

## Organic matter quantity and quality, metals availability and foraminiferal assemblages as environmental proxy applied to the Bizerte Lagoon (Tunisia)



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### ABSTRACT

This study analyzes the benthic trophic state of Bizerte Lagoon (Tunisia) based on the total organic matter and the bioavailability of biopolymeric carbon including proteins (PTN), carbohydrates (CHO), lipids (LIP), chlorophyll *a*, as well as bacteria counts. The overall simultaneously extracted metals (SEM), and acid volatile sulfides (AVS) as well as the SEM/AVS ratio indicative of the toxicity of the sediments also were analyzed aiming to study their impact in the dimension, composition and structure of both dead and living benthic foraminiferal assemblages.

In the studied sites TOC content is relatively high and the PTN/CHO values indicate that they can be considered as meso-eutrophic environments. The CHO/TOC and C/N values suggest that the OM which accumulated on the sediments surface has mainly natural origin despite the introduction of municipal and industrial effluents in the lagoon and the large bacterial pool.

The living assemblages of benthic foraminifera of Bizerte Lagoon are quite different to other Mediterranean transitional systems studied until now. They are composed of typical lagoonal species but also include several marine and opportunistic species including significant numbers of bolivinids, buliminids, *Nonionella/Nonionoides* spp. and *Cassidulina/Globocassidulina* spp. These assemblages seem to benefit from the physicochemical parameters and the sediment stability. They may however face environmental stress in the lagoon related to the AVS production as a result of the organic matter degradation and toxicity in some areas due to the available concentrations of metals. Nonetheless statistical results evidence that the structure and dimension of assemblages are being controlled mostly by OM quantity and quality related mainly to the availability of PTN, CHO and chlorophyll *a*. Results of this work support the importance of considering OM quantity and quality in studies of environmental impact in coastal systems.

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### 1. Introduction

Humans have always relied on the oceans and their resources, first as a food source (fishing), later for transportation of raw materials. Today, our oceans are also exploited for mineral, gas, oil, and other natural resources of great economic importance. It is noticeable that 50% of the world population lives in coastal areas or nearby regions (Sherbinin et al., 2007). Such a high population density along the coastal areas results in high environmental stress due to the multiple activities that take place there (Agardy et al., 2005). The discharge of municipal and agriculture effluents, for instance, may result in functional changes of coastal environments due to the entry of excessive nutrients that

might stimulate the primary production and influencing food chain thereof (Voss et al., 2011). However, when these inputs exceed the limit of the environmental consumption capacity, a bloom of phytoplankton might occur and lead to dissolved oxygen (DO) depletion and an alteration of the ecosystems structure (Diaz, 2001). In coastal environments, the contents of contaminants and organic matter in sediment, which might be capable of damaging biota, are mainly ascribed to human activities (Karlson et al., 2007; Diaz and Rosenberg, 2008). Rivers, estuaries, and marine waters have mainly served for final repositories of anthropic waste both organic and inorganic (Ludwig and Gould, 1988; Islam and Tanaka, 2004) and where the sediment represents the final sink (Long et al., 1995; Goher et al., 2014). In order to assess the environmental quality, physicochemical parameters of waters and the sediment characteristics are commonly considered (Barbour et al., 1999). More recently, the scientific community has emphasized the importance of biological organisms (biological indicators commonly referred as bioindicators) in the assessment of the environmental quality (Cairns and Pratt, 1993; Walley and Hawkes, 1997).

Benthic foraminifera are an important component of oceanic systems and may even account for 50% of eukaryotic biomass (Gooday et al., 1992). These organisms are excellent indicators for assessing the exported productivity on the ocean floor (van der Zwaan et al., 1999) and for cycling the organic matter (Gooday et al., 1992). They are also widely used in marine environments, mainly transitional and coastal marine settings, as bioindicators of environmental quality (i.e., Alve, 1995; Arminot du Châtelet and Debenay, 2010; Frontalini and Coccioni, 2011; Martins et al., 2013).

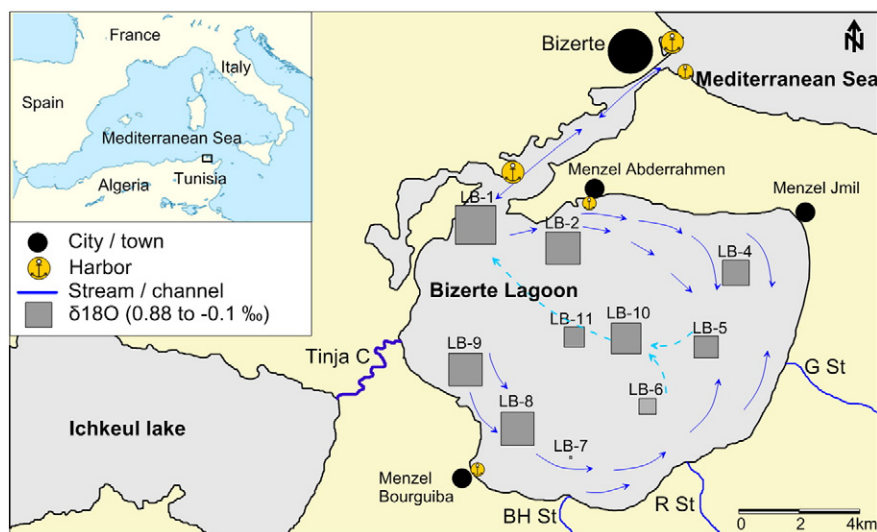
The distribution, abundance and diversity of benthic foraminifera are mostly controlled by physicochemical properties of water and sediment (Sen Gupta, 1999). These parameters encompassing salinity, pH, currents, temperature, DO in water and sediment, type of substrate, and organic carbon content represent the main factors regulating the foraminifera distribution (Murray, 2006). More recently, the quality of the organic matter has been inferred to play an important role in determining the distribution of benthic foraminiferal assemblages in both open and marginal settings (i.e., Schönfeld, 2001; Fontanier et al., 2002; Clemente et al., 2014; Martins et al., 2015a, 2015b). In light of it, the characterization of the sources (marine vs. continental), the quantity and quality of the organic matter is considered crucial for benthic foraminifera ecological inference, particularly in the highly dynamic settings like transitional marine environments.

The living assemblages of benthic foraminifera, several physicochemical parameters and the sediments characteristics, namely available metals and TOC content, have been analyzed in Bizerte Lagoon to assess the environmental quality of bottom sediment in this coastal system (Martins et al., 2015b). The main objectives of this work are therefore to unravel if also changes in organic matter quality, considering its enrichment in biopolymers, are reflected both in living and dead foraminifera assemblages' variations in the Bizerte Lagoon.

### 1.1. Study area

The Bizerte Lagoon located northeast of Tunisia, (37°08'–37°17'N/9°46'–9°55'E) is a semi-closed ecosystem in association with the central Mediterranean Sea (Fig. 1). The lagoon has a surface area of 121.6 km<sup>2</sup> and average depth of 8 m. Its northeast extremity communicates with the sea through a 6-km-long channel and with the Ichkeul Lake to the west through Tinja Channel (Fig. 1). The lagoon exchanges with these connections, saline and fresh waters, respectively (Ben Ismail et al., 2012). The lagoon also receives fresh water input from non-permanent watercourses such as Rharek, Ben Hassine, and Guenich streams (Fig. 1). The Bizerte Lagoon has a complex settings formed by the marine zone affected by the navigation system and densely populated areas as for instance along the channel that connects the lagoon with the Mediterranean and at the southwest part and is affected by municipal, agricultural and industrial activities (Fertouna-Bellakhal et al., 2014).

The lagoon hydrodynamics is mainly driven by wind forces that induce seasonal changes (Sakka Hlaili et al., 2008). The strongest tidal currents are located on the northern border of the lagoon and near the inlet (INSTM, 2002), whereas currents are considerably reduced inside the lagoon (Fertouna-Bellakhal et al., 2014). A greater water mixture in the vertical column occurs in winter due to increased rainfall, and the highest input of fresh water from Ichkeul Lake through the Tinja Channel and the other streams (Fertouna-Bellakhal et al., 2014). On the other hand, the influence of saline water in summer is increased and the higher temperatures promote the stratification of the water column (Sakka Hlaili et al., 2003). These seasonal changes influence the nutrient flow along the water column (Sakka Hlaili et al., 2008). Despite the biological abundance of phytoplankton (Sakka Hlaili et al., 2006); metazooplankton (Hamdi et al., 2002), metazoan (Zaabar et al., 2015) and microorganisms (Sakka Hlaili et al., 2008), the Bizerte Lagoon



**Fig. 1.** Location of the study area, in Tunisia (a), Bizerte Lagoon (b). The studied stations in Bizerte Lagoon (LB) are labeled with numbers. The main towns are signed: MA – Menzel Abderrahaman; MJ – Menzel Jmil; MB – Menzel Bourguiba. The main streams are also indicated: G St – Guenich stream; R St – Rharek Stream; BH St – Ben Assine Stream; Tinja C – Tinja Channel which connects the Ichkeul Lake with the lagoon. Arrows represent the main pattern of water circulation (adapted from Fertouna-Bellakhal et al., 2014). Data of *A. parkinsoniana*  $\delta^{18}\text{O}$  are mapped (b).

represents a fragile environment due to its enclosed nature and the long residence time (Harzallah, 2003). In addition, it is influenced by harmful algal blooms (Afli et al., 2008; Sahraoui et al., 2009; Fertouna-Bellakhal et al., 2014, 2015) and to pollutants' inputs (Harzallah, 2003; Barhoumi et al., 2014). Pollutants' inputs are due to municipal effluents coming from the bordering cities (Dellali et al., 2001), from the naval port and the metallurgic factory and several other industries such as iron and steel plant, cement factory and refinery (Essid and Aissa, 2002) and are adsorbed by the sediments (Zaaboub et al., 2015). The aquaculture of the mussel *Mytilus galloprovincialis* and other bivalves such as oysters (Afli et al., 2008) also contributes to the increase of the organic matter flux to the bottom and to the establishment of anoxic conditions (Zaaboub et al., 2015). Anoxic conditions and toxicity caused by metals generate damage benthic organisms (Martins et al., 2015b; Zaaboub et al., 2015).

## 2. Materials and methods

This work is based on the analysis of sediment samples collected in 10 stations in the Bizerte Lagoon on 22 March 2013 (Fig. 1). The  $\delta^{18}\text{O}$  data represented in this figure were obtained in living organisms of *Ammonia parkinsoniana*, according to the methodology described by Martins et al. (2015b). The methodology of sampling, data acquisition related to physicochemical parameters (e.g. DO content in water and sediments), benthic foraminifera and bacterial analysis, including total coliform (TC), fecal enterococci (FE), total mesophilic counts (TMC), as well as granulometric and geochemical data, such as total organic carbon (TOC), acid volatile sulfides (AVS), simultaneously sequential extracted metals (SEM) and chlorophyll *a* (Chl *a*) also are described by these authors.

About 20 living specimens of *A. parkinsoniana* were collected from the sediment fraction 300–350  $\mu\text{m}$  and were used for stable isotope analyses. The analysis ran in the “Kiel IV Carbonate Device” equipment coupled with a “Delta V Plus – Isotope Ratio MS” analyzer (Thermo Scientific). The foraminiferal isotopic values are reported in  $\delta$ -notation versus Vienna Pee Dee Belemnite standard (VPDB). The accuracy of the measurements is controlled by the IAEA-CO-1 (Marble) standard reference, which the standard deviation for  $\delta^{13}\text{C}$  is 0.030 and for  $\delta^{18}\text{O}$  is 0.1.

The new benthic foraminifera data presented in this work were analyzed by coupling but keeping separated living (rose Bengal stained) and dead foraminifera specimens in the same amount of sediment (>125  $\mu\text{m}$ ) to evaluate and compare the foraminifera density (FD, as number of specimens in 10 mg of sample), absolute abundance of specimens per species (no./10 mg), and composition of the living and dead assemblages. The Shannon index ( $H'$ ) was used as a measure of diversity for the living and dead assemblages. Most of the identified foraminifera species were imaged with a digital Scanning Electron Microscope EVO MA10 (Zeiss).

Moreover, the total biopolymer (TBP) concentrations consisting of lipids (LIP), proteins (PTN) and carbohydrates (CHO) it includes. These variables were analyzed in the sediment according to the methodology described by Martins et al. (2015a) and were carried out in the Laboratory of Organic Facies and Palynofacies of the Federal University of Rio de Janeiro (UFRJ). The relative enrichment of biopolymers was evaluated through several parameters: TBP/TOC, CHO/TOC, PTN/CHO, PTN/LIP + CHO, LIP/CHO + PTN, CHO/LIP + PTN and Chl *a*/TOC.

### 2.1. Statistical analyses

An additive logarithmical-transformation  $\log(1 + x)$  of the biotic and abiotic data was carried out before applying the multivariate statistical analysis. A Q-mode cluster analysis (CA) based on the absolute abundance of living and dead foraminifera identified in the study area, considering the Euclidean distances and Ward method for samples agglomeration was applied to evaluate the similarity between the dead

and living assemblages in each station. A Principal Components Analysis (PCA) was also applied to explain the variance of the main species, taxa/groups of species of living and dead foraminifera in relation to other data analyzed in the studied stations. Both analyses were performed in Statistica 13.0 software.

A DCA in PC-ORD 5.3 program was also applied taking into account the most abundant and frequent species in the study area. The purpose of the application of this analysis was to check ecologically the influence of the abiotic factors related to OM quantity and quality in the pattern of distribution of the biocenosis found in each studied site.

## 3. Results

The complete datasets are presented in the Appendices 1–3. The *A. parkinsoniana*  $\delta^{18}\text{O}$  values ranging from 0.88 to  $-0.1\text{‰}$  exhibit the highest values in the northern sector and eastern and western lagoonal margins and are the lowest in the southern region (Fig. 1b). The dissolved oxygen (DO) content varies between 0.16 and 0.55 mg/l with relatively higher values in the outer part of the lagoon except in LB-1 which exhibits a low oxygen content (Fig. 2a). Salinity values ranged between 28 and 33.3 with lower values in the inner part of the lagoon and near the river's mouth and temperature at the bottom from 14.7 °C to 15.8 °C (Appendix 1). The TOC (2.54–5.93%) and TBP (9.3–11.9 mg g C<sup>-1</sup>) contents show similar patterns of distribution with higher values in the outer and eastern parts of the lagoon (Fig. 2 b, c). The LIP (3.5–5.3 mg g C<sup>-1</sup>) and PTN (2.5–3.1 mg g C<sup>-1</sup>) contents tend to be higher in the northern and central areas of the lagoon (Fig. 2 d, f). The highest values of CHO (3.0–4.4 mg C g<sup>-1</sup>) are found in LB9 (near the Tinja Channel), LB4 and LB5 (closed to the Guenich Stream) stations (Fig. 2e).

The TBP/TOC (1.9–3.7) and CHO/TOC (5.1–13.8) ratios show the highest values in the western part of the lagoon (Fig. 3 a, b). The PTN/CHO (0.67–0.95), PTN/LIP + CHO (0.33–0.43) and LIP/CHO + PTN (0.55–0.82) ratios reach the highest values in the northern and central parts of the lagoon whereas higher values of CHO/LIP + PTN (0.43–0.63) ratio are mostly found in stations located in both the eastern and the western margins of the lagoon (Fig. 3c, d, e, f).

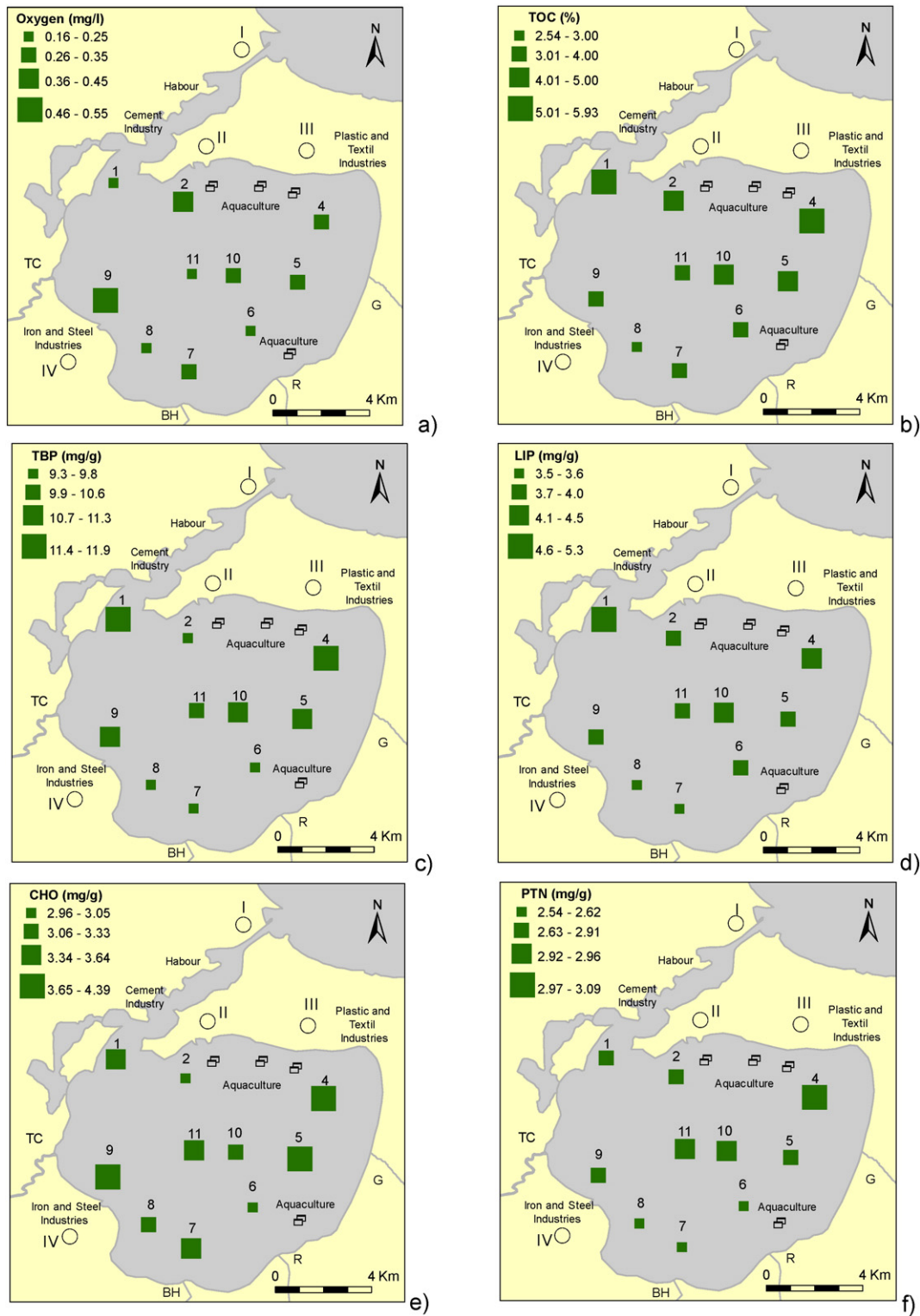
The values of C/N ratio, which are related to organic matter quality, vary between 1.5 and 6.9. The maximum concentrations of Chl *a* (69.3–699 mg/m<sup>3</sup>), TC (1–40 NPP/100 mg) and TMC (800–9200 CFU/g) are observed near the Guenich Stream whereas the highest abundance of *Enterococcus faecalis* (FE: 1–110 NPP/100 mg) is found in the LB7 station located in front of the Ben Hassine Stream.

The SEM (80.1–171.7 mg/g) is higher in the central and southern parts of the lagoon whereas the highest values of AVS (0–695.2 mg/g) are documented in stations LB2 and LB11, which are associated with high TOC and biopolymers contents. The SEM/AVS ratio is higher >1 in the stations LB6 and LB9.

### 3.1. Benthic foraminiferal assemblages

The FD in the dead assemblage is higher (D-FD: 2012 to 23,913) than the living counterpart (L-FD: 549 to 15,020). Both densities reach the highest values in the central lagoonal area and minimum ones in LB1 located in the channel connecting the lagoon with the Mediterranean Sea (Fig. 4a, b). Living and dead foraminifera diversities (L-H: 1.70–2.78 and D-H: 1.57–2.97, respectively) have an overall similar patterns of distribution being higher in the northern and central areas of the lagoon and with the lowest values recorded in stations LB1 and LB5 (Fig. 4c, d).

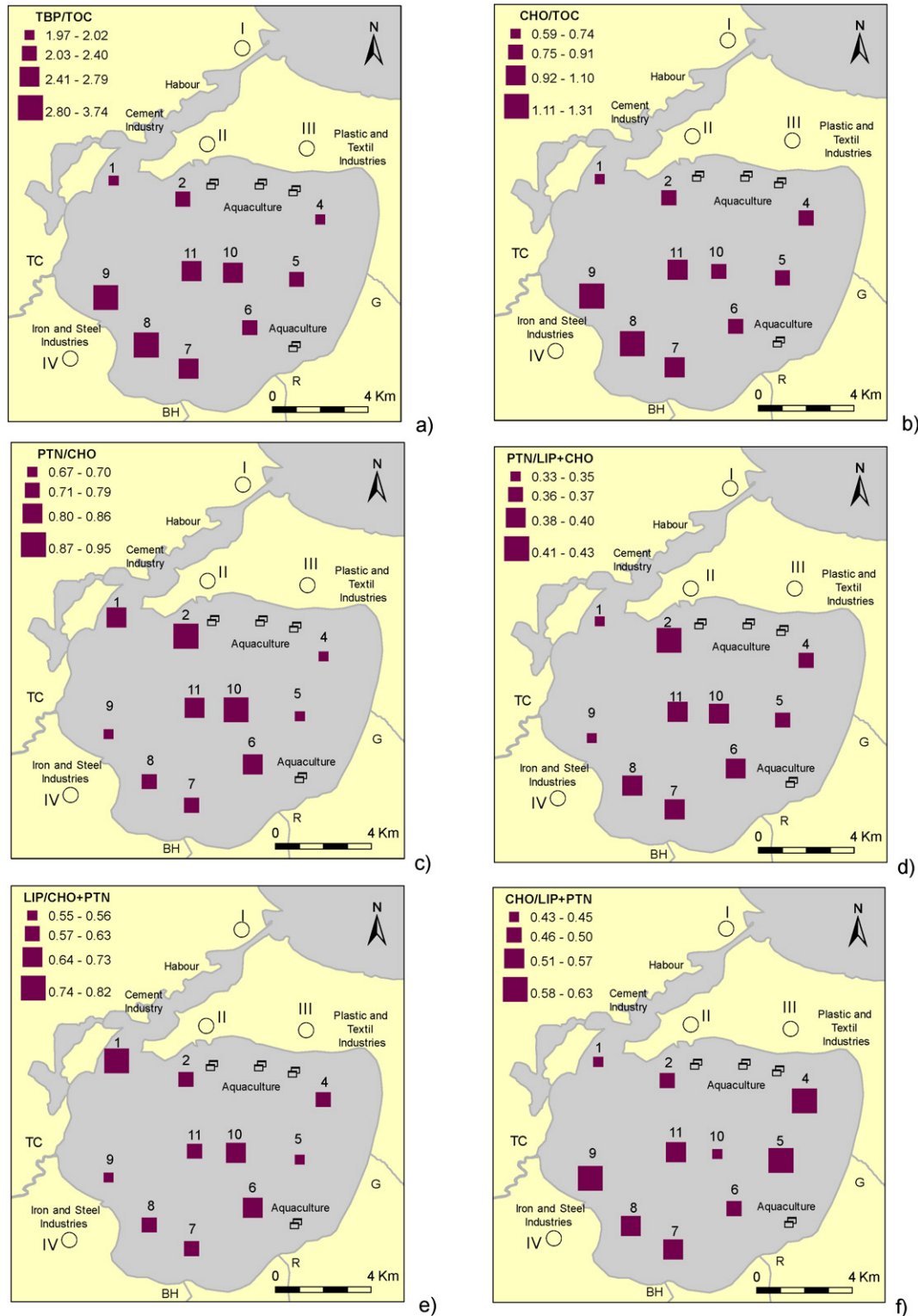
On overall 89 taxa have been identified in the benthic foraminiferal assemblages in the dead and living assemblages (Appendix 2). Specimens of most of the species found in Bizerte Lagoon were photographed (Appendix 3; Plates 1–7). The most abundant species in terms of absolute abundance (specimens/10 mg) is *A. parkinsoniana* in both the dead (384–12,885) and living (357–6166) assemblages (Fig. 5a, b). The dead



**Fig. 2.** Distribution maps of: a) Oxygen – surface sediment dissolved oxygen content (mg/l); b) TOC – total organic carbon (%); c) TBP – total of biopolymers (mg/g); d) LIP – lipids (mg/g); e) CHO – carbohydrates (mg/g); f) PTN – proteins (mg/g). Legend. Some of the main cities (indicated with open circles): 1 – Bizerte City; 2 – Menzel Abderrhaman City; 3 – Menzel Jemil City; 4 – Menzel Bourguiba City. Main Streams: TJ – Tinja Stream; BH – Ben Hassine Stream; R – Rharek Stream; G – Guenich Stream. Stations (LB1, LB2 to LB4-LB11) indicated with numbers above the filled squares.

assemblages are also represented (>500 specimens/10 mg), in decreasing order of abundance, by *Bolivina striatula*, *Bolivina ordinaria*, *Elphidium gunteri*, *Hopkinsina atlantica*, *Nonionella atlantica*, *Bolivina dilatata*, *Globocassidulina crassa*, *Rosalina bradyi* and *Triloculina trigonula*. The main taxa/groups in the dead assemblages, in decreasing

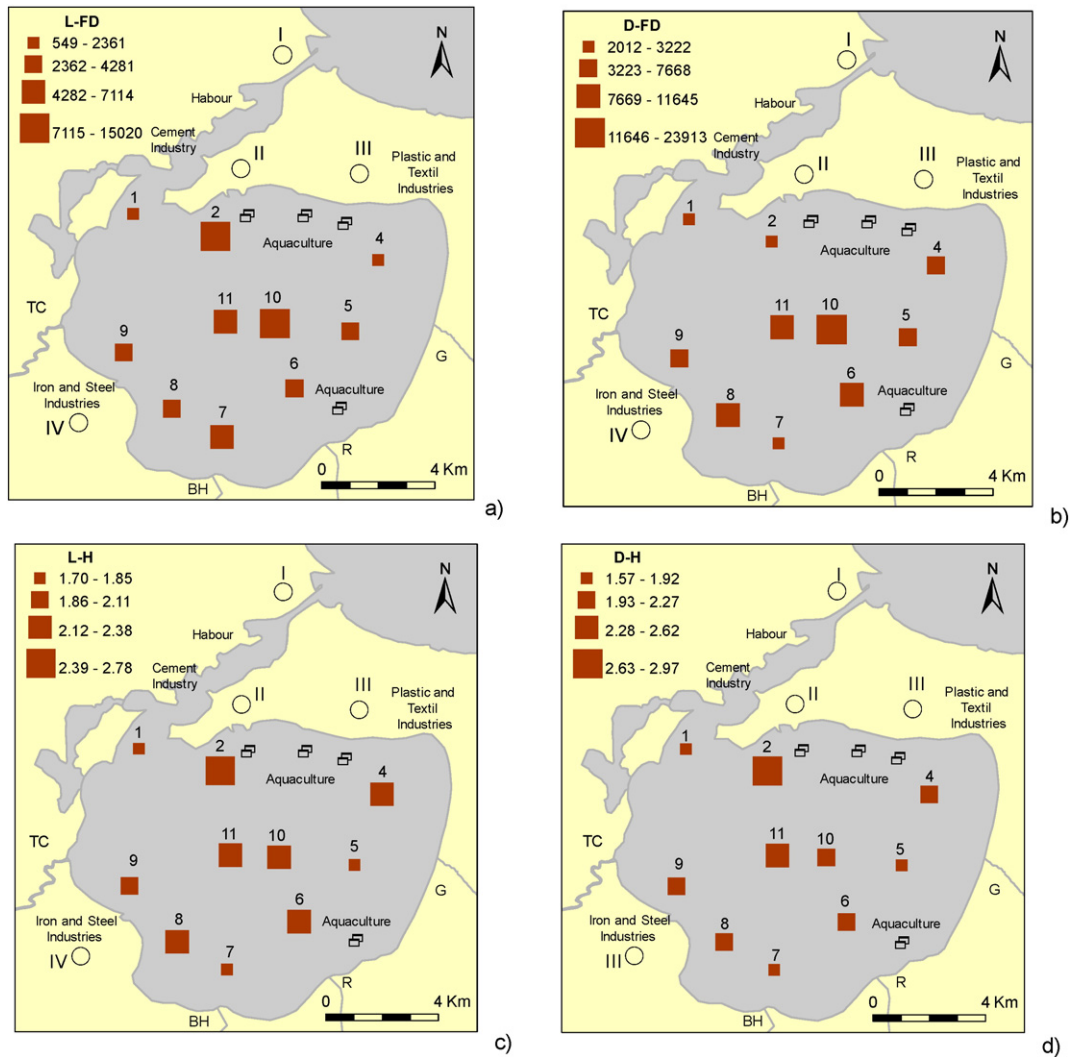
order of abundance, are Bolivinids/Buliminids (100–6047; Fig. 5c), Miliolids (144–1937; Fig. 5e), *Elphidium* (27–949; Fig. 5g), *Nonionella/Nonionoides* (25–632) and *Cassidulina/Globocassidulina* (0–474; Fig. 5i). Agglutinated foraminifera show a relatively low abundance (<80).



**Fig. 3.** Distribution maps of the following ratios: a) TBP/TOC; b) CHO/TOC; c) PTN/CHO; d) PTN/LIP + CHO; e) LIP/CHO + PTN; f) CHO/LIP + PTN. Legend. Some of the main cities (indicated with open circles): 1 – Bizerte City; 2 – Menzel Abderrhman City; 3 – Menzel Jemil City; 4 – Menzel Bourguiba City. Main Streams: TJ – Tinja Stream; BH – Ben Hassine Stream; R – Rharek Stream; G – Guenich Stream. Stations (LB1, LB2 to LB4-LB11) indicated with numbers above the filled squares.

In addition to *A. parkinsoniana*, the living assemblages are mostly represented (>500 specimens/10 mg), in decreasing order of abundance, by *B. striatula*, *B. ordinaria*, *H. atlantica*, *T. trigonula*, *Quinqueloculina seminula*, *Bulimina aculeata*, *Bolivina compacta*, *B. dilatata* and *Stainforthia fusiformis*. Agglutinated foraminifera also occur in

relatively low abundance (<55) in the living assemblages. The main taxa/groups of the living assemblage are *Cassidulina/Globocassidulina* (582–16,680; Fig. 5i), *Bolivina/Buliminids* (42–5375; Fig. 5d), *Miliolids* (116–2292; Fig. 5f), *Elphidium* (25–711; Fig. 5h) and *Nonionella/Nonionoides* (0–356).



**Fig. 4.** Distribution maps of: a) L-FD – living foraminifera density; b) D-FD – dead foraminifera density; c) L-H – Shannon Index values of living assemblages; d) D-H – Shannon Index values of dead assemblages. Legend. Some of the main cities (indicated with open circles): 1 – Bizerte City; 2 – Menzel Abderhaman City; 3 – Menzel Jemil City; 4 – Menzel Bourguiba City. Main Streams: TJ – Tinja Stream; BH – Ben Hassine Stream; R – Rharek Stream; G – Guenich Stream. Stations (LB1, LB2 to LB4-LB11) indicated with numbers above the filled squares.

### 3.2. Statistical analyses

The results of Q-mode CA based on the absolute abundance of living and dead foraminifera identified in the studied sites separate two main clusters (Fig. 6): cluster 1 includes stations LB10 and LB11 for both dead and living foraminifera assemblages and stations LB9 and LB6 only for the dead assemblages; whereas cluster 2 includes all the other sites. In cluster 2 it is possible to recognize two sub-clusters (2.1 and 2.2), in which station LB1 for both dead and living assemblages is separated from the other and it represents sub-cluster 2.1.

The plot of factor 1 against factor 2 of the PCA, that account together for 54% of the data variability, separates four groups of variables (Fig. 7): Group I, composed of TOC, TBP, namely CHO and LIP, and TMC and FE bacteria, is in opposition to Group II containing the following variables PTN/LIP + CHO, CHO/TOC, CHL a/TOC, TBP/TOC and D-FD, L-FD, D-H, L-H, L-A. *parkinsoniana*, L-*Elphidium* as well as living and dead Bolivinids/Buliminids, Miliolids, *Nonionella/Nonionoides* and *Cassidulina/Globocassidulina*. Group III joins TC bacteria, TBP/TOC, CHO/TOC, CHO/LIP + PTN and CHL a/TOC and is opposed to Group IV containing TOC, PTN, LIP, LIP/CHO + PTN, AVS, D-H and living and dead agglutinated species.

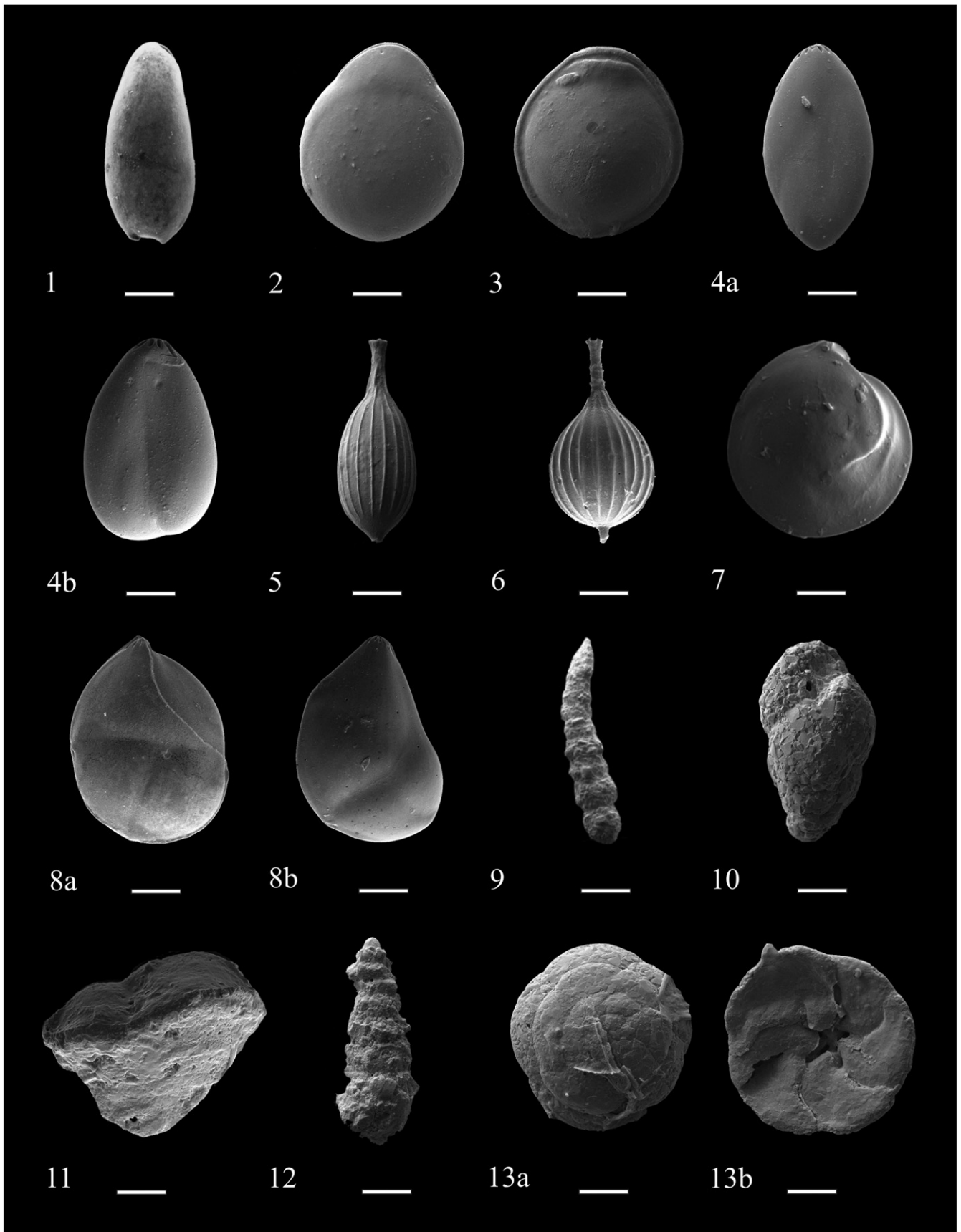
The most important results presented in Fig. 8 are related particularly to axis 1 which explains most of the data variability (84.2%).

Considering axis 1 of the DCA, the species *E. gunteri*, *A. cliarensis*, *F. squamosa*, *A. parkinsoniana*, *B. pseudoplicata*, *P. rotunda* and *S. fusiformis* respond positively to TOC, LIP/CHO + PTN, FE, TMC and negatively to PTN/LIP + CHO, CHL a/TOC, CHO/TOC, CHO/LIP + PTN and TBP/TOC. In contrast, *B. compacta*, *Q. seminula*, *H. atlantica*, *B. aculeata*, *B. ordinaria*, *B. striatula* and *C. quinquecarinata* respond inversely to the above-mentioned parameters. In relation to axis 2 it is observed that the species *A. parkinsoniana*, *P. rotunda*, *B. pseudoplicata* and *S. fusiformis* also are related positively to the TBP.

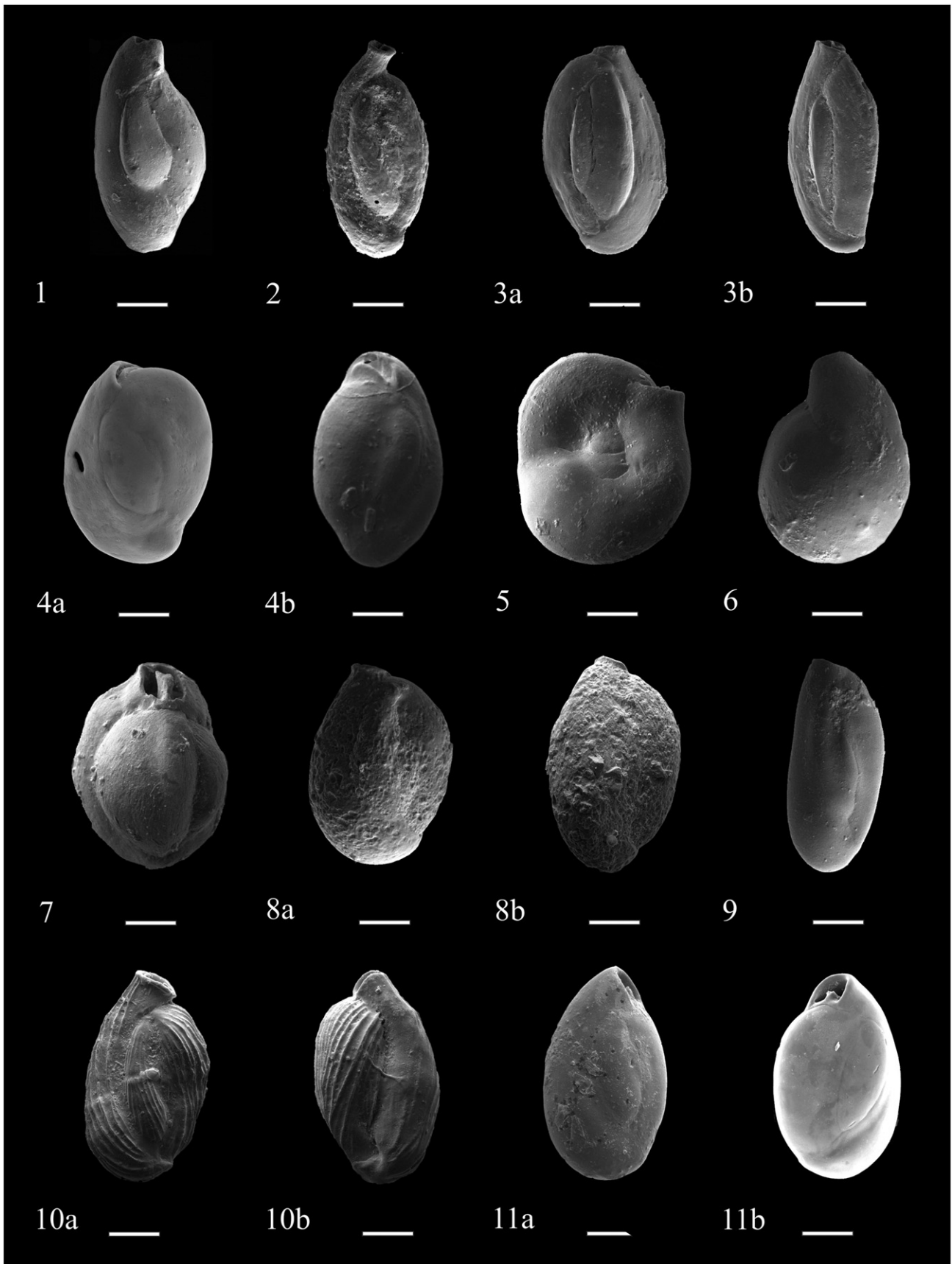
## 4. Discussion

### 4.1. Environmental settings

According to Béjaoui et al. (2008), the southern sector of Bizerte lagoon is under the influence of the rivers' discharge draining the southern watershed as well as the outflow of the Ichkeul Lake supplied through the Tinja Channel that originates from the southeast due to the predominant currents' pattern circulation (Frisoni et al., 1986; Harzallah, 2003; Béjaoui et al., 2008). In contrast, the northern sector, without an influence of major streams, is largely influenced by the sea-water input. An indication of the water circulation and Mediterranean/fresh water influence in Bizerte Lagoon, near the bottom, is given by the

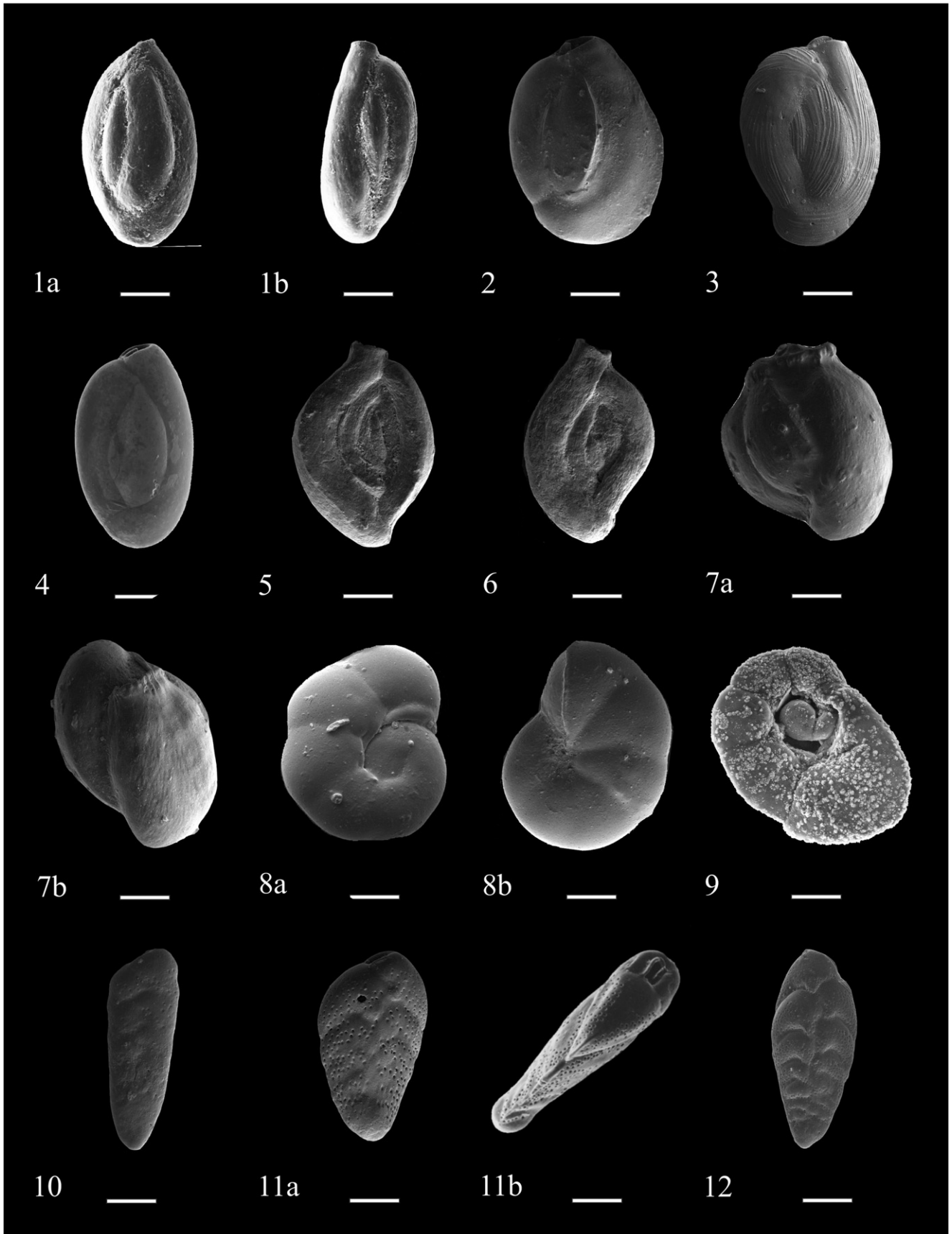


**Plate 1.** 1. *Fissurina globosocaudata* Albani and Yassini, scale bar 50 µm. 2. *Fissurina laevigata* Reuss, scale bar 50 µm. 3. *Parafissurina inaequalabiata* Yassini and Jones, scale bar 50 µm. 4 a, b. *Laryngosigma lactea* (Walker and Jacob), scale bar 50 µm. 5. *Lagena spicata* Cushman and McCulloch, scale bar 50 µm. 6. *Lagena strumosa* Reuss, scale bar 50 µm. 7. *Lenticulina inornata* (d'Orbigny) scale bar 50 µm. 8 a, b. *Neolenticulina variabilis* (Reuss), scale bar 50 µm. 9. *Hormosina bacillaris* (Brady), scale bar 50 µm. 10. *Eggerelloides scaber* (Williamson), scale bar 50 µm. 11. *Sahulina conica* (d'Orbigny), scale bar 50 µm. 12. *Reophax communis* Lacroix, scale bar 50 µm. 13 a, b. *Rotaliammina concava* (Seiglie), scale bar 50 µm.



**Plate 2.** 1. *Adelosina cliarensis* (Heron-Allen and Earland), scale bar 50 µm. 2. *Cycloforina exsculpta* (Heron-Allen and Earland), scale bar 50 µm. 3 a, b. *Cycloforina quinquecarinata* (Collins), scale bar 50 µm. 4 a, b. *Miliolinella subrotunda* (Montagu), scale bar 50 µm. 5. *Ophthalmidium balkwilli* Macfadyen, scale bar 50 µm. 6. *Planispirina cliarensis* Heron-Allen and Earland, scale bar 50 µm. 7. *Pseudotriloculina rotunda* (Schlumberger), scale bar 50 µm. 8 a, b. *Quinqueloculina agglutinans* d'Orbigny, scale bar 50 µm. 9. *Quinqueloculina bosciiana* d'Orbigny, scale bar 50 µm. 10 a, b. *Quinqueloculina boueana* d'Orbigny, scale bar 50 µm. 11. a, b. *Quinqueloculina dimidiata* Terquem, scale bar 50 µm.





**Plate 3.** 1 a, b. *Quinqueloculina laevigata* d'Orbigny, scale bar 50  $\mu\text{m}$ . 2. *Quinqueloculina lamarckiana* d'Orbigny, scale bar 100  $\mu\text{m}$ . 3. *Quinqueloculina poeyana* d'Orbigny, scale bar 50  $\mu\text{m}$ . 4. *Quinqueloculina seminula* (Linné), scale bar 200  $\mu\text{m}$ . 5. *Spiroloculina depressa* d'Orbigny scale bar 100  $\mu\text{m}$ . 6. *Spiroloculina eximia* Cushman, scale bar 100  $\mu\text{m}$ . 7 a, b. *Triloculina inflata* d'Orbigny, scale bar 100  $\mu\text{m}$ . 8 a, b. *Alliatinella differens* (McCulloch), scale bar 50  $\mu\text{m}$ . 9. *Lamarckina haliotideae* (Heron-Allen and Earland), scale bar 50  $\mu\text{m}$ . 10. *Bolivina compacta* Sidebottom, 1905. 11 a, b. *Bolivina dilatata* Reuss, 1850. 12. *Bolivina ordinaria* Pheleger and Parker, 1952.

distribution of the *A. parkinsoniana*  $\delta^{18}\text{O}$  data. The map presented in Fig. 1 shows more positive values of *A. parkinsoniana*  $\delta^{18}\text{O}$  in areas influenced by the colder and saltier water entrance from the Mediterranean Sea. The *A. parkinsoniana*  $\delta^{18}\text{O}$  is more positive near the opening of the channel which connects the Bizerte Lagoon to the Mediterranean sea (larger gray squares – Fig. 1). This region (at the northern area of the lagoon) is strongly influenced by the Mediterranean sea's saltier water. In contrast, relatively more negative values (smaller gray squares – Fig. 1) of *A. parkinsoniana*  $\delta^{18}\text{O}$  in the central and particularly in the interior zone of the lagoon are probably associated with the progressive mixture of the Mediterranean water with low saline waters due to the rivers' outflow which induces the isotope fractionation. The isotope distribution reflects the internal lagoon current circulation and the progressive dilution of the high salinity Mediterranean water in the interior of the lagoon and high residence time of the water inside the lagoon (Harzallah, 2003; Béjaoui, 2009).

The water circulation and the hydrodynamic regime still influence the transportation, accumulation and the structure of the sediment (Genovesi et al., 2013) as well its granulometry, composition and organic matter content (Martins et al., 2015a). The prevalence of relatively dynamic currents near the margins influences the distributional pattern of size fractions of the sediments, which are composed by relatively high sand particles content (29%, on mean) in the depth range between 0 and 4 m (Zaaboub et al., 2015). Calmer currents' activity prevailing in the central area allow the deposition of high amount of clay and silt particles which reach an average of 41% and 30%, respectively, at a depth between 4 m and 12 m (central part) (Zaaboub et al., 2015). For the studied stations, on the basis of mostly fine-dominated sediments, a lower hydrodynamic bottom regime can be inferred for the central part of the Bizerte Lagoon whereas a somewhat more active one can prevail in the northern sector, near the channel connecting the lagoon to the sea and in the eastern and western margins of the lagoon.

The TOC contents are relatively high (4.2%, on mean) with the highest values in the northern and eastern parts of the lagoon. According to Mendonça Filho et al. (2003), the TOC content between 2.5% and 5.9% may be associated with dysoxic–anoxic environments and with a high rate of organic matter accumulation. High supply of organic matter and its breakdown can lead to a depletion of DO that ultimately might affect the proliferation and viability of marine organisms (Borja et al., 2012). However, in the studied sites the surface sediments are in general oxygenated as suggested by our data but become low oxie or anoxic some millimetres below the sediment–water interface as indicated in dark grey or black colors. As referred by Martins et al. (2015b), the low depth and good transparency of the lagoon allow the development of benthic microalgae on the bottom. The presence of these microalgae is also related to the presence of chlorophyll *a* in all the stations. This algae favor allows the oxygenation of the surface sediment, which might favor benthic organisms that live on the sediments surface.

However, the high accumulation of OM and its decomposition in deeper sedimentary layers can lead to a sharp decrease in DO and eventually to the establishment of very shallow anoxia. Once anoxic conditions are established, the bacterial activity employs the sulfate as an oxidizing agent, with a consequent increase in the concentration of its reduction product (sulfide) (Heitmann and Blodau, 2006). This process is indicated by the AVS values which are  $>1$  in most of the studied sites. The highest values of AVS are recorded mainly in the central, northern and eastern areas of the lagoon (LB2, LB11, LB4, LB5 and LB9). These areas receive effluents from industrial and urban activities which coupled with products provided by mytilicol and ostreicol deposits may induce this effect.

In oxygen-depleted environments, the metals introduced in the lagoon through the anthropic activities react with sulfide produced by bacteria and fungi to form insoluble metal sulfides (Zaaboub et al., 2015). However the metals become adsorbed to the sediments in ionic form, and during changes of oxidation/reduction potential, they can be released and become bioavailable (Simpson et al., 2012). Microbial degradation of organic matter can also release adsorbed metals in the interstitial water. Certain bacteria are capable of methylate metals in some organic forms that are more bioavailable than inorganic ones (Apostoli, 2006).

Chong and Wang (2000) reported that the AVS interacts with metals in a different way in the anoxic and suboxic environments, conditioning the metals' bioavailability. The ratio SEM/AVS values are  $>1$  near the mouths of the Tinja Channel (LB9) and Rharek Stream (LB6), where also the DO values were found at the minima, indicating the presence of toxic sediments due to the metals' availability (Martins et al., 2015b).

#### 4.2. Quality of organic matter

Concentrations of LIP and CHO are higher than those of PTN in all the studied stations, and in most of the samples, the concentrations of LIP are higher than CHO except in LB4, LB5 and LB9 stations that are located near the mouth of the Guenich Stream and Tinja Channel. High amounts of LIP are commonly present in estuarine sediments (Salot, 1984; Ittekkot, 1988) being well preserved in anoxic sediments (Pinturier-Geiss et al., 2002) and seem to be also the situation in Bizerte Lagoon. The highest concentrations of CHO near the mouths of the Tinja Channel and Guenich Stream might be related to a phytoplankton and plant debris origin.

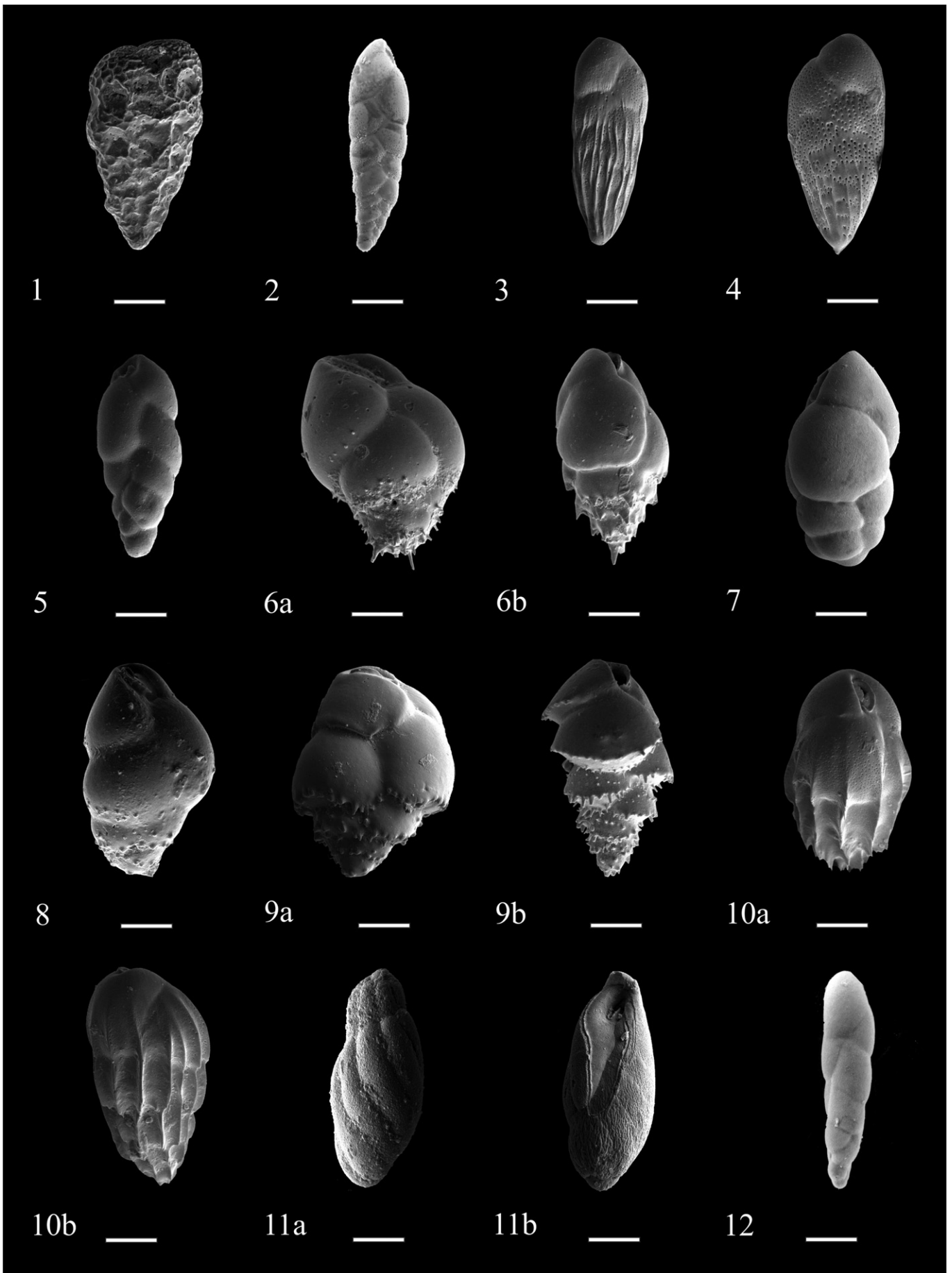
Several ratios among biopolymers are useful proxies for assessing the origin of the sedimentary organic matter and have been widely used in (e.g. Martin et al., 1987; Fabiano et al., 1999; Pusceddu et al., 1999; Dell'Anno et al., 2002). The TBP/TOC ratio can provide useful information about the overall labile enrichment in organic matter. The highest values of this variable in Bizerte Lagoon are found in the south-western marginal area and in the central part probably due to the flow of nutrients supplied by the Ichkeul Lake through the Tinja Channel.

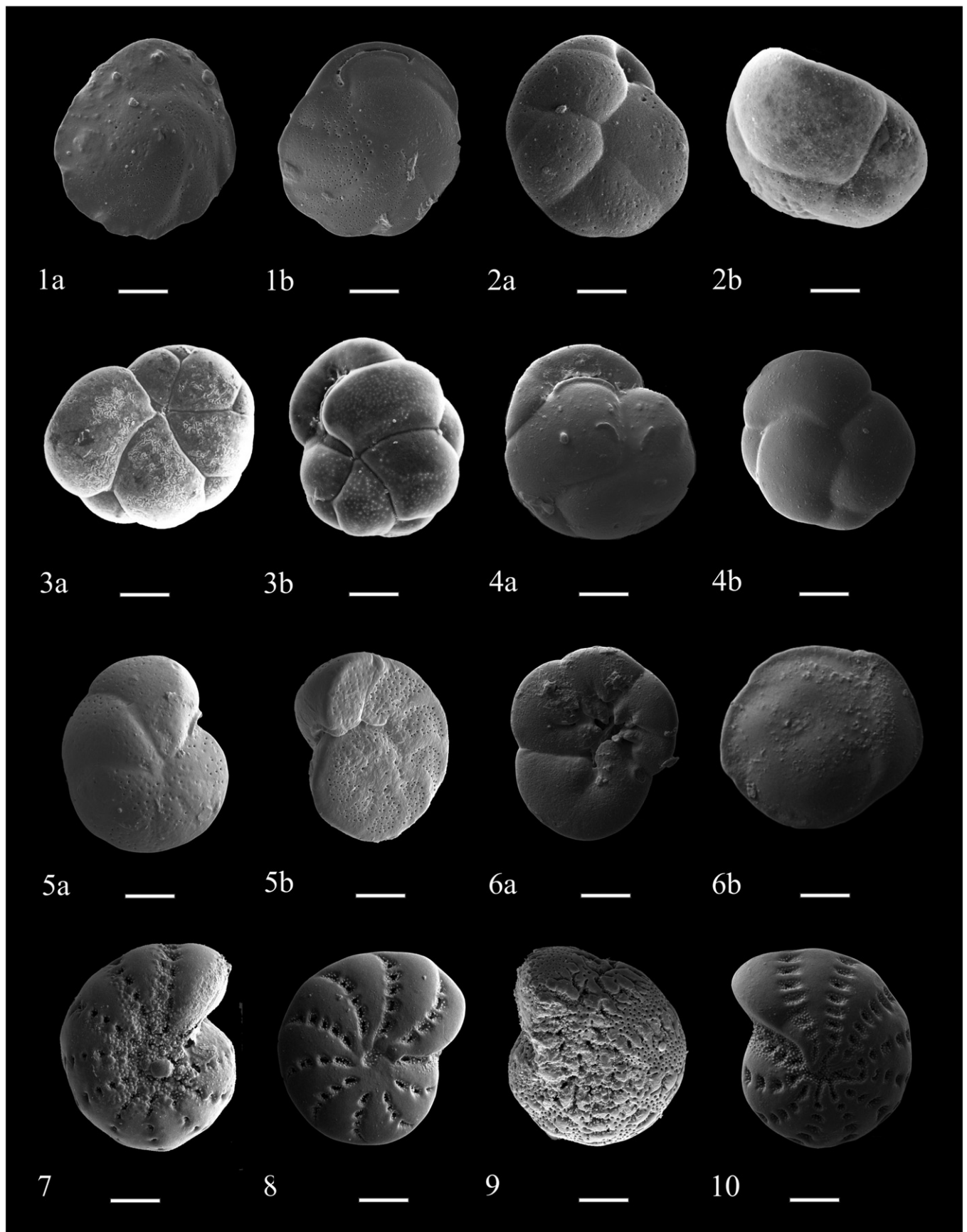
The highest values of LIP/CHO + PTN near the channel connecting the lagoon with the Mediterranean and closed to some streams outflow suggest that this enrichment is probably related to the increase of recalcitrant substances originated by the fluvial input and sewage caused by anthropogenic activities.

The PTN/CHO ratio is directly linked to the importance of nitrogen fraction in organic matter (Martin et al., 1987; Fabiano et al., 1999). Productive areas, such as estuaries and coastal regions tend to have high values of the PTN/CHO ratio as those found in the Biscay estuary (Cotano and Villate, 2006). According to Pusceddu et al. (1999) and Dell'Anno et al. (2002), the PTN/CHO ratio can be applied as an indicator of the trophic level of coastal systems which can be classified as: meso-oligotrophic (protein  $< 1.5 \text{ mg} \cdot \text{g}^{-1}$ ; carbohydrate  $< 5 \text{ mg} \cdot \text{g}^{-1}$ ), eutrophic (protein  $1.5\text{--}4 \text{ mg} \cdot \text{g}^{-1}$ ; carbohydrate  $5\text{--}7 \text{ mg} \cdot \text{g}^{-1}$ ) and hypertrophic (proteins  $> 4 \text{ mg} \cdot \text{g}^{-1}$ ; carbohydrate  $> 7 \text{ mg} \cdot \text{g}^{-1}$ ). According to this classification, the studied lagoon (PTN:  $2.54\text{--}3.09 \text{ mg} \cdot \text{g}^{-1}$ ; CHO:  $2.96\text{--}4.39 \text{ mg} \cdot \text{g}^{-1}$ ) should be considered as a meso-eutrophic environment. This agrees with the observations of Grami et al. (2008) that classified the Bizerte Lagoon as an ecosystem tending to eutrophication due to the increased load of nutrients from industrial expansion, urban and agricultural developments.

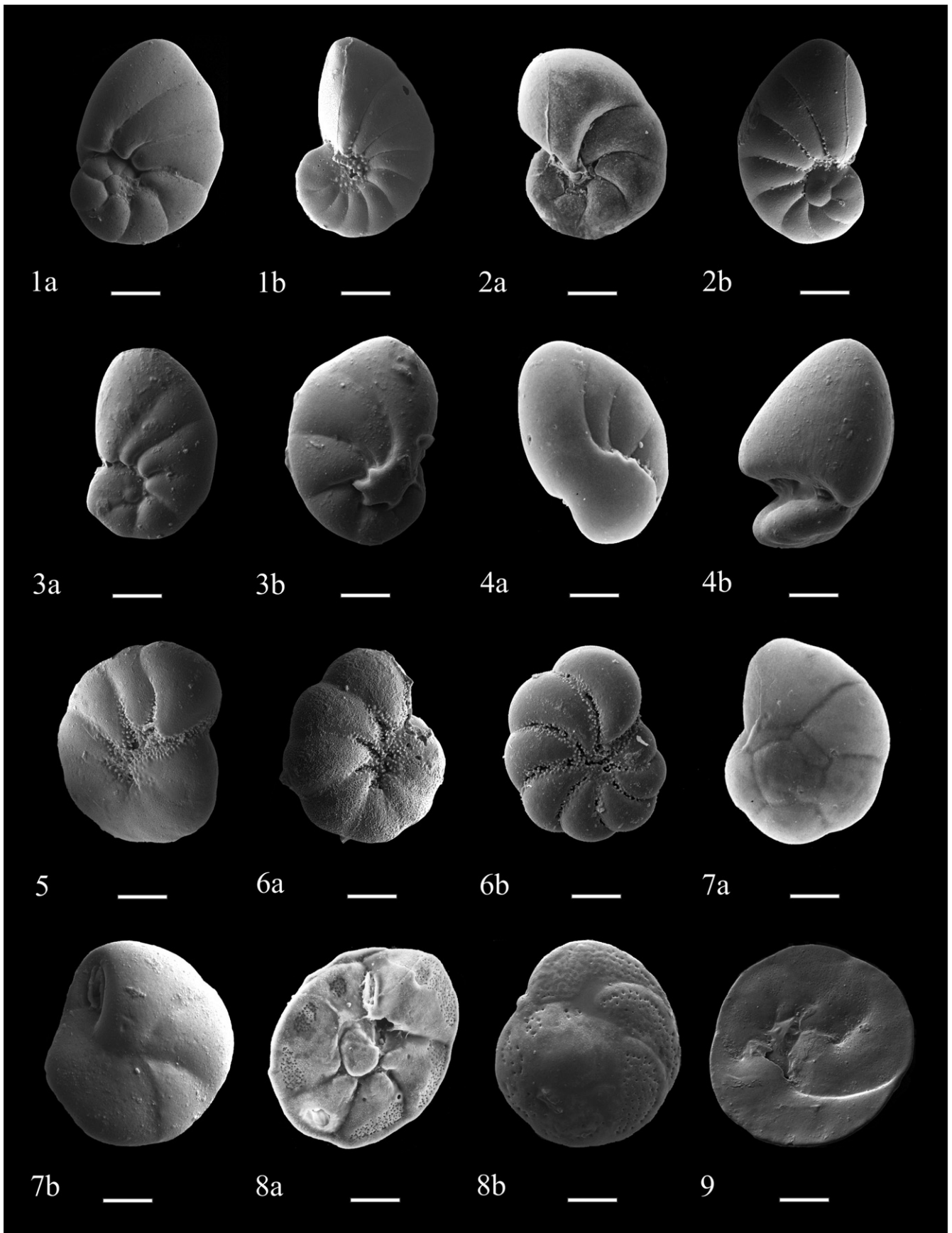
In addition, it was suggested that PTN/CHO  $> 1$  should be linked to the presence of fresh organic matter, since protein degradation is faster

**Plate 4.** 1. *Bolivina pseudoplicata* Heron-Allen and Earland, scale bar 50  $\mu\text{m}$ . 2. *Bolivina seminuda* Cushman, scale bar 50  $\mu\text{m}$ . 3. *Bolivina striatula* Cushman, scale bar 50  $\mu\text{m}$ . 4. *Bolivina subaenariensis* Cushman, scale bar 50  $\mu\text{m}$ . 5. *Fursenkoina squamosa* (d'Orbigny), scale bar 50  $\mu\text{m}$ . 6 a, b. *Bulimina aculeata* d'Orbigny, scale bar 50  $\mu\text{m}$ . 7. *Bulimina elongata* d'Orbigny, scale bar 50  $\mu\text{m}$ . 8. *Bulimina gibba* Fornasini, scale bar 50  $\mu\text{m}$ . 9 a, b. *Bulimina marginata* d'Orbigny, scale bar 50  $\mu\text{m}$ . 10 a, b. *Bulimina striata* d'Orbigny, in Guérin-Méneville, scale bar 50  $\mu\text{m}$ . 11 a, b. *Buliminella elegantissima* (d'Orbigny), scale bar 50  $\mu\text{m}$ . 12. *Buliminella tenuata* (Cushman), scale bar 50  $\mu\text{m}$ .

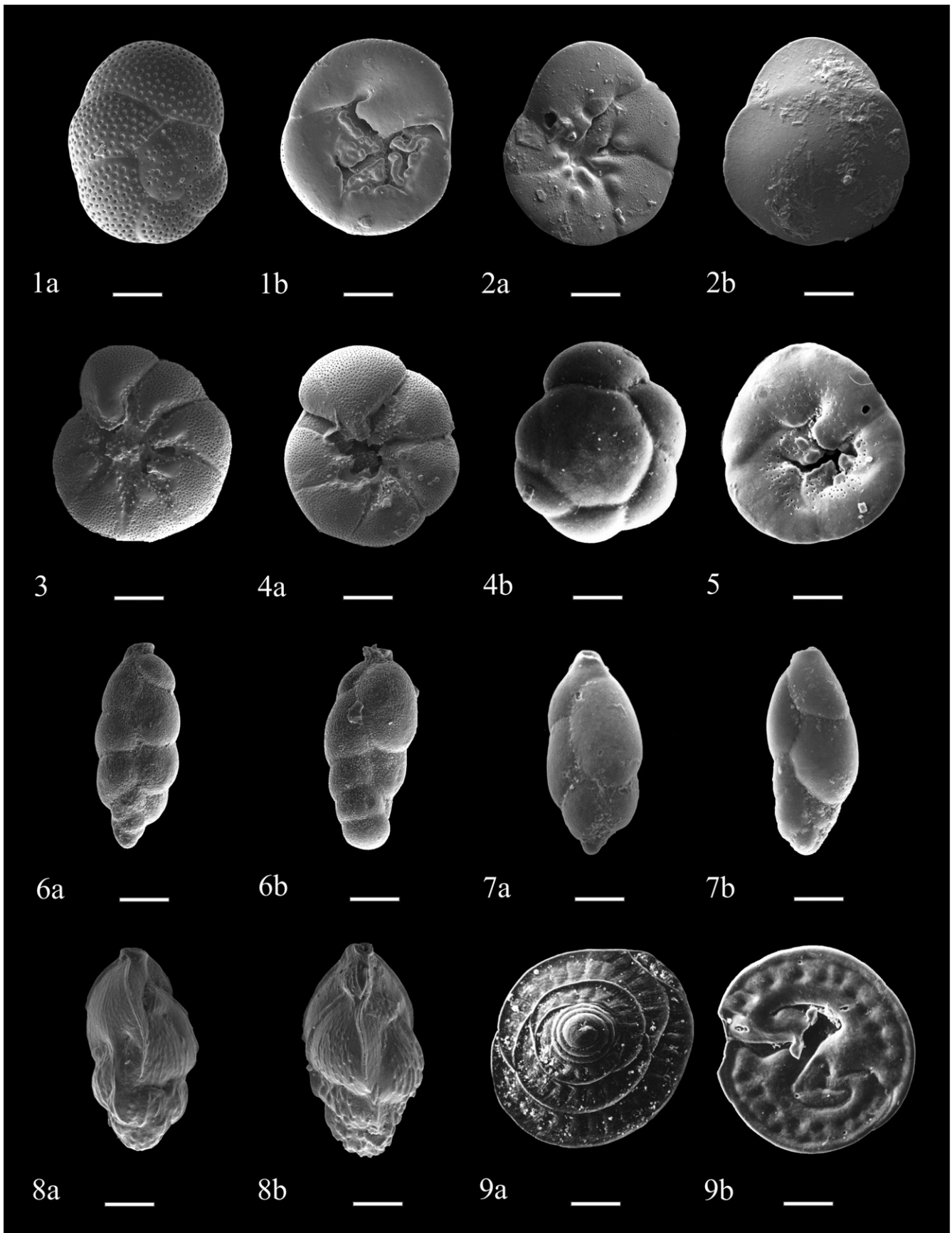




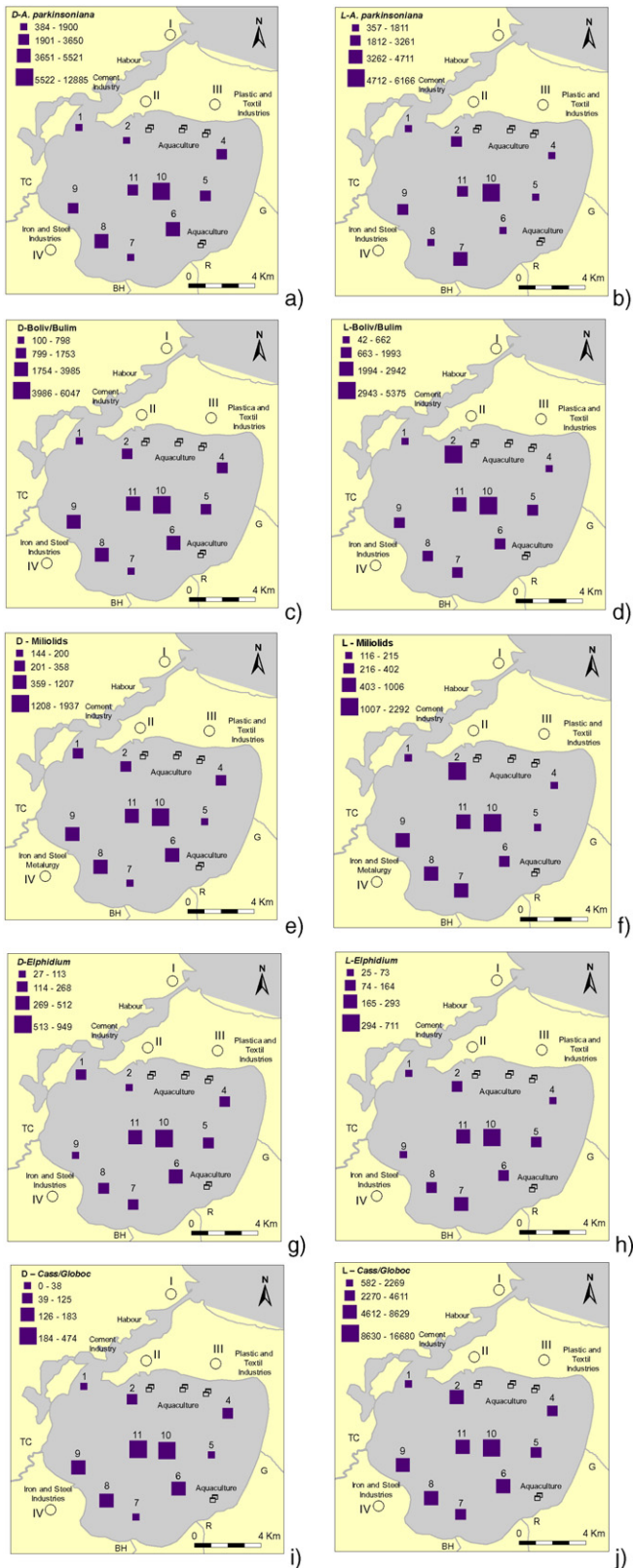
**Plate 5.** 1 a, b. *Cassidulina carinata* Silvestri, scale bar 50 µm. 2 a, b. *Globocassidulina crassa* (d'Orbigny), scale bar 50 µm. 3 a, b. *Globocassidulina crassa rossensis* Kennett, scale bar 50 µm. 4 a, b. *Globocassidulina minuta* (Cushman), scale bar 50 µm. 5 a, b. *Lobatula lobatula* (Walker and Jacob, 1798), scale bar 50 µm. 6 a, b. *Discorbis parkeri* Natland, scale bar 50 µm. 7. *Criboelphidium excavatum* (Terquem), scale bar 50 µm. 8. *Elphidium gerthi* van Voorthuysen, scale bar 50 µm. 9. *Elphidium gunteri* Cole, scale bar 50 µm. 10. *Elphidium williamsoni* Haynes, scale bar 50 µm.



**Plate 6.** 1 a, b. *Nonionella atlantica* Cushman, scale bar 50  $\mu\text{m}$ . 2 a, b. *Nonionella iridea* Heron-Allen and Earland, scale bar 50  $\mu\text{m}$ . 3 a, b. *Nonionella stella* Cushman and Moyer, scale bar 50  $\mu\text{m}$ . 4 a, b. *Nonionoides turgida* (Williamson), scale bar 50  $\mu\text{m}$ . 5. *Haynesina depressula simplex* (Cushman), scale bar 50  $\mu\text{m}$ . 6 a, b. *Haynesina germanica* (Ehrenberg), scale bar 50  $\mu\text{m}$ . 7 a, b. *Eilohedra vitrea* (Parker), scale bar 50  $\mu\text{m}$ . 8 a, b. *Gavelinopsis praegeri* (Heron-Allen and Earland), scale bar 50  $\mu\text{m}$ . 9. *Neoconorbina terquemi* (Rzehak), scale bar 50  $\mu\text{m}$ .



**Plate 7.** 1 a, b. *Rosalina bradyi* (Cushman), scale bar 50  $\mu\text{m}$ . 2 a, b. *Rosalina williamsoni* (Chapman and Parr), scale bar 50  $\mu\text{m}$ . 3. *Ammonia parkinsoniana* (d'Orbigny), scale bar 50  $\mu\text{m}$ . 4 a, b. *Ammonia tepida* (Cushman), scale bar 50  $\mu\text{m}$ . 5. *Tretomphaloides concinna* (Brady), scale bar 50  $\mu\text{m}$ . 6 a, b. *Hopkinsina atlantica* Cushman, scale bar 50  $\mu\text{m}$ . 7 a, b. *Stainforthia fusiformis* (Williamson), scale bar 50  $\mu\text{m}$ . 8 a, b. *Trifarina angulosa* (Williamson), scale bar 50  $\mu\text{m}$ . 9 a, b. *Patellina corrugata* Williamson, scale bar 50  $\mu\text{m}$ .



**Fig. 5.** Distribution maps of the density of the following species taxa/groups of species in dead assemblages (d) and living one (L) such as: a), b) *Ammonia parkinsoniana*; c), d) Boliv/Bulim – Bolivinids and Buliminids; e) f) *Miliolids* – Miliolids; g), h) *Elphidium* spp.; i), j) Cass/Globoc – *Cassidulina/Globocassidulina* spp. Some of the main cities (indicated with open circles): 1 – Bizerte City; 2 – Menzel Abderrhman City; 3 – Menzel Jemil City; 4 – Menzel Bourguiba City. Main Streams: TJ – Tinja Stream; BH – Ben Hassine Stream; R – Rharek Stream; G – Guenich Stream. Stations (LB1, LB2 to LB4-LB11) indicated with numbers above the filled squares.

than the others biopolymers, while the proportion  $PTN/CHO < 1$  might evidence a predominance of aged organic matter in the sediments (Danovaro et al., 1993). Despite the values of  $PTN/CHO$  ratio remained below the unit in all the studied stations what might suggest a relatively high accumulation of aged organic matter, some stations, namely LB2 and LB10, exhibit values approaching 1. Interestingly, these stations also revealed the highest values of L-FD.

Regarding the functional role of proteins, Dell'Anno et al. (2002) related the high concentrations of PTN to the primary productivity that supports all the food web and the heterotrophic development of the benthic ecosystem. According to our interpretation supported by the large bacterial pool in the sediments and benthic foraminifera and ostracods (observed in high number in all sites) a rapid recycling of labile organic matter compounds and a relatively high heterotrophic activity of microbenthic organisms, seem to take place in most of the studied sites of the Bizerte Lagoon. So the  $PTN/CHO < 1$  in the studied stations does not necessarily mean aged organic matter, but it should be related to high OM recycling due to intense heterotrophic activity carried out mainly by microorganisms since the macrofaunal organisms, such as mollusks, polychaeta and crustaceans are in general present in low abundance in most part of Bizerte Lagoon (Afi et al., 2009).

The ratio  $CHO/TOC$  has been used as a semi-quantitative signal of sewage-derived organic matter by Paez-Osuna et al. (1998). These authors suggested that values  $< 20$  indicate organic matter of natural origin (sea or land), while values around 30 point to a sewage origin. According to this classification, the values of  $CHO/TOC$  ratio (5.1–13.8) of the studied stations indicate a prevalence of a mainly natural origin of the organic matter supplied to the bottom of Bizerte Lagoon. The same conclusion was inferred through the C/N ratio by Martins et al. (2015b) despite the introduction of organic matter and other materials from industrial and municipal waste waters that also should stimulate natural productivity in the lagoon.

Some OM difference should result from the differential influence generated by the Mediterranean/streams' water entrance in the lagoon as suggested by the distribution the  $CHO/LIP + PTN$  ratio. These differences should be, to some extent, associated with the two different trophic chains distinguished by Sakka Hlaili et al. (2007, 2008) in the outer and inner sectors of Bizerte Lagoon.

#### 4.3. Relationship of foraminifera with the quantity and quality of organic matter

The foraminifera assemblage found in the Bizerte Lagoon is composed mostly by *A. parkinsoniana*, common in Mediterranean transitional biotopes under significant influence of sea water (Frontalini et al., 2011) but also includes species commonly found in the marine realm such as bolivinids, buliminids, *Nonionella/Nonionoides*, *Cassidulina* and *Globocassidulina* (Murray, 1991, 2006; Wang et al., 1988). In the Bizerte Lagoon the last group of species was found in waters with temperature and salinity close to the marine one (Martins et al., 2015b). Foraminifera assemblages' composition of Bizerte Lagoon is quite different from other Mediterranean lagoons, as reported by Frontalini et al. (2011) and Martins et al. (2015b). Thus we can deduce that these species or/group of species are being able to populate the lagoon due to physicochemical (such as temperature and salinity) adequate properties. Due to the character opportunistic of these species which are able to live in fine sediments rich in organic matter and with oxygen-limited (e.g. Bernhard and Sen Gupta, 1999; Loubere and Fariduddin, 1999) the living assemblage was compared in this work with the dead one, since the first may reflect a seasonal answer of the organisms to the environmental conditions, while the second one should constitute a longer memory of it.

As suggested by the results of Q-mode CA the living and dead assemblages have a similar composition in most of the studied sites. Thus considering the estimations of the sedimentation rate of Garali et al. (2013) who determined values of 2 to 2.5 mm/year in the western and central

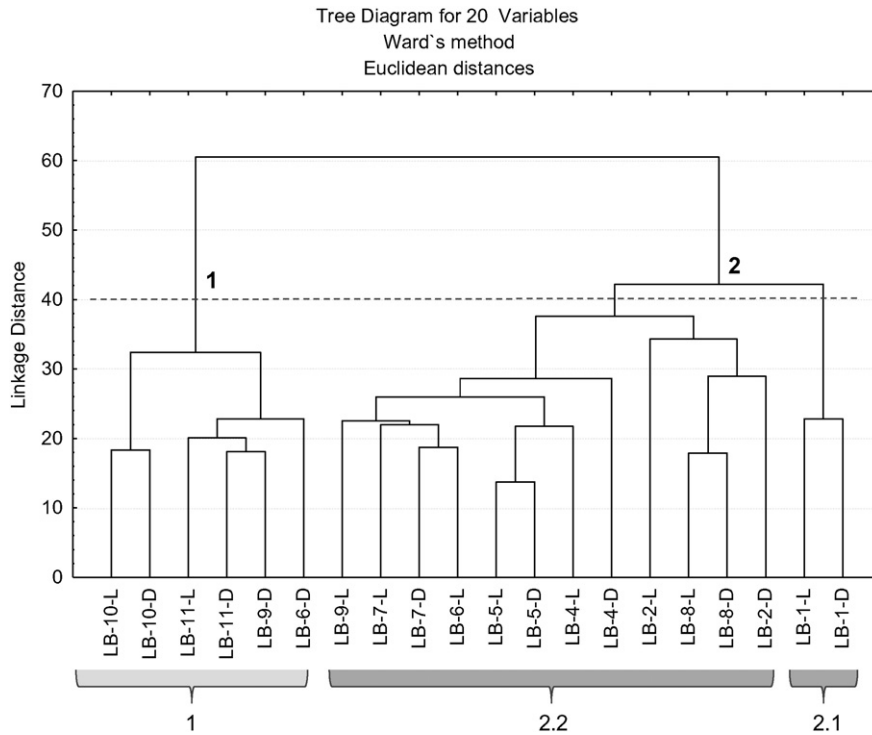


Fig. 6. Q-mode cluster analysis based on the absolute abundance of living (L) and dead foraminifera (D) identified in the studied sites (LB).

sectors of the lagoon and up to 0.3 mm/year on the northern and eastern sectors, our dead assemblages would represent a roughly average information about the living organisms' production during several decades. In this case both assemblages are similar in general, this is, the dead assemblage largely reflects the living one. Hydrodynamic forces or physicochemical processes (tests dissolution, for example) may contribute to

change the original composition of the dead assemblage. But in this case the changes of the dead assemblages appear to have not been significant in the studied sites. The living assemblage can change along the year due to seasonal environmental changes and differences in the reproduction rate of the species. However, the similarity between both assemblages should indicate a relative stability in the sedimentary

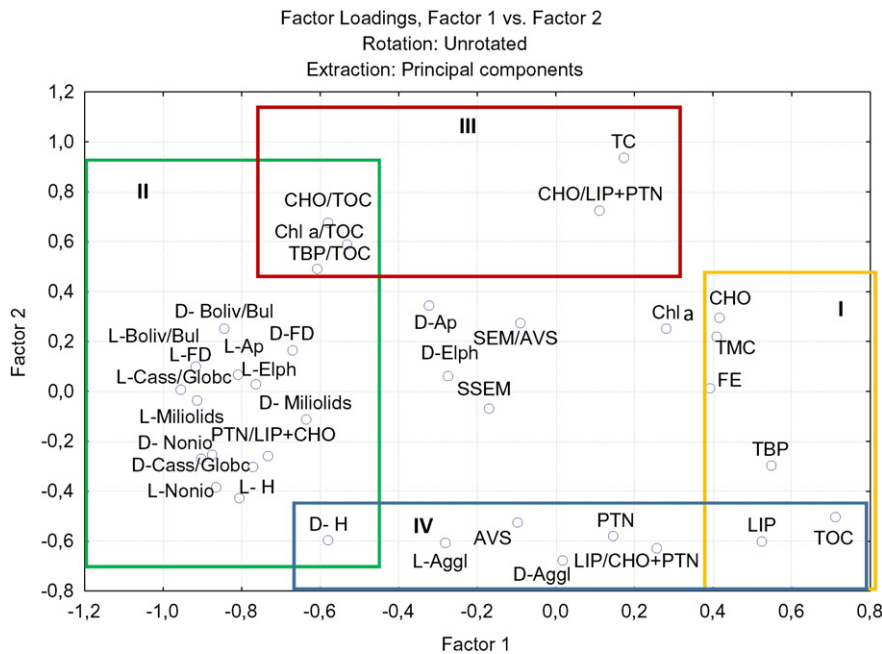
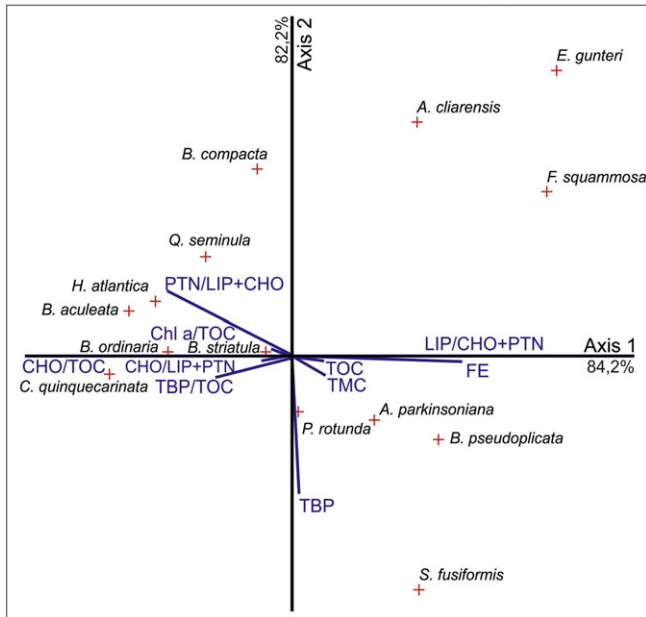


Fig. 7. Results of PCA for the first four factors. Legend: TOC – Total organic carbon; Chl a – chlorophyll a; SEM – simultaneously sequential extracted metals total concentrations; AVS – acid volatile sulfides; TC – total coliform; FE – fecal enterococci; TMC – total mesophilic counts; LIP – Lipids; PTN – proteins; CHO – carbohydrates; TBP – total of biopolymers; L-H – Shannon Index values of living foraminifera; D-H – Shannon Index values of dead foraminifera; L-DF – density of living foraminifera; D-FD – density of dead foraminifera; D-Ap – dead *Ammonia parkinsoniana*; L-Ap – living *A. parkinsoniana*; D-Elph – dead *Elphidium* spp.; L-Elph – living *Elphidium* spp.; D-Cass/Globc – dead *Cassidulina/Globocassidulina*; L-Cass/Globc – living *Cassidulina/Globocassidulina*; D-Aggl – dead agglutinated foraminifera, L-Aggl – living agglutinated foraminifera; D-Nonio – dead *Nonionella/Nonionoides*; L-Nonio – living *Nonionella/Nonionoides*; D-Miliolids – dead Miliolids; L-Miliolids – living Miliolids; D-Boliv/Bul – dead Boliviniids/Buliminids; L-Boliv/Bul – living Boliviniids/Buliminids.





**Fig. 8.** Results of DCA relating the most abundant and frequent species of the living assemblages of benthic foraminifera of Bizerte Lagoon with parameters associated with quantity and quality of organic matter.

environment of Bizerte Lagoon for a relatively long period of time (considering that 1 cm of the sediment should represent a record until about 20 to 25 years).

However, CA essentially results in the separation of stations located at the margins of the lagoon (cluster 2) than that located at or close to the central area (cluster 1). In cluster 2, station LB1 (sub-cluster 2.1) can be considered distinct from all the others probably due to the influence of the relatively high hydrodynamism of the channel that connects the lagoon with the Mediterranean Sea and by its location near the influence of densely urbanized, industrialized and harboring areas. This area exhibits even high TOC and TBP contents, namely of LIP and PTN. In Cluster 1 also includes stations LB9 and LB6 for dead assemblages. Both stations should have toxic sediments as referred by Martins et al. (2015b) that perhaps determined a reduction in living foraminiferal diversity but not necessarily a deep decrease in assemblage's dimension.

The biochemical composition of sedimentary organic matter, in terms of biopolymers, can be considered as an estimate of labile organic materials potentially available for benthic consumers (Fichez, 1991; Danovaro et al., 1993; Fabiano et al., 1995; Tselepidis et al., 2000). Benthic foraminiferal assemblages are strongly shaped in terms of the density and diversity by the organic carbon and nutrient concentrations (Moodley et al., 2000; Schönfeld, 2001; Fontanier et al., 2002).

Results of PCA indicate that most part of the main species and groups of species of benthic foraminifera distribution, such as *A. parkinsoniana*, bolivinids, buliminids, *Nonionella*, *Cassidulina*, *Globocassidulina*, is generically explained by organic matter quantity and quality than for metals' availability or AVS. However the increasing TOC content and TBP mainly in LIP are negatively related to dead and living foraminiferal density and diversity and to the referred main taxa/groups of foraminifera but linked positively with bacterial pool related to sewage (TCM and FE). On the other hand, the dead and living benthic foraminifera density, diversity and most of the species, taxa and groups of species seem to be more related to the enrichment in PTN/LIP + CHO, CHO/TOC, Chl a/TOC and TBP/TOC. Bacterial TMC and FE are positively related to the enrichment in organic matter and TBP but mostly LIP, and negatively to foraminifera abundance and diversity and species/taxa distribution. Bacterial TC are positively linked with CHO/TOC, Chl a/TOC and TBP/TOC.

The PCA results also evidence another cause of stress for benthic faunas. The rising of organic matter and AVS contents is associated with a relatively increasing agglutinated foraminifera both in dead and living assemblages as they are not only less prone to dissolution, but they can biomineralize in such conditions. The metals' availability associated with AVS at least in some areas can contribute to the decline of some sensitive species and favors the rise of the most tolerant to both stressing factors. In a similar study, Cherchi et al. (2012), revealed a connection between heavy metal dispersion, decrease in pH of the sea-water and bioerosional processes on calcareous foraminifera.

This might suggest that foraminifera are negatively influenced by the excessive increase of organic matter and even if with relatively good quality, or significantly enriched in biopolymers. A similar observation was also done by Koho et al. (2014) in an oxygen-minimum zone where the sediments had abundant organic matter of moderately high quality and exhibited a surprisingly poor microbial degradability (bioavailability) with a negative impact in benthic foraminifera faunas.

The results of the DCA confirm the PCA findings and further show that the species that best answered the organic quality of natural origin, especially enriched in proteins, carbohydrates and chlorophyll *a* were some bolivinids such as *B. compacta*, *B. aculeata*, *B. ordinaria*, *B. striatula*, some miliolids such as *Q. seminula*, *C. quinquecarinata* as well as *H. atlantica*. Otherwise *A. parkinsoniana*, *E. gunteri*, *F. squamosa*, *B. pseudoplicata* and *S. fusiformis* for instance responded positively to the total organic matter increment, in sites influenced by anthropogenic contamination, associated with the enrichment of lipids which can represent a more recalcitrant fraction of OM and with the action of bacteria found in sewage.

Some benthic foraminifera species are known to use herbivory and bactivory such as *Ammonia*, *Bolivina*, *Rosalina*, *Elphidium*, *Quinqueloculina*, whereas some species are detritivorous, such as *Globobulimina* and *Reophax*, or feed on phytodetritous as for instance *Cassidulina*, *Globocassidulina* and *Fursenkoina*, or are predators such as some *Ammonia* species (Murray, 2006 and references therein).

Results of this work highlight characterization of a coastal system using bioindicators. It is also important not only to use physicochemical parameters and metals' concentrations or other kind of pollutants but also to access the quantity and the quality of organic matter. This could be also important to understand the tolerance of opportunistic species to the environmental stress.

## 5. Conclusion

The assemblages' composition of benthic foraminifera in the Bizerte Lagoon is different from the other Mediterranean lagoons, including not only species such as *A. parkinsoniana* which are common in lagoonal environments but also marine ones such as *Cassidulina/Globocassidulina*, Bolivinids/Buliminids and *Nonionella/Nonionoides* that are present in high numbers both in dead and living assemblages. These species known for opportunistic adaptive strategy seem to be favored by physicochemical characteristics of bottom water, similar to those found in the nearby marine environment and abundance of food of relatively high quality. However, they found in the lagoon some stressful factors.

One of the main determining factors of the generic size and structure of living communities of benthic foraminiferal assemblages inside the lagoon seem to be the supply of organic matter of high quality to the benthic compartment. The accumulation of OM on the sediments may cause the oxygen decline and the AVS production which is observed in most areas of the lagoon. These adverse conditions and the relatively high availability of metals in some zones should be harmful for the benthic organisms. In spite of that some opportunistic species which are more common in oceanic environments populate the lagoon probably attracted by the quality of organic matter. The results of this work also indicate that it should be important to study the quality of organic matter to access the trophic state and the environmental quality of coastal

ecosystems and to understand the organisms answers to environmental stress.

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