

X-ray Radiography of a Spraying Stick Insect (Phasmatodea)

Röntgenradiographie einer sprühenden Stabschrecke (Phasmatodea)

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Summary: We have visualized the discharge of defensive secretions from the prothoracic glands of the phasmid species *Peruphasma schultei* using X-ray radiography. The secretion may be ejected as droplets, especially, however, as extremely thin jet no longer visible to the naked eye. During this process the unsculptured, wrinkled cuticle surrounding the gland's mouth will be unfolded, probably by hemolymph pressure allowing perhaps a focused spraying to some extent.

Keywords: *Peruphasma schultei*, Phasmatodea, defensive glands, X-ray radiography

Zusammenfassung: Wir haben die Abgabe von Wehrsekret aus den Prothoraxdrüsen von *Peruphasma schultei* mit Hilfe der Röntgenradiographie visualisiert. Das Sekret kann in Tropfen, vor allem aber als dünner, feiner Strahl, der nicht mehr mit dem unbewaffneten Auge sichtbar ist, abgegeben werden. Während des Vorgangs wird die die Drüsenmündung umgebende, nicht skulpturierte Cuticula, möglicherweise durch Hämolympdruck, vorgestülpt; dies erlaubt vielleicht sogar bis zu einem gewissen Grad ein zielgerichtetes Spritzen.

Schlüsselwörter: *Peruphasma schultei*, Phasmatodea, defensive glands, X-ray radiography

1. Introduction

Many phasmids have well developed defensive glands in their prothorax. These glands are invaginations of the integument and therefore consist of a secretion reservoir lined by a thin cuticle, the secretory epithelium and a thick musculature surrounding the reservoir (HAPP et al. 1966; NOIROT & QUENNEDY 1974). Secretions are highly variable and may contain glucose, monoterpene cyclopentanoids as anisomorphal, and nepetalacton, monoterpene alkaloids such as actinidin and quinolone, alkyldimethylpyrazines or spiroketals (see e.g. EISNER et al 1997; DOSSEY et al. 2006, 2007, 2009, 2012, and further readings therein), which mainly serve as a defence against potential predators (e.g. EISNER

1965; EISNER et al. 1997; BEIN & GREVEN 2006; BRADLER 2009). After disturbances secretions of these glands may be ejected in form of a drop, a spray, or as a fine jet of liquid. Spray and fine jet are hardly visible with the naked eye, and often one smells only that the secretion has been discharged, but the target item generally is wetted (e.g. Strong 1975; EISNER et al. 1997; BEIN & GREVEN 2006). Non-visible discharge of secretions and wetting of the target has been attributed to an ejection in form of affine spray (EISNER 1965; STRONG 1975; EISNER et al. 1997).

In the present note, we used synchrotron X-ray radiography to make this jet and the immediate process of discharge visible. This method was already successfully employed to examine physiological pro-

cesses in insects such as tracheal movements (e.g. WESTNEAT et al. 2003, SOCHA et al. 2007) feeding kinematics (BETZ et al. 2008) or explosion-induced defensive spray pulsation in bombardier beetles (ARNDT et al. 2015) and facilitates the real-time visualization of both external and internal movements with high speed and micrometre resolution.

The experimental animal was a male of *Peruphasma schultzei*, a black colored phasmid species with yellow eyes and deep red or pink anal fields of the hind wings that are raised when the insect is excited or disturbed (CONLE & HENNEMANN 2005, VAN DE KAMP 2011). The defensive secretion of this species consists of glucose and a stereoisomer of anisomorphal and dolichodial called peruphasmal (DOSSEY

et al. 2006).

2. Material and Methods

2.1. Histology

Parts of the defensive gland were excised from a male specimen of *Peruphasma schultzei*, fixed in 2,5 % glutaraldehyde in 0,1 mol/l cacodylate buffer for several hours, postfixed in 1 % osmium tetroxide and embedded in Spurr's medium (SPURR 1969). Semithin sections (approx. 1 μm thick) were stained with toluidine blue/borax.

2.1. Scanning electron microscopy (SEM)

Heads and prothoraces of further species were either used in toto or were opened

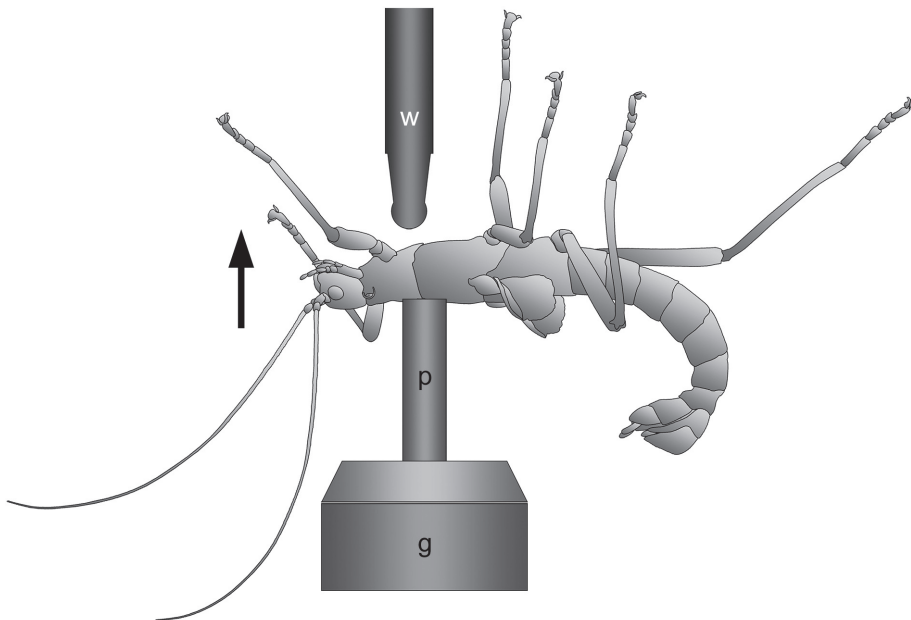


Fig. 1: For radiography the stick insect was glued to a paper stub (p) that was fixed in a goniometer head (g) mounted on a motorized stage. During image acquisition the insect was gently pushed against a wrench (w, arrow) to provoke the defensive reaction.

Abb. 1: Die Stabschrecke wurde für die Radiographie auf ein Papierstäbchen (p) geklebt und in einem Goniometerkopf (g) fixiert. Um die Verteidigungsreaktion auszulösen, wurde das Tier mit Hilfe eines motorisierten Tisches behutsam gegen einen Inbusschlüssel (w, Pfeil) gedrückt.

to expose the defensive glands. Then, they were fixed as described (see 2.1.), dehydrated, critical-point dried, mounted on stubs, sputtered with gold and examined in the Leitz AMR 1000 SEM.

2.3. X-ray radiography

A paper stub was glued on top of the thorax of a male specimen and fixed to a goniometer head, mounted on a motorized stage. During image acquisition, the insect was gently pushed against a wrench to provoke the defensive reaction (Fig. 1). The radiographic sequence was recorded at the TOPO-TOMO beamline of the ANKA Synchrotron Radiation Facility (VAN DE KAMP et al. 2013), using a parallel polychromatic X-ray beam at a spectrum peak of about 15 keV. An indirect detector system composed of a 300 μm LuAG:Ce scintillator, diffraction limited optical microscope (Optique Peter) and 12 bit pco.dimax high speed camera with 2016 x 2016 pixels resolution (DOS SANTOS ROLO et al. 2014) was employed to capture 125 frames per second over a total scan duration of 66 seconds. A 3.6 x optical magnification led to an effective pixel size of 5.55 μm .

3. Results

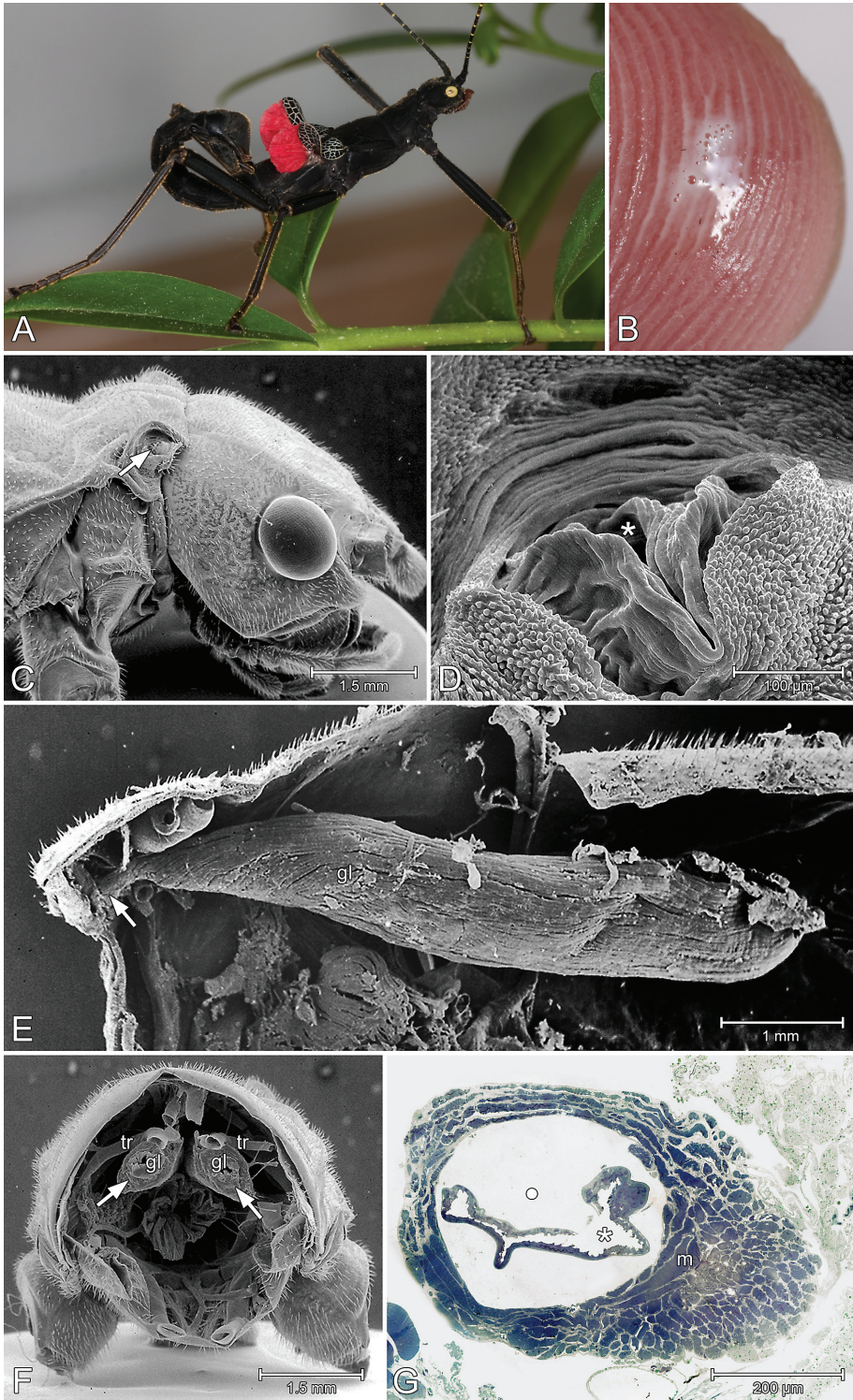
Fig. 2 A shows an adult male of *Peruphasma schultzei* raising its wings. When removing the animal from cage or irritating it by other means it sprays a jet (hardly visible with the naked eye) that leaves a milky substance on the target (Fig. 1C). The secretion comes from two large, paired glands located on the right and left side just beneath the anterolateral corners of the pronotum (see Fig. 2F). The glands extend far into the mesothorax and open at the front corners of the pronotum (Fig. 2C, E). The opening of the gland is a slit surrounded by a wrinkled, not sculptured cuticle (Fig. 2D). Above these glands large

tracheae are situated (Fig. 2F). Histologically, the glands are composed of a thin secretory epithelium covered by a delicate cuticle, and enclosed by a strong layer of muscles that is more pronounced on the outer face (Fig. 2E, F).

The process of the release of secretion as visualized by means of X-ray radiography is as follows: The specimen was glued with his back on the paper stub. It constantly moved its legs; and, thus, also the internal organs, such as the glands, were moved more or less rhythmically. At the point of the gland there was a small transparent tubercle (Fig. 3A). After slightly pressing the lower surface of thorax far beyond the gland opening; the pumping movement continued (Fig. 3B), but then the tubercle protruded further; and from its tip a fine secretion jet with a diameter of ca. 50 μm was ejected backwards (Fig. 3C). The jet hit the paper stub; the secretion drop adhering here contained small bubbles giving it a foamy appearance (Fig. 3C, F). Further, secretions accumulated at the gland opening (Fig. 3E). A second shot from the same gland followed 10 sec later (Fig. 3F).

4. Discussion

Using X-ray radiography it was possible to visualize (twice in succession) the discharge of secretions from a defence gland of *Peruphasma schultzei* that was fixed on a metal support. The secretion was ejected as an extremely thin jet and not as spray, but obviously also small droplets may be exuded in course of ejection. We think that many spraying phasmids eject their secretions of the defensive glands in form of a very fine jet rather than a spray. Discharge in several forms corresponds to other studies, e.g. of *Oreophoetes peruana*, a phasmid species that delivers its secretion even consecutively from both openings and this in different ways, i.e. with slowly exuding, relatively



large droplets or with a fine jet (see BEIN & GREVEN 2006), obviously depending on the kind of stimulus, e.g. unilateral or bilateral (EISNER 1965).

Certainly, this discharge is mainly effected by the strong muscle layer surrounding the glands, which in *O. peruana*, however, becomes thinner at the distal end (excretory ductule). Width of the ductule part as well as the shape and size of the gland opening may vary among species. This is probably true also for the surrounding muscles (STRONG 1975; HAPP et al 1966; EISNER et al 1997; own observations).

Regarding the behaviour of the cuticle that surrounds the gland opening in *P. schultei* the process of releasing secretions from the reservoir appears a little more complex. Surely, the contraction of the muscles ejects the secretions with high pressure. Just before ejection, however, this contraction may press the hemolymph against the wrinkled and obviously rather flexible cuticle that in turn protrudes slightly backwards forming a kind of launching pad allowing a shoot backwards. We do not think that the protrusion was due to a too high pressure, as discharge of secretions started with some delay.

Whether and to what extent *P. schultei* and other phasmid species may perform an aimed ejection of secretions is largely unknown. Aimed “sprays” in various direc-

tions were shown for *Anisomorpha buprestoides* (EISNER 1965). Indeed, knowledge of the organisation of the opening and the surrounding tissue of defence glands in phasmids is anecdotal, if any. In *Extatosoma tiaratum*, a species that produces a secretion not obnoxious (to man), the gland opening and their environment was studied by SEM and histology. Here the aperture opens on a cuticular tubercle, the posterior part of which is considerably thicker forming a hump, whereas the anterior part forms a thin lip covering the aperture. The cuticle around the base of the tubercle appeared flexible and “can be distorted by internal muscles” (not further identified) suggesting movements of the tubercle and thus some aiming (STRONG 1975, p. 68). Similar conditions are found in *O. peruana* (unpublished). A more detailed comparative analysis of the gland openings and their surrounding in several phasmids may contribute to questions related to the accuracy and direction of secretion release.

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Fig. 2: **A** *Peruphasma schultei*, adult male. **B** Fingertip with the milky secretion from the defensive gland. **C** SEM: Head and prothorax; gland opening (arrow). **D** Detail from C. Note the gland opening (asterisk) surrounded by a smooth and wrinkled cuticle. **E** SEM: Length expansion of a prothoracal gland (gl). **F** SEM: Thorax, cross section showing the paired defensive glands (gl) and their asymmetric muscles (arrows); above the glands are large tracheae (tr). **G** Histological cross section (1 µm thick) shows the empty reservoir (asterisk) of the gland and the surrounding asymmetrically arranged muscles (m). The space (circle) is artificial.

Abb. 2: **A** Männchen von *Peruphasma schultei*. **B** Fingerbeere mit milchigem Sekret der Wehrdrüsen. **C** REM: Kopf und Prothorax; Drüsenmündung (Pfeil). **D** Ausschnitt aus C. Man beachte die unskulpturierte, runzlige Cuticula um die Austrittsöffnung (Stern). **E** REM: Längsausdehnung einer Wehrdrüse (gl). **F** REM: Thorax, quer, mit den paarigen Wehrdrüsen (gl) und ihrer asymmetrischen Muskulatur (Pfeile); darüber jeweils große Tracheen (tr). **G** Histologischer Querschnitt (1 µm) durch die Drüse. Man beachte die asymmetrische Muskulatur (m) und das leere Sekretreservoir (Stern). Der Raum (Kreis) zwischen Muskulatur und Drüsenepithel ist artifiziell.

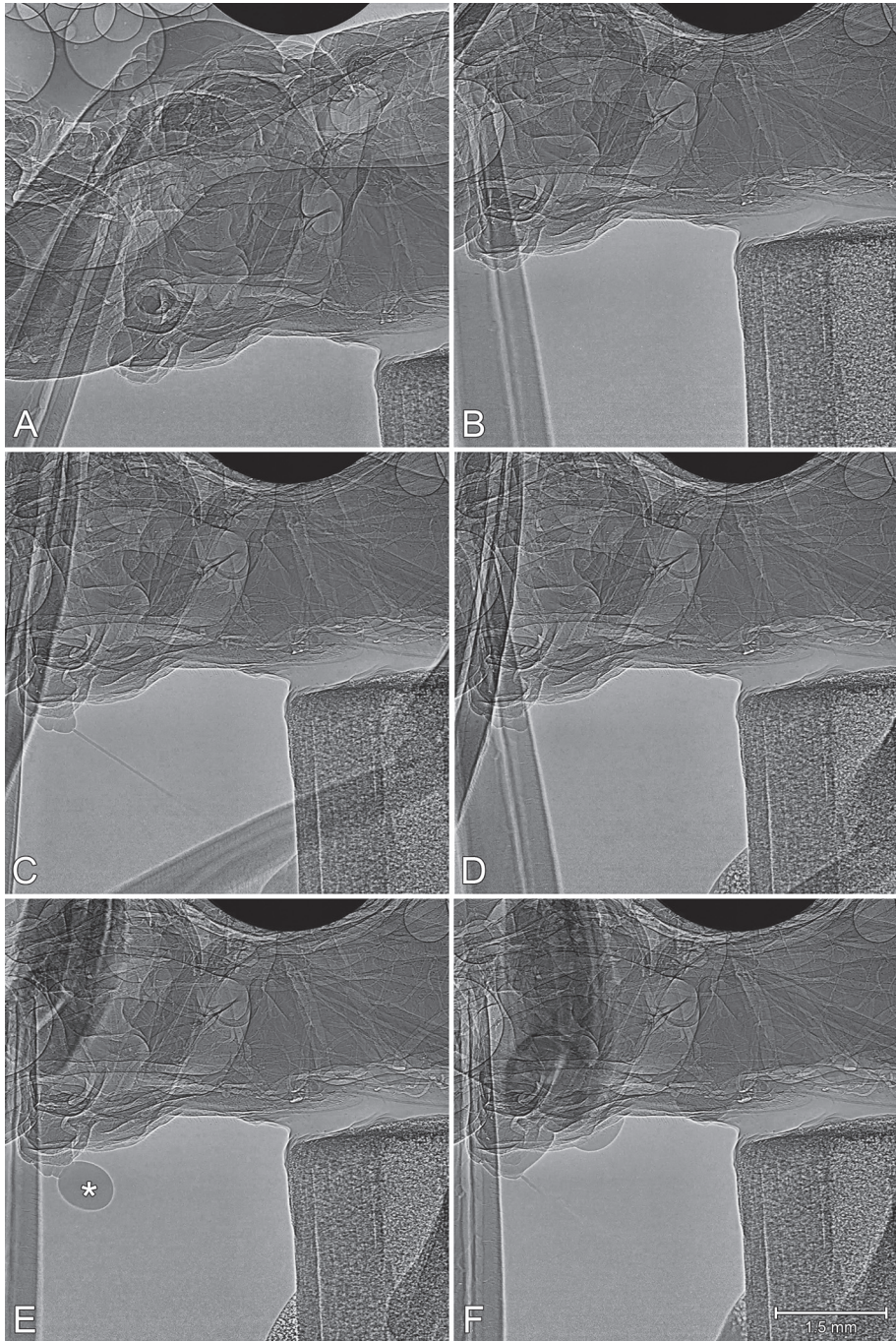


Fig. 3. **A** Specimen before stimulation. **B** The specimen is gently pushed against a wrench (black, on top) (19.4 s). **C** A fine jet of secretion is sprayed from the gland (first shot) (44.8 s). **D** The discharged secretion has a foamy appearance (bottom right) (45.6 s). **E** Secretion droplet attached to the gland opening (asterisk) (46.6 s). **F** Second shot with a visible jet hitting the drop discharged before (46.8 s).

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Abb. 3: **A** Das Tier vor der Stimulation. **B** Tier wird gegen einen Inbusschlüssel (oben, schwarz) gedrückt (19.4 s) **C** Ein feiner Sekretstrahl verlässt die Drüsenöffnung (erster Schuss) (44.8 s). **D** Das abgegebene Sekret wirkt schaumig (unten rechts) (45.6 s). **E** Sekrettropfen an der Drüsenmündung (Stern) (46.6 s) **F** Zweiter Schuss; der jetzt sichtbare Strahl trifft den alten Tropfen (46.8 s).

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