Journal of the Marine Biological Association of the United Kingdom, 2016, 96(2), 313–322. © Marine Biological Association of the United Kingdom, 2015
doi:10.1017/S0025315415001642

Morphological and molecular systematics of the '*Cliona viridis* complex' from south-eastern Brazil

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Bioeroding sponges of the Cliona viridis *species complex play a large role in carbonate cycling and reef health. In the present study we provide the first record and a description of a Mediterranean lineage of* C. viridis *(Schmidt, 1862) in the south-western Atlantic. Specimens were collected in Maricás Archipelago, Rio de Janeiro State in September 2010 by scuba diving at 10–12 m depth and deposited in the Porifera collection of Museu Nacional, Universidade Federal do Rio de Janeiro. Morphologically, the specimens presently examined are very similar to those described in the beta and gamma growth form from the Mediterranean. The Brazilian and Mediterranean specimens share large and irregular papillae over 2 cm in diameter, megasclere tylostyles up to 500 µm long and microsclere spirasters with up to five twists and 34 µm long. A Maximum Likelihood analysis of 28S rDNA of* C. viridis, C. aprica, C. jullieni, C. schmidti *and* C. varians *was performed for a genetic identification of the Brazilian specimens. The Brazilian material is phylogenetically closer to the Mediterranean* C. viridis *than to the Caribbean and Indian Ocean members of this species complex included in the present analysis. Our results suggest that* C. viridis *is a cryptogenic species with a distribution extending from the Mediterranean to the eastern Atlantic and in the SE Brazilian coast or further.*

Keywords: Porifera, excavating sponges, *Cliona viridis*, cryptogenic species, 28S rRNA, molecular taxonomy, distribution, Brazil

Submitted 30 October 2014; accepted 11 September 2015; first published online 12 October 2015

INTRODUCTION

Bioeroding sponges inhabit and corrode calcium carbonate materials and can significantly affect the carbonate balance of many marine habitats (Schönberg, 2008; Wisshak *et al.*, 2014). Within the family Clionaidae (Demospongiae, Clionaida) the genus *Cliona* Grant, 1826 is best studied and comprises mostly shallow-water marine sponges (Rützler, 2002). *Cliona* species occur worldwide (Van Soest *et al.*, 2015), in warm waters often representing the dominant endolithic organisms (e.g. MacGeachy, 1977; Mallela & Perry, 2007). Their bioerosion activity reworks especially skeletons of molluscs and reef-building corals (Schönberg, 2008).

Their important ecological role requires a sound understanding of their distributions and abundances, and in that context knowledge about local biodiversities and faunistics is essential (e.g. Carballo *et al.*, 1994). Unfortunately, many bioeroding sponges are challenging to identify (e.g. Rosell, 1994; Barbieri *et al.*, 1995; Bavestrello *et al.*, 1996a; Schönberg & Beuck, 2007). The most important features for differentiating *Cliona* species are growth forms (alpha: endolithic-papillate, beta: endolithic-encrusting, gamma: free-living massive; Schönberg, 2008), papillar characters, the size and morphology of spicules and excavation patterns (e.g. Pang, 1973; Rosell, 1994; Rosell & Uriz, 1997; Schönberg, 2001, 2008; Rützler, 2002), but there are variable characters and similarities between species. For example, sponges are well-known for their high degree of phenotypic plasticity, creating morphological dissimilarities in the same and similarities between different species, and clionaid sponges are no exception (e.g. Rützler, 1974; Wiedenmayer, 1977; Hill, 1999; Bell *et al.*, 2002; Hill & Hill, 2002). Moreover, spicule dimensions overlap between species belonging to Clionaidae, and the size and the shape of their tylostyles and abundance of microscleres can occasionally vary with individual specimens, which apparently can be caused by environmental conditions, patchy distribution in the tissues, developmental stages, cryptic speciation, misidentification or contamination (e.g. Rosell & Uriz, 2002; Xavier *et al.*, 2010; De Paula *et al.*, 2012). Identification is especially difficult in species in which microscleres are rare or absent (Rützler, 2002; Schönberg *et al.*, 2006). At genus or family level some distinguishing information can be obtained from erosion

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scars on the substratum (Calcinai *et al.*, 2003, 2004, but see Schönberg, 2008), but size and shape of galleries and sponge chips resulting from the boring activity may be affected by the nature of substratum and the state of advance into the substrate (Schönberg, 2008), and their usefulness in taxonomy was occasionally questioned (e.g. Schönberg, 2000a; Fromont *et al.*, 2005; Calcinai *et al.*, 2007). Taxonomic studies on bioeroding sponges are further hampered by poor species descriptions, scarcity of comparative material in scientific collections or their inaccessibility, and difficulties with usual methods of collection (e.g. Schönberg & Beuck, 2007).

As a result the systematics of the Clionaidae is partly confused, and their biodiversity is underestimated at many locations (e.g. Xavier *et al.*, 2010; De Paula *et al.*, 2012). Currently there are 77 accepted species in the genus *Cliona* (Van Soest *et al.*, 2015). *Cliona* species are fairly well known from the Mediterranean Sea and neighbouring areas of the Eastern Atlantic (14 species: Von Lendenfeld, 1897; Rützler & Bromley, 1981; Topsent, 1932; Van Soest, 1993; Carballo *et al.*, 1994, 1997; Corriero & Scalera-Liaci, 1997; Rosell & Uriz, 2002; Corriero & Nonnis Marzano, 2006; Calcinai *et al.*, 2011), Australia (15 species: Carter, 1886; Topsent, 1888, 1932; Hooper & Wiedenmayer, 1994; Schönberg, 2000a; Fromont *et al.*, 2005; Schönberg *et al.*, 2006; Van Soest *et al.*, 2015), and best-studied in the Tropical Western Atlantic, especially in the Caribbean region (24 accepted species: De Laubenfels, 1950; Pang, 1973; Rützler, 1974; Duchassaing & Michelotti, 1864; Sollas, 1878; Carter, 1882; Topsent, 1888; Leidy, 1889; Van Soest, 1993; Holmes, 2000; Zea & Weil, 2003; Miloslavich *et al.*, 2010; Friday *et al.*, 2013; Van Soest *et al.*, 2015), while subareas of the above and other regions are under-represented. Nine *Cliona* spp. were reported for Brazil and neighbouring waters from early expeditions such as the 'HMS Alert' (Hechtel, 1976; Ridley, 1881) and the 'Calypso' (Boury-Esnault, 1973) and contemporarily, by Brazilian researchers (e.g. Muricy & Hajdu, 2006; Muricy *et al.*, 2008).

Species united in the *Cliona viridis* (Schmidt, 1862) species complex are difficult to distinguish from each other and have historically caused much confusion (e.g. Bavestrello *et al.*, 1996a, b; Schönberg, 2000b; Zea & Weil, 2003). Schönberg (2002) defined *C. viridis* species complex species by having tylostyles and mostly delicate spirasters, and being in symbiosis with dinoflagellate zooxanthellae that cause a brownish colour in many species. Members of this species complex that can develop to beta or gamma growth forms have been recognized as very good space competitors that can aggressively invade and kill live corals (e.g. Schönberg & Wilkinson, 2001; López-Victoria *et al.*, 2006; Márquez & Zea, 2012), and that they will acutely react to ocean acidification with accelerated bioerosion rates (Wisshak *et al.*, 2012, 2013, 2014; Fang *et al.*, 2013; Stubler *et al.*, 2014). They can increase in abundance after disturbance events or with decreasing water quality (e.g. Schönberg & Ortiz, 2009), and because the most destructive *C. viridis* complex species share a global distribution with reef-forming corals they have been recommended to be monitored at regular intervals (Schönberg, 2015). This highlights the importance of faunistic and eco-physiological knowledge about this group.

Recent expeditions carried out to Maricás Archipelago, central coast of Rio de Janeiro, Brazil, have yielded specimens of sponges of *C. viridis* complex very similar to those described for its type locality in the Mediterranean Sea. This work identifies and describes the sampled material using morphological and molecular approaches and will discuss findings in context of present knowledge of the *C. viridis* species complex.

MATERIALS AND METHODS

Study area

Maricás Archipelago is located 30 km east of Rio de Janeiro city and 3.6 km off mainland (23°00′S 42°55′E), on the central coast of Rio de Janeiro state, south-east Brazil (Figure 1). The archipelago is formed by Maricá Island (the largest island, 1.6 km long by 300 m wide) and two smaller rocky islets aligned in a SW–NE direction, with the mean depth in this area being 17 m (Monteiro-Neto *et al.*, 2013). The rocky bottom is composed of granitic boulders of varying size (Monteiro-Neto *et al.*, 2013). A preliminary survey at Maricás Archipelago conducted by the first and last authors in 2010 recorded 15 sponge species (E. Esteves, personal observation). Water quality is poor in this area due to the effect of untreated domestic and industry sewage from Guanabara Bay (Kjerfv *et al.*, 1997) and runoff from small estuaries nearby. Maricás Archipelago is also subject to introduction of exotic species due to intense traffic related to oil platforms next to the Guanabara Bay entrance (Ignacio *et al.*, 2010). The area experiences common upwelling events, occasionally reducing temperatures to <20°C (Campos *et al.*, 2000).

Sampling and morphological analysis

Sponge specimens were collected on Maricás Archipelago on 26 September 2010. Specimens were photographed *in situ* prior to collections with an 8MP digital camera (Sealife DC800, Moorestown). Oscula and ostial papillae measurements were estimated from digital underwater photos of specimens of the new Brazilian material. Fragments of three specimens were collected by knife on scuba in a depth of 10–12 m. Samples were fixed in 80% ethanol and deposited in the Porifera Collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ) after respective analyses. Comparative material was obtained for morphological analysis. A schizolectotype of *Cliona varians* (Duchassaing & Michelotti, 1864) from the British Museum of Natural History, London (BMNH) was examined, as well as a fragment of an unpublished specimen of *C. viridis* collected in the Mediterranean Sea, deposited in the MNRJ collection. Procedures for preparing slides of spicules followed Rützler (1978). Cross sections were obtained with a diamond saw from epoxy resin-embedded fragments of specimens and substrate. Thirty spicules for each specimen were randomly selected and measured at magnification 40–400× under an Olympus BX 50 light microscope equipped with an eyepiece micrometer. Bioerosion traces and spicules were studied by scanning electron microscopy (SEM microscope JEOL JSM6390LV). Spicule and bioerosion sponge scars dimensions are presented in micrometres as minimum–*mean*–maximum (±standard deviation).

Molecular analysis

DNA extractions were conducted as described by De Paula *et al.* (2012). PCR amplifications of the D1–D2 region of

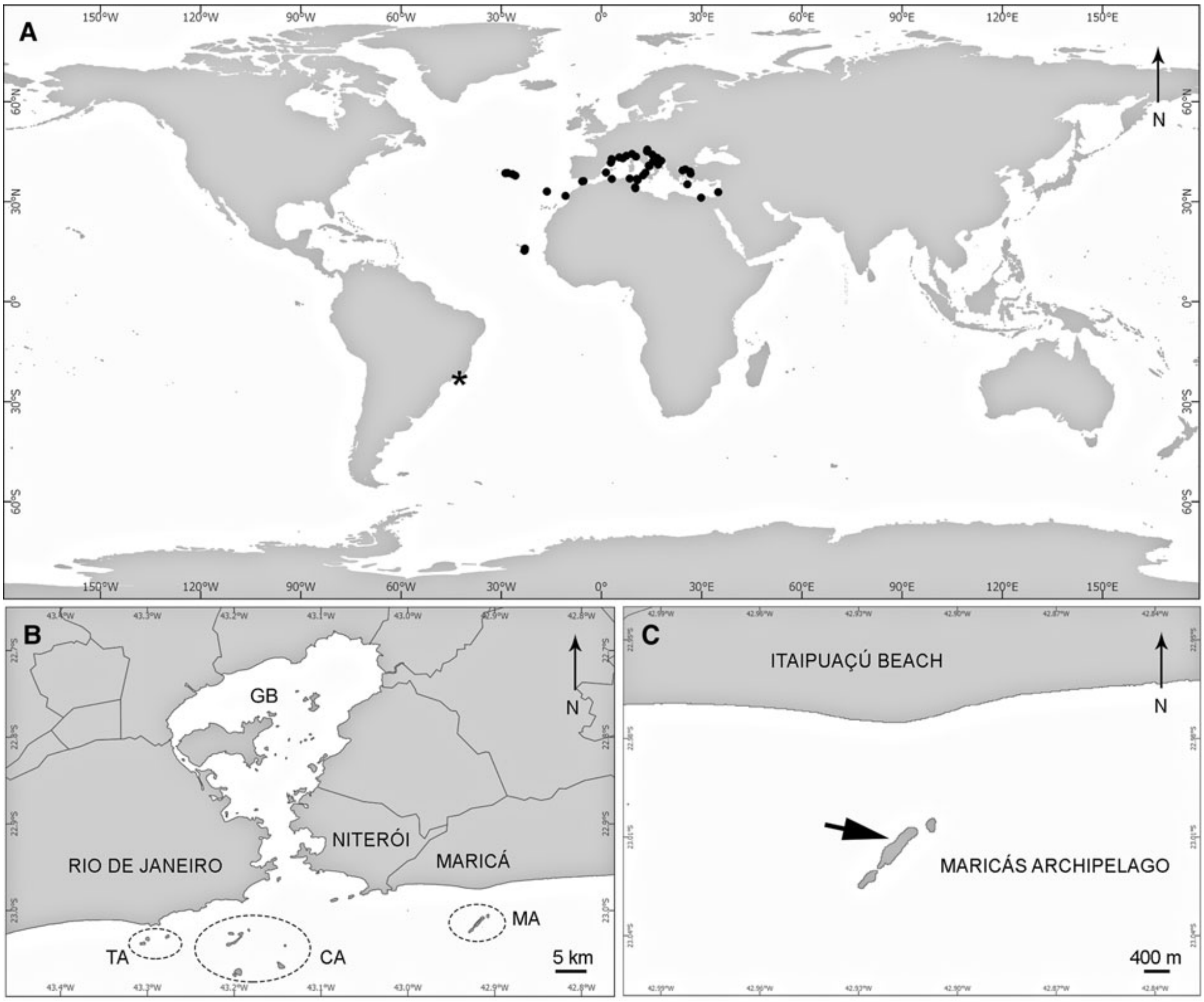

Fig. 1. Geographic distribution of *C. viridis* (Schmidt, 1862): (A) map with the confirmed distribution of *C. viridis* in the world, according to Van Soest *et al.* (2015) (black circles) and the new record of this species from Brazil (asterix); (B) map of the central coast of Rio de Janeiro state and continental islands in its surroundings; (C) precise collection locality of *C. viridis* in Maricás Archipelago, SE Brazil (arrow). CA, Cagarras Archipelago; GB, Guanabara Bay; MA, Maricás Archipelago; TA, Tijucas Archipelago.

the 28S rRNA gene (~800 bp) were carried out following protocol employed by Morrow *et al.* (2012), using the primers Por28S-15F and Por28S-878R. PCR products were sequenced by third party service (Macrogen Inc., South Korea) in both directions. The sequences were aligned with MAFFT v6.935 (Katoh *et al.*, 2005) using the E-INS-i algorithm for local alignment searches with default parameters, and positions containing gaps or missing data were excluded from the phylogenetic analyses. The phylogenetic reconstruction was performed through the Maximum Likelihood (ML) method, implementing the CAT-GTR model. The analysis was conducted with RAxML v7.0.4 (Stamatakis, 2006), and the best-scoring ML tree was inferred after rapid Bootstrap analysis with 1000 replicates. Specimens from *Cliona aprica* Pang, 1973 (MNRJ 15685; the Caribbean) and *C. varians* (MNRJ 10724; North-east Brazil) were included for comparison, in addition to the following sequences obtained from GenBank (accession numbers in parentheses): *C. viridis* (AF062606 and AM293637) and its junior synonym *C. nigricans* (AM293635 and AM293636); *C. jullieni* Topsent, 1891 (AM293625, AM293626 and AM293627); *C. schmidti* (Ridley, 1881) (AM293632 and AM293633); and *Spirastrella hartmani* Boury-Esnault *et al.*, 1999 (KC869504), used as outgroup. All sequences generated in this work were deposited in GenBank under accession numbers KP400590–400594.

RESULTS

SYSTEMATICS

Class DEMOSPONGIAE Sollas, 1885
Subclass HETEROSCLEROMORPHA Cárdenas *et al.*, 2012
Order CLIONAIDA Morrow & Cárdenas, 2015
Family CLIONAIDAE D'Orbigny, 1851
Genus *Cliona* Grant, 1826

DIAGNOSIS

Clionaidae in alpha, beta or gamma growth forms. Megascleres tylostyles or subtylostyles, and raphides in some species as accessory spicules. Microscleres spirasters and derivatives, as straight, bent, kinked, helical, spiny or rarely smooth rhabds, including amphiastrose forms supposedly derived from true spirasters. Microscleres occasionally rare or entirely absent (Rützler, 2002). Type species: *Cliona celata* Grant, 1826.

Cliona viridis (Schmidt, 1862)
(Figures 1–4; Table 1)

SYNONYMY

Vioa viridis Schmidt, 1862, p. 77
Papillina nigricans Schmidt, 1862, p. 69
Papillina suberea Schmidt, 1862, p. 69
Osculina polystomella Schmidt, 1868, p. 3
Cliona copiosa Sarà, 1959, p. 8
Cliona tremitensis Sarà, 1961, p. 38

For additional synonyms see: Van Soest *et al.* (2015).

DIAGNOSIS (based on Carballo *et al.*, 1994; Rosell & Uriz, 2002; Schönberg, 2000b).

Cliona species occurring in all three growth forms: alpha, beta and gamma. Moss green, brown, blackish or whitish colour in life. Specimens in alpha or beta growth form with tiny papillae, up to 4 mm in diameter, circular to oval in shape. Specimens in gamma growth form exhibiting circular, oval or irregular ostial papillae and fleshy oscules over 2 cm wide. Spicules: megasclere tylostyles up to 400 µm long in alpha and beta growth forms, up to 600 µm long in gamma form sponges. Two categories of spirasters in all growth forms: spiraster I, with straight shaft 13–30 µm long and relatively large spines concentrated terminally, resembling amphiasters; and spiraster II, with 2–5 bends, 10–50 µm long. Type locality: Zadar Channel, Adriatic Sea (Schmidt, 1862).

MATERIAL EXAMINED

MNRJ 14016, 14017 and 14019, Maricás Archipelago, next to the 'Moreno' shipwreck (23°00′41″S 042°55′11″W), Maricá, Rio de Janeiro, south-eastern coast of Brazil, 10–12 m depth, E. L. Esteves coll., 26 September 2010.

COMPARATIVE MATERIAL FOR MORPHOLOGICAL STUDIES

MNRJ 13458 (unpublished specimen of *C. viridis*), Mediterranean Sea, S. Ribeiro and A. Villamor colls. Date of collection and precise collection locality not available.

BMNH 1928.11.12.49 (schizolectotype of *C. varians* (Duchassaing & Michelotti, 1864)), St. Thomas, US Virgin Islands. Date of collection and collector not available.

DESCRIPTION

External morphology

Thickly encrusting (beta growth form) to massive (gamma growth form), forming patches of over 20 cm in diameter and up to 5 cm thickness. Ostial papillae irregular to oval, numerous, 2 cm wide on average *in vivo* (Figure 2A), barely distinguishable after fixation in ethanol (Figure 2B). Oscular papillae more pronounced than ostial ones, slightly elevated, 3 cm in diameter, oval in shape (Figure 2A). Colour in life moss green externally and internally, changing to brownish green after fixation in ethanol. Surface rough by large amounts of encrusting and embedded sand debris on ectosome, markedly undulated after fixation in ethanol due to papillar contraction. Consistency firm, only slightly compressible.

Internal anatomy

Ectosomal skeleton composed of tylostyle palisade (Figure 2C). Choanosome cavernous with wide channels and abundant coarse calcareous debris loosely incorporated in inner parts and close to ectosome. Choanosomal skeleton formed by subtylostyles disorganized in inner parts and arranged in bundles close to ectosome (Figure 2C). Spirasters, when present, dispersed in the choanosome (Figure 2C).

Spicules

Megascleres: No apparent size classes, but tylostyles to subtylostyles size-variable, straight or slightly curved, with rounded, elongated or occasionally subterminal tyle, and sharply pointed (Table 1; Figure 2D–E, G–H). Microscleres: spirasters observed in two specimens in beta and gamma growth forms, with 1–5 twists, the smaller with stout and almost straight axis and with scarce spines, the larger with up to five twists and slender axis, with abundant and bifurcated spines (Table 1; Figure 2I).

Excavation pattern

Bioerosion sponge scars on calcareous substrate in erosion chambers made in encrusting coralline algae convex, pentagonal or hexagonal depressions (Figure 3A–D) measuring 42.0–*55.0*–65.0 (±1.8) µm in width (N = 30). Chambers connected by apertures ~300 µm wide.

Ecology

Cliona viridis was one of the most common sponge species in the north-western portion of the Maricás Archipelago during a visit in September 2010 and could not be relocated at the same locality in February 2013 and 2015 (E. Esteves, personal observation). Putative specimens of *C. viridis* were observed and collected in July 2015 at Maricás and Cagarras Archipelago (Figure 1B), but their identification needs to be confirmed (F. Moraes, personal communication). *Cliona viridis* is a photosymbiotic species, covering the uppermost portion of rocks, and was found excavating crusts of calcareous algae. *Cliona viridis* was observed frequently associated with epizoic green macroalgae and was usually covered by coarse sand (Figure 2A).

GEOGRAPHIC AND BATHYMETRIC DISTRIBUTION

Cliona viridis is distributed in the Mediterranean and Eastern Atlantic, occurring from zero to 365 m depth (Carballo *et al.*, 1994; Rosell & Uriz, 2002; Van Soest *et al.*, 2015), and in south-eastern Brazil in 10–12 m depth (present study; Figure 1).

MOLECULAR ANALYSIS

ML phylogenetic reconstruction using 28S sequences recovered the Brazilian specimens together with Mediterranean *C. viridis* sequences in a highly supported monophyletic clade (Figure 4). The phylogenetic relationship among sequences within this clade became apparent without any geographic structure. The *C. viridis* clade was clustered closely to *C. aprica*, followed by *C. varians* and *C. schmidti* in a basal polytomy. *Cliona jullieni* formed the most basal clade among the sequences of the *C. viridis* complex analysed.

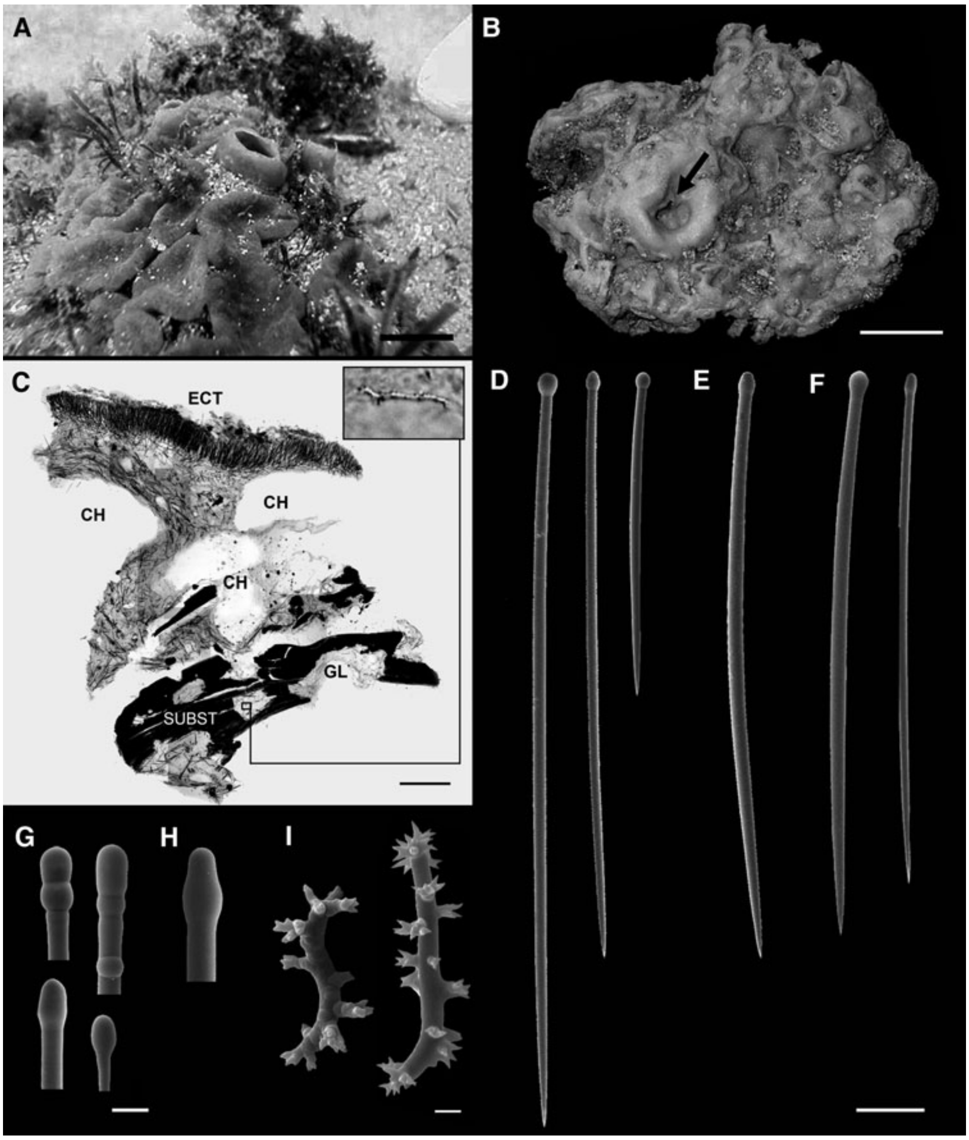

Fig. 2. *Cliona viridis* (Schmidt, 1862) from Maricás Archipelago, south-eastern Brazil: (A) photo *in situ*; (B) fixed specimen; note the markedly undulated surface due to the contraction of conspicuous irregular ostial papillae and an oscule (arrow); (C) cross-section of skeleton obtained with a diamond saw with a spiraster 28 μm long in detail; (D–E) tylostyles of a specimen from Maricás Archipelago, south-eastern Brazil; (F) Tylostyles of a specimen from the Mediterranean Sea; (G–H) base of tylostyles of specimens from Maricás Archipelago in detail; (I) Spirasters from a specimen in beta growth form from Maricás Archipelago. Scale bars: A, 2 cm; B, 3 cm; C, 1 mm; D–F, 50 μm; G–H, 10 μm; I, 2 μm. (A, E, H) MNRJ 14019; (B, D, G) MNRJ 14016; (C, I) MNRJ 14017; (F) MNRJ 13458. CH, channels; ECT, ectosome; GL, gallery; SUBST, substrate.

REMARKS AND DISCUSSION

The Brazilian specimens examined in the present study were conspecific with *Cliona viridis* and thus recognized as belonging to the *C. viridis* species complex, as indicated by the presence of a series of characters, i.e. the brownish green colour, gamma or beta growth form, typical subtylostyles and slender spirasters, and the presence of photosymbionts (Carballo *et al.*, 1994; Rosell & Uriz, 2002; Schönberg, 2000b). The complex comprises an unknown number of species mostly distributed in warm waters and largely follows the distribution of coral reefs (Van Soest *et al.*, 2015). Five or six species have previously been assigned to the *C. viridis* complex from the Tropical Western Atlantic: *C. aprica, C. caribbaea* Carter, 1882, *C. tenuis* Zea & Weil, 2003, *C. tumula* Friday *et al.*, 2013 and *C. varians* (Duchassaing & Michelotti, 1864) (Zea & Weil, 2003; Escobar *et al.*, 2012; Friday *et al.*, 2013). *Cliona varians* is well known from the north-east and south-east coast of Brazil (Muricy *et al.*, 2008; Hajdu *et al.*, 2011). However, this list is expected to be incomplete, as some massive species should be part of it (presently *Spheciospongia* spp.), and in addition less typical species were not formally included in the complex, e.g. *C. schmidti* and *C. jullieni* from the Mediterranean and Indo-Pacific region (see Barucca *et al.*, 2007). These two species were included in our phylogenetic analysis, because they possess similar skeletal characters and molecular properties, and at least *C. jullieni* has photosymbionts (e.g. Barucca *et al.*, 2007; Schönberg, unpublished data).

We excluded most of the known brown-coloured, symbiotic Indo-Pacific species typical for the *C. viridis* species complex from the present comparison as they are spatially

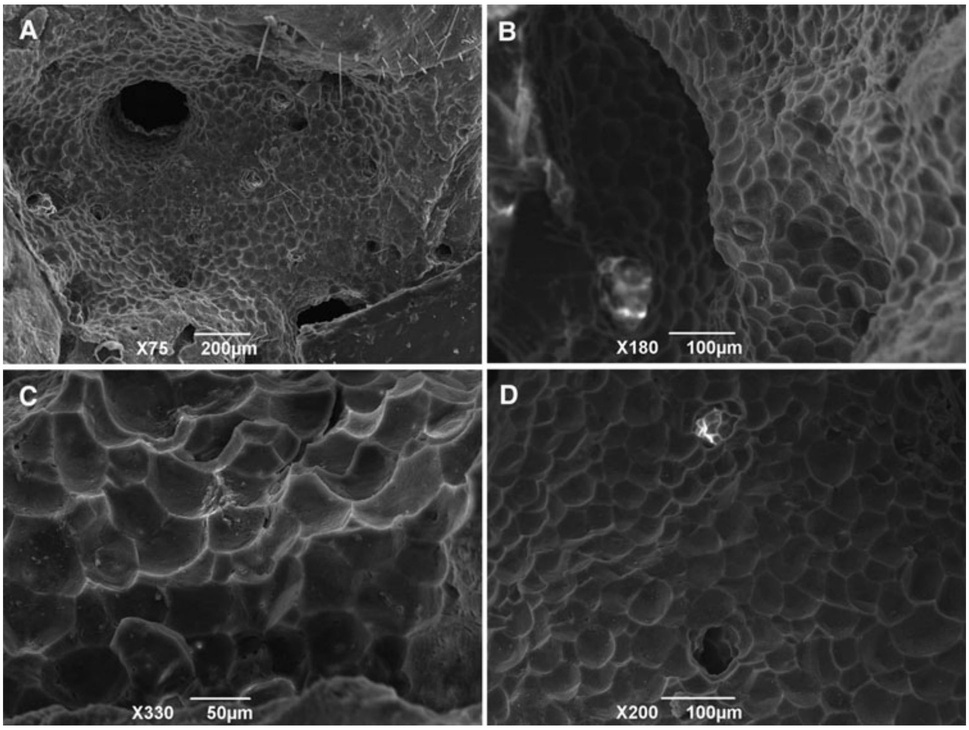

Fig. 3. Scanning electron micrographs of sponge-generated bioerosion chambers found in substrate inhabited by *C. viridis* (Schmidt, 1862) from Maricás Archipelago, south-eastern Brazil: (A–B, D) Chambers and apertures; (C) Pentagonal and hexagonal pits, the so-called 'sponge scars', on the substratum walls resulting from the excavation activity. All micrographs were taken from the substratum excavated by the specimen in beta growth form (MNRJ 14017).

removed from our sample area and largely differ in morphology from our samples (e.g. *C. orientalis* Thiele, 1900, *C. albimarginata* Calcinai *et al*., 2005, *C. caesia* (Schönberg, 2000a)). We considered it most likely that our material derived from the Western Atlantic and might have originated from the larger Caribbean Region. However, the Caribbean species *C. caribbaea* and *C. tenuis* can attain only alpha or beta growth forms (Rützler, 1974; Zea & Weil, 2003), and *C. aprica* only occurs in alpha form (Zea & Weil, 2003), while our material was sampled as beta and gamma sponges. Additionally, these three species have tiny (1–4 mm), circular or oval oscules and shorter tylostyles, up to 400 µm (Schönberg, 2002; Zea & Weil, 2003), whereas our specimens have larger papillae and longer tylostyles (Table 1). According to its original description, *C. paucispina* Rützler, 1974 from Bermuda also belongs into the *C. viridis* species complex and was thus considered. It develops small colonies in beta form and has zooxanthellae, but has tylostyles of a mean length of 340 µm and spirasters with reduced spination and thus differs from our material (Rützler, 1974). *Cliona varians* can develop a similar habit compared with our specimens, forming thick crusts to gamma form specimens (Hill, 1999). Schönberg (2000b) included this species in the *C. viridis* complex. Nevertheless, it was clearly different from our material for possessing typically C-shaped spirasters (anthosigmas; confirmed after examination of a schizolectotype of this species in the present study). Indeed, *C. varians* never develops ostial papillae rising above the surface (e.g. Muricy *et al*., 2008; Hajdu *et al*., 2011).

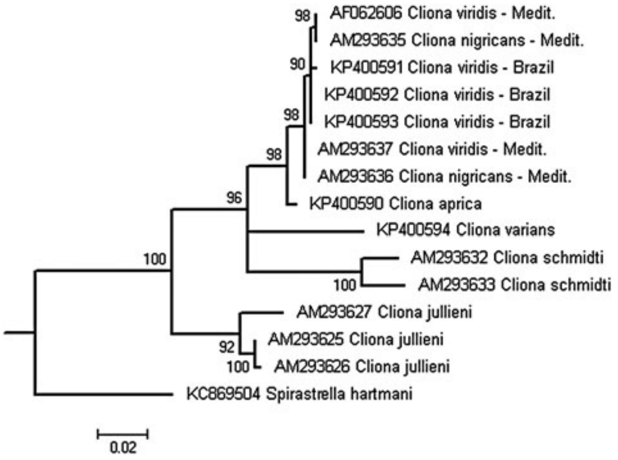

Fig. 4. Cladogram reconstructed from partial 28S sequences of species belonging to the *C. viridis* complex. Tree topology recovered through the Maximum Likelihood method, implementing the CAT-GTR model. Numbers above branches are the support values obtained by 1000 rapid bootstrap replicates. Sequences are listed by their accession numbers, followed by taxon name and locality. See text for details.

The next best connectivity was assumed to Mediterranean and Central East Atlantic species, which yielded a good match. The specimens described in the present study are very similar to those belonging to *C. viridis* in gamma or beta growth from the Mediterranean by their large and irregular papillae over 2 cm in diameter (Carballo *et al*., 1994, Figure 8B; Rosell & Uriz, 2002, Figure 17D–E), moss green to brownish colour in life and long tylostyles up to 500 µm long, and slender spirasters with up to five twists and 34 µm long (Table 1). Spirasters were relatively common in the specimen MNRJ 14017 in beta growth form, and were apparently absent in one specimen in gamma growth form (MNRJ 14019). However, spirasters were probably overlooked in the latter

Table 1. Dimensions of spicules of *C. viridis* in micrometres from Maricás Archipelago, Rio de Janeiro State, south-eastern Brazil. Values are presented as minimum–*mean*–maximum (±standard deviation). N = 30 per specimen.

Specimens	Tylostyle			Spirasters	
	Length	Width	Tyle width	Length	Turns
MNRJ 14016	267.5–*414.2*–500.0 (±68.2)	2.5–*7.7*–11.3 (±1.8)	6.3–*10.5*–13.8 (±1.9)	–	–
MNRJ 14017	182.5–*447.6*–543.8 (±67.5)	2.5–*7.0*–11.3 (±2.3)	6.3–*10.1*–13.8 (±2.0)	5.8–*16.9*–34.0 (±6.0)*	0–5
MNRJ 14019	272.5–*400.7*–482.5 (±70.3)	6.3–*9.0*–12.5 (±1.4)	8.8–*11.3*–15.0 (±1.4)	–	–

*Measurements of microscleres taken from cross sections.

specimen, since they are usually very scarce in specimens of this species in gamma growth form (Carballo *et al.*, 1994). An additional specimen in gamma form from the Mediterranean Sea examined in the present study for comparison (MNRJ 13458) exhibited the same convoluted surface due to the contraction of conspicuous papillae, a pale brown colour after fixation, and similarly, did not show spirasters. Hence, we assume that the Brazilian *C. viridis* specimens in beta and gamma form represent different stages of growth or ecophenotypes of the same species. Our morphological identification was confirmed by molecular analysis in this study (Figure 4).

The size and shape of erosion chambers and pits or scars caused by the Brazilian specimens were very similar to those described for Mediterranean specimens of this species (Rosell & Uriz, 2002). Nevertheless, they were also similar to the traces created by other unrelated excavating species, i.e. *Siphonodictyon labyrinthicum* (Hancock, 1849) and *Spiroxya levispira* (Topsent, 1898) (see Rosell & Uriz, 2002) and hence were not useful in species recognition in the present study. Chamber morphology can vary and is not usually distinctive for a single sponge species (e.g. Bromley & D'Alessandro, 1984, 1990). Notwithstanding, the microanatomy of the wall pitting in galleries created by *Cliona* spp. was described for only a few species and should be better investigated (e.g. Bromley, 1978; Calcinai *et al.*, 2003, 2004; Schönberg & Shields, 2008).

Overall, the Brazilian specimens are morphologically very similar to beta and gamma forms of the Mediterranean *C. viridis* complex. Indeed, our specimens were phylogenetically closer to the Mediterranean members of this species complex than to the Caribbean *C. aprica* and Brazilian *C. varians*. Furthermore, we showed that in our selection of samples *C. jullieni* and *C. schmidti* were only distantly related to the clade formed by *C. aprica* and *C. viridis*. Following White's (1977) proposal on the species complex concept – 'A species complex consists of sibling species which, by definition, are species with obscure morphological differences. What, then, is the possible role of morphology in understanding species complexes? It is to find out if the seemingly undifferentiated members of a supposed complex are, in reality, a group of species differentiated sufficiently well for some or all of them to be identified morphologically' – we have tentatively identified our samples as *C. viridis*. Nevertheless, only a detailed revision of the *C. viridis* complex at least in its type-locality (the Mediterranean) and connective areas (i.e. Eastern Atlantic and Tropical Western Atlantic) would possibly allow a better explanation for the morphological affinities between the Brazilian and Mediterranean populations of this species and the taxonomic implications of our findings for unravelling this highly productive, reef shaper complex of species.

This is not the first time an eastern Atlantic/Mediterranean lineage of *Cliona* was found in south-eastern Brazil, since De Paula *et al.* (2012) reported an Irish lineage of *C. celata* in south-eastern Brazil. However, this is the first record of a Mediterranean lineage of *C. viridis* in the south-western Atlantic. The south-eastern coast of Brazil and the western Mediterranean are warm temperate and temperate ecoregions, respectively (Spalding *et al.*, 2007), which would possibly allow the development of sponge populations of a same species from both geographic regions (e.g. Klautau *et al.*, 2004; Longo *et al.*, 2007). Given the intense harbour activities near the sampling site (e.g. Ignacio *et al.*, 2010), we believe that our *C. viridis* specimens may have their origin in the Mediterranean Sea, and they were possibly brought to Brazil by ballast water, associated with traffic around oil platforms or other man-made structures. Nevertheless, as we have no confirmed evidence of introduction of Mediterranean lineages of this species in south-eastern Brazil we assumed that *C. viridis* is a cryptogenic species with a distribution extending from the Mediterranean to the eastern Atlantic and down to south-eastern Brazil. Recent expeditions conducted in February 2013, 2015 to the same locality failed to find additional specimens of this species, suggesting that *C. viridis* reached the south-eastern coast of Brazil, but apparently disappeared before firmly established (E. Esteves, personal observation) and re-colonized the continental islands off Rio after February 2015 (if specimens recently collected prove to be *C. viridis*). Otherwise, the Brazilian population of this zooxanthellate and thus temperature-dependent species (e.g. Schönberg & Suwa, 2007) might have reduced at south-east Brazil, caused by low temperatures stress occasioned by upwelling events in this area (Campos *et al.*, 2000). Nevertheless, there is no evidence linking the absence or scarcity of the sponge in this area to thermal stress. A long-term study monitoring *C. viridis* at south-east Brazil is strongly recommended given its possible negative impacts on reef health and wide potential distribution. Additionally, phylogeographic, ecological and reproductive studies including other species belonging to this complex are necessary to elucidate the evolutionary history of *C. viridis* in the Tropical Western Atlantic.

ACKNOWLEDGEMENTS

We thank Cecília Pascelli, Fabiana Fernandes, Fener Abdalla, Filipp Soares, Kady Coelho and Wellington Vieira for help during collections. Kady Coelho is also thanked for underwater photographs. Eduardo Hajdu is acknowledged for providing facilities, including the use of laboratory space and scanning electron microscopy at Museu Nacional (UFRJ). Thanks to Fernando Moraes, Guilherme Muricy and the team of 'Projeto Ilhas do Rio', sponsored by Petrobras through the Program Petrobras Ambiental, for providing additional information and access to newer samples collected on continental islands off Rio de Janeiro. Sula S. Mota and Phillip Willenz gave us access to a schizolectotype of *C. varians*. Three anonymous referees gave us valuable comments on this manuscript.

FINANCIAL SUPPORT

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior supported CVL with a PhD fellowship and ELE with a fellowship to attend the 9th International Porifera Conference, held in Fremantle (Western Australia). ELE and GLH received grants from the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Rio de Janeiro and the Conselho Nacional de Desenvolvimento Científico e Tecnológico.

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