

On the Architecture of Beetle Elytra

Zur Architektur von Käferelytren

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Summary: We examined the elytron cuticle of various beetle species histologically and by scanning electron microscopy (SEM). All cuticles examined appear to be of pseudorthogonal type, i.e., single orthogonal layers of microfibrils are delineated by intervening helicoidal microfibrils. In the endocuticle two “types” of “lamellae” (i.e. cuticular layers visible in histological sections) can be distinguished. One type is characterised by successive plies of unidirectional arranged microfibrils, in the other the plies are subdivided in paralleling rods consisting of thick bundles (macrofibres) of microfibrils that are unidirectional oriented along the bundle. Transitional stages between the two types are expected to occur. Successive plies and successive bundles cross at various angles. The “type” with the macrofibres corresponds to the “balken cuticle” of the classical German literature. Further, data from the elytra of 40 beetle species of 24 families point to the considerable variation regarding the total thickness of an elytron, the thickness of the dorsal and ventral cuticle of an elytron and the width of the hemolymph space within elytra.

Key words: insect cuticle, elytra, “balken”, pseudorthogonal cuticle

Zusammenfassung: Wir haben die Elytrencuticula verschiedener Käferarten histologisch und mit Hilfe des Rasterelektronenmikroskops (REM) analysiert. Wir gehen davon aus, dass alle untersuchten Cuticulae vom pseudorthogonalen Typ sind, d. h., dass die einzelnen orthogonalen Lagen aus Mikrofibrillen von helicoidal angeordneten Mikrofibrillen begrenzt werden. In der Endocuticula sind zwei „Typen“ von „Lamellen“ (einzelne Lagen der Cuticula im histologischen Schnitt) zu unterscheiden, die wahrscheinlich durch Übergänge miteinander verbunden sind. Ein „Typ“ ist durch Lagen mehr oder weniger flächiger Schichten einheitlich ausgerichteter Mikrofibrillen charakterisiert, im zweiten „Typ“ bestehen diese Lagen aus parallel liegenden, weitgehend getrennten, dicken Bündeln (Makrofibrillen) von Mikrofibrillen, die einheitlich längs des Bündels ausgerichtet sind. Aufeinander gestapelte Lagen und Bündel kreuzen sich unter verschiedenen Winkeln. Der „Typ“ mit den parallelen Makrofibrillen entspricht der „Balkencuticula“ der klassischen deutschsprachigen Literatur. Zudem belegen wir bei 40 Käferarten aus 24 Familien eine beträchtliche Variation in der Dicke der gesamten Elytre, auffallende Unterschiede in der Dicke der Cuticula der ventralen und dorsalen Cuticula sowie der Ausdehnung des Hämolymphraums zwischen beiden.

Schlüsselwörter: Insekten-cuticula, Elytren, Balken, pseudorthogonale Cuticula

1. Introduction

The insect cuticle is a multifunctional lightweight composite material, which has over the years attracted much attention in various fields (summarized e.g. by RICHARDS 1951; NEVILLE 1975, 1993, 1998; VINCENT & WEGST 2004). As seen throughout the arthropods, the adult insect cuticle is

multilayered, being divided into an outermost epicuticle preceded by the chitin containing procuticle, in which a dense exo- (= preecdysal procuticle) and a thicker endocuticle may be distinguished. This holds also for the elytra, fore wings in the Coleoptera that cover the membranous hind wings, when the latter are folded up at rest. Elytra develop from the imaginal discs and are principally evaginations

of the epidermis. Therefore they exhibit an outer (dorsal) and an inner (ventral) cuticle, which enclose the haemolymph space traversed by trabeculae, which connect upper and lower elytral surfaces. Elytra of beetles are “hard” and highly sklerotised (see textbooks of entomology).

Exo- and endocuticle often show unit layers termed “lamellae” when viewed under the light microscope, but more so when studied with the transmission electron microscope. In sections through the cuticle of insects, parabolic arcs (BOULIGAND 1965) or spiral patterns of microfibrils (e.g. MEYER-ROCHOW 1975) are frequently visible under both LM and TEM, but as explained in detail by BOULIGAND (1972) and others, these patterns are optical artifacts. The cuticle consists of laminae of chitin microfibrils that run parallel to the cuticle surface and are embedded in a protein matrix. Within each lamina the microfibrils are oriented in the same direction, but in successive laminae there is a slight rotation in orientation, and for every 180° a lamella is observed in sections normal to the surface.

In cuticles of other species, however, unidirectional oriented microfibrils form a preferred layer (“ply”), which represents strictly speaking a thick lamina. Most of them do not consist exclusively of unidirectional “plies”, i.e. they are not strictly orthogonal, but “plies” exhibit a thin intervening layer of helicoids. Therefore, these systems have been called pseud-orthogonal (see the two-system model for chitin-protein complexes in insect cuticles by NEVILLE & LUKE 1969; see also NEVILLE 1975, 1993, 1998):

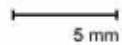
When checking the literature, we became aware of two “types” of pseudorthogonal cuticles. One “type” has successive layers (“plies”) made up of microfibrils with a unidirectional orientation (for the first time described in *Tenebrio molitor*: NEVILLE & LUKE 1969; NEVILLE 1975). The other “type” shows parallel bundles (macrofibres) of tightly packed microfibrils orientated unidirectional along the bundle, which are separated from each other by a small gap, or macrofibres may form a network (see below). Macrofibres change their direction in successive layers (e.g. *Pachnoda marginata*, HEPBURN 1972; HEPBURN & BALL 1973; DENNELL 1976; GREVEN & SCHWINGER 2005). The bundles of macrofibres correspond to the “balken” described in the classical German literature (summarized in RICHARDS 1951). MEYER (1842; fide BIEDERMANN 1903) was the first, who found that each “lamella” in the cuticle of *Lucanus cervus* was composed of parallel and partly anastomosing “clear rods” (“glas-helle Stäbchen”) and that successive lamellae crossed at various angles. In *Oryctes nasicornis* these rods, however, form a network (see also KAPZOV 1911). The term “balken” or “balkenlage” was introduced by KAPZOV (1911), who noticed that “balken” might fuse to form a more or less continuous “lamella” (see also KÜHNELT 1928).

























In later articles, authors did not clearly differentiate between continuous plies and “balken”. STEGEMANN (1930: p. 11) wrote that the “balkenlage” of the elytra in Cicindelae (sic!) consisted of clear plates with parallel fibrils and similarly SPRUNG (1932: p. 447) described for the elytra of Carabidae “Balkenlagen”(p. 447) consisting of fibrous chitin

Fig. 1: Some beetles, whose elytra were studied (shown to scale).

Abb. 1: Einige Käferarten, deren Elytren untersucht wurden (im richtigen Größenverhältnis zueinander).

A *Pterostichus niger* (Carabidae). **B** *Cetonia aurata* (Cetoniidae). **C** *Anoplotrupes stercorosus* (Scarabaeidae). **D** *Tenebrio molitor* (Tenebrionidae). **E** *Chrysolina fastuosa* (Chrysomelidae). **F** *Ceutorhynchus pallidactylus* (Curculionidae). **G** *Trigonopterus nasutus* (Curculionidae). **H** *Sitophilus granarius* (Curculionidae). **I** *Phyllobius betulinus* (Curculionidae).



	species	el	uns	ups	ex	lay	ty	hs
	<i>Acanthoscelides obtectus</i> (Chrysomelidae)	52.1 – 100.7	17.9 – 30.5	3.7 – 5.3	8.8 – 14.5	13	balken	huge
	<i>Adelocera murina</i> (Elateridae)	73.1 – 98.4	31.1 – 42.9	11.4 – 12.3	8.3 – 10.6	11	balken	huge
	<i>Anoplotrupes stercorosus</i> (Scarabaeidae)	91.2 – 96.3	52.0 – 53.7	5.0 – 10.1	6.3 – 8.3	19	plies	huge
	<i>Anthaxia fulgurans</i> (Buprestidae)	35.6 – 46.7	16.5 – 23.9	2.7 – 3.3	3.0 – 4.1	9	plies & balken	medium
	<i>Byrrhus</i> sp. (Byrrhidae)	45.5 – 78.5	27.5 – 42.2	8.1 – 9.5	3.7 – 4.8	20	balken	medium
	<i>Byturus ochraceus</i> (Byturidae)	33.3 – 37.8	15.0 – 19.7	2.4 – 2.8	2.4 – 3.1	15	plies	medium
	<i>Cetonia aurata</i> (Cetoniidae)	211.4 – 242.6	68.0 – 89.1	29.0 – 33.8	12.4 – 19.8 2.1 – 2.3*	11	balken	huge
	<i>Ceutorhynchus pallidactylus</i> (Curculionidae)	4.7** – 45.0	16.4 – 23.3***	3.4 – 4.2	1.8 – 4.2	12	balken	medium
	<i>Chrysolina fastuosa</i> (Chrysomelidae)	47.6 – 59.2	33.8 – 35.8	9.3 – 11.5	4.2 – 5.9	14	balken	medium
	<i>Coccinella septempunctata</i> (Coccinellidae)	32.4 – 38.4	22.5 – 31.9	1.8 – 2.2	2.4 – 2.7	10	balken	medium
	<i>Colydium elongatum</i> (Colydidae)	19.9 – 51.5	11.3 – 30.9	3.3 – 6.1	9.2 – 15.5	10	balken	medium
	<i>Cryptorhynchidius lapathi</i> (Curculionidae)	40.3 – 148.5	69.1 – 74.5	31.5 – 36.7	5.0 – 20.2	22	balken	medium
	<i>Curculio glandium</i> (Curculionidae)	8.6 – 58.4	24.6 – 29.6	8.3 – 10.4	2.9 – 14.1	18	balken	medium
	<i>Dermestes peruvianus</i> (Dermestidae)	36.9 – 49.8	18.9 – 24.2	8.1 – 14.6	6.0 – 8.6 1.3 – 2.0*	9	plies	medium
	<i>Donacia versicolorea</i> (Chrysomelidae)	31.7 – 45.7	15.6 – 21.7	4.2 – 5.7	5.1 – 6.0	9	balken	medium
	<i>Dromaeolus barnabita</i> (Eucnemidae)	26.7 – 39.3	13.0 – 18.5	4.0 – 6.0	7.3 – 8.5	8	balken	medium
	<i>Endomychus coccineus</i> (Endomychidae)	47.2 – 54.4	18.7 – 22.8	5.6 – 7.6	8.0 – 10.4	8	balken	huge
	<i>Glischrochilus hortensis</i> (Nitidulidae)	68.9 – 88.2	38.4 – 41.6	3.8 – 5.6	11.7 – 17.6	13	balken	medium
	<i>Hololepta plana</i> (Histeridae)	41.4 – 48.6	21.1 – 26.7	1.6 – 2.6	12.5 – 14.9	8	balken	huge
	<i>Julodis onopordi</i> (Buprestidae)	97.0 – 142.3	55.5 – 64.1	15.4 – 21.1	2.3 – 3.7	12	balken	medium
	<i>Lamia textor</i> (Cerambycidae)	135.5 – 164.5	38.1 – 66.7	6.7 – 8.8	7.4 – 8.8	15	balken	medium
	<i>Phyllobius betulinus</i> (Curculionidae)	6.7 – 38.0	12.7 – 18.9	4.2 – 5.9	1.0 – 3.4	13	balken	medium
	<i>Platyrhinus resinus</i> (Anthribidae)	77.8 – 162.2	42.4 – 74.7	12.5 – 34.7	4.2 – 6.9	20	balken	medium
	<i>Rhynchites auratus</i> (Attelabidae)	43.9 – 57.6	18.5 – 35.1	4.3 – 7.8	1.3 – 4.1	17	balken	medium
	<i>Salpingus ruficollis</i> (Salpingidae)	16.5 – 18.1	9.7 – 10.8	1.4 – 2.9	2.5 – 2.9	10	balken	medium
	<i>Spondylis buprestoides</i> (Cerambycidae)	112.6 – 199.4	46.7 – 67.7	18.0 – 23.9	7.8 – 9.6	12	balken	medium
	<i>Tenebrio molitor</i> (Tenebrionidae)	33.3 – 86.7	21.8 – 32.5	8.6 – 13.5	5.7 – 8.9	10	plies	medium
	<i>Thanasimus formicarius</i> (Cleridae)	26.0 – 59.0	9.6 – 19.7	7.5 – 5.7	2.6 – 6.3	12	balken	medium
	<i>Tritoma bipustulata</i> (Erotylidae)	54.5 – 65.8	26.4 – 33.6	7.8 – 9.7	6.1 – 7.8	13	balken	huge
	<i>Uleiota planata</i> (Silvanidae)	16.5 – 28.1	8.0 – 10.7	5.3 – 7.2	1.0 – 1.7	8	balken	small
	<i>Adexius scrobipennis</i> (Curculionidae)	49.9 – 119.2	63.7 – 89.1	16.9 – 19.3	1.3 – 2.6	32	balken	small
	<i>Gymnopholus ovatus</i> (Curculionidae)	201.1 – 250.0	91.7 – 97.8	48.9 – 57.2	1.4 – 2.8	26	balken	small
	<i>Leiosoma deflexum</i> (Curculionidae)	34.5 – 59.1	43.4 – 47.0	2.6 – 4.1	1.6 – 2.8	23	balken	small
	<i>Otiorynchus ovatus</i> (Curculionidae)	55.9 – 93.0	40.3 – 49.9	8.5 – 11.9	3.6 – 4.1	19	plies & balken	medium
	<i>Pterostichus niger</i> (Carabidae)	28.0 – 88.1	26.2 – 36.7	9.1 – 15.1	2.8 – 3.4	26	plies	medium
	<i>Rutera hypocrita</i> (Curculionidae)	50.9 – 143.2	48.6 – 108.3	7.2 – 14.0	3.6 – 14.6	23	balken	small
	<i>Sitophilus granarius</i> (Curculionidae)	31.4 – 56.9	36.9 – 40.3	4.6 – 5.7	5.7 – 9.6	13	balken	small
	<i>Timarcha metallica</i> (Chrysomelidae)	57.9 – 91.2	42.8 – 49.6	4.6 – 7.8	3.3 – 4.3	19	balken	medium
	<i>Trachodes hispidus</i> (Curculionidae)	29.9 – 44.2	22.9 – 28.1	7.6 – 10.6	1.8 – 5.0	15	balken	small
	<i>Trigonopterus nasutus</i> (Curculionidae)	56.3 – 91.7	46.8 – 60.8	5.0 – 16.6	6.0 – 8.6	25	balken	small

Tab. 1: Thicknesses of the elytra and widths of cuticular layers, occurrence of plies and “balken”, size of the hemolymph space and capability of flight (indicated by symbols) of the beetle species examined. All measurements in μm . *Exocuticle of the ventral cuticle; **the small values refer to the elytral grooves, ***measurements above the haemolymph space. el = overall thickness of

layers (“fibrilläre Chitinschichten”; p. 447). Later, DENNELL (1976; p. 162) distinguished clearly between “layers of bundles” (= balken; see also DENNELL 1978) and “layers of horizontally disposed fibres”. In the more recent literature, especially studies focusing on the mechanical properties, only pseudorthogonal cuticles without further differentiation are mentioned and occasionally the cuticle structure is insufficiently described (e.g. CHEN & FAN 2004; YANG et al. 2010 and references therein).

Apart from the histological studies mentioned above and a histological study comparing the thickness of the various lamellae and the proportions of exo - and endocuticle in the elytra of 36 beetle species (KRZELJ 1969), detailed comparative studies of beetle elytra using transmission and scanning electron microscopy, are largely missing.

From a wider study concerning the possible phylogenetic significance of the architecture of the elytron cuticle (in preparation) we selected the elytra of 14 beetle species to illustrate the classical “balken cuticle” and other pseudorthogonal cuticles employing histological sections and scanning electron microscopy (SEM). Further, we give some measurements of the elytra from altogether 40 beetle species of 24 families and to call attention to some topics insufficiently studied or not at all studied.

2. Material and methods

Elytra of the 40 beetle species examined herein (some of them are shown in Fig. 1; see Table 1)

were from the collection of the State Museum of Natural History in Karlsruhe (SMNK). Most of them were stored in ethanol.

For light microscopy small pieces of the elytra were dehydrated and embedded in epoxy resin (SPURR 1969). Semithin sections, approx. 0.5 µm thick, were made with a Reichert OM U3 microtome using glass knives and stained with toluidin- blue-borax. Photos were edited with Adobe Photoshop CS3. Measurements (see Table 1) were taken from sections of a single specimen of each species.

For scanning electron microscopy (SEM) dried elytra were broken vertically with two fine forceps. The pieces were glued to stubs, sputtered with gold and examined under a Zeiss LEO 1430VP scanning electron microscope. In some preparations the angle between two successive plies or “balken” could be estimated by viewing the samples directly from above.

Abbreviations used in the figures 2 and 3: en = endocuticle, ep epicuticle, ex = exocuticle, hs = hemolymph space, tr = trabecula.

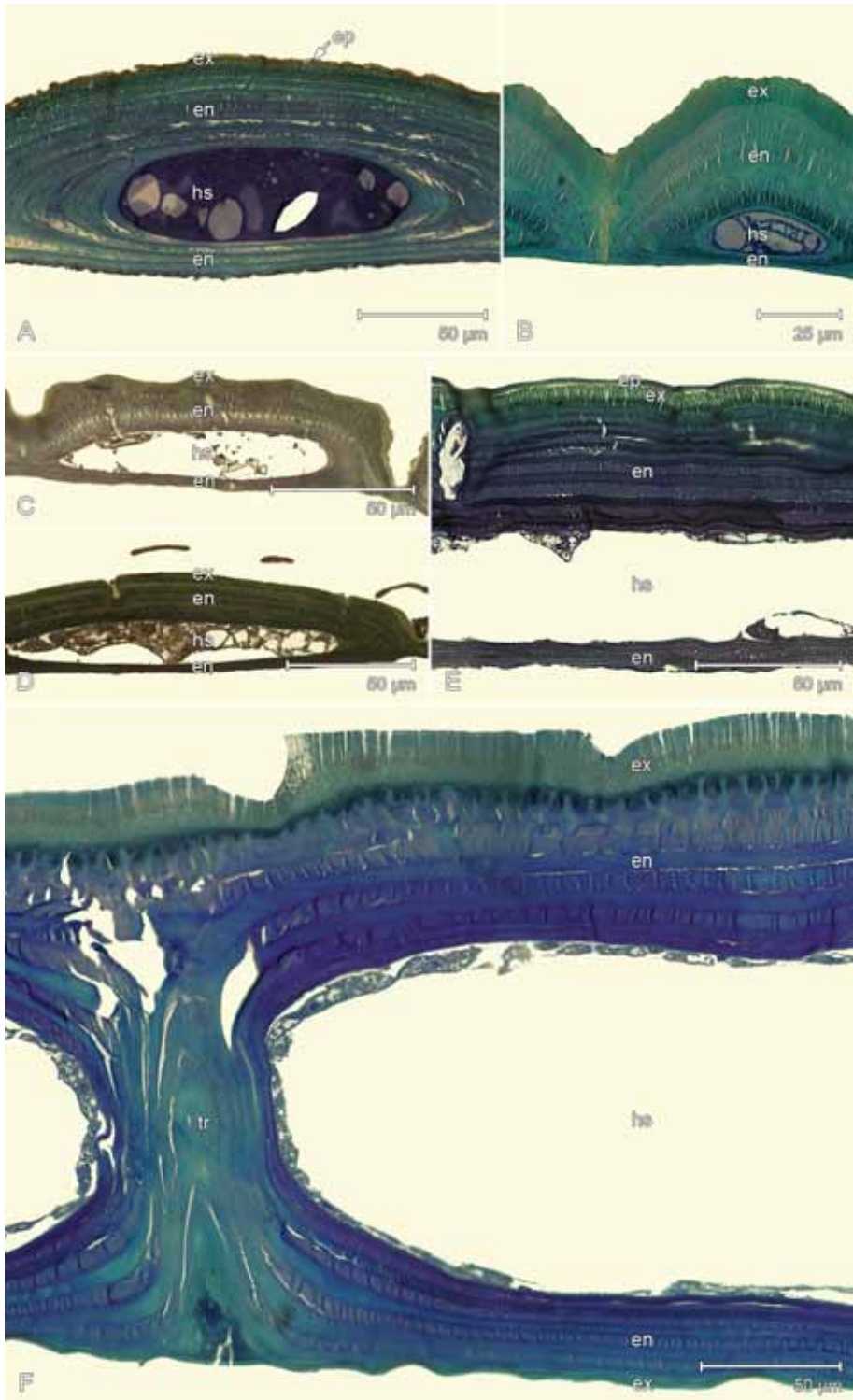
3. Results

Dorsal (upper) and ventral (lower) parts of an elytron are separated by a haemolymph space created by cuticular columns, the trabeculae. Figure 3 shows sections of the elytra of some of the beetles examined. Table 1 gives some measurements of the dimensions of the elytron cuticles and some of their constituents in 40 beetle species.

The thickness of the total elytron varies considerably across species, but also in

elytron; uns = thickness of under side; ups = thickness of upper side; ex = exocuticle thickness; lay = unidirectional endocuticle layers counted (upper side); ty = endocuticle type; hs = size of hemolymph space.

Tab. 1: Dicke der gesamten Elytre und einzelner Cuticulaschichten, Vorhandensein von Lagen und Balken, Größe des Hämolympfraumes und Flugfähigkeit (durch Symbole gekennzeichnet) der untersuchten Käferarten. Alle Angaben in µm. *Exocuticula der Unterseite, **die kleinen Werte beziehen sich auf die Rillen in den Elytren; ***Messung über dem Hämolympfraum. el = Gesamtdicke der Elytren; uns = Dicke der Unterseite; ups = Dicke der Oberseite; ex = Dicke der Exocuticula; lay = Unidirektionale Lagen in der Endocuticula (Oberseite); ty = Typ der Endocuticula; hs = Größe des Hämolympfraums.



different regions of an elytron. Further, the ventral cuticle is always thinner than the dorsal one. Also the extension of the haemolymph space is extremely variable (Fig. 2). This space is very small in the compact elytron of the weevil *Sitophilus granarius* (Fig. 2 A), somewhat wider in the elytron of other weevils such as *Ceutorhynchus pallidactylus* (Fig. 2 B) and *Phyllobius betulinus* (Fig. 2 C), medium-sized in the carabid *Pterostichus niger* (Fig. 2 D), and considerably larger in the scarabaeid *Anoplotrupes stercorosus* (Fig. 2 E) and the cetoniid *Cetonia aurata* (Fig. 2 F).

In *S. granarius* (Fig. 2 A), *C. pallidactylus* (Fig. 2 B), *P. niger* (Fig. 2 D) and *A. stercorosus* (Fig. 2 E), semithin sections allow distinguishing the thin brown-stained epicuticle from the largely lamellate procuticle in both, the ventral and dorsal part of an elytron. The procuticle could be clearly divided in exo- and endocuticle by means of the narrower “lamellae” in the former. In some cases – *A. stercorosus* (Fig. 2 E) and *C. aurata* (Fig. 2 F) –, the exocuticle does not show typical “lamellae”. The course of “lamellae” is more or less horizontal; in the trabeculae “lamellae”

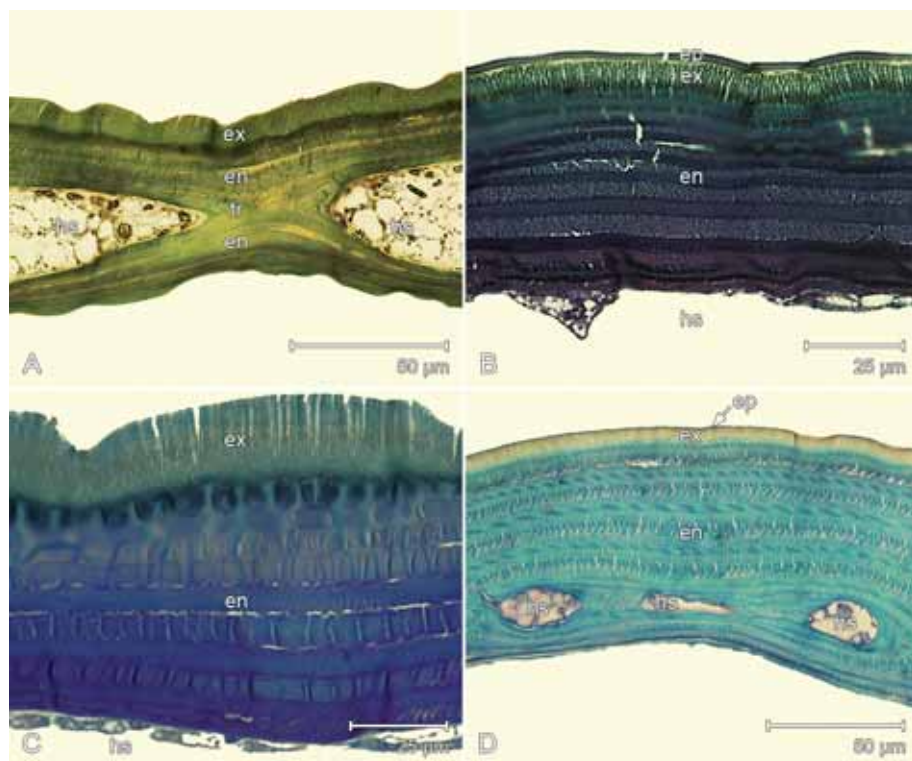


Fig. 3: Pseudorthogonal cuticles of elytra with “plies” (A, B) and “balken” (C, D). Semithin sections.

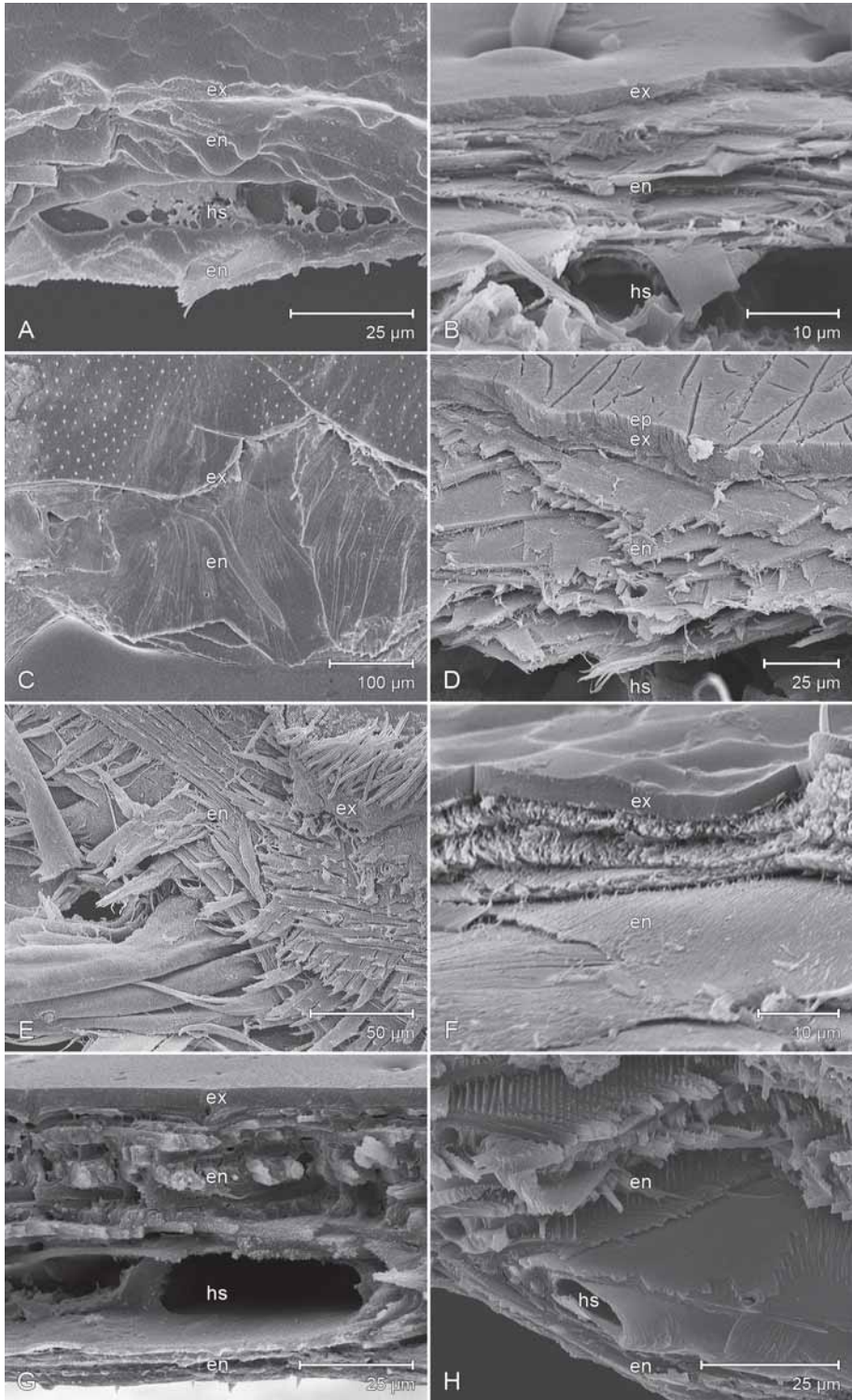
Abb. 3: Pseudorthogonale Cuticulae der Elytren mit Platten (A, B) und Balken (C, D). Semidünnschnitte.

A *Tenebrio molitor*. **B** *Anoplotrupes stercorosus*. **C** *Cetonia aurata*. **D** *Trigonopterus nasutus*.

Fig. 2: Various sized hemolymph spaces of the elytra. Semithin sections.

Abb. 2: Unterschiedlich große Hämolympfräume der Elytren. Semidünnschnitte.

A *Sitophilus granarius*. **B** *Ceutorhynchus pallidactylus* (Curculionidae). **C** *Phyllobius betulinus*. **D** *Pterostichus niger*. **E** *Anoplotrupes stercorosus*. **F** *Cetonia aurata*.



are arranged vertically (Fig. 2 F). “Lamellae” are variously thick in a given elytron (Tab. 1). At least in the endocuticle two “types” of cuticle can be distinguished already by LM (Fig. 3). In “type” 1 the lamellae consist of more or less continuous “plies” as in *P. niger* (Fig. 2 D), *T. molitor* (Fig. 3 A) and *A. stercorosus* (Fig. 3 B). In “type” 2 the “plies” seem to be broken down in single parallel strands (= “balken”)

or rods of different sizes. These rods clearly change their direction in successive layers either abruptly at an angle of 90°, which leads to alternating cross- and longitudinal sectioned “balken” in successive layers such as in *C. aurata* (Fig. 3 C), or at smaller angles, which results in variously obliquely sectioned “balken” limited by longitudinal sectioned “balken” after a rotation of the layers of 180°

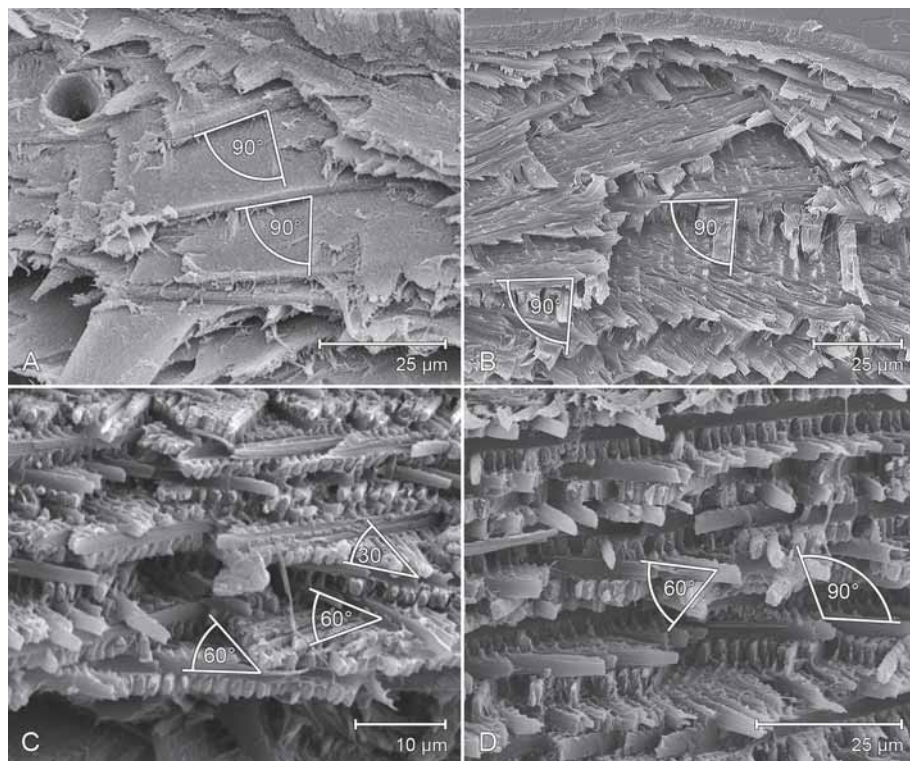


Fig. 5: Angles between the successive “plies” (A) and “balken” (B-D) in the elytron cuticle. Angles do not correspond exactly to the angles really determined (see “Material and methods”). SEM images.

Abb. 5: Winkel zwischen den übereinanderliegenden Platten (A) und Balken (B-D) in der Elytren-cuticula. Die Winkel entsprechen nicht den tatsächlich gemessenen (s. „Material und Methoden“). REM-Aufnahmen.

A *Anoplotrupes stercorosus*. **B** *Timarcha metallica* (Chrysomelidae). **C** *Trigonopterus nasutus*. **D** *Gymnopholus subnaereus* (Curculionidae).

Fig. 4: Pseudorthogonal cuticles of elytra with “plies” (A-D) and “balken” (E-H). SEM images. **Abb. 4:** Pseudorthogonale Cuticulae der Elytren mit Platten (A-D) und Balken (E-H). REM-Aufnahmen.

A *Tenebrio molitor*. **B** *Byturus ochraceus* (Byturidae). **C** *Pterostichus niger*. **D** *Anoplotrupes stercorosus*. **E** *Cetonia aurata*. **F** *Anthaxia fulgurans* (Buprestidae). **G** *Chrysolina fastuosa*. **H** *Otiorhynchus ovatus* (Curculionidae).

as in the weevil *Trigonopterus nasutus* (Fig. 3 D; see also Fig. 2 A, B).

Fractures of the elytra examined by SEM confirm and broaden the histological analysis. The outermost layer appears as a dense solid material, which bears the surface micro-sculpture of the elytron (e.g. Fig. 4 C). Although this layer is likely to be the epicuticle, it cannot clearly be differentiated from the underlying exocuticle (Fig. 4 B, G). Only in *A.*

stercorosus the layer shows a fine vertical striation (Fig. 4 D), which is seen also in the histological section (Fig. 3 B). The bulk of procuticles is organized either in “plies” (Fig. 4 A-D, 5 A) or in more or less compact strands or balken (Fig. 4 E-H, 5 B-D). In some cases also “plies” and “balken” occur in a given endocuticle such as in the buprestid *Anthaxia fulgurans* (Fig. 4 F) and in the weevil *Otiorhynchus ovatus* (Fig. 4 H).

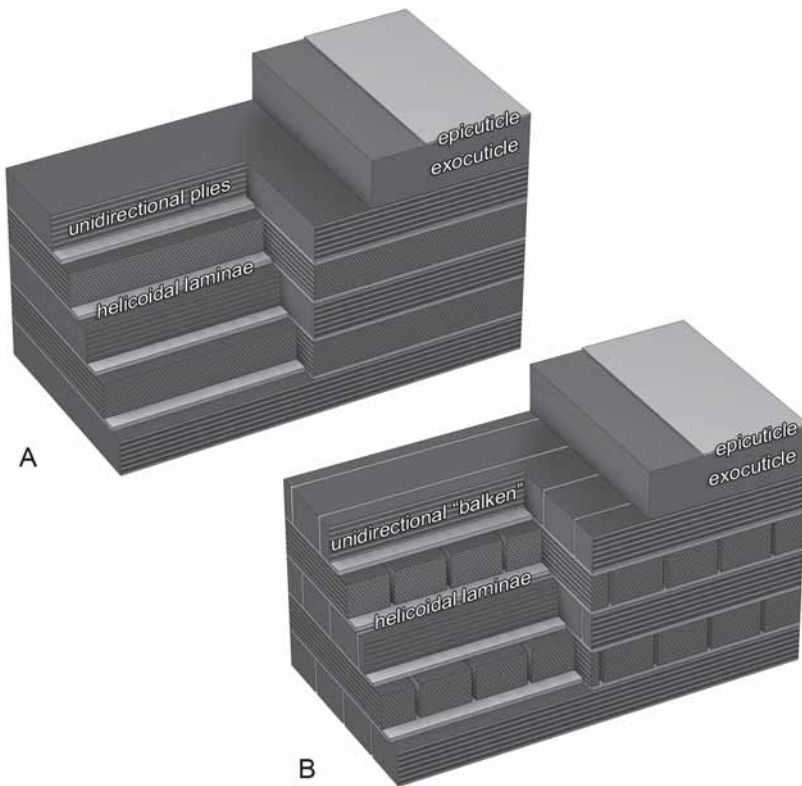


Fig. 6: Diagram of the variation of pseudorthogonal endocuticles of beetle elytra. “Plies” and “balken” cross at 90°. **A** Pseudorthogonal cuticle with continuous “plies”. **B** Pseudorthogonal cuticles with single “balken”. The organisation of the exocuticle (ex) is not pseudorthogonal and variable. Presence of helicoids between “plies” and “balken” was not documented in the herein examined elytra. For further explanations see text.

Abb. 6: Schema von zwei unterschiedlichen Ausprägungen der pseudorthogonalen Endocuticula von Käferelytren. Die Lagen und die Balken sind jeweils um 90° versetzt. **A** Pseudorthogonal mit mehr oder weniger flächigen Lagen. **B** Pseudorthogonale Cuticula mit einzelnen Balken. Die Organisation der Exocuticula (ex) ist nicht pseudorthogonal und variabel. Die helicoidalen Laminae zwischen den Platten und Balken sind bei den hier untersuchten Elytren noch nicht nachgewiesen. Weitere Erklärungen s. Text.

Angles at which the plies or “balken” cross are difficult to determine in the fractures. In a few cases a reliable estimation was possible. In *C. aurata* (Fig. 4 E) and *Timarcha metallica* (Fig. 5 B) „balken” cross at angles of approx. 90°. The same holds for the plies of *A. stercorosus* (Fig. 5 A), whereas “balken” in *Trigonopterus nasutus* (Fig. 5 C) cross at angles of 30 to 60° and in *Gymnopholus ovatus* (Fig. 5 D) of 60 to 90° (for further data see Table 1).

4. Discussion

Elytra of beetles are lightweight, multifunctional structures. They behave mechanically as a composite material (as insect cuticle in general) with a good resistance to bending and compression, serve among others to protect the hind wings and the body and produce significant aerodynamic forces in those beetles, which fly with their elytra extended laterally (e.g., NACHTIGALL 1964; SCHNEIDER & HERMES 1976; NEVILLE 1993; DE SOUZA & ALEXANDER 1997).

Certainly, saving of material contributes to the light-weight construction of elytra. This may be achieved by reducing their overall thickness, the thickness of the ventral cuticle and/or the enlargement of the haemolymph space. Figures 2 and 3 show sections through the elytra of three species, which are flightless and exhibit relatively small haemolymph spaces (*Pterostichus niger*, *Trigonopterus nasutus*, *Sitophilus granarius*) and five species, which are able to fly having relatively large haemolymph spaces (*Cetonia aurata*, *Anoplotrupes stercorosus*, *Tenebrio molitor*, *Ceutorhynchus pallidactylus*, *Phyllobius betulinus*). These very rough estimations and the additional measurements given in table 1 are highly suggestive. Surprisingly, we did not find reliable data in the literature to substantiate our assumptions. KRZELJ (1969) gave numerous measurements of the thicknesses of the various layers of the elytron cuticle of beetles, but focused the discussion on the proportion of exo- and endocuticle in the elytra. We think comparative

studies would be promising, which, however, should consider a variety of other parameters like for instance, the spacing and dimensions of the trabeculae crossing the haemolymph space, size and weight of the beetle, size of the flight muscles etc.

Elytra of Coleoptera are denoted as heavily sclerotized. Generally it is the exocuticle that has a dense chitin-protein structure and becomes hard and stiff due to sclerotization (e.g. NEVILLE 1975, 1993). KRZELJ (1969) observed in the elytra of 36 beetle species a layer dyed red after Mallory-Heidenhain, which he denominated exocuticle and which accounted for 65-85% of the cuticle thickness. The “typical” fully sclerotized cuticle after Mallory staining is amber, brown or black in the outer portions (exocuticle), red in the central layers (mesocuticle = exocuticle in the “soft” state) and blue in the inner portions (endocuticle) (for staining and terminology see RICHARDS 1951; NEVILLE 1975). However, in the few studies on beetle elytra using TEM and SEM, authors denominate the bulk of elytron cuticles as endo- or mesocuticle (e.g. HEPBURN 1972; LEOPOLD et al. 1992). Exocuticles may be exclusively helicoidal throughout their thickness and “lamellae” are generally narrower than in the endocuticle, but other configurations may also occur, i.e. division in a superficial part where fibres are oriented nearly perpendicular to the surface and a deeper part with fibres parallel to the surface (NEVILLE 1967, 1998; BARBAKADZE et al. 2006). The LM and SEM-techniques applied herein did not allow a clear classification of this layer.

The bulk of the cuticle of the beetle elytra examined appears to be organized in the pseudorthogonal fashion which has been described for the cuticle of other Coleoptera (e.g., HEPBURN 1972; ZELAZNY & NEVILLE 1972; NEVILLE 1975, 1993). The thin postulated intervening layers of helicoidal lamellae between the layers could not be adequately resolved by the techniques used herein (light microscopy, scanning electron

microscopy). However, to our knowledge these helicoids have not been adequately documented in any beetle elytron. HEPBURN (1972) and HEPBURN & BALL (1973) have shown by LM, TEM and SEM that in *Pachnoda sinuata* the parallel macrofibres of one horizontal layer are connected by “intraply” cross linking fibres and those of successive layers by “interply” fibres. On the TEM pictures of the “balken cuticle” of the weevil *Anthonomus grandis* shown by LEOPOLD et al. (1992) no helicoids between the often tightly adjacent successive “balkenlagen” can be recognized.

Our survey confirms the existence of two “types” of pseudorthogonal cuticles (summarized in Figure 6). One “type” has “lamellae” (as identified in histological sections) formed by more or less continuous plies; in the second “type” lamellae are formed by parallel macrofibres.

We think that there is some evidence that both may be the endpoints of a ply-balken continuum”. Contrary to DENNELL (1976), we found in *Geotrupes stercocorus* (now *Anoplotrupes stercorosus*) plies instead of “balken”. Further, occasionally the innermost “lamellae” of endocuticles are not broken down in “balken”, but form a more or less continuous ply as shown in *Anthaxia fulgurans* and *Otiorhynchus ovatus*. Moreover, adjacent “balkens” may anastomose and may be organized in networks of macrofibrils (see citations above) and often the differentiation between “balken” and plies seems rather subjective as a “balken” can reach a considerable width (not shown). However, as studied by TEM and SEM the macrofibres of the “balken cuticle” in the weevil *Anthonomus grandis* develop as discrete units and not as plies. Macrofibres are secreted after eclosion as a lattice-like endocuticle in the form of layers of parallel rod- or beam-shaped branching macrofibres, which are joined to adjacent macrofibres above and below. The spaces between the macrofibres are assumed to be filled with a probably proteinaceous

“fibrous matrix” that was extractable with KOH (LEOPOLD et al 1992).

Angles, at which plies or “balken” cross, may vary. Very common are angles of 90°, but smaller ones have also been reported (BIEDERMANN 1903; KÜHNELT 1928; STEGEMANN 1929; ZELAZNY & NEVILLE 1972; HEPBURN 1972, HEPBURN & BALL 1973; DENNELL 1976; 1978; GREVEN & SCHWINGER 2005); angles may even depend on the cuticular region. For example, “balken” in the elytral cuticle of *Cybister* sp. cross at 90° throughout the bulk of the cuticle, at approximately 60° in the innermost region, but do not cross in deeper layers of the femur cuticle (DENNELL 1978). A computer-aided determination showed that each successive layer of macrofibres in the sclerites of the weevil *Anthonomus grandis* is rotated with respect to the overlying layer by an angle of about 72° (LÉOPOLD et al. 1992). Fibre patterning is discussed as being controlled by the epidermal cells and/or induced mechanically or is disposed by self assembly (e.g., RICHARDS 1951; HEPBURN 1972; ZELAZNY & NEVILLE 1972; 1975; DENNELL 1978; LÉOPOLD et al. 1992). The possible mechanical differences between “balken” versus “plies”, however, has, to our knowledge, not been considered as yet.

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