## How to Improve Soil Anti-adhesion by Studying the Micro Relief of the Cuticle Surface of Digging Beetles: Exploring the Sulcophanaeus batesi Pronotum Using Translucent Replicas

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## **Meeting-report**

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For some years, we have been studying the microtopography of soil diggers beetles from Argentina to find the anti-adhesion pattern to decrease a soil particle adhesion for agricultural machinery components. In 2018, we designed a macro topographic pattern for the upper surface of a steel shovel for tilling (agricultural tool) from the study of the microtopography (microrelief) of the cuticular surface of the pronotum of Diloboderus abderus (Coleoptera, Scarabaeidae), whose main feature is the presence of dimples randomly distributed. The proposed macro topography pattern reduces the drawing force on the tool with a reduction of the average traction demand of approximately 7% for the shovel [1]. In 2020, we started to explore the micro relief of the cuticle surface of other beetle species living in Argentina that are considered true soil diggers with the aim of finding new non-stick patterns [2]. A fundamental step for the design of these patterns is to characterize the biological surface micro relief using different microscopy techniques. In the two previous works, scanning electron microscopy (SEM) images were used for the study. In this case, we have used translucent replicas for the observation of the thorax cuticle of female and male specimens with pronotum of similar size of Sulcophanaeus batesi (Coleoptera, Scarabaeidae) (Fig. 1), a true dung beetle, collected from Tucumán (Argentina). This technique has one main advantage. The thorax has a convex shape, which would prevent, in a traditional SEM image, measuring the real dimensions of the micro relief. The flexibility of the thorax replica makes it possible to flatten the surface and get better measurements of the dimples. It is a simple, non-destructive, fast and low-cost method, which allows a more precise measurement [3]. The replica technique used a two-component high-precision dental silicone (Lastic 90 Fine + Lastic Universal) to make a first mold (negative), then a transparent nail polish (positive) was deposited on the first mold for the final product [3]. The pronotum of female and male specimens were studied using replicas, observed by light microscopy (DMRX, Leica) and the images were analyzed with Image J (Fig. 2). Only one half of the pronotum was analyzed. It was divided into six sections following the curvature of the pronotum laterally and anteroposterior direction. The cuticle surface presents dimples. These were not perfect circles, so two perpendicular dimensions were considered (d1 and d2). Figure 3 shows the mean dimensions (Mean d1, Mean d2), as well as the circle eccentricity (Mean d1/Mean d2) for the six sections (Fig. 4). In total, more than 500 dimples were measured on each specimen. The results indicate that there are differences between the female and male specimens. The size of the female dimples is bigger than the male's, however dimple eccentricity is similar for both specimens. Within the same sexual gender, there are differences between sections. In summary, the method of translucent replicas is good and simple for measuring the real size, morphology of dimples, which will allow to improve the design of macro topography patterns in surfaces of agricultural tools.



Fig. 1. Sulcophanaeus batesi specimens. A- Female; B- Male.



**Fig. 2.** Light micrographs of translucent replicas of the thorax cuticle of *Sulcophanaeus batesi*. **A** - **F**: female specimen (one per section 1 - 6). **G** - **L**: male specimen (one per section 1 - 6). The images are shown following the arrangement of the sectors defined in the pronotum. (Scale bar: 200 μm).



Fig. 3. Perpendicular mean dimensions (d1; d2) of the pronotum dimples of female and male specimens of Sulcophanaeus batesi.



Fig. 4. Dimple eccentricity (Mean d1/Mean d2) of the pronotum dimples of female and male specimens of Sulcophanaeus batesi.

## References

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- 2. Setten, et al., Microsc. & Microanal. 26(2) (2020), p. 306.
- 3. Setten, et al., 7º Congreso Argentino de Microscopía SAMIC 2022. (2022), p. 106.