Arthropod Structure & Development 47 (2018) 131–143

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

Jumping mechanisms and performance in beetles. II. Weevils (Coleoptera: Curculionidae: Rhamphini)

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ARTICLE INFO

Article history:
Received 2 January 2018
Accepted 23 February 2018
Available online 6 March 2018

Keywords:
Coleoptera
Functional morphology
Jump
Kinematics
Locomotion
Resilin

ABSTRACT

We describe the kinematics and performance of the natural jump in the weevil *Orchestes fagi* (Fabricius, 1801) (Coleoptera: Curculionidae) and its jumping apparatus with underlying anatomy and functional morphology. In weevils, jumping is performed by the hind legs and involves the extension of the hind tibia. The principal structural elements of the jumping apparatus are (1) the femoro-tibial joint, (2) the metafemoral extensor tendon, (3) the extensor ligament, (4) the flexor ligament, (5) the tibial flexor sclerite and (6) the extensor and flexor muscles. The kinematic parameters of the jump (from minimum to maximum) are 530–1965 m s^{-2} (acceleration), 0.7–2.0 m s^{-1} (velocity), 1.5–3.0 ms (time to take-off), 0.3–4.4 μJ (kinetic energy) and 54–200 (g-force). The specific joint power as calculated for the femoro-tibial joint during the jumping movement is 0.97 W g^{-1}. The full extension of the hind tibia during the jump was reached within up to 1.8–2.5 ms. The kinematic parameters, the specific joint power and the time for the full extension of the hind tibia suggest that the jump is performed via a catapult mechanism with an input of elastic strain energy. A resilin-bearing elastic extensor ligament that connects the extensor tendon and the tibial base is considered to be the structure that accumulates the elastic strain energy for the jump. According to our functional model, the extensor ligament is loaded by the contraction of the extensor muscle, while the co-contraction of the antagonistic extensor and flexor muscles prevents the early extension of the tibia. This is attributable to the leverage factors of the femoro-tibial joint providing a mechanical advantage for the flexor muscles over the extensor muscles in the fully flexed position. The release of the accumulated energy is performed by the rapid relaxation of the flexor muscles resulting in the fast extension of the hind tibia propelling the body into air.

1. Introduction

The role of jumping as a mean of locomotion is considered as an escape response and a locomotion pattern for bridging short distances. It has been evolved among insects numerous times during their evolutionary history and has resulted in highly specialized jumping insects such as those of the Siphonaptera, Orthoptera and many other orders.

The jumping mechanism of insects is based on the principle of a power amplifier, i.e. a so-called “catapult mechanism“. To achieve high velocity and acceleration prior to take-off, insects accumulate elastic strain energy and store it in specialized structures in their limbs or body. Finally, the accumulated energy is abruptly released and translated into the movement of the jumping limbs, catapulting the body into the air (Gronenberg, 1996).

Specialized jumping mechanisms have been established for a number of insects. Their jumping mechanics, kinematics, performance, anatomy, physiology, behaviour and neural regulation are relatively well studied in specialized jumpers as diverse as grasshoppers and locusts (Orthoptera) (Heitler, 1974; Bennet-Clark, 1975; Burrows, 1995), fleas (Siphonaptera) (Bennet-Clark and Lucey, 1967; Sutton and Burrows, 2011), leafhoppers (Cicadellidae) (Burrows, 2007a, b), froghoppers (Cercopoidea) (Burrows, 2006a, b; 2007c), planthoppers (Fulgoridae) (Burrows, 2009a), and shore bugs (Heteroptera: Saldidae) (Burrows, 2009b). Jumping is also known to occur in some stick insects (Phasmatodea: Timematidae) (Burrows, 2008) and snow fleas (Mecopera: Boreidae) (Burrows, 2011).

Beetles with specialized jumping apparatus have been recorded in the Curculionidae (weevils), Chrysomelidae (leaf beetles),

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https://doi.org/10.1016/j.asd.2018.02.006
1467-8039/

Buprestidae (jewel beetles) and Scirtidae (marsh beetles) (Furth and Suzuki, 1992). A distinguishing feature indicative of the ability of jumping in beetles is the presence of the modified extensor tendon (MET, also known as the metafemoral spring, metafemoral apodeme or Maulik's organ) in the hind femora. This structure is found in leaf beetles, jewel beetles and weevils and anatomically represents the attachment site for the extensor muscle.

The jumping mechanism and performance in flea beetles (Coleoptera: Chrysomelidae: Galerucinae: Alticini) has recently been studied and described (Nadein and Betz, 2016). We found that the elastic strain energy for the jump accumulates in the resilin-bearing elastic extensor ligament that connects the tibial base and the metatibial extensor tendon in the hind femora.

The jumping mechanism and performance found in some weevils has however never been studied and the mechanics of this mechanism remain unknown. Furth and Suzuki (1992) discovered the MET in three subfamilies of Curculionidae, i.e. the Curculioninae (tribe Rhamphini: genera *Isochnus*, *Orchestes*, *Rhamphus*, *Tachyerges*), Erirhininae (tribe Derelomini, genus *Pedetinus*) and Ceutorhynchinae (tribe Hypurini: genus *Hypurus*; tribe Mononychini: genus *Mononychus*, tribe Cnemogonini: genera *Acanthoscelidius*, *Auleutes*, *Craponius*, *Cnemogonus*, *Dietzella*, *Hypocoeliodes*, *Perigaster*, *Panophtalmus*; tribe Scleropterini: genera *Homosoma* and *Amalus*; tribe Phytobiini: genera *Eurhychiopsis*, *Neophytobius*, *Parenthis*, *Pelenomus*, *Phytobius*, *Rhinoncus*). We present herein the results of our studies on the structure and function of the jumping apparatus of the weevil *Orchestes fagi*.

2. Material and methods

2.1. Study species

The jumping mechanism, structure of the jumping apparatus, jumping performance, and kinematics were examined and analysed in the weevil *O. fagi* (Fabricius, 1801) (Curculionidae: Curculioninae: Rhamphini) (Fig. 1). The beetles were collected by a sweep net from the leaves of *Fagus sylvatica* Linnaeus, 1753 (Fagaceae) in a mixed forest in the vicinity of Rottenburg (Baden-Württemberg, Germany) during the spring and summer season (April to August of 2014 and 2015).

2.2. High-speed videography

Jumping performance was examined by using a high-speed video camera (Photron Fastcam SA3 120K-M2, Photron, Germany) combined with a stereomicroscope (Leica MZ7.5, Leica Microsystems GmbH, Germany). Beetles were observed in a transparent plastic cube of 2 × 2 cm; their jumps were recorded without stimulation at a rate of 2000 and 3000 frames s^{-1}. In total, 21 records for *O. fagi* were acquired, with 10 of them being chosen for the calculation of kinematic parameters (Table 1) by using Tracker v4.87 software (Brown, 2014, https://physlets.org/tracker/). For the calculation of the kinematic parameters, the freshly anaesthetized beetles were weighed on a microbalance (Sartorius BP211D, Sartorius AG, Göttingen, Germany); the average mass of a single individual was found to be 2.2 mg (±0.1). The length of beetles ranged from 2.5 to 2.9 mm; the average length of a single individual was calculated to be 2.7 mm.

Image processing and preparation for publishing were carried out by Adobe Photoshop® CE6 EXD (Adobe Systems Inc., U.S.A.) and CorelDraw® X8 (Corel Corp., Canada).

2.3. Material preparation

All material preparation was carried out under a stereomicroscope (MZ7.5, Leica Microsystems GmbH, Germany). Dry-pinned museum material or fresh samples fixed in 70% ethanol were examined. Fine needles and razor blades were used for dissections, which were carried out under distilled water or 70% ethanol. Slides for microscopy were treated with glycerin. The calculation of the mechanical quantities (Table 2) of the jumping movement of the femoro-tibial joint of *O. fagi* followed the methodology described in Betz and Mumm (2001). The morphological sectional planes used to describe the legs always refer to the isolated leg.

Fig. 1. External appearance (dorsal habitus) of the weevil *Orchestes fagi*.

The mass of the extensor muscles and the telopodite (femur + tibia + tarsus) of the hind leg were measured as dry mass. The extensor muscles were extracted from 10 femora of alcohol-preserved specimens, allowed to dry for two days and weighed together on a microbalance (BP211D, Sartorius AG, Göttingen, Germany); the value obtained was divided by 10 to calculate the extensor muscles mass for a single femur. The same method was applied for the measurement of the mass of the telopodite. The obtained dry extensor muscle mass was corrected for fresh mass, assuming a correction factor 1.75 (follows Betz and Mumm, 2001; Nadein and Betz, 2016), and this value was used in the calculations.

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

2.5. Scanning electron microscopy (SEM)

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

Table 1
Kinematic parameters of jumping in the weevil *Orchestes fagi*[a] (Coleoptera: Curculionidae) and other jumping insects.

Name	Take-off time (ms)	Velocity ($m\ s^{-1}$)	Acceleration ($m\ s^{-2}$)	Kinetic energy (μJ)	g-force	Reference
Weevil *Orchestes fagi* (Coleoptera: Curculionidae: Curculioninae)	1.5–3.0 (2.2)	0.7–2.0 (1.3)	530–1965 (1048)	0.3–4.4 (2.2)	54–200 (106)	Present paper
Flea beetles (Coleoptera: Chrysomelidae)	1.1–7.7	0.7–2.9	100–2660	0.9–19.7	83–342	Brackenbury and Wang (1995), Nadein and Betz (2016)
Grasshoppers (Orthoptera: Tridactylidae, Tettigoniidae)	1.8–2.2	1.5–5.4	69–3000	100–11000	213–306	Burrows and Morris (2003), Burrows and Picker (2010)
Fleas Siphonaptera	1.2–1.6	1.1–1.9	727–1600	0.4–1.8	75–160	Sutton and Burrows (2011)
Leafhoppers (Hemiptera: Cicadellidae)	2.3–6.4	1.1–2.9	188–1055	0.6–77	19–225	Burrows (2007a)
Froghoppers (Hemiptera: Cercopoidea)	0.8–1.5	2.5–4.7	1667–5400	28–238	170–550	Burrows (2006a)
Shore bugs (Hemiptera: Saldidae)	3.4–3.9	1.3–1.8	335–529	3.4	34–54	Burrows (2009a)
Planthoppers (Hemiptera: Issidae)	0.7–1.6	2.2–5.5	1295–7051	75–303	133–719	Burrows (2009b)
Stick insects (Phasmatodea: Timematidae)	12–14.9	0.5–0.9	36–75	7–19	4–8	Burrows (2008)
Snow fleas (Mecoptera: Boreidae)	6.2–7.4	0.7–0.9	106–121	0.9–1.3	11–16	Burrows (2011)

[a] Parameters are calculated based on 10 high-speed video records. Values are given from recorded minimum to maximum; the arithmetic mean is given in parentheses.

Table 2
Mechanical quantification of jumping movement with special regard to the femoro-tibial joint of *Orchestes fagi*.

Attributes of hind leg	Performance	Power output
(1) Telopodite length[a] (m) 1.7×10^{-3}	(5) Angular velocity[a] ($rad\ s^{-1}$) 994 (1200)	(8) Joint power (W) =(5) × (7) 2.1×10^{-5} (3.1×10^{-5})
(2) Telopodite mass[a] (kg) 2.2×10^{-8}	(6) Angular acceleration ($rad\ s^{-2}$) = (5)/0.001 s 9.9×10^{5} (1.2×10^{6})	(9) Specific joint power ($W\ g^{-1}$) =(8)/(4) 0.66 (0.97)
(3) Hind leg moment of inertia ($kg\ m^2$) $= 1/3\ (2) \times (1)^2$ 2.1×10^{-14}	(7) Torque (N m) =(3) × (6) 2.1×10^{-8} (2.5×10^{-8})	
(4) Extensor muscles of femur mass[a] (g) 3.1×10^{-5}		

All calculations were made for (i) the arithmetic means and (ii) the observed maximum value (in parentheses).
[a] Determined by direct measurements or directly established from high-speed video films sequences; all other quantities were calculated by using the given equations (after Betz and Mumm, 2001).

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

The freshly caught weevils were preserved in 70% alcohol. The hind legs were removed, stepwise dehydrated in increasing ethanol concentrations and critical-point dried (Polaron 3100, Quorum Technologies, United Kingdom). The hind legs were glued onto the tips of plastic stubs (1.2 cm long; 3.0 mm in diameter). SR-μCT was carried out in the ID19 beamline at the European Synchrotron Radiation Facility (ESRF, Grenoble; experiment LS-2342) at 19 keV (wavelength of $8 \cdot 10^{-11}$ m) and an effective detector pixel size of 0.65 μm with a corresponding field of view of 1.43 mm by 1.43 mm; 6000 projections were recorded over the 180° 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).

3. Results

3.1. Jumping performance and kinematics

According to our high-speed videography footage (Figs. 2–4), the jump is performed by the hind legs. The movements of the body and the hind legs during the preparation and the beginning of the jump can be subdivided into three phases.

Phase 1, preparation: usually, the position of the body prior to the jump is horizontal with respect to the substrate (Figs. 2A and 3), although upward or downward inclined positions can also occur (Figs. 2B and 4A). The jumping hind legs may be fully flexed (Figs. 2A and 3 frame 1) or partially flexed (Figs. 2B frame 1, 4A), or the two legs might be differently flexed (Fig. 2B frame 1). The positions of the fore and the middle legs can vary during this phase, either touching the substrate (Figs. 2A frame 1, 4A) or being lifted up (Fig. 3 frame 1). The duration of the preparatory phase varies in a great extent from minutes to seconds. Sometimes a beetle sits motionlessly for several minutes and then jumps abruptly. Sometimes a beetle does jump for several times one by one with a very short preparatory stage.

Phase 2, take-off: this phase starts with the extension of the hind tibiae and the simultaneous lifting of the body (Figs. 2A and 3). The hind tibial apices always touch the substrate (Figs. 2A frame 7, 2B frame 6, 3 frame 6, 4C) immediately before the moment when the body is propelled into the air. The hind tarsi either contact the substrate (Fig. 4B) or are raised (Fig. 4C). The jump is performed with closed (Figs. 2, 3 and 4A), partially (Fig. 4B) or fully open (Fig. 4D and E) hind wings and can finally be turned into flight for relocation. The extension of both hind tibiae usually occurs simultaneously, although asynchronous movements or jumps performed by one leg only have also been recorded (Fig. 4F). In the fully flexed position prior to the jump, the angle between the hind tibia and the femur is 21–31° (Fig. 3). The hind tibia is able to extend to an angle of 116–147° (Fig. 3). The full extension takes 1.8–2.5 ms and the angular velocity of the hind tibia extension ranges between 42.8 and 69.4 deg ms^{-1}.

Phase 3, post-take-off: the trajectory of the jump after the loss of contact with the substrate depends on the body orientation and movements during the previous phases. Within the initial 10–20 ms, the body moves along an almost straight trajectory

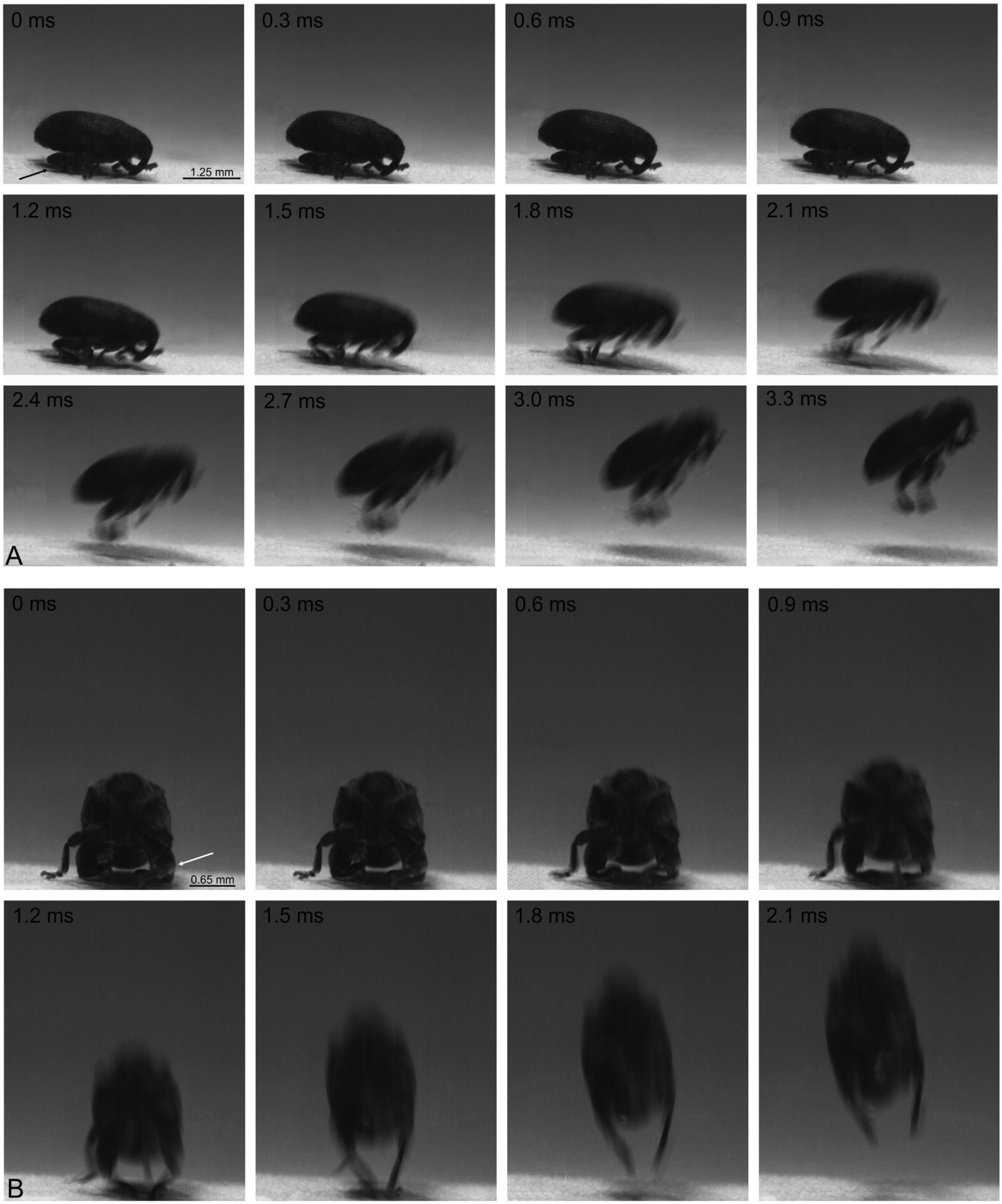

Fig. 2. Jumping performance of the weevil *Orchestes fagi*. (A) Frame-by-frame depiction of a jump viewed from the side; arrow shows the fully flexed hind leg. (B) Frame-by-frame depiction of a jump viewed from the anterior; white arrow shows partially flexed hind leg. Frame rates amount to 3000 frames s^{-1}; frames are numbered according to their sequence.

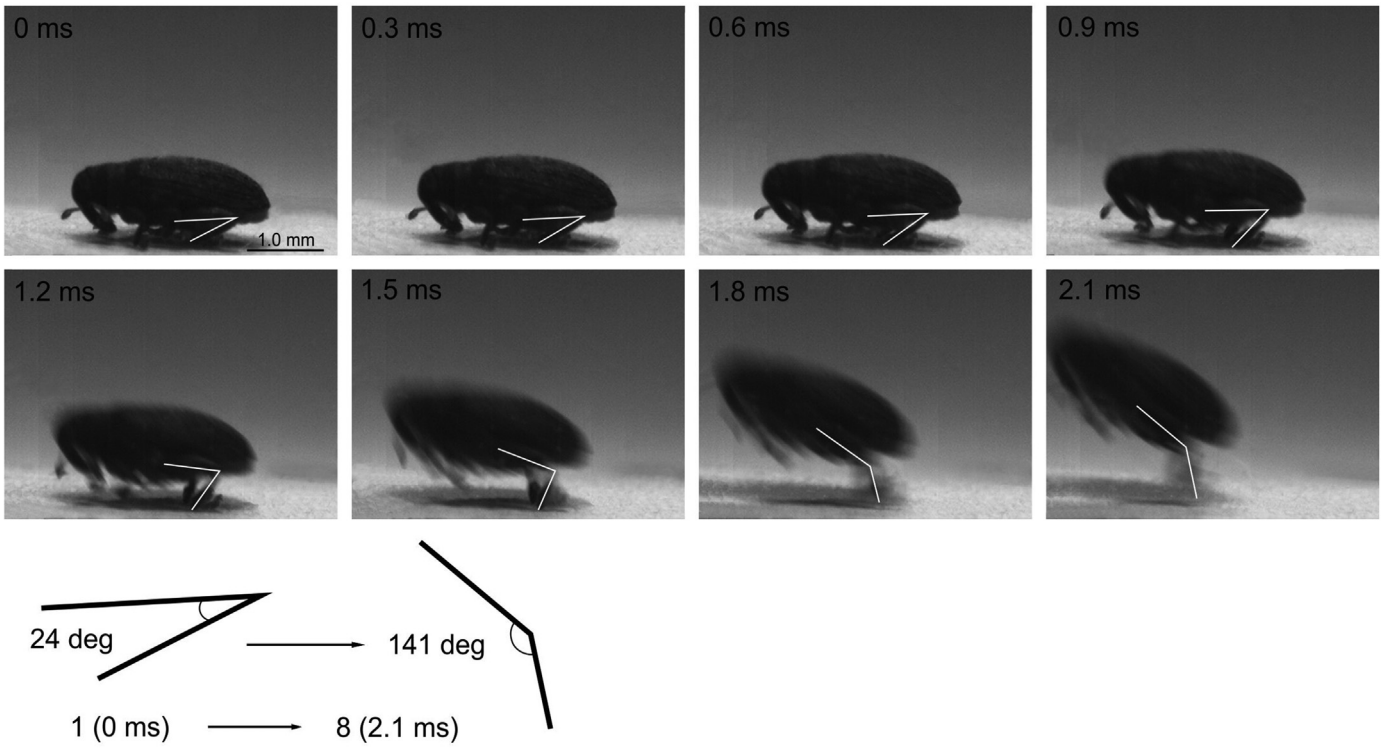

Fig. 3. Frame-by-frame depiction of the jump of *Orchestes fagi* showing the extension of the hind tibia; images were captured at a rate of 3000 frames s^{-1}; frames are numbered according to their sequence. The diagram shows the extension of the tibia during phase 2 of the jump until just before take-off.

ahead and can experience rotations along the transverse or longitudinal axis.

Kinematic parameters of the natural jump have been calculated for *O. fagi* and are summarized in Table 1 in comparison with other jumping insects. The highest value of acceleration (maximum recorded: 1965 m s^{-2}) occurs in phase 2 during the initial 1–2 ms of the hind tibia extension. In contrast, the highest values of the velocity and kinetic energy of the jump (recorded maxima of 2.0 m s^{-1} and 4.4 μJ, respectively) occur immediately at the moment of take-off in Phase 3.

3.2. Morphology of jumping hind leg

3.2.1. General morphology and structure of jumping leg

The metafemur in jumping weevils is represented by two types, i.e. (1) swollen (Fig. 5A) with a length/width ratio of 2.1–2.3 (characteristic of species in the tribe Rhamphini) and (2) non-swollen with a width/length ratio of 3.3–4.2 (characteristic of species in the tribe Phytobiini). The comparison of the metatibia in jumping curculionids and their non-jumping relatives demonstrates their overall principal similarity and absence of external special structural adaptations for jumping. Of note, representatives of the tribe Rhamphini (to which *O. fagi* belongs) show a curved metatibia that corresponds to the shape of the swollen metafemur (Fig. 5A).

The principal structural elements of the jumping leg in *O. fagi* (Figs. 5 and 6) comprise (1) the femoro-tibial joint, (2) the metafemoral extensor tendon, (3) the extensor ligament, (4) the tibial flexor sclerite, (5) the flexor tendon and (6) the extensor and flexor muscles.

A peculiar feature found in the joint of legs of both jumping and non-jumping Curculionidae is an internal protrusion that is present at the anterior part of the ventral femoral wall (Fig. 5B and C) and that resembles the so-called 'lumps' in grasshoppers (Heitler, 1974).

3.2.2. Metafemoral extensor tendon (MET)

In jumping weevils, the metafemoral extensor tendon is morphologically modified (Figs 5B, D, 6A–G) and represents a sclerotized structure having a hook-like shape (Furth and Suzuki, 1992). The MET is significantly enlarged in comparison with the extensor tendon of non-jumping weevils, presumably to provide an increased attachment surface area for the increased volume of the extensor muscle (see 3.2.4 Musculature of hind femur of O. fagi). Two principal parts were distinguishable in the examined *O. fagi*. The *distal elongate part* (Figs. 5B and 6B, C) is nearly straight and dorsoventrally flattened, its distal apex ends near the extensor ligament (terminology of Furth, 1988; Zombori and Steinmann, 1999). The *proximal curved part* (Figs. 5D and 6E–G) is ventrally bent; its basal part lies under the *distal elongate part* and reaches nearly half the length of the MET.

3.2.3. Tibial flexor sclerite (TFS)

The tibial flexor sclerite (TFS) is a part of the tibial flexor system (Furth and Suzuki, 1990a, b) and is represented in *O. fagi* by a small but thick sclerotized plate having a rectangular shape (Fig. 6H–K). The apex of the TFS is connected to the tibial base by an elastic flexor ligament (Fig. 6B, D); the ventral side of TFS attaches to the internal femoral protrusion by a broad and short ligament (Fig. 8), whereas the flexor muscle is attached to the TFS by the string-like flexor tendon attached to the basal part of TFS (Figs. 5B and 8D f.t.). The TFS is found in both jumping and non-jumping Curculionidae. The functional aspects of the TFS suggested by Furth and Suzuki (1990a) and Gorb (1995) include: (1) mechanical strengthening of the base for the flexor tendon, (2) increasing the working angle of the leg flexor system and (3) protecting the ventral side of the exposed femoro-tibial joint.

3.2.4. Musculature of hind femur of Orchestes fagi

The volume of the muscles in the femur of the jumping hind leg is greatly increased (Fig. 7) in weevils of the tribe Ramphini

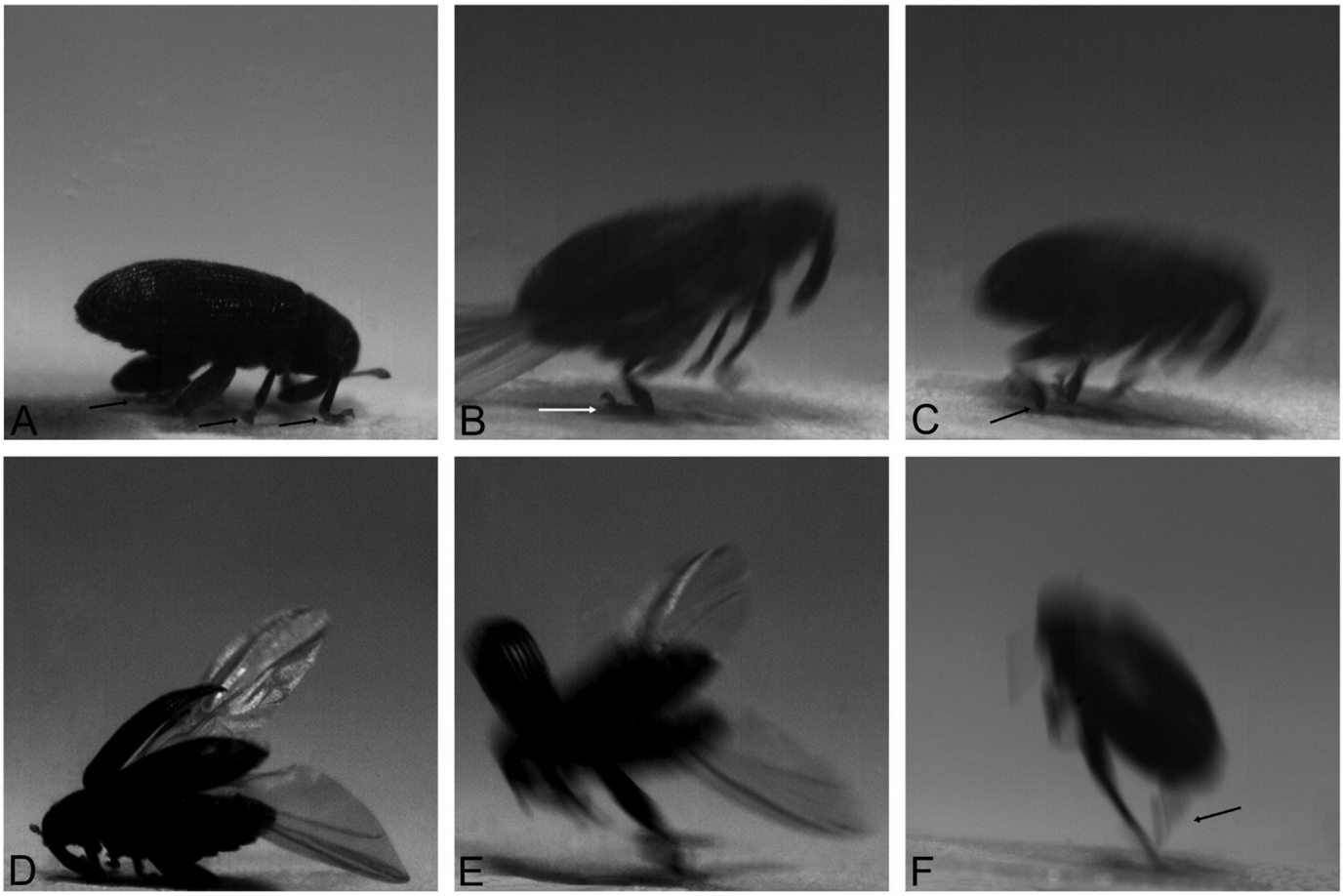

Fig. 4. Images of the weevil *Orchestes fagi* during various phases of a jump. (A) Moment prior to take-off; the arrows show partially flexed hind leg and the position of the fore and middle tarsi touching the substrate. (B) Moment of take-off; the arrow shows the position of the hind tarsi contacting the substrate. (C) Moment of take-off; the arrow shows the position of the hind tibia contacting the substrate by the tibial apices. (D, E) Jumps with wings opened: D, Moment prior to take-off; E, Moment of take-off. (F) Moment of take-off propelled by one hind leg; the arrow shows the flexed right leg.

(those showing swollen metafemora), whereby the volume of the extensor muscle is increased to the largest extent (Fig. 7A and B). This is probably in contrast with jumping weevils with non-swollen metafemora (e.g. the tribe Phytobiini). Several bundles of fibers of extensor muscle attach at the MET (Fig. 7A–B). The bundles of fibers attach dorsally at the distal elongate part of the MET (Fig. 7A–C, F dm). The largest group of fibers of extensor muscle having a pennate appearance is attached to the proximal curved part of the MET and to the string-like tendon (see below) connected to it (Fig. 7A, B, E–G em, Fig. 8A e.t.). These muscles affix at the dorso-posterior wall of the metafemur. Another group of muscle fibers inserts at the outer side of curvature of the MET and originates at the external lateral side of the femoral wall (Fig. 7E elm).

The flexor muscle (Fig. 7A–B, E fm) is smaller than the extensor muscle. It is represented by several bundles of fibers inserting at the proximal side of TFS and originating on the ventral and postero-ventral femoral wall.

3.2.5. Resilin in legs of jumping weevils

Samples of the jumping legs of *O. fagi* were examined for the presence of resilin. This rubber-like protein can be revealed by its bright blue fluorescence under UV illumination. Resilin was observed in the following structures of the hind leg: (1) the extensor ligament connecting the tibial base and the MET (Fig. 8 e.l.), (2) the string-like extensor tendon connected to the distal part of the MET (Fig. 8A–B e.t.), (3) the flexor ligament connecting the TFS and the tibial base (Fig. 8 f.l.), (4) the short and broad ligament connecting the ventral side of the TFS and the internal protrusion of the metatibia (Fig. 8C–F b.l.), (5) the string-like flexor tendon connecting the flexor muscle and the basal part of the TFS (Fig. 8D f.t.) and (6) the arthrodial membranes connecting the tibial base and the femoral wall (Fig. 8C–F m). No resilin was detected in the MET (Fig. 8B, D, F).

The extensor ligament (1) can be reversibly deformed, i.e. elastically stretched to a great extent. It is at least two times longer in the flexed position (Fig. 8C–F e.l.) compared with the extended position of the metatibia (Fig. 8A and B e.l.).

The flexor ligament (3) is also highly elastic, being stretched at the extended position of the tibia (Fig. 8A and B f.l.) and shorter at the flexed position (Fig. 8 CeF f.l.). The ventral side of the TFS is connected to the internal (ventral) femoral wall at the internal femoral protrusion via the short and broad resilin-bearing ligament (4) (Fig. 8C–F b.l.). This ligament stretches when the TFS moves backwards and the tibia is in the flexed position (Fig. 8C–F b.l.).

3.3. Mechanics of the jump and mechanical lever system of the femoro-tibial joint of Orchestes fagi

The extension of the hind tibia is the most crucial movement of the jump, pushing the body upwards. The basic mechanical quantities for the description of the tibial movement and the initial

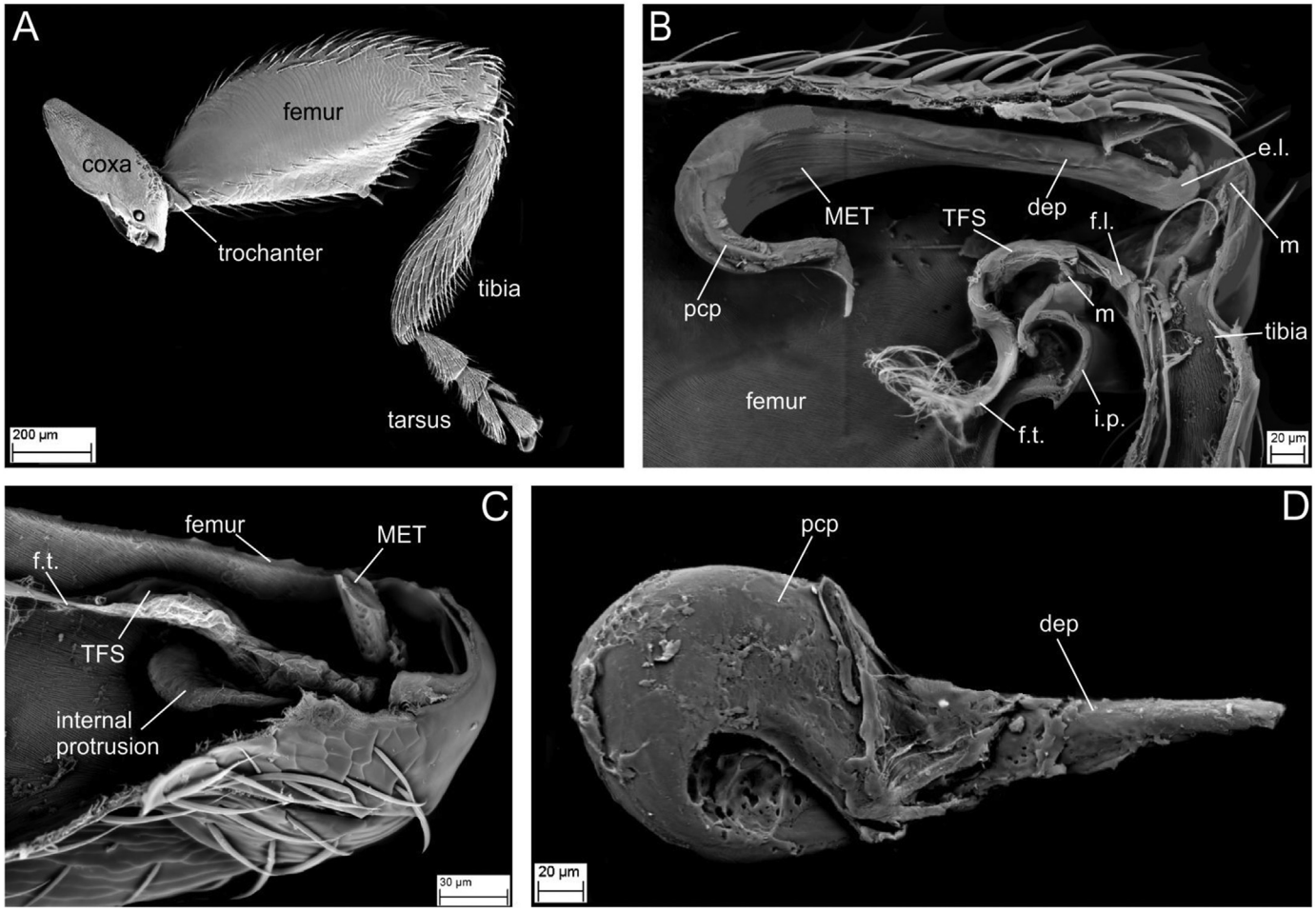

Fig. 5. Images obtained by scanning electron microscopy (SEM) showing the morphological structures of the jumping leg of *Orchestes fagi*; the section plane names follow those in Fig. 7D. (A) External morphology of the hind leg. (B) Sagittal section through the femoro-tibial joint of the jumping hind leg. (C) Frontal section through the femoro-tibial joint of the jumping hind leg. (D) Metafemoral extensor tendon, ventrolateral view. Abbreviations: dep, distal elongate part; e.l., extensor ligament; f.l., flexor ligament; f.t., flexor tendon; i.p., internal protrusion; m, membrane; MET, metafemoral extensor tendon; pcp, proximal curved part; TFS, tibial flexor sclerite.

phase of the jump (prior to take-off) are summarized in Table 2. The specific joint power value calculated for the recorded maximum was 0.97 W g^{-1} (average: 0.66 W g^{-1}).

The hind femur and tibia in the femoro-tibial joint are represented by simple two-armed class 1 levers with the pivot slightly shifted from the centre towards the insertion of the extensor ligament (Fig. 9A). The effective lengths of the mechanical extensor and flexor levers and their ratio were calculated for the totally flexed position of the tibia to represent the natural leverage conditions of the jumping leg prior to the jump. The measurements were made on the basis of the results of the virtual reconstruction and fluorescence microscopy representing the natural conditions of the anatomical elements of the levers (Fig. 9D and E). Both the leverage and ratio of the effective flexor and extensor levers change during the flexion of the tibia because of the presence of the internal protrusion (Fig. 5B and C; Fig. 9A). Since the flexor ligament is placed directly dorsally to the internal protrusion, its course towards its insertion at the tibia is redirected as shown in Fig. 9. Consequently, the angle at which it is connected to the tibia changes during extension and flexion: in the extended position of the tibia, the flexor ligament is connected to the tibia at an acute angle ($\beta = 21°$, Fig. 9C), whereas at the fully flexed position, it becomes nearly a right angle (98°, Fig. 9B). In the fully flexed position, the effective extensor lever (Fig. 9B, arm e) is shorter than the effective flexor lever (Fig. 9B, arm f). The effective extensor leverage at this position is 'a x sin (α) = 22.2 µm', whereas the effective flexor leverage is 'b x sin (β) = 51.9 µm'. Consequently, the mechanical advantage of the flexor over the extensor in the fully flexed position of the tibia is 2.3. In comparison, in the extended position of the tibia, the ratio of the effective flexor and extensor leverage is 1.2 (Fig. 9C).

4. Discussion

Among the jumping beetles, the mechanism of jumping has previously been studied in the flea beetles Alticini (Nadein and Betz, 2016), in which the jump occurs via the modified hind legs with their especially swollen metafemora and with enlarged volume of musculature. The actual jumping apparatus consists of the femoro-tibial joint, the modified metafemoral extensor tendon, the extensor ligament, the tibial flexor sclerite and the extensor and flexor muscles. The kinematic parameters (Table 1) calculated for selected genera of flea beetles represent some of the best in terms of acceleration and velocity among jumping insects. The specific joint power of the femoro-tibial joint of the hind leg during the jumping movement has also been shown to be high; a recent calculation has corrected its value to a maximum of 2.4 W g^{-1}, instead of 0.714 W g^{-1} as previously

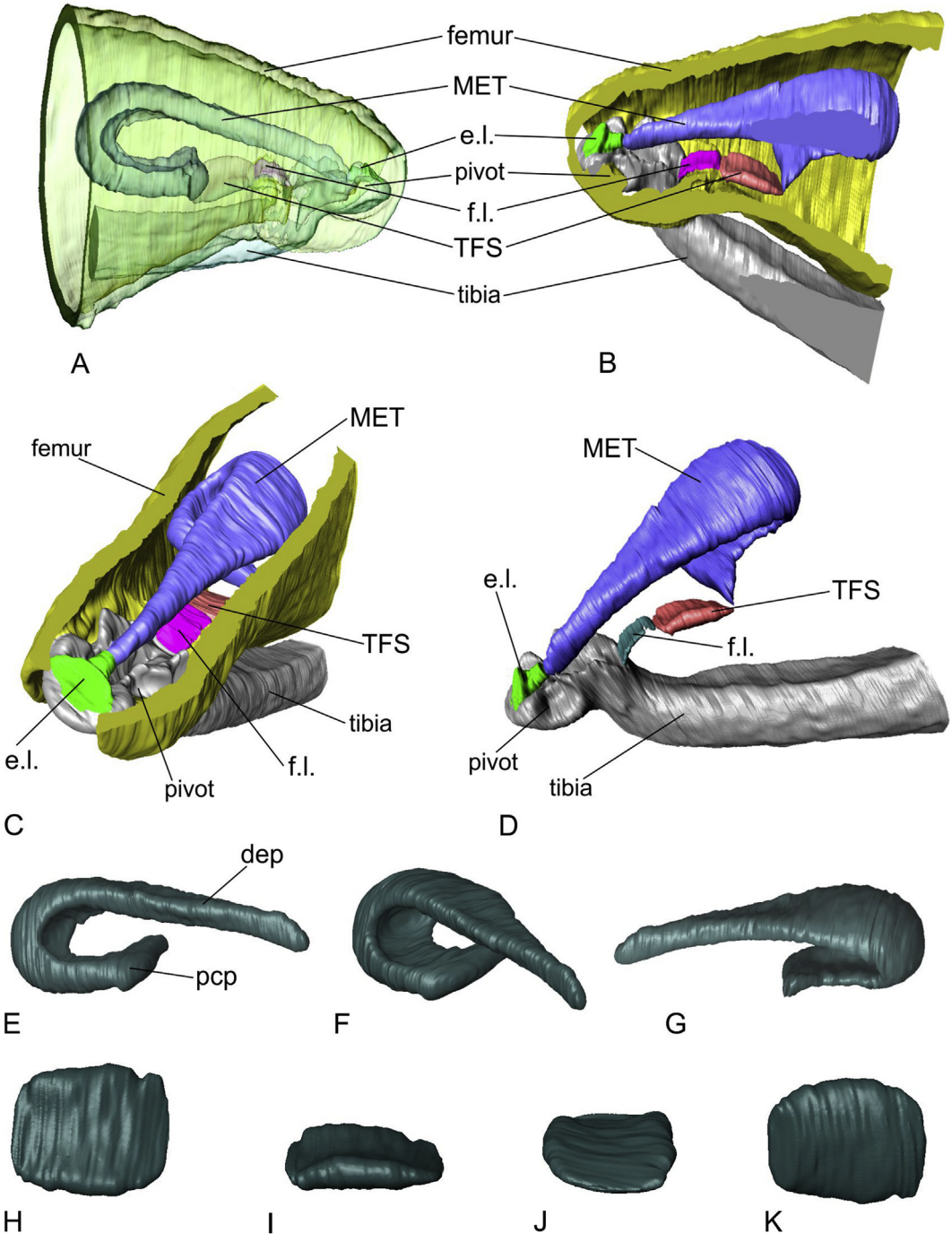

Fig. 6. Femoro-tibial joint in the jumping hind leg of *Orchestes fagi* based on synchrotron X-ray micro-computed tomography (SR-μCT) data reconstruction, the section plane names follow those in Fig. 7D. (A) Joint structure inside the femur. (B) Sagittal plane section of the femoro-tibial joint. (C) Frontal plane section of the femoro-tibial joint. (D) Lateral aspect of tibia and internal structures of the femoro-tibial joint with femoral wall removed. (E, F, G) Metafemoral extensor tendon, E, lateral view; F, antero-lateral view; G, dorso-lateral view. (H, I, J, K) Tibial flexor sclerite, H, dorsal view; I lateral view; J, anterior view; K, ventral view. Abbreviations: dep, distal elongate part; e.l., extensor ligament; f.l., flexor ligament; MET, metafemoral extensor tendon; pcp, proximal curved part; TFS, tibial flexor sclerite.

reported by Nadein and Betz (2016). In their publication, Nadein and Betz (2016) have suggested that jumping is performed via a catapult mechanism: elastic strain energy is stored prior to the jump in the resilin-bearing extensor ligament when it is stretched by the extensor muscle during the co-contraction of the flexor and extensor muscles.

4.1. Functional morphological analysis of the jumping mechanism in weevils

Our high-speed footage suggests that the jump in *O. fagi* is performed exclusively by the hind legs. The most crucial act of the jumping movement is the extension of the hind tibia from its flexed

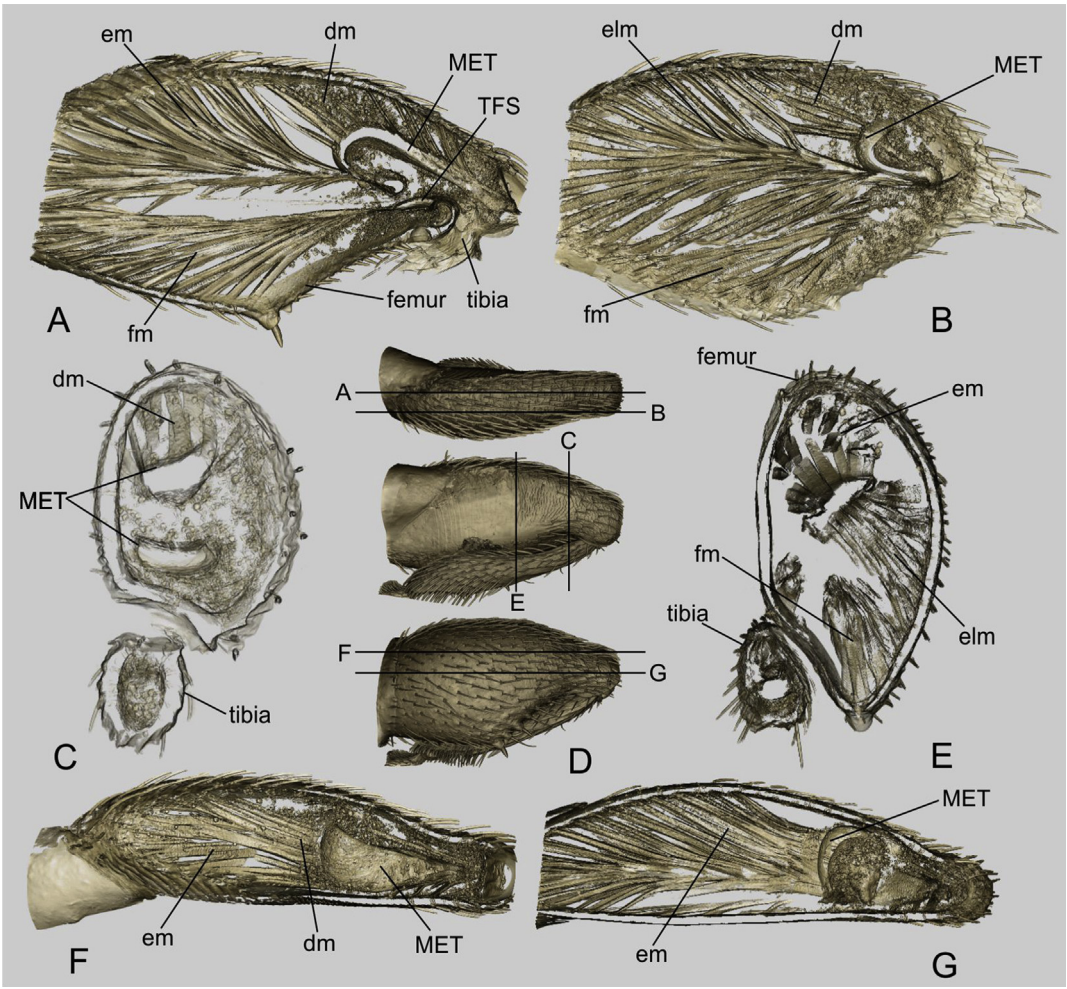

Fig. 7. Muscular system of the metafemur of the jumping hind leg of *Orchestes fagi* based on SR-μCT data volume reconstruction. (A, B) Sagittal plane section. (C, E) Transverse plane section. (D) Hind leg in various positions illustrating the section planes. (F, G) Frontal plane section. Abbreviations: dm, fibers of the dorsal part of extensor muscle; em, extensor muscle; elm, fibers of the lateral part of extensor muscle; fm, flexor muscle; MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

position to its full extent. Kinematic parameters and their values of the natural jump in *O. fagi* (Table 1), such as take-off time (up to 3.0 ms), velocity (up to 2.0 m s^{-1}), acceleration (up to 1965 m s^{-2}) and kinetic energy (up to 4.4 μJ), are within the scope of other specialized jumping insects such as flea-beetles, grasshoppers, fleas and froghoppers, whose jumping performance has been attributed to a catapult mechanism with power amplification (meaning that the same energy is expended but within a shorter time span that is impossible to achieve by direct muscle contraction).

The maximum specific joint power output that insect muscles can exert by direct muscle contraction has been reported to reach about 0.1 W g^{-1} (Weis-Fogh, 1956; Machin and Pringle, 1959; Josephson, 1975; Ellington, 1985). The specific joint power calculated for the jumping movement of the hind leg in *O. fagi* (Table 2) has a maximum of 0.97 W g^{-1} (average: 0.66 W g^{-1}), which is thus significantly higher than the maximum reported for direct muscle contraction. The full extension of a hind tibia until take-off takes up to 2.5 ms with an angular velocity of up to 42.8 deg ms^{-1} (the best records are 1.8 ms and 69.4 deg ms^{-1}, respectively), values considerably exceeding the temporal limitations known for insect muscle contractions (Neville and Weis-Fogh, 1963; Usherwood, 1962; Josephson, 1975; Gronenberg, 1996). We can thus conclude that the jumping movement of the hind tibia cannot be performed by direct muscle contraction alone and that the jump might also involve a catapult mechanism. As pointed out by Gronenberg (1996), 'catapult mechanisms are exploited by many small animals that need to overcome the temporal limitation of muscle action'.

Catapult mechanisms are based on the storage of elastic strain energy. This requires elastic, reversibly deformable structures. Energy can be stored in specialized resilin-bearing structures (e.g. Andersen and Weis-Fogh, 1964; Lyons et al., 2011). Resilin has been found in many jumping insects (Gronenberg, 1996); e.g., in the semi-lunar processes of the hind femoro-tibial joint of locusts (Burrows and Sutton, 2012), the pleural arch of froghoppers and planthoppers (Burrows et al., 2008; Burrows, 2010), special patches in the metathorax of fleas (Sutton and Burrows, 2011) and the extensor ligament of the hind legs of flea beetles (Nadein and Betz, 2016). In the jumping hind leg of *O. fagi*, resilin occurs in both the extensor ligament and the flexor tendon (Fig. 8). Results from our fluorescence microscopy indicate that the extensor ligament stretches when the tibia is flexed, and relaxes once the tibia is extended (Fig. 8). We can assume that the extensor ligament is a major elastic structure involved in the storage of elastic strain energy prior to the catapult-like jump.

In insects, several options have been taken for the loading of elastic 'springs' (i.e. deformable elastic structures for the storage of elastic strain energy) and the sudden release of the accumulated energy. Such mechanisms involve (1) passive locking mechanisms (Burrows, 2006b), (2) active latches (Gronenberg, 1996; Sutton and

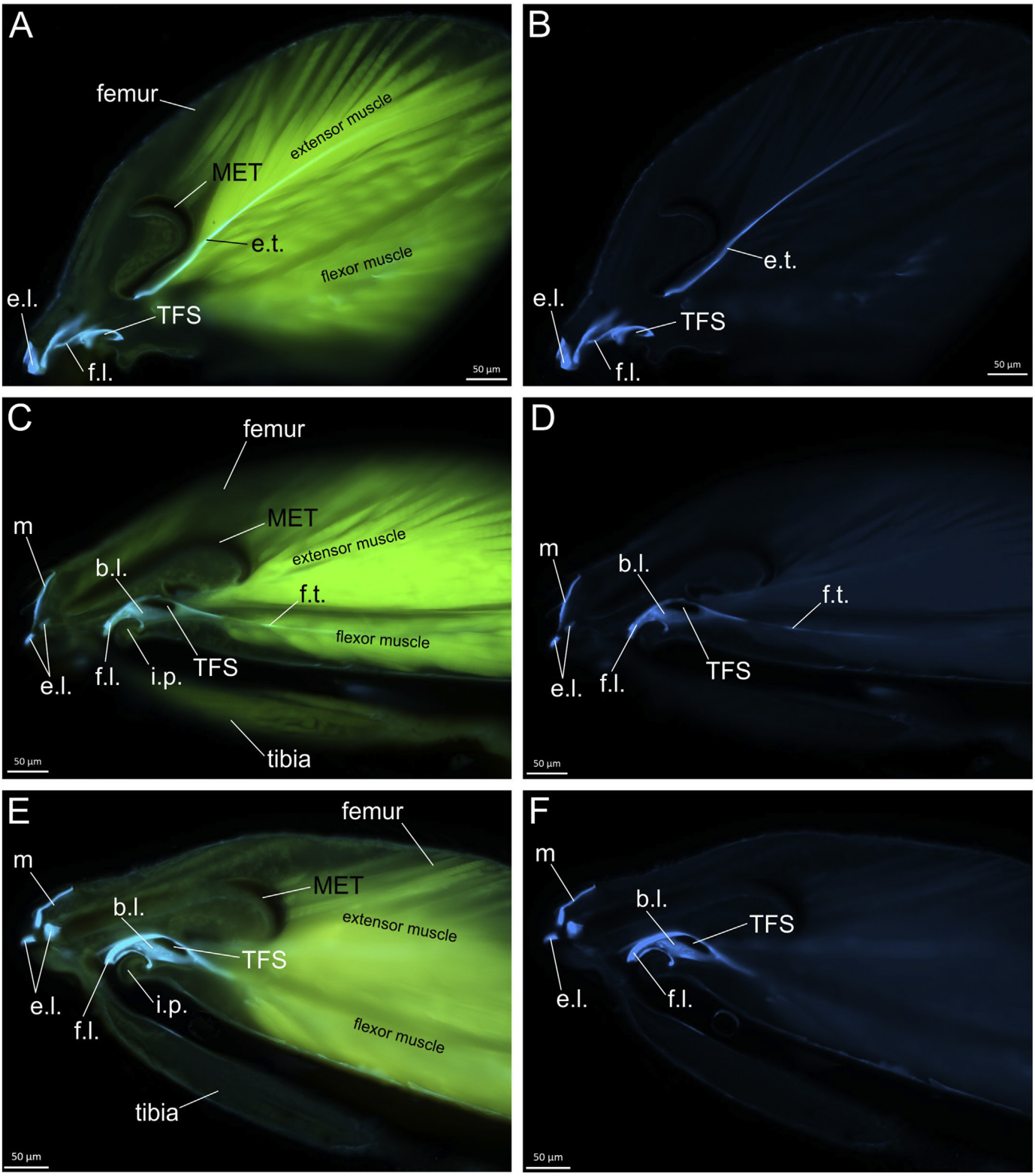

Fig. 8. Occurrence of resilin in the femoro-tibial joint of the hind jumping leg of a weevil *Orchestes fagi* based on fluorescence microscopy. The presence of resilin is indicated by the blue colour. (A, C, E) Combined image of the images taken under the different filters (DAPI, Alexa488, Cy5). (B, D, F) Images corresponding to the images A, C, E but taken under the DAPI filter. (A, B) Extended position of the tibia; relaxed resilin-bearing extensor ligament. (C–F) flexed position of the tibia; stretched resilin-bearing extensor ligament. Abbreviations: b.l., broad ligament; e.l., extensor ligament; e.t., extensor tendon; f.l., flexor ligament; f.t., flexor tendon; i.p., internal protrusion; m, membrane; MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

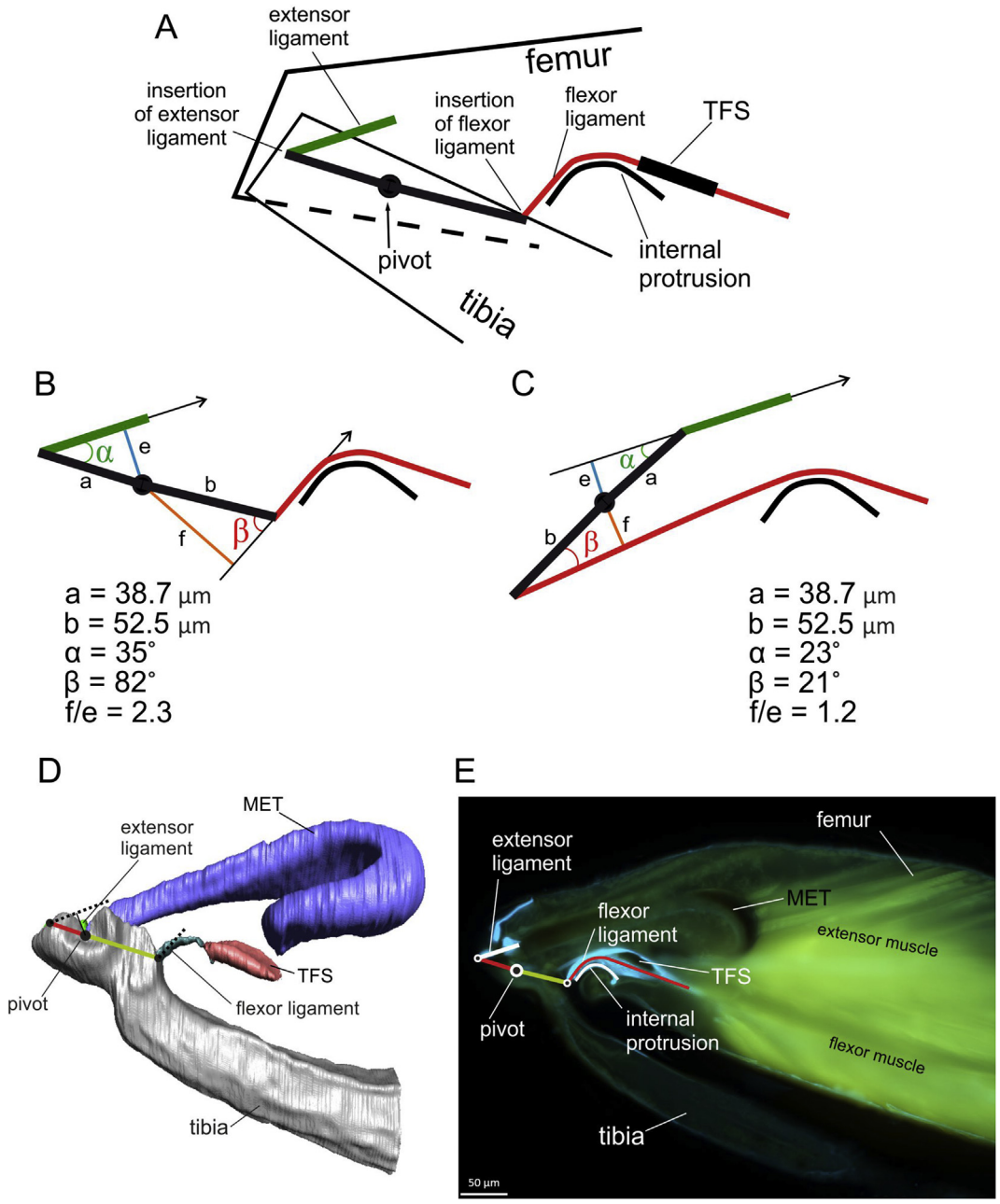

Fig. 9. Leverage in the femoro-tibial joint of the hind leg of *Orchestes fagi*. (A) Schematic mechanical model of the levers in the femoro-tibial joint based on data obtained by SR-µCT (D) and fluorescence microscopy (E). (B, C) Mechanical model of the femoro-tibial joint illustrating its crucial functional elements; (B) fully flexed position; (C) extended position. (D, E) Femoro-tibial joint of the hind leg in the fully flexed position with scheme of the leverage used for the coordination of the positions of the elements of the leverage and the measurement of the lengths of the lever arms for the mechanical schemes A, B, and C; (D) SR-µCT data reconstruction; (E) Fluorescence microscopy image (interpolation of the filters DAPI, Alexa488, Cy5). Abbreviations: a, anatomical arm of the extensor lever; b, anatomical arm of the flexor lever; e, effective extensor lever; f, effective flexor lever; MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

Burrows, 2011) and (3) co-contractions of antagonistic muscles (Heitler, 1974, 2007; Gronenberg, 1996; Betz and Mumm, 2001). As has been emphasized, direct locking and triggering mechanisms are energetically expensive and include additional muscles and accessory structures plus the energy to maintain the movements (Gronenberg, 1996). Our anatomical studies of the jumping hind legs in *O. fagi* have failed to discover latches, locking mechanisms or specialized trigger muscles analogous to those of fleas, some ants, froghoppers and click-beetles. Therefore, we suggest that the accumulation of elastic strain energy is performed by the co-contraction of the antagonistic flexor and extensor muscles. In *O. fagi*, the extensor muscle pulls the MET backwards and simultaneously stretches the extensor ligament, whereas the flexor muscle is antagonistic to it. As shown by the example of locusts, the smaller antagonistic flexor muscle must have an advantage over the much more powerful extensor muscle (Heitler, 1974, 2007). The leverage system of the femoro-tibial joint of the jumping hind leg of the weevil *O. fagi* (Fig. 9) is comparable. The internal protrusion in the antero-ventral wall of the femur changes the angle with which the flexor ligament pulls on the tibia and changes the ratio of the lever's arms (Fig. 9A–C, e, f). In the fully flexed position, the effective flexor leverage has the mechanical advantage over the

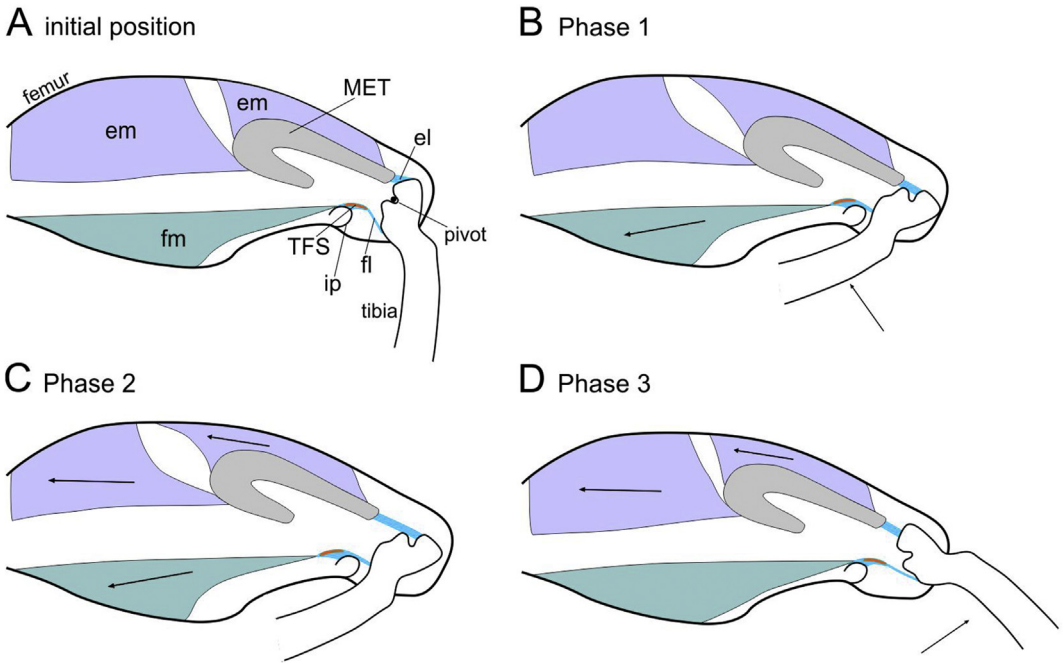

Fig. 10. Schematic model of the crucial functional elements in the hind leg and depiction of the various phases of their movement during a jump. (A) Initial position of the tibia. (B) Phase 1: flexion of the tibia; arrows indicate the direction of the flexor muscle contraction and flexion of the tibia. (C) Phase 2: co-contraction of the antagonistic muscles and stretching 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 extensor muscle and extension of the tibia. Abbreviations: em, extensor muscle; el, extensor ligament; fm, flexor muscle; fl, flexor ligament; ip, internal protrusion; MET, metafemoral extensor tendon; TFS, tibial flexor sclerite.

extensor, a value that amounts to 2.3 (Fig. 9B). This corresponds to the almost perpendicular angle at which the flexor ligament is connected to the tibia in the fully flexed position (Fig. 9B) allowing the flexor muscle to perform its maximal work.

We further suggest that elastic strain energy accumulates in the extensor ligament loaded by the contraction of the extensor muscle, while the co-contracting antagonistic flexor muscle holds the tibia in its flexed resting position. In concert with the short input extensor lever (i.e. the lever that amplifies the input force of the extensor muscle) (Fig. 9A), this would also contribute to the velocity and power gain that is necessary for the jump.

The jumping apparatus in weevils and flea beetles are similar in principle. They share the same structural and functional elements, such as modified MET and the resilin-bearing extensor and flexor ligaments. Nevertheless, some differences are apparent: (1) the leverage condition that makes possible the co-contraction of antagonistic muscles and the mechanical advantage of the flexor leverage in weevils are based on the presence of the internal protrusion of the femur, whereas in contrast, the femur in flea beetles lacks an internal protrusion and the mechanical advantage of the flexor leverage is achieved by the large difference in the lengths of the input and output arms (Nadein and Betz, 2016, Fig. 13A); (2) the absence of an additional locking mechanism that is assumed in some cases of flea beetles; (3) the presence of jumping in weevils without swollen metafemora (e.g. from the tribe Phytobiini) and without an enlarged extensor muscle volume that is supposedly correlated with less powerful jumps, which still remains to be tested.

4.2. Functional model of the jumping mechanism of Orchestes fagi

We propose the following functional model for the jumping mechanism of *O. fagi* as based on the data presented here (Fig. 10). According to this model, the jumping mechanism can be subdivided into three phases:

Phase 1: flexion of the hind tibia by the contraction of flexor muscle; the resilin-containing extensor ligament stretches over a short distance (Fig. 10B).

Phase 2: contraction of extensor muscle; flexor muscle is active and holds the tibia in the flexed position according to its effective lever advantage resulting in the co-contraction of antagonistic muscles; the extensor muscle stretches the resilin-containing extensor ligament still further, thus accumulating elastic strain energy (Fig. 10C).

Phase 3: abrupt relaxation of the flexor muscle; rapid extension of the hind tibia powered by the pre-stored elastic strain energy in the extensor ligament; propulsion of the body into the air (Fig. 10D).

Funding

The work of K. Nadein was funded by an Alexander von Humboldt Foundation Fellowship for Experienced Scientists (award no. 3.3-UKR/1151783STP, 2014–2016). Our experiments at the ESRF (European Synchrotron Radiation Facility, Grenoble, experiment LS-2342) were funded by the European Union.

Author's 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.

Acknowledgments

The authors are indebted to the following colleagues for their assistance: M. Meinert (Evolutionary Biology of Invertebrates, Tübingen University) with SEM, Y.-D. Stierhof and L. Henneberg (Center for the Plant Molecular Biology, Tübingen University) with

fluorescence microscopy; Alexander Rack (ESRF, Grenoble) with ID19 beamline. The authors express their sincere gratitude to D. Furth (Smithsonian Institution, Washington DC, USA) and R. T. Jones (Germany) for their linguistic checking of the English of the manuscript and valuable comments.

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