The Evolution of a Capacity to Build Supra-Cellular Ropes Enabled Filamentous Cyanobacteria to Colonize Highly Erodible Substrates

Ferran Garcia-Pichel*, Martin F. Wojciechowski

School of Life Sciences, Arizona State University, Tempe, Arizona, United States of America

Abstract

Background: Several motile, filamentous cyanobacteria display the ability to self-assemble into tightly woven or twisted groups of filaments that form macroscopic yarns or ropes, and that are often centimeters long and 50–200 µm in diameter. Traditionally, this trait has been the basis for taxonomic definition of several genera, notably Microcoleus and Hydrocoleum, but the trait has not been associated with any plausible function.

Method and Findings: Through the use of phylogenetic reconstruction, we demonstrate that pedigreed, rope-building cyanobacteria from various habitats do not form a monophyletic group. This is consistent with the hypothesis that rope-building ability was fixed independently in several discrete clades, likely through processes of convergent evolution or lateral transfer. Because rope-building cyanobacteria share the ability to colonize geologically unstable sedimentary substrates, such as subtidal and intertidal marine sediments and non-vegetated soils, it is also likely that this supracellular differentiation capacity imparts a particular fitness advantage in such habitats. The physics of sediment and soil erosion in fact predict that threads in the 50–200 µm size range will attain optimal characteristics to stabilize such substrates on contact.

Conclusions: Rope building is a supracellular morphological adaptation in filamentous cyanobacteria that allows them to colonize physically unstable sedimentary environments, and to act as successful pioneers in the biostabilization process.

Introduction

It has long been known that certain filamentous cyanobacteria can form tightly-woven, rope-like bundles of trichomes (cohesive rows of cells) that remain held together in a common, tubular, extracellular polysaccharide sheath [1,2], even though this trait has not been related to any particular ecophysiological function (Fig. 1). It has, however, been used in taxonomy as the main morphological character to define several traditional genera, such as Microcoleus, Schizothrix or Hydrocoleum [3], with the tacit phylogenetic implication that rope-building is a synapomorphy, a case of shared inheritance of this trait in the present-day descendants of a common ancestor that built such ropes. While the genetic basis of rope-building has not been studied directly, due to the lack of genetically modifiable strains, it is likely a genetically controlled trait, inasmuch as it appears easily lost in spontaneous genetic variants upon continued cultivation [4]. We use the term rope to denote the presence of tightly woven filaments in the multilament structure, as a special case of the more inclusive terms bundle or fascicle, that only imply a multiplicity of filaments more or less tightly held together by a common sheath, and would include cyanobacterial general like Trichodesmium or Aphanizomenon.

Rope-building cyanobacteria today thrive in habitats as disparate as desert soils [3,6], marine subtidal stromatolites [7,8], and intertidal sediments [4]. In natural habitats, multifilament ropes are the preferred configuration, and single filaments or trichomes are rare. The nature of the evolutionary advantage conferred by rope-building, however, is all but evident. In fact, according to size-scaling models, growth as large aggregated bundles instead of separate, single trichomes, must certainly bring about at least some negative physiological effects. For example, self-shading effects among the filaments in a bundle configuration will decrease significantly the overall photosynthetic efficiency with respect to incident radiation, and these effects become particularly noticeable as cyanobacteria reach sizes larger than some 10 µm in diameter [9]. Nutrient and metabolite exchange is generally thought to be the main constraint for attaining large sizes in organisms without internal transport systems [10]. A bundle configuration is likely to hamper the efficiency of nutrient uptake systems, and will likely create significant local accumulation of metabolic by-products, such as the photosynthetically-derived molecular oxygen. It is thus only logical to expect some trade-off, an advantage conferred by rope-building determinant enough as to overcome the competitive burdens brought about by crowding.

We contend that the correct interpretation of the rope-building phenomenon can be informed by knowledge of the evolutionary history of the organisms that display this faculty. Particularly if the phylogenetic distribution of the trait indicates that it was gained,
and retained, independently in several evolutionarily distant groups of cyanobacteria, then one can seek evidence for the nature of its adaptive value in environmental characteristics that are common to the habitats typical of those particular groups, but absent in those lacking it. Once this is achieved, and as a means to validate the correlation, one should seek functional models that explain the fitness value of the phenotypic trait in terms of a mechanism exclusively relevant to the shared environments. We used a phylogenetic investigation of pedigreed strains or field samples of demonstrated rope-builders to explore this hypothesis, and provide a fitness value model based on the application of sedimentary physics to this phenomenon.

For the initial phylogenetic investigation, at least three alternative hypotheses can be considered, the implications of which are quite different. If all rope-building cyanobacteria form a coherent monophyletic group, this implies that the trait evolved only once and its continued presence in a variety of habitats may be related to its fitness value, but it may also be a simple legacy of shared ancestry, similar to the fact that the exact number of legs in tetrapods is four. Any inferences made from this situation would be weak (as one cannot infer that four legs are required for locomotion). Alternatively, we may find that rope-builders constitute a polyphyletic group, distributed in two or more, well-separated clades. In this case two explanations are possible. In one, rope-building appeared in the common ancestor of all filamentous cyanobacteria, but was subsequently lost in one or more lineages over time as they evolved and adapted to different habitats. This situation would not allow us to distinguish strictly between fitness value vs. evolutionary legacy either, precluding further inferences. A second, simpler explanation for polyphyly, is that the trait was not inherited from a common ancestor, but rather gained independently in each clade during the course of cyanobacterial evolution. In this scenario, evolutionary legacy can play no role in determining the trait’s distribution, only positive selection. Here one can seek evidence for the possible fitness value of rope building in environmental characteristics common to the rope-building clades, but absent in other cyanobacteria. We note that two distinct evolutionary mechanisms may result in the independent gain of a trait: horizontal gene transfer or convergent evolution. Simple phylogenetic analyses cannot easily distinguish between them. The inference about the need for positive selection is the same, however, regardless of the mechanism.

Results and Discussion

Polyphyly of rope building

To test our hypothesis that rope-building evolved independently in several groups of cyanobacteria, we reconstructed the phylogenetic relationships of bona fide rope-building strains and field samples based on sequence comparisons of two genes (16S rRNA and kacC), newly obtained for this work or from traceable sequences available in public databases. These two genes were chosen because the number of sequences available from other genes in relevant cyanobacterial representatives is very limited. We focused our analyses on rope-building cultures and field samples from intertidal marine microbial mats and desert soils, for which we had the largest database coverage, and for which we had easy access to field samples. Our choice was based on the logical tenet that if polyphyly could be demonstrated in any one subgroup of rope-builders, then the results would be necessarily applicable to the whole. Bayesian analyses of 16S sequences obtained from cultures and field samples from terrestrial and marine origin, show that rope formers are indeed polyphyletic, and resolved into several separate, distinct, and statistically well-supported clades.
within the cyanobacterial phylogenetic tree (Fig. 2a). While these correspond roughly, if not absolutely, to traditional taxonomic entities, one should resist the temptation of nomenclatural distractions: the phylogenetic analysis clearly supports the fact that pedigreed rope-builders are polyphyletic. In fact, to propose that the two main clades (purple and green in Fig. 2a) are monophyletic would require one to break six nodes with statistical support $>0.9$ each (Bayesian posterior probabilities), a scenario with a combined probability $<1/10^6$. Having established this point, its taxonomic implications may be noteworthy. Our results are in line with findings of previous studies [4] and a recent report that proposes a revision of the genus *Microcoleus* into at least two genera based on analyses of molecular, morphological, and structural characters [11]. We can clearly extend the need for revision to the species *M. steenstrupii* and possibly *M. sociatus*.

The most logical explanation for this 16S rRNA tree is that rope building among cyanobacteria has evolved independently at least in each of these four clades. However, the ancient trait explanation, where the common ancestor of all of these distinct, well-defined clades possessed the trait but it was repeatedly lost through evolution in most intervening lineages, cannot be rejected as such. Because of the large evolutionary distance spanned by the clades of rope-builders, this would require that rope building was a truly ancestral trait dating back to the early diversification of the filamentous cyanobacteria.

In order to find independent confirmation of polyphyly and to narrow our interpretations, we used a second, independent marker, *katC*, from a more limited set of samples. The *katC* gene is a member of a small cluster of genes that are important for maintaining circadian rhythms in cyanobacteria [12,13]. In general, distinct, well-resolved, and supported clades were also found for strains and field samples of the terrestrial *M. vaginatus* and the marine *M. chthonoplastes* based on Bayesian analyses of this gene (Fig. 2b), a result congruent with our initial explanation of polyphyly based on 16S sequences. In the case of *M. sociatus*, we note the possibility of a case of horizontal transfer of the *marker gene*, since the two sequences of *katC* available from this species are clearly nested within a clade otherwise comprised of the more distantly related heterocystous cyanobacterium *Nostoc* (Fig. 2b). To propose a monophyletic origin of the two major clades would require breaking 5 well-supported nodes (probability $<1/10^6$). The *katC* tree however, offered an interesting additional piece of information: the ancient trait interpretation would require postulating that a very ancient, rope-building, filamentous, ancestor existed that gave rise to most extant clades of cyanobacteria. It also would necessitate that a large and diverse group composed exclusively of unicellular or colonial, but not filamentous, cyanobacteria (*Synechococcus*, *Xenococcus*, *Acaryochloris*, *Microcystis*; see Fig. 2b) had a filamentous ancestor that made ropes, which is logically untenable.

Thus, our combined analyses speak for a scenario where either through convergent evolution or horizontal transfer (or both) across already well-separated evolutionary lineages, rope-building ability has been retained in phylogenetically separate groups of filamentous cyanobacteria, presumably by offering fitness contributions important enough. The main implication of this finding for our work is that the fitness value of rope building has to do with some ecological constraint(s) shared by these ecologically diverse cyanobacteria.

**A common denominator**

A survey of the literature reveals what is common to all of these rope-builders: they are pioneers in the colonization of unstable sedimentary substrates such as sandy soils (*M. vaginatus* [5,14], *M. steenstrupii* [15], *M. sociatus* [6]), intertidal sand flats (*M. chthonoplastes* [5,13]), and subtidal marine carbonate sediments (*Schizothrix* spp., *Hydrocoleum canthanidum* [16]). It is important to point out that these are not the only microorganisms that can stabilize unconsolidated sediments, since many different microbes are well-known biofostilization agents in sedimentary environments [17,18,19]. Two traits are noteworthy about rope builders among microorganisms involved in biofostilization; on the one hand they are typically pioneers, and on the other, they are comparatively very efficient in stabilizing their own habitat, in both terrestrial (*M. vaginatus* [20,21]), and marine (*M. chthonoplastes* [22]) environments. It is thus reasonable to hypothesize on the basis of these correlations that rope building may somehow impart or promote their biofostilization abilities. The key, however, is to find a mechanistic explanation as to why a rope should be superior to a mesh or a web of single filaments.

The mechanisms of sediment erosion are varied and complex as soon as sedimentary particles have been set in motion, but the conditions necessary for initial particle movement are relatively easy to model [23]. When a fluid such as water or air flows over a sedimentary bed, it transmits to it part of its momentum, exerting a tangential shear stress, $\tau_0$, over the contact surface that can cause movement of protruding particles and, aided by lift, the entrainment of sedimentary particles into the flow stream. Opposing this stress, we have the bed's shear strength, composed of gravity and internal cohesive forces. An erosion threshold occurs when stress over-whelms strength, a critical point commonly used to characterize the erodibility of sedimentary beds [24,25]. Erosive and cohesive forces act on sedimentary particles and thus the “unit” of erosion is in principle a particle or a cohesive group of particles that the forces at play cannot break apart. The agency of microbes in biofostilization of sedimentary environments can result from their weaving of sedimentary particles and biological materials [8], from gluing those particles together with extracellular polymeric substances [25], or from particle cementation with biominerals formed in situ as a result of microbial metabolic activity [8]. In all these cases the net effect is an increase in the effective particle size subject to erosive forces. Alternatively, decreasing the surface roughness by means of copious amounts of polymeric substances can also ameliorate shear stress on the beds [18].

**A physical mechanism for fitness**

By virtue of braiding fibers into yarns or ropes, three major overall parameters are increased: diameter, overall length span, and tensile strength. The tensile strength of a rope is actually less that the sum of the tensile strengths of all the fibers that compose it, since some strength is lost by fiber obliquity necessary for twisting [26]. In this sense, rope building does not represent an improvement in a microbial population’s ability to stabilize sediment over the combined actions of single filaments, unless the sedimentary particles are so large that they cannot be spanned without a rope conformation. But this size (cm range) would imply gravel-sized sediments, which are practically non-erodible under most relevant (non-catastrophic) wind or current regimes anyway [24]. Thus, only the size increase remains a possible avenue for improvement. What is then the relationship between size of particles and the stability of sediments? Particle size is an important factor, since it affects particle weight and inter-particle cohesion (Fig. 3; adapted from reference [27]). For particles larger than 75–100 $\mu$m, gravity dominates the resistance to erosion so that the larger and heavier, the harder they are to lift. In this domain, microbial stabilization can be easily achieved through gluing or weaving particles together into a larger effective particle. But below 75–100 $\mu$m, in the silt and clay ranges, cohesive forces become increasingly important, sediments becoming more stable as particle size diminishes (Fig. 3). Gluing or weaving together of
Figure 2. Phylogenetic relationships of bona-fide, rope-building cyanobacteria based on 16S rRNA (A) and kaiC (B) sequences. Topologies shown are a Bayesian consensus of 200 trees sampled from stationarity (post burnin) derived from each analysis. Branches with posterior probabilities >0.95 are indicated by bold lines; all other branches have posterior probabilities of 0.50–0.95. Different, congruent clades of rope-builder are indicated in color. Gloeobacter violaceus was designated as the outgroup for all analyses. Entries are not all labeled for clarity, and some clades are given names based on the overall composition. Complete trees are available as additional information. Results from maximum parsimony analyses of both the 16S rRNA and kaiC sequences were largely congruent with Bayesian analyses shown here, although many clades receive only low to moderate support by non-parametric bootstrap analysis in the most parsimonious trees identified (data not shown).
doi:10.1371/journal.pone.0007801.g002
related to bed shear stress as the velocity gradient and the roughness of the surface against flow, occur. Friction velocity \( u^* \) is an integrated measure of the steepness of the velocity gradient and the roughness of the surface against flow, related to bed shear stress as \( \tau_0 = \rho_0 \left( \frac{u^*}{d} \right)^n \), where \( \rho_0 \) is fluid density. Threshold friction velocities are a direct function of particle size for large particles where gravitational forces are dominant, but an inflexion point and minimum occurs around roughly 75 \( \mu \)m, where cohesive forces become more important and below which friction velocities actually decrease with particle size. The particle size dependence holds also for water erosion over sediments, and the values of the minimum range between 50 and 200 \( \mu \)m under a wide variety of conditions and sediment bulk densities [23].

Figure 3. Erosivity by wind of sedimentary beds as a function of particle size. Threshold shear stress is estimated here as the threshold friction velocity \( u^* \) above which particle movement will occur. Friction velocity \( u^* \) is an integrated measure of the steepness of the velocity gradient and the roughness of the surface against flow, \( \tau_0 \) being the shear stress. Threshold friction velocities are a direct function of particle size for large particles where gravitational forces are dominant, but an inflexion point and minimum occurs around roughly 75 \( \mu \)m, where cohesive forces become more important and below which friction velocities actually decrease with particle size. The particle size dependence holds also for water erosion over sediments, and the values of the minimum range between 50 and 200 \( \mu \)m under a wide variety of conditions and sediment bulk densities [23].

doi:10.1371/journal.pone.0007801.g003

Table 1. Sources for assignment of rope-building ability.

| Sample/Strain                  | Genetic marker(s) | Correlated by            |
|-------------------------------|-------------------|--------------------------|
| M. vaginatus field sample BW   | kaiC              | direct observation       |
| M. vaginatus field sample BW2  | kaiC              | description, ref. [5]    |
| M. vaginatus field sample BW3  | kaiC              | direct observation       |
| M. vaginatus field sample BW4  | kaiC              | direct observation       |
| M. vaginatus field sample BW5  | kaiC              | direct observation       |
| M. vaginatus field sample GR2  | kaiC              | direct observation       |
| M. vaginatus ASU LT04          | kaiC              | direct observation       |
| M. vaginatus OTA32c150 6A      | 16S rRNA          | description, ref. [15]   |
| M. vaginatus B5 lac149 48      | 16S rRNA          | description, ref. [15]   |
| M. vaginatus F5SMC4 c1821A     | 16S rRNA          | description, ref. [15]   |
| M. vaginatus OTA21c1521B       | 16S rRNA          | description, ref. [15]   |
| M. chthonoplastes SAG3898      | kaiC              | direct observation       |
| M. chthonoplastes Ney3 289     | kaiC              | description, ref. [13]   |
| M. chthonoplastes Ney3 407     | kaiC              | description, ref. [13]   |
| M. chthonoplastes Ney4 350     | kaiC              | description, ref. [13]   |
| M. chthonoplastes Ney5 361     | kaiC              | description, ref. [13]   |
| M. chthonoplastes K27FWS       | kaiC              | description, ref. [13]   |
| M. chthonoplastes FWS 17       | kaiC              | description, ref. [13]   |
| M. chthonoplastes NDN-1        | kaiC              | description, ref. [4]    |
| M. chthonoplastes PCC7420      | kaiC, 16S rRNA    | description, ref. [4]    |
| M. chthonoplastes GNS          | 16S rRNA          | description, ref. [4]    |
| M. chthonoplastes MEL1         | 16S rRNA          | description, ref. [4]    |
| M. sociatus SAG2692            | kaiC              | description, ref. [4]    |
| M. sociatus MPI96 MS KID       | 16S rRNA          | description, ref. [4]    |
| M. steenstrupii 73 2E          | 16S rRNA          | description, ref. [15]   |
| M. steenstrupii 94 2B          | 16S rRNA          | description, ref. [15]   |
| M. steenstrupii 52 2A          | 16S rRNA          | description, ref. [15]   |
| M. steenstrupii 150 3A         | 16S rRNA          | description, ref. [15]   |
| M. steenstrupii 177 7B         | 16S rRNA          | description, ref. [15]   |

doi:10.1371/journal.pone.0007801.t001

consistent with the successional dynamics observed in some ecosystems initially dominated by rope builders, such as stromatolites [9] or desert biological soil crusts [20].

Materials and Methods

Criteria for rope-formation in samples, microbial strains and public DNA sequences

Given the confusion between taxonomic systems for the cyanobacteria, the high level of entry errors found in public databases, and the facility with which rope building is lost upon cultivation, we included in our phylogenetic analyses only those sequences originating from cyanobacteria known to make ropes in a traceable and explicit manner. For field samples this meant sequences from isolated filaments obtained after direct micromanipulation of large ropes, either by us, or from the literature, when explicitly described as such in the original publication. For cultivated strains it meant isolates that form ropes presently, or those that have been documented to form ropes at one time, if an explicit reference was available. Table 1 lists the criterion for each sequence in detail.
Sequencing and phylogenetic reconstruction. New sequences of 16S rRNA gene and kaC were obtained from either cultures or from field-collected samples (see Supplementary Table S1). DNA was extracted from culture samples using a kit (MoBio plant extraction kit). For field samples, ropes of filaments were excised from the habitat by micromanipulation under a dissecting microscope, and cleaned by dragging over agar surfaces. After morphological identification under the compound microscope, each rope was submerged in TE buffer (pH 8.0), frozen and thawed three times. Aliquots of each sample were used as a template for standard PCR amplification using previously described specific primers for the kaC gene [10] and 16S rDNA [4]. All PCR products were purified and sequenced on both strands using the same primers. New sequences were edited using Sequencher 4.1 (GeneCodes, Ann Arbor, MI) and then aligned with relevant sequences available from Genbank using ClustalX [29]. Phylogenetic analyses were performed as described previously [30]. Best fitting models for the two molecular sequence data sets were selected using the Akaike Information Criterion implemented in the program ModelTest v. 3.07 [31]: a GTR+I+Γ model for the 16S rDNA sequences; and a TVM+I+Γ model for kaC. Phylogenetic relationships were estimated with Bayesian inference using MrBayes v. 3.1 [32], in which two independent Markov chain Monte Carlo runs of 2–2.5 × 10^6 generations were conducted using variable rate priors, with sampling every 1000 generations. Post burn-in sampled topologies (200) from each analysis were summarized as a majority rule consensus tree to obtain the posterior probability (PP) credibility interval for each clade.

Supporting Information

Table S1 List of taxa included in phylogenetic analyses and Genbank accession number for molecular sequences. Found at: doi:10.1371/journal.pone.0007801.s001 (0.16 MB DOC)

Author Contributions
Conceived and designed the experiments: FGP MFW. Performed the experiments: FGP MFW. Analyzed the data: FGP MFW. Contributed reagents/materials/analysis tools: FGP MFW. Wrote the paper: FGP MFW.

References
1. Castenholz RW (1989) Subsection III, order Oscillatoriales. In: Staley JT, Bryant MP, Pfennig N, Holt JG, eds. Bergey's Manual of Systematic Bacteriology. Baltimore: Williams & Wilkins Co, pp 1771–1780.
2. Garcia-Pichel F (2000) Cyanobacteria. In: Lederberg J, ed. Encyclopedia of Microbiology. 2nd ed. San Diego: Academic Press.
3. Geitler L (1932) Cyanophyceae. Leipzig: Akademische Verlagsgesellschaft.
4. Garcia-Pichel F, Prufert-Bebout L, Muyzer G (1996) Phenotypic and phylogenetic analyses show Microcoleus chthonoplastes to be a cosmopolitan cyanobacterium. Applied and Environmental Microbiology 62: 3294–3291.
5. Garcia-Pichel F, López-Cortés A, Nübel U (2001) Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. Applied and Environmental Microbiology 67: 1902–1910.
6. Lange OL, Kidron GJ, Bidel B, Meyer A, Kilian E, et al. (1992) Taxonomic composition and photosynthetic characteristics of the “biological soil crusts” covering sand dunes in the western Negev Desert. Functional Ecology 6: 519–527.
7. Abed RMM, Golubic S, Garcia-Pichel F, Gamoin SF, Sprachta S (2003) Characterization of microbialite forming cyanobacteria in a tropical lagoon: Tikehau Atoll, Tuamotu, French Polynesia. J Phycol 39: 862–873.
8. Keiv RP, Visscher PT, Decho AW, Stolz JF, Bebout BM, et al. (2000) The role of microbes in accretion and early lithification of modern marine stromatolites. FEMS Microbiology 60: 73–79.
9. Schulz H, Jørgensen B (2001) Big Bacteria. Ann Rev Microbiol 55: 105–137.
10. Wiegesmund MA, Johansen JR, Karsten U, Friedl T (2008) Characterization of microbialite forming cyanobacteria in a tropical lagoon: Tikehau Atoll, French Polynesia. J Phycol 39: 862–873.
11. Grant J, Gast G (1987) Prediction of coastal sediment stability from photopigment content of mats of purple sulfur bacteria. Nature 330: 244–246.
12. Paterson DM, Black KS (1999) Water flow, sediment dynamics and benthic biology. Advances in Ecological Research 29: 153–193.
13. Stal L (2003) Microphytobenthos, their extracellular polymeric substances and the morphogenesis of intertidal sediments. Geomicrobiology J 28: 463–470.
14. Behnap J, Gillette BA (1998) Vulnerability of desert biological soil crusts to wind erosion: the influences of crust development, soil texture, and disturbance. Journal of Arid Environments 39: 133–142.
15. Hu C, Li Y, Song L, Zhang D (2002) Effect of desert soil algae on the stabilization of fine sands. J Applied Phycology 14: 281–292.
16. Abed RMM, Golubic S, Garcia-Pichel F, Carnon G (2003) Characterization of microbialite forming cyanobacteria in a tropical lagoon: Tikehau Atoll, French Polynesia. J Phycol 39: 862–873.
17. Greely R, Iversen JD (1985) Wind as a geological process on Earth, Mars, Venus and Titan. Cambridge: GBR Woodheal Publishing.
18. Paterson D, Black K (1999) Water flow, sediment dynamics and benthic biology. Adv Ecol Res 29: 155–193.
19. McKenna H, Hearle J, O'Hear N (2004) Handbook of fiber rope technology. New York: John Wiley. pp 221.
20. Tikehau Atoll, Tuamotu, French Polynesia. J Phycol 39: 862–873.
21. Garcia-Pichel F (2000) Cyanobacteria. In: Lederberg J, ed. Encyclopedia of environmental microbiology. New York: John Wiley. pp 1196–1197.
22. McKenna H, Hearle J, O'Hear N (2004) Handbook of fiber rope technology. New York: John Wiley. pp 221.
23. Hu C, Li Y, Song L, Zhang D (2002) Effect of desert soil algae on the stabilization of fine sands. J Applied Phycology 14: 281–292.
24. Addison W, Adlkinson Wesley Longman Ltd. pp 221.
25. Paterson D, Black K (1999) Water flow, sediment dynamics and benthic biology. Adv Ecol Res 29: 155–193.
26. McKenna H, Heade J, O’Hear N (2004) Handbook of fiber rope technology. Cambridge: GBR Woodheal Publishing.
27. Greely R, Iversen JD (1985) Wind as a geological process on Earth, Mars, Venus and Titan. Cambridge: Cambridge University Press. pp 333.
28. Garcia-Pichel F (2002) Desert environments: biological soil crusts. In: Bittgen G, ed. Encyclopedia of environmental microbiology. New York: John Wiley. pp 1019–1023.
29. Thompson JD, Gibson TJ, Pfenning F, Jammolkchin H, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24: 4876–4882.
30. Wojciechowski MF, Lavin M, Sanderson MJ (2004) A phylogeny of legumes (Leguminosae) based on analysis of the plastid matK gene resolves many well-supported subclades within the family. Ann J Bot 91: 1046–1062.
31. Posada D, Crandall KA (1998) ModelTest: testing the model of DNA substitution. Bioinformatics 14: 817–818.
32. Huelsenbeck JP, Ronquist FR (2001) MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 734–735.