

**Three-dimensional structures of the tracheal systems of *Anopheles sinensis*
and *Aedes togoi* pupae**

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Supplementary materials and methods

Olive oil (Sigma-Aldrich, St. Louis, MO, USA) was used to test compare respiration of *An. sinensis* and *Ae. togoi* pupae. 300 ul of olive oil was applied to 700ml of water in PYREXTM Spot plate (Fisher Scientific, Pittsburgh, PA, catalog 722085). The movements of the respiratory trumpets of both mosquito pupae were observed using a stereoscopic microscope (Stemi DV4, Carl Zeiss, Germany).

Supplementary results

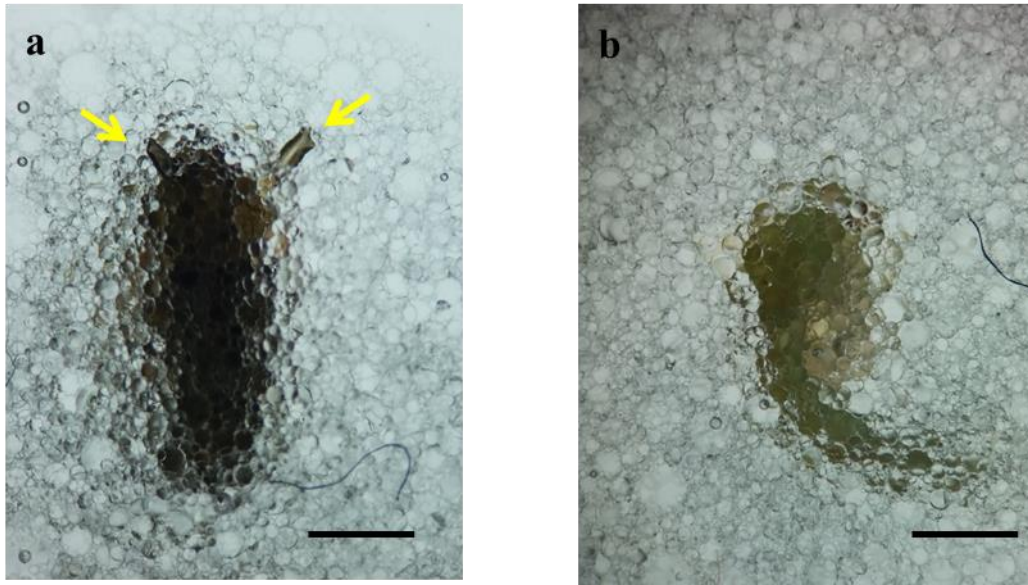
Supplementary Video 1. This video shows the cross-sectional view of –X-ray image stack from the cephalothorax to the 3rd abdomen of *An. sinensis* pupa. The purple line indicates the tracheal system.

Supplementary Video 2. This video shows the cross-sectional view of –X-ray image stack from the cephalothorax to the abdomen of *Ae. togoi* pupa. The purple line indicates the tracheal system.

Supplementary Video 3. This video shows the movement of tracheal organ of *Ae. togoi* pupa. This movie exhibits the peristaltic motions of dorsal longitudinal trunks.

Supplementary Figure 1. The effect of surface tension of water are investigated using olive oil. Under the same condition, the respiratory trumpets of *Ae. togoi* pupa penetrates the water surface mixed with olive oil (Fig. S1a). However, the respiratory trumpets of *An. sinensis* pupa can not penetrate the oil-treated water surface (Fig. S1b).

Supplementary figure



Supplementary video legends

Supplementary video. V1. The cross-sectional views of the cephalothorax to the 3rd abdomen of *An. sinensis* pupa.

Supplementary video. V2. The cross-sectional views of the cephalothorax to the abdomen of *Ae. togoi* pupa.

Supplementary video. V3. The movement of tracheal organ of *Ae. togoi* pupa.

Supplementary Figure S1. Photographs of *Ae. togoi* and *An. sinensis* Pupae at the oil-treated water surface. Axial image of *Ae. togoi* (a) and *An. sinensis* (b) pupae. The yellow arrows indicate the respiratory trumpets. Scale bars represent 1 mm.