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Inter- and intraspecific variability in the chemical composition of the mineral phase of cements from several tube-building polychaetes[☆]

Variabilité inter- et intraspécifique de la composition chimique de la phase minérale des ciments utilisés dans la construction du tube de plusieurs polychètes bioconstructeurs

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Abstract

Several species of marine polychaetes reside in individual protective tubes. These tubes may be agglomerated in patches of varied sizes with very high densities, or form massive reef-like mounds which can be stretch over several kilometers. These polychaetes can thus be considered as the most important building organisms after corals in coastal environments. We especially focused on several species belonging to the families of Sabellariidae, Terrebiliidae and Pectinariidae. Tubes grains are glued together with biomineralized cement secreted from a building organ connected to specialized glands. Different methods of micro-analysis were used to analyze the biomineral components of these cements for four varyingly gregarious tube-building polychaetes: *Lanice conchilega*, *Pectinaria koreni*, *Sabellaria alveolata* and *Phragmatopoma caudata*. The aim of this study is to describe and compare the inter- and the intraspecific variability in the main biomineral components of cement at local, regional and continental scales. Scanning Electron Microscopy confirms an identical structure of the cement within species of Sabellariidae in Europe and America. Electron Probe Micro-Analysis (EPMA) confirms the presence of calcium, phosphorus, and magnesium plus traces of manganese in all cements, with varying concentrations of these elements at different locations for the same species or for different species at the same location. Finally, our dataset is compared with previous studies from literature.

Keywords: Biogeomorphology; Reefs; Polychaetes; Tube-worms; Cement; Micro-analysis

Résumé

Plusieurs espèces de polychètes marins vivent isolées dans des tubes protecteurs. Ces bioconstructions peuvent être non jointives, mais réparties sur de grands espaces, et pouvant atteindre de très fortes densités individuelles ; d'autres construisent des formations récifales massives étendues parfois sur plusieurs kilomètres carrés. Ces polychètes peuvent ainsi être considérés comme les plus importants organismes bioconstructeurs de la zone littorale après les coraux hermatypiques. Les tubes des Sabellariidae, des Terrebiliidae et des Pectinariidae, par exemple, sont élaborés à partir de grains de sables et de fragments de coquilles d'une taille et d'une forme bien particulières, adaptées à l'organe préhensile du ver constructeur. L'organisation des clastes dans les parois des tubes est variable : celle des Sabellariidae ressemble à l'agencement des tuiles d'un toit alors que les fragments de coquilles utilisés par les Terrebiliidae sont assemblés de manière aléatoire. Les grains sont collés à l'aide d'un ciment biominéralisé sécrété par un organe spécialisé connecté aux glandes cémentaires. Différentes méthodes de micro-analyse ont été utilisées pour analyser la composante biominérale de ces ciments pour quatre espèces de polychètes tubicoles : *Lanice conchilega*, *Pectinaria koreni*, *Sabellaria alveolata* et *Phragmatopoma caudata*. L'objectif de cette étude est de décrire et de mesurer la variabilité inter- et intraspécifique des principaux éléments du ciment aux échelles locale, régionale et continentale. La Microscopie Électronique à Balayage montre que la structure du ciment entre les espèces de Sabellariidae en Europe et en Amérique est identique. L'analyse par micro-sonde électronique (EPMA) confirme la présence de calcium, de phosphore et de magnésium, avec des traces de manganèse dans tous les ciments. La teneur de ces

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éléments varie selon l'environnement pour la même espèce et selon les espèces dans un même environnement. Une comparaison des données acquises avec celles de la littérature est présentée.

Mots clés : Biogéomorphologie ; Récifs ; Polychètes ; Vers tubicoles ; Ciments ; Micro-analyse

1. Introduction

Several species of marine polychaetes reside within self-created protective tubes. Some of these worms construct isolated tubes in variously large patches with very high tube densities (Rabaut et al., 2008). Others form massive reef-like mounds which can reach several kilometers in length (Caline et al., 1988; Kirtley, 1994; Naylor and Viles, 2000). These species of polychaetes can be considered as the most important building organisms after corals in coastal environments. We especially focused on several species belonging to the families of Sabellariidae, Terrebiliidae and Pectinariidae. Their tubes are made from sand clasts and bits of calcareous shell fragments of a given size and shape gathered in the water column. The organisation of clasts in the tube walls of Sabellariidae resembles roof tiles whereas those of Terrebiliidae are randomly attached. Grains are glued with a biomineralized cement secreted from a building organ connected to specialized glands (Vovelle, 1965; Truchet and Vovelle, 1977; Vovelle and Grasset, 1990).

For biogeomorphologists, the study of these species of marine worms is of primary importance because annelid reefs and beds that do not produce carbonate may however concentrate carbonate-rich sediments (Fournier et al., 2005, 2007a,b). These colonies actively control the texture and the distribution of intertidal sands because of their ability to trap and selectively concentrate particles of specific mineralogy and grain-size. These bioconstructions provide a spectacular example of modern carbonate-rich reefs or beds living under temperate and tropical climates.

Pectinaria (= *Lagis*) *koreni* (Malmgren, 1866) [Pectinariidae] is an infaunal polychaete widely spread in the Northern European sandflats (Fig. 1(a)). This intertidal species displays a clear preference for fine sediments. Nevertheless, *P. koreni* can also be found in coarser sediment with a median grain size up to 500 μm (Vovelle, 1973). The slightly curved tube (diameter = 0.8 cm, total length up to 8 cm) is constructed with medium-sized sand grains and is buried in the sediment with the worm upside down. This species has an aggregative distribution with patches reaching densities of several hundreds of individuals per square meter. But the gregariousness of this species is relative. This sub-surface deposit-feeder is responsible for important bioturbation of the surrounding sediments (Nichols, 1974; Busch and Loveland, 1975; Dobbs and Scholly, 1986).

Lanice conchilega (Pallas, 1766) [Terrebiliidae] is one of the most common tube-building polychaetes in the Northern European sandflats (Strasser and Pieloth, 2001; Reise, 2002; Fig. 1(b)). This amphiboreal species ranges from intertidal to subtidal (up to 100 m depth) environments and inhabits fine to

coarse sediments (Rabaut et al., 2007). Populations are mainly concentrated below the low water neap tide level. This tube-builder has an aggregative distribution with patches reaching densities of several thousands of individuals per square meter (up to 5000 ind. m^{-2} in the bay of Mont-Saint-Michel; Godet et al., 2008, 2009; Fig. 1(c)). The *L. conchilega* tube (diameter = 0.5 cm, total length up to 65 cm) is prolonged by sand-fringes which protrude from the sediment and impact the local hydrosedimentary environment (Toupoint et al., 2008). Above a threshold density, current velocities decrease within clusters, deposition of fine sediment particles is facilitated and mounds (up to 1 m high) are created (Féral, 1989; Zühlke, 2001).

Sabellaria alveolata Linnaeus, 1767 [Sabellariidae] is well known for the building of extended reefs along the shores of Northwestern Europe, from Wales to Morocco. Reefs are found on the lower shore and below on hard substrates. In the bay of Mont-Saint-Michel, colonies form reefs over 1.8 m in thickness (Fig. 1(d, e)). This species can form important colonies by the juxtaposition of their sand-tubes which form dense and solid mounds of various shapes and sizes (Dubois et al., 2002, 2003). The average grain-size increases toward the distal end of the tube. By showing preference for shell fragments, *S. alveolata* reinforce the rigidity of their tubes at a reduced cost since the bioclastic grains have a large surface area per unit of mass. This species uses a large spectrum of sediment class sizes and actively controls the texture of the surrounding sediments.

Phragmatopoma caudata (Krøyer) Mörch, 1863 [Sabellariidae] constructs massive reef-like mounds in one major form such as ball-shaped structures over 0.5 m thick (Kirtley and Tanner, 1968; Fig. 1(f, g)). This species is common on rocky platforms and uses a large spectrum of sediment class sizes (Main and Nelson, 1988). *P. caudata* reefs are found over a wide area of the western Atlantic coastline from Florida (USA) to the Santa Catarina State in Brazil. Reefs are located in high-energy environments. Factors which control the abundance and distribution of the reefs of Sabellariidae include requirements such as the availability of a stable substrate (rocky platforms, beach-rocks), appropriate levels of wave- and current-induced turbulence and the availability of sediment with well-defined class-sizes and shape.

The nature of the sand masonry of several marine polychaetes is now well known. *Phragmatopoma* spp. cement consists of proteinic substances (glycine, serine, alanine) that bear similarities to sericin, the silk protein (Waite, 1987; Jensen and Morse, 1988; Waite et al., 1992; Zhao et al., 2005), and significant levels of phosphorus, calcium and magnesium (Gruet et al., 1987). This proteinaceous cement has a microporous foam structure and seems to be made up of hollow spheroids in a matrix (Stewart et al., 2004). It is

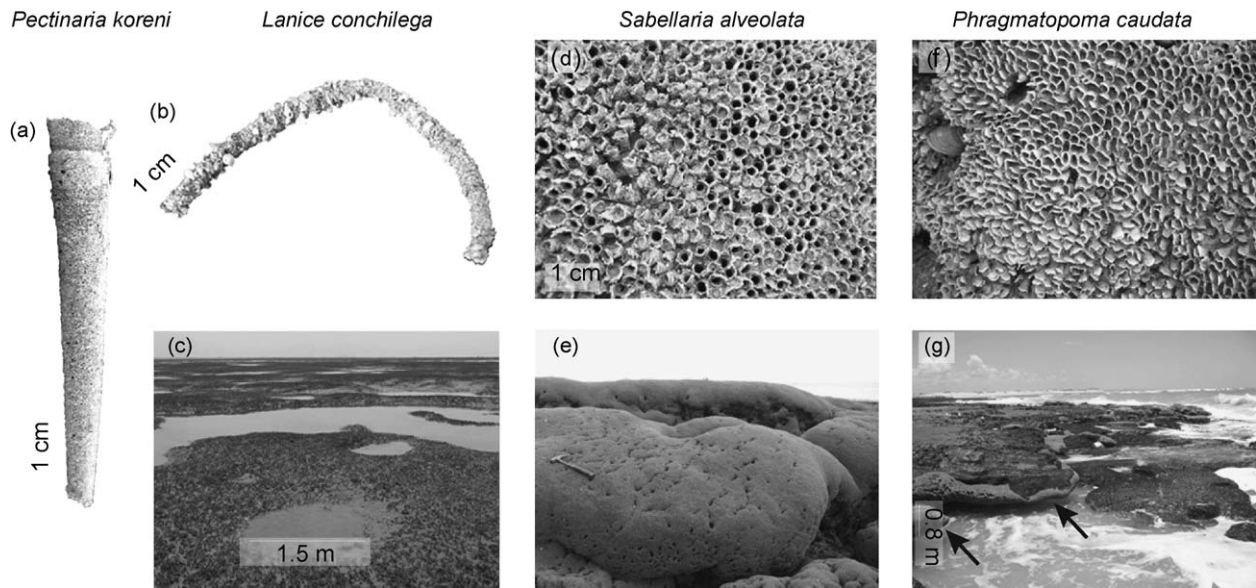


Fig. 1. Photographs of tubes and reefs. (a) Close-up of one tube of *Pectinaria koreni* sampled in the Bay of Le Croisic, France; (b) Close-up of one tube of *Lanice conchilega* sampled in the Bay of Mont-Saint-Michel, France; (c) General view of a typical bed of *L. conchilega* with thousands individuals per square meter taken in the central part of the Bay of Mont-Saint-Michel; Close-up of the tubes of *Sabellaria alveolata* (d) and reef (e) taken in Champeaux, eastern part of the Bay of Mont-Saint-Michel; Close-up of the tubes of *Phragmatopoma caudata* (f) taken in Mel Island, State of Paraná, Brazil, and reef (g, arrows) taken in Tibau do Sul, State of Rio Grande do Norte, Brazil.

produced by two types of large unicellular glands, situated in the thorax of the worm, containing homogeneous or heterogeneous secretory granules (Vovelle, 1965). Homogeneous material is used to form the matrix whereas spheroids derive directly from inclusion of the heterogeneous granules (Flammang and Lambert, 2008). Differential pH between secretory glands and seawater might explain the drying out reaction mechanism (coacervation model) involving, first, a rapid (30 s) change in the nature of the bonding of divalent cations with protein Pc3, and then, a second reaction characterized by a slower hardening of the cement (Zhao et al., 2005; Stevens et al., 2007). Stability of the cement is achieved, for *S. alveolata*, by internal quinone tanning (Gruet et al., 1987), but calcium or magnesium depletion can affect the structural and mechanical properties of the cement by lowering pull-out forces and tube compressive strength (Sun et al., 2007). Most studies undertaken on these cements have been mono-specific and examined their nature and properties; comparative works between these tube-building species have never been undertaken.

The aim of this study is to compare the inter- and intraspecific variability in the main biomineral components of cement at local, regional or continental scales by testing the following hypotheses:

- Are the components of the cement the same between isolated tube-building (*P. koreni*, *L. conchilega*) and reef-building (*S. alveolata*, *P. caudata*) species and between scattered (*P. koreni*) and gregarious species (*L. conchilega*, *S. alveolata* and *P. caudata*)?
- Are the concentrations of the pre-identified elements the same at different locations for the same species and for different species at the same location?

2. Materials and methods

2.1. Study sites and sampling method

The study was carried out both in the Bay of Mont-Saint-Michel (Southwestern part of the Channel, France), whose main characteristic is the presence of well developed reefs of *S. alveolata* and beds of *L. conchilega* and along the whole coast of Brazil from Ceará State (3°S) in the North to Santa Catarina State (28°S) in the South for *P. caudata* reefs.

Several tubes of *L. conchilega* were collected individually in the main patch located in the central part of the bay of Mont-Saint-Michel (Table 1). Tubes of *P. koreni* were collected individually in the eastern part of the bay of Le Croisic, located on the southern coast of Brittany, because this species is very rare in the bay of Mont-Saint-Michel and resides only in the subtidal area. Tubes of *S. alveolata* were collected in Champeaux reef located in the eastern part of the bay of Mont-Saint-Michel, in Ste-Anne reef located in the central part of the bay of Mont-Saint-Michel and in Planguenoual reef located in the eastern part of the bay of St-Brieuc (Fig. 2).

Samples of *P. caudata* were collected from seven sites in Brazil (Florianópolis, Santa Catarina [SC]; Ilha do Mel, Paraná [PR]; Ubatuba, São Paulo [SP]; Trindade, Rio de Janeiro [RJ]; João Pessoa, Paraíba [PB]; Tibau do Sul, Rio Grande do Norte [RN] and Fortaleza, Ceará [CE]; Fig. 3). When they were present, worms were removed from the tubes. Individual tubes or reef fragments (for Sabellariids species) were washed carefully with distilled water and then stored into individual hermetic plastic bags. Samples were air-dried before analysis (Gruet et al., 1987; Stewart et al., 2004).

Table 1

Location of sampled reefs.

Species	Location	Geographical coordinates	Sampling date
<i>L. conchilega</i>	France, Mont-St-Michel, central part	48°39'N – 1°38'W	August 2006
<i>S. alveolata</i>	France, Mont-St-Michel, Champeaux	48°43'N – 1°33'W	September 2006
	France, Mont-St-Michel, Ste-Anne reef	48°39'N – 1°39'W	February 2007
	France, St-Brieuc, Planguenoual reef	48°33'N – 2°36'W	February 2007
<i>P. koreni</i>	France, Le Croisic	47°17'N – 2°29'W	September 2006
<i>P. caudata</i>	Brazil, Florianópolis, Santa Catarina	27°48'S – 48°32'W	October 2005
	Ilha do Mel, Paraná	25°34'S – 48°18'W	November 2006
	Ubatuba, São Paulo	23°22'S – 44°50'W	November 2006
	Trindade, Rio de Janeiro	23°21'S – 44°43'W	November 2006
	João Pessoa, Paraíba	7°08'S – 34°47'W	February 2006
	Tibau do Sul, Rio Grande do Norte	6°10'S – 35°05'W	February 2006
	Fortaleza, Ceará	3°48'S – 38°24'W	March 2006

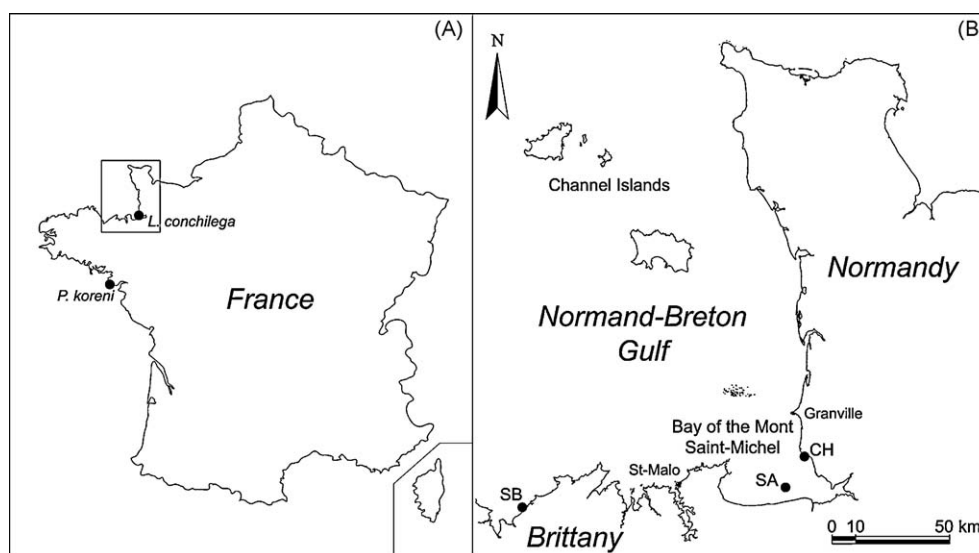


Fig. 2. Localisation map of the samples of *L. conchilega* (Bay of Mont-Saint-Michel) and *P. koreni* (Le Croisic) (A) and of *S. alveolata* (B). CH: Reef of Champeaux (Manche); SA: Reef of Sainte-Anne (Ille-et-Vilaine); SB: Reef of Planguenoual (Côtes-d'Armor).

2.2. Procedures for Electron Probe Micro-Analysis (EPMA)

Analyses were made on polychaetes tube slices impregnated with Epofix, a cold-setting resin based on two fluid epoxy components. It is specially developed for impregnation of porous specimens like *Sabellaria* or *Phragmatopoma* tubes. Owing to its low viscosity, Epofix penetrates into all porosities and cracks of the tubes. In order to facilitate penetration and degassing, impregnated samples were maintained in a vacuum chamber during the solidification phase. Epofix blocks were sectioned into two pieces using a STRUERS Minitom automatic precision cut-off machine. Lower pieces were conserved and manually polished using 1200 µm to 200 µm silicon carbide paper. After manual polishing, samples have been cleaned using a FISHER Bioblock Scientific ultrasonic cleaning machine. Mechanical polishing has been performed with a PRESI Mecapol P-200 polishing machine using three PRESI Mecaprex polishing solutions in succession (6 µm, 3 µm and 0.25 µm). Samples were then coated with carbon and

cement composition was analysed using a CAMECA SX100 Electron Probe Micro-Analyser. Calcium (Ca), Phosphorus (P), and Magnesium (Mg) contents were measured (calibration: calcite, apatite, and dolomite, respectively) according to Gruet et al. (1987), Stewart et al. (2004) and Sun et al. (2007). Focused electron beam width was 8 nm. After the mounting procedure, 19 samples consisting of several leveled tubes (at least 10 tubes per sample) were analyzed. Each species was represented by 3 samples, except *P. caudata* for which 10 samples were processed, allowing intraspecific analysis. Eight to 15 cement sites have been selected per sample and each site was analyzed 4 times (60 seconds per counting).

2.3. Procedures for Scanning Electron Microscopy Analysis

Both rough and impregnated samples were observed under a JEOL-JSM 5810LV scanning electron microscope. Rough samples were air-dried for several weeks, mounted on an aluminium plate and coated with carbon. Observations were



Fig. 3. Localisation map of the samples of *P. caudata*.

restricted to secondary electron imagery which allows relief characterization of rough samples. Indeed, SEI allows the observation of the external structure of tubes in three dimensions. Sliced samples previously analyzed with EPMA were observed under back-scattered electron mode. Image resolution is higher under SEM than EPMA, thus allowing characterization of the internal structure of the tube and the observation of grain/cement relationships (Truchet and Vovelle, 1977).

2.4. Data analysis

Differences between element contents of the cement were tested using one-way ANOVA. The assumptions of normality and homoscedasticity were verified by direct observation of residuals, Kolmogorov-Smirnov tests, Lilliefors tests and Levene tests or Brown-Forsythe tests, respectively, with Statistica v.6.1 software. In multivariate analysis based on quantitative data, transformations are used to weight the relative contribution of each element contents (Zar, 1999). As a result, data were square-root transformed before using one-way ANOVA (Field et al., 1982). Finally, post-hoc comparisons were performed through HSD Tukey tests (Scherrer, 1984). To compare cement component variability, we followed the methods of Clarke and Warwick (1994) and used the Plymouth Routines in Multivariate Ecological Research (PRIMER) version v6.1.6 software (Clarke and Gorley, 2006). Non-metric

multidimensional scaling ordinations (nMDS) and analysis of similarity (one-way ANOSIM) were performed after computation of a Bray-Curtis similarity matrix (Legendre and Legendre, 1998). The nMDS technique takes a distance matrix and plots all the samples in the configuration that best satisfies all the rank constraints of the matrix. Therefore, samples with the greatest similarity will be plotted closest together. Stress values related to nMDS plots indicate the goodness of representation of dissimilarities between samples. A stress value < 0.1 corresponds to an excellent representation of the data with little chance of a misleading diagram. Stress value between 0.1 and 0.3 still provide a potentially useful picture, although the detail of the plot cannot be considered truly accurate. Moreover, the similarities within and between the cement of species were identified by the SIMPER subroutine of the PRIMER software.

3. Results

3.1. Interspecific variability

Differences for Ca, P and Mg concentrations between samples of each species were tested. Fig. 4 shows the mean of each element and 95% confidence intervals for all studied species. No significant differences were found for samples of *L. conchilega* and *S. alveolata*. Significant differences appear only for Mg content of *P. koreni* ($F_{(2,18)} = 6.384$; $p < 0.01$) and *P. caudata* ($F_{(2,22)} = 5.907$; $p < 0.01$) cements.

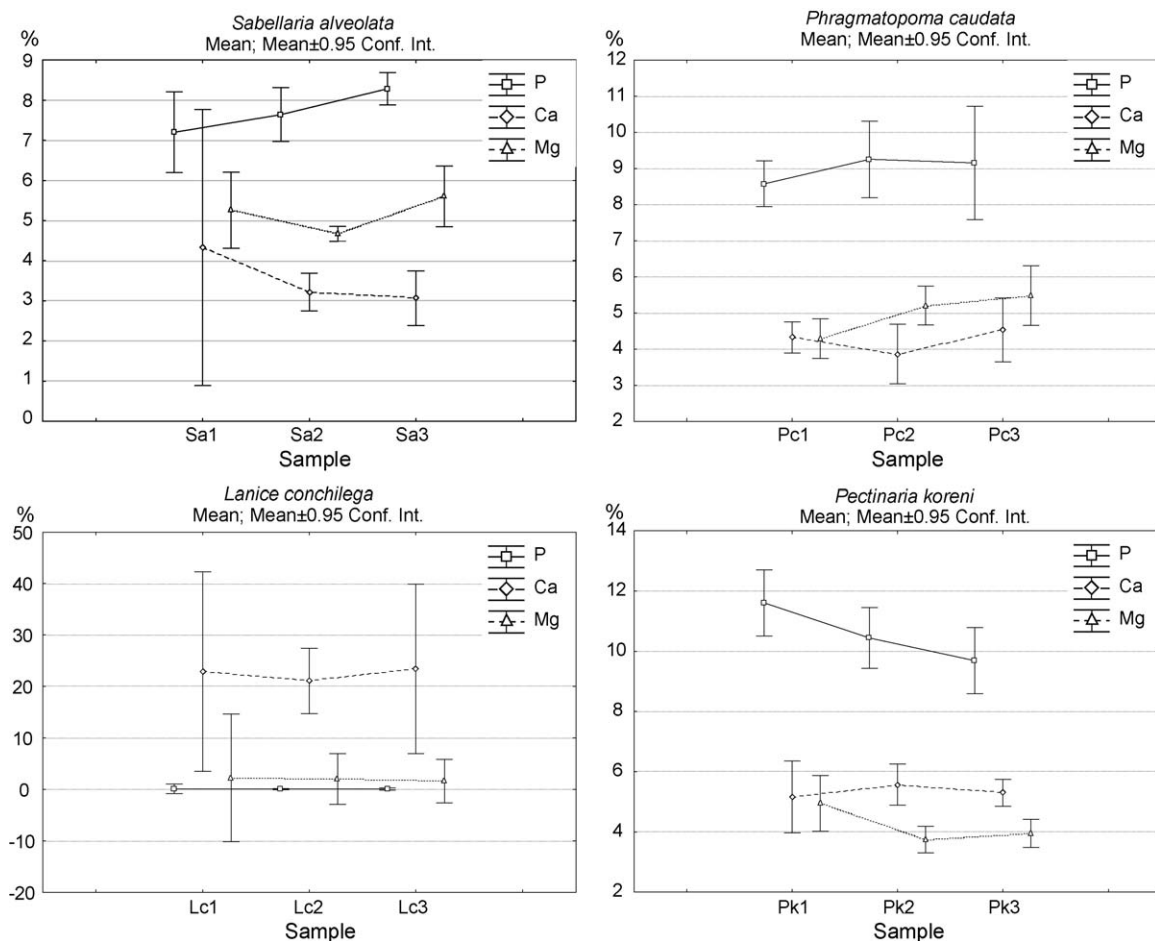


Fig. 4. Means and 95% confidence intervals of P, Ca and Mg contents for the cement of *S. alveolata*, *P. caudata*, *L. conchilega* and *P. koreni*.

Correlations between elements were performed and showed a significant relation between P and Mg for *P. koreni* ($r = 0.764$; $p < 0.001$), *P. caudata* ($r = 0.699$; $p < 0.001$) and *S. alveolata* ($r = 0.716$; $p < 0.005$). A negative correlation was found between Ca and Mg ($r = -0.677$; $p < 0.01$) and between Ca and P for *S. alveolata* ($r = -0.731$; $p < 0.005$) (Clark, 1965). On the other hand, a positive correlation was found between Ca and P for *P. caudata* ($r = 0.490$; $p < 0.05$). The SIMPER analysis shows that the average similarity of the cement for each species is high (88.74% for *P. caudata*; 91.78% for *S. alveolata*; 91.57% for *P. koreni* and 94.00% for *L. conchilega*).

Comparisons between species show that the concentration of elements are of high significant difference (Ca: $F_{(3,61)} = 373.550$ $p < 0.001$; Mg: $F_{(3,61)} = 28.039$ $p < 0.001$; P: $F_{(3,61)} = 394.780$ $p < 0.001$). Post-hoc tests do not reveal any difference between *S. alveolata* and *P. caudata* for Ca and Mg content, but a significant difference for P appears between both species (HSD Tukey test $p < 0.01$). The concentration of elements of the cement between *L. conchilega* and *P. koreni* is of high significant difference (Ca and Mg: HSD Tukey test $p < 0.001$; P: HSD Tukey test $p < 0.001$) as well as for the two species of Sabellariidae (Ca and Mg: HSD Tukey test $p < 0.05$; P: HSD Tukey test $p < 0.01$). The SIMPER analysis confirms that the average dissimilarity is low between the two species of

Sabellariidae (11.00%) and can be attributed mainly to the P content (total contribution = 40.58%). The average dissimilarity between the two tube-building species, *L. conchilega* and *P. koreni*, is very high (66.59%) and can be attributed mainly to the Ca content (total contribution = 57.88%). The highest average dissimilarity was found between the two reef-building species (*P. caudata*, *S. alveolata*) and *L. conchilega* (71.93% and 74.76%, respectively) and can be attributed to the Ca content (total contribution = 63.33% and 65.13%, respectively). The average dissimilarity between the two species of Sabellariidae and *P. koreni* was low (*P. caudata* 14.26%, *S. alveolata* 18.13%) and can be attributed to the P content (45.85% and 44.64%, respectively).

The dendrogram and the nMDS plot of the hierarchical cluster analysis reveal two groups isolated at a similarity level of ca. 74% (Fig. 5). At a 74% similarity threshold, *L. conchilega* and the three other species are separated. ANOSIM pairwise tests show that both species of Sabellariidae are not significantly different (ANOSIM $p = 12.4\%$; $R = 0.074$).

Others pairwise tests, illustrated by nMDS plot (Fig. 6), show significant differences between *P. caudata* and *P. koreni* (ANOSIM $p = 0.01\%$; $R = 0.320$) and between *S. alveolata* and *P. koreni* (ANOSIM $p = 0.01\%$; $R = 0.894$, fourth-root transformed data). The differences highlighted can be explained both by interspecific differences and differences in

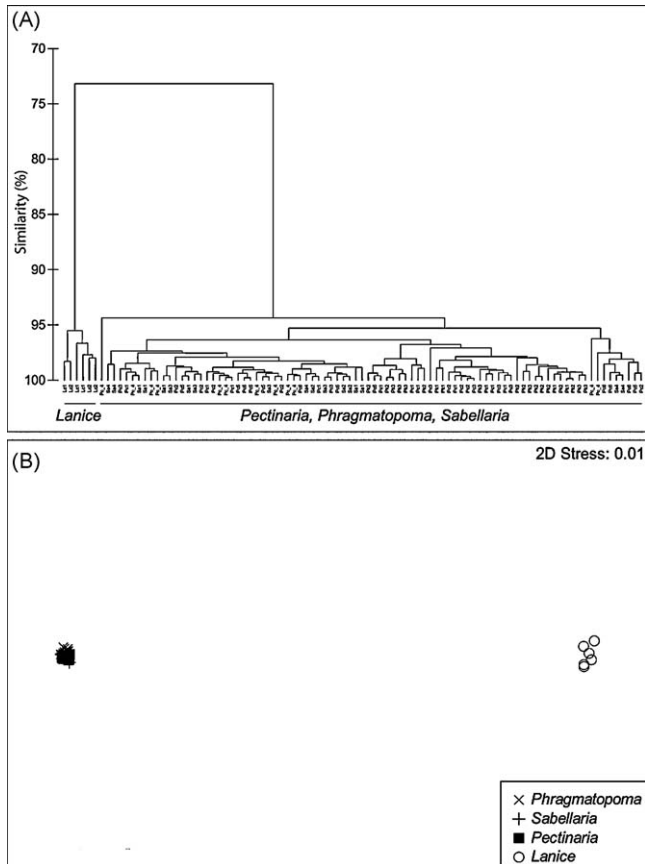


Fig. 5. Dendrogram of the hierarchical cluster analysis (fourth root-transformed) obtained from the P, Ca and Mg contents of the cement for the four species (A) and corresponding non-metric multidimensional scaling (nMDS) ordination plot based on the Bray-Curtis similarity (B).

environmental conditions. Complementary analysis will be necessary to determine it.

3.2. Intraspecific variability

Intraspecific variability has been tested for both species of Sabellariidae: *S. alveolata* in three stations located in the Bay of Saint-Brieuc and in the Bay of Mont-Saint-Michel, and *P. caudata* in seven stations along the Brazilian coast from Ceará State in the North to Santa Catarina State in the South. Comparisons between stations for *S. alveolata* show that the concentration of elements are significantly different for P ($F_{(2,16)} = 3.864$; $p < 0.05$) and Mg ($F_{(2,16)} = 16.501$; $p < 0.001$). Post-hoc tests do not reveal any difference between stations for P ($p > 0.05$). A significant difference for Mg has been found between the station of the Bay of Saint-Brieuc (Planguenoual [SB]) and the two others from the Bay of Mont-Saint-Michel (Sainte-Anne reef [SA] $p < 0.05$; Champeaux reef [CH] $p < 0.001$). However, the SIMPER analysis shows that the average dissimilarity is very low between the stations (2.36% for CH and SA; 2.96% for CH and SB and 2.45% for SA and SB) and can be attributed mainly to the Mg content (total contribution = 41.75% for CH and SA; 64.21% for CH and SB; 42.27% for SA and SB). ANOSIM pairwise tests, illustrated by

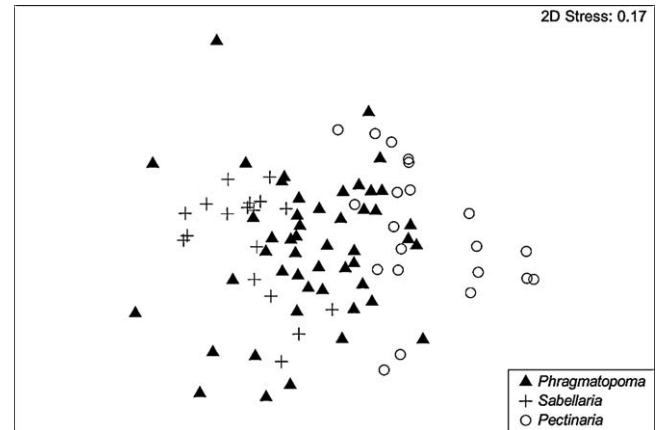


Fig. 6. Non-metric multidimensional scaling (nMDS) ordination plot based on the Bray-Curtis similarity (fourth root-transformed data) showing the interspecific differences of the cements between *P. caudata*, *S. alveolata* and *P. koreni*.

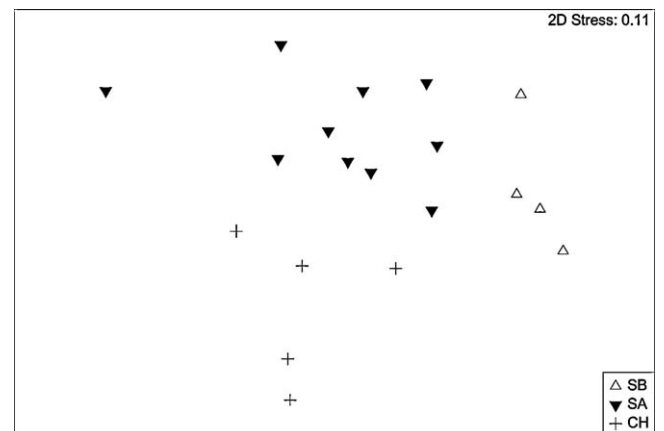


Fig. 7. Non-metric multidimensional scaling (nMDS) ordination plot based on the Bray-Curtis similarity (fourth root-transformed data) showing the intraspecific differences of the cement of *S. alveolata* between stations of the Bay of Saint-Brieuc and the Bay of Mont-Saint-Michel. CH: Reef of Champeaux (Manche); SA: Reef of Sainte-Anne (Ille-et-Vilaine); SB: Reef of Planguenoual (Côtes-d'Armor).

nMDS plot (Fig. 7), show significant differences between CH and SA (ANOSIM $p = 0.2\%$; $R = 0.557$) and between SA and SB (ANOSIM $p = 0.2\%$; $R = 0.573$).

The composition of the cement between stations for *P. caudata* along the whole Brazilian coast are of a high significant difference for each studied element: P ($F_{(6,42)} = 22.532$; $p < 0.001$); Mg ($F_{(6,42)} = 19.320$; $p < 0.001$); and Ca ($F_{(6,42)} = 9.416$; $p < 0.001$). Post-hoc tests show that two main groups are significantly different and can be separated for P ($p < 0.05$) and Mg ($p < 0.05$). The first one clusters stations from the Southern coast of Brazil (SC, PR, SP and RJ) and the second one stations from the Northern coast of Brazil (RN and CE). The State of Paraíba (PB) seems to be in an intermediate position between the two groups. For Ca, post-hoc tests were computed and showed significant differences between three groups ($p < 0.05$): SC and PR (Southern coast of Brazil), SP, RJ and PB (Central part of Brazilian coast), and RN and CE

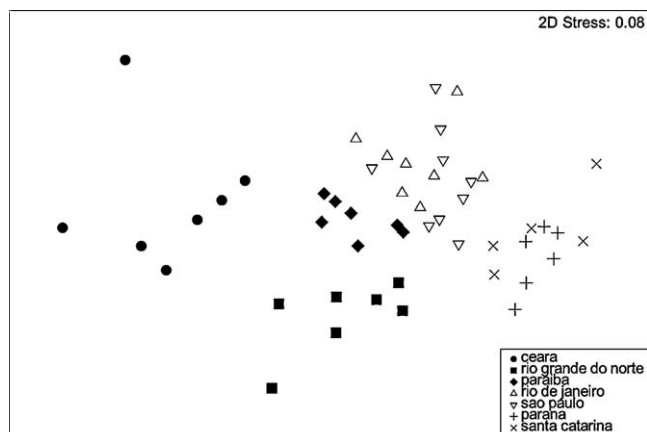


Fig. 8. Non-metric multidimensional scaling (nMDS) ordination plot based on the Bray-Curtis similarity (fourth root-transformed data) showing the intra-specific differences of the cement of *P. caudata* between stations along the whole coast of Brazil, from the State of Ceará in the north to the State of Santa Catarina in the south.

(Northern coast of Brazil). The SIMPER analysis shows that the average dissimilarity between stations from the extreme South (SC, PR) and North (RN, CE) (5.00%) can be attributed to the P content (total contribution = 36.92%) and Mg content (total contribution = 36.92%). The average dissimilarity between stations from the Central and Southern regions is lower (2.66%; Ca total contribution = 51.20%) than between stations from the Central and Northern regions (3.50%; Mg total contribution = 44.28%). The best average dissimilarity was obtained with SC vs. CE (6.58%; Mg total contribution = 36.94%) and PR vs. CE (6.60%; P total contribution = 34.05%). ANOSIM pairwise tests, illustrated by nMDS plot (Fig. 8), show significant differences between each region: North vs South (ANOSIM $p = 0.01\%$; $R = 0.745$); Central vs South (ANOSIM $p = 0.01\%$; $R = 0.749$); Central vs. North (ANOSIM $p = 0.01\%$; $R = 0.685$). The best R was obtained with SC/SP/RJ vs. CE (ANOSIM $p = 0.1\%$; $R = 0.994$, $p = 0.04\%$; $R = 0.947$, $p = 0.02\%$; $R = 0.907$, respectively) and SC vs. PB (ANOSIM $p = 0.1\%$; $R = 0.954$).

4. Discussion and conclusion

The study of polychaete cements has encountered a renewed interest in recent years due to their particular mechanical properties (Sun et al., 2007; Shao et al., 2009). Proteinic characteristics of the cement have focused nearly all interests (Jensen and Morse, 1988; Waite et al., 1992; Stewart et al., 2004; Zhao et al., 2005; Stevens et al., 2007), whereas its mineral phase has been largely neglected (Gruet et al., 1987; Sun et al., 2007). Cement generated by tube-building species is essential to understand biogeomorphological processes in Sabellariidae reefs or Terrebiliidae beds (Murray et al., 2002). Resistance and flexibility are required to allow these bioconstructions to manage varying hydrodynamical stress. Sun et al. (2007) have demonstrated that Ca and Mg play a premium role in the resistance of the cement to physical stress. This study shows that the mineral phase of the cement of the

four studied species is mainly composed of P, Ca and Mg; all elements being strongly correlated. Mn and Fe are additional minor elements encountered in the cement whose presence is common in Ca-Mg concretions of marine invertebrates (Simkiss, 1983). Our results confirm those of Gruet et al. (1987) for *S. alveolata*, Truchet and Vovelle (1977) and Vovelle and Grasset (1990) for *P. koreni*, and Lafon (1959) for *L. conchilega*. *Phragmatopoma caudata* cement has never been studied before, whereas *Phragmatopoma californica* has been studied by Stewart et al. (2004), Sun et al. (2007), and Zhao et al. (2005). Our results show that the cement of both species is very close in term of mineral composition and physical structure.

Cements have a foam-like structure with micrometric voids (Fig. 9). SEM images also reveal that *P. koreni* cement (this study and Vovelle and Grasset, 1990) is similar to that produced by reef-building species such as those of the Sabellariidae family: *S. alveolata* (this study and Gruet et al., 1987), *P. caudata* (this study) and *P. californica* (see pictures in Stewart et al., 2004; Stevens et al., 2007). The same mineral phase (P, Ca, Mg) is encountered in these tube-building polychaetes, but proportions of the three studied elements vary greatly between species. Different analytical procedures of the samples were used in the studies, so quantitative data could not be compared here. Statistical tests show that both reef-building sabellariid species (*S. alveolata* and *P. caudata*) cannot be differentiated from other tube-building engineer species, i.e. *L. conchilega* and *P. koreni*, the latter having a less-pronounced influence on surrounding sediment features. Hence, the chemical signature of the organic cement does not typify the studied tube-building species.

Considering inter-specific variations, *L. conchilega* cement differs significantly from the three other cements for the three elements analysed. The cement of the three other species is very similar, although P content variation is statistically significant to discriminate the three species. Considering intra-specific variations, we have tested the mineral phase of the cement as a discriminating element between isolated populations. Statistical analyses show that significant differences exist between studied populations, even though these differences are weak.

On a regional scale, it has been possible to distinguish *S. alveolata* populations from Saint-Brieuc Bay from those of the Mont-Saint-Michel Bay, isolation being based mainly on Mg content, secondly on P content. Considering the present knowledge, it is not possible to know if the environment (e.g., physical and chemical composition of seawater) is responsible for the differences recorded, although it has been demonstrated recently that, on the genetic scale, both populations belong to the same meta-population (Farcy, 2003; Rigal, 2005).

On a continental scale, it has been possible to separate *P. caudata* populations distributed along the Brazilian coast. P and Mg contents are significant variables, allowing a discrimination between southern populations (Santa Catarina, Paraná, São Paulo and Rio de Janeiro States) and northern populations (Rio Grande do Norte and Ceará States). The population of Paraíba State has an intermediate position between both groups. Ca, although less distinctive, reinforces the three-fold separation. The first group gathers southern populations (Santa Catarina

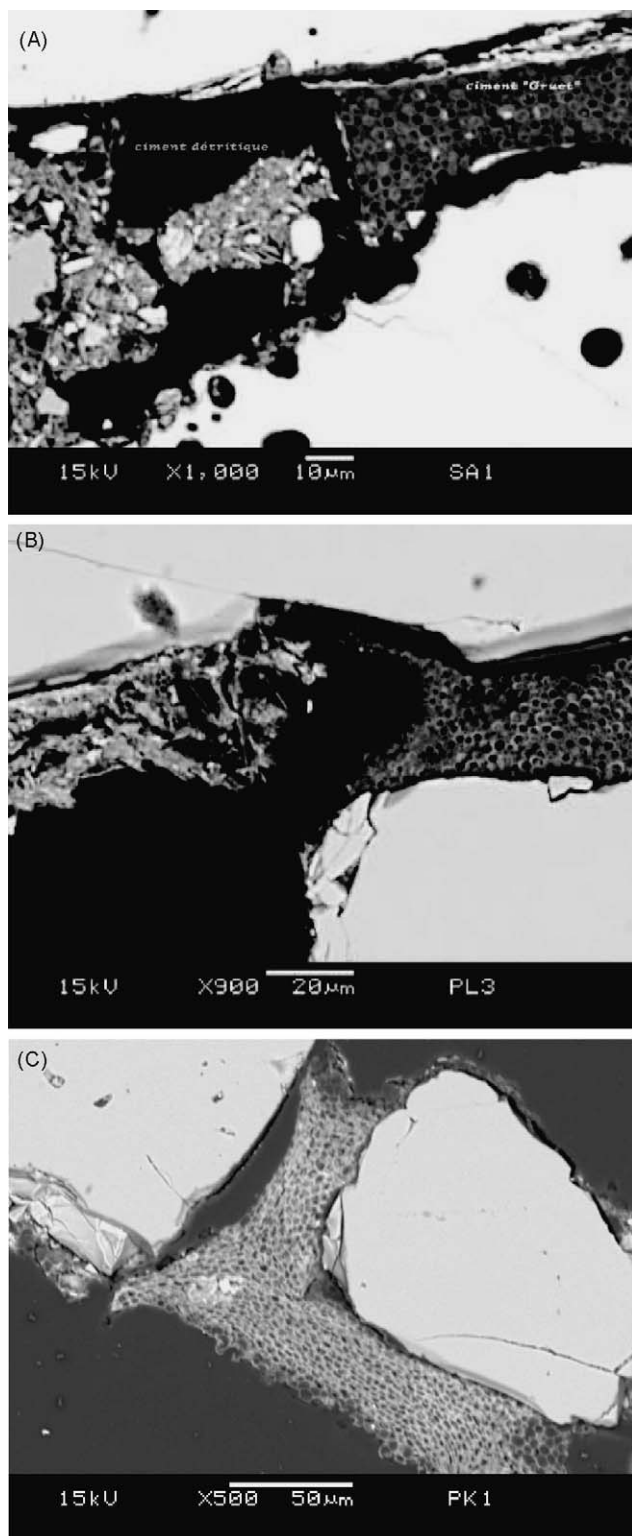


Fig. 9. Representative SEM micrographs of the structure of the cements of *Sabellaria alveolata* (A), *Phragmatopoma caudata* (B) and *Pectinaria koreni* (C) (back-scattered electron mode).

and Paraná), the second one São Paulo, Rio de Janeiro and Paraíba populations, and the third one northern population (Rio Grande do Norte and Ceará). Thus P, Ca and Mg content variations in the mineral phase of the cement enables the identification of different populations of *P. caudata*. This result

is not in accordance with genetic analysis which shows that all specimens belong to the same population (Drake et al., 2007). Once again, the importance of environmental variations in the nature of the cement has not been well studied yet and might be a premium factor. It should be considered in the future, as polychaete cement may appear as an environmental marker, which potentially could be very useful for palaeo-environmental reconstruction. The discovery of Miocene sabellarian reefs (Garcin and Vachard, 1987) and Oligocene reef-building polychaete worm (Fisher et al., 1989) provide the first unequivocal examples of fossil terrigenous annelid reefs. The scarcity of fossil sabellarian reefs contrasts with the abundance of gregarious tube-like trace fossils, commonly considered as worm burrows (e.g., *Sabellarifex*, *Skolithos*, *Terebellina*, *Tigillites*), that occur in Palaeozoic sandstones. This is partly due to the poor preservation potential of these fragile sandy-reefs but mainly reflects the limited attention given to recognising biosedimentological features in shallow water marine sands and sandstones.

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