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RESEARCH ARTICLE

Jumping mechanisms and performance in beetles. I. Flea beetles (Coleoptera: Chrysomelidae: Alticini)

Konstantin Nadein* and Oliver Betz

ABSTRACT

The present study analyses the anatomy, mechanics and functional morphology of the jumping apparatus, the performance and the kinematics of the natural jump of flea beetles (Coleoptera: Chrysomelidae: Galerucinae: Alticini). The kinematic parameters of the initial phase of the jump were calculated for five species from five genera (average values from minimum to maximum): acceleration 0.91–2.25 ($\times 10^3$) m s^{-2}, velocity 1.48–2.80 m s^{-1}, time to take-off 1.35–2.25 ms, kinetic energy 2.43–16.5 μJ, *g*-force 93–230. The jumping apparatus is localized in the hind legs and formed by the femur, tibia, femoro-tibial joint, modified metafemoral extensor tendon, extensor ligament, tibial flexor sclerite, and extensor and flexor muscles. The primary role of the metafemoral extensor tendon is seen in the formation of an increased attachment site for the extensor muscles. The rubber-like protein resilin was detected in the extensor ligament, i.e. a short, elastic element connecting the extensor tendon with the tibial base. The calculated specific joint power (max. 0.714 W g^{-1}) of the femoro-tibial joint during the jumping movement and the fast full extension of the hind tibia (1–3 ms) suggest that jumping is performed via a catapult mechanism releasing energy that has beforehand been stored in the extensor ligament during its stretching by the extensor muscles. In addition, the morphology of the femoro-tibial joint suggests that the co-contraction of the flexor and the extensor muscles in the femur of the jumping leg is involved in this process.

KEY WORDS: Locomotion, Jump, Kinematics, Functional morphology, Resilin, Coleoptera

INTRODUCTION

Jumping activity is traditionally considered an anti-predator mechanism that originated as a result of predator–prey interactions or as a response to disturbance, i.e. a defense reaction (Chapman, 2013). This mechanism is highly effective and can be considered a successful example among the diverse defense mechanisms of insects. The uniqueness of this type of locomotion is thought to be, foremost, that it provides an effective defense mechanism for comparatively low energetic outlays accompanied by relatively simple structure (Bennet-Clark, 1976; Furth, 1988).

Jumping in insects generally involves spring-loaded mechanisms for the storage of elastic energy in order to transform muscular action into rapid movement at the appropriate moment (Gronenberg, 1996). Such systems are called 'catapult mechanisms' and represent

power amplifiers. The force needed to deform the 'spring' can be recovered further in its elastic recoil. The spring is usually composed of the rubbery protein resilin, providing elasticity and preventing fractioning and fatigue of the material. The slow muscle contraction performs a mechanical reversible deformation of the spring, energy is stored in the form of elastic strain energy and finally very rapidly converted into kinetic energy. The morphological structures for the accumulation and storage of the elastic energy in jumping insects are variable, taking the form of semilunar plates in grasshoppers, locusts and crickets (Burrows and Sutton, 2012; Burrows and Morris, 2003), resilin pads in fleas (Sutton and Burrows, 2011), thoracic pleural arches in froghoppers (Burrows et al., 2008) and planthoppers (Burrows, 2010), and an abdominal appendage in springtails (Brackenbury and Hunt, 1993). Some jumpers employ a 'locking mechanism' working as passive or active latches (e.g. specialized sclerites or various projections of the cuticle on the limbs or body) preventing the springs from premature recoiling (Burrows, 2006b). The jumping apparatus is so effective that it allows the insects to perform a jump at a distance that greatly exceeds its body length (up to 289 times), is at high velocity (up to 5.5 m s^{-1}) and acceleration (from 70 to about 7000 m s^{-2}), has a very short time to take-off (0.8–14 ms) and has a high *g*-force (up to 700 in the best jumpers; Brackenbury and Wang, 1995; Burrows, 2006a, 2007a, 2008, 2009a,b, 2011; Burrows and Morris, 2003; Schmitt, 2004; Burrows and Picker, 2010; Sutton and Burrows, 2011). This mechanism is a characteristic of insect orders as diverse as grasshoppers and locusts (Orthoptera) (Bennet-Clark, 1975; Burrows, 1995; Heitler, 1974; see 'How Grasshoppers Jump' by W. J. Heitler, http://www.st-andrews.ac.uk/~wjh/jumping/index.html), and fleas (Siphonaptera) (Bennet-Clark and Lucey, 1967; Sutton and Burrows, 2011), and occurs in some groups of Hemiptera like leafhoppers (Cicadellidae) (Burrows, 2007b), froghoppers (Cercopoidea) (Gorb, 2004; Burrows, 2006b), planthoppers (Fulgoridae) (Burrows, 2009b) and shore bugs (Heteroptera: Saldidae) (Burrows, 2009a). It is also known to stick insects (Phasmatodea: Timematidae) (Burrows, 2008) and snow fleas (Mecoptera: Boreidae) (Burrows, 2011).

Although jumping beetles are well known, the specialized jumping apparatus is not widespread (Furth and Suzuki, 1992) and has been observed so far only in the families Curculionidae (weevils), Chrysomelidae (leaf beetles), Buprestidae (jewel beetles) and Scirtidae (marsh beetles). In addition, the jump (but without a specialized jumping apparatus) has been recorded for a variety of other beetle families (e.g. Mordellidae, Melandryidae, Eucinetidae, Anthribidae, Elateridae; Furth and Suzuki, 1992).

The best-known jumpers among Coleoptera are flea beetles (Chrysomelidae: Galerucinae: Alticini), whose ability to jump is reflected in their name (Fig. 1). Study of the jumping apparatus of flea beetles has a long history beginning with the work of Maulik (1929), who discovered that the swollen hind femur contains a peculiar three-dimensional convoluted structure, which he
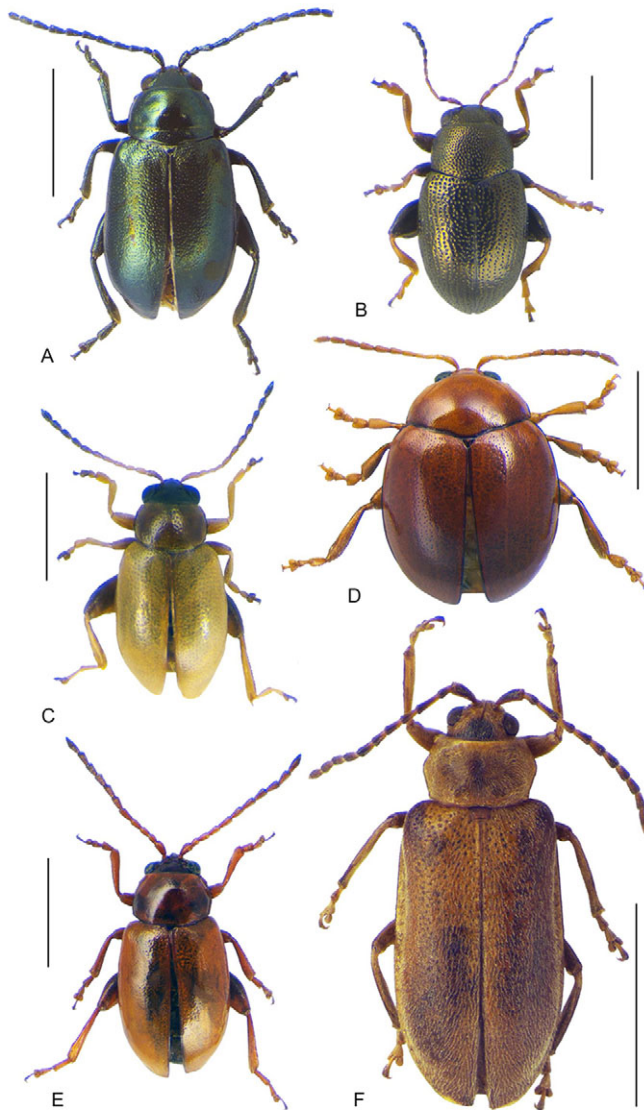
Fig. 1. External appearance of the leaf beetles examined. (A–E) Flea beetles, Galerucinae: Alticinae: (A) *Altica oleracea*, (B) *Chaetocnema aridula*, (C) *Longitarsus pratensis*, (D) *Sphaeroderma testaceum*, (E) *Aphthona cyparissiae*. (F) *Galerucella lineola* (Galerucinae: Galerucini). Scale bars: A, 1.6 mm; B, 1 mm; C, 0.9 mm; D, 1.6 mm; E, 1.7 mm; F, 2.6 mm.

referred to as a 'chitinized endoskeletal tendon', also known as 'Maulik's organ'. Anatomically, it provides attachment points for extensor muscles (Maulik, 1929; Furth, 1980; Schmitt, 2004). Hypotheses about the mechanism of jumping in flea beetles were presented in the works of Barth (1954) and Ker (R. F. Ker, Some structural and mechanical properties of locust and beetle cuticle, PhD thesis, Oxford University, 1977), and summarized in Furth (1988). The studies of Furth (1982, 1988) and Furth et al. (1983) were focused on the intergeneric comparative morphology and the anatomy of the modified metafemoral extensor tendon (MET), the so-called 'metafemoral spring' or 'metafemoral apodeme'. It was supposed that extensor muscles dilate the metafemoral spring (where the tension energy accumulates), which then is released during recoil, transferring the stored energy into the rapid extension of the tibia. Brackenbury and Wang (1995) measured the kinematics of jumping for seven species of flea beetles. They suggested that the high take-off acceleration, high velocity and short

take-off time are compatible with jumping based on a spring-driven mechanism.

The model of the jumping mechanism in flea beetles proposed by Betz et al. (2007) is the most detailed one and is based on modern micro-computed tomography (μCT) studies of the jumping apparatus as a whole. In brief, the flexor muscles adduct the tibia, then the flexor and the extensor muscles co-contract, the extensor muscles contract the MET and the energy of contraction is accumulated in the MET. A triangular flexor sclerite (Lever's triangular plate) connected to the tibial base serves as a locking mechanism (corresponding to the functional model proposed by Barth, 1954) to prevent the extension of the tibia during the co-contraction of the more powerful extensor muscles over the flexor. According to this model, the locking mechanism might be accomplished by pressing Lever's triangular plate against the distal margin of the posterior femoral wall that forms an abutment (Betz et al., 2007, fig. 12E–G). The subsequent rapid release of the flexor and the extensor muscles leads to the fast dilation of the MET, and the stored energy is released and converted into kinetic energy of the extension of the hind tibia, so that the beetle performs a jump by pushing off the hind tibia from the substrate surface, which propels the body into the air. Nevertheless, the above-mentioned authors share the opinion that the exact mechanism of energy storage and release in flea beetles is still not fully understood.

The present study is an attempt to describe the morphological, kinematic and functional aspects of jumping in flea beetles and to propose an overall model of the jumping mechanism. This model considers the possible role of the elastic protein resilin in energy storage, the exact lever conditions and joint power output at the femoro-tibial articulation, and comparative data on the jumping performance attained by high-speed videography.

MATERIALS AND METHODS
Study species

The jumping mechanism, structure of the jumping apparatus, jumping performance and kinematics were analyzed for seven species from seven genera of the flea beetle tribe Alticini (Fig. 1A–E) (Chrysomelidae: Galerucinae): *Altica oleracea* (Linnaeus 1758), *Chaetocnema aridula* Gyllenhal 1827, *Longitarsus pratensis* Panzer 1784, *Sphaeroderma testaceum* (Fabricius 1775), *Aphthona cyparissiae* Koch 1803, *Crepidodera aurata* Marsham 1802, *Podagrica fuscicornis* (Linnaeus 1766). For comparison with non-jumping relatives, two species from two genera of the tribe Galerucini (Chrysomelidae: Galerucinae) were included in the study: *Galerucella lineola* (Fabricius 1781; Fig. 1F) and *Luperus flavipes* (Linnaeus 1767). The beetles were collected by a sweep net from the glades at the forest edges in the vicinity of Rottenburg (Baden-Württemberg, Germany) during the spring–summer season (April to August of 2014 and 2015). A species of jumping seed beetle, *Eubaptus scapularis* (Pic 1900) (Chrysomelidae: Bruchinae), was borrowed from the collection of the Smithsonian Institution, National Museum of Natural History, Washington, DC, USA.

Material preparation

All material preparation and identification were carried out under a stereomicroscope (MZ7.5, Leica Microsystems GmbH, Germany). Dry-pinned museum material or fresh samples fixed in 70% ethanol were used. Fine needles and razor blades were used to make the preparations; dissections were carried out in distilled water or 70% ethanol. The slides for microscopy were fixed in glycerin. Calculation of the mechanical quantities of the jumping movement of the femoro-tibial joint of *Sphaeroderma testaceum* follows the

Table 1. Kinematic parameters of jumping in flea beetles

Name (N)	Length (mm)	Mass (mg)	Take-off time (ms)	Velocity (m s⁻¹)	Acceleration (×10³ m s⁻²)	Kinetic energy (µJ)	*g*-force
Aphthona cyparissiae (4)	3.3–3.6	4.2–4.6	2.0–2.5 (2.25)	2.49–2.96 (2.70)	1.42–1.63 (1.51)	13.9–19.7 (16.5)	144–166 (154)
Crepidodera aurata (3)	2.8–3.2	2.5–2.8	1.5–3.0 (2.10)	1.38–1.77 (1.60)	0.81–1.06 (0.95)	2.5–4.2 (3.5)	83–107 (97)
Chaetocnema aridula (3)	2.0–2.2	1.3–1.5	1.2–2.0 (1.47)	1.58–2.17 (1.78)	1.33–1.57 (1.46)	1.7–3.3 (2.43)	135–159 (143)
Longitarsus pratensis (13)	2.1–2.5	1.6–1.9	1.2–2.0 (1.35)	2.03–3.60 (2.80)	1.03–3.36 (2.25)	3.7–11.1 (7.3)	105–342 (230)
Sphaeroderma testaceum (4)	3.0–3.4	4.3–4.8	1.5–2.5 (2.08)	1.25–1.61 (1.48)	0.72–1.14 (0.91)	3.6–7.8 (5.64)	74–115 (93)

N, number of records. Values are given from recorded minimum to maximum; the arithmetic mean is given in parentheses.

methodology described in Betz and Mumm (2001). The mass of the extensor muscles and the telopodite (femur+tibia+tarsus) of the hind leg are given in dry mass. The extensor muscles were extracted from 10 femora of alcohol-preserved specimens, dried out for 2 days, and weighed together on a microbalance (BP211D, Sartorius AG, Göttingen, Germany); the value obtained was divided by 10 to calculate the extensor muscle mass for a single femur. The same method was applied for measuring the mass of the telopodite.

Fluorescence microscopy

The dissected parts of hind legs were mounted on glycerin slides without dyes and observed under an epifluorescence microscope (Zeiss AxioImager M2, Carl Zeiss AG, Germany). Resilin detection was based on autofluorescence under ultraviolet illumination (Haas et al., 2000; Burrows et al., 2008; Donoughe et al., 2011; Burrows and Sutton, 2012). The following filters were used: DAPI (excitation 353 nm, emission 465 nm), Alexa488
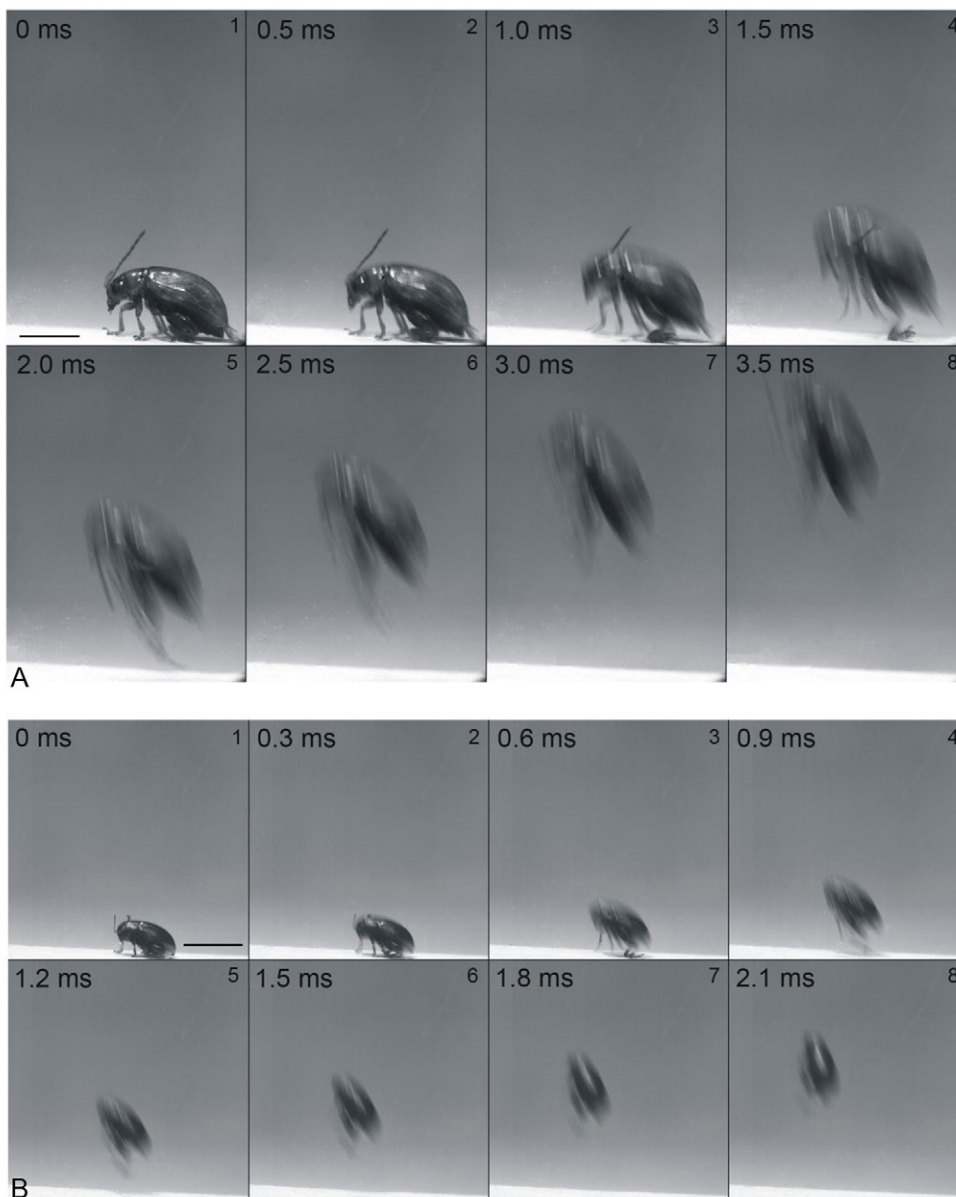

Fig. 2. Jumping performance of flea beetles. (A) *Aphthona cyparissiae*; frame-by-frame depiction of a jump viewed from the side. Images were captured at a rate of 2000 frames s⁻¹; frames are numbered according to their sequence. Scale bar, 1.75 mm. (B) *Chaetocnema aridula*; frame-by-frame depiction of the jump viewed from the side. Images were captured at a rate of 3000 frames s⁻¹; frames are numbered according to their sequence. Scale bar, 2.1 mm.
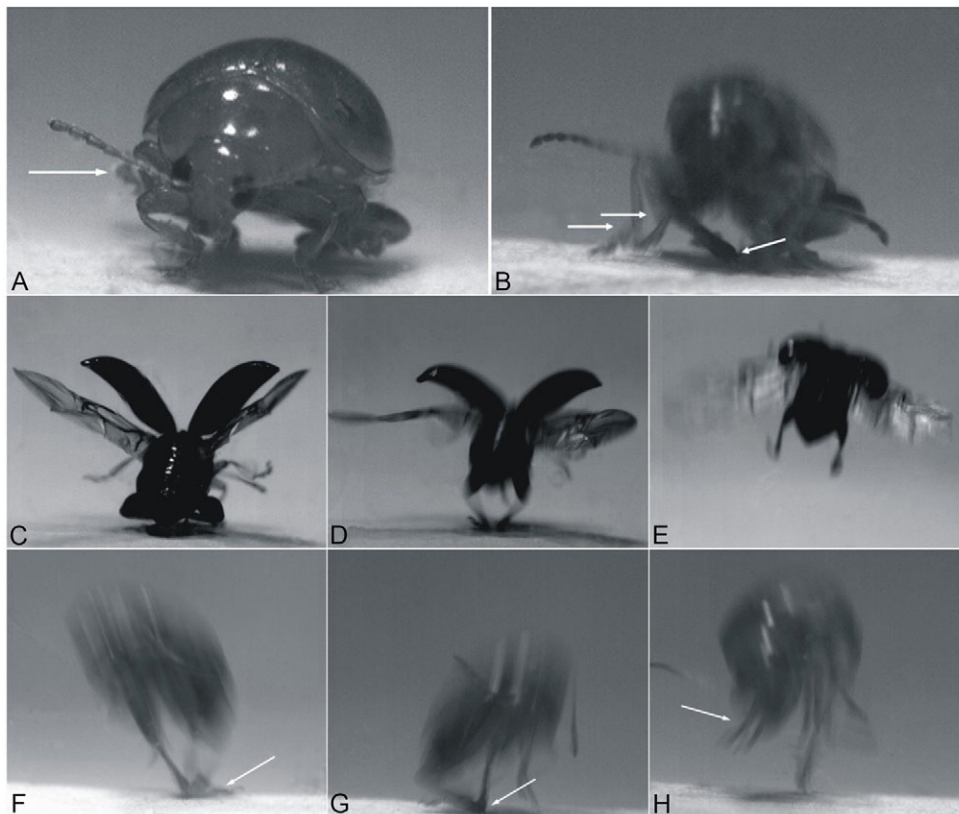
Fig. 3. Photographs of flea beetles during the different phases of a jump. (A) *Sphaeroderma testaceum*, phase 2; the arrow indicates the position of the lifted middle leg; frame rate 3000 frames s^{-1}. (B) *Longitarsus pratensis*, phase 2; the arrows indicate the position of the fore and middle legs; frame rate 3000 frames s^{-1}. (C–E) Jump by *Crepidodera aurata* in which the wings were opened and moved; frame rate 3000 frames s^{-1}: C, before the take-off; D, at the moment of take-off; E, after take-off. (F) *Aphthona cyparissiae*, phase 2; moment of take-off; the arrow shows the position of the hind tarsi contacting the substrate; frame rate 2000 frames s^{-1}. (G) *Longitarsus pratensis*, phase 2; the arrow shows the position of the hind tibia contacting the substrate by the tibial apices; frame rate 3000 frames s^{-1}. (H) Jump by *L. pratensis* at the moment of take-off propelled by one hind leg; the arrow shows the flexed right leg; frame rate 3000 frames s^{-1}.

(excitation 500 nm, emission 525 nm), Cy5 (excitation 646 nm, emission 664 nm).

Scanning electron microscopy (SEM)

Hind legs were removed from the body of a beetle, dissected with a razor blade, immersed overnight in 10% KOH if necessary, cleaned in distilled water, stepwise dehydrated in ethanol, critical-point dried (Polaron 3100, Quorum Technologies, UK), fixed onto stubs, coated with gold (Emitech K550X, Quorum Technologies) and investigated with a scanning electron microscope (Carl Zeiss EVO LS10).

Synchrotron X-ray micro-computed tomography (SR-µCT)

The freshly caught specimens of beetles were preserved in 70% alcohol; the hind legs were removed and stepwise dehydrated in increasing ethanol concentrations, and critical-point dried (Polaron 3100, Quorum Technologies); dry specimens from museum collections were left intact. The hind legs were glued onto the tips of plastic stubs (1.2 cm long, 3.0 mm in diameter). For SR-µCT, we used the ID19 beamline at the European Synchrotron Radiation Facility (ESRF, Grenoble; experiment LS-2342) at 19 keV (wavelength 8×10^{-11} m) and an effective detector pixel size of 0.65 µm with a corresponding field of view of 1.43×1.43 mm; 6000 projections were recorded over the 180 deg rotation. The detector-to-sample distance was 12 mm. For 3D reconstruction, we used the graphic segmentation tool software Amira® 6.0 (FEI Company, Visage Imaging, Germany) and the volume graphics visualization Drishti 2.5.1 (Limaye, 2014).

High-speed video recording

Jumping performance was examined using a high-speed video camera (Photron Fastcam SA3 120K-M2, Photron, Germany) combined with a stereomicroscope (Leica MZ7.5). In total, 63 records for five species from five genera were acquired (Table 1) and
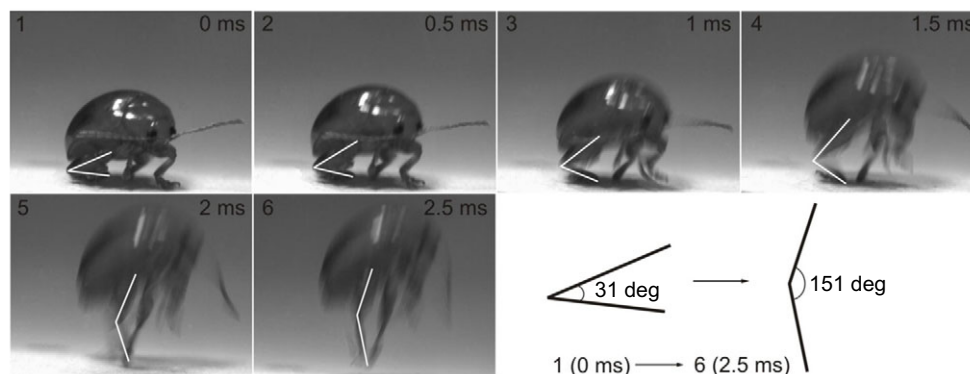

Fig. 4. Natural jump by the flea beetle *Sphaeroderma testaceum* showing extension of the hind tibia. Frame-by-frame depiction of the jump; frames are numbered according to their sequence; images were captured at a rate of 3000 frames s^{-1}. The schematic diagram shows extension of the tibia during phase 2 of the jump.
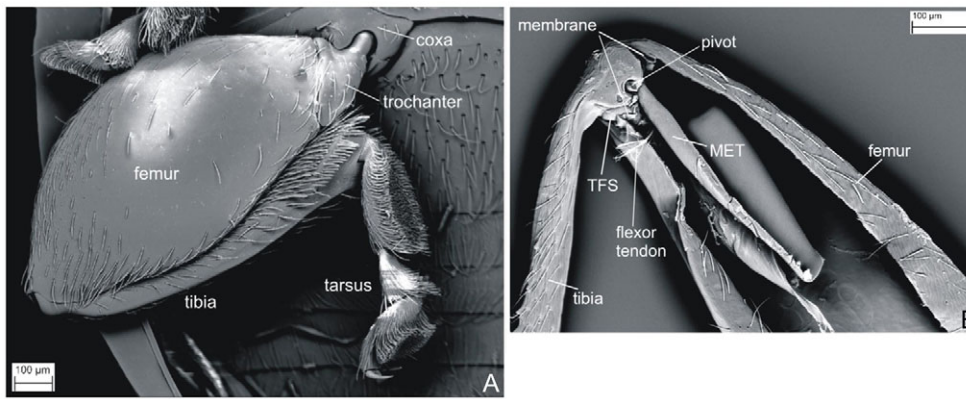
Fig. 5. Scanning electron microscopy (SEM) images showing the structure of the jumping leg in flea beetles. (A) External morphology of the hind leg of *Sphaeroderma testaceum*. (B) Sagittal section of the hind jumping leg of *Longitarsus pratensis*. MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

27 of them were chosen for calculation of kinematic parameters using Tracker v4.87 software (www.cabrillo.edu/~dbrown/tracker). Beetles were captured in a transparent plastic cube of 2×2 cm; the jumps were recorded without stimulation at a rate of 2000 and 3000 frames s^{-1}.

The image processing and preparation for publishing were carried out by Adobe Photoshop® CE6 EXD (Adobe Systems Inc., USA) and CorelDraw® Graphic Suite 12 (Corel Corp., Canada).

RESULTS
Jumping performance and kinematics
The jumping process and performance
The jump is performed by the hind legs and starts while the hind tibiae are beginning to extend (Fig. 2). Three phases of the jump are distinguishable, as described below.

Phase 1, preparation: the body is oriented in the jumping direction and its position is close to horizontal (Figs 2, 3A). The hind tibiae are fully flexed; sometimes partly flexed tibiae perform the jump. Usually, the fore legs are in full contact with the substrate (Fig. 3B), while one or both middle legs might be lifted (Fig. 3A).

Phase 2, take-off: the hind tibiae start to extend, simultaneously lifting the body into the air. The jump is performed either with closed wings (mostly as a defense reaction) or with fully open hind wings (Fig. 3C–E) and flight occurs right after take-off (mostly for relocation to another place, e.g. from one leaf to another one). During the extension, the hind legs are in contact with the substrate either through the hind tarsi (Fig. 3F) or through the tibial apices (Fig. 3G). The two hind legs usually move together but sometimes can move asynchronously, and some jumps are propelled by just one

hind leg (Fig. 3H); however, such jumps are weaker and poorly directed. The angle between the hind tibia and the femur in the fully flexed position is about 30–35 deg (Fig. 4). After full extension, the angle reaches up to 145–155 deg (Fig. 4). The full extension of hind tibia takes on average about 1–3 ms.

Phase 3, post-take-off: right after loss of contact with the substrate, the body moves according to the initial trajectory of the jump, which is close to straight, and with the velocity vectors corresponding to the trajectory (Fig. S1). The body might experience a rotation in the longitudinal or the transverse axis.

Kinematic parameters
The kinematic parameters of the jumps of five species of five genera of flea beetles were calculated (Table 1). The rate of acceleration, velocity and kinetic energy for two of the species, *Aphthona cyparissiae* and *Chaetocnema aridula*, are presented in Fig. S2, which corresponds to the frame-by-frame depiction of jumps shown in Fig. 2. The highest value of acceleration was reached in the first 1–2 ms after the nominal starting point (phase 2: during take-off) and sharply decreased in phase 3 right after take-off. The greatest velocity and kinetic energy of jump were achieved in phase 3 within 1–2 ms of take-off, with a subsequent gradual decrease.

Morphology of the jumping hind leg
External morphology
The jumping hind leg in flea beetles is a walking (cursorial) type of leg (Fig. 5A). The general structure of the primary elements of the legs, such as the transverse coxa, small trochanter, elongated femur and tibia, femoro-tibial joint and tarsus, is principally identical to
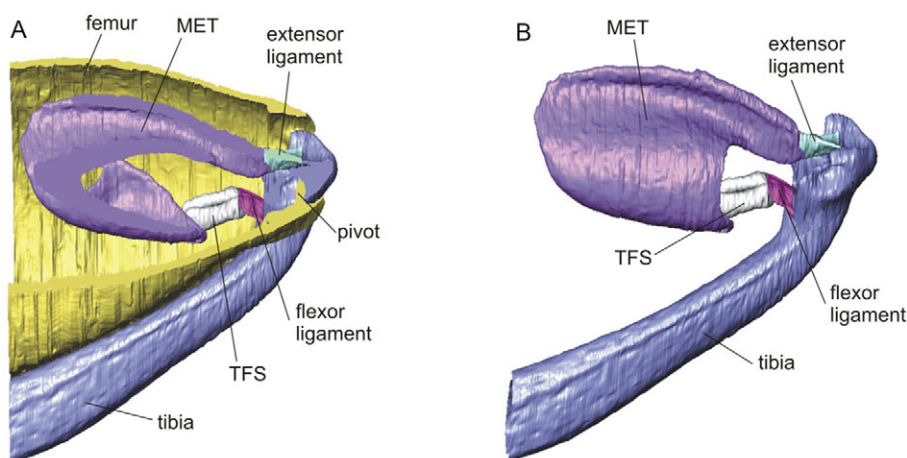

Fig. 6. Femoro-tibial joint of the hind leg of *Sphaeroderma testaceum* based on synchrotron X-ray micro-computed tomography (SR-μCT) data reconstruction. (A) Sagittal section of the femur, lateral view. (B) Femoro-tibial joint with femoral wall removed. MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.
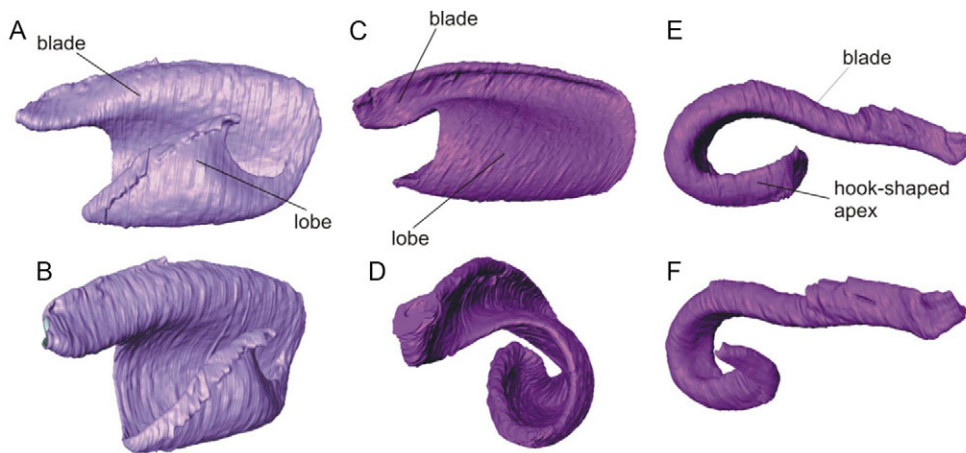
Fig. 7. Shape and structure of the modified MET in leaf beetles based on SR-μCT data reconstruction. (A) *Sphaeroderma testaceum*, lateral view. (B) *Sphaeroderma testaceum*, antero-lateral view. (C) *Hyphasis unicolor*, lateral view. (D) *Hyphasis unicolor*, anterior view. (E) *Eubaptus scapularis*, lateral view. (F) *Eubaptus scapularis*, antero-lateral view.

those of non-jumping leaf beetles. The peculiar feature of flea beetles is a swollen metafemur whose length/width ratio varies from 1.5 to 3.5 (Fig. 5A), whereas typical ratios in other leaf beetles do not exceed 3.5 and are usually lower. The general structure and shape of the metafemur is typical of other leaf beetles. The surface is usually covered with short hairs of different density; denticles, spines or other projections are absent (a large projection is known in the genus *Podontia* Dalman 1824); the ventral surface shows a longitudinal concavity for the insertion of the tibia in the fully flexed position (Fig. 5A). The metatibia in jumping leaf beetles of the subfamily Galerucinae shows a large intergeneric variability in morphology (Furth, 1980) that has possible functional consequences for jumping. The length of the metatibia varies from being shorter than the metafemur (e.g. in genera of the subtribes Monoplatina and Oedionychina) to being equal to it (remaining genera). The distance of the tarsal articulation varies from the apical position (in the majority of genera) to a position shifted up to the mid-length of the tibia (e.g. in *Psylliodes* Latreille 1829). The apical spur is considered an important structure to ensure contact of the tibia with the substrate surface, especially during jumping. Whereas in the majority of genera the apical spur is very short and acute, there are some extreme cases with largely shortened metatibia and strongly elongated apical spurs (in *Serraphula* Jacoby 1897 and *Aphthonoides* Jacoby 1885, see Biondi and D'Alessandro, 2010, figs 1–20; Döberl, 2005, figs 2, 3, 31, 75) or with long and bilobate apical spurs (e.g. in *Dibolia* Latreille 1829).

Metafemoral extensor tendon (MET)

The primary morphological elements of the jumping apparatus in flea beetles are: MET, tibial flexor sclerite (TFS), extensor ligament, flexor ligament and femoro-tibial joint (Fig. 5B). In jumping leaf

beetles (subfamilies Galerucinae and Bruchinae), the metafemoral extensor tendon (MET) is enlarged and morphologically modified (Figs 5B, 6–8). In non-jumping leaf beetles, the extensor tendon is represented by a long, narrow and flexible strip or string, or a bunch of thin fibers. The distal apex of the MET is connected to the tibial base by a ligament (terminology by Furth, 1988; Zombori and Steinmann, 1999) (Fig. 6). The main body of the MET forms the attachment site of several bunches of extensor muscles. Two principal types of the MET characteristic to jumping leaf beetles are revealed. Type 1 – folded, consisting of a central longitudinal blade and a ventrally curved lobe (Figs 7A–D, 8A); the lobe is a folded, broad, flat projection of the central blade, with a configuration and curvature that vary greatly from genus to genus (Furth, 1982, 1988). This type is characteristic to flea beetles (Galerucinae: Alticini). Type 2 – hook-shaped (Fig. 7E,F), with a narrow to slightly broadened central longitudinal blade and a distal hooked apex; there is no curved ventral lobe. This type is characteristic to some flea beetles of uncertain taxonomic position (*Hespera* Weise 1889, *Sphaerometopa* Chapuis 1875, *Acrocrypta* Baly 1862, *Chaloenosoma* Jacoby 1893, *Licyllus* Jacoby 1885, *Sjoestedtinia* Weise 1910; see also Furth and Suzuki, 1998) and to seed beetles of the genus *Eubaptus* Lacordaire 1845 (Bruchinae: Eubaptini) (see also Furth and Suzuki, 1990b, 1992). It is also found in the jumping weevils Curculionidae (subfamilies Curculioninae and Ceutorhynchinae) (Furth and Suzuki, 1992).

The macrostructure of the MET is composed of several layers of cross-oriented fibers (Fig. 8B). Previous tests for resilin in the MET have been negative (Furth et al., 1983), which corresponds to the findings of the present investigation. Fluorescence microscopy data revealed an autofluorescence of the MET material in the wavelength spectrum covered by Alexa488 and Cy5 filters (see 'Resilin in the
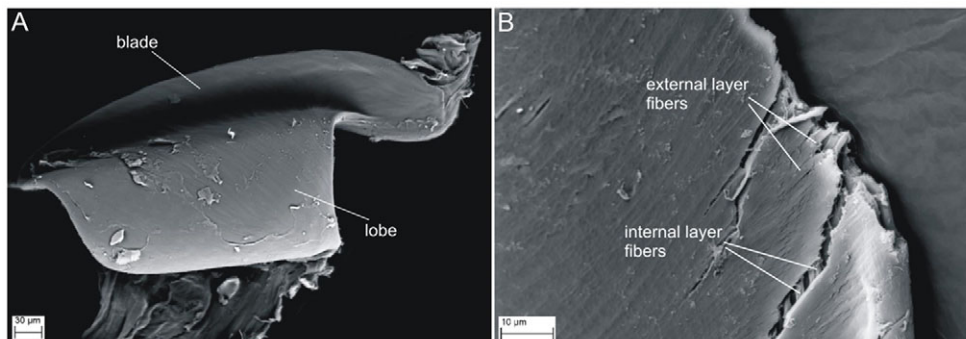

Fig. 8. Shape and structure of the modified MET in flea beetles. (A) MET of *Sphaeroderma testaceum*. (B) Cross-section of the MET of *Crepidodera aurata* showing the external and internal layers of cross-oriented fibers.
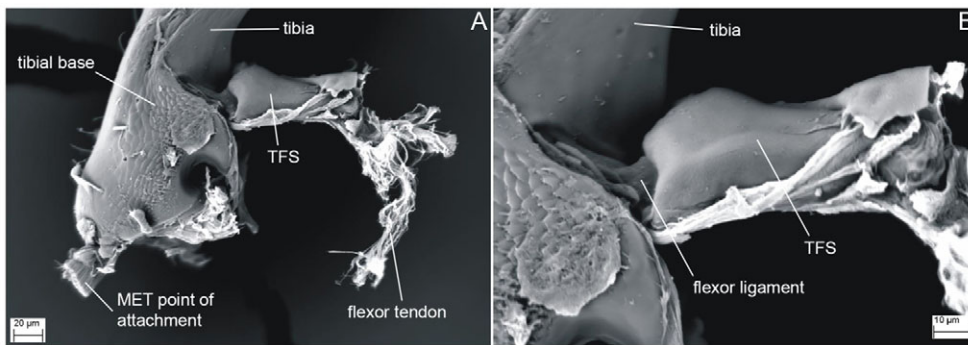
Fig. 9. Tibial base and femoro-tibial joint of the flea beetle *Longitarsus pratensis*. (A) Tibial base with spatial positions of the TFS and MET (major part removed). (B) Lateral view of the TFS. MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

legs of jumping leaf beetles'; see below). The same autofluorescence was discovered in the external and internal cuticle of the femur and the tibia, which confirms the similarity of the material of the MET to a chitin-based cuticle of the exoskeleton. It should also be noted that the most intensive autofluorescence was revealed by the Cy5 filter, corresponding to the material of the harder outer cuticle.

Tibial flexor sclerite (TFS)

The TFS (Furth and Suzuki, 1990a,b) is a part of the tibial flexor system and is represented in flea beetles by a thick sclerotized plate of triangular shape (Figs 9 and 10). In the fully flexed position of the tibia, the TFS is completely drawn into the femoral capsule (Fig. 6A), whereas it is entirely exposed in the fully extended tibia. The TFS has three points of attachment: (1) the distal apex is joined to the tibia by a short resilin-bearing ligament (Fig. 9B); (2) the proximal edge is connected to the flexor muscles by thin fibers (Fig. 9A); and (3) the ventral side is joined to the internal side of the femoral wall by an elastic membrane (Fig. 5B).

Musculature

One of the most conspicuous structural differences between the jumping and the non-jumping leaf beetles is the enlarged volume of the extensor muscles in the hind femur (Fig. 11). The muscular system of the metafemur of the jumping leg consists of a group of extensor muscles and a bunch of flexor muscles. Extension of the tibia is powered by a group of large muscles that occupy most of the space within the femur. The extensor group is presented by three main muscles: (1) a dorsal muscle attached to the longitudinal blade of the MET and to the dorsal side of the femoral capsule (Fig. 11A,E, G,H: dm); (2) an internal lateral muscle attached to the outer side of the curved lobe of the MET and to the surface of the inner side of the femur (Fig. 11B,G,J: ilm); and (3) an external lateral muscle attached to the curved lobe of the MET and to the surface of the outer side of the femur (Fig. 11B,D,E,F: elm). The flexor muscle is attached to the TFS and to the ventral and posterior surface of the

femoral capsule (Fig. 11C: fm). The extensor muscles are oriented mostly in a longitudinal axis corresponding to that of the femur; thus, they can move the MET backward only along the longitudinal axis.

Resilin in the legs of jumping leaf beetles

To detect the possible presence of resilin in the jumping apparatus of flea beetles, we examined the species *Sphaeroderma testaceum*, *Podagrica fuscicornis* and *Altica oleracea* (Fig. 12), representing different phylogenetic lineages of Alticini (Ge et al., 2012). The resilin was revealed by the intense blue fluorescence under UV illumination in the following structures of the jumping leg: (1) the extensor ligament connecting the metafemoral extensor tendon with the base of the hind tibia (Fig. 12A–H); (2) the flexor ligament connecting the tibial flexor sclerite with the base of the hind tibia (Fig. 12B–H); (3) the membranes surrounding the femoro-tibial joint and the membranes connecting the TFS and the femoral cuticle (Fig. 12B–D); (4) the extensor tendon attached to the ventral curved lobe of the MET (Fig. 12B); and (5) the flexor tendon attached to the base of the TFS.

The extensor ligament between the MET and the tibial base is a compound structure. Two structural parts of this ligament revealed by the SEM observation are distinguishable: (1) a basal part that covers the very tip of the MET from which it is separated by a visible border (Fig. 12A) and (2) an apical part that is connected to the tibial base and consists of numerous convoluted and sinuous fibers (Fig. 12I). The extensor ligament also has two material components distinguishable and revealed by their autofluorescence in two spectral regions covered by DAPI and Alexa488 filters, correspondingly. The first material component indicates the presence of resilin (Fig. 12A), whereas the second corresponds with the autofluorescence of other parts of the leg, especially the internal surface of the cuticle of the femoral capsule, and the cuticle of the tibia (Fig. 12A,C). The extensor ligament is an elastic structure demonstrated by the results of the fluorescence microscopy (Fig. 12C–H). An approximate estimation revealed that the length of this ligament is about three times greater in the longest (fully flexed
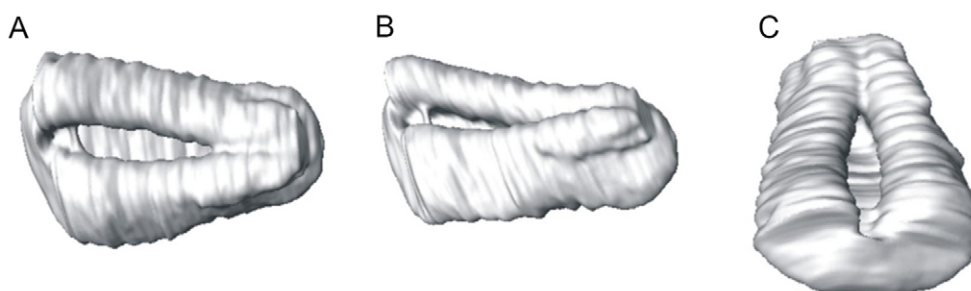

Fig. 10. External structure of the TFS of *Sphaeroderma testaceum* based on SR-μCT data reconstruction. (A) Dorsal view. (B) Dorso-lateral view. (C) Antero-dorsal view.
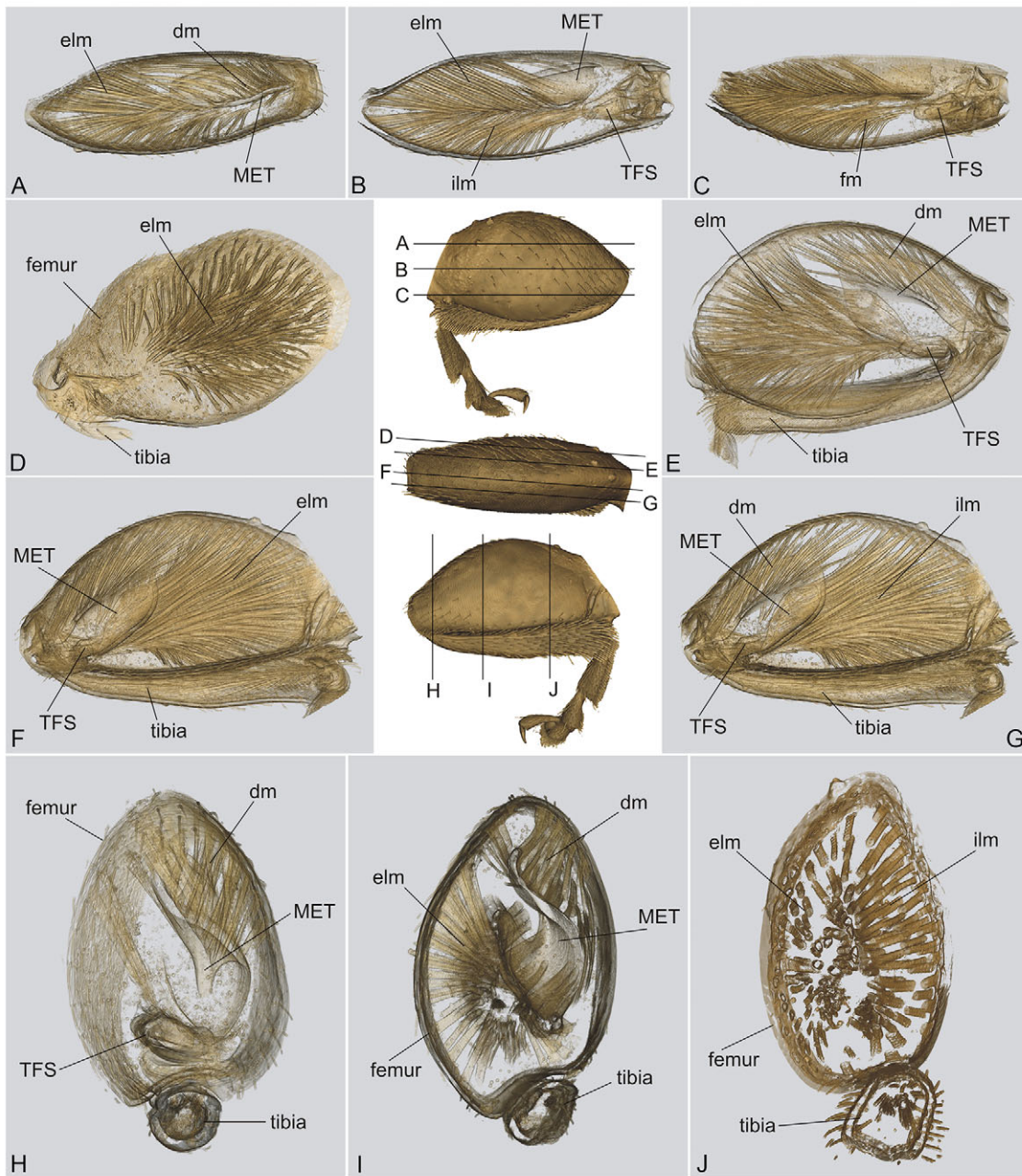
Fig. 11. Muscular system of the metafemur of the jumping hind leg of *Sphaeroderma testaceum* based on SR-µCT data volume reconstruction. (A–C) Frontal plane sections. (D–G) Sagittal plane sections. (H–J) Transverse plane sections. The sections in A–J are indicated in the central panel. dm, dorsal extensor muscle; elm, external lateral extensor muscle; ilm, internal lateral extensor muscle; fm, flexor muscles; MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

tibia; Fig. 12C,F) compared with the shortest (fully extended tibia; Fig. 12E,H) condition.

Mechanics of the jump in flea beetles
Mechanics of the jump

The basic mechanical quantities for describing the initial phase of a jump during extension of the hind tibia were measured and calculated for *Sphaeroderma testaceum* (Table S1) and followed the methodology described in Betz and Mumm (2001). The calculated joint power output of the jumping movement of the femoro-tibial joint was $13.5×10^{-5}$ W (max. $25.01×10^{-5}$ W) and the specific joint power amounted to 0.387 W (max. 0.714 W).

The mechanical lever system of the femoro-tibial joint of *Sphaeroderma testaceum*

The femoro-tibial joint is a simple mechanical system in which the tibia is a two-armed class 1 lever (Fig. 13). Two levers are distinguishable in this system: (1) an extensor lever (anatomical arms A_1 and A_2) that maintains the extension of the tibia and (2) a flexor lever (anatomical arms A_2 and A_3), maintaining the flexion of the tibia.

In the totally flexed condition, the ratio of arms (measured and calculated based on the scheme shown in Fig. 13B) of the mechanical flexor lever (L_2 and L_3) is about 1:5, so that the value of the mechanical advantage of the flexor lever over the extensor lever
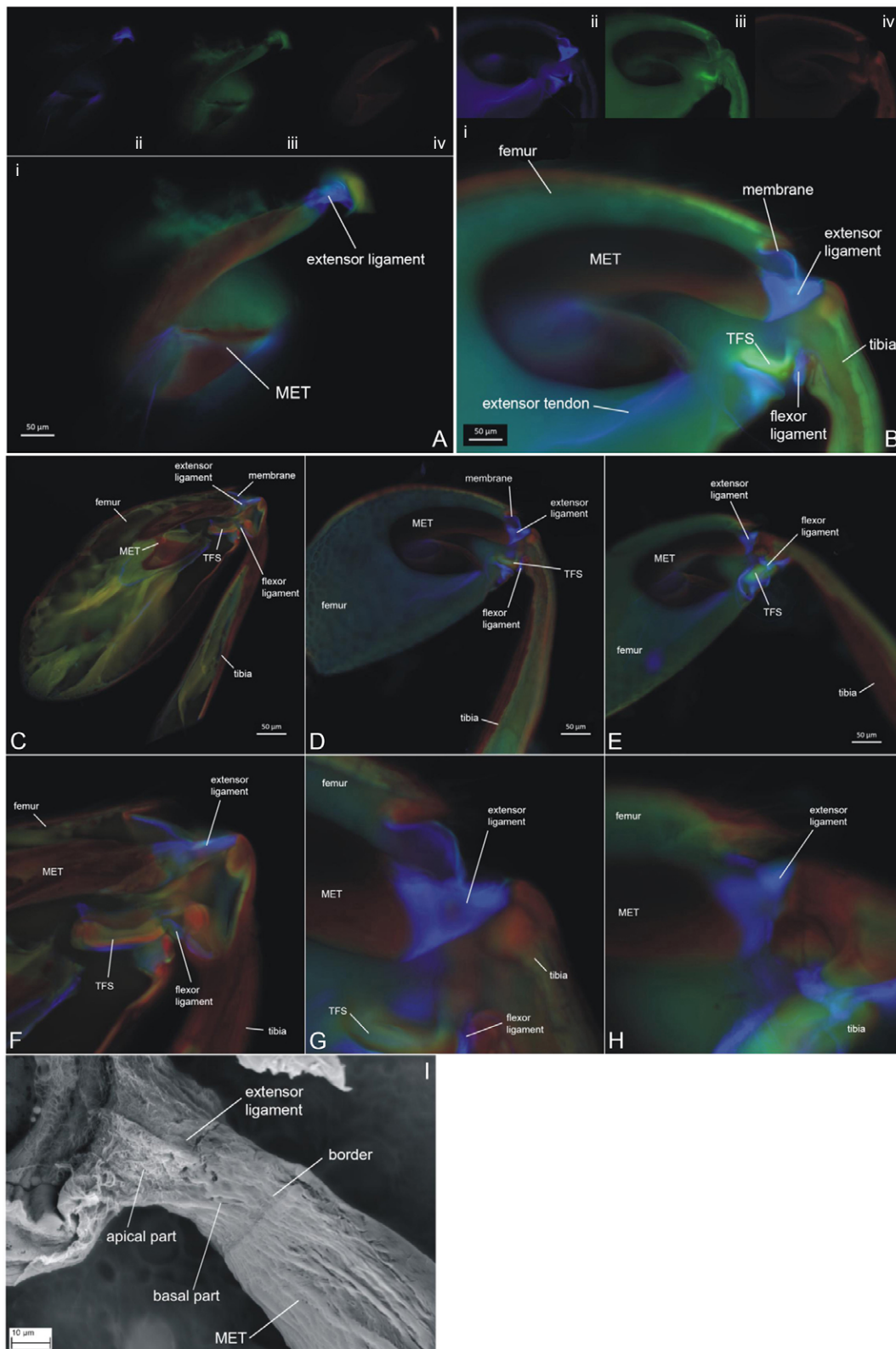
Fig. 12. Resilin in the hind leg of flea beetles based on fluorescence microscopy. The presence of resilin is indicated by the blue color. (A, B) Photographs taken under the different filters: i, combined image of the photographs taken under the different filters; ii, DAPI filter (blue); iii, Alexa488 filter (green); iv, Cy5 filter (red). (A) Modified metafemoral extensor tendon of *Podagrica fuscicornis*. (B) Femoro-tibial joint of *Sphaeroderma testaceum*. (C–H) Compression and extension of the resilin-bearing extensor ligament in the femoro-tibial joint of the hind leg of *Sphaeroderma testaceum* (combined images taken under DAPI, Alexa488 and Cy5 filters). C–E: the hind leg showing the different positions of the tibia: C, flexed; D, intermediate; E, extended. F–H: the region of the femoro-tibial joint of the corresponding photograph in C–E under greater magnification, showing the extensor ligament: F, extended; G, half-compressed; H, compressed. (I) SEM photograph of the extensor ligament in the femoro-tibial joint of the hind leg of *Longitarsus pratensis*. MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.
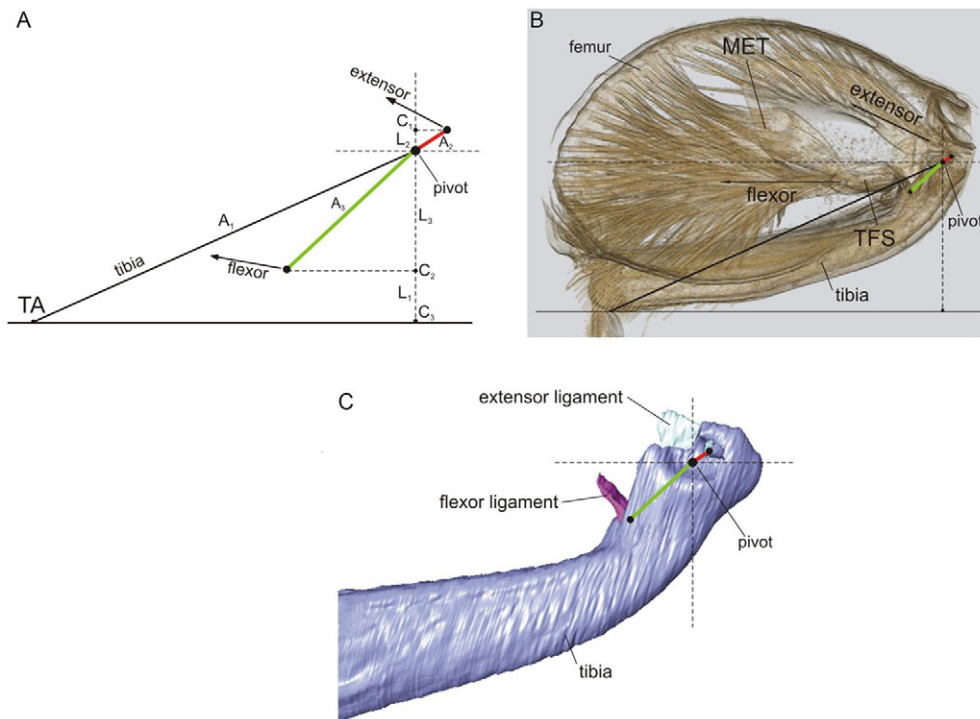
Fig. 13. Leverage scheme in the femoro-tibial joint of the hind leg of a flea beetle exemplified by the visualization and measurement of the hind leg of ***Sphaeroderma testaceum*** **in its fully flexed position.** (A) Scheme of the mechanical model of the levers in the femoro-tibial joint. The proportions of the arms have been changed intentionally to fit the size of the image and do not represent natural conditions. A_1, output arm of the anatomical extensor lever (black line); A_2, input arm of the anatomical extensor lever (red line); A_3, input arm of the anatomical flexor lever (green line); L_1, output arm of the mechanical extensor lever (from the pivot to point C_3); L_2, input arm of the mechanical extensor lever (from the pivot to point C_1); L_3, input arm of the mechanical flexor lever (from the pivot to point C_2). TA, tibial apex. (B) Reconstruction of the virtual sagittal plane section of the hind leg (based on SR-µCT data) with coordinated scheme of the leverage which was used for measurement of the lengths of the lever arms. MET, metafemoral extensor tendon; TFS, tibial flexor sclerite. (C) Tibial base with spatial positions of the extensor and flexor ligaments, with corresponding positions of the flexor (green line) and extensor (red line) arms of levers (based on SR-µCT data).

amounts to a factor of 5 (Fig. 13A). At the same time, the ratio of arms of the mechanical extensor lever (L_2 and L_1) is about 1:25, so that the value of the mechanical disadvantage of the extensor lever amounts to a factor of 25 (Fig. 13A).

DISCUSSION
The role of the metafemoral extensor tendon in the jumping mechanism
The morphology, functional role and phylogenetic relevance of the MET (also known as the metafemoral spring, metafemoral apodeme or Maulik's organ) have been extensively studied for flea beetles, tribe Alticini (Furth, 1980, 1982, 1988, 1989; Furth and Suzuki, 1990b, 1992, 1994, 1998; Furth et al., 1983; Schmitt, 2004; Betz et al., 2007). It was proposed (see Introduction) that the MET is a spring-like specialized structure for the storage of elastic strain energy performed by its compression and dilation, resulting in a fast extension of the tibia. However, we now suggest that the MET itself cannot store large amounts of energy and work as a spring for the following reasons: (1) the MET is composed of several layers of fibers arranged in a cross-oriented direction (Fig. 8B), so that the longitudinal fibers might prevent significant distortion or recoil in the transverse direction and vice versa, and (2) the MET does not contain resilin (Fig. 12A,B) and appears rigid and non-elastic upon mechanical testing with forceps. The modification of the MET is a peculiar feature in beetles that is exclusively found in jumping life forms. Its peculiarly folded morphology (Fig. 7, 8A) can be considered a compromise between the limited volume of the

metafemur and the demand to provide an increased surface area for muscle attachment.

The role of the tibial flexor sclerite in the jumping mechanism
The TFS or Lever's triangular plate is a modification of the tibial flexor tendon as found in Hemiptera, Neuroptera, Megaloptera, Hymenoptera and Coleoptera (Furth and Suzuki, 1990a,b). The TFS is found in both jumping and non-jumping Chrysomelidae. Functional aspects of the TFS proposed by Furth and Suzuki (1990a) and Gorb (1995) are: (1) strengthening of the base for the flexor tendon; (2) increasing the working angle of the leg flexor system; and (3) protection of the ventral side of the exposed femoro-tibial joint.

Barth (1954), based on the studies of the flea beetle *Homophoeta sexnotata* Harold 1876, and later Betz et al. (2007: figs 11, 12), based on SR-µCT findings in *Altica* sp., proposed that the TFS may serve as a key part of a catching mechanism preventing the premature extension of the tibia during the co-contraction of both the flexor and the extensor muscles. According to this model, the catching mechanism might be accomplished by pressing the TFS against the distal margin of the posterior femoral wall that forms an abutment (Betz et al., 2007: figs 11 and 12E–G). Although in most of our examined genera the TFS was placed far from the femoral wall (Fig. 11E), it cannot be excluded that the TFS might at least in some species function as a catch, which momentarily prevents the extension of the tibia, whereas the co-contracting extensor and
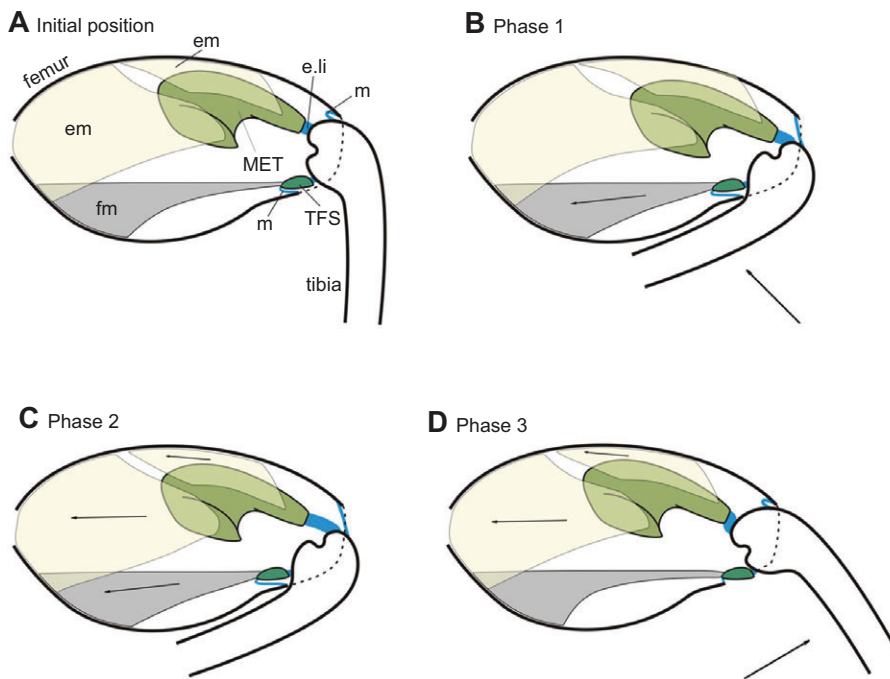
Fig. 14. Schematic model of the crucial functional elements in the hind leg and depiction of the different phases of their movement during a jump. (A) Initial position of the tibia. (B) Phase 1, flexion of the tibia; arrows indicate the direction of the flexor muscle contraction and flexion of the tibia. (C) Phase 2, co-contraction of the antagonistic muscles and extension of the extensor ligament; arrows indicate the direction of contraction of the extensor and flexor muscles. (D) Phase 3, extension of the tibia; arrows indicate the direction of contraction of the extensor muscle and extension of the tibia. em, extensor muscles; fm, flexor muscles; e.li., extensor ligament; m, membrane; MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

flexor muscles build up the energy necessary for the jump (see below).

Functional morphological analysis of the jumping mechanism in flea beetles

The jumping movement in terms of the attainment of velocity and acceleration is connected to the rapid extension of the hind tibiae in the femoro-tibial joint during phase 2 of the jump (Figs 2 and 4). This fastest part of the jump is reached when the tibia extends in 1–3 ms by about 110–115 deg (from about 30–35 to about 145–150 deg; Fig. 4). The recorded maximum velocity in one *Longitarsus pratensis* individual reached 3.6 m s^{-1} within the initial 1.2 ms. The kinematic parameters of jumping flea beetles were also calculated by Brackenbury and Wang (1995). The following kinematic parameters were obtained by these authors for seven species of six genera (*Aphthona*, *Psylliodes*, *Longitarsus*, *Crepidodera*, *Podagrica* and *Altica*; values are given from minimum to maximum): take-off time 1.1–7.7 ms, velocity 0.72–2.93 m s^{-1}, acceleration 0.1–2.66 ($\times 10^3$) m s^{-2}, kinetic energy 0.96–17.60 µJ. These authors also investigated visual targeting and the ability to rule out and to correct both jumping direction and jumping distance. The kinematic parameters in jumping insects, e.g. fleas, grasshoppers, froghoppers, planthoppers, leafhoppers, shore bugs, stick insects and snow fleas, vary in a broad range (values are given from minimum to maximum): take-off time 0.7–14.9 ms, velocity 0.5–5.5 m s^{-1}, acceleration 36–7000 m s^{-2}, kinetic energy 0.4–11,000 µJ, *g*-force 4–719 (from Burrows, 2006a,b, 2007a, 2008, 2009a,b, 2011; Burrows and Morris, 2003; Burrows and Picker, 2010; Sutton and Burrows, 2011). As follows from Table 1, the kinematic parameters of flea beetles are comparable with those of other jumping insects. Such values are characteristic to jumpers for which a catapult mechanism was established and the source of the additional energy for the jump is the elastic energy stored in the spring-like structures prior to the jump.

The question is: can the femoral extensor muscles generate the propulsive movement by direct muscle contraction or by a catapult

mechanism allowing for abrupt release of energy that has been stored? Although the short input extensor lever (see Fig. 13) already entails a velocity gain (see Gronenberg, 1996), the anatomical structure and the mechanical conditions of the femoro-tibial joint of flea beetles indicate that power amplification must be involved in jumping.

There exist some indirect indications of the presence of an energy storage mechanism in the rapid tibial extension during phase 2 of the jump: (1) the examined duration of the full extension of a hind tibia until take-off is very short (on average 1.5–3 ms; Table 1), which is well below the temporal limitations known for insect muscle contractions (Gronenberg, 1996; Josephson, 1975; Neville and Weis-Fogh, 1963; Usherwood, 1962); (2) the calculated maximum specific joint power developed by the extensor muscles required for the recorded jumps reached 0.714 W g^{-1} (see Results, 'Mechanics of the jump'; Table S1). This exceeds the reported specific power output of about 0.1 W g^{-1} that insect muscles can exert by direct action (Ellington, 1985; Josephson, 1975; Weis-Fogh, 1956; Machin and Pringle, 1959). Similar conditions were revealed in the raptorial fore limbs of the rove beetle *Philonthus marginatus* (Stroem 1768), suggesting that a catapult mechanism is involved in their rapid unfolding (Betz and Mumm, 2001).

In the flea beetles examined here, the extensor ligament between the modified metafemoral extensor tendon and the tibial base (Fig. 6) is the only elastic structure directly associated with the extensor system, where potentially additional energy for the jump can be stored. According to its resilin content (Fig. 12A–H), this is a structure with high elasticity and the ability to reversibly deform. It is subjected to extension when the tibia is flexed (Fig. 12C,F) and to compression when the tibia is extended (Fig. 12E,H). We suppose that, prior to the jump, the extensor ligament becomes loaded via stretching through the contracting extensor muscles. This requires a fully flexed position of the hind tibia that simultaneously must be held by the contraction of the weaker flexor muscles. Such a co-contraction of antagonistic muscles for the accumulation of elastic energy has been established in the raptorial limb extension in rove beetles (Betz and Mumm, 2001), the jumping legs of

locusts and crickets (Heitler, 1974; Burrows and Morris, 2003; W. J. Heitler, http://www.st-andrews.ac.uk/~wjh/jumping/index. html), and the coxo-trochanteral articulation of leafhoppers (Burrows, 2007b), for example. In some cases (Betz and Mumm, 2001; W. J. Heitler, http://www.st-andrews.ac.uk/~wjh/jumping/ index.html), this mechanism functions by giving a mechanical force advantage to the weaker flexor muscles over the stronger extensor muscles as a result of the ratio of the lever's arms. As follows from the measurements for the leverage system of the femoro-tibial joint exemplified by *Sphaeroderma testaceum*, in the fully flexed position, the flexor lever has about five times the mechanical advantage over the extensor lever (see Results, 'MET'; Fig. 13). Thus, it can be supposed that the contracted flexor muscle is able to hold the tibia in a fully flexed position while the extensor muscle is co-contracting concomitantly, extending the resilin-bearing extensor ligament still further. It can be assumed that the elastic energy stored in this way in the resilin-containing extensor ligament, the string-like extensor tendon (Fig. 12B) and the muscles is rapidly released upon relaxation of the flexor muscles.

Model for the jumping mechanism in flea beetles

Based on the data presented here, we propose the following functional model of the jumping mechanism of flea beetles (Fig. 14).

Phase 1: contraction of the flexor muscle; flexion of the hind tibia with simultaneous short dilation of the resilin-containing extensor ligament (between the tendon and the tibial base; Fig. 14B).

Phase 2: co-contraction of extensor muscles; nevertheless, the flexor muscle holds the tibia in its flexed position according to its effective lever advantage; extensor muscles slowly extend the ligament still further, with accumulation of the additional amount of elastic energy (Fig. 14C).

Phase 3: rapid relaxation of the flexor muscles; extension of the tibia via the energy pre-stored in the resilin-bearing ligament, contracting extensor muscles and extensor tendon (Fig. 14D).

An additional argument in favor of this model is the ability of flea beetles to carry out visual targeting (Brackenbury and Wang, 1995) and the jumping behavior observed in the laboratory and in the field (K.N. and O.B., original unpublished data). It was demonstrated that the beetles are able to control both the speed and the trajectory of a jump with high accuracy. The beetles may use a jump for bridging short distances (on a plant) for which the precise control over the distance, trajectory and speed is relevant. Alternatively, jumps can be used as escape reactions when high-speed reactions are needed. The take-off velocity and acceleration might depend on the accumulated and released energy during phase 2 of the jump. Such a control might be realized by the combination of fast and slow motor neurons associated with the extensor muscle (Hoyle, 1978; Hughes and Salinas, 1999) regulating its degree of contraction and the extension of the extensor ligament.

Author contributions
Both K.N. and O.B. developed the scientific question and prepared the study design. K.N. carried out the experiments, observations and calculations, and prepared the manuscript and the figures. O.B. discussed the results and revised the manuscript.

Supplementary information
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