COMPRESSION RESISTANT DESIGNS FROM THE EXOSKELETON OF A TOUGH BEETLE

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ABSTRACT

A sub class of arthropods dating back over 300 million years, beetles possess diverse predator avoidance responses and structural adaptations with exceptional mechanical properties across flying, terrestrial and aquatic variants. One example being the family of terrestrial beetles the Zopherinae, ironclad beetles, are known for their compression resistant nature. Here we focus on the multi-functional exoskeleton of the diabolical ironclad beetle, *Phloeodes diabolicus*. We report an encompassing study of the structural architecture of the exoskeleton of *P. diabolicus*, an oak dwelling fungivore primarily residing on the western coast of North America. This desert insect exhibits an outstanding adaptation to feign death along with an extremely predator resistant exoskeleton, able to withstand high compressive loads that would prove fatal to most insects. This study focuses on the abdominal portion of the exoskeleton, consisting of the elytra and ventral cuticle. We investigate this robust design, forged by ecological pressures, to reveal structure-property relationships. Our work highlights that this structure is a non-mineralized composite that is not only light and extremely compression resistant, but also demonstrates the capability to collect, transport and store water in an arid environment. We investigated the elytra (forewing), which consists of a hierarchical arrangement of unidirectional alpha-chitin fiber sheets assembled in a helicoidal arrangement. This model system represents a tough, damage tolerant biological material that exemplifies a departure from mineralized structures and further enhances our knowledge of natural multi-functional composites.

INTRO

There remains a demand for a new generation of tough, lightweight, environmentally friendly structural materials. Exhibiting unique or superior performance versus engineering materials, natural systems have developed tough composites designs and processes yielding a variety of hierarchical structures from available resources. The structure-function relationship of these materials drives their performance through the incorporation of specific material components with controlled gradients and specific hierarchical designs assembled in controlled environmentally friendly processes.

Exemplifying these traits, beetle cuticles provide a prominent example of a multifunctional tough composite. One noteworthy structure is the exoskeleton of the diabolical ironclad beetle, *Phloeodes diabolicus*, known for its compression resistant nature. This beetle remains notorious amongst entomologists for its ability to bend steel pins. We have determined that its exoskeleton can resist crushing from an automobile that proves fatal to other insects. Noted as one of the toughest insects on record and reported to be both crush and penetration resistant, the ironclad beetle can survive compressive forces as high as 150 N and numerous predator attacks.

We investigate this robust design, forged by ecological pressures, to reveal structure-property relationships. Our work highlighted that this structure is a non-mineralized composite that is not only light-weight and extremely compression resistant, but also demonstrates the capability to collect transport and store water in a dry environment. In beetles (the order is *Coleoptera*), the elytra represents the sclerotized forewings of the insect that cover its membranous hindwings. We investigated the elytra (forewing), which consists of a hierarchical arrangement of unidirectional alpha-chitin fiber sheets assembled in a helicoidal arrangement. To date, structure-mechanical property relationships have not yet been fully realized for the ironclad beetle's exoskeleton. Unlike the diabolical ironclad beetle, many species are not equipped with the adaptation of a robust exoskeleton able to withstand extensive external forces. This model system represents a tough, damage tolerant biological material that exemplifies a departure from mineralized structures and further enhances our knowledge of natural multi-functional composites.

Here we highlight the novel architecture within the exoskeleton of *P. diabolicus* that varies from the conventional terrestrial beetles and contributes to enhanced mechanical performance. This exoskeleton primarily consists of alpha chitin fibers in layers of assembled bundles (Balken) that are joined together by a proteinaceous matrix. We further reveal toughening mechanisms resulting from the macrostructural architecture along with microstructural and mechanical interfacial features that provide insight into the fabrication of tough, impact resistant composite materials.

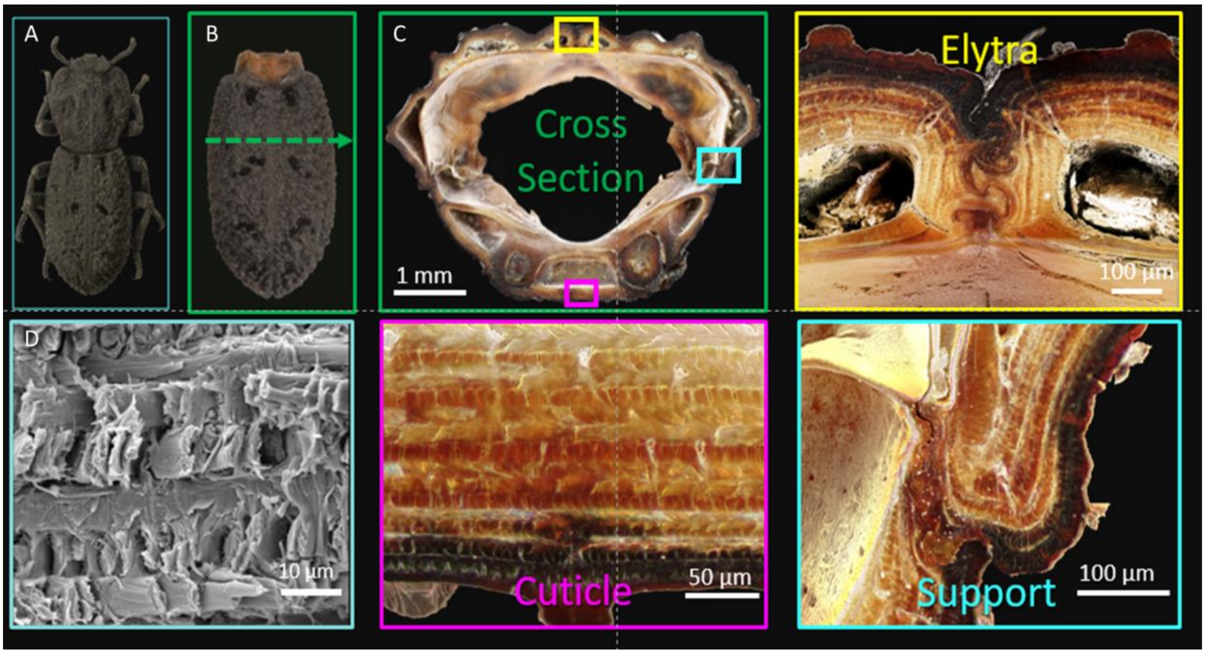

Figure 1: A) The diabolical ironclad beetle along with an isolated section of B) the abdomen section of the insect. C) A cross section highlighting: the medial suture, lateral support and ventral cuticle. D) Further examination of the microstructure reveals the layers of bundles in a pseudo-helicoidal arrangement.

Interfacial Features

To identify the origins of compression resistance, we isolated the features of interest. Analyzing a transverse cross section of the abdomen, the exoskeleton can be divided into three distinct regions (Fig. 1C): the elytra (comprised of two sclerotized hardened forewings), the lateral supports and the ventral cuticle. With stress typically concentrating between interlocking segments, the mechanical responses between the interfaces of these sections remains the focus of this examination. Joining the two elytron, the medial suture spanning the length of the abdomen (Fig 1C) is composed of layers of alpha chitin fibers creating elliptical interlocking regions that interface the hardened forewings. When stressed the dorsal region of the suture is under compression while the ventral segment remain under tension. Upon loading, the wrapped fibrous design promotes delamination of the interdigitated segments as a mode of failure instead of fracture at the thinnest segment as observed in bulk materials [21]. Similarly, the lateral support consists of alpha chitin fibers wrapped around the interfacial regions between the elytra and the ventral cuticle. The overall size of interdigitations in the lateral interfaces ranges from 20-7 µm in diameter. Due to the variation of the applied load on the region, the lateral support exhibits a variation in geometry, circular instead of elliptical, but a similar number of interdigitated segments to the suture. Working in tandem with these interfacial features, *P. diabolicus* possesses a relatively thick elytron (ranging from 250- 350 µm) with specialized design elements including helical fiber orientation, structural gradients, cellular organization and overlapping architectures that attribute to this organism's resistance to crushing and pecking attacks. [10].

Elytra Fiber Arrangement

Developing from imaginal discs and representing evaginations of the epidermis the elytra exhibits an outer (dorsal) and inner ventral cuticle connected by trabecula and enclosing the hemolymph space (Fig. 2) [10,8]. The exocuticle (5-7 µm) consists of unit layers (lamellae) of non-mineralized alpha chitin fibrils organized in a periodic arrangement penetrated by perpendicular pore canal fibers extending from the endocuticle [22]. Recent publications affirm fiber alignment in a spiral pattern or Bouligand arrangement of parabolic arcs in the exocuticle using TEM and AFM but fail to fully address through layer pore canal fibers visible in the fractured cross sections[23]. In the ironclad, the endocuticle incorporates a helicoidal design at the nanoscale with trough ply porecanal fibers improving flexural strength and torsional rigidity. These pore canal fibers can serve to strengthen the interface between layers reducing the effects of shear forces that would cause delamination upon an applied load [24]. The rotated layered structure increases fracture toughness by introducing numerous interfaces that provide a torturous path for crack propagation while providing rigidity under torsion and bending due to the additional isotropy of layers. Transitioning into the endocuticle, graded structures observed my nanoindentation maps relieve stress between interfaces of dissimilar materials and may arrest crack propagation resulting from a mismatch in elastic modulus.

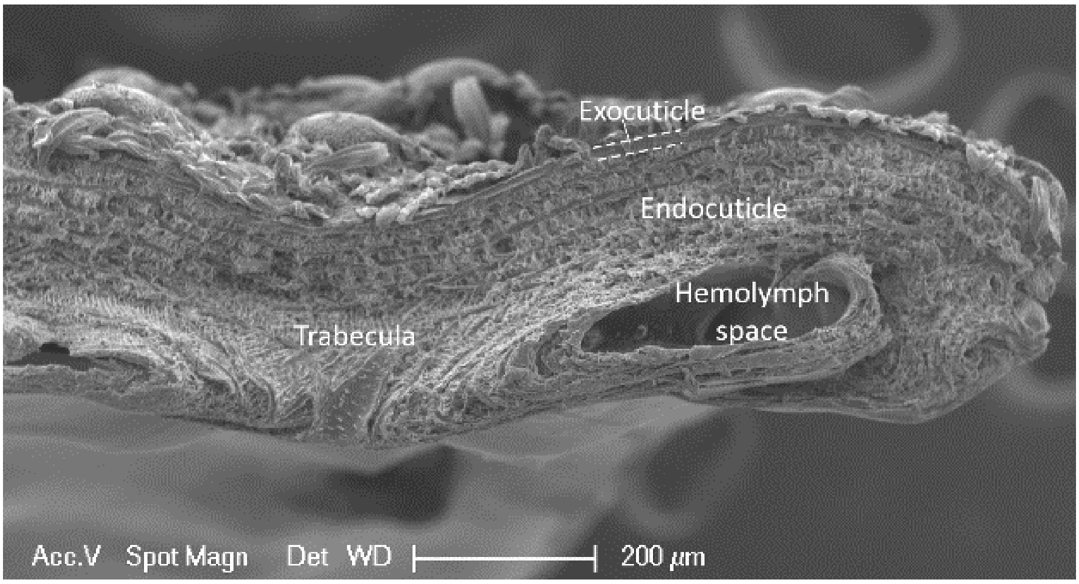

Fig. 2. SEM micrograph of a fractured cross section of the elytron of a *P. diabolicus* highlighting the regions of the elytra denoted by the exocuticle, endocuticle, hemolymph space and trabecula.

The microstructural complexity of the elytra further bestows the structure with several mechanisms to resist failure. An architectural translon between the exo and endocuticle represents a pseudo Bouligand arrangement incorporating a larger pitch between layers resulting in a graded behavior between sections. [26,27,23]. The bulk of the structure, the endocuticle, 200 – 300 µm, represents a balken (layers of bundles) structure comprised of parallel fiber bundles, macrofibres, consisting of unidirectional microfibrils[28,8]. Each balken possesses a shift in orientation with each successive layer, providing enhanced mechanical properties when bending stresses are high [28]. Resultantly, the same region also possesses the highest modulus but this trend shifts along with fiber orientation and subsequent layers do not follow this trend. Similar trends are visible for the modulus values of the region except when looking at the out of plane fiber bundles. Between successive plies fiber direction and angles appear to shift by 30 or 60 degrees but perception depend on body region and may vary between successive layers of the cuticle (e.g. Dennell, 1978; Zelazny and Neville, 1972). The ovular nature of the macro fibers result from inter ply penetrating microfibers strands emanate from the microfibers oriented perpendicular to balkens [29,11]. Fibrillar strands through the thickness of the cuticle add vertical support through successive layers to provide stiffening to the non-mineralized structure while augmenting resistance to shear forces that would cause delamination [29]. Further variations can be observed from the transition to a balken structure between the exocuticle to the endocuticle.

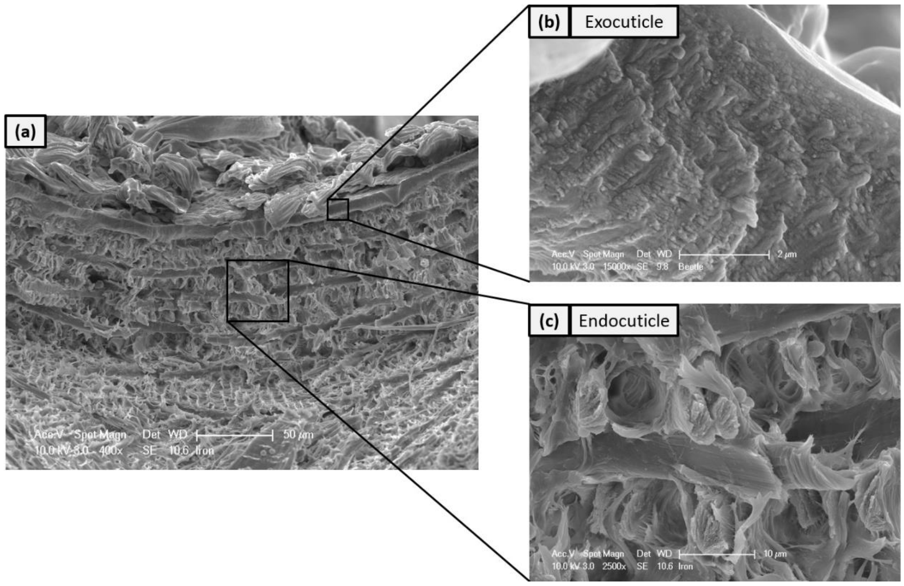

Fig. 3. Fractured section of the elytron of a *P. diabolicus* highlighting an (a) overview of the cross-section; (b) structure of the exocuticle (~ 4-µm thick), and (c) stacking of multi directional layers is seen in the endocuticle.

Fibrous structures composed of hierarchical sets of aligned fibrils provide asymmetric behavior, high tensile strength but suffer in compression, requiring an architectural variation to distribute compressive load. Augmenting the compressive performance of the structure through evolution, a series of through ply penetrating fibers have been introduced adding isotropy between layers and assisting in compression where the primary failure mode is buckling and delamination between layers. Similar to what is seen in enamel and horns, these columnar structures may infer common design themes for compression resistance [30,31,32]. Further examination of the structure reveals the absence of such fibers in the ventral portion of the elytra primarily subjected to tensile stress. Further incorporation of a pseudo helical design in the endocuticle provides in-plane isotropy and typically increases toughness and strength by incorporating fibrillar reinforcement in various angles. Departing from the idealized constant shift of angles comprising a full periodic rotation. The addition of a helicoidal architecture to this non-mineralized structure provides an increase in isotropy through fiber stacking, toughness by providing a tortuous path for cracks to propagate and compressive strength due to isotropy but the outer surface remains the interface between harsh climates and predator attacks.

Possessing a mountainous topography that possibly assist in the distribution of forces, the elytra is populated with overlapping structures creating an armored surface. Analysis of the elytra by scanning electron microscopy (SEM, Fig. 2) highlights the leaf like setae, sporadically located granular warts and epicuticle coating the elytra surface. Leaflike setae coating the exoskeleton are reminiscent of scales and may serve as an armor layer to deter predator attacks. Further analysis reveals networks of ruffled on the leaf like setae. Granular warts (~50 µm) bearing an elongated posterior seta and dimpled in nature are theorized to enhance water collection [33]. Serving as structural color, variance in color change response to humidity serve as a camouflage mechanism around woodchips that experience a similar hydroscopic property. Further analysis remains to be conducted to identify further fluid transport.

CONCLUSION

Our studies reveal exoskeleton of the diabolical ironclad beetle exhibits a departure from previously investigated Coleoptera due to its fibrous architecture that allows it to withstanding loads that would prove fatal to most insects. This work brings to light a hierarchical ultrastructure utilizing varying microstructural features that permit the application of a staggering load while diminishing the internal fractures within the cuticle. These toughening mechanisms include varying supports to resist lateral expansion along with a densely interdigitated suture and balken cuticle of tightly ordered non-mineralized alpha chitin fibers in a three-dimensional array. Architectural analysis highlights an enhanced resistance to external loads through ordered fiber orientation that stiffens the non-mineralized composite. Assessing the multifunctional nature of the cuticle revealed an intricate hydration network starting from the setae and traveling through the pore canals to the hemolymph space. Questions remain on the surface coatings of the insect along with the development and evolutionary conditions that resulted in such a robust cuticle. Furthermore, lessons learned from the study of this biological composite can be implemented to additive manufacturing techniques to create biomimetic structures that represent the next generation of impact resistant composites.

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