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
Introduction

Since they have been discovered and studied by E.C. Herdman (1921, 1922, 1924a, b), benthic dinoflagellates stayed poorly studied for several decades (Balech, 1956; Dragesco, 1965). They gained a new interest for scientists when the epiphytic species producing maitotoxin and ciguatoxin, namely *Gambierdiscus toxicus* was associated with ciguatera disease in the tropical Pacific (Adachi & Fukuyo, 1979; Taylor, 1979).

Then, several other benthic taxa were found to be harmful and involved in the complex mechanism of ciguatera (Bomber & Aikman, 1989; Litaker *et al.*, 2010). Because of this potential toxicity, several taxonomic studies were subsequently realized in the tropical regions (Fukuyo, 1981; Besada *et al.*, 1982; Berland *et al.*, 1992; Grzebyk *et al.*, 1994; Chinain *et al.*, 1999) but also in temperate areas worldwide (e.g. Horiguchi & Chihara, 1983; Saunders & Dodge, 1984; Larsen, 1985; Hoppenrath, 2000b; Aligizaki & Nikolaidis, 2006; Murray, 2009; Chomérat *et al.*, 2010b; Fraga *et al.*, 2011).

In the Caribbean Sea where ciguatera is known from the late 18th century and has caused health problems for many years (Bagnis, 1981; Olsen *et al.*, 1984; Tosteson, 2004; Tester *et al.*, 2010), studies on benthic dinoflagellates were mostly focused on epiphytic species responsible for this disease, in order to better understand their distribution and assess the associated risk. Several investigations were made in the northern and eastern parts of the basin (Ballantine *et al.*, 1985; Taylor, 1985; Ballantine *et al.*, 1988; Litaker *et al.*, 2010). On the western side, other studies have later been realized on Belizean coast (e.g. Faust & Gulledge, 2002; Faust, 2009), and around the Mexican and Cuban coasts (Hernández-Becerril & Almazán Becerril, 2004; Delgado *et al.*, 2006). Comparatively, southern Caribbean sea has been only scarcely investigated by Grzebyk *et al.* (1998) who collected samples in a Panamian island and Rodriguez *et al.*
al. (2010) who made a survey in San Andrés Island (Caribbean Colombia). In French Antilles (also known as French West Indies) where ciguatera intoxications have been recurrently documented (Olsen et al., 1984; Vernoux, 1988; Pottier et al., 2001; Rosine et al., 2008; Tester et al., 2010), only a few surveys have been undertaken to check the presence and identify toxigenic species (Besada et al., 1982; Taylor, 1985; Litaker et al., 2010).

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

Since no taxonomic survey has been realised to date in French Antilles, we undertook to assess the diversity of benthic dinoflagellates in Martinique Island from occasional samples. During our study, we encountered a very atypical and interesting taxon, which is distinct from any armoured dinoflagellate genus hitherto described. Cells are strongly flattened laterally, and thecal plates are delicate and arranged with a
unique pattern. In the present paper, we aim to describe its morphology using light and scanning electron microscopy, and attempt to establish its phylogenetic position among other dinoflagellates using molecular data from environmental samples and clonal cultures.

Material and methods

Sampling and cultivation

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

For cultivation, single cells from the live sediment subsamples were identified and isolated with a micropipette under an IX41 (Olympus, Tokyo) inverted microscope. Then, they were rinsed in several drops of seawater and placed in 96-well culture plates containing seawater and medium. After some divisions, each clonal strain was
transferred to culture plates with increasing well volume. Several cultures were established but only two were kept and grown in 50 ml culture flasks. The strain IFR-MLO-01M was grown in K medium (Keller et al., 1987) while the second strain IFR-MLO-02M was grown in f/2 medium (Guillard & Ryther, 1962; Andersen et al., 2005). Both strains were maintained in a growth chamber set up at 22 ± 1.0 °C and 12:12 light:dark illumination cycle with ~ 50 µmol photons m⁻² s⁻¹ provided by white fluorescent tubes.

**Observations**

**LM**

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

**Scanning electron microscopy**
Cells from the cultures were obtained after a vigorous shaking of the flask, and then fixed with 2% formaldehyde. Then, the specimens were processed according to the methods described in Couté (2002) and Chomérat & Couté (2008). They were dehydrated and critical point dried, and then they were observed with a Quanta 200 (FEI, Eindhoven, The Netherlands) scanning electron microscope with an acceleration voltage of 5 kV and a secondary electrons detector. Cells were measured from SEM digital micrographs using ImageJ software (Rasband, 1997–2006). SEM images were presented on a uniform background using Adobe Photoshop CS5 (v. 12.1, Adobe Systems).

**DNA amplification and sequencing**

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

**Molecular analysis and phylogeny**

The SSU and LSU sequences obtained were aligned with other dinoflagellates sequences and *Perkinsus marinus* (perkinsoid, Alveolate) as external group, using MAFFT software version 7 (Katoh & Standley, 2013) with selection of the Q-INS-i algorithm which considers the secondary structure for the alignment. This step was followed by refinement by eye with MEGA software version 5.2.1 (Tamura *et al.*, 2007).
2011). For SSU a dataset of 77 taxa and 1691 aligned positions has been used. For LSU, a matrix of 49 taxa and 860 positions was used. Ambiguous parts of the alignment (including the D2 domain) were excluded from the analysis using gblocks software version 0.91b, with less stringent parameters. Genbank accession numbers of all sequences used are available in the supplementary material.

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

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

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

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

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

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

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

**TYPE SPECIES:** Madanidinium loirii

**HOLOTYPE:** Fig. 11 (cell from the culture IFR–MLO–02M, SEM stub IFR-13H6 has been deposited to the Centre of Excellence for Dinophytes Taxonomy (CEDiT) with the accession reference CEDiT2013H22).
ISOTYPES: Figs 12–13, fixed culture CEDrT2013I23

TYPE LOCALITY: Anse Dufour (14°31.538′ N, 61°05.446′ W), Martinique Island, eastern Caribbean Sea.

DNA SEQUENCE INFORMATION: Sequences have been deposited in Genbank under accession numbers KF751599, KF751600, KF751601, KF751602, KF751603 and KF751604.

The cells are laterally flattened, with their depth (i.e. dorso-ventral width) larger than lateral width. Hence, they are mostly observed in lateral view and their shape is almost circular (Figs 2–3, 5–6). They are 25.2–31.0 µm long (mean 28.9 µm, s.d. 1.4 µm, 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 (n=2). The length to depth ratio varies from 1.05 to 1.21 (mean 1.12, S.D. 0.05, n=16).

The cingulum is anterior and descending (left-handed) (Figs 4, 11, 13). Seen from the left side (Figs 3, 12), it is straight, anterior, and the epitheca is very small, emerging of 1.7–2.7 µm (n=5) above the cingulum. In contrast, in the right lateral view (Figs 2, 11), the epitheca is higher (4.1–7.6 µm, n=7), and the cingulum is conspicuously oblique, descending towards the ventral area (Figs 11, 24, 27).

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

The thecal plate pattern is 2′ 1a 7" 5c 3s 5′′ 1′′′. The epitheca comprises 10 plates and does not have an apical pore (Figs 16, 26). Since the application of the Kofoid nomenclature of thecal plates was not straightforward, we decided that the apical plates were those in contact with the apex (geometrically speaking) of the cell.
and the unique plate actually not in contact with the apex and the cingulum is considered as an intercalary plate. In apical view, the epitheca is roughly pear-shaped, and tapers ventrally (Fig. 16). Plates are arranged asymmetrically and those on the right side are higher than those inserted on the left side (Figs 16–18, 26). The 1’ and 2’ plates are medium-sized, pentagonal and located at the apex of the slightly dome-shaped epitheca (Figs 16–17). The 1” plate is elongated, five sided and located ventrally (Figs 16, 19). The 2” and 3” plates are pentagonal and border the left side of the epitheca (Figs 16, 18). The 4” and 5” plates are very small, rectangular, four-sided, and located on the dorsal side of the epitheca (Figs 17, 20). The 6” plate which is the largest of the epitheca, is six-sided (Figs 16–17). The 7” plate is roughly trapezoidal and four-sided (Figs 16, 19), although it has a very short contact with the Sd plate ventrally (Figs 9, 10, 19). The unique intercalary plate 1a is pentagonal and in line with the two apical plates, but it is located more dorsally (Figs 16, 18, 20).

The cingulum completely encircles the cell and is composed of five plates unequal in size (Figs 16–18). The c2 plate is large and runs along the left side of the theca, with its distal end facing the suture 2′′′/3′′′ on the hypotheca (Fig. 12). The c3 plate is small and located dorsally, and is running along the width of the 3′′ plate (Fig. 20). The sulcus is moderately long, and slightly oblique with respect to the longitudinal axis of the cell (Fig. 13). In SEM, we partially observed the flagellar pore, which is elongated oval in shape and located ventrally (Fig. 19). It is bordered by three major sulcal plates Sa, Sd, and Sp (Figs 13, 19). Our observations of the sulcus using epifluorescence microscopy on several specimens confirm that the sulcus is composed of three plates (Figs 9, 10). The Sa plate is hook-shaped and in contact with the c1 plate.
The Sd plate forms the end of the cingulum and connects the epitheca. The Sp plate is the largest of sulcal plates, and is posteriorly pointed (Figs 9–10).

The hypotheca is formed of 6 major plates. The first postcingular plate ′′′ is ventral and folds in order to form a flange covering the left side of the sulcus (Figs 11, 13). The 2′′′ plate which is the largest of the hypotheca, is trapezoidal and four-sided, covering most of the left lateral side (Fig. 12). The 3′′′ plate is rectangular and is located on the dorsal side of the hypotheca (Fig. 14). The 4′′′ plate is large and four sided (Fig. 11). The 5′′′ plate is the smallest of postcingular plates and contacts six plates, namely 1′′, 4′′, 1′′′, $c_5$, Sd and Sp (Fig. 13). The antapical plate 1′′′ is pentagonal and elongated (Fig. 15).

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

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

Molecular phylogeny

The results of the SSU and LSU phylogenetic analyses show that the sequences acquired from cultures and environmental specimens group together within a well supported clade (Figs 29, 30). In the phylogeny inferred from SSU, the position of
Madanidinium clade is not supported and no clear relationships with other genera emerge (Fig. 29). In the LSU analysis, Madanidinium appears as a sister-clade to Adenoides eludens (Fig. 30), albeit without support (bootstrap value of 51 in ML and posterior probability of 0.90 in BI). In addition, the clade formed by Madanidinium and Adenoides forms a sister group with Prorocentrum species but without support.

Discussion

Morphologically, Madanidinium has features closely related to other strongly laterally compressed sand-dwelling genera with a reduced epitheca like Plagiodinium, Planodinium, Sabulodinium, Cabra, and Pileidinium (Table 1) but also some Thecadinium species (Hoppenrath 2000a, Yoshimatsu et al., 2006). In addition, a morphological resemblance can be found with the genus Sinophysis Nie et Wang (Dinophysales), that is also strongly laterally compressed and possesses a reduced-epitheca (Hoppenrath 2000b), but the thecal plate organization of Madanidinium is not of the dinophysoid type and no further comparison is possible. In Plagiodinium belizeanum, the epitheca is atypical, very small and slightly inclined to the ventral side (Faust & Balech, 1993), which differs from M. loirii. The left-handed displacement of the cingulum in M. loirii is peculiar and reminds that of Thecadinium yashimaense (Bolch & Campbell, 2004; Hoppenrath et al., 2004; Yoshimatsu et al., 2004; Hoppenrath et al., 2005), but also the planktonic taxa Thecadiniopsis tasmanica and Pseudothecadinium campbellii (Croome et al., 1987; Hoppenrath & Selina, 2006). This is the reverse situation in the benthic genus Cabra where the epitheca is higher on the left side than on the right lateral side. When seen in the left lateral view, M. loirii outline is very similar to that of Sabulodinium, because the epitheca is almost not visible
and the cingulum is short and very anterior. However, in *Sabulodinium*, the cingulum is not displaced, as well as in *Planodinium* (Saunders & Dodge, 1984; Hoppenrath *et al.*, 2007). And in contrast with *Pileidinium*, the cingulum is complete in *Madanidinium*.

Hence, owing to its peculiar overall morphology and position of the cingulum, *M. loirii* can be easily distinguished from most other benthic genera with the light microscope.

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

The presence of five cingular plates in *Madanidinium* is a feature found also in *Plagiodinium*, *Sabulodinium*, *Thecadiniopsis* and *Thecadinium*. Croome *et al.* (1987) emphasized that this is a character similar with freshwater peridinioids, while most of gonyaulacoids have six plates. The reduced number and very simple arrangement of the sulcus of *Madanidinium* is remarkable and to date it is the minimum number of sulcal
plates observed in a benthic genus. In other taxa, four or more sulcal plates have been described. Nevertheless, although we have used epifluorescence microscopy and plate staining, it cannot be excluded that some very small platelets have been overlooked in our study, since the sulcus is a difficult part to study. In addition, since the 1” plate seems to have a short contact with the flagellar pore, it could be alternatively interpreted as a fourth sulcal (Sa) plate. However, as it is not part of the furrow and is actually completely in the epitheca, we considered that it fits better with the definition of a precingular plate. Moreover, the plate that we interpreted as Sa is hook-shaped, as in some gonyaulacoid genera like Alexandrium.

The arrangement of plates on the hypotheca of M. loirii is not distinctive and many benthic dinoflagellates like Cabra, Plagiodinium, Sabulodinium, Pileidinium (Table 1), Thecadinium pro parte and the planktonic genera Thecadiniopsis and Pseudothecadinium have a similar pattern of five postcingular and one antapical plate. However, the presence of an area of densely arranged pores near the centre of the 2” plate on the left lateral side of the hypotheca is a very uncommon feature among the genera (Table 1). An area of grouped pores (or deep areolae) has been reported in Cabra and some other benthic genera such as Rhinodinium, Roscoffia and in some benthic Prorocentrum species. However this area is antapical and located on the 1”” plate in Cabra, Rhinodinium and Roscoffia (Hoppenrath & Elbrächter, 1998; Murray et al., 2006; Chomérat et al., 2010a), which differs from Madanidinium where it is lateral as in Prorocentrum species. In Prorocentrum panamense and P. pseudopanamense, a roundish depression with a sieve-like bottom is present on the posterior dorsal side of the right lateral plate (Hoppenrath et al., 2013) while in P. glenanicum, a group of closely arranged pores, very similar to that observed in M. loirii, is found just above the
centre of the right lateral plate (Chomérat et al., 2011). To date, the role of these
structures has not been ascertained, but from observations of a live culture of *P.
* *panamense*, it seems that cells can extrude mucous from the pores of this area, and
attach to the subtrate (M. Saburova, pers. comm.). Such fixation can be very efficient,
and this can explain the strong adherence of cells of *M. loirii* in culture flasks. This is
likely an adaptation to the benthic way of life to resist to water flow but further
ultrastructural studies are required to confirm this hypothesis.

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

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

**Molecular phylogeny**

Molecular data support that *Madanidinium loirii* corresponds to a new dinoflagellate
taxon, since its SSU and LSU sequences diverge from all other known genera.
However, as previously shown by several authors, the resolution and support of deeper
branches in the phylogenies inferred from ribosomal genes is inexistent or very low, and
no clear relationship between Madanidinium and other taxa can be found from our analyses. With SSU, the position of this new genus is not stable in the trees, which indicates that this ribosomal gene lacks a good phylogenetic signal which would allow to place it within a higher taxonomic rank (family, order). This problem has already pointed out with several other ‘unusual’ and monotypic genera of benthic dinoflagellates (Tamura & Horiguchi, 2005; Hoppenrath et al., 2007; Yamada et al., 2013). Moreover, no relationship was found with any of the morphologically related taxa with a lateral compression for which SSU rDNA sequences are available, such as Sabulodinium, Pileidinium and Thecadinium. Although the position of Sabulodinium and Pileidinium is uncertain in the SSU tree due to the lack of support, they are widely divergent from Madanidinium. From LSU, there is an indication that Madanidinium could be related to Adenoides eludens, another benthic and phototrophic genus, but this is almost unsupported. Morphologically, Adenoides is also compressed laterally, but less than M. loirii, and no similarities in the thecal plate arrangement can be found between these two genera. Thus, the phylogenetic relationship result should be treated with caution because this affinity (not supported) has not been observed in the SSU phylogeny although the sequence of this species was included in the tree. Moreover, there are almost no LSU sequences of the morphologically related taxa compressed laterally available in Genbank, which can bias our analyses. The dataset should be improved with the addition of more taxa. As a consequence, the evolution of benthic and laterally compressed dinoflagellates is still unclear. It is not yet possible to infer whether these genera derived from a common benthic ancestor or if they resulted from a convergent evolution of similar traits well adapted to the benthic life. Hence, a considerable work of sequence acquisition remains to be done for benthic
dinoflagellates, and it is absolutely necessary in order to get a better understanding of the evolution within this very diverse and complex group of protists. This task is rendered difficult by the rarity of these organisms and the difficulty to keep them in cultures. In case of phototrophic taxa, as with Madanidinium, the use of strains in culture can allow extensive ultrastructural, genetic and biochemical studies, which represents a great opportunity to increase the knowledge and understanding of the biology of benthic dinoflagellates.

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Figure legends:

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

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

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

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

**Fig. 29** Maximum likelihood (ML) phylogenetic tree inferred from SSU rDNA (matrix of 77 taxa and 1691 aligned positions). The tree was rooted using *Perkinsus marinus* sequence as outgroup. Model selected GTR + I + Γ4. Log likelihood =-19792.7. Substitution rate matrix: A ↔ C = 1.52090, A ↔ G = 4.15185, A ↔ T = 1.43273, C ↔ G = 0.81766, C ↔ T = 9.38294, against G ↔ T = 1.00000. Assumed nucleotide frequencies: f(A)=0.24690, f(C)=0.19272, f(G)=0.25795, f(T)=0.30243. Among site rate variation: assumed proportion of invariable sites I = 0.317. Rates at variable site assumed to be gamma distributed with shape parameter α = 0.511. Bootstrap values (1,000 pseudoreplicates) > 65 (in ML) and posterior probabilities > 0.5 (in BI) are shown at nodes, thick lines indicate full support of the branch (100/1.00). ‘+’ indicate
nodes present but unsupported. Asterisks indicate benthic taxa with a lateral compression related to *M. loirii* by morphology.

**Fig. 30** Maximum likelihood (ML) phylogenetic tree inferred from partial LSU rDNA (matrix of 49 taxa and 860 aligned positions). The tree was rooted using *Perkinsus marinus* sequence as outgroup. Model selected TN93 + \( \Gamma_4 \). Log likelihood = -14250.35343. Transition/transversion ratio for purines = 2.860; transition/transversion ratio for pyrimidines = 7.812. Nucleotides frequencies \( f(A) = 0.23690, f(C) = 0.18977, f(G) = 0.28854, f(T) = 0.28479 \). Rates at variable site assumed to be gamma distributed with shape parameter \( \alpha = 0.528 \). Only bootstrap values (1,000 pseudoreplicates) > 65 (in ML) and posterior probabilities > 0.5 (in BI) are shown at nodes; thick lines indicate full support of the branch (100/1.00); ‘+’ indicates a node present but unsupported and ‘-’ indicates an irresolution (in BI). Benthic taxa with a lateral compression are highlighted with asterisks.
Fig. 29
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 *Thecadiopsis* and *Pseudothecadinium*.

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<tr>
<th></th>
<th>Madanidinium</th>
<th>Cabra</th>
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<th>Plagiodinium</th>
<th>Sabulodinium</th>
<th>Pileidinium</th>
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<tr>
<td></td>
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<td>Saunders et Dodge</td>
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<td>shape (in lateral view)</td>
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<td>32–51</td>
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<td>6–9**</td>
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<td>Croome et al. ⁸</td>
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|                |                |       |
| 42–48          | 36–53          |       |
| 25–30          | 31–45          |       |
| 36–41          | –              |       |

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