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An effective method for the close up photography of insect genitalia during dissection: a case study on the Lepidoptera

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Abstract. Characters of male and female genitalia in insects in general, especially in Lepidoptera, are essential for species identification as they display extensive morphological variation. In embedded genitalia, due to the positioning of the genitalia and the pressure of the cover glass, the appearance of some diagnostic characters might be confusing. This potentially leads to taxonomic misinterpretation. Additionally, the photography of genitalia structures in ethanol is difficult, due to drift or hardening of genitalia. A method is presented here to fix the position of the genitalia in ethanol, which allows comparative close up photography. The advantage of the method is demonstrated by illustrating the sacculus projection of three *Triphosa* species.

Introduction

Reproductive organs of insects are extremely diverse in form and function and they are a valuable source of information for taxonomic purposes. The complex genitalia, especially the sclerotized male genitalia in the Lepidoptera, have been extensively used in taxonomic revisions (Scoble 1992; Hausmann 2001).

Unfortunately, during the embedding process of the genitalia, some important diagnostic characters may become hardly visible, due to the pressure of the cover glass on the genitalia. As a result, the arrangement of some diagnostic characters might be confusing. Additionally, studying the three-dimensional structures in an embedded position may lead to taxonomic misinterpretation. In most, if not all, cases a comparison and photography of these structures in fluid (e.g. ethanol or glycerol) and three-dimensional arrangement is necessary to obtain reliable results. However, drift and movement of the genitalia is a major problem during the stacking photography. Su (2016) suggested an effective method for photography of genitalia using a "Hand-sanitizer" to solve this problem. Unfortunately, this is not possible for fixing some foldable structures (e.g. the sacculus projection in male genitalia of *Triphosa* species (Geometridae: Larentiinae)), because in such cases the viscosity of the "Hand-sanitizer" is not high enough to keep it in fixed position. Therefore, a quick and easy solution for the photography of the genitalia during dissection in a fixed position is presented here.

In the genus *Triphosa*, the sacculus projection of the valva (Figs 1, 2) is an important diagnostic character in species level taxonomy (Hausmann and Viidalepp 2012). The interspecific differences of this character are mostly not comparable, when the valvae are open and placed in the embedded slides (Fig. 2, black arrows). For solving this problem, a new, inexpensive and simple method is proposed.

Material and methods

Material examined

Triphosa dubitata (Linnaeus, 1758)
1 ♂, Georgia: Borjomi Kharaguali NP, Borjomi District, vic. Likani, trail 1 near ranger shelter, 1850 m, 22.VII.2006, 41°51'01,6"N, 43°15'39,1"E, at light, leg. C. Häuser, D. Bartsch, g. prep. 0016/2018 D. Wanke. 1 ♂, Iran, Elburs, vic. Kendevan, 2500 m-3000 m, 7.-9.VIII.1977, leg. W. Thomas, coll. W. Schäfer, Stuttgart, g. prep. 0015/2018 D. Wanke.

Triphosa sabaudiata (Duponchel, 1830)
1 ♂, Gempenhöhle, 23.11.47, g. prep. 0024/2018 D. Wanke. 1 ♂, Gempen, Jan. 59, S. Blattner, g. prep. 0052/2018 D. Wanke.

Triphosa tauteli Leraut, 2008
1 ♂, Hispania, Lerida, Boixols Umgeb., 1300 m, 2.7.1976, leg. Aistleitner, coll W. Schäfer, Stuttgart, g. prep. 0053/2018 D. Wanke.

All examined material is deposited in **SMNS** (State Museum of Natural History, Stuttgart).

Methods

Required material: Petri dish, plastic pipette, razor blade, super glue (transparent, highly fluid glue, based on Ethyl cyanoacrylate (ECA); Trade name: "UHU Sekundenkleber blitzschnell").

Preparation procedure of the mounting system:

1. Cut the tip of plastic pipette (about 2–3mm, diameter of the tip must be chosen according to the size of the genitalia capsule, Fig. 1A). As an alternative, tips of plastic syringes may be used.
2. Split the tiny piece of pipette into two halves (Fig. 1B).
3. Attach one of the halves on the bottom of the petri dish using super glue (fig. 1C), to generate a half tunnel-shaped holder.
4. Dissect the genitalia following standard methods (Robinson 1976).
5. Place the genitalia capsule in the half tunnel shaped holder (see Figs 1C and 3A) in the desired position.
6. Cover the half tunnel and genitalia with 70% ethanol.
7. Photograph the sample (here we used a Keyence microphotography system (Model: VHX-5000) and a Visionary Digital System (Model: LK Imaging System)).

Case study: Photography of fixed sacculus projection in the genus *Triphosa*

The sacculus projection of *Triphosa* species is heavily sclerotized and distally forked. Size and shape of sacculus projection contains valuable diagnostic information (Leraut 2008, Hausmann and Viidalepp 2012). These structures are easily visible, when the valvae are not spread open (Fig. 3). The new technique holds the structures in a fixed position allowing to photograph and compare them in natural position. Later, the valvae can be spread open and embedded in Euparal, to allow comparison with other dissections.

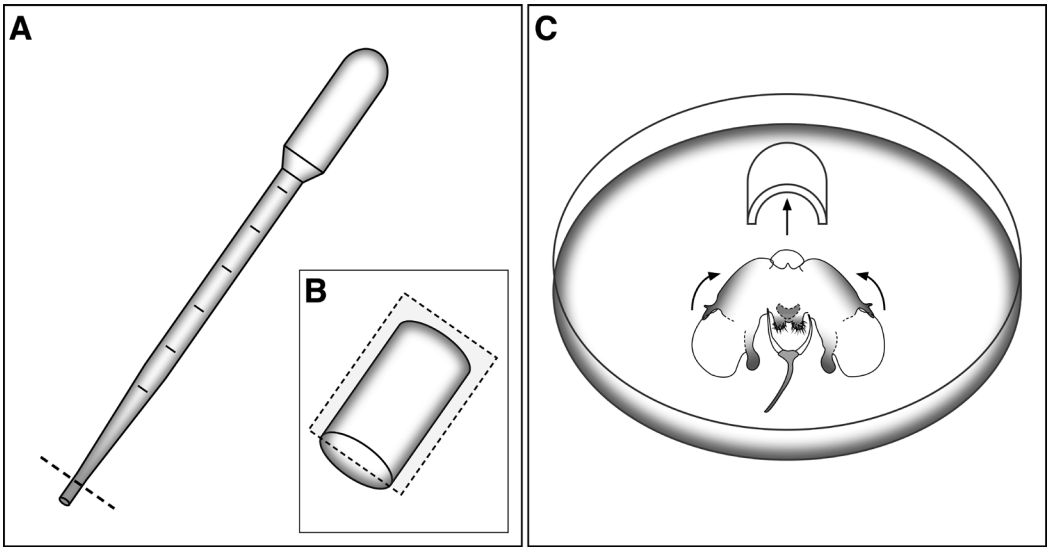

Figure 1. Preparation procedure of the mounting system. **A.** Cut off the tip of the plastic pipette; **B.** Split the small piece of pipette into two halves; **C.** Attach one of the halves on the bottom of the petri dish and fix the genitalia in the generated tunnel. Dashed lines indicate the cuts.

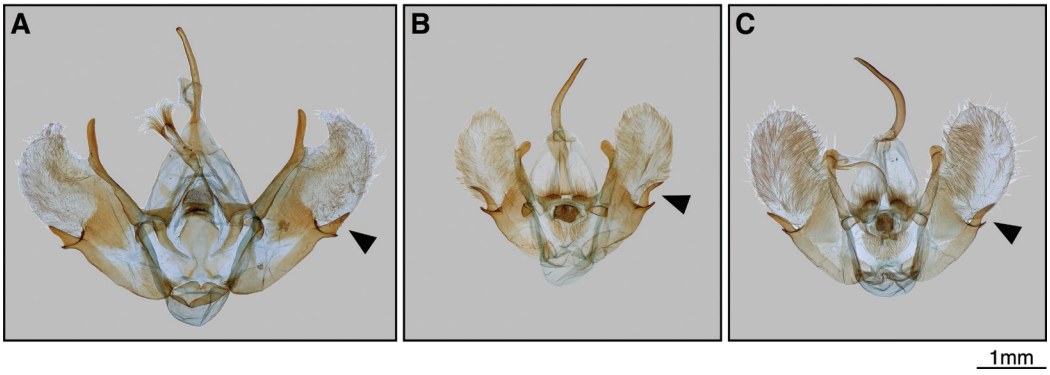

Figure 2. Embedded male genitalia with opened valvae in ventral view. **A.** *Triphosa sabaudiata*; **B.** *T. dubitata*; **C.** *T. tauteli*. Arrows indicate the sacculus projection of each species, which appear similar from this angle.

Here we compared the embedded genitalia of three species: *Triphosa sabaudiata*, *T. dubitata* and *T. tauteli* (Fig. 2). In the embedded slides, the differences between sacculus projection of *T. sabaudiata* and the other two species (*T. dubitata* and *T. tauteli*) may be slightly recognizable (Fig. 2A–C). In contrast, the differences of the forked tip in *T. dubitata* and *T. tauteli* are hardly visible (Fig. 2B, C).

Using the new method suggested in this paper, we fixed the genitalia capsule (with valvae unspread) in the tunnel-shaped holder. As is visible in the figures 3A–C, the shape of sacculus projection is distinctly different in all three listed species. The fork-shaped tip of the sacculus projection in *T. sabaudiata* consists of two identical processes 'thorns' (Fig. 3B), whereas in *T. dubitata* (Fig. 3C) and *T. tauteli* (Fig. 3D) the lower 'thorn' is shorter in length than the upper one. Addi-

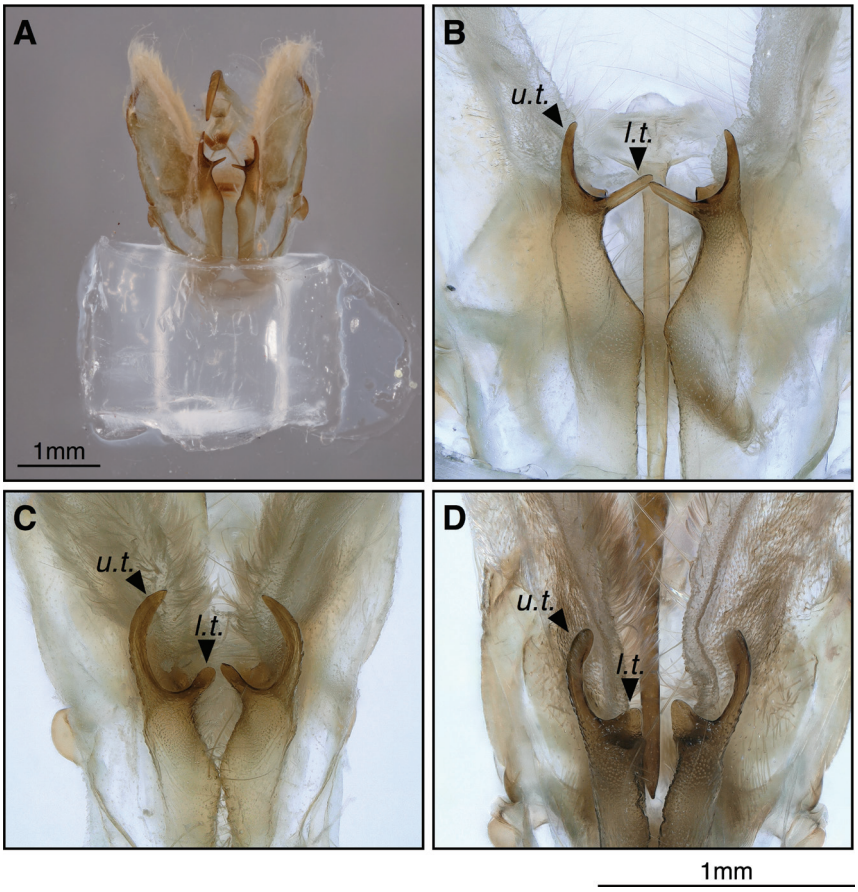

Figure 3. Unmounted genitalia capsule with closed valvae in ventral view in the tunnel-shaped holder. **A.** Overview of a *T. sabaudiata* genitalia placed in the tunnel; **B–D:** Close up photography of the sacculus projection, **B.** *T. sabaudiata*; **C.** *T. dubitata* and **D.** *T. tauteli*. Abbreviations: *u.t.* upper 'thorn' of sacculus projection; *l.t.* lower 'thorn' of sacculus projection. The sacculus projections appear diagnostic in this angle.

tionally, the differences between the 'thorns' of the sacculus projection in the species *T. dubitata* and *T. tauteli* are now visible (Fig. 3; these differences are nearly invisible in embedded genitalia capsule; Fig. 2). In *T. dubitata* the lower 'thorn' is thin and slight elongated ('thorn' shorter, and clearly thicker in *T. tauteli*, see Fig. 3).

Using the method explained above, or a modification of it, photography of the genitalia structures of the male and female in different angles and views could easily be obtained before mounting them on permanent slides.

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