TRILOBITE LARVA SENSILLA AS INDICATORS OF THE SOUNDSCAPES IN COASTAL ZONES DURING THE JURASSIC

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INTRODUCTION

During the SARS-COVI-2 Pandemic, research work was curtailed at the Smithsonian Institution's U. S. National Museum of Natural History. During this interval, the author took the opportunity to study archived specimens of trilobite larva, collected in the nineteenth century, and juvenile specimens of the marine chelicerate *Limulus polyphemus* (Linnaeus, 1758). A summary of these studies placed in the context of previous research is presented here.

There are a handful of marine organisms which have maintained their external morphology for prolonged periods of time, spanning periods of major extinction. These creatures are termed "Stabilomorphs" One such creature is the Horseshoe crab, genus *Limulus.* This genus has survived morphologically unchanged for 142 million years. Fossils of *Limulus Darwini from the Jurassic* and the extant *Limulus polyphemus* (Linnaeus,1758) are anatomically indistinguishable. Of interest to bio-acousticians is the fact that members of the genus *Limulus* have well developed mechanoreceptors whose morphology and associated neuron connections indicate that they can detect particle motion, near field sound. In addition to proprioceptors in the walking legs capable of detecting vibrations in the solid substrate, three types of innervated sensilla, tactile sensilla, "Peg sensilla" optimized for responding to particle motion and water currents, and a third type with a double helix structure found on the trilobite larva of *Limulus.* The latter mechanoreceptors are not found in other marine chelicerates, the Pycnogonida, or any other marine arthropod. It is proposed that they are vestigial organs.

Sensilla, which are used to detect near field sound energy, particle motion must protrude through the boundary layer (δ) for particle motion in water. The size of the helical sensilla indicates that they are functional in detecting near field sounds in water of frequencies ≥ 0.1 kHz. The Peg sensilla on the prosoma are believed to be active in detecting both near field sound energy at higher frequencies than the helical sensilla and water currents.

The horseshoe crab *L. polyphemus* is often referred to as a "living fossil." The genus *Limulus* O.F. Müller, 1785, has been present in the fossil record for 142 million years with no change in its external morphology[1-2]. The term "stabilomorphs" has been used for such taxa that have survived unchanged through one or more mass extinction events, for which *L. polyphemus* qualifies[2]. The genus represents a window into life in the distant past. This study describes the mechanoreceptor organs in the trilobite larva of *L. polyphemus* and considers their potential functions. Because the function of sensilla is closely related to their morphology and these animals have not significantly changed morphologically since the Jurassic we may also make inferences as to the soundscape in that geological era.

Horseshoe crabs have an amphibious lifestyle with the adults coming ashore to lay their eggs on sandy beaches at the high-water mark of a spring tide. The fertilized eggs undergo embryonic development and molt four times before hatching[3]. On subsequent Spring Tides when the eggs hatch the trilobite larva crawl to the surface of the sand and move into the ocean[3]. The first instar after hatching is called the trilobite larva because of its resemblance to the members of the class Trilobita which became extinct at the end of the Paleozoic Era.

Peg sensilla like those found on the dorsal carapace of the trilobite larva are adapted to sense water currents and near-field sound energy in the form of particle movements [4 -8]. Such mechanoreceptors have been shown to be capable of functioning in near-field sound communication between animals[8]. Kaplan et al.[9] established the presence of mechanoreceptors on the dorsal carapace of *Limulus* with a morphology and innervation pattern comparable to those found on crustaceans. Sensilla with a helical form were present on the pedipalps, second and third walking legs. and pusher on the ventral side of the prosoma of trilobite larva[4 & 5]. Some of these helical sensilla form a double helix structure by twisting around adjacent sensilla (Fig. 3).

Materials and Methods

Fifty trilobite larvae of *L. polyphemus* were collected from Reeds Beach, Cape May, New Jersey, USA (39.1186°N, 74.89°W) in 1888 and fixed in 5% formaldehyde at the time of collection, stored in 70% ethyl alcohol. The specimens were dehydrated in 75%, 90%, and 100% ethyl alcohol. After being critical-point dried, the larvae were coated with gold palladium alloy[4 & 5]. Samples of the prosomal carapace of a juvenile specimen, 160 mm in length, which had also been preserved in 70% EtOH were prepared in the same fashion for study with the Scanning Electron Microscope. The images were produced In the Imaging laboratory of the U. S. National Museum of Natural History using a Zeiss EVO MA15 Electron Microscope (Zeiss, Tokyo, Japan). All measurements were made from the electron micrographs[4 & 5].

Results and Discussion

The mechanoreceptors of adult horseshoe crabs, *L. polyphemus*, received close attention in the twenty-year period from 1956 to 1976. The existence of proprioception organs in the adult legs was shown to exist and be associated with leg joints[9]. Pressure sensing organs in the chelae at the ends of the first five prosomal appendages were shown to be present[10]. The lateral margin of the prosomal carapace was shown to have tactile sensilla along its margin [11]. The presence of sensilla capable of detecting water currents and particle motion dispersed over the prosomal carapace were also shown to be present[9]. The mechanoreceptors, however, have received little attention for a half century until recently[4 & 5].

Three forms of mechanoreceptors were described on the Trilobite larvae of *L. polyphemus*, (1) tactile sensilla on the lateral margins of the prosomal carapace, (2) peg sensilla on the dorsal surface of the prosomal carapace (Figure 1) and (3) helical sensilla (Figure 2)[4 & 5]. The tactile sensilla were 80 µm long and a maximum of 5 µm in diameter at their base.

Peg sensilla 30 µm to 100 µm long with a diameter of 1 µm to 2µm arising from a socket of about 5 µm in diameter were found on the dorsal surface of the carapace near the lateral ridge and compound eye[4 & 5]. The peg sensilla are capable of detecting near field sound energy at frequencies ≥ 0.5 kHz. The helical sensilla arose from a socket about 10 µm in diameter and were 123 µm to 170 µm long and 5 µm in dimeter at their base. The length of a single helix varied from 17 µm to 55 µm[4 & 5]. The helical sensilla are capable of detecting near field sound energy at frequencies ≥ 0.1 kHz (Graph 1).

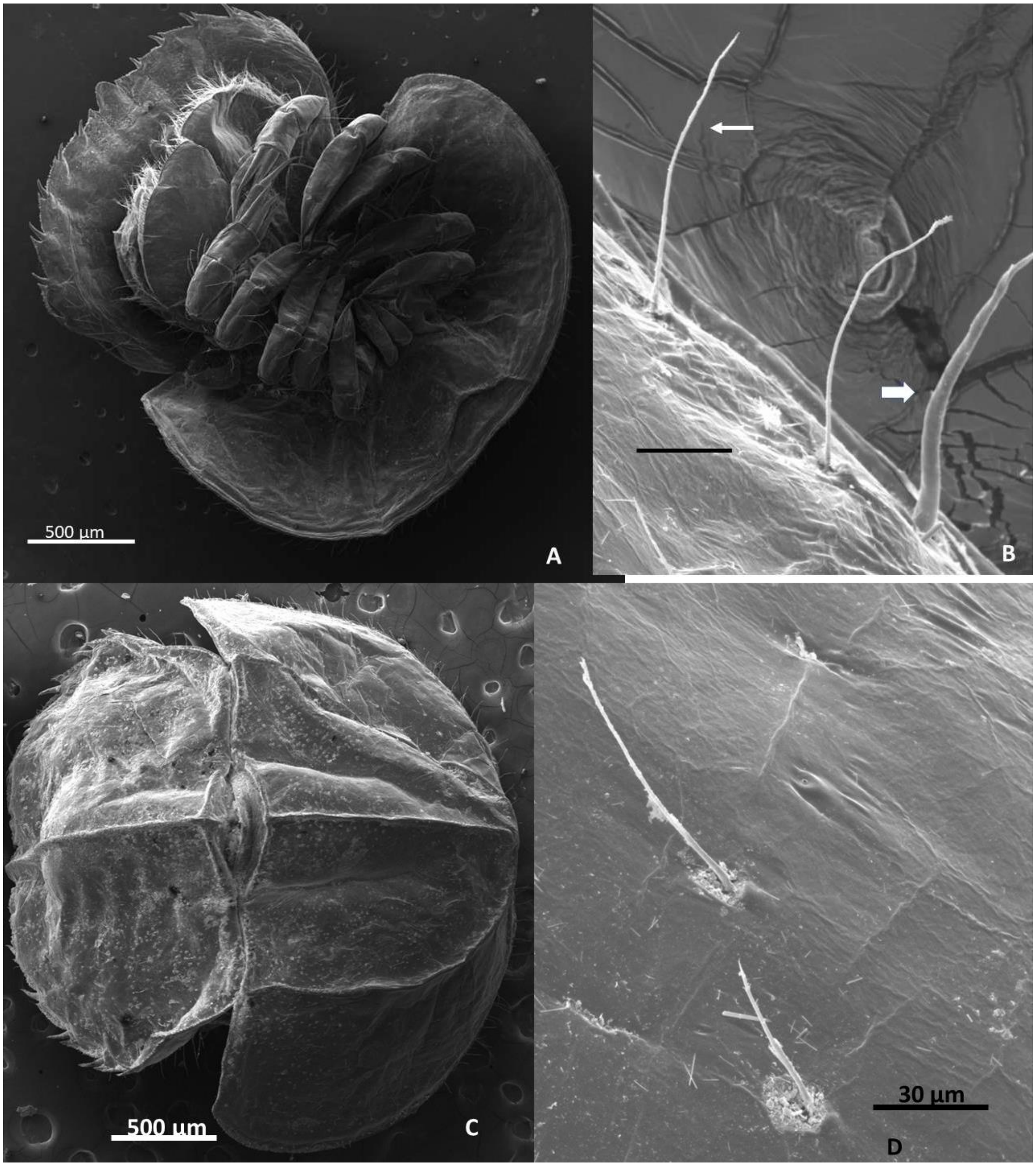


Figure 1. (A) A ventral view of the trilobite larva of *Limulus.* (B) peg sensilla and tactile sensilla on the carapace of the prosoma. The thin arrow is a peg sensilla capable of sensing near field sound and the thick arrow is a tactile sensilla (C) a dorsal view of the trilobite larva of *Limulus.* (D) An enlarged view of the peg sensilla on the dorsal carapace of the prosoma showing the sensilla and the socket in the carapace's exoskeleton.

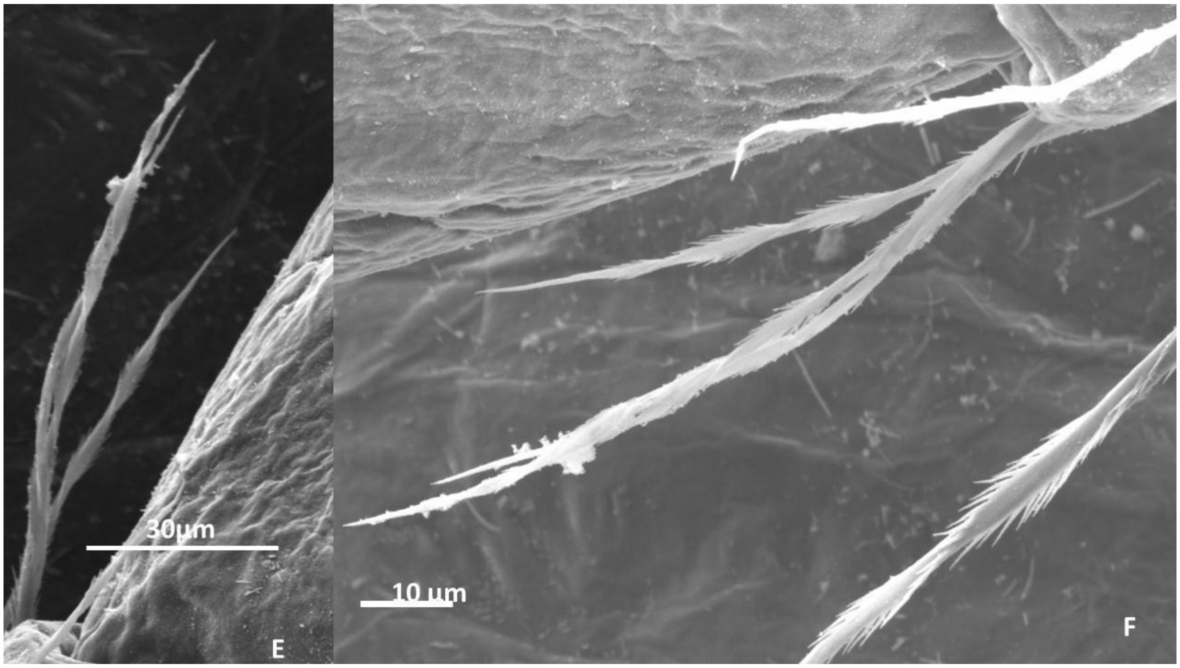


Figure 2. [E] An enlarged view of a double helix sensilla on the walking legs of trilobite larvae. [F] A detailed view of a double helix sensilla and a single spiral helix sensilla.

The “Peg Sensilla” are sensitive to near field sound energy. The frequencies v which they respond to are related to the length of the sensilla[12 & 13]. Two different models for the response of peg sensilla to near field sound frequencies, v. The first by Barth[14] holds that the sensilla must extend out of the boundary layer of the frequency, v, being detected. This boundary layer is defined by the equation [kinematic viscosity/ v]$^{1/2}$ (Graph 1). This was derived for nearfield sound energy detection by spiders [14]. A second model derived by Fields, & Weissburg[15] for copepod sensilla proposed that $v \sim L^{-3}$, where L is the length of the sensilla. In both models long sensilla detect low frequencies and short sensilla detect high frequencies.

The peg sensilla and tactile sensilla are present on the Pycnogonida, Xiphosura, and Crustacea, in short, all marine arthropods[4 – 8, 11, & 15]. As such they represent a primitive structure. We may infer therefore that to a certain extent, that the near field sound scape of the Jurassic seas, was much like that of the present coastal seas. The presence of the helical and double helical sensilla which are interpreted here as vestigial organs tells us that there was a component of the Mesozoic near field sound scape that was not present today.

Most studies of ocean soundscapes are conducted using pressure sensitive hydrophones. That is, they are far field soundscapes. The nearfield soundscape is rarely measured and while particle motion may be calculated from the pressure fluctuations of far field soundscapes. This is only possible in open ocean conditions, and not in the shallow coastal waters where *Limulus* is found[16]. The soundscapes experienced by arthropods, such as *Limulus* are near field soundscapes where particle motions are the dominant form of acoustic energy, not pressure fluctuations. They are consequently sensitive to sound sources which are close to the animal, centimeters to tens of meters.

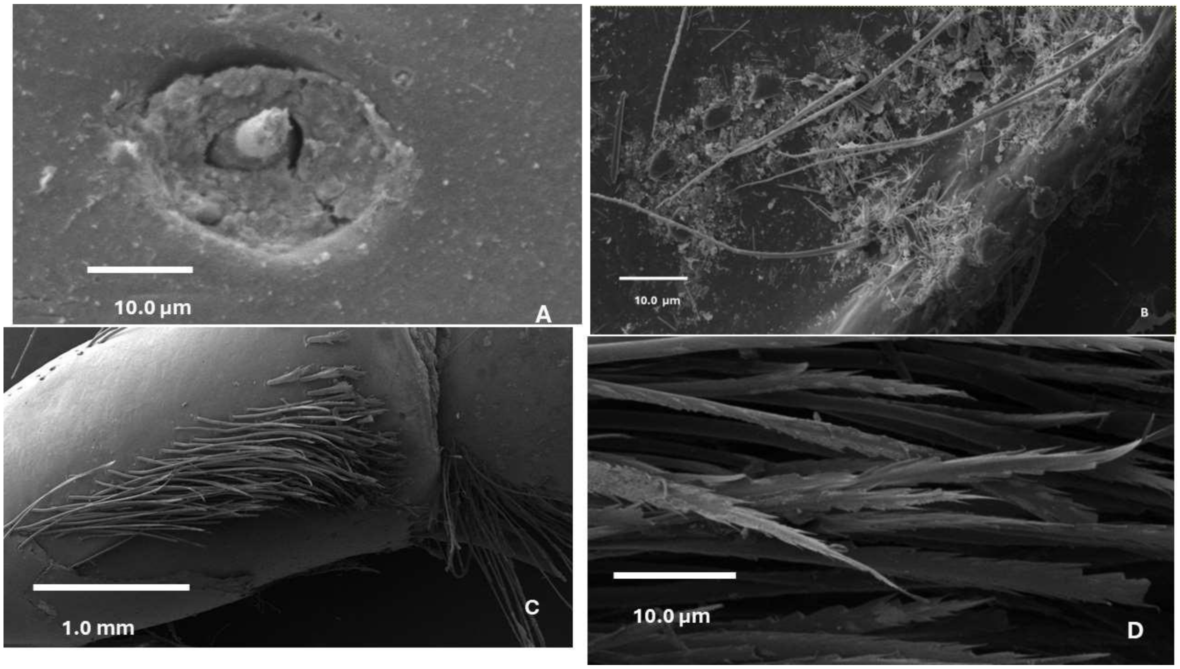
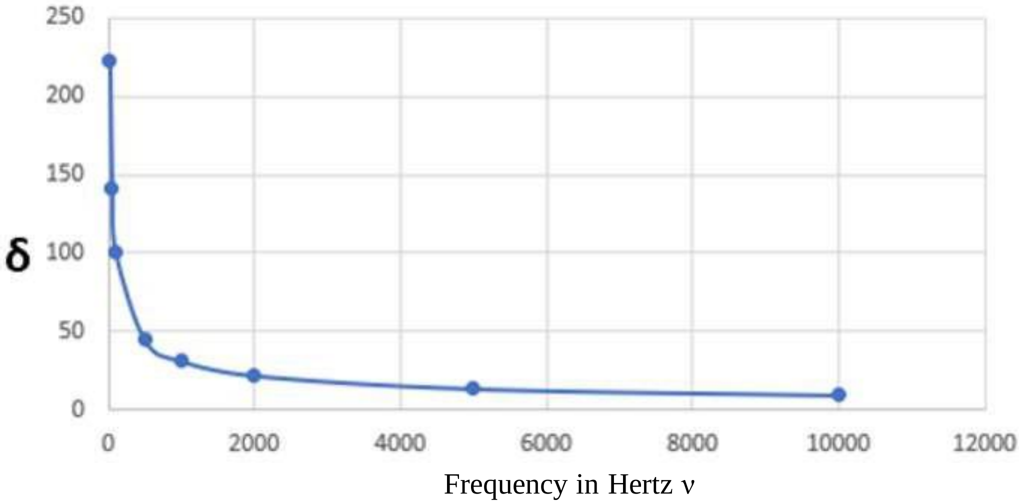


Figure 3. These are mechanoreceptors found on a subadult *Limulus polyphemus.* [A] is a large sensilla of the type reported by Kaplan et al[9]. [B] these are peg sensilla like those found on the trilobite larva. {C] these are large sensilla found in the location of the helical sensilla found on the larva. [D] this is an enlargement of the sensilla in [C].



Graph 1. The boundary layer δ as a function of frequency in an aqueous medium. $\delta = [\text{kinematic viscosity}/ \nu]^{1/2}$ [after Barth[14]].

The mechanoreceptors found on the juvenile specimen include the large sensilla first reported by Kaplan et al. (Figure 3 A)[9]. These large sensilla are found near the median eyes and the larger lateral compound eyes. Kaplan et al. showed these mechanoreceptors to be sensitive to particle motion and water currents[9]. First reported in the current study are the “Peg Sensilla” many tens of µm long and thus potentially capable of detecting high frequency near field sound energy. The sensilla present on the juvenile pusher legs (Figure 3 C & D) are typical of those found on many marine crustaceans[11-14]. These sensilla are morphologically distinct from the helical and double helical sensilla found on the pusher legs of the trilobite larval stage. This an indication that the helical and double helical sensilla found on the trilobite pusher appendages are vestigial organs.

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