An anti-slip underlay for the investigation and documentation of arthropod structures in liquid

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TECHNICAL NOTE

An anti-slip underlay for the investigation and documentation of arthropod structures in liquid

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Abstract

The investigation, preparation, and photography of small natural history samples in liquids require reliable methods to ensure the object remains still, thus maximizing results and minimizing potential damage. Here, we present a method for fixing specimens or specimen parts, designed to enhance stability and immobility during their study and documentation in liquids. This method utilizes an anti-slip underlay made of silicone rubber, which can be supplemented by a purpose-made hollow for containing smaller objects. The advantages of this method are illustrated with some examples.

Keywords: Arthropoda, beetle, dissection, insect, mounting, photography, preparation, silicone, spider, stacking, tarantula.

Zusammenfassung

Die Untersuchung, Präparation und Fotografie kleiner naturkundlicher Proben in Flüssigkeiten erfordert zuverlässige Methoden, die sicherstellen, dass das Objekt in Position gehalten wird, um so die Ergebnisse zu maximieren und mögliche Schäden zu minimieren. Hier stellen wir eine Methode zur Fixierung von Proben oder Probenteilen vor, die die Stabilität und Unbeweglichkeit während ihrer Untersuchung und Dokumentation in Flüssigkeiten verbessern soll. Bei dieser Methode wird eine rutschfeste Unterlage aus Silikongummi verwendet, die durch eine speziell angefertigte Kuhle zur Aufnahme kleinerer Objekte ergänzt werden kann. Die Vorteile dieser Methode werden anhand einiger Beispiele erläutert.

Looking back at the long history of naming species, most species were differentiated and described based on morphological characters (Wiens 2007; Cook et al. 2010; Poulin et al. 2022). During taxonomic investigations, it is therefore essential to have photographs and drawings which help emphasize the diagnostic value of these characters (Wanke & Rajaei 2018). In arthropods, liquids (e.g., ethanol, glycerol) are used to prevent diagnostic characters from dehydrating and collapsing during their examination, dissection, or photography, and specimens must be carefully kept in position to avoid damage or distortion which could lead to character misinterpretation or make identification impossible (Ungureanu 1972; Schauff 1986; Krogmann & Vilhelmsen 2006; Wanke et al. 2019). When it comes to extended depth of field photography (stacking photography) in liquids, difficulties such as the evaporation of ethanol or vibrations can have a negative effect on the quality of the photograph or change the position of the structure to be photographed (Su 2016; Haselböck et al. 2018). To avoid this, taxonomists have come up with all kinds of ideas for keeping specimens or specimen parts in position (e.g., Gurney et al. 1964; Su 2016; Haselböck et al. 2018; Wanke & Rajaei 2018; Wanke et al. 2019, 2021).

Wanke et al. (2021) showcased the use of silicone rubber as a translucent and soft underlay for the Fixator. However, this type of underlay has proven to be far more helpful than expected in the everyday examination and imaging of arthropods, as the silicone also has anti-slip properties when liquids are added. Here, the advantages of a silicone rubber underlay are highlighted through two examples: (1) extended depth of field photography of the tarantula bulbus in apical view and (2) dissection and preparation of a small beetle.

Preparation of the anti-slip underlay

Material. Petri dish (we used a 60 mm glass Petri dish), silicone rubber (SILIXON10, formerly known as EROSIL10; ordered at https://www.silikonfabrik.de/), an Eppendorf tube (we used a 0.5 ml tube), and insect or sewing pins.

Preparation. The silicone rubber should be mixed according to the manufacturer's instructions and poured into the Petri dish to the desired level (here, the Petri dish was half filled). After solidification (2–3 hours at room temperature), the silicone rubber is ready for use and can be wetted with liquids. In this condition, it has good anti-slip properties that facilitate the examination of samples.

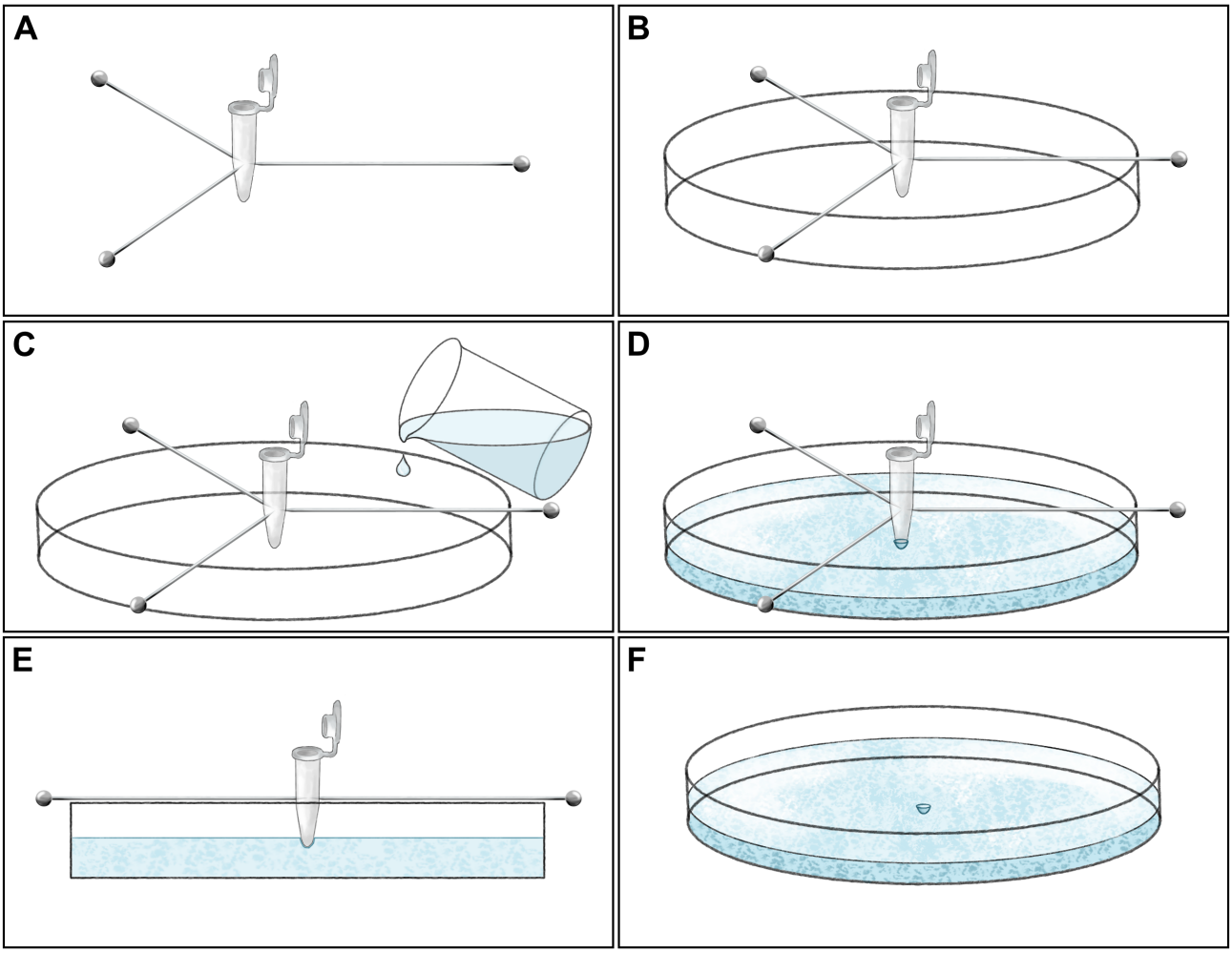

Fig. 1. Preparation of the anti-slip silicone underlay. **A**. Insertion of three pins in an Eppendorf tube at the desired height. **B**. Placement of the Eppendorf tube in the Petri dish in the desired position. **C–E**. Mixing of the silicone according to the manufacturer's instructions and filling of the Petri dish to the desired level. **F**. Removal of the Eppendorf tube from the solidified silicone. The silicone underlay is now ready for use and liquids (e.g., ethanol) can be added.

Note. When examining very small samples or specimens, a hollow can be created in the surface of the underlay to further hold the sample in place. For this, the tip of an Eppendorf tube can be placed in the Petri dish before adding the liquid silicone and removed after solidification (Fig. 1). The size of the hollow can be adjusted by regulating the height of the Eppendorf tube and the level of the silicone (the hollow presented here was 3 mm in diameter). A hollow can also be manually excavated using forceps. The silicone rubber underlay is now ready for use, and liquids (e.g., ethanol) can be added to the Petri dish. The underlay can be easily removed and replaced if necessary.

Photography of the tarantula bulbus in apical view

In spiders, the copulatory organs are important structures for the identification and differentiation of species and genera, and therefore are almost always included in descriptions (Grasshoff 1968; Jäger 2016). In the tarantula genera *Phormingochilus* Pocock, 1895, *Omothymus* Thorell, 1891, and *Lampropelma* Simon, 1892, the apical view of the bulbus is considered crucial for genus-level differentiation (Gabriel & Sherwood 2019). However, the shape of the basal part of the male bulbus impedes its positioning for examination or photography in apical view. Use of the underlay described above (Fig. 1) facilitates the positioning of the bulbus in the hollow of the underlay in apical view (Fig. 2), and its anti-slip properties prevent the sample from drifting and blurring during photography.

Dissection and preparation of a small beetle

Small insects have a high tendency to drift when placed in a liquid medium, hence complicating their morpho-

logical examination and handling (Su 2016). The anti-slip properties of the silicone underlay have proved to be of great benefit for the examination, dissection, and preparation of small insect specimens such as beetles of the subfamily Pselaphinae (Fig. 3).

Morphological examination of a specimen. The silicone underlay allows the sample to be precisely positioned for examination and prevents it from drifting. In cases where additional stabilizing friction is needed (e.g., when the specimen is positioned in lateral view), the beetle can be held in place within a purpose-made hollow (Fig. 3).

Dissection of a specimen with enhanced precision. As illustrated in Fig. 3, one pin is used to lock the specimen in place by pressing it against the silicone underlay, while a second pin is used to remove the specimen's abdomen. The pins used in this example are minutien pins, with one pin modified into a hook that facilitates detachment of the body parts and is especially helpful for extracting internal diagnostic structures such as the aedeagus.

Mounting of entire specimen or its dissected parts. The entire specimen can be mounted efficiently by gently pressing it, ventral side up, against the silicone underlay. Its appendages can be placed in roughly their desired position before slowly removing the liquid medium with a pipette. Once the specimen is semi-dry (about 5 minutes for the average Pselaphinae beetle), the appendages can be adjusted to their final position. After drying completely, the specimen can be carefully picked up from the silicone underlay with a fine brush and glued to a card mount. This method ensures easy mounting of the specimen and avoids the sometimes complicated arrangement of the appendages on the adhesive.

Re-articulation and mounting of dissected body parts. For the dissection of the male genitalia in beetles, hooked minutien or insect pins are often used depending on the size of the insect, but this differs among groups (e.g., Smith 1979; Hanley & Ashe 2003). In the subfamily Pselaphinae, the use of this method is not always possible, as in many cases the entire abdomen can be destroyed by pulling the aedeagus out. It has therefore been shown that it is easier to separate the abdomen in order to preserve its structures, even if other intermediate steps, e.g., DNA extraction, have to be carried out. Subsequent mounting procedures after dissection also differ among groups. While very small beetles (3–5 mm) can be embedded on permanent slides, the abdomen of larger beetles can be glued next to the specimen (Smith 1979; Hanley & Ashe 2003). Here, we present an alternative, easier way to reattach body parts of small beetles using the anti-slip underlay. The thorax is gently pressed against the silicone underlay and the remaining body parts (e.g., the head, abdomen, or, in some cases, legs) can be carefully re-attached to the thorax via cohesion. Once the liquid medium is slowly removed and the specimen is left to dry, the body parts remain attached, and the specimen can be picked up carefully with a fine brush and glued to a card mount.

Use of the silicone underlay presented here has proved to be of special importance for the handling of small beetles, especially cavernicolous taxa, which have very elongated, thin, and fragile appendages. The anti-slip properties of the silicone underlay make handling much more effective and gentler, thus minimizing the risk of damage to delicate specimens.

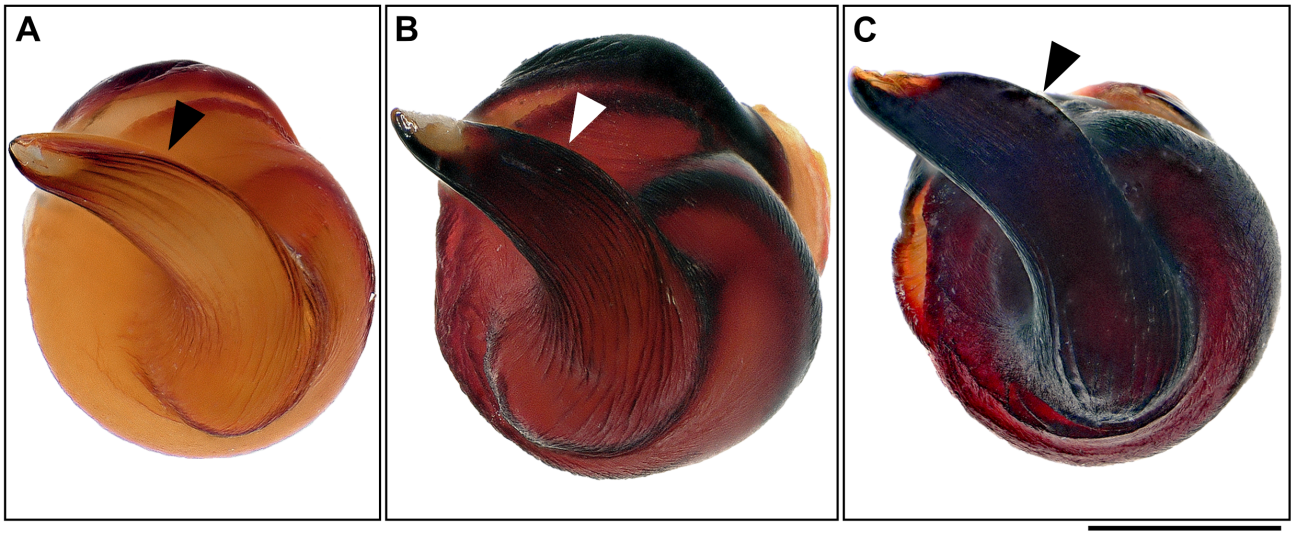

Fig. 2. Bulbi of three tarantula genera photographed after placement in the hollow of the anti-slip underlay. **A**. *Phormingochilus* Pocock, 1895 (accession number: SMNS-Aran-004016). **B**. *Omothymus* Thorell, 1891 (accession number: SMNS-Aran-004028). **C**. *Lampropelma* Simon, 1892 (accession number: SMNS-Aran-004099). The arrows point to an important diagnostic character necessary for the differentiation of these genera. Scale bar: 1 mm.

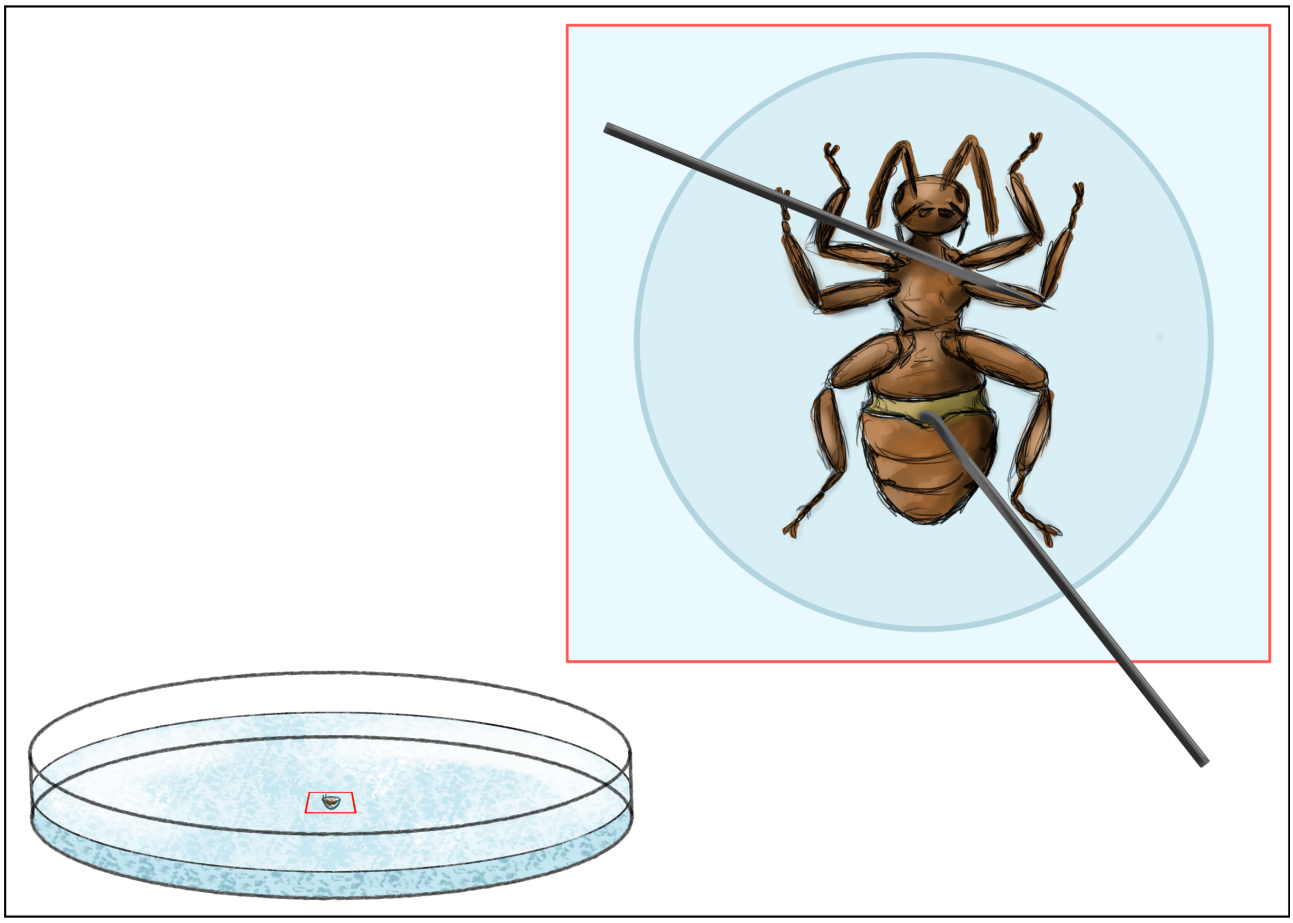

Fig. 3. Example of handling and dissecting of a small beetle on the silicone underlay.

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