



## Short Communication

## It's time to consider the Arcellinida shell as a weapon

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## ABSTRACT

The shells of testate amoebae are morphologically diverse and persistent in the environment. Accordingly, the examination of the morphology and composition of shells became a standard tool in ecological, palaeoecological, and evolutionary studies. However, so far the function of the shell remains poorly understood and, although based on limited evidence, the shell was considered as a defense mechanism. Based on recent evidence, we propose that the shell of arcellinid testate amoebae is a crucial component facilitating the amoebae's attack of large prey. Accordingly, the shell is not purely protective, but must be considered also as a weapon. This change in perspective opens up numerous new avenues in protistology and will lead to a substantial change in ecological, palaeoecological, and evolutionary research.

## 1. Arcellinida - amoebae with a 'protective' shell

Testate amoebae are amoeboid organisms enclosed in a shell. The shell usually exhibits one opening, the aperture, from which pseudopodia protrude for locomotion and feeding (Fig. 1). The morphology, dimensions and composition of the shell and its aperture are species-specific (Dumack et al., 2017; González-Miguéns et al., 2022b, 2022a; Kosakyan et al., 2016; Nikolaev et al., 2005). Shells are composed of durable components like agglutinated silica scales, quartz grains, repurposed diatom frustules, or entirely proteinaceous material. They can be self-secreted, built out of agglutinated material of mineral elements or derived from their prey (Dumack et al., 2018a; Lahr et al., 2015). The remarkable persistence of the shell in the environment allows its isolation and identification from desiccated or aged environmental samples. Due to their ease of observation and manipulation, shell-bearing amoebae are an excellent model group to study protistan diversity, ecology, and evolution. Accordingly, they have long captivated protistologists, ecologists and palaeontologists (Harnisch, 1927;

Lahr et al., 2019; Laminger et al., 1982, 1980; Schönborn, 1992, 1975; Volz, 1929). Shells preserved as subfossils have become a standard tool in palaeoecological studies of lake sediments and peat sequences (Marcisz et al., 2020). Even older fossils dating back to the Neoproterozoic provide key evidence for studying long-term ecology and evolution (Porter, 2011; Porter and Knoll, 2000; Porter et al., 2003).

Since these shells consist of such durable, persistent compounds, the shell has traditionally been viewed as an essentially defensive structure, safeguarding testate amoebae against predators, parasites and environmental stresses like drought (Bobrov and Mazei, 2004; Collins et al., 1990; Marcisz et al., 2020; Medioli et al., 1990; Schönborn, 1962). However, recent findings have revealed the vital role of the shell as a tool for predation in arcellinid testate amoebae (Amoebozoa, summarized below). Consequently, reimagining the shell not merely as a defense mechanism but also as an offensive weapon is a paradigm shift and presents promising avenues for the study of the ecology and evolution of testate amoebae, which we will discuss in the following paragraphs of this opinion paper.

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## 2. The shell's crucial involvement in predation

The most common way of predation in protists is the phagocytosis-facilitated engulfment of entire prey cells (Martin et al., 2017). This predation mechanism should impose size limitations on protists, and especially enclosed organisms like testate amoebae, which need their prey to fit through the aperture of their shell. It was thus long believed that the aperture size represents an upper size limit for prey ingestion and thus a potential indicator of the amoeba's trophic level (Jassey et al.,

2013; Marcisz et al., 2020). Nonetheless, over the many years of exploration of testate amoebae, several observations were made reporting arcellinidan testate amoebae to prey on larger preys (Gilbert et al., 2000; Jassey et al., 2012). For instance, *Apodera* vas, *Diffflugia* sp. and *Cryptodiffflugia oviformis* were shown to prey on nematodes (Geisen et al., 2015; Yeates and Foissner, 1995). *Pseudonebela africana* was shown to perforate the cell walls of desmid prey algae, like *Closterium* (Hoogenraad and Groot, 1941; Siemensma and Opitz, 2014). *Lesquerusia spiralis*, *Lagenodiffflugia* vas, *Diffflugia constricta* and *D. lobostoma*



**Fig. 1.** An overview of the morphological diversity of Arcellinida shells. *Pseudonebela* (*Diffflugia*) *rubescens* feeding on its green algal prey (A); *Netzelia lobostoma* attached to a cyanobacterial filament, lateral view (B) and apertural view (C); *Hyalosphenia cuneata* (D), note the prominent epipodia anchoring the amoeba to its shell; *Heleopera sphagni* – lateral view and frontal view (E); *Netzelia oviformis* (F); *Lamtopyxis* sp. (G); *Arcella mitrata* (H); *Netzelia corona* (I); *Netzelia oviformis* (J); *Cyclopyxis leidyi* (K); *Bullinularia indica* (L). Scale bars: 50 µm, except F = 20 µm.

were reported to perforate filamentous green algae prior to consuming their cell contents (Stump, 1935). However, due to a lack of cultures and controlled conditions, the predation mechanism was not explored.

Recent studies by Dumack et al. (2018b) and Estermann et al. (2023) shed further light on the feeding mechanisms of the order Arcellinida, focusing on microscopical investigation of cultured *Phryganella paradoxa* and *Cryptodiffugia oviformis*. Both species belong to the Phryganellina, one of the most basal branching suborders of the Arcellinida. In contrast to the general trend observed in other protistan species, both Phryganellina species investigated in these studies exhibited a remarkable ability to feed on larger preys. *Cryptodiffugia oviformis* could be considered omnivorous, consuming a diverse range of preys, including bacteria, yeasts, hyphae, and even microscopic animals. *Phryganella paradoxa* appeared to be highly specialized, with its diet primarily consisting of pennate diatoms. Although bacteria were found in food vacuoles of *P. paradoxa*, bacteria alone did not support culture growth. The mechanistic basis of how Phryganellina, and other Arcellinida, handle larger preys warrants investigation. We posit that this predation mechanism is underpinned by cytoskeletal elements, with the shell playing a pivotal role in the feeding process:

*Cryptodiffugia oviformis* was shown to perforate or rupture chitin cell walls during predation. Feeding experiments revealed distinct behaviors in its consumption of different fungi: small yeasts are consumed as a whole, thin hyphae are broken, and thick hyphae are perforated (Estermann et al., 2023). Detailed observation showed that while *C. oviformis* initiates predation, pseudopodia retract, followed by the formation of circular, pulsating cytoplasmic protrusions known as 'blebs' (Fig. 2). Bleb formation is a common feature observed in various eukaryotic cells and results from the retraction of actin filaments from the cell cortex (Charras, 2008). Upon closer investigation of the cytoskeleton during feeding, the F-actin was found to be repurposed during predation. F-actin is typically associated with locomotion, but during predation conical bundles of F-actin are formed which extend deep into the cell body of *C. oviformis* and which connect to the prey (Fig. 2). These actin bundles are often found in association with structures known as epipodia. Epipodia have traditionally been recognized as pseudopodia-like structures responsible for anchoring testate amoebae in their shell (Figs. 1, 2). Observations of Estermann et al. (2023) suggest that actin is used to exert force on the cell walls of the amoeba's prey while being anchored to the inner shell surface. Supporting this idea, observations show that the flexible chitin wall of the prey is considerably deformed during an attack (Fig. 2B). Consequently, small prey like yeasts squeeze through the slightly narrower aperture, thin hyphae are broken before uptake and hyphae which are too large to be pulled through the shell's aperture, but sturdy enough to be only perforated and not broken, are

perforated. Considering that an anchoring of the actin seems crucial to feeding, the shell emerges as a critical component in *Cryptodiffugia's* eukaryovorous feeding strategy.

Unlike the omnivorous *C. oviformis*, *P. paradoxa* is a specialized predator of diatoms (Dumack et al., 2018b). When attacking its prey, *P. paradoxa* bends and breaks its prey's tough siliceous shells, called frustules. While no actin stainings were made during the investigation of *P. paradoxa*, Dumack et al., (2018b) revealed another intriguing aspect of how *P. paradoxa* uses its shell during these attacks. Interestingly, *P. paradoxa* first inserts the tip of the diatom's frustule into its shell's aperture just before breaking it. Food-range experiments showed that only diatoms thin enough to be partially inserted into the amoeba's aperture were successfully broken and consumed, suggesting that this point of leverage is a mandatory requirement to break the thick and sturdy siliceous frustules of diatoms. Accordingly, the rigid shell wall around the opening must play a vital role in *P. paradoxa's* feeding strategy. This feeding mechanism effectively counters the diatom's typical defense of increased cell length, which is usually effective against most predators (Van Donk et al., 2011). While *P. paradoxa* can successfully prey on diatoms that are several hundred micrometers long, the requirement to partially insert the tips into the aperture means that filaments, for instance by *Fragillaria* spp. or *Melosira* sp., serve as an effective defense against *P. paradoxa*.

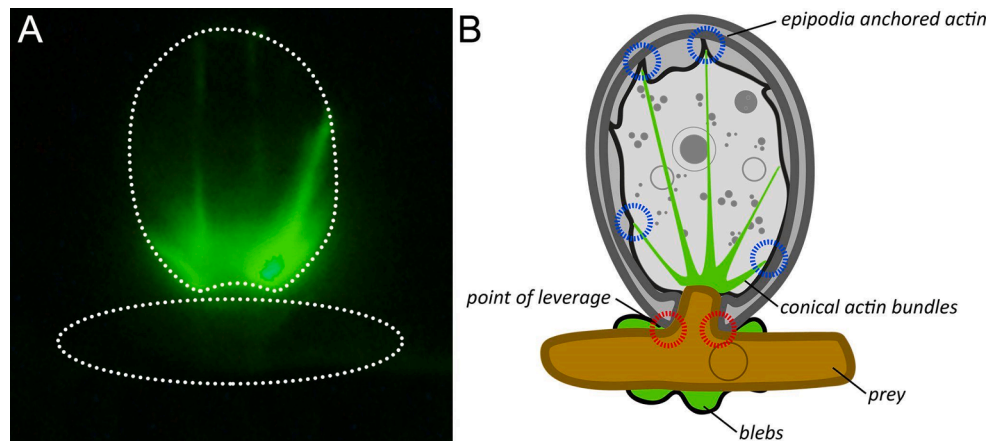
In summary, arcellinidan shell's rigidity, the shell walls around the aperture, and anchoring points for actin collectively seem to contribute to the effective mechanical manipulation of prey, enabling the amoeba to overcome their prey's protective cell walls (Fig. 2). Although there is no evidence yet, it is reasonable to presume that the apertures' shape, size and position contribute to the specificity and performance of this predation mechanism.

### 3. A potential change in perspective

In the following section, we will outline several ideas, hypotheses, and questions resulting from considering the shell of Arcellinida as a weapon that merit exploration in future investigations.

#### 3.1. Can shell morphological traits be explained by specific prey spectra?

The shells of Arcellinida exhibit diverse shapes and sizes. Their shapes range from spherical through ovoid to bottle-shaped and more specialized forms (Fig. 1). Their sizes range from around 10  $\mu\text{m}$  to several hundred  $\mu\text{m}$ . The apertures can be slit-like, circular, or star-shaped. This variation in shell morphology proved to be crucial for ecological investigation, and thus protistologists wondered for centuries



**Fig. 2.** Arcellinida utilize their cytoskeleton and shell to break their prey. (A) Actin staining of *Cryptodiffugia oviformis*. Conical tubules of actin are seen inside the cell body, reaching from the contact zone with the prey to the epipodia. (B) Schematic overview of an Arcellinida amoeba feeding on its prey. Red circles indicate the point of leverage where the prey will be fractured if enough force is exerted. Blue circles indicate the anchor points of the green actin.

how these various shapes and sizes evolved (Lahr et al., 2019). Considering the shell as purely defensive, research so far failed to explain the large observed morphological diversity in arcellinidan shells. We predict that the morphological diversity in arcellinidan shells can be better explained by their prey spectra and the accompanying differences in the deployed predation mechanism. Detailed investigation may reveal that the lobed apertures of *Pseudonebela* spp. or *Netzelia* spp. could concentrate actin-derived force, facilitating the fracture of their green algal prey's cell walls, while collar thickening in *Cryptodiffugia* spp., *Netzelia* spp., *Heleopera* spp., or *Nebela* spp. might reinforce the aperture against the forces exerted during prey manipulation (Figs. 1, 2). A potential secondary loss of a thickened collar, a thick shell wall, or the ovoid shell shape as a result of increased bacterivory and reduced eukaryvory might explain reduced shell morphologies in taxa such as *Galeripora* spp. or *Microchlamys* spp. Investigating the involvement of actin in the feeding mechanisms of various Arcellinida taxa can substantiate or refute these hypotheses.

### 3.2. How strong do prey communities determine the community composition of testate amoebae and vice versa?

The high variation in shell morphology enabled palaeoecologists and ecologists to link the composition of microfossils to changes in the environment (Marcisz et al., 2020). Under the perspective that shell morphology is linked to defense, it is reasonable to presume that mainly abiotic and predation pressures affect the community composition of testate amoebae. Accordingly, this is how these branches of palaeoecology and ecology developed. The community composition of testate amoebae was used to determine changes in the environment, for instance, drought. Nonetheless, abiotic variables merely explain a fraction of the found variation in community data (Marcisz et al., 2020). We predict that biotic variables affect the testate amoeba community profoundly. We therefore propose to investigate the full spectrum of potential prey, perhaps starting with typically cell wall bearing taxa such as algae and fungi, to explore how it affects the community composition of testate amoebae. *Vice versa*, once biotic effects are further explored, it might be possible to determine past prey communities by determining the community composition of testate amoebae in aged samples.

### 3.3. What is the function of non-arcellinid shells?

In testate amoeba research, shell-bearing amoebae of the Cercozoa (mainly Euglyphida), Stramenopiles (mainly Amphitrematidae), and Amoebozoa (mainly Arcellinida) are often investigated together. Indeed, all three taxa evolved shells that are morphologically similar, but still specific enough to be differentiated. Since there is no evidence yet for cercozoan or stramenopilan shell-bearing amoebae to rupture their prey, like we presented for the Arcellinida, the function of stramenopilan and cercozoan shells might be different. We predict that the evolutionary pressures leading to the diversification of cercozoan and stramenopilan shell bearing amoebae result from defense and protection purposes. A consequence of this thought is that the explained variation in (palaeo)ecological data on the Cercozoa and Stramenopiles should substantially differ from the variation in the Amoebozoa. Biotic factors, i.e. the community composition of large prey, should affect amoebozoan shell-bearing amoebae more, while the community composition of shell-bearing amoebae in general should be equally affected by potential predators.

### 3.4. Notes on shell evolution

Shell-bearing protists lived and diversified in the shallow oceans during the late Tonian era (800–700 Ma (mega annum)) of which some can be recovered as microfossils (Morais et al., 2021). These organisms called VSM (for Vase-shaped Microfossils) pre-dated animals and

multicellular organisms in general (Lahr et al., 2019). The resemblance between VSM and the extant Arcellinida suggests that these fossils could actually belong to that group (Lahr et al., 2019). Another hypothesis would be that the Arcellinida did not originate in shallow oceans, but in continental systems, when freshwater ecosystems became widespread at the end of the Cryogenian, after Snowball Earth glaciations (Useros et al., 2023). Whether in the oceans or on continental systems, potential prey organisms with thick cell walls were present, such as the predominantly marine *Bangiomorpha*, the early limnic Zygnematophyceae, or continental fungi (Butterfield, 2000; Loran et al., 2019; Žárský et al., 2022). Some fossil morphotypes, such as *Trigonocyrrillium*, had complex apertures resembling the extant *Trigonopyxis* (Porter et al., 2003), which seems to be associated with fungal hyphae (Vohník et al., 2009) and could also feed on different filamentous eukaryotes protected by cell walls analogous to fungi.

Given that Amoebozoa and thus Arcellinida are considered to be one of the oldest protistan taxa even basic eukaryvorous predation through mechanical manipulation of large or cell-wall-bearing prey likely offered substantial evolutionary advantages. Ancestral Arcellinida would prey on cell wall-bearing preys such as algae and early fungi, using their tests as a tool, and would therefore be apex predators in these ancient, animal-lacking ecosystems. If the shell did not evolve from a protective cell coating, as often assumed, but as an optimization of initially poor eukaryvory, early shell precursors might have had distinct, simpler, shapes from modern ones. An internal, simple, slightly-bent rod, may have provided an anchor point for actin and a rigid breaking point on the other end. Subsequently, this internal structure may have evolved into a more spherical and eventually external shell. While highly speculative, this idea merits further exploration.

## 4. Conclusion

In summary, we conclude that the shell is crucial for eukaryvory in the Arcellinida. Acknowledging this change in perspective opens up numerous exciting avenues substantially changing the testate amoeba research.

### CRediT authorship contribution statement

**Kenneth Dumack:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Enrique Lara:** Writing – review & editing. **Clément Duckert:** Writing – review & editing. **Elizaveta Ermolaeva:** Writing – review & editing. **Ferry Siemensma:** Visualization, Writing – review & editing. **David Singer:** Writing – review & editing. **Valentyna Krashevskaya:** Writing – review & editing. **Mariusz Lamentowicz:** Writing – review & editing. **Edward A.D. Mitchell:** Writing – review & editing.

### 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.

### Data availability

No data was used for the research described in the article.

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