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***Madanidinium loirii* gen. et sp. nov. (Dinophyceae), a new marine benthic dinoflagellate from Martinique Island, Eastern Caribbean**

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Abstract:

A new benthic phototrophic dinoflagellate is described from sediments of a tropical marine cove at Martinique Island and its micromorphology is studied by means of light and electron microscopy. The cell contains small golden-brown chloroplasts and the oval nucleus is posterior. It is laterally compressed, almost circular in shape when viewed laterally. It consists of a small epitheca tilted toward the right lateral side and a larger hypotheca. In the left view, the cingulum is more anterior and the epitheca is reduced. The cingulum is displaced and left-handed. This organism is peculiar in having no apical pore and its thecal plate arrangement is 2' 1a 7'' 5c 3s 5''' 1'''''. The plates are smooth with small groups of pores scattered on their surface. An area with 60–80 densely arranged pores is found near the centre of the 2''' plate, on the left lateral side. Morphologically, these features are different from all other laterally compressed benthic genera. In addition, molecular genetic sequences of SSU and partial LSU form a distinct and well-supported clade among dinoflagellates and support the erection of a new genus. However, molecular phylogenies inferred from ribosomal genes failed to confirm any clear relationship with other benthic taxa and affinity with other laterally compressed dinoflagellates has not been demonstrated. Hence, the taxonomic affinity of *Madanidinium loirii* with a defined order and family is unclear at the moment.

Keywords: benthic ; Caribbean ; Dinophyceae ; Martinique ; Morphology ; Phylogeny ; rDNA ; SEM ; taxonomy

46 **Introduction**

47 Since they have been discovered and studied by E.C. Herdman (1921, 1922, 1924a, b),
48 benthic dinoflagellates stayed poorly studied for several decades (Balech, 1956;
49 Dragesco, 1965). They gained a new interest for scientists when the epiphytic species
50 producing maitotoxin and ciguatera toxin, namely *Gambierdiscus toxicus* was associated
51 with ciguatera disease in the tropical Pacific (Adachi & Fukuyo, 1979; Taylor, 1979).
52 Then, several other benthic taxa were found to be harmful and involved in the complex
53 mechanism of ciguatera (Bomber & Aikman, 1989; Litaker *et al.*, 2010). Because of
54 this potential toxicity, several taxonomic studies were subsequently realized in the
55 tropical regions (Fukuyo, 1981; Besada *et al.*, 1982; Berland *et al.*, 1992; Grzebyk *et*
56 *al.*, 1994; Chinain *et al.*, 1999) but also in temperate areas worldwide (e.g. Horiguchi &
57 Chihara, 1983; Saunders & Dodge, 1984; Larsen, 1985; Hoppenrath, 2000b; Aligizaki
58 & Nikolaidis, 2006; Murray, 2009; Chomérat *et al.*, 2010b; Fraga *et al.*, 2011).

59 In the Caribbean Sea where ciguatera is known from the late 18th century and has
60 caused health problems for many years (Bagnis, 1981; Olsen *et al.*, 1984; Tosteson,
61 2004; Tester *et al.*, 2010), studies on benthic dinoflagellates were mostly focused on
62 epiphytic species responsible for this disease, in order to better understand their
63 distribution and assess the associated risk. Several investigations were made in the
64 northern and eastern parts of the basin (Ballantine *et al.*, 1985; Taylor, 1985; Ballantine
65 *et al.*, 1988; Litaker *et al.*, 2010). On the western side, other studies have later been
66 realized on Belizean coast (e.g. Faust & Gullede, 2002; Faust, 2009), and around the
67 Mexican and Cuban coasts (Hernández-Becerril & Almazán Becerril, 2004; Delgado *et*
68 *al.*, 2006). Comparatively, southern Caribbean sea has been only scarcely investigated
69 by Grzebyk *et al.* (1998) who collected samples in a Panamian island and Rodriguez *et*

70 *al.* (2010) who made a survey in San Andrés Island (Caribbean Colombia). In French
71 Antilles (also known as French West Indies) where ciguatera intoxications have been
72 recurrently documented (Olsen *et al.*, 1984; Vernoux, 1988; Pottier *et al.*, 2001; Rosine
73 *et al.*, 2008; Tester *et al.*, 2010), only a few surveys have been undertaken to check the
74 presence and identify toxigenic species (Besada *et al.*, 1982; Taylor, 1985; Litaker *et*
75 *al.*, 2010).

76 Harmful species apart, taxonomic studies focused on the diversity of benthic
77 dinoflagellates are relatively scarce in the Caribbean. The first major contribution was
78 made by Carlson (1984) who collected samples in several places in Virgin Islands and
79 identified 38 benthic taxa. Then, M. A. Faust investigated extensively the western coast
80 and published a remarkable series of papers with descriptions or reinvestigations of taxa
81 from coral-reefs mangrove embayments in Belize (Faust, 1990, 1993a, b, c, 1994, 1996;
82 Faust *et al.*, 1996; 2008). In the course of her study, she described the very atypical and
83 intriguing genus *Plagiodinium* (Faust & Balech, 1993), which has been then found very
84 infrequently in other areas (M. Saburova, pers. comm.) and still needs further
85 investigation. Indeed, most of these taxa are known only from their morphology, and it
86 would now be of a great importance to complement their knowledge with DNA
87 sequences to better understand their phylogenetic position within dinoflagellates
88 lineages (Hoppenrath *et al.*, 2013).

89 Since no taxonomic survey has been realised to date in French Antilles, we
90 undertook to assess the diversity of benthic dinoflagellates in Martinique Island from
91 occasional samples. During our study, we encountered a very atypical and interesting
92 taxon, which is distinct from any armoured dinoflagellate genus hitherto described.
93 Cells are strongly flattened laterally, and thecal plates are delicate and arranged with a

94 unique pattern. In the present paper, we aim to describe its morphology using light and
95 scanning electron microscopy, and attempt to establish its phylogenetic position among
96 other dinoflagellates using molecular data from environmental samples and clonal
97 cultures.

98

99 **Material and methods**

100 *Sampling and cultivation*

101 Martinique Island is a French volcanic island of the Lesser Antilles archipelago, located
102 in the eastern part of the Caribbean Sea (Fig. 1). It is about 70 km long and 30 km wide.
103 Samples of upper sediments were collected by snorkelling (1 to 3 m below the surface
104 of water) at Anse Dufour (coordinates 14° 31.538' N, 61° 05.446' W), a cove located on
105 the Caribbean side of the island (Fig. 1), the 16 March 2010, and 22, 26, 30 March and
106 6 April 2013. All samples of March 2010 and March 2013 were immediately preserved
107 with acidic Lugol's solution (~5% final concentration) and stored in the dark at 4 °C
108 before further examination. The 6 April 2013, aliquots were fixed and stored in the
109 same conditions, and aliquots were kept fresh for algal isolation and cultivation.
110 Immediately after collection, they were carefully packed to limit thermal variations in
111 the baggage compartment and transferred to Ifremer laboratory in Concarneau
112 (mainland France) by plane and train. Because of travel duration, the isolation of living
113 cells was carried out two days after sampling (8 April).

114 For cultivation, single cells from the live sediment subsamples were identified
115 and isolated with a micropipette under an IX41 (Olympus, Tokyo) inverted microscope.
116 Then, they were rinsed in several drops of seawater and placed in 96-well culture plates
117 containing seawater and medium. After some divisions, each clonal strain was

118 transferred to culture plates with increasing well volume. Several cultures were
119 established but only two were kept and grown in 50 ml culture flasks. The strain IFR-
120 MLO-01M was grown in K medium (Keller *et al.*, 1987) while the second strain IFR-
121 MLO-02M was grown in f/2 medium (Guillard & Ryther, 1962; Andersen *et al.*, 2005).
122 Both strains were maintained in a growth chamber set up at 22 ± 1.0 °C and 12:12 light
123 : dark illumination cycle with ~ 50 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ provided by white fluorescent
124 tubes.

125

126 *Observations*

127 LM

128 Observations in light microscopy were performed on isolated cells put on standard slide
129 with a coverslip, using a BX41 (Olympus, Tokyo) upright microscope. It was equipped
130 with brightfield, differential interference optics, epifluorescence filter sets U-MWU2 for
131 DAPI stain (excitation: BP330-385; beamsplitter: DM400; emission: BA 420) and U-
132 MWIB2 for chlorophyll autofluorescence (excitation: BP460-490; beamsplitter:
133 DM505; emission: BA510IF), an Osram mercury short arc HBO 100W lamp as the
134 light source for epifluorescence, and a DP72 (Olympus, Tokyo) color digital camera.
135 To visualize the nuclei, some cells from the culture were isolated and fixed with 2%
136 glutaraldehyde for 10–20 min at 4 °C, and then stained with 4',6-diamidino-2-
137 phenylindole (DAPI) according to Chomérat *et al.* (2012). In addition, thecal plates
138 were observed using Calcofluor White M2R (Sigma Aldrich) as fluorescent dye.

139

140 Scanning electron microscopy

141 Cells from the cultures were obtained after a vigorous shaking of the flask, and then
142 fixed with 2% formaldehyde. Then, the specimens were processed according to the
143 methods described in Couté (2002) and Chomérat & Couté (2008). They were
144 dehydrated and critical point dried, and then they were observed with a Quanta 200
145 (FEI, Eindhoven, The Netherlands) scanning electron microscope with an acceleration
146 voltage of 5 kV and a secondary electrons detector.

147 Cells were measured from SEM digital micrographs using ImageJ software (Rasband,
148 1997–2006). SEM images were presented on a uniform background using Adobe
149 Photoshop CS5 (v. 12.1, Adobe Systems).

150

151 *DNA amplification and sequencing*

152 Single cells from the fixed sediment sample of the 16 March 2010 were isolated with a
153 capillary pipette under the IX41 inverted microscope. They were rinsed in several drops
154 of distilled water and then placed in a 0.2 mL PCR tube containing 5 µl of distilled
155 water. Living cells from the two cultures were isolated similarly and 1 to 5 cells were
156 placed into PCR tubes. All tubes were stored at -20 °C prior to analysis. For PCR, tubes
157 were thawed and processed as described previously in Chomérat et al. (2010b).

158

159 *Molecular analysis and phylogeny*

160 The SSU and LSU sequences obtained were aligned with other dinoflagellates
161 sequences and *Perkinsus marinus* (perkinsoid, Alveolate) as external group, using
162 MAFFT software version 7 (Kato & Standley, 2013) with selection of the Q-INS-i
163 algorithm which considers the secondary structure for the alignment. This step was
164 followed by refinement by eye with MEGA software version 5.2.1 (Tamura *et al.*,

165 2011). For SSU a dataset of 77 taxa and 1691 aligned positions has been used. For LSU,
166 a matrix of 49 taxa and 860 positions was used. Ambiguous parts of the alignment
167 (including the D2 domain) were excluded from the analysis using gblocks software
168 version 0.91b, with less stringent parameters. Genbank accession numbers of all
169 sequences used are available in the supplementary material.

170 For each data set, evolutionary models were examined using maximum
171 likelihood (ML) and Bayesian Inference analysis (BI). The evolutionary model was
172 selected using jModelTest version 0.1.1 (Posada, 2008). According to Akaike
173 information criterion (AIC) and Bayesian information criterion (BIC), a general time
174 reversible (GTR) model with a gamma correction (Γ) for among-site rate variation and
175 invariant sites was chosen for the SSU dataset while a Tamura-Nei model with no
176 invariant sites was chosen for the LSU dataset.

177 Maximum likelihood analyses were performed using PhyML version 3.0
178 (Guindon *et al.*, 2010), and Bayesian analyses were run using Mr Bayes version 3.1.2
179 (Ronquist & Huelsenbeck, 2003). Bootstrap analysis (1000 pseudoreplicates) was used
180 to assess the relative robustness of branches of the ML tree. Initial Bayesian analyses
181 were run with a GTR model (nst=6) with rates set to invgamma (gamma for LSU
182 dataset). Each analysis was performed using four Markov chains (MCMC), with two
183 millions cycles for each chain. Trees were saved every 100 cycles and the first 2000
184 trees were discarded. Therefore, a majority-rule consensus tree was created from the
185 remaining 18000 trees in order to examine the posterior probabilities of each clade.

186 The consensus trees were edited using MEGA 5 software. The best ML
187 phylograms are shown with robustness values for each node (ML/BI).

188 **Results**

189 ***Madanidinium loirii* gen. et sp. nov. Chomérat (Figs 2–28)**

190 DIAGNOSIS GENERICO-SPECIFICA (art. 38.5, McNeill *et al.*, 2012)

191 Genus repositum in Dinophyta ; in incertum ordinem et incertam familiam ; solitarium ;
192 marinum ; cum theca et in arena vivens. Cellulae fere circulares in latere visu valdeque
193 compressae a latere in ventrali visu. Longitudo : 25.2–31.0 μm ; latitudo : 16.7–18.7 μm
194 ; dorsoventralis altitudo : 22.1–28.8 μm . Epitheca inclinata et deminuta ; altior in
195 dextero aspectu. Porus apicalis absens. Cingulum cellulam perfecte cingens et
196 descendens ; transversus in sinistro visu et obliquus in dextero visu. Hypotheca major.
197 Thecae laminarum tabulatio : 2' 1a 7'' 5c 3s 5''' 1'''' . Laminae thecae laeves cum poris in
198 parvo numero aggregatis aequabiliterque dispersi in tota theca. Lamina 2''' cum parva
199 regione praebenti poros dense compressos. Parvi chloroplasti numerosi. Nucleus ovalis
200 in posteriore cellulae parte positus.

201

202 ETYMOLOGY: the genus is named after *Madanina*, the ancient local name of Martinique
203 Island (du Tertre, 1667-1671; Daney de Marcillac, 1846) and *-dinium* suffix for
204 Dinophyceae. The specific epithet *loirii* commemorates Maurice Loir (French
205 diatomist) who collected many samples from Martinique Island, and who kindly offered
206 to the authors those used in the present study.

207

208 TYPE SPECIES: *Madanidinium loirii*

209 HOLOTYPE: Fig. 11 (cell from the culture IFR–MLO–02M, SEM stub IFR-13H6 has
210 been deposited to the Centre of Excellence for Dinophytes Taxonomy (CEDiT) with the
211 accession reference CEDiT2013H22).

212 ISOTYPES: Figs 12–13, fixed culture CEDiT2013I23
213 TYPE LOCALITY: Anse Dufour (14°31.538' N, 61°05.446' W), Martinique Island, eastern
214 Caribbean Sea.
215 DNA SEQUENCE INFORMATION: Sequences have been deposited in Genbank under
216 accession numbers KF751599, KF751600, KF751601, KF751602, KF751603 and
217 KF751604.

218

219 The cells are laterally flattened, with their depth (i.e. dorso-ventral width) larger than
220 lateral width. Hence, they are mostly observed in lateral view and their shape is almost
221 circular (Figs 2–3, 5–6). They are 25.2–31.0 μm long (mean 28.9 μm , s.d. 1.4 μm ,
222 $n=16$), 22.1–28.8 μm deep (mean 25.9 μm , s.d. 1.8 μm , $n=16$) and 16.7–18.7 μm wide
223 ($n=2$). The length to depth ratio varies from 1.05 to 1.21 (mean 1.12, S.D. 0.05, $n=16$).
224 The cingulum is anterior and descending (left-handed) (Figs 4, 11, 13). Seen from the
225 left side (Figs 3, 12), it is straight, anterior, and the epitheca is very small, emerging of
226 1.7–2.7 μm ($n=5$) above the cingulum. In contrast, in the right lateral view (Figs 2, 11),
227 the epitheca is higher (4.1–7.6 μm , $n=7$), and the cingulum is conspicuously oblique,
228 descending towards the ventral area (Figs 11, 24, 27).

229 Cells contain small yellow-brown chloroplasts. The oval nucleus is located
230 posteriorly (Figs 5, 7, 24). Some cells have a large pusule located on the anterior ventral
231 side, near the sulcal area (Figs 3, 24).

232 The thecal plate pattern is 2' 1a 7'' 5c 3s 5''' 1'''''. The epitheca comprises 10
233 plates and does not have an apical pore (Figs 16, 26). Since the application of the
234 Kofoid nomenclature of thecal plates was not straightforward, we decided that the
235 apical plates were those in contact with the apex (geometrically speaking) of the cell

236 and the unique plate actually not in contact with the apex and the cingulum is
237 considered as an intercalary plate. In apical view, the epitheca is roughly pear-shaped,
238 and tapers ventrally (Fig. 16). Plates are arranged asymmetrically and those on the right
239 side are higher than those inserted on the left side (Figs 16–18, 26). The 1' and 2' plates
240 are medium-sized, pentagonal and located at the apex of the slightly dome-shaped
241 epitheca (Figs 16–17). The 1'' plate is elongated, five sided and located ventrally (Figs
242 16, 19). The 2'' and 3'' plates are pentagonal and border the left side of the epitheca
243 (Figs 16, 18). The 4'' and 5'' plates are very small, rectangular, four-sided, and located
244 on the dorsal side of the epitheca (Figs 17, 20). The 6'' plate which is the largest of the
245 epitheca, is six-sided (Figs 16–17). The 7'' plate is roughly trapezoidal and four-sided
246 (Figs 16, 19), although it has a very short contact with the Sd plate ventrally (Figs 9, 10,
247 19). The unique intercalary plate 1a is pentagonal and in line with the two apical plates,
248 but it is located more dorsally (Figs 16, 18, 20).

249 The cingulum completely encircles the cell and is composed of five plates
250 unequal in size (Figs 16–18). The c_2 plate is large and runs along the left side of the
251 theca, with its distal end facing the suture 2'''/3''' on the hypotheca (Fig. 12). The c_3 plate
252 is small and located dorsally, and is running along the width of the 3''' plate (Fig. 20).
253 The sulcus is moderately long, and slightly oblique with respect to the longitudinal axis
254 of the cell (Fig. 13). In SEM, we partially observed the flagellar pore, which is
255 elongated oval in shape and located ventrally (Fig. 19). It is bordered by three major
256 sulcal plates Sa, Sd, and Sp (Figs 13, 19). Our observations of the sulcus using
257 epifluorescence microscopy on several specimens confirm that the sulcus is composed
258 of three plates (Figs 9, 10). The Sa plate is hook-shaped and in contact with the c_1 plate.

259 The Sd plate forms the end of the cingulum and connects the epitheca. The Sp plate is
260 the largest of sulcal plates, and is posteriorly pointed (Figs 9–10).

261 The hypotheca is formed of 6 major plates. The first postcingular plate 1''' is
262 ventral and folds in order to form a flange covering the left side of the sulcus (Figs 11,
263 13). The 2''' plate which is the largest of the hypotheca, is trapezoidal and four-sided,
264 covering most of the left lateral side (Fig. 12). The 3''' plate is rectangular and is located
265 on the dorsal side of the hypotheca (Fig. 14). The 4''' plate is large and four sided (Fig.
266 11). The 5''' plate is the smallest of postcingular plates and contacts six plates, namely
267 1''', 4''', 1''''', c₅, Sd and Sp (Fig. 13). The antapical plate 1'''' is pentagonal and elongated
268 (Fig. 15).

269 Thecal plates are thin, delicate, and smooth. They are covered by small groups
270 of pores, and some isolated pores (0.1–0.2 µm in diameter) (Fig. 21). On the large
271 lateral plate 2''', an area of closely arranged pores (68–86 in number; $n = 4$) of 0.08–0.1
272 µm in diameter is present nearly in the centre (Figs 12, 22–23). This area is variable in
273 shape, being circular to elongated (Figs 22–23).

274 In culture, cells of *M. loirii* are almost always attached to the bottom of the
275 container, and swimming cells are observed occasionally. The cells are strongly
276 adherent to the substrate by their lateral sides and they appear almost always in lateral
277 views. However, no particular structures such as stalks have been observed.

278

279 *Molecular phylogeny*

280 The results of the SSU and LSU phylogenetic analyses show that the sequences
281 acquired from cultures and environmental specimens group together within a well
282 supported clade (Figs 29, 30). In the phylogeny inferred from SSU, the position of

283 *Madanidinium* clade is not supported and no clear relationships with other genera
284 emerge (Fig. 29). In the LSU analysis, *Madanidinium* appears as a sister-clade to
285 *Adenoides eludens* (Fig. 30), albeit without support (bootstrap value of 51 in ML and
286 posterior probability of 0.90 in BI). In addition, the clade formed by *Madanidinium* and
287 *Adenoides* forms a sister group with *Prorocentrum* species but without support.

288

289 **Discussion**

290 Morphologically, *Madanidinium* has features closely related to other strongly laterally
291 compressed sand-dwelling genera with a reduced epitheca like *Plagiodinium*,
292 *Planodinium*, *Sabulodinium*, *Cabra*, and *Pileidinium* (Table 1) but also some
293 *Thecadinium* species (Hoppenrath 2000a, Yoshimatsu *et al.*, 2006). In addition, a
294 morphological resemblance can be found with the genus *Sinophysis* Nie et Wang
295 (Dinophysales), that is also strongly laterally compressed and possesses a reduced-
296 epitheca (Hoppenrath 2000b), but the thecal plate organization of *Madanidinium* is not
297 of the dinophysoid type and no further comparison is possible. In *Plagiodinium*
298 *belizeanum*, the epitheca is atypical, very small and slightly inclined to the ventral side
299 (Faust & Balech, 1993), which differs from *M. loirii*. The left-handed displacement of
300 the cingulum in *M. loirii* is peculiar and reminds that of *Thecadinium yashimaense*
301 (Bolch & Campbell, 2004; Hoppenrath *et al.*, 2004; Yoshimatsu *et al.*, 2004;
302 Hoppenrath *et al.*, 2005), but also the planktonic taxa *Thecadiniopsis tasmanica* and
303 *Pseudothecadinium campbellii* (Croome *et al.*, 1987; Hoppenrath & Selina, 2006). This
304 is the reverse situation in the benthic genus *Cabra* where the epitheca is higher on the
305 left side than on the right lateral side. When seen in the left lateral view, *M. loirii*
306 outline is very similar to that of *Sabulodinium*, because the epitheca is almost not visible

307 and the cingulum is short and very anterior. However, in *Sabulodinium*, the cingulum is
308 not displaced, as well as in *Planodinium* (Saunders & Dodge, 1984; Hoppenrath *et al.*,
309 2007). And in contrast with *Pileidinium*, the cingulum is complete in *Madanidinium*.
310 Hence, owing to its peculiar overall morphology and position of the cingulum, *M. loirii*
311 can be easily distinguished from most other benthic genera with the light microscope.

312 Concerning the plate pattern, *Madanidinium* is also very atypical. The number
313 and arrangement of epithecal plates is the major discrepancy with other genera (Table
314 1). The absence of an apical pore on the epitheca is a striking and uncommon feature
315 which has been reported to date only in *Planodinium striatum* (Saunders & Dodge,
316 1984) and a few *Thecadinium* species, as shown first by Hoppenrath (2000a) and then
317 by Yoshimatsu *et al.* (2004). Comparatively, in *Plagiodinium belizeanum*, the authors
318 reported an unusual, minute plate provisionally named Po, which has been seen only at
319 high magnification with the light microscope (Faust & Balech, 1993). Unfortunately, it
320 has not been studied in SEM and no detailed information about this pore is available. In
321 *Pileidinium ciceropse*, a simple circular pore has been found on the epitheca (Tamura &
322 Horiguchi, 2005) and it is considered as homologue of the apical pore present in other
323 taxa. Interestingly, the asymmetric epitheca of *Madanidinium* with precingular plates
324 larger on the right side and smaller plates on the left side is an unusual character not
325 found in other genera with a displaced cingulum such as *Cabra* or *Thecadiniopsis*.

326 The presence of five cingular plates in *Madanidinium* is a feature found also in
327 in *Plagiodinium*, *Sabulodinium*, *Thecadiniopsis* and *Thecadinium*. Croome *et al.* (1987)
328 emphasized that this is a character similar with freshwater peridinioids, while most of
329 gonyaulacoids have six plates. The reduced number and very simple arrangement of the
330 sulcus of *Madanidinium* is remarkable and to date it is the minimum number of sulcal

331 plates observed in a benthic genus. In other taxa, four or more sulcal plates have been
332 described. Nevertheless, although we have used epifluorescence microscopy and plate
333 staining, it cannot be excluded that some very small platelets have been overlooked in
334 our study, since the sulcus is a difficult part to study. In addition, since the 1'' plate
335 seems to have a short contact with the flagellar pore, it could be alternatively interpreted
336 as a fourth sulcal (Sa) plate. However, as it is not part of the furrow and is actually
337 completely in the epitheca, we considered that it fits better with the definition of a
338 precingular plate. Moreover, the plate that we interpreted as Sa is hook-shaped, as in
339 some gonyaulacoid genera like *Alexandrium*.

340 The arrangement of plates on the hypotheca of *M. loirii* is not distinctive and
341 many benthic dinoflagellates like *Cabra*, *Plagiodinium*, *Sabulodinium*, *Pileidinium*
342 (Table 1), *Thecadinium* pro parte and the planktonic genera *Thecadiniopsis* and
343 *Pseudothecadinium* have a similar pattern of five postcingular and one antapical plate.
344 However, the presence of an area of densely arranged pores near the centre of the 2'''
345 plate on the left lateral side of the hypotheca is a very uncommon feature among the
346 genera (Table 1). An area of grouped pores (or deep areolae) has been reported in *Cabra*
347 and some other benthic genera such as *Rhinodinium*, *Roscoffia* and in some benthic
348 *Prorocentrum* species. However this area is antapical and located on the 1'''' plate in
349 *Cabra*, *Rhinodinium* and *Roscoffia* (Hoppenrath & Elbrächter, 1998; Murray *et al.*,
350 2006; Chomérat *et al.*, 2010a), which differs from *Madanidinium* where it is lateral as in
351 *Prorocentrum* species. In *Prorocentrum panamense* and *P. pseudopanamense*, a
352 roundish depression with a sieve-like bottom is present on the posterior dorsal side of
353 the right lateral plate (Hoppenrath *et al.*, 2013) while in *P. glenanicum*, a group of
354 closely arranged pores, very similar to that observed in *M. loirii*, is found just above the

355 centre of the right lateral plate (Chomérat *et al.*, 2011). To date, the role of these
356 structures has not been ascertained, but from observations of a live culture of *P.*
357 *panamense*, it seems that cells can extrude mucous from the pores of this area, and
358 attach to the substrate (M. Saburova, pers. comm.). Such fixation can be very efficient,
359 and this can explain the strong adherence of cells of *M. loirii* in culture flasks. This is
360 likely an adaptation to the benthic way of life to resist to water flow but further
361 ultrastructural studies are required to confirm this hypothesis.

362 *Madanidinium* is a phototrophic genus that can be maintained in culture, like
363 *Plagiodinium* and *Pileidinium* also reported with plastids (Faust & Balech, 1993;
364 Tamura & Horiguchi, 2005). Interestingly, these two genera are from tropical areas, like
365 *Madanidinium*. Among *Thecadinium* species, the type species *T. kofoidii* has
366 chloroplasts (Hoppenrath, 2000a) and *T. yashimaense* and *T. arenarium* are
367 phototrophic (or mixotrophic), as well as *Pseudothecadinium* (Hoppenrath & Selina,
368 2006). In contrast, the genera *Cabra*, *Planodinium*, *Sabulodinium* and most
369 *Thecadinium* species are colourless and strictly heterotrophic (Saunders & Dodge, 1984;
370 Chomérat *et al.*, 2010a).

371 As a consequence, morphological features of *Madanidinium* are different
372 enough from all described genera and justify the establishment of a new genus.

373

374 *Molecular phylogeny*

375 Molecular data support that *Madanidinium loirii* corresponds to a new dinoflagellate
376 taxon, since its SSU and LSU sequences diverge from all other known genera.
377 However, as previously shown by several authors, the resolution and support of deeper
378 branches in the phylogenies inferred from ribosomal genes is inexistent or very low, and

379 no clear relationship between *Madanidinium* and other taxa can be found from our
380 analyses. With SSU, the position of this new genus is not stable in the trees, which
381 indicates that this ribosomal gene lacks a good phylogenetic signal which would allow
382 to place it within a higher taxonomic rank (family, order). This problem has already
383 pointed out with several other ‘unusual’ and monotypic genera of benthic
384 dinoflagellates (Tamura & Horiguchi, 2005; Hoppenrath *et al.*, 2007; Yamada *et al.*,
385 2013). Moreover, no relationship was found with any of the morphologically related
386 taxa with a lateral compression for which SSU rDNA sequences are available, such as
387 *Sabulodinium*, *Pileidinium* and *Thecadinium*. Although the position of *Sabulodinium*
388 and *Pileidinium* is uncertain in the SSU tree due to the lack of support, they are widely
389 divergent from *Madanidinium*. From LSU, there is an indication that *Madanidinium*
390 could be related to *Adenoides eludens*, another benthic and phototrophic genus, but this
391 is almost unsupported. Morphologically, *Adenoides* is also compressed laterally, but
392 less than *M. loirii*, and no similarities in the thecal plate arrangement can be found
393 between these two genera. Thus, the phylogenetic relationship result should be treated
394 with caution because this affinity (not supported) has not been observed in the SSU
395 phylogeny although the sequence of this species was included in the tree. Moreover,
396 there are almost no LSU sequences of the morphologically related taxa compressed
397 laterally available in Genbank, which can bias our analyses. The dataset should be
398 improved with the addition of more taxa. As a consequence, the evolution of benthic
399 and laterally compressed dinoflagellates is still unclear. It is not yet possible to infer
400 whether these genera derived from a common benthic ancestor or if they resulted from a
401 convergent evolution of similar traits well adapted to the benthic life. Hence, a
402 considerable work of sequence acquisition remains to be done for benthic

403 dinoflagellates, and it is absolutely necessary in order to get a better understanding of
404 the evolution within this very diverse and complex group of protists. This task is
405 rendered difficult by the rarity of these organisms and the difficulty to keep them in
406 cultures. In case of phototrophic taxa, as with *Madanidinium*, the use of strains in
407 culture can allow extensive ultrastructural, genetic and biochemical studies, which
408 represents a great opportunity to increase the knowledge and understanding of the
409 biology of benthic dinoflagellates.

410

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417

418

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653

654 Figure legends:

655

656 **Fig. 1.** Maps showing the localization of the Martinique Island in the Caribbean Sea
657 (Atlantic Ocean), and the sampling area (Anse Dufour) on the western coast of the
658 island.

659

660 **Figs 2–10.** Light micrographs of *Madanidinium loirii* gen. et sp. nov. **2.** Right lateral
661 view of a living cell with the longitudinal flagellum (lf) visible. **3.** Left lateral view of a
662 living cell with a pusule (pu) visible. **4.** Dorsal view of a living cell showing the
663 epitheca inclined toward the right side. **5.** Right view of a cell with focus on the nucleus
664 (n). **6.** Right lateral view of a fixed environmental specimen used for single-cell
665 molecular analysis (isolate IFR 12–200). **7.** Left lateral view of a DAPI-stained
666 specimen showing the posterior position of the nucleus (n). **8.** Right lateral view of a
667 living cell seen in epifluorescence (blue excitation) showing chlorophyll
668 autofluorescence and the presence of small discoid chloroplasts. **9–10.** Detail of sulcal
669 plates of two specimens stained with Calcofluor white. Except in Fig. 6, all specimens
670 are from strain IFR–MLO–02M. Scale bars: 10 μ m.

671

672 **Figs 11–15.** SEM micrographs of *Madanidinium loirii* gen. et sp. nov. from strain IFR-
673 MLO-02M. **11.** Right lateral view (holotype specimen). **12.** Left lateral view, note the
674 reduced epitheca and area of densely arranged pores (arrowhead). **13.** Ventral view
675 showing the tilted epitheca. **14.** Dorso-lateral view (arrowhead pointing to the area of
676 densely arranged pores). **15.** Antapical view. Scale bars: 10 μ m.

677

678 **Figs 16–23.** Details of the theca of *Madanidinium loirii* gen. et sp. nov. in SEM. **16.**
679 Apical view. **17.** Right lateral side of the epitheca. **18.** Left lateral side of the epitheca.
680 **19.** Ventral view of the epitheca, note the flagellar pore (fp) visible partially. **20.** Dorsal
681 view. **21.** Detail of thecal surface with groups of pores and some isolated pores. **22.**
682 Oval area of densely arranged pores on the 2^{'''} plate. **23.** Area of pores on the 2^{'''} plate
683 of another specimen, note that the shape is elongated. Scale bars: 5 μm in Figs 16–20; 1
684 μm in Figs 21–23.

685

686 **Figs 24–28.** Line drawings of *Madanidinium loirii* gen. et sp. nov. **24.** Representation of
687 a live cell in right lateral view (n: nucleus, pu: pusule). **25.** Ventral view of the theca.
688 **26.** Apical view. **27.** Right lateral view. **28.** Left lateral view. Scale bars: 10 μm in Figs.
689 24, 25, 27, 28 and 5 μm in Fig. 26.

690

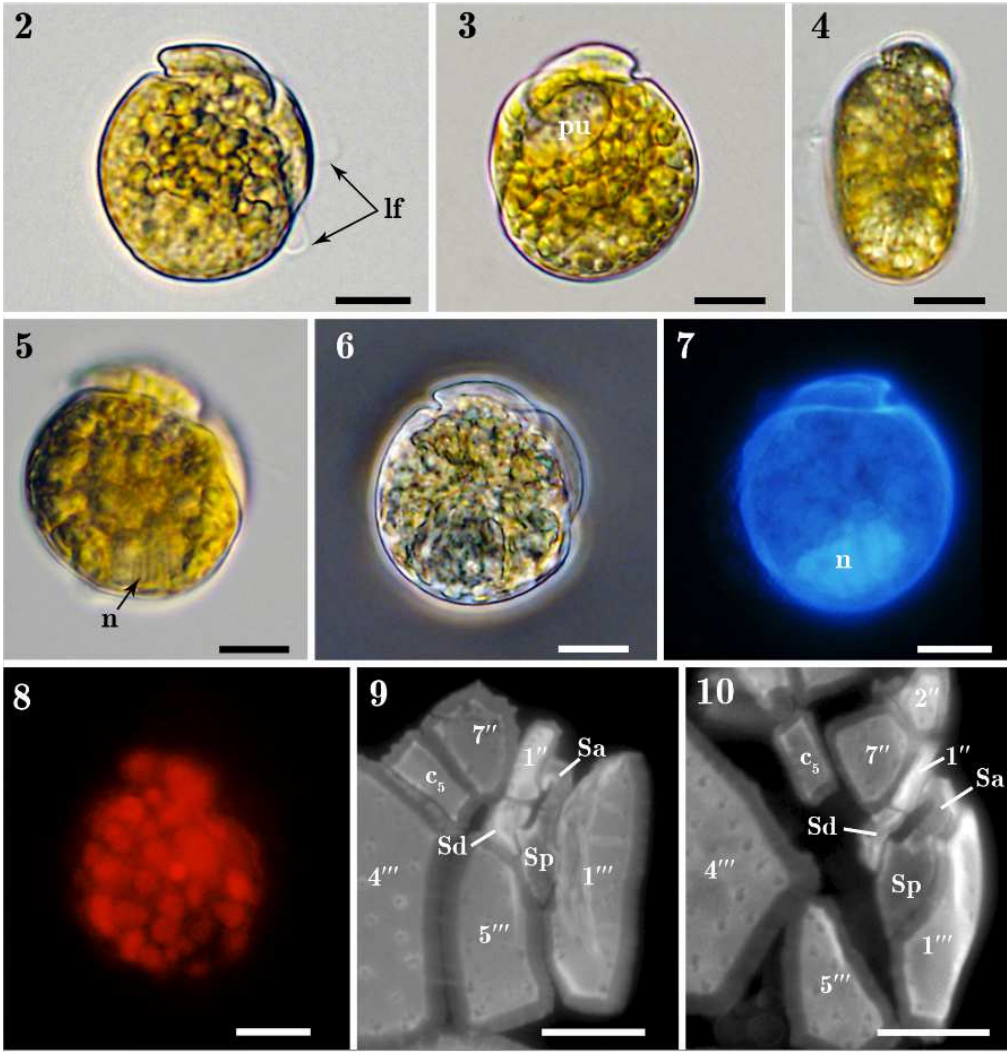
691 **Fig. 29** Maximum likelihood (ML) phylogenetic tree inferred from SSU rDNA (matrix
692 of 77 taxa and 1691 aligned positions). The tree was rooted using *Perkinsus marinus*
693 sequence as outgroup. Model selected GTR + I + Γ_4 . Log likelihood = -19792.7.
694 Substitution rate matrix: A ↔ C = 1.52090, A ↔ G = 4.15185, A ↔ T = 1.43273, C ↔
695 G = 0.81766, C ↔ T = 9.38294, against G ↔ T = 1.00000. Assumed nucleotide
696 frequencies: f(A)=0.24690, f(C)=0.19272, f(G)=0.25795, f(T)=0.30243. Among site
697 rate variation: assumed proportion of invariable sites I = 0.317. Rates at variable site
698 assumed to be gamma distributed with shape parameter $\alpha = 0.511$. Bootstrap values
699 (1,000 pseudoreplicates) > 65 (in ML) and posterior probabilities > 0.5 (in BI) are
700 shown at nodes, thick lines indicate full support of the branch (100/1.00). '+' indicate

701 nodes present but unsupported. Asterisks indicate benthic taxa with a lateral
702 compression related to *M. loirii* by morphology.
703
704 **Fig. 30** Maximum likelihood (ML) phylogenetic tree inferred from partial LSU rDNA
705 (matrix of 49 taxa and 860 aligned positions). The tree was rooted using *Perkinsus*
706 *marinus* sequence as outgroup. Model selected TN93 + Γ_4 . Log likelihood =
707 -14250.35343. Transition/transversion ratio for purines = 2.860; transition/transversion
708 ratio for pyrimidines = 7.812. Nucleotides frequencies f(A)=0.23690, f(C)=0.18977,
709 f(G)=0.28854, f(T)=0.28479. Rates at variable site assumed to be gamma distributed
710 with shape parameter $\alpha = 0.528$. Only bootstrap values (1,000 pseudoreplicates) > 65
711 (in ML) and posterior probabilities > 0.5 (in BI) are shown at nodes; thick lines indicate
712 full support of the branch (100/1.00); '+' indicates a node present but unsupported and
713 '-' indicates an irresolution (in BI). Benthic taxa with a lateral compression are
714 highlighted with asterisks.
715



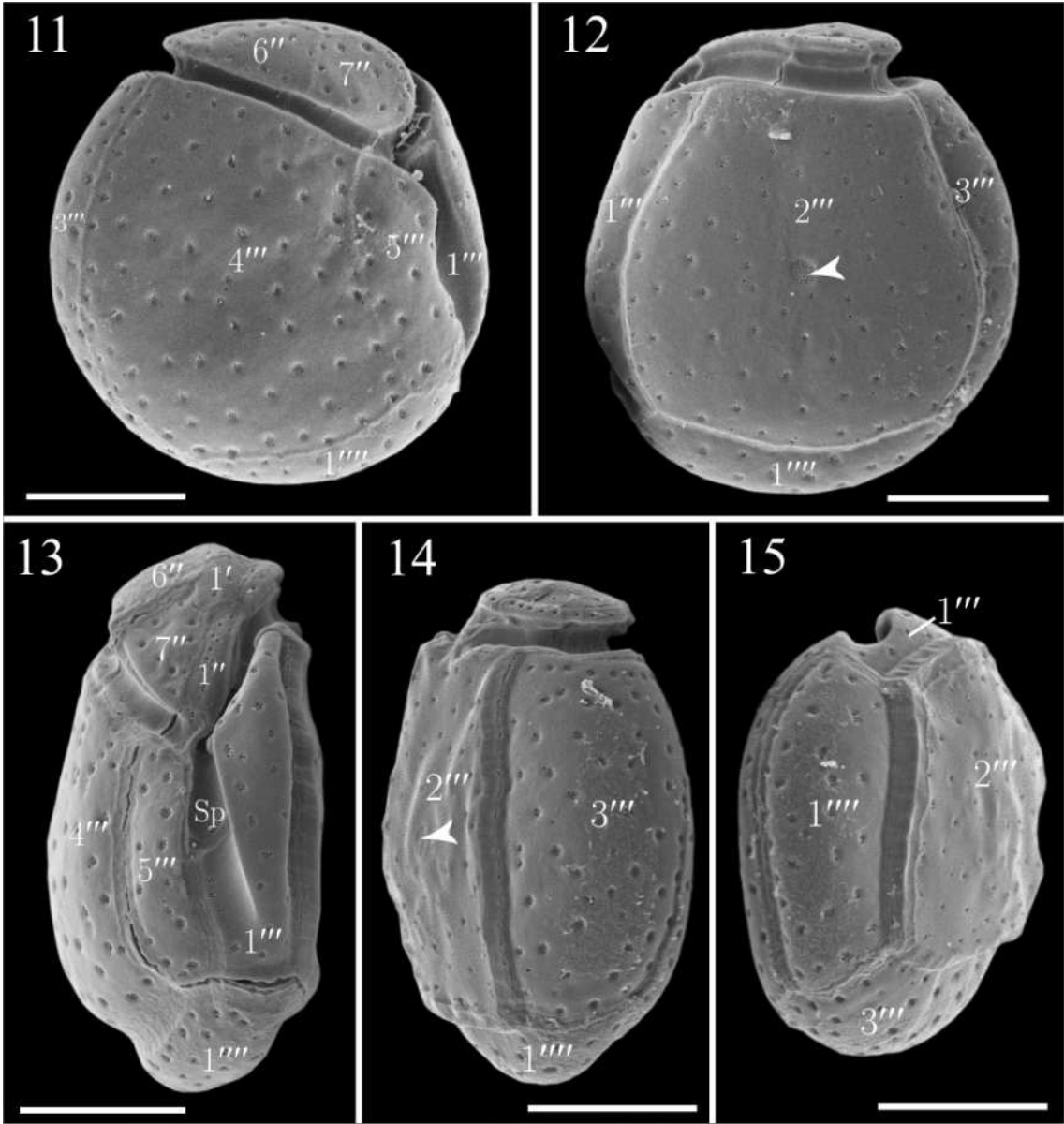
716

717 Fig. 1

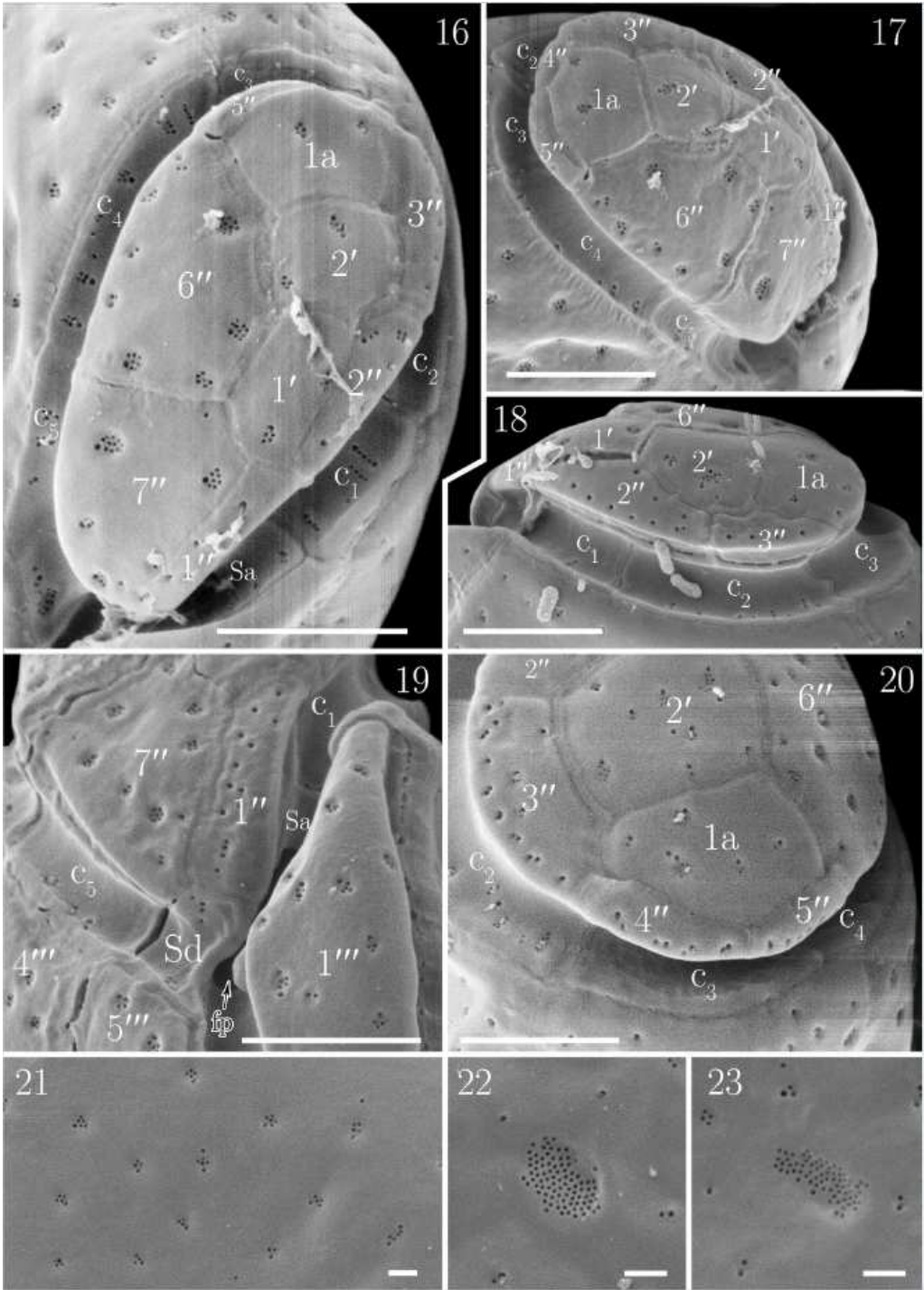


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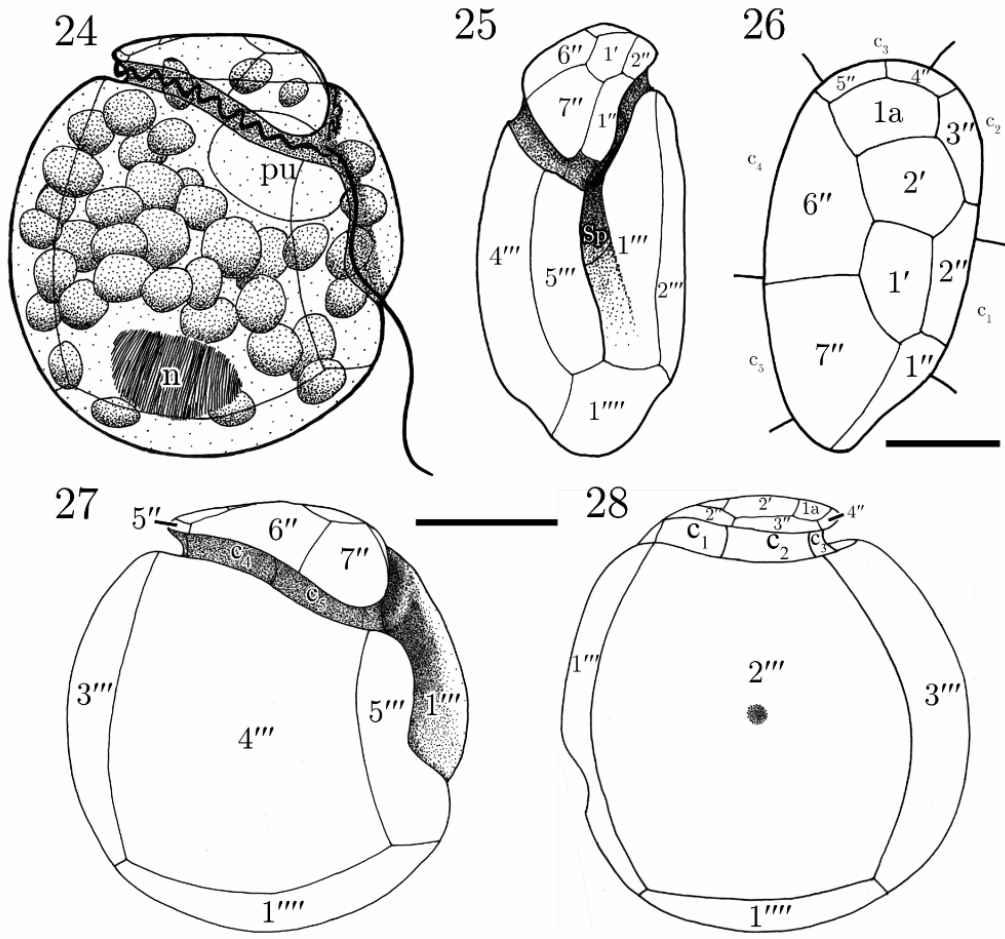


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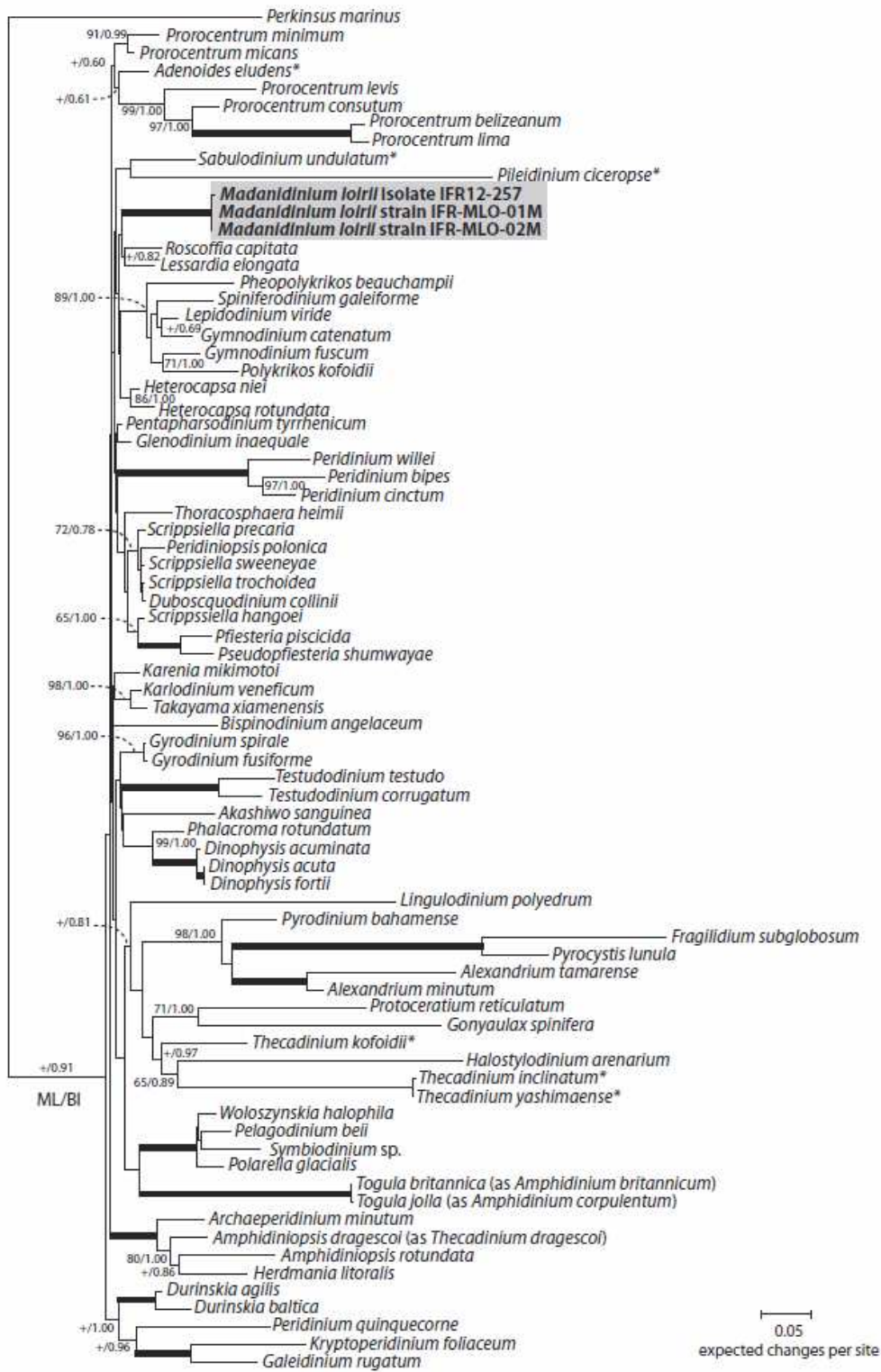
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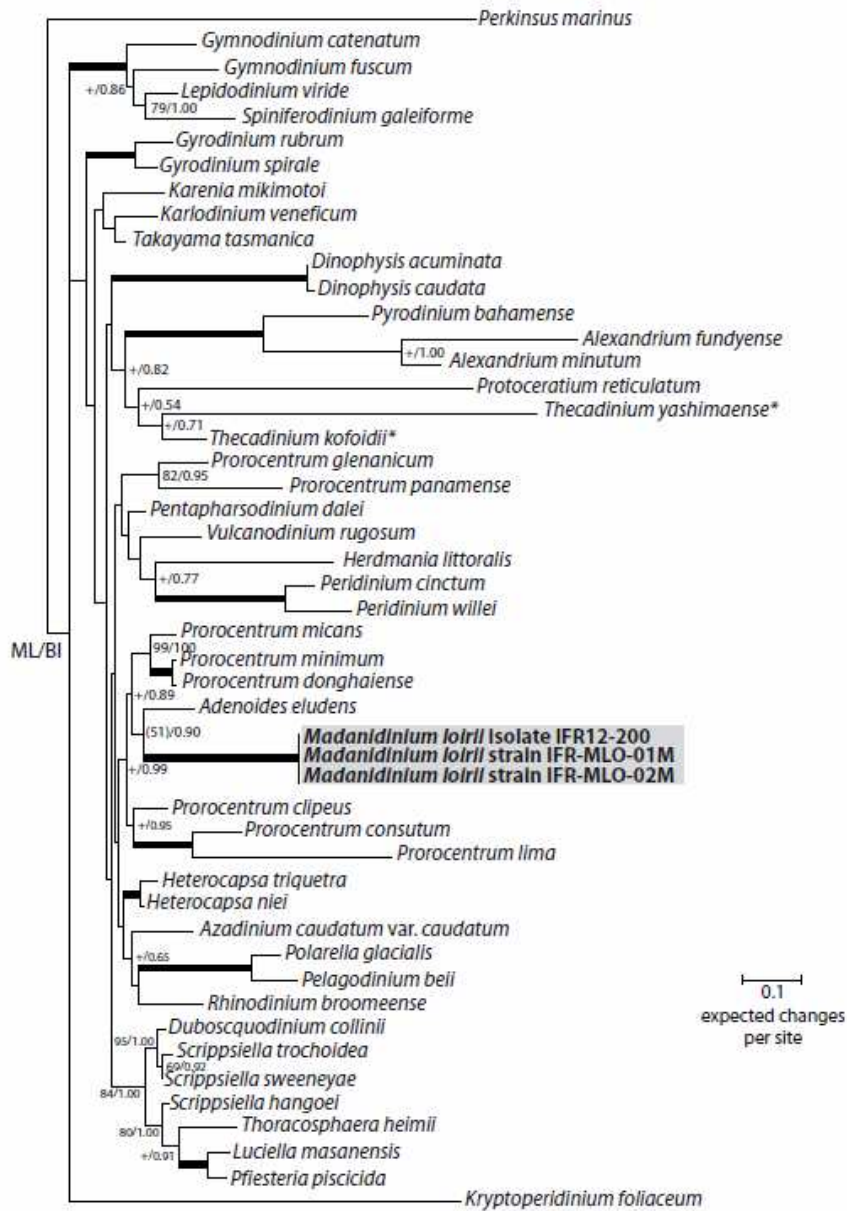
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728

729 Fig. 30

Table 1: Comparative features of *Madanidinium loirii* and other selected sand-dwelling dinoflagellate genera with a laterally-compressed body (*Thecadinium* excluded), and the planktonic genera *Thecadiniopsis* and *Pseudothecadinium*.

	<i>Madanidinium</i> gen. nov. Chomérat ¹	<i>Cabra</i> Murray et Patterson emend. Chomérat <i>et al.</i> ^{2,3}	<i>Planodinium</i> Saunders et Dodge ⁴	<i>Plagiodinium</i> Faust et Balech ⁵	<i>Sabulodinium</i> Saunders et Dodge ^{4,6}	<i>Pileidinium</i> Tamura et Horiguchi ⁷
shape (in lateral view)	roughly circular	polygonal	roughly quadrangular	oblong	more or less oval	trapezoidal
cingulum	descending	ascending	not displaced	not displaced	not displaced	incomplete
apical pore	absent	present, APC	absent	present, APC?	present, APC	present, simple
apical plates (')	2	3	3	5	5	1
anterior intercalary (a)	1	1	0	0	1	0
precingular plates (")	7	5	7	0	6	5
cingular plates (c)	5	3	6	5	5	4
sulcal plates (s)	3	?	?	5	4	4
postcingular plates (""')	5	5	3	5	5	5
antapical plates	1	1	1	1	1	1

('''')						
size (µm)						
length	25–31	32–51	27–30	26–31	27–43	14–26
depth	22–29	23–43	–	20–25**	18–36	14–20
width	16–19	17*	10–11	6–9**	–	10–14
ornamentation						
plates	smooth	foveate, reticulate or areolate	with ridges, reticulate	smooth	smooth	reticulate
thecal pores	arranged in small groups	scattered, two kinds	surrounded by smaller pores	some small pores	simple, scattered	not described
special area of pores	present, lateral (on 2''' plate)	present, antapical (on 1'''' plate)	absent	absent	absent	absent
nucleus	ovoid, posterior	ovoid, dorsal	ovoid, posterior	spherical, posterior	posterior	ovoid, posterior
habitat	benthic, marine	benthic, marine	benthic, marine	benthic, marine	benthic, marine	benthic, marine
trophic mode	phototrophic	heterotrophic	heterotrophic	phototrophic	heterotrophic	phototrophic

Table 1 (cont.)

<i>Thecadiniopsis</i> Croome <i>et al.</i> ⁸	<i>Pseudothecadinium</i> Hoppenrath et Selina ⁹
broadly ovoid	ovoid
descending	strongly descending
present	present
4	4
1	2
4	4
5	4
5?	5?
5	5
1	1
42–48	36–53
25–30	31–45
36–41	–
smooth	smooth
scattered, large	scattered, large

absent	absent
?	ovoid,
	posterior
planktonic,	planktonic,
freshwater (coastal)	marine and brackish
phototrophic	phototrophic

¹present study; ²Murray & Patterson (2004); ³Chomérat *et al.* (2010a); ⁴Saunders & Dodge (1984); ⁵Faust & Balech (1996); ⁶Hoppenrath *et al.* (2007); ⁷Tamura & Horiguchi (2005); ⁸Croome *et al.* (1987); ⁹Hoppenrath and Selina (2006); * measured only in *C. matta*; ** depth and width values are reversed in Faust & Balech (1993).