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Compartmentalization of microbial communities that inhabit the hindguts of millipedes

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ABSTRACT

The gut lumen of the arthropod detritivore provides hospitable and multifaceted environments for diverse assemblages of microbes. Many microbes, including trichomycetes fungi, bacteria, and archaea establish stable, adherent communities on the cuticular surface secreted by the hindgut epithelium. Regional differences in the surface topography within the hindgut of a given millipede are reflected in differing and diverse microbial assemblages. The spirostreptid millipede *Cambala speobia* is a detritivore found on the floors of Texas caves. This millipede species has a very circumscribed distribution in North America and a diet confined to the limited litter that accumulates on floors of these caves while the common julid millipede *Cylindroiulus caeruleocinctus,* an introduced European species, feeds on the diverse litter found in organic soils throughout North America. In both millipedes, the gut lumina are inhabited along their entire lengths by microbes, with the highest microbial densities in the hindguts. The anterior third of the hindgut with its distinctive six-fold symmetry is lined by cuticle having fine polarized scales, and the posterior-most third is lined by smooth cuticle. Trichomycetes only inhabit the anterior third of the hindgut, and scattered patches of filamentous bacteria along with their smaller adherent microbes occupy the posterior third. The densest populations of microbes inhabit the central region of the hindgut. Over the cuticular surface of this hindgut region, uniformly distributed indentations mark possible channels for nutrient and water exchange between the hindgut lumen and host hemolymph. Films of microbes are adherent to the cuticle that lines the hindgut while those microbes in the remainder of the gut (*i.e.,* foregut + midgut) represent mostly unattached inhabitants.

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1. Introduction

Arthropod detritivores consume diets that are often low in nitrogen and protein yet high in the complex polysaccharides and lignin polymers of phenylpropane found in plant cell walls and the chitin polymers of N-acetylglucosamine found in fungi and invertebrate cuticles. Digestion of these plant polymers and chitin polymers requires the action of a variety of cellulases, chitinases, and lignin-degrading enzymes that are produced primarily – or entirely – by microbial sources, probably making the symbiotic microbes essential partners in utilizing the lignocellulose substrates of their arthropod hosts. If the nutritional requirements of these detritivores are not entirely dependent on the enzymatic activities of their microbial symbionts, the diets of detritivores are at least supplemented with nutrients generated by cellulase and chitinase activity of their resident microbes (Geib et al., 2008). Some of these bacteria hosted by detritivores, in addition, fix atmospheric nitrogen and provide a source of nitrogen to an otherwise nitrogen-deficient diet (Brune and Dietrich, 2015; Ceja-Navarro et al., 2014; Nardi et al., 2002). This descriptive study reveals host landscapes within the alimentary canals of a diverse order of arthropods – the millipedes – that offer spatially differentiated habitats for their microbial inhabitants. The specific spatial distributions of diverse microbial assemblages within the millipede hindgut offer an appreciation for the multitrophic interactions that exist among microbes inhabiting this complex landscape.

While insect detritivores, such as termites, beetles, crickets, and cockroaches, have guts that are longer than their body lengths and are often folded and convoluted to fit within their body cavities

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(Brune, 2014, 1998; Nardi et al., 2006; Ulrich et al., 1981), the alimentary canals of millipedes are straight along their entire lengths and do not exceed the lengths of their bodies. Like all arthropods guts, millipede guts are delineated into three distinct regions – foregut, midgut, hindgut – with the midgut exceeding both the hindgut and foregut in length. The foregut represents the shortest of the three gut regions. The midgut epithelial cells and their associated regenerative cells have been described for several millipede species (Sosinka et al., 2014). The special relationship between midgut epithelial cells and hepatic storage cells appears to be a novel feature of millipede guts observed in no other arthropods (Nardi et al., 2016; Hopkins and Read, 1992). The midgut-hindgut interface is marked by the transition from a lumen lined by microvilli to a lumen lined by cuticle and is the site for attachment of the two long Malpighian tubules. The hindgut cuticle offers the gut habitat and substrate that is most densely and diversely populated by microbes (Byzov, 2006; Nardi et al., 2002; Cazemier et al., 1997; Cruden and Markovetz, 1987).

The common troglobitic cave millipede *Cambala speobia* (Spirostreptida: Cambalidae) has been shown to occupy a basal trophic position in cave ecosystems (Taylor et al., 2007), presumably feeding upon detritus represented by guano of cave crickets (Taylor et al., 2005) and bats along with the microbial community growing upon this guano. In addition to having access to this circumscribed diet, this millipede species has a restricted distribution in the caves of central Texas. By contrast, the widely distributed julid millipede *Cylindroiulus caeruleocinctus* (Julida: Julidae) is a common detritivore that occurs in habitats offering detritus from a wide range of sources and is also known to feed on certain root crops and seeds (Koprdova et al., 2010). These two species of millipedes from different orders differ dramatically in the range of their habitats and the diversity of their diets.

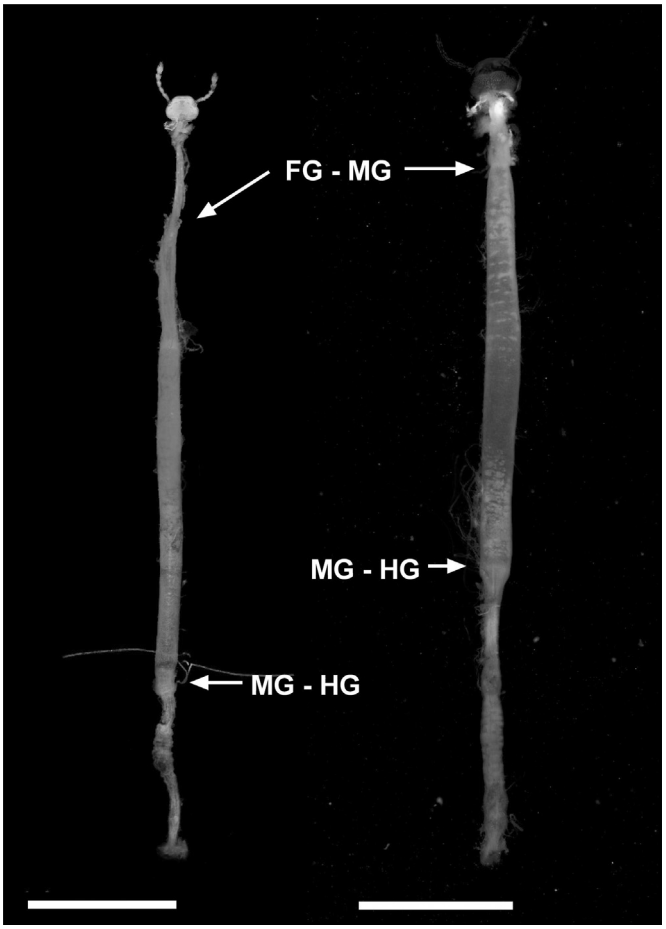

Fig. 1. The relative proportions of the alimentary canals devoted to foregut (FG), midgut (MG) and hindgut (HG) are displayed in these whole mounts of the gut epithelia of *Cambala speobia* (left) and *Cylindroiulus caeruleocinctus* (right). Bars = 5.0 mm.

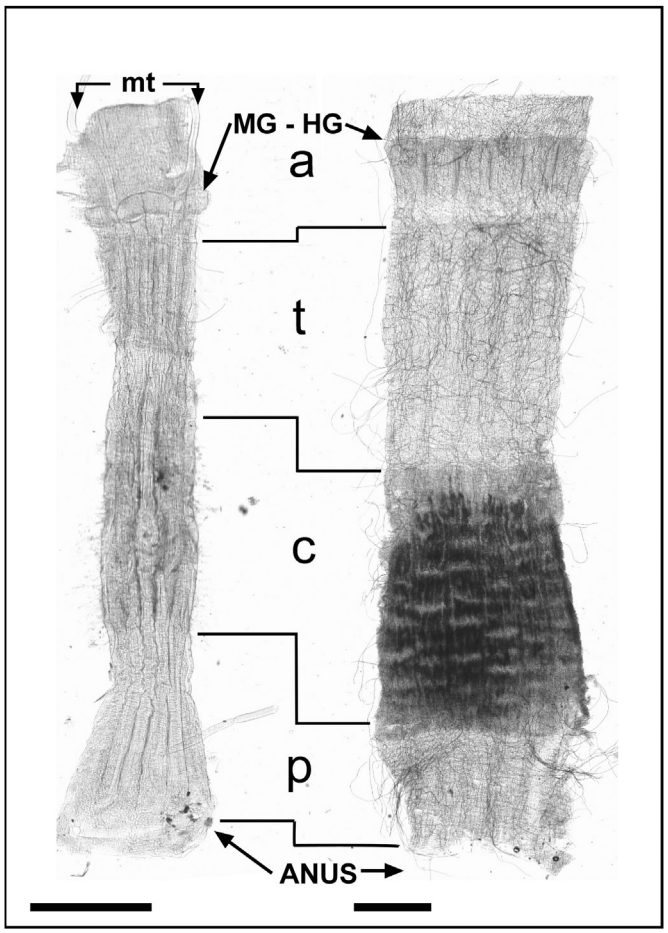

Fig. 2. Luminal surfaces of cylindrical hindgut epithelia have been exposed as a two-dimensional sheet by cutting the cylindrical epithelia along their anterior-posterior (a-p) axes. Anterior is at top. The midgut-hindgut (MG-HG) interface lies at the top of the figure; the anus lies at the bottom. Compartmentalization of microbial inhabitants into clearly delineated anterior (a), central (c) and posterior (p) regions along each a-p axis corresponds to a compartmentalization of landscape features of hindgut cuticle. A less clearly defined transition region (t) lies between the anterior and central compartments. *Ca. speobia* (left) and *Cy. caeruleocinctus* (right). Malpighian tubules (mt) are marked in left specimen. Bars = 1.0 mm.

Microbes are known to be associated with the guts of the almost 30 species of millipedes that have been examined (Šustr et al., 2014; Thompson et al., 2012; Knapp et al., 2010; Byzov, 2006; Nardi et al., 2002: Cazemier et al., 1997; Hackstein and Stumm, 1994). These millipedes include species in several orders, including the two orders (Spirostreptida, Julida) represented in this manuscript. This manuscript represents the first ultrastructural description of the hindgut where the majority of microbes reside. These microbes inhabit a sheltered, highly structured environment that is regionally differentiated along its anterior-posterior axis. In both species of millipedes examined in this study, the global distribution of gut microbes is reflected in regional differences in topography of the hindgut cuticle.

An abundance of studies catalog the diverse microbial composition of entire guts of arthropods. An ultrastructural study

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