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RESEARCH ARTICLE

Kinematics of burrowing by peristalsis in granular sands

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ABSTRACT

Peristaltic burrowing in muds applies normal forces to burrow walls, which extend by fracture, but the kinematics and mechanics of peristaltic burrowing in sands has not been explored. The opheliid polychaete *Thoracophelia mucronata* uses direct peristalsis to burrow in beach sands, with kinematics consistent with the 'dual anchor system' of burrowing described for diverse organisms. In addition to expansions associated with a constrictive direct peristaltic wave, worms alternately expand the head region, which is separated by septa from the open body cavity, and expansible lateral ridges that protrude from the 10th setiger. Tracking of chaetae with fluorescent dye showed that the body wall advances while segments are thin, then stationary segments expand, applying normal forces to burrow walls. These normal forces likely compact burrow walls and serve as anchors. Perhaps more importantly, peristaltic movements minimize friction with the burrow wall, which would expand dilatant sands. Considerable slipping of worms burrowing in a lower-density sand analog suggests that this dual-anchor peristaltic burrowing may be limited to a narrow range of mechanical properties of substrata, consistent with the limited habitat of *T. mucronata* in a narrow swash zone on dissipative beaches.

KEY WORDS: Invertebrate locomotion, Biomechanics, Hydrostatic skeleton, Functional morphology, Polychaete, Opheliidae

INTRODUCTION

Recent work has highlighted the mechanical differences between muddy and sandy sediments: marine muds are cohesive, elastic solids through which diverse animals extend burrows by fracture, whereas sands are non-cohesive granular materials, yet these mechanically different environments are inhabited by many of the same or morphologically similar organisms (Dorgan, 2015; Dorgan et al., 2006). Many burrowing animals have soft, elongate, vermiform bodies, lack limbs, and use hydrostatic skeletons to move. Peristalsis, waves of muscle contraction moving anteriorly or posteriorly along the body, is used in conjunction with differential friction to achieve forward movement by burrowers and crawlers from numerous phyla (Elder, 1980). In muddy sediments, peristaltic movements apply forces to the walls of burrows that extend by fracture (Che and Dorgan, 2010; Dorgan et al., 2005). Peristaltic burrowers inhabit sandy sediments as well as muds, but the mechanisms by which peristalsis achieves burrow extension in granular rather than elastic media have not been explored.

The mechanisms of burrow extension in granular sands have been shown for several different burrowing behaviors. Burrowing sandfish lizards use body undulations to move through granular media by bulk fluidization (Maladen et al., 2009), whereas other undulatory burrowers plastically deform bulk sediment or rearrange and compact grains (Dorgan et al., 2013; Gidmark et al., 2011; Herrel et al., 2011). For undulatory locomotion, wave efficiency, η, the ratio of the animal's forward velocity to the velocity of the posteriorly traveling undulatory wave, has been used to distinguish between movement through fluidized versus solid granular media, with $\eta=1$ indicating no backward slipping and a solid substrate and $\eta<1$ indicating swimming through a fluidized medium (Dorgan et al., 2013; Maladen et al., 2009). Rather than reflecting behavioral or kinematic differences, backward slipping results from different mechanical properties of the media, with fluidization and sand-swimming appearing to be limited to dry, loosely packed surface sands (Dorgan et al., 2013; Maladen et al., 2009; Sharpe et al., 2015b). Employing a very different strategy, the mole crab, *Emerita*, uses rapid leg movements to pick up and resuspend surface grains when burrowing into beach sands, which limits mole crabs to shallow burrowing when submerged so that the higher density of water facilitates sand resuspension (Trueman, 1970).

Similarity in burrowing behaviors for bivalves in sands and muds is well established, but the underlying mechanics are less well understood. Trueman (1971) described burrowing behavior by the surf clam *Donax* as being similar to that of other bivalves in using a 'dual anchor system' in which alternating body expansions anchor part of the animal, e.g. the bivalve shell, while a dilated region, e.g. the foot, moves forward (Trueman, 1971, 1966, 1968). While most of Trueman's studies on burrowing bivalves were conducted in sands, he did find that the same behavioral stages occurred across a broad range of taxa in both sands and muds (Trueman, 1966). This 'dual anchor system' is consistent with the mechanism of burrow extension by fracture in muddy sediments, with the expansions serving a primary function of applying forces to burrow walls (Dorgan et al., 2006). These forces are amplified at the tip of the crack, resulting in burrow extension by fracture. The similar dual anchor system behavior of burrowers in non-cohesive sands that clearly do not fail by fracture is intriguing. Winter et al. (2012) showed localized fluidization around the valves of burrowing bivalves, but their suggestion that valve closure draws pore water through the sand is refuted by Trueman's (1967) data showing increased pore-water pressure around *Ensis arcuatus* corresponding with valve closure (Trueman, 1967; Winter et al., 2012). The small fluidized region around the shell likely instead results from ejection of water from the mantle cavity as described by Trueman (1968). While fluid ejection from the mantle cavity may reduce drag, the fluidized region does not extend to the anterior of the burrow (if it did, the foot would not be able to anchor to pull the shell down). Thus, this localized fluidization is not the mechanism of burrow extension by bivalves in sand.

Although many of the common sandy beach macrofauna are hard-bodied, e.g. mole crabs and bivalves, some polychaetes can be found in high abundance in the swash zone of sandy beaches. Notably, the opheliid polychaete *Thoracophelia mucronata*

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(Fig. 1A) reaches very high levels of abundance on some beaches along the California coast, exceeding 40,000 individuals m^{-2} or 7 tons (6350 kg) of worms along a mile of beach (McConnaughey and Fox, 1949). Although worms are found along a narrow band of the upper intertidal region, substantial movements of *Thoracophelia* spp. have been documented: burrows are mostly horizontally oriented, consistent with worms migrating toward and away from the water with the tidal cycle (Dafoe et al., 2008; Seike, 2008). Preliminary observations of *T. mucronata* burrowing showed that worms burrow using a direct peristaltic wave, consistent with having an open body cavity (Fig. 1B) (Elder, 1980; Law et al., 2014).

Soft-bodied animals have also been described as burrowing using a dual anchor system with an eversible pharynx or proboscis as the 'terminal anchor' and a dilated posterior region as the 'penetration anchor', analogous to the dilated foot and expanded shell of bivalves (Clark, 1964). Peristaltic burrowing involves a wave of muscle contraction traveling either in the direction of locomotion (direct peristalsis) or opposite to the direction of locomotion (retrograde peristalsis), in which moving expansions have been compared to the dilated regions of a dual anchor system (Elder, 1980). In muddy sediments, these 'anchors' have been reinterpreted as serving a primary function of applying force to the walls of the burrow, which extends by fracture (Dorgan et al., 2006). Kinematic analysis of peristaltic burrowing in gelatin, a transparent analog for muds, by the cirratulid polychaete *Cirriformia moorei* showed that worms exhibited clear and rhythmic burrowing cycles – anterior stretching to reach the front of the burrow, burrow extension by fracture, anterior body expansion and lateral crack extension, then peristaltic wave progression (Che and Dorgan, 2010). Worms applied forces normal to the burrow wall sufficient to extend the burrow by fracture.

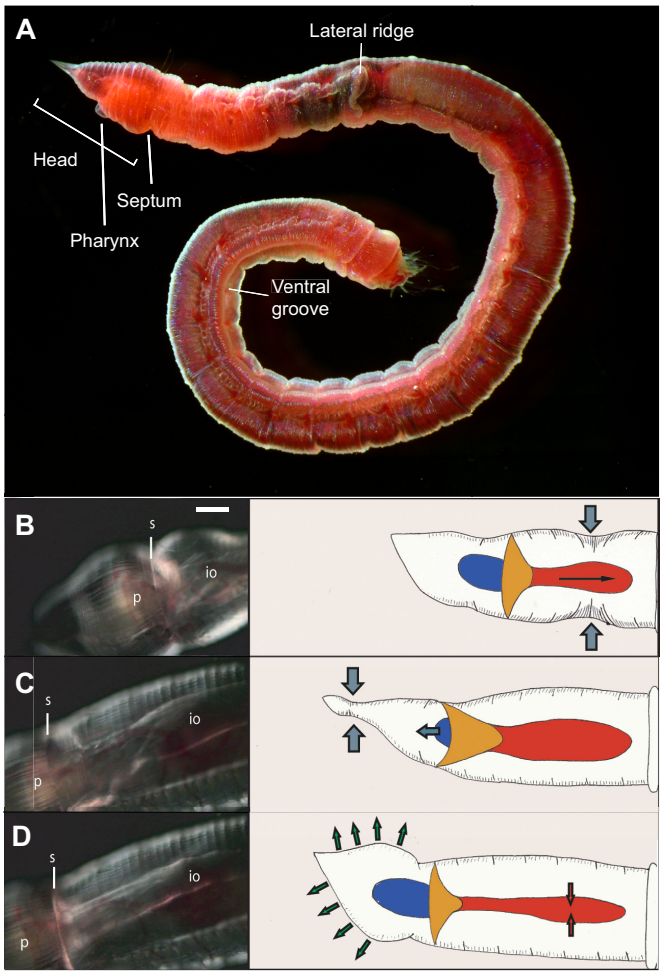

Fig. 1. *Thoracophelia mucronata*. (A) Photo of *T. mucronata*, showing the expansible head region (distinguished from the rest of the body by the narrower position where the septum attaches to the body wall), the lateral ridge and the ventral groove. (B–D) Image sequence and schematic diagram of head expansion showing the pharynx (p; blue), septum (s; yellow) and injector organ (io; red). (B) The peristaltic wave (gray arrows) travels anteriorly toward the head. Scale bar: 200 µm. (C) As it passes the septum, fluid in the head region is pushed posteriorly, fully expanding the injector organ and extending the septum posteriorly (1.6 s). (D) The injector organ and septum contract to push the pharynx anteriorly and expand the head region (2.2 s). Photo credit (A): Greg Rouse; B–D were reproduced from fig. 9B,G,I of Law et al. (2014) with permission.

Elder (1980) identifies four taxa that use direct peristalsis in burrowing and points out that all of these animals are limited to fairly low body fluid pressures compared with those of other burrowers and that the peristaltic wave is primarily used to move the body wall forward, while alternative methods are employed to extend the burrows. Interestingly, these species tend to live either in very soft sediments, in which applying a small force over a large area of the body is effective because resistance to sediment deformation is so low (e.g. *Priapulus caudatus* and *Polyphysia crassa*, as well as the related *Scalibregma inflatum*, see Dorgan et al., 2016), or in sandy sediments, through which locomotion is slow (e.g. the anemone *Peachia hastata*) or aided by tentacles (e.g. the holothurian *Leptosynapta* spp.) (Elder, 1980). Although *T. mucronata* burrows through beach sands with a direct peristaltic wave, these worms move much more quickly than burrowing anemones or holothurians (K.M.D., personal observation).

This study aimed to describe the kinematics of peristaltic burrowing in sandy sediments to determine the mechanism by which peristaltic burrowers extend burrows in sands. I focus on distinguishing behaviors of burrowers in sands from those in muds to understand how infauna with similar morphologies (and presumably burrowing behaviors) successfully burrow through media with very different mechanical properties. Qualitative observations suggest at least a superficial similarity in burrowing behaviors between peristalsis by *T. mucronata* in granular sands and by *C. moorei* in cohesive, elastic muds (Che and Dorgan, 2010), but peristaltic expansions are not used for fracturing this granular material. I specifically tested two hypotheses. (1) Like peristaltic burrowers in muds, *T. mucronata* uses peristaltic movements that would apply normal forces to burrow walls (Fig. 2). Body expansions that occur while segments are stationary apply normal forces, achieved by forward movement of narrow segments (Dorgan et al., 2006). (2) Alternatively, *T. mucronata* applies shear forces to burrow walls, which is achieved by anterior–posterior movements of expanded, rather than narrow, segments (Fig. 2). Normal forces would potentially compact the surrounding sediment, whereas shear forces may dislodge grains for excavation or ingestion. The low pressures and slow movements associated with most burrowers using direct peristalsis are inconsistent with the well-packed sand grains in the *Thoracophelia* zone on beaches and more rapid movements of *T. mucronata*: this study describes behaviors and morphological features that may allow this species to dominate sandy beach habitats.

MATERIALS AND METHODS

Animals

Thoracophelia mucronata (Treadwell 1914) were collected from the intertidal sandy beach near the pier at Scripps Institution of

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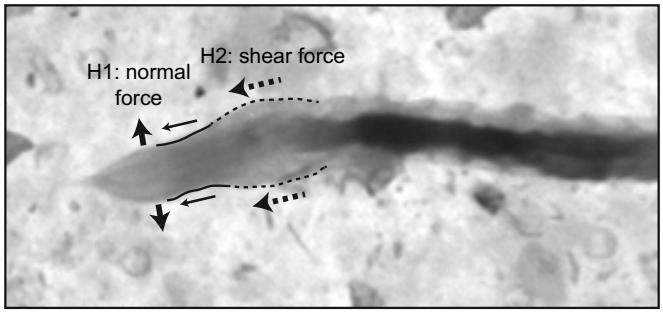

Fig. 2. Image of *T. mucronata* burrowing in cryolite, with annotation illustrating the two alternative hypotheses being tested. H1: narrow segments (solid outline) move forward (solid arrows parallel to body), then expand when stationary to apply normal forces (solid arrows perpendicular to body) to burrow walls. H2: dilated segments (dashed outline) move forward, applying shear forces (dashed arrows) to the burrow walls.

Oceanography, La Jolla, CA, USA. Worms were used within 1 day of collection, from March 2011 to May 2013.

Burrowing observations in analog sands

Burrowing observations were conducted in several different media: sand grains of the mineral cryolite, spherical 500 µm glass beads (Ceroglass, Columbia, TN, USA), low-density gelatin grains, and a mixture of gelatin grains and glass beads. Cryolite, a transparent mineral with similar refractive index to seawater, not only enabled the best visualization but also most closely matched natural sands in density, particle shape and size (Francoeur and Dorgan, 2014; Josephson and Flessa, 1972). Cryolite was obtained from Ward Scientific (Rochester, NY, USA) as cobble and was ground with mortar and pestle and sieved to obtain grains <500 µm. Cryolite grains were tumbled in a rock tumbler (Lortone 3A, Lortone Inc., Mukilteo, WA, USA) overnight to smooth jagged edges, as worms had difficulty burrowing during preliminary experiments, presumably due to jamming of irregular grains. Gelatin grains were produced from 6× concentrated gelatin (85 g l^{-1} seawater) by chopping in a food processor until grains were roughly 500 µm diameter (see Dorgan et al., 2013). Mixtures of gelatin and glass beads were obtained by alternately layering (~1–3 cm depth) the two media – mixtures were not homogeneous, but whereas worms had difficulty burrowing in gelatin grains alone, they were more successful in burrowing in this mixture, and videos were used for qualitative observations only.

Videos were recorded of >45 worms burrowing in several different media in narrow aquariums (1.5 cm×7 cm×8 cm high). Aquariums were fabricated to be thick enough so that worms could displace grains and not be too confined, but thin enough that light could pass through and worms could be visualized. Wall effects were undoubtedly present, and would have potentially made grain displacement and thus burrowing movements more difficult. Videos were recorded using a CCD video camera (Basler A622f, Exton, PA, USA) with a 6× close-focus zoom lens (Edmund Optics #52-274, Barrington, NJ, USA) at 15 frames s^{-1} and custom-written LabView software (v.7.1.1, National Instruments, Austin, TX, USA), following methods of Dorgan et al., (2013). Dorsal- and lateral-view videos were observed in all media, and 16 individuals burrowing in cryolite were further analyzed: key positions (anterior of worm, lateral ridge, gut position) were tracked using the MTrackJ plugin for ImageJ v.1.44o (Meijering et al., 2012). The width of the widest position in the head (defined as the region anterior of the septa–body wall connection) was measured in every other frame using ImageJ. Distance traveled of each region was plotted as a function of time to assess patterns and periodicity of movements.

Body wall tracking experiment

Videos of worms with fluorescent staining of chaetae were analyzed to determine the relationship between segment width and movement of that segment – if worms predominantly applied normal forces to burrow walls, segments would be narrow when moving forward and wide when stationary, but if worms applied larger shear forces to burrow walls, segments would be wide when moving forward (Fig. 2). The positions at which the fluorescent chaetae extended from the body wall could be easily visualized and were used to calculate segment width and velocity. Forward movements of the central position between the paired chaetae over time were used to measure segment velocity.

Chaetae were dyed with a fluorescent dye, and worms were observed (dorsal view) burrowing in a shallow dish of cryolite using a stereo microscope (Fig. 3). Worms were first placed in a dish of seawater with 0.1% Direct Yellow 96 (Sigma-Aldrich, St Louis, MO, USA) for approximately 5 min, following methods modified from Hoch et al. (2005) developed for visualization of chitinous fungal cell walls. Preliminary experiments using Calcofluor White (Fluorescent brightener 28) showed that although chaetae did appear fluorescent, the effect only lasted for a short period of time, consistent with the findings of Hoch et al. (2005); visualization was much improved with Direct Yellow 96. The top of a plastic Petri dish was filled with cryolite and seawater to a depth of about 4–5 grain diameters, then a

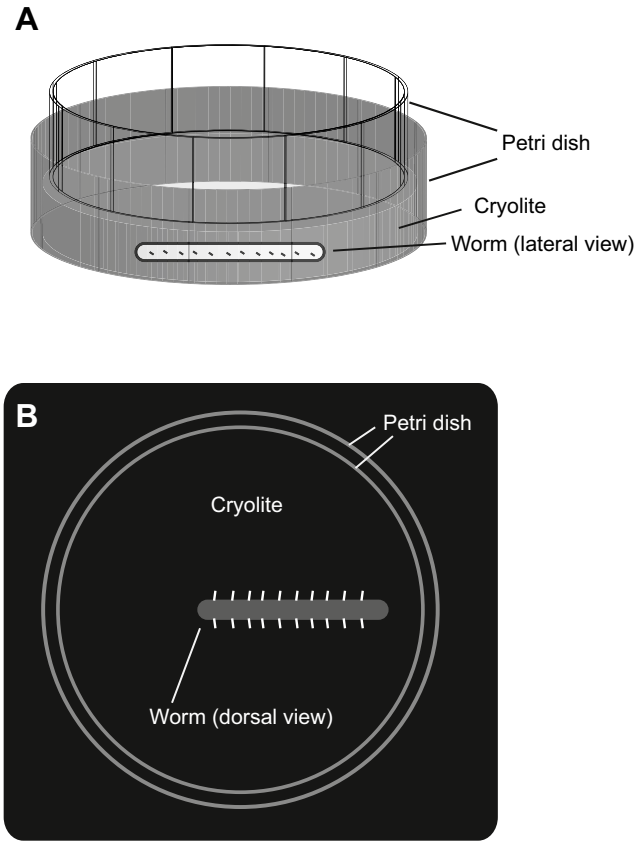

Fig. 3. Schematic diagram of experimental set-up for chaetae tracking observations. (A) Side view showing a shallow Petri dish of cryolite with a glass cover. (B) Top (camera) view showing the dorsal view of a worm. The body of the worm and Petri dish walls (gray) were barely visible, but chaetae (white) fluoresced green visually and appeared white in videos.

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worm with dyed chaetae was placed in a shallow depression in the cryolite and the bottom part of the Petri dish was placed on top of the cryolite (open side up) to gently compress the cryolite containing the worm (Fig. 3). This step was necessary to prevent the worm from burrowing up out of the cryolite. Dorsal view videos of worms burrowing were recorded with a QImaging Retiga 2000R camera with a stereo microscope with CFP filter (436 nm). Direct Yellow 96 has an excitation of 470–490 nm and emission of 510–550 nm, so chaetae appeared green visually, but because of low light levels appeared white in recorded videos.

To calculate segment width (lateral distance between chaetae–body wall contacts), first the proximal end of each chaetal bundle (where it contacts the body wall) from the anterior-most to posterior-most setigers for which chaetae were clearly visible was tracked using the MTrackJ plugin for ImageJ v.1.44o (Meijering et al., 2012). Segment width was calculated for each setiger in each frame as the distance between corresponding chaetae positions using custom-written Matlab script (The MathWorks, Natick, MA, USA) and the midpoint was used to calculate segment speed. Speed in the forward direction was calculated as the dot product between the vector of positions from successive frames and a unit vector in the forward direction. The forward direction vector was calculated from a line fitted through the midpoints of each chaetal pair for all frames of the video segment. Segment velocity was plotted as a function of segment width for individual segments over the duration of a video segment.

RESULTS

Burrowing in different media

Cryolite grains around burrowing worms showed very little movement and worms slipped backward only minimally, indicating that sands are not fluidized during burrowing and suggesting little effective shearing by the body wall (Fig. 4A–G; Movie 1).

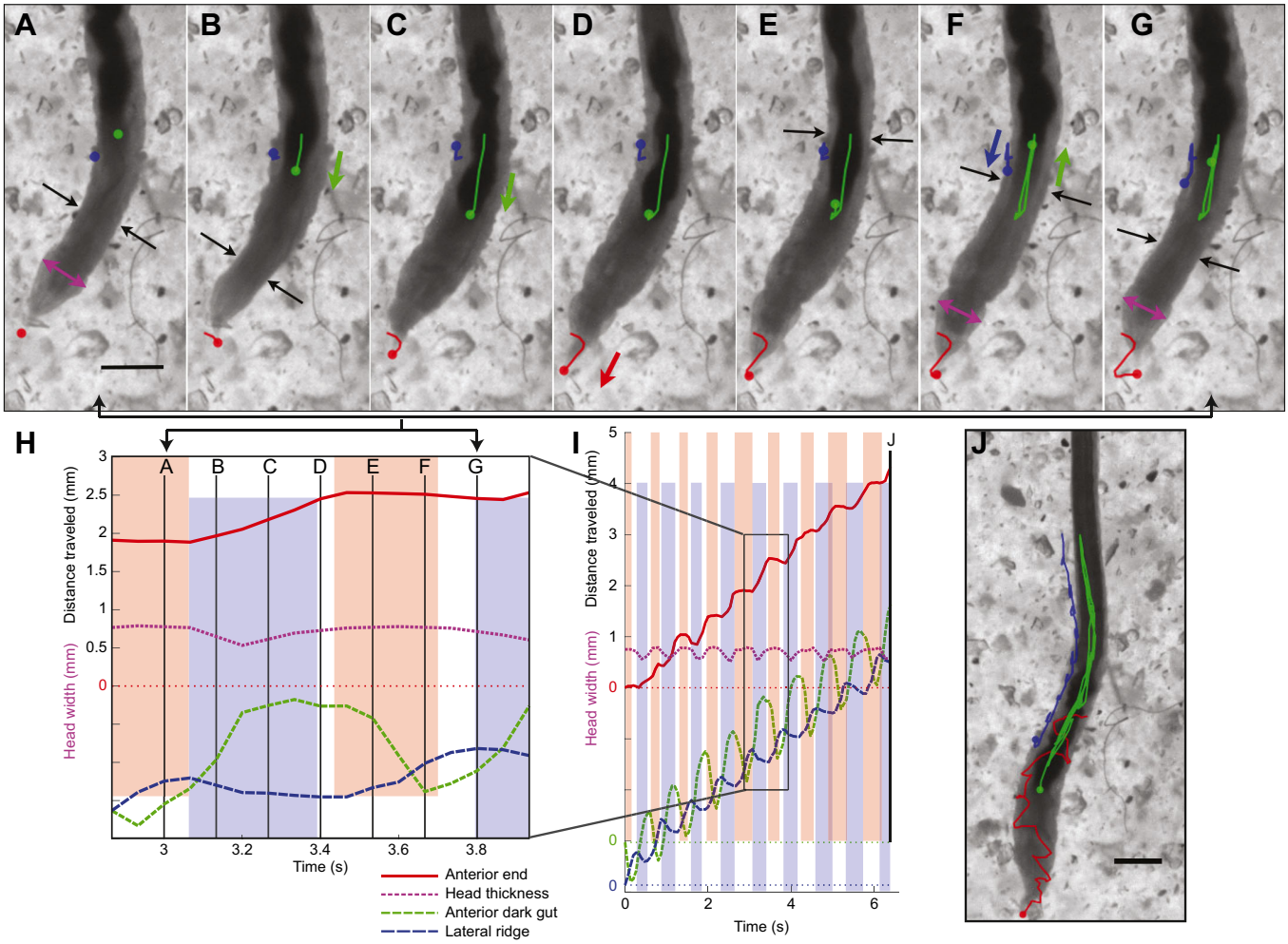

Fig. 4. Distance traveled by an individual *T. mucronata* burrowing in aquariums of cryolite. (A–G) Dorsal view image sequence of one cycle of *T. mucronata* burrowing (0.13 s between subsequent images). Black arrows indicate the contractile peristaltic wave moving anteriorly; dots show the positions of the anterior end (red), anterior of the dark region in the gut visible through the body wall (green), and the lateral ridge on one side (blue), and associated thin colored lines show the positions in previous frames. Colored (red, green, blue) arrows highlight movements of each body position, and magenta double-headed arrows show the position at which head width was measured on frames with expanded head regions. (H) Distance traveled by the head (red), gut (green) and lateral ridge (blue) of the worm shown in A–G, with head width overlaid (magenta). Black vertical lines show the time of each of the images in A–G. (I) Distance traveled by the three regions over a longer sequence including that shown in A–H (boxed region). Absolute numbers are shown for distance traveled by the head (red); gut and lateral ridge positions are plotted at their respective distances from the head with '0' indicating initial position and forward movement following the same scale as that of the head. Thin dotted lines show 0 position for each body region. Shaded pink (higher) and blue (lower) rectangles highlight the duration of head and lateral ridge expansion (anchoring), respectively, and show alternating expansion of these two regions. (J) Image of *T. mucronata* in the last frame of the sequence shown in I with the full tracked sequence of the head, lateral ridge and gut shown in colored lines. Note the irregular back-and-forth movement of the head, the slight backward slipping of the lateral ridge, and the much larger anterior–posterior movement of the gut. Scale bars: 1 mm.

In contrast, worms were unable to burrow in low-density gelatin particles, which were more easily displaced or deformed. Worms in a mixture of gelatin particles and glass beads were able to burrow through regions dominated by glass beads, but exhibited considerable backward and lateral slipping in regions with more gelatin particles (Movie 2). Although slipping occurred, this slipping was not accompanied by the bulk movement of grains resulting from fluidization (Movie 2). Comparison of worms burrowing in glass beads versus cryolite showed considerably more movement of glass beads than cryolite around burrowing worms (Movie 2).

Worms burrowed through all media using a direct peristaltic wave that progressed between two distinct expansible regions (Movies 1 and 2). Dorsal views of worms burrowing in cryolite showed that the lateral ridges at the 10th segment are expanded throughout the burrowing cycle, contracting to varying extents only when that segment is moving forward (Fig. 4; Movie 1). As the contractile peristaltic wave moves anteriorly toward the head region, the head expands (Figs 1B and 4F,G). This head expansion is achieved by contraction of the septum and injector organ (Law et al., 2014), and presumably aided by increased internal pressure as coelomic fluid is pushed anteriorly by the peristaltic wave. Head expansions generally alternate with expansions of the lateral ridge (Fig. 4H,I). Coincident with the cyclical expansion and forward movement of the head and lateral ridge is a periodic anterior–posterior movement of the gut, which is visible through the body wall (Fig. 4; Movie 1). These gut movements can be substantial, extending to a distance greater than the diameter of the worm (Fig. 4I,J).

In a general burrowing cycle, the contractile peristaltic wave progresses anteriorly, advancing the lateral ridge forward as it passes and then expanding the head (Fig. 4A,H). When the peristaltic wave reaches the head (Fig. 4B,H), the head becomes narrow and begins to move forward (Fig. 4B–D,H), while the lateral ridge is expanded and stationary. Forward head movement is generally coincident with or occurs slightly after forward movement of the gut (Fig. 4C,D,H). A new peristaltic wave begins and progresses anteriorly past the lateral ridge as the gut moves posteriorly and the head expands (Fig. 4E–H).

Whereas some worms exhibited a regular, periodic alternation between head and lateral ridge expansions (pink and blue bars in Fig. 4H,I), sometimes the lateral ridge remained stationary while the head expanded (Fig. 5A–C). These events (red arrows pointing at overlapping pink and blue bars in Fig. 5A–C) generally coincided with large anterior movements of the gut (Fig. 5A), and expansion of not only the head (Fig. 5A) but also the entire body anterior of the lateral ridge (Fig. 5D,E). Expansions resulted in sediment grain displacements that appeared to occur in bulk and extend several grain diameters away from the worm (Movie 1). Very little anterior–posterior grain displacement was visible around burrowing worms when they were not exhibiting this anterior region expansion behavior (Movie 1).

Chaetae staining experiment

Observations of 21 individual worms with fluorescent chaetae burrowing in cryolite showed that segments were thinnest when moving forward and expanded once they reached their anterior-most position (Fig. 6; Movie 3). Observations were much clearer than videos, which were blurred by low light levels and worm movements, but videos clear enough to track the chaetae–body wall contact point were recorded for 8 individuals (with 2 separate segments for 2 individuals). All worms observed showed the same behavior, using a direct peristaltic wave to move forward (Fig. 6A–I). Anterior body expansions were not observed in these short video segments. Worms in recorded videos all burrowed very close to the surface of the thin layer of cryolite, in contact with the upper Petri dish, but their behaviors were indistinguishable from those of worms burrowing in the middle or lower part of the cryolite that could be visually observed but did not produce useable videos. The peristaltic wave was most distinguishable in the anterior 6–7 segments (shown in red and green in Fig. 6) and became less pronounced in posterior segments closer to the lateral ridge (blue and magenta in Fig. 6). Speed in the forward direction was negatively correlated with segment width for segments 3–7 ($P<0.01$; Fig. 6J), indicating that segments moved forward when they were narrow and slipped backwards when widest. This correlation disappeared for segment 8 (Fig. 6J), which showed less change in width than the other segments (Fig. 6I). This relationship was consistent across all 8 individuals, with variability in fit (R^2) for segments 3–7 likely attributable to varying quality of video as well as variability in periodicity of the burrowing movements (Fig. 6K). Pauses in movement would increase variability in this relationship, thus decreasing the R^2 of the regression (Fig. 6K). There was no correlation between segment width and speed for segment 8 (Fig. 6K).

DISCUSSION

Burrowing in different media

Observations of *T. mucronata* burrowing in different media suggest that the granular material remains solid rather than being fluidized around the burrowing worm and that the worms operate in a material with a narrow range of resistance to forces applied by burrowers. That slipping in the gel-bead mixture was not accompanied by bulk movement of fluidized grains indicates that the gelatin particles behaved like a solid that deformed through rearrangement of grains under the forces applied by the worms (see Dorgan et al., 2013). Considerably more movement of beads than cryolite grains was consistent with observations of burrowing in these two media by several species of orbiniid polychaetes (Francoeur and Dorgan, 2014). Increased bead movement is likely due to decreased friction between the spherical smooth glass beads, compared with the more irregularly shaped cryolite grains. Although I did not specifically test for the impact of grain shape on burrowing, qualitative differences in burrowing ability both between the cryolite grains before and after being smoothed in a rock tumbler and between cryolite grains and the smoother glass beads suggests that grain shape is likely important in burrowing in sands.

More generally, behaviors of burrowing animals in media that do not match their natural sediments should be interpreted with caution (see Dorgan et al., 2006). I attempted to compare kinematics of burrowing by *T. mucronata* with those described by Hunter et al. (1983) for *Polyphysia crassa* (Scalibregmatidae), which burrows in soft muds by direct peristalsis. The images show a direct contractile peristaltic wave with a narrower contracted region than that of *T. mucronata* (Hunter et al., 1983), but my attempts to determine from these images whether body wall movements of *P. crassa* occurred while segments were narrow or dilated were confounded by what appears to be backward slipping of the worm while burrowing. Worms were photographed burrowing in methylcellulose (Hunter et al., 1983), a highly viscous non-Newtonian fluid that behaves differently from the elastic muds in which these worms live. I have observed worms burrowing in methylcellulose, and although they do make forward progress, they exhibit considerable slipping and appear to struggle much more than in gelatin (K.M.D., unpublished data). Qualitative observations of the related *Scalibregma inflatum* burrowing in gelatin show forward head movements following

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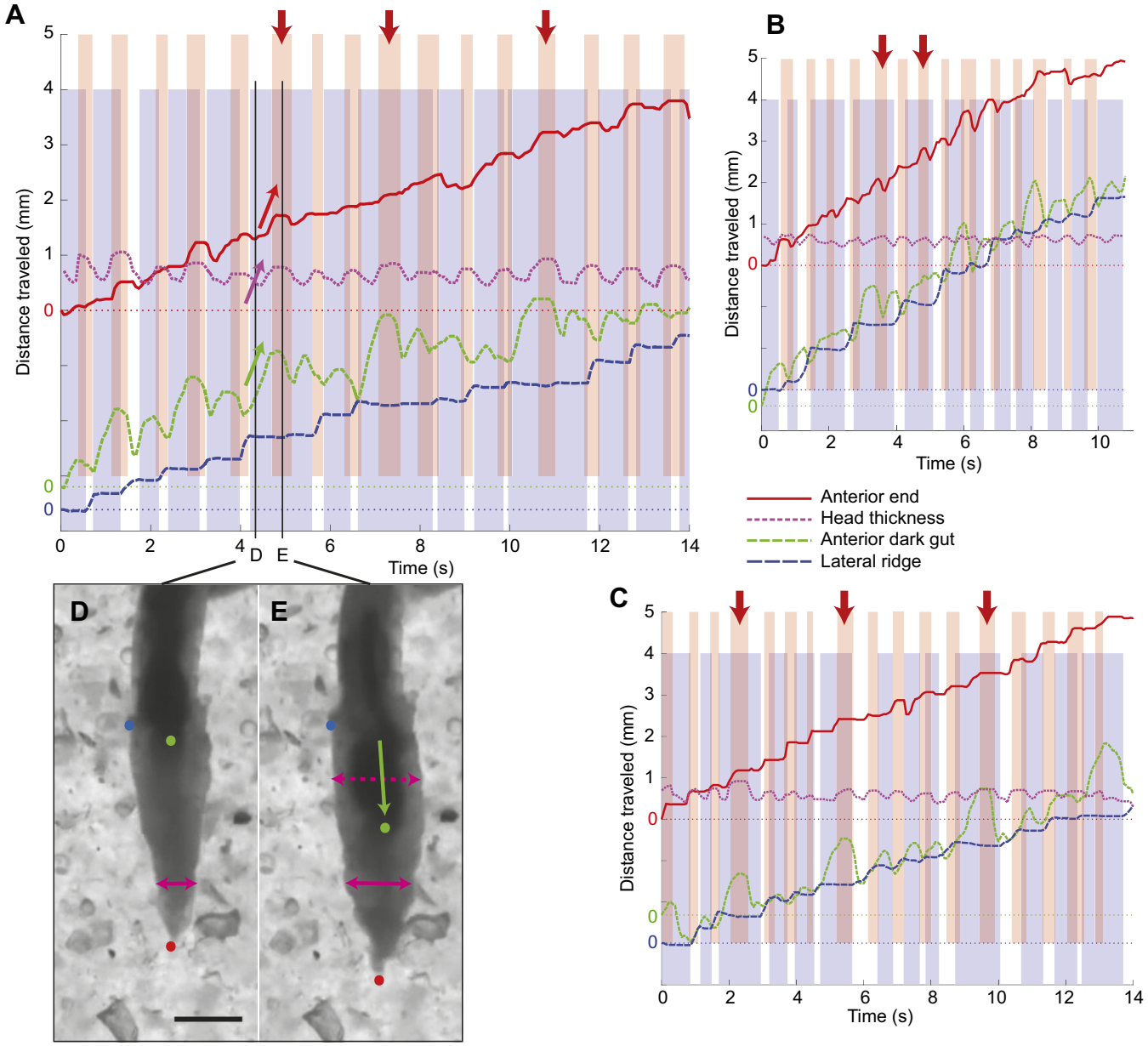

Fig. 5. Distance traveled by three individual *T. mucronata* burrowing in aquaria of cryolite and displaying occasional anterior body expansions. (A–C) Plots as described in Fig. 4H,I for three different individuals, showing distance traveled by the head (red), gut (green) and lateral ridge (blue) of the worm, with head thickness overlaid (magenta). Shaded pink (higher) and blue (lower) rectangles highlight the duration of head and lateral ridge expansion (anchoring), respectively. In contrast to the individual in Fig. 4, which alternated expansion of these two regions, at several times in each of these sequences (indicated by red arrows at the top) head expansions occurred (shaded pink) while the lateral ridge was stationary (shaded blue). Black vertical lines in A show times before and during the periods in which the head and lateral ridge were stationary, with corresponding images shown in D and E. Colored arrows on the graph highlight simultaneous anterior gut movement, head expansion and forward movement of the head preceding the period of stationary expansion shown in E. (D) Image of *T. mucronata* with narrow head and gut starting to move anteriorly. Colored dots show positions and arrow shows head width indicated in A. (E) Image of *T. mucronata* with lateral ridge and head stationary, gut at its anterior-most position (green arrow indicates movement from D) and head fully expanded (solid magenta arrow). The anterior region between the septum and lateral ridge is also expanded (dotted magenta arrow). Scale bar: 1 mm.

progression of the direct peristaltic wave to the head, consistent with the body wall moving forward when the segments are contracted (see movies 1 and 2 in Dorgan et al., 2016). Although forces were not measured, this behavior resulted in burrow extension by fracture, consistent with normal forces applied against the burrow wall (Dorgan et al., 2016).

The anterior expansions exhibited by *T. mucronata* (Fig. 5D,E) are similar to those exhibited by the orbiniid polychaete *Orbinia johnsoni* burrowing in cryolite (Francoeur and Dorgan, 2014). In both species, these expansions occur occasionally but not in every burrowing cycle and are accompanied by anterior movement of the gut (compare Fig. 5D,E in this study with fig. 5I–L of Francoeur and Dorgan, 2014). Although observations suggested that worms used these anterior region expansions more frequently when burrowing at greater depths or slower speeds (cf. Fig. 5A versus Fig. 4I), limitations of my experimental design prevented quantitative comparisons of expansion frequencies. Increased frequency of expansions with increased depth would indicate that these

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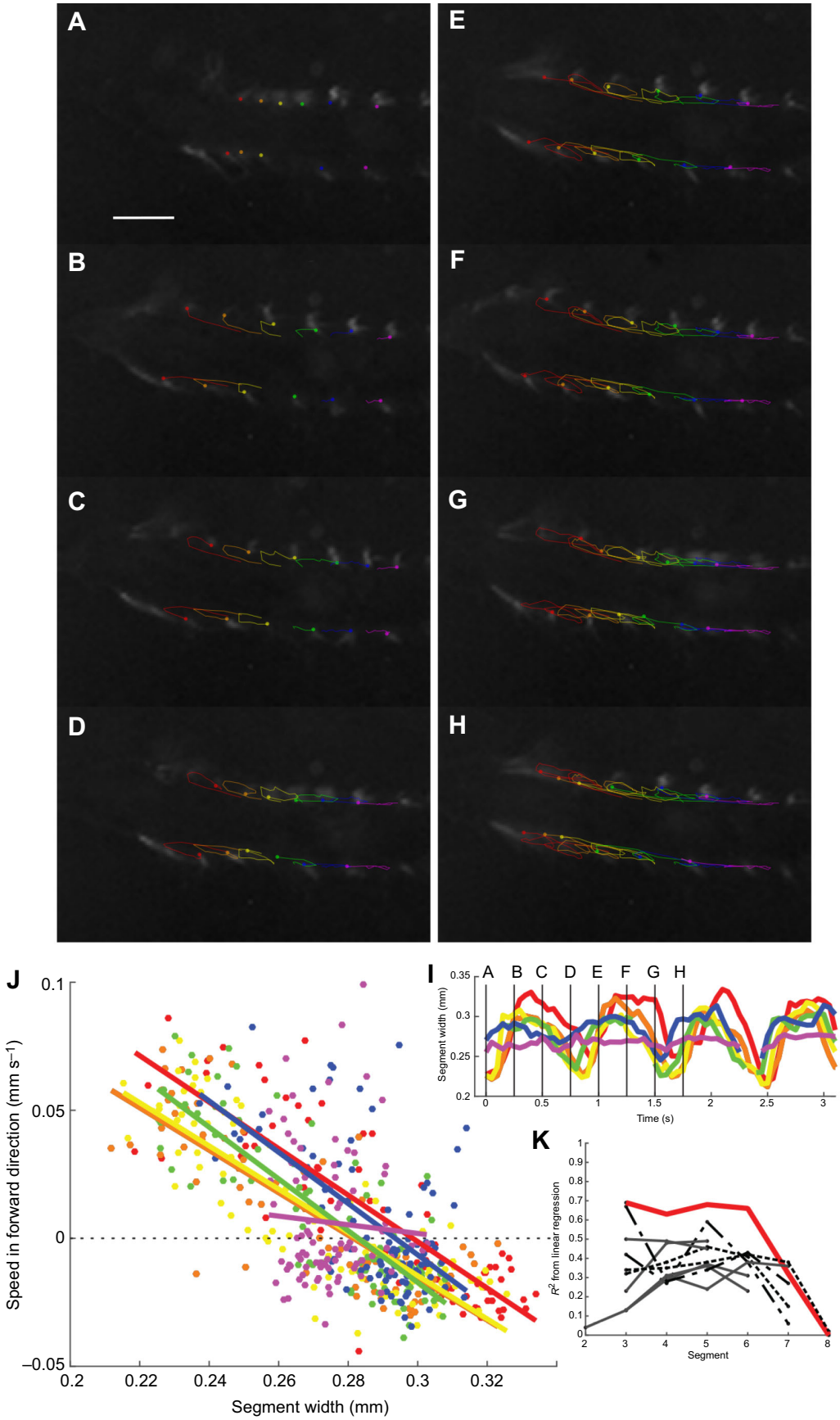

Fig. 6. See next page for legend.

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Fig. 6. Chaetae tracking. (A–I) Sequence of dorsal view images of *T. mucronata* burrowing in a Petri dish of cryolite (set-up shown in Fig. 3) with chaetae–body wall contact tracked (see Movie 3). Chaetae fluorescence appears white against a dark background of cryolite. Colored dots show the position of chaetae on the body wall in the current frame, with lines showing the tracked positions in previous frames. Different colored tracked positions correspond to different setigers, with setiger 3 in red and setiger 8 in magenta. (A) Initial image (0 s) showing setiger 3 (red) about to move forward (scale bar: 0.25 mm); (B) setiger 3 reaches its anterior-most position for this peristaltic wave and begins to expand (0.25 s); (C) setiger 3 slips backward and is wider than when moving forward (0.5 s); (D) setigers 5–8 (yellow–magenta) moving forward, with greater movement by setiger 8 corresponding to the direct (forward-moving) peristaltic wave (0.75 s); (E–H) setiger 6 (green) expanding (E, 1.0 s; F, 1.25 s; G, 1.5 s; H, 1.75 s). (I) Variations in segment width over time show setiger expansion progressing from the posterior-most setiger (magenta) to the anterior-most setiger (red), with a direct (anterior traveling) peristaltic wave. Colors correspond to those in tracked images and vertical black lines indicate the time of each image (A–H). (J) Speed in the forward direction was negatively correlated with setiger width for setigers 3–7 ($P<0.01$) but not for setiger 8 (magenta, $P>0.05$). Colored dots represent each setiger in a single frame of video, and solid lines show the linear regression for each setiger. (K) The negative correlation between speed in the forward direction and segment width shown for one worm in J was consistent with data for 7 additional worms measured. R^2 values for the negative correlation between forward speed and width of each segment from the plot in J are shown as a thicker red line. Gray and black lines represent 7 additional individual worms for which setigers could be tracked in video segments; all showed a negative relationship between speed and segment width. Two video segments were analyzed for each of two individuals (black dotted lines).

expansions are important in overcoming force resistance, which increases with depth. Force resistance through the cryolite, however, showed strong dependence on the history of disturbance (e.g. removing a worm after burrowing, which was achieved by fluidizing the cryolite with a pipet of seawater) (K.M.D., unpublished data), consistent with previous experiments showing a need for rigorous control of granular media for repeatable measurements (Sharpe et al., 2015b). Thus, linking the use of anterior region expansions with the material properties of the granular media is not possible based on these data. A link between expansion frequency and force resistance would be intriguing, but would not fully address the question of how these expansions are used to extend a burrow; specifically, whether these expansions apply normal forces to pack the burrow walls or are used to shear the burrow walls and displace grains. These body expansions do, however, appear to be a potentially mechanically important deviation from a periodic burrowing cycle, distinguishing peristaltic burrowing in sand from the more periodic burrowing cycles in mud (cf. *Cirriformia moorei*; Che and Dorgan, 2010).

Although *T. mucronata* burrows using a direct peristaltic wave, these results suggest that the classic description of peristalsis as the alternating contraction and relaxation of antagonistic circular and longitudinal muscles in the body wall around a hydrostatic skeleton (Elder, 1980; Kier, 2012) oversimplifies the kinematics of locomotion by *T. mucronata*. Alternating expansions of the head and lateral ridge (Fig. 4I) are consistent with descriptions of the dual-anchor burrowing mechanism described for burrowers across numerous phyla (Clark, 1964; Elder, 1980). The lateral ridge has long been an obvious character in identifying *Thoracophelia* spp. but the function has not been described (Rouse and Pleijel, 2001). These results show a clear anchoring function for the lateral ridge and raise questions about possible analogous structures in other soft-bodied burrowers. Considerable movement of coelomic fluid occurs during peristalsis (and can be visualized through movements of eggs in the coelomic fluid of reproductive females; data not shown). This is consistent with the limitation of direct peristalsis to aseptate worms: achieving forward movement with a forward-traveling peristaltic wave requires segments to change shape, with fluid traveling among segments as the peristaltic wave progresses (Elder, 1980). Many aseptate worms that use direct peristalsis do have one or a few anterior septa, associated with pharynx eversion (e.g. the gular membrane of arenicolids; Wells, 1954) or burrowing (e.g. scalibregmatids; Dorgan et al., 2016). However, in *T. mucronata*, the periodicity and extent of gut movements suggest that the gut may play an important role in increasing internal pressure in the anterior of the worm that drives head expansion and forward movement and that the gut musculature may work with body wall musculature during burrowing.

Peristalsis applies normal forces rather than shear

Chaetae tracking shows clearly that the body wall of *T. mucronata* moves forward while segments are narrow (Fig. 6J), and that segments expand once stationary to apply normal forces while burrowing. These normal expansions are similar to those of burrowers in muds that apply normal forces to extend a burrow by fracture (Dorgan et al., 2005). This is consistent with the idea that anterior expansions may be important in overcoming resistance to burrowing that increases with depth. These normal forces likely pack sand grains around the burrow walls, which increases both burrow space and stability. Peristaltic movements that apply normal forces also minimize shear forces and consequently minimize friction. Although I did not directly measure shear forces, that no shear strain was observed suggests that shear forces were at least low enough to not exceed the yield stress in the sand. Sands have larger grains and therefore larger surface roughness than muds and have less organic material that may act as a lubricant. More importantly, however, many sands are dilatant (Duran, 2000), so shear stresses applied along the burrow wall large enough to result in shear strain would cause expansion of the sand around the worm. This expansion is required for previously packed grains to slide past each other as the surface layer of sand is sheared past the stationary subsurface layer. Expansion of the surrounding sand is clearly counterproductive to burrow formation. Friction reduction may be important for other sand burrowers as well: ejection of water from the mantle cavity of bivalves (see Trueman, 1968) may be more important in lubricating shell movement than fluidizing sand. The production of mucus by soft-bodied burrowers in both muds and sands likely reduces friction in both media. Although I did not explicitly consider mucus in this study, I did observe that burrows of *T. mucronata* are lined with a very thin layer of mucus. Worms were kept in a dish with a thin layer of sand between collection and experiments, and they often left behind very fragile burrow structures held together by mucus that disintegrated when touched (K.M.D., personal observation). This thin layer of mucus presumably prevents burrow collapse and may also reduce friction. Although I did not measure internal body pressures of or forces applied by *T. mucronata*, the head expansion and anterior expansion behaviors seem ideally suited to apply large forces, in contrast to other direct peristaltic burrowers, e.g. Scalibregmatidae, that apply fairly low pressures over a large region of their body in very soft sediments (Elder, 1973).

Application of normal rather than shear forces clearly distinguishes peristaltic burrowers from the undulatory burrowers in sands whose mechanics and kinematics have been much more extensively studied. Application of resistive force theory (RFT) to undulatory burrowing has allowed prediction of optimal locomotory performance based on variables such as the extent of body curvature

(amplitude:wavelength) (Maladen et al., 2011) and body length (Sharpe et al., 2015a). The RFT model is based on the assumption that the body can be divided into infinitesimal elements that act independently, and applicability of the superposition principle means that the net force on the body (drag+thrust) can be calculated by summing the perpendicular and parallel components of force acting on each segment, knowing the orientation of the segment (Maladen et al., 2009). Application of RFT to peristaltic burrowing is challenging for several reasons. Kinematics of *T. mucronata* suggest that as the peristaltic wave travels, forces applied by individual segments vary considerably, with narrow moving segments applying much smaller forces than stationary expanded segments. The aperiodic anterior expansions contribute to the variability in forces applied along the body. This variability would complicate application of measured drag forces to locomotory kinematics as has been done in previous applications of RFT to burrowing in granular media (see Maladen et al., 2009). Thrust is likely generated by a small backward-oriented component of the normal force that prevents backward slipping during peristalsis, but measuring this component would require novel methods beyond the scope of this study. Similarly, drag forces are likely small and depend on the proximity to and thickness of dilated stationary segments relative to the narrow moving segments. The traveling peristaltic wave indicates that forces on one segment are not independent of those on other segments. Thus, these results show that peristaltic burrowing by *T. mucronata* violates several assumptions of RFT and that practical application of RFT to peristaltic burrowing is complicated by the variable and small forces applied. Understanding the kinematics of peristaltic burrowing in sands is a critical first step, however, in either applying RFT or developing new theory to assess peristaltic locomotory performance and to quantitatively compare the locomotory performances of peristaltic and undulatory burrowers.

The strategy and limitations of burrowing by peristalsis in sands suggested by these data are consistent with the abundance of *T. mucronata* in a narrow habitat range in the upper intertidal of dissipative (flat) beaches where sands are compacted (McConnaughey and Fox, 1949; McLachlan, 1990). This zone remains fairly constant over tidal cycles, with worms burrowing deeper as the tide comes in and shallower as the tide goes out (McConnaughey and Fox, 1949), but over longer periods (months) as beach morphology changes, worms move horizontally seaward or landward to remain in the swash zone (Seike, 2008). Comparison between burrowing performance in cryolite and gelatin grains suggests that peristaltic burrowing is limited to a narrow range of force resistances of sands that allows for both compaction as the anterior expands and anchoring by an expanded region without slipping. In contrast, undulatory burrowing is effective in both media because forces applied in the direction opposite to locomotion are effective over a much broader range of resulting deformations (Dorgan et al., 2013). The advantage of peristalsis over undulatory locomotion, however, is a reduction of friction and dilatant expansion of sand, suggesting that peristalsis may be a more energetically efficient but less versatile mode of locomotion in granular media. For deposit-feeders on very low quality sand that require substantial movements to maintain high gut throughput times (Kemp, 1986), minimizing the energetic cost of locomotion may outweigh being limited to sands with a higher force resistance. The narrow, mid- to high-intertidal zone of compacted sand in which *T. mucronata* are found experiences the highest volume of seawater filtered through the sands and therefore the highest influx of particulate organic material (Kemp, 1986; McLachlan, 1990; McLachlan and Turner, 1994). The high abundance of *T. mucronata* applying normal forces to compact sand in this compacted region raises questions about potential feedback between burrowing activities and the filtering function of beach sands. It would be interesting to test whether the combination of compaction and burrow formation in sands inhabited by *T. mucronata* results in higher permeability but lower porosity and therefore greater stability than sands without worms.

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