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 representing 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 additionally enables assessment of within-group variability and the identification of biological and environmental elemental signatures characteristic of specific taxa. Elemental incorporation in tests of six co-occurring taxa was 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, including differences in calcifying-fluid regulation, Rayleigh-type enrichment, Mg exclusion, and element-specific transport processes. Within each

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order, element/Ca ratios show distinct taxon-specific patterns: in some taxa, variability appears to be biologically controlled through biomineralization processes, while in others it appears to be partly environmentally driven, reflecting the chemical composition of the surrounding microhabitat.

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; Lea, 2006). The direct coupling between ambient seawater chemistry, temperature and the biomineralization pathways that lead to the precipitation of their calcitic test allows for the incorporation of metals and non-metal elements 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 values observed in inorganically precipitated calcite (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., 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; 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; Toyofuku et al., 2000). These group-specific signatures reflect intrinsic physiological controls linked to the evolutionary origin of their calcification pathways rather than

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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 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, may complicate the calibration and inter-species comparability of geochemical proxies (de Nooijer et al., 2009, 2023; Pawlowski et al., 2013; Sierra et al., 2022).

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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 elemental ratios, including rare earth elements (REEs), in the tests of six representative rotaliid and miliolid taxa collected simultaneously from an oligotrophic nature reserve on 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 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. It strengthens their application as reliable geochemical recorders in both modern and ancient marine systems.

2 Materials and methods

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). Environmental conditions at Dor HaBonim were considered representative of the shallow Israeli Mediterranean coast. Regional monitoring of the Israeli shelf indicates typical near-surface temperatures of approximately 16–18°C in winter and 28–30°C in summer, salinity of ~38.8–39.6, and slightly alkaline seawater pH of ~8.1–8.2. These values are consistent with normal eastern Levantine coastal seawater

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(Ozer et al., 2022), characterized by strong seasonal temperature variability, persistently high salinity, and stable alkaline carbonate-system conditions. Samples hosting live benthic foraminifera were collected during October 2022 from the macroalgal mats covering the abrasion platforms where specimens 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* spp., and *Lachlanella* sp.) 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.

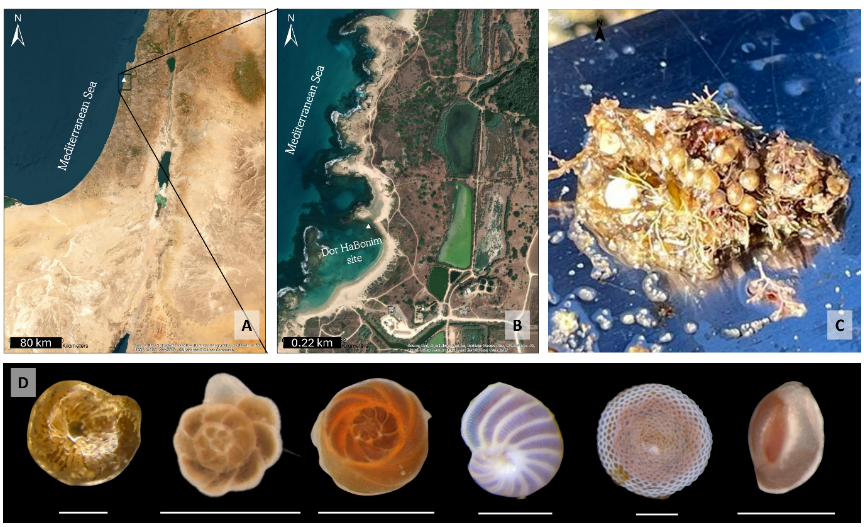

Figure 1: A-B Location map (imagery©2026 NASA, Map data©2026 Google, Mapa GISrael) B. Dor HaBonim study site C. Macroalgal mats from Dor HaBonim, showing high densities of the studied benthic foraminifera (mostly *Amphistegina lobifera*). D. the 6 studied species. From left to right: the rotaliids: *A. lobifera*, *P. calcariformata*, *R. globularis*, and the miliolids: *Peneroplis* spp., *S. orbiculus*, and *Lachlanella* sp. 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 implemented to ensure a robust statistical assessment of intra-taxon variation. Following

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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, and centrifuged for 5 minutes to remove any residual solid particles.

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Whole tests were measured using a triple-quadrupole ICPMS (Agilent 8900; nebulizer flow rate 1 L/min) at the Institute of Earth Sciences, Hebrew University of Jerusalem. The following isotopes were monitored: ^{24}Mg, ^{55}Mn, ^{63}Cu, 88Sr, ^{111}Cd, ^{139}La, ^{146}Nd, ^{147}Sm, ^{208}Pb, and ^{238}U. Calcium ^{43}Ca was used as an internal standard for normalization to El/Ca ratios. All solutions were spiked with internal standards (50 µg/L Sc, and 5 µg/L Re & Rh) and analyzed in He collision mode. The oxide formation rate (< 1% for Ce) was checked by using a 1 µg/L Ce tune solution and sensitivity was optimized before analyses. 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 (Mix 16-8), and (2) *Amphistegina lobifera* tests collected from the Mediterranean coast for this study (Table S1a. 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 size and composition.

Procedural blanks consisting of 3% HNO_3 digestion solution were included with each analytical batch. The average blank, standard deviation and limit of detection (LOD) signal for each element was subtracted from the sample measurements (Table S1b). 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).

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 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. Principal Component Analysis (PCA) was performed on El/Ca ratios of individual foraminiferal samples using R (version 4.1.1). Elements with more than 50% missing values were excluded prior to analysis. The remaining data were mean-centered and scaled to unit variance, and PCA was conducted using the correlation matrix.

3. Results

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.

The El/Ca ratios reveal a clear contrast between miliolids (M) and rotaliids (R), with miliolids generally exhibiting higher elemental incorporation across most measured elements (M > R; Fig. 2). This inter-order pattern is strongest for the rare earth elements (REEs), which are enriched by a factor of 33–45 in miliolids relative to rotaliids, and is also pronounced for Mn, Pb, and U, with miliolid El/Ca ratios 10–16 times higher (Table S4). More moderate miliolid enrichments, up to fourfold, are observed for Cd, Mg, Cu, and As.

Several elements deviate from this general pattern. Fe, V, and Sr show either no significant differences between groups or broadly overlapping values, whereas Li/Ca shows the opposite trend, with rotaliids displaying values approximately two times higher than those of miliolids (M < R; Fig. 2). Thus, while the overall dataset supports a strong miliolid enrichment for most El/Ca ratios, the exceptions indicate element-specific controls on incorporation.

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Deleted: 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. 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.

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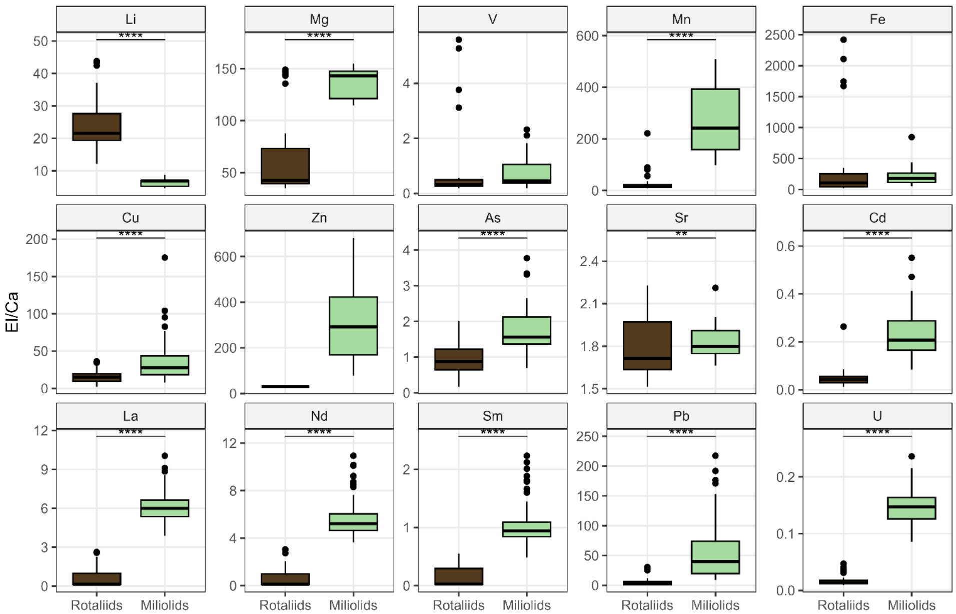

Figure 2: Comparison of element/Ca ratios in rotaliids and miliolids calcite tests. Values shown in µmol/mol, except for Mg/Ca and Sr/Ca, which are expressed in mmol/mol. Asterisks indicate different levels of statistical significance based on Mann–Whitney U tests with FDR correction.

To further assess differences in the elemental distribution patterns between orders and their possible relationship to biomineralization, a principal component analysis (PCA) was performed on El/Ca ratios of the six taxa (Fig. 3). 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 Mn, Pb, Zn, Cd, and the REEs, which showed elevated ratios in miliolids, particularly *Lachlanella* sp. and *Peneroplis* spp.), reinforcing the overall M > R dichotomy. In contrast, rotaliids, especially *A. lobifera* and *P. calcariformata*, plot toward the opposite side of PC1, reflecting their generally lower incorporation of these elements. At the same time, the distribution of rotaliid taxa indicates species-level variability within the broader inter-order separation.

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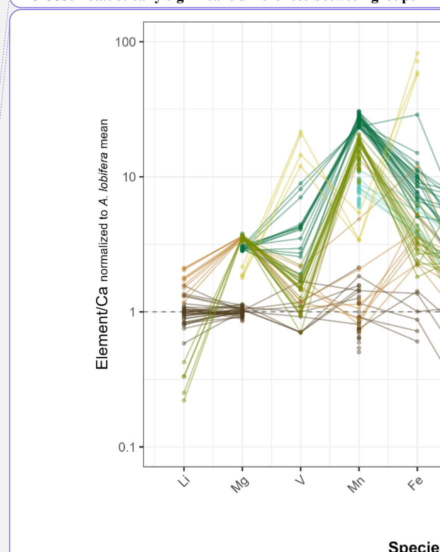

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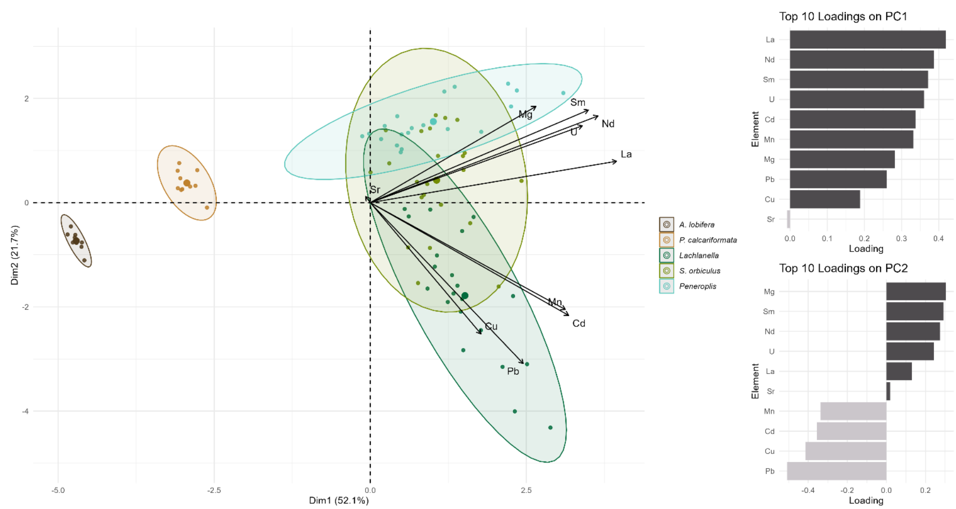

Figure 3. PCA of standardized El/Ca ratios in foraminiferal species. Points represent individual samples colored by species, larger symbols indicate species centroids (multivariate means), and ellipses denote the 95% confidence region. Bar plots show the top contributing elements to PC1 and PC2. *Rosalina* samples were excluded from the PCA due to insufficient elemental coverage after data filtering.

3.2 Intra-order variability

Superimposed on the overall M > R pattern, substantial intra-order variability is observed within both miliolids and rotaliids, as reflected by the distribution of El/Ca values among taxa (Fig. 3, 4). Among the rotaliids, the El/Ca values of *A. lobifera* are significantly lower than those of the two other rotaliid taxa, except for Zn/Ca and Mn/Ca, which overlap among rotaliids. The El/Ca records of *R. globularis* are limited due to analytical constraints (Table S2). 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 partly overlap with those of the miliolids. The three miliolid taxa generally exhibit broader El/Ca distributions than rotaliids, although Mg/Ca and Sr/Ca are comparatively more constrained. Among the miliolids, *Lachlanella* sp. shows the highest Zn/Ca, Pb/Ca, Cd/Ca, Mn/Ca, and As/Ca, but the lowest Mg/Ca and U/Ca values, indicating a distinct taxon-specific signature within the miliolids.

The second principal component (PC2; 19.8% variance; Fig. 3) captures intra-group differences. Within the miliolids, PC2 separates *Lachlanella* sp. from the other taxa, mainly due to elevated Zn/Ca, Pb/Ca, and Cd/Ca. Among the rotaliids, *R. globularis* and *P. calcariformata* also display distinct geochemical profiles. Together, these patterns indicate that species-

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specific elemental incorporation is superimposed on the broader inter-order separation. Thus, the PCA highlights both the first-order miliolid–rotaliid contrast and the taxon-specific variability within each order.

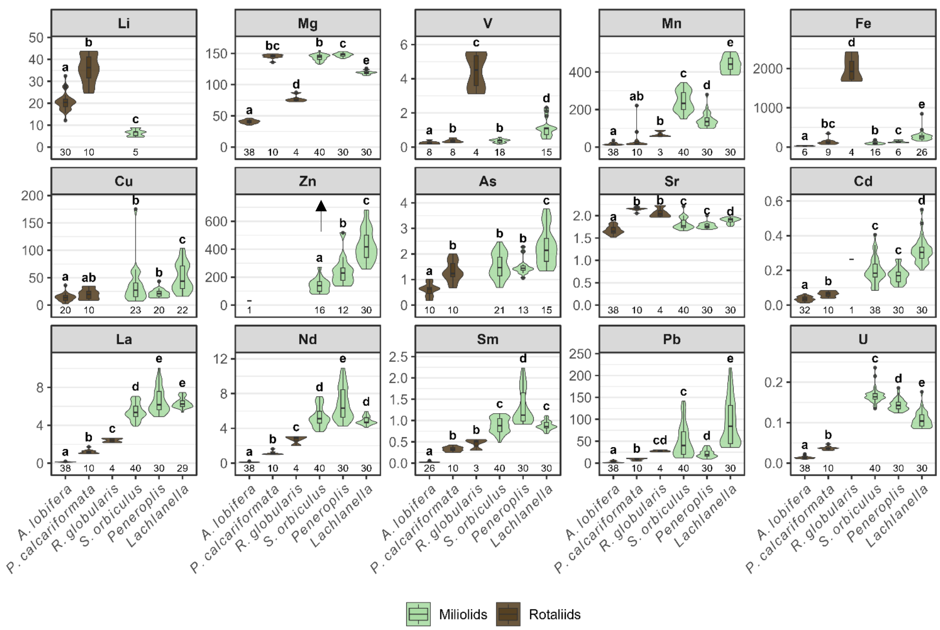

Figure 3: Distribution El/Ca between different taxa. Values are expressed as µmol/mol except for Mg/Ca and Sr/Ca that are mmol/mol. The colors separate the species into two orders; rotaliids (brown) and miliolid (green) foraminifera. Letters above each distribution indicate statistically significant differences between species in each order. Numbers at the x-axes represents the number of replicate analyses. The arrow marks an outlier Zn/Ca value (4,063 µmol/mol) that was excluded from the plot.

4 Discussion

4.1 Elemental dichotomy between miliolids and rotaliids

Deleted: separating *Lachlanella* from the other miliolids due to elevated Zn/Ca, Pb/Ca, and Cd/Ca, while *Rosalina* and *P. calcariformata* display distinct 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.

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Deleted: 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.
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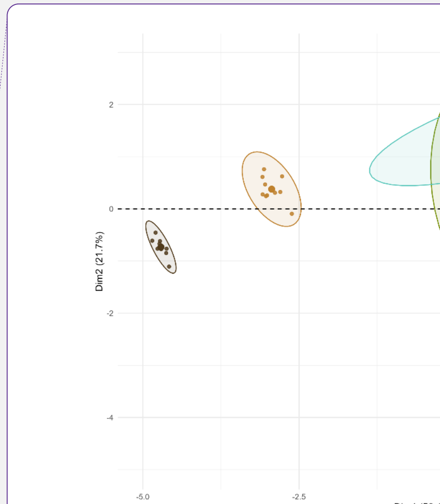

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Figure 4. PCA of standardized El/Ca ratios in foraminiferal species. Points represent individual samples colored by species, larger symbols indicate species centroids (multivariate means), and ellipses denote the 95% confidence region. Bar plots show the top contributing elements to PC1 and PC2. *Rosalina* samples were excluded from the PCA due to insufficient elemental coverage after data filtering.Figure 5: Principal Component Analysis and top loadings of El/Ca ratios in foraminifera species.

Analysis of multiple, co-occurring rotaliid and miliolid taxa expands the comparative geochemical dataset for these two main calcifying orders of foraminifera, particularly for miliolids and for REEs, for which published data remain limited. The dataset reveals a hierarchical structure, with (1) a first-order inter-order contrast between miliolids and rotaliids, (2) consistent species-level ranking within each order, and (3) element and environment-specific deviations. To place our results in a broader context, we compiled available published El/Ca data for each measured element and present these comparisons in the Supplementary Material (Figs. S1–S15). Together, our results and the published datasets reveal a strong first-order inter-order pattern, with higher El/Ca ratios in miliolids relative to rotaliids for most measured elements (M > R; Fig. 2; Figs. S1–S15). This contrast is most pronounced for REEs, Mn, Pb, and U, which show order-of-magnitude differences, and is moderate but consistent for Zn, Cd, Mg, Cu, and As. Lithium is a notable exception (M < R), a trend not previously reported, indicating that the miliolid–rotaliid contrast is element-specific rather than universal.

Titelboim et al., (2018) reported a similar M > R pattern between *Lachlanella* sp. and *P. calcariformata*, sampled monthly from the eastern Mediterranean. *Lachlanella* sp. consistently exhibited higher Zn/Ca, Pb/Ca, Mn/Ca, Cu/Ca, and Ba/Ca across all months, demonstrating a stable, taxon-dependent control on elemental incorporation. Together with the present co-occurrence dataset, these observations suggest that the miliolid-rotaliid contrast is not simply a local environmental signal, but reflects differences in biomineralization and ion regulation between the two orders. The elemental dichotomy between miliolids and rotaliids likely reflects fundamental differences in their biomineralization pathways. Miliolids originated 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 and mode of crystal formation likely contribute to variation in elemental uptake.

Following the conceptual synthesis of Branson and de Nooijer, (2025) and the biomineralization-reservoir models for foraminifera (Elderfield et al., 1996), the miliolid-rotaliid contrast can be viewed in terms of the balance between biological modification of the calcifying fluid and precipitation-driven evolution of that fluid. In this view, shell El/Ca ratios reflect processes such as Ca addition, ion transport, pH regulation, and Rayleigh-type enrichment during calcite precipitation. For many trace elements (TE), apparent biogenic partition coefficients (D_{TE}) exceed inorganic partition coefficients (K_{TE}), suggesting enrichment relative to inorganic calcite. Such enrichment may develop when Ca is progressively removed into $CaCO_3$ while other elements remain relatively enriched in the residual calcifying fluid, producing coherent increases in multiple TE/Ca ratios.

In this context, the higher TE/Ca values observed in miliolids may reflect stronger Rayleigh-type enrichment of the calcifying fluid, potentially related to their porcelaneous calcification pathway, restricted crystal-forming microenvironments, longer residence time of the calcifying fluid, or lower renewal rates during needle formation. By contrast, the generally lower TE/Ca

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values of rotaliids may reflect stronger Ca addition, more frequent renewal of the calcifying fluid, or tighter biological regulation, all of which would limit Rayleigh-type enrichment. This interpretation is consistent with models of foraminiferal calcification that involve biologically regulated calcifying reservoirs, seawater-derived vesicles or vacuoles, pH elevation, and active ion transport (Bentov et al., 2009; Bentov and Erez, 2006; de Nooijer et al., 2009, 2014a).

Mg/Ca requires a separate but related interpretation. Although miliolids show high Mg/Ca relative to most rotaliids, Mg incorporation in foraminiferal calcite remains biologically suppressed relative to inorganic calcite, such that D_{Mg} is lower than K_{Mg}. Thus, the high Mg/Ca values of miliolids do not indicate the absence of biological control, but rather weaker Mg exclusion, or a calcifying fluid with higher Mg/Ca, compared with many rotaliids. This explains why Mg/Ca can show a continuum between rotaliids and miliolids while still contributing to the broader inter-order pattern.

Structural differences in the tests may also account for the geochemical differences between the two orders by influencing 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.

The opposite behavior of Li/Ca further emphasizes that the miliolid-rotaliid pattern is element-specific. At present, we cannot assign this pattern to a single mechanism. Branson and de Nooijer, (2025) noted that some elements may be enriched through specific transport pathways rather than by Rayleigh-type enrichment; for example, Li may be incidentally transported alongside HCO_3^-. In foraminifera, calcification is known to involve strong carbonate-system regulation, including pH modulation at the calcification site (de Nooijer et al., 2009), proton removal by V-type H^+ ATPase during chamber formation (Toyofuku et al., 2017), carbonic anhydrase activity in *Amphistegina lessonii* biomineralization (De Goeyse et al., 2021)), and DIC-sensitive Li incorporation in *Amphistegina* (Charrieau et al., 2023; Vigier et al., 2015). However, the direct pathway linking these processes to Li incorporation remains unresolved. In addition, because Li^+ is a small monovalent ion, its incorporation into calcite requires charge compensation and may be more sensitive to crystal-surface, organic-matrix, or carbonate-system conditions than divalent or trivalent elements. The higher Li/Ca values observed in rotaliids may therefore reflect DIC-related regulation, pH modulation, charge-balance effects, or crystal-growth pathways rather than the Rayleigh-type enrichment that appears to dominate many other TE/Ca ratios in miliolids.

Thus, the M > R dichotomy represents a strong first-order taxonomic signal, reflecting divergent biomineralization pathways and ion-regulatory mechanisms between the two orders. At the same time, Li/Ca and the observed intra-order variability show that this signal is modified by element-specific and taxon-specific controls, including Ca addition, Mg exclusion, calcifying-fluid renewal, Rayleigh-type enrichment, organic-matrix interactions, and element-specific transport. Clarifying the relative contribution of these processes will require targeted physiological and experimental studies, but the co-occurrence comparison

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at Dor HaBonim demonstrates that taxonomy, both between and within orders, exerts a major control on foraminiferal El/Ca signatures.

4.2 Variability within Foraminiferal Orders

Although the M > R pattern represents a strong first-order taxonomic signal, it does not imply uniform elemental incorporation within each order. Instead, substantial intra-order variability indicates that the broader miliolid-rotaliid dichotomy is modulated by species-specific physiological, phylogenetic, and ecological controls. The comparison with published data further shows that such variability is especially pronounced among rotaliids, whereas the available miliolid dataset remains comparatively limited for many elements, particularly REEs (Figs. S1–S15). In the context of the calcifying-fluid model discussed above, such variability may reflect differences in the balance between Ca addition, Mg exclusion, fluid renewal, Rayleigh-type enrichment, calcification kinetics, organic-matrix interactions, and microhabitat conditions.

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* spp. and *S. orbiculus* show the highest 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. Their similar El/Ca patterns may therefore reflect a shared mode of porcelaneous calcification and comparable regulation of the calcifying fluid, including similar degrees of Mg exclusion and trace-element enrichment during calcite needle formation.

In contrast, *Lachlanella* sp. which is phylogenetically distant from the other two taxa and belongs to a highly diverse family of small miliolids, displays a partially distinctly different geochemical signature, with differences that are element-specific rather than systematic across all elements. This is also reflected in the PCA, where *Lachlanella* overlaps with other miliolids along the primary axis of variability (PC1), but is offset along PC2 (Fig. 3). This secondary separation is mainly driven by elements such as Mg, Mn, Cu, Cd and Pb versus REEs, indicating that differences are controlled by specific elemental groups rather than a uniform shift in overall chemistry. While most differences between *Lachlanella* sp. compared 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* sp. 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* sp. 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+}.

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Deleted: The overall consistency of the M > R signal among most elements indicates that the calcification pathways of both orders are 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.

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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* sp. representing a notable high-end outlier (van Dijk et al., 2020) and in the current study (Fig. 3).

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Although all miliolid specimens were collected from turf samples, *Lachlanella* sp. 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* sp. occupies more reducing microenvironments, and that its geochemical signature partially records environmental redox variability rather than purely taxon-specific biomineralization. Thus, *Lachlanella* sp. illustrates how the general miliolid enrichment pattern can be further amplified or modified by microhabitat conditions, particularly for redox-sensitive elements.

4.2.2 Rotaliids

The three rotaliid taxa exhibit substantial interspecific variation in most elemental ratios, yet the general M > R pattern remains evident. Importantly, this variability is not random, but follows a consistent species-level ranking across many elements: *A. lobifera* generally has the lowest El/Ca ratios, *P. calcariformata* shows higher values for several elements, including Mg/Ca, and *R. globularis* is distinct in its enrichment of Fe/Ca and V/Ca (Fig. 4). This pattern suggests that rotaliid calcification should not be treated as a single uniform mechanism, but as a shared perforate biomineralization pathway modified by species-specific physiological controls.

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 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.

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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 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) (Fig S2). 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., 2023), our data suggest that species-specific biological regulation exerts a dominant control. For example, *P. calcariformata*, which evolved in the Quaternary, exhibits higher Mg/Ca than *Amphistegina*, which originated earlier, during a period when seawater Mg/Ca was lower than today, although the precise timing of its rise remains debated (Evans et al., 2026). Thus, Mg incorporation appears primarily governed by species-specific biological regulation rather than by ambient seawater chemistry.

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Beyond Mg, the same species-level ordering is also observed for many other elements. Such inter-element correlation points to shared physiological controls on elemental incorporation, rather than independent behavior of each element. Similar correlations have been interpreted as evidence for common controls on calcifying-fluid chemistry and element partitioning, including Ca transport, precipitation dynamics, and Rayleigh-type evolution of the calcifying reservoir (Branson and de Nooijer, 2025; Marchitto et al., 2018).

A. lobifera consistently exhibits the lowest El/Ca ratios for most elements, indicating strong biological discrimination against elemental incorporation despite its thick, multilayered test that integrates multiple growth phases. Because *A. lobifera* and *P. calcariformata* occur in the same habitat and both host diatom symbionts, their contrasting El/Ca signatures are unlikely to reflect ambient seawater chemistry or symbiosis alone. The low values in *A. lobifera* may instead reflect stronger or more persistent physiological regulation, such as more effective Ca addition, more frequent renewal of the calcifying medium, reduced precipitation-driven enrichment, or slower and more regulated calcification. Its thick test may further average numerous chamber-formation and secondary-calcification events toward a stable, species-specific low-El/Ca signature.

By contrast, the higher El/Ca values of *P. calcariformata* may indicate lower renewal, weaker trace-element exclusion, stronger precipitation-driven evolution of the calcifying fluid, or faster calcification kinetics. Faster precipitation from a semi-isolated calcifying fluid could enhance Rayleigh-type enrichment by rapidly removing Ca relative to many trace elements, while also reducing the time available for selective ion regulation during crystal growth. This interpretation is consistent with its unusually high Mg/Ca values relative to many other rotaliids and with previous evidence for large Mg/Ca variability among rotaliid taxa. Thus, intra-order variability in rotaliids appears to reflect lineage-specific modifications of a shared perforate calcification strategy.

Rosalina 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 to calcification dynamics rather than redox conditions. This taxon-specific enrichment further supports the view that rotaliid intra-order variability is structured by species-level physiological controls, superimposed on the broader miliolid-rotaliid pattern.

4.3 Implications for biomonitoring of dissolved elements in seawater

The pronounced miliolid-rotaliid first-order pattern (M > R) highlights the critical importance of species selection when using benthic foraminifera as bioindicators. Miliolids, owing to their higher elemental uptake, may serve as more sensitive recorders of trace-element enrichment in seawater or microhabitats, particularly for Pb, Cd, Mn and Zn. However, their weaker ion-selectivity control means incorporation which could result in non-linear uptake at elevated concentrations as suggested by evidence for Rayleigh-type modification of the calcifying fluid during precipitation. Such processes may complicate quantitative reconstructions and calibration experiments. Rotaliids, by contrast, generally exhibit lower uptake for many elements and may therefore provide more conservative records, but their strong species-level variability means that they should not be treated as a single uniform biomonitoring group.

For environmental monitoring, this means that miliolids may be advantageous for detecting subtle enrichment trends but require careful calibration at the species and element level due to possible non-linear uptake. Rotaliids may provide more stable baselines, especially in long-term or low-variability studies, though they may miss minor perturbations or respond differently depending on the selected species. A dual-order, multi-species approach may therefore maximize detection sensitivity while providing cross-validation of environmental signals and helping to distinguish environmental enrichment from taxon-specific biomineralization effects.

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 taxonomically resolved framework can improve trace-metal reconstructions, refine species selection for biomonitoring, and guide future experimental work aimed at separating environmental signals from biomineralization controls on elemental incorporation.

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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.; 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

No additional disclaimer is required.

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