



# Comparative anatomy of pupal tarsi in caddisflies (Insecta: Trichoptera) with focus on the claw system

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## Abstract

The decticious pupa of Trichoptera is an unusual case, as the larvae pupate in a silk cocoon under water. This leads to the problem that the pharate adult (i.e. the imago prior to eclosion within the pupal exuviae) has to cut through the cocoon and actively swim to land. To solve the latter problem, pupal legs are specifically modified. The midlegs are usually equipped with rows of hairs and are used as swimming legs to bring the insects to the water surface or the shore. Some species shed the pupal exuviae while floating on the water surface, others after crawling on stones or plants. It was assumed that this is assisted by attachment structures, especially the pupal claws. Pupal claws can differ distinctly in trichopteran lineages. However, detailed information on this character system is very limited in the literature. Furthermore, the functional principle of the pupal claw system is not well understood. Here, we present detailed data on the pupal tarsus of 15 species (14 families) using confocal laser scanning microscopy and histology. The results are discussed in terms of functional morphology, relations to larval habitat, pupal behavior, and phylogenetic implications.

**Keywords** Trichoptera · Tarsus · Claws · Pupa · Eclosion · Pharate adult · Attachment structures

## Introduction

The pupal stage is the most prominent feature in the life cycle of holometabolan insects. Different types and subtypes can be distinguished morphologically based on the condition of the appendages, the type of cocoon, and other features (see Hinton 1946). The two main types (after Hinton 1946, 1949) are the pupa dectica and the pupa adectica, which primarily differ in having functional (= moveable) mandibles or not. Furthermore, decticious pupae usually also possess articulated legs and a comparatively thin cuticle (Hinton 1949). This type represents the ancestral condition within Holometabola and occurs in Neuropterida, Trichoptera, basal Lepidoptera, and Mecoptera. The decticious pupae use their large and well-sclerotized, moveable mandibles to cut through the cocoon prior to eclosion (Hinton 1946,

1949). Close to eclosion, the moveable appendages of decticious pupae are operated by the pharate adult, i.e., the imago enclosed in the pupal exuviae (Hinton 1946, 1949; Kubiak et al. 2015).

The pupae of Trichoptera are unique among decticious forms for several reasons. Trichopterans are the only group with decticious pupae and larvae pupating under water (e.g., Wichard et al. 1995). Other holometabolan lineages with aquatic larvae (e.g., Megaloptera, some Neuroptera, some groups of Coleoptera) generally leave the water for pupation on land (e.g., New and Theischinger 1993). Underwater pupation is also known in some dipteran and coleopteran groups (adecticious pupae; e.g., Simuliidae, Culicidae; Noteridae), but this feature is definitely not part of the ground plan of the orders. The pharate adults of Trichoptera show a series of adaptations to the unusual pupation habitat. As the adult is not able to swim and has open spiracles for breathing, it cannot shed its pupal exuviae under water. Therefore, the pharate adult has to swim towards the surface or the shore by using the midlegs, which bear rows of well-developed swimming hairs on the pupal exuviae (e.g., Wesenberg-Lund 1910; Tobias 1971). The shedding of the pupal exuviae can take place after crawling out of the water to the shore or plants (e.g., Hydropsychidae, Schumacher

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1970; Limnephilidae, Tobias 1971) or while floating on the water using the shed pupal skin as raft (e.g., Leptoceridae, Phryganeidae; Wesenberg-Lund 1910; Solem 1976). It was assumed that the pharate adult uses attachment structures of the pupal exuviae, i.e., the claws to crawl out of the water (Betten 1934; Hinton 1946).

Anatomical differences of the pupal tarsus, i.e., the presence or absence of claw-shaped projections, were already recognized more than a century ago (e.g., Ulmer 1903; Thienemann 1905; Betten 1934). Unfortunately, pupae are often neglected or treated very briefly in taxonomic descriptions. Even if the pupa is described, information on the presence of claws is often missing—a fact already mentioned by Ulmer (1903: p. 261). Therefore, the condition of this conspicuous feature is only well known for some trichopteran families (e.g., Ulmer 1903; Thienemann 1905). The lack of data impedes an evolutionary interpretation and the recognition of potential phylogenetic signal and patterns.

Besides the very limited knowledge of external features, the fine anatomy of the pupal tarsus is almost completely undescribed. The condition in *Rhyacophila nubila* was illustrated by Kluge (2004: Fig. 11), but an adequate description of the anatomy is lacking. Detailed information on the anatomical interconnections between the tarsi of the pharate adult and the surrounding pupal exuviae is essential for the reconstruction of the functional principle. Comparative analyses are necessary to detect potential evolutionary changes due to the morphological modifications visible externally.

To close massive gaps in the knowledge of pupal tarsi in Trichoptera, we investigated 15 species of 14 families using confocal laser scanning microscopy. In addition, the internal structure of the tarsus is studied in detail for three species representing different morphotypes (with/without claws) using histological sections and 3D-reconstruction. The results are compared with the data available in the literature for other trichopteran, basal lepidopteran, and mecopteran taxa. Finally, the functional morphology of the pupal tarsus is reconstructed, and the pattern of presence and absence of pupal claws is discussed in comparison with the pre-eclosion behavior in different groups.

## Materials and methods

### Taxa examined

Pharate adults of representatives of four spicipalpian, three annulipalpian, and seven integripalpian families were investigated (see below). Specimens were fixed and stored in 70% ethanol. Species identification of the European material was carried out using Malicky (2004). Taxa of family and superfamily level are named according to Holzenthal et al. (2011).

“Spicipalpia”:

Glossosomatidae: *Agapetus fuscipes* Curtis, 1834  
 Hydrobiosidae: *Taschorema* sp.  
 Hydroptilidae: *Orthotrichia atraseta* Wells, 1979; *Ugan-datrichia maliwan* Malicky & Chantaramongkol, 1991  
 Rhyacophilidae: *Rhyacophila fasciata* Hagen, 1859  
 Annulipalpia:  
 Hydropsychidae: *Hydropsyche incognita* Pitsch, 1993  
 Polycentropodidae: *Neureclipsis bimaculata* (Linnaeus, 1758)  
 Philopotamidae: *Philopotamus ludificatus* MacLachlan, 1878  
 Integripalpia:  
 Brachycentridae: *Brachycentrus subnubilus* Curtis, 1834  
 Phryganeidae: *Trichostegia minor* (Curtis, 1834)  
 Goeridae: *Silo nigricornis* (Pictet, 1834)  
 Leptoceridae: *Ceraclea aurea* (Pictet, 1834)  
 Limnephilidae: *Limnephilus flavicornis* (Fabricius, 1787)  
 Odontoceridae: *Odontocerum albicorne* (Scopoli, 1763)  
 Sericostomatidae: *Sericostoma personatum* (Kirby & Spence, 1826)

## Methods

### Confocal laser scanning microscopy (CLSM)

To visualize anatomical details and the grade of sclerotization, detached forelegs were investigated using CLSM. Whole specimens or detached foretarsi were embedded in drops of glycerin or 70% ethanol between two cover glasses separated by glass spacers. The autofluorescence of the cuticle induced at 488 nm laser light was carried out using a Leica TCS-SPE confocal laser scanning microscope. The emitted light was recorded in two separate channels (500–575, 580–695 nm) and colored green and red, respectively. The overlay of both channels in volume renderings resulted in brown coloration of sclerotized structures, whereas non-sclerotized (membranous) parts of the exoskeleton remain green (see, e.g., Deans et al. 2012; Michels and Gorb 2012; Friedrich et al. 2014). Volume renderings were produced with Bitplane Imaris 8 software (MIP projection).

### Histology and 3D-reconstruction

The foretarsi of the pharate adult of *R. fasciata*, *L. flavicornis*, and *H. incognita* were cut off proximally. The samples were dehydrated and embedded in LR-White resin. Semi-thin sagittal or horizontal sections were produced with a Reichert-Jung Ultracut E microtome with a thickness of 0.99 µm using a diamond knife. Sections were stained with 1% toluidine blue and pyronin G and subsequently sealed with DPX (Fluka).

A semiautomatic slide scanner (Leica DM6000 operated by MetaMorph software) was used for the

digitalization of the section series at 20× magnification (pixel size = 0.3125 μm). Local contrast adjustments of the images were performed using Adobe® Photoshop® CS5. Images were rigidly aligned with VSG Amira 6.0.1 software. To reduce the impact of sectioning deformations on the slices, Elastic Alignment was applied by importing the pre-aligned Amira stack in Fiji software (freeware: <https://fiji.sc/>; Schindelin et al. 2012). The data were processed with the TrakEM2 module (Cardona et al. 2010) using the workflow described by Saalfeld et al. (2012).

The final data set was re-transferred into Amira to manually segment the different parts of the pupal and the imaginal tarsus. Volume visualizations of the segmented data set were carried out using Volren and VolumeRendering modules of Amira software.

### Scanning electron microscopy (SEM)

Detached pupal legs of *P. ludificatus*, *L. flavicornis*, and *H. incognita* were dehydrated in an ethanol series and critical point dried (Balzers CPD 020). Samples were glued to metal pins and placed on a rotatable specimen holder (Pohl 2010). After coating with platinum (Polaron SC7650 Sputter Coater), pictures were taken using an LEO 1525 at 5 keV.

### Post-processing

Images were adjusted (contrast, color) using Adobe® Photoshop® CS5 software and image tables assembled with Adobe® Illustrator® CS5.

## Results

In the following, we focus on the detailed description of the distal tarsomeres of the pupae of *Philopotamus ludificatus* (Fig. 1) and *Rhyacophila fasciata* (Figs. 2a, 3) as both species possess well-developed pupal claws and an overall very similar anatomical composition. Comparisons with other species are presented in “Comparative analysis”.

### General organization

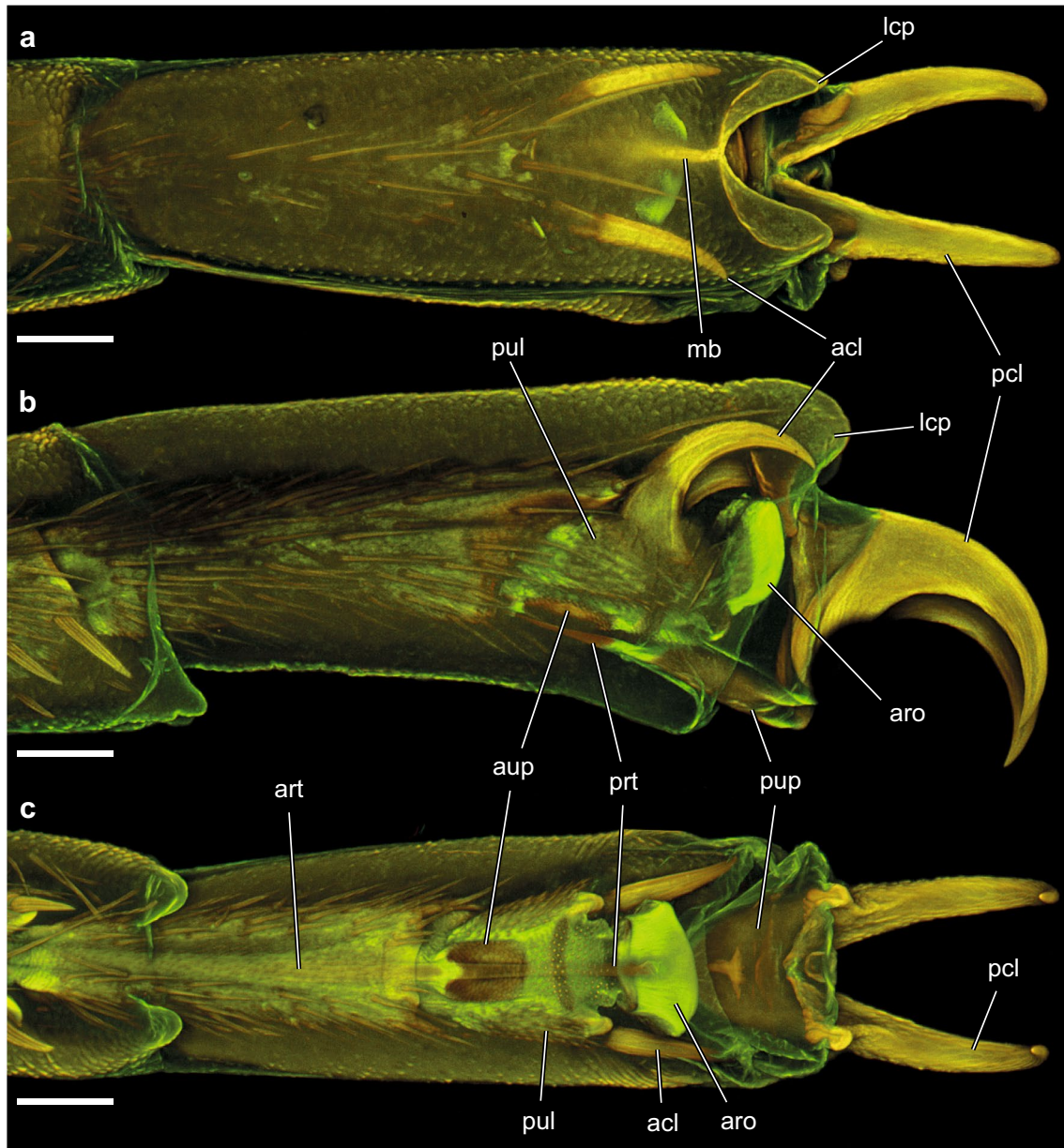
The pupal tarsus is composed of six segments. Five correspond to the five tarsomeres of the adult and a shorter, terminal one forms a cover for the adult claws (Figs. 1b, 2a). The pupal tarsomeres are thin-walled and rather weakly sclerotized (Fig. 3: pe). The ultimate tarsomere bears a pair of curved, strongly sclerotized claws. The bases of the pupal claws almost contact each other dorsally in the midline (Fig. 1a). They are interconnected by a thickened, elastic cuticle (Fig. 1a), which continues dorsally and is anchored in the thin cuticle of the fifth pupal tarsomere by a distinctly

developed median band (Figs. 1a, 3a: mb). Ventrally, the bases of the claws are movably connected to a broad, sclerotized plate, which resembles the unguitactor plate of the adult tarsus and is, therefore, named pupal unguitactor plate (Figs. 1b, c, 2a, 3a, c: pup). The tapering, posterior end of the plate is directed towards the adult tarsus, and reaches deeply into the invagination between the fifth and the sixth pupal tarsomere (Figs. 1b, 2a). Posteriorly, the plate is continuous with a strong retractor tendon interconnecting the pupal and adult tarsus (Figs. 1c, 2a, 3: prt). It runs in the midline below the adult arolium and inserts onto the hollow unguitactor tendon of the adult (Figs. 1b, c, 3). The opening is located at the base of the adult unguitactor plate and the pipe-like adult tendon forms a sheath for its pupal counterpart over the complete length of the tarsus (Fig. 3b: oart).

The tarsus of the encased adult is clearly visible through the semi-transparent pupal skin. As the pharate adult in the specimen is close to ecdysis, its tarsomeres show the usual adult sclerotization and coloration pattern. The tips of the adult claws are not inserted into their pupal counterparts but positioned above the base of the latter (Fig. 1b). The corresponding area of the pupal exuviae forms small lateral pockets and is slightly more sclerotized than the surrounding cuticle (Fig. 1c). Further tarsal attachment devices are not developed. The surface structure of the pupal tarsomeres is generally smooth and without protuberances. SEM investigations on *Philopotamus* revealed small fields of thorn-shaped microtrichia (length 6–12 μm, width 1 μm) in the disto-lateral areas of the pupal tarsomeres (Fig. 4b). The tips of these microstructures are distally directed.

### Comparative analysis

The pupal tarsi are quite similar among trichopteran taxa. Therefore, only observed differences will be noted in the following. The most striking difference is the presence or absence of claws. All species with well-developed claws (*Rhyacophila*, *Agapetus*, *Taschorema* and *Philopotamus*) have also a strongly developed retractor tendon, which is easily recognizable in CLSM images. It connects these pupal structures to the adult retractor system (Figs. 1b, c, 2a–c, 3). The tendon is not distinctly visible in CLSM data of clawless species (*Hydropsyche*, Hydroptilidae, Integripalpia; Figs. 2d, 5). However, histological sections of *Limnephilus* and *Hydropsyche* revealed the presence of the structure in these species. The tendon is comparatively thin in both cases, flattened and weakly sclerotized. It originates mesally from the ventrodistal part of the last pupal tarsomere. Like in the pupae with claws, it is inserted in the adult unguitactor tendon (Fig. 3b). In clawless species, the entire pupal cuticle is weakly sclerotized. The lateral pockets housing the adult claws are reduced as small, pointed outgrowths



**Fig. 1** Pharate adult of *Philopotamus ludificatus* (Philopotamidae), distal tarsomeres of the foreleg. CLSM images. **a** dorsal view; **b** lateral view; **c** ventral view. *acl* adult claw, *aro* arolium, *art* adult retrac-

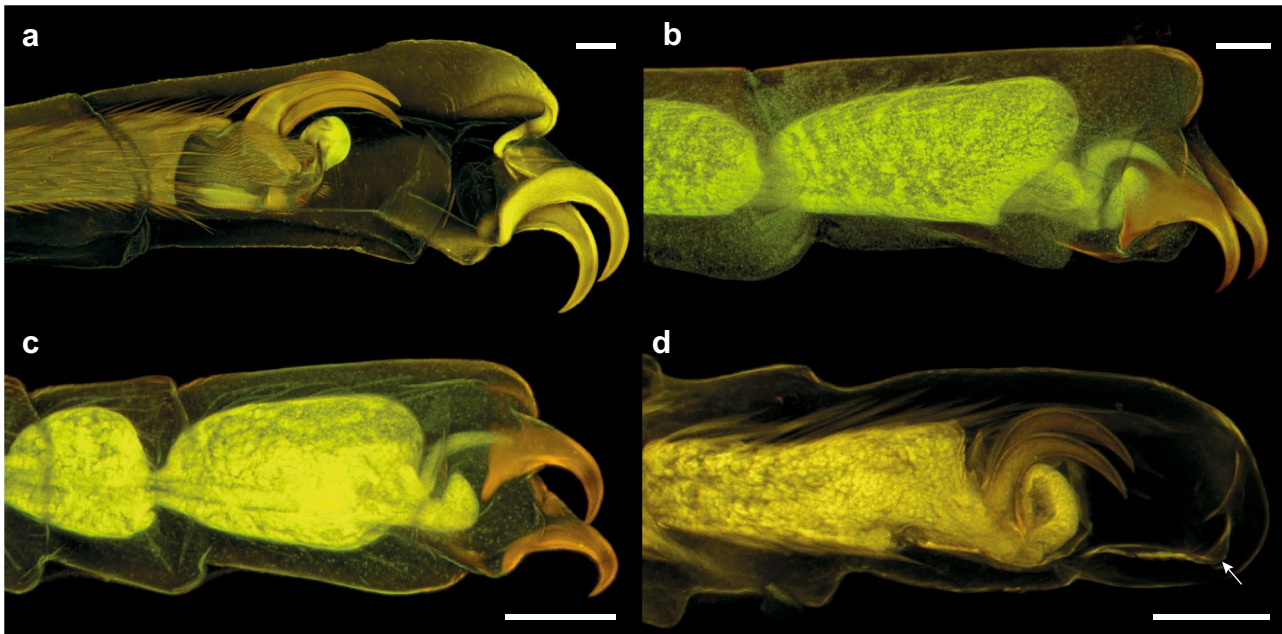
tor tendon, *aup* adult unguitractor plate, *lcp* lateral claw pouches, *mb* median band, *pcl* pupal claw, *prt* pupal retractor tendon, *pul* pulvillus, *pup* pupal unguitractor plate. Scale bars: 50  $\mu$ m

in the dorsolateral areas (Fig. 5b–d, g, h). They are usually weakly sclerotized.

A very specific condition of these pockets in odonotocerid and phryganeid species is the presence of similar hook-shaped structures (Fig. 5e, f). These appendages differ strikingly from the typical claws found in other species (compare Figs. 3a and 5f) and are, therefore, addressed as hooks in the following. They are less curved and distinctly less sclerotized than claws. In contrast to claws, they are not articulated with the pupal tarsus. The adult claws are placed at the base of the hooks in our specimens and do not enter

the hook. However, it has to be noted that the position of the adult tarsus within the pupal exuviae is often artificially shifted, during the primary fixation of the insect or during preparation for CLSM imaging (see, e.g., Figs. 2a, d, 5g). Therefore, an artificial retraction of the adult leg cannot be excluded in some cases.

A unique configuration of reduced pupal claws was found in the polycentropodid *Neureclipsis*. A vestigial pair of short, slightly curved, and moderately sclerotized processes resembles the apical parts of typical claws (Fig. 5a: rc). Similar to well-developed claws, these processes emerge from



**Fig. 2** Distal tarsomeres of the foreleg of the pharate adult in spicipalpi species. CLSM images. **a** *Rhyacophila fasciata* (Rhyacophilidae); **b** *Taschorema* sp. (Hydrobiosidae); **c** *Agapetus fuscipes*

(Glossosomatidae); **d** *Ugandatrichia maliwan* (Hydroptilidae). Arrow indicates claw pockets. Scale bars: 50  $\mu$ m

a comparatively well-sclerotized apical area of the tarsus, which is dorsally anchored with a short but distinct median band (Fig. 5a: mb). Typical ventrolateral claw joints are missing. Instead, the ventral region below the claw remnants bears a straight, ventrally directed process, which is apically less sclerotized (Fig. 5a: vp). The configuration of this process differs distinctly from the hooks described above. The adult claws were never directly inserted into the ventral processes in the two specimens investigated.

The microstructure of the tarsal surface is very similar in all species studied. However, the microtrichia are shorter and less numerous in the annulipalpi representatives compared to *Limnephilus* (Fig. 4).

## Discussion

### Functional interpretation of claw movement

A placement of well-sclerotized pupal claws in the otherwise thin and membranous cuticle of the pupal exuviae raises the question how these structures are moved and stabilized when used as attachment structures. The functional principle can be reconstructed based on a combination of the sclerotisation pattern revealed by CLSM and histological data.

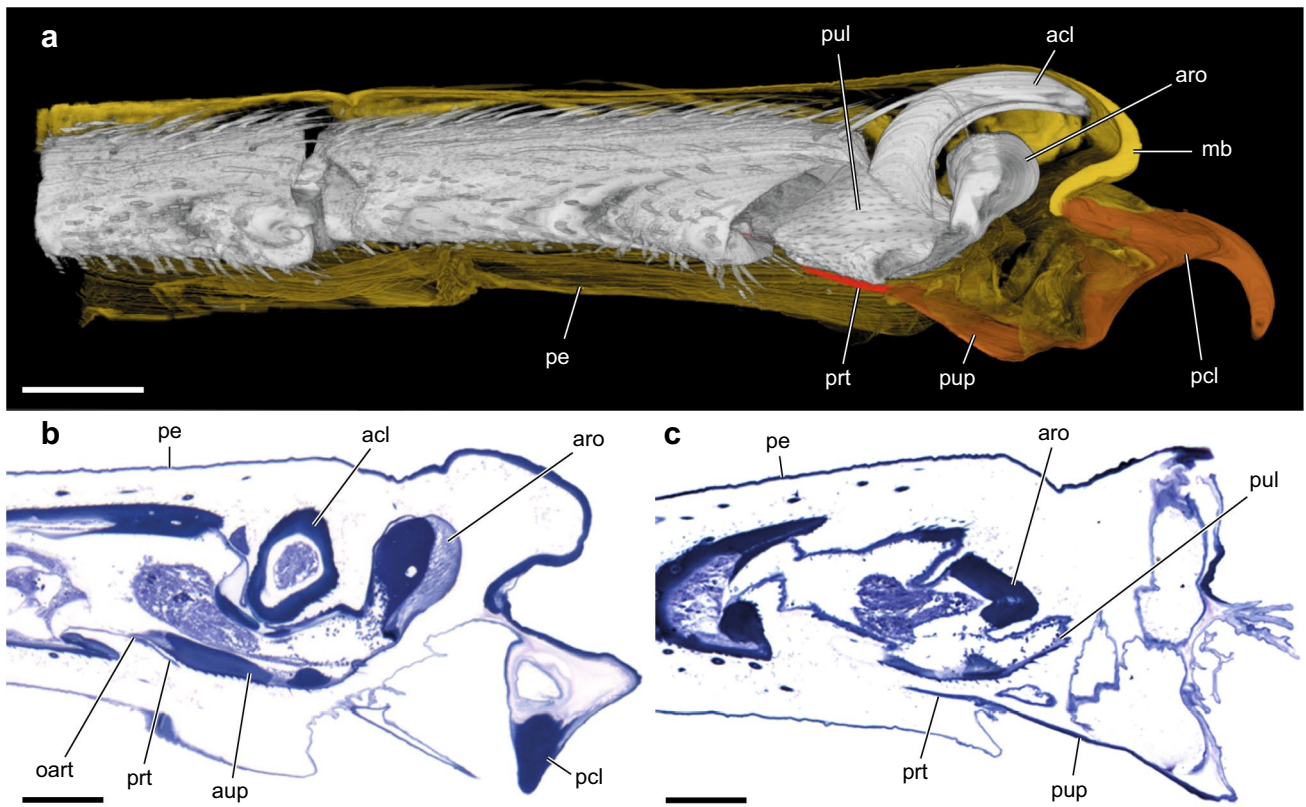
In all insects, the adult claws are moved by a retractor muscle via a long tendon attached to the proximal end of the unguitactor plate (Snodgrass 1935; Beutel et al. 2014).

As the adult claws are not directly inserted in their pupal counterparts in caddisflies, a direct interaction and joint movements are unlikely. The pupal claws are pulled downwards by the force of the adult retractor tendon. However, the transmission is achieved by an additional pupal tendon and not by the adult claws. This interconnection between the adult retractor tendon and the pupal unguitactor plate is the key for understanding operations of the pupal claws. Joints at the ventrolateral sides of the claws and a thickened, semimembranous area above them are well suited to guide the movements. The adult claws housed in dorsolateral pockets potentially stabilize the pupal tarsus and might indirectly support the flexion of the entire pupal tarsus. The movements of the tarsus and its claws are not independent as both unguitactor plates are simultaneously operated by the retractor tendon.

This general mode of interconnection between pupal and adult tarsus is still preserved in forms lacking claws. The tips of the adult claws are usually housed in small pouches and the pupal tendon is attached to the ventromedian area of the tarsal tip. In combination, both structures support the flexion of the entire tarsus.

### Distribution of pupal claws

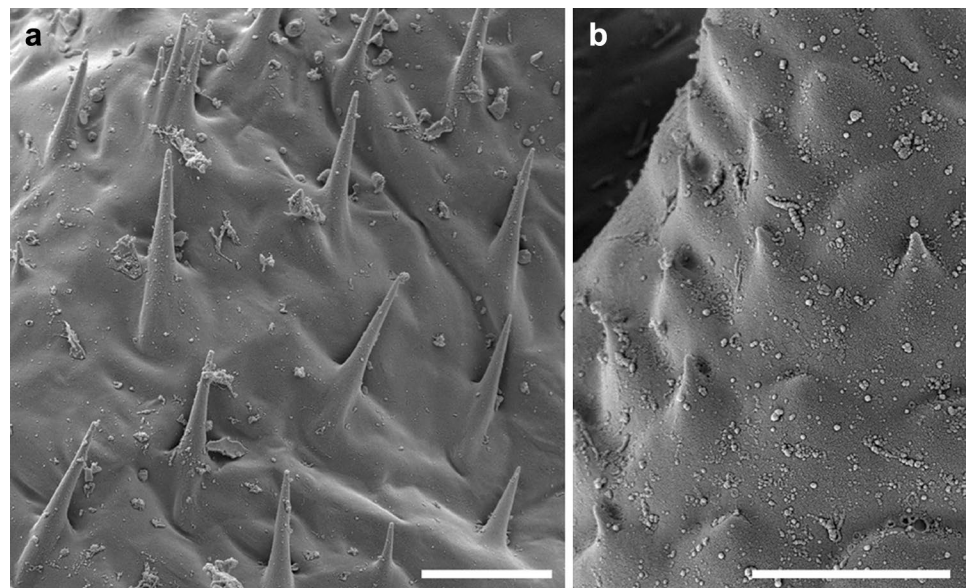
The morphology of the sixth pupal tarsomere differs strikingly among trichopteran lineages. Well-developed and sclerotized claws are present in some groups, whereas the

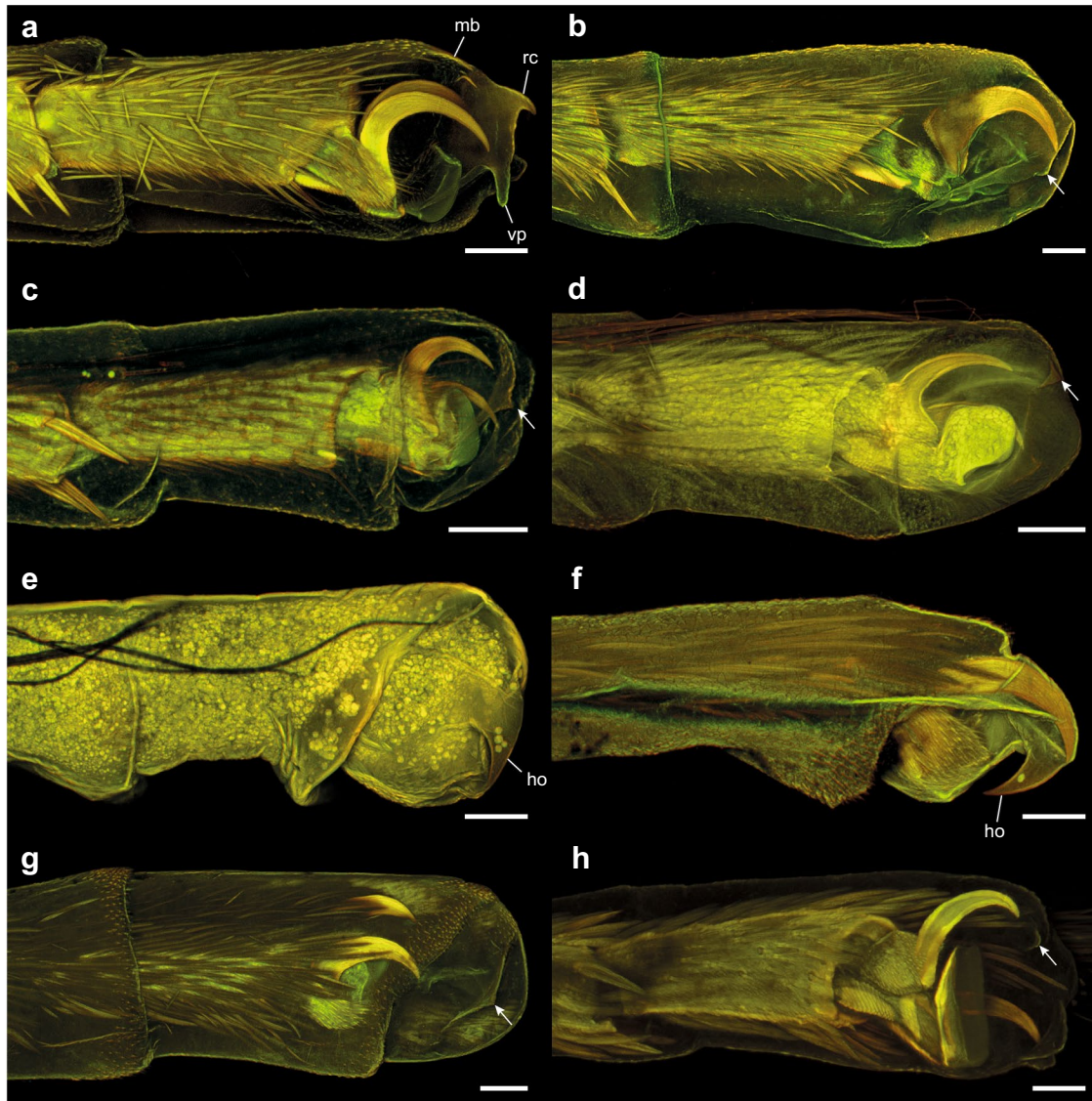


**Fig. 3** Pharate adult of *Rhyacophila fasciata* (Rhyacophilidae), distal tarsomeres of the foreleg. **a** Volume rendering of digitalized histological longitudinal sections. Coloration: grey—leg of the adult, yellow—pupal exuviae, brown—pupal claws, red—pupal retractor tendon. **b** Histological longitudinal section showing the pupal retractor tendon leaving the adult tendon at the base of the adult unguitrac-

tor plate. **c** Histological longitudinal section showing the insertion of the pupal retractor tendon on the pupal unguitactor plate. *acl* adult claw, *aro* arolium, *aup* adult unguitactor plate, *mb* median band, *oart* opening of adult retractor tendon, *pcl* pupal claw, *pe* pupal exuviae, *prt* pupal retractor tendon, *pul* pulvillus, *pup* pupal unguitactor plate. Scale bars: **a** 100  $\mu\text{m}$ ; **b**, **c** 50  $\mu\text{m}$

**Fig. 4** Distally directed microtrichia in the ventrolateral part of the distal tarsomere of the foreleg. SEM micrographs. **a** *Limnephilus flavicornis* (Limnephilidae) **b** *Philopotamus ludificatus* (Philopotamidae). Scale bars: 10  $\mu\text{m}$





**Fig. 5** Distal tarsomeres of the foreleg of the pharate adult or pupa in annulipalpien and integripalpien species. CLSM images. Arrows indicate claw pockets. **a** *Neureclipsis bimaculata* (Polycentropodidae); **b** *Hydropsyche incognita* (Hydropsychidae); **c** *Silo nigricornis* (Goeridae); **d** *Brachycentrus subnubilus* (Brachycentridae); **e** *Tri-*

*ostegia minor* (Phryganeidae); **f** *Odontocerum albicorne* (Odonotoceridae); **g** *Sericostoma personatum* (Sericostomatidae); **h** *Ceraclia aurea* (Leptoceridae). *ho* hooks, *mb* median band, *rc* remnant of pupal claw, *vp* ventrad process. Scale bars: 50  $\mu$ m

tarsi of others bear short, poorly sclerotized hooks or lack them completely. Besides the species described above, the presence of well-developed pupal claws is recorded in the literature for Ptilocolepidae (Thienemann 1904; Gonzáles et al. 2000), Psychomyiidae (Thienemann 1905), and *Holocentropus* (Polycentropodidae; Ulmer 1903). The combination of shortened claws with secondary, ventrally directed processes in the polycentropodid *Neureclipsis bimaculata* (Fig. 5a) is a new type and has not been described so far.

Distinct claws are lacking in Apatiniidae, Molannidae, Leptoceridae, Beraeidae, Sericostomatidae, and some polycentropodids (Ulmer 1903; Thienemann 1905; Hamilton

1985). Weakly sclerotized hooks are described for Odonotoceridae and Phryganeidae (Ulmer 1903; Thienemann 1905). In contrast to Ulmer (1903), Thienemann (1905: 69) found “long, strongly curved, well-sclerotized, pointed claws” (translation from German) in the lepidostomatid species *Lepidostoma basalis* and *Crunoecia irrorata*.

There are different options for interpreting this pattern:

1. Correlation with the larval habitat: fast-running streams (pupal claws present) vs. stagnant water (without or with strongly reduced claws) (e.g., Ulmer 1903; Thienemann 1905).

2. Relation of tarsal morphology and climbing habits of the pupae (e.g., Ulmer 1903; Hickin 1967).
3. Phylogenetic constraints: The presence of claws is an ancestral (plesiomorphic) feature, whereas the absence is a derived feature.

The first option was already discussed critically by Ulmer (1903). Even though many species living in fast-running streams (e.g., *Rhyacophila*, *Philopotamus*) have well-developed claws, pupae of co-occurring taxa such as *Hydropsyche* or *Brachycentrus* completely lack these structures. Furthermore, the tarsi of some species inhabiting stagnant waters also bear strong claws (e.g., *Holocentropus*; Ulmer 1903). In this context, it has to be noted that different species developing in the same habitat might display different behavioral patterns between leaving the cocoon and shedding the pupal skin. Hickin (1967: p. 44) mentioned casually that “The relation between the tarsal claws of the pupae and the habit of climbing from the water before emergence of the adult is easily seen”. Unfortunately, the precise behavior of the pharate adults leaving the water is only described for few species. Ulmer (1903) proposed that claw-bearing pupae crawl or climb out of the water, whereas those without claws have to swim using the rows of swimming hairs on the midlegs (option 2). This is true for some taxa, as for instance of Ptilocolepidae, which do not swim but climb on moss or other plants out of the water (Thienemann 1904). However, as shown above, representatives of the closely related Hydroptilidae lack pupal claws despite living in similar hygropetric habitats (Wells 1985). Several other examples do not fit with this explanation. It is widely known that the pupae of most limnephilids are excellent swimmers (e.g., Wiggins 2004). Our own observations show that they are also good climbers—despite the lack of claws. Leaving the water using smooth vertical sticks always worked during a rearing experiment with more than 100 specimens. In contrast, the claw-less pupae of *Hydropsyche pellucidula* failed in the same setup. They were only able to crawl out of the water on more or less horizontal surfaces. These tests showed that the lack of claws is not an indirect proof for a poor climbing performance. The fields of distally directed microtrichia positioned ventrolaterally on the tarsomeres of *Limnephilus* (Fig. 4a) very likely support the climbing on this kind of plant material. Microtrichia are distinctly shorter in *Hydropsyche* and *Philopotamus* (Fig. 4b) and the areas are smaller. It has to be noted that the adult claws had pierced through the pupal exuviae in some specimens prior to eclosion in our rearing experiments. This could be an artefact or might have been used to enhance the climbing capacity, compensating for the lack of pupal claws.

## Phylogenetic implications

In contrast to Trichoptera, which usually pupate underwater, pupation takes place in terrestrial habitats in most representatives of other holometabolous orders (e.g., Kalténbach 1978; Crowson 1981; Kristensen 1999). In the sister group, Lepidoptera pupal claws are present in Agathiphagidae (Kristensen 1999: Fig. 4.2.D), but seem to be absent from Micropterigidae (Lorenz 1961) and Eriocraniidae (Hinton 1946). Within Mecoptera, “a pair of minute recurved hooks” is described for Nannochoristidae by Pilgrim (1972: p. 162). Similar tarsal structures are absent from the pupae of Boreidae (*Boreus*; Cooper 1974: Fig. 3J), Bittacidae (Currie 1932; Setty 1940), and Panorpidae (Byers 1963). Different conditions are also present in neuropteroid insects. In Raphidioptera and Megaloptera (see, e.g., Kluge 2004; Kalténbach 1978), claw-like structures are developed. The detailed drawing of *Raphidia* by Kluge (2004: Fig. 44) shows that the pupal claws house the adult ones like a glove. The same seems to be true for Megaloptera (e.g., Barnard 1931: Fig. 5). Unfortunately, there is no information given on the grade of sclerotization of these pupal claws. They appear to be used as a sheath for the adult claws rather than as a climbing device. In Neuroptera, prominent tarsal claws are generally missing. The description of a *Chrysopa* pupa by Kluge (2004: Figs. 39, 40) fits perfectly with our observations on an undetermined chrysopid species. Both show tiny, weakly sclerotized hooks, which do not house the tips of the adult claws. A very similar condition is found in Myrmeleontidae (pers. obs.). This suggests that claws are a plesiomorphic feature of decteous pupae. It is also evident that the pupal claws were independently reduced within the orders.

The data at hand suggest that the presence of pupal claws is a groundplan feature of Trichoptera. The claws were apparently lost several times independently within the order, at least two times within Annulipalpia, i.e. in *Hydropsychidae* and within *Polycentropodidae*. However, the evolution of this character in the latter family cannot be reconstructed with the presently available data. Well-developed claws are reported from *Holocentropus* (Ulmer 1903), whereas other species of other genera lack these structures (Thienemann 1905). *Neureclipsis bimaculata* possess distinctly reduced, claws in combination with a unique ventrally directed process (Fig. 5a). Detailed investigations on further species are necessary to elucidate this issue.

Despite the independent loss of pupal claws within Annulipalpia, the consistent lack of these structures in Integripalpia could be an autapomorphy of the suborder. Candidates for the sister group of Integripalpia were extensively discussed over the last decades (see, e.g., Malm et al. 2013). Although there are still competing hypotheses, the majority of morphological analyses favored Hydroptiloidea (= Hydroptilidae + Ptilocolepidae sensu Malicky



2001, 2005) as potential candidate (e.g., Ross 1956, 1967; Frania and Wiggins 1997; Ivanov 2002). The monophyly of Hydroptiloidea was never seriously questioned. This and the presence of claws in Ptilocolepidae (Thienemann 1904; González et al. 2000) clearly contradict the interpretation of lacking claws as synapomorphy of Integripalpia and Hydroptiloidea. Based on the current knowledge, it has to be assumed that the pupal claws were independently lost in the last common ancestor of Integripalpia, and again within the hydroptiloid lineage.

The loss of claws results in distinctly simplified distal tarsomeres in Integripalpia and other groups. However, tiny pouches are present in all these taxa, pointed in some cases and housing the tips of the adult claws. These structures are likely used for covering the tips of the adult claw to prevent the penetration of pupal exuviae under water. It is also conceivable that they enhance the climbing ability.

The secondary hook-like structures of Phryganeidae and Odontoceridae have very likely evolved independently in both groups, as a close relationship can be excluded. Phryganeidae are part of Plenitentoria, whereas Odontoceridae belong to the Brevitentoria lineage of Integripalpia (e.g., Kjer et al. 2002; Holzenthal et al. 2007; Malm et al. 2013).

## Conclusion

The morphology of the pupal claw system shows an unexpected variability within Trichoptera. The loss of claws and the formation of secondary hooks and processes are very likely results of common ancestry in some groups (e.g., Integripalpia). However, it is also certain that claws were reduced several times independently (e.g., in Hydropsychidae, Hydroptiloidea and within Polycentropodidae). The reasons for the loss of claws are very likely not the same in different groups. A simple link to a single behavioral characteristic might apply for some groups, but a more complex functional background appears more likely in most cases. Unfortunately, the behavior of the pharate adult is not well-covered in the literature. The steps between leaving the cocoon and shedding the pupal exuviae are documented for only few species (e.g., *Hydropsyche*; Schumacher 1970; *Ptilocolepus*; Thienemann 1904). Comparative analyses of closely related species (e.g., Polycentropodidae) are not available. In summary, more information on the behavior of the pharate adults is needed to unravel the mix of phylogenetic, functional, and behavioral constraints leading to the diversity of trichopteran pupal attachment structures. Furthermore, a standardized treatment of the pupal pretarsus in taxonomic descriptions of this life stage (as already done with mandibles and abdominal hooks) would help to increase the understanding of this character system.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or living animals performed by any of the authors.

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