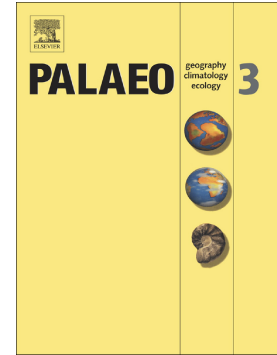# Journal Pre-proof

Improving the detection of shell alteration: Implications for sclerochronology

Rute Coimbra, Stefan Huck, Niels J. de Winter, Ulrich Heimhofer, Philippe Claeys

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1   **Improving the detection of shell alteration: implications for sclerochronology**
2
3   Rute Coimbra[a,*] rcoimbra@ua.pt; Stefan Huck[b] huck@geowi.uni-hannover.de; Niels J. de Winter[c,d]
4   niels.de.winter@vub.be; Ulrich Heimhofer[b] heimhofer@geowi.uni-hannover.de; Philippe Claeys[c]
5   phclaeys@vub.be
6   a) GeoBioTec, Departamento de Geociências, Universidade de Aveiro, Portugal
7   b) Institut of Geology, Leibniz University Hannover, Germany
8   c) Analytical, Environmental and Geochemistry Department, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels,
9   Belgium
10 d) Department of Earth Sciences, Faculty of Geosciences, Utrecht University, the Netherlands
11 *Corresponding author at: Rute Coimbra, Dpto. Geociências, Universidade de Aveiro, Campus de
12 Santiago, 3810-193 Aveiro, Portugal

13
14 **Abstract**
15   Sclerochronology makes use of (fossil) shell-archives to establish records

16 allowing for investigation of high-resolution environmental dynamics. Nevertheless,

17 this potential can often not be fully exploited due to the interplay between

18 paleoenvironmental variability, vital effects and the potential diagenetic modification of

19 skeletal materials, which often results in highly complex records. A novel dynamic

20 approach, aiming to separate pristine from altered shell material for paleoclimate and

21 paleoenvironmental reconstructions is proposed.

22   Seventeen fossil bivalve shells (requieniid rudists, pectinids and chondrodonts)

23 from two neighbouring Lower Cretaceous (Albian) shallow-water sections (Lusitanian

24 Basin, western Portugal) were analysed for their major and trace elemental

25 compositions using high-resolution quantitative μXRF line scans. Their complex

26 records were subject to a novel statistical analysis protocol, which tested mono- and

27 multi-species datasets, as well as comparing shells from both locations.

28   Characteristic elemental associations reveal the differential impact of early and

29 late diagenetic alteration processes. The incorporation of elements associated with

30 detrital contribution (Fe, Si, Al) is attributed to syn-depositional bioerosion (shell-

31 boring). In clear contrast, shell-portions showing a strong correlation between Fe and

32 Mn are indicative of later diagenetic alteration. The influence of each process is

33 different at each site, revealing local differential alteration pathways. Mono-specific

34 comparisons provides identical geochemical responses, suggesting that intra-specific

35 differences do not control the observed elemental patterns. In contrast, inter-species

36 tests rendered a clear separation in the way elements are incorporated in the shells of

37 pectinids and requieniids (e.g., as evidenced by differences in Mg or Sr content). Such

38 differences can be linked to differential biomineralization pathways, easily detected by

39 the applied method.

40   We present a new, dynamic method for distinguishing pristine from altered shell

41 material, not relying on arbitrary diagenetic thresholds for trace element content. By

42 clearly identifying shell-alteration pathways, syn- to post-depositional processes are

43 recognized. A progressive cleaning of the elemental dataset allows paleoenvironmental

44 studies to be based on the most pristine data, contributing to unravelling the complex

45 interplay between climate, environmental dynamics and their impact on

46 biomineralization processes and sclerochronological archives.

47

48 Keywords: Shells, geochemistry, diagenesis, statistical analysis, paleoenvironment

49
50 **1- Introduction**

51   The study of past climate and environmental change yields crucial information

52 about Earth's climate system and how global perturbations can affect the biosphere (e.g.

53 Zachos et al., 2001). Most of these studies aim to characterize perturbations on a

54 geological time scale (thousands to millions of years), identifying long term trends in

55 climate, environment and ecology. Contrarily, the field of sclerochronology uses

56 mineralogical, structural and chemical changes in accretionary biogenic archives to

57 study changes in environment that take place on the scale of lifespans of the organisms

58 (e.g. several years) that form these archives (e.g. Jones, 1983). These studies

59 complement longer timescale reconstructions and have the potential to yield snapshots

60 of climate and environmental variability on a shorter timescale which can be placed in

61 the context of longer timescale perturbations (e.g. Steuber et al., 2005). Bivalve shells

62 have been an especially popular archive for sclerochronology work, because their

63 growth rates are comparatively high (reported values up to 4 cm/yr; e.g. Batenburg et

64 al., 2012; Schöne et al., 2005; Nedoncelle et al., 2013) and because the calcite shells of

65 some species of bivalves (e.g. oysters and pectinids) are more resistant to diagenesis

66 than their aragonitic counterparts (e.g. corals and gastropods, e.g. Brand and Veizer,

67 1980; 1981).

68   In addition, growth lines and increments in the shells of fossil bivalves have

69 been successfully applied to construct independent shell chronologies which allow

70 researchers to accurately assess the timing and duration of shell formation and link shell

71 chemistry to environmental variability in modern species (e.g. Richardson et al., 2004;

72 Schöne et al., 2005; Gillikin et al., 2008). However, the complex interplay of multiple

73 environmental, physiological and post-depositional (diagenetic) parameters on the

74 chemical composition of bivalve shells has hampered the interpretation of the above-

75 mentioned chemical variations in terms of environmental change. As a result,

76 sclerochronologists often combine several chemical tracers in a multi-proxy approach in

77 an attempt to disentangle the effects of different parameters on shell composition (e.g.

78 Surge et al., 2001; de Winter et al., 2017a; 2018).

79   Elemental records obtained from biogenic calcite and/or aragonite have been

80 used to reconstruct (paleo) environmental parameters (temperature, salinity, primary

81 productivity, current patterns; e.g. Nürnberg et al., 1996; Halfar et al., 2000; Surge et

82 al., 2001; Gillikin et al., 2008; Chan et al., 2011; Schöne et al., 2011; de Winter et al.,

83 2017a; 2018; Ullmann et al., 2018; Huyghe et al., 2019; Markulin et al., 2019).

84 Nevertheless, strong differences in biomineralization pathway are often found to disrupt

85 an already naturally complex signal, further hampered by the effects of diagenesis in

86 fossil shell materials. The cautious use of statistical tools provides a valuable solution

87 for this limitation.

88   Principal component analysis (PCA) is among the most popular multivariate

89 statistical techniques for dealing with such large datasets, and is widely used across

90 many scientific disciplines (Cordella, 2012; Yao et al., 2012; Coimbra et al., 2015,

91 2018; Cai et al., 2019). PCA aims to extract, compress, simplify and analyse the

92 structure of multivariate datasets. The inter-correlation of several dependent variables is

93 tested and expressed as a set of new orthogonal variables (Principal Components- PC),

94 evidencing the degree of similarity between observations and variables (Abdi and

95 Williams, 2010 and references therein). In this study, as an innovative approach to

96 sclerochronological data, a double PCA approach is applied (Coimbra et al., 2017), with

97 a new automated adaptation (density analysis) for higher precision in isolating samples

98 of interest.

99   The proposed data reduction approach simplifies the evaluation of elemental

100 variations in shell archives, highlighting taxon and habitat specific variations in trace

101 element content. Environmental and diagenetic influence on original elemental

102 composition can be addressed efficiently, allowing the identification of growth rhythms

103 or responses of shell microstructure. This contribution provides significant advances in

104 the detection of shell alteration, with the potential of refining sclerochronological

105 interpretations for a variety of skeletal remains from a wide range of geological time

106 periods.

107

108 **2- Geological and paleoenvironmental setting**

109   The well-exposed sections at São Julião (38.9319776ºN, 9.4219073ºW) and

110   Praia das Maçãs (38.8297059ºN, 9.468163ºW) are located along the western coast of

111   Portugal, within the southern sector of the Lusitanian Basin (Figs. 1 and 2). During the

112   Cretaceous, the western Iberian plate was located at about 30ºN (Stampfli and Borel,

113   2002), under the influence of two major climatic zones: the mid-latitude warm humid

114   belt and the northern hot arid belt (Chumakov et al., 1995). Throughout the Lusitanian

115   Basin, a major unconformity marks the Aptian-Albian transition (Dinis and Trincão,

116   1995; Heimhofer et al., 2007; Dinis et al., 2008). This regional unconformity is overlain

117   by   coarse-grained   continental   siliciclastics   (Rodízio   Formation)   deposited

118   diachronously throughout the basin in fluvial-deltaic settings. These deposits are

119   covered by nearshore strata and shallow-water platform carbonates of the Galé

120   Formation. This formation is overlain by lagoonal and terrestrial deposits– the Caneças

121   Formation.

122   The   studied   sedimentary   succession   comprises   shallow-water   carbonate-

123   siliciclastic deposits (Figs. 2 and 3) assigned by previous workers to cover Albian to

124   Early Cenomanian age (Rodízio and Galé Formations; Hasenboehler, 1981; Medus,

125   1982; Berthou, 1984; Rey, 1992; Horikx et al., 2014). The Albian Galé Formation is

126   subdivided into the Água Doce and the Ponta da Galé Members (Rey, 1992). The

127   lowermost Água Doce Member is mainly composed of alternating marly, carbonate-

128   and sandstone-rich coastal-marine strata. More marine conditions are evidenced by

129   increasingly thicker limestone beds towards the top of this member and mark the

130   transition to the overlying carbonate-rich Ponta da Galé Member (Rey 1992). This work

131   will focus on the stratigraphic interval covering the Ponta da Galé Member—the

132   carbonate-rich upper portion of the Galé Formation, defined at the base by the regional

133   occurrence of the first rudist beds, evidencing an overall deepening trend (Rey, 1992).

5

134   An overview of the main sedimentological, stratigraphical, and paleontological features

135   of the São Julião and Praia das Maçãs sections is given in Fig. 3A. Based on previous

136   work performed on these sections, the stratigraphic intervals under scope are well-

137   known for not having experienced pervasive deep-diagenetic influence (Horikx et al.

138   2014, 2016; Coimbra et al., 2017), justifying their use for exploring sclerochronological

139   aspects. The regional stratigraphic correlation between both sections has been well-

140   established using C-isotope stratigraphy, here partially reproduced after Horikx et al.

141   (2014) (Fig. 3A). Accordingly, the base of the Praia das Maçãs section can be correlated

142   to the second rudist-bearing bed at Sao Julião (165 m; Fig. 3A). Both sections are

143   characterised by decreasing trend in C isotopes from bottom to top, despite minor

144   differences in absolute value. Sampled shell materials retrieved from these deposits

145   (examples in Fig. 3B) are investigated to explore the potential and evaluate the

146   limitations of these shells as archives of paleoenvironmental change.

147

148   **3- Studied shell materials**

149   3.1- *Pectinidae*

150   Nowadays, bivalves belonging to the superfamily Pectinidae (Rafinesque, 1815)

151   (scallops) occupy a huge variety of habitats in polar, temperate and tropical seas,

152   ranging from the intertidal zone to water depths up to 7000 m (Brand, 2006; Serb,

153   2006). They can be byssally attached, free-living or encrusted and their life habit

154   changes ontogenetically (Stanley, 1970). Largely, ancient pectinid shells such as those

155   of *Amussiopecten baranensis* comprise an outer layer composed of (crossed, regular or

156   irregular) foliated low-Mg calcite (LMC), a middle aragonitic layer and an inner

157   foliated LMC layer (Zamarreño et al., 1996; Carter 1990). Commonly, shells of

158   Cretaceous pectinids such as *Prohinnites favrinus* are predominantly composed of

6

159　foliated calcite (Harper et al., 1996). In fossil specimens, the inner crossed-lamellar

160　aragonite layers are often replaced by coarse, sparry low-Mg calcite (Harper et al.,

161　1996).

162

163　3.2- Rudists

164　　　　Rudist bivalves (superfamily Hippuritoidea) are epibenthic sessile suspension-

165　feeders that inhabited a variety of carbonate-dominated shallow water settings in the

166　(sub-) tropical Tethyan-Atlantic-Pacific belt during late Mesozoic times (Gili et al.,

167　1995; Skelton, 2003; Skelton, 2018; Gili and Götz, 2018). During the Cretaceous, they

168　evolved to one of the most important neritic carbonate producers, with maximum

169　carbonate production rates ranging between 2.2 and 35.7 kg per square meter per year

170　(Steuber, 2000). In contrast to reef-building corals, rudists were typically loosely

171　arranged in low-relief bioconstructions referred to as bouquets (n <12), clusters (n=

172　>12) or thickets (n= >100; Philip, 1972; Gili et al., 1995). Significant differences in

173　growth geometries (morphotypes) indicate that rudists were able to adapt to various

174　habitats. Recumbent forms lay prone but unattached on mobile substrates in current-

175　swept settings (Ross and Skelton, 1993). In contrast, the left valve of so-called

176　'clingers' was attached (cemented) to solid substrates (Skelton, 1978). During ontogeny,

177　the elongated and initially cemented valve of predominantly cylindrical 'elevators'

178　stabilized by surrounding sediment or by neighbouring rudists (clustering; Gili and

179　Götz, 2018).

180　　　　In general, the shell of rudists comprises two layers: a rarely preserved inner

181　layer originally composed of crossed lamellar aragonite and an outer low-Mg calcite

182　layer composed of fibrous prisms (Skelton and Smith, 2000; Skelton, 2018). The

183　mesostructural properties of the outer layer, however, show significant differences

184   among the rudist families (Skelton, 2018). Whereas Hippuritidae and Requieniidae

185   provide relatively compact and thick calcitic shells, the right valves of most Radiolitidae

186   typically shows a complex celluloprismatic structure composed of numerous cells, often

187   spar-filled after fossilization (Pons and Vicens, 2008).

188

189   **4. Methods**

190   4.1- Sample collection and preparation

191   Shells were cut to obtain thin sections and polished surfaces (Fig A1 for high-

192   resolution images of all studied specimens). Selected shells were embedded in resin

193   (Araldite® 2020, Huntsman, Basel, Switzerland) and subsequently cut along their major

194   growth axis using a slow rotating rotary saw. A parallel slab was cut out of one-half of

195   the shell, while the other half was preserved (archive half). For micro-XRF scanning,

196   slabs were polished using silicon carbide polishing discs (up to P2400).

197

198   4.2- Micro X-Ray Fluorescence

199   All polished shell surfaces were subject to non-destructive trace elemental

200   analyses using micro-X-ray fluorescence (micro-XRF). Analyses were carried out with

201   a Bruker M4 Tornado micro-XRF scanner (Bruker nano GmbH, Berlin, Germany) at

202   the AMGC research group of the Vrije Universiteit Brussel (VUB, Brussels, Belgium).

203   The Bruker M4 Tornado is equipped with a Rh metal-ceramic X-ray source operated at

204   maximum energy settings (50 kV, 600 µA). The X-ray beam was focused on a 25 µm

205   diameter circular spot (calibrated for Mo-Kα radiation) and the intensity of returning X-

206   rays was measured using two silicon drift detectors (see de Winter and Claeys, 2016).

207   The sample position was controlled by a high-precision (±1µm) XYZ sample stage that

208   can be moved relative to the focused X-ray beam. Details on the setup and methodology

209   of the M4 Tornado XRF scanner can be found in de Winter and Claeys (2016) and de

210   Winter et al. (2017a). Two types of analyses were carried out: semi-quantitative

211   elemental mapping and quantitative point-by-point line scanning.

212   Elemental mapping was carried out by stitching together horizontal line scans

213   that were produced by moving the sample through the focused X-ray beam in

214   continuous motion, using short acquisition times per 25 μm wide spot (20 ms). This

215   acquisition time is insufficient for full quantitative analyses of individual points.

216   Therefore, 2D-grids of relative trace element abundance were constructed by integrating

217   the intensity under element X-ray fluorescence peaks and plotting differences in XRF

218   intensity over the entire sample surface. These maps serve as a qualitative assessment of

219   the nature of the material, guiding the position of quantitative XRF line scans (Fig. 4B

220   to G). In this way, the exact path to follow during line scans was established ensuring

221   minimal contribution of heavily altered shell portions (Fig. 4D and H).

222   Quantitative point-by-point micro-XRF line scans were carried out using longer

223   integration times (60 s per point). Contrary to map analyses, line scans were carried out

224   point by point rather than by means of continuous scanning. This approach allows the

225   X-ray beam to stay on the same spot for enough time to reduce the signal to noise ratio

226   of the XRF spectrum sufficiently for point-by-point quantification. The minimum time

227   required for quantitative point analyses was determined by repeated analyses of

228   carbonate reference materials following the protocol detailed in de Winter et al.

229   (2017b). Spectra were quantified using the Bruker Esprit software calibrated using the

230   matrix-matched BAS-CRM393 limestone standard (Bureau of Analyzed samples,

231   Middlesbrough, UK), after which individual measurements were calibrated offline using

232   seven matrix-matched international certified reference materials (CCH1, COQ1,

233   CRM393, CRM512, CRM513, ECRM782, and SRM1d; see de Winter et al., 2018 for

234   details), which were treated as samples. Untreated trace element results are reported in

235   Data_Appendix. The applied set of standards collectively contained enough certified

236   values to allow concentrations to be quantified (calibration line R^2>0.98) for the

237   elements Mg, Al, Si, K, Ca, Ti, Cr, Mn, Fe, Cu, Sr, Y and Ba. Other elements with less

238   well constrained calibration lines (R^2 values between 0.9 and 0.98) include P, Zn, Zr,

239   Nb and Pb. These elements are more difficult to quantify because their XRF peaks tend

240   to overlap with elements which are more common in bivalve carbonate (see de Winter

241   and Claeys, 2016). These latter elements did not meet strict standards for quantification,

242   but their semi-quantitative results can still be used to assess relative differences in

243   elemental composition using the proposed double PCA and density analysis method.

244   Details on reproducibility errors and detection limits are provided in the Appendix

245   (Table A1).

246

247   4.3- Statistical analysis: the double PCA+ approach

248   The double Principal Component Analysis was previously proven to be useful in

249   shallow-marine contexts (Coimbra et al., 2017; details in Appendix). To determine the

250   selection of elements to include in the analysis, an exploratory PCA is performed in

251   order to detect the most significant (loadings <-0.5 and >0.5) geochemical variables for

252   each considered dataset (see Fig. A2 in Appendix). This ensures that the following

253   double PCA method is carried out exclusively for the variables showing higher degree

254   of affinity, using the statistical software package XLSAT, an add-in to Excel (detailed

255   steps in Appendix). Here it is adapted to process the dense sclerochronological

256   elemental datasets produced by micro-XRF in order to provide clues on the processes

257   acting upon these mid-Cretaceous shells during their lifetime and after deposition. We

258   expand the double PCA method by adding density analysis, performed using PAST

259   statistic package (Hammer et al., 2001, version 3.25)—an automated criterion for

260   accurately delimiting different clusters of samples within a dataset (see Fig. A2 in

261   Appendix for stepwise description and Figs. A3 and A4 in for further

262   recommendations). Accordingly, data points included in the range of 2/3 of the

263   maximum data density and within delimiting regular shaped contour lines are accepted

264   as valid (Fig. A2C). This implies that data points plotting further away from the main

265   cluster, which also generate distortion along the contour lines, are excluded from further

266   computation. This improved version—double PCA+—can be applied to various

267   combinations of datasets (one shell-transect, several transects, several sites, etc). The

268   information generated by the double PCA+ is threefold: (i) it explores the array of

269   possible mechanisms accounting for shell-alteration, (ii) provides arguments to isolate

270   the best-preserved data, unlocking masked paleoenvironmental fluctuations; (iii)

271   provides customized thresholds of shell-preservation, highlighting particularities of

272   given sites or species. As the complete dataset comprises several geochemical variables

273   extracted from seventeen shells belonging to two localities, the possibilities for

274   comparison are large The reasoning followed during this work was to select sets of

275   specimens suitable to perform mono-specific and multi-species analysis for each

276   location; followed by mono-specific analysis comparing both locations. Any other

277   selection would be equally valid, depending on the aim of the study (e.g., targeting

278   specific elements of interest; ancient specimens with better constrained ecological

279   requirements; ancient versus modern examples; modern examples only).

280

281   **5- Results**

282   5.1- Conventional multi-proxy analysis

283   The large volume of information gathered during this research surpassed

284   200.000 individual elemental values. The conventional approach of plotting the

285   elemental data against shell length (example given in Fig. 5) can only be used when

286   comparing a low number of transects amongst each other, otherwise plots become too

287   large and confusing. For the pectinid shells from both locations, the obtained range of

288   absolute values was different (Fig. 5). For example, baseline Mn values for the pectinid

289   specimen from Praia das Maçãs are much higher than those obtained for the São Julião

290   specimen, as well as the maximum peak values. This also holds true for all other

291   presented elements (Fe, Sr and Mg). The temporal trends are hard to deduce due to

292   frequent disruptions by sharp maximum peak values (noted in Fig. 5). Portions of shell

293   with persistently high or low elemental values were also observed (e.g., high Sr values

294   in Fig. 5). In order to compare the 17 specimens under scope, an alternative data-

295   reduction method was required—the double PCA+ (see Appendix). Such a dense data

296   cloud would distract from the differences between specimens, species and sites or

297   concentration differences arising from differences in shell preservation.

298

299   5.2- Praia das Maçãs: mono-specific versus multi-species

300   For the locality of Praia das Maçãs, two groups of specimens were tested. The

301   first group is mono-specific (Fig. 6A), with six (taxonomically undifferentiated)

302   requiniid shells showing tightly clustered data, without evidence of major differences in

303   elemental content. This clustering of samples forms a high-density area in the PCA-

304   space (Fig. 6A), including 65% of the samples included in this group of shells. These

305   were then used to establish the customized elemental threshold values of the most

306   significant elemental proxies (see Fig. 6A and Appendix for detailed workflow). A

307   rather large number of samples (35% of the total) fall out of this main cluster, largely

308 following a common trend towards higher Fe, Mn and Mg content (Fig. 6A, see also

309 Fig. A5). In fact, these samples even seem to form a smaller cluster in the density plot.

310 Only a very small group of samples does not follow this trend, plotting towards higher

311 Si and S abundance (lowermost portion of the PCA space, Figs. 6A and A5).

312   The second set of samples from Praia das Maçãs is composed of 7 shells

313 belonging to 4 different species (Chondrodont, hippuritid elevator rudist, pectinid and

314 radiolitid; Figs. 6B and A4). A main data density area (see details on density analysis in

315 Appendix) is defined by 85% of the samples of this group leading to a tight cluster

316 despite the presence of different species. Only a minority of samples plot towards

317 negative values of PC1, in response to higher concentration of Fe, Mn and Mg; and

318 towards the more positive range of PC2 pointing at higher abundances of Si. When

319 considering the sample distribution per specimen, the elevator rudist and pectinid shells

320 are both slightly shifted towards higher Sr content. Additionally, the pectinid shell

321 presents more samples responsible for the trend towards higher Si values.

322   When considering both sets of samples (mono- and multi-species), no major

323 differences in elemental trends were observed (Figs. 6 and A5 in Appendix).

324 Nevertheless, it is noteworthy that the mono-specific set denoted more variability in Fe,

325 Mn and Mg (PC1 in Fig. 6A) as well as Si (PC2 in Fig. 6A); whilst the multi-species

326 group revealed slight differences in Sr content, but overall good agreement in elemental

327 abundance of the measured proxies (Fig. 6B).

328

329 5.3- São Julião: mono-specific versus multi-species

330   Specimens from the São Julião section were initially tested as a multi-species

331 group including two *Apricardia carentonensis* shells and two pectinid shells (Figs. 7

332 and A5 in Appendix). The double PCA+ provided two clearly differentiated clusters

13

333   defined by both species, largely attributable to significant differences in Sr and Pb

334   content (Fig. 7A). In fact, by applying the density analysis criteria, the selected set of

335   samples was largely composed of datapoints belonging to pectinid shells (ca. 78%),

336   imposing an unwanted bias to this analysis. A mono-specific analysis was therefore

337   preferred for these shells.

338   The dataset was consequently divided into two mono-specific sets: two

339   *Apricardia* shells (Fig. 7B) and two pectinid shells (Fig. 7C). The *Apricardia* shells

340   clustered tightly within a high-density area comprising ca. 80% of the samples. This set

341   of samples was used to establish the elemental thresholds for this setting and species.

342   Samples showing a clear trend towards lowered Ca abundance, along with higher Mg,

343   Fe and Al content (PC1; Fig. 7B) fall out of the main cluster. Only a minor number of

344   samples revealed the influence of trace Br and P concentration, showing an opposite

345   trend (PC2; Fig. 7B). The two pectinid shells revealed a similar elemental profile when

346   compared to *Apricardia* specimens: a main cluster generated by 65% of the samples,

347   disrupted by samples denoting higher Mg, Fe, Si and S across PC1; and less significant

348   incorporation of trace elements such as Pb, Br and Zn (Fig. 7C).

349

350   5.4- São Julião versus Praia das Maçãs

351   Two pectinid shells retrieved at each of the studied sites were compared using

352   the double PCA+ protocol (Figs. 8 and A5 as Appendix). Samples from both shells

353   form a high-density cluster containing 80% of the total dataset, but samples from São

354   Julião clearly dominate this accumulation (Fig. 8). This pattern is due to the higher

355   variability in elemental composition evidenced by samples from the Praia das Maçãs,

356   denoting a clear trend towards higher Mg, Fe, Mn and Br (PC1), along with lower Sr

357   abundance (PC2). An alternative minor trend is also observed in pectinid data from

358   Praia das Maçãs, with a small set of samples responding to higher Si (and P) content

359   (Fig. 8).

360

361   **6- Discussion**

362   In general, the PCA results are characterized by a high number of samples

363   clustering tightly in all cases. As such, on average 75% of the total tested samples

364   revealed a higher data density (see Fig. A2c in Appendix), forming a main cluster (Figs.

365   6 to 8 and Fig. A5 as Appendix). In contrast, a small number of samples depart from

366   this main cluster, forming different dispersion patterns (Figs. 6 to 8 and Table 1).

367   Accordingly, the main cluster corresponds to the best-preserved samples, as evidenced

368   by their low content in elements indicative of diagenetic alteration (e.g., Mn, Fe; Brand

369   and Veizer, 1980; Al-Aasm and Veizer, 1986). Their high Sr content is also an

370   indication of pristine shell material. This cluster contains information which can be

371   interpreted in terms of paleoenvironmental conditions (Table 2). Contrarily, samples

372   scattering away from the main cluster correspond to secondary processes (alteration)

373   which occurred at any given state of the evolution of these biogenic materials. Persistent

374   patterns of sample distribution were identified in the sets of tested shells (Figs. 6 to 8;

375   summarized in Fig. 9). These patterns may respond to a wide array of variables,

376   including biomineralization pathways, paleoenvironmental conditions and syn- to post-

377   depositional alteration. Geochemical profiles can be interpreted in terms of species-

378   dependence, site-specific processes and general disruption of the original signals; and

379   the potential for isolating noise from paleoenvironmental information (Fig. 10). As an

380   example of the benefits resulting from the Double PCA+ approach, Sr elemental values

381   are addressed in Figure 11. The unprocessed raw elemental data obtained for the mono-

382   specific set of six Requiniid shells (see also Figs. 3 and A1) shows sharp variations,

383   largely towards very low abundance values (minimum value of 50 ppm; Fig. 11A). The

384   dynamic elemental threshold values of 1204 to 1852 ppm obtained by the Double PCA+

385   approach (Figs. 6 and 12; Table 1) provided a range of best-preserved datapoints,

386   representing 80% of the unprocessed data. By applying this threshold, the resulting Sr

387   plots for each shell now show a clearer variation patterns, denoting certain cyclicity in

388   the sclerochronological record (Fig. 11B). The focus of this study is to improve the

389   detection of shell alteration, hence the significance of short-term (seasonal/ontogenetic)

390   elemental fluctuations enclosed in the "clean" sclerocronological record are beyond the

391   scope of this contribution.

392   Focusing on the "clean" dataset generated by the application of the Double

393   PCA+ method provided further validation, namely when dealing with mean elemental

394   values of selected geochemical proxies (Mg and Sr; Table 2). Once shell alteration is

395   detected and excluded from the dataset the comparison between specimens, species and

396   sites becomes straightforward. At São Julião, Mg concentrations are evidently higher

397   for both Pectinid shells, when compared to *Apricardia* specimens. In fact, *Apricardia*

398   shells at São Julião show the lowest Mg concentration of all tested shells (905 and 973

399   ppm; Table 1). At Praia das Maçãs, Mg abundance is higher for Chondrodont specimens

400   (3384 to 3823 ppm), also showing low Mg variability amongst them. This low intra-

401   species Mg variability is also evident for requiniid specimens. Regarding Sr abundance,

402   this element was not computed for some specimens from São Julião due to low

403   statistical significance (Table 2 and Fig. 7). For the remaining shells, the obtained Sr

404   record is rather stable, denoting lowered concentration only in the radiolithid shell (994

405   ppm). For a better assessment of the good-preservation of the final dataset, Fe and Mn

406   are also shown in Table 2, showing a narrow range of fluctuation in all shells and

407   absolute values of Fe from 56 to 342 ppm and Mn from 46 to 102 ppm (Mn was not

408   computed for some specimens due to low statistical significance; Table 2).

409

410   6.1- Main geochemical trends: processes and elemental responses

411   At Praia das Maçãs, both intra and inter-species datasets provided similar PCA

412   patterns (Figs. 6 and 9A). Samples deviating towards the PCA region denoting higher

413   Fe, Mn and Mg abundance (average values in Fig. 6) are interpreted as diagenetically

414   compromised, recrystallized shell-portions (examples in Fig. 4A and E). Fe and Mn are

415   typically enriched in calcite during diagenesis due to their solubility and high

416   distribution coefficients (Veizer, 1983; Rimstidt et al., 1998; Swart, 2015).

417   Accompanying the described Fe and Mn enrichment, Mg abundance also increases in

418   these samples, with absolute values still within LMC range (<4000 ppm; Fig. 6A). This

419   reflects the loss of biogenic signature of the analysed calcite samples. Accordingly,

420   originally lower Mg calcite portions of the shell (ca. 2500 ppm Mg; Fig. 6A and also

421   chapter 3), also denote lower abundance in Sr (and Ca) due the recrystallization process

422   (Brand and Veizer, 1980; Al-Aasm and Veizer, 1982, 1986). These interpretations are

423   based on the combined elemental features, which are more informative than reading

424   isolated elemental values. The few samples deviating towards higher Si (and S) are

425   interpreted as the result of the incorporation of siliciclastic material (pyrite not

426   excluded) in borings by epifaunal organisms (Fig. 4A and B). The portions of the record

427   remaining after the double PCA+ approach can then be interpreted in terms of

428   environmental change. The double PCA+ therefore provides solid elemental threshold

429   values (Fig. 6). When comparing intra and inter-species PCA results, most altered

430   samples are contained in the sample set consisting of six requiinid shells (Fig. 6A). This

431   effect is due to the higher degree of similarity between the elemental records of each

432   shell within a mono-specific dataset, yielding higher-density data distribution along

433   PCA plots (see section 4.3 and Fig. A2c for details on density analysis). This trend

434   translates into narrower threshold values. In contrast, inter-species datasets generate

435   lower density data distribution due to generation of more diffuse main clusters (example

436   in Fig. 6B). For the latter, it also becomes evident that within the best-preserved data,

437   certain species have higher Sr content (ca. 1500 versus 1200 ppm; Fig. 6B), as is the

438   case of pectinid and radiolitid shells (Fig. 6B). This effect likely relates to a differential

439   incorporation of Sr during shell growth and/or differences in shell microstructure (and

440   microporosity), as all these specimens belong to the same site and geological time

441   interval and were therefore subject to the similar diagenetic history (Fig. 3A). A

442   species-dependent process may be envisaged, suggesting main differences in growth

443   rate and/or calcification rate controlled Sr incorporation. The proposed calcification rate

444   control on Sr operates in modern skeletal materials (Stoll and Schrag, 2000; Stoll et al.,

445   2002; Rickaby et al., 2002; Carré et al., 2016), as well as in inorganic calcite (Lorens,

446   1981; Tesoriero and Pankow, 1996). Under higher calcification rates, Sr uptake occurs

447   under kinetic control such that the animal cannot discriminate effectively against Sr,

448   resulting in a higher distribution coefficient for this element—D_{Sr} (Elderfield et al.,

449   2002). The reported ontogenetic decrease in growth rates for rudists (Steuber, 1996) and

450   oysters (Ullmann et al., 2010) supports the notion that shell-growth influences Sr

451   incorporation.

452   At the São Julião section, inter-species differences were larger than in Praia das

453   Maçãs, generating a clear separation between *Apricardia* and pectinid specimens (Fig.

454   7A). It is noteworthy that pectinid shells, at this different location, also show a

455   distinctive Sr signature (Fig. 7A). The most distinctive trait between *Apricardia* and

456   pectinid shells is the concentration in Sr, which is on average 350 ppm higher in

457   *Apricardia* shells (1578 versus 1226 ppm). This feature supports the previous notion

458   that ontogenic and/or metabolic control has a strong influence on Sr incorporation.

459   When species are analysed separately, similar PCA patterns are found (Fig. 7B and C,

460   see also Fig. 9B), with shell-alteration characterized by elevated concentration of the

461   elemental association of Si, Fe, Al and Mg attributed to the influence of detrital

462   siliciclastics included in bore holes (quartz and aluminosilicate minerals). Despite well-

463   known difficulties related to the measurement of heavy metals with XRF, the minor

464   incorporation of heavy metals (Pb, Zn) and Br is nevertheless relevant in both species

465   (Fig. 7B, C), in part also related to an inverse trend of decreasing phosphorous

466   abundance (Fig. 9B). Cautious comparison with modern examples may shed light into

467   this trend. Bioaccumulation of naturally present trace elements is a well-known process

468   in modern settings, of extreme relevance in the context of anthropogenic contamination

469   on coastal areas and related mitigation efforts (Ferreira et al., 2004; Du et al., 2011;

470   Markulin et al., 2019; VanPlantinga and Grossman, 2019). This process is of major

471   concern regarding the soft tissue of bivalves (potentially for human consumption), but

472   also affecting shell components. Primarily, the calcified layer of the shells is composed

473   of a mineral phase, constituting over 95% of the shell mass. The remainder is the

474   organic matrix (chitin, silk fibroin protein and acidic macromolecules) present as a thin

475   envelope or sheet surrounding each mineral unit (Gregoire, 1972 in Heuer et al., 2002;

476   Furuhashi et al., 2009). Intra-crystalline proteins occluded in the mineral lattice can also

477   occur, potentially distorting the mineral lattice (Pokroy et al., 2006). This structure

478   provides structural support, exerting control over the mineralization process (Jacob et

479   al., 2009; Immenhauser et al., 2018). Proteins and carbohydrates have high affinity for

480   heavy metals (Jacob et al., 2009), which can be remobilized during organic matrix

481   degradation of ancient shells. Heavy metal and Br enrichment via the diagenetic

482   stabilization of organic components can potentially explain the presence of the

483   persistent PCA trend observed on ancient shells of different species (Fig. 7B and C and

484   Fig. 9B).

485   By combining data from the same species (pectinid shells) at different locations

486   (Praia das Maçãs and São Julião), a combination of all the above processes becomes

487   apparent (Fig. 8). The specimen from Praia das Maçãs shows clear evidence of more

488   significant late diagenetic alteration, with more samples deviating towards the PCA

489   space corresponding to higher Mn and Fe concentration. Significant shell-boring

490   evidence (higher Al and Si values) is also detected at this location (Fig. 8). This result

491   may hint at higher prevalence of syn-depositional bioerosion at Praia das Maçãs

492   compared to São Julião and highlights the potential of the double PCA+ method for

493   highlighting changes in paleoenvironment and –ecology. Conversely, the specimen

494   collected at São Julião shows a tighter cluster along the PCA space, with only a few

495   samples deviating from the main group of samples.

496   From all the tested shell combinations, three main patterns of sample distribution

497   were identified on the PCA plots (Fig. 9C to E). When dealing with mono-species

498   analysis, altered samples deviate from a main cluster, responding differently to a variety

499   of processes (Fig. 9C). For analysis including different species, two possibilities arise:

500   there are no evident elemental differences between species, forming one single main

501   cluster and altered samples respond similarly (Fig. 9D); or inter-species elemental

502   incorporation is significantly different, generating more than one main cluster from

503   which altered samples deviate (Fig. 9E). In a next step, separate analysis (per species) is

504   recommended (as described in Fig. 7).

505

506   6.2- Disruption of background signal

507   Raw elemental data corresponding to single shell transects (e.g., Fig. 5) typically

508   present baseline values corresponding to environmentally relevant information, but

509   often masked by noise. These interruptions of the background signal may relate to

510   intervals of several consecutive samples showing moderately higher or lower values in

511   respect to the baseline (Fig. 10A; trend1); or to sharp peaks generated by a small

512   number of samples presenting higher/lower values regarding the baseline (Fig 10A;

513   trend2). In the first case, recrystallized portions of the measured transects (e.g.,

514   cemented veinlets; Fig. 4A and E) are often the cause for this effect and the double

515   PCA+ approach is effective in separating syn to post-depositional processes from the

516   primary elemental signals (Fig. 10B). In the case of conspicuous peaks, corresponding

517   to the elemental signal of small fractures and/or borings (Fig. 4A) or even instrumental

518   noise, the impact of such a small number of samples on the overall trend is minimal,

519   easily corrected when smoothing the paleoenvironmental data obtained after the double

520   PCA+ (Fig. 10C).

521

522   6.3- Dynamic thresholds: filtering-out altered elemental signals

523   Commonly, elemental cut-off values—ranges of values considered for well-

524   preserved ancient materials— are obtained from previous cases discussed in the

525   literature (e.g. Brand and Veizer, 1980, 1981; Al-Aasm and Veizer, 1986a; b), based on

526   well-preserved specimens tested by a wide array of techniques (petrographic inspection,

527   geochemistry and others) and contrasted with closely related modern specimens (when

528   available). Valuable paleoclimatic interpretations have also been produced by more

529   sophisticated thresholds for diagenesis (Jones et al., 2009; de Winter et al., 2017a;

530   2018). For the present case of Cretaceous rudists, early contributions by Al-Aasm and

531   Veizer (1986a; b) and Steuber (1996) form the backbone of sclerochronological

21

532   research on these materials. For a better detection of shell-alteration, the threshold

533   values in this study (Fig. 12 and Table 1) were obtained by performing the double

534   PCA+ approach and confidently excluding altered samples from paleoenvironmental

535   considerations. Applying these dynamic thresholds resulted in an average of 75% of the

536   initial datasets for each specimen being preserved (Figs. 6 to 8 and A5), a good

537   indication of the careful selection of specimens and overall good preservation state.

538   When compared to available literature for examples of similar (geological) age (Steuber

539   et al., 1999; Steuber, 2000; Tibljaš et al., 2004; Damas-Molla et al., 2006; Higuera et

540   al., 2007), no major differences were detected in terms of the elemental range of well-

541   preserved shell-portions (Fig. 12 and Table 1). More interestingly, traditionally used

542   cut-off values were improved by using the case-sensitive approach—the double PCA+.

543   This was the case for the majority of frequently used elemental thresholds, in general

544   narrower than literature values (Fig. 12 and Table 1).

545   Additionally, because these are dynamic thresholds, based on each specific case

546   under scope, they can be applied to different sets of shells, adapting to other studies

547   with specific aims (e.g., targeting a specific element on different shells, comparing

548   intra-shell variations; ancient *versus* modern comparions). This means that each time

549   the Double PCA+ is applied, different thresholds can be derived, hence the designation

550   of "dynamic" elemental thresholds. Also, they are applicable to other carbonate archives

551   (belemnites, oysters, corals, and many others). The application of the double PCA+

552   allows researchers to work with more unconventional elemental data (Si, Pb, Br, among

553   others), shedding light into their paleoenvironmental significance. To our knowledge,

554   this is the first attempt to use such a wide array of geochemical proxies, opening up the

555   possibility for exploring their use as proxies in future studies.

556

557

558 **7- Conclusions**

559   Dense and complex elemental archives of seventeen bivalve shells belonging to

560 two neighbouring Upper Albian shallow-water sections (western Portugal) were

561 explored using a thorough statistical analysis protocol, comparing mono- and multi-

562 species datasets, as well as shells from different locations.

563   Several inferences can be learned from applying the double PCA+ approach:

564 (i) Syn- to post-depositional processes can easily be identified based on the

565 characteristic elemental associations revealed by PCA results. These include the

566 influence of diagenesis (Fe, Mn and Mg); shell-borings filled by terrigenous materials

567 (persistent coupling of Si, Al, Fe and Mg); bioaccumulation of heavy metals (Pb, Zn)

568 and Br due to stabilization of organic shell components;

569 (ii) Different elemental patterns may arise, depending on the variability of the sampled

570 materials. For mono-species groups, the tight clustering of less-altered samples provides

571 a very clear decoupling of samples responding to syn and post-depositional processes.

572 This may also be the case for multi-species datasets, but if the original differences in

573 elemental incorporation are significant, several main clusters arise, which should be

574 analyzed separately to disentangle species-specific from depositional effects on trace

575 element content;

576 (iii) Background elemental signals (calcification mechanism and/or environmental) are

577 typically interrupted by two main types of disruption: one locally affecting only a minor

578 portion of the shell, thus characterized by sharp and very significant changes in

579 elemental composition (e.g., boring); the second affecting a larger portion of the shell,

580 but in a less prominent elemental shift (e.g., recrystallizations);

581   (iv) Regardless the degree of shell-alteration, our PCA+ approach successfully isolated

582   paleoenvironmental signals. The less-altered portions of the shells provided the

583   establishment of dynamic cut-off values, customized for each set of shells and in overall

584   agreement with elemental data retrieved from the literature. More unconventional

585   elemental data also responded well to the double PCA+ approach, contributing with

586   new clues for unravelling their incorporation mechanisms in ancient shells (Cu, P, S, Cl,

587   K, Br, Zn, Pb).

588   This research provides new and relevant methodological advances, underlining

589   the need to explore this and other tools that counterbalance the increasing technical

590   ability to obtain dense, but highly intricate sclerochronological datasets and the most

591   efficient way to unveil hidden, but relevant paleoenvironmental information. Expanding

592   the use of the presented double PCA+ approach to other skeletal materials and time

593   slices is thus a promising path towards a deeper understanding of past climatic

594   dynamics, biomineralization processes and shell-archives and diagenetic pathways over

595   time, promoting their use as proxies in future studies.

596

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605

606   Conflict of Interest

607 All authors declare that no conflict of interest exists regarding the content on this
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609

610 **References**

611 Abdi, H., Williams, L.J., 2010. Principal component analysis. Wiley Interdisciplinary

612   Reviews: Computational Statistics 2, 433-459.

613 Al-Aasm, I.S. and Veizer, J.J., 1982. Chemical stabilization of low-magnesium calcite:

614   an example of brachiopods. Journal of Sedimentary Petrology, 52, 1101-1109.

615 Al-Aasm, I.S., Veizer, J., 1986a. Diagenetic stabilization of aragonite and low-Mg

616   calcite, II. Stable isotopes in rudists. Journal of Sedimentary Research 56, 763-770.

617 Al-Aasm, I.S., Veizer, J., 1986b. Diagenetic stabilization of aragonite and low-Mg

618   calcite, I. Trace elements in rudists. Journal of Sedimentary Research 56, 138-152.

619 Batenburg, S.J., Reichart, G.-J., Jilbert, T., Janse, M., Wesselingh, F.P., Renema, W.,

620   2011. Interannual climate variability in the Miocene: High resolution trace element

621   and stable isotope ratios in giant clams. Palaeogeography, Palaeoclimatology,

622   Palaeoecology 306, 75-81.

623 Berthou, P.-Y., 1984. Albian-Turonian stage boundaries and subdivisions in the

624   western Portuguese basin, with special emphasis on the Cenomanian-Turonian

625   boundary in the ammonite facies and rudist fades. Bulletin of the Geological

626   Society of Denmark 33, 41-45.

627 Brand, R., 2006. Scallop ecology: distributions and behaviour. *In*: Shumway, S.E.,

628   Parsons, G.J. (Eds.), Scallops: Biology, Ecology and Aquaculture. Elsevier B.V,

629   Amsterdam, The Netherlands, 651-744.

630 Brand, U., Veizer, J., 1980. Chemical diagenesis of a multicomponent carbonate system

631   - 1: Trace elements. Journal of Sedimentary Research 50, 1219-1236.

632 Brand, U., Veizer, J., 1981. Chemical diagenesis of a multicomponent carbonate

633   system-2: stable isotopes. Journal of Sedimentary Research 51, 987-997.

634   Cai, T., Wu, H., Qin, J., Qiao, J., Yang, Y., Wu, Y., Qiao, D., Xu, H., Cao, Y., 2019. In

635   vitro evaluation by PCA and AHP of potential antidiabetic properties of lactic acid

636   bacteria isolated from traditional fermented food. LWT 115, 108455.

637   Carter, J.G., 1990. Skeletal biomineralization: patterns, processes and evolutionary

638   trends. Van Nostrand & Reinhold, New York, 832 pp.

639   Chan, P., Halfar, J., Williams, B., Hetzinger, S., Steneck, R., Zack, T., Jacob, D.E.,

640   2011. Freshening of the Alaska Coastal Current recorded by coralline algal Ba/Ca

641   ratios. Journal of Geophysical Research: Biogeosciences 116 (G1).

642   Carré, M., Bentaleb, I., Bruguier, O., Ordinola, E., Barret, N.T., Fontugne, M., 2006.

643   Calcification rate influence on trace element concentrations in aragonitic bivalve

644   shells: Evidences and mechanisms. Geochimica et Cosmochimica Acta 70, 4906-

645   4920.

646   Christ, N., Immenhauser, A., Amour, F., Mutti, M., Tomás, S., Agar, S.M., Alway, R.,

647   Kabiri, L., 2012. Characterization and interpretation of discontinuity surfaces in a

648   Jurassic ramp setting (High Atlas, Morocco). Sedimentology 59, 249-290.

649   Chumakov, N.M., Zharkov, M.A., Herman, A.B., Doludenko, M.P., Kalandadze, N.N.,

650   Lebedev, E.A., Ponomarenko, A.G., Rautian, A.S., 1995. Climate belts of the

651   mid Cretaceous time. Stratigraphy and Geological Correlations 3, 241-260.

652   Coimbra, R., Azerêdo, A.C., Cabral, M.C., 2018. Cretaceous coastal lagoon facies:

653   Geochemical insights into multi-stage diagenesis and palaeoclimatic signals.

654   Cretaceous Research 85, 60-77.

655   Coimbra, R., Horikx, M., Huck, S., Heimhofer, U., Immenhauser, A., Rocha, F., Dinis,

656   J., Duarte, L.V., 2017. Statistical evaluation of elemental concentrations in shallow-

657   marine deposits (Cretaceous, Lusitanian Basin). Marine and Petroleum Geology 86,

658   1029-1046.

659   Coimbra, R., Immenhauser, A., Olóriz, F., Rodríguez-Galiano, V., Chica-Olmo, M.,

660   2015. New insights into geochemical behaviour in ancient marine carbonates (Upper

661   Jurassic Ammonitico Rosso): Novel proxies for interpreting sea-level dynamics and

662   palaeoceanography. Sedimentology 62, 266-302.

663   Cordella, C.B.Y., 2012. PCA: The Basic Building Block of Chemometrics. In: Krull, I.

664   S. (director), Analytical Chemistry, pp. 1-44.

665   Damas-Mollá, L., Aranburu-Artano, A., García-Garmilla, F., 2006. Resistencia a la

666   alteración diagenética de conchas de Chondrodonta sp. en las calizas rojas del

667   Aptiense-Albiense Inferior de Ereño (Bizkaia). Geogaceta, 40, 195-198.

668   de Winter, N. J., & Claeys, P. (2016). Micro X-ray fluorescence (μXRF) line scanning

669   on Cretaceous rudist bivalves: A new method for reproducible trace element profiles

670   in bivalve calcite. Sedimentology, 64(1), 231-251.

671   de Winter, N. J., Goderis, S., Dehairs, F., Jagt, J. W., Fraaije, R. H., Van Malderen, S.

672   J., et al. (2017a). Tropical seasonality in the late Campanian (late Cretaceous):

673   Comparison between multiproxy records from three bivalve taxa from Oman.

674   Palaeogeography, Palaeoclimatology, Palaeoecology, 485, 740-760.

675   de Winter, N. J., Sinnesael, M., Makarona, C., Vansteenberge, S., & Claeys, P. (2017b).

676   Trace element analyses of carbonates using portable and micro-X-ray fluorescence:

677   performance and optimization of measurement parameters and strategies. Journal of

678   Analytical Atomic Spectrometry, 32(6), 1211-1223.

679   de Winter, N. J., Vellekoop, J., Vorsselmans, R., Golreihan, A., Soete, J., Petersen, S.

680   V., et al. (2018). An assessment of latest Cretaceous Pycnodonte vesicularis

681   (Lamarck, 1806) shells as records for palaeoseasonality: a multi-proxy investigation.

682   Climate of the Past, 14(6), 725-749.

683   Dinis, J. L., Rey, J., De Graciansky, P. C., 2002. The Lusitanian Basin (Portugal) during

684   the late Aptian- Albian: Sequential arrangement, proposal of correlations, evolution.

685   Comptes Rendus Geoscience 334 (10), 757-764.

686   Dinis, J. L., Trincão, P., 1995. Recognition and stratigraphical significance of the

687   Aptian unconformity in the Lusitanian Basin, Portugal. Cretaceous Research 16 (2-

688   3), 171-186.

689   Dinis, J.L., Rey, J., Cunha, P.P., Callapez, P., Pena dos Reis, R., 2008. Stratigraphy and

690   allogenic controls of the western Portugal Cretaceous: an updated synthesis.

691   Cretaceous Research 29, 772-780.

692   Du, Y., Lian, F., Zhu, L., 2011. Biosorption of divalent Pb, Cd and Zn on aragonite and

693   calcite mollusk shells. Environmental Pollution 159, 1763-1768.

694   Elderfield, H., M. Vautravers, and M. Cooper, 2002. The relationship between shell size

695   and Mg/Ca, Sr/Ca, $\delta^{18}O$, and $\delta^{13}C$ of species of planktonic foraminifera.

696   Geochemistry, Geophysics, Geosystems 3(8), 1-13.

697   Ferreira, A.G., Machado, A.L.S., Zalmon, I.R., 2004. Temporal and spatial variation on

698   heavy metal concentrations in the bivalve Perna perna (LINNAEUS, 1758) on the

699   northern coast of Rio de Janeiro State, Brazil. Brazilian Archives of Biology and

700   Technology 47, 319-327.

701   Furuhashi, T., Schwarzinger, C., Miksik, I., Smrz, M., Beran, A., 2009. Molluscan

702   shell evolution with review of shell calcification hypothesis. Comparative

703   Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 154,

704   351-371.

705   Gili, E., Götz, S., 2018. Part N, Volume 2, Chapter 26B: Paleoecology of rudists.

706   Treatise Online 103: 1-29.

707 Gili, E., Masse, J.P., Skelton, P.W., 1995. Rudists as gregarious sediment-dwellers, not

708   reef-builders, on Cretaceous carbonate platforms. Palaeogeography,

709   Palaeoclimatology, Palaeoecology 118, 245-267.

710 Gillikin, D.P., Lorrain, A., Paulet, Y.-M., André, L., Dehairs, F., 2008. Synchronous

711   barium peaks in high-resolution profiles of calcite and aragonite marine bivalve

712   shells. Geo-Marine Letters 28, 351-358.

713 Gillikin, D.P., Lorrain, A., Paulet, Y.M., André, L., Dehairs, F., 2008. Synchronous

714   barium peaks in high-resolution profiles of calcite and aragonite marine bivalve

715   shells. Geo- Marine Letters 28 (5-6), 351-358.

716 Gillikin, D.P., Wanamaker, A.D., Andrus, C.F.T. 2019. Chemical sclerochronology.

717   Chemical Geology 526, 1-6.

718 Grégoire, C., 1972. Structure of the molluscan shell. In: Florkin, M., Scheer, B.T. (Eds.)

719   Chemical Zoology. Academic Press, New York, Vol. 7, pp. 45-102.

720 Halfar, J., Zack, T., Kronz, A., Zachos, J.C., 2000. Growth and high-resolution

721   paleoenvironmental signals of rhodoliths (coralline red algae): a new biogenic

722   archive. Journal of Geophysical Research: Oceans 105 (C9), 22107-22116.

723 Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological statistics

724   software package for education and data analysis. Palaeontolologia Electronica 4(1),

725   9pp.

726 Harper et al., 1996. Early Cretaceous cementing pectinid bivalves. Cretaceous Research

727   17, 135-150.

728 Hasenboehler, B., 1981. Étude paleobotanique et palynologie de l'Albien et du

729   Cénomanien du "Bassin Occidental Portugais" au sud de l'accident de Nazaré

730   (Portugal). Université Pierre et Marie Curie, Paris, Thése 3éme Cycle.

731   Unpublished PhD thesis, 348 pp.

732 Heimhofer, U., Hochuli, P. A., Burla, S., Weissert, H., 2007. New records of Early

733   Cretaceous angiosperm pollen from Portuguese coastal deposits: Implications for the

734   timing of the early angiosperm radiation. Review of Palaeobotany and Palynology

735   144(1), 39-76.

736 Heuer, A.H., Su, X., Kamat, S., Ballarini, R., Buschow, K.H.J., Cahn, R.W., Flemings,

737   M.C., Ilschner, B., Kramer, E.J., Mahajan, S., Veyssiüre, P., 2001. Shell: Properties.

738   In: Jürgen Buschow, K. H., Cahn, R.W., Flemings, M. C., Ilschner, B., Kramer, E.,

739   Mahajan, S., Veyssière, P. (Eds.), Encyclopedia of Materials: Science and

740   Technology. Elsevier, Oxford, pp. 8462-8469.

741 Higuera, I.R., Garmilla, F.G., Skelton, P.W., 2007. Sclerochronology and diagenesis of

742   Late Cretaceous radiolitids (Bivalvia, Hippuritoidea), Spain. In: Scott, R.W. (Ed.),

743   Cretaceous rudists and carbonate platform: environmental feedback. SEPM Special

744   Publication, 87, pp. 115-139.

745 Horikx, M., Heimhofer, U., Dinis, J., Huck, S., 2014. Integrated stratigraphy of shallow

746   marine Albian strata from the southern Lusitanian Basin of Portugal. Newsletters on

747   Stratigraphy 47, 85-106.

748 Horikx, M., Hochuli, P. A., Feist-Burkhardt, S., Heimhofer, U., 2016. Albian

749   angiosperm pollen from shallow marine strata in the Lusitanian Basin, Portugal.

750   Review of Palaeobotany and Palynology 228, 67-92.

751 Huyghe, D., de Rafelis, M., Ropert, M., Mouchi, V., Emmanuel, L., Renard, M.,

752   Lartaud, F., 2009. New insights into oyster high-resolution hinge growth patterns.

753   Marine Biology 166, 48.

754 Immenhauser, A., Schöne, B.R., Hoffmann, R., Niedermayr, A., 2018. Mollusc and

755   brachiopod skeletal hard parts: Intricate archives of their marine environment.

756   Sedimentology 63, 1-59.

757 Jacob, D.E., Soldati, A.L., Wirth, R., Huth, J., Wehrmeister, U., Hofmeister, W., 2008.

758 Nanostructure, composition and mechanisms of bivalve shell growth. Geochimica et

759 Cosmochimica Acta 72, 5401-5415.

760 Jones, D.S., 1983. Sclerochronology: Reading the Record of the Molluscan Shell:

761 Annual growth increments in the shells of bivalve molluscs record marine climatic

762 changes and reveal surprising longevity. American Scientist 71, 384-391.

763 Jones, K., Bronson, S., Brink, P., Gordon, C., Mosher-Smith, K., Brown, M., Chaudhry,

764 S., Rizzo, A., Sigismondi, R., Whitehurst, M., Lukaszewski, A., Kranz, D., Bland,

765 K., Gordan, D., Lobel, J., Sullivan, J., Metzger, M., Shea, C., Harris, C.,

766 Kambhampati, M., 2009. Bivalve Characterization Using Synchrotron Micro X-Ray

767 Fluorescence. Acta Physica Polonica A 115.

768 Kullberg, J. C., Rocha, R. B., Soares, A. F., Rey, J., Terrinha, P., Azerêdo, A. C.,

769 Callapez, P., Duarte, L. V., Kullberg, M. C., Martins, L., Miranda, J. R., Alves, C.,

770 Mata, J., Madeira, J., Mateus, O., Moreira, M., Nogueira, C.R., 2013. A Bacia

771 Lusitaniana: Estratigrafia, Paleogeografia e Tectónica. In Geologia de Portugal (eds.

772 R. Dias, A. Araújo, P. Terrinha and J-C. Kullberg). Volume 2, Escolar Editora,

773 Lisbon, Portugal, pp 195-347.

774 LNEG-LGM, 2010. Carta Geológica de Portugal à escala 1:1000000, edição de 2010.

775 Laboratório Nacional de Energia e Geologia, Lisbon, Portugal.

776 Lorens, R. B., 1981. Sr, Cd, Mn, and Co distribution coefficients in calcite as a function

777 of calcite precipitation rate. Geochimica et Cosmochimica Acta, 45, 553-561.

778 Markulin, K.i., Peharda, M., Mertz-Kraus, R., Schöne, B.R., Uvanovič, H., Kovač, Ž.,

779 Janeković, I., 2019. Trace and minor element records in aragonitic bivalve shells as

780 environmental proxies. Chemical Geology 507, 120-133.

781   Medus, J., 1982. Palynofloristic correlations of two Albian sections of Portugal.

782   Cuadernos Geología Ibérica 8, 781-809.

783   Nedoncelle, K., Lartaud, F., de Rafelis, M., Boulila, S., Le Bris, N., 2013. A new

784   method for high-resolution bivalve growth rate studies in hydrothermal

785   environments. Marine Biology 160, 1427-1439.

786   Nürnberg, D., Bijma, J., Hemleben, C., 1996. Assessing the reliability of magnesium in

787   foraminiferal calcite as a proxy for water mass temperatures. Geochimica et

788   Cosmochimica Acta 60, 803-814.

789   Philip, J., 1972. Paléoécologie des formations à rudistes du Crétacé supérieur -

790   l'exemple du sud-est de la France. Palaeogeography, Palaeoclimatology,

791   Palaeoecology 12, 205-222.

792   Pokroy, B., Fitch, A., Zolotoyabko, E., 2006. The Microstructure of Biogenic Calcite: A

793   View by High-Resolution Synchrotron Powder Diffraction. Advanced Materials

794   18, 2363-2368.

795   Pons, J.-M., Vicens, E. , 2008. The structure of the outer shell layer in radiolitid rudists,

796   a morphoconstructional approach. Lethaia 41, 219-234.

797   Rauch, M., 2005. Geochemie von Rudistenschalen - Beiträge zur Meerwasserchemie

798   (Sr/Ca, Mg/Ca, δ^{13}C) und Paläoklima der Kreide. PhD thesis, 198 pp., Ruhr-

799   University Bochum, Bochum, Germany.

800   Rey, J., 1992. Les unités lithostratigraphiques du Crétacé inférieur de la région de

801   Lisbonne. Comunicações dos Serviços Geológicos de Portugal 78, 103-124.

802   Richardson, C.A., Peharda, M., Kennedy, H., Kennedy, P., Onofri, V., 2004. Age,

803   growth rate and season of recruitment of Pinna nobilis (L) in the Croatian Adriatic

804   determined from Mg: Ca and Sr: Ca shell profiles. Journal of Experimental Marine

805   Biology and Ecology 299, 1-16.

806   Rickaby, R. E. M. , Schrag, D. P. , Zondervan, I. and Riebesell, U. (2002): Growth-rate

807   dependence of Sr incorporation during calcification of Emiliania huxleyi, Global

808   Biogeochemical Cycles, 16 (1), 1-8.

809   Rimstidt, J.D., Balog, A., Webb, J., 1998. Distribution of trace elements between

810   carbonate minerals and aqueous solutions. Geochimica et Cosmochimica Acta 62,

811   1851-1863.

812   Ross, D.J., Skelton, P.W., 1993) Rudist formations of the Cretaceous: A

813   palaeoecological, sedimentological and stratigraphical review. Sedimentology

814   Review 1, 73-91.

815   Schöne, B. R., Zhang, Z., Radermacher, P., Thébault, J., Jacob, D. E., Nunn, E. V., &

816   Maurer, A.-F. (2011). Sr/Ca and Mg/Ca ratios of ontogenetically old, long-lived

817   bivalve shells (Arctica islandica) and their function as paleotemperature proxies.

818   Palaeogeography, Palaeoclimatology, Palaeoecology, 302(1-2), 52-64.

819   Schöne, B.R., Fiebig, J., Pfeiffer, M., Gleβ, R., Hickson, J., Johnson, A.L., Dreyer, W.,

820   Oschmann, W., 2005. Climate records from a bivalved Methuselah (Arctica

821   islandica, Mollusca; Iceland). Palaeogeography, Palaeoclimatology, Palaeoecology

822   228, 130-148.

823   Schöne, B.R., Houk, S.D., Castro, A.D.F., Fiebig, J., Oschmann, W., Kröncke, I.,

824   Dreyer, W., Gosselck, F., 2005. Daily growth rates in shells of Arctica islandica:

825   assessing sub-seasonal environmental controls on a long-lived bivalve mollusk.

826   Palaios 20, 78-92.

827   Serb, J.M., 2006. Reconciling morphological and molecular approaches in developing a

828   Phylogeny for the Pectinidae (Mollusca: Bivalvia). In: Shumway, S.E., Parsons,

829   G.J. (Eds.), Scallops: Biology, Ecology and Aquaculture. Elsevier B.V,

830   Amsterdam, The Netherlands, 1-29.

831   Skelton, P.W., 1978. The evolution of functional design in rudists (Hippuritacea) and its

832   taxonomic implications. Royal Society of London, Philosophical Transactions

833   284, 305-318.

834   Skelton, P.W., 2003. The Cretaceous World. The Open University and Cambridge

835   University Press. Cambridge, UK. 360 pp.

836   Skelton, P.W., 2018. Part N, Volume 1, Chapter 26A: Introduction to the Hippuritida

837   (rudists): Shell structure, anatomy, and evolution. Treatise Online 104: 1-37.

838   Skelton, P.W., Smith, A.B., 2000. A preliminary phylogeny for rudist bivalves: Sifting

839   clades from grades. *In*: Harper, E.M, Taylor, J.D., Crame, J.A. (Eds.): The

840   Evolutionary Biology of the Bivalvia. Geological Society, London, Special

841   Publications 177, 97-127.

842   Stampfli, G.M., Borel, G.D., 2002. A plate tectonic model for the Paleozoic and

843   Mesozoic constrained by dynamic plate boundaries and restored synthetic oceanic

844   isochrons. Earth and Planetary Science Letters 196, 17-33.

845   Stanley, S.M., 1970. Relation of shell form to life habits of the Bivalvia (Mollusca).

846   Geological Society of America Memoires 125, 1-296.

847   Steuber, T., 1996. Stable isotope sclerochronology of rudist bivalves: Growth rates and

848   Late Cretaceous seasonality. Geology, 24, 315-318.

849   Steuber, T., 1999. Isotopic and chemical intra-shell variations in low-Mg calcite of

850   rudist bivalves (Mollusca-Hippuritacea): disequilibrium fractionations and late

851   Cretaceous seasonality. International Journal of Earth Sciences 88, 551-570.

852   Steuber, T., 2000. Skeletal growth rates of Upper Cretaceous rudist bivalves:

853   implications for carbonate production and organism-environment feedbacks. In:

854   INSALCO, E. et al. (eds.): Carbonate Platform Systems: Components and

855   Interactions. Geol. Soc. London, Spec. Publ., 178, 21-32.

856   Steuber, T., Rauch, M., Masse, J.-P., Graaf, J., Malkoč, M., 2005. Low-latitude

857   seasonality of Cretaceous temperatures in warm and cold episodes. Nature 437,

858   1341-1344.

859   Stoll, H.M., Klaas, C.M., Probert, I., Ruiz Encinar, J., Garcia Alonso, J.I., 2002.

860   Calcification rate and temperature effects on Sr partitioning in coccoliths of multiple

861   species of coccolithophorids in culture. Global and Planetary Change 34, 153-171.

862   Stoll, H.M., Schrag, D.P., 2000. Coccolith Sr/Ca as a new indicator of coccolithophorid

863   calcification and growth rate. Geochemistry, Geophysics Geosystems (1), 1006.

864   Surge, D., Lohmann, K. C., & Dettman, D. L. (2001). Controls on isotopic chemistry of

865   the American oyster, Crassostrea virginica: implications for growth patterns.

866   Palaeogeography, Palaeoclimatology, Palaeoecology, 172(3), 283-296.

867   Swart, P.K., 2015. The geochemistry of carbonate diagenesis: the past, present and

868   future. Sedimentology 62, 123-e1304.

869   Tesoriero, A. J., and J. F. Pankow, 1996. Solid solution partitioning of Sr^{2+}, Ba^{2+} and

870   Cd^{2+} to calcite. Geochimica et Cosmochimica Acta, 60, 1053-1063, 1.

871   Tibljaš, D., Moro, A., Ostroš, Z., 2004. Mineral and Chemical Composition of Rudist

872   Valves from Upper Cretaceous Limestones of Southern Istria, Croatia. Geologia

873   Croatica, 57 (1), 13-19.

874   Ullmann, C.V., Gale, A.S., Huggett, J., Wray, D., Frei, R., Korte, C., Broom-Fendley,

875   S., Littler, K., Hesselbo, S.P., 2018. The geochemistry of modern calcareous barnacle

876   shells and applications for palaeoenvironmental studies. Geochimica et

877   Cosmochimica Acta 243, 149-168.

878   Ullmann, C.V., Wiechert, U., Korte, C., 2010. Oxygen isotope fluctuations in a modern

879   North Sea oyster (Crassostrea gigas) compared with annual variations in seawater

880   temperature: Implications for palaeoclimate studies. Chemical Geology 277, 160-

881   166.

882   VanPlantinga, A.A., Grossman, E.L., 2019. Trace elements in mussel shells from the

883   Brazos River, Texas: environmental and biological control. Biogeosciences

884   Discussions 2019, 1-27.

885   Veizer, J., 1983. Chemical analysis of carbonates: theory and application of trace

886   element technique. In: Arthur, M.A., Anderson, T.F., Kaplan, I.R., Veizer, J. (Eds.),

887   Stable Isotopes in Sedimentary Geology. SEPM Short Course No.10, pp. 1-100.

888   Yao, F., Coquery, J., Lê-Cao, K.-A., 2012. Independent Principal Component Analysis

889   for biologically meaningful dimension reduction of large biological data sets. BMC

890   bioinformatics 13, 24-24.

891   Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and

892   aberrations in global climate 65 Ma to present. Science 292, 686-693.

893   Zamarreño et al., 1996. The shell microstructure, mineralogy and isotopic composition

894   of Amussiopecten baranensis (Pectinidae, Bivalvia) from the Miocene of Spain:

895   A valuable paleoenvironmental tool Geobios 29, 707-724.

896
897   **Fig. 1.** Geographical setting of the studied locations. A) Map of Iberia showing the

898   location of the western Portuguese coast. B) Regional distribution of Cretaceous

899   outcrops (adapted from LNEG-LGM, 2010), along with delimitation of the Lusitanian

900   Basin (after Kullberg et al., 2013). C) Location of study sites at São Julião and Praia das

901   Maçãs (indicated by stars), south of Ericeira (Portugal).

902

903   **Fig. 2.** Field photographs and particular aspects of sampled beds and shells. A and B)

904   Aerial photographs of Praia das Maçãs and near São Julião (extracted from

905   http://portugalfotografiaaerea.blogspot.com/); C) Field aspect of one specific sampled

906   bed; D) Field aspect of the pectinid bearing bed at Praia das Maçãs (PM4); E) Field

907   aspect of a requiniid-rich horizon at Praia das Maçãs (PM12); F) Detail of an

908   *Apricardia carentonensis* shell from the São Julião section.

909

910   **Fig. 3.** Stratigraphic context, selected specimens and comparison scheme. A) Synthetic

911   lithological log of São Julião and Praia das Maçãs showing height (in meters),

912   stratigraphy, C-isotope values as complementary lateral correlation tool and the main

913   sedimentological features (see Horikx et al., 2014 and Coimbra et al., 2017 for more

914   detailed explanations). B) List of selected specimens and scheme illustrating the logic of

915   comparison followed during this work (see text for further details and Fig. A1 in

916   Appendix for photographs of all used specimens). Numeral tags (in blue) link each shell

917   (or group of shells) specimen to their stratigraphic position, used throughout this

918   contribution.

919

920   **Fig. 4.** Shell structure and preliminary assessment of shell-preservation state. A to D)

921   Example from Praia das Maçãs, including microscope detail of small borings and small

922   cemented (sub-vertical) veinlet and elemental mapping of Mn and Sr, as well as the

923   chosen transect for further geochemical line scanning. E to H) example from São Julião,

924   with microscope detail of small borings and fractures affecting the shell and elemental

925   mapping of Fe and and Mn, as well as the selected transect for further line scanning (red

926   line). Shell length ca. 8 cm in both cases, numeral tags (in blue) are according to Figure

927   3 and Fig. A1 as Appendix).

928

929   **Fig. 5.** Selected raw elemental data (Mn, Fe, Sr, Mg) for two pectinid specimens

930   collected at different locations (Praia das Maçãs and São Julião) illustrating the need of

931 an efficient data treatment approach. Note the high complexity of each elemental record,

932 differences in absolute value and fluctuations along shell-length, as well as the

933 difficulties on comparing the records of both shells. Numeral tags (in blue) are

934 according to Figure 3 and Fig. A1 as Appendix.

935

936 **Fig. 6.** Principal component analysis results (scores and loadings), combined with

937 density analysis (see Appendix for further details) for the specimens collected at Praia

938 das Maçãs. Sample distribution by shell is also represented by using coloured dots,

939 corresponding to the contribution of each shell to the cloud of data generated by the

940 PCA procedure (in white dots). This representation is proportional to the main PCA

941 plot, respecting the position of each sample along the PCA space. A) mono-specific

942 analysis including six requiniid shells; B) multi-species analysis including seven

943 different shells belonging to four species. Note tight clustering of a significant

944 percentage of the samples delimiting a range of elemental thresholds, as well as similar

945 elemental trend of deviation from the main cluster (see text for detailed explanation).

946 Numeral tags (in blue) are according to Figure 3 and Fig. A1 as Appendix.

947

948 **Fig. 7.** Principal component analysis results (PCA scores and loadings), combined with

949 density analysis (double PCA+; see Appendix for further details) for the specimens

950 collected at São Julião. Sample distribution by shell is also represented by using

951 coloured dots, corresponding to the contribution of each shell to the cloud of data

952 generated by the PCA procedure (in white dots). This representation is proportional to

953 the main PCA plot, respecting the position of each sample along the PCA space. A)

954 multi-species analysis including four different shells belonging to two species; B and C)

955 mono-specific analysis of samples included in A), separated by species (*Apricardia* and

956   pectinid, respectively). Note persistent elemental trends when considering different

957   species (see text for detailed explanation). Numeral tags (in blue) are according to

958   Figure 3 and Fig. A1 as Appendix.

959

960   **Fig. 8.** Principal component analysis results (PCA scores and loadings), combined with

961   density analysis (double PCA+; see Appendix for further details) for pectinid shells

962   collected at São Julião and Praia das Maçãs (mono-specific analysis). Sample

963   distribution by shell is also represented. Note a tighter clustering of samples belonging

964   to the pectinid shell collected at São Julião (see text for further explanations). Numeral

965   tags (in blue) are according to Figure 3 and Fig. A1 as Appendix.

966

967   **Fig. 9.** Representative scheme of main elemental trends as depicted by double PCA+

968   approach (see also Fig. A4). A and B) Summary of the persistent elemental trends and

969   associations identified along the tested shells, with their respective process (diagenesis,

970   boring, a combined effect of both and bioaccumulation); C to E) Example of sample

971   distribution across the PCA space in response to the identified processes. Sample

972   distribution will depend on which processes are involved (single or multiple) and on the

973   variability of the biogenic record (mono vs. multi-species analysis).

974

975   **Fig. 10.** Schematic workflow for improving the detection of shell-alteration. A) Possible

976   disruptions of background signal by significantly altered portions and/or cracks; B) fast

977   detection of altered samples allows their elimination from further analysis, in order to

978   obtain a cleaner and relevant paleoenvironmental signal; C) Isolating the clean

979   background record allows the identification of intra-shell fluctuations, attributable to

980   seasonal climate in ancient times. Numeral tags (in blue) are according to Figure 3 and

981   Fig. A1 as Appendix.

982

983   **Fig. 11.** Comparison between unprocessed elemental data and the resulting information

984   after the Double PCA+ approach. A) Sr values for the set of 6 requiniid shells (tag

985   numeral as in Figs. 3 and A1). White shaded area delimits the range of best-preserved

986   Sr values, when Double PCA+ is computed for this specific group of shells (see statistic

987   results in Fig. 6). B) Best-preserved Sr values for all requinid shells, after excluding

988   20% of the altered data by the Double PCA+ method (see text for details). The clean

989   dataset of each shell now delineates discernible cyclic variations in Sr abundance. Note

990   that the obtained elemental threshold values are customized for this set of mono-speficic

991   shells (see Fig. 12 and Table 1 for obtained values of other sets of shells).

992

993   **Fig. 12.** Dynamic elemental threshold values obtained by the application of the double

994   PCA+ screening method (see Appendix for details) and data retrieved from available

995   literature. Note overall agreement with published cut-off elemental values, as well and

996   the establishment of new threshold for more unconventional elements (Cu, Zn, Pb, Cu).

997   Numeral tags (in blue) are according to Figure 3 and Fig. A1 as Appendix.

998

999   **Table 1.** Dynamic elemental threshold values obtained by the application of the double

1000   PCA+ screening method (see Appendix for details) and threshold values extracted from

1001   literature. Groups 1 to 6 refer to used literature and tested shell combinations (same as

1002   in Figure 11). Min=minimum; max=maximum. Numeral tags (in blue) are according to

1003   Figure 3 and Fig. A1 as Appendix.

1004

1005 **Table 2.** Average elemental values (in ppm) resulting from the application of the double

1006 PCA+ approach (see Appendix for details). Shell tags as in Fig. 3 and A1.

1007
1008 Table 1- Dynamic elemental threshold values obtained by the application of the double PCA+ screening
1009 method (see Appendix for details) and threshold elemental values extracted from literature. Groups of
1010 shells 1 to 6 refer to used literature and tested shell combinations (same as in Figure 11; min=minimum;
1011 max=maximum).

	Ca (min; max)	Mg (min; max)	Sr (min; max)	Fe (min; max)	Mn (min; max)	Al (min; max)	Si (min; max)	Cu (min; max)	S (min; max)	P (min; max)	Br (min; max)	Zn (min; max)	Pb (min; max)
1	a)	1200; 4600	700; 7230	0; 420	0; 410	a)	a)	0; 24	a)	a)	a)	0; 20	0; 10
2	387666; 395206	359; 4911	888; 2000	0; 689	0; 170	--	1573; 6209	0; 50	--	--	--	--	--
3	392627; 397951	631; 3080	1204; 1852	0; 459	0; 266	--	821; 1985	--	71; 987	--	--	--	--
4	390535; 396651	568; 2892	986; 1734	0; 578	0; 130	138; 290	941; 3885	--	--	3; 27	0; 39	--	--
5A	392024; 395914	498; 1492	--	0; 310	--	225; 391	--	--	--	15; 29	10; 88	--	--
5B	393869; 395633	1031; 1821	--	0; 255	--	--	1783; 3223	--	307; 838	--	9; 20	15; 42	4; 30
6	a)	277; 1337	879; 1428	0; 46	0; 25	a)	a)	a)	a)	a)	a)	a)	a)

(a) Not found, presented, measured and/or assumed as constant value; -- Not computed due to low statistical significance (see text)

Group 1- literature*; Group 2- Different species; same location (Praia das Maçãs) 5 6 7 8; Group 3- Same species; same location (Praia das Maças) 9
Group 4- Same species; different location (pectinid shells) 1 5; Group 5A- Same species, same location (Apricardia, São Julião) 3 4
Group 5B- Same species, same location (pectinid shells, São Julião) 1 2 ; Group 6- Different species; different locations (ICP-powder samples)

1012
1013
1014 Table 2. Average elemental values (in ppm) resulting from the application of the double PCA+ method
1015 (see Appendix for details). Shell tags as in Figs. 3 and A1.

Location	Shell Tag	Mg	Sr	Fe	Mn
São Julião section	Tag 1_pectinid 1	1597[2] 1366[3]	1284[2] --	141[2] 82[3]	50[2] --
	Tag 2_pectinid 2	1465	--	111	--

	Tag 3_*Apricardia* 1	905	--	56	--
	Tag 4_*Apricardia* 2	973	--	58	--
	Tag 5_pectinidA	2212[1] 2222[2]	1512[1] 1501[2]	159[1] 147[2]	88[1] 59[2]
	Tag 6_Chondrodont 1	3668	1285	342	99
	Tag 6_Chondrodont 2	3823	1460	269	70
	Tag 6_Chondrodont 3	3384	1363	134	52
Praia das Maçãs section	Tag 6_Chondrodont 4	3795	1397	120	61
	Tag 7_elevator rudist	1174	1474	85	68
	Tag 8_radiolithid	3810	994	279	102
	Tag 9_Requiniid 1	1786	1640	58	83
	Tag 9_Requiniid 2	1801	1543	85	47
	Tag 9_Requiniid 3	1554	1465	92	51
	Tag 9_Requiniid 4	2283	1540	70	46
	Tag 9_Requiniid 5	1784	1530	111	46
	Tag 9_Requiniid 6	1805	1453	83	53

(1) computed in Group 2, see Figs. 6B and 12; (2) computed in Group 4, see Figs. 8 and 12; (3) computed in Group 5B, see Figs. 7C and 12; -- Not computed due to low statistical significance (see text)

Highlights

- A novel dynamic method aims to separate pristine from altered shell material;

- Syn- to post-depositional processes were easily identified;

- PCA+ approach successfully isolated paleoenvironmental signals;

- Different elemental patterns arise from mono- or multi-species sets of shells;
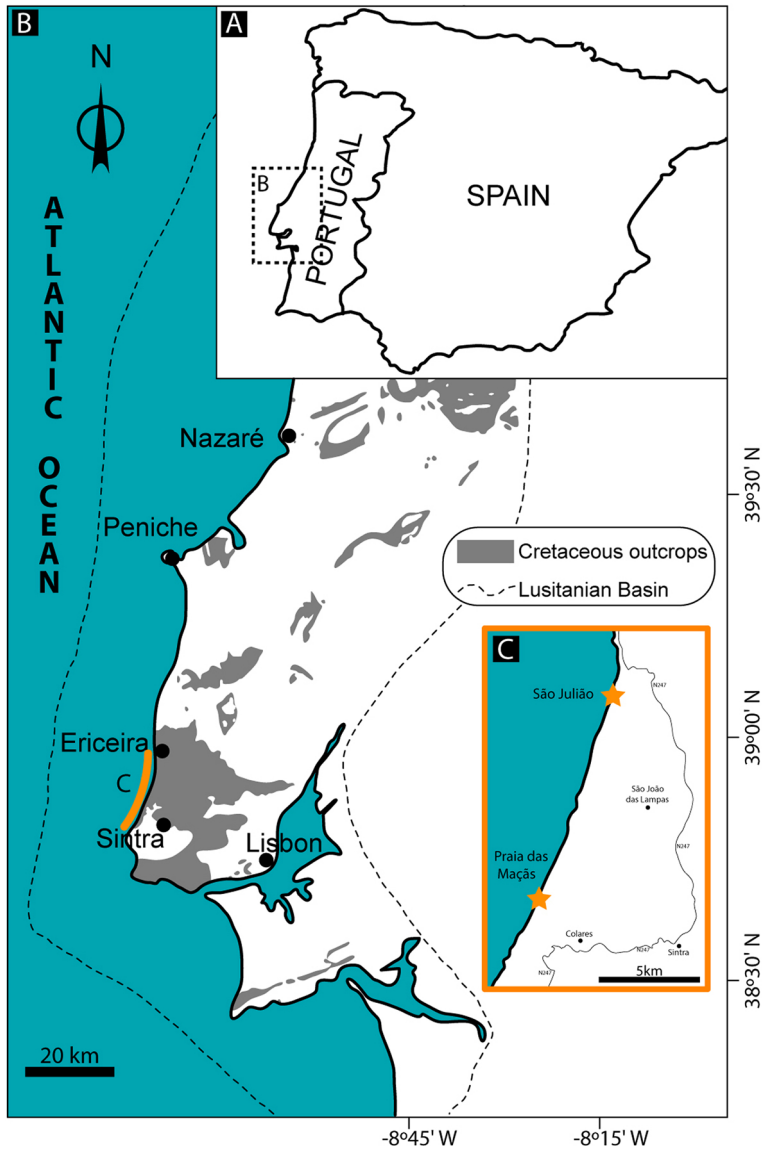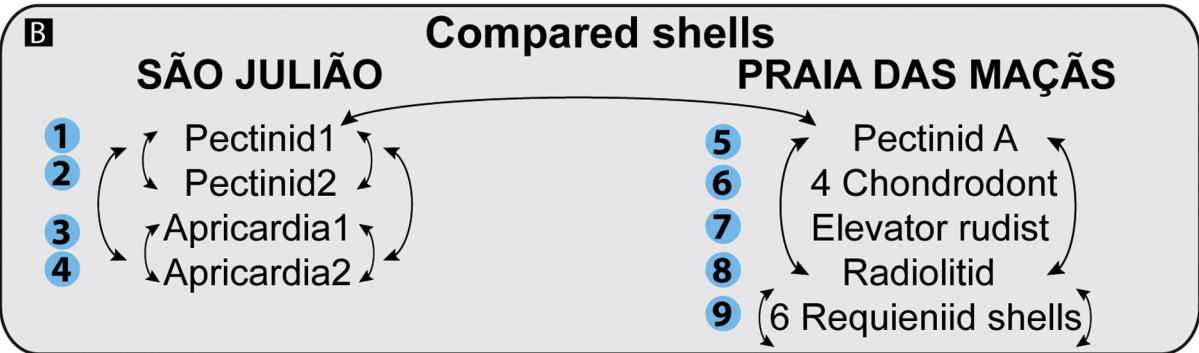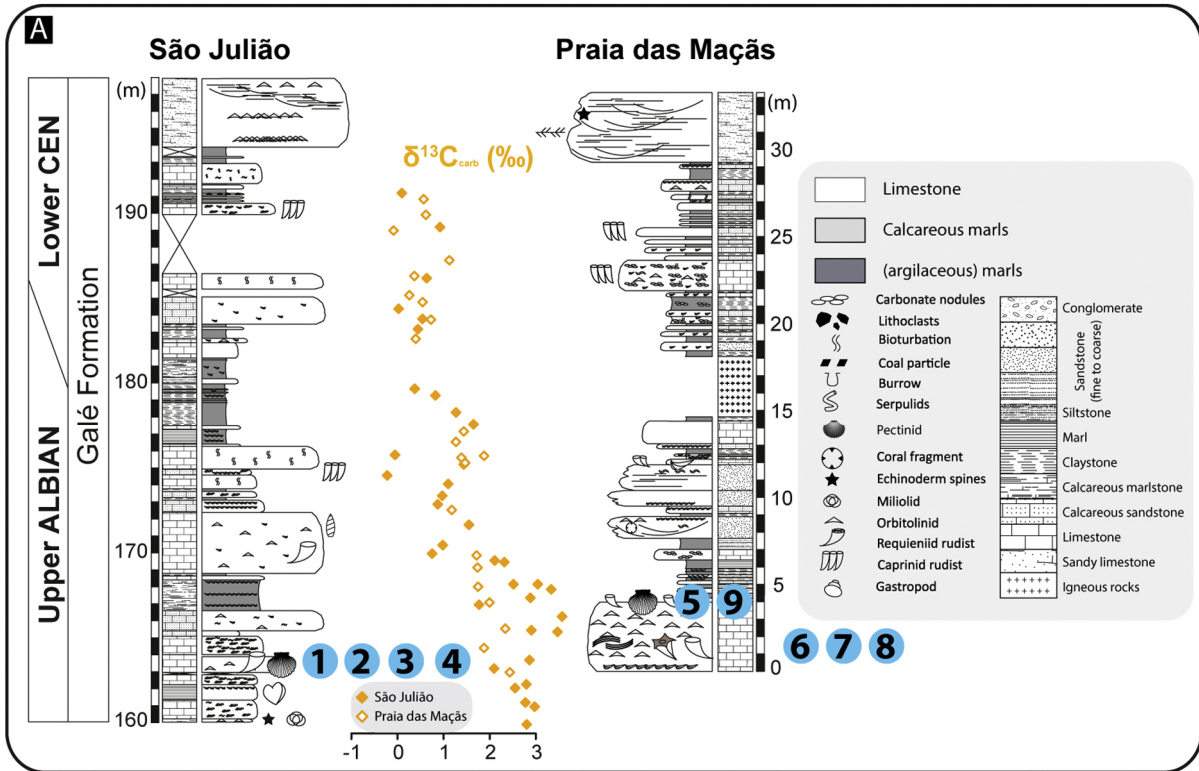
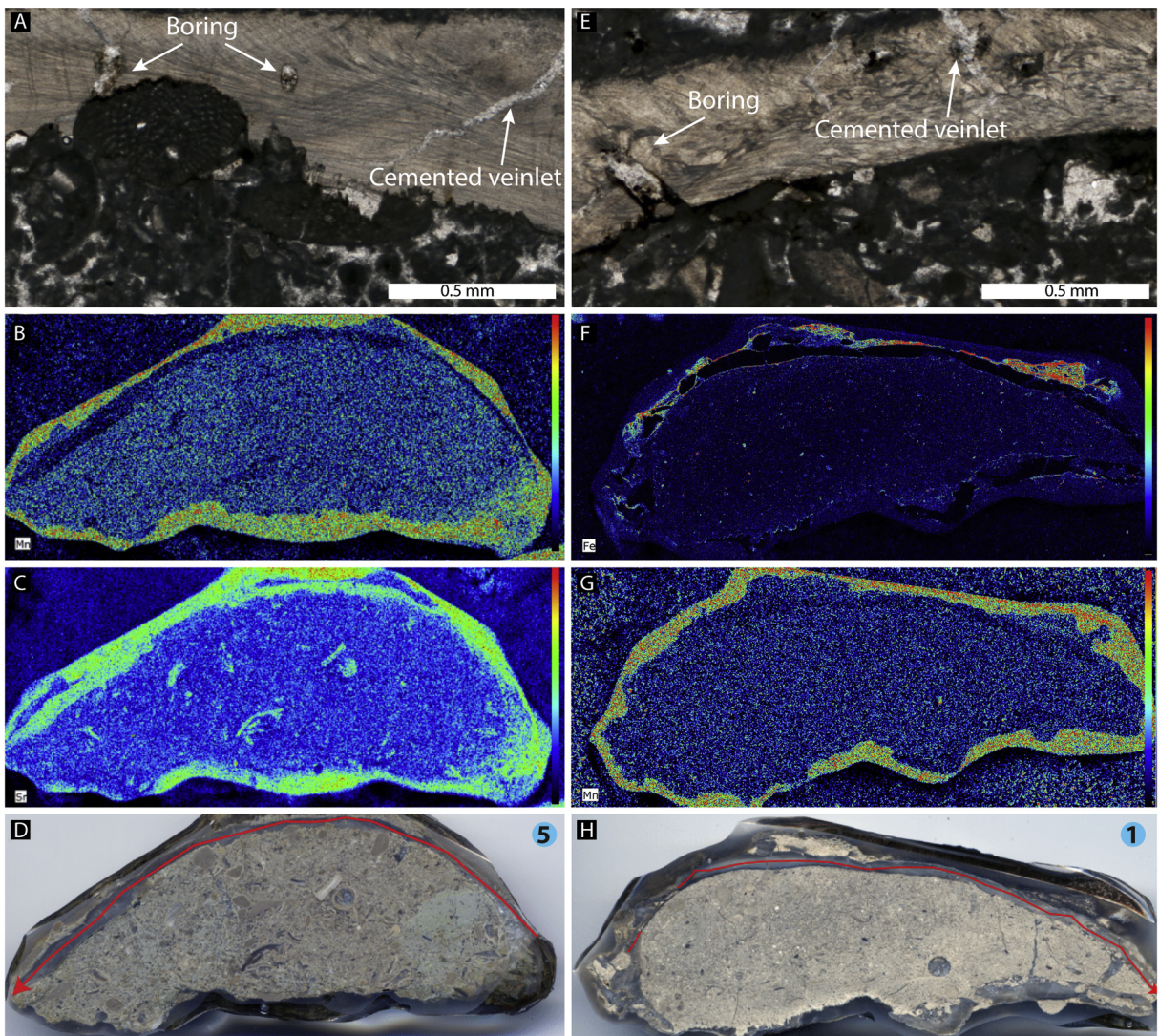- Dynamic elemental cut-off values are customized for each set of shells.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Panel A:

São Julião: different species
(2Apricardia+2Pectinid)
① ② ③ ④

PC1+PC2=54 %

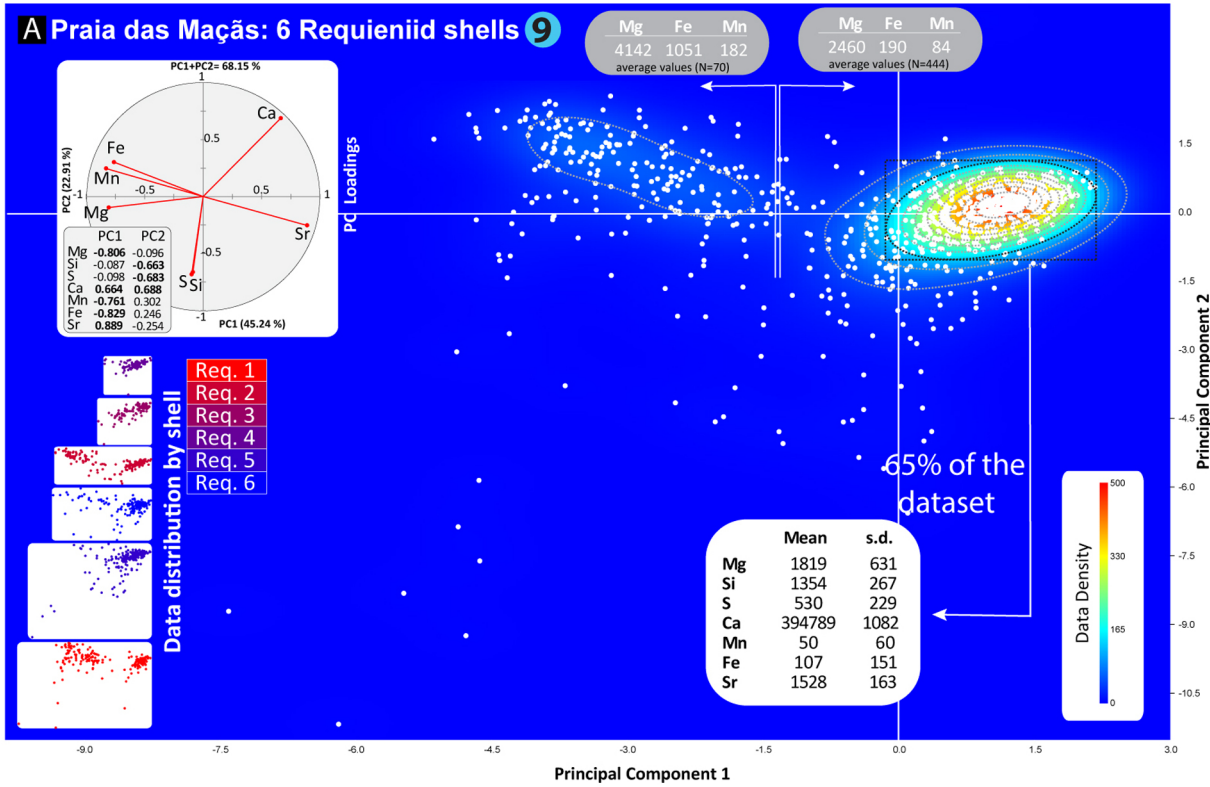|	PC1	PC2
Mg	-0.380	-0.725
Al	-0.695	0.193
Si	-0.566	-0.021
P	-0.414	0.470
Ca	0.815	0.387
Fe	-0.507	-0.485
Sr	-0.437	0.642
Pb	-0.455	0.525

Data distribution by shell

Apricardia1
Apricardia2
Pectinid1
Pectinid2

Contains 78% of Pectinid shell data

Panel B:

PC1+PC2= 61 %
③ ④ Two Apricardia shells (São Julião)

	PC1	PC2
Mg	-0.847	0.041
Al	-0.619	-0.129
P	0.177	-0.784
Ca	0.806	-0.083
Fe	-0.743	-0.171
Br	0.042	0.795

Data distribution by shell

Apricardia1
Apricardia2

	Mean	s.d.
Mg	995	497
Al	262	38
P	22	6.8
Ca	393970	1946
Fe	105	205

Mg	Fe	Al
4160	2139	346

average values (N=83)

Mg	Fe	Al
1044	130	263

average values (N=870)

79% of the dataset

Panel C:

PC1+PC2= 50 %
① ② Two Pectinid shells (São Julião)

	PC1	PC2
Mg	-0.703	-0.079
Al	-0.621	-0.125
Si	-0.465	-0.249
Ca	0.934	0.157
Fe	-0.528	0.037
Pb	-0.056	0.713
Zn	-0.248	0.697
Br	-0.233	0.702

Data distribution by shell

Pectinid1
Pectinid2

	Mean	s.d.
Mg	1426	395
Si	2504	720
S	573	266
Ca	394752	882
Fe	127	129
Pb	17	13
Zn	28	14
Br	15	6

Mg	Fe	Si	S
2089	436	3875	906

average values (N=244)

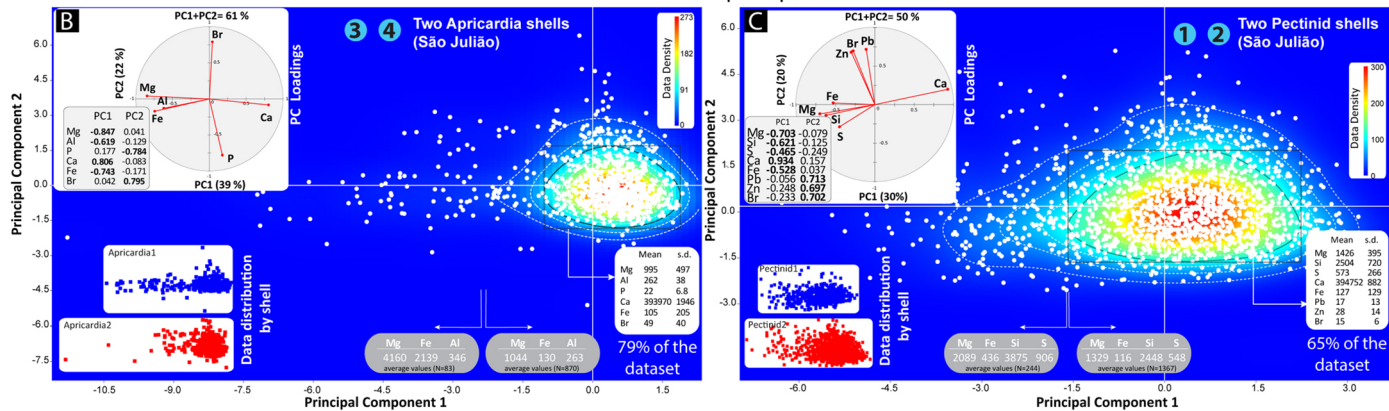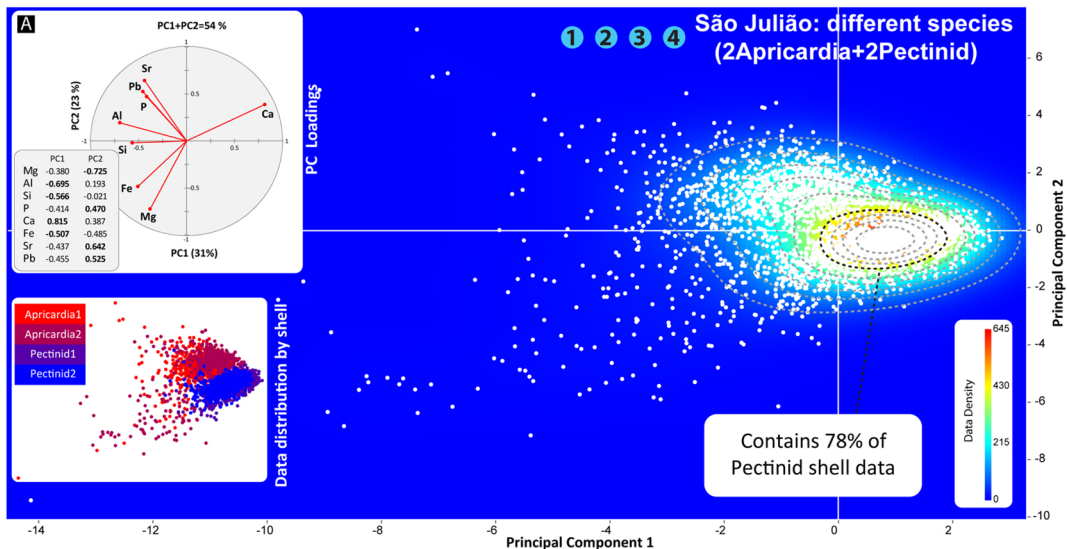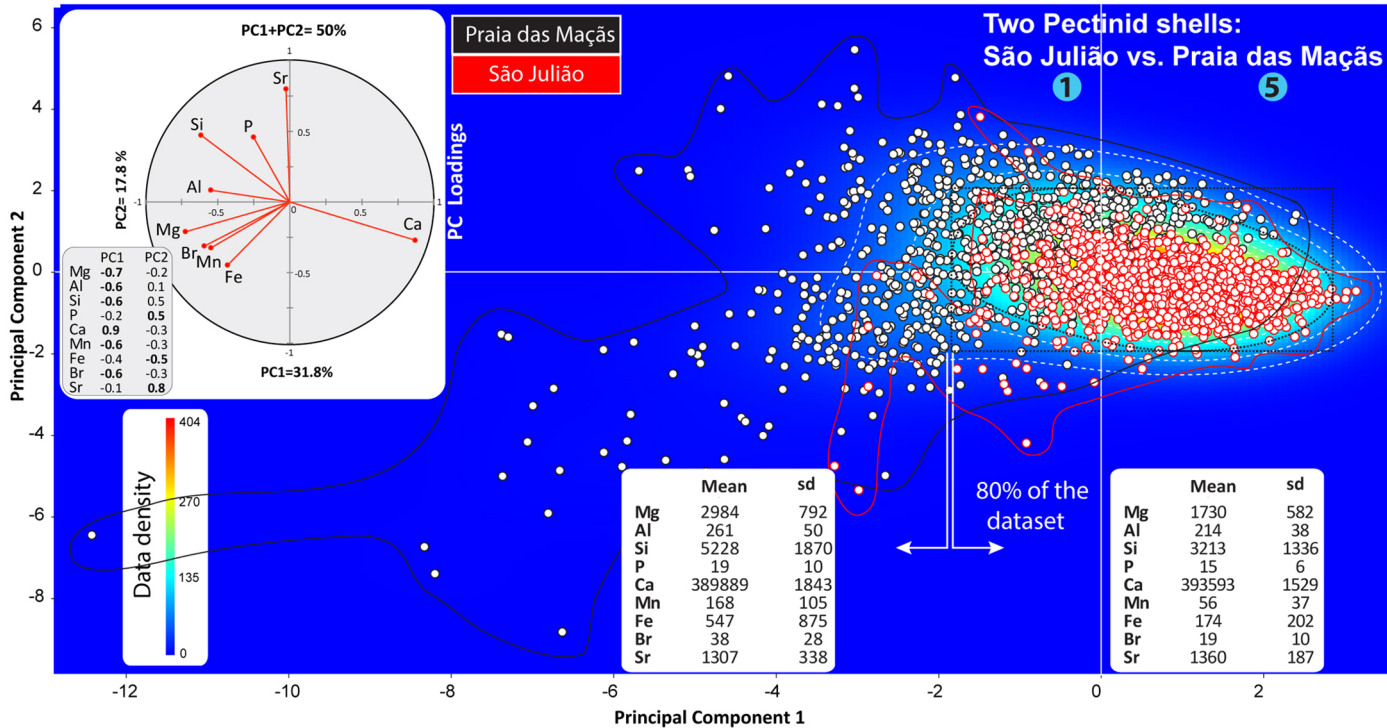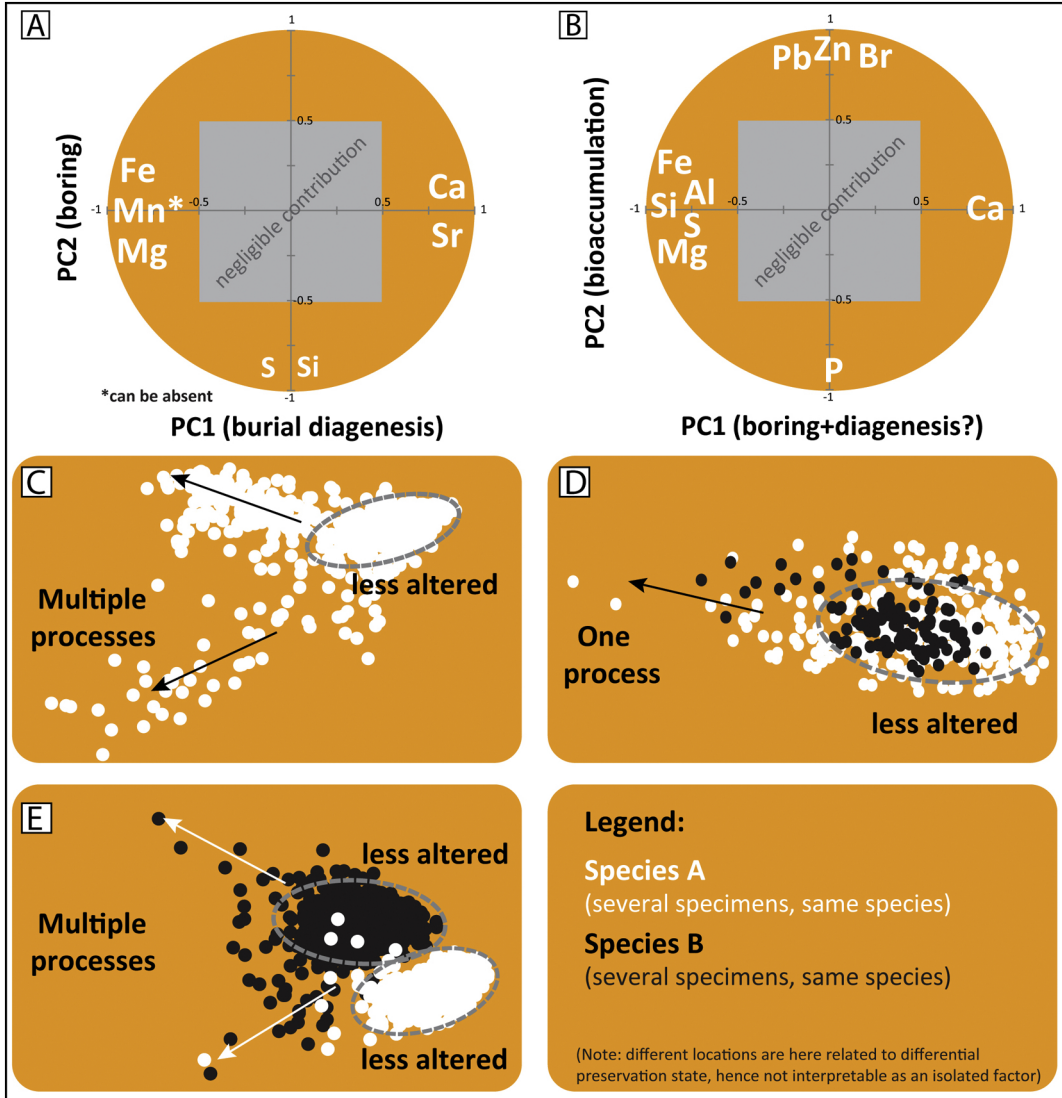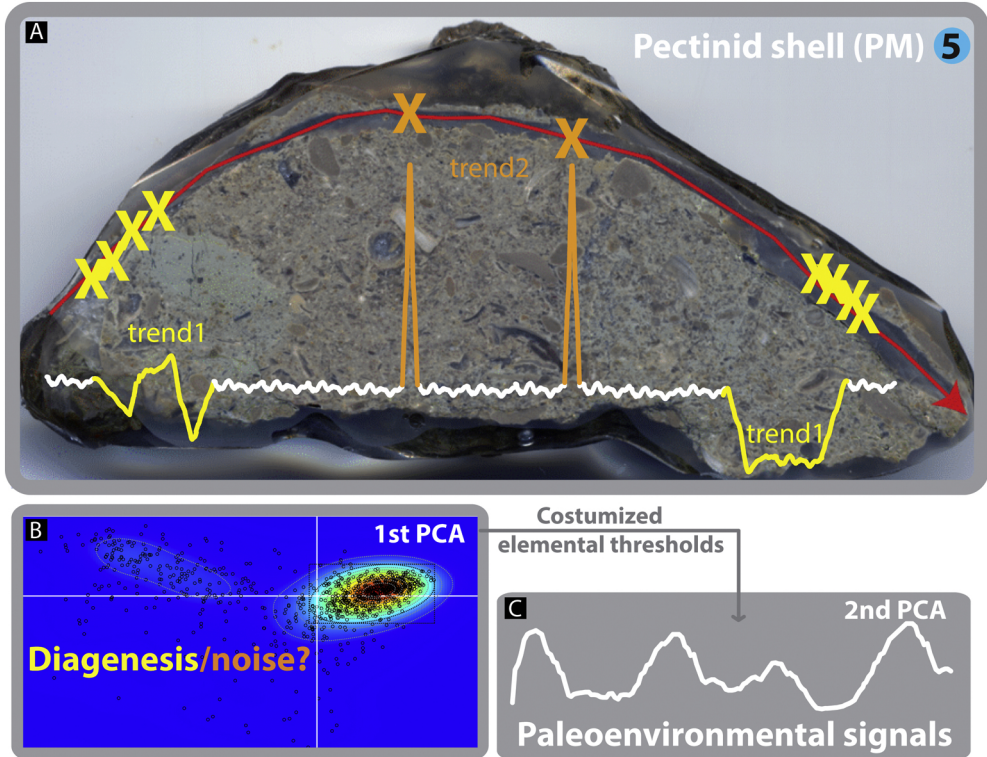| Mg	Fe	Si	S
1329	116	2448	548

average values (N=1367)

65% of the dataset

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12