A Multilayer Micromechanical Model of the Cuticle of *Curculio longinasus* Chittenden, 1927 (Coleoptera: Curculionidae)

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Abstract

Curculio longinasus Chittenden, 1927 (Coleoptera: Curculionidae), is a weevil species common throughout the southwestern United States that uses its rostrum – a very slender, curved, beak-like projection of the head – to excavate tunnels in plant organs (such as acorns) for egg laying (oviposition). Once the apical portion of the rostrum has been inserted into the preferred substrate for oviposition, the female begins rotating around the perimeter of the hole, elevating her head by extending the fore-legs, and rotating the head in place in a drilling motion. This action causes significant elastic deformation of the rostrum, which will bend until it becomes completely straight. To better understand the mechanical behavior of the cuticle as it undergoes deformation during the preparation of oviposition sites, we develop a comprehensive micro/macro model of the micromechanical structure and properties of the cuticle, spanning across all cuticular regions, and reliably mirroring the resultant macroscale properties of the cuticle. Our modeling approach relies on the use of multi-scale, hierarchical biomaterial representation, and employs various micromechanical schemata - e.g., Mori-Tanaka, Effective Field, and Maxwell - to calculate the homogenized properties of representative volume elements at each level in the hierarchy. We describe the configuration and behavior of this model in detail, and discuss the theoretical implications and limitations of this approach with emphasis on future biomechanical and comparative evolutionary research. Our detailed account of this approach can thereby serve as a methodological template for exploring the biomechanical behavior of new insect structures.

Keywords: Curculionidae, Beetle Cuticle, Chitin, Homogenization, Elastic Properties, Multilayer

1. Introduction

The purpose of this study is to establish a modeling approach to study how weevil (snout beetle) species respond to the mechanical stresses they face during the process of preparing sites for oviposition (egg-laying). Our project is to create a multilayer, hierarchical, micromechanical model of insect cuticle. To do so, we mathematically characterize the microstructure of the exoskeleton of beetles in general, while using certain parameters specific to an individual species, as “proof of concept” in establishing the validity of the model. The formulation of this modeling approach is a critical step towards understanding the way in which cuticular structures are optimized in form and function to accommodate mechanical loading. The methods employed herein will ultimately enable us to analyze the material response of an entire head to load, across multiple species. Thus, our study serves as a key element of a larger research sequence and is foundational to a future comparative, multi-taxon program.

The aims of this work are to (1) model the hierarchical complexity of beetle cuticle to recover the bulk elastic

constants for the different regions of cuticle, (2) verify that application of the model to a common species produces robust and valid results, and (3) didactically present the results (and more critically, the methods) to be accessible to biologists with minimal knowledge of materials science. We strongly hope that “democratizing” these methods for biologists will encourage their use more widely in studying functional morphology and will enable biologists to strengthen inferences of the function and evolution of anatomical structures. Likewise, we feel that a deeper understanding of the mechanical behavior of biological materials will ultimately permit others to unlock their potential for biomimicry. Hence, we elaborate on the use of the models and homogenization schemes herein, including their assumptions, parameters, and outputs, in an effort to make this work more accessible to other biologists.

1.1. Biology and Rostral Adaptations of *Curculio longinasus* Chittenden, 1927

The insect species examined herein, *Curculio longinasus* Chittenden, 1927 (Fig. 1), is an exemplary species of the genus *Curculio* Linnaeus, 1758 that is common throughout the southwestern United States and northern Mexico (Gibson, 1969). *Curculio* is the type (name-giving) genus

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Figure 1: **A** Lateral habitus photograph of a female *Curculio longinasus* Chittenden, 1927 **B** Head of female *C. longinasus*; green line indicates location of cross-section **C** Scanning electron micrograph (SEM) of rostrum in cross-section; detail frames indicate size and location of subsequent magnified SEMs **D** Cross-section with chitin macrofibers ("balken") indicated **E** Cross-section with regions of cuticle indicated.

of the family Curculionidae, which are a group of beetles (order Coleoptera) commonly known as weevils. Weevils are readily identified by a structure known as the rostrum (Fig. 1), which is a rigid extension of the cuticle (exoskeleton) of the head that bears the terminal chewing mouthparts (Morimoto and Kojima, 2003). Many weevils, in-

cluding all members of genus *Curculio*, use the rostrum to excavate or otherwise prepare sites for oviposition (the process of laying eggs) in their respective host plants. This group is considered to be megadiverse, with over 62,000 species described to date (Oberprieler et al., 2014).

The biology of *C. longinasus*, as well as its congeners in North America, is described generally in a monographic revision by Gibson (1969), and has been expanded upon by others (Aguirre Uribe, 1978; Hughes and Vogler, 2004; Toju and Sota, 2006b; Toju, 2008). Many species of *Curculio* are impactful pest species, the most notable of which are *C. caryae* (Horn, 1873), the pecan weevil, *C. elephas* (Gyllenhal, 1835), the chestnut weevil, and *C. nucum* Linnaeus, 1758, the hazelnut weevil (Aguirre Uribe, 1978; Gibson, 1969). With respect to *C. longinasus*, Gibson (1969) lists the only confirmed host plant for this species as the southern red oak *Quercus falcata* Michaux, 1801. *Curculio* females, including those of *C. longinasus*, use their rostra to excavate a hole into the kernel of an acorn or other fruiting body into which they subsequently oviposit. The developing larva feeds upon the tissue of the acorn and remains inside until pupation. During the final instar stadium, the larva will exit the acorn and burrow into the soil, where it will pupate. Eclosion generally takes place 2-3 weeks thereafter (Gibson, 1969).

In preparation of a site for oviposition, the adult female will excavate a bore-hole into the fruit (usually an acorn) using her rostrum and mandibles. These are unusual (and phylogenetically derived) for weevils, and insects in general, in that they resemble shears and articulate along a vertical plane (Gibson, 1969; Ting, 1936, 1933). The rostrum is strongly curved (up to 90°) in most species of *Curculio*, including *C. longinasus* (Fig. 1), and in some species, can exceed the length of the remainder of the body (Gibson, 1969; Hughes and Vogler, 2004; Toju and Sota, 2006a,b). As the bore-hole is excavated, the female ingests the material removed by the action of the mandibles. Once the apical portion of the rostrum has been inserted into the acorn, the female begins rotating around the perimeter of the hole and elevate her head by straightening the fore-legs. This action causes significant elastic deformation of the rostrum, which will bend until it becomes completely straight. The rostrum is kept straight by maintaining the head capsule directly above the bore-hole, and by rotating the head from side to side about the longitudinal axis of the rostrum in a (slow) drilling motion. The female will continue to move around the perimeter of the hole, using a side-stepping gait, while simultaneously maintaining an upright stance and performing the drilling motion of the head, often until the entire rostrum is buried in the acorn, up to the anterior portion of the eyes. This action enables the strongly curved rostrum to excavate a perfectly straight, narrow hole in the acorn (fruit), into which she may deposit one or more eggs. This behavior has been described (and is easily observed) in several species of *Curculio*, and is likely manifested throughout the entire genus (Aguirre Uribe, 1978; Moffett, 1989; Toju

and Sota, 2006a,b). This behavior has been recorded in the supplementary videos of Toju and Sota (2006b).

1.2. Research Goals and Challenges

Here we focus on the mechanical behavior of the cuticle of the *C. longinasus* rostrum as it undergoes remarkable deformation during the preparation of oviposition sites. Upon removal from the bore hole, the rostrum instantly flexes back to its original, curved shape, without any apparent damage, and in spite of the extreme degree of bending that occurs. Indeed, a single female may excavate many such holes in her adult life period, with or without eggs, and with no evident harm (Aguirre Uribe, 1978; Hughes and Vogler, 2004). The way in which this is achieved is of great interest from both a functional-mechanical and an evolutionary perspective. There has been no prior attempt to characterize the behavior of the cuticle of *Curculio* weevils in this context. Absent a proper material model and/or extensive mechanical testing, it is impossible to perform *in-silico* modelling of the rostrum during deformation to assess the response of the cuticle under load. We are therefore unable to make informed statements about the biomechanical optimization and evolution of this adaptive structure as a whole (Dumont et al., 2009).

Our principle aim is to create a micromechanical model of beetle cuticle, based on our specimens and the general structure of the cuticle, that will provide us with accurate estimates of the elastic parameters of the cuticle. Combined with a precise reconstruction of the physical dimensions of the head, this model will enable us to predict the response of the rostrum to simulated loads in future work.

Additionally, a better understanding of the physical response of this material to applied strains will permit us to eventually investigate whether the differential performance demands of regions of the weevil rostrum during oviposition are met by chemical/compositional changes in the cuticle, or by structural adjustments of the cuticular components themselves. In keeping with previous findings regarding the mechanical behavior of arthropod cuticle (see for example (Nikolov et al., 2011, 2010)), we predict that the structural arrangement and relative proportions of the components of the cuticle will most greatly affect its resulting macroscale properties. With these challenges and goals in mind, we present below a brief summary of the structure of insect cuticle and review prior research that has expanded our understanding of the mechanical behavior of this complex material.

1.3. Microstructure of Insect Cuticle

The insect cuticle is comprised of three general regions (Figs. 1 and 2), including (1) endocuticle, which is the most compliant and innermost region; and (3) exocuticle, which is the outermost region, usually the hardest, and has a thin waxy covering (epicuticle) that is not of great mechanical significance in most groups. Between these

Figure 2: **A** Reconstruction of cuticle regions (not to scale), with pores **B** Manifestation of cuticle layering in cylindrical structures (as realized in the *C. longinasus* rostrum), without pores.

regions lies (2) the mesocuticle, which is similar to exocuticle, but is less compact and exhibits a lesser degree of tanning (sclerotization) (Klocke and Schmitz, 2011; Vincent, 1982; Vincent and Wegst, 2004). In general, these regions are confluent, and not necessarily sharply defined (Fig. 4). The cuticle of arthropods, including insects, is made up of chitin and numerous uncharacterized proteins, which effectively act as a matrix into which the chitin is embedded (Nikolov et al., 2011, 2010; Vincent and Wegst, 2004). In this way, we may consider the cuticle as a fiber-reinforced composite material. Furthermore, the cuticle is laminate, and has numerous laminae of unidirectional chitin fibers embedded in a proteinaceous matrix, which are stacked at a more or less constant angle to each other in a helicoidal arrangement (Fig. 3). This ply arrangement is referred to as the Bouligand structure (Blackwell and Weih, 1980; Bouligand, 1972; Neville et al., 1976).

The cuticle is generally porous, with pore canals that have a twisted ribbon configuration and often a central pore canal fiber. These fibers likely enhance the shear stiffness of the composite by providing a form of cross-stitching (Cheng et al., 2009). Beetles in particular have a highly modified endocuticle made up of large unidirectional bundles of chitin macrofibers, called “balken” (German for beam, strut). These layers are generally pseudo-orthogonal (Figs. 2 and 5), although some other configurations have been observed (Hepburn and Ball, 1973; van de Kamp and Greven, 2010). A thorough review of the balken structure can be found in the work of van de Kamp and Greven (2010) and van de Kamp et al. (2015). In addition to its micro-structural properties, the cuticle is also strongly influenced by its chemical properties, especially the degree of tanning and overall water content, as discussed in great detail by Klocke and Schmitz (2011) and Vincent and Wegst (2004).

1.4. Review of Prior Studies and Modeling Approach

Prior mechanical simulation studies of the arthropod exoskeleton and the insect cuticle as highly structured, hierarchically organized biomaterials have greatly advanced our knowledge of the behavior of these structures. Of particular significance is the modeling approach of (Nikolov et al., 2011, 2010) as applied to the endocuticle of *Homarus americanus* Edwards, 1873. We emulate this approach for modeling hierarchical materials using the micro/macro method (see below). Some prior analyses of the arthropod cuticle have failed to consider the layering of the exoskeleton, and have furthermore posited (for pragmatic modeling reasons) that the cuticle is homogeneously isotropic, thereby neglecting the mechanical implications of its microstructure (Flannigan, 1998; Herbert et al., 2000; Hörschemeyer et al., 2013; Mccullough et al., 2014). However, in actuality the cuticle is a highly complex, hierarchically layered, and textured material, whose configuration and properties can vary within an individual organism and across species or higher-level taxa, and which displays a correspondingly complex response to mechanical loads (Raabe et al., 2005; Vincent, 1982). Our ability to apply previous material models to simulate this behavior is limited by our knowledge of the exact makeup of the cuticle, and especially knowledge of the relative water and protein content, which generally prohibits modeling of the viscoelastic response of this material (Nikolov et al., 2011, 2010). All mechanical simulations of the cuticle (including this work) have thus far been limited to conditions of small strain and assume linear elasticity.

We adopt the micro/macro method to achieve balance between model complexity and performance. To explain our modeling approach, we first outline the basic principles of the micro/macro method. We then develop the specific formulation, notation, and relevant assumptions

Figure 3: **A** Reconstruction of Bouligand structure of mesocuticle (approx. to scale), without pore canals **B** Typical “twisted-ribbon” pore canal in mesocuticle (bisected), without pore canal fiber; in reality, pores are often larger with nanofibrils wrapping around canals.

of the models used to calculate the effective properties of the different regions of the cuticle observed in our specimens. Considerable space is allocated to explain our modeling approach for each region of the rostrum, focusing on decisions related to the inclusion and specific configuration of parameters due either to mathematical contingencies, input from relevant literature, or experimental observations. We thoroughly characterize the outputs of the micromechanical models at each hierarchical level of complexity, with the aim to reach both biologist and biomechanical engineering audiences, both of which might find our methods relevant to their respective research programs.

2. Methods

2.1. Micro/Macro Method

The micro/macro approach enables us to take into account the integrated rostrum microstructure to calculate its overall macroscopic properties (Doghri, 2000; Mura, 1987; Nikolov et al., 2011, 2010). This method relies on the use of representative volume elements (RVE), which can be considered as the smallest volume of material that has the same mechanical properties as the material at the macroscale (Doghri, 2000). In composite materials, a single RVE shows a heterogeneous composition, having two or more constituents with different properties (Reddy, 2004). The effective macroscale properties of the RVE are calculated using an appropriate homogenization scheme that takes into account the relevant material properties of each component, and yields the mechanical properties of the RVE as if it were of homogeneous composition (Doghri, 2000; Mura, 1987). By describing the homogenized properties of a RVE for each level of microstructure in the cu-

ticle, we are able to find the bulk mechanical properties of the different types of cuticle present in the exoskeleton.

2.2. Symbols and Notation

In this section we summarize the definitions of terms and symbols used throughout this study. Additional information for parameters specific to each model are provided in subsequent sections.

E_i Young’s modulus of elasticity along axis i

ν_{ij} Poisson’s ratio corresponding to displacement along axis j under load along axis i

G_{ij} Shear modulus along axis j in the plane normal to axis i

K Bulk modulus

θ Angle of rotation

V Volume

n Number of constituent elements within representative volume element (RVE)

φ_e Volume fraction of constituent element e (e.g. matrix or fiber)

r_f Fiber radius

s_f Lateral separation distance of fibers embedded in a matrix

C_m Fourth-order stiffness tensor of a material m

S_m Used to represent either the fourth-order compliance tensor or the Eshelby’s tensor of a material m , as specified in the respective context.

290	A_m	Strain concentration tensor of a material m	335
	I	Identity matrix	
	R	Rotation matrix	
	T_{ijkl}^α	Elements of standard tensor basis	340
295	H_i	Fourth-order compliance contribution tensor for an inhomogeneity i	
	P_m	Fourth-order Hill's tensor of a material m (typically used for fibers embedded in a matrix)	
	Q_m	Fourth-order Hill's tensor of a material m (typically used for voids embedded in a matrix)	345
300	:	Indicates the tensor product contracted over two indices	
	M^{-1}	Indicates the inverse of a matrix M	
	M^T	Indicates the transpose of a matrix M	

2.3. Homogenization Schemes and Limitations

In this section, we specify the models and homogenization schemes utilized to calculate the effective properties of the weevil rostrum cuticle. We list each in order of its simplicity and appearance in the study. For each, we give a summary of the parameters of the model, conditions under which the model may be considered valid, and the mathematical formulation of the model. In general, the six models presented herein can be classified as belonging to one of three higher “families” of models: (1) numerical/volumetric averaging of RVEs, specifically the Voigt model and isotropic approximation of anisotropic media; (2) homogenization of a single inclusion (or multiple non-interacting inclusions) in a matrix, specifically the Mori-Tanaka model and non-interaction approximation; and (3) homogenization of multiple interacting inclusions in a matrix, specifically the effective field method¹ and Maxwell scheme. The application of each method is discussed in Section 3.

2.3.1. Voigt Model

Assumptions: The Voigt model assumes that an imposed macro strain will result in a uniform strain field within a RVE (Hill, 1952). Under conditions of linear elasticity, this model is generally taken as the upper bound for the calculation of a homogenized stiffness tensor for a material, while the Reuss model, which assumes uniform stress, is taken as the lower bound (Doghri, 2000; Hill, 1952). The Reuss model is not considered herein, as it consistently underestimates cuticle elastic constants and is thus of limited utility. The Voigt model relies upon the assumption of linear elasticity (i.e. linear relationship between stress

resulting from strain imposed on a material) so that the macro strain is equal to the average strain within the RVE (Doghri, 2000). We calculate the effective tensor, C_{eff} , of an RVE by taking the volumetric average of the stiffness tensors of the individual constituents of the RVE.

Parameters: The following are parameters specific to this model.

C_{eff} Effective stiffness tensor of the RVE

V_i Volume of the i^{th} element of the RVE

V_{RVE} Volume of the RVE

C_i Stiffness tensor of the i^{th} element of the RVE

Formulation: Below we present the formulation of this model:

$$C_{eff} = \sum_{i=1}^n \frac{V_i}{V_{RVE}} C_i \quad (1)$$

2.3.2. Mori-Tanaka Model

Assumptions: The Mori-Tanaka model was developed for to calculate the effective properties of two phase composites containing dilute, aligned, ellipsoidal inclusions, under conditions of linear elasticity, where the inclusions have the same properties as the matrix and are isotropic (Mori and Tanaka, 1973; Mura, 1987). This model generally treats each inclusion as if it were isolated in an infinite matrix so that the inclusions do not interact with each other, and thus produces optimal results when the volume fraction of inclusions is less than 20-30% (Doghri, 2000; Nikolov et al., 2011). See also Buryachenko et al. (2008) for problems associated with this method. When the inclusions have different properties than the matrix material, they are referred to as inhomogeneities, and may be simulated by an equivalent inclusion with the properties of the matrix with a uniform eigenstrain, using the equivalent inclusion method (Benveniste, 1987; Mura, 1987).

By incorporating the equivalent inclusion method, a reformulation of the Mori-Tanaka model was created to accommodate the calculation of the effective properties of transversely orthotropic or anisotropic inclusions (such as cracks or fibers) embedded in an isotropic matrix, depending solely on the properties of the matrix material and the shape of the inhomogeneities (Benveniste, 1987). We therefore assume that the material is a linear elastic, two-phase composite with perfect bonding between an isotropic matrix material and the inclusions, which are identical, dilute, aligned, and ellipsoidal in shape.

Parameters: The following are parameters specific to this model.

¹Note that this collapses to a specific subset of possible solutions that coincides with the Mori-Tanaka model, when the “correlation hole” is concentric and of the same shape as the inclusions.

C_{eff} Effective stiffness tensor of the RVE

φ_M Volume fraction of the isotropic matrix

φ_F Volume fraction of the fibrous inhomogeneities

ν_M Poisson's ratio of the isotropic matrix

C_M Stiffness tensor of the isotropic matrix

C_F Stiffness tensor of the fibrous inhomogeneities

A_F Strain concentration factor for the fibrous inhomogeneities

S_F Eshelby's tensor for a cylindrical inclusion

Formulation: Below we present the formulation of this model.

$$C_{eff} = C_M + \varphi_F [(C_F - C_M) : A_F] [\varphi_M I + \varphi_F A_F]^{-1} \quad (2)$$

Where:

$$A_F = [I + S_F : (C_M)^{-1} : (C_F - C_M)]^{-1} \quad (3)$$

For an infinite isotropic elastic matrix, with a single embedded circular cylinder, whose longitudinal axis is aligned with axis 1, the non-zero components of the Eshelby's tensor, S_F , are as follows:

$$\begin{aligned} S_{2211} &= S_{3311} = \frac{\nu_M}{2(1-\nu_M)} \\ S_{2222} &= S_{3333} = \frac{1}{2(1-\nu_M)} \left[\frac{3}{4} + \frac{(1-2\nu_M)}{2} \right] \\ S_{2233} &= S_{3322} = \frac{1}{2(1-\nu_M)} \left[\frac{1}{4} - \frac{(1-2\nu_M)}{2} \right] \\ S_{2323} &= \frac{1}{2(1-\nu_M)} \left[\frac{1}{4} + \frac{(1-2\nu_M)}{2} \right] \\ S_{1212} &= S_{1313} = \frac{1}{4} \end{aligned} \quad (4)$$

*note that: $S_{1111} = S_{1122} = S_{1133} = 0$

Where:

$$S_F = \begin{bmatrix} S_{1111} & S_{1122} & S_{1133} & 0 & 0 & 0 \\ S_{2211} & S_{2222} & S_{2233} & 0 & 0 & 0 \\ S_{3311} & S_{3322} & S_{3333} & 0 & 0 & 0 \\ 0 & 0 & 0 & 2S_{2323} & 0 & 0 \\ 0 & 0 & 0 & 0 & 2S_{1313} & 0 \\ 0 & 0 & 0 & 0 & 0 & 2S_{1212} \end{bmatrix} \quad (5)$$

2.3.3. Non-Interaction Approximation

Assumptions: For our purposes, the non-interaction approximation is similar to the Mori-Tanaka model in its assumptions; namely, that we are finding the effective properties of a linear elastic two-phase composite containing a matrix phase and identical inclusions that are ellipsoidal in shape and do not interact with each other (Kachanov, 1999; Sevostianov and Kachanov, 2002; Sevostianov et al., 2001, 2005). As in the Mori-Tanaka model, this method simulates the effect of adding a single inclusion to the matrix

material, thus assuming no interaction between inclusions. The effective properties of the composite are assumed to be equal to the sum of the stiffness (or compliance) of the matrix and the effect of the inclusions either as a stiffness or compliance contribution tensor, respectively (Kachanov, 1999; Sevostianov et al., 2005). Because we use this method in a matrix that is transversely isotropic, with inclusions (specifically pores) whose longitudinal axes are aligned with the axis of transverse isotropy, we use the formulation of Sevostianov et al. (2005), which utilizes explicit inversions made simpler by representation of fourth-order tensors in "tensorial basis", as indicated below. See Kachanov (1999) and Sevostianov et al. (2005) for more information.

Parameters: The following are parameters specific to this model.

S_{eff} Effective compliance tensor of the RVE

S_M Compliance tensor of the matrix

V_i Volume of the i^{th} inhomogeneity

V_{RVE} Volume of the RVE

φ_I Volume fraction of the inclusions

H_i Compliance contribution tensor of the i^{th} inhomogeneity

H_{NI} Compliance contribution tensor of all inhomogeneities for the non-interaction model

h_α Components of compliance contribution tensor H_i in standard tensor basis

C_M Stiffness tensor of the matrix

C_α Components of C_M expressed in standard tensor basis

T_{ijkl}^α Tensors for expression in standard tensor basis

Formulation: Below we present the formulation of this model.

In general, the non-interaction approximation for a two-phase composite is given by:

$$S_{eff} = S_M + H_{NI} \quad (6)$$

Where H_{NI} is given by the sum of the compliance contribution tensors for n inhomogeneities in the RVE, such that:

$$H_{NI} = \sum_{i=1}^n H_i \quad (7)$$

The i^{th} inhomogeneity has a compliance contribution tensor H_i , expressed in tensorial basis as:

$$H_i = \frac{V_i}{V_{RVE}} \sum_{\alpha=1}^6 h_\alpha T_{ijkl}^\alpha \quad (8)$$

By substitution of Eq. (8) into Eq. (7):

$$H_{NI} = \sum_{i=1}^n \left(\frac{V_i}{V_{RVE}} \sum_{\alpha=1}^6 h_{\alpha} T_{ijkl}^{\alpha} \right) \quad (9)$$

However, assuming all inhomogeneities are identical, this can be expressed as:

$$H_{NI} = \frac{nV_i}{V_{RVE}} \sum_{\alpha=1}^6 h_{\alpha} T_{ijkl}^{\alpha} \quad (10)$$

Therefore, because $\frac{nV_i}{V_{RVE}} = \varphi_I$:

$$H_{NI} = \varphi_I \sum_{\alpha=1}^6 h_{\alpha} T_{ijkl}^{\alpha} \quad (11)$$

If we assume the general form of a 6×6 matrix, then a symmetrical tensor M , expressed as a 6×6 matrix, has the following components (Doghri, 2000):

$$M = \begin{bmatrix} M_{1111} & M_{1122} & M_{1133} & M_{1123} & M_{1113} & M_{1112} \\ M_{2211} & M_{2222} & M_{2233} & M_{2223} & M_{2213} & M_{2212} \\ M_{3311} & M_{3322} & M_{3333} & M_{3323} & M_{3313} & M_{3312} \\ M_{2311} & M_{2322} & M_{2333} & M_{2323} & M_{2313} & M_{2312} \\ M_{1311} & M_{1322} & M_{1333} & M_{1323} & M_{1313} & M_{1312} \\ M_{1211} & M_{1222} & M_{1233} & M_{1223} & M_{1213} & M_{1212} \end{bmatrix} \quad (12)$$

Any symmetrical fourth-order tensor expressed with components expressed in 6×6 matrix form can also be represented in a “standard tensor basis” as:

$$M = \sum_{\alpha=1}^6 M_{\alpha} T_{ijkl}^{\alpha} \quad (13)$$

Where M_{α} are of the same form as C_{α} , given in Eqs. (16), and the non-zero components of tensors⁴⁴⁵ T_{ijkl}^{α} are as follows:

$$\begin{aligned} T_{1111}^1 &= T_{2222}^1 = T_{1122}^1 = T_{2211}^1 = 1 \\ T_{1212}^2 &= T_{2121}^2 = T_{1221}^2 \\ &= T_{2112}^2 = T_{1111}^2 = T_{2222}^2 = \frac{1}{2} \\ T_{1122}^2 &= T_{2211}^2 = -\frac{1}{2} \\ T_{1133}^3 &= T_{2233}^3 = 1 \\ T_{3311}^4 &= T_{3322}^4 = 1 \\ T_{1313}^5 &= T_{2323}^5 = T_{1331}^5 = T_{2332}^5 = T_{3113}^5 \\ &= T_{3223}^5 = T_{3131}^5 = T_{3232}^5 = \frac{1}{4} \\ T_{3333}^6 &= 1 \end{aligned} \quad (14)$$

The coefficients h_{α} are:

$$\begin{aligned} h_1 &= \frac{2C_3C_4 - C_6(2C_1 + C_2)}{4C_2(C_3C_4 - C_1C_6)} \\ h_2 &= \left(\frac{1}{C_1} + \frac{2}{C_2} \right) \\ h_3 &= h_4 = \frac{C_3}{2(C_3C_4 - C_1C_6)} \\ h_5 &= \frac{8}{C_5} \\ h_6 &= \frac{C_1}{C_3C_4 - C_1C_6} \end{aligned} \quad (15)$$

Where C_{α} are given in terms of the 6×6 matrix components of the stiffness tensor C_M such that:

$$\begin{aligned} C_1 &= \frac{C_{1111} + C_{1122}}{2} \\ C_2 &= 2C_{1212} \\ C_3 &= C_{1133} \\ C_4 &= C_{3311} \\ C_5 &= 4C_{1313} \\ C_6 &= C_{3333} \end{aligned} \quad (16)$$

2.3.4. Effective Field Method

Assumptions: With the effective field method, all preceding assumptions for the non-interaction approximation are applicable, except the inhomogeneities are expected to interact with each other (the interaction is assumed to be geometrical, see Sevostianov (2014)). This method simulates the interaction between inclusions by placing them into a local strain field. Additionally, it is assumed, for simplicity, that the strain field of each inclusion is identical to that of any other inclusion, and that this field does not depend on the geometry or elastic properties of the inclusions, but rather, is determined by the elastic properties of the matrix and orientation of the inclusions (Sevostianov et al., 2005). By additionally assuming that there is symmetry to the distribution of inhomogeneities, and that the “correlation hole” (the region within which it is unlikely that the centers of other inclusions will be found) is of the same shape and concentric with the inhomogeneity, it is possible to provide an explicit solution for this method that coincides with the Mori-Tanaka Model (Sevostianov et al., 2005). This is the case if the inclusions under consideration are aligned, identical, and arranged such that the distance between any two neighboring fibers is identical (Buryachenko et al., 2008). We use the formulation of Sevostianov et al. (2005), which utilizes explicit inversions made simpler by representation of fourth-order tensors in “tensorial basis”, in the form indicated by Eq. (13). It is assumed that axis 3 is the axis of transverse

isotropy, aligned with the axes of rotation of the inclusions.

Parameters: The following are parameters specific to this model.

C_{eff} Effective stiffness tensor of the RVE

C_M Stiffness tensor of the matrix

C_α Components of C_M expressed in standard tensor basis

C_D Inverse of S_D

C_D^α Components of C_D expressed in standard tensor basis

S_I Compliance tensor of an inclusion

S_M Compliance tensor of the matrix

S_D Difference of the compliance tensors of the inclusions and matrix, respectively

φ_I Volume fraction of the inclusions

φ_M Volume fraction of the matrix

P Hill's tensor

p_α Components of P expressed in standard tensor basis

Δ_1 Coefficient of tensor inversion defined in Eq. (19)

Δ^* Coefficient of tensor inversion defined in Eq. (20)

T_{ijkl}^α Tensors for expression in standard tensor basis

Formulation: Below we present the formulation of this model.

$$C_{eff} = \sum_{\alpha=1}^6 C_{eff}^\alpha T_{ijkl}^\alpha \quad (17)$$

Where C_{eff}^α are the components of C_{eff} given by:

$$\begin{aligned} C_{eff}^1 &= C_1 + \frac{\varphi_I}{2\Delta^*} \left[\frac{2C_D^1}{\Delta_1} + \varphi_M P_6 \right] \\ C_{eff}^2 &= C_2 + \varphi_I \left[\frac{1}{C_D^2} + \varphi_M P_2 \right]^{-1} \\ C_{eff}^3 &= C_{eff}^4 = C_3 + \frac{\varphi_I}{\Delta^*} \left[\frac{C_D^3}{\Delta_1} - \varphi_M P_3 \right] \\ C_{eff}^5 &= C_5 + 4\varphi_I \left[\frac{4}{C_D^5} + \varphi_M P_5 \right]^{-1} \\ C_{eff}^6 &= C_6 + \frac{\varphi_I}{2\Delta^*} \left[\frac{2C_D^6}{\Delta_1} + \varphi_M P_1 \right] \end{aligned} \quad (18)$$

Where²:

$$\Delta_1 = 2 [C_D^1 C_D^6 - C_D^3 C_D^4] \quad (19)$$

²Note that in [Sevostianov et al. \(2005\)](#), this is erroneously produced as: $\Delta_1 = C_D^1 C_D^6 - (C_D^3)^2$; see [Sevostianov et al. \(2015\)](#), Eq. A.4, for the correct formulation of Δ for tensor inversion in tensorial basis.

$$\Delta^* = 2 \left[\frac{C_D^6}{2\Delta_1} + \varphi_M P_1 \right] \left[\frac{2C_D^1}{\Delta_1} + \varphi_M P_6 \right] - 2 \left[\frac{C_D^3}{\Delta_1} + \varphi_M P_3 \right]^2 \quad (20)$$

Given:

$$C_D = C_I - C_M \quad (21)$$

Additionally, C_D^α and C_α are components of the stiffness tensors C_D and C_M , respectively, that may be derived from Eqs. (16). Furthermore, p_α are the components of Hill's tensor, and are given by:

$$\begin{aligned} p_1 &= \frac{1}{2(2C_1 + C_2)} \\ p_2 &= \frac{1}{2(2C_1 + C_2)} + \frac{1}{C_2} \\ p_3 &= p_4 = p_6 = 0 \\ p_5 &= \frac{2}{C_5} \end{aligned} \quad (22)$$

2.3.5. Isotropic Approximation of Anisotropic Media

Assumptions: For slightly anisotropic media, it is possible to create an isotropic approximation of the material from its elastic constants ([Saadat et al., 2012](#); [Sevostianov and Kachanov, 2008](#)). For this particular formulation, we assume that the material in question is transversely isotropic.

Formulation: Below we present the formulation of this model. From Hook's Law, we may derive the elastic constants of orthotropic media from their stiffness and compliance tensors as follows:

$$S_m = [C_m]^{-1} = \begin{bmatrix} \frac{1}{E_1} & -\frac{\nu_{21}}{E_2} & -\frac{\nu_{31}}{E_3} & 0 & 0 & 0 \\ -\frac{\nu_{12}}{E_1} & \frac{1}{E_2} & -\frac{\nu_{32}}{E_3} & 0 & 0 & 0 \\ -\frac{\nu_{13}}{E_1} & -\frac{\nu_{23}}{E_2} & \frac{1}{E_3} & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{1}{G_{23}} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{G_{31}} & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{1}{G_{12}} \end{bmatrix} \quad (23)$$

For a transversely isotropic material, the isotropic approximations of the shear and bulk moduli may be calculated, respectively, as:

$$\begin{aligned} G^{-1} &= 4 [(1 + \nu_{12}) E_1^{-1} + (1 + \nu_{23}) E_2^{-1} + (1 + \nu_{31}) E_3^{-1}] \\ &\quad + 3 (G_{12}^{-1} + G_{23}^{-1} + G_{31}^{-1}) \\ K^{-1} &= (1 - 2\nu_{12}) E_1^{-1} + (1 - 2\nu_{23}) E_2^{-1} + (1 - 2\nu_{31}) E_3^{-1} \end{aligned} \quad (24)$$

From these, we may also find the isotropic approximations of Poisson's ratio and Young's modulus:

$$\begin{aligned} \nu &= \frac{3K - 2G}{2(3K + G)} \\ E &= \frac{9KG}{3K + G} \end{aligned} \quad (25)$$

The stiffness tensor of the isotropic approximation may then be calculated, by Hook's Law, such that:

$$C = \frac{E}{(1+\nu)(1-2\nu)} \times \begin{bmatrix} (1-\nu) & \nu & \nu & 0 & 0 & 0 \\ \nu & (1-\nu) & \nu & 0 & 0 & 0 \\ \nu & \nu & (1-\nu) & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{(1-2\nu)}{2} & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{(1-2\nu)}{2} & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{(1-2\nu)}{2} \end{bmatrix} \quad (26)$$

2.3.6. Maxwell Homogenization Scheme

Assumptions: For the Maxwell homogenization scheme, we observe the same assumptions *shared* between the previous models. The approach of Maxwell was to remove a domain Ω from a composite material and insert it into the matrix, where its effect will invariably be described by either the sum of the compliance (or stiffness) contribution tensors of the inclusions, or by the compliance (or stiffness) contribution tensor of the entire domain, which is assumed to have unknown properties. By equating these quantities, it is possible, given the aforementioned assumptions of our system, to explicitly calculate the effective properties of Ω as our RVE, while accounting for the interactions between inhomogeneities (Sevostianov and Giraud, 2013; Sevostianov, 2014; Sevostianov et al., 2015; Vilchevskaya and Sevostianov, 2015). We follow the formulation of Sevostianov (2014), which has been successfully used to calculate the effective properties of cortical bone, another biomaterial with a complex microstructure (Vilchevskaya and Sevostianov, 2015).

Parameters: The following are parameters specific to this model.

S_{eff} Effective compliance tensor of the RVE

S_M Compliance tensor of the matrix

S_F Compliance tensor of a fiber

φ_F Volume fraction of the fibers

G_M Shear modulus of elasticity of the matrix

ν_M Poisson's ratio of the matrix

H_F Compliance contribution tensor of all fibers in the RVE

Q Hill's tensor

q_α Components of Q expressed in standard tensor basis

κ Coefficient defined in Eq. (30)

f_0 Coefficient defined in Eq. (31)

f_1 Coefficient defined in Eq. (32)

γ Aspect ratio of an inhomogeneity

$g(\gamma)$ Shape function for an inhomogeneity with aspect ratio γ

Formulation: Below we present the formulation of this model.

$$S_{eff} = S_M + \left\{ [H_F]^{-1} - Q \right\}^{-1} \quad (27)$$

Where:

$$H_F = \varphi_F \left[(S_F - S_M)^{-1} + Q \right]^{-1} \quad (28)$$

The Hill's tensor for the fibers, Q , has the components q_α , in standard tensor basis:

$$\begin{aligned} q_1 &= G_M [4\kappa - 1 - 2(3\kappa - 1)f_0 - 2f_1] \\ q_2 &= 2G_M [1 - (2 - \kappa)f_0 - f_1] \\ q_3 &= q_4 = G_M [(2\kappa - 1)f_0 + 2f_1] \\ q_5 &= 4G_M [f_0 + 4f_1] \\ q_6 &= 8G_M [\kappa f_0 - f_1] \end{aligned} \quad (29)$$

Given:

$$\kappa = \frac{1}{2(1 - \nu_M)} \quad (30)$$

$$f_0 = \frac{\gamma^2 [1 - g(\gamma)]}{2(\gamma^2 - 1)}; \text{ for cylinders, } f_0 = \frac{1}{2} \quad (31)$$

$$f_1 = \frac{\kappa\gamma^2}{4(\gamma^2 - 1)^2} [(2\gamma^2 + 1)g(\gamma) - 3] \quad (32)$$

for cylinders, $f_1 = 0$

Additionally, the shape function $g(\gamma)$ is expressed in terms of the aspect ratio, γ , of the inhomogeneities. We assume that the inhomogeneities are fibers, and therefore are cylindrical and can be considered as prolate spheroids with an infinite aspect ratio ($\gamma \rightarrow \infty$). The general form of the shape function for prolate spheroids is:

$$g(\gamma) = \frac{1}{2\gamma\sqrt{\gamma^2 - 1}} \ln \frac{\gamma + \sqrt{\gamma^2 - 1}}{\gamma - \sqrt{\gamma^2 - 1}} \quad (33)$$

2.4. Chitin-Protein Nanofibrils

The effective properties of a single chitin-protein nanofibril were determined using (1) a Mori-Tanaka model (Section 2.3.2) and (2) the ab-initio calculations of Nikolov et al. (2011, 2010) for the effective properties of α -chitin and fiber proteins. We assume, based on previous research on beetles and other arthropods, that the α -chitin crystals are wrapped in cylindrical proteins in a hexagonal arrangement and are of uniform diameter (Blackwell and Weih, 1980; Giraud-Guille et al., 1990; Neville et al., 1976). In some arthropod species, the resulting chitin-protein nanofibrils are tightly packed in a uniform, quasi-hexagonal arrangement to form a single chitin-protein microfibril, while in others, such as *C. longinasus*, the chitin-protein nanofibrils are embedded into a protein matrix

Figure 4: Interface of Bouligand structure between mesocuticle (bottom layers) and exocuticle (upper layers), showing gradual decrease in number of laminae per 180° alternation (decreasing pitch) and a gradual increase in the number of chitin-protein nanofibrils per lamina (increasing volume fraction).

(Figs. 3 and 4) to form layers in the Bouligand structure (Blackwell and Weih, 1980; Chen et al., 2008; Cheng et al., 2008; Kaya et al., 2015; Raabe et al., 2005). For fibers embedded in a matrix with a hexagonal arrangement (Nikolov et al., 2010)

$$\varphi_F = \frac{2\pi r_f^2}{\sqrt{3}(2r_f + s)^2} \quad (34)$$

Because the microfibers are comprised solely of parallel nanofibrils, and given the assumptions of the Mori-Tanaka model, we may consider the effective properties of a homogenized microfiber and a homogenized nanofibril to be equivalent, physically differing only in radius. We therefore use data from Neville et al. (1976) for chitin-rich structures in beetles to calculate the volume fractions of chitin and protein from which we can derive, via the Mori-Tanaka model, the effective properties of a single chitin-protein nanofibril. We note that the values for the effective properties of the protein matrix come from relevant literature, as discussed below, and are different for the exocuticle, to reflect the increased cross-linking between the nanofibrils and the protein matrix that results from the process of tanning (sclerotization); also see Sections 3.1 and 3.3.

2.5. Mesocuticle

The effective properties of the helicoidal ply (Bouligand) structure of the mesocuticle can be ascertained in a three-step procedure that generally follows the method of Nikolov et al. (2011, 2010):

1. We first calculate the effective properties of a single, imperforate lamina by using the Mori-Tanaka

method. We assume that a single ply (the RVE) in the Bouligand structure can be effectively modelled as a layer consisting of aligned, identical, evenly spaced, cylindrical inclusions (fibers) embedded in a matrix. As input, we take the effective stiffness tensor of a single chitin-protein nanofibril, the effective stiffness tensor of the protein matrix into which the nanofibrils are embedded, and the volume fraction of the nanofibrils, as calculated from Eq. (34). The exact separation distance between the nanofibrils is unknown, but it is possible to estimate this value based on the number of layers in the Bouligand structure, the size of the chitin-protein nanofibrils, and the pitch of the ply in our samples. We outline this method in greater numerical detail below (see Section 3.2).

2. Next, we simulate the helicoidal stacking of these layers to generate a transversely isotropic approximation of the Bouligand structure with a Voigt estimate (Section 2.3.1). At the macroscale, the Bouligand structure can be considered a transversely isotropic composite, so long as the number of plies is large (and can thus be considered infinitesimally thin) and the pitch of the ply (number of layers per 180° rotation of the ply) is correspondingly small. Given the very large number of layers in the cuticle and relatively small pitch, the Bouligand structure can be considered transversely isotropic at small strains. We simulate this by considering n layers (per 180° alternation) stacked at θ degrees relative to each other along the normal axis, then taking a Voigt estimation (see Eq. (1)) over each layer where the properties of the i^{th} layer are given by Reddy (2004):

$$C_i = R : C : R^T \quad (35)$$

Where C is the stiffness tensor for a single lamina, and R is given in terms of θ (Reddy, 2004):

$$R = \begin{bmatrix} \cos^2 \theta & \sin^2 \theta & 0 & 0 & 0 & \sin 2\theta \\ \sin^2 \theta & \cos^2 \theta & 0 & 0 & 0 & -\sin 2\theta \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & \cos \theta & -\sin \theta & 0 \\ 0 & 0 & 0 & \sin \theta & \cos \theta & 0 \\ -\sin \theta \cos \theta & \sin \theta \cos \theta & 0 & 0 & 0 & \cos^2 \theta - \sin^2 \theta \end{bmatrix} \quad (36)$$

The Voigt estimate is therefore given, from Eqs. (1), (35), and (36):

$$C = \frac{1}{n} \sum_{i=1}^n C_i \quad (37)$$

3. Finally, we model the effect of adding pore canals to the structure. To calculate the effective properties of the helicoidal ply including pore canals, we utilize a non-interaction approximation (Section 2.3.3) from Sevostianov et al. (2005) rather than the three-point approximation scheme of Hyun and Torquato (2000). We assume that the pores are cylindrical, rather than helico-ellipsoidal (Fig. 3), and have the same cross sectional area throughout. While sufficient for Nikolov et al. (2011, 2010), the three-point

approximation scheme is not optimal for our purposes. Our scheme requires estimation of a complete effective stiffness tensor; yet the three point approximation only generates the in-plane elastic constants, not addressing the remaining constants. [Sevostianov et al. \(2005\)](#) found an explicit solution for calculating the effective properties of a material consisting of aligned spheroidal inclusions in a transversely isotropic matrix, when the axis of rotation of the inhomogeneities is aligned with the axis of transverse isotropy. For this reason we use their method and a non-interaction approximation to estimate the properties of the Bouligand structure with pores, based on the low volume fraction of pores in the cuticle of our samples, as explained below (see Section 3.2).

2.6. Exocuticle

The process for calculating the effective stiffness tensor of exocuticle is the same as that of mesocuticle, where we consider the material in three hierarchical levels: a single (imperforate) lamina, a laminate composite of stacked plies, and finally the Bouligand structure with pores. In our model, there are three main structural differences between the mesocuticle and exocuticle (see Section 3.3):

1. The packing of the laminae of the Bouligand structure is estimated to be approximately three times closer than mesocuticle, based on the pitch of the ply in exocuticle of our samples and data from other arthropods ([Bouligand, 1972](#); [Chen et al., 2008](#); [Cheng et al., 2009](#)). The reduced spacing also greatly increases the fiber fraction of the exocuticle so that the Mori-Tanaka method is no longer effective. To accommodate a high volume fraction of fibers and account for interactions between them, we utilize the effective field method (Section 2.3.4) for calculating the macroscale properties of the resulting individual ply ([Sevostianov et al., 2005](#)).
2. We estimate the effective Young’s modulus of the fiber proteins to be significantly higher than in the endocuticle and mesocuticle, due to increases in cross-linking between the individual chitin-protein nanofibrils present in the exocuticle, as well as decreased water content. We use value presented by [Vincent \(1982\)](#), who provides measurements for the protein matrix of anatomical structures that exhibit large degrees of fiber cross-linking.
3. The effective Young’s modulus of the protein matrix in which the nanofibrils are embedded is also estimated to be higher than in the mesocuticle, in keeping with the findings of [Ker \(1977\)](#), for highly sclerotized cuticle.

2.7. Endocuticle

Beetle endocuticle differs significantly in microstructural arrangement when compared to other layers of cuticle; namely, the endocuticle is characterized by the presence of unidirectional chitin macrofibers or balken ([van de](#)

Figure 5: Typical “Balken-type” endocuticle, represented as orthogonally-stacked layers of chitin macrofibers embedded in a porous protein matrix; the pores exhibit a square arrangement between the macrofibers (not illustrated to scale).

[Kamp and Greven, 2010](#)). In these structures, the individual chitin cores surrounded by protein (the chitin-protein nanofibrils) are embedded in a protein matrix to form chitin-protein microfibers (Fig. 5). The latter, in turn, are tightly conjoined to form the macrofibers ([van de Kamp and Greven, 2010](#); [Kaya et al., 2015](#); [Hepburn and Ball, 1973](#)). The endocuticle generally exhibits the smallest degree of fiber cross-linking in the cuticle as a whole, and is the least sclerotized portion of this tissue ([Vincent, 1982](#); [Vincent and Wegst, 2004](#)). We therefore adjust the properties of the protein matrices accordingly in our formulation of a micromechanical model for the endocuticle, as described in Section 3.4.

Among the Coleoptera, there are two main types of structural arrangements of the macrofibers that can occur ([van de Kamp and Greven, 2010](#)). The first type is considered the “classical” balken-type cuticle, where the macrofibers are arranged unidirectionally and are connected by a thin layer of protein matrix with interspersed pores. The second type is referred to as “reticulate” balken-type cuticle, which is characterized by cross-linking fibers between the balken that connect the macrofibers and also reinforce the pores. The latter type creates a linear mesh, where the gaps are filled by protein matrix and have central pores ([Hepburn and Ball, 1973](#)). In between these there are cuticles that exhibit only a small amount of cross-linking between macrofibers, and are very similar to the classical type; this was observed in *Anthonomus grandis* by [Leopold et al. \(1992\)](#). To simulate the micromechanical properties of a layer comprised of multiple balken, protein matrix, and pores, it is necessary to replicate the arrangement and volume fractions of these components within a RVE. We describe our general approach for endocuticle in

four steps:

1. We first compute the effective properties of a single chitin macrofiber (our RVE at this step), given the properties of a homogenized chitin-protein nanofibril, the diameter and separation of the nanofibrils within the microfibrers, and the properties of the protein matrix in which the nanofibrils are embedded. The lateral separation of the nanofibrils is known from recent work in weevils by [van de Kamp et al. \(2015\)](#) (see Section 3.4), thus leaving the effective properties of the matrix as the only unknown. We therefore take these properties from [Nikolov et al. \(2010\)](#). Although their data relate to the lobster endocuticle, we presume that their estimations are similar enough to be applicable the weevil endocuticle. This reasoning is supported both by the results outlined below and by findings for other insects ([Ker, 1977](#); [Vincent, 1982](#); [Vincent and Wegst, 2004](#)). Based on the volume fraction of microfibrers observed in our samples, we utilize the effective field method to calculate the effective properties for a single, unidirectional macrofiber.
2. We assume, based on the observations of weevil balken by [Leopold et al. \(1992\)](#) and [Davis \(2014\)](#), that the even spacing of the macrofibers in our samples is due to a reduced amount of cross-linking between macrofibers, such that they can be considered effectively separated along most of their length by protein matrix. We may thus model a single balken layer by assuming that the overall architecture is that of a unidirectional, fiber-reinforced composite with a transversely isotropic fiber fraction and an isotropic matrix embedded with cylindrical pores. This enables us to calculate the volume fractions of the layers of protein matrix, pores, and macrofibers, respectively, from the cross sectional areas of the respective elements of the structure and based on our samples. Furthermore, we assume a square arrangement of the pores due to the linear mesh arrangement of the balken layers, wherein:

$$\varphi_F = \frac{2\pi r_f^2}{(2r_f + s)^2} \quad (38)$$

Because the volume fraction of pores in our sample is quite low (see Section 3.4), we utilize a non-interaction approximation to homogenize the pore-embedded matrix.

3. Our strategy then follows the approach of [Vilchevskaya and Sevostianov \(2015\)](#) for two-phase composites. We do not attempt to simultaneously calculate the combined effect of pores and fibers, which are assumed to be cylindrical, aligned, identical (within each respective phase, pore or fiber), and orthogonal to each other in the matrix, since it is possible that the combined effect of a stiff phase and a compliant phase can lead to numerical singularity at cer-

tain volume fractions ([Sevostianov, 2014](#)). Rather, we first calculate the effective properties of the pore-embedded matrix, and then create an isotropic approximation of the resulting transversely isotropic, homogenized material. Because the resulting degree of transverse isotropy is correspondingly low, an isotropic approximation (Section 2.3.5) can be used with reasonable accuracy to calculate the Hill's tensor for the macrofibers³, as explained below.

4. Given an isotropic approximation for the protein matrix embedded with cylindrical pores, we can now simulate the effect of adding macrofibers to the (porous) matrix via a Maxwell homogenization scheme (Section 2.3.6). The isotropic approximation is used to calculate the Hill's tensor for the macrofibers, Q , used in Eq. (28), and whose components are given in Eqs. (29). We assume that the interaction between pores and the fiber fraction is essentially negligible, though we utilize the compliance tensor of the transversely isotropic, pore-embedded matrix, rather than the isotropic approximation, to calculate the effective compliance tensor of the balken layer in Eq. (27).

3. Results

In this section, we examine the explicit numerical solutions of the models as outlined in the preceding section. We begin at the lowest hierarchical level for each region of cuticle, building up to the complete structure sequentially, at each level in the structural hierarchy. We also report, where appropriate, values obtained from relevant literature for certain parameters that we were not able to measure experimentally. With this foundation, we are able to present the macroscale properties of each layer of the cuticle, as well as examine the robustness of the resultant elastic constants with respect to literature-extracted model parameters.

3.1. Chitin-Protein Nanofibrils

[Nikolov et al. \(2011, 2010\)](#) specify the stiffness tensor for crystalline α -chitin, with the longitudinal axis of the fiber aligned with axis 1, in 6×6 matrix notation configured as:

$$C_{CH} = \begin{bmatrix} 119 & 0.10 & 1.1 & 0 & 0 & 0 \\ 0.10 & 28 & 2.0 & 0 & 0 & 0 \\ 1.1 & 2.0 & 24 & 0 & 0 & 0 \\ 0 & 0 & 0 & 5.0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 8.0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 2.0 \end{bmatrix} \quad (39)$$

Assuming that the fiber protein matrix is isotropic and that Young's modulus and Poisson's ratio are $E_{FP} = 0.004$

³This crucial step enables us to approximate the effective properties of this two-phase composite, while simultaneously sidestepping two particular complications: potential numerical singularity during tensor inversion ([Sevostianov, 2014](#)), and the problem of explicitly calculating the effective properties of fibers embedded in a transversely isotropic matrix, where the fibers are aligned *orthogonal* to the axis of transverse isotropy ([Saadat et al., 2012](#); [Vilchevskaya and Sevostianov, 2015](#)).

GPa and $\nu_{FP} = 0.49$, respectively [Nikolov et al. \(2011, 2010\)](#), and given the general form of a stiffness tensor for isotropic media from Eq. (26), the stiffness tensor of the fiber proteins is specified as:

$$C_{FP} = \begin{bmatrix} 0.068 & 0.066 & 0.066 & 0 & 0 & 0 \\ 0.066 & 0.068 & 0.066 & 0 & 0 & 0 \\ 0.066 & 0.066 & 0.068 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.0013 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.0013 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.0013 \end{bmatrix} \quad (40)$$

In Coleoptera belonging to two different families (Scarabaeidae and Tenebrionidae), the α -chitin crystallites in chitin-rich structures show a quasi-hexagonal arrangement [Neville et al. \(1976\)](#). The diameter of individual α -chitin crystallites have been measured, on average, as $2r_f = 2.71, 2.86$ nm, with the center to center distances as 5.9, 5.3 nm respectively, and translating into lateral separation distances of $s = 3.19, 2.44$ nm, respectively [Neville et al. \(1976\)](#). [Nikolov et al. \(2011\)](#) reported that these values can vary without noticeable influence on the resultant macro-scale properties of the cuticle. Hence we apply averages of these measures such that $2r_f = 2.785$ nm and $s = 2.815$ nm, and consequently (given Eq. [34]), $\varphi_{CH} = 0.22$ and $\varphi_{FP} = 0.78$. Because the volume fraction is less than 30%, we use the Mori-Tanaka model described in Section 2.3.2.

The Eshelby's tensor for the α -chitin crystallites is dependent only on Poisson's ratio of the matrix ($\nu_{FP} = 0.49$), and can be found by Eqs. (4) and (5):

$$S_{CH} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0.45 & 0.75 & 0.24 & 0 & 0 & 0 \\ 0.45 & 0.24 & 0.75 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.51 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.50 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.50 \end{bmatrix} \quad (41)$$

We can thus calculate the strain concentration factor from Eq. (3):

$$A_{CH} = \begin{bmatrix} 1.0 & 0 & 0 & 0 & 0 & 0 \\ 0.0019 & 0.0024 & 0.0022 & 0 & 0 & 0 \\ -0.043 & 0.0026 & 0.0028 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.00053 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.00034 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.0013 \end{bmatrix} \quad (42)$$

Given Eq. (2), the stiffness tensor of a chitin protein nanofibril is specified as:

$$C_{NF} = \begin{bmatrix} 27 & 0.067 & 0.067 & 0 & 0 & 0 \\ 0.067 & 0.089 & 0.085 & 0 & 0 & 0 \\ 0.067 & 0.085 & 0.089 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.0021 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.0021 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.0021 \end{bmatrix} \quad (43)$$

3.2. Mesocuticle

Here we present the numerical results of the four-step procedure outlined in Section 2.5.

1. To calculate the effective properties of a single layer of the Bouligand structure as a fiber-reinforced matrix, we need to account for the stiffness tensor of the chitin-protein nanofibrils and protein matrix, as well as their respective volume fractions. The stiffness tensor of the chitin-protein nanofibrils is given

by Eq. (43). The volume fractions may be calculated from Eq. (34), assuming a quasi-hexagonal arrangement of the fibers. The exact separation between the nanofibrils is unknown, but it is possible to estimate their separation based on the number of layers in the Bouligand structure, the size of the chitin-protein nanofibrils, and the pitch of the ply. In our samples, there are (on average) approximately ten 180° alternations per $1\mu\text{m}$. We therefore estimate approximately 100 nm per 180° alternation. The nanofibrils have an estimated diameter of 5.6 nm each, based on Section 3.1. If we assume a separation distance of 1.5 times the radius of a nanofibril ($s = 4.2$ nm), the resulting structure has roughly 10 layers per 180° alternation. This number agrees with experimentally measured stacking in the mesocuticle of beetles ([Cheng et al., 2009](#)). Moreover, these parameters give the model macroscale properties that show very good correspondence with fresh mesocuticle ([Klocke and Schmitz, 2011](#)).

The above specifications yield $\varphi_{NF} = 0.30$ as an upper bound for the volumetric fraction of nanofibrils in the apparent mesocuticle and therefore $\varphi_{PM} = 0.70$. The volume fraction of chitin is therefore nearly 6.5%, which agrees with experimentally measured values of chitin inclusion ([Vincent, 1982](#); [Vincent and Wegst, 2004](#)). Given this result, the Mori-Tanaka model may be used to find the effective stiffness tensor of a single ply within the Bouligand structure of the apparent mesocuticle, without pore canals. This volume fraction is generally considered to be at the upper limit (30%) for this method; in this instance, however, we find that estimation of the effective tensor for mesocuticle using the Maxwell scheme yields nearly identical results.

Young's moduli, E_{PM} , for matrix proteins have been reported by [Ker \(1977\)](#) to vary from 0.12 GPa in tendons to 1.7 GPa in the highly sclerotized tissue (tibia). Poisson's ratio, ν_{PM} , of these proteins is unknown for insects, but was found by [Nikolov et al. \(2011\)](#) to be 0.45 for lobsters. To calculate the stiffness tensor of the protein matrix, we assume $E_{PM} = 1.2$ GPa and $\nu_{PM} = 0.45$ because of the degree of tanning and sclerotization in the mesocuticle. We therefore specify:

$$C_{PM} = \begin{bmatrix} 4.6 & 3.7 & 3.7 & 0 & 0 & 0 \\ 3.7 & 4.6 & 3.7 & 0 & 0 & 0 \\ 3.7 & 3.7 & 4.6 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.41 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.41 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.41 \end{bmatrix} \quad (44)$$

Accordingly, Eshelby's tensor for a chitin-protein nanofibril is:

$$S_{NF} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 \\ 0.41 & 0.73 & 0.18 & 0 & 0 & 0 \\ 0.41 & 0.18 & 0.73 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.55 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.50 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.50 \end{bmatrix} \quad (45)$$

The strain concentration factor for the nanofibrils is modeled as:

$$A_{NF} = \begin{bmatrix} 1.0 & 0 & 0 & 0 & 0 & 0 \\ 3.7 & 5.6 & 3.5 & 0 & 0 & 0 \\ 3.7 & 3.5 & 5.6 & 0 & 0 & 0 \\ 0 & 0 & 0 & 2.2 & 0 & 0 \\ 0 & 0 & 0 & 0 & 2.0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 2.0 \end{bmatrix} \quad (46)$$

Lastly, the stiffness tensor of a single lamina of the Bouligand structure is:

$$C_L = \begin{bmatrix} 9.5 & 0.82 & 0.82 & 0 & 0 & 0 \\ 0.82 & 1.1 & 0.71 & 0 & 0 & 0 \\ 0.82 & 0.71 & 1.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.22 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.23 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.23 \end{bmatrix} \quad (47)$$

2. A Voigt estimate (Section 2.3.1) can be applied to find the effective upper bound of the stiffness tensor of the entire Bouligand structure according to Eqs. (35)-(37). The rotation matrix is necessary to find the effective properties of all three plies in the same coordinate system, as they are rotated relative to each other. Axis 3 is used as the transverse normal to the laminae, while axes 1 and 2 are the in-plane laminar axes. The simplest laminate composite conforming to the generalized Bouligand structure is a stack of three laminae each rotated 60° to each other along the normal axis. We therefore assume that $i = 1$ represents the instance of the lamina whose properties are already represented in the appropriate coordinate system, where $\theta = 0^\circ$, and given in Eq. (47). Thus, given that $\theta = 60^\circ, 120^\circ$, when $i = 2, 3$, respectively:

$$\begin{aligned} C_1 &= C_L \\ C_2 &= \begin{bmatrix} 1.7 & 2.3 & 0.80 & 0 & 0 & 0.93 \\ 2.3 & 5.9 & 0.80 & 0 & 0 & 2.7 \\ 0.80 & 0.80 & 1.1 & 0 & 0 & 0.050 \\ 0 & 0 & 0 & 0.22 & 0.0042 & 0 \\ 0 & 0 & 0 & 0.0042 & 0.22 & 0 \\ 0.93 & 2.7 & 0.050 & 0 & 0 & 1.7 \end{bmatrix} \\ C_3 &= \begin{bmatrix} 1.7 & 2.3 & 0.80 & 0 & 0 & -0.93 \\ 2.3 & 5.9 & 0.80 & 0 & 0 & -2.7 \\ 0.80 & 0.80 & 1.1 & 0 & 0 & -0.050 \\ 0 & 0 & 0 & 0.22 & -0.0042 & 0 \\ 0 & 0 & 0 & -0.0042 & 0.22 & 0 \\ -0.93 & -2.7 & -0.050 & 0 & 0 & 1.7 \end{bmatrix} \end{aligned} \quad (48)$$

Therefore, for the Bouligand structure without pores⁴:

$$C_{PW} = \begin{bmatrix} 4.3 & 1.8 & 0.77 & 0 & 0 & 0 \\ 1.8 & 4.3 & 0.77 & 0 & 0 & 0 \\ 0.77 & 0.77 & 1.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.22 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.22 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1.2 \end{bmatrix} \quad (49)$$

3. Sevostianov et al. (2005) calculated the effective properties of a transversely isotropic matrix embedded with spheroidal inhomogeneities whose rotational axes are aligned with the axis of symmetry in the matrix. If the Bouligand structure can be effectively represented as a transversely isotropic matrix, and the pore canals as cylinders, rather than helices, then

we can calculate the additional compliance added to the material by an arbitrary number of pores. In general, this is a two-step procedure: first, the additional compliance imbued by a single pore must be ascertained, then the influence of multiple pores can be calculated, based on the shape and number of pores.

Unlike the cuticle of other arthropods, such as lobsters, beetle cuticle does not have an extensively developed pore canal system, and often appears in SEM or TEM to have a solid surface area, φ_S , in excess of $\varphi_S = 0.96$ ((Cheng et al., 2009; Hepburn and Ball, 1973; Hyun and Torquato, 2000; McNamara et al., 2012)). In keeping with their findings, we assume a pore fraction of $\varphi_{PC} = 0.04$ as an upper bound, although the exact area fraction of the pores is not known for any member of the genus *Curculio*, and is quite possibly much lower. Because the pore canal fraction is very low, we can assume that the interaction between pore canals is essentially negligible. We therefore use a non-interaction approximation, described in Section 2.3.3, to simulate the effect of introducing pore canals into the Bouligand structure (Kachanov and Sevostianov, 2005; Sevostianov and Kachanov, 2002; Sevostianov et al., 2005).

We begin by calculating the compliance tensor of the stacked laminae, S_{PW} , from the stiffness tensor presented in Eq. (49):

$$S_{PW} = [C_{PW}]^{-1} = \begin{bmatrix} 0.30 & -0.10 & -0.13 & 0 & 0 & 0 \\ -0.10 & 0.30 & -0.13 & 0 & 0 & 0 \\ -0.13 & -0.13 & 1.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 4.5 & 0 & 0 \\ 0 & 0 & 0 & 0 & 4.5 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.81 \end{bmatrix} \quad (50)$$

From Eq. (11)-(16), we derive:

$$H_{NI} = \begin{bmatrix} 0.027 & -0.019 & -0.0053 & 0 & 0 & 0 \\ -0.019 & 0.027 & -0.0053 & 0 & 0 & 0 \\ -0.0053 & -0.0053 & 0.042 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.090 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.090 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.023 \end{bmatrix} \quad (51)$$

Therefore, given Eq. (6), the compliance tensor for mesocuticle is:

$$S_M = \begin{bmatrix} 0.32 & -0.12 & -0.14 & 0 & 0 & 0 \\ -0.12 & 0.32 & -0.14 & 0 & 0 & 0 \\ -0.14 & -0.14 & 1.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 4.6 & 0 & 0 \\ 0 & 0 & 0 & 0 & 4.6 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.83 \end{bmatrix} \quad (52)$$

And, the stiffness tensor for mesocuticle is specified as:

$$C_M = \begin{bmatrix} 4.1 & 1.8 & 0.74 & 0 & 0 & 0 \\ 1.8 & 4.1 & 0.74 & 0 & 0 & 0 \\ 0.74 & 0.74 & 1.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.22 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.22 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1.2 \end{bmatrix} \quad (53)$$

3.3. Exocuticle

In our model, we identify three primary differences between mesocuticle and exocuticle (see also Section 2.6):

⁴This result is accurate to at least twelve decimal places regardless of the number of laminae used.

1. The packing of the laminae is estimated to be approximately 2-3 times closer than mesocuticle based on the pitch of the ply in exocuticle of our samples, and data from other arthropods (Bouligand, 1972; Chen et al., 2008; Cheng et al., 2009; Klocke and Schmitz, 2011). We posit that the diameter of the nanofibrils remains the same, but that the pitch of the ply and separation distance between nanofibrils are reduced. Assuming a lateral separation equal to one-quarter of the radius ($s = 0.7 \text{ nm}$) and half as many laminae per 180° alternation, the resulting structure has three times as many alternations as mesocuticle. The reduced spacing also greatly increases the fiber fraction of the exocuticle, such that $\varphi_{NF} = 0.72$ (total volume of chitin rises to 16.1%). This volume fraction violates the assumption of dilute inclusions used by the Mori-Tanaka model, and we therefore use the effective field method presented in Sevostianov et al. (2005) to derive the effective properties of a single lamina of the exocuticle.
2. We estimate the effective Young's modulus of the fiber proteins as $E_{FP} = 0.12 \text{ GPa}$, on the basis of Vincent (1982), who provides measurements for the protein matrix of anatomical structures that exhibit large degrees of fiber cross-linking. Changing this parameter of the Mori-Tanaka model presented in Section 3.1 yields:

$$C_{NF} = \begin{bmatrix} 28 & 1.9 & 1.9 & 0 & 0 & 0 \\ 1.9 & 2.6 & 2.4 & 0 & 0 & 0 \\ 1.9 & 2.4 & 2.6 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.063 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.063 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.062 \end{bmatrix} \quad (54)$$

3. The effective Young's modulus of the protein matrix in which the microfibers are embedded is estimated as $E_{PM} = 1.7 \text{ GPa}$, in keeping with the findings of Ker (1977), for highly sclerotized cuticle.

To use the effective field method, it is necessary to first transform the tensors used as input so that the axis of rotation of an inclusion and the axis of transverse isotropy of the matrix are aligned with the global axis 3. The stiffness tensor presented above has the longitudinal axis of the fiber aligned with global axis 1. The transformed matrix representation can be specified as:

$$C_{NF} = \begin{bmatrix} 2.6 & 2.4 & 1.9 & 0 & 0 & 0 \\ 2.4 & 2.6 & 1.9 & 0 & 0 & 0 \\ 1.9 & 1.9 & 2.8 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.062 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.063 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.063 \end{bmatrix} \quad (55)$$

The protein matrix is isotropic and does not need to be rotated; however, given the higher Young's modulus of the matrix, we determine:

$$C_{PM} = \begin{bmatrix} 6.4 & 5.3 & 5.3 & 0 & 0 & 0 \\ 5.3 & 6.4 & 5.3 & 0 & 0 & 0 \\ 5.3 & 5.3 & 6.4 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.59 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.59 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.59 \end{bmatrix} \quad (56)$$

And, from Eq. (21):

$$C_D = \begin{bmatrix} -3.9 & -2.8 & -3.4 & 0 & 0 & 0 \\ -2.8 & -3.9 & -3.4 & 0 & 0 & 0 \\ -3.4 & -3.4 & 22 & 0 & 0 & 0 \\ 0 & 0 & 0 & -0.52 & 0 & 0 \\ 0 & 0 & 0 & 0 & -0.52 & 0 \\ 0 & 0 & 0 & 0 & 0 & -0.52 \end{bmatrix} \quad (57)$$

Therefore, for a single lamina we can specify (following rotation back to the original coordinate system)⁵:

$$C_L = \begin{bmatrix} 22 & 2.5 & 2.5 & 0 & 0 & 0 \\ 2.5 & 3.2 & 2.9 & 0 & 0 & 0 \\ 2.5 & 2.9 & 3.2 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.15 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.16 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.16 \end{bmatrix} \quad (58)$$

For the Bouligand structure (using a Voigt estimate):

$$C_{PW} = \begin{bmatrix} 10 & 4.8 & 2.7 & 0 & 0 & 0 \\ 4.8 & 10 & 2.7 & 0 & 0 & 0 \\ 2.7 & 2.7 & 3.2 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.15 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.15 & 0 \\ 0 & 0 & 0 & 0 & 0 & 2.6 \end{bmatrix} \quad (59)$$

Assuming the same porosity as the mesocuticle, the effective tensor for the exocuticle is:

$$C_X = \begin{bmatrix} 9.5 & 4.7 & 2.6 & 0 & 0 & 0 \\ 4.7 & 9.5 & 2.6 & 0 & 0 & 0 \\ 2.6 & 2.6 & 3.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.15 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.15 & 0 \\ 0 & 0 & 0 & 0 & 0 & 2.5 \end{bmatrix} \quad (60)$$

3.4. Endocuticle

Here we present the results of the general approach outlined for the derivation of the effective properties of endocuticle, as outlined in Section 2.7:

1. Beetle endocuticle is characterized by the presence of unidirectional chitin macrofibers (van de Kamp and Greven, 2010). In these structures, the individual chitin cores surrounded by protein (the chitin-protein nanofibrils) form chitin-protein microfibers that can vary in size from 20-90 nm in diameter (Kaya et al., 2015). These microfibers are tightly conjoined to form the macrofibers. Hepburn and Ball (1973) found the approximate size of microfibers in *Pachynoda sinuata* (Fabricius 1775) to be approximately 30 nm. This is close to the diameter and separation of fibers that can be seen in *Anthonomus grandis* Boheman 1843 (Leopold et al., 1992). In our samples, we observed that these fibers are of a similar size on average. Because these microfibers appear to be tightly bound, and are not visibly separated in transmission electron micrographs [see van de Kamp et al. (2015)], we treat the macrofibers as if they are solely comprised of aligned nanofibrils embedded in a matrix.

⁵It is worth noting that the formulation of the effective field method presented herein is for *transversely isotropic* fibers, rather than fully orthotropic ones. We treat the nanofibrils as transversely isotropic; however, reformulating to allow for orthotropic fibers does change the resultant stiffness tensor very slightly. Because these numerical differences are beyond the ninth decimal place for the values used herein, and essentially disappear with the Voigt estimate, we feel that this is not a critical factor in the calculation of the effective properties of the exocuticle.

We can readily calculate the effective tensor for a single macrofiber in a manner similar to calculating the effective properties of a single ply in the exocuticle or mesocuticle, without canals. If we assume that the nanofibrils are separated by a distance equal to their own radius [as seen in [van de Kamp et al. \(2015\)](#)] in a quasi-hexagonal arrangement, we find, given Eq. (34), $\varphi_F = 0.40$. We also assume $E_{FP} = 0.004$ GPa, to reflect the reduced cross-linking in the endocuticle, and that $E_{PM} = 1.0$ GPa, in keeping with the value used for endocuticle by [Nikolov et al. \(2010\)](#). With these values, we find the stiffness tensor for a the protein matrix as:

$$C_{PM} = \begin{bmatrix} 3.8 & 3.1 & 3.1 & 0 & 0 & 0 \\ 3.1 & 3.8 & 3.1 & 0 & 0 & 0 \\ 3.1 & 3.1 & 3.8 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.34 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.34 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.34 \end{bmatrix} \quad (61)$$

And the effective stiffness tensor for a single chitin macrofiber:

$$C_{MF} = \begin{bmatrix} 12 & 0.50 & 0.50 & 0 & 0 & 0 \\ 0.50 & 0.71 & 0.43 & 0 & 0 & 0 \\ 0.50 & 0.43 & 0.71 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.14 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.15 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.15 \end{bmatrix} \quad (62)$$

2. In order to calculate the properties of a single balken layer, we posit that the layer is comprised of macrofibers, with effective stiffness C_{MF} , aligned in an isotropic protein matrix with stiffness identical to C_{PM} . In addition we assume that the layer is porous, and that the pores are embedded in the protein matrix between the macrofibers. From our samples, we find that the area fraction (and, equivalently, the volume fraction) of protein matrix layers varies, by layer, from roughly 14-18%. We therefore take the average and set $\varphi_L = 0.16$ and $\varphi_{MF} = 0.84$. Additionally, the ratio of the diameter of a pore and its separation distance to the closest pore is equal to the area fraction of the protein layers in cross-section. We assume a square arrangement of the pores due to the linear mesh arrangement of the balken layers; thus, given Eq. (38), we find $\varphi_p \approx 0.015$.

We now consider an isotropic protein matrix with effective properties equal to C_{PM} . We assume a global coordinate system where axis 1 is the long axis of the macrofibers, axis 2 is the in-plane axis that, along with axis 1, defines the plane of a single layer of balken, and axis 3 is the cuticle normal. We then introduce into this matrix a system of identical, cylindrical pores with the same arrangement of our samples, whose long axis is aligned with global axis 3. Because the pores in our samples are effectively embedded in the protein matrix between the macrofibers and occupy a very small volume, we can estimate the properties of the homogenized matrix, before introducing the macrofibers, using a non-interaction approximation (Section 2.3.3).

From the non-interaction approximation outlined in Section 2.3.3, we derive the following:

$$H_{NI} = \begin{bmatrix} 0.031 & -0.017 & -0.0067 & 0 & 0 & 0 \\ -0.017 & 0.031 & -0.0067 & 0 & 0 & 0 \\ -0.0067 & -0.0067 & 0.015 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.022 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.022 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.028 \end{bmatrix} \quad (63)$$

Thus, given C_{PM} and Eq. (6), the effective compliance tensor for a layer of protein separating the macrofibers is obtained as: particular sets of factors

$$S_L = \begin{bmatrix} 1.0 & -0.47 & -0.46 & 0 & 0 & 0 \\ -0.47 & 1.0 & -0.46 & 0 & 0 & 0 \\ -0.46 & -0.46 & 1.0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 2.9 & 0 & 0 \\ 0 & 0 & 0 & 0 & 2.9 & 0 \\ 0 & 0 & 0 & 0 & 0 & 2.9 \end{bmatrix} \quad (64)$$

And, therefore:

$$C_L = \begin{bmatrix} 3.6 & 2.9 & 3.0 & 0 & 0 & 0 \\ 2.9 & 3.6 & 3.0 & 0 & 0 & 0 \\ 3.0 & 3.0 & 3.6 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.34 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.34 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.34 \end{bmatrix} \quad (65)$$

3. Because the pores occupy a small volume, the anisotropic generated by their inclusion will be relatively small. We are therefore able to generate an isotropic approximation of the matrix to calculate the stiffness contribution tensor N_{MF} and Hill's tensor Q_{MF} for a macrofiber. Considering a transversely isotropic layer of porous protein matrix, with stiffness tensor C_L , the isotropic approximation of the shear modulus G and bulk modulus K , as well as Young's modulus E and Poisson's ratio ν are given by Eqs. (24) and (25), respectively:

$$\begin{aligned} G &= 0.34 \text{ GPa} \\ K &= 3.2 \text{ GPa} \\ E &= 0.98 \text{ GPa} \\ \nu &= 0.45 \end{aligned} \quad (66)$$

From these elastic constants, and through application of Eq. (23), we can calculate the isotropic approximations of the effective stiffness and compliance tensors of the protein matrix:

$$S_{IL} = \begin{bmatrix} 1.0 & -0.46 & -0.46 & 0 & 0 & 0 \\ -0.46 & 1.0 & -0.46 & 0 & 0 & 0 \\ -0.46 & -0.46 & 1.0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 2.9 & 0 & 0 \\ 0 & 0 & 0 & 0 & 2.9 & 0 \\ 0 & 0 & 0 & 0 & 0 & 2.9 \end{bmatrix} \quad (67)$$

4. Using the isotropic approximation of the porous matrix, we now calculate the effective properties of a single balken layer by simulating the effect of embedding macrofibers into the matrix, using the Maxwell homogenization scheme described in Section 2.3.6. Given the elastic constants in Eq. (66) and the components of Hill's tensor, Q , presented in Eqs. (29),

we obtain⁶:

$$Q_{MF} = \begin{bmatrix} 1.2 & 0.28 & 0.28 & 0 & 0 & 0 \\ 0.28 & 0.46 & 0.15 & 0 & 0 & 0 \\ 0.28 & 0.15 & 0.46 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.15 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.17 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.17 \end{bmatrix} \quad (68)$$

Thus, given Eq. (28), the compliance contribution tensor for the macrofibers is:

$$H_{MF} = \begin{bmatrix} -9.0 & 4.0 & 4.0 & 0 & 0 & 0 \\ 4.0 & -1.1 & -2.1 & 0 & 0 & 0 \\ 4.0 & -2.1 & -1.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 2.1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1.9 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1.9 \end{bmatrix} \quad (69)$$

Using Eq. (27), the compliance stiffness tensors for a single balken layer are, respectively:

$$S_B = \begin{bmatrix} 0.12 & -0.057 & -0.047 & 0 & 0 & 0 \\ -0.057 & 1.9 & -1.2 & 0 & 0 & 0 \\ -0.047 & -1.2 & 1.9 & 0 & 0 & 0 \\ 0 & 0 & 0 & 6.1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 5.8 & 0 \\ 0 & 0 & 0 & 0 & 0 & 5.8 \end{bmatrix} \quad (70)$$

$$C_B = \begin{bmatrix} 9.0 & 0.63 & 0.60 & 0 & 0 & 0 \\ 0.63 & 0.86 & 0.53 & 0 & 0 & 0 \\ 0.60 & 0.53 & 0.86 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.16 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.17 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.17 \end{bmatrix} \quad (71)$$

3.5. Summary of Results

In Eq. (72) [see following page] we present the effective stiffness tensors (in 6×6 matrix notation) for the exocuticle, mesocuticle, and a single balken layer of the endocuticle, as C_X , C_M , and C_B , respectively. Additionally, we list the elastic constants that can be calculated from the inverse of these tensors in Tab. 1 and Fig. 6, and summarize the models from which these tensors were derived in Tab. 2. Accordingly, the in-plane (axes 1 and 2) Young's moduli of exocuticle and mesocuticle are 6.39 GPa and 3.08 GPa, respectively, while the transverse (axis 3) moduli for these regions are 2.14 GPa and 0.91 GPa, respectively. Interestingly, Young's modulus of endocuticle along the major axis of the macrofibers is much stiffer than either of the other regions, at 8.47 GPa; however, Young's moduli for endocuticle along axes 2 and 3 are substantially lower, at 0.52 GPa. Due to this anisotropy, cuticular stiffness will depend upon the stacking angle of the balken layers (Reddy, 2004).

We note that the values for several constants were extracted from relevant literature, as we were unable to accurately measure these quantities in the species considered herein. These values do not necessarily represent the actual values present in *C. longinasus*, and we consider these estimates as useful and necessary for the construction of this model, while considering the possible error introduced by their inclusion. We therefore investigate the potential error introduced by the following variables: (1) Young's modulus of fiber proteins, E_{FP} ; (2) Poisson's ratio of fiber

Young's Moduli of *C. longinasus* Cuticle

Figure 6: Summary of Young's moduli of elasticity along each global axis for each region of cuticle.

proteins, ν_{FP} ; (3) Young's modulus of matrix proteins, E_{PM} ; (4) Poisson's ratio of matrix proteins, ν_{PM} ; (5) volume fraction of chitin-protein nanofibrils, φ_{NF} , and; (6) volume fraction of pore canals, φ_{PC} . We do not examine the volume fraction of α -chitin in a nanofibril, which was investigated by Nikolov et al. (2010), and found to have minimal effects on the resultant macroscopic elastic moduli with relatively large variation.

In Fig. 7, we graphically represent estimates of the macroscopic Young's moduli of each cuticular layer as these parameters change individually, with all others kept constant. The in-plane macroscopic Young's moduli exhibit little or no change with respect to the elastic constants of the fiber and matrix proteins, given the range of values used in the model. We observe that the structural arrangement of the chitin-protein nanofibrils has a strong influence on the resulting macroscopic properties of the cuticle, and influences which factors will contribute most to the overall stiffness of each region. For instance, the Bouligand structure greatly reduces the anisotropy of the cuticle compared to the parallel bundles of chitin in the endocuticle; increasing the volume fraction of nanofibrils in the mesocuticle to levels seen in the exocuticle results in an increase of the in-plane Young's modulus of about 3 GPa, while doing the same for the endocuticle results in an increase of almost 5 GPa (along axis 1). Due to the parallel arrangement of chitin fibers in the endocuticle, smaller variations in the volume fraction of nanofibrils or pores elicit a larger change in the longitudinal Young's modulus compared to either mesocuticle or exocuticle.

The Bouligand structure is also responsible for the sensitivity of the normal Young's moduli to the properties of the fiber proteins. In Fig. 8 we present the point-wise elasticity of the model with respect to each of these variables. We observed that the macroscale Young's moduli are most sensitive to the volume fraction of nanofibrils (transverse)

⁶The formulation of Q presented in Eqs. 29 assumes that the longitudinal axis of the inhomogeneities is aligned with global axis 3. We therefore rotate the resultant tensor to align the macrofibers with global axis 1, and assign the transverse normal to global axis 3.

Table 1: Summary of elastic constants for the layers of cuticle. Young's and bulk moduli are given in GPa.

Constant	Exocuticle	Mesocuticle	Endocuticle*
E_1	6.4	3.1	8.5
E_2	6.4	3.1	0.52
E_3	2.1	0.91	0.52
G_{12}	2.5	1.2	0.17
G_{31}	0.15	0.22	0.17
G_{23}	0.15	0.22	0.16
ν_{12}	0.36	0.37	0.49
ν_{21}	0.36	0.37	0.03
ν_{23}	0.54	0.42	0.63
ν_{32}	0.18	0.13	0.63
ν_{31}	0.18	0.13	0.06
ν_{13}	0.54	0.42	0.42

*Single layer of chitin macrofibers

Table 2: Homogenization schema used for cuticle layers of *C. longinasus*.

RVE	Exocuticle	Mesocuticle	Endocuticle*
Chitin Nanofibril	Mori-Tanaka	Mori-Tanaka	Mori-Tanaka
Single Lamina	Effective Field	Mori-Tanaka	—
Laminae <i>sans</i> Pores	Voigt Estimate	Voigt Estimate	—
Laminae with Pores	Non-Interaction	Non-Interaction	—
Chitin Macrofiber	—	—	Effective Field
Porous Protein Matrix	—	—	Non-Interaction
Balken Layer	—	—	Maxwell Scheme

*Single layer of chitin macrofibers

$$\begin{aligned}
 C_X &= \begin{bmatrix} 9.5 & 4.7 & 2.6 & 0 & 0 & 0 \\ 4.7 & 9.5 & 2.6 & 0 & 0 & 0 \\ 2.6 & 2.6 & 3.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.15 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.15 & 0 \\ 0 & 0 & 0 & 0 & 0 & 2.5 \end{bmatrix} \\
 C_M &= \begin{bmatrix} 4.1 & 1.80 & 0.74 & 0 & 0 & 0 \\ 1.8 & 4.1 & 0.74 & 0 & 0 & 0 \\ 0.74 & 0.74 & 1.1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.22 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.22 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1.2 \end{bmatrix} \\
 C_B &= \begin{bmatrix} 9.0 & 0.63 & 0.60 & 0 & 0 & 0 \\ 0.63 & 0.86 & 0.53 & 0 & 0 & 0 \\ 0.60 & 0.53 & 0.86 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0.16 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0.17 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0.17 \end{bmatrix}
 \end{aligned} \tag{72}$$

Figure 7: Macroscopic Young's moduli for exo-, meso-, and endocuticle (as indicated by subscripts X, M, B, respectively), along transverse axes 1 and 2, and normal axis 3, given variation in: **A** fiber protein modulus **B** fiber protein Poisson's ratio **C** protein matrix modulus **D** protein matrix Poisson's ratio **E** volume fraction of nanofibrils **F** volume fraction of pore canals.

*Vertical lines are placed at values used in the model, for each cuticle region, as indicated; a single, unlabeled line indicates that the same value was used for all regions

Figure 8: Point-wise elasticity of the macroscopic Young's moduli for exo-, meso-, and endocuticle (as indicated by subscripts X, M, B, respectively), along transverse axes 1 and 2, and normal axis 3, with respect to literature extracted parameters. Elasticity is highest with respect to Poisson's ratio of the fiber proteins (for normal moduli) and the volume fraction of nanofibrils (for transverse moduli).

and Poisson’s ratio of the fiber proteins (normal). Due to the significantly denser arrangement of the chitin-protein nanofibrils, the normal Young’s modulus of the exocuticle is highly sensitive to changes in Poisson’s ratio of the fiber proteins, with an elasticity of 26.8, as compared to mesocuticle (5.5) and endocuticle (2.2). However, given the comparatively low elasticities of the normal Young’s moduli for the other variables (the highest value is for exocuticle at 1.5 for ν_{PM}), and the transverse Young’s moduli to all parameters (the highest value is for exocuticle at 0.87), we infer that the model is generally quite robust over a wide range of input conditions. This observation corroborates the findings of [Nikolov et al. \(2011\)](#), who came to a similar conclusion.

4. Discussion

4.1. Major Insights

Our analysis indicates that the substantial differences between the regions of cuticle along the *C. longinasus* rostrum appear to be largely due to structural modifications, rather than chemical/compositional changes. In particular, the structural arrangement and volume fraction of the chitin nanofibrils appear to have the greatest influence on the macroscale properties of the corresponding cuticular region. We observed that significant variation in certain properties, such as the elastic modulus of the protein matrix, have comparatively little effect on the macroscale moduli. These results are largely consistent with previous studies that demonstrate the importance of microstructure in determining the macroscopic properties of biomaterials ([Cheng et al., 2009](#); [Nikolov et al., 2011, 2010](#); [Vincent and Wegst, 2004](#)). Furthermore, as noted in Section 3.5, the endocuticle is much stiffer along axis 1 (corresponding to the longitudinal axis of the macrofibers) than either the exocuticle or mesocuticle. By contrast, in other arthropods, where the innermost region of the cuticle also conforms to the Bouligand structure, the endocuticle is often the most compliant region ([Klocke and Schmitz, 2011](#); [Nikolov et al., 2011, 2010](#)). While the stiffness of beetle endocuticle is balanced by the comparatively low Young’s modulus along the other axes, the stiffness of any cuticular structure in beetles is most likely dependent upon the stacking angle of the balken layers, due to the high degree of anisotropy of this material.

Additionally, we notice an inverse correlation between the in-plane and normal Young’s moduli of the exocuticle and mesocuticle, with respect to fiber fraction. This is a result of the high degree of anisotropy of the chitin-protein nanofibrils, the effect of which is even more pronounced in the endocuticle. Within the chitin macrofibers, the properties of the protein matrix largely determine the macroscale moduli normal to the longitudinal axis (axis 1), especially at lower volume fractions of nanofibrils. As the volume fraction of nanofibrils increases, we observe a strong decrease in these moduli, as the chitin nanofibrils

are significantly more compliant than the protein matrix along axes 2 and 3, while simultaneously an increase in stiffness is seen along axis 1. In the exocuticle and mesocuticle, this effect is somewhat mitigated by the arrangement of nanofibrils in the Bouligand structure, which results in a degree of averaging of the moduli in the 1 and 2 axes. In general, the mesocuticle and endocuticle are of similar composition; however, the Bouligand structure creates a greater degree of isotropy in the mesocuticle due to the lower volume fraction of chitin.

How then do our outcomes explain the differences in elasticity between exocuticle and mesocuticle? We observe that differences between the elastic constants of exocuticle and mesocuticle are primarily correlated with the volume fraction of nanofibrils and the elastic properties of the fiber proteins. For the in-plane moduli, the volume fraction of nanofibrils is the primary contributing factor of the increased stiffness of the exocuticle, which is not strongly affected by variation of other parameters. In contrast, we note that the properties of the fiber proteins have a strong influence on the elastic constants of the exocuticle in the normal direction, due to the high volume fraction of the nanofibrils. The values of Young’s modulus and Poisson’s ratio of the fiber proteins must therefore be carefully considered, due to their influence on the properties of the exocuticle in the normal direction.

If we presume that these values are the same for both exo- and mesocuticle, then the resulting exocuticle would be significantly weaker in the normal direction than the mesocuticle. Based on the data reviewed in the work of [Klocke and Schmitz \(2011\)](#), this is unlikely for insects. We instead consider the reduced water content and increased cross-linking between nanofibrils and matrix (due to sclerotization), as well as the work of [Vincent \(1982\)](#); [Vincent and Wegst \(2004\)](#) and [Ker \(1977\)](#), in estimating Young’s modulus and Poisson’s ratio of the fiber proteins. This enables us to utilize more appropriate and realistic values that account for these factors, and provide results that closely match what is observed in insects⁷ [i.e., the exocuticle is stiffer than the mesocuticle in the normal direction ([Klocke and Schmitz, 2011](#))]. Further investigation is needed to more accurately determine these parameters for members of the genus *Curculio*.

4.2. Modeling Limitations

Our model suffers from two critical limitations. The first is the pragmatic constraint of approximating certain values based on the literature. Generally, these values can vary by nearly an order of magnitude without creating a correspondingly large variation in the model output. We are also able to mitigate the potential for significant error

⁷It is interesting to consider that increasing the fiber fraction of the exocuticle results in transverse (in-plane) stiffening at the expense of stiffness in the normal direction. We speculate that sclerotization might occur more strongly in the exocuticle to counter this effect, and thereby increase stiffness in the normal direction.

by comparing the results of the model to the known elastic properties of insect cuticle. The second limitation concerns the scope of the model, which is restricted to conditions of small strains and linear-elastic mechanical behavior. The viscoelastic and viscoplastic behavior of the cuticle during significant deformation has been explored in lobster cuticle, but has not been accurately described by any micromechanical model, mainly because of the numerous and difficult-to-measure factors that require assessment in order to accurately formulate such a model. For example, we would require a full characterization of the makeup of the protein matrix, degree of interconnection between chitin fibers, and presence of interply fibers (Nikolov et al., 2011; Sachs et al., 2008; van de Kamp et al., 2015). In spite of these limitations, our model yields useful insights into the interplay of structural and compositional factors in relation to the macroscale properties of each region of cuticle. Specifically, we provide a predictive framework within which we may systematically compare the optimization of the female *C. longinasus* cuticle in response to the mechanical stresses to that of different species in this genus, as well as in other phylogenetically related groups. Refining our approach to accommodate experimentally validated data for multiple species can produce novel insights into the evolutionary and developmental trade-offs of this system.

Future research should focus in particular on the degree of optimization that has occurred in related *Curculio* species in response to mechanical stress, and consider the evolutionary trajectory of this genus as a whole. The elastic constants generated by our approach can now be applied to full-scale three-dimensional models of the head of *C. longinasus*. In conjunction with data about the orientation angles of the endocuticular macrofibers in this species, these constants will permit us to perform finite element analysis to simulate mechanical loads encountered by females during oviposition. By examining the responses of different species and the localization of stress and strain on their heads during deformation, and aligning these variations phylogenetically, we hope to better mechanistically understand the process of structural adaptation and optimization that has occurred during the evolution of this group of weevils (Toju, 2008; Toju and Sota, 2006a,b).

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