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1 **First insights on the diversity of the genus *Ostreopsis* (Dinophyceae,**
2 **Gonyaulacales) in Guadeloupe Island, with emphasis on the phylogenetic**
3 **position of *O. heptagona***

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19 Abstract:

20 The present study aims to identify epiphytic *Ostreopsis* cells collected in Guadeloupe between 2017
21 and 2018 using a morpho-molecular approach. This method combined microscopical observations
22 of wild specimens (light and scanning electron microscopy) with a phylogenetic analysis inferred
23 from concatenated sequences of ribosomal operon (SSU + ITS + LSU) of Ostreopsidoideae. Four
24 distinct morphotypes were identified in our samples and studied by SEM. Molecular data obtained
25 from single-cell PCR for the four morphotypes were consistent with observations and confirmed the
26 presence of three *Ostreopsis* species resolved in well characterized genotypes (*O. cf. ovata*, *O.*
27 *lenticularis* and *O. siamensis*) and an unidentified clade. Detailed morphological characters
28 including sulcal plates confirmed the identification of the last morphotype as *O. heptagona*
29 D.R.Norris, J.W.Bomber & Balech, which forms a new basal clade in the genus, not previously
30 reported. Observations highlighted overlapping sizes for *O. lenticularis*, *O. siamensis* and *O.*
31 *heptagona*. Direct sequencing of PCR products obtained for some *O. lenticularis* and *O. heptagona*
32 collected at one site revealed unexpectedly the presence of the parasitoid dinoflagellate
33 *Amoebophrya*. Some *Ostreopsis* cells were found partially emptied and exhibiting a compact mass.
34 Further analyses are needed to understand the ecological role of *Amoebophrya* on blooms of
35 epiphytic *Ostreopsis* species.

36

37 Keywords:

38 Concatenated phylogeny, morpho-molecular taxonomy, *Ostreopsis* cf. *ovata*, *Ostreopsis*
39 *lenticularis*, *Ostreopsis siamensis*, ribosomal operon

40

41 Highlights:

42 Identification of four *Ostreopsis* species and characterization of a novel basal clade

43 Detailed SEM observations and rDNA sequencing of *Ostreopsis heptagona*

44 Detection of infected *Ostreopsis* spp. cells by the parasitoid *Amoebophrya*

45 **Introduction**

46 The dinoflagellate genus *Ostreopsis* Johs.Schmidt, 1901 had first been observed in plankton
47 samples collected in the Gulf of Thailand (Schmidt, 1901) but has seldomly been reported since
48 then, due to its predominantly benthic habitat (Besada et al., 1982). Owing to its co-occurrence with
49 the genus *Gambierdiscus* in tropical regions (Fukuyo, 1981; Ballantine et al., 1985; Berland et al.,
50 1992), this genus benefited from the interest on microalgae associated with the ciguatera poisoning
51 (CP) which allowed the description of several species between the 1980's and the 1990's (Fukuyo,
52 1981; Norris et al., 1985; Faust and Morton, 1995; Faust, 1999) and initiated the assessment of their
53 toxicity (Ballantine et al., 1988; Tosteson et al., 1989; Berland et al., 1992). The toxins synthesized
54 by *Ostreopsis* species are palytoxin-like molecules that can cause health issues especially in
55 temperate areas (Gallitelli et al., 2005; e.g. Durando et al., 2007; Tichadou et al., 2010; Vila et al.,
56 2016) where some species form recurrent blooms (Shears and Ross, 2009; Cohu et al., 2013;
57 Ninčević Gladan et al., 2019; Açaçaf et al., 2020). Reports of *Ostreopsis* have become more and more
58 frequent over the past decades (Shears and Ross, 2009; Shah et al., 2014; Park et al., 2020).
59 Furthermore, palytoxin analogues can bio-accumulate in marine organisms (Biré et al., 2013, 2015)
60 and constitute a significant risk for human health because of their potentially high toxicity and their
61 thermostability (Katikou, 2007). Blooms are less frequently reported in tropical areas (Shears and
62 Ross, 2009), but it has been shown that some species can reach high abundances and sometimes
63 form benthic blooms (Chomérat et al., 2020b). Although palytoxin-like molecules have not been
64 directly linked-with CP, they may be involved in poisoning derived from the ingestion of tropical
65 marine organisms causing various symptoms reported in ciguateric endemic areas such as
66 palytoxicosis and clupeotoxism (Alcala et al., 1988; Onuma et al., 1999; Randall, 2005).

67 In terms of species diversity, the Caribbean basin appears as a particularly rich area where
68 nine of the eleven currently described species included in the genus *Ostreopsis* (Guiry and Guiry,
69 2021) have been reported from the Gulf of Mexico and the Caribbean Sea. Three species described
70 from the Indo-Pacific area have been mentioned in the Caribbean, such as *O. siamensis*
71 Johs.Schmidt (Bomber et al., 1988, 1989; Faust and Gullede, 1996), *O. ovata* Y.Fukuyo (Besada et
72 al., 1982; Faust and Gullede, 1996; Faust, 2004) and *O. lenticularis* Y.Fukuyo (Ballantine et al.,
73 1985, 1988; Tosteson et al., 1986, 1989; Faust, 1995, 2004; Faust et al., 1996; Delgado et al., 2006).
74 Interestingly, five other species have been described from various areas of the Caribbean basin,
75 including *O. heptagona* D.R.Norris, J.W.Bomber & Balech (Norris et al., 1985), *O. labens*
76 M.A.Faust & S.L.Morton (Faust and Morton, 1995), *O. belizeana* M.A.Faust, *O. caribbeana*
77 M.A.Faust and *O. marina* M.A.Faust (Faust, 1999). While the presence of *O. heptagona*, *O. labens*
78 and *O. mascarenensis* Quod (Quod, 1994) has been suggested in various tropical areas (Faust and
79 Morton, 1995; Faust et al., 1996), the other three species have never been unambiguously identified
80 elsewhere than in their type localities. For instance the putative identification of *O. marina* in the

81 Indian Ocean (Carnicer et al., 2015) was unclear, and morphological and genetic data allowed to
82 conclude that the species present in that region actually belongs to *O. lenticularis* (Chomérat et al.,
83 2019b).

84 As stated previously, the morphological plasticity in *Ostreopsis* species led to some
85 ambiguous identifications (Parsons et al., 2012; Hoppenrath et al., 2014; Chomérat et al., 2019b;
86 Rhodes et al., 2020) and the existence of some *Ostreopsis* morphospecies has recently been
87 questioned (Borsato et al., 2020; Chomérat et al., 2020b). In the Caribbean area, health risks
88 associated with the toxicity of some *Ostreopsis* species have been reported (Norris et al., 1985;
89 Tosteson et al., 1986; e.g. Ballantine et al., 1988), the identification of toxic species, however,
90 remained unclear (Chomérat et al., 2019b), and further studies based on molecular data appear now
91 necessary to resolve such issues (Sato et al., 2011; Tawong et al., 2014; Zhang et al., 2018). The use
92 of molecular data from ribosomal DNA or internal transcribed spacers regions has proven to be
93 extremely powerful to discriminate genotypes within the genus (e.g Penna et al., 2010; Sato et al.,
94 2011; Tawong et al., 2014; Lee and Park, 2020). Still, association of genotypes with existing
95 taxonomic descriptions was not always clear, since morphological data were lacking or not detailed
96 enough for accurate characterization (Sato et al., 2011; Chomérat et al., 2020b). Only two species,
97 *O. fattorussoi* Accoroni, Romagnoli & Totti and *O. rhodesiae* Verma, Hoppenrath & S.A.Murray
98 include molecular data with their formal description (Accoroni et al., 2016; Verma et al., 2016), and
99 recent re-investigations of *O. lenticularis*, *O. mascarenensis* and *O. siamensis* allowed to clarify
100 their genetic identity (Chomérat et al., 2019b, 2020a; Nguyen-Ngoc et al., 2021). Combining
101 morphological and genetic data from specimens acquired at or near the type localities appears as a
102 major task to provide reliable taxonomic reference data for unambiguous identification of species
103 (Parsons et al., 2012).

104 To date, there are no molecular studies on the genus *Ostreopsis* available from the Caribbean
105 and there is a serious need of data to confirm the putatively high diversity inferred from
106 morphological reports. Therefore, in the framework of a program aiming to explore the diversity of
107 benthic dinoflagellates in the Caribbean Sea, the present study focuses on clarifying the taxonomic
108 diversity of *Ostreopsis* in Guadeloupe Island based on a morpho-genetic approach. For the first
109 time, single-cells of different morphotypes isolated from samples collected around the island were
110 used for molecular sequencing of LSU rDNA. In addition, detailed morphological features were
111 studied with high resolution scanning electron microscopy to assess species identifications. By
112 combining morphological and molecular data, the present study allowed to better interpret the
113 diversity around Guadeloupe Island, which constitutes a prerequisite for ecological studies of
114 benthic communities associated with CP. Moreover, this study showed, in an unexpected manner,
115 the infection of *Ostreopsis* cells by the parasitoid *Amoebophrya*.

116 **Material and Methods**

117 ***Sampling***

118 Samples were collected between July and October 2017 and in July 2018 at four sites in various
119 parts of Guadeloupe Island: Bois Jolan, Chapelle, Le Gosier and Rivière Sens (Fig. 1). During the
120 sampling, several macrophytes were carefully collected with surrounding water in 50 ml plastic
121 tubes avoiding the resuspension of epiphytic microalgae growing on the thalli (Table 1). Acidic
122 Lugol solution at 1% (v/v) was added to all samples to preserve the microalgae and 10 seconds
123 agitation allowed benthic dinoflagellates to detach from the macrophyte (Jauzein et al., 2018).
124 Then, the epiphytic suspension was passed through a 500 µm mesh to remove larger organisms and
125 detritus. Samples were stocked in the dark at 4°C.

126

127 ***Light and scanning electron microscopy***

128 For scanning electron microscopy (SEM), cells were first individually isolated and concentrated in
129 2 ml tubes containing water and a drop of formaldehyde to prevent the development of fungi. Then,
130 cells were filtered on polycarbonate membrane filters (Millipore RTTP Isopore, 1.2 µm pore size,
131 Millipore, Billerica, USA), rinsed in deionized water, and prepared according to Chomérat & Couté
132 (2008). Dehydration was carried out in ethanol baths 15%, 30%, 50%, 70%, 95% vol. ethanol for
133 about 20 min in each bath, and then several baths of absolute ethanol. Cells were then dried using
134 an EMS 850 (Electron Microscopy Science, Hatfield, PA, USA) critical point drier. After gold-
135 coating as in Chomérat et al. (2020b), SEM examinations were carried out using a Sigma 300 field-
136 emission SEM (Carl Zeiss Microscopy GmbH, Jena, Germany) in full vacuum mode and with an
137 electron acceleration of 2 kV. Cells were measured on SEM digital micrographs using ImageJ
138 software (Rasband, 1997). Depth corresponds to dorso-ventral length (DV) and height to antero-
139 posterior (AP) length. SEM images were presented on a uniform background using GNU Image
140 Manipulation Program v.2.10.18. For dissection of thecal plates, isolated cells were placed in a
141 small drop of water on a slide, covered with a coverslip, and a drop of sodium hypochlorite (5%
142 final concentration) was deposited on the border. The coverslip was then gently pressed using a
143 stick and dissociated thecal plates were observed at high magnification using a BX41 (Olympus,
144 Tokyo, Japan) upright microscope equipped with DIC optics. The terminology used for thecal plates
145 in this paper follows the hybrid system of Hoppenrath et al. (2014) and Norris et al. (1985) for some
146 sulcal plates.

147

148 ***Single-cells isolation, DNA amplifications and sequencing***

149 For molecular characterization, single-cells were isolated from preserved field-samples as described
150 in Chomérat et al. (2019a). In order to focus on the diversity of *Ostreopsis* species in each site,

151 samples were analysed by sampling site, indistinctively of the macrophyte substratum. To resuspend
152 epiphytic cells, the tube containing Lugol-fixed material was vigorously shaken and a subsample of
153 30 µl was diluted in 4 ml of filtered seawater in a small Petri dish. Each cell of interest was isolated
154 with a micropipette under an inverted microscope (Olympus IX51, Tokyo, Japan) measured and
155 photographed with a camera (Olympus E-300 digital, Tokyo, Japan). Then, the cells were rinsed in
156 four drops of distilled water before being transferred into a 200 µl PCR tube containing 5 µl of PCR
157 grade distilled water. This process allowed to associate the morphology of a specimen with its
158 sequence (Chomérat et al., 2019a). Tubes containing single-cells were stored at -20 °C until further
159 analysis. A nested Polymerase chain reaction (PCR) was used for the amplification of one or two
160 regions of the large subunit of ribosomal DNA (rDNA) (D1–D3 and D8–D10 domains). The first
161 round of PCR used ITS-FW and RB primers (Table S1) then a second round of PCR was carried out
162 using 1 µl of the amplicon produced in the first round, allowing the separate amplification of the
163 D1–D3 and D8–D10 regions. PCR reactions were carried out in 20 µl using KOD Hot Start Master
164 Mix (Novagen-Merck KgaA, Darmstadt, Germany), according to the manufacturer's instructions.
165 The PCR cycling comprised an initial 2 min heating step at 95 °C to activate the polymerase,
166 followed by 35 cycles of 95 °C for 20 s, 56 °C for 20 s, and 70 °C for 2 min. Primers used were
167 D1R and D3B for D1–D3 domain and D8 and OstD10R for D8–D10 domain (Table S1). To obtain
168 sequences of the parasitoid, a specific primer (AmoebR) has been designed from preliminary
169 sequence results obtained with universal primers, and used to amplify specifically SSU–ITS region
170 of the parasitoid (Table S1). PCR products were visualized on a 1% agarose gel after
171 electrophoresis and the positive samples were purified using the ExoSAP-IT PCR Product Cleanup
172 reagent (Affymetrix, Cleveland, OH, USA).

173 To confirm parasitoid and host sequences from infected specimens, a cloning step has been
174 added. To allow a better ligation of the amplicon, the second PCR round was carried out using Taq
175 polymerase (Promega PCR master mix) and primers ITS-FW–D3B with an initial 2 min heating
176 step at 95 °C to activate the polymerase, followed by 20 cycles of 95 °C for 20 s, 60 °C for 20 s,
177 and a final extension at 70 °C for 2 min. After confirmation by agarose gel electrophoresis, positive
178 PCR products were purified using NucleoMag kit (Macherey-Nagel) and cloning was performed
179 using pGEM[®]-T Easy Vector Systems kit and JM109 Competent Cells (Promega) according to the
180 manufacturer's instructions. Positive clones were extracted with PureLink Quick Plasmid Miniprep
181 kit (Invitrogen).

182 The Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan)
183 was used for sequencing purified PCR products. Primers and excess dye-labeled nucleotides were
184 first removed using the Big Dye X-terminator purification kit (Applied Biosystems, Foster City,
185 CA, USA). Sequencing products were run on an ABI PRISM 3130 Genetic Analyzer (Applied
186 Biosystems). Forward and reverse reads were obtained.

187

188 ***Sequences alignment and phylogenetic analyses***

189 *Ostreopsis* sequences of the LSU D1-D3, D8-10 and additionally ITS region for morphotype 4
190 acquired in the present study were used in a multi-loci phylogenetic analysis following the most
191 recent taxonomic treatment of gonyaulaceans by Tillmann et al. (2021), and including sequences of
192 the three genera encompassed in subfamily Ostreopsidoideae Gottschling, Tillmann and Elbrächter.
193 For that purpose, 138 sequences of *Ostreopsis* spp., *Coolia* spp. and *Alexandrium* spp. from the
194 analysis by Tillmann et al. (2021) served as a basis to construct our matrix, using *Triadinium*
195 *polyedricum* (2 sequences) and *Pyrodinium bahamense* (3 sequences) as outgroup. Sequences
196 acquired on 25 different specimens in the present study and 21 *Ostreopsis* spp. sequences retrieved
197 from GenBank were added to complete the genus diversity. Full voucher information of the
198 systematically representative set comprising 184 gonyaulacean Ostreopsidoideae (84 *Ostreopsis*
199 spp., 30 *Coolia* spp., 70 *Alexandrium* spp.) and outgroup (2 Gambierdiscoideae Fensome,
200 F.J.R.Taylor, G.Norris, Sarjeant, Wharton & G.L.William, 3 Pyrodinioideae F.J.R.Taylor, G.Norris,
201 Sarjeant, Wharton & G.L.William) are provided in Table S2. As described in Tillmann et al. (2021),
202 for alignment constitution, separate matrices of the rRNA operon (i.e. SSU, ITS region and LSU)
203 were constructed, aligned using MAFFT v. 7 (Kato and Standley, 2013) and concatenated
204 afterwards using Seaview v. 5.05 software (Gouy et al., 2010). After concatenation, the matrix was
205 refined by eye with Linux version of MEGA software v. 10.1.7 (Kumar et al., 2018).

206 A second dataset was prepared to analyse the phylogenetic position of the parasitoid
207 *Amoebophrya* sp. infecting some *Ostreopsis* specimens. A matrix including small subunit of
208 ribosomal RNA (SSU) and internal transcribed spacer 1 was prepared, using closely related
209 sequences retrieved in GenBank, some sequences covering only SSU. They were aligned with
210 MUSCLE algorithm (Edgar, 2004) followed by refinement by eye.

211 Prior to phylogenetic analyses, jModeltest2 v. 2.1.7 (Darriba et al., 2012) was used to search
212 for the most appropriate model of sequence evolution. Two methods of phylogenetic reconstruction
213 were used. Maximum Likelihood analysis (ML) was performed using PHY-ML v. 3 software
214 (Guindon et al., 2010), and a bootstrap analysis (500 pseudoreplicates) were to assess the relative
215 robustness of branches of the ML tree. Bayesian Inference analysis (BI) was carried out using
216 MrBayes 3.1.2 software (Ronquist and Huelsenbeck, 2003). Parameters used to run phylogenetic
217 calculations are given in supplementary Table S3. Nomenclature of clades (genotypes) follows that
218 proposed by Sato et al. (2011) and subsequently by Tawong et al. (2014) and Chomérat et al.
219 (2019b, 2020b).

220

221

222 Results

223 *Morphological observations*

224 Based on their size and appearance, assignment to four distinct morphotypes was possible. Cells of
225 the morphotype 1 were small and conspicuously tear-shaped (Figs 2, 3A–B, 7A). The morphotype 2
226 included broadly large oval cells with a regular margin (Figs 2, 3–I, 7B–E) while the morphotype 3
227 corresponded to cells with a similar size and shape as morphotype 2 but with an irregular undulated
228 margin and a twisted aspect (Figs 2, 4A–B, 7F–J). Finally, cells of morphotype 4 were large and
229 tear-shaped (Figs 2, 5A–B, 6A, 7K–O). Morphotype 2 was found at CH, BJ and RS sites,
230 morphotype 3 was only found at GO and RS sites, while morphotypes 1 and 4 were only present at
231 RS and CH sites, respectively. Owing to these morphological peculiarities and their observation at
232 certain stations, cells of the four morphotypes could be retrieved in the samples analysed by SEM,
233 allowing further detailed observations of their characters. All cells were found to possess the typical
234 plate pattern of the genus *Ostreopsis*, APC 3' 7'' 6?c ?s 5''' 2'''''. In the following descriptions, only
235 significant distinctive morphological features are emphasized for each morphotype.

236

237 Morphotype 1 - *Ostreopsis* cf. *ovata* Y. Fukuyo

238 Cells were ovate (Figs 3A–B, 7A), 43.2–52.3 μm (mean \pm SD: $48.1 \pm 3.5 \mu\text{m}$, $n = 10$) deep (dorso-
239 ventral length), 24.1–34.4 μm wide (mean $28.2 \pm 3.0 \mu\text{m}$, $n = 10$). The length to width ratio varied
240 from 1.52 to 1.87 (mean 1.71, $n = 10$) (Figs 3A– B). The apical pore complex (APC) was composed
241 of a slightly curved Po plate of about 8 μm that was supported by a long 2' plate reaching the 4''
242 plate dorsally (Fig. 3C). The cingulum was almost straight and deep (Figs 3D–E). The thecal
243 surface was smooth and covered by thecal pores of 0.25–0.32 μm in diameter without collar rim
244 (Figs 3F–G). Interestingly, some rare smaller thecal pores (*ca.* 50 nm in diameter) were visible on
245 the surface of plates (Figs 3F–G).

246

247 Morphotype 2 - *Ostreopsis lenticularis* Y. Fukuyo

248 Cells were large, lenticulate in shape and broadly ovate (Figs 3H–I, 7B). Cells were 65.0–119.9 μm
249 deep (mean \pm SD: $95.8 \pm 12.8 \mu\text{m}$, $n = 25$) and 56.2–96.9 μm wide (mean \pm SD: $77.8 \pm 11.2 \mu\text{m}$, n
250 $= 25$). The length to width ratio varied from 1.11 to 1.39 (mean \pm SD: 1.23 ± 0.08 , $n = 25$). The
251 APC was composed of a slightly curved Po plate of 15.8–18.2 μm (mean \pm SD: $17.0 \pm 1.2 \mu\text{m}$, $n =$
252 5) (Fig. 3J). The first apical plate 1' was rather large and six-sided (Fig. 5H). The second apical
253 plate 2' was short and did not reach the 4'' plate (Fig. 3J) . The cingulum was straight in lateral view
254 (Figs 3K–L). The thecal surface was covered with two kinds of pores without collar rim (Fig. 3M).
255 The large pores were 0.3–0.4 μm in diameter and the small pores were 90–150 nm in diameter.
256 They were both present densely on the thecal surface.

258 Morphotype 3 - *Ostreopsis siamensis* Johs. Schmidt

259 Cells were broadly ovoidal (Figs 4A–B, 7F). They were 58.6–97.6 μm deep (mean \pm SD: 80.4 \pm
 260 11.1 μm , n = 16) and 43.3–82.5 μm wide (mean \pm SD: 66.1 \pm 11.7 μm , n = 16). The length to width
 261 ratio varied from 1.04 to 1.50 (mean: 1.23, n = 16). The APC was composed of a slightly curved Po
 262 plate whose length varied from 14.8 to 17.9 μm (mean \pm SD: 16.8 \pm 1.0 μm , n = 8) (Fig. 4C). The
 263 first apical plate was six-sided (Fig. 4A).. The second apical plate 2' was narrow, extending to
 264 contact the 4'' plate (Fig. 4C). Ventrally, the cingulum appeared oblique and formed a V-shape (Fig.
 265 4D). Cells appeared bulging in the median part of the epitheca (height: 30.6–41.7 μm , n = 5) and
 266 thinner near the edges (Figs 4E–F, G). In lateral view, the cingulum was conspicuously undulated,
 267 giving a somewhat twisted aspect to the cells (Figs 4E–F). In the sulcal area, cells exhibited the
 268 ventral opening (Vo), the anterior left sulcal plate (Ssa), the anterior right sulcal plate (Sda) and the
 269 posterior sulcal plate (Sp) (Fig. 4H). On the hypotheca, plate 2'''' had typical curved shape (Figs 6B,
 270 F) due to the cell twist. The thecal surface was covered with large pores (*ca.* 0.3 μm in diameter)
 271 surrounded by a shallow elevated rim and some very small pores (*ca.* 50–60 nm in diameter) were
 272 also present at a lower density (Fig. 4I).

273

274 Morphotype 4 - *Ostreopsis heptagona* D.R.Norris, J.W.Bomber & Balech

275 Cells were large, and typically tear-shaped (Figs 5A–B, 6A, 7K– O, S1). They were 77.9–110.5 μm
 276 deep (mean \pm SD: 95.1 \pm 6.2 μm , n = 45) and 45.7–75.2 μm wide (mean \pm SD: 60.0 \pm 6.8 μm , n =
 277 43). The length to width ratio varied from 1.39 to 1.80 (mean \pm SD: 1.56; n = 43). The APC was
 278 composed with a slightly curved Po plate 14.6–17.2 μm long (mean \pm SD: 16.1 \pm 0.9 μm ; n = 8)
 279 with the apical pore consisting in a slit encircled by a row of thecal pores partially visible (Figs 5D,
 280 6B). The epithelial surface examination of this morphotype revealed a conspicuous seven-sided (i.e.
 281 heptagonal) 1' plate making contacts with plates Po, 3', 1'', 2'', 5'', 6'' and 7'' (Figs 5A, C). The
 282 second apical plate 2' was narrow and elongated and reached the 4'' plate dorsally (Fig. 5D). Plate 3'
 283 was pentagonal (Fig. 5A). Precingular plates were unequal in size, with plates of the right side (i.e.
 284 4'', 5'', 6'' and 7'') higher than those of the left side (Figs 5A,F). On the left side, plate 3'' was the
 285 smaller of the series. Among precingular plates, 1'', 3'', 6'' and 7'' were four-sided, in contrast with
 286 2'', 4'' and 5'' which were pentagonal.

287 The cingulum appeared almost closed and straight in all studied specimens, and no cingular
 288 plates could be distinguished in SEM (Figs 5E–F, S1). In LM, some cingular plates (c1; c2)
 289 appeared very narrow (Figs 6B, D, E). The sulcus was small and narrow (Figs 5F–G). Ventrally, the
 290 ventral opening (Vo) was visible in contact with 1'' plate (Figs 5F–G, 6A, D). From SEM
 291 observations, only three sulcal plates could be resolved and some were obscured by the overlap of
 292 1'''' plate (Fig. 5G). Observation of dissected cells at high magnification in LM allowed to complete

293 the sulcus description and four additional plates could be observed (Figs 6C–I). The most
294 conspicuous plates both in LM and SEM were the anterior left sulcal plate (Ssa) which appeared
295 elongated and in contact with Vo and 1'' on the epitheca, while it extended on the left within the
296 cingular furrow (Figs 5G, 6D, H) and the anterior right sulcal (Sda), elongated located below Ssa
297 and forming a prominent list (Figs 5G, 6D, H). These two plates had a complex three dimensional
298 structure, and appeared conspicuously curved when seen from top (Figs 6D–E, H–I). On the left
299 side of Ssa, a deeply recessed plate with a squared shape on the right and a rounded indentation
300 with a thick edge on the left margin, was present and interpreted as a 't' plate (Fig. 6G). Just below
301 this plate, a rectangular posterior left sulcal (Ssp) plate was observed in contact with Ssa, Sda (Fig.
302 6H). A small elongated and almost triangular (Fig. 6E) posterior right sulcal plate (Sdp) was in
303 inserted between Ssp and the posterior sulcal plate (Sp) which was six-sided, and with a hyaline
304 part where it was overlapped by 1'''' (Fig. 6F). Finally, the dissection revealed that a ring-shaped
305 plate delimiting Vo could detach from the Ssa (Fig. 6I), and it could be interpreted as an anterior
306 sulcal plate (Sa).

307 The hypotheca comprised seven major plates unequal in size (Fig. 5B). Seen antapically, the
308 2''/3'' and 4''/5'' sutures appeared roughly at the same level (Fig. 5B). Plate 1'''' was in contact
309 with 1'', 2'', 2'''' and overlapped the sulcal area (Fig. 5B, G, S1). Plate 2'''' was pentagonal with
310 contacts with 1'', 3'', 1'''' and 2'''' plates and the cingulum. Furthermore, this plate was broader on its
311 dorsal part, giving an asymmetrical shape (Fig. 5B, S1). Plates 3'' and 4'' were both four-sided and
312 roughly of the same size (Fig. 5B, S1). Four-sided plate 5'' had the most distinctive shape with its
313 long and curved suture in contact with plate 2'''' and its narrower size ventrally (Figs 5B, S1).

314 Interestingly, in the population from sample CH1, we noticed some variations of the
315 hypothecal pattern of some specimens, while no variation was seen on epithecae. On these
316 particular cells, a small extra plate was found on the ventral part of 2'''' plate, in contact with 1''''
317 and Sp plates (Fig. S1). Plate 2'''' of these specimens appeared to be split obliquely on its ventral
318 left side, with the larger part (2'''' α) dorsal and six sided, and the smaller part (2'''' β) ventral and
319 four-sided and more or less reduced (Fig. S1).

320 The thecal surface was homogeneously covered with a single type of pores *ca.* 0.3–0.4 μ m
321 in diameter (Fig. 5H). Some larger pores up to 0.5 μ m were occasionally observed on some
322 specimens (e.g. on plates 2'' and 3'', Fig. 5B).

323

324 *Single-cells isolation and phylogenetic analysis*

325 For the phylogenetic analysis of Ostreopsidoideae, the alignment comprised 189 OTUs including 25
326 specimens from Guadeloupe Island (Figs 7A–O) and was 6382 bp (1896 + 815 + 3671) long
327 including gaps. It comprised 631 + 637 + 1352 parsimony-informative sites and 3785 distinct
328 PhyML alignment patterns.

329 The ML majority-rule consensus tree is shown on Fig. 8 and topologies were largely
330 congruent regardless of whether ML or bayesian inference methods were used. The genus
331 *Ostreopsis* was monophyletic and fully supported among other Ostreopsidoideae (Figs 8, S2).
332 *Coolia* formed a sister clade to *Ostreopsis* while *Alexandrium* appeared more basal (Figs 8, S2).
333 Among *Ostreopsis*, 13 major lineages that corresponded to established taxa at species rank were
334 resolved and well to fully supported (Fig. 8): *O. cf. ovata*, *O. rhodesiae*, *O. mascarenensis*, *O.*
335 *fattorussoi*, *O. lenticularis*, *O. siamensis*, *O. heptagona* and *Ostreopsis* sp. 1/2-4, 7-10 (Fig. 8).
336 Sequences acquired from the four different morphotypes in the present study (Figs 7A–O) were
337 resolved within four major clades (Fig. 8). One sequence was recovered in *O. cf. ovata* clade, 11 in
338 *O. lenticularis* clade, 6 in *O. siamensis* clade, and 7 in *O. heptagona* clade. Sequences of *O. cf.*
339 *ovata*, *O. lenticularis* and *O. siamensis* from Guadeloupe Island were closely related to sequences
340 already available in GenBank and thus represent known genotypes (Fig. 8). Within *O. lenticularis*
341 clade, all sequences from Guadeloupe Island were closely related to those from French Polynesia
342 (type locality) (Fig. 8). Among sequences of *O. siamensis*, the 6 sequences from the present study
343 were resolved with a strong support with sequences from the Gulf of Thailand (TF25OS,
344 VNPQ218) and Tahiti Island (PNA19-6, PNA19-8 and IFR20-173). By contrast, sequences of *O.*
345 *heptagona* were all similar and clustered in a new clade, sister of all other *Ostreopsis* lineages,
346 which did not include any sequence from GenBank (Fig. 8). This clade was basal to all other
347 *Ostreopsis* sequences but it was fully recovered within *Ostreopsis* genus based on the concatenated
348 analysis (100BS, 1.00PP). Phylogenetic analyses inferred from single locus sequences (ITS and
349 LSU D1-D3 + D8-D10) are given in supplementary figures S3 and S4. While the exact same
350 position was found for this clade, the support was less robust than in the concatenated analysis
351 presented herein.

352 353 ***Detection of infected Ostreopsis spp. cells by the parasitoid Amoebophrya sp.***

354 Direct sequencing of PCR products obtained for some specimens from sample CH1 (Table 1)
355 unexpectedly revealed the presence the parasitoid dinoflagellate genus *Amoebophrya* which has not
356 been previously recognized when isolating single-cells. This preliminary result indicated a possible
357 contamination or the presence of this parasitoid in the sample, but contamination was excluded as
358 several independent PCR analyses revealed the same finding. Since partial LSU sequences obtained
359 were not useful enough for identification and phylogenetic analysis of the parasitoid, a more
360 thorough investigation has been undertaken to confirm this result. New specimens have been sought
361 in the Lugol-fixed sample CH1 for amplification of SSU–ITS region. Observed carefully with light
362 microscope, some *Ostreopsis* cells were found to have a peculiar appearance and seemed partially
363 emptied with gaps in their cytoplasm, and they exhibited a compact mass, more or less developed
364 and located dorsally (Figs 9A–F). This typical feature has been observed both in *O. lenticularis*

365 (Figs 9A–D) and *Ostreopsis heptagona* (Figs 9E–F). For putatively infected specimens of *O.*
366 *lenticularis* (IFR18-618, IFR18-622, IFR18-624), only sequences of the parasitoid were obtained.
367 In case of the specimen IFR17-685 of *Ostreopsis heptagona* (Fig. 7N), sequences of both the
368 parasitoid and the host were obtained. The four sequences of *Amoebophrya* sp. infecting *Ostreopsis*
369 spp. (accession numbers MW363873–MW363876) were identical. In the ML-tree (Fig. 10), they
370 were resolved with strong support with the sequence MK752531.1 of an isolate (AT5) for which the
371 host was not identified. From the phylogenetic analysis, it appeared that among the great genetic
372 diversity within *Amoebophrya*, the parasitoid identified in this study belongs to a clade distantly
373 related to other genotypes known to infect other planktonic dinoflagellates genera such as
374 *Akashiwo*, *Alexandrium*, *Ceratium* (=Tripos), *Cochlodinium*, *Dinophysis*, *Gonyaulax*,
375 *Gymnodinium*, *Karlodinium*, *Phalacroma*, *Prorocentrum* and *Scrippsiella* (Fig. 10).

376

377 **Discussion**

378 *Phylogeny and species identities*

379 Results from morphotype observations and phylogenetic analysis confirm the presence of four
380 *Ostreopsis* species in the samples studied from Guadeloupe Island. The concatenated tree reveal a
381 similar topology than found in the recent study by Tillmann et al. (2021), and *Ostreopsis* is a well
382 resolved genus among subfamily Ostreopsidoideae. Within *Ostreopsis* clade, the topology is also
383 congruent with previous studies based on single locus data (e.g. Chomérat et al., 2019b, 2020b;
384 Sato et al., 2011; Tawong et al., 2014) and using a longer dataset provides a stronger support for
385 several clades. While three of the genotypes from Guadeloupe Island cluster unambiguously within
386 already known clades of *Ostreopsis* (*O.* cf. *ovata*; *O.* *lenticularis* and *O.* *siamensis*), the analysis
387 reveals a novel basal clade not previously identified in any other study and for which the identity
388 needs to be clarified.

389 As confirmed by molecular data, smaller cells of morphotype 1 correspond to *O.* cf. *ovata*,
390 and compared with existing morphological data for *O.* cf. *ovata*, cells from Guadeloupe Island are
391 in the size range reported for this species from various areas (e.g. Fukuyo, 1981; Penna et al., 2010;
392 Zhang et al., 2018; Junqueira de Azevedo Tibiriçá et al., 2019; Nascimento et al., 2020). The
393 detailed morphological analysis by SEM also confirms the plate pattern and characters observed
394 previously (Fukuyo, 1981; Junqueira de Azevedo Tibiriçá et al., 2019). Nevertheless, observations
395 at high magnification show the presence of very small thecal pores (*ca.* 50 nm) on the surface of
396 thecal plates, as recently reported by Tibiriçá et al. (2019). Hence, this confirms that this species
397 possesses a few small pores, as in other species such as *O.* *lenticularis* (Fukuyo, 1981; Chomérat et
398 al., 2019b), *O.* *rhodesiae* (Verma et al., 2016) or in *O.* *siamensis* (Chomérat et al., 2020b; Nguyen-
399 Ngoc et al., 2021). These small pores are however rare and quite difficult to observe, and they may

400 be present in certain strains only, which may explain the variability reported in different studies
401 (e.g. Hoppenrath et al., 2014). Genetically, the unique sequence from Guadeloupe is not identical
402 with any strain from GenBank and it might constitute a different ribotype.

403 Cells of morphotype 2 identified as *O. lenticularis* have the typical morphology of this large
404 species, with wide and sometimes almost round cells. The size variation observed in specimens
405 from Guadeloupe is larger than reported in any other study, exceeding the ranges 60–100 μm in
406 depth and 45–80 μm in width given by Fukuyo (1981) in the original description. Interestingly,
407 Faust et al. (1996) reported larger sizes for *O. lenticularis*, although it was obviously misidentified
408 as '*O. siamensis*', as emphasized by Chomérat et al. (2019b). Using specimens from various
409 populations worldwide including the Caribbean Sea, the Indian Ocean and the Japanese Pacific
410 Ocean, these authors reported 108–123 μm in depth and 76–86 μm in width, but in absence of
411 location data it is impossible to know if there was a pattern linked with geographical origin. From
412 these values, the size variation appears quite low, in contrast with the observations by Zhang et al.
413 (2018) who reported specimens 68.0–113.5 μm deep and 56.5–97.3 μm wide in their study from
414 Hainan Island, or in the present study. Faust et al. (1996) apparently did not find cells smaller than
415 108 μm deep which is already a very large size for this species, compared with its original
416 description (Fukuyo, 1981; Chomérat et al., 2019b). However, it is noteworthy that such large
417 specimens as observed by Faust et al. (1996) are present in Guadeloupe Island, and the present
418 study reveals that they are genetically identical to other sequences of *O. lenticularis*, which suggests
419 that all belong to the same species. As mentioned by Fukuyo (1981) and later by Chomérat et al.
420 (2019b), the major thecal characters of this species are a straight cingulum, and the typical presence
421 of large and small pores, the latter being very abundant. All these features are present in the
422 specimens from Guadeloupe Island and no difference has been found with other data.

423 The last species clearly identified by morphology (morphotype 3) and molecular data is *O.*
424 *siamensis*, which has been recently reinvestigated by Nguyen-Ngoc et al. (2021). Prior to this study,
425 this genotype was referred as to *Ostreopsis* sp. 6 (Sato et al., 2011; Tawong et al., 2014; e.g.
426 Chomérat et al., 2020b; Lee and Park, 2020). Cells are large, undulated with a sigmoid cingulum
427 and large thecal pores are surrounded by a shallow elevated rim (Chomérat et al., 2020b; Nguyen-
428 Ngoc et al., 2021). All these features correspond well to the observations in Guadeloupe Island, but
429 the cell undulation was even more pronounced, giving an asymmetrical shape of the cells, visible
430 even with light microscope. Regarding size, cells from Guadeloupe Island are almost in the same
431 range than in Tahiti Island (cells 58.0–82.5 μm deep and 45.7–61.2 μm wide), but larger specimens
432 have been found in the present study. Compared with data for *O. siamensis* (excluding *O. cf.*
433 *siamensis* which is another species, Chomérat et al. 2020b; Nguyen-Ngoc et al. 2021), cell sizes
434 from Guadeloupe are remarkably similar to measurements given by Fukuyo (1981) from Japanese
435 populations. Compared with data by Faust et al. (1996), considering the misidentification between

436 *O. lenticularis* and *O. siamensis* by these authors (Chomérat et al., 2020b), a wider size range is
437 observed in Guadeloupe Island. However, if data of *O. labens* and *O. siamensis* in Faust & Morton
438 (1995) and Faust et al. (1996), are merged to consider a single species, the resulting size range
439 becomes remarkably similar with our data, with cells 65–98 μm deep and 57–80 μm wide. As
440 already pointed out by Chomérat et al. (2020b), larger sizes reported by Faust & Morton (1995) for
441 *O. labens* must be regarded cautiously as the sizes measured on some micrographs using scale bars
442 are outside the range given in the text. In addition, Chomérat et al. (2020b) questioned the existence
443 of *O. labens* because morphology was not significantly different from *O. siamensis*, but it may be
444 cryptic and belongs to one of the subclades within *O. siamensis* (Chomérat et al., 2020b).
445 Additional sequences from the Caribbean region are necessary to resolve this question and present
446 data from Guadeloupe Island, eastern Caribbean, bring new elements to help clarifying this issue.
447 The fact that all sequences obtained in this study are almost identical and cluster with strong support
448 with sequences from the Gulf of Thailand (TF39OS, VNPQ218) and Tahiti Island indicate
449 unambiguously that all belong to *O. siamensis* since strain VNPQ218 from Phu Quoc has been
450 recently proposed as epitype by Nguyen-Ngoc et al. (2021). Nevertheless, a different genotype
451 putatively corresponding to *O. labens* may be present in Belize and absent in Guadeloupe Island,
452 and molecular data from Belize (type locality of *O. labens*) are absolutely necessary to clarify its
453 existence or its junior synonymy with *O. siamensis*.

454

455 ***Identification of the morphotype 4 as O. heptagona***

456 The morphology of morphotype 4 in Guadeloupe Island fits almost perfectly with the description of
457 *O. heptagona* from Knight Key, Florida (Norris et al., 1985). Regarding size, Norris et al. (1985)
458 described it as a large species, 96–122 μm (average 108 μm) deep and 62–84 μm (average 70 μm)
459 wide. Cell sizes in the present study are in the same range, although no specimen reached the higher
460 values reported by Norris et al. (1985), while smaller cells have been observed in Guadeloupe
461 Island. By comparison, Faust et al. (1996) reported smaller cells of *O. heptagona* in Belize, where
462 size was 80–108 μm deep and 46–59 μm wide, and their lower values are in agreement with
463 smaller specimens in our observations. Overall morphology and plate pattern of cells from
464 Guadeloupe Island are similar to the description by Norris et al. (1985). The most striking character
465 of this species is the presence of a conspicuous suture between 1' and 5'' plates, giving a heptagonal
466 shape to 1' plate. Although this pattern has sometimes also been observed in teratological specimens
467 of cultured *O. cf. ovata* (Besada et al., 1982; Penna et al., 2010), our observations reveal that it is an
468 unambiguous character in all observed specimens. The shape and the number of sulcal plates
469 observed match with the description of *O. heptagona* made by Norris et al. (1985) which confirm
470 the identification of this species. Another conspicuous feature is the very narrow cingulum, almost

471 closed, which is also a character mentioned by Norris et al. (1985) who reported a width of *ca.*
472 1.5–3.0 μm .
473 In contrast with *O. lenticularis* or *O. siamensis* recently observed in detail with SEM (Chomérat et
474 al., 2019b, 2020b), the small posterior right sulcal plate (Sdp) which inserts between Sda and Sp
475 plates has not been observed in any specimen from Guadeloupe Island, and if present, it is
476 completely hidden by the overlap of 1''' plate. Previous SEM observations of this species are
477 scarce. Faust et al. (1996) provided illustrations showing only partially the morphology of *O.*
478 *heptagona*, and they observed one type of thecal pores (0.3 μm in diameter) which is similar with
479 our data. Surprisingly, Accoroni et al. (2020) recently mentioned the use of SEM for identification
480 of this species in Florida, but they did not provide any image nor molecular data preventing from
481 any comparison. From all aforementioned, morphological characters of cells from Guadeloupe
482 Island are in good agreement with the description of *O. heptagona*, and to our knowledge, the
483 present study is the first to combine detailed SEM observations and DNA sequences of this species.
484 However, since our material from Guadeloupe Island was relatively distant from type locality (*ca.*
485 2,250 km southeast from Knight Key), it would be important to obtain sequences from this area.

486 Interestingly, our observations of many cells from the population of Chapelle sample reveal
487 some specimens with an aberrant hypothecal pattern, in particular with a split 2''' plate, which has
488 not previously been reported from field specimens of *O. heptagona*. Variations in the thecal plate
489 pattern of dinoflagellates and existence of split plates is not unusual and this has been mentioned in
490 several genera (e.g. Besada et al., 1982; Chesnick and Cox, 1985; Chomérat and Couté, 2008). Such
491 variations concern more generally the epitheca than the hypotheca that is generally considered as
492 more stable (Chesnick and Cox, 1985), so the present result seems contradictory. Since the sample
493 in which specimens with additional plates have been found is also containing numerous infected
494 cells by the parasitoid *Amoebophrya* (Syndiniales), it can be hypothesized that thecal variations
495 occur more frequently in parasited host cells, but further investigations would be necessary confirm
496 this fact.

497

498 ***Diversity and comparison with data from the Caribbean***

499 As discussed previously by Chomérat et al. (2019b), several confusions have been made regarding
500 *O. siamensis* and *O. lenticularis* in different studies, and in most reports the correct identification
501 was not ascertained. For instance, the name change from *O. siamensis* in Carlson (1984) to *O.*
502 *lenticularis* in Carlson & Tindall (1985) in the interpretation of the same dataset introduced some
503 confusions which have been encouraged by the speculative statement by Norris et al. (1985) who
504 considered the two species as possibly conspecific without any detailed argument. Some subsequent
505 reports from the Caribbean area (e.g. Ballantine et al., 1985; Tosteson et al., 1986, 1989) do not
506 provide a clear identification of *Ostreopsis* species and the identity of the studied species remains in

507 question. It cannot be excluded that several co-existing species have been misidentified and referred
508 as to a single name. In 1990, Tindall et al. (1990) examined toxic clonal strains from U.S. Virgin
509 Islands ascribed to '*O. lenticularis*' with SEM and realized that morphologically cells better
510 correspond with the description of *O. siamensis*, but considering that this species had not been
511 mentioned from the Caribbean, they intentionally continued with the misidentification of *O.*
512 *lenticularis*, making the identity of the toxic species doubtful. Interestingly, using LM only,
513 Gamboa Márquez et al. (1994) were able to accurately identify both *O. siamensis* and *O.*
514 *lenticularis* from Los Roques archipelago in the Caribbean Sea of Venezuela, and they precisely
515 represented some differences such as the cingulum undulation and the long 2' plate in *O. siamensis*
516 which are absent in *O. lenticularis* (cf. Figs 14, 16. in Gamboa-Márquez et al. 1994). In spite of
517 these differences, Faust et al. (1996) surprisingly made a major confusion between the two species
518 in their taxonomic study and provided mistaken descriptions, perpetuating confusions between these
519 species (Chomérat et al. 2019a, b). These studies however revealed a great diversity and emphasize
520 the presence of both *O. siamensis* and *O. lenticularis* in Caribbean samples, which we can confirm
521 in the present study using molecular data. On the light of this evidence, and since previous studies
522 showed an absence of toxicity in *O. lenticularis* in contrast with *O. siamensis* (Sato et al., 2011;
523 Chomérat et al., 2019b, 2020b), several reports of toxic '*O. lenticularis*' from the Caribbean
524 (Tosteson et al., 1986; Ballantine et al., 1988; Mercado et al., 1994; Meunier et al., 1997; e.g.
525 Ashton et al., 2003; Pérez-Guzmán et al., 2008) appear now doubtful and need to be reinvestigated.

526 Moreover, descriptions of morphospecies with poor morphological distinctive features have
527 from the Caribbean added sources of confusions. For instance, the descriptions of *O. labens* (Faust
528 and Morton, 1995), *O. belizeana*, *O. caribbeana*, and *O. marina* (Faust, 1999) introduced new
529 names in an existing context of confused taxonomy. Size ranges of these species considerably
530 overlap with other species and considering large variations observed in populations observed in the
531 present study, it cannot be excluded that some of these species are junior synonyms of existing taxa,
532 as aforementioned for *O. labens*, likely a synonym for *O. siamensis*. A similar situation occurs with
533 *O. marina* which does not seem to be different from *O. lenticularis* except that it has a larger size,
534 as putatively identified by Irola-Sansores et al. (2018). Molecular data from the present study reveal
535 that larger specimens with this morphology are genetically identical with *O. lenticularis*, making
536 the existence of *O. marina* as a separate species in question.

537 Reports of *O. ovata* all over the Caribbean [St. Barthelemy Island Besada et al.(1982);
538 Virgin Islands, Carlson (1984); Venezuela, Gamboa Márquez et al. (1994); Belize and Puerto Rico,
539 Faust et al. (1996), Cuba, Delgado et al. (2006)] are based exclusively on morphology.
540 Nevertheless, recent studies demonstrated that morphology of *O. cf. ovata* is not distinctive and
541 divergent genotypes such as *O. cf. ovata* complex (Nascimento et al., 2020), *Ostreopsis* sp. 1 (Sato
542 et al., 2011) or *Ostreopsis* sp. 7 (Tawong et al., 2014) are cryptic. Hence our molecular

543 characterization confirms the presence of a genotype of the *O. cf. ovata* complex in Guadeloupe
544 Island, but, as it was infrequently found only at Rivière Sens, other genotypes may be present in
545 other sites. In contrast with larger species such as *O. lenticularis* and *O. siamensis*, this species
546 received little attention in the Caribbean area, probably because it was less abundant (Carlson,
547 1984) and it has not been studied thoroughly. Since *O. cf. ovata* is now a widely common species
548 worldwide, with some strains producing high levels of toxins (e.g. Nascimento et al., 2012;
549 Tartaglione et al., 2017) causing health concerns and beaches closures (Tester et al., 2020), studies
550 focussing on its genetics, distribution and potential toxicity should be further addressed in
551 Guadeloupe Island and more generally in the Caribbean Sea.

552 By contrast, *O. heptagona* has been mentioned several times in the Gulf of Mexico where it
553 has been originally described (Norris et al., 1985; Bomber et al., 1988, 1989; Okolodkov et al.,
554 2007, 2014; Aguilar-Trujillo et al., 2017) and in the Caribbean area (Faust et al., 1996; Morton and
555 Faust, 1997; Almazán-Becerril et al., 2015; Irola-Sansores et al., 2018). The presence of *O.*
556 *heptagona* in Guadeloupe Island constitutes the southernmost report to date. In spite of its regular
557 presence in various parts of this area, the potential toxicity of this species remains unclear, and the
558 limited toxicity to mice assessed with an unclear $LD50 > 5 \times 10^6$ cells·kg⁻¹ (according to Babinchak
559 in Norris et al. 1985) needs to be re-evaluated.

560 Species identification is hardly possible by light microscopy due to large variations in size.
561 Poor distinctive morphological characters lead to considerable confusions in the past and the use of
562 new identification tools based on molecular data appears to be essential for ecological studies and
563 monitoring purposes. This work is however essential to generate a reference dataset of sequences,
564 which constitutes a prerequisite for further development of molecular techniques based on
565 environmental DNA such as probes for fluorescent *in situ* hybridization assay (Pitz et al., 2021),
566 RT-PCR assays and metabarcoding analyses. While light microscopic observations can accurately
567 estimate the abundance of the genus *Ostreopsis* (Boisnoir et al., 2018, 2019, 2020) studying species
568 distribution, dynamics and phenology will be facilitated by implementation of such tools at the
569 scale of the Caribbean.

570

571 ***Evidence of parasitism on Ostreopsis cells***

572 Parasitoids belonging to the genus *Amoebophrya* are known to infect various planktonic organisms
573 such as radiolarians or dinoflagellate populations (Cachon, 1964) as *Akashiwo*, *Alexandrium*,
574 *Ceratium*, *Cochlodinium*, *Dinophysis*, *Gymnodinium*, *Gyrodinium*, *Karlodinium*, *Phalacroma*,
575 *Prorocentrum*, and *Scrippsiella* (Coats and Park, 2002; Chambouvet et al., 2008; Chambouvet,
576 2009; Alves-de-Souza et al., 2012). Interestingly, and in spite of being described as a generalist
577 parasitoid which can infect a great variety of hosts, *Ostreopsis* species have never been associated
578 with *Amoebophrya* infection, and thus present data constitute the first evidence that such events

579 occur in natural populations. From our data, the same parasitoid species can infect at least two
580 different *Ostreopsis* species, namely *O. lenticularis* and *O. heptagona*, which confirms a low
581 specificity. The compact mass within dinoflagellate cells could correspond to the trophont stage, an
582 advanced stage of the infection caused by the parasitoid *Amoebophrya* (Cachon, 1964; Chambouvet
583 et al., 2008). In addition, infections of other dinoflagellate genera present in the same samples (e.g.
584 *Gambierdiscus* spp., *Prorocentrum* spp.) have not been investigated and are possible. From a
585 molecular point of view, the genotype of the parasitoid found in the present study is rather distant
586 from *Amoebophrya* strains isolated from other dinoflagellates and only one sequence of this
587 genotype was already available in GenBank (MK752531, *Amoebophrya* sp. isolate AT5), without
588 any indication of the putative host and origin.

589 This unexpected finding is an illustration of serendipity, and as it was not the focus of the study,
590 several aspects regarding parasitism remain in question due to inappropriate sampling. The presence
591 of *Amoebophrya* infecting populations of *Ostreopsis* spp. could impact their dynamics and
592 probably constitute an important controlling factor of the blooms (Chambouvet et al., 2008;
593 Mazzillo et al., 2011; Park et al., 2019). Further analyses should focus on determining the host
594 specificity, the prevalence/infectivity, and the generation time of *Amoebophrya* sp. (Coats and Park,
595 2002). As suggested by Kim and Park (2016), parasitism may play a role in the toxicity of
596 dinoflagellates, and such interactions should probably better taken into consideration in studies of
597 benthic toxigenic taxa.

598

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609

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612

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616

617 **Authors contributions**

618 A. Boisnoir: original concept, sampling, microscopy, drafting and editing manuscript; G. Bilien:
619 molecular analysis, sequencing and editing manuscript, R. Lemée: original concept, editing
620 manuscript; N. Chomérat: original concept, microscopy, analysis of molecular data, drafting and
621 editing manuscript.

622

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- 910

911 **Figure legends**

912 **Fig. 1.** Maps showing the location of Guadeloupe Island in the Caribbean Sea and sampling sites
913

914 **Fig. 2.** Size ranges observed for the four different morphotypes calculated using all available data
915 from LM and SEM. Crosshairs show the minimum, maximum and mean (at intersection) values for
916 depth (i.e. DV length) and width. Individual symbols correspond to single-cells used in the
917 phylogenetic analysis (isolate numbers given in italics) and measured from LM.

918

919 **Figs 3.A–M** SEM micrographs of morphotype 1 (*O. cf. ovata*) from Rivière-Sens (**A–G**) and
920 morphotype 2 (*O. lenticularis*) from Chapelle (**H–M**). **A:** Apical view. **B:** Antapical view. **C:** Detail
921 of 2' and 3' plates and apical pore complex (APC). **D:** Left latero-apical view. **E:** Right lateral view
922 showing the straight cingulum. **F–G:** Detail of thecal pores, with some small pores (arrows) visible.
923 **H:** Apical view. **I:** Antapical view. **J:** Detail of 2' (short) and 3' plates and apical pore complex
924 (APC). **K:** Left lateral view showing the straight cingulum. **L:** Right lateral view showing the
925 straight cingulum. **M:** Detail of the smooth thecal surface with thecal pores of two sizes classes.
926 Bars: 10 µm (**A–B, H–I, K–L**); 5 µm (**D–E**); 2 µm (**C, J**), 1 µm (**F, M**); 200 nm (**G**).

927

928 **Figs 4. A–I** SEM micrographs of morphotype 3 (*O. siamensis*) from Rivière-Sens. **A:** Apical view.
929 **B:** Antapical view. **C:** Detail of 2' and 3' plates and apical pore complex (APC). **D:** Ventral view. **E:**
930 Right lateral view showing the undulated cingulum. **F:** Oblique antapical view showing the strong
931 undulation of a cell. **G:** Oblique apical view showing the cell undulation and depression of the
932 hyptheca. **H:** Detail of the ventral area with the 'ventral opening' (Vo) and sulcal plates visible. **I:**
933 Detail of cell surface with two kinds of thecal pores visible, the smaller indicated with arrows.
934 Bars, 10 µm (**A–B, D–G**); 5 µm (**C**); 2 µm (**H**); 1 µm (**I**).

935

936 **Figs 5A–H** SEM micrographs of morphotype 4 (*O. heptagona*) from Chapelle site. **A:** Apical view
937 with the suture 1'/5'' highlighted (arrowhead). **B:** Antapical view. **C:** Detail of the seven-sided first
938 apical plate 1', note the sutures 1'/5'' (white arrowhead) and 5''/6'' (black arrowhead). **D:** Detail of
939 the apical pore complex (APC) and long 2' plate contacting 4'' plate. **E:** Left lateral view of a
940 specimen showing the almost straight and narrow cingulum. **F:** Ventro-apical view. **G:** Detail of the
941 sulcal area with the ventral opening (Vo) and three sulcal plates visible. **H:** Detail of cell surface
942 with one type of thecal pores. Bars: 10 µm (**A–B, E–F**); 5 µm (**C**); 2 µm (**D–G**); 1 µm (**H**).

943

944 **Figs 6.A–I** Light micrographs of dissected cells and sulcal plates of morphotype 4 (*O. heptagona*)
945 from Chapelle **A:** Apical view of an epitheca showing the peculiar shape of 1' plate. **B:** Detail of Po

946 and 2' plates. **C:** Detached sulcal area from a broken theca. **D–E:** Views of a sulcal area with
947 different level of focus, showing some small plates Sp, Sdp and the complex three dimensional
948 shape of Ssa and Sda. **F:** Detail of an isolated Sp (with a hyaline area) and 1'''. **G:** Detail of the 't'
949 plate at the left end of Ssa. **H:** Isolated Ssa, Sda bearing a conspicuous list, and Ssp. **I:** Isolated Ssa
950 and Sda with a detached annular platelet (Sa) encircling Vo. Bars: 20 µm (**A**); 10 µm (**C**); 5 µm (**B**,
951 **D–I**).

952

953 **Figs 7.A–O.** Light micrographs of *Ostreopsis* cells with different morphologies used for single-cell
954 PCR analyses. Codes of each isolate (IFR-) and origin sample (see Table 1) are given in the lower
955 left corner. **A:** Morphotype 1 (*O. cf. ovata*); **B–E:** Morphotype 2 (*O. lenticularis*); **F–J:**
956 Morphotype 3 (*O. siamensis*); **K–O:** Morphotype 4 (*O. heptagona*), note that specimen IFR17-685
957 (**N**) was infected by *Amoebophrya* sp. Bars = 20 µm.

958

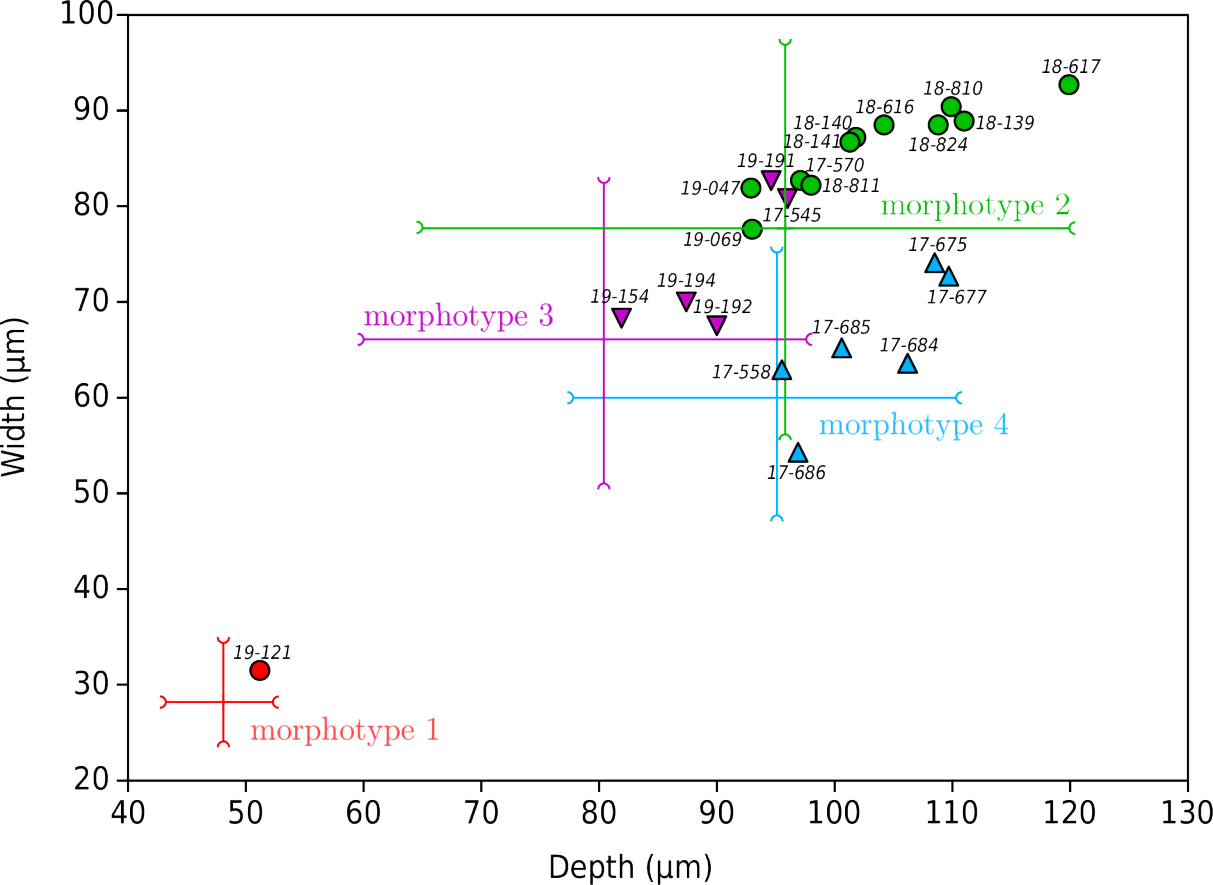
959 **Fig. 8.** Maximum-Likelihood phylogenetic tree based on 189 concatenated sequences of ribosomal
960 operon (SSU + ITS + LSU D1-D3 and D8-D10) (6382 aligned characters) of different strains of
961 *Ostreopsidoideae* and other gonyaulaceans as outgroup. *Alexandrium/Centrodinium* subclades are
962 collapsed for readability (Full tree in supplementary figure S2). Sequences acquired in the present
963 study are indicated in bold type. Branch robustness was indicated by bootstrap values (ML) and
964 posterior probabilities (BI). Bootstraps values below 65 and posterior probabilities below 0.9 are
965 shown with '-'. On the right, vertical bars delimit major clades corresponding to the different
966 species/genotypes.

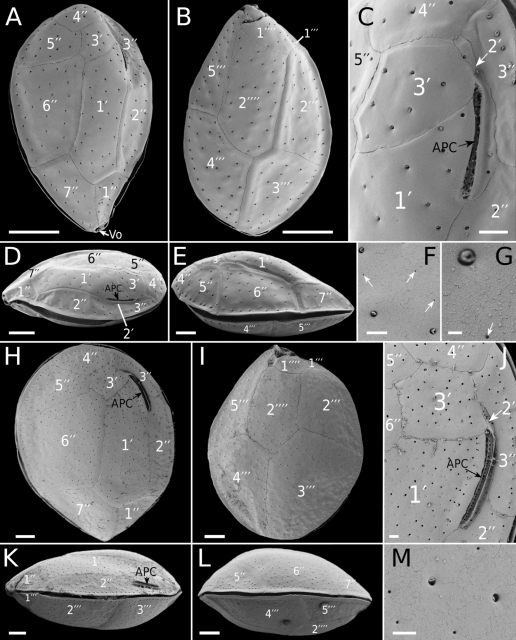
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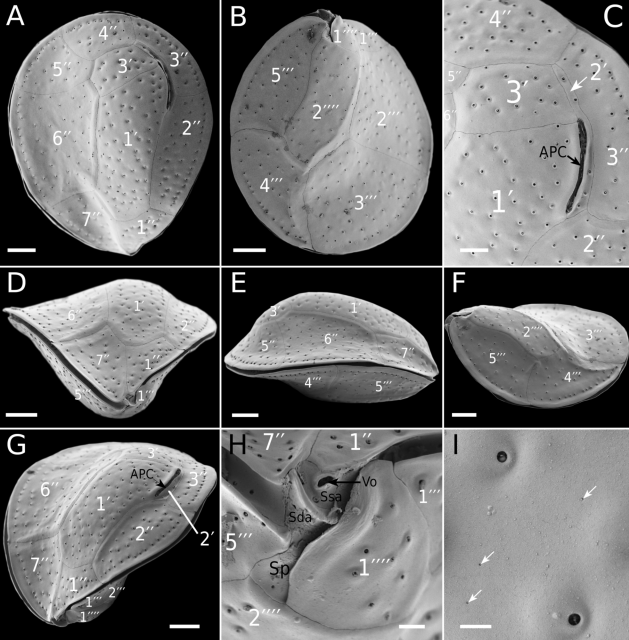
968 **Figs 9 A–F** Light micrographs of Lugol-fixed *Ostreopsis* cells infected by the parasitoid
969 *Amoebophrya* sp. **A–D:** Cells of *O. lenticularis*, note that three specimens have been used for
970 sequencing the parasitoid (isolate codes indicated in the lower left corner). **E–F:** Cells of *O.*
971 *heptagona*. The white arrowheads show the more or less developed trophont stage of *Amoebophrya*
972 within *Ostreopsis* cells. Bars = 10 µm.

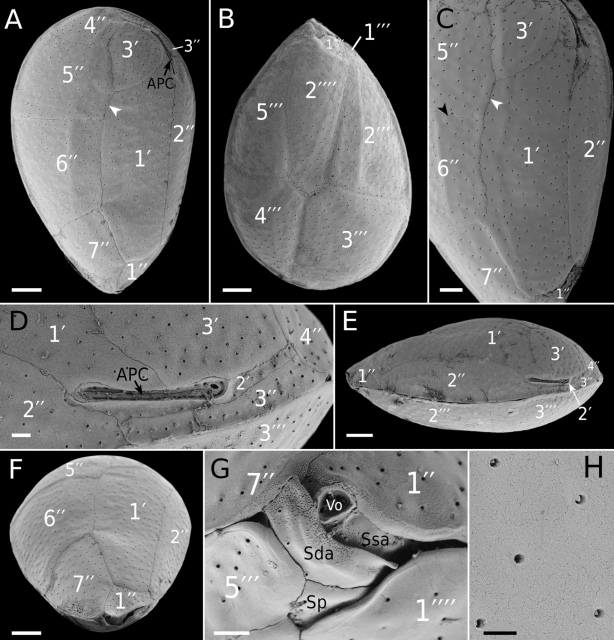
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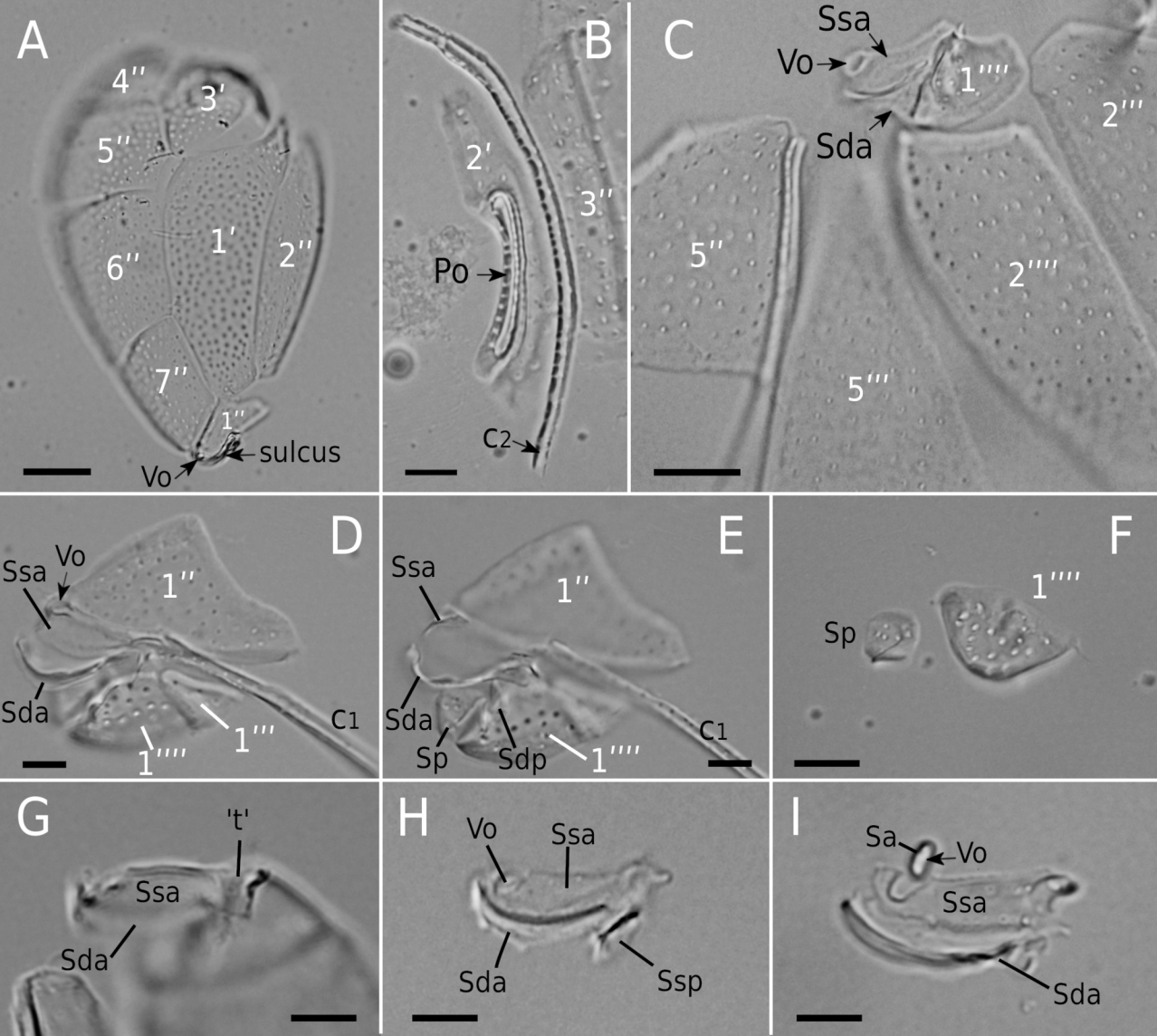
974 **Fig. 10.** Maximum-Likelihood phylogenetic tree based on 49 sequences of small ribosomal SSU
975 and internal transcribed spacer 1 (2354 aligned characters) of *Amoebophrya* spp. and parasitoid
976 dinoflagellates (Syndiniales). The clade including the four new sequences found in infected
977 *Ostreopsis* cells (in bold type) is highlighted with a grey background. Branch robustness was
978 indicated by bootstrap values (ML) and posterior probabilities (BI). Bootstraps values below 65 and
979 posterior probabilities below 0.9 are indicated with '-'.

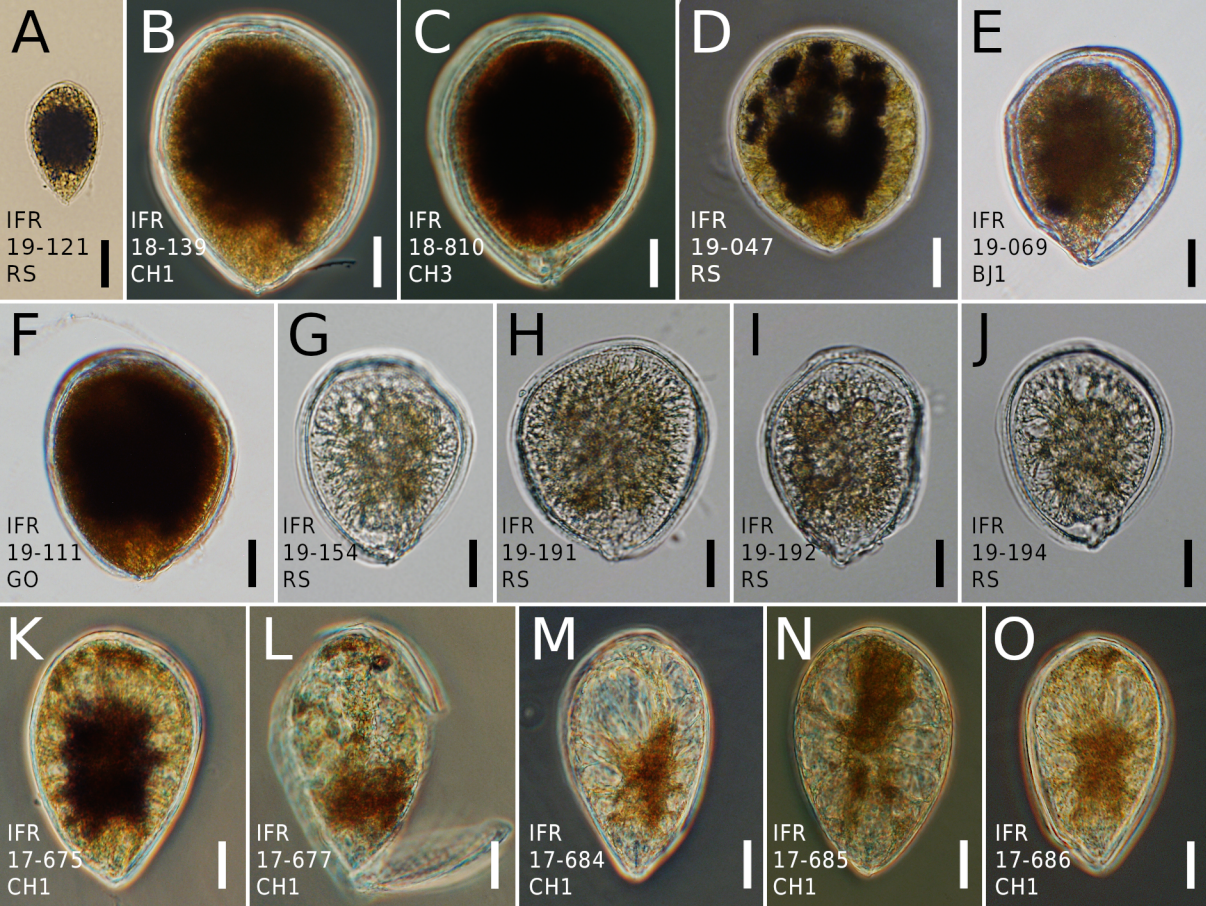






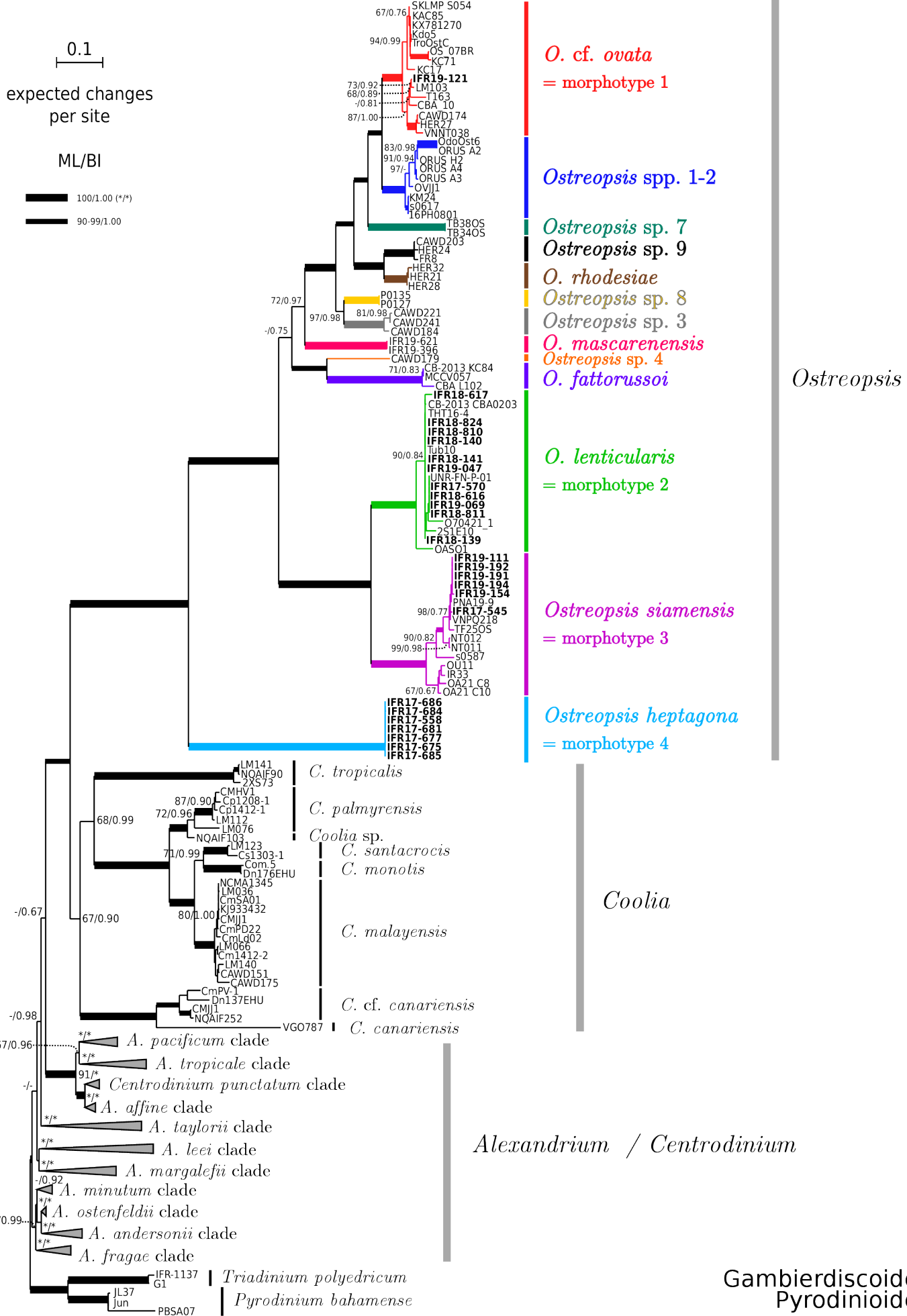




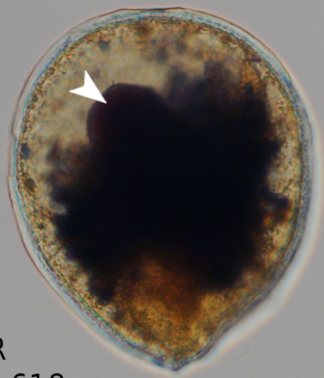
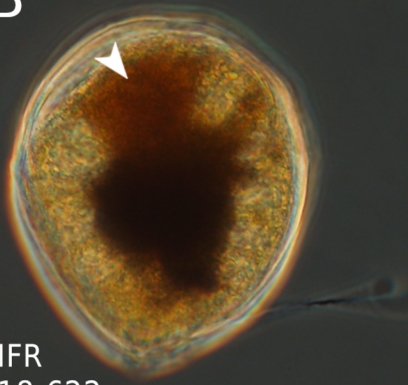
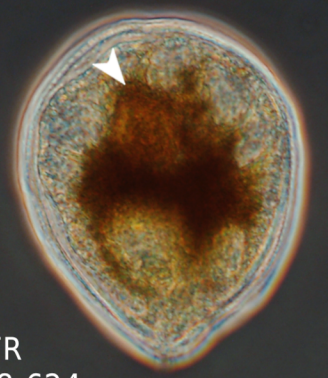
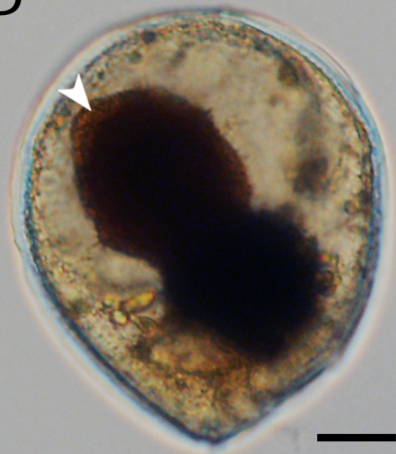
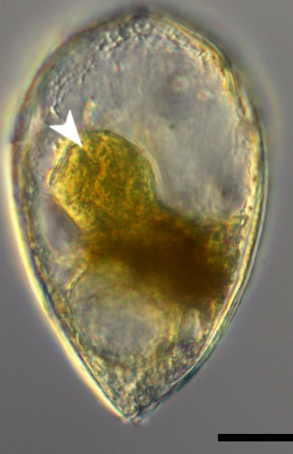


0.1
 expected changes
 per site

ML/BI
 100/1.00 (**)
 90-99/1.00



Gambierdiscoideae
 Pyrodinioideae

AIFR
18-618**B**IFR
18-622**C**IFR
18-624**D****E****F**