Phylogeny of the coral genus *Plesiastrea* (Cnidaria, Scleractinia)

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Abstract

Until coral molecular phylogenies were published, the genus *Plesiastrea* was traditionally part of the family Faviidae and considered by several authors to be closely related to the genus *Montastraea*. However, genetic data has shown that *Plesiastrea versipora*, the genus type species, is evolutionarily distinct within the Robust clade of the Scleractinia and does not belong to the large clade grouping most representatives of the families Faviidae, including *Montastraea*, Mussidae, Merulinidae, Trachyphylliidae, and Pectiniidae. Instead, *P. versipora* is closely related to non reef-dwelling taxa currently ascribed to the Oculinidae (*Cyathelia axillaris*) and Caryophylliidae (*Trochocyathus efateensis*). However, no discussion on the morphologic features of *P. versipora* compared to other taxa has been published yet. Moreover, no information is available about the phylogenetic placement of *Plesiastrea devantieri*, the only other species in the genus. The phylogeny of both *Plesiastrea* species was addressed through molecular analyses (COI and rDNA) and morphological analysis. Morphological differences between the two species included number of septa, cycles of vertical structures in front of the septa and septal micromorphology. On the basis of these data and nuclear and mitochondrial markers, *P. devantieri* belongs to the Faviidae-Merulinidae-Pectiniidae-Trachyphylliidae clade (Clade XVII *sensu* Fukami *et al*., 2008) and is most closely related to *Goniastrea aspera* and *G. palauensis*. The type species of the genus *Goniastrea*, *G. retiformis*, however, is not closely related to either *G. aspera* and *G. palauensis*, or to *P. devantieri*. Taxonomic implications of these findings and morphologic affinities between the two species and closely related taxa are discussed.

Contents

Introduction

Scleractinian corals (Cnidaria, Scleractinia) belong to two main molecularly defined clades (Romano and Palumbi, 1996), the Robust and the Complex clade, and most families and genera as traditionally defined on the basis of skeleton macromorphologic characters are polyphyletic (Fukami *et al*., 2004, 2008; Benzoni *et al*., 2007; Nunes *et al*., 2008; Huang *et al*., 2009, 2011; Kitahara *et al*., 2010a). Moreover, Kitahara *et al*. (2010a) recently showed that azooxanthellate solitary corals from deep-water form a clade basal to both the Robust and the Complex corals. In other words, molecular phylogenies of scleractinian corals and traditional taxonomy and systematics based on skeleton morphology are, to a large extent, incompatible. Reconciliation of these two approaches is ongoing, but presently the need for an integrative approach which includes molecular, macrostructural, microstructural, reproductive and ecological analyses is required (Budd and Stolarski, 2009; Budd *et al*., 2010). In addition,

shallow water, deep water, colonial, solitary, tropical and temperate taxa should all be included in the analyses to determine the evolutionary history of this ancient group (Romano and Cairns, 2000; Le Goff-Vitry *et al*., 2003; Barbeitos *et al*., 2010; Kitahara *et al*., 2010b).

One of the most challenging tasks in the taxonomy of the shallow water zooxanthellate corals is the revision of the family Faviidae Gregory, 1900 which, together with the Merulinidae Verrill 1866, Mussidae Ortmann, 1890, Trachyphylliidae Verrill, 1901, and Pectiniidae Vaughan and Wells, 1943, are found in the Robust clade (Fukami *et al*., 2004, 2008; Kitahara *et al*., 2010b). Despite a deep divergence between Atlantic and Indo-Pacific taxa in both the Mussidae and Faviidae (Fukami *et al*., 2004), most genera from these families were retrieved in several closely related clades. The genera *Cladocora* Ehrenberg, 1834, *Oulastrea* Milne-Edwards and Haime 1848, *Leptastrea* Milne-Edwards and Haime 1848, and *Plesiastrea* Milne-Edwards and Haime 1848, however, are not closely related to the remainder of the Faviidae, nor to one another (Fukami *et al*., 2008; Barbeitos *et al*., 2010). *Plesiastrea* is clearly distinct and divergent from the rest of the Faviidae (Fukami *et al*., 2008). Despite strong morphologic affinities (Vaughan and Wells, 1943; Wells, 1956; Chevalier, 1971; Wijsman-Best, 1977; Veron *et al*., 1977; Chevalier and Beauvais, 1987), no close phylogenetic relationship has been found between *Plesiastrea* and the genus *Montastraea* de Blainville, 1830, the latter actually being polyphyletic (Fukami *et al*., 2004, 2008; Huang *et al*., 2009).

Different vertically developed skeletal structures are found in the corallites of scleractinian corals. Their origin, structure, number, size, and shape have been used for taxonomy and systematics (Vaughan and Wells, 1943). The principal ones are the septa (radially arranged around the centre of the corallite) and, if present, the columella (found at the centre of the corallite) (Vaughan and Wells, 1943; Chevalier and Beauvais, 1987). In certain taxa a third type of vertical skeletal structure can exist between the inner margin of the septa and the columella. These, generally referred to as palar structures (PS), are arranged in one or more crowns surrounding the columella. In transverse section PS can be of different shape and size within the same corallite. Their origin and the process of formation can be different. The term palus (pali) is used to refer to a pillar-like palar structure composed of its own system of divergent centers of rapid accretion (Stolarski, 2003) (formerly trabeculae), while a pali-form lobe is a PS formed by a single center of rapid accretion stemming from the inner end of a septum (Chevalier and Beauvais, 1987).

The genus *Plesiastrea* was originally described by Milne-Edwards and Haime (1848) on the basis of a specimen from the Indian Ocean named *Astrea versipora* by Lamarck (1816). The main characters of the genus were described as plocoid corallites, presence of pali *sensu stricto*, and the extracalicinal mode of corallite budding. The species *Plesiastrea urvillei* Milne-Edwards and Haime, 1849, and *P. quatrefagiana* Milne-Edwards and Haime, 1849, were also ascribed to the genus but both may be junior synonyms of *P. versipora* (Veron *et al*., 1977). According to Chevalier (1971) the main skeletal character distinguishing *Plesiastrea* from the other genera is the presence of pali as opposed to paliform lobes in the other Faviidae such as, among the others, *Montastraea* and *Goniastrea*. *Plesiastrea* has presumably remained monotypic for decades until the description of *P. devantieri* on the basis of specimens from Socotra Island, Yemen (Veron, 2000, 2002). In his original description Veron (2002) did not provide details on the micromorphological characters of *P. devantieri* and species phylogenetic relationships were not examined.

Morphological affinities between *Plesiastrea* and *Montastraea* and nomenclatural confusion between the two taxa and *Orbicella*, a junior synonym of *Montastraea* (Wijsman-Best, 1977) on the one hand, and *Favia* on the other hand, have been discussed by Wijsman-Best (1977) and Veron *et al*. (1977) who provided detailed accounts of the history of nomenclatural confusion between those genera. Although *Plesiastrea* is superficially similar to *Cyphastrea* (Veron, 2002) no nomenclatural confusion occurred between the two taxa. Chevalier (1971) described transitional morphs between *P. versipora* and *M. curta*. The latter species was first ascribed to *Orbicella* by Dana (1846) and later moved to the genus *Montastraea* whose type species, *Astrea guettardi* Defrance, 1830, is a fossil. According to Wijsman-Best (1977) some specimens identified as *Orbicella* by Yabe *et al*. (1936) would actually belong to *Plesiastrea* while specimens identified by Wells (1954) as *Plesiastrea* would belong to *Montastraea*. Moreover, Wijsman-Best (1977) recognised transitional forms between *P. versipora* and another *Montastraea* species, *M. annuligera* (*cf*. holotype illustrated in Veron *et al*., 1977, p. 141, Fig. 267). Vaughan and Wells (1943) in their description of the genus, indicated that *Plesiastrea* was like *Favia*, but with extracalicinal budding, as opposed to intracalici-

nal. It was Chevalier (1971) who finally suggested that the presence of true pali instead of paliform lobes and the absence of directive mesenteries would be sufficient to separate *Plesiastrea* from any species ascribed to the genus *Montastraea*. Later, molecular data supported this separation (Fukami *et al.*, 2004).

No close relative of *Plesiastrea versipora* was apparent until Kitahara *et al.* (2010b) included several azooxanthellate and deep water taxa in their phylogenetic analyses of the Scleractinia. In their COI phylogeny a close phylogenetic relationship between *P. versipora* and *Trochocyathus efateensis* Cairns, 1999 (traditionally in the Caryophylliidae Dana, 1846) and *Cyathelia axillaris* (Ellis and Solander, 1786) (currently in the Oculinidae Gray, 1847) was suggested. To date, no morphological analyses have been performed to revise the micro-morphology of *P. versipora* in light of these molecular results. Finally, the phylogenetic relationships between *P. versipora* and *P. devantieri* Veron, 2002 have not been investigated.

In this paper morphologic and molecular analyses of *P. versipora* and *P. devantieri* were performed to

ascertain the phylogenetic relationships, first, between the two congeneric species, and, second, with the Faviidae in general.

Material and methods

Sampling

Sampling took place at several localities in the Indian Ocean and Gulf of Aden (Table 1). Specimens of *Plesiastrea versipora* for morphological and molecular analyses were sampled along the Gulf of Aden coasts of Yemen, in Socotra and Mayotte. *Plesiastrea devantieri* was sampled in Socotra (type locality) and in Mayotte. The *Goniastrea retiformis* (Lamarck, 1816) and *Montastraea curta* (Dana, 1846) specimens included in the molecular analyses and illustrated in Fig. 1 were collected in Balhaf, Yemen, and Socotra, respectively. Sampling in Yemen and Socotra was performed between 2007 and 2010 within the frame of the *Yemen Scleractinia Biodiversity Project* supported by TOTAL SA.

Table 1. List of the material collected for this study and housed at the University of Milano-Bicocca (UNIMIB) collection. BI = Bir Ali (Gulf of Aden, Yemen); SO = Socotra Island (Indian Ocean, Yemen); KA = Kamaran Island (Red Sea, Yemen); MY = Mayotte Island (Indian Ocean, France); FB = F. Benzoni; MP = M. Pichon; SM = S. Montano; AC = A. Caragnano.

| UNIMIB code	Genus	species	collector	rDNA	COI
BAL 178	*Plesiastrea*	*versipora*	FB, MP		
BAL 214	*Plesiastrea*	*versipora*	FB, MP		
Y719	*Plesiastrea*	*versipora*	FB	FR837995	FR837985
MU010	*Plesiastrea*	*versipora*	FB, MP		
MU111	*Plesiastrea*	*versipora*	FB, MP		
MU146	*Plesiastrea*	*versipora*	FB, MP	FR837996	FR837986
MU169	*Plesiastrea*	*versipora*	FB, MP		
BA123	*Plesiastrea*	*versipora*	FB, SM	FR837994	FR837984
AD021	*Plesiastrea*	*versipora*	FB, MP		
KA124	*Plesiastrea*	*versipora*	FB, AC		
SO115	*Plesiastrea*	*versipora*	FB, MP		
MY343	*Plesiastrea*	*versipora*	FB		
SO024	*Plesiastrea*	*devantieri*	FB, MP	FR837997	FR837987
SO031	*Plesiastrea*	*devantieri*	FB, MP	FR837998	FR837988
SO093	*Plesiastrea*	*devantieri*	FB, MP	FR837999	FR837989
MY003	*Plesiastrea*	*devantieri*	FB		
MY004	*Plesiastrea*	*devantieri*	FB		
MY053	*Plesiastrea*	*devantieri*	FB		
MY064	*Plesiastrea*	*devantieri*	FB		
MY139	*Plesiastrea*	*devantieri*	FB		
SO041	*Montastraea*	*curta*	FB	FR838000	FR837990
BA024	*Goniastrea*	*retiformis*	FB, SM	FR837991	FR837981
BA077	*Goniastrea*	*retiformis*	FB, SM	FR837992	FR837982
SO070	*Goniastrea*	*retiformis*	FB, MP	FR837993	FR837983

Type material　　　　　　　Examined material
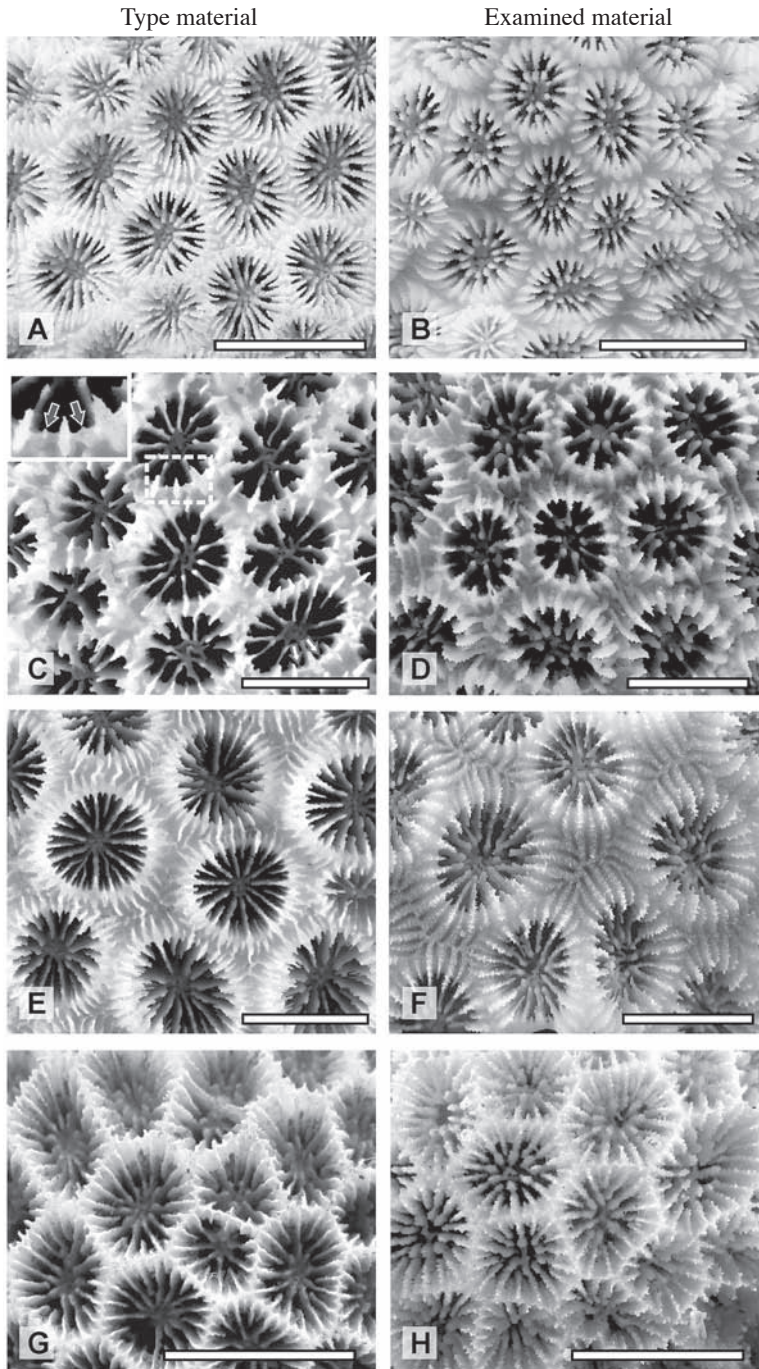
Fig. 1. Morphology of type material and examined specimens of: *Plesiastrea versipora* A) holotype MNHN 36, and B) specimen MU146; *Plesiastrea devantieri* C) holotype MTQ G 55847 (Picture by P. Muir, MTQ), and D) specimen SO031; *Montastraea curta* E) syntype USNM 14 (Picture by the National Museum of Natural History, Smithsonian Institution), and F) specimen SO041; *Goniastrea retiformis* G) Holotype MNHN 86, and H) specimen BA077. Scale bars = 1 cm. Grey filled arrows in inset of C show third order septa (S_3) in *P. devantieri* holotype.

Sampling in Mayotte took place in May 2010 during the third Reef leg of the *Tara Oceans* scientific expedition.

Digital images of living corals in the field were taken with a Canon G9 in an Ikelite underwater housing system. Coral specimens were collected, tagged, and for each specimen 1 cm^2 was broken off the colony and

preserved in absolute ethanol for further molecular analysis. The remaining corallum was placed for 48 hours in sodium hypochlorite to remove all soft parts, rinsed in freshwater and dried for microscope observation. Images of the cleaned skeletons were taken with a Canon G9 digital camera.

Specimen identification

The material collected was identified referring to the genera and species descriptions and illustrations by Lamarck (1816), Dana (1846), Milne-Edwards and Haime (1848), Vaughan and Wells (1943), Wijsman-Best (1976, 1977), Veron *et al*. (1977), Chevalier and Beauvais (1987), and Veron (2000, 2002). Moreover, the type specimens of *Plesiastrea versipora* (Fig. 1A), *P. devantieri* (Fig. 1C), *Montastraea curta* (Fig. 1E), and *Goniastrea retiformis* (Fig. 1G) were examined and their morphology compared with that of the material sampled for this study (Fig. 1B, D, F, and H, respectively).

Museum collections and other examined specimens

Type material and specimens examined for this study are deposited in different institutes.

Abbreviations:
EPA	Environment Protection Authority, Sana'a and Socotra, Yemen
IRD	Institut de Recherche pour le Développement, Nouméa, New Caledonia
MNHN	Muséum National d'Histoire Naturelle, Paris, France
MTQ	Museum of Tropical Queensland, Townsville, Australia
SMF	Senckenberg Museum, Frankfurt, Germany
UNIMIB	Università di Milano-Bicocca, Milan, Italy
USNM	United States National Museum of Natural History, Washington, U.S.A.

The holotype of *Plesiastrea devantieri* (MTQ G 55847) was examined at the Museum of Tropical Queensland. The *P. versipora* specimens in the Lamarck and Milne-Edwards collections hosted at MNHN, including the species holotype, as well as the type material of *Plesiastrea urvillei* (MNHN 773), *Astrea retiformis* (MNHN 86), and *Heliastrea annuligera* Milne-Edwards and Haime, 1848 (MNHN 674), were examined during a visit in October 2010. Images of the latter were kindly taken by A. Andouche (MNHN). The U.S Exploring Expedition specimens at the USNM were examined during a visit in July 2009. The illustration of the one of the syntypes of *Astraea (Orbicella) curta* (USNM 14) was provided with the permission of the National Museum of Natural History, Smithsonian Institution, (http://www.nmnh.si.edu/). *Plesiastrea* spp. and *Cyathelia* cf *axillaris* specimens

in the SMF Socotra collection currently deposited at the Yemen EPA Socotra were studied in February 2010. The images of *Trochocyathus efateensis* were kindly provided by M. Kitahara (James Cook University).

Morphological analyses

Both macro and micromorphological characters (*sensu* Budd and Stolarski, 2009) of the two *Plesiastrea* species were examined using light microscopy (Zeiss Stemi DV4 stereo-microscope) and SEM, respectively. For SEM, specimens were mounted using silver glue, sputter-coated with conductive gold film and examined using a Vega Tescan Scanning Electron Microscope at the SEM Laboratory, University of Milano-Bicocca.

DNA extraction, amplification and sequencing

Extraction of coral DNA was performed using DNeasy® Tissue kit (QIAGEN, Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocol. Each extracted sample was quantified using a Nanodrop 1000 spectrophotometer (Thermo Scientific) and diluted to a final DNA concentration of 3ng/µl. The mitochondrial gene COI and a portion of rDNA (spanning the entire ITS1, 5.8S, ITS2 and a portion of 28S and 18S) were amplified and sequenced. COI and rDNA have been extensively used to reconstruct phylogenetic relationships among scleractinians corals (Odorico and Miller, 1997; Lam and Morton, 2003; Fukami *et al*., 2004, 2008; Moothien Pillay *et al*., 2006; Benzoni *et al*., 2007, 2010; Stefani *et al*., 2008; Huang *et al*., 2009; Kitahara *et al*., 2010b). The use of both markers allowed inference of the phylogeny at higher and lower systematic levels, due to their different evolutionary rates (Shearer *et al*., 2002; Chen *et al*., 2004; Wei *et al*., 2006; Huang *et al*., 2008). The mitochondrial marker was amplified using specific primers for Scleractinia, MCOIF (5' TCT ACA AAT CAT AAA GAC ATA GG 3') and MCOIR (5' GAG AAA TTA TAC CAA AAC CAG G 3') (Fukami *et al*., 2004). The 50 µl PCR mix was composed of 1X PCR buffer, 2 mM $MgCl_2$, 0.3 µM for both of each primer, 0.01 mM dNTP, 3 U taq polymerase (Sigma-Aldrich Co., St. Louis, MO, USA) and 8 µl of DNA solution. The thermal cycle consisted of an initial denaturation phase of 94°C for 2 min followed by 30 cycles composed of 94°C for 45 sec, 55°C for 45 sec, 72°C for 90 sec, and finally an extension phase at 72°C for 5 min.
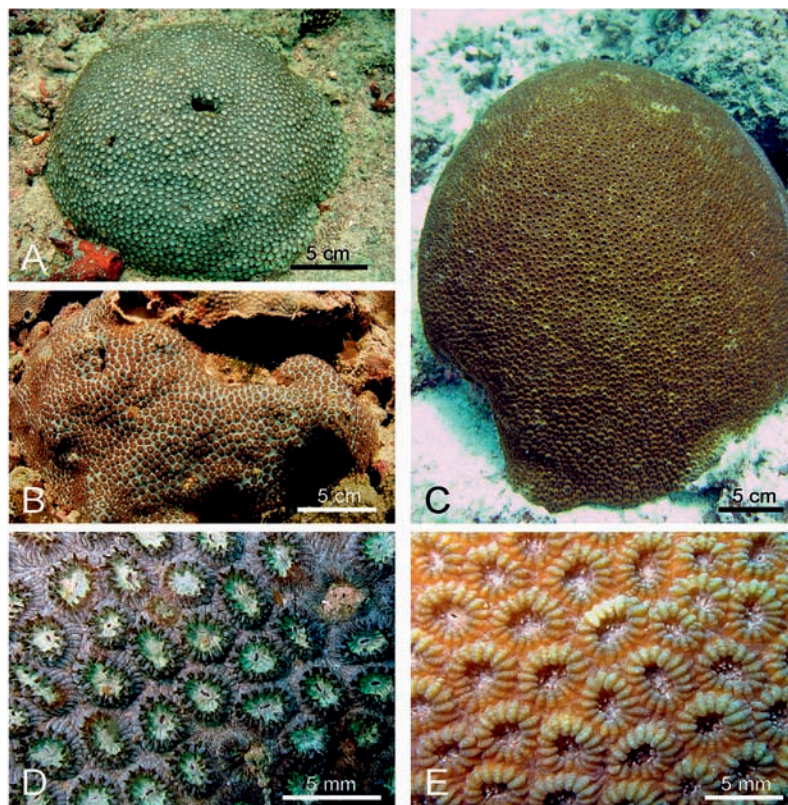
Fig. 2. In situ images of living colonies of: *Plesiastrea versipora* A) massive colony with typical green colouration, Kamaran Island, Yemen, 8 m; B) encrusting colony with brown colouration, Balhaf, Yemen; *Plesiastrea devantieri* C) massive colony, Socotra Island, 10 m. Close ups of the corallites of D) *P. versipora* with expanded polyps and E) *P. devantieri*.

Amplification of nuclear marker was performed using the coral-specific primer A18S (5' GAT CGA ACG GTT TAG TGA GG 3') (Takabayashi *et al*., 1998) and the universal primer ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') (White *et al*., 1990). Reactions were conducted with 1X PCR buffer, 2 mM MgCl$_2$, 0.4 μM for both of each primer, 0.1 mM dNTP, 2 U taq polymerase (Sigma-Aldrich Co., St. Louis, MO, USA), 8 μl of DNA solution and water to 50 μl, using the protocol of 96°C for 2 min, 30 cycles of 96°C for 10 sec, 50°C for 30 sec, 72°C for 4 min, ending with 72°C for 5 min. PCR products were sent to Macrogen Inc. (Seoul, South Korea) for further purification and sequencing.

Phylogenetic analyses

Chromatograms were manually checked using Codon-Code Aligner 2.0.6 (CodonCode Corporation, Dedham, MA, USA). Sequences were aligned with BioEdit Sequence Alignment Editor 7.0.9.1 (Hall, 1999). Invariable, polymorphic and parsimony informative sites were detected with DnaSP 5.10.01 software (Librado and Rozas, 2009). Genetic distances and their standard deviation were calculated as p-distance with MEGA 4.0.2 software (Tamura *et al*., 2007). Phylogenetic inference was conducted using Bayesian Inference (BI) with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), Maximum Likelihood (ML) using PhyML 3.0 (Guindon and Gascuel, 2003) and Maximum Parsimony (MP) based on PAUP* 4.0b10 (Swofford, 2003). For BI and ML analyses evolutionary models were assessed with Akaike Information Criterion (AIC) as implemented in MrModeltest 2.3 software (Nylander, 2004). As most suitable models AIC selected the HKY+I+Γ for the mitochondrial marker (gamma=1.0033 and p-invar=0.5341) and the GTR+I+Γ for the nuclear marker (gamma=1.0079 and p-invar=0.2315). BI analysis consisted of four parallel Markov chains implemented for 10500000 generations for COI (1000000 generations for rDNA), saving a tree every 100 generations for both mitochondrial and nuclear markers and discarding the first 26251 trees as burn-in for COI (2501 for rDNA). For both markers BI analyses were stopped when the standard deviations of split frequencies were <0.01. The software Tracer 1.5 (Drummond and Rambaut, 2007) was also used in order to correctly estimate

the burn-in and to verify the convergence. Best ML tree was obtained with PhylML (HKY85 model for COI and GTR model for rDNA, 4 substitution rate categories, setting the proportion of invariable sites and gamma distribution parameter to the values estimated with MrModeltest 2.3) using Shimodaira and Hasegawa (SH-like) test to check the support of each internal branch. MP analysis was performed using a heuristic search and the tree-bisection-reconnection branch swapping algorithm producing a strict consensus tree. Node supports were obtained with 500 bootstrap replicates in which the maximum number of trees was set to 40000.

Results

Macromorphology

Plesiastrea versipora and *P. devantieri* share macromorphological traits such as a flattened to massive colony (Fig. 2A-C), plocoid corallite arrangement (Fig. 1A-D, 2D-E), the presence of three orders of laminar septa continuing over the wall as costae (Fig. 3, 4A-B) and well developed palar structures (Figs 3-4).

The two species can be distinguished, however, on the basis of several macro and micromorphological features (Table 2). Corallites are smaller in *P. versipora* (2 to 3.5 mm in diameter) (Fig. 1A-B) than in *P. devantieri* (3 to 5 mm in diameter) (Fig. 1C-D). Although both species have three cycles of septa (S_{1-3}) and well developed palar structures, their number and development is substantially different in the two taxa (Table 2). In *P. versipora* S_1 (6 to 8 septa) and S_2 (6 to 8 septa) can be equal (Fig. 3A), or S_2 variably shorter than S_1 (Fig. 1A). Although in the holotype S_1 and S_2 can be 8 in most corallites (Fig. 1A), specimens bearing 6 S_1 and S_2 are commonly observed (Fig. 1A; [Figs. 284-292] Veron and Pichon, 1976). S_3 can be up to ¼ the length of S_1 (Fig. 3C), or extremely reduced ($S_1 \geq S_2 > S_3$). S_1 always reaches the columella, S_2 only if as developed S_1. Two crowns of pali (P_{1-2}) (12 - 16 pali in total) are present between S_{1-2} and the columella (Figs 3A, C, 4A, C). P_1 and P_2 can be equal (Fig. 4C) or sub-equal (Veron *et al.*, 1977) and are very variable in width and length (see Veron *et al.*, 1977, p. 151, Figs. 284-292). P_{1-2} flush with, or slightly higher than, the corallite wall upper margin (Fig. 4A).

In *P. devantieri* S_1 usually number 6 to 8 in mature corallites and reach the columella (Fig. 3B). In this species palar structures are actually paliform lobes, not pali, and number 8 in most corallites (Figs 3B, D, 4D). S_2 are often incomplete, usually ¼ to ½ of the first order in length. No paliform lobe development occurs between S_2 and the columella (Fig. 3B, D). Occasionally,

Table 2. Morphological characters distinguishing *Plesiastrea versipora* from *P. devantieri*.

|	Character	*P. versipora*	*P. devantieri*
Macromorphology	Corallite diameter	2 - 3.5 mm	3 - 5 mm
	Orders of septa (S)	3	3
	S_1	6 - 8 reaching the fossa	6 - 8 reaching the fossa
	S_2	6 - 8 £ S_1	6 - 8, £ S_1
	S_3	12 - 16 < S_{1-2}	12 - 16 < S_{1-2}
	Type of palar structures	Pali	Paliform lobes
	Crowns of palar structures (PS)	2	1
	PS_1	6 - 8	6 - 8
	PS_2	6	-
	Columella	Papillose	Spongy
Micromorphology	Septum margin ornamentation	Rounded to pointed granules regularly arranged along the main axis	Paddle-shaped teeth
	Shape of palar structures (transverse section)	Round to oval	Oval to wedge shaped
	Granulation arrangement of palar structures	Linearly arranged along the main axis	Radially arranged
	Height of the palar structures	Flush with the corallite wall upper margin	Between the columella and the corallite wall upper margin
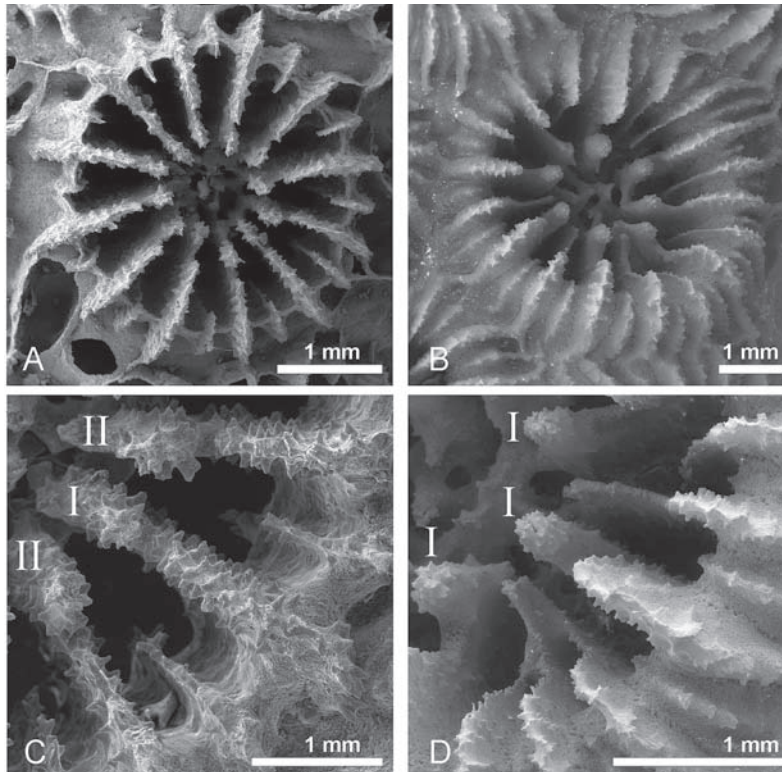
Fig. 3. Scanning Electron Micrograph of a corallite of A) *Plesiastrea versipora* (BA123) and of B) *Plesiastrea devantieri* (TO-MAY053) showing septal arrangement. Details of septa and palar structures (PS) in C) *P. versipora* (MU146) and D) *P. devantieri*. I = PS in front of first order septa; II = PS in front of second order septa.
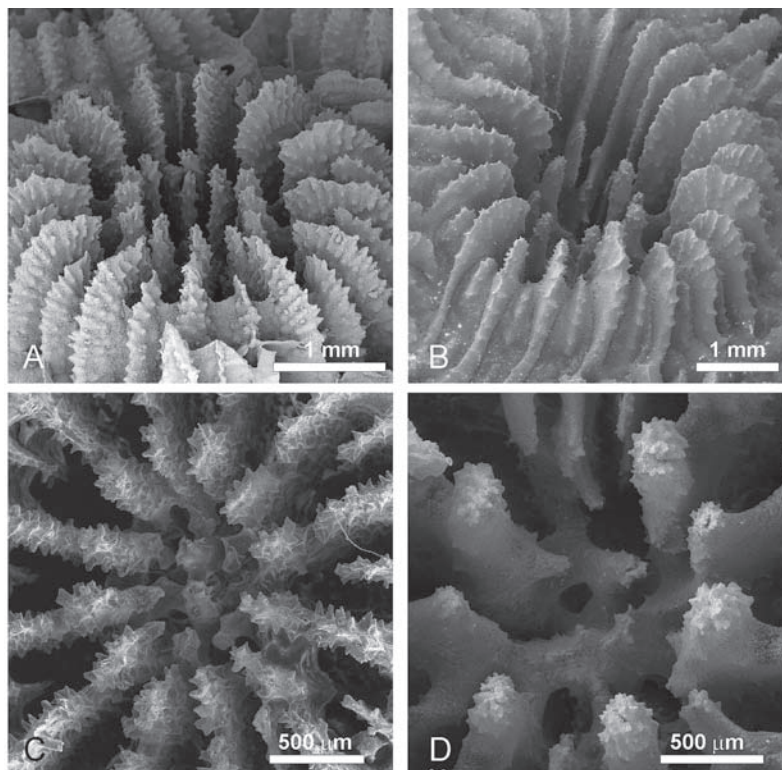

Fig. 4. Scanning Electron Microscope side view of a corallite of A) *Plesiastrea versipora* (BA123) and of B) *Plesiastrea devantieri* (TO-MAY053) showing different height of the palar structures in the two species. Arrangement and shape of C) pali *P. versipora* (BA123) and D) paliform lobes in *P. devantieri*.

one or two longer secondary septa can almost reach the columella but these remain devoid of paliform lobes. Although not mentioned in the species original description, a third order of septa is also present in the holotype (inset in Fig. 1C) as well as in the rest of the examined material. S_3 generally much reduced (Fig. 3B, D) and hard to detect with the naked eye (Fig. 1C-D). Although Veron (2002) indicated in the *P. devantieri* original description that costae do not extend over the coenosteum in the holotype, this was observed in parts of the specimen, and in a number of specimens where the coenosteum between corallites is more developed costae show a tendency to extend over it (Fig. 1D). The paliform lobes between S_1 and the columella sit in the fossa, lower than the corallite wall upper margin level (Fig. 4B).

The columella in *P. versipora* is always well developed although small and papillose (Fig. 4C). In *P. devantieri* the columella is made of a few interwoven threads (Fig. 4D).

Micromorphology

SEM observations of septal micromorphology showed remarkable differences between the two examined species. In *P. versipora* minute, regularly spaced septal teeth are arranged along the septum direction (Fig. 5A, C). Conversely, in *P. devantieri* the typical paddle-like structures or fans described in the Atlantic Faviidae (Cuif and Perrin, 1999; Cuif *et al.*, 2003) but not in the Pacific Faviidae (Budd and Stolarski, 2011) are observed (Fig. 5B, D). These distinct spiked ridges perpendicular to the septal plane (Budd and Stolarski, 2009) were also observed on the costae (Fig. 5B).

The septa centers of rapid accretion in *P. versipora* are formed by calcification axes inclining outwards from the centers of rapid accretion axis and producing a granulation pattern on the septal sides (Fig. 6A). The granules are arranged in vertical lines and the degree to which they protrude may vary between corallites and specimens (Fig. 3C) or less (Fig. 5A, C). In *P. devantieri* granulations on the septa sides are similar though less regularly arranged and the lines are further apart (Fig. 6B).

Finally, shape and ornamentation of the palar structures differ between *P. versipora* and *P. devantieri*. In *P. versipora* pali can be elongate to wedge-shaped in transverse section (Fig. 4C), their main axis following the same direction as the septum behind it. Granulations are regularly arranged along the palus main axis (Fig. 6A, C) in the same fashion as along the septum.

Paliform lobes in *P. devantieri* are round to oval in transverse section (Fig. 4D) and their ornamentation is star-like (Fig. 6B, D), closely resembling in shape and ornamentation the septal teeth formed by calcification axes in multiple directions as illustrated by Budd and Stolarski (2009, *cf.* Fig. 6) in the Mussidae.

COI phylogeny

Three specimens of *Plesiastrea versipora* and three of *P. devantieri* were sequenced and used for phylogenetic reconstruction, together with 51 sequences from clades XI to XXI delineated in Fukami *et al.* (2008) and other sequences from Kitahara *et al.* (2010b). In particular, the available sequences of *Trochocyathus efateensis*, *Cyathelia axillaris* and *Plerogyra* sp. were added. A sequence of *Galaxea fascicularis* (AB441201) was included as an outgroup. Finally, three sequences of *Goniastrea retiformis* and one of *Montastraea curta* from the Indian Ocean were also obtained during this study and added to the dataset.

The final alignment consisted of 580 positions. No indels were observed and a total of 185 polymorphic sites (112 informative) were identified. The phylogenetic trees obtained under the three criteria are largely congruent between each other and with the main available phylogenies. In detail, clades XII, XIII, XIV, XV, XVIII, and XXI *sensu* Fukami *et al.* (2008) are resolved. Clade XI is partially unresolved, due to the basal unresolved position of *Oulastrea crispata*. Clade XVII, also includes subclade XVIII. Finally, clade XIX and XX merged in a single clade. The phylogeny shows low resolution at genus and species levels, as extensive polytomies affect clade XVII in particular. The three examined specimens of *P. devantieri* are not resolved within this main clade. Nevertheless, *Plesiastrea versipora* samples from the Gulf of Aden and the Indian Ocean cluster together with the other specimens from the Western Pacific, with *C. axillaris*, and *T. efateensis* in a sister clade, thus confirming the strong affinity between these genera as indicated by Kitahara *et al.* (2010b). Overall, the genus *Plesiastrea* results polyphyletic and a strong divergence between the two species ascribed to this genus is supported by the mitochondrial marker.

rDNA phylogeny

A total of three specimens of *Plesiastrea versipora* and three of *P. devantieri* were amplified, sequenced and used in the phylogenetic analysis. No intraindividual
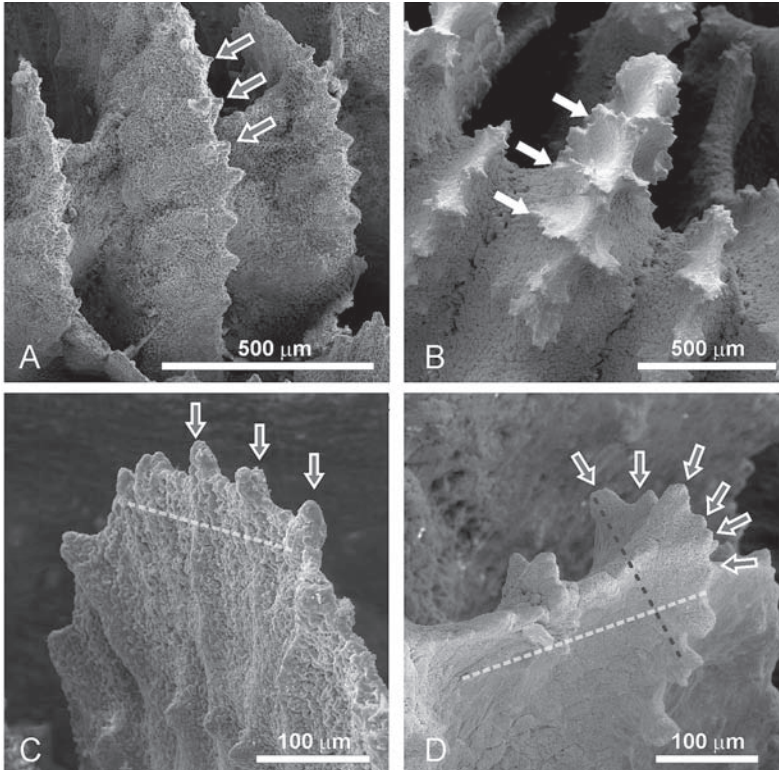
Fig. 5. Scanning Electron Microscope images of A, C) *Plesiastrea versipora* (MU146) and B, D) *Plesiastrea devantieri* (TO-MAY053) septa showing different types of ornamentation in the two species. Grey filled arrows point at granules; white filled arrows point at paddle-shaped teeth; white dashed lines indicate the septal plane direction; grey dashed line indicates the paddle-shaped teeth main axis direction.
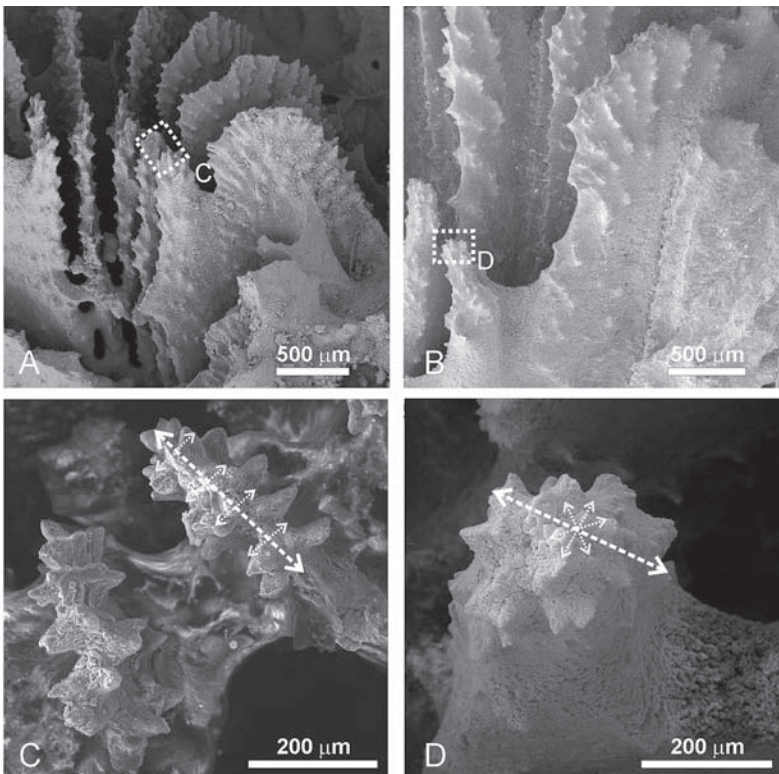

Fig. 6. Scanning Electron Microscope lateral views of septa and palar structures of A) *Plesiastrea versipora* (MU146) and B) *Plesiastrea devantieri* (TO-MAY053). White dotted insets indicate the position of elements shown in C and D, respectively. Top view of C) two pali of *P. versipora* and D) one paliform lobe in *P. devantieri* showing different arrangement of granules in the two species. Larger dashed white arrows indicate the septal plane direction, smaller dashed grey arrows indicate the granules growth directions.

polymorphisms were observed in the chromatograms. Although direct sequencing of rDNA in corals may hide some rare intra-individual variants (Vollmer and Palumbi, 2004), this loss of information may be relevant only in a limited number of cases in the Complex clade (Marquez *et al*., 2003; Vollmer and Palumbi, 2004; Chen *et al*., 2004), and direct sequencing prevents the risk of introducing PCR artefacts into the analysis, unlike methods using cloning (Bradley and Hillis, 1997; Flot *et al*., 2006).

The phylogenetic relationships between the obtained sequences and 42 other sequences, congruent with those employed for the mitochondrial phylogeny, were then inferred. Unfortunately, no sequences of *C. axillaris* or *T. efateensis* were available. Sequences of *Goniastrea retiformis* were obtained and included, together with the homolog and congeneric sequences of *Goniastrea palauensis* and *Goniastrea aspera* available in Genbank. The final alignment consisted of 763 bp, with 384 positions showing indels. A total of 177 polymorphic sites (127 parsimony informative) were detected.

The reconstructions obtained according to the three criteria are largely congruent. Minor variations are due to the lack of resolution in a few nodes shown by the MP analysis. Overall, the clades XI, XIV, XV *sensu* Fukami *et al*. (2008) detected in the mitochondrial tree are retained in the rDNA phylogeny, but here a higher resolution at species and genus levels is observed. Clades XIX and XX *sensu* Fukami *et al*. (2008) merge in a single clade, while clade XVIII form a subclade within the main XVII clade.

Plesiastrea versipora clusters with *P. lichtensteini*, thus supporting the relationships shown by the mitochondrial phylogeny. *Plesiastrea devantieri* falls within clade XVII, as seen in the COI phylogeny. A close affinity of *P. devantieri* with a clade clustering together *G.palauensis* and *G. aspera* is shown. The type species of the genus *Goniastrea*, *G. retiformis*, groups together with *Scapophyllia cylindrica* and is more closely related to *Montastraea curta* and *Platygyra sinensis* than to the other two congeneric species.

Discussion

Polyphyly of the genus Plesiastrea

Plesiastrea versipora is a well known widely distributed Indo-Pacific species that has been recognised and identified for almost two centuries (*inter alia* Lamarck,

1816; Milne-Edwards and Haime, 1849; Yabe *et al*., 1936; Wells, 1954; Chevalier, 1971; Wijsman-Best, 1977; Veron *et al*., 1977; Scheer and Pillai, 1983; Carpenter *et al*., 1997; Veron, 2000; Pichon *et al*., 2010). The nomenclatural history of *P. versipora* is well summarised in Veron *et al*. (1977 [Figs 284-292]), together with a series of illustrations of the species morphological variability (see also Scheer and Pillai, 1983 [Plate 33, Figs 2-5]). The specimens examined in this study fall within the range of variability previously documented. Conversely, *P. devantieri* is a relatively newly described species recorded from Socotra and Madagascar (Veron, 2002). Only a few illustrations (Veron, 2000, 2002) and deposited specimens of *P. devantieri* are available. Sampling for this study in the type locality, as well as in another locality of the Indian Ocean, Mayotte (new geographic record for the species), provided material to evaluate the extent of intraspecific variability of certain characters and the stability of others. On the basis of the material examined there are superficial similarities between the two species. However, closer examination of the septal and palar macro and micromorphology together with molecular analyses using two independent markers indicates that *P. versipora* and *P. devantieri* are not phylogenetically closely related, and the latter shares more characters with the so-called 'Bigmessidae' (*sensu* Huang *et al*., 2011) than with *Plesiastrea*.

Our molecular results indicate a divergence level, expressed as p-distance, of 22.1 ± 1.7 % (mean ± s.d.) for the rDNA marker and 5.4 ± 0.9 % for the COI marker. In the case of rDNA the divergence is comparable to the estimates obtained for some of the most polyphyletic and divergent genera, such as *Montastraea* and *Platygyra* (Wei *et al*., 2006). Also the p-distance obtained from the COI region is close to the upper distribution limit of confamilial divergences within the Scleractinia (Shearer and Coffroth, 2008).

From a morphological standpoint, the differences between *P. versipora* and *P. devantieri* are consistent and exceed the variation normally observed between closely related (*i.e.* congeneric) species of hard corals (*i.e.* variations in corallite diameter, length of septa, number of cycles of septa, number of septa in a cycle). Differences in the septal margin ornamentation and palar structures between these species support the non-monophyly of the two taxa at the genus level.

The distribution of the calcification centres controls the septal external micro-morphology and the formation of teeth (Cuif and Perrin, 1999). In the Faviidae the teeth shape and structure, and the organization of
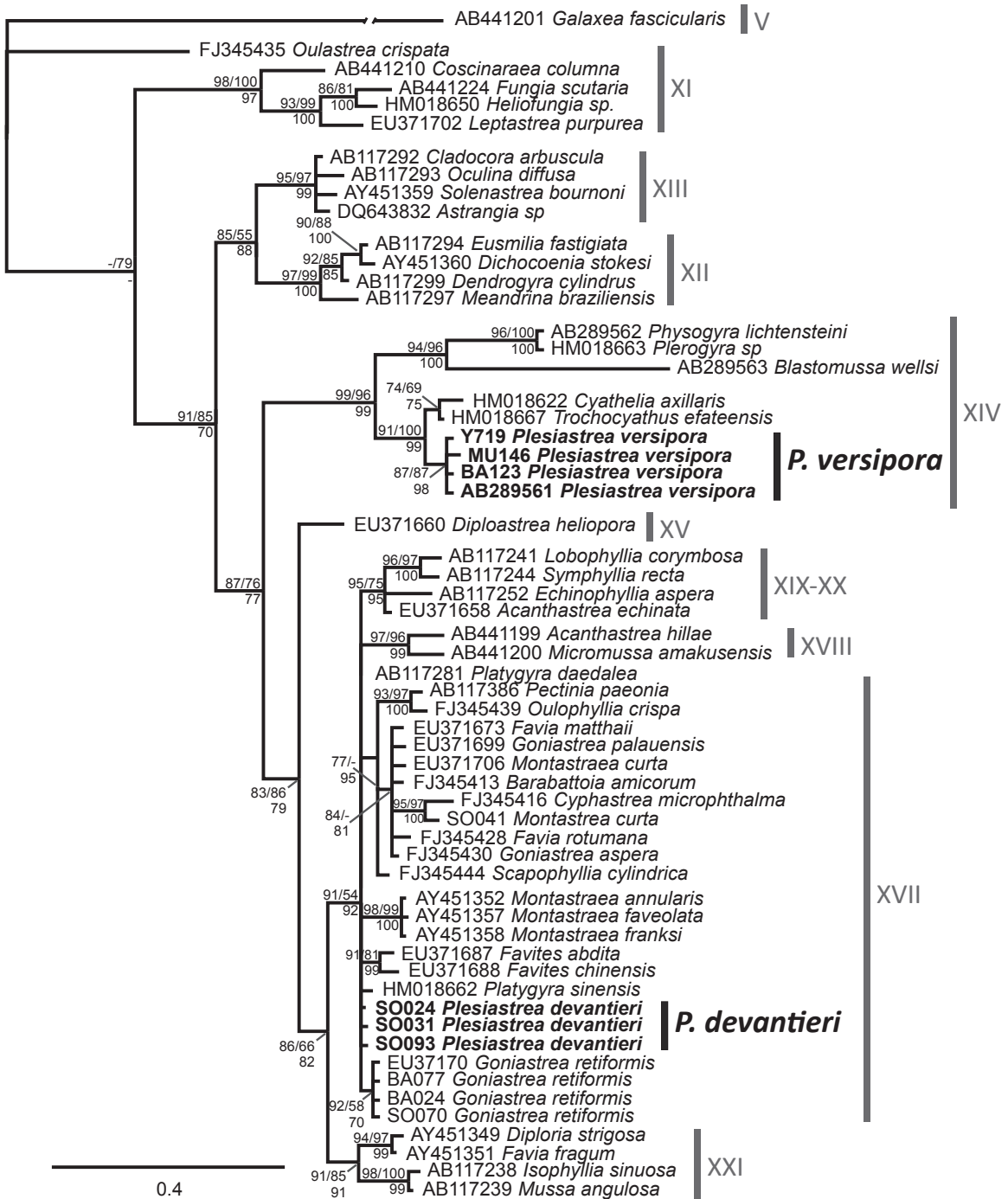
Fig. 7. Phylogenetic tree of mitochondrial gene COI reconstructed with Bayesian Inference. Maximum Likelihood and Maximum parsimony detected similar topologies. Numbers at each node show percentages of (above) Sh-like support (>70%), and MP bootstrap (>50%), and (below) Bayesian posterior probability (>70%); - = no support. Clades of *Plesiastrea versipora* and *Plesiastrea devantieri* are in bold. Clade numbers refer to the same clades previously identified by Fukami *et al.* (2008).
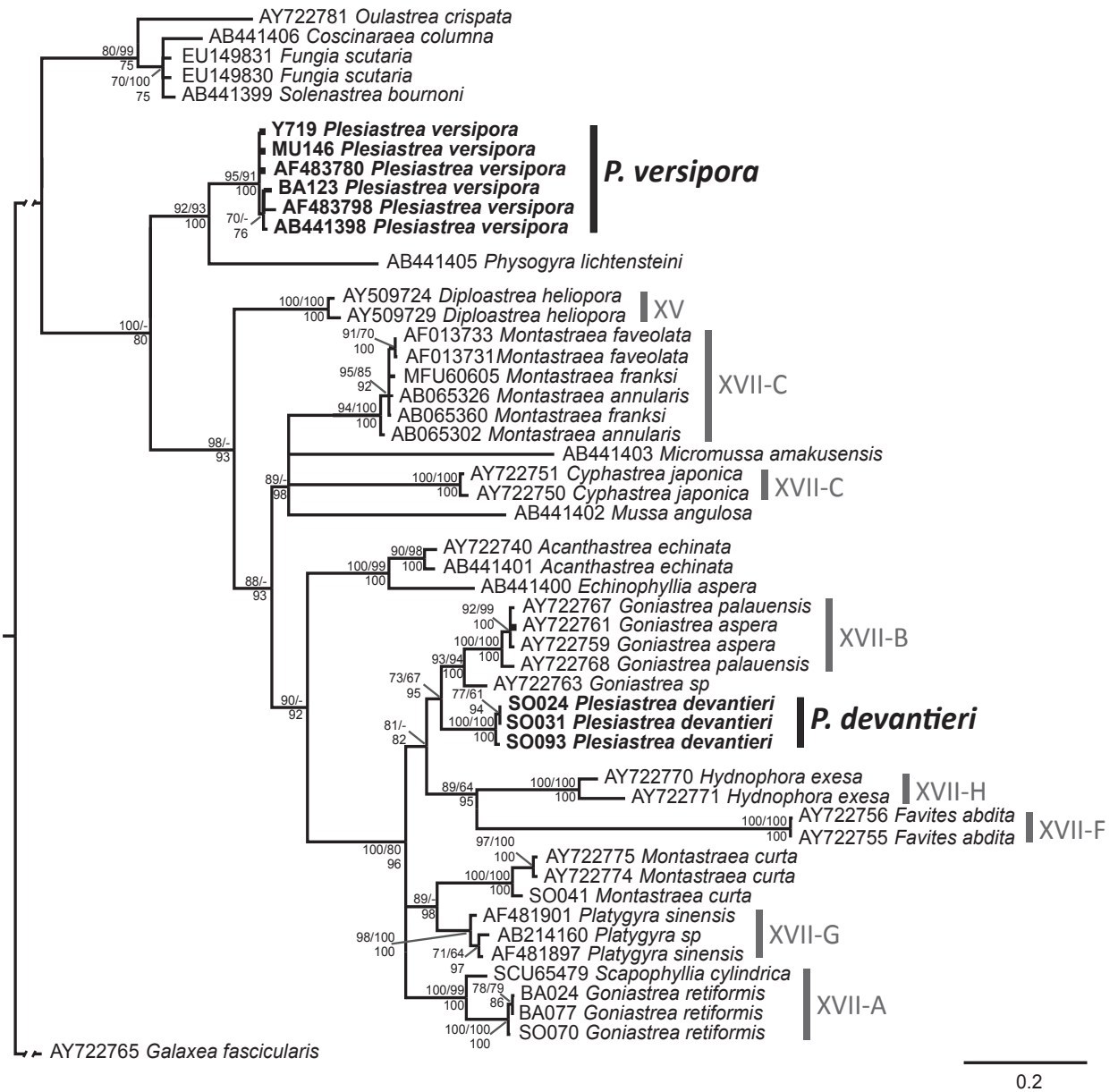
Fig. 8. Phylogenetic tree of a portion of rDNA (spanning the entire ITS1, 5.8S, ITS2 and a portion of 28S and 18S) reconstructed with Bayesian Inference. Maximum Likelihood and Maximum Parsimony detected similar topologies. Numbers at each node show percentages of (above) SH-like support (>70%), and MP bootstrap (>50%), and (below) Bayesian posterior probability (>70%); - = no support. Clades of *Plesiastrea versipora* and *Plesiastrea devantieri* are in bold. Clade codes refer to the same sub-clades previously identified by Huang *et al.* (2011).

the granules are variable and different between Atlantic and Pacific taxa (Budd and Stolarski, 2011). In the Atlantic Faviidae the septal calcification centres are arranged in clusters giving typical 'paddle-shaped' teeth (Figs 4-6 in Cuif and Perrin, 1999; Budd and Stolarski, 2011). Following the definition given by Budd and Stolarski (2009) 'a *paddle-shaped* tooth is formed by a short series of calcification centres (a secondary calcification axis) that is transverse to the direction of the septal plane'. Paddles are separated by short septal zones in which centres occur in the median septal plan. In the Pacific Faviidae septal teeth are multidirectional, more irregular and spine-like or 'lacerate' (Budd and Stolarski, 2011 [Fig. 2]). In *P. versipora* septal teeth are regularly arranged along the septum direction. In this species, the septal ornamentation and micro-structure are devoid of septal paddles, and similar to that described by Cuif *et al.* (2003) for *Cladocora caespitosa*, another taxon previously included by some authors in the Faviidae and currently in need of revision. Conversely, paddle-shaped teeth like those described in the Atlantic Faviidae were observed in *Plesiastrea devantieri*, an Indian Ocean species recovered within clade XVII. Although this may seem surprising, *Favia stelligera*, a Pacific faviid also found in clade XVII, sometimes has discrete centers of rapid accretion as well (Budd and Stolarski, 2011).

As remarked by several authors (Vaughan and Wells, 1943; Wells, 1954; Veron *et al.*, 1977; Chevalier and Beauvais, 1987), it is not always easy to differentiate between pali and paliform lobes. They are ontogenetically and structurally different. Pali are defined as vertical structures developed along the inner axial margin of certain entosepta (Wells, 1956) and structurally identical to them. They are formed as the result of a substitution process of certain exosepta during the formation of entosepta (Wells, 1956 [Figs. 238, 241]) and composed of divergent centers of rapid accretion (or trabeculae in Chevalier and Beauvais, 1987 [Figs. 322A, 332F]). Paliform lobes are the result of the detachment of offsets of the centers of rapid accretion from the inner edge of certain septa (Wells, 1956) and are formed from a single simple or compound center of rapid accretion (Chevalier and Beauvais, 1987 [Fig. 322C]). In practical terms, however, the distinction is not straightforward. For example, the genus *Goniastrea* typically presents a well developed crown of palar structures. According to Wells (1956) these should be referred to as paliform lobes. However, Chevalier and Beauvais (1987) call these pali. Moreover, microstructural analyses have revealed that similar palar morphologies are structurally different (e.g. between the genus *Goniastrea* and *Montastraea*) (Budd and Stolarski, 2011). The palar structures in *P. devantieri* described as paliform lobes by Veron (2000) and as pali (Veron, 2002) and observed in the holotype as well as in the other specimens examined are similar to the paliform lobes typically found in the genera *Favia* and *Montastraea* rather than the pali observed in *P. versipora* (Milne-Edwards and Haime, 1851; Vaughan and Wells, 1943; Chevalier and Beauvais, 1987).

Micro-structural and molecular data provided concordant evidence indicating that *P. devantieri* is closer to, *inter alia*, the genera *Montastraea*, *Goniastrea* and *Favia*, rather than *P. versipora*. In other words, *P. devantieri* is a valid species which does not belong to the genus *Plesiastrea*. In addition *P. versipora* is morphologically and genetically distant from the Faviidae and monophyletic with two apparently very different taxa, *Cyathelia axillaris* and *Trochocyathus efateensis*. Both statements require further discussion and are addressed hereafter.

Phylogenetic relationships of Plesiastrea versipora

On the basis of macro and microstructural features, *Plesiastrea versipora* is clearly distinct from the rest of the taxa presenting typical faviid micromorphology, including, as discussed, *P. devantieri*. Thus, the results of the species macro and micromorphology substantiate the previously published molecular results indicating that *P. versipora* is not closely related to the 'traditional' family Faviidae. What remains to be explained, at least from a morphological standpoint, is the strongly supported clade including *P. versipora* (formerly Faviidae), *Cyathelia axillaris* (traditionally Oculinidae) and *Trochocyathus efateensis* (traditionally Caryophylliidae) (Kitahara *et al.*, 2010b).

It is not surprising that molecular phylogenies recover well supported clades including taxa with very different corallum organization and ecology (Barbeitos *et al.*, 2010). However, some clades are more readily interpretable than others. To date, perhaps the most obvious case of a monophyletic group of Scleractinia including solitary and colonial, zooxanthellate and azooxanthellate, tropical and temperate, shallow and deep species is that of the Dendrophylliidae Gray 1847. This family, however, is well characterised by a number of skeletal characters supporting the monophyly of this well studied group (Cairns, 2001). Conversely, the morphological characters shared by other strongly supported clades including unexpectedly

closely related taxa such as the one including *P. versipora*, *C. axillaris*, and *T. efateensis*, are less immediate.

Although one of the main goals of this study was to evaluate the monophyly of *Plesiastrea* rather than to examine in detail the morphologic affinities between this genus and *Trochocyathus* and *Cyathelia*, some macro morphologic features of the three genera (Fig. 9) are perhaps worth discussing in light of their close phylogenetic relationships (Kitahara *et al*., 2010b). The three taxa are strikingly different in terms of corallum condition and organization: solitary and cyathiform in *Trochocyathus* (Fig. 9A), colonial and encrusting to massive in *Plesiastrea* (Fig. 9B), and colonial and dendroid formed by regular and alternate budding in *Cyathelia* (Fig. 9C). Corallites of *T. efateensis* generally present 5 cycles of septa ($S_{1-2}>S_3>S4>S_5$) (Cairns, 1999), those of *C. axillaris* 4 ($S_{1-2}>S_3>S_4$) (Cairns, 1994), and of *P. versipora* 3 ($S_1 \geq S_2>S_3$) (Veron *et al*., 1977; Wijsman-Best, 1976). From an ecological point of view, while both *T. efateensis* and *C. axillaris* are azooxanthellate deep water corals, *P. versipora* is a zooxanthellate reef dwelling species. However, the latter is also well known for being well adapted to high latitude environments (Kevin and Hudson, 1979; Veron *et al*., 1977; Rodriguez-Lanetty and Hoegh-Guldberg, 2002; Thomson and Frisch, 2010), to conditions of extreme temperature variations as in the Gulf (Carpenter *et al*., 1997; Burchard, 1979), and to the pseudo-high latitude conditions caused by cold water upwelling (Pichon *et al*., 2010).

Besides the presence of a papillose columella and well developed costae, the main character shared by *P. versipora*, *C. axillaris*, and *T. efateensis* is the number and disposition of discrete crowns of pali with respect to the number of cycles of septa (Vaughan and Wells, 1943; Chevalier and Beauvais, 1987; Cairns, 2004). In these species pali are well developed in front of each septal cycle before the last following the rule given by Milne-Edwards and Haime (1848) for the genus *Plesiastrea* ('*Des pali bien développés devant tous les cycles cloisonnaires qui précèdent le dernier*'). In *Plesiastrea* P_1 and P_2 can be more (Fig. 284 in Veron *et al*., 1977) or less (Fig. 3A, 4C) differentiated as also remarked by Milne-Edwards and Haime (1851) ('*ceux* [pali] *des secondaires un peu plus larges et un peu moins rapprochés du centre que ceux des primaires*'). Overall, a comparison of the corallites of *P. versipora* with well developed and sub-equal pali with the corallites of the other two closely related taxa does indeed suggest similarities between the three species (Fig. 9D, E, F).

As mentioned above, pali are usually interpreted as the result of the substitution of exosepta during the formation of entosepta (Wells, 1956 [Fig. 238]). This process explains their disposition in corals where those are only found in front of the penultimate cycle of septa, such as in the genus *Caryophyllia* Lamarck, 1816, and most of its close allies (Kitahara *et al*., 2010a, b). However, it does provide a satisfactory explanation in the case of corals with pali in front of each
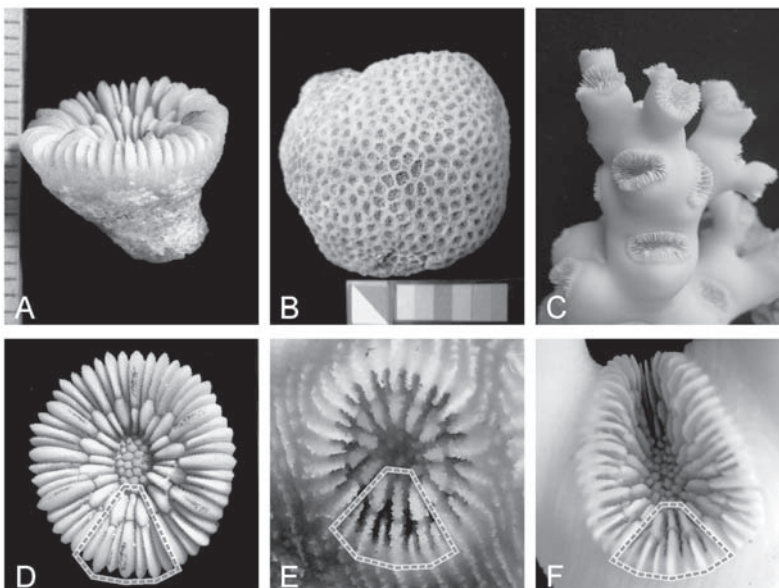

Fig. 9. Coralla of *Trochocyathus efateensis* [A) side view; D) top view, images kindly provided by Marcelo V. Kitahara], *Plesiastrea versipora* [B) whole colony of the Holotype MNHN 36; E) detail of a corallite (AD021)], and *Cyathelia axillaris* [C) ramified colony (SMF C318S at EPA Socotra); F) detail of a corallite of the same specimen]. Dashed polygons in D, E, and F contour similar septa and pali arrangement in *T. efateensis*, *P. versipora*, and *C. axillaris*, respectively.

septal cycle except for the last. In fact, the substitution model calls for a number of crown of pali equal to the number of cycles of exosepta minus one (Wells, 1956), and, hence, it would not be possible to find a crown of pali in front of S_1. Rather, a second model of formation of pali described by Cuif (1968) and Chevalier and Beauvais (1987) seems to apply to the corals with pali in front of each septal cycle except for the last. According to this model, pali are produced by successive isolation from the septa inner margin, regardless of their ento or exocoelic nature, and acquisition of a system of divergent centers of rapid accretion. A different model involving a change of pali position with the formation of new septa was also proposed by Lacaze-Duthiers (1897).

Further analyses are needed to determine the relationship between *Plesiastrea*, *Cyathelia*, and *Trochocyathus*, and possibly other taxa, on the one hand, and to clarify if further morphologic affinities can be found in the septal micromorphology on the other hand. For example, Kitahara *et al.* (2010a) have proposed that, on the basis of 16S rDNA, *Trochocyathus* and *Tethocyathus* Kühn, 1933, may be sister genera. If this was confirmed, then another deep water taxon characterised by pali in front of each septal cycle before the last would be part of the *Plesiastrea-Cyathelia-Trochocyathus* clade. A new family would perhaps have to be described. However, other genera following the same plan may well not be closely related to *Plesiastrea*, and, hence, additional morphological characters will have to be considered.

Phylogenetic relationships of Plesiastrea devantieri *and implications for taxonomy*

According to the rDNA phylogeny in this study *Plesiastrea devantieri* is closely related to *Goniastrea aspera* Verrill, 1905 and *G. palauensis* (Yabe, Sugiyama & Eguchi, 1936). However, COI results indicate that its position is unresolved in a clade including most of the Pacific faviids. Although similarities between *Plesiastrea* and *Montastraea* have been discussed at length in the literature, and despite the fact that a few authors have also indicated similarities with *Favia*, no author has gone as far as discussing similarities between *Plesiastrea* and *Goniastrea*.

From purely morphologic standpoint, the taxa looking closest to *P. devantieri* would be some species in the genus *Montastraea*, such as *M. curta* (Fig. 1E-F) and *M. annuligera*. Phylogenetic relationships between *P. devantieri* and the former species have been

investigated in this study and revealed that the two taxa are not closely related (Figs 7-8). Moreover, on the basis of the results by Huang *et al.* (2011) *M. annuligera* is more closely related to *M. valenciennesi* and different species of *Favia*, rather than to *G. aspera* and *G. palauensis*. Finally, the genus *Montastraea* is deeply polyphyletic and awaiting formal taxonomic revision (Huang *et al.*, 2011).

One of the long-standing paradigms of coral taxonomy and systematics is that cerioid and plocoid corallite arrangement are informative characters (Wells, 1956). Although both *P. devantieri* and *Goniastrea* spp. are characterized by well developed crowns of paliform lobes, the plocoid condition of the former, the cerioid condition of the latter, and the different mode of calicinal budding would not have suggested phylogenetic affinities. A revision of the genus *Goniastrea* does require a separate study. However, on the basis of both molecular phylogenies presented in this study, as well as in previous studies by Huang *et al.* (2009, 2011), the genus *Goniastrea* is not monophyletic, and the type species of the genus, *G. retiformis*, is not closely related to the two *Goniastrea* species which are closest to *P. devantieri*, namely *G. aspera* and *G. palauensis*. Instead, *G. retiformis* is closely related to *Scapophyllia cylindrica* (Milne-Edwards and Haime, 1848). Thus, while it has been shown in the present study that *P. devantieri* can no longer be assigned to the genus *Plesiastrea*, and that it should be included into the molecularly defined clade XVII (*sensu* Fukami *et al.*, 2008), its assignation to a different genus is not a straightforward nomenclatural process. Until a complete revision of the Robust clade, or, at least, of clade XVII, is performed and phylogenetic relationships are fully understood, the taxonomic position of *P. devantieri* remains *incertae sedis*.

It is likely that once more taxa are added to the existing molecular phylogenies of the Scleractinia, more cases of monophyletic and well supported clades of taxa with very different morphologies will be discovered. Then, once more, the quest for evolutionarily informative characters, most likely to be found or rediscovered at the skeleton micro rather then the macro scale, will be open.

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