
Supplementary information

**Toughening mechanisms of the elytra of the
diabolical ironclad beetle**

In the format provided by the
authors and unedited

Supplementary Information

Toughening Mechanisms of the Elytra of the Diabolical Ironclad Beetle

Authors: Jesus Rivera¹, Maryam Sadat Hosseini², David Restrepo^{2,3}, Satoshi Murata⁴, Drago Vasile⁵, Dilworth Y. Parkinson⁶, Harold S. Barnard⁶, Atsushi Arakaki⁴, Pablo Zavattieri², David Kisailus^{1,5,7*}

Affiliations:

¹Materials Science and Engineering Program, University of California, Riverside, CA 92521.

²Lyles School of Civil Engineering, Purdue University, West Lafayette, IN 47907

³Department of Mechanical Engineering, The University of Texas at San Antonio. Texas 78249

⁴Institute of Global Innovation Research, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan

⁵Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521.

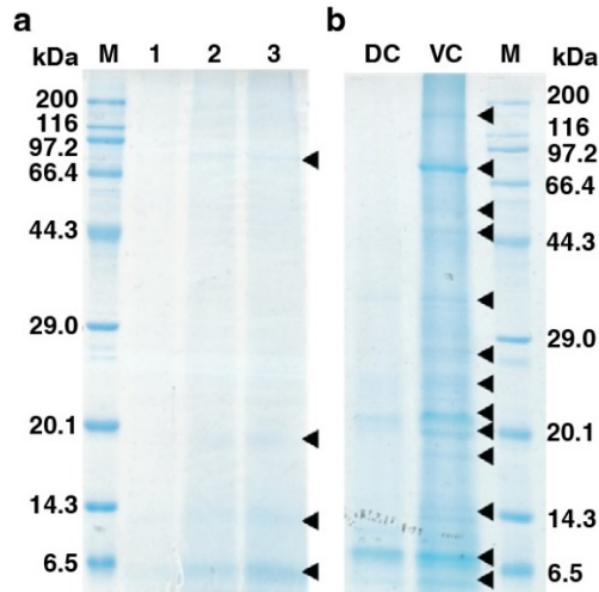
⁶Advanced Light Source, Berkeley Labs, Berkeley, CA

⁷Department of Materials Science and Engineering, University of California, Irvine, CA 92697.

*Correspondence to: david.k@uci.edu

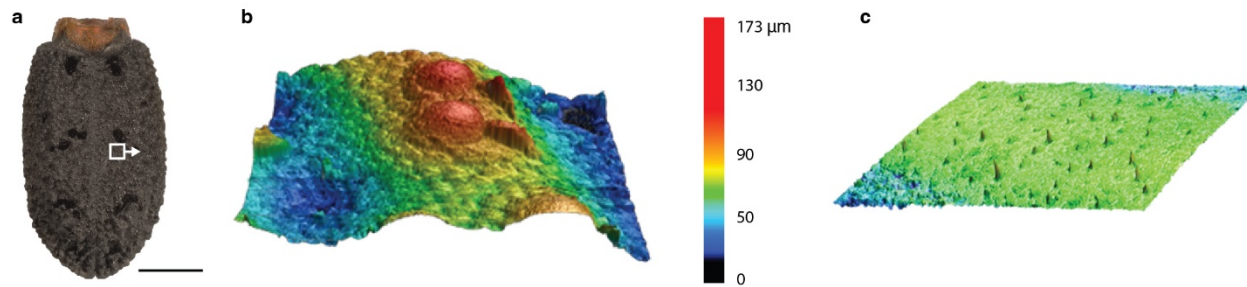
1. Elytral mechanical and structural characterization. *P. diabolicus*' high compression resistance likely results from not only the matrix components, but also the assembly of remarkable architectures across multiple length scales¹. Cross-sectional analysis (Extended Data Fig. 1b) of the elytra reveals a bouligand-like architecture (Extended Data Fig. 1d) typically observed in arthropods² and found to provide significant impact resistance not only in biological structures but also in engineered materials³⁻⁵. These bundled, balken, layers are joined together via z-oriented fibers (Extended Data Fig. 1c) within the exocuticle, providing additional compressive strength and toughening⁶. Computed Tomography analysis revealed z-oriented pore canals (Extended Data Fig. 1e), which similar to those found in antlers, teeth and bone, provide transport pathways for nutrients during structural assembly and development, but are also known to mechanically arrest crack propagation by blunting the crack tips⁷⁻¹⁰.

The exoskeletons of these beetles are non-mineralized and consist of an orchestrated hierarchical assembly of alpha-chitin fibers embedded within a proteinaceous matrix that provides both strength and toughness¹¹⁻¹⁴. Chemical analysis of the elytra of *P. diabolicus* revealed a mixture of approximately 32.6 wt% chitin, 52.2 wt% protein, and 15.1 wt% lipid. In contrast, the elytra from the flying beetle, *Trypoxylus dichotomus*, contained 42.0 wt%, 43.4 wt%, and 15.0 wt% of chitin, protein and lipid, respectively. The relatively high protein content found in the elytra of *P. diabolicus* is likely correlated to its significantly thicker endocuticle as well as the amount of matrix between chitin fibrils¹⁵, which not only plays a key role in the elytra morphogenesis¹⁶ and microstructural formation¹⁷, but also enables significant energy absorption. Unlike crustaceans, which add mineral to their cuticles to provide strength, stiffness and hardness, arthropods (e.g., insects), rely on the chemical modification of proteins (e.g., tanning/sclerotization) to stiffen and harden the exoskeleton¹⁶ (see Nanoindentation section below). The degree of this cross-linking is directly correlated to the modulus of the cuticle¹⁷. Tanning of the insect exoskeleton is a complex process, which involves hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and decarboxylation of DOPA to dopamine. Through several additional steps, DOPA and dopamine are converted to quinone metabolites, which undergo cross-linking reactions with side chains of elytral proteins such as histidyl residues for cuticle sclerotization¹⁸. Thus, the relatively high protein content found in the elytra of *P. diabolicus* is likely correlated to its enhanced mechanical properties, including those resulting from a high degree of cross-linking¹⁹. In addition to the relative concentration of protein in the elytra, SDS-PAGE analysis revealed differences in elytral protein constituents between *P. diabolicus* and *T. dichotomus* (Supplementary Figure 1). The major protein bands in *P. diabolicus* appeared at approximately 6 and 13 kDa (Supplementary Figure 1A), while those in *T. dichotomus* were approximately 7, 21, and 80 kDa (Supplementary Figure 1B). Proteins from the elytra of *P. diabolicus* were less extractable than that of *T. dichotomus*, suggesting its elytral proteins are highly cross-linked providing an enhanced stiffness. In addition, these protein constituents and their association with chitin fibers at molecular levels may also play a role in regulating the mechanical properties of elytra of beetles. Further evaluation is underway.



Supplementary Figure 1. Difference of protein profile between *P. diabolicus* (A) and *T. dichotomus* (B) elytra. Proteins were extracted by 1% SDS solution. Lane M, molecular size marker; lane 1, 10 µl of extracts were loaded; lane 2, 20 µl of extracts were loaded; lane 3, 30 µl of extracts were loaded; lane DC, protein extracts from dorsal elytral cuticle; lane VC, protein extracts from ventral elytral cuticle. Arrow heads indicate major protein bands appeared in each sample.

Laser profilometry scans of the elytra surface (Supplementary Figure 2) highlight the dull, matt-like elytra of *P. diabolicus* that has a roughness of $(27.26 \pm 2.77\mu\text{m})$. This is due to an extensive coating of leaf-like setae and a large tubercles (Supplementary Figure 2b) which may serve as storage for nutritive material¹⁸. Exterior setae can also serve as camouflage as the dark coloration matches with the appearance of small rocks and bark. Furthermore, the increased surface area of the elytra due to these features may assist with possible water collection. Conversely, the elytra of *T. dichotomus*, which appears smooth (roughness of $9.77 \pm 2.47\mu\text{m}$) has few features, with a sparse coating of setae (Supplementary Figure 2c). This smooth and waxy-looking coating prevents excess moisture from condensing on its surface and deterring flight^{19,20}. For the ironclad beetle, the elevated topography likely assists in distributing any biting or pecking forces from predators, similar to how arches or domes distribute normal forces. This addition of undulations for load dissipation has been observed in other arthropod exocuticles such as the carinae in the telson of the mantis shrimp²¹.



Supplementary Figure 2. Laser profilometry scans of the elytra of *P. diabolicus* and *T. dichotomus*. (a) Coronal image of the elytra (scalebar 5 mm) highlighting (b) the topography of the surface. (c) Surface topography of *T. dichotomus*.

Common predators to beetles include birds, reptiles and mammals, whose impact strain rates during biting or pecking ranges from $10^0 - 0.5 \times 10^2 \text{ (s}^{-1}\text{)}$ ^{22–24}. Crows can peck at a rate of up to 0.5×10^2 and with a force in excess of 20N ²³. Smaller aviary predators typically strike with a peak force of 11 N at a strain rate of $\sim 10^1$ when interrogating prey ²⁵. Small reptiles, which try to eat the ironclad beetle, are at a relatively lower strain rate ($\sim 10^0$), and have an average biting force of $12.05 \pm 2.41 \text{ N}$ compared to that of small mammals $13.704 \pm 14.6 \text{ N}$ ^{22,24,26}. This relatively high resistance to compression seen in the ironclad beetle suggests its need for a robust exoskeleton capable of resisting the interrogatory biting and pecking forces from these predators. Although there is a variation in the strain rate from these predators (ranging from quasi-static to $0.5 \times 10^2 \text{ (s}^{-1}\text{)}$), a small increase in stiffness and peak load is expected ²⁷. However, at these relatively low strain rates ($< 10^3$) exhibited by the natural predators, no significant post-yield strain hardening is expected ²⁸.

2. Nanoindentation. Interrogating the regional hardness and modulus of the elytra, nano indentation maps were conducted on polished transverse cross sections. Indentation maps including the exo and endocuticle of *P. diabolicus* are shown in Extended Data Figure 2. The exocuticle of *P. diabolicus* has a hardness and reduced elastic modulus of 0.59 ± 0.15 and $9.85 \pm 2.19 \text{ GPa}$, respectively, that decrease to 0.37 ± 0.16 and $9.28 \pm 3.37 \text{ GPa}$, when moving into the endocuticle. This change in hardness and modulus suggest a variation in sclerotization of the cuticle further supported by the visibly darker color of the exocuticle transitioning to the lighter endocuticle (Extended Data Figure 2b). The reduced elastic modulus is dependent on fiber bundle orientation as indicated in the endocuticle, where indentation maps show the out of plane balkens have the greatest stiffness (Extended Data Figures 2 d, e)

3. Mechanical response of lateral supports. A three-dimensional FE model of the cuticle under compressive load confirms that the stress significantly drops at the lateral supports for the free-standing variant versus those in the interdigitated case (Extended Data Fig. 4). We surmise that the free-standing and interlocking supports enable deflection of the elytra and therefore, increase the energy absorption during crushing events by providing compliance, while the interdigitated supports increase the stiffness.

Models of the entire abdomen, elytra and ventral cuticle under compression highlight the distribution of the principal stresses as the first and second support transfer stress to the ventral cuticle. Further analysis reveals a repetition of the second support at the posterior of the abdomen to prevent collapse of the elytra. In addition, models show that there are stresses developing in the top of the supports, but they are 2 times lower than those at the side supports and are thus, not as apparent as the limits of the stress fields are fixed to be consistent with the stress shown between the three supports. This also suggests the presence of a bending component to the lateral supports.

4. Medial Suture: Microstructural effects. Finite element models and 3D printed prototypes were implemented in two parametric case studies (capacity and quantity variation) to evaluate variations in stiffness, toughness, and peak tensile load versus number and geometry of the sutural blades. For the first set of tensile tests: capacity variation, the number of blades was increased while keeping the contact area between two elytra constant, resulting in a decrease of feature sizes (Fig. 3d). For quantity variation, the number of blades (with the naturally observed geometry) was varied from one to five blades (Extended Data Fig. 6a). Moreover, the FE models suggest that the initial stiffness increases with the number of blades, yet the 3D printed tests result in a stiffness plateau at 3 blades. This could result from air bubbles or uncured resin weakening the relatively small 3D printed blades²⁹. Printing larger samples would most likely circumvent size-related defects resulting in the linear trend in stiffness, toughness, and normalized peak load calculated in the FE models.

5. References

1. Chen, Q. & Pugno, N. M. Bio-mimetic mechanisms of natural hierarchical materials: A review. *J. Mech. Behav. Biomed. Mater.* **19**, 3–33 (2013).
2. Bouligand, Y. Twisted fibrous arrangements in biological materials and cholesteric mesophases. *Tissue Cell* **4**, 189–217 (1972).
3. Weaver, J. C. *et al.* The stomatopod dactyl club: A formidable damage-tolerant biological hammer. *Science* (80-.). **336**, 1275–1280 (2012).
4. Grunenfelder, L. K. *et al.* Bio-inspired impact-resistant composites. in *Acta Biomaterialia* **10**, 3997–4008 (Elsevier Ltd, 2014).
5. Suksangpanya, N., Yaraghi, N. A., Pipes, R. B., Kisailus, D. & Zavattieri, P. Crack twisting and toughening strategies in Bouligand architectures. *Int. J. Solids Struct.* **150**, 83–106 (2018).
6. Andrew Jansen, M., Singh, S. S., Chawla, N. & Franz, N. M. A multilayer micromechanical model of the cuticle of *Curculio longinasus* Chittenden, 1927 (Coleoptera: Curculionidae). *J. Struct. Biol.* **195**, 139–158 (2016).
7. Chen, P. Y., Lin, A. Y. M., McKittrick, J. & Meyers, M. A. Structure and mechanical properties of crab exoskeletons. *Acta Biomater.* **4**, 587–596 (2008).
8. Yang, W. *et al.* Structure and fracture resistance of alligator gar (*Atractosteus spatula*) armored fish scales. *Acta Biomater.* **9**, 5876–5889 (2013).
9. Kasapi, M. A. & Gosline, J. M. Design complexity and fracture control in the equine hoof wall. *J. Exp. Biol.* **200**, 1639–1659 (1997).
10. Chen, P. Y., Stokes, A. G. & McKittrick, J. Comparison of the structure and mechanical properties of bovine femur bone and antler of the North American elk (*Cervus elaphus canadensis*). *Acta Biomater.* **5**, 693–706 (2009).
11. Foley, I. A. & Ivie, M. *A phylogenetic analysis of the tribe Zopherini with a review of the species and generic classification (Coleoptera: Zopheridae)*. Zootaxa (Magnolia Press, 2008).
12. Vincent, J. F. V & Wegst, U. G. K. Design and mechanical properties of insect cuticle. *Arthropod Struct. Dev.* **33**, 187–199 (2004).
13. Cribb, B. W. *et al.* Hardness in arthropod exoskeletons in the absence of transition metals. *Acta Biomater.* **6**, 3152–3156 (2010).
14. Dennell, R. The structure and lamination of some arthropod cuticles. *Zool. J. Linn. Soc.* **58**, 159–164 (1976).
15. Sun, J. & Bhushan, B. Structure and mechanical properties of beetle wings: a review. *RSC Adv.* **2**, 12606 (2012).
16. Noh, M. Y., Muthukrishnan, S., Kramer, K. J. & Arakane, Y. Cuticle formation and pigmentation in beetles. *Curr. Opin. Insect Sci.* **17**, 1–9 (2016).
17. Li, C., Gorb, S. N. & Rajabi, H. Cuticle sclerotization determines the difference between the elastic moduli of locust tibiae. *Acta Biomater.* **103**, 189–195 (2020).
18. Ayari, A., Raimond, M., Souty-Grosset, C. & Nasri-Ammar, K. Hierarchical organization of the cuticle of the subsocial desert isopod, *Hemilepistus reaumurii*. *J. Struct. Biol.* **193**, 115–123 (2016).
19. Klocke, D. & Schmitz, H. Water as a major modulator of the mechanical properties of insect cuticle. *Acta Biomater.* **7**, 2935–2942 (2011).
20. Gibbs, A. G. Water-proofing properties of cuticular lipids. *Am. Zool.* **38**, 471–482

- (1998).
21. Yaraghi, N. A. *et al.* The Stomatopod Telson: Convergent Evolution in the Development of a Biological Shield. *Adv. Funct. Mater.* **1902238**, 1–13 (2019).
 22. Freeman, P. W. & Lemen, C. A. Measuring Bite Force in Small Mammals with a Piezo-resistive Sensor. *J. Mammal.* **89**, 513–517 (2008).
 23. Kamata, N., Yamada, R. & Sugita, S. Maximum peck and pull forces exerted by Jungle and Carrion Crows. *Japanese J. Ornithol.* **60**, 191–199 (2011).
 24. Kristopher Lappin, A. & Jones, M. E. H. Reliable quantification of bite-force performance requires use of appropriate biting substrate and standardization of bite out-lever. *J. Exp. Biol.* **217**, 4303–4312 (2014).
 25. Asoegwu, S. N., Obasi, A. U., Ohanyere, S. O. & Nwakuba, N. R. Analytical development of bird pecking force equation for a self-metering poultry feeder. *Agric. Eng. Int. CIGR J.* **19**, 183–188 (2017).
 26. Aguirre, L. F., Herrel, A., Van Damme, R. & Matthysen, E. Ecomorphological analysis of trophic niche partitioning in a tropical savannah bat community. *Proc. R. Soc. B Biol. Sci.* **269**, 1271–1278 (2002).
 27. Arola, D., Ghods, S., Son, C., Murcia, S. & Ossa, E. A. Interfibril hydrogen bonding improves the strain-rate response of natural armour. *J. R. Soc. Interface* **16**, 1–12 (2019).
 28. Siviour, C. R. & Jordan, J. L. High Strain Rate Mechanics of Polymers: A Review. *J. Dyn. Behav. Mater.* **2**, 15–32 (2016).
 29. Li, Y., Ortiz, C. & Boyce, M. C. A generalized mechanical model for suture interfaces of arbitrary geometry. *J. Mech. Phys. Solids* **61**, 1144–1167 (2013).