

**Dinoflagellate Cysts and
Chattonella Resting Stages from
Recent Sediments of the Southeast
Coast of Iran**

by

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**School of Aquaculture
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*In The Name of God,
The Compassion and The Merciful*

DECLARATION

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ABSTRACT

Many harmful microalgae, including dinoflagellates and raphidophytes, produce long-lived, resistant, resting stages called resting cysts. Mapping the presence and abundance of these stages in coastal sediments combined with germination studies can provide valuable information on the distribution of potentially harmful species in an area. The resting cyst flora of equatorial and tropical regions of the world is poorly known, particularly in coastal waters. This thesis examines the dinoflagellate resting cyst flora in coastal marine sediments collected along the southern coast of Iran. The aim of the study was twofold. Firstly, to determine whether potentially harmful dinoflagellates or raphidophytes are present in the area. Secondly, to document the species diversity of dinoflagellate resting cysts in a little studied tropical region.

Nine sediment samples were collected at three locations (i.e. the Bahoo-kalat estuary, Pasabandar coast and Chabahar Bay) along the southeast coast of Iran. Dinoflagellate cyst species abundance and diversity was examined by light and scanning electron microscopy, and the resting cyst identity and affinity confirmed by cyst germination, where possible. Established cultures were further characterised by PCR amplification and DNA sequencing of the large subunit ribosomal RNA gene (LSU-rDNA) and internal transcribed spacer (rDNA-ITS) regions.

Over forty (40) distinct cysts morphotypes were identified in the samples examined. The most common groups identified were: *Scrippsiella* species (79%), *Protoperidinium* species (10%) and, at three sites (Pasabandar) an unusual angular, trapeziform resting cyst was common (7%). Potentially harmful species were also found in some sites; for example, the paralytic shellfish poisoning (PSP) causative species, *Alexandrium tamarense*, yessotoxin producing species *Lingulodinium polyedrum*.

Cyst incubation and germination studies established over 30 dinoflagellate cultures that were further characterised by detailed morphological (LM and SEM) and molecular approaches. The unusual trapeziform resting cysts were successfully germinated and identified as a previously undescribed dinoflagellate. Morphological studies of the resting cysts showed a microreticulate paratabulation, with the pattern reflecting amphiesmal patterning of the motile cell, including the cingulum and sulcus. Using cultures established from cyst germination the phylogenetic affinities of the species was examined by comparison of partial LSU-rDNA sequences with a range of other Gymnodinioids. The resulting analyses indicated a clear relationship to the other three known microreticulate cyst-forming Gymnodinoid species, *Gymnodinium catenatum*, *G. nolleri* and *G. microreticulatum*. Identical resting cysts were also successfully produced in nutrient depleted laboratory cultures, and the pattern of successful crosses indicated a homothallic mating system for this species. This dinoflagellate is described as a new species *G. trapeziforme* Attaran-Fariman & Bolch sp. nov.

The majority of the remaining cultures established from single cyst incubations were found to be allied with the Calciodinelloidean genera *Scrippsiella*, *Calciodinellum*, *Calciodinellum*. Of these cultures, four distinct morphotypes were identified and

subjected to DNA sequencing of the rDNA-ITS regions. Three morphotypes were allied with *Scrippsiella trochoidea* var. *aciculifera*, *Scrippsiella* sp., and *Scrippsiella trochoidea* respectively. The fourth morphotype, possessing a small second anterior intercalary plate, was allied with but distinct from the similarly tabulated *Scrippsiella precaria* and *S. ramonii*. Morphological examination shows that its overall size and shape is similar to *S. precaria*, but with a more equatorially placed cingulum, an antapically placed nucleus and a larger, rounded 2nd anterior intercalary plate similar to *S. ramonii*. This species is described here as *Scrippsiella irregularis* Attaran-Fariman & Bolch sp. nov.

Mixed sediment incubations from site 6 (Pasabandar coast) also commonly released a raphidophyte belonging to the genus *Chattonella* Biecheler. Uni-algal cultures established from incubations were examined and determined to be allied with *Chattonella subsalsa* Biecheler. Analysis of the LSU-rDNA and rDNA-ITS regions sequences compared to other known *Chattonella* species showed that this species is related to *C. subsalsa*, but is genetically distinct, and may represent a new species.

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Table of Contents

ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
Table of Contents.....	vi
List of Plates	x
List of Figures	xi

CHAPTER 1 Introduction.....1

1. General Introduction	1
1.1. History of Dinoflagellate Cyst Studies	3
1.2. Taxonomy and Classification of Dinoflagellate Cysts.....	4
2. Cyst Morphology	9
2.1. Overall Body Shape of Cysts	11
2.2. Cyst Wall Structure and Colour	11
2.2.1. <i>Cyst Wall Processes</i>	12
2.2.2. <i>Cyst Wall Paratabulation</i>	14
2.3. Archeopyle	17
2.4. Cell Contents.....	18
3. Molecular Approach to Study Dinoflagellate Cysts	20
4. Dinoflagellate Life Cycles	21
4.1. Encystment.....	23
4.1.1. <i>Cyst Formation</i>	23
4.1.2. <i>Cyst Formation in the Laboratory</i>	24
4.1.3. <i>Encystment in the Natural Environment</i>	26
4.1.4. <i>Encystment in Tropical and Sub-tropical Areas</i>	27
4.2. Dormancy and Quiescence.....	28
4.3. Excystment and Quiescence.....	30
5. Biological Importance of Resting Cysts	33
6. Resting Cysts, Harmful Algal Blooms and Shellfish Toxicity Monitoring.....	36
7. Research Aims	38
8. References	39

CHAPTER 2 Dinoflagellate Cysts from Recent Marine **Sediment of the Southeast Coast of Iran.....** **(Northeast of the Gulf of Oman).....52**

1. Introduction	52
2. Material and Methods	53

2.1. Study Area.....	53
2.2. Sediment Processing	56
2.3. Quantitative Analyses of Cysts	56
2.4. Cyst Germination Experiment.....	57
2.5. Microscopy.....	57
2.5.1. <i>Light Microscopy</i>	57
2.5.2. <i>Scanning Electron Microscopy</i>	58
3. Results	58
3.1 Cyst Composition and Relative Abundance	58
3.2. Systematics.....	60
4. Discussion	115
4.1. Dinoflagellate Cyst Composition.....	115
4.2. Comparisons with Adjacent Tropical Areas	116
4.3. Cysts of Toxic Dinoflagellates.....	119
5. Conclusion	120
6. Further Study.....	121
7. References	122

**CHAPTER 3 Morphology and Phylogeny of a New
Reticulate Cyst-Forming Dinoflagellate,
Gymnodinium trapeziforme sp. nov.126**

1. Introduction.....	126
2. Materials and Methods.....	128
2.1. Sediment Collection.....	128
2.2. Sediment Processing	128
2.3. Cyst Germination Experiments.....	129
2.3.1. <i>Encystment and Crossing Experiments</i>	129
2.4. Microscopy.....	130
2.4.1. <i>Light Microscopy</i>	130
2.4.2. <i>Scanning Electron Microscopy (SEM)</i>	130
2.5. Toxin Analysis	131
2.6. DNA Extraction and Polymerase Chain Reaction (PCR).....	132
2.6.1. <i>DNA Sequencing</i>	133
2.6.2. <i>Alignment and Phylogenetic Analyses</i>	133
3. Results	136
3.1. Description and Observations	136
3.1.1. <i>Vegetative Cell Description</i> (Figs 1-15)	137
3.1.2. <i>Resting Cyst Description</i> (Figs 15-37).....	143
3.2. Nutrient Deficiency and Crossing Experiment	149
3.3. Toxins.....	150
3.4. Phylogenetic Analyses of rRNA Genes	151
4. Discussion	157
4.1. Morphological Comparison	157
4.2. Comparisons with Microreticulate Cyst-producing Species	158
4.2.1. <i>Vegetative Cell Comparisons</i>	159
4.2.2. <i>Cyst Comparisons</i>	162

4.3 Phylogenetic Relationships	164
4.4 Crossing and Cyst Formation.....	164
5. Conclusion	165
6. Further Study.....	165
7. References	166

**CHAPTER 4 Morphological and Molecular Analysis of
Scrippsiella species Isolated from the
Southeast Coast of Iran.....170**

1. Introduction.....	170
2. Material and Methods	173
2.1. Sediment Collection and Processing.....	173
2.2. Cyst Germination Experiments	173
2.3. Encystment.....	173
2.4. Microscopy.....	174
2.4.1. <i>Light Microscopy</i>	174
2.4.2. <i>Scanning Electron Microscopy (SEM)</i>	174
2.5. DNA Extraction and Polymerase Chain Reaction (PCR).....	175
2.5.1. <i>DNA Sequencing</i>	176
2.5.2. <i>Alignment and Phylogenetic Analyses</i>	177
3. Results.....	179
3.1. Morphology.....	179
3.1.1. <i>Species Description</i>	181
3.1.2. <i>Scrippsiella</i> sp. 1 (Figs 20-39)	188
3.1.3. <i>Scrippsiella</i> sp. 2 (Figs 40-51)	193
3.1.4. <i>Scrippsiella trochoidea</i> (Figs 58-76)	199
3.2. Phylogenetic Analyses	206
4. Discussion	210
4.1. Phylogenetic Relationship and Morphological Comparisons among the Calciadinellaceae	210
4.2. Phylogenetic Relationship and Morphological Comparison of the Species from the Present Study.....	213
5. Conclusion	221
6. Further Study.....	222
7. References	223

**CHAPTER 5 Phylogenetic and Morphological Analysis
of *Chattonella* cf. *subsalsa* strain CHP36
from Southeast Coast of Iran227**

1. Introduction.....	227
2. Material and Methods	229
2.1. Cell Isolation and Culture	229

2.2. Microscopy.....	230
2.2.1. <i>Light Microscopy</i>	230
2.2.2. <i>Scanning Electron Microscopy (SEM)</i>	230
2.3. DNA Extraction, PCR and DNA Sequencing.....	230
2.3.1. <i>Alignment and Phylogenetic Analyses</i>	231
3. Results	234
3.1. Morphology.....	234
3.2. Phylogenetic Analyses	238
4. Discussion	243
4.1. Morphology of <i>Chattonella</i> cf. <i>subsalsa</i> CHPI36	243
4.2. Molecular Analyses.....	247
5. Conclusions	249
6. Further study	249
7. References.....	251
CHAPTER 6 Discussion and Summary	255
References	262
Appendices	265
Appendix 1	266
Appendix 2	270
Appendix 3	273

List of Plates

Plate 1: Gonyaulacoid resting cysts from the southeast coast of Iran.....	66
Plate 2: Gonyaulacoid resting cysts and thecae from the southeast coast of Iran.....	71
Plate 3: Resting cysts of <i>Alexandrium</i> , <i>Pyrophacus</i> and <i>Scrippsiella</i> from southeast coast of Iran.....	76
Plate 4: <i>Scrippsiella</i> spp. resting cysts and germinated motile cells from southeast coast of Iran.....	79
Plate 5: <i>Scrippsiella</i> spp. resting cysts and germinated thecae from southeast coast of Iran.....	83
Plate 6: <i>Scrippsiella</i> resting cysts from southeast coast of Iran.....	85
Plate 7: <i>Protoperidinium</i> spp. resting cysts and thecate cells from southeast coast of Iran.....	92
Plate 8: <i>Protoperidinium</i> spp. resting cysts and theca cells from southeast coast of Iran.....	99
Plate 9: <i>Protoperidinium</i> spp. resting cysts from southeast coast of Iran.....	101
Plate 10: <i>Protoperidinium</i> spp. and <i>Diplosalis</i> spp. resting cysts and thecae from southeast coast of Iran.....	107
Plate 11: Gymnodinioid resting cysts and thecate cells from southeast of Iran coast.....	110
Plate 12: Unidentified putative resting cysts from southeast coast of Iran.....	113

List of Figures

Chapter 1

Fig. 1. Main features of cyst and motile stage.....	10
Fig. 2. The morphological terms for dinoflagellate cysts processes.....	13
Fig. 3. Different type of dinoflagellate cyst based on processes length.....	14
Fig. 4. Kofoid tabulation system in a thecate dinoflagellate.....	16
Fig. 5. General morphology of athecate dinoflagellates (Gymnodiniaceae) species.....	17
Fig. 6. Archeopyle types in recent dinoflagellate cysts.....	19
Fig. 7. A general sexual life cycle of dinoflagellate.....	22

Chapter 2

Fig. 1. Map of Study Area.....	55
--------------------------------	----

Chapter 3

Figs 1-4. LM. <i>Gymnodinium trapeziforme</i>	139
Figs 5-8. LM. <i>Gymnodinium trapeziforme</i>	140
Figs 9-10. SEM. Cells of <i>G. trapeziforme</i>	141
Figs 11-15. SEM. <i>Gymnodinium trapeziforme</i>	142
Figs 16-21. LM. Resting cyst of <i>G. trapeziforme</i> from Iranian sediments.....	144
Figs 22-27. Resting cyst of <i>G. trapeziforme</i> produced in laboratory cultures.....	145
Fig. 28. SEM. Ventral view of <i>G. trapeziforme</i> wild cyst.....	146
Fig. 29. SEM. Typical microreticulate cyst (<i>Gymnodinium catenatum</i>).....	146
Figs 30-33. SEM. Cultured cysts of <i>G. trapeziforme</i>	147
Figs 34-38. SEM. <i>Gymnodinium trapeziforme</i> paratabulation details.....	148
Fig. 39. High performance liquid chromatograms (HPLC) of postcolumn oxidation for the three classes of paralytic shellfish toxins: C-toxins, gonyautoxins (GTX) and saxitoxins (STX). (a) standard, (b) <i>G.</i> <i>trapeziforme</i>	150
Fig. 40. Phylogenetic tree of 59 gymnodinioid taxa based on LSU-rDNA (ME-LgD).....	153
Fig. 41. Single most parsimonious tree including 59 gymnodinioid taxa based on partial LSU rRNA (D1-D3) sequences.....	155
Fig. 42. Line drawing of single cell of four microreticulate cyst-producing gymnodinioids.....	161

Chapter 4

Figs 1-4. SEM. Vegetative cell of <i>Scrippsiella irregularis</i>	183
Figs 5-9. SEM. Theca cell of <i>Scrippsiella irregularis</i>	184
Figs 10-15. LM. <i>Scrippsiella irregularis</i>	185

Figs. 16-19. Resting cyst of <i>Scrippsiella irregularis</i>	187
Figs. 20-25. SEM. <i>Scrippsiella</i> sp. 1, thecate cells.....	189
Figs 26-29. SEM. <i>Scrippsiella</i> sp. 1 thecal cell.....	190
Figs 30-34. LM. <i>Scrippsiella</i> sp. 1 thecal cell.....	191
Figs 35-39. LM. <i>Scrippsiella</i> sp. 1 resting cysts.....	192
Figs. 40-45. SEM. <i>Scrippsiella</i> sp. 2.....	194
Figs 46-51. LM. <i>Scrippsiella</i> sp. 2.....	195
Figs 52-53. LM. Resting cyst of <i>Scrippsiella</i> sp. 2 from culture.....	197
Figs 54-57. LM. Resting cyst of <i>Scrippsiella</i> sp. 2.....	198
Figs 58-62. SEM. <i>Scrippsiella trochoidea</i> (strain SCBC18).....	200
Figs 63-65. SEM. <i>Scrippsiella trochoidea</i> thecate cells.....	201
Figs 66a-d. SEM. Thecate cell of <i>Scrippsiella trochoidea</i> strain SCPC73.....	202
Figs 67-71. LM. Resting cysts of <i>Scrippsiella trochoidea</i>	203
Figs 72-76. LM. Resting cysts of <i>Scrippsiella trochoidea</i> produced in cultures.....	204
Figs 77-80. SEM. Resting cyst of <i>Scrippsiella trochoidea</i> isolated from sediment.....	205
Fig. 81. Phylogeny of 56 taxa of Calciodinellaceae inferred from 5.8s and both ITS1 and ITS2 of rRNA regions (NJ Tree).....	207
Fig. 82. Phylogeny of 26 taxa of Calciodinellaceae inferred from 5.8s and both ITS1 and ITS2 of rRNA region (MP tree).....	208
Fig. 83. Schematic drawing of <i>Scrippsiella irregularis</i> (left shapes) theca cell, compared with <i>Scrippsiella precaria</i>	216

Chapter 5

Figs 1-6. LM. Motile cells of <i>Chattonella</i> cf. <i>subsalsa</i> CHPI36.....	235
Figs 7-12. <i>Chattonella</i> cf. <i>subsalsa</i> , vegetative cell.....	236
Figs 13-18. LM. <i>Chattonella</i> cf. <i>subsalsa</i> putative resting cysts and non-motile cells.....	237
Fig. 19. Phylogenetic relationship among Chattonellates genera inferred from phylogenetic of partial LSU of rDNA gene (MP tree).....	239
Fig. 20. Phylogenetic relationship among Chattonellates genera based on partial LSU rDNA gene sequences (domains D1-D3) (NJ tree).....	240
Fig. 21. Relationship of <i>Chattonella</i> cf. <i>subsalsa</i> CHPI36 to other Chattonellates species inferred from the phylogeny analysis of ITS regions of rDNA gene (MP tree).....	241
Fig. 22. Relationship of <i>Chattonella</i> cf. <i>subsalsa</i> CHPI36 to other Chattonellates species inferred from the phylogenetic analysis of the ITS regions of rDNA gene (ME-LgD tree).....	242
Fig. 23. Morphological comparison of Chattonellate species.....	244
Figs 24-29. LM. Comparing the morphology of vegetative cells of <i>C. cf. subsalsa</i> CHPI36 with <i>C. subsalsa</i> CCMP217.....	245

Chapter 6

Fig. 1. General pattern of monsoon wind and water circulation in Arabian Sea.....	258
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