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Lewis, J., Taylor, J., Neale, K. and Leroy, S.A.G.

This is a copy of the final version of an article published in 61 (1), pp. 21-31. It is available from the publisher at:

https://dx.doi.org/10.1515/bot-2017-0041

Jane Lewis*, Joe D. Taylor, Klaire Neale and Suzanne A.G. Leroy

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https://doi.org/10.1515/bot-2017-0041
Received 2 May, 2017; accepted 14 November, 2017; online first 19 December, 2017

Abstract: To investigate the disparity between plankton and cyst records, sediment slurry cultures were used to isolate the motile stage of dinoflagellates from Caspian Sea sediment. This has resulted in new records for this area of *Kryptoperidinium foliaceum*, *Gymnodinium aureolum* and *Woloszynskia* sp. and for the cyst record, *Scrippsiella acuminata*. Two *Gonyaulax* species were isolated, one was identified as *Gonyaulax baltica* and the other an unknown species. Cultures of *Lingulodinium polyedra* were also isolated. The approach of using slurries was useful to provide cultures from sediments that were relatively poor in dinoflagellate cysts with contents.

Keywords: Caspian Sea; *Gonyaulax baltica*; *Gymnodinium aureolum*; *Kryptoperidinium foliaceum*.

Introduction

The Caspian Sea is the largest inland body of water in the world with a surface area of 3.5 million km^2. It is a complex lake system with three distinct basins, of which the southern one is the most saline (13), the warmest (surface waters 10–28°C) and the deepest (1025 m) (Kosarev and Yablonskaya 1994). The hydrography of the lake is driven by riverine inputs (largely in the north) and seasonal climatic changes over its long latitudinal range (36°33′–47°07′N). The biota of the lake is dominated by freshwater/brackish species and is characterised by a large amount of endemism (Dumont 1998). Particular ecological pressures have been experienced in the lake system from pollution, oil and water exploitation and also, more recently, introduced species through shipping and the development of the Volga-Don canal. A recent driver of change in the plankton community has come from the introduced ctenophore *Mnemiopsis leidyi* A. Agassiz (Kideys et al. 2008, Roohi et al. 2010, Nasrollahzadeh et al. 2014). Phytoplankton communities have changed over the long term with fluctuation of freshwater input, changes in nutrient inputs and introduction of new species (Kosarev and Yablonskaya 1994). From 1994 to 2005, the productivity of the southern basin changed from oligotrophic to meso-eutrophic (Nasrollahzadeh et al. 2008a,b, Bagheri et al. 2011). Phytoplankton diversity for the Caspian is dominated by cyanophytes (147 taxa) and diatoms (238 taxa) (Gogorev 2006). However, with respect to dinoflagellates, it is notable that in the various studies of the phytoplankton flora of the Caspian most papers list only some 20–30 taxa (Table 1 and references therein). The total number of species recorded is just under 50 contrasting with a checklist for the Black Sea comprising 267 species (Gómez and Boicenco 2004) and for the Mediterranean comprising 673 species (Gómez 2003). Although low in species diversity, dinoflagellates are an important component of the phytoplankton community in terms of abundance and biomass (Kideys et al. 2005, Bagheri et al. 2012b, Nasrollahzadeh et al. 2014) with *Prorocentrum* Ehrenberg species being the most abundant (Kosarev and Yablonskaya 1994, Kideys et al. 2005). Blooms of *Prorocentrum cordatum* (Ostenfeld) Dodge (Bagheri and Fallahi 2014) and *Heterocapsa* F. Stein (Bagheri et al. 2012a) have been recorded and *Lingulodinium polyedra* (Stein) Dodge contributed to a bloom dominated by *Nodularia* Mertens *ex* Bordet *et* Flahault in 2009 (Nasrollahzadeh et al. 2011).

The sediment of the Caspian Sea has been the focus of a number of recent palynological studies investigating the history and geography of the basin (Leroy et al. 2006, 2007, 2011, 2013a,b,c, 2014), with descriptions and taxonomy of

***Corresponding author: Jane Lewis,** Faculty of Science and Technology, Department of Life Sciences, University of Westminster, 115 New Cavendish Street, London, W1W 6UW, UK, e-mail: lewisjm@westminster.ac.uk
Joe D. Taylor: Faculty of Science and Technology, Department of Life Sciences, University of Salford, 115 New Cavendish Street, London, W1W 6UW, UK; and School of Environment and Life Sciences, University of Salford, Peel Building, Salford, M5 4WT, UK
Klaire Neale: Faculty of Science and Technology, Department of Life Sciences, University of Westminster, 115 New Cavendish Street, London, W1W 6UW, UK
Suzanne A.G. Leroy: Centre Européen de Recherche et d'Enseignement en Géosciences de l'Environnement, Technopôle de l'Arbois, 13545 Aix-en-Provence cedex 4, France

Table 1: Brackish/marine dinoflagellates[a] recorded in the Caspian Sea in the phytoplankton and in recent sediments (linkages between the two are recorded where known).

Phytoplankton[b]	Sediments
Amphidinium cf. *rhynchocephalum* Annisomova[1]	
Diplopsalis acuta Paulsen[2,3,7,9]	
D. lenticula Bergh[7,8,9]	
Exuviaella cordata Ostenfeld[2]	
Glenodinium behningii (Lindemann) Kisselev[2,3,5,6,7,9]	
G. caspicum (Ostenfeld) Schiller[3,7,8,9]	
G. obliquum (Pouchet)[1]	
G. paululum Lindemann[1]	
G. penardii Lemmermann[2,9]	
G. pilula (Ostenfeld) Schiller[9]	
Gonyaulax baltica Ellegaard, Lewis *et* Harding[15]	*Impagidinium caspienense* Marret[10,11,12,15]
G. digitale Kofoid[2,7,8,9]	
G. minima Matzenauer[2]	
G. polygramma Stein[1,4]	
G. spinifera Diesing[2,7,8,9]	
Gonyostomum depressum Herdman[2]	
G. semen Diesing[7,8]	
Gymnodinium fuscum (Ehrenberg) Stein[1]	
G. lacustre Schiller[7,8,9]	
G. sanguineum Hirasaka[1]	
G. variabile Herdman[2,5,6,7,8,9]	
G. wulfii Schiller[1]	
Gyrodinium fusiforme Kofoid *et* Swezy[1]	
G. pingue (Schütt) Kofoid *et* Swezy[1]	
Heteraulacus polyedricus (Pouchet) Drugg *et* Loeblich[6]	
Heterocapsa rotundata (Lohmann) Hansen[9]	
H. triquetra (Ehrenberg) Stein[6]	
Kolkwitziella acuta (Apstein) Elbrächter[14]	Cysts of *Kolkwitziella acuta*[14]
Lingulodinium polyedra (Stein) Dodge[2,6,7,8,9]	*Lingulodinium machaerophorum* (Deflandre *et* Cookson) Wall[10,11,12]
Oblea rotunda (Lebour) Balech *ex* Sournia[1,9]	
Peridiniella danica (Paulsen) Okolodkov *et* Dodge[2,7,8,9]	
Peridiniopsis berolinense[9]	
Peridinium minusculum Pavillard[1]	
P. subsalum Ostenfeld[2]	
Preperidinium meunieri (Pavillard) Elbrächter[2,5,9]	
Prorocentrum compressum (Bailey) Abé *ex* Dodge[1]	
P. cordatum (Ostenfeld) Dodge[2,3,4,5,6,7,8,9]	
P. lima (Ehrenberg) Stein[9]	
P. micans Ehrenberg[1,2,7,9]	
P. obtusum Ostenfeld[2,7,8,9]	
P. proximum Makarova[2,5,6,9]	
P. scutellum Schröder[1,2, 3,5,6,7,8,9]	
Protoperidinium achromaticum Levander[3,6,7,9]	
P. granii Balech[7]	
P. leonis Pavillard[7,8]	
P. pallidum Balech[7]	
Pyrophacus horologicum Stein[9]	
Scrippsiella trochoidea (Stein) Loeblich[1,2,6,9]	
	Caspidinium rugosum Marret[10,11,12]
	Pyxidiniopsis psilata (Wall *et* Dale) Head[10,15]
	Spiniferites belerius Reid[11]
	Spiniferites cruciformis Wall *et* Dale[10,11,12]
	Cysts of *Pentapharsodinium dalei* Indelicato *et* Loeblich III[10,11,12]
	Cysts of *Scrippsiella plana* Luo, Mertens, Bagheri *et* Gu[13]
	Brigantedinium Reid[10,11,12]

[a]Gogorev (2006) includes a further 14 freshwater species found in the north of the Caspian Sea.

[b]Species names updated according to AlgaeBase (Guiry and Guiry 2017).

[1]Kideys et al. (2005); [2]Ganjian et al. (2010); [3]Bagheri et al. (2012b); [4]Pautova et al. (2015); [5]Nasrollahzadeh et al. (2008a); [6]Nasrollahzadeh et al. (2014); [7]Bagheri and Fallahi (2014); [8]Bagheri et al. (2010); [9]Gogorev (2006); [10]Marret et al. (2004); [11]Leroy et al. (2013c); [12]Kazancı et al. (2004); [13]Luo et al. (2016); [14]Mertens et al. (2015a,b); [15]Mertens et al. (2017).

recent dinoflagellate cysts in Marret et al. (2004), Leroy et al. (2006) and Leroy (2010). Again the relative paucity of species is of note. A comparison of the literature from the plankton and sediment (Table 1) reveals that, with the exception of *Gonyaulax baltica* Ellegaard, Lewis *et* Harding, *L. polyedra* and *Scrippsiella plana* Luo, Mertens, Bagheri *et* Gu, little congruity exists between the two lists. This study was undertaken to explore this disparity and this paper documents preliminary investigations of southern Caspian Sea sediment designed to test the diversity of living dinoflagellates held in the sedimentary record.

Materials and methods

Grab samples were collected from a motorboat and the top 1-cm sediment was removed and placed in airtight, dark containers and kept in the cool and dark until processing. Stations 1–7 were taken along the Gorgan transect on 9 February 2014 with station 1 nearest to the shore (2 m deep) and station 7 furthest from the shore (13 m deep). Sample HCGA09 was taken 1.8 m depth in the Anzali Lagoon at 37°26′56.6N and 49°22′49.8E on 26 June 2008 (Figure 1).

Initial work revealed that, apart from *Lingulodinium polyedra*, very low number of cysts with contents were found in the Caspian Sea sediment samples. Therefore, the approach of using slurry cultures to establish viability and diversity was initiated. For comparison, a single sediment sample from the Black Sea was also treated in the same way. Slurry cultures were established with approximately 1 cm^3 wet sediment from each sample (Caspian Sea stations 1–7 and HCGA09, and Black Sea sample). These were briefly sonicated (ca. 2 min), sieved through a 80- and 20-µm mesh and the material retained on the 20-µm mesh was processed using the sodium polytungstate density gradient method as described in Bolch (1997). The recovered cysts were incubated as slurry cultures at 10°C, under fluorescent tubes, ~160 µmol photons m^{-2} s^{-1}, 14:10 h light:dark cycle in 30-mm sterile Petri dishes with ca. 4 ml modified f/20 or f/2 medium without silicate (Guillard 1973). Medium was modified by the addition of sodium selenite (Na_2SeO_3, Sigma, St. Louis, MI, USA, final concentration 10^{-8} M) and reducing copper sulfate ($CuSO_4 \cdot 5H_2O$, BDH, Poole, UK, final concentration 10^{-8} M) and prepared with 0.2-µm filtered natural seawater and distilled water – final salinity ca. 12). Single cells were isolated from the slurries using 96-well plates with 200 µl of modified f/20 or f/2 and incubated at 15°C, under fluorescent tubes, ~160 µmol photons m^{-2} s^{-1}, 14:10 h light:dark cycle. Successful isolations were progressively scaled up through small Petri dishes to f/2 in 25-ml tissue culture flasks.

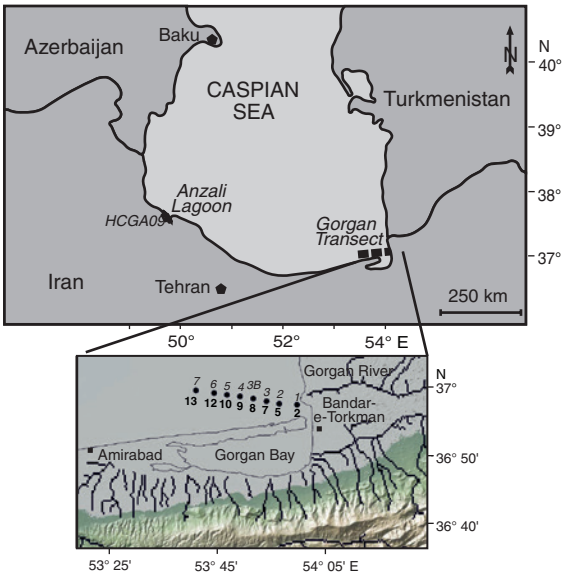


Figure 1: Location of grab samples in the Caspian Sea. In the Gorgan transect, the station numbers are in italics and the water depths (m) in bold.

Cultures were observed on an inverted (Olympus IMT-2, Olympus, London, UK) or a dissecting microscope (Olympus SZH-ILLK, Olympus, London, UK). Detailed light microscopy of cells was carried out with an Olympus BH2 microscope (Olympus, London, UK) and photographed using a Zeiss Axioskop 2 microscope (Carl Zeiss Ltd., Cambridge, UK) with a Leica DFC290HS camera (Leica, London, UK) or a Nikon Eclipse Ci-L microscope, Nikon, Tokyo, Japan, fitted with a Nikon digital sight DS-Fi2 camera (Nikon, Tokyo, Japan) using NIS elements software (Nikon, Tokyo, Japan). Cell dissections were facilitated by the use of 2% sodium hypochlorite (Sigma-Aldrich, Gillingham, UK) and the plates were stained with Trypan blue (Sigma-Aldrich, Gillingham, UK) and examined under phase contrast optics. Dodge (1982), Steidinger (1997) and Hoppenrath et al. (2009) were used for initial identifications with follow up in primary literature as indicated in the species descriptions below.

For molecular work, cultures were harvested in the exponential growth phase. Approximately 15 ml of culture was centrifuged at 1537 *g* for 15 min. The supernatant was removed and the Deoxyribonuleic acid (DNA) was extracted using an Invisorb® Spin Plant Mini Kit (Invisorb, Berlin, Germany), where 0.4 ml of lysis buffer from the kit was used to re-suspend the cell pellet and the lysis buffer was added to screw cap micro-centrifuge tubes containing 0.2 g glass beads (600 µm), the tubes were placed in a

BioSpec 3110BX Mini-BeadBeater-1 (BioSpec, Bartlesville, USA) and the machine was run for 60 s at 4800 oscillations min^{-1} to disrupt the cell membranes and lyse the DNA into solution. The remaining protocol was carried out in accordance with the manufacturer's instructions and the DNA was re-suspended in 50 µl nuclease free water (Ambion, Thermo Fisher Scientific, Waltham, MA, USA). The primers D1R (5′ACCCGCTGAATTTAAGCATA 3′; Lenaers et al. 1989) and DC3Ca (5′ACGAACGATTTGCACGTCAG 3′; Scholin and Anderson 1994) were used to amplify a ca. 900 base pair region of the large-subunit (LSU) rRNA gene. The polymerase chain reaction (PCR) reactions contained a final volume of 50 µl consisting of 25 µl MYTAQ 2× master mix (Bioline, UK), 1–2 µl of template DNA (ca. 50 ng), 2 µl of each primer (0.5 µM final concentration) and made up to 50 µl with diethyl pyrocarbonate-treated water (Ambion, Thermo Fisher Scientific, Waltham, MA, USA). The reaction was then subjected to the following conditions in a thermo-cycler: an initial denaturation of 5 min at 95°C then 30 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and then a final extension step of 72°C for 3 min. The products were checked by electrophoresis using 1% agarose gel (prepared with 1× tris-borate-ethylenediaminetetraacetic acid) and then purified using an Invisorb® Fragment Cleanup. Sequences obtained in this study were subjected to an European Bioinformatics Institute (EMBL-EBI) Fasta 33 search (Pearson and Lipman 1988).

Phylogenetic trees were constructed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7 (Kumar et al. 2016). Sequences from this study along with the matching top hits from the EMBL database and representative phylogenetic sequences from major groups of dinoflagellates were included in tree construction, *Oxyrrhis marina* Dujardin was used as an outgroup. The sequences were first aligned using ClustalW (Thompson et al. 1994). Phylogenetic analysis was performed using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei 1993). Bootstrap analysis (Felsenstein 1985) was also carried out with 500 replicates to provide confidence limits for tree branches. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 43 nucleotide sequences. There were a total of 503 positions in the final dataset.

Results

Using the slurry culture approach, several hundred cells were isolated which resulted in some 30 successful cultures. Motile cells of *Kryptoperidinium* Lindemann, *Gonyaulax* Diesing, *Gymnodinium* F. Stein emend. Hansen *et* Moestrup, *Lingulodinium* Wall, *Scrippsiella* Balech and *Woloszynskia* Thompson were isolated (Table 2, Figures 2–31) and sequenced (Figure 32).

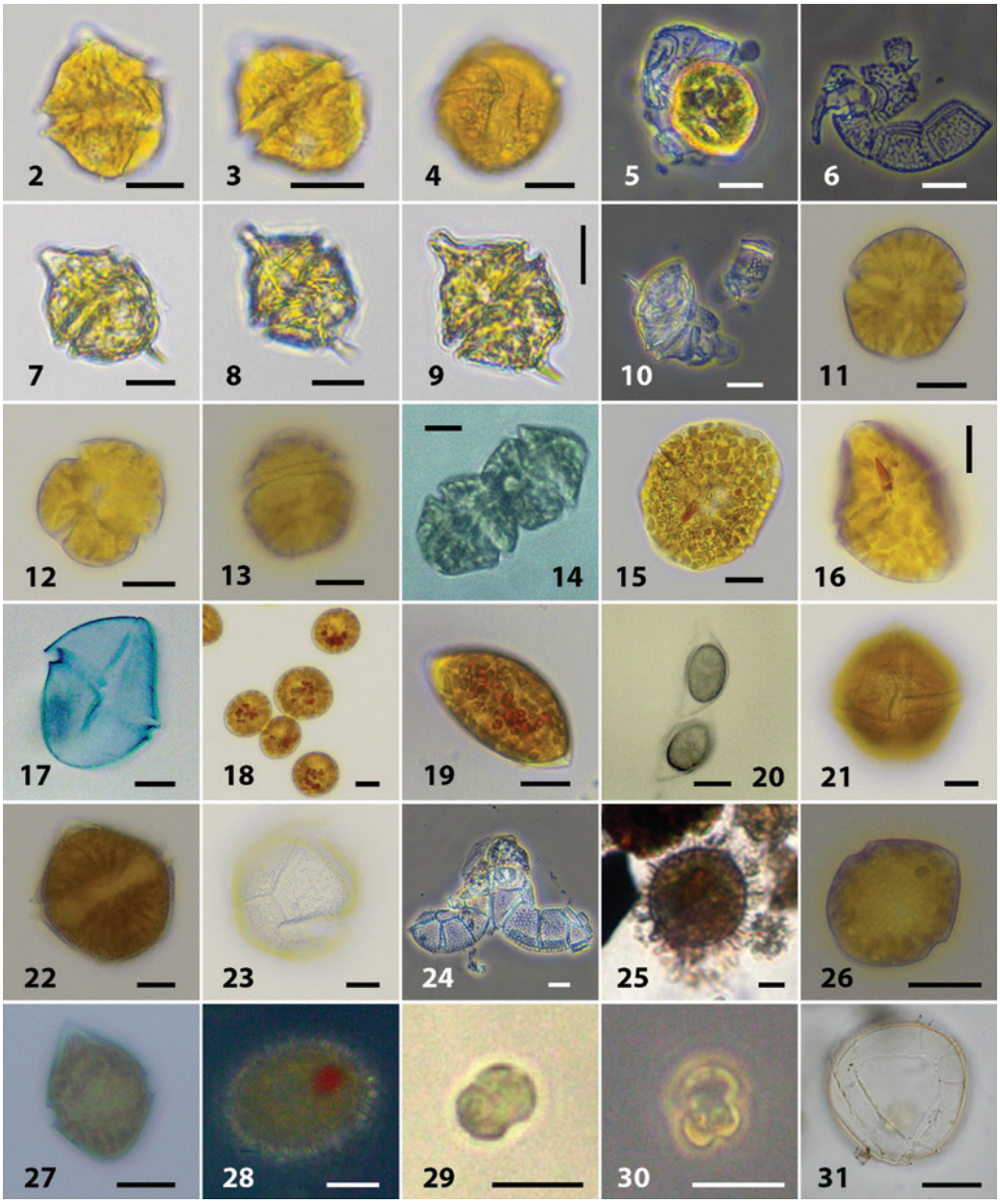
Gonyaulax Diesing 1866

Gonyaulax baltica Ellegaard, Lewis *et* Harding (Figures 2–6)

Motile cells were brownish-yellow in colour, 30–32 µm long and 25–28 µm wide. The cells had a conical epitheca with a short apical horn with slight shoulders and a rounded

Table 2: Dinoflagellate cultures isolated from the Caspian and Black Seas.

Code	Station	Identification	Accession no.
CS-ST1-005	Caspian Sea Station 1	*Gonyaulax baltica*	KY921621
CS-ST2-001	Caspian Sea Station 2	*Gonyaulax* sp.	
A3	Caspian Sea HCGA09	*Gymnodinium aureolum*	KY921616
CS-ST7-009	Caspian Sea Station 7	*Gymnodinium aureolum*	KY921624
CS-ST1-001	Caspian Sea Station 1	*Kryptoperidinium foliaceum*	KY921622
CS-ST1-007	Caspian Sea Station 1	*Kryptoperidinium foliaceum*	
A6	Caspian Sea HCGA09	*Kryptoperidinium foliaceum*	KY921623
CS-ST1-002 – 004	Caspian Sea Station 1	*Lingulodinium polyedra*	
CS-ST3-001	Caspian Sea Station 3	*Lingulodinium polyedra*	
CS-ST4-001 – 004	Caspian Sea Station 4	*Lingulodinium polyedra*	
CS-ST6-002 – 004	Caspian Sea Station 6	*Lingulodinium polyedra*	
D10	Caspian Sea HCGA09	*Scrippsiella acuminata*	KY921618
B1	Black Sea	*Scrippsiella acuminata*	KY921618
CS-ST2-004 and 006	Caspian Sea Station 2	*Scrippsiella acuminata*	
D4	Caspian Sea HCGA09	*Woloszynskia* sp.	KY921615
D5	Caspian Sea HCGA09	*Woloszynskia* sp.	KY921617
D6	Black Sea	*Woloszynskia* sp.	KY921620



Figures 2–31: Light micrographs of Caspian Sea dinoflagellates grown in culture from sediments.
(2–6) *Gonyaulax baltica*, culture CS-ST1-005. (2) Lateral view of cell showing cingulum offset. (3) Dorsal view of cell showing general shape and wide cingulum. (4) Antapical view of cell showing broad sulcus. (5) Ecdysed cell showing antapical spines. (6) Hypotheca of cell showing plate reticulation, smooth sulcus and intra plate growth bands. (7–10) *Gonyaulax* sp., culture CS-ST2-001. (7) Lateral view of cell showing general shape, cingulum offset, apical horn and antapical spine. (8) Ventral view of cell showing broad offset cingulum.
(9) Dorsal view of cell in outline showing pronounced apical horn and definite shoulders. (10) Ecdysed theca showing solid antapical spine and heavy plate reticulation. (11–14) *Gymnodinium aureolum*. (11–13) Culture CS-ST7-009. (14) Culture A3. (11) Cell showing overall shape, central nucleus and radiating chloroplasts. (12) Ventral view of cell showing sulcal-cingulum arrangement. (13) Dorsal view of cell. (14) Cells in duplet. (15–20) *Kryptoperidinium foliaceum*. (15 and 19) Culture CS-ST1-007. (16 and 18) Culture CS-ST1-001. (17 and 20) Culture A6.
(15) Ventral view of cell showing leaf-like curvature, central nucleus, median cingulum and eyespot. (16) Lateral view of cell showing dorso-ventral flattening and eyespot. (17) Theca stained with trypan blue. (18) Cysts in ventral view. (19) Cyst in lateral view. (20) Cysts in mucoid capsule. (21–25) *Lingulodinium polyedra*. (21 and 23) Culture CS-ST1-002. (22) Culture CS-ST1-004. (25) Culture CS-ST1-003. (21) Ventral view of cell showing cingulum offset and first apical plate. (22) Dorsal view of cell showing horse-shoe shaped nucleus. (23) Ecdysed hypotheca showing distinctive polyhedral shape and plate reticulation. (24) Squashed cell showing thecal plates. (25) Cyst from Station 1 sediment.
(26–28) *Scrippsiella acuminata*. (26) Culture CS-ST2-006. (27–28) Culture D10. (26) Outline view of cell. (27) Outline view of cell. (28) Cyst formed in culture. (29–30) *Woloszynskia* sp. (29) Culture D3 showing cell with eyespot. (30) Cell showing general outline from culture D4.
(31) *Impagidinium caspienense* from palynological preparation (core CS03/1 at 32 cm) showing archeopyle. Scale bars = 10 μm.

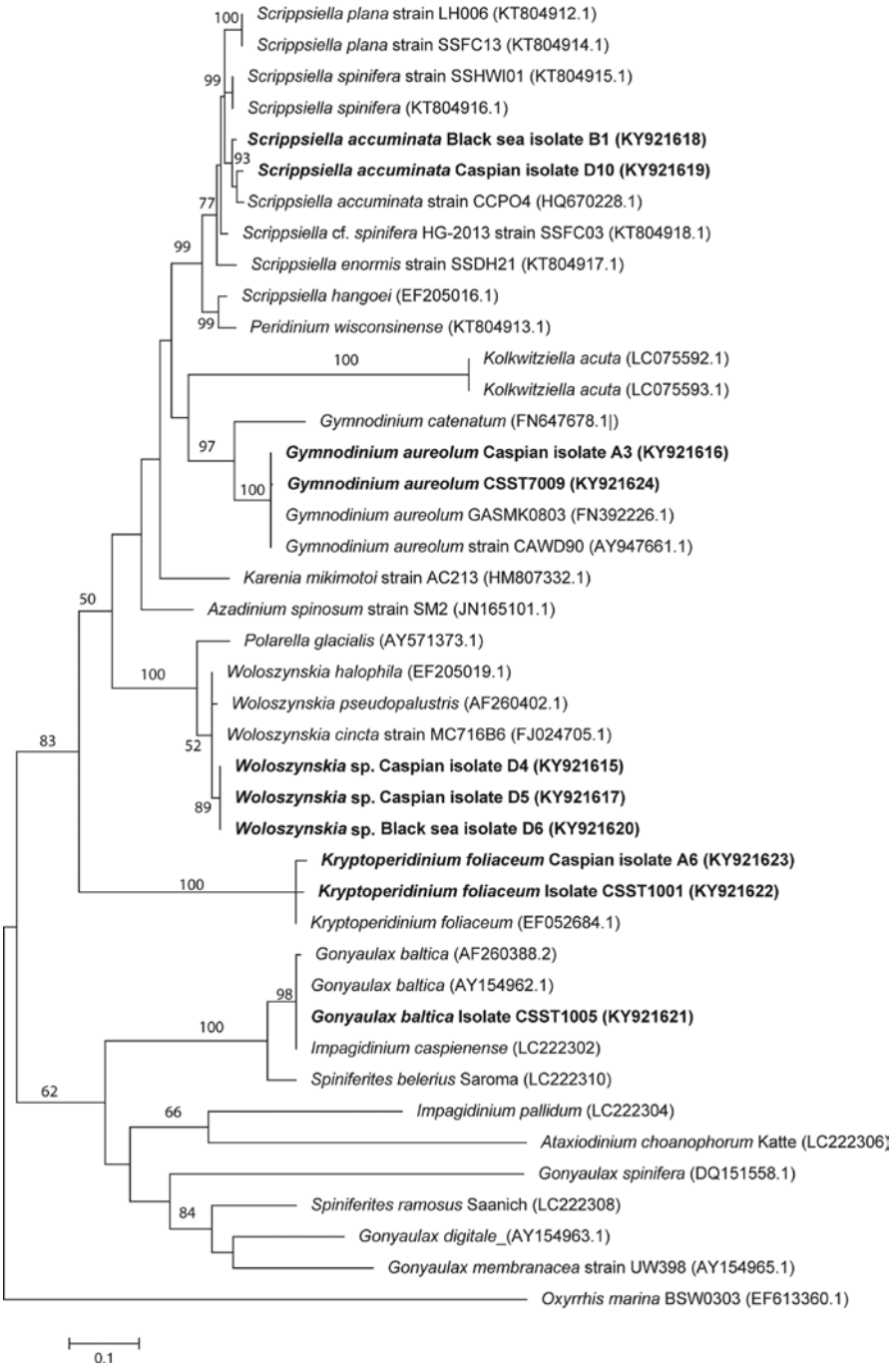


Figure 32: Molecular phylogeny of dinoflagellates isolated from the Caspian and Black Sea sediments inferred from partial large-subunit rDNA (LSU rDNA) sequences based on the maximum likelihood (ML) method.
Oxyrrhis marina was used as an outgroup. Numbers on branches represent ML bootstrap values for that node; bootstrap values >50% are shown. Sequences from isolates in this study are indicated in bold. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Scale bar = nucleotide substitutions per site. The analysis involved 42 nucleotide sequences. Sequences generated in this study are available from GenBank, Accessions KY921615-KY921624. There were a total of 503 positions in the final dataset.

hypotheca (Figure 2). The wide median cingulum was offset by two cingulum widths (Figures 2 and 3). The sulcus is broad and smooth (Figures 4–6). Other thecal plates had clear reticulation (Figures 5 and 6). On some cells, short acuminate processes could be discerned on the hypotheca (Figure 5). Overall, the thecal morphology was attributable to *G. baltica* (Ellegaard et al. 2002). One strain was isolated from Caspian Sea material and was sequenced, emerging as identical to *G. baltica* isolated from the Baltic Sea and *Impagidinium caspienense* Marret isolated from the Caspian Sea (Figure 32).

***Gonyaulax* sp. (Figures 7–10)**

Motile cells were brownish-yellow in colour, 35–40 μm long and 25–30 μm wide. Cells had a conical epitheca with a pronounced apical horn arising from distinct shoulders (Figures 7–9). The hypotheca was slightly flattened with a long (5–8 μm) single antapical horn. The wide median cingulum was offset by two cingulum widths. Thecal plates were strongly reticulate (Figure 10). This poorly growing strain did not survive to sequencing so observations as to identity can only be made on the basis of light microscopy. The cells can be compared to *Gonyaulax digitale* Kofoid and *Gonyaulax elongata* (Reid) Ellegaard, Daugbjerg, Rochon, Lewis *et* Harding for which cyst-theca relationships have been described (Lewis et al. 2001, Ellegaard et al. 2003). They resemble most closely the former with the exception of the single prominent antapical spine – no specimens being seen with two or more spines as is diagnostic for this species. *Gonyaulax elongata* is characterised by a single antapical flange but has a rather less pronounced apical horn with only weak shoulders in contrast to the cells observed here. Neither have either of these cyst types been recorded from Caspian Sea sediment. Table 1 shows there are several other gonyaulacoid cysts recorded in the sediment – *Spiniferites belerius* Reid, *Spiniferites cruciformis* Wall *et* Dale and *Caspidinium rugosum* Marret. From this study, it is not possible to determine which, if any, of these are linked to this *Gonyaulax*. Comparison to some 25 other described *Gonyaulax* species does not yield any clear affinities. At this point, we do not consider we have sufficient information to fully describe this species and prefer to wait to provide a description that would also include the cyst stage.

Gymnodinium F. Stein 1878 emend. Hansen *et* Moestrup 2000

***Gymnodinium aureolum* (Hulburt) Hansen (Figures 11–14)**

Motile cells were yellow-brown in colour and were spherical in outline with a flattened antapex and slight dorsoventral flattening (Figures 11–13). Cells were 30–38 μm long and 25–30 μm wide. The cingulum surrounded the middle of the cell with a slight offset (Figures 11 and 12). The nucleus was centrally placed (Figure 12). Divided cells were occasionally seen as duplets (Figure 14). Cells matched the description given for this species in Hansen et al. (2000). Two strains isolated from Caspian Sea material were successfully sequenced and are placed in the tree alongside strains of *Gymnodinium aureolum* from South Korea and New Zealand (Figure 32).

Kryptoperidinium Lindemann 1924

***Kryptoperidinium foliaceum* (F. Stein) Lindemann (Figures 15–20)**

Cells were pale brown in colour with a red eye spot and a central nucleus (Figures 15 and 16). Cells were 30–50 μm long and 28–45 μm wide. Cells were strongly dorso-ventrally flattened and broadly circular in dorsal view (Figures 15 and 16). The cingulum was median and not offset (Figure 15). Cells had very thin thecae on which it was very difficult to discern any thecal tabulation (Figure 17) although it has been reported by Figueroa et al. (2009). Cysts were formed within our cultures – these were ovoid to spherical in dorsal view (Figure 18) and narrowly elliptical in apical view (Figures 19 and 20). Two strains from the Caspian were successfully sequenced which match those for *Kryptoperidinium foliaceum* (Figure 32).

Lingulodinium Wall 1967

***Lingulodinium polyedra* (F. Stein) Dodge (Figures 21–25)**

Cells were brown in colour (Figure 21), 28–45 μm long and 28–45 μm wide. Cells showed a characteristic angular outline with flattened hypotheca and angular epitheca with a very small apical horn (Figure 22). Cingulum was median and offset by one cingulum width (Figure 21). Strong thecal plates with circular ridges around the trichocyst pores and ridges along plate boundaries (Figures 23 and 24). The nucleus was U-shaped and lying across the middle of the cell (Figure 22). Cysts were found in the sediment samples examined (Figure 25), and various spine lengths were noted, probably driven by the low salinities in the Caspian, as has been recorded by Mertens et al. (2012). Cultures of *Lingulodinium* were the most numerous in this study indicating their common occurrence in the sediment as well as ease of culturing.

Scrippsiella Balech 1959

***Scrippsiella acuminata* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S. Soehner, Kirsch, Kusber *et* Gottschling (Figures 26–28)**

Cells were brown in colour with a pale central nucleus (Figure 26) and 21–26 μm long and 20–28 μm wide. Cells had a conical epitheca and a rounded hypotheca

and were circular when seen in apical view (Figures 26 and 27). The cingulum was median and only slightly offset. Characteristic cysts were formed in one isolate (Figure 28). One Caspian and one Black Sea isolate were successfully sequenced and placed in the tree alongside *Scrippsiella trochoidea* (F. Stein) Loeblich III (considered a heterotypic synonym of *S. acuminata* by Kretschmann et al. 2015) from the Lafeyette River in the USA (Figure 32). Two other *Scrippsiella* species are of note to review here – *S. plana* and *S. spinifera* Honsell *et* Cabrini. In their paper describing *S. plana* from the Caspian Sea for the first time, Luo et al. (2016) clearly illustrate both species which each have characteristic motile cells. *Scrippsiella plana* has a distinctive flattened morphology and *S. spinifera* an elongate morphology with small antapical spines. The cultures developed in this study did not show these thecal morphologies; furthermore, a sequenced isolate formed characteristic *S. trochoidea* cysts. The taxonomy of *Scrippsiella sensu lato* remains enigmatic with further resolution awaiting sequencing of key species (Luo et al. 2016).

Woloszynskia Thompson 1951

Woloszynskia sp. (Figures 29 and 30)

Cells were small in comparison to other isolated species, averaging 9–10 µm long and 7–8 µm wide, and gymnodinioid in shape (Figures 29 and 30). Cells were orange/brown in colour. Eyespots were visible in the centre of cells (Figure 29) as has been reported in other *Woloszynskia* species (Siano et al. 2009). Cells swam fast with a distinctive whip-like movement. Cysts were ovoid in shape, brown in colour and ca. 9 µm long and 7–8 µm wide. Two isolates were obtained from the Caspian and one from the Black Sea and all three were successfully sequenced being placed in the phylogenetic tree alongside other *Woloszynskia* species (Figure 32).

Discussion

Gonyaulax baltica, Kolkwitziella acuta (Apstein) Elbrächter and *Lingulodinium polyedra* are the only species that have previously been recognised in both planktonic and sediment samples (Table 1). Of these, we have confirmed the presence of *G. baltica* and *L. polyedra* in Caspian Sea sediment. The cyst-theca relationship for *Impagidinium caspienense* (Figure 31) was recently elucidated by Mertens et al. (2017) as being linked to *G. baltica*. In common with other spiny gonyaulacoid cysts, the cyst morphology of *G. baltica* is strongly influenced by salinity and the form found in the Caspian is at one end of the spectrum of spine bearing with no process development evident. Thus when first described from Caspian sediment, rather than attribution to *Spiniferites* Mantell, it was attributed to the genus *Impagidinium* Stover *et* Evitt (Marret et al. 2004). However, the cyst form of *G. baltica* in its original description by Ellegaard et al. (2002) from Baltic sediment was of a more typical *Spiniferites* morphology although description was provided in that paper for substantial variation in form. Attribution to *Impagidinium* is questioned by Mertens et al. (2017) on the basis of some morphological features and also geography (other *Impagidinium* species being typically found offshore rather than in more coastal habitats). Further, molecular and detailed taxonomic studies involving both cysts and thecae in the wider *Gonyaulax* group provide evidence for the polyphyletic nature of *Gonyaulax sensu lato*. For now, however, they suggest the simplest solution is to retain the current dual classification of the cyst and motile stage, awaiting a more fundamental overhaul of *Gonyaulax* in the future. That there remains a great deal of work to clarify species in the Gonyaulacales has previously been highlighted (e.g. Lewis et al. 2001, Mertens et al. 2015a) and the presence of another unidentified *Gonyaulax* in this study also confirms that further work on the cyst-theca relationships in the Gonyaulacales is necessary.

Scrippsiella acuminata is recorded from Caspian sediment for the first time. *Scrippsiella acuminata* has previously been recognised in the plankton but not recorded in sediment samples. *Scrippsiella* species are common constituents of coastal sediment (Lewis 1991). Caspian sediment has largely been investigated using palynological techniques (references in Table 1). As a calcareous cyst with a thin inner organic wall, it seems likely that these cysts have been destroyed by the processing techniques used for these studies and hence the previous lack of records. This is the first record for *Kryptoperidinium foliaceum, Gymnodinium aureolum* and *Woloszynskia* sp. from the Caspian Sea. *Woloszynskia pascheri* (Suchlandt) von Stosch has been recorded from the Iranian rivers which flow into the Caspian (Zarei Darki 2009). It is possible that all of these species have been previously overlooked in the plankton samples – a number of *Glenodinium* Ehrenberg and *Gymnodinium* species have been recorded (see Table 1) some of which might be confused with these species. It can be especially difficult to speciate *Gymnodinium* species because of the difficulties of preserving them successfully. This was illustrated by Sundström et al. (2009) describing a new species of *Gymnodinium* from the extensively studied Baltic Sea

which had not been recognised despite it being a relatively common member of the spring phytoplankton flora. Cysts have been described for both *K. foliaceum* (Figueroa et al. 2009) and *G. aureolum* (Tang et al. 2008), but neither has been recorded from Caspian sediment. Cysts are also known from the *Woloszynskia* genus (e.g. Kremp et al. 2005) but none has been recorded from Caspian sediment. Previous studies in the Caspian have been for geological purposes and so sediment has been processed by palynological means. It seems likely that the hyaline cysts of these species did not survive such harsh techniques or if they did, given their lack of paratabulation, they might not be recognised as such. However, the presence of cysts in sediment can be a useful way of providing an integrated record of occurrence of species that might be missed in the plankton and indeed *K. foliaceum* was recognised for the first time in the Mediterranean Sea by this route (Satta et al. 2013). Given the variety of *Gymnodinium* species recorded in the plankton, it would seem likely other cysts in this genus might also be present in the sediment.

Reviewing Table 1, other gaps can be highlighted between the two lists. Species of the following genera, *Oblea* Balech *ex* Loeblich *et* Loeblich III, *Diplopsalis* Bergh and some *Protoperidinium* Bergh, recorded in the plankton could be represented by the *Brigantedinium* Reid recorded in the dinocyst record. However, the presence of *Protoperidinium leonis* Pavillard in the plankton would suggest the distinctive *Quinquecuspis* Harland might also be recorded in the dinocyst record. It is hard to reconcile this lack – unless it is due to rarity. *Heterocapsa triquetra* (Ehrenberg) F. Stein recorded in the plankton also is not recorded in sediments. The presence of *Pentapharsodinium dalei* Indelicato *et* Loeblich III in the sedimentary record would suggest it should similarly be recorded in the plankton. Here there could be confusion with *S. acuminata* that is similar in morphology in the motile stage.

The approach of using sediment slurries has been invaluable for investigating the diversity of dinoflagellates in a region where intact cysts were relatively rare. Single cyst isolations are very helpful but, where there is a paucity of intact cysts, they are challenging. Using slurries offers the opportunity to develop cultures that can provide a wealth of taxonomic information as well as the possibility of cyst formation as demonstrated in this study. Our results also highlight the need for culturing to learn more about the interactions between motile stages and cysts, which would allow understanding of species origination over time linked to the palaeohydrological history of the Caspian Sea.

Acknowledgements: We thank M. Naderi-Beni (INIOAS, Tehran, Iran) for taking the grab samples; Fabienne Marret and Lee Bradley for stimulating our interest in the Caspian and for initial provision of sediment, and Sarah Rowing for assistance with culturing at the University of Westminster.

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Bionotes

Jane Lewis
Faculty of Science and Technology, Department of Life Sciences, University of Westminster, 115 New Cavendish Street, London, W1W 6UW, UK,
lewisjm@westminster.ac.uk

Jane Lewis is a Professor in the Department of Life Sciences at the University of Westminster. Her research is focussed on the ecology and taxonomy of dinoflagellates. There have been two main themes for this research – the use of dinoflagellate cysts to interpret the fossil record and the role of microalgal life cycles in harmful algal blooms. Current research includes the role of temperature in modulating life cycle transitions in *Alexandrium tamarense* in the Gulf of Maine and the morphological variation of cysts in various species within the Gonyaulacales.

Joe D. Taylor
Faculty of Science and Technology, Department of Life Sciences, University of Westminster, 115 New Cavendish Street, London, W1W 6UW, UK; and
School of Environment and Life Sciences, University of Salford, Peel Building, Salford, M5 4WT, UK

Joe D. Taylor is an independent research fellow at the University of Salford, UK. He was awarded a PhD in Environmental Microbiology by the University of Essex, UK, working on microbial degradation of algal polysaccharides in estuarine littoral zones. He has worked on across multiple habitats and with multiple taxonomy groups. His current research is focused on micro-eukaryote diversity and function in oceanic, coastal and terrestrial food-webs.

Suzanne A.G. Leroy
Centre Européen de Recherche et d'Enseignement en Géosciences de l'Environnement, Technopôle de l'Arbois, 13545 Aix-en-Provence cedex 4, France

Suzanne A.G. Leroy was Professor at Brunel University London (UK) from 2000 to 2016. She is now an associate researcher at the CEREGE, France. She is a palynologist, specialising in the Caspian Sea palaeo-environmental history since 1994. She has pioneered the work on dinocysts from Caspian sediment and has stimulated their taxonomical and phylogenetical analyses. Other areas of current research include palaeo-climates and lake-level changes in the Middle East and Central Asia.