Polyphasic characterization of *Kastovskya adunca* gen. nov. et comb. nov. (Cyanobacteria: Oscillatoriales), from desert soils of the Atacama Desert, Chile

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Abstract

Recent taxonomic revisions within the cyanobacteria have shown that the traditional simple filamentous genera often represent large polyphyletic clusters of not-so-closely-related taxa. In this study, the new cyanobacterial genus *Kastovskya* is described based on a combination of morphological, molecular, and ecological evidence. *Kastovskya* was first described as *Schizothrix adunca*, a morphospecies discovered in the Atacama Desert, Chile more than 50 years ago. This species has been transferred to *Kastovskya* and serves as the generitype. *Kastovskya adunca* currently represents a unique and probably endemic taxon for the soils of the Atacama Desert region. Description of this new genus contributes to the revision of the Phormidiaceae by providing a clear taxonomic definition to one of the clades within the *Schizothrix/Microcoleus/Phormidium* cluster.

Introduction

Deserts provide harsh habitats (saline lakes, arid soils) habitable only by extremophiles, such as Cyanobacteria. However, species-level diversity is poorly known for this group. In particular, arid deserts such as the Atacama Desert are greatly understudied.

The Atacama Desert forms a narrow but long strip of arid land along the Pacific coast in South America. It extends from La Serena, Chile in the south to the Peruvian-Chilean border in the north (Rundel et al. 1991). Two main regions can be distinguished in this desert. First is the hyperarid core, located in the vicinity of Yungay, Chile. This part is considered to be one of the driest places of the world with soil water potential reaching as low as -166 Mpa, and thus has soils posing similar challenges to conditions found on Mars (McKay et al. 2003, Navarro-González et al. 2003). Some studies have shown that parts of this Atacama core may be even too dry for microbes to survive (Navarro-González et al. 2003, Lester et al. 2007). The second region is the coastal zone adjacent to the Pacific Ocean. In comparison with the hyperarid core, the climate is milder here, and is characterized by the presence of microorganisms and higher plants. The crucial factor influencing the environmental conditions is regular formation of fog (called “camanchaca” in Chile) which serves mostly as the only source of water (Rundel et al. 1991, Cereceda et al. 2008). A remarkable number of endemic plants have been discovered in the fog oases (Rundel et al. 1991, Larrain et al. 2002, Cereceda et al. 2008); however, much less is known about the potential endemism of microorganisms living in this desert.

Research focusing on cyanobacteria inhabiting soils of the Atacama Desert started more than 50 years ago near the cities of Antofagasta and Caldera, Chile. The author of the very first study confirmed the presence of living blue-green algae by finding four cyanobacterial species: *Calothrix desertica* Schwabe (1960: 282), *Schizothrix adunca* Schwabe (1960: 293), *S. atacamensis* Schwabe (1960: 296), and *Plectonema polymorphum* var. *viridis* Schwabe (1960: 291). All of them except *P. polymorphum* were species new to science (Schwabe 1960). Six years later, Forest & Weston (1966) reported three additional species of cyanobacteria from the Atacama Desert. Nevertheless, due to their use of the oversimplified classification system of Drouet (1968), it is difficult to determine which species were actually present (Büdel 2001).
More recent studies focused predominantly on the ability of cyanobacteria to withstand and survive challenging desert conditions. In the hyperarid core several hypolithic (= beneath rocks) cyanobacteria were observed, with Chroococcidiopsis-like morphotypes representing the most common taxa (Warren-Rhodes et al. 2006, Lacap et al. 2011). Besides hypolithic habitats, Chroococcidiopsis Geitler (1933: 625) was found to inhabit new unique microhabitats inside of halite evaporite rocks, where an organic layer up to 5 mm thick sometimes formed (Wierzchos et al. 2006, de los Ríos et al. 2010, Vítek et al. 2010). Because evaporite rocks can retain water within their crystalline and porous structure, they provide more favorable conditions than the surrounding, drier environment (Wierzchos et al. 2006). In the coastal zone, water from condensed fog often gathers under quartz rocks, and subsequently evaporates more slowly than from bare soil. Azúa-Bustos et al. 2006, 2010) detected five cyanobacterial genera in these hypolithic habitats: Anabaena Bory de Saint-Vincent ex Bornet & Flahault (1886: 180, 224); Chroococcidiopsis; Microcoleus Desmazières ex Gomont (1892: 350); Nostoc Vaucher ex Bornet & Flahault (1886: 181); and Scytonea Agardh ex Bornet & Flahault (1886: 85).

Despite the fact that the Atacama Desert represents a very unique environment, the identity of the cyanobacteria present remains mostly unknown. This situation has changed recently, and several studies have discovered additional algological α-level diversity in this area (Osorio-Santos 2011, Baldarelli 2012). The current work is part of this recent effort to identify algae living in the soils of the Atacama Desert, and focuses on simple filamentous cyanobacteria that create multiple trichomes within a common sheath.

Traditionally, simple filamentous cyanobacteria with multiple (more than three) trichomes in a sheath were thought to represent any of several recognized genera: Hydrocoleum Kützing ex Gomont (1892: 332), Microcoleus, Schizothrix Kützing ex Gomont (1892: 292), Blepharix Kützing ex Anagnostidis & Komárek (1988: 429), and Symplacostrum (Gomont 1892: 314) Kirchner (1898: 69). According to more recent definitions of these genera, Symplacostrum differs from the others by the presence of a tuft-like thallus formation and Blepharix by remarkably larger trichome size (8–40 μm). Based on the cellular ultrastructure, Schizothrix was placed in the order Pseudanabaenales and its maximum trichome width was defined as 4 μm. Hydrocoleum and Microcoleus are morphologically very similar, with oscillatoriacean cellular ultrastructure and overlapping trichome size (~3–8 μm). Thus, until recently, most of the cyanobacteria exhibiting oscillatoriacean characteristics, containing multiple trichomes in a sheath, and creating flat mats were classified in one of these two genera (Komárek & Anagnostidis 2005).

Not surprisingly, recently undertaken revisions of Microcoleus, based on molecular data, have shown that this genus is clearly a polyphyletic cluster of organisms. Several examples of genera already split from Microcoleus include: Wilmottia Strunecký, Elster & Komárek in Strunecký et al. (2011: 62) containing Phormidium murrayi (West & West 1911: 289) Anagnostidis & Komárek (1988: 408) and Microcoleus glaciei Johansen & Casamatta in Casamatta et al. (2005: 432); Coleofasciculus Siegesmund, Johansen & Friedl in Siegesmund et al. (2008: 1575) created from the cluster around Microcoleus chthonoplastes (Mertens in Hornemann 1813: 6) Zanardini (1840: 200); and Trichocoleum Anagnostidis (2001: 369) containing several Microcoleus species exhibiting pseudanabaenalean ultrastructural characteristics. The recent taxonomic fracturing of Microcoleus suggests that the diversity of simple filamentous cyanobacteria is much larger than traditionally recognized and still awaits further discovery and description. The well-defined phylogenetic position of M. vaginata (Vaucher 1803: 200–201) Gomont ex Gomont (1892: 355) (Garcia-Pichel et al. 2001, Starkenburg et al. 2011), the type species of Microcoleus, makes the ongoing revisions of this genus much easier. However, Hydrocoleum and all the other cyanobacterial genera defined traditionally without 16S rRNA gene sequences remain unexamined and unevaluated.

In this paper, Kastovskya gen. nov. is described based on its morphological, ecological, and molecular characteristics. This genus has been recorded so far exclusively from the Atacama Desert and contains the single species K. adunca, originally described as S. adunca Schwabe (1960). This new cyanobacterial genus corresponds morphologically to a certain extent with M. steenstrupii Petersen (1928: 292) as described in Boyer et al. (2002) from desert soils in North America. However, the phylogeny based on 16S rRNA sequence analyses shows that Kastovskya is clearly distinct from the forms placed in that taxon from North America. Microcoleus steenstrupii, as currently described (Boyer et al. 2002), is not monophyletic, does not belong to the same clade as the type species of Microcoleus, and is in urgent need of revision. This work is the first step towards a better understanding of this complex.
Materials and methods

Origin and morphological characterization of strains:—Strains used in this study were isolated from the soils of the Atacama Desert (Table 1). Their relatives, as revealed from phylogenetic analyses, were also used as strains for comparison of 16S rRNA similarity and 16S-23S ITS secondary structures.

TABLE 1. Origin of strains belonging to *Kastovskya* and closely allied relatives (ND = not determined).

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>GPS Coordinates</th>
<th>Elevation</th>
<th>Collection</th>
<th>Site Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATA3-4Q</td>
<td>Atacama, Chile</td>
<td>27° 50' 49&quot; S, 71° 00' 34&quot; W</td>
<td>190 m a.s.l</td>
<td>13-May-09</td>
<td>Quartz rocks, lichens, plants very sparse.</td>
</tr>
<tr>
<td>ATA3-5Q</td>
<td>Atacama, Chile</td>
<td>27° 43' 42&quot; S, 71° 02' 05&quot; W</td>
<td>9 m a.s.l</td>
<td>13-May-09</td>
<td>Quartz rocks, sandy soil near the coast with cacti covered in lichens. Algae present.</td>
</tr>
<tr>
<td>ATA6-11</td>
<td>Atacama, Chile</td>
<td>26° 06' 42&quot; S, 70° 38' 52&quot; W</td>
<td>335 m a.s.l</td>
<td>26-Jun-11</td>
<td>Soil fine with consolidation, partly moist from fog. Large rocks, many cacti, lichens present.</td>
</tr>
<tr>
<td>WJT32</td>
<td>Mojave, USA</td>
<td>34° 05' 59&quot; N, 115° 27' 18&quot; W</td>
<td>624 m a.s.l</td>
<td>22-Jun-06</td>
<td>Granitic soil, well developed algal crust with lichens and mosses.</td>
</tr>
<tr>
<td>JO1*</td>
<td>Chihuahuan, USA</td>
<td>32° 30' 57&quot; N, 106° 47' 20&quot; W</td>
<td>1353 m a.s.l</td>
<td>ND</td>
<td>Jornada LTER, arid soils.</td>
</tr>
<tr>
<td>ANT,LPE**</td>
<td>Antarctic</td>
<td>ND</td>
<td>ND</td>
<td>1997–1999</td>
<td>Antarctic lake.</td>
</tr>
<tr>
<td>B-Tom***</td>
<td>Mata Atlantica, Brazil</td>
<td>23° 28'51&quot; S, 45° 04'15&quot; W</td>
<td>0 m a.s.l</td>
<td>20-Nov-04</td>
<td>Wet rock on the sea shore.</td>
</tr>
</tbody>
</table>

*Boyer et al. (2002), **Taton et al. (2006), ***Lokmer (2007) and Jan Kaštovský (pers. communication).

The surface layer of the soil, including soil crusts or the under-surface of rocks inhabited by microorganisms, was sampled. Dilution plating and the Moistened Soil Method (Johansen *et al*. 1984) as described in Mühlsteinová *et al*. (2014) were used to isolate unialgal strains of cyanobacteria on to Z-8 (Carmichael 1986) agar slants. Cultures were maintained in a growth chamber with 16:8 h light:dark cycle at 15–18 °C.

To investigate strain morphology throughout the cyanobacterial life cycle, observations of cultures using an Olympus BH-2 photomicroscope with Nomarski DIC optics equipped with a digital camera (Olympus DP25) were made between the 2nd and 18th week (2, 3, 4, 6, 8, 13, 14, 18), and at 17 months. Measurements of filament, trichome, cell, and apical cell sizes were taken, and characteristic properties were noted. Images were composited into a photographic plate using Adobe Photoshop CS5.1.

Molecular characterization:—Genomic DNA was extracted using UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc.; Carlsbad, CA, USA). The 16S rRNA gene (bp 325–1487) and the associated 16S–23S internal transcribed spacer (ITS) region were amplified using polymerase chain reaction (PCR) with primers 1 and 2 *sensu* Boyer *et al*. (2001, 2002). The reaction was prepared according to the protocol in Lukešová *et al*. (2009) and run in a S1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA). The size of amplified DNA product (~1600 bp long) was checked on 1% agarose/ethidium bromide gel, and then cloned into plasmids using the StrataClone PCR Cloning Kit (La Jolla, CA, USA). Plasmids from transformed cells were purified using QIAprep Spin Kit (QIAGEN, Carlsbad, CA, USA). Digestion reaction with *Eco* R I was used to verify the presence of the expected insert. Subsequently, two or three plasmids containing the insert of interest were sequenced by Functional Biosciences, Inc. (Madison, WI, USA), together with primers M13 forward, M13 reverse, and internal primers 5, 7, and 8, according to Boyer *et al*. (2001, 2002). Sequencher (v. 4.9, Ann Arbor, MI, USA) was used to assemble contigs.

Phylogenetic analyses:—Consensus sequences of the 16S rRNA genes (~1162 nucleotides) of each strain were aligned with other GenBank (April 2013) sequences manually using Microsoft Word. These sequences were added either based on Blast Search executed against our sequences, or based on genus names known to belong to the order Oscillatoriales. This 16S rRNA gene alignment contained 239 sequences together with *Gloeobacter violaceus* Rippka, Waterbury & Cohen-Bazire in Rippka *et al*. (1974: 436) (FR798294) as an outgroup. PAUP v.4.02b (Swofford 1998) was used to construct a 16S rRNA tree based on parsimony. The criteria of the heuristic search were set as: multrees = no; branch-swapping algorithm = TBR; gapmode = newstate; steepest descent = yes; and nreps = 1000. For the bootstrap values calculation, 1000 replicates were run. Subsequently, the Bayesian
analyses using the GTR-Γ model was performed using supercomputing facilities available within MetaCentrum (www.metacentrum.cz). Eight Markov chains were executed in two runs for 15 million generations with default parameters, sampling every 100 generations (the final average standard deviation of split frequencies was lower than 0.01). The first 25% of sampled trees were discarded as burn-in. All trees were viewed using FigTree (Rambaut 2007).

Similarity of the 16S sequences of our strains was determined as 100(1-(P-distance)) as calculated in PAUP. To analyze intrageneric variability among our strains, the ITS secondary structures—D1-D1’, Box-B, V2, and V3 helices, were folded using M-fold (Zuker 2003) and compared. The secondary structures together with the 16S tree were re-drawn in Adobe Illustrator CS5.1.

Herbarium specimens and accession numbers:—Herbarium specimens, together with fresh biomass preserved in 4% formaldehyde of each strain, were placed in the Herbarium for Nonvascular Cryptogams at the Monte L. Bean Museum in Provo, Utah, with accession numbers BRY37741-37748. Cultures of investigated strains are kept in Dr. Johansen’s algal collection at John Carroll University, University Heights, Ohio, USA and selected strains were deposited in the Culture Collection of Autotrophic Organisms (CCALA) at the Institute of Botany, Academy of Sciences of the Czech Republic, Treboň, CZ (strains CCALA 1025–27). Sequences obtained as part of this work were submitted to the GenBank database with numbers KF312340–312350.

Results

Class Cyanophyceae
Subclass Oscillatoriophycideae
Order Oscillatoriales
Family Phormidiaceae

Kastovskya Mühlsteinová, Johansen et Pietrasiak, gen. nov. (Fig. 1)

Akin to a sister clade of soil-inhabiting Phormidiacean strains fitting M. steenstrupii sensu Boyer et al. (2002: 1225), but differing in having very strongly constricted cells that are nearly quadratic rather than mostly longer than wide, and apical cells that are irregularly shaped.

Description:—Thallus in form of long, dark or bright blue-green filaments creeping on agar, becoming yellowish to pale orange with age. Filaments containing one to multiple trichomes. Sheath colorless, usually thick, lamellated, sometimes wavy with undulated edges, containing one to several intertwined or parallel arranged trichomes, occasionally narrowed at the ends. Trichomes motile, long, blue-green to pale blue-green, distinctly constricted at cross-walls, slightly attenuated at the ends, unbranched, less than 6 μm wide. Cells mostly quadratic, sometimes longer or shorter than wide, often with clearly fasciculated thylakoids and phormidiacean type of division. Apical cells longer than wide, conical to pointed, sometimes with strangely curved shape or bulge on either side, without calyptra. Necridia present.

Habitat:—Desert soils.

Etymology:—Genus is named in honor of the important Czech algological scientist, mentor, and friend Jan Kaštovský.

Type species:—Kastovskya adunca (Schwabe) Mühlsteinová, Johansen et Pietrasiak, comb. nov.

Basionym:—Schizothrix adunca Schwabe (1960: 293, fig. 7).

Emended description of the type species:—Thallus in the form of long, dark or bright blue-green filaments creeping on agar, becoming yellowish or orange with age, composed of creeping fascicles up to 7 mm long. Filaments containing one to multiple trichomes, 4.2–28.3 μm wide. Sheath colorless, usually thick, lamellated, sometimes wavy with undulated edges, containing one to several intertwined or parallelly arranged trichomes, occasionally narrowed at the ends. Trichomes motile, long, blue-green to pale blue-green, distinctly constricted at cross-walls, slightly attenuated at the ends, unbranched, (2.3) 2.5–5.0 (5.3) μm wide. Cells mostly quadratic, but can be longer or shorter than wide, often with distinct pattern of thylakoids and phormidiacean type of division, (1.8) 2.0–5.8 (10) μm long. Apical cells longer than wide, conical to pointed, sometimes with irregularly curved shape or bulge on either side, without calyptra, (1.8) 2.0–3.3 (3.8) μm wide and 4.0–15.8 (16.3) μm long. Necridia present.
Reference strain:—Kastovskya adunca ATA6-11-RM4, CCALA 1025 (Algal Collection at John Carroll University, Cleveland, USA and Culture Collection of Autotrophic Organisms at the Institute of Botany, Třeboň, CZ).

Observations:—One of the most noticeable features of Kastovskya is the characteristic shape of the apical cells. They range from bluntly conical to sharply pointed (Figs. 1I, M) and are often irregularly curved with a bulge on either side of the cell (Figs. 1B–E). The distinctive irregularly-shaped apical cells were present throughout the entire life cycle. Another rather unique morphological characteristic is the presence of distinct thylakoid fascicles in the cells (Figs. 1B–M). This feature was observed in cultures of all investigated ages (2 weeks–17 months) as well. Morphology of Kastovskya did not seem to be substantially affected by age of culture since transfer.

Morphologically, strains isolated in this study corresponded closely with the species described from the Atacama Desert by Schwabe (1960) as S. adunca. Schwabe named this species according to the characteristic shape of the apical cells (from Latin *adunca* = “curved”). Our isolates had a slightly larger range in width (2.3–5.3 μm compared to 2.7–5.0 μm), although the majority of our specimens were a near exact match in width (2.5–5.0 μm). Presence of the distinct apical cells together with correspondence of trichome measurements led to identification of our strains with *S. adunca*. However, because of the width of its trichomes, distinct thylakoid pattern, and position of its phylogeny based on 16S rRNA sequence, this morphotype clearly belongs to the order Oscillatoriales, while *Schizothrix* is placed in the Pseudanabaenales based on morphological characteristics of the type species *S. fusescens* Kützing ex Gomont (1892: 324).

In the order Oscillatoriales, *K. adunca* is the most similar to *M. steenstrupii* and *C. chthonoplastes* (Gomont) Siegesmund, Johansen & Friedl in Siegesmund *et al* (2008: 1575). All these taxa share the ability to create multiple trichomes in a common sheath, constrictions at the crosswalls, and trichome widths within 2–7 μm (Boyer *et al*. 2002, Siegesmund *et al*. 2008). Nevertheless, the combination of the distinct pattern of thylakoids, irregular curves and bulges on the apical cells, strong constriction of trichomes, motility, and presence of necridia are so far unique for *K. adunca*.

Phylogenetic analyses:—Based on the 16S rRNA sequence analyses, Kastovskya is a distinct clade well separated from the other taxa in Oscillatoriales (Fig. 2). The clade was highly supported in both Bayesian and parsimony analyses. “*M. steenstrupii* JO1-MC1” was the most closely related cyanobacterium by Bayesian analysis; however, this relationship was not supported by the parsimony analysis. The next relatively closely related, well defined group contained taxa assigned in GenBank to *M. steenstrupii*. This clade needs revision, as the type species of *Microcoleus (M. vaginatus)* is clearly in a clade distant from all other taxa designated as *M. steenstrupii* (Fig. 2). Furthermore, *M. steenstrupii* appears scattered throughout the clade containing *Geitlerinema* (Anagnostidis & Komárek 1988: 404) Anagnostidis (1989: 35), *Wilmoitia* and *Kastovskya*, so it is polyphyletic as currently defined, and consequently represents several as-yet undescribed genera. Finally, *M. steenstrupii* was originally described from soils surrounding a hot spring in Iceland; therefore, the desert soil strains given this placeholder name may all be distinct from that species.

Another related taxon is *W. murrayi* (West & West 1911: 289) Strunecký, Elster & Komárek in Strunecký *et al*. (2011: 63). This recently described genus is narrowly and well-defined using modern criteria and is thus free of the problems rampant in *Microcoleus*. Because *Wilmoitia* represents a rare case of a well-defined clade in the proximity of *Kastovskya*, and is fairly distantly related to *Kastovskya*, it was used as an outgroup (strain ANT.LPE.2, AY493631) for following analyses. As another outgroup *Phormidium* sp. B-Tom (EU196672), representing an as yet undescribed taxon that belongs to the sister clade of *Kastovskya* (Fig. 2), was used.

According to the comparison of the 16S sequence similarities, the most closely related taxon to *Kastovskya* was “*M. steenstrupii*” JO1-MC1 (AF355395), with a similarity as high as 96.2% to strain *K. adunca* ATA3-4-K001 (Table 2). The undefined sister clade had similarities of 94.5–95.5% with *Kastovskya* strains, a level of similarity low enough that most cyanobacteriologists would consider this evidence alone to be sufficient to define the sister clade as a separate species. *Wilmoitia murrayi* was even more distantly related, with similarity values 94.3–95.7% to *Kastovskya*. The similarity within *Kastovskya* was fairly high with a range of 98.8%–100% (Table 2).

Secondary structure of the 16S–23S ITS:—Only rRNA operons containing the tRNA^Ile^ gene in the 16S–23S ITS were recovered in *Kastovskya*. For the WJT32-NPBGF strain in the sister clade, operons with no tRNAs as well as the one with the tRNA^Ile^ gene were sequenced. Unfortunately, ITS sequence was not available for “*M. steenstrupii*” JO1-MC1. *Kastovskya adunca* ATA6-11-RM4 was the only strain for which a consensus sequence of the operons could not be obtained, and thus, letters A and C are used to distinguish the different operons. All comparisons that follow are based on operons with the tRNA^Ile^ gene in the ITS region.
The comparison of lengths of each ITS region resulted in a highly conserved pattern in *K. adunca* (Table 3). None of the strains differed from each other in the size of their helices or spacers; only the sequences varied among *Kastovskya* strains. The D1-D1’ helix was very consistent in *Kastovskya*, with a single structure shared by all the strains.
Kastovskya strains except ATA6-11-RM10 (Figs. 3A, B). In ATA6-11-RM10 the middle part of the helix differed by having an unpaired A-C instead of an A-U linkage (Fig. 3B). The sister group represented by WJT32-NPBGF and Phormidium sp. B-Tom seemed to share a unique feature in the D1-D1’ helix: two guanines on the 5’ side of the helix in opposition to the unilateral bulge on the corresponding side (Figs. 3C–D). However, WJT32-NPBGF and Phormidium sp. B-Tom differed from each other in length and structure of the terminal part of the D1-D1’ helix. All the outgroups differed from Kastovskya by absence of a small terminal loop subtended by a bilateral bulge, and by the number of bases creating the basal unilateral bulge (Kastovskya: 8 bases, outgroups: 6 or 9 bases).

**TABLE 2.** Comparison of the 16S rRNA gene sequence similarity among Kastovskya and outgroup taxa.

<table>
<thead>
<tr>
<th>Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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<tbody>
<tr>
<td>W. murrayi ANT.LPE.2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Phormidium sp. B-Tom</td>
<td>95.1</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>“M. steenstrupii” WJT32-NPBGF</td>
<td>94.1</td>
<td>95.9</td>
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<td>94.9</td>
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<td>K. adunca ATA6-11-RM4</td>
<td>94.3</td>
<td>95.5</td>
<td>95.2</td>
<td>96.0</td>
<td>99.6</td>
<td>99.8</td>
<td>99.7</td>
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<td>95.3</td>
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<td>99.3</td>
<td>99.8</td>
<td>99.4</td>
<td>99.4</td>
<td>100</td>
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<td>94.3</td>
<td>95.4</td>
<td>95.2</td>
<td>96.0</td>
<td>99.3</td>
<td>99.9</td>
<td>99.4</td>
<td>99.4</td>
<td>100</td>
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</tr>
</tbody>
</table>

**TABLE 3.** Lengths of the 16S–23S ITS region in Kastovskya strains and outgroup taxa.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Leader</th>
<th>D1-D1’ helix</th>
<th>D2 with spacer</th>
<th>D3 with spacer</th>
<th>tRNA Ile gene</th>
<th>Pre-Box-B spacer</th>
<th>Box-B helix</th>
<th>Post-Box-B</th>
<th>Box-A</th>
<th>D4</th>
<th>V3</th>
<th>D5</th>
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<tbody>
<tr>
<td>W. murrayi ANT.LPE.2</td>
<td>8</td>
<td>64</td>
<td>35</td>
<td>18</td>
<td>74</td>
<td>45</td>
<td>53</td>
<td>19</td>
<td>11</td>
<td>38</td>
<td>47</td>
<td>?</td>
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<tr>
<td>Phormidium sp. B-Tom EU196672</td>
<td>9</td>
<td>58</td>
<td>32</td>
<td>17</td>
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<td>80</td>
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<td>18</td>
<td>11</td>
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<td>“M. steenstrupii” WJT32-NPBGF</td>
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<td>74</td>
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<td>32</td>
<td>18</td>
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<tr>
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<td>72</td>
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<td>17</td>
<td>11</td>
<td>15</td>
<td>59</td>
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<tr>
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<td>11</td>
<td>15</td>
<td>59</td>
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<td>74</td>
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<td>17</td>
<td>11</td>
<td>15</td>
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</tbody>
</table>
The Box-B helix was the most conserved helix represented in *Kastovskya*, all strains having a single structure (Fig. 3F). The three comparison taxa shared the basal helix of four base pairs, but differed significantly in the remainder of the helix from *Kastovskya* and each other (Figs. 3G–I).


The V3 region was the most variable helix within *Kastovskya*, having four different secondary structures (Figs. 4A–D). However, these structures were highly similar with only minor differences in the middle part, but clearly different from the V3 helices of the comparison taxa (Figs. 4E–G).

**Discussion**

Komárek & Anagnostidis (2005) indicate that *Schizothrix*, as currently described, is a large heterogenous genus that in the strict sense should contain only those species with multiple trichomes in a common, widened, closed sheath, with parietal thylakoids, and no necridia. The maximum width of trichomes is given as 3 (4) µm (Komárek & Anagnostidis 2005). The morphotype described from the Atacama Desert as *S. adunca* does not meet the criteria...
for the trichome width (with trichomes as wide as 5.3 μm), and information on the position of thylakoids and necridia formation are not given (Schwabe 1960). However, the characteristic shape of the apical cells, corresponding measurements, and geographical origin clearly suggest that Kastovskya belongs to the same lineage as S. adunca. With detailed morphological investigation of newly isolated strains utilizing Nomarski DIC optics, missing information on the cellular ultrastructure could be added. Because the position of thylakoids is obviously fasciculated and necridia are present (Fig. 1), Schizothrix is not the correct genus for this morphospecies.

In addition to the morphological features suggesting placement of Kastovskya in the Oscillatoriales rather than the Pseudanabaenales, the position within Oscillatoriales was confirmed by phylogenetic analysis. A large phylogeny of all non-heterocytous cyanobacteria showed that Kastovskya is in the Oscillatoriophycidae rather than the Synechococcophycidae (tree not shown). Kastovskya is, to a certain extent, morphologically similar with “M. steenstrupii” and Coleofasciculus, but it is phylogenetically distinct from both taxa.

The analyses of the 16S–23S ITS region, allowing assessment of intrageneric variability, did not reveal any major differences among K. adunca strains investigated in this study (Table 3, Figs. 3–4). Even though the strains were isolated from three different sites (Table 1), the morphology was identical among all of them and agreed nearly exactly with Schwabe’s (1960) description. Furthermore, the variance in ITS sequence and structure was very minor and consistent with interpopulational variability. Based on the invariance in both morphology and ITS structure, only a single species of Kastovskya could be recognized, K. adunca.

V3 helix


Based on the 16S rRNA sequence phylogeny, the most related taxon was represented by the strain identified as “M. steenstrupii” (J01-MC1). However, the position of this sequence in close proximity to the Kastovskya cluster was supported only by Bayesian analyses (Fig. 2). The 16S similarity of this strain to Kastovskya was 96.2% which represents a fairly high value. Nevertheless, comparison of 16S rRNA gene similarities has been shown as an unreliable technique for cyanobacterial taxonomy when applied as the sole factor in taxonomic assignment (Johansen & Casamatta 2005). Unfortunately, the 16S rRNA sequence together with three pictures from the original study by Boyer et al. (2002) represented the only available information about this strain; however, the morphology of J01-MC1 strain (based on three original pictures, not shown) corresponded exactly with the description of M. steenstrupii given by Boyer et al. (2002). J01-MC1 strain did not exhibit any distinct pattern of thylakoids, the curved shape of apical cells, or the degree of constriction observed in Kastovskya (cf. Fig. 1 and Boyer et al. 2002: fig. 2f–h). Based on these morphological differences and sequence dissimilarity, we decided not to transfer the J01-MC1 strain to Kastovskya at this time, but to leave it with its original place-holder name until more data are available.
As can be seen in the example of the re-investigated phylogeny of strains from Boyer et al. (2002), morphology in the order Oscillatoriales is insufficient for discrimination of the different lineages. In fact, strains falling within the morphological description given by Boyer et al. (2002) for *M. steenstrupii* are distributed in the 16S rRNA tree among taxa with clearly different morphology (e.g. *Wilmottia, Kastovskya*). However, morphology in the original study was not investigated throughout the whole life cycle, so it is possible that some morphological variability within the *M. steenstrupii* morphotype exists. The polyphyletic nature of this group highlights the need for using large datasets for phylogenetic studies. Today, 11 years after Boyer et al. (2002), many more cyanobacterial sequences are available in databases. Consequently, much more reliable results in terms of phylogenetic position of strain groups can be obtained.

Recent investigations have shown that the Atacama Desert possesses an interesting cyanobacterial flora, which definitely deserves more attention (Osorio-Santos 2011, Baldarelli 2012). *Kastovskya adunca* studied in this work represents a stable morphotype unique for this desert. Since the description of this cyanobacterium more than 50 years ago (Schwabe 1960) it has not been recorded from any other location. This suggests that the Atacama Desert contains endemic cyanobacterial species. By thorough investigation of cyanobacteria from different parts of the world, not only will microfloristics be better understood, but cyanobacterial diversification will be better characterized. The simple filamentous cyanobacteria need further revision and many new genera and species probably will need to be described and defined in order to obtain a taxonomic system comprised only of monophyletic taxa.

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http://dx.doi.org/10.1007/BF00940428


http://dx.doi.org/10.1007/s00248-010-9784-5

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