

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

Article

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1 | ~~The potentially toxic~~ *Pentaplacodinium saltonense* gen. et sp. nov. (Dinophyceae), and its
2 | relationship to the cyst-defined [genus](#) *Operculodinium psilatam* and ~~the~~ yessotoxin-producing
3 | *Protoceratium reticulatum*

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90

91

92 **Abstract**

93

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

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

120

121 | **Keywords**

122 | ~~yessotoxins, *Protoceratium*, *Pentaplacodinium*, *Protoceratium*~~, precingular plates, Salton Sea,
123 | *Ceratocorys*, *Operculodinium*, Cribroperidinioideae

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126

127

128 1. Introduction

129

130 The dinoflagellate genus *Protoceratium* was erected by Bergh (1881, p. 242) with
131 *Protoceratium aceros* as the type species (fig. 36), which was recovered from Strib, Denmark.
132 Bütschli (1885, p. 1007, plate 52, fig. 2) considered *Peridinium reticulatum* as described
133 earlier by Claparède and Lachmann (1858) from Bergen Fjord, Norway, as a senior synonym,
134 and he proposed the combination *Protoceratium reticulatum*. He also considered *Clathrocysta*
135 *reticulata* as described by Stein (1883) a junior synonym. The plate formula for *P.*
136 *reticulatum*, 4', 0a, 6'', 6''', 1p, 1''''', was first provided by Wołoszyńska (1929) through the
137 study of Baltic [Sea](#) specimens. Reinecke (1967) erected the name *Gonyaulax grindleyi* for
138 specimens from Elands Bay in Cape Town, South Africa, with the tabulation 3', 1a, 6'', 6''',
139 1p, 1'''''. Based on a detailed study of the theca of *Protoceratium reticulatum* from the North
140 Sea, Stosch (1969) considered *G. grindleyi* to be a junior synonym of *P. reticulatum*, although
141 he considered it assignable to the genus *Gonyaulax*. Dodge (1989) agreed with the tabulation
142 of Reinecke (1967), but retained the genus *Protoceratium* because he considered it different
143 from his emendation of the genus *Gonyaulax*, by having only one intercalary plate. Hansen et
144 al. (1997) restudied specimens close to the type locality of *P. aceros*, and based on the plate
145 analysis concluded that *P. reticulatum*, *P. aceros* and *G. grindleyi* were conspecific, and
146 agreed with the tabulation of Wołoszyńska (1929). Paez-Reyes and Head (2013) [reviewed](#)
147 [the morphological variability reported for *P. reticulatum* and](#) concurred with Dodge (1989) in
148 maintaining *Protoceratium* as a distinct genus from *Gonyaulax*.

149 ~~Since the early 1900's, s~~Seven other *Protoceratium* species have been described [since](#)
150 [the early 1900s, and](#) the latest review of these taxa [was having been](#) performed by Schiller
151 (1937 p. 322–326). Kofoid (1907) described *P. areolatum* from the tropical Pacific and
152 emended the genus for the first time. Meunier (1910) described a very similar species from

153 the Kara Sea that he named *Protoceratium splendens*, which is possibly a junior synonym, as
154 suggested by Gómez (2012). Later, Kofoid in Kofoid and Michener (1911) emended
155 *Protoceratium* once more to include several new species from the eastern tropical Pacific that
156 were described without illustration (*P. cancellorum*, *P. globosum*, *P. pellucidum*, *P. pepo*, *P.*
157 *promissum*), and he suggested a tabulation formula for the genus: 2', 0a, 6" (?c), 6"', 0p, 3''''.

158 Schiller (1937) transferred *Clathrocysta aculeata* as described by Stein (1883) to
159 *Protoceratium aculeatum*, presumably based on the fact that Bütschli (1885) [had](#) considered
160 the genus *Clathrocysta* described by Stein 1883 as a junior synonym of *Protoceratium*.

161 Schiller (1937) transferred *Peridinium spinulosum* as described by Murray and Whitting
162 (1899) to the genus *Protoceratium*. Later, Balech (1988) rediscovered this species in the
163 South-~~W~~west Atlantic and suggested yet [another variation on the](#) tabulation for
164 *Protoceratium*, 3', 0a, 6", 6"', 2''''', based on his observations of *Protoceratium spinulosum*.

165 *Protoceratium reticulatum* (Claparède et Lachmann) Bütschli 1885 is a very common
166 dinoflagellate found in cold and warm waters, as well as in oceanic and neritic environments
167 (e.g., [as *Operculodinium centrocarpum* in Zonneveld et al., 2013](#)). [-Its resting cyst distribution](#)
168 [today reveals a strong link with the North Atlantic Current, an association traceable through](#)
169 [the upper Cenozoic fossil record \(Hennissen et al., 2017 and references therein\)](#).

170 *Protoceratium reticulatum* is considered potentially toxic because of its production of
171 yessotoxins (e.g. Paz et al., 2008; Sala-Pérez et al., 2016). It has been successfully isolated
172 [and cultured](#) from many parts of the world, and grown into cultures. Cysts of *P. reticulatum*
173 were first observed in cultures established from motile cells from the inner Oslofjord-~~↵~~
174 Norway) by Braarud (1945). This cyst was related by Wall and Dale (1966, 1967, 1968) to the
175 cyst-defined species described from the Miocene of Australia, *Operculodinium centrocarpum*
176 (Deflandre et Cookson 1955) Wall 1967. [That assignation was challenged by Head and](#)
177 [Wrenn \(1992\) and Head \(1996a\) on the grounds that *Operculodinium centrocarpum* was](#)

178 [larger and more robust than the cysts recorded by Wall and Dale \(1966\) from modern](#)
179 [sediments.](#) ~~However, a~~ restudy of the holotype of *Operculodinium centrocarpum* ~~refuted~~
180 ~~this assignment~~ [confirmed this](#), and the name “cyst of *Protoceratium reticulatum*” was
181 recommended (Matsuoka et al., 1997). Wall and Dale (1968) proposed that *P. reticulatum*
182 was also related to the cyst-defined *Operculodinium psilatatum* Wall 1967 and furthermore
183 possibly to *Operculodinium israelianum* (Rossignol 1962) Wall 1967. The cyst-defined
184 *Pyxidinosia psilata* (Wall et Dale in Wall et al., 1973) Head 1994 was subsequently also
185 linked to *Protoceratium reticulatum* (Dale, 1996, as *Tectatodinium psilatatum*) although this
186 connection was [later](#) questioned ~~later~~ (Mertens et al., 2011). Because of uncertainty regarding
187 the links between the cysts produced by *P. reticulatum* ~~(see Head, 2006)~~, and ~~cysts~~ ~~cyst-~~
188 ~~defined species named based on~~ [from the](#) fossil records, [Head \(1996a, 1996b\) and subsequent](#)
189 [authors used the](#) ~~the~~ term “*Operculodinium centrocarpum* sensu Wall and Dale, 1966” ~~was~~
190 ~~used~~ to describe the cysts that had [first](#) been observed by Braarud (1945) and Wall and Dale
191 (1966). With the removal of *Pyxidinosia psilata* as a potential cyst of *Protoceratium*
192 *reticulatum*, Paez-Reyes and Head (2013) argued on the basis of non-overlapping geographic
193 distribution that the “cyst of *Protoceratium reticulatum*” was now unambiguous and should
194 replace the term “*Operculodinium centrocarpum* sensu Wall and Dale, 1966”. That approach
195 is followed here. Recent studies of variation in the process length of cysts of *Protoceratium*
196 *reticulatum* have been related to variations in sea surface salinity and other parameters (e.g.,
197 Mertens et al. 2011; Jansson et al., 2014), and the cyst wall appears to be composed of
198 cellulose glucan (Bogus et al. 2014). Resting cyst production through sexual reproduction has
199 recently been demonstrated by Salgado et al. (2017).

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

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

221 | The Salton Sea is the largest saline lake in California with a surface area of 980 km²
222 | (Reifel et al., 2002). It has a mean depth of 8 m and a maximum depth of 15 m (Ferrari and
223 | Weghorst, 1997). Although originally composed of relatively freshwater, it has become saline
224 | due to a lack of outflow and high evaporation rates. During 1997–1999, the salinity was
225 | between 41 and 45 g l⁻¹ (Watts et al., 2001), while the temperature varied between about 12
226 | and 40°C seasonally (Watts et al., 2001; Holdren and Montaña, 2002). Oxygen at times was
227 | supersaturated due to phytoplankton photosynthesis, but was also often severely depleted,

228 occasionally even in surface waters (Watts et al., 2001; Holdren and Montaña, 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~~ were compared (Table 1, Suppl. Table 1). In addition, ~~we studied the~~ phylogenies are
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 Microscopemicroscope~~ (Germany). ~~When the~~
267 ~~s~~ Samples were ~~was~~ filtered, ~~by placing an 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 Light m~~ Microscope ~~(Germany) with~~
273 ~~magnification 10x5x~~. 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.

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

282 For scanning electron microscopy (SEM) of culture CCMP 3243 by K.N.M., the
283 culture was filtered and washed with distilled water and dehydrated in a graded ethanol series
284 (30 to 100% in six steps). The filters were encased in metallic baskets, critical-point dried
285 with CO₂ (CPD Bal-Tec 030), glued onto stubs, sputter coated with platinum/palladium for 90
286 s (JEOL JFC-2300 HR) and examined in a JEOL 6330F scanning electron microscope (JEOL,
287 Tokyo, Japan) at the University of Copenhagen.

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

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

297

298 2.2. Germination experiment of cysts of *P. saltonense*

299

300 Sediment samples were collected from the Salton Sea ~~(CA, U.S.A.) at on the same time day~~
301 ~~of as plankton sample collection on, during the same field campaign, on 24 October, 2013,~~
302 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 cm³ of wet sediment was immersed in filtered seawater and, after one
306 | ~~minute~~ 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~~scientific 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 *Pentaplagodinium 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

328 ~30 sec and finally collected on a 15 µm mesh. Aliquots of residue were mounted on
329 microscope slides using glycerine jelly.

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

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

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

350

351 *2.4. Single-cell polymerase chain reaction (PCR) amplification and sequencing of culture of*
352 *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'- TGATCCTTCTGCAGGTTACCTAC-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 MgCl₂, 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-HCl pH 8.3 (at 25 °C), 50 mM KCl, 2 mM MgCl₂, 0.001 % w/v
412 gelatin, 400 µM dNTP's, 1 µM of each primer, and 1 U Taq polymerase. Amplifications were
413 performed using the following parameters: 94 °C for 5 min followed by 35 cycles of 95 °C for
414 30 s, 50 °C for 45 s, 72 °C for 1 min, and a final extension of 72 °C 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 beads [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 TGATCCTTCTGCAGGTTACCTAC-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 MgCl₂, 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 ~~XXXXX~~[MG646283-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

477 | Division DINOFLAGELLATA (Bütschli 1885) Fensome et al. 1993**b**
478 | Class DINOPHYCEAE Pascher 1914
479 | Subclass PERIDINIPHYCIDAE Fensome et al. 1993**b**
480 | Order GONYAULACALES Taylor 1980
481 | Suborder Gonyaulacineae autonym
482 | Family uncertain
483 | Genus *Pentaplacodinium* Mertens, Carbonell-Moore, Pospelova et Head gen. n. (Plate 3)
484 | Type: [Plate 3A, the holotype of *Pentaplacodinium saltonense*](#) gen. et sp. nov.
485 | *Diagnosis:* A [gonyaulacoid-gonyaulacinean](#) genus with roundish to [slightly](#)-polyhedral thecae
486 | [with bearing](#) heavily reticulated plates without [appendicesappendages](#). The tabulation is Po,
487 | Pt, [4' or 2'+*2'](#), 5'', ~~6C6c~~, ~~6S6s~~, ~~*65'''~~, 1p, 1''''', cover plate is oval.
488 | *Etymology:* The name is derived from the Greek words *penta* meaning five, *plax* plate, and
489 | *dino* whirling; with reference to the five precingular plates that characterize this dinoflagellate
490 | genus.
491 | *Pentaplacodinium saltonense* Mertens, Carbonell-Moore, Pospelova et Head gen. et sp. n.
492 | (Plates 3, 4, Figs 2A, 3, 4A)
493 | *Synonymy:*
494 | 1970 *Protoceratium reticulatum* (Claparède et Lachmann); Steidinger and Williams, p. 62,
495 | plate 38, fig. 140a–c.
496 | 1991 *Protoceratium reticulatum* (Claparède et Lachmann); Al-Muftah, pp. 180–181, figs.
497 | 246–247.
498 | ? 2002 *Protoceratium reticulatum* (Claparède et Lachmann); Reifel et al., p. 275.
499 | 2005 *Gonyaulax grindleyi* Reinecke; Faust et al., p. 110, figs. 2–4.
500 | ? 2007 *Gonyaulax grindleyi* Reinecke; Tiffany et al., p. 582.

501 | ? 2009 "Protoceratium globosum" Kofoid ~~et~~ Michener; Morquecho et al., p. 18, 20, figs.
502 | 13–17.

503 | *Diagnosis:* Theca roundish to somewhat polyhedral with tabulation Po, Pt, 2'+*2', 35''+*2'',
504 | 6C6c, 6S6s, *65''', 1p, 1''', with 3'' interpreted as *(3''+4''). The theca has an L-type ventral
505 | organization and dextral torsion. The plates are heavily reticulated with one pore inside each
506 | reticulation, although two or more pores might be found in reticulations next to a suture. The
507 | ends of the descending cingulum are displaced by ~2.0 widths. The cysts have an
508 | approximately roundish-spherical central body with a thin pedium and thicker spongy-fibrous
509 | luxuria. ~~Processes-Process distribution apparently have a nonintran~~tabular ~~distribution,~~ are
510 | Processes fibrous and distally tapering, and have acuminate to minutely expanded distal ends.
511 | The archeopyle corresponds to the ~~third~~ *(3''+4'') precingular plate and has a smooth margin
512 | with rounded angles. The operculum is free.

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

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

515 | *Gene sequence:* The 28S and 18S gene sequence of the cell isolated from culture 2E3,
516 | established from a cyst extracted from surface sediment from station 2 in the Salton Sea

517 | (Table 1). —GenBank Accession No. ~~XXXXXX~~ MG646301 (18S) and ~~XXXXXX~~
518 | MG646323 (28S). Several other strains are considered to belong to the same species (Suppl.
519 | Table 1).

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

523 | *Description:* Motile cells observed in the Salton Sea plankton samples (Plate 3, except D).

524 | ~~The cell shape varies from~~ Thecae have a roundish to somewhat polyhedral shape (Plate 3A,
525 | C). ~~The thecae have and~~ a typical sexiform gonyaulacoid tabulation (sensu Fensome et al.,

526 | 1993**b**, ~~t~~Text-Fig. 64B) with an L-type ventral organization (sensu Fensome et al., 1993**b**,
527 | Text-Figs. 82A, C) and dextral torsion (sensu Fensome et al., 1993**b**, 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 | ~~occur~~be 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,~~properly 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. 1993**b**, 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) on the epitheca, and *4''' forming the keystone plate on 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 spherical~~roundish~~. 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 $\sim 1.0 \mu\text{m}$ 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 [eyst](#)
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, †~~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.

601 | *Dimensions:* The ~~holotype~~-cell [illustrated in Plate 3A, the holotype figure](#), is 44 μm in length,
602 | 41 μm in width and 38 μm in depth. Germinated motile cells: length, 48.1 (53.7) 63.4 μm
603 | (SD=6.0, n=5); width, 38.5 (42.4) 47.5 μm (SD= 3.2, n=5). Cells observed in plankton from
604 | 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
605 | μm (SD=4.2, n=28).

606 | Two single cysts germinated to give the identifiable thecae: maximum central body diameter,
607 | 52.3 (53.5) 54.7 μm (SD=1.7, n=2); minimum central body diameter, 51.1 (52.2) 53.3 μm
608 | (SD=1.6, n=2); average length of three randomly chosen processes per cyst, 2.4 (3.0) 3.6 μm
609 | (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)
610 | 2.1 (SD=0.3, n=6). Palynologically treated cysts from surface sediments of the Salton Sea:
611 | maximum central body diameter, 48.6 (56.3) 70.9 μm (SD=5.3, n=23); minimum central body
612 | diameter, 45.7 (52.1) 61.4 μm (SD=3.8, n=22); average length of three processes per cyst, 1.0
613 | (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
614 | thickness 0.9 (1.6) 2.4 (SD=0.4, n=66).

615 | *Comments:* *Pentaplacodinium saltonense* n. gen et sp. is defined [primarily](#) from the characters
616 | of the motile stage, these distinguishing it from species of the genus *Protoceratium*. The
617 | morphology of several thecae observed from off Yucatan (Gulf of Mexico), the Indian River
618 | Lagoon (Florida, USA), and off Qatar (Persian Gulf) (Table 1; Plate 2) and from cultures
619 | established from cells from Biscayne Bay (Florida, USA) (CCMP1720, CCPM1721), the
620 | Indian River Lagoon (Florida, USA) (CCMP3241, CCMP3243) and the Salton Sea
621 | (California, USA) (CCMP404) ([Suppl. Table 13](#), Plate 1) agree with the description of *P.*
622 | *saltonense* given above. Cysts formed from cultures established from a strain from the Indian
623 | River Lagoon (Florida, USA) (CCMP3243) have the same morphologies (Plate 6). The
624 | observed cysts correspond [most closely](#) to the fossil based ~~taxon-species~~ [Operculodinium](#)
625 | [israelianum](#) (Rossignol 1962) Wall 1967 described from the Pleistocene of Israel, and

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

631

632 3.3. Phylogenetic position of *P. saltonense* and other studied strains

633

634 The SSU rDNA sequences for all *P. reticulatum* strains analysed were identical, forming a
635 distinct clade separated from the *P. saltonense* sequences which were identical to the
636 unidentified Malaysian sequences (Fig. 5). *P. reticulatum* and *P. saltonense* sequences shared
637 92% nucleotide identity for the V9 region analysed.

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

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

649 The three phylogenies ([Figures 5–7](#)) show that strains identified as *Protoceratium*
650 *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 ~~eingulum-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

676 | between Sa and 1' is very narrow ~~whilst~~ whereas in *P. reticulatum* this contact is wide — this
677 | causes an ~~evident~~ conspicuous separation between 1'' and 6'' in *P. reticulatum*, when in *P.*
678 | *saltonense* there is ~~an apparent~~ almost a small point of contact between those two plates (Plate
679 | ~~4D3D~~). *Gonyaulax grindleyi* Reinecke 1967 is ~~here considered~~ shown to be a synonym of *P.*
680 | *reticulatum*, as ~~it has been~~ already suggested by von Stosch (1969) and Hansen et al. (1997),
681 | and ~~is~~ now confirmed by the LSU rDNA phylogeny in this study (see below).

682 | Several other *Protoceratium* species have been described (e.g. Schiller, 1937, p. 322–
683 | 326). *Protoceratium splendens* Meunier 1910 from the Kara Sea has six precingular plates; it
684 | is possibly a junior synonym of *Protoceratium reticulatum*, as suggested by Gómez (2012).
685 | *Protoceratium aculeatum* (Stein 1883) Schiller 1937 bears antapical spines and an apical
686 | horn. *Protoceratium areolatum* Kofoid 1907 and *Protoceratium spinulosum* (Murray and
687 | Whitting 1899) Schiller 1937 have fewer reticulations in both the epitheca and hypotheca than
688 | *P. saltonense*. Of the five species described by Kofoid and Michener (1911~~:-~~), *Protoceratium*
689 | *cancellorum*, *Protoceratium pellucidissimum*, *Protoceratium pepo*, *Protoceratium globosum*
690 | and *Protoceratium promissum*, none ~~have~~ has illustrations and it is therefore ~~it is~~ impossible
691 | to compare them to *P. saltonense*.

692 | *Pentaplacodinium saltonense* differs from *Ceratocorys anacantha* Carbonell-Moore
693 | 1996 because it is not as polyhedral. In addition, in contrast to the insert epithecal
694 | configuration of *P. saltonense*, *C. anacantha* has an episert type I epithecal configuration,
695 | meaning that 1' does not contact the anterior sulcal plate and that 1'' and 6'' are in contact
696 | (Paez-Reyes and Head, 2013).

697 |

698 | 4.2. Comparison of the cyst of *P. saltonense*

699 |

700 The cyst of *Pentaplacodinium saltonense* ~~corresponds to~~ compares with *Operculodinium*
701 *psilatum* because its cysts display an alignment of processes along the cingulum, it bears short
702 processes (1.0–5.7 μm ~~vs. 2 μm (Wall, 1967) or 0.0–2.9 μm (Head, 1996)~~), its body diameter
703 is of similar size (45.7–70.9 μm vs. 50–60 μm ~~(, Wall, 1967);~~ or 62–79 μm ~~(, Head, 1996b)~~)
704 and its wall thickness (0.9–2.4 vs. 1.4–2.2 μm ~~(; Head, 1996b)~~) is similar. *Operculodinium*
705 *psilatum* differs, however, in having processes that in general are shorter (2 μm , Wall, 1967;
706 0.0–2.9 μm , Head, 1996b) and sparsely distributed. The cingulum and sulcus are also more
707 conspicuously expressed in *Operculodinium psilatum* (Wall, 1967; Head et al., 1996b), and *O.*
708 *psilatum* lacks the wide archeopyle of *P. saltonense*.

709 The cyst of *P. saltonense* is also similar to *Operculodinium israelianum* (Rossignol
710 1962) Wall 1967 as described by Rossignol (1964, as *Baltisphaeridium israelianum*);
711 ~~however,~~ although the processes of the latter species are longer (6–10 μm) than ~~of the~~
712 ~~specimens from Salton Sea~~ for *P. saltonense* (1.0–5.7 μm). ~~At this time, we do not~~ It is not
713 presently known whether variation in process length is related to variations in ecology, similar
714 ~~to~~ as demonstrated for the cysts of *Lingulodinium polyedra* (= *Lingulodinium*
715 *machaerophorum*) (Mertens et al., 2009), cysts of *Protoceratium reticulatum* (Mertens et al.,
716 2011) and cysts of *Pyrodinium bahamense* (= *Polysphaeridium zoharyi*) (Mertens et al., 2015).
717 ~~In addition,~~ The process distribution appears to be ~~is~~ intratabular for the cysts of *P.*
718 *saltonense* and often shows parallel alignments, which is not readily visible and this is likely to
719 be the case also for ~~on~~ *O. israelianum* (e.g., *O. cf. israelianum* of Head, 1997, fig. 17.2),
720 although the study of topotype material will be needed for confirmation. The rounded angles
721 of the archeopyle in *O. israelianum*, *O. psilatum* and the cysts of *Pentaplacodinium*
722 *saltonense*, and the shared presence of a spongy-fibrous to fibroreticulate luxuria, accentuate
723 the overall similarities between these cysts, although the relatively wider archeopyle in *P.*
724 *saltonense* cysts distinguishes them from these other species.

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

738 The cysts of *P. saltonense* differs from ~~the cysts~~ of *P. reticulatum*; ~~due to the in~~
739 ~~having a~~ thick spongy-fibrous luxuria ~~wall~~ (vs. thin, fibrous luxuria), less developed distal
740 ends of the processes, ~~larger central its-body size-diameter~~ (48.6–70.9 μm vs. 33–48 μm;
741 Rochon et al., 1999), and generally shorter process length (1.0–5.7 μm vs. typically 7–14 μm;
742 Rochon et al., 1999) (48.6 (56.3) 70.9 μm (SD=5.3, n=23)) for *P. saltonense*, as opposed to
743 33–48 μm for *P. reticulatum* (Rochon et al., 1999) and its process length (1.0 (3.1) 5.7 μm
744 (SD=1.2, n=66)) for *P. saltonense*, as opposed to typical 7–14 μm for *P. reticulatum* (Rochon
745 et al., 1999); although the cysts of *P. reticulatum* shows very wide variation vary widely sizes,
746 with ~~formation of some being~~ completely bald ~~eysts~~ (e.g., Mertens et al., 2012a; Jansson et al.,
747 2014).

748 ~~Several~~ Numerous other *Operculodinium* species have been described and a detailed
749 comparison is given by ~~we refer to~~ Marret and Kim (2009) ~~for their detailed comparison, as;~~
750 none of these closely resembles the cysts of *P. saltonense*.

751

752 4.3. Phylogenetics, evolution and position and relationships of *Protoceratium*,
753 *Pentaplagodinium*, and *Ceratocorys*

754

755 ~~There are s~~ Several morphological characteristics of the theca ~~that~~ are important ~~to~~ in
756 understanding the evolution of *Protoceratium*, *Pentaplagodinium*, and *Ceratocorys* ~~the three~~
757 genera in question (Plate 7). The shape of the cover plate of *Ceratocorys* is more similar to
758 that of the cover plate of *Pentaplagodinium*, but less similar to the sigmoidal cover plate of
759 *Protoceratium*.

760

Pentaplagodinium and *Ceratocorys* can be considered closer to *Gonyaulax* than
761 *Protoceratium*, because the anterior intercalary is always well-separated from the apical pore
762 plates, ~~whilst~~ whereas in *Protoceratium reticulatum* it is closer and has even been suggested
763 to contact the apical pore plates (Hansen et al. 1997). ~~However,~~ It should ~~however be noted,~~
764 however, ~~that~~ *Protoceratium-reticulatum* and *Gonyaulax* have six precingular plates, whereas
765 ~~whilst~~ *Ceratocorys* and *Pentaplagodinium* have five precingulars. So it is not surprising that
766 in the molecular phylogenies, *Pentaplagodinium* has an intermediate position between
767 *Ceratocorys* and *Protoceratium* (Figs. 5–7); the relation to other gonyaulacoids at this time is
768 unclear and further molecular studies of related genera are required, particularly to understand
769 how to resolve the position of *Protoceratium* at family level. Another issue regards a conflict
770 in the dual nomenclature: the cyst of *P. reticulatum* and *P. saltonense* both are considered to
771 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

797 later toxin analysis by LC-MS of the same strains was negative (Paz et al., 2007), and the
798 authors considered that these strains had lost their toxicity after a number of years in culture.
799 The ~~produced~~ toxins produced by these strains of *Pentaplacodinium saltonense* are similar to
800 toxins produced by strains ~~we~~ identified as *Protoceratium reticulatum*, all of which are
801 yessotoxin producers, such as strains from Chile (Alvarez et al., 2011), Jervis Inlet, British
802 Columbia, Canada (Cassis, 2005), German Bight, ~~the~~ North Sea (Röder et al., 2011, 2012),
803 Okkirai Bay, Japan (Koike et al., 2006) and Spain (Paz et al., 2007, 2013). ~~The presence of *P.*~~
804 ~~*saltonense* in the Salton Sea has been considered a potential causative agent of mortality~~
805 ~~events in the Salton Sea indicates that it is potentially toxic~~ (Reifel et al., 2002, whom
806 identified it as *Protoceratium reticulatum*). ~~However, t~~However, there have not however not
807 been ~~no~~ reports of toxic events knowingly involving *P. saltonense*.

808 Several other studies have investigated the toxicity of strains ~~that~~ they designate as
809 *Protoceratium reticulatum*, but for which we could not verify the identifications could not be
810 verified (e.g., Satake et al., 1999; Ciminiello et al., 2003; Samdal et al., 2004; Finch et al.,
811 2005; Eiki et al., 2005; Mitrovic et al., 2005; Guerrini et al., 2007; Suzuki et al., 2007).

812

813 5. Conclusions

814

815 *Pentaplacodinium saltonense* gen. et sp. nov. is described from the Salton Sea (CA, USA).
816 The distinct cover plate (similar to *Ceratocorys*, but sigmoidal in *Protoceratium*), five
817 precingular plates (also five as in *Ceratocorys*, but six in *Protoceratium*), the very narrow
818 contact between 1' and Sa (wide contact in *Protoceratium*, no contact in *Ceratocorys*), a more
819 roundish-rounded eell-thecal shape, the displacement of the cingulum by two widths (~~vs.~~ one
820 width in *Protoceratium*), as well as the clear separation and distances seen in the three
821 phylogenies, justifies the creation of a new genus. The chorate cysts of *P. saltonense* bear

822 short processes often with parallel alignments. These cysts correspond to the cyst-defined
823 species-genus *Operculodinium* Wall 1967, and are most similar to *O. israelianum* and *O.*
824 *psilatum* with short processes and often parallel alignments. The geographic distribution of
825 mMotile stages of *Pentaplacodinium saltonense* wasare confirmed infrom four widely
826 dispersed locations and its distribution is thereforeis considered, suggesting a subtropical to
827 tropical distribution for this species., and does not overlap with that of *Protoceratium*
828 *reticulatum* and *Pentaplacodinium saltonense* are not known to inhabit the same
829 environments. Similar toAs with the -yessotoxin-producing *Protoceratium reticulatum*,
830 *Pentaplacodinium saltonense* is potentially a yessotoxin producer, as shown by previous
831 studies.

832

833 Note added: While this paper has been going through the process of final acceptance to
834 Harmful Algae, another study was accepted (Salgado et al., accepted) that addresses similar
835 scientific questions.

836

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851 | SEM images of *P. saltonense* from cultures established from the Indian River Lagoon.

852

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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 epithelial 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
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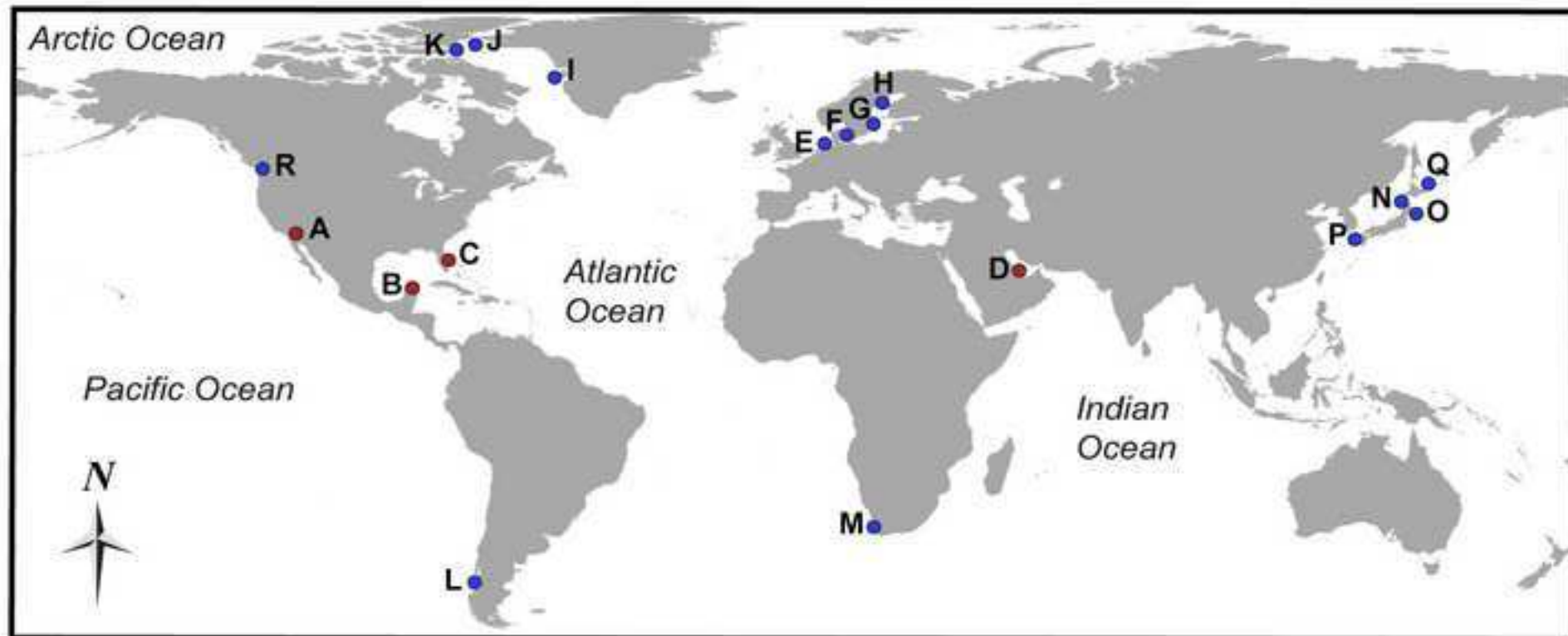


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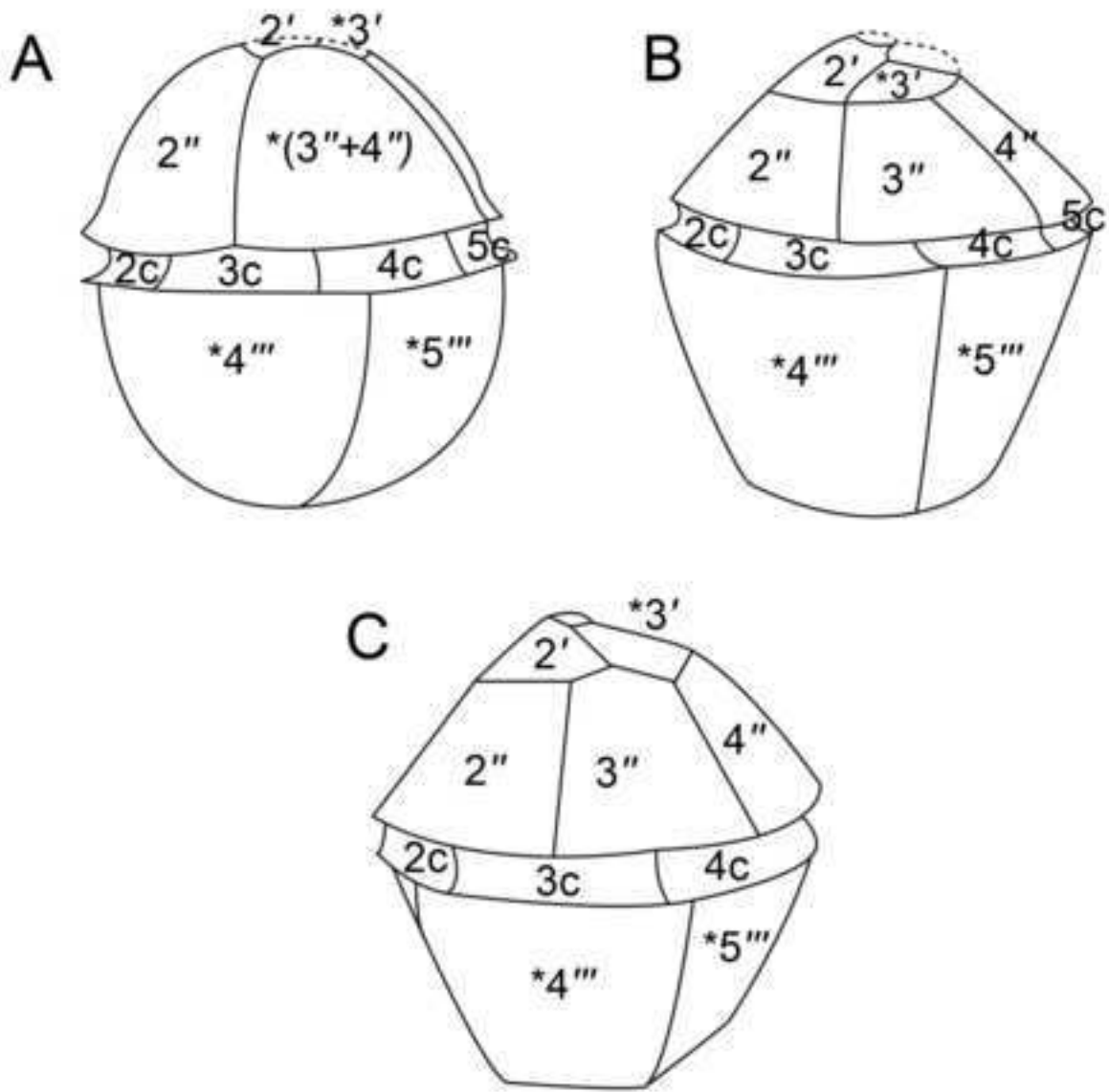


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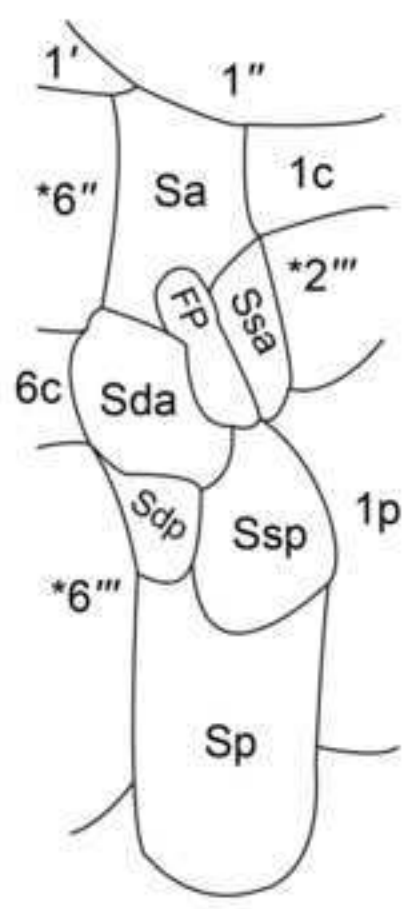


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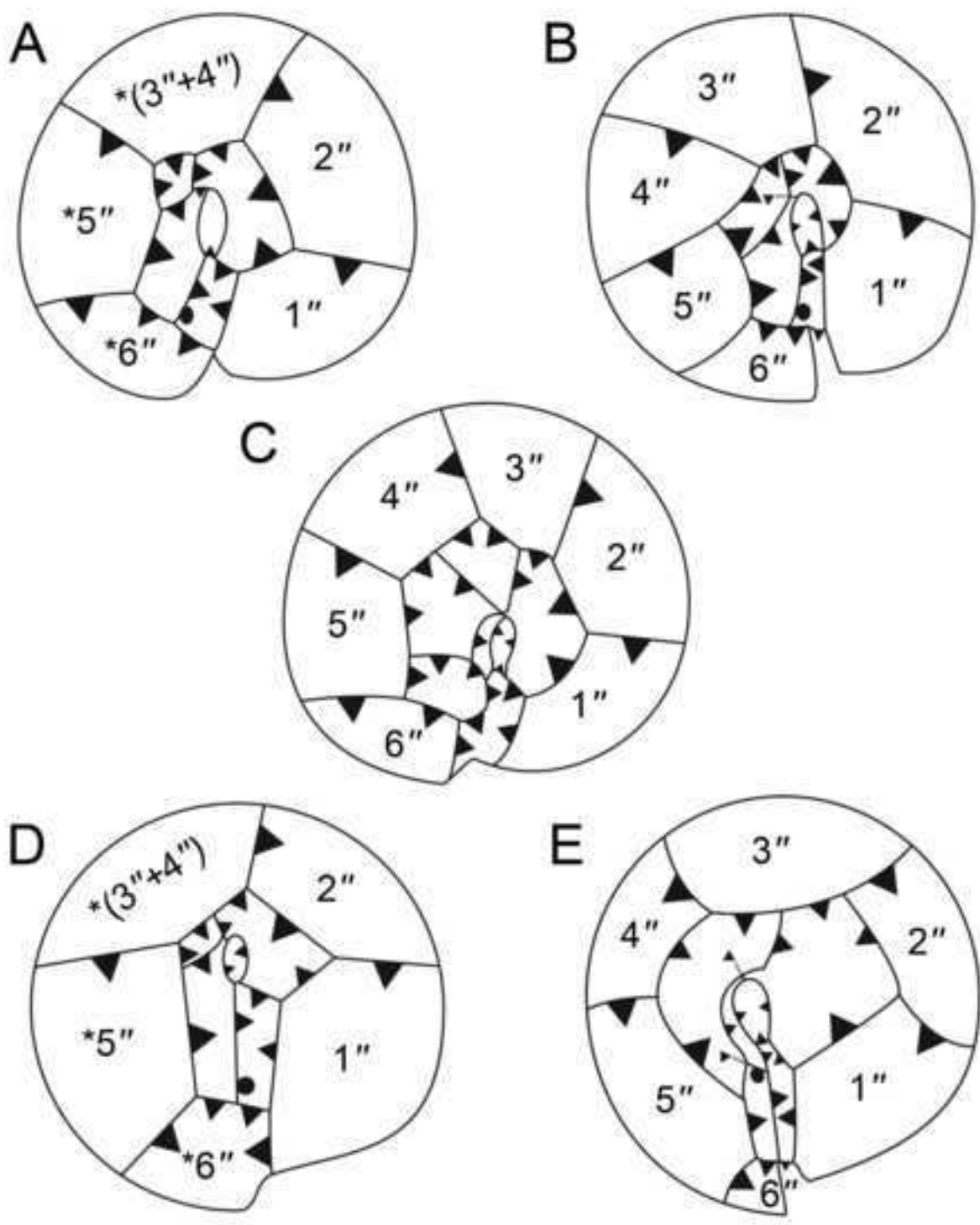


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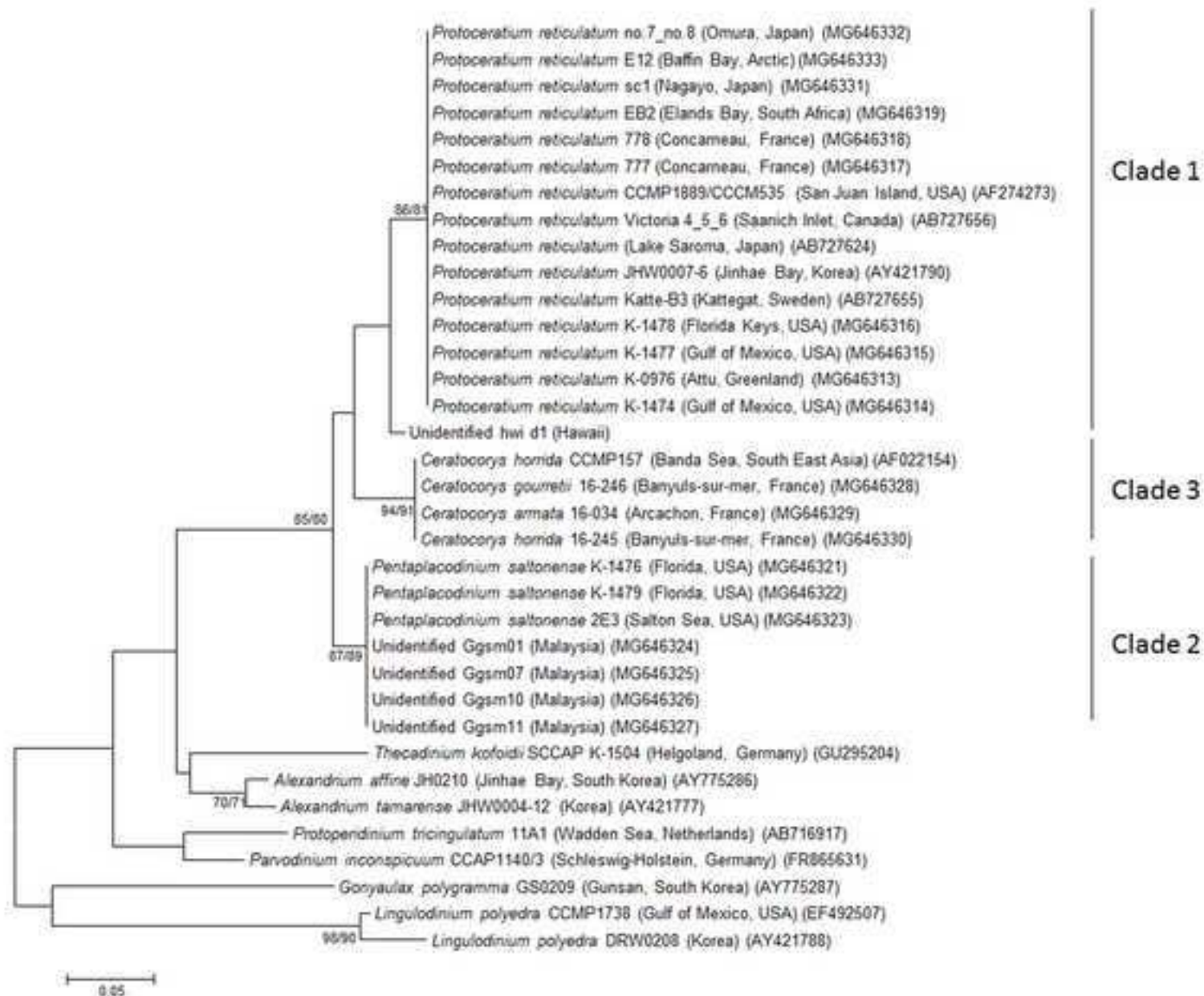


Figure 5

Figure 6
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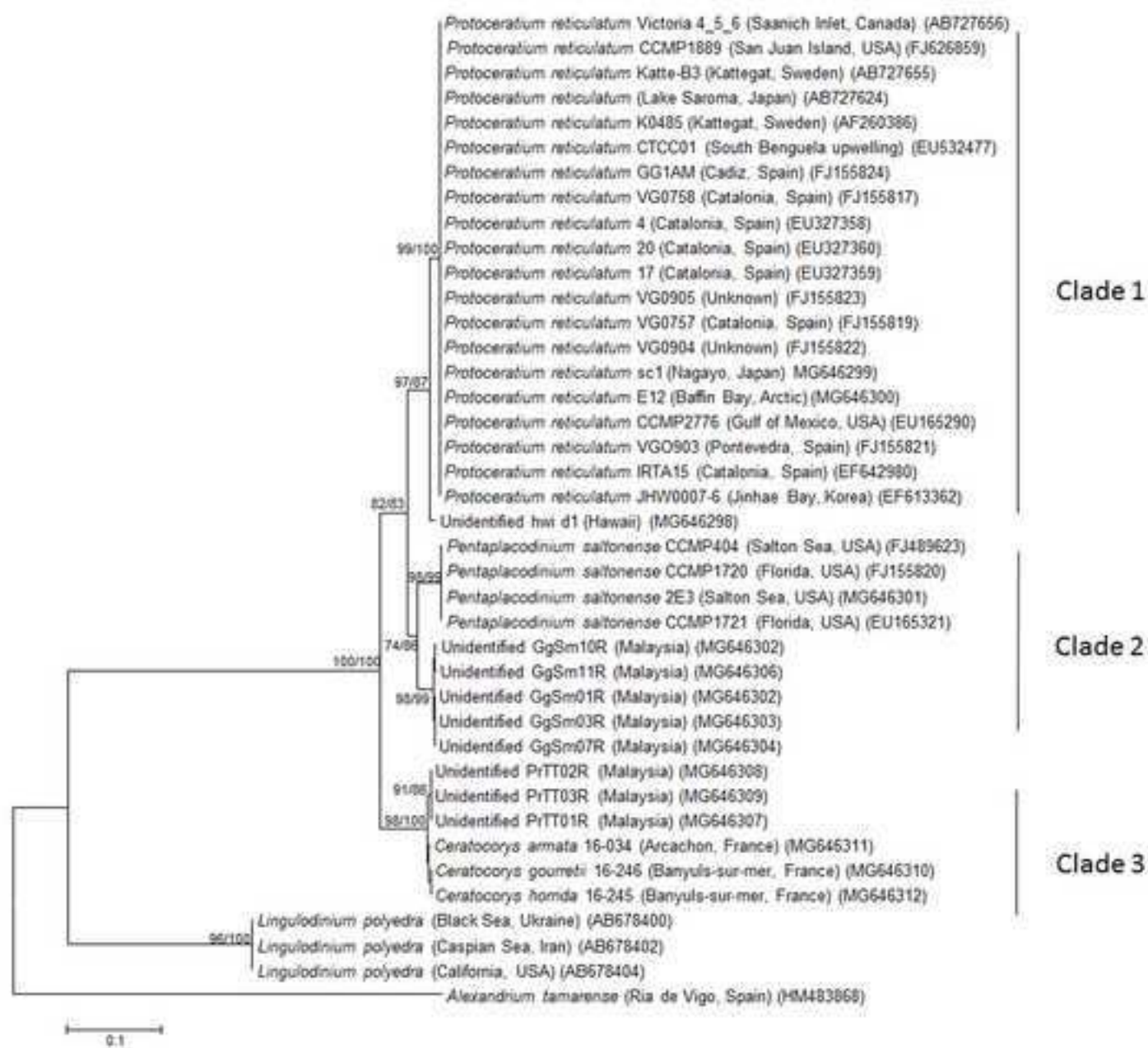


Figure 6

Figure 7
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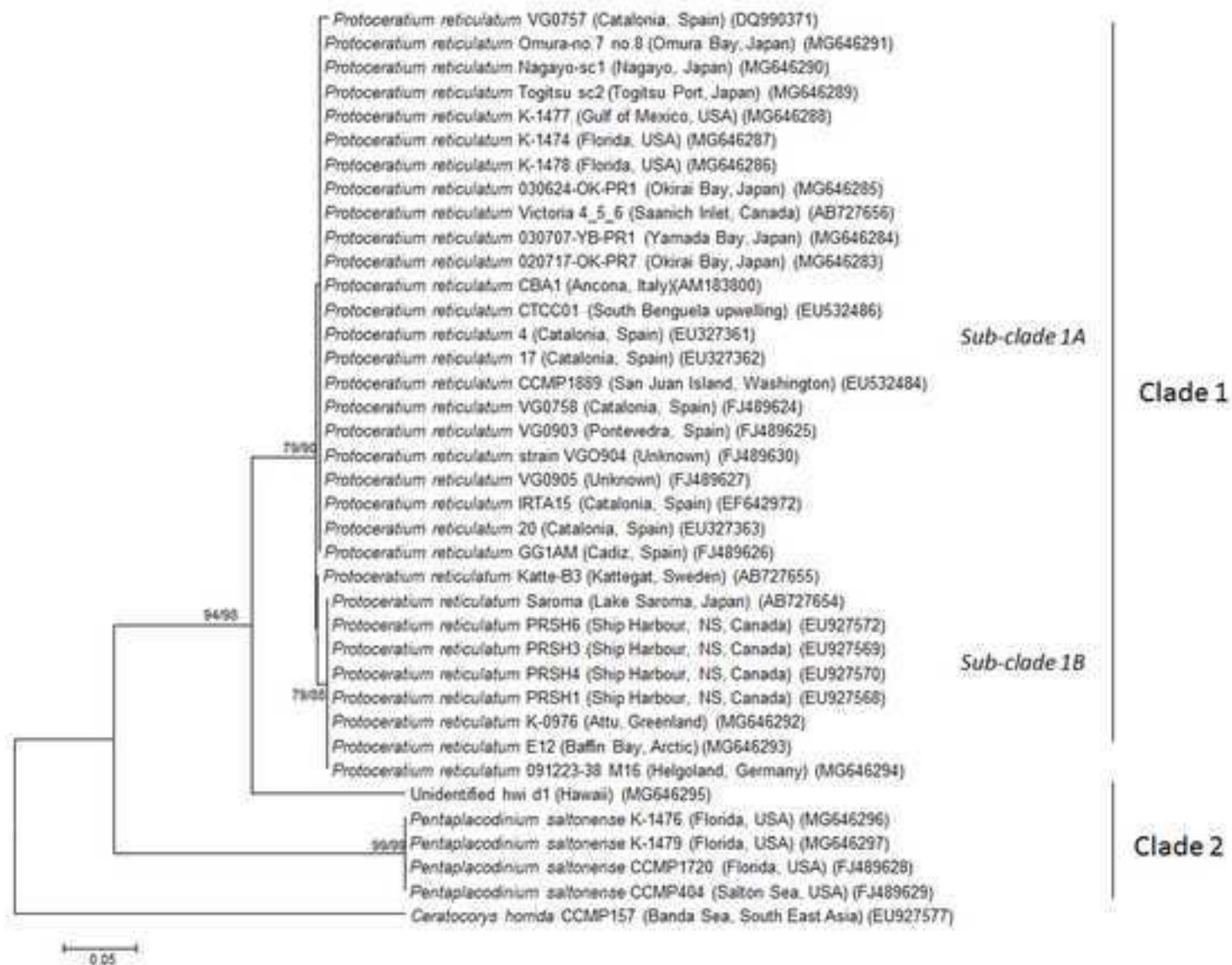
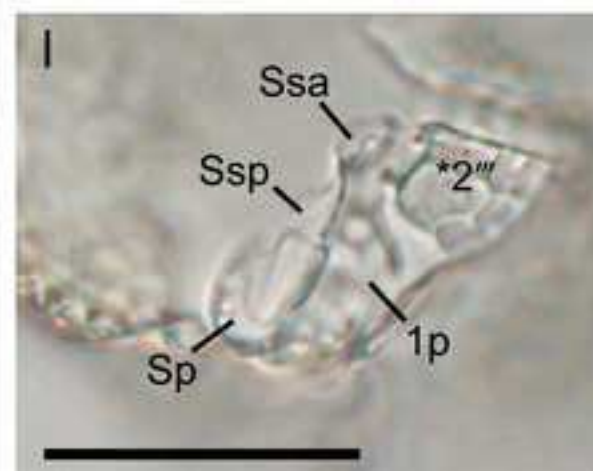
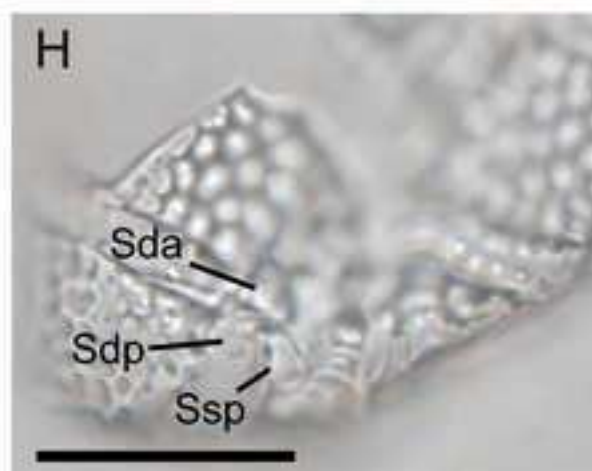
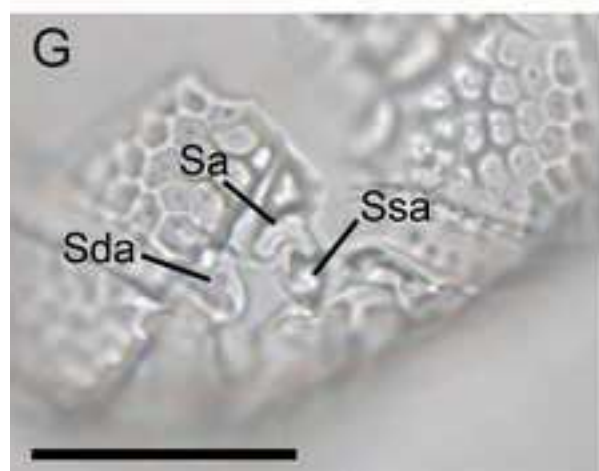
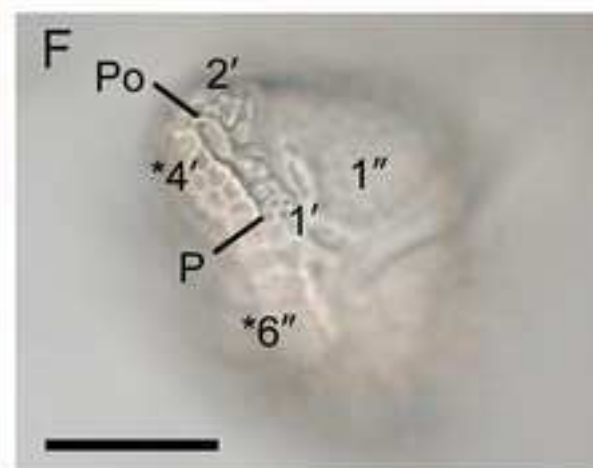
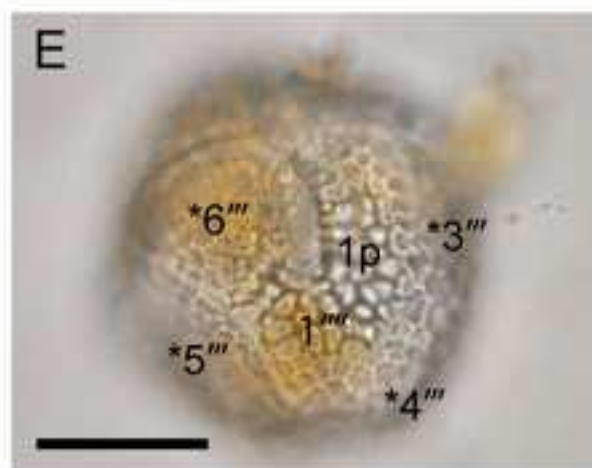
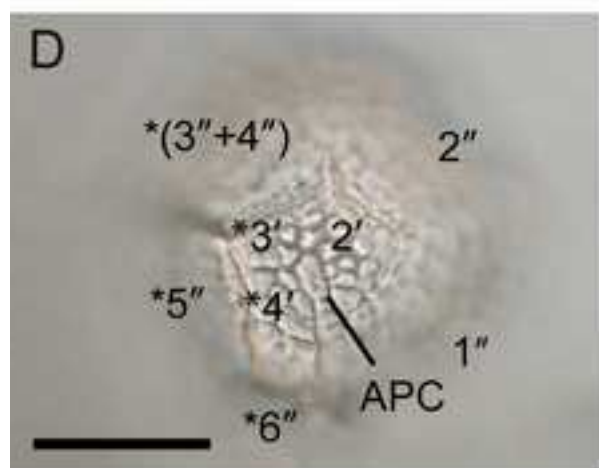
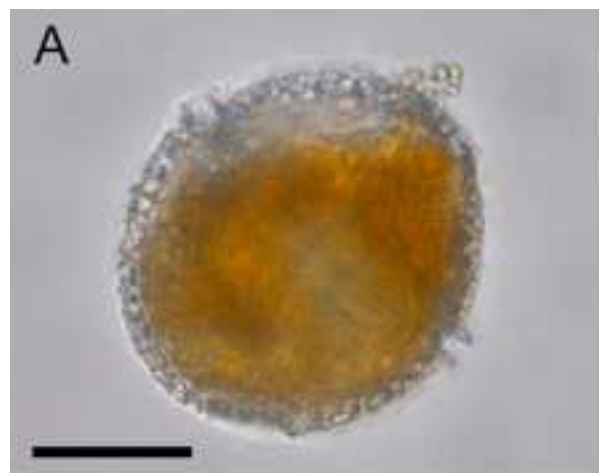
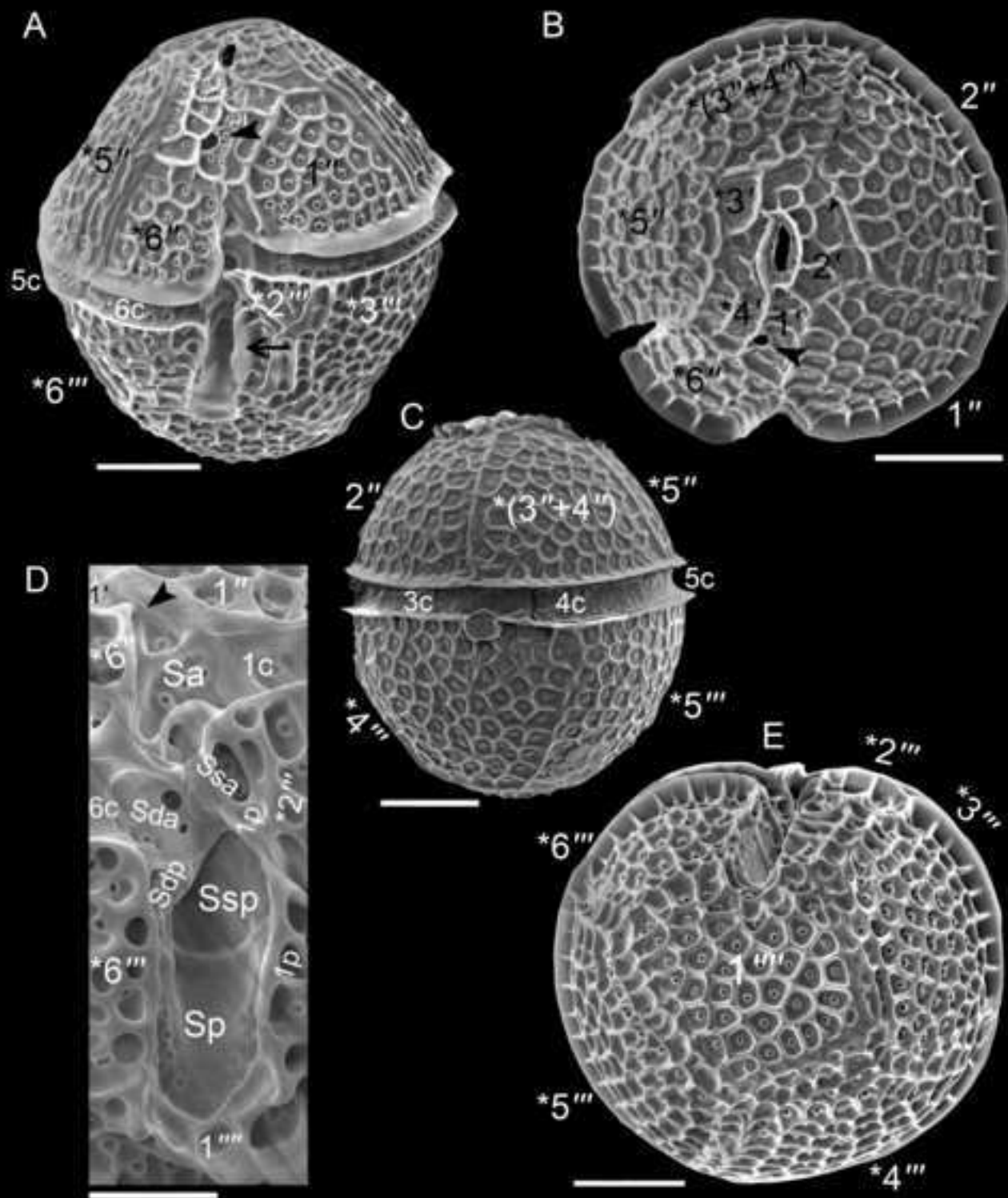
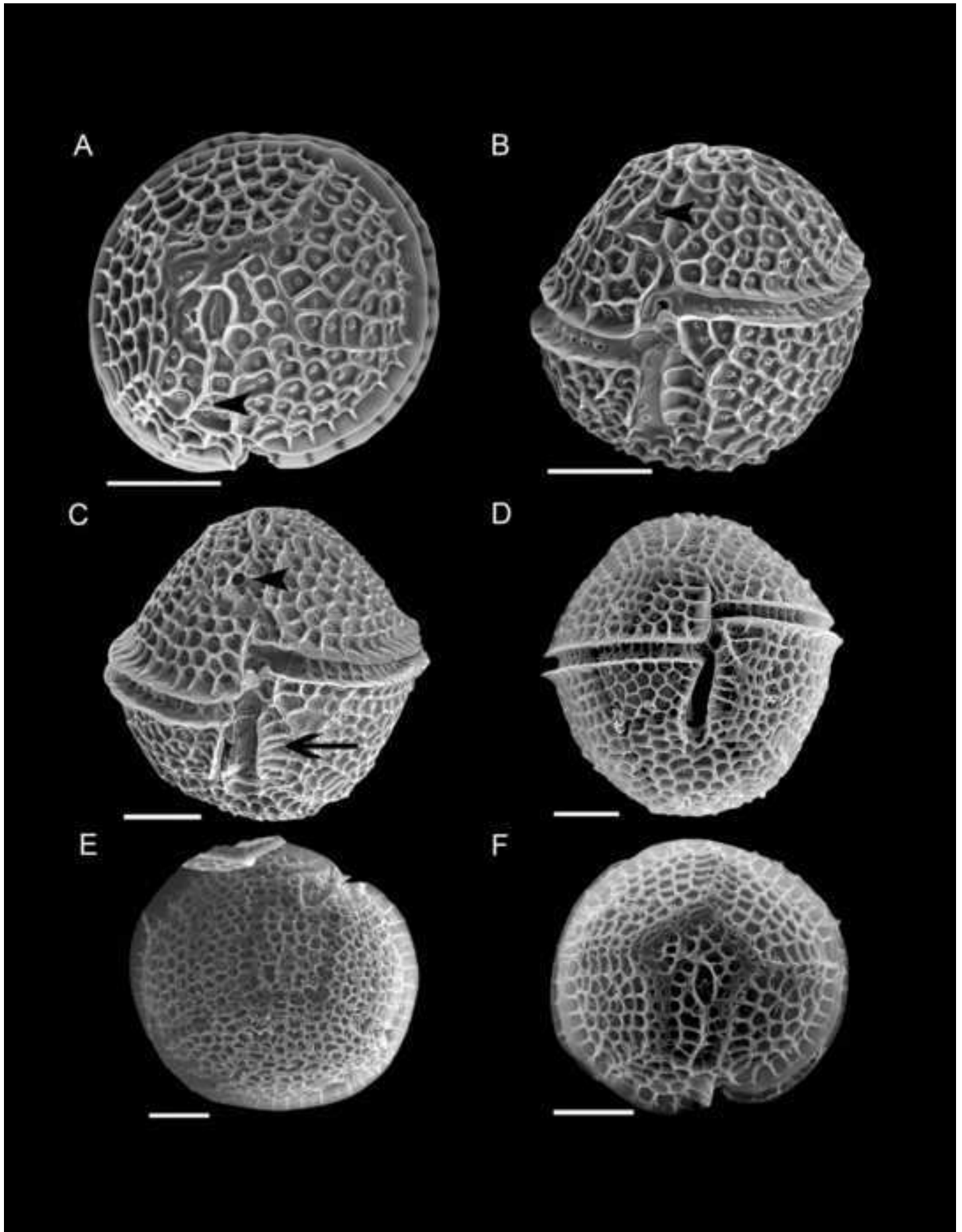


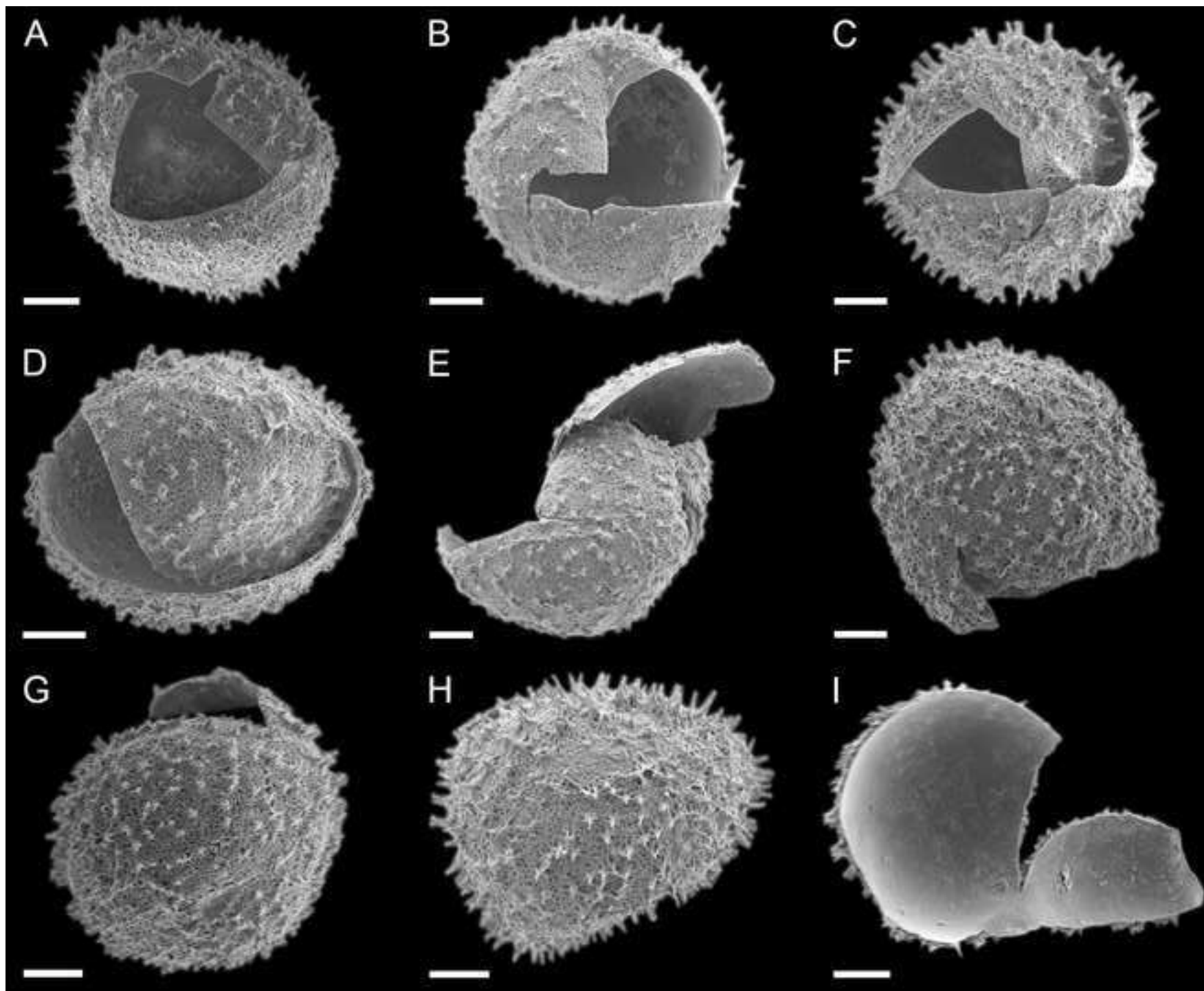
Figure 7

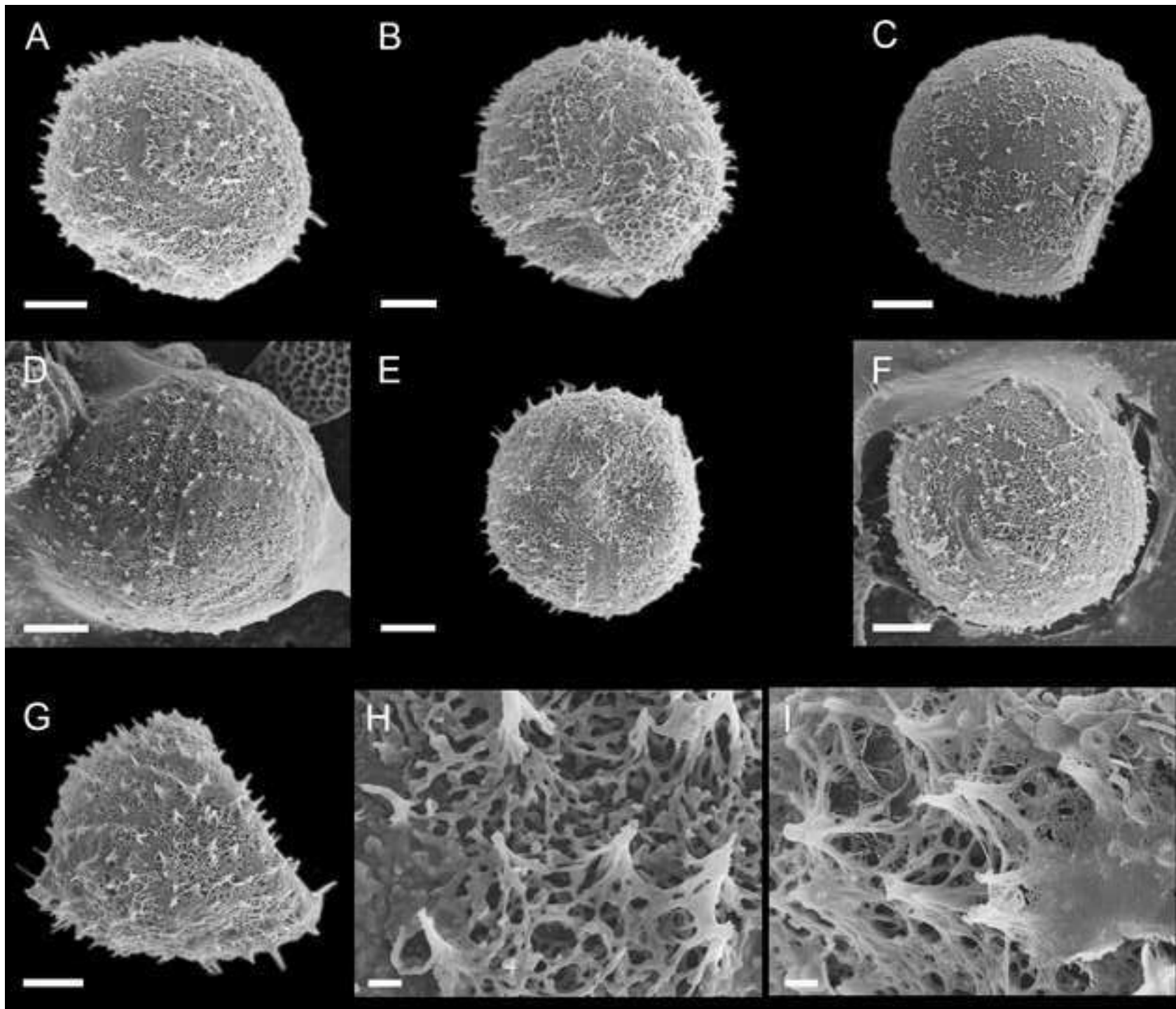












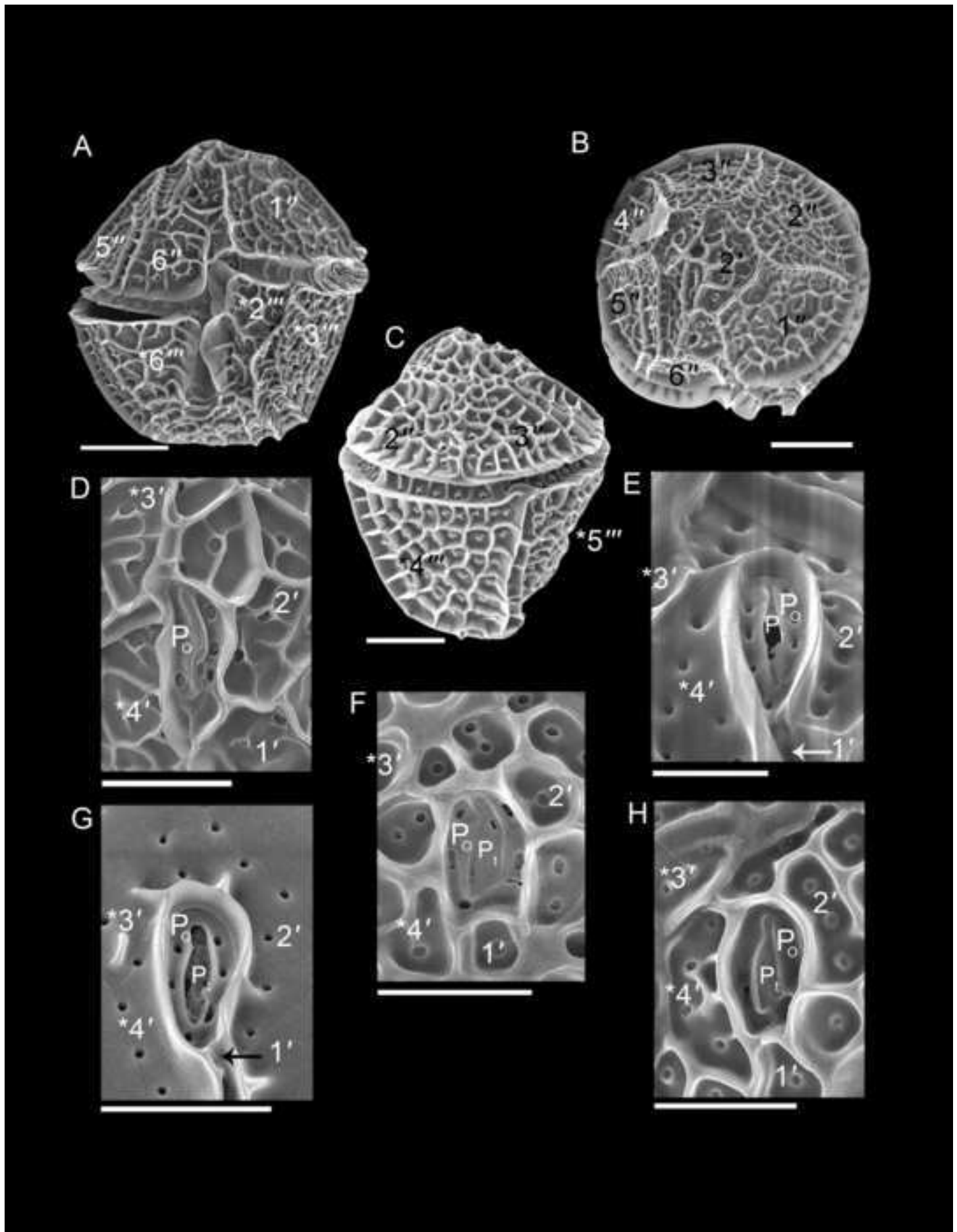


Table 1

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	1% Glutaraldehyde	PR	KK
Okkirai Bay, Iwate, Japan	O	16-Jul-03	39,08	141,85	32,6	15,8	Plankton net >20 µm	5% Glutaraldehyde	PR	KK
Okkirai Bay, Iwate, Japan	O	28-Aug-03	39,08	141,85	32,3	20,0	Plankton net >20 µm	5% Glutaraldehyde	PR	KK
Okkirai Bay, Iwate, Japan	O	23-Jun-04	39,08	141,85	32,7	16,0	Plankton net >20 µm	5% Glutaraldehyde	PR	KK
Okkirai Bay, Iwate, Japan	O	12-Aug-04	39,08	141,85	33,5	23,0	Plankton net >20 µm	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 = *Pentaplecodinium saltonense*, PR = *Protoceratium reticulatum*, AK = Anke Kremp, AR = André Rochon, AA = Abdulrahman Al-Muftah, MCCM = Consuelo Carbonell-Moore, JL = Jacob Larsen, KK = Kazuhiko Koike, KM = Kenneth Neil Mertens, KMA = Kazumi Matsuoka, MH= Martin J. Head, MHO = Mona Hoppenrath, MHU=Majja Huttunen, MP = Maya Pfaff, PH = Paul Hargreaves, UT = Urban Tillmann, VP = Vera Pospelova, XV = Ximena Vivanco, YO = Yuri B. Okolodkov

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	<i>P. saltonense</i>	Salton Sea (California)	33.375	-116.0	1966	Dodson, A.	EU532476	FJ489629	FJ489629***	
Salton Sea 2E3 *	<i>P. saltonense</i>	Salton Sea (California)	33°					Not sequenced		
CCMP1720 = K-1475 = ALO013	<i>P. saltonense</i>	Biscayne Bay (Florida)	30.122'	-115° 54.879'	19/11/2013	Mertens, K.	MG646301		MG646323	Aika Yamaguchi
CCMP1721 = K-1480 = ALO012	<i>P. saltonense</i>	Biscayne Bay (Florida)	25.8	-80.3333	15/02/1995	Hargraves, P.	FJ155820	FJ489628	FJ489628***	
CCMP3241 = K-1479	<i>P. saltonense</i>	Biscayne Bay (Florida)	25.8	-80.3333	01/06/1994	Hargraves, P.	EU165321	Not sequenced	Not sequenced	
CCMP3243 = K-1476	<i>P. saltonense</i>	Indian River Lagoon (Florida)	27.83	-80.45	11/06/2008	Hargraves, P.	Not sequenced	MG646297	MG646322	Andrea Highfield / Declan Schroeder
		Indian River Lagoon (Florida)	27.83	-80.45	05/06/2008	Hargraves, P.	Not sequenced	MG646296	MG646321	Andrea Highfield / Declan Schroeder
CCMP3031 = K-1474	<i>P. reticulatum</i>	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 *	<i>P. reticulatum</i>	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	<i>P. reticulatum</i>	Gulf of Mexico, U.S.A.	25.0167	-81.4003	NA	Sinigalliano, C.	EU165290	MG646288	MG646315	Andrea Highfield / Declan Schroeder
CCMP3113 = K-1478	<i>P. reticulatum</i>	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	<i>P. reticulatum</i> **	Alfacs Bay, Ebro Delta (Catalonia Spain)	NA	NA	NA	Fernandez-Tejedor, M.	FJ155817	FJ489624	FJ489624***	
VGO903 = ALO014	<i>P. reticulatum</i> **	Ría de Pontevedre (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	<i>P. reticulatum</i>	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 *	<i>P. reticulatum</i> (cyst-based)	Kattegat, Swedish coast	57.5	11.8	Nov. 2011	Mertens, K.	AB727655	AB727655	AB727655	
091223-38_M16_Protocer3-ITS1	<i>P. reticulatum</i>	Helgoland, Germany	54.19	7.9	avr-03	Hoppenrath, M.	Not sequenced	MG646294	Not sequenced	Karin Röder
Lake Saroma *	<i>P. reticulatum</i> (cyst-based)	Lake Saroma, Japan	44.12	143.87	Aug. 2011	Mertens, K.	AB727654	AB727654	AB727654	
K-0976	<i>P. reticulatum</i>	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 *	<i>P. reticulatum</i>	Station 323, Northern Baffin Bay	74.12	79.45	02-mai-11	Mertens, K.	MG646300	MG646293	MG646333	Yoshihito Takano
K-0485	<i>P. reticulatum</i>	Southern Kattegat, Bouy St.	56.20	12.04	03-avr-89	Hansen, G.	AF260386	Not sequenced	Not sequenced	
PRSH6 (NRC Halifax)	<i>P. reticulatum</i>	Ship harbour, NS, Canada	NA	NA	NA	Ferrell, J. F.	Not sequenced	EU927572	EU927572***	
PRSH3 (NRC Halifax)	<i>P. reticulatum</i>	Ship harbour, NS, Canada	NA	NA	NA	Ferrell, J. F.	Not sequenced	EU927569	EU927569***	
PRSH4 (NRC Halifax)	<i>P. reticulatum</i>	Ship harbour, NS, Canada	NA	NA	NA	Ferrell, J. F.	Not sequenced	EU927570	EU927570***	
PRSH1 (NRC Halifax)	<i>P. reticulatum</i>	Ship harbour, NS, Canada	NA	NA	NA	Ferrell, J. F.	Not sequenced	EU927568	EU927568***	
CTCC 01	<i>P. reticulatum</i>	Southern Benguela upwelling region	NA	NA	NA	NA	EU532477	EU532486	EU532486***	
Elands Bay 2013 (EB2) *	<i>P. reticulatum</i>	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

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	<i>P. reticulatum</i>	Okkirai Bay, Iwate, Japan	39.08	141.85	17-Jul-02	Koike, K.	Not sequenced	MG646283	Not sequenced	Kazuhiko Koike
030707-YB-PR1	<i>P. reticulatum</i>	Yamada Bay, Iwate, Japan	39.46	141.97	7-Jul-03	Koike, K.	Not sequenced	MG646284	Not sequenced	Kazuhiko Koike
030624-OK-PR1	<i>P. reticulatum</i>	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 *	<i>P. reticulatum</i>	Omura Bay, Japan	32.85	129.87	2 may 2011	Takano, Y.	Not sequenced	MG646291	MG646332	Yoshihito Takano
Nagayo-sc1 *	<i>P. reticulatum</i>	Nagayo, Japan	32.85	129.87	24 May 2011	Takano, Y.	MG646299	MG646290	MG646331	Yoshihito Takano
Togitsu-sc2 *	<i>P. reticulatum</i>	Togitsu Port, Japan	32.85	129.87	23 May 2011	Takano, Y.	Not sequenced	MG646289	Not sequenced	Yoshihito Takano
777	<i>P. reticulatum</i>	Concarneau large, France	47.83	-3.95	16 July 2008	Nézan, E.	Not sequenced	Not sequenced	MG646317	Gwenaël Bilien
778	<i>P. reticulatum</i>	Concarneau large, France	47.83	-3.95	16 July 2008	Nézan, E.	Not sequenced	Not sequenced	MG646318	Gwenaël Bilien
16-034	<i>Ceratocorys armata</i>	Bouée 7, Arcachon, France	44.54	-1.26	18 Dec 2015	Nézan, E.	MG646311	Not sequenced	MG646329	Gwenaël Bilien
16-246	<i>Ceratocorys gourretii</i>	Station B70, Banyuls-sur-mer, French Mediterranean	42.48	3.18	26 Sept 2016	Nézan, E.	MG646310	Not sequenced	MG646328	Gwenaël Bilien
16-245	<i>Ceratocorys horrida</i>	Station B70, Banyuls-sur-mer, French Mediterranean	42.48	3.18	26 Sept 2016	Nézan, E.	MG646312	Not sequenced	MG646330	Gwenaël Bilien
CCMP157	<i>Ceratocorys horrida</i>	Banda Sea, South East Asia	-5,00	130,00	22 April 1975	Sweeney, B.	Not sequenced	EU927577	AF022154	
SCCAP K-1504	<i>Thecadinium kofoidii</i>	Helgoland, Germany	54.19	7.9	Aug. 2002	Hoppenrath, M.	GU295207***	Not sequenced	GU295204	
JH0210	<i>Alexandrium affine</i>	Jinhae Bay, South Korea	NA	NA	NA	NA	Not sequenced	Not sequenced	AY775286	
JHW0004-12	<i>Alexandrium tamarense</i>	Korea	NA	NA	NA	NA	Not sequenced	Not sequenced	AY421777	
CCMP116	<i>Alexandrium tamarense</i>	Ria de Vigo, Spain	42.23	-8.8	1 June 1984	Yentsch, C.M.	HM483868	Not sequenced	Not sequenced	
CCMP1738	<i>Lingulodinium polyedra</i>	Gulf of Mexico, USA	27.8	-97.13	NA	Buskey, E.	Not sequenced	Not sequenced	EF492507	
DRW0208	<i>Lingulodinium polyedra</i>	Korea	NA	NA	Aug. 2001	NA	Not sequenced	Not sequenced	AY421788	
BLACK1	<i>Lingulodinium polyedra</i>	Black Sea, Ukraine	49.90	30.29	2011	Takano, Y.	AB678400	AB678399***	AB693195***	
CASP1	<i>Lingulodinium polyedra</i>	Caspian Sea, Iran	37.51	49.91	2011	Takano, Y.	AB678402	AB678401***	AB693194***	
SANPEDRO1	<i>Lingulodinium polyedra</i>	San Pedro Harbor, California, USA	33.74	-118.24	2011	Takano, Y.	AB678404	AB678403***	AB693196***	
GS0209	<i>Gonyaulax polygramma</i>	Gunsan, South Korea	NA	NA	NA	NA	Not sequenced	Not sequenced	AY775287	
11A1	<i>Protoperidinium tringulatum</i>	Wadden Sea, Netherlands	53.60	6.58	2006	Kawami, H.	Not sequenced	Not sequenced	AB716917	
CCAP1140/3	<i>Parvodinium inconspicuum</i>	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-aout-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 sequenced	Not sequenced	Haifeng Gu



* = 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.