

## Masters of miniaturization: Convergent evolution among interstitial eukaryotes

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Marine interstitial environments are teeming with an extraordinary diversity of coexisting microeukaryotic lineages collectively called “meiofauna.” Interstitial habitats are broadly distributed across the planet, and the complex physical features of these environments have persisted, much like they exist today, throughout the history of eukaryotes, if not longer. Although our general understanding of the biological diversity in these environments is relatively poor, compelling examples of developmental heterochrony (*e.g.*, pedomorphosis) and convergent evolution appear to be widespread among meiofauna. Therefore, an improved understanding of meiofaunal biodiversity is expected to provide some of the deepest insights into the following themes in evolutionary biology: (i) the origins of novel body plans, (ii) macroevolutionary patterns of miniaturization, and (iii) the intersection of evolution and community assembly – *e.g.*, “community convergence” involving distantly related lineages that span the tree of eukaryotes.

### Keywords:

■ body plan; community; convergence; meiofauna; miniaturization

### Miniaturization, meiofauna, and evolution

Marine sediments form the most widespread habitats on Earth, and a multitude of single-celled eukaryotes (“protists”) and relatively tiny metazoans, collectively known as meiofauna

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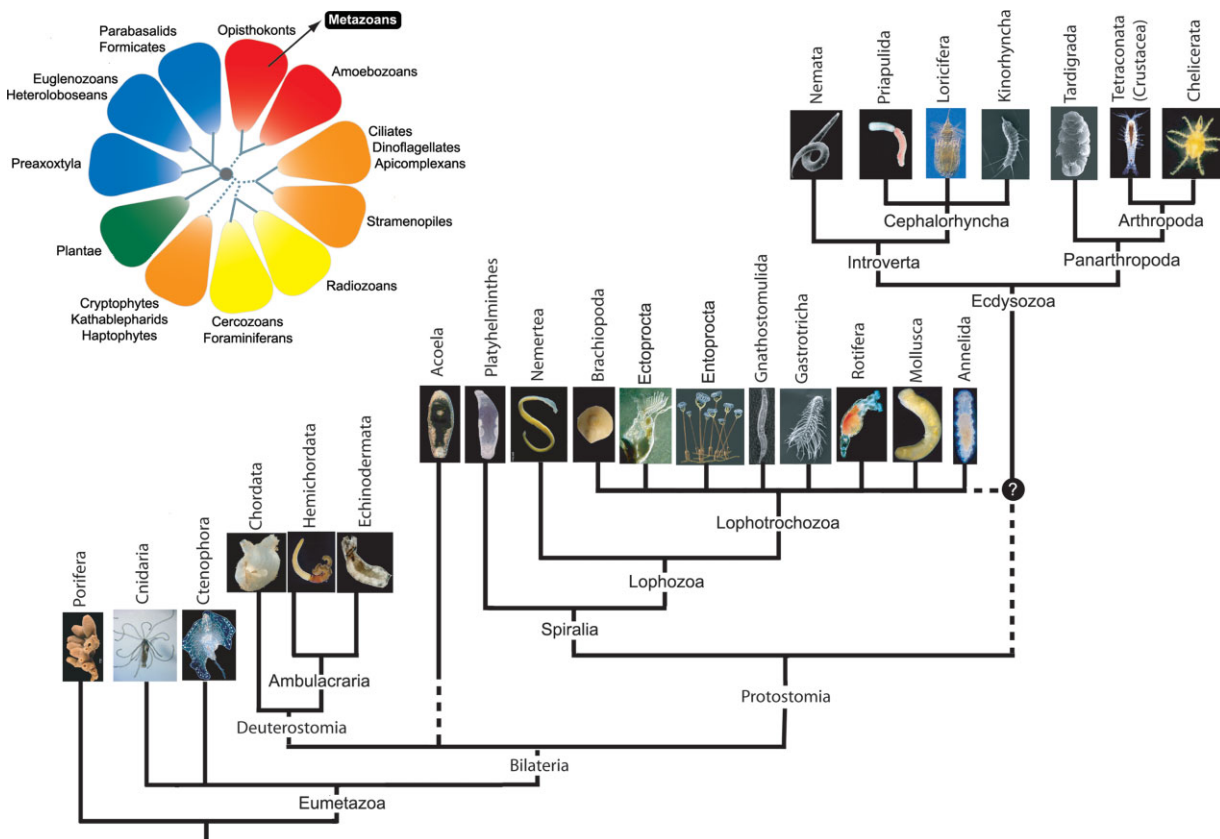
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(*i.e.*, organisms between 60  $\mu\text{m}$  and 2 mm in size), have diversified within the spaces between individual grains of sand. Dozens of very distantly related eukaryotic lineages can simultaneously inhabit a teaspoon of sand, sometimes possessing convergent morphologies and representing a variety of feeding modes, including but not limited to predators, grazers, suspension feeders and bacterivores [1–3]. Miniaturization, or the evolution of extremely small body size,[4] is the rule rather than the exception among meiofauna; nearly all of the 38 major lineages of metazoans have meiofaunal representatives (Fig. 1), many of which were only discovered recently (*e.g.*, gnathostomulids, loriciferans, meiofaunal entoprocts, and tunicates described in the mid to late 20th century[2] and micrognathozoans described in this century[5]). Some meiofaunal lineages are ancestrally miniature, often comprising only meiofaunal species (*e.g.*, gastrotrichs, gnathostomulids, kinorhynch, loriciferans), and others have secondarily evolved miniature morphologies from macroscopic ancestors (*e.g.*, annelids, arthropods, molluscs, nemerteans, and priapulids). All of these lineages have independently exploited the substantial prokaryotic and microeukaryotic biomass present in interstitial environments. The fact that miniaturized lineages are well represented in both the deep and shallow nodes of the tree of eukaryotic life suggests that the investigation of meiofauna will contribute to our understanding of important themes of evolution. Meiofaunal lineages have received insufficient study relative to their macrofaunal counterparts, particularly from a phylogenetic perspective, and therefore many patterns and mechanisms of miniaturization in meiofauna remain largely unexplored.

Miniaturization is best documented in vertebrates [4] such as fish [6, 7], frogs [8–10], and salamanders [11, 12] (but also see examples in terrestrial arthropods [13, 14]), where it illustrates evolutionary themes such as the origin of morphological novelty [15], heterochrony [7], and convergence [6]. Here, we suggest that meiofauna might provide some of the deepest insights into these fundamental evolutionary processes, and, because these miniature species are ubiquitous throughout the metazoan tree of life, we suggest that meiofauna are uniquely poised to shed light on the origins and diversification of animals.



**Figure 1.** An illustration showing the phylogenetic distribution of meiofaunal lineages within the Metazoa; inset: the phylogenetic position of metazoans within the context of the tree of eukaryotes. Metazoans are one branch within the Opisthokonta, which is part of a supergroup known as “unikonta” (shown in red; blue, excavates; green, green algae and red algae; yellow, rhizarians; orange, “chromalveolates”).[78] Photos illustrate meiofaunal representatives of each phylum, where appropriate. The metazoan tree topology is a

composite based on accepted relationships based on comparative morphology, and the most recent molecular phylogenetic evidence.[77, 79] Note that annelids comprise several groups, some of which were once considered phyla (e.g., sipunculans).[80] Also note that the relationships among some phyla, e.g., loriciferans and some other blastocoelomate taxa, are unresolved, either because they were not included in any recent phylogenetic analyses, or because branch statistical support is weak.

The common theme between the current agenda in meiofaunal research and the first studies of meiofauna is species discovery. The earliest meiofaunal work of the mid 19th to early 20th centuries[2, 16] focused on exploratory sampling methods and individual species taxonomy; later research shifted toward more detailed ecological approaches and more comprehensive taxonomic schemes.[17, 18] With the exception of relatively recent high-profile discoveries of new lineages, such as the Loricifera[19], the preponderance of meiofaunal research has involved isolated taxonomic studies and the use of relative abundances of common meiofaunal groups for ecological assessments, such as measuring nematodes and harpacticoid copepods as indicators of coastal pollution.[20, 21] Nonetheless, it is likely that most meiofaunal species still await discovery and form cryptic species complexes within geographically restricted areas.[2, 22–25] Widespread convergence and extreme morphological reduction among meiofaunal species present a massive challenge for accurate phylogenetic reconstruction, because morphological and reproductive evidence on their own can mislead inferences of meiofaunal interrelationships and

evolutionary history.[24, 25] Molecular phylogenetic approaches provide great potential to circumvent these issues and help avoid the circularity intrinsic to using the same morphological and reproductive characters one is trying to understand.[26]

We anticipate that improved understanding of meiofaunal diversity will provide important insights into (i) the origin and early evolutionary stages of metazoans, (ii) the origins of miniaturization through heterochronic developmental mechanisms, and (iii) the patterns and causes of convergent evolution at both organismal and community levels. Each of these lines of research will be discussed in turn, helping to provide a framework for future research on the evolutionary history of meiofauna.

## Origins of meiofauna

Meiofauna are usually concentrated in the upper 10–20 mm of different grain-sized sediments, ranging from the continental shelves to the deep sea, and including biogenic

sands.[2, 27–29] Although biogenic sands have only been around as long as their source, namely several groups of skeletonized invertebrates (*e.g.*, corals and molluscs), coralline algae and unicellular eukaryotes (*e.g.*, foraminiferans, coccolithophorids, and diatoms), the physical properties of rock-based sediments have likely persisted relatively unchanged for most of Earth's history. Once sufficient oxygen was available in the very upper reaches of sand (*e.g.*, in oxygen oases of shallow seas[30]), meiofaunal animals could thrive in these habitats and exploit the rich source of bacterial and microeukaryotic prey that was undoubtedly already present within the sediments.[31] This set of circumstances suggests that meiofaunal species were involved in the earliest stages of the diversification of metazoan life.

The microscopic and soft bodies of most meiofauna severely limit the representation of these lineages in the fossil record. However, a deep evolutionary origin of meiofauna in marine benthic habitats is suggested by both molecular phylogenetic evidence[32–34] and paleontological evidence.[35, 36] For instance, some lower Ediacaran microfossils (>100  $\mu\text{m}$ ) from anoxic sediments have comparable ultrastructure and size to the resting cyst stages of extant metazoans, including crustaceans.[37] Moreover, deeply diverging lineages in molecular phylogenetic analyses, such as acelomorphs and placozoans, include mainly meiofaunal representatives and were likely integral players in the early diversification of animals.[33, 38–40] The widespread representation of meiofauna throughout the tree of metazoans (Fig. 1) and the fossil evidence mentioned above support the hypothesis that animals with meiofaunal lifestyles were thriving long before the Cambrian.[32, 41, 42] Therefore, it is possible that the backbone of the animal phylogenetic tree consists mainly of ancestral lineages with diminutive body sizes, and that relatively large body sizes evolved independently within several of the major animal phyla (*e.g.*, the Cnidaria, Nemertea, Mollusca, Annelida, Arthropoda, and Chordata), potentially contributing to the apparent “explosion” of novel body plans during the Cambrian period.[43]

### Mechanisms of miniaturization: Pedomorphosis and reduction

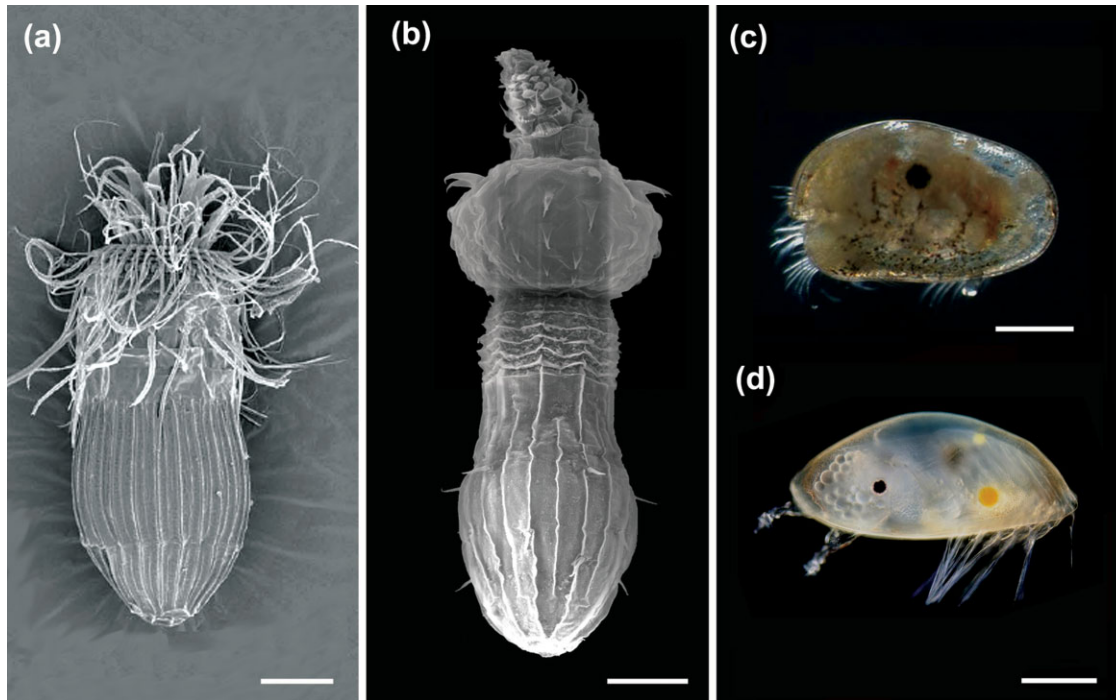
Marine invertebrates in general have diverse early developmental stages and subsequent larval forms,[36, 44] which are likely independently evolved.[45] Some of this diversity can be explained by the reduction or loss of ancestral characters, which is a reoccurring theme in the evolution of miniaturization in marine meiofauna. Coeloms, for instance, are important body cavities in relatively large infaunal animals that rely on robust hydrostatic skeletons for locomotion (*e.g.*, polychaetes, sipunculids, echiurans, and holothuroideans). Coeloms are among the first features to vanish during the evolution of miniaturized lineages from coelomate ancestors (*e.g.*, minute lineages of annelids and possibly gastrotrichs and rotifers), a consequence of the different body size and locomotory requirements of interstitial environments (*e.g.*, the size of meiofauna precludes the need for burrowing and

instead most rely on ciliary gliding between sand grains). Other meiofaunal lineages, by contrast, evolved directly from acoelomate ancestors or retained an acoelomate larval condition (*e.g.*, gnathostomulids and acoels).

The origins of several miniaturized lineages probably involved the retention of features found in the larval stages of closely related lineages, a phenomenon called “pedomorphosis” (Fig. 2). In these instances, development is truncated so that meiofaunal lineages appear to complete their sexual life cycles without metamorphosing into a distinct adult stage that differs significantly from the larval stage in size, morphology, and behavior.[2] In other words, many meiofaunal lineages may have acquired direct developmental strategies through the loss of (larger) adult stages present in their ancestors and close relatives.

The two examples shown in Fig. 2 illustrate some of the inherent challenges associated with the reconstruction of pedomorphic change and its discrimination from convergent evolution. Figures 2a and b show that several features are shared between the larvae of priapulids and the adults of loriciferans, suggesting that loriciferans evolved through pedomorphosis of an ancestral priapulid-like larval stage.[46–49] Such pedomorphic change might occur through progenesis, the acceleration in the maturation of gonads relative to the duration of somatic development, or neoteny, the retardation of somatic development relative to gonadal maturation. In practice, it is difficult to distinguish between progenesis and neoteny. This requires comparative knowledge of developmental timing in several closely related lineages, which is particularly problematic in meiofauna because so few meiofaunal groups have been subjected to detailed phylogenetic or ontogenetic investigation.[50–52] Figures 2c and d illustrate the close resemblance between adult meiofaunal (and planktonic) ostracods and the Cypris larval stages of barnacles. Although the close correspondence between larval and adult morphologies in these different lineages is suggestive of pedomorphosis, current hypotheses of crustacean phylogeny based on molecular data do not reinforce this inference.[53] Therefore, the similarities between ostracods and Cypris larval stages may instead be indicative of convergent evolution between the larval stage in one lineage and the adult stage in a distantly related lineage. If so, then this example provides an excellent opportunity for exploring the selective pressures and developmental underpinnings associated with the independent evolution of similar traits (*e.g.*, dorsally hinged carapace) in two entirely different life history stages.

Ultimately, different genetic and developmental architectures may underlie superficially similar adult morphologies.[54, 55] Homology statements about pedomorphic characters can be complicated by cryptic convergent evolution, which underscores the importance of building robust molecular phylogenetic frameworks for the meiofaunal lineages of interest.[56, 57] Acoels, *e.g.*, were only recently hypothesized to be separate and distinct from the Platyhelminthes[38, 39, 58] because these lineages share a large suite of ancestral bilaterian features. Overall, convergent evolution and the highly reduced body plans of meiofauna make the discovery, delineation, and identification of these lineages difficult.



**Figure 2.** Two examples suggesting that the miniaturization of some meiofaunal lineages evolved via pedomorphosis – the retention of larval features in the sexually mature adult form. Adult loriferans (A) share several characters with the larval stages of priapulids (B). Adult ostracods (image courtesy of A. Anker) (C) share several features

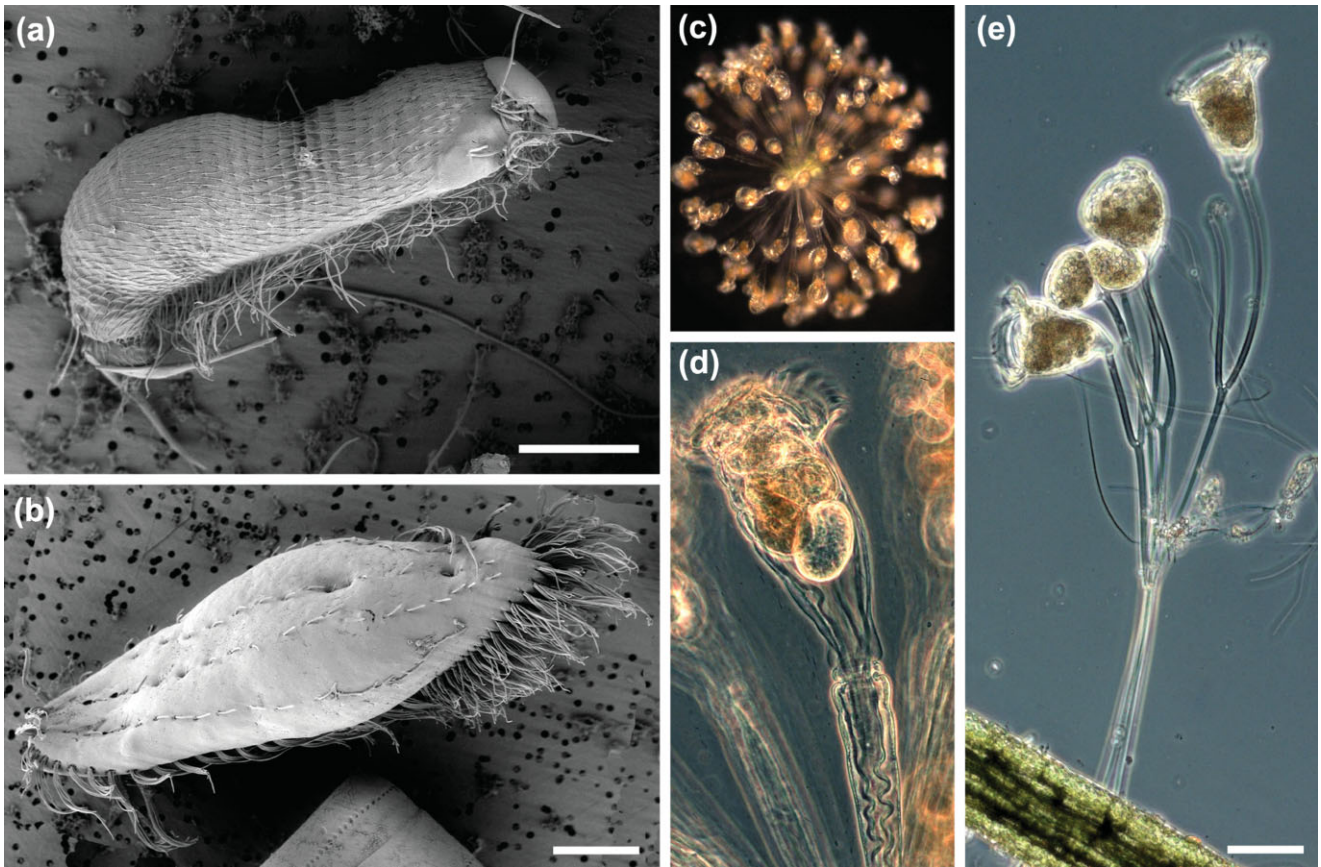
with the Cypris larval stages of barnacles (image courtesy of W. van Egmond/Visuals Unlimited) (D). Scale bars: 30  $\mu\text{m}$  (A); 100  $\mu\text{m}$  (B); 100  $\mu\text{m}$  (C, D). Image (A) adapted from Heiner and Kristensen (courtesy of Elsevier LTD); [81] image (B) adapted from Todaro and Shirley (courtesy of Taylor and Francis Group). [82]

## Convergent evolution in meiofauna

Adaptations for interstitial modes of life not only include miniature body forms but also features like highly differentiated cilia, vermiform shape, adhesive structures, and direct development. Perhaps the most widespread feature of meiofauna is a tiny vermiform body plan (Fig. 1), and this can make the initial assignment of species to major eukaryotic phyla challenging for specialists and non-specialists alike. Understanding the order of evolution of frequently convergent morphological characters also remains problematic, [2] and underscores the importance of independent datasets in exploring morphological evolution. [26] In some groups, it has been suggested that miniaturization arose multiple times independently, even within clades of closely related species (e.g., nerillid polychaetes [59]), although this remains to be tested using independent character sets (e.g., by mapping morphological characters onto molecular-derived phylogenetic trees). Convergent evolution in general, appears to be common in meiofauna, [2] and species representing very distantly related groups of eukaryotes also possess similar features related to the interstitial milieu. Some meiofaunal animals (supergroup “opisthokonts”) and ciliates (supergroup “chromalveolates”), for instance, provide some of the most compelling examples of ultimate convergence involving multicellular analogs to unicellular systems (and *vice versa*) [60, 61] (Fig. 3). In other words, these distantly related taxa possess analogous characters comprising few, if any, homologous subcomponents.

For example, some gastrotrichs (multicellular animals) and ciliates (unicellular alveolates) have very similar overall body plans associated with microphagous (sometimes predatory) modes of life: both are about the same size, have differentiated anteroposterior axes, have dense bands of cilia on the ventral surface, and have longitudinal rows of short projections (cuticular spicules in the former and cilia in the latter) on the dorsal surface (Fig. 3). The ventral bands of cilia are locomotory, and the dorsal projections, presumably, have defensive, adhesive and sensory functions. Moreover, very distantly related lineages of sessile microeukaryotes can be almost indistinguishable from one another. Some colonial rotifers (multicellular animals) and colonial ciliates (unicellular alveolates) have very similar overall body plans associated with suspension feeding: both are about the same size, have stalks capable of rapid contraction, and have radially arranged oral cilia that both generate highly coordinated water currents and discriminate between different kinds of particles suspended in the water column (Fig. 3).

Interestingly, meiofaunal animals, like gastrotrichs and rotifers, reach some of the smallest sizes known for multicellular organisms, and meiofaunal ciliates reach some of the largest sizes known for unicellular organisms. These lineages have essentially converged on the same size, by starting from opposite ends of the organismal size spectrum; some meiofaunal animals appear to have reached a critical minimum size, while some ciliates (and many other groups of eukaryotes) appear to have reached a critical maximum size within the confines of a single cell membrane. Meiofaunal animals



**Figure 3.** Two specific examples of convergent evolution between (multicellular) animals and (single-celled) ciliates living in freshwater interstitial environments. **A:** Scanning electron micrograph of a gastrotrich showing a dense ventral band of cilia and rows of cuticular spicules on the dorsal surface. **B:** Scanning electron micrograph of a ciliate showing a dense ventral band of cilia and sparse rows of short

cilia on the dorsal surface. **C, D:** Light micrographs showing the colonial, stalked rotifer *Conochilus*. **E:** Light micrograph showing the colonial, stalked ciliate *Epistylis*. Scale bars = 10  $\mu\text{m}$ . **C–E** reproduced under license from microscope.mbl.edu and with permission from D. Patterson.

at their “critical minimum” (e.g., nematodes, tardigrades) sometimes employ eutely, wherein growth occurs through increase in cell size rather than cell number. Eutely functionally renders these animals unicellular (and complex transport mechanisms obsolete), by reducing the number of partitions within the organism’s body, which facilitates efficient transport of oxygen, nutrients and metabolic waste. “Large” meiofaunal unicellular eukaryotes on the other hand, can elongate and flatten to maintain optimal diffusion distances (e.g., karyorelictid ciliates). Investigation of constraint in nutrient transport at the unicellular-multicellular organism boundary might provide important clues on the emergence and diversification of metazoan life.[62]

### A hierarchical view of community convergence

Although “marine sand” conjures up an image of a uniform underwater dune, devoid of life, meiofauna occur throughout

the ocean (intertidal to deep sea), sometimes in great abundance, and often dozens of distantly related taxa inhabit the same small area. This is complicated by patchiness among meiofaunal communities, where species richness and abundance of different species in different environments can fluctuate greatly in space and time.[63, 64] However, growing evidence suggests that the number of microbial species involved and the degree of species’ geographic restriction might be much greater than previously assumed[23–25, 65, 66] and this has opened up new research on microbial community assembly and diversity.[67] Meiofaunal communities in different localities seem to have comparable niche structures, but the composition of lineages in these separate communities can be very different.[2, 68] This observation is perhaps best explained with the concept of community convergence,[69, 70] wherein corresponding sets of different species fulfill similar roles in geographically separate localities. In terrestrial environments, the corresponding species tend to be closely related to one another (e.g., different species of Caribbean *Anolis* lizard[71] or Hawaiian *Tetragnatha* spider[72]). Community convergence in interstitial

environments, by contrast, seems to differ in one significant respect: the corresponding species in different interstitial environments can be very distantly related to one another. For example, the niche occupied by predatory gastrotrichs (opisthokonts) in one interstitial habitat can be occupied by predatory ciliates (alveolates) in another similar, but geographically distinct, interstitial habitat. Very distantly related lineages within these geographically distinct interstitial habitats can look remarkably similar to one another through the convergent evolution of similar adaptations (Fig. 3), just as closely related species representing the same “ecomorph” on different islands do (e.g., *Tetragratha perreirai* and *T. kamakou* representing the maroon ecomorph on the islands of Oahu and Maui, respectively [72]). Some examples of convergent evolution between distantly related lineages living in the same habitat might have first arisen in association with community convergence, followed by the subsequent migration of some lineages to a geographically distinct but corresponding habitat.

It is unclear whether convergent communities involving very distantly related lineages are exclusive to interstitial

environments or are also found in other hyper-diverse ecosystems, like coral reefs or tropical rainforests. The presence of many very distantly related lineages occurring at one place, at one time, in marine sand might reflect the fact that interstitial environments and representatives of their corresponding lineages have persisted longer on Earth than scleractinian-based coral reefs (mid-Triassic origins) or tropical rainforests (post-Silurian origins). [35] Nonetheless, the most obvious limitation for understanding the assembly and evolution of meiofaunal communities is our ignorance about the overall diversity, basic ecology (e.g., feeding preferences), and biogeography of many meiofaunal groups.

## Conclusions

Interstitial environments are some of the most ancient and persistent ecosystems on Earth, and microbial life has dominated the niches contained therein for eons. Ancestral lineages that could not compete in this microbial world either vanished forever or were able to successfully exploit new

## Glossary

**Body plan:** an assemblage of morphological features shared among many members of a phylum-level group. [35]

**Cilia:** thread-like locomotory organelles containing a highly conserved (9 + 2) arrangement of microtubules; homologous with flagella, but generally shorter and more numerous.

**Coelom:** an internal body cavity positioned between the gut and the outer body wall musculature that is lined with derivatives of the embryonic mesoderm.

**Convergent evolution:** independent evolution of similar features from different ancestors, usually from different antecedent features or by different developmental pathways.

**Direct development:** reproductive strategy where offspring are released as miniature adults, rather than going through a (planktonic) larval stage; this life history strategy also usually involves internal fertilization.

**Eutely:** species-specific constancy of cell numbers or nuclei; growth occurs through increased cell size rather than increased cell number.

**Heterochrony:** changes in the rate or timing of developmental events over evolutionary time.

**Interstitial:** the habitat in between sand grains and on the facets of individual sand grains.

**Larva:** a small free-living or parasitic developmental stage in the life history of many animals that is significantly different in morphology and behavior to the sexually mature adult form.

**Meiofauna:** organisms that fit between 60 and 1000  $\mu\text{m}$  mesh sizes. Meiofauna are also generally restricted to the interstitial environment throughout their life cycle.

**Microeukaryotes:** lineages of eukaryotes that require a microscope to observe; although some are multicellular, most consist of a single eukaryotic cell (syn., “protists”). Refers to a polyphyletic assemblage of lineages that comprises most of the tree of eukaryotes and spans all of the supergroups.

**Miniaturization:** extreme reduction in body size (e.g., relative to other members of their respective clade; note that some taxa do not have any obvious larger bodied ancestors).

**Neoteny:** a retardation in somatic development that results in the retention of larval or juvenile traits in the sexually mature adult form.

**Pedomorphosis:** the retention of larval or juvenile traits in the sexually mature adult form.

**Parallel evolution:** the evolution of similar or identical features independently in related lineages, usually based on modifications of the same (homologous) developmental pathways.

**Polychaete:** a species-rich group of marine annelid worms, usually with elaborate parapodia and chaetae. It is the largest (paraphyletic) group within the Annelida, with over 10 000 species.

**Progenesis:** an acceleration in the maturation of gonads and gamete production relative to the duration of somatic development, resulting in the retention of larval characters in the sexually mature adult form.

**Supergroup:** a level of taxonomic organization that is conceptually above a “kingdom” in traditional nomenclature, and representing phylogenetic cohesion of large taxonomic subgroups. For example, animals are a single branch within the supergroup “opisthokonts,” which includes roughly fifteen other major branches, including the Fungi.

**Vermiform:** worm-shaped (*i.e.*, soft-bodied and substantially longer than wide).

opportunities for resources by becoming larger, mainly through the independent origins of multicellularity (e.g., brown algae, red algae, green algae/land plants, animals, and fungi). Some lineages of animals with relatively large ancestors were able to successfully re-exploit the abundant resources (e.g., prokaryotic and microeukaryotic biomass) in interstitial environments through miniaturization. Miniature animals living within the spaces between sand grains – i.e., meiofauna – evolved several times independently, but some of these lineages might also reflect a persistent ancestral state that forms the backbone of the overall phylogenetic tree of animals. Shared features between certain lineages of meiofauna and the larval stages of their closest relatives suggest a central role for pedomorphosis in the evolution of meiofaunal animals. Several miniaturized lineages of animals also have acquired adaptations that are similar to those found in very distantly related lineages of microeukaryotes living in the same environments, such as some ciliates (i.e., divergence times over 1 billion years ago). These examples of ultimate convergence might reflect broader ecological patterns that are exclusive to interstitial environments, such as community convergence involving distantly related lineages that occupy corresponding niches in different localities.

Future research on meiofauna should utilize several different molecular phylogenetic approaches to explore new habitats, estimate overall biodiversity, and characterize novel species (e.g., DNA barcoding from individually isolated animals, environmental PCR clone libraries, and 454 pyrosequencing assemblies [73–75]). 454 sequencing, e.g., can be used for metagenomics to characterize the genomic content of species-rich communities, whose component species are often not easily culturable [73]. Additional model systems for evolutionary developmental biology are also necessary for understanding enigmatic lineages, many of which are meiofaunal. We have comparatively larger developmental and molecular datasets for the enigmatic *Trichoplax* [76] than we do for meiofaunal metazoan phyla, which might be just as important in elucidating early metazoan evolution. Moreover, increasing taxon sampling within meiofaunal lineages (e.g., gnathostomulids) is important for resolving deeper nodes in metazoan phylogenies. [77] Although a great deal of uncertainty remains in our understanding of meiofaunal biodiversity, ecology, and evolutionary history, it is clear that this area of research remains among the most challenging, the most neglected, and potentially the most enlightening frontiers of discovery in biology.

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