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The Fixator—A simple method for mounting of arthropod specimens and photography of complex structures in liquid

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Abstract

Mounting and preparing arthropods in liquids for photography and further investigations is a challenging task and may lead to unsatisfactory results and, in the worst case, to damage to specimens. A new method is presented here, which allows the fixation of specimens of different sizes under various degrees of pressure. The method is illustrated by three case studies from different groups of insects and arachnids.

Keywords: fixation, genitalia dissection, maceration, specimen handling

Introduction

A major aspect of taxonomy is the description and interpretation of external and internal morphological characters (Dayrat 2005, Mutanen & Pretorius 2007, Padial et al. 2010). Many arthropod dissections are therefore carried out in liquid environments (e.g. ethanol, glycerol) to avoid dehydration and collapsing of structures (Robinson 1976, Ungureanu 1972, Krogmann & Vilhelmsen 2006).

During and after dissection, photography of macro- and microstructures in ethanol or glycerol can be challenging, as in most cases photos from specific angles or in natural positions are mandatory for useful comparisons (Wanke & Rajaei 2018). Proper positioning of specimens in liquids can be frustrating, and results may be not satisfactory (Su 2016) due to the following disruptive factors, which can lead to artifacts during stacked photography: first the evaporation of ethanol, which can generate a flow causing the specimen or dirt particles around it to drift and secondly, any kind of vibrations (Haselböck et al. 2018).

To overcome these problems, different methods have been published so far. Flat objects can easily be fixed by placing them, within the liquid, on a slide covered with a cover glass. However, the pressure of the cover glass can also deform important structures (Wanke & Rajaei 2018). Su (2016) showed that drift and movement of specimens can be minimized by placing them in hand sanitizer gel. Similarly, different concentrations of agarose gel can be used (Haselböck et al. 2018). Wanke & Rajaei (2018) used a fixed tunnel-shaped holder to document important characters in the male genitalia of lepidopterans. However, when specimens or structures need to be photographed from a specific angle, all of the listed methods may be inefficient.

Here, we present the Fixator, an easy and low-priced method to fix arthropod specimens of all sizes in almost any position. The method is described and demonstrated through three case studies: (1) documentation of a special structure of the male genitalia of geometrid moths; (2) genitalia dissection of a spider wasp and (3) photography of the tibial apophysis of a spider from different angles.

Specimens used for case study

Lepidoptera: Geometridae ♂♂: *Peribatodes secundaria* (Denis & Schiffermüller, 1775), *P. umbraria* (Hübner, 1809), *P. rhomboidaria* (Denis & Schiffermüller, 1775)

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Hymenoptera: Pompilidae ♂: *Agenioideus usurarius* (Tournier, 1889)
Arachnida: Theraphosidae ♂: *Chaetopelma* sp. Ausserer, 1871

Material

A Petri dish (preferably made of plastic for a more durable glueing), a plastic pipette, some nylon thread, a razor blade, super glue (based on Ethyl cyanoacrylate (ECA)).

Step by step guide for building the Fixator

1. Cut two 3-mm pieces from the tips of two plastic pipettes (as thread holders). The nylon thread has to fit through the hole without being too loose (Fig. 1A).

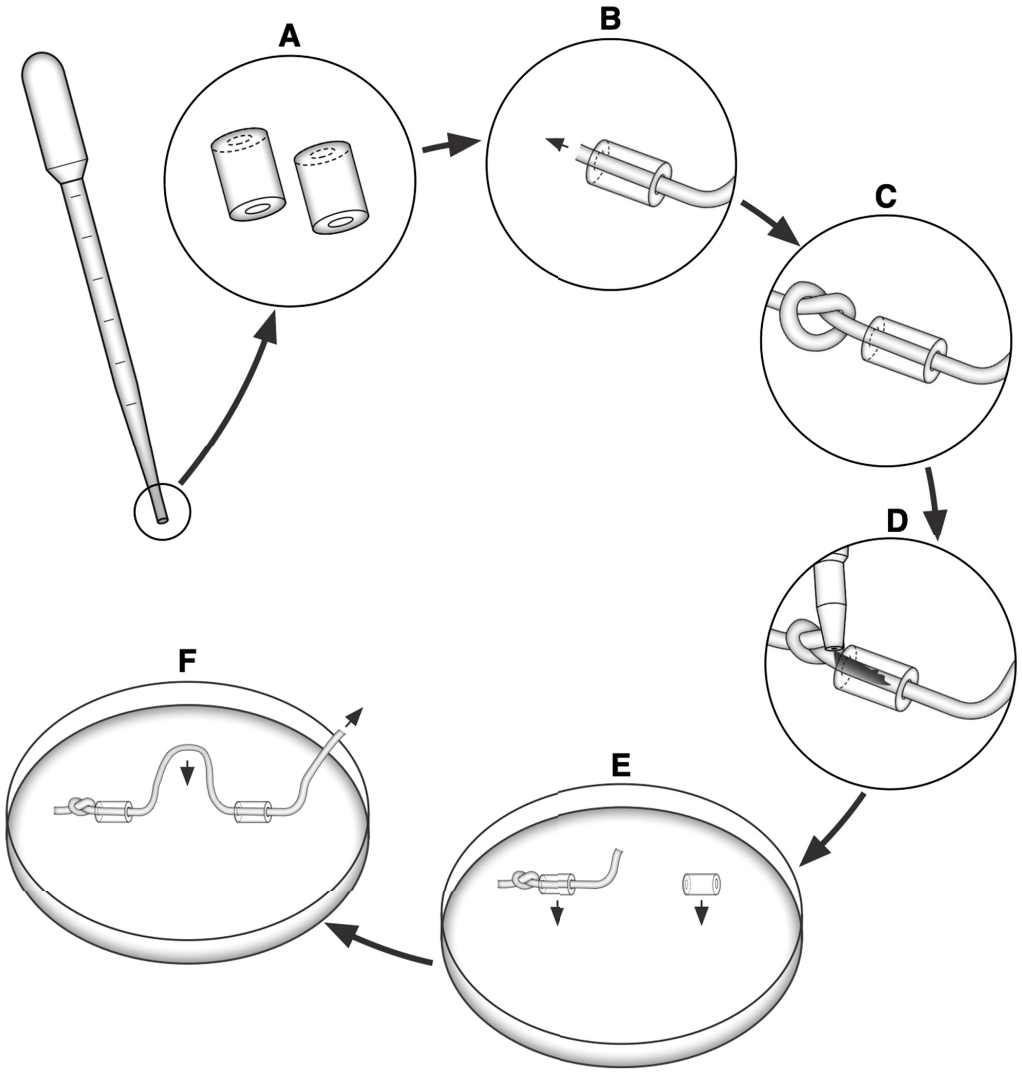

FIGURE 1. Construction of the Fixator. **A.** Cut pipette tips as thread holders of similar size. **B.** Pull the nylon thread through one thread holder. **C.** Tie a knot in one end of the nylon thread. **D.** Put a drop of super glue on the thread so that it runs into the tip, pull the thread at the free end. **E.** Glue both tips onto surface of Petri dish. **F.** Pull the nylon thread through the other thread holder. The gadget is now ready to use.

2. Thread the nylon thread through one of the pipette tips, tie a knot and glue the tip and nylon thread together (Figs 1B-D).

3. Glue the thread and thread holder to the floor of the Petri dish, to one side, and the other thread holder on the opposite side (the space left between the two holders can vary depending on the size of the object to be examined) (Fig. 1E).

4. Thread the nylon thread through the second thread holder (this forms a loop under which the object to be photographed will be placed) (Fig. 1F).

5. A small piece of plastic with a slit can be glued to the edge of the Petri dish (in case the structure needs to be very firmly fixed) (Fig. 2).

6. By pulling the nylon thread, the strength of fixation can be adjusted.

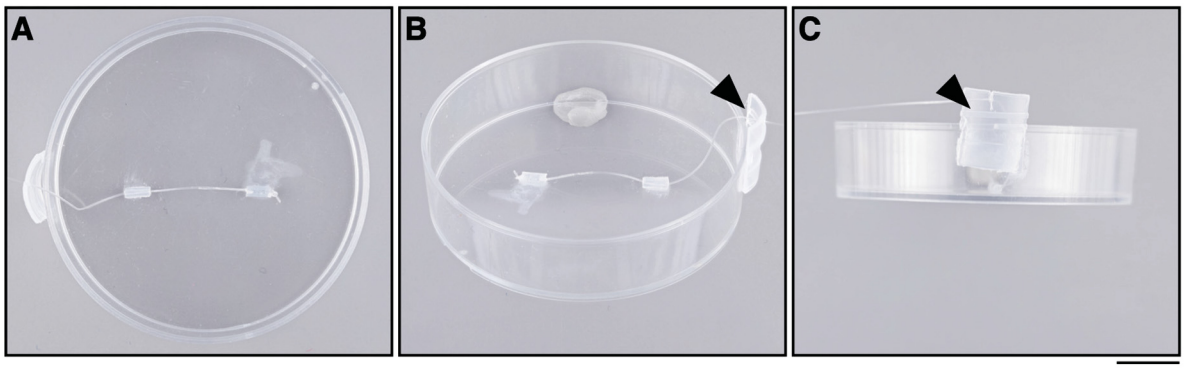

FIGURE 2. Overview of the fixing gadget. **A.** Top view; **B.** Angled view; **C.** Lateral view. Arrows indicate the thread-fixing plastic card; scale bar 1cm.

Important note: depending on the size of the object, an underlay (a piece of plastic, plastazote or Styrofoam) should be placed beneath the thread, to enable a strong tension.

Photography. Objects and specimens were photographed using a Keyence VHX-5000 and Visionary Digital photography system (LK Imaging System, Dun.Inc.).

Results & Discussion

(1) Case study: Fixation of male genitalia for photography in Lepidoptera

Genitalia structures in lepidopterans display species-specific diagnostic characters, which are important for their diagnosis (Scoble 1992, Hausmann & Viidalepp 2012). After dissection, the genitalia are often embedded on permanent slides (Robinson 1978), where the pressure of the cover glass may lead to rotation of the structure to an unnatural position and to an altered shape and/or position of several important characters (Wanke & Rajaei 2018). It is therefore necessary to photograph these structures before embedding takes place.

In *Peribatodes* species, the uncus displays an important species-specific character, which is only visible in lateral view (Müller et al. 2019) (Fig. 3A). However, once the genitalia have been embedded, this character is not visible anymore (Fig. 3B). Unfortunately, photography of the genitalia capsule in ethanol is often very difficult, due to variation of the angle or drift. For photography in ventral view, Wanke & Rajaei (2018) suggested fixation in a tunnel-shaped holder, which is in some cases also possible in lateral view. However, this is unfeasible for *Peribatodes*, whereas using the Fixator allows more comparable results.

Here, the genitalia capsules of three *Peribatodes* species, *P. secundaria*, *P. umbraria* and *P. rhomboidaria*, were mounted using the suggested method (Fig. 3C-E), which allowed an easy comparison of the uncus across the different species (see Fig. 3): slightly swollen in *P. secundaria*, dorsally well extended and rectangular in *P. umbraria*, dorsally extremely extended in *P. rhomboidaria* (see Müller et al. 2019). The results show that mounting the genitalia capsule with the Fixator allows an easy comparison in lateral view. Furthermore, the suggested method allows a fast mounting of male and female genitalia in almost all positions before embedding in a permanent slide takes place.

(2) Case study: Removal of subgenital plate and genitalia in male Hymenoptera for subsequent study

In male Hymenoptera, the morphology of the genitalia and subgenital plate provides useful information and has

been proven especially useful for species identification in Pompilidae (Day 1988, Wiśniowski 2009, Krogmann & Austin 2011). However, the removal of male genitalia, especially in small specimens, usually requires dexterity and practice (Day 1988). The specimen must be kept fixed in place while the genitalia structures are carefully prepared, avoiding any damage to the genitalia structures or to important external morphological structures. The Fixator facilitates the dissection without damaging the specimen during the fixation process. Figure 4 shows the extracted genitalia and subgenital plate of a specimen fixed, in ethanol, on the presented fixing device. The Fixator can be used to hold specimens in place to facilitate the genitalia dissection, but also for photography of external and internal morphological structures in general.

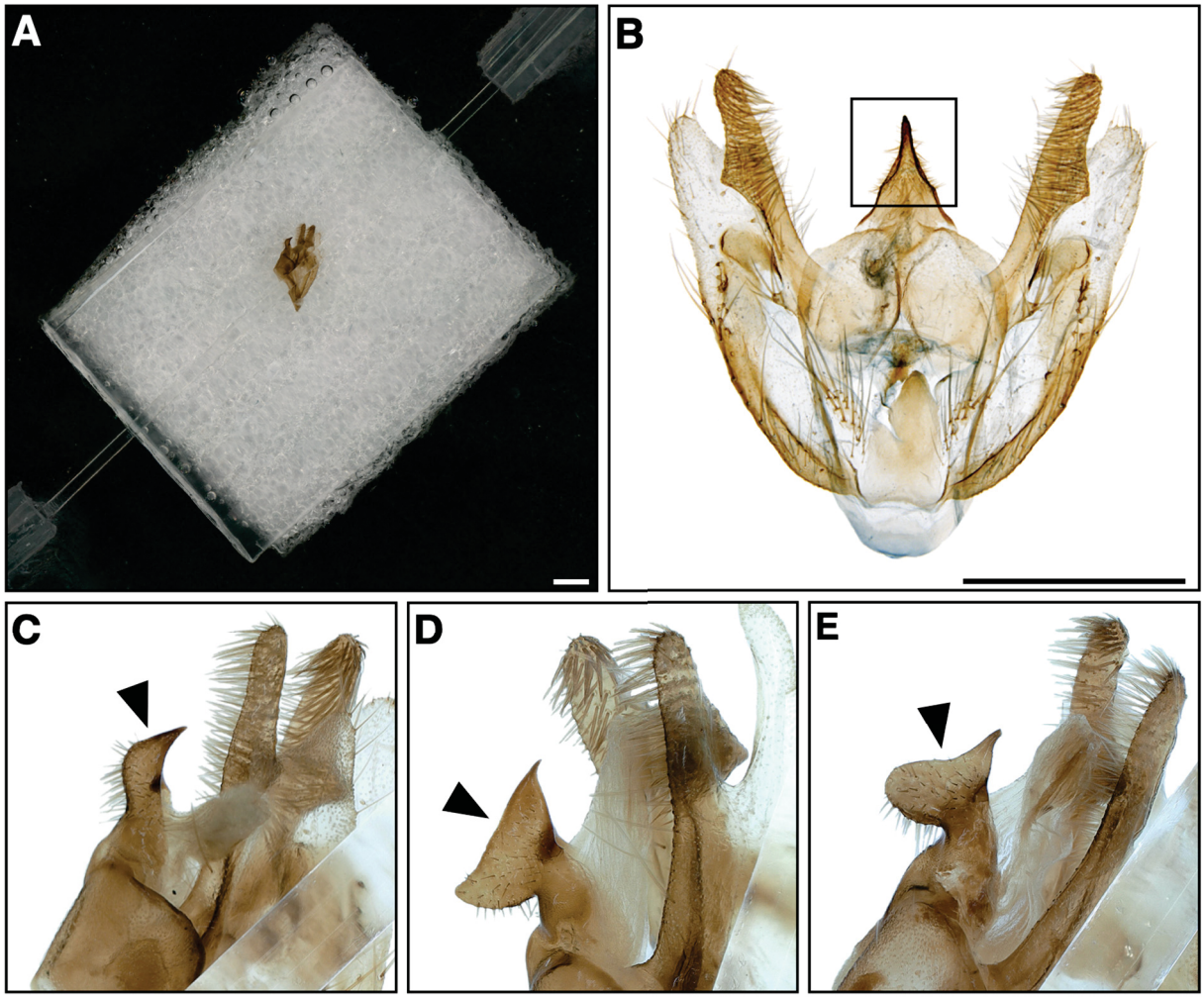

FIGURE 3. Use of the Fixator for genitalia photography in *Peribatodes* spp. (Geometridae). **A.** Genitalia of *P. secundaria* fixed in lateral view. The capsule in this case is placed on a serrated plastic plate glued to a piece of plastazote. **B.** Embedded genitalia capsule of *P. secundaria*; the diagnostic shape of the uncus (framed) is not visible. **C-E.** Close-up photography of the uncus in lateral view (black arrows). **C.** *P. secundaria*; **D.** *P. umbraria* and **E.** *P. rhomboidaria*. Scale bars 1mm.

(3) Case study: Fixation of spiders to image selected body parts

Spiders in collections are usually conserved in ethanol (Martin 1977). Morphological features need to be photographed in a liquid environment to prevent desiccation of the specimen or setae from adhering to the body surface, especially in very hirsute species. Furthermore, for identification or species descriptions it is crucial to study several characters from a preassigned angle. For this purpose, small species or parts of specimens can easily be locked in position using agarose gel or a similar viscous liquid (Haselböck et al. 2018), but large specimens are often too refractory and unmanageable for efficient use of this method.

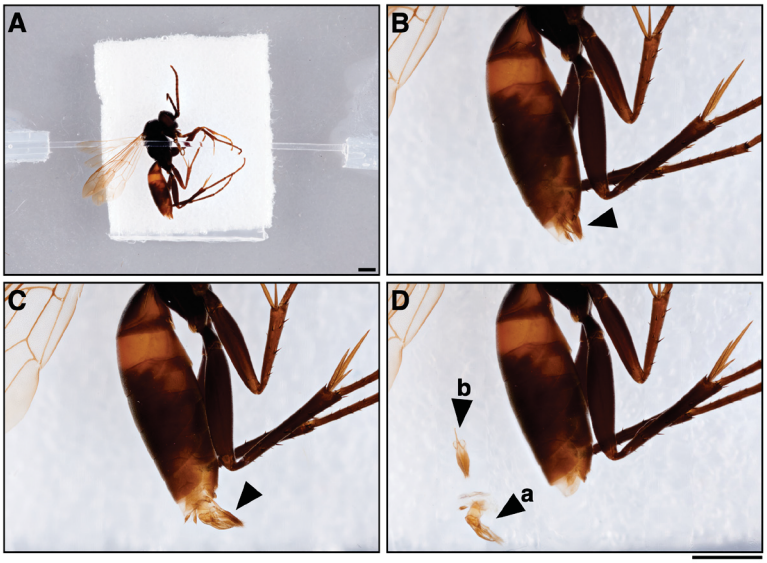

FIGURE 4. Lateral fixation of a male spider wasp for genitalia dissection (Hymenoptera: Pompilidae: *Agenioides usurarius*). Use a pair of sharp Dumont 5 forceps to apply pressure and draw out the genitalia capsule and subgenital plate. **A.** Overview of the specimen mounted in the Fixator. The specimen is placed on a serrated plastic plate on plastazote; **B.** Genitalia in the natural position; **C.** Partly removed genitalia capsule; **D.** Fully removed genitalia capsule (a) and subgenital plate (b); black arrows indicate the genitalia. Scale bars 1mm.

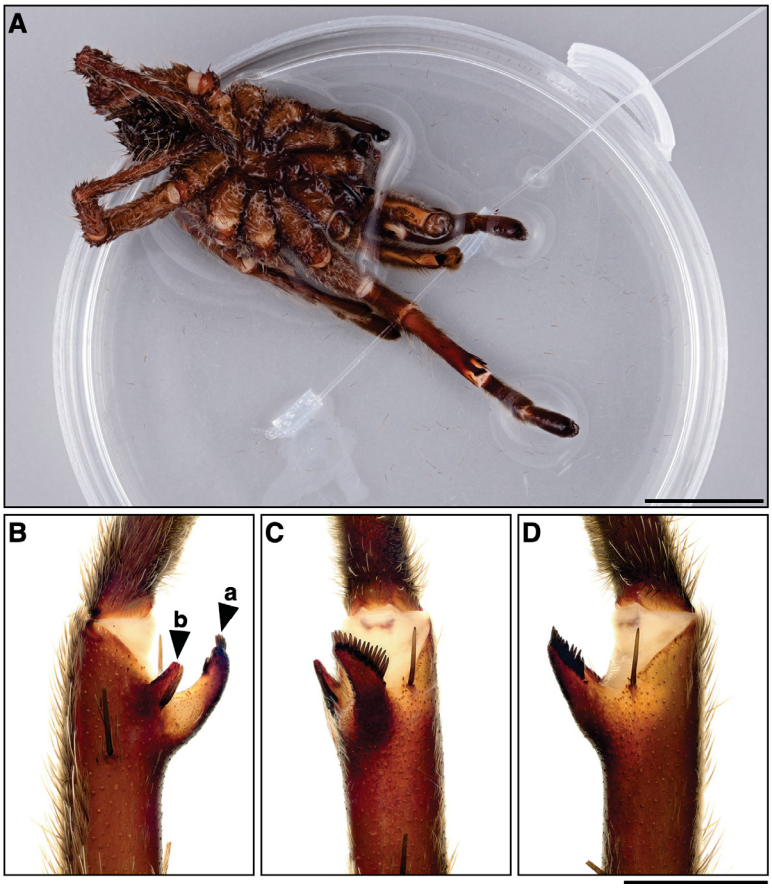

FIGURE 5. Fixation of a male spider (*Chaetopelma* sp.: Theraphosidae) to capture images of diagnostic structures situated on its appendages. **A.** Overview of fixed specimen in ventral view: leg-I is fixed, interfering appendages are positioned out of the way; scale bar 10 mm. **B-D:** Close-up images of the tibial apophysis showing two branches: (a) a long and curved retrolateral branch, wider in its distal portion and with an apical row of 15 spines, and (b) a short prolateral branch with an adjacent spine at its base; **B.** prolateral view; **C.** ventral view; **D.** retrolateral view. Scale bar 2,5 mm.

In tarantulas (Araneae: Theraphosidae), many taxonomically important characters are situated on the legs. Males of many species possess tibial apophyses on their first pair of legs, which are used to hold the females chelicerae during mating. In *Chaetopelma* and closely related genera, the presence and shape of the tibial apophysis are diagnostic features (Guadanucci & Gallon 2008, Guadanucci & Wendt 2014). Using the Fixator described here, we were easily and rapidly able to take images of this structure on leg-I of a male *Chaetopelma* from three angles, prolateral, ventral and retrolateral, without removing it from the specimen (Fig. 5). Altering the position of the leg can be easily accomplished without damaging the specimen.

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