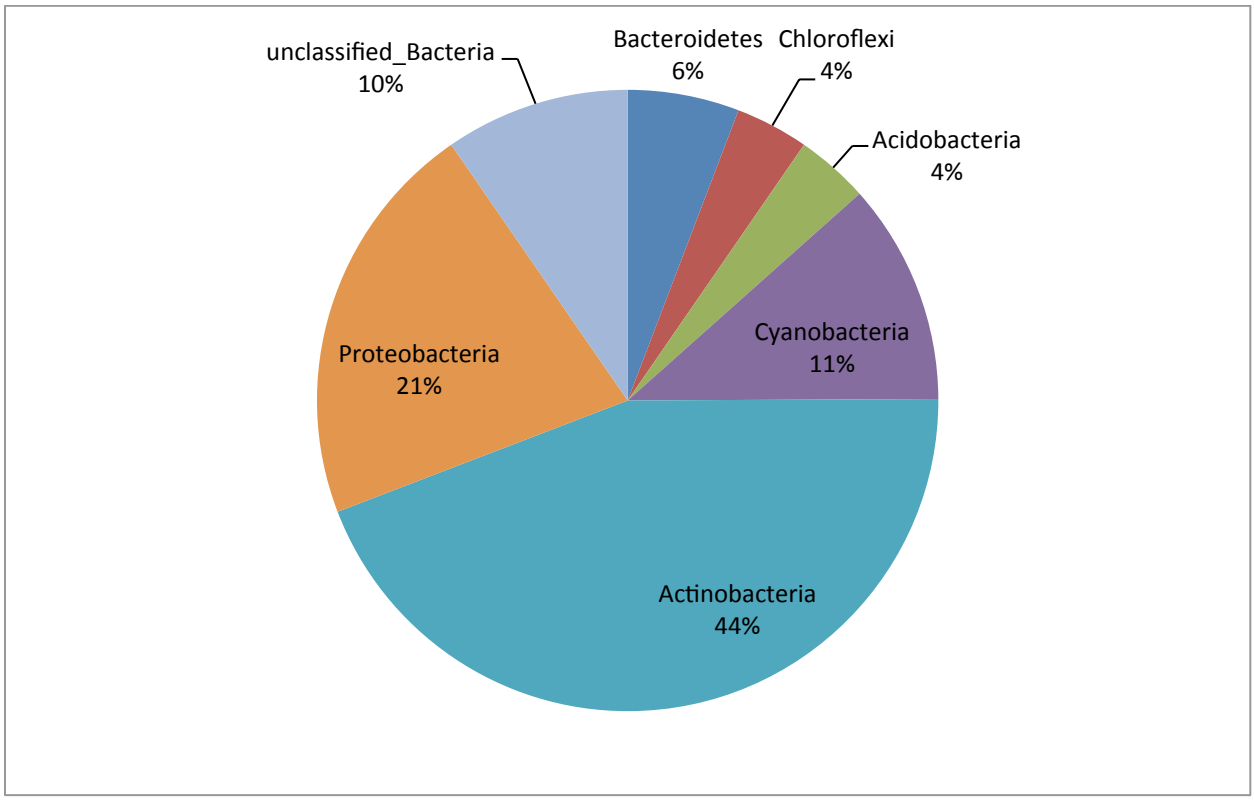
3%
"Proteobacteria"
3%
"Actinobacteria"
3%
Cyanobacteria
85%

B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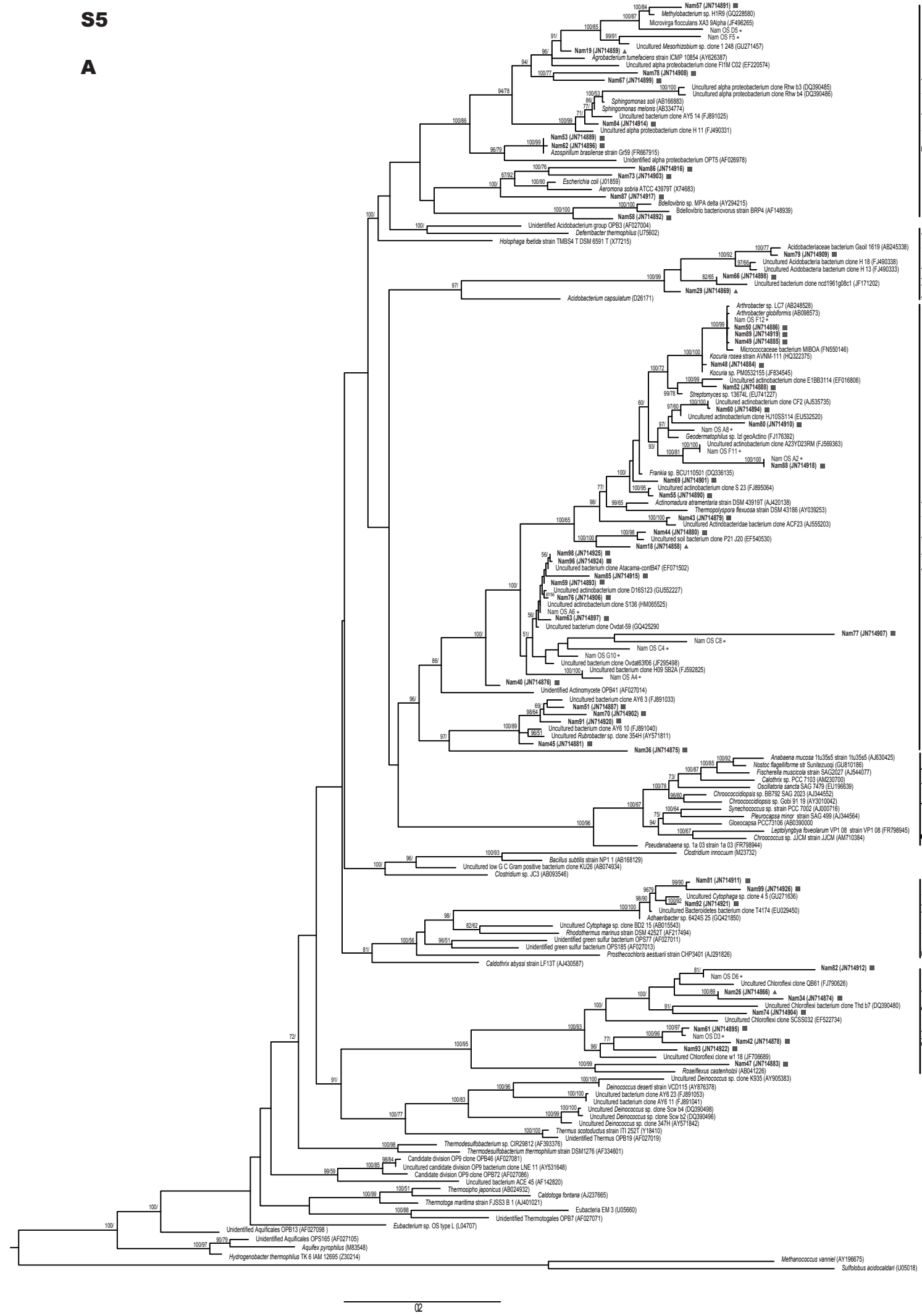
6%
Chloroflexi
4%
Acidobacteria
4%
Cyanobacteria
11%
Proteobacteria
21%
Actinobacteria
44%

S5

A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Actinobacteria
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Bacteroidetes
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Bacillus subtilis strain NP1 1 (AB168129)
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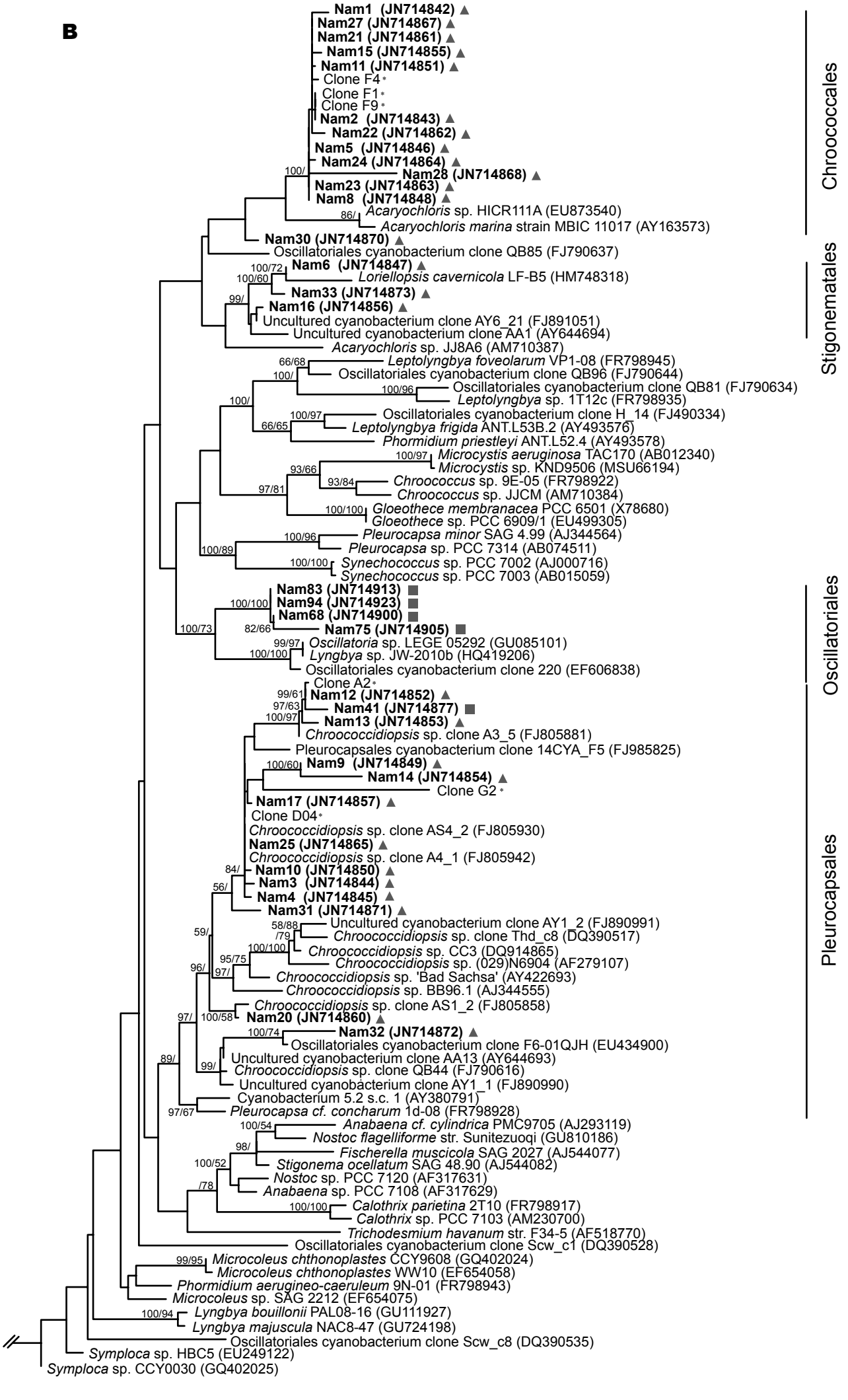
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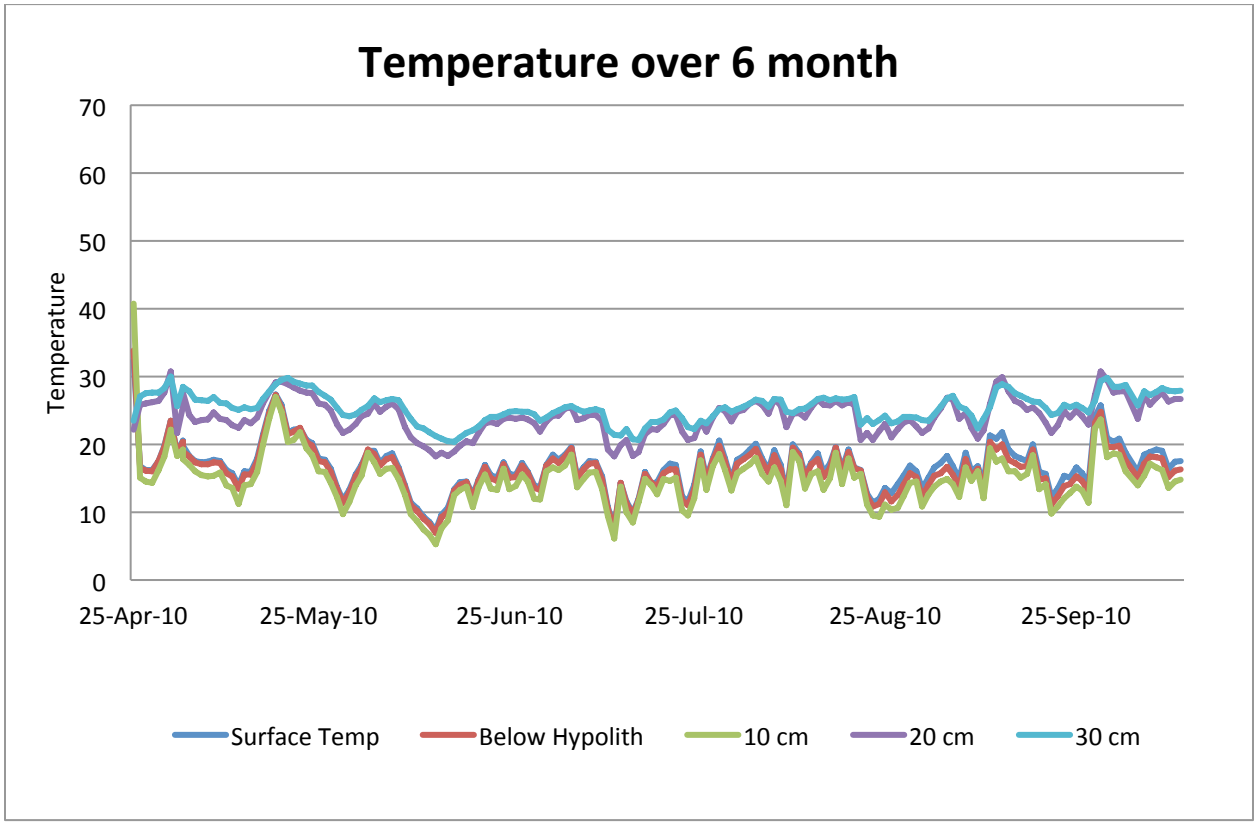
Uncultured cyanobacterium clone AA13 (AY644693)
Chroococcidiopsis sp. clone QB44 (FJ790616)
Uncultured cyanobacterium clone AY1_1 (FJ890990)
Cyanobacterium 5.2 s.c. 1 (AY380791)
Pleurocapsa cf. concharum 1d-08 (FR798928)
Anabaena cf. cylindrica PMC9705 (AJ293119)
Nostoc flagelliforme str. Sunitezuoqi (GU810186)
Fischerella muscicola SAG 2027 (AJ544077)
Stigonema ocellatum SAG 48.90 (AJ544082)
Nostoc sp. PCC 7120 (AF317631)
Anabaena sp. PCC 7108 (AF317629)
Calothrix parietina 2T10 (FR798917)
Calothrix sp. PCC 7103 (AM230700)
Trichodesmium havanum str. F34-5 (AF518770)
Oscillatoriales cyanobacterium clone Scw_c1 (DQ390528)
Microcoleus chthonoplastes CCY9608 (GQ402024)
Microcoleus chthonoplastes WW10 (EF654058)
Phormidium aerugineo-caeruleum 9N-01 (FR798943)
Microcoleus sp. SAG 2212 (EF654075)
Lyngbya bouillonii PAL08-16 (GU111927)
Lyngbya majuscula NAC8-47 (GU724198)
Oscillatoriales cyanobacterium clone Scw_c8 (DQ390535)
Symploca sp. HBC5 (EU249122)
Symploca sp. CCY0030 (GQ402025)
Chroococcales
Stigonematales
Oscillatoriales
Pleurocapsales
0.2

S7

A


Temperature over 6 month
Temperature
70
60
50
40
30
20
10
0
25-Apr-10
25-May-10
25-Jun-10
25-Jul-10
25-Aug-10
25-Sep-10
Surface Temp
Below Hypolith
10 cm
20 cm
30 cm

B

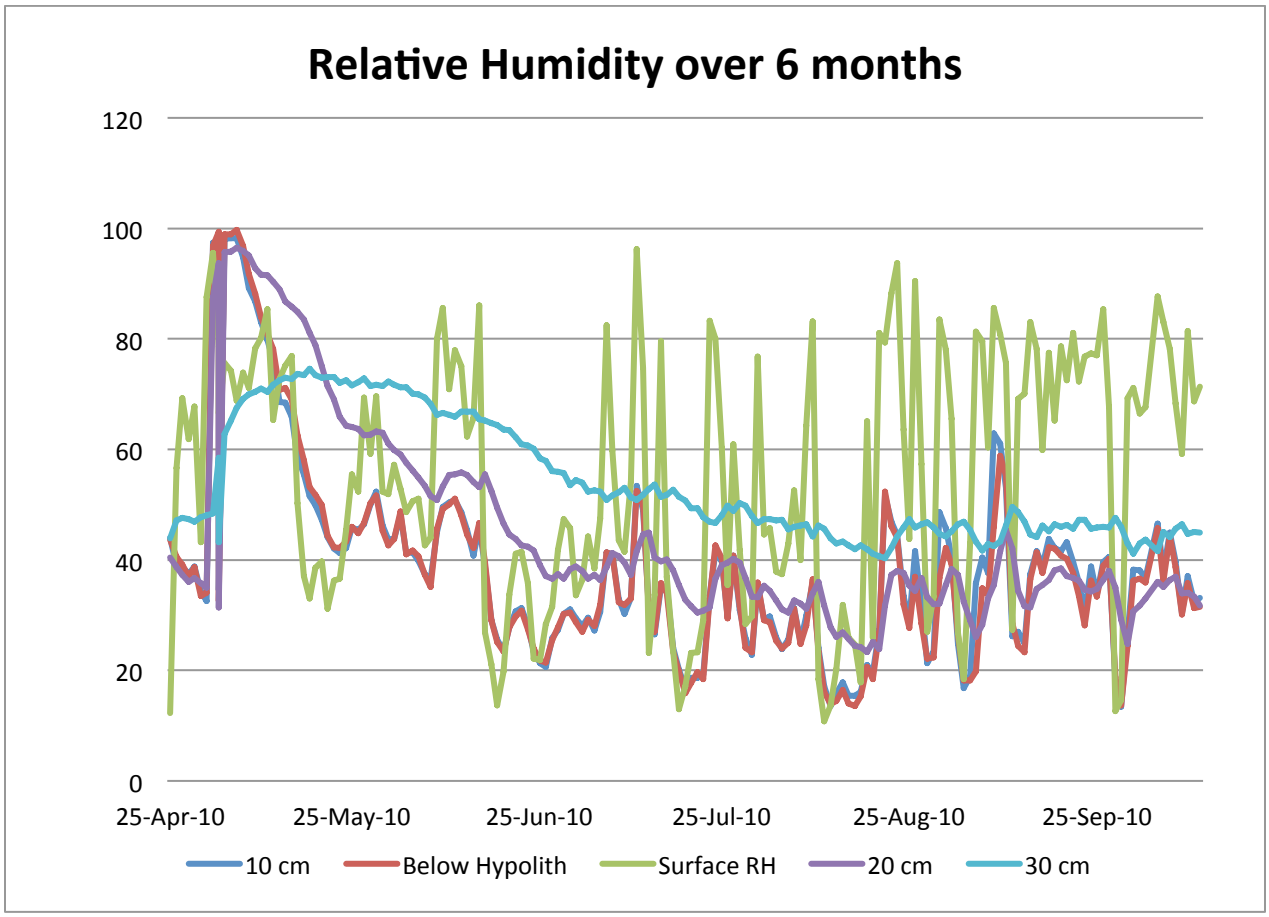

Relative Humidity over 6 months
120
100
80
60
40
20
0
25-Apr-10
25-May-10
25-Jun-10
25-Jul-10
25-Aug-10
25-Sep-10
10 cm
Below Hypolith
Surface RH
20 cm
30 cm

Table S1

Seq. Identification	Accession number	Closest Homologous in GenBank	%	Origin -country	Isolation source	Source clone library
NamSP1	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP2	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP3	AF493850.1	Uncultured bacterium clone 11	99	Southern Mojave Desert	Surface of desert rocks	Hypolith
NamSP4	FJ805942.1	Uncultured Chroococcidiopsis sp. clone A4_1 16S	97	Botswana: Kalahari	Desert quartz	Hypolith
NamSP5	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP6	FJ891051.1	Uncultured cyanobacterium clone AY6_21	97	Atacama Desert	Quartz	Hypolith
NamSP7	FN813975.1	Uncultured bacterium 16S rRNA gene, clone 26B1-D3	91		*Lactuca sativa* (phyllosphere)	Hypolith
NamSP8	FJ230783.1	Uncultured bacterium clone agateC2	92	Australia	Hypolithic soil	Hypolith
NamSP9	AY615380.1	Uncultured bacterium clone AP18	96	Atacama Desert	Rock	Hypolith
NamSP10	AF493850.1	Uncultured bacterium clone 11	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP11	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic soil	Hypolith
NamSP12	AF493842.1	Uncultured bacterium clone 3	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP13	FJ230828.1	Uncultured bacterium clone Prehnite44	98	Australia	Hypolithic slime	Hypolith
NamSP14	FJ230827.1	Uncultured bacterium clone	95	Australia	Hypolithic slime	Hypolith
NamSP15	FJ230783.1	Uncultured bacterium clone agateC2	98	Australia	hypolithic slime	Hypolith
NamSP16	FJ891051.1	Uncultured cyanobacterium clone AY6_21	99	Atacama Desert	Rock	Hypolith
NamSP17	HM241076.1	Uncultured bacterium clone 211	98	Global hypolith study	Hypoliths from desert	Hypolith
NamSP18	FR849426.1	Uncultured bacterium clone B16S-XJcc-2-29	95	Xinjiang Province	Soil	Hypolith
NamSP19	JF295649.1	Uncultured bacterium clone Ovdat61c11	100	Xinjiang Province	Soil	Hypolith
NamSP20	HM241001.1	Uncultured bacterium clone 136	96	Global hypolith study	Hypoliths from desert	Hypolith
NamSP21	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith
NamSP22	FJ230783.1	Uncultured bacterium clone agateC2	98	Australia	Hypolithic slime	Hypolith
NamSP23	FJ230783.1	Uncultured bacterium clone agateC2	98	Australia	Hypolithic slime	Hypolith
NamSP24	FJ230783.1	Uncultured bacterium clone agateC2	96	Australia	Hypolithic slime	Hypolith

NamSP25	AF493850.1	Uncultured bacterium clone 11	99	Mojave Desert	Surface of desert rocks	Hypolith
NamSP26	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	95	China	Uncultured Chloroflexi	Hypolith
NamSP27	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith
NamSP28	FJ230783.1	Uncultured bacterium clone agateC2	99	Australia	Hypolithic slime	Hypolith
NamSP29	FJ230783.2	Uncultured bacterium clone agateC2	98	Australia	Hypolithic slime	Hypolith
NamSP30	FJ230783.2	Uncultured bacterium clone agateC2	95	Australia	Hypolithic slime	Hypolith
NamSP31	HM241076.1	Uncultured bacterium clone 211	97	Australia	Hypolithic slime	Hypolith
NamSP32	JF173381.1	Uncultured bacterium clone ncd1994h09c1	96	china	Soil	Hypolith
NamSP33	FJ891051.1	Uncultured cyanobacterium clone AY6_21	97	Yungay, Atacama Desert	Quartz	Hypolith
NamSP34	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	89	China	Soil	Soil
NamSP35	FJ478825.1	Uncultured bacterium clone p7i15ok	92	Oklahoma, Kessler Farm	Undisturbed tall grass prairie, top 5 cm	Soil
NamSP36	AY923081.1	Uncultured bacterium clone DRV-B011	86	Whipple Mountains	Rock varnish	Soil
NamSP37	FJ592827.1	Uncultured bacterium clone G01_SB3A	97	Atacama	Socompa Volcano, Andes	Soil
NamSP38	GQ495419.1	Uncultured bacterium clone Bas-7-62	99	Iceland	Hnausahraun lava flow	Soil
NamSP39	FR687056.1	Uncultured bacterium clone d21h4b13	95	China	Paddy soil	Soil
NamSP40	GU219537.1	Uncultured bacterium clone Obs1-15	94	Iceland	Bsidian outcrop, Valafell	Soil
NamSP41	AF493842.1	Uncultured bacterium clone 3	99	Mojave Desert	Surface of desert rocks	Soil
NamSP42	HM240933.1	Uncultured bacterium clone 068	95	Global hypolith study		Soil
NamSP43	AJ555203.1	Uncultured Actinobacterium	98	Lower Austria, Marchfeld	Agricultural soil	Soil
NamSP44	EF540530.1	Uncultured soil bacterium clone P21_J20 16S	97	Estonia	Semi-coke	Soil
NamSP45	JN037870.1	Uncultured Actinobacterium clone UHAS5.5	99	India	Saline-alkaline soil	Soil
NamSP46	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP47	DQ906857.1	Uncultured bacterium clone 10D-4	95	Oman	Subsurface soil	Soil
NamSP48	JF834545.1	Kocuria sp. PM0532155	99	India	Environmental sample	Soil
NamSP49	AB248528.2	Arthrobacter sp. LC7 gene for 16S rRNA,	99	Niigata (Japan)	Soil	Soil
NamSP50	FN550146.1	Micrococcaceae bacterium isolate MI-BOA	98	Marion Island	Soil	Soil
NamSP51	GQ425963.1	Uncultured bacterium clone Adulam-209	99	Adulam (Israel)	Soil	Soil
NamSP52	EF016806.1	Uncultured actinobacterium clone E1B-B3-11	95	Atacama Desert	Soil	Soil

NamSP53	FR667915.1	*Azospirillum brasilense* 16S rRNA gene, strain Gr59	99	Greece	Soil	Soil
NamSP55	GU552232.1	Uncultured actinobacterium clone D-16S-130	99	Atacama Desert	Desert soil	Soil
NamSP57	JF295718.1	Uncultured bacterium clone Lehavim48d08			Soil	Soil
NamSP58	AB205958.1	Uncultured bacterium clone OS-27	97	Niigata (Japan)	Activated sludge	Soil
NamSP59	GQ495419.1	Uncultured bacterium clone Bas-7-62	99	Iceland	Hnausahraun lava flow	Soil
NamSP60	AJ535735.1	Uncultured actinobacterium clone CF2	99	Marchfeld (Austria)	Soil	Soil
NamSP61	GQ425251.1	Uncultured bacterium clone Ovdat-20	96	Israel: Ovdat	Soil	Soil
NamSP62	FR667915.1	*Azospirillum brasilense* strain Gr59	99	Greece		Soil
NamSP63	HM565047.1	Uncultured Actinomycetales bacterium clone N-35	98	China	Concrete	Soil
NamSP66	AY923081.1	Uncultured bacterium clone DRV-B011	86	Whipple Mountains	Rock varnish	Soil
NamSP67	GQ425251.1	Uncultured bacterium clone Ovdat-20	96	Israel: Ovdat	Soil	Soil
NamSP68	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP69	DQ336134.1	Frankia sp. strain BCU110345	96	Argentina	Soil	Soil
NamSP70	FR849478.1	Uncultured bacterium clone B16S-XJrs-3-8	99	Xinjiang Province		Soil
NamSP73	JN684205.1	Uncultured bacterium clone H144	100	China	Environmental sample	Soil
NamSP74	HM565054.1	Uncultured Chloroflexi bacterium clone N-229	89	China	Environmental sample	Soil
NamSP75	FJ230801.1	Uncultured bacterium clone QuartzC15	92	Australia	Hypolithic slime	Soil
NamSP76	EU440648.1	Actinomycetales bacterium clone Plot17-A07	99			Soil
NamSP77	FR849480.1	Uncultured bacterium clone B16S-XJrs-3-61	99	China	Desert	Soil
NamSP78	HQ910327.1	Uncultured bacterium clone P-8_B22	97	Utah (USA)	Desert soil	Soil
NamSP79	FR852514.1	Uncultured bacterium clone W3-199	98	China	Red soil	Soil
NamSP80	JF295697.1	Uncultured bacterium clone Lehavim48g01	93	Israel	Soil	Soil
NamSP81	EU029450.1	Uncultured Bacteroidetes bacterium clone T4174	95	Israel	Environmental sample	Soil
NamSP82	JF707601.1	Uncultured Chloroflexi bacterium clone HKTK7-4	93	India	Desert soil	Soil
NamSP83	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP84	HM240929.1	Uncultured bacterium clone 064	98	Global hypolith study	Hypoliths from desert	Soil
NamSP85	GQ425235.1	Uncultured bacterium clone Ovdat-4	99	Ovdat (Israel)	Soil	Soil
NamSP86	HM584296.1	Acinetobacter sp. CJ-S-MA3	99	Korea	Environmental sample	Soil
NamSP87	EF651023.1	Uncultured beta proteobacterium clone AUVE_03A05	98	Australia	Cropland	Soil

NamSP88	FJ478825.1	Uncultured bacterium clone p7i15ok	92	Oklahoma (USA)	Tall grass prairie, top 5cm	Soil
NamSP89	AB622776.1	Uncultured bacterium clone: IMCUGWBC9-1	99	China	High arsenic aquifer	Soil
NamSP91	FJ790550.1	Uncultured bacterium clone VB29	99	Tibet	Soil	Soil
NamSP92	FM209314.1	Uncultured bacterium 16S rRNA gene, clone 230	96	Israel:Negev desert	Soil	Soil
NamSP93	JF295619.1	Uncultured bacterium clone Ovdat61h02	92	Israel	Soil	Soil
NamSP94	FJ230801.1	Uncultured bacterium clone QuartzC15	94	Australia	Hypolithic slime	Soil
NamSP96	JF706662.1	Uncultured actinobacterium clone w3-15	99	Atacama Desert	Hypolith	Soil
NamSP98	JF706662.1	Uncultured actinobacterium clone w3-15	98	Atacama Desert	hypolith	Soil
NamSP99	FM209314.1	Uncultured bacterium clone 230	91	Negev desert (Israel)	Desert	Soil