

Abrasive Wear in The Leg Joints of Insects

Konstantin Nadein* and Stanislav N. Gorb

The presence of wear of the joint cuticle of the beetle legs is demonstrated experimentally and is represented by abrasive wear. Presence of the abrasive wear in the natural-habiting beetles of various families is also established. Abrasive wear comprises of both two-body and three-body wear as a result of the action of particles that have entered the joint. The presence of the sliding wear in the absence of abrasive particles is not found. The wear effects are represented by microcuttings, indentations, cracking, delamination, brittle fracturing, and destruction of the surface layer (epicuticle). The correlation of quantitative indicators of wear between the types of experimental substrates (paper, wood, sand) and the length of exposure of beetles on a particular substrate turned out to be very weak. It is hypothesized that the lubricating material in the leg joints of beetles may provide an anti-wear function by binding particles and facilitating their removal thereby reducing surface damage by sharp asperities, and protecting the damaged surface to prevent further wear.

1. Introduction

The wear resistance studies on biological and bio-inspired composite materials, which include the cuticle of insects, is of great applied interest, especially in the fields of biotribology and biomimetics, and is being actively developing.^[1,2] The cuticle of insects, a fibrous composite material, is potentially subject to wear being the outermost material of the body that is in constant contact with environmental objects.^[3–6] Wear and tear of the cuticle can lead to the loss of the body integrity to some limitations of vital activity.^[7,8] At the same time, the restoration and regeneration of the cuticle in insects is limited.^[9,10]

Wear effects of insect cuticle have been previously demonstrated by observations for various body parts of insects, for example in mouthparts of water inhabiting insects, such as Ephemeroptera, Plecoptera, Coleoptera, Diptera, grazing

epilithic algae,^[11] mandibles of ants,^[8] and beetles.^[12,13] Experimental data on the wear effects of insect cuticle are still very scarce and so far, limited only by studies of mouthparts abrasion in some Ephemeroptera and Orthoptera.^[11,14–16] It is represented primarily by the abrasive wear due to friction between surfaces of the contact pair (two-body wear) or between those surfaces and particles between them (three-body wear).

Wear affects the material by becoming deformed or being torn away as a consequence of friction of the contacting surfaces.^[17–19] Friction is inevitably present as a result of the interaction of contacting surfaces, which in the body of insects are represented by joints connecting segments. This is especially true for the joints of insect legs with their intense cyclic loading. In contrast to

the vertebrates, the insect leg joints are open: they are not enclosed into an isolated chamber and exposed to the environment.^[20] Structurally, insect leg joints resemble mechanical micro-joints and micro-hinges^[21,22] and presumably have similar wear challenges. These include primarily frictional wear and abrasive wear due to the penetration of contaminant particles into the open joint.

Both the wear process and wear control in insect leg joints remain largely unexplored. Studies of leg joints in some Orthoptera and Coleoptera have suggested that frictional wear is reduced there by a very low coefficient of friction and a combination of the surface texture of the contacting surfaces and their stiffness.^[23–27] However, these studies have not aimed to experimentally establish the presence or absence of frictional or/and abrasive wear. Thus, so far it is unknown, whether these types of wear really occur in leg joints of insects. Hence, it seems reasonable that the studies on the wear resistance should be preceded by a study of the wear effects in the leg joints. Therefore, in the present study, we are aiming at answering the following questions: (i) which type of wear (frictional or/and abrasive wear) is present in the insect leg joints, (ii) which underlying mechanisms of wear and which wear effects are occurred in the joints, (iii) under which conditions wear can occur in the joints.

2. Experimental Section

2.1. Study Specimens and Preparation

Darkling beetle *Zophobas morio* (Coleoptera: Tenebrionidae) and Congo rose chafer *Pachnoda marginata* (Coleoptera: Scarabaeidae) were purchased at the larval stage from commercial

K. Nadein, S. N. Gorb
Functional Morphology and Biomechanics
Zoological Institute
Christian-Albrechts University of Kiel
Am Botanischen Garten 1–9, 24118 Kiel, Germany
E-mail: knadein@zoologie.uni-kiel.de

The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/admi.202300743>

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suppliers (www.reptilienkosmos.de, Germany) and then kept and reared in the colony at the Laboratory of Functional Morphology and Biomechanics at the Kiel University. Ground beetle *Abax parallelepipedus* (Piller & Mitterpacher, 1783) (Coleoptera: Carabidae) (2 specimens collected at 20.09.2019), carrion beetle *Oiceoptoma thoracicum* (Linnaeus, 1758) (Coleoptera: Silphidae) (3 specimens collected at 15.08.2020), dung beetle *Geotrupes stercorarius* (Linnaeus, 1758) (Coleoptera: Scarabaeidae) (5 specimens collected at 10.09.2019), longhorn beetle *Leptura maculata* (Poda, 1761) (Coleoptera: Cerambycidae) (2 specimens collected at 02.10.2019) were collected in Kiel, Germany.

The following numbers of *Zophobas morio* individuals were examined: immobilized beetles: 22; beetles kept with no substrate: 5; paper substrate: age of 30 and 60 days, 8 each; wood substrate: age of 50, 70, and 110 days, 12 each; sand substrate: 10 and 40 days, 10 each. Fore, middle and hind legs from the right side of the body from ethanol-preserved individuals were cut off and dissected with a razor blade into two halves through the femoro-tibial joint in a sagittal plane. In total, six samples of the tibia and six samples of the femur for each individual were attached to the aluminium stub for the further scanning electron microscopy (SEM) observations. In total 1212 halves of femur and tibia were examined under the SEM, for which 1618 images were acquired and analyzed.

The cuticle surface of the leg joints in representatives of four families of beetles (see above) from natural habitats with different lifestyles was studied. All the animals were collected at the end of summer or in autumn, to represent the result of interactions of joints with environment during the life of an individual. Additionally, leg joints of the burrowing Congo Rose chafer *Pachnoda marginata*, kept in laboratory culture for at least six months with a conventional soil substrate, were also examined.

2.2. Scanning Electron Microscopy (SEM)

The aluminium stubs with glued legs were air dried at room temperature for at least 24 h and then coated (thickness 10 nm) with gold-palladium using a Leica EM SCD500 sputter coater (Leica Microsystems GmbH, Wetzlar, Germany). The observations were made at an accelerating voltage of 3 kV using a Hitachi S4800 (Hitachi High-Technologies Corp., Japan) scanning electron microscope.

2.3. X-Ray Micro-Computed Tomography (micro-CT)

For the anatomical studies, micro-CT scanning of the dry fore leg was carried out using a SkyScan 1172 (Bruker Corp., Billerica, U.S.A.) at 40 kV and 250 μ A, with camera pixel size of 8.8 μ m, image pixel size 2.0 μ m. 1573 projections were recorded over the 180° rotation. For 3D reconstruction, the graphic segmentation tool software Amira 6.2 (FEI Company, Visage Imaging, Germany) has been used. For the experiments with metallic particles, the scanning has been done in a transfusion mode without the specimen rotation.

2.4. Experiments

It was hypothesized that both sliding and abrasive types of wear affect the cuticle surface in leg joints of beetles having active movements and a contact with a substrate which can be a source of contaminant abrasive particles, whereas wear was presumably absent in joints of beetles just emerged from the pupa (before active movements). These immobilized beetles were acquired by placing the pupas into small glass tubes (with diameter corresponding to the width of a pupa) until the beetles were hatched, matured, and remained in the tubes without movement of legs. Then the tubes were filled out with ethanol and samples of their femoro-tibial joints were used as a control.

To detect the presence or absence of a sliding wear (i.e., two-body wear in the absence of contaminant particles), the beetles were kept in an empty plastic box, where only food (jelly) was provided. To detect the possible presence of the abrasive wear in the presence of contaminant abrasive particles, the specimens of *Zophobas morio* were kept in plastic boxes (40 cm \times 30 cm \times 20 cm) with three types of substrates of different hardness and thus serving as a source of different contaminating abrasive particles: (i) shredded and crumpled paper; (ii) shavings and shredded pieces of wood; (iii) fine aquarium sea sand (particles size from 1 μ m to \approx 0.5 mm). To estimate the wear rate, the specimens were fixated in ethanol at the determined time of exposure (see above).

2.5. Experiments with Contamination by Metallic Particles

Fresh samples of the fore legs of *Zophobas morio* were tested in two separate experiments: contamination by metallic particles through the dorsal gap and that of through the ventral gap. Metallic particles (iron, Fe), whose hardness significantly exceeds the hardness of the cuticle in the joint, were chosen to visualize wear patterns, simulating the entry of contaminant particles into the joint cavity and making visible wear traces on the cuticular surface. The particles of the size 2–5 μ m were placed on the dorsal surface of the tibial base or ventral surface of the tibial base and apex of the femur. The tibia was set in motion by hand, allowing extension and flexion of 1500 times to the maximum possible opening angle (named here cycles). Every 50 times, a portion of particles was applied on the corresponding surface. The penetration of particles into the joint cavity was recorded using micro-CT after 50, 100, 200, 500, 700, 1000, and 1500 movements of tibia. Metallic, electron-dense particles were clearly visible in X-rays and contrast sharply against the background of a less dense cuticle. Each sample of the femoro-tibial joint after completion of the full number of movements was dissected in sagittal plane and examined in SEM as described above.

2.6. Data Acquisition, Treatment, and Statistical Analysis

The dissected femoro-tibial joints were examined in SEM. The following areas were analyzed with corresponding image acquisition, in order to collect the data on the wear effects: for a femoral counterpart – femoral condyle and femoral

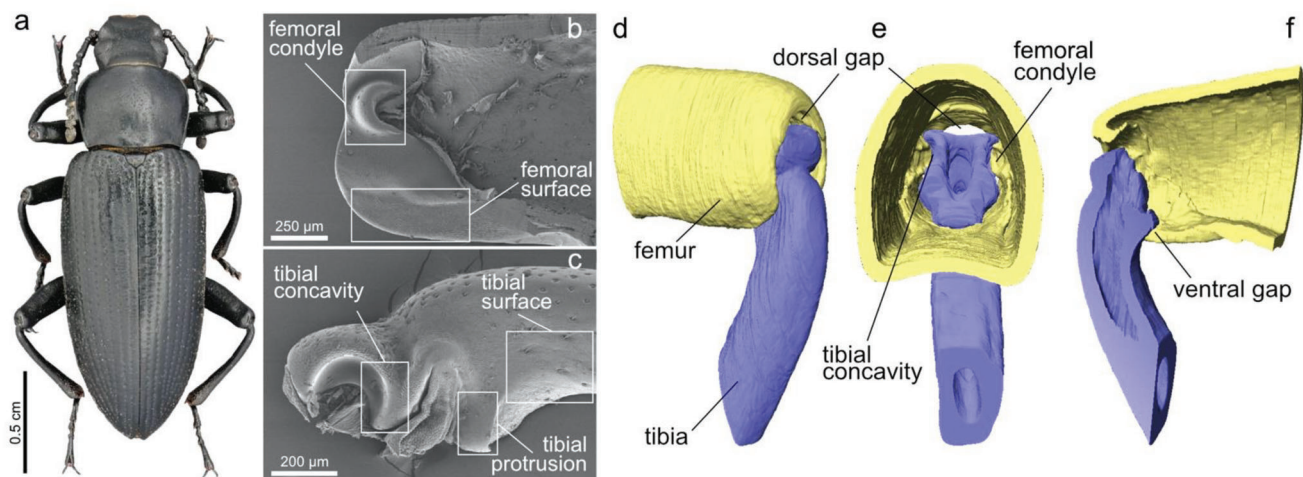


Figure 1. *Zophobas morio* and structure of the femoro-tibial joint. a) Darkling beetle *Zophobas morio*, general appearance; b,c) SEM micrographs; b, femoral counterpart, sagittal section; c) tibial counterpart, lateral view; d–f) micro-CT volume reconstruction: d) femoro-tibial joint, external antero-lateral view; e) the same, view through femur; f) the same, sagittal section. White rectangles indicate the areas analyzed in the course of experiments.

surface (Figure 1b); for a tibial counterpart – tibial concavity, tibial protrusion, tibial surface (Figure 1c). Additionally, the cuticle surface around the coxal opening, coxal protrusion and trochanter were examined to detect the wear effects. Comparison between the fore, middle and hind legs was not performed.

SEM micrographs were analyzed in ImageJ software (ver. 1.53s, Rasband, W.S., U.S. National Institutes of Health, Bethesda, Maryland, USA). The following wear effects were counted and measured: for a tibial concavity and a femoral condyle – (a) quantity of scratches larger than 10 μm and (b) a length of scratches; for a tibial protrusion, tibial surface and femoral surface – area (in μm^2) of the worn surface.

The obtained values were summarized (number of scratches, their length and area of the worn surface) for each individual and for each type of the substrate (tibial concavity, tibial protrusion, tibial surface, femoral condyle, femoral surface). The arithmetical mean was calculated based on the values from each individual and from each type of surface and represented as an average for an individual insect for each type of the substrate (Table 1).

For the statistical treatment of data, emerging of the wear signs (scratches or worn surface) was considered random and with equally probability for each leg of an individual animal and for each individual animal. One-way ANOVA was performed using software SigmaPlot 12.5 (Systat Software Inc. San Jose, U.S.A.) for the results of experiments.

Table 1. Wear effects characteristics in the femoro-tibial joint of *Zophobas morio* beetles kept on various substrates. the values are given as an average for an individual. maximal values are those recorded for an individual taken from the entire recorded data. TC: tibial condyle, number (N) and length of scratches; TP: tibial protrusion, destructed area; TS: tibial surface, destructed area; FC: femoral condyle, number (N) and length of scratches; FS: femoral surface, destructed area.

	TC		TP		TS		FC		FS	
	N, average [max]	Length average, μm [range]	Area, average, mm^2	max	Area, average, mm^2	max	N, average [max]	Length average, μm [range]	Area, average, mm^2	max
Paper substrate										
30 days	9.2 (22)	32.7 (11.8–60.6)	0.0034	0.0084	0.025	0.054	18.8 (53)	47.0 (10.3–172.0)	0.028	0.060
60 days	11.1 (24)	50.5 (22.0–128.8)	0.0026	0.0043	0.037	0.090	24.5 (44)	55.1 (25.0–160.9)	0.027	0.060
Wood substrate										
50 days	3.0 (5)	28.3 (14.5–59.4)	0.0026	0.005	0.023	0.047	7.7 (14)	43.6 (15.0–108.3)	0.011	0.023
70 days	4.7 (16)	25.4 (11.1–50.9)	0.0023	0.004	0.037	0.096	9.2 (32)	41.7 (10.8–126.6)	0.016	0.037
110 days	8.0 (15)	26.6 (11.1–85.2)	0.0028	0.004	0.034	0.088	14.4 (38)	35.2 (10.3–167.4)	0.042	0.096
Sand substrate										
10 days	5.4 (11)	42.8 (21.8–82.2)	0.0067	0.011	0.053	0.096	20.2 (54)	58.9 (9.8–236.5)	0.074	0.137
40 days	7.1 (21)	49.9 (25.9–102.5)	0.0071	0.016	0.043	0.093	20.2 (48)	71.4 (11.4–250.8)	0.083	0.142

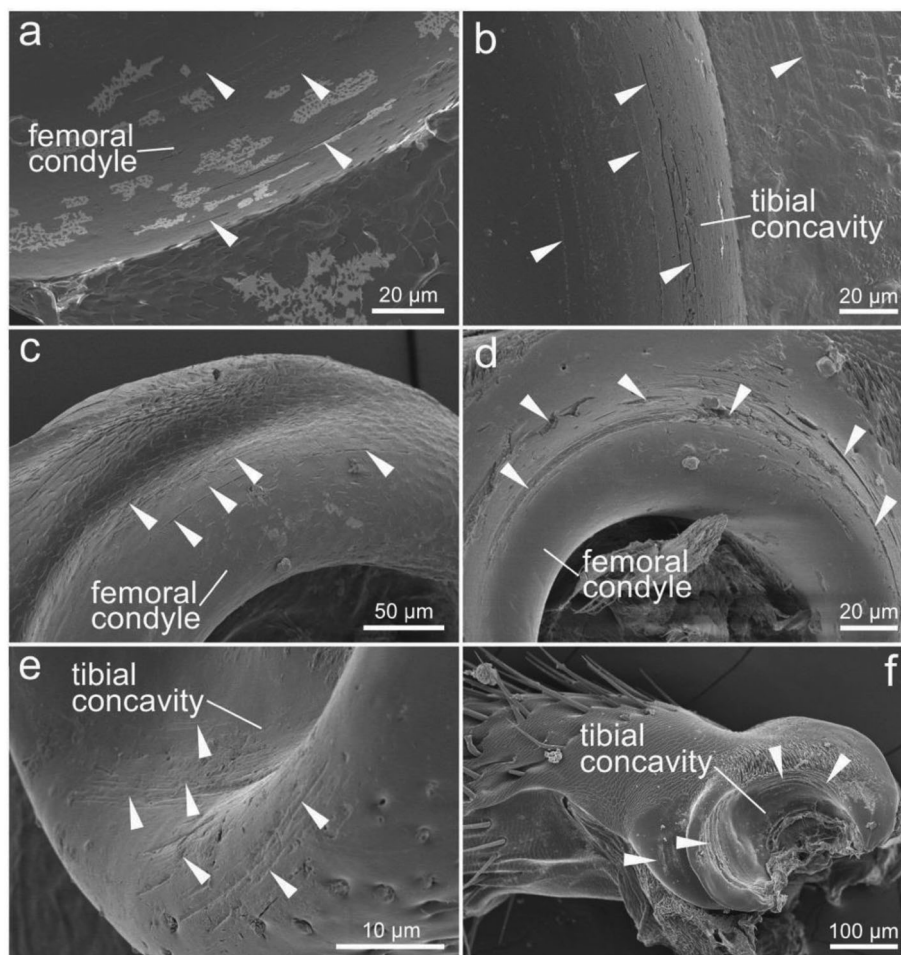


Figure 2. Abrasive wear of cuticle in the femoro-tibial joint of beetles (Coleoptera) from natural habitats and laboratory culture. White arrowheads indicate the areas with wear marks. a,b) Congo rose chafer *Pachnoda marginata*: a) femoral condyle, b) tibial concavity; c) Dung beetle *Geotrupes stercorarius*, femoral condyle; d) Ground beetle *Abax parallelepipedus*, femoral condyle; e) Carrion beetle *Oiceoptoma thoracicum*, tibia; f) Longhorn beetle *Leptura maculata*, tibia.

3. Results

3.1. Structure of the Femoro-Tibial Joint in *Zophobas morio*

The femoro-tibial joint in the darkling beetle *Zophobas morio* (Tenebrionidae) (Figure 1a) comprises two main counterparts, tibial and femoral ones correspondingly connected by arthroidal membrane (Figure 1b–f). The tibial counterpart (Figure 1c) occupies the basal part of tibia and is inserted into the joint cavity made by the femoral counterpart. The principal structural elements of the tibial counterpart that are relevant for the present study are (a) a pair of semi-circular invaginations called here “tibial concavities” (Figure 1c), (b) the convex area of approximately rectangular shape situated proximally from concavities and called here “tibial protrusion” (Figure 1c), and (c) moderately flat area situated proximally from “tibial protrusion” and called here “tibial surface” (Figure 1c). The femoral counterpart is situated at the apical part of the femur. The femoral counterpart bears a pair of semicircular convex condyles (Figure 1b) inserted into corresponding tibial concavities, form-

ing a frictional pair, and connected by elastic ligaments. This construction allows the joint rotation along a single axis. The area situated between the femoral condyle and the border of joint cavity (made by membrane) called here “femoral surface” (Figure 1b). The most of the surfaces within the joint cavity, except the femoral condyle and tibial concavity, are covered with regular microstructure resembling flattened folds or scales. These surfaces are covered with numerous pores of $\approx 1 \mu\text{m}$ in diameter delivering the semi-solid lubricant to the cuticular surface.^[28,29]

3.2. Wear in the Leg Joints of Natural-Habiting Beetles

Traces of wear of various appearances were found in all individuals of the studied beetle species. In the digging-legged *Pachnoda marginata*, signs of wear are represented predominantly by scratches and indentations (Figure 2a,b), as is the case with the digging dung beetle *Geotrupes stercorarius* (Figure 2c). In the running-legged ground beetle *Abax parallelepipedus*, signs of

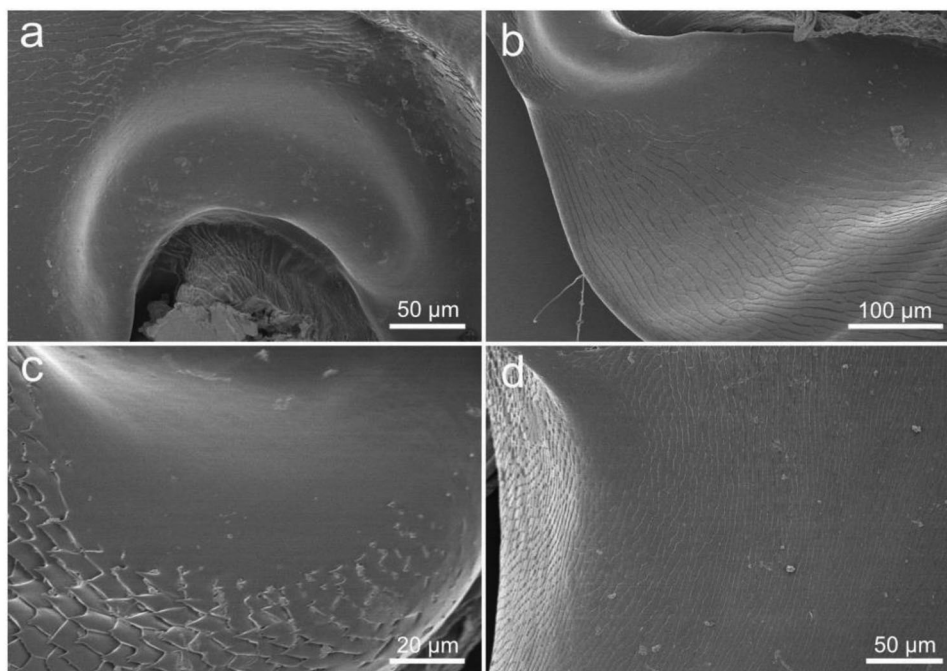


Figure 3. Contacting surfaces of the femoro-tibial joint of *Zophobas morio*, control; immobilized, and specimens kept without substrate. a) Femoral condyle, immobilized specimen; b) Femoral surface, without substrate specimen; c) Tibial concavity, immobilized specimen; d) Tibial surface, immobilized specimen.

wear are represented by scratches, indentation, cracks, and brittle fractures (Figure 2d). In the carrion beetle *Oiceoptoma thoracicum*, having walking legs, the wear marks were found in the form of grooves and scratches (Figure 2e). The same type of wear (Figure 2f) was found in the longhorn beetle *Leptura maculata*, having walking legs.

3.3. Experimental Detection of the Cuticle Wear in Leg Joints

Control examination of the cuticle surface of the leg joints in immobilized specimens did not reveal signs of wear. No scratches, surface destructions or other signs of the surface destruction were found on the cuticle surface (Figure 3; Figure S1, Supporting Information). The same result was obtained, when examining the cuticle surface of leg joints in beetles kept without substrate for ≈ 30 days (Figure 3; Figure S2, Supporting Information).

Experiments with Contamination by Metallic Particles clear traces of wear were found on the cuticle surface by treatment of legs joints with 2–5 μm size iron particles penetrated through the ventral and dorsal gaps (Figure 1d,e). Wear marks and metal particles were found inside the joint cavity on the tibial concavity, femoral condyle, tibial protrusion, femoral and tibial surfaces (Figure 4; Figure S3, Supporting Information). Wear traces are represented by numerous deep grooves, scratches and cracks that have a regular shape of arcs of various lengths.

Experimental Substrates wear marks of various appearances were found on the cuticle surface of the leg joints of freely mobile beetles kept on all three types of experimental substrates (Figure 5; Figures S4–S6, Supporting Information). The marks

are represented by (1) scratches, (2) indentation marks, (3) cracks, (4) grooves, (5) brittle fracturing and (6) removal of the external layer of the cuticle. Scratches of different lengths (from a few micrometres to over a hundred micrometres), widths and depths are observed. The direction of the scratches coincides with the direction of movement of the contact surfaces. Indentation marks are represented by a regular series of shallow and minute indents arranged in a line. Cracks of different lengths and widths, from very short to very long (from 1 μm to reaching over 60 μm). The location of scratches either coincides with the direction of movement of the contact surfaces or differs. Often cracks have an irregular shape or resemble the form of a broken line. Also, there is a series of short cracks of similar form separated by smooth intervals and lined up along one line. Often the edges of the cracks can be uneven, and the width of the crack can vary in its different parts. Both scratches and cracks occur only on the smooth surface of the articular surfaces of the tibia and femur.

Grooves of different lengths, thicknesses, and depths were observed. They are often the result of removal of the surface layer of the cuticle. The direction of the grooves always coincides with the direction of movement of the contact surfaces. The width of the grooves always exceeds the width of scratches and cracks. The edges of the grooves are often gently sloping. Usually, the grooves are situated next to another. The grooves were found on both smooth and textured surfaces of the joint. Brittle fracture, which is presented in the form of surface areas covered with cracks of various shapes and directions, exfoliating sections of the cuticle, partially destroyed and crumbled sections of the cuticle. Often brittle fracturing contains traces of grooves. Areas with brittle fracturing are usually found on smooth surfaces of

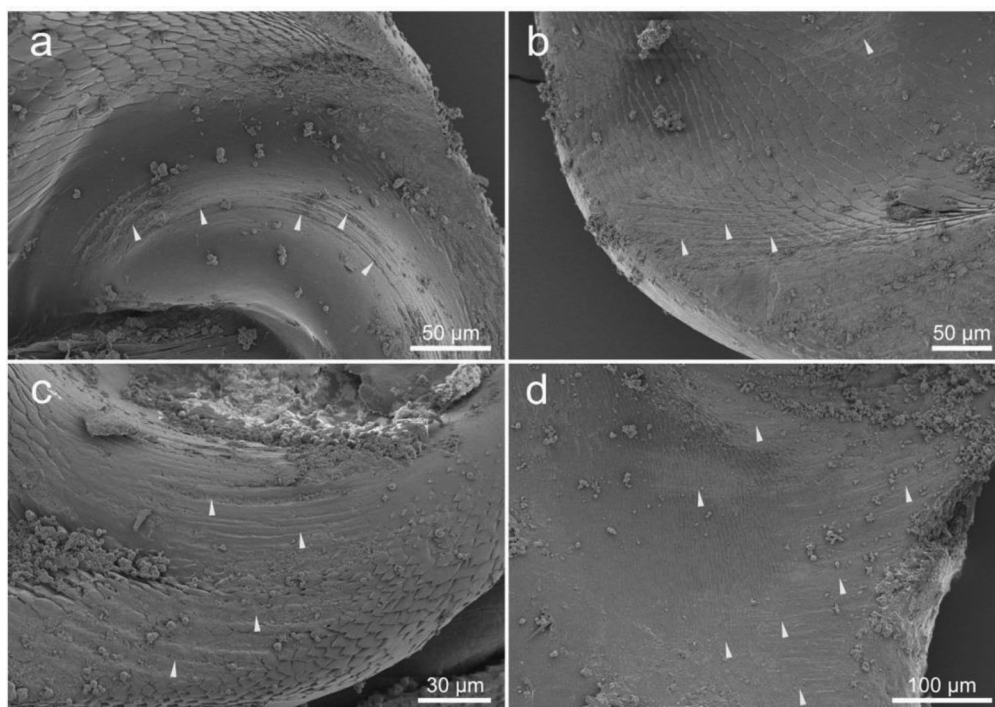


Figure 4. Abrasive wear in the femoro-tibial joint of *Zophobas morio* contaminated with 2–5 μm metallic particles. White arrowheads indicate the areas with wear marks. a) femoral condyle, b) femoral surface, c) tibial concavity surface, and d) tibial surface.

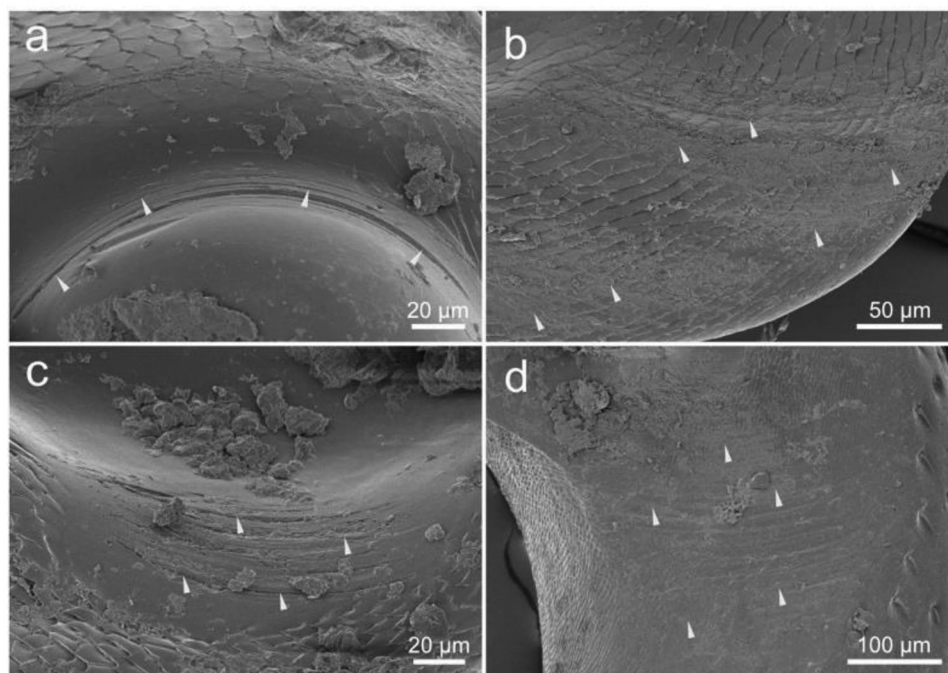


Figure 5. Contacting surfaces of the femoro-tibial joint of *Zophobas morio*; experimental substrates, examples. White arrowheads indicate the areas with wear marks. a) femoral condyle, b) femoral surface, c) tibial concavity, and d) tibial surface.

the tibia and tibial protrusion. *Removal of the external layer* of the cuticle, mostly entirely, occurs mainly on the textured surfaces of the tibia and femur. Although all of the above traces of wear were found on the cuticle of the leg joints of animals kept in different experimental environments, nevertheless, the most pronounced traces are characteristic of beetles kept on a sandy substrate.

To compare the quantitative characteristics of the wear process and its results, the three following parameters were selected: the number and length of scratches on the femoral condyle (Figure 1b) and tibial concavity (Figure 1c) and the area of the destructed surface on the tibial surface and protrusion and on femoral surface as well (Figure 1b,c). The results of measurements are summarized in the Table 1 and Figure 6.

Statistical treatment of the data was performed for each type of substrate, as well as comparison between the time of exposure. In this case, only the results of comparison of the same structures were taken into account. The analysis using one-way ANOVA ($F(13,111) = 4.195$, $P < 0.0001$) for the number of scratches in all three types of substrates (paper, wood, and sand) reveals no statistically significant difference neither within or between the types of substrates nor for the time of exposure. For the destructed surface, the corresponding analysis by one-way ANOVA ($F(20,155) = 9.734$, $P < 0.0001$) revealed statistically significant difference for the following substrates (given the comparable time of exposure only): femoral surface (paper, 30 days) versus femoral surface (sand, 40 days), femoral surface (paper, 60 days) versus femoral surface (sand, 40 days), and femoral surface (wood, 50 days) versus femoral surface (sand, 40 days). No statistically significant differences were revealed for the different time of exposure within a type of substrate.

4. Discussion

4.1. Experimental Evidence of the Abrasive Wear in the Leg Joint Cuticle

Abrasive wear is represented by two main modes – two-body wear and three-body wear. Two-body wear takes place, when constrained hard particles or in the absence of particles one surface (usually harder than the other) eliminate material from the opposing surface. In three-body wear, this occurs, when the particles are unconstrained and remain able to slide down and roll on a surface. To test the hypothesis of the two-body wear in the absence of abrasive particles (i.e., sliding wear), a control experiment was performed with the beetles kept in an empty clean plastic container (without substrate). The results of the experiment showed that the surfaces of the joint cuticle did not contain traces of the wear (Figure 3; Figure S2, Supporting Information). This may indicate that the hardness of the cuticle material of both surfaces is comparable, and the roughness of both surfaces is too small to create visible wear marks.

Abrasive wear of the cuticle surface was found in the leg joints of beetles kept on all three types of substrates: paper, sawdust, and sand. Beetles were kept on the various substrates, in order to investigate the impact of substrate on the occurrence of wear. Thus, it can be concluded that abrasive particles of appropriate sizes can easily penetrate from various substrates

into the joint and initiate the process of abrasive wear. Since the physical characteristics of the used substrates and, accordingly, their particles differ, a set of quantitative parameters of the abrasive wear was used: (1) number of scratches, (2) length of scratches, (3) area of the destructed surface. This made it possible, to compare the wear of different parts of the joint surfaces and in turn estimate the rate of wear within the same substrate depending on the exposure times, and also to compare the quantitative characteristics of wear in beetles kept on different substrates.

Although, in general, no statistically significant differences in the quantitative characteristics of wear were found between the substrates (this may be partly due to the number of beetle specimens), nevertheless, certain trends can be observed. Since the substrates were selected according to the relative hardness of their material, it was expectable that the wear in beetles kept on sand and wood substrate is high. Surprisingly, the abrasive wear found in leg joints of beetles kept on the paper substrate was also relatively high. The only explanation of this effect is that paper substrate represented by the recycled, shredded and crumpled paper is contaminated by hard particles during the fabrication process. It is noteworthy that such quantitative characteristics of wear as the number of scratches and the area of the destructed surface for the paper and sand substrates (in tibia protrusion and femoral surface) turned out to be higher than for the wood substrate (Figure 6, Table 1). Apparently, this is due to the fact that the particle sizes in the paper and sand substrates are smaller than in the wood substrate and they can presumably more easily penetrate into the joint cavity. It is also obvious that the hardness of the particles of these substrates (especially that of sand) is higher than that of wood.

In general, it can be seen that for the sand substrate, the average values of wear are generally higher than for other substrates (Figure 6, Table 1), which can be explained both by the abundance of fine particles and their high hardness. There are certain differences (including statistically significant) in the quantitative values for different areas of the cuticle, such as femoral surface for the sand substrate and all other substrates. These results can also be explained by the higher hardness of particles, and possibly also by their higher abundance, compared to both paper and wood substrates. At the same time, it is obvious that the length of the scratches does not correlate with the type of substrate and is determined by the specific conditions of interaction between the surfaces and the abrasive.

No statistically significant difference was found in the quantitative characteristics of wear depending on the time of exposure of the beetles on the substrate. Although in general, it can be seen that a number of values tend to increase with an increasing exposure time, in some cases there is an inverse situation. For example, for the sand substrate, the area of tibial surface destruction decreased, as did the average number of scratches for the femoral condyle. This can be explained by the fact that the beetles received abrasive damage to the cuticle of the leg joints in the first days, and subsequently reduced their locomotor activity, which is confirmed by observations of beetles in cages. Since the sandy substrate itself is not characteristic for the darkling beetle *Zophobas morio*, it is not adapted to life on such a surface and, as a behavioral response to unusual condi-

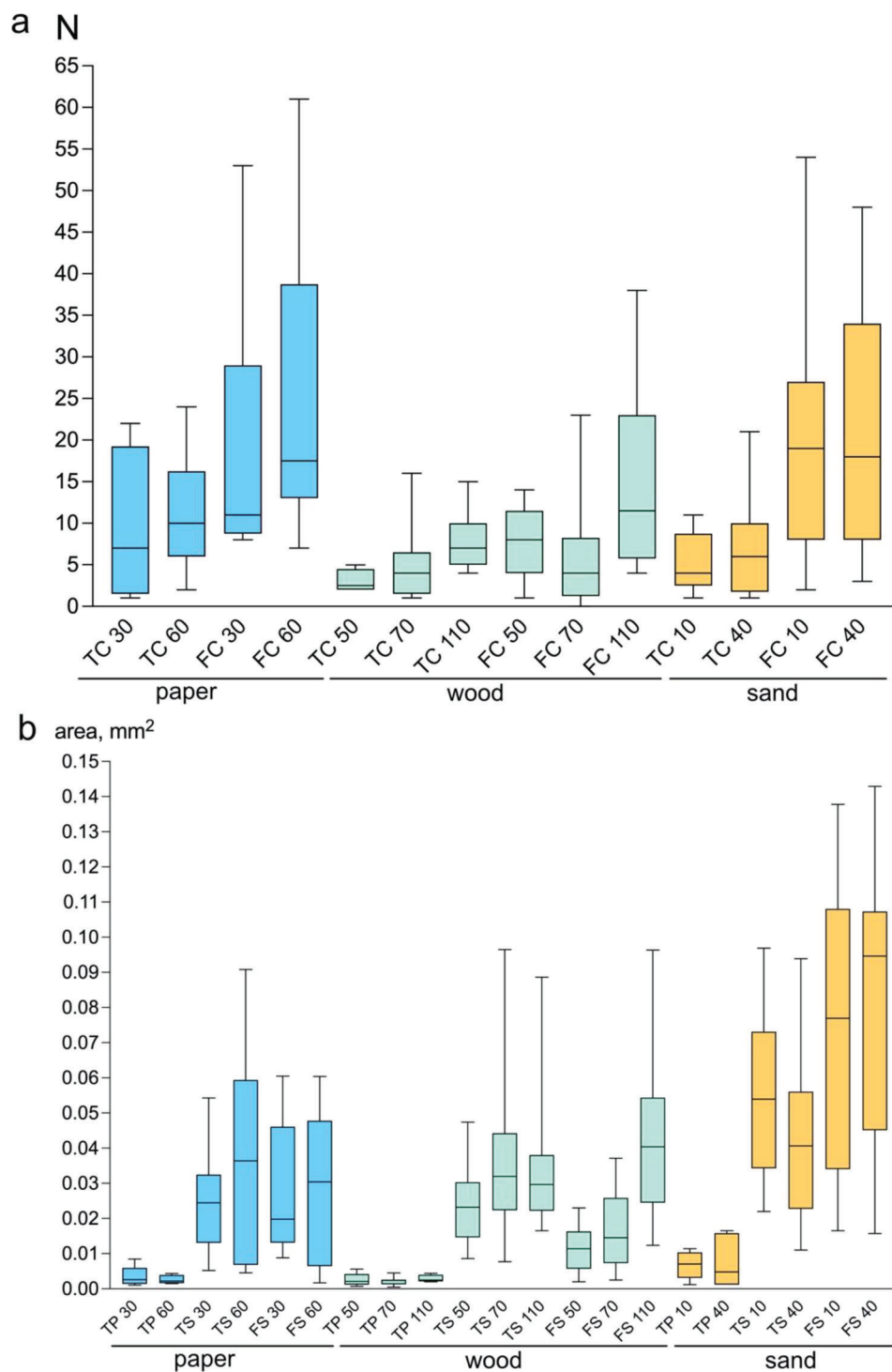


Figure 6. Comparison of the wear effects for various parts of the femoro-tibial joint of *Zophobas morio*. Error bars show standard deviations. Number after abbreviation shows time of exposure in days (see Material & Methods). a) Number of scratches (N); b) Area of destructed surface, mm². Abbreviations: FC, femoral condyle; FS femoral surface; TC, tibial condyle; TP, tibial protrusion; TS, tibial surface.

tions, reduces its activity. The same seems to apply to the paper substrate, where, for example, the wear values of the tibial protrusion surface were lower after 60 days than after 30 days.

4.2. Contamination by Metallic Particles

In order to reveal (1) the places of penetration of abrasive particles into the joint and (2) the areas of the joint mainly subjected to wear, and (3) to identify the direction of the relative movement of the articular surfaces, experiments were performed with contamination of the joints with iron particles of 2–5 μm in size. The results showed that the two main ways of penetration of particles are the dorsal and ventral gaps (Figure 1d,e). Examination of the areas of the articular surfaces, where signs of wear were found, namely the ventral surface of the tibial concavity, the edges of the femoral condyle, the lateral surfaces of the tibia and the ventral surface of the femur, completely coincide with those found in specimens kept on experimental substrates. Wear marks in the form of scratches and large number of grooves, as well as their orientation on the surface, are identical to those in the joints of beetles living on the experimental substrates.

4.3. Abrasive Wear in the Leg Joints of Natural-Habiting Beetles

Signs of wear on the joint cuticle have been found in representatives of several families of beetles, namely Carabidae, Scarabaeidae, Silphidae, and Ceramycidae. The wear marks are represented mainly by scratches, grooves, cracks, and brittle fractures (Figure 2). This pattern of traces is typical for abrasive wear due to the penetration of the particles into the joints. This probability is quite high, since most of the studied beetles lead a terrestrial lifestyle on the soil surface, from where particles of sand and clay can penetrate into their joints as in the case of two species, *Pachnoda marginata* and *Geotrupes stercorarius*, that are specialized diggers. The other two species, *Abax parallelepipedus* and *Oiceoptoma thoracicum*, live primarily on the surface of the soil and may also burrow under rocks or tree trunks. At the same time, the presence of wear marks in the form of deep grooves on the tibial cavity of the longhorn beetle *Leptura maculata*, which lives mainly on flowers and often uses flight for movement, seems somewhat unusual. A possible explanation for this may be the assumption that pollen particles with a hard shell of sporopollenin (elasticity modulus of 9.5–16 GPa),^[30] which significantly exceeds that of the insect cuticle,^[5] as well as the presence of sharp protrusions on the pollen grains, can serve as an abrasive and damage the cuticle surface. The presence of traces of abrasive wear in beetles that live in natural conditions and differ in their way of life indicates that this phenomenon is natural and inevitably occurs upon contact with a substrate containing an abrasive material. Known protection and cleaning systems in leg joints^[31] apparently minimize the damaging effects of abrasive wear to a certain extent, although they cannot eliminate it completely. At the same time, in most cases, the damages are of a superficial nature and presumably are not critical for the locomotor activity of the beetles with relatively short longevity at an adult stage.

4.4. Abrasive Wear of the Insect Cuticle and Its Underlying Mechanisms

The abrasive wear is defined as the loss of material due to hard particles or hard protuberances that are forced against and move along a solid surface.^[32] It occurs, when a hard rough surface slides across a softer surface. The harder material may be one of the rubbing surfaces or hard particles that have found their way between the mating surfaces. As stated above, frictional wear was not found in the joints, so the only cause of observed wear is particles from outside.

The particles found in the experimental substrates are represented by the great variety of shapes and sizes: from the rounded, nearly spherical barrel-shaped to angular with sharp edges and asperities as well as irregular-shaped (Figures 7a,c, 8a,d). Their sizes vary from >1 μm to dozens of micrometers. The shape of particles and the way how their asperities slide over a surface determines the nature and intensity of abrasive wear.^[33] Rounded particles or particles without sharp asperities deform the surface by the blunt indentation resulting in the formation of yielding with subsequent cracking. They also lead to fatigue destruction and wear as a result the multicycling deformation by the impact of rounded particles (with possible formation of internal microcracking) with a subsequent removing of the material in the process of cracking, delamination and microcutting by particles with sharp asperities. The action of particles with sharp asperities leads to a variety of consequences that are discussed below based on the results of observations on the leg joint cuticle (Figures 7 and 8; Figures S5–S7, Supporting Information).

Scratches and microcutting (Figures 5a,c,7b,d, and 8c; Figures S4d,f, S5d,f, and S6d,f, Supporting Information) occurs as a result of the action of particles with sharp asperities and edges, whose hardness is much higher than that of the cuticle, as, for example, in the sand particles with hardness reaches 10.7–11.7 GPa. In this case, the particles are fixed on one of the surfaces, which leads to the effect of two-body wear (as the particles are constrained in this case)

Indentation occurs due to the action of the free-moving particles that roll between sliding surfaces. Sharp edges or asperities of particles leave traces on the surface of the cuticle in the form of a series of small pits or depressions.

Cracking (Figure 7b,d; Figures S4f and S5f, Supporting Information) is usually formed as a result of the action of particles without sharp edges or protrusions due to the local pressure with a high stress concentration at one or few points. Particles in this case can be both fixed and freely moving between surfaces. Indentation by sharp particles can also lead to the formation of cracks as an initiator of the process, after which microcracks can be developed at the indentation site as a result of, for example, the subsequent pressure of rounded particles (without sharp edges). Often, such cracks can be oriented at different angles to the direction of relative motion of the surfaces. Long longitudinal cracks corresponding to the direction of movement are also often encountered and, apparently, can be the result of microcutting with subsequent deepening and development of a crack under high local stress.

Brittle fracture and chipping (Figures 5c and 7d,f; Figures S4f and S6f, Supporting Information). Irregular or repetitive impacts of freely moving angular and rounded particles may causes

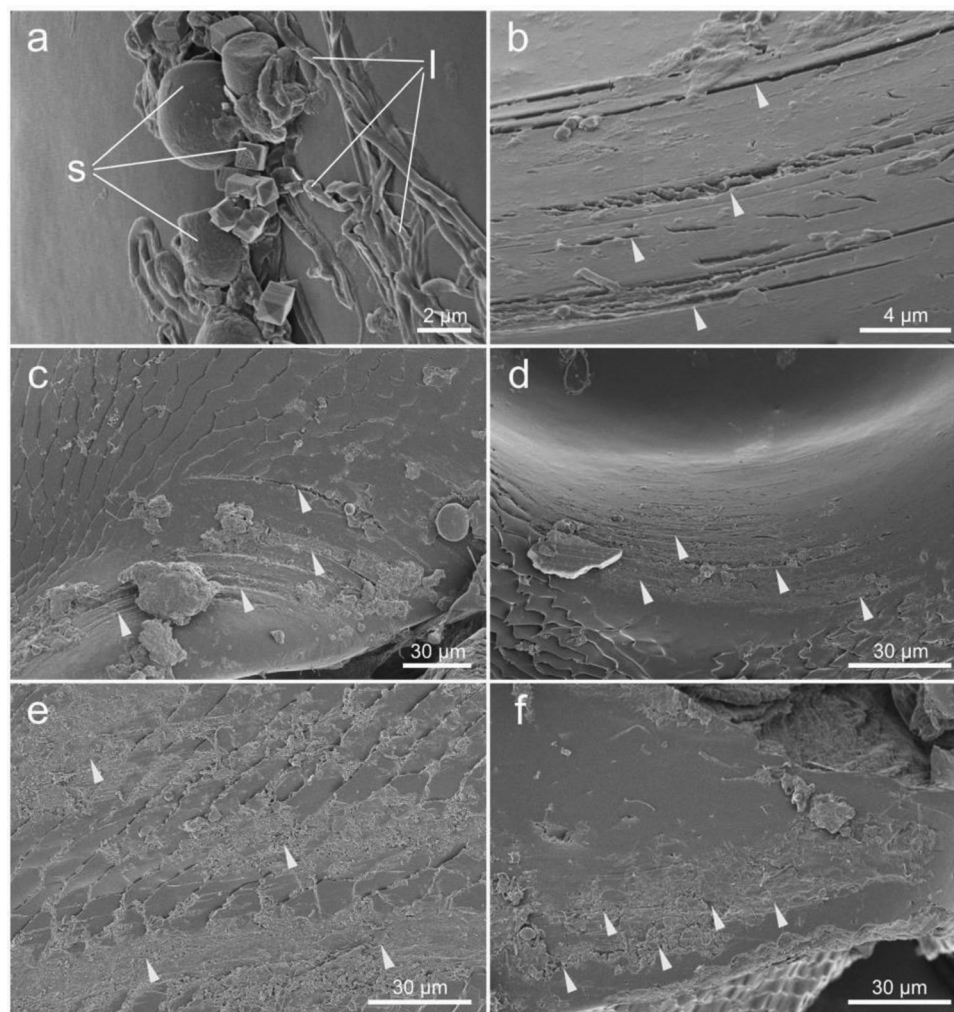


Figure 7. Abrasive wear in the femoro-tibial joint of *Zophobas morio*. White arrowheads indicate the areas with wear marks. a) Various shapes of the abrasive particles from sea sand (s) and lubricant (l); b) Tibial concavity surface with the wear marks in form of scratches and cracks; c) Femoral condyle surface with the wear marks in form of scratches, grooves and cracks; d) tibial concavity surface with the wear marks in form of scratches, indentations, cracks and brittle fracture; e) Femoral surface with the wear marks in form of scratches and removing of external layer of cuticle; f) Tibial projections surface with the wear marks in form of scratches, cracks, delamination and brittle fracture.

brittle fracture, where material is removing from the surface by the formation and intersection of cracks, short scratches, microcuttings, and indentations. Thus, brittle fracture is a result of integrative impact of fatigue wear and elastic deformation accompanying by puncturing (indentation) and short microcuttings resulting in development of a propagating cracking with a subsequent material removing.

Delamination (Figure 7f; Figures S4f and S6f, Supporting Information) is a result of the integral action of such forms of wear as microcutting, cracking, indentation, fatigue wear, and in this sense, it is quite similar to brittle fracture, of which it is a part. During delamination, the outer layers of the cuticle, which has a layered structure, are separated and removed from the surface.

Grooving (Figures 5a, 7c, and 8a; Figures S4a,d, S5a and S6a,c, Supporting Information). The wear marks in the form of grooves (superficially similar to ploughing, which is typical for technical materials, such as metals or polymers where material remains on

the surface at the edges of the groove and is the result of plastic deformation) in the case with insect cuticle is characterized by the surface removed completely and looks like series of deep and broad grooves directed along the direction of the relative movement of contacting surfaces. Usually, such grooving is the result of the action of large fixed particles with sharp edges.

External surface destruction (Figures 7e and 8b; Figures S5c,h, S6c,h, Supporting Information). Its appearance is partly similar to the grooving, but differs in the process leading to it. Destruction is an integral process that includes microcutting, indentation, cracking, brittle fracture, fatigue wear, and delamination. Thus, the cumulative action of these types of abrasive wear leads to the complete removal of the upper layer of the cuticle (epicuticle) over a certain area.

Wear marks are unevenly distributed on the contact surfaces of the joint, as, for example, in the case of tibial concavity (Figure 1c), where wear marks are mainly occurred in its ventral

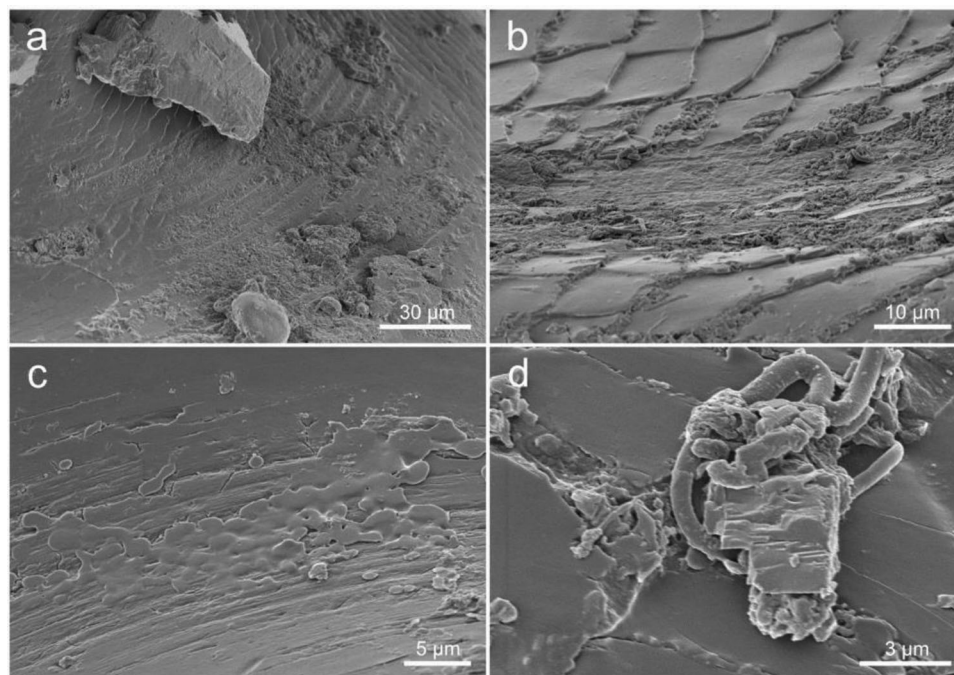


Figure 8. Wear effects and wear resistance in the femoro-tibial joint of *Zophobas morio*. a) Trochanteral surface covered with grooves; b) Area of the femoral surface with destructed external layer; c) Scratched surface of femoral condyle covered with lubricant; d) Abrasive particle covered with lubricant. Scale bar: a) 30, b) 10, c) 5, and d) 3 μm .

part (Figures 5c and 7d). This can be explained as follows. In the normal position of the body of the beetle, it rests with its femora on the tibiae in such a way that the tibiae serve as a support for the body, while in the joint the femoral condyle is inserted within the tibial concavity and, accordingly, rests on the ventral part of the cavity. Thus, it is the ventral part of the tibial concavity that is the contact zone subjected to both static (when the beetle rests on its legs) and dynamic loads (when the beetle moves along horizontal or slightly inclined surfaces) to the maximum extent. This correlates with our observation that the greatest number of wear marks on the femoral condyle was also found on its ventral part. The larger amount of wear signs (mostly scratches) in the femoral condyle surface in comparison with tibial one can be explained in the following way. The scratches on the femoral condyle are situated at the marginal area of the condyle, but not on the top of convexity. The whole surface of the femoral condyle is smooth including marginal areas around the condyle. The counterpart, tibial concavity, has two type of surfaces, smooth and textured, covered with microstructures of regular shape. The textured surface is situated at the sides (vertical walls) of the concavity, whereas the bottom is smooth. The textured sides of the tibial concavity are in contact with marginal areas of femoral condyle, while smooth bottom is in contact with smooth top of the condyle. It can be supposed that contaminant abrasive particles can penetrate between the surfaces and stuck more or less firmly among the folds of textured microstructures of tibial concavity. Then while moving, the tibia with stuck abrasive particles can scratch the surface of femoral condyle making two-body abrasive wear. In this case, the femoral condyle would be scratched, whereas the tibial concavity would not. Besides this, these microstructures on the tibial condyle are oriented by their

sharp edges and margins outside the concavity and can supposedly be considered as an anti-contamination mechanism, i.e., the “catcher” of particles, in order to prevent them of entering the inner smooth surfaces of the joint.

Summarizing, it can be stated that the abrasive wear of the cuticle in the leg joints of beetles is represented by a wide range of effects from small scratches to the total removal of the surface layer of the cuticle (**Figure 9**). In most cases, the visible effects of wear, such as brittle fracture and removal of the surface layer, are complex in nature, due to the integral action of several mechanisms. In turn, this is determined both by the tribological properties of the contact surfaces and by the variety of abrasive particles, their sizes, shapes, and material properties.

4.5. Wear Resistance: Possible Solutions

As stated by Briscoe & Sinha (2006)^[34]: “Despite several potential advantages, there are still many challenges, which remain for the effective and economic use of polymers for specific tribological applications. For example, the wear rate can be extremely high, if the contact pressure is slightly raised, and low friction is not necessarily associated with low wear rate”. This is perhaps attributable for the insect cuticle composed mainly by such polymers as chitin and proteins.

It is known that the cuticle, specialized in performing heavy mechanical work and potentially subjected to abrasion, is usually additionally strengthened. Wear resistance has been studied for mandibles of Orthoptera, Phasmida, Lepidoptera, Hymenoptera, and Coleoptera^[35–41] and ovipositors of Hymenoptera and Auchenorrhyncha^[42–44] revealed their reinforcement among

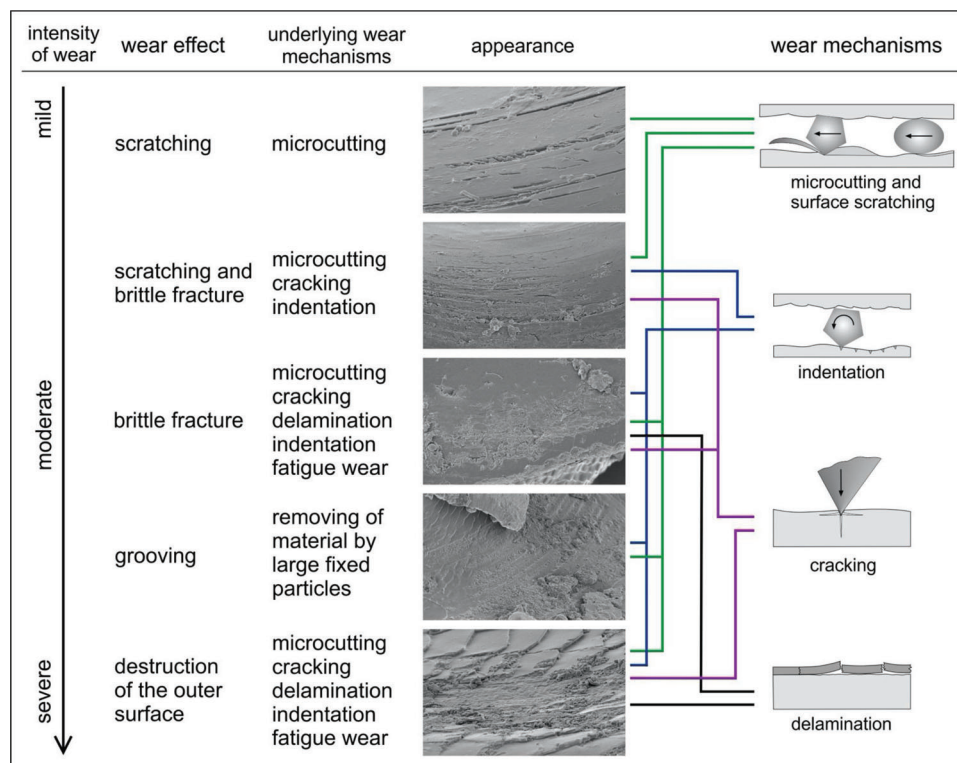


Figure 9. Abrasive wear of insect cuticle with underlying wear mechanisms.

others by crosslinking the cuticle with metal ions, such as Mn, Zn, or Fe. The presence of metals in the cuticle of insect leg joints is not known, although no such studies have been specifically conducted.

Lubrication is another obvious mechanism for minimizing wear. Lubricants have been found in the joints of the legs of beetles and cockroach,^[28,29] and in addition to their actual lubricating function, their participation in the cleaning of the joint cavity from contaminating soil particles was shown using the example of a digging beetle *Pachnoda marginata*.^[31] Our observations show that in *Zophobas morio*, the lubricant can perform anti-wear function (1) by covering particles with sharp asperities, to prevent scratching and indentations (Figure 8d), (2) by gluing particles together, to enable their removing from the joint cavity (Figure 7a) and (3) by covering the destructed and damaged surface, to prevent its further damage (Figure 8c).

5. Conclusion

The wear of the cuticle in the beetle leg joints was detected and its presence was confirmed experimentally in laboratory conditions. Cuticle wear was also found in beetles living in natural conditions. Wear of the cuticle of the leg joints is represented by abrasive wear, while friction wear was not detected. The abrasive wear appears as a result of the action of particles that have entered the joint. The abrasive wear is represented by such types of damage as: scratches, microcuttings, indentations, cracking, chipping, delamination, grooving (ploughing), brittle fracture, and destruction of the surface layer. Quantitative characteristics of wear, such as the number and length of scratches, as well as the

area of the destructed surface, in general, correlate rather weakly with the type of substrate (paper, wood, sand), and very weakly with the exposure time of beetles (the time they stay on the substrate). It is assumed that the semi-solid lubricant present in the joint of the legs prevents wear to a certain extent by trapping particles, sticking them together, and leveling out the effects of wear by covering damaged surfaces.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

K.N. and S.G. developed the scientific question and prepared the study design. K.N. carried out the experiments, calculations, and observations

and prepared the manuscript and figures. Both co-authors discussed the results and revised the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

beetles, biotribology, coleoptera, cuticles, lubrication, three-body wear, two-body wear

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