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## Method Article

# A stationary tweezer platform for high throughput dissections of minute arthropods and extirpation of their minute organs



Joseph M. Cicero\*, Judith K. Brown

*University of Arizona, Tucson, United States*

## A B S T R A C T

A homemade platform satisfied the need for fast, efficient dissection of minute arthropods and extirpation of their key organs, such as salivary glands and midguts, involved in agricultural disease transmission pathways. With its implementation, ~200 organs could be extirpated per 8 h workday while the subjects are submerged in protein or transcript protectant. A vacuum wand is used to capture insects and position them in the field of view. Two stationary tweezers are positioned on an adjustable scaffold that spans the microscope stage transversely such that their tips, and the insects they immobilize, can be submerged in select dissection media. High tensile strength fishlines are attached to the stationary tweezers for opening and closing with the 5th fingers while hand-held dissection tweezers load insects from the wand to their tines, then extirpate the target organs. Organs are lifted out with glass splints or plastic toothpicks into a final tube of select preservation media for freezing at session end.

- Constructed from common retail materials
- Adjustable design fits many microscopes
- Can also be used in a wide variety of applications, including materials science

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## A R T I C L E I N F O

*Method name:* Microdissection, mass extirpation

*Keywords:* Minute arthropods, Mass dissection, Proteomics, Transcriptomics, Micromanipulation, Salivary glands, Midguts

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\* Corresponding author.

*E-mail address:* [jmc6@email.arizona.edu](mailto:jmc6@email.arizona.edu) (J.M. Cicero).

## Specifications table

|  |   |
|--|---|
| Subject Area:                          | Agricultural and Biological Sciences  |
| More specific subject area:            | <i>Molecular anatomy of small arthropods</i>  |
| Method name:                           | <i>Microdissection; mass extirpation</i>  |
| Name and reference of original method: | <i>Authors claim full originality for conception and design of the device herein.</i> |
| Resource availability:                 | <i>Common retail hardware store and biological supply house materials</i>             |

## Methods

### Method validation

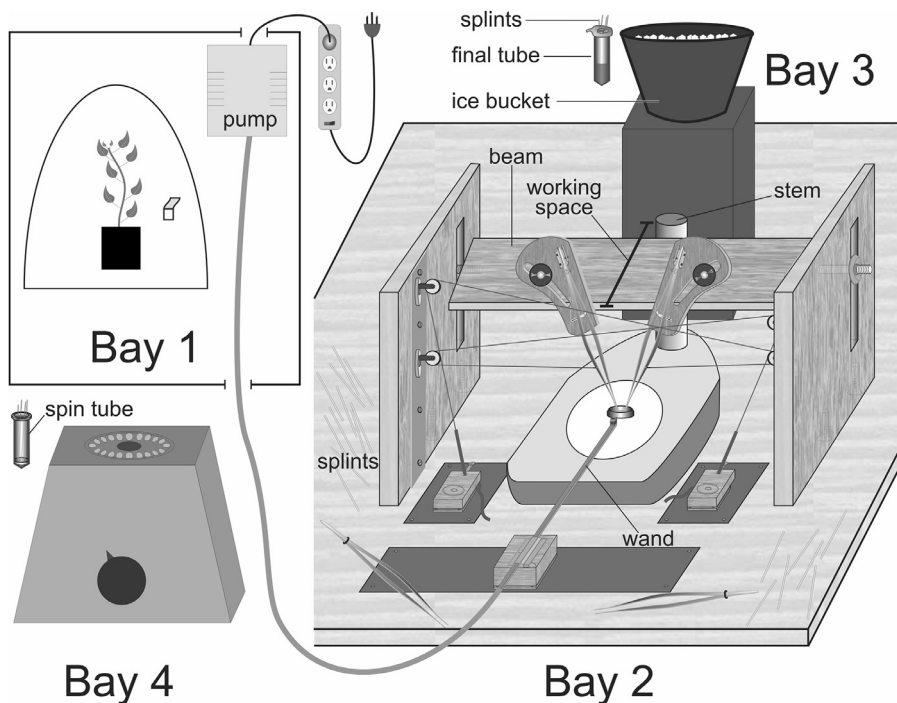
Numerous papers have been published on isolation of specific organs from insects and other arthropods for molecular studies, especially those involved in human and agricultural crop disease transmission [1,2]. Depending on desired results, large (e.g. [3,4]) and small (e.g. [5,6]) numbers of individually extirpated organs fulfill the need for determining their respective transcriptomes, as well as proteomes and metabolomes. Extirpation is also needed to determine an organ's configuration and ultrastructure [7–9], and for immuno- and *in situ* localization of molecules with light and electron microscopy [10,11]. Collection of large numbers of minute organs such as salivary glands from minute insects can be especially challenging [3] and researchers have resorted to dissecting on dry ice [6], in a buffer with no nucleic acid protectant [3,12] and isolating and processing the body section that includes the glands instead of the glands themselves [12].

In recent and past research goals, several thousand salivary glands and midguts of infected and uninfected (control) individuals of three major hemipteran pests, were needed to study the effector molecules involved in transmission of agricultural crop diseases. These insects were: mitotypes of the silverleaf whitefly *Bemisia tabaci* (Genn.) cryptic species group (Aleyrodidae), reared on cotton, *Gossypium hirsutum* L.; the potato psyllid *Bactericera cockerelli* (Sulc) (Triozidae), on tomato, *Lycopersicon* sp.; and the Asian citrus psyllid *Diaphorina citri* Kuwayama (Liviidae), on orange, *Citrus* sp. and mock orange, *Murraya paniculata* (L.) Jack (Rutaceae). The demand for rapid and efficient extirpation of such numbers of organs from such minute insects for isolation and purification of their transcriptomes led to invention of the stationary tweezer platform. This article describes the construction of the platform and work area, and illustrates how the operator can quickly capture and immobilize insects between the tines of two pairs of stationary tweezers submerged in nucleic acid protectant. The hands are then free to use two other tweezer pairs to precision dissect, extirpate, discard, and replace with fresh specimens.

### Construction

The work area consisted of four bays (Fig. 1): Bay 1- The specimen capture bay, with caged insects and a suction **wand** connected to a vacuum **pump**. Bay 2- The stationary tweezer platform (Fig. 2), built using common retail materials, and adjustable to accommodate many dissecting microscopes. Bay 3- The **final tube** (Fig. 3D) with a molecular protectant of choice to receive **splints** used to lift out extirpated organs from the dissection media. Splints were made by heating and stretching solid glass rods with an acetylene torch. Common plastic toothpicks were also used. Both were trimmed to a length appropriate for the microfuge tubes that received them (Figs. 1, 3Db, Ee). These were tested for degradation and leaching by soaking in Trizol LS<sup>®</sup> (ThermoFisher Scientific), the nucleic acid protectant of choice, for 24 h. Bay 4- The **spin tube** (Fig. 3E, F) and countertop centrifuge (Fig. 1) to collect any protectant adhering to the splints for recycling back to Bay 2.

*Specimen capture bay* (Fig. 1, Bay 1). BugDorm<sup>®</sup> cages (BioQuip Products, Rancho Dominguez, CA) held the insects and their respective host plants. The suction **wand** (Fig. 3A) was a 5/32" square metal tube (K&S Precision Metals, Chicago, IL) connected at one end to a vacuum pump by way of gum rubber **bunsen burner tubing** (Thermo Fisher Scientific, Waltham, MA), chosen for its flexibility and firmness under vacuum. A short length of the same metal tube was modified with a wire saw

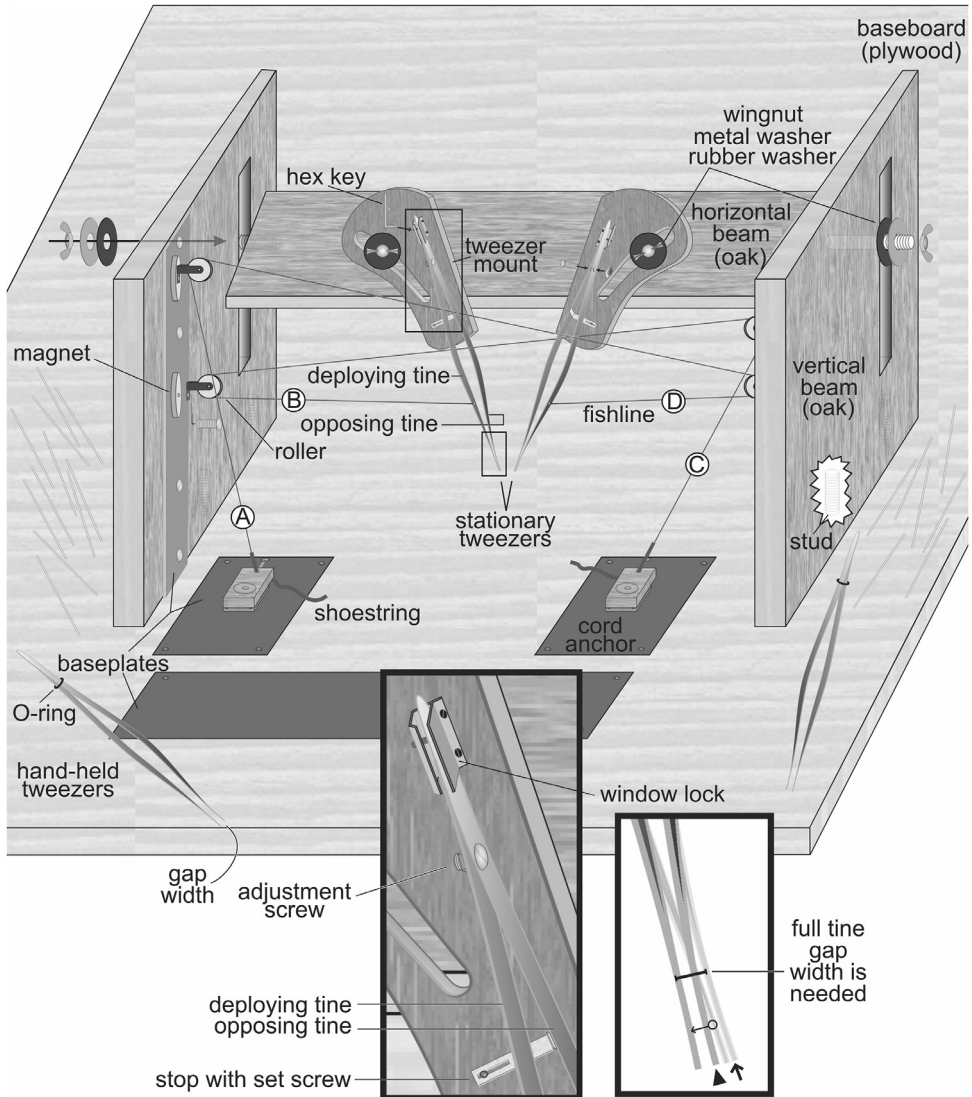


**Fig. 1.** Schematics of the four bays of the work area. Not drawn to scale. The actual platform size depends on the operator's microscope and its **working space** (clearance) between objective lens and **stem** through which the horizontal **beam** passes. Bay 1 is for specimen capture and includes a suction **wand** (cf. Fig. 3A) that can reach through an access hole into the cage, arrest specimens, and position them next to the dissection vessel in the microscope's field of view (cf. Fig. 4). Bay 2 is the stationary tweezer platform. Bay 3 is an **ice bucket** and pedicel that hold the **final tube** high above the microscope as needed to avoid breathing fumes from the nucleic acid protectant, Trizol LS<sup>®</sup>. Organs are fished out of the dissection media with glass **splints** and dropped into the final tube. Bay 4. **Splints** are then transferred to the spin tube and spun to remove any adherent protectant (cf. Fig. 3E, F).

(Electron Microscopy Sciences, Hatfield, PA) and glued to the other end to form a **perpendicular** bend. Mosquito **netting** was fitted over the orifice with **heat shrink tubing**. The vacuum pump was turned on and off from the switch of a power strip located where convenient. A rectangular piece of sheet aluminum was fashioned into a **clip** that the wand would snap under securely, and mounted on a wooden **support block** next to the microscope stage. The block was constructed the same way as the cord anchors (Fig. 3B, C; see below) but without the overhang. The height of the block was tailored to the height of the microscope stage. The clip was held with only one screw so that it could rotate in association with the adjustable position of the block.

**Stationary tweezer platform** (Fig. 1, bay 2; Fig. 2). The platform's baseboard was made with **plywood**, and its beams were made with **oak** wood. Two vertical beams, held immobile by **studs** screwed in from underneath the base, supported a **horizontal beam** that was adjustable in height and rotation by its own two studs.

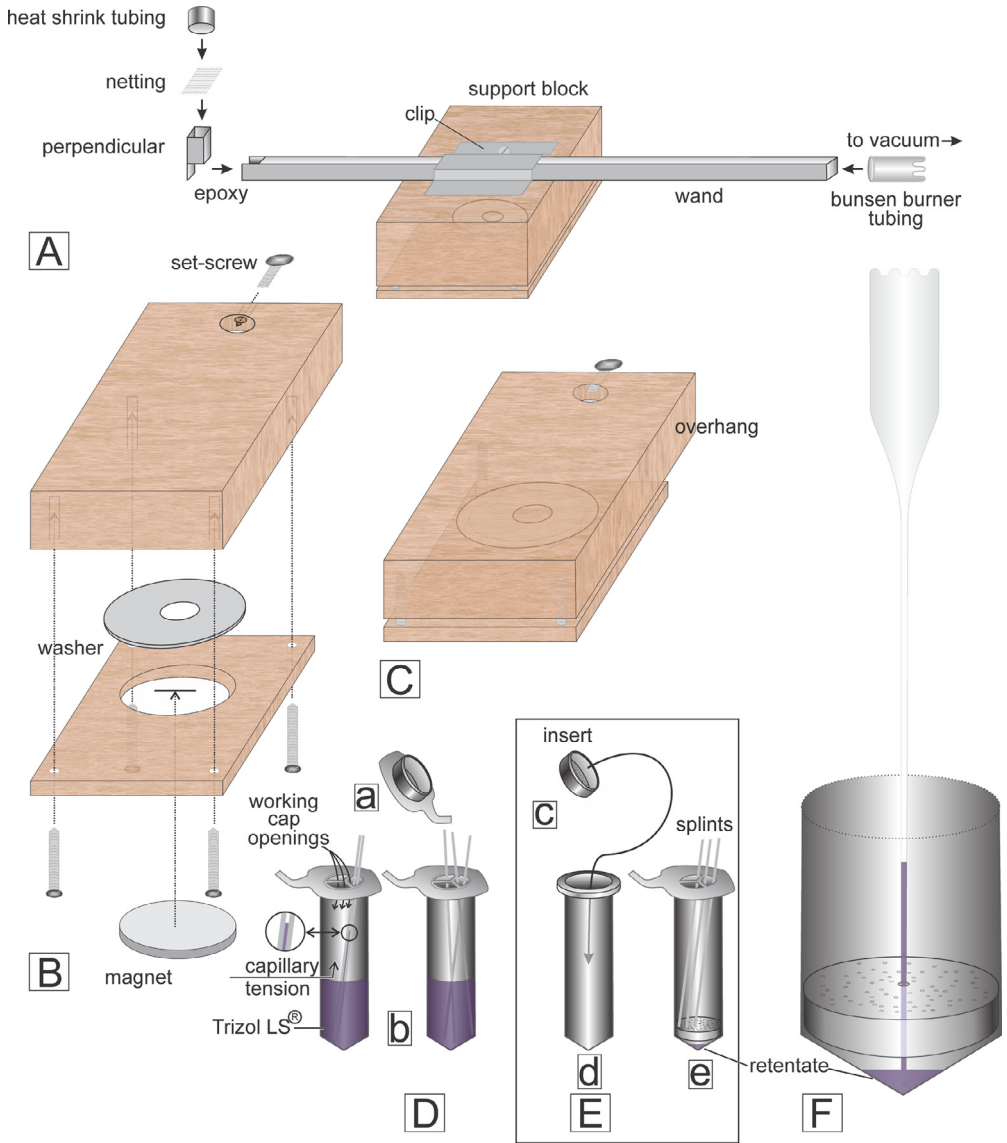
The horizontal beam was fitted with two adjustable **tweezer mounts**. **Window locks** were screwed down firmly onto the mounts (Fig. 2, left inset). The ones selected were provided with hexagonal set-screws. These were tightened to lock the **stationary tweezers** into their slots using a **hex key**, or "Allen Wrench" as they are also called. Choice of stationary tweezer dimensions depended on shaft length and tip width, the latter for a sufficiently firm hold on the insects (Fig. 4). **Adjustment screws** and nuts were tightened to the extent that the tine tips pressed against each other with a minimal firmness capable of holding the insects (Fig. 2, left inset). Braided, high tensile-strength



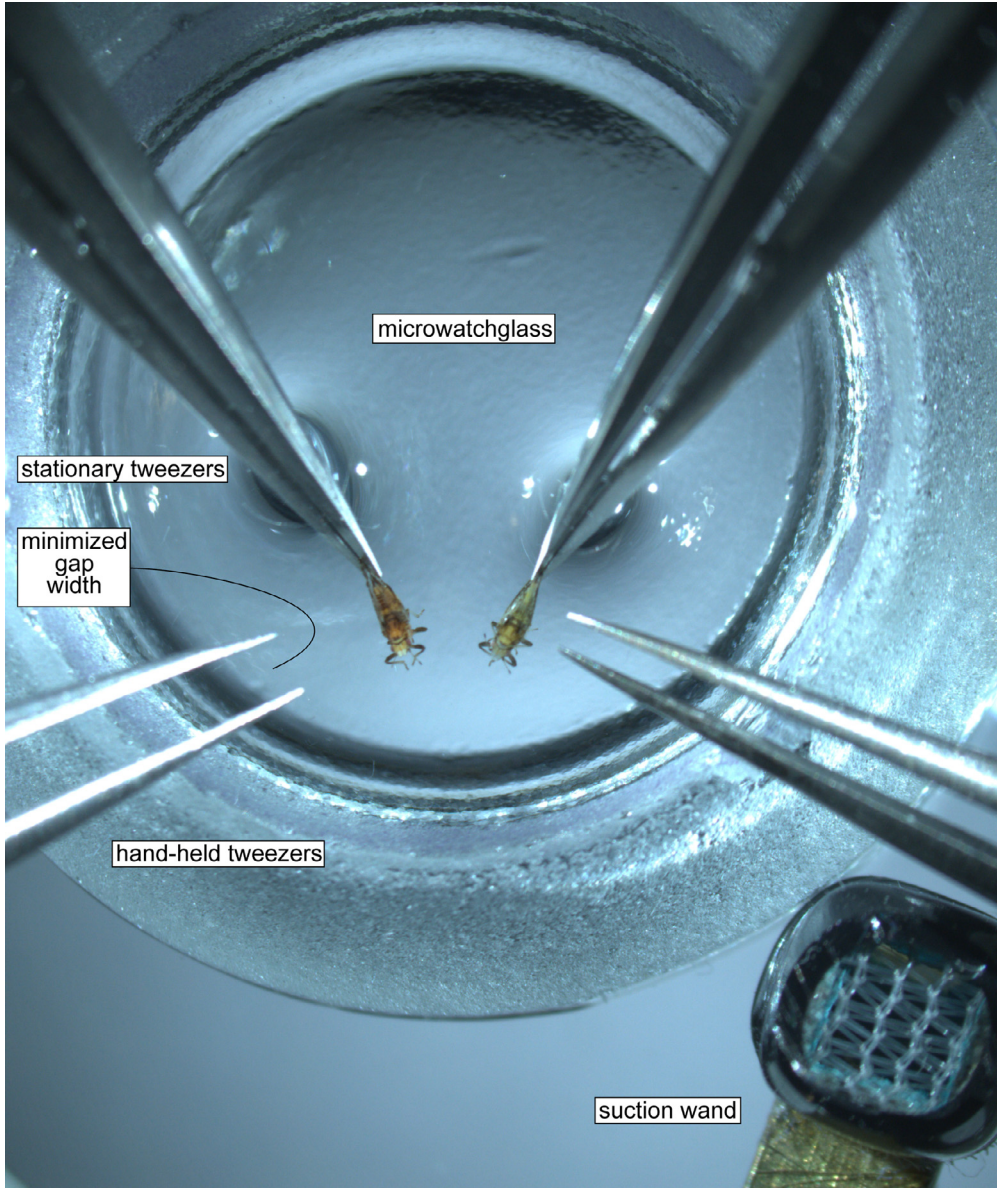
**Fig. 2.** Schematic of the stationary tweezer platform in greater detail. Microscope and wand mount not shown. See text for full descriptions. (A) and (B) operate either tweezer pair with the left hand as needed. (C) and (D) operate either tweezer pair with the right hand as needed. Left inset- Magnification of left tweezers mount. When fishline (B) or (C) is pulled, the **deploying tine** of the left tweezers will bend about the **adjustment screw**. Right inset- However, although the deploying tine (circled) will bend to an open position, this bending will cause the **opposing tine** (arrow) to move with the pull too (from arrow to arrowhead), such that there will be a loss of **full gap width** between tine apices. The **stop** prevents this loss by arresting the opposing tine position at the arrow.

**fishline** was passed through a hole drilled into the **deploying tine** and secured with a fishhook knot. An adjustable metal **stop** restrained the **opposing tine** from moving with the deploying tine when the fishline was pulled (Fig. 2, right inset).

Sheet metal **baseplates** were screwed to the vertical beams and to the platform base. Screen door **rollers** were screwed to neodymium disc **magnets** (Master Magnetics Inc.<sup>®</sup>, Castle Rock, CO)



**Fig. 3.** Schematics of accessories. (A) Suction wand. The **wand** is fitted with mosquito **netting** to capture and immobilize insects for transfer to the stationary tweezer tines. The wand **clip** is affixed to a **support block** of wood fashioned in the same way as the **cord anchor** (B). The support block holds the wand at the same height as the microscope stage so that immobilized insects and stationary tines are in the same field of view. (B) Cord anchor. A **washer** is sandwiched between two blocks of wood which, when screwed together, press it against a neodymium disc **magnet** tightly fitted into a hole in the lower block. (C) Assembled cord anchor with **overhang**. (D) **Final tube**. Organs are fished out of the dissection media (RNA Later<sup>®</sup>) with a glass splint (Fig. 1) or plastic toothpick and dropped into the final tube containing Trizol LS<sup>®</sup>. (a) Cap for storing the harvest between sessions. (b) Three **openings** were cut out of the **working cap** to retard evaporation and keep splints separate. Left- splints close to, or in contact with, each other will draw Trizol upward by **capillary tension**. Right- splints kept separate will have minimal Trizol clinging to them and, after several splints accumulate in the final tube, they can be transferred to the spin tube. (E) **Spin tube**. (c) A cap is cropped of its rim with a razor blade, perforated with an insect pin, and pressed tightly into the bottom of the spin tube (d) so that when spun in the centrifuge, media adherent to the splints will be isolated below it. These splints can then be reused. All perforations in the spin tube insert are made at a diameter smaller than that of the splints except for one in the center that allows access to the retentate. (F) Retrieval of retentate with a heat-stretched borosilicate pipette.



**Fig. 4.** Two Asian citrus psyllids held submersed in media by stationary tweezer tines. Both hands are free to dissect with the hand-held tweezer pair. **O-rings** (Fig. 2) constrain **gap width** to a minimum.

and adjusted on their respective baseplates to route the fishline to the **cord anchors** (Fig. 2A–D) with a tautness weak enough to keep the tines at minimal closure. Each cord anchor consisted of a neodymium disc magnet pressed tightly into a hole drilled into a block of wood with a height equal to that of the magnet. A second block was cut longer so that it overhung the first block when the two were screwed together. A metal **washer** was sandwiched between the two directly above the magnet. In this configuration, continuity of magnetic attraction was established from washer to

baseplate, and the magnet would not pull out of its hole should the operator want to lift the block off of the baseplate. The fishline was tied to a low-stretch **shoestring** (Fig. 2) of a wide enough diameter to pass snugly through a hole in the overhang and locked in place with a thumb **set-screw** (Fig. 3B, C).

## Operation

Moving the cord anchors forward or loosening their set-screws to release the tension of the cord allowed for adjustment of the height and roll of the horizontal beam. The position of the screen door rollers and the cord anchors could then be adjusted upon their metal baseplates so that the fishlines were taut and their paths did not conflict with any other parts. These various adjustment features, and placement of the microscope, allowed the operator to position the dissection vessel, a **microwatchglass**, the submerged tine tips, and the wand orifice together in the field of view for fast transfer of the insects to the stationary tines (Fig. 4). They also allowed left-handed and right-handed operators to open and close either of the stationary tines with either hand (Fig. 2A–D).

The microwatchglass was filled with a starter solution of 50% RNA Later<sup>®</sup> in nuclease free H<sub>2</sub>O (Ambion, Foster City, CA). As sessions progressed, evaporation would increase protection until the RNA Later<sup>®</sup> concentrated to a point that caused tissue degradation, disallowing extirpations to continue. The expired media was then replaced with fresh 50% media. Methods and finesse below were developed to minimize duration and complexity of each manual motion for high throughput yield with no muscle fatigue.

With vacuum on, the wand was used to reach through an access hole into the cage and capture insects, then withdrawn and snapped under the **clip** (Fig. 3A). Stationary tweezer tines were opened by pulling fishline with the 5th finger of either hand as needed while two **hand-held tweezers** were used by the thumb and forefingers (Figs. 2 and 4) to transfer insects to them from the wand. These tweezers were fitted with an **O-ring** to minimize the **gap width** between tines and prevent muscle fatigue.

The platform was designed to facilitate both left- and right-handed operators by installing left and right redundant fishlines. As operators of either hand develop the finesse required for high throughput, they will find the need to use the fishlines for both sides. For example, occasionally, specimens will need to be repositioned for better address or tighter grip. Occasionally, operators may find it easier to grasp a fishline on one side or the other with several fingers, taking care not to bump the fragile hand-held tweezer tine tips (see below).

For salivary glands, the insect was mounted dorsum up, with the stationary tweezers clamped onto the abdomen. The hand-held tweezers were used to pull the head and prothorax away from the mesothorax and away from each other at the same time, revealing the tentorial side-arms [13], which were landmarks for locating the salivary glands. During a separate run for the guts, the insect was mounted venter up, with the stationary tweezers clamped onto the head and thorax. Hand-held tweezers grasped the basal abdominal cuticle between the hind coxae and tore it away in a posterior direction. After both insects were opened for penetration of protectant, the glands were lifted out by clamping onto the salivary ducts with one of the hand-held tweezers or, in the case of the guts, clamping onto the esophagus and hindgut. For both these organ pulls, hand-held tweezer pairs were ultrasharp (0.01 mm x 0.05 mm tine tips, Electron Microscopy Supplies). Such ultrasharpness rendered the tips very fragile, and therefore a finesse was developed to minimize their movement at the watchglass, and to use a focused escort by gently returning them back to the platform after each dissection is completed.

The glands and guts were released and allowed to float free while the tweezers were set down and a glass splint was picked up to retrieve them, then dropped into the final tube (Fig. 3D).

The final tube contained 200  $\mu$ l of **Trizol LS**<sup>®</sup> (Fig. 3Db), and resided in an ice bath placed on a **pedicel** behind and well above the microscope to avoid breathing the protectant's vapor (Fig. 1). Three **openings** were cut out of the final tube's working cap to retard evaporation (Fig. 3Db, arrows). Several splints could be accumulated but they needed to be kept separate from each other (Fig. 3Db, right), otherwise **capillary tension** would lift the media between them (Fig. 3B, left) and carry it excessively to the **spin tube** (Figs. 1, Bay 4; 3Ed, e). An uncut cap (Fig. 3Da) was reserved for storing the final

tube. After a number (~9–12) of splints accumulated in the final tube, the splints were transferred to the spin tube. Trizol LS<sup>®</sup> was determined to be the best media by direct observation of spontaneous dissolution of the organs, which eliminated any possible clinging of organs or parts of organs to the splint when the splint was transferred to the spin tube.

The spin tube was fitted with an insert made by cropping the rim of a microfuge tube (Fig. 3Ec) and perforating it with an insect pin. The insert was pressed down to the bottom of the spin tube cylinder, leaving a space for **retentate** to accumulate (Fig. 3F). The spin tube resided in a centrifuge and was spun with the addition of each splint to collect residual, adherent Trizol. These splints were then reused without back-contamination to the RNA Later<sup>®</sup> in the microwatchglass. A larger hole was made in the center of the insert for a heat-stretched borosilicate pipette to pass through and retrieve the retentate for transfer back to the final tube (Fig. 3F). The centrifuge model chosen was one of many that allowed spinning with the lid open.

## Results

Using the platform, ~200 organs, whether salivary glands or guts, were extirpated per day. Trizol LS<sup>®</sup> was determined to be the best media by direct observation of spontaneous dissolution of the organs. Organs or parts of organs therefore did not cling to the splint when the splint was transferred to the spin tube. As sessions progressed, evaporation would increase protection until the RNA Later<sup>®</sup> concentrated to a point where tissue degradation disallowed extirpations to continue and expired media had to be replaced with fresh 50% media.

## Discussion

The homopterans mentioned herein are only exemplary. Methods and finesse for harvesting must be developed for specific organs of specific subjects. The platform can also be used for electronics and other non-arthropod subjects. The dimensions of the platform- length, width, height - as well as the length of the wand, are all dependent on the corresponding dimensions of user's microscope. Also, the adjustable features of the platform can be dispensed with if its dimensions are customized for a specific microscope in the user laboratory. The microscope of choice would depend primarily on the range of magnification, the diameter of its field of view, the size of the **working space** between the microscope body and the stem (Fig. 1, Bay 2), and whether transmitted light is desired. The working space is very important because the horizontal beam must pass through it. Zoom rather than step magnification is preferable in order to track the free-floating organs in the Z-axis of the field of view while reaching for a splint to fish them out.

## Declaration of Competing Interest

The first author declares retention of full originality of conception, design, and operation of the platform. No competing or collaborating interests were involved other than the need of the research team for high throughput yield of specimen material. The second author declares no conflict of interest.

## Acknowledgments

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## References

- [1] D.G. Heckel, Genomics in pure and applied entomology, *Annu. Rev. Entomol.* 48 (2003) 235–260, doi:[10.1146/annurev.ento.48.091801.112624](https://doi.org/10.1146/annurev.ento.48.091801.112624).
- [2] T.W. Fisher, M. Vyas, R. He, W. Nelson, J.M. Cicero, M. Willer, R. Kim, R. Kramer, G.A. May, J.A. Crow, C.A. Soderlund, D.R. Gang, J.K. Brown, Comparison of potato and Asian citrus psyllid adult and nymph transcriptomes identified vector transcripts with potential involvement in circulative, propagative *Liberibacter* transmission, *Pathogens* 3 (2014) 875–907, doi:[10.3390/pathogens3040875](https://doi.org/10.3390/pathogens3040875).
- [3] Y-L. Su, J-M. Li, M. Li, J-B. Luan, X-D. Ye, X-W. Wang, S-S. Liu, Transcriptomic analysis of the salivary glands of an invasive whitefly, *PLoS ONE* 7 (6) (2012) e39303, doi:[10.1371/journal.pone.0039303](https://doi.org/10.1371/journal.pone.0039303).
- [4] C.A. Stafford-Banks, D. Rotenberg, B.R. Johnson, A.E. Whitfield, D.E. Ullman, Analysis of the salivary gland transcriptome of *Frankliniella occidentalis*, *PLoS ONE* 9 (4) (2014) e94447, doi:[10.1371/journal.pone.0094447](https://doi.org/10.1371/journal.pone.0094447).
- [5] M. Ramalho-Ortigão, I.V. Coutinho-Abreu, V.Q. Balbino, C.A.S. Figueiredo, R. Mukbel, H. Dayem, H.A. Hanafi, S.S. El-Hossary, E.E.Y. Fawaz, M. Abo-Shehada, D.F. Hoel, G. Stayback, M. Wadsworth, D.A. Shoue, J. Abrudan, N.F. Lobo, A.R. Mahon, S.J. Emrich, S. Kamhawi, F.H. Collins, M.A. McDowell, *Phlebotomus papatasi* SP15: mRNA expression variability and amino acid sequence polymorphisms of field populations, *Parasites Vectors* 8 (2015) 298, doi:[10.1186/s13071-015-0914-2](https://doi.org/10.1186/s13071-015-0914-2).
- [6] K.C. Showmaker, A. Bednarova, C. Gresham, C.Y. Hsu, D.G. Peterson, N. Krishnan, Insight into the salivary gland transcriptome of *Lygus lineolaris* (Palisot de Beauvois), *PLoS ONE* 11 (1) (2015) e0147197, doi:[10.1371/journal.pone.0147197](https://doi.org/10.1371/journal.pone.0147197).
- [7] J.M. Cicero, E. Hiebert, S.E. Webb, The alimentary canal of *Bemisia tabaci* and *Trialeurodes abutilonea* (Homoptera, Sternorrhynchi): histology, ultrastructure and correlations to function, *Zoomorphology* 115 (1995) 31–39, doi:[10.1007/BF00397932](https://doi.org/10.1007/BF00397932).
- [8] J.M. Cicero, J.K. Brown, Anatomy of accessory salivary glands of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) and correlations to begomovirus transmission, *Ann. Entomol. Soc. Am.* 104 (2) (2011) 280–286, doi:[10.1603/AN10171](https://doi.org/10.1603/AN10171).
- [9] J.M. Cicero, D. Sammataro, The salivary glands of adult female *Varroa destructor* (Acari: Varroidae), an ectoparasite of the honey bee, *Apis mellifera*, *Int. J. Acarol.* 36 (5) (2010) 377–386, doi:[10.1080/01647951003757961](https://doi.org/10.1080/01647951003757961).
- [10] M. Ghanim, M. Brumin, S. Popovski, A simple, rapid and inexpensive method for localization of tomato yellow leaf curl virus and potato leafroll virus in plant and insect vectors, *J. Virol. Methods* 159 (2009) 311–314, doi:[10.1016/j.jviromet.2009.04.017](https://doi.org/10.1016/j.jviromet.2009.04.017).
- [11] E.D. Ammar, R.G. Shatters Jr, D.G. Hall, Localization of *Candidatus Liberibacter asiaticus*, associated with citrus huanglongbing disease, in its psyllid vector using fluorescence *in situ* hybridization, *J. Phytopathol.* 159 (2011) 726–734, doi:[10.1111/j.1439-0434.2011.01836.x](https://doi.org/10.1111/j.1439-0434.2011.01836.x).
- [12] P. Thorpe, P.J.A. Cock, J. Bos, Comparative transcriptomics and proteomics of three different aphid species identifies core and diverse effector sets, *BMC Genom.* 17 (2016) 172–190, doi:[10.1186/s12864-016-2496-6](https://doi.org/10.1186/s12864-016-2496-6).
- [13] J.M. Cicero, P.A. Stansly, J.K. Brown, Functional anatomy of the oral region of the potato psyllid, (Hemiptera: Psylloidea: Trioziidae), *Ann. Entomol. Soc. Am.* 108 (5) (2015) 743–761, doi:[10.1093/aesa/sav059](https://doi.org/10.1093/aesa/sav059).