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Universal locking mechanisms in insect legs: jumping and grasping

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Complete List of Authors:	Földvari, Mihaly; Oslo and Akershus University College of Applied Sciences, Natural History Museum Mikó, István; Pennsylvania State University, Entomology Ulmer, Jonah; Pennsylvania State University, Entomology dos Santos Rolo, Tomy; Karlsruhe Institute of Technology Institute of Radiation Research, Institute for Photon Science and Synchrotron Radiation (IPS) Csösz, Sándor; MTA-ELTE-MTM Ecology Research Group, Ecology Research Group Pomiankowski, Andrew; University College London, Centre for Computation, Mathematics and Physics in the Life Sciences and Experimental Biology (CoMPLEX) Baumbach, Tilo; Karlsruhe Institute of Technology, ANKA / Institute for Photon Science and Synchrotron Radiation; Karlsruhe Institute of 15 Technology (KIT), Institute for Photon Science and Synchrotron Radiation van de Kamp, Thomas; Karlsruhe Institute of Technology, ANKA / Institute for Photon Science and Synchrotron Radiation
Please choose a section from the list:	Morphology and Ontology
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Overall, the two reviewers recommend “major revision”, but to be clear, this is primarily revision of the writing, which should be feasible to complete in time to include for the special collection. An action that could be taken in terms of major methodological revision would be to document the skeletomuscular anatomy of at least one taxon which is closely related to each study organism (the gryllid, the torymid, the diopsid, and the ephydrid) for comparison. In the stead of this, direct comparison of literature descriptions of legs may be done either explicitly (side-by-side in the results) or implicitly via a more-detailed description of leg skeletomuscular anatomy in the introduction. The balance is up to the discretion of the authors.

The focus of this paper is on the ventral locking mechanism at the femoro-tibial joint, and hence we do not think a detailed description of the entire leg musculature is needed. We have altered the Introduction and discussion as well as Figure 1 accordingly.

The current description in the result section focuses on the two muscles that are involved in the movement of the femoro-tibial joint, which has been considered as a “simple mechanical system” by Nadein and Betz 2016. We have rewritten the introduction accordingly.

Importantly, the two reviewers disagree about whether the manual dissection is convincing. While Reviewer 2 accepts the conclusions based on the manipulations in glycerol, Reviewer 1 is skeptical based on three primary points: (1) There is more variation in locking mechanisms than implied by the authors; (2) the observations are based on manipulation of dead specimens; and (3) the study is qualitative, rather than quantitative. Addressing these three points will substantially improve the work.

1. ***We have to give a big “Thank you” for reviewer 1 for her/his first comment. The examined grasping legs share the presence of a ventral femoral lock with jumping taxa and we suspected that the fine details of the locking systems in these legs are different. However, we felt that a more thorough comparison with jumping legs, including flea beetles would be above the scope of the present paper. We simply wanted to describe a phenomenon (presence of a lock in grasping legs) and a new (old) way to show that a lock is present. However, after reading the comment, we did some further discussion and gained a much better understanding about the different locks and include these new information into the manuscript, that substantially improved it.***
2. ***It would be really hard to do similar observations on live specimens. We have provided more details about how we did our dissections and also about how certain observations support the presence of a lock.***
3. ***Yes, it is a quantitative study. We did not dissect hundreds of specimens, however, we dissected more than five grasping/jumping legs for each species and these dissection are supported our hypothesis.***

Toward the first point, Reviewer 1 helpfully recommends Betz & Mumm (2001), Gronenberg (1996), and a series of articles by Sutton and Burrows, all of these these being easily retrievable via Google Scholar, and some of which are already cited by the authors. A brief review of these works (or a

selection thereof) as it pertains to the study question would be appropriate for the introduction, and would also provide grist for discussion.

We have included sections in the introduction with references of the above mentioned papers.

However, based on the critique, it is probably necessary to adjust the generality of the claim, but this is not fatal. In a positive light, this may be used as a fulcrum for the benefit of the authors, as they may emphasize the convergence of the mechanism and explain why it is important to know (for example, “exaptation” is listed in the keywords, but isn’t used anywhere else in the text).

We have erased exaptation from the keywords.

There is also an outstanding question to address, even if hypothetically: Are the TFS and HL homologous across the Pterygota? Reviewer 1 suggests otherwise.

We have expanded the section about TFS and the genuiflexor sclerite and their relative position to the apodeme (site of origin of the tibial flexor apodeme). Homology is at least as ambiguous concept as important. The TFS and and locking mechanism is obviously not homologous as they have evolved in numerous insect taxa independently.

The second and third points are bound together as the main crux, and relate to the exactness and certainty of the conclusions. The reviewer asks (paraphrased) “How do you know exactly (a) how strong and how far the muscles contract, exactly (b) which muscles need to be contracted or relaxed, and (c) how do you really know that in the live insect [the] TFS presses against [the] HL?”. A theme of the manuscript as written is the demonstration of function in dead insects, but there isn’t a cogent argument for or against this approach (e.g., lines 65, 66 in the Introduction), and the qualitative evidence isn’t clearly separated from the conclusion (both reviewers commented on the mixing of “Results” and “Discussion”).

To address these two points, provide a justification for your approach and methods (Introduction), describe how the manipulation was done (Methods),

We have justified our approach and described our methods in the Materials and Methods section. Briefly, if an insect dies with fully contracted tibial flexor muscles, the femoro-tibial joint is often resists against any extension. If the tibia can not be opened after the detachment of the flexor muscles from the femur, it is likely, that a locking mechanism is involved, that can be further examined by moving the joint and observing the movement/relationship of the sclerotic element.

explicitly relate the results of the manipulation (Results), and clearly outline the reasoning that led to the conclusion (Discussion). With regard to the discussion, explain why alternative mechanisms are unlikely, make clear the limits of what can be inferred, and address the additional comments of Reviewer 1 (e.g., how would an insect avoid damage from friction between the TFS and the HL?).

We don’t really understand how a fully flexed tibia would be involved in walking, insects usually walk with at least partially expanded tibiae. Functional analysis of walking is beyond the scope of the recent paper.

Once the authors address Reviewer 2’s three critiques above, the remaining issues are the organization of the writing (see Reviewer 1’s comments on the Discussion, plus Reviewer 2’s notes),

and the vagueness plus brevity with which certain topics are treated. This latter point is more-or-less about oversimplification, which is most clearly shown in the opening sentence “Arthropod legs are simple anatomical structures.”

We have changed the opening sentence.

One could argue that the biramous appendages of non-hexapodan Pancrustacea are very complex in their form and ornamentation, and that insect legs can have complex patterns of ornamentation as well as surprising mechanical adaptations (e.g., screws or gears). For this reason, perhaps it is best to emphasize the relative biomechanical simplicity. (Even this is debatable, given the complexity of the extrinsic coxal musculature.) Further, there are some word choice issues—proof versus demonstration versus evidence, conclusion versus inference, etc. Careful writing will address this issue.

We have corrected the MS accordingly.

Additional actions which will improve the writing are as follows:

- Introduction Section

o Explicitly outline the skeletomuscular anatomy of walking legs, rather than simply relying on the generalization provided in Figure 1. For the description, specify which muscles generally occur in the limb, name them according to convention (Snodgrass 1935 is the most stable foundation), and specify sources of alternative nomenclature.

We have corrected the introduction and narrowed down its focus on the femoro-tibial joint and muscles that move this joint. We followed the terminology of Snodgrass 1956 as we did in the first version and included an appendix for anatomical structures that are linked to the Hymenoptera Anatomy Ontology using ontology class URI-s. It would be really sad if the convention would be Snodgrass 1935.

Reviewer 2 helpfully provides a list toward this end derived from the “Muscles of the Telopodite” section of Chapter IX of Snodgrass (1935). This action will not only provide the necessary information for understanding the modifications, but will be enriching for the general reader, who may not—and is probably not—a morphologist. Moreover, Snodgrass’s muscle names for the leg are primarily functional, which eases the task of explaining the mechanics. It is not necessary to discuss the extrinsic limb muscles here (i.e., those originating in the thorax and inserting on the coxa).

As described above, this paper is focusing on a locking mechanism in the femoro-tibial joint. Discussion of “Muscles of Telepodite” is beyond the scope of this paper. We have followed the terminology from Snodgrass 1956.

o Provide a brief review of the variety of locking mechanisms in insect legs (i.e., address the first point of Reviewer 1’s critique). This would be a good place to address Reviewer 2’s question about the primary function of the tibial flexor sclerite (see the last sentence of R2’s “Major notes” section).

We have cited Gronenberg 1996, who provided a really thorough review for locking mechanisms in insect legs and added a small section to the Introduction about the possible function of the tibial flexor sclerite.

o State the objective of the study, and outline the methods and approach—with justification.

What was the objective of the study? What guided taxon selection? How do the methods justify the results? (Why were certain microscopical methods chosen?)

We have clearly described the objectives of this study in the Introduction and have added explanations for our taxon selection, microscopic and dissection techniques in the Materials and methods section.

- Methods Section

o Add a table with abbreviations, as recommended by Reviewer 2.

Abbreviations are available from Appendix 1 (URI Table)

o Move the table of specimens examined out of the Supplement into the Methods (as recommended by Reviewer 1), and split column 1 (“group”) into three columns (Order, Family, Subfamily). For this table, also put the Diptera together, and the Diopsidae together within the Diptera. An additional column which would be useful would state which figures and supplementary files are associated with each specimen.

We have corrected the table accordingly and moved to the main text.

- Results Section

o Provide explicit descriptions of the skeletomuscular anatomy of each taxon addressed. An approach to this to which would reduce the work involved would be to provide a description of the shared features, then specify observed differences for each taxon. This would address Reviewer 1’s question “is [this a] generalized description of the legs for Diptera, Hymenoptera, and Orthoptera?” This is also an opportunity to clarify whether one or more than one locking mechanism was observed, as wondered by Reviewer 1.

We have rewritten the Results section, in which we focus on the description of anatomical structures that are involved in the ventral lock of the femoro-tibial joint. Only one locking mechanism has been described in the femoro-tibial joint in insecta. A second lock has been described in mantis shrimps in a joint that might or might not be homologous with the femoro-tibial joint in insects.

- Discussion & Conclusion Sections

o Address Reviewer 1’s questions.

o Provide an explanation of alternative mechanisms, if any, and outline what needs to be done experimentally in future studies.

We have largely rewritten the Discussion section and provided a section about future directions. Other possible functions for the enlarged tibial flexor sclerite and the genuflexor sclerite has been proposed by Furth and Suzuki 1990a and mentioned in the introduction.

- For the figures:

o Move text from the figure captions into the main text (where appropriate), and explicitly note which limb is being examined and the view at which the image has been taken. 22

We have simplified the figure legends, provided view information and defined explicitly which limb has been examined.

o Provide scale bars consistently.

We have provided scale bars consistently

o Note that Reviewer 2 requests that a series of screencaptures from the manual dissection be presented. I recognize that Fig 4 A¬–D represent such “screencaps”. State explicitly that these are derived from the manual dissection in the “Locking mechanisms” section of the results.

We think that one of the major issues with the submission was that the reviewers were not able to access the video files where locking and unlocking of the catch mechanism can be seen in action. The journal allows us to embed video files in the online version of the article. For the printed version, we would like to hear the suggestions from the editorial board for representing data from the video files on a more static way that coud be incorporated into a pdf file.

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author

In general the manuscript and presented data are interesting, especially those for the presence of tibial flexor sclerite and Heitler’s lump in the grasping legs of Diptera and Hymenoptera. However the major problem is a so-called experimental data that are unconvincing being based on the dead objects. There are also others major and minor problems listed separately. Thus the paper is in need of a major revision and in the present state cannot be recommended to publication.

Reviewer: 2

Comments to the Author

General estimate

This submission excites dual impressions. On the one hand, the Authors thoroughly selected several rare cases of probable passive leg grasping (without continuous expense of force and energy), applied elaborated and most recent methods to reveal internal morphology of joints in rather small insects, used simple but convincing manipulation on glycerol-stored leg specimens in order to demonstrate the role of a passive lock which ensures grasping. On the other hand, the text and figures are produced astonishingly carelessly by 50%, and the present work may be accepted only after major revision.

The Authors revealed homology between mechanisms of energy storage and triggering in jumping and kicking arthropods (which have been studied for almost a century in various unrelated taxa) and

in some grasping insects, despite the different relative power of two antagonist muscles in two object sets. I think, however, that jumping in adult insects of different taxa is performed almost predominantly with hind legs (despite variability of loci of energy storage); on the contrary, passive retention of object grasping by the legs is achieved by several methods: suckers on the fore tarsi in male diving beetles; spurs on ventral edges of the tibia and the femur in mantids and Mantispa (Neuroptera); lethal injections in bugs (Nepa, Belostoma, Phymata) and robber flies (Asilidae); special processes on male tarsi in several species of Lispe (Diptera, Muscidae). Therefore it was so difficult to select and obtain objects with grasping, fixed with the Heitler's lock.

Really important to note hear that we have never stated that the structures in the leg of grasping taxa are homologous to that if jumping taxa, in fact we did not used the term 'homology'. **Our work helps to reveal similarities and aids the research of future morphologists to establish whether there is a homology or not.**

Major notes

The most convincing part of the Text is description of manipulated and triggered flexion on amputated legs. Astonishingly, this story is shortly mentioned not in Results, but in Discussion, without any material confirmation by tables, schemes, pictures: thus one must take this tale on trust. These experiments were filmed and attached as movies in Supplementary materials, but alas! I had no access to supplements in the download for my review.

These videos will be embedded in the online version of the manuscript.

Manipulated movements of glycerol-soaked legs were forgotten in the Methods section.

We have provided a much cleaner explanation for our method, as well as, our interpretations of the results.

Meanwhile manipulations are the strongest and single evidence about the hypothetical mechanism of passive grasping and its triggering release, because the Authors lack any observations on live insects. I suggest to dedicate a good few of Results for (i) a list or a table with numbers of tested leg specimens and successful responses/faults for each tested species;

We have altered our specimen table accordingly

number of film records or notes of visual observations and (ii) stripping of representative frames at least for 2-4 episodes. Add these illustrations on account of, say, Fig. 2.

The journal allows us to embed video files in the online version of the article. For the printed version, we would like to hear the suggestions from the editorial board for representing data from the video files on a more static way that could be incorporated into a pdf file.

I think that the journal "Insect Systematics and Diversity" is exacting in nomenclature standards. Please indicate for insects in Table 1: order, family, genus, specific name, author's name and year – look other articles in ISD as a template. If you know the genus, you certainly know the family. You spent so much efforts for SR-μCT, CLSM, dissection, video and TEM, that a task to identify the specific name of Ochthera – using the "Manual of Nearctic Diptera" or cited Clausen (1977) would be a sort of relax.

We fully agree with the reviewer and added these information to our specimen table. Unfortunately, we still are unable to identify the Ochhera species. Manual of Nearctic Diptera is a generic level treatment and the Ochthera species that we examined belongs to the mantis species complex whose members can not be identified based on female specimens.

More difficult is determination of *Podagrion* sp. –apply to local professionals in Torymidae.

It is really hard to identify the Podagrion specimens and, we rather use sp. than provide a false identification.

The other problem is the anatomical nomenclature. I suggest to follow Snodgrass (1935) as the authoritative standard (with few later changes). Let us look at Fig. 1: <Two muscles connect the four proximal leg segments, the flexors (blue) close and the extensors (brown) open the joint.> First of all, it is not clear, what means “open or close” for joints between short podomeres. Secondly, all muscles have names according to movements they exert relative to the body: promotor, remotor, adductor, abductor, rotator (subcoxal muscles), levator, depressor (onto the trochanter from different sclerites in the thorax and the coxa), reductor femoris (without any antagonists!), recent terms extensor and flexor (onto the tibia), depressor(s) and sometimes levator (onto the first tarsomere), recent term retractor unguis (also without antagonists) from the tibia (and sometimes from the femur) onto the unguitractor. So you see, that muscles are not always arranged in pairs.

We have used Snodgrass 1956 for the two tibial muscles. We have changed the text and Figures and annotated only the tibial flexor and extensor muscles that were the focus of our paper.

Muscles in the femur are painted correctly, but in the caption the enlarged brown muscle in the jumping leg (1A) is described as the “enlarged tibial flexor”, the same error is in 1C, whereas in D, E the flexor sclerite and the flexor painted blue are named properly. Muscles are named according to insertion onto a distal podomere. Check the same names in the Text. The nonsclerotized (flexible) cuticle in joints is called articular membrane (by Snodgrass), whereas your “conjunctiva” has its main application in the ophthalmology.

We have corrected Figure 1 accordingly. Conjunctiva is used in entomology for any membranous structures that are continuous with sclerites (Ronquist and Nordlander 1989).

Errors in muculature in Fig. 1 are bad, but the scheme of a leg and its muscles is quite acceptable as the standard orientation with respect to the body: head to the left, femur horizontally, knee to the right, tibia downward. Sorry, orientation in following figures is so variable that it is difficult to compare figures together: one meets turns about the right angle clockwise or anticlockwise, mirror orientation (flips). A Reader must guess first of all, where is the head and the heart in each figure. I recommend to decide, which orientation is standard throughout all small figures, use primary originals without inscriptions, rotate or flip canvas and mount each multiple panel in a common standard. Insert pairs of arrows, e.g. dors-dist, ant-prox etc. Scale bars were mostly neglected in different figures of the same panel.

We have made the orientations of the figures uniform and added orientation to figure legends (e.g. distal to the left).

The evidence of independent parallelism of the Heitler's lump in remote taxa is interesting, but first of all I would like to know the primary function of the tibial flexor sclerite, which provides preadptation for jumping or passive grasping.

There are two sclerites involved in the ventral lock of the femoro-tibila joint. In Orthopterans and in flea beetles, the genuflexor sclerite and in Grasping taxa and Orchestes, the tibial flexor sclerite. These sclerotic elements are not homologous structures. We have discussed the possible function of these elements in the discussion. The GFS might protect the ventral side of the femoro-tibial joint, but not only in this taxa where the sclerite is atrophied.

Minor notes

Move

<295 We have found accessory tibial flexor muscles only in Diopsidae. These muscles are

296 composed of 10–15 muscle fibres and seemingly behave differently compared to the more

297 proximal muscles. In specimens where the femoro-tibial joint is locked, these muscles do not

298 seem to be in a contracted state (fe-tifld: Figs 3D, 7E, F). Based on their orientation, it is possible

299 that the accessory flexor muscles disentangle the lock between the TFS and the HL.>

to Results.

We have moved this information to the Results section

Move long explanations in captions to the text in Results.

We have corrected figure legends accordingly.

Collect all abbreviations, used in Results and captions, into a table or a list in the alphabetic order and place it either before the Text, or before Results. Insert an abbreviation at the first mention in the Text, it would be enough.

We have added abbreviations to the URI table.

The role of resilin as an accumulator of the potential energy is partly reconsidered in the last decade: the main role is ascribed to composite sclerotized structures with hypothetical participation of resilin as a protector against break-up of chitin elements. The semilunar process is one of candidates fir composites. Sorry, I don't know about ultrastructural studies of hypothetical composites. The story on resilin is not wanted in Fig. 2, because resilin is not detectable by X-ray tomography.

The section about resilin has been removed from Figure 2.

Section "Materials and Methods" lists a lot of equipment and software, but only in few cases the producer, city and country are quoted – check this style according to the journal standard.

We have added producer, city and country for equipments.

<42 Arthropod legs are simple anatomical structures.> it is not true, including your own structural observations, which state quite contrary assertion.

We have rephrased the introduction accordingly.

Reviewer 2.

Review ‘Universal locking mechanisms in insect legs: jumping and grasping’ General comments. In general the manuscript and presented data are interesting, especially those for the presence of tibial flexor sclerite and Heitler’s lump in the grasping legs of Diptera and Hymenoptera. However the major problem is a so-called experimental data that are unconvincing being based on the dead objects. There are also others major and minor problems listed below. Thus the paper is in need of a major revision and in the present state cannot be recommended to publication.

Title ‘Universal locking mechanisms in insect legs: jumping and grasping’

Comment. How did the universality of the locking mechanism proved? The observation have been done on a few specimens of Diptera, Hymenoptera and Orthoptera. There are insects without locking mechanism (leaf beetles, weevils, jewel-beetles) and insects with another type of locking mechanism (marsh beetles, staphylinids, some homopterans).

Even though they are not homologous, the ventral locks of the femoro-tibial joints are universal in insecta. We have expanded our observations to Orthoptera, and some beetle taxa including flea beetles and proved that Betz was right in his 2007 paper as locking mechanisms are present in this taxon.

One cannot be agreed with universality based on the presented data. It is recommended to change a title in the following way, e.g. ‘Locking mechanisms in the jumping and grasping legs of Diptera, Hymenoptera and Orthoptera’. For more information it is recommended to read the important paper of Gronenberg, 1996 which is missing in the Reference list (Gronenberg, W. 1996. Fast actions in small animals: springs and click mechanisms. J. Comp. Physiol. A, 178, 727-734).

Gronenberg provides a review for different catch (locking) mechanisms in his paper, we are focusing in the present study the locking mechanism of the femoro-tibial joint that is achieved by the interaction between the TFS and the HL.

Abstract

Line 33. ‘’We were able to prove the presence of a locking mechanism in all of the studied

grasping legs by simple manipulation of dead specimens in glycerol.''

Comment. It looks very doubtful that it is possible to imitate the muscles' contractions by ''simple manipulation of dead specimens in glycerol''. How do you know exactly how strong and how far the muscles contract? How do you know exactly which group of muscles need to be contracted or relaxed in such a movement? How do you really know that in the live insect TFS presses against HL? Are there any confirmations based on the live insect?

Thank you for this comment! We made it clear in the new version of our paper which part of our experiments support the presence of a lock:

"We assumed that a locking mechanism is present in the femoro- tibial joint only if we have seen two interacting sclerites preventing the joint to open in fully flexed legs after the flexor muscle origin was detached from the femur. The ability to relock the joint by pulling the flexor muscle is only an additional evidence for the presence of the lock, and we have to acknowledge, that by pulling the flexor muscles, we can not model properly the natural contraction of the muscles. The flexor muscle fibres are grouped in multiple, distinct bundles, whose neural control, strength and speed are remained to be described. "

We see the TFS (genuflexor sclerite) pressed against the femoral wall in dead freshly killed insects which fully contract their tibial flexor muscle. This is also clearly visible in earlier papers (i.e. Nadein and Betz 2016). To examine these locks in living insects would be great, and perhaps we should do this in the future (insects would certainly survive some bleaching in their cuticle), but right now the quality of observation of internal structures in living insects is very limited.

Introduction

Line 61. ''… HL have been reported in numerous other jumping and grasping insects

(Jumping: Chrysomelidae…''

Comment. Legs of jumping leaf beetles lack Heitler's lump. Instead, the internal

invagination similar to Heitler's lump is found in the legs of jumping weevils (Nadein &

Betz, 2018). This is one more evidence of the absence of 'universality' even for comparatively related group of insects.

We have changed the introduction according to the reviewer comments.

Line 77. ''… structure and function of locking mechanisms (TFS and HL)…''

Comment. TFS and HL are not the locking mechanisms but anatomical structures. It is better to write ''In this study, we explored the structure and role of TFS and HL in the locking mechanisms in the above mentioned taxa''.

We have corrected the text accordingly.

Line 79. '' …observation of function…''

Comment. It is hard to investigate the function by the simple dissections and observation. Moreover, this is in the contradiction with your statement in Conclusion ''Descriptive analysis of the relative position of different anatomical structures cannot provide proof of the presence of any locking mechanism.''

Thus, the data presented in this manuscript are doubtful as well as their reliability.

We have corrected the Introduction section, so this paragraph is not included in the introduction in the new version. We also clarified that our observation is different from that of made on static images in that although we used dead specimens, we were able to observe anatomical structures in motion. One might criticize that these observations are not in live specimens. This is a valid criticism. However, our observations provide definitely more information than the ones that were made on static images (e.g. not in-vivo microCT-data)

The Introduction chapter lacks well-formulated goal.

It is recommended to write clearly the list of model objects.

We have provided goals and the range of taxa we dissected in the introduction.

Material and Methods

Line 83. The Table with model objects is better to place directly here.

We have inserted the table in the materials and methods section.

Results

Line 146. ‘’Integument’’, Line 167. ‘Muscles’

Comment. Is that generalized description of the legs for Diptera, Hymenoptera and Orthoptera? If so it is anatomically incorrect. The legs of such different insects cannot be described so shortly and in a generalized form.

We provided description of anatomical structures that are involved in the ventral lock of the femoro-tibial joint in the new version of the manuscript and described our observations that were backed up with video shootages.

Moreover there is no mention which pair of legs are described – fore, middle or hind, neither in the Material and Methods chapter nor in the Results chapter.

We have added the described legs in the specimen table in the Materials and method section.

Line 179 ‘Locking mechanisms’

Comment. Is there just one locking mechanism or more than one revealed? If there are more than one mechanism all of them need to be separately described in the chapter

Discussion but not in the chapter Results. In fact here, in the chapter Locking mechanisms, only the results of manipulations with legs have been described but nothing deal with locking mechanism. Therefore the title of the chapter is incorrect and need to be Changed.

We have corrected the Results section and treated these issues.

Discussion

The Discussion represents the chapters mixed up with results of the study and review of published data. It should be better structured in a logical order. A lot of information written here could be transferred to the Introduction chapter. It is not clear which type of locking mechanism is described – active or passive sense Gronenberg (1996). If it is an active locking mechanism the additional muscles are required to control the locking function of TFS. Could you please provide an explanation and evidences in what way the same flexor muscles can participate both in locking function and in a simple flexion of tibia while walking?

To examine how the flexor muscles are involved in walking is beyond the scope of the present paper.

Obviously, the different muscles are required for different movements. In other words, how the locking mechanism can be activated in the absence of specialized muscles? Nothing of that has been found in the text of MS.

We have added a section that explains why the presence of a trigger (or release) muscle is not required for the release of a locking mechanism.

When walking does TFS rubs against HL, isn't it?

No.

How does insect avoid such adamageable friction?

See previous response.

If no, again, how does insect avoid that contact when walking?

See previous response.

If the locking mechanism is called ‘universal’ how does it works in the absence of Heitler’s lump in many insects?

These observations clearly demonstrate that, albeit the presence of a ventral lock in the tibio-feoral joint of enlarged legs is universal in insects, different lineages achieve this mechanical function using a myriad of different solutions.

Nothing is written about that.

The detailed review of the variety of locking mechanisms in the insects’ legs is highly

recommended (see the papers of Betz & Mumm, 2001, many papers of Burrows and

Sutton & Burrows devoted to jumping insects, Gronenberg (1996), etc.).

This paper is focusing on the anatomical structures that are involved in the ventral lock of the femoro-tibial joint.

Conclusions

Line 312-313. ‘’... building blocks of this system are present in every insect…’’

Comment. Naturally, it is not. TFS and HL are not presented in every insect.

All insects have a ventral femoral wall, the tendon of the tibial flexor muscle and most if not all of them possess the genuflexor sclerite.

Supplementary Table 1

Comment. The Table is incomplete. 1. ‘group’ – there is no group like ‘stalk-eyed fly’, this is an informal name. Need to add the Latin names of the order, family and subfamily. ‘Specimen data’ – uncertain meaning; better to name it ‘Locality’; 3. The collecting data from Germany are missing.

We have corrected the specimen table accordingly.

Universal locking mechanisms in insect legs: jumping and grasping

Mihály Földvári[1], István Mikó[2,3], Jonah M. Ulmer[3], Tomy dos Santos Rolo[4,5], Sándor Csősz[6], Andrew Pomiankowski[7,8], Tilo Baumbach[4,9], Thomas van de Kamp[4,9]

[1]Natural History Museum, University of Oslo, PO Box 1172 Blindern, NO-0316 Oslo, Norway

[2]UNH Collection of Insects and other Arthropods, Department of Biological Sciences, University of New Hampshire, Durham, NH, USA

[3]Department of Entomology, The Pennsylvania State University, University Park, PA, USA

[4]Institute for Photon Science and Synchrotron Radiation (IPS), Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

[5]Department of Electrical and Electronic Engineering, Southern University of Science and Technology, Shenzhen 518055, China

[6]MTA-ELTE-MTM, Ecology Research Group, Pázmány Péter sétány 1C, H-1117 Budapest, Hungary

[7]Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK

[8]Centre for Computation, Mathematics and Physics in the Life Sciences and Experimental Biology (CoMPLEX), University College London, Gower Street, London WC1E 6BT, UK

[9]Laboratory for Applications of Synchrotron Radiation (LAS), Karlsruhe Institute of Technology (KIT), Kaiserstr. 12, 76131 Karlsruhe, Germany

Abstract

Thickened femora of insects are correlated to enlarged muscle masses and serve two basic purposes: jumping/kicking and grasping/holding. Modifications on the ventral femoral wall and the tibial flexor tendon that are possibly involved in catch mechanisms have been described in multiple insect taxa with both jumping and grasping legs. Our comparative study aims to explore the functional and structural similarities of these modification in jumping and grasping leg types from Coleoptera, Hymenoptera, Diptera and Orthoptera with the combination of cutting edge, non-invasive imaging methods and classical dissections techniques. Our data indicate that locking mechanisms are present in the jumping and grasping legs of insects. We describe three femoro-tibial lock types based on the location of the interacting sclerites relative to the site of origin of the tibial flexor tendon. All of the three types can be found in jumping insect legs, while only one type is present in grasping legs. The locking mechanism might aid to keep the femoro-tibial joint in a flexed position for an extended period of time. Our data indicate that morphologically similar modifications in the femoro-tibial joint are involved in energy-saving mechanism both in jumping and grasping legs in insects.

Keywords: courtship, sexual selection, convergence, CLSM, SR-μCT, 3D reconstruction, TEM, SEM.

Introduction

The insect femoro-tibial joint is a relatively simple mechanical system as it is composed of two cylindrical leg segments (tibia and femur) that are movably connected to each other with a

ring-like conjunctiva, two antagonistic muscles - tibial flexor and tibial extensor - and with a dicondylic joint composed of a pair of lateral articulations (Fig. 1). Straightening (extension) and bending (flexion) of the leg is achieved by the alternate movement of the two tibial muscles which arise from the femur with wide, fan shaped site of attachments and inserts at the base of the tibia with elongate tendons (Figs 1A–C). The ratio of the size of the tibial flexors and extensors is highly variable and corresponds to the adequate function of the leg that serves a broad variety of motion or grasping related behaviors (Furth and Suzuki 1990a). The muscles are equivalent in normal walking legs while the flexor muscle is enlarged in grasping and the extensor in jumping/kicking legs (Figs 1A–C, Furth and Suzuki 1990a).

Changes in the muscle mass ratio is often accompanied with tendonal modifications, which have been reported from the enlarged fore, middle and hind legs of numerous insect taxa. The tibial flexor sclerite, atrophied basal sclerotization at the tibial flexor tendon, is perhaps the most common tendonal modification in jumping and grasping legs. In locusts, the tibial flexor sclerite is involved in a lock (catch) and plays an important role in the energy releasing catapult mechanism (Heitler 1974, Gronenberg 1996). While the tibial flexor muscle contracts, and bents (flexes) the tibia, the tibial flexor sclerite is pulled over an internal projection (invagination) of the ventral femoral wall (Heitler's lump) into a locked position (Figs 1D, F, 2A–G). When the tibial extensor muscle starts to contract, the lock prevents the flexor tendon to move and straighten (extend) the femoro-tibial joint and it requires an extra power from the extensor muscle to eventually overcome the lock. During the time of release, energy from the contracting extensor muscle is stored in an apical, resilin rich structure of the femur, the semilunar process (SLP: Figs 2A–C, 10A, B). The stored energy from the semilunar processes allow the locusts to jump multiple times of their body length.

Albeit it was suspected, it has never been shown that the tibial flexor sclerite of other jumping insects would participate in similar locks and catapult mechanisms (Barth 1954, Furth and Suzuki 1990a, Betz 2007). Instead, it is now speculated that the tibial flexor sclerite in these taxa might be involved in strengthening the tendon, altering the working angle of the flexor system or simply protect the ventral portion of the femoro-tibial joint (Nadein and Betz 2016, 2018). These hypotheses are also supported by the fact that the sclerite is not only found in jumping but also in grasping insect legs, which would not utilize a catapult mechanism (Furth and Suzuki 1990a, b).

Although the tibial flexor sclerite across jumping and grasping insects seems structurally equivalent, its relative position to the site of origin of the tibial flexor tendon tendon is variable (Furth and Suzuki 1990a). The tibial flexor tendon is an invagination of the single layer epithelium at the femoro-tibial conjunctiva and is connected to the ventral femoral wall with a resilin rich ligament, the genuflexor sclerite (Snodgrass 1956). In Alticinae leaf beetles and in Orthoptera, the tibial flexor sclerite is the atrophied genuflexor sclerite (Snodgrass 1956, Furth and Suzuki 1990a, Nadein and Betz 2016) while in jumping curculionids, the tibial flexor sclerite is the atrophied basal region of the tibial flexor tendon (Nadein and Betz 2018).

While examining ethanol preserved beetle, fly and hymenopteran specimens with enlarged fore or hind femora, we discovered, that if the specimen died with fully flexed legs, we were not able to open (unflex) the femoro-tibial joint easily, while in specimens that died with not fully flexed legs, the joints could usually be easily moved. We also observed in specimens with transparent femoral cuticle that the tibial flexor sclerite and the ventral tibial wall is locked together in some specimens preventing the straightening of the femoro-tibial joint. I this study, we examined the femoro-tibial joint of 13 grasping and jumping insect taxa combining simple

dissection and cutting edge 3D visualization techniques, to reveal if a lock is present in the modified femoro-tibial joints and to better understand structural equivalencies of the anatomical structures that might be involved in these locks. This study demonstrates that simple observations using classical dissection techniques still play an important and unavoidable role in insect morphology even in the age of non-invasive 3D reconstruction techniques.

Materials and Methods

We have examined grasping legs with enlarged femora in taxa where modifications on the tibial flexor tendon have never been reported (diopsid flies, shore flies and torymid wasps) and reexamined jumping (Alticini, Chrysomelidae) and a grasping (Bruchidae) beetles in which the tendon sclerotizations have been reported (Furth and Suzuki 1990a, b) but their involvement in the ventral femoral lock has been dubious (Alticini) or were never proposed (Bruchidae). We have recorded our dissections with bright field microscopy and visualized dissection results with Confocal Laser Scanning Microscopy. For 3D reconstruction, we applied synchrotron based Micro-CT and to explore the fine structure of the tibial flexor sclerite, we applied scanning and transmission electron microscopy.

Specimens for the present study (Table 1) were stored in 75% ethanol and were transferred to anhydrous glycerol on a concave coverslip for dissection and CLSM and are deposited in the UNH Collection of Insects and Arachnids (UNHC).

Terminology for cuticular elements follows Klass and Matushkina (2012) and Ronquist and Nordlander (1989). We used the term sclerite for less flexible areas of the exoskeleton that are connected to each other by more flexible conjunctivae (=arthrodial membrane, =membrane). We identified these elements by manipulating the exoskeleton using insect pins and forceps.

Terminology of anatomical structures in the femoro-tibial joint follows Furth and Suzuki (1990a), Snodgrass (1956) and Betz (2009). We have classified sclerites on the ventral region of the femoro-tibial joint based on their relative position to the site of origin of the tendon of the tibial flexor apodeme, which corresponds to an invagination on the distal femoral margin. We used the term tibial flexor sclerite (TFS, Furth and Suzuki 1990a) for slcerotized elements on the tibial flexor tendon and the term genuflexor sclerite (GFS, Snodgrass 1956, =Lever's triangular plate, =tibial flexor sclerite sensu Furth and Suzuki 1990a, b, Betz 2009, Nadein and Betz 2016) for slcerotized elements between the site of origin of the tibial flexor tendon and the proximal tibial margin. We used the term Heitler's lump for the flattened invagination on the ventral femoral wall proximal to the anterior margin and femoral abutment for the resilin rich distal projection at the distal margin of the ventral femoral wall (=of 'Lever's triangular plate, Nadein and Betz 2018). We have introduced the new term genuflexor apodeme for the invagination on the distal tibial end of the genuflexor sclerite that is sclerotized and is adjacent to the external wall of the tibia (GFS: Figs 4A, D, Fig. 6A) and the ventral lock of the femoro-tibial joint that refers to a lock between the ventral femoral wall and a sclerite that originates from the femoro-tibial conjunctiva (genuflexor sclerite or tibial flexor sclerite). The terminology for muscles follows Snodgrass 1956. We use the term lock to refer to two sclerite surfaces that are involved in a locking mechanism.

We ***dissected*** ethanol preserved and dried (card mounted) specimens. One part of the ethanol preserved specimens were transferred to anhydrous glycerol and longitudinally bisected with Personna razor blades (Edgewell Operations, Allendale). Another part of ethanol stored specimens and all dried specimens were bleached and rehydrated in 35% H2O2 (Sigma Aldrich, Burlington Massachusetts) for 24 hours and then transferred to anhydrous glycerol (Mikó et al.

2016). Specimens were dissected in glycerol with Dumont 5# forceps (Fine Science Tools, Foster City, California), insect pins (#2), Vannas Spring Scissors with 2mm cutting edge (Fine Science Tools, Foster City, California) and Personna razor blades on concavity slides in anhydrous glycerol using an Olympus SZX16 stereomicroscope equipped with a 2X objective providing a 230× magnification (Olympus Corporation of the Americas, Center Valley, PA) and a Huvitz HSZ-ZB700 stereo-microscope (Huvitz BD, Gyeonggi-do, Republic of Korea).

We ***observed the movement/interaction*** between the proximal tibial flexor tendon and the ventral femoral wall while moving (straightening and bending) the femoro-tibial joint through the bleached cuticle of H2O2 treated specimens or viewing the internal side of bisected specimens. Then we detached (severed) muscle sites of origin and repeated the observations while moving the joint. If we found a lock mechanism between the tibial flexor tendon and the ventral femoral wall, we tried to unlock/relock the catch by straightening the joint or by using an insect pin as a lever to dislodge the locking sclerites.

Videos were taken on an Olympus SZX16 stereo-microscope and a Huvitz HAZ-ZB700 stereo-microscope with a Canon EOS 70D and a Canon Rebel DSLR camera (Canon USA Inc. Melville, New York), respectively. Stacks of bright field images were taken manually on an Olympus CX41 microscope (Olympus Corporation of the Americas, Center Valley, PA) with a Canon EOS 70D DSLR camera attached and the images were combined using the Align and Stack All (DMap) algorithm of ZereneStacker (Version 1.04 Build T201404082055; Zerene Systems LLC, Richland, WA).

Sample preparation for ***confocal laser scanning microscopy*** (CLSM) followed Mikó and Deans (2013). Specimens were imaged between two #1.5 coverslips with an Olympus FV10i confocal laser-scanning microscope (CLSM, Olympus Corporation of the Americas, Center

Valley, PA) at the Microscopy and Cytometry Facility at the Huck Institute of Life Sciences at the Pennsylvania State University and with a Nikon A1R-HD CLSM at the University of New Hampshire Instrumentation Center. With the Olympus FV10i we used three excitation wavelengths, 405 nm, 473 nm, and 559 nm, and detected the autofluorescence using two channels with emission ranges of 490–590 nm, and 570–670 nm (Fig. 2). On the Nikon A1R-HD, we either used a preset (confocal) with 3 excitation wavelengths, 408.9 nm, 487.4 nm and 559.9 nm and 3 emission ranges of 435–470 nm, 500–540 nm and 570–645 nm (Fig. 1) or used one excitation wavelength 487 nm laser with emission ranges defined using the A1-DUS spectral detector, 500–560 nm and 570–630 nm (Figs 3E, F, 4–6). The resulting image sets were assigned pseudo-colors that reflected the fluorescence spectra. Volume-rendered micrographs and media files were created using FIJI (Schindelin et al. 2012) and Nikon NIS-Elements AR v. 5.02.01.

Synchrotron X-ray tomography (SR-μCT) was performed at the UFO imaging station of the Karlsruhe Institute of Technology (KIT) light source. The specimens were either critical point dried (*Gryllus campestris* & *T. dalmanni*) or scanned in 70% ethanol (*Podagrion* sp.). For each scan, 2,500 (*G. campestris* & *Podagrion* sp.) or 3,000 (*T. dalmanni*) equiangularly spaced radiographic projections were acquired in a range of 180°. A parallel polychromatic X-ray beam was spectrally filtered by 0.2 mm Al to obtain a peak at about 15 keV. The detector consisted of a thin, plan-parallel lutetium aluminum garnet single crystal scintillator doped with cerium (LuAG:Ce), optically coupled via a Nikon Nikkor 85/1.4 photo-lens to a pco.dimax camera with a pixel matrix of 2008x2008 pixels (dos Santos Rolo et al., 2014). The magnification was set to 10X (*Gryllus* sp. & *Podagrion* sp.) and 20X (*T. dalmanni*), resulting in effective pixels sizes of 1.22 and 0.61 μm. Tomographic reconstruction was performed with the GPU-accelerated filtered back projection algorithm implemented in the software framework UFO (Vogelgesang et al.,

2012). 3D reconstruction of tomographic data was performed using Amira (version 5.4.3, FEI) for volume segmentation and rendering.

For ***TEM***, legs were removed from adult flies and fixed in 2% paraformaldehyde (PFA), 1.5% glutaraldehyde in 0.1M phosphate buffered solution (PBS) for 1.5 hours at room temperature . After three 10 minute washes in 0.1M PBS, the fixed tissue was transferred to 1% osmium oxide (OsO4) for 45 minutes, followed by a 10 minute buffer (PBS) wash and two 10 minute washes in double distilled H2O (ddH2O) and then to 2% uranyl acetate (UO2(CH3COO)2·2H2O) for 15 minutes, followed by three 10 minutes washes in ddH2O. The legs were then dehydrated through an ethanol (EtOH) series (5 minutes at 25% EtOH, 5 minutes at 75% EtOH, 5 minutes at 90% EtOH, 5 minutes at 100% EtOH). This was followed by four 10 minute washes in 100% EtOH and three in propylene oxide (C3H6O). The legs were then embedded in the epoxy resin, Agar 100 ® (Agar Scientific, UK) in a stepwise manner, being transferred to 2 parts propylene oxide: 1 part Agar 100® resin for 1.5 hours and then 1 part propylene oxide: 2 parts Agar 100® resin for 1.5 hours. The samples were left in 100% Agar 100® for 8-16 hours at room temperature before the Agar 100® was replaced and the samples placed in resin in moulding blocks at 60ºC, to harden for 48 hours.

Results

Fully flexed femoro-tibial joints with enlarged femora in the studied taxa were locked and difficult or impossible to open even if the site of origin of the tibial flexor muscle has been destroyed. Based on the involved sclerotic elements and their interaction with the ventral femoral wall and the femoro-tibial conjunctiva, we identified three major lock types at the ventral portion of the femoro-tibial joint.

208 *Type I* (Figs 3A, B, 4–8, Videos 1–8). In taxa with grasping legs and in the jumping
209 curculionid, the lock is between the tibial flexor sclerite and the Heitler's lump. In the locked
210 position, the anteroventral portion of the convex ventral surface of the sclerite is in physical
211 contact with the Heitler's lump. The ventral surface of the tibial flexor sclerite is not connected
212 to the ventral femoral wall.
213 We were able to release the lock after extending the multiple times in *Orchestes* and in
214 *Schletterius*. In the diopsids, *Ochthera*, *Caryobruchus* and *Podagrion*, we were not able to open
215 the joint without forcing the tibial flexor sclerite over the Heitler's lump with an insect pin.
216 While moving the sclerite over the lump, we observed that it stuck multiple times at different
217 positions of the lump (as if they were two sides of a velcro tape). By pulling the tibial flexor
218 muscle, we were able to move the sclerite over the Heitler's lump, and thereby secure it in a re-
219 engaged locked position in all taxa. We were able to unlock and lock the joint multiple times.
220 The proximal portion of the femoro-tibial conjunctiva between the site of origin of the tibial
221 flexor tendon and the distoventral margin of the femur is not located in-between the tibial flexor
222 sclerite and the Heitler's lump when the joint is in a locked position. In the hymenopteran and
223 dipteran specimens, the genuflexor apodeme is well developed, and the external tibial wall is
224 angled at the point of its attachment with the apodeme (Figs 4A, D, Figs 6A).
225 The tibial flexor sclerite has a melanized center that is covered ventrally by a transparent
226 (glass-like) ventral layer that is in physical contact with the dorsal surface of the Heitler's lump
227 in all grasping taxa and in *Orchestes*. In *T. dalmanni*, The melanized center of the tibial flexor
228 sclerite is electron dense (darker on TEM images) while the transparent ventral layer is electron
229 lucent (core, covp, covd: Figs 7A–D), the ventral surface of the TFS is heavily sculptured (Figs
230 6B–F), the Heitler's lump is T-shaped in cross section (HL: Figs 3A, D, E, 4A, C, 5G, 6A, 7E, F)

and lack enlarged epithelial cells on its internal (dorsal) surface. The genuflexor sclerite is resilin-rich while the tibial flexor sclerite and the Heitler's lump are not containing resilin based on the presence/absence of blue autofluorescence in response to UV light (407 nm, Figs 4E–H) in the diospids, *Podagrion* and *Ochthera*. In diopsids, 10–15 fibres of the tibial flexor muscle (inserting on the internal surface of the genuflexor sclerite) are oriented vertically and arise to reach the femoral wall distally to the tibial flexor sclerite when the femoro-tibial joint is fully bent (flexed) while these fibres are oriented proximodistally similarly to more proximal fibres in not fully bent legs (fe-tifld: Figs 4A, D, Video 8).

Type 2 (Figs 3C, D, 9). In Alticini, the lock is between the genuflexor sclerite and the ventral femoral wall distal to the site of origin of the posterior portion of the femoro-tibial conjunctiva. Only the distal end of the genuflexor sclerite is in physical contact with the femoral abutment. The femoral abutment contains a distal sclerite and bends ventrally apically when the genuflexor sclerite is unlocked (s: Figs 9A–F). We were able to unlock the joint by extending the tibia multiple times. By pulling the tibial flexor muscle, we were not able to relock the joint. The femoro-tibial conjunctiva is not in between the interlocking sclerite surfaces and the ventral surface of the genuflexor sclerite is not connected to the ventral femoral wall.

Type 3. In the locust (Figs 2, 3E, F, 10), similarly to flea beetles, the lock is between the external surface of the genuflexor sclerite and a process on the internal surface of the ventral femoral wall (Heitler's lump). The external surface of the genuflexor sclerite is concave and limited proximally by a ridge. We were able to release the lock after extending the tibia multiple times. We were able to relock the joint by pulling the tibial flexor sclerite. The proximal portion of the femoro-tibial conjunctiva (proximad to the site of origin of the tibial flexor tendon) is in-between the genuflexor sclerite and the Heitler's lump when the joint is in a locked position

(Figs 2D, E, Figs 3 E, F). The internal surface of the Heitler's lump is covered with enlarged epithelial cells (HL: Fig. 2D). The elements of the locking mechanism (Figs 10A–F) are present in *Gryllus*, we did not find specimens with fully flexed and locked femoro-tibial joint and similarly to the locust, we were not able to lock the joint by pulling the tibial flexor muscle.

Discussion

Besides locusts, the presence of ventral locks in the femoro-tibial joints have never been undoubtedly evinced in insects (Furth and Suzuki 1990a, Burrows and Wolf 2002, Hustert and Baldus 2010, Betz et al. 2007). Using simple manipulations in glycerol stored specimens we were able to show that locking mechanisms are present in the atrophied legs of the examined jumping and grasping insects except in *Gryllus*. These locks are either (i) between the external surfaces of the ventral femoral wall and the genuflexor sclerite (Figs 3E, F), or (ii) between the external surface of the genuflexor sclerite and the internal surface of the ventral femoral wall (Figs 3C, D) or (iii) between the internal surface of the ventral femoral wall and the internal surface of the tibial flexor sclerite (Figs 3A, B). These types also differ in the position and the size of the locking surfaces, the presence or absence of the tibio-femoral membrane in between the locking surfaces, and numerous other modifications. The first two types occur in jumping (Orthoptera, Chrysomelidae) and the third type in both jumping (Curculionidae) and grasping insects (Diptera, Hymenoptera, Bruchidae). These observations clearly demonstrate that, albeit the presence of a ventral lock in the tibio-femoral joint of enlarged legs is universal in insects, different lineages achieve this mechanical function using different solutions.

Furth and Suzuki (1990b) has observed that the tendon of the tibial flexor muscle is enlarged in some bruchid and oedemerid taxa with grasping (holding) hind legs. They did not

discover the ventral femoral lock and concluded that the atrophied tendon might be related to the increased stress caused by the extended contraction of the enlarged tibial flexor muscle (Furth and Suzuki 1990b). According to our study, the enlarged portion of the tibial flexor muscle (the tibial flexor sclerite) of bruchids is involved in the ventral femoro-tibial locking mechanism and helps to keep the femoro-tibial joint in a flexed position for an extended period of time. We found similar locks in grasping Hymenoptera and Diptera taxa where holding for an extended period of time might play a crucial role in their biology.

In their paper, de la Motte and Burkhardt (1983) describe diopsid males, where the larger opponent (*Diopsis subnotata*) catches the smaller one (*Megalabops rubicunda*) by the eye stalk through the use of "tibia-femur pincers" as a grasping mechanism that is capable of locking an object. They also observed numerous *Cyrtodiopsis* (in literature sometimes referred to as *Teleopsis*) individuals with absent eye-stalks and leg segments and they suspect aggressive encounters as reasons i.e. they are capable of breaking off each other's eye stalks. Based on our observations, this behavior occurs rarely and the few individuals seen with broken eye stalks are dying soon; stalk-eyed flies rather reach out to try to grasp the supporting legs of conspecific males as reported by Wickler and Seibt (1972) during fight. It is also reported that they grab and flip each other off surface – in particular off root hairs where they accumulate in the evenings, and they also jab each other with their extended legs (Panhuis and Wilkinson 1999). Diopsid females are often competing for nesting sites or food resources and although not as expressed as in males, they also exhibit aggressive behavior with the involvement of striking with fore legs (Burkhardt and de la Motte 1983, Al-khairulla et al. 2003, Bath et al. 2015).

Females of multiple distantly related chalcidoid taxa use their hind legs to secure their body position (Cowan 1979, Grissell and Goodpasture 1981) while depositing eggs in the host.

Perhaps the most intriguing of them is the example of *Lasiochalciia igiliensis* (Chalcidoidea: Chalcididae) as in this species the female holds the mandible of antlion larvae apart while depositing her eggs through the less sclerotized regions between the head and pronotum (Steffan 1961). Other species use their legs for securing their body on their host during dispersal. Phoresis has been reported in torymid *Podagrion* species, where the females are grasping the wing of their mantid hosts (Bordage 1913, Xambeu 1881). Although grasping has never been described in *Podagrion* males, they often kick each other as part of their aggression behavior similarly to chalcidid females (Cowan 1979, Grisell and Goodpasture 1981).

Ochthera species are well characterized by their enlarged fore femur and sickle shaped tibia representing typical raptorial legs (Clausen 1977). They are predators of smaller aquatic insect larvae and have been reported as important natural enemies of black flies and mosquitoes (Travis 1947, Minakawa et al. 2007). *Ochthera* flies use their "prehensive" fore legs to secure their prey items while they are probing and consuming them (Deonier 1972), but the enlarged fore femur is also used as a waving device during their courtship and aggressive interactions (Eberhard 1992).

Grasping behavior have never been reported from Stephanidae (Hausl-Hofstätter and Bojar 2016), but the presence of robust teeth on the ventral surface of their hind femora indicate that they might be used for grasping. Both males and females of stephanids have been reported to kick with their middle and hind legs during intraspecific fights (Hausl-Hofstätter and Bojar 2016).

Genuflexor sclerite, tibial flexor sclerite, Heitler's lump and femoral abutment

The key components of the ventral femoro-tibial locks are atrophied sclerites at the tibial flexor tendon (tibial flexor sclerite) or the femoro-tibial conjunctiva distal to the tendon (genuflexor sclerite). In flea beetles and in orthopterans, the atrophied sclerite that participates in the lock is the genuflexor sclerite, that is located distal to the site of insertion of the tibial flexor tendon. The genuflexor sclerite can be found in almost all insects, it is more or less sclerotised and in numerous cases it is not involved in any locking mechanism (e.g. *Apis mellifera*; Snodgrass 1956). The genufexor sclerite is continuous to the tibial flexor tendon and connects the tibial flexor sclerite to the tibial base, and has an important mechanical function (the tendon that arises from the femoro-tibial conjunctival would perhaps destroy the conjunctiva without the presence of the genuflexor sclerite). Furth and Suzuki (1990a) proposed that the tibial flexor sclerite (in their paper they used this term for both the genuflexor sclerite and the tibial flexor sclerite) might protect the ventral side of the femoro-tibial joint. The protective function might be possible, but this function is not restricted to taxa with atrophied genuflexor sclerite.

The Heitler's lump on the ventral femoral wall is a cuticular invagination in grasping insects, jumping curculionids and orthopterans that is more or less flattened (pressed against the femoral wall), while the femoral abut is a resilin rich and flexible apical region of the ventral femoral wall that has an apical sclerotic component in Alticini beetles. The Heitler's lump has largely been ignored in grasping insects, as the focus has been on the gross morphological description of tibial flexor sclerites in earlier works (Furth and Suzuki 1990a). and has only been mentioned on a single illustration for grasping heteropterans (*Ranatra* sp., Gorb 1995, fig. 11, d).

We found that the femoral abutment in flea beetles are more complex than has been described, as it is movable and has a sclerotic component. In the dissection experiment of the bisected femoro-tibial joint (Videos 10, 11), it is clearly visible, that when we unlocked the

genuflexor sclerite, the pivot changed its shape and this might explain why we were not able to relock this joint: proper backfolding of the pivot most likely requires an orchestrated movement of the tibial flexor muscle, and perhaps even the extensor muscles and the tibial extensor apodeme.

Friction enhancing modifications on the lock surfaces

Friction between the interacting sclerite surfaces must play an important role in keeping the femoro-tibial joint locked. Consequently, understanding the mechanical properties of the included sclerite surfaces should be the requisite of any studies that aim to understand the biomechanics of the systems that involves these locks. Surprisingly, earlier studies mostly failed to provide a detailed description of the fine structure of the interacting sclerite surfaces, including the perhaps most well studied Heitler's lump of the locust. A pad of soft tissue has been reported from the ventral surface of the genuflexor sclerite in bush crickets (Burrows and Morris 2003) that is suspected by the authors to enhance the impact of the Heitler's lump on the lever of the tibial flexor muscle. We found that the genuflexor sclerite in *Gryllus* have a thick ventral pad (GFS: Figs 10.) similar to bush crickets. We did not find a similar pad in the locust, however, the surface of the Heitler's lump is covered with a thick layer of columnar epithelial cells with unknown mechanical properties (Fig. 2G).

We did not find cricket specimens with fully locked femoro-tibial joints, neither were able to relock the joint, supporting the hypothesis that these insects, unlike locusts, do not possess the ventral lock in the femoro-tibial joint. A locust can only kick and jump if the femoro-tibial joint is fully flexed and the lock is activated, while crickets are able to kick and jump even with partially flexed femoro-tibial joints (Burrows and Morris 2003). Better understand the

368 biomechanical impact of the above described differences between locusts and crickets certainly
369 would help us to better understand evolutionary differences that led to cardinally different
370 jumping behaviors in these taxa.
371 In grasping taxa and in jumping curculionids, the ventral ⅓ of the tibial flexor sclerite is
372 transparent, lack resilin and has cardinally different electron microscopy properties than the
373 melanized core of the sclerite. The surface of the sclerite is heavily sculptured. Since unlike in
374 orthopterans, the proximal region of the femoro-tibial conjunctiva is not stuck in between the
375 tibial flexor sclerite and the Heitler's lump, better understanding of surface friction in these taxa
376 is especially important. Unlike Alticini and Orthoptera, in many grasping taxa, the joint cannot
377 be unflexed only by pulling the tibia away from the femur, but we have to actively move over the
378 tibial flexor sclerite through the Heitler's lump as an obstacle. This joint can be relocked multiple
379 times while we were forcing the sclerite over the lump indicating that larger surface area of both
380 the lump and the slcerite has an increased surface frictional property.
381
382 *Presence or absence of trigger muscles*
383 Burrows (1969) mentioned that releasing a lock not necessary requires the presence of
384 newly evolved trigger or release muscles to carry the additional load because slight
385 modifications of the already present antagonistic muscles can act as triggers in mantis shrimps.
386 Similarly, Heitler (1974) concluded that, although putative release accessory muscles can be
387 found in locust, these muscles are most likely not involved in the release of the lock. Rather, he
388 proposed, that contraction of the antagonistic extensor might be enough to release the lock. It has
389 been proposed (based on their innervation pattern), that these muscles do not lift the TFS out
390 from the locked position, but might have a stabilizing function in jumping systems (Nishino

2004). The differently oriented 10–15 muscle fibres that inserts on the genuflexor sclerite in diopsid flies (*fe-tifld*: Figs 3D, 7E, F) and clearly for a separate, fan shape morphological unit in fully flexed femoro-tibial joint, might represent trigger portions of the tibial flexor muscle. This band can not be separated and thus observed in not-fully-flexed legs, indicating the possibility that similar muscles might have been simply overlooked in other taxa examined, and we should perhaps put more emphasis to properly describe these patterns in future works.

How to confirm the presence of a lock

In earlier studies, the presence/absence of locking mechanisms in the femoro-tibial joint were inferred indirectly based on slow motion video recordings, and spatial relationships between anatomical structures on static images. Heitler (1974) suspected the presence of a lock in locust legs based on his experiment in which he pulled the flexor muscles until femoro-tibial joint was fully flexed and then measured the force required to reopen the joint.

H2O2 bleaching is perhaps the most crucial part of our dissection based approach as it let us to see how different sclerties interact while we move the joint. We assumed that a locking mechanism is present in the femoro-tibial joint only if we have seen two interacting sclerites preventing the joint to open in fully flexed legs after the flexor muscle origin was detached from the femur. The ability to relock the joint by pulling the flexor muscle is only an additional evidence for the presence of the lock, and we have to acknowledge, that by pulling the flexor muscles, we can not model properly the natural contraction of the muscles. The flexor muscle fibres are grouped in multiple, distinct bundles, whose neural control, strength and speed are remained to be described.

Importance of simple observations in the 21st century morphology

The advent of Micro-CT based, high resolution 3D reconstruction tremendously accelerated the collection of morphological data and made insect morphology accessible for a broader range of students (Betz 2007, Deans et al. 2012). However, micro-CT based methods, at least today, did not substitute perfectly traditional dissection based techniques and histology. Albeit there is some convincing development in in-vivo X-ray imaging techniques (dos Santos Rolo et al. 2014, Xu et al. 2016), dissections are still the most available and perhaps the most accurate methods to visualize the motion of anatomical systems both in live and dead specimens. Besides observing motion of elements, to define functional units of the skeleton also requires classical methods as the tissue specific contrast of X-ray based methods are usually not sufficient enough to separate more and less flexible cuticular elements (sclerites and conjunctivae) from each other.

Nadein and Betz (2016, 2018) have used highly sophisticated, noninvasive imaging techniques to analyse the femoro-tibial complex. However, using basic dissections techniques, we hereby revealed two key elements of this system that they were not able to capture properly: the presence of a ventral lock in the femoro-tibail joint and the lack of the connection between the internal surface of the ventral femoral wall and the genuflexor sclerite.

They proposed that in jumping curculionids the genuflexor sclerite/tibial flexor apodeme is connected to the ventral femoral wall, when the joint is fully flexed (bl, broad ligament: figs 8E, F, 10 *in* Nadein and Betz 2018). We did not find this connection in our specimens, and it would be logically impossible to have this connection if we properly consider the simple nature of the insect exoskeleton, that is the product of a single cell layer. The tibial flexor sclerite is on the tibial flexor tendon in *Orchestes*. The site of origin of the tendon is continuous with the

genuflexor sclerite distally and with the proximal portion of the femoro-tibial conjunctiva proximally and is not connected to the ventral wall of the femur with any other structures. Nadein and Betz (2018), most likely, considered the ventral, transparent layer on the tibial flexor sclerite on their CLSM micrograph as the connection between the sclerite and the internal surface of the ventral femoral wall. This would be interesting to further explore as we did not find resilin in this structure in the newly examined grasping taxa.

Conclusion and future directions

The ventral lock of the femoro-tibial joint reveals a remarkable parallel implementation of the physical mechanism to create a grasping and a jumping function. The building blocks of this system, genuflexor sclerite, ventral femoral wall and tibial flexor tendon are obviously present in most insects. Descriptive analysis based on static images can suggest the presence of a locking mechanism and our dissection-based experimental technique, by studying moving parts under the microscope, can reveal the workings of a locking apparatus and describe its functioning in detail. Due to its simplicity it offers a chance to the wide scientific community to test various species representing diverse clades of insects. Until micro-CT techniques can be applied to live animals in sufficient resolution the best solution is to utilize the wide variety of existing anatomical techniques (CLSM, SEM, TEM, etc.) and combine it with the traditional dissections-under-the-microscope technique that allows to manipulate e.g. joints in a manner that provides information on their live role while in motion. These may range from jumping leaf beetles to leafhoppers and grasping (predatory) water scorpions, heteropterans and robber flies.

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609 **Table 1.** Specimens examined.

Order	Taxon(number of specimens), Family	Function	Specimen data	Sclerite involved in lock	Study technique	Unlocking /locking
Diptera	*Teleopsis dalmanni* (15), Diopsidae	grasping, kicking	UCL lab culture (MALAYSIA: KL)	TFS	fore, middle and hind legs, dissection, video, CLSM, SR-µCT	+/+
Diptera	*Sphyracephala brevicornis* (Say, 1817) (6), Diopsidae	grasping, kicking (?)	USA: New Hampshire Durham 43.135, -70.933	TFS	fore and middle legs, dissection, video, CLSM	+/+
Diptera	*Ochthera* sp. *mantis*-group (6♀), Ephydridae	grasping	USA: Texas Bracketville 29312, -100637 III.20-22.2010 YPT	TFS	fore and middle legs, dissection, video, CLSM	+/+
Hymenoptera	*Podagrion* sp. 1. (6), Torymidae	grasping, kicking	GERMANY	TFS	Hind legs SR-µCT of hind legs	+/+
Hymenoptera	*Podagrion* sp. 2. (3), Torymidae	grasping, kicking	USA: Texas Bracketville 29312, -100637 III.20-22.2010 YPT	TFS	hind and middle legs, dissection, video	+/+
Orthoptera	*Gryllus campestris* Linnaeus, 1758 (3), Gryllidae	jumping	HUNGARY: Hortobágy	GFS	hind legs, dissection, SR-µCT	-/-
Orthoptera	*Omocestus (Omocestus) haemorrhoidalis* (Charpentier, 1825) (5), Acrididae	jumping, kicking	HUNGARY: Bács-Kiskun Bugacpusztaháza 46.696945°, 19.601822° Aug.10.2014 alkaline meadow sweeping Deans and Mikó	GFS	hind legs, dissection, video	+/-
Coleoptera	*Disonycha xanthomelas* (Dalman, 1823) (5),Chrysomelidae	jumping	USA: NH, Dover, Bellamy Rd. 43.172, -70.809 v.17-v.19.2019, YPT I. Miko	GFS	hind legs, dissection, video	+/-
Coleoptera	*Chaetocnema minuta* F. E. Melsheimer, 1847 (6), Chrysomelidae	jumping	USA: NH, Dover, Bellamy Rd. 43.172, -70.809 v.17-v.19.2019, YPT I. Miko	GFS	hind legs, dissection, CLSM	+/-
Coleoptera	*Longitarsus sp.* (3), Chrysomelidae	jumping	HUNGARY: Bács-Kiskun Bugacpusztaháza 46.696945°, 19.601822° Aug.10.2014 alkaline meadow sweeping Deans and Mikó	GFS	hind legs, dissection	+/-

Coleoptera	*Caryobruchus gleditsiae (*Linnaeus, 1763) (2), Bruchidae	grasping	USA: FL:Coll. Co. Wiggins Pass Rec. Area 10 mi N Naples.XII-3, 1-1992, R.M. Reeves, rotten wood on beach	TFS	hind legs (dry specimens), dissection, video	+/+
Coleoptera	*Orchestes mixtus* Blatchley & Leng , 1916 (2), Curculionidae	jumping	USA, VT. Lamoille Co. Wolcott, Lamoille Riv. 5-26-2009. T. Murray	TFS	hind legs (dry specimens), dissection, video	+/?
Hymenoptera	*Schletterius cinctipes* (4), Stephanidae	kicking, grasping (?)	USA: CA: S. BRDO. Co. Jenks LK. Rd., 2105m, 34,9'48"N: 116,51'43"W; ex. Abies log coll. 28.I.06. Emerge iv.06, F. Reuter Schletterius cinctipes	TFS	Hind legs, dissection, video	+/+

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Universal locking mechanisms in insect legs: jumping and grasping

Mihály Földvári[1], István Mikó[2,3], Jonah M. Ulmer[3], Tomy dos Santos Rolo[4,5], Sándor Csősz[6], Andrew Pomiankowski[7,8], Tilo Baumbach[4,9], Thomas van de Kamp[4,9]

[1]Natural History Museum, University of Oslo, PO Box 1172 Blindern, NO-0316 Oslo, Norway

[2]UNH Collection of Insects and other Arthropods, Department of Biological Sciences, University of New Hampshire, Durham, NH, USA

[3]Department of Entomology, The Pennsylvania State University, University Park, PA, USA

[4]Institute for Photon Science and Synchrotron Radiation (IPS), Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

[5]Department of Electrical and Electronic Engineering, Southern University of Science and Technology, Shenzhen 518055, China

[6]MTA-ELTE-MTM, Ecology Research Group, Pázmány Péter sétány 1C, H-1117 Budapest, Hungary

[7]Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK

[8]Centre for Computation, Mathematics and Physics in the Life Sciences and Experimental Biology (CoMPLEX), University College London, Gower Street, London WC1E 6BT, UK

[9]Laboratory for Applications of Synchrotron Radiation (LAS), Karlsruhe Institute of Technology (KIT), Kaiserstr. 12, 76131 Karlsruhe, Germany

Abstract

Thickened femora of insects are correlated to enlarged muscle masses and serve two basic purposes: jumping/kicking and grasping/holding. ~~Energy storing mechanisms amplifying the power of muscle contractions are well known and understood in jumping legs, but have never been described in grasping/holding legs. Our research explored the energy storing mechanisms of grasping legs in diopsid flies, chalcidoid wasps and shore flies. Using synchrotron micro-CT and confocal microscopy we revealed the presence of modifications in in the tibial flexor tendon and on the ventral femoral wall - the tibial flexor sclerite (TFS) and the Heitler's lump (HL) - that resembles structures involved in energy storing mechanisms of jumping legs of locusts. This is, to our knowledge, the first mention of TFS in the order of Diptera. We were able to prove the presence of a locking mechanism in all of the studied grasping legs by simple manipulation of dead specimens in glycerol. The locking mechanism in jumping and grasping legs is an example of seemingly very different kinetic implementations using the same morphological trait.~~ Modifications on the ventral femoral wall and the tibial flexor tendon that are possibly involved in catch mechanisms have been described in multiple insect taxa with both jumping and grasping legs. Our comparative study aims to explore the functional and structural similarities of these modification in jumping and grasping leg types from Coleoptera, Hymenoptera, Diptera and Orthoptera with the combination of cutting edge, non-invasive imaging methods and classical dissections techniques. Our data indicate that locking mechanisms are present in the jumping and grasping legs of insects. We describe three femoro-tibial lock types based on the location of the interacting sclerites relative to the site of origin of the tibial flexor tendon. All of the three types can be found in jumping insect legs, while only one type is present in grasping legs. The locking mechanism might aid to keep the femoro-tibial joint in a flexed position for an extended period

of time. Our data indicate that morphologically similar modifications in the femoro-tibial joint are involved in energy-saving mechanism both in jumping and grasping legs in insects.

Keywords: courtship, sexual selection, ~~exaptation,~~ convergence, CLSM, SR-µCT, 3D reconstruction, TEM, SEM.

Introduction

~~Arthropod legs are simple anatomical structures.~~ The insect femoro-tibial joint is a relatively simple mechanical system as it is composed of two cylindrical leg segments (tibia and femur) that are movably connected to each other with ~~flexible cuticle, usually with~~a ring-like conjunctiva, two antagonistic muscles - tibial flexor and tibial extensor - and with a dicondylic joint composed of a pair of lateral articulations (~~the tarsus is connected to the tibia with a single muscle and a monocondylic joint).~~Fig. 1). Straightening (extension) and bending (flexion) of the leg is achieved by the alternate movement of the ~~extensor and flexor~~ two tibial muscles~~.~~

which arise from the femur with wide, fan shaped site of attachments and inserts at the base of the tibia with elongate tendons (Figs 1A–C). The ratio of the size of the tibial flexors and extensors ~~are~~is highly variable and ~~correspond~~corresponds to the adequate function of the leg~~.~~ that serves a broad variety of motion or grasping related behaviors (Furth and Suzuki 1990a). The muscles are equivalent in normal walking legs~~,~~ while the flexor muscle is enlarged in grasping and the extensor in jumping ~~legs (Fig.1). Besides the increased muscle mass, energy storing mechanisms play key roles in some specialized movement types, such as the ability for~~

~~jumping several times farther than the body length. In locusts (Orthoptera: Acrididae), this extreme jumping performance is achieved through a lock that prevents the femoral extensor muscles from opening the femoro-tibial joint to the sudden release of the lock (Heitler 1974, 1977). This lock is composed of a sclerite on the tendon of the tibial flexor muscle (tibial flexor sclerite: TFS) and an internal ridge on the ventral femoral wall (Heitler's lump: HL, Figure 2, Supplementary Figure 1.). In the locked position, energy produced by the contracting extensor muscles is stored in the resilin-rich distal regions of the femur, the semi-lunar processes (slp: Figure 2; Burrows 2016). When the lock is released, this energy and the contraction of the extensor muscles, together, result in the unparalleled jumping ability.~~/kicking legs (Figs 1A–C, Furth and Suzuki 1990a).

~~Although both the TFS and HL have been reported in numerous other jumping and grasping insects (Jumping: Chrysomelidae, Coleoptera, Furth and Suzuki 1990a, 1990b, 1992, Nadein and Betz 2016; Proscopiidae, Orthoptera, Burrows and Wolf 2002; Gryllidae, Orthoptera, Hustert and Baldus 2010. Grasping: multiple insect orders, Furth and Suzuki 1990b; aquatic Heteroptera, Gorb 1995), the presence of a lock between the flexor muscle and the femoral wall has been proved only in locusts, which might be ascribed to the difficulties in unambiguously demonstrating the presence of locks in dead insects.~~

~~Locking mechanisms could play an important role in grasping legs, especially if an extended time of grasping (holding) is required. In diopsid flies (*Teleopsis dalmanni* and *Sphyracephala brevicornis*, Diptera: Diopsidae), *Podagrion* wasps (Hymenoptera: Chalcidoidea) and *Ochthera* shore flies (Diptera: Ephydridae) holding is crucial. *Diopsid* fly males use their first leg to attack and potentially hold their competitor's legs during fights, the foretic chalcidoid *Podagrion* females use their hind legs to hold on the body of female *Mantis* specimens (Cowan~~

~~1979, Mantodea: Mantidae) and *Ochthera* shore flies use their first pair of raptorial legs to hold their preys (Deonier 1972). These behaviors all require an extreme amount of muscle power and the majority of invested energy can be spared by making use of a lock mechanism that mechanically holds the leg in the required position even when the muscles are not contracted.~~

~~In this study, we explored the structure and function of locking mechanisms (TFS and HL) and their presence in the above mentioned taxa. Structural analysis was done through the use of 3D reconstruction, however observation of function, or lack thereof, in the locking mechanism was achieved through classical dissection techniques in glycerol.~~

Changes in the muscle mass ratio is often accompanied with tendonal modifications, which have been reported from the enlarged fore, middle and hind legs of numerous insect taxa. The tibial flexor sclerite, atrophied basal sclerotization at the tibial flexor tendon, is perhaps the most common tendonal modification in jumping and grasping legs. In locusts, the tibial flexor sclerite is involved in a lock (catch) and plays an important role in the energy releasing catapult mechanism (Heitler 1974, Gronenberg 1996). While the tibial flexor muscle contracts, and bents (flexes) the tibia, the tibial flexor sclerite is pulled over an internal projection (invagination) of the ventral femoral wall (Heitler's lump) into a locked position (Figs 1D, F, 2A–G). When the tibial extensor muscle starts to contract, the lock prevents the flexor tendon to move and straighten (extend) the femoro-tibial joint and it requires an extra power from the extensor muscle to eventually overcome the lock. During the time of release, energy from the contracting extensor muscle is stored in an apical, resilin rich structure of the femur, the semilunar process (SLP: Figs 2A–C, 10A, B). The stored energy from the semilunar processes allow the locusts to jump multiple times of their body length.

Albeit it was suspected, it has never been shown that the tibial flexor sclerite of other jumping insects would participate in similar locks and catapult mechanisms (Barth 1954, Furth and Suzuki 1990a, Betz 2007). Instead, it is now speculated that the tibial flexor sclerite in these taxa might be involved in strengthening the tendon, altering the working angle of the flexor system or simply protect the ventral portion of the femoro-tibial joint (Nadein and Betz 2016, 2018). These hypotheses are also supported by the fact that the sclerite is not only found in jumping but also in grasping insect legs, which would not utilize a catapult mechanism (Furth and Suzuki 1990a, b).

Although the tibial flexor sclerite across jumping and grasping insects seems structurally equivalent, its relative position to the site of origin of the tibial flexor tendon tendon is variable (Furth and Suzuki 1990a). The tibial flexor tendon is an invagination of the single layer epithelium at the femoro-tibial conjunctiva and is connected to the ventral femoral wall with a resilin rich ligament, the genuflexor sclerite (Snodgrass 1956). In Alticinae leaf beetles and in Orthoptera, the tibial flexor sclerite is the atrophied genuflexor sclerite (Snodgrass 1956, Furth and Suzuki 1990a, Nadein and Betz 2016) while in jumping curculionids, the tibial flexor sclerite is the atrophied basal region of the tibial flexor tendon (Nadein and Betz 2018).

While examining ethanol preserved beetle, fly and hymenopteran specimens with enlarged fore or hind femora, we discovered, that if the specimen died with fully flexed legs, we were not able to open (unflex) the femoro-tibial joint easily, while in specimens that died with not fully flexed legs, the joints could usually be easily moved. We also observed in specimens with transparent femoral cuticle that the tibial flexor sclerite and the ventral tibial wall is locked together in some specimens preventing the straightening of the femoro-tibial joint. I this study, we examined the femoro-tibial joint of 13 grasping and jumping insect taxa combining simple

dissection and cutting edge 3D visualization techniques, to reveal if a lock is present in the modified femoro-tibial joints and to better understand structural equivalencies of the anatomical structures that might be involved in these locks. This study demonstrates that simple observations using classical dissection techniques still play an important and unavoidable role in insect morphology even in the age of non-invasive 3D reconstruction techniques.

Materials and Methods

We have examined grasping legs with enlarged femora in taxa where modifications on the tibial flexor tendon have never been reported (diopsid flies, shore flies and torymid wasps) and reexamined jumping (Alticini, Chrysomelidae) and a grasping (Bruchidae) beetles in which the tendon sclerotizations have been reported (Furth and Suzuki 1990a, b) but their involvement in the ventral femoral lock has been dubious (Alticini) or were never proposed (Bruchidae). We have recorded our dissections with bright field microscopy and visualized dissection results with Confocal Laser Scanning Microscopy. For 3D reconstruction, we applied synchrotron based Micro-CT and to explore the fine structure of the tibial flexor sclerite, we applied scanning and transmission electron microscopy.

Specimens for the present study (~~Supplementary~~ Table 1) were stored in 75% ethanol and were transferred to anhydrous glycerol on a concave coverslip for dissection and CLSM~~.~~ ~~Specimens for TEM were processed as outlined below. Specimens~~ and are deposited in the UNH Collection of Insects and Arachnids (UNHC).

Terminology for cuticular elements follows Klass and Matushkina (2012) and Ronquist and Nordlander (1989). We used the term sclerite for less flexible areas of the exoskeleton that are connected to each other by more flexible conjunctivae (=arthrodial membrane, =membrane).

We identified these elements by manipulating the exoskeleton using insect pins and forceps. Terminology of anatomical structures in the femoro-tibial joint follows Furth and Suzuki (1990a), Snodgrass (1956) and Betz (2009). We have classified sclerites on the ventral region of the femoro-tibial joint based on their relative position to the site of origin of the tendon of the tibial flexor apodeme, which corresponds to an invagination on the distal femoral margin. We used the term tibial flexor sclerite (TFS, Furth and Suzuki 1990a) for slcerotized elements on the tibial flexor tendon and the term genuflexor sclerite (GFS, Snodgrass 1956, =Lever's triangular plate, =tibial flexor sclerite sensu Furth and Suzuki 1990a, b, Betz 2009, Nadein and Betz 2016) for slcerotized elements between the site of origin of the tibial flexor tendon and the proximal tibial margin. We used the term Heitler's lump for the flattened invagination on the ventral femoral wall proximal to the anterior margin and femoral abutment for the resilin rich distal projection at the distal margin of the ventral femoral wall (=of 'Lever's triangular plate, Nadein and Betz 2018). We have introduced the new term genuflexor apodeme for the invagination on the distal tibial end of the genuflexor sclerite that is sclerotized and is adjacent to the external wall of the tibia (GFS: Figs 4A, D, Fig. 6A) and the ventral lock of the femoro-tibial joint that refers to a lock between the ventral femoral wall and a sclerite that originates from the femoro-tibial conjunctiva (genuflexor sclerite or tibial flexor sclerite). The terminology for muscles follows Snodgrass 1956. We use the term lock to refer to two sclerite surfaces that are involved in a locking mechanism.

We ***dissected*** ethanol preserved and dried (card mounted) specimens. One part of the ethanol preserved specimens were transferred to anhydrous glycerol and longitudinally bisected with Personna razor blades (Edgewell Operations, Allendale). Another part of ethanol stored specimens and all dried specimens were ~~dissected~~ bleached and rehydrated in 35% H2O2 (Sigma

Aldrich, Burlington Massachusetts) for 24 hours and then transferred to anhydrous glycerol (Mikó et al. 2016). Specimens were dissected in glycerol with Dumont 5# forceps ~~and insect pins (#2)~~(Fine Science Tools, Foster City, California), insect pins (#2), Vannas Spring Scissors with 2mm cutting edge (Fine Science Tools, Foster City, California) and Personna razor blades on concavity slides in anhydrous glycerol using an Olympus SZX16 stereomicroscope equipped with a 2X objective providing a 230× magnification~~.~~ (Olympus Corporation of the Americas, Center Valley, PA) and a Huvitz HSZ-ZB700 stereo-microscope (Huvitz BD, Gyeonggi-do, Republic of Korea).

~~Glycerine-dissected~~We ***observed the movement/interaction*** between the proximal tibial flexor tendon and the ventral femoral wall while moving (straightening and bending) the femoro-tibial joint through the bleached cuticle of H2O2 treated specimens ~~were examined~~or viewing the internal side of bisected specimens. Then we detached (severed) muscle sites of origin and repeated the observations while moving the joint. If we found a lock mechanism between the tibial flexor tendon and the ventral femoral wall, we tried to unlock/relock the catch by straightening the joint or by using an ~~Olympus SZX16 stereomicroscope with an Olympus SDF PLAPO 1XF objective (115×) and an Olympus SDF PLAPO 2XPFC objective (230× magnification).~~ insect pin as a lever to dislodge the locking sclerites.

Videos were taken on ~~this~~ an Olympus SZX16 stereo-microscope and a Huvitz HAZ-ZB700 stereo-microscope with a Canon EOS 70D and a Canon Rebel DSLR camera (Canon USA Inc. Melville, New York), respectively. Stacks of bright field images were taken manually on an Olympus CX41 microscope (Olympus Corporation of the Americas, Center Valley, PA) with a Canon EOS 70D ~~and a Canon Rebel~~ DSLR camera attached~~. Images~~ and the images were ~~subsequently aligned and stacked~~combined using ~~Zerene Stacker~~the Align and Stack All (DMap)

algorithm of ZereneStacker (Version 1.04 Build T201404082055; Zerene Systems LLC, Richland, WA~~, USA). Plates~~).

Sample preparation for ***confocal laser scanning microscopy*** (CLSM) followed Mikó and Deans (2013). Specimens were imaged between two #1.5 coverslips with an Olympus FV10i confocal laser-scanning microscope (CLSM, Olympus Corporation of the Americas, Center Valley, PA) at the Microscopy and Cytometry Facility at the Huck Institute of Life Sciences at the Pennsylvania State University and with a Nikon A1R-HD CLSM at the University of New Hampshire Instrumentation Center. With the Olympus FV10i we used three excitation wavelengths, 405 nm, 473 nm, and 559 nm, and detected the autofluorescence using two channels with emission ranges of 490–590 nm, and 570–670 nm (Fig. 2). On the Nikon A1R-HD, we either used a preset (confocal) with 3 excitation wavelengths, 408.9 nm, 487.4 nm and 559.9 nm and 3 emission ranges of 435–470 nm, 500–540 nm and 570–645 nm (Fig. 1) or used one excitation wavelength 487 nm laser with emission ranges defined using the A1-DUS spectral detector, 500–560 nm and 570–630 nm (Figs 3E, F, 4–6). The resulting image sets were assigned pseudo-colors that reflected the fluorescence spectra. Volume-rendered micrographs and media files were created ~~in Adobe Photoshop 6™ (Adobe Systems, San Jose, CA, USA).~~ using FIJI (Schindelin et al. 2012) and Nikon NIS-Elements AR v. 5.02.01.

Synchrotron X-ray tomography (SR-μCT) was performed at the UFO imaging station of the Karlsruhe Institute of Technology (KIT) light source. The specimens were either critical point dried (*Gryllus campestris* & *T. dalmanni*) or scanned in 70% ethanol (*Podagrion* sp.). For each scan, 2,500 (*G. campestris* & *Podagrion* sp.) or 3,000 (*T. dalmanni*) equiangularly spaced radiographic projections were acquired in a range of 180°. A parallel polychromatic X-ray beam was spectrally filtered by 0.2 mm Al to obtain a peak at about 15 keV. The detector consisted of

a thin, plan-parallel lutetium aluminum garnet single crystal scintillator doped with cerium (LuAG:Ce), optically coupled via a Nikon Nikkor 85/1.4 photo-lens to a pco.dimax camera with a pixel matrix of 2008x2008 pixels (dos Santos Rolo et al., 2014). The magnification was set to 10X (*Gryllus* sp. & *Podagrion* sp.) and 20X (*T. dalmanni*), resulting in effective pixels sizes of 1.22 and 0.61 μm. Tomographic reconstruction was performed with the GPU-accelerated filtered back projection algorithm implemented in the software framework UFO (Vogelgesang et al., 2012). 3D reconstruction of tomographic data was performed using Amira (version 5.4.3, FEI) for volume segmentation and rendering.

For ***TEM***, legs were removed from adult flies and fixed in 2% paraformaldehyde (PFA), 1.5% glutaraldehyde in 0.1M phosphate buffered solution (PBS) for 1.5 hours at room temperature . After three 10 minute washes in 0.1M PBS, the fixed tissue was transferred to 1% osmium oxide (OsO_4) for 45 minutes, followed by a 10 minute buffer (PBS) wash and two 10 minute washes in double distilled H_2O (ddH_2O) and then to 2% uranyl acetate ($UO_2(CH_3COO)_2 \cdot 2H_2O$) for 15 minutes, followed by three 10 minutes washes in ddH_2O. The legs were then dehydrated through an ethanol (EtOH) series (5 minutes at 25% EtOH, 5 minutes at 75% EtOH, 5 minutes at 90% EtOH, 5 minutes at 100% EtOH). This was followed by four 10 minute washes in 100% EtOH and three in propylene oxide (C_3H_6O). The legs were then embedded in the epoxy resin, Agar 100 ® (Agar Scientific, UK) in a stepwise manner, being transferred to 2 parts propylene oxide: 1 part Agar 100® resin for 1.5 hours and then 1 part propylene oxide: 2 parts Agar 100® resin for 1.5 hours. The samples were left in 100% Agar 100® for 8-16 hours at room temperature before the Agar 100® was replaced and the samples placed in resin in moulding blocks at 60ºC, to harden for 48 hours.

~~Sample preparation for confocal laser scanning microscopy (CLSM) followed Mikó & Deans (2013): male genitalia were temporarily mounted between two coverslips (1.5 µm, 22 × 60) in a glycerin droplet, which did not reach the edge of the coverslip. We used Blu-tack (Bostik, Wauwatosa, WI, USA) as spacer as this material does not interact with glycerol and provides an adjustable, appropriate distance between the coverslips. Specimens were examined with an Olympus FV10i desktop CLSM using a 60X objective. Soft and sclerotized anatomical structures in arthropods tend to fluoresce with different intensities at different wavelength intervals (Mikó & Deans, 2013). CLSM tissue-specific contrast is gained by exciting specimens using multiple excitation wavelengths and/or recording the fluorescence on multiple channels assigned to different laser wavelength intervals. In previous research (Mikó et al., 2013; Popovici et al., 2014; Ernst, Mikó & Deans, 2013), specimens were excited with only one blue laser (480 nm) and the auto-fluorescence was detected with two channels (500–580 and 580–800 nm). Although the resulting micrographs had differences in their intensity patterns, data from the two channels largely overlapped. In the present paper, we use two different lasers (631 and 499 nm) and set filters (644 and 520 nm, respectively; narrow green and narrow red presets in Olympus Fluoview viewer software version 4.2) with narrow wavelength windows that result in a much higher tissue-specific contrast, almost perfectly separating muscle tissue and skeletal components (Fig. 1).~~

Results

~~*Integument*~~

~~The tibia and~~ Fully flexed femoro-tibial joints with enlarged femora in the ~~femur are articulated by two lateral hinges (pivot points) that define a horizontal rotational axis (con: Figs 5B, C, H). The genuflexor sclerite (GFS) is ventromedian~~studied taxa were locked and difficult

or impossible to open even if the site of origin of the tibial flexor muscle has been destroyed. Based on the involved sclerotic elements and their interaction with the ventral femoral wall and the femoro-tibial conjunctiva ~~and is continuous with~~ , we identified three major lock types at the ventral portion of the femoro-tibial ~~wall distally~~joint.

Type I (Figs 3A, B, 4–8, Videos 1–8). In taxa with grasping legs and in the jumping curculionid, the lock is between the tibial flexor sclerite and the Heitler's lump. In the locked position, the anteroventral portion of the convex ventral surface of the sclerite is in physical contact with the Heitler's lump. The ventral surface of the tibial flexor sclerite is not connected to the ventral femoral wall ~~proximally (GFS: Figs 4E–H). The GFS is invaginated distally~~.

We were able to release the lock after extending the multiple times in *Orchestes* and ~~is adjacent to the dorsal wall of the tibia (GFS: Figs 3A, D, 5A).~~in *Schletterius*. In the diopsids, *Ochthera*, *Caryobruchus* and *Podagrion*, we were not able to open the joint without forcing the tibial flexor sclerite over the Heitler's lump with an insect pin. While moving the sclerite over the lump, we observed that it stuck multiple times at different positions of the lump (as if they were two sides of a velcro tape). By pulling the tibial flexor muscle, we were able to move the sclerite over the Heitler's lump, and thereby secure it in a re-engaged locked position in all taxa. We were able to unlock and lock the joint multiple times. The proximal portion of the femoro-tibial conjunctiva between the site of origin of the tibial flexor tendon and the distoventral margin of the femur is not located in-between the tibial flexor sclerite and the Heitler's lump when the joint is in a locked position. In the hymenopteran and dipteran specimens, the genuflexor apodeme is well developed, and the external tibial wall is angled at the point of its attachment with the ~~GFS. An elongate external pit corresponds to the site of origin of the tibial flexor tendon (pit: Figs 4F, 6C). The distal region of the tibial flexor tendon is thickened just~~

~~after its site of origin and forms an elliptic tibial flexor sclerite (TFS: Figs 3A, D, E,~~ apodeme (Figs 4A, ~~C, F–H, 5D–H, 6B–F).~~ D, Figs 6A).

The ~~TFS~~tibial flexor sclerite has a melanized ~~(dark brown) and~~ center that is covered ventrally by a transparent (glass-like) ventral layer that is in physical contact with the dorsal surface of the Heitler's lump in all grasping taxa and in *Orchestes*. In *T. dalmanni*, The melanized center of the tibial flexor sclerite is electron dense (darker on TEM images) ~~core that is surrounded by a~~while the transparent ~~and~~ ventral layer is electron lucent ~~external coating~~ (core, covp, covd: Figs 7A–D~~). The~~), the ventral surface of the TFS is heavily sculptured (Figs 6B–F~~). The ventral femoral wall, just proximal to the site of origin of the tibial flexor tendon bears an invagination that is~~), the Heitler's lump is T-shaped in cross section~~, the Heitler's lump~~ (HL: Figs 3A, D, E, 4A, C, 5G, 6A, 7E, F~~). The ventral surface of the TFS is adjacent to the ventral tibial wall distal to the HL in open (extended) joint position, and it is proximal to the HL in closed position (3A, D, 4A–D). The ventral, transparent segment of the sclerite is seemingly deformed by the HL when compared to the TFS of open legs (TFS: Figs 4B–D, Supplementary Video Files). The GFS~~) and lack enlarged epithelial cells on its internal (dorsal) surface. The genuflexor sclerite is resilin-rich while the ~~TFS~~tibial flexor sclerite and the ~~HL~~Heitler's lump are not containing resilin based on the presence/absence of blue autofluorescence in response to UV light (407 nm, Figs 4E–H) in the diospids, *Podagrion* and *Ochthera*. In diopsids, 10–15 fibres of the tibial flexor muscle (inserting on the internal surface of the genuflexor sclerite) are oriented vertically and arise to reach the femoral wall distally to the tibial flexor sclerite when the femoro-tibial joint is fully bent (flexed) while these fibres are oriented proximodistally similarly to more proximal fibres in not fully bent legs (fe-tifld: Figs 4A, D, Video 8).

Muscles

The tibial flexor muscle (fe-tifl: 3F, G, 4B, G) is attached to the tibial flexor tendon along the dorsal surface of the TFS and along all surfaces of the tendon proximal to the TFS (muscle fibres are not arising from the ventral surface of the TFS, Figs F, H). The fibres of the muscle are oriented proximodorsally and arise to reach the femoral wall proximal to the TFS in all taxa except in the two examined diopsids, where 10–15 fibres of the tibial flexor muscle (inserting on the GFS) are oriented vertically and arise to reach the femoral wall distally to the TFS (fe-tifld: Figs 3A, D, E, 6E, 7E, F). The fibres of the tibial extensor muscle arise from the proximal section of the femur and insert at the proximal margin of the tibial wall dorsal to the rotational axis (*fe-tiex*: Figs 2E, F). The tibial flexor muscle is distinctly larger and composed of 2–3 times as many fibres than the tibial extensor muscle.

Locking mechanisms

Supplementary Videos 1–5

We were not able to open (extend) the closed (flexed) tibia of dead insects without breaking the proximal femoral wall or rupturing the tibial flexor muscle and/or its apodeme in the examined grasping legs. We observed, that by forcing the closed legs to an open position, the TFS is pressed against the HL and it responded to each opening movement with changing the shape of its ventral, transparent coating. On the other hand, we were able to move the tibia of insects with an open (extended) leg at death without damaging anatomical structures.

In closed legs, we were able to push the TFS over the HL with an insect pin. After this manipulation, we were able to open and fully extend the tibia similarly to legs that were open at death.

~~By pulling the proximal portion of the tibial flexor muscle, we were able to move the TFS over the HL and close the joint into a fully flexed position in legs that were open at death; as well as we could open legs that were closed at death by moving the TFS distal to the Heitler's lump. After locking the mechanism by moving the TFS proximally to the HL we, again, were not able to open (extend) the legs without breaking the proximal tibial wall or rupturing the tibial flexor muscle and/or its apodeme. We were able to repeat opening and closing multiple times in many specimens (for the entire experiment see Supplementary Video 5). The locking mechanism is present between the TFS and the HL in all dissected species with grasping legs (Supplementary Video Files), but absent from the examined cricket leg.~~

Type 2 (Figs 3C, D, 9). In Alticini, the lock is between the genuflexor sclerite and the ventral femoral wall distal to the site of origin of the posterior portion of the femoro-tibial conjunctiva. Only the distal end of the genuflexor sclerite is in physical contact with the femoral abutment. The femoral abutment contains a distal sclerite and bends ventrally apically when the genuflexor sclerite is unlocked (s: Figs 9A–F). We were able to unlock the joint by extending the tibia multiple times. By pulling the tibial flexor muscle, we were not able to relock the joint. The femoro-tibial conjunctiva is not in between the interlocking sclerite surfaces and the ventral surface of the genuflexor sclerite is not connected to the ventral femoral wall.

Type 3. In the locust (Figs 2, 3E, F, 10), similarly to flea beetles, the lock is between the external surface of the genuflexor sclerite and a process on the internal surface of the ventral femoral wall (Heitler's lump). The external surface of the genuflexor sclerite is concave and limited proximally by a ridge. We were able to release the lock after extending the tibia multiple times. We were able to relock the joint by pulling the tibial flexor sclerite. The proximal portion of the femoro-tibial conjunctiva (proximad to the site of origin of the tibial flexor tendon) is in-

between the genuflexor sclerite and the Heitler's lump when the joint is in a locked position (Figs 2D, E, Figs 3 E, F). The internal surface of the Heitler's lump is covered with enlarged epithelial cells (HL: Fig. 2D). The elements of the locking mechanism (Figs 10A–F) are present in *Gryllus*, we did not find specimens with fully flexed and locked femoro-tibial joint and similarly to the locust, we were not able to lock the joint by pulling the tibial flexor muscle.

Discussion

~~We often make assumptions about function based on structure. In locusts, the Heitler's lump (HL) and the tibial flexor sclerite (TFS) are key components of a locking mechanism that allows these organisms to jump multiple times of their body size (Heitler 1974, 1977). One might suspect that well developed HL and TFS in other insects indicate the presence of locking mechanisms. However, besides locusts, locking mechanisms have never been described in taxa with well developed TFS and HL (Furth and Suzuki 1990a, Burrows and Wolf 2002, Hustert and Baldus 2010, Betz et al. 2007). In crickets (Orthoptera: Gryllidae), it has been proved that ballistic movements in the leg - kicking, swimming, jumping - are achieved without a lock but in other taxa with TFS and HL, the presence/absence of a lock is still questionable (Nadein and Betz 2016, 2018).~~

~~Furth and Suzuki (1990a) have shown that the TFS is present in multiple grasping taxa (e.g. Reduviidae) but the HL has never been described from any grasping insects. We have found that both the HL and the TFS are present in two diopsid fly species, a chalcidoid wasp and a shore fly, taxa that possess grasping legs to serve a wide variety of life history strategies, predation (shore fly), intraspecific competition (diopsid flies) and phoresis (chalcidoid wasps).~~

~~*Life history strategies associated with femoro-tibial system*~~

Besides locusts, the presence of ventral locks in the femoro-tibial joints have never been undoubtedly evinced in insects (Furth and Suzuki 1990a, Burrows and Wolf 2002, Hustert and Baldus 2010, Betz et al. 2007). Using simple manipulations in glycerol stored specimens we were able to show that locking mechanisms are present in the atrophied legs of the examined jumping and grasping insects except in *Gryllus*. These locks are either (i) between the external surfaces of the ventral femoral wall and the genuflexor sclerite (Figs 3E, F), or (ii) between the external surface of the genuflexor sclerite and the internal surface of the ventral femoral wall (Figs 3C, D) or (iii) between the internal surface of the ventral femoral wall and the internal surface of the tibial flexor sclerite (Figs 3A, B). These types also differ in the position and the size of the locking surfaces, the presence or absence of the tibio-femoral membrane in between the locking surfaces, and numerous other modifications. The first two types occur in jumping (Orthoptera, Chrysomelidae) and the third type in both jumping (Curculionidae) and grasping insects (Diptera, Hymenoptera, Bruchidae). These observations clearly demonstrate that, albeit the presence of a ventral lock in the tibio-femoral joint of enlarged legs is universal in insects, different lineages achieve this mechanical function using different solutions.

Furth and Suzuki (1990b) has observed that the tendon of the tibial flexor muscle is enlarged in some bruchid and oedemerid taxa with grasping (holding) hind legs. They did not discover the ventral femoral lock and concluded that the atrophied tendon might be related to the increased stress caused by the extended contraction of the enlarged tibial flexor muscle (Furth and Suzuki 1990b). According to our study, the enlarged portion of the tibial flexor muscle (the tibial flexor sclerite) of bruchids is involved in the ventral femoro-tibial locking mechanism and helps to keep the femoro-tibial joint in a flexed position for an extended period of time. We

found similar locks in grasping Hymenoptera and Diptera taxa where holding for an extended period of time might play a crucial role in their biology.

In their paper, de la Motte and Burkhardt (1983) describe diopsid ~~flies~~males, where the larger opponent (*Diopsis subnotata*) catches the smaller one (*Megalabops rubicunda*) by the eye stalk through the use of "tibia-femur pincers" as a grasping mechanism that is capable of locking an object. They also observed numerous *Cyrtodiopsis* (in literature sometimes referred to as *Teleopsis*) individuals with absent eye-stalks and leg segments and they suspect aggressive encounters as reasons i.e. they are capable of breaking off each other's eye stalks. Based on our observations, this behavior occurs rarely and the few individuals seen with broken eye stalks are dying soon; stalk-eyed flies rather reach out to try to grasp the supporting legs of conspecific males as reported by Wickler and Seibt (1972) during fight. It is also reported that they grab ~~each other~~ and flip each other off ~~surfaces~~surface – in particular off root hairs where they accumulate in the evenings, and they also jab each other with their extended legs (Panhuis and Wilkinson 1999). Diopsid females are often competing for nesting sites or food resources and although not as expressed as in males, they also exhibit aggressive behavior with the involvement of striking with fore legs (Burkhardt and de la Motte 1983, Al-khairulla et al. 2003, Bath et al. 2015).

Females of multiple distantly related chalcidoid taxa use their hind legs to secure their body position (Cowan 1979, Grissell and Goodpasture 1981) while depositing eggs in the host. Perhaps the most intriguing of them is the example of *Lasiochalciia igiliensis* (Chalcidoidea: Chalcididae) as in this species the female holds the mandible of antlion larvae apart while depositing her eggs through the less sclerotized regions between the head and pronotum (Steffan 1961). Other species use their legs for securing their body on their host during dispersal. Phoresis has been reported in torymid *Podagrion* species, where the females are grasping the wing of their

mantid hosts (Bordage 1913, Xambeu 1881). Although grasping has never been described in *Podagrion* males, they often kick each other as part of their aggression behavior similarly to chalcidid females (Cowan 1979, Grisell and Goodpasture 1981).

Ochthera species are well characterized by their enlarged fore femur and sickle shaped tibia representing typical raptorial legs (Clausen 1977). They are predators of smaller aquatic insect larvae and have been reported as important natural enemies of black flies and mosquitoes (Travis 1947, ~~Travis et al. 2003,~~ Minakawa et al. 2007). *Ochthera* flies use their "prehensive" fore legs to secure their prey items while they are probing and consuming them (Deonier 1972), but the enlarged fore femur is also used as a waving device during their courtship and aggressive interactions (Eberhard 1992).

~~Although it is sometimes more exaggerated in male specimens, both sexes of the examined species possess enlarged grasping femora and locking mechanism. In *Ochthera* flies, grasping is related to their predaceous lifestyle that is sex independent. In diospid flies,~~Grasping behavior have never been reported from Stephanidae (Hausl-Hofstätter and Bojar 2016), but the presence of robust teeth on the ventral surface of their hind femora indicate that they might be used for grasping. Both males and females of stephanids have been reported to kick with their middle and hind legs during intraspecific fights (Hausl-Hofstätter and Bojar 2016).

Genuflexor sclerite, tibial flexor sclerite, Heitler's lump and femoral abutment

The key components of the ventral femoro-tibial locks are atrophied sclerites at the tibial flexor tendon (tibial flexor sclerite) or the femoro-tibial conjunctiva distal to the tendon (genuflexor sclerite). In flea beetles and in orthopterans, the atrophied sclerite that participates in the lock is the genuflexor sclerite, that is located distal to the site of insertion of the tibial flexor

tendon. The genuflexor sclerite can be found in almost all insects, it is more or less sclerotised and in numerous cases it is not involved in any locking mechanism (e.g. *Apis mellifera*; Snodgrass 1956). The genufexor sclerite is continuous to the tibial flexor tendon and connects the tibial flexor sclerite to the tibial base, and has an important mechanical function (the tendon that arises from the femoro-tibial conjunctival would perhaps destroy the conjunctiva without the presence of the genuflexor sclerite). Furth and Suzuki (1990a) proposed that the tibial flexor sclerite (in their paper they used this term for both the genuflexor sclerite and the tibial flexor sclerite) might protect the ventral side of the femoro-tibial joint. The protective function might be possible, but this function is not restricted to taxa with atrophied genuflexor sclerite.

The Heitler's lump on the ventral femoral wall is a cuticular invagination in grasping insects, jumping curculionids and orthopterans that is more or less flattened (pressed against the femoral wall), while the femoral abut is a resilin rich and flexible apical region of the ventral femoral wall that has an apical sclerotic component in Alticini beetles. The Heitler's lump has largely been ignored in grasping insects, as the focus has been on the gross morphological description of tibial flexor sclerites in earlier works (Furth and Suzuki 1990a). and has only been mentioned on a single illustration for grasping heteropterans (*Ranatra* sp., Gorb 1995, fig. 11, d).

We found that the femoral abutment in flea beetles are more complex than has been described, as it is movable and has a sclerotic component. In the dissection experiment of the bisected femoro-tibial joint (Videos 10, 11), it is clearly visible, that when we unlocked the genuflexor sclerite, the pivot changed its shape and this might explain why we were not able to relock this joint: proper backfolding of the pivot most likely requires an orchestrated movement of the tibial flexor muscle, and perhaps even the extensor muscles and the tibial extensor apodeme.

Friction enhancing modifications on the lock surfaces

Friction between the interacting sclerite surfaces must play an important role in keeping the femoro-tibial joint locked. Consequently, understanding the mechanical properties of the included sclerite surfaces should be the requisite of any studies that aim to understand the biomechanics of the systems that involves these locks. Surprisingly, earlier studies mostly failed to provide a detailed description of the fine structure of the interacting sclerite surfaces, including the perhaps most well studied Heitler's lump of the locust. A pad of soft tissue has been reported from the ventral surface of the genuflexor sclerite in bush crickets (Burrows and Morris 2003) that is suspected by the authors to enhance the impact of the Heitler's lump on the lever of the tibial flexor muscle. We found that the genuflexor sclerite in *Gryllus* have a thick ventral pad (GFS: Figs 10.) similar to bush crickets. We did not find a similar pad in the locust, however, the surface of the Heitler's lump is covered with a thick layer of columnar epithelial cells with unknown mechanical properties (Fig. 2G).

We did not find cricket specimens with fully locked femoro-tibial joints, neither were able to relock the joint, supporting the hypothesis that these insects, unlike locusts, do not possess the ventral lock in the femoro-tibial joint. A locust can only kick and jump if the femoro-tibial joint is fully flexed and the lock is activated, while crickets are able to kick and jump even with partially flexed femoro-tibial joints (Burrows and Morris 2003). Better understand the biomechanical impact of the above described differences between locusts and crickets certainly would help us to better understand evolutionary differences that led to cardinally different jumping behaviors in these taxa.

In grasping taxa and in jumping curculionids, the ventral ⅓ of the tibial flexor sclerite is transparent, lack resilin and has cardinally different electron microscopy properties than the melanized core of the sclerite. The surface of the sclerite is heavily sculptured. Since unlike in orthopterans, the proximal region of the femoro-tibial conjunctiva is not stuck in between the tibial flexor sclerite and the Heitler's lump, better understanding of surface friction in these taxa is especially important. Unlike Alticini and Orthoptera, in many grasping taxa, the joint can not be unflexed only by pulling the tibia away from the femur, but we have to actively move over the tibial flexor sclerite through the Heitler's lump as an obstacle. This joint can be relocked multiple times while we were forcing the sclerite over the lump indicating that larger surface area of both the lump and the slcerite has an increased surface frictional property.

Presence or absence of trigger muscles

Burrows (1969) mentioned that releasing a lock not necessary requires the presence of newly evolved trigger or release muscles to carry the additional load because slight modifications of the already present antagonistic muscles can act as triggers in mantis shrimps. Similarly, Heitler (1974) concluded that, although putative release accessory muscles can be found in locust, these muscles are most likely not involved in the release of the lock. Rather, he proposed, that contraction of the antagonistic extensor might be enough to release the lock. It ~~females are often competing for nesting sites or food resources and although not as expressed as in males, they also exhibit aggressive behavior with the involvement of striking with fore-legs (Burkhardt and de la Motte 1983, Al-khairulla et al. 2003, Bath et al. 2015). Although grasping has never been described in *Podagrion* males, they often kick each other as part of their aggression behavior similarly to chalcidid females (Cowan 1979, Grisell and Goodpasture 1981).~~

Although this motion does not require increased extensor muscle mass, the presence of the locking mechanism might be essential.

Diversity of insect tendons and the function of TFS

Insect tendons are invaginations of the single layer epidermis, their site of origin is usually marked by an external pit and they are hollow on the inside. The cuticle of tendons are usually rich in resilin and exhibit a great diversity of cuticular specializations. In some cases, epidermal cells of a tendons function as glands and extracts substances important in communication (Jarau et al. 2012, exocrine glands) or digestion (Rivera-Vega et al. 2017).

The TFS is a modification on the tibial flexor tendon that clearly has a mechanical function. Furth and Suzuki (1990a,b) state that TFS (Lever's triangular plate) are present in Hemiptera, Neuroptera, Megaloptera, Hymenoptera and Coleoptera. This is to our knowledge the first mention of TFS in the order of Diptera. It has been hypothesized that the sclerite in these taxa might simply strengthen the tendon of the enlarged tibial flexor muscles, protect the ventral side of femoro-tibial joint or just alter the working angle of the leg flexor system (Nadein and Betz 2018, Gorb 1995). 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). Nadein and Betz (2016) found TFS placed far from the femoral wall, therefore they conclude it most probably does not prevent the tibia from extending, but they

do not exclude this possibility. Tendon sclerites seem to be involved in click/locking mechanisms even outside the order of Insecta. Burrows (1969) and Burrows and Hoyle (1972) described the mechanics of catching in the mantis shrimps that involves sclerites developed at the distal portions of both the flexor and extensor muscles of the propus (leg segment topologically similar to tibia).

Proof of presence of the lock via dissections

Although static images and 3D models can indicate the presence of the features of a locking mechanism, true evidence of the presence of a locking mechanism could be achieved only through observations of the lock in function, i.e. to observe the unlocking and locking of the structures in situ. Our present study shows that the TFS and the HL are involved in the locking mechanisms in the examined taxa. The lock can be fastened and unfastened in legs by forcing the TFS against the HL in glycerol stored specimens. Mechanically the system works because of the angle of the flexor tendon, which makes it possible to pull the TFS beyond the Heitler's lump and pull it "down" (adpress it) simultaneously to the inner surface of the femur. In this position the friction between the TFS and the femoral cuticle prevents sliding and therefore it fixes the position of the tibia. The ventral, transparent and electron lucid sheet of the TFS might play an important role in generating the required friction. Although the flexibility that is required to fulfill such requirements could be provided by a resilin-rich structure; we were not able to locate this material from the TFS.

We have found accessory tibial flexor muscles only in Diopsidae. These muscles are composed of 10–15 muscle fibres and seemingly behave differently compared to the more proximal muscles. In specimens where the femoro-tibial joint is locked, these muscles do not

~~seem to be in a contracted state (*fe-tifld*: Figs 3D, 7E, F). Based on their orientation, it is possible that the accessory flexor muscles disentangle the lock between the TFS and the HL.~~

~~An alternative triggering mechanism can be activated by the contraction of the resilin-rich GFS, which action would pull on the *fe-tifld* cluster of fibres. If these, however, are in a fully extended state the pulling could not stretch them further, and as a result the TFS would move in the direction of the tibia (fixing the locked state), but rather towards the pivot point of the joint, thereby releasing the lock.~~

~~Similar accessory muscles have been described from the locusts and crickets, and although their position suggests their lock release function (Heitler 1974) it~~ has been proposed (based on their innervation pattern), that these muscles do not lift the TFS out from the locked position, but might have a stabilizing function in jumping systems (Nishino 2004). The differently oriented 10–15 muscle fibres that inserts on the genuflexor sclerite in diopsid flies (*fe-tifld*: Figs 3D, 7E, F) and clearly for a separate, fan shape morphological unit in fully flexed femoro-tibial joint, might represent trigger portions of the tibial flexor muscle. This band can not be separated and thus observed in not-fully-flexed legs, indicating the possibility that similar muscles might have been simply overlooked in other taxa examined, and we should perhaps put more emphasis to properly describe these patterns in future works.

How to confirm the presence of a lock

In earlier studies, the presence/absence of locking mechanisms in the femoro-tibial joint were inferred indirectly based on slow motion video recordings, and spatial relationships between anatomical structures on static images. Heitler (1974) suspected the presence of a lock

in locust legs based on his experiment in which he pulled the flexor muscles until femoro-tibial joint was fully flexed and then measured the force required to reopen the joint.

H2O2 bleaching is perhaps the most crucial part of our dissection based approach as it let us to see how different sclerties interact while we move the joint. We assumed that a locking mechanism is present in the femoro-tibial joint only if we have seen two interacting sclerites preventing the joint to open in fully flexed legs after the flexor muscle origin was detached from the femur. The ability to relock the joint by pulling the flexor muscle is only an additional evidence for the presence of the lock, and we have to acknowledge, that by pulling the flexor muscles, we can not model properly the natural contraction of the muscles. The flexor muscle fibres are grouped in multiple, distinct bundles, whose neural control, strength and speed are remained to be described.

Importance of simple observations in the 21st century morphology

The advent of Micro-CT based, high resolution 3D reconstruction tremendously accelerated the collection of morphological data and made insect morphology accessible for a broader range of students (Betz 2007, Deans et al. 2012). However, micro-CT based methods, at least today, did not substitute perfectly traditional dissection based techniques and histology. Albeit there is some convincing development in in-vivo X-ray imaging techniques (dos Santos Rolo et al. 2014, Xu et al. 2016), dissections are still the most available and perhaps the most accurate methods to visualize the motion of anatomical systems both in live and dead specimens. Besides observing motion of elements, to define functional units of the skeleton also requires classical methods as the tissue specific contrast of X-ray based methods are usually not sufficient

enough to separate more and less flexible cuticular elements (sclerites and conjunctivae) from each other.

Nadein and Betz (2016, 2018) have used highly sophisticated, noninvasive imaging techniques to analyse the femoro-tibial complex. However, using basic dissections techniques, we hereby revealed two key elements of this system that they were not able to capture properly: the presence of a ventral lock in the femoro-tibail joint and the lack of the connection between the internal surface of the ventral femoral wall and the genuflexor sclerite.

They proposed that in jumping curculionids the genuflexor sclerite/tibial flexor apodeme is connected to the ventral femoral wall, when the joint is fully flexed (bl, broad ligament: figs 8E, F, 10 *in* Nadein and Betz 2018). We did not find this connection in our specimens, and it would be logically impossible to have this connection if we properly consider the simple nature of the insect exoskeleton, that is the product of a single cell layer. The tibial flexor sclerite is on the tibial flexor tendon in *Orchestes*. The site of origin of the tendon is continuous with the genuflexor sclerite distally and with the proximal portion of the femoro-tibial conjunctiva proximally and is not connected to the ventral wall of the femur with any other structures. Nadein and Betz (2018), most likely, considered the ventral, transparent layer on the tibial flexor sclerite on their CLSM micrograph as the connection between the sclerite and the internal surface of the ventral femoral wall. This would be interesting to further explore as we did not find resilin in this structure in the newly examined grasping taxa.

Conclusion and future directions

~~The studied TFS/HL system~~The ventral lock of the femoro-tibial joint reveals a remarkable parallel implementation of the physical mechanism to create a grasping and a

jumping function. The building blocks of this system, genuflexor sclerite, ventral femoral wall and tibial flexor tendon are obviously present in ~~every insect, as their ground plan contains the necessary structural components in the leg joints, especially in femorotibial (femur to tibia) connections.~~most insects. Descriptive analysis ~~of the relative position of different anatomical structures cannot provide proof of~~based on static images can suggest the presence of ~~any~~a locking mechanism~~. These studies only raise the suspicion that such a mechanism may exist. Our~~ and our dissection-based experimental technique, ~~on the other hand~~by studying moving parts under the microscope, can reveal the workings of a locking apparatus and describe its functioning in detail. Due to its simplicity it offers a chance to the wide scientific community to test various species representing diverse clades of insects. Until micro-CT techniques can be applied to live animals in sufficient resolution the best solution is to utilize the wide variety of existing anatomical techniques (CLSM, SEM, TEM, etc.) and combine it with the traditional dissections-under-the-microscope technique that allows to manipulate e.g. joints in a manner that provides information on their live role while in motion. These may range from jumping leaf beetles to leafhoppers and grasping (predatory) water scorpions, heteropterans and robber flies.

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Figure legends

Figure 1. Generalized representation of an insect leg and modifications for jumping and grasping. Two muscles connect the four proximal leg segments, the flexors (blue) close and the extensors (brown) open the joint. A, the tibial flexor is enlarged in a jumping leg; B, the two femoro-tibial muscles are similar in mass in walking legs; C, the tibial extensor is enlarged in grasping legs; D, E, the locking mechanism between the tibial flexor muscle tendon and the ventral wall of the tibia is formulated by the Heitler's lump and the tibial flexor sclerite in locusts (closed and open position).

Figure 2. Synchrotron-based micro-CT micrographs of the femoro-tibial joint in the cricket, *Gryllus campestris*. A, C, closed joint; B, D, Opened joint; E, F, cross sections of the proximal femoral regions in a closed joint. Lines on C indicate planes of cross sections. Although the semilunar process (slp), tibial flexor sclerite (TFS, =cushion) and the Heitler's lump (HL, internally a convex region on the ventral femoral wall) are present in crickets, the energy storing mechanism found in locusts is apparently absent. In locusts, the TFS is locked proximal to the HL when the tibial flexor is contracted and energy is stored in the resilin-rich semilunar processes during the contraction of the extensor muscle in a locked position. The lock is absent in crickets and the semilunar process is rigid, not resilin-rich (Hustert and Baldus 2010; tib=tibia, fem=femur, *fe-tifl*=tibial flexor muscle, *fe-tiex*=tibial extensor muscle). Although the HL and the TFS are present in numerous other insects taxa, without observing in action, we can not predict the presence of an energy storage locking mechanism.

Figure 3. Synchrotron based micro-CT micrographs of the femoro-tibial joints of *T. dalmanni*. A–D, male fore leg; E, female fore leg; F, G, male middle and hind legs. In an open joint, all fibres of the femoro-tibial extensor muscle (*fe-tifl*) are oriented proximally and muscle contraction moves the TFS proximally. In a closed leg, some distal fibres (*fe-tifld*)) of the muscle that attach proximally to the genuflexor sclerite (GFS) are oriented anterodorsally, a position that makes it a potential trigger muscle, which releases the locking mechanism (triggering could be initiated by the contraction of the resilin-rich GFS). This distal cluster of fibres of the tibial flexor is absent in other taxa examined.

Figure 4. Bright field images and CLSM micrographs of the tibial flexor sclerite (TFS) of grasping insect legs. A–D, *T. dalmanni*; E–F, *Ochthera* sp;, G, *Podagrion* sp.; H, *T. dalmanni*. The ventral surface of the TFS is in contact with the Heitler's lump (HL) when the mechanism is in a locked position. The ventral margin of the sclerite is covered with a glassy, transparent cuticular coating, resembling resilin that is slightly deformed when it is pressed against the Heitler's lump. The region is not resilin-rich (autofluorescence tested with 407 nm excitation), however, the genuflexor sclerite (GFS) shows strong blue autofluorescence when tested with the same UV light. The TFS is the origin of the tibial flexor tendon (ten), a cuticular invagination

marked by a distinct, elongate pit (pit) that is adjacent to the proximal end of the GFS and partially surrounded by the femoro-tibial conjunctiva (con) (tib: tibia, fe-tifl: femoro-tibial flexor muscle).

Figure 5. Synchrotron-based micro-CT micrographs showing the femoro-tibial joint of grasping legs. The medioventral region of the femoro-tibial conjunctiva is sclerotized composing the genuflexor sclerite (GFS) and possesses two invaginations at the proximal and distal ends of the GFS. The distal invagination extends to and is connected to the dorsal tibial wall (GFS: A), while the proximal invagination forms the tibial flexor apodeme (GFS: D). The femur and the tibia are articulated by two lateral hinges (pivot points): condyles on the tibia (con: B, C) that insert into fossae on the distal margin of the femur and thereby create the horizontal axis of the femoro-tibial joint. The tibial flexor sclerite (TFS) is positioned at the most basal section of the tibial flexor tendon. The Heitler's lump (HL) is a complex flattened invagination of the ventral femoral wall. The TFS is adjacent to the ventral femoral wall.

Figure 6. SEM micrographs showing anatomical structures at the femoro-tibal joint of the fore leg of *T. dalmanni*. The Heitler's lump (HL) is a flattened invagination of the ventral femoral wall providing a convex surface essential for the locking mechanism (A). The tibial flexor sclerite (TFS) is at the basal section of the tibial flexor tendon, an invagination at the proximal end of the femoro-tibial conjunctiva. The tendon's site of origin is marked by an elongate pit (pit, C). The genuflexor sclerite (GFS) is a median sclerotized area on the ventral wall of the femoro-tibial joint. The surface of the TFS is highly sculptured, supposedly increasing the friction between the TFS and HL on the basal section, leaving the apical section smooth for better sliding (B, C, D, F). The tibial flexor muscle is subdivided into a proximal (*fe-tiflp*) and a distal cluster of muscle fibres (*fe-tifld*). Fibres of the proximal cluster insert to the dorsal surface of the TFS, while the distal cluster fibres insert on the GFS.

Figure 7. TEM and CLSM micrographs showing the femoro-tibial joint in *T. dalmanni*. The TFS has an electron dense core (*core*) that is surrounded by an electron lucent coating. The ventral section of the coating (*cov*) is more electron lucent than the dorsolateral (*col*) portion. The coating is transparent (not melanized) on brightfield images (B). The core is composed of radiating cuticular layers (C, D). The Heitler's lump is an invagination on the ventral wall of the femur (HL: E, F). The tibial flexor is composed of a distal and a proximal cluster of fibres. The orientation of these clusters is the same in open legs, but in closed legs the proximal cluster (*fe-tiflp*) is oriented proximally, the distal cluster (*fe-tifld*, E, F) is oriented distodorsally and might function as a trigger muscle enabling the release of the locking mechanism.

Supplementary Xu, L., Chen, R., Du, G., Yang, Y., Wang, F., Deng, B., Xie, H. and Xiao, T. (2016) Anisotropic shrinkage of insect air sacs revealed in vivo by X-ray microtomography. *Scientific reports* 6, p.32380.**Table 1.** Specimens examined.

group	Species	Specimen data	Study technique
stalk-eyed fly	*Teleopsis dalmanni*	UCL lab culture (MALAYSIA: KL)	SR-μCT, CLSM, dissection, video, TEM
chalcidoid wasp	*Podagrion* sp.	GERMANY,	SR-μCT
chalcidoid wasp	*Podagrion* sp.	GERMANY,	dissection, CLSM
chalcidoid wasp	*Podagrion* sp.	USA: Texas Bracketville 29312, -100637 III.20-22.2010 YPT	dissection
stalk-eyed fly	*Sphyracephala brevicornis*	USA: New Hampshire Durham 43.135, -70.933	dissection
cricket	*Gryllus campestris*	HUNGARY: Hortobágy	SR-μCT
cricket	*Gryllus campestris*	HUNGARY: Hortobágy	dissection
shore fly	*Ochthera* sp.	USA: Texas Bracketville 29312, -100637 III.20-22.2010 YPT	dissection

Table 1. Specimens examined.

Glycerol

Order	Taxon(number of specimens), Family	Function	Specimen data	Sclerite involved in lock	Study technique	Unlocking /locking
Diptera	*Teleopsis dalmanni* (15), Diopsidae	grasping, kicking	UCL lab culture (MALAYSIA: KL)	TFS	fore, middle and hind legs, dissection, video, CLSM, SR-μCT	+/+

Diptera	*Sphyracephala brevicornis* (Say, 1817) (6), Diopsidae	grasping, kicking (?)	USA: New Hampshire Durham 43.135, -70.933	TFS	fore and middle legs, dissection, video, CLSM	+/+
Diptera	*Ochthera* sp. *mantis*-group (6♀), Ephydridae	grasping	USA: Texas Bracketville 29312, -100637 III.20-22.2010 YPT	TFS	fore and middle legs, dissection, video, CLSM	+/+
Hymenoptera	*Podagrion* sp. 1. (6), Torymidae	grasping, kicking	GERMANY	TFS	Hind legs SR-µCT of hind legs	+/+
Hymenoptera	*Podagrion* sp. 2. (3), Torymidae	grasping, kicking	USA: Texas Bracketville 29312, -100637 III.20-22.2010 YPT	TFS	hind and middle legs, dissection, video	+/+
Orthoptera	*Gryllus campestris* Linnaeus, 1758 (3), Gryllidae	jumping	HUNGARY: Hortobágy	GFS	hind legs, dissection, SR-µCT	-/-
Orthoptera	*Omocestus (Omocestus) haemorrhoidalis* (Charpentier, 1825) (5), Acrididae	jumping, kicking	HUNGARY: Bács-Kiskun Bugacpusztaháza 46.696945°, 19.601822° Aug.10.2014 alkaline meadow sweeping Deans and Mikó	GFS	hind legs, dissection, video	+/-
Coleoptera	*Disonycha xanthomelas* (Dalman, 1823) (5),Chrysomelidae	jumping	USA: NH, Dover, Bellamy Rd. 43.172, -70.809 v.17-v.19.2019, YPT I. Miko	GFS	hind legs, dissection, video	+/-
Coleoptera	*Chaetocnema minuta* F. E. Melsheimer, 1847 (6), Chrysomelidae	jumping	USA: NH, Dover, Bellamy Rd. 43.172, -70.809 v.17-v.19.2019, YPT I. Miko	GFS	hind legs, dissection, CLSM	+/-
Coleoptera	*Longitarsus sp.* (3), Chrysomelidae	jumping	HUNGARY: Bács-Kiskun Bugacpusztaháza 46.696945°, 19.601822° Aug.10.2014 alkaline meadow sweeping Deans and Mikó	GFS	hind legs, dissection	+/-
Coleoptera	*Caryobruchus gleditsiae (*Linnaeus, 1763) (2), Bruchidae	grasping	USA: FL:Coll. Co. Wiggins Pass Rec. Area 10 mi N Naples.XII-3, 1-1992, R.M. Reeves, rotten wood on beach	TFS	hind legs (dry specimens), dissection, video	+/+
Coleoptera	*Orchestes mixtus* Blatchley & Leng , 1916 (2), Curculionidae	jumping	USA, VT. Lamoille Co. Wolcott, Lamoille Riv. 5-26-2009. T. Murray	TFS	hind legs (dry specimens), dissection, video	+/?
Hymenoptera	*Schletterius*	kicking,	USA: CA: S. BRDO. Co.	TFS	Hind legs,	+/+

	cinctipes (4), Stephanidae	grasping (?)	Jenks LK. Rd., 2105m, 34,9'48"N: 116,51'43"W; ex. Abies log coll. 28.I.06. Emerge iv.06, F. Reuter Schletterius cinctipes		dissection, video	

Supplementary Video 1. Fastening and unfastening of the femoro-tibial lock in *Teleopsis dalmanni*, male, by forcing the TFS against the HL and pulling the tibial flexor muscle in glycerol stored specimens.

Supplementary Video 2. Fastening and unfastening of the femoro-tibial lock in *Sphyracephala brevicornis*, male, by forcing the TFS against the HL and pulling the tibial flexor muscle in glycerol stored specimens.

Supplementary Video 3. Fastening of the femoro-tibial lock in *Podagrion* sp., female, by forcing the TFS against the HL and pulling the tibial flexor muscle in glycerol stored specimens.

Supplementary Video 4. Unfastening of the femoro-tibial lock in *Podagrion* sp., female, by forcing the TFS against the HL and pulling the tibial flexor muscle in glycerol stored specimens.

Supplementary Video 5. Fastening and unfastening of the femoro-tibial lock in *Ochthera* sp., female, by forcing the TFS against the HL and pulling the tibial flexor muscle in glycerol stored specimens.

Supplementary Video 6. Surface rendered model of the femoro-tibial joint of *Teleopsis dalmanni*, male.

Supplementary 3D pdf. Surface rendered model of the femoro-tibial joint of *Teleopsis dalmanni*, male.

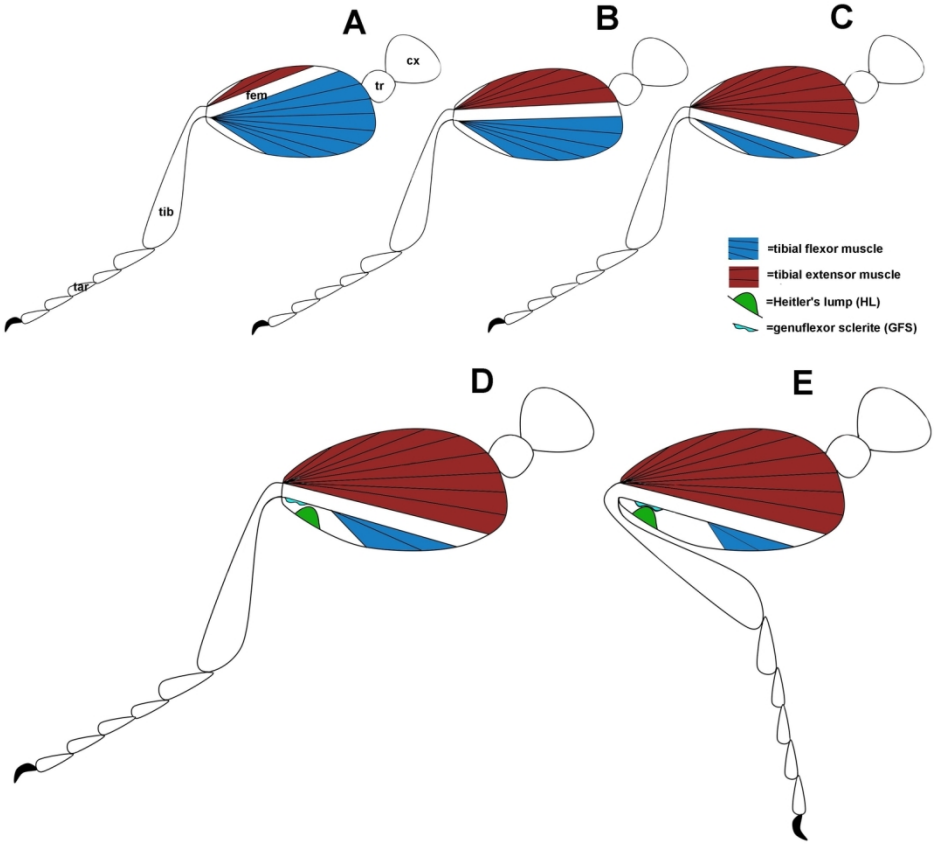

Figure 1. Generalized representation of an insect leg and modifications in the tibio-femoral joint for jumping and grasping. Two muscles connect the tibia and the femur. The tibial flexor (blue) bends (flexes) while the tibial extensor (brown) straightens (extends) the femoro-tibial joint. The tibial extensor is enlarged in a jumping legs (A), the two muscles are similar in their mass in walking legs (B), and the tibial flexor is enlarged in grasping legs (C). The locking mechanism between the tibial flexor muscle tendon and the ventral wall of the tibia is composed of the Heitler's lump (HL) and the genuflexor sclerite (GFS) in orthopterans (flexed (D) and extended (E) positions, modified after Gronenberg 1996; cx=coxa, tr=trochanter, tib=tibia, fem=femur, tar=tarsus; distal to the left).

145x128mm (300 x 300 DPI)

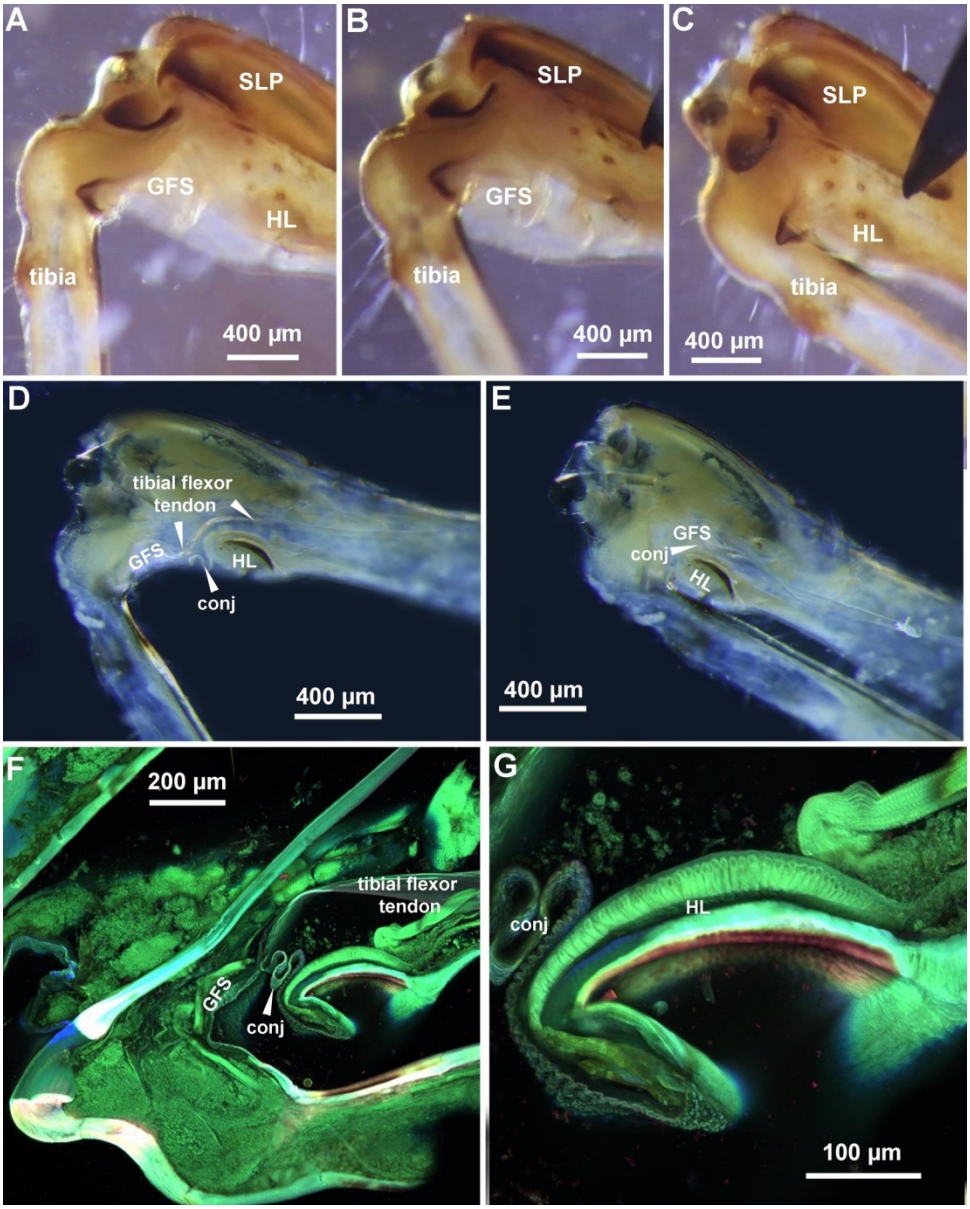

Figure 2. The femoro-tibial joint of the hind leg of the locust, *Omocestus haemorrhoidalis* (Acrididae); A–C closing of the joint, D open joint, E closed joint (conjunctiva is located between Heitler's lump and GFS), F open joint (CLSM), G same at greater magnification (GFS=genulexor sclerite, conj=conjunctiva between the site of origin of the tibial flexor tendon and the distoventral margin of the femur, HL=Heitler's lump, SLP=semilunar process, distal to the left).

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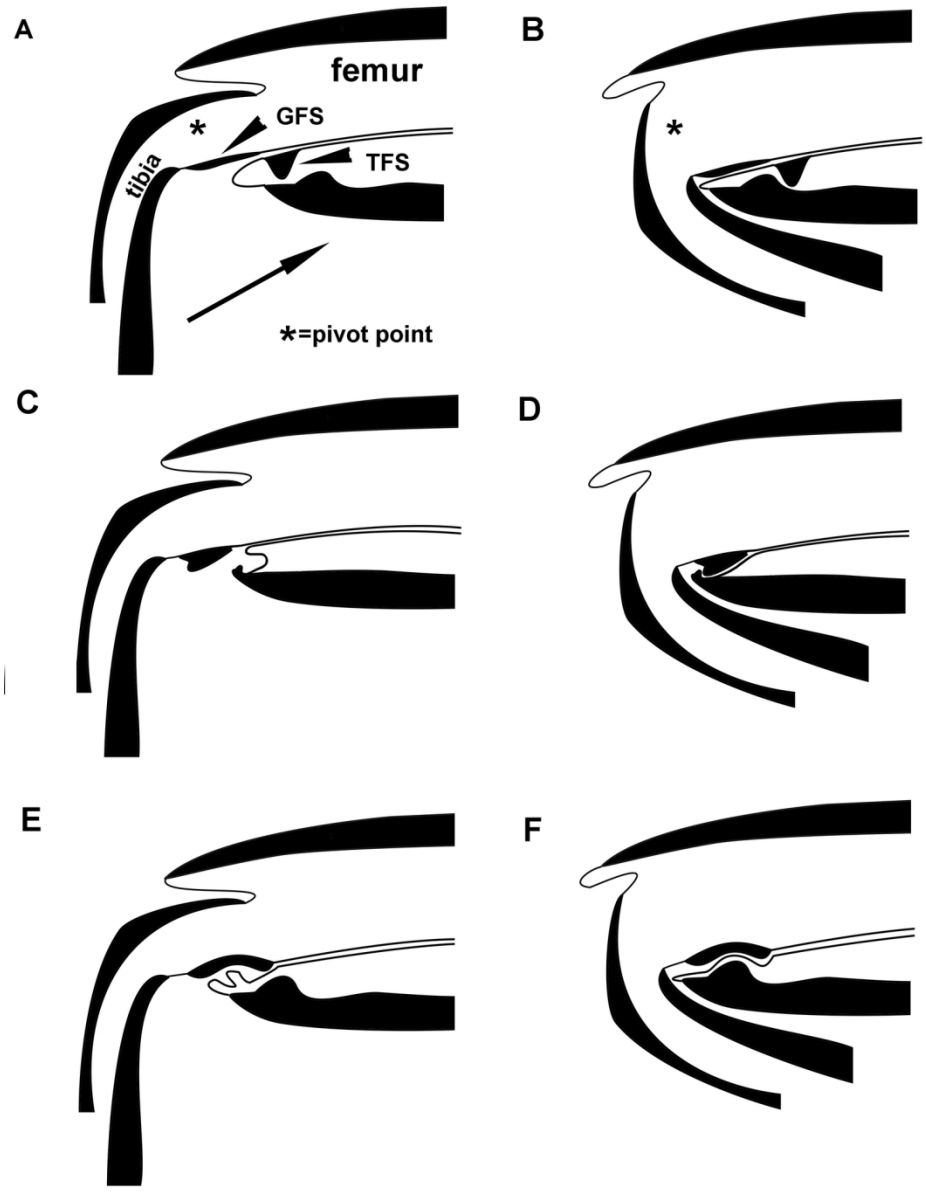

Figure 3. Line drawings showing the three major types of locking mechanisms in the ventral portion of the femoro-tibial joint. Left: extended position; Right: flexed position. A, B TFS over Heitler's lump without conjunctiva in-between (grasping legs and Orchestes jumping leg); C, D GFS locked at tip of femur, no conjunctiva in-between (Alticini); E, F GFS over Heitler's lump with conjunctiva in-between (Orthoptera; GFS=genuflexor sclerite, TFS=tibial flexor sclerite, distal to the left).

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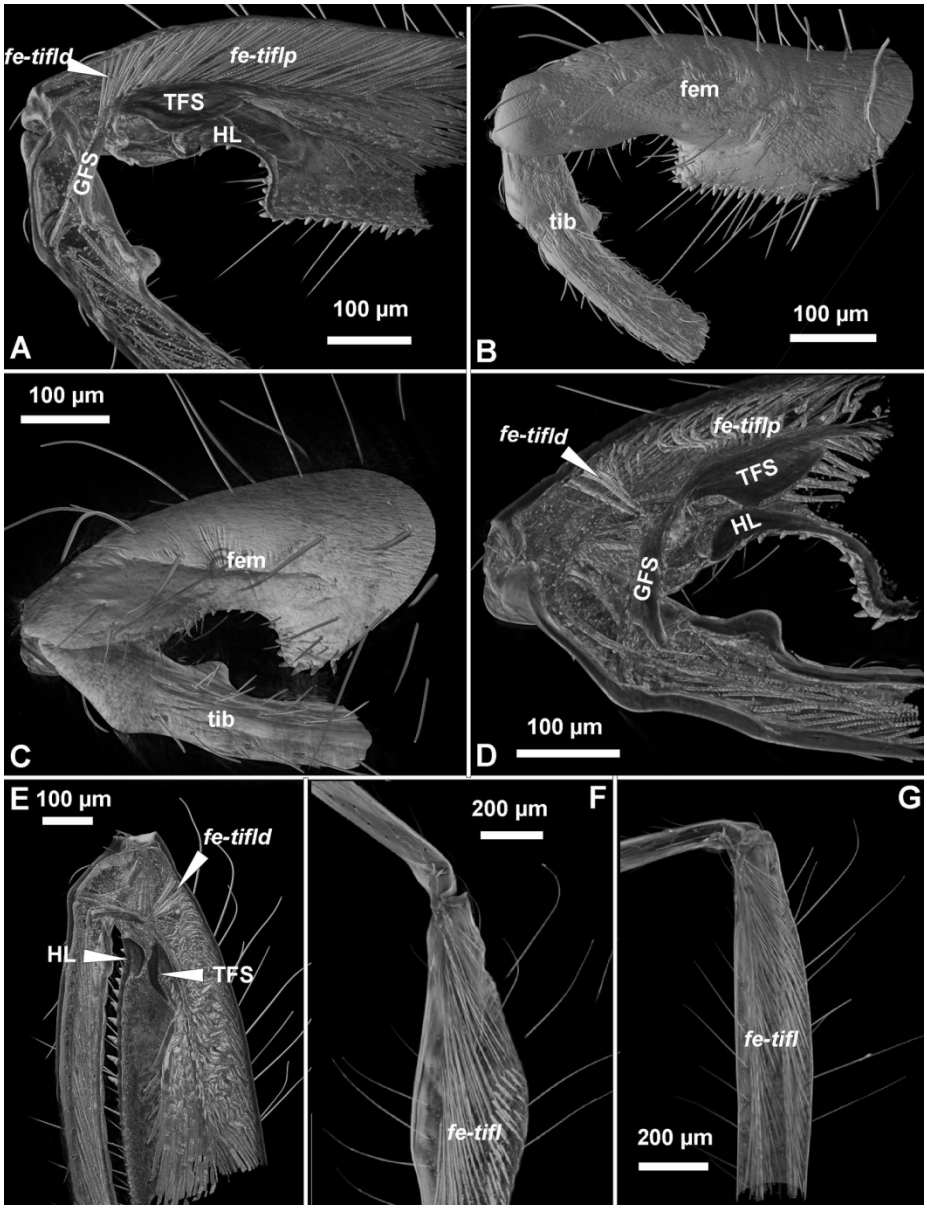

Figure 4. Synchrotron based micro-CT micrographs of the femoro-tibial joints of the stalk-eyed fly, Teleopsis dalmanni. A–D, male fore leg; E, female fore leg; F, G, male middle and hind legs (GFS=genuflexor sclerite, TFS=tibial flexor sclerite, HL=Heitler's lump, fe-tifld=distal tibial flexor muscle (potential trigger or release muscle), fe-tiflp=proximal tibial flexor muscle, fem=femur, tib=tibia, A–D, distal to the left; E–F, distal to the top).

145x190mm (300 x 300 DPI)

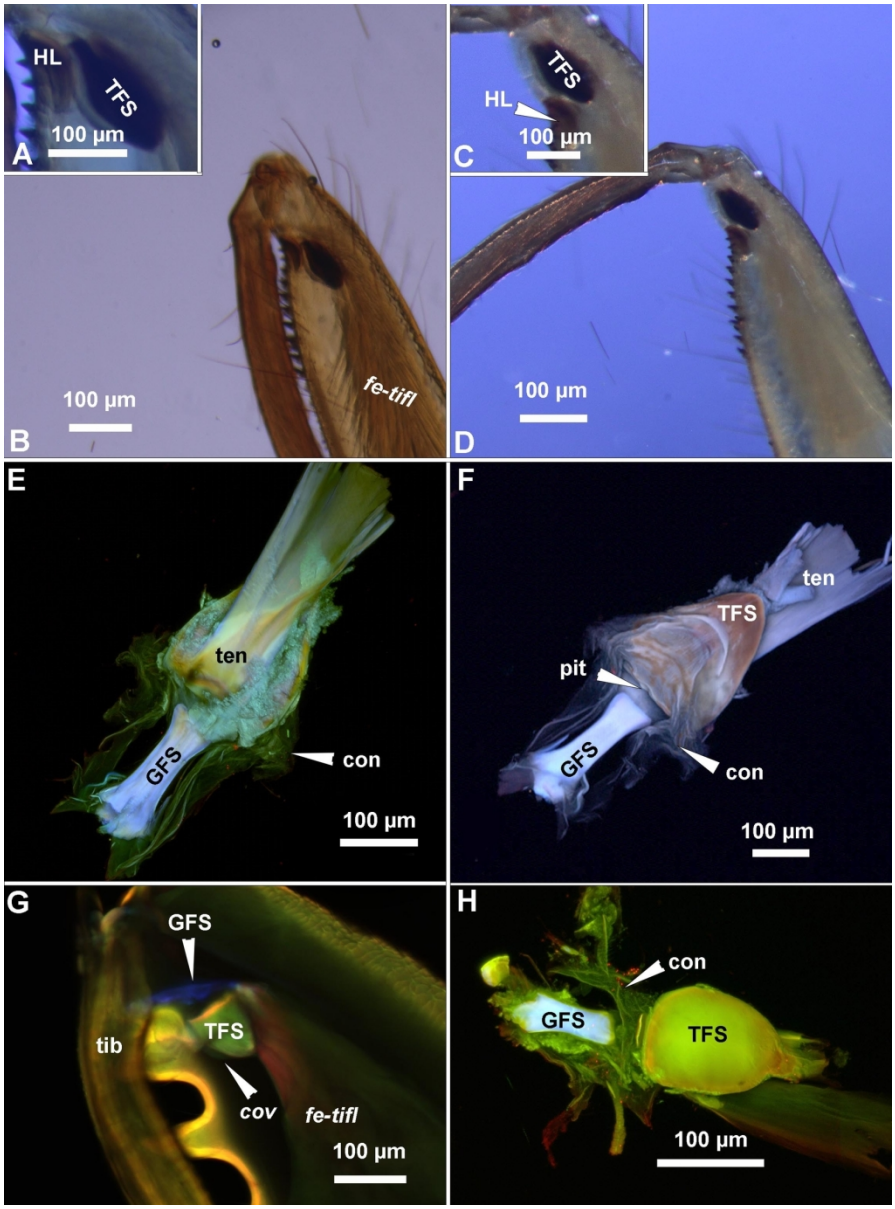

Figure 5. Bright field images and CLSM micrographs of the femoro-tibial joint of grasping insect legs. A–D, *T. dalmanni*, female, fore leg; E–F, *Ochthera* sp., female, fore leg; G, *Podagrion* sp., female, hind leg; H, *T. dalmanni*, male, fore leg (GFS=genuflexor sclerite, TFS=tibial flexor sclerite, HL= Heitler's lump, ten=tendon of the femoro-tibial muscle, cov=glassy ventral layer of the tibial flexor sclerite; *fe-tifl*=tibial flexor muscle, pit=pit corresponding to the invagination of the femoro-tibial flexor tendon, tib=tibia).

145x196mm (300 x 300 DPI)

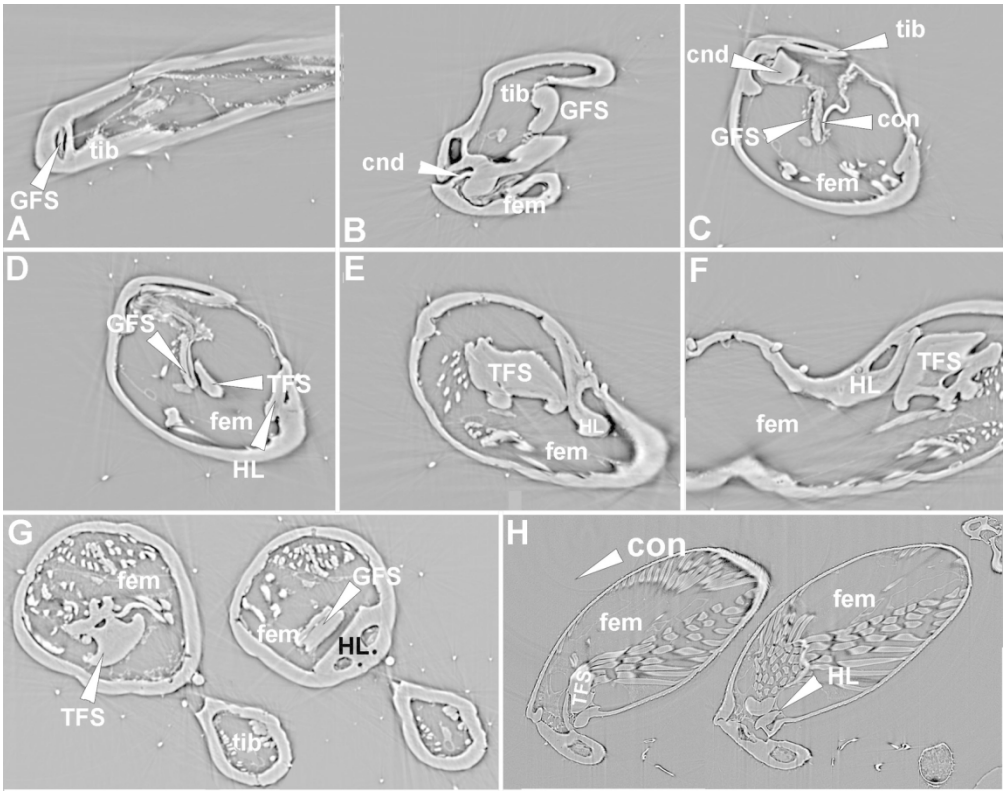

Figure 6. Synchrotron-based micro-CT micrographs showing the femoro-tibial joint of grasping legs. A–F, *T. dalmanni*, male, fore leg; G, H, *Podagrion* sp., female, hind leg (GFS=genuflexor sclerite, TFS=tibial flexor sclerite, HL=Heitler's lump, con=femoro-tibial conjunctiva, cnd=condyles (pivot points), tib=tibia, fem=femur, A–F, distal to the right, H, distal to the left).

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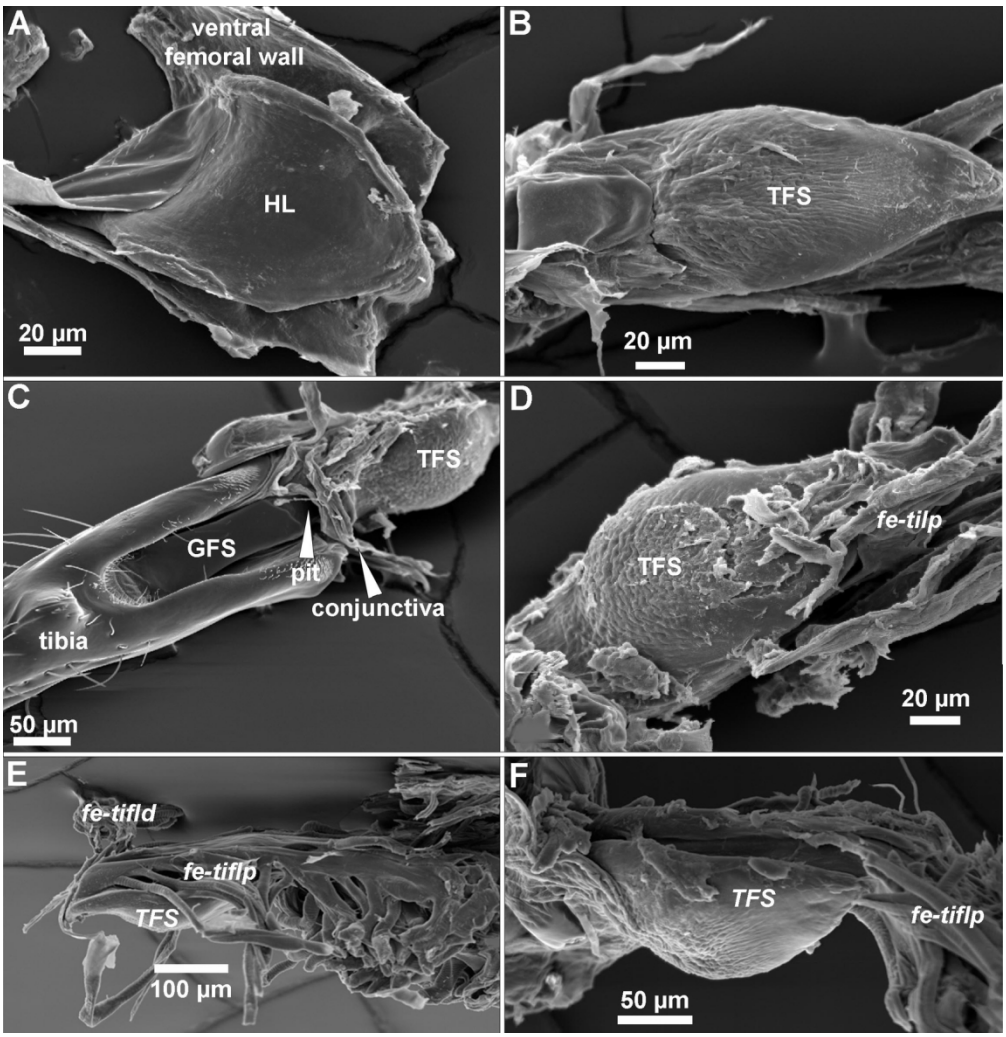

Figure 7. SEM micrographs showing anatomical structures at the femoro-tibal joint of the fore leg of male *T. dalmanni*. A, internal (dorsal) view, B, ventral view, C, external (ventral view), D, ventral view, E, F, lateral view (GFS=genuflexor sclerite, TFS=tibial flexor sclerite, HL=Heitler's lump, pit=pit corresponding to the invagination of the femoro-tibial flexor tendon, fe-tifld=distal tibial flexor muscle (potential trigger or release muscle), fe-tiflp=proximal tibial flexor muscle, distal to the left).

145x149mm (300 x 300 DPI)

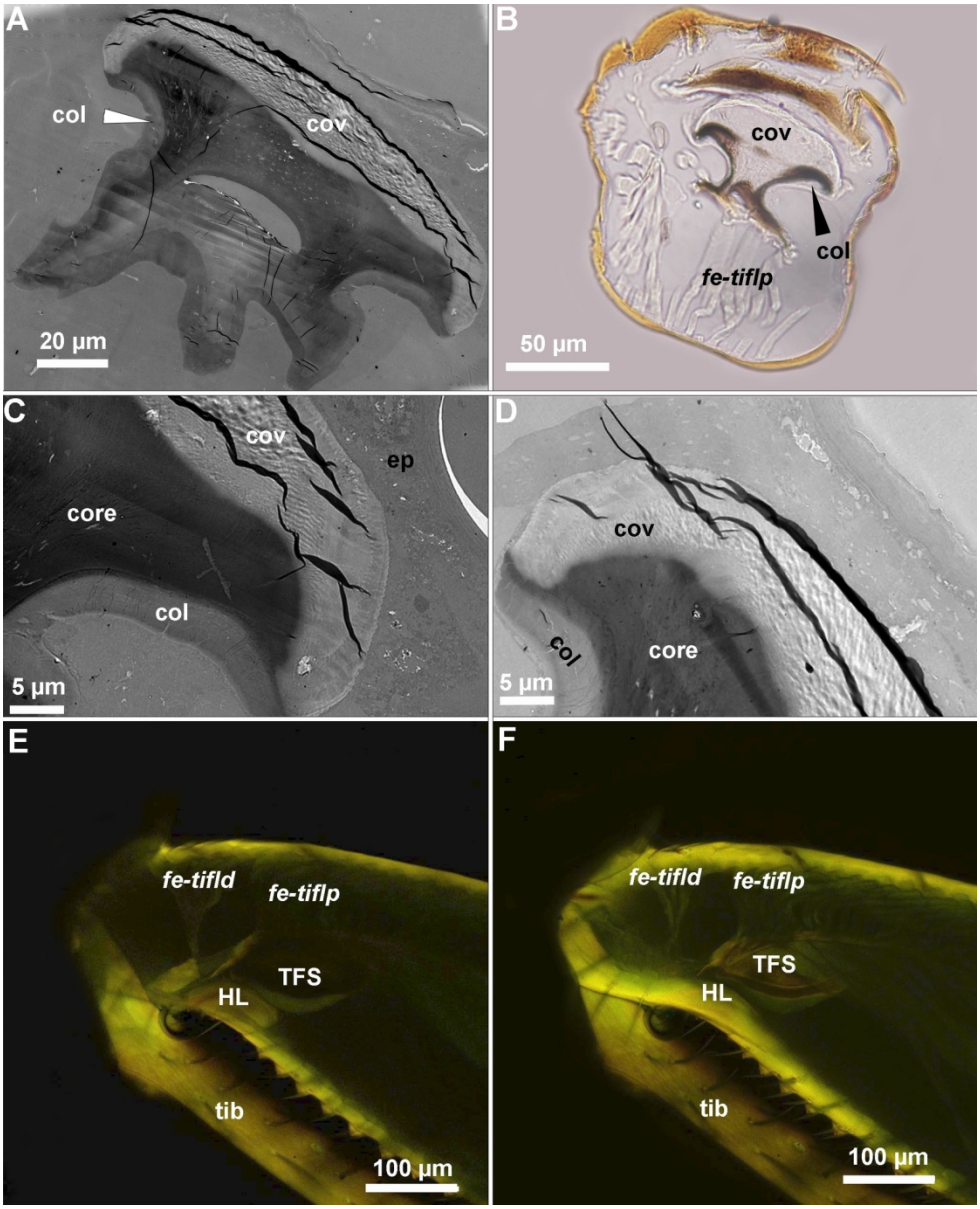

Figure 8. TEM and CLSM micrographs showing the femoro-tibial joint in *T. dalmanni* (TFS=tibial flexor sclerite, core=electron dense core of TFS, cov=electron lucent external surface (coating) on the ventral portion of the TFS, col=electron dense external region (coating) on the lateral portion of the TFS, tib=tibia, fe-tifld=distal tibial flexor muscle (potential trigger or release muscle), fe-tiflp=proximal tibial flexor muscle, distal to the left).

145x179mm (300 x 300 DPI)

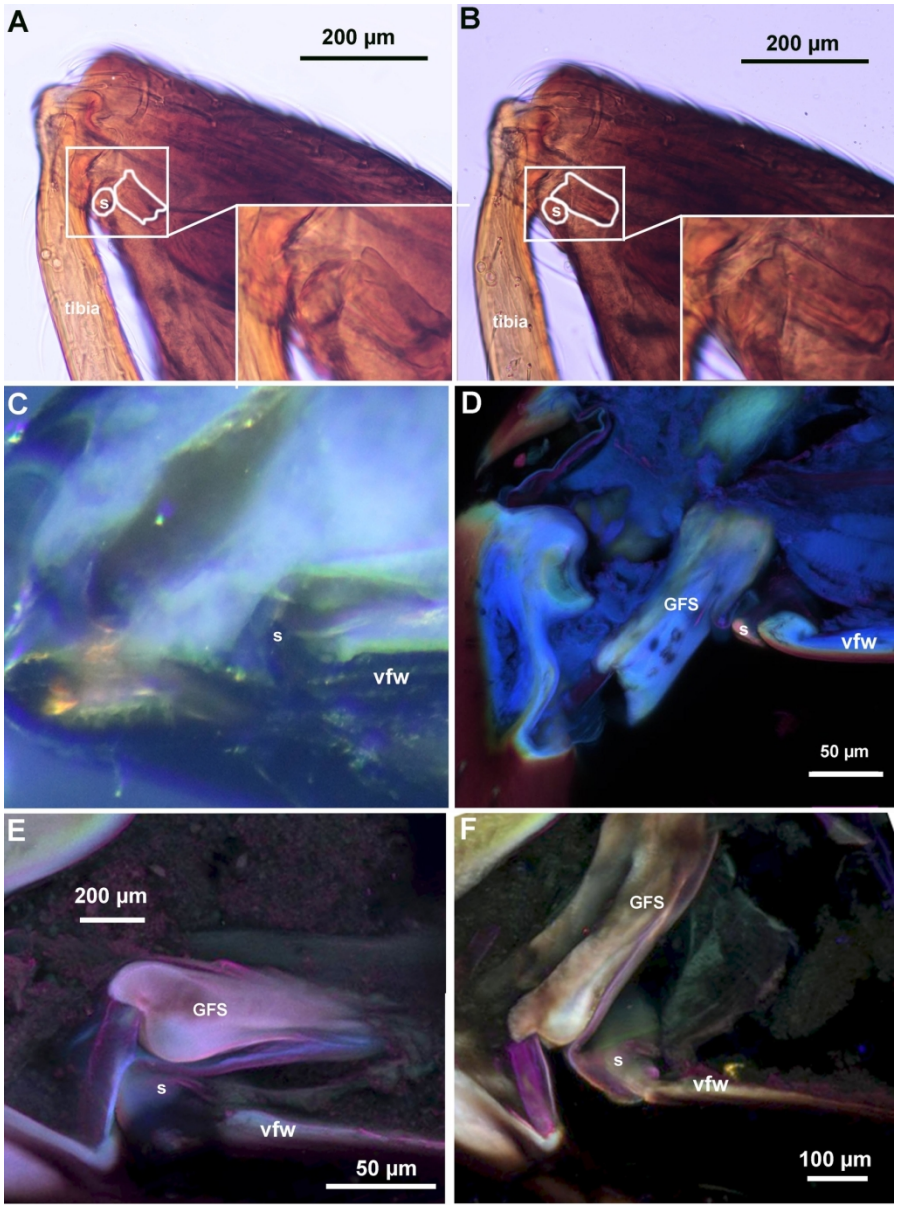

Figure 9. Femoro-tibial joint of the hind legs in Alticini (Chrysomelidae). A, B, *Longitarsus* sp., A, genuflexor sclerite in a locked position, B, genuflexor sclerite in an unlocked position, C, *Disonycha xanthomela*, D, *Chaetocnema minute*, genuflexor sclerite in unlocked position, E, F, *D. xanthomela*, E, genuflexor sclerite in a locked position, F, genuflexor sclerite in an unlocked position (GFS=genuflexor scelite, s=distal sclerotic element of the femoral abutment (=femoral abutment of Lever's trinagular plate), vfw=ventral femoral wall, distal to the left).

145x196mm (300 x 300 DPI)

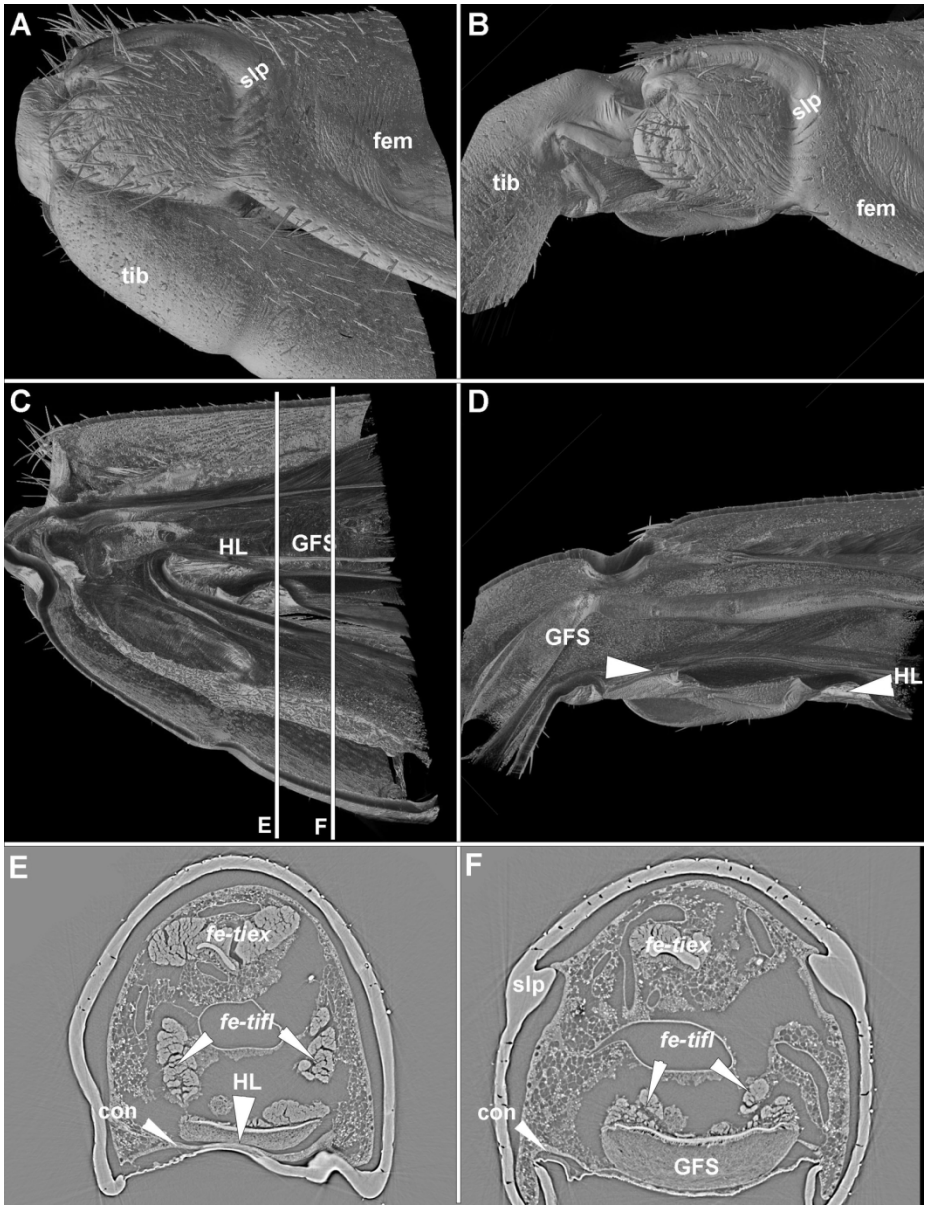

Figure 10. Synchrotron-based micro-CT micrographs showing the hind leg femoro-tibial joint of *Gryllus campestris* (slp=semilunar process, fem=femur, tib=tibia, HL=Heitler's lump, GFS=genuflexor sclerite, con=femoro-tibial conjunctiva, fe-tifl=tibial flexor muscle, fe-tiex=tibial extensor muscle, lines marked with E, F show the sites of sections on figures E and F, distal to the left).

145x190mm (300 x 300 DPI)

Term	Abbr.	Concept	URI
apodeme		The process that is internal.	http://purl.obolibrary.org/obo/HAO_0000142
conjunctiva	conj	The area of the cuticle that is more flexible than	http://purl.obolibrary.org/obo/HAO_0000221
femoro-tibial joint		The dicondylic joint that is composed of the femur	http://purl.obolibrary.org/obo/HAO_0001517
femur	fem	The leg segment that is distal to the trochanter and proximal to the tibia.	http://purl.obolibrary.org/obo/HAO_0000327
genuflexor apodeme		The invagination at the distal end of the genuflexor sclerite that is adjacent to (articulated with) the outer	http://purl.obolibrary.org/obo/HAO_0002542
genuflexor sclerite	GFS	The sclerite that is located posteriorly along the proximal margin of the tibia and corresponds to the site of insertion of the medial femoro-tibial muscle.	http://purl.obolibrary.org/obo/HAO_0001529
Heitler's lump	HL	The distal invagination of the ventral femoral wall that is in contact with the tibial flexor sclerite or with the genuflexor sclerite when the tibial flexor muscle is fully contracted and the femoro-tibial joint is fully	http://purl.obolibrary.org/obo/HAO_0002536
lock		The anatomical cluster that is composed of two sclerite surfaces, that are adjacent to each other and	http://purl.obolibrary.org/obo/HAO_0002539
ridge		The apodeme that is elongate.	http://purl.obolibrary.org/obo/HAO_0000899
sclerite	s	The area of the cuticle that is less flexible than adjacent conjunctivae.	http://purl.obolibrary.org/obo/HAO_0000909
tendon	ten	The cuticular invagination on which a muscle is attache	http://purl.obolibrary.org/obo/HAO_0000996
tibia	tib	The leg segment that is proximal to the tarsus and distal to the femur.	http://purl.obolibrary.org/obo/HAO_0001017
tibial extensor muscle	fe-tiex	The intrinsic leg muscle that arises laterally (dorsally) of the femur and inserts on the genuflexor plate.	http://purl.obolibrary.org/obo/HAO_0002544
tibial flexor muscle	fe-tifl	The intrinsic leg muscle that arises medially (ventrally) of the femur and inserts on the genuflexor	http://purl.obolibrary.org/obo/HAO_0002538
tibial flexor sclerite	TFS	The proximal sclerite of the tibial flexor tendon.	http://purl.obolibrary.org/obo/HAO_0002537
tibial flexor tendon		The tendon that is connected to the distal end of the tibial flexor muscle.	http://purl.obolibrary.org/obo/HAO_0002541
ventral lock of the femoro-tibial joint		The lock between a sclerite that is on or continuous	http://purl.obolibrary.org/obo/HAO_0002540