# 3D reconstruction of tracheal systems in one-eared and two-eared praying mantises

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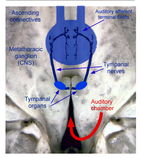
## 1. Most praying mantises have a single ear

Approximately 80% of praying mantis species, like the *Sphodromantis aurea* to the left, possess a single ear in the midline of the ventral metathorax. Wing length and hearing are tightly correlated. With only a few exceptions, long-winged mantises have sensitive hearing. All mantises with reduced wings have minimal hearing or are deaf.

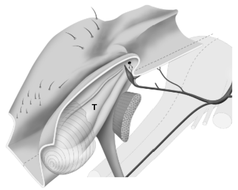
The ear can be seen between the metathoracic coxae. Externally, it is a 1-2 mm slit with two cuticular knobs at the rostral end (the * in the middle photograph). The slit is actually the opening to an auditory chamber whose walls contain the tympana. The acoustics of the chamber double the sensitivity and shape the tuning of the ear.




Despite its unique location, structure, and appearance, the mantis ear has the same fundamental components shared by all pressure-detecting ears, vertebrates included. Sound pressure waves cause the movement of a thin membrane (tympanum). Sensory neurons in a tympanal organ (TO) convert the movement to neural signals that go to the CNS for processing. This mechanism will not work, however, without an airspace behind the tympanum to allow the membrane free motion.

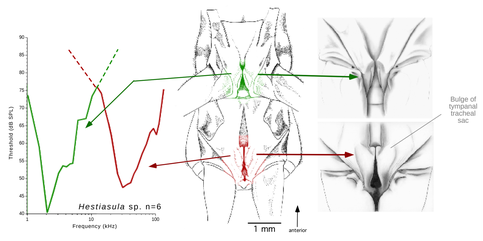



The metethoracic ear has all three: the thinned oval tympanum (T), the TO and tympanal nerve (black), and a large, apposed tracheal sac (shown partially cut away. The bulges in the ventral cuticle around the ear outline the tympanal tracheal sacs (see next box).



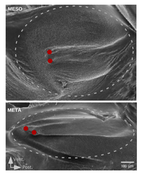

## 2. A few mantises have two ears, but not a pair

Several mantis lineages have independently evolved in the mesothorax a second complete auditory system. It is serially homologous, but does dot nave an auditory chamber. Instead of ultrasound, it hears only 2-4 kHz sounds. The MESO ear is anatomically, bioacoustically, and functionally independent from the META ear. For mantises, the ventral midline must be uniquely suited as a site for an ear.




The separate evolution of two auditory systems in the same animals provides a unique opportunity to study the precursors of auditory structures and the pathways by which they become specialized for hearing. The two mantis ears specialize in two very different frequency ranges, which adds potentially revealing bioacoustic constraints to the process.

The tympana (dotted boundaries) of the two ears differ primarily in shape. The MESO is broad, almost-drum-like, and the TO attachments (red dots) are near the center. The META is a narrow oval, and the TO attachments are at the rostral end.



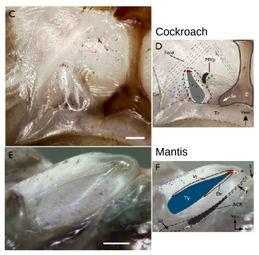

These are SEMs of casts of the tympana made by fillling the auditory chamber with Mercox. Therefore, the hills in the casts are actually valleys in the tympana. The casts provide <2 µm resolution with virtually no distortion.

## 3. What do mantises have that cockroaches don't?

Cockroaches are mantises' closest relatives, but the do not hear sounds above 1 kHz and do not have midline ears. Are there homologous precursors to the components of the mantis' ear?

Region of the cockroach methorax homologous to the ear region of mantises.

A close examination of the cockroach metathoracic midline shows an organization parallel to the wall of the mantis' auditory chamber. A key feature is the single chordotonal organ, the necessary precursor to the TO (attachment at red dots).

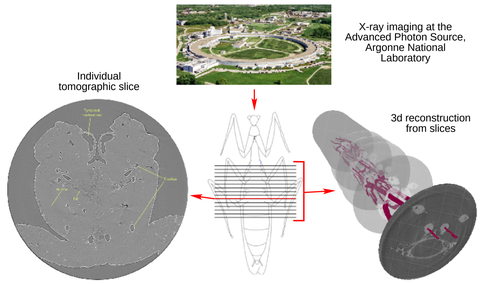



A rotation and of the existing structures and folding toward the midline would make an auditory chamber. The neural component is already present as is the thinned membrane necessary for the tympanum. However, the third component necessary for sensitive hearing, an air space behind the tympanum, is missing in the cockroach.

Tracheal system modification is a critical element in the transition from earless to eared. The goal of this project is to define the the tracheal system of praying mantises, focusing on the adaptations associated with hearing.

## 4. Synchrotron x-ray micro-CT scanning to study mantis tracheal systems

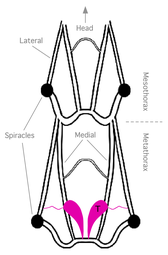
The gross anatomy of the tracheal system is difficult to study because of the distortion introduced by all conventional methods. Linear and volume measurements are problematic. We have used synchrotron x-ray micro-CT imaging to overcome those limitations. At the Advanced Photon Source at Argonne National Laboratory, the mantis is imaged at 0.125° increments as it rotates in an x-ray beam. The 1440 projection images are converted to a series of tomographic slices (akin to traditional histological sections) with 3-6 µm resolution and minimal distortion. From these we can generate 3D reconstructions of the tracheal system.

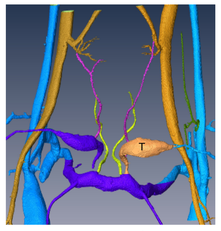



We focused specifically on comparisons between the MESO and META ear regions of one-eared (*Parasphendale agrionina*) and two-eared species (*Creobroter* sp. and *Pseudocreobotra ocellata*). We also examined changes in the tracheal systems during development for both types of mantis.

## 5. The thoracic tracheal system and metathoracic ear

The common pattern of tracheal distribution in the thorax of all mantises is a series of large longitudinal tubes (ventral, lateral, and dorsal groups) with periodic cross-connections between left and right. All of the longitudinal tracheal connect directly or indirectly with openings to the outside (spiracles) for gas exchange.



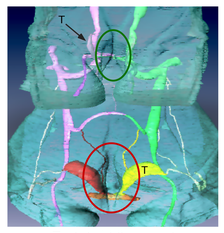




Early nymph

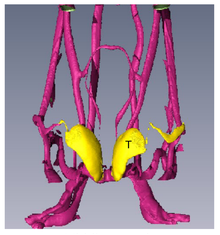
Adult tympanal tracheal sacs (T) are predominantly longitudinal and at the midline. They do not differ in one-eared and two-eared mantises. Ear length is 1.0-1.5 mm; body lengths are 35-45 mm.

Developing tympanal air sacs are evident in 2nd instar nymphs as bulges in transverse tubes connecting lateral longitudinal trachea to the large transverse trachea between the spiracles. The thin purple tubes merge with the connection between the tympanal sac and the transvers trachea.

The auditory chamber has not yet formed in early/middle instar nymphs (red circle). The tympanal sacs become progressively more longitudinal, increase in proportional size, and start to take on their distinctive 'bean-shape.' The volume of a sac is 0.10 - 0.15 µl. The length of the ear region is ca. 200 µm with body lengths of 10-15 mm.




Early/middle nymph




Middle nymph

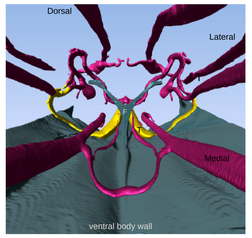
Overlaying the cuticle on the tracheal reconstruction (right) provides correlation of cuticular and tracheal auditory changes.

The transitions through later nymphal development involve gradual enlargement of the tracheal sacs and reorientation to become longitudinal. The orientation change puts the medial face of the sacs up against the tympanum as the auditory chamber forms. In late instars sac volume is ca. 0.25 µl with ear lengths of 300-600 µm.

The 'fly-through' view (far right) emphasizes the ventral midline location of the ear and tympanal tracheal sacs (gold). It shows the thin tubes connecting the tympanal sacs with the lateral trachea. The furcasternum (F) forms the floor of the auditory chamber and is an internal muscle attachment site.

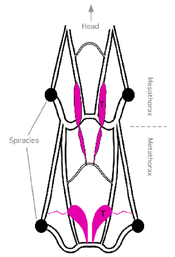
3D reconstruction used the Amira software package (Visage Imaging, Inc.) running on a custom graphics workstation. Trachea of interest were automatically traced in every slice; accuracy was confirmed manually. Amira assembled the traced profiles in each slice to create a 3D image. Useful resolution was 7-12 µm.


Late nymph/adult



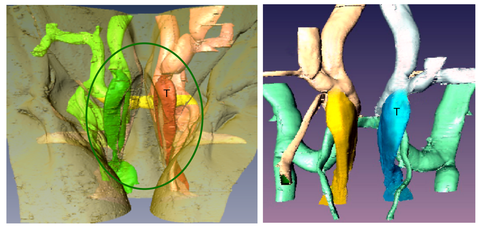

A 'fly-through' view inside the metathorax looking from rostral (head) toward the ear with the longitudinal tracheal groups labeled.

## 6. Two-eared mantises have a different mesothoracic traceal system




Although they serve the same function (increasing sensitivity through impedence-matching), the META and MESO auditory tracheal systems differ markedly in structure and development.

The adult MESO tympanal sacs are flattened expansions of large medial tracheal tubes. The sacs have large diameter openings at both ends, although there is a characteristic constriction rostrally. (MESO ear circled)



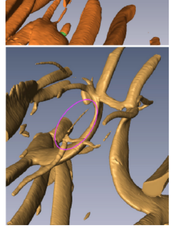

The sacs contact the inner side of the tympanum only at the floor of the groove rather than across the entire tympanum as in the META ear (histological sections). However, the mesothorax is packed with large trachea so that much of the internal volume close to the ear is air as shown in the histological sections below (upper, longitudinal; lower, transverse. The stars indicate the attachment sites of the TOs to the tympanum.


The histological sections through the MESO ear region are 10 µm thick and stained with Masson's trichrome.

Doesn't hear

Hears


Middle/late nymphs



The MESO ear region of one-eared and two-eared mantises has the same basic configuration, but is broader in thew latter. The tracheal systems differ dramatically. For *P. agrionina* (left) there is a simple tube and minimal air space. The internal volume of the ear region of *Creobroter* sp. is almost all air space.

The MESO tympanal sac is evident in the early nymphs as a bulge in the tracheal tube (above; see also Box 5, early/middle nymph). It enlarges during nymphal development, but there is little change in shape or position. In the one-eared *P. agrionina* (below), there is never more than a small tracheal in the area (in red circle; too small to be resolved throughout).

## 7. Conclusions and Questions

- The mesothoracic auditory system remains a puzzle in several respects. Its input is distributed widely throughout the CNS and the latencies are almost as short as those of the ultrasound-sensitive auditory system of the metathorax. The latter triggers a very effective evasive response. Does this imply that the LF system does something similar?

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