Pentaplacodinium saltonense gen. et sp. nov. (Dinophyceae) and its relationship to the cyst-defined genus Operculodinium and yessotoxin-producing Protoceratium reticulatum

1   ~~The potentially toxic~~ *Pentaplacodinium saltonense* gen. et sp. nov. (Dinophyceae)~~,~~ and its

2   relationship to the cyst-defined genus *Operculodinium* ~~*psilatum*~~ and ~~the~~ yessotoxin-producing

3   *Protoceratium reticulatum*

4

5   Kenneth Neil Mertens*

6   Research Unit for Palaeontology, Ghent University, Krijgslaan 281 s8, 9000 Ghent, Belgium

7   Now at: Ifremer, LER BO, Station de Biologie Marine, Place de la Croix, BP40537, F-29185

8   Concarneau Cedex, France, Tel: (0033)2 98 10 42 82

9

10   M. Consuelo Carbonell-Moore*

11   Oregon State University, Department of Botany and Plant Pathology, College of Agricultural

12   Sciences, 2082 Cordley Hall, Corvallis, OR 97331-2902, U.S.A.

13

14   Vera Pospelova

15   School of Earth and Ocean Sciences, University of Victoria, OEASB A405,

16   P.O. Box 1700 STN CSC, Victoria, BC, V8W 2Y2, Canada

17

18   Martin J. Head

19   Department of Earth Sciences, Brock University, 1812 Sir Isaac Brock Way~~500 Glenridge~~

20   ~~Avenue~~, St. Catharines, Ontario, L2S 3A1, Canada

21

22   Andrea Highfield

23   The Marine Biological Association of the United Kingdom, Citadel Hill, Plymouth PL1 2PB,

24   United Kingdom

25

26   Declan Schroeder

27   The Marine Biological Association of the United Kingdom, Citadel Hill, Plymouth PL1 2PB,

28   United Kingdom

29   School of Biological Sciences, University of Reading, Reading RG6 6AJ, United Kingdom

30

31   Haifeng Gu

32   Third Institute of Oceanography, SOA, Xiamen 361005, China

33

34   Karl B. Andree

35   Margarita Fernandez

36   IRTA, Crta. Poble Nou, Km 5.5, 43540-Sant Carles de la Rápita, Spain

37

38   Aika Yamaguchi

39   Kobe University Research Center for Inland Seas, Kobe 657-8501, Japan

40

41   Yoshihito Takano

42   Kazumi Matsuoka

43   Institute for East China Sea Research (ECSER), Nagasaki University, 1551-7, Taira-machi,

44   Nagasaki, 851-2213, Japan

45

46   Elisabeth Nézan

47   Ifremer, LER BO, Station de Biologie Marine, Place de la Croix, BP40537, F-29185

48   Concarneau Cedex, France

49

50   Gwenael Bilien

51   Ifremer, LER BO, Station de Biologie Marine, Place de la Croix, BP40537, F-29185

52   Concarneau Cedex, France

53

54   Yuri Okolodkov

55   Universidad Veracruzana, Instituto de Ciencias Marinas y Pesquerías, Calle Hidalgo núm.

56   617, Colonia Río Jamapa, Boca del Río, 94290 Veracruz, México

57

58   Kazuhiko Koike

59   Graduate School of Biosphere Science, Hiroshima University, Kagamiyama 1-4-4, Higashi-

60   Hiroshima, Hiroshima 739-8528, Japan

61

62   Mona Hoppenrath

63   Senckenberg am Meer, Deutsches Zentrum für Marine Biodiversitätsforschung (DZMB),

64   Südstrand 44, D-26382 Wilhelmshaven, Germany

65

66   Maya Pfaff

67   Marine Biology Research Center, Ma-RE Institute, Zoology Department, University of Cape

68   Town, Rondebosch 7701, South Africa

69

70   Grant Pitcher

71   Marine and Coastal Management, Private Bag X2, Rogge Bay 8012, Cape Town, South

72   Africa

73

74   Abdulrahman Al-Muftah

75   Department of Biological and Environmental Sciences, Qatar University, Doha, Qatar

Rochon, Andrée

Institut des sciences de la mer de Rimouski (ISMER), Université du Québec à Rimouski,

310 allée des Ursulines, Rimouski, QC, Canada G5L 3A1

Po Teen Lim, Chui Pin Leaw, Zhen Fei Lim

Institute of Ocean and Earth Sciences, University of Malaya, 16310 Bachok, Kelantan,

Malaysia

Marianne Ellegaard

Department of Plant and Environmental Sciences, University of Copenhagen,

Thorvaldsensvej 40, DK-1871, Frederiksberg, Denmark

(*both authors contributed equally)

Abstract

Strains of a dinoflagellate from the Salton Sea, previously identified as *Protoceratium reticulatum* and yessotoxin producing, have been reexamined morphologically and genetically and *Pentaplacodinium saltonense* n. gen. et sp. is erected to accommodate this species. *Pentaplacodinium saltonense* differs from *Protoceratium reticulatum* (Claparède et Lachmann 1859) Bütschli 1885 in the number of precingular plates (five vs. six), cingular displacement (two widths vs. one), and distinct cyst morphology. Incubation experiments (excystment and encystment) show that the resting cyst of *Pentaplacodinium saltonense* is morphologically most similar to the cyst-defined species *Operculodinium israelianum* (Rossignol 1962) Wall 1967 and *Operculodinium. psilatum* Wall 1967. Collections of comparative material from around the globe (including *Protoceratium reticulatum* and the genus *Ceratocorys*) and single cell PCR were used to clarify molecular phylogenies. Variable regions in the LSU (three new sequences), SSU (12 new sequences) and intergenic ITS 1-2 (14 new sequences) were obtained. These show that *Pentaplacodinium saltonense* and *Protoceratium reticulatum* form two distinct clades. *Pentaplacodinium saltonense* formed a monophyletic clade with several unidentified strains from Malaysia. LSU and SSU rDNA sequences of three species of *Ceratocorys* (*C. armata, C. gourreti, C. horrida*) from the Mediterranean and several other unidentified strains from Malaysia form a well-supported sister clade. The unique phylogenetic position of an unidentified strain from Hawaii is also documented and requires further examination. In addition, based on the V9 SSU topology (bootstrap values >80%), specimens from Elands Bay (South Africa), originally described as *Gonyaulax grindleyi* by Reinecke (1967), cluster with *Protoceratium reticulatum*. The known range of *Pentaplacodinium saltonense* is tropical to subtropical, and its cyst is recorded as a fossil in upper Cenozoic

sediments. *Protoceratium reticulatum* and *Pentaplacodinium saltonense* seem to inhabit different niches: motile stages of these ~~species~~ dinoflagellates have not been found in the same plankton sample.

Keywords

1. Introduction

The dinoflagellate genus *Protoceratium* was erected by Bergh (1881, p. 242) with *Protoceratium aceros* as the type species (fig. 36), which was recovered from Strib, Denmark. Bütschli (1885, p. 1007, plate 52, fig. 2) considered *Peridinium reticulatum* as described earlier by Claparède and Lachmann (1858) from Bergen Fjord, Norway, as a senior synonym, and he proposed the combination *Protoceratium reticulatum*. He also considered *Clathrocysta reticulata* as described by Stein (1883) a junior synonym. The plate formula for *P. reticulatum*, 4′, 0a, 6″, 6‴, 1p, 1⁗, was first provided by Wołoszyńska (1929) through the study of Baltic Sea specimens. Reinecke (1967) erected the name *Gonyaulax grindleyi* for specimens from Elands Bay in Cape Town, South Africa, with the tabulation 3′, 1a, 6″, 6‴, 1p, 1⁗. Based on a detailed study of the theca of *Protoceratium reticulatum* from the North Sea, Stosch (1969) considered *G. grindleyi* to be a junior synonym of *P. reticulatum,* although he considered it assignable to the genus *Gonyaulax.* Dodge (1989) agreed with the tabulation of Reinecke (1979), but retained the genus *Protoceratium* because he considered it different from his emendation of the genus *Gonyaulax*, by having only one intercalary plate. Hansen et al. (1997) restudied specimens close to the type locality of *P. aceros*, and based on the plate analysis concluded that *P. reticulatum, P. aceros* and *G. grindleyi* were conspecific, and agreed with the tabulation of Wołoszyńska (1929). Paez-Reyes and Head (2013) reviewed the morphological variability reported for *P. reticulatum* and concurred with Dodge (1989) in maintaining *Protoceratium* as a distinct genus from *Gonyaulax*.

Since the early 1900's, sSeven other *Protoceratium* species have been described since the early 1900s, and the latest review of these taxa was having been performed by Schiller (1937 p. 322–326). Kofoid (1907) described *P. areolatum* from the tropical Pacific and emended the genus for the first time. Meunier (1910) described a very similar species from

the Kara Sea that he named *Protoceratium splendens,* which is possibly a junior synonym, as

suggested by Gómez (2012). Later, Kofoid in Kofoid and Michener (1911) emended

Protoceratium once more to include several new species from the eastern tropical Pacific that

were described without illustration (*P. cancellorum, P. globosum, P. pellucidum, P. pepo, P.*

promissum), and he suggested a tabulation formula for the genus: 2′, 0a, 6″ (?c), 6‴, 0p, 3⁗.

Schiller (1937) transferred *Clathrocysta aculeata* as described by Stein (1883) to

Protoceratium aculeatum, presumably based on the fact that Bütschli (1885) had considered

the genus *Clathrocysta* described by Stein 1883 as a junior synonym of *Protoceratium*.

Schiller (1937) transferred *Peridinium spinulosum* as described by Murray and Whitting

(1899) to the genus *Protoceratium.* Later, Balech (1988) rediscovered this species in the

South Wwest Atlantic and suggested yet another another variation on the tabulation for

Protoceratium, 3′, 0a, 6″, 6‴, 2⁗, based on his observations of *Protoceratium spinulosum*.

Protoceratium reticulatum (Claparède et Lachmann) Bütschli 1885 is a very common

dinoflagellate found in cold and warm waters, as well as in oceanic and neritic environments

(e.g., as *Operculodinium centrocarpum* in Zonneveld et al., 2013). Its resting cyst distribution

today reveals a strong link with the North Atlantic Current, an association traceable through

the upper Cenozoic fossil record (Hennissen et al., 2017 and references therein).

Protoceratium reticulatum is considered potentially toxic because of its production of

yessotoxins (e.g. Paz et al., 2008; Sala-Pérez et al., 2016). It has been successfully isolated

and cultured from many parts of the world, and grown into cultures. Cysts of *P. reticulatum*

were first observed in cultures established from motile cells from the inner Oslofjord (,

Norway) by Braarud (1945). This cyst was related by Wall and Dale (1966, 1967, 1968) to the

cyst-defined species described from the Miocene of Australia, *Operculodinium centrocarpum*

(Deflandre et Cookson 1955) Wall 1967. That assignation was challenged by Head and

Wrenn (1992) and Head (1996a) on the grounds that *Operculodinium centrocarpum* was

larger and more robust than the cysts recorded by Wall and Dale (1966) from modern sediments. ~~However, a~~A restudy of the holotype of *Operculodinium centrocarpum* ~~refuted this assignation~~confirmed this, and the name "cyst of *Protoceratium reticulatum*" was recommended (Matsuoka et al., 1997). Wall and Dale (1968) proposed that *P. reticulatum* was also related to the cyst-defined *Operculodinium psilatum* Wall 1967 and furthermore possibly to *Operculodinium israelianum* (Rossignol 1962) Wall 1967. The cyst-defined *Pyxidinopsis psilata* (Wall et Dale in Wall et al., 1973) Head 1994 was subsequently also linked to *Protoceratium reticulatum* (Dale, 1996, as *Tectatodinium psilatum*) although this connection was later questioned ~~later~~(Mertens et al., 2011). Because of uncertainty regarding the links between the cysts produced by *P. reticulatum* ~~(see Head, 2006),~~ and ~~cysts~~cyst-defined species ~~named based on~~from the fossil record~~s~~, Head (1996a, 1996b) and subsequent authors used the ~~the~~ term "*Operculodinium centrocarpum* sensu Wall and Dale, 1966" ~~was used~~ to describe the cysts that had first been observed by Braarud (1945) and Wall and Dale (1966). With the removal of *Pyxidinopsis psilata* as a potential cyst of *Protoceratium reticulatum,* Paez-Reyes and Head (2013) argued on the basis of non-overlapping geographic distribution that the "cyst of *Protoceratium reticulatum*" was now unambiguous and should replace the term "*Operculodinium centrocarpum* sensu Wall and Dale, 1966". That approach is followed here. Recent studies of variation in the process length of cysts of *Protoceratium reticulatum* have been related to variations in sea surface salinity and other parameters (e.g., Mertens et al. 2011; Jansson et al., 2014), and the cyst wall appears to be composed of cellulose glucan (Bogus et al. 2014). Resting cyst production through sexual reproduction has recently been demonstrated by Salgado et al. (2017).

Protoceratium reticulatum was assigned questionably to the subfamily Cribroperidinioideae by Fensome et al. (1993b) based on the presence of six precingular plates, L-type ventral organization and possible dextral torsion, which at the time had not

been documented. This assignment was confirmed by Paez-Reyes and Head (2013). ~~However,~~ ~~t~~The description of the ~~very~~ similar cyst-defined *Operculodinium bahamense*, with neutral torsion and modified L-type ventral organization, ~~which would be placed~~allowing placement in the subfamily Leptodinioideae, either ~~challenges~~challenges ~~such~~the present subfamilial classification of the Gonyaulacaceae, or implies that *Operculodinium* is polyphyletic, with both outcomes being possible (Paez-Reyes and Head, 2013). Furthermore, molecular phylogenetics show that *Protoceratium reticulatum* is closely related to the family Ceratocoryaceae but not to the other extant cribroperidinean, *Lingulodinium polyedra* (Saldarriaga et al., 2004). It should also be noted that morphological variation and sequencing of cysts has suggested pseudocryptic speciation in *P. reticulatum* (Mertens et al., 2012a). Howard et al. (2009) investigated the phylogenetic relationships of yessotoxin-producing dinoflagellates, including several strains of *P. reticulatum* from different localities. Using Large Sub Unit (LSU) and Internal Transcribed Spacer (ITS) ribosomal DNA (rDNA) sequencing, they showed that the *P. reticulatum* strains formed a monophyletic clade in both phylogenies. ~~However, o~~One particular strain (CCMP404) isolated from the Salton Sea (California) in 1966 showed significant genetic differences from the other strains in both phylogenies. Despite these genetic differences, Howard et al. (2009) considered all the strains to belong to the species *P. reticulatum*.

The Salton Sea is the largest saline lake in California with a surface area of 980 km^2 (Reifel et al., 2002). It has a mean depth of 8 m and a maximum depth of 15 m (Ferrari and Weghorst, 1997). Although originally composed of relatively freshwater, it has become saline due to a lack of outflow and high evaporation rates. During 1997–1999, the salinity was between 41 and 45 g l^{-1} (Watts et al., 2001), while the temperature varied between about 12 and 40°C seasonally (Watts et al., 2001; Holdren and Montaño, 2002). Oxygen at times was supersaturated due to phytoplankton photosynthesis, but was also often severely depleted,

228 occasionally even in surface waters (Watts et al., 2001; Holdren and Montaño, 2002). Reifel

229 et al. (2002) reported *P. reticulatum* from the Salton Sea without illustration or description.

230   In the present study, through reevaluation of the CCMP404 strain originated from the

231 Salton Sea and observations of recently collected plankton samples from the Salton Sea, it

232 is~~we~~ demonstrated that specimens living in the Salton Sea that ~~have~~ had ~~been~~ previously been

233 identified as *P. reticulatum*, have a ~~dissimilar~~ different tabulation to that of *P. reticulatum*. To

234 resolve this issue and accommodate these organisms, ~~we have erected a new genus and new~~

235 ~~species,~~ *Pentaplacodinium saltonense* n. gen. et sp. is erected. From ~~the~~ Salton Sea plankton

236 samples, ~~we describe~~ the morphology of the thecate stage is described, showing significant

237 differences with *P. reticulatum*. Similarly, through incubation of cysts from Salton Sea

238 surface sediments, ~~we describe~~ the corresponding cyst is described. Phylogenetic relationships

239 are explored, including those with several unpublished sequences of *P. reticulatum,*

240 *Ceratocorys armata* (Schütt 1895) Kofoid 1910*, Ceratocorys gourretii* Paulsen 1931*,*

241 *Ceratocorys horrida* Stein 1883, and several unidentified strains. In addition, both the

242 autecology and fossil record of *Pentaplacodinium saltonense* are examined.

243

244 **2. Material and Methods**

245

246   The cyst–theca relationship of *P. saltonense* was established through a germination

247 experiment of a sample from the Salton Sea (CA, USA). To identify differences and

248 similarities between *P. reticulatum* and *P. saltonense,* ~~we compared~~ the morphology of

249 thecate stages of strains present in culture collections and other cells used for sequencing,

250 are~~we~~ compared (Table 1, Suppl. Table 1). In addition, ~~we studied the~~ phylogenies are~~we~~

251 constructed ~~of~~ using LSU, ITS and SSU rDNA based sequences of *P. saltonense* and *P.*

252 *reticulatum* from several of the same cells or cultured strains, as well as three *Ceratocorys*

253 species isolated from the Mediterranean, and several unidentified strains from Hawaii and

254 Malaysia (Table 1, Suppl. Table 1).

255

256 *2.1. Morphological ~~study~~ imaging of cells ~~in~~ from plankton samples and strains present in*

257 *culture collections ~~with microscopy~~*

258

259 Plankton samples were obtained from the Salton Sea (California, U.S.A.; 33.50 °N, 115.91

260 °W) on 24 Oct. 2013 using a plankton net with a 20 µm mesh size. These samples were fixed

261 with ethanol (50% final concentration) and stored cold. Several strains from previously

262 sequenced strains from culture collections established from several other locations were also

263 studied using transmitted light or scanning electron microscopy (Figure 1, Table 1).

264 For scanning electron microscopy (SEM) of thecate stages by M.C.C.~~-~~M., samples

265 were prepared either by filtering a plankton sample or culture, or isolating a single cell under

266 a Leica™ ~~Inverted~~ inverted ~~Light~~ light ~~Microscope~~microscope (Germany). ~~When the~~

267 ~~s~~Samples were ~~was~~ filtered~~,~~ by placing a~~n aliquot of~~ ~300 µL aliquot ~~was placed~~ on a

268 Millipore™ 0.25 mm diameter~~,~~ 5 µm pore~~,~~ polycarbonate filter at the bottom of a

269 Millipore™ column. Approximately 7 mL of distilled water were added to remove the

270 fixative (ethanol, lugol or formaldehyde) and seawater. A gentle manual vacuum with a 60 cc

271 syringe was used to speed filtration. ~~Individually isolated ce~~Cells were removed using a glass

272 micropipette under a Leica ~~Inverted~~ inverted l~~L~~ight m~~M~~icroscope ~~(Germany) with~~

273 ~~magnification 10x~~5x. Individual cells were washed six times with distilled water in double

274 depression microscope slides~~)~~. After the cells were clean, they were placed on the same kind

275 of filter as for the filtered samples. All filters were air-dried, then ~~adhered~~ affixed to 25 mm

276 diameter aluminium stubs with adhesive tabs (7/16" diameter). The mounted filters were then

277 coated with a mixture of gold-palladium in a Cressington Sputter Coater (U.S.A.) for 60 s.

Observations were performed with a FEI Quanta 3D Dual Beam SEM (Clackamas, Oregon, U.S.A.), at 5 kV. Tilts up to 52° were applied. Digital images were saved in Tiff format (2048 x 1768 pixels). Adobe Photoshop™ software was used to remove the background while maintaining the integrity of the original image.

For scanning electron microscopy (SEM) of culture CCMP 3243 by K.N.M., the culture was filtered and washed with distilled water and dehydrated in a graded ethanol series (30 to 100% in six steps). The filters were encased in metallic baskets, critical-point dried with CO_2 (CPD Bal-Tec 030), glued onto stubs, sputter coated with platinum/palladium for 90 s (JEOL JFC-2300 HR) and examined in a JEOL 6330F scanning electron microscope (JEOL, Tokyo, Japan) at the University of Copenhagen.

Measurements of thecate thecae stages of the newly described species were done conducted by M.C.C.-M. under SEM. For each motile cell, the length was measured along the center of the longitudinal axis, the width was measured along the middle of the cingulum, perpendicular to the longitudinal axisfrom one lateral margin to the other. All motile cell measurements in the species descriptions cite the minimum, average (in parentheses) and maximum values (in µm), in that order. The standard deviation (SD) is also provided where appropriate.

Labelling of tabulation follows a modified Kofoid system that recognizes homologs (e.g., Fensome et al. 1993b). The sulcal plate labelling is according tos with Balech (1980).

2.2. Germination experiment of cysts of P. saltonense

Sediment samples were collected from the Salton Sea (CA, U.S.A.) aton the same timeday ofas plankton sample collectionon,during the same field campaign on 24 October. 2013, using a Petite Ponar Grab at shallow water depths (<0.5 m). All samples were stored in plastic

303 bags in a refrigerator at 4°C. *In~~-~~-situ* sea surface salinities and sea surface temperatures were

304 measured during sampling (Table 1).

305   About 0.5–1.0 cm^3 of wet sediment was immersed in filtered seawater and, after one

306 min~~ute~~ of ultrasonication using an ultrasonic bath, the sediment was rinsed through a 20 µm

307 nylon-_ _mesh sieve using filtered seawater. From this residue, the cyst fraction was separated

308 using the heavy-liquid sodium polytungstate (SPT) at a density of 1.3 g cm^{-1} (Bolch, 1997).

309 Single cysts were then transferred to Orange S~~s~~cientific 0.5 mL microwells subjected to an

310 irradiance of 100 µmol photons m^{-2} s^{-1} and 24-hour light, and filled with f/2 medium at room

311 temperature and a salinity of 35 psu. Cysts were regularly checked for germination, and

312 observations were performed under a Leitz DM IL inverted light microscope. Encysted and

313 excysted cysts, as well as motile cells, were photographed and measured using a Leica

314 DM5000B light microscope with 100x oil immersion objectives.

315

316 *2.3. Morphological study of cysts extracted from surface sediments ~~with~~ using light*

317 *microscopy and SEM*

318

319 Surface sediment samples were collected from several ~~sites in the~~ Salton Sea sites ~~were~~

320 ~~obtained for~~to study ~~of~~ cysts of *Pentaplacodinium saltonense* (Table 1). Palynological

321 techniques were used for processing (e.g., Pospelova et al., 2010; Mertens et al., 2012b).

322 Material was rinsed twice with distilled water to remove salts. The samples were oven-dried

323 at 40°C and then treated with ~~room-temperature~~ 10% hydrochloric acid (HCl) at room

324 temperature to remove calcium carbonate particles. To dissolve ~~siliceous~~ silicate particles,

325 samples were treated with 48–50% ~~room-temperature~~ hydrofluoric acid (HF) at room

326 temperature for two days, and then treated for 10 min with room-temperature HCl (10%) to

327 remove fluorosilicates. The residue was rinsed twice with distilled water, ultrasonicated for

~30 sec and finally collected on a 15 µm mesh. Aliquots of residue were mounted on microscope slides using glycerine jelly.

All measurements and light photomicrographs were obtained by K.N.M. and V.P., respectively using an Olympus BX51 with a Nikon digital sight DS-1L 1 module, and a Nikon Eclipse 80i transmitting light microscope with a DS-L2 module, all with 100x oil immersion objectives.

For each cyst, the lengths of the three longest visible processes with the corresponding widths at their base were measured within the focal plane. Process length was measured from the middle of the process base to the process tip. The average distance between processes was determined by measuring the distance between a process near the center of the cyst on the upper surface of the cyst near the centreer and the five processes nearest to it, as measured between the middle of the process bases as seen from the surface of the cyst. The central body wall thickness was measured at two to three positions around the cross section of each cyst. The central body maximum and minimum diameters were also measured unless specimens were overly compressed or broken. Fragments representing less than half of a cyst, and cysts with mostly broken processes, were not measured. All cyst measurements in the species descriptions cite the minimum, average (in parentheses) and maximum values (in µm), in that order. The standard deviation (SD) is also provided where appropriate.

For SEM observation of cysts at Geotop (the Université du Québec à Montréal, Canada), single specimens were picked under an inverted microscope with a micropipette, sputter coated with platinum/palladium for 60 s and observed using a scanning electron microscope (Hitachi S-3400N SEM).

2.4. Single-cell polymerase chain reaction (PCR) amplification and sequencing of culture of Salton Sea culture

353

354   Isolated cells were washed three times in serial drops of 0.22 µm filtered and sterilized

355   seawater by micropipette. Each cell was transferred to a 200 µm PCR tube containing 10 µL

356   of Quick Extract FFPE DNA Extraction Solution (Epicentre, Madison, WI, USA) and

357   incubated for 1h at 56˚C, then for 2 min at 90˚C. The resulting extract was used as a DNA

358   template for the initial PCR amplification. Sequences of SSU and partial LSU rDNA were

359   determined from single cells of *P. saltonense*. The PCR was performed with EconoTaq 2X

360   Master Mix (Lucigen, Middleton, WI, USA) following the manufacture's protocols. The

361   external primers (SR1 and LSU R2) were used for the initial PCR. The first PCR product was

362   used as a DNA template for the second PCR. The following combinations of primer pairs

363   were used separately for the second PCR: SR1 and SR12, 25F1 and LSU R2. Using the

364   second PCR products as the template DNA, the third PCR ~~were~~ was performed by the

365   following combinations of primer pairs: SR1b and SR3, SR1b and SR5TAK, SR4 and

366   SR7TAK, SR6 and SR9p, SR8p and SR12, 25F1 and 25R1, D3A and LSU R2. ~~The d~~Details

367   of the primers are described in Takano and Horiguchi (2004) and Yamaguchi et al. (2016).

368   The PCR protocols and sequencing are described in Yamaguchi et al. (2016).

369

370   *2.5. Sequencing of single cells of* Protoceratium reticulatum *from Elands Bay (South Africa),*

371   *originally described as* Gonyaulax grindleyi *by Reinecke (1967)*

372

373   Isolated cells were washed three times in serial drops of 0.22 µm filtered and sterilized

374   distilled water and then transferred to a 0.2 mL PCR tube. Cells were subjected to three

375   rounds of heating to 95°C for 5 min~~utes~~ and cooling on ice for 5 min~~utes~~ to induce cellular

376   lysis. 5 µL of the cell lysate was then used as a template for PCR using primers to amplify a

377   168 bp region of the SSU, encompassing the V9 region, V9 For (5'-

378 GTACACACCGCCCGTC-3') V9 Rev (5'- TGATCCTTCTGCAGGTTCACCTAC-3')

379 (Lane et al., 1991; Medlin et al., 1988). PCR reactions were carried out in 25 µL volumes

380 containing 5 µL DNA template, 10 pmol each primer, 1 x buffer, 1 mM MgCl$_2$, 0.0025 mM

381 dNTPs, 0.5 Unit Gotaq polymerase (Promega). PCR reactions proceeded with an initial

382 denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30

383 seconds, annealing at 54°C for 20 seconds and extension at 72°C for 20 seconds and a final

384 extension step of 72°C for 5 minutes. PCR products were sequenced directly in both

385 directions using the respective primers (Source Bioscience).

386

387 *2.6. Sequencing of unidentified cultured strains*

388

389 For strains from Hawaii and Malaysia, single cells were isolated from plankton samples

390 (Suppl. Table 1) and washed three times with sterilized bi-distillate water and were used as

391 the template to amplify about 1,430 bp of the LSU rRNA gene (D1-D6 domains), using the

392 primers D1R (forward, 5′ -ACCCGCTGAATTTAAGCATA-3′) (Scholin et al., 1994), 28-

393 1483R (reverse, 5′ -GCTACTACCACCAAGATCTGC-3′) (Daugbjerg et al., 2000), 1740

394 bp of the SSU rRNA gene, using the primers SR1(forward, 5′ -

395 TACCTGGTTGATCCTGCCAG-3′) and SR12b (reverse, 5′ -

396 CGGAAACCTTGTTACGACTTCTCC-3′) (Takano & Horiguchi, 2006), and 600 bp of the

397 total ITS1–5.8S–ITS2, using the primers ITSA (forward, 5′ -CCTCGTAAC

398 AAGGHTCCGTAGGT-3′), ITSB (reverse, 5′ -CAGATGCTTAARTTCAGCRGG)

399 (Adachi et al., 1996). A 50 µL PCR cocktail containing 0.2 µM forward and reverse primer,

400 PCR buffer, 50 µM dNTP, 1U of Taq DNA polymerase (Takara, Dalian, China) was

401 subjected to 35 cycles using a Mastercycler PCR (Eppendorf, Hamburg, Germany). The PCR

402 reaction procedure was 4 min at 94 °C, followed by 25 cycles of 1 min at 94 °C, 2 min at 45

403 °C, 3 min at 72 °C, and final extension of 7 min at 72 °C. PCR products were sequenced

404 directly in both directions using the ABI Big-Dye dye-terminator technique (Applied

405 Biosystems, Foster City, CA, USA), according to the manufacturer's recommendations.

406   DNA extracts from strains collected in Spain (processed at IRTA) were prepared

407 according to the protocol described in Andree et al. (2011). The extracted DNA was used in

408 the amplification of ITS-1, 5.8S, ITS-2 sequences utilizing primers described in Andree et al.

409 (2011), and a partial LSU sequence was amplified utilizing the primers described ~~previously~~

410 in Hansen et al. (2000). The amplification reactions were carried out in 25µL volume

411 containing: 10 mM Tris-HC1 pH 8.3 (at 25 ºC), 50 mM KCl, 2 mM MgC1$_2$, 0.001 % w/v

412 gelatin, 400 pM dNTP´s, 1 µM of each primer, and 1 U Taq polymerase. Amplifications were

413 performed using the following parameters: 94 ºC for 5 min followed by 35 cycles of 95 ºC for

414 30 s, 50 ºC for 45 s, 72 ºC for 1 min, and a final extension of 72 ºC for 5 min. The PCR

415 products were purified using Qiagen spin columns (Qiagen PCR Purification Kit) and sent for

416 bi-directional sequencing by a commercial company (Sistemas Genomicos, Valencia, Spain)

417 utilizing the same primers as those used in the original amplification. The resulting nucleic

418 acid sequence data was manually proofed using BioEdit (Hall et al., 1999) to confirm the

419 consensus sequence.

420   The strain 091223-38_M16 from Helgoland (North Sea) was sequenced by M.H.. The

421 Epicentre MasterPure complete DNA & RNA Purification Kit was used for the DNA

422 extraction. ~~We use~~ puReTaq ready-to-go PCR beats ~~are~~were used; annealing temperature was

423 50°C; 33 cycles; primers: ITS1 (forward) 5' GGTGAACCTGAGGAAGGAT 3'; ITS4

424 (reverse) 5' TCCTCCGCTTATTGATATGC 3'. The PCR product of the ~~right~~ correct size

425 was gel isolated (QIAquick Gel Extraction Kit). Sequencing was done by Macrogen with the

426 ITS1 primer.

427   Strains and single cells from Japan, sequenced by Yoshihito Takano and Kazuhiko

428 Koike, were sequenced using methods mentioned in Mertens et al. (2012a).

429   DNA was extracted from cultures (strain references K1474, 1476, 1477, 1478, 1479

430 and 0976) acquired from the NCMA (National Centre for Marine Algae) using the DNeasy

431 DNA extraction kit (Qiagen) according to manufacturers' instructions. The 760 bp region of

432 the LSU rRNA gene was amplified using 2 μL DNA in PCR reactions spanning the D1-D2

433 variable region D1R (forward, 5'-ACCCGCTGAATTTAAGCATA-3'), D2C (reverse, 5'-

434 GCTTGGTCCGTGTTTCAAGA-3') (Scholin et al., 1994) a 168 bp region of the SSU rRNA

435 gene (V9) V9 For (5'-GTACACACCGCCCGTC-3') V9 Rev (5'-

436 TGATCCTTCTGCAGGTTCACCTAC-3') (Lane et al., 1991; Medlin et al., 1988) and a 710

437 bp intergenic region ITS1, 5.8S, ITS2, EITS2 For (5'-GTAGGTGAACCTGCVGAAGA-3')

438 EITS2 Rev (5'-TGGGGATCCTGTTTAGTTTC-3') (Guillou et al. 2002). PCR for V9 is

439 detailed in section 2.5. For LSU and ITS, PCR reactions were carried out in 50 μL volumes

440 containing 2 μL DNA, 20 pmol each primer, 1 x buffer, 1.5 mM $MgCl_2$, 0.0025 mM dNTPs,

441 1 Unit Gotaq polymerase (Promega). PCR reactions proceeded with an initial denaturation at

442 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing

443 at 60°C for 45 seconds and extension at 72°C for 1 min and a final extension step of 72°C for

444 5 minutes. PCR products were sequenced directly in both directions using the respective

445 primers (Source Bioscience) and sequences were manually verified using Chromas

446 (Technelysium Pty Ltd) prior to phylogenetic analysis.

447   Novel sequences were deposited in Genbank under accession numbers

448 XXXXXMG646283-MG646333.

449

450 *2.7. Sequence alignments and phylogenetic analyses*

451

452 Multiple sequence alignments were constructed for sequences generated for the variable

453 regions V9 (SSU), D1-D2 (LSU) and partial ITS1, 5.8S, ITS2 (intergenic region),

454 respectively, in BioEdit 7.0 (Hall 1999) using ClustalW along with other available sequences

455 from Genbank. Alignments were trimmed accordingly based on the lengths of the sequences

456 acquired and to allow for a sufficient number of sequences to be included in the phylogeny.

457 Phylogenetic analysis based on neighbour-joining and maximum likelihood was undertaken

458 using MEGA 6 (Tamura et al., 2013) using the default parameters. Bootstrap values were

459 retrieved from 1000 replicates and are indicated on the nodes of the trees.

460

461 **3. Results**

462

463 *3.1. Study of plankton samples, culture strains, germination experiments, and surface*

464 *sediments*

465

466 Investigation of plankton samples from the Salton Sea revealed the presence of a species that

467 is superficially similar to *P. reticulatum* and is here assigned to *Pentaplacodinium saltonense*

468 gen. et sp. nov.~~n. sp.~~ Three process-bearing cysts (Plate 1) were isolated from surface

469 sediments of the Salton Sea~~, California, USA~~ (Table 1) and identical morphologies emerged

470 from these cysts (Plate 2). These cells started dividing after germination, and one strain was

471 maintained. The cells were identical in morphology to specimens observed in plankton

472 samples from the Salton Sea (Plate 3), as well as to specimens from several culture strains

473 (Plate 4, Suppl. Table 1), as described below.

474

475 *3.2. Systematics*

476

Division DINOFLAGELLATA (Bütschli 1885) Fensome et al. 1993<u>b</u>

Class DINOPHYCEAE Pascher 1914

Subclass PERIDINIPHYCIDAE Fensome et al. 1993<u>b</u>

Order GONYAULACALES Taylor 1980

Suborder Gonyaulacineae autonym

Family uncertain

Genus *Pentaplacodinium* Mertens, Carbonell-Moore, Pospelova et Head gen. n. (Plate 3)

Type: <u>Plate 3A, the holotype of </u>*Pentaplacodinium saltonense* gen. et sp. nov.

Diagnosis: A ~~gonyaulacoid~~ <u>gonyaulacinean</u> genus with roundish to ~~slightly~~ polyhedral theca~~e~~ ~~with~~ <u>bearing</u> heavily reticulated plates without ~~appendices~~<u>appendages</u>. The tabulation is Po, Pt, <u>4′ or </u>2′+*2′, 5″, ~~6C~~<u>6c</u>, ~~6S~~<u>6s</u>, ~~*6~~<u>5</u>‴, 1p, 1⁗, cover plate is oval.

Etymology: The name is derived from the Greek words *penta* meaning five, *plax* plate, and *dino* whirling; with reference to the five precingular plates that characterize this dinoflagellate genus.

Pentaplacodinium saltonense Mertens, Carbonell-Moore, Pospelova et Head gen. et sp. n. (Plates 3, 4, Figs 2A, 3, 4A)

Synonymy:

1970 *Protoceratium reticulatum* (Claparède et Lachmann); Steidinger and Williams, p. 62, plate 38, fig. 140a–c.

1991 *Protoceratium reticulatum* (Claparède et Lachmann); Al-Muftah, pp. 180–181, figs. 246–247.

? 2002 *Protoceratium reticulatum* (Claparède et Lachmann); Reifel et al., p. 275.

2005 *Gonyaulax grindleyi* Reinecke; Faust et al., p. 110, figs. 2–4.

? 2007 *Gonyaulax grindleyi* Reinecke; Tiffany et al., p. 582.

? 2009 "~~"~~*Protoceratium globosum*~~"~~" Kofoid ~~et~~and Michener; Morquecho et al., p. 18, 20, figs. 13–17.

Diagnosis: Theca roundish to somewhat polyhedral with tabulation Po, Pt, 2′+*2′, ~~35~~5″+*2″, ~~6C~~6c, ~~6S~~6s, *65‴, 1p, 1⁗, with 3″ interpreted as *(3″+4″). The theca has an L-type ventral organization and dextral torsion. The plates are heavily reticulated with one pore inside each reticulation, although two or more pores might be found in reticulations next to a suture. The ends of the descending cingulum are displaced by ~2.0 widths. The cysts have a~~n~~ approximately ~~roundish~~ spherical central body with a thin pedium and thicker spongy-fibrous luxuria. ~~Processes~~ Process distribution apparently ~~have a no~~intran~~tabular~~tabular ~~distribution, ~~. ~~are~~ Processes fibrous and distally tapering, and have acuminate to minutely expanded distal ends. The archeopyle corresponds to the ~~third~~ *(3″+4″) precingular plate and has a smooth margin with rounded angles. The operculum is free.

Etymology: The specific epithet refers to the type locality for this species.

Type locality: The Salton Sea, California, U.S.A. (station 1 at 33°30.192′ N, 115°54.869′ W).

Gene sequence: The 28S and 18S gene sequence of the cell isolated from culture 2E3, established from a cyst extracted from surface sediment from station 2 in the Salton Sea (Table 1). —GenBank Accession No. ~~XXXXXX~~ MG646301 (18S) and ~~XXXXXX~~ MG646323 (28S). Several other strains are considered to belong to the same species (Suppl. Table 1).

Holotype: ~~Illustrated on~~ Plate 3A. The specimen illustrated is on an SEM stub (designated CEDiT2017H62) ~~will be deposited~~ curated at the Senckenberg Research Institute and Natural History Museum, Centre of Excellence for Dinophyte Taxonomy, Germany.

Description: Motile cells observed in the Salton Sea plankton samples (Plate 3, except D). ~~The cell shape varies from~~Thecae have a roundish to somewhat polyhedral shape (Plate 3A, C)~~. The thecae have~~ and a typical sexiform gonyaulacoid tabulation (sensu Fensome et al.,

526 1993b, tText-Fig. 64B) with an L-type ventral organization (sensu Fensome et al., 1993b,

527 Text-Figs. 82A, C) and dextral torsion (sensu Fensome et al., 1993b, Text-Fig. 83C). The

528 epitheca was is often somewhat shorter in length than the hypotheca. The plates are

529 reticulated with one pore inside each reticulation, although two or more pores could may

530 occurbe found in reticulations next to a suture. All pores each contain ~3 minute pores (small

531 arrowhead in Plate 3B). The reticulations are faintly expressed on the sulcus and cingulum

532 (Plate 3C). The cell content is brownish-red owing to the presence of chloroplasts (Plate 2A).

533 Several red bodies are present (Plate 2A–C).

534   The apical pore complex consists of a cover plate surrounded by a pore plate (Plate 7F,

535 H). The oval cover plate, which is often absent (Plate 3B), is relatively broad and is

536 surrounded by the pore plate. The pore plate is perforated by 5–7 large pores. A low apical

537 collar may encompass the pore plate and is formed by the raised edges of the first and second

538 apical plates, and the fourth apical homolog as well (Plate 3F, H). The first and second apical

539 plates (1′ and 2′) and the fourth apical homolog (*4′) are elongated. The first apical plate (1′)

540 is rectangular, while whereas the second apical plate (2′) and the fourth apical homolog (*4′)

541 are six-sided and irregularly shaped (Plate 3B). The third apical homolog (*3′) is small and

542 contacts 2′ and *4′, but in the specimens that were observed it never contacted the apical pore

543 plates (Plate 3B). There is a large ventral pore located posteriorly between 1′ and *4′ (Plates

544 3A, B, 4A–C). The precingular series consists of five large plates, where 2″ is the largest,

545 *(3″+4″) forms the keystone plate, and *6″ is the smallest. Plates 1″, *(3″+4″), and *5″ are

546 five-sided, 2″ is four-sided, while *6″ is six-sided (the suture with the anterior right sulcal is

547 very small (Fig. 3) (Plates 3B, 4A–C). External views of the theca can could suggest that

548 there would be no contact between the anterior sulcal plate and 1′ (e.g., Plate 3A, B).

549 However, pProperly oriented external views and internal views, however, show however a

550 narrow contact between both plates (Plate 3D). This contact between the anterior sulcal plate

551   and 1′, in combination with the contact between *6″ and 1′ therefore results in an insert

552   configuration (sensu Fensome et al. 1993b, Text-Fig. 62A). The cingulum is left-handed

553   (descending), lined with narrow lists, and comprises six cingular plates. The ends of the

554   cingulum do not overhang, and are displaced by ~2.0 widths (Plates 3A, 4B).

555   The sulcus is narrow anteriorly and slightly widens posteriorly. It consists of six plates

556   (Plate 3D, Fig. 3) — the first postcingular plate 1‴ is treated as a sulcal and labeled the

557   anterior left sulcal plate (Ssa). The anterior sulcal plate (Sa) is relatively large and anteriorly

558   intruded between plates 1″ and *6″ and barely contacts 1′ (Plate 3D). The anterior left sulcal

559   plate (Ssa) is similar in size to the anterior right sulcal plate (Sda). Immediately below these

560   two plates, lay the small posterior right sulcal (Sdp) and a much larger plate, the left posterior

561   sulcal. Finally, there occurs the large posterior sulcal (Sp) is found, which presents lines of

562   pores around its sutures with the adjacent non-sulcal plates (Plate 3D, Fig. 3).

563   The hypotheca is asymmetrical as a consequence of dextral torsion (Plates 3A). There

564   are five homolog postcingular plates. Plate *2‴ is irregularly shaped and the smallest in the

565   series. All other postcingular plates are large, though *6‴ is relatively smaller; in addition,

566   they are trapezoidal and four-sided (Plates 3E, 4E). The posterior intercalary plate (1p) bears a

567   conspicuous flange on its right margin (Plates 3A, 4C). The plate overlap is typical for

568   gonyaulacoids, with 3″ (in our case *(3″+4″)) forming the keystone plate (the plate that

569   overlaps all adjacent plates) oin the epitheca, and *4‴ forming the keystone plate oin the

570   hypotheca (Fig. 4, Plate 3E).

571

572   *Cysts from the Salton Sea surface sediments* (Plates 1, 5). The central body is approximately

573   sphericalroundish. The wall is thick, consisting of a thin, solid pedium that has a smooth inner

574   surface, and a thicker spongy-fibrous luxuria that appears loosely granular in surface view.

575   Processes are numerous and are solid and fibrous along their entire length, often loosely

576   fibrous at the base. Process bases are expanded, and larger processes may be concave in

577   lateral profile for at least half of their length. Some closely adjacent processes are joined at the

578   base. Most processes usually have a minute distal expansion, observed under SEM as a

579   concave platform ~1.0 µm or less in diameter with strongly irregular margins that may be

580   approximately perpendicular to the shaft. Alongside these, some processes on most specimens

581   taper to distal points, and such processes occasionally predominate on ~~cyst~~

582   ~~specimens~~individual specimens. Processes are mostly of even height, but shorter and thinner

583   processes may be interspersed. The process length/central body ratio is about 0.06. Processes

584   are not evenly spaced, and their parallel alignment and bands devoid of processes observed in

585   many specimens suggest intratabular distribution. ~~However, t~~There is however no clear

586   evidence of tabulation except for the archeopyle and often parallel alignment along the

587   ~~cingulum~~cingular margins~~. The archeopyle is formed from the loss of plate *(3″+4″), is~~

588   ~~reduced, and has slightly rounded angles and straight margins~~. The ~~usual~~ archeopyle ~~that~~ is

589   moderately wide and reflects the precingular thecal plate *(3″+4″), whereas the operculum is

590   released as a single piece, and has well defined to moderately rounded angles and straight

591   margins, as illustrated on Plate 1G-H. A reentrant angle along the anterior margin of the

592   archeopyle, signaling the fusion of plates 3″ and 4″, was not seen in the thecal or cyst

593   tabulation of *P. saltonense* although this might not ~~in fact~~ be expected (see Below, 1987, p.

594   36, fig. 18a; translated in Fensome et al., 1993a, p. 844). An unusually wide archeopyle that

595   seems to reflect two adjacent precingular thecal plates, *(3″+4″) and 2″, where the operculum

596   is again released as a single piece, is illustrated on Plate 1C-E. If this interpretation is correct,

597   then the component representing 2″ in the archeopyle/operculum is reduced in size, because

598   on the theca the second precingular plate is actually similar or larger in size than the *(3″+4″)

599   plate.

600

Dimensions: The ~~holotype~~ cell illustrated in Plate 3A, the holotype figure, is 44 µm in length, 41µm in width and 38 µm in depth. Germinated motile cells: length, 48.1 (53.7) 63.4 µm (SD=6.0, n=5); width, 38.5 (42.4) 47.5 µm (SD= 3.2, n=5). Cells observed in plankton from St. 2 in the Salton Sea: length, 37.8 (46.1) 59.8 µm (SD = 5.5, n=28); width, 31.0 (39.5) 48.5 µm (SD=4.2, n=28).

Two single cysts germinated to give the identifiable thecae: maximum central body diameter, 52.3 (53.5) 54.7 µm (SD=1.7, n=2); minimum central body diameter, 51.1 (52.2) 53.3 µm (SD=1.6, n=2); average length of three randomly chosen processes per cyst, 2.4 (3.0) 3.6 µm (SD=0.4, n=6); process width at base 1.4 (2.2) 2.7 (SD=0.6, n=6) and wall thickness 1.3 (1.7) 2.1 (SD=0.3, n=6). Palynologically treated cysts from surface sediments of the Salton Sea: maximum central body diameter, 48.6 (56.3) 70.9 µm (SD=5.3, n=23); minimum central body diameter, 45.7 (52.1) 61.4 µm (SD=3.8, n=22); average length of three processes per cyst, 1.0 (3.1) 5.7 µm (SD=1.2, n=66); process width at base 1.0 (2.2) 3.9 (SD=0.6, n=66) and wall thickness 0.9 (1.6) 2.4 (SD=0.4, n=66).

Comments: Pentaplacodinium saltonense n. gen et sp. is defined primarily from the characters of the motile stage, these distinguishing it from species of the genus *Protoceratium.* The morphology of several thecae observed from off Yucatan (Gulf of Mexico), the Indian River Lagoon (Florida, USA), and off Qatar (Persian Gulf) (Table 1; Plate 2) and from cultures established from cells from Biscayne Bay (Florida, USA) (CCMP1720, CCPM1721), the Indian River Lagoon (Florida, USA) (CCMP3241, CCMP3243) and the Salton Sea (California, USA) (CCMP404) (Suppl. Table 1~~3~~, Plate 1) agree with the description of *P. saltonense* given above. Cysts formed from cultures established from a strain from the Indian River Lagoon (Florida, USA) (CCMP3243) have the same morphologies (Plate 6). The observed cysts correspond most closely to the fossil based ~~taxon~~ species *Operculodinium israelianum* (Rossignol 1962) Wall 1967 described from the Pleistocene of Israel, and

Operculodinium psilatum Wall 1967 described from the postglacial (Holocene) of the Caribbean, p. 111–112, Plate 6, figs. 6–8. However, *Operculodinium israelianum* has longer processes (6–10 µm; Rossignol, 1964), and *O. psilatum* has a psilate surface interrupted by minute and sparsely distributed processes, and a pronounced cingulum (Wall, 1967). Both have archeopyles that are less wide than for the cyst of *P. saltonense*.

3.3. Phylogenetic position of P. saltonense *and other studied strains*

The SSU rDNA sequences for all *P. reticulatum* strains analysed were identical, forming a distinct clade separated from the *P. saltonense* sequences which were identical to the unidentified Malaysian sequences (Fig. 5). *P. reticulatum* and *P. saltonense* sequences shared 92% nucleotide identity for the V9 region analysed.

For the LSU rDNA V4 analysis (Figure 6), *P. reticulatum* sequences were identical apart from a couple of sporadic nucleotide substitutions which were called identified as ambiguous bases by the sequencing software. The unidentified strain from Hawaii had 12 nucleotide substitutions across the 570 bp multiple sequence alignment compared to *P. reticulatum*. *P. saltonense* sequences shared more similarity with the unidentified GgSm strains from Malaysia (96%) compared to that of *P. reticulatum* (94%).

The ITS (intergenic region between ITS1 and 2) was the only marker to resolve intraspecific diversity within the *P. reticulatum* species, with strain E12 (Baffin Bay, Arctic) sharing 98% nucleotide similarity with strain VG0757 isolated from Spain. The phylogeny separates *P. reticulatum* into two large subclades: subclade 1A that regroups several strains from warmer waters, and subclade 1B that regroups several strains from colder waters (Fig 7).

The three phylogenies (Figures. 5—7) show that strains identified as *Protoceratium reticulatum* form a monophyletic group (Clade 1), as well as strains identified as *P.*

651   *saltonense* that form a clade with the unidentified GgSm strains from Malaysia (Clade 2)*,* as

652   well as the *Ceratocorys* species that form a clade with PrTT strains from Malaysia (Clade 3)

653   supported by high bootstrap values (>70). The unidentified strain from Hawaii does not group

654   with the *Protoceratium reticulatum* or *Pentaplacodinium saltonense* clades. The topology of

655   the trees ~~are~~ is not consistent between the three phylogenies (i.e. the relatedness between

656   clades), ~~however~~ but the three clades identified are consistently formed. The trees furthermore

657   highlight the unexplored diversity within this group of dinoflagellates, and further incubation

658   and plankton studies from these locations should reveal whether the unidentified strains are

659   new species or not.

660   In addition, the phylogenies show that V9 SSU sequences from cells from Elands Bay

661   (South Africa)~~,~~ (bootstrap values >80%)~~,~~ that have been previously identified as *G. grindleyi*

662   by Reinecke (1967), clusters with *Protoceratium reticulatum* (Fig~~.~~ure 5).

663   The three studied species of *Ceratocorys* (*C. armata, C. gourreti, C. horrida*) share

664   high nucleotide similarity for the SSU (100%) and LSU sequences (>99% identity) (Figure 6).

665

666   **4. Discussion**

667

668   *4.1. Comparison of the ~~cell~~ theca of* P. saltonense

669

670   *Pentaplacodinium saltonense* differs from *Protoceratium reticulatum* because it bears five

671   precingular plates, whereas *P. reticulatum* has six ~~precingular plates~~. Furthermore, *P.*

672   *saltonense* has a larger ~~cingulum~~ cingular displacement (2 widths vs. 1 width respectively)

673   and an oval cover plate, as opposed to a sigmoidal cover plate in *P. reticulatum*. In addition,

674   the theca of *P. saltonense* is mostly roundish, whereas in *P. reticulatum* it is always

675   polyhedral. Both species have an insert configuration, but in *P. saltonense* the contact

between Sa and 1′ is very narrow ~~whilst~~ whereas in *P. reticulatum* this contact is wide ~~—~~ this

causes a~~n evident~~ conspicuous separation between 1″ and 6″ in *P. reticulatum*, when in *P.*

saltonense there is ~~an apparent~~almost a small point of contact between those two plates (Plate

~~1D~~3D). *Gonyaulax grindleyi* Reinecke 1967 is ~~here considered~~ shown to be a synonym of *P.*

reticulatum, as ~~it has been~~ already suggested by von Stosch (1969) and Hansen et al. (1997),

and ~~is~~ now confirmed by the LSU rDNA phylogeny in this study (see below).

Several other *Protoceratium* species have been described (e.g. Schiller, 1937, p. 322–

326). *Protoceratium splendens* Meunier 1910 from the Kara Sea has six precingular plates; it

is possibly a junior synonym of *Protoceratium reticulatum*, as suggested by Gómez (2012).

Protoceratium aculeatum (Stein 1883) Schiller 1937 bears antapical spines and an apical

horn. *Protoceratium areolatum* Kofoid 1907 and *Protoceratium spinulosum* (Murray and

Whitting 1899) Schiller 1937 have fewer reticulations in both the epitheca and hypotheca than

P. saltonense. Of the five species described by Kofoid and Michener (1911~~:~~), *Protoceratium*

cancellorum, Protoceratium pellucidissimum, Protoceratium pepo, Protoceratium globosum

and *Protoceratium promissum*, none ~~have~~ has illustrations and it is therefore ~~it is~~ impossible

to compare them to *P. saltonense*.

Pentaplacodinium saltonense differs from *Ceratocorys anacantha* Carbonell-Moore

1996 because it is not as polyhedral. In addition, in contrast to the insert epithecal

configuration of *P. saltonense*, *C. anacantha* has an episert type I epithecal configuration,

meaning that 1′ does not contact the anterior sulcal plate and that 1″ and 6″ are in contact

(Paez-Reyes and Head, 2013).

4.2. Comparison of the cyst of P. saltonense

The cyst of *Pentaplacodinium saltonense* ~~corresponds to~~compares with *Operculodinium*

psilatum because its cysts display an alignment of processes along the cingulum, it bears short

processes (1.0–5.7 µm ~~vs. 2 µm (Wall, 1967) or 0.0–2.9 µm (Head, 1996)~~), its body diameter

is of similar size (45.7–70.9 µm vs. 50–60 µm ~~(,~~ Wall, 1967~~)~~; or 62–79 µm ~~(,~~ Head, 1996b~~)~~)

and its wall thickness (0.9–2.4 vs. 1.4–2.2 µm ~~(;~~ Head, 1996b~~)~~) is similar. *Operculodinium*

psilatum differs, however, in having processes that in general are shorter (2 µm, Wall, 1967;

0.0–2.9 µm, Head, 1996b) and sparsely distributed. The cingulum and sulcus are also more

conspicuously expressed in Operculodinium psilatum (Wall, 1967; Head et al., 1996b), and O.

psilatum lacks the wide archeopyle of P. saltonense.

The cyst of *P. saltonense* is also similar to *Operculodinium israelianum* (Rossignol

1962) Wall 1967 as described by Rossignol (1964, as *Baltisphaeridium israelianum*)~~;~~

~~however,,~~ although the processes of the latter species are longer (6–10 µm) than ~~of the~~

~~specimens from Salton Sea~~for *P. saltonense* (1.0–5.7 µm). ~~At this time, we do not~~It is not

presently ~~kno~~wn whether variation in process length is related to variations in ecology, ~~similar~~

~~to~~as demonstrated for the cysts of *Lingulodinium polyedra* (=*Lingulodinium*

machaerophorum) (Mertens et al., 2009), cysts of *Protoceratium reticulatum* (Mertens et al.,

2011) and cysts of *Pyrodinium bahamense* (=*Polysphaeridium zoharyi*) (Mertens et al., 2015).

~~In addition, t~~The process distribution appears to be ~~is~~ intratabular for the cysts of *P.*

saltonense~~and often shows parallel alignments~~, ~~which is not readily visible~~and this is likely to

be the case also for~~on~~ *O. israelianum* (e.g., *O.* cf. *israelianum* of Head, 1997, fig. 17.2),

although the study of topotype material will be needed for confirmation. The rounded angles

of the archeopyle in *O. israelianum*, *O. psilatum* and the cysts of *Pentaplacodinium*

saltonense, and the shared presence of a spongy-fibrous to fibroreticulate luxuria, accentuate

the overall similarities between these cysts, although the relatively wider archeopyle in *P.*

saltonense cysts distinguishes them from these other species.

~~We t~~Therefore, it is not consider~~ed it not~~ to be a morphological variant of *Operculodinium israelianum.* ~~There is reasonable doubt that Operculodinium israelianum is related to another species: Wall and Dale (1968) (and subsequently Dale, 1983) have suggested that O. israelianum can be related to Protoceratium reticulatum.~~ *Operculodinium israelianum* ~~is very similar to~~resembles the Miocene *Operculodinium centrocarpum* (Deflandre et Cookson 1955) Wall 1967, which ~~is~~ also has a spongy-fibrous luxuria, although it is somewhat larger (54–80 μm)~~ (;~~ Deflandre and Cookson, 1955) and has longer processes. Head (1996b) noted an intergradation in size and process length between *O. israelianum* and *O. centrocarpum* in Pleistocene assemblages of eastern England. ~~It is of interest to remark that specimens from the Ludham borehole intergrade between O. israelianum and O. centrocarpum Deflandre et Cookson 1955 (Head, 1996).~~ A restudy of ~~both holotypes~~topotype material ~~ as well as the~~is needed to confirm the range of variability within each species~~ would be needed to resolve this issue~~.

The cyst~~s~~ of *P. saltonense* differs from ~~the cyst~~those of *P. reticulatum*~~, due to the~~ in having a thick spongy-fibrous luxuria ~~wall ~~(vs. thin, fibrous luxuria), less developed distal ends of the processes, larger central ~~its ~~body ~~size ~~diameter (48.6–70.9 μm vs. 33–48 μm; Rochon et al., 1999), and generally shorter process length (1.0–5.7 μm vs. typically 7–14 μm; Rochon et al., 1999)~~(48.6 (56.3) 70.9 μm (SD=5.3, n=23)) for P. saltonense, as opposed to 33–48 μm for P. reticulatum (Rochon et al., 1999) and its process length (1.0 (3.1) 5.7 μm (SD=1.2, n=66)) for P. saltonense, as opposed to typical 7 14 μm for P. reticulatum (Rochon et al., 1999)~~; although the cyst~~s~~ of *P. reticulatum* ~~shows very wide variation~~vary widely ~~sizes~~, with ~~formation of~~some being completely bald ~~cysts ~~(e.g., Mertens et al., 2012a; Jansson et al., 2014).

Several Numbered ~~Several~~ Numerous other *Operculodinium* species have been described and a detailed

comparison is given by ~~we refer to~~ Marret and Kim (2009) ~~for their detailed comparison, as~~;

none of these closely resemble~~s~~ the cysts of *P. saltonense*.

4.3. Phylogenetics, evolution and position and relationships of Protoceratium*,*

Pentaplacodinium*, and* Ceratocorys

~~There are s~~Several morphological characteristics of the theca ~~that~~ are important ~~to~~ in

understand~~ing~~ the evolution of *Protoceratium, Pentaplacodinium, and Ceratocorys* ~~the three~~

~~genera in question~~ (Plate 7). The shape of the cover plate of *Ceratocorys* is more similar to

that of the cover plate of *Pentaplacodinium*, but less similar to the sigmoidal cover plate of

Protoceratium.

Pentaplacodinium and *Ceratocorys* can be considered closer to *Gonyaulax* than

Protoceratium, because the anterior intercalary is always well- separated from the apical pore

plates, ~~whilst~~ whereas in *Protoceratium reticulatum* it is closer and has even been suggested

to contact the apical pore plates (Hansen et al. 1997). ~~However,~~ It should ~~however~~ be noted,

however, that *Protoceratium* ~~reticulatum~~ and *Gonyaulax* have six precingular plates, whereas

~~whilst~~ *Ceratocorys* and *Pentaplacodinium* have five ~~precingulars~~. So it is not surprising that

in the molecular phylogenies, *Pentaplacodinium* has an intermediate position between

Ceratocorys and *Protoceratium* (Figs. 5–7); the relation to other gonyaulacoids at this time is

unclear and further molecular studies of related genera are required, particularly to understand

how to resolve the position of *Protoceratium* at family level. Another issue regards a conflict

in the dual nomenclature: the cyst of *P. reticulatum* and *P. saltonense* both are considered to

belong to the cyst-defined genus *Operculodinium,* whereas ~~whilst~~ the thecate stages belong to

772 two different genera; further cyst—theca experiments within this group of related species

773 should help to understand how the ~~genus~~ generic concepts can be ~~made conform~~rationalized.

774   In addition, the ITS marker was able to separate two large subclades within *P.*

775 *reticulatum*: strains that are predominantly associated with warmer waters (Sub-clade 1A),

776 and other strains largely associated with colder waters (Sub-clade 1B) (Fig. 7). Do these

777 subclades reflect pseudocryptic speciation in *Protoceratium reticulatum* as previously

778 suggested by Mertens et al. (2012a)?

779   Other morphological characteristics of the theca are conserved in *Protoceratium*,

780 *Pentaplacodinium*, and *Ceratocorys* and other gonyaulacoids. For instance, there is no

781 difference in overlap pattern between *Protoceratium, Pentaplacodinium, Ceratocorys,*

782 *Gonyaulax* and *Lingulodinium* (Fig. 4).

783

784 *4.4. Biogeography and ecology of* P. saltonense

785

786 According to ~~the~~ plankton observations, *P. saltonense* can be found in tropical to subtropical

787 regions. ~~We have not observed~~ *P. saltonense* and *P. reticulatum* have not been observed in the

788 same samples, which suggests that ~~both~~ these species ~~possibly~~ inhabit different niches, where

789 *P. saltonense* has a preference for higher temperatures and salinities, and *P. reticulatum* ~~has a~~

790 ~~preference~~ for somewhat lower temperatures and salinities. This difference would need to be

791 ~~established~~ quantified through culture experiments.

792

793 *4.5. Toxicity*

794

795 Strains identified as *Pentaplacodinium saltonense* (CCMP404, CCMP1720 and CCMP1721),

796 have been identified as yessotoxin producers using fluorescence HPLC (Paz et al., 2004). A

later toxin analysis by LC-MS of the same strains was negative (Paz et al., 2007), and the

authors considered that these strains had lost their toxicity after a number of years in culture.

The ~~produced~~ toxins produced by these strains of *Pentaplacodinium saltonense* are similar to

toxins produced by strains ~~we~~ identified as *Protoceratium reticulatum*, all of which are

yessotoxin producers, such as strains from Chile (Alvarez et al., 2011), Jervis Inlet, British

Columbia, Canada (Cassis, 2005), German Bight, ~~the~~ North Sea (Röder et al., 2011, 2012),

Okkirai Bay, Japan (Koike et al., 2006) and Spain (Paz et al., 2007, 2013). ~~The presence of~~ *P.*

saltonense ~~in the Salton Sea~~ has been considered a potential causative agent of mortality

events in the Salton Sea~~indicates that it is potentially toxic~~ (Reifel et al., 2002, who~~m~~

identified it as *Protoceratium reticulatum*). ~~However, t~~THowever, there have not ~~however not~~

been ~~no~~ reports of toxic events knowingly involving *P. saltonense*.

Several other studies have investigated the toxicity of strains ~~that~~ they designate as

Protoceratium reticulatum, but for which ~~we could not verify~~ the identifications could not be

verified (e.g., Satake et al., 1999; Ciminiello et al., 2003; Samdal et al., 2004; Finch et al.,

2005; Eiki et al., 2005; Mitrovic et al., 2005; Guerrini et al., 2007; Suzuki et al., 2007).

5. Conclusions

Pentaplacodinium saltonense gen. et sp. n~~ov~~. is described from the Salton Sea (CA, USA).

The distinct cover plate (similar to *Ceratocorys,* but sigmoidal in *Protoceratium*), five

precingular plates (~~also five~~as in *Ceratocorys*, but six in *Protoceratium*), the very narrow

contact between 1′ and Sa (wide contact in *Protoceratium*, no contact in *Ceratocorys*), a more

~~roundish~~ rounded ~~cell~~ thecal shape, the displacement of the cingulum by two widths (~~vs.~~ one

width in *Protoceratium*), as well as the clear separation and distances seen in the three

phylogenies, justifies the creation of a new genus. The chorate cysts of *P. saltonense* bear

short processes often with parallel alignments. These cysts~~,~~ correspond to the cyst-defined

~~species~~ genus *Operculodinium* Wall 1967, and are most similar to *O. israelianum* and *O.*

psilatum ~~with short processes and often parallel alignments~~. ~~The geographic distribution of~~

~~m~~Motile stages of *Pentaplacodinium saltonense* ~~was~~are confirmed ~~in~~from four widely

dispersed locations ~~and its distribution is therefore~~~~is considered~~, suggesting a subtropical to

tropical distribution for this species. ~~, and does not overlap with that of~~ *Protoceratium*

reticulatum and *Pentaplacodinium saltonense* are not known to inhabit the same

environments. ~~Similar to~~As with the ~~-~~yessotoxin-producing *Protoceratium reticulatum,*

Pentaplacodinium saltonense is potentially a yessotoxin producer, as shown by previous

studies.

Note added: While this paper has been going through the process of final acceptance to

Harmful Algae, another study was accepted (Salgado et al., accepted) that addresses similar

scientific questions.

Acknowledgements

References

Adachi, M., Sako, Y., Ishida, Y., 1996. Analysis of *Alexandrium* (Dinophyceae) species using

sequences of the 5.8S ribosomal DNA and internal transcribed spacer regions. J. Phycol. 32,

424–432.

Al-Muftah, A.R., 1991. Dinoflagellates of Qatari ~~Waters~~ waters (PhD thesis), vol. 2.

University College of North Wales, Bangor University, pp. 261.

Alvarez, G., Uribe, E., Díaz, R., Braun, M., Mariño, C., Blanco, J., 2011. Bloom of the

Yessotoxin producing dinoflagellate *Protoceratium reticulatum* (Dinophyceae) in ~~Northern~~

northern Chile. J. Sea Res. 64, 427–434.

Andree, K.B., Quijano-Scheggia, S., Fernández, M., Elandaloussi, L.M., Garcés, E., Camp,

J., Diogene, J. 2011. Quantitative PCR ~~Coupled~~ coupled with ~~Melt~~ melt ~~Curve~~ curve

~~Analysis~~ analysis for ~~Detection~~ detection of ~~Selected~~ selected *Pseudonitzschia* spp.

(Bacillariophyceae) from the ~~Northwestern~~ northwestern Mediterranean Sea. App. and Env.

Micro, 77(5) 1651–1659.

Balech, E., 1980. On thecal morphology of dinoflagellates with special ~~emphases~~ emphasis on

circular and sulcal plates. Anales del Centro de Ciencias del Mar y Limnología, UNAM 7(1),

57–68.

Balech, E., 1988. Los dinoflagellados del Atlantico sudoccidental. Publicaciones Especiales

Instituto Español de Oceanografia 1, 1–310.

Below, R., 1987. Evolution und Systematik von Dinoflagellaten-Zysten aus der Ordnung

Peridiniales. I. Allgemeine Grundlagen und Subfamilie Rhaetogonyaulacoideae (Familie

Peridiniaceae). Palaeontographica, Abteilung B 205, 1–164.

Bergh, R.S., 1881. Der Organismus der Cilioflagellaten. Eine phylogenetische Studie.

Morphologisches Jahrbuch 7(2), 177–288.

Bogus, K. Mertens, K.N., Lauwaert, J., Harding, I.C., Vrielinck, H., Zonneveld, K.A.F.,

Versteegh, G.J.M., 2014. Differences in the chemical composition of organic-walled

dinoflagellate resting cysts from phototrophic and heterotrophic dinoflagellates. J. Phycol. 50,

254–266.

Bolch, C.J.S., 1997. The use of polytungstate for the separation and concentration of living

dinoflagellate cysts from marine sediments. Phycologia 37, 472–478.

Braarud, T., 1945. Morphological observations on marine dinoflagellate cultures (*Porella*

perforata, Goniaulax tamarensis, Protoceratium reticulatum). Avh. Utgit. Nor. Vidensk.

Akad. Oslo Mat.–Naturvidensk. Kl. 11, 1–18.

897

Bütschli, O. 1885. Erster Band. Protozoa. 3. Unterabtheilung (Ordnung) Dinoflagellata. In:

Dr. H.G. Bronn's Klassen und Ordnungen des Thier-Reichs, wissenschaftlich dargestellt in

Wort und Bild. C.F. Winter'sche Verlagshandlung, Leipzig und Heidelberg. p. 906–1029.

901

Cassis, D., 2005. The effect of harmful algae on the summer mortality of juvenile Pacific

~~Oysters~~ oysters (*Crassostrea gigas*). MSc. Thesis, University of British Columbia, 73 pp.

904

Ciminiello, P., Dell' Aversano, C., Fattorusso, E., Forino, M., Magno, S., Guerrini, F.,

Pistocchi, R., Boni, L. 2003. Complex yessotoxins profile in *Protoceratium reticulatum* from

north-western Adriatic sea revealed by LC-MS analysis. Toxicon 42, 7–14.

908

Claparède, E., Lachmann, J., 1858. Etudes sur les infusoires et les rhizopodes. Mem. Inst. Nat.

Genev. 6, 261–482.

911

Dale, B., 1983. Dinoflagellate resting cysts: "benthic plankton". In: Fryxell, G.A. (ed.),

Survival Strategies of the Algae, Cambridge University Press, Cambridge, U.K., pp.69–136.

914

Dale, B., 1996. Dinoflagellate cyst ecology: modelling and geological applications. In:

Jansonius, J., McGregor, D.C. (eds.), Palynology: Principles and Applications. Volume 3.

American Association of Stratigraphic Palynologists Foundation, Dallas, Texas, pp. 1249–

1275.

919

Daugbjerg, N., Hansen, G., Larsen, J., Moestrup, Ø., 2000. Phylogeny of some of the major

genera of dinoflagellates based on ultrastructure and partial LSU rDNA sequence data,

922 including the erection of three new genera of unarmoured dinoflagellates. Phycologia 39,

923 302–317.

924

925 Deflandre, G., Cookson, I.C., 1955. Fossil microplankton from Australian Late Mesozoic and

926 Tertiary sediments. Australian Journal of Marine and Freshwater Research 6, 242–313.

927

928 Dodge, J.D., 1989. Some revisions of the family Gonyaulaceae (Dinophyceae) based on a

929 scanning electron microscope study. Bot. Mar. 32, 275–298.

930

931 Eiki, K., Satake, M., Koike, K., Ogata, T., Mitsuya, T., Oshima, Y., 2005. Confirmation of

932 yessotoxin production by the dinoflagellate *Protoceratium reticulatum* in Mutsu Bay. Fish.

933 Sci. 71, 633–638.

934

935 Faust, M.A., Litaker, R.W., Vandersea, M.W., Kibler, S.R., Tester, P.A. 2005. Dinoflagellate

936 diversity and abundance in two Belizean coral-reef mangrove lagoons: a test of Margalef's

937 mandala. Atoll Research Bulletin 534, 105-132.

938

939 Fensome, R.A., Gocht, H., Stover, L.E., Williams, G.L., 1993a. The Eisenack Catalog of

940 Fossil Dinoflagellates. New Series. Volume 2. p.829–1461; E. Schweizerbart'sche

941 Verlagsbuchhandlung, Stuttgart, Germany.

942

943 Fensome, R., Taylor, F., Norris, G., Sarjeant, W., Wharton, D., Williams, G., 1993b. A

944 classification of fossil and living dinoflagellates. Micropaleontology Special Publication **7**, 1–

945 245.

946

947 Ferrari, R.L., Weghorst, P., 1997. Salton Sea 1995 hydrographic GPS survey. U.S. Bureau of

948 Reclamation, Water Resources Services. Denver, Colorado, 23 pp.

949

950 Finch, S.C., Wilkins, A.L., Hawkes, A.D., Jensen, D.J., MacKenzie, A.L., Beuzenberg, V.,

951 Quilliam, M.A., Olseng, C.D., Samdal, S.A., Aasen, J., Selwood, A.I., Cooney, J.M., Sandvik,

952 M., Miles, C.O., 2005. Isolation and identification of (44-R,S)-44,55-dihydroxyyessotoxin

953 from *Protoceratium reticulatum*, and its occurrence in extracts of shellfish from New

954 Zealand, Norway and Canada. Toxicon 46, 160–170.

955

956 Gómez, F., 2012. A checklist and classification of living dinoflagellates (Dinoflagellata,

957 Alveolata). CICIMAR Océanides 27(1), 65–140.

958

959 Guerrini, F., Ciminiello, P., Dell'Aversano, C., Tartaglione, L., Fattorusso, E., Boni, L.,

960 Pistocchi, R., 2007. Influence of temperature, salinity and nutrient limitation on yessotoxin

961 production and release by the dinoflagellate *Protoceratium reticulatum* in batch-cultures.

962 Harmful Algae 6, 707–717.

963

964 Guillou, L., Nezan, E., Cueff-Gauchard, V., Barbier, G., 2002. Genetic diversity and

965 molecular detection of three toxic dinoflagellate genera (*Alexandrium*, *Dinophysis* and

966 *Karenia*) from French coasts. Protist. 153, 223-238

967

968 Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis

969 program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41, 95–98.

970

Hansen, G., Moestrup, Ø. and Roberts, K.R., 1997. Light and ~~Electron~~ electron ~~Microscopical~~ microscopical ~~Observations~~ observations on *Protoceratium reticulatum* (Dinophyceae). Arch. Protistenk. 147, 381–391.

Hansen, G., Daugbjerg, N., Henriksen, P., 2000. Comparative study of *Gymnodinium mikimotoi* and *Gymnodinium aureolum*, comb. nov. (= *Gyrodinium aureolum*) based on morphology, pigment composition, and molecular data. J. Phycol., 36, 394– 410.

Head, M.J., Wrenn, J.H. (eds.), 1992. A forum on Neogene–Quaternary dinoflagellate cysts: The edited transcript of a round table discussion held at the Second Workshop on Neogene dinoflagellates. In: Head, M.J., Wrenn, J.H. (eds.), *Neogene and Quaternary Dinoflagellate Cysts and Acritarchs*. American Association of Stratigraphic Palynologists Foundation, Dallas, Texas, pp. 1–31.

Head, M.J., 1996a. Modern dinoflagellate cysts and their biological affinities. In: Jansonius, J., McGregor, D.C. (eds.), *Palynology: principles and applications*. American Association of Stratigraphic Palynologists Foundation, Dallas, Texas, vol. 3, pp. 1197–1248.

Head, M.J., 1996b. Late Cenozoic dinoflagellates from the Royal Society borehole at Ludham, Norfolk, eastern England. J. Paleontol. 70, 543–570.

Head, M.J., 1997. Thermophilic dinoflagellate assemblages from the mid Pliocene of eastern England. J. Paleontol. 71, 165–193.

Hennissen, J.A.I., Head, M.J., De Schepper, S., Groeneveld, J., 2017. Dinoflagellate cyst paleoecology during the Pliocene–Pleistocene climatic transition in the North Atlantic. Palaeogeogr., Palaeoclimatol., Palaeoecol. 470, 81–108.

Holdren, G.C., Montaño, A., 2002. Chemical and physical characteristics of the Salton Sea, California. Hydrobiol. 473, 1–21.

Howard, M.D.A., Smith, G.J., Kudela, R.M., 2009. Phylogenetic relationship of yessotoxin-producing dinoflagellates based on the Large Subunit and Internal Transcribed Spacer Ribosomal DNA Domainsdomains. Appl. Environ. Microbiol. 75, 54–63.

Jansson, I.-M., Mertens, K.N., Head, M.J. with contributions from de Vernal, A., Londeix, L., Marret, F., Matthiessen, J., Sangiorgi, F., 2014. Statistically assessing the correlation between salinity and morphology in cysts produced by the dinoflagellate *Protoceratium reticulatum* from surface sediments of the North Atlantic Ocean, Mediterranean–Marmara–Black Sea region, and Baltic–Kattegat–Skagerrak estuarine system. Palaeogeogr. Palaeoclimatol. Palaeoecol. 399, 202–213.

Kofoid, C.A., 1907. Reports on the scientific results of the expedition to the eastern tropical Pacific, in charge of Alexander Agassiz, by the U.S. Fish Commission steamer "„Albatross„", from October, 1904, to March, 1905, Lieut-. Commander L.M. Garrett, U.S.N., commanding. IX. New species of dinoflagellates. Bull. Mus. Comp. Zool. 50(6), 163–207.

Kofoid, C.A., Michener, J.R., 1911. Reports on the Scientific Results of the Expedition to the Eastern Tropical Pacific, in Charge of Alexander Agassiz, by the U.S. Fish Commission

Steamer "Albatross", from October 1904, to March, 1906, Lieut. L.M.

Garrett, U.S.N., Commanding. XXII. New genera and species of

dinoflagellates. Bull. Mus. Comp. Zool. 54(7), 267–302.

Koike, K., Horie, Y., Suzuki, T., Kobiyama, A., Kurihara, K., Takagi, K., Kaga, S.N.,

Oshima, Y., 2006. *Protoceratium reticulatum* in northern Japan: environmental factors

associated with seasonal occurrence and related contamination of yessotoxin in scallops. J.

Plankton Res. 28, 103–112.

Lane, D. J., 1991. 16S/23S sequencing. *In:* Nucleic Acid Technologies in Bacterial

Systematic. Stackebrandt E, Goodfellow M (Eds) *pp.* 115–175, *Wiley, NY.*

Marret, F., Kim, S.-Y., 2009. *Operculodinium aguinawense* sp. nov., a dinoflagellate cyst

from the late Pleistocene and recent sediments of the east Equatorial Atlantic ocean.

Palynology 33, 125–139.

Matsuoka, K., McMinn, A., Wrenn, J.H., 1997. Restudy of the Holotype of *Operculodinium*

centrocarpum (Deflandre & Cookson) Wall (Dinophyceae) from the Miocene of Australia,

and the taxonomy of related species. Palynology 21, 19–33.

Medlin, L. K, Elwood, H. J., Stickel, S., Sogin, M. L., 1988. The characterization of

enzymatically amplified eukaryotic 16S-like rRNA-coding regions. Gene 71, 491–499.

Mertens, K.N., Ribeiro, S., Bouimetarhan, I., Caner, H., Combourieu Nebout, N., Dale, B., de

Vernal, A., Ellegaard, M., Filipova, M., Godhe, A., Goubert, E., Grøsfjeld, K., Holzwarth, U.,

1045   Kotthoff, U., Leroy, S.A.G., Londeix, L., Marret, F., Matsuoka, K., Mudie, P.J., Naudts, L.,

1046   Peña-Manjarrez, J.L., Persson, A., Popescu, S.-M., Pospelova, V., Sangiorgi, F., van der

1047   Meer, M., Vink, A., Zonneveld, K.A.F., Vercauteren, D., Vlassenbroeck, J., Louwye, S.,

1048   2009. Process length variation in cysts of a dinoflagellate, *Lingulodinium machaerophorum*,

1049   in surface sediments: Investigating its potential as salinity proxy. Mar. Micropaleontol. 70,

1050   54–69.

1051

1052   Mertens, K.N., Dale, B., Ellegaard, M., Jansson, I.-M., Godhe, A., Kremp, A., Louwye, S.,

1053   2011. Process length variation in cysts of the dinoflagellate *Protoceratium reticulatum*, from

1054   surface sediments of the Baltic-Kattegat-Skagerrak estuarine system: a regional salinity

1055   proxy. Boreas 40, 242–255.

1056

1057   Mertens, K.N., Bringué, M., Van Nieuwenhove, N., Takano, Y., Pospelova, V., Rochon, A.,

1058   de Vernal, A., Radi, T., Dale, B., Patterson, R.T., Weckström, K., Andrén, E., Louwye, S.,

1059   Matsuoka, K., 2012a. Process length variation of the cyst of the dinoflagellate *Protoceratium*

1060   *reticulatum* in the North Pacific and Baltic-Skagerrak region: calibration as an annual density

1061   proxy and first evidence of pseudo-cryptic speciation. J. Quaternary Sci. 27, 734–744.

1062

1063   Mertens, K.N., Price, A., Pospelova, V., 2012b. Determining the absolute abundance of

1064   dinoflagellate cysts in recent marine sediments II: further tests of the *Lycopodium* marker-

1065   grain method. Rev. Palaeobot. Palynol. 184, 74–81.

1066

1067   Mertens, K.N., Wolny, J., Carbonell-Moore, C., Bogus, K., Ellegaard, M., Limoges, A., de

1068   Vernal, A., Gurdebeke, P., Omura, T., Mohd. A Al-Muftah, A., Matsuoka, K., 2015.

1069   Taxonomic re-examination of the toxic armoured dinoflagellate *Pyrodinium bahamense* Plate

1906: can morphology or LSU sequencing separate *P. bahamense* var. *compressum* from var. *bahamense*? Harmful Algae 41, 1–24.

Meunier A., 1910. Microplancton des mers de Barents & de Kara. In: Duc d'Orléans Campagne Arctique de 1907. Bulens, Bruxelles, 355 pp.

Mitrovic, S.M., Hamilton, B., McKenzie, L., Furey, A., James, K.J., 2005. Persistence of yessotoxin under light and dark conditions. Mar. Environ. Res. 60, 397–401.

Morquecho, L., Góngora-González, D.T. & Okolodkov, Y.B. 2009. Cyst-theca relationships of Gonyaulacales and Peridiniales (Dinophyceae) from Bahía Concepción, Gulf of California. Acta Botanica Mexicana 88, 9–29.

Murray, G., Whitting, F.G., 1899. New Peridiniaceae from the Atlantic. Trans. Linn. Soc. Lond., Bot. 5, 321–342.

Paez-Reyes, M., Head, M.J., 2013. The Cenozoic ~~Gonyaulacacean~~ gonyaulacacean ~~Dinoflagellate~~ dinoflagellate ~~Genera~~ genera *Operculodinium* Wall, 1967 and *Protoceratium* Bergh, 1881 and ~~Their~~ their ~~Phylogenetic~~ phylogenetic ~~Relationships~~relationships. J. Paleo. 87(5), 786–803.

Paz, B., Riobó, P., Fernández, M.L., Fraga, S., Franco, J.M., 2004. Production and release of yessotoxins by the dinoflagellates *Protoceratium reticulatum* and *Lingulodinium polyedrum* in culture. Toxicon 44, 251–258.

Paz, B., Riobó, P., Ramilo, I., Franco, J.M., 2007. Yessotoxins profile in strains of *Protoceratium reticulatum* from Spain and USA. Toxicon 50, 1–17.

Paz, B., Daranas, A.H., Norte, M., Riobó, P., Franco, J.M., Fernández, J.J., 2008. Yessotoxins, a Group group of Marine marine Polyether polyether Toxinstoxins: an Overviewoverview. Mar. Drugs 6, 73–102.

Paz, B., Blanco, J., Franco, J.M., 2013. Yessotoxins production during the culture of *Protoceratium reticulatum* strains isolated from Galician Rias Baixas (NW Spain). Harmful algae 21–22, 13–19.

Pospelova, V., Esenkulova S., Johannessen S.C., O'Brien O'Brien M. C. & , Macdonald R.W., 2010. Organic-walled dinoflagellate cyst production, composition and flux from 1996 to 1998 in the central Strait of Georgia (BC, Canada): a sediment trap study. *Mar. Micropaleontol.* 75:, 17–37.

Reifel, K.M., McCoy, M.P., Rocke, T.E., Tiffany, M.A., Hurlbert, S.H., Faulkner, D.J., 2002. Possible importance of algal toxins in the Salton Sea, California. Hydrobiologia 473, 275–292.

Reinecke, P., 1967. *Gonyaulax grindleyi* sp. nov.: a dinoflagellate causing a red tide at Elands Bay, Cape Province in December 1966. S. Afr. J. Bot. 33, 157–160.

Rochon, A., de Vernal, A., Turon, J.-L., Matthiessen, J., Head, M.J., 1999. Distribution of Recent recent dinoflagellate cysts in surface sediments from the North Atlantic Ocean and

1120   adjacent areas in relation to sea-surface parameters. American Association of Stratigraphic

1121   Palynologists, Contributions Series, no. 35, 146 p.

1122

1123   Röder, K., Fritz, N., Gerdts, G., Luckas, B., 2011. Accumulation and ~~Depuration~~ depuration

1124   of ~~Yessotoxin~~ yessotoxin in ~~Two~~ two ~~Bivalves~~bivalves. J. Shellfish Res. 30, 167–175.

1125

1126   Röder, K.,Hantzsche, F.M., Gebühr, C., Miene, C., Helbig, T., Krock, B., Hoppenrath, M.,

1127   Luckas, B., Gerdts, G., 2012. Effects of salinity, temperature and nutrients on growth, cellular

1128   characteristics and yessotoxin production of *Protoceratium reticulatum*. Harmful Algae 15,

1129   59–70.

1130

1131   Rossignol, M., 1964. Hystrichosphères du Quaternaire en Méditerranée orientale, dans les

1132   sédiments Pléistocènes et les boues marines actuelles. Revue de micropaléontologie 7, 83–99.

1133

1134   Sala-Pérez, M., Alpermann, T.J., Krock, B., Tillmann, U., 2016. Growth and bioactive

1135   secondary metabolites of arctic *Protoceratium reticulatum* (Dinophyceae). Harmful Algae 55,

1136   85–96.

1137

1138   Saldarriaga, J.F., Taylor, F.J.R., Cavalier-Smith, T., Menden-Deuer, S., Keeling, P.J., 2004.

1139   Molecular data and the evolutionary history of dinoflagellates. Eur. J. Protistol. 40, 85–111.

1140

1141   Salgado, P., Figueroa, R.I., Ramilo, I., Bravo, I., 2017. The life history of the toxic marine

1142   dinoflagellate *Protoceratium reticulatum* (Gonyaulacales) in culture. Harmful Algae 68, 67–

1143   81.

1144

Salgado, P., Fraga, S., Rodríguez, F., Riobó, P., accepted. *Ceratocorys mariaovidiorum* sp. nov. (Gonyaulacales), a new dinoflagellate species previously reported as *Protoceratium reticulatum*. Journal of Phycology, accepted.

Samdal, I.A., Naustvoll, L.J., Olseng, C.D., Briggs, L.R., Miles, C.O., 2004. Use of ELISA to identify *Protoceratium reticulatum* as a source of yessotoxin in Norway. Toxicon 44, 75–82.

Satake, M., Ichimura, T., Sekiguchi, K., Yoshimatsu, S., Oshima, Y. 1999. Confirmation of Yessotoxin and 45,46,47-Trinoyessotoxin production by *Protoceratium reticulatum* collected in Japan. Natural toxins 7, 147–150.

Schiller, J., 1937. Dinoflagellatae (Peridineae) in monographischer Behandlung. In: Dr. L. Rabenhorst's Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Bd. 10(3). Teil 2(3), pp. 321–480.

Scholin, C.A., Herzog, M., Sogin, M., Anderson, D.M. 1994. Identification of group- and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. J. Phycol. 30, 999–1011.

Steidinger K.A. & J. Williams (1970) Dinoflagellates. Memoirs of the Hourglass Cruises 2, 1–251. Published by Marine Research Laboratory, Florida Department of Natural Resources, St. Petersburg, Florida.

1168   Stein, F. von, 1883. Der Organismus der Infusionsthiere. 3. Abt. Der Organismus der

1169   Arthrodelen Flagellaten nach eigenen Forschungen in systematischer Reihenfolge bearbeitet.

1170   2. Hälfte. Einleitung und Erklärung der Abbildungen, W. Engelmann, Leipzig, pp. 1–30.

1171

1172   Stosch, H.A. von, 1969. Dinoflagellaten aus der Nordsee I. Über *Cachonina niei* Loeblich

1173   (1968), *Gonyaulax grindleyi* Reinecke (1967) und eine Methode zur Darstellung von

1174   Peridineenpanzern. Helgoländ. Wiss. Meer. 19, 558–568.

1175

1176   Suzuki, T., Horie, Y., Koike, K., Satake, M., Oshima, Y., Iwataki, M., Yoshimatsu, S., 2007.

1177   Yessotoxin analogues in several strains of *Protoceratium reticulatum* in Japan determined by

1178   liquid chromatography–hybrid triple quadrupole/linear ion trap mass spectrometry. Journal of

1179   Chromatography A 1142, 172–177.

1180

1181   Takano, Y., Horiguchi, T., 2004. Surface ultrastructure and molecular phylogenetics of four

1182   unarmoured heterotrophic dinoflagellates, including the type species of the genus *Gyrodinium*

1183   (Dinophyceae). Phycol. Res. 52, 107–116.

1184

1185   Takano, Y., Horiguchi, T. 2006. Acquiring scanning electron microscopical, light

1186   microscopical and multiple gene sequence data from a single dinoflagellate cell. J. Phycol. 42,

1187   251–256.

1188

1189   Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. 2013 MEGA6: Molecular

1190   Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol. 30: 2725–2729

1191

Tiffany, M.A., González, M.R., Swan, B.K., Reifel, K.M., Watts, J.M., Hurlbert, S.H. 2007. Phytoplankton dynamics in the Salton Sea, California, 1997-1999. Lake and Reservoir Management 23, 582–605.

Wall, D., 1967. Fossil microplankton in deep-sea cores from the Caribbean Sea. Palaeontology 10, 95–123.

Wall, D., Dale, B., 1966. "Living fossils" in western Atlantic plankton. Nature 211 (5053), 1025–1026.

Wall, D., Dale, B., 1968. Modern dinoflagellate cysts and evolution of the Peridiniales. Micropaleontology 14, 265–304.

Watts, J.M., Swan, B.K., Tiffany, M.A., Hurlbert, S.H., 2001. Thermal, mixing and oxygen regimes of the Salton Sea, California, 1997–1999. Hydrobiol. 162, 159–176.

Wołoszyńska, J., 1929. Dinoflagellatae Polskiego Baltyku i blot nad Piasnica. Archivum Hydrobiologji i Rybactwa 3, 153–278 [In Polish].

Yamaguchi, A., Yoshimatsu, S., Hoppenrath, M., Wakeman, K.C., Kawai, H. 2016. Molecular phylogeny of the benthic dinoflagellate genus *Amphidiniopsis* and its relationships with the family Protoperidiniaceae. Protist. 167, 568–583.

Zonneveld, K.A.F., Marret, F., Versteegh, G.J.M., Bogus, K., Bonnet, S., Bouimetarhan, I., Crouch, E., de Vernal, A., Elshanawany, R., Edwards, L., Esper, O., Forke, S., Grøsfjeld, K.,

1217   Henry, M., Holzwarth, U., Kielt, J.-F., Kim, S.-Y., Ladouceur, S., Ledu, D., Chen, L.,

1218   Limoges, A., Londeix, L., Lu, S.-H., Mahmoud, M.S., Marino, G., Matsouka [sic], K.,

1219   Matthiessen, J., Mildenhal [sic], D.C., Mudie, P., Neil, H.L., Pospelova, V., Qi, Y., Radi, T.,

1220   Richerol, T., Rochon, A., Sangiorgi, F., Solignac, S., Turon, J.L., Verleye, T., Wang, Y.,

1221   Wang, Z., Young, M., 2013. Atlas of modern dinoflagellate cyst distribution based on 2405

1222   datapoints. Rev. Palaeobot. Palynol. 191, 1–197.

1223

1224 **Figure captions**

1225

1226 **Figure 1.** Sites of studied plankton samples and cultured strains containing thecate stages of

1227 *Pentaplacodinium saltonense* (in red) and *Protoceratium reticulatum* (in blue). The locations

1228 are listed in Table 1.

1229

1230 **Figure 2.** Line drawings of extant members of the subfamily *Cribroperidinioideae* in dorsal

1231 view to show the dextral torsion typical of these gonyaulacoids. A. *Pentaplacodinium*

1232 *saltonense*. B. *Protoceratium reticulatum*. C. *Lingulodinium polyedra.* Labeling of tabulation

1233 follows a modified Kofoid system that recognizes homologs.

1234

1235 **Figure 3.** Line drawing of sulcal area of *Pentaplacodinium saltonense*. FP: flagellar pore; Sa:

1236 anterior sulcal plate; Sda: right anterior sulcal plate; Sdp: right posterior sulcal plate; Ssa:

1237 anterior left sulcal plate; Ssp: posterior left sulcal plate; Sp: posterior sulcal plate; c: cingular

1238 plates.

1239

1240 **Figure 4.** Line drawings of epithecal overlapping plate patterns of gonyaulacoids discussed in

1241 this paper. Arrows indicate direction of overlap. A. *Pentaplacodinium saltonense.* B.

1242 *Protoceratium reticulatum.* C. *Lingulodinium polyedra.* D. *Ceratocorys horrida.* E.

1243 *Gonyaulax spinifera.*

1244

1245 **Figure 5**. Neighbour-joining tree of *P. reticulatum, P. saltonense* and related strains

1246 sequenced in this study and sequences from Genbank based on an 80 bp alignment of the V9

1247 region of the SSU gene. Bootstrap values were retrieved from 1000 replicates and those

1248 >70% are indicated at the nodes for neighbour-joining and maximum likelihood respectively.

1249   Strain names are indicated followed by their geographic origin and accession number

1250   (Genbank).

1251

1252   **Figure 6.** Neighbour-joining tree of *P. reticulatum, P. saltonense* and related strains

1253   sequenced in this study and sequences from Genbank based on a 571 bp alignment of the V4

1254   region of the LSU gene. Bootstrap values were retrieved from 1000 replicates and those

1255   >70% are indicated at the nodes for neighbour-joining and maximum likelihood respectively.

1256   Strain names are indicated followed by their geographic origin and accession number

1257   (Genbank).

1258

1259   **Figure 7.** Neighbour-joining tree of *P. reticulatum, P. saltonense* and related strains

1260   sequenced in this study and sequences from Genbank based on a 356 bp alignment of the ITS

1261   1–2 region. Bootstrap values were retrieved from 1000 replicates and those >70% are

1262   indicated at the nodes for neighbour-joining and maximum likelihood respectively. Strain

1263   names are indicated followed by their geographic origin and accession number (Genbank).

1264

1265   **Plate Captions**

1266

1267   **Plate 1.** Light microscope images of *Pentaplacodinium saltonense* based on cyst-theca

1268   experiment from the Salton Sea. A. Living cyst from the Salton Sea St. 1. B–F. Germinated

1269   cyst from St. 2 (culture 2E3 used for single-cell PCR). B. Cross section, showing attached

1270   operculum. C. Focus on elongated simple operculum reflecting plates 2″+*(3″+4″). D–E.

1271   Focus on archeopyle, after removal of operculum. F. Cross section showing processes. G–I.

1272   Germinated cyst from St. 2 (culture 1A7 used for single-cell PCR). G. Focus on precingular

1273   archeopyle reflecting plate *(3″+4″), showing attached operculum. H. Focus on operculum. I.

1274   Cross section, showing opened operculum. Scale bars = 20 μm.

1275

1276   **Plate 2**. Light microscope images of cyst-theca experiment from the Salton Sea. A–I. Images

1277   of living cells of *Pentaplacodinium saltonense* germinated from cyst depicted in Plate 1, Figs.

1278   B–F (culture 2E3). A. Globular cell. B. Angular cell. C. Fusiform cell. D. Epitheca. E.

1279   Hypotheca. F. Ventral view showing configuration of apical plates. G–I. Sulcal plates. Scale

1280   bars = 20 μm.

1281

1282   **Plate 3.** Scanning electron microscope images of *Pentaplacodinium saltonense*, all different

1283   cells from the Salton Sea, except D. A. Holotype. Ventral view. Arrowhead points to ventral

1284   pore between plates 1′ and *4′. Arrow shows flange on plate 1p. B. Apical view, missing the

1285   cover plate. Small arrowhead points to small pores inside the thecal pores. Large arrowhead

1286   points to ventral pore between plates 1′ and *4′. Small arrowhead points to the three minute

1287   pores inside most pores. C. Dorsal view, showing dextral torsion. Note the cell roundness. D.

1288   Sulcal plates of a cell from culture SSCAP K-1479 (Indian River Lagoon, Florida).

1289   Arrowhead shows the narrow point of contact between the Sa and 1′ plates. E. Antapical

1290   view. Scale bars A–C, E = 10 μm; D = 5 μm.

1291

1292   **Plate 4.** Scanning electron microscope images of *Pentaplacodinium saltonense* from the

1293   Indian River Lagoon. A. Apical view of a cell from culture SSCAP K-1479. Arrowhead

1294   points to ventral pore between plates 1′ and *4′. B. Same specimen as in A. Ventral view.

1295   Arrowhead points to ventral pore between plates 1′ and *4′. C. A different cell from a

1296   plankton sample courtesy of Paul Hargraves. Ventral view. Arrowhead points to ventral pore

1297   between plates 1′ and *4′. Arrow shows flange on plate 1p. D. Ventral view of a cell from a

1298   culture established by Paul Hargraves. E. Antapical view of a cell from the same culture as in

1299   D. E. Apical view of a cell from the same culture as in D. D–F: SEMs by Paul Hargraves.

1300   Scale bars = 10 μm.

1301

1302   **Plate 5.** Scanning electron microscope images of cysts of *Pentaplacodinium saltonense*

1303   extracted from Salton Sea sediment (St. 2) using palynological methods. A–C. Views showing

1304   shape of archeopyle, reflecting plate *(3″+4″). D. Specimen that is torn along the cingulum.

1305   E. Specimen showing alignment of processes along the cingulum. F. Specimen with relatively

1306   large openings in cyst wall. G. Specimen with distinct intratabular processes. H. Specimen

1307   with relatively coarsely reticulated wall surface. I. Internal view of smooth cyst wall. Scale

1308   bars = 10 μm.

1309

1310   **Plate 6.** Scanning electron microscope images of cysts of *Pentaplacodinium saltonense*

1311   formed in culture of strain 3243 (Indian River Lagoon). A. Specimen showing preformed

1312   archeopyle and margins of principal archeopyle suture with reduced ornament. B. Specimen

1313   with attached thecal plate. C. Specimen with partly developed processes. D. Specimen with

1314   processes clearly reflecting tabulation. E. Specimen with preformed archeopyle. F. Specimen

1315   showing reflection of the sulcus. G–H. Specimen with well-developed wall texture. I. Wall

1316   texture of specimen with 'spider-web' microreticulation. Scale bars = 10 μm, except H, I,

1317   scale bars = 1 μm.

1318

1319   **Plate 7.** Scanning electron microscope images of *Protoceratium reticulatum* cells and of the

1320   apical pore plates of the gonyaulacoids discussed in this study. A. *Protoceratium reticulatum.*

1321   Cell from Greenland, ventral view. B. Same cell in apical view. C. *Protoceratium reticulatum.*

1322   Cell from Elands Bay, South Africa. Dorsal view, note the dextral torsion. D. Apical pore

1323 plates of a different cell of *Protoceratium reticulatum* (Greenland). E. Apical pore plates of a

1324 cell of *Ceratocorys horrida* (Central equatorial Pacific). F. Apical pore plates of a cell of

1325 *Pentaplacodinium saltonense* from culture SSCAP K-1479 (Indian River Lagoon). G. Apical

1326 pore plates of a cell of *Ceratocorys gourretii*. H. Apical pore plates of another cell of

1327 *Pentaplacodinium saltonense* from culture SSCAP K-1479 (Indian River Lagoon). Scale bars

1328 A–C = 10 μm; D–H = 5 μm.

1329

Figure 1
Click here to download high resolution image

Figure 2
Click here to download high resolution image

Figure 3
Click here to download high resolution image

Figure 4
Click here to download high resolution image

Figure 5

Figure 6
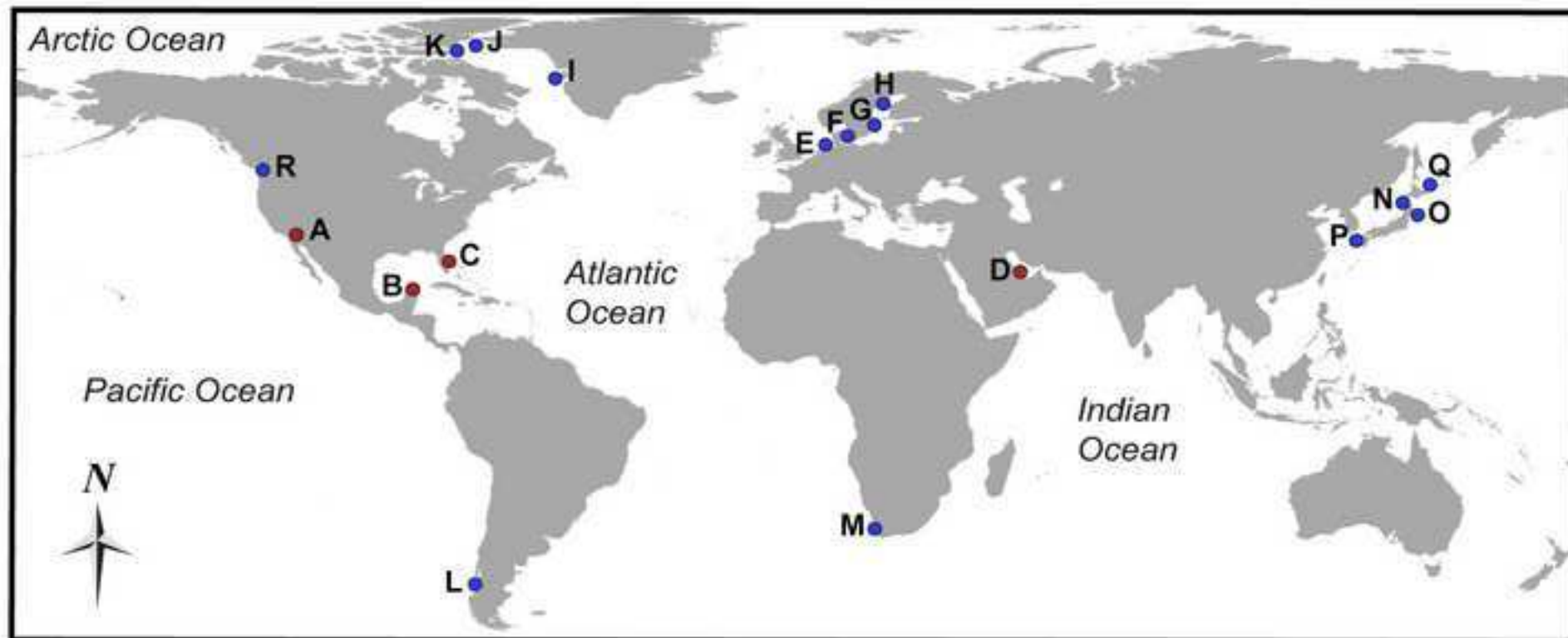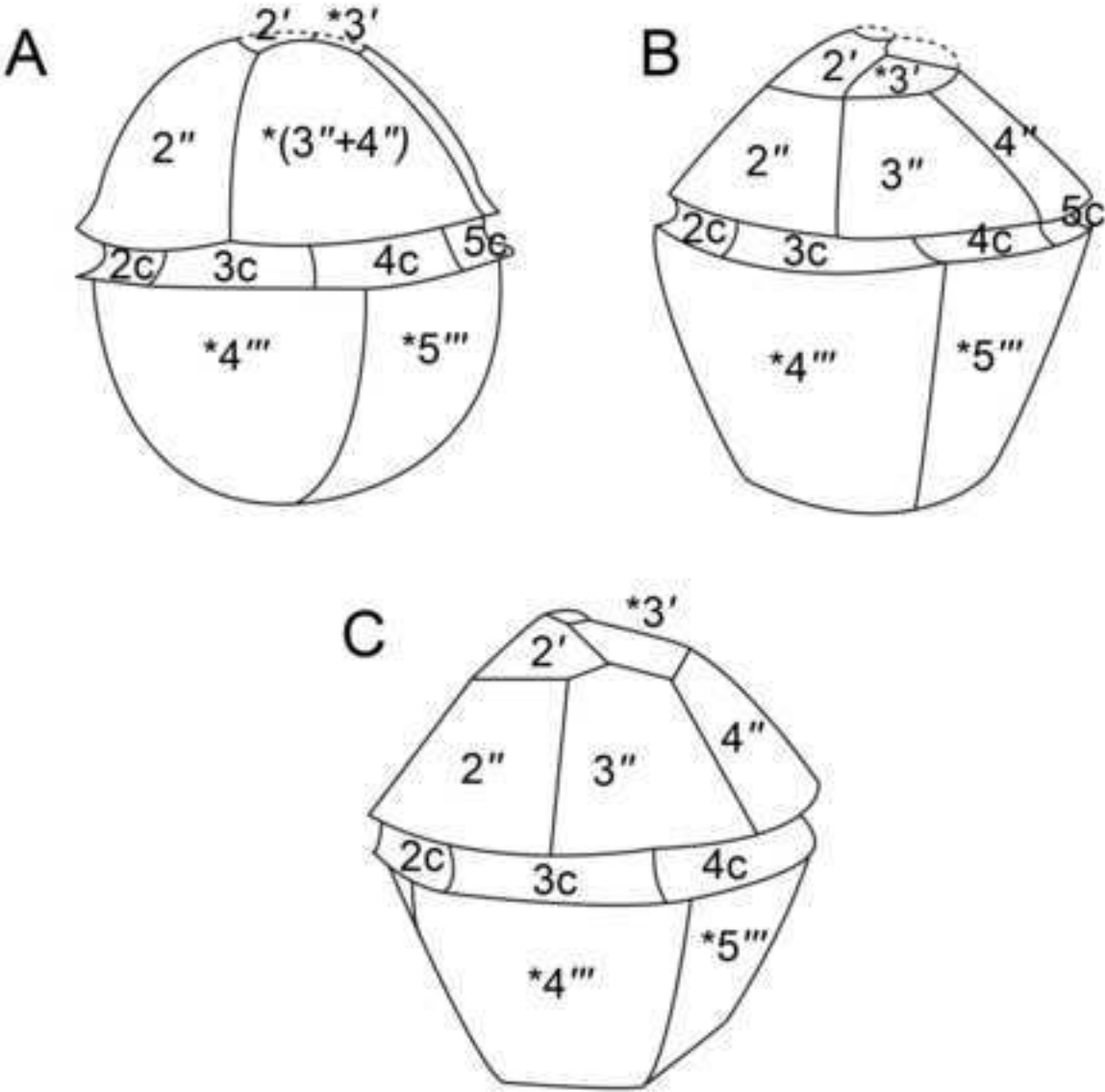**Click here to download high resolution image**

Figure 6

Figure 7

Plate 4
[Click here to download high resolution image](#)

Plate 5
[Click here to download high resolution image](#)

Plate 6
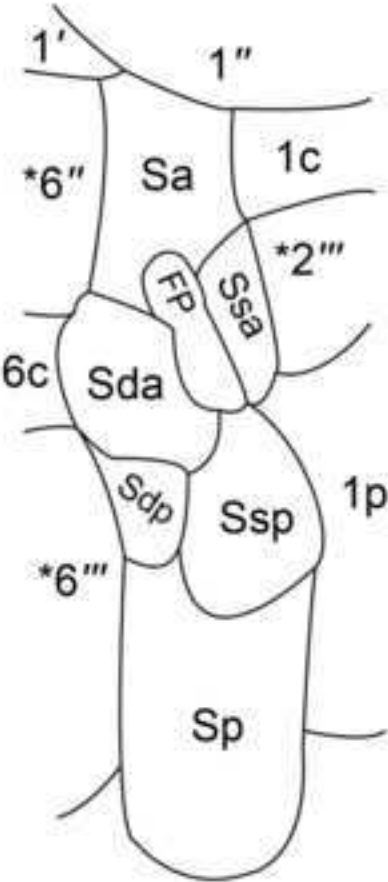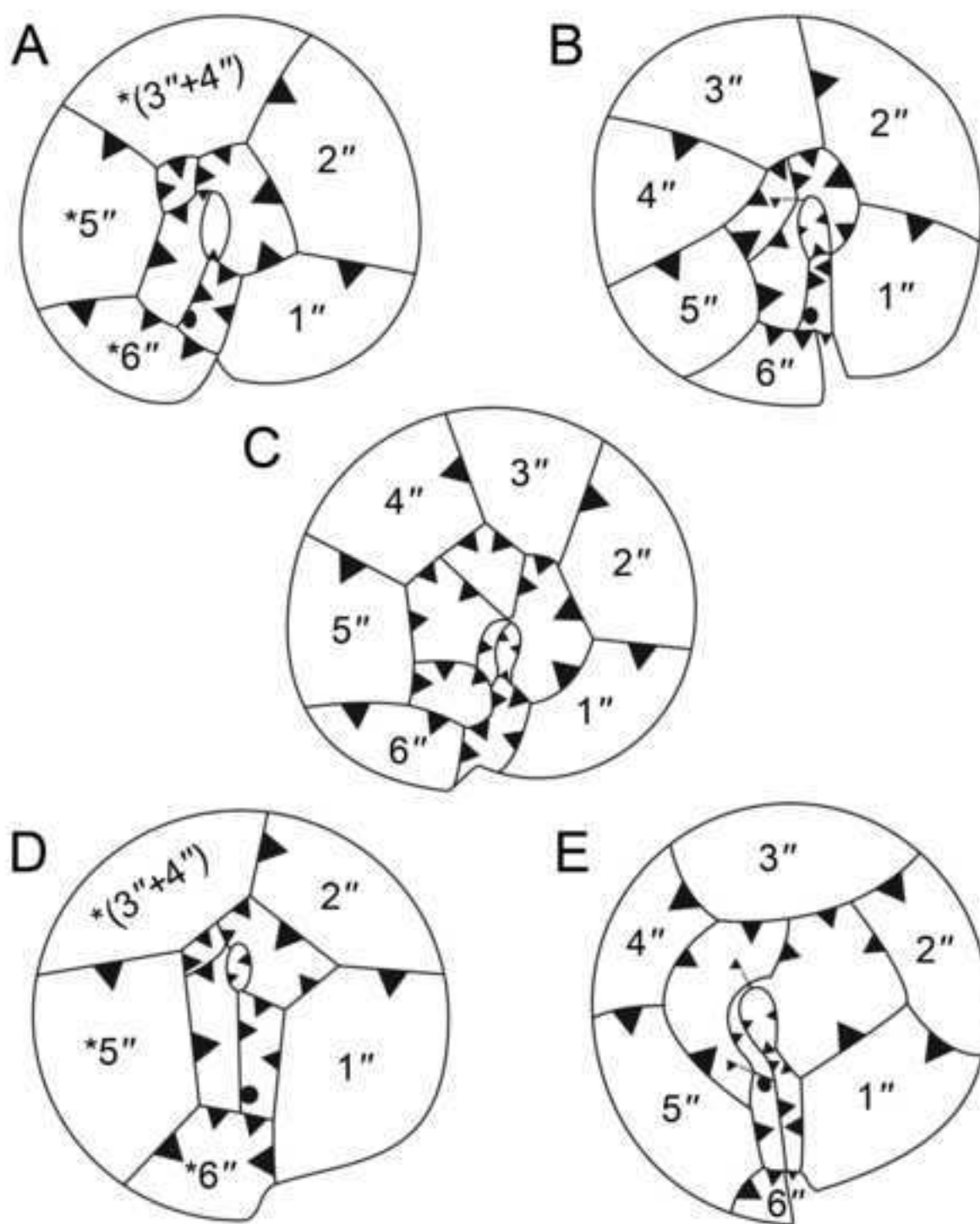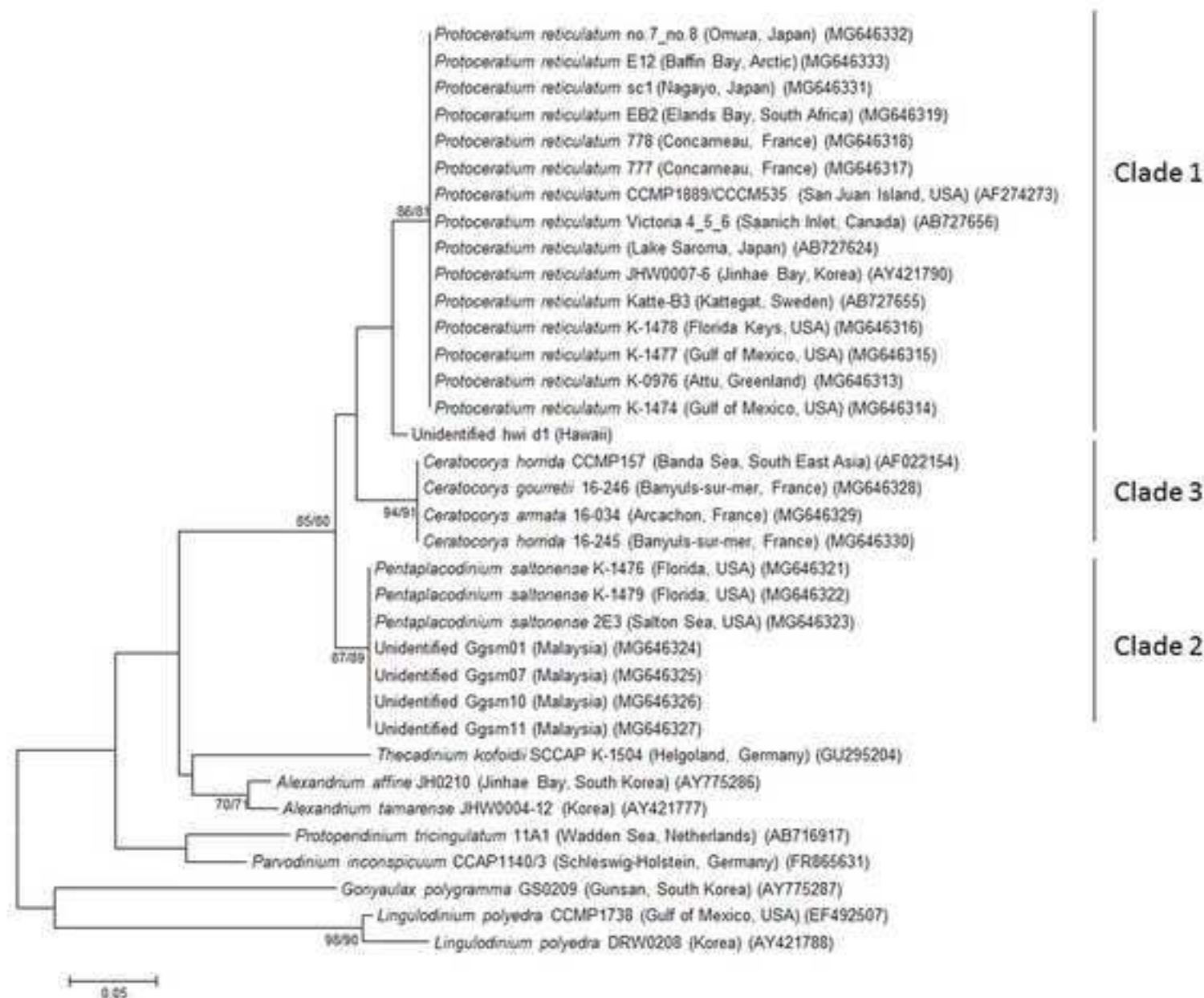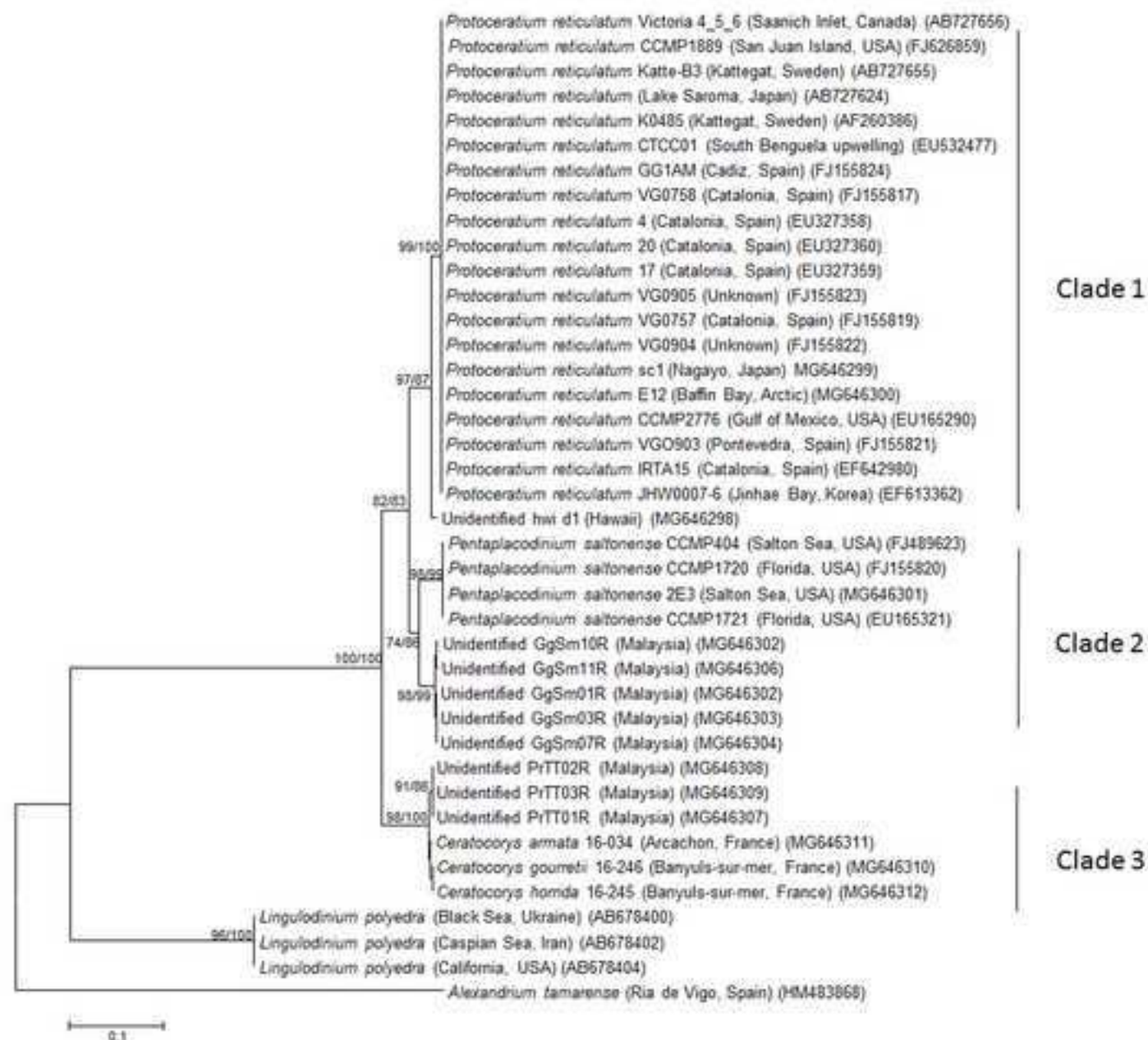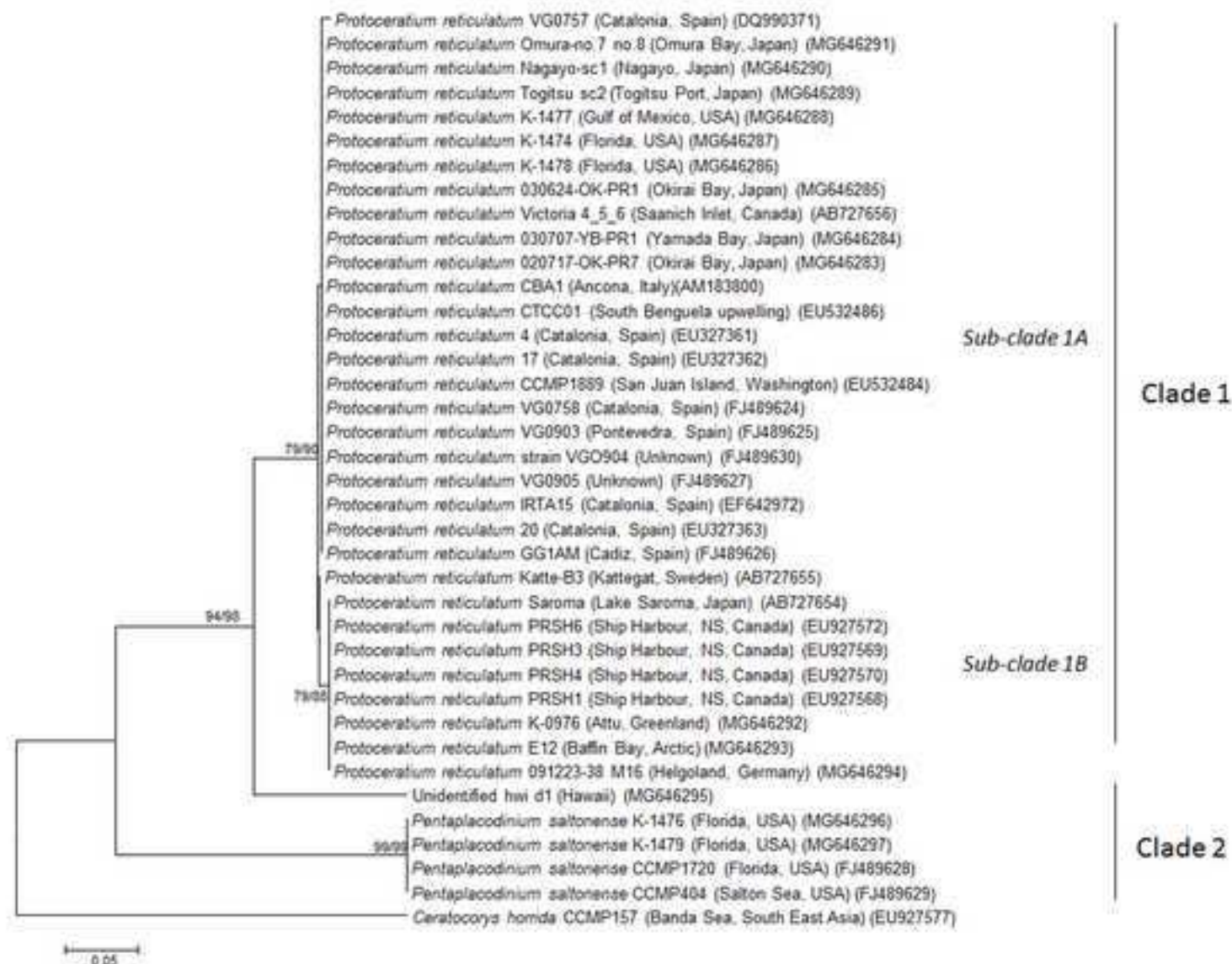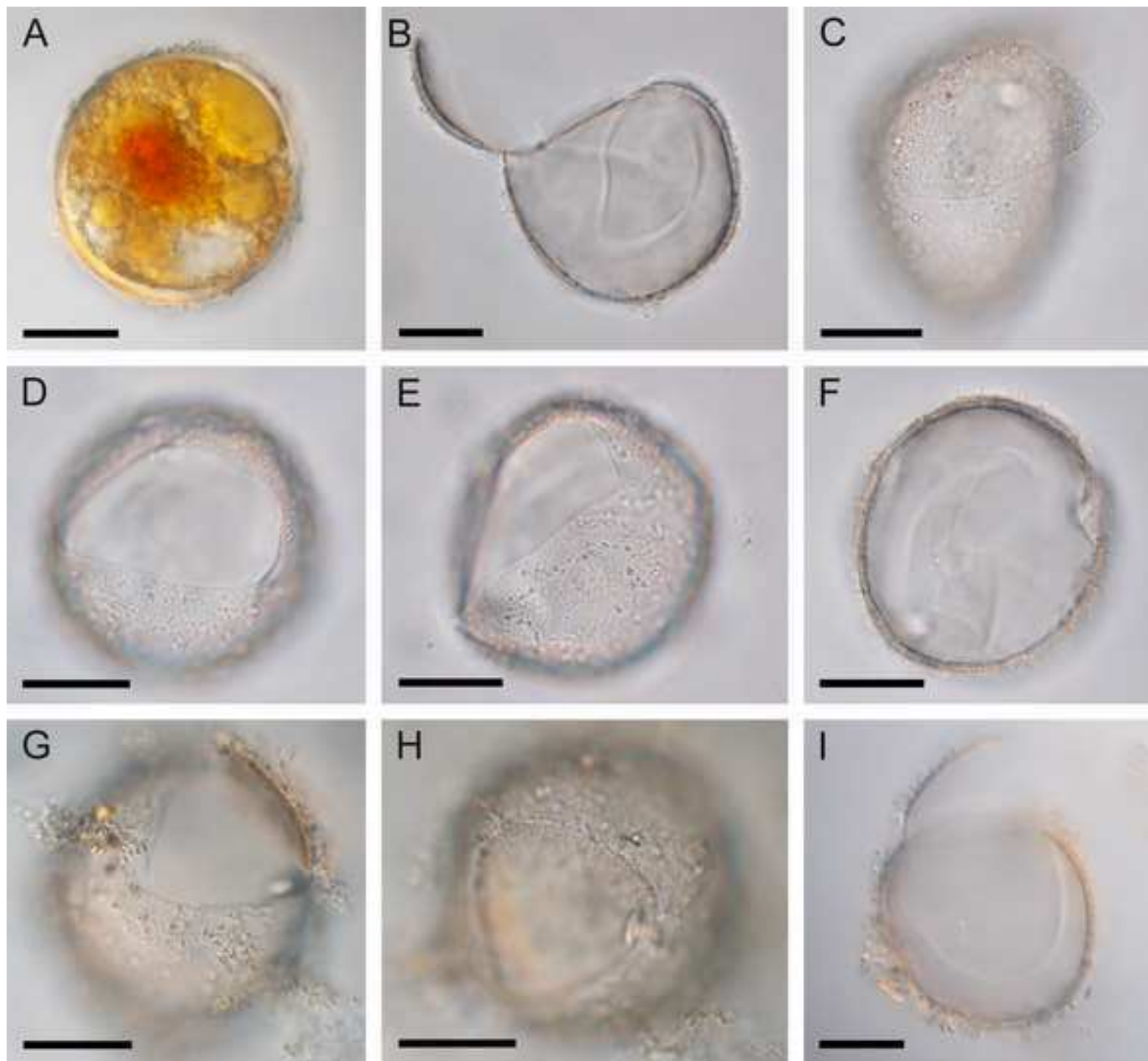[Click here to download high resolution image](#)

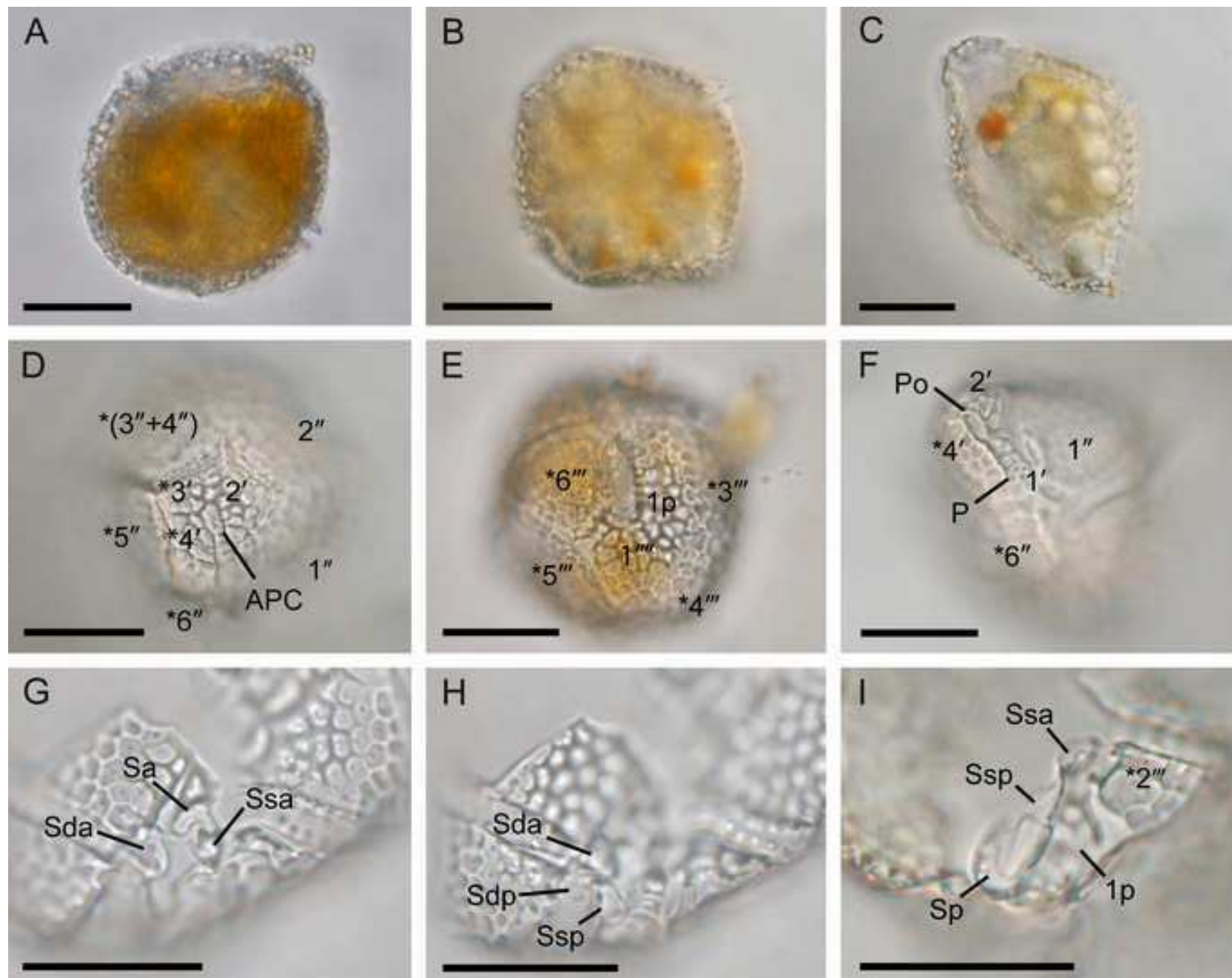**Plate 7**
[Click here to download high resolution image]
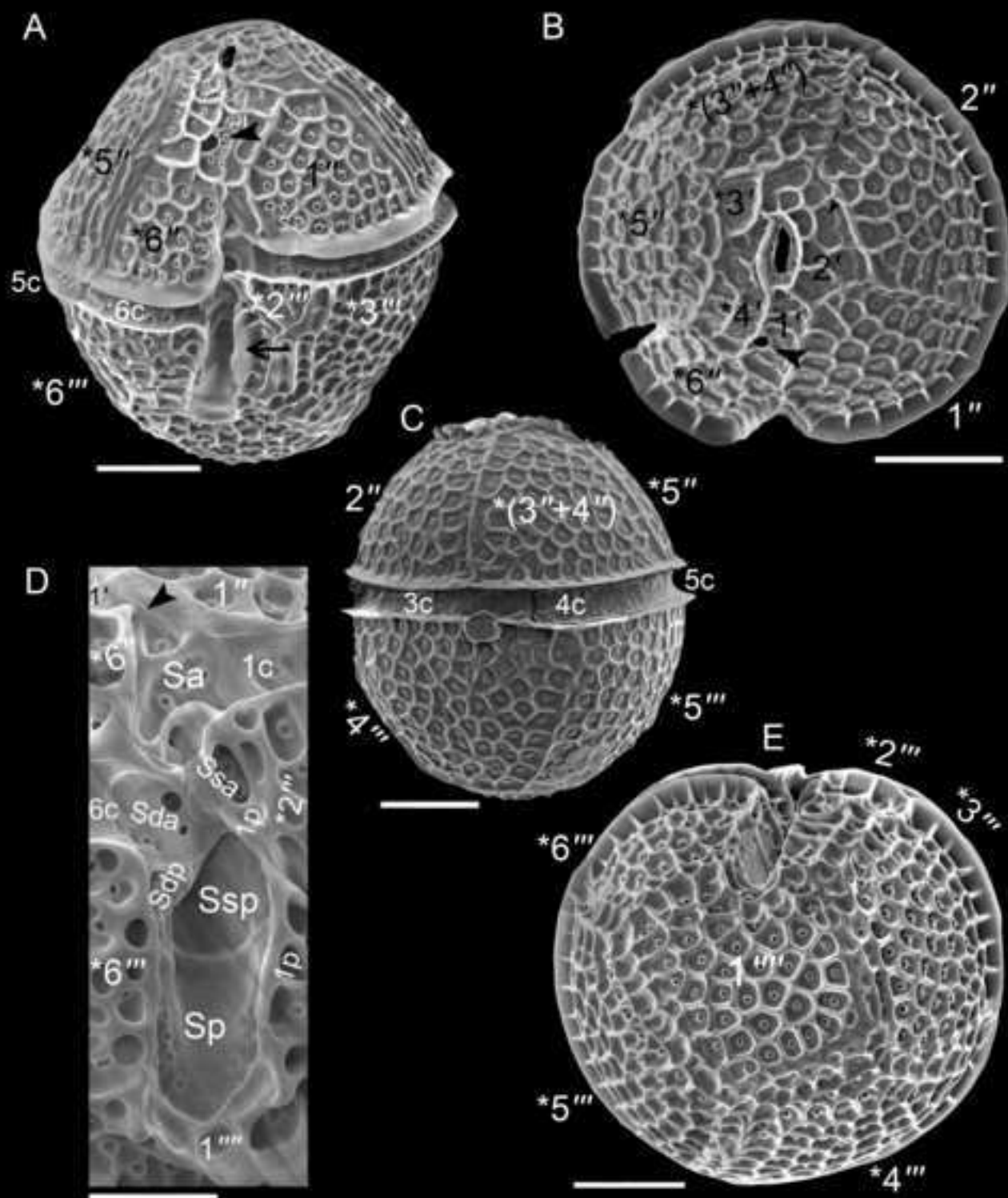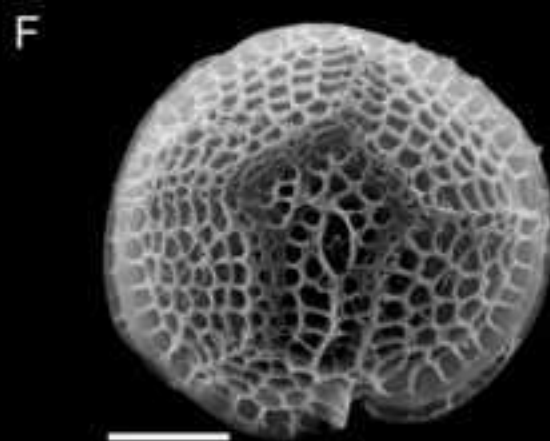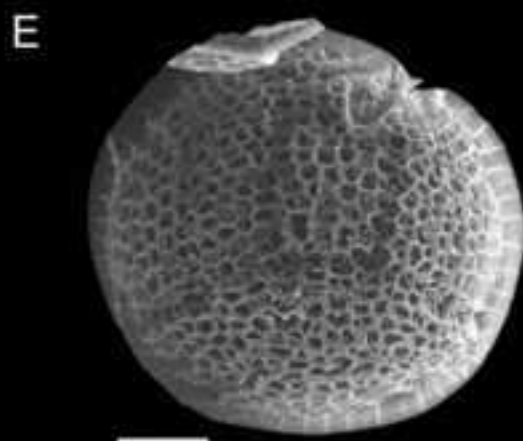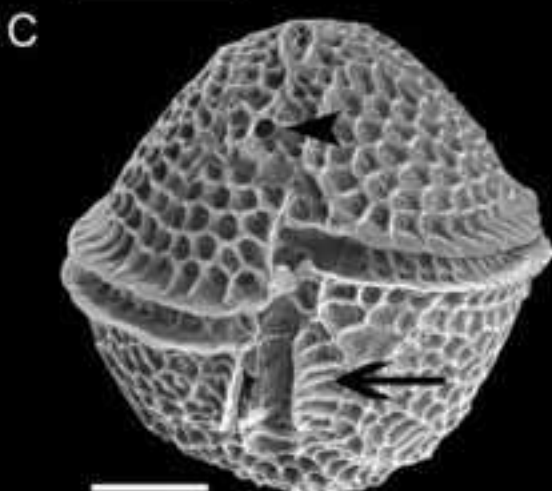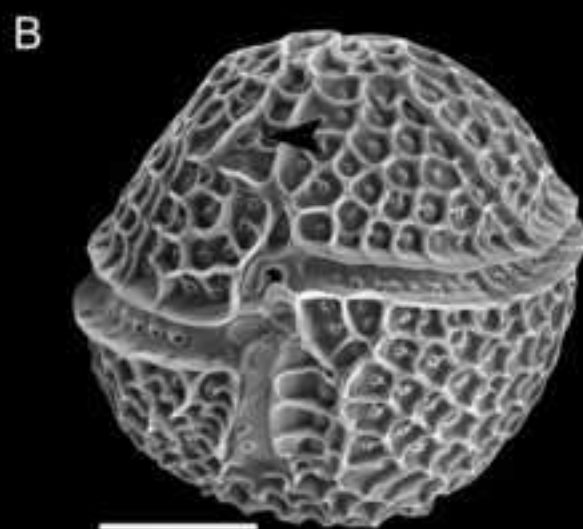
Table 1

Table 1. Site location of plankton samples investigated, location mark on Figure 1, sampling date, latitude, longitude, sea surface salinity (psu), sea surface temperature (°C), sampling device, used fixative, and name of persons who did the sampling.

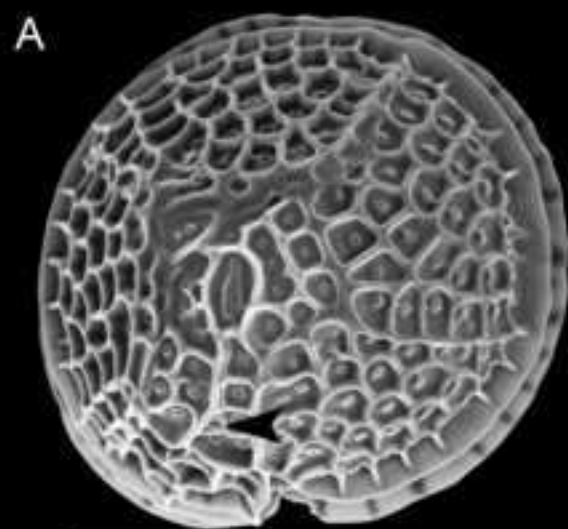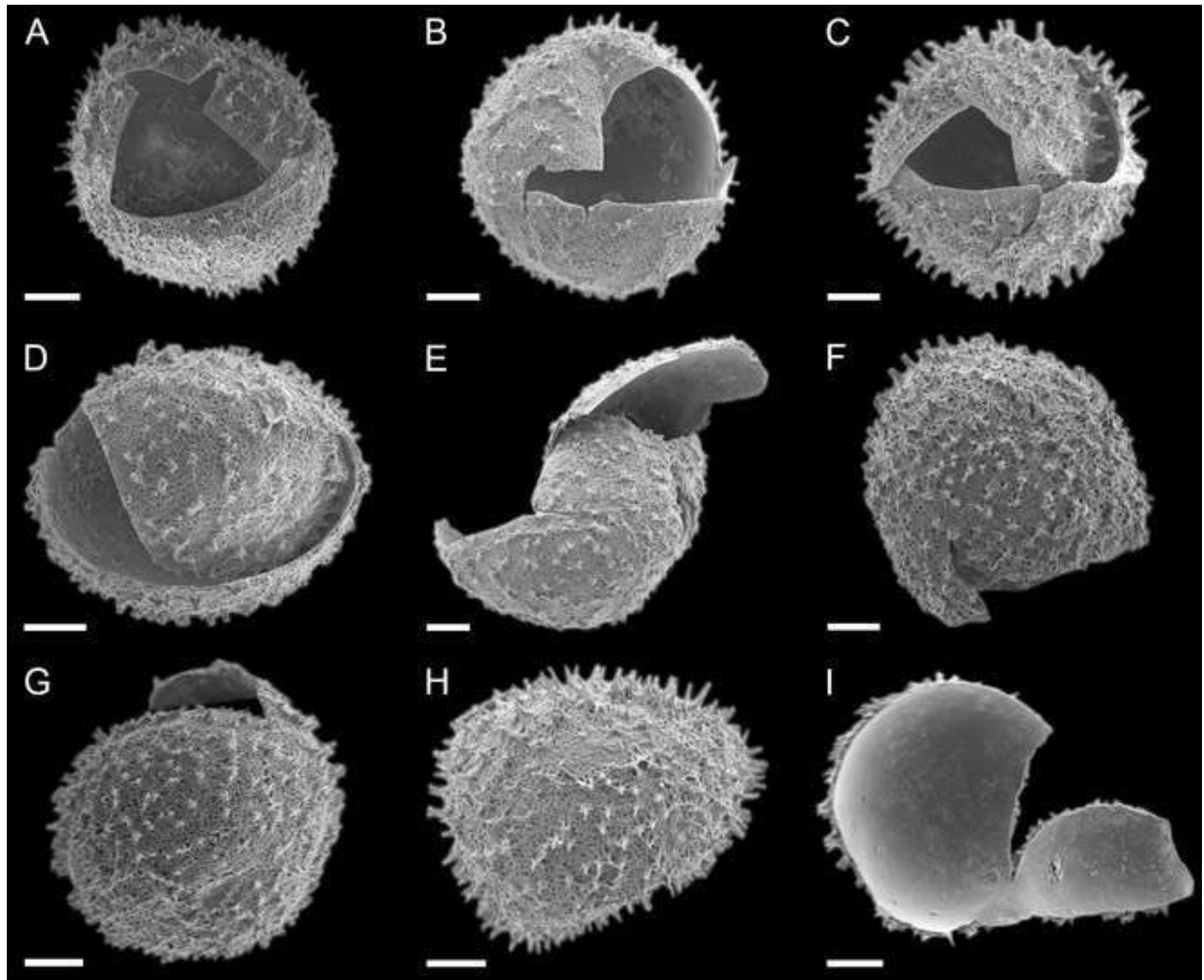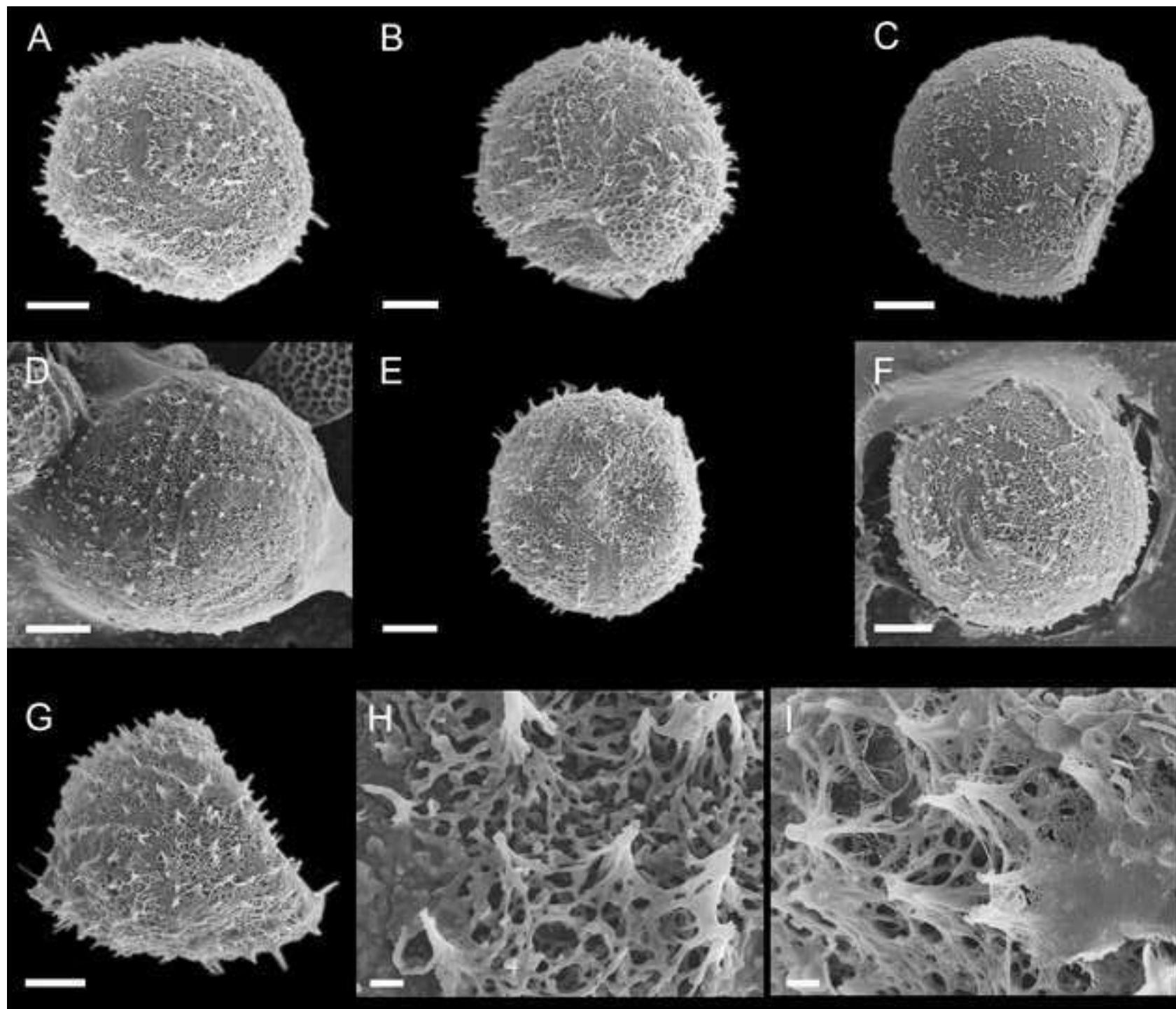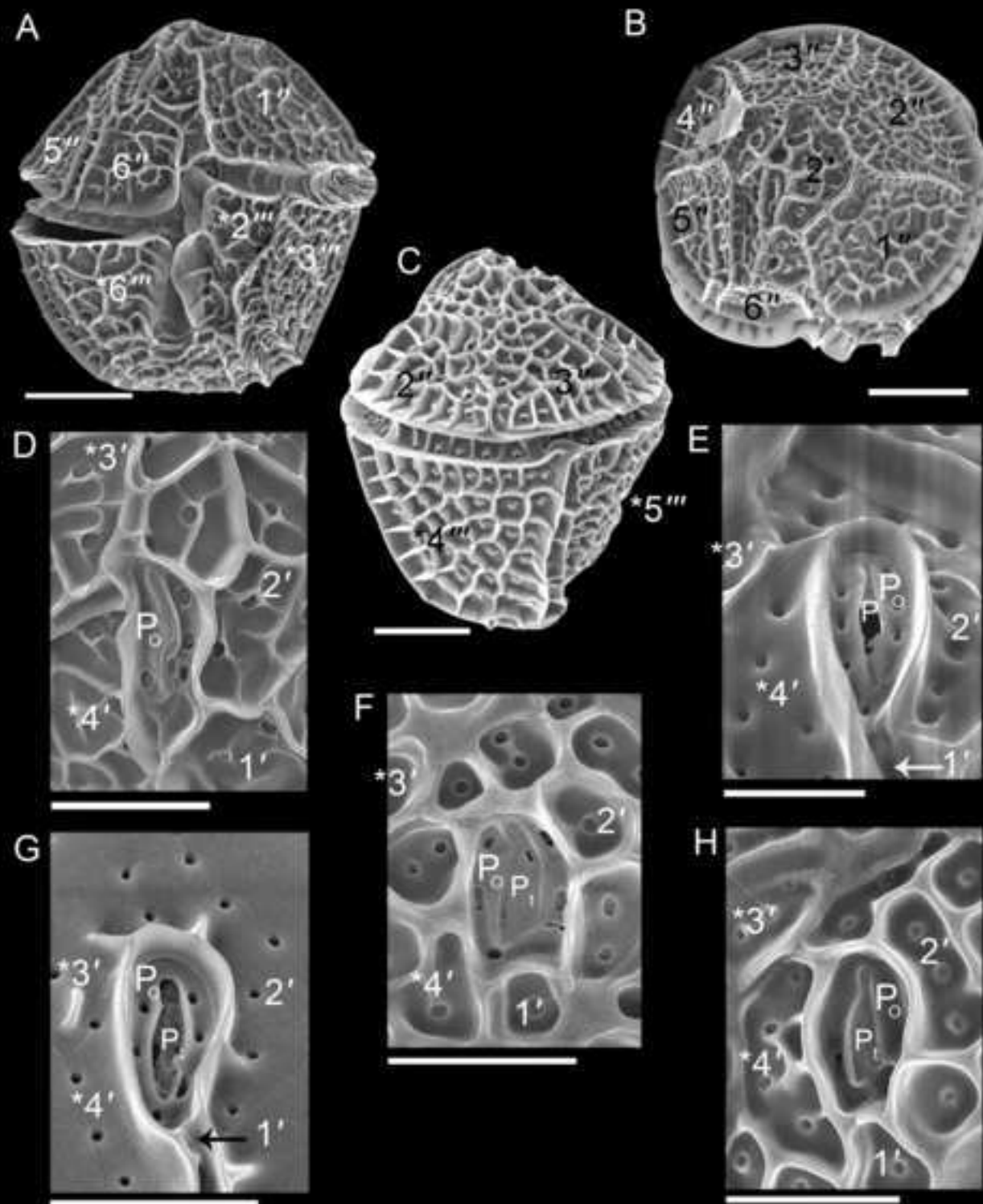| Sampling site	Location mark on Figure 1	Sampling date	Latitude (°)	Longitude (°)	SSS (psu)	SST (°C)	Sampling device	Fixative used	Species present	Sampled by
Salton Sea, St. 1, California, USA	A	24-Oct-09	33,50	-115,91	>40.2 (62)	23,1	Plankton net > 20 µm	Ethanol 100%	PS	KM, VP, MH, MCCM
Salton Sea, St. 2, California, USA	A	24-Oct-09	33,50	-115,91	>40.2 (64)	25,5	Plankton net > 20 µm	Ethanol 100%	PS	KM, VP, MH, MCCM
Salton Sea, St. 3, California, USA	A	24-Oct-09	33,50	-115,92	>40.2 (65)	25,5	Plankton net > 20 µm	Ethanol 100%	PS	KM, VP, MH, MCCM
Salton Sea, St. 4, California, USA	A	24-Oct-09	33,50	-115,91	>40.2 (56)	30,3	Plankton net > 20 µm	Ethanol 100%	PS	KM, VP, MH, MCCM
Salton Sea, St. 5, California, USA	A	24-Oct-09	33,50	-115,91	>40.2 (65)	~30	Plankton net > 20 µm	Ethanol 100%	PS	KM, VP, MH, MCCM
Off Yucatan, St. 4, Gulf of Mexico, Mexico	B	19-May-09	21,39	-88,08	31,0	24,1	Plankton tow >20 µm	Formaldehyde	PS	YO
Off Yucatan, Gulf of Mexico, Mexico	B	19-May-09	21,40	-88,84	31,0	24,1	Plankton tow >20 µm	Formaldehyde	PS	YO
Indian River Lagoon, St. TR, Florida, USA	C	28-May-08	27,50	-80,34	37,4	29,5	Plankton tow >20 µm	Formalin 2%	PS	PH
Off Qatar, Persian Gulf	D	Sept. 1991	25,29	51,54	38-43	20-35	Plankton net	Formalin 5%	PS	AA
North Sea, Helgoland, Germany	E	3-Jun-02	54,19	7,90	32,0	12,5	Plankton net >20 µm	Lugol	PR	MHO
Kattegat, St. Central, Denmark	F	NA	56,92	11,28	NA	NA	Plankton net	Lugol & Formalin	PR	JL
Kattegat, St. 431, Denmark	F	22-Jun-05	55,77	12,75	NA	NA	Plankton net	Lugol	PR	JL
Kattegat, St. 925, Denmark	F	17-Aug-00	56,08	11,02	NA	NA	Plankton net	Lugol	PR	JL
Baltic Sea, St. F64, Finland	G	20-Aug-10	60,18	19,13	5,7	17,6	Plankton net	Lugol	PR	AK, MHU
Gulf of Bothnia, Baltic Sea, St. US5B, Finland	H	20-Aug-10	62,58	19,98	5,1	16,0	Plankton net	Lugol	PR	AK, MHU
Western Greenland, St. 516, Denmark	I	30-Jul-12	69,20	-54,10	33,2	6,9	Water Bottle-CTD	Formaldehyde	PR	UT
Baffin Bay, St. 2008-029-0039/9/0039A, Canada	J	6-Sep-08	76,57	-73,96	31,0	2,2	Plankton net >20 µm	Formaldehyde	PR	AR
Baffin Bay, St. 2008-029-027A, Canada	J	5-Sep-08	77,29	-74,34	30,8	2,2	Plankton net >20 µm	Formaldehyde	PR	AR
Baffin Bay, St. 2008-029-0043A, Canada	K	7-Sep-08	75,58	-78,63	31,0	2,0	Plankton net >20 µm	Formaldehyde	PR	AR
Baffin Bay, St. 2008-029-0035A, Canada	J	6-Sep-08	76,33	-71,43	31,0	4,5	Plankton net >20 µm	Formaldehyde	PR	AR
Off Puerto Aguirre, Chile	L	21-May-06	-44,99	-73,53	28,8	10,0	Plankton net > 26 µm	Formaldehyde	PR	XV
Elands Bay, South Africa	M	16-Mar-13	-32,31	18,32	NA	NA	Plankton net	Formaldehyde	PR	MP
Off Cape Town, South Africa	M	NA	-33,89	18,42	NA	NA	Plankton net	Lugol + formalin	PR	JL
Mutsu Bay, Aomori, Japan	N	5-Apr-10	40,92	141,12	32,7	5,9	Plankton net >20 µm	Glutaraldehyde 1%	PR	KK
Okkirai Bay, Iwate, Japan	O	16-Jul-03	39,08	141,85	32,6	15,8	Plankton net >20 µm	Glutaraldehyde 5%	PR	KK
Okkirai Bay, Iwate, Japan	O	28-Aug-03	39,08	141,85	32,3	20,0	Plankton net >20 µm	Glutaraldehyde 5%	PR	KK
Okkirai Bay, Iwate, Japan	O	23-Jun-04	39,08	141,85	32,7	16,0	Plankton net >20 µm	Glutaraldehyde 5%	PR	KK
Okkirai Bay, Iwate, Japan	O	12-Aug-04	39,08	141,85	33,5	23,0	Plankton net >20 µm	Glutaraldehyde 5%	PR	KK
Omura Bay, Nagayo-ura, Japan	P	9-May-11	32,85	129,87	32,7	20,2	Plankton net >20 µm	Formalin	PR	KMA
Omura Bay, Inoura, Japan	P	12-May-11	33,05	129,74	25,6	18,5	Plankton net >20 µm	Formalin	PR	KMA
Omura Bay, Togitsu Port, Japan	P	23-May-11	32,85	129,87	32,6	19,5	Plankton net >20 µm	Formalin	PR	KMA
Kagoshima Bay, St. 1, Japan	P	20-Jun-11	31,55	130,57	23,8	22,9	Plankton net >20 µm	Formalin	PR	KMA
Saroma Lake (Lagoon), St. 1, Japan	Q	22-Jul-11	44,12	143,82	32,2	17,1	Plankton net	Ethanol	PR	KM

Saanich Inlet, St. S2, BC, Canada	R	13-Jul-10	48,55	-123,53	27,8	14,8	Plankton net	Formaldehyde	PR	VP
Saanich Inlet, St. S5, BC, Canada	R	13-Jul-10	48,71	-123,46	27,8	13,6	Plankton net	Formaldehyde	PR	VP
Saanich Inlet, St. S3, BC, Canada	R	13-Jul-10	48,59	-123,48	27,8	14,8	Plankton net	Formaldehyde	PR	VP
Saanich Inlet, St. S4.5, BC, Canada	R	14-Jul-10	48,67	-123,49	28,0	13,7	Plankton net	Formaldehyde	PR	VP
Saanich Inlet, St. S11, BC, Canada	R	14-Jul-10	48,73	-123,54	28,0	13,7	Plankton net	Formaldehyde	PR	VP
Saanich Inlet, St. S4, BC, Canada	R	13-Jul-10	48,63	-123,49	27,4	15,1	Plankton net	Formaldehyde	PR	VP
Saanich Inlet, St. Pat UVic 262, BC, Canada	R	10-Aug-11	48,65	-123,44	29,0	17,8	Plankton net	Formaldehyde	PR	VP
Saanich Inlet, St. Pat UVic 264, BC, Canada	R	21-Aug-11	48,65	-123,44	31,0	17,9	Plankton net	Formaldehyde	PR	VP

Abbreviations: NA = Not available, PS = *Pentaplacodinium saltonense,* PR = *Protoceratium reticulatum,* AK = Anke Kremp, AR = André Rochon*,* AA = Abdulrahman Al-Muftah, *M*CCM = Consuelo Carbonell-Moore, JL = Jacob Larsen, KK = Kazuhiko Koike, KM = Kenneth Neil Mertens, KMA = Kazumi Matsuoka, MH= Martin J. Head, MHO = Mona Hoppenrath, MHU=Maija Huttunen, MP = Maya Pfaff, PH = Paul Hargreaves, UT = Urban Tillmann, VP = Vera Pospelova, XV = Ximena Vivanco, YO = Yuri B. Okolodkow

Suppl. Table 1. Culture strains and cells or cyst picked for SEM and/or phylogenetic analysis.

CODI (Strain ID)	Identified here by its morphology as	Geographic Origin	Latitude (°N)	Longitude (°E)	Isolation date	Isolated by	LSU (28S) Genbank	ITS Genbank	SSU (18S) Genbank	Sequenced in this study by
CCMP404 = ALO011	*P. saltonense*	Salton Sea (California)	33.375	-116.0	1966	Dodson, A.	EU532476	FJ489629	FJ489629***	
Salton Sea 2E3 *	*P. saltonense*	Salton Sea (California)	33° 30.122'	-115° 54.879'	19/11/2013	Mertens, K.	**MG646301**	Not sequenced	**MG646323**	**Aika Yamaguchi**
CCMP1720 = K-1475 = ALO013	*P. saltonense*	Biscayne Bay (Florida)	25.8	-80.3333	15/02/1995	Hargraves, P.	FJ155820	FJ489628	FJ489628***	
CCMP1721 = K-1480 = ALO012	*P. saltonense*	Biscayne Bay (Florida)	25.8	-80.3333	01/06/1994	Hargraves, P.	EU165321	Not sequenced	Not sequenced	
CCMP3241 = K-1479	*P. saltonense*	Indian River Lagoon (Florida)	27.83	-80.45	11/06/2008	Hargraves, P.	Not sequenced	**MG646297**	**MG646322**	**Andrea Highfield / Declan Schroeder**
CCMP3243 = K-1476	*P. saltonense*	Indian River Lagoon (Florida)	27.83	-80.45	05/06/2008	Hargraves, P.	Not sequenced	**MG646296**	**MG646321**	**Andrea Highfield / Declan Schroeder**
CCMP3031 = K-1474	*P. reticulatum*	Off coast Florida, Gulf of Mexico	25.0167	-81.4003	NA	NA	Not sequenced	**MG646287**	**MG646314**	**Andrea Highfield / Declan Schroeder**
Victoria-no.4_5_6 *	*P. reticulatum*	Brentwood Bay, Saanich Inlet	48.57	123.47	Oct. 2011	Mertens, K.	AB727656	AB727656	AB727656	
CCMP1889 = K-0634 = CCCM535	NA	Friday Harbor, San Juan Island, Washington USA	48.544	-123.01	1983	Taylor, F.J.R.	EU532475	EU532484	FJ626858***	
CCMP2776 = K-1477	*P. reticulatum*	Gulf of Mexico, U.S.A.	25,0167	-81,4003	NA	Sinigalliano, C.	EU165290	**MG646288**	**MG646315**	**Andrea Highfield / Declan Schroeder**
CCMP3113 = K-1478	*P. reticulatum*	Marquesa Keys, Florida Keys	-24.58	-82.1	NA	NA	Not sequenced	**MG646286**	**MG646316**	**Andrea Highfield / Declan Schroeder**
VGO757	NA	Ebro Delta (Catalonia, Spain)	NA	NA	NA	Fernandez-Tejedor, M.	FJ155819	DQ990371	DQ990371***	
VGO758	*P. reticulatum***	Alfacs Bay, Ebro Delta (Catalonia Spain)	NA	NA	NA	Fernandez-Tejedor, M.	FJ155817	FJ489624	FJ489624***	
VGO903 = ALO014	*P. reticulatum***	Ría de Ponteverde (Bueu)	NA	NA	NA	Fernandez-Tejedor, M.	FJ155821	FJ489625	FJ489625***	
VGO904	NA	NA	NA	NA	NA	Fernandez-Tejedor, M.	FJ155822	FJ489630	FJ489630***	
VGO905	NA	NA	NA	NA	NA	Fernandez-Tejedor, M.	FJ155823	FJ489627	FJ489627***	
IRTA015	*P. reticulatum*	Ebro Delta (Catalonia Spain)	NA	NA	NA	Fernandez-Tejedor, M.	EF642980	EF642972	EF642972***	
20	NA	Catalan Coast	NA	NA	NA	Fernandez-Tejedor, M.	Not sequenced	EU327363	EU327363***	
GG1AM	NA	La Atunara (Cádiz, Spain)	NA	NA	NA	Fernandez-Tejedor, M.	FJ155824	FJ489626	FJ489626***	
17	NA	Catalan Coast	NA	NA	NA	Fernandez-Tejedor, M.	Not sequenced	EU327362	EU327362***	
4	NA	Catalan Coast	NA	NA	NA	Fernandez-Tejedor, M.	Not sequenced	EU327361	EU327361***	
Katte-B3 *	*P. reticulatum* (cyst-based)	Kattegat, Swedish coast	57.5	11.8	Nov. 2011	Mertens, K.	AB727655	AB727655	AB727655	
091223-38_M16_Protocer3-ITS1	*P. reticulatum*	Helgoland, Germany	54,19	7,9	avr-03	Hoppenrath, M.	Not sequenced	**MG646294**	Not sequenced	**Karin Röder**
Lake Saroma *	*P. reticulatum* (cyst-based)	Lake Saroma, Japan	44.12	143.87	Aug. 2011	Mertens, K.	AB727654	AB727654	AB727654	
K-0976	*P. reticulatum*	Attu, Greenland	67.924068	-53.649824	21.08.2005	Moestrup, Ø.	Not sequenced	**MG646292**	**MG646313**	**Andrea Highfield / Declan Schroeder**
Arctic E12 = K1-1-1 - K-1-3 *	*P. reticulatum*	Station 323, Northern Baffin Bay	74.12	79.45	02-mai-11	Mertens, K.	**MG646300**	**MG646293**	**MG646333**	**Yoshihito Takano**
K-0485	*P. reticulatum*	Southern Kattegat, Bouy St.	56.20	12.04	03-avr-89	Hansen, G.	AF260386	Not sequenced	Not sequenced	
PRSH6 (NRC Halifax)	*P. reticulatum*	Ship harbour, NS, Canada	NA	NA	NA	Ferrell, J. F.	Not sequenced	EU927572	EU927572***	
PRSH3 (NRC Halifax)	*P. reticulatum*	Ship harbour, NS, Canada	NA	NA	NA	Ferrell, J. F.	Not sequenced	EU927569	EU927569***	
PRSH4 (NRC Halifax)	*P. reticulatum*	Ship harbour, NS, Canada	NA	NA	NA	Ferrell, J. F.	Not sequenced	EU927570	EU927570***	
PRSH1 (NRC Halifax)	*P. reticulatum*	Ship harbour, NS, Canada	NA	NA	NA	Ferrell, J. F.	Not sequenced	EU927568	EU927568***	
CTCC 01	*P. reticulatum*	Southern Benguela upwelling region	NA	NA	NA	NA	EU532477	EU532486	EU532486***	
Elands Bay 2013 (EB2) *	*P. reticulatum*	Elands Bay, South Africa	32°18.618'	-18°19.267	16 March 2013	Carbonell-Moore, C.	Not sequenced	Not sequenced	MG646319	**Andrea Highfield / Declan Schroeder**

Strain	Species	Location	Lat	Long	Date	Collector				Contributor
JHW0007-6	NA	Jinhae Bay, Korea	NA	NA	juil-00	Kim, K.-Y.	EF613362	Not sequenced	AY421790	
CBA-1	NA	Adriatic Sea, Ancona, Italy	NA	NA	NA	Totti C.	Not sequenced	AM183800	AM183800***	
020717-OK-PR7	*P. reticulatum*	Okkirai Bay, Iwate, Japan	39.08	141.85	17-Jul-02	Koike, K.	Not sequenced	**MG646283**	Not sequenced	**Kazuhiko Koike**
030707-YB-PR1	*P. reticulatum*	Yamada Bay, Iwate, Japan	39.46	141.97	7-Jul-03	Koike, K.	Not sequenced	**MG646284**	Not sequenced	**Kazuhiko Koike**
030624-OK-PR1	*P. reticulatum*	Okkirai Bay, Iwate, Japan	39.08	141.85	24-Jun-03	Koike, K.	Not sequenced	**MG646285**	Not sequenced	**Kazuhiko Koike**
Omura-no.7_no.8 *	*P. reticulatum*	Omura Bay, Japan	32.85	129.87	2 may 2011	Takano, Y.	Not sequenced	**MG646291**	**MG646332**	**Yoshihito Takano**
Nagayo-sc1 *	*P. reticulatum*	Nagayo, Japan	32.85	129.87	24 May 2011	Takano, Y.	**MG646299**	**MG646290**	**MG646331**	**Yoshihito Takano**
Togitsu-sc2 *	*P. reticulatum*	Togitsu Port, Japan	32.85	129.87	23 May 2011	Takano, Y.	Not sequenced	**MG646289**	Not sequenced	**Yoshihito Takano**
777	*P. reticulatum*	Concarneau large, France	47.83	-3.95	16 July 2008	Nézan, E.	Not sequenced	Not sequenced	**MG646317**	**Gwenael Bilien**
778	*P. reticulatum*	Concarneau large, France	47.83	-3.95	16 July 2008	Nézan, E.	Not sequenced	Not sequenced	**MG646318**	**Gwenael Bilien**
16-034	*Ceratocorys armata*	Bouée 7, Arcachon, France	44.54	-1.26	18 Dec 2015	Nézan, E.	**MG646311**	Not sequenced	**MG646329**	**Gwenael Bilien**
16-246	*Ceratocorys gourretii*	Station B70, Banyuls-sur-mer, French Mediterranean	42.48	3.18	26 Sept 2016	Nézan, E.	**MG646310**	Not sequenced	**MG646328**	**Gwenael Bilien**
16-245	*Ceratocorys horrida*	Station B70, Banyuls-sur-mer, French Mediterranean	42.48	3.18	26 Sept 2016	Nézan, E.	**MG646312**	Not sequenced	**MG646330**	**Gwenael Bilien**
CCMP157	*Ceratocorys horrida*	Banda Sea, South East Asia	-5,00	130,00	22 April 1975	Sweeney, B.	Not sequenced	EU927577	AF022154	
SCCAP K-1504	*Thecadinium kofoidii*	Helgoland, Germany	54.19	7.9	Aug. 2002	Hoppenrath, M.	GU295207***	Not sequenced	GU295204	
JH0210	*Alexandrium affine*	Jinhae Bay, South Korea	NA	NA	NA	NA	Not sequenced	Not sequenced	AY775286	
JHW0004-12	*Alexandrium tamarense*	Korea	NA	NA	NA	NA	Not sequenced	Not sequenced	AY421777	
CCMP116	*Alexandrium tamarense*	Ria de Vigo, Spain	42.23	-8.8	1 June 1984	Yentsch, C.M.	HM483868	Not sequenced	Not sequenced	
CCMP1738	*Lingulodinium polyedra*	Gulf of Mexico, USA	27.8	-97.13	NA	Buskey, E.	Not sequenced	Not sequenced	EF492507	
DRW0208	*Lingulodinium polyedra*	Korea	NA	NA	Aug. 2001	NA	Not sequenced	Not sequenced	AY421788	
BLACK1	*Lingulodinium polyedra*	Black Sea, Ukraine	49.90	30.29	2011	Takano, Y.	AB678400	AB678399***	AB693195***	
CASP1	*Lingulodinium polyedra*	Caspian Sea, Iran	37.51	49.91	2011	Takano, Y.	AB678402	AB678401***	AB693194***	
SANPEDRO1	*Lingulodinium polyedra*	San Pedro Harbor, California, USA	33.74	-118.24	2011	Takano, Y.	AB678404	AB678403***	AB693196***	
GS0209	*Gonyaulax polygramma*	Gunsan, South Korea	NA	NA	NA	NA	Not sequenced	Not sequenced	AY775287	
11A1	*Protoperidinium tricingulatum*	Wadden Sea, Netherlands	53,60	6,58	2006	Kawami, H.	Not sequenced	Not sequenced	AB716917	
CCAP1140/3	*Parvodinium inconspicuum*	Kl. Ukleisee, Schleswig-Holstein, Germany	NA	NA	NA	Meyer	Not sequenced	Not sequenced	FR865631	
hwi d1	Unidentified	Hawaii	21,59	-158,10	04-mars-14	Anne de Vernal and Geneviève Vautour	**MG646298**	**MG646295**	**MG646320**	**Haifeng Gu**
GgSm10R	Unidentified	Malaysia	1,60	110,32	17-janv-13	Bao Juan Kam	**MG646305**	Not sequenced	**MG646326**	Haifeng Gu
GgSm11R	Unidentified	Malaysia	1,60	110,32	17-janv-13	Bao Juan Kam	**MG646306**	Not sequenced	**MG646327**	Haifeng Gu
GgSm01R	Unidentified	Malaysia	1,60	110,32	10-août-10	Toh Hii Tan	**MG646302**	Not sequenced	**MG646324**	Haifeng Gu
GgSm03R	Unidentified	Malaysia	1,60	110,32	22-sept-10	Toh Hii Tan	**MG646303**	Not sequenced	Not sequenced	Guat Ru Liow
GgSm07R	Unidentified	Malaysia	1,60	110,32	17-janv-13	Toh Hii Tan	**MG646304**	Not sequenced	**MG646325**	Zhen Fei Lim
PrTT02R	Unidentified	Malaysia	1,92	109,77	28-mars-13	Sing Tung Teng	**MG646308**	Not sequenced	Not sequenced	Haifeng Gu
PrTT03R	Unidentified	Malaysia	1,92	109,77	28-mars-13	Sing Tung Teng	**MG646309**	Not sequenced	Not sequenced	Haifeng Gu
PrTT01R	Unidentified	Malaysia	1,92	109,77	28-mars-13	Sing Tung Teng	**MG646307**	Not	Not sequenced	Haifeng Gu

						sequenced	

* = single cells or cysts sequenced through single-cell PCR; Accession numbers in bold denote sequences from this study. ** = these cultures showed presence of 5 precingular plates and are considered aberrant. *** = sequence not used in phylogenies. NA = Not acknowledged.