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Morphology and Molecular Phylogenetics of *Sphaerospermopsis oumiana* (M.Watan.) Tuji et Niiyama Compared with *Sphaerospermopsis torques-reginae* (Komárek) Werner *et al.*

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Abstract The morphology and the 16S rDNA sequences of *Shaerospermopsis oumiana* (M.Watan.) Tuji et Niiyama from Japan and *S. torques-reginae* (Komárek) Werner *et al.* from South America are compared and their phylogenetic relationship are discussed. The results show that *S. oumiana* and *S. torques-reginae* are distinct species.

Key words: *Anabaena oumiana*, *Anabaena torques-reginae*, 16S rDNA sequences, morphology, *Sphaerospermopsis oumiana*, *Sphaerospermopsis torques-reginae*.

Introduction

Polyphasic study using the morphological, ecological, physiological and molecular methods is important to solve the phylogeny of cyanobacteria and several new genera have been established for planktonic taxa of traditional *Anabaena* and *Aphanizomenon* based on the polyphasic approach (Komárek, 2005; Rajaniemi *et al.*, 2005; Tuji and Niiyama, 2010; Wacklin *et al.*, 2009; Zapomělová *et al.*, 2009).

Zapomělová *et al.* (2009, 2010) described a new genus, *Sphaerospermopsis*, based on the morphological features and 16S rDNA sequences. The trichome of *Sphaerospermopsis* is free-floating, solitary, coiled or straight, akinetes are spherical or nearly spherical and adjacent to heterocytes, and terminal cells elongated and tapered or undifferentiated. Zapomělová *et al.* (2009) pointed out the necessity to confirm the affiliation of *Anabaena oumiana* M.Watan. and *A. torques-reginae* Komárek to genus *Sphaerospermopsis* using analysis of 16S rDNA sequences. *A. torques-reginae* was originally described by Komárek (1984) from Cuba. Komárek (2005) stated that *A. torques-reginae* occurs in Cuba, Brazil and San Salvador. *A. oumiana* was observed for the first time in 1994 from Lake Biwa in Japan and described as a new species by Watanabe (1996). The trichome morphology of *A. torques-reginae* and *A. oumiana* corresponds well to that of *Sphaerospermopsis*. Tuji and Niiyama (2010, 2012) analyzed 16S rDNA and rbcLX sequences of many Japanese planktonic *Anabaena* strains and proposed the new combination of *Sphaerospermopsis oumiana* (M.Watan.) Tuji et Niiyama for *Anabaena oumiana* based on both morphological characteristics (Watanabe *et al.*, 2004) and 16S rDNA and rbcLX DNA sequences. Werner and Laughinghouse IV (2009) reported *A. oumiana* for the first time from South America. Later Werner *et al.* (2012) reidentified this taxon as *A. torques-reginae* according to the opinion of Komárek and Zapomělová (2007), who treated *Anabaena oumiana* as a synonym of *Anabaena torques-reginae*, and then transferred to *Sphaerospermopsis torques-reginae* (Komárek) Werner *et al.*

from the morphological and molecular studies.

The aim of this study is to compare the morphology and the 16S rDNA sequences of *S. oumiana* from Japan and *S. torques-reginae* from South America and discuss the phylogenetic relationship of these taxa.

Materials and Methods

The history of cultured strains of Japanese *Sphaerospermopsis oumiana* used in this study and the method of DNA extraction and amplification are stated in Tuji and Niiyama (2010).

The Basic Local Alignment Search Tool (BLAST) at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and some data of our own strains were used to find sequences similar to the sequence of *S. oumiana* and *S. torques-reginae*. Short sequences in NCBI were not used. Molecular evolutionary analyses for obtained sequences were conducted using MEGA 5 computer program (Tamura *et al.*, 2011). Alignments were checked manually. Maximum Likelihood (ML) tree was calculated using the software with the best fits model by BIC scores (Bayesian Information Criterion) and the substitution nucleotide matrix parameters were calculated by the software. One thousand bootstraps were generated. Neighbor-joining (NJ) analysis was performed using the same model as for the ML. Bootstrapping values for the NJ tree was also generated using 1000 replicates. All positions containing gaps and missing data were eliminated.

Results and Discussion

Sphaerospermopsis oumiana and *S. torques-reginae* have similar morphology as following, trichomes are free-floating and solitary, akinetes are spherical and at one or both sides of the heterocytes and heterocytes are spherical (Komárek, 1984; Watanabe, 1996). However, the trichomes of *S. torques-reginae* are irregularly coiled and two trichomes are arranged in parallel (Komárek, 1984, 2005) or irregularly or regularly coiled (Werner and Laughinghouse IV, 2009; Werner *et al.*, 2012), while those of *S. oumiana* are regularly coiled with thick mucilage but not arranged in parallel (Watanabe, 1996; Watanabe *et al.*, 2004) (see Table 1). The size of akinetes of

Table 1. Morphological comparison and locality of *Sphaerospermopsis oumiana* and *S. torques-reginae* according to Komárek (1984), Watanabe (1996), Watanabe *et al.* (2004), Werner and Laughinghouse IV (2009) and Werner *et al.* (2012)

	S. oumiana	*S. oumiana*	*S. torques-reginae*	*S. torques-reginae*	*S. torques-reginae*
Reference	Watanabe (1996)	Watanabe *et al.* (2004)	Komárek (1984)	Werner and Laughinghouse IV (2009)	Werner *et al.* (2012)
Trichome morphology	regularly coiled	regular or slightly irregular coils	irregulaly coils, parallel filaments in coils	slightly irregular coils	irregular or regular coils
width	28–46 μm	20–40 μm		20–46 μm	(17.5) 20–54.5 μm
distance	10–20 μm	10–30μm		(8.3) 9–20 μm	9–22.5 μm
mucilage	thick			thick (1–10.2 μm)	thick (1–10.2 μm)
Cell morphology	subspherical or barrel-shaped	spherical	spherical	rounded	rounded
width	6.0–7.1 μm	5.0–7.5 μm	5.5–7.6 μm	5–7.6 μm	(4) 4.3–7 (8) μm
length	3.0–6.5 μm	3.8–7.5 μm		4.5–7.5 μm	4–6.4 (8) μm
Heterocyte morphology	spherical	spherical	spherical	rounded	rounded
width	6.8–8.5 μm	7.5–8.8 μm	6.8–10 μm	(6) 7–9 (9.5) μm	(4–5) 5.4–9.5 μm
length	6.4–8.0 μm			6.8–8 μm	(5) 5.5–9 μm
Akinete morphology	spherical	spherical	spherical	rounded	rounded
width	10–12 μm	9.5–12.5 μm	14–14.5 μm	9–13 μm	(7) 7.9–13 μm
Locality	Japan	Japan	Cuba	Brazil	Colonbia, Brazil, Argentina

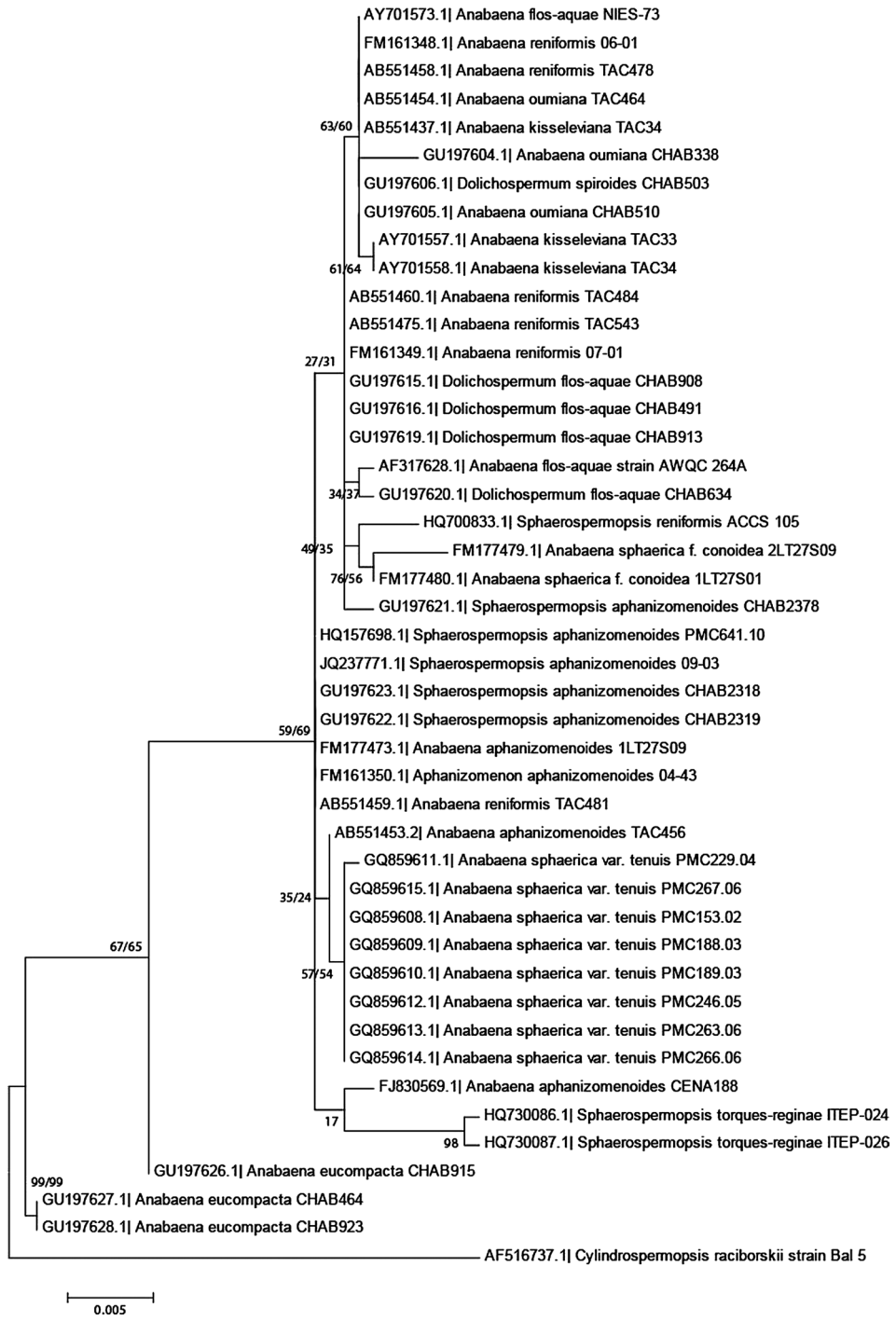

Fig. 1. Phylogenetic position of *Shaerospermopsis oumiana* and *S. torques-reginae* and related taxa determined by Maximum Likelihood (ML) method applied to 16S rDNA sequences. Originally deposited scientific names were used. The analysis involved 46 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1239 positions in the final dataset. Numbers at branches indicate NJ (Neighbor Joining)/ML bootstrap support values. Deposited taxonomic names in gene bank, were used.

Table 2. Estimates of evolutionary divergence among the 16S rDNA gene sequences of *Shaerospermopsis oumiana* and *S. torques-reginae* and related taxa. The numbers of base differences per sequence from between sequences are shown. The analysis involved 46 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1239 positions in the final dataset.

		1	2	3	4	5	6	8	9	13	14	15	19	21	22	26	30	31	39	40	41	42	43	45
1	AB551454.1|_Anabaena_oumiana_TAC464																							
2	GU197605.1|_Anabaena_oumiana_CHAB510	0																						
3	GU197604.1|_Anabaena_oumiana_CHAB338	4	4																					
4	AB551437.1|_Anabaena_kisseleviana_TAC34	0	0	4																				
5	AB551458.1|_Anabaena_reniformis_TAC478	0	0	4	0																			
6	AB551459.1|_Anabaena_reniformis_TAC481	3	3	7	3	3																		
8	AB551475.1|_Anabaena_reniformis_TAC543	1	1	5	1	1	2																	
9	FM161348.1|_Anabaena_reniformis_06–01	0	0	4	0	0	3	1																
13	AY701558.1|_Anabaena_kisseleviana_TAC34	1	1	5	1	1	4	2	1															
14	AY701573.1|_Anabaena_flos-aquae_NIES-73	0	0	4	0	0	3	1	0	1														
15	AF317628.1|_Anabaena_flos-aquae_strain_AWQC_264A	3	3	7	3	3	4	2	3	4	3													
19	GU197620.1|_Dolichospermum_flos-aquae_CHAB634	3	3	7	3	3	4	2	3	4	3	2												
21	FJ830569.1|_Anabaena_aphanizomenoides_CENA188	7	7	11	7	7	4	6	7	8	7	8	8											
22	AB551453.2|_Anabaena_aphanizomenoides_TAC456	4	4	8	4	4	1	3	4	5	4	3	5	5										
26	GU197621.1|_Sphaerospermopsis_aphanizomenoides_CHAB2378	3	3	7	3	3	4	2	3	4	3	4	4	6	5									
30	FM177480.1|_Anabaena_sphaerica_f._conoidea_1LT27S01	3	3	7	3	3	4	2	3	4	3	4	4	8	5	4								
31	GQ859609.1|_Anabaena_sphaerica_var._tenuis_PMC188.03	5	5	9	5	5	2	4	5	6	5	2	4	6	1	6	6							
39	FM177479.1|_Anabaena_sphaerica_f._conoidea_2LT27S09	8	8	12	8	8	9	7	8	9	8	9	9	13	10	9	5	11						
40	HQ730086.1|_Sphaerospermopsis_torques-reginae_ITEP-024	12	12	16	12	12	11	11	12	13	12	13	13	11	12	13	9	13	14					
41	HQ730087.1|_Sphaerospermopsis_torques-reginae_ITEP-026	12	12	16	12	12	11	11	12	13	12	13	13	11	12	13	9	13	14	2				
42	GU197626.1|_Anabaena_eucompacta_CHAB915	14	14	18	14	14	11	13	14	15	14	13	15	13	10	13	15	11	20	22	22			
43	GU197627.1|_Anabaena_eucompacta_CHAB464	23	23	27	23	23	20	22	23	24	23	20	22	18	19	22	22	18	27	19	19	9		
45	AF516737.1|_Cylindrospermopsis_raciborskii_strain_Bal_5	40	40	44	40	40	37	39	40	41	40	39	39	34	38	37	37	37	42	34	34	38	31	

S. torques-reginae is 14–14.5 μm (Komárek, 1984) or 7.9–9–13 μm (Werner and Laughinghouse IV, 2009; Werner *et al.*, 2012) and those of *S. oumiana* is 9.5–12.5 μm (Table 1). The maximum size of akinetes of *S. torques-reginae* is larger than those of *S. oumiana*. The cells of *S. oumiana* are spherical or barrel-shaped and the cells of *S. torques-reginae* are spherical. Distribution of both taxa is also so different, as *S. oumiana* is found only in Japan, while *S. torques-reginae* is found from Central and South America.

Phylogenetic tree based on 16S rDNA (Fig. 1) suggests that *S. oumiana* is genetically far from *S. torques-reginae* in the genus *Sphaerospermopsis* though supported values on this tree are almost very low. The number of base differences between these two species are bigger than the differences between *S. oumiana* and other *Sphaerospermopsis* species such as *S. kisseleviana* (Elenkin) Zapomělová *et al.* and *S. aphanizomenoides* (Forti) Zapomělová *et al.* These morphological (Table 1) and molecular (Fig. 1 and Table 2) results indicate that *S. oumiana* and *S. torques-reginae* are not synonyms and should be differentiated at species level.

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