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MULTIPROXIES (BENTHIC FORAMINIFERA, OSTRACODS AND BIOPOLYMERS) APPROACH APPLIED TO IDENTIFY THE ENVIRONMENTAL PARTITIONING OF THE GUADIANA RIVER ESTUARY (IBERIAN PENINSULA)

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Abstract

The Guadiana River is the fourth longest river in Europe and is a natural frontier between southern Portugal and Spain. This river was historically used to transport minerals exploited in the region since the Roman Empire and therefore suffered human interventions that have been intensified after the industrial revolution. The Guadiana River has in its limits the Guadiana Valley Natural Park, which is of great value for the Conservation of Geobiodiversity. This study mainly aims to identify zones with the environmental characteristics in the estuarine area of the Guadiana River based on the distribution and ecology of microorganisms (ostracods and foraminifera) associated with physicochemical parameters and sedimentological and geochemical (carbohydrate, lipid, protein, total organic carbon and total sulfur) data. Fifty-five foraminifera taxa were identified along the estuary with dominance of *Ammonia tepida* and *Miliammina fusca* and 13 ostracods taxa with dominance *Leptocythere lacertosa* and *Loxoconcha elliptica*. Detrended correspondence analysis (DCA) performed using biotic and abiotic variables indicated that pH, grain size, total organic carbon (TOC) and lipids were the most influent factors in the distribution of these organisms. Four zones were identified in the Guadiana River estuary: i) Low estuary - region with the largest marine influence with sandy sediment, higher salinity and total sulfur and mainly represented by the dominance of estuarine species of foraminifera (*Ammonia tepida* and *Cribroelphidium vadescens*) and by the ostracods (*Darwinula stevensoni*, *Semicytherura sulcata* and *Urocythereis oblonga*); ii) Intermediate estuary - region characterized by neutral pH and sandy sediment enriched in carbohydrates; this region is characterized by the presence of the ostracods species *Cytherois fischeri* and *Neocytherideis subulata* and by calcareous and agglutinated species in foraminiferal assemblages; iii) Upper estuary - silt, high TOC, proteins and lipids content, and by the presence of *Loxoconcha elliptica*; iv) Freshwater environment - has similar characteristics to the upper estuary, but do not display foraminifera and was composed of ostracod species such as *Cyclocypris ovum*, *Heterocypris incongruens* and *Ilyocypris* sp. These species are commonly found in freshwater environments or in waters with very low salinity. The predetermined limit in previous studies for the intermediate/upper estuary shifted approximately 5 km further north. This effect may be due to human influence, since a dam was built on the river decreasing the freshwater flow and allowing marine water entrance to an inner area of the estuary.

Keywords: Microfossils. Bioindicators. Meso-tidal estuary. Estuarine hydrodinamic.

1. Introduction

Estuaries represent one of the most biologically productive natural habitat of the planet and offer countless ecosystem services that rely on the overall environmental quality (Costanza et al., 1997). The rapid world population growth particularly in coastal areas has resulted in a great ecological stress on aquatic ecosystems, like estuaries (Flemer and Champ, 2006). The effects on the local biota depend on the nature and the volume of the effluents, whether they are discharged directly into the estuary from a point source or, indirectly, through river system and, eventually, on the hydrographical and geomorphological properties of the estuary (Alve, 1995). Every estuarine ecosystem is unique and represents a complex buffer zone between fresh and salt water environments.

The Guadiana River crosses extensive rural area of massive sulphide deposits, the Iberian Pyrite Belt, with intense mining extraction since Roman Age (Palanques et al., 1995; Leistel et al., 1998). Despite the pyrite extraction has ceased in the last decade, the estuary is still affected by the input of domestic sewages from two cities located near the mouth (Chícharo et al., 2001; Domingues et al., 2005). Since 2002, more than 80% of the freshwater flow has been regulated by the Alqueva Dam that is 140 km upstream of the estuarine mouth and minimizes the abrupt river discharges to the estuary. This dam has a water storage capacity of ca. 4150 hm^3 (Cravo et al., 2006). The impounding of freshwater behind dams causes a significant reduction in freshwater discharge in such areas (Humborg et al., 1997) that results in a decrease in suspended particulate matter and nutrient concentrations (Wetsteyn and Kromkamp, 1994; Humborg et al., 1997). This can lead to dramatic shifts in phytoplankton productivity and composition (Justic et al., 1995; Humborg et al., 1997), as nutrients play a key role in controlling primary production (Libes, 1992; Mortimer et al., 1998; Cruzado et al., 2002). These changes originated marked effects on the biogeochemical cycling of nutrients, in the structure of the food web in coastal areas, and in turn affects biological productivity as well as the ecological balance of these ecosystems (Wright and Worrall, 2001; Cruzado et al., 2002).

The use of benthic organisms such as foraminifera and ostracods as proxies of environmental conditions (hydrodynamic and/or pollution) in coastal environments has been intensified in recent years (Souza et al., 2010). Benthic foraminifera are particularly useful for classifying estuaries (e.g., types of estuaries, salinity gradients, hydrodynamic conditions, sedimentological compartments) and to identify anthropic impacts (Nichols, 1974; Laut et al. 2010, 2011, 2012; Souza et al., 2010). Each estuary has its own characteristic assemblages that mirror specific environment parameters and conditions (Nichols, 1974).

Coimbra et al. (2007) indicated that ostracods can also be used as bioindicators in estuaries like foraminifera because they have high sensitivity to environmental changes and high potential for preservation in the sediment. However, the studies on ostracods fauna in coastal regions started only in 1960. According to Laut et al. (2014), the current knowledge of the distribution of foraminiferal and ostracod assemblages and their environmental constraints in estuarine regions can represent the starting point for monitoring and environmental management and serve as the background for studies of sea level changes and Quaternary evolution of coastal regions. Muñoz et al. (1995) used for the first time foraminiferal and ostracod assemblages to identify estuarine compartments by using only qualitative parameters in the Iberian Peninsula.

The aims of this study is to characterize the quality of organic matter, physicochemical parameters and benthic

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foraminiferal and ostracod assemblages distribution after Alqueva Dam construction using multivariate analyses to identify different environmental sectors of Guadiana River estuary.

2. Study area

The Guadiana River headland is located in Lagoas de Ruidera (Spain) at 1,700 m altitude. The river runs 810 km until reaching the Atlantic Ocean between the Portuguese town Vila Real de Santo António and Spanish town Ayamonte. The Portuguese stretch of the river is 260 km long, of which 110 km delimit the border with Spain (INAG, 2001; Chícharo et al., 2006). The Guadiana River estuary is located in SW Portugal and in physical terms embraces the 70 km of the total length of Guadiana River (Wolanski, 2006). Boski et al. (2008) sets the Guadiana Estuary as a rich wetland zone, where saltmarshes dominate and salt pans, lagoons, tidal creeks, intertidal flats, barrier islands and sandy spits are widespread. Many other habitats of high ecological value are also found in this region. The hydrologic regime of the Guadiana River reflects the regional Mediterranean climate that is characterized by dry and hot summers as well as mild and wet winters (Morales, 1993).

The Guadiana River estuary is a mesotidal system with semi-diurnal tides with a mean tidal range of 2 m. The tidal wave along the coast moves from east to west, and produces slow velocity currents, when the flood is 0.40 m/s to the west and ebb is 0.30 m/s to the east during a mean spring tide (Instituto Hidrográfico, 1998). In the estuary, waves propagate following a synchronic model (floods can reach 0.80 m/s and ebb can reach 0.90 m/s during a mean spring tide) (Morales et al., 2006). The estuary is prolonged offshore by a submerged delta (Morales, 1997), where fluvial sediment is mixed with the marine material arriving from longshore sediment transport (Gonzalez et al., 2001). The Guadiana River discharge shows a marked inter-annual and seasonal variability (Morales et al., 1997). During periods of maximum discharge, tidal currents can rework and carry all of the sediments that are moved by the river to the sea (Morales et al., 1997).

Fig. 1. Location of the studied stations along the Guadiana River estuary, Algarve, Portugal.

3. Material and methods

3.1. Sampling and sample preparation

In September of 2010, ten sediment samples were collected in the subtidal zone of the Guadiana River estuary in the low tide period (Fig. 1). The sampling points were selected to identify estuarine gradients (Tab. 1).

In each station, a total of 50 ml of sediment were collected in triplicate using a van Veen Grab for foraminiferal and ostracod analyses (Fig. 1). The sediment was stored in plastic containers with alcohol 70% and Rose Bengal (2 g L^{-1}) to avoid the tests degradation by bacterial activity and for the identification of living organisms during sampling. A total of 100 g of sediment in each station was sampled and stored in plastic bags to grain size and biopolymer analyses.

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The physiochemical parameters of the water, such as salinity (refractometer, model 10419, American Optical), temperature, pH (pH-meter CG837, Schott Gerate) and dissolved oxygen (O_2 Meter CG867, Schott Gerate) were measured *in situ*.

3.2. Laboratory analyses

3.2.1. Grain size analysis

Grain size analyses were accomplished according to the methodology described by Suguio (1973), by wet sieving separating mud (silt and clay) and sand fractions through a 0.062 mm sieve. The finest fractions were analyzed using the pipette method (Suguio, 1973).

3.2.2. Geochemical analysis

The percentages of total organic carbon (TOC) and total sulfur (S) were analyzed in dry sediment with a LECO SC 144 device, after acidification to remove carbonates, according to ASTM D 4239 (American Society for Testing and Materials - ASTM, 2008) and NCEA-C-1282 (United States Environmental Protection Agency-US EPA, 2002) methodologies. These analyses were performed in triplicate in Laboratório de Palifacies & Fácies Orgânicas from Universidade Federal do Rio de Janeiro.

Biopolymers were determined according to Silva et al. (2013) and Martins et al. (2015a). The CHO, PTN and LIP concentrations were converted to carbon equivalents by using the following conversion factors: 0.49, 0.40 and 0.75 μg C/g, respectively.

3.2.3. Microfaunal analysis

Benthic foraminiferal and ostracod assemblages were studied in the dry sediment fraction 63-1000 μm. In this study was considered only living (stained) foraminifera and ostracods. The foraminiferal and ostracod density, expressing as the number of stained tests (foraminifera) and carapace (ostracods) per volume (50 ml of sediment), was determined. Foraminiferal density (FD) is evaluated in number of test/50 ml.

Foraminiferal genera were taxonomically classified in accordance with Loeblich and Tappan (1988). The taxonomic identification, at species level, was based on several publications, notably Todd and Brönnimann (1957), Boltovskoy et al. (1980), Debenay et al. (2002) and Martins and Gomes (2004). The ostracods were taxonomically classified in accordance with Yassini and Jones (1995) and Ruiz et al. (1997, 2000, 2005). The taxonomy has been checked in World Marine Species Database (Appendix 1) (Hayward et al., 2014).

Ecological indexes such as species richness (S), constancy (c), diversity (*H'*), and equitability (*J'*) were used in the data interpretation. The S is the number of species recognized in each sample. The *H'* is an index proposed by Shannon–Weaver (1948), which is appropriate for random samples of a community of species or sub-community of interest, and it is estimated by: $H' = \Sigma pi \ln pi$, where pi represents the portion of i- species in the sample and ln is the natural (base e) logarithm. Equitability (*J'*) is related to the distribution of individuals among the species, being proportional to the *H'* and inversely proportional to the dominance. It compares the *H'* index with the distribution of the observed species, maximizing the diversity. This index is obtained through $J' = H'/\ln(S)$. The software MVSP 3.1 was used for calculation of *H'* and *J'*.

3.3. Statistical analysis

Only samples with at least 100 foraminifera or ostracods specimens were considered for statistical analyses because they may provide reliable results concerning the species representativeness in the assemblage composition (Fatela and Taborda, 2002). The GD09 (less than 100 foraminifera) and GD10 stations (without foraminifera) were excluded from statistical analysis. The relative abundances of the foraminiferal and ostracod taxa were analyzed by detrended correspondence analysis (DCA) and used to show their relationship with grain size, salinity, pH, TOC and biopolymers (CHO, LIP and PTN). All data were normalized with square root of 0.5. The DCA analysis computed as relative Euclidean distance was performed with PCord 5.0 software.

4. Results

4.1. Abiotic parameters

Salinity showed a gradient ranging from 28.06 (GD01) close to the estuary mouth to 0.18 in the upper part of the estuary (GD 09 and GD10). The higher values were measured in the tributary channel (GD04: 29.35 and GD05: 23.43). The pH values ranged from 6.6 to 7.78 along the estuary (Tab. 1).

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The TOC values remained low throughout the river (Tab. 1), the highest value (1.88%) was found in the innermost area of the river, near Alcoutim town (GD10) and the lowest (0.52%) near the river mouth (GD01).

The concentration of biopolymers showed a similar pattern of increasing gradient (GD01 to GD10), where the lowest values were largely identified at river mouth whereas relatively higher values in the inner part of the estuary (Tab. 1). The LIP, PTN and CHO ranged from 4.42 to 16.29 mg/C g, from 1.24 to 4.4 mg/C g and from 1.31 to 3.39 mg/C g, respectively. Sulfur concentrations varied from 0.04% in the inner portion (GD10) to 0.11% in the outermost stations (GD02 and GD03).

Tab. 1. Physicochemical parameters, sedimentological and geochemical data of the Guadiana River estuary (TOC - Total Organic Carbon, S - Sulphur, CHO - Carbohydrates, LIP - Lipids, PTN - Proteins).

Stations	Lat North	Long West	pH	salinity	TOC (%)	S (%)	CHO ($mg.g^{-1}$)	LIP ($mg.g^{-1}$)	PTN ($mg.g^{-1}$)	Sand (%)	Silt (%)	Clay (%)
GD01	37º 11' 41.0"	07º 24' 21.5"	7.38	28.06	0.52	0.07	1.31	4.42	1.24	100	0	0
GD02	37º 15' 39.1"	07º 25' 40.5"	7.26	25.6	1.37	0.11	2.27	9.14	2.85	77	16	7
GD03	37º 18' 29.5"	07º 26' 35.7"	6.9	11.85	1.36	0.11	2.28	10.59	1.92	48	24	28
GD04	37º 10' 26.4"	07º 23' 21.7"	7.34	29.35	1.33	0.1	1.92	11.33	2.53	30	46	24
GD05	37º12'53.5"	07º25'55.9"	6.89	23.43	1.54	0.1	2.54	15.61	3.27	10	66	24
GD06	37º12'53.4"	07º25'55.9"	7.78	6.23	1.58	0.07	2.41	11.77	3.52	23	63	14
GD07	37º12'53.2"	07º25'55.9"	7.16	2.09	1.87	0.08	3.39	15.81	3.88	32	56	12
GD08	37º 20' 59.3"	07º 26' 41.0"	7.6	1.43	1.84	0.07	3.37	14.01	4.17	65	29	6
GD09	37º 24' 14.7"	07º 27' 31.8"	6.76	0.18	1.88	0.06	3.30	14.00	4.25	36	10	54
GD10	37º 28' 30.3"	07º 28' 13.9"	6.60	0.18	1.88	0.04	1.43	16.29	4.4	63	31	6

Grain-size analysis indicated that the substrate at the stations in the mouth of the estuary was mostly composed of sand (GD01 – 100% and GD02 – 74%), with a marked reduction of the sandy fraction in the inner part of the estuary up to 10% at GD05). In the station GD09 the dominant textural fraction was the clay (54%) (Tab. 1).

4.2. Microfaunal parameters

A total of 57 benthic foraminiferal taxa was identified along the Guadiana River estuary, 42 and 15 of which were calcareous and agglutinated, respectively (Appendix 1). Selected taxa are represented in Plates 1 and 2. All the benthic foraminiferal data including FD, S, *H'* and *J'* are reported in Appendix 1. Foraminiferal density varied from zero (GD10) to 712 (GD01). The most abundant species were *Ammonia tepida* (mean of stations 28%) followed by *Miliammina fusca* (mean of stations 34%). Other calcareous species like *Ammonia parkinsoniana*, *Cribroelphidium excavatum*, *Elphidium gunteri*, *Haynesina germanica*, *Miliolinella subrotunda*, *Quiqueloculina lamarckiana* and *Rosalina bradyi* were constant in the samples but showed low values of relative abundance (Appendix 1).

The *H'* index was relatively lower in the GD01 and GD07 stations and the highest values (2.28) was recorded in the GD08 station. The *J'* showed values ranging from 0.4 (GD01) to 0.9 (GD09) (Appendix 1).

Along the Guadiana River estuary 13 ostracods taxa were found (Appendix 2; Plate 3). The density of living ostracods (number of specimens/50 ml sediment) ranged from 100 (GD05) to 1092 (GD02). The GD01 and GD10 stations with seven recognized taxa showed the highest S. The *H'* index ranged from 0.33 (GD04) to 1.64 (GD01), the *J'* index showed the lowest value in GD04 (0.30) and the highest in GD03 (0.97). *Loxoconcha elliptica* (ranging from 14.3%, in GD01, and 50.3%, in GD06) and *Leptocythere lacertosa* (ranging from 4.3% ,in GD10, and 91.2%, in GD04) were the most abundant ostracod species in the estuary (Appendix 2).

4.3. Statistical analysis

Multivariate DCA analysis was based on sediments granulometry, salinity, pH, TOC and biopolymers (CHO, LIPs and PTN) and the relative abundance of the main foraminiferal and ostracods species. The axis 1 of the DCA explains 57% of data variability and the axis 2 only 6% (Fig. 2). Environmental variables with larger vectors and with lower angle with the axis 1 is pH, grain size fractions (sand, silt and clay) and the percentage of TOC and LIP. For the axis 2, the most important environmental variable was CHO content.

The DCA analysis distributed the species and stations into five groups. Group I is composed by the species of ostracods *Darwinula stevensoni*, *Semicytherura sulcata* and *Urocythereis oblonga* and by the station GD01. These variables responded positively mainly to pH and sand increasing and negatively to PTN, TOC and LIP.

Group II has not ostracods representatives but solely encloses benthic foraminifera, in particular *Trochammina inflata* as the only agglutinated foraminiferal taxon and the following calcareous species *A. tepida*, *Ammonia* sp., *Astrononion stelligerum*, *Cribroelphidium excavatum*, *Cribroelphidium vadescens*, *Elphidium complanatum*, *Elphidium crispum*, *Elphidium gunteri* and *Patellina corrugata*. These variables answered positively to the increasing of salinity. Group II also contains the stations GD02, GD03, GD04 and GD05.

Group III contains *Cytherois fischeri* and *Neocytherideis subulata* as ostracods taxa and the agglutinated species of foraminifera: *Arenoparrella mexicana*, *Entzia macrescens*, *Miliammina fusca*, *Siphotrochammina lobata*, *Trochamminita salsa* and *Textularia earlandi*. The following calcareous species also belong to this group: *Adelosina longirostra*, *Ammonia parkinsoniana*, *Bolivina variabilis*, *Elphidium discoidale*, *Entzia polystoma*, *Haynesina germanica*, *Quinqueloculina lamarckiana*, *Quinqueloculina seminula* and *Rosalina bradyi*. Group III also encompasses the stations GD06, GD07 and GD08 which seem to be positively associated with pH, and CHO increase.

Group IV embraces the ostracod species *L. elliptica* and the station GD09 whereas Group V encompasses the species of ostracods *Cyclocypris ovum*, *Heterocypris incongruens* and *Ilyocypris* sp. as well as the station GD10. Both Groups IV and V had no foraminifera and responded positively to finer grained sediments, TOC and PTN and LIP content (biopolymers) and negatively to salinity, pH and sand fraction. The species of ostracod *Leptocythere lacertosa* is isolated in the DCA and seemed to positively respond to fine fraction increasing (Fig. 2).

5. Discussion

Most of the analyzed abiotic parameters showed gradients from the mouth to the inner area of the river. The sedimentary dynamic processes within this estuary are the result of the balance between discharges of freshwater and tides providing sediments that may be altered by climate, sea level fluctuations and human action (Camacho et al., 2014, 2015).

The studied stations are characterized by sand to very silty sand substrates. This distribution can be related to changes in the energy resulting from different tidal conditions and seasonal hydrodynamic (Dias et al., 2001). Grain-size analysis also indicated a gradient with coarser sediments at the mouth which became progressively finer towards inner zone of the estuary, though the sandy fraction is prevalent in the innermost stations (GD08 and GD10). This pattern of particle size distribution evidences the importance of marine hydrodynamic processes on the external part of the estuary (Machado et al., 2007).

Salinity decreased along the river. The stations GD01, GD02, GD03 and GD04, nearest the mouth, showed the highest values of salinity due to the proximity to the sea. The stations GD06-GD10 have lowest salinity, but also show the influence of the tides in the upper course. The hydrodynamic forces associated to a mesotidal regime, with an average range of 2 m, and wave action that propagate into the interior of the Guadiana River estuary, lose progressively the energy (Morales, 1993; Muñoz et al., 1995). The action limit of the salt wedge in the estuary can be seen in the stations GD09 and GD10. The average values of the salinity found along the estuary were similar to those recorded by Muñoz et al. (1995) and Camacho et al. (2014, 2015). The station GD05 has a relatively high salinity despite being in the intermediate compartment of the river. This station is located in a tributary channel that receives a lower contribution of fresh water relative to the amount of saltwater and where the action of winds intensifies the evaporation. These processes increase salinity in this area (Morales, 1993; INAG, 2001). The pH values display low variability through the estuary suggesting minor change as also observed by Muñoz et al. (1995) and Camacho et al. (2014, 2015).

Values of TOC and biopolymers in the studied stations are relatively low and presents an opposite gradient to that of salinity. Values of TOC and biopolymers increase towards the river inland, probably due to an increase of continental organic matter contribution as noted by Cravo et al. (2006). According to Mendonça-Filho et al. (2003), when TOC concentrations exceed 4%, the environment is dysoxic/anoxic. The higher values of TOC and biopolymers are observed in coastal environments with greater anthropic influence in tropical regions such as Guanabara Bay and Bertioga Channel (Brazil) and impacted regions at Iberian

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Peninsula such as in Ria de Aveiro (Mendonça-Filho et al., 2003; Eichler et al., 2003; Clemente et al., 2015; Martins et al., 2015a, b, c). Low TOC concentrations founded in Guadiana River Estuary were generally be observed in other coastal regions that are affected by wave action, such as in the Arade river in Portugal (Laut et al., 2015) and in the Paraiba do Sul River delta in Brazil (Laut et al., 2012). These results indicate that the hydrodynamic conditions in Guadiana River estuary avoids the deposition of organic matter and its preservation in surface sediments.

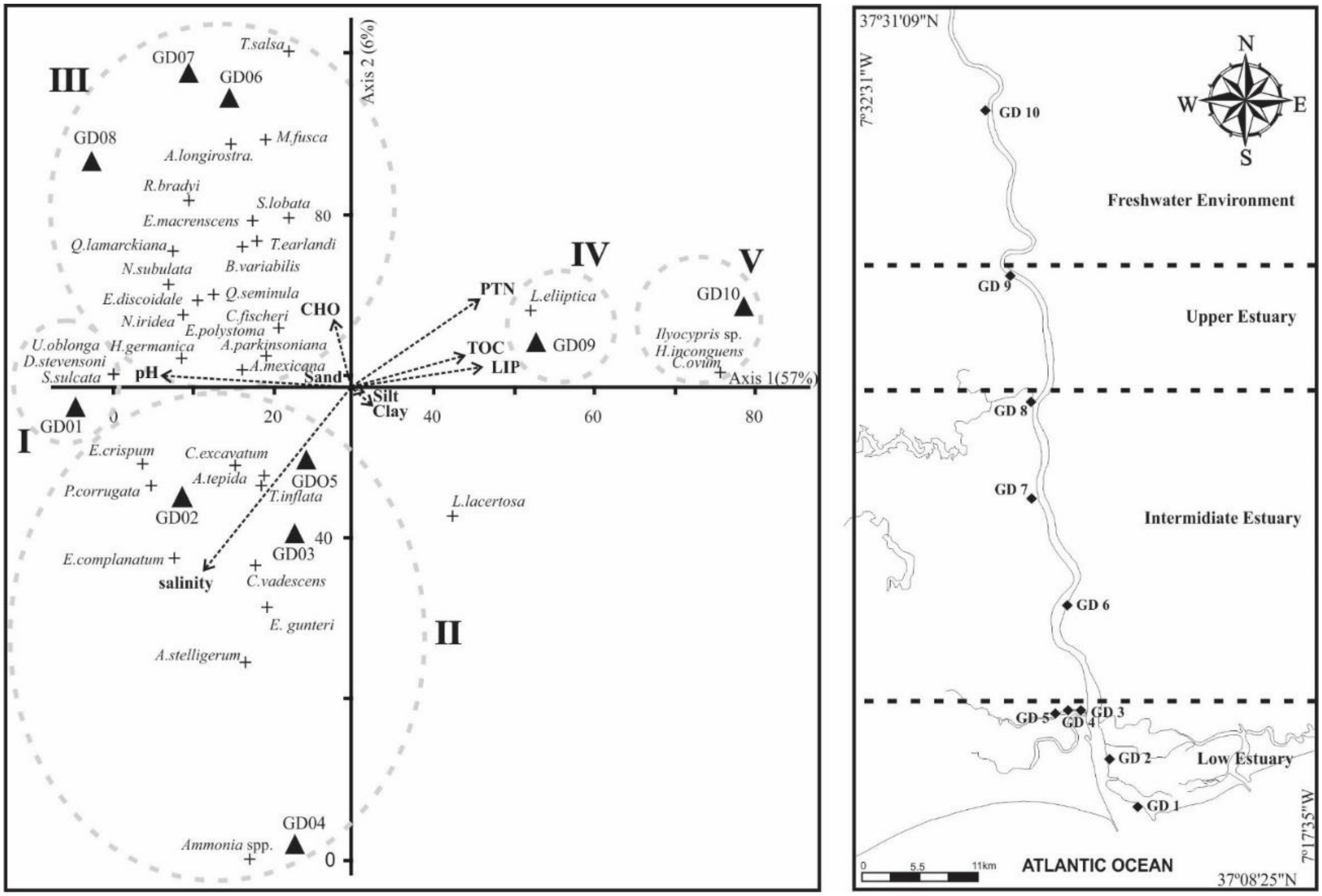

Fig. 2. DCA multivariate analysis of the stations and foraminiferal and ostracods species, the distribution of stations groups and estuarine compartments proposed to Guadiana River Estuary (PTN - proteins, CHO - carbohydrates; LIP - lipids; TOC - total organic carbon).

5.1. Ecological parameters

The studied stations of the Guadiana River estuary showed higher number of species of foraminifera (57 species) than those described by Muñoz et al. (1995). However, the specific richness was comparable to the values documented by Camacho et al. (2014, 2015). This value is higher than that found in other estuaries of the south of Iberian Peninsula such as that of Arade River (Laut et al., 2014) and Tinto-Odiel system (Ruiz et al., 2005). In the Guadiana River estuary, mostly cosmopolitan and calcareous species (42 species) were found such as *A. tepida*, *A. parkinsoniana*, *C. excavatum*, *E. gunteri*, *H. germanica*, *M. subrotunda*, *Q. lamarckiana*, *Q. seminula* and *R. bradyi* that are considered characteristic of mixohaline and brackish environments (Muñoz et al., 1995; Ruiz et al., 2005; Laut et al., 2014). Agglutinated species, such as *A. mexicana*, *E. macrescens*, *E. polystoma*, *T. inflata* and *M. fusca*, are relatively abundant in the studied stations.

They are cosmopolitan and occur in other Iberian Peninsula estuaries and lagoons, such as the Arade river (Laut et al., 2014) and Ria de Aveiro (Martins et al., 2010, 2013, 2014, 2015 a, c). These species have high tolerance to salinity variations and tend to live in brackish environments

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such as estuaries, mangroves and salt marshes (Souza et al., 2010).

The number of ostracod species recognized in the Guadiana River estuary are similar to those described by Muñoz et al. (1995) in this estuary and also in the River Arade estuary, also located in Algarve, Portugal (Laut et al., 2015). The most abundant taxa were *Loxoconcha elliptica*, *Leptocythere lacertosa* and *Cytherois fischeri*. These species are considered cosmopolitan, indigenous and are adapted to eurihaline conditions in the estuaries of the Iberian Peninsula (Muñoz et al., 1995; Laut et al., 2015). The density, specific richness and diversity of foraminifera and ostracoda reflect a similar pattern to that found in the same area in previous studies, such as Muñoz et al. (1995) and Camacho et al. (2014, 2015). These authors suggest that factors such as salinity, grain size and the input of organic matter are the main factors driving the pattern of distribution of these organisms. The same is suggested by the results of DCA analysis included in Figure 2.

5.2. Ecological parameters

The DCA analysis (Fig. 2) indicated that the foraminiferal and ostracod species respond, to some extent, to abiotic variables such as salinity, grain size and organic matter (TOC and biopolymers) suggesting an influence of hydrodynamic factors along the river. These variables allowed to distinguish four distinct regions.

Low Estuary. Region with the largest marine influence that presents coarser sediment and highest salinity. This part comprises the region of the mouth represented by the Group I of the DCA (GD01 station and the following species of ostracod *D. stevensoni*, *S. sulcata*, *U. oblonga*) and the Group II of the DCA (GD02, GD03, GD04 and GD05) (Fig. 2). The latter includes calcareous foraminifera species such as *A. tepida*, *A. stelligerum*, *C. excavatum*, *C. vadescens*, *E. crispum*, *E. complanatum*, *E. gunteri*, *P. corrugata*, and the agglutinated species *T. inflata* that have high tolerance to changes in physicochemical parameters (Murray, 1991). These species were also found by Mendes et al. (2004) on the nearby continental shelf. Of these species, the most common in worldwide estuaries is *Ammonia tepida* (Murray, 1991). This species occurs due to its high tolerance to environmental variations (Martins et al., 2010, 2013, 2014, 2015 a, c). The same was observed by Muñoz et al. (1995) and Camacho et al. (2014, 2015) in this estuary. The ostracod species *S. sulcata* and *U. oblonga* live in marine and coastal brackish water environments (Perçin-Paçal et al., 2015). These species have also been identified in previous studies in this region (Muñoz et al., 1995) and also at the mouth of Arade River (Laut et al., 2015).

Intermidiate Estuary. Transitional region with sandy sediments and enriched in carbohydrates. This region is represented by the Group III of DCA including stations GD06, GD07 and GD08, and ostracods *Cytherois fischeri* and *Neocytherideis subulata* (Fig. 2) that are reported as estuarine species and commonly found in this area of the Guadiana River estuary (Muñoz et al., 1995) and at the mouth of the Arade River (Laut et al., 2015). This group also contains the agglutinated foraminifera species *A. mexicana*, *E. macrescens*, *M. fusca*, *S. lobata*, *T. earlandi* and *T. salsa*. Calcareous taxa such as *Adelosina longirostra*, *Ammonia parkinsoniana*, *Bolivina variabilis*, *Elphidium discoidale*, *Entzia polystoma*, *Haynesina germanica*, *Quinqueloculina lamarckiana*, *Quinqueloculina seminula* and *Rosalina bradyi* are also grouped in it. Carbohydrates enrichment suggests that the organic matter has phytoplanktonic or detrital origin (Cotano and Villate, 2006). The source areas of detrital organic materials are probably the salt marsh estuary margins. The species *A. mexicana*, *E. polystoma*, *E. macrescens*, *S. lobata* and *T. salsa* should be also linked with this contribution (Souza et al., 2010). The great predominance of *M. fusca* species in this group is related to an increase of fluvial influence (Debenay et al., 2003; Camacho et al., 2014, 2015; Laut et al., 2014). The miliolids such as *Q. seminula* have been found in several transitional ecossystems associated with sandy sediment and basic to neutral pH (Eichler et al., 2003; Martins et al., 2013, 2014, 2015 a, c; Laut et al., 2014).

The region defined as intermediate estuary agrees with the previously defined in the Guadiana River (Muñoz et al. 1995). However, the boundary between this compartment and the upper estuary differs in about 5 km further north to the limits set by Muñoz et al. (1995). This effect may be related to the construction of the Alqueva Dam that began operating in 2002, this is after the study performed by Muñoz et al. (1995) for the Guadiana River estuary. According to Gonzalez et al. (2001), after the construction of the Alqueva Dam the river flow volume was greatly reduced. The anthropic change and the dry and arid climatic conditions during the sampling period of this work, promoted the reduction of river flow which may have facilitated the saline wedge entrance by some kilometers further inland.

Upper Estuary. This region is the innermost area of the Guadiana River estuary (Fig. 2). This zone is characterized by finer sediments (silt and clay), relatively high TOC content as well as by proteins and lipids enrichment. Group

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IV of the DCA (Fig. 2) represents this zone, composed of station GD09 and the species of ostracoda *L. elliptica*. The occurrence of the species *L. elliptica* along the Guadiana River estuary was also observed by Muñoz et al. (1995). This species is considered an indicator of eurihaline confined environments with high organic matter content enriched in lipids (Loureiro et al., 2009; Laut et al., 2015). This region was considered the area limit of the upper estuary (Fig. 2). Living foraminifera, which are known to avoid or be absent in freshwater environments, were yet found in this area, however with low density (<100 specimens/50 ml). Foraminifera of this station were not considered in the statistical treatment, however, the presence of these living organims suggests that the salt wedge also are affecting this region and are moving each more northward.

Freshwater region. A region with characteristics similar to the upper estuary but with greatest influence of freshwater (Fig. 2). Group V represents this region and is composed by the station GD10 and by the ostracod species *C. ovum*, *H. incongruens* and *Ilyocypris* sp. Living foraminifera were not found in the station GD10. The identified ostracods species are commonly found in freshwater environments or in environments with very low salinity values (Muñoz et al., 1995; Külköylüoglu, 2003). The species *L. lacertosa* is isolated in quadrant IV of the DCA (Fig. 2) and might be associated with the enrichment of organic matter in lipids and proteins. This species is commonly found in environments with high concentrations of organic matter (Loureiro et al., 2009) and was observed in most stations, but had higher density values in the stations GD04 and GD05, located in a tributary channel. In these stations, a large number of ostracods juveniles were observed, possibly because they are more sheltered areas that favor the reproduction of this mesohalin species (Meric et al., 2010).

6. Conclusion

The Guadiana River estuary from the mouth to the innermost portion displayed a decreasing gradient of physicochemical parameters (i.e., salinity, pH) and an increasing one of biochemical factors (TOC and biopolymers). On the basis of these factors coupled with benthic foraminiferal and ostracod assemblages, the estuary can be divided into four main regions (low estuary, intermediate estuary, upper estuary and freshwater environment) similar to those identified in previous studies. This study evidences a displacement of approximately 5 km further north of the intermediate/upper estuary limit. This may be related to human influence due to the construction of the Alqueva Dam that contributed to the decrease, at least seasonally, the freshwater flow, which allowed a strong salt wedge penetration in the innermost area of this river.

The distribution of ostracod assemblages confirm the identification and the separation of different environments in the Guadiana River Estuary. The appearance/disappearance of the ostracod species *Neocytherideis subulata*, *Semicytherura sulcata* and *Urocythereis oblonga* at the downstream region and *Cyclocypris ovum*, *Cypridopsis vidua*, *Heterocypris incongruens* and *Ilyocypris* sp. agrees with the suggested compartmentalization of this estuary.

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Appendix 1. Foraminiferal density (FD: number of test/50 ml), species richness (S: number of species per sample), diversity (*H'*), Equitability (*J'*) and percentage of species in the studied stations of the Guadiana River estuary.

Variables/Stations	GD01	GD02	GD03	GD04	GD05	GD06	GD07	GD08	GD09
Living foraminifera density (number of test/50 ml)	712	304	207	248	300	199	252	141	27
Specific Richness (number of species per sample)	27	25	10	10	9	19	15	21	8
Diversity (*H'*)	1.12	2.16	1.28	1.25	1.32	1.42	1.06	2.26	1.78
Equitability (*J'*)	0.4	0.7	0.6	0.5	0.6	0.5	0.4	0.7	0.9
Adelosina longirostra (d'Orbigny, 1846)	0.1	-	-	-	-	0.5	0.4	-	-
Adelosina cliarensis (Heron-Allen & Earland, 1930)	0.1	-	-	-	-	-	-	-	-
Ammonia parkinsoniana (d'Orbigny, 1839)	0.1	1.3	-	53.2	2.0	3.0	-	2.8	-
Ammonia sp.	-	2.0	-	1.6	-	-	-	-	-
Ammonia tepida (Cushman, 1926)	2.8	31.6	58.9	-	61.7	21.1	16.7	17.7	14.8
Arenoparrella mexicana (Kornfeld, 1931)	-	0.3	1.4	-	-	1.0	-	5.7	-
Asterigerinata mamilla (Williamson, 1858)	0.7	-	-	0.8	-	-	-	-	-
Astrononion stelligerum (d'Orbigny, 1839)	0.6	7.9	16.9	3.2	-	-	-	2.1	-
Aubignyna planidorso (Atkinson, 1989)	-	-	-	-	-	-	-	0.7	-
Bolivina sp.	-	-	-	-	-	-	-	0.7	-
Bolivina variabilis (Williamson, 1858)	-	1.6	-	-	-	2.0	-	0.7	-
Cribroelphidium excavatum (Terquem, 1875)	-	2.0	-	-	2.0	-	-	0.7	-
Cribroelphidium vadescens Cushman & Brönniman, 1948	-	21.1	2.4	-	4.0	0.5	0.8	-	25.9
Discorbis parkeri Natland, 1950	-	-	-	-	-	-	-	0.7	-
Eggerelloides scaber (Williamson, 1858)	-	0.7	-	-	-	-	-	-	-
Eilohedra vitrea (Parker, 1953)	0.1	-	-	-	-	-	-	-	-
Elphidium advenum (Cushman, 1922)	1.0	-	-	-	-	-	-	-	-
Elphidium complanatum (d'Orbigny, 1839)	0.3	0.7	-	-	-	-	-	-	-
Elphidium crispum (Linnaeus, 1758)	2.1	0.3	-	-	-	-	-	-	-
Elphidium discoidale (d'Orbigny, 1839)	1.7	0.5	-	-	-	-	0.4	6.4	-
Elphidium gerthi Van Voorthuysen, 1957	-	1.0	-	-	-	-	-	-	-
Elphidium gunteri Cole, 1931	-	1.3	13.0	0.4	3.3	0.5	1.6	7.1	18.5
Entzia macrescens (Brady, 1870)	-	0.3	0.5	-	-	1.0	0.8	1.4	3.7
Entzia polystoma (Bartenstein & Brand, 1938)	-	-	5.3	29.8	1.3	2.0	0.4	2.1	-
Eoeponidella pulchella (Parker, 1952)	-	0.3	-	0.4	-	-	-	-	-
Haynesina germanica (Ehrenberg, 1840)	78.1	5.3	-	-	7.3	-	2.4	8.5	3.7
Hoeglundina elegans (d'Orbigny, 1878)	1.0	-	-	0.8	-	-	-	-	-
Lamarckina haliotidea (Heron-Allen & Earland, 1911)	0.4	-	-	-	-	-	-	-	-
Lenticulina limbosa (Reuss, 1863)	-	-	-	-	-	-	-	0.7	-
Lepidodeuterammina eddystonensis Brönnimann & Whittaker, 1990	-	-	-	-	-	-	-	0.7	-
Lepidodeuterammina ochracea (Williamson, 1858)	-	-	-	-	-	0.5	-	-	-
Lobatula lobatula (Walker & Jacob, 1798)	2.0	-	-	-	-	-	-	-	-

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Appendix 1 (cont.). Foraminiferal density (FD: number of test/50 ml), species richness (S: number of species per sample), diversity (*H'*), Equitability (*J'*) and percentage of species in the studied stations of the Guadiana River estuary.

Variables/Stations	GD01	GD02	GD03	GD04	GD05	GD06	GD07	GD08	GD09
Melonis sp.	-	0.1	-	-	-	-	-	-	-
Miliammina fusca (Brady, 1870)	-	-	0.5	-	16.0	60.3	71.0	34.0	25.9
Miliolinella sp.	0.1	-	-	8.5	-	-	-	-	-
Miliolinella subrotunda (Montagu, 1803)	-	-	-	-	-	0.5	-	-	-
Nonion spp.	-	4.0	-	-	-	-	-	-	-
Nonionella iridea Heron-Allen & Earland, 1932	0.1	0.7	-	1.2	-	-	-	1.4	-
Patellina corrugata Williamson, 1858	1.0	0.3	-	-	-	-	-	-	-
Planorbulina mediterranensis d'Orbigny, 1826	0.1	-	-	-	-	-	-	-	-
Quinqueloculina lamarckiana d'Orbigny, 1839	2.1	-	-	-	-	0.5	-	-	-
Quinqueloculina seminula (Linnaeus, 1758)	3.2	14.5	-	-	-	1.0	3.4	1.4	3.7
Quinqueloculina poeyana d'Orbigny, 1839	0.1								
Quinqueloculina spp.	0.3	-	-	-	-	2.5	-	2.8	-
Reophax nana Rhumbler, 1913	-	0.1	-	-	-	-	-	-	-
Rosalina bradyi (Cushman, 1915)	0.6	-	-	-	-	-	0.4	-	-
Siphotrochammina lobata Saunders, 1957	-	-	0.5	-	-	0.5	0.4	-	-
Sphaeroidina bulloides d'Orbigny, 1826	0.1	-	-	-	-	-	-	-	-
Textularia earlandi Parker, 1952	-	1.0	-	-	-	0.5	0.4	-	-
Textularia pseudogramen Chapman & Parr, 1937	0.6	-	-	-	-	-	-	-	-
Tiphotrocha comprimata (Cushman & Brönnimann, 1948)	-	-	-	-	-	-	-	1.6	-
Tritaxis squamata (Jones & Parker, 1860)	-	-	-	-	-	0.5	-	-	-
Trochammina inflata (Montagu, 1808)	-	1.0	0.5	-	2.3	-	0.4	-	3.7
Trochamminita salsa (Cushman & Brönnimann, 1948)	-	-	-	-	-	1.5	0.4	-	-
Wiesnerella auriculata (Egger, 1893)	0.7	-	-	-	-	-	-	-	-

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Appendix 2. Ostracods density (number of test/50 ml), species richness (S: number of species per sample), diversity (*H'*), Equitability (*J'*) and percentage of species in the studied stations of the Guadiana River estuary.

Variables/Stations	GD01	GD02	GD03	GD04	GD05	GD06	GD07	GD08	GD09	GD10
Number of living organisms (50 ml)	1020	1092	549	103	100	141	210	954	948	927
Species Richness (S)	7	5	3	3	3	4	3	3	3	7
Diversity (*H'*)	1.64	1.07	1.06	0.33	0,99	0.83	0.84	0.70	0.84	1.55
Equitability (*J'*)	0.84	0.66	0.97	0.30	0.90	0.63	0.76	0.64	0.77	0.80
Cyclocypris ovum (Jurine, 1820)	-	-	-	-	-	-	-	-	-	12.8
Cypridopsis vidua (O.F. Muller, 1776)	-	-	-	-	-	-	-	-	-	2.1
Cytherois fischeri (Sars, 1866) Brady & Norman, 1889	-	50.9	28.2	1.3	18.0	4.9	27.9	71.0	31.0	-
Darwinula stevensoni (Brady & Robertson, 1870)	5.7	-	-	-	-	-	-	-	-	2.1
Heterocypris incongruens (Ramdohr, 1808)	-	-	-	-	-	-	-	-	-	40.4
Ilyocypris sp.	-	-	-	-	-	-	-	-	-	23.4
Leptocythere lacertosa (Hirschmann, 1912)	8.6	27.6	26.9	91.2	55.1	44.5	7.7	-	6.9	4.3
Loculicytheretta pavonia (Brady, 1866) Ruggieri, 1954	2.9	-	-	-	-	-	-	-	-	-
Loxoconcha elliptica Brady, 1868	14.3	20.6	45.0	7.5	26.9	50.3	64.5	25.8	62.1	14.9
Neocytherideis subulata (Brady, 1868) Wagner, 1957	7.1	0.6	-	-	-	0.3	-	3.2	-	-
Palmoconcha guttata (Norman, 1865) Penney, 1987	-	0.3	-	-	-	-	-	-	-	-
Semicytherura sulcata (Mueller, 1894) Ruggieri, 1959	21.4	-	-	-	-	-	-	-	-	-
Urocythereis oblonga (Brady, 1866) Wagner, 1957	40	-	-	-	-	-	-	-	-	-

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Plate 1. A - *Miliammina fusca* (GD05); B - *Arenoparrella mexicana* (GD03); C – *Siphotrochammina lobata* (GD03); D - *Tiphotrocha comprimata* (GD08); E - *Trochammina inflata* (GD05); F - *Adelosina cliarensis* (GD01); G - *Quinqueloculina seminula* (GD05); H - *Quinqueloculina poeyana* (GD01); I - *Quinqueloculina lamarckiana* (GD01); J - *Lobatula lobatula* (GD01); K - *Rosalina bradyi* (GD01); L - *Planorbulina mediterranensis* (GD01); M - *Asterigerinata mamilla* (GD01); N - *Asterigerinata mamilla* (GD01); O - *Astrononion stelligerum* (GD03); P - *Haynesina germanica* (GD02).

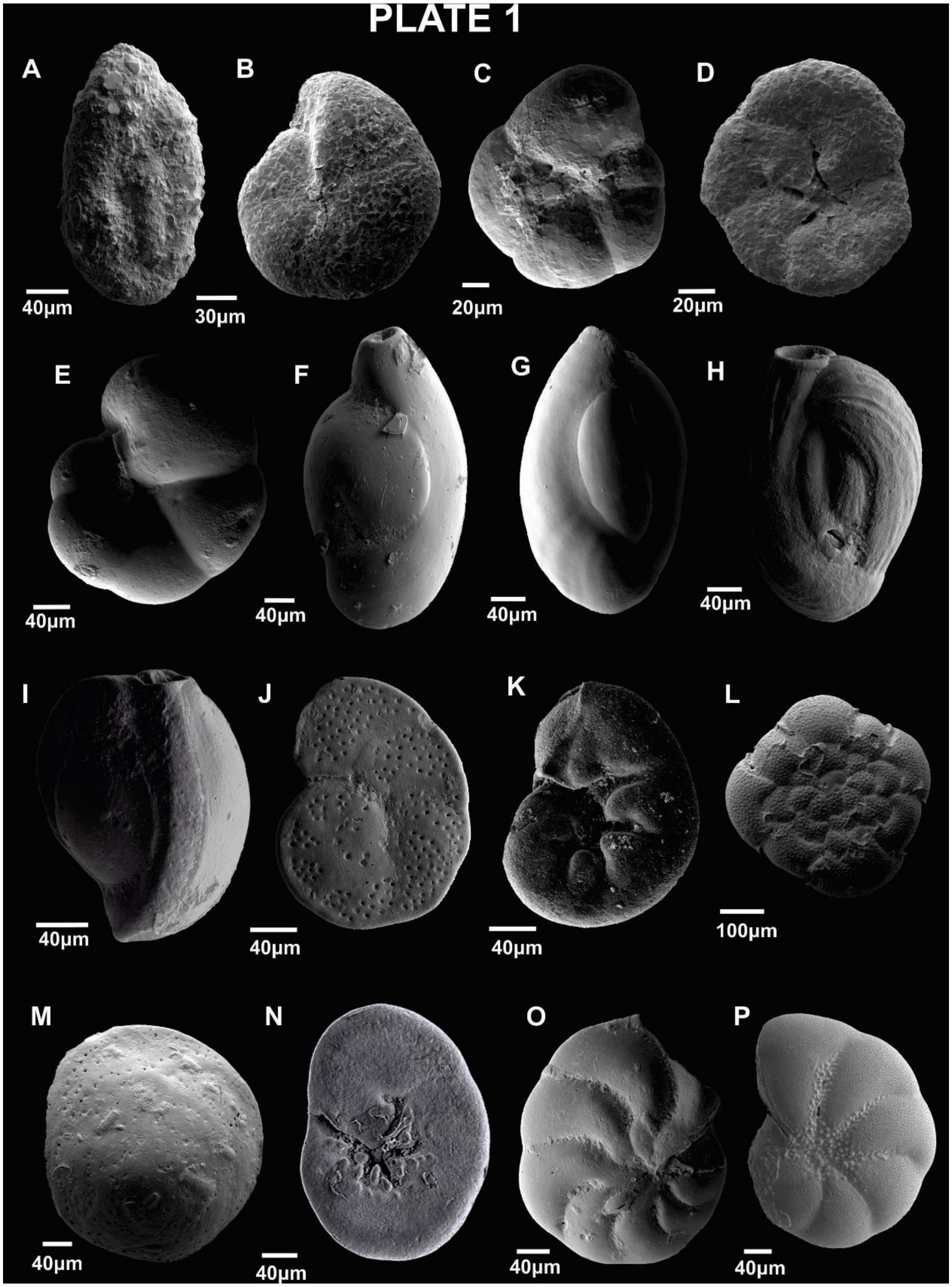

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Plate 2. A – *Bolivina variabilis* (GD08); B - *Elphidium gerthi* (GD02); C - *Elphidium complanatum* (GD01); D - *Elphidium crispum* (GD01); E - *Elphidium gunteri* (GD04); F - *Elphidium excavatum* (GD05); G - *Ammonia tepida* (GD02); H - *A. tepida* (GD02); I - *Ammonia parkinsoniana* (GD05); J - *A. parkinsoniana* (GD05); K - *Entzia polystoma* (GD05).

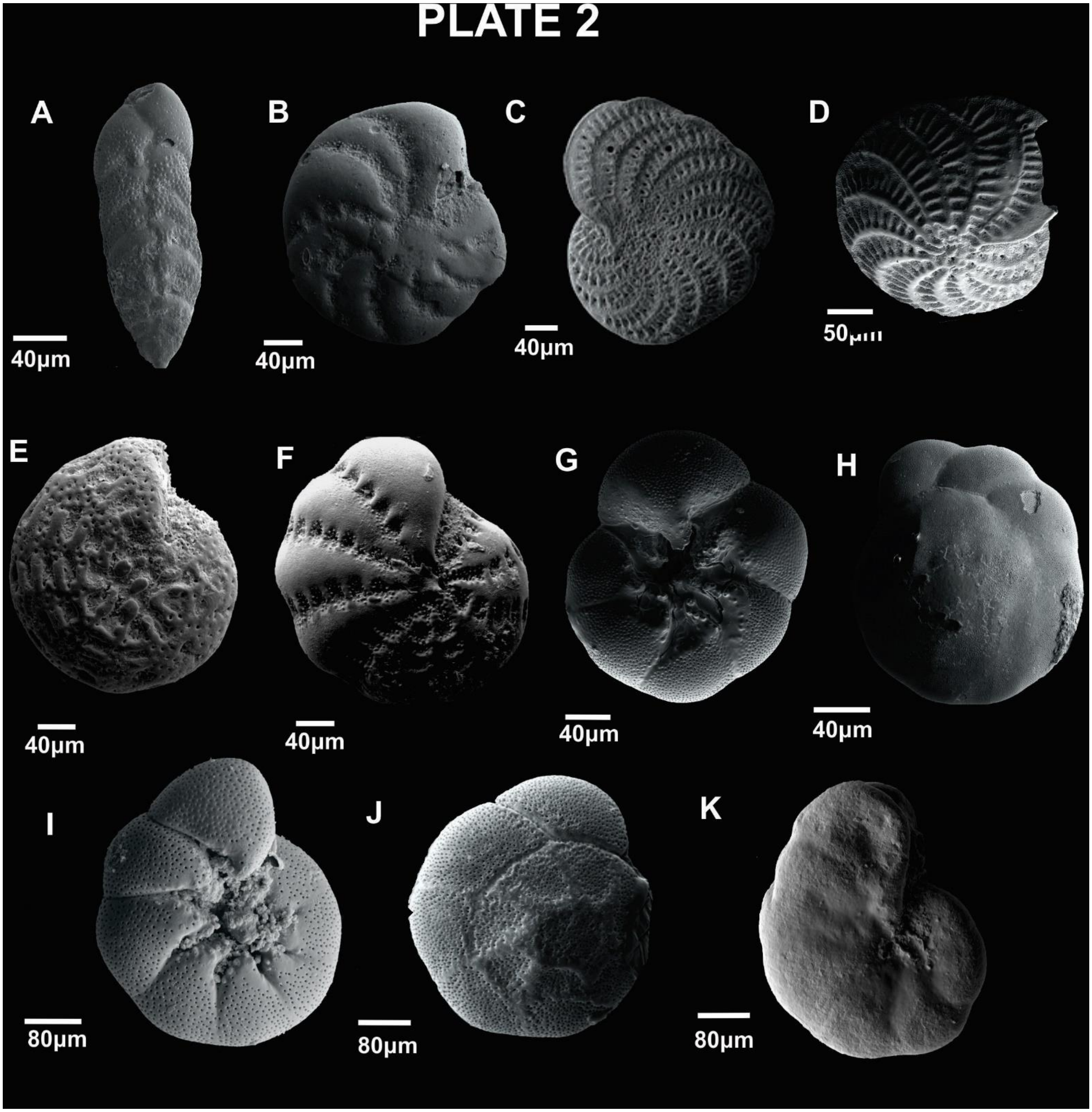

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Plate 3. A - *Loxoconcha elliptica* (GD01); B - *Cytherois fischeri* (GD02); C - *Leptocythere lacertosa* (GD02); D - *Palmoconcha guttata* (GD02); E - *Neocytherideis subulata* (GD01); F - *Semicytherura sulcata* (GD01); G - *Urocythereis oblonga* (GD01); H - *Darwinula stevensoni* (GD01); I - *Loculicytheretta pavonia* (GD01); J - *Heterocypris incongruens* (GD10); K - *Cypridopsis vidua* (GD10L); L - *Cyclocypris ovum* (GD10); M - *Ilyocypris* sp. (GD10).