Biogeochemical Dichotomy and Intra-Order Variability in Miliolid and Rotaliid Foraminifera

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Abstract. Foraminiferal geochemical records reflect both environmental and biological influences. Disentangling these factors is essential for improving their application in marine monitoring and contributing valuable insights into the evolution across major foraminiferal lineages. Calcifying foraminifera evolved independently, with miliolids and rotaliids represent the most widespread and ecologically dominant calcifying foraminiferal groups. Most geochemical studies to date have focused on rotaliids, despite the importance of miliolids in ecological and environmental roles as prolific calcifiers. This study leverages the unique southeastern Mediterranean Israeli coastal waters, where dominant representatives of both groups co-occur in the same habitats, allowing for a direct comparison of bioincorporation differences, known as the vital effect. This setting also allowed for within-group variability and the identification of biological and environmental elemental signatures characteristic of specific taxa. Elemental incorporations in tests of six co-occurring taxa were analyzed: three rotaliids and three miliolids, from an oligotrophic Mediterranean marine reserve using whole-test ICP-MS analyses. Results reveal a clear geochemical dichotomy, with miliolids exhibiting consistently higher element/Ca ratios than rotaliids for nearly all measured elements, except Li, which shows the opposite trend. The contrast is strongest for rare earth elements (REEs) with order of magnitude differences (up to 45 times), and moderate but systematic for other elements (e.g., Zn, Cd, Fe). This dichotomy likely reflects fundamental differences in biomineralization pathways between the two orders. Within each order, element/Ca ratios show

distinct patterns: in some taxa, variability appears to be biologically controlled through biomineralization processes, while in others it seems environmentally driven, reflecting the chemical composition of the surrounding water.

1 Introduction

Calcifying foraminifera, single-celled eukaryotes, have long served as key recorders of marine environmental conditions through their geochemical record (Erez, 2003; Katz et al., 2010; de Nooijer et al., 2009). The direct coupling between ambient seawater chemistry and the biomineralization pathways that lead to the precipitation of their calcitic test allows for the incorporation of metals in proportions that reflect both environmental concentrations and biological regulation (Boehnert et al., 2020; Hauzer et al., 2025; de Nooijer et al., 2007, 2017a; Sagar et al., 2021b, a; Smith et al., 2020; Titelboim et al., 2017).

However, elemental incorporation in foraminiferal tests does not solely mirror environmental conditions. It is strongly influenced by vital effects, intrinsic biological factors that cause deviations of test geochemistry (elemental ratios and stable isotopes) from equilibrium with seawater (e.g., Elderfield et al., 1996; Nehrke et al., 2013; de Nooijer et al., 2023). These vital effects vary widely among foraminiferal lineages, reflecting their distinct evolutionary and physiological pathways. Calcification in foraminifera evolved independently in at least 6 lineages, with miliolids and rotaliids representing the most widespread and ecologically dominant calcifying orders characterized by multichambered tests (de Nooijer et al., 2009, 2023; Pawlowski et al., 2013).

Recent molecular phylogenies confirm the monophyly of miliolids and rotaliids, each nested within one of the two main classes of multichambered foraminifera, Tubothalamea (miliolids) and Globothalamea (rotaliids). They differ substantially in morphology and calcification strategies, reflecting their deep evolutionary separation (Dubicka et al., 2018; Dubicka and Gorzelak, 2017; Pawlowski et al., 2013; Sierra et al., 2022). Rotaliids produce bi-lamellar hyaline calcite tests with crystallites precipitated extracellularly upon a primary organic sheet (Anderson and Faber, 1984; Erez, 2003; ter Kuile et al., 1989; de Nooijer et al., 2009, 2014a). In contrast, miliolids form porcelaneous tests composed of densely packed calcite needles embedded in an organic matrix. Their calcite crystallites are first precipitated intracellularly within cytoplasmic vesicles and subsequently assembled outside the cell to form the chamber wall (Angell, 1980; Debenay et al., 1996; Dubicka et al., 2024; Erez, 2003; Hemleben et al., 1986; de Nooijer et al., 2009; Toyofuku et al., 2000).

The geochemical consequences of these differences are profound: miliolids typically exhibit higher Element/Ca (El/Ca) ratios than rotaliids, as documented primarily for Mg/Ca ratios (Bentov and Erez, 2006; de Nooijer et al., 2009; Toyofuku et al., 2000). These group-specific signatures reflect intrinsic physiological controls linked to the evolutionary origin of their calcification pathways rather than environmental variation. In contrast, variation within each group is often driven by more

specific biological factors such as photosymbiont presence, metabolic activity, or growth rate (e.g Evans et al., 2015; Mewes
65   et al., 2015; van Dijk et al., 2017, De Goeyse et al., 2024).

Despite the clear dichotomy between miliolid and rotaliid calcification, geochemical studies on foraminifera are heavily biased
toward a small set of rotaliid species, while leaving miliolids comparatively understudied, and direct lineage-to-lineage
comparisons are rare (Pacho et al., 2023). This gap limits our understanding of how fundamental differences in
biomineralization influence elemental incorporation and, consequently, the interpretation of geochemical proxies (de Nooijer
70   et al., 2009, 2023; Pawlowski et al., 2013; Sierra et al., 2022).

The Southeastern Mediterranean marine coast of Israel offers an exceptional natural laboratory for deciphering the
geochemical dichotomy between miliolid and rotaliid foraminifera and for exploring intra-order variability. This region is
currently undergoing rapid tropicalization, resulting in the establishment of benthic foraminiferal hotspots characterized by
high abundances of diverse miliolid and rotaliid species (Manda et al., 2024). The primary objective of this study is to quantify
75   elemental ratios, including rare earth elements (REEs), in the tests of representative six rotaliid and miliolid taxa collected
simultaneously from an oligotrophic nature reserve at the northern Israeli coast. This setting provides a unique opportunity to
compare elemental incorporation under identical environmental conditions, thereby isolating biological ("vital") effects from
environmental influences.

By analyzing multiple taxa across the two major calcifying orders, we specifically aim to (1) evaluate the magnitude and
80   consistency of the miliolid–rotaliid geochemical divergence and (2) assess intra-group variability that may reflect biological
or microenvironmental factors. This comparative approach yields new insights into the evolutionary and physiological controls
on trace-element incorporation in foraminifera and strengthens their application as reliable geochemical recorders in both
modern and ancient marine systems.

2 Materials and methods

85   ### 2.1 Field sampling and selected species

To establish species-specific geochemical records, we chose a protected national reserve site of Dor HaBonim beach
(coordinates: 32°37'23.07" N, 34°55'12.18" E) that is not directly impacted by nearby coastal industries (Figure 1.A, B). This
site has been referred to as Nachsholim in previous studies (e.g., Titelboim et al., 2018). Samples hosting live benthic
foraminifera were collected during October 2022 from the macroalgal mats covering the abrasion platforms where specimens
90   of both groups are found in high densities (Figure 1C). The samples were transferred to the lab, and live specimens were picked
during the week of collection.

Six taxa were selected for geochemical analyses, representing miliolid (*Sorites orbiculus*, *Peneroplis* and *Lachlanella*) and rotaliid taxa *(Amphistegina lobifera, Rosalina globularis,* and *Pararotalia calcariformata*) (Figure 1.D). The specimens were isolated and picked from the macroalgae under a stereomicroscope. Their living status was validated by the distinctive coloration indicative of the presence of cytoplasm or symbionts and by active motility following picking.
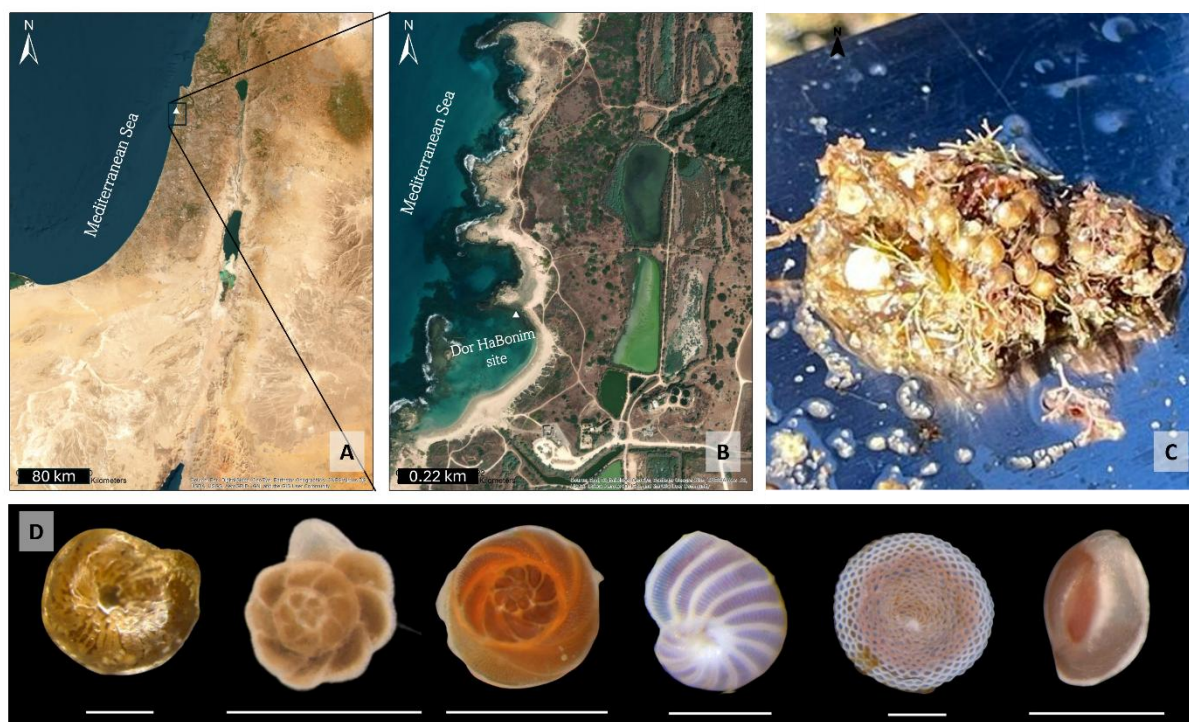
Figure 1: A-B Location map (imagery©2026 NASA, Map data©2026 Google, Mapa GISrael) B. Dor HaBonim study site C. Macroalgal mats from Dor HaBonim, showing high densities of the studied benthic foraminifera (mostly *Amphistegina lobifera*). D. the 6 studied species. From left to right: *A. lobifera*, *P. calcariformata*, *R. globularis*, *Peneroplis*, *S. orbiculus*, and *Lachlanella*. Scale bar = 500 µm.

2.2 Specimens cleaning and whole test ICP-MS analyses

Live specimens of each taxon were subdivided into replicate groups, each comprising between 3 and 50 individuals, depending on species sizes. For most taxa, 10 replicates were analyzed, although the number of specimens per replicate varied among species. This replicated design was done to ensure a robust statistical assessment of intra-taxon variation. Following the Fehrenbacher et al. (2015) cleaning protocol, the specimens were placed in Eppendorf tubes to thoroughly remove organic matter from the tests. Briefly, specimens were rinsed with Milli-Q water and methanol, oxidized with a mixture of H_2O_2 and NaOH, and finally dissolved in 3 mL of 3% HNO_3 solution, then centrifuged for 5 minutes to remove any residual solid particles.

Whole tests were measured using a triple-quadrupole ICPMS (Agilent 8900; nebulizer flow rate 1 L/min) at the Institute of
110   Earth Sciences, Hebrew University of Jerusalem. All solutions were spiked with internal standards (50 µg/L Sc, and 5 µg/L
Re & Rh) and analyzed in He collision mode. Two in-house standards were analyzed for every ten samples for quality control:
(1) a long-term drift solution prepared from local Red Sea sediments, and (2) *Amphistegina lobifera* tests collected from the
Mediterranean coast for this study. Procedural blanks were processed similarly to the samples through the cleaning steps and
analysis. Elemental concentrations are presented normalized to calcium (Ca) to standardize for differences in carbonate test
115   size and composition.

Procedural blanks consisting of 3% HNO_3 digestion solution were included with each analytical batch. The average blank
signal for each element was subtracted from the sample measurements (Table S1). Samples with blank-to-signal ratios
exceeding 10% were excluded to minimize analytical noise; only samples below this threshold were retained for analysis
(Table S2).

120   Statistical analyses were conducted in R. Data normality was assessed using the Shapiro–Wilk test, and homogeneity of
variances was evaluated with Levene's test. Because most El/Ca distributions deviated from normality and/or exhibited
unequal variances, non-parametric tests were primarily applied. Differences between foraminiferal groups (rotaliids vs.
miliolids) were evaluated using two-sided Mann–Whitney U tests (Table S3a). Comparisons among multiple species were
assessed using Kruskal–Wallis tests, and when significant, followed by pairwise Wilcoxon rank-sum tests (Table S3b). P
125   values were adjusted for multiple testing using the Benjamini–Hochberg False Discovery Rate (FDR) procedure. Elements
with FDR-adjusted p values < 0.05 were considered statistically significant. For the limited subset of elements that met
assumptions of normality and homogeneity of variances after log_{10} transformation, independent two-sample t-tests were
additionally applied with FDR correction.

3. Results

130   ### 3.1 Differences in elemental incorporation between miliolids and rotaliids

Elemental ratios were compared using boxplots that illustrate both the distribution and variability within each group (Fig. 2).
Zinc was excluded from this comparison due to an insufficient number of reliable measurements in rotaliids. To further resolve
interspecific differences in elemental enrichment, El/Ca ratios of 15 metals from the six studied taxa were normalized to the
average values of *A. lobifera* and presented as a spider diagram (Fig. 3). As the most abundant rotaliid in the study area, *A.*
135   *lobifera* consistently exhibited the lowest El/Ca ratios across most elements and among the three analyzed rotaliid species. It
was therefore selected as a reference baseline, allowing relative elemental enrichment patterns in other species to be
emphasized and facilitating comparison between miliolids and rotaliids.
The El/Ca ratios reveal a clear contrast between miliolids (M) and rotaliids (R), with miliolids generally exhibiting higher
elemental incorporation (M > R; Figs. 2–3). This pattern is observed for all analyzed elements except Fe, V, and Sr, which

140   either show no significant differences between groups or overlapping values, and Li, which is enriched in rotaliids (M < R). The largest contrasts between the two groups occur in the rare earth elements (REEs), which are enriched by a factor of 33–45 in miliolids relative to rotaliids (Table S4). Substantial enrichments are also observed for Mn, Pb, and U, with miliolid El/Ca ratios 10–16 times higher. In contrast, Cd, Mg, Cu, and As show more moderate enrichments, reaching up to fourfold higher values in miliolids. A notable exception to the overall pattern is Li/Ca, for which rotaliids display values approximately

145   two times higher than those of miliolids (M < R; Fig. 2).
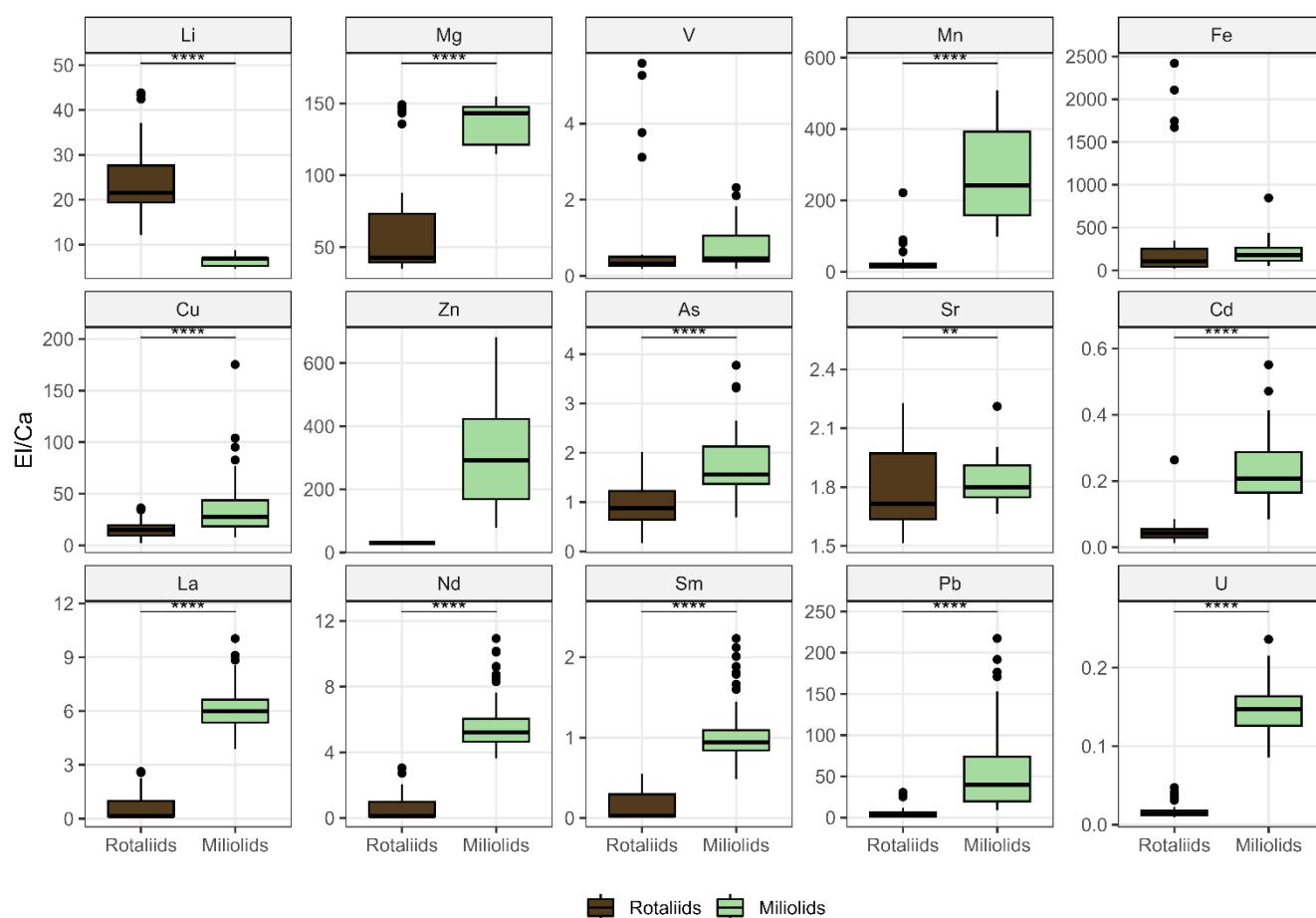
Figure 2: Comparison of element/Ca ratios in rotaliids and miliolids calcite tests. Values shown in µmol/mol, except for Mg/Ca and Sr/Ca, which are expressed in mmol/mol. Asterisks indicate statistically significant differences between groups based on Mann–Whitney U tests with FDR correction.

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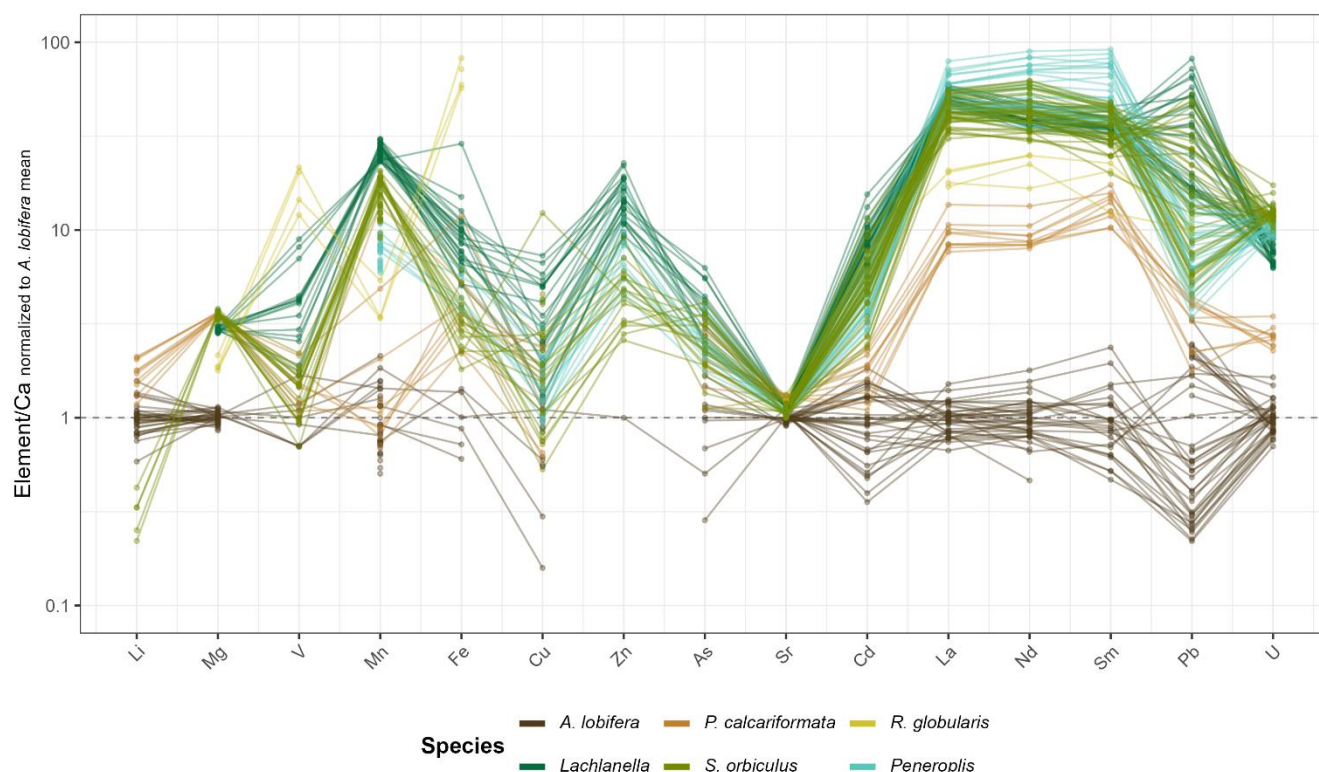
Figure 3: Spider plot showing Element/Ca (El/Ca) ratios in six benthic foraminiferal species, each normalized to the average El/Ca ratio of *A. lobifera*. Individual replicates are represented by colored lines corresponding to each species. This visualization emphasizes the differences in elemental incorporation between species and between the two main foraminiferal groups, highlighting relative enrichment patterns rather than absolute concentrations.

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To further assess differences in the elemental distribution patterns between species and potential connection to biomineralization pathways, a principal component analysis (PCA) was performed on El/Ca ratios of the six taxa (Fig. 4). The PCA ordination reveals a distinct separation between miliolids and rotaliids along the first principal component (PC1), which explains 37.4% of the total variance. The PC1 pattern is primarily driven by metals such as Mn, Pb, Zn, Cd, and the REEs,

160 which showed elevated ratios in miliolids (particularly *Lachlanella* and *Peneroplis*), reinforcing the M > R dichotomy. In contrast, rotaliids (especially *A. lobifera* and *P. calcariformata*) cluster tightly, reflecting their lower incorporation of these elements.

3.2 Intra-order variability

165 Underlying the cases of M > R pattern is intra-order variability observed within both miliolids and rotaliids noted by the distribution of El/Ca values across the different taxa (Fig. 4). Among the rotaliids, the El/Ca values of *A. lobifera* are significantly lower than those of the two other rotaliid taxa, except Zn/Ca and Mn/Ca ratios that overlap with the others. The El/Ca records of *R. globularis* are limited due to analytical constraints (Table 1). Notably, *R. globularis* exhibits the highest V/Ca and Fe/Ca values among all taxa, and *P. calcariformata* stands out for its elevated Mg/Ca ratios, which partially overlap

170 with those of the miliolid taxa. The three miliolid taxa generally exhibit broader El/Ca distributions than rotaliids, except for Mg/Ca and Sr/Ca values, which are more constrained. Among the miliolids, *Lachlanella* shows the highest Zn/Ca, Pb/Ca, Cd/Ca, Mn/Ca, and As/Ca, and the lowest Mg/Ca and U/Ca values.

The second principal component (PC2; 19.8% variance; Fig. 5) captures intra-group differences, separating *Lachlanella* from the other miliolids due to elevated Zn/Ca, Pb/Ca, and Cd/Ca, while *Rosalina* and *P. calcariformata* display distinct

175 geochemical profiles among rotaliids. The PCA loadings indicate taxa-specific vital effects on elemental incorporation, reflecting lineage-specific biological traits and, in some cases, microhabitat influences.

Table 1: Median El/Ca ± standard deviation (SD) in foraminiferal species. Each reported value represents the number of replicate analyses (n). * Indicates that 1 outlier was removed from the calculation.

Taxa	Li/Ca µmol/mol	Mg/Ca mmol/mol	V/Ca µmol/mol	Mn/Ca µmol/mol	Fe/Ca µmol/mol	Cu/Ca µmol/mol	Zn/Ca µmol/mol	As/Ca µmol/mol
A. lobifera	20.0 ± 4.1 (n=30)	40.76 ± 2.91 (n=38)	0.25 ± 0.09 (n=8)	14.6 ± 6.3 (n=38)	28.0 ± 9.9 (n=6)	13.8 ± 7.4 (n=20)	30.0 ± NA (n=1)	0.63 ± 0.25 (n=10)
P. calcariformata	36.0 ± 6.6 (n=10)	145.68 ± 3.81 (n=10)	0.34 ± 0.10 (n=8)	18.2 ± 65.4 (n=10)	110 ± 87 (n=9)	19.5 ± 9.4 (n=10)	NA	1.20 ± 0.42 (n=10)
R. globularis	NA	75.77 ± 6.64 (n=4)	4.52 ± 1.18 (n=4)	57.0 ± 19.0 (n=3)	1900 ± 350 (n=4)	NA	NA	NA
Peneroplis	NA	148.19 ± 3.02 (n=30)	NA	135.7 ± 39.0 (n=30)	120 ± 32 (n=6)	21.0 ± 7.5 (n=20)	229.8 ± 105.9 (n=12)	1.40 ± 0.34 (n=13)
S. orbiculus	6.9 ± 1.7 (n=5)	144.47 ± 5.01 (n=40)	0.38 ± 0.10 (n=18)	233.0 ± 54.9 (n=40)	95 ± 34 (n=16)	25.9 ± 18.0* (n=23)	143.5 ± 51.8* (n=16)	1.5 ± 0.5 (n=21)
Lachlanella	NA	119.21 ± 2.69 (n=30)	1.08 ± 0.55 (n=15)	440 ± 38.1 (n=30)	260 ± 130 (n=26)	42.5 ± 24.8 (n=22)	416.7 ± 113.2 (n=30)	2.10 ± 0.74 (n=15)

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Taxa	Sr/Ca mmol/mol	Cd/Ca µmol/mol	La/Ca µmol/mol	Nd/Ca µmol/mol	Sm/Ca µmol/mol	Pb/Ca µmol/mol	U/Ca µmol/mol

A. lobifera	1.65 ± 0.09 (n=38)	0.03 ± 0.01 (n=32)	0.13 ± 0.02 (n=38)	0.12 ± 0.03 (n=38)	0.02 ± 0.01 (n=26)	1.54 ± 2.29 (n=38)	0.013 ± 0.003 (n=38)
P. calcariformata	2.15 ± 0.04 (n=10)	0.06 ± 0.02 (n=10)	1.10 ± 0.22 (n=10)	1.14 ± 0.22 (n=10)	0.33 ± 0.06 (n=10)	9.75 ± 2.55 (n=10)	0.037 ± 0.005 (n=10)
R. globularis	2.04 ± 0.12 (n=4)	0.26 ± NA (n=1)	2.40 ± 0.23 (n=4)	2.90 ± 0.48 (n=4)	0.50 ± 0.13 (n=3)	27.39 ± 2.30 (n=4)	NA
Peneroplis	1.76 ± 0.06 (n=30)	0.17 ± 0.04 (n=30)	6.27 ± 1.33 (n=30)	6.47 ± 1.86 (n=30)	1.10 ± 0.41 (n=30)	19.35 ± 8.77 (n=30)	0.140 ± 0.015 (n=30)
S. orbiculus	1.77± 0.12 (n=40)	0.18 ± 0.07 (n=38)	5.36 ± 0.86 (n=40)	5.11 ± 1.14 (n=40)	0.88 ± 0.18 (n=40)	40.22 ± 36.69 (n=40)	0.160 ± 0.018 (n=40)
Lachlanella	1.91 ± 0.06 (n=30)	0.30 ± 0.07 (n=30)	6.20 ± 0.54 (n=29)	4.70 ± 0.47 (n=30)	0.86 ± 0.10 (n=30)	84.20 ± 52.43 (n=30)	0.104 ± 0.020 (n=30)
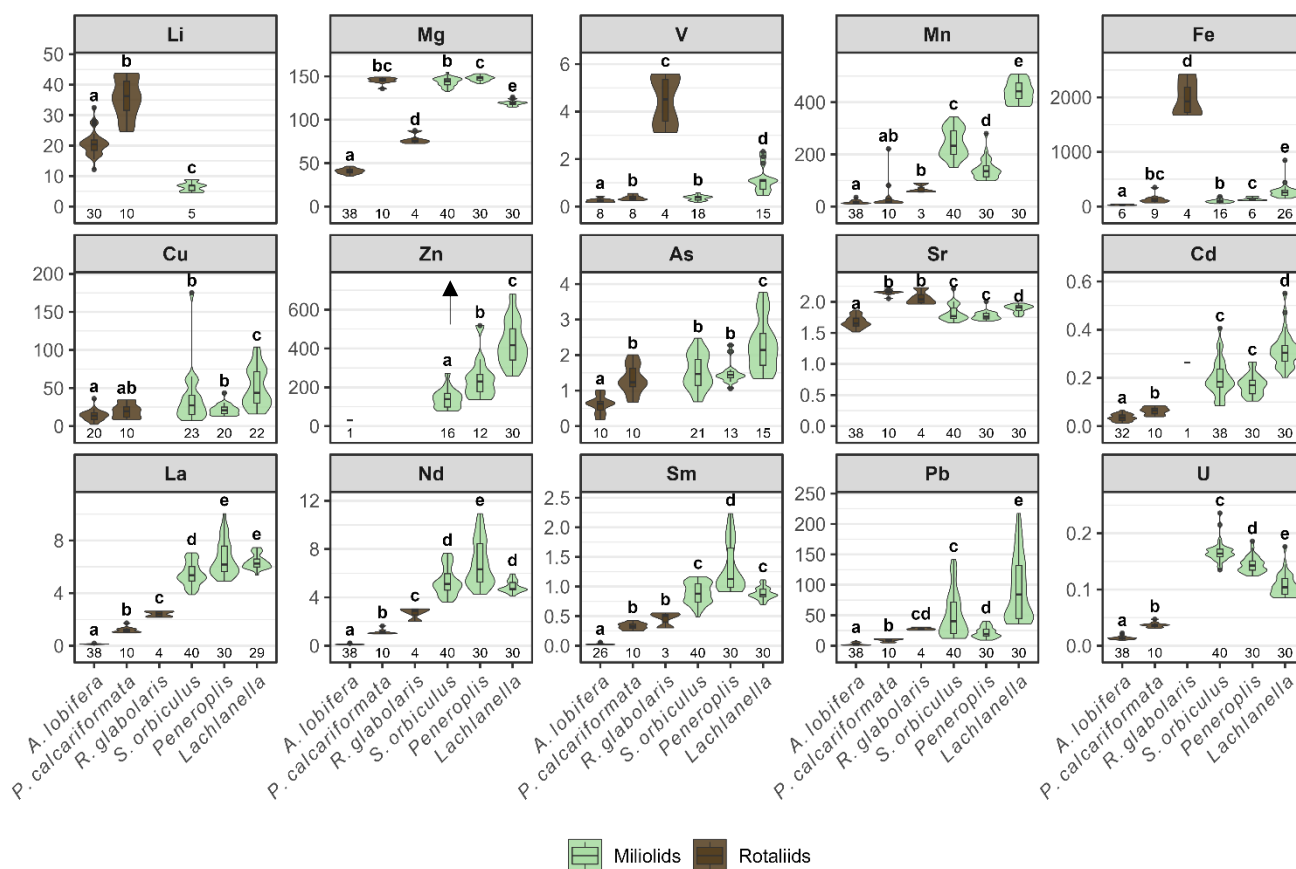
Figure 4: Distribution El/Ca between different taxa. Values are expressed as µmol/mol except for Mg/Ca and Sr/Ca that are

mmol/mol. The colors separate the species into two orders; rotaliids (brown) and miliolid (green) foraminifera. Letters above each

185 distribution indicate statistically significant differences between species in each order. The arrow marks an outlier Zn/Ca value

(4,063 µmol/mol) that was excluded from the plot.
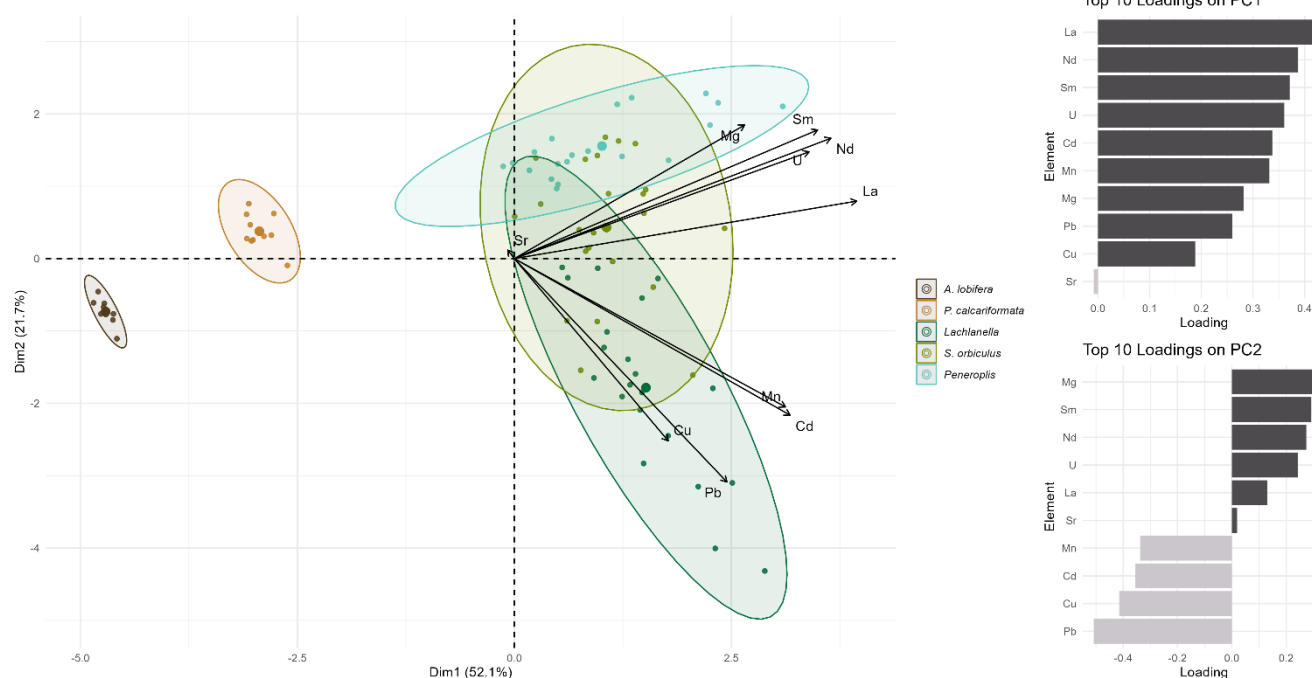
Figure 5: Principal Component Analysis and top loadings of El/Ca ratios in foraminifera species.

4 Discussion

4.1 Elemental dichotomy between miliolids and rotaliids

Analysis of multiple, co-occurring rotaliid and miliolid taxa expands the comparative geochemical dataset for these two main calcifying orders of foraminifera. The results confirm a robust, systematic dichotomy, with higher El/Ca ratios in miliolids relative to rotaliids (M > R; Fig. 2) with almost no overlap. The contrast is most pronounced for REEs, Mn, Pb, and U (order-

195 of-magnitude differences), and moderate but consistent for Zn, Cd, Mg, Cu, As. Lithium is a notable exception (M < R), a trend not previously reported. Titelboim et al., (2018) reported a similar M > R pattern between *Lachlanella* and *P. calcariformata*, sampled monthly from the Eastern Mediterranean. *Lachlanella* consistently exhibited higher Zn/Ca, Pb/Ca, Mn/Ca, Cu/Ca, and Ba/Ca in all months, demonstrating a stable, biologically driven lineage effect.

The elemental dichotomy between miliolids and rotaliids likely reflects fundamental differences in their biomineralization

200 pathways. Miliolids appeared at least 100 million years before rotaliids and probably evolved under different oceanic

conditions (Loeblich and Tappan, 1987), leading to calcification mechanisms adapted to distinct seawater chemistries and different ion-partitioning behaviours in the calcifying medium (de Nooijer et al., 2023). Although both groups elevate pH at the calcification site (de Nooijer et al., 2009), differences in the location of crystal formation likely contribute to variation in elemental uptake.

205 Structural differences in the tests may also account for the geochemical differences between the two orders by influencing the interactions with interlocked organic matter, potentially involved in binding trace elements during or after calcification (Bentov et al., 2009; Bentov and Erez, 2006; Erez, 2003; de Nooijer et al., 2014a). Elevated element concentrations in miliolids may therefore, in some cases, reflect enhanced adsorption to organic substrates or mineral crystals, facilitated either by a greater abundance of interlocking organic material or by the higher surface area of calcitic needles.

210 Thus, the M > R dichotomy represents a pronounced set of vital effects arising from divergent biomineralization pathways and ion-regulatory mechanisms between the two orders. The mechanistic basis likely includes differences in active ion transport (e.g., proton pumps and other membrane transporters) that determine the composition of the calcifying fluid. In some cases, the M > R dichotomy may also reflect differences in organic matrix composition and architecture, which influence element binding and incorporation during or after crystal growth (Dubicka et al., 2018; de Nooijer et al., 2009; Schmidt et al., 2022).
215 Clarifying the relative contributions of these processes will require targeted physiological and experimental studies, but the co-occurrence comparison at Dor HaBonim clearly demonstrates that taxonomy (between and within order) exerts a first-order control on foraminiferal El/Ca signatures.

4.2 Variability within Foraminiferal Orders

The overall consistency of the M > R signal among most elements indicates that the calcification pathways of both orders are
220 rooted in their monophyly (synapomorphy). Nevertheless, within each order, El/Ca ratios can range from relatively low to high, reflecting taxon-specific vital effects and, in some cases, environmental influences.

4.2.1 Miliolids

Miliolids are generally known as high-Mg/Ca foraminifera, with similar values displayed across taxa in the order (de Nooijer et al., 2017b). In this study, the two large benthic foraminifera miliolids taxa *Peneroplis* and *S. orbiculus* show the highest
225 degree of geochemical similarity across most elements, consistent with their close evolutionary relationship within the same subfamily, as supported by molecular phylogeny (Holzmann et al., 2001). This reinforces the idea that geochemical similarity is more likely among closely related lineages within the same family.

In contrast, *Lachlanella*, which is phylogenetically distant from the other two taxa and belongs to a highly diverse family of small miliolids, displays a distinctly different geochemical signature. While most differences between *Lachlanella* compared

230 to the two large benthic foraminifera taxa are likely evolutionarily related, specific elemental patterns, including Mg/Ca, may reflect environmental influences rather than purely vital effects. For example, the relatively low Mg/Ca in *Lachlanella* compared to other miliolids has previously been interpreted as a record of colder winter temperatures (Titelboim et al., 2017).

The Mn/Ca ratio in *Lachlanella* provides one of the clearest examples of an environmentally influenced elemental signal. Foraminiferal Mn/Ca ratios are primarily controlled by redox conditions, which regulate the availability of dissolved Mn^{2+}.

235 Under oxic conditions, Mn exists as insoluble Mn (IV) oxides, whereas under reducing conditions, these oxides dissolve, releasing Mn^{2+} into porewaters or the water column. As a result, Mn/Ca in benthic foraminifera typically reflects the redox state of the surrounding environment (Glock et al., 2012; Groeneveld and Filipsson, 2013; Koho et al., 2017). Published data from both rotaliids and some miliolids show a broad range of Mn/Ca values, typically spanning from <1 to ~300 μmol/mol, with *Lachlanella* representing a notable high-end outlier (van Dijk et al., 2020) and in the current study (Fig. 4).

240 Although all miliolid specimens were collected from turf samples, *Lachlanella* appears more frequently associated with the deeper, denser algal matrices within the turf, where microhabitats may experience periodic oxygen depletion. This habitat association likely explains its elevated Mn/Ca values, as well as its significantly higher ratios of other redox elements, Fe/Ca and V/Ca, compared to the other miliolids. The co-enrichment of these elements supports the interpretation that *Lachlanella* occupies more reducing microenvironments, and that its geochemical signature partially records environmental redox

245 variability rather than purely taxon-specific biomineralization.

4.2.2 Rotaliids

The three rotaliid taxa exhibit substantial interspecific variation in most elemental ratios, yet the general M > R pattern remains evident. The best-documented exception is of Mg/Ca, which shows large differences among rotaliids, from low to high values that can overlap with those of miliolids (Dueñas-Bohórquez et al., 2011; Oron et al., 2021; Titelboim et al., 2018). Previous

250 LA-ICPMS and NanoSIMS studies also reported strong intra-individual variability in Mg/Ca, occasionally linked to the diurnal cycle (Wit et al., 2012). Our approach, based on whole-test analyses of multiple specimens and numerous replicates, averages out such variability and highlights taxon-level trends consistent with earlier observations.

Extant planktonic foraminifera and small benthic taxa such as *Ammonia* typically have low Mg/Ca (Dueñas-Bohórquez et al., 2011; de Nooijer et al., 2014b). In contrast, *Pararotalia calcariformata* displays unusually high Mg/Ca values, comparable to

255 those of miliolids, likely reflecting its high-Mg calcarinid ancestry (Titelboim et al., 2018, Fig. 5). Large benthic rotaliids range from mid-Mg (*A. lobifera*) to high-Mg (*Heterostegina depressa*) species, again overlapping with miliolids (Segev and Erez,

2006). The marked difference in Mg/Ca between the diatom-bearing *A. lobifera* and *P. calcariformata* implies that endosymbionts are not a major factor in this vital effect.

Although Mg/Ca has been proposed to reflect evolutionary adaptation to changing seawater composition (de Nooijer et al.,
260   2023), our data suggest otherwise. For example, *P. calcariformata*, which evolved in the Quaternary, exhibits higher Mg/Ca than *Amphistegina*, which originated in the Eocene after seawater Mg/Ca rose. Thus, Mg incorporation appears primarily governed by species-specific biological regulation rather than by ambient seawater chemistry.

Beyond Mg, our data reveal broader geochemical heterogeneity among rotaliids. *A. lobifera* consistently exhibits the lowest El/Ca ratios for most elements, indicating strong biological discrimination against elemental incorporation despite its thick,
265   multilayered test that integrates multiple growth phases. In contrast, *R. globularis* shows markedly elevated Fe/Ca and V/Ca ratios. Although both elements are redox-sensitive and could, in principle, indicate episodic oxygen depletion, this interpretation is unlikely given the species' association with epiphytic macroalgal habitats, shared with *P. calcariformata*. Moreover, the lack of concomitant Mn/Ca enrichment in *R. globularis* further argues against a purely environmental control. We therefore propose that the enrichment of Fe and V in *R. globularis* reflects an as-yet-unknown biological process related
270   to calcification dynamics rather than redox conditions.

4.3 Implications for biomonitoring of dissolved elements in seawater

The pronounced miliolid–rotaliid dichotomy (M > R) highlights the critical species choice when using benthic foraminifera as bioindicators. Miliolids, owing to their higher elemental uptake, can serve as more sensitive recorders of changes in seawater concentrations, particularly for Pb, Cd, Mn and Zn. However, their weaker ion-selectivity control means incorporation may be
275   non-linear at elevated concentrations, complicating quantitative reconstructions and calibration experiments. Rotaliids, by contrast, exhibit lower uptake and tighter biological regulation, making them more conservative recorders, but potentially less responsive to low concentrations.

For environmental monitoring, this means: miliolids have the advantage for detecting subtle enrichment trends but require careful calibration due to possible non-linear uptake. Rotaliids may provide more stable baselines, especially in long-term or
280   low-variability studies, though they may miss minor perturbations. Dual-order approaches can maximize detection sensitivity while providing cross-validation of environmental signals.

Beyond monitoring, these results expand the benthic foraminiferal elemental-proxy toolkit by establishing baseline element ranges for both orders, including several understudied elements in miliolids. This framework can improve trace-metal reconstructions, refine species selection for biomonitoring, and guide future experimental work to understand
285   biomineralization processes and environmental controls on elemental incorporation.

Author contributions

Conceptualization, S.A., B.H., N.T., S.A.P., and A.T.; methodology, S.A., and A.T.; validation, L.H., S.A., B.H., N.T., and A.T.; formal analysis, L.H., S.A., B.H., N.T., and A.T.; investigation, L.H.; resources, S.A. and A.T.; writing - original draft preparation, L.H.; writing-review and editing, L.H., S.A., B.H., N.T., S.A.P., and A.T.; visualization, L.H.; supervision, S.A.;
290   project administration, S.A. and A.T.; funding acquisition, S.A. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Disclaimer

295   No additional disclaimer is required.

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