Locomotory Palp Function in Interstitial Annelids

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Abstract

The interstitial environment of marine sediments is a complex network of voids and pores that is inhabited by a diverse and abundant fauna. Animals living within these interstitial spaces show widespread functional adaptations to this environment and have developed many strategies for moving and navigating through small spaces. Interstitial annelids demonstrate a remarkable level of morphologic diversity, and some possess dexterous, filiform palps (tentacle-like appendages common across Annelida). The function(s) of these palps in interstitial spaces has not been closely examined, and we propose that they serve a sensory role in the navigation of interstitial spaces. We investigated the locomotory function of long, dexterous palps in three families of interstitial annelids to determine their role in interstitial navigation. We observed two species of protodrilids (Protodrilidae), Pharyngocirrus eroticus *(Saccocirridae), and* Protodorvillea recuperata *(Dorvilleidae), as they moved through two transparent sand analogs: cyolite and glass beads. All four species of annelids consistently used their palps to probe the interstitial environment while locomoting, and the distance probed with their palps was greater than the distance traveled with their heads, indicating a sensory form of palp-based navigation. The functionality of palps as sensory organs in the interstitial environment raises interesting questions about interstitial navigation and how fauna without appendages map their surroundings. The discovery of this previously undocumented function was possible only through the direct observation of interstitial behavior and emphasizes the importance of developing new techniques to study these animals in more natural habitats.*

Introduction

Meiofauna are diverse organisms that live within the interstitial spaces of marine sediments. They include representatives from many extant metazoan lineages (Cerca *et al.*, 2018; Giribet and Edgecombe, 2020; Schmidt-Rhaesa, 2020), and interstitial fauna can be found in marine sediments worldwide. A unifying feature of the interstitial environment is that the space between individual sediment grains is limited, so successful exploitation of interstitial spaces requires that an organism not only become small enough to fit but also develop appropriate strategies for locomotion, reproduction, feeding, and navigation in these confined spaces. Meiofauna have responded to this selective pressure by evolving specialized adaptations such as small size, direct gamete transfer and development, elongation, reduced photoreceptors, and adhesive organs (Giere, 2009). Here we focus on the challenge of navigating the interstitial environment and present new observations that demonstrate how some interstitial annelids have adapted to explore these spaces.

We define navigation in this context as the process of an organism sensing its local environment, evaluating and orienting itself to that environment, and then using that information to plan and follow a route. When this definition is applied to the interstitial realm, an organism must use its sensory equipment to evaluate the surrounding pore space in three dimensions, determine which pores are viable for entry, and, if multiple options exist, make some decision about which pore to enter. In marine sediments, however,

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certain sensory tools may be more useful than others. For example, natural sediments are opaque, light is generally low, and any given field of view is limited by the distribution of void sizes; thus, photoreceptors are of reduced utility. While light cues have been shown to affect the vertical movements and migrations of meiofauna (Palmer, 1984; Higgins and Thiel, 1988; Manley and Shaw, 1997; Buffan-Dubau and Carman, 2000), photoreceptors are not likely to play a crucial role in the lateral navigation of interstitial spaces. This limited utility is demonstrated by the reduction of photoreceptors observed across many phyla of meiofauna (Giere, 2009). Conversely, chemosensing has been shown to be an important aspect of interstitial navigation, with sensory cues from food, oxygen, and sulfide content driving patterns of aggregation and patchiness in marine sediments (Lee *et al.*, 1977; McLachlan *et al.*, 1977; Höckelmann *et al.*, 2004; Giere, 2009). This "long-range" navigation allows interstitial animals to locate resources and avoid dangers that are not in their immediate vicinity, and chemosensing has been shown to be a crucial ecological tool at most biological scales in the marine environment (Hay, 2009). However, chemosensing can be used only when navigating in relation to a chemical source, and it may not be a useful tool for navigation without a specific chemical source (*e.g.*, rapidly escaping a predator). Moreover, detection of a chemical cue in the interstitial environment does not necessarily indicate a viable path to its source. A given cue may have permeated through a pore that is too small for the detecting organism to move through; therefore, successfully locating the source of any given cue likely requires the use of multiple sensory modalities. Given the three dimensionality and constrained physical structure of the interstitial environment, mechanosensing is likely also important in interstitial navigation. In addition to the demonstrated ability of interstitial animals to detect and respond to overlying water movement (Boaden, 1968; Palmer, 1984; Palmer and Molloy, 1986; Armonies, 1988), mechanosensing allows individuals to evaluate the sediment grains and pores in their immediate vicinity and determine the viability of potential paths through the interstitial environment. Effective "short-range" navigation such as this is likely crucial to the successful survival of interstitial animals. Some interstitial annelids possess dexterous palps (tentacle-like appendages; Fig. 1) that appear well suited to this task.

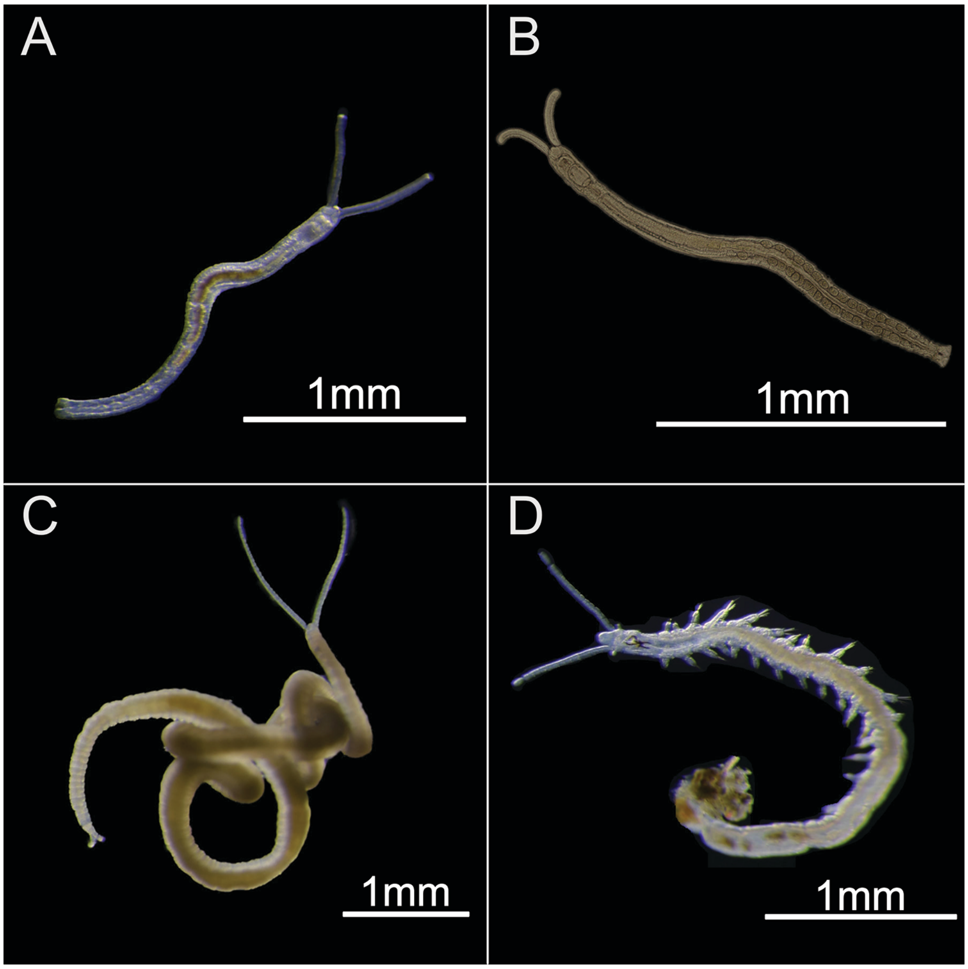

Figure 1. Photos of each species studied. (A) Protodrilidae collected from Washington (80×). (B) Protodrilidae collected from Alabama (100×). (C) *Pharyngocirrus eroticus* (20×). (D) *Protodorvillea recuperata* (64×).

Palps are widespread across Annelida (though have been lost in some lineages) and usually appear as two appendages protruding from somewhere near the anterior end of the worm. They are considered homologous and an important physical trait in the ground pattern of the annelid body plan (Orrhage, 2001; Orrhage and Müller, 2005; Struck, 2011; Weigert *et al.*, 2014; Parry *et al.*, 2016; Chen *et al.*, 2020). Palps are highly adaptable sensory appendages whose early origin has allowed for extensive morphological and functional variation across Annelida (Rouse *et al.*, 2022). Palps are traditionally associated with either sensory or feeding functions, depending on whether they are tapering ventral palps or grooved ciliated palps, respectively (Rouse *et al.*, 2022). This distinction is more illustrative of a worm's ability to collect food with its palps than of its use of palps as a sensory appendage. To effectively capture, retain, and transport a food particle to the mouth, feeding palps must be effective sensors as well, and annelids have been documented using their palps as both sensory and feeding appendages (Levin, 1981; Shimeta and Koehl, 1997; Ferner and Jumars, 1999). The anterior positioning, functional plasticity, and utility as sensory appendages of annelid palps make them a compelling potential tool for navigating the pore spaces of the interstitial environment. Despite their early origin and potential utility in navigation, however, long (relative to segment width), dexterous, filiform palps are present in only some lineages of interstitial annelids (*e.g.*, members of the clade Protodriliformia, some dorvilleids and sigalionids) and are completely absent in many others (see Worsaae *et al.*, 2021). The loss of appendages in interstitial fauna is generally considered an adaptive response to the physically restrictive interstitial environment (Swedmark, 1964; Giere, 2009). Thus, the continued presence of dexterous palps in some lineages raises interesting questions about their potential ecological function. Investigating the function of these palps is challenging, as it requires direct behavioral observations of their use in small interstitial spaces. Here we examine the locomotory function of morphologically similar palps in three different families of interstitial annelids: two species of Protodrilidae (one from the Pacific coast, Washington, and one from the Gulf of Mexico, Alabama), *Pharyngocirrus eroticus* (Saccocirridae), and *Protodorvillea recuperata* (Dorvilleidae). We hypothesize that a primary function of long, dexterous palps in interstitial annelids is mechanosensory and that these palps are used during locomotion to navigate and explore interstitial spaces.

The four species we observed represent two different clades of annelids, Protodriliformia and Eunicida, that are grouped within the larger clade Errantia (Struck *et al.*, 2015). The two species of protodrilids and *P. eroticus* belong to Protodriliformia, while *P. recuperata* belongs to Eunicida. All members of Protodriliformia inhabit the interstitial environment, which they likely entered through stepwise miniaturization (Weigert *et al.*, 2014; Struck *et al.*, 2015). *Protodorvillea recuperata* (Dorvilleidae) belongs to Eunicida, a diverse clade of annelids that contains predominantly macrofaunal worms, but some families, such as Dorvilleidae, include interstitial taxa. Palp morphology is highly variable within Eunicida and among dorvilleids; while some interstitial dorvilleid genera lack palps completely (*e.g.*, *Parapodrilus, Apodotrocha*), others have long, dexterous palps like those of *Protodorvillea* (*e.g.*, *Coraliotrocha*) (Schmidt-Rhaesa, 2020).

Protodrilids, including both species observed (Fig. 1A, B), are small translucent worms that lack chaetae and external segmentation and have relatively simple musculature that is dominated by longitudinal fibers (Martínez *et al.*, 2018). The most prominent feature of protodrilids is their pair of anterior, filiform palps common to all species within this family. These palps are ciliated but not grooved and are connected to the prostomium *via* small coelomic channels that connect posterior to the brain (Purschke, 1993). The function of palps in interstitial protodrilids is thought to be sensory, as they are adorned with many sensory cells equipped with differing number of sensory cilia of varying lengths (Purschke, 1993), though explicit observations of interstitial palp function are lacking. Nearly all protodrilids live interstitially and locomote using a midventral ciliary band (Jägersten, 1954; Martin, 1978), with known exceptions of a cave-dwelling species that swims (*Megadrilus pelagicus*) (Martínez *et al.*, 2018) and one species that appears to specialize on the bones of whale falls (*Protodrilus puniceus*) (Sato-Okoshi *et al.*, 2015). These two noninterstitial species are two of the only species with described palp function: *M. pelagicus* has been observed suspension feeding while drifting through the water column of an anchialine lava tube (Martínez *et al.*, 2017), while *P. puniceus* has been observed in a petri dish using its palps to gather organic particles to its mouth (Worsaae *et al.*, 2021). Aside from these observations, most protodrilids appear to be subsurface deposit feeders that are thought to consume the bacteria, diatoms, and detritus attached to sediment grains by using oral ciliary bands and their pharyngeal apparatus to separate labile particles from mineral grains (Jägersten, 1952; Westheide, 1990; Rouse and Pleijel, 2001; Jumars *et al.*, 2015). The two species of protodrilids examined here are morphologically similar but were both included in this study because they come from locations with different sandy environments. One was collected from coarser, subtidal sand in the Pacific Northwest (referred to as the Pacific protodrilid), while the other was collected from finer beach sands in the northern Gulf of Mexico (referred to as the Gulf protodrilid). While these worms were not identified further than family, they are assumed to be different species because of the presence of eyes in the Pacific protodrilid and the lack of eyes in the Gulf protodrilid, in addition to their large geographical separation.

Saccocirrids, including *P. eroticus* (Fig. 1C; Gray, 1969; Di Domenico *et al.*, 2014b), are long, slender worms that resemble protodrilids but are generally larger and have more obvious external segmentation. Small parapodia with chaetae appear on most segments but are reduced and then completely absent near the pygidium. The pygidium is bilobed, highly adhesive, and heavily involved in locomotion. The head of *P. eroticus* is adorned with two eyes and two muscular palps. Much like those of protodrilids, the palps of saccocirrids have been considered sensory in function (Purschke, 1993, 2005) and have received little investigation. One species of *Saccocirrus* has been observed in petri dishes, using its palps to gather food particles from the water column and guide them to its mouth (Di Domenico *et al.*, 2014a). All saccocirrids move primarily by gliding *via* their midventral ciliary band, although some are capable of muscular swimming for short distances (Di Domenico *et al.*, 2014a). While locomoting, *P. eroticus* attaches its pygidium to a sediment grain, allowing the worm to rapidly retract or change direction by contracting its longitudinal muscles. *Pharyngocirrus eroticus* is a deposit feeder, consuming primarily detritus (du Bois-Reymond Marcus, 1946), but because of its small size likely selects for labile material (Jumars *et al.*, 2015).

Protodorvillea recuperata (Fig. 1D) is small and transparent with obvious external segmentation (Banse and Nichols, 1968). It possesses a set of complex, sclerotized jaws that are visible through the transparent body, and, unlike the other species observed, it has distinct parapodia with long, simple, and compound chaetae. The prostomium features two eyespots, two small antennae, and two ventral, ungrooved palps with oval terminal palpodes. Palp function has not previously been investigated in this species or in other interstitial dorvilleids. *Protodorvillea recuperata* moves *via* muscular crawling using its body wall muscles, parapodia, and chaetae. Like other dorvilleids, *P. recuperata* is thought to consume primarily diatoms, bacteria, and other organic material, which it presumably rasps from sediment grains using its jaws (Jumars *et al.*, 2015), although no explicit studies of diet have been made.

While the similarities between the palps of protodrilids and *Pharyngocirrus* are likely the result of their close common ancestry, *Protodorvillea* is likely too distantly related for its palp characteristics to represent homologies in any way other than the homology that underlies all annelid palps. A similar navigatory function of these independently evolved palps would further support their utility to an interstitial lifestyle.

Materials and Methods

Sample collections and preparation

Pacific protodrilid, *Pharyngocirrus eroticus* (Gray, 1969), and *Protodorvillea recuperata* Banse & Nichols, 1968 individuals were all extracted from sediments collected at ~28-m depth by Van Veen grab at ~ 48°32′42.2″ N, 122° 59′26.7″ W, near the Friday Harbor Laboratories on San Juan Island, Washington, in May 2019. All mean grain size measurements represent the geometric mean, following the methods of Folk and Ward (1957) and were calculated using the GRADISTAT V0.1 software package (Blott and Pye, 2001). Sediments consisted of coarse shell, gravel, and sand with a mean grain size of 767 μm. Sediments were taken back to the lab and maintained under running seawater for 3 weeks. Gulf protodrilids were extracted from sediments collected at ~1-m depth by hand at 30°14′48.4″ N, 88°07′19.8″ W, on Dauphin Island, Alabama, in September 2021. Sediments consisted of fine to medium beach sand with a mean grain size of 294.9 μm. Sediments were taken back to the Dauphin Island Sea Lab, where they were maintained for 1 week under recirculating seawater.

All worms were extracted from sediments in small batches; sediment was placed in a 1-L flask and mixed with 50% $MgCl_2$ (isotonic with seawater) and 50% seawater to anesthetize meiofauna (Giere, 2009). The flask rested for ~10 min, then its contents were decanted over a 100-μm sieve. This was repeated three times, then the particulates retained on the sieve were rinsed into a petri dish of seawater. Worms were selectively sorted out of the sample as needed.

Observations

To investigate locomotory palp function in interstitial annelids, we observed their behaviors in two different media that re-created the interstitial environment while allowing for detailed observations. Individuals were placed in petri dish covers containing either cryolite or a monolayer of 500-μm glass beads and were covered with the bottom of the corresponding petri dish. Cryolite (mean grain size: 226 μm) is a transparent mineral that has a refractive index similar to water; when fully submerged, it becomes functionally transparent (Josephson and Flessa, 1972). Glass beads (500 μm) were used because they were larger than the grains of cryolite and they created larger interstitial spaces, although their refractive index (~1.5) was higher than that of seawater (~1.33), which distorted the line of sight, making observations deeper than the first layer of beads impossible. Both media have been used in previous burrowing studies (Francoeur and Dorgan, 2014; Dorgan, 2018), and the combination of observations is beneficial because the animals can be more clearly observed in cryolite; however, the grain interfaces are hard to distinguish, whereas glass beads allow for better observation of faunal-grain interactions. Animals were left to acclimate for ~10 min. Dishes were then placed under a stereomicroscope (Leica M150, 2× objective, 20–200× magnification) with a red-light filter, and worms were observed and filmed using a Nikon D5300 camera attached to a PC running digiCamControl software (digiCamControl, 2023; Table 1). No organic matter was present in either media, and worms did not appear to engage in any feeding activities during these observations.

Videos of worms moving through interstitial spaces were first analyzed using the BORIS behavioral tracking software (Friard and Gamba, 2016) to determine how frequently palps were active during locomotion and the percentage of

Table 1

Summary of observations and morphological measurements made for each species

	Pacific protodrilid		Gulf protodrilid		*Pharyngocirrus eroticus*		*Protodorvillea recuperata*	
	Cryolite	Glass beads	Cryolite	Glass beads	Cryolite	Glass beads	Cryolite	Glass beads
Individuals observed	10	6	9	6	13	8	6	6
Individuals recorded	4	4	6	4	5	4	6	3
Seconds filmed in substrate	607	540	929	226	1047	410	1741	494
Retractions observed	5	28	31	4	56	32	33	4
Observations encountered	4	3	7	0	14	1	8	0
Observations retracted	2	1	3	0	7	0	0	0
Observations circumvented	2	2	4	0	7	1	5	0
Observations dislodged	0	0	0	0	0	0	5	0
Palp length (mm)	0.42 ± 0.11		0.26 ± 0.03		0.90 ± 0.090		0.68 ± 0.12	
Palp width (mm)	0.025 ± 0.004		0.029 ± 0.003		0.052 ± 0.009		0.036 ± 0.007	
Head width (mm)	0.097 ± 0.01		0.075 ± 0.004		0.13 ± 0.010		0.14 ± 0.025	
Segment width (mm)	0.11 ± 0.011		0.08 ± 0.006		0.15 ± 0.002		0.19 ± 0.032	
Palp length/segment width (mm)	3.92 ± 0.88		3.10 ± 0.39		6.12 ± 0.64		3.63 ± 0.49	
Palp width/segment width (mm)	0.23 ± 0.042		0.34 ± 0.056		0.35 ± 0.067		0.19 ± 0.021	

Behavioral data are broken into observations made in cryolite and glass beads. Morphological measurements were made and averaged for the cryolite observations, so the number of individuals filmed in cryolite is the sample size used in the calculations. The error represented is the standard deviation.

time the worms spent moving. An ethogram of observed behaviors was generated from initial viewing of videos (*e.g.*, moving, stationary, encountering obstacle, retraction; for more detail see Table A1, available online), then videos were analyzed for the defined behaviors. Worms occasionally escaped the substrate or wedged themselves between the petri dish lid and bottom; palp and locomotory activity were analyzed only when worms were in the substrate. Palp length and width, head width (measured just behind the palps), and segment width (one of segments 4–8 depending on visibility) were measured for each worm filmed in cryolite, using ImageJ (National Institutes of Health, Bethesda, MD). To further investigate whether these worms were using their palps to navigate interstitial spaces, the location of the palp tips and the tip of the prostomium were tracked in ImageJ using the MTrackJ plugin (Meijering *et al.*, 2012) at 5 frames s^{-1}. Video segments in which the field of view did not change, or in which static features were present that allowed for the correction of palp and prostomium coordinates as the field of view shifted, were subsampled for analysis, using the software FFMPEG (Bellard and Bingham, 2000).

Analysis

Durations of locomotion and palp activity calculated using BORIS were normalized by the time the individual was observed in the substrate, then averaged across replicate individuals. To illustrate how palps were used to navigate interstitial space, the coordinates for palp and prostomium tips were used to re-create the paths traveled by individual worms. These paths could then be compared between species. The distance traveled by the prostomium and the tip of each palp was determined by taking the x- and y-coordinates of the prostomium and palps in subsequent frames and applying the standard distance formula $D = ((x_2 - x_1)^2 + (y_2 - y_1)^2)^{1/2}$, where (x_1, y_1) and (x_2, y_2) were the coordinates of corresponding features from subsequent frames. Cumulative distance traveled was then plotted against time. To re-create the track taken by each worm through cryolite, palp and prostomium locations were plotted in an x-y plane, with corresponding palps and prostomiums connected by simplified straight lines.

Results

Observations on movement and palp use

All four species of annelids observed in this study actively used their palps during most of their locomotion (Fig. 2; see standard deviations in Table A2, available online). Worms used their palps to probe the spaces and substrate around them, both while stationary and while in motion (Videos S1–S4, available online). This probing activity occurred primarily with the palps oriented in front of or lateral to the prostomium, although occasionally a single palp was dragged behind during a change in direction. All species encountered diverging paths that were explored with palps before one was entered. Palps were actively probing during nearly all locomotion and are therefore interpreted to be related primarily to the navigation of interstitial spaces. Detailed descriptions of the locomotion and probing activity of each species are discussed below.

Protodrilidae locomotion

The Pacific and Gulf protodrilids both had extremely similar locomotory behaviors with a few notable differences. The Pacific protodrilid exhibited the lowest activity of the four species when observed in cryolite (Fig. 2), with prolonged periods of inactivity, while the Gulf protodrilid was active in both substrates. The Pacific protodrilid appeared to struggle to move through the pore space of the cryolite, seemingly stuck in small voids and pores. This did

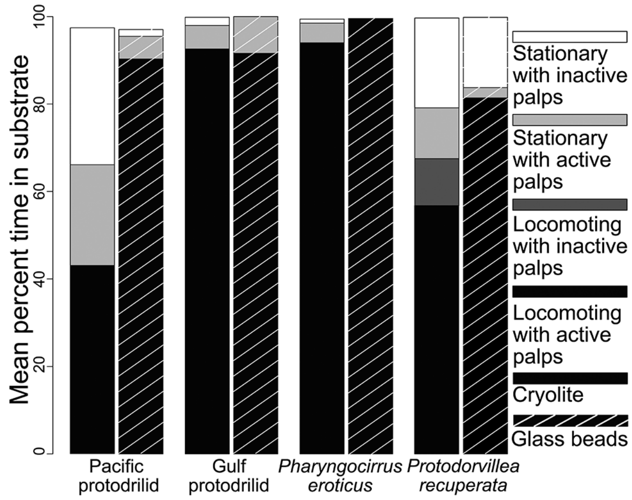

Figure 2. Mean worm activity of each species while observed in cryolite and glass beads.

not appear to be a problem for individuals in glass beads, and the percent of the time they were stationary was much lower (Fig. 2). Both the Pacific and Gulf protodrilids moved through available pore space while actively probing their surroundings with their palps (Videos S1, S2). The palps of both species were oriented either anteriorly or laterally to the prostomium, extending down diverging paths and probing against grains of cryolite or glass beads (Figs. 3, 4A–F). When an individual of either species encountered diverging paths, it extended a palp in each possible direction (Figs. 3, 4A), selected a path, then glided down that path, leading with the palp that was initially extended in that direction. Once a path had been selected, both palps were brought together, and the individual continued forward with both palps oriented anteriorly. When the Pacific protodrilid encountered an obstructing grain of cryolite (Fig. 3C), both palps were generally used to probe the obstruction, leading the worm to either find an alternate route or retract out in the opposite direction (Table 1; Fig. 3G, H; Video S1, 25 s). Both species actively probed spaces that were not ultimately traveled down (Figs. 3, 4H). This active probing behavior led the palps to move a much greater cumulative distance than the prostomium over the course of the observation (Figs. 3, 4I). Difficulties in achieving a perfect monolayer of glass beads in petri dishes led to some beads becoming more dispersed near the edges. While individuals occasionally entered this area (Video S2, glass), no obvious changes in behavior were observed in these areas.

Saccocirridae locomotion

Pharyngocirrus eroticus was the most active of the three species observed, rarely stopping locomotion for more than a few seconds at a time (Fig. 2). Despite being larger than the protodrilids, *Pharyngocirrus* did not appear to have any difficulty moving though the cryolite. *Pharyngocirrus eroticus* glided through the interstitial spaces by using its ciliary band and appeared to use its chaetae and small parapodia to aide in locomotion by pushing against cryolite grains as it moved around individual grains (Video S3, cryolite). The palps of *P. eroticus* actively probed the surrounding environment both during active locomotion and while the individuals were stationary. When an obstruction was encountered, both palps were used to probe the obstruction and surrounding area (Fig. 5A–C). Following this probing, worms appeared to locate and select an alternate path to circumvent the grain of cryolite (Fig. 5D–F). If an obstruction was encountered that could not be circumvented, *P. eroticus* generally retracted its body and began probing alternative routes (Table 1). *Pharyngocirrus* showed a modest increase in activity, encountered fewer obstructions, and retracted less when placed in glass beads, but its activity was very similar in both media. *Pharyngocirrus eroticus* consistently led forward movement with its palps (Fig. 2). No worms were observed moving forward without at least one palp extended directly ahead of the prostomium (Fig. 5G, H) constantly probing and exploring the interstitial spaces ahead of them, including paths that were not followed by the prostomium (Fig. 5H). This constant exploration of the surrounding void spaces caused the palps to move a much greater cumulative distance than the prostomium over the course of the observation (Fig. 5I).

Dorvilleidae locomotion

Protodorvillea recuperata was moderately active during observations, with longer durations of inactivity than *Pharyngocirrus* but fewer than the Pacific protodrilid (Fig. 2). Unlike the Pacific protodrilid, these periods of inactivity did not seem to be caused by an inability to move through the cryolite; *Protodorvillea* appeared to move through both media with ease, and activity levels were similar in cryolite and glass beads. These worms used their parapodia and chaetae to move through the interstitial environment, crawling

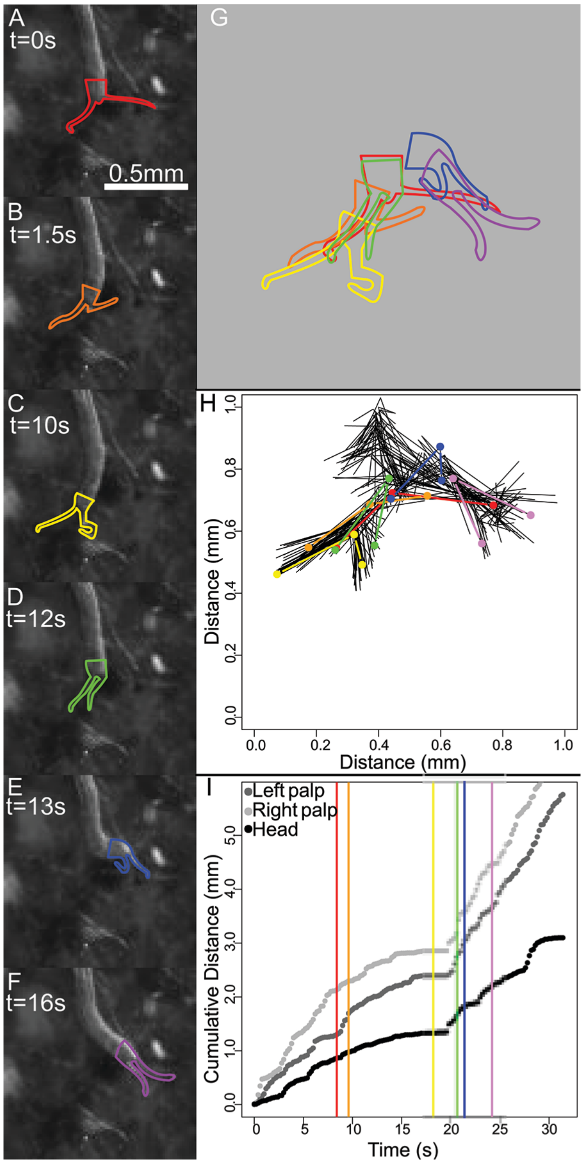

Figure 3. Pacific protodrilid movement diagrams and patterns. (A–F) Pacific protodrilid navigating through cryolite. (A) Encounters divergent paths and evaluates with left and right palps. (B) Moves down the frame and leads with lower palp. (C) Continues forward; right palp encounters obstruction and folds. (D) Left palp encounters obstruction, and worm withdraws. (E) Moves into alternate route that was initially probed in (A). (F) Extends second palp into pore space. (G) Outlines of the Pacific protodrilid's head and palps from (A–F) overlaid to demonstrate the area explored. (H) The same path taken as in (A–F) but showing all time points. Three-point lines represent the right palp, the head, and the left palp, respectively. Colored lines correspond to the head and palp locations of the corresponding frames; black lines correspond to other time points. (I) The cumulative movement (mm) of the left palp, right palp, and head over time. Vertical lines indicate the time points of the movement panels to the left and progress from left to right (A–F), respectively.

through spaces large enough to accommodate their bodies. The palps of *P. recuperata* actively probed diverging paths in the pore space (Fig. 6A, D), and worms followed one of the paths explored by the palps (Fig. 6B, C, E, F). Unlike the other species observed, however, *Protodorvillea* would occasionally lead forward locomotion with its prostomium, with the palps oriented posteriorly along its body. This generally occurred when worms encountered an obstructing piece of cryolite: the obstruction was located and explored with the palps, then the palps were retracted and the prostomium was used to dislodge and move the obstruction (Video S4). *Protodorvillea recuperata* was the only species

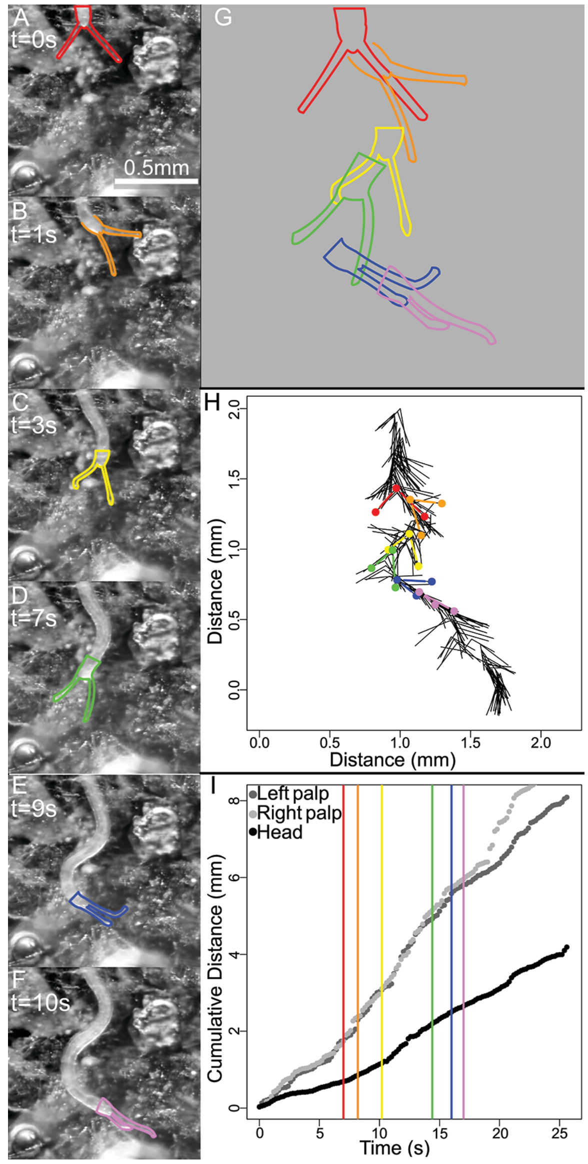

Figure 4. Gulf protodrilid movement diagrams and patterns. (A–F) Gulf protodrilid navigating through cryolite. (A) Extends its palps down diverging paths. (B) Moves down the path to its left. (C) Turns right following its right palp. (D) Again probes two divergent paths. (E) Moves down the path to its left. (F) Continues down this path with palps together. (G) Outlines of the worm's head and palps from (A–F) overlaid to demonstrate the area explored. (H) The same path taken as in (A–F) but showing all time points. Three-point lines represent the right palp, the head, and the left palp, respectively. Colored lines correspond to the head and palp locations of the corresponding frames; black lines correspond to other time points. (I) The cumulative movement (mm) of the left palp, right palp, and head over time. Vertical lines indicate the time points of the movement panels to the left and progress from left to right (A–F), respectively.

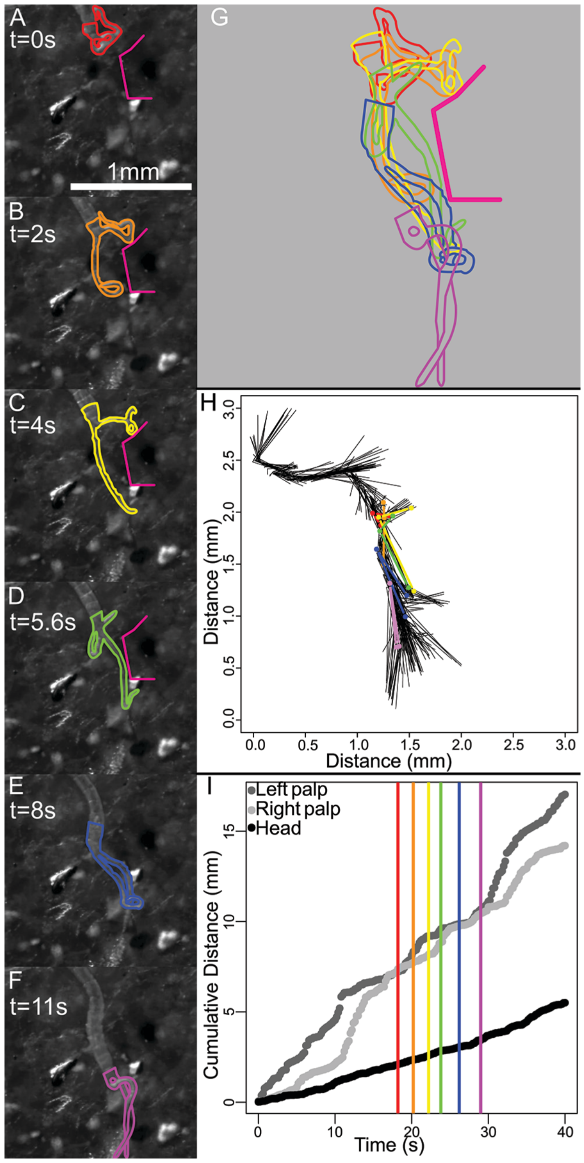

Figure 5. *Pharyngocirrus eroticus* movement diagrams and patterns. (A–F) *Pharyngocirrus eroticus* navigating through cryolite. (A) Left and right palps are folded back while encountering obstruction. The pink line outlines the edge of an obstructing grain of cryolite. (B) Left palp extends past the obstruction and probes ahead. (C) Left palp is fully extended and *P. eroticus* begins to follow its leading palp. (D) Rotates its head, bringing its right palp beneath its left. (E) Extends its left (previously right) palp ahead and continues to move forward. (F) Moves forward with palps extended. (G) Outlines of the *P. eroticus* head and palps from (A–F) overlaid to demonstrate the area explored. (H) The same path taken by *P. eroticus* but showing all time points. Three-point lines represent the right palp, the head, and the left palp, respectively. Colored lines correspond to the head and palp locations of the corresponding frames; black lines correspond to other time points. (I) The cumulative movement (mm) of the left palp, right palp, and head over time. Vertical lines indicate the time points of the movement panels to the left and progress from left to right (A–F), respectively.

that was observed dislodging cryolite during locomotion (Table 1)—an ability likely due to its large prostomium and muscular crawling style of locomotion—but this behavior was only rarely observed, and most of its locomotion was interstitial. Aside from the ability to dislodge grains of cryolite, the locomotory palp activity of *Protodorvillea* resembled that of the other species observed. *Protodorvillea* generally led forward locomotion with at least one palp, actively probing and exploring the voids and pores in its path, including

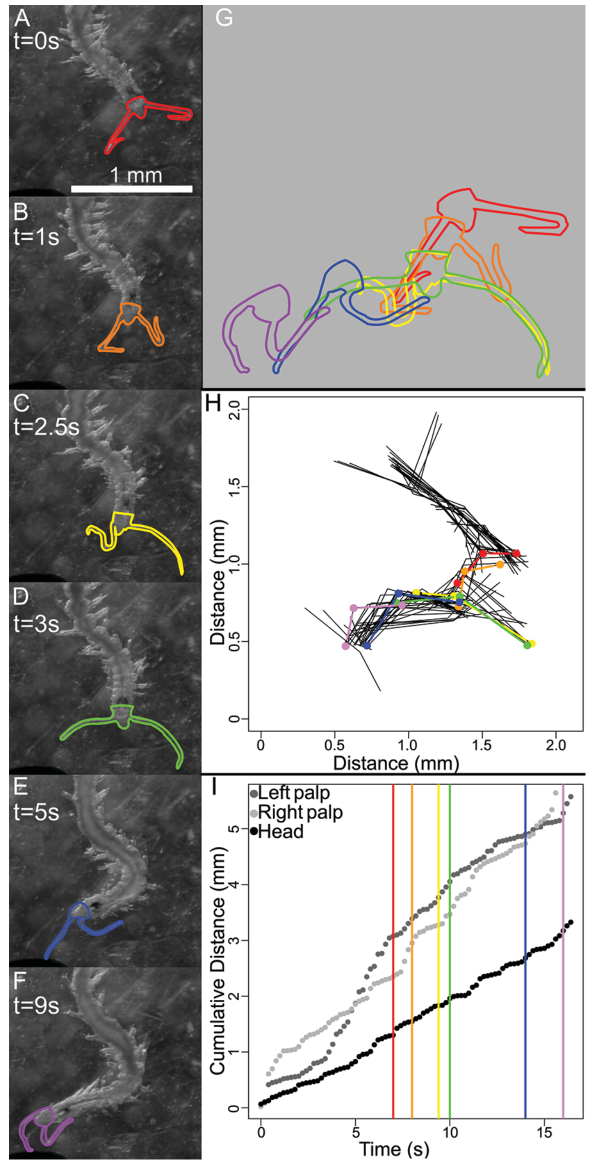

Figure 6. *Protodorvillea recuperata* movement diagrams and patterns. (A–F) *Protodorvillea recuperata* navigating through cryolite. (A) Encounters diverging paths and probes both with palps. (B) Moves to the left of the obstruction and begins to retract its right palp. (C) Encounters more diverging paths and extends its right palp in one direction while probing a path with its left. (D) Palps evaluate two possible paths. (E) Moves to the left and begins to follow its left palp. (F) Encounters diverging paths and brings its right palp forward. (G) Outlines of the *P. recuperata* head and palps from (A–F) overlaid to demonstrate the area explored. (H) The same path taken by *P. recuperata* but showing all time points. Three-point lines represent the right palp, the head, and the left palp, respectively. Colored lines correspond to the head and palp locations of the corresponding frames; black lines correspond to other time points. (I) The cumulative movement (mm) of the left palp, right palp, and head over time. Vertical lines indicate the time points of the movement panels to the left and progress from left to right (A–F), respectively.

paths it did not ultimately occupy (Fig. 6G, H). This active probing and exploration caused the palps to move a much greater cumulative distance than the prostomium over the course of the observation (Fig. 6I).

Discussion

Our observations and results are consistent with our hypothesis that the palps of these interstitial annelids serve a mechanosensory function during locomotion, specifically, the navigation and evaluation of interstitial spaces. The consistent forward orientation of the palps during locomotion, in addition to the constant probing activity and the large area explored by palps over time, suggests that palps are used to survey spaces before the worm enters. These behaviors were documented in all species observed, and the similarities in palp use between the two different clades of annelids (Protodriliformia and Eunicida) is indicative of the broad utility that palps offer in interstitial spaces.

While we only observed behaviors consistent with a mechanosensory function of palps, we cannot exclude the possibility that they may serve other functions. It is reasonable that this probing activity may also be used to evaluate predation risks or to sense chemical cues from food and environmental conditions or in the collection and ingestions of food. Our experimental setup allowed for the creation of a transparent interstitial environment but notably lacked pore water flow, predators or competing organisms, labile organic matter, and chemical stimuli. Without such a complete re-creation of the interstitial environment, a full description of interstitial palp functions is difficult to make. Several observations and studies suggest that the palps of some protodrilids and saccocirrids are utilized in the capture and ingestion of food particles (Di Domenico *et al.*, 2014a; Martinez *et al.*, 2017; Worsaae *et al*, 2021), and it is possible that they are involved in interstitial feeding as well. Additionally, the palps of protodrilids are frequently lost (WMB, pers. obs.) and may be an example of autotomy, the defensive and intentional shedding of appendages as a result of external stimuli (Fleming *et al.*, 2007). Leading forward locomotion with an appendage that may be shed in a predator interaction could be advantageous, given the close proximity inherent in encountering another organism in the interstitial environment, but further observations of species interactions are required to definitively determine whether the palps of protodrilids are used in this way.

The Pacific protodrilid had more difficulty moving through the cryolite than any of the other species observed. We believe this is a result of a slight mismatch between the available interstitial space in the natural sediments where the Pacific protodrilids were collected and the spaces within the cryolite. The Pacific protodrilids were extracted from natural sediments that consisted of a mix of shell hash and gravel from Friday Harbor; this heterogeneous mixture likely led to more variable void and pore sizes (Nolan and Kavanagh, 1994). This contrasts with the more homogeneous cryolite, which likely had a tighter distribution of void and pore sizes that may have been smaller than those the Pacific protodrilid usually encounters. This resulted in the weakly muscled worm struggling to move through the interstitial environment. This hypothesis is supported by the increase in activity observed when the Pacific protodrilids were placed in glass beads. The larger void spaces present in the monolayer of glass beads allowed the Pacific protodrilids to glide through the spaces largely unimpeded. *Pharyngocirrus* and *Protodorvillea* were collected from the same sediments as the Pacific protodrilids but experienced less difficulty moving through the cryolite despite its composition. Both species utilize their musculature (as opposed to only ciliary bands) during locomotion (Gray, 1969; WMB, pers. obs.) and were observed using their chaetae when moving, which likely aided their locomotion through cryolite. The Gulf protodrilids also experienced less difficulty than those from the Pacific when moving through the cryolite, despite their similar morphology and overall behavior. The Gulf protodrilids observed were smaller than those from the Pacific (Fig. 1; Table 1) and were extracted from sediments with a mean grain size (294 μm) much closer to that of the cryolite (226 μm) than the sediments from which the Pacific protodrilids were extracted (767 μm). This likely resulted in an interstitial environment in the cryolite that was more like the Gulf protodrilids' natural habitat, allowing them to locomote well. This further supports that the Pacific protodrilid's lower activity in the cryolite was a result of limited interstitial space and an inability to move grains to create more space. The importance of grain size and, as a result, pore size to the distribution, locomotion, and general ecology of interstitial animals has been well documented (Wieser, 1956, 1959; Boaden, 1962; Lombardi and Ruppert, 1982; Giere, 2009) and is further supported here. This close association between interstitial fauna and the pores in which they live emphasizes the importance of these animals' ability to sense their environment in a tactile way and supports our hypothesis that palps are useful mechanosensory tools in addition to any other functions they may serve (*e.g.*, chemosensing, feeding). Navigating with palps would allow these worms to evaluate the volume of an adjacent void and determine whether it was feasible for entry, helping to prevent individuals from becoming stuck in small voids and improving their energetic efficiency by decreasing the time and energy spent moving down dead-end paths.

Protodorvillea was the only species observed that had the ability to dislodge individual grains while moving through the cryolite. This is an advantage when moving through granular material because individuals are less restricted to preexisting voids and pores than the other species studied, potentially increasing the percentage of the interstitial environment available to them. However, dislodging grains in this way may be energetically costly, as *Protodorvillea* almost constantly uses its palps to explore and evaluate voids and pores and was observed circumventing as many obstructions as dislodging. Although these worms can dislodge grains when needed, this did not happen frequently and is probably not their preferred form of locomotion, so we consider these animals primarily interstitial.

The differing ability of these species to move through the same substrate illustrates the importance that morphology, musculature, and locomotory style play in interstitial life. The ciliary gliding and small size of the protodrilids suggest that their primary strategy is being small enough to fit in the available interstitial spaces, and our observations indicate that when they struggle to fit, they struggle to move. This has broad implications for their dispersal potential because the Pacific protodrilid observed could only colonize sediments that contained the appropriate interstitial environment. Conversely, *Pharyngocirrus* and *Protodorvillea* did not struggle to move through the cryolite, despite being larger than the Pacific protodrilid (Table 1) and living in the same coarser sands. The musculature and chaetae of *Pharyngocirrus* and *Protodorvillea* allow them to better anchor and push through tight spaces, possibly enabling them to inhabit a larger range of sediments with a larger distribution of void sizes. Despite these differences, the palps of all four worms were observed being used to navigate interstitial spaces during locomotion, indicating that these appendages are useful sensory appendages, regardless of a worm's ability to muscle through a void.

The demonstrated function of palps in the interstitial environment raises an interesting question about the presence or absence of palps in interstitial annelids. Because palps are thought to be a pleiomorphic character in annelids (Orrhage, 2001; Orrhage and Müller, 2005; Struck, 2011; Weigert *et al.*, 2014; Parry *et al.*, 2016; Chen *et al.*, 2020), their absence in some lineages is proposed to represent a secondary loss. Many interstitial annelids do not possess palps (*e.g.*, apharyngtids, dinophilids, diurodrilids, lobatocerebrids, parergodrilids, psammodrilids, some dorvilleids, *etc.*), and the reduction or loss of appendages in interstitial organisms is frequently considered an adaptation to the restricted space in interstitial environments (Swedmark, 1964; Giere, 2009). The results of this study demonstrate that palps play an active role in the locomotion of the worms observed and indicates that palps are useful in interstitial navigation. The absence or reduction of palps in some other interstitial annelid lineages may therefore be attributed to ancestral loss. The families Apharyngtidae, Diurodrilidae, and Parergodrilidae have been proposed to be grouped within Orbinida (Struck *et al.*, 2015; Martín-Durán *et al.*, 2021), a clade whose macro- and meiofaunal members all lack palps. Alternately, a lack of palps may simply be the result of a progenetic origin (Westheide, 1987), that is, that palps develop after the larval or juvenile stage of development. The demonstrated role of palps in interstitial navigation raises questions about whether other interstitial families such as Dinophilidae and Lobatocerebridae lack palps as a result of ancestry or interstitial origin. If palps were lost in the transition to an interstitial lifestyle, what navigational strategies do these animals use instead?

The precise control the worms examined in this study display over their palps allows them to probe extremely small spaces and explore areas that might otherwise be out of reach for the worm. From the perspective of these meiofaunal worms, the interstitial environment is an endless maze of voids and pores. The ability to evaluate these voids for available space, a dead end, or a predator before entry would seem extremely valuable to any meiofaunal organism. While the results of this study show that palps are useful to interstitial navigation, the absence of palps on many meiofaunal animals shows that they are clearly not necessary. Other annelid families not investigated here, such as Sigalionidae, also contain interstitial genera with dexterous palps. While these worms may be using their palps in similar ways, the vast majority of meiofauna do not possess such appendages, and even some worms that do have palps (protodrilids; see above) shed them easily, demonstrating that all groups can likely navigate without palps. How do phyla without external appendages of any kind (nematodes, platyhelminths, acoels, *etc.*) navigate the interstitial environment? Further investigations of interstitial navigation, and the sensory strategies and modalities employed, could become an interesting and fruitful avenue of research within meiobenthology.

The need to navigate interstitial spaces and the utility of a palp-like appendage for that function are not unique to the meiofauna. The field of soft robotics is already developing fluid-filled, tentacle-like, soft robots to explore anthropogenic environments such as the rubble of collapsed buildings (Hawkes *et al.*, 2017; Blumenschein *et al.*, 2020). While these robots extend and retract differently, their overall pattern of exploration is extremely reminiscent of annelid palps and demonstrates that such strategies are efficient at exploring tight spaces. Further research on meiofaunal strategies of navigating their interstitial space could help improve the effectiveness and efficiency of bioinspired robots.

These results highlight the importance of observing interstitial animals in conditions similar to their natural environment in linking form to function in the meiofauna. In the present study, relatively simple observations revealed that at least three families of interstitial annelid utilize their palps to navigate the interstitial environment. This discovery would not have been possible without observing these animals in a substrate that accurately re-created a true interstitial environment while still allowing for direct observation (cryolite). Meiofaunal morphology has been well documented across many phyla to the family level (Schmidt-Rhaesa, 2020), but few direct observations of faunal behavior have been made. Many species await descriptions of their locomotion, feeding, and general behavior, and many more novel behaviors like the ones documented here await observation. Further studies of meiofauna behavior will likely result in many more insights into how the interstitial environment has shaped meiofaunal evolution and how the physical constraints of an environment may breed functional adaptations across and within phyla.

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