European Journal of Protistology 100 (2025) 126160

Contents lists available at ScienceDirect

European Journal of Protistology

journal homepage: www.elsevier.com/locate/ejop

Trait-based predictors of feeding ecology patterns in shelled microorganisms

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ARTICLE INFO

Keywords:
Functional morphology
Holarctic thecamoebians
Morphometric data
Protist ecology
Trophic niches

ABSTRACT

Functional traits provide key insights into ecological strategies and evolutionary diversification. In this study, we analyzed a comprehensive trait dataset to investigate morphological predictors of feeding ecology in testate amoebae from the Northern Holarctic realm, focusing on variability across 372 species. We also examined whether trait diversity mirrors taxonomic richness at the family level. Morphological traits included shell length, shell width, aperture dimensions, shape, and covering. The Principal Component Analysis (PCA) indicated that Axis 1 predominantly represented variation in shell and aperture size, while Axis 2 was associated with differences in overall shape proportions. Bacterivorous species exhibited the greatest morphological and taxonomic diversity, spanning 21 families and 48 genera, with mixotrophs and predators occupying nested subsets of their broader morphospace. The regression analyses demonstrated significant associations between species richness and variation in protective features, including aperture rim morphology and the presence of spines. Decision tree models identified the aperture width-to-length ratio as a key predictor of feeding strategy, although classification accuracy was lower for mixotrophs and predators. Future research should integrate shell morphology with phylogenetic data to enhance ecological strategy predictions in testate amoebae and explore hypotheses regarding functional diversification across a broader geographical scale and within different environments.

1. Introduction

Traits are morphological, physiological, or phenological characteristics that can be measured at the level of an individual organism and are defined independently of environmental context or higher levels of biological organization (Rodrigues-Filho et al., 2023; Violle et al., 2007). Although trait-based ecology has made significant progress, persistent challenges continue to limit the design of relevant research goals and hypotheses across a more diverse set of organisms, as well as the ability to link easily measurable traits to their impacts on ecosystems (de Bello et al., 2025). Trait-centered research on microorganisms still lags behind that of macroorganisms. This gap is likely driven by geographically biased databases, incomplete trait information, and the vast diversity of microbial lifestyles and functional traits, many of which remain undocumented (de Bello et al., 2025; Krause et al., 2014; Lajoie and Kembel, 2019).

Testate amoebae are a morphologically diverse group of protists that build protective extracellular shells (tests). These tests vary in shape, size, and composition, and serve both as defensive structures and tools for prey capture (Dumack et al., 2024; Meisterfeld, 2002; Su et al., 2024). For instance, recent studies suggest that the shells of arcellinid testate amoebae, particularly their rigidity and aperture structure, facilitate prey capture by enabling mechanical force application, indicating a functional adaptation for predation on larger prey (Dumack et al., 2024). Although the identification of testate amoeba functional traits and the use of trait-based indices have enabled inferences about past water table fluctuations and environmental factors such as disturbance and light availability (Marcisz et al., 2014, 2016; van Bellen et al., 2017), and despite the growing availability of molecular data, further studies are still needed to rigorously test trait–function relationships in testate amoebae.

Morphological traits of testate amoeba are often linked to ecological functions such as nutrient cycling, decomposition, and ecosystem stability (Branco et al., 2023; Gilbert and Mitchell, 2006; Lamentowicz et al., 2020; Wilkinson and Mitchell, 2010), and are widely used as indicators of environmental change over both short and long timescales (Charman, 2001). Some taxa have been proposed as bioindicators of ammonia deposition (Evans et al., 2025), while mixotrophic species

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https://doi.org/10.1016/j.ejop.2025.126160
Received 24 March 2025; Received in revised form 21 July 2025; Accepted 22 July 2025
Available online 23 July 2025
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containing endosymbiotic algae may reflect shifts in light and nutrient availability (Herbert et al., 2019; Marcisz et al., 2014; Payne et al., 2016). The early emergence of diverse thecamoebian morphologies in the Neoproterozoic—and their relative morphological conservatism since—suggests that distinct functional morphotypes aligned with ecological niches and feeding strategies were established early in their evolutionary history (Lahr et al., 2019).

Investigating functional diversity in microorganisms presents considerable challenges due to the large number of measurable traits and the difficulty of collecting standardized data across diverse taxa (Macumber et al., 2020; Su et al., 2024). Progress in developing comprehensive trait databases depends on precise, individual-level quantitative measurements conducted under consistent protocols and using standardized tools, although these efforts are often time-consuming and hampered by limited cross-laboratory standardization. Leveraging the newly published database of testate amoeba functional traits from the Northern Holarctic region (Su et al., 2024), this study explores multidimensional trait relationships in testate amoebae. We specifically assess whether shell and aperture morphology exhibit distinct morphospace patterns linked to feeding strategies (bacterivory, mixotrophy, and predation), identify which traits best explain variation in feeding modes, and examine whether morphological diversity scales with taxonomic richness.

2. Material and methods

We used a recently compiled list of species and relevant morphological traits influencing ecological functions, comprising 19 quantitative and 8 categorical traits (Fig. 1; for further details, see Su et al., 2024). The list includes 376 morphospecies of testate amoebae compiled from three quantitative databases encompassing diverse habitats across Eurasia, Europe, and North America. The first database consisted of 1635 samples collected across Eurasia between 2004 and 2020 from mires (n = 1115), soils (n = 429), and lakes (n = 91). The second included 1799 samples from 113 peatland sites in 18 European countries, originally assembled for a pan-European palaeohydrological transfer function. The third comprised 1956 samples from 137 North American peatlands used to develop a similar transfer function. Feeding types were assigned based on multiple sources: primary literature, online databases, and taxonomic keys (Su et al., 2024). The family level was assigned to all species. For all analyses, data were log-transformed using the formula $\log_{10}(x + 1)$.

We assessed differences in quantitative shell traits, which included continuous morphological measurements expressed in micrometers (μm) or as dimensionless numerical ratios (R1–R4) among feeding types (bacterivory, mixotrophy, and predatory). To do this, we used analysis of variance (ANOVA) followed by post hoc pairwise comparisons. We performed a Principal Component Analysis (PCA) on the continuous shell and aperture morphological traits that were not highly autocorrelated ($r < 0.9$). PCA was conducted using the 'prcomp' function in R. Feeding types (bacterivory, mixotrophy, and predatory) were visualized using convex hulls around point clouds in PCA space to show functional clustering. Convex hulls were converted to spatial polygons using the sf package (Pebesma and Bivand, 2023) and pairwise intersections were calculated. Overlap percentages were expressed as the proportion of one group's morphospace covered by another group's polygon, allowing for asymmetrical comparisons.

We assessed whether trait distributions among taxonomic families of testate amoebae deviate from randomness, indicating taxa-specific constraints. First, we calculated the number of species per family (species richness) and the number of distinct states per categorical trait (trait diversity). Categorical trait variables considered were: position of the aperture, degree of invagination of the aperture, aperture rim morphology, presence/absence of a collar, shell covering type, presence/absence of internal partitions, presence/absence of spines or horns, shell test shape, and feeding type. Linear regressions were performed between log-transformed species richness and log-transformed trait diversity to test whether more speciose families exhibit greater morphological diversification. To statistically assess whether the distribution of trait states across families differed from random expectations, we constructed contingency tables for each trait and applied Pearson's chi-square tests with Monte Carlo permutation (1000 permutations) to avoid approximation errors due to small expected frequencies. This test allowed us to compare the observed distributions of each trait state across families against the null hypothesis of random distribution.

To identify which morphological variables are most important in discriminating feeding types, we fitted classification decision trees using species-level continuous trait data. We implemented a cross-validation-based hyperparameter tuning approach using the caret and rpart packages in R. We varied minbucket (the minimum number of observations in terminal nodes; values: 5, 7, 10, 12, 15, 20, 25, and 30) and tree complexity (cp). For each combination, models were evaluated via 5-fold cross-validation. The optimal model parameters were selected based on minimizing the root mean square error (RMSE) and maximizing classification accuracy. We used the R packages dplyr (Wickham et al., 2025a), tidyr (Wickham et al., 2025b), stringr (Wickham, 2023), ggplot2 (Wickham, 2016), and rpart.plot (Milborrow, 2024) for data manipulation and visualization. All analyses were conducted in R ver. 4.4.2 (R Core Team, 2025).

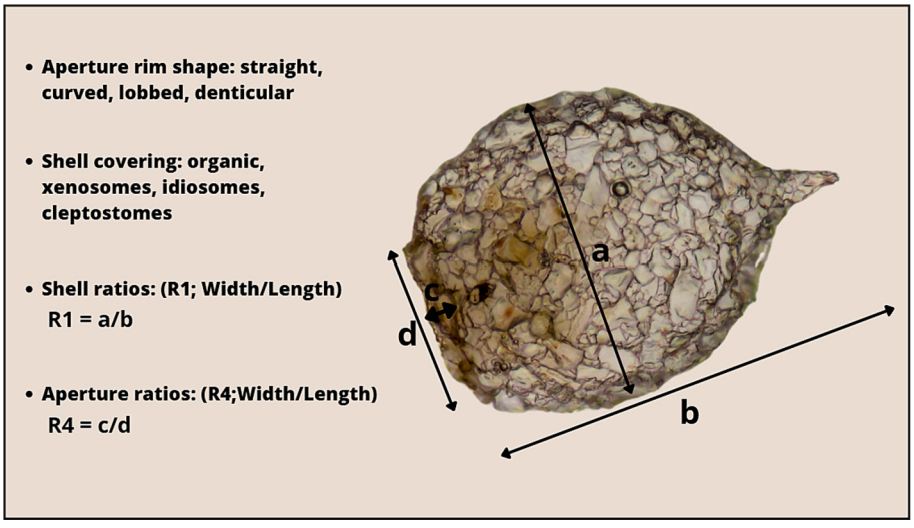

Fig. 1. Examples of morphological traits used in the present analyses.

3. Results

The majority of species richness was concentrated within a few families, notably Difflugiidae (96 species) and Euglyphidae (57 species). At the genus level, Hyalospheniidae and Euglyphidae stood out with the highest counts, while most families contained only one or two genera (Supplementary Fig. 1A, B). When grouped by feeding strategy, bacterivory dominated taxonomic diversity, comprising 21 families and 48 genera. In contrast, mixotrophic and predatory groups exhibited much lower taxonomic breadth, each represented by only seven families and nine genera (Supplementary Fig. 1C, D). These findings suggest that predatory and mixotrophic species are more taxonomically constrained.

The multivariate dispersion analysis showed notable differences in trait variability among feeding types. Bacterivorous species exhibited the highest dispersion (measured as distance from the centroid), indicating greater heterogeneity in morphological traits within this group (Figs. 2, 3). A post hoc test showed that the dispersion of predatory species was significantly lower than that of bacterivorous species ($p = 0.0017$), highlighting a tighter clustering of trait values within the predatory group. Differences between mixotrophs and the other feeding types were not statistically significant ($p > 0.29$), although a trend toward lower dispersion in predatory species compared to mixotrophs was observed. The first two principal components explained 39.4 % and 28.5 % of the total morphological trait variation, respectively, accounting for a cumulative 67.9 %. The first principal component (PC1) was primarily driven by the minimum shell width and the maximum aperture length, while the second prrincipal component (PC2) was influenced by the morphological ratio R1. Convex hulls highlighted clear, though partially overlapping, separations between feeding types.

The overlap analysis showed that 18.8 % of the bacterivore morphospace overlapped with mixotrophs, and almost the entire mixotroph morphospace (98.9 %) was contained within the bacterivore space. For predatory species, 7.7 % of the bacterivore space overlapped with their morphospace, but 100 % of the predatory morphospace was nested within that of bacterivores. The overlap between mixotrophs and predators was moderate, with 31.5 % of mixotroph morphospace overlapping with predators, and 77.5 % of the predator morphospace overlapping with mixotrophs.

Eight of the 19 traits showed significant differences among feeding types (Fig. 4). Mixotrophic species generally exhibited larger shell sizes than bacterivorous and predatory species. Predators showed distinct morphological patterns, with significantly lower values in the shape ratios R2 and R4 (both $p < 0.0001$), reflecting adaptations in shell shape. These results indicate that feeding types are strongly associated with distinct morphological profiles (Supplementary Fig. S2; Supplementary Table S1).

More speciose families exhibited greater morphological diversification, particularly in protective and structural features (Fig. 5A). We found strong positive relationships between family species richness and diversity in certain morphological traits, particularly the presence of spines or horns ($R^2 = 0.691$), aperture rim ($R^2 = 0.613$), and position of the aperture ($R^2 = 0.609$). Moderate relationships were observed for feeding type ($R^2 = 0.448$), shell covering ($R^2 = 0.427$), and shell test shape ($R^2 = 0.465$). In contrast, weak relationships were found for the degree of invagination of the aperture ($R^2 = 0.223$) and the presence of internal partitions ($R^2 = 0.121$). Specifically, families with higher species richness, such as Difflugiidae and Hyalospheniidae, also showed greater feeding type diversity. Some families (e.g., Amphitremidae and Netzeliidae) deviated from the general trend, exhibiting higher feeding diversity relative to their species richness (Fig. 5B).

The chi-square analyses confirmed that the distribution of trait states across families is not random. All categorical traits showed highly significant deviations ($p < 0.001$), indicating that certain structural and morphological characteristics are concentrated within specific families rather than evenly distributed.

The decision tree illustrating the hierarchical structure of feeding type prediction is shown in Fig. 6. The resulting classification tree showed four clear decision paths. The first split occurred based on the shell shape ratio R4 (threshold: 0.56), the variable that best explained the variance, followed by splits on maximum shell width and average shell width. The confusion matrix of the selected model demonstrated high accuracy in classifying bacterivorous species (dominant in the dataset), while mixotrophic and predatory species had lower classification rates, reflecting their lower representation and potentially overlapping morphological features. When the shell aperture ratio (R4) was equal to or greater than 0.56 and the maximum shell width was less than 141 μm, the probability of bacterivory was 94 %. However, if under these conditions the average shell width was equal to or greater than 123 μm, the probability shifted toward mixotrophy (80 %). In contrast, when R4 was less than 0.56 and the maximum shell width was less than 81 μm, the model predicted a 69 % likelihood of predatory feeding. In cases where the maximum shell width exceeded these thresholds,

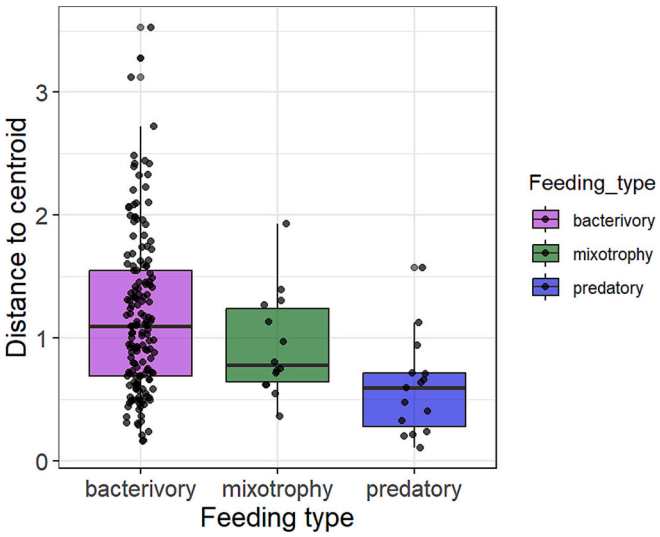

Fig. 2. Dispersion of species in morphological traits. Greater distance from the centroid indicates higher heterogeneity in trait variability within each feeding type group.

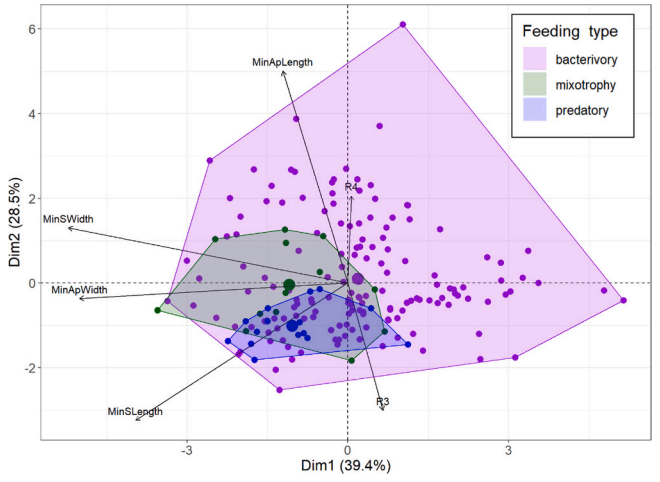

Fig. 3. Principal Component Analysis (PCA) of six continuous shell and aperture traits selected to minimize multicollinearity ($r < 0.9$).The first principal component (PC1) is primarily influenced by the minimum shell width (loading = −0.62), minimum aperture width (−0.60), and minimum shell length (−0.47). The second principal component (PC2) is driven by the minimum aperture length (0.70), the minimum shell length (−0.46), and the ratio R3 (−0.42).

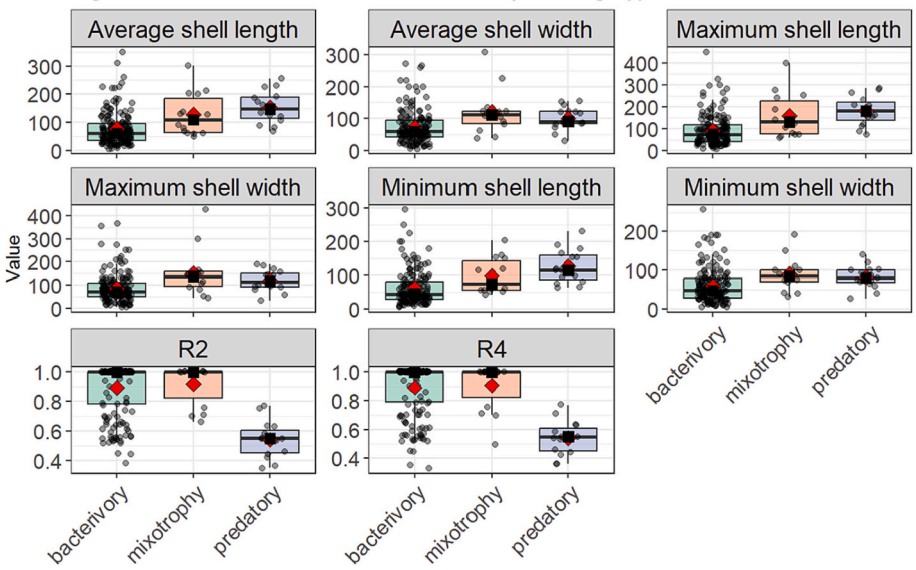

Fig. 4. Boxplots showing the distribution of quantitative morphological traits (expressed in micrometers [μm] or as dimensionless ratios R1–R4) that differ significantly among feeding types (bacterivory, mixotrophy, and predatory). Traits without significant differences are shown in Supplementary Figure S2.

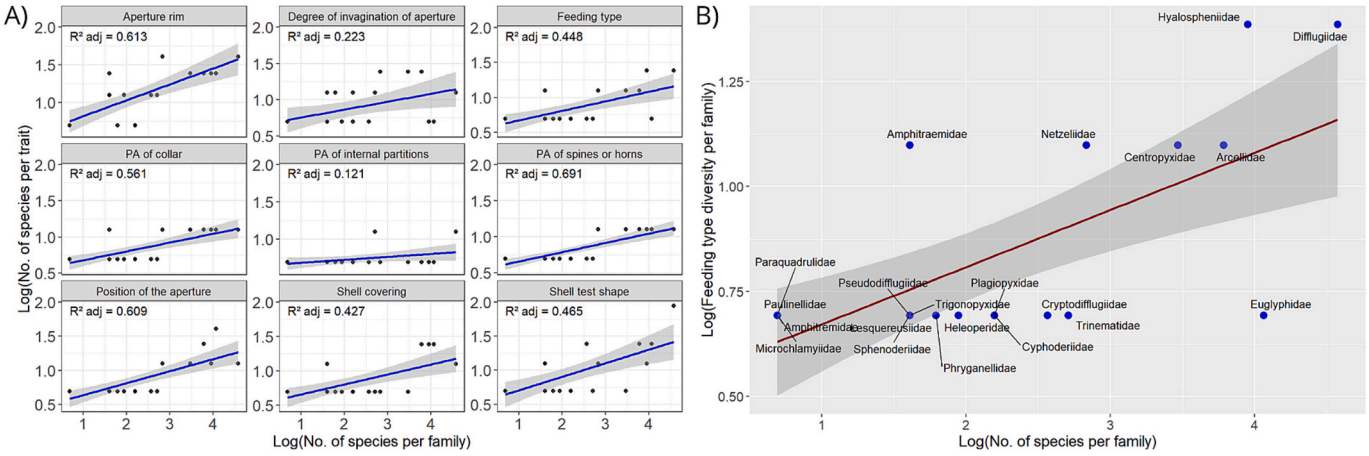

Fig. 5. (**A**) Relationship between the number of species per family and the number of species exhibiting specific trait states. Both axes are log-transformed. PA indicates presence/absence. Adjusted R^2 values for each linear regression are shown. The shaded area represents the 95 % confidence interval of the linear regressions. **(B)** Detailed view of the relationship between family-level species richness and feeding type diversity. Each point represents a family, labeled to highlight variation in feeding type diversity relative to species richness.

bacterivory remained the dominant feeding strategy.

4. Discussion

Our results reveal strong taxonomic biases in testate amoeba shell trait diversity across feeding types, with bacterivorous species showing significantly greater taxonomic breadth than mixotrophic and predatory groups. The dominance of bacterivory, represented by 21 families and 48 genera, aligns with previous studies highlighting the ecological flexibility and evolutionary success of bacterivorous testate amoebae in diverse microhabitats (Lamentowicz et al., 2015; Mitchell and Gilbert, 2004; Pratt and Cairns Jr, 1985). Ecologically, these patterns reflect different functional constraints: predatory species exhibit more convergent traits due to selective pressures associated with prey capture and hunting efficiency, while bacterivorous species display broader trait diversity, possibly linked to generalist feeding strategies and a wider range of ecological niches (Gilbert et al., 2003; Jassey et al., 2013; Wanner and Xylander, 2005). Environmental context can shape morphological traits through habitat-specific selective pressures, potentially confounding interpretations that attribute trait differences solely to feeding strategies. For example, testate amoebae may show trait convergence in response to water-level fluctuations (Fournier et al., 2012) and are generally sensitive to peat moisture content and pH (Lamentowicz and Mitchell, 2005; van Bellen et al., 2017). Thus, our results raise an open question: are morphological traits (e.g., shell size, aperture features) highly conserved among bacterivorous species across diverse environments (peatlands, mires, soil, lakes), suggesting functional convergence, or is there environmental adaptation masked by a shared feeding strategy?

Life strategies adapted to widely available and generalized resources often promote broad geographic distributions and the formation of superspecies complexes, in which member species tend to be largely allopatric with limited ecological divergence (Kennedy et al., 2018). In contrast, lineages that rely on highly specialized or rare resources are frequently restricted in their geographic ranges, as these resources are unevenly distributed across space (Ricklefs, 2005). Such ecological specialization can also drive the evolution of specific morphological traits that may limit dispersal potential and reduce opportunities for

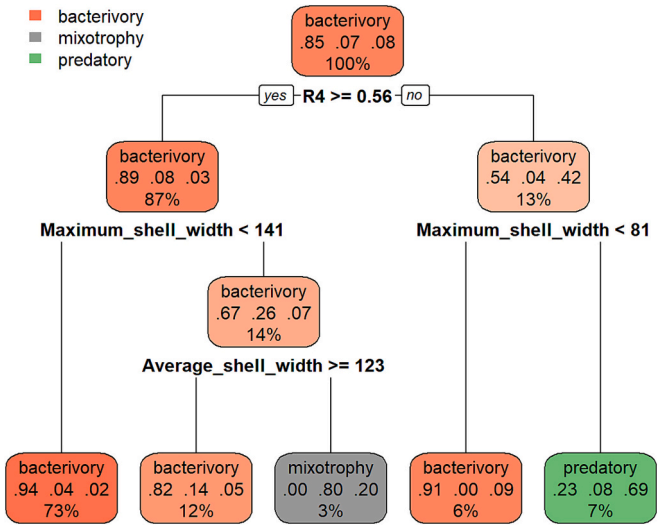

Fig. 6. Decision tree model predicting feeding type (bacterivory, mixotrophy, and predatory) based on morphological traits. Splits are defined by thresholds in R4, shell width, and shell depth. Terminal nodes show the proportion and dominant class of feeding types. The tuning procedure identified that models with moderate complexity (minbucket = 7, cp = 0.0569) provided optimal predictive performance. Box color intensity reflects the dominance of a feeding type, with darker shades indicating higher proportions.

range expansion. As a result, generalist lineages are expected to display higher taxonomic and functional trait diversity, while specialist groups are likely to remain both taxonomically and morphologically constrained (Day et al., 2016; Roland et al., 2017; Sriswasdi et al., 2017). Applying these concepts to testate amoebae provides an opportunity to investigate whether taxonomic richness aligns with trait diversity, or whether particular traits become disproportionately concentrated within certain groups.

Our results show that bacterivorous species occupy the broadest morphospace, encompassing nearly the entire morphospaces of mixotrophic and predatory groups. This challenges the intuitive expectation that mixotrophs (capable of both photosynthesis and heterotrophy) would display the most versatile morphology. Instead, mixotrophic species may be morphologically constrained by structural demands of hosting photosynthetic endosymbionts and maintaining shell architectures suitable for both light capture and feeding (Creevy et al., 2018; Marcisz et al., 2020; Payne et al., 2016). For example, light availability has long been recognized as a key factor shaping the distribution of mixotrophic testate amoebae, particularly those harboring zoochlorellae, which often dominate in nutrient-poor, *Sphagnum*-dominated peatlands (Heal, 1964; Schönborn, 1965). Several studies have shown that as water levels decline, test size tends to decrease (in length, width, and range), often accompanied by the disappearance of mixotrophic species (Jassey et al., 2013; Koenig et al., 2018; Marcisz et al., 2014). This pattern suggests that mixotrophs are generally larger and likely specialized for wetter, light-rich habitats. Supporting this, our decision tree analysis identified aperture ratio and shell width as strong predictors of feeding strategies, with the aperture ratio (R4) serving as a key axis of ecological differentiation. Nonetheless, morphospace coincidence between mixotrophs and predators may indicate potential morphological overlap, or that morphology alone may not fully capture ecological function. Given that environmental constraints could limit morphological diversification, mixotrophy may be a specialized adaptation to stable, light-penetrated environments rather than a mark of generalists. The complete nesting of predatory and mixotrophic morphospaces within that of bacterivores suggests that morphological adaptations for bacterivory formed the foundation from which more specialized feeding strategies evolved. Thus, bacterivory appears likely to be the ancestral feeding mode and may represent the morphological baseline from which more specialized strategies emerged (Tekle et al., 2022). This highlights the importance of incorporating environmental and molecular data to improve predictive power and ecological inference.

Morphological variation in testate amoebae strongly aligns with habitat type, making certain morphotypes useful indicators of ecological conditions and evolutionary morphology (Schönborn, 1989). For example, species exhibiting a terminal and relatively large aperture, such as acrostomic and trachelostomic forms, are prevalent in aquatic environments and moist moss habitats. However, this functional classification has seen limited application in ecological research (Marcisz et al., 2020; Wilkinson and Mitchell, 2010). Most testate amoebae function as predators and consume a wide range of prey, including bacteria, which helps explain the overlap between predatory and bacterivorous morphospaces (Coûteaux and Pussard, 1983). While aperture size generally constrains prey size, some amoebae are capable of feeding on organisms larger than themselves, such as filamentous algae and even sizeable nematodes, which have been reported to be sometimes hunted in groups (Geisen et al., 2015; Stump, 1935). Larger aperture sizes are associated with higher trophic levels, reflecting broader diets and higher trophic positions (Jassey et al., 2016). Additionally, smaller bacterivorous species, such as *Assullina muscorum*, *Corythion dubium*, and *Trinema lineare*, appear more adept at recolonizing habitats after drought disturbances compared to larger taxa (Koenig et al., 2018).

Most traits exhibit a positive linear relationship between species richness and trait diversity. Families with more species tend to diversify in traits associated with structural complexity and defense, such as spines, aperture features, and shell coverings. This suggests that variation in these features may drive niche differentiation and species radiation (Mitchell et al., 2008). Moderate scaling with feeding type and shell covering indicates that trophic adaptations and external shell modifications accompany diversification but are constrained by ecological pressures. The positive correlation aligns with theories of adaptive radiation, where lineage diversification leads to the expansion of functional traits (Lahr et al., 2019; Porfirio Sousa et al., 2024). However, our results support a more nuanced interpretation. Outliers, such as Amphitremidae, may represent evolutionary specialization or historical ecological shifts that promoted disproportionate trait diversity. Conversely, the speciose family Euglyphidae displays low trait diversity, possibly due to niche conservatism or recent diversification. The limited reports of cryptic diversity within Euglyphida, so far documented in only two morphospecies (Wylezich et al., 2002), suggest that its lower functional diversity may reflect true evolutionary history rather than unresolved taxonomy. This contrasts with Arcellidae, where high species richness coupled with only moderate functional diversity exemplifies how general trends are shaped by evolutionary convergence and cryptic diversity. These phenomena are widespread in Arcellinida (González-Miguéns et al., 2022; Kosakyan et al., 2013; Singer et al., 2018), where ecological pressures often lead to convergent morphologies. The overall positive trend is nuanced by family-specific dynamics, where ecological convergence may constrain trait diversification. The non-random distribution of traits, confirmed by significant chi-square tests, supports the concept of trait conservatism, in which morphological and ecological characteristics remain stable within lineages due to functional advantages or phylogenetic inertia (Blomberg and Garland, 2002). For instance, families with high species richness, such as Difflugiidae and Hyalospheniidae, exhibited high diversity in aperture structures and shell coverings, suggesting that these features promote ecological flexibility and evolutionary success. Conversely, some smaller families, such as Amphitremidae and Paraquadrulidae, display disproportionately broad feeding-type diversity relative to their species richness. This decoupling between morphological and functional traits suggests that functional diversity can arise independently of species number, potentially driven by niche specialization or historical ecological shifts. Alternatively, biogeographic constraints may also

contribute to these deviations.

Overall, while morphological traits offerr substantial insight into ecological strategies, their predictive accuracy diminishes for less common feeding types. The difficulty in distinguishing mixotrophic and predatory species underscores the limitations of morphology in capturing the full spectrum of functional diversity. This aligns with broader concerns that morphological traits in microorganisms may obscure cryptic species, leading to inflated perceptions of widespread distributions and ecological uniformity (Fontaneto et al., 2008). Rather than simply recommending that future studies complement morphology-based analyses with molecular and environmental data, we emphasize the importance of improving trait extraction and classification by prioritizing those traits with the highest predictive power. This approach minimizes confounding factors and reduces unnecessary laboratory work. Such refinement is essential for fully resolving functional diversity and enhancing the applicability of trait-based approaches at the ecology–policy interface (Mitushasi et al., 2025; Van den Ende et al., 2023), as well as for deepening our understanding of how shell traits shape testate amoeba biodiversity.

CRediT authorship contribution statement

Rafael L. Macêdo: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Odete Rocha:** Writing – review & editing, Validation, Supervision, Investigation.

Funding sources

Rafael L. Macêdo is supported by a postdoctoral fellowship funded by the CAPES–Humboldt Research Fellowship Program.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Jiahui Su, Basil N. Yakimov, and their collaborators for providing the valuable dataset on testate amoeba traits, which greatly facilitated this study and will support future research on functional diversity. We are grateful to the editors and anonymous reviewers for their thoughtful comments and suggestions, which significantly improved the quality and clarity of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejop.2025.126160.

Data availability

Data available online.

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