Morphology and Phylogeny of *Scrippsiella trochoidea* (Dinophyceae**) a potentially harmful bloom forming species isolated from the sediments of Iran's south coast**

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Received: July 2011 Accepted: November 2011

Abstract

Phytoplankton cells and resting cysts of the species *Scrippsiella trochoidea* are regular and dominant components of the dinoflagellate flora of coastal marine waters and sediments around the world. This species is a common harmful bloom forming species in coastal waters. In this study, for the first time cyst of S. *trochoidea* were isolated from the sediments of southeast coast of Iran. Five strains from the germination of a single cyst belonged to *S. trochoidea*. In order to confirm identification of the species an excystment and encystment experiment, cyst and germinated cell morphology and plate pattern by light and electron microscopy (SEM) have been described. The nucleotide sequences of two highly diverse regions, the rDNA-ITS 1,2 and 5.8S-rDNA have been sequenced for all strains. Homologous sequences from GenBank with five Iranian strains were compared to find their phylogenetic relationship. Both NJ and MP phylogenetic and morphological analysis showed five strains of *S. trochoidea* from Iran were clustered with previously described *S. trochoidea* and *Calciodinellum levantinum* species, and its closest relationship was with *Scrippsiella* sp. strain with a 1.2-1.4% sequence divergence. Results indicate that molecular studies of rDNA if combined with morphological cyst and vegetative cells could be a valuable approach to identification and taxonomy of calciodinelloideae dinoflagellate.

Keywords: Cyst, Iran south coast, Molecular analysis, Morphology, Phylogeny, *Scrippsiella trochoidea,* Sediment, Vegetative cell

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[DOR: 20.1001.1.15622916.2012.11.2.2.0]

Introduction

The marine dinoflagellate *Scrippsiella* Balech ex A.R and Loeblich III belonging to the subfamily of Calciodinelloideae (order peridinales Ehrenberg) is morphologically conservative but ecologically a diverse group of thecate photosynthetic marine phytoplankton. *S. trochoidea* is a common bloom forming species (e.g. Juárez, 2008; Wang et al., 2008; Gárate-Lizárraga et al., 2009; Spatharis et al., 2009; Zhuo-Ping et al., 2009) that has been listed by UNESCO. The resting cyst acts as seed population for initiation and decline of bloom. The genus comprises 20 species including *S. trochoidea* (Stein and Leoblich III) that produce calcareous cysts. The cysts are common and cosmopolitan species (Tang et al., 2010). Cyst wall composition and structure, archeopyle type, cyst shape, and the number of thecal plates on the vegetative cell are the main taxonomic features used in the classification of calcareous dinoflagellates cyst species (Fensome et al., 1993; D'Onofrio et al., 1999). According to the resting cysts, the shape and arrangement of calcareous crystalline processes are important features for taxonomy (Lewis, 1991). Many calcareous resting cysts germinate to produce small motile cells with a typical *Scrippsiella* plate pattern and have generally been referred to as *S. trochoidea* (Blanco, 1995; Montresor et al., 1998; Godhe et al., 2000). However, Montresor et al. (2003) has demonstrated high morphological, physiological and genetic variability within this single morph type, suggesting the presence of several unresolved cryptic species. The separation of cryptic species within the group based only on cell morphology can be difficult or impossible; therefore, integrated molecular data and morphological studies of cyst and germinated cell could be valuable for distinguishing *Scrippsiella* species.

Nucleotide sequences of internal transcribed spacers (ITS) regions containing the 5.8S rRNA gene are considered to be less conserved than the small and large subunit rRNA genes and are therefore useful for phylogenetic inference at the inter- and intraspecific level (Adachi et al., 1994; Adachi et al., 1996; Adachi et al., 1997; Penna and Magnani, 1999). This region has also been shown to be suitable for evaluation of genetic relationship among *Scrippsiella* species (Montresor et al., 2003; Gottschling et al., 2005a, b). In this study, we have described morphology of cyst and germinated *S. trochoidea* cell from south coast of Iran using light and scanning electron microscopy and also compared rDNA-ITS sequences with those of the related species. This work is the first record of *Scrippsiella trochoidea* from Iran.

Materials and methods

Cyst Isolation and Germination experiment

Sediment samples were collected by Ekman grab from Pasabandar along the southeast coast of Iran in 2007 (Fig. 1). Pasabandar is a coastal area in which usually harmful algae bloom and cause water discoloration and an increase in fish and shellfish mortality. Sediment from grab was stored at 20°C in anoxia

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condition prior to further processing. In the laboratory 3g of stored sediment was mixed with filtered sea water and sonicated for 3 min using a microson ulterasonic cell disruptor (small probe 200W). The samples were processed by sieving through a 125 µm sieve and collected ona 20 µm sieve (Attaran-Fariman et al., 2011). After washing the suspension , density gradient centrifuged test was conducted using sodium polytungstate (SPT) with specific gravity of 1.3 g cm^3 (Attaran-Fariman and Bolch, 2007). Cysts from processed material were collected into suitable containers for analysis.

Single cysts were isolated by micropipette under a Leica stereomicroscope and after washing with distilled water they were placed in a well containing 20 ml f2 medium (Guillard,1962). The isolated cysts were incubated at 27°C±0.5 under light 70-90µmol photon $m^{-2}s^{-1}$ with a 12:12 light: dark cycle. The plates were checked regularly for germination. Germinated cells were allowed to grow for a week and then they were transferred to Erlenmeyer flasks containing 100 culture medium. For encystment studies *Scrippsiella trochoidea* strains were incubated in nitrate-phosphate deficient f2 medium under the above conditions (Attaran-Fariman and Bolch, 2007).

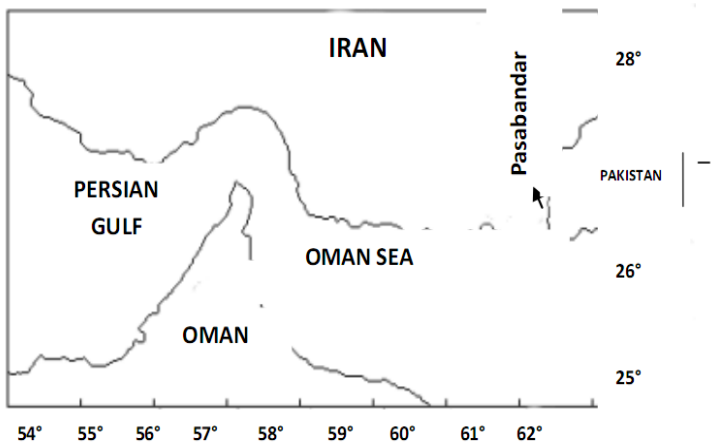


Figure 1: Map of sampling site

Light and SEM Microscopy

Wild and cultured cysts and germinated cells were photographed by an Olympus BH-2 light microscope equipped with a Leica DC300F digital imaging system. For SEM, two methods were utilized. In the first method, the cells were prepared using the cell stripping technique of Mason et al. (2003). In the second method, 10-ml of cultured cells was concentrated by centrifugation collected on poly-lysine coverslips (Marchant and Thomas, 1983) and using a graded methanol series for

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dehydration. Mounted specimens were then dried from hexamethyldisilazane (Nation, 1983) and placed on SEM stubs for examination by SEM. Air dried cysts on Nuceopore filters were mounted on SEM stubs. Stubs were coated with gold and examined with JEOL JSM-840 scanning electron microscope.

DNA extraction and phylogenetic analysis

Genomic DNA of *S. Trochoidea* unialgal cultures in exponential growth phase were extracted using phenol:chloroform:isoamyl alcohol gentle-lysis method (Bolch et al., 1998). The internal transcribed spacer and 5.8S rRNA gene (rDNA-ITS) region was amplified using the primers ITSA (5-CCA AGC TTC TAG ATC GTA ACA AGG (ACT)TCC GTA GGT-3 and ITSB(5-CCT GCA GTC GAC ATG ATG CTT AA(AG) TTC AGC (AG)GG-3 (Adachi et al. 1994). The PCR reactions contained Bioline NH4 PCR reaction buffer, 3 mM MgCl2, 200 mM dNTPs,10 pM of each primer, 1 U BioTaq DNA polymerase (Bioline,UK), and 10 ng of DNA template. The Thermocycling program consisted of 94°C for 2 min, followed by 35 cycles of 1 min at 94°C, 1 min at 60°C and 2 min at 72°C. Montage^{TM} PCR Devices (Millipore, Billerica, MA, USA) were used to purify successful PCR products according to manufactures protocol. DNA was quantified by a Turner TBS-380 DNA fluorometer. Purified PCR products were sequenced in both directions with the forward or the reverse primer using a Dye Terminator Sequencing Kit (Beckman-Coulter, Fullerton, CA, USA) and completed reactions were electrophoresed on a Beckman-Coulter CEQ2000, USA capillary sequencer. Base-calling errors of nucleotide sequences of electropherograms in both directions were corrected using the BioEdit (Hall, 1999). Sequences were aligned using ClustalX version 1.83 (Jeanmougin et al., 1998). For the phylogenetic analysis PAUP* 4.0b10 (Swofford, 2002) was used. Analysis included 30 rDNA-ITS sequences from calciodinellaceaean dinoflagellate (table 1) to create main branching patterns and clusters between taxa. The peridinioid taxa *Heterocapsa triquetra* Ehrenberg 1840 (Stein, 1883) was used as out groups to root the analyses. This species is close enough to *S. throchoidea* to allow inference from sequence and far enough to be a clear outgroup. Both species belong to thecate dinoflagellate but with clear differences in hypotheca tabulation. Significant phylogenetic structure in the data set was estimated by the random tree method and probability tables using the critical values of g1 (Hillis and Huelsenbeck, 1992). Phylogenetic trees were made using neighbor-joining (NJ) using mean distance and logDet-Paralinear distance matrices and maximum parsimony (MP) using the branch and bound algorithm. All characters were equally weighted, and gaps were treated as missing data, with multistate characters (DNA ambiguities) interpreted as uncertainty Bootstrap analyses of NJ and MP trees (Felsenstein, 1985) utilized 1000 replicates of the heuristic search algorithm.

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Table 1: List of taxa and strains and their GenBank accession used in this study

Taxon	Strain No.	GenBank No.
Scrippsiella precaria	CS-294	AY499518
Scrippsiella ramonii	SZN7	AF527820
Scrippsiella infula	GeoB 110	AY499523
Scrippsiella rotunda	SZN66	AF527821
Scrippsiella trifida	GeoB 109	AY499521
Scrippsiella lachrymosa	D192	AY728078
Scrippsiella sp.	M34-*25/5	AY499531
Scrippsiella sp.	GeoB 138	AY499525
S. trochoidea var. *aciculifera*	SZN63	AF527078
S. trochoidea var. *aciculifera*	GeoB*213	AY676164
S. trochoidea	GeoB*214	AY676160
S. trochoidea	SZN33	AF527070
S. trochoidea	SZN61	AF527075
Calciodinellum sp.	GeoB 120	AY676148
Calciodinellum albatrosianum	GeoB 149	AY676143
Calciodinellum albatrosianum	M34-*26/4	AY676145
S. lachrymosa	D192	AY676150
S. sweeneyae	NIES 684	AY499520
Pentapharsodinium dalei	SZN19	AF527817
Calciodinellum levantinum	GeoB 122	AY676146
Ensiculifera cf. *imariensis*	JB3	AF527814
Scrippsiella hangoei	SHTV1	AY499515
Pernambugia tuberosa	GeoB 61	AY499519
Heterocapsa triquetra	NIES 7	AB084101
S. irregularis	SCBC17	EF584460
Scrippsiella sp.	SCPC21	EF584453
Scrippsiella sp.	SCPC116	EF584459
S. trochoidea	SCBC18	EF584454
S. trochoidea	SCPC36	EF584455
S. trochoidea	SCPC39	EF584458
S. trochoidea	SCPC51	EF584457
S. trochoidea	SCPC73	EF584456

Note: The last 8 strains are from Iranian coastal area; SCPC stand for S; *Scrippsiella* C; Chabahar; P; Pasabandar, C; excystment from Cysts, B;Bahoo-Kalat

Results

Morphology

The thecate cells established from five resting cysts and all uni-algal cultures possessed the same morphology and thecal plate pattern as *S. trochoidea*, as described by Lewis (1991). Strains are named as SCBC18, SCPC36, SCPC39, SCPC51, SCPC73 and have been submitted in the Genbank with the same name. Most strains possessed a conical epitheca with straight sides and a well pronounced apical horn (Figs 2, 7, 9). Cell sizes range from 18-34µm in length (average=24µm, n=50) and 17-29µm in width (average =22µm, n=50). Numerous simple trichocyst pores are scattered on the thecal plates. The epitheca is slightly larger than the hypotheca.

The plate tabulation is Po, X, 4', 3a, 7", 5C+1 transitional plate, 5S, 5'", 2"" (Figs 2-10). The first apical plate is relatively broad and rhomboidal and slightly asymmetrical (Figs 4, 7a, 10). The

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pore plate is circular and surrounded by a high collar (Figs 2-10, 9, 10) connected to the first apical plate by a long well-defined canal plate. Five sulcal plates are present. The Sm plate is not completely hidden by the Sd plate (Fig. 6). The transverse flagellum originates is between Sa and Ss plates and both contact with the T plate (Fig. 6). The longitudinal flagellum originates at the connection between the plates Sd and Sm which is functionally confined by the groove of the Sp plate. The sulcus is deeply excavated and broadens antapically (Figs 4, 3c, 9). The nucleus is spherical and centrally positioned (Fig.15b). The globular to ribbon shaped chloroplasts are peripherally placed (Fig.15b).

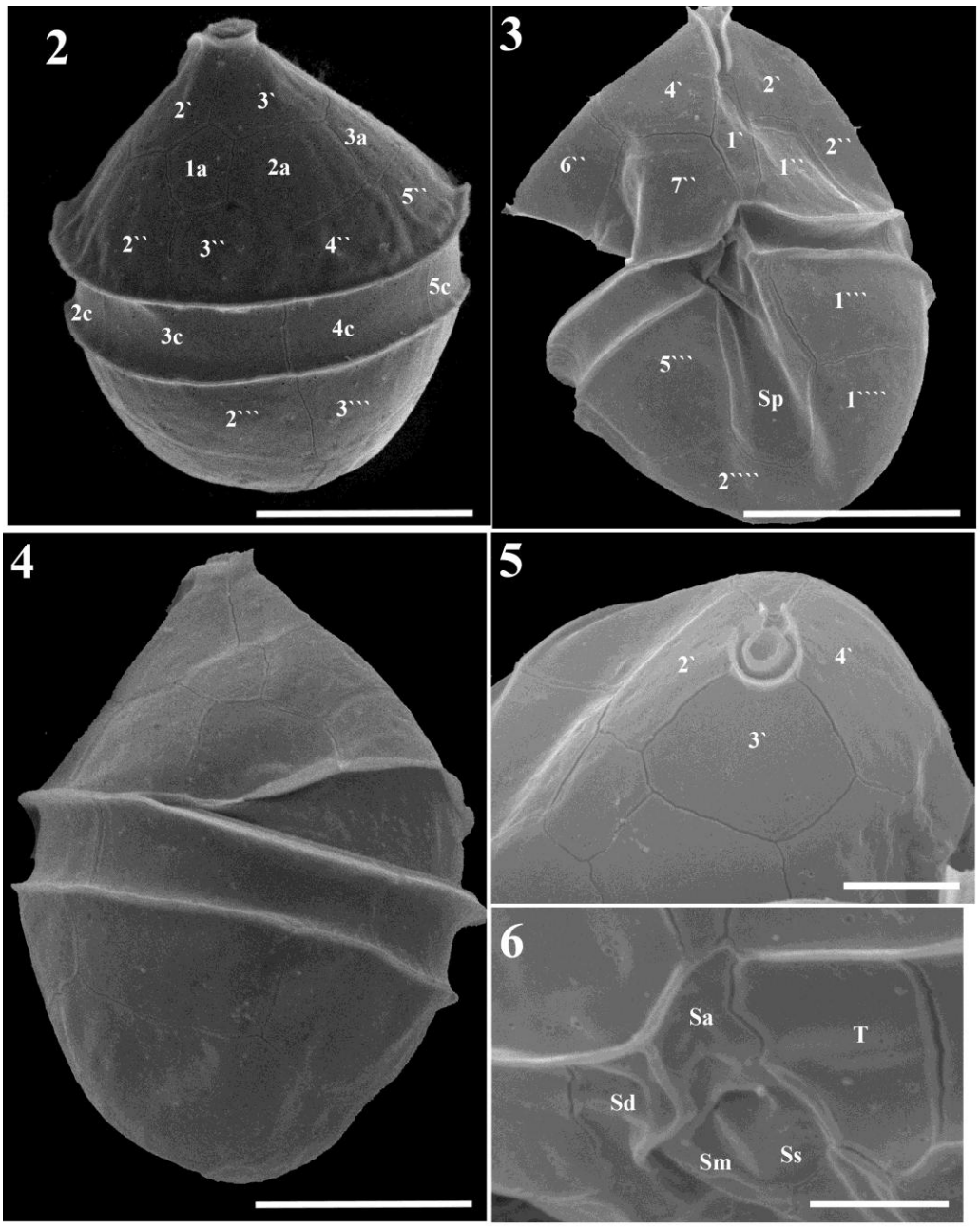
Resting cysts are spherical to oval in shape, and range from 18-37µm in diameter. The cyst wall is covered by calcareous spines that extend from a polygonal basal plate and are triangular in cross-section. The spines range from 1.6-4µm in length. All cysts successfully germinated did not possess spines (Figs 11, 12, 14). The archeopyle is an irregular split about 1/4 of the cyst diameter, with a cap-shaped operculum remaining attached (Figs 17, 19). Cysts are dark grey-brown in color. A red accumulation body is visible in intact cysts (Fig. 16).

Phylogenetic analysis

Based on sequencing of the rDNA-ITS region, sequence alignment contained 31 taxa and 757 characters (including gaps introduced into the alignment). Five sequences of *S. trochoidea* from this study, with 25 other Scrippsiella-like species available from GenBank were used in the phylogenetic analysis. Significant negative skewness was observed in the tree length distribution of random maximum parsimony with 30 taxa ($g1=-0.787$, $P< 0.01$), suggesting that the datasets were phylogenetically informative and more structured than random data (Hillis and Huelsenbeck, 1992). Both NJ and MP analyses produced trees with similar branching topology therefore only MP tree is shown in this study. The Mp analysis showed two major clades; the first clade consisted of *Pentapharsodinium dalei* Indelicate and Loeblich and *Ensiculifera* Balech species; the second clade included all *Scrippsiella*-like species (i.e. this clade is referred to as SCR clade hereafter). Both clades were highly supported by bootstrap analyses (100% and 93% respectively). *Scrippsiella hangoei* occupied a basal position in first clade. The SCR clade included *Scrippsiella, Calciodinellum* Deflandre and *Pernambugia* Janofske & Karwath.

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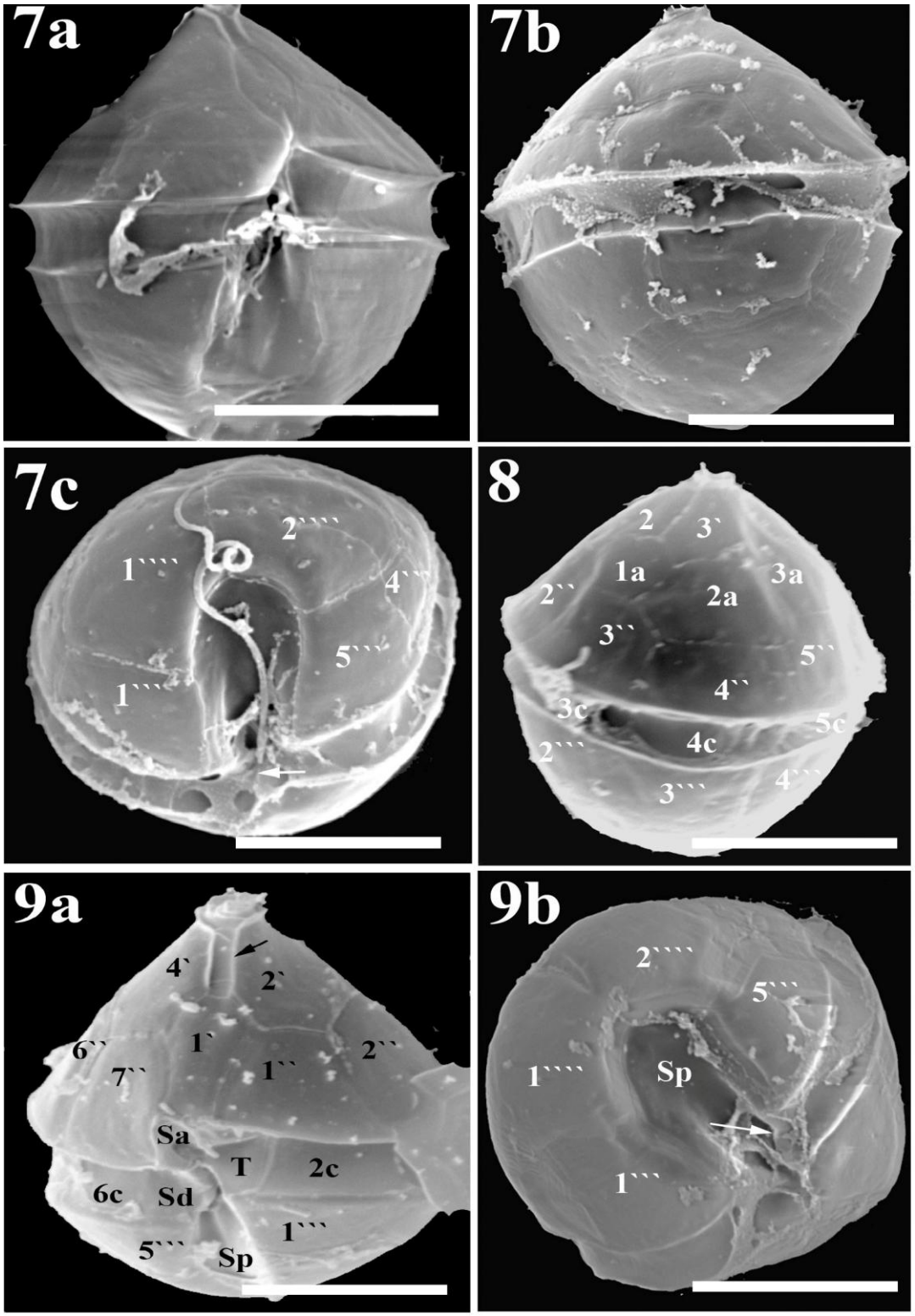
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Figures 2-6: SEM. *Scrippsiella trochoidea* (strain SCBC18). Fig. 2. Dorsal view showing the plate pattern. Fig. 3. Dorso-lateral view of the cell. Fig. 4. Ventral view showing the plate pattern. Fig. 5. Detail of the apical region. Fig. 6. Detail of sulcal plates. All scale bars=10µm, except Fig. 5 and 6 scale bars=5µm and 2µm respectively.

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Figures 7-9: SEM. *S. trochoidea* thecate cells. Fig. 7a. Ventral view of strain SCPC36. Fig. 7b. Dorsal view of strain SCPC36. Fig. 7c. Antapical view of strain SCPC36. Fig. 8. Dorsal view of strain SCPC39. Fig. 9a. Ventral view of strain SCPC51. Note pronounced canal plate (arrow). Fig. 9b. Antapical view of the same strain. Arrow showing S_m plate. All scale bars=10µm.

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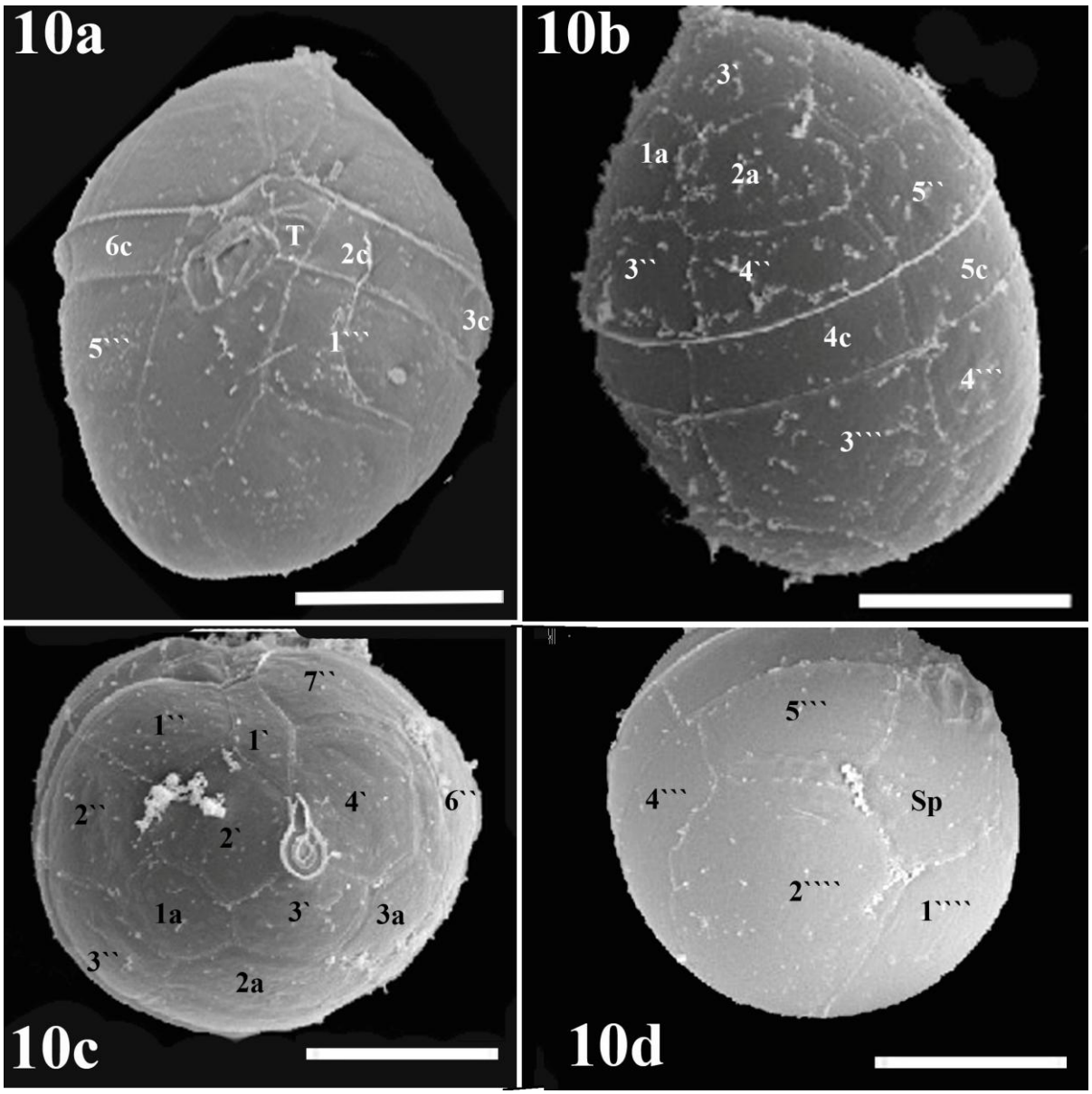
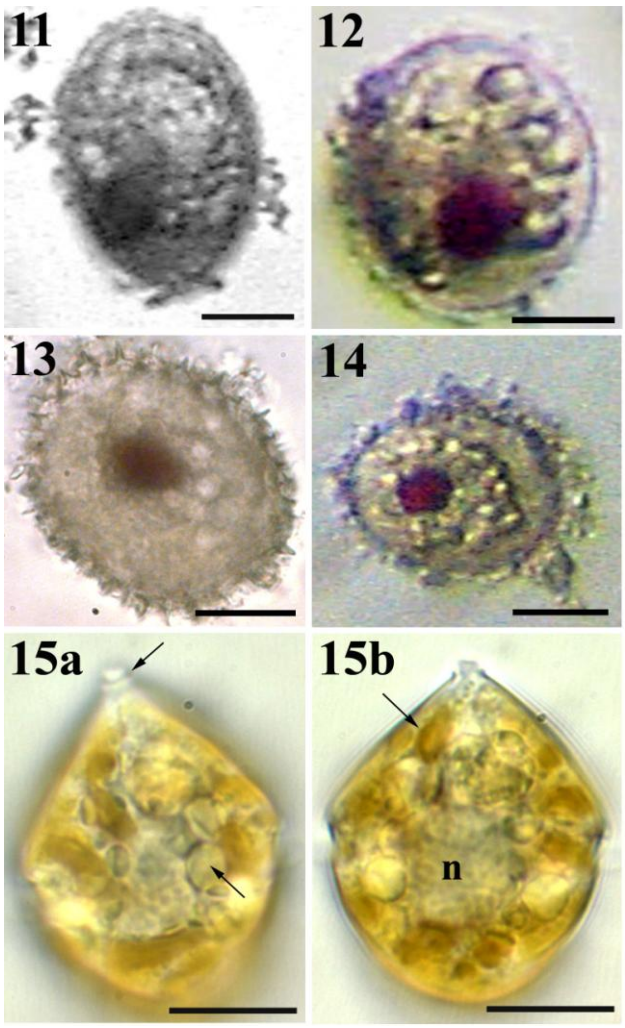


Figure 10a-d: SEM. Thecate cell of *S. trochoidea* strain SCPC73. Stripping and cell swelling technique was used. Fig. 10a. Ventral view of the cell. Fig. 10b. Dorsal view of the cell. Fig. 10c. Apical view of the cell. Fig. 10d. Antapical view of the cell. All scale bars=10µm

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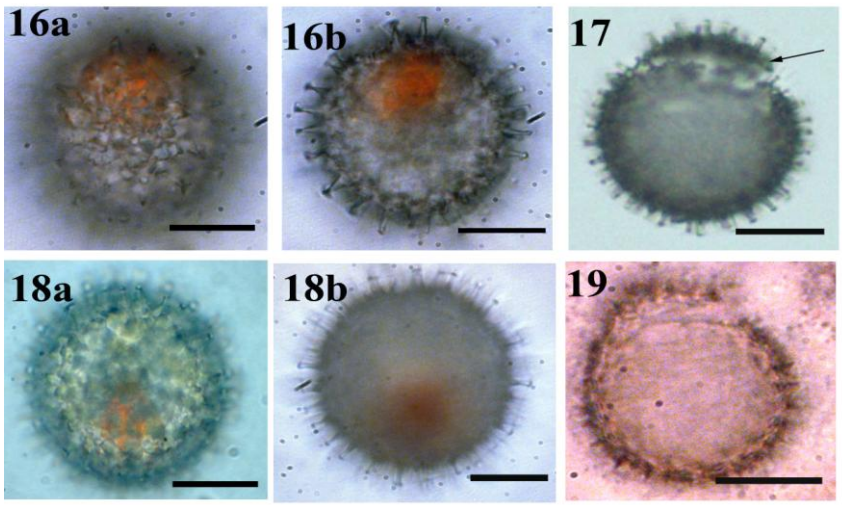
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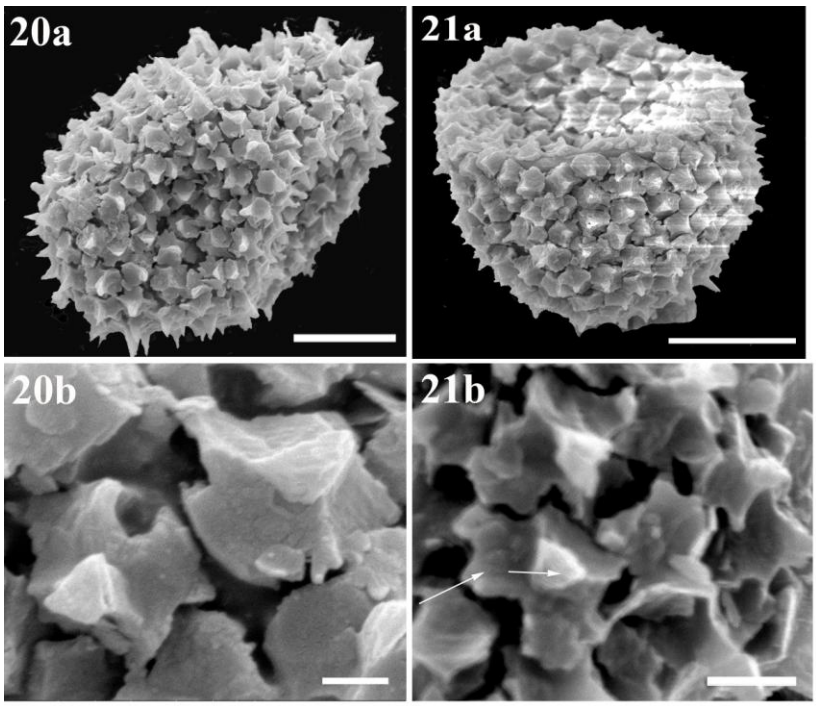
Figures 11-15: LM. Resting cysts of *Scrippsiella trochoidea*.. Fig. 11. Wild resting cyst germinated to establish strain SCBC18. Fig. 12. Wild resting cyst germinated to establish strain SCPC36. Note lack of spines. Fig. 13. Resting cyst of *Scrippsiella trochoidea* isolated from sediment. Fig. 14. Wild resting cyst germinated to establish strain SCPC73. Fig. 15a. Vegetative cell of strain SCPC51. Note pronounced apical horn (top arrow) and globular chloroplasts (bottom arrow).Fig. 15b. Dorsal view of cell showing nucleus (n) and chloroplasts (arrow). All scale bars=10µm

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Figures 16-19: LM. Resting cysts of *S. trochoidea* produced in cultures. Figs 16a-b. Resting cyst with calcareous spines strain SCBC18. Fig. 16a. Spines in deep focus. Fig. 16b. Note the spines in different focus. Fig. 17. Resting cyst of strain SCPC39. Note archeopyle (arrow). Fig.18a. Resting cyst of strain SCPC73, surface focus. Fig. 18b. Same cyst in deep focus showing spines. Fig. 19. Resting cyst of strain SCPC51. Note archeopyle (arrow).



Figures 20-21. SEM. Resting cyst of *S. trochoidea* isolated from sediment. Fig. 20a. Calcareous cyst with triangular spines. Fig. 20b. Detail of spines showing irregular base. Fig. 21a. Calcareous oval cyst. Fig. 21b. Note base of spine and calcareous tip (arrows). All scale bars=10µm, except Fig. 77b and Fig. 79b=5µm.

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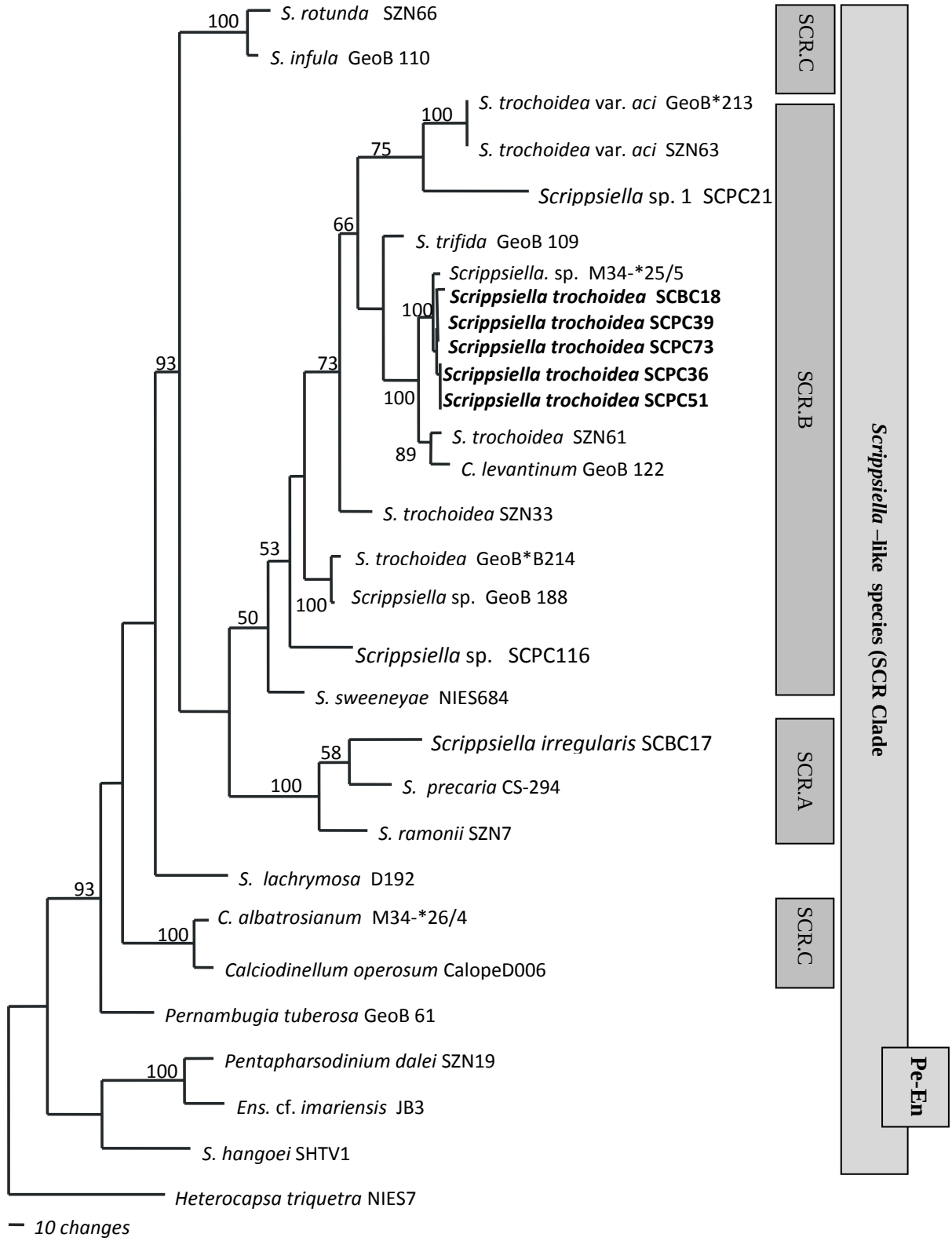


Figure 22: Most parsimonious tree from a maximum parsimony analysis of 30 taxa from the Calciodinellaceae inferred from sequences of the 5.8S rRNA gene and ITS1 and ITS2 regions. Values at branch points indicate bootstrap support (>50%). *Heterocapsa triquetra* is outgroup taxon. Iranian strains are in bold

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Discussion

Taxonomy of *Scrippsiella* is complicated in some aspects; *S. trochoides* is somewhat a complex species, strains of the same ribotype shows significant morphological variability and the species are genetically (not morphologically) different (same plate pattern and same spiny resting cysts) (Montresor et al., 2003; Gottschling et al., 2005b; Tang et al., 2010; Zinssmeister et al., 2011). This study is in agreement with previous ones (e.g. D'Onofrio et al., 1999; Montresor et al., 2003; Gottschling et al., 2005a,b; Craveior et al., 2011; Zinssmeister et al., 2011) on molecular phylogeny of Calciodinellaceae which shows that the primitive lineage is *Pentapharsodinium* and *Ensiculifera* which are sister groups to the *Scrippsiella*-like species. The present study also shows that all species possessing *Scrippsiella* plate patterns form a monophyletic lineage, confirming the taxonomic value of the number/arrangement of thecal plate at the supra-specific level within this group (Montresor et al., 2003). The taxonomy of vegetative cells of these three genera is based on the number of singular plates and the shape of the first singular plate (Kobayashi and Matsouka 1995; Steidinger and Tangen, 1996). The genus *Scrippsiella* possesses six singular plates (including transitional plate), whereas the other two genera have five singular plates (including T-plate). The *Ensiculifera* species can also be distinguished from *Pentapharsodinium* genus by the long spine on the first singular plate (T-plate). The four groups resolved within the *Scrippsiella* clade, are mostly supported by morphology of the vegetative cells. The most primitive branch in the *Scrippsiella* lineage is the SCR.A group which comprises the *S. precaria*-like species (*S. precaria* Montresor & Zingone, *S. ramonii* Montresor and *S. irregularis* Attaran & Bolch). This monophyletic group is genetically and morphologically distinct from the remaining *Scrippsiella* group. The similarity in the morphology of vegetative cells within these species is highly supported by the phylogenetic analyses. All members in the SCR.A clade possess an asymmetrical arrangement of intercalary plates that is distinct from the symmetrical arrangement of other *Scrippsiella* species (Montresor and Zingone, 1988; Montresor et al., 1997; Attaran-Fariman and Bolch, 2007). The SRC.B subclade, which is a paraphyletic group with weak bootstrap support (50%), includes *S. trochoidea* complex, and the phylogenetic relationship among these groups is still unresolved (Montresor et al., 1993; Montresor et al., 2003; Gottschling et al., 2005a, 2005b; Tang et al., 2010; Zinssmeister et al., 2011). However, species with similarity in cell or resting cysts morphology are grouped together in phylogenetic analyses. *Pernambugia tuberosa* is clearly distinct from the remaining *Scrippsiella* because of its archeopyle shape (i.e. complete epicyst) (Karwath et al., 2000; Gottschling et al., 2005a). While the cell morphology of *S. lachrymosa* Lewis is mostly similar to the other members in the subclade SCR.B, the cysts morphology is distinct due to the elongate shape cysts and irregular crystalline processes (Lewis, 1991) The presence of *Calciodinellum levantinum*

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(Meier, Janofske and Willems), within *S. trochoidea* complex group is also not surprising as its thecal tabulation is essentially identical to other *Scrippsiella* species (Meier et al., 2002).

In subclade SCR.C *Scrippsiella rotunda* Lewis and *Scrippsiella infula* form a monophyletic group with *Scrippsiella operosa* and *C. albatrosianum* (Kamptner) Janofske & Karwath with weak bootstrap support. Motile cells of these species possess very similar morphology and their plate patterns exactly match the plate patterns of *Scrippsiella* (Montresor et al., 2003). All three have poorly developed apical horns, smooth thecal plates with a scattered pore, and a similar nucleus position (Lewis, 1991; Montresor et al., 1997). All species in this subclade produce smooth cysts without spines. They can therefore be only distinguished by the morphology of their cysts. *S. operosa* produces cysts with pronounced paratabulation. Although Gottschling et al. (2005a) found variability in producing calcareous cysts in culture of *S. operosa* and *C. albatrosianum*, both species produce cysts with and without clear paratabulation. However, they stated that most strains of *C. albatrosianum* are without clear paratabulation, whereas most strains of *S. operosa* produce cysts with clear paratabulation.

S. trochoidea (strains SCBC18, SCPC36, SCPC39, SCPC51, and SCPC73) from Iranian waters shows high similarity to the described *S. trochoidea* (Lewis, 1991; Janofske, 2000; Montresor et al., 2003; Zinssmeister et al., 2011). They all comprise alike vegetative cells of almost similar cell size, bipesioid arrangement of intercalary plates, a narrow to medium width first apical plate, smooth plate surface with scattered pores, a conical epitheca and rounded hypotheca and a central position of the nucleus. The number, shape and arrangement of the sulcal plates are conservative features in species and valuable characters for the taxonomy of the armored dinoflagellates (Balech, 1980). The number of sulcal plates for *S. trochoidea* does not agree with Janofske (2000) who described six sulcal plates and an overall plate pattern of Po, X, 4', 3a, 7", 6C, 6S, 5"', 2"". The extra plate appears to be the anterior flagellar pore plate (af) placed between the anterior sulcal plate (S_a), and the T-plate, slightly overlapped by the right sulcal (rs; S_d). In the present study, five sulcal plates were identified: the anterior (S_a), left (S_s), right (S_d), posterior (S_p) and a small median sulcal plate (S_m). The S_m plate corresponds to the posterior flagellar pore of Janofske (2000). However, the left sulcal plate (S_s), in *S. trochoidea* (Fig. 62, strain SCBC18) appears to be folded over the transverse one. Five sulcal plates (S_a, S_s, S_d, S_p & S_m) are also reported in other descriptions for *S. trochoidea* with slightly unstable arrangement of these plates between individuals (e.g. Lewis 1991, Steidinger and Tangen, 1996; Montresor et al., 2003; Zinssmeister et al., 2011). In some individuals S_a, S_s, and S_m are largely covered by S_d plate (Zinssmeister et al., 2011).

The rDNA-ITS sequence data indicate Iranian strains are closely related to *Scrippsiella* sp. (strain M34-*25/5). This monophyletic group is a sister group to *S.*

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trochoidea (SZN61). *Scrippsiella* sp strain M34-*25/5 is not well characterised (Gottschling et al. 2005a) and the only figure of the motile cell (fig 33, dorsal view, Gottschling et al., 2005a), shows a cell outline similar to the strains in the present study). The cyst of strain M34-*25/5 is ovoid and covered with regular-shaped crystals without any spines (Gottschling et al., 2005a). Previous studies on the phylogeny of calcareous cyst-producing dinoflagellate (e.g. D'Onofrio et al., 1999; Montresor et al., 2003) have also found that species with obviously different cyst morphology may group together in the phylogenetic trees.

The resting cysts of *S. trochoidea* in the present study correspond with those previously described for the same species, possessing capitated calcareous spines with triangular cross-sections, emerging from an irregular base plate (Janofske, 2000; Zinssmeister et al., 2011). The archeopyle of the Iranian cyst is also identical with those of *S. trochoidea*. Therefore, molecular data, morphology of the cyst and vegetative cell suggest that this species can be considered as *S. trochoidea.*

Acknowledgements

We thank Sharareh Khodami from the Iranian Fisheries Research Institute (IFRI), Dr. David Steele and Geraldine Nash from Australia for their assistance.

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مورفولوژی و فیلوژنی(Dinophyceae) *Scrippsiella trochoidea* با پتانسیل تشکیل بلومهای مضرفیتوپلانکتونی جدا شده از رسوبات سواحل جنوب ایران

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چکیده

سیست و سلولهای پلانکتونی *Scrippsiella trochoidea* از گونه های متداول و غالب فلور داینو فلاژلاها درآبها و رسوبات نواحی ساحلی در همه مناطق دنیا می باشد. این گونه یکی از گونه های بسیار رایج تشکیل دهنده بلومهای مضر فیتوپلانکتونی در مناطق ساحلی می باشد. در این بررسی برای اولین بار سیست گونه *S. trochoidea* از رسوبات جنوب شرق ایران جدا سازی گردید. 5 ایزوله از گونه های کشت داده شده متعلق به گونه *S. trochoidea* بودند. به منظور تایید شناسایی گونه ها آزمایشات اگزسیتمنت و این سیستمنت انجام گردید و ریخت شناسی سیست و الگوی صفحات سلولهای شکوفا شده با استفاده از میکروسکوپ نوری و الکترونی(SEM) مورد بررسی قرار گرفت. توالی نوکلئوتیدهای DNA ریبوزومی در دو ناحیه بسیار متنوع ژنی ITS1,ITS2 و همینطور ناحیه 5.8S برای همه ایزوله ها بررسی گردید. برای تعیین روابط فیلوژنتیک توالی نوکلئوتیدی 5 ایزوله ایرانی با توالی های همولوگ در بانک ژن مقایسه گردید. هر دو آنالیزهای NJ و Mp و بررسیهای ریخت شناسی نشان دادند که هر پنج ایزوله ایرانی متعلق به گونه *S. trochoidea* و با گونه های توصیف شده قبلی *S. trochoidea* و همینطور با گونه *Calciodinellum levantinum* کلاستر می شوند و رابطه خویشاوندی نزدیکی با گونه *Scrippsiella* sp. با میزان ٪1.2 –٪1.4 تنوع ژنی را نشان دادند. نتایج این این مطالعه نشان داد که اگر مطالعات مولکولی DNA ریبوزومی با ریخت شناسی سیست و سلول پلانکتونی شکوفا شده گونه همراه باشد می تواند به عنوان دستاورد با ارزشی در شناسایی و طبقه بندی داینوفلاژلاهای calciodinelloideae باشد.

واژگان کلیدی: رسوبات ، *ریخت شناسی، سواحل جنوب ایران، سلول پلانکتونی، سیست، Scrippsiella trochoidea، فیلوژنی*

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